PE-ERS Spinning Disc Confocal Microscope Standard Operation Protocol

**Basic Operation**

**Turn on system (For confocal user)**
- Compress Air (if necessary)
- Power switch (for microscope) ①
- Power switch (for computer and shutter) ②
- Microscope switch ③
- EMCCD A
- Laser Power Supply B
- Laser ON/OFF Switch C
- Laser Key D
- 488nm Laser switch E1 and/or 561nm Laser switch E2 (Turn on the one you need only)
- Fluorescent lamp ④
- Temperature control ⑤ (Live sample only)
- CO2 control and CO2 tank ⑥ (Live sample only)
- Turn on computer and log in Windows with “user” ⑦

**Turning on system (For widefield user)**
- Compress Air (if necessary)
- Power switch (for microscope) ①
- Power switch (for computer and shutter) ②
- Turn on microscope ③
- Fluorescent lamp ④
- Temperature control ⑤ (Live sample only)
- CO2 control and CO2 tank ⑥ (Live sample only)
- Turn on computer and log in Windows with “user” ⑦
Start the MetaMorph software by double clicking the **Widefield** or **Confocal** icon on desktop

Select **Magnification** from drop down list and click OK

Select CCD Camera and click OK
- **EMCCD** for confocal image acquisition
- **ORCA03G** for widefield image acquisition

**Locating the sample with the microscope**

Put the sample on the stage (Coverslip faces down)

Select objective by pressing the touch screen

Note: Please pay attention to the cable connecting to objective turret. The cable will get entangled around objective turret if it keeps turning the same direction.

Click **Binocular Port**

Click **BF** tab for Brightfield observation
- Switch On the LED in the LED controller
- Adjust condenser annulus and Köhler illumination if phase contrast image is needed (Seek help from technician if necessary)

Click **FL GFP** tab for green emission channel observation

Click **FL Alexa546** tab for red emission channel observation

Click **FL DAPI** tab for blue emission channel observation

Focus with the coarse and fine adjustment knob

Find the right field of view for imaging with the stage controller
Image Acquisition

Click “Multi Dimensional Acquisition” on the task bar

Set up acquisition configuration step by step

Click “Saving”

- Click “Select Directory” to set data saving directory.
- Note: All data should be saved in your own folder in D drive. No data is allowed in C 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…. )

Click “Wavelengths”

- Select number of channels in “Number of Wavelengths”
Select each wavelength to set the required “Illumination”.

For widefield imaging:
- Select “Camera Blue” for Blue emission (such as DAPI) channel
- Select “Camera Green” for Green emission (such as GFP) channel
- Select “Camera Red” for Red emission (such as mCherry) channel
- Select “Camera Phase” for widefield phase contrast channel

For confocal imaging:
- Select “Confocal Green” for green emission (such as, GFP) channel
- Select “Confocal Red” for red emission (such as, mCherry) channel
- Select “Confocal Phase” for widefield Phase contrast channel

Widefield Image Acquisition
- Click “Camera port” and “ORCA-03G” on task menu
- Select “W1” to adjust the first channel
- Click “Live” at the bottom of “multi dimensional acquisition” panel to have real time image
- Click “Full chip” button to utilize full size of the sensor
- Adjust Exposure time to have the optimal intensity
- Adjust Gain if necessary (1x or 5x)
- Select “W2” and repeat the same procedure to adjust the second channel
- Click “Acquire” in the bottom to start acquisition
Confocal Image Acquisition

- Click “Confocal port” and “Evolve EMCCD” on main menu
- Select “W1” to adjust the first channel
- Click “Live” at the bottom of “multi-dimensional acquisition” panel to have real-time image
- 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 adjust the second channel
- Click “Acquire” in the bottom to start acquisition

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.
- 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 and then click “**Set to current**”. Click **Stop** and then “**Resume**” for continuing the acquisition.
Multi stage position

- Give a Label for your stage positions; (Label name should be ended with digit “1”. The number will be automatically updated to record the subsequence position.)
- Use “Live” mode to find the right position (x, y) and focus level (z)
- Click “+” to add the position (x, y, z) in position list
- To overwrite recorded stage position, highlight the one to be overwrote and click “+”.

Z Series

For Spherical object, use “Range around current” mode:

- Tick “Range around current”
- Focus the centre of your object
- Set up “Step Size” for distance between each focus plane
- Set up “Number of Steps” for the total number of planes

Otherwise, 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
Review Acquired Images

- Click **Review Multi Dimensional Data** in the Task Bar after Images Acquisition
- Choose your folder in **Select Directory** and select a 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.
Turn off system (For confocal user)

- Clean oil objective (if applicable)
- Exit “MetaMorph” software, transfer your data to server and turn off computer
- Turn off CO2 control and CO2 tank (if applicable)
- Turn off temperature control (if applicable)
- Turn off fluorescent lamp and sign on log-sheet
- Turn off E1 and/or E2 Laser channel ON/Off Switch
- Turn the Laser Key D anticlockwise to OFF position
- Turn off Laser Power
- Turn off Laser Power Supply Switch B
- Turn off EMCCD A
- Turn off microscope switch
- Turn off power switch
- Turn off power switch

Turn off system (For widefield user)

- Clean oil objective (if applicable)
- Exit “MetaMorph” software, transfer your data to server and turn off computer
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- Turn off temperature control (if applicable)
- Turn off fluorescent lamp and sign on log-sheet
- Turn off microscope switch
- Turn off power switch
- Turn off power switch
LAST User Check list:

- Sign on log book
- Clean oil objective with lens paper (if applicable)
- Move objective to 10x
- Adjust focus to lowest position
- Turn off system
- Cover microscope
- Turn off CO2 supply (if applicable)
- Turn off compress air supply
- Clean the table
- Take all your stuff
- Turn off room light
- Lock the door