Nikon

NIS-Elements AR

(Advanced Research)

User's Guide

(Ver. 4.50)

Thank you very much for choosing Nikon.
This manual explains installation and use of the NIS-Elements Advanced Research. For trouble-free operation, read this manual before using the program.

To run NIS-Elements Advanced Research optimally, the following specifications are required.
Minimum PC Specification:
   CPU: Intel Core 2 Duo or higher
   RAM for 32bit OS: 2GB or higher
   RAM for 64bit OS: 4GB or higher
   Operating system: Windows 7 Professional SP1 64bit (English)
   HDD: 2GB or more of available hard disk space for installation
   Display: 1280x1024 pixel (True Color mode)
   User rights: Administrators for installation

* Operation cannot be guaranteed on all computer models. For further information, contact your nearest Nikon representatives.

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1. Command Line Startup Options

See also 2.1.3. Command Line Installation Options.

When starting *NIS-Elements* from the command line (or when editing the desktop shortcut properties), you may append some switches with parameters to the main command and thereby modify the startup behavior.

> Note
>
> *Some switches do not have parameters.*

Example 1.1. Command line switches syntax

" *NIS-Elements* executable" *switch#1 parameter#1 switch#2 parameter#2* etc.

For example, to run *NIS-Elements AR* and open the *starting_image.jp2* at straight away, run.:

```
"c:\Program Files\NIS-Elements\nis_ar.exe" -f "C:\Images\starting_im-
age.jp2"
```

Startup Switches

-? Displays a help screen with the description of switches (basically this page).

-c "Command" The application runs the specified macro *Command*.

-cam "Camera Name" The specified Camera will be used. When using this option you must also specify the *Grabber Name* and the *HW Unit Name*. To find the camera name, search the application log file for "Camera Name". It is also possible to use only a part of the Camera Name, but the part of the name must be unique among all the available cameras.

-cw "Command Wait" The application runs the specified macro command and waits until it is completed

-f "Filename" The application tries to open the image pointed to by the *Filename* parameter.

-g "Grabber Name" The image grabber (driver) of the specified *Grabber Name* will be used and no camera-selection window will show at startup. Set the parameter value to "last" and the most recently used grabber/camera will be loaded. As the grabber name, you must use internal name of the grabber, not the one you see in the startup dialog window.

Either use one of the following names or search the application log file for the phrase "Grabber Name" (see Fixed Grabber Startup).

-gn No grabber (camera) driver will be loaded. Image acquisition will not be possible.

-h "HW Unit Name" The specified HW Unit will be used. When using this option you must also specify the Grabber Name. Two HW Units (e.g. two DS-U2 cameras) can be run by one grabber/driver. The available HW Units are listed in the *Acquire > Select *camera name** window. Set the parameter value to "select" and the selection box will be shown. When using this option you must also use the *-g* switch. To find the HW Unit Name, please search through the application log file for "HW Unit Name".

-i "Config Name" A user can specify the configuration to be used by *NIS-Elements*. The default config-uration is saved in the "C:\ProgramData\Laboratory Imaging\Platform" folder. If you want to create a

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custom configuration, use the *-i* switch, e.g.: `nis_ar.exe -i "My_Configuration"`. The configuration of this instance will be saved to the `"C:\ProgramData\Laboratory Imaging\My_Configuration"` folder.

This is useful when using two cameras on one microscope for example. You can create a separate program shortcut which uses the -i switch to load a different configuration.

-l "Language" The application will run in the specified language (if available). Use three-letter language codes according to the ISO 639 [http://en.wikipedia.org/wiki/List_of_ISO_639-2_codes] standard.

-m "Macro File" The application will execute the macro file (*.mac) pointed to by the *Macro File* parameter.

-mw "Macro File" The application will execute the macro file (*.mac) pointed to by the *Macro File* parameter and wait until it is completed.

-p "Command" ["Param"] The application window will be placed according to the *Command*. Possible values are:

- *left*, *right*, *top*, *bottom* - the window is placed on the corresponding monitor screen.

- *monitor N* - the window is placed on the Nth monitor.

- *rectangle (x0,y0,x1,y1)* - the window is placed in the specified rectangle (in workspace coordinates). The coordinates must be in parenthesis without any spaces.

-passive The application will not connect to any device on startup (*NIS-Elements AR* will start with blank Device Manager). Camera selection is not affected by this option.

-q A new instance of the application will run. By default, only one instance of *NIS-Elements* is allowed to run.

-s "Settings File" The application will use the specified *Settings File* to load and save its settings. The settings file can be created from MS Windows Start menu by the *NIS Settings Utility*.

-var, -vbr, -vd, -vf For testing purposes, you can switch the application to run as if you have a different version (AR/BR/D/Freeware) installed. However, you have to have the corresponding licence written in your key (Even if you normally run NIS-AR, the *-vd* switch will not work to run NIS-D unless you have also the NIS-D licence).

> Note
>
> *Mind that the set of functions you will get once the software is run depends on the setup file used for installation (for example, some functions of NIS-D are not included in the NIS-AR setup so these will not appear in the software run by:* `nis_ar.exe -vd`

Fixed Grabber Startup

Each time *NIS-Elements AR* is run or the grabber / camera / HW unit is changed, four parameter values are written to the application log file (C:\ProgramData\Laboratory Imaging\Platform\Logfiles).:

- Grabber Name

- HW Unit Name

- HW Unit Connection String

- Camera Name

Let's say we want to set the last used configuration for every *NIS-Elements AR* start:

1. Lookup the parameters in the most recent log file. E.g.:

  Grabber Name CLxGrabberDriverSim

  HW Unit Name SimGrabber

  HW Unit Connection String (empty)

  Camera Name Sim_Camera_Color

2. Modify the shortcut, E.g.:

  ```
  "c:\Program Files\NIS-Elements\nis_ar.exe" -g CLxGrabberDriverSim -h
  SimGrabber -cam Sim_Camera_Color
  ```

3. Double-click the shortcut. *NIS-Elements* will start without asking for grabber selection

2. Installation and Settings

2.1. Installation and Updates

2.1.1. The Installation DVD-ROM Content

- *NIS-Elements* software setup file

- Drivers and utilities for the HASP Key

- Drivers for selected cameras

- User's Guide in the PDF file format

- Sample image database

- Sample ND2 image sequences

2.1.2. NIS-Elements Installation Steps

Caution

You have to possess the administrator rights to your computer to be able to install NIS-Elements AR successfully.
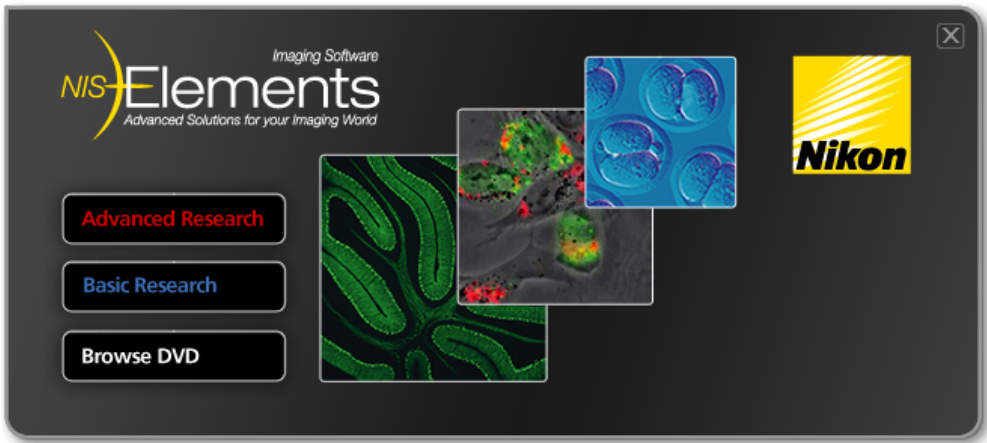
Quick Guide

- Insert the installation DVD in the DVD-ROM drive. A window automatically appears.

- Install the selected *NIS-Elements* software version, additional modules and device drivers.

- Plug the provided HASP key into the USB port of your PC.

- Run *NIS-Elements*.

1)   When the installation DVD is inserted, a selection window appears automatically.

Select the software package to be installed. Select the one you have got the license for and which is properly coded in your HASP key. The installation wizard welcome-window appears. Click *Next* to continue.

2)   **Install Local Options**

Figure 2.2. Local Options installation
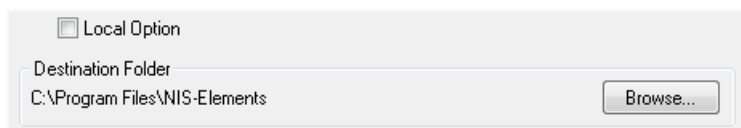
Select whether to install *Local Option* or not. Define the folder where *NIS-Elements AR* should be installed. We recommend to use the predefined directory. If you want to change the directory anyway, press the *Browse* button and select a new one. Otherwise click *Next*.

Note

The Local Option installation provides some advanced features which did not pass the quality assurance procedure yet. We recommend to wait until they are released officially.

3)
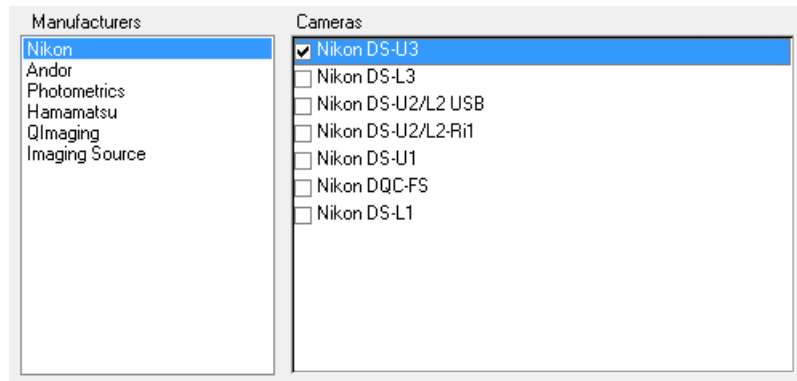
Now, select the cameras which will be used with *NIS-Elements AR*.

4)
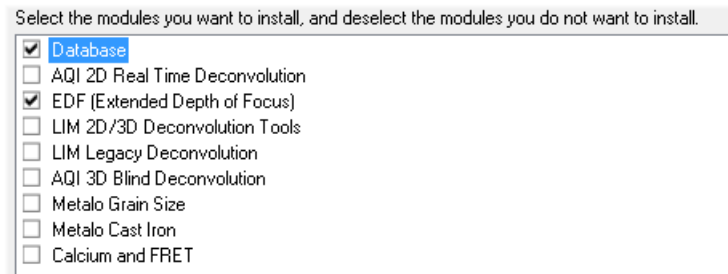
If your licence contains some additional modules besides the *NIS-Elements AR* core software, please select them in this window.

Note
_____

Any module selected will be installed along with NIS-Elements AR automatically. However, you might not be licensed to use it. The module will run after you get the corresponding code registered in your HASP key.

5)

Select the devices which will be used with *NIS-Elements AR*. Finish the installation by clicking the *Install* button.

Note

NIS-Elements may not be connected to devices after installation depending on the PC status at installation. If so, execute the Modify installation command of the Windows Start menu (see the next step) to perform Repair that corrects the previously installed status.

Warning

For Windows7 users: Some devices do not work correctly after recovery from Sleep mode. Please turn off the Windows 7 Sleep Mode to prevent possible problems:
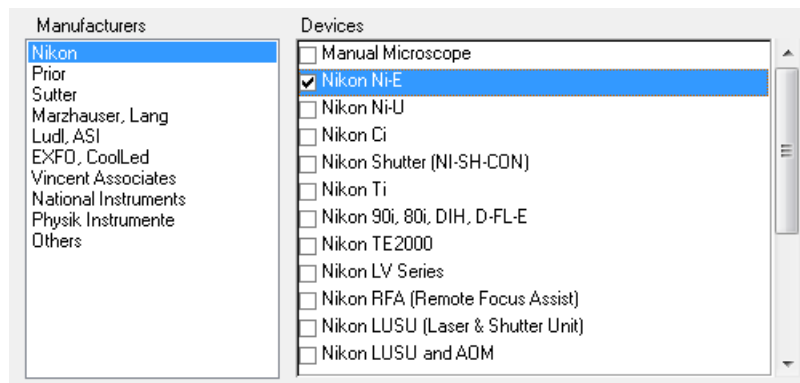
1. *Run the Start > Control Panel command on the main Windows toolbar.*

2. *Make sure the control panel is displayed in the Category mode (View by in the top-right corner).*

3. *Click Hardware and Sound*

4. *In the Power Options section, click Change when the computer sleeps*

5. *Set the Put the computer to sleep option to Never*

6. *Confirm the settings by the Save changes button and close the Control Panel window.*

6)
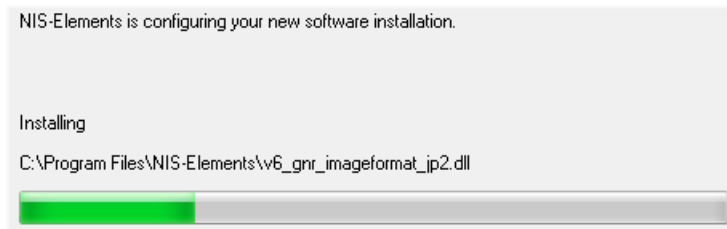
The setup creates a new program group in the *Start* menu containing the following items: *NIS-Elements AR* application shortcut, the HASP key information shortcut, the *Modify Installation* shortcut (for adding hardware drivers, modules, etc.), the *Uninstall* procedure, and the Send Info Tool. A shortcut to *NIS-Elements AR* is created on the desktop too. These changes affect all user profiles of your local Windows operating system.

Figure 2.7. Start menu

Note

Clicking the Uninstall command deletes all installed files from disk, and removes the NIS-Elements AR program group from the Start menu as well as it removes the desktop icon.
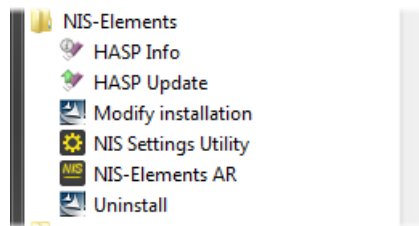
2.1.3. Command Line Installation Options

See 1. *Command Line Startup Options* for details about how to use command line switches. The following switches are to be used with the *NIS-Elements* installation file to modify behavior of the installation wizard.

-all This parameter unhides options in the setup which are hidden by default. It concerns mainly drivers of devices which were formerly supported but are no longer available.

-new If this command line parameter is appended, the setup checks if any other setup has already been installed. If so, the setup restarts and runs in an independent mode. The update mode is disabled. This enables to install two or more builds together on one operating system.

-xp This parameter enables the user to install *NIS-Elements* on Windows XP even though this operating system is NOT supported officially.

2.1.4. Additional Module/Device Installation

You may need to install a device or an additional module after the *NIS-Elements AR* main system installation.

• Go to [Start menu > Programs > *NIS-Elements AR*] program group.

• Select the *Modify Installation* command.

• Follow the installation wizard instructions. Select the check-boxes by the items you would like to add.

• Finish the installation.

2.1.5. Sample Database Installation

If you chose to install the Sample Database, a new subdirectory "Databases" is created inside the *NIS-Elements AR* installation directory (e.g. C:\Program files*NIS-Elements AR* \Databases\...). The "Sample_Database.mdb" file is copied to there along with database images (stored in subdirectories). An administrator username/password to access this database is set to:

• Username: "sa"

• Password: "sa"

2.1.6. Software Copy Protection

The *NIS-Elements AR* software is delivered with a hardware key (also called HL = hardware licence).

Figure 2.8. Hardware key

The key contains information about the software licence and allows users to run the corresponding software. A warning message is displayed when user starts NIS-Elements with incorrect HASP. Please connect the USB HASP after the *NIS-Elements AR* installation is finished. The utility called HASPinfo is

installed to the *NIS-Elements AR* directory. It enables the user to view information about the software licence and is accessible via the   *Hasp Info* menu command.

2.1.7. Device Updates

The main goal of the Device Updates setup is to add new devices/cameras or to fix problems that are localized in the drivers only and do not affect any other *NIS-Elements* functionality.

Device Update setup requires installation of the corresponding *NIS-Elements* version. The setup contains only .dll files supporting a new device or some corrections and upgrades of already existing drivers. This update form keeps *NIS-Elements* stable and the update file size small.

Device Update numbering is consecutive and appropriate to its *NIS-Elements* version until a new full setup of *NIS-Elements* is released (Major version, Service Pack or HotFix) - then the numbering starts from the beginning.

Device Updates are cumulative and contain all the changes from the previous updates. Also full *NIS-Elements* setups (Major versions, Service Packs and HotFixes) will contain all the changes from the previously released Device Updates.

2.1.8. Fixes

Fixes represent driver changes which serve for testing purposes or for correcting problems occurring only under special conditions. Fixes are packed into a zip file containing modified driver files. Fixes are usually user requested, therefore they are not considered as Device Update candidates.

2.1.9. Installing the Database Module on 64-bit Systems

The *NIS-Elements* installation file contains 64bit MDB drivers which improve the speed of the database module on 64bit systems. However, 32bit version of MS Office 2010 cannot be used along with these 64bit drivers. You can install these drivers, if:

- you run 64bit version of MS Office 2010

- you use other version of MS Office such as Office 2013

- you do not use MS Office at all

Find the drivers in the *NIS-Elements* folder, typically: "C:\Program Files*NIS-Elements*\Drivers\Database_MDB\"

2.2. Additional Modules Available in NIS-Elements AR

2.2.1. Acquisition and Analysis Modules

3D Measurement If you have this module enabled in HASP, some extra commands and features for analysis of multiple binary layers over Z become available in the *Binary* menu.

6D This module enables users to combine all 6 dimensions (X, Y, Z, time, wavelength, large image) within a single ND acquisition experiment.

Advanced 2D Tracking This module extends the basic *Object Tracking* module by the ability to track binary objects and by other features.

Bio Analysis *(requires: 64bit NIS-Elements)* This module enables to perform bio-analytical tasks. The Bio Analysis module measures and analyses time-lapse and multipoint ND images (Z-stack data sets are not supported).

CA FRET With this module, you can capture/create FRET images, measure Calcium ion concentration or make automatic FRET calibrations.

Extended Depth of Focus The EDF module allows you to combine an existing Z-stack of images into one focused image by picking the focused regions from each frame and the pieces together.

HC Template It is a simple-to-use acquisition and high content analysis module. Using a pre-defined template, you can acquire image data-sets, perform high content analysis and browse the resulting data.

High Dynamic Range This module brings the functionality of capturing and creating HDR images within *NIS-Elements AR*.

Illumination Sequence This module provides an easy graphical user interface for setting and running advanced real-time (triggered) acquisition experiments.

JOBS Editor JOBS is an environment aiming at complex acquisition and data analysis with easy to use and comprehensive user interface. JOBS is a "visual programming" tool enabling non-programmers to design their own automated acquisition / analysis experiments. This module allows user to create, edit and run JOBs.

JOBS Remote Database With this module, you can easily share the JOBS database. Typically, it is used on multi-workstation systems where one PC is used for data acquisition and another for performing analyses.

JOBS Viewer This module enables user to browse the data acquired via *JOBs*.

ND Advanced Acquisition *(requires: Local Option)* This module enables users to perform complex timelapse/multipoint ND acquisitions and also adds some other features. Please ask your local dealer of *NIS-Elements* for details.

Object Classifier Provides the ability to classify binary objects.

Object Tracking Motion characteristics of objects such as living cells can be measured automatically or manually using the *Tracking* module. Tracking is based on ROIs.

RT Acquisition This module user to perform fast hardware-driven (triggered) acquisition experiments.

Signature *(requires: Local Option)* With this module, you can measure multiple features in the image consecutively.

Single Particle Tracking Single Particle Tracking (SPT) is used to track single molecules and for analysing parameters of these tracks.

Time Measurement The time measurement tool enables to record average pixel intensities within Regions Of Interest (ROIs) during a time interval. Please see 8.4. Time Measurement.

2.2.2. Deconvolution Modules

2D Deconvolution Image deconvolution module suitable for standard 2D images. Apart from the 2D deconvolution, it provides a *Fast Deconvolution* and *Live De-Blur* (applicable on live image).

3D Deconvolution Image deconvolution module suitable for image sequences containing a Z dimension.

Blind Deconvolution AutoQuant blind deconvolution module.

LIM Deconvolution (Legacy) A simplified predecessor of the new *2D/3D Deconvolution* modules.

RT Deconvolution AutoQuant deconvolution module aimed at real-time deconvolution.

2.2.3. Industrial Modules

Filters Particle Analysis *(requires: 64bit NIS-Elements)* This industrial module is aimed at analysis of filters according to the ISO 16232 standard.

Metalo - Cast Iron Analysis After the module installation, a new item called *Metallography* appears in the *Applications* menu. Select the *Cast Iron* command to display the cast iron measurement layout.

Metalo - Grain Size Analysis After the module installation, a new item called *Metallography* appears in the *Applications* menu. Select the *Grain Size* command to display the grain size measurement layout.

2.2.4. Device Modules

Slide Loader This module enables *NIS-Elements AR* connect and control a slide loader

Stage This module enables to connect and control motorized XY stages.

Stage Incubator This module enables to connect and control gas and temperature-controlling chambers.

TTL/Analog IO This module enables *NIS-Elements AR* to send and receive TTL and analog signals via a DAQ board.

Wavelength Switcher This module enables *NIS-Elements AR* connect and control wavelength switchers.

Well Plate Loader This module enables *NIS-Elements AR* connect and control a well plate loader.

Z Drive This module enables connect and control motorized Z drives.

See also 4.1.4. Connecting a Device to NIS-Elements .

2.2.5. Other

64bit NIS-Elements Some functions and applications run only under the 64bit version of *NIS-Elements*. Unlike with 32bit applications, the size of memory available to a single application is not restricted on 64bit.

Database (DBASE) If the database module was installed, the system can create and control image databases. A database can help you to efficiently organize your image archives and manage additional information related to the images.

Local Option When Local Option is installed (see Step 2), *NIS-Elements AR* will provide some advanced features which did not pass the quality assurance procedure yet. We recommend to wait until they are released officially.

2.3. User Rights

User management is very useful when a single workstation is shared by number of users. Some user accounts may have administrator rights to *NIS-Elements* while other's privileges are quite restricted. A per-person system may be established or user accounts may be shared. The following principles are utilized within *NIS-Elements AR*:

MS Windows accounts support *NIS-Elements AR* can assign privileges to MS Windows accounts. Whoever is logged in the operating system and runs *NIS-Elements* obtains a default set of privileges. System administrator can then further restrict or extend the user's rights.

NIS-Elements AR password protection It may be not comfortable enough to log the current user off from MS Windows when a user change is required in *NIS-Elements*. For such case a completely independent list of user accounts, which is not connected with MS Windows users, can be created. Then just restart of *NIS-Elements* is needed to switch the user.

Private vs. Shared storage Every user-created item (setting) important in the work-flow can be protected from unwanted change in two levels. First, the creator of the item (optical configuration, layout, objective, etc.) can save it as *private*. Unless its status is changed to *shared*, no other user will even see this item within the lists throughout the application. The second level of protection concerning all *shared* items is provided by assigning users to *Groups* and *Privileges* groups.

Groups Every user is a member of a group. The group enumerates shared items (optical configurations, objectives, etc.) which will be visible for its members. *NIS-Elements* administrators can create any number of groups and select the items to be visible.

Privileges There are basic groups of privileges which allows users or restrict them to perform certain actions. This concerns for example modifying macros, reports, selecting cameras, etc. There are four levels of privileges by default, but further can be created by an administrator:

- Admin

- Common

- Guest

- Default

2.3.1. NIS-Elements authentication

If you have chosen " *NIS-Elements* (Password protected)" user authentication method, a login dialog window appears at every start-up of *NIS-Elements*. Enter a user name and a password for existing user account. If the option *Allow to Create a New User at Start-up* is enabled, you can create a new user account without needing an administrator. This account is assigned to the *Default* group of users and *Default* group of privileges automatically.

2.3.2. Creating a Shared Layout

Please read the following example on user management. Step by step, we describe the creation of a shared layout which can not be modified by members of the *Common* and *Guest* groups.

1)   Run the *View > Layout Manager* command. Set at least one of the defined layouts as *Shared*. (see 3.3. Arranging User Interface)

2)   In the left column of the window, select *User Rights*.

3)   Make sure you are logged as a user with the *Privilege* to *Modify user rights*. If not, use the Log As... button to log in under a different account which has the privilege.

4)   **Set layout visibility**

Select the *Groups* tab. Here you can see at least one group of items called *Default*. Either select one a group or create a new one by the *New* button and choose *Layout* within the list of items. All layouts set as *shared* will be listed on the right side within the *Accessible items* field. Make sure the check boxes next to the layouts which you allow to be used by the group are selected.

5)   **Disable layout modification**

Select the *Privileges* tab. Select the group of privileges which should have not the permission to modify the selected shared layouts. In the *Privileges* field on the right, make sure you de-select the *Modify Shared Layouts* option.

6)   **Apply the new policies**

Select the *Users* tab. In the list of users, select the ones which you would like to apply the policy to (users can be selected one by one or by multi-selection with the Shift or Ctrl key

pressed). Then, assign the appropriate *Group* and *Privileges* by selecting them from a pull-down menu on the right side of the window.

7)   Confirm the changes by the Apply button.

2.3.3. Closing Another Instance of NIS-Elements

When more users want to work with *NIS-Elements* on the same computer and one of them did not close his *NIS-Elements* running copy, other users cannot use the program, because only one *NIS-Elements* copy can be running at a time. The possibility to close the running program started by a different user during a new *NIS-Elements* launch is available in the following dialog window. This applies only if there are no experiments running.

Figure 2.9. Another instance running
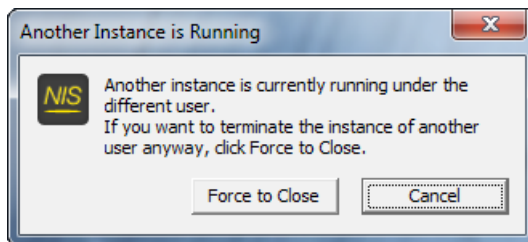
2.3.4. User Rights Options

User Authentication The *Windows* authentication utilizes Windows accounts to automatically log in to *NIS-Elements AR*.

The *NIS-Elements AR (Password protected)* authentication utilizes application user accounts and passwords at the start-up of *NIS-Elements AR*. Check the *Allow to Create a New User at Start-up* option to enable creating of a new user in the *Login* dialog window.

Current user Displays currently logged in user.

Log As Opens the *Login As* dialog which enables you to login as a different user e.g. an administrator with more user rights for the purpose of changing *User rights* settings.

Figure 2.10. Login As
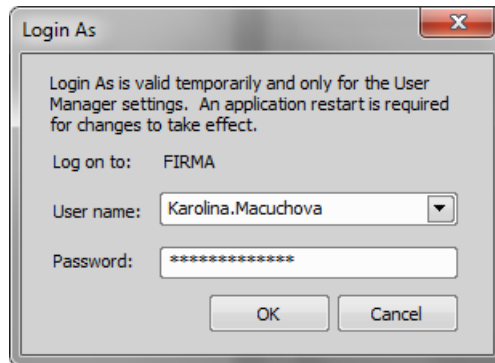
Select the user name you want to log-in as from the drop-down menu. Enter a valid password and press *OK*. The new user identity is available only while the User manager is opened.

Statistics Edit path and file name of a database file for saving statistics.

Export/Import user rights These buttons enable anyone to save/load complete settings of user accounts and their privileges to/from an external XML file. Standard *Open* and *Save As* windows appear. Having the data exported may be useful when copying the settings to other computers.

2.3.5. Users Tab Options

New Enables to create new *NIS-Elements AR* user account. You have to posses the privilege Modify User Rights.

Figure 2.11. Creating new user
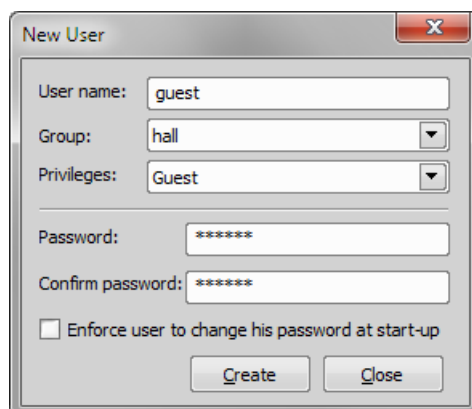
Enter a new user name, assign him to one of the existing Groups and group of Privileges and set the password. Check the *Enforce user to change his password at start-up* option to make the user change his password at his first login into the *NIS-Elements AR*.

User name and password properties

Length of the user name and password is limited to 50 characters. You can use any combination of characters, symbols or numbers (except the Windows ones: *:, *, ?, ", \, /, <, >, |*).

Remove Removes selected user account. You can select multiple accounts and delete them at once.

Copy To Choose a user from the pull down menu whose group and group of privileges will be changed according to settings of a selected user.

Duplicate You can easily create a new user by duplicating the existing. This preserves all account settings under a different name. If you have selected to duplicate more then one user, enter a name prefix, which is added in front of the original name for all selected duplicated accounts. User which was created as a duplicate has an empty password and is forced to change his password at next application startup.

User Accounts List This table displays all existing user accounts. You can sort them according to any of four columns. The first column displays type of the account. The second one shows user names. The third one displays assigned group of users. The fourth one displays assigned group of privileges. Select one or more users whose settings needs modification.

Set password To change password of a selected *NIS-Elements AR* user account, press the *Set password* button. You have to posses the Privilege Modify User Rights. Otherwise you can change only password of the current user. Following window appears:

Figure 2.12. Reset of password
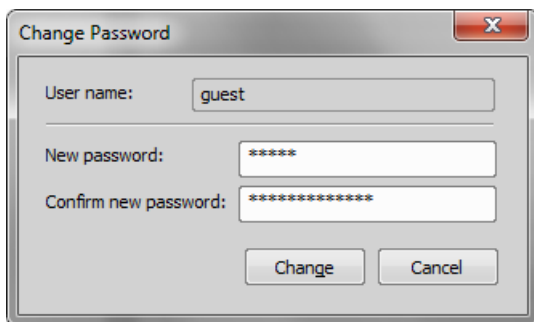
Name of the user is displayed. Enter and confirm new password. When done, press the *Change* button.

Enforce user to change his password at start-up Check this option to make the user change his password at his first login into the *NIS-Elements AR*.

Enumerate All Windows Users This button starts copying MS Windows accounts to *NIS-Elements AR*. The accounts are assigned to user groups (admin, common, guest) according to their Windows permission settings.

2.3.6. Groups Tab Options

Figure 2.13. Groups tab
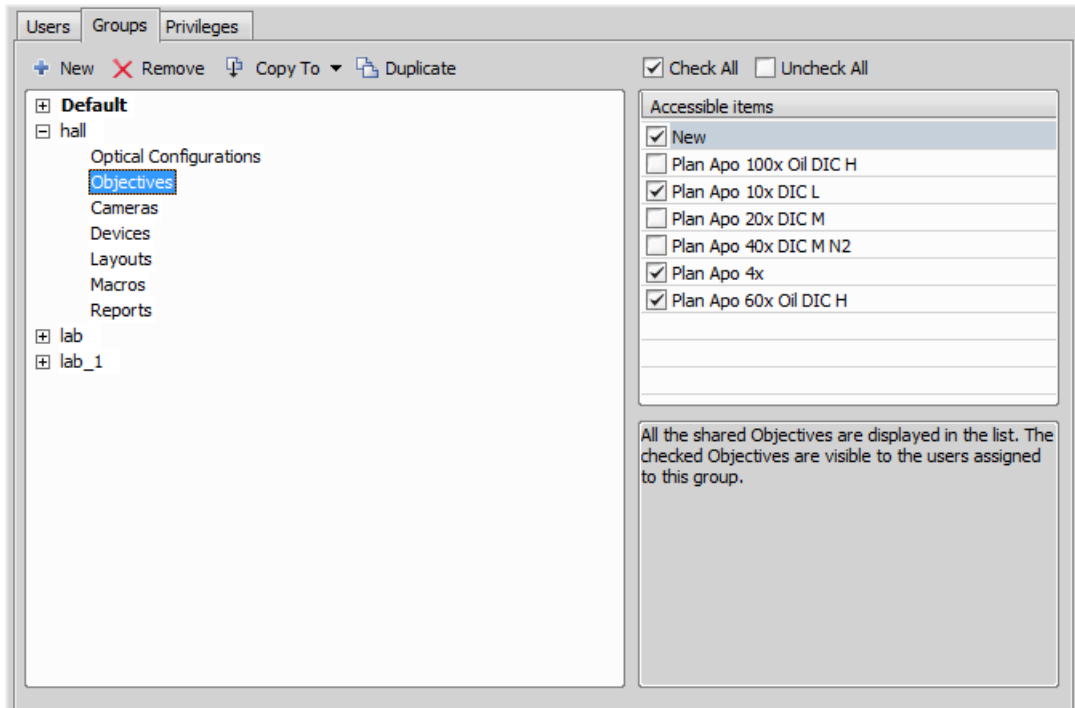
New Enables to create new group of users. You have to posses the privilege Modify User Rights. Enter a name of new group.

Remove Removes selected groups. You can select multiple groups and delete them at once.

Copy To Choose a group from the pull down menu which settings will be changed according to selected group.

Duplicate You can easily create a new group of users by duplicating the existing. This preserves all group settings under a different name.

List of Groups All defined groups are listed in this window. Each group contains subsets. If you click on a subset, its items appear in the *Accessible items* list.

Accessible items This list shows items of a selected subset. Every item has its own check box. If the check box is checked, all users assigned to the corresponding group will see the item. Otherwise they will not see the item.

    Optical Configuration Displays all shared optical configurations.

    Objectives Displays all shared objectives.

Cameras Displays all installed cameras. From the pull down menu below select a camera which is used as default for users without the privilege Select Camera.

Devices Displays all installed devices.

Layouts Displays all shared layouts.

Macros Displays all shared macro commands. Check a macro to make the content of it visible to this Group.

Reports Displays all shared reports and report templates.

2.3.7. Privileges Tab Options

Figure 2.14. Privileges tab
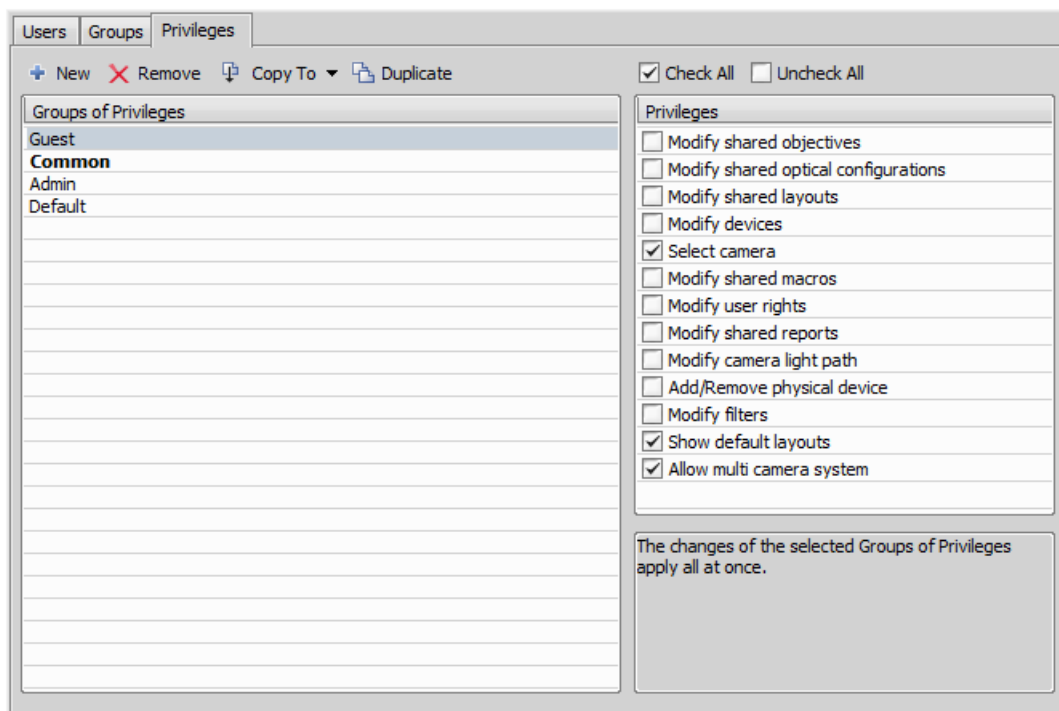
New Enables to create new group of privileges. You have to posses the privilege Modify User Rights. Enter a name of new group.

Remove Removes selected groups. You can select multiple groups and delete them at once.

Copy To Choose a group from the pull down menu which settings will be changed according to selected group.

Duplicate You can easily create a new group of privileges by duplicating the existing. This preserves all group settings under a different name. If you have selected to duplicate more then one group of privileges, enter a name prefix, which is added in front of the original name for all selected duplicated accounts.

Groups of Privileges A list of all groups of privileges is displayed.

Privileges A list of privileges is displayed. Every item has its own check box. If the check box is checked, it grants all users assigned to the corresponding group relevant privilege to access, change, modify, etc.

> Note
>
> *Users that do not have the privilege "Modify Shared Optical Configuration" can temporarily change the brightness settings (e.g. exposure time, gain, etc.) in a shared optical configuration and use such adjusted optical configuration for example in Multichannel acquisition. But all such modifications will not be stored and they will disappear when the application is restarted.*

2.4. NIS-Elements Preferences

2.4.1. Adjusting Program Preferences

1) Run the ⚙ *Edit > General Options* command. The *Options* dialog window appears.

2) Select a tab which contains requested options. Options are sorted into several groups:

General Options concerning basic image operations. See 2.4.2. General.

Appearance Options concerning the graphical user interface. See 3.9. Appearance Options.

Open Next Options concerning the *File > Open/Save Next > Open Next* menu command. See 6.1.1.3. Options for the Open Next Command.

Save Next See 6.1.2.4. Save Next Options for more information.

Macro Configures key shortcuts to macros and sets macros to run automatically on startup See 10.5. Macro Preferences.

Measurement See 8.1.4. Measurement Options for more information.

Data export See Data Export Options for more information.

User rights See 2.3. User Rights for more information.

Layout Manager See 3.5. Layout Manager for more information.

3) Make any changes you need in preferences and use the following buttons to manage them:

Defaults for this Page Restores default settings of the currently displayed options tab.

OK Confirms and saves changes made to the preferences. The dialog window is closed afterwards.

Cancel Cancels all changes made to the preferences. The dialog window is closed afterwards.

Apply Applies changes made in preferences but keeps the *Options* dialog window open. You can apply changes individually on each tab.

Help Displays relevant help page.

2.4.2. General

Documents and History

(requires: Local Option)

Use fix path for images The defined directory is always used when using the 📂 *File > Open* or 💾 *File > Save As* command.

Limit number of opened documents When this options is checked, the user is limited to a single opened image at one time.

Capture always creates a new document If checked, every acquisition creates a new image.

Use last LUTs on image open When a new image is opened, this option turns the look up tables automatically on, and copies the settings from the current or the last opened image. Some image formats (jp2, ND2) can contain the LUTs settings. If such image is opened, the saved LUTs are loaded instead of the most recently used.

Use AutoLUTs on image open for images without LUTs information This option keeps the AutoLUTs feature of the 📂 *File > Open* window always ON, and it applies AutoLUTs to the image after opening. This option is *ignored* if LUTs settings are saved in the image, or if the *Use last LUTs on image open* option is applied.

Show mapping dialog in organizer after drag and drop Displays the *Mapping* window every time an image is inserted to a database table. It enables you to check/modify mapping of the image info values to the database table fields.

Enable saving ND experiment to TIFF series Displays the *Save As TIFF Series* option within the *ND Acquisition* control panel. It enables you to save single frames of the ND experiment as tiff images. You can modify the order of the dimensions and the images format (mono, color, merged). Also you choose to convert multichannel images to RGB, or to convert images to 16 bit.

Open new ND views to new window Displays each newly generated ND view in a separate window.

Show image info window on save Displays the *File > Image Properties* window every time an image is saved via the *Save As* command.

Detect sequence on image open When you select to open an image that is a part of image sequence such as 001.jp2, 002.jp2..., it is recognized automatically and you are offered to convert it to an ND2 file.

Show binary layers' contours Check this item to display contours of binary layers.

Use zero based time scale for ND documents Select this option to ensure that the first frame of a time sequence will always start at 0.0s.

State of saturation indicator after start Select what to do with the saturation indicator settings () after *NIS-Elements AR* is restarted. Turn it ON, OFF, or remember the last setting.

Rotation Flips and Shifts

Apply to overlaid binary layer If checked, in overlay mode both binary and color images are rotated, shifted and flipped.

Optical Configuration

Save all camera settings to optical configuration automatically If the currently selected optical configuration contains Camera Settings and this option is checked, any change of the settings is written to the optical configuration immediately.

Save brightness to optical configuration automatically If the currently selected optical configuration contains Camera Settings, the Brightness setting is being updated continuously according to the current state. Also functionality of saving confocal brightness setting(laser power, detector gain and scan frame rate) is contained in this option.

Select corresponding optical configuration when filter changed... *(requires: Local Option)* Enabled if "Unselect" in the option below is chosen. Allows *NIS-Elements* to automatically change the optical configuration based on the filter selected on the microscope.

When Optical Configuration setting changed

Keep Selected The Opt. configuration name is marked by the "*" sign and the change is offered to be saved.

Unselect Every change of Optical configuration setting results in its deselecting and switching to user settings.

Save all changes All changes regarding the Opt. configuration settings are updated automatically according to the current state.

Temp *NIS-Elements AR* uses the Temp directory for storing temporary data when there is not enough RAM available. You can redirect the system to use a directory on another harddisk, which may speed up the whole system.

Defaults for this Page Restores the default setting for General Options.

2.5. NIS-Elements ER Package

Requires new HASP license *Enhanced Resolution*.

NIS-Elements ER package consists of *NIS-Elements* C Package and Deconvolution package (2D, 3D and automatic deconvolution).

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2.5.1. Automatic Deconvolution

Automatic deconvolution button displayed in the image toolbar can be used to easily and quickly deconvolve the currently opened image. By default, each time you execute automatic deconvolution, the deconvolution dialog window is shown. This behaviour can be enabled/disabled in the drop-down menu next to the icon of the function (*Show deconvolution GUI*).

1) Open the image file to be processed.

2) Click ⬤ *Automatic Deconvolution* on the top of the image toolbar to perform automatic deconvolution on the current image.

> Note
>
> *If the source image does not contain any deconvolution metadata, the deconvolution dialog window is opened enabling to specify the metadata and run the deconvolution process.*

3. User Interface

Figure 3.1. The NIS-Elements Main Window
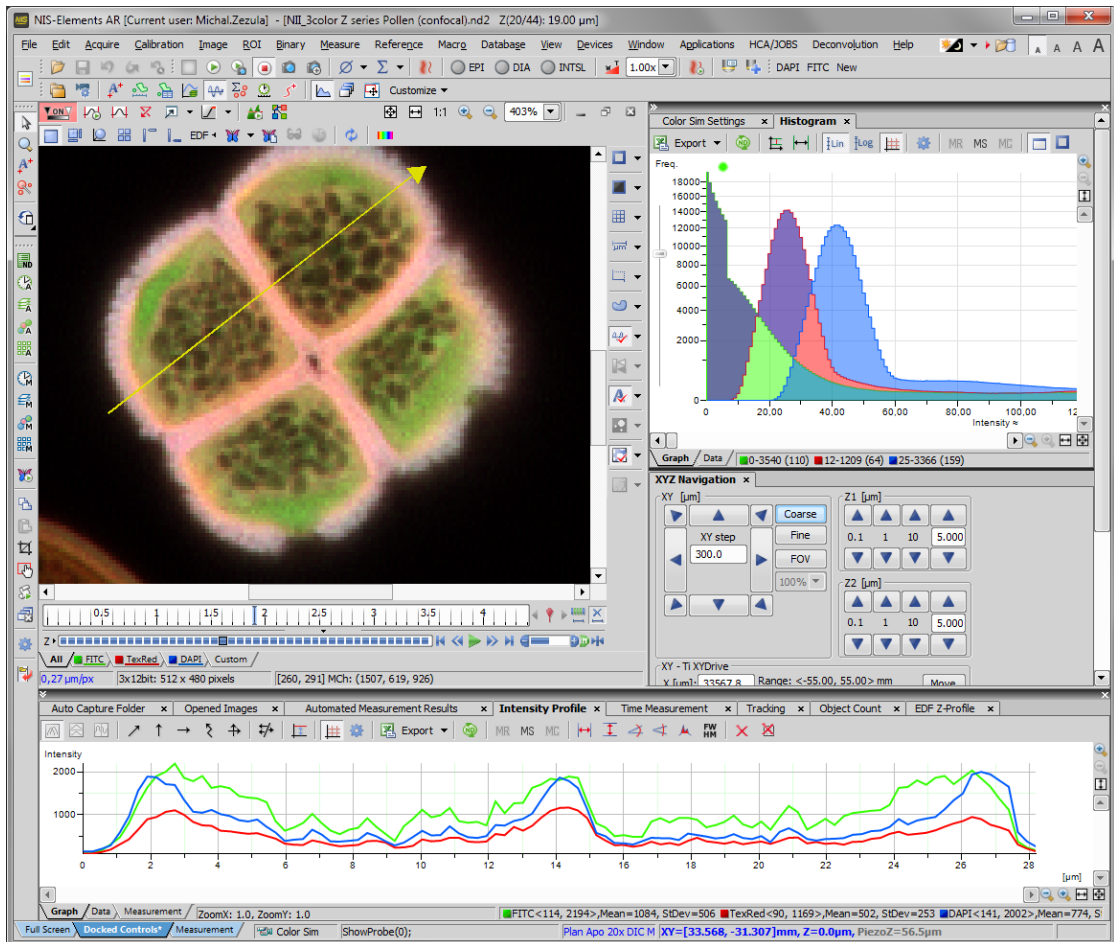
3.1. Main Window Components

3.1.1. Main Menu

All basic *NIS-Elements* functions are accessible from the main menu at the top of the screen. Menu commands are grouped according to their purpose.

3.1.2. Toolbars

There is a default set of toolbars, each toolbar containing number of buttons. There is also one fully customizable toolbar - the main left toolbar - to which any button can be added. Every button or whole toolbar can be hidden by user. Please see 3.3. Arranging User Interface for further details.

3.1.3. Status Bar

The status bar at the bottom of the screen displays the following information:

Figure 3.2. The application status bar
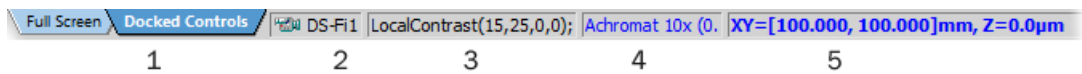
1. This part of the status bar displays available layouts.

   > Note
   >
   > *The layout Tabs may be hidden when the Show Layout Tabs option in the 3.3. Arranging User Interface window is deselected.*

2. This status bar section displays the type of the currently selected camera.

3. Here you can get information about the most recently performed command. The FPS / Exposure / Focus info is shown in case of live image. The black bar indicates the focus rate. Longer black bar represents more of the image in focus.

4. This section show the name of the current objective.

5. Current coordinates of XY (Z) stage are shown in this part of the status bar.

3.1.4. Docking Panes

Docking panes are square spaces inside the application window, where you can place (dock) any of the control panels. There is one docking pane available at the Right, Bottom, and Left side of the application screen.

To Display a Docking Pane

1. Go to the ▭ *View > Docking Panes* sub-menu and select the pane you would like to display.
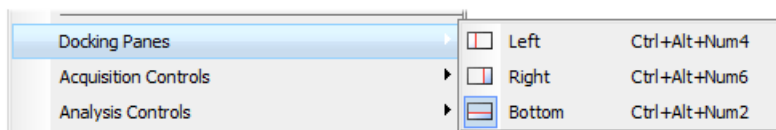
Note

The Docking Panes sub-menu can be also displayed by right-clicking into the empty application screen.

2. The docking pane appears, either empty or with some window(s) docked inside.

3. Repeat this procedure to display more docking panes.

Handling Control Panels

Various control panels can be displayed docked within the docking panes or they can be floating. See the following picture:

Figure 3.4. The Docked Control Panel Caption

To handle the control panels (CPs), you can:

Open recently closed CP Locate the ⬚ button on the main left toolbar. When you click it, the list of recently closed CPs appears. Pick one to display it again.

Add CP to a docking pane Right click inside a docking pane (3) to display the context menu. Select the control panel to be displayed. If the window is already opened somewhere else (in another docking pane or floating), it closes and moves to the new destination.

Close CP Click the *cross* button (2) in the right top corner of the tab.

Drag CP Drag any CP by the tab and drop it somewhere. If you drop it by the edge of a docking pane, it will create another column of this pane. If you drop it over the caption of another CP, it will be docked in the same pane as a new tab. If dropped somewhere else, the CP will be floating.

A color frame appears when you place the mouse cursor dragging a CP over the edge of a docking pane or a caption of another CP. It indicates that if you drop it, its placement will be handled automatically.

Minimize the docking pane Click the arrows in the top left corner. The pane minimizes to a stripe by the edge of the screen. It can be restored to its original position by double clicking this stripe or by clicking the arrows again.

Close the docking pane Click the *cross* button (4) in the docking pane caption. Or you can right click the pane and unselect the *Docking View* option.

Dock/undock CP To dock (and undock) a CP, double click its tab.

Display CP Another way to display a CP is to go to the *View* menu and select the desired control panel. After that, the CP appears on the screen - floating or docked. Positions of the windows are being saved by the system so that each control panel appears in the same position as it was before it being hidden. The controls are sorted to several groups.
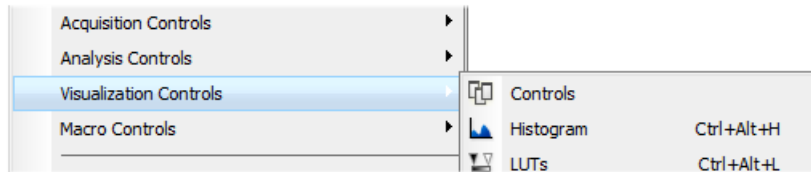
Shrink/Expand dockers Having more docking panes opened, a situation where there is not enough room for the control panels can occur. In such case, the *Shrink* and *Expand* commands shall be used.
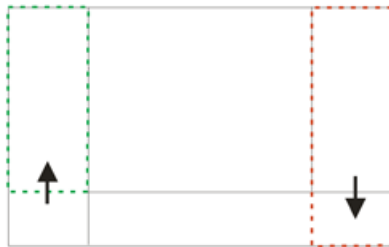
1. Right click the pane you would like to shrink/expand. A context menu appears.

2. Select the *Expand/Shrink* command. When one of the panes shrinks, the neighbouring pane expands to the emptied corner and vice-versa.

3.2. Image Window

Tools affecting the appearance of the current image are gathered within the image window toolbars (the top image toolbar and the right image toolbar) . There are the following buttons by default:

Figure 3.7. Image Window
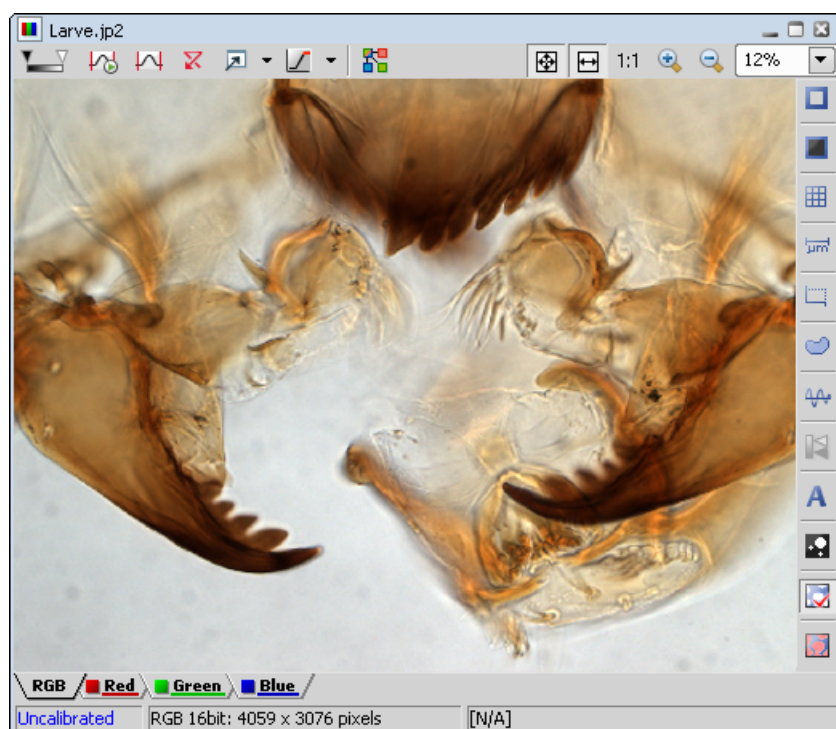
Enable LUTs Applies LUTs to the image. See 6.5. LUTs - Non-destructive Image Enhancement.

Keep Auto Scale LUTs Applies the AutoScale command to the image continuously.

Auto Scale Performs automatic setting of LUTs.

Reset LUTs Discards the LUTs settings.

Show LUTs window Opens the window with LUTs.

Pixel Saturation Indication Turns on / off pixel saturation indication without setting on / off LUTs. Select the highlighting color from the nearby pull-down menu for *Oversaturated* and/or *Undersaturated* pixels. See 6.2.3. Channel Coloring.

Open Attachment Opens a file previously attached and saved with the ND2 file. To attach any file to an image, right-click inside the image, select *Add Attachment* and choose a file to be attached. Maximum size of the attached file is 64 MB. Context menu over the image also enables to *Open Attachment...* (same functionality as the button), *Save Attachment As...* (saves the attached file separately) or *Remove Attachment* (attachment is removed from the ND2 file).

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Split Components Turns ON a special view, where color channels of the image are displayed separately (tiled). This mode is also supported in 6.3.3. Volume Viewer.

Fit to screen Adjusts zoom to view the whole image within the *NIS-Elements AR* screen.

Best Fit Adjusts zoom to fit the image window in one direction and fill the available area with the image.

1:1 **1:1 Zoom** Adjust zoom so that one pixel of the image matches one pixel of monitor.

Zoom In Increases magnification of the image.

Zoom Out Decreases magnification of the image.

Show Probe This button activates the probe. The probe affects histograms, auto exposure and auto white balance functions.

Show Background Probe Activates the background probe. Some commands use the BG probe data as reference.

Show Grid Displays the grid for rough measurements.

Show Scale Displays the image scale.

Show Frame Displays and applies the measurement frame.

Turn ROI On/Off Displays the Measurement Region Of Interest.

Show Profile Displays the **⋀** *Measure > Intensity Profile* control panel. It allows you to specify a linear section in the image of which the pixel intensities graph will be created.

View LUT Intensity Displays the scale of intensities used inside the image. It works on monochromatic images or a single image channel.

A Show Annotations Displays the vector layer which typically consists of annotation objects (text labels, arrows) and measurement objects.

View Binary Displays the binary layer of the image.

View Color Displays the color layer of the image.

View Overlay Displays the color layer and the binary layer in overlay.

Tip

Right click the icons to invoke a context menu where properties of each tool can be modified.

Channel Tabs

*Figure 3.8. Channel tabs of an RGB image*

Channel tabs at the bottom left corner of the image window enable switching between image channels. You can also edit their properties using commands available via a context-menu. See also 6.2. Image Layers.

Status Bar

The status bar at the bottom of the image window displays the following information:

Figure 3.9. Status bar of the image window
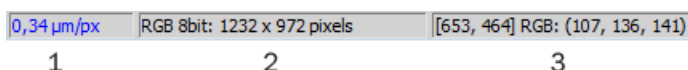
1. The first field of the image window status bar Image displays the calibration. See also 8.1.1. Calibration , 8.1.1.3. Units.

2. Image bit depth (8bit, 12bit, 16bit, etc.) followed by Image size. You can change the displayed units from the context menu.

3. Pixel coordinates of the mouse cursor along with channel intensities, Binary layer value (0 or 1) and the Color mode (RGB, Monochromatic, etc.).

3.3. Arranging User Interface

Having a well organized application layout can help you make the work with *NIS-Elements AR* very effective. There are the following options on customizing the appearance of *NIS-Elements AR*:

Custom window placement All control panels (Camera Settings, Measurement, Histogram, LUTs, etc.) can be arranged inside or outside of the main application window.

Compact window or multiple windows The control panels as well as toolbars can be floating or docked on sides of the application screen.

Multiple monitor support The *NIS-Elements AR* window can be stretched to occupy two monitors. When you switch from different application, *NIS-Elements AR* will be activated on both monitors.

Customized toolbars Toolbar buttons may be added and removed from toolbars. See 3.6. Modifying Toolbars.

Maximizing the Image Area You can hide some of the GUI elements which are displayed by default:

- The channel tabs and the layout tabs may be hidden to save some screen-space. Display the 3.9. Appearance Options window and de-select the *Show Channel Tabs* and the *Show Layout Tabs* options.

- Image controls and the image status bar may be hidden. Use the *Auto hide bottom toolbar* option of the 3.9. Appearance Options window.

- When an image is displayed in great magnification, scroll bars automatically appear by the sides of the image window. You can hide them by de-selecting the *Show Scrollbars* context menu option.

3.4. Layouts

A layout in the context of *NIS-Elements AR* is a set of options describing the arrangement of control panels, toolbars, and menu items. Blue tabs representing active layouts appear in the application status bar. The following layouts are placed there by default:

- *Full Screen*

- *Docked Controls*

- *Measurement*

Other layouts can be added and managed via the Layout Manager. To hide/show the layout tabs within the status bar, go for   *View > Layout Manager* and (de)select the *Show Layout Tabs* option.

To Create a New Layout

Figure 3.10. Layout tabs

1. Modify the current layout so that it suits your concept of work.

2. An asterisk * appears next to the layout name (to indicate it has been modified).

3. Right click the layout tab and select the *Save Current Layout As* or *Save As Default* command. If you do not need to create a new layout but would like to save the changes made, just right click the current (asterisk-marked) tab and select the *Save* command from the menu.

4. Write the new layout name and confirm it by OK .

5. A new tab appears and the layout is saved to the list of layouts.

To Reload Previous Layout Settings

You may want to undo the changes made to the layout. Mostly, it can be done by the *Reload* command. Or by selecting the *Load Default* command.

- Right click the asterisk-marked (recently modified but not saved) tab and choose the *Reload* command. The application restores the last saved state of the layout.

• Right click the asterisk-marked tab and choose the *Load Default* command. The application loads the previously saved default layout.

3.5. Layout Manager

Run *View > Layout Manager* to display the Layout Manager. The list of currently available layouts is placed on the left side of the layout manager. Each layout may contain information about controls, toolbars, menu, and commands to be performed when switching between layouts.

Figure 3.11. List of Layouts
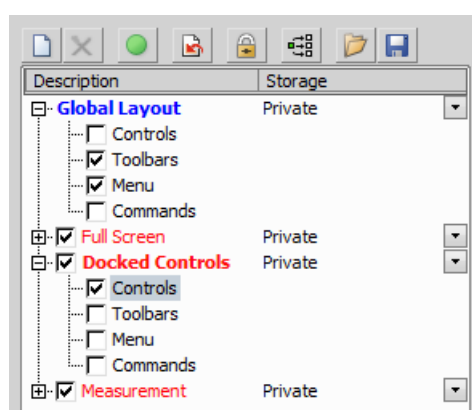
Modifying the Layout Settings

1. Select items within the *Global Layout* list which you want to be shared by all layouts.

2. The check marks on the left of the layout names indicate the layout visibility. Select the ones you want to display in the application status bar.

3. If an item within some particular layout is not selected, it means you do not want to customize it and the global settings - if selected within the *Global Layout* - will be used. If the item is not selected within the Global Layout either, the settings of the most recently active layout will be used.

4. Set whether the layout is *Private* or *Shared*. If set *Private*, it will not be visible for other users. See 2.3. User Rights.

5. Customize each item according to your needs (see below).

Layout Manager Tools

New Adds a new layout to the list of layouts.

Remove Deletes the selected layout. The first two layouts cannot be deleted.

🟢 **Activate** Makes the selected layout active.

🖼️ **Load Default** Loads original settings of the selected pre-defined layout (*Full Screen*, *Docked Controls*, *Measurement*) so that it looks just like after the program installation.

🔒 **Lock Layout** Select a layout component and click this button to lock these elements so that they cannot be moved or closed. To unlock these components, click the button again or right-click the locked layout tab at the bottom and click *Unlock Layout*.

⊞ **Apply to All** Ensures that the executed changes will be applied to all layouts.

📂 **Import** Enables to load a previously saved set of layouts from an XML file. When you try to import a layout with already existing name, you will be prompted to choose whether: the imported layout replaces the existing colliding layout, or is not imported, or is imported and renamed.

💾 **Export Layouts** The settings of layouts can be saved to an external XML file. Use this *Export* button. In the windows that appears, define the destination file name and check which layouts will be included in the exported xml file.

3.6. Modifying Toolbars

Hiding Toolbar Buttons
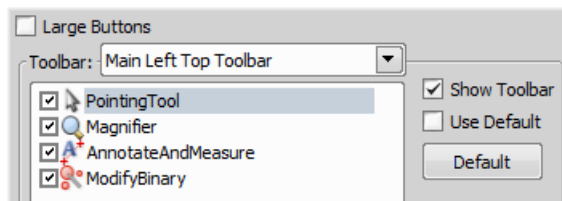
1. Display Layout Manager by the   *View > Layout Manager* command.

2. Select the *Toolbars* item within the list of layouts. The right-side part of the window changes.

3. Choose one of the toolbars which you would like to modify from the *Toolbar* pull-down menu.

4. If selected, de-select the *Use Default* option on the right.

5. Any button of the toolbar may be hidden by de-selecting it. No buttons can be added to any of the toolbars except the *Main Left Toolbar*.

6. The whole toolbar can be hidden by de-selecting the *Show Toolbar* check box.

7. There are two sizes of buttons available. Select the *Large Buttons* option to use the larger one. This setting is shared by all toolbars.

Adding Buttons to the Left Toolbar

Custom user buttons can be added to the main left toolbar. You can define your own buttons which run single macro functions or execute macros . Select the *Main Left Toolbar* from the pull-down menu.
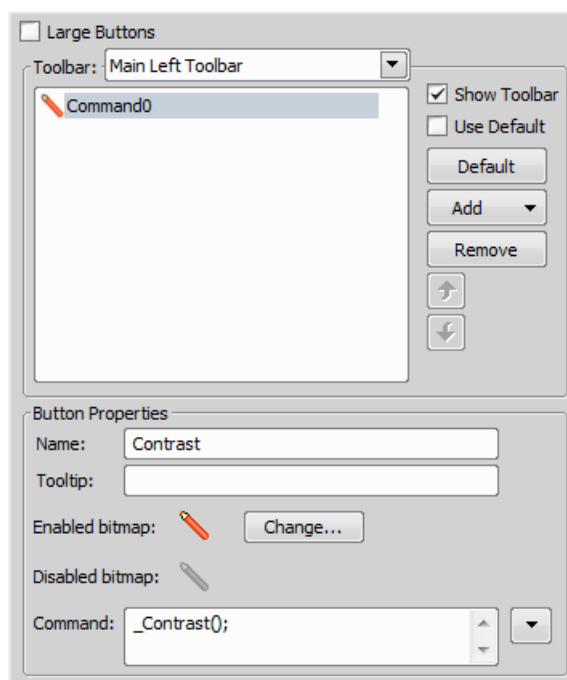
Let's say that we very often use the ⬤ *Image > Contrast* command. It is useful to add a shortcut button to the toolbar.

1. Press the *Add* button, and choose *Command* from the pull-down menu:

2. A new command - *Command0* - is added to the list.

3. Now, assign a macro command: Open the pull-down menu on the right side of the *Command* edit box and click *Command List*.

4. A list of commands appears. Choose *_Contrast()*.

5. Confirm the selection by *OK*.

> Note
>
> *It is possible to assign a sequence of commands to a single button by repeating this procedure.*

If you are not satisfied with the default icon, you can change it by pressing the Change... button. A window for selecting the icon appears. You can select the image from the *NIS-Elements AR* icon set or load some other from any file containing icons (ico, dll). You can define another icon for the command in a disabled state, too.

It is handy to define a tool-tip (a text that appears when the mouse cursor is placed over the icon) for your command. Simply write the text into the *Tooltip* box. You can change the position of the command in the toolbar using the arrow buttons. The Default button discards your changes and inserts the default set of commands to the toolbar.

3.7. Modifying Menus

The *Main Menu* and some of the *Context Menus* within the application window may be modified. Items of the context menus can be hidden by de-selecting them similarly to the toolbars. The main menu can be modified as follows:

Modifying the Main Menu

1. Display Layout Manager by the *View > Layout Manager* command.

2. Select the *Main Menu* in the topmost pull-down menu.
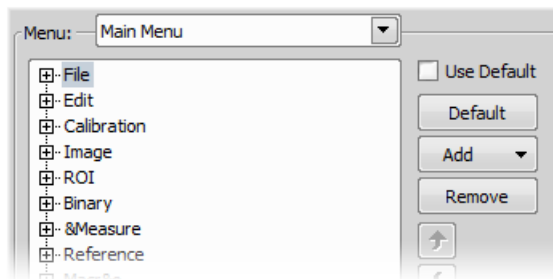
3. Any item may be added to the main menu - a *Separator*, a *Menu Command*, a sub-menu (*Menu Popup*), and even a new menu (*Main Menu Popup*) - by the *Add* button.

4. Select the existing menu item under which you would like to place the new item.

5. Click the *Add* button and select the item to be added from the pull-down menu.

6. Edit the *Item Properties* .
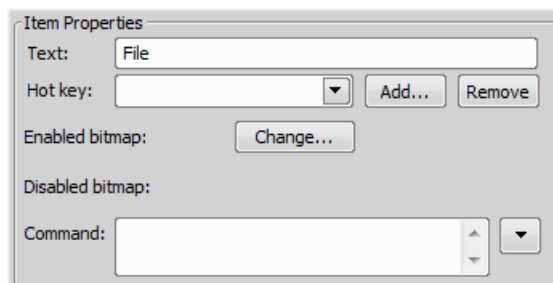
Text This is the text which appears in the pull-down menu. You may add "&" before any letter - such letter will be considered a keyboard shortcut when browsing the menu.

Hot key One or more hot key shortcuts may be assigned to the command. Just press the *Add* button and press the key combination. Press *Remove* to remove the selected hot key.

Enabled/Disabled bitmap, Command These fields serve for assigning a bitmap image and a macro function to the menu command. It works the same way as when modifying the Main Left Toolbar (described above).

Note

The Default button discards all changes and loads the main menu original configuration. The Remove button deletes the selected item. The arrow buttons move the selected item up/down. The Use Default check box, when selected, applies the default settings to the menu.

3.8. Running a Macro Upon Layout Change

A macro command or a macro can be run upon layout change.

1. Display the layout manager by the *View > Layout Manager* command.

2. Select the layout by displaying of which the macro function will run and select the *Commands* check box.

3. The following box appears on the right side:

4. Select the timing. The "before" option will run the command when you click on the layout tab, but before actually changing the layout. The "after" option runs the command right after the layout is changed.

5. When the field is enabled, type the command or insert it via the pull-down menu.

3.9. Appearance Options

General appearance adjustments can be made in the *Options* window. Run the ⚙ *Edit > General Options* command and switch to the *Appearance* tab.

Background Background of the main screen may use default tiles or custom color.

Color scheme There are the following color schemes predefined within the application: Light scheme, Dark scheme, Black scheme.

Language Select the language to be used in GUI. The language pack is a part of the Local Option feature set.

Prompts on Image Save dialog Actual words used on buttons of the window which appears if an image has been modified and is about to be closed.

The Z value displayed in statusbar Having two Z devices, select whether to display Z1, Z2 or both on the main status bar.

Close ND Acquisition window after Run The *View > Acquisition Controls > Capture Timelapse* window will be closed automatically upon acquisition start.

Enable Z intensity control This option enables the *View > Acquisition Controls > Z Intensity Correction* within *NIS-Elements* user interface.

Image window toolbars You can decide whether the top and side image toolbar is integrated into the main toolbar (*Common for all Images*) or whether each image has its own set of toolbars (*Visible for each image*). The most space-saving option is to use shared toolbars and hide the image status bar and the ND2 control bar (6.3.1.1. Control Bar) automatically (*Common for all images, auto hide bottom toolbar*). It will show up only when the mouse cursor rolls over the bottom part of the image.

In the *Right Toolbar* section you can select the appearance of buttons having more sub-options. See 3.2. Image Window.

Auto hide bottom toolbar This option hides the image status bar and the ND2 control bar (6.3.1.1. Control Bar) automatically. It is displayed only when the mouse cursor rolls over the bottom part of the image picture.

Show channel tabs Show channel names and colors in the image window status bar.

Show Layout tabs Show layout tabs in the main status bar.

Show Task Bar If unselected, the Windows taskbar will be hidden.

Default vertical docker on the *(requires: Local Option)*

Select the preferred side for the default vertical docking pane.

Keep text size while zooming When selected, text annotations will not be zoomed with the image. See ⚬ *View > Analysis Controls > Annotations and Measurements* .

Allow zoom factors lower than best fit When selected, the image can be resized to be smaller than displayed using the ⊞ *View > Zoom > Fit to Screen* command.

Lock camera magnification If you are used to switch between two camera modes (resolutions), this option ensures that the scene observed does not change its size or position.

Keep picture window aspect If checked, the image window respects the image size while zooming.

Initial Zoom You can select the zoom factor of the newly opened images. The options are Best Fit, 200, 100, 50, 25%.

Initial zoom 100% for Live and Capture Whenever a new Live or Captured window is opened, the zoom will be automatically set to 100%.

Default Volume View Rendering Engine Switch between the Old/New engine used in the Volume Viewer.

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4. Cameras & Devices

4.1. Basic Workflows

4.1.1. Camera Selection on Startup

Let's assume the camera works properly, is connected to the PC with proper system drivers installed and running (if required by the camera).

Setting Up the Camera

**1)**

Select Camera Driver

You will be asked to select the camera driver every time you launch *NIS-Elements AR*. You can change the driver later using the *Acquire > Select Driver* command. Choose the driver that matches your camera:

Multiple Cameras

Two cameras can be connected to one system. If this is your case and you intend to use both cameras, check the *Enable Multi Camera* option and select the *Second driver* from the list.
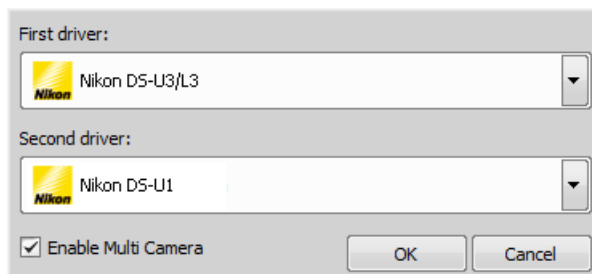
The following set of buttons appears in the top toolbar when multiple cameras are used:

2)

Selecting a Camera

Color cameras can be used in a monochromatic mode. The actual camera type (color/mono) can be selected by the *Acquire > Select *camera name** command.

> Note
> ___
> *The emulated monochromatic mode is optimized for use with fluorescence specimen where often only a single-color signal is being captured. The following formula is used to compute intensity of the emulated mono image:*
>
> *I = (Ir * Wr) + (Ig * Wg) + (Ib *Wb)*
>
> *Where: W is channel weight calculated from the channel histogram and I is channel intensity. W ensures, that channels containing some signal are accentuated while channels without signal are suppressed. As a result, depending on conditions, the brightness of a monochrome image might not change even if the lamp light intensity is changed.*

3)

Adjust the Camera Settings

Exposure time, camera resolution, and other camera-specific features are adjustable from the *Camera Settings* window. To invoke it, use the *Acquire > Camera Settings* command.

4.1.2. Optical Configurations

4.1.2.1. Introduction to Optical Configurations

Typically, a laboratory computer image analysis system consists of a computer, a camera, and a microscope equipped with certain accessories (objectives, filters, shutters, illumination, rotary changers, etc). Most of the mentioned microscopic hardware can be motorized and therefore can be controlled via *NIS-Elements AR*. In addition, it is possible to integrate single settings of all these devices into one compact set called *Optical Configuration*. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the current hardware configuration.

4.1.2.2. Creating New Optical Configuration

1)   Please check that all the devices (microscopes, cameras, etc.) which you want to associate with the new optical configuration are properly attached to the system and working.

2)   Choose the 🔧 *Calibration > New Optical Configuration* command. In the window which appears, adjust the settings of the devices to match the intended state which will be saved to the optical configuration.

3)   Type the name of the new optical configuration to the *Name* field. Use a short descriptive name, the name is used on the button in the main toolbar when you select the *Show on toolbar* option.

4)   In the left column, select which device settings to associate with the Optical configuration.

Camera setting A list of the current camera properties appears on the right. It is being updated dynamically.

If you want to use stored ROIs for Camera ROI setting (turn ROI on/off and use the stored ROI value), tick "Use Stored ROI" checkbox in the Camera features box.

Channel setup These settings determine how channels of newly captured images will be named and what color will be assigned to them. The available properties depends on the current camera setup (color/mono, triggered acquisition engaged or not, dual view engaged or not, etc.). In the mono camera mode, you can either assign the name, the emission length and color to the channel *Manually*, or leave this task to *NIS-Elements AR* (the *Automatically* option). In such case, information of the light path (emission wavelength) will be used to determine the channel name and color.

Microscope setting If there is more than one shutter available and you would like to associate a shutter with the optical configuration, select which one is the *Active Shutter* from the pull-down menu. Select which parts of the microscope shall be included in the configuration by checking them in the *Used devices* dialog box.

Note

Active shutters remember their aperture setting and display it in the Microscope setting section.

Objective An objective mounted to a motorized nosepiece can be included in the configuration. Select the objective from the pull-down menu. Objectives which are currently assigned to any position of the nosepiece are listed. See 4.1.3. Objectives.

Note

The objective must be assigned to a nosepiece position via the microscope control pad or the nosepiece control panel beforehand.

5)   If some of the device settings still need to be adjusted, click the `Camera & Devices Controls`
button and select the appropriate control panel from the pull-down menu. Adjust the settings
within the control panel, the optical configuration will be updated automatically.

6)   Click *Finish* to save the new optical configuration and to close the window.

7)   You can create more optical configurations by repeating the procedure. The optical configurations
are saved to registry immediately. A backup of optical configurations can be made by running
the 🖼️ *Calibration > Optical Configurations* command and clicking the *Export* button.

4.1.2.3. Managing Optical Configurations

To display the optical configurations manager window, run the 🖼️ *Calibration > Optical Configurations*
command. You can make the following actions from the window:

• Create, duplicate, rename, delete, copy settings and switch between optical configurations.

• Modify optical configuration properties.

• Import and export optical configurations to/from an XML file.

4.1.2.4. Operations with Optical Configurations

Once created, the configuration appears in the list and can be shared with other users by changing the
Private option to *Shared* (see 2.3. User Rights). The following operations can be performed on the selected
configuration:

• It can be deleted by pressing the *Remove* button. A confirmation dialog box appears.

• Its name can be changed by the *Rename* button. A button with the configuration name appears in
the toolbar (if the *Show on toolbar* option is selected).

• The configuration settings may be transferred to another optical configuration by the `Copy to` button.
Press the button and select the optical configuration to be overwritten with the current one.

• A copy of the configuration can be made via the *Duplicate* button.

• The configuration can be applied to by the *Set As Active* button.

• The settings of all optical configurations can be exported to an external XML file using the *Export*
button.

• The previously exported optical configurations settings can be loaded from the XML file via the *Import*
button.

• The list of optical configurations can be ordered manually using the arrow-up and arrow-down buttons.

• Each configuration can be arbitrarily modified within the right-side portion of the window.

The optical configuration buttons are available in the main toolbar when *Show on toolbar* option was selected during the setting process.

4.1.2.5. Using Auto Exposure while Switching between Optical Configurations

You can use one optical configuration when working with live image and switch to another optical configuration just for the image acquisition, very often, this is performed automatically i.e. by a macro command. If the "on-acquisition" optical configuration has the *Auto Exposure* camera mode turned *ON*, it may produce over- or under-saturated images.

Since the Auto Exposure algorithm estimates the optimal exposure time by analyzing several last frames, it is not able to make the estimation correctly if the change of optical configurations would cause significant change in image brightness. To prevent this, we recommend to use *Manual Exposure* mode in the optical configuration used for capturing.

4.1.3. Objectives

If you want to perform measurements on captured images it is wise to calibrate all objectives used to capture images. Whenever an image is captured through a calibrated objective, the image inherits its calibration.

4.1.3.1. Managing Objectives

Run the *Calibration > Objectives* command. The *Objectives* window appears:
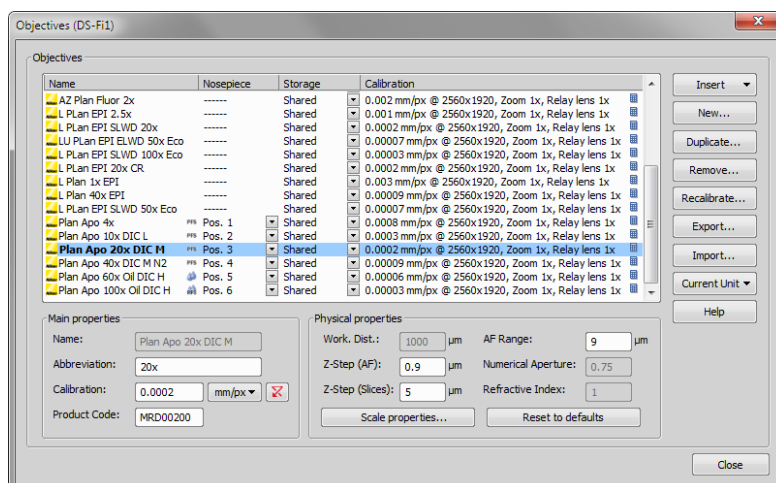
A list of used objectives can be created within the Objectives window. For each objective, the objective name, position in the changer, storage status and the calibration are displayed. A *Calculator* icon next to the calibration value indicates that the calibration has been calculated from objective properties.

If the calculator icon is missing, the calibration has been performed manually. Use the buttons on the right side to manage the objectives:

Insert Click the button and select one of the objectives from the database (ordered by magnification). Custom objectives can be added to the database via an INI file.

New Press this button to create a custom objective. The new objective will be added to the list. Then, define the *Main properties* and the *Physical properties* of the objective.

Duplicate If zoom is used, the objective calibration must be re-calculated accordingly. Use this button to make a copy of the objective and define the zoom factor in the window which opens.

Remove This button deletes the selected objective.

Recalibrate Starts the calibration of the selected objective. See 4.1.3.3. Objective Calibration.

Export Enables you to export the complete list of objectives to an XML file. The standard *Save As* window appears.

Import This button enables you to import a complete list of objectives from an external XML file previously created by the *Export* button.

Current Unit This button invokes a pull-down menu where units for the whole application can be selected.

Help Displays a help page to the *Calibration > Objectives* command.

Edit the properties of the selected objective in the bottom part of the window. Press the *Close* button to finish this window.

4.1.3.2. Assigning Objective to a Nosepiece Position

1. To assign an objective to a position or to change an attached objective assignment, click the setup button in the *nosepiece* section of the microscope control pad.

2. A window appears which enables you to select one of the available objectives to the corresponding position.

3. Each objective has its specifications displayed in the table. These specifications are not editable.

4.1.3.3. Objective Calibration

After you press the *Recalibrate* button in the *Objectives* window or when creating a new custom objective, the following window appears:
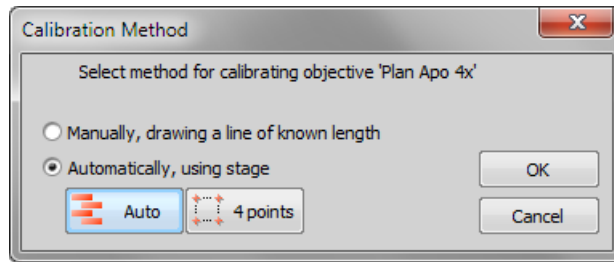
Select one of the calibration methods:

- The *Manual* calibration lets you draw a distance into a picture and assign real length to it (see below).

- If a motorized XY stage is available, the *Auto* and *4 points* automatic methods appear.

Press *OK* to continue...

Manual Calibration

1)   When performing a manual calibration, the live image starts automatically and the following window appears:
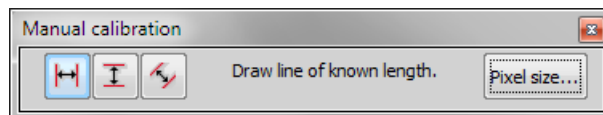
2)   Select one of the icons to draw a distance to the image. If you know the precise calibration value (px/unit), press the *Pixel Size* button to enter the real size of one pixel:
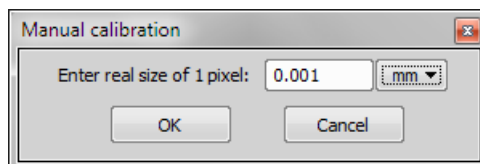
Enter the calibration value, select units, and confirm the calibration by OK. If you do not know the pixel size, you will have to continue with the calibration on live image:

3)   Insert a calibration slide to the microscope stage.

4)   The distance is defined by placing lines (Horizontal, Vertical, Parallel) to the image. Choose the orientation of the lines by pressing the appropriate button.

If you are sure the camera angle is 0, 90, 180, or 270°, it is recommended to select either vertical or horizontal lines. Otherwise, select the parallel lines.

5) Click into the image to place the first line. Place the second line in the intended position by another click.

Note

You can modify the line position while holding the primary mouse button. After you release the button no further changes can be made. When "Parallel lines" were selected, draw the first line by clicking twice inside the image. The line can be moved and adjusted arbitrary by mouse. When satisfied, finish the first line by right-click. The second line can be placed by another click to the image, this time only to adjust the distance from the first line. The process is completed by right-click.

6) The following dialog box appears:

Manual calibration

Enter real size of line: 25   µm ▼

OK   Cancel
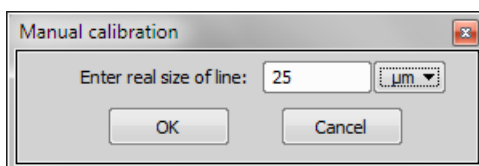
7) Enter the distance between the two lines and select correct units.

8) Press *OK*. The objective is calibrated now.

Automatic Calibration

The automatic calibration requires a motorized stage. Select one of the following methods:

Auto The *Auto* method is fully automatic. Calibration is performed on a part of the live image marked by red square that is shown before the calibration is launched. You can also choose the channel to be used by auto-calibration.

Figure 4.9. Auto-calibration window
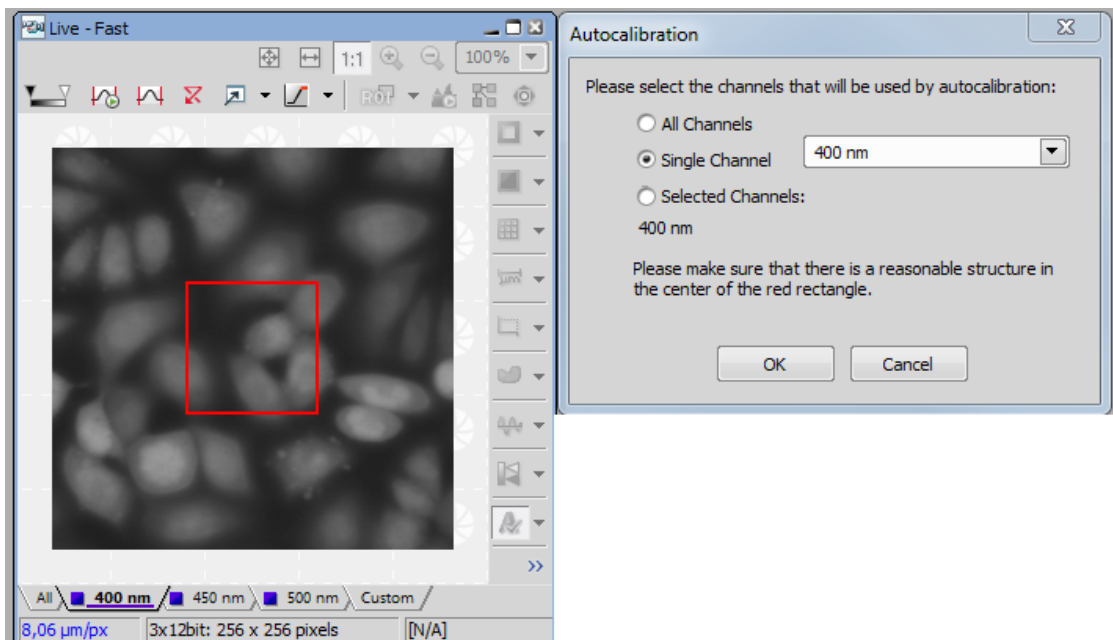
NIS-Elements AR moves the motorized stage, acquires two images, and calculates the calibration from the shift of the images. The auto-calibration performs an estimation of the real relay lens zoom factor. If the estimation does not match the value specified within the *Acquire > Camera Light Path* window, a warning appears.

Figure 4.10. Example of the warning message
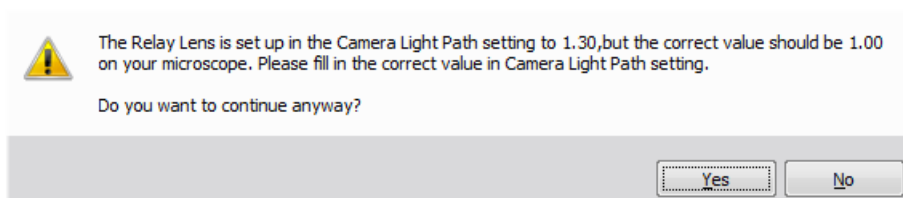
It is recommended to cancel the auto calibration (click No), check the real zoom factor of your relay lens and check and correct the relay lens settings within the *Acquire > Camera Light Path* command window.

The success of this method depends on the texture of the specimen, its contrast, illumination, etc. If the combination of these factors is unsuitable, the auto calibration may fail. If it fails, try the following:

- *Move the stage to another area of the specimen to get a better texture.*
- *Improve contrast by setting LUTs.*
- *Check focus, re-focus if needed.*
- *Turn on the Acquire > Shading Correction > Shading Correction command ON.*

4 points If you select the *4 points* method, the system draws four points on the screen (subsequently) and asks user to move one significant part of the specimen to each position. After all four steps are completed, the calibration is calculated from the moves of the stage.

Superresolution Calibration

This type of calibration is available after successful manual or automatic calibration. It triples the camera's resolution and uses fine stage movements to improve the resulting objective calibration. Click the `Run SR Calibration` button and wait till the objective calibrates.

Calibrate Using Objective

When you have an uncalibrated image, you can use an already calibrated objective to calibrate it. Right-click the *Uncalibrated* field on the image status bar and choose one of the objectives from the *Calibrate using Objective* sub-menu. Of course, the same objective which was used to capture the image shall be selected.

4.1.4. Connecting a Device to NIS-Elements

Before you get to work with *NIS-Elements AR*, all hardware accessories should be connected properly to the system. In most cases, the following basic procedure is sufficient to connect a device successfully:

1. Install *NIS-Elements AR*, and select the appropriate device(s) during the installation.
2. Connect the device to the PC and switch the device ON.
3. Run *NIS-Elements AR*, and run the *Devices > Manage devices* command to open the Device Manager.
4. Use the *Add* button to add the device to *NIS-Elements AR*.
5. Select the device from the list of installed devices and press the *Connect* button.
6. Select logical devices to be activated.
7. Configure device-specific settings using the *Configure (physical) Device* and the *(logical) Device Parameters* buttons.
8. *Close* the Device Manager.

Note

MULTIZOOM AZ100M, ECLIPSE LV series, ECLIPSE MA200, or ECLIPSE L200N/300N microscopes can not be connected from NIS-Elements AR while the setup tool of each microscope is active. To connect any of these microscopes to NIS-Elements AR, exit the setup tool and then run the NIS-Elements AR.

Renaming of devices

Either logical or physical device can be renamed by user. Just right-click the device name and select the Rename Device command from the context menu. The user-defined names can be handy in two cases:

- *You are using two logical devices having matching names, but you need to uniquely identify them (e.g. from macro).*

- *You are used to call a device with another name and would like to rename it in order not to be confused by the predefined name any more.*

4.1.5. What are "Logical Devices"?

NIS-Elements AR handles hardware accessories using the concept of logical devices. There are features of different hardware devices which equal and therefore can be controlled equally. Such features are called "logical devices". A typical logical device is *Stage XY*. Different microscopes can be equipped with different XY motorized stages, although - regarding the user interface - they behave equally. One physical device (a piece of hardware) can contain one or more logical devices the list of which appear in the Device Manager after the connection is established.

Available Logical Devices

Analyzer The analyzer is a polarizing filter placed in the optical path between the specimen and the lamp. The logical device offers two states: ON (inserted) and OFF (extracted).

Aperture This logical device is used for controlling apertures in the light path. It is used in complex microscopes rather than as a standalone device. Two parameters can be typically set for aperture devices, the state (ON/OFF) and aperture size.

Condenser A condenser is a two-lens combination located next to the illumination source in the optical path. Its purpose is to collect light and direct it to the specimen being examined. The corresponding logical device relates to a changer of different condensers.

Filter This logical device controls filter changer movements. There can be several filter changers connected to *NIS-Elements AR* at a time. Each filter changer needs to be set up - filter types shall be assigned to positions of the changer:

1. Display the filter changer control panel (*Devices > Filters and shutters* or *Devices > Microscope Control Pad*).

2. Click the settings button ⬈, a window appears.

3. Select one of the available positions which the filter will be assigned to.

4. Click the [...] button, a list of available filters appears.

5. Select the filter name from the list and confirm it by [OK].

6. The filters can be moved within the already defined positions using the *Up/Down* arrow buttons.

> Note
>
> *When browsing the list of filters, details about the currently selected filter are displayed on the right side of the window.*

Illuminator This logical device is used for controlling the specimen illumination remotely. There is no standard dialog box for the illuminator control. Each device handles this logical device via a user interface specially designed for it - typically containing one button for switching it ON/OFF and a slider for regulating intensity.

Light Path Some microscopes have more than one port where it is possible to attach a light source or a camera. This logical device can switch the illumination between these ports.

Microscope This logical device is used to group standalone logical devices used in certain microscopes. To control the logical devices of a microscope from one control panel, select the *Devices > Microscope Control Pad* command.

Nosepiece This logical device serves for controlling microscope objective changers. There can be three nosepiece types attached to a microscope:

- *Manual* - it can not be controlled via the software.

- *Intelligent* - the current nosepiece position is read by the application, but can not be controlled.

- *Motorized* - such nosepieces can be fully controlled via the *Microscope Control Pad* or the *Nosepiece* control panel.

See 4.1.3.2. Assigning Objective to a Nosepiece Position .

ND Filter A neutral-density filter is a light absorbing filter whose absorption spectrum is moderately flat. It is used to reduce the illumination intensity within the optical path. The logical device offers two states: ON (inserted) and OFF (extracted).

PFS Perfect Focus System - this logical device corresponds to the PFS physical device available with Nikon TE2000/TI microscopes.

Shutter This logical device can control shutters installed in your system. This device is handled via the *Devices > Filters and shutters* control panel or straight from the microscope control pad. You can select the type and rename the shutter by running a contextual menu command either within the *Filters & Shutters* control panel, *Device Manager* window, or in the main toolbar.

> Note
>
> *There was a restricted number of shutters which could be connected to the system in previous releases of NIS-Elements AR and they were identified by Type (DIA, EPI, Aux1, etc...). The current version supports identification of shutters by custom Names, but the Type attribute can be still found in some windows (or used in some macro functions). This is to ensure backward compatibility.*

Zoom This logical device is used for controlling the zoom factor. Run the *Devices > Zoom Configuration* command to adjust the zoom settings.

Stage XY Stage XY enables the movement of specimen within X and Y axes. The system offers user to control stage movements.

Stage Z The Z Drive device enables movement throughout the Z axis direction.

TTL/Analog Input, Output There are six more logical devices available when the NIDAQ controller set is installed:

TTL Input
TTL Output
Analog Input
Analog Output
Calibrated Analog Input and Calibrated Analog Output.
Each of these logical devices represents a connection to an external device which can receive (send) signals from (to) *NIS-Elements*. The number of available connections depends on the actual NIDAQ card.

4.1.6. XY Stages and Z Drives Tips

Please read the following tips for using motorized XY Stages and Z Drives.

Motorized Stage Initialization

A motorized stage being initialized could strike the objective. Before initializing the stage, make sure that the objective is away from the stage. If a motorized Z drive is available, use the *Devices > Objective Clearance* command to prevent this.

Setting Software Limits to Stage Movement

Some microscopes enable you to reduce the range of movement of the motorized stage by setting limits within the configuration window.

1) Display the configuration dialog window (within the *Devices > Manage devices* window).

2) Move the stage to the position where the limit shall be set.

3) Click the appropriate button.

4) Set all the limits by repeating this procedure.

> Caution
>
> *This procedure can not be used if you already set some limits and would like to broaden them at the same time. NIS-Elements AR will not allow you to move the stage to any out-of-limits position. What you need to do is to reset the limits within the configuration window beforehand.*

Using two independent Z Drive devices

Workstations can be equipped with two independent Z drive systems, one coarse (slow) and the other fine (fast). Typically, the first one is used for specimen manipulation in Z axis, when changing objectives, etc. , the second one is used for auto-focusing. The following text explains how *NIS-Elements* handle the two Z drives.

Absolute Z The current positions of both Z drives (Z1, Z2) and the absolute Z position (sum of Z1 and Z2) are displayed in the main status bar. If there is not enough space to display all these values, only the *absolute Z* value is displayed (Z1 and Z2 still appear in a tool-tip). The absolute Z value appears in the Z Series Setup.

Active Z

The concept of *Active Z* enables user to select the preferred Z drive:

- The active Z drive is used when performing auto-focus.

- The *Devices > Enable Mouse Joystick Z in Live (*current value* um)* command applies to the active Z.

You can select the Active Z device in the *Devices > Mouse Joystick and Auto Focus Z* menu.

Regular Z drive + Piezo Z drive

If you have a regular Z drive and a Piezo Z drive installed, some functionality is added:

- The *Move Piezo Z to* button is added to the *Z Series Setup* window when Piezo Z is selected.

- The *Devices > Keeps Z position and centers Piezo Z* and the *Devices > Move Piezo Z to Home Position* commands appear in the *Devices* menu

The button and the *Devices > Keeps Z position and centers Piezo Z* command provide corresponding functionality. They move the Piezo Z device to its home position, and compensate this shift by moving the second Z drive in order to maintain the absolute Z position.

4.1.7. Camera Settings

A digital camera mounted to a microscope port records images of the observed scene on a light-sensitive sensor, and transfers them to a computer. *NIS-Elements AR* supports various cameras differing from each other in resolution, frame-rate, sensor type, etc. Despite these differences, controlling different cameras is similar. The following features are included in the *View > Acquisition Controls > Mono Sim Settings* window depending on a particular camera type.

Note

The complete list of cameras and devices supported by NIS-Elements is available in a separate document.

1 frame for Fast Timelapse The camera head is equipped with 4GB of memory available for fast capturing. Use this button to run acquisition with maximum available frame rate. However, the size of image sequence is limited to 4GB.

Note

The actual frame rate depends on other settings (readout mode, sensor mode) and is limited by the read-out speed of the camera sCMOS chip. Maximum frame rate can be achieved with the combination of Rolling shutter and Overlap sensor mode.

Note

When using the Fast Timelapse acquisition, maximum number of frames which fit to PC memory can be determined by the Max *button. However, due to specifics of Andor NEO this number is overestimated. The real number of frames is a sum of frames which fit to the 4GB memory plus frames which are sent to the PC during the acquisition. It is impossible to predict this number correctly.*

AE Compensation In automatic exposure modes, the compensation affects how optimum exposure settings (Exposure time and Gain) are calculated. The Compensation value is expressed in Exposure Values (EV). Setting the compensation to + 1.0 EV makes the image twice brighter (e.g. doubles the Exposure time or Gain).

AE Lock This option makes the automatic exposure mode to lock the current exposure settings (Exposure time and Gain).

Analog Gain Controls the Gain (Sensitivity) of the camera and strength of the camera analog signal before it is digitized. This setting affects image brightness.

Auto White Balance (AWB) This button performs an automatic white balancing. It calculates the right values and then adjusts the red, green, and blue image components in order to get a neutral white color. The Auto White gives the best results on color neutral (gray) scenes.

Averaging Averaging is a commonly used technique of decreasing noise in the image. In this method 2, 4, 8 or 16 consecutive frames are averaged together.

Binning The binning mode provides considerably enhanced camera chip sensitivity by integrating more elements (pixels) together. E.g.: *binning 4x4* integrates the signal from the area of 4x4 chip elements to one pixel of the resulting image. Smaller resolutions and faster frame rates are achieved using the binning modes.

Capture Bit Depth Channel Select per-channel bit depth used for capturing.

Clear Cycles This option enables you to decide, how many times the CCD chip should be cleared (reset). The set *Clear Mode* determines how often it is performed.

Clear Mode Sets how often the CCD chip will be reset. Please, see the camera user manual for further details. These options are available:

• Automatic

• Never

• Pre-exposure

• Pre-sequence

• Post-sequence

- Pre and Post-sequence

- Pre-Exposure and Post-Sequence

Commands > ROI > Use Current ROI Turns the camera ROI ON/OFF. See 5.3. Camera ROI.

Contrast Affects dynamics of how the luminosity is rendered. There are several modes for different illumination (contrast) scene situation.

Conversion Gain This is a hardware pre-amplifier gain.

Cooling The Qi1/Ri1 camera heads are equipped with a cooling system that enables to cool down the CCD chip by -5° or -10° of the current room temperature.

Warning

When cooling by -10°, there might be a risk of water condensing inside the camera head (depending on humidity inside the room).

Desired Temperature Sets the target temperature of the CCD chip.

Detector Mode

Only in 12bit mode

> **Timed** A common mode for capturing images.

> **Strobe** If the camera controller is equipped with *DS-RC* switch, this mode needs to be selected to use the switch for capturing images.

Dynamic Range Dynamic range of a camera sensor is defined by the largest possible signal that it can generate divided by the smallest possible signal. Higher bit dynamic ranges bring wider ranges of grayscale levels from each camera pixel.

EM Gain Multiplier EM gain allows weak signals to be multiplied before any readout noise is added by the output amplifier, hence rendering the read noise negligible. There is a complex relationship (approximated to an exponential) between the value set and the actual level of multiplication. Typically, optimal signal-to-noise ratio and dynamic range is achieved between x1 to x300 EM Gain. When the setting exceeds the value of 300, the field gets highlighted red in order to notify you.

Exposure, Exposure Mode Exposure mode determines how the Exposure time is calculated. Usually, the user can select between the automatic and the manual mode:

> **Auto Exposure** Automatically calculates the exposure time in order to achieve optimal brightness of the scene. Image quality is the priority, therefore longer exposure times are preferred.

Note

Depending on particular light conditions, the result of auto exposure may not be optimal for image acquisition.

Warning

Under- over-saturation may occur when switching between optical configurations. Please see 4.1.2.5. Using Auto Exposure while Switching between Optical Configurations.

Available exposure time range depends on the selected Format and Readout Speed .

Manual Exposure User selects both *Exposure* time and *Gain* manually.

Continuous AE When selected, the automatic exposure time is calculated continuously. If brightness of the scene changes, the exposure time is adjusted so that the live image is not over- or under-saturated.

Exposure Time *Exposure time* is the time of charge accumulation in a camera chip between two adjacent frames. Prolonging the exposure time increases brightness of the image as well as its quality (there will be less noise).

Fast (Focus), Quality (Capture) The software enables to create two resolution presets - *Fast* used for live image and *Quality* used for capturing. The formats then differ in image size, and consequently in frame rate (number of frames per second - fps). The higher the resolution is, the lower frame rate can be achieved. Available resolutions depend on the camera type.

Note

When switching between the formats while observing the live image, the size of the image on the screen is maintained - the zoom setting is changed instead. Only in some special cases, the behavior changes and the image size is changed instead of the zoom setting which is maintained.

Gain Controls the camera sensitivity. Increasing the gain increases brightness of the image, but decreases quality (the more random noise, the more streaky noise and color unevenness), and increases the frame rate indirectly by enabling shorter exposure times.

Gamma The gamma correction maps the intensity of live signal exponentially to the gamma parameter. For gamma < 1, dark portions of the image are enhanced whereas for gamma > 1, image parts of higher intensities are enhanced.

High Quality Capture When this option is checked, images are not processed by the camera control unit, but are sent to the computer in a RAW format. The computer then processes the images into the RGB format. This mode produces images with less noise and better color fidelity. Of course, transfer of the RAW data is slower than the normal mode which causes a lower maximal frame-rate.

Hue Hue shifts image colors across the rainbow.

Internal Shutter This option sets behavior of the camera internal shutter.

Opened Internal shutter is opened upon NIS-Elements and stays opened through the whole session. Only with one exception: if the *Readout Mode* is set to *Normal 80kHz at 16bit*, the shutter stays closed and opens only for exposures.

Closed Internal shutter is closed upon NIS-Elements startup and gets opened only for exposures.

Automatic Shutter is closed when the NIS-Elements is running. It opens only for acquisition.

Isolated Crop Mode This option turns on the *Isolated Crop Mode* described within the Andor documentation. In this special mode, the size of chip is reduced and very high frame-rates can be achieved.

Keep Overlapped If checked. The Clear Mode and Sensor Mode boxes are set to Automatic and become disabled. This mode produces maximal frame rate and decreases the list of exposure times (in the camera settings window). Only the times of 1 frame and longer become available.

Limit Max. FPS to Insert maximum frame rate (frames per second) you would like to use, this will prolong the total duration of the time-sequence in exchange for the reduced frame rate. This option may be useful especially when using the *1 frame for Fast Timelapse* button with a small region of interest. Then the frame rate could get unreasonably high.

> Note
>
> *The Limit MAX FPS is possible only when camera is in Normal mode (not overlapped), the maximum fps in normal mode is shown next to the edit box.*

Live Acceleration If the exposure time is too long, *Live Acceleration* helps you to make it shorter. The system automatically shortens the exposure time (so the frame rate rises), and the loss of intensity is compensated by increasing the gain (software multiplication). This procedure is not used upon *Capture*.

Maintain Temperature on Camera Shutdown If this option is checked, the camera maintains its temperature until the computer workstation shut down. The temperature will be maintained for example during NIS-Elements restart or when another camera is selected.

Maximum Exposure This is a safeguard of the time of the Auto Exposure. For quick exposure, it is convenient not to set this value too high.

Metering Mode If this option is available, auto exposure can be calculated with an emphasis on over-exposed peaks (*Peak*) or average pixel intensity (*Average*).

Mode Defines how the Auto Exposure is performed. Average is suitable for bright field and Peak is better used for dark field.

Mode This option selects the mode of the camera. In the *Extended NIR* mode, the camera chip sensitivity in the near-infrared region of spectra is raised.

Multiplier Controls on-chip multiplication gain. There is complex relationship (approximated to an exponential) between the multiplier value and the level of multiplication - in other words, the value 2 does not mean the signal is multiplied two times.

Noise Reduction If activated, it reduces noise generated by higher gain. NR works on interframe basis so you may notice some shadows on moving objects.

Offset Sets the brightness of the image. It is a constant additive (positive or negative) changing all pixel values of the image. With negative offset value the dark image areas become pure black. Considering fluorescence microscopy, an appropriate offset setting can create continuous black background and thus help to enhance (together with gain or illumination enhancement) the contrast.

Overillumination Tolerance Sets how many pixels should be white after the Auto exposure is performed. Use lower values (0.01%) for very bright (shining) objects (fluorescence). For common bright field, even 1% may be a good value.

Readout Mode Within this option you can select a combination of these parameters - readout speed and bit depth. The settings influences frame rate and image quality. Higher frequencies increase frame rate, lower frequencies offer better image quality.

Readout Rate Select the readout speed 100 or 280 MHz. If speed is not crucial, leave *100 MHz*.

Readout Speed The speed of digitization - influences frame rate and image quality. Higher frequencies increase frame rate, lower frequencies provide better image quality.

Saturation The amount of saturation determines how colors are rendered. More saturation produces richer colors. Less saturation makes the colors gray.

Scene Mode, Preset There are presets of camera settings optimized for the specific usage. You can reset the settings by the *Reset* button.

> **Neutral** Neutral preset.

> **Industrial microscopy:**

>> **W (Water/IC)** Wafer IC-Chip.

>> **M (Metal)** Metal or ceramic.

>> **C** Circuit boards.

>> **FPD** Flat panel displays.

> **Bioscience/Biological microscopy:**

>> **DF/FL, F** Dark-field fluorescence.

>> **DIC/PH** DIC or phase contrast.

>> **LED-Bright Field, B** LED illuminated bright field.

>> **HAL-Bright Field** Halogen illuminated bright field.

>> **HAL-HE, H** Halogen illuminated Hematoxylin and eosin stain.

>> **HAL-ELA, E** Halogen illuminated enzyme labeled antibody method.

> **Asbestos** Asbestos.

Sensor Mode This option sets the method of reading the CCD chip.

> **Automatic** This option makes the system to choose the best mode.

> **Normal** A standard mode where the exposure phase is followed by the readout phase. Please, see the camera user manual for further details.

> **Alternate Normal** Please, see the camera user manual for further details.

> **Overlap** This mode enables the camera to achieve higher frame rates by reducing the exposure+readout time (the readout is performed simultaneously with the exposure of the next frame). Please, see the camera user manual for further details.

> **Frame Transfer** This mode enables the camera to achieve higher frame rates by reducing the exposure+readout time (the readout is performed simultaneously with the exposure of the next frame). Please, see the camera user manual for further details.

Separate Channel Settings Having a color camera, influence of the settings described above on each color channel can be adjusted. In such case, three edit boxes with the default values set to 1.0 are displayed below the feature setting. These values determine the influence of the feature on each color channel.

Sharpness Some camera settings provide the sharpness control which affects how sharp edges in the image appear. Too much sharpness leads to over-saturated edges.

Speed up Triggered Acquisition + Different Exposures Connect the *Arm* and the *Ext. Trig* connectors of the camera and enable this option. When you run triggered acquisition with the *Use different exposures*

option enabled, it will speed up the performance (the camera will not have to wait for software triggers but will be controlled by the *Arm* triggering signal).

Spurious Noise Filter This option reduces spurious noise. Try whether it has desirable influence on image quality.

Target Maximum Intensity Restricts the maximum of image intensity after the auto exposure was applied. The value represents a percentage of the whole camera dynamic range.

Trigger Mode Sets the exposure method.

> **Internal** The exposure time and the beginning of each frame acquisition is controlled by the settings the software.

> **Strobe** In this mode, the beginning of each frame exposure is being controlled by external signal.

> **Bulb** The exposure time and frames timing are controlled by external signal connected to the camera.

Trigger Mode Sets the exposure method.

> **Internal** The exposure time and the beginning of each frame acquisition is controlled by the NIS-Elements AR settings.

> **Strobe** In this mode, the beginning of each frame exposure is being controlled by external signal.

> **Bulb** The exposure time and frames timing are controlled by external signal connected to the camera.

> **Triggered by master camera** Having a dual camera system (or a special triggering device connected to the camera), you can use this mode. If the cameras *Interval* setting is set to *No delay* within an experiment or if the Live mode is run, the slave camera will be triggered only once so that both cameras will run simultaneously while single frames will not be synchronized. If the settings are different, the trigger will be engaged once and all the subsequent frames will be controlled by the NIS-Elements AR settings as in the *Internal* mode.

Use Current ROI The currently defined region of interest, set by the *Define ROI* command, can be switched ON/OFF by checking this item.

Using Probe The probe ▢ enables you to determine a small image area that serves as the data source area for LUTs, histogram etc. It also affects the AWB (auto white balance) and the AE (Auto Exposure) features of the cameras which support it. When the probe functionality is not supported, the AWB and AE algorithms are computed from the whole image.

Vertical Pixel Shift

> **Shift Speed** Please, see the camera user manual for further details

> **Vertical Clock Voltage Amplitude** Please, see the camera user manual for further details

White Balance There are Red, Green and Blue Gain properties that control how colors are rendered. It is used to eliminate a color cast from white areas.

4.1.8. Controlling Illumination Devices

The substance of an illumination device is the light source. The light emission is produced mainly by a laser, diode or a light bulb. *NIS-Elements AR* does not distinguish between wavelength modules of these light sources and all are controlled in a similar manner. Basically you select which wavelength(s) you want to use for illumination and set its light intensity. The selected wavelength modules are turned off until you activate the ⬤ shutter button usually placed in the bottom left corner of the control panel.

5. Image Acquisition

5.1. Introduction to Image Acquisition

Having *NIS-Elements AR* installed and all hardware accessories set up, you can start capturing images. Let's begin with the simplest case.

How to capture a single image

1)   Turn the connected camera and other devices ON and start *NIS-Elements AR*.

2)   Run   *Acquire > Camera Settings* to display the *Camera Settings* control panel.

3)   Switch camera to the *Live-Fast* mode (*Acquire > Live - Fast*)

4)   Adjust resolution of the *Live Fast* mode to get a continuous Live image. It is recommended to set a low resolution in order to achieve high frame rates. This is good when searching the specimen or when focusing manually.

5)   Adjust *Exposure time* to get a nice image of the scene.

6)   Focus on the scene.

7)   Optionally, turn on the camera ROI. The *Define ROI* button appears on the toolbar if the camera ROI function is supported by your camera. See also 5.3. Camera ROI.

8)   Capture the image by running the   *Acquire > Capture* command

9)   A new image is opened automatically and named "Captured".

Tools for Handling the Live Image

Live - Quality, `Ctrl` + `+` If a high frame rate is not crucial or if you would like to view the live image exactly as it will be captured, run the   *Acquire > Live - Quality* command. It displays the live image in the *Quality (Capture)* resolution. This resolution is used whenever an image is captured.

Capture, `Ctrl` + `-` When pressed, the camera exposure runs till the end of the current frame, and the next frame is captured and displayed on the screen (it is the first frame with the complete exposure after you have pressed the *Capture* button).

If you are using the *Live-Fast* mode and click   *Acquire > Capture* , *NIS-Elements* will automatically switch to the quality mode to capture the image. When the image is captured, it is opened on the screen in a new image window.

Freeze, - The *Freeze* button interrupts the camera exposure, and displays the very last complete frame.

5.2. Shading Correction

Shading correction is a method which can correct illumination inhomogenities of the captured images. How does this work? First of all, a "correction image" must be captured. It is an image which represents illumination intensities within the field of view. Such image is created by capturing a blank scene and can look like this:

Figure 5.1. Inhomogeneous illumination

As you see in the image, the illumination intensity is 100% in the center but gets darker near the edges. This can be corrected by applying the shading correction which will - based on the correction image - equalize intensities of the resulting image:

Figure 5.2. Image acquired without the shading correction
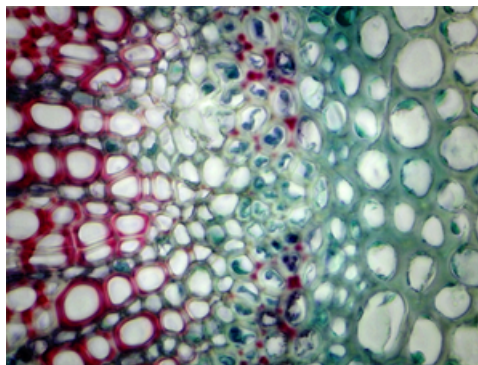
Figure 5.3. Image acquired and corrected
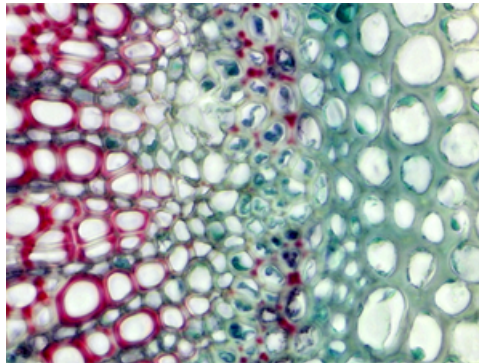
The shading correction can minimize the illumination and background inhomogenities by subtracting a blank scene from the current image.

How to setup a shading correction

1) Decide whether to use just a single shading correction image or different image for each optical configuration. Make the selection via the *Acquire > Shading Correction > Shading per Optical Configuration* commands.

2) Get the correction image. You can either acquire a new image by the *Acquire > Shading Correction > Capture Correction Image* command or use an existing one. To use some existing image, open it and call the *Acquire > Shading Correction > Use Current Image As Shading Correction* command. In both cases the correction image will be kept in memory. To view the correction image, run *Acquire > Shading Correction > Show Correction Image* .

3) In case of "per optical configuration" shading correction, repeat this procedure for with each optical configuration turned ON.

4) The *Acquire > Shading Correction > Shading Correction* command is enabled if only the correction image is available for the current acquisition settings. For example, if the *Shading per Optical Configuration* is chosen but the correction image for the current OC has not been captured yet, the *Acquire > Shading Correction > Shading Correction* stays disabled.

5.3. Camera ROI

You can specify a camera ROI on a live image by selecting the *Acquire > Camera ROI > Define ROI* command. The live image is then restricted to the defined area whenever the ROI button is pressed.

Figure 5.4. Turn Camera ROI ON/OFF

The camera ROI can be also saved to a file for later use. This can be done by the *Acquire > Camera ROI > Save ROI* command. When the time comes, load the saved camera ROI by the *Acquire > Camera ROI > Load ROI* command. The current ROI will be overwritten.

The same Define ROI button appears in the toolbar of a captured image too. It does not matter whether you define the ROI on the captured or live image, the setting is shared.

<u>**Camera ROI Definition**</u>

1)   Run *Acquire > Camera ROI > Define ROI* . The following window appears:
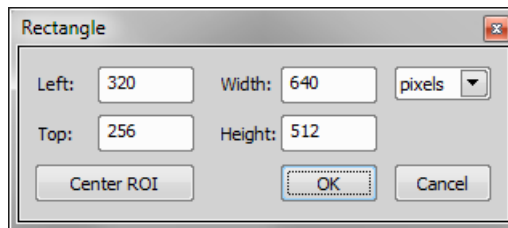
2)   A ROI frame appears in the image at the same time. You can either change ROI dimensions and/or position using a mouse cursor, or you can enter precise numerical values - position from the *Left* and *Top* and dimensions of the ROI - *Width* and *Height*. Units selected from the combo box on the right side of the dialog window (*pixels*, *μm*, *mm*) are automatically applied to the ROI definition and preview image.

3)   If you would like the ROI to be placed in the center of the camera chip, click Center ROI . The ROI will move to the center of the live image.

4)   Confirm the action by the OK button.

> Note
>
> *NIS-Elements may adjust the ROI size automatically after the user sets it. Such automatic adjustment is needed in order for the ROI to work in different resolutions of the Live Fast and Quality Capture formats.*

Caution

Nikon DS cameras have fixed-sized camera ROI, therefore width and height of the ROI cannot be changed. Only its position can be adjusted within this window.

<u>Predefined Camera ROI</u>

If you are using one of the following cameras, you can choose a predefined camera ROI from the pull-down menu which offers several predefined frame sizes.

- Andor Neo

- Andor Zyla

- Hamamatsu ORCA Flash 2.8

- Hamamatsu ORCA Flash 4.0
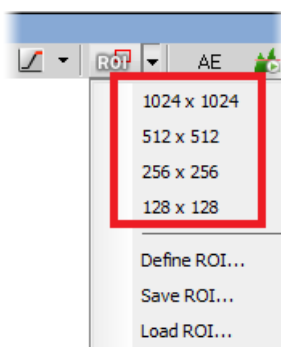
5.4. About ND Acquisition

NIS-Elements AR being a multi-purpose imaging system can be used as a handy tool to study objects, live organisms, processes, etc. The universal ND2(N-dimensional) file format is what makes this possible. One *ND2* file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types:

Note

Each acquisition type gives a name to the corresponding dimension of the resulting ND2 file. For example, we can speak about two-dimensional TZ ND2 file, which means the file contains the Time-lapse dimension and the Z-series dimension.

Time-lapse - T A sequence of images can be captured over a period of time and create a time-lapse image. See 5.5. Time-lapse Acquisition.

Multi-point - XY Images from different coordinates of the XY stage can be acquired. See 5.6. Multi-point Acquisition.

Z-series - Z Several techniques utilize Z-stacks of images. Such Z-stack can be for example converted to a 3D model of the specimen. See 5.7. Z-series Acquisition.

Multi-channel - λ Fluorescence signals can be captured selectively using excitation an emission filters, each of them creating a different color channel. See 5.8. Multi-channel Acquisition.

Large image - M *(requires: 6D)*

When the area of interest exceeds the camera field of view, you can capture a large image composed of multiple image frames and let it be stitched by an automatic algorithm. See 5.9. Large-image Acquisition.

An nd2 file can be created (captured) either containing one dimension or combining several of them.

- Single-dimensional documents can be created automatically (using motorized accessories) or manually using commands from the *Acquire* menu.

- To create a multi-dimensional image, use the *Applications > 6D > Define/Run ND Acquisition* command (ND module required).

<u>Common ND Experiment Options</u>

The following options can be applied to all types of ND experiments:

Experiment You can enter a custom name of the experiment. Also name of the phase is editable.

Path Browse to a folder where your ND2 images will be stored.

Save to File Please see 5.11.2. Save to File.

Custom Metadata *(requires: Local Option)*

Check this option to add custom metadata. The created metadata can be changed after acquisition. Display image properties (*File > Image Properties*), display the *Custom Metadata* tab and click the Modify Description button.

Record Data Please see 5.11.3. Recorded Data.

Auto Focus Automatic focusing can be used during the experiment. You can select the auto-focus method that best meets your needs. It can be determined whether the focusing shall be performed at the beginning of the whole experiment or at the beginning of each phase. The *Define* button shows a window where you can define parameters of the selected focusing method.

> Note
> ___
> *If the auto-focus is set to be performed Before each Phase and the ND Experiment contains just a single time phase, the automatic focus will not be performed. To run auto focus, use the At the Beginning option.*

Closing Shutter The active shutter can be closed between single image acquisitions. Just select the *Close active Shutter...* option in the experiment window.

Running Macro Commands You can define a command (or a macro) to be run in various stages of the experiment. Select the timing in the *Advanced* section of the experiment window and enter the command to be run.

In/Out signals *(requires: 6D)*

An ND2 experiment can control some accessory devices and can be controlled by another accessory devices at the same time. This can be achieved by sending/receiving Analog/TTL signals through an NIDAQ card. If the NIDAQ PCI card is installed, an extra tab called *Inputs/Outputs* appears within the ND experiment window.

Load Use this button to for a configuration XML file to be loaded. The file with the current configuration can be created by the *Save* button.

Save Click this button to save the current configuration of this dialog to an XML file. A standard *Save As* window appears.
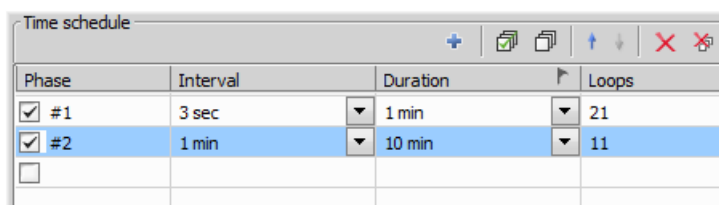
ND Sequence Options

The following options can be applied to time, multipoint and multichannel sequences in ND experiments:

✛ **New** This button adds new step into the sequence.

▥ **Select All** Use this button to select all steps in the sequence.

▭ **Clear Selection** Use this button to clear all steps selection.

↑ **Move Up,** ↓ **Move Down** These buttons moves the current step up/down in the sequence.

✗ **Remove Current** This button removes current step from the sequence.

▧ **Remove All** This button removes all steps from the sequence.

5.5. Time-lapse Acquisition

Detailed studying of long-lasting processes is enabled by the time lapse acquisition mode of *NIS-Elements AR*. The achievable experiment duration is limited by the hardware abilities of your PC only. Invoke the *Acquire > Capture Timelapse > Capture Automatically* command to set the experiment up:

Phase	Interval	Duration	Loops
☑ #1	3 sec	1 min	21
☑ #2	1 min	10 min	11
☐			

The *Time schedule* table enables you to define consecutive time phases where duration, interval between single images, and number of images of the phase can be adjusted. The *Interval*, *Duration*, and *Loops* settings are bound together, so you just need to set two of these parameters. The remaining parameter is calculated automatically. Only the selected time phases will be captured.

The flag mark indicates the Duration/Loops priority. For example: if the camera exposure time exceeds the defined *Interval* between frames, than the experiment settings are not achievable - only the marked column will be pushed to be correct. You can set the priority column by clicking on its caption.

Special Options

The following options can be used with this type of ND acquisition. Options common to all types are described here.

Incubation *(requires: Stage Incubator)*
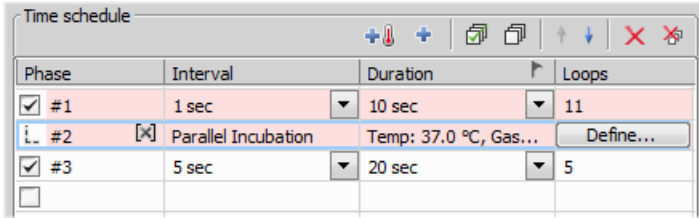
This option is available when an incubator is properly connected. Press the ✚🌡 *Add New Incubation* button to add incubation phase to the time schedule list. You can connect the incubation phase to the previous time phase which makes the incubation run parallel with the selected time phase. To connect an incubation phase, press the up arrow button that appears in the Phase column next to the phase name. To cancel the connection, press the cross button that has replaced the arrow button.

Figure 5.8. Connected Incubation Phase

The *Duration* column displays value defined in the Incubation Phase Settings window that appears after you press the *Define* button. Incubator parameters (temperature and gas) can be set within this window.
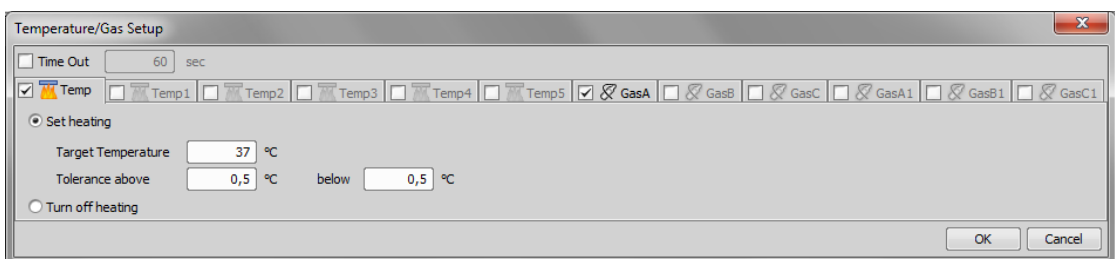
Switch Transmitted Illuminator off When Idle If this option is selected, the lamp is turned off whenever there is a long-enough gap between two acquisitions.

Warning

It takes a little time for some lamps to reach the set intensity. In such case, we recommend to use the `Wait();` *macro function before the capture is performed.*

FRET Checking this item turns on the FRET View for nd2 file which will be acquired. You can open the *FRET View* dialog by pressing the *Define FRET* button.

Use HW sequencer This option is available only for A1 confocal microscope. If checked, A1 HW sequencer will be used for acquisition. It provides the ability to define two experimental setups for HW Sequencer on and off.

Events Press the Events... button to display the following window:
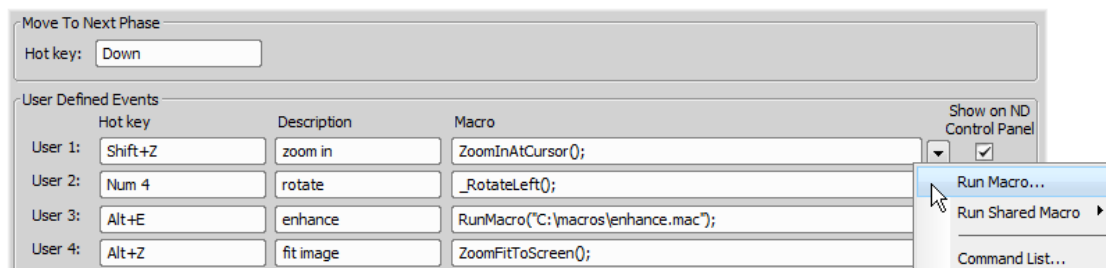
Define user hot keys which run user events during time-lapse acquisition. Simply click inside the *Hot key* field of the User events window and press any key combination you want to assign to the event. Then you can enter the event description. A macro command can be specified which will be executed when the event occurs (triggered by user). When the event occurs, the specified command is run and a marker is placed to the image sequence.

Tip

* *Check the Show on ND Control Panel option for any event to be displayed it in the   View > Acquisition Controls > ND Control Panel window.*

* *In the Selected User Event pull down menu at the bottom of this window, choose one of the events which will be inserted to an ND2 image sequence every time the* 📍 *Insert Default User Event button is pressed. See Event and Selection Controls.*

Note

When the time measurement runs on live image, an ND acquisition is in progress, or the Time ND document is active, the hot keys of the defined events take priority over all other hot keys.

Keep Object in View *(requires: Local Option)*

This function automatically finds the object of interest based on the correlation between the previous and the next frame and always keeps it in the center of view using the microscope stage to compensate the object's motion.

Start by checking the *Keep Object in View* check box and clicking Settings to specify the channel on which the object of interest is visible and will be followed. Move your object into the center of your field of view. Adjust your camera and illumination setting to highlight the object. High contrast and low noise image bring the best results. Now you can run the ND Acquisition. The stage is automatically moved in between capturing frames so that the object of interest is always centered.

5.5.1. Timing Explanation

When performing fast Time-lapse acquisition (capturing images in short intervals), it is important to understand how the acquisition with digital camera works.

Case #1: No delay If the interval in the experiment is set to *No delay*, the camera runs in "internal hardware trigger mode" (i.e.: Timed mode, Streaming mode). The camera sends maximum frames to the computer. This may result in a situation where the computer is not capable of receiving all the frames the camera sends. If so, depending on the camera type, the overflowing frames may be omitted.

Case #2: Interval is too short The time needed to receive one frame consists of three phases: exposure time, readout time and software overhead delay.
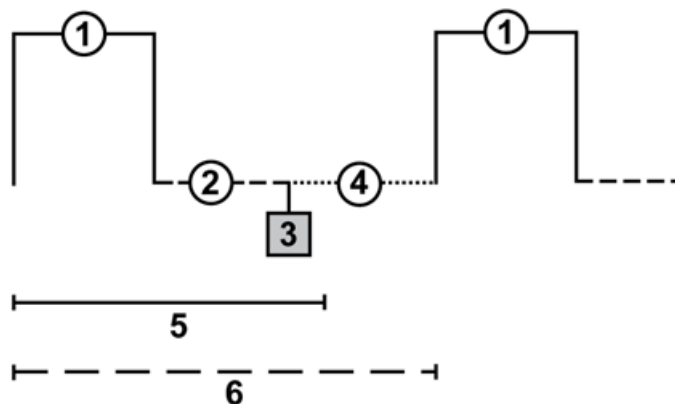
Legend:

1. exposure time

2. readout time

3. camera sends data to PC

4. software overhead time

5. specified interval

6. resulting interval

The camera runs in a "software triggered mode" where it gives the frames much slower than in *No delay* because it is being synchronized with other events. In this case it is synchronized with timing of the time-lapse acquisition. If the interval between frames is set to a shorter time than is the sum of the three phases (e.g. 50 msec), some latency will occur. As a result, the interval in the image sequence is pro-longed to (exposure time + readout time + SW overhead).

Case#3: Interval is sufficient The camera runs in a "software triggered mode". If the interval is sufficient, the timing in the resulting image sequence will equal the timing specified within the acquisition window.
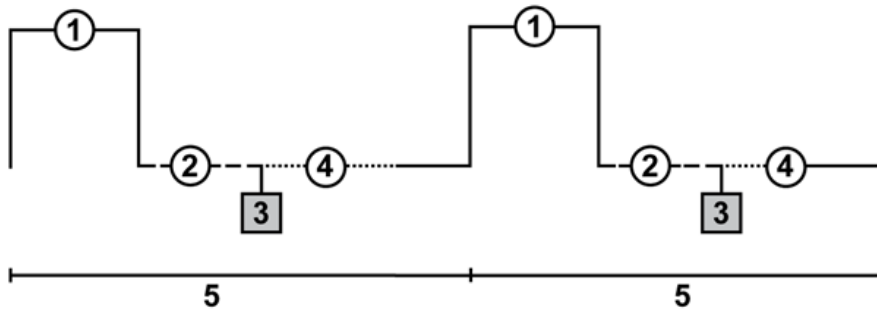
Legend:

1. exposure time

2. readout time

3. camera sends data to PC

4. software overhead time

5. specified interval

5.6. Multi-point Acquisition

This window serves for defining XY(Z) points to be scanned during the multipoint capture experiment. This feature is available when a motorized stage XY(Z) is present in the system. Check the *Include Z* box to display the Z column. The list of defined points can be saved (and loaded later) to an XML file by the *Save* (*Load*) button. Run the *Acquire > Capture Multipoint > Capture Automatically* command.
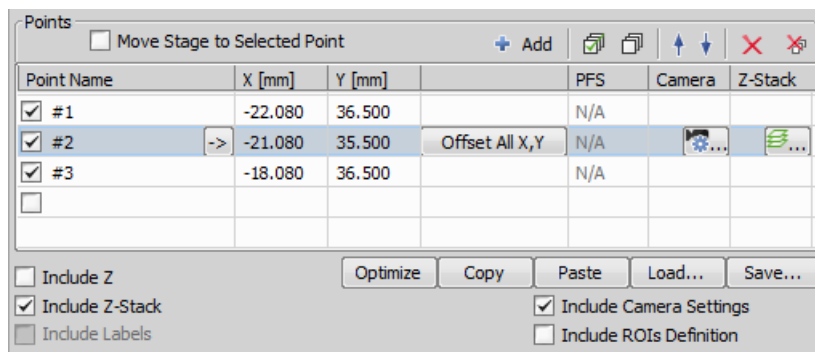
5.6.1. Point by Point (Manual) Multi-Point

1. Move the stage to the first position.

2. Press the ✛ *Add New* button. A new line containing current coordinates of the stage appears in the list.

3. Move to the next position and repeat the steps until you have all the intended points defined.

5.6.2. Well Plate (Rectangular) Multi-Point

You can insert a pattern designed to cover a wellplate. Of course it can be used to create any rectangular pattern.

1) Click the Custom button. The following window appears

2) Select *Manual* if you know the distances between wells. Otherwise select the *Interactive* and continue.

Manual This method will create a pattern which will scan *Rows* x *Columns* fields. The *Distance X* and *Y parameters* specify distances between two fields. The scanning will begin at the current position.

Interactive The interactive method lets you specify the number of fields to be scanned and starts a wizard where you will specify the top-left and the bottom-right corner of the well plate. The distances between wells are calculated automatically.

3) Click Finish.

5.6.3. Large Image (Covering) Multi-Point

A Multi-point which will cover certain area can be created:

1) Click the Custom button and select the *Large Image* tab

2) Set the large image settings:

Scan Area Select whether to define the *Scan Area* by number of fields or the actual field size.

Camera Select the camera to be used. This field is enabled only if you have a dual-camera setup.

Objective Select the objective for which the multi-point will be calculated. The resulting number of fields / area size changes depending on the selected objective magnification.

Overlap Overlap of neighboring images in %.

3) Click Finish .

5.6.4. Random Multi-Point

(requires: Local Option)

Specify the area where a random set of points will be generated.

1) Click the Custom button and select the *Random* tab.

2) Select shape of the area to be filled with random points

Rectangle The area will be defined by a rectangle. Define X and Y coordinates of left top corner of the area, width and height of the area.

Radius The area will be defined by a circle. Define X and Y coordinates of center of the circle.

3) Specify *Number of points* in the multi-point set.

4) Click Finish .

5.6.5. To Change a Single Z Coordinate

1. Make sure the *Move stage to selected point* is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.

2. Click inside the line you would like to change.

3. Move the Z drive to the new position.

4. Click the <- button.

Note

The XY coordinates of one point cannot be adjusted (unless you delete it and add a new point).

5.6.6. Special Options

Autofocus None, Steps in Range, Steps in Continuous.

Custom Displays a tool for creating predefined multi-point patterns.

See 5.6.2. Well Plate (Rectangular) Multi-Point, 5.6.3. Large Image (Covering) Multi-Point, 5.6.4. Random Multi-Point.

Execute Command after Capture You can either Run Macro, or select a command from the Command list. The command is executed after capturing.

Execute Command before Capture You can either Run Macro, or select a command from the Command list. The command is executed before capturing.

Include Z If selected, also the Z coordinate will be taken into account during XY(Z) acquisition.

> Note
>
> *This option is disabled if the Relative XY option is used.*

Leave PFS offset ON between points Check this item to keep PFS on while moving between the points.

Move Stage to Selected Point This button ensures that the motorized stage moves to the coordinates of the currently selected point.

Offset All

The offset all button can shift the XY coordinates of all points in the same way:

1. Make sure the *Move stage to selected point* is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.

2. Select one point of the list.

3. Move the XY(Z) stage to a new position (define the offset).

4. Press the *Offset All* button.

5. The coordinates of all points change - the same shift which you made with the stage is applied to them.

Optimize If the *Optimize* button is pressed, the system will re-order the defined points in order to minimize the XY stage trajectory.

Point name Displays the name of the point. You can change the name (default names are #1, #2 #3, etc.).

Redefine reference Z after Auto Focus/PFS Check this option to redefine reference Z position after performing autofocus or PFS (if AF or PFS is turned on).

Relative XY Check the *Relative XY* item to consider all coordinates as relative with respect to the current stage position. Any of the points may be used as the reference point, just right-click it and select *Set this point as a reference position*.

Split Multipoints Select this box to perform the *File > Import/Export > Split Multipoints* command directly after the ND2 experiment.

> Note
>
> *When you apply the Split Multipoints command on ND acquisition that contains only the XY dimension, the information about the ND dimensions in the File > Open dialog becomes unavailable.*

Use Focus Surface Uses Focus Surface for capturing.

X, Y, Z Displays X, Y and Z coordinates of the point. The arrow buttons `->` `<-` assign the current XYZ position to the point. The `Offset All X, Y, Z` button shifts the XYZ coordinates of all points by the same offset which is defined as a difference between the current stage coordinates and coordinates of the selected point.

5.7. Z-series Acquisition

Automatic capturing of images from different focal planes using a motorized Z drive can be performed. Run the *Acquire > Capture Z-Series > Capture Automatically* command to display the setup window. There are three different approaches of how to set the experiment:

Top and Bottom, Step

1) Press the ⊥ button, the cube turns blue.

2) Run the Live camera signal. In case you have two Z drives, select the *Z device* from the pull-down menu.

3) Set the Z range: move the Z drive to the top position and press the *Top* button, move it to the bottom position and press the *Bottom* button.

4) Define the *Step* size in µm or the number of steps to be captured.

> Note
>
> *In this mode the Home position is assigned automatically to the middle step (e.g. the third step out of the whole 5 steps). The Reset button discards the Top, Home, and Bottom positions settings.*
>
> *Depending on the direction of Z-acquisition, the last Z position (top or bottom) may slightly differ from the user setting. However, you can select which position will be preserved exactly. Right-click one of the Top/Bottom buttons and select Keep exact Bottom/Top Position. The setting is indicated by underlining the button text.*

Home Position, Range

1) Press one of the following buttons, the cube turns yellow. The range is defined by the home-position and the scanning range:

Symmetric mode Define the *Range* by inserting a value in µm.

Asymmetric mode The range is specified by two values, *Below* - the distance below the home position, and *Above* - the distance above the home position.

The ✛ *Relative* button resets the *Home* position value and the Z range is calculated relatively.

2)   Run the Live camera signal. In case you have two Z drives, select the *Z device* from the pull-down menu.

3)   Move the Z drive to the position which you would like the Z drive to move around. Press the *Home* button to define the home position.

4)   Specify the scanning *Range* .

5)   Define the *Step* size in µm or the number of *steps* to be captured.

> Note
>
> *When you set the Home Position in the Top/Bottom or Symmetric mode, its value is kept after switching to the Asymmetric mode.*

Special Options

Defining TIRF position When the TIRF system is enabled, the TIRF option appears in the right portion of the window. To set the TIRF position:

- Select the *TIRF* check box to enable TIRF.

- Move the Z drive to the correct TIRF Z position.

- Click the *Set TIRF Pos...* button.

Using TIRF

- Enable TIRF by selecting the *TIRF* check box within the Z-Series experiment settings

- At least one value of the *Z Pos* column of the Multi-channel acquisition setup has to be set to *TIRF* (See 5.8. Multi-channel Acquisition.).

Piezo Z If a Piezo Z drive device is connected, the *Piezo* button appears in the panel. Use the pull-down menu to select the action which will be performed upon pressing the button:

> 📊 **Keeps Z position and centers Piezo Z** This option moves the Piezo Z drive to the home position, but compensates the movement by the second Z drive in order to keep the original absolute Z position(sum of Z1 and Z2).

> 🏠 **Move Piezo Z to Home position** Moves the Piezo Z drive to the home position regardless of the absolute Z drive position.

Direction You can define the Z-stack scanning direction - either from *Top to Bottom*, or vice versa (*Bottom to Top*).

Two Z devices Two Z devices (Main Z + Piezo Z) can be used for Z-Series acquisition and 🔾Autofocus task in Jobs. Z device combination is used automatically if AF/Z-Stack range exceeds the range of Piezo Z. Combining two Z devices speeds up the Z stack acquisition, however the user can still decide whether or not to use the Z device combination. Only the Main Z device is used if it is selected in the *Z*

device combo box. Only the Piezo Z device is used if it is selected in the *Z device* combo box and the defined *Range* is within the device range. Combination of both Main Z and Piezo Z is used if Piezo Z is selected in the *Z device* combo box and the defined *Range* is bigger than the device range.

5.8. Multi-channel Acquisition

Fluorescence (multi-channel) pictures can be acquired. Run the *Applications > 6D > Define/Run ND Acquisition* command and select the *Lambda* tab. There you can set the multi-channel experiment.
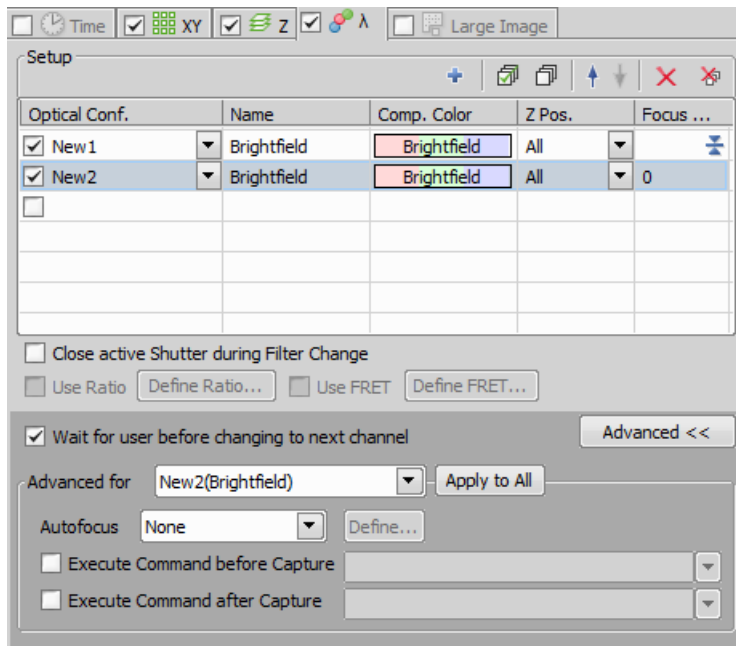
Channels Setup

Specify how many channels are to be captured. Set a descriptive name of each channel and select the optical configuration that is to be used for its acquisition. If no suitable configuration exists, you can create a new one by selecting the *<define new>* option from the pull-down menu in the Optical Configuration column. The *Comp. Color* column specifies the color tone, in which the channel will be displayed. The *Focus Offset* column enables parfocality adjustments for different filters (wavelengths).

Special Options

Camera When you are in a multi camera mode, you can select the camera to capture each channel.

T pos. If there are T dimensions engaged in the experiment, another column called T Phase appears in the table. You can reduce the number of captured channels by selecting *First* or *n-th* (the channel is included in the first or every n-th time phase only).

Z Pos. If there is a Z dimension engaged in the experiment, another column called *Z Pos.* appears in the table. You can reduce the number of captured channels by selecting the *Home* option - the channel will be captured within the home position of each Z series only. Also, if a *TIRF* system is available, the *TIRF* option appears as an option. If selected, the channel will be captured within the TIRF position of each Z series only (the TIRF position can be set within the Z-Series acquisition setup window).

Merge Cameras Available only in multi camera mode. When using two cameras, two nd2 files will be created by default. If the *Merge Cameras* option is selected, only one nd2 file is created. This nd2 file contains the maximum parameters from the two cameras (width, height and bit depth per component).

Stretch Camera images to Same Size Available only for multi camera mode. When the two cameras differ in resolution, the camera images will be stretched to the same size.

> Note
> _____
>
> *The settings of the T phase and Z Pos. columns can be combined in any way, but at least one channel must be included in every T or Z loop. In other words, the All option must be selected at least once in each of the columns.*

Advanced Options

Wait for user before changing to next channel The application waits for the user confirmation before changing to next channel.

Advanced for Choose OC onto which the Advanced setting is applied. Apply to All button applies the advanced setting to all OCs used in the Setup area.

> Caution
> _____
>
> *If the experiment contains Lambda tab with just one OC (one channel) in combination with other dimensions (e.g. T, XY, Z) the Advanced options are not applied because of optimization.*

Manual Capture

The *Acquire > Capture Multichannel Image > Capture Manually* command runs the live image and displays a new toolbar with the *Capture* button. After pressing the *Capture* button one channel is acquired. The number of channels depends on the settings in the *Acquire > Capture Multichannel Image > Multichannel Setup* window. After all channels are acquired, the *Recapture* button replaces the *Capture* button. It enables you to capture the multichannel image again. All previously obtained multichannel data will be lost. To recapture a single channel, select it by mouse and press the *Recapture* button. When satisfied, press the *Finish* button.

Capture >> This command opens a new image window with the live image and the Capture button. After pressing the Capture button one channel is captured. The number of channels depends on the settings of the *Acquire > Capture Multichannel Image > Multichannel Setup* window.

Recapture After all channels are acquired, the Recapture button replaces the Capture button. It enables you to capture the multichannel image again. All previously obtained multichannel data will be lost. When a single channel is selected, it can be recaptured separately.

Finish Press the *Finish* button to complete the multichannel capturing.

5.9. Large-image Acquisition

There is a special option in the ND2 acquisition window, to capture a Large Image. A motorized XY stage is required to have this option enabled. When set to work, it captures several frames within a defined area and stitches them together, so you get an extra large image.

> Note
>
> *When camera ROI on Live Image is on (see: 5.3. Camera ROI), only its frame defining the sensor area is used during the scanning.*

Scan Area

The area can be defined in three ways:

Define by setting an array of frames Check the first radio button and define the number of rows and columns of the scanning grid.

Set the Area Size Check the second radio button and define the size of the scanned area in millimeters.

Pattern Click the Browse button and select an XML file containing a large-image pattern. This XML can be created using the *Acquire > Grab Large Image Free Shape* command.

> Caution
>
> *Do not mistake it with similar XMLs created by other functions. For example the XML files saved from the Acquire > Capture Multipoint > Capture Automatically command window would not work.*

Stitching

Define the stitching method:

Stitch The system automatically stitches the images together to form a large image using *precise stitching (Image Registration)*. If there are more channels available, select which one to use from the pull down menu.

Do not Stitch The system will stitch the images together without using the *Image Registration* algorithm. Stitching can also be done manually after the acquisition finishes via the *File > Sequences > Stitch Large Image from Files* command.

Use ... for Stitching Select which channel will be used for Large image stitching.

Overlap Enter the percentage of image overlapping which is used for image stitching.

Close Active shutter during stage movement Check this item to close the shutter during stage movement.

Wait before each Capture This delay between captures can be used for sample stabilization, e.g. after XY movement or autofocus.

5.10. Inputs/Outputs Control

This special option in ND acquisition enables to control the experiment from outside (section *When Device Changes Then Do Action*) and also allows the experiment to give feedback (section *On Experiment Event Set Device Output*). A special device (NI DAQ controller set) is required to run this option.

Figure 5.16. Input/Output Example
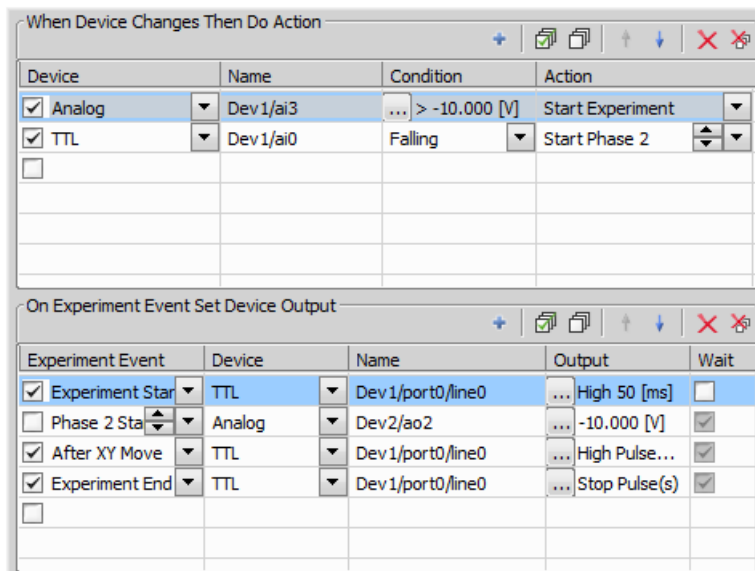
An example of an input: When voltage in dev1/ai3 line of an analog input is higher than -10 V, then the experiment starts.

An example of an output: When experiment starts, a high TTL type of signal which lasts 50 ms is given through Dev1/port0/line0 line.

5.11. Combined ND Acquisition

The dimensions can be combined together creating an N-dimensional image as the result:

1. Run the *Applications > 6D > Define/Run ND Acquisition* command.

2. The following control panel appears:
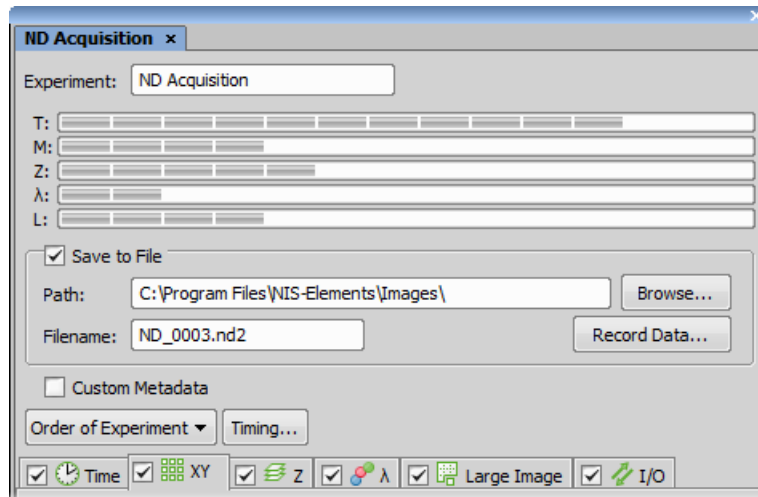
3. Select one or more dimensions (tabs) which should be included in the resulting ND2 file. Above the tabs, there is a preview of how the nd2 file structure will look like.

4. Press the *Order of Experiment* button and select the item which defines the desired order of loops in the nd2 file.

5. Set the parameters of each dimension acquisition.

6. Select whether to save the nd2 file directly to a file or just to keep it in memory to be saved afterwards. To do so, select the *Save to File* check-box. The path and the file name shall be defined if saving to a file is preferred.

7. Click the *Run now* button to start the acquisition.

    Note
    _____

    When the experiment is finished, a window may appear saying "Please wait. Finishing ND experiment, writing image data to the harddisk". The Cancel button within this window enables you to abort saving of the file. Only the data which has already been physically written to the resulting ND2 file will be available (this also concerns the Recorded Data).

5.11.1. Z Intensity Correction

The Run Z corr button works similar to the Run now button, but the acquisition uses Z intensity correction Defined within the *View > Acquisition Controls > Z Intensity Correction* window.

5.11.2. Save to File

An image can be captured and saved manually after the experiment is finished, or it can be saved to harddisk "on the fly":

• Check the *Save to File* box. The window reveals an extra section.

• Insert the *Path* or use the *Browse* button to define the destination folder.

• Specify the *Filename*.

5.11.3. Recorded Data

The nd2 file contains various *Recorded Data* which carry e.g. acquisition times of single frames. Some of them can be recorded optionally:

• Press the *Record Data* button. The following window appears:
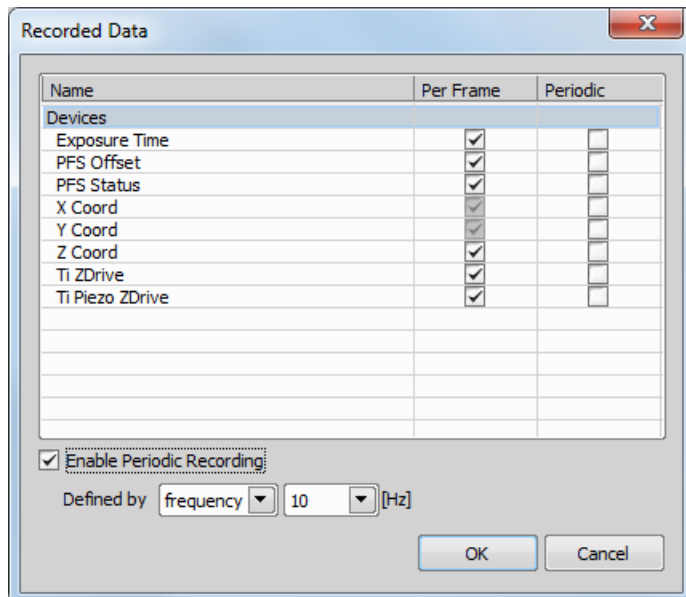
• Select whether to record the data for each frame (*Per Frame*) or with a certain *Frequency* or *Time*.

- The data to be recorded shall be check-marked.

- To view the recorded data of an opened ND2 file, invoke the *File > Image Properties* command and switch to the *Recorded Data* tab.

5.11.4. Experiment Timing

This button shows the window with expected acquisition duration, total number of frames and number of timelapse frames.

Figure 5.19. Experiment Timing window
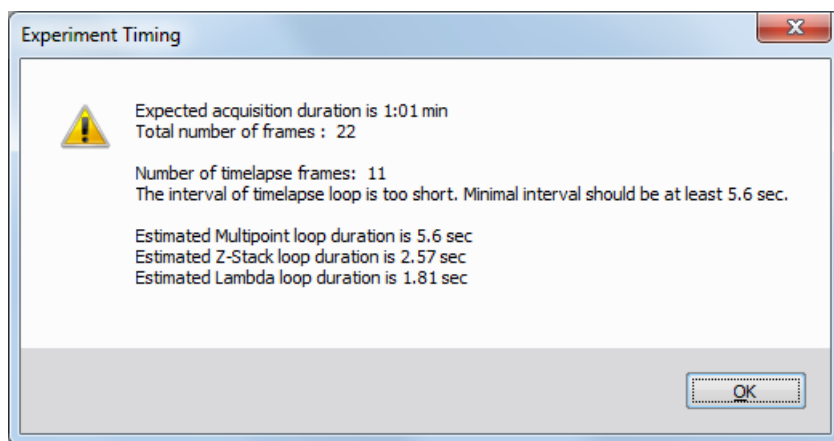
5.11.5. ND Experiment Progress

The following window appears while the experiment is running:
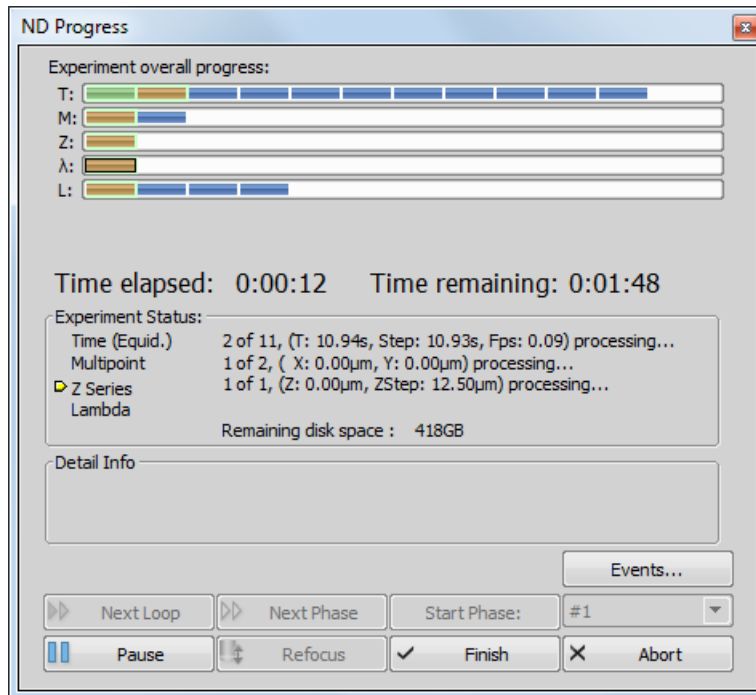
The window displays the experiment overall progress, time elapsed from start of the experiment, estimated remaining time, remaining free space on the disk where the captured nd2 file is being saved, and detailed messages about the experiment work-flow such as: "waiting for next loop", "stage moving" etc.

You can control the running experiment by the following buttons:

Next Loop When the time interval of one time loop is long enough, the system captures all the included dimensions and waits for the scheduled beginning of the next loop. In such case the *Next Loop* button becomes enabled and you can click it to start the next time loop ahead of the schedule.

Next Phase After you press this button, the experiment jumps to the beginning of the next time *Phase* after the current loop is finished.

Start Phase Use this button to start the time phase selected in the combo box. Select the phase first and press the *Start Phase* button.

Pause/Continue The experiment can be paused by the *Pause* button. Let it go on by pressing the same button.

Refocus If there is a need to focus manually, press the *Refocus* button. It pauses the ND experiment and runs *Live* camera signal.

Finish This button stops the experiment and saves captured data.

Abort Use this button to stop the experiment without saving the captured data.

Events If a remarkable event occurs during the experiment, the user can press a shortcut key and the information will be saved to the resulting nd2 file. You can define the key-shortcuts after you press the Events button.

See Special Options.

5.12. Capturing to Ring Buffer

The Capturing-to-RAM technique enables user to record sequences displaying very quick actions - lasting tens of milliseconds. Make sure that the RAM Capturing buttons are displayed on the *Grabber & Devices ToolBar* (see 3.3. Arranging User Interface), or use the corresponding commands.

- *Acquire > RAM Capture > Circular Buffer ON*

- *Acquire > RAM Capture > Circular Buffer OFF*

- *Acquire > RAM Capture > Capture*

- *Acquire > RAM Capture > Settings*

The technique uses a ring buffer to store temporary data. The ring buffer is a part of virtual memory that is being constantly (and repeatedly) filled with the live image data. Depending on how fast the action to be captured is, you should set the ring buffer size (in seconds).

1. Run the *Acquire > RAM Capture > Settings* command. The following window appears:
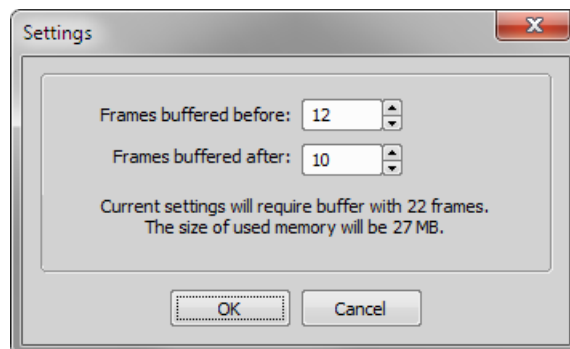
2. Set the *Time buffered before/after* values. These values represent the time interval before and after you run the *Acquire > RAM Capture > Capture* command. Image frames from this time interval will be included in the resulting image sequence.

3. Confirm the settings by the *OK* button.

Turn the Buffer ON

- Turn the circular buffer ON by the *Acquire > RAM Capture > Circular Buffer ON* command to enable buffering of the Live image to RAM.

- The ring buffer functionality gets activated. The circular buffer button ⊙ (if displayed on the toolbar) indicates the buffer activity.

Capture the Image Sequence

- Press the *RAM Capture* ⊙ button or run the *Acquire > RAM Capture > Capture* command.

- A new image window containing the captured image sequence opens.

Note

If you press the Ram Capture ⊙ *button while in the Frozen mode or with the circular buffer turned OFF, only the frames of the Time buffered after interval will be captured.*

6. Displaying Images

6.1. Open, Save, Close Images

6.1.1. Opening Image Files

6.1.1.1. Different Ways to Open an Image File

NIS-Elements AR offers several ways to open an image file, using either:

The 📂 *File > Open* **command** To invoke the Open dialog box where you can select the file to be opened, run the 📂 *File > Open* command. This command is also called when you click the *Open* button on the main toolbar

Organizer An image can be opened by double clicking its filename within *Organizer*. Run the 📖 *View > Organizer Layout* command `F10` to switch to the Organizer. See the 6.6. Organizer.

Recent Files List You can quickly access the last opened images using the *File > Recent Files* menu.

Open next/previous/first/last Commands These commands enable you to continuously open the subsequent images from a particular directory or a database table. The *File > Open/Save Next > Open Previous* , *File > Open/Save Next > Open Next* , *File > Open/Save Next > Open First* , *File > Open/Save Next > Open Last* commands may be used.

Auto Capture Folder The *Auto Capture Folder* is a control panel that can be displayed by calling the *View > Acquisition Controls > Auto Capture Folder* command. It displays images within a selected folder. To change the folder click the 📁 button in the top left corner of the control panel and browse for another folder. Any image of this folder can be opened by a double-click.

Any File Manager During installation, *NIS-Elements AR* creates file associations to files that are considered its native format for storing images (JPEG2000 , ND2). The JPEG2000 (JP2) and ND2 image files can be then opened in *NIS-Elements AR* just by double clicking their names within any file manager or the desktop.

6.1.1.2. Switching Between Loaded Images

Commands for managing the opened images are grouped in the *Window* menu. The recently opened files are listed in its bottom part. The currently displayed image is indicated by the selected radio button. To change the current image, select it from the list or use the *Next* or the *Previous* commands (represented by `Ctrl + Tab` and `Ctrl` + `Shift` + `Tab` shortcuts).
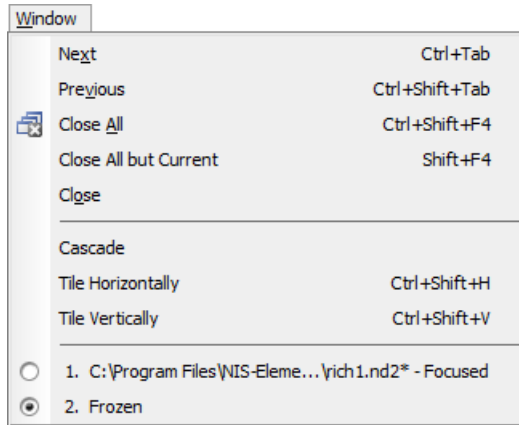
Image windows can be also arranged automatically using the *Tile horizontally* or *Tile vertically* commands. This will change the size and position of the opened documents and they will be arranged next to each other in the selected direction.

6.1.1.3. Options for the Open Next Command

This window enables you to configure the *File > Open/Save Next > Open Next* command properties. Run the ⚙ *Edit > General Options* command and switch to the *Open Next* tab.

Open from file

> **Directory** Specifies the directory with files to open.
>
> **Files of Type** Filters the files to open by the image format.
>
> **Order By** This pull-down menu enables you to select one of the image properties as the ordering criterion. The button toggles the alphabetical order.
>
> **Prefix** Filters the files to open by prefix. You can click ⟱ to show advanced filtering options window. The ▽ button turns the advanced filter on/off.
>
> **Next file** Defines the name of the file that will be automatically opened after pressing OK. You can see its name on the right side of the box.

Open from Database These options equals the ones described above.

Limit Number of Image Limit the number of documents, which will be opened and accessible in the Opened Images tab. The maximum is 24 opened documents.

Defaults for this Page Pressing this button restores the default settings of this window. All your changes will be lost.

6.1.1.4. Automatic Image Sequence Recognition

When you select to open an image that is a part of image sequence such as 001.jp2, 002.jp2..., it is recognized automatically and you are offered to convert it to an ND2 file.

6.1.2. Saving Image Files

6.1.2.1. Basic Workflows

NIS-Elements AR offers several ways to save an image file, using either:

The 💾 *File > Save* **command** Saves changes in the current image. If the current image is new (was never saved before), the command is redirected automatically and the 💾 *File > Save As* command is called instead.

The 💾 *File > Save As* **command** The most common way of saving the current image. You can select image format in the *Save as type* pull-down menu. See 6.1.2.3. 💾 *File > Save As* Options.

The 💾⁺ *File > Open/Save Next > Save Next* **command** This command saves the current image (live or static) automatically according to the settings defined within the general options window. See 6.1.2.4. Save Next Options.

During image-capturing experiments Images and image sequences can be saved automatically during experiments such as ND Acquisition (5.4. About ND Acquisition) .

6.1.2.2. Saving Images with UAC

Because of the security enhancement with the UAC (user account control) of *Windows Vista* and *Windows 7*, it is not possible to save images in those folders that require Windows administrator user rights. Those folders are:

• *C:\Windows* and subsequent folders

• *C:\Users* and subsequent folders excluding C:\Users\[login-user-name]

• *C:\Program Files* and subsequent folders excluding C:\Program Files*NIS-Elements AR*\Images

6.1.2.3. 💾 *File > Save As* Options

Save as Type Specifies the file type, in which the image will be saved. See 6.1.4. Supported Image Formats.

Save Color Image Check this option to save the color image into the file.

Save Binary Image Check this option to save the binary layer into the file. If the selected file format does not support layers, the option turns in *Burn Binary Image*. The binary data are burned into the color data before saving the file.

Save Annotations Check this option to save the annotation layer and the scale layer into the file. If the selected file format does not support layers, the option turns in *Burn Annotations*. The currently displayed annotation/scale data are burned into the color image before saving the file.

Compression Choose the image compression level (only some image formats support different compression levels).

Save When pushing the *Save* button, the Archive information dialog box appears (For LIM and JPEG2000 formats only). Confirm it to save the image.

TIF Compatibility Options When saving other than 8- or 16-bit tif file, you can choose to keep the original bit depth of the new file, or to rescale it to 8- or 16-bits. For all new tif files you can also select the option to convert a multichannel image to RGB.

Note

If you are saving 16bit tiff images, the Scale 16bit to 8bit option is available while the Scale 16bit to 16bit option is not available. If you are saving 8bit tiff images, the Scale 8bit to 16bit and Scale 8bit to 8bit options are not available.

Saving to AVI/MOV files AVI and MOV movie files can be created from a nd2 file. When the *.avi/*.mov format is selected in the Save As Type combo box, the following options appear:
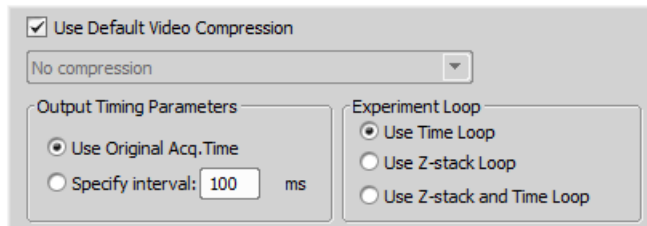
Compression Select which video compression is applied on created movie files. See 11.4. About Video Compression.

XY Points When the ND document contains XY points, you can decide how these are saved. Either only the current point can be saved into the file, or all XY points can be saved in separate files which are recognizable by *"_number"* suffix.

Output Timing Parameters Timing of frames of the resulting AVI movie may equal the Original Acquisition Time. If this does not suit your needs, define a custom interval in *ms* .

Experiment Loop Select the dimension, frames of which will be used to create the AVI movie. The dimension must be present in the ND2 file necessarily.

> **Use Time Loop** The T dimension frames of the current Z stack position will be used.

> **Use Z-stack Loop** The Z dimension frames of the current Time position will be used.

Use Z-stack and Time Loop All frames of the ND2 file will be used in the AVI movie.

When the Multipoint dimension is present, only frames of the current Multipoint position are used automatically.

6.1.2.4. Save Next Options

This window enables you to configure the ⊞⁺ *File > Open/Save Next > Save Next* command properties. Run the ⚙ *Edit > General Options* command and switch to the *Save Next* tab.

Save to File

> **Directory, Prefix, Digits, File Format, Compression** Defines name of the file that will be automatically generated. Let's assume, that you have selected - Directory: c:\images; Prefix: seq; Digits: 4; File Format: JPEG2000. Then, by pressing *Enter* (calling the *Save Next* command), an automatic file named "seq0001.jp2" is generated and saved to the "c:\images" directory. When pressing *Enter* again, the file "seq0002.jp2" is saved to the same directory etc.
>
> **Define Image Info** Displays a dialog box, where you can enter some description (jp2 meta-information), that will be saved with every image:
>
> **Overwrite/Skip already existing files** Allow or deny rewriting of the images already existing in the default save directory.

Save to Database When selected, images are being saved to a chosen database table, instead of saving them directly onto disk.

> **Database** If you are already connected to some database, select it from the pull-down menu. Else a dialog window appears, which enables you to connect to any database.
>
> **Table** Select the database table which the images will be stored to.
>
> **Autoincremental field** Choose which field of the database will be used to store the generated image descriptions.
>
> The image descriptions are generated according to the *Prefix, Digits, Number* values. Let's assume, that you define - Prefix: seq; Digits: 4; Number: 20. Then, by pressing *Enter* , the current image is saved to the selected database table and the automatic image description is put into the *Autoincremental field* of the table. Its name would be "seq0020" in our case. When pressing *Enter* again, the second record signed "seq0021" is created.
>
> **External mapping** You may create an external *.txt or *.ini file containing mappings that assign table field names to some particular values. These values are automatically filled into the database records when the *Save Next* command is called. Please, see the comments below for an example.
>
> ---
> Note
> ---
>
> *External mapping example - The *.ini file content may be:*
>
> ```
> [Table 1]
> Author=Jack Sparrow
> ```

93

```
    Experiment number=12
    Sample=Malus silvestris
```

It means: When saving images to the database table named "Table 1", the fields of Author, Experiment number, and Sample will be filled with the specified values.

Options

> **Show grabbing dialog before saving** Before an image is saved the Grabbing dialog box is shown. Selecting this option enables you to define the way of grabbing (with/without a shading correction, averaged etc).
>
> **When saving, display Image Info dialog box** You can change the default image information (jp2 meta-information) right before saving.
>
> **Sound Alert** If checked, a short tone from your PC speaker is played every time the *Save Next* command is used.
>
> **Save with annotation and binary layers** If checked, images are saved together with the binary and annotation layer.
>
> **Change to live after saving an image** After saving a single image, a Live image is displayed immediately.

Defaults for this Page Pressing this button restores the default settings of this window. All your changes will be lost.

6.1.3. Closing Images

- The currently displayed image can be quickly closed by pressing the cross button in the top-right corner of the image window.

- The image can be also closed by invoking the *File > Close* command.

- If you want to close all images, use the *File > Close All* command.

- If you try to close an image that has been changed, *NIS-Elements AR* will display a confirmation dialog box, offering to save the changes.

- Use the *Window > Close All but Current* command to close all opened documents but keep the current one opened.

6.1.4. Supported Image Formats

NIS-Elements AR supports a number of standard file formats. In addition, *NIS-Elements AR* uses its own image file format (ND2) to fulfil specific application requirements.

JPEG2000 Format (JP2) An advanced format with optional compression rates. Image calibration, text descriptions, and other meta-data can be saved together with the image in this format.

ND2 Format (ND2) This is the special format for storing sequences of images acquired during ND experiments. It contains various information about the hardware settings and the experiment conditions and settings. It also maintains all image layers of course.

Joint Photo Expert Group Format (JFF, JPG, JTF) Standard JPEG files (JPEG File Interchange Format, Progressive JPEG, JPEG Tagged Interchange Format) used in many image processing applications.

Tagged Image File Format (TIFF) This format can save the same amount of meta-data as JPEG2000. TIFF files are larger than JPEG2000 files but are loaded faster. TIFF files have several ways to store image data, therefore there are many versions of TIFF. *NIS-Elements AR* supports the most common TIFF modalities. TIFF image format in *NIS-Elements* supports also floating point images.

CompuServe Graphic Interchange Format (GIF) This is a file format commonly used on the Internet. It uses a lossless compression and stores images in 8-bit color scheme. GIF supports single-color transparency and animation. GIF does not support layers or alpha channels.

Portable Network Graphics Format (PNG) This is a replacement for the GIF format. It is a full-featured (non-LZW) compressed format intended for a widespread use without any legal restraints. *NIS-Elements AR* does not support the interlaced version of this format.

Windows Bitmap (BMP) This is the standard Windows file format. This format does not include additional image description information such as author, sample, subject or calibration.

LIM Format (LIM) Developed for the needs of laboratory image analysis software. Nowadays, all its features (and more) are provided by the JPEG 2000 format.

ICS/IDS image sequence ICS/IDS image sequences are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

> Caution
>
> *ND Images containing multi-point XY dimension cannot be saved to a file in the ICS/IDS format. See also 5.4. About ND Acquisition.*

NanoZoomer files (NDPI, VMS) *(requires: Local Option)*

We can open these files produced by Hamamatsu devices and save the image information to an ND2 file.

ShuttlePix files *(requires: Local Option)*

Nikon ShuttlePix Digital Microscopes produce standard TIFF and JPEG files and save the calibration info to a separate file. *NIS-Elements* recognizes the calibration file and loads it along with the image data.

6.2. Image Layers

6.2.1. Introduction to Image Layers

The following image layers can be saved within a *NIS-Elements AR* image.
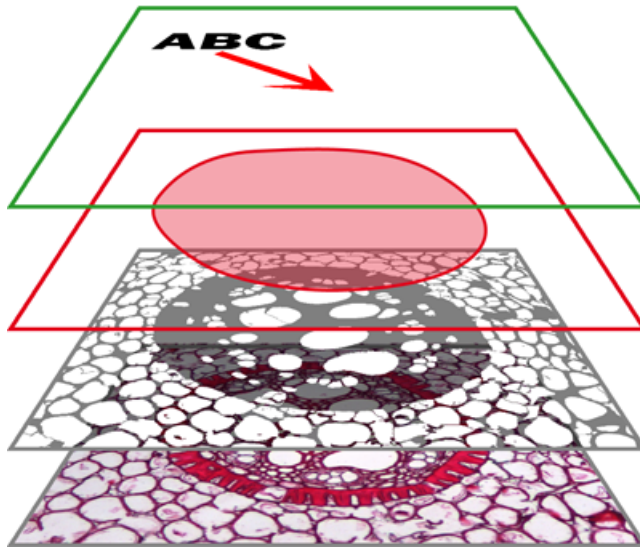
Annotation layer In this layer, vector objects are stored. The results of manual and automatic measurements, text labels and other annotations can be included.

Binary layer(s) The binary layer is usually the result of thresholding. By thresholding, you distinguish objects of interest from background. A picture of night sky can make a nice example - shining stars may be "thresholded" and compose the binary layer. Then, by means of automatic measurement, you can e.g. count them. More than one binary layer can be placed over one image.

ROI layer ROIs (regions of interest) is a strong tool for distinguishing objects from background similar to the binary layer. The advantage over the binary layer is that ROIs are vector objects and therefore provide different way of manipulation. See 7.4.1. Introduction to ROIs.

Color layer The color layer contains the image data captured by a camera. It can handle images with the depth of up to 16 bits per color component. The dimensions of this layer determines the dimensions of the other layers.

Note

When saving an image, only some file formats are capable of saving all the layers. The other image formats will save the content of the color layer only. See 6.1.4. Supported Image Formats.

6.2.2. Image Types

RGB Images

Images acquired by a color camera typically consist of three components that represent red, green and blue channel intensities. You can display a single color channel using the tabs located in the bottom-

left corner of the image window. Or, an arbitrary combination of them can be selected while holding the `Ctrl` key down.

Multi-channel Images

These documents usually arise from fluorescence microscopy. Instead of 3 color components (RGB), multichannel images can be composed of arbitrary number of user-definable color channels.

> Note
>
> *If there is a image that contains more than 8 components, the tabs in the bottom left corner of the image are replaced by the wavelength dimension, similarly to how other dimension loops of nd2 files are displayed.*

Click the *Treat As Spectral* button to use the current image as a spectral image and to reveal more channel switching features in the bottom control bar.
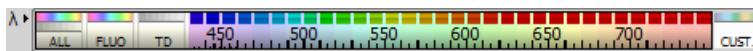
ALL Selects the full spectrum of the image including spectral (FLUO) and transmitted detector (TD) channels.

FLUO Selects only spectral channels. This button is useful for quick switching between FLUO and TD.

TD Displays only the transmitted detector (TD) image.

CUST. This button displays custom channels. To set a channel (multiple channels) as custom, make a channel selection in the control bar, right-click over the selection and choose *Set selected channels as Custom*.

ND2 (multi-dimensional) files / datasets

An nd2 file is usually a sequence of images. One *ND2* file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types, also called dimensions available: Time-lapse, Multi-point, Z-series, Multi-channel, Large-image. The dimensions can be combined together.

Please see 6.3.1. Navigation in ND2 Files, 5.4. About ND Acquisition

6.2.3. Channel Coloring

Single channels and monochromatic images can be visualized using a predefined color scale. Right-click the "channel tab" in the bottom left corner of the image window and select a color scale from the *Channel Coloring...* sub-menu.
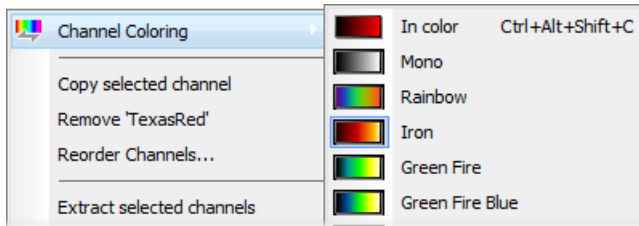
Brightfield and Fluorescence channel image

If your nd2 image contains only one brightfield channel with its *Modality* set to *Brightfield* in *Channel Properties*, the behaviour of viewing colors of these channels slightly changes. The brightfield channel is set to *Mono* color by default but can be switched to any other pseudocolor. The fluorescence channels are displayed in their native fluorescence color by default and can by switched to any other pseudocolor. Once the brightfield and fluorescence channels are displayed together in an overlay, the pseudocolor of the fluorescence channel is ignored and is shown in its true color.

6.2.4. Assigning Colors to Channels

Despite the color in which a channel is displayed, other per-channel colors can be assigned to it. This regards colors for:

- indication of oversaturated pixels

- indication of undersaturated pixels

- indication of binary pixels

In the context menu over a channel, select *Colors for...* and choose a color for oversaturated pixels, undersaturated pixels (these colors are used globally for all images in *NIS-Elements* until next change) and for binary pixels. Selected color for binary pixels is linked to the channel name and is shown in all images having the same channel name.
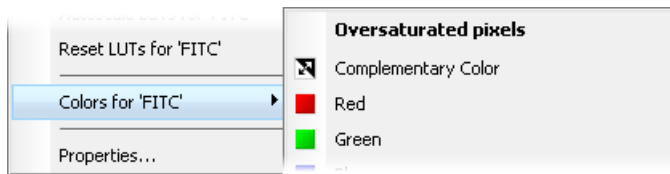
Color of oversaturated and undersaturated pixels may also be selected from the pull down menu next to the *Pixel Saturation Indication* ⬛ button shown in the image toolbar.

Figure 6.8. Pixel Saturation Indication sub-menu
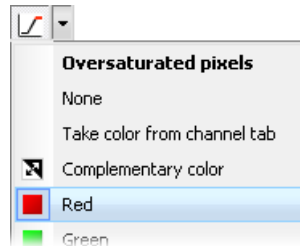
None The pixel saturation will not be indicated.

Take color from channel tab Color which has been previously assigned to oversaturated and undersaturated pixels in the menu over the channel tab will be used. See 6.2.4. Assigning Colors to Channels.

Complementary color Colors complementary to the display color of each channel will be used in order to ensure good visibility of the highlighted pixels.

6.2.5. Copying Channels by Drag and Drop

Drag one of the channel tabs and drop it:

- to another image, so it will become a multichannel image;

- inside the *NIS-Elements AR* application window in order to create a new image

  Note

  - *Even the All and RGB tab can be copied.*

  - *When extracting a channel from Live image, it does not freeze the camera signal.*

  - *Any user events (6.3.1.2. Events) present in the document are automatically transferred during the channel extraction.*

ND2 files

1)   Drag one of the channel tabs and drop it by the *primary mouse button*. It behaves the same way as if performed on a single image.

2)   Drag one of the channel tabs by the *secondary mouse button* and drop it to the application screen. A context menu appears that enables you to select whether to create the new image from all frames or just a single frame.

3)   *Two nd2 files* that have matching structure can be *merged into one* easily. E.g. two single channel Z stacks with the same number of Z positions can be merged into a single two component ND2 Z-stack by dragging one into the other.

4)   Drag one of the channel tabs of a *single image* and drop it *to an nd2 file*. The channel will be copied to every loop of the ND2 file.

> Note
> ___
>
> *This behavior, too, takes place when copying channels that do not have matching structure (just the current frame is copied to every position of the target ND2)*

6.2.6. Shifting Image Channels

The currently selected channel(s) can be shifted using the `CTRL + SHIFT + Arrow` keys. All channels are visible during the operation so you can see the complete image.

The procedure also works on nd2 files equally to other image processing performed on ND2 files:

1. Select the image frame and the channels to be shifted.

2. Perform the shift using `Ctrl+Shift+Arrows` keys.

3. A standard ND2 image processing window appears (see 7.1.2.2. ND2 Files Processing).

4. Select which part of the nd2 file to apply the shift to, and confirm it by *OK* .

6.3. ND Dimensions

6.3.1. Navigation in ND2 Files

6.3.1.1. Control Bar

When an ND2 file is opened, its structure is pictured at the bottom of the image window. There is a time line with all captured images indicated by gray markers. The blue-highlighted marker indicates the currently observed image. Below the time line, loops of each dimension are indicated by rectangles. In case the multi-channel dimension is included and the number of channels does not exceed 10, the channels are indicated by the color tabs at the very bottom of the image window. Selection in the time line and in the loops is highlighted green.

Browse the nd2 file by clicking inside the time line. You can also display a single loop by selecting the corresponding blue rectangle. There are some examples, how the nd2 file control bar can look like in different cases:

Figure 6.10. A T/Z/multi-channel image with some loops selected.
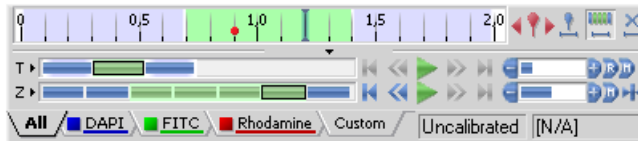
Figure 6.11. A T/Z/multi-channel image containing 11 channels and the time line hidden

Figure 6.12. An HDR multi-exposure image

Playing Controls

Play Sequence Plays all images of the dimension at selected speed. If a selection is applied, only the selected images will be included in the playback.

Stop Playing Stops playing the sequence at the last displayed frame.

Previous Position Displays the previous frame of the dimension.

Next Position Displays the next image of the dimension.

Decrease/Increase Playing Speed Changes the playback speed by one step down/up

Real Time Playing Speed Sets the playing speed to real-time (as the image was captured).

Maximum Playing Speed for Every Frame Sets the playing speed to maximum while the display of every frame is guaranteed (when the speed is set to maximum by the + button, some frames are usually omitted when playing the sequence depending on your graphic card).

Home Position Displays the frame of the Z dimension that was set as "home" during the acquisition.

Tips

- Right-click the selection to invoke a context menu. The selection can be adjusted, deleted, or the nd2 file can be cropped.

- Time Axis can be hidden by unchecking *Show Time Axis* in the context menu over a single dimension frame.

- Detailed info about dimensions will be displayed after you click the leftmost button of the control bar (T>, Z>, ...).

- Place the cursor over one of the dimensions. A tool-tip which displays statistics of the dimension appears.

Playing options

Right-click the speed bar to display the following context menu and select the playing mode.
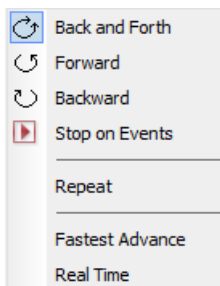
Backward/Forward/Back and Forth commands define the direction how the image sequence will be played. When you check the *Stop on Events* option and then press the ▶ *Play Sequence* button, a dialog window appears once the first user event is reached. The dialog window displays information about the reached user event. Use the *Continue* button to continue playing to the next event, or press the *Stop* button to stop the playback at the current frame. Within the window, you can also select the *Do not ask again in this session (Always stop)* option. Playing will then stop automatically on every user event but the window will not be shown. *Repeat* command sets the repetitive infinite playback. *Fast Advance* command corresponds to the ▶ Maximum Playing Speed for Every Frame button described above. *Real Time* command corresponds to the ▶ Real Time Playing Speed button described above.

6.3.1.2. Events

User events can be pinned to any of the frames of the image in order to highlight a remarkable scene or action. There is number of event types. Some of them are inserted automatically while the ND experiment is running, others must be defined by user (by the 📍 button). You can manage the events from several context menus which appear when you right-click:

- The time axis

- The 📍 *Insert Default User Event* button

- One of the event markers

All the context menus contain the *Event Manager* command. Open the *Event Manager* to manage events within the current nd2 file. The time can be put in, and the event type selected from a combo box. See 5.5. Time-lapse Acquisition.
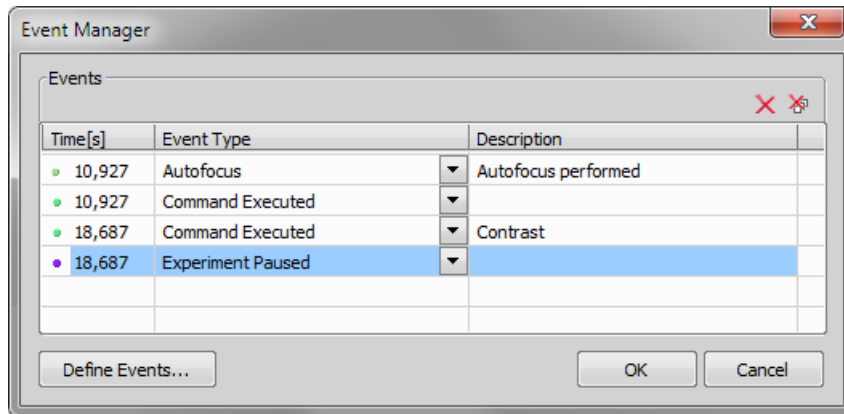
Event and Selection Controls

Insert Default User Event This button inserts an event marker to the current frame.

Move to Previous/Next User event Click these arrows to browse through the frames where user events occur.

User Event Selection This button selects frames of the ND2 file according to the *Event Playing Range* settings - right click this button and pick the *Event Selection Range* command from the context menu to view/modify them. A simple window appears. Define the number of frames/seconds which will be selected around each event.

Range Selection Clicking this button selects a user defined range of frames. The selection is done by mouse. A range can also be selected by mouse while holding the `Shift` key down. Single frames can be added/removed while holding the `Ctrl` key down. Right-click the navigation bar to invoke the context menu. A single frame can be selected (*Select Frame*) or deleted (*Delete Frame*). Multiple selected frames can be deleted (*Delete Selected Frames*) or extracted from the rest (*Keep Selected Frames*).

> Note
>
> *ND2 files may contain extremely large number of frames so it may be helpful to omit some of the frames from the selection. Right click the Range Selection button and a context menu appears. It enables you to select every 2nd, 5th, 10th, 20th, 50th, 100th frame. The Select All Frames option selects the whole range of frames as if you have selected the first and the last time phase with the* `Shift` *key down, but preserves the Select Every... setting. Invert selection selects only the frames that were not selected before executing this command.*

Disable Selection Press this button to de-select all frames of the nd2 file.

6.3.2. ND Views

There are several views which can display the nd2 files in various ways. Some views are available for some dimensions only. If a view is available for two or three dimensions of the nd2 file, a pull-down menu appears in the top image toolbar. There you can select the dimension to be displayed.

Note

When you switch the view, a new image window opens by default. This behavior can be changed within the ⚙ Edit > General Options command window so that only one view of an image will be opened at a time.

Main View When you open an ND2 file, it opens in this view.

Slices View This view displays orthogonal XY, XZ, and YZ projections of the image sequence. (Requires Z or T dimension)

Figure 6.15. Slices View
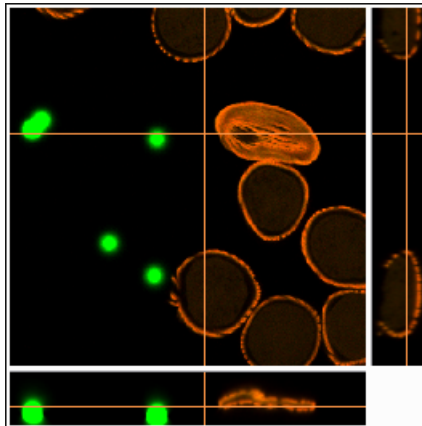
Volume View This view creates a 3D model of the acquired object. (Requires Z dimension)

Figure 6.16. Volume View
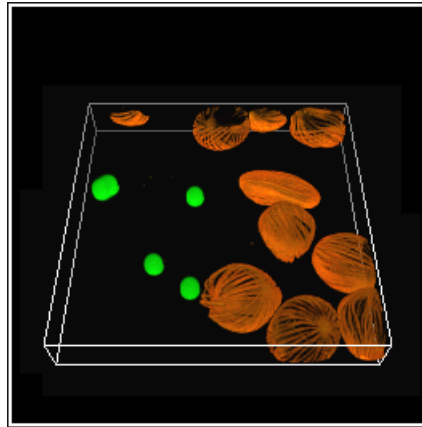
Tiled View This view displays frames of the selected dimension arranged one next to other. (Requires Z, T or XY dimension)

One or two dimensions can be viewed at a time.

Figure 6.17. Tiled View
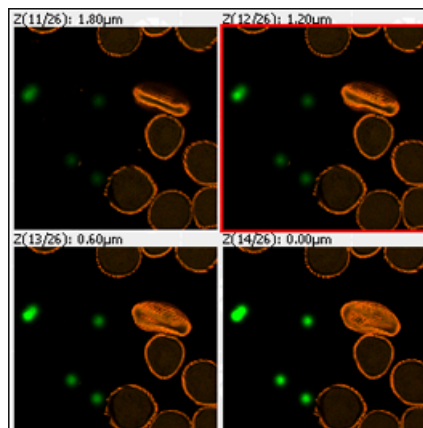
Maximum/Minimum Intensity Projection View These projections analyse all frames of one dimension and pick pixels with the maximum/minimum intensity values. These pixels are used in the resulting image. (Requires Z or T dimension)

Figure 6.18. Main View, Max/Min Intensity Projection View
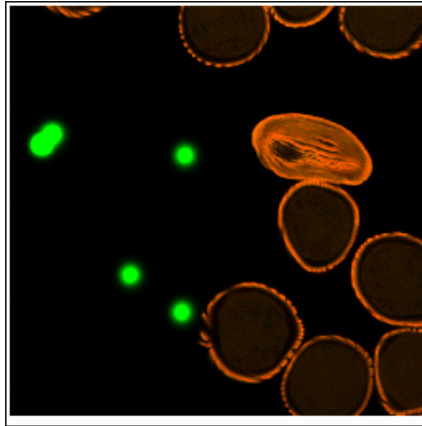
6.3.2.1. ND2 Information

Information about current frame of an ND2 document or live image can be displayed using the *Show ND Information* command from the image contextual menu. It displays information about the current frame of the ND2 document or live image. All possible information (metadata) are displayed by default.

You can edit which information is displayed in the *Label Properties* dialog window. Right click the ND2 information field and select *ND Info Properties...*. Select displayed features from the *Available ND information* area and use the ^ button to insert them into the *ND information text scheme* area which defines the visible information. Use *Format options* tools to specify the data format (e.g. numeric precision, date format, etc.) and adjust the text appearance in the *Text Box* tab. *Always show Acq. Time* forbids removing the Acquisition Time information from the ND information text box.

6.3.3. Volume Viewer

6.3.3.1. Introduction

Run the *View > Image > ND View > Volume View* command to display the current Z-stack as a 3D model using perspective projection. If your graphical card supports DirectX 10 or newer, the model automatically opens in the *New Engine*. Otherwise the *Old Engine* is used.

6.3.3.2. New Engine

The New Engine (requires graphical card supporting DirectX 10 or newer) brings additional functionality and better rendering performance than the Old Engine. To switch between the new/old engine, execute

the ⚙ *Edit > General Options* command, click on the *Appearance* tab and use the combo box in the *3D rendering* area (see: 3.9. Appearance Options) or use the *3D Settings* ⚙ icon on the main toolbar and the *Renderer* combo box.

Window Toolbar Options

▼ **Volume Options** Displays the *Volume Options* panel containing more volume controls and features. The most useful features are already listed in the main toolbar (see: Volume Options Tab).

Free rotation control mode Click and drag over the model for free rotation.

X-axis rotation control mode Rotate the model only in the X axis.

Y-axis rotation control mode Rotate the model only in the Y axis.

Z-axis rotation control mode Rotate the model only in the Z axis.

View Plane Select which plane will be facing towards your perspective. The *Default* view is slightly rotated so that the structure of the sample can be overviewed. The six other views display side views and horizontal projections.

Blending Each blending mode mixes the Z slices together using different transparency and interpolation patterns. Choose which one fits your sample the best.

> **Alpha (Alpha Blending)** Uses the physical principles of light absorbing, so that the object surfaces are accentuated.

> **MaxIP (Maximum Intensity Projection)** Only the pixels with the highest intensity values of the Z-sequence are displayed in the image.

> **MinIP (Minimum Intensity Projection)** Only the pixels with the lowest intensity values of the Z-sequence are displayed in the image.

> **AccumulatedIP (Accumulated intensity projection)** Sums up (accumulates) all pixel intensity values of the Z-sequence. This blending mode can be fine tuned in the *3D Renderer Settings* dialog window (see: 3D Settings).

> **Depth Coded Alpha (Depth Coded Alpha Blending)** A color gradient perpendicular to the Z-stack planes is applied to the volume. Lower part of the volume is colored with different colors than the middle and upper section enabling to easily distinguish the position of examined objects.

> **Depth Coded MaxIP (Depth Coded Maximum Intensity Projection)** Applies a color gradient to the pixels with the highest intensity values of the Z-sequence.

> **Shaded (Shaded Volume)** Uses the physical principles of light absorbing and reflectance. The object surface becomes more distinguishable. The *shading volume lightning* type can be altered in the *3D Renderer Settings* dialog window (see: 6.3.3.2. New Engine).

Note

Depth Coded Alpha and Depth Coded MaxIP blending modes support Z coloring. Choose a predefined Z color gradient by clicking on the gradient button. Four options at the bottom of the sub-menu define the gradient color direction and value order (Low to High or High to Low). The same menu

can be evoked when right-clicking over the gradient scale bar in the bottom left part of the image window.

Show Box Displays a box bounding the 3D image.

Show/Hide Grid Enables to show or hide the graticule. Define visible graticule planes in the *Grid & Scale* section of the *Volume Options* panel.

Show/Hide Scale Displays/hides a scale around the bounding box.

Show volume info This button displays/hides the *navigation axis/cube* and information about the calibration and volume dimensions which are displayed at the bottom of the image window.

> Note
>
> *To display ND information and metadata, right-click into the image and select Show ND information.*

Show Hi/Low Resolution Rendering *Low resolution* is recommended for basic volume previewing. When precise static details are to be visualized, switch to *High resolution*. The most noticeable changes between these two modes are observed in large images (beyond the graphic card capabilities).

If the image is fully loaded into the memory of the graphical card, *High resolution* is turned on, the viewer shows the maximum quality and the button is automatically disabled. If the memory capabilities are exceeded, two more modes appear - *Highest Possible Resolution* (optimized for the highest resolution without downsampling; can be slower) and *Balanced Resolution vs Speed* (resolution can be lower; speed performance should be sufficient).

3D Settings This button enables you to fine-tune the 3D renderer performance. The following window appears:
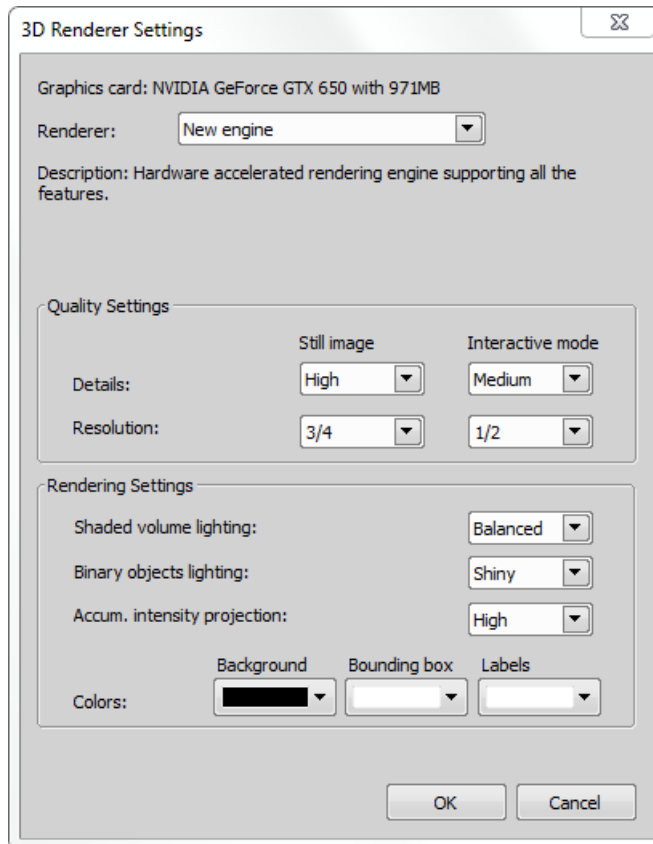
Renderer Enables to switch between the *New Engine* and the *Old Engine*.

Quality Settings

> **Still image / Interactive mode** *Still image* settings will be used for static viewing (which usually allows the highest quality) while the *Interactive mode* settings will be used when moving with the 3D model.

> **Details** Select the desired quality settings.

> **Resolution** Select the resolution of the 3D scene. Available options express the ratio of the current image window size. The *Limited* option actually equals 1/4 but differs when the image window size is reduced so that the image would lack details in the 1/4 resolution.

Rendering Settings

Enables to set further settings for the rendering modes.

> **Shaded volume lighting** Choose one of lighting modes.

> **Binary objects lighting** Lighting presets are available in this combo box. Illumination is adapted according to the selected binary surface. *Balanced* mode automatically balances light irrespective of the surface type.

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Accum. intensity projection Modifies (multiplies by a coefficient) the pixel intensity sums created by the *AccumulatedIP* blending mode.

Colors Using these color combo boxes you can define the background color, bounding box color and color of the labels.

🖥 **Switch to full screen mode rendering** This option switches the volume viewer to fullscreen mode. You can abort this mode by pressing `Esc`. When using two monitors, the *Volume Options* window can be controlled from one monitor and the 3D Volume can be in full screen on the other monitor.

🎥 **Create Movie** This button runs the *Movie Maker* application. It enables to create AVI movies of the 3D model. See 11.3. Creating a Movie for further information.

🔑 **Show Help** Displays the help page.

Figure 6.20. Navigation axis and navigation cube
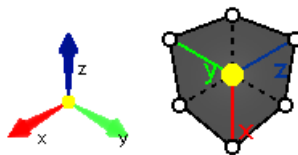
3D Navigation tools

Navigation in the 3D image can be facilitated by the navigation axis or navigation cube. Switching between these two is done from the context menu over the axis/cube. The graphic not only shows you the exact position of the 3D volume in xyz dimensions but it can be used to align the volume with one of the axes (click on the desired axis in the axis mode), with a cube plane (click on the cube wall) or to rotate to a node projection (click on the white ball of the cube). To reset the view into default, double-click directly into the 3D image.

Figure 6.21. Volume Options tab
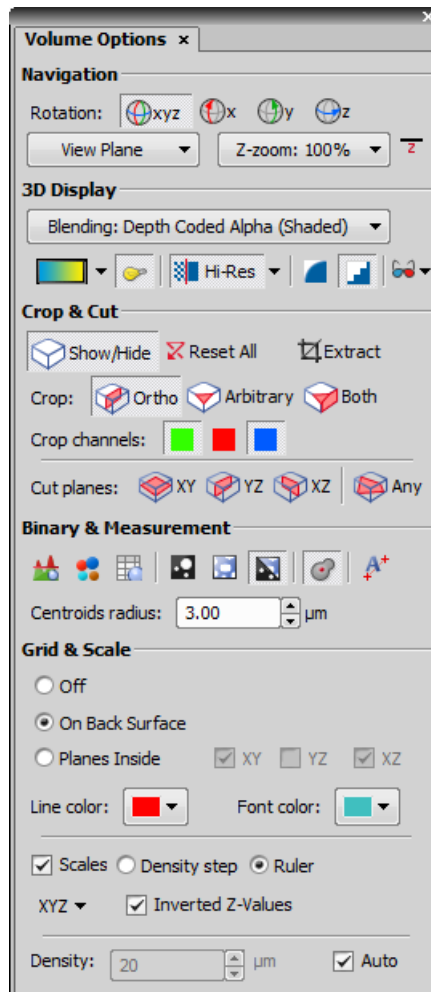
Volume Options Tab

Z-zoom Adjusts the Z-step height of the image Z sequence.

The first frame is highest/lowest Use these two buttons to set the first frame as the highest/lowest one in the sequence.

Toggle light visibility This option is supported with *Alpha* and *Depth Coded Alpha* blending. Press the button to switch the light on. Move the light freely around using the left mouse button. Double-click on the light to pin it to the *Scene* (stays in its place, 3D volume moves) or *Volume* (light moves together with the 3D volume). Change the light intensity with the mouse wheel when the cursor is over the light. Context menu over the light can be used to *Reset Light Settings*.

Show smooth volume Click on this button to smooth the 3D volume.

Show non-interpolated volume Click on this button to display raw, non-interpolated 3D volume.

Anaglyph 3D stereo This button enables/disables the anaglyph 3D view. Press the arrow next to the button to display the menu with possible color combinations (depends on the type of your anaglyph glasses).

Open Z-LUTs for Volume View *(requires: Local Option)* Opens the Z-LUTs for Volume View panel.

Show/Hide Displays or hides any cropping and cutting made on the 3D image.

Reset All Removes any crop changes made on the image and returns the cut planes into their default position.

Extract Extracts a new document from the current view limited by the crop/cut tools.

Ortho Orthogonal crop enabling to crop the 3D image perpendicularly. Hold down `Ctrl` and move the desired plane with the left mouse button.

Arbitrary Arbitrary crop enabling to crop the 3D image freely. Hold down `Ctrl` and move the yellow ball to position the center of the cropping plane and then move the yellow cone to tilt the plane which is illustrated by the white circular grid.

Both Both orthogonal and arbitrary crops are enabled at the same time (see above).

Note

If you restrict the volume size using the cropping tools, you can shift the result inside the original volume in all three directions. Press `Ctrl` + `Shift` and then click and drag over a plane determining the shift direction. Color lines with crosses indicate the shift axis.

XY Plane delimited by x and y axis can be used for cutting through the 3D image. Hold down `Ctrl` and drag the cutting plane in Z direction.

YZ Plane delimited by y and z axis can be used for cutting through the 3D image. Hold down `Ctrl` and drag the cutting plane in X direction.

XZ Plane delimited by x and z axis can be used for cutting through the 3D image. Hold down `Ctrl` and drag the cutting plane in Y direction.

Any Any plane can be used to cut through the 3D image. Hold down `Ctrl` and move the yellow ball to position the center of the cutting plane and then move the yellow cone to tilt the plane.

Crop channels Select which channels will be cropped. This option is available only with *Orthogonal* cropping.

Threshold 3D Opens the 3D threshold window used for visualizing 3D binary objects.

Spot Detection 3D *(requires: Local Option)* Enables to detect bright/dark circular objects within a 3D space. Please see: 7.2.2. Spot Detection for more information.

Colorize Binary by 3D Objects This function is used to distinguish between binary objects by colorizing each 3D object with a different color. This function can be turned on/off in the context menu over the 3D image where it is also possible to change the color of each binary 3D object (*Change Binary 3D Object Color*).

Objects 3D Opens the *3D Object Measurement* tab displaying values of measured features for each 3D object in the image.

View Binary Displays the binary layer of the 3D image. Use the key shortcut Ctrl + B .

View Color Displays the color layer of the 3D image. Use the key shortcut Ctrl + R .

View Overlay Displays the color layer and the binary layer of the 3D image at once. Use the key shortcut Ctrl + V .

Show/hide centroids Displays/hides a centroid for each binary object in the 3D image. Adjust the *Centroid radius* in the combo box below.

Measure 3D Enables to measure distances inside the 3D image. *Annotations and Measurements* tab is opened where ⚲ *Length 3D* is automatically selected and measurement results are shown in the table below. When measuring centroids, the start/end of the measuring line automatically snaps to the center of the closest centroid.

> Note
>
> *To measure distances more precisely, switch to Slices View , make the measurement in the partic-ular Z-plane and switch back to Volume View.*

Grid & Scale This section enables to display additional grids and scales. Activate *On Back Surface* to view a grid on the bottom and distant sides of your current volume rotation. Color of the grid can be altered in the *Line color* combo box. Density of the grid can be adjusted manually in the *Density* edit box or switched to *Auto* for automatic calculation of suitable spacing. Turn on *Scales* to show the grid dimension - *Density step* shows the grid size over one square whereas *Ruler* marks each grid line with its distance from 0. Which axes will be labeled is defined in the combo box (*XYZ* or *Z*). Direction of the Z ruler can be inverted using the *Inverted Z-Values* check box. Color of the scale and its labels can be changed in the *Font color* combo box.

Planes Inside enable to display/hide the XY, YZ and XZ planes (use the check boxes). Each plane is carrying an automatic or manually defined grid. Hold down Shift to evoke a small cube. Drag the yellow ball of the cube to alter the zero grid position. Drag the arrow ends to move the grid just in the selected axis direction.

Time volume projection

Timelapse images can be projected inside the volume viewer to illustrate any time displacements. If more dimensions are available, select *Time* from the first drop-down menu and choose a suitable blending type.

6.3.3.3. Old Engine

Window Toolbar Options

View Select one of the predefined views to display the model. The *Default X, Y, Z* view displays the model in the most suitable perspective. The six other views display side views and horizontal projections.

Z-zoom Adjusts the Z-step height of the image Z sequence.

Blending Choose which of the following types of projection will be used:

Alpha Blending Uses the physical principles of light absorbing, so that the object surfaces are accentuated.

Maximum Intensity Only the pixels with the highest intensity values of the Z-sequence are displayed in the image.

Minimum Intensity Only the pixels with the lowest intensity values of the Z-sequence are displayed in the image.

Z Depth Coding Color is associated to Z depth.

Z Depth Coding (Shaded) Color is associated to Z depth with light and shade options.

Shaded Volume Uses the physical principles of light absorbing and reflectance. The object surfaces are more distinguishable.

Accumulated intensity projection Accumulates all pixel intensity values of the Z-sequence. The projection is similar to X-ray imaging technique.

Z-depth code This option appears when the Z Depth Coding method is selected. It opens a menu where you can select Z Depth Coding color scheme from a list of predefined gradient color sets. Check the *Reversed Z-Depth Values* option to reverse the association between Gradient color and Z depth. The *Show Depth Code Scale* option shows/hides the Z Depth Code scale in the image window.

Show Box Toggles the model outlines visibility.

Show Axis Displays the XYZ axis model orientation.

Anaglyph 3D This button enables/disables Anaglyph 3D view. Press the arrow button to display the menu with possible color combinations for Anaglyph 3D (depends on Anaglyph glasses type).

Graticules

Figure 6.22. Graticule Visibility - Halved bounding box (on bottom)
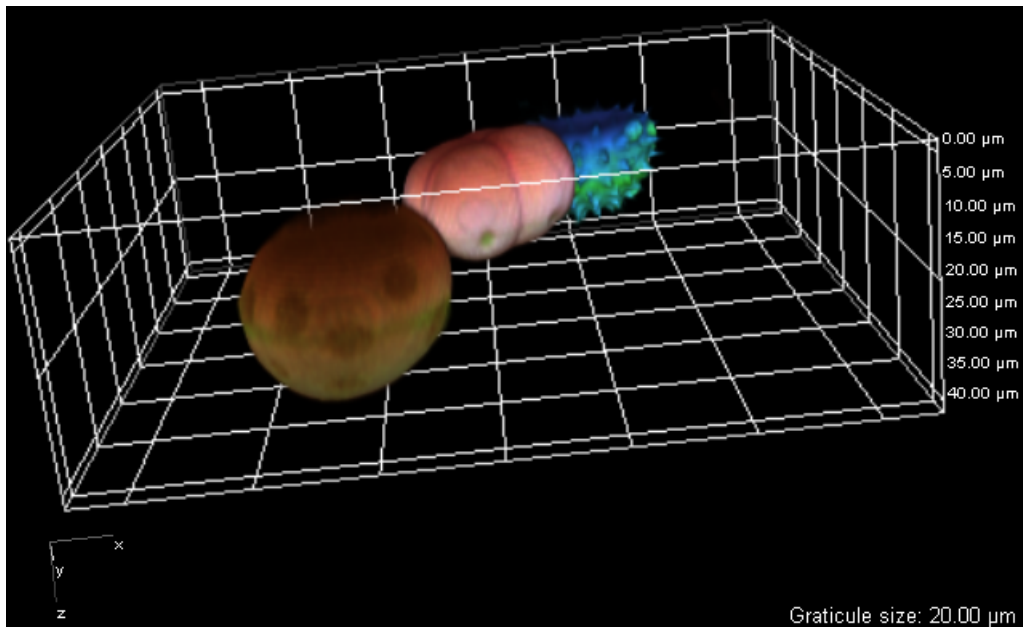
⊞ **Graticule/ Hide Graticule** Enables to show or hide the graticule. Select visible graticule planes from the pull-down menu.

Graticules pull down menu options

Planes Select which graticule planes will be visible. You can freely combine the first three of them (the *XY* , *YZ* or *XZ plane*). The *Bounding box* option shows graticules on all sides of the box. The *Halved bounding box* shows graticules on three sides of the box. How the graticules are displayed depends also on additional settings.

Always on Top Adjust the graticule visibility with this button. The graticules are shown always on the background unless you switch on this option.

Graticules Properties Displays a window with the graticules settings. The top part of the window serves for faster visible plane definition. The color of the lines as well as their top/background appearing can be adjusted in the middle part of the window. Set the graticule *Density* in the bottom part of the window or use *Automatically adjust density*.

Figure 6.23. Graticule Properties Window
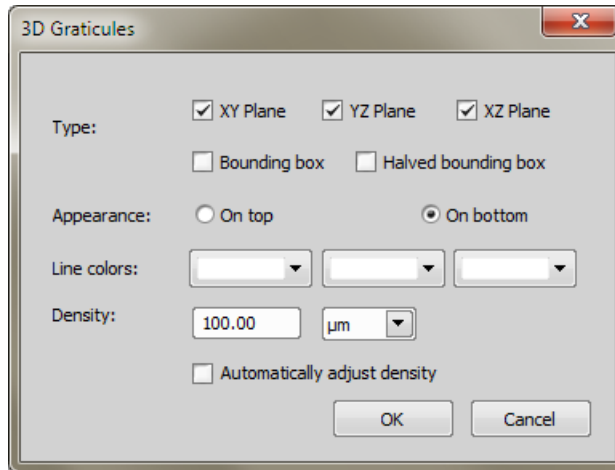
Type Select which planes will be visible. You can combine them with other planes. Check the *Bounding box* options to show the graticules on all sides of the box. The *Halved bounding box* displays graticules on three sides of the box.

Appearance Adjust the graticule visibility. The graticules can be shown on the top or on the background.

Line colors Select a color for each axis individually.

Density Specify value and units of graticule density. Check the *Automatically adjust density* option to let the system set the graticule density automatically.

Show Z Scale This ↕ button displays/hides Z scale in the image window. Press the arrow button to open a menu with additional options: the *Reversed Z values* option reverses Z values. The *Z Scale Properties* opens the *Tick marks density* dialog window:
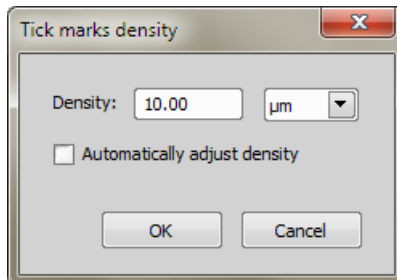
Define density value of tick marks for Z scale or let the application automatically adjust by checking the *Automatically adjust density* option.

Show volume dimensions This button displays/hides information about the calibration and volume dimensions which are displayed at the bottom of the image window.

The first frame is highest/lowest Use these two buttons to set the first frame as the highest/lowest one in the sequence.

Calculate Additional Slices This button enables to enhance the quality of undersampled datasets by calculating additional slices in the Z-axis.

Create Image Cache for Time Loop Use this button if your image sequence contains the time dimension and you would like to observe it. The visualization will be pre-loaded to memory and therefore smooth and fast-enough. *(requires: 64bit NIS-Elements)*

Create Movie This button runs the *Movie Maker* application. It enables to create AVI movies of the 3D model. See 11.3. Creating a Movie for further information.

Switch to fullscreen mode rendering This option switches the volume viewer to fullscreen mode. You can abort it by pressing `Esc`.

Show Help Displays the help page.

3D Renderer Settings This button enables you to fine-tune the 3D renderer performance. The following window appears:
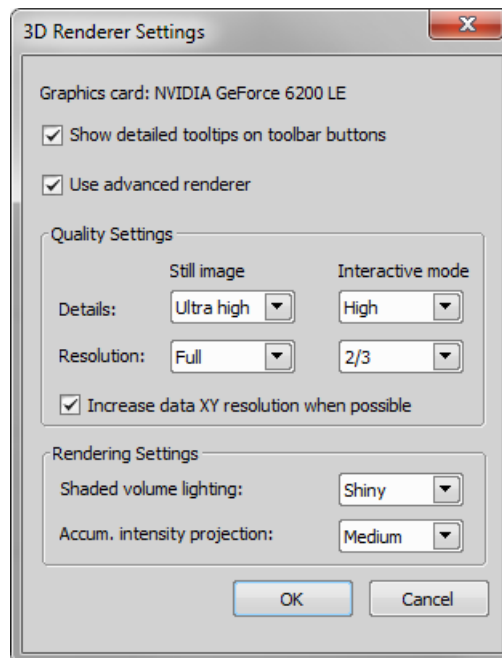
This button appears only if you have a high-performance graphic card with DirectX 9.0c support, Shader Model 3.0. Recommended cards are Nvidia GeForce 6800 and higher or ATI Radeon X1900 and higher.

Show detailed tooltips on toolbar buttons Check this item to display detailed tooltips.

Use advanced renderer If an advanced graphic card is detected in your computer, this options becomes available. Choose it in order to make use of the capabilities of the card. The resulting image will be nicer and the performance faster.

When using the basic render, a stripe pattern may appear in the volume render. Selecting this option will correct this behaviour.

Quality Settings

Still image / Interactive mode *Still image* settings will be used for static viewing (which usually allows the highest quality) while the *Interactive mode* settings will be used when moving with the 3D model.

Details Select the desired quality settings. You can opt between the *Ultra High*, *High*, *Medium*, *Low* values.

Resolution Select the resolution of the 3D scene. *Full*, *2/3*, *1/2*, *1/4*, *Limited*. These options express the ratio of the current image window size. The *Limited* option actually equals 1/4 but differs when the image window size is reduced so that the image would lack details in the 1/4 resolution.

Rendering Settings

Enables to set further settings for rendering modes.

Shaded volume lighting Choose one of lighting modes: Balanced, Matt or Shiny.

Accum. intensity projection Select the intensity of accumulating the pixel values: High, Medium, Low

Working with Objects

Rotating the 3D model Use the primary mouse button to freely rotate the image.

Or, hold down the *A* key. An axis system scheme appears. Move the mouse cursor near the chosen axis. Press and hold the primary mouse button and rotate the model by moving the mouse in right-left direction. Release the primary mouse button and choose another axis, if necessary. Release the *A* key to finish.
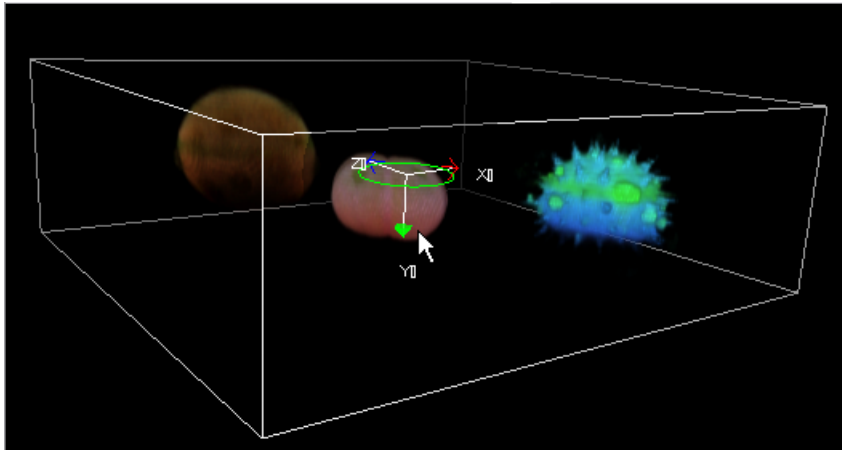
Or you can use commands placed in the right vertical image toolbar. These commands enable primarily to select an axis easily.

<u>Rotation Controls</u>

Rotation via screen axes Use this ⊕ button to freely rotate the model.

Rotation via dataset axes Use one of these ⊕, ⊕, ⊕ buttons to rotate the model according to the selected axis.

Moving the 3D model Use the secondary mouse button to move the model.

Zooming the 3D model Use the mouse wheel to zoom the model.

Cropping the 3D model Press the ⊕ *Crop Control Mode* button to switch to the Cropping Mode. We recommend to have the *Show Box* option ON. Move the mouse cursor to the desired plane outline. Outline of the selected plane becomes red highlighted. Now press and hold the primary mouse button and move the mouse. The cropping plane will move accordingly. Release the mouse button when you are satisfied with the result. Select another cropping plane and continue cropping.

Or you can press and hold the `Ctrl` key during the whole cropping procedure. After you release the `Ctrl` key cropping is finished.
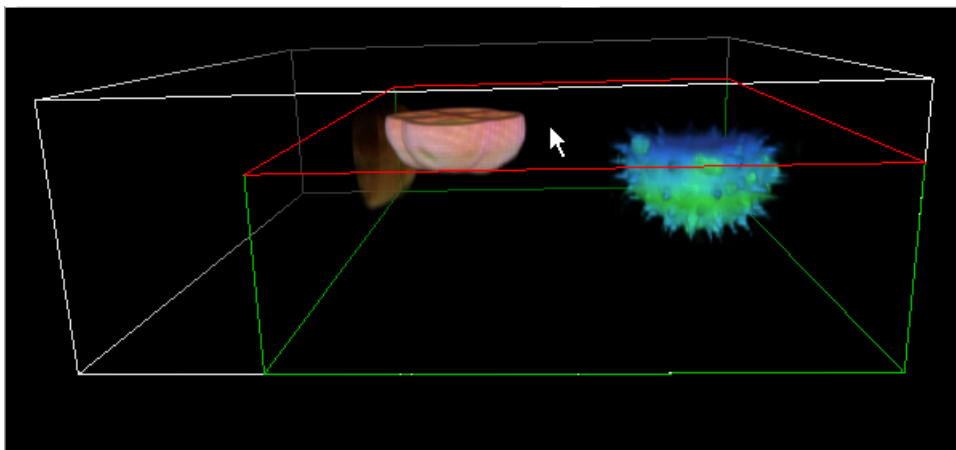
Note

The cropping planes form a box. If you need to move the whole box, instead of moving a single plane, press and hold the secondary mouse button. Now simply move the box to a new location.

Or you can enable the arbitrary cropping plane mode. Switch on the arbitrary cropping plane by the ▽ *Arbitrary Cropping Plane* button in the right image toolbar. Hold down Shift and click the primary mouse button to move the cropping plane, or hold hold down Shift and use the secondary mouse button to rotate the plane. Note that this mode will crop all channels and a binary layer together.

You can also use the ⊡ *3D Crop* button: current image is cropped according to current bounding box and a new image containing the 3D crop is created.

Shortcuts in Cropping Plane Mode

> **Primary mouse button** Moves orthogonal cropping plane.
>
> **Primary mouse button + Shift** Enables to move the graticules (only if the *Automatically adjust density* option in the *Graticules properties* window is unchecked).
>
> **Primary mouse button + A** Rotates the 3D model around a selected axis.
>
> **Secondary mouse button** Move parallel orthogonal cropping planes in *Crop Control Mode*. Rotate orthogonal cropping plane in *Arbitrary Cropping Plane Mode*.
>
> **Mouse wheel** Zooms the 3D model.

Scaling the graticule Run the *Graticules Properties...* window and set the graticule *Density* value or switch *Automatically adjust density* off, confirm it by clicking OK and hold down the Shift key and move the mouse wheel to scale the graticules. The information about the current graticule size is displayed in the bottom right corner of the image window.

Moving the graticule To move the graticule freely, hold down the Shift key, grab the graticule and drag it to new position. If you need to adjust the graticule position only in some directions, hold down the Shift key. An axis system scheme appears. Move the mouse cursor near to one of the axes or planes you want to fix. The selected plane (or axis) becomes highlighted. Now hold the primary mouse

button and move the mouse cursor. The graticule will move accordingly in the selected plane or axis direction.

Figure 6.28. The XY plane is selected so the graticules will move only in the X and Y axis direction
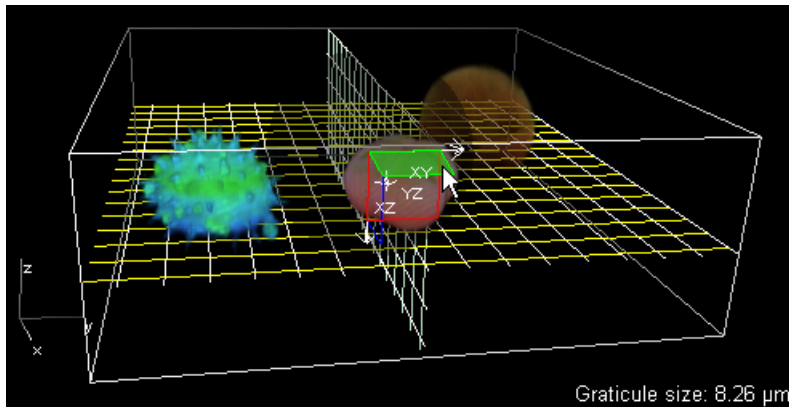
<u>Shortcuts</u>

Primary mouse button + Shift Enables to move the graticules.

Subvolume Settings You can crop the whole model or you can select one or more channels to crop. Any combination of channels can be cropped. Select the corresponding subvolume buttons holding the `Ctrl` key.

Subvolume on All Channels Applies the subvolume (crop) settings on all channels of the image.

Subvolume on a Channel Applies the subvolume (crop) settings on the selected channel.

Reset Crop Discards the subvolume cropping.

Note

This selection is ignored when cropping in Arbitrary Cropping Plane Mode (i.e. when the ⬙ Arbitrary Cropping Plane button is pushed).

Light This option is supported in binary isosurface view or in *Shaded volume lighting Mode* (Advanced Renderer). Press the 💡 *Toggle Light Visibility* button to switch the light on. If rendering mode supports light then a light object is shown. Pick the light with primary mouse button and move it freely around. Light can be fixed to dataset or camera. Double click on light object to toggle fixation mode. Change light intensity with mouse wheel when cursor is over the light object.

<u>Time volume projection</u>

Timelapse images can be projected inside the volume viewer to illustrate any time displacements. If more dimensions are available, select *Time* from the first drop-down menu and choose a suitable blending type.

6.3.4. Synchronizer

Synchronizer enables to compare (run and view) two or more nd2 files at the same time. It automatically synchronizes view of all documents added to the list. Run   *View > Visualization Controls > Synchronizer* :
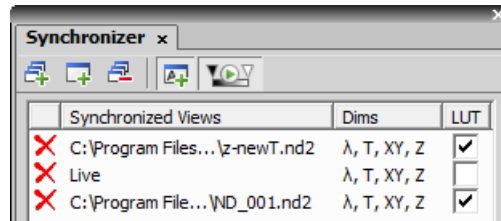
- Open at least two nd2 files you would like to observe.

- Use the *Add All Views* button to add them to the synchronizer table. In case there are more images opened which you do not want to add, use the *Add Current View* button on each of the images. You can also select the *Add New Views Automatically* button to have all newly opened ND2 files synchronized.

- The names of all added images appear in the *Synchronizer* window.

All documents added to the Synchronizer are automatically being synchronized.

- Set one of the selected images active.

- Any action regarding the view (Z position, displayed frame, etc.) is performed on the synchronized images as well as on the active one.

- Besides synchronization of dimensions, you can also synchronize LUTs settings of the images by checking the *LUT* check box. LUTs synchronization works only between LUT-compatible images (images must have the same number of channels etc.). If the images are not LUT compatible, only the *Keep AutoScale* and *AutoScale* actions are synchronized.

  You can select the *Enable LUT synchronization by default* button. All newly added documents will have the option to synchronize LUTs selected by default.

- To remove an image from synchronizer, click the reset button ✕ placed before its name. To remove all images from synchronizer, press the *Remove All Views* button.

6.4. Large Images

6.4.1. Opening Files in Progressive Mode

If a single image (or one ND2 frame) is too big and it can not be loaded to RAM in one piece - you will be asked whether to open it in a *Progressive Mode*. This means that only a thumbnail of the image will be loaded to the RAM. When zooming-in to view image details, the image data of the particular portion of the image will be loaded to RAM progressively (piece by piece).

Many image processing functions and commands are disabled in this mode. This problem usually occurs with *Large Images* created by stitching several frames together. In case you need to process such an image, a smooth solution is to split it into tiles and create a multi-point ND2 file out of it. Please see 6.4.2. Splitting Large Images.

6.4.2. Splitting Large Images

You can split an existing large image to tiles easily.

1)   Open the large image to be split.

2)   Run the   *Image > Split Image*   command. A dialog window appears.

3)   Set split options:

   Split to separate files - output format Select the output format (ND2 or TIFF). A separate file will be saved for each tile. Define *Output folder* and *Prefix* where the files will be saved.

   Create multipoint ND Document Only one multi-point ND2 file will be created and opened after the split.

   Set measurement frame after splitting The measurement frame will be turned ON after the split in order to exclude overlapping areas. This is to prevent distortion of data in case of measurement.

   Tile Size, Number of tiles Define width and height of a single tile in pixels or set the number of columns and rows to which to split.

   Overlap Set overlap value of neighboring tiles either in % or the current calibration units (presumably µm). Overlap up to 50% of the tile size is allowed, higher values are reduced to the maximum .

   Fill background Select a color to fill empty spaces which will appear the total area of tiles exceeds the image. Use Optimize to prevent the empty spaces.

4)   If you do not require the tiles to have exact size, click Optimize . The system will arrange/resize the tiles automatically so that the whole image is covered precisely.

5)   Click OK .

6.4.3. Locating XY Positions Between Images

If two images have matching XY coordinates you can locate the exact position in one image inside the other. This would be the case especially after using the *Image > Split Image* command. Right click the location within the first (multi-point) image and select *Find this Point in Paired Document* from the context menu.

If you do this right after splitting the large image (the two images are "paired"), the system displays it and highlights the XY position by a flashing cross. If it is not clear to the system which images shall be paired (e.g. three or more images with matching coordinates are open), the user will be prompted to select the second image to the pair.

6.4.4. Methods Used for Stitching Large Images

Some *NIS-Elements* functions for creating large images by stitching multiple image frames enables the user to select a stitching method. Select the one you serves better your purpose:

Blending Overlapping image parts will be blended.

Figure 6.30. Stitching via "Blending" (pixel intensity is changed in the overlapped region)
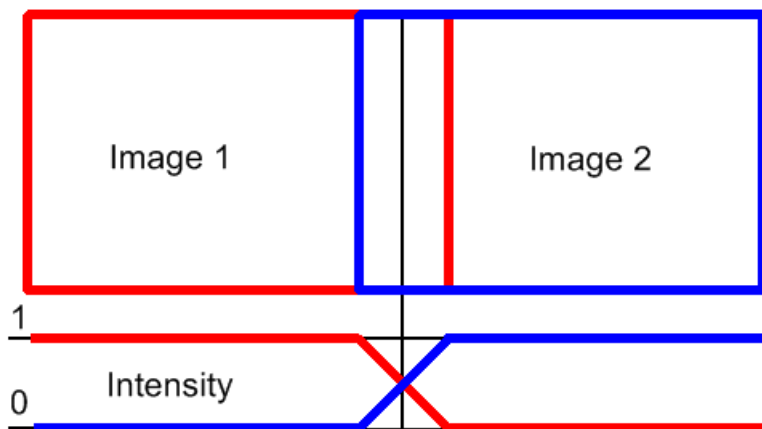
Optimal Path A contour where two overlapping images are least different will be computed. The images will be stitched copying this contour.

Figure 6.31. Stitching via "Optimal path" (pixel intensity is preserved)
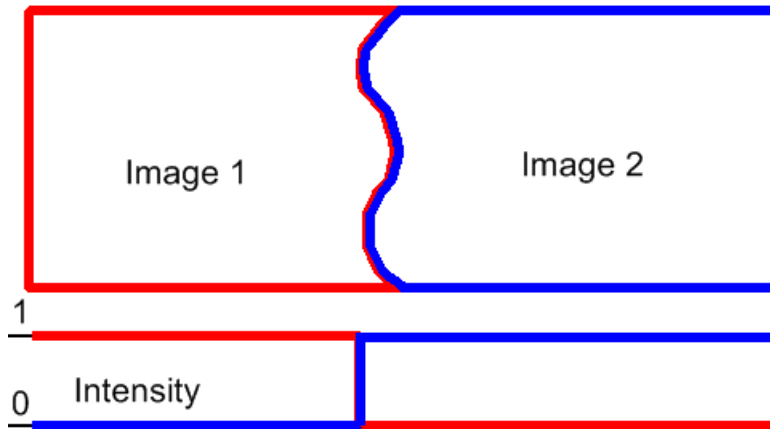
6.5. LUTs - Non-destructive Image Enhancement

6.5.1. Introduction to Look-Up Tables (LUTs)

LUTs is a useful tool for image color and brightness modifications. You can use LUTs to enhance images for observation purposes so the color modifications will be non-destructive to the image data. LUTs settings are saved along with the image file. If required, the LUTs settings can be applied to the image data by pressing the ▮◆▮ button. Use the ⬈ *Show LUTs window* button in the image toolbar to display the LUTs window.

The following actions are available within the LUTs window:

- Adjusting the *Gamma parameter*. The gamma curve is drawn in gray over each graph. You can drag the middle point and move it up or down, or enter an exact value to the *G:* field at the top of the graph area.

- Adjusting the input intensity range. The input intensity range can be restricted by moving the black and white triangular sliders to the center. All pixels with values outside of the modified input range will be set to maximum/minimum values and the remaining pixels will be approximated in order to fill the output (full) range.

  Note

  This is the way how images can be equalized. For example, if the image is very dark and most of the histogram is located to the left while the right part of it is flat, you may move the white slider(s) to the position where the histogram line starts to rise. This will incredibly brighten the image and reveal details which were previously hidden in a dark.

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- You can adjust the histograms height non-proportionally by moving the slider on the left side of the window up and down.

Tips

- Right-click the graph area and (de)select *Draw trend style*. When ON, the LUTs curves will be smoothed to display the data trends rather then represent the actual image data values.

- The position of the black, white and Gamma sliders can be reset by a double click.

Different controls will be available when applying LUTs to monochromatic, RGB, multi-channel or spectral image. When LUTs are active, the LUTs button in the top-left corner of the image window is highlighted red.

6.5.2. LUTs on RGB Images
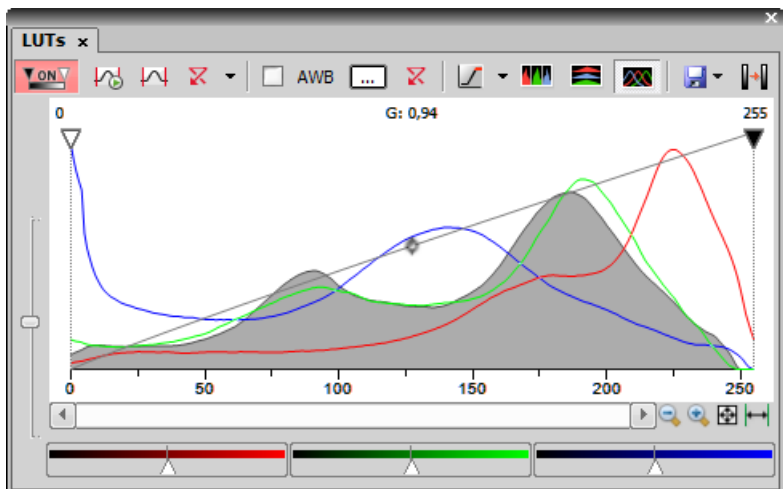
There are 3 separate windows for each of the RGB channels. All channels are controlled at the same time by default. To control each channel separately, press and hold the `Shift` key while you drag the sliders or adjust Gamma.

LUTs Tools

Enable/Disable LUTs This button applies LUTs to the current image.

Keep Auto Scale Press this button to run the auto scale procedure permanently (on the live image). When you turn this button OFF, the settings remain as if the *Auto Scale* button was pressed only once.

Auto Scale This button adjusts the white slider position of all channels automatically with the purpose to enhance the image reasonably. If you have selected the *Use Black Level* option from the *Settings* pull-down menu, the black slider will be affected too.

Reset All Components Discard all LUTs settings and turn LUTs OFF by pressing this button.

Settings Display the pull down menu and select one of the following commands:

- *Brightfield Opacity* - You can change opacity of a brightfield channel. Define the opacity in the *Brightfield Opacity* dialog window that appears after you run this command.

- *Use Black Level* - Check this item to ensure that the black slider will be affected by the auto scale functions .

- *Settings* - opens the *Auto Scale Settings* window. See Auto Scale Settings.

Keep Auto White Balance Check Box Check this option to switch the Auto White Balance functionality permanently (on the live image).

AWB Press this button to perform the Auto White Balance operation once.

Auto White Balance Color (...) This button Opens the *AWB Color* window and enables you to select a color shade which the system shall eliminate (make white). See AWB.

Reset AWB Discard all AWB settings with this button.

Color Oversaturation Switch this button ON and the system highlights all pixels with values reaching maximal values. Select colors to highlight the pixels with in the pull-down menu. See also 6.2.3. Channel Coloring.

These buttons arrange histograms of single channels next to each other horizontally or vertically.

This button arranges histograms of single channels overlapped. Use sliders at the bottom of the graph window to amplify or reduce the green/red/blue component display in the view.

Save/Load LUTs This pull-down menu enables user to handle LUTs settings in various ways. They can be saved to a **.lut* file and loaded later. Or, LUTs settings saved along with an image can be loaded directly from this image by the *Reuse LUTs from File* command. Or you can copy/paste the settings within the documents opened in *NIS-Elements AR*.

Modify Image Through LUTs Press this button to apply the LUTs settings to the image data - the original image will be overwritten. Until you press this button, no changes are made to the image data.

Reset Zoom Zooms the histogram to fit the preview window.

Auto Range zooms the histogram so that the "high" and "low" limits are distinguishable. For example, if a small intensity range is defined on a 16bit image the low and high lines are displayed as one-pixel line. Pressing this button will stretch the histogram in order to display the lines separately.

Auto Scale Settings

Press the arrow next to the *Reset LUTs* button and a pull-down menu appears. Invoke the *Settings* command.
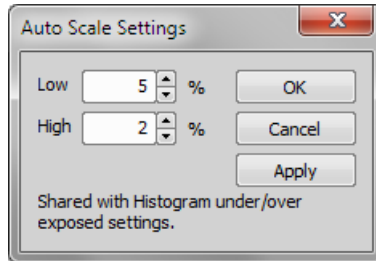
The *Low* and *High* fields determine how many of all pixels of the picture are left outside the sliders when *Auto Scale* is applied (0-10%).

AWB

White color in some images may suffer from having a color tint. The AWB (Auto White Balance) mode adjusts the image to get pure white instead of this tint. Similarly to LUTs auto scale, the AWB function can be used once, or permanently on the live image. If you know the tint your "white" color has, you can select this color by the color picker that appears after pressing the *...* button:
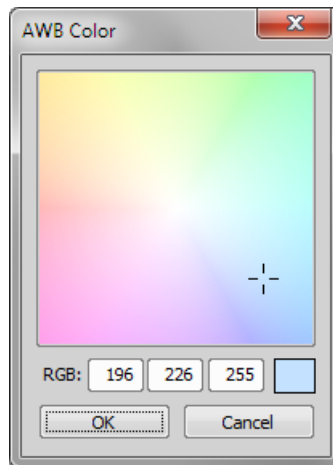
Choose the color by mouse. You can also enter the RGB values to the fields below. A preview of the chosen color appears in the rightmost rectangle.

6.5.3. LUTs on Monochromatic Images
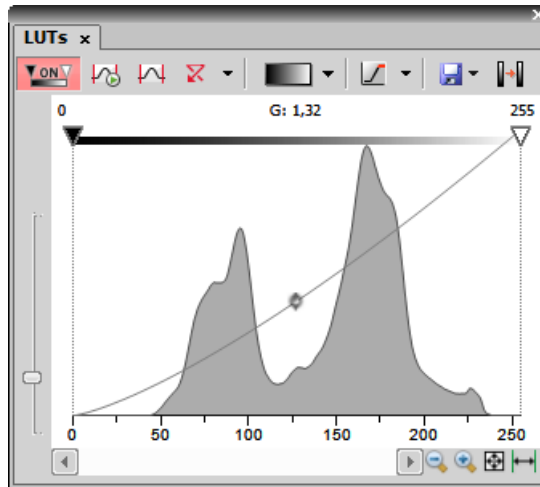
All features mentioned above are also valid for monochromatic images, except that the *AWB* function is not available. There are some other features added when in "mono" mode.

Gradient Mapping monochromatic images to pseudo-color gradients is often used to enhance details in the image which would otherwise not be obvious. This button indicates the currently selected gradient. When pressed, a pull-down menu appears where you can select the color scheme you would like to use. Try a few of the schemes to see which one suits best.
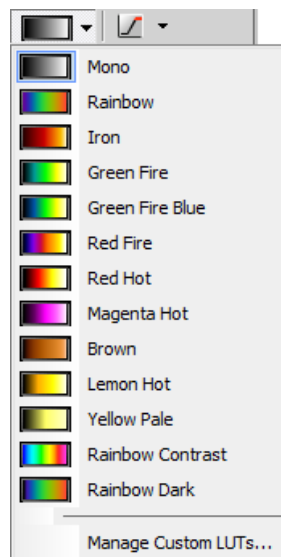
Manage Custom LUTs *(requires: Local Option)*

The *Manage Custom LUTs* command in the pull down menu enables to define a custom LUTs gradient. Use the tools available in the dialog window to define your custom gradients. They then appear in the pull down menu ready to be applied. Please see *View > Image > LUTs > Create Custom LUTs* for details about the custom LUTs definition.

6.5.4. LUTs on Multichannel Images

Figure 6.37. LUTs window on two-channel image
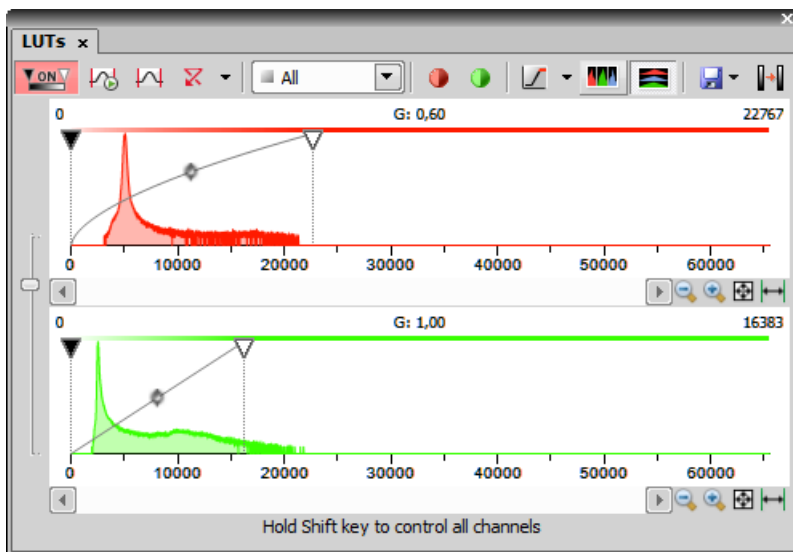
Most of features mentioned above are also valid for multi-channel images. There are some other features added when in "multi-channel" mode. Each channel is controlled separately by default. To control all channels at the same time, press and hold the `Shift` key while you drag the sliders or adjust Gamma.

When the image contains up to three channels, all channels can be visible at one time and you can handle them similarly to the RGB mode. When there are more than three channels, only one channel is displayed at one time. A pull-down menu in the toolbar enables you to select the channel to be displayed.

◗ Next to the channel selection pull-down menu, there is this *Auto Scale* button. By pressing it, the system will automatically adjust only the current channel settings.

6.5.5. LUTs on Spectral Images

When observing a spectral image, the graph inside the LUTs window does not show the image histogram(s), but reveals another information about the image. Intensity values are on the Y axis and spectra is on the X. There are two curves in the graph. The top (white) curve displays the maximal intensity and the color curve displays the mean intensity of each channel.

Note

Spectral image consists of many channels, each representing a single wavelength.

The LUTs behavior depends on the selected displaying mode. There are four displaying modes. You can select the mode either by a pull-down menu in the LUTs toolbar, or within the image top toolbar.

• *True Color*

• *Custom Color*

• *Grouped*

• *Gray Scale*

In the bottom part of the LUTs window, *Gain* and *Black Level* settings can be adjusted either for all channels together or for each group separately - depending on the displaying mode. Gain brightens the image and the Black Level works just like the left (black) on RGB/mono images.

Note

Each slider (gain or black level) offers a range of values to be set by moving it, but any higher value can be entered in the field next of the sliders.

TD channel

A spectral image can contain additional TD (transmitted detector) channel. Its basically a standard monochromatic image. LUTs settings of this channel appear on the right edge of the LUTs window.

True Color Displaying Mode

You can switch to this displaying mode by clicking the button located in the image toolbar.
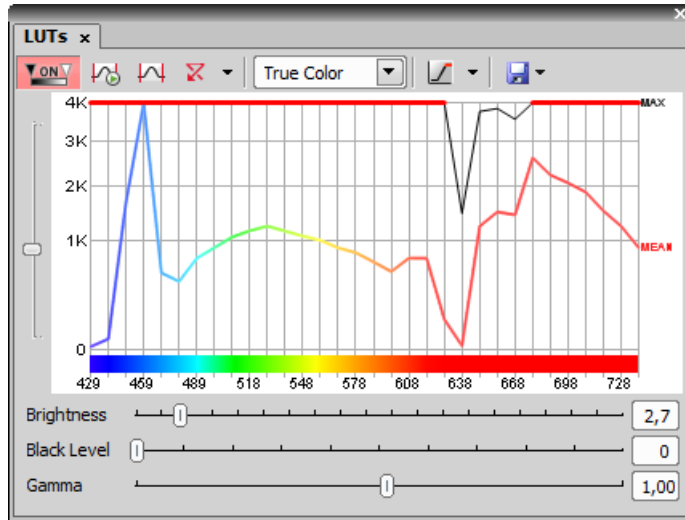
Custom Color Displaying Mode

You can switch to this displaying mode by clicking the 🖼 button located in the image toolbar. You can assign custom colors to sections of the spectrum. Sections (colors) can be added/removed by clicking the + and - buttons placed below the spectral graph. To change the section color, click the button located in the color stripe. A window appears where you can select the color from a palette or define it by *Hue* or *Wavelength* settings.
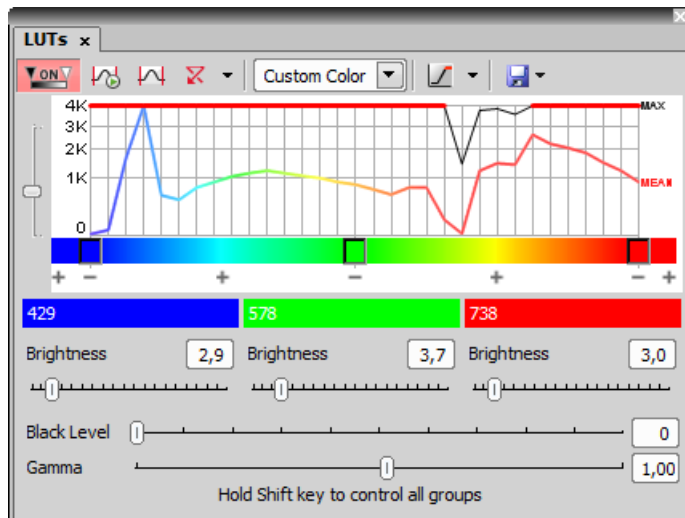
Grouped Displaying Mode

You can switch to this displaying mode by clicking the ▮▮ button located in the image toolbar. This view enables you to reduce the number of channels multiple-times. You can adjust the number of groups by the + and - buttons and also change each channel width by moving the white horizontal dividers. Gain and Black Level for each group can be adjusted.
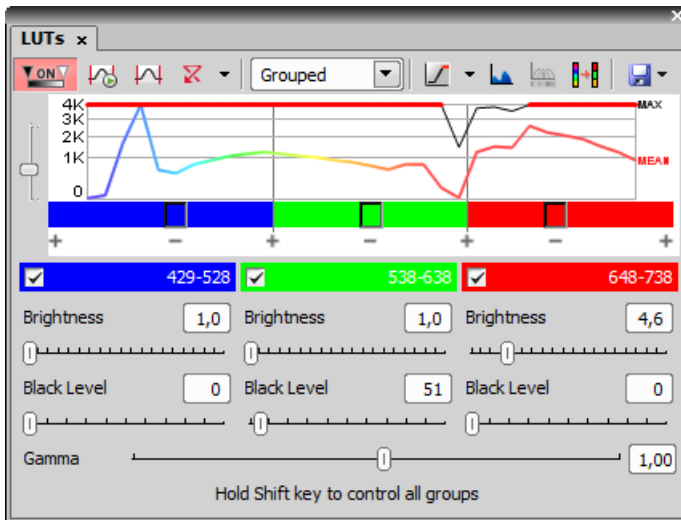
Gray Scale Displaying Mode

You can switch to this displaying mode by clicking the ▬ button located in the image toolbar. The image is displayed in gray scale, but various color gradients can be applied to it.
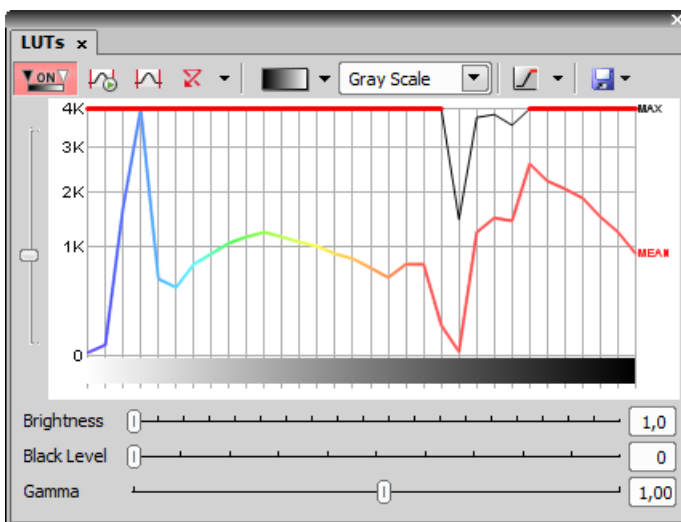
6.6. Organizer

6.6.1. About Organizer

Apart from the main application layout used for capturing and image analysis, *NIS-Elements AR* provides an extra layout called *Organizer*. Organizer makes the work with image files and databases as easy as possible. To activate it, run the ⬛ *View > Organizer Layout* command or click the ▸ ⬛ button located in the top right corner of the application window. The screen opens divided into two identical panes. To switch from one pane to the other, use the *View > Next Pane (F6)* command. To copy files between the panes, simply drag the images from one side to the other side.

Figure 6.42. The organizer layout.
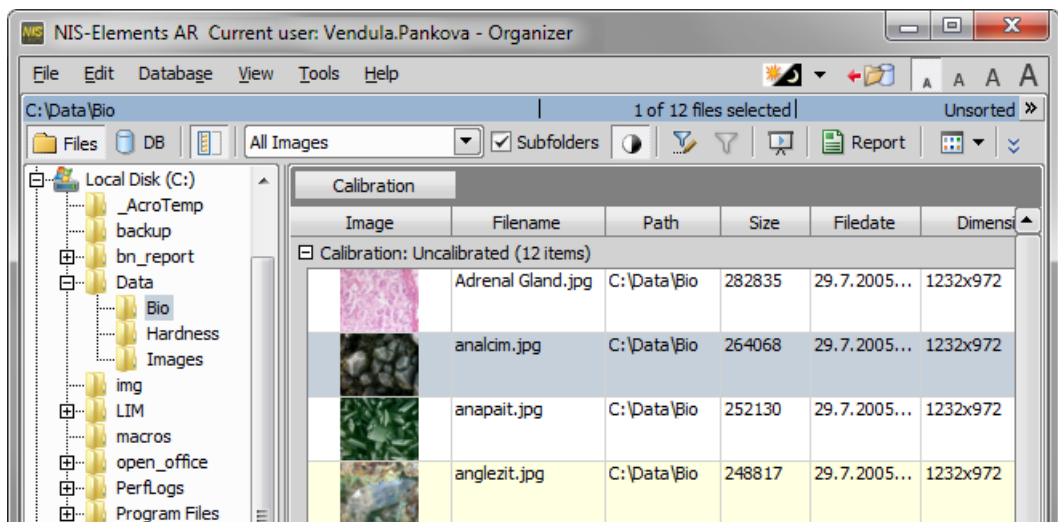
- ⬛ This button toggles the display of the directory tree. You can switch it off to get additional space to display images.

- In the nearby pull-down menu, it is possible to set the file type and only the one will be displayed. Or, *All Images* can be displayed.

- If the *Subfolders* check-box is selected, all images from included sub-folders are displayed.

6.6.2. Image Filter

You can define a filter which enables you to display only images which fit the defined conditions. One or two conditions may be applied.

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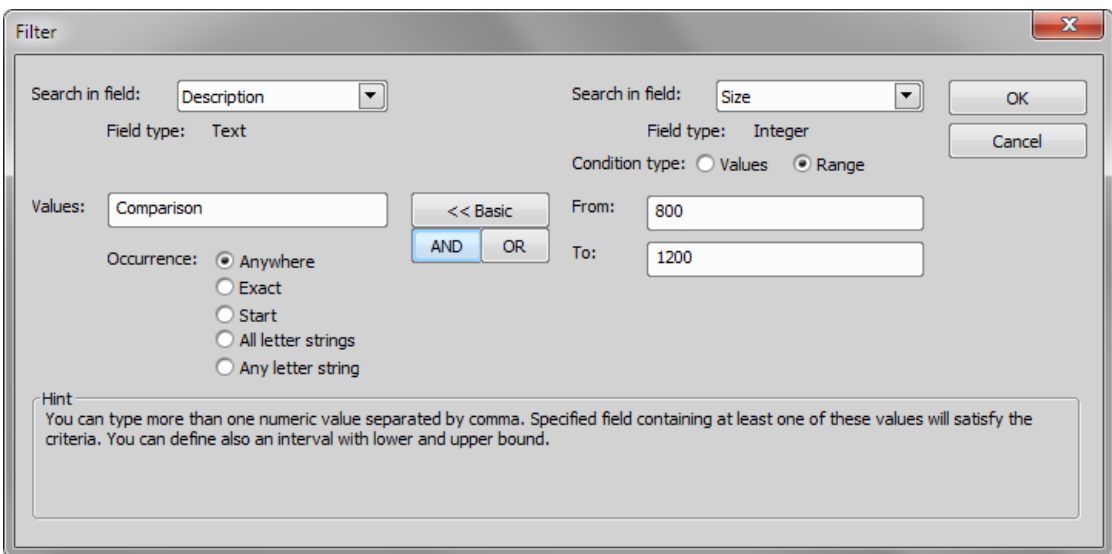
- Pressing this button invokes the filter setup window.

- This button activates the filter.

Basic Mode

This mode enables you to filter files according to one condition.

Advanced Mode

This mode enables you to define two conditions with a relationship between them. Either select *OR* to display files matching at least one condition, or select *AND* to display files which match both conditions.

1. *Search in field* - select the field where *NIS-Elements AR* should search for a given expression.

2. If the selected field is of a numerical type (e.g. Size, Calibration, File date etc.) you can specify, whether you want to find the exact *value* or a value in a given *range*. This is selected by the *Condition type* radio button.

3. If the field type is *Text* , the *Occurrence* setting determines the way of evaluating the expression:

   - *Anywhere* - If the given sequence of characters is found anywhere in the field, the system will evaluate it as a match. For example: you have entered "set" to the *Values* field. The filter will select records with the following field values: "set", "reset", "settings", "preset", etc.

   - *Exact* - If the given sequence of characters exactly equals to the content of the field, it is evaluated as a match. Fields containing the "set" value only will match.

   - *Start* - If the entered string is found at the beginning of a field, it passes the condition. For example: fields containing "set", "settings", "setup" will match.

- *All letter strings* - It is possible to search for more expressions. These should be entered separated by commas. If you want to enter an expression with a space, insert it quoted. If this option is selected, only records where all of those expressions appear (anywhere) will match.

- *Any letter string* - This option is for entering multiple expressions as above, but this time every field with an occurrence of at least one from the given expressions is matched.

6.6.3. Operations with Images within Organizer

- To open an image double click its thumbnail. *NIS-Elements AR* will close *Organizer* and display it in the main window.

- To select multiple images, either click on the first and the last image holding the `Shift` key (continuous group selection) or click individual images with the `Ctrl` key down.

- You can copy one or more images from one folder to another by "drag and drop".

- To delete selected images press the `Delete` key.

All these operations and some other can be invoked also from the context menu, which appears after you right-click on the image thumbnail:
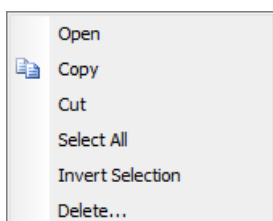
Thumbnail Displaying Options

You can adjust the way images are shown in the organizer.

Thumbnail View Press this button and select the size of displayed image thumbnails. Selecting the *Details with preview* option will display images below each other with all available information aside.

Rotating Images There is a possibility to rotate images from within the organizer. It affects not only the image thumbnails, but the image data too. Press the corresponding buttons.

Autocontrast Press this button to turn the *Apply autocontrast to thumbnails* option ON. It enhances the image thumbnails automatically. Dark images gain details.

Sorting of Images

To order displayed images, right click anywhere in the pane - a context menu will appear. Move to the *Sort by* sub-menu which offers several ordering criteria. If the ordering is turned ON already, the icon is displayed on the left side of the applied criterion.
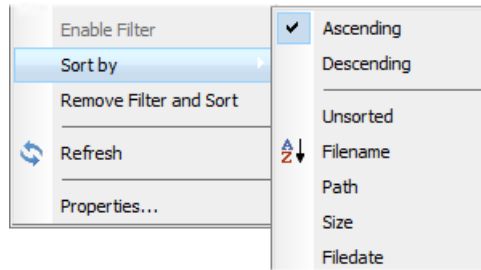
Grouping of Images

To arrange the view of images efficiently, you can use the capability of grouping of images. Drag the column caption to the grouping bar right above the table. All files with matching field values of the selected column will be grouped together. This can be undone by dragging the column caption back to the others. See Figure 6.42, "The organizer layout." (the Calibration column is grouped).

> Note
>
> *If you have the Database module installed, the Organizer becomes switchable between two modes: the Files View and the Database View.*

6.6.4. Resizing the Organizer Panes

The pane size is adjustable. To resize it, you can either:

- 1. Place the mouse cursor over the dividing line in the middle.

  2. The cursor becomes an arrow with two tips.

  3. Drag it left or right to the new position.

- ⊡ Resizes the panes to achieve the same size for both of them.

- « » Resizes the pane to its maximal/minimal size (one pane is then displayed on the whole screen).

7. Image Analysis

7.1. Preparation

7.1.1. Histogram

A histogram is a graphical representation of pixel intensity distribution in the image. It is a basic tool which is used during all phases of an experiment to assess the image information. It is especially useful to make sure there are not too many over- or under-saturated (overrun) pixels. Run the ◿ *View > Visualization Controls > Histogram* command to display the histogram control panel:
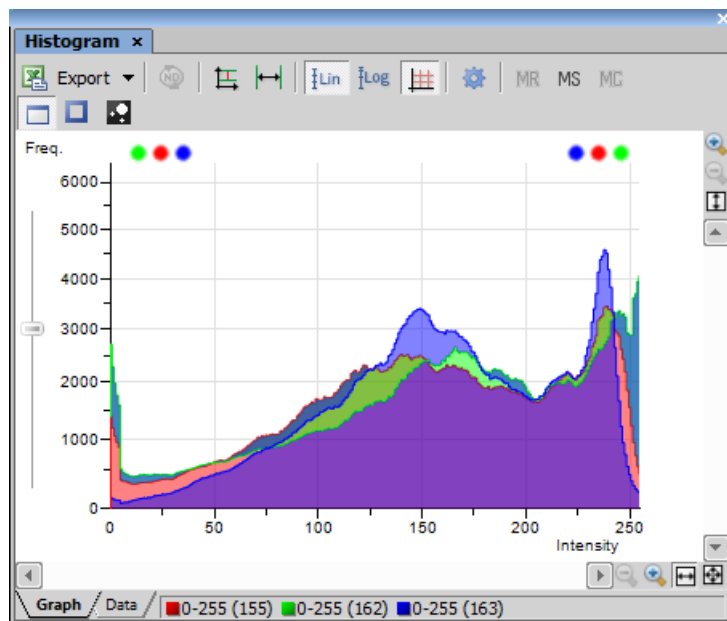
7.1.1.1. Source Data

Source data of the histogram can be viewed if you swap to the *Data* tab in the bottom-left corner of the window. The area from where the histogram is calculated can be restricted according to the selected histogram mode:

☐ The data are obtained from the whole image.

◼ The data are obtained from within the probe.

The data are obtained from within the current ROI.

The data are obtained from the image parts under the binary layer.

7.1.1.2. Export

The source data or the histogram image can be exported to an external file. Display the *Export* pull-down menu and select a suitable destination. Click the button to perform the export. The *Export ND histogram* button enables to export histogram data of all frames of the current nd2 file. The same destination which is selected in the *Export* pull-down menu is applied.

> Note
>
> *Please, see the 8.1.7. Exporting Results chapter for further details.*

7.1.1.3. Histogram Scaling

The histogram can be zoomed in and out using the zoom buttons on sides of the window. There are also other options to adjust the graph appearance:

Left slider There is a slider along the Frequency axis. Drag it in order to stretch the view of either lower or upper values of the axis.

Auto Scale Vertical Zooms the graph of each channel separately to fit the available area. When this function is ON, the histogram is not proportional.

Auto Scale Horizontal Zooms the graph so that the marginal zero frequencies, if there are some, are excluded from display.

Graph Linear Displays the linear scale on the Y axis.

Graph Logarithmic Displays logarithmic scale on the Y axis.

Show Grid Turns ON/OFF the grid in the background.

7.1.1.4. Overrun Indication

If certain amount of pixels with maximum/minimum intensity values (black/white pixels) are spotted in the image, color dots appear above the graph. The color indicates which channel is affected. When you place the mouse cursor over the dots, a tool tip message appears with details about the percentage of under/overexposed pixels. The percentage limit setting is loaded from the LUTs settings (the color dots do not appear unless the set percentage of black/white pixels is exceeded). If the overrun concerns more than three channels (considering multi-channel images), only one white dot is displayed instead of many color dots.

Note

Intensity ranges of all components present in the image are displayed in the Histogram status bar at the bottom of the window.

7.1.1.5. Graph Memorizing

You can save the current graph to memory and display it later for comparison with another graph.

MR Displays memorized graph.

MS Memorizes the current graph. Only one graph can be memorized at a time. If any graph has already been memorized before, it will be overwritten by the current graph.

MC Clears the memory.

7.1.1.6. Histogram Options

Drawing Style

There are two ways the histogram can be drawn:

- *Raw Data* - tries to draw the source data precisely to the graph

- *Trend Style* - interpolates the data so that the histogram lines appear smoother.

Histogram Options Window

The graph appearance can be modified. Press the Options ⚙ button - a window appears where the following settings can be adjusted:

Colors Graph background and axes colors can be selected.

Pen Width Set the width of the histogram(s) line to 1, 2, or 3 pixels.

Fill Graph Area The area below the histogram line can be filled with the channel color.

Graph Area Opacity Select the opacity of the Graph Area color in %.

Vertical/Horizontal AutoScale, Show Grid These options equals the corresponding buttons of the histogram toolbar.

Interpolation method Select the way of drawing the graph line. The *Linear* (smooth) and *Quick* (precise) options are available.

Show grid Shows the grid of the graph.

AntiAlias Smooths the edges of the graph line.

Horizontal axis always visible If checked, the axis does not leave the graph area while zooming in the graph.

7.1.2. Image processing

7.1.2.1. Processing On: Intensity/RGB/Channels

If you work with RGB images, you can decide whether to perform the processing on single RGB channels of the image, or on the intensity component. The RGB/Intensity setting is global so it is applied to all the other processing commands automatically. When processing a multichannel image, this option is disabled - the operation is applied to channels automatically.

> Note
>
> *In case the command does not open a window where it would be possible to make the choice, the setting from the last performed processing command (the global setting) is applied.*

7.1.2.2. ND2 Files Processing

Most of the image processing and binary image processing commands can be applied to one frame, all frames, or selected dimension of the nd2 file. When you use such command with an ND2 file, the following options are added to the command dialog window:
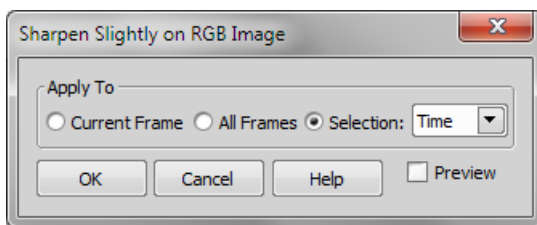
Current Frame The operation will be performed on the current frame of the nd2 file.

All Frames The operation will be performed on all frames of the current nd2 file.

Selection The operation will be performed on all frames of the selected dimension. If you selected some of the frames/loops of the nd2 file by mouse, the *Selection* option appears in the pull-down menu. Opt for it to process only these selected (green highlighted) frames/loops.

When you process an image containing T and Z dimensions, selecting the Z-Series dimension performs the operation on all Z frames of the current T loop.

7.1.2.3. RGB/Mono/Multichannel Image Processing

Options of some of the image processing commands may vary slightly depending upon the type of image being processed. See 6.2.2. Image Types for the more detailed explanation of the image types.

RGB

- When the image processing function works with the image components, you can select whether the *Red*, *Green*, or *Blue* component is used.

Mono

- You cannot select which component is used, because the image contains only one component, which is then selected automatically.

Multichannel images

- When the image processing function works with the image components, you can select which component is used from the pull down menu or other selection tool.

7.2. Image Segmentation

7.2.1. Thresholding

7.2.1.1. Basic Way to Threshold an Image

Specifying correct threshold limits is a crucial procedure of the automated image analysis. The point is to determine which pixels will and which will not be included in the binary layer and thereby distinguish objects to be analyzed from background. Thresholding can be performed in the following modes:

1) Open or capture the image to be thresholded.

2) Run the *View > Analysis Controls > Thresholding* command to display the *Thresholding* panel:

Figure 7.3. Thresholding panel
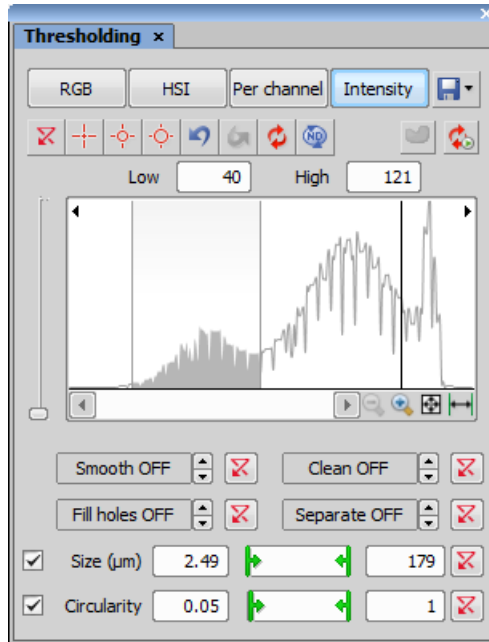
3) In the top toolbar, select the color mode which will be used for thresholding. According to the type of image opened, the following modes could be available: *RGB*, *HSI*, *Intensity* and *MCH*. See 7.2.1.2. Color Modes.

4) Select one of the pixel-picking tools (e.g. ✛) and start clicking on objects in the image you want to detect. Color values of each of the clicked pixel(s) are used to adjust the low and the high threshold values so that the clicked pixel would stay inside the thresholded interval.

Apart from this "clicking" method, the thresholds can be adjusted directly within the histogram by dragging the vertical lines which highlight the thresholded interval.

5) All pixels having their numeric values inside the thresholded interval become a part of the resulting binary layer.

6) Very often, also other binary objects appear in the image which you did not intend to detect, for example dust particles with intensity similar to the specimen, etc. To eliminate these mis-detected object, use one or more tools available at the bottom of the panel. You can either apply some morphology functions to the binary layer or apply size or circularity restrictions to detected objects. See 7.2.1.3. Tips and Tricks.

7.2.1.2. Color Modes

RGB The *RGB* thresholding mode is available only if an RGB image is opened. There are two ways how to define the threshold limits, by choosing reference points within the image, or by defining the limit values for each color channel.

HSI The *HSI* thresholding mode is available only if an RGB image is opened. The thresholding procedure works the same way as in the RGB mode except that the pixel values are displayed in the HSI (Hue, Saturation, Intensity) color space and that the *Saturation* and *Intensity* channels can be switched OFF. Therefore you can threshold over the *H*, *HS*, or *HI* channels only. Switch the channel off by deselecting the check box in the top-left corner of the channel histogram.

Per channel This mode is dedicated to perform threshold on multichannel images, but can be applied to RGB images as well. It is not available when working with a monochromatic (single channel) image.

> Note
>
> *If there are too many channels, only one channel histogram is displayed. Switch between the channels by selecting their names from the bottom pull-down menu.*

Intensity The thresholding procedure works the same way as in the RGB mode except that it is performed on the intensity pixel values.

7.2.1.3. Tips and Tricks

Threshold Adjustments

Thresholding parameters can be adjusted very precisely in the following way:

- Threshold limits of each channel can be adjusted by rewriting the values in the top-left/top-right corner of each channel histogram.

- Threshold range of each channel (the colored stripe) can be shifted by mouse. Place the cursor in the middle of the range (a circle appears) and drag it left or right.

- Threshold limits of each channel can be adjusted by mouse. Place the cursor on the edge of the thresholding range and drag it left or right.

The two last behaviors can be further modified by pressing additional keys:

- Move the threshold using the right mouse button - the binary layer in the image is not updated continuously, but is updated only once after the mouse button is released. Such operation saves some computing and therefore is quicker.

- Hold `Shift` while moving the threshold limits - the threshold adjustment is performed on all channels together (RGB images only).

- Hold `Ctrl` while moving the threshold limits - both the low and the high threshold limits move in opposite directions.

<u>**Binary Operations**</u>

Four basic operations can be performed on the binary layer before it is displayed on screen. To turn the operation on, click on the up arrow button in order to define the number of its iterations:

Clean Removes small objects from binary image.

Smooth Smooths the binary image contours.

Fill Holes Fills holes within binary objects.

Separate Separates objects.

<u>**Restrictions**</u>

Size Define size range using min/max value in the edit boxes or using the slider.

Circularity Define circularity range using min/max value in the edit boxes or using the slider.

Thresholding Large Images

If thresholding images larger than 5000 x 5000 pixels, a new check box called *Preview on selected area only* is displayed. This feature enables to display the threshold preview just on a limited area to speed up the thresholding adjustment.

Changing Appearance of the Binary Layer

To change the color or transparency of the layer, right-click the *Overlay* button in the right image toolbar. A contextual menu appears, where you can select one of the predefined transparency levels, or run the *Colorize Binary Objects* command. This command displays the binary objects in several different colors. The algorithm ensures that two neighboring objects are never colored by similar colors.

See 7.3.4. Mathematical Morphology Basics.

7.2.2. Spot Detection

This function opens the Spot Detection dialog window. It is used mainly for detecting circular objects with similar sizes. Bright spot and dark spot detection methods are split into individual dialog windows.

Figure 7.4. A typical, suitable sample for Spot Detection.
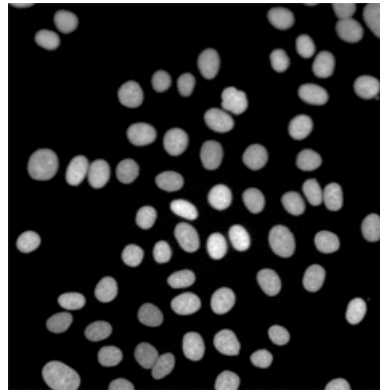
Channel Select a channel on which you want to detect objects. If multichannel file is opened, spectral group selection is available.

Method For *Bright Spots* detection choose ⎯⋏⎯ *Bright, Clustered* detection method if you want to detect clustered bright objects on a dark background or ⎯⋀⎯ *Bright, Different Sizes* for detecting differently sized bright objects on a dark background.

For *Dark Spots* detection choose ⎯⋁⎯ *Dark, Clustered* detection method if your clustered objects are dark and their background is light or ⎯⋎⎯ *Dark, Different Sizes* method if your objects have different sizes and your background is light.

Output Binary output defines in which form the detected objects will be saved. *Circular Area* saves one circle for each detected spot whereas *Spot* saves a center point with a defined pixel size.

Growing The *Growing* check box can be used to save grown regions with borders between adjacent spots. The function itself finds local threshold values for each of the spots detected. Therefore it matches the exact shape of the object. The numeric value represents image intensity degrees. ┼ *Pick intensity* function can be used to get a reference pixel intensity from the picture by a single mouse click. Use the slider to adjust the *Growing* value.

Typical Diameter Adjust the typical size of your objects using the slider or edit box.

Contrast Adjust the contrast value of your spots using the slider or edit box.

Object symmetry: detect ... objects. Can be used to add yet undetected/remove already detected spots based on their symmetry.

Remove Dark Objects Check this option and adjust the position of the slider to exclude dark objects. The more left you move the slider, the more objects appear.

OK Uses the actual settings for performing Spot Detection.

Close Closes the Spot Detection window.

Reset Resets all settings to default.

Help Opens this help file.

Preview Shows the changes directly on the actual frame of your opened image. Previewing progress is shown below this check box.

How to detect Circular Areas

1) Start by opening your image for the Circular Area detection. Select the proper detection method. For bright spots on a dark background choose Bright Spots. For dark spots on a bright background choose Dark Spots.

2) If you can't see any detected objects in your image yet, and your *Preview* button is checked, you should press the *Reset* button.

3) Now choose the *Bright / Dark* vs. *Clustered / Different Sizes* detection *Method* based on your image. Continue by adjusting the *Typical Diameter* values to fit our objects tightly inside the circles. Now adjust the *Contrast* slider to remove unwanted spots. Optionally adjust the *Remove Dark Objects* slider to remove undesirable dark spots.

4) If you are satisfied with the object detection, choose where to apply the current settings (Current Frame, All Frames or a Selection) and click *OK*. Now you can save the new binary layer together with your file.

Note

Circular areas detected close to each other are automatically separated.

Figure 7.5. Circular Area output
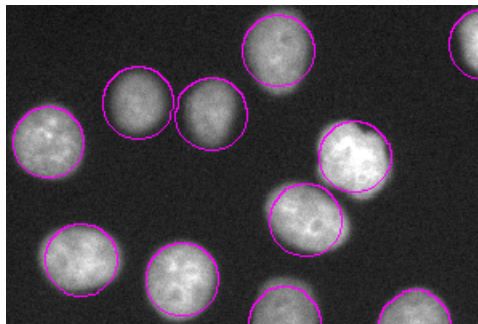
Note

To see the binary layers as shown in the image above, right-click the ⬛ View Overlay icon on the Image window toolbar and select Transparency 100% or use Ctrl *+up/down arrow keys.*

How to detect Spots

1)   Do steps 1-3 from the How to detect Circular Areas procedure.

2)   Switch the radio button from *Circular Area* to *Spot* and adjust the pixel size of the spots.

3)   Choose where to apply the current settings (Current Frame, All Frames or a Selection) and click *OK*.

How to detect Growing

1)   Do steps 1-3 from the How to detect Circular Areas procedure.

2)   Check the *Growing* check box.

3)   Adjust the slider position to augment/diminish the regions. Optionally use the ┼ *Pick intensity* feature to grab the pixel intensity threshold directly from your image.

4)   Choose where to apply the current settings (Current Frame, All Frames or a Selection) and click *OK*.

7.3. Binary Layers

7.3.1. Introduction to Binary Layers

7.3.1.1. How Do I Get a Binary Layer?

Commands for creating and editing binary layers are placed in the Binary menu. Basic methods of binary layer creation are:

Thresholding This is the most common way of creating a binary layer for image analysis purposes. Use the *View > Analysis Controls > Thresholding* command to specify threshold limits, a binary layer will be created automatically. See 7.2.1. Thresholding.

Drawing Manually in Binary Editor A binary layer can be edited or even created manually using the built-in binary layer editor. To enter it, click *Binary > Binary Editor* . See 7.3.3. Binary Editor.

Spot Detection Spot detection is a dedicated segmentation method designed to detect circular objects and can be started by the *Binary > Spot Detection* command. See 7.2.2. Spot Detection.

7.3.1.2. How Do I Display the Binary Layer?

There are the following buttons at the bottom of the right image toolbar.

View Binary This button displays the binary layer only of the image.

View Overlay Binary layers can be displayed together with the color layer using an overlay mode.

View Color This button switches to view the color image (only).

To manage display of multiple binary layers, use the *View > Analysis Controls > Binary Layers* control panel. See 7.3.2. Managing Binary Layers.

7.3.2. Managing Binary Layers

7.3.2.1. Binary Layers Control Panel

Run the *View > Analysis Controls > Binary Layers* command to display the following panel.
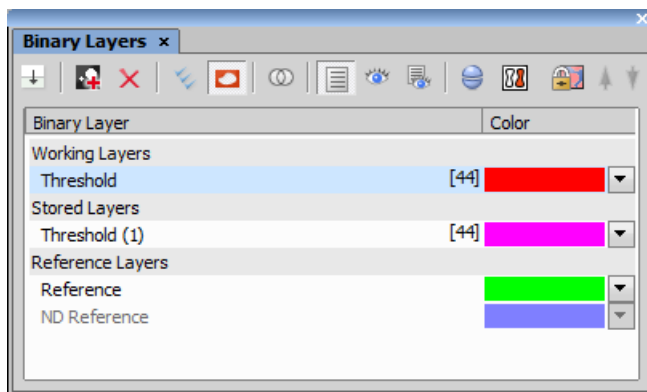
Each time you create a new binary layer, it is automatically placed into the *Working Layers* area of the *Layer List*. Working Layers may be overwritten by the application each time a new binary process is performed. Therefore once you are satisfied with the layer content it is highly recommended to move it into the *Stored Layers* section using the *Store Selected Working Layers*.

7.3.2.2. Operations with Binary Layers

Change color Color of a binary layer can be changed using the color pull down menu. It offers a list of predefined colors. Besides the list of predefined colors it is possible to create a custom color (*More...*) or to colorize objects in a single layer using multiple colors (*By Object/By 3D Object*).

Change order Use the arrow buttons (⬆ ⬇) to change the order of the layers in the list.

Combine Layers using Binary Operations If you drag-drop a binary layer name over another binary layer name, the *Binary operations* window appears. The same window can be displayed by the ◎ *Binary Operations Dialog* button.

> Note
>
> *If multiple layers are selected in the layer list and drag-dropped over another layer, the Binary Operation dialog calculates with multiple (more than two) layers. Available functions are dependent on the number of selected layers.*

See *Binary > Binary Operations* .

Create 3D Binary Objects To create 3D objects in the selected binary layer, click ● *Connect objects to 3D*. If the binary layers are not defined for all frames of the ND document having more than one frame (e.g. only the current frame contains binary information), you can use 🟦 *Fill missing binary frames* to copy the binary from the current frame to all frames where the binary layer is missing.

Duplicate To duplicate one or more binary layers, select them by mouse and drag-drop them to the empty area below the layer list, or click the 🟥 button.

> Note
>
> *If you drag and drop a binary layer to some already existing reference layer, content of the dragged layer is copied to it.*

Make Selection Binary layers can be selected by mouse (pick more layers holding `Ctrl` or select a range holding `Shift`) or you can select all layers by clicking ⟱ *Select All Layers*.

Remove Select the binary layers to be removed and click the ✕ button.

Rename Double click a layer name to rename it. Only name of the *Reference Layers* can not be edited.

7.3.2.3. Attaching Binary Layers to Channels

1)   If a binary layer is created by thresholding in the Per channel mode, such binary layer is automatically attached to the channel on which it has been created. Other binary layers can be attached to a channel manually:

  1. Right-click a binary layer in the *Binary Layers* panel.

2. Select the *Attach to Component* sub-menu and select to which channel/component, the layer should be attached.

2) Visibility of binary layers attached to channels can be synchronized with channels. Turn ON the *Synchronize binaries with channels* button. Now if a single channel is selected for display, only binary layers attached to it are displayed and vice versa.

3) To make an attached binary layer global again, right click the binary layer name or thumbnail and select *Detach from Component*.

7.3.3. Binary Editor

The binary layer, as a result of thresholding, can be modified by hand using the binary layer editor. It is a built-in application providing various drawing tools and morphology commands. Go for the *Binary > Binary Editor* command or press *Tab*. New buttons appear on the toolbars:

7.3.3.1. Drawing tools

The binary image can be modified using various drawing tools. Although the way of use of some tools differs, there are some general principles:

- Make sure you are in the right drawing mode (drawing background /foreground)

- Drawing of any object which has not been completed yet can be canceled by pressing *Esc* .

- The polygon-like shapes are drawn by clicks of the primary mouse button. The right button finishes the shape.

- The automatic drawing tools (threshold, auto detect) have a changeable parameter. It can be modified by $+$ and $-$ keys or by mouse wheel.

- The scene can be magnified by the UP/DOWN arrows when mouse wheel serves another purposes.

- You can drag a magnified image by secondary mouse button.

- A line width can be set in the top toolbar.

- Hints are displayed in the second top toolbar.

Binary Layer Color and Transparency

When in overlay mode:

- The *Insert* key switches between predefined overlay colors.

- `Ctrl + Up/Down` increases/decreases the binary layer transparency.

Erasing Single objects

Single binary objects can be erased in the following way:

- Run *View > Analysis Controls > Binary Toolbar* .

- Select the *Delete Object* ✖ tool from the toolbar.

- Click inside the objects to be erased.

Multiple Binary Layers

An arbitrary number of binary layers can be created within one image. Click this *Create New Binary Layer* 🔳 button in the image toolbar to add a new binary layer. The binary layer that you are currently editing can be selected in the nearby pull-down menu. Binary layers can be managed from the 🔳 *View > Analysis Controls > Binary Layers* control panel.

7.3.4. Mathematical Morphology Basics

The binary image as a result of thresholding often needs to be modified before a measurement is performed. Edges of the objects can be smoothed, holes in the objects filled etc. by using the mathematical morphology commands.

> Note
>
> *"Image Analysis and Mathematical Morphology" by J. Serra (Academic Press, London, 1982) was used as a reference publication for the following overview.*

The basic processes of mathematical morphology are: erosion, dilation, open, close and homotopic transformations.

🔴 **Erosion** After performing erosion, the objects shrink. Marginal pixels of the objects are subtracted. If an object or a narrow shape is thinner than the border to be subtracted, they disappear from the image.

🔵 **Dilation** After performing dilation, the objects enlarge. Pixels are added around the objects. If the distance between two objects is shorter than twice the thickness of the border to be added, these objects will be merged. If a hole is smaller than twice the thickness of the border, it disappears from the image.

🔴 **Open** Open is erosion followed by dilation so the size of the objects is not significantly affected. Contours are smoothed, small objects are suppressed and gently connected, particles are disconnected.

🔵 **Close** Closing is a dilation followed by erosion so the size of objects is not significantly affected. Contours are smoothed, small holes and small depressions are suppressed. Very close objects may be connected together.

🔴 **Clean** This transformation is also called geodesic opening. The image is eroded first so small objects disappear. Then, the remaining eroded objects are reconstructed to their original size and shape. The advantage of this algorithm is that small objects disappear but the rest of the image is not affected.

Fill Holes Fills the holes inside objects. This transformation is handy when objects have a rich inner structure with intensities typical for background. After applying the *Fill Holes* transformation, objects become homogeneous.

Contour This transformation converts objects into their contours.

Smooth Smooth affects rough edges of the objects makes them smooth.

Morpho Separate Objects This transformation detects standalone objects that are connected together and isolates them.

7.3.4.1. Homotopic Transformations

Homotopic transformations preserve topological relations between objects and holes inside them. Using a homotopic transformation, an object with 5 holes will be transformed to another object with 5 holes. Two objects without any holes, even if they are very near each other, will become again two objects without holes, but likely with a different shape and size. Opening, Closing, Erosion and Dilation are not homotopic transformations. Typical homotopic transformations in *NIS-Elements* are: *Skeletonize*, *Homotopic Marking* and *Thickening*.

7.3.4.2. Connectivity

Applying the above mentioned transformations has some limitations due to digital images rasterization.

When speaking about binary image processing, a binary image is a set of pixels where values 1 represent objects and values 0 represent background. In the square grid of the image, two possible connectivities can be used for processing - the 8-connectivity or the 4-connectivity. Look at the picture below. If the 8-connectivity is used, the two pixels represent one object. If the 4-connectivity is applied, there are two objects in the picture. *NIS-Elements AR* works with the 8-connectivity model, so all pixels neighboring by the corner belong to one object.

7.3.4.3. Structuring Element = Kernel = Matrix

When applying Erosion, Dilation, Opening or Closing, one of the parameters which determines the transformation result is the selection of kernel (structuring element, matrix) type. There are the following kernels used in *NIS-Elements AR*:
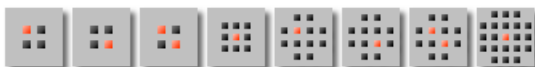
The bright pixel in the center or near the center of the kernel represents its "midpoint".

Example 7.2. Erosion

Let's assume 1 and 0 represent object(1) and background(0) pixels of the binary layer. You can imagine the erosion as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel and make the following decision:

- If there are object(1) pixels in all the positions of the kernel, set the midpoint to object(1).

- If there is at least one background(0) pixel in the kernel, set the midpoint to background(0).

Example 7.3. Dilation

You can imagine the dilation as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel and make the following decision:

- If there is at least one object(1) pixel in any position of the kernel, set the midpoint to object(1).

- If there are background(0) pixels in all the positions of the kernel, set the midpoint to background(0).

Example 7.4. Open and Close

Open is performed by eroding the image and then applying a dilation to the eroded image. On the contrary, Closing is performed as a dilation followed by erosion.

Repetition issues

If the midpoint is not in the center, applying erosions or dilations by odd number of iterations causes image to shift by 1 pixel. Normally, the total image shift would be determined by the number of Iterations (in pixels). *NIS-Elements AR* eliminates this shift: it changes the position of the midpoint 1 pixel down-rightwards within the kernel for even operations. For opening and closing it is possible to eliminate this shift totally. However, if you run the erode or dilate processes again and again using the kernel with even dimensions and odd number of iterations, then the shift becomes significant.

7.3.4.4. Mathematical Morphology Examples

Please see the following examples of some of the binary functions applied to an image. In the following sequence of images, the functions were applied subsequently:

Example 7.5. Functions applied subsequently
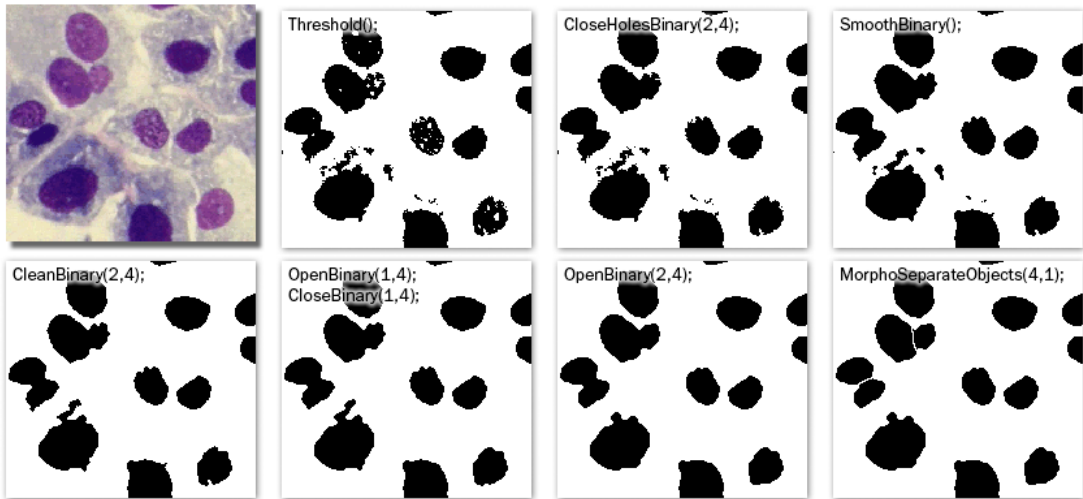
Example 7.6. Single function applied to the original

The following examples were created by calling each of the functions separately:
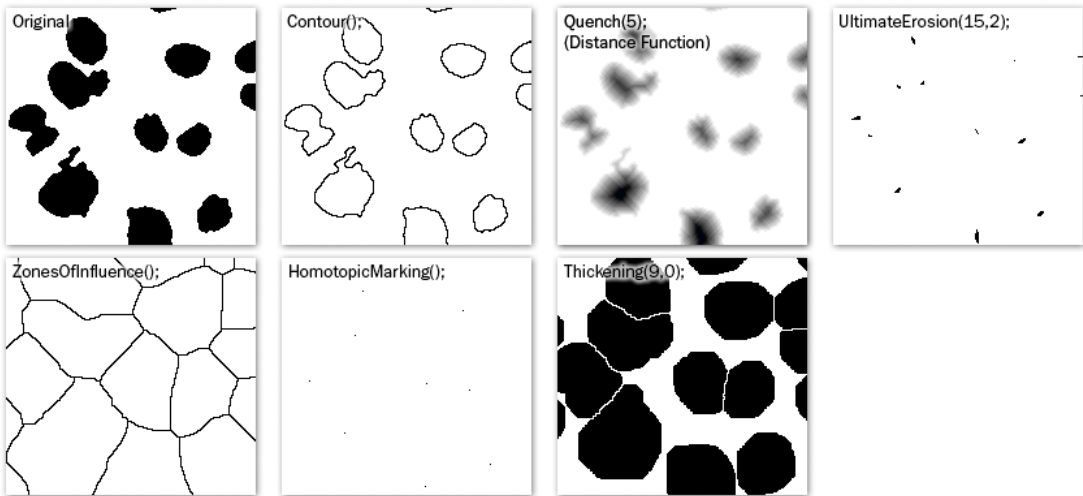
Example 7.7. Other functions applied subsequently
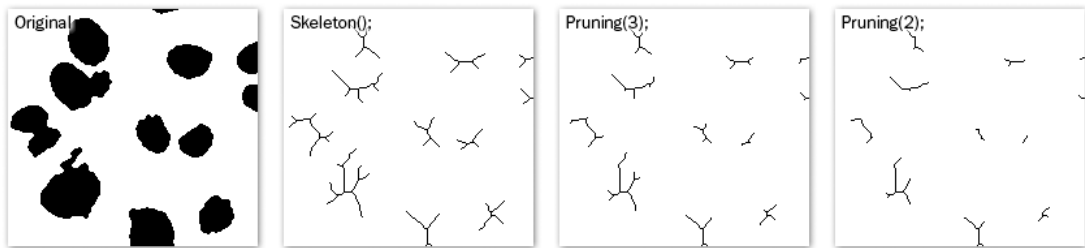
7.4. Regions of Interest - ROIs

7.4.1. Introduction to ROIs

In laboratory imaging experiments, users are often interested in just a part of the image. To define such an interesting part of the image we use one or more Regions of Interest (ROIs). These regions are used later in analysis and measurements.

Note

Do not confuse image ROIs with Camera ROI which is used to reduce the active area to just a part of the camera sensor (CCD or CMOS). Such reduction usually results in higher frame rate. See 5.3. Camera ROI.

7.4.1.1. Creating and editing ROIs

ROIs can be created and edited using commands gathered in the ROI menu. ROIs can be also created from binary objects, graticules (for equally spaced shapes) and from binary layers created by tracking.

Figure 7.12. Available ROI tools
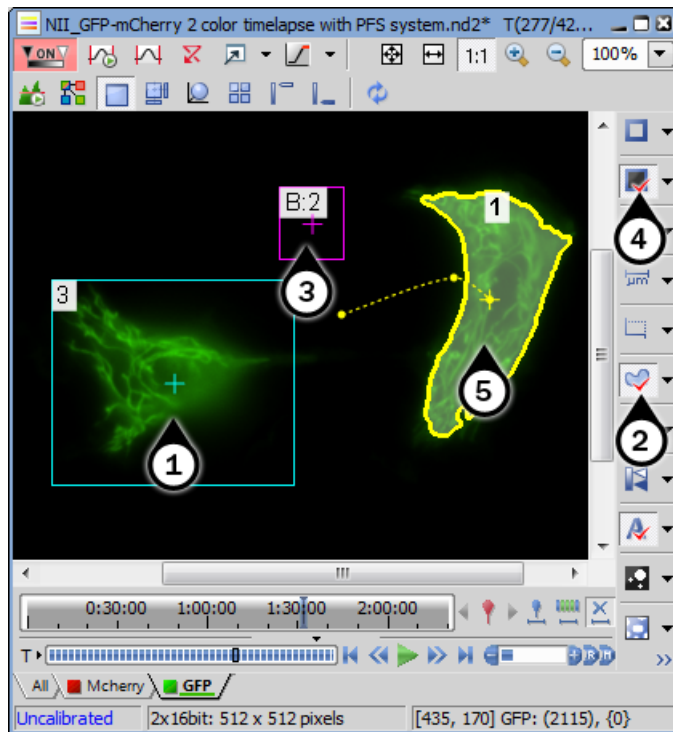
1. Global ROI

2. Turn ROI On

3. Background ROI

4. Turn Background ROI On

5. Moving ROI

7.4.1.2. Types of ROI

Individual ROIs (ROI objects) are 2D objects. They can have different shape: rectangular, elliptical polygonal or bezier. One shape can be created with one or more tools. For instance polygon ROI can be defined using both "Draw Polygonal ROI" and "Autodetect ROI".

In case of an ND file which has time axis (e.g. time-lapse) individual ROI objects can be defined as "Global " or as "Changing over time". Global objects do not change any of their characteristics over time. Objects that are defined as changing over time can change their position and shape in time (however objects cannot change their shape - e.g. from ellipse to polygon). This feature is useful for track moving or warping objects.

To track moving objects use the "Edit ROIs in Time" editor or just change any global ROI into changing over time from the objects' context menu and move it or change its' shape (see interactive edit) on two or more frames. Such frames are called "key-frames" (2 shows trajectories with little boxes representing the key-frames). All frames in between key-frames are interpolated from these two key-frames. Frames before and after first and last frame respectively are taken from that first or last frame. Therefore it is not necessary to reposition or redraw the ROI on every frame.

In case of Multi Point nd2 file there is one more option: ROI can be defined per Multi Point (which is the default). It means that ROIs from one point are completely independent of ROIs defined on the other point (even if they have the same number). This applies also to ROIs defined as "Changing over Time" (in case the image has time axis too). On the other hand if the ROI is changed to a "Global" it will be shared among all points of the Multi Point set.

Depending on the application purposes ROI objects can have different usage. NIS can handle "Background ROIs", "Reference ROIs", "Stimulation ROIs" and "Standard ROIs".

7.4.1.3. Interaction with ROIs

ROIs can be manipulated anytime directly in the image window. Many of the following techniques work in ROI editors too. Objects cannot be moved or resized when they are Locked (some analysis tools or users may lock them to prevent accidental modification). They must be unlocked (from the ROIs' context menu) before any manipulation. Note that the mouse cursor changes over the ROI to indicate which action will be started after mouse button is pressed (it changes when modification keys like Alt and Ctrl are pressed).

Selection ROI objects can be selected by clicking on an unselected object (other previously selected are unselected). Clicking on a ROI object with Ctrl key pressed results in altering the selection state. More than one object can be selected in this way. Double-clicking on any object results in only that object being selected. All objects can be selected by pressing Ctrl+A or by choosing "Select All ROIs" from the menu over any ROI. Alternatively, it is also possible to select a set of objects by dragging a rectangle around them with Ctrl key being pressed.

Moving Objects are moved by simply dragging them (cursor indicates that). When more objects are selected, only the one below the cursor position is moved by default. This can be changed by pressing the *Alt* key and move all selected objects. When object is changing over time, any moving results in a new key-frame. Individual dots representing the key-frames can be moved too. It is possible to duplicate existing objects by Shift dragging. It works on selected objects. In case of a key-framed object, object is duplicated with all its' key-frames unless Alt is pressed.

Resizing Resizing a ROI is done by dragging its' contour (the cursor changes accordingly). Note that the hot-zone (the area where mouse cursor changes into different tool) for resizing is outside of the object contour while the hot-zone for moving is inside. Resizing always affects only one object (selection is not taken into account). The behavior slightly varies depending on the object shape. By default resize does not hold aspect of the object - width and height can be changed independently. By holding Shift key aspect is maintained (useful for circles) and the center is not moved. Rectangle can be also rotated by using Alt key while dragging.

Exact values can be entered in "ROI Properties" dialog which is accessible from the ROI context menu.

7.4.1.4. Simple ROI Editor

When creating more than one ROI, the Simple ROI editor is the tool of choice. With this tool it is possible to make all possible shapes with different methods. All interaction techniques work here as well.

Figure 7.13. Simple ROI Editor toolbar

Note that many actions have a hot-key (P for Pointing tool, R for Rectangle, E for ellipse, L for Polygon, B for Bezier , A for Auto detection, H for drawing holes and others) to speed up the process of interaction.

7.4.1.5. Auto-Detect Tool

Auto-detect tool is available from ROI menu, Simple ROI editor, Edit ROIs in time editor and some other places. It produces a Polygon ROI. Using the tool is two step process: The first click on an image structure results in preliminary object contour, that is drawn on the screen. After that, user can further tweak the object contour and then finish whole auto-detect by confirming with secondary mouse button. User can always cancel auto-detect by pressing Esc key.

The very first click is important, because these pixels under the cursor determine intensities from which the algorithm guesses the whole object. As a general rule it is better to choose brightest pixels in the bright objects (fluorescence) and dimmest in the dark object. It is also good to click near the center of the object.

After the click the algorithm presents its guess of the object contour and goes into the interactive phase. In this phase it is possible to:

Redefine the first point by clicking again in the image

Cancel auto-detect by pressing Esc key

Grow or shrink the object area (changes the intensity range of pixels making the object) by scrolling mouse wheel or pressing PageUp/PageDown (for finer steps use Ctrl + mouse wheel)

Erode or Dilate morphologically the object by 'E'/'D' or Down/Up keys

Open or Close morphologically the object by 'O'/'C' keys

Morpho-separate portions of the object by 'S' or 'P' key (if it is not possible to separate the key does nothing)

7.4.1.6. Using ROIs for analysis

ROIs are used mainly in Time Measurement to measure varying image intensity inside the ROIs, Automated Measurement to measure ROIs features or number of binary objects inside each ROI , Object Count to restrict binary objects to areas of interest only, ROI statistics to measure intensities interactively.

In all these cases ROI selection is taken into account as well as intersection options in Measurement Options.

Additionally the Time measurement interprets the ROI usage type. Background ROIs are used for offset subtraction and Reference ROIs can be used for bleaching correction.

7.4.2. ROIs in Time - Moving ROIs

7.4.2.1. Introduction to Moving ROIs

To define a moving ROI:

1. Open or capture a time-lapse nd2 file.

2. Display the first frame of the image and open the vector ROI editor by the *ROI > Simple ROI Editor* command.

3. With one of the editor tools, draw the ROI around an object. Close the ROI editor.

4. Right-click the ROI and select *ROI Changes Over Time* from the context menu.

5. Play the image sequence and if the object of interest starts to move out of the ROI, pause the replay.

6. Now it is possible to drag the ROI to the new position. If the ROI is created by the vector editor, its size and rotation can be also modified.

7. Play the sequence further and drag the ROI to a new position each time the object in the image moves.

> Note
>
> *ROI positions between two user-defined frames are interpolated. If the object of interest was moving at constant speed with a linear trajectory, then it would be sufficient to define the ROI positions only in the first and the last frame of the image sequence.*

Tips on Moving ROI

ROI Tracking Motion characteristics of a manually defined moving ROI can be measured using the *View > Analysis Controls > Tracking* control panel. Just click the *Add Moving ROIs* button to add the current ROIs to the table of objects.

Automatic ROI Definition Automatic *Tracking* features may be utilized to define moving ROIs. Already tracked objects may be converted to moving ROIs by clicking the *Binary to ROI* button.

ROI Multiple Selection Use the Ctrl key to select/unselect more than one ROI. When you have selected more ROIs, press and hold the `Alt` key while dragging a single ROI. Other selected ROIs will not be moved but remain selected.

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8. Measurement

8.1. Basic Workflows

8.1.1. Calibration

8.1.1.1. Introduction

Calibration of the image is of crucial importance to measurement. On calibrated images, realistic measurements can be performed and objects of different images could be compared to each other. A correct calibration has to be made before you begin to measure.

There are two ways how to obtain a calibrated image:

- To capture images with a calibrated system (objective). Please see the 4.1.3.3. Objective Calibration chapter which explains how to calibrate the *NIS-Elements AR* system.

- To calibrate an uncalibrated image manually:

8.1.1.2. Calibrating an Uncalibrated Image

1)   Run the *Calibration > Recalibrate Document* command.

2)
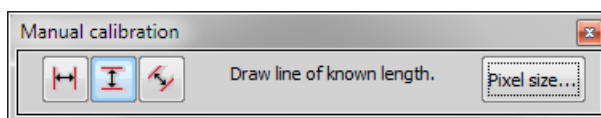
3)

    a)   If you know the size of one pixel, click the `Pixel size...` button.

    b)   If you do not know the pixel size, draw a distance to the image and set its real length. For this purpose, you should use an image of a calibration slide or a ruler captured with your system.

There are three modes: horizontal, vertical and parallel lines, select one of them. Click into image to place first line, then second line appears on your mouse cursor. Place this line and the following dialog box appears.

4)

Figure 8.2. Pixel Size Calibration
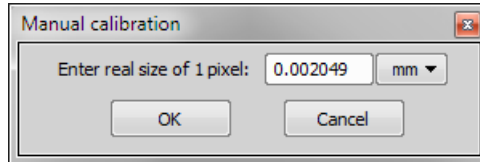
Figure 8.3. Line-length Calibration
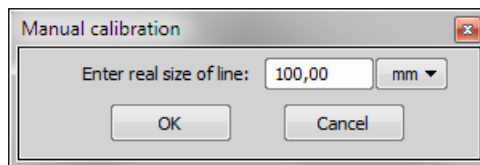
5) Click OK to finish the calibration.

8.1.1.3. Units

NIS-Elements AR supports the following units:

pixels
nanometers
micrometers
millimeters
centimeters
decimeters
meters
kilometers
inches
mils

If the image is uncalibrated, pixels are the only units available. In case of a calibrated image, it is possible to select other units which are then used to display all values (e.g. measured length/area). There are two ways of how to select the desired units:

- Right click the image status bar where the calibration is displayed. Select the units and precision from the context menu.

- Or, click the *Current unit* button located in the *Calibration > Objectives* command window and select the units.

8.1.2. Rough Measurement

Quick and approximate measurements can be performed utilizing graticules. They behave like adjustable floating rulers. User can simply align a graticule with the measured object and read the distance value (e.g. the diameter). To activate the graticule, press the *Graticules* button.

1)   Select Graticule Type

The type of the graticule ruler is indicated by a picture on the *Graticules* button placed on the right image toolbar. To change the graticule type, click on the graticules button with the secondary mouse button and select the appropriate item from the context menu:

 Rectangular Grid

 Circle

 Simple Circle

 Cross

 Industrial Cross

 Simple Cross

 Vertical Ruler

 Horizontal Ruler

2)   Define Graticule Properties

Right click the *Graticules* button and select the *Graticules properties* command from the pull-down menu. A window appears where display parameters (shape, color, line width, density of lines) of all graticules can be adjusted.

Graticules Density

The density value - the closest distance between two line intersections of the graticule - can be set, or you can let *NIS-Elements AR* adjust it automatically according to the current zoom factor. The units selection depends on the image calibration (calibrated/uncalibrated).

> Note
>
> *Concerning the Cross graticule. The distance defined by Density is divided into smaller sections automatically. However, for odd density values greater than 10, the markings remain hidden because it is not possible to display them accurately.*

3)   Measure the Image

The graticule measurement provides the following options:

- The graticule can be moved by mouse arbitrary.

- The graticule position can be reset by using the *Move Graticules to Center* command from the context menu.

- A binary layer can be created from the current graticule using the *Copy Graticules to Binary* command.

- A *Graticule Mask* can be created from the current binary layer using the *Copy/Add Binary to Graticule Mask* command.

- A *Graticule Mask* can be stored/loaded to/from an external file via the *Save Graticule Mask As* and *Open Graticule Mask* commands.

- A new image containing graticules can be created by the *Edit > Create Full View Snapshot (8bit RGB)* command.

8.1.3. Object Count

The *Object Count* tool is designed for automated object detection and counting. It enables users to threshold the image, automatically measure the binary objects, and export the measured data to a file in a straightforward way. Object count can be performed even on Live image. Run the *View > Analysis Controls > Object Count* command to display the control panel.

The recommended workflow through the dialog is to move from left to right, starting with thresholding, then using restrictions and finally reviewing the result and export out of *NIS-Elements AR* to a text file or an MS Excel spreadsheet.

8.1.3.1. Thresholding the Image
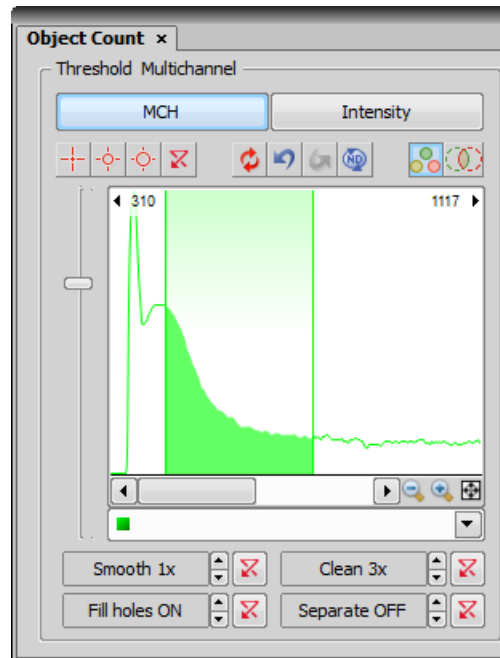
The threshold limits shall be defined by picking object-typical colors from the image. Select one of the following tools:

- ┼ *1 point threshold tool*

- ⌖ *3 points circle threshold tool*

- ⌖ *6 points circle threshold tool*

Click inside the image to define typical object areas. The system will detect similar parts of the image and highlight it by color. The threshold limits are indicated in the histogram and can be further modified by dragging the limit lines by mouse.

Please, refer to the 7.2.1. Thresholding chapter for further details on thresholding.

8.1.3.2. Applying Restrictions

The number of objects that are included in the results table can be restricted by defining limits. Objects which do not fit these limits will be left out of the result table.
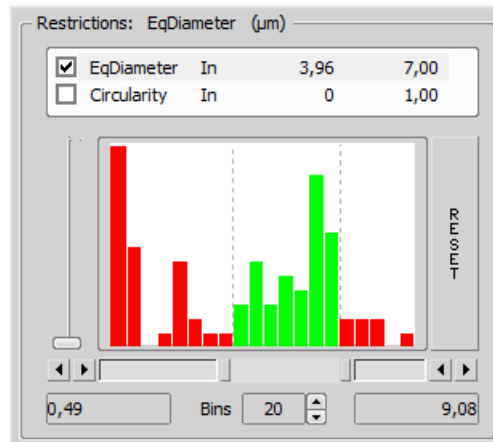
1. Right click to the restrictions field to select one or more of the available measurement features.

2. Select the restriction feature you would like to define.

3. Move sliders below the histogram to set the limits. The limit values are indicated next to the feature name, and can be modified by double clicking the indicated value.

4. Decide whether the defined interval will be excluded or included - this is done by setting the *Inside/Outside* option next to the feature name.

5. The nearby check box indicates whether the restriction is currently being applied or not. If applied, the histogram below is color, otherwise it is gray.
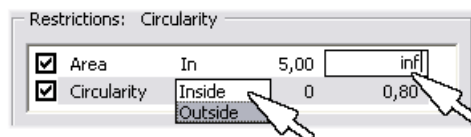
Note

Infinitude can be defined by entering "oo" or "inf".

Reset The *Reset* button on the right side discards restriction settings of all features.

Bins This setting determines the number of columns in the histogram.

Restrictions from the Image

A reference object can be picked from the image and used to set the limit values.

- Select the restriction feature you would like to set by mouse. Let's assume you have selected *Circularity*.

- Right click the thresholded object which is going to be used as source. A context menu appears.

- Select whether to *INCLUDE* or *EXCLUDE* the selected object and all others with higher/lower circularity.

- The restriction will be applied and indicated by colors within the image.
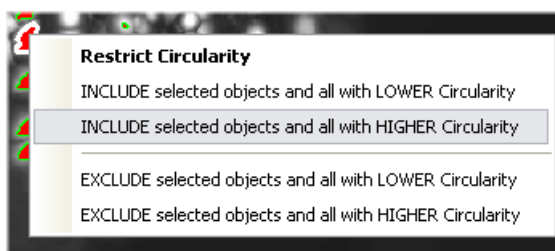
8.1.3.3. Results

The following results appear in the results table:

- *Total Area* is the area of the ROI. It is shown in square pixels or square *calibration units*.

- *Measured Area* is the area of the binaries inside the ROI.

- *Number of objects* - number of objects after applying restrictions.

- *Table of results* - measurement results for each object are displayed in this table. Select the columns to display/hide from the context menu which appears when you right-click one of the column captions.

- *#* - number of selected objects is displayed in the left part of the toolbar. One or more objects can be selected by mouse while holding the `Ctrl` or the `Shift` key.

Toolbar

There is a toolbar above the results table which contains the following buttons:

✕ **Delete selected objects** Removes the selected objects. The objects can be selected by mouse while holding `Ctrl`.

↩ **Invert selection** Deselects objects selected by mouse and vice versa.

⊞ **Generate binary** Erases binary objects which do not meet the restrictions settings.

⊞ **Show Object Calatog** Opens the *Object Catalog* control panel with data source automatically set to "Object Count".

Export The measured data can be exported to clipboard or a file using the *Export* button. Please refer to the 8.1.7. Exporting Results chapter for further details.

⊕ **Export ND data** When an ND2 file is opened, the thresholding, measurement, and restrictions are applied to a single frame. Pressing this button will apply it to all frames of the ND2 document and export the results to a file or clipboard according to the current export settings.

⊟ **Save/Load Object Count configuration** Invokes a pull-down menu which enables you to load/save the current object count settings from/to an external file (*.counting).

Use Standard EqDia Check this option to enable counting of objects using standard EqDiameter. Value of the standard EqDiameter parameter can be edited in the field nearby.

Note

Please be aware that the number of objects calculated by this function is only an approximation and not an accurate count of objects.

⊕ **Keep updating count** Refreshes the measured results each time the binary layer changes (e.g. when the threshold is modified).

8.1.3.4. Object Count Procedure Example

1. Open the *Object Count* control panel. See 8.1.3. Object Count.

2. Threshold the image. Use the thresholding picking tools and click on the image to sample areas of interest. These pixels selected will determine what intensity/ part of histogram is considered accepted. The threshold will detect several specs or smaller objects such as noise or background. If desired, the *Clean* and *Smooth* filters will exclude smaller objects.

3. You can also measure using the defined region of interest (the ⌣ button) or measurement frame (the ⬚ button). Check the options are properly set in the *Measure > Measurement Options* dialog window.

4. Apply restrictions. Select *Area* from the list of available restriction features. Use the sliders in the histogram to specify the lower limit and the upper limit. The lower and upper limit will update in the display.
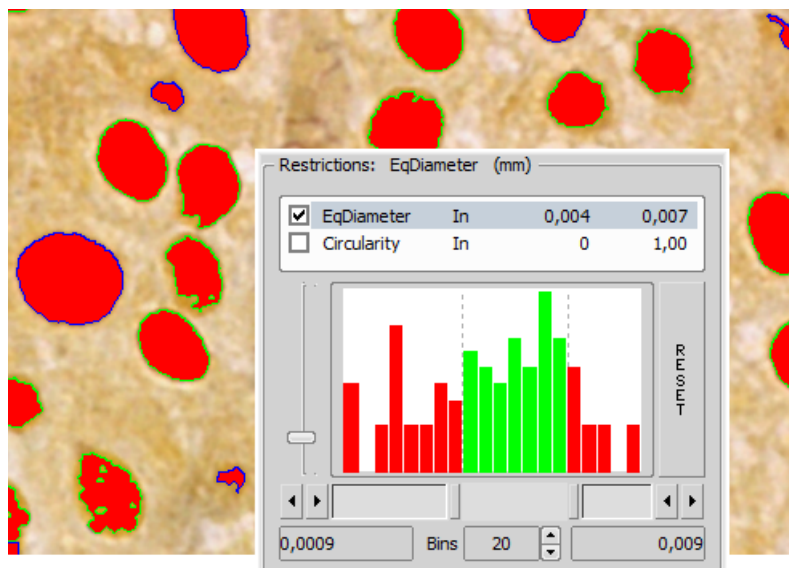
The green area of the histogram represents the accepted range areas. Any object with an area in the green range will be considered an object. Alternatively, any object with an area in the red ranges will not be considered an object.

The outlines of objects on the image differ according to whether the object is restricted or accepted. In this case, accepted objects have a green outline and restricted objects have a blue outline.

To ensure that that count is updating with any change, click the 🔄 icon:

5. Remove any unwanted object from the object count by selecting the Delete Selected Objects ✕ icon from the Result section. The object will lose its thresholded overlay and will be excluded from the measurement.

> Warning
>
> *You cannot Undo this action. To restore an object repeat the original threshold procedure or load a saved Object Count configuration.*

6. View the result data and export them to a file or spreadsheet application. See 8.1.7. Exporting Results for further information.

8.1.4. Measurement Options

Run the ⚙ *Edit > General Options* command and select the *Measurement* tab.

Rules for Excluding Objects This section regards automatic measurement. Select the options how to treat the objects touching the area borders when you are performing the automatic measurement:

> **Using Measurement Frame** Pick up the option how to treat the object touching the measurement frame. Run the automatic measurement by the *Measure > Use Measurement Frame* command.

> **Using ROI** Pick up the option how to treat the object touching the ROI frame. Run the automatic measurement by the *View > Layers > ROI* command.

> **Without Measurement Frame and ROI** Pick up the option how to treat the object touching the image border. Turn off the measurement frame and ROI and run the automatic measurement.

These options do not affect the field measurement results (field measurement is always applied inside the defined area without exceptions).

Object Colors After the automatic measurement is performed, all measured objects are highlighted by color borders. Here you can select colors for the objects excluded from measurement according to the ROI/measurement frame settings. The *Out of limits* color will be used to highlight objects which do not fit the applied restrictions (*View > Analysis Controls > Automated Measurement*).

Manual Measurement

> **Automatically zoom in Length manual measurements** This option zooms the image while placing the measurement points to the image. Only the indicated measurement tools are affected.

> **Copy manual measurement objects from live image to captured** *NIS-Elements AR* enables to measure manually on live camera signal (*View > Analysis Controls > Annotations and Measurements*). After the *Acquire > Capture* command is used, the measurement objects in the image are copied (or not) to the captured image according to this setting.

> **Label** The manual measurement objects can be labeled. Select what information will be attached to every manual measurement object in an image.

8.1.5. Measurement on Graph
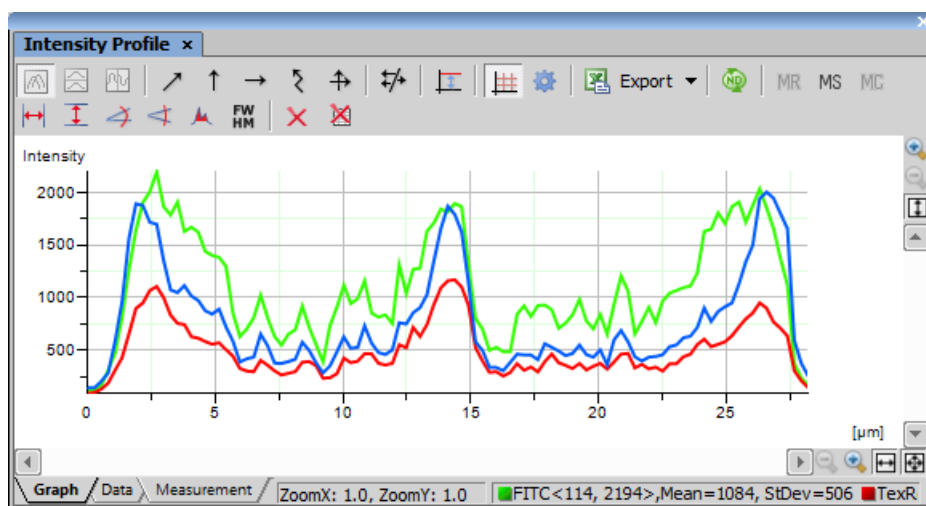
The following interactive measurements can be performed within graphs.

Vertical, **Horizontal** Pick this tool to measure distances within the graph.

Angle This tool enables selecting an angle and its both line lengths. The resulting number represents a ratio between the projection of the y arrow on y axis and projection of the x arrow on x axis. The resulting number is a dimensionless value. This tool does not allow adjusting the x line length.

Free Angle This tool measures the same features as the previous angle tool but allows to adjust all of the angle's anchor points.

Area of Graph This tool enables you to draw a rectangle in the graph. Area will be measured of intersection of the rectangle with the area below the graph curve.

FWHM This tool measures the *Full Width at Half Maximum* value on the given graph range.

Clear Measurement Objects Deletes all the measured objects in the graph.

Reset Data Clears the table with the measurement results.

8.1.6. Pixel Classifier

A typical task for Pixel Classifier may be counting the area of a cross-section of a blood vessel. Since there are at least three different types of tissue whose area should be calculated while background should not be counted at all.

1. Eliminate the background. You can:

   - define a region of interest. See 7.4.1. Introduction to ROIs for more information.

   - use the method of accepted region. Run the *Edit > Region Settings* command first. Choose color of the background in the *Region Settings* dialog that appears. You can fill in the value *0*, which is easy to threshold. Next run the *Edit > Region > Accepted Region* command. Draw the boundary around the area to keep.
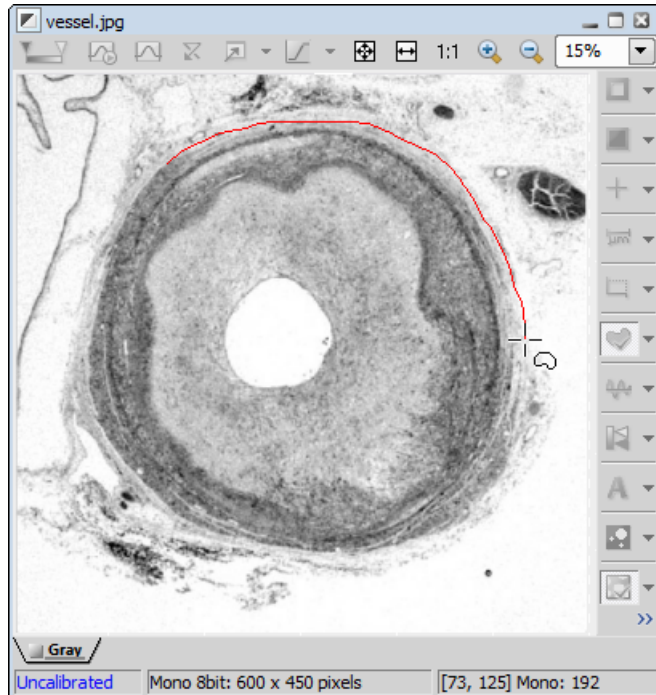
Finish drawing with a right click. The image is cropped according to the boundary. Area of image which remained outside the border is deleted and filled with the set background color.
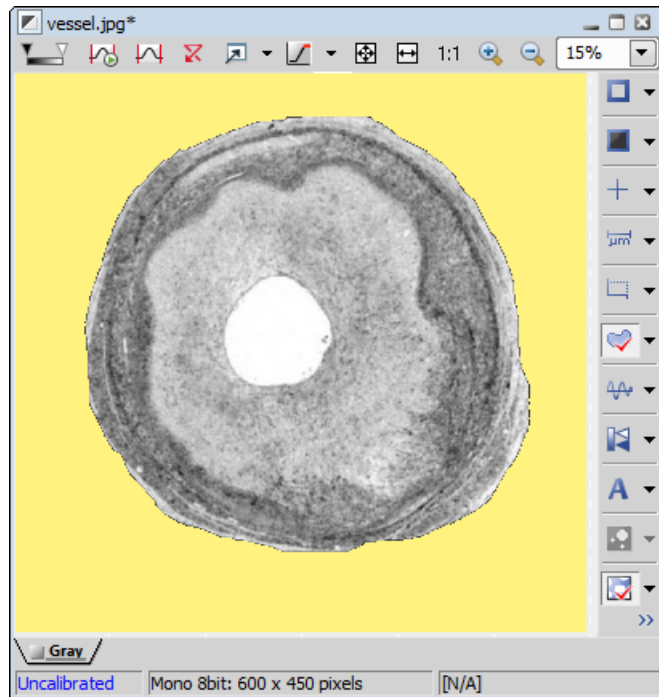
2. Run *View > Analysis Controls > Pixel Classifier* to display the classifier panel. Press the *Define* button and edit all phases you want to detect separately. Use the ⊕ and ⊖ buttons and commands in the contextual menu to *Add*, *Rename* or Delete any class.

   If you choose the *Manual* method of classifying, you can manually choose the intensity ranges of all phases.
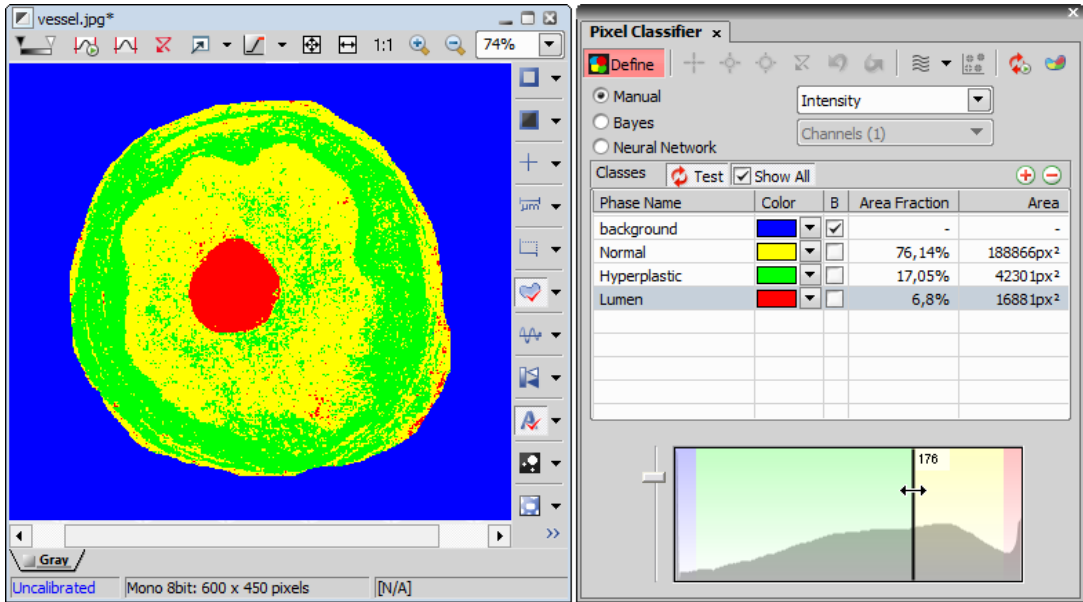
Alternatively, you can sample areas and train the other algorithms to find phase boundaries.

3. Once all phases have been defined properly, the settings can be re-used to classify another images.

8.1.7. Exporting Results

Some image analysis data and measurement results can be transferred out of *NIS-Elements AR* so they could be used further. There is a standard *Export* pull-down menu in some of the control panels (intensity profile, histogram, measurement, etc...) which enables the export.
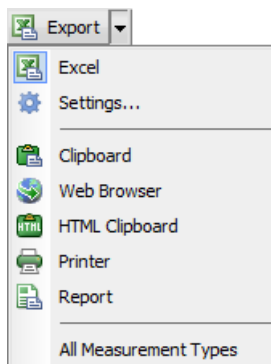
The set of commands of the menu differs according to the type of data (tables or graphs) which can be exported. Click the arrow button to display the pull-down menu. When you select the destination, the

pull down menu hides and the icon of the selected export type appears on the *Export* button. The export action is performed after the button is pressed once more.

Export Destinations

It is possible to export tabular data or a graph:

To MS Excel Tabular data can be exported to MS Excel. A new XLS sheet opens and the table is copied to it automatically.

To File Tabular data can be exported to an MS Excel sheet (xls) or a text file (tab delimited txt).

To Clipboard Data tables and graphs can be exported (copied) to MS Windows clipboard. Then the data or the image can be inserted into any appropriate application (text editor, spreadsheet processor, graphics editor) typically by the *Paste* command.

To Report Tabular data and graphs can be exported to *NIS-Elements AR* report. If a report is already opened for editing, the data/graph will be appended to it. Otherwise, a new report will be created and the exported data inserted to it.

To Web Browser, To HTML Clipboard Some tabular data can be exported as a HTML table. It can be displayed in a default Internet browser (the *Web Browser* option), or the HTML code can be copied to Windows clipboard - ready to be inserted to any HTML/text editor.

To Printer Tabular data can be printed directly. This option opens a standard *Print* dialog window where it is possible to select a printer and print the data.

Data Export Options

Run the *Options* command and switch to the *Data export* tab or press the *Settings* ⚙ button within the export pull-down menu. There are two tabs available:

Global Settings Options

Export into Microsoft Excel application Select this option if you want to export the data to MS Excel application. You can choose if the data will be exported into currently opened file or a new file will be created. Or you can set to export the data to a specific file. If this file does not exist, it will be created. Choose the path and name of the file to be created/opened.

Set the *Start at column* values (column and row indexes). These values mark the first cell in the MS Excel application which will hold the exported data.

Export text files into folder Select this item if you prefer to export the data to a text file. Press the *...* button and specify the target folder and the file name.

Data delimiter Pick up the type (tabulator, space, semicolon) of data delimiter which will be used when exporting data to MS Excel or clipboard.

Append exported data after previous exports If you check this item the existing data will not be over-written by the new data.

Export also column headers if not appending Include the titles of columns in the files which are being created or overwritten. If the *Append exported data after previous exports* is selected, no headers will be included in the export.

Activate target application after export If you check this item the exported file is opened in the default application (MS Excel or the default text editor) automatically.

Insert empty lines Check this item to allow insertion of empty lines in the exported data.

Export ROI data per channel If this function is checked, ROI data are exported for each channel separately.

Data export Options

You can modify the amount of data included in each export type.The control panels which contain the Export to file functionality are listed in the top pull-down menu. Select the control panel of which the export shall be modified.

Export Name A control panel such as A^+ *View > Analysis Controls > Annotations and Measurements* stores information about the measurement in multiple tables according to the measurement type (area, length, etc.). In this column, you can select the table you would like to modify.

Sheet name In this column you can define an arbitrary name which will be used to name a sheet or a txt file to which the data will be exported.

Options for: This section of the window enables you to select the items to be included/excluded from the selected table during export.

8.2. Manual Measurement

8.2.1. Introduction
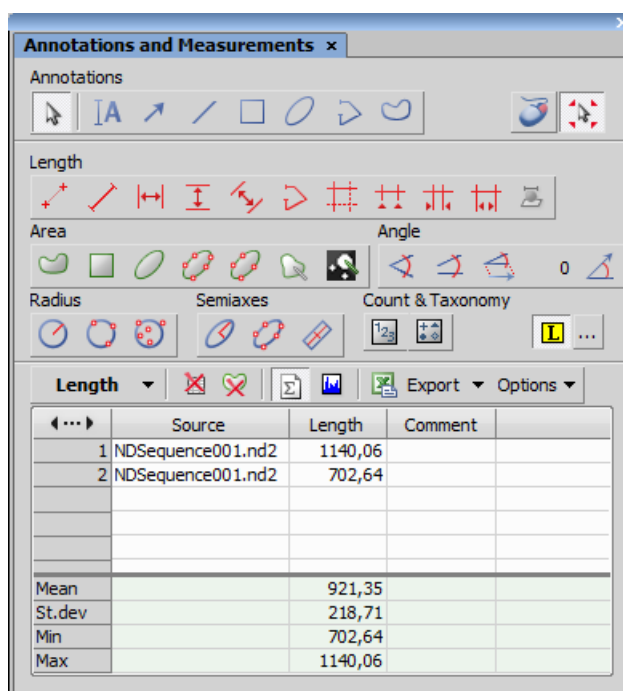
Length, area, angles, taxonomy, counts, circle radius, and ellipse semiaxes can be measured manually over an image. The results are being recorded to a simple statistics table, which can be exported to a file or clipboard. Also, the data can be presented as a graph.

1. Run the A^+ *View > Analysis Controls > Annotations and Measurements* command. The manual measurement control panel appears.

2. Select a tool corresponding to the feature you are going to measure. There are several tools for measuring each feature.

3. Measure the objects in the image using mouse.

4. Select where to export results in the *Export* pull-down menu.

5. Export the results using the *Export* button.

Measurement of the image of a grain:
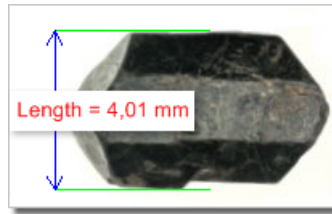
1) Select the *Vertical parallel lines* ⊥ tool.

2) Place the first line on the top edge of the crystal by clicking into the image. The position of the line can be adjusted while you hold the primary mouse button down. After you release it, the line is positioned.

3) Repeat this to place the second line on the bottom edge of the crystal.

4) When finished, an arrow is drawn between the lines, and the result of the measurement is attached. A record with the measurement type and the measured value is added to the results table.

8.2.2. Working with the Measurement and Annotation Objects

There are two types of vector objects which can be placed over the image: annotation objects and measurement objects.

A This button of the right image toolbar turns the visibility of annotation layer ON/OFF. Right-clicking the button displays a context menu with the following options:

- *Clear All Objects* will delete annotation and measurement objects.

- *Clear Annotation Objects* will delete all annotation objects.

- *Clear Measurement Objects* will delete all measurement objects.

- *Select Annotation Objects* will select all objects that are not the measurement ones.

- *Select Measurement Objects* will select all measurement objects.

- *Select All Objects* and *Deselect* will select/deselect all annotation and measurement objects.

Visibility of annotation/measurement objects In an ND document that contains a time dimension, you can set visibility of any annotation object. Right-click on an object and select *Object Visibility...*. In the opened dialog, you can choose that the *Object is Always Visible in Time* or that the *Object is Visible in a Time Range* specified in the edit boxes below. Current/First/ Last buttons fill in the current/first/last

value automatically. If you have already made a frame selection in the time line, use Get Range Selection . If you are working with a multi point document, you can also *Limit Object Visibility to the Current Multi Point Frame*.

8.2.3. Measurement Tools

8.2.3.1. Angle

Measure Free Angle Place the first line in the image, adjust its position, and finish it by right-click. Place the second line anywhere in the image, adjust its position, and finish it by right-click. The two lines connects to make the measured angle.

Measure 2 lines Draw the first line and then draw the second line. These lines do not need to visibly intersect. You can adjust their mutual position, then confirm the measurement by right-click.

Measure via Reference Angle Use the *Define Reference Angle* tool first. Then, select this tool, and define a line in the image. The angle between this line and the reference angle will be measured.

Define Reference Angle Draw a line in the image or put the precise value in degrees. Finish the definition by *OK* or the right-click.

8.2.3.2. Area

Polygon Draw a polygon by clicking the mouse inside the image, the secondary mouse button will place the last node of the polygon and finish the measurement.

Rectangle Draw a rectangle holding the primary mouse button down.

Ellipse The first step is to draw a circle holding the primary mouse button down. The second step is to stretch it to the shape of ellipse.

5-point ellipse - The system draws an ellipse interpolating five points defined by mouse.

4-point ellipse Draws an ellipse interpolating four points defined by mouse.

Auto Detect The system detects a homogeneous area around the clicked point. Tolerance (size of the area) can be adjusted by rolling the mouse wheel or the *PgDown/PgUp* keys.

Select binary Makes new interactive measurement object according to selected binary object.

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8.2.3.3. Length

2 Points Distance between two points. Use the primary mouse button to define the start and the end point.

Simple Line The line length. Use the primary mouse button to draw the line to be measured.

Horizontal The distance between two vertical lines. Use the primary mouse button to insert the line markers for the start and the end of the measured distance.

Vertical The distance between two horizontal lines. Use the primary mouse button to insert the line markers for the start and for the end of the measured distance.

Parallel Lines The distance between two parallel lines. Use the primary mouse button to draw the first marker. Adjust its position by mouse and click the right mouse button. Place the second line using the primary mouse button again. Press the right mouse button to complete the measurement.

Polyline The polyline length. A polyline consists of several line segments. Use the primary mouse button to draw the line segments. Each mouse-click creates a new node. Complete the total length definition by the right mouse button.

Crosses The distance between two crosses. The cross-like cursor helps to position the start and the end points of the measured distance.

Auto Default The distance between two points estimated by computer. The system detects two sharpest changes of the pixel intensities and places the points in there.

Auto Outer The distance between two points estimated by computer. The system detects several sharp changes of the pixel intensities and selects the first and the last one as the measurement points.

Auto Inner The distance between two points estimated by computer. The system detects several sharp changes of pixel intensities and selects the second and the next to the last one as the measurement points.

8.2.3.4. Length 3D

Length 3D This tool enables to perform interactive 3D measurement on nd2 files that include the Z dimension.

Measurement on Slices View

1. Open an nd2 file containing a Z dimension

2. Run the *View > Image > ND View > Slices View* command to display the image in *Slices View* .

3. Select the *Length 3D* tool, the cursor gets changed.

4. Select the Z position where the first point lies, place the crosshair, and click the right mouse button.

5. When the crosshair color changes, select the Z position of the second point and place the cursor there.

6. Confirm the measurement by right click. The measured value is written to the control window results table.

Measurement on focused Image and Surface View

(requires: Extended Depth of Focus)

1. Open an nd2 file containing a Z dimension and run the 🦋 *Applications > EDF > Create Focused Image* command.

2. You may switch to Surface View or stay in the Focused Image view that appears automatically. The 3D Measurement works the same way in both of them.

3. Invoke the 3D Measurement command. The rest of the procedure equals the interactive 2D measurement:

4. Define both points by mouse and the measurement is performed automatically using the EDF 3D surface model.

8.2.3.5. Radius

Circle Click into the image to define the center and define the perimeter holding the primary mouse button down. Adjust the size and finish the measurement by right-click.

3-points circle The circle is defined by three points inserted to the image by mouse.

N-points circle This tool creates the circle by interpolating any number of defined points. Finish the points definition by right-click.

8.2.3.6. Semiaxes

Ellipse The first step is to draw a circle holding the primary mouse button down. The second step is to stretch it to the shape of ellipse.

5-points ellipse The system draws an ellipse interpolating five points defined by mouse.

Free Rectangle Draw the rectangle in the image. If you would like to rotate it, select [Pointing Tool], and drag the green point above the top of the rectangle.

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8.2.3.7. Count and Taxonomy

Count Click inside the image several times. The positions and the number of clicked points is recorded and you see the actual count in the tooltip. Finish the measurement by right-click. The counted points are marked by colour in the image.

Taxonomy This tool enables to manually count objects of up to 70 classes.

1. Click the icon.

2. Right-click to the results table and select *Taxonomy Options* . A window appears.

3. In the window, define the number of classes (from 2 to 70) and the way of inserting markers:

> **On left button down** Inserts a marker to the current cursor position after a mouse click. If this option is selected, the function keys enable to switch between the classes. In this mode, the *Fn* keys works for switching between classes.

> **On Fn key at cursor position** After the *Fn* key is pressed, the count is performed and the corresponding marker is placed to the current cursor position.

> **On Fn key at screen center** After the *Fn* key is pressed, the count is performed and the corresponding marker is placed to the center of the image view.

> **On Fn key at cursor position, no marker inserted** After the *Fn* key is pressed, the count is performed and no marker is placed.

4. Start the count according to the *Taxonomy Options* setting.

> ---
> Note
> ---
> *The keyboard function keys F1 - F10 can be used as a shortcut for classification/class selection. Since there are 70 classes available, certain key combinations must be pressed in order to select/classify the right class:*
>
> | Key Combination | Class Number |
> |---|---|
> | Fn | n |
> | Ctrl + Fn | 10 + n |
> | Alt + Fn | 20 + n |
> | Shift + Fn | 30 + n |
> | Ctrl + Alt + Fn | 40 + n |
> | Ctrl + Shift + Fn | 50 + n |
> | Shift + Alt + Fn | 60 + n |

5. Perform the measurement until all classes are counted.

8.3. Automated Measurement

Automated measurement is the most powerful of image analysis features. In combination with user macros, *NIS-Elements AR* can become a semi- or fully-automatic image analysis tool. There are several key procedures which a proper automated measurement should involve:

1. Optical system calibration (see the 4.1.2. Optical Configurations chapter)

2. Image acquisition (see the 5. *Image Acquisition* chapter)

3. Defining threshold, creating the binary layer (see the 7.2.1. Thresholding chapter)

4. Selection of measured features. Viz 8.5. Measurement Features.

5. Performing the automated measurement

6. Results presentation

Image Layers Involved in Measurement

The binary layer and also the color image can get involved in the automated measurement:

- *Binary layer* - is typically used for shape and size measurements (area, perimeter, surface fraction).

- *Color layer* - intensity or hue measurements are carried out on this layer. The area covered with the binary layer is used as the source data.

Object/Field Measurement

NIS-Elements AR distinguishes two types of automated measurements: object and field.

Object Measurement Groups of neighbouring pixels of the binary layer are called objects. Results of the object measurement are usually the object data such as Length, Area, CentreX/Y (the X,Y position of the central pixel of an object), etc.

Field Measurement Field measurements produce information regarding the whole measurement area (measurement frame, ROI). Such information can be Area Fraction, Mean Brightness, Density Variation, etc.

Please see the complete list of measurement features in the 8.5. Measurement Features chapter.

Restricting the Area of Measurement

The area of measurement can be restricted by the measurement frame or by a user defined region of interest (ROI).

Region of Interest - ROI ROI is a user defined area of the image. Whenever the ROI is displayed (by the *View > Layers > ROI* command), it is also applied to restrict the area of measurement.

Measurement Frame The measurement frame is a re-sizable rectangular frame which serves for bounding the area accepted for measurement. Turn the measurement frame ON via the *Measure > Use Measurement Frame* command.

Binary objects touching edges of ROI or the measurement frame can be treated differently (excluded from the measurement or included). This behavior can be specified within the *Measure > Measurement Options* window.

Statistics and Data Presentation

NIS-Elements AR calculates basic statistics - mean value, standard deviation and distribution of all measured features. The features to be measured can be selected in the *Measure > Object Features* and the *Measure > Field and ROI Features* windows. Results of all automated measurements are presented and can be exported from the *View > Analysis Controls > Automated Measurement Results* control panel.

8.4. Time Measurement

The time measurement tool enables to record average pixel intensities within Regions Of Interest (ROIs) during a time interval. The measurement can be performed on Live camera signal or on a saved nd2 file. Before the measurement is started, regions of interest shall be defined and turned ON. When no ROI is defined, Time Measurement is performed on the whole image automatically.

> Note
>
> *Time measurement is not supported for multichannel ND documents containing a brightfield channel.*

Run the *View > Analysis Controls > Time Measurement* command to open the Time Measurement control panel.

Figure 8.17. Time measurement window

The control panel consists of three parts: measurement tools, ROI definition tools and data display.

8.4.1. Quick Guide

- Enable the Measurement ROI by the *View > Layers > ROI* command.

- Run the *Acquire > Live - Fast* command to display the live camera signal.

- Define ROI(s) within the image. Time measurement will be performed under the ROI areas. Use the *ROI > Simple ROI Editor* command or the *Define* toolbar button to open the vector ROI editor and use the *ROI > Raster ROI Editor* command to display the raster ROI editor. See 7.4.1. Introduction to ROIs.

- Once the ROIs are defined, press the ▷ *Measure* button - the measurement will start.

- When the current ND document contains combination of time dimension with Z dimension or multi-points, press the ⬛ᴺᴰ *Measure All* button to measure all frames across all multipoints and/or Z stacks at once.

- Press the ⬛ *Stop* button to finish the measurement.

- Export the results via the 📊 *Export* button. See 8.1.7. Exporting Results for further details.

- A snapshot of the graph will be created if you press the 📷 *Create Snapshot* button.

Note

If there are too many ROIs defined and the graph gets confusing, a single ROI (and the corresponding graph) can be highlighted. Right click the ROI name and select Bookmark this ROI from the context menu.

Click >> next to Color to reveal Mean and Maximal Intensity columns which are useful for filtering out ROIs/objects with insufficient intensity.

ROI	Color «	Mean Int.	Max Int. ▽
#4 - Standard	🟨 ▼	526,27	637,69
#2 - Background	🟩 ▼	477,33	559,97
#3 - Standard	🟦 ▼	460,43	532,14
#1 - Reference	🟥 ▼	397,03	413,57

8.4.2. Options

Click the *Options* ⚙ button. A dialog appears where you can specify the time measurement details:
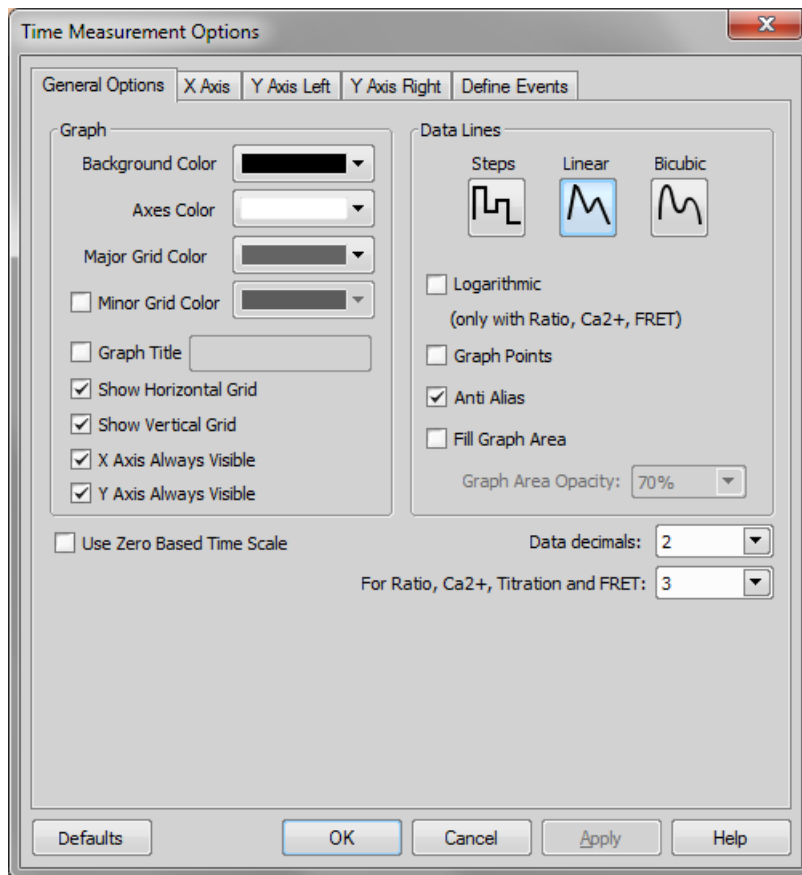
Graph Choose background, axes and grid colors from the palette. Check the *Graph Title* option to display a title, whose text you can enter in the adjacent field. Then check the *Show ... Grid* items if you want to make them visible in the graph. If *.. Axis Always Visible* item is checked, the axes do not leave the graph area while zooming in the graph.

Data Lines Select interpolation method or drawing the graph lines by pressing the relevant button. You can choose Steps (rough), Linear (smoother), or Bicubic (really smooth) interpolation method.

 Logarithmic Check to use logarithmic scale in the graph.

 Graph points Check this item to display data points. Small dots indicating the actual data values position can be displayed on the graph line. The points appear only if the distance between them is big enough for them to be recognized (they usually appear when you zoom in the graph).

 Anti Alias Turning this option ON will make the graph lines look smooth.

 Fill Graph Area Check this item to fill in the area under the line chart with a color. Select the amount of opacity used from the list of predefined values.

Show point coordinates Check this item to display or hide X, Y coordinates of pixels in the Data tab of the *Intensity Profile* control panel.

Display During Measurement Select one of two options:

Fit graph to measured data If you select this option, displayed area of graph during measurement is fit to measured data.

Fixed time range If you select this option, displayed area of graph during measurement is fixed to defined values. Then define time range of displayed area of graph.

Use Zero Based Timescale Select this option to ensure that the first frame of a time sequence will always start at 0.0s.

Data decimals Define decimal precision of the results in the data table.

For ratio, calcium titration and FRET Define decimal precision of data in data table for ratio, calcium titration and FRET.

X axis
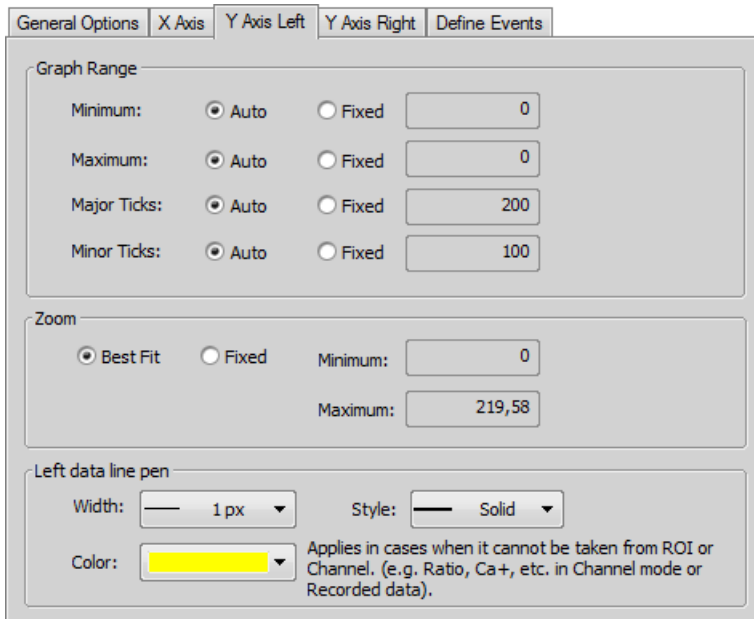
Graph Range The *Auto* option enables that the range values are set automatically. On the contrary the range values can be set on *Fixed* values also. The range values can be set for minimum of X axis, maximum of X axis, major and minor grid of X axis.

Zoom If you select the *Best Fit* option, zoom of the X axis will be set to best fit. Otherwise you can set the *Fixed* range.

Y axis left/right

The *Graph Range* and *Zoom* options are described above.

Left/Right Data line pen Set the line appearance - line color (in case color of image component is not set), thickness and style (solid, dot, dashed, dash-dot).

Define Events

This tab enables to define user hot keys which run special user events during time measurement. Simply click inside the *Hot key* field of the User event and press any key combination you want to assign to the event. Then you can arbitrary enter an event description. Finally select or enter command or macro which should be executed when you press the set hot key.
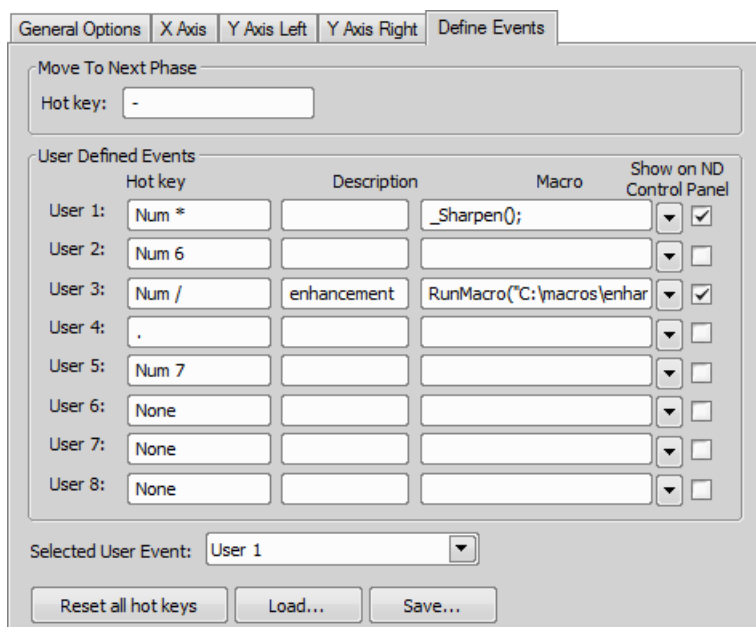
When these events are performed on a document, they are also displayed in the graph. These events are marked on the time axis and identified by their name. For more information see Special Options.

8.5. Measurement Features

The following list describes all the features that can be measured within *NIS-Elements AR* Measurements. There are the following. Different measurement tools measure different features.

OBJ Object features - measured under binary objects (layers)

ROI ROI features - measured within ROIs

FLD Field features - measured over the whole image area

MAN Features available in manual measurement

EDF EDF features - can be measured only after EDF focused Image was created.

3D 3D features - measured on 3D binary objects (a thresholded Z-series data set is needed)

TRC Tracking features - measured on tracked binary objects or ROIs.

Accel.N **TRC**

Normal acceleration is the part of acceleration vector that is perpendicular to the velocity vector incoming to the current object position.

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Accel.T `TRC`

Tangential acceleration having the same direction as the velocity vector.

Acceleration `TRC`

Total force that is acting on an object calculated using speed difference between the next and the current segment.

AcqTime `OBJ` , `ROI` , `FLD`

Calling the Grab Sequence command, the AcqTime is set to zero at the beginning. Then, *NIS-Elements AR* assigns the AcqTime to every newly grabbed image file header, which denotes time elapsed from the beginning of the grabbing. If the Grab Sequence command is not called, than the AcqTime is the time elapsed since the start of *NIS-Elements AR* .

Angle `MAN`

Angle between two user-defined lines. See 8.2.3. Measurement Tools.

Area `OBJ` , `MAN` , `TRC` *(requires: Advanced 2D Tracking)*

Area is a principal size criterion. In a non-calibrated system, it expresses the number of pixels; in a calibrated one, it expresses the real area.

Figure 8.23. Area
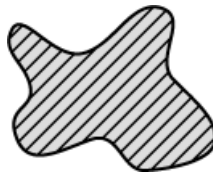
Axis A, Axis B, Axis A/B `MAN`

Lengths of the major (A) and minor (B) axes of an ellipse and their ratio. See 8.2.3. Measurement Tools.

BinaryArea `FLD`

Sum of areas of all binary objects.

BinaryAreaFraction `FLD`

$$BinaryAreaFraction = \frac{BinaryArea}{MeasuredArea}$$

BinID `TRC`

Binary layer identifier.

Blue MAN

Arithmetic mean of pixel intensities of the blue component.

BoundsAbsLeft, BoundsAbsRight, BoundsAbsTop, BoundsAbsBottom OBJ

These are absolute distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle within a motorized stage range. This feature can be measured only when a motorized stage is connected.

Figure 8.25. BoundsAbs
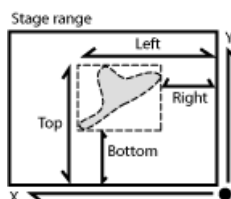
BoundsLeft, BoundsRight, BoundsTop, BoundsBottom OBJ

These are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle measured in the current calibration units. If the image is not calibrated, the coordinates are in pixels.

Figure 8.26. Bounds
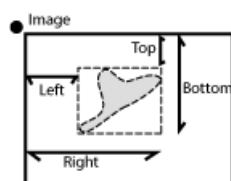
BoundsPxLeft, BoundsPxRight, BoundsPxTop, BoundsPxBottom OBJ

These are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle measured in pixels.

BrightVariation OBJ , ROI , FLD

It is the usual standard deviation of brightness values.

CentreX, CentreY OBJ , ROI , FLD , MAN

Coordinates of the center of gravity measured in the current calibration units. If the image is not calibrated, the coordinates are in pixels.

CentreXabs, CentreYabs `ROI` , `FLD`

Absolute coordinates of the center of gravity in the scope of the stage XY range. These features can be measured only when a motorized stage is connected.

CentreXpx, CentreYpx `OBJ` , `ROI` , `FLD`

Coordinates of the center of gravity measured in pixels.

Channel `OBJ` , `ROI` , `FLD` , `MAN`

Comma delimited list of channel names on which the measurement has been performed.

Circularity `OBJ` , `TRC` *(requires: Advanced 2D Tracking)*

Circularity equals to 1 only for circles; all other shapes are characterized by circularity smaller than 1. It is a derived shape measure, calculated from the area and perimeter. This feature is useful for examining shape characteristics.

$$Circularity = \frac{4 \times \pi \times Area}{Perimeter^2}$$

For tracking: *(requires: Advanced 2D Tracking)*.

Comment `OBJ` , `ROI` , `FLD` , `MAN`

This feature adds an editable column to the results table.

Convexity `OBJ`

Indicates convexity of the object edges.

$$Convexity = \frac{Area}{Convex\ Hull\ Area}$$

DensityVariation `OBJ` , `ROI` , `FLD`

DensityVariation is derived from density values. It is the usual standard deviation of density values.

Diameter `MAN`

Diameter of a circle measured manually. See 8.2.3. Measurement Tools.

Diff Coef `TRC`

The diffusion coefficient characterizes how a substance diffuses in time. It is a feature of a track. Having a track over *k* frames, we get *k* value pairs ((MSD_1, T_1), ..., (MSD_k, T_k)), from which *k* equations are created:

$$MSD_i = 2 \times n \times D \times T_i + E$$

D Diffusion coefficient

MSD Mean Squared Displacement of the object in each frame (time *T*)

n number of dimensions. 2 for 2D tracking, 3 for 3D tracking

T Time

Then, the method of *least squares* is used to calculate a single diffusion coefficient (*D*) from the set of equations.

Diff Coef R2 `TRC`

Indicates how well the calculated diffusion coefficient (Diff Coef) reflects the data. *Diff Coef R2 = 1* means that the *Diff Coef* matches the data. If *Diff Coef R2 = 0*, *Diff Coef* does not match the data at all.

$$Diff\,Coef\,R2 = 1 - \frac{SSres}{SStot}$$

$$SSres = \sum_{i=1}^{k}(MSD_i - f(T_i))^2$$

$$f(T) = 2 \times n \times D \times (T - \overline{T_i}) + \overline{MSD_i}$$

$$SSTot = \sum_{i=1}^{k}(MSD_i - \overline{MSD_j})$$

Duration `TRC`

Time duration of the track.

EdfRoughness `OBJ` , `ROI` , `EDF` , `FLD`

Based on the 3D EDF surface model, it indicates how much the surface is rough. Its value for a flat surface is *1*.

$$Edf\,Roughness = \frac{Edf\,Surface}{Area}$$

EdfRoughnessRA `OBJ` , `ROI` , `EDF` , `FLD`

Based on the 3D EDF surface model, it indicates how much the surface is rough. It is a sum of absolute values of differences between Z value of each pixel and mean of all Z values divided by Area. Its value for a flat surface is 0. It is the most widely used one-dimensional roughness parameter

$$Edf\,Roughness\,RA = \frac{1}{n}\sum_{i\in\Omega}|Z_i - EZ|$$

$$Area = |\Omega| = n \qquad EZ = \frac{1}{n}\sum_{i\in\Omega}Z_i$$

EZ The mean height of the Z-map (mean of all Z coordinates of the EDFSurface).

n, Area, Ω Number of pixels in the measured area (image/object/ROI).

Z_i EDFSurface Z coordinate of a particular pixel.

EdfSurface `OBJ` , `ROI` , `EDF` , `FLD`

Based on the 3D surface model, it is an approximation of the surface area. Its value for a flat surface = Area.

Figure 8.33. EdfSurface highlighted red, Area of four Edf surfaces highlighted yellow
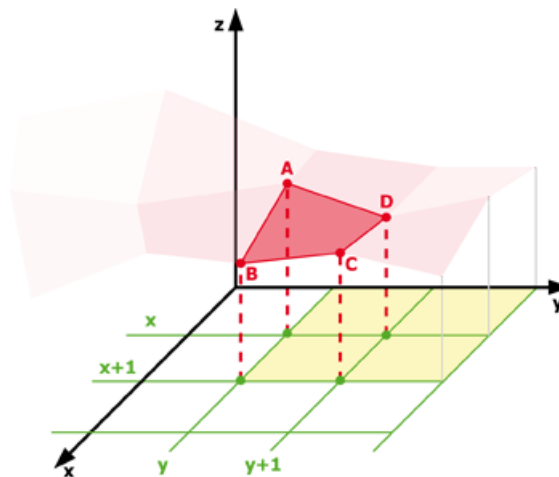
$$A(x, y, z1)$$
$$B(x + 1, y, z2)$$
$$C(x + 1, y + 1, z3)$$
$$D(x, y + 1, z4)$$

$$a = |AB|$$
$$b = |BC|$$
$$c = |CD|$$
$$d = |DA|$$

$$EdfSurface = \frac{a \times b}{2} + \frac{c \times d}{2}$$

$$EdfSurface \geq Area$$

Elevation `TRC`

Elevation is the angle between the direction of the velocity vector and the XY plane. Positive elevation is towards positive Z coordinates. The values range from -90 to 90.

(requires: Advanced 2D Tracking) and *(requires: 3D Measurement)*.

Elongation `OBJ` , `TRC` *(requires: Advanced 2D Tracking)*

Characterizes the object shape. Ratio of MaxFeret and MinFeret features.

$$Elongation = \frac{MaxFeret}{MinFeret}$$

Elongation `3D`

Characterizes the object shape. Elongation is calculated as a ratio of axes lengths.

$$ElongationM1M2 = \frac{Major}{Minor}$$

$$ElongationM2M3 = \frac{Minor}{Minor2}$$

$$ElongationM1M3 = \frac{Major}{Minor2}$$

EqDiameter `OBJ` , `MAN` , `TRC` *(requires: Advanced 2D Tracking)*

A size feature derived from the area. It determines the diameter of a circle with the same area as the measured object:

$$EqDiameter_{2D} = \sqrt{\frac{4 \times Area}{\pi}}$$

Figure 8.41. EqDiameter
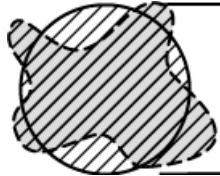
EqDiameter `3D`

A feature derived from the volume. It determines the diameter of a sphere with the same volume as the measured object:

$$EqDiameter_{3D} = \left(\frac{6 \times Volume}{\pi}\right)^{\frac{1}{3}}$$

ExPurity `MAN`

This feature specifies the amount of white in the measured color, if the measured color can be composed of white and a pure spectral color.

FieldID `OBJ`, `FLD`, `ROI`

Automatically generated field ID. Each measurement per field generates a new ID.

File Name `TRC`

Name of the source file.

FillArea `OBJ`

If an object contains holes, FillArea remains the same while Area is reduced by the area of the holes. In case an object does not contain holes, *FillArea = Area*.

Figure 8.43. FillArea
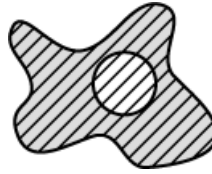
FillRatio `OBJ`

FillRatio is the ratio of Area and FillArea:

$$FillRatio = \frac{Area}{FillArea}$$

If an object does not contain holes the FillRatio is equal 1. If an object contains holes, FillRatio is less than 1. This feature can distinguish objects with and without holes.

Generation `TRC`

When track splitting is performed, this feature displays the generation of each track. The parent track has Generation = 1, tracks after first splitting have Generation = 2, etc.

Green `MAN`

Arithmetic mean of pixel intensities of the Green component.

Heading `TRC`

Angle between the direction of the velocity vector and the X axis. Zero degrees is in the direction of X axis (to the right) and increases counterclockwise until 360.

Height `MAN`

Projection of 3D length measurement to the Z axis. See 8.2.3. Measurement Tools.

HueTypical `OBJ` , `ROI` , `FLD`

HueTypical (H) is the hue value with maximum frequency in a hue value histogram. This feature describes the most frequent hue (color) in an object or field.

$$\alpha = \frac{1}{2}(2R - G - B)$$

$$\beta = \frac{\sqrt{3}}{2}(G - B)$$

$$H = atan2(\beta, \alpha)$$

HueVariation `OBJ` , `ROI` , `FLD`

HueVariation is the usual standard deviation of hue values. This feature describes hue (color) distribution of inner structure of an object or field.

$$\alpha = \frac{1}{2}(2R - G - B)$$

$$\beta = \frac{\sqrt{3}}{2}(G - B)$$

$$H = atan2(\beta, \alpha)$$

ID `TRC`

Unique binary object identifier.

IntensityVariation `OBJ` , `ROI` , `FLD`

IntensityVariation is derived from an intensity histogram. It is an usual standard deviation of intensity values. This feature describes the inner structure of an object or a field.

Length `OBJ` , `MAN` , `TRC`

Length is a derived feature appropriate for elongated or thin structures. As based on the rod model, it is useful for calculating length of medial axis of thin rods.

$$Length = \frac{Perimeter + \sqrt{(Perimeter^2 - 16 \times Area)}}{4}$$

Figure 8.48. Length

Width
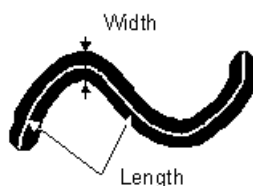
Length

Length xyz, Length xy `MAN`

3D length measured manually. *Length xyz* is the actual length measured on *Slices View* or *Volume View*. *Length xy* is length of a projection of the measured line to the XY plane. See 8.2.3. Measurement Tools.

LineLength `OBJ` , `TRC`

Line length is defined as the length of the object with elongated shape. For tracking it is the length of a straight line from the track origin to the current point.

LineSpeed `TRC`

Line Length divided by the time elapsed. It represents the speed of progression of a given object.

MaxFeret, MaxFeret90 `OBJ` , `MAN` , `TRC`

The MaxFeret is the maximal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle α equals the projected length of object at angle α , α (0,180); *NIS-Elements AR* calculates Feret's diameter for α =0, 10, 20, 30, ..., 180.

The MaxFeret90 is a length projected across the MaxFeret diameter.

Figure 8.49. MaxFeret

MaxFeret90

MaxFeret
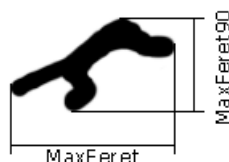
For tracking: *(requires: Advanced 2D Tracking)*.

MaxIntensity `OBJ` , `ROI` , `MAN` , `FLD`

MaxIntensity value is derived from the intensity histogram. It is the maximal of pixel intensity values (I).

$$I = \frac{1}{3}(R + G + B)$$

Mean Sq. Displacement `TRC`

Mean Square Displacement of the track calculated as a sum of squared distances from the beginning of the track.

Mean*ChannelName*, MeanRed, MeanGreen, MeanBlue `OBJ` , `ROI` , `FLD`

Arithmetic mean of pixel intensities of one image component.

MeanBrightness `OBJ` , `ROI` , `MAN` , `FLD`

Arithmetic mean of brightness values of pixels.

MeanChord `OBJ` , `ROI` , `FLD`

MeanChord is the mean value of secants in the 0, 45, 90 and 135 degrees directions. It is a derived feature and is calculated from the Area and mean projection according to the following formula.

$$MeanChord = \frac{4 \times Area}{(Pr_0 + Pr_{45} + Pr_{90} + Pr_{135})}$$

MeanDensity `OBJ` , `ROI` , `MAN` , `FLD`

Arithmetic mean of density values of pixels.

MeanIntensity `OBJ` , `ROI` , `MAN` , `TRC` *(requires: Advanced 2D Tracking)* , `FLD`

MeanIntensity value is derived from the intensity histogram. It is the arithmetic mean of pixel intensities (I).

$$I = \frac{1}{3}(R + G + B)$$

MeanIntensity `3D`

Arithmetic mean of intensities inside the object.

$$MeanInt = \frac{\frac{SumIntensity}{Volume}}{VoxelVolume}$$

MeanRatio, MeanCa2+, MeanCorrFRET, MeanFRETEff, MeanTitration `OBJ` , `ROI` , `FLD`

Arithmetic mean of intensities of the *Ratio / Ca2+ / Corr FRET / FRET Eff / Ph* channel. This feature is activated upon creation of the channel view.

MeanSaturation `OBJ` , `ROI` , `FLD`

Arithmetic mean of saturation values of pixels (S).

$$M = max(R, G, B)$$
$$m = min(R, G, B)$$
$$C = M - m$$

$$S_{HSI} = \begin{cases} 0, & \text{if } C = 0 \\ 1 - \frac{m}{I}, & \text{otherwise} \end{cases}$$

MeasuredArea `OBJ` , `ROI` , `FLD`

MeasuredArea value is the area of the measurement frame or of a mask within the measurement frame, if the mask status is on.

MinDistance `3D`

Distance between the centroid of the object and the centroid of the nearest object.

MinFeret `OBJ` , `MAN` , `TRC`

The MinFeret value is the minimal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle α equals the projected length of object at angle α , α (0,180); *NIS-Elements AR* calculates Feret's diameter for α =0,10,20, 30, ..., 180.

For tracking: *(requires: Advanced 2D Tracking)*.

MinIntensity `OBJ` , `ROI` , `MAN` , `FLD`

MinIntensity value is derived from the intensity histogram. It is the minimum of intensity values of pixels.

Figure 8.56. Calculation of Intensity (I)

$$I = \frac{1}{3}(R + G + B)$$

ND.M `FLD` , `ROI` , `TRC` , `MAN`

Number of the Multipoint.

ND.T `FLD` , `ROI` , `TRC` , `MAN`

Frame index in time.

NearestObjDist `OBJ`

The smallest distance to another object (measured between centers of gravity).

> **Note**
>
> *If ROIs are used during the measurement, NearestObjDist is calculated only between the objects within the specific ROI (objects having the same ROI ID).*

No.Segments `TRC`

Number of segments in a track.

NumberObjects `FLD` , `ROI`

NumberObjects value returns the number of objects in the measurement frame. Exclusion rules for counting objects are taken into account.

NumberObjectsRestricted `FLD`

Number of objects left after applying restrictions.

Numbers `MAN`

Number of points clicked within one *Count* measurement. See 8.2.3.7. Count and Taxonomy

ObjectAreaFraction `ROI`

$$ObjectAreaFraction = \frac{BinaryArea}{ROIArea}$$

ObjectsArea `ROI`

Area of all binary objects within ROIArea.

ObjID `OBJ`

Binary object ID. Each binary object has a unique ID

Orientation `OBJ` , `3D`

> **Orientation** `OBJ` Orientation is the angle at which Feret's diameters have their maximum. The diameters are calculated with 5 degrees angle increment.

Figure 8.57. Orientation
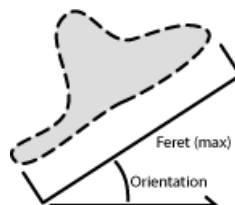
Orientation `3D` Angle (O) between the X axis and the projection of the Major (M) axis in the XY plane. Orientation [0, 180).

Figure 8.58. Orientation (O) and Pitch (P)
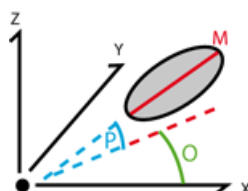
OuterPerimeter `OBJ`

Length of the outer perimeter (unlike in *Perimeter*, holes inside the object do not count).

PathLength `TRC`

Sum of the segment distances from the first frame to the current frame.

PathSpeed `TRC`

Length of the path divided by the amount of time elapsed from the beginning to the current position.

Perimeter `OBJ` , `ROI` , `MAN` , `FLD` , `TRC`

Perimeter is the total boundary length. It includes both the outer and inner boundary (if there are holes inside an object). The perimeter is calculated from four projections in the directions 0, 45, 90 and 135 degrees using Crofton's formula

$$Perimeter = \frac{\pi \times (Pr_0 + Pr_{45} + Pr_{90} + Pr_{135})}{4}$$

Figure 8.59. Perimeter

For tracking: *(requires: Advanced 2D Tracking)*.

PerimeterContour `OBJ`

Total boundary length computed from object contours. Compared to *Perimeter*, it is more accurate (especially on elongated objects) but slower to get the result.

Pitch `3D`

Angle between the Major axis and its projection in the XY plane. Pitch [0, 90].

Figure 8.60. Orientation (O) and Pitch (P)
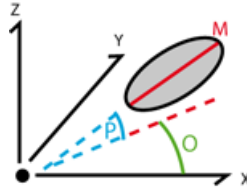
PositionX, PositionY, PositionZ `TRC`

X,Y,Z coordinates of the object's centroid.

Position Z: (requires: Advanced 2D Tracking), (requires: 3D Measurement).

Radius `MAN`

Radius of a circle measured manually. See 8.2.3. Measurement Tools.

Red `MAN`

Arithmetic mean of pixel intensities of the Red component.

RefLength `3D`

Distance from the object centroid to the reference object. Any of the 3D object can be set as reference from context menu in the *Measure > 3D Object Measurement* window.

RefLineLength `TRC`

Length of a straight line from the Reference Point defined by the user to the current point.

RefObjLineLength `TRC`

Length of a straight line from the Reference Object (e.g. Track) defined by the user to the current point.

ROIArea `ROI`

Area of each ROI measured in the calibration units.

RoiID `OBJ` , `ROI`

ROI ID. Each ROI has a unique ID.

Roughness `OBJ` , `MAN`

This feature indicates how much the object is rough. "1" means the object roughness is minimal (the object is circular). The feature may acquire values in range <0;1>.

$$Roughness = \frac{Convex\ hull\ perimeter}{Perimeter}$$

RoughnessInf `OBJ`

This feature indicates how much the object is rough. "1" means the object roughness is minimal (the object is circular). The feature may acquire values in range <1;inf>.

$$RoughnessInf = \frac{1}{Roughness}$$

Seg.Length `TRC`

Length between two neighboring frames.

ShapeFactor `OBJ` , `MAN`

This parameter is to define whether the object is rough or not.

$$ShapeFactor = \frac{4 \times \pi \times Area}{Convex\ hull\ perimeter^{\,2}}$$

Figure 8.64. ShapeFactor
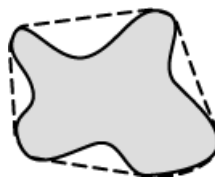
Source `OBJ` , `FLD` , `ROI` , `MAN`

Name of the measured image file.

Speed `TRC`

Segment length divided by the amount of time elapsed between the two positions.

Sphericity `3D`

Similarity to a sphere, computed as the ratio of the object surface to the surface of a sphere with matching volume. A sphere has the maximum Sphericity = 1.

$$Sphericity = \frac{\sqrt[3]{\pi(6V)^2}}{S}$$

V = volume, *S* = surface

StartX, StartY `OBJ`

When you scan the image from the origin as indicated (left->right), the first pixel of the object you hit is the one with StartX and StartY coordinates.

Figure 8.66. StartX, StartY
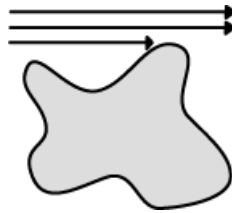
StartXpx, StartYpx `OBJ`

StartX and StartY coordinates measured in pixels (see above).

StgPosX, StgPosY `FLD` , `ROI`

StgPos features are coordinates of the absolute position of the measured field. It is available only for systems equipped with an XY stage.

Figure 8.67. StgPos
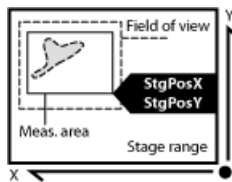
Straightness `TRC`

Feature which shows overall straightness of the object trajectory calculated by formula: End Points Distance / Trajectory Length. Values range is 0.0 - 1.0.

Sum*ChannelName* `OBJ` , `ROI` , `FLD`

The sum of intensity of all pixels of the object/ROI/field.

SumBrightness `OBJ` , `ROI` , `MAN`

SumBrightness is defined as the sum of brightness in every pixel of the object.

SumDensity `OBJ` , `ROI` , `MAN` , `FLD`

SumDensity is the sum of individual optical densities (O.D.) of each pixel in the area being measured. This feature describes, for instance, the amount of a substance in biological sections. Optical density is evaluated according to the following formula:

$$O.D. = -log\frac{PixelIntensityValue + 0.5}{MaxIntensityValue}$$

SumIntensity `OBJ` , `ROI` , `3D` , `FLD` , `MAN` , `TRC` *(requires: Advanced 2D Tracking)*

Sum of intensity in every pixel/voxel of the object.

Surface `3D` , `TRC` *(requires: Advanced 2D Tracking)*

Surface of the object.

SurfVolumeRatio `ROI` , `FLD`

SurfVolumeRatio is a feature with a strong stereological interpretation: if you measure on fields that are sampled systematically and independently of the content of the sections, then the feature is an unbiased estimator of the surface area of objects (inner structure) per volume of the whole sample.

$$SurfVolumeRatio = \frac{\frac{4}{\pi} \times Perimeter}{MeasuredArea}$$

Time `TRC` , `MAN`

> **Time** `TRC` Time assigns the time to a field (object) after a measurement has been performed. See the `SetReferenceTime` function help.

> **Time** `MAN` Time interval measured on a 1DT document. See also Velocity.

TrackID `TRC` , `OBJ`

(requires: Object Tracking)

Objects measured with the Object Tracking module provide this feature. If branching of tracks is allowed, each branch has its own *TrackID*.

Figure 8.70. TrackID
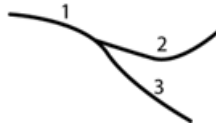
Type `TRC` *(requires: Advanced 2D Tracking)*

Name of the binary layer (created by thresholding) or ROI with its number.

Velocity `MAN`

This feature can be measured on a 1DT image using length measurement tools. 1DT image can be created from a timalapse ND file.

1. Open a timelapse ND2 file and run *View > Image > ND View > Slices View* .

2. Rightclick one of the side views and select *Create New Document from this View*. A new image will be created.

3. Perform length measurement on the new image.

Volume `3D` , `TRC` *(requires: Advanced 2D Tracking)*

Volume of the object.

$$Volume = (number\ of\ voxels \times voxel\ volume)$$

VolumeEqCylinder `OBJ`

This parameter is based on the rod model. Length is interpreted as height and Width as the base diameter of a cylinder. Bases are considered to be spherical.

$$VolumeEqCylinder = \frac{(\pi \times d^2)(l - d)}{4} + \frac{\pi \times d^3}{6}$$
$$l = max(MaxFeret, Length)$$
$$d = min(MinFeret, Width)$$

Figure 8.73. VolumeEqCylinder
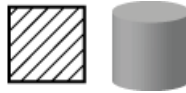
VolumeEqSphere `OBJ`

Volume of a sphere whose diameter is equal to EqDiameter of the measured 2D object.

$$VolumeEqSphere = \frac{\pi \times EqDiameter^3}{6}$$

Figure 8.74. VolumeEqSphere
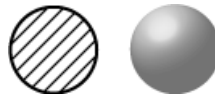
Width `OBJ` , `TRC` *(requires: Advanced 2D Tracking)*

Width is a derived feature appropriate for elongated or thin structures. It is based on the rod model and is calculated according to the following formula:

$$Width = \frac{Area}{Length}$$

Figure 8.76. Width
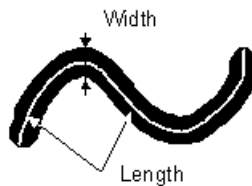
X, Y, Z `3D`

Coordinates of the binary centroid.

9. Creating Reports

9.1. Report Generator

Report Generator allows the user to create a print-ready document which can contain measured data, images, graphs etc. Once you start it by the ▤ *File > Report > New Blank Report* command, a graphical editor appears.
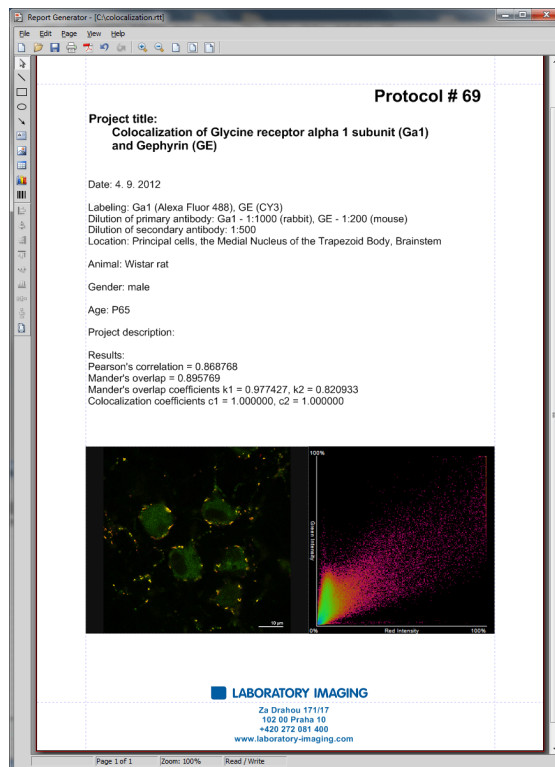
Creating of a report in *Report Generator* is very similar to creating any document in a common text editor. You can insert text fields, simple shapes, images, tables or graphs. As well as in text editors, you can create templates containing objects which do not change such as headers and footers, but also dynamic objects to be filled with data (e.g.: image name, author, etc) filled upon creation of the report. This comes especially handy in connection with the *Database* module where database fields are loaded to the report(s) automatically.

9.2. Report Objects

A report is composed from report objects. In the left toolbar, there are tools for inserting different objects.

1. Select one of the tools

2. Click and move the mouse inside the report to determine the area reserved for the object. Release the mouse button.

3. Double-click the report object to open a window where its properties can be adjusted.

4. Confirm the changes by OK.

> **Tip**
>
> *Vector objects (report objects, annotations, interactive measurement objects, ROIs) can be copied by "drag and drop" while holding the* `Shift` *key down.*
>
> *1. Select the object(s).*
>
> *2. Press* `Shift` *and drag the object somewhere.*
>
> *3. A copy of the object is placed to where you release the mouse button.*

9.2.1. Aligning Objects

Align Objects

You can align two objects to the same horizontal or vertical level.

1. Select more objects (e.g. by holding down CTRL and clicking with the primary mouse button).

2. Right-click one of the selected objects and select the *Align or Distribute > Align …* command from the context menu.

3. The objects will be aligned as indicated on the command icon.

4. If the *Relative to Page* ( ) option is turned *ON*, the objects will be aligned to the edges or the center of the page.

Distribute Objects

Objects of similar size can be distributed uniformly in the horizontal/vertical direction.

1. Select three or more objects.

2. Right click one of the objects and select the *Align or Distribute > Distribute…* command from the context menu.

3. Distances between the objects will be adjusted to equal.

4. If the *Relative to Page* option (⬜) is turned *ON*, the outer objects will be moved to the edges of the page.

Match Object Sizes

Sizes of objects can be unified too.

1. Select two or more objects.

2. Right-click the "master" object to the size of which you want to resize the other objects.

3. Select one of the *Resize Objects* sub-menu commands.

9.2.2. Dynamic Data

Results of automatic-interactive measurement, graphs, or the current image can be inserted to reports.

1. Insert an object which can contain dynamic data (text, picture, table, or graph).

2. Right-click the object and select *Insert Dynamic Data/Insert Dynamic Picture* from the context menu.

3. A window appears.

4. Select one of the available sources, and click *Next* .

5. Finish the source definition and click *OK* . The data appear on the report page.

List of Available Dynamic Data Sources:

• *Data inserted by user* (*available for: image, text, table*) - the system will ask you to type a text or browse for an image to insert during the report creation. When creating a dynamic object of this type, a query text which will be used to prompt you for the data can be defined.

• *System data* (*available for: text, table*) - enables you to insert some general data such as date, the name of the user account currently logged in, the page number, or the page count.

• *Macro* (*available for: text, table*) - enables you to insert expressions, values, or results of a macro.

• *Measurement* (*available for: text, table, graph*) - enables you to insert results of automatic/interactive measurement.

• *Database* (*available for: image, text, table*) - this data source is displayed only when creating a database report template (see below). It enables you to insert a link to any of the database records.

The real purpose of the dynamic data turns up when creating reports using report templates.

9.3. Report Templates

A report template is a layout defining the appearance of future reports, which is ready to be filled with data. The data can be inserted by the user "on demand" or automatically (dynamic data).

To Create a Report Template

1. Run the *File > Report > New Template* command. An empty report appears.

2. Edit the report template in the same way as a common report.

3. Insert the dynamic data where it is appropriate - measurement results to text boxes or tables, the current image to the image field, etc.).

4. Save the report template (*.rtt) via the *File > Save* command.

To Create a Report FROM Template

1. If the template is opened inside the Report Generator, run the *File > Create Report* command. Otherwise, use the *File > Report > New Report from Template > Browse* command inside the main application window to open a template from harddisk.

2. A report opens and the dynamic data is inserted automatically.

3. Save the report, print it, or export the page(s) to PDF using the commands from the *File* menu.

10. Macros

10.1. Creating Macros

A macro - an executable sequence of commands - can make the work very effective. *NIS-Elements AR* provides a C-like programming language utilizing its internal set of functions. The sequence of functions can be created either by recording the performed actions, by writing the functions within the macro editor, or by modifying the command history (the history is recorded automatically during the work). The macro can be saved to an external (*.mac) file for later use.

Recording a Macro

The fastest way to create a macro is to record it.

- Start the *NIS-Elements AR* macro recorder by selecting the *Macro > Record* command.

- Perform the series of actions you would like to record.

- Finish the recording by the same command again (its name changes to "Stop Recording")

- It is recommended to check the macro in the macro editor before saving. Run the ⬚ *Macro > Edit* command to display it.

- Save the macro to a file via the *Macro > Save As* command.

Creating a Macro from History

You can create a macro using the list of recently performed commands.

1) Run the *Macro > History* command to display the *Command History* control panel:
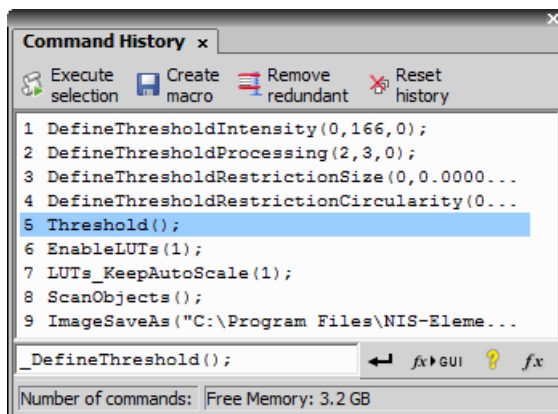
2) Push the *Create Macro* button.

3) Select what portion of the command history will be used in the macro:
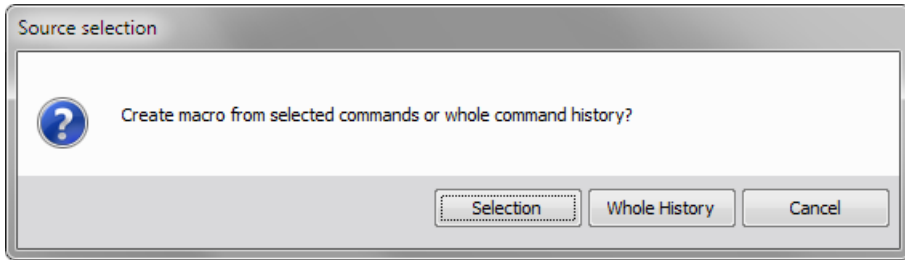
Selection This option will use only commands which you have previously selected by mouse within the *Command History* window. Group selection (holding `Shift` or `Ctrl`) is available.

Whole History All commands listed in the *Command History* window will be used for the new macro.

4) After you selected one of the options, the macro editor appears containing the new macro source code. Use the editor to fine-tune it.

Note

The Remove Redundant command is automatically performed before the macro is opened in the editor.

Writing/Editing a Macro

If you posses programmers skills, a macro can be written by hand in the built-in macro editor. Run the *Macro > Edit* command to display it.
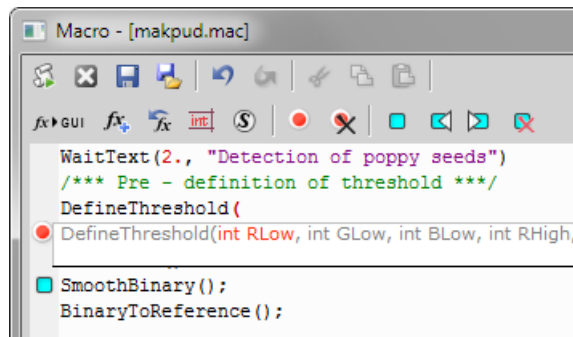
The macro editor provides:

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- Undo/Redo functionality.

- Command insertion from the list of available commands.

- Interactive command names list. Press `Ctrl+Space` to display a simplified list of all commands while typing the macro.

- Syntax hints (parameter types and names) appear as you type a command name.

- Bookmarks ⬜ can be placed to the code so you can easily roll to the important parts of the macro.

- Breakpoints 🔴 can be placed to the code. A breakpoint forces the macro to stop the execution at a certain point so you can check the state of variables and therefore inspect the macro functionality thoroughly.

- Syntax highlighting.

- Help on commands with detailed description.

- Use the *Save Macro* 💾 and the *Save Macro As* 🗂 commands in the macro editor to save the created macro.

  NIS-Elements automatically remembers the folder from where the macros were run the last time and will open this folder when user clicks on Open/Save command in Macro menu.

10.2. Running a Macro

NIS-Elements AR provides several ways to run a macro.

- You can run the current macro loaded to *NIS-Elements AR* by choosing the 🏃 *Macro > Run* command or by pressing *F4*.

- A *hotkey* combination (Ctrl+Alt+1,2,3 ... 9) can be assigned to it in the ⚙ *Macro > Macro Options* window.

- You can run a macro at the beginning of the *NIS-Elements AR* session, by assigning the *StartUp* flag to the macro in the ⚙ *Macro > Macro Options* window.

- You can run a macro saved to a disk by using the *Macro > Run From* command.

- A toolbar button can be assigned to run a macro. See 3.6. Modifying Toolbars.

Breaking the Macro

The macro execution can whenever be stopped by pressing the `Ctrl+Break` key shortcut.

10.3. Macro Language Syntax

Specifies the *NIS-Elements AR* Macro Language features.

10.3.1. Variable types

The following data types are implemented:

char text

char8 <-128, 127>

byte <0, 255>

int <-32768, 32767>

word <0, 65535>

long <-2 147 483 648, 2 147 483 647>

dword <0, 4 294 967 295>

double <1.7E +/- 308 (15 digits)>

10.3.2. Structures and Unions

Structures and unions are not supported.

10.3.3. Arrays

One and two dimensional arrays are supported.

10.3.4. Local and Global Variables

You should declare local variables at the beginning of macro or function only. You should declare global variables only at the beginning of macro. You can run two nested macros declaring the same global variables, but they must be of the same type. You can declare global variables by prefixing the declaration *global*. Eg.:

```
global int Number_Rows;
global char buffer[200];
```

The "global" keyword in front of the variable definition assigns the variable to the global scope. Such variable is then accessible from all function scopes within the macro interpreter.

10.3.5. Statements

Supported Statements

for The *for* statement lets you repeat a statement a specified number of times.

```
Syntax
```

```
for([init-expr]; [cond-expr]; [loop-expr])
        statement
```

First, the initialization (init-expr) is evaluated. Then, while the conditional expression (cond-expr) evaluates to a non zero value, Statement is executed and the loop expression (loop-expr) is evaluated. When cond-expr becomes 0, control passes to the statement the following the for loop.

while The *while* statement lets you repeat a statement until a specified expression becomes false.

```
Syntax
```

```
while(expression) statement
```

First, the expression is evaluated. If expression is initially false, the body of the while statement is never executed, and control passes from the while statement to the next statement in the program. If expression is true (nonzero), the body of the statement is executed and the process is repeated.

if, else Conditionally executes a statement or group of statements, depending on the value of an expression.

```
Syntax
if(expression) statement1
    [else statement2]
```

The *if* keyword executes statement1 if expression is true (nonzero); if *else* is present and expression is false (zero), it executes statement2. After executing statement1 or statement2, control passes to the next statement.

goto Transfers control of the program execution.

```
Syntax
```

```
goto name;
    . . .
    name: statement
```

You cannot use goto to jump inside the block from outside. E.g. following is not allowed:

```
goto label;
    if(k>5)
      {
    label:
        DilateBinary(3, 5);
```

```
    FillHoles();
    }
```

You cannot use goto to jump out from the block more then 2 block levels down. E.g. the following is not allowed:

```
for(i=0; i<64; i=i+1)
    {
    for(j=0; j<64; j=j+1)
        {
        if(a[i] > b[i])
            {
            value = i;
            goto end;   // crossing 3 right brackets
            }
        }
    }
  end
```

break Terminates the execution of the nearest enclosing statement.

```
Syntax
```

```
break;
```

The *break* keyword terminates the execution of the smallest enclosing *for* or *while* statement in which it appears. Control passes to the statement that follows the terminated statement.

continue Passes control to the next iteration of the statement in which it appears.

```
Syntax
continue;
```

The *continue* keyword passes control to the next iteration of the *for* or *while* statement in which it appears. Within a while statement, the next iteration starts by reevaluating the expression of the while statement. Within a for statement, the first expression of the for statement is evaluated. Then the compiler reevaluates the conditional expression and, depending on the result, either terminates or iterates the statement body.

Statements which are not supported

do, switch, case, default, typedef These statements are not supported.

10.3.6. Directives

The following directives are supported by the system:

define The #*define* directive assigns a meaningful name to a constant in a program.

```
//Syntax

    #define identifier token-string
```

The directive substitutes token-string for all subsequent occurrences of the identifier in the source file. Token-string can be a value or a string (only for 32-bit version of *NIS-Elements AR*).

```
//Example

    #define ERROR_SPRINTF 0
    #define MAINDIR "c:\Images"

    int main()
    {
    char buf[256];
    int retval;

    retval = sprintf(buf, "%s", "MAINDIR");
    if(retval == ERROR_SPRINTF)
    Beep();
    else WaitText(0., buf);
    return TRUE;
    }  \
```

include Specifies the name of the file to be included.

```
//Syntax

    #include filename
```

The #*include* directive includes the contents a file with a specified filename in the source program at the point where the directive appears.

```
Example
    // if you do  not specify the full path, NIS-Elements assumes, that \
it is a relative path to a main directory

    #include "macros\my_macro.h"
    #include "c:\NIS-Elements\macros\my_macro1.h"
```

import The #*import* directive is used to incorporate information from an external library.

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```
//Syntax

    #import("DLLname");
    #import function_declaration
```

NIS-Elements can call functions from external DLL's. You should import the DLL, where the functions resides and then make a declaration of the functions. You should not import the following system DLL's: kernel32.dll, user32.dll, gdi32.dll, com32.dll, comdlg32.dll. This feature is available only for 32 bit version of *NIS-Elements*.

```
//Example

    #import("luc_13.dll");
    #import int RTF_ReplaceVariables(LPSTR destfile, LPSTR sourfile);
    #import int RTF_FindQuestion(LPSTR sourfile, LPSTR question, long \
*length, LPSTR defvar);
    #import int RTF_ReplaceQuestion(LPSTR destfile, LPSTR sourfile, LPSTR \
replacement);
```

__underC The *__underC* directive assigns a function to be interpreted by the UnderC engine instead of the standard interpreter.

```
//Syntax
//works only on 32bit operating systems

__underC int inter_sharpen(int cols, int rows) { }
```

#importUC The *#importUC* directive imports an API function to the UnderC engine so that it can be used from within there.

```
//Syntax
//works only on 32bit operating systems

#importUC DisplayCurrentPicture;
```

10.3.7. Operators

The following operators are supported by the system. If an expression contains more than one operator, the order of operations is given by the priority of operators. Following operators have higher priority then the others: */ * %*. You should use brackets to define evaluation order other than implemented in *NIS-Elements*, which is: from right to the left.

arithmetic operators

+ Addition

- Subtraction

__*__ Multiplication

/ Division

assignment operators

The assignment operator assigns the value of the right operand to the left operand.

= Addition

bitwise operators

The bitwise operators compare each bit of the first operand to the corresponding bit of the second operand. The following bitwise operators are supported.

& Bitwise AND

| Bitwise OR

~ One's complement

pointer operators

& Address of ?

__*__ Indirection

relational operators

< Less than

<= Less than or equal to

> Greater than

>= Greater than or equal

== Equal

!= Not equal

logical operators

The logical operators perform logical operations on expressions. The following logical operators are supported:

&& Logical AND

|| Logical OR

! Logical NOT

10.3.8. Expression evaluator

Expression evaluator supports the precedence of operators ∕ * %. It evaluates the expression strictly from right to left, so you need to use brackets.

10.3.9. C-like functions

The system can interpret your own C - like functions. An entry point to program is the main() function in your macro. If main() is not presented, for backward compatibility, the body of the macro is also considered as "main function". The new macros should use main() as an entry point.

The general C-like functions (also called interpreted functions, as opposed to basic, system functions from *NIS-Elements* or LUC32_1.DLL) have the following syntax.

```
Syntax

    int MyFunction(int a, LPSTR str, double d)
    {
    int retval;
    . . .
    return retval;
    }
```

Return Value The return value can be any basic data types (char, int, word, dword, int, double or pointer).

Parameters Parameters can be any basic data types (char int, word, dword, int, double or pointer).

> Note
>
> *Int and double parameters are automatically converted to string type, when they are assigned to text variable, or are one of the parameters of a function.*

```
Example
    int main()
    {
    char buf[256];
    my_function1(buf);
    WaitText(0., buf);
    return TRUE;
    }

    int my_function1(char *buf)
    {
    strcpy(buf, "This function has a pointer to char array as a \
```

```
parameter");
    return TRUE;
}
```

10.4. Controlling Cameras by Macro

Every camera can be controlled from within a macro by changing its properties. There is a CameraGet_*
and CameraSet_* macro function available for each camera property (e.g. CameraGet_ExposureTime();).
The number of functions (beginning with CameraSet_ and CameraGet_) and their actual names depend
on the type of camera(s) you have currently connected to *NIS-Elements AR*.

CameraGet_ The *CameraGet_...* functions retrieve current values of the properties determined by the
function name postfix. So the `CameraGet_Exposure(int Mode, double *Exposure);`
function retrieves the current value of Exposure of the specified Mode.

CameraSet The *CameraSet_...* functions allows you to adjust camera properties. So the `CameraSet_Exposure(int Mode, double *Exposure);` function sets a new Exposure value for the specified
Mode.

How To Set Camera Properties

Instead of describing each property function, it is better to show you a universal way to handle a camera
by macro:

1) Display the *View > Acquisition Controls > Mono Sim Settings* control panel and find the property
you would like to control.

2) Make a change of the property from within the control panel. For example, change the exposure
time.

3) Display the *View > Macro Controls > Command History* window and see the last function which
has been called and its parameters (e.g. CameraSet_ExposureTime(1, 500);). This is the function
you will control the exposure time with.

4) Experiment with the camera control panel in order to determine the right parameter values of
the functions to be called within your macro.

10.5. Macro Preferences

This window enables you to configure key shortcuts to macros and set a macro to run automatically on
startup. Run the ⚙ *Edit > General Options* command and switch to the *Macro* tab.

Macros Lists of macros that can be executed using hot keys or automatically at the beginning of the *NIS-Elements AR* session.

> **Start Up** To launch a macro automatically after starting the *NIS-Elements AR* program, select it and press the StartUp button. StartUp field of the selected macro in the list box is filled.

> **Hot Key** To assign a hot key to a macro, select the macro in the macro list box and press the Hotkey button. Select one of the pre-defined key combination and press OK button.

> **Full Path** Shows the full pathname of the selected macro.

> **Add** Adds a macro to the macro list box. Select Macro dialog box appears and you can search the disk for the macro.

> **Remove** Removes a selected macro from the macro list box.

> **Edit** Enables to edit a selected macro from the macro list box.

Filename substitution These lines display the current values of substitution strings available in macro commands.

> **#** Letter representing a drive e.g. "C"

> **##** A customizable path e.g. "C:\Images"

> **###** Path to a directory according to the macro function used. It leads to the folder where *NIS-Elements* EXE file is placed and to a subdirectory - "Images" for functions concerning image files or "macros" for functions concerning macros. E.g.:

```
ImageOpen("###\agnor.tif");        //leads to the IMAGES subdir
RunMacro("###\macro_001.mac");     //leads to the MACROS subdir
```

> **####, #####** The current filename including/excluding the path. These substitutions can be used with the **Sequences** or **SequencesEx** macro commands.

Information Shown in Caption Optionally, the name of the current macro and an arbitrary user text can be displayed in the *NIS-Elements AR* caption.

Defaults for this Page Restores the default setting for Macro Options.

11. Movies

11.1. Capturing AVI Movie

1. Display the AVI Acquisition control panel by the *Camera > AVI Acquisition* command.

2. Adjust the advanced settings, especially define the file name and the destination folder.

3. Click the *Record* button. The live image appears and recording starts according to the settings.

4. Stop recording by the *Stop* button, or wait until the time defined in the *Duration* field passes.

5. When stopped, the AVI file remains opened in a new image window.

11.2. Save ND2 as AVI

You can easily create an AVI movie from an nd2 file. Run the 💾 *File > Save As* command and select the AVI format in the *Save As Type* pull-down menu. The *Save As* window is extended by the following options:

Use Default Video Compression Either leave this box checked or select which video compression will be applied to the movie file. See 11.4. About Video Compression.

XY Loop When the ND document contains XY points, you can decide how these are saved.

   Current XY Only the currently selected XY loop will be saved as a movie.

   All XY in separate files A movie will be saved for each XY loop present in the ND2 file. An automatic number will be appended to the file name.

Output Timing Parameters Timing of frames of the resulting AVI movie may equal the Original Acquisition Time. If this does not suit your needs, define a custom interval in *ms* .

Experiment Loop Select the dimension, frames of which will be used to create the AVI movie.

   Use Time Loop The T dimension frames of the current Z stack position will be used.

   Use Z-stack Loop The Z dimension frames of the current Time position will be used.

   Use Z-stack and Time Loop All frames of the ND2 file will be used in the AVI movie.

Note

If you have the QuickTime Player installed on your PC, a QuickTime movie (.mov) can be created as well.*

11.3. Creating a Movie

11.3.1. Creating a Movie Step by Step

1. Display the current volumetric data set (an ND file containing Z) using the *View > Image > ND View > Volume View* or the 🌐 *Applications > 6D > Show Surface View* command.

2. Run the Movie Maker by the 🎥 button.

3. Adjust the movie settings by the *Settings* button a window appears where the movie duration and frame rate can be defined.

4. You can use one of the predefined presets or import some previously defined key frames by the *Export/Import* button.

5. Either enable or disable the Director's mode by the clapper button. See 11.3.3. Directors Mode.

6. Go to the beginning of the time line and define the initial zoom, position, rotation or component appearance of the object. This step is not necessary, but very much recommended. Press the *Add/Update key frame* button to fix the initial appearance.

7. Browse the time line, modify the object position each time, and set key frames by clicking the *Add/Update key frame* button. Press the *Play Movie* button for a preview.

8. When all the key frames are properly set, press the *Create Movie* button. The movie is created and the AVI file will open in a new image window.

9. Save the new file by the standard 💾 *File > Save As* command.

11.3.2. Movie Maker Controls

When you start the movie maker, the following bar appears at the bottom of the image view. The user is supposed to create a set of key-frames. The rest of the movie will be created by interpolation between these key frames. This gives the user absolute freedom in how the movie will look like.
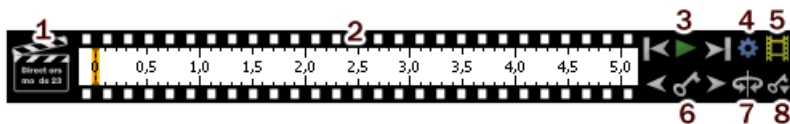
1. The *Clapper* button. By this button, the director's mode can be turned ON/OFF. See 11.3.3. Directors Mode.

2. Time line. By clicking to the time line you can browse the movie frames. When a key frame is added, a bold line appears in the time line to indicate its position.

3. *First Frame*, *Play*, *Last Frame* buttons.

4. *Settings* - by this button, you can define the movie length and the frame rate.

5. *Create Movie*

6. The key frame buttons. The existing key frames may be browsed by pressing the *Previous/Next Key Frame* buttons. The *Add/Update Key Frame* button inserts a key frame to the current position on the time line.

7. *Presets* - a predefined rotation can be loaded. When you press the button and select the rotation type, the existing key frames settings will be overwritten.

8. *Import/Export Key Frames* - this enables you to save and load the key frame settings. A file with the **.KEY* extension will be created during the export.

11.3.3. Directors Mode

In the directors mode it is not needed to press the *Add key frame* button every time the position of the object is changed. The director's mode detects all changes made to the object position and adds a key frame to the time line whenever such change is detected. A typical work-flow would look like this:

1. Turn ON the directors mode by the *Clapper* button. The time line gets highlighted with red color.

2. Click to the time line where a certain position of the object shall occur.

3. Move the object by mouse or adjust it in some way (zoom, crop, etc.) - at this moment, the key frame will be added to the time line automatically.

4. Click to the time line where another position shall occur, and so on...

The director's mode shall be used when you know precisely how the movie shall look.

11.4. About Video Compression

It is common to encode (compress) movies in order to save some disk capacity. This can be done using various types of codecs. A video codec is a software tool which can code (compress) and decode (de-compress) video files. Remember that the codec you use to save the video will be required for playback as well (when playing the video on other computers).

Look at the list of codecs coming with *NIS-Elements AR* by default. If other codecs are installed on the computer, they become also available to *NIS-Elements AR*.

Caution

32bit operating systems provide better choice of codecs because a 64bit codec must be used on a 64bit operating system, but there are not many of those in the meantime.

We performed a test by converting a 1 GB / 4000 frames ND2 file to AVI using different codecs. The results are displayed in the list:

No compression Original quality, file size: 787 MB.

DV Video Encoder This codec produces only videos with 640 x 480 resolution. Videos with other resolutions are stretched to fit, therefore we do not recommend to use this codec.

MJPEG Compressor Very good quality, file size: 240 MB.

Cinepack Codec by Radius Average quality, file size: 70 MB.

Intel IYUV codec Very good quality, file size: 390 MB. Provides the best quality / compression rate.

Microsoft RLE Very poor quality, file size: 90 MB.

Microsoft Video 1 Very poor quality, file size: 6 MB.

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NIS-Elements AR

Manual

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