

3i W1 Spinning disk

User manual

Cell Imaging & Cytometry

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1. Information

The Cell Imaging and Cytometry Core is responsible for maintenance of this microscope.

Each user must follow the CIC [user policy](#).

More information: <https://bioscience.fi/cic>

- **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 the image quality. You can use ethanol to aid the cleaning. Do not use the microscope lens tissue. Clean your slides beforehand in your own 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

The immersion medium should match the objective.

The 100x and 63x oil objectives need oil with a refraction index of 1.518.

The image will be suboptimal when incorrect oil is applied. Mixing immersion oils is prohibited.

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

Start imaging with the objective in the lowest position. Then, focus the objective upwards until the just oil touches the coverslip.

Next, focus the sample visually through the eyepieces.

After imaging, wipe the oil off from the objective softly with a dry lens tissue. Then, finish the cleaning by wiping softly with a new lens tissue moistened with isopropanol.

Only lens tissue may touch the objective lens.

Lower the objective when changing the sample to view with an immersion objective

- Press **load position on the touchpad** to move the objective down.
- Remove the sample and add oil if needed
- Place the sample and raise the objective back into contact with the sample.
- If you are changing from an oil objective in between samples, wipe the objective clean with a dry lens tissue. Otherwise, oil might spill into the microscope.

4. System start-up

- Switch on button labelled “1” , **wait 60 seconds**
- Switch on “2” and **wait 30 seconds**
- To use the Evolve EMCCD camera switch on button labelled Evolve
- Switch on buttons “3” and “4”



Start the computer and log in



- Switch on the lasers you will use:
 - 405
 - 488
 - 561
 - 640
 - 730
- Turn the **laser lock key** to **ON** from the top panel of the laser rack (arrow).

Note: Photo manipulation is available for 405/488/561/640

5. System shut down

- Move objective to the load position and clean carefully.
- **Switch off all laser lines** and turn the **laser lock key to OFF** from the top panel.
- Close the SlideBook software, take your data and shut down the Windows/PC.

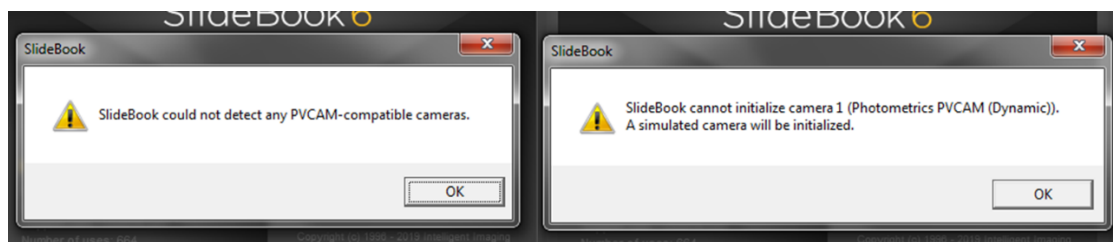


- Turn off the switches **starting at 4 and finishing at 1**.

Note: Remember to turn off the Evolve camera if it has been used.

6. Starting the software

Click the Slidebook icon on the Windows desktop

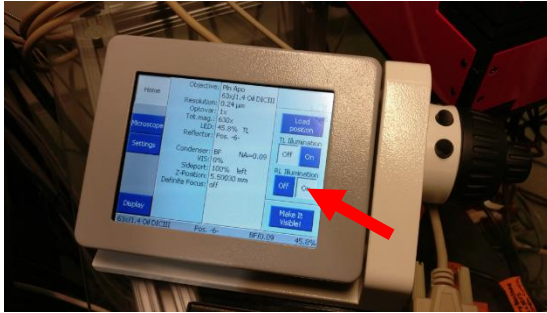


NOTE: If the Evolve camera is OFF you will get two errors. Click OK to continue start up. If there are more errors than these, please notify us.

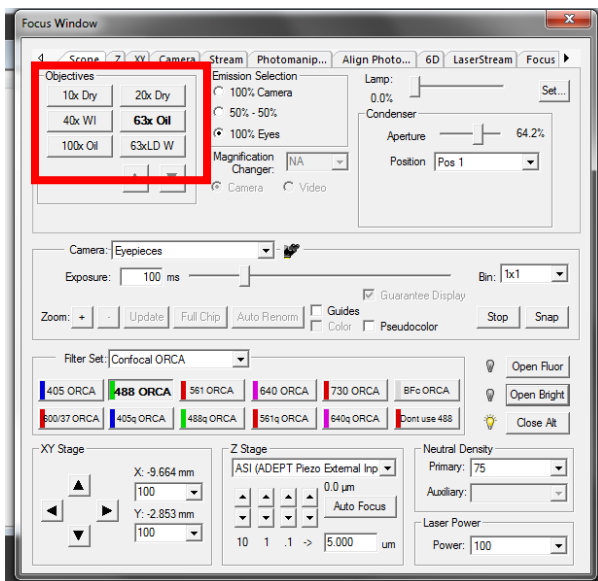


- Open a new slide (and save it to your folder) from icons
- Open Focus and Capture windows

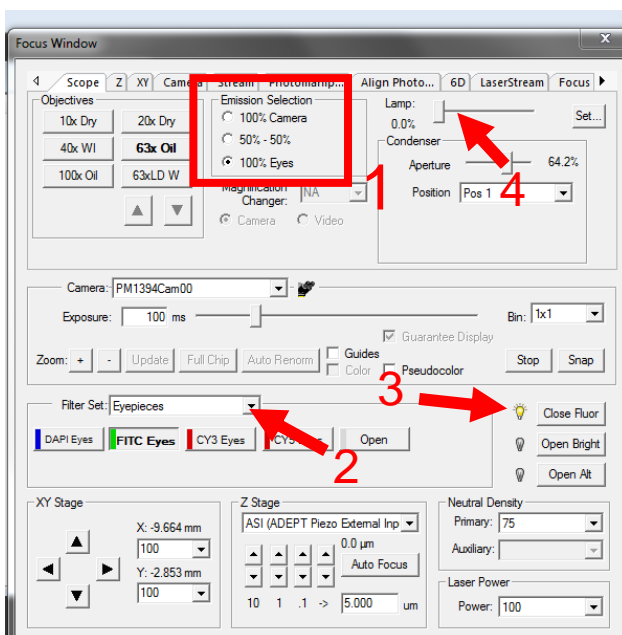
7. Finding sample using oculars



NOTE: the first user must open the RL shutter on the touchpad to allow fluorescence through the eyepieces!



- In the **Focus window**, the objectives are displayed in the upper left corner:
 - 10x/0.45 Dry
 - 20x/0.8 Dry
 - 40x/1.1 Water
 - 63x/1.4 Oil
 - 100x/1.4 Oil
- Available by request:
 - 63x/1.15 Water, long working distance
 - 100x/1.46 Oil

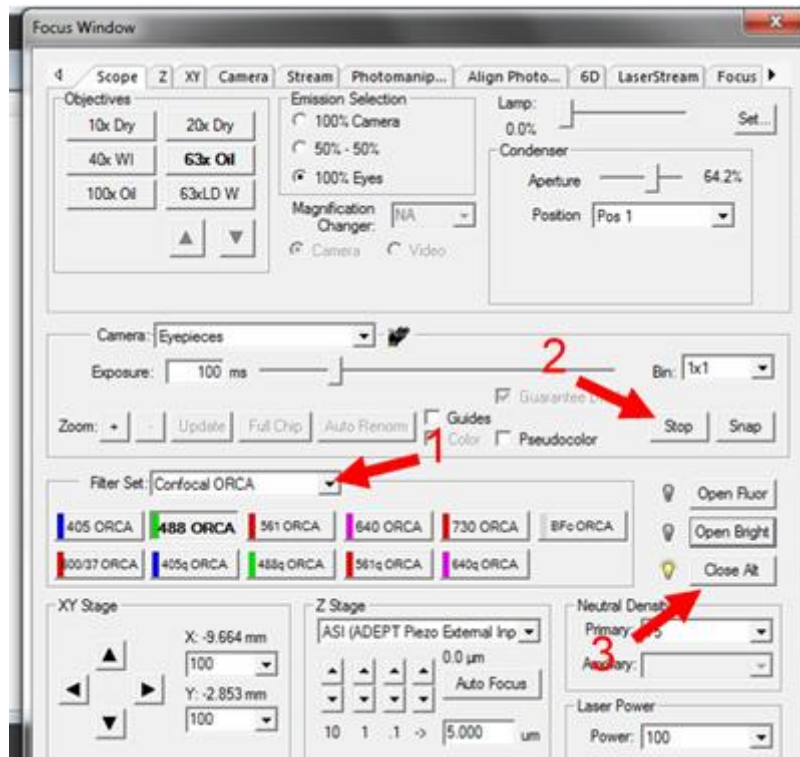


- **Emission selection (1)** determines the direction the fluorescence is sent:
 - 100% Camera
 - 100% Eyes

Note: 50% - 50% is not used

- From **Filter Set (2)**: select **Eyepieces** and select the channel you want to visualise e.g. **FITC Eyes**
- For fluorescence, you must select **Open Fluor (3)** to open the shutter.
- For transmitted light, select the **Open filter** and **Open Bright**. Transmitted light intensity is adjusted by using the Lamp slider (**4**) and the top right of the focus window.

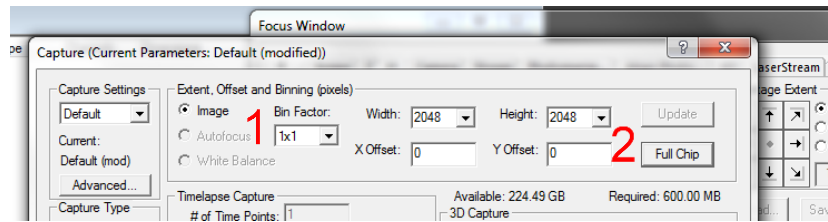
8. Sample visualisation using confocal



- Confocal mode can be used by selecting **Filter set (1)** and choosing either the **Confocal Orca** or **Confocal Evolve** camera set up.
- Select the filter set such as **488 Orca**.
- **Note:** A quad band filter is available for fast imaging (405q/ 488q/ 560q/ 640q) However, these are not recommended for most imaging as channel cross talk is highly likely.
- Pressing the **live/stop (2)** button will open/close the **Open Alt (3)** laser shutter and start imaging. Camera exposure time can be adjusted with the slider.

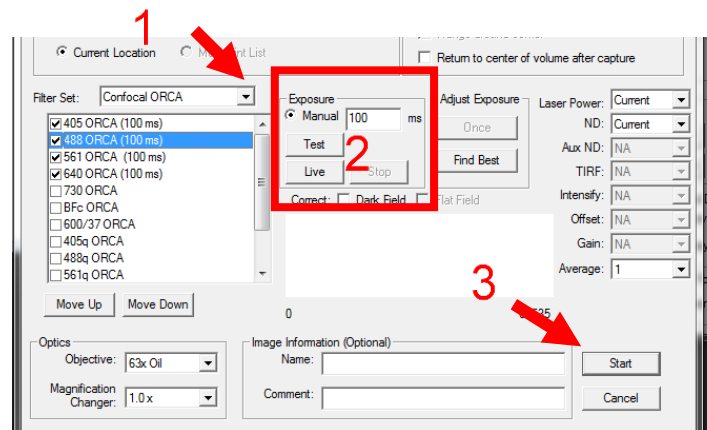
Note: Image optimization for confocal capture is better to do in the Capture tab.

9. Imaging Acquisition using Capture window



- The top part of the window allows selection of **Bin Factor (1)** to increase sensitivity by combining local pixels: Binning setting available are: 1x1, 2x2 and 4x4
- Chip size width and height: Orca has 2048x2048 and Evolve 512x512 pixels. Press **full chip (2)** to using the whole camera chip when imaging.

Note: If you select binning 4x4 and have selected Orca full chip (2048x2048) the actual frame size will be 512x512.



- To set channels for capture select Filter set **e.g. Confocal Orca (1)** and check the channel in the box e.g. 405 Orca and 488 Orca.
- Exposure times (milliseconds) are adjustable by typing into the box.
- Below are **Test** and **Live** buttons (2). Test takes one single frame and live will continually update the image.

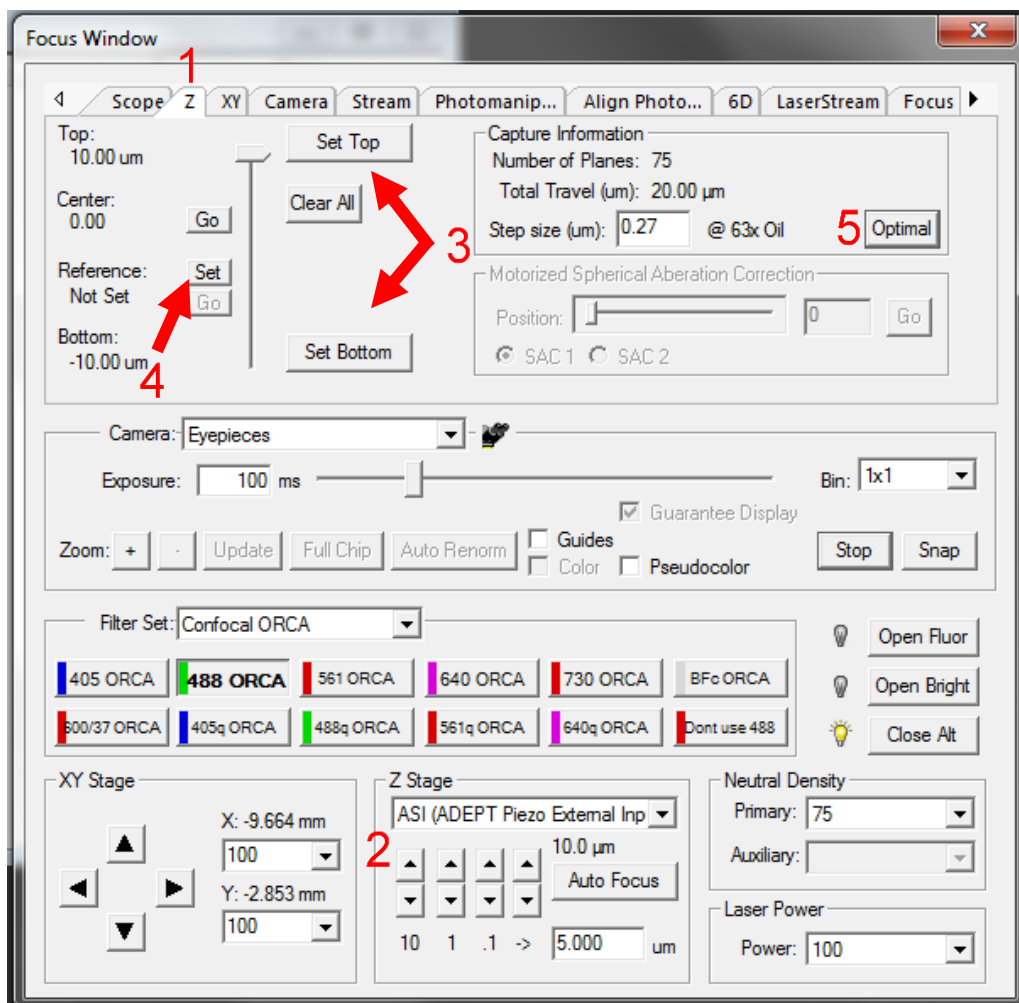
Note: The test setting allows you to optimise exposure time with greatly reduced risk of bleaching the sample.

- The test and live button will only capture the channel highlighted in blue.

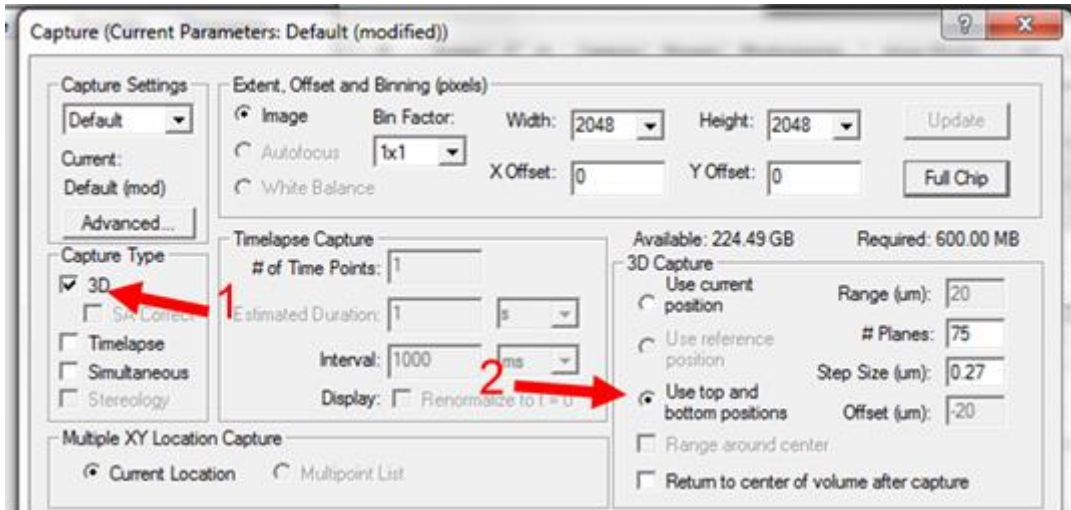
Note: **Laser Power**, and **Averaging** settings are adjusted on the right side of the tab. The "Current" setting for the Laser Power is 100%. In addition, if you define the laser power for one channel, all other selected channels must be manually defined.

- Once all your channels are optimised, **name** your file and press **start (3)**

10. Capturing a Z stack



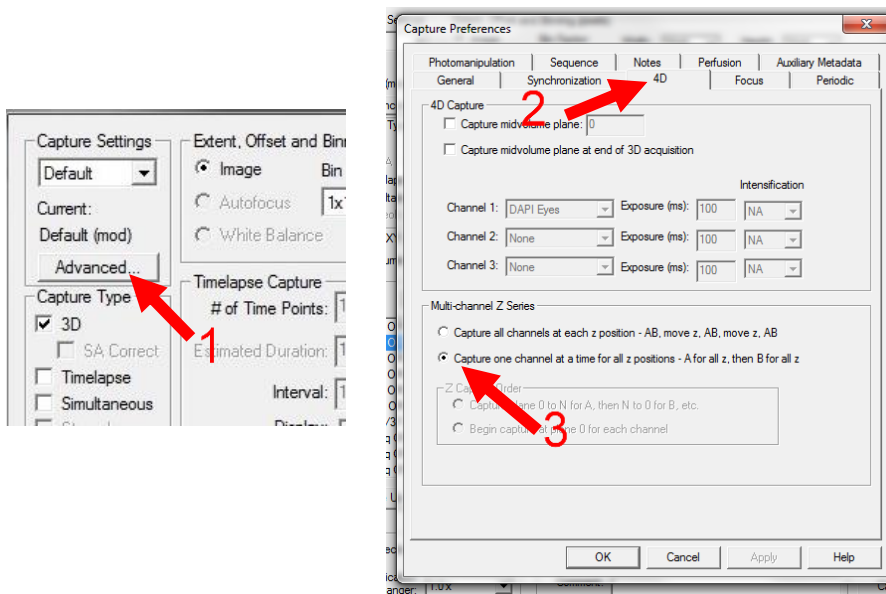
- A range must be set to capture a Z stack. In the **Focus window**, select the **Z tab (1)** from the top.
- Using the **Z Stage controls (2)**, set the **top and bottom limits (3)** of your stack. The Z controls use the ASI Piezo stage to move through your sample.
- Alternatively, you can also set a **reference position with the Set button (4)** and it can be used to centre a Z stack around it. This is useful for multi position imaging.
- **Optimal (5)** z step size can be selected and is based on Nyquist sampling for the objective used.



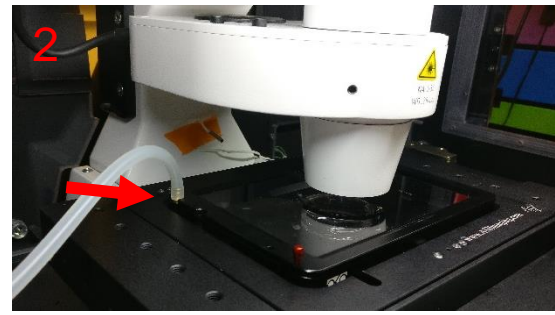
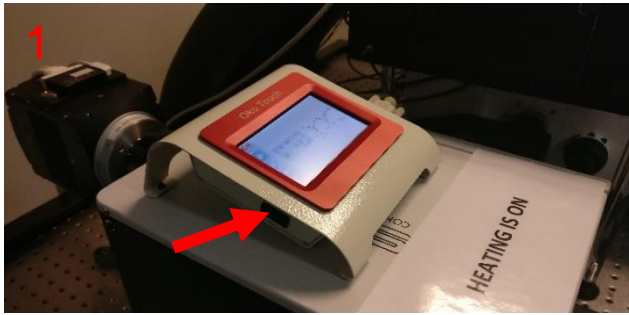
- In the Capture window activate **3D (1)**
- Select **Use top and bottom positions (2)** if you set a top and bottom limit.
- If you set a reference position slices can be set to range around it.
- Range, # planes and Z step size are adjust on the right side of under **3D Capture**.

10.1. Capturing one channel at a time for whole Z stack

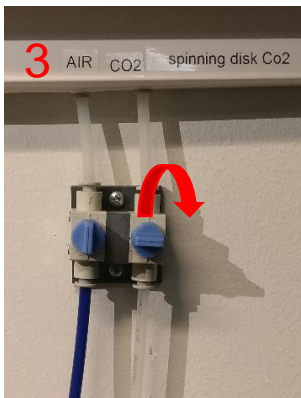
- By default, the system will capture each channel per slice, then move to the next slice.
- You can capture one channel for the whole stack then repeat for the following channels. This is faster but is less accurate for checking colocalization for example
- To capture one channel for the whole stack select **Advanced** and go to the **4D tab**.
- Under Multi-channel Z series select capture one channel at a time for all z positions



11. Live cell imaging



- Turn on the heating **(1)** and set the desired temperature using the Oko touch pad. If possible, set the heating on at least 2 hours before the imaging begins.
- Insert the live cell holder and connect the air tube **(2)**. Be extremely careful when lowering the condenser so you do not smash into the glass lid of live cell chamber.



- Before you bring your sample, open the CO2 valve **(3)**.
- The CO2 valve is open when it is turned to the 12 and 6 direction.

Note: Do not touch the air valve beside the CO2.



- Turn on the air pump **(4)**.
- Check that the correct air and CO2 levels are set on the mixing unit **(5)**. These are marked on the unit itself.
- To adjust the levels turn the knobs clockwise or counter clockwise.



12. Troubleshoot

12.1. No excitation light to a sample in widefield mode (Eyepieces)

Check that light path is correct and open the RL-shutter of the microscope manually from the touch pad (see page 7).

12.2. Cannot get sample into focus

- Check that your sample and a sample holder are properly installed.
- Use focus knob of the microscope and focus to a sample, and then continue focusing from software (with piezo focus steps).

12.3. Cameras do not work or no driver found

Close the Slidebook software, wait 2 min, then open the software again. If the cameras are still unavailable, restart the whole system and ensure that the cameras are on.

12.4. Photomanipulation is misaligned

The calibration is camera and objective depended. Contact CIC personnel to do full calibration.