TURKU BIOSCIENCE

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# DeltaVision OMX SR (SIM and TIRFM)

## User manual

Cell Imaging & Cytometry

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# 1. Information

The Cell Imaging and Cytometry Core is responsible for the maintenance of this microscope.
Each user must follow the CIC user policy.
More information: https://bioscience.fi/services/cell-imaging

- **Only authorized users may use the CIC instruments.**
- **Users must report any malfunction or problem to the CIC personnel.**
- **The user is responsible for removing their data from the hard drive and should do so immediately.**
- **Files older than 30 days are automatically wiped from the system without prior notice.**

## 2. Before Imaging

### Check your sample with an ordinary fluorescence microscope

It is a good practice to check the quality of your sample before making use of a more expensive instrument.

### Clean your slides

Clean the remaining salt and mounting medium off the coverslip. Dirty coverslip compromises image quality. You can use ethanol to aid the cleaning. Do not use the microscope lens tissue. Clean your slides beforehand in your lab, as it is impractical to use microscopy time for cleaning.

### Check the environment

Switch on the lights and check if the microscope environment is tidy.
If there are oil spills or other issues, please inform CIC personnel.
(microscopy@bioscience.fi)

### Start the heating earlier if possible

When doing live cell imaging, it is good to switch on the heating at least two hours in advance. If someone is using the instrument, ask whether it is possible to switch it on. If no one is using the instrument, start the instrument and switch on the heating.

### Cancellation

Cancellation must be done 12 hours before the reservation starts. However, if you suddenly cannot use your time, contact us by email or come to the office. Late cancellations are only accepted under exceptional circumstances.

If you are the last user of the day and cannot come, you are responsible for the instrument shutdown.

### Unsure?

If you feel that you need support, please contact CIC personnel.

## 3. Working with immersion objectives

Please follow our common rules on how to work with immersion objectives, but **note the special rules concerning the OMX**:

The immersion medium should match a sample refractive index and used laser lines. Use the **Immersion Oil Calculator** application by Ge Healthcare to find a correct immersion oil (selection of 15 grades with refractive indices between 1.510 and 1.534):
https://www.gelifesciences.com/en/us/support/online-tools/cell-imaging-and-microscopy/immersion-oil-calculator

A small drop of oil is enough, adding too much can make a mess and damage the instrument.

Contrary to our normal instructions, **move the objective to the wanted working level before loading the sample on the OMX**. Place the sample carefully, and monitor how the sample surface touches the immersion oils. Move the sample gently by hand if air bubbles have formed in the immersion.

After imaging, wipe the oil off from the objective softly with dry lens tissue. Then, finish the cleaning by gently wiping with a new lens tissue moistened **with Abs. EtOH**.
Only lens tissue may touch the objective lens.

Inform CIC personnel immediately, if you can not clean the objective fully. Structured illumination is possible only with an absolutely clean lens.

# 4. Start-up and standby

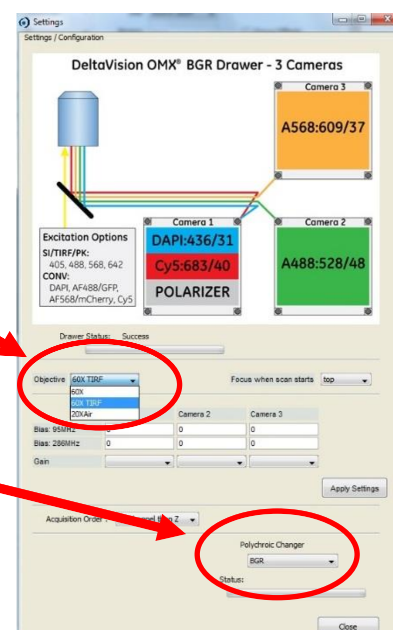
## 1. Start-up

1. Switch on the lasers you need, and turn ON the laser interlock key.

2. Switch on the monitors

3. Start the OMX software

4. Ensure that the correct objective is installed
**(Objectives can be changed only by CIC personnel)**
* 60x 1.42 SI objective
* 60x 1.49 TIRF objective (check the temperature collar!)




5. Check the configuration settings:

- **The objective setting** must match with a chosen objective
  - 60x SI
  - 60x TIRF
- Choose a **Polychroic Changer** according to laser lines in use.
  - BGR for 405/488/568/642
  - CYR for 445/514/568

**** Remember to follow special rules concerning working with immersion oils, the page 3.***

## 2. Standby mode

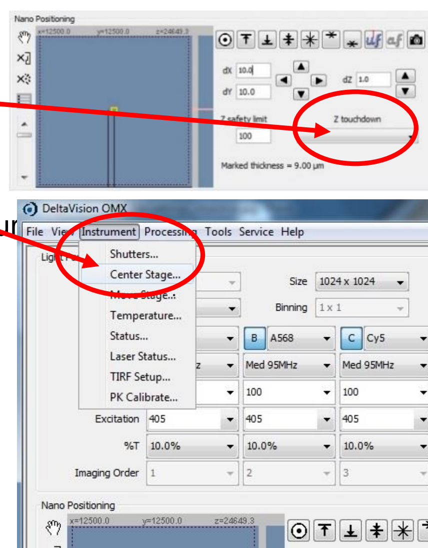
The system is kept on 24/7. Full system start-up/shut down is done only by CIC personnel.

**Standby instructions:**

1. Remove the sample
2. Clean the objective
3. Centre the stage
4. Move the stage up “Top Position”
5. Switch off lasers, and turn off the laser interlock key
6. Switch off screens

*You can leave workstation to run image processing for overnight but remove your data as soon as possible.*

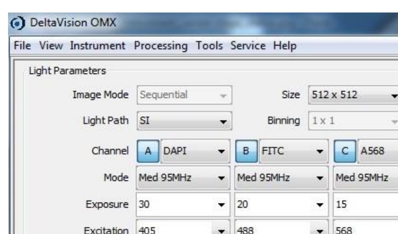
# 5. Starting the software




1. Move the stage up: Z touchdown "Top 24500"
2. Centre the stage
3. Set the stage to a Z touchdown according to your
4. sample type before adding a sample
5. Apply an immersion oil type
6. Place the sample

# 6. Imaging settings (Light parameters)

See the ***DeltaVision OMX Quick reference 1, Startup and Acquisition*** to find more information on loading a sample, navigation, defining the light parameters and experiment settings.




***Note the following points:***

Our OMX has three sCMOS cameras with a full-frame of 1024x1024 pixels.
Complete SI image reconstruction over full FOV must have maximum 512x512

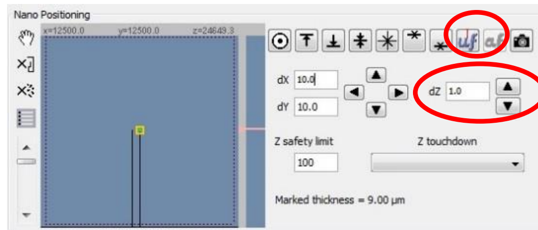
**Mode:** use medium frequency (fast mode when exposure is less than 10ms).

**Excitation (light source):** numbers describe lasers for TIRF and SI, and fluorophore names (GFP, Cy5 etc.) refers to the conventional mode. DIC can be used for any channel

**Excitation power:** monitoring of photobleaching is highly important for SIM!

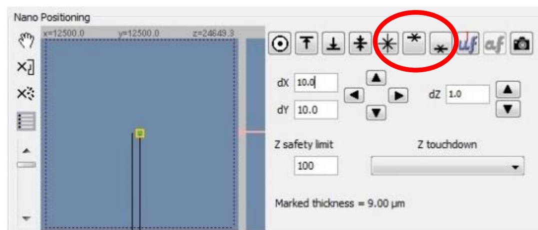
**Note! The 405 and 642 channels use the same camera. The light path is currently optimized for 405-dichroic to match with 488 and 568 channels, therefore the 642 channel is slightly out of focus. SI Image reconstruction in 3D corrects this error, but in 2D this is not possible. Therefore z- offsets must be set manually to make the focal plane equal between 4 channels.*

***Focusing:***
Use Ultra Focus system (UF) to find the sample surface. Continue focusing with small Z-steps to find the sample (dZ 1-5 um depending on a sample). Fine-tune a focus by using step size 0.1 - 0.5 um.




***Navigation in XY***
Use arrow steps, or drag and drop the navigation square, or “go to position” on a mosaic image.

***3D stack***
3D volume can be set with upper and lower marks or a middle point, but then the stage must be moved to a starting level before an imaging acquisition.



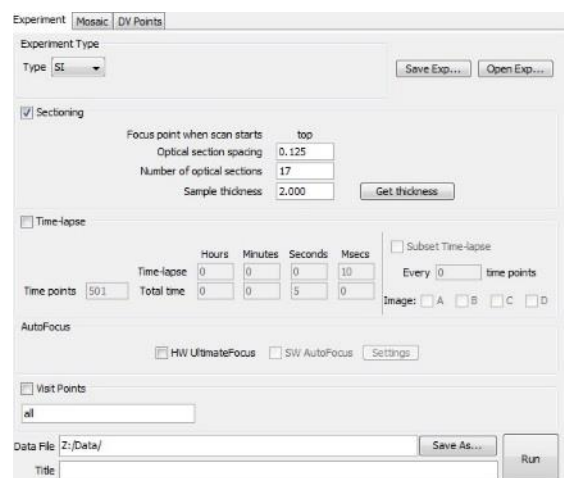

Optionally you can just move to the bottom/top of the cell and let sectioning to start from this level without Z-marks.

In both cases, **you must know the travel direction** that you can move to the correct starting level: check this from **instrument settings.**

**Sectioning:** Use 0.125 um step size

***Data File:***
SAVE AS: Save your data on Data1 –Drive, into your group folder.

Create a work folder by naming a file with prefix YYMMDD_.




**NOTE!**
**Folders and file names can not include space. Use (–) or (_) for separation!**

# 7. TIRFM

Open the TIRF menu: *Instrument → TIRF setup*

Focus to a sample and find a cell to the middle of FOV. Calibrate the ring TIRF illumination by choosing "Calibrate TIRF". Accept and save new values.

In case of simultaneous imaging, there has to be one TIRF angle for all the channels, and Z offset is unavailable.

The TIRF objective has a very small working distance from the cover-glass. In a multiposition experiment, this can cause instability in Z levels if there's any tilting of a sample: the objective can push the sample upwards when moving in XY, and it drops back in some point. UF can't always correct this. Try to choose imaging positions from near of each other (from the area of max 5x5 or 10x10 frames). Note that too much oil can cause similar drifting problems.

# 8. Image processing

Please follow instructions from the DeltaVision ***OMX Quick reference 2, SI Image Reconstruction***. The instructions describe how to use ***Task Builder*** for SI and TIRF image processing.

TIRF images need to be procced to align the channels.

In the case of TIRF deconvolution an OTF file has to be chosen manually:
**"OLYMPUS 60x 142.10612.otf"**

# 9. About sample preparation

Discuss with CIC personnel about your experiment and possible IF staining in advance.

Use high precision 1.5H cover glasses only (1.5H, 0.170 mm ± 0.005mm).

Coverslips must be extremely clean: acid wash is highly recommended.

The stage can move within a restricted area of 25x25 mm, therefore it is crucial to mount the cover glass onto the middle of a slide.

IF staining and the mounting medium have unique recommendations. Please contact CIC personnel before you start to plan sample preparation

Clean coverslips careful with Abs. EtOH before loading to the microscope.

# 10. Troubleshooting

## 1. The stage does not work

**Known issues:**

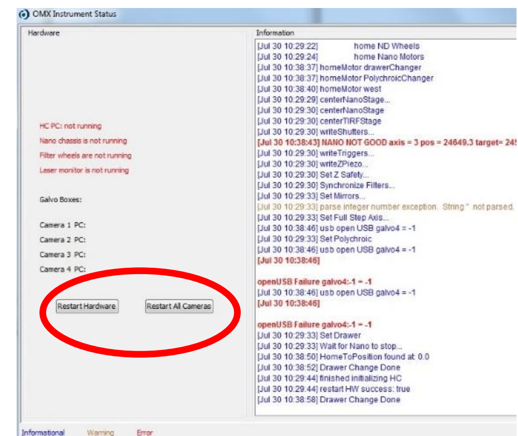
1. The stage does not travel to the expected position (x.y.z)
2. The stage can travel in the z-axis but does not reach the correct level

**Solution:**

1. Remove the sample and sample holder
2. Close the OMX software
3. Switch off the Nanomotion Chassis and Piezojena Controller from the component rack.

4. Wait 2 mins, and then switch on the Nanomotion Chassis and Jena Piezo Controller
5. Wait 1 min and launch OMX software
6. Restart the hardware and cameras from the OMX software:
   *Instrument → Instrument Status*
   - Restart the Hardware (wait until done)
   - Restart Cameras

## 2. Lasers do not work




Check settings and laser safety lock:

1. Laser switched on and the key turned to on position?
2. The microscope cabin door is closed and the red laser safety light lit normally?
3. The objective and the DIC slider are in the correct position and do block the laser beam?
4. Imaging mode and settings ok?
5. Do you have the same problem with other lasers?
6. Report CIC staff!

## 3. 488-laser stop working

488-laser can overheat, which cuts its power. In this case, the laser must be cooled:

1. Switch of all the lasers and turn the laser lock key off.
2. Wait at least 20-60 minutes before trying again.

## 4. Deconvolution or image reconstructions do not start:

1. File naming: check folder and file names from the file path that there are no spaces
2. Objective setting can be wrong (the setting will be written to metadata, which is used in image processing).