User manual openSIM software

software version: with integrated camera control

Contents

1. Structure openSIM user manual:

- The main part of the user interface describes operating the openSIM software in normal operating mode which includes all the controls and indicators necessary for SIM image acquisition.
- For testing and troubleshooting purposes the expert mode can be used (see appendix) during which additional status and log information are displayed.
- The user interface is categorized in controls (user can enter information) and indicators (information are displayed to user)

- Currently the control for two different cameras (Andor Zyla and Andor iXon) is implemented in the software. As camera setting are mostly specific for one camera type, the corresponding descriptions are marked in grey

2. Introduction to the openSIM software

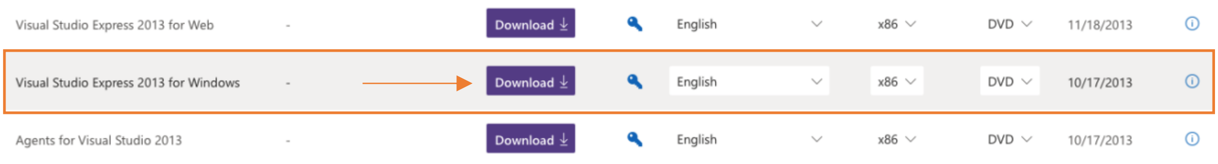
The openSIM software is written with LabView. It controls the openSIM device itself and interfaces additional components such as the camera and piezo focus positioner if available. It includes a user interface to enter the necessary parameters and to display currently executed commands and error messages. The part of the software operating the openSIM device controls the LEDs, provides the pattern sequence for the spatial light modulator (SLM), and actuates the fan. The projected pattern sequences can be selected either manually from a list of pattern sequences or automatically based on parameter inputs from the user. The software handles the image mode selection, saving and formatting of the acquired data and the corresponding metadata. In addition, the software controls the camera for the image acquisition. If a device necessary for z-stacking, such as a piezo focus positioner or a motorized focus controller, is available, the software can be used to perform z-stacking. The z-stacking function automatically changes the focus of the objective by providing an analog output to the focus adjusting device.

The software is written in a modular structure to enable a customization and adaption to different peripheral components such as different camera models or focus adjustment. The current version of the software includes an integration for an Andor Zyla sCMOS, an Andor iXon EMCCD camera (information specifically relevant for these two camera types are marked in grey in the following text) and Piezosystem Jena Mipos 100 piezo focus positioner. For controlling the Andor cameras a software development kit provided by Andor (SDK 3 for LabView) is used. The SDK contains a dynamic library from which we used functions to enable and execute the configuration and data acquisition process of the camera.

See the documentation of openSIM software (provided within the distributed openSIM documentation folder) for more detailed information on the LabView code.

3. Installation

1. **Microsoft Visual Studio**
   Install Microsoft Visual Studio Express 2013. You may encounter compatibility issues if you attempt to run the openSIM software with a different version of Visual Studio. A key is not required for the installation of the Express version.
   Download: https://visualstudio.microsoft.com/vs/older-downloads/



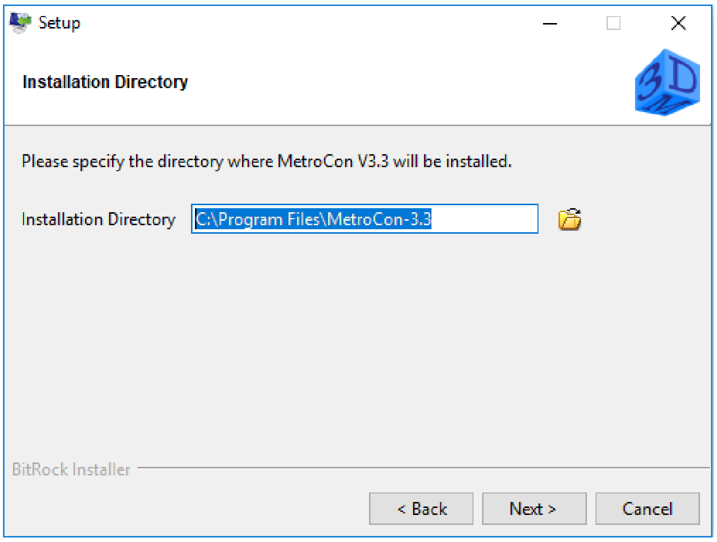
Visual Studio Express 2013 for Web	-	Download		English	x86	DVD	11/18/2013
Visual Studio Express 2013 for Windows	-	Download		English	x86	DVD	10/17/2013
Agents for Visual Studio 2013	-	Download		English	x86	DVD	10/17/2013

   This will download an ISO file. To access to setup.exe file in order to install the software you can either mount (right-click on ISO file → mount command) the file (opens a virtual drive that is visible in the file explorer) or extract the files (right-click on ISO file → extract)

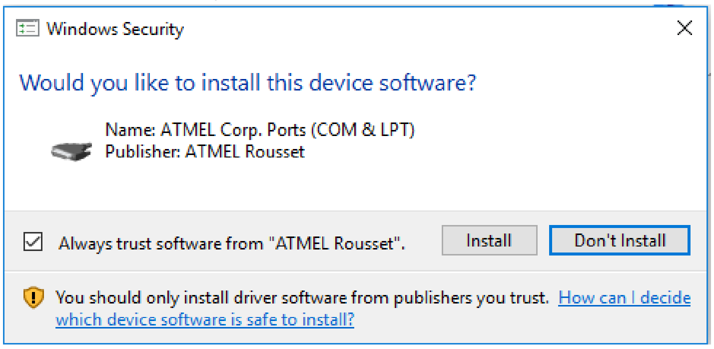
2. **LabView**
   Install LabView 2018 SP1 including the vision development module and DAQmx module. The openSIM software was created with LabView 2018 SP1.
   Download: https://www.ni.com/en-us/support/downloads/software-products/download.labview.html#329059
   - ➔ Vision development module: https://www.ni.com/en-us/support/downloads/software-products/download.vision-development-module.html#329460

➔ DAQmx module: https://www.ni.com/en-us/support/downloads/drivers/download.ni-daqmx.html#333268

3. **MetroCon**
   Run the MetroCon 3.3 application from ...\drivers\ForthDD_QXGA\MetroCon within the distributed openSIM folder
   Check that MetroCon is installed in C:\Program Files\MetroCon-3.3
   (or C:\Program Files (x86) \MetroCon-3.3 for the 36-bit version or the corresponding path to the program folder for other operating systems than windows)



Install ATMEL Corps. Ports (COM & LPT)

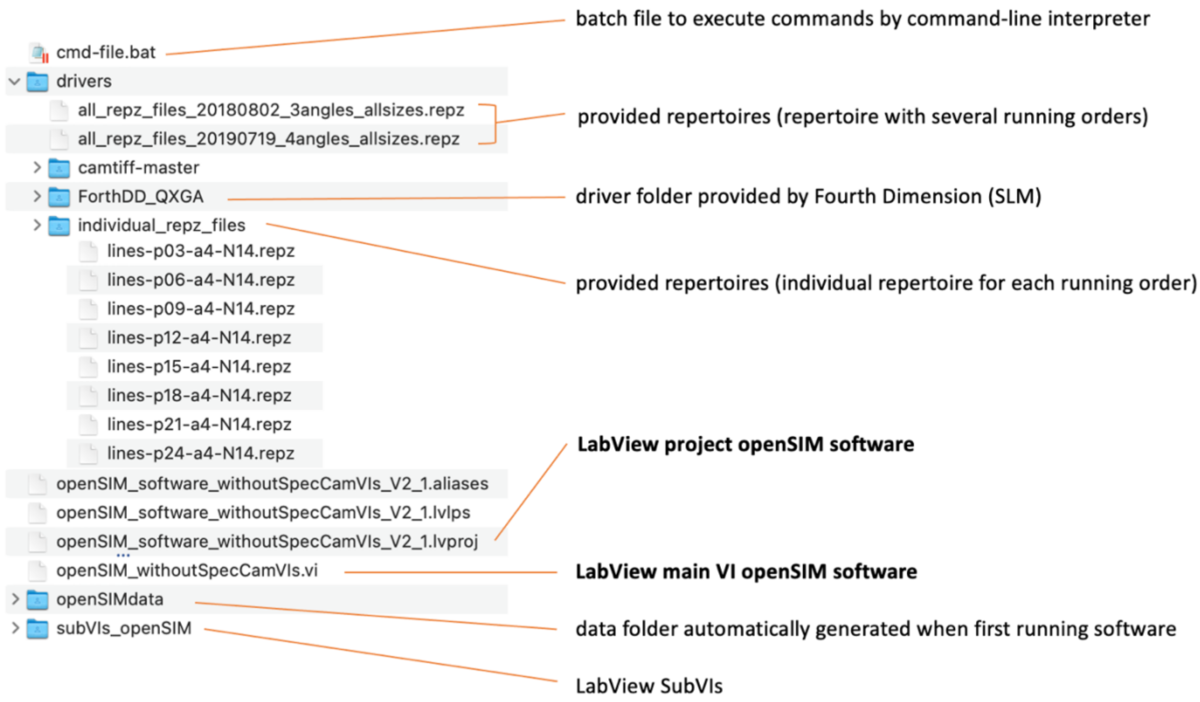


4. Restart the computer after completing the installation steps
5. Copy the openSIM software folder to a local directory and make sure that the folder structure looks like below

4. Distributed files and required folder structure

These are the distributed files in order to run the openSIM application (software version without camera control).

Please ensure that the folder structure is retained when copying the distributed openSIM folder to your local directory as the openSIM software used this folder structure as base directory.



5. Illumination patterns

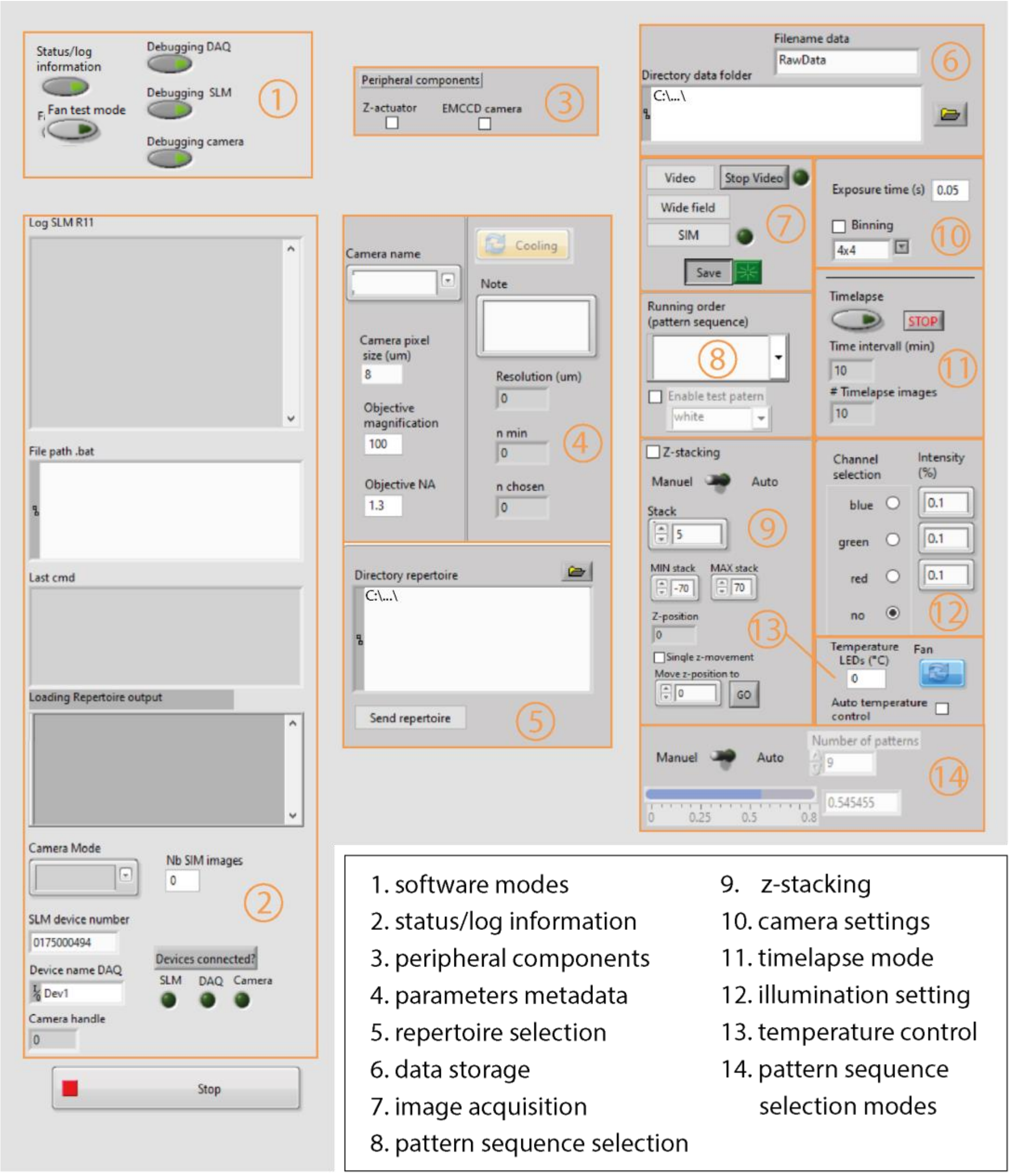
For SIM image reconstruction, the sample needs to be illuminated with a set of patterns with different phases and angles. We generated different line illumination patterns (according to SIMToolbox – supplementary documentation and user's guide) which are distributed with the openSIM software.
For a better understanding we provide here some background information and explain the used terminology.

Pattern sequence:

- sequence of patterns projected by the SLM during the SIM image acquisition
- each pattern of the sequence has a different phase and angle → the number of patterns in the sequence defines the total number of images during one SIM image acquisition (one image is acquired per pattern and the total amount of images is saved as an image stack)
- pattern sequence is stored as repertoire (.repz file format), a zip file containing:
  - images of the pattern (.bmp file format)
  - timing schedule (.seq file format, supplied by Forth Dimension, defines the bright and dark phases of the SLM)
  - running order (.rep file format, describes the order of the projected pattern images in the sequence and the and trigger mode)
- a pattern sequence can contain several running orders (rep file with description of several running orders)
- we provide the generated pattern sequences in two ways:
  - repertoire with several running orders (repz files: all_patternSequences_3angles.repz; all_patternSequences_4angles.repz): repertoire to be uploaded to the SLM, the running orders define the different available individual sequences of pattern which can be projected by the SLM
  - repertoire with one running order: when using the SIMToolbox for SIM image reconstruction, the software requires information about the pattern sequence projected during the SIM image acquisition, the software can automatically read in the pattern information when stored in a specifically formatted text file (.yaml file format)
    → to facilitate the automatic read in of the pattern description file, we provide a folder (individual_patternSequences) which contains an individual repertoire (repz files: lines-p03-a4-N14.repz, ….) for each running order (lines-p03-a4-N14,…) available in the provided repertoires descripted above (all_patternSequences_3angles.repz; all_patternSequences_4angles.repz), when acquiring a SIM image the individual repertoire corresponding to the used running order is saved together with the acquired data

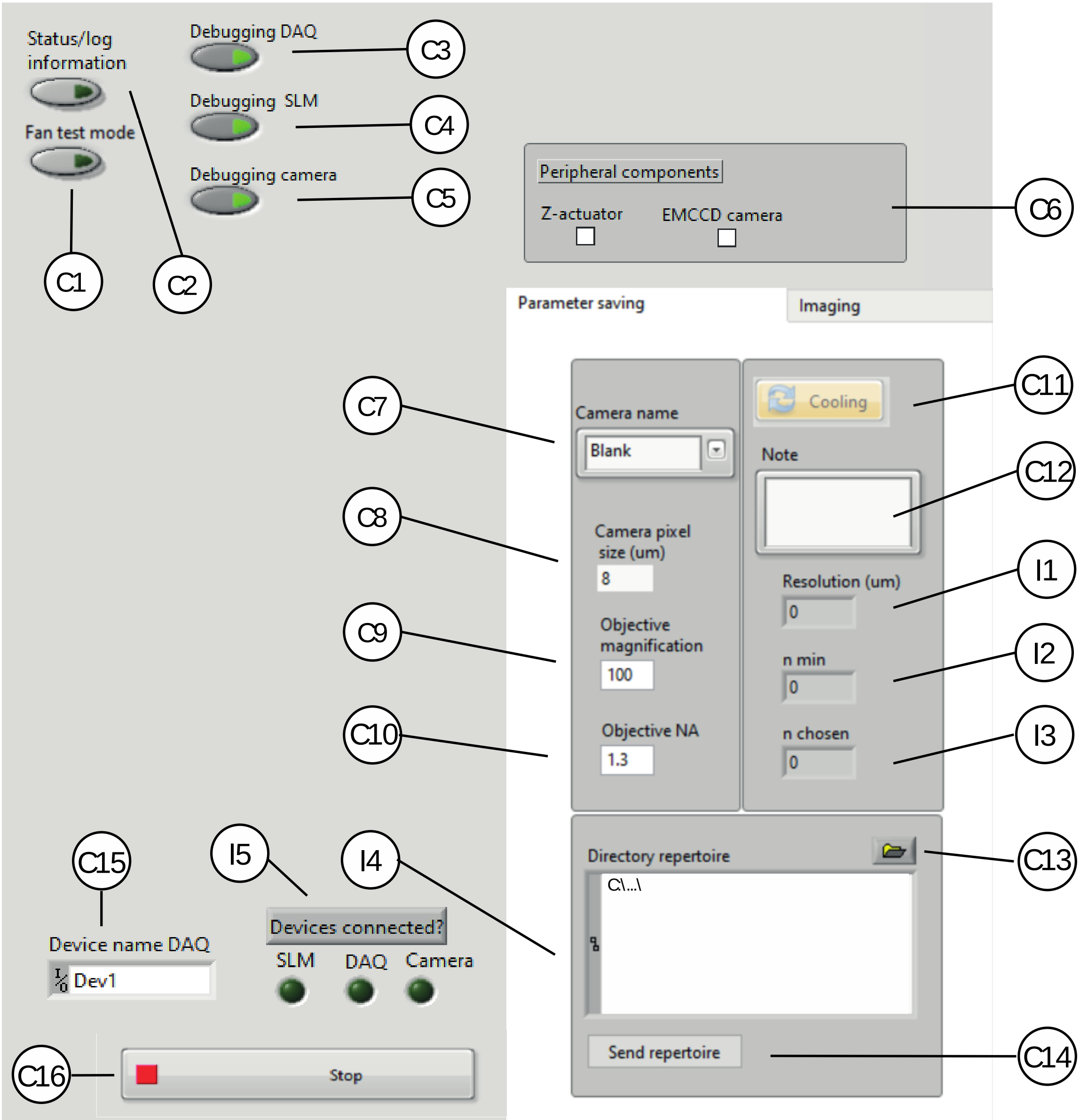
Terminology for openSIM user interface and user manual:
→ for a better differentiation we use the term 'repertoire' for .repz files and the term 'pattern sequence' for the actually, by the running order defined and by the SLM projected, sequence of patterns during a single SIM image acquisition

6. Control of the openSIM software



Overall structure of complete openSIM user interface

6.1. User interface in normal operating mode

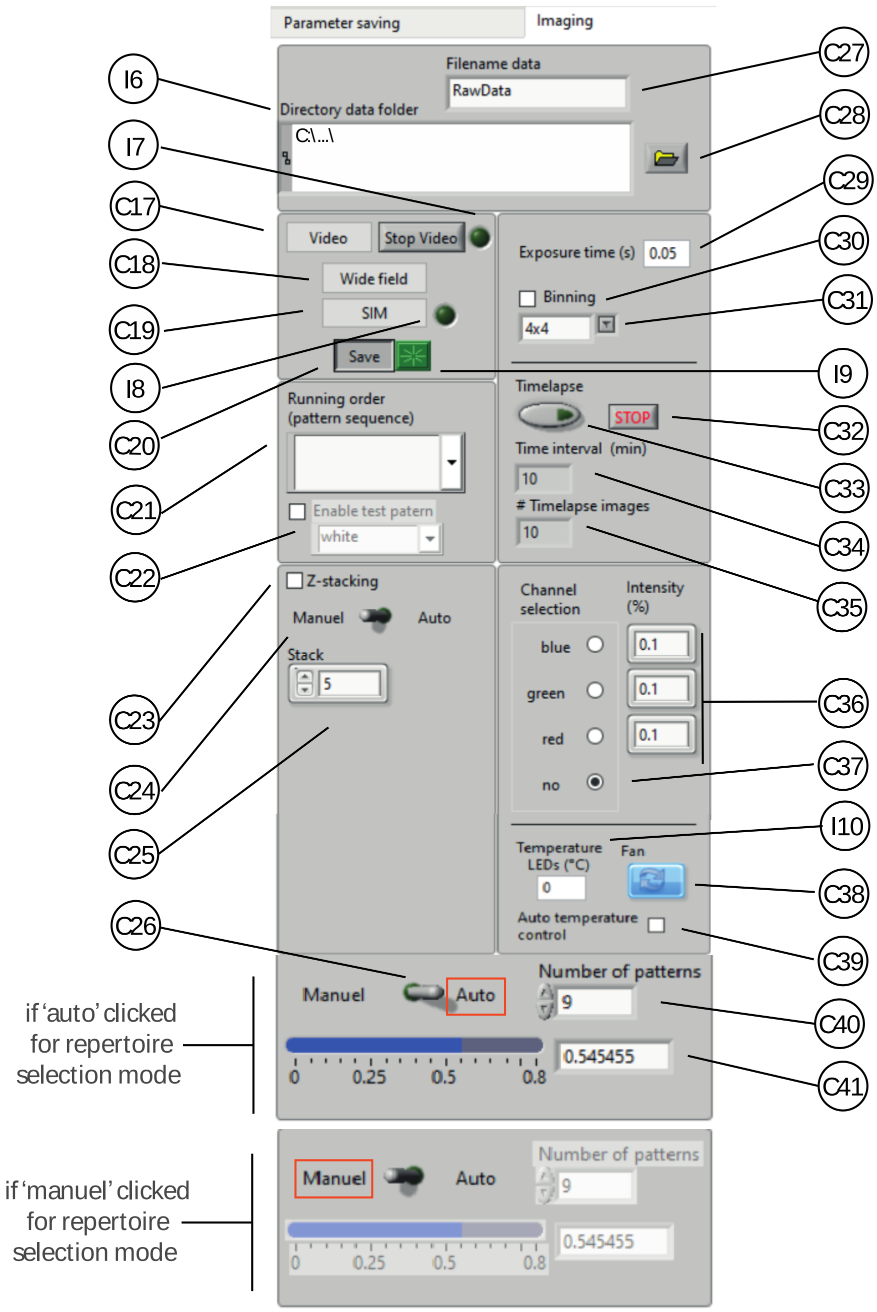


C1: Enabling fan test mode
C2: Displaying status / log information
C3: Enabling DAQ debugging mode
C4: Enabling SLM debugging mode
C5: Enabling camera debugging mode
C6: Selection of available peripheral components
C7: Selection of camera
C8: Pixel size of selected camera
C9: Magni cation of microscope objective
C10: Numerical Aperture (NA) of microscope objective
C11: Enabling camera cooling system

C12: Note added to meta data
C13: Browse directory to select reper-toire directory
C14: Load repertoire to SLM
C15: Selection of device name of DAQ
C16: Stop program execution

I1: Calculated resolution
I2: Minimal possible period of grating
I3: Selected numer of patterns
I3: Selected number of patterns
I5: Indication if devices are connected

User interface in normal mode (part 1)



C17: Selection of acquisition mode (video)
C18: Selection of acquisition mode (Wide eld)
C19: Selection of acquisition mode (SIM)
C20: Enabling data saving (default: enabled)
C21: Selection of running order (pattern sequence)
C22: Enabling test pattern
When enabled: selection of test pattern below
C23: Enabling z-stacking
C24: Selection of manual/automatic z-stacking mode
C25: Number of images within the stack
C26: Selection repertoire selection mode
C27: File name for saved data
C28: Browse directory data folder
C29: Setting camera exposure time
C30: Enabling binning of camera

C31: Setting binning size of camera
C32: Stop timelapse mode
C33: Timelapse mode enable
C34: Timelapse interval (min)
C35: Number of timelapse images
C36: Selection LED channel
C37: Selection LED intensity
C38: Turn on/o openSIM fan
C39: Enabling automatic temperature control
If auto repertoire selection mode is selected:
C40: Number of desired patterns
C41: Slider to select period of grating

I6: Selected directory data folder
I7: Indicator video aquistion on

User interface in normal mode (part 2)

6.2. Input parameters

→ Depending on certain selections/modes some of the indicators and controls are greyed out.
These controls and indicators are clickable when the corresponding mode is selected.

INDICATORS:

I1: Calculated resolution in µm
→ based on wavelength of selected channel and NA of objective *(C37, C10)*

I2: Calculated minimal spatial period
→ only relevant for auto running order (pattern sequence) selection mode
→ based on calculated resolution *(I1)*, camera pixel size *(C8)* and magnification of objective *(C9)*

I3: Selected spatial period
→ only relevant for auto running order (pattern sequence) selection mode

I4: Directory of SLM repertoire
→ path to chosen repertoire (.repz file)
→ default: path to the .repz file which is included in the distributed openSIM software folder

I5: Indicator showing if devices (SLM, DAQ, Camera) are connected

I6: Display showing the selected folder where the acquired data is saved in

I7: Indicator showing if video acquisition is currently running

I8: Indicator showing if SIM acquisition is currently running

I9: Indicator showing if saving of the acquired images is currently enabled
→ images are saved in directory selected with *C28* and showed in *I6*

I10: Temperature measured by thermistor at illumination block holding the PT54 LEDs
→ temperature is used as input for the temperature control system
→ if the temperature is too high, the two fans at the aluminum heat sink extender are turned on

CONTROLS:

C1: Enabling/Disabling expert mode (normal mode is default)
→ expert mode includes additional indicators which support debugging
(in the expert mode indicator *I10-I17* are visible)
→ see appendix for more information about the expert mode

C2: Enabling/Disabling fan test mode
→ if test mode is enabled, only the fan is controlled

Debugging modes:

- In order to facilitate testing and debugging of the software and the individual devices (i.e. SLM, NI DAQ, camera) it can be useful to connect only some of the devices (e.g. only NI DAQ is connected and tested with the software, while the SLM and camera are not connected). However, in the normal (non-debugging) mode the openSIM software will return an error message and stops when devices are not connected (e.g. because of missing device handles when the device is not connected which are needed in the initialization step). In the debugging mode, the software can be used despite the corresponding device not being connected.
- Debugging mode can be used for only one device or several devices at the same time

C3: DAQ debugging mode
→ facilitates the testing of functions independent of the DAQ devise
→ if enabled, the software does not try to connect to the DAQ device, software can be used even if the NI DAQ is not connected

C4: SLM debugging mode
→ facilitates the testing of functions independent of the SLM devise
→ if enabled, the software does not try to connect to the SLM device, software can be used even if the SLM is not connected

C5: Camera debugging mode
→ facilitates the testing of functions independent of camera
→ if enabled, the software does not try to connect to the camera, software can be used even if the camera is not connected

C6: Selecting the available peripheral components
→ specific controls are visible/invisible depending on the availability of the corresponding peripheral component
→ Z-actuator: should be selected if an actuator for changing the z-position of the microscope such as a piezo focus positioner, stepper motor or automated microscope is available and implemented. If enabled, the controls *C38-42* are visible
(see section 5 for more information how to implement a z-actuator and see appendix for more information about the user interface for automated z-positioning)
→ EMCCD camera: should be selected if a EMCCD camera, where the EM gain can be changed, is implemented and used. If enabled, the controls *C43-C44* are visible
(see section 5 for more information how to implement a camera and see appendix for more information about the additional control for a EMCCD camera)

C7: Selection of camera from list of implemented camera interfaces
→ see appendix for information how to include an interface for a different camera
→ select *Andor iXon* for Andor iXon EMCCD camera
→ select *Andor Zyla* for Andor Zyla sCMOS camera

C8: Pixel size of selected camera in µm
→ see the specification sheet of your camera for information about the pixel size
→ sCMOS camera Andor Zyla 4.2: pixel size is 6.5µm
→ EMCCD camera Andor iXon: pixel size is 13µm

C9: Magnification of microscope objective

C10: Numerical aperture (NA) of microscope objective

C11: Enabling/Disabling cooler (relevant if the camera includes a cooling system)
→ see the specification sheet of your camera to see whether it includes a cooling system
→ see appendix for information about the control of a camera cooling system when including a different camera
→ EMCCD camera Andor iXon has a deep vacuum cooling
→ sCMOS camera Andor Zyla 4.2 has no cooling option

C12: saved note in SIM mode
→ when acquiring SIM images, a text file containing meta data such as the imaging parameter settings are saved
→ the inserted note is added to the saved text file in SIM mode

C13: Browse directory to select file path to the repertoires (.repz file)
→ selected repertoire which is loaded to the SLM
→ default: path to the file 'all_patternSequences_4angles.repz'

C14: Loading repertoires to SLM (selected with *C13* and displayed at *I4)*

→ repertoires (.repz file) have to be loaded only once to the SLM

C15: Device name of connected data acquisition (DAQ) device
→ device name is shown when USB cable of DAQ device is connected to the PC (see operation instructions for more information)

C16: Stop button to stop the program execution

C17: Selection of acquisition mode → video: mainly for test purpose, continuous acquisition of widefield images without a saving option

C18: Selection of acquisition mode → widefield: acquisition of widefield images, default: one image is acquired when bottom is clicked, if z-stacking is enabled: number of total images equals number of stacks *(C25)*

C19: Selection of acquisition mode → *SIM*: acquisition of SIM image stack, number of total images depends on the number of patterns in selected pattern sequence and number of stacks *(C25)* if z-stacking is enabled

C20: Enabling/Disabling data saving
→ acquired data is saved as .tiff file
→ in the SIM mode, the metadata (number of SIM datasets, number of pixels (x,y), camera pixel size, exposure time, EM gain, microscope magnification, channel selection) is saved as .txt file located at the file path specified by saving path *(I6),* saving is enabled by default

C21: Selection of running order (pattern sequence) selection mode
→ manual running order selection mode: the user manually selects the running order (pattern sequence from the list of running orders available for uploaded repertoire in the dropdown menu
→ auto running order selection mode: the control serves as a display which shows the automatically chosen running order (see *C21* for more information about the running order selection mode)

C22: Enabling/Disabling test pattern
→ default: test pattern disabled (greyed out when test pattern is disabled)
→ if test pattern is enabled, different projected test patterns can be selected
→ possible test pattern: black, white, vertical bars

C23: Enabling/Disabling z-stacking
→ widefield mode: the z-position of the microscope objective is changed in steps and one image is acquired per step, the number of images in the stack is defined by *C25*
→ SIM mode: the z-position of the microscope objective is changed in steps and for every step several SIM images are acquired, the number of images in the stack in defined by *C25* and *C36*
(default: z-stacking disabled)

C24: Selecting manual or automatic z-stacking mode
→ manual mode: the user has to select the starting z-position of the microscope objective and has to manually change the z-position by using the focus adjustment knob of the microscope before acquiring the next image of the z-stack
→ automatic mode: the focus is automatically changed by an actuator according to the z-stacking input parameter during the image acquisitions
(see appendix for more information about the automatic stacking mode)

C25: Number of images in the stack

C26: Running order (pattern sequence) selection mode
→ default: manual mode (if manual mode selected *C36, C37* are greyed out)
→ manual mode: the user has to select the desired running order (pattern sequence) from the list of running orders *(C21)* available for the uploaded repertoire *(I4)*
→ auto mode: the running order (pattern sequence) is automatically selected based on the selected period *(I3)* and the desired number of projected patterns in the sequence*(C36)*

C27: File/folder name of saved data

→ The file name is automatically appended with an XXX-digit number for consecutive images

→ widefield acquisition mode: inserted name defines the name of the image (.tiff file) which is saved at the location defined by *C24* and showed in *I6*

→ SIM acquisition mode: inserted name defines the name of an automatically created folder where the image (.tiff file), the metadata (.txt file) and the projected pattern (.repz file) are saved in

C28: Browse directory to select path to folder where the data is saved in (default: path to folder 'openSIMdata' the directory containing the application)

C29: Exposure time of camera in seconds

C30: Enabling/Disabling binning

→ only implemented for Andor Zyla sCMOS camera

→ sCMOS camera Andor Zyla: if binning is disabled the area of interest (AOI) is set back to 1048 pixel, if binning is enabled the pixel binning is set to the binning size defined by *C31*

C31: Binning size of camera

→ possible options are *2x2, 3x3, 4x4, 8x8*

C32: Stop timelapse mode

→ stops timelapse imaging before last timelapse image is acquired

→ normally timelapse mode stops after acquisition of last timelapse image

C32: Enable timelapse mode

→ only for SIM image acquisition mode

→ if enabled, SIM image stacks are acquired in an interval defined by *C34*

→ first image is acquired when SIM image acquisition mode is selected *(C19)*

→ number of acquired SIM images is defined by *C35*

→ each SIM image stack is saved individual and not as a time stack since individual SIM image stacks are required for image reconstruction

C34: Time interval

→ defines the time period in min between two subsequent SIM image acquisitions

C35: # Timelapse images

→ defines the number of total timelapse images are acquired

→ total timelapse session: time interval * (# Timelapse images – 1)

C36: LED excitation intensity in %

C37: Selection of channel

→ indicates color of excitation light (*blue, green, red LED*)
(default setting is no LED selected)

C38: Manually turning the two fans which are mounted to the SIM device on/off

→ are also controlled by an automatic temperature control

→ fans are at the aluminum heat sink extender

C39: Enabling automatic temperature control

C40: Number of desired patterns in pattern sequence

→ relevant for auto running order selection mode

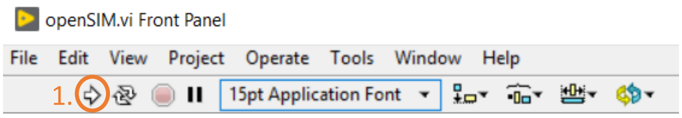
C41: Slider in % (maximum 80% possible)

→ determines how close the period of the grating is to the minimal possible value *(I2),* selected value is displayed on the right (relevant for auto running order selection mode)

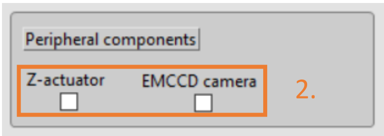
6.3. Operating instructions

Running the software with default settings

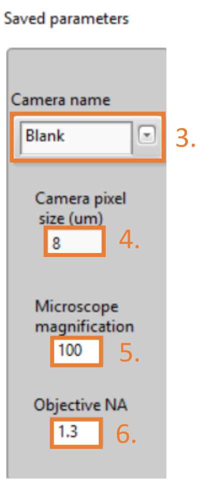
1. Run the Labview VI ‘openSIM’



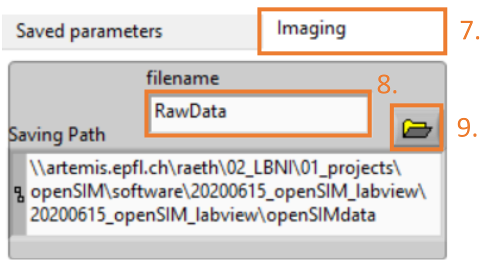
2. Select the available peripheral components
   → see appendix for information about possible peripheral components and corresponding differences in the user interface and operational instructions
   → do only select Z-actuator and EMCCD camera if you are sure that the appropriate components are connected



3. Select camera from the list of implemented cameras by clicking on the drop-down menu *(C7)*
   → select *Andor iXon* for Andor iXonUltra EMCCD camera
   → select *Andor Zyla* for Andor Zyla sCMOS camera
   → select *Blank* for testing purposes if no camera is connected
   → see appendix for information how to include an interface for a different camera



4. Insert camera pixel size in µm *(C8)* of selected camera
5. Insert magnification of used microscope objective *(C9)*
6. Insert NA of used microscope objective *(C10)*
7. Select ‘Imaging’ tab
8. Insert file name *(C27)*



9. Select saving path *(C28)* (saving is enabled by default)

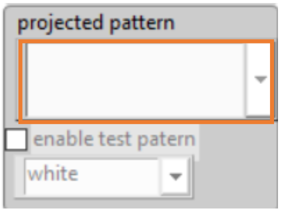
10. Select running order (pattern sequence) from list of running orders *(C21)* by clicking on the drop-down menu

    → naming scheme of running orders:

    lines-p45-a4-N14

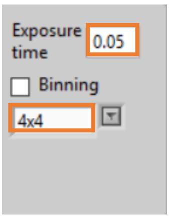
    grating type   period of grating   number of angles   number of patterns

11. Set exposure time in seconds *(C29)*



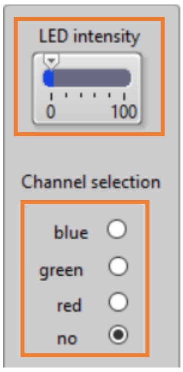
11.

12. (optional) Enable binning if desired *(C31)* and select binning



12.

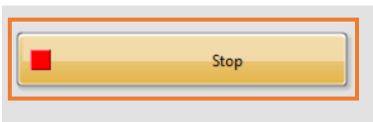
13.



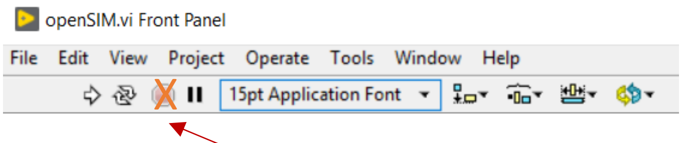
14.

15.

13. Set LED intensity in % *(C36)*
14. Select the LED channel *(C37)* to choose the color of the excitation light
15. Image acquisition (see instructions below)
16. Close the program by clicking stop button on front panel *(C16)*
    → Important to stop execution by stop button on front panel and not by 'Abort Execution' button in the LabView toolbar



17.



! Do not stop the program here !

Note:

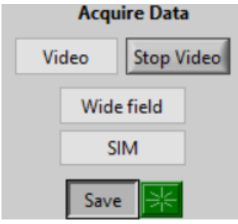
Repertoires (.repz file) have to be loaded only once to the SLM

→ Selecting repertoire path *(C13)* and sending repertoire path *(C14)* should be performed only when using the software for the first time after assembling the openSIM or if the user wants to load additional repertoires to the SLM

Default settings
- Expert mode: disabled
- Test mode: disabled
- Repertoire path: to the file 'all_patternSequences_4angles.repz' in the directory containing the application
- Saving path: to folder 'openSIMdata' in the directory containing the application
- Saving: enabled (data is saved with the file 'RawData' at the default saving path)
- Fan: turned on
- Running order (pattern sequenc) selection mode: manual
- Test pattern: disabled
- EM gain: disabled

Acquire Data: widefield image

1. Adjust microscope focus to the desired imaging plane
2. Click on the 'Widefield' button *(C18)*



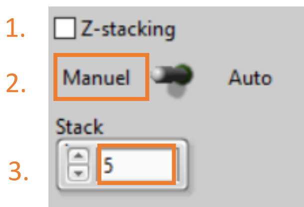
Acquire Data: SIM image

1. Adjust microscope focus (manually or via microscope interface) to the desired imaging plane
2. For timelapse imaging the timelapse imaging mode has to be enabled before next step (Clicking 'SIM' button acquires the first timelapse image, the subsequent images are acquired in a sequence defines by *C34* and *C35*)
3. Click on the 'SIM' button to acquire a SIM image stack *(C19)*

Manual z-stacking

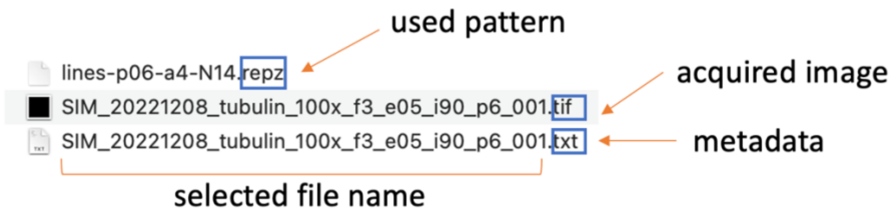
→ stacking disabled: one single SIM/widefield image is acquired
→ stacking enabled: several SIM/widefield images are acquired

1. Enable z-stacking
2. Select manual z-stacking mode
3. Select the number of desired images within the stack
4. Manually select the starting z-position of the microscope objective
5. Click either the Widefield *(C18)* or the SIM button *(C19)* depending on the desired imaging mode in order to start the acquisition
6. After every acquired image, a dialog window pops up asking if the next image can be acquired. Before clicking on *acquire*, change manually the z-position of the objective by using the focus adjustment knob of the microscope. When clicking acquire, the next image will be acquired
7. Repeat step six until all images are acquired



7. Data handling

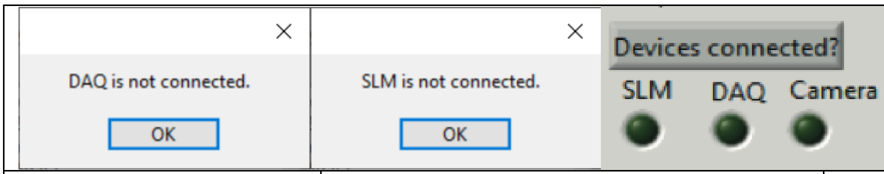
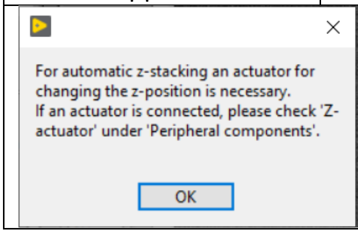
- The acquired data is saved in the selected folder (file name can be seleted with *C27*, path of folder where data is saved in can be selected with *C28* and is shown in *I6*)
- The data is saved in tiff format (widefield: tiff file with one frame, SIM: tiff file with image stack where the number of frames corresponds to the chosen number of patterns in pattern sequence (shown in *I18*)
- Along with the tiff image file, the repertoire (repz file with single running order) corresponding to the used running order (pattern sequence) selected with *C21* and a file containing the metadata (.txt) (same file name as image file but different file format) is saved
- The metadata file contains information about the used parameters during the image acquisition (e.g., image type, pixel size, number of patterns, LED channel, exposure time)
- The tiff format is a commonly used file format (lossless file format) compatible with ImageJ and the image reconstruction software *SIMToolbox* (MATLAB toolbox for structured illumination fluorescence microscopy)



Example how data is saved during image acquisition

8. Troubleshooting

Problem	Possible reason	Possible Solution
It is not possible to connect to the NI DAQ, SLM or camera → error message(s), indicator does not light green and software automatically stops execution) → if the openSIM software cannot establish the communication with the SLM and/or DAQ, camera an automatic error message will appear and the software execution is stopped	The openSIM software was closed by clicking the 'Abort execution' at the Labview toolbar instead of clicking the stop bottom *(C16)*. By clicking the user interface stop bottom it is assured that the NI DAQ, SLM and camera are set back to their default settings and that the devices are closed properly. Not doing so can lead to reconnection problems when subsequently restarting the software.	Close the complete openSIM LabView project and reopen. If this does not solve the problem close the software and dis- and reconnect the individual components. Then start the applications again. If it is still not possible to connect to the devices, close the applications and restart the computer.

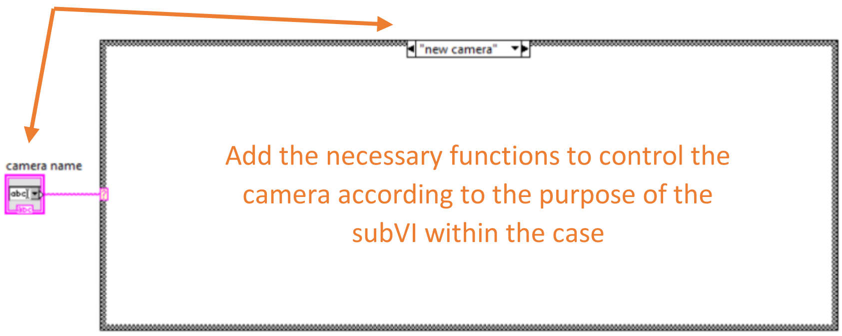
		
Error message: 'Error 5006 occurred at AT Open A.vi: OUT OF RANGE'	Camera is physically not connected to the PC	Connect the Andor Zyla camera to the PC via the USB cable
	The openSIM software cannot connect to the camera. This problem can occur if the 'Abort execution' at the Labview toolbar is used for stopping the program execution instead of the stop button in the user interface *(C16)*. If the camera is not closed properly, the data structures which are held internally by the SDK are not cleaned up which can lead to problems when reconnecting to the camera.	Disconnect and reconnect the USB cable from the camera to the PC. If this does not solve the problem restart the camera first and if the error message still occurs, then also restart the PC. One can also check if the camera can be accessed via via AndorSolis (Andor's camera control system)
When enabling z-stacking error message appears	Z-actuator at peripheral component selection is not enabled *(C6)*	Enable z-actuator and try to enable z-stacking again
		

9. Software adaptation

9.1. Integration of a different camera

The current version of the software includes an integration for an Andor Zyla sCMOS, an Andor iXon EMCCD camera. In order to integrate another camera to the openSIM software the subVIs listed below have to be adapted. All of these VIs contain a main case structure to differentiate between the implemented cameras. An additional case has to be added to include the necessary code within the subVIs. The camera name has to be added to the Combo Box *Camera name* to select the corresponding case in the subVIs. Many scientific camera manufacturers provide Labview SDKs for their camera systems which are often structured as libraries containing several subVIs to control the camera. The exact integration of the camera highly depends on the camera type and the camera manufacturer. In the section below the general approach how to implement a different camera into the openSIM software is descripted. For more specific information about the camera-specific SDK please see the information provided by the manufacturer. They are often distributed together with the SDK installation program or accessible online.

Add a new case to the structure which is selected by the camera name



subVi_Initialyse_Camera
- input: *Camera name, Em gain enable, Em gain, Exp Time, binning enable, binning size*
- output: *camera connected?, IMAQ Buffer, Handle, Xpixel, Ypixel*

→ in this subVI, the camera/camera specific library has to be initialized and all general and camera specific parameters have to be set in order to run the camera (for more specific information about the camera initialization and the necessary parameters please refer to the information about the corresponding SDK often provided by the camera manufacturer)
→ the indicator camera connected? has to be set true at the end of the subVI if everything was initialized and set correctly
→ EM gain only relevant for EMCCD cameras
→ handle, area of interest (AOI) width, AOI height, Auxiliary (AUX) output source (configures the signal which appears on the AUX output pin of the camera) is relevant when using SDK3 to control sCMOS cameras from Andor

Internal settings for Andor Zyla 4.2 sCMOS camera:
- Pre-amplification gain control: 12-bit (low noise)
- Auxiliary output source: Fire all
- AOI height: 1048px, AOI width: 1048px

Internal settings for Andor iXonUltra EMCCD camera:
- EM gain mode: 8-bit
- Acquisition mode: 1
- Read mode: 4
- Trigger mode: internal
- Acquisition timing: TTL high
- Shutter: auto

EM_gain_enable
- input: *EM gain, Handle, Camera name, EM gain enable*
→ only relevant when using a EMCCD camera
→ if EM gain is enabled the value for the EM gain has to be set to the value determined by *C44*

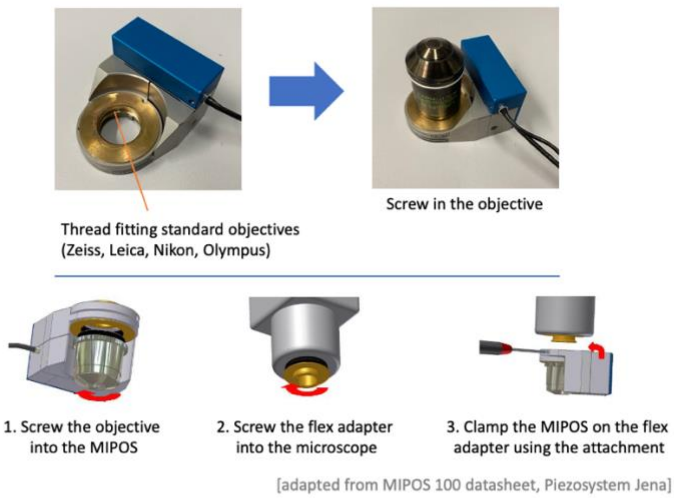
sub_vi_exp_time
- input: *Handle, Camera name, Exp time*
→ in this subVI the exposure time of camera in seconds has to be set to the value determined by *C29*

subVI_GetImage_Camera
- input: *Camera name, IMAQ buffer, Handle, Xpixel, Ypixel*
- output: *array, array 2D, Display 2*
  → in this subVI, the image acquisition has to be implemented including triggering start of image acquisition by the camera, readout data from camera, if necessary, caching in an additional buffer, conversion of byte data into pixel data according to the selected pixel encoding
  → Xpixel / Ypixel are used to create 2D array of pixel data to save the data afterwards (saving has not to be included in the subVI)
  → IMAQ buffer is used to display data

subVI_Close_CamerasubVI_Close_Camera
- input: *Camera name, Handle*
  → closes camera
  → within the subVI it has to be ensured that the camera is closed properly to ensure a bugless start at the next execution (for many cameras the data structures which are held internally by the SDK have to be not cleaned up)

9.2. Integration of a different focus positioning

The implemented z-stacking/single z-movement uses an analog signal via the DAQ device to control a piezo focus positioner (piezo focus positioner Jena Mipos 100 from Piezosystem). The piezo focus lens positioner can move objectives in a precise and accurate way. To do so, the piezo positioner is attached to the standard optical microscope. The objective is then screwed into the piezo positioner instead of the objective turret. In order to use a piezo focus positioner with a different motion range or voltage range but with the same operation principle (see figure 1), the initialization values for the motion and voltage range has to be changed in the initialization frame (first frame in Labview block diagram). If a different actuator type or a different operating principle should be implemented, the subVI *subVI_Zstacking_piezo* and the cases within the consumer loop have to be changed. Corresponding cases are *Z-auto, stacking_off, checking stacking input, single z-movement_on, single z-movement_off*.

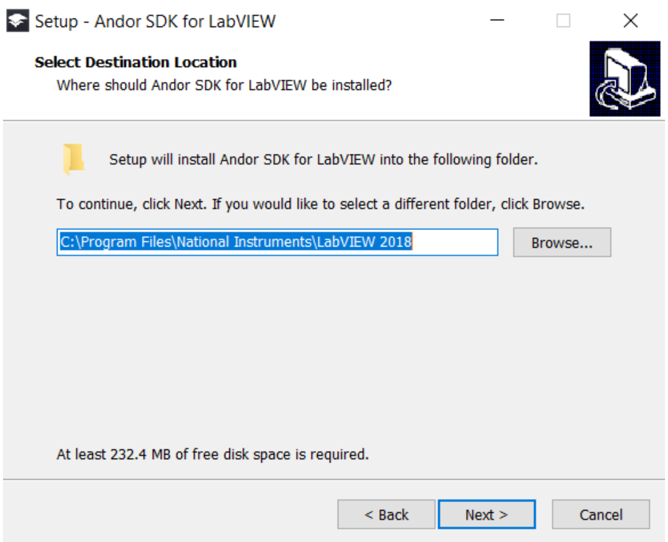


Appendix

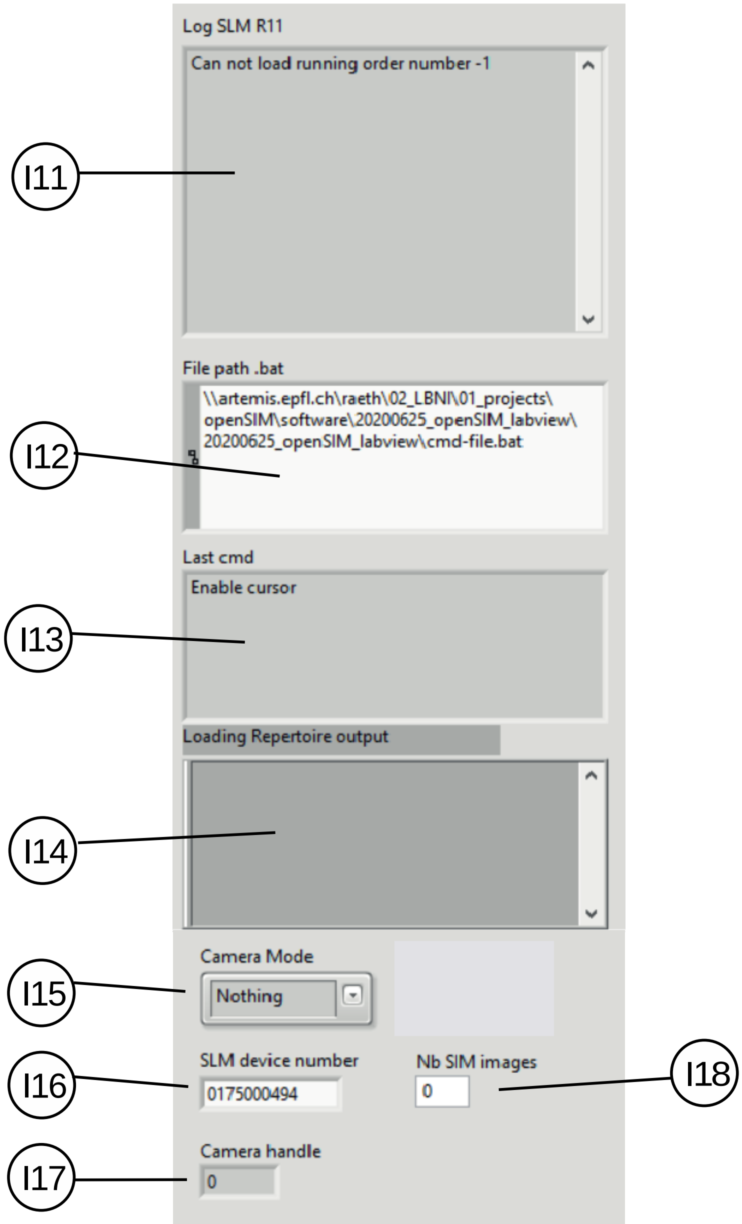
Installation of Andor SDK3 for LabView

→ LabView has to be installed before installing SDK3 for LabView

1. Run the Andor SDK3 for LabView installer (the SDK can be downloaded at https://andor.oxinst.com/products/software-development-kit/software-development-kit if it is not provided when purchasing the camera)
2. Choose the folder where LabView is installed as folder to install the SDK3 (program files → National Instruments → LabView XXXX) - if several LabView applications are installed (version, application bitness), make sure that the correct one is chosen
   → e.g. C:\Program Files\National Instruments\LabVIEW 20xx
   → e.g. C:\Program Files (x86)\National Instruments\LabVIEW 20xx
   → or corresponding path to the program folder for other operating systems than windows



Status / log information



I11: Dislaying current status of SLM
I12: Path to bat le
I13: Last executed command
I14: Text which is passed to command line
I15: Current acquistion mode
I16: Device number of SLM
I17: Handle of camera
I18: Number of projected patterns

Figure 1: User interface with status / log information

Additional Indicators:

I11: Display showing the current status of the SLM R11
→ e.g. bat file cleared/loaded, repertoire loaded, load running order number, cannot load running order number, pattern send, device closed

I12: File path to bat file
→ the bat file contains commands for the SLM in order to load the repertoire
→ see *I4* for more information about the repertoire
→ the path should not be changed from the default value.

I13: Display showing the last executed command in the openSIM program

I14: Display showing the text which is passed to the command line in order to run a system command sent to the SLM (bat file to load repertoire)

I15: Current acquisition mode (i.e. video, widefield, SIM)
→ selection via controls *C17* (for video), *C18* (for widefield), *C19* (for SIM)
→ video: mainly for test purpose, continuous acquisition of widefield images, without a saving option
→ widefield: acquisition mode for widefield imaging, default: one image is acquired when clicking *C18,* number of acquired images depends on number of stacks *(C25)* if z-stacking is enabled
→ SIM: acquisition mode for SIM imaging, number of total images depends on number of patterns in pattern sequence *(I17)* and number of stacks *(C25)* if z-stacking is enabled

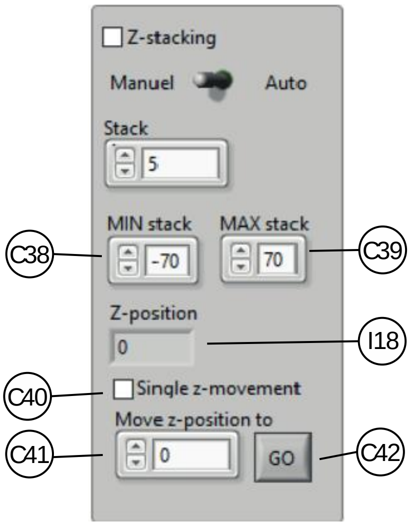
I16: Device number of connected SLM

I17: Handle to connected camera (relevant for sCMOS camera Andor Zyla)

I18: Number of SIM images/patterns
→ is automatically indicated based on the chosen running order (pattern sequence)

Automatic z-positioning

This section applies only if the piezo focus positioner Jena Mipos 100 (Piezosystem) is used according to the operating principle depicted in figure 5. If a piezo positioner with a different motion and voltage range or a different actuator type is used, please refer to the section 5 for more information about the integration.



C38: Motion range of z-actuator (min)
C39: Motion range of z-actuator (max)
C40: Enabling single z-movement
C41: Desired z-position within motion range of z-actuator
C42: Start single z-movement

I18: current z-position of acutuator during z-stacking

C43: Enable EM gain for EMCCD camera
C44: Set EM gain for EMCCD camera

Figure 2: User interface for automatic z-stacking

Additional Indicator and Controls

I16: Current position of z-actuator in µm during z-stacking if z-stacking is enabled

C38/C39: stack range in µm

→ min: z-position of first stack relative to mid-position
→ max: z-position of last stack relative to mid-position
(values have to be within motion range of z-actuator, for MIPOS 100 -70µm – 70µm)

C40: Enabling/disabling single adjustment of z-position by z-actuator

→ if enabled, piezo focus positioner is set to mid-position as initial position (half of its motion range)

C41 Desired z-position for single z-movement

→ focus positioner moves to inserted z-position in µm relative to mid-position by clicking the *GO* button (value has to be within motion range of piezo focus positioner, for MIPOS 100 -70µm – 70µm)

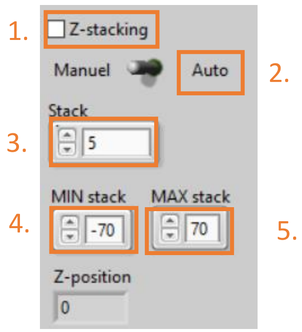
C42: Executing single z-movement

Operational instructions for automatic z-positioning

Z-stacking:

→ relative to mid-position (half of the piezo motion range), mid-position is used as initial position

1. Enable z-stacking *(C23)* → piezo is set to mid-position (order important!)
2. Adjust microscope focus (manually or via microscope interface) to the object of interest
3. Set stack size *(C25)*
4. Set z-position of first stack in µm relative to mid-position (value has to be within motion range of piezo z-positioner) *(C38)*
5. Set z-position of last stack in µm relative to mid-position (value has to be within motion range of piezo z-positioner) *(C39)*
6. Acquire either widefield or SIM images

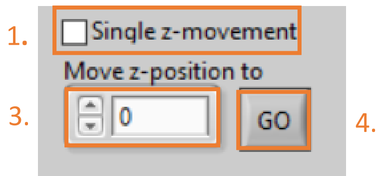


Single z-movement:

→ relative to mid-position (half of the piezo motion range), mid-position is used as initial position

→ independent of z-stacking: if z-stacking is enabled, the piezo focus positioner will be set back to its initial mid-position

1. Enable focus adjustment *(C40)* → piezo is set to mid-position (order important!)
2. Adjust microscope focus to the object of interest
3. Set desired z-position in µm relative to mid-position *(C41)*
4. Click the *GO* button *(C42)* to change z-position



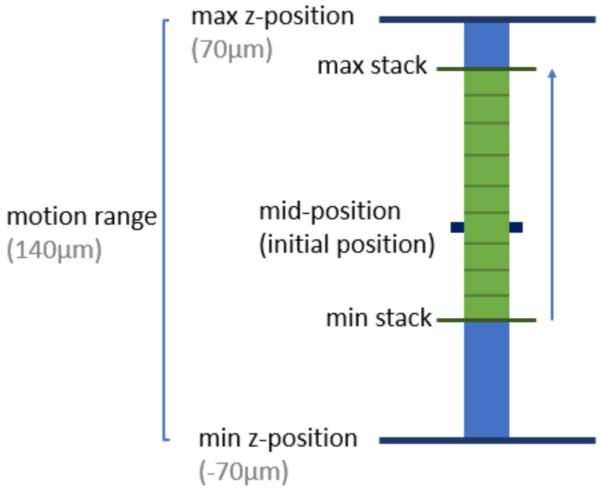


Figure 3: Schematic illustrating z-stacking via piezo focus positioner

EMCCD camera

C38: Enable EM gain for EMCCD camera
C39: Set EM gain for EMCCD camera

Figure 4: User interface for EMCCD cameras

Additional controls

C38: Enabling/Disabling electron multiplying (EM) gain of EMCCD

→ if EM gain is enabled, the gain is set according to the value specified with *C39*

→ if EM gain is disabled, the gain is set to zero

C39: EM gain

Troubleshooting Andor Zyla integration:

Problem	Possible reason	Possible Solution
Error message: 'Error 5006 occurred at AT Open A.vi: OUT OF RANGE'	Camera is physically not connected to the PC	Connect the Andor Zyla camera to the PC via the USB cable
	The openSIM software cannot connect to the camera. This problem can occur if the 'Abort execution' at the Labview toolbar is used for stopping the program execution instead of the stop button in the user interface *(C16)*. If the camera is not closed properly, the data structures which are held internally by the SDK are not cleaned up which can lead to problems when reconnecting to the camera.	Disconnect and reconnect the USB cable from the camera to the PC. If this does not solve the problem restart the camera first and if the error message still occurs, then also restart the PC. One can also check if the camera can be accessed via via AndorSolis (Andor's camera control system)