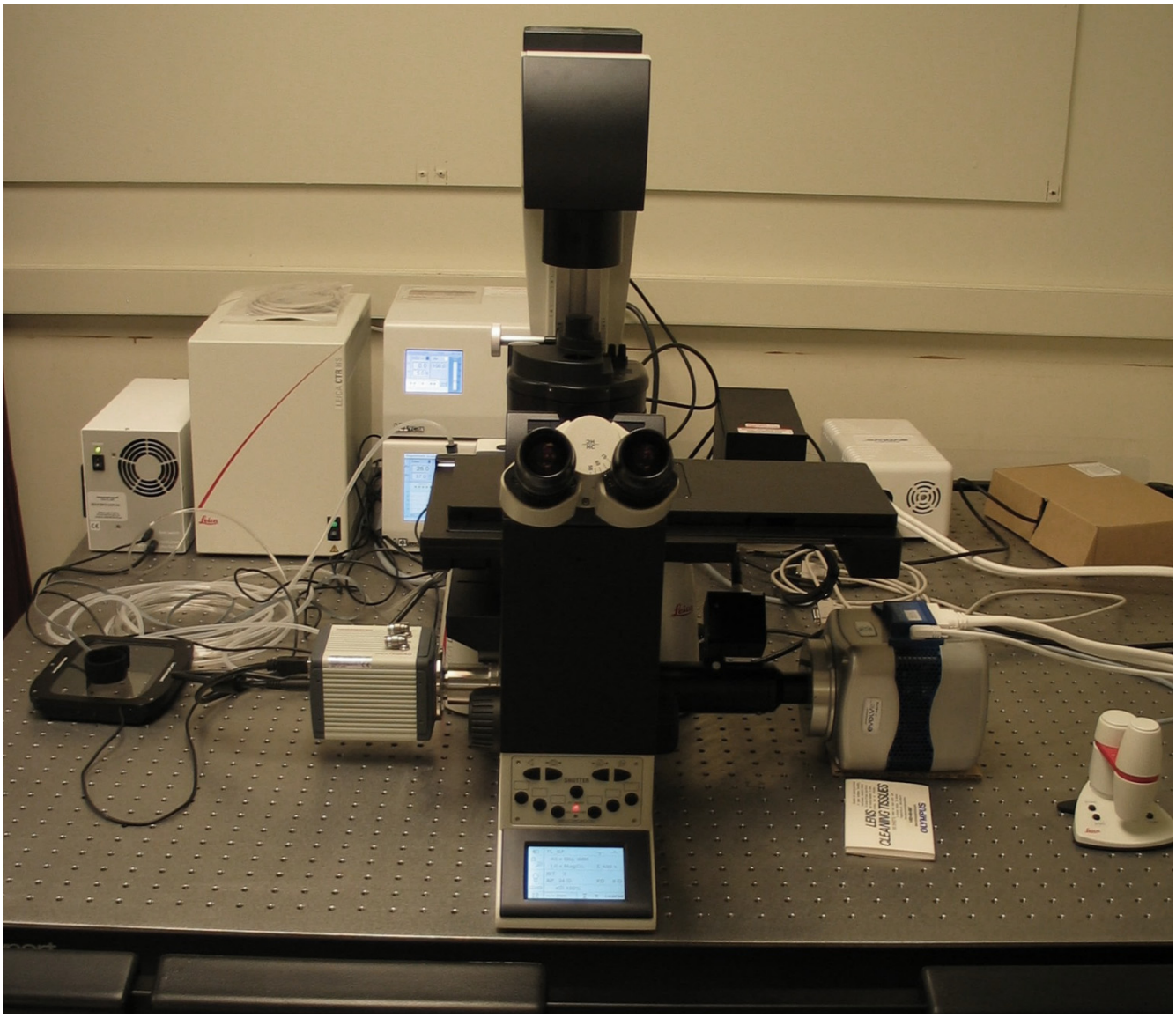
Heit/Kerfoot/Dikeakos

Leica DMI6000 B



Western

User Manual

Schulich
MEDICINE & DENTISTRY

WARNING

This microscope is a multi-lab resource that many individuals are dependent on. As such the following rules must be followed in order to minimize the risk of damage and downtime to the instrument.

Users who fail to follow these guidelines **will lose their access to this microscope**. In the event of damage to the microscope caused by a failure to follow these instructions, **the user and/or their supervisor will be responsible for the repair costs.**

Key Rules

1) This microscope is to be used only by approved users who have been trained by an approved trainer. Trained users are not considered to be approved trainers.
2) Under no circumstances are you to attempt to fix an issue with the microscope. Always seek the help of Drs. Heit or Kerfoot if you encounter an equipment problem.
3) Under no circumstances are you to remove lenses, turret plugs, filters or any other component of the microscope.
4) Follow the sign-up and sign-in procedures.
5) Rigorously follow the start-up, shut-down and safe use procedures.
6) If using the EVOLVE camera, or other specialized components of the microscope make sure that you restore the microscope to the default configuration when your experiment is complete.
7) Always return stage-inserts and other removable components to the storage drawer when your experiment is complete – these must never leave the lab.
8) **Always clean lenses, stages, stage-inserts and any spills when you are done.**

Data Storage

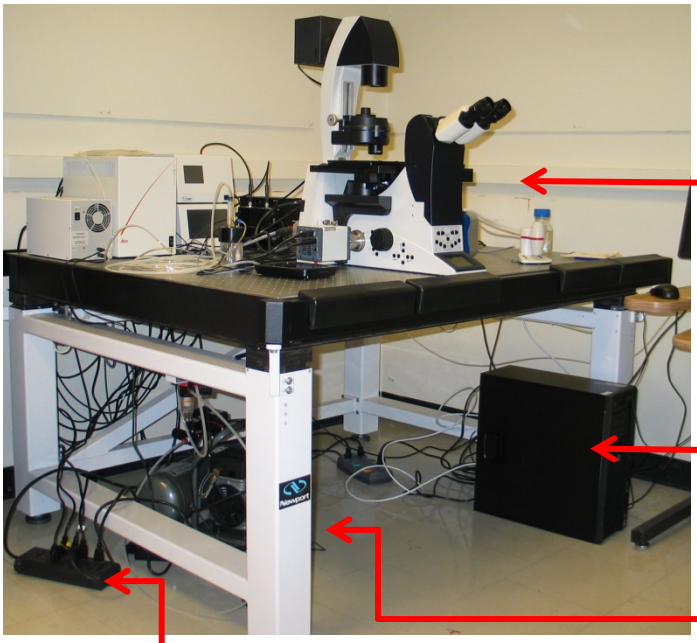
Many of the experiments performed on this microscope create extremely large data sets. To ensure good computer performance and sufficient storage for all experiments, data should be removed from the computer immediately upon completing your experiment.

Data on the microscope more than 48 hours old is subject to deletion without notification to the owner. Data stored anywhere but the E: (HDD2) will be deleted immediately.

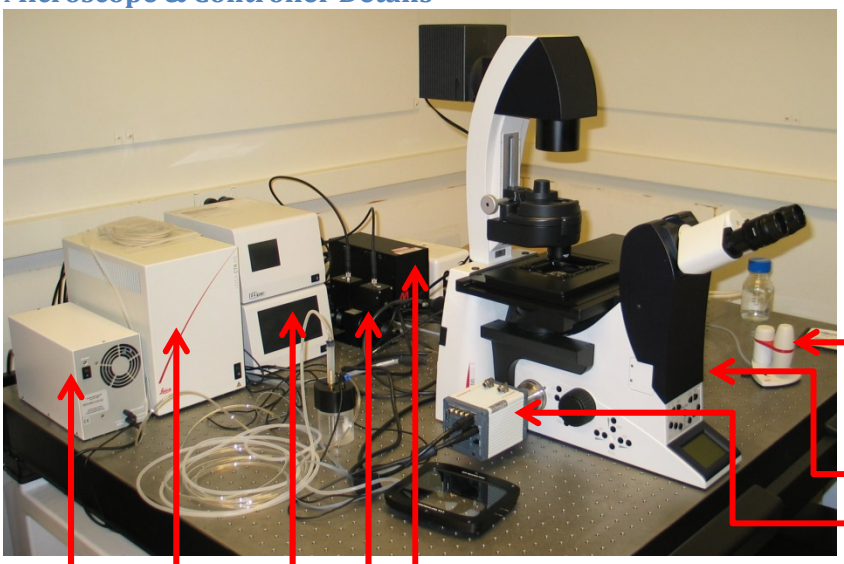
Table of Contents

Microscope Configuration

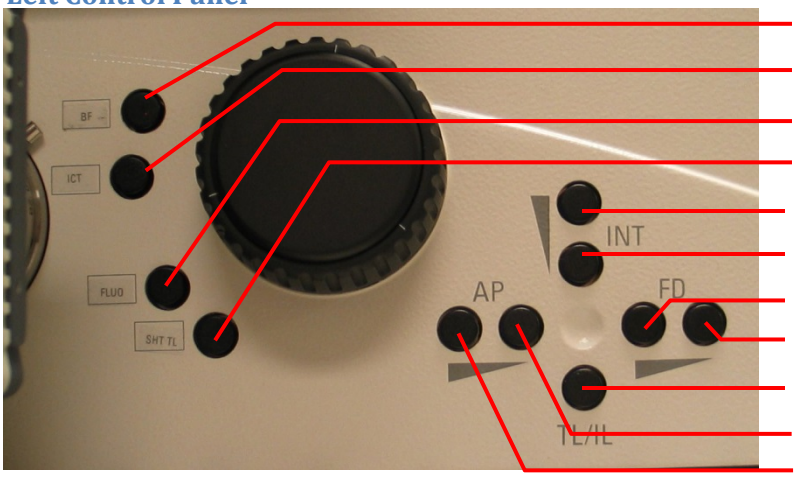
Overview



Microscope & Controller Details

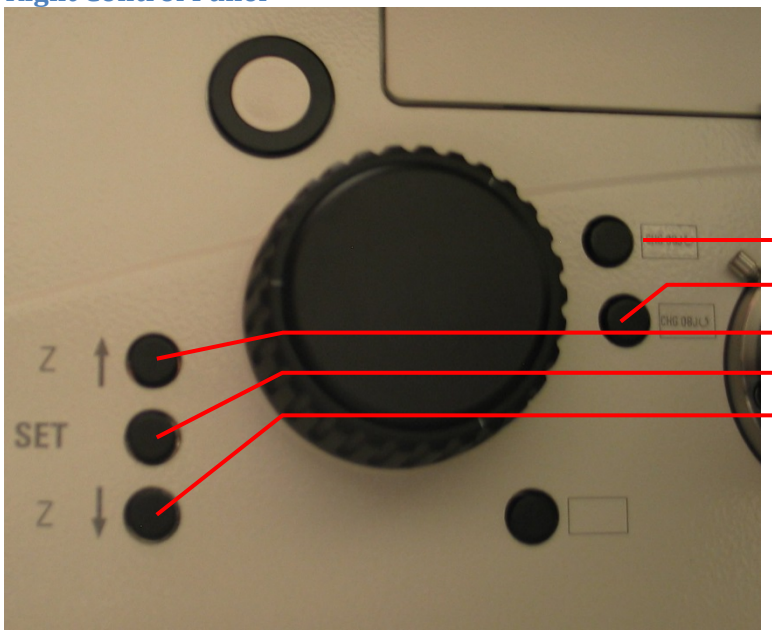


Left Control Panel



- Activate Brightfield Illumination
- Activate DIC (AKA ICT) Illumination
- Activate Florescence Illumination
- Transmitted Light Shutter
- Increase Brightfield Brightness
- Decrease Brightfield Brightness
- Open Field Diaphragm
- Close Field Diaphragm
- Brightfield Shutter
- Close Brightfield Aperture
- Open Brightfield Aperture

Right Control Panel

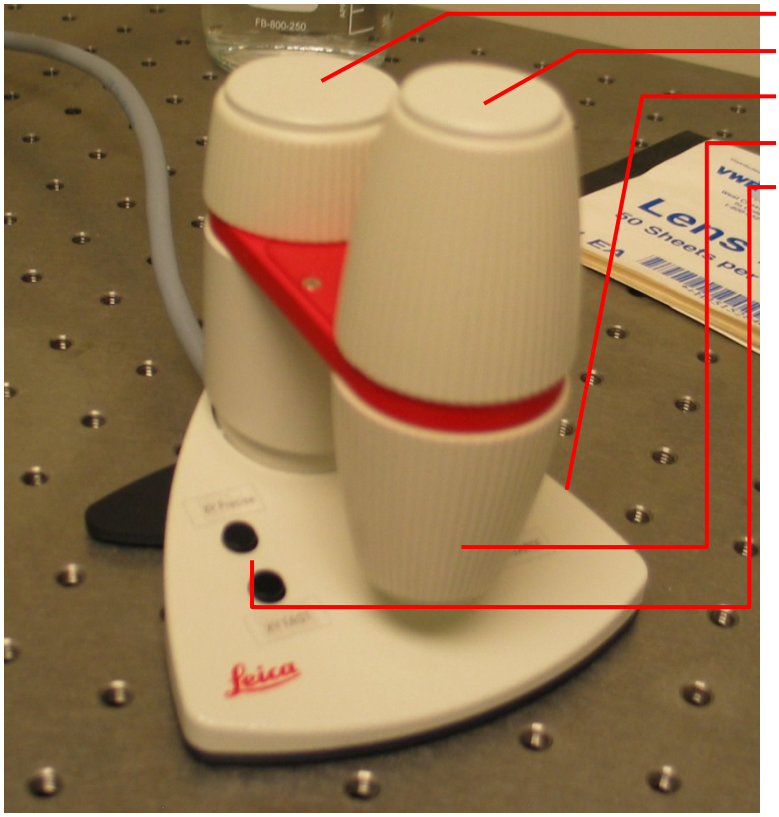


- Change lens (clockwise)
- Change lens (counter-clockwise)
- Set/Move to top Z-position
- Set top/bottom Z-position
- Set/Move to bottom Z-position

Front Control Panel

- Direct light to oculars (eyepiece)
- Add 1.5X magnifier to light-path
- Remove 1.5X magnifier from light-path
- Direct light to left/right camera
- Turn on/off Shutter

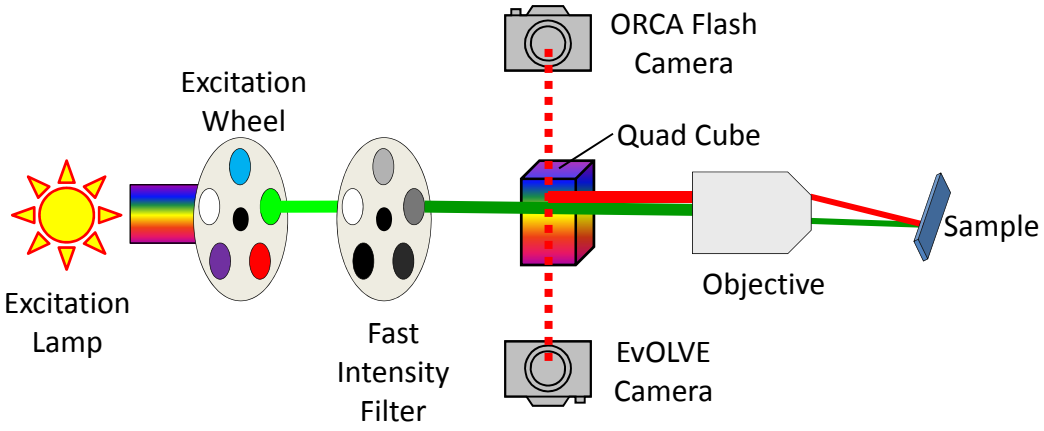
Stage Controller



Microscope Light Paths

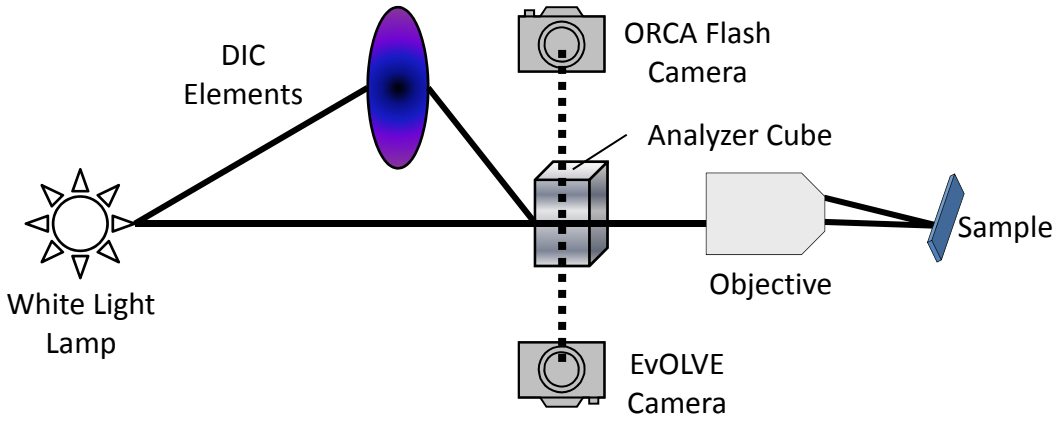
This microscope has multiple optical configurations that enable a range of different experiments. In some cases multiple light paths can be used in a single image acquisition. For example, the DIC light path can be used with all other light paths.

Light Path #1 – Conventional Fluorescence Microscopy



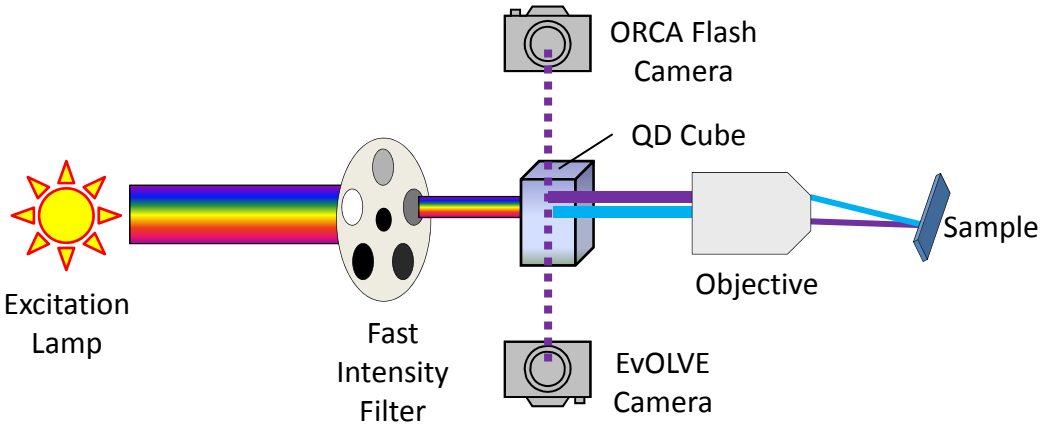
This is the default fluorescence configuration for the microscope and is used for the majority of experiments. In this setup the excitation light passes first through the default excitation filter wheel which contains filters for DAPI (405/460nm), FITC/GFP (490/505nm), Cy3/mCherry (550/570nm) and Cy5 (650/670nm). The filtered light then passes through a fast intensity filter for control of excitation intensity. The excitation light then excites the sample and the emission light filtered by the quad-pass filter cube. This light path can be used with either the ORCA-FLASH 2 or EvOLVE cameras.

Light Path #2 – DIC/Brightfield Imaging



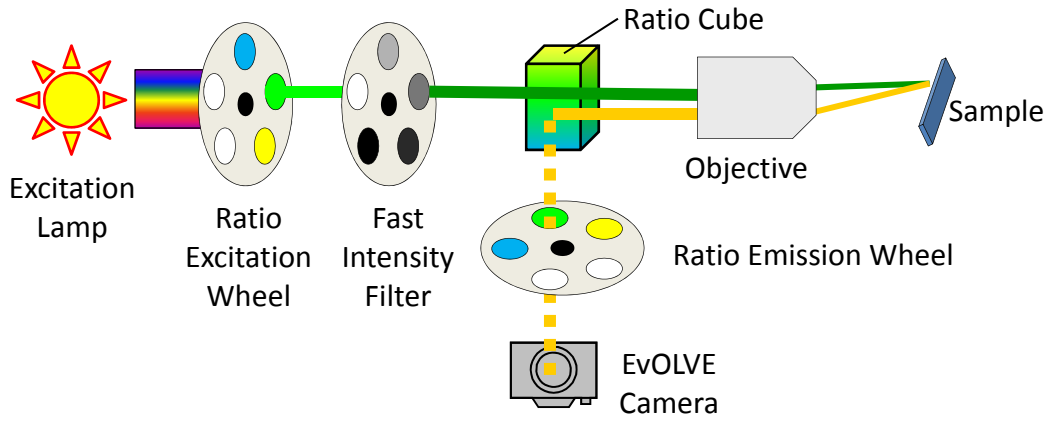
This is the light-path setup that enables for white-light (DIC or conventional brightfield) imaging. This light path can be combined with any of the other light paths on the system, but requires a cube-change and therefore should be avoided if performing high-speed imaging using another light path. This light path can be used with either the ORCA-FLASH 2 or EvOLVE cameras.

Light Path #3 – Quantum Dot Imaging



This light path is intended for high-speed tracking of 655nm quantum dot labeled samples. This light path only requires the quantum dot cube, although the use of the DAPI filter on the default excitation wheel is highly recommended. This light path can be used with either the ORCA-FLASH 2 or EvOLVE cameras.

Light Path #4 – FITC pH Ratiometric and CFP/YFP FRET



This light path is intended for pH measurements using ratiometric imaging (EX 434/490; EM 505) of FITC or Oregon Green dyes, and for FRET measurements using a CFP/YFP FRET-pair (or equivalent). This light path may only be used on the EvOLVE camera and requires the use of the specialized "Ratio – EX" excitation and "Ratio – EM" emission wheels. If using this configuration, please swap the "Ratio _EX" excitation filter for the default excitation wheel when your experiment is complete.

Start-Up Procedure

Note: Live Cell Imaging

If using the heated imaging chamber for live cell imaging insert the desired sample holder into the chamber, and turn on the heater (A), CO_2 mixer (B) and CO_2 tank [at 5 PSI] (C) for 30 to 60 minutes prior to use. If the microscope is in use the stage can be placed to the side of the microscope, but an additional 10-15 minutes of equilibrium time is required once the chamber is placed on the stage. See "Incubated Stage" section for details.

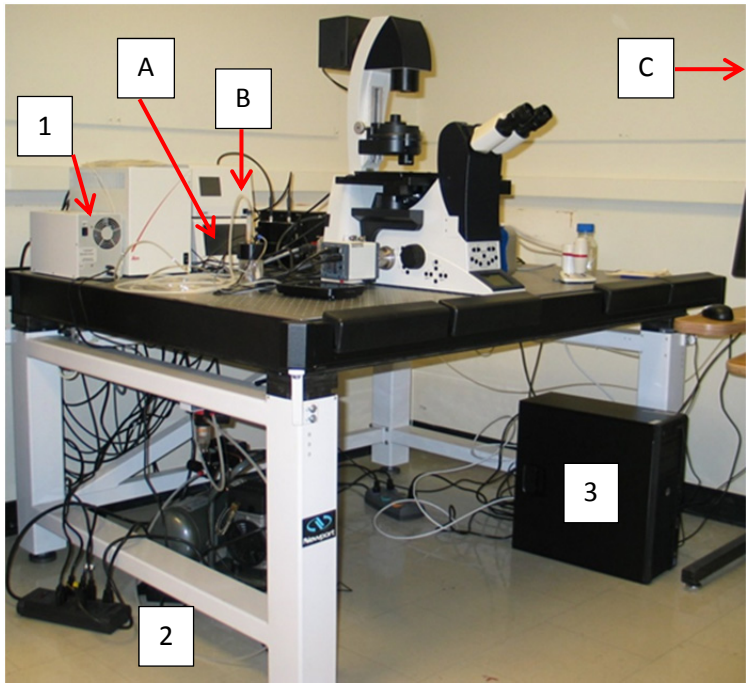
System Start-Up

1) Turn on fluorescent light source

2) Turn on power-bar; this will activate:

- Compressor for the vibration table
- Microscope controller
- Stage controller
- EvOLVE camera power supply
  - This does not turn on the camera itself; see "EvOLVE Camera" section for how to use this camera.

Note: If one of the above components does not start, this may be because someone turned off the component. Check the power switches of each component; if this is not the issue seek help from Drs. Heit or Kerfoot



3) Turn on the computer, log into your labs account, and start Leica LAS-AF software

4) Select the "DefaultDynamicWidefiledTree" configuration and "Dynamic" sub-setting

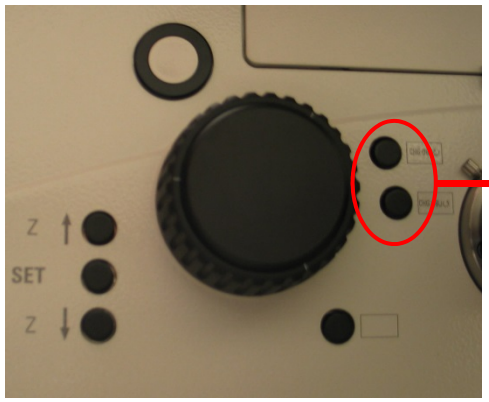
5) If requested, run the stage initialization.

WARNING: Use the focus drive to position the lens as low as possible before initializing the stage. This is required to ensure the lens is not struck and damaged by the stage during initialization.

Prepare the Lens

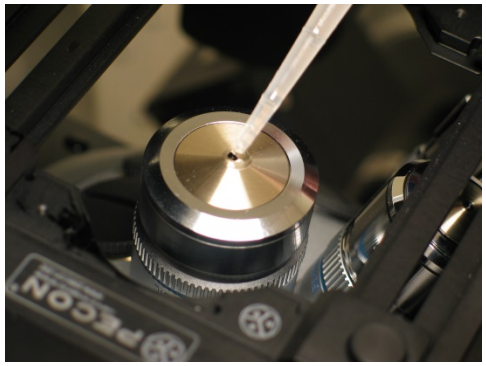
1) Using the focus wheel or z-controller, lower lens to its lowest point. Make sure the lens turret is clear of obstructions

2) Select the required lens using either the select button located on the right-side of the microscope body, or using the lens selection buttons in the Leica LAS AD software. The lens should swing into place

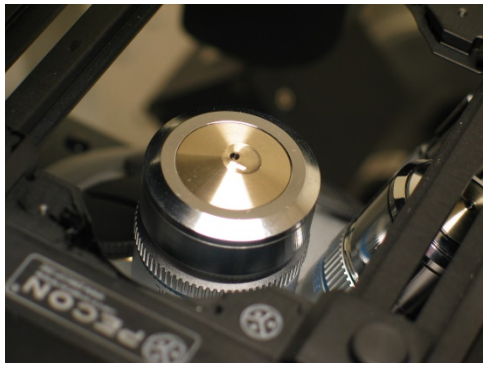


Lens Select

3) Add a small drop of lens oil to the objective. Use only the minimal amount required. The drop of oil should not be larger than the glass potion of the lens. **Clean excess oil off the lens immediately using a lens cloth or cotton swab.**



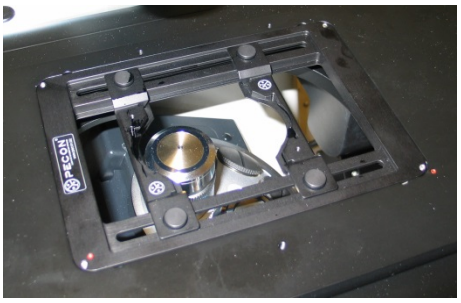
Correct amount of oil



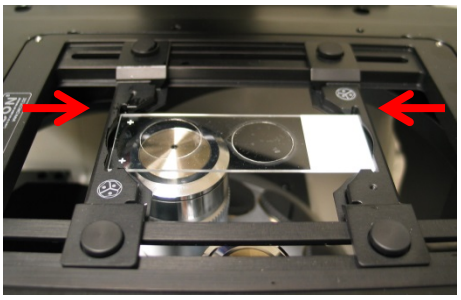
Too much oil

Mounting a Slide or Dish (Fixed Samples)

1) If not already inserted, insert the slide/dish holding insert into the stage.



2) Place the slide/dish on the stage and close the sliders to firmly clamp the slide/disk.

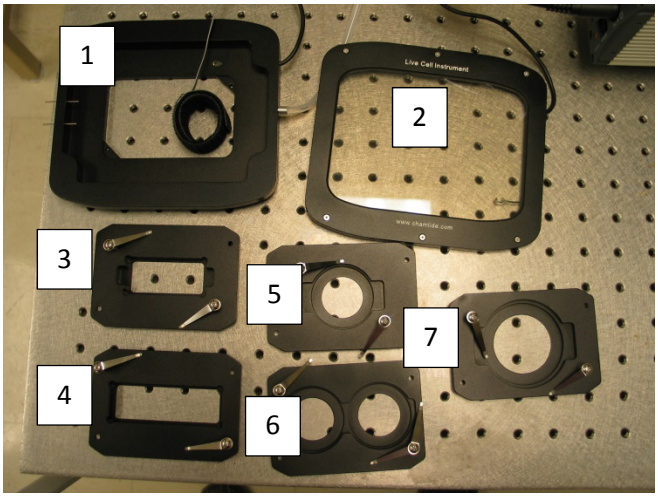


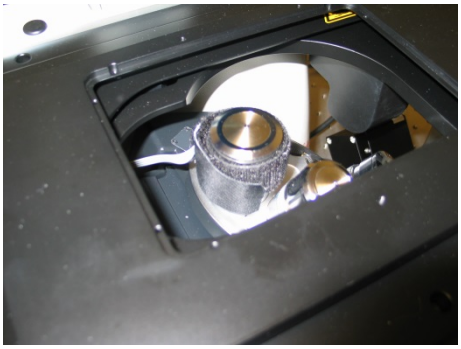
3) Use the stage controls to centre the sample on the stage. Slowly bring the lens up until it makes contact with the sample; this will appear as a spreading of the oil.

Incubated Stage (Live Cell Imaging)

Incubated Stage Components:

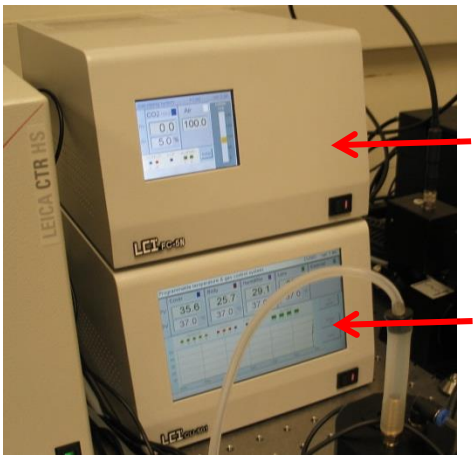
1. Incubated stage insert & lens warmer
2. Incubated stage heated lid
3. Chambered coverslip sample holder
4. Chambered slide sample holder
5. 35mm dish sample holder
6. Dual 35mm dish sample holder (note shown: adaptor rings for 22mm dishes)
7. 50mm/60mm dish sample holder





1) After selecting the desired lens, wrap the lens warmer around lens. Run the lens warmer between the stage and lens turret. Remove when the experiment is complete to avoid tangling of the cable during lens changes.

2) Assemble the stage insert by placing the required sample holder into the stage, and then insert the combined insert/sample holder into the stage. Place the lid on the assembled stage and begin equilibrating the stage.



3) To equilibrate the stage, turn on the temperature controller, and if required, the CO_2 mixer and CO_2 tank. Allow to equilibrate for 30 to 60 minutes.

If required the assembled stage can be warmed separately from the microscope stage.

4) If required, the temperature of the stage and CO_2 concentration can be changed using the touch-screens on the temperature controller & CO_2 mixer.

5) Place sample into the heated stage and place the lid back onto the incubated stage. **Note:** The incubated stage should not be used without the lid in place.

6) Use the stage controls to centre the sample on the stage. Slowly bring the lens up until it makes contact with the sample; this will appear as a spreading of the oil.

Once sample is mounted on the microscope proceed with imaging, as covered later in the manual.

Ocular Setup

1) Rotating the eyepieces of the oculars adjusts their focus; this can be used to equalize the focus through they eye pieces for people with uneven vision between their eyes.

2) Start by adjusting the ocular for your dominant eye such that the edge of the rotating ring is aligned with the silver ring (red arrows) on the eyepiece body.

3) Focus on your sample using your dominant eye

4) Without adjusting the microscope focus, adjust the ocular for your non-dominant eye until the image through that eye is also in focus.

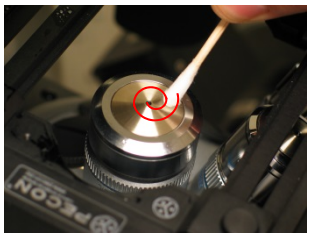
NOTE: When the oculars are aligned with the silver bands the eyepieces are parfocal to the camera, meaning that the focal plane you observe through they eyepieces will be the same as that observed at the camera. If you find the focal plane of the camera is consistently off compared to the eyepieces, you have likely misadjusted the oculars.

Shut-Down Procedure

Note: If you used the FITC ratiometric/FRET filter sets, or otherwise altered the microscope configuration, please return it to the default setup when your experiment is complete.

1) Save all your files and exist the Leica LAS software. Do not proceed to step 2 until the software has exited completely. You can begin transferring your data to an external hard drive or server at this time.
2) If used, turn off the Evolve EM-CCD camera.
3) Remove your sample from the microscope and remove the stage insert to give you clear access to the lens.

4) Using lens paper or a kim-wipe, dab the bulk of the oil off of the lens.

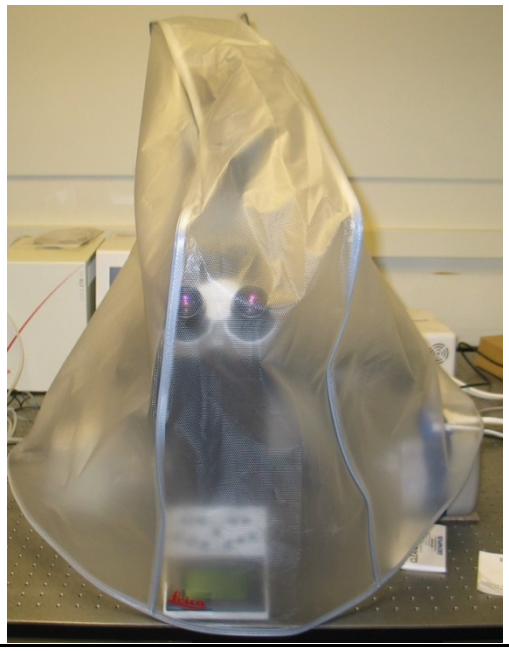


5) Whet a cotton swab (damp, not dripping-wet) in isopropyl alcohol. Clean the lens by placing the cotton swab over top of the lens, and then wiping off the oil by moving the swab in an expanding-circle pattern.

5) Repeat step 5, using a clean swab every time, until the lens is oil-free.

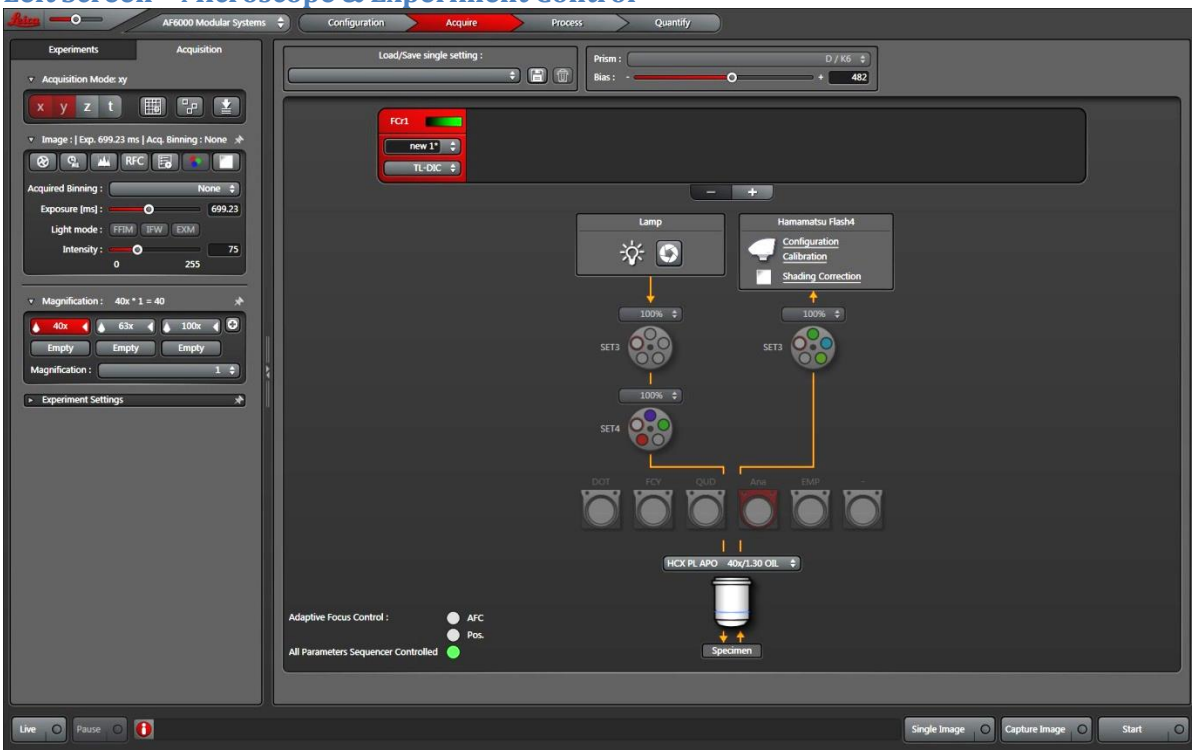
Stop at this point if another user will immediately be using the scope, otherwise continue:

1. If used, turn off the heated stage, CO_2 mixer and CO_2 tank.
2. Turn off the power-bar.
3. Turn off the fluorescent light source.
4. Cover the microscope with the dust-cover, making sure to spread the cover such that both cameras are protected (picture below).



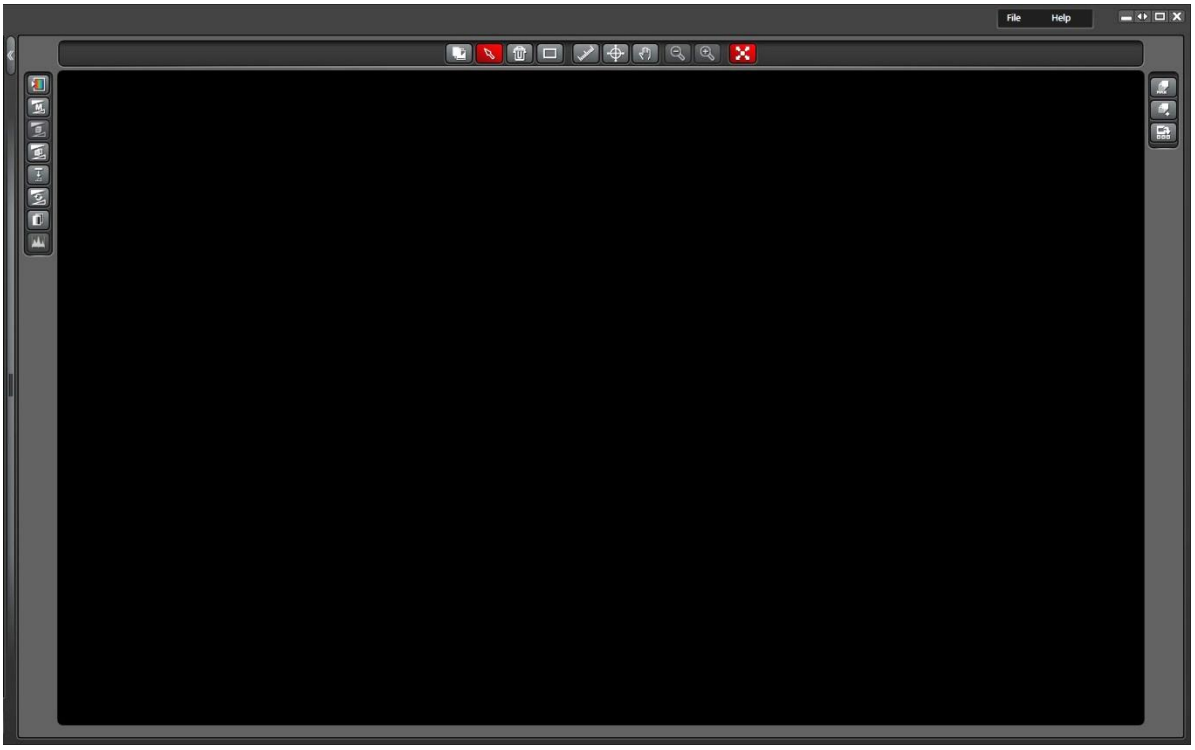
Basic Image Capture

Left Screen – Microscope & Experiment Control



The left screen contains the bulk of the controls for the microscope and experiment acquisition. The various controls will be covered in-depth later in the manual.

Right Screen – Image Display & Measurements



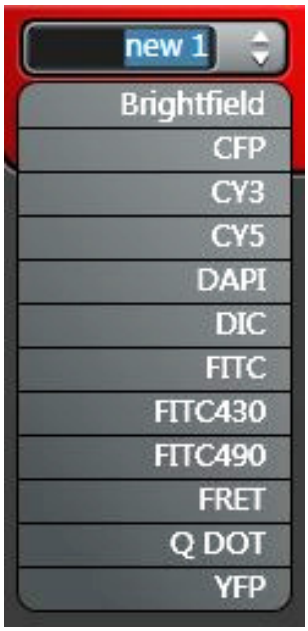
The right screen displays the current image and contains a number of controls to alter image appearance and to take measurements such as distance measurements. These controls are generally intuitive and thus are not covered in this manual. In brief, the buttons on the left side adjust picture aspects such as display range and false colouring. The top buttons control image zoom and allow for basic measurements. The buttons on the right alter display modes (e.g. side-by-side, time series, colour overlay, etc).

Basic Acquisition

Begin by ensuring the default excitation filter wheel is inserted in the excitation filter wheel holder. If it is not, follow the instructions in "FRET and pH Imaging" to replace it. If you require the EvOLVE camera you need to take additional steps to make it available, as outlined later in the manual.

1) Using the drop-down menu on the channel configuration button select bright-field or DIC mode. Then, click the "Start" button on the left screen; this will turn on the white-light lamp and being imaging the sample.

2) Using either the camera or the oculars, find the cell(s) you wish to image and center them in the frame. You can switch between the oculars and cameras using the buttons on the front of the scope.



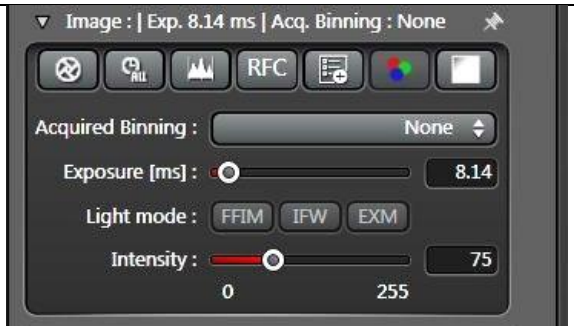
3) If your sample is labeled with a stable, visible fluorophore, you can use one of the fluorescent channels to find your sample instead of while light.

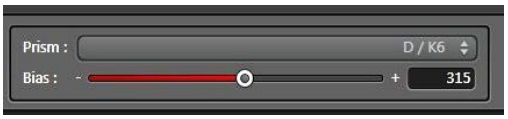
Note: All available fluorophores are listed on this list; it is not necessary to configure your own.

Warning: Not all channels are available at the same time. If the default filter wheel is in place you can rapidly (>10/second) switch between DAPI, FITC, Cy3 and Cy5 fluorescence, as well as combine this with slower acquisition routines including Brightfield, DIC and Q-Dot imaging. All of these imaging modes can be used with both cameras

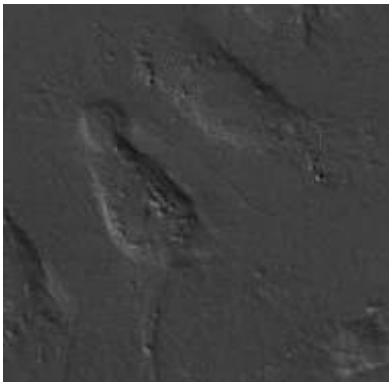
CFP, YFP, FRET, FITC430 and FITC490 channels are only available using the specialized ratiometric emission/excitation filters and the EvOLVE camera.

4) For DIC/bright field imaging, adjust the camera exposure time and white-light intensity using the sliders on the camera panel. If using DIC a small window will open (below) allowing you to adjust the bias angle of the DIC optics, which can alter the contrast of the resulting image.





5) A properly configured DIC image will have strong contrast and no areas of saturation.

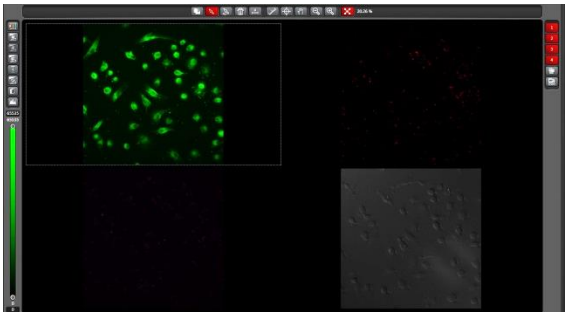


6) Additional channels can be added or removed as needed using the "+" and "-" buttons below the channels window. Each channel's acquisition time and intensity can be adjusted independently. Note: for fluorescent channels you do not directly control the light source's intensity, and instead select between one of 5 fast filter wheels which provide from 0% to 99% attenuation of the excitation signal.

7) The "Live" button will excite the same using the active channel and display the image on the screen. "Pause" will stop excitation and keep the last image on the screen. <u>These images are not saved</u>.

8) The "Single Image" button will capture single image of the active channel and place it in your experiment library. The "Capture Image" button will capture images all active channels and place them in your experiment library. The "Start" button will perform al configured steps in the imaging sequence – e.g. all channels, Z-stacks, time-lapse, tiling, etc - and place them in your experiment library.

9) An example of the view window after the “Capture Image” button was pressed with the configuration shown in step 6. Four channels have been captured – FITC (top-left), Cy3 (top-right), Cy5 (bottom-left) and Brightfield (bottom-right)



9) Captured images can be viewed in the “Experiments” tab. The can be re-named to be more intuitive. Note that these are not saved until you save the experiment to the hard-drive or manually export the images.

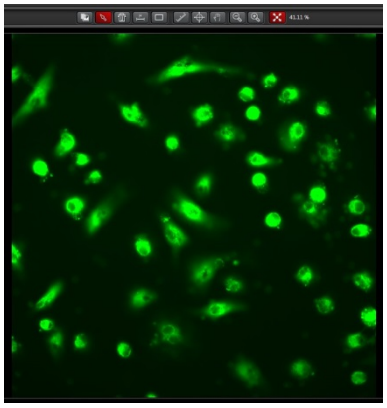
Intermediary Image Capture

Intermediary image capture options are activated using the sub-panel located on the left-side screen. Clicking the 'z' button activates z-stacking, the 't' button activates time-lapse imaging, the grid activates tiling, the 3-box icon activates mark & find, and the arrow activates auto/adaptive focus.

Z-stacks

Z-stacks capture a sequential series of images through the thickness of the cell. These can later be deconvolved to improve image quality, and also be used to build 3D images of the cell. Configuring the microscope for Z-stacks is very easy:

1) Setup your channels and exposures as described on pages 15 & 16. Focus where you want either the stack to start (e.g. the top or bottom of the cells).



2) Click the "begin" button in the Z-stack panel, located on the left-side of the screen. Then re-focus the microscope to where you want the stack to end and click the "end" button. Adjusting the zoom slider allows you to better visualize your stack. The current position of the stage is indicated by the grey box.

Alternatively, you can manually enter Ending and Beginning points around a central Z-position.

3) Select your Z-step size, this can be:

- System optimized – the ideal step-size for your lens
- Nr. of Steps – you set how many z-sections are taken
- Z-step Size – you define the number of steps imaged

You can also choose the direction the stack is imaged; it is customary to go from bottom-to-top, which is the default (upward, red-highlighted arrow in the image to the right).

Clicking the "Start" button will capture the Z-stack.

Mark & Find (Point-Visiting)

Point visiting allows you to mark the positions of multiple regions you wish to image, and then re-visit these points whenever you wish (so long as you don't remove the sample from the stage). This is particularly useful during time-lapse imaging of live samples (see "time-lapse imaging, bottom of this page).

1) Find and center your first region you wish to enter. Click on the plus (+) button to save the position.

2) Move to the next position and mark it with the plus button. Repeat until all positions are marked.

3) Positions can be deleted if required (trash can), saved and loaded, and otherwise manipulated using the buttons at the top of the panel.

4) You can move between positions using the position drop-down list.

5) If performing a z-stack at each point you can choose to use the same stack for all points, or to set each stack separately. Configure z-stacks as per usual.

6) Clicking the "Start" button will begin the point-visiting process.

Time-Lapse Imaging

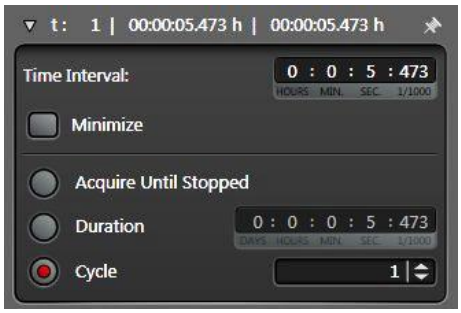
Time-lapse imaging allows you to capture images over time. This can be combined with all other capture modes – e.g. Mark & Find, Z-sectioning, etc, to produce complex image acquisitions. **Warning:** it is easy to photo-damage your samples when performing time-lapse, especially if performing z-stacking. Take this into account when configuring your acquisition routine.

1) Open the time-lapse window.

2) Select the time interval you wish between images. Clicking minimize will auto-configure for the fastest possible acquisition.

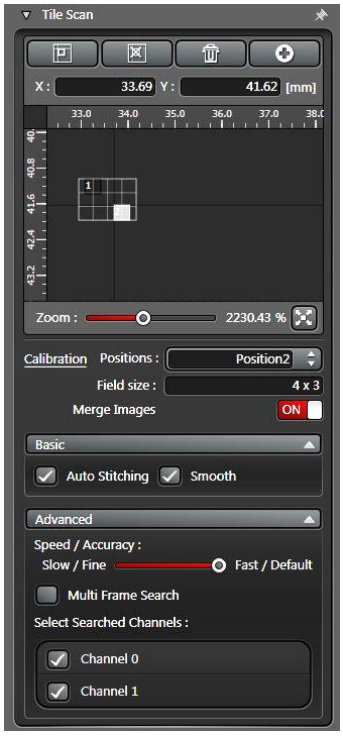
Note: If performing complex acquisitions make sure you leave sufficient time between intervals for all capture processes.

3) Select how long you wish the acquisition to run – this can be until stopped, for a set period of time, or for a set number of frames (cycles)



Tiling

Tiling allows the user to capture a series of overlapping regions and then assemble them into large images covering a larger area than can be imaged with a single picture.



1) Locate one corner of the area you wish to image and mark using the buttons on the tile scan window; marking is done in the same manner as with Mark & Find.

2) Locate the opposite corner of the image you wish to form and mark it. The software should automatically draw a grid of all the images required to produce the desired tiled image.

3) Select if you want the images to be merged or saved separately; if you choose to merge the images you will need to select between a number of stitching options. These options are quite complex; please see the built-in help file for details of using these features. For most users the "Basic" mode with "Auto Stitching" and "Smoothing" provides a suitable output.

4) Under the advanced tab the user can control the quality of the stitching process, the channels used for stitching and other advanced features.

Autofocus & Adaptive Focus Control

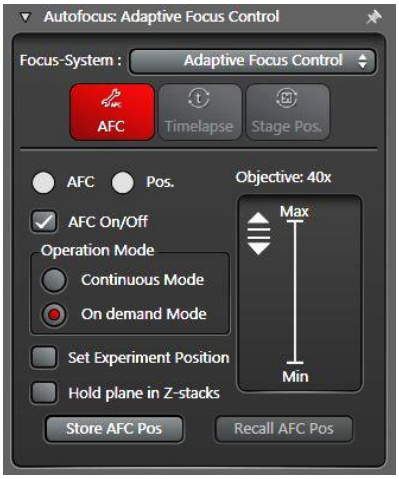
This microscope features autofocus and adaptive focus; both useful for time-lapse, mark & find and tiling acquisitions. Autofocus uses a quick z-stack to focus the image every frame/stage position/tile. Adaptive Focus Control maintains focus by directly measuring the distance between the lens and coverslip with an infrared interferometer. These systems can work independently or together to maintain focus.

Autofocus:

1) Select "High-speed AF" in the autofocus window to activate.

2) Various settings can be used depending on whether you are doing timelapse or tiling/mark & find imaging. For example, you can choose to re-focus every frame or every x^{th} frame, or at every point in a mark & find series.

Warning: Autofocus will increase the photobleaching of your sample; adjust your imaging settings and frequency of autofocusing to account for this.



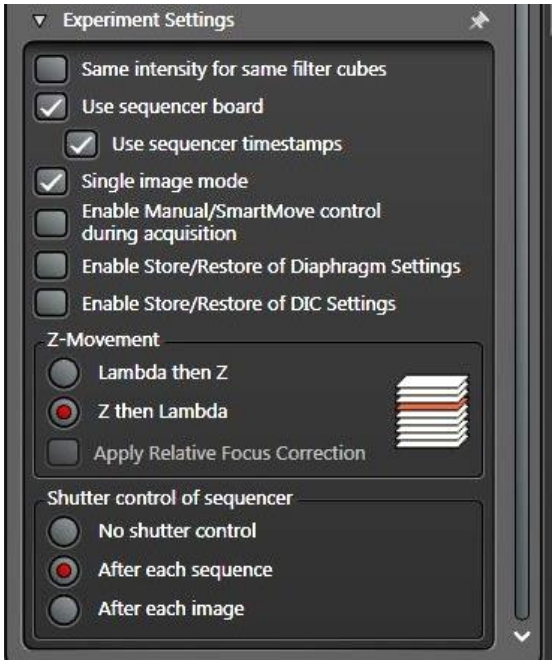
Adaptive Focus Control:
1) Adaptive focus control is configured much like autofocus, with different options available for timelapse versus point-visiting.

2) You can choose to keep the system continually active (Continuous Mode) or to only turn under the conditions set under the Timelapse and Stage Position options (On demand Mode). Continuous provides greater focal stability, but the interferometer may overheat the sample on long acquisitions.

Additional Imaging Controls

The "Experiment Settings" drop-down provides access to a number of useful options

Enable Manual/SmartMove control during acquisition: Activating this option allows you to use the joystick to change focus or move the stage during acquisition. Useful for tracking migrating cells or altering focal planes during time-lapse acquisitions, but can cause problems if accidentally bumped.



Z-Movement: Allows you to configure whether the system captures all the z-slices for a channel (one channel = one lambda) before switching to the next channel, or captures all the channels on one z-plane before moving onto the next z-plane. If your configuration does not require switching light paths the "Lambda then Z" will be faster; if switching light paths (e.g. combining fluorescence and DIC), the "Z then Lambda" option will be faster.

Shutter control: Allows you to set how the shutter is controlled. "After each sequence" maximized capture speed but increases photobleaching as the shutter is not closed during a sequence (a z-stack would be considered a sequence). "After each image" protects the sample by closing the shutter after every image capture, but slows acquisition. "No shutter control" should not be used outside of single particle tracking, as it leaves the shutter open throughout the entire acquisition procedure. This maximizes acquisition speed but causes a large degree of photobleaching and other photo-damage.

Advanced Image Capture

EvOLVE EM-CCD Camera

The Photometrics Delta EvOLVE EM-CCD camera is a high sensitivity camera with nearly single-photon sensitivity. Its purpose is the imaging of faint subjects and high-speed imaging (e.g. single particle tracking). The high sensitivity of this camera comes from an electron multiplier, which if mis-used can permanently damage the camera. As such, it is critical that this camera be used only when necessary, and using with the best of practices to ensure its long life.

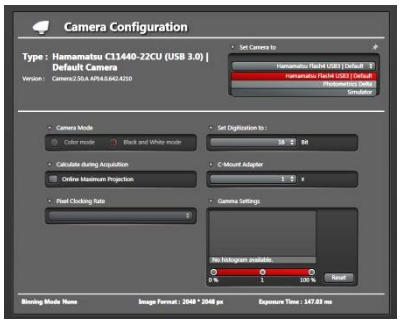
1) Before starting the computer, turn on the EvOLVE camera using the on-off button located on the top of the camera.

2) Start the LAS-AF software as per normal.

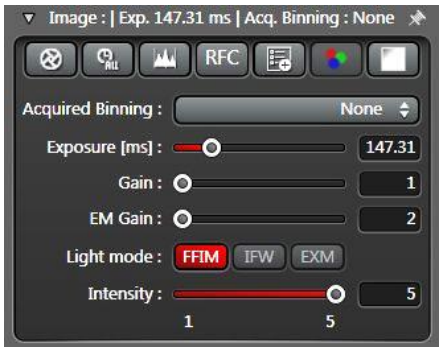
3) Click on the configuration button at the top of the LAS AF window

4) Click on the 'camera' options button

5) Select the "Photometrics Delta" camera from the drop-down menu; then select "apply". The EvOLVE camera should now be initialized & loaded into the software.



6) The camera controls will change, to give you access to the additional features of the EvOLVE camera.



Important Note: The "EM Gain" slider is what controls the gain of the camera. Setting this to too high a value can damage the camera. When adjusting the camera:

1) First set the exposure & FFIM intensity filter to reasonable values for your experiment.
2) Only after the exposure and FFIM are set should the EM Gain be adjusted to provide a reasonable image.
3) Unless absolutely necessary the EM gain should never go above 50% maximum.

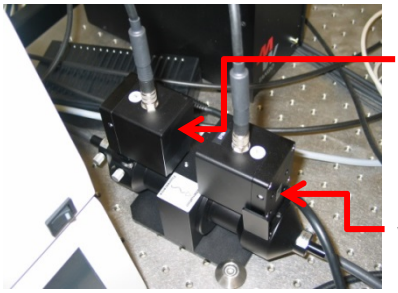
7) At the end of your experiment adjust the EM Gain to zero.

8) Turn off the EvOLVE camera after exiting the Leica LAS AF software

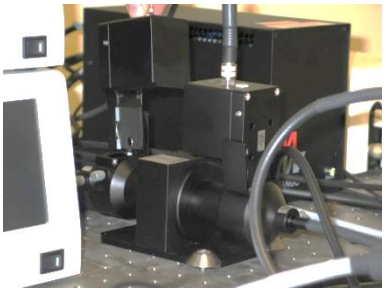
FRET and pH Imaging

1) FRET/pH (FITC ratiometric) imaging requires the default excitation filter be swapped for the "Ratio – EX" excitation filter and requires the "Ratio – EM" emission filter be in place.

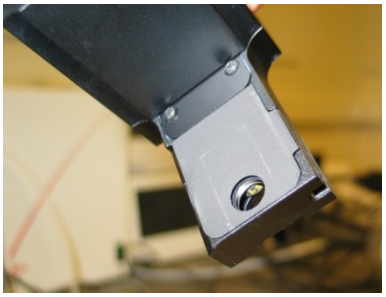
Note: FRET/pH imaging can only be performed using the EvOLVE camera



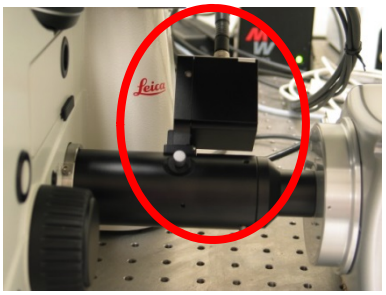
2) Gently lift the default excitation filter out of the filter holder; a door will snap closed to protect the light path from dust and debris. Place the excitation filter into its protective container located at the back of the microscope table.



3) Insert the "Ratio – EX" excitation filter; the notch (visible on the right) goes to the right side of the filter holder. The filter should drop into place – do not apply pressure; the filter unit will not insert backwards.



4) Confirm the "Ratio – EM" emission filter is inserted into the filter holder located on the connecting tube between the microscope body and EvOLVE camera. If it is missing, insert it following the instructions in step 3.



5) For FITC ratiometric pH imaging, use the FITC430 (pH insensitive) and FITC 490 (pH sensitive) settings

6) For FRET imaging use the CFP, YFP and FRET settings; it may be necessary to manually create additional channels depending on the form of FRET you are using

5) When your experiment is complete ensure that you turn off the EvOLVE camera and that you replace the Ratio – EX excitation filter with the default excitation filter set.

Live Data Mode

Live data mode allows for the development of complex acquisition routines including variable-rate time lapse imaging, imaging of different channels at different points in the experiment, and even external control of TTL-compatible instruments.

1) Select “Live Data Mode” from the drop-down menu.

2) Jobs (imaging routines), pauses, patterns and other acquisition tasks can be added from the “LDM Pattern” drop-down menus.

- Camera jobs = conventional acquisitions. Set up as you would any acquisitions - time-lapse, z-stacks and point visiting can all be contained within a single camera job
- Pause = place a pause between jobs
- Pattern = control TTL triggers
- Reference to Existing Record = load a previously used pattern
- Record From File = load a previously saved live data mode session

3) Added jobs will appear as a list in the control window

4) Right-clicking on a job will access additional features such as pause lengths and naming options

5) Loops can be added by shift-selecting the start and end-point of a loop (red tiles) and then clicking the “Define Loop” button

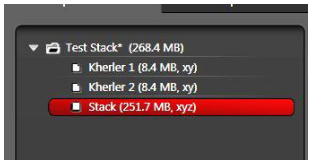
6) The number of loops can be changed by changing the loop number value (red circle); loops can be deleted by clicking the ‘x’ on the right side of the loop symbol.

7) Once all steps are configured, click the ‘Start” button to run the complex acquisition. This example acquisition:

1) Job 1 captures a 4-channel (FITC/Cy3/Cy5/DIC) image sequences 6 times; 30 minutes between image sequences, using point-visiting.
2) Then captures the same image sequence 8 times, with 15 minutes between images.

Handling Data

Saving Files & Exporting Images



1) Files are saved in the experiments tab; note that files in this tab are not saved on the hard drive until you manually save them yourself.

2) To save the file, click the "Save" button at the bottom of the experiment tab.

3) Always save data to HDD2 (E:), as the other hard drives must be kept clear to maximize instrument performance.

WARNING

HDD2 is for temporary storage only. At the end of your experiment please remove your data files from the computer.

Files more than 48 hours old are subject to removal without prior warning.

4) Leica uses a proprietary format to save their images which is not compatible with many down-stream applications. As such, export in common file formats is often required. This can be done by right-clicking on the image you wish to export, select "Export" from the pop-up list, and then the desired export format.

Viewing Images

If your image is a time series or Z-stack you have a few additional display options in the viewing window:

Right Buttons:

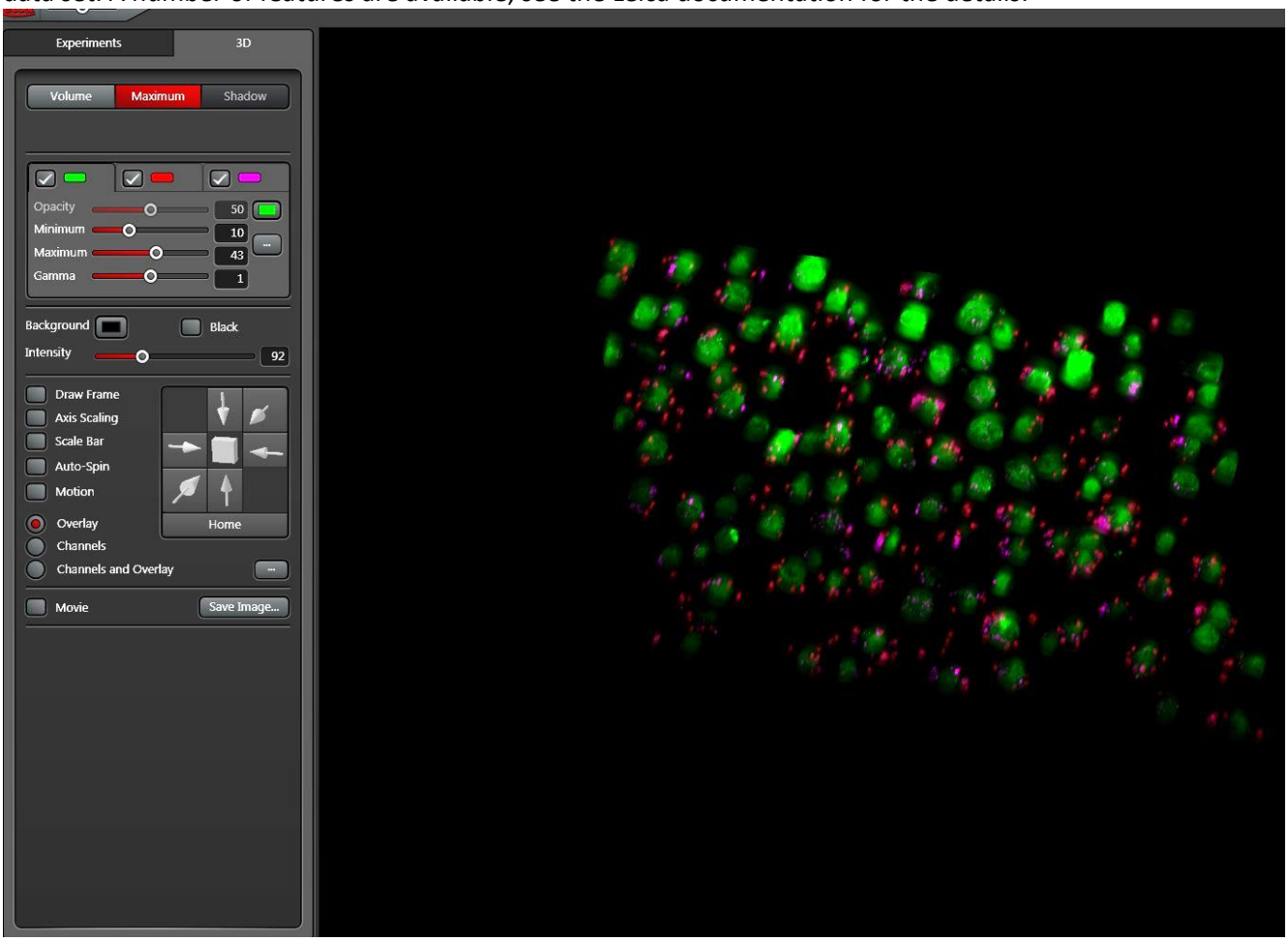
- Numbers: toggle on/off individual channels
- MAX: view a maximum-projection image instead of a stack (z-stacks only)
- Rotate view mode: select between viewing overlays and various arrangements of the individual channels
- Crop: crop an image
- 3D: Activate 3D viewer (see next section, z-stacks only)
- Album mode

Left Buttons:

- Playback options for animating stacks and time-series
- Z- or time sliders for scrolling through z-stacks and time series

3D Mode

For z-stacks the 3D mode can be activated. This allows for the 3D modeling, rotation and animation of your data set. A number of features are available; see the Leica documentation for the details:



The major controls are, from top-to-bottom:

- Rendering mode: Volume rendering, Maximum intensity rendering and shadows
- Channel colour select and intensity adjustments
- Image background colour and average image brightness
- Animation and display options
- Movie mode

Additional Analysis

Additional forms of basic analysis are available on the main microscope. However, it is strongly recommended that all analysis be conducted on the stand-alone Leica software, available on the Tux workstation.

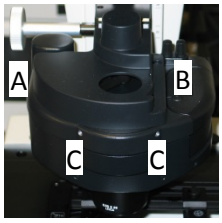
Configuring Koehler Illumination

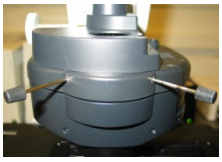
Good DIC/ICT imaging requires proper alignment of the white-light optics. This alignment is termed Koehler Illumination. You should not need to do this often, but if DIC quality is poor this is the first thing to try as a correction. If you are not comfortable doing this, ask Drs. Heit or Kerfoot for assistance.

1) Place a sample on the microscope and find a good focal plane; using this plane adjust the oculars such that they are parfocal (see Ocular Setup, page 12). It is easiest to configure this using a lower magnification lens.

2) Switch to brightfield illumination, using the BF button on the left side of the scope (red circle).

3) Close the field diaphragm using the FD buttons, located on the left side of the microscope. A hexagon-shaped diaphragm should become visible through the oculars or on the screen.





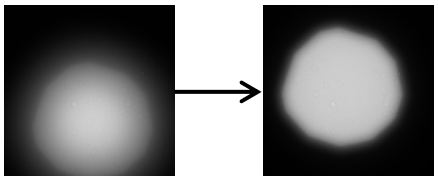
Left: Locations of condenser adjustments:

A. Condenser Focus
B. Allen Keys
C. Condenser X/Y Adjustments

Right: Proper positioning of the Allen keys

4) Using the focus knob (A) on the brightfield condenser, move the head until the edges of the diaphragm are focused (will appear sharp)

5) Using the Allen keys (B) stored in the DIC head, and the upper screws (C), center the diaphragm. Once centered, expand the field diaphragm until the edges of the diaphragm are visible at the edges of the oculars.



Out of focus & off-centre

Correct

6) Put the Allen keys back into their storage slots

7) Switch to ICT (DIC) using the ICT button; the wheel on the back-left side of the lens turret (circled) can be used to adjust the DIC shear angle (also controlled in the software). Adjust to give good contrast across the image.