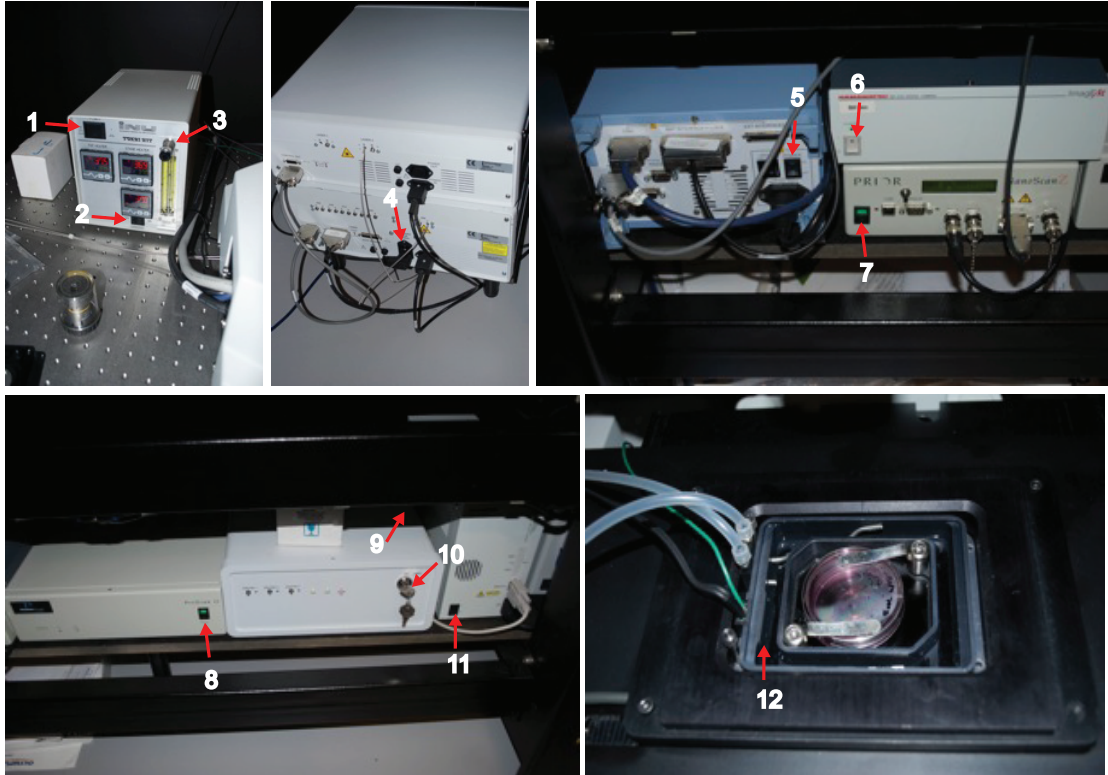


Using the Ultraview Vox spinning disk confocal

Switching the system on

1. It is important to pre-heat the environment chamber (1) and objective (2) before use. Switch the heater on at least 3 hours before use, preferably the night before.
2. Switch on the laser launcher (4), and then all the control boxes mounted under the table in order half an hour before your experiment.



- 1 - Chamber environment heater
- 2 - Objective heater
- 3 - CO₂ feed regulation
- 4 - Laser launcher
- 5 - Controller
- 6 - Hamamatsu digital Camera

- 7 - NanoscanZ
- 8 - Proscan II
- 9 - Shutter controller (switch on the back)
- 10 - Laser safety (switch on back)
- 11 - Olympus microscope
- 12 - Water chamber

Don't switch the Exfo lamp on, we do not use it for confocal imaging. Add some water to the humidity chamber (12). Open the CO₂ feed on the bottle next to the entrance door (do not touch the regulator) and turn the CO₂ supply (3) until the pressure is approximately 0.4 barr.

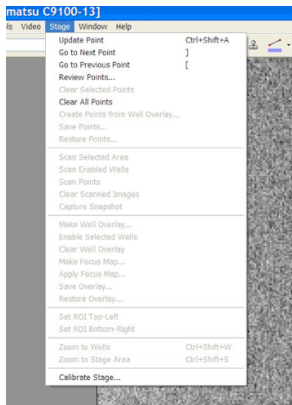
Check hard drive space – you will need 40-60 GB free space. Start Volocity 64-bit version on the PC.

Performing a live-cell imaging experiment

Check hard drive space – you will need 40-60 GB free space. Start Volocity 64-bit version found on the desktop of the PC.



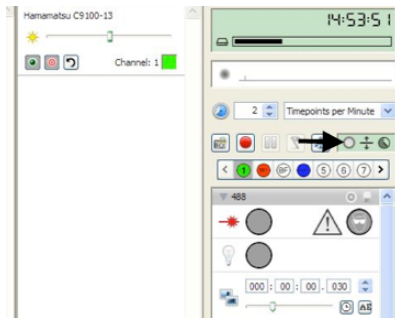
1. Setup a new library name or continue a previous experiment.
2. Save your files into a folder with your name and date
3. The library will contain all your data from the experiment in the form of a package.



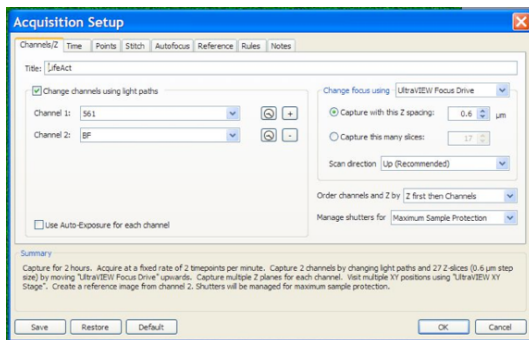
Before you start the setup of your experiment it is a good idea to clean all the previous stage positions from the previous user.

Select stage – Clear all points

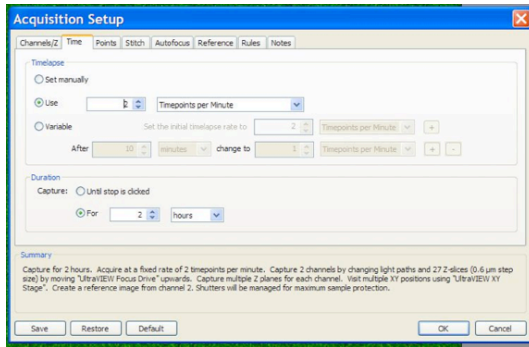
You will need to set up the settings for your experiment to fit your specific needs.



Press the icon highlighted by the arrow. Here you can setup channels, Z, time

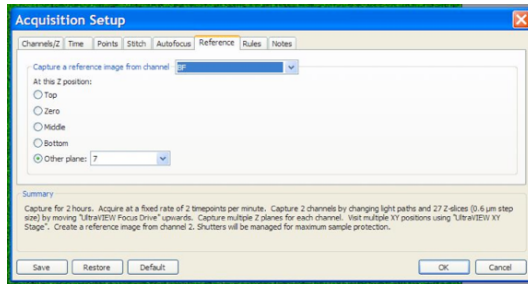


Input the channels you need, and the Z spacing (if any needed). As a default we use the ultraVIEW focus drive (piezo) for Z acquisition (which is fast and reliable) and scan direction (UP).

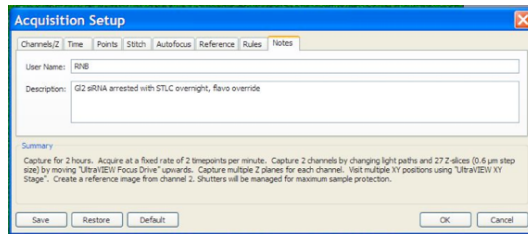


Adjust the time lapse acquisition setup.

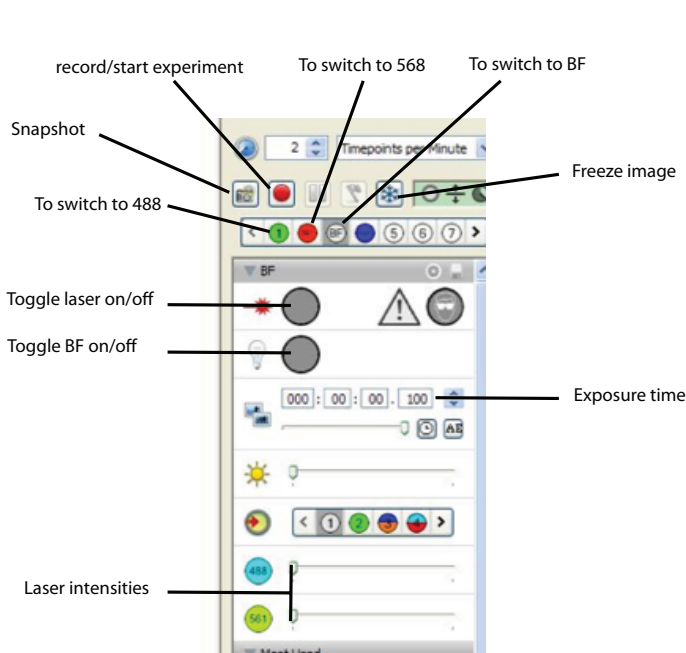
In the points Tab UltraVIEW XY stage should be selected



The reference tab allows you to take a single image. In our case we generally use (BF) this allows maximal sample protection by avoiding having to take a Z stack. The position is flexible



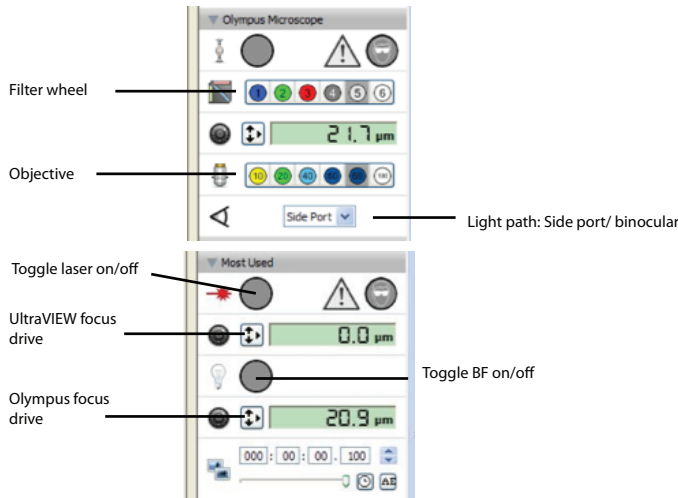
Add a small description of the experiment in the note tab



Description of the most important buttons used

For standard imaging we use 2-3% laser power for the eGFP and mCherry channels. Exposure time is typically 30 msec, but shorter times may be possible for strong signals such as histone H2B stable cell lines.

For the spinning disk to be fully functional the following settings have to be selected.

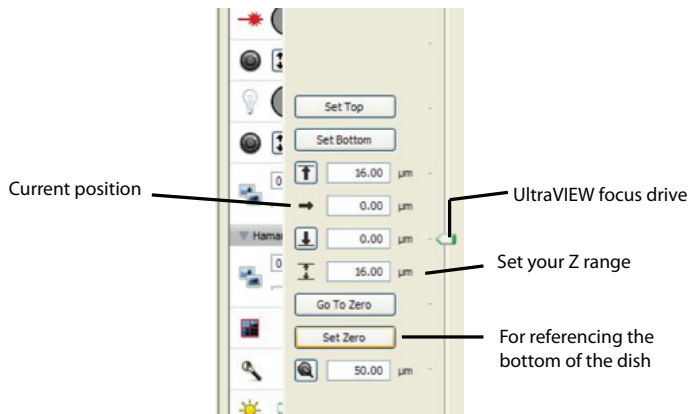


Please do not change them!
 Filter wheel has to be selected on an empty position (5 by default)
 Light path should be set to Side port.

We use the ultraview focus drive for Z acquisition

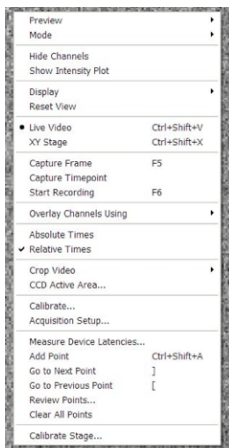
Starting your experiment:

Once you dish of cells is set in the environment chamber and has left to equilibrate for 30 min you can start your experiment. Switch the appropriate laser/BF to find your cells. Focus with the Olympus focus drive on the microscope.

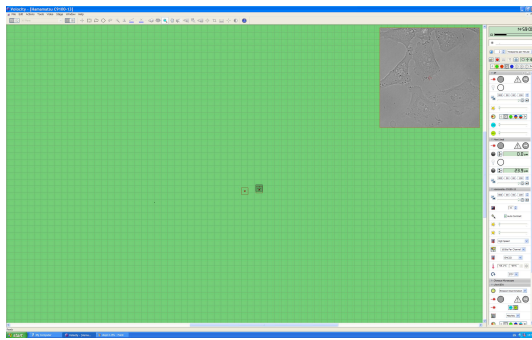


The system does not now where the dish is so you need to tell it where is the bottom of the dish and reference it. Do to this find the bottom of the dish and then press the ultraVIEW focus drive button and press zero

You can now start setting up the stage positions (if any). Try to find positions relatively close to each other to avoid shift and drifts during acquisitions but make sure they do not overlap since this will cause double exposures and the cells will die. To add new positions go to the stage dialog and press add position.

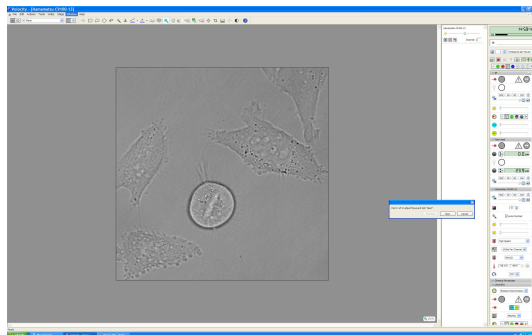


Once you are happy you can review them by pressing the right mouse button and selecting XY stage.



Here you can delete positions you do not want by selecting them with the selection tool and then going to the stage tab and pressing clear selected positions

Once all the positions are set, go to the stage tab and press review points.



Switch the appropriate laser/BF to find your cells. Focus with the Olympus focus drive on the microscope body. Once you are happy press next. Repeat this for the next steps and at the end press finish. To start your experiment press the record (red button)

Xy resolution of 60x objective 0.221237 μm

Objectives:	xy resolution
20x NA 0.5 air	0.666667
40x NA 0.75 oil	0.332226
60x NA 1.2 water	0.221237
60x NA 1.42 oil	0.221237
100x NA 1.4 oil	0.133331

Run regedit

To change user settings in volocity