Zeiss Elyra Microscope Standard Operation Protocol

Basic Operation

Machine Startup

1. Turn on the “Incubator” power to warm up the incubator for a couple of hours prior to sample acquisition as image could be drifted easily when temperature fluctuates. You may request FCF operators to help turning on the incubator if you have a booking on the following morning.
2. Switch on the main power “2”.
3. Switch on the computer power “3”.
4. If you are using oil or water objective lens, apply one drop of 30°C oil or water onto the tip of the objective lens.
5. Take out the sample holder and place your desired slide onto it followed by putting the lever to hold the slide firmly.
6. DO NOT hold the “Alignment screw” when placing the stage holder to the stage.
7. Use the stage controller to locate the region of interest to the center of the objective lens.
8. Close the lids of all compartments that are labeled with YELLOW laser sign.
9. Log on to “LSM user” window.
10. Double click the “ZEN” software → “Start System”.

11. Select the objective lens which you would like to use by clicking the drop down button or select it from the touch screen of microscope controller.
   a. Use 63x oil lens for fixed sample.
   b. Use 63x water lens for live sample.

12. Go to Locate → find a region of interest using either the BF or fluorescent channel. Three bandpass filters are used for fluorescence channel viewing. When a target is found → click All Off.
SR-SIM Workflow:

A. Acquisition: i) 2D image acquisition  
   ii) 3D/z-stack acquisition

i) 2D image acquisition:
   1. Go to **Acquisition** → load the acquisition parameter which suits your experiment.
   2. Just click **Yes** to allow the laser switch to turn **On**.
   3. Click on one fluorescence channel at a time to adjust the laser power and exposure time for the best image intensity. The laser power and exposure time should not be lower than 20.0 and 50.00 respectively.
4. Select **Live** for continuous fast scanning or **Continue** for different grating (e.g. 3 or 5 rotations).

5. Click on the **Min/Max** for the optimum intensity of signal display for each channel.

6. Repeat step 16-18 for the other channels/tracks.

7. Check all the required boxes for the channels which need to be captured.
8. You may set a frame size of a desired area (please note that the Frame size is for region/area adjustment but not for image pixel adjustment). **DO NOT change** the parameters for the Number of the Averaging and the position (left/right or up/down) of the square box under the Scan Area.

9. You can select the number of rotation (either 3 or 5 rotations) under the Grating section prior to image acquisition.

10. Click Snap.
ii) 3D/Z-stack acquisition:

1. Check the box of Z-stack → Expend the dropdown button of the Z-stack under Multidimensional Acquisition.

2. Select Centre, focus at a desired focal plane → click Center → click Optimal → key in the desired number of Slices or Range of sample thickness.

3. Select fluorescent tracks of interest --> click Start Experiment.
B. Processing: i) Structure Illumination  
   ii) Channel Alignment

i) Structure Illumination

1. Go to Processing ➔ scroll down to Structured Illumination ➔ select a desired image ➔ Click Select ➔ Apply.

2. Save both the original and Structured Illumination image files.
ii) Channel Alignment

1. Under the **Processing** tab → Select **Channel Alignment** → Select the image after performing Structured Illumination → Uncheck Fit → Select **Affine** → check the **ID number** (If the ID sequence of your image is different from that stated in the software, please input the correct sequence by clicking the dropdown icon) → click **Load**.
2. Go to Computer → Data (D) → Maintenance → there are different combinations (select a bin file which suits your image requirement e.g. 1024x1024 frame size/3 rotations) → ok → click Apply.

3. Save the Channel Alignment file. You may proceed with further data processing using ZEN offline software or export the image in TIFF format.
Summary

A. Acquisition: i) 2D image acquisition
   ii) 3D image/Z-stack acquisition

A. Acquisition: i) 2D image acquisition

1. Select the area of ROI
   - Either 512 sq. or 1024 sq.

2. Select number of rotation

3. Select desired channel of acquisition according to:
   - D420 - D405-405

4. Laser power: 10-20%

5. Exposure time: 100ms

6. Check the box if Z-stack imaging is required

7. Start the experiment

A. Acquisition: ii) 3D image/Z-stack acquisition

11. Key in the desired number of slides or thickness

10. Click the smallest value

8. Click Interval

9. Find a desired focal plane
   - Click center

12. Set the center

13. Set the Offset

14. Set the pixel units

15. Set the interval
B. Processing:

i) Structure Illumination
   - 1. Click processing
   - 2. Select structured illuminated image
   - 3. Select the acquired image
   - 4. Click Select
   - 5. Select Automatic
   - 6. Select the desired output files

ii) Channel Alignment
   - 8. Make sure the channel ID sequence in the software is corresponding to your image channel
   - 9. Load the latest bin file saved under the Maintenance folder
Data Exportation

1. Double click the “Computer” Icon on desktop

2. Go to “Tools” → Click “Map Network Drive…”

3. Type in “\192.168.10.292\your HKU portal ID”

4. Click “different user name” → OK

5. Key in hkuπe\Z Portal ID → Portal Password → OK

6. A network drive is mapped to your computer system

7. Transfer your data from D Drive to the Network drive:

8. Right Click

Data Exportation
Machine Shutdown

1. Exit the ZEN software → 
   shutdown the computer → 
   turn off main switch → 
   turn off incubation system power.