Light sheet microscopy

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1 Works for me
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ABSTRACT

Protocol documents how to operate light sheet microscope (UltraMicroscope II, LaVision BioTec)

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PROTOCOL CITATION

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19227

GUIDELINES

Please note that this protocol should be used as a supplement to the protocol provided by the manufacturer as the light sheet microscope offers extensive capabilities that are not described here.

MATERIALS TEXT

MATERIALS

Benzyl ether (Dibenzyl ether) **Sigma Aldrich Catalog #108014-1KG**

SAFETY WARNINGS

Exercise caution and use proper PPE (lab coat, gloves) when handling cuvette filled with dibenzyl ether (DBE). Any traces of DBE should be cleaned up using a 70% ethanol solution.

Microscope set-up

1 1. Open Impspector Pro software

Must turn laser and microscope on before starting software
(Version: Imspector Pro 328)

2 Remove lid from cuvette

3 Handling only frosted portion of cuvette, place cuvette onto extended stage mounting apparatus and slowly lower into position. The two frosted sides should face the back and front of the microscope (Figure 1)

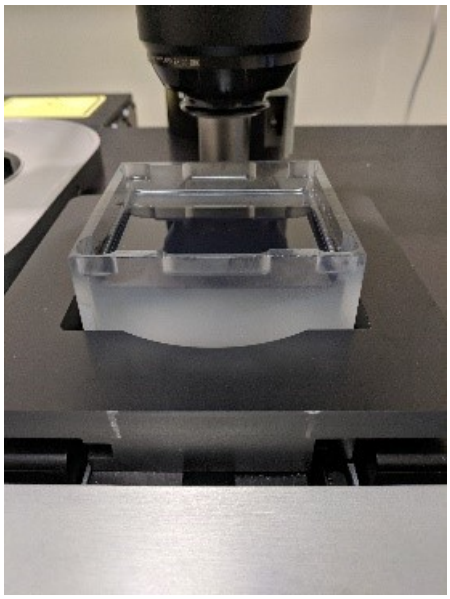

Figure 1

4 Fasten the metal coverpiece over cuvette (Figure 2)

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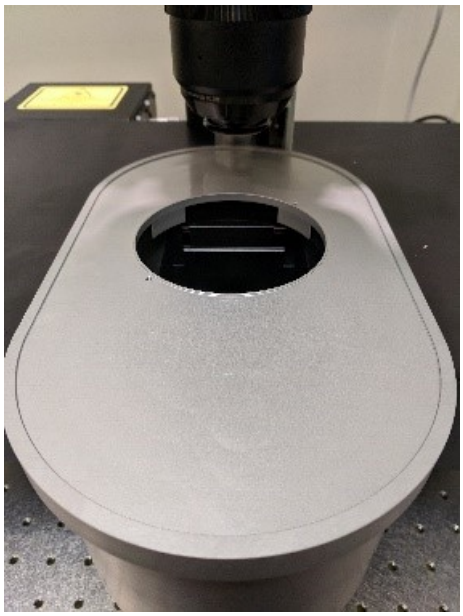

Figure 2

5 Mount tissue sample with region of interest facing upwards

a. Gently push sample onto prongs of metal piece (called "hedgehog") inside mounting apparatus (Figure 3).

b. The surface you wish to image should be facing up.

Samples need very little pressure to remain fastened during imaging and you want to inflict as little damage to the sample as possible

Figure 3. For imaging of ventral structures (i.e. sympathetic chain ganglia), the specimen is mounted ventral side up. When imaging dorsal structures (i.e. intermediolateral bundle (IML) in the spinal cord), the specimen is mounted dorsal side up.

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6 Place mounting apparatus into cuvette

7 Position objective piece over cuvette
a. If using Corrected lens turn to halfway position (Figure 4)

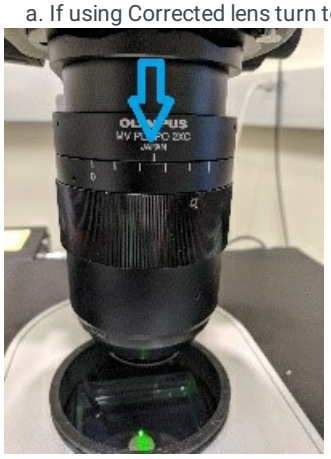

Figure 4.

Software set-up

8 Within computer software, set instrument mode to CDC 2x (Figure 5)

We use CDC2x because of the type of corrective cap that is attached to the front of the zoom objective

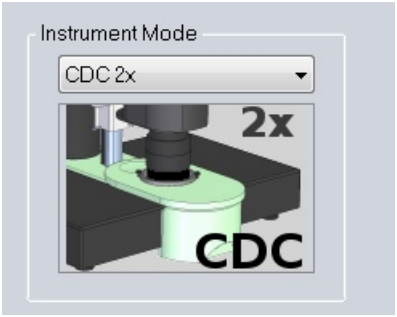

Figure 5

9 To appropriately visualize the region of interest, select the desired zoom factor (Figure 6). You will need to select the zoom factor both on the program and on the microscope (clearly labeled knob on right side of microscope).

To create overview images of the thoracic cavity, a zoom factor of 0.63 is used. For imaging individual sympathetic chain ganglia, we increase the zoom factor to 2.0 or higher.

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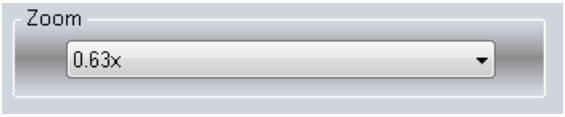

Figure 6.

10 Set measurement parameters in table 2 (Figure 7).
a. Xyz-Table Z
b. Ultrafilter
c. Xyz-Table X
d. Xyz-Table Y

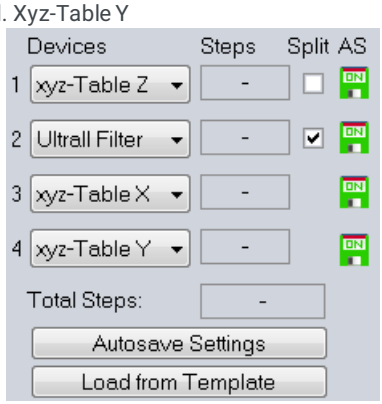

Figure 7. Make sure all devices have the "on" feature displayed and only the "ultrall filter" is checked

11 Select desired laser wavelengths (according to your fluorescent labeling) by checking them on the left side. Here you can also pseudocolor the wavelengths by clicking on the color gradient shown. A dropdown menu will appear and you can select another color (Figure 8).

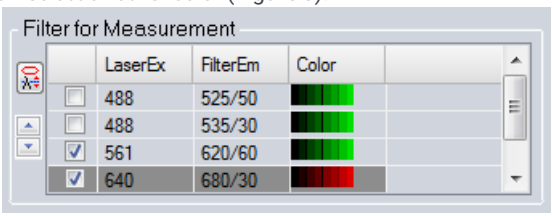

Figure 8.

12 In the "Sample" window, select the liquid used in the cuvette from the dropdown menu (Figure 9).

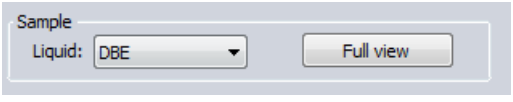

Figure 9. Since our samples are stored in DBE, the cuvette is also filled with DBE.

13 Click the video icon – a window will appear that displays a real-time view of the sample (Figure 10).

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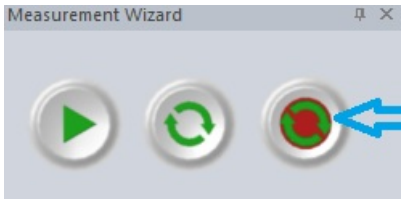

Figure 10.

14 Set initial laser percentages (Figure 11).

Figure 11. Start with a low laser intensity (10%). It is bright enough to visualize the tissue, but low enough to minimize photobleaching of samples while adjusting all the various settings

15 Using the Z knob on the joystick (which will move the mounting apparatus up and down, not the objective) adjust the position of the sample until it comes into the plane of the lasers. Figure 12 is demonstrating optimal illumination of the sample wherein the majority of the tissue is being illuminated by the lasers.

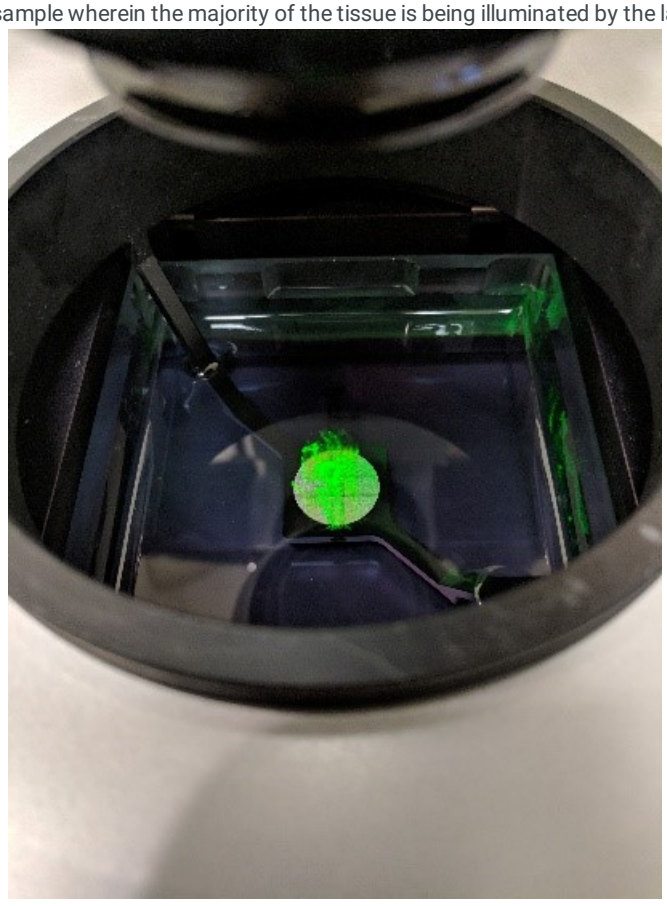

Figure 12.

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16 Use the coarse adjustment knob to lower objective into the cuvette (Figure 13).

a. While lowering objective, direct attention to the computer to make sure the objective actually submerges into the DBE, but does not hit your sample - you will be able to visualize the objective submerging into DBE.

b. Use the X and Y knobs on the joystick to move tissue left/right and forward/backward, respectively.

Figure 13.

Figure 14. Continuously click the blue icon with the yellow star to adjust brightness on the screen as you lower objective

c. At this time you can also use the fine adjustment knob to create a clear image, BUT do not spend too much time on fine adjustment at this point because you still need to customize the laser settings

17 1. Select appropriate laser percentage MUST CLICK APPLY OR SETTINGS WILL BE LOST!

a. Select the laser you will want to visualize first. Use fine adjustment knob during this time to create crisp image.

b. If using more than one laser, each laser will need its own specific percentage setting, and you need to click apply for each laser to save the settings.

c. Make coarse and fine adjustment corrections on ***only one laser wavelength***. To ensure all lasers will match in visual clarity, select the "chromatic correction" button and slide the arrow to the desired setting.

Example: I am going to use wavelengths 561 and 640 for this sample. I will make all coarse and fine adjustments on 561 only. Once I'm satisfied with how the sample looks, I will select wavelength 640. You will see that it appears out of focus. Select the chromatic correction button and use arrow to bring sample into focus (Figure 15).

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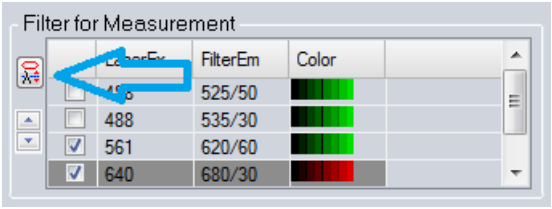

Figure 15.

18 1. In the “Scan Range” window, set the Z-stack – this will define the depth of your specimen (Figure 16)
a. The “start” is the top of the tissue and “end” is the bottom of your tissue
b. Rotating the Z-knob counterclockwise will bring you to the top, or “start” of your sample. Once at the top, click the button “set as zero” and then click the compass button (Figure 16).

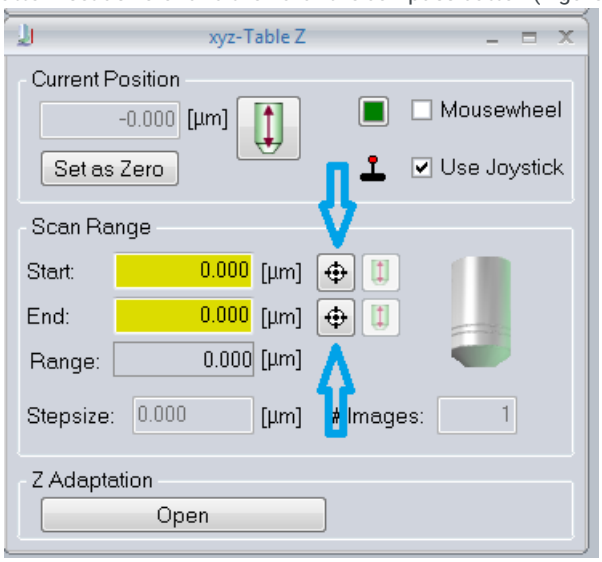

Figure 16.

c. Rotating the Z-knob clockwise will bring you to the bottom, or the “end” of your sample, then click the compass button (Figure 16).

19 In same window, determine desired stepsize. Once selected, #Images will automatically populate and you can then decide if you want to increase or decrease stepsize.

20 Click “autosave settings” on left side of screen to name and save image

21 Click “play” button to begin imaging process (Figure 17).

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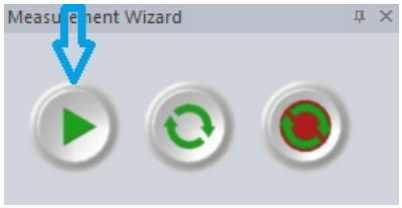

Figure 17.

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