User manual openSIM software

software version: without integrated camera control

Contents

1. Introduction to the openSIM software

The openSIM software is written with LabView 2018. It controls the openSIM add-on and openSIM interface box including LEDs, data acquisition device (DAQ), spatial light modulator (SLM) and temperature control. It comprises of a user interface to enter the necessary parameters, execute commands and to display currently used parameters, executed commands and error messages. Additionally, it can optionally interface additional peripheral components such as a piezo focus positioner, if available.
The main functionalities of the openSIM software are controlling the LEDs (channel selection, intensity), selecting the pattern sequence projected by the spatial light modulator (SLM), actuating the fan and handling the metadata storage. The projected patterns can be selected either manually from a list of patterns or automatically based on parameter inputs from the user. The software saves the used parameters and the used projected pattern sequence the facilitate the image reconstruction. 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.

Difference to software versions with integrated camera control:
It is important to note that there are distinct differences between the software version without integrated camera control (described here in the user manual) compared to the software versions with integrated camera control.
This software version (software version without integrated camera control) is an executable (stand-alone application) which can be distributed without needing the full LabView development version for which a chargeable license is needed. In order run a LabView executable the free LabView Runtime Engine corresponding to the LabView version with which the application was developed is sufficient. To avoid the need of deployment licenses for LabView modules (some are subject to charges) we only integrated LabView modules into the openSIM software for which the software drivers are free of charge.
In this software version, we intentionally did not integrate the camera control into the openSIM software in order to avoid the need for chargeable and camera-specific SDK (e.g. SDK 3 LabView for control of Andor Zyla, iXon camera). This requires the parallel use of the camera control software (for image acquisition) and the openSIM software (for control of the openSIM add-on and interface box). As the imaging data handling (such as displaying and saving acquired image) is linked to the image acquisition, this functionality was removed from this software version is should be handled by the camera software. Besides avoiding the need of chargeable licenses, this openSIM software version has the advantage of being completely independent of the used camera type or manufacturer whereas a software with integrated camera control is inherently camera specific.
The openSIM application in combination with the camera software of the used camera, fulfills the same functionalities than the other software versions.

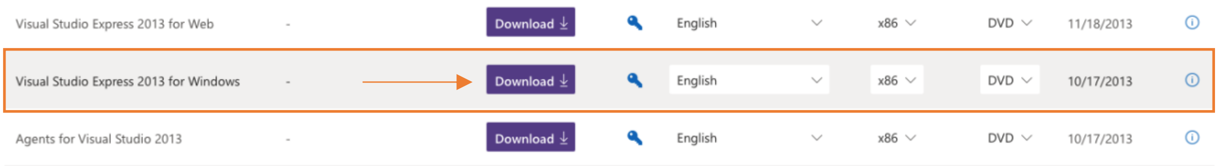
2. Structure of user manual

- The main part of the user interface describes operating the openSIM software using the default settings.
- The appendix contains description of the control of peripheral components, the automatic pattern selection mode and the optional display of status and log information.
- The user interface is categorized in controls (user can enter information, execute commands; indicated with C) and indicators (information are displayed to the user, indicated with I)

3. Installation

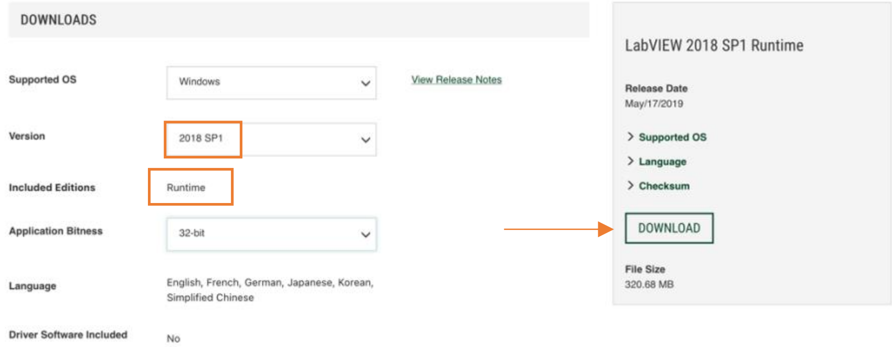
→ for none of the software which are required to be installed in order to run this openSIM software version (without camera control) a chargeable key/license is required

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/

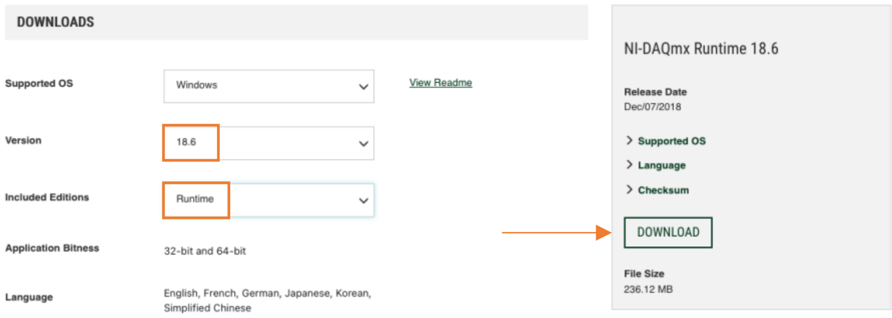


   This will download an ISO file. To access to setup.exe file in order to install the software you can either mount (right-click on ISO file → mount command) the file (opens a virtual drive that is visible in the file explorer) or extract the files (right-click on ISO file → extract)

2. **LabView**
   Install the LabView runtime engine 2018 SP1. The openSIM software was created with LabView 2018 SP1. The version of the runtime engine has to be the same than the LabView version used to create the application.
   Download: https://www.ni.com/en/support/downloads/software-products/download.labview-runtime.html#484336



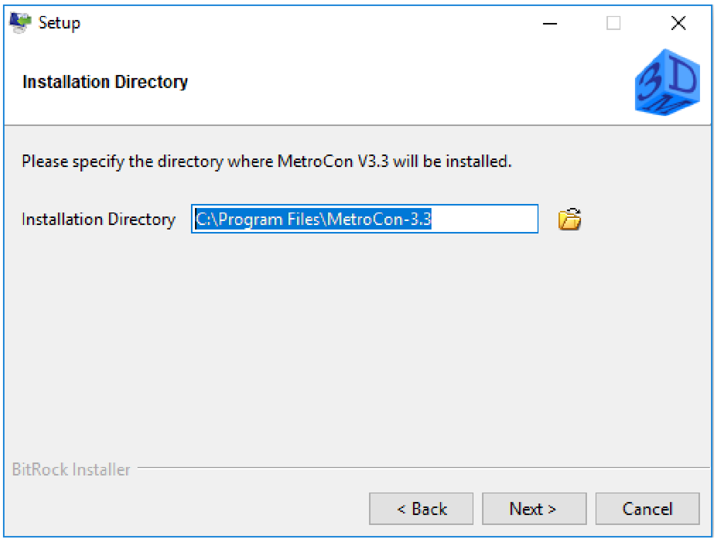
   Install the NI-DAQmx 18.6 driver software.
   Download: https://www.ni.com/en/support/downloads/drivers/download.ni-daq-mx.html#291872



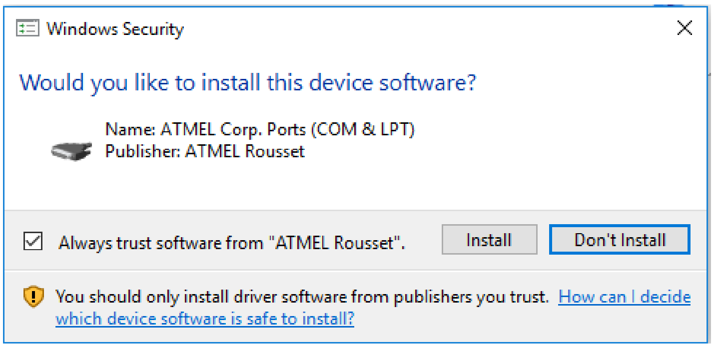
(If you already have NI Package Manager installed, both LabView runtime engine and NI-DAQmx can be also directly installed there. For more information see: https://knowledge.ni.com/KnowledgeArticleDetails?id=kA03q000000YGvpCAG&l=en-CH)

3. **MetroCon 3.3**

   Install 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) (if the window pops up)

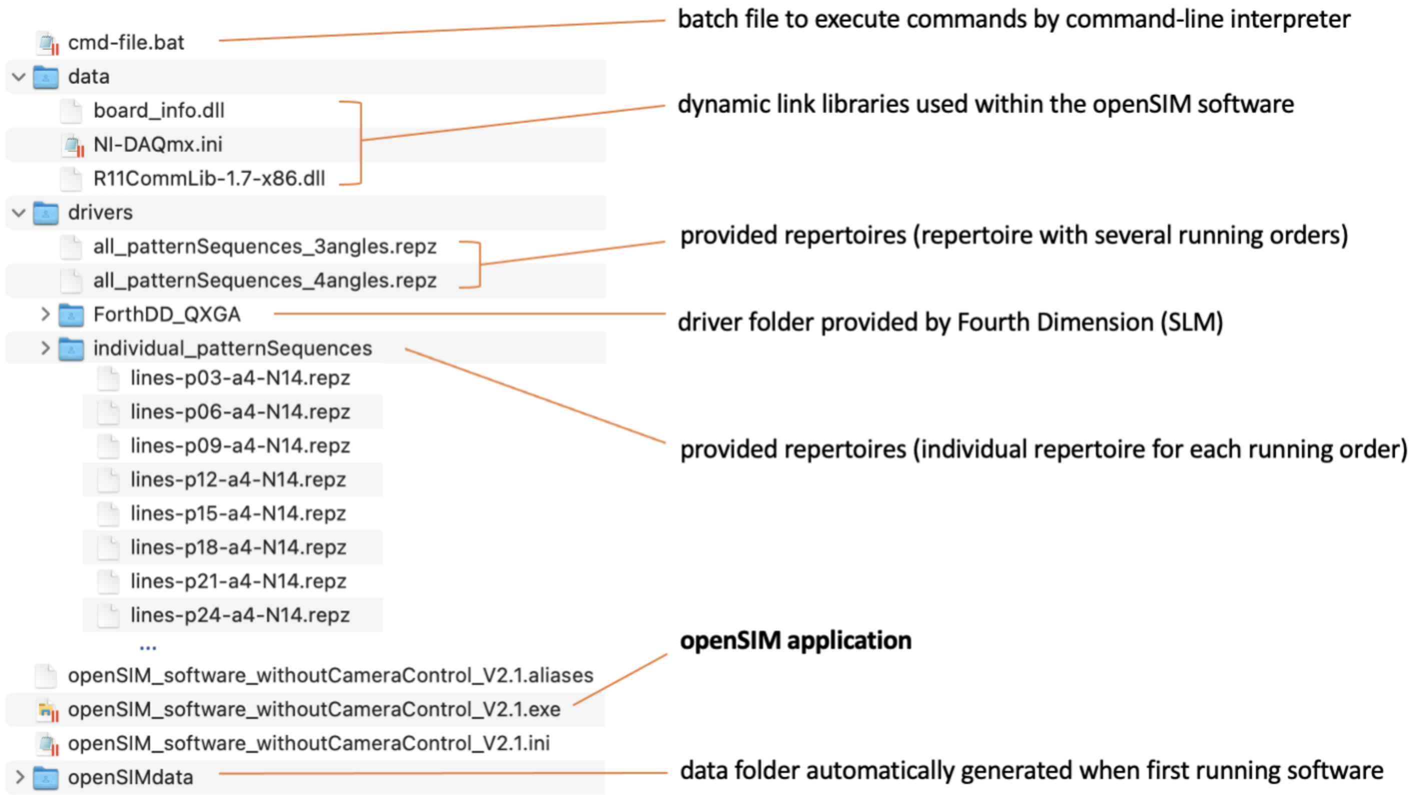


4. Restart the computer after the installation steps
5. Copy the openSIM software folder to a local directory and make sure that the folder structure looks like below

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.

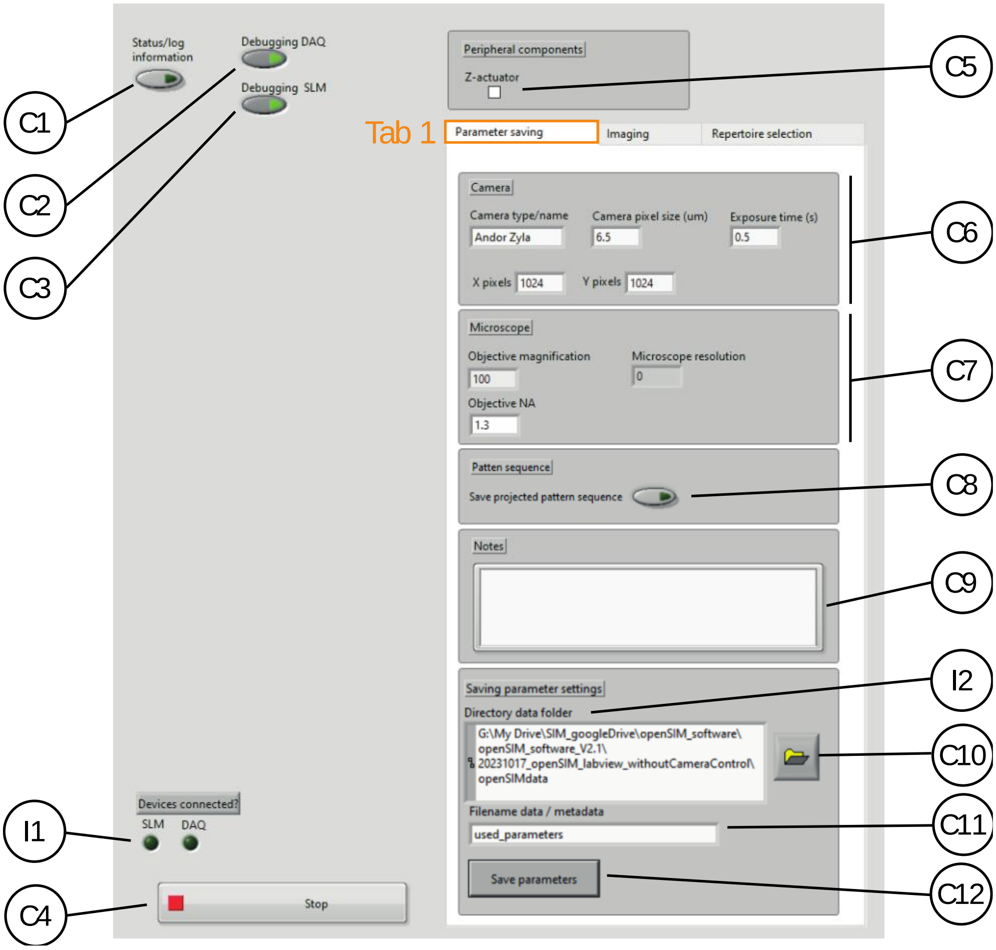
Pattern sequence:

- 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

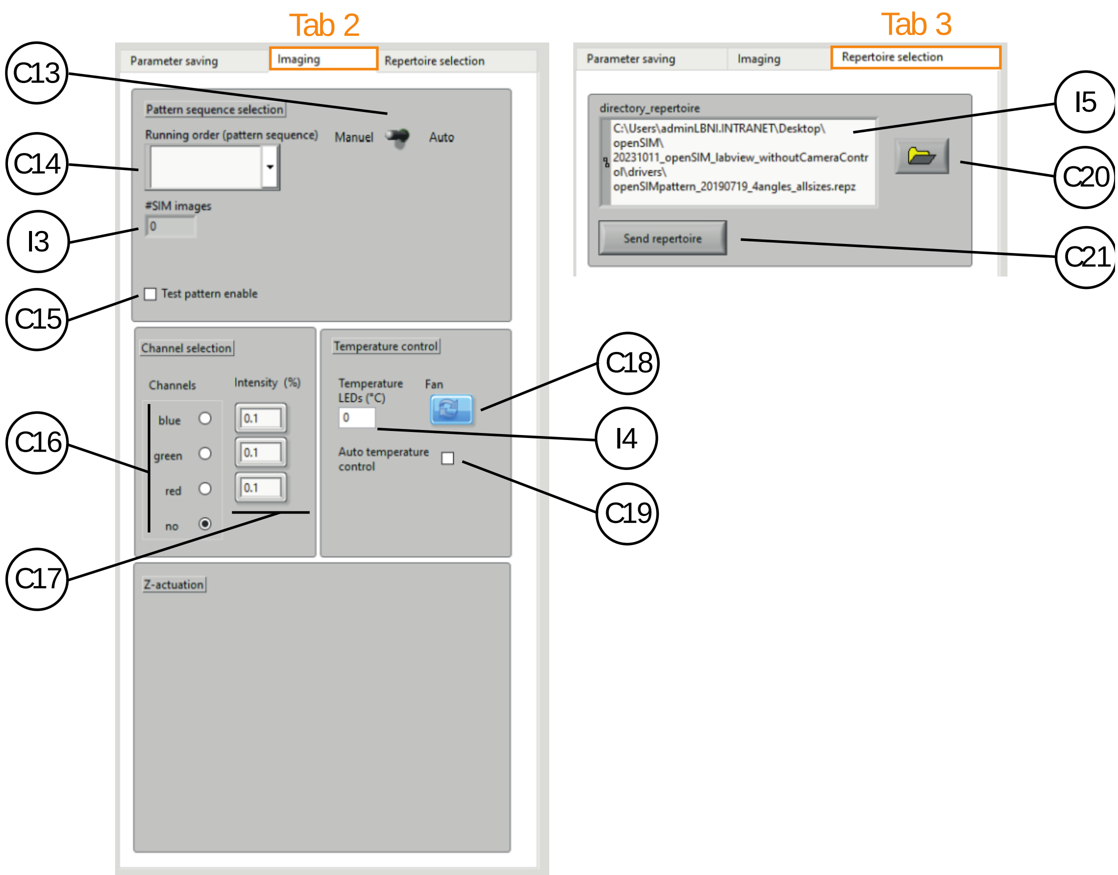
6. Control of the openSIM software with default parameter

6.1 User interface



User interface with default parameter (part 1)

C1	Displaying status / log information
C2	Enabling DAQ debugging mode
C3	Enabling SLM debugging mode
C4	Stop program execution
C5	Selection of available peripheral components
C6	Parameter saving: used camera parameter settings
C7	Parameter saving: used microscope parameter settings
C8	Enable saving used repz file
C9	Parameter saving: additional notes
C10	Selection directory of data folder
C11	Filename txt file with used parameter settings
C12	Saving parameter settings
I1	Indication if devices are connected
I2	Display of selected directory of data folder



User interface with default parameter (part 2)

C13	Selection of automatic/manual pattern selection mode
C14	Selection of running order (pattern sequence)
C15	Enable test pattern
C16	Selection LED channel
C17	Selection of LED intensity
C18	Turn on/off fan
C19	Enable automatic temperature control
C20	Selection directory of repertoire
C21	Load repertoire to SLM
I3	Number of SIM images
I4	Current LED temperature
I5	Display of selected repertoire directory

6.2 Input parameters

→ This section covers only the indicators and controls for the operation mode with default settings
→ For information about the additional indicators and control see the appendix
→ Depending on certain selections/modes some of the indicators and controls are not visible or greyed out. These controls and indicators become visible and enabled when the corresponding mode is selected.

INDICATORS:

I1: Indicator showing if devices (SLM, DAQ) are connected
→ indicator is green if the communication between the openSIM software and the devices is established

I2: Display of the selected directory of data folder
→ shows the path to the data folder
→ folder can be selected/changed with *C10*

I3: Number of SIM images
→ number of SIM images based on selected running order *(C14)*
→ corresponds to the number of patterns in the selected set of patterns and thus the number of images which need to be acquired during the SIM image acquisition

I4: Temperature measured by thermistor at illumination block holding the 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

I5: Display of selected repertoire directory
→ directory selected with *C20*

CONTROLS:

C1: Enabling/disabling displaying of status and log information
→ this mode includes additional indicators which support debugging
→ see appendix for more information about the indicators

Debugging modes:

- In order to facilitate testing and debugging of the software and the individual devices (i.e. SLM, NI DAQ) 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 is not connected). However, in the default (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
- In debugging mode the software does not try to establish a connection to the devices (SLM, DAQ) and device depended functions (e.g. DAQ: change channel, SLM: change running order are not executed)

C2: 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

C3: 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

C4: Stop button to end the program execution
→ the program has to be stopped here and not with the button in the LabView task line (otherwise devices such as SLM and DAQ are not closed properly and some parameters settings are not set back correctly)

C5: Selecting available peripheral component (Z-actuator)
→ specific controls are visible/invisible depending on the availability of the corresponding peripheral component
→ <u>Z-actuator:</u> can be selected if an actuator for changing the z-position of the objective such as a piezo focus positioner is available and implemented (see appendix for more information about the parameter settings and use of automated z-positioning)

Tab1: Parameter saving

- This tab handles the saving of the used parameter settings during image acquisition.
- The information entered here are saved in a txt file and the used .repz file is saved in addition on request. The saved txt file with the parameter description and the .repz file enables the automatic import of information, necessary for the image reconstruction by the SIMToolbox. The txt and repz file structure is created in a way to be compatible with the automatic SIMToolbox reading in of parameter settings.
- The txt file can save additional information which can be useful as metadata of the imaging data.

C6: Used camera parameter settings

- camera type/name
- camera pixel size in µm
- exposure time in seconds
- X and Y camera pixels

→ see the specification sheet of your camera for the corresponding information

C7: Used microscope parameter settings

- magnification of used objective
- NA of used objective
- Microscope resolution (calculated resolution in µm, based on wavelength of selected channel and NA of objective)

C8: Enable/Disable saving of used repz file
→ if enabled the repz file corresponding to the used pattern selected with *C14* is saved in the same folder than the txt file
→ only relevant for SIM image acquisition

C9: Additional notes
→ additional notes added to the txt file together with the parameter settings

C10: Selection directory of data folder
→ default: folder 'openSIMdata' in same directory than openSIM software
→ when using the openSIM software for the first time 'openSIMdata' folder is generated automatically

C11: Filename of txt file containing the parameter settings used during image acquisition
→ acquired image via camera software (tiff file) has to have same file name as txt file for automatic upload of metadata by SIMToolbox

C12: Saving parameter settings
→ the values from *C6, C7, C9* are saved in a txt file with the filename defined by *C11*

→ when save button clicked a new folder in the directory of the data folder *(I2)* is generated with the corresponding date as filename
→ within this folder the txt file is saved with the file name selected with *C10*
→ if *C8* is enabled also the used .repz file is saved in this folder

Tab 2: Imaging

C13: Selection of automatic/manual 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 the uploaded repertoire in the dropdown menu (default)
→ auto running order selection mode: the running order (pattern sequence) is automatically selected based on certain input parameter (see appendix for more information about the automatic running order selection mode)

C14: Selection of running order (pattern sequence)
→ selection from a list of all running orders which are available in the repertoire currently uploaded onto the SLM
→ selected running order defines the pattern sequences actually projected by the SLM

C15: Enabling/Disabling test pattern
→ the test pattern are static patterns which can be used for testing purposes
→ video acquisition mode in camera software should be selected for testing
→ if test pattern is enabled, the control with the list of available test pattern will be displayed
→ different projected test patterns can be selected available test pattern: white, black, bars1, bars2 (pattern with vertical lines), default: test pattern disabled

C16: Selection LED channel
→ color of excitation light (blue, green, red LED)
→ default: no LED selected

C17: Selection of LED intensity
→ LED intensity in %

C18: Manually turning on/off the two fans for LED cooling
→ can be automatically controlled when enabling automatic temperature control *(C19)*
→ fans support the cooling of the LED which are mounted onto an aluminum heat sink extender

C19: Enabling automatic temperature control
→ if the heat sensor at the heat sink measures a temperature above 30°C the fans are turned on and if the temperature falls below 30°C the fans are turned off

C20: Selection of directory of repertoire
→ directory to repertoire in order to send/upload onto SLM

Tab 3: Repertoire selection
→ the repertoire can also be selected and uploaded to the SLM via the MetroCon software

C13: Browse directory to select file path to the repertoires
→ selected .repz file which is loaded to the SLM
→ default: all_repz_files_20190719_4angles_allsizes.repz (distributed in driver folder in the directory containing the application; directory of repz file should not be changed)

C14: Sending repertoire to SLM
→ uploads the repertoire selected with *C13* and displayed at *I5* onto the SLM
→ repertoire has to be sent to SLM only once, afterwards it is uploaded onto SLM
→ sending repertoire has to be done only when using the SLM for the first time or when wishing to change the current repertoire

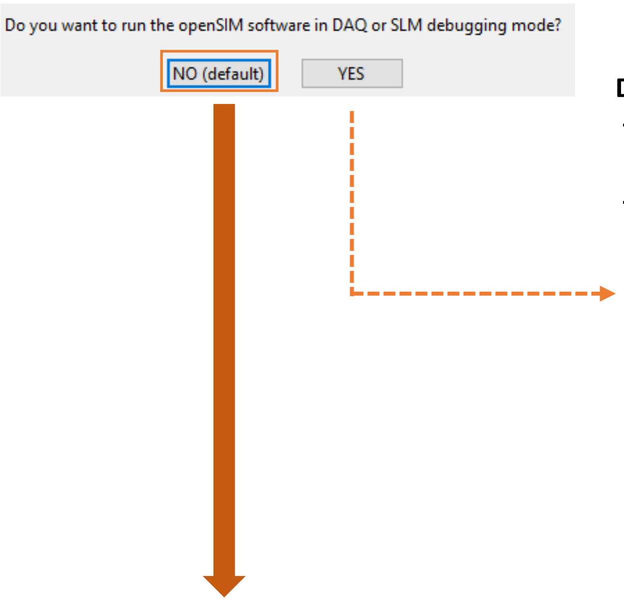
6.3 Operating instructions

Running the software with default settings

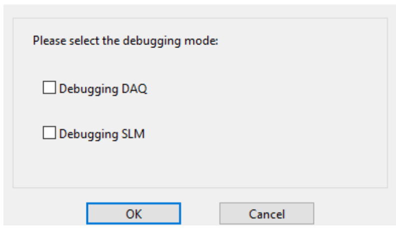
Start the openSIM software

1. Execute the openSIM software application (openSIM_software_withoutCameraControl_V2_1.exe)

2. Select the software mode
→ right after the software user interface opens a window opens in which the software mode (either normal mode or debugging mode) has to be selected
→ default: normal mode (see section 4.1 for more information about the debugging mode)



Debugging software mode:
→ communication to DAQ and/or SLM is not established
→ debugging mode for either one or both devices can be selected



Normal software mode:

Enter and save the used parameter settings

3. Insert the used parameter settings *(C6, C7, C9)* in the fields under 'Parameter saving' (tab 1) based on the microscope and objective used for the image acquisition session
→ the fields camera pixel size, x/y pixel, objective magnification are mandatory information needed for the correct image reconstruction, the other fields are optional

4. If the data should be saved in another folder than the default directory 'openSIMdata', the directory can be changed with *C20*

5. If another file name for the txt file with the metadata than the default 'used_parameter' is wished for, the filename can be changed with *C11*

6. Select the running order in order to define the pattern sequence is going to be projected during the SIM image acquisition in the drop-down menu *C14* under 'Imaging' (tab 2); the corresponding number of images which need to be acquired during the SIM image acquisition is displayed in *I3*

→ naming scheme of running order:

lines-p45-a4-N14

pattern type   period of grating   number of angles   number of patterns

7. click *C12* to create the data folder for this imaging session and save the used parameter and projected pattern in the generated folder

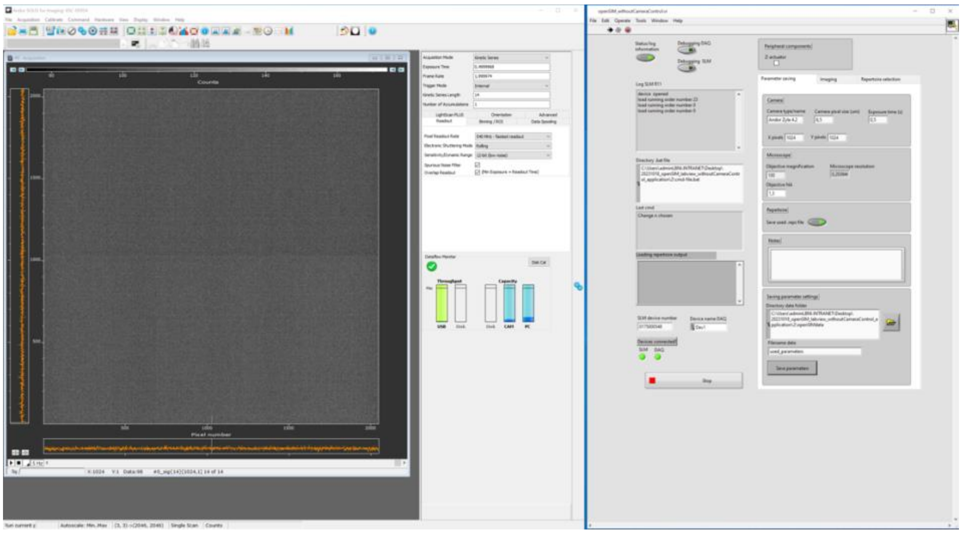
Set LED channel settings

8. Select the desired LED channel based on the used fluorophore *(C16)*
9. Set the desired intensity of the corresponding channel *(C17)*

Start the camera software

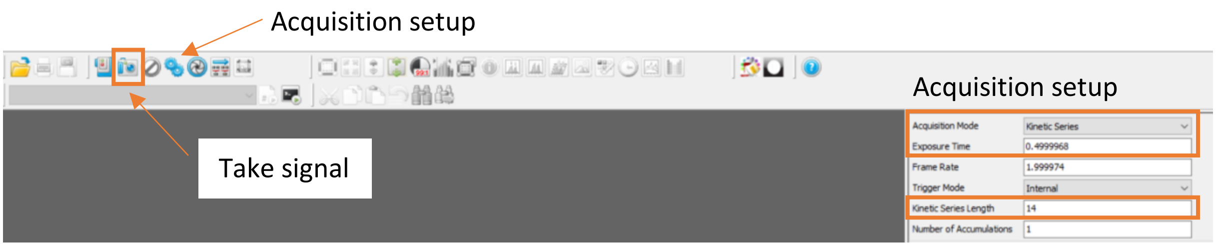
→ we used the Andor control software Solis in parallel to the openSIM software
→ parameters specific to the used camera software are indicated in grey

10. Start the control software of the corresponding camera in use
    → Andor Solis
11. Arrange the openSIM software and camera software next to each other



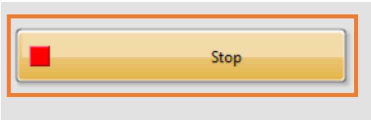
Select the camera parameters

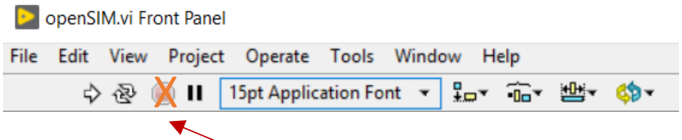
10. Select the triggering mode
    → 'Hardware' → 'Auxiliary Output Configuration' → 'Fire all'
11. Select the data folder generated in step 6 as imaging data directory
    → 'Acquisition Setup'
12. Change the image file name to the file name used for the txt file *(C11)*
    → 'Acquisition Setup'
12. Change the acquisition type from acquisition of a single image to acquisition of a series of images (series/stack of subsequent images saved as one file)
    → 'Acquisition Setup' → 'Acquisition mode' → 'Kinetic series'
13. Set the image series length (amount of subsequently acquired images) according to the selected projected set of patterns *(I3)*
    → 'Acquisition Setup' → 'Kinetic series length' → insert same number as number of SIM images
14. Set desired exposure time
    → 'Acquisition Setup' → 'Exposure time'
15. Acquire a series of SIM images
    → 'Take Signal'



Close the software

16. Close the camera software
17. Stop the openSIM software by clicking the stop button *(C4)* and close the software
    → Important to stop execution by stop button on front panel and not by 'Abort Execution' button in the LabView toolbar





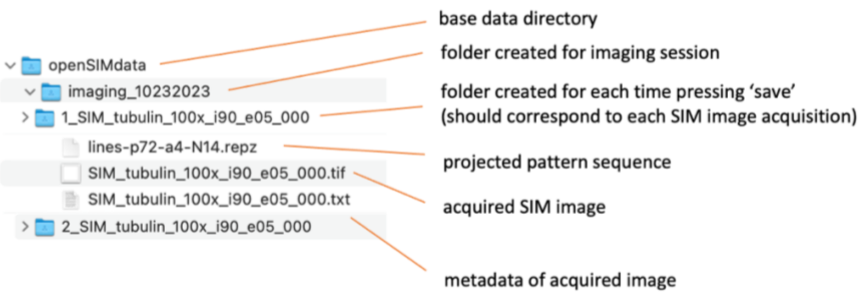
Do not stop the program here!

Note: Repertoire

Repertoires (.repz file) have to be loaded only once to the SLM
→ Selecting repertoire path *(C20)* and sending repertoire path *(C21)* 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

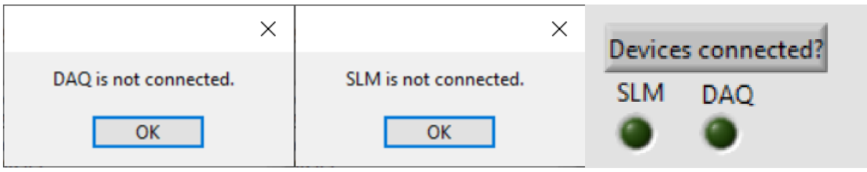
Note: Data handling with default settings

- The base data directory is the folder 'openSIMdata' in the same directory than the openSIM application (when running the openSIM software for the first time this folder is generated)
- Folder for imaging session: When clicking *C12* (to save the parameter) for the first time after starting the application a folder for all the data generated during this imaging session (defined by start and stop of the application) is automatically created with the current date as file name ('imaging_date')
- Folder for individual image acquisition: Within the imaging session folder, a folder with the data of each SIM image acquisition is generated with *C11* and an incrementing number (e.g. '1_SIM_data', '2_SIM_data', …) as file name, such a folder is generated each time *C12* is clicked (having a folder for each SIM image acquisition with corresponding metadata and used pattern sequence ensures the automatic and correct linkage between data and metadata needed for the image reconstruction and facilitates the read in of the data with corresponding metadata with the SIMToolbox)
- → clicking *C12* should correspond to one SIM image acquisition with the camera software (i.e. *C12* should be clicked before each SIM image acquisition execution with the camera software)
  → within this folder the corresponding txt with the metadata and the used pattern sequence are saved besides the imaging data
  → this folder should be selected as directory for the imaging data in the camera software
- The acquired images should be saved in tiff format (camera software acquisition settings)
- 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 of the data structure

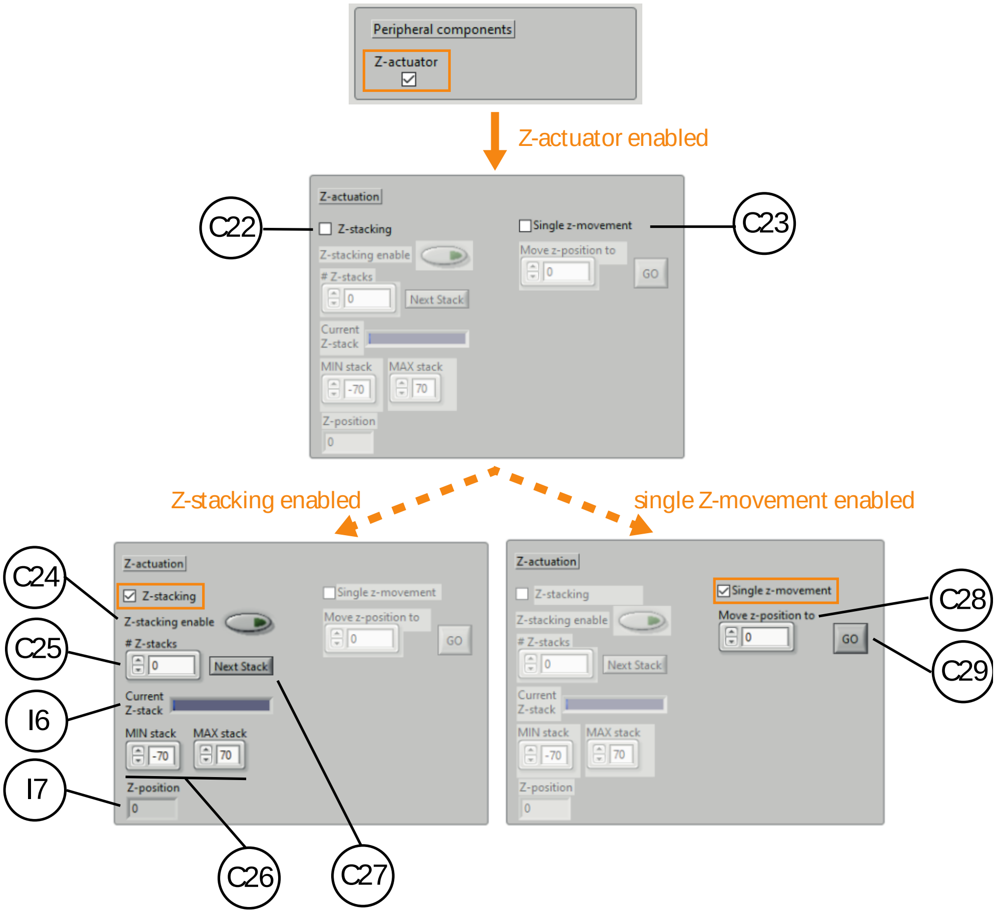
7. Troubleshooting

Problem	Possible reason	Possible Solution
It is not possible to connect to the NI DAQ and/or SLM → error message(s), *I2* does not light green and software automatically stops execution) → if the openSIM software cannot establish the communication with the SLM and/or DAQ 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 *(C4)*. By clicking the user interface stop bottom it is assured that the NI DAQ and SLM 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 openSIM LabView application and camera software and reopen them. If this does not solve the problem close the applications and dis- and reconnect the USB connection between the individual devices (SLM, DAQ) and PC. Then start the applications again. If it is still not possible to connect to the devices, close the applications and restart the computer.
 		
When executing the openSIM application for the first time, it is not possible to connect to the DAQ despite the device being connected	The NI DAQmx software driver is not installed The NI DAQmx version is older than the LabView runtime engine version	Download the free NI package manager and check if the driver is installed Check in the NI package manager if the NI DAQmx version 18.1 (or newer) is installed In order to check if the NI DAQ USB 6001 is detected by the PC, the 'Windows Device Manager' or 'NI Measurement & Automation Explorer – NI max' (free software which provides access to NI hardware; information how to download NI Max can be found here). NI max can also be used to troubleshoot the DAQ control independent of the openSIM software via If the NI DAQ does not appear in the NI DAQmx, you can find further support here.

When executing the openSIM application for the first time, it is not possible to connect to the SLM	Another version than Microsoft Visual Studio 2013 is installed.	You may encounter compatibility issues if you attempt to run the openSIM software with a different version of Visual Studio. You can find the Visual Studio Express 2013 download here. A license/key is not needed for the Express version. If this does not solve the problem, try to connect to the SLM via the MetroCon software itself. There one can test connecting to the SLM and uploading a repertoire independent of the openSIM software.
It is not possible to load a repertoire to the SLM	The directory of the .bat file is not correct (see section 3), or the bat file is missing.	The repertoire can alternatively be selected and uploaded to the SLM via the MetroCon software instead of using the openSIM software.

Appendix

Peripheral components (Z-actuation)



C22	Selection z-stacking as z-actuation mode
C23	Selection single z-movement as z-actuation mode
C24	Enabling z-stacking
C25	Total number of z-stacks
C26	Motion range of z-stacking
C27	Go to next stack position
C28	Desired z-position within motion range of z-actuator
C29	Execute single z-movement
I6	Display current z-stack number
I7	Display z-position

An external objective positioning system can be used to precisely adjust the z-position of the objective. In the openSIM software we implemented options to control a z-actuation device in order to automatically move the objective to a certain position ('single z-movement') or automatically subsequentially move the z-position of the objective to specified positions in order to acquire a z-stack of the sample ('Z-stacking'). We used the piezo actuation system MIPOS 100 (Piezosystem Jena) as z-actuator. The implemented z-stacking/single z-movement uses an analog signal via the DAQ device to control the piezo focus positioner. 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. The currently implemented motion range limits are based on the MIPOS 100 system. Please see schematic below on how to mount the MIPOS 100 actuator.

→ When 'Z-actuator' *(C5)* is selected under 'Peripheral components' the parameter settings for 'Z-stacking' *(C22)* and 'Single z-movement' *(C23)* become visible but greyed out
→ Depending on the selection of 'Z-stacking' *(C22)* or 'Single z-movement' *(C23)* the corresponding settings get enabled

Additional Indicator and Controls

I7: Display z-position
→ current position of z-actuator in µm during z-stacking

C22: Selection z-stacking as z-actuation mode
→ when selected corresponding settings *(C24, C25, C26, C27, I6, I7)* are enabled and possible to set
→ relative to mid-position (half of the piezo motion range), mid-position is used as initial position

C23: Selection single z-movement as z-actuation mode
→ when selected corresponding settings *(C28, C29)* are enabled and possible to set
→ 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

C24: Enabling z-stacking
→ enables the z-stacking mode
→ after inserting the necessary parameter for z-stacking *(C25, C26)* z-stacking should be enabled in order to subsequently execute the z-movements for the z-stack

C25: Total number of z-stacks
→ Defines the number of desired z-positions for the z-stack

C26: Motion range of z-stacking (minimum, maximum) in µm
→ defines the first (at minimum position of motion range) and last (at maximum position of motion range) position of the z-stack

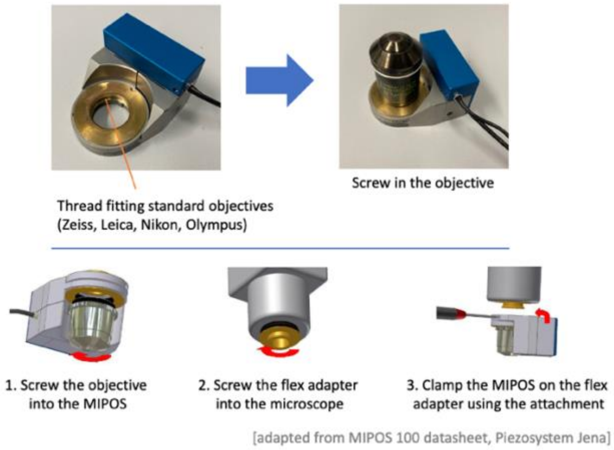
- min: z-position of first stack relative to mid-position
- max: z-position of last stack relative to mid-position

→ depending on the selected number of stacks *(C25)* the distance between the individual stack is evenly distributed (operating principle is depicted in schematic below)
→ the selected motion range has to lie in the physical limits of the used z-actuator
→ the current limits are set to -70µm and 70µm based on the used MIPOS 100

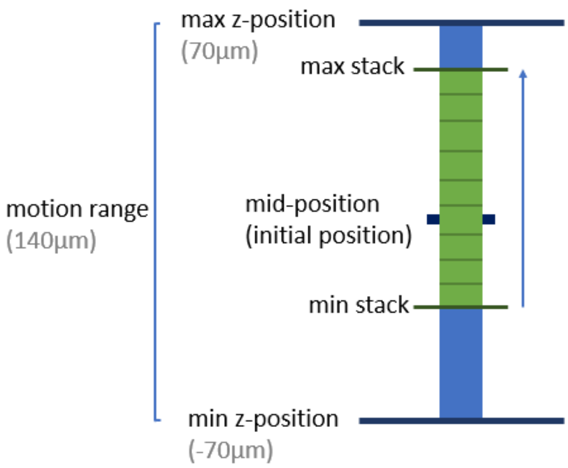
C27: Go to next stack position
→ as the image acquisition is initiated externally with the camera software, the command for the next step has to be issued manually
→ after each acquired SIM image (via the used camera software) the next stack (subsequent z-position) has to be initiated by clicking the button (also for first stack at *C26* min)

C28: Desired z-position within motion range of z-actuator
→ focus positioner moves to inserted z-position in µm relative to mid-position by clicking *C29*
→ value has to be within motion range of piezo focus positioner, for MIPOS 100 -70µm –70µm

C29: Execute single z-movement

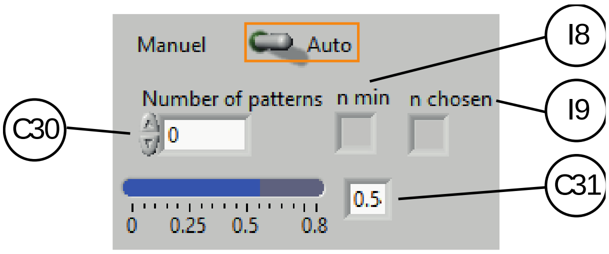


Images depicting how to mount MIPOS and objective



Schematic illustrating z-stacking via piezo focus positioner

Automatic pattern selection mode



C30	Desired number of patterns
C31	Slider to select period of grading
I8	Minimal possible period of grading
I9	Selected period of grading

Running order (pattern sequence) selection modes
→ default: manual mode (if manual mode is selected the parameter settings necessary for automatic running order selection mode are not visible, as soon as automatic mode is selected the parameter settings become visible and enabled)
→ manual mode: the user has to select the desired running order (pattern sequence) from the available list of running orders *(C14)* of the currently uploaded repertoire *(I5)* manually, the selectable pattern sequences are defined by the repertoire uploaded to the SLM
→ auto mode: the running order (pattern sequence) is automatically selected based on the selected period *(I9)* and the desired number of projected patterns within the sequence *(C36)*

I8: Calculated minimal period
→ minimal spatial period of pattern which can be applied with the used microscope and objective
→ based on calculated microscope resolution, camera pixel size and magnification of objective *(C7)*

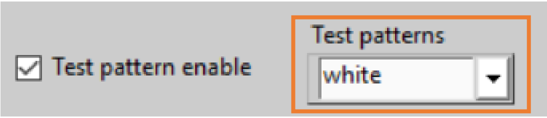
I9: Selected spatial period
→ running order (pattern sequence) is automatically selected from list of available running orders based on the selected spatial period
→ selected based on minimally usable spatial period *(I8)* and slider *(C31)*

C30: Desired number of patterns in the set of patterns
→ defines the number of SIM images per SIM image acquisition *(I3)*

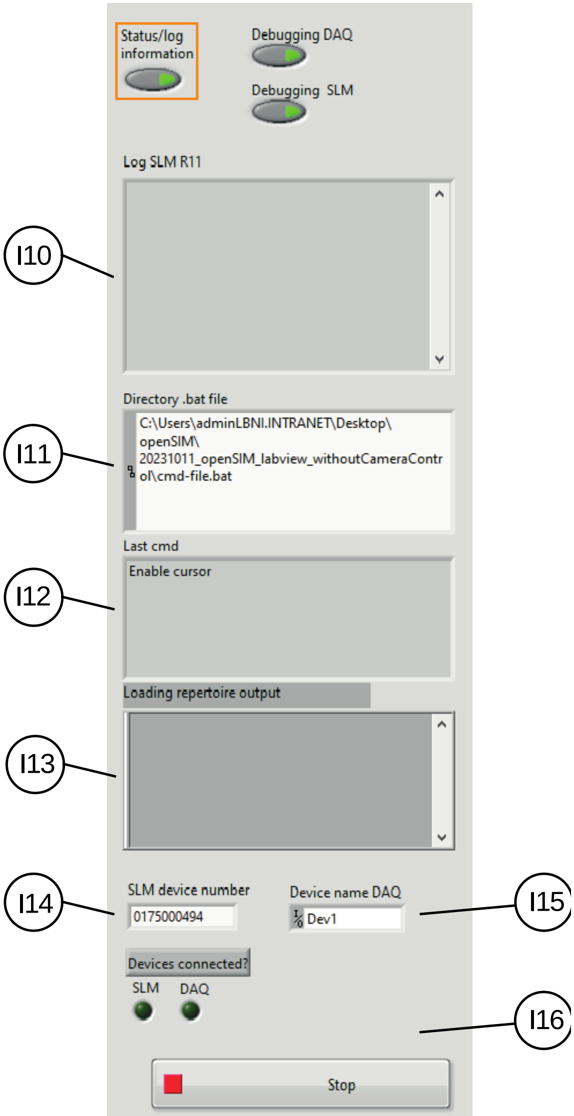
C31: Slider in %
- → determines how close the period of the grating is to the minimal possible value *(I8),* selected value is displayed on the right (relevant for auto running order selection mode)
- → maximal 80% possible

Test pattern

- For test purposes the SLM can project static patterns. Currently four test pattern are implemented (white, black, bars1, bars2 (pattern with vertical lines))
- When test pattern (C15) is enabled, the dropdown list of available patterns becomes visible and when test pattern is disabled the list becomes greyed out
- In contrast to the sets of patterns for SIM imaging, the pattern is static and does not change
- To project the pattern the camera acquisition mode of the used camera software should be used



Status/log information



I10	Display current status of SLM
I11	Directory of .bat file
I12	Last executed command
I13	Text which was passed to command line
I14	Device number SLM
I15	Device name of DAQ
I16	Indicator if software is running

Additional Indicators:

I10: 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

I11: Directory of .bat file
→ the bat file (distributed with the openSIM software) contains commands for the SLM in order to load the repertoire
→ the directory of the bat file should not be changed

I12: Display showing the last executed command in the openSIM program

I13: 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)

I14: Device number of connected SLM

I14: Device name of connected data acquisition (DAQ) device
→ device name is shown when USB cable of DAQ device is connected to the PC