# Live-SR Super Resolution/TIRF Standard Operation Protocol



HKU LKS Faculty of Medicine Centre for PanorOmic Sciences 香港大學泛組學科研中心

IMAGING AND FLOW CYTOMETRY CORE CENTRE FOR PANOROMIC SCIENCES THE UNIVERSITY OF HONG KONG

# Contents

| Turn on System  |
|---|
| Set the Temperature and CO2 Control for Live Cell Imaging |
| Sample Locating and Focusing4                             |
| Image Acquisition   |
| Live SR(Super-resolution) Imaging                         |
| TIRF Imaging7   |
| FRAP9   |
| Ablation10  |
| Targeted Laser Calibration10                              |
| FRAP/FLIP/Ablation Experimental Protocol11                |
| Timelapse13   |
| Multi Positions   |
| Perfect Focus System (PFS)14                              |
| Z Series15  |
| Review Acquired Images16                                  |
| Turn off System17   |
| 6   |

# Live-SR Super Resolution/TIRF Standard Operation Protocol Basic Operation

### **Turn on System**

Please sign on the log sheet before switching on system.

- Switch on main power control on the wall (1), leave 10 seconds before next step
- Switch on main power control on the wall 2, leave 10 seconds before next step
- Switch on main power control on the wall (3), leave 1 mins before next step
- Turn on computer power (4),



- Click to log into **USER** account at the startup screen. The password is below the PC screen. leave 1mins after Windows startup.
- Start the MetaMorph software

1 min

### Set the Temperature and CO2 Control for Live Cell Imaging

(Only applicable for live cell imaging, please skip this step if it is not needed):

- Switch on the **Power** of Tokai Hit incubation system controller. Temperature can be altered via pressing the green button of each heating parts on the touch screen.
- Make sure the CO<sub>2</sub> sliding button is turned ON.
- Key in the temperature of interest, then press Enter. (Please do change temperature back to default setting as shown blow if any modification is made)



Tokai Hit incubation system controller

- Turn on CO<sub>2</sub> tank by turning the main switch **anticlockwise**.
- Turn on CO<sub>2</sub> regulator by turning regulator **clockwise** to set output pressure at 100kPa, till the indicator points to red line which represent 100 kPa.
- Put on objective heater on objective if oil objective is used.
- Metal ball floats is an indication of the presence of gas supply.
- Add MilliQ water into the water chamber and covered if overnight(s) acquisition is required.



20x Ablation

60x TIRF

100x CSU

100x TIRF

100x Ablation

100

### Sample Locating and Focusing

- Select objective
  - select  $100 \times \text{TIRF}$  or  $60 \times \text{TIRF}$  for TIRF imaging
  - select 100×ablation or 20×ablation for ablation
  - select 100×CSU for super-resolution imaging
- Apply a drop of immersion oil if oil objective is used
- Place your sample, make sure the coverslip of the slide is facing down
- To view Brightfield under the eyepiece, click Eyepiece →select Trans or DIC → select Current Shutter → push the Bright Field knob up/down to increase/decrease the light brightness.



• To view Fluorescence under the eyepiece, click Eyepiece → select WF LED Tri → select Current Shutter → to adjust intensity, choose XCite 6%, XCite 12%, XCite 25%, XCite 50%, XCite 75% or XCite 100%. Or click XCite%, input number into the space and click OK.

| Comercia           |                     | XCite Intesnity %    | ×       |
|--------------------|---------------------|----------------------|---------|
| Camera             | WF LED Tri          | XCite Intensity:     |         |
| •••                | Current Shutter     | 5                    | 6       |
| en cyepiece        | Current Shutter     | ОК                   | Cancel  |
| XCite 6% XCite 12% | XCite 25% XCite 50% | XCite 75% XCite 100% | XCite % |

- Press the right arrow of the DISP button 4 times to find X: Y: and Z: position panel.
- Move the Stage Controller to adjust XY position (XY speed can be adjusted by XY<sup>2</sup> button: <sup>(A</sup> > <sup>A</sup> > <sup>A</sup>)
- Focus the sample with the focusing knob → Clockwise\_Down; Anti-clockwise\_Up (Focusing speed can be adjusted by Z<sup>^</sup> button:



### **Image Acquisition**

#### Live SR(Super-resolution) Imaging

- Select 100xCSU objective. Apply one drop of immersion oil on the objective.
- Click MDA on the task bar
- Go to Main>check Load State
- Click Select All>check Load > choose CSU.MDS from Desktop>NDSTATE folder> check Open



- Click **Saving** → **Select Directory** (all data should be saved in D drive/USER under your name)
  - Type in the base name of your file (experiment or date or etc.) in Base Name.
  - Do not use digits at the end of the base name, a digit will be added by the system according to the acquisition sequence.
  - Another suffix will be added for record time series image (t1, t2....) or multi-stage-position image (s1, s2....).

| lain         | Description: *In | nages automatically saved with base file* |  |
|--------------|------------------|---|--|
| Saving       |                  | ~   |  |
| Wavelengths  |                  |   |  |
| W1: CSU DAPI |                  | ~   |  |
| W2: CSU GFP  | Select Directory | D:\llear Data                             |  |
| W3: CSU mChe | Sciect Directory | Increment base name if file exists        |  |
| W4: Trans    | Base Name:       | experiment13                              |  |
| Display      |                  | -   |  |

- If multiple fluorescence channels are required,
  - Check the box of "Multiple wavelengths" in the main menu
  - Click Wavelengths
- Key in the **number** of channels in "Number of Wavelengths
- Select the proper wavelength to set the required "Illumination" from the illumination dropdown list.
  - Select "CSU DAPI" for Blue emission (e.g. BFP) channel
  - Select "CSU GFP" for Green emission (e.g. GFP) channel
  - Select "CSU mCherry" for Red emission (e.g. mCherry) channel
  - Select "CSU Cy5" for Farred emission (e.e. Cy5) channel.







- Preview the image on screen by clicking Live review the intermedium image (image before processing)
- Adjust below parameter for each channel
  - Laser power (Click S button on Modular to open laser power
  - adjustment panel) Exposure time in Exposure

|   | /lult                                      | i Dimensional Acc | quisition     |                         |
|---|--|-------------------|---------------|-------------------------|
| м | ain  |                   | Mumination :  |                         |
|   | Sa   | ving              | illumination. | CS0 DAPI V              |
|   | Wavelengths<br>W1: CSU DAPI<br>W2: CSU GFP |                   | Gain:         | Gain 1 (HDR) V          |
|   |  |                   | Digitizer:    | 100 MHz (16-bit) $\sim$ |
|   |  |                   | Exposure:     | 150 🜩 ms 🗸              |
|   |  | W2: CSU mChe      |               |                         |

Laser power adjustment panel

S

?

ð

• Two methods as below to acquire SR image

#### Method 1

- Set up 2.5times higher laser power or elongate exposure time to 2.5 times compare to intermedium image.
- Click Acquire Acquire on MDA to get image.
- Open ND file in Review MDA
- Select intermedium images (which has no SR at the end of file name) and click Process to get the SR images.

#### Method 2

- Click live to review intermedium image for each channel.
- Set up 2.5times higher laser power if the sample is not easy to be bleached.
- If the sample is easy to be bleached, elongate exposure time to 2.5 times.
- Click Setup MDA.
- Click Snap to take a processed Live SR image for current channel.
- Click Acquire Acquire on MDA. Both intermedium image and SR image will be acquired and save to your selected directory.

#### **TIRF Imaging**

- Select 100xTIRF objective. Apply one drop of immersion oil on the objective.
- Click MDA (Multi Dimensional Acquisition) on the task bar
- Go to Main>check Load State
- Click Select All>check Load to choose TIRF.MDS from Desktop>NDSTATE folder> check Open

| File Edit Regions   | Stack Acquire Devices Display  | Process Log I   | Saving<br>Wavelengths<br>W1: TIRF mCher<br>W2: TIRF GFP<br>W3: Trans<br>Display<br>Summary | Timelapse Multiple Stage Positions Multiple Wavelengths Z Series Stream Run Journals Use Dual Z Motors Con   | figure   |  | Summa<br>Save St<br>Load St |
|---|--|---|--|--|--|--|-----------------------------|
| Load MDA State<br>Camera Regions and Image Positions<br>Main: Series to be performed<br>Main: File name and directory<br>Time Lapse: Timelapse<br>Stage: Stage Positions<br>Wavelengths: Illumination and Algoment<br>Wavelengths: Exposures and Collection<br>Wavelengths: Z Series / Auto Focus |  | Image: Second state of the second | ate<br>top > NDSTATE<br>ADS<br>t MDA.MDS<br>scen.MDS<br>IDS                                | ✓      ✓ | Search N<br>Type<br>MDS File<br>MDS File<br>MDS File<br>MDS File | X<br>IDSTATE<br>Size<br>Size<br>15 KB<br>15 KB<br>15 KB<br>15 KB<br>15 KB<br>15 KB |                             |
|   | Stream: Burnation Consignation     Stream: Dimensions to stream     Stream: Dimensions to stream     Stream: Burnination     Stream: Storage and Camera Mode     Journals     Select All Clear All Load     Cancel | File name: *.MDS  |  | ~<br>  | *.MDS  | Cance  |                             |



Image and Flow Cytometry Core Centre for PanorOmic Sciences, HKU

- If multiple fluorescence channels are required,
  - Check the box of "Multiple wavelengths" in the main menu
  - **Click Wavelengths** \_
  - Key in the number of channels in "Number of Wavelengths" \_
- Select the proper wavelength to set the required "Illumination" from
  - Select "TIRF DAPI" for Blue emission (e.g. BFP) channel
  - Select "TIRF GFP" for Green emission (e.g. GFP) channel \_
  - Select "TIRF mCherry" for Red emission (e.g. mCherry) channel
  - Select "TIRF Cy5" for Farred emission (e.e. Cy5) channel.
- Click Saving  $\rightarrow$  Select Directory (all data should be saved in D drive/USER under your name)
  - Type in the base name of your file (experiment or date or etc.) in **Base Name**.
  - Do not use digits at the end of the base name, a digit will be added by the system according to the acquisition sequence.
  - Another suffix will be added for record time series image (t1, t2....) or multi-stage-position image (s1, s2....).

| Saving       Images addomatically saved with base me         Timelapse       Wavelengths         W1: TIRF GFP       Select Directory         W2: TIRF mChei       D:\User Data         W3: TIRF Cy5       Base Name:         W4: TIPE DAPI       Eest | Main                               | Description: *Ir | nagoo automatical             | h any od with hone file* |  |
|---|------------------------------------|------------------|-------------------------------|--------------------------|--|
| W1: TIRF GFP       Select Directory       D:\User Data         W2: TIRF mChei       Increment base name if file exists         W3: TIRF Cy5       Base Name:       test   | Saving<br>Timelapse<br>Wavelengths |                  | nages automatical             |                          |  |
| W3: TIRF Cy5 Base Name: test  | W1: TIRF GFP<br>W2: TIRF mCher     | Select Directory | D:∖User Data<br>☑ Increment b | ase name if file exists  |  |
| WE HAD DEFI   | W3: TIRF Cy5<br>W4: TIRF DAPI      | Base Name:       | test                          |                          |  |

🗯 Bin: 1

Gain:

Digitizer:

Exposure:

Bin: 1 + Gain 1 (HDR)

100

100 MHz (16-bit)

\$

ms

1:TIRF GFP

V

~

• Preview the image on screen by clicking Live  $\square$  at the bottom of "multi-dimensional acquisition" and adjust the focus and parameters (Exposure Time, Laser Power, TIRF angle).



Main



Target Intensiti

Carline Acquire

•

- Click double double arrow glyph icon over the "360 TIRF" on the right panel
- Adjust laser power by moving the slider bar for each laser
- Adjust TIRF angle for each laser by dragging the edge of the circle from center to edge. The actual Angle and Penetration Depth are shown below the circle.



• Click Acquire at the bottom of MDA right to start acquisition.

#### FRAP

- If you are using **TIRF with FRAP**, please load **TIRF configuration** in MDA. Then choose **TIRF objective**.
- If you are using CSU with FRAP, please load CSU configuration in MDA. Then choose FRAP cube and CSU objective.



Ablation (Only applicable for ablation, please skip this step if it is not needed):

- Choose 100xablation objective 🦉 100x Ablation
- Choose ablation cube
   Ablation Cube
- Turn on 355nm laser.



Please be noted that 355nm lasers are hazardous for eye/skin exposure. For safety reason, the 355nm will go standby mode (shutter will switch off) when change to eyepiece mode.

- Switch on laser by turning key clockwise for 90 degrees (5)
- The lights of POWER ON will flash for a few seconds.
- Press red button before acquisition. \*

\*Two lights of LASER ON will on continuously means 355nm laser is ON. Laser can only be switched ON under camera mode and go standby under eyepiece mode. You need to press red button to start laser every time when change back to camera mode.



#### **Targeted Laser Calibration**

- Replace with plastic fluorescence slide.
- Preview the focused image on screen by clicking Live at the bottom of MDA
- Select "Calibration" on the right black bar below the "TARGET"



#### Image and Flow Cytometry Core Centre for PanorOmic Sciences, HKU

- Set exposure time to 100 ms and laser power of FRAP to 0% or 0.5% (lower than 1%).
  - Click on the icon

to activate the targeted laser.

- Through MetaMorph live window, move the light spot in the blue grid calibration area to place the laser spot to the top left corner by dragging the Grey Point 2. Then dragging the Grey Point 3 to bring the laser spot to the bottom right corner.
- Click on the calibration button to begin calibration.
- When calibration is done, click on the save icon to save the calibration setting to your folder.



#### FRAP/FLIP/Ablation Experimental Protocol

- is a tool which enables to interactively target the laser while being in live mode.
- Click and expand the function of
- Click on the certain position in the MetaMorph live window to activate the laser.
- Set the certain laser power to 5% for a trail
- Select to define the duration length of targeted laser.
- Select b to define the replication times of targeted laser. Each time takes 0.65 ms. If you set replication times as 2, the total duration shown under LIVE will be 1.30 ms.
- Set the proper diameter of laser spot and duration.



- Preview the image on screen by clicking Live at the bottom of MDA and adjust the focus and parameters (Exposure Time and Laser Power).
- Mark the region of interest (ROI) through using the region tools  $\boxed{ROS \land V \land C \textcircled{C}}$  at the top of MetaMorph, then add one ROI by click + button or add multiple ROIs by click + button.

- Adjust bleaching parameters.
  - No. of repetitions
  - Laser power (405nm/488m/561nm/639nm for FRAP, **355nm** for ablation)
  - Line Thickness (for Line only)
  - The testing bleaching could be done by click laser activation button



- To setup FRAP, choose FRAP from the dropdown list<sup>1</sup>, set up Interval (time interval between two time points) and N Time Points (number of time points) <sup>2</sup> for Pre-sequence and Post-sequence acquisition (maximum 2 different post-sequences are allowed) <sup>3</sup>. The Interval and N Time Points of 2 post-sequences can be different.
- Click Setup MDA to import the parameters into MDA window in metaMorph



- Click on the Acquire icon on MDA to begin acquisition.
- To set up FLIP, choose FLIP from the dropdown list (1), set up Interval (time interval between two time points) and N Time Points (number of time points) for Pre-sequence(2) and Post-sequence acquisition (3), and repetition time **4**.
- Click Setup MDA **5**to import the parameters into MDA window in metaMorph
- Click on the Acquire icon on MDA to begin acquisition.

acquisition time point  $\rightarrow$  Set the

• Give a Position Label for your stage

positions; (Label name should be

• Click Start Contiguous Focusing tab

or press PFS button on the control

panel to start perfect focus system.

subsequence position.)

(x, y) and focus level (z)

#### Timelapse

do

**Multi Positions** • Choose Stage

>> 😳 🗢 IARGEI 0 % 0% 561 639 15000 10000 5000 time [ms] FLIP N Time Points 2 0% 0% Þ 0 ms FRAP/PA N Time Points 5 🐥 Post-sequence 1 1 0 % for 3 🗘 times (4)

488

- Multi Dimensional Acquisition • Set up "Time interval" between each Main Experiment Length Saving Number of time points ÷ **Duration** of the entire experiment or Timelapse  $\sim$ Duration 1 🜲 ms Number of time points, either one will Stage Wavelengths Time Interval 1 ÷  $\sim$ ms W1: TIRF mChe Estimated minimum interval: 2860.00 ms W2: TIRE GEP Interval specified is below calculated minimum W3: TIRF DAPI W4: Trans - • × Multi Dimensional Acquisition ended with digit "1". The number will Main Position Labe be automatically updated to record the Saving Position 1 Timelapse X٠ -4575 ÷. Stage -35.2 ÷ AF Offset Wavelengths 4737.96 🜲 7. 9753 1 Stop Continuous Focusing W1: TIRF mChe -0.0031 🖨 Z2: W2: TIRF GFP • Use "Live" mode to find the position Offset Z for travel to this position 0 ÷ W3: TIRF DAPI Stage list is empty W4: Trans Distance: None Positions: • Click "+" to add the position (x, y, z) Display Summary
- to the end of position list. • To overwrite recorded position, change the Position Label to the same as existing one, highlight the one to be overwrote and click " $\rightarrow$ ".
- Use advanced stage position/wavelength table acquisition para • To insert a new position between two existing positions, change a distinct Position Label, highlight the one to be inserted afterward and click " →".

٠

×

t

Move to Position

Sort

Load.

- To delete recorded position, highlight the one to be deleted and click " $\times$ ".
- Click Acquire in the bottom to start acquisition of necessary.

#### Perfect Focus System (PFS)

|                |      | Nikon               | LOF  | RESET |        |
|----------------|------|---------------------|------|-------|--------|
| ⊷-ݢֶׂ-<br>DISP | XVZR | EL/PFs:ER1          | Waza | Q     |        |
|                | Z    | 483.72um            |      | (XY)  |        |
| UP PFS         |      | 3 4<br>XY 🖹 Sp      |      |       | buttor |
| Zŝ             |      | (i)                 | . (  | 21    |        |
|                |      |                     | Spe  | ed    | - A.   |
|                |      | Stage<br>Controller |      |       |        |

front panel



• The status of the PFS is displayed on the PFS indicator on the front panel and the LCD of the joystick:

| PFS indicator    | PFS     | Shown on    | PFS operating       | Details   |
|------------------|---------|-------------|---------------------|---|
|                  | on/off  | the display | status              |   |
| On               | PFS on  | PFS: ON     | Perfect focusing is | The PFS is maintaining the focal point.                           |
|                  |         |             | in progress         |   |
| Blinking at      | PFS on  | PFS: DIS    | Waiting for         | When the interface is detected within the allowable focusing      |
| slow intervals   |         |             | interface detection | range by moving the focusing position, the PFS is                 |
|                  |         |             |                     | automatically turned on to start perfect focusing.                |
| Blinking at fast | PFS off | PFS: OFF    | Perfect focusing is | The interface is detected within the allowable focusing range.    |
| intervals        |         |             | off.                | Turn on the PFS to start perfect focusing.                        |
| Off              | PFS off | PFS: OFF    | Perfect focusing is | The interface is not detected within the allowable focusing       |
|                  |         |             | off.                | range. In this case, turning on the PFS places it in an interface |
|                  |         |             |                     | detection waiting state.  |

Principal of Perfect Focus System (PFS)

The PFS is used to keep focus for long-term observations by using near-infrared rays.

The allowable PFS focusing range refers to the range defined for each objective (where PFS is usable). When using an air objective, the boundary surface between cover glass and air will be detected. When using oil immersion objectives, the PFS will define the reference interface between liquid and coverslip. There must be culture solution in the imaging chamber.

If sample cells attached to the cover glass of a glass-bottomed dish, the thickness of cover glass should be ranged between 150 to 180  $\mu$ m, and refractive index is 1.5. The culture solution should be at least 3 mm and 1.33 refractive index.

#### Z Series

- Click "Nikon Z" in the tool bar.
- Tick "Z Series" in main menu
- Choose Acquire wavelength set at each Z or Acquire Z series for one wavelength at a time

Method 1: use "Range around current" mode

- Tick "Range Around Current"
- Focus the center of your object
- Set up "Step Size" for distance between each focus plane
- Set up "Number of Steps" for the total number of planes
- Click Acquire

| Multi Dimensional Acc  | quisition   |
|--|---|
| Main   | Interactive settings  |
| Saving   | Current Position: 2981.7 🜩 um Increment: 0.5 束                                    |
| Wavelengths  | Cattions for any idition and a  |
| W1: CSU GFP  | Loop order  |
| Z Series   | ○ Acquire wavelength set at each Z  |
| Display  | Acquire 2 series for one wavelength at a time     Keen shutter open between stens |
| Summary  |   |
|  | Range: 20 🔄 🗹 Range Around Current  |
|  | Top: 2991.7 🜩 Set Top To Current  |
|  | Bottom: 2971.7 🜲 Set Bottom To Current  |
|  | Step Size: 0.2 🜩 Center Around Current  |
|  | Number of Steps: 101  |
|  |   |
|  |   |
| 100 Bn: 1 🐳 🏴 E  | Acquire Ctr   |
| Bin: 1 : E   | Acquire Ck  |
| Bin: 1 : E<br>Multi Dimensional Ac<br>Main   | Sin: 1  CSU GFP  Kext  Cx  Cx  Cx  Cx  Cx  Cx  Cx  Cx  Cx                         |
| Bin: 1 C Bin | Acquire Ck  |
| Bin: 1 C Bin |   |

Range Around Current

Previous

1:CSU GFP

 2991.7 
 ➡
 Set Top To Current

 2971.7 
 ➡
 Set Bottom To Current

0.2 🗘 Center Around Current

Range

Top:

Botton

🗯 Bin: 1 🛊 🎬 Bin: 1 🛊 🔳 🔳

Step Size

Number of Steps: 101

nmended Step Size: 0.2 um

Version 1.1 2023

#### Method 2: use "Top" and "Bottom" mode

- Tick off "Range Around Current"
- Find any one end of your sample with fine focus, click "Set Top To Current"
- Find the other end of your sample with fine focus, click "Set Bottom To Current"
- Set up "Step Size" or "Number of Steps" for distance between each focus plane
- Click Acquire

Acquire Close

### **Review Acquired Images**

- Click **Review Multi Dimensional Data** in the Task Bar after Images Acquisition
- Choose your folder in **Select Directory** and select an image **Data set (base name +suffix. nd)** and then click **View**
- Select the Wavelength acquired to be displayed.
- Display a single image by clicking any single grid.
- Select Stage position in the pull down menu.
- To review series images, left click the header number of the **Row** or **Column** for displaying images of **Time series** or **Z-series** respectively.
- Then click Load Image (s)
- To export series images as movie, please refer to MetaMorph analysis software protocol.



- To Overlay images of different channels, check the Color Composite box in the Display tab and then assign corresponding channel to the RGB color to composite an overlay image.
- To stack all plans in a z-series to create a single 2D image, choose Maximum projection in Z Projection tab and check the Z Projection box.

| Selections [X's] Display Z Projection   Event marks |               |     |                |               |   |               |   |
|---|---------------|-----|----------------|---------------|---|---------------|---|
| Full Image  | Active Reg    | ion |                |               |   |               |   |
|   | Red:          |     | Green:         | Blue:         |   | Gray:         |   |
| Color Composite                                     | <none></none> | *   | Confocal Blı 💌 | <none></none> | * | <none></none> | ~ |
|   |               |     |                |               |   |               |   |

| Selections [X's] Display Z Project | tion Event marks                                 | 3  |
|------------------------------------|--|--|
| Z Projection                       |  |  |
| Type: Maximum 🗸                    | Orientation                                      | Border Color: 0 Create Rotation          |
| Angle: 0                           | <ul> <li>Horizontal</li> <li>Vertical</li> </ul> | Z Distance<br>Calibrated Ouser Specified |
| Planes: 1 0 to: 2 0                | All Planes                                       | Z Dist.: 1.5 🛟                           |

### **Turn off System**

Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you.

- If you used live cell chamber and CO<sub>2</sub> controller. (Please skip this step if it is not needed)
  - Take off objective heater on objective
  - Switch off temperature and CO2 controller.
  - Turn off CO2 tank by turning the main switch clockwise
  - Turn off CO2 regulator by turning regulator clockwise to the end

# Temperature and CO2 controller



- If oil objective lens is USED, it must be cleaned thoroughly with the LENS **PAPER** instead of Kimwipes.
  - Oil residue from the objective lens should firstly be removed using a DRY lens tissue.
  - Repeat this step with a new area/piece of the lens cleaning tissue until no oil Streaks are seen on the tissue.
- Switch objective to empty position in the hardware and set to "Lower Z-limit".
- Exit MetaMorph software.
- Transfer data to CPOS imaging and cytometry storage server and shut down the computer.
- Switch off laser power (5) by turning key anticlockwise for 90 degrees if you used ablation. (Please skip this step if it is not needed)
- Switch off laser power (3), leave 10 seconds before next step.
- Switch off power (2), leave 10 seconds before next step.
- Switch off main power (1).
- Sign on the logbook and put on the microscope cover.







