Optical Tweezer Standard Operation Protocol
Basic Operation

Centre for PanorOmic Sciences

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

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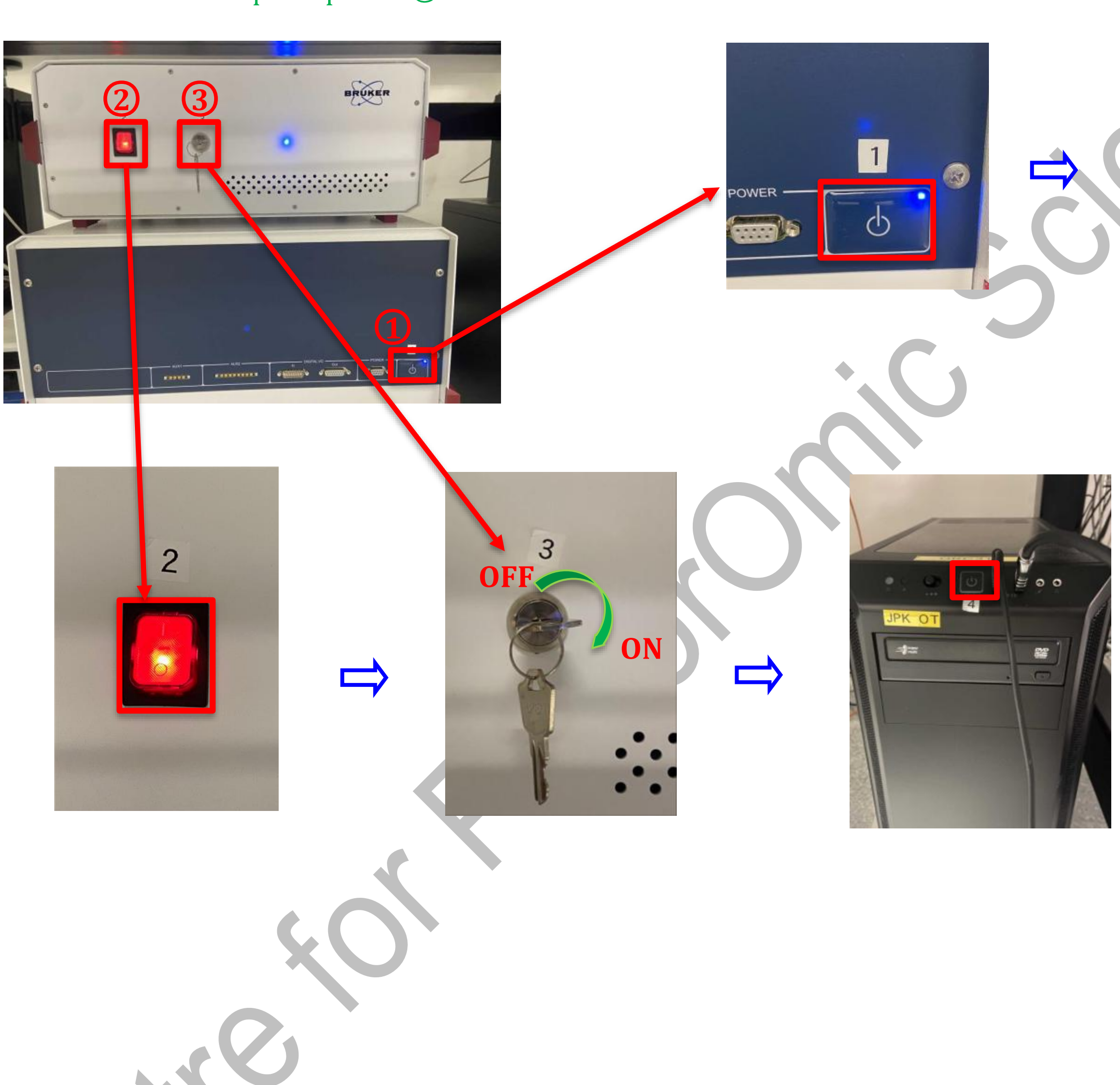
Turn on System

Please sign on the log sheet before switching on system.

1. Turn on main power control ①
2. Switch on main power control ②
3. Turn on laser power by switching key clockwise for 90 degrees ③
   (Please leave 3 minutes gap before turning on the computer)
4. Turn on computer power ④



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5. Turn on another main power control 5 on the wall ⑤
6. Press the button 6 to turn on the power of laser 488 and 561 ⑥
7. Press the buttons at the back of laser suppliers. (B1 for laser 488, C1 for laser 561)
8. Wait for 30 seconds, until the lasers warmed up and shown standby, turn the keys clockwise for 90 degrees. (B2 for laser 488, C2 for laser 561)
9. When actual value achieves 100% and the Laser status turns to READY, turn on the TIRF computer ⑦
10. Press the button to turn on the vibration isolation.

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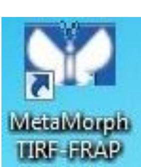
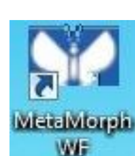
Software Initialization

1. Log on computer with "User" account (Please find User account password posted under the computer screen)
2. Double click the JPK NT Desktop to open the software on Linux (left computer)

3. Start the MetaMorph software on Windows (right computer)

– For TIRF users, click **MetaMorph TIRF FRAP** icon
– For widefield users, click **MetaMorph WF** icon





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Set the Temperature (Optional)

(Please skip this step if it is not needed)
To heat the whole acoustic enclosure,

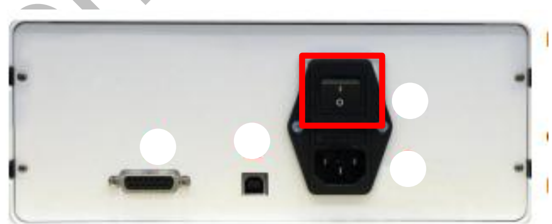
- Turn on the power of T-controller (the power button is on the wall at the left back of the equipment, and T-controller is on the acoustic enclosure) ⑨
- Press the **red button** of the T-controller for turning on the temperature ⑩
- Turn the **black switcher** clockwise to max for turning up the wind before reach to the target temperature.
- Set the temperature on the black screen by pressing the arrow keys below **the LED display**
- When temperature reaches expected value and becomes stable, turn down the wind to min by turning anticlockwise.

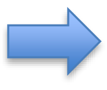
To heat the driver on the stage (for culture dish only),

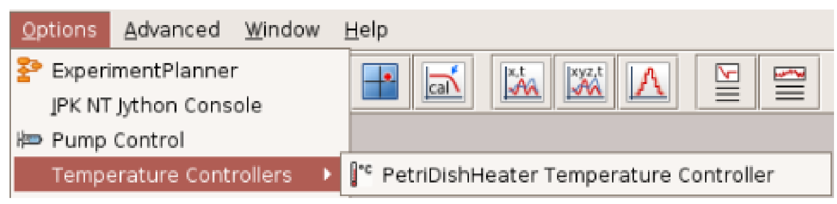
- Turn on the Petridish heater (on the vibration isolation left side of microscope) by pressing the **button** on the backside
- Press the red button near the temperature screen to start heating
- If the different temperature is applied, go to software interface open the temperature control panel:

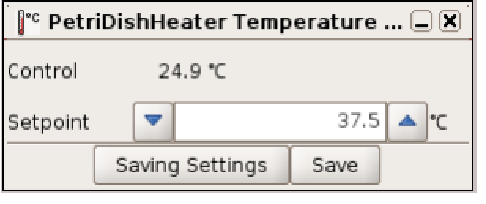
Options -> Temperature Controllers ->PetridishHeater Temperature controller -> Set the expected temperature -> Save

- Please set back to default temperature (37 °) after using.









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Lightpath Selection

If fluorenscence is applied under wide field, turn switcher (behind the microscope) to LED WF mode.

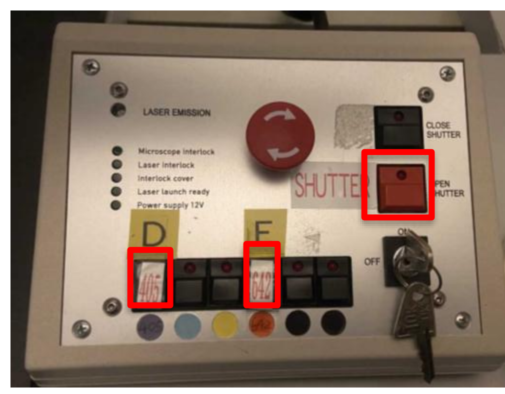
- Then turn the filter wheel to the proper position
  - If DAPI channel is applied, turn the filter wheel to Position 2
  - If GFP channel is applied, turn the filter wheel to Position 3
  - If RFP channel is applied, turn the filter wheel to Position 4
  - If Cy5 channel is applied, turn the filter wheel to Position 5

Figure 1 Example of Position 3 for GFP channel

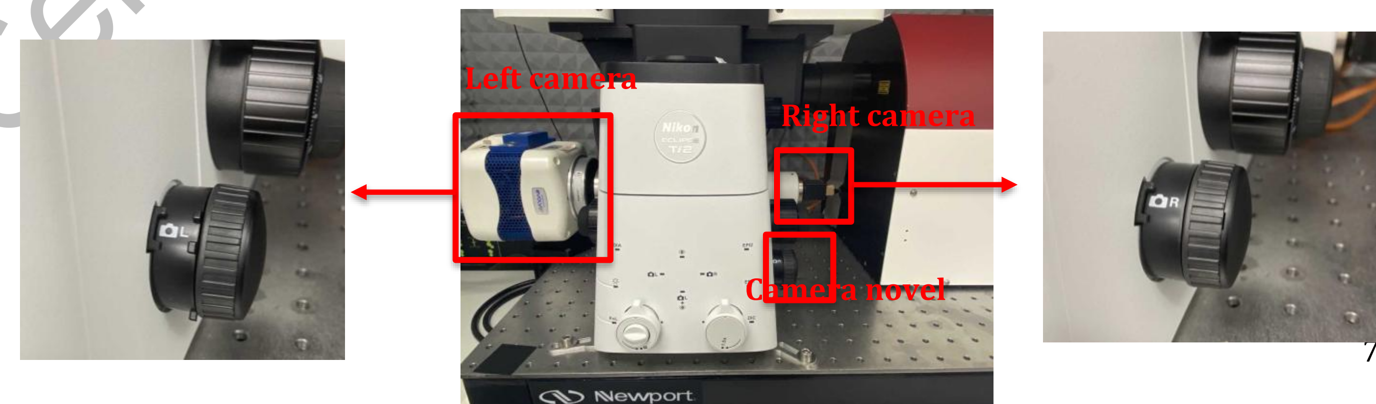
Figure 2 Example of Position 4 for RFP channel

If TIRF laser is applied, please turn switcher to TIRF mode.

- Turn the filter wheel to Position 1
- Press the red shutter to turn on the laser 488 nm and 561 nm
- If laser 405 nm is applied, press button D to turn on DAPI channel. If laser 642 nm is applied, press button E to turn on Cy5 channel.



To control cameras,
If the signal is detected by right camera (Optical tweezer/JPK), spin camera novel to R mode.
If the signal is detected by left camera (TIRF/Metamorph), spin camera novel to L mode.



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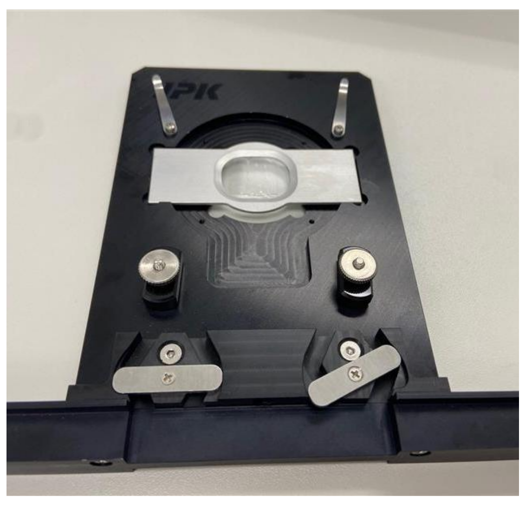
Sample Preparation

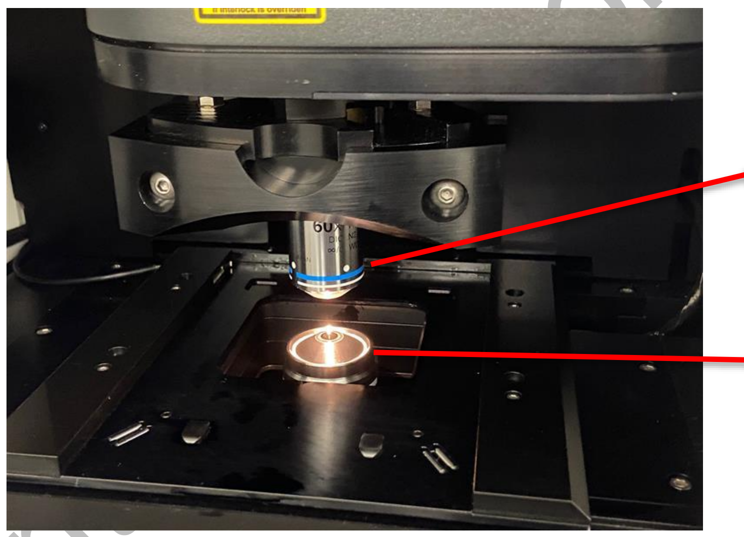
The sample should be sealed in channels by nail polish or paste.

- Make sure the cover slip face down to the objective.
- Mount the sample onto the slider with holder. The left one below is used for glass slides, and the right one below is applied for culture dishes.

(Please keep the clamps away from the central region to avoid against the detection objectives when shifting)



- Apply a drop of immersion oil on the trapping objective
- Slid the slider into NanoTracker head



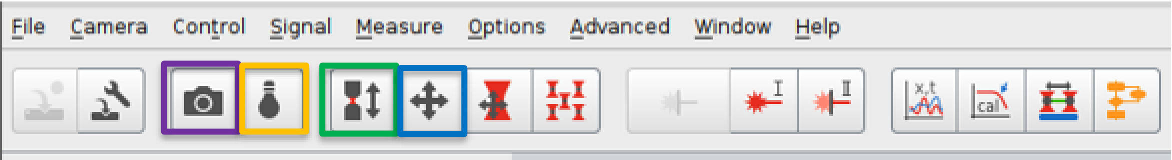
Detective Objectives

Trapping Objectives

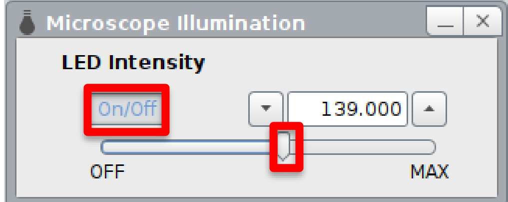
Objective Info:

Detective objective		**Trapping objective**	
Water Dipping		Oil Objective	
Magnification 60x		Magnification 100x	
NA 1		NA 1.49	
Working Dist. 2.8		Working Dist. 0.16-0.09	

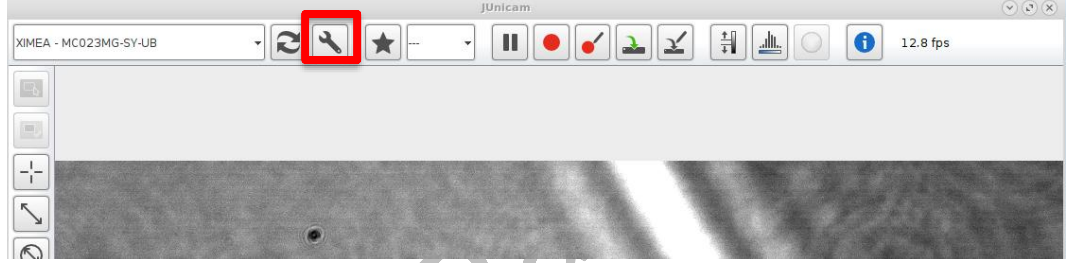
- Open up **Junicam** , **Microscope Illumination** , **Objective Positioning** and **Sample Positioning** the menu bar.



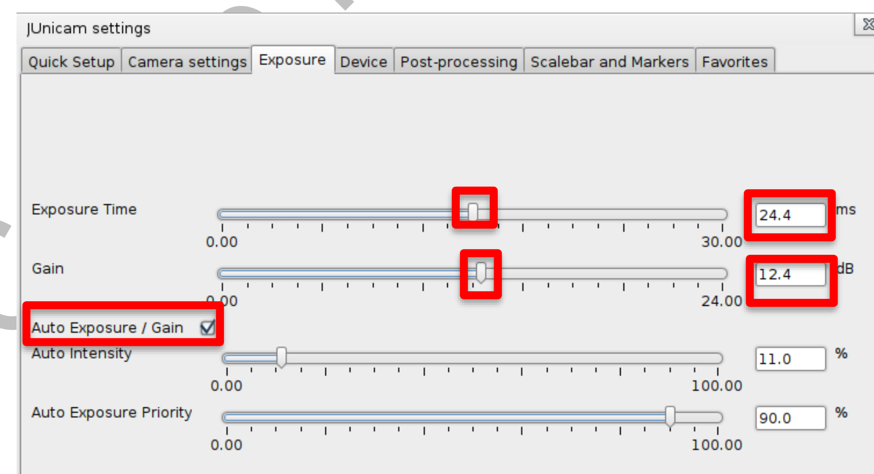
• Drag the bar of the **microscope illumination** towards MAX (right side) to increase LED intensity and drag the bar to the left side to turn down LED intensity. To turn off the LED, drag the bar to OFF or click **On/Off**.



• If the image is still not obvious with maximum LED intensity, exposure time can be set for display. Click the **spanner** in the **JUnicam** to open Junicam settings.



• Click **Exposure** to set exposure time and gain. Tick off Auto Exposure/ Gain before adjusting exposure time and gain. Drag **the slider of exposure** to right side to incrase the exposure time. Oppsite direction will decrease the exposure time. Otherwise, type the **exposure time** in ms into box.
• Drag **the slider of gain** to right side to incrase the gain. Oppsite direction will decrease the gain. Otherwise, type **Gain** into box.



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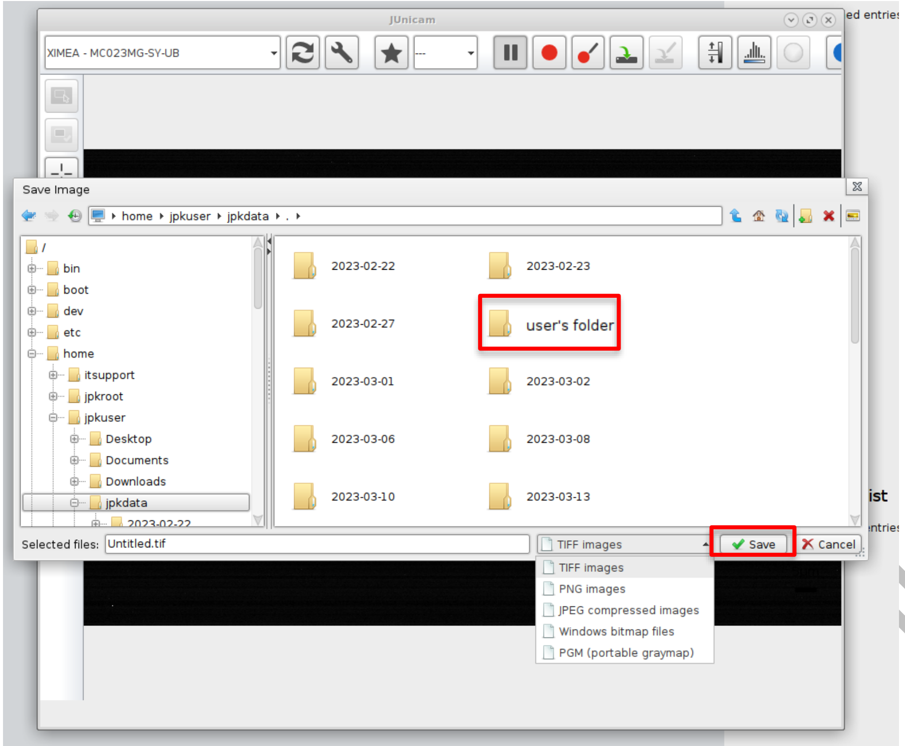
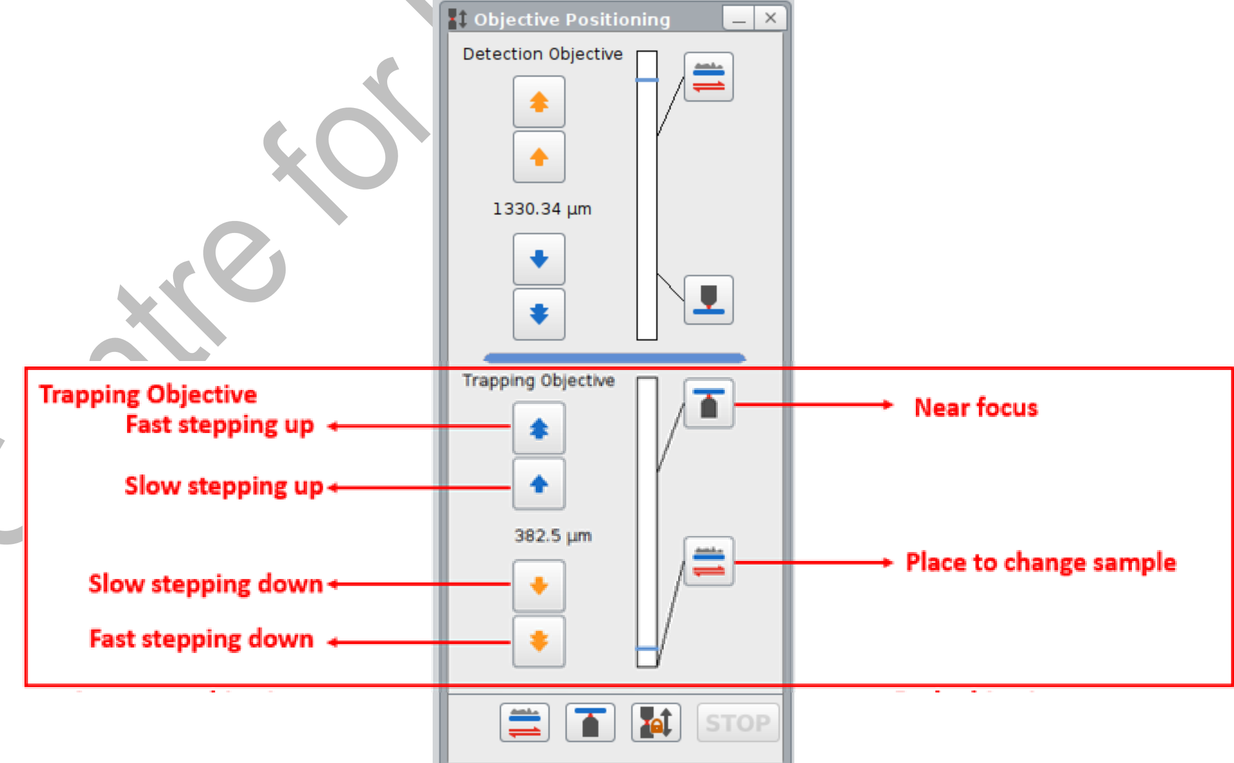
- Images from JUnicam can be saved by **Save** and **Save as** buttons in the toolbar.
- Create a folder named as [Your name] in jpkuser. Then select the proper file format and click **save**.



- Click **fast stepping up** of trapping objective to move up the trapping objective close to the sample. Until the lens attached to the slide bottom, switch to **slow stepping up**. Keep moving up trapping objective, the image of sample will become clear when focus is arrived.



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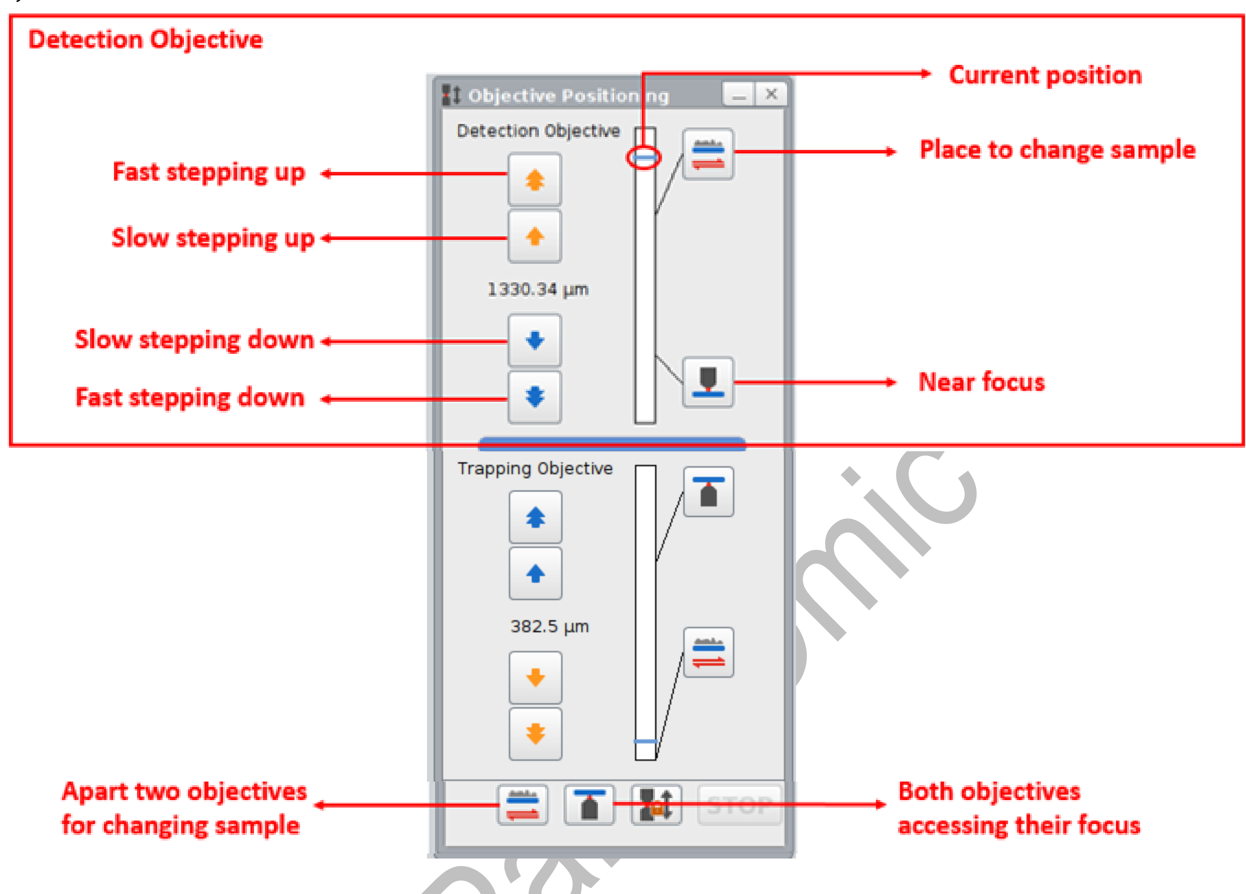
• If the glass slide is applied, drop water on the sample surface, then move down detection objective by **fast stepping down** until it accesses the water on the cover slip. If the culture dish is applied, ensure the objective is in the water or media.



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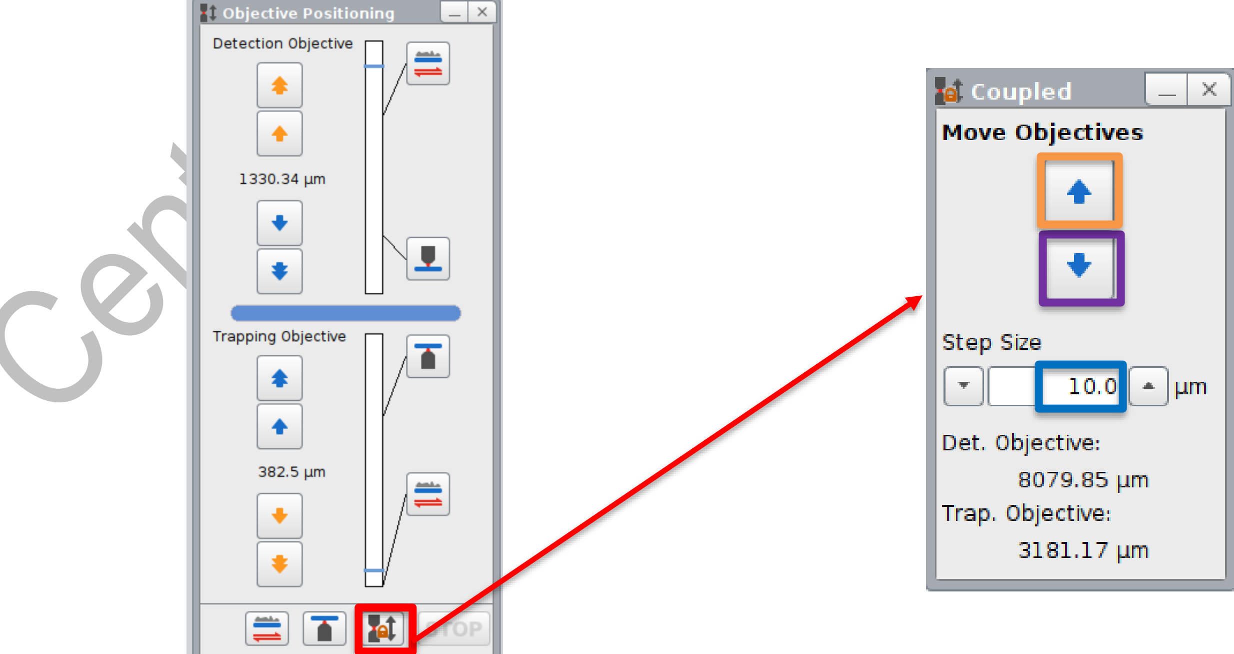
• Close the **field aperture diaphragm** (switch to Min) on the NanoTracker head to visualize it in the field of view ①.

• Next, rise detection objective until the edges of hexogen turn clear ② and open the field aperture (move back to Max) ③.

• If the confocal dish is applied, ensure the liquid in dish over 2ml. Move down detection objective by **fast stepping down** until it accesses the water. Close the **field aperture diaphragm** (switch to Min) on the NanoTracker head to visualize it in the field of view ①. Then, keep dropping down detection objective with **slow stepping down** until the edges of hexogen turn clear ② and open the field aperture (move back to Max) ③.

Please do not lift, drop or move anyone of the objectives in objective positioning after the steps of defining the focus.

• If the objective position must be adjusted, move the **coupled objectives** together by **typing step size** then click arrows for **moving up** or **down**.



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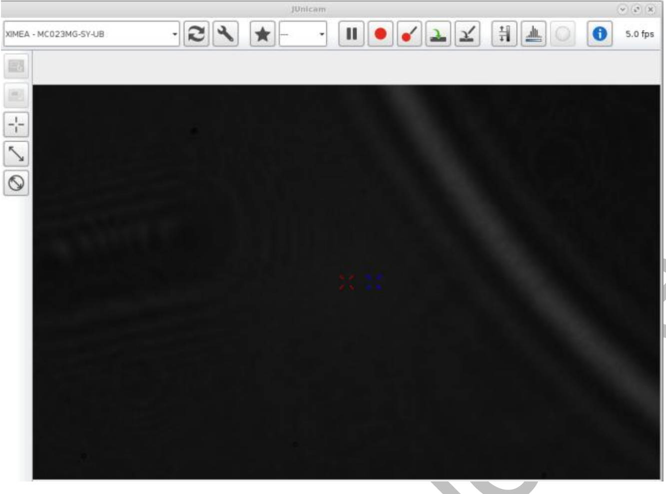
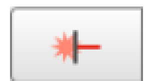
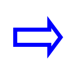
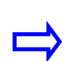
Beads Trapping

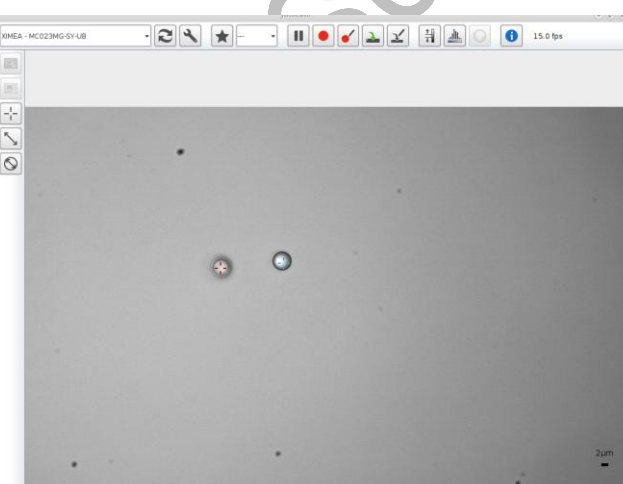


• In live image window, the red cross represents Trap 1, and the blue cross is for Trap 2.
• Put the cross on the beads and turn on the laser
• Switch on the main laser shutter

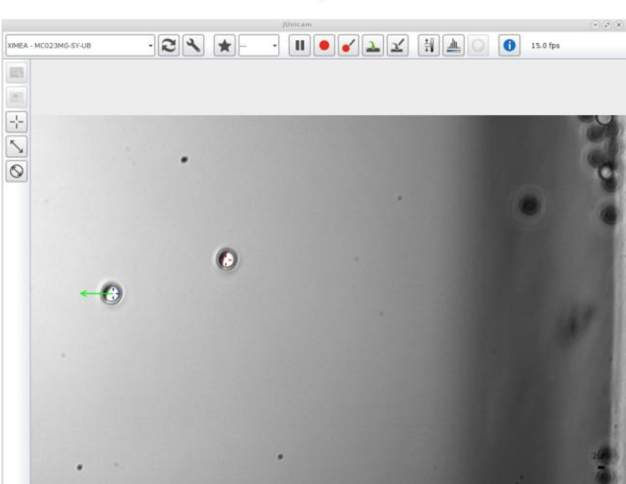



• Turn on the laser shutter of Trap 1 and Trap 2 respectively





• The sign of laser changes from “X” to “+” when laser is on.



• The particle can be trapped in two ways, dragging one of the traps to the particle or double-clicking onto the particle.
• To move the trapped particle, it can be dragged to position of interest or double-click a new position.

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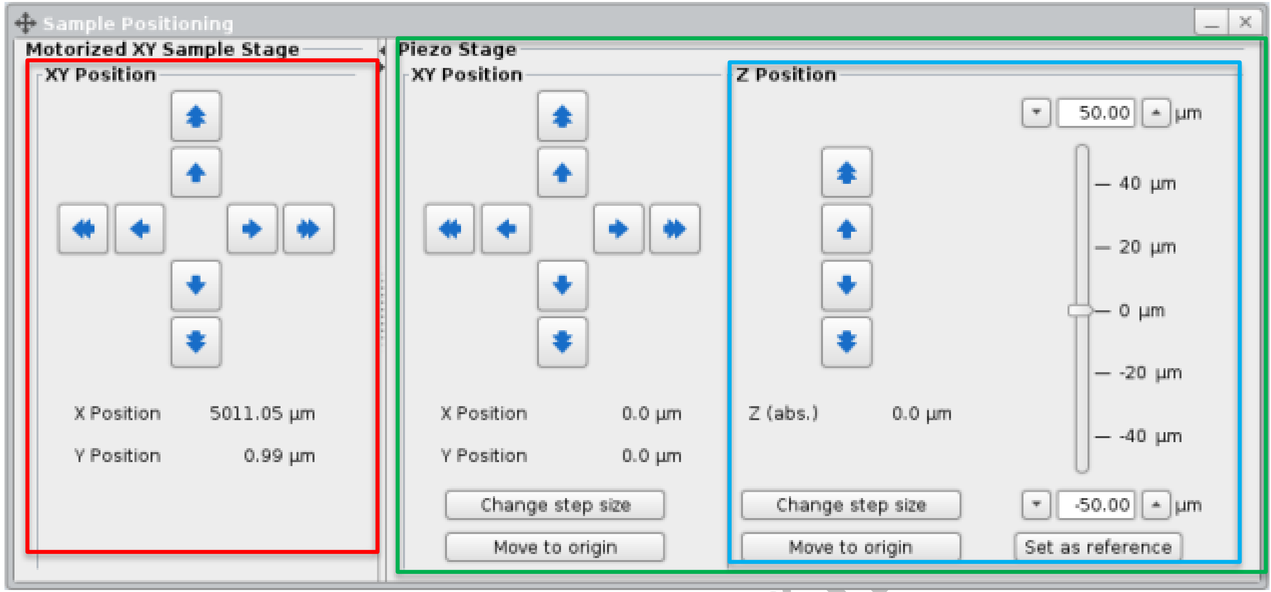
- Use **XY position** in **sample positioning** to monitor the motor stage, moving sample into the field of view
- The **piezo stage** in the middle has a range of 100 µm in X, Y and Z direction for fine positioning.
- Using blue arrow or slider bar of **Z position** to set a reference z position in the range of -50 um to 50 um.



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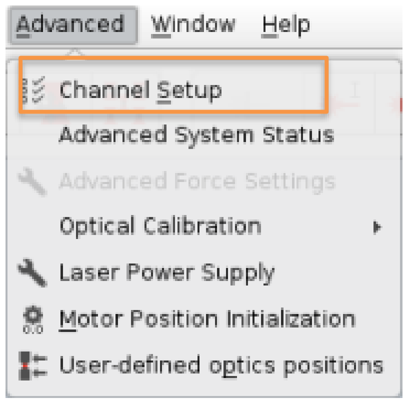
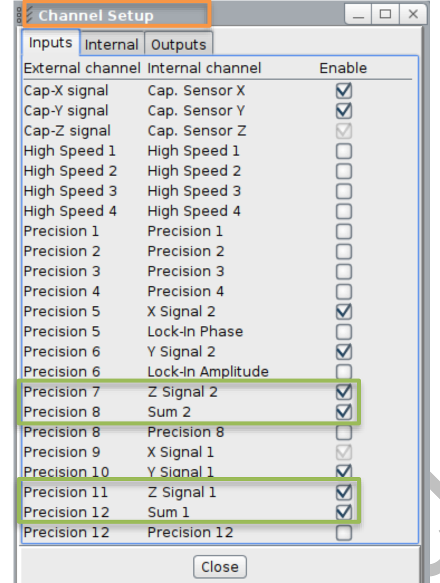
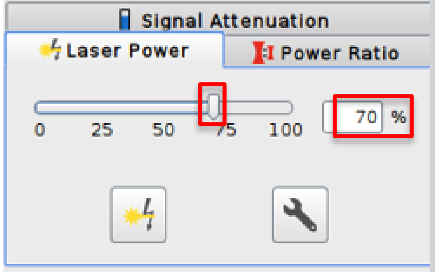
Calibration of Trapping

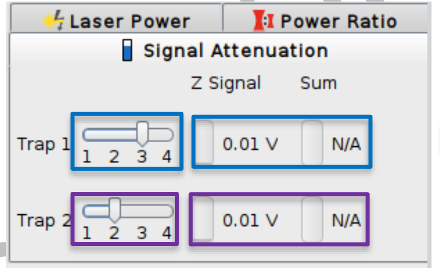
- Open **Advanced** → **Channel Setup**
- Tick **Z signal 1, 2 and sum 1, 2**

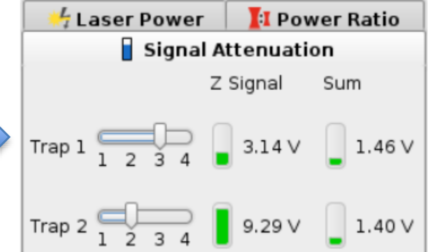




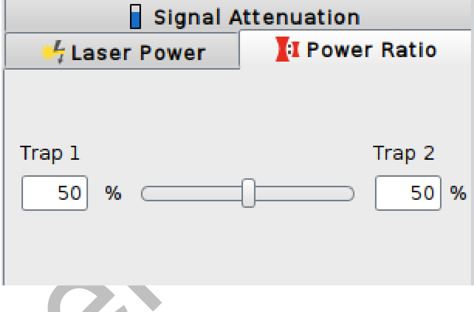
- Turn up the laser power by dragging the **slider** to right or **typing the value** into description field until Z signal and sum of Trap 1 and Trap 2 are all green (1-10 V in z)
- If **Z signal and sum values of Trap 1** are red or over 10 V, increase **the signal attenuation of Trap 1** (from level 1 to level 4). If **Z signal and sum values of Trap 2** are red or over 10 V, increase **the signal attenuation of Trap 2** (from level 1 to level 4).







- Adjust the power ratio of Trap 1 and Trap 2. Increasing the power of one value will automatically decrease another one. The trap with larger stiffness would take larger ratio of power.



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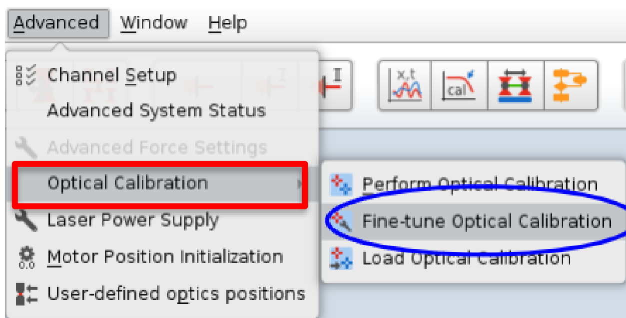
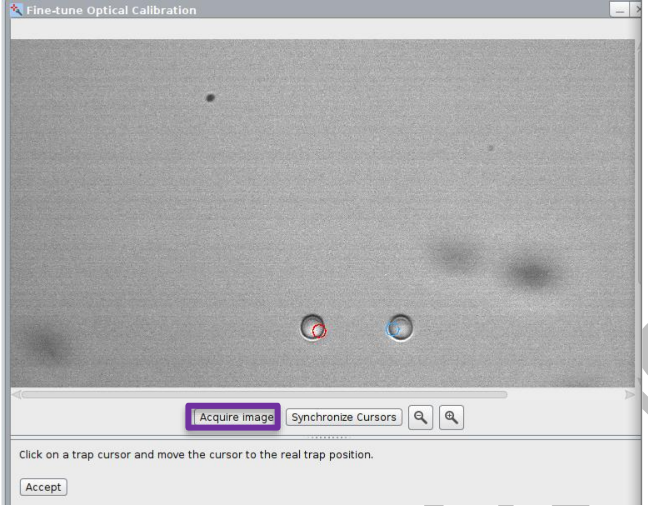
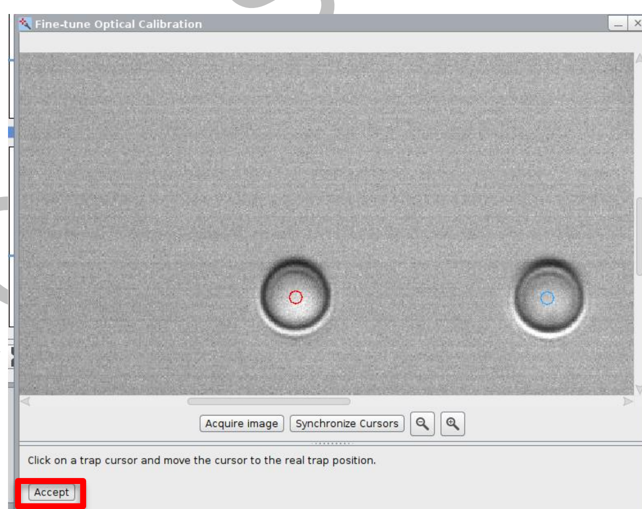
- Open **Advanced** → **Optical Calibration** → **Fine-tune Optical Calibration**



- Press **Acquire image** to capture an actual image from Junicam
- Position the red and blue circles as accurate as possible to center of two beads separately. Use the zoom function (or mouse wheel) to enlarge the field of view to make the alignment easier and more precise.
- Then click **Accept** to update optical calibration and close the window





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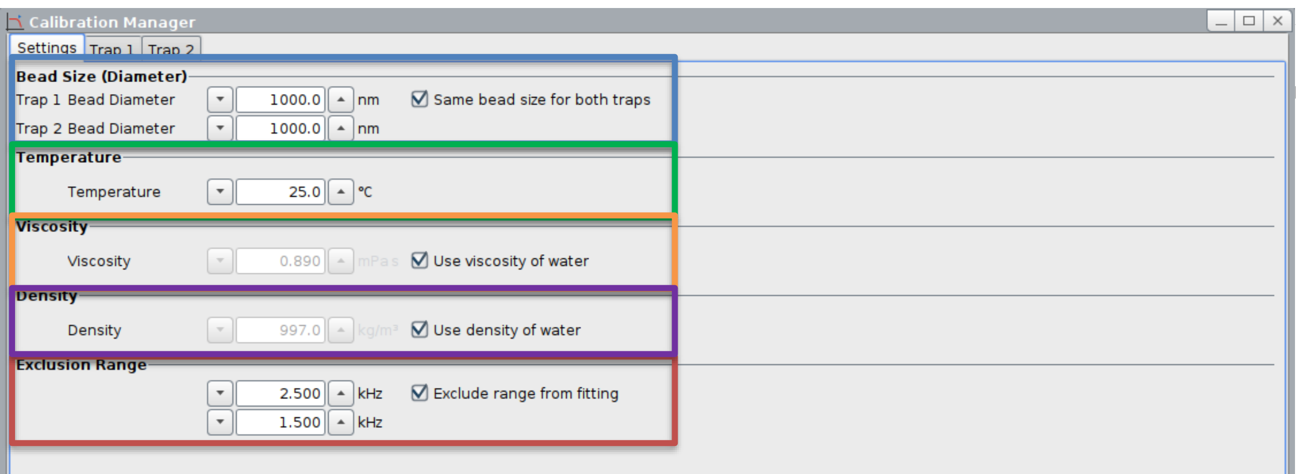
- Open the **Calibration Manager** in the task bar to set each trap.
- In the setting panel, set up the **diameter of the beads**, **solvent temperature**, **viscosity of the media** and **density of the media.**
- If the media is not water, tick off “Use viscosity of water” and “Use density of water”, type the absolute value of media’s viscosity and density into the description field.
- The **exclusion range of frequency** will be defined by trap signal later

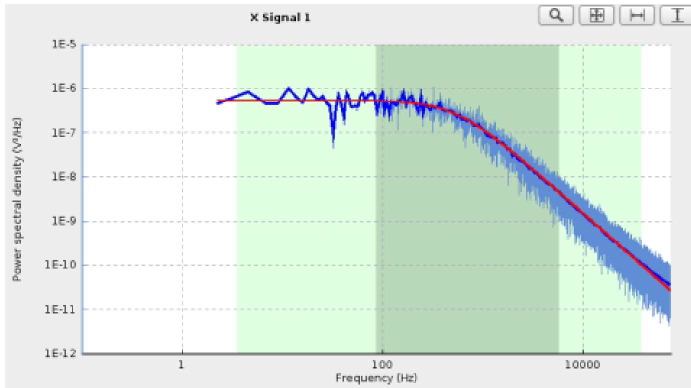


- Click **Trap 1/Trap 2** in Calibration Manager

- Set Scan Averaging to all scans or No. of spectra moving average
- Click **Run** to plot a single power spectrum (power spectral density vs. Frequency). **Reset** erases the current spectrum.

  Light blue: original power spectrum of the thermal motion of the particle
  Dark blue: a logarithmically average version of spectrum
  Green bar: selected area
  Red line: the best fit of the original spectrum
  Grey area: exclusion range

- Select the corresponding frequency range with corner frequency, excluding certain peaks or unwanted features.
- Press the **Accept values**, the calibration data of sensitivity and stiffness will be fitted.
- Then, tick **use it** for both sensitivity and stiffness.



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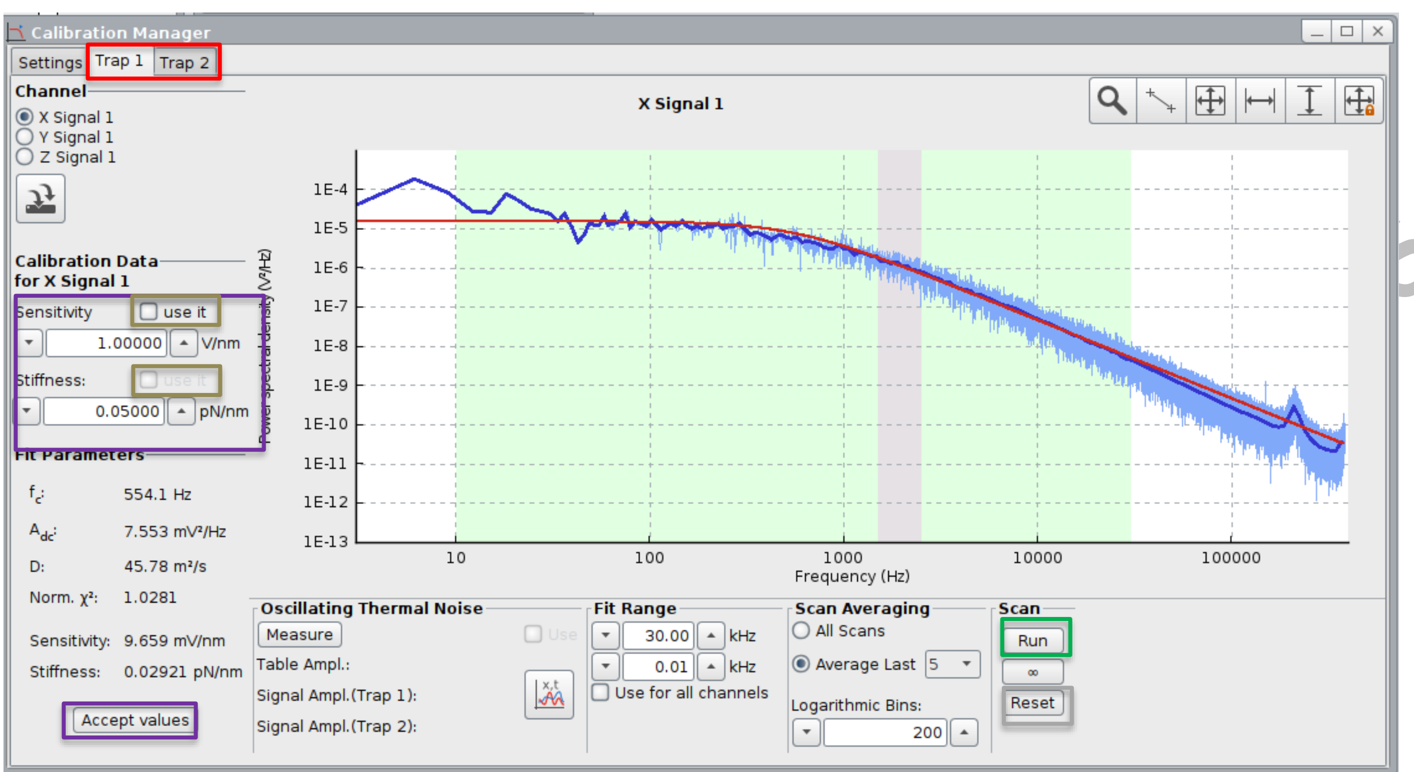

- Go back to **Calibration manager** and tick **exclude range from fitting** in **exclusion range**

Exclusion Range

2.500 kHz ☑ Exclude range from fitting

1.500 kHz

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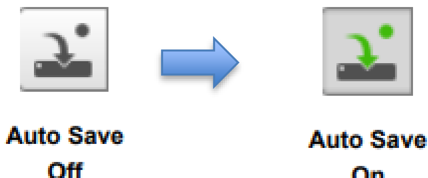
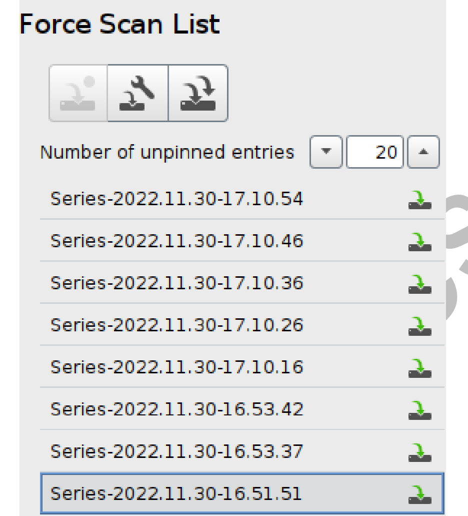
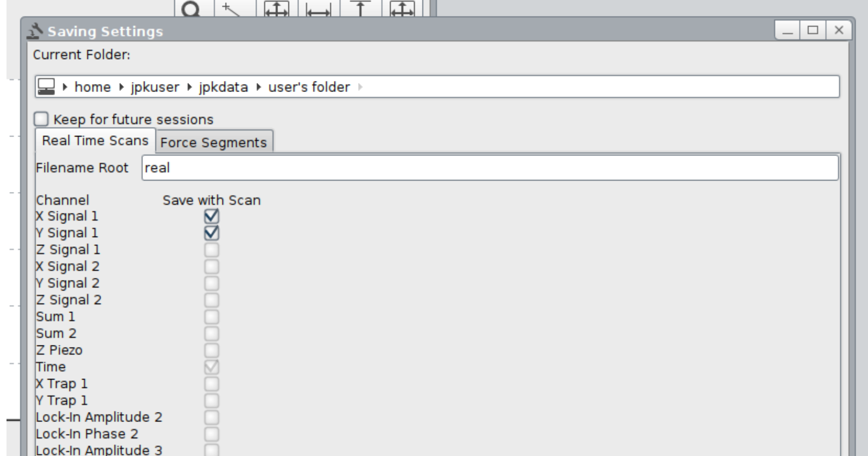
Data Recording of Force spectroscopy

• Turn on the Auto Save in pre-setting. Every Force curve will be directly saved into hard drive.





• Open **File -> Saving Settings**
• Tick the appropriate selection in **saving setting**. All the data should be saved in user's folder with correct filename root.



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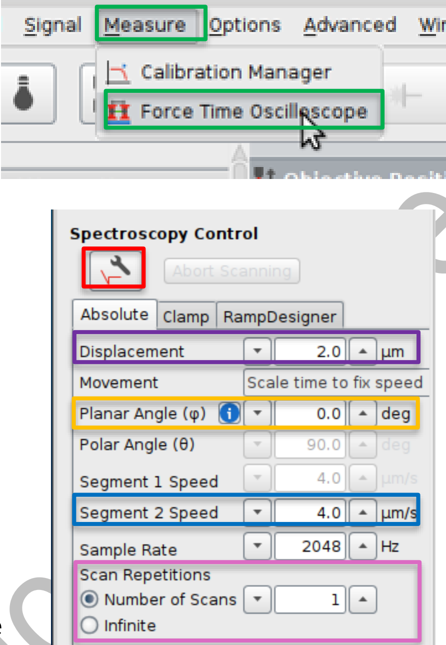
Force Spectroscopy and Movement Setting

Force Spectroscopy oscilloscope

- Click **Measure** in the tool bar, and select **Force Time Oscilloscope**
- The control panel of spectroscopy is on the left side of software



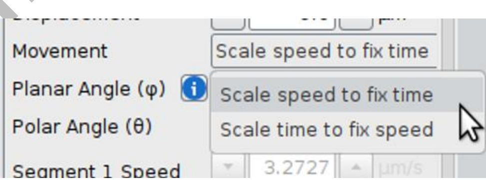
Method 1 Absolute Force control

- Set the relative **displacement** and the **direction** in **planar angle**.
- Planar angles of 0º, 90º, 180º, 270º (or -90º) correspond to the +X, +Y, -X and -Y directions, respectively.

For example,

0° (right)	90° (up)	180°(left)	270°(-90°) (down)	160° (up left)

- Determine the **speed of segment 2**. Segment 1 speed will generate the same speed as segment 2.
- Sample rate refers to the speed of recording data. Normally, apply default setting which is 2048 Hz.
- Select Scale time to fix speed for movement mode with controlled speed
- Select Scale speed to fix time for movement mode with controlled time
- To repeat the movement, type number of scans into **scan repetition**
- Choose infinite if keep the same movement



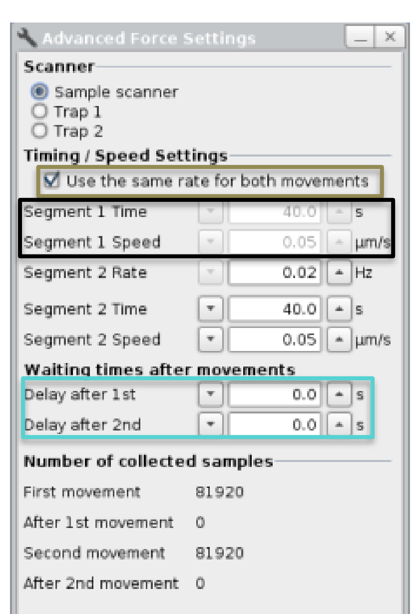
- Click [icon] to open **advanced force setting** to check controlled time or variable time (or Setup → Advanced Force Settings)

  - Select sample scanner or trap 1, trap 2 to switch target

  - Check the time, speed, and rate of segment 2. There values are updated automatically when any one of them is altered.

  - If apply the different extent and retract rates, tick off "**Use the same rate for both movement**". Type in the **time and speed of Segment 1**.

  - Waiting time (s) at the extended (**Delay after 1st**) or retracted point (**Delay after 2nd**) can also be set by typing into description field.



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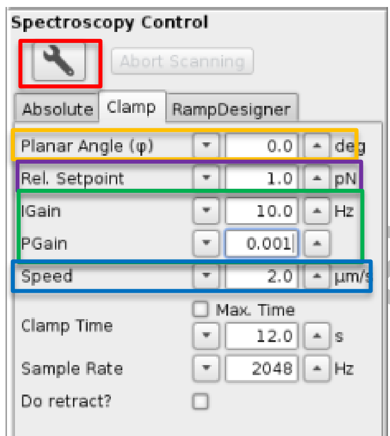
Method 2 Force clamp

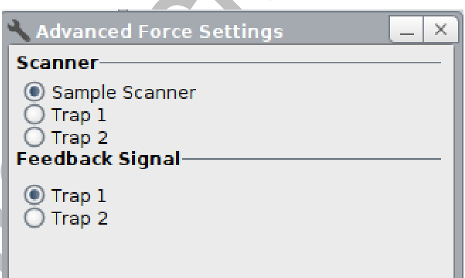
- Set up the **planar angle** to defines the direction of force clamping.
- Planar angles of 0º, 90º, 180º, 270º (or -90º) correspond to the +X, +Y, -X and -Y directions, respectively.

For example,

0° (right)	90° (up)	180°(left)	270°(-90°) (down)	160° (up left)

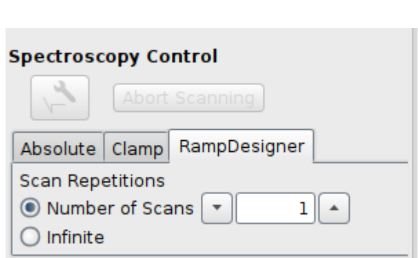


- **Rel. Setpoint** parameter refers to the value of force, which should be kept constant by the feedback system during clamping.
- **IGain and PGain** are kept in default setting, respectively 10 Hz and 0.001.
- After the start of clamping, the moving velocity is defined by **speed** in direction adjusted by Planar Angle until force Setpoint is achieved.
- Click to open **advanced force setting** for switching scanner target among Sample Scanner, Trap 1, and Trap 2.



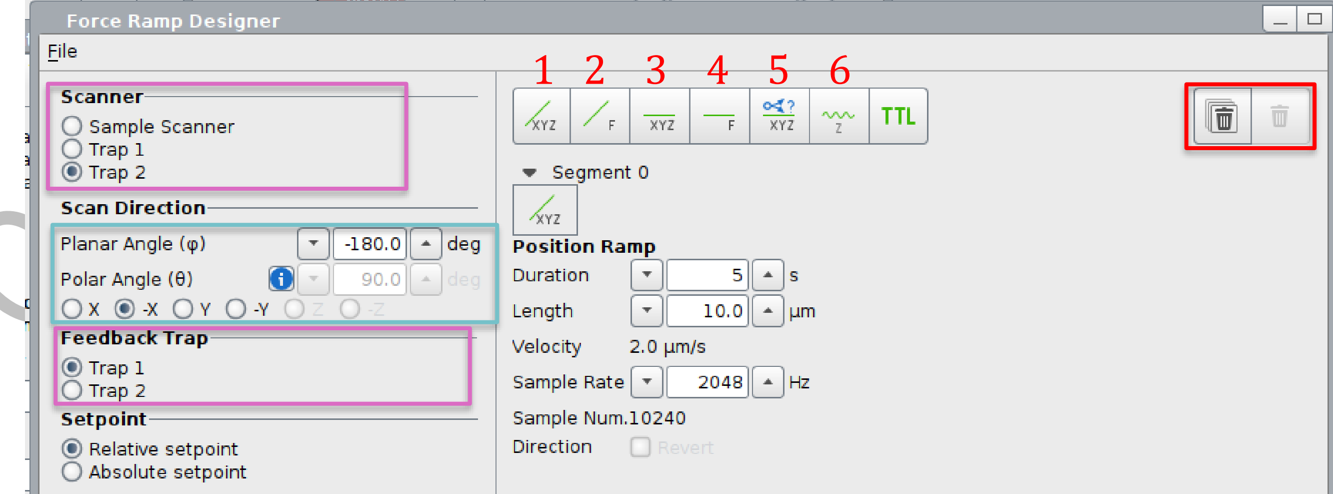
Method 3 RampDesigner

- Click **RampDesigner** in Spectroscopy control, then **Force Ramp Designer** will be open automatically
- On the left part, switch scanner target among Sample Scanner, Trap 1, and Trap 2 in **Scanner** and **Feedback trap**
- Set up the **planar angle** to defines the movement direction or select X, -X, Y or -Y as movement **direction**.



- If clear all the movements, click .
- If delete the certain step, select the certain segment, and click .

- On the right side, all the different movements can be applied in the adjacent window
- Select relative setpoint as Setpoint



Core for PanorOmic Sciences

1. Position Ramp

- Adding an ongoing movement by determining the length, time, and rate.
- Set up **duration** for the entire movement time or the whole **length** of the movement distance. **Velocity** will be calculated.
- The direction is defined on the left side of window (seen Page 19)
- **Sample rate** refers to the number of data points recorded in one second. Normally, set into default value 2048 Hz.
- If retract the sample to process the revert movement, tick **revert** in direction and the movement will be in the opposite direction

2. Force Ramp

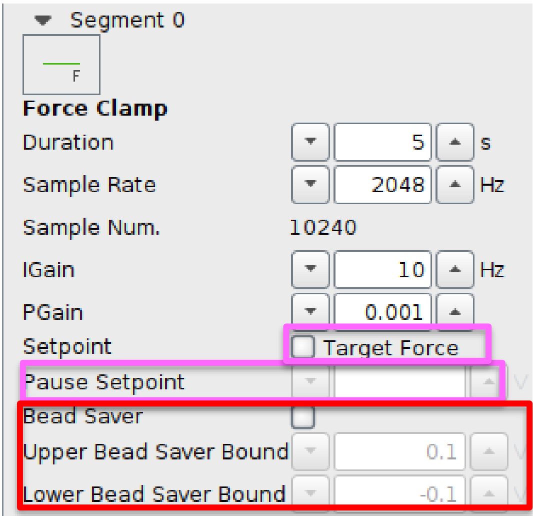
- Set up the **duration** and **distance length** like Position Ramp
- **Length** is an expected value to help set **velocity** and **sample rate**
- The scanner will move for the distance **Length**, and stop when the **setpoint** is reached
- If the setpoint is not reached during Length, the scanner continues to move until it is reached

3. Pause

- Set up the **duration** for pause.
- Check the **sample rate** is same as other segments.

4. Force clamp

- Tick **Target force** and define **Pause setpoint** for the force of feedback trap is clamped.
- **IGain** and **PGain** refer to the integral and proportional gains of feedback trap. Use the default value 10 Hz and 0.001.
- If the **Target Force** is not checked then the Force Clamp segment expects to receive the Setpoint value from previous segment.
- Tick **Bead Saver**, the values of **Upper** and **Lower Bound** will define at which levels relative to the setpoint the RampDesigner will stop the experiment in emergency case, avoiding lost beads or molecule bond broken.



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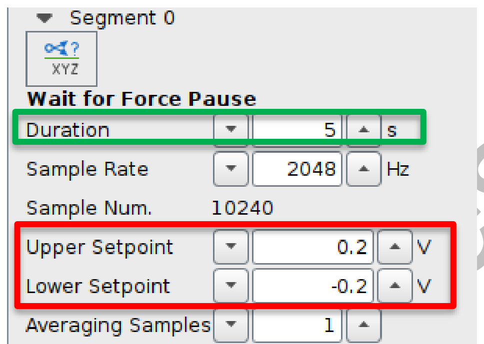
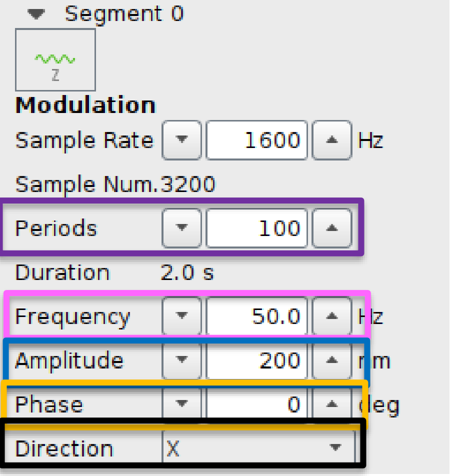
5. Wait for force Pause

- Set up the **duration** in second
- Set up the range of force by **Upper Setpoint** and **Lower Setpoint**.
- When the force ranged between upper setpoint and lower setpoint in set duration, the particle will stop movement.



6. Modulation

- Input **period**, **frequency**, **amplitude**, and **phase** to investigate the sinus-oscillations.
- Fill the periods for duration length.
- The parameter **Direction** can be X or Y for trap scanners and X,Y and Z for the sample-scan.



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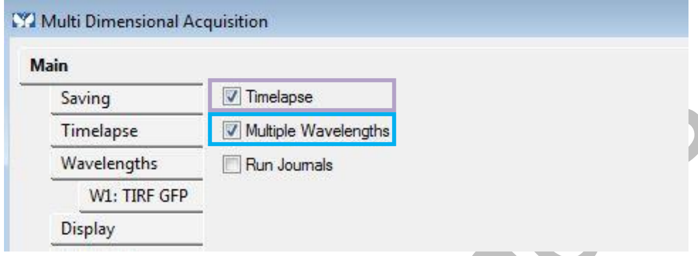
Metamorph

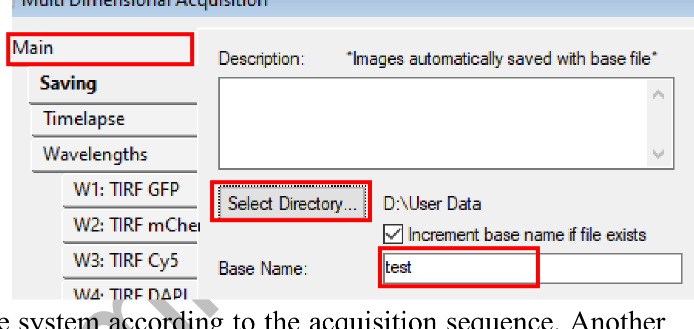
Multi Dimensional Acquisition

Image Acquisition

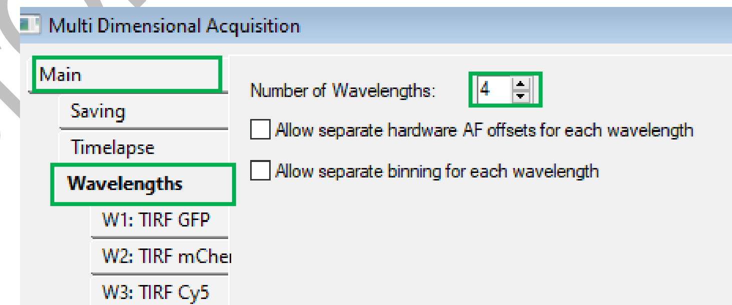
- Click “**Multi Dimensional Acquisition**” on the task bar
- Select the applied function in the main list
  - Tick “**Timelapse**”, if take different time points
  - Tick “**Multiple wavelengths**”, if apply multiple channels



- To Set the **Saving** path
  - Select “**Saving**”
  - Click “**Select Directory**” to set data saving directory.
  - Note: All data should be saved in your own folder in E drive/USER. No data is allowed in C or D drive.
  - Type in the base name of your file (experiment or date or etc.) in “**Base Name**”. Do not use digit 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….).



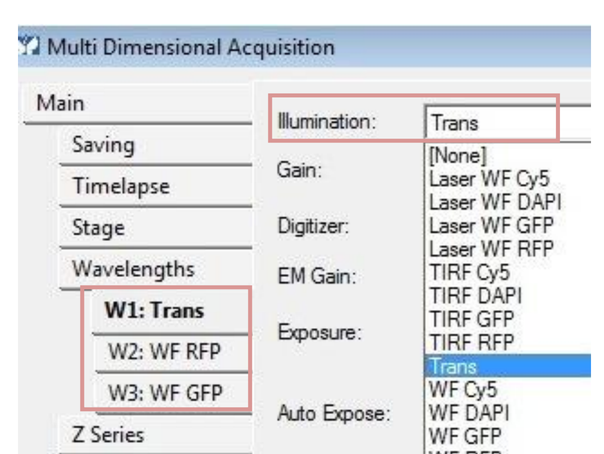
- Set **Wavelengths**
  - After ticking “**Multiple wavelengths**” in the main menu, select **Wavelengths**
  - Set number of channels in “**Number of Wavelengths**” by typing in number of wavelength or click ^ to increase wavelength, click v to decrease wavelength.



- Select each wavelength (W1,W2...) to set the required “**Illumination**”.

For Widefield Imaging:

- Select “WF DAPI” for Blue emission (such as DAPI)
- Select “WF GFP” for Green emission (such as GFP)
- Select “WF RFP” for Red emission (such as mCherry)
- Select “WF Cy5” for FarRed emission (such as Cy5)
- Select “Trans” for brightfield channel



For TIRF Imaging:

- Select “TIRF DAPI” for Blue emission (such as, BFP) channel
- Select “TIRF GFP” for Green emission (such as, GFP) channel
- Select “TIRF RFP” for Red emission (such as, mCherry) channel
- Select “TIRF CY5” for Farred emission (such as, Cy5) channel
- Select “Trans” for brightfield channel

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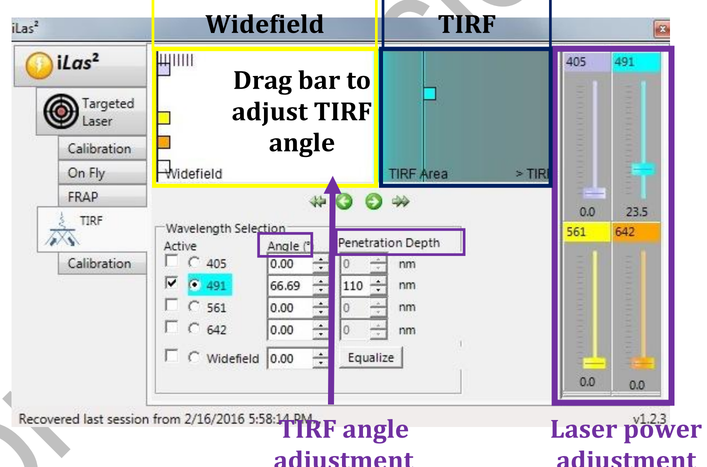
Image Adjustment

In MDA setting,

- Select "W1" to set the first channel
- Adjust **EM Gain** and **Exposure** time to have optimal signal intensity
- Adjust **Gain** if necessary (1x, 2x or 4x)
- Select "W2" and repeat the same procedure to set the second channel
- Click **Live** at the bottom of "multi-dimensional acquisition" panel to have real time image

In iLas 2 setting:

- Preview the image on screen by clicking Live at the bottom of "multi-dimensional acquisition" and adjust the focus in piezo z stage and parameters (EM Gain, Exposure Time and Laser Power) to achieve a well-focused and properly illuminated image.
- Click "TIRF" in ILas2 software panel
- Adjust **laser power** by move the slider bar for each laser
- Adjust TIRF angle for each laser by move the slider into the TIRF area. The actual Angle and Penetration Depth are shown on the panel.



405nm 491nm 561nm 642nm

- Click **Acquire** at the bottom to start acquisition of necessary.

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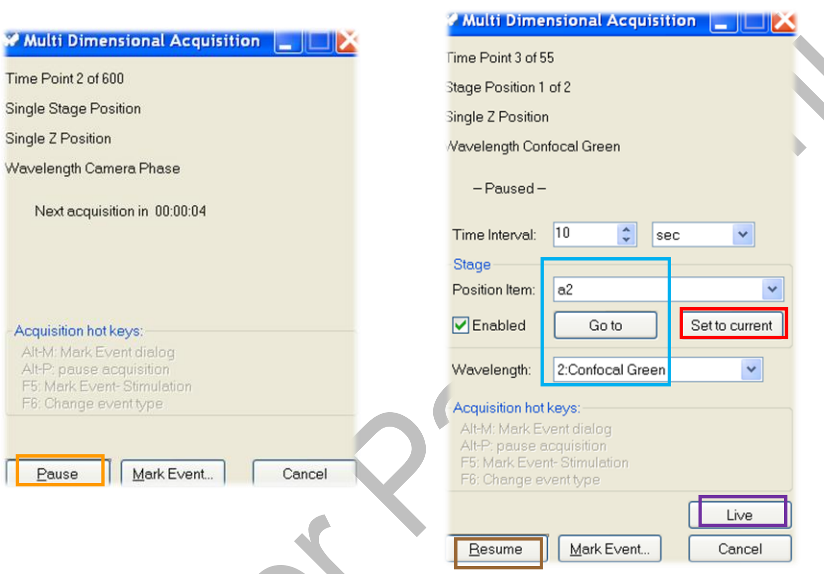
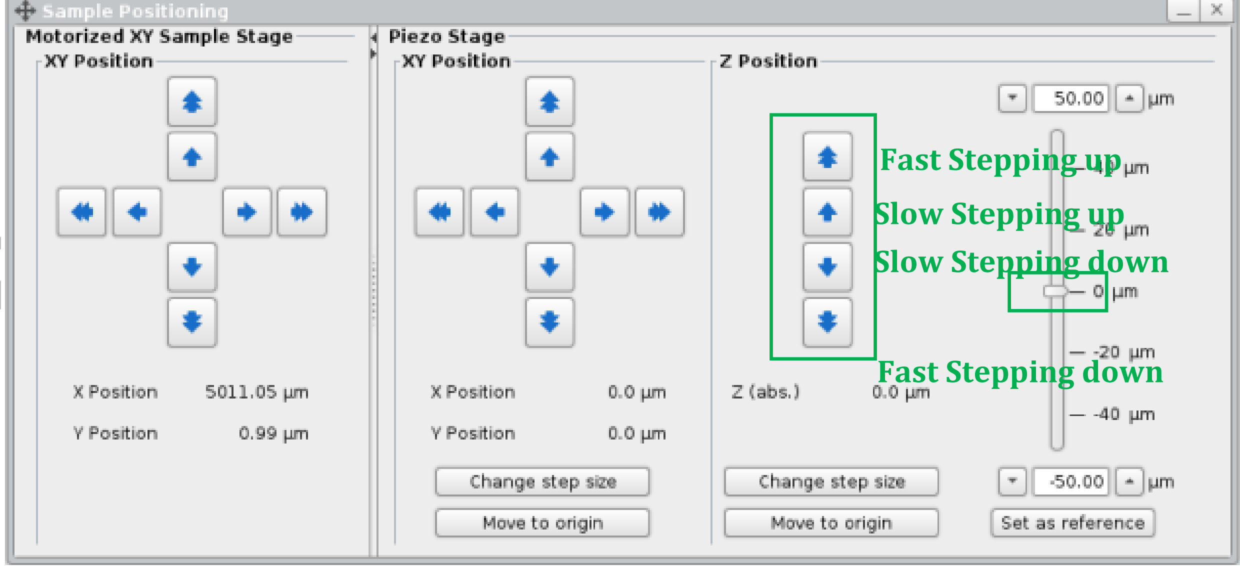
Timelapse

- Set up "**Time interval**" between each acquisition time point
- Set up "**Duration**" for the whole experiment length or "**Number of time points**", the other one will be calculated automatically.
- Click **Acquire** in the bottom to start acquisition of necessary
- Click **Acquire** in the bottom to start acquisition of necessary

Adjust Focus during Time Lapse Acquisition

- After the start of acquisition, you can "**Pause**" the acquisition to adjust the position and time interval. Click "**Live**", choose a **Position** and click "**Go to'**. Choose a suitable **Wavelength**, adjust the position and focus on JPK NT software by **clicking the arrows or dragging the bar** between 50 μm to -50 μm and then click "**Set to current**". Click "**Resume**" for continuing the acquisition.



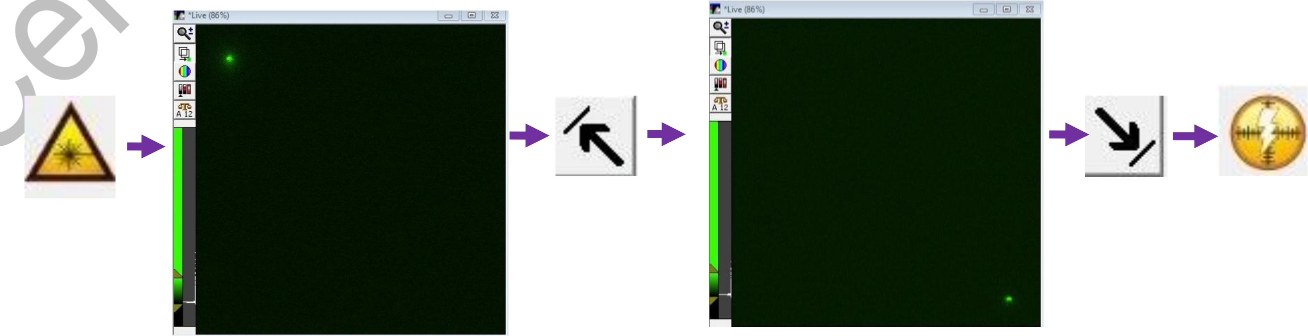


FRAP

- Targeted Laser Calibration
  - Preview the image on screen by clicking **Live** at the bottom of “multi-dimensional acquisition”
  - Select “Calibration” in “Targeted Laser” on iLas panel
  - Load the latest FRAP calibration setting

Load calibration setting
Save calibration setting
Laser Power adjustment for FRAP

  - Click on the icon to activate the targeted laser. Adjust the focus and parameters (EM Gain, Exposure Time and laser power) to achieve a highly contrasted laser spot image in MetaMorph Live window.
  - Move the red cross in the grey calibration area to bring the laser spot to the top left corner and press
  - Bring the laser spot to the bottom right corner and press
  - Click on the calibration button to begin calibration
  - When calibration is done, click on the save icon to save the calibration setting



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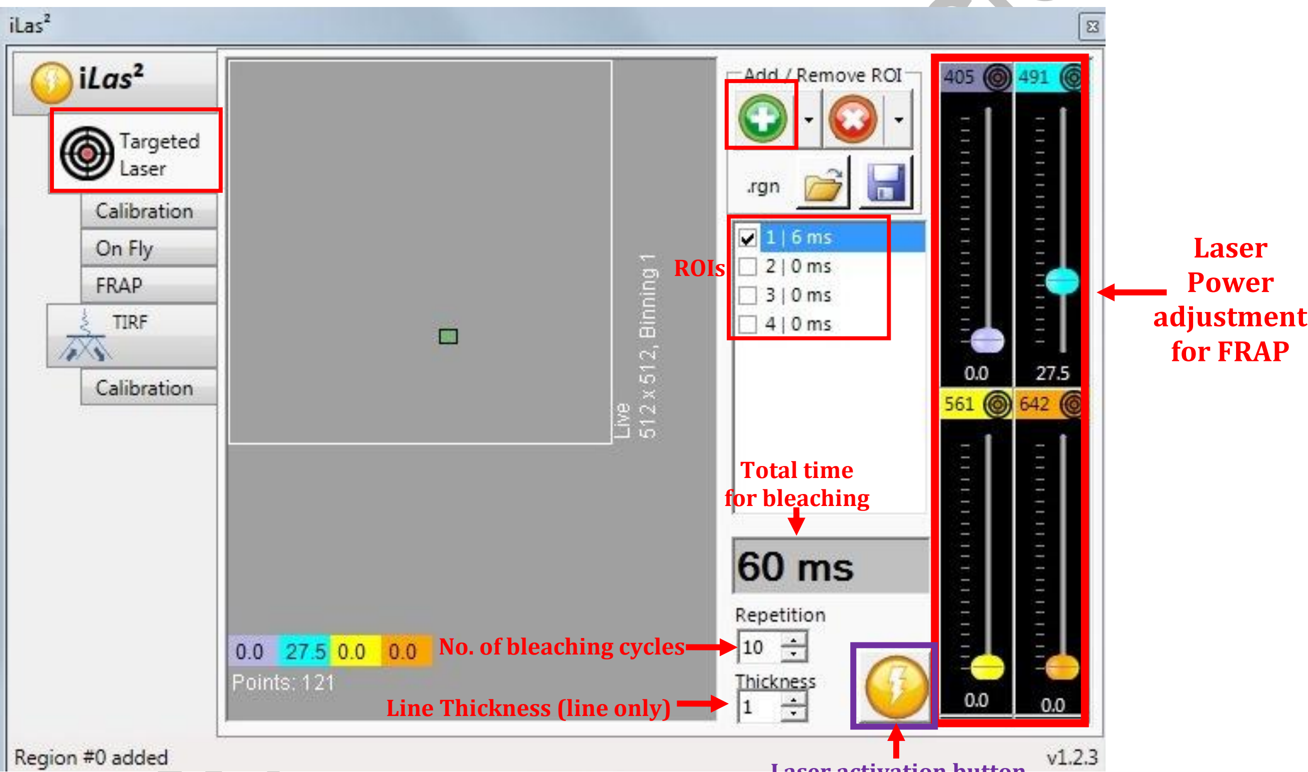
- FRAP Experimental Protocol
  - Select FRAP MDA template in main iLas window
  - Preview the image on screen by clicking **Live** at the bottom of "multi-dimensional acquisition" and adjust the focus and parameters (EM Gain, Exposure Time and Laser Power) to achieve a well-focused and properly illuminated image.
  - Click "Targeted Laser" in iLas window
  - Mark the region of interest (ROI) using the region tools in MetaMorph and ROI(s) by click
  - Adjust bleaching parameters (No of repetitions, laser power)
  - The testing bleaching could be done by click laser activation button



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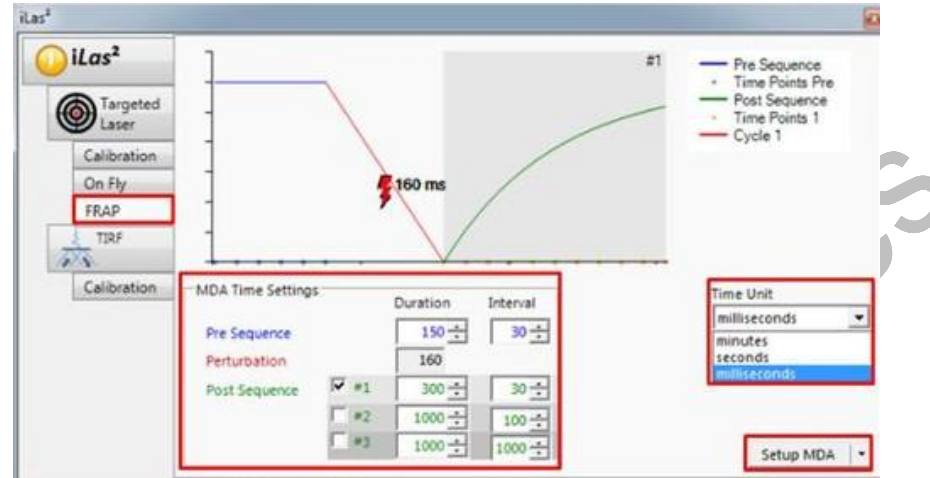
- Click FRAP Tab, set up time interval and duration for Pre & Post sequence acquisition
- Click Setup MDA to import the parameters into Mulit Dimensional Acquisition widow in metaMorph
- Click on the Acquire icon to begin acquisition



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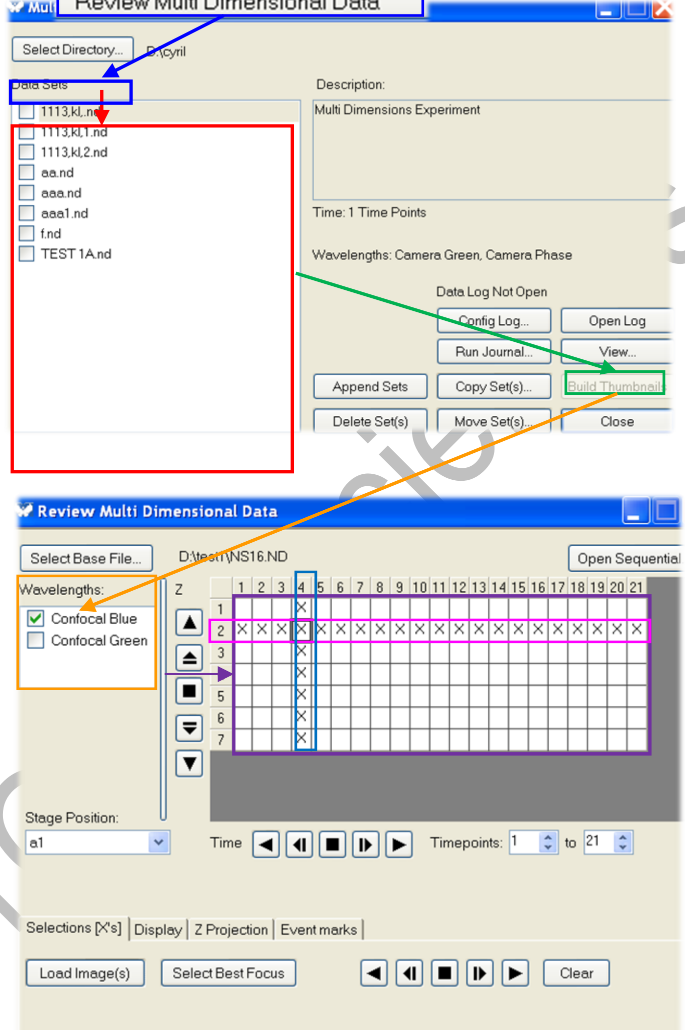
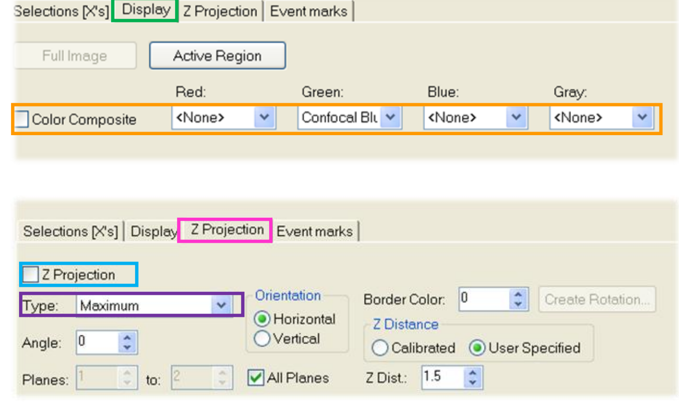
Review Acquired Images

- 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 a 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.



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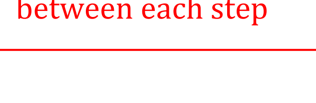
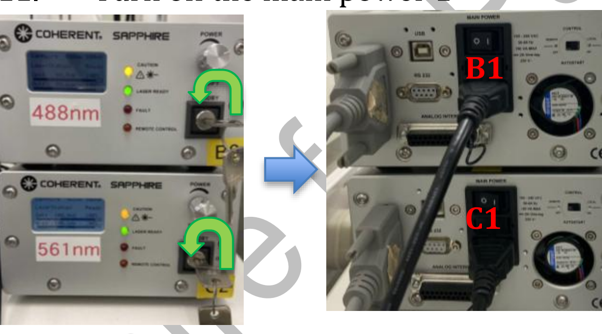
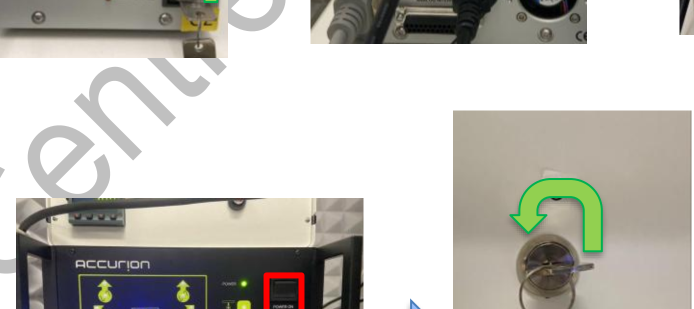
Turn off System

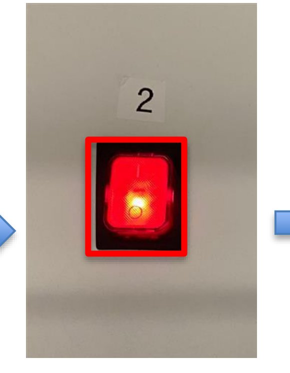
Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (1h) after you.

1. Please retract two objectives to the original position by clicking [up] / [down].
2. Remove water on your sample, then retract the sample carrier from the NanoTracker head.
3. Clean the oil objective with lens cleaning tissue only but NOT Kimwipe.
   a. Remove immersion oil from the trapping objective with lens cleaning tissue.
   b. Repeat this step with a new area/piece of the lens cleaning tissue until no oil streaks are seen on the lens paper.
4. If you are using TIRF,
   a. Exit MetaMorph software
   b. Transfer data to Faculty Core Facility storage server and shut down the computer ⑧.
   c. Switch off laser power (488 nm) key
   d. Switch off laser power (561 nm) key
   b. Wait the laser output decreases to 0, then switch off C1 (561nm)
   c. Wait the laser output decreases to 0, then switch off B1 (488nm)
   d. Turn off Power button 6
   e. Turn off Power button 5
5. Turn off the heater on the top of outside box if it is used.
6. Turn off PetriDish heater if it is used.
7. Turn off vibration isolation.
8. Turn off the computer of JPK NT
9. Switch off the key of laser power for step 3
10. Turn off the main power 2
11. Turn off the main power 1


Please keep 5 seconds between each step







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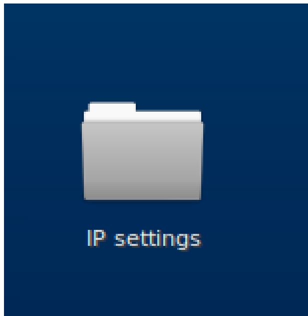
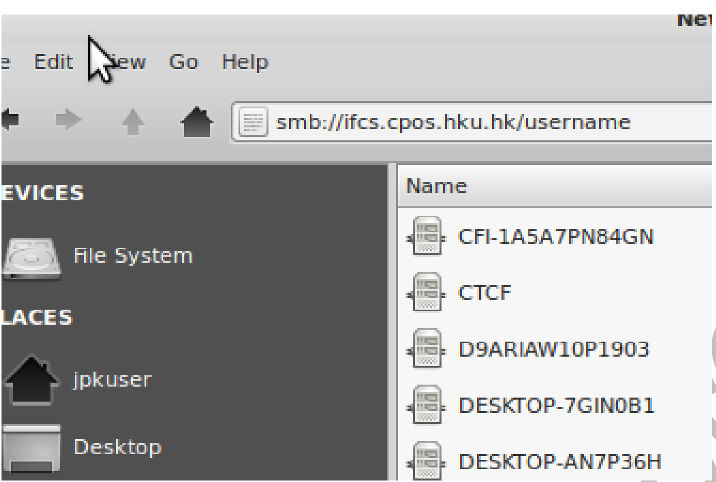
CPOS IFC Server Connection and Data Export (Linux)

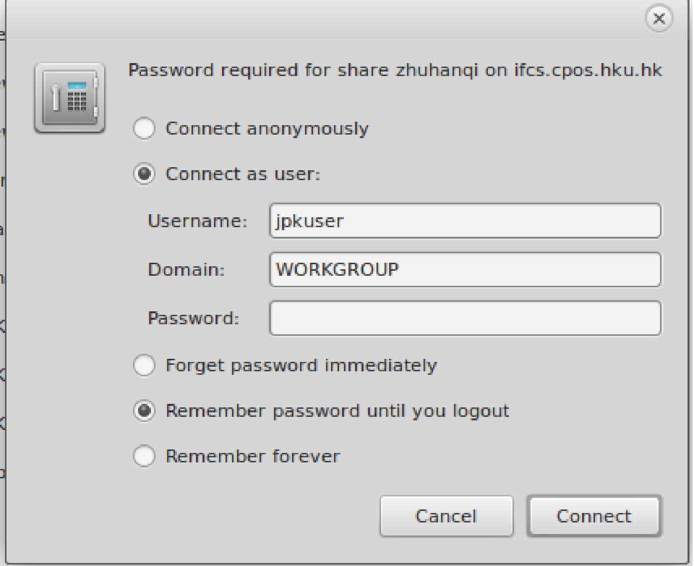
① Click IP setting on the desktop



② Type smb://ifcs.cpos.hku.hk/username, then Enter



③ Enter User name and Password, and click **Connect**



④A network drive is mapped to the computer. Transfer your data from computer to the Network drive: copy →Paste

⑤Right click network drive and disconnect

PanorOmic Sciences