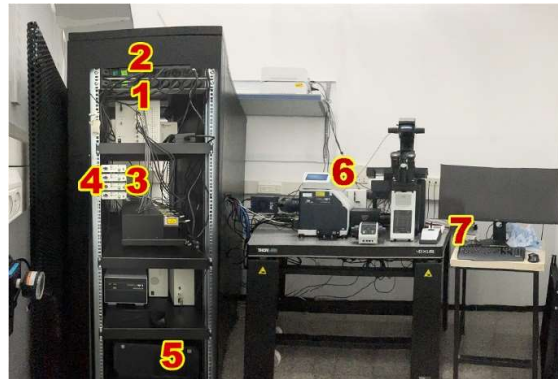


Olympus IXplore Spinning Disk Confocal Multidimensional Experiment Setup



Switch on in the order on the attached labels:

- Air condition
- (1), (2), extension cord switches
- (3) laser switches
- (4) laser keys (1/4 turn CW)
- (5) computer – the password is 123456
- (6) microscope touch-pad



- (7) LED on/off button to show "on" on its display
- (8) If applicable, switch on incubator tablet

The incubator inserts accept slide chambers and 35mm petri. The incubator settings should read:

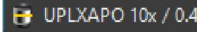
CO₂: 6.8%
Flow: 170cc/min
Cover: 37.5°C
Body: 37.0°C
Humidifier: 37.0°C

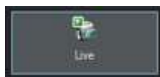



Open the OLYMPUS cellSens Dimension software by activating your reservation on BookItLab.



The software will ask to calibrate the stage. Agree and continue to define the XY limits. Do not calibrate Z when asked.

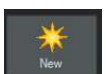
Select  Layout


On the **Microscope Control** toolbar choose the x10 objective  to facilitate location of sample (only objectives x4 and x10 accept plastic bottom dishes (long working distance); objective x4 does not support ZDC function)

In **Camera control** tool press . Exposure in confocal channels should be in multiples of 5ms.


Select **Observation methods** tool → **transmitted** →  (or another) channel and in the **Shutters** tool open the relevant shutter (transmitted/reflected). (It is preferable not to use the oculars to see the sample; you can only see DAPI and GFP by the oculars.) The installed filters are: **Cy3**: ex 546/12, T565 lpxr, em 617/73; **Cy5** tx 620/60, T660 lpxr, em 685/40.

Navigate to a well, focus manually, in **ZDC control** tool → , find focus again manually, press  so that it will give you the difference between the actual focus and what it finds.


Press **Experiment manager** tool →  in order to open the settings of an experiment (.oex extension file) or make one yourself:

First press time stack  and drag a frame. Select it, go to the **Time lapse loops** tool and choose number of cycles and interval.

Next press stage loop (i.e., positions)  and drag a frame inside the previous one.

Next press channels  and drag a frame inside the inner one.



Choose channels from the list:  and connect them with connectors

DAPI cf, GFP cf, mCherry cf, Cy5cf are the spinning disk confocal channels.

DAPI WF, GFP WF, mCherry WF, Cyf WF are the widefield channels (usually only to locate the cells)

BF and BF_FreeIntensity are transmitted light channels.

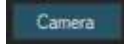


You can also add autofocus and Z drift compensation

To set channel parameters:

Select each channel and set its parameters:

- In **Laser/LED combiner** set laser intensity or in **Device Units** set LED intensity and press



- In the **Camera Control** toolbar set exposure



- <Right-click> → Get settings

In live experiments in particular you want to have minimal laser intensity and exposure; check saturation

by pressing  in **camera control**.

You can add Z sections to each channel by dragging around it after grabbing .

Note: channels inside an encompassing frame should have the same settings in z and bit depth.


Select each channel and change image bit depth in the experiment in **Camera Settings** (on the right panel)

To set Z sections:


Select the frame, and in **Z-stack loop** tool, choose range (from center) and select range and step size.

To scan positions/tiles:


First set the overview range:

In **Stage Navigator** in 4x objective tool press  and follow directions (confirm top left and bottom right corners – be generous when you define the borders).

You may find convenient to acquire an overview image with the x4 objective, low camera resolution by



pressing acquire overview [] (you can cancel by pressing cancel on the bottom left corner).

To set positions:

In stage Navigator choose positions 

To set tiles:


Choose a way to define the area to be scanned, e.g., .

Choose supporting points to focus  (use the arrow to the right to choose at which concentration – you can add additional supporting points by right click). You can manually go from one to the next with the arrows  to set the z value or use autofocus. Exit the function. Make sure in **Stage Loop** tool, with the stage loop rectangle of the experiment selected that [use focus map] is selected.


To set positions in a multiwell dish:

First calibrate the dish:

In **Well Navigator** tool in 4x objective press , and follow the instructions to calibrate the dish.

To define a pattern of positions, go to required objective, press  and give the numbers of rows and columns and press **Spread** and **OK**. Click or drag to define wells where you wish to apply this pattern.

To set the ZDC as a starting point for autofocus:

In **Stage navigator** tool press position list  to read the ZDC offset of the positions: in the ensuing dialog, go to the first position “go to position”, you focus manually, press “update Z”, “read offset”, “ok”, you check another two or three and see if they are quite close, then you go to another, “go to position”, focus manually, “update Z”, “read offset”, “apply to all”, “ok”.

Autosave, naming and location of files

In Experiment Manager click Acquisition Settings → Saving → Process/experiment → Destination [File system]; File type [Virtual Slide Image *.vsi] and choose the directory path D:\Users Data\[Your PI's name]; create subdirectory as it suggests – leave underscores where they are → apply to all

Also: Document name → process/experiment → customize text: [file name of your choice] → apply to all → ok

Save files in the recommended .vsi filetype which includes metadata with the experiment's parameters (raw data file). You can open this filetype with ImageJ (by Plugins → BioFormat Importer) or with [OlyVIA](#) free software.

Press Start Process



At the end of the experiment copy the files to the server which is accessible from any computer in the building after obtaining permission from Masha (mdmasha@technion.ac.il).

Clean up and shut down the system in the opposite order of starting up.