

Diskovery Spinning Disk

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Spinning Disk Confocal Microscope - *Discovery* -

An inverted Leica DMI8 microscope connected to a Quorum Discovery Spinning Disk system with Super-resolution capabilities (STORM and PALM). Equipped with UV and two visible light lasers. Image acquisition with Volocity for regular confocal imaging and with MetaMorph for Super-resolution.

Microscope: Inverted Leica DMI8 microscope stand with AFC (Adaptive Focus Control) and a fully automated ASI xyz controlled stage (scanning, stitching, multiple points, z-stacks); brightfield and DIC optics

Objectives: 10x (Dry), 40x Long Working Distance (LWD, dry), 40x (Oil, DIC), 63x (Oil, DIC), 63xTIRF (Oil, NA 1.47)

Fluorescence filters: Chroma - ET-DAPI (UV), ET-GFP (blue), ET-Rhodamine (green)

Laser system: 405 nm ILE Laser (200 mW)
488 nm ILE Laser (150 mW)
560 nm ILE Laser (150 mW)

Confocal filter blocks: ET450/50M, ET 525/50M, ET600/50M, ET 620/60M

Fluorescence as well as transmitted light detection

Imaging and image processing software: Volocity™ (version 6.3, regular confocal imaging) and Metamorph™ (version 7.8.13.0, super-resolution imaging)

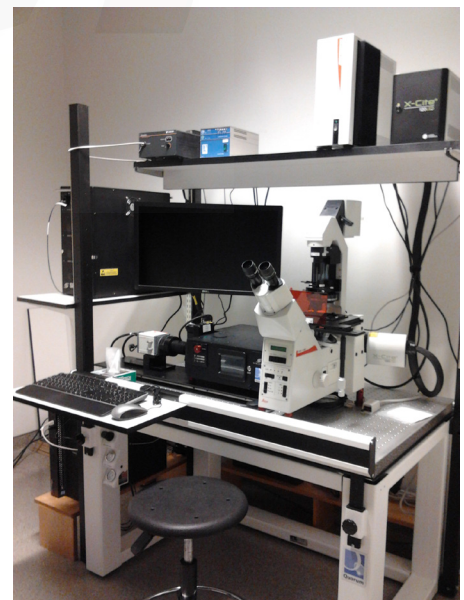
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Tel: (519) 824-4120 ext. 52737

E-mail: confocal@uoguelph.ca



User Guidelines

1. When you begin to use any system in the facility please provide a Billing Authorization Sheet including the supervisor's signature and the Trust Fund Account number.
2. Before any unsupervised access is granted, users must enroll in supervised training sessions during which they will review with the confocal manager how to operate the equipment properly and safely. The time required for the training sessions will vary depending upon the user's demonstrated competency with the equipment. Billing will be at the "Training" fee rate.
3. When using these facilities you must clearly write in the sign-up sheet the date, your name, department, log on and log off time, and total number of hours you used the equipment.
4. For any planned after hours use of the confocal system, please make arrangements with the confocal manager for access to the hallways.
5. Users are expected to bring all their own supplies including pipets, slides, coverslips, computer disks, etc. However, the facility will provide immersion oil and lens paper.
6. Files saved to computer hard drives must be removed as soon as possible. All computer hard drives will be cleared on a regular basis - it is the users' responsibility to manage their own image files. USB keys are not allowed on any of the instrument computers! The facility offers a variety of other options for data transfer.
7. Please notify the confocal manager immediately of any problems that you encounter with the equipment - it is essential that we work together in taking care of the facility. Improper care of the equipment will result in rejection of access to the facility.

Laser Safety

Please be aware that the confocal microscope is Class 3B laser equipment!

This laser equipment may be operated only by persons who have been trained in the use of the system and the potential dangers of laser radiation.

The University of Guelph offers a Radiation Safety Course, please visit the Occupational Health & Wellness webpage for details.

As it is not possible to anticipate every potential hazard, please take care and apply common sense to the operation of the confocal microscope.

- Observe all safety precautions relevant to Class 3b lasers.
- **Avoid exposing eyes or skin to direct radiation!**
- Do not deviate from the operating instructions provided.

The failure to observe these instructions shall be exclusively at the user's own risk!

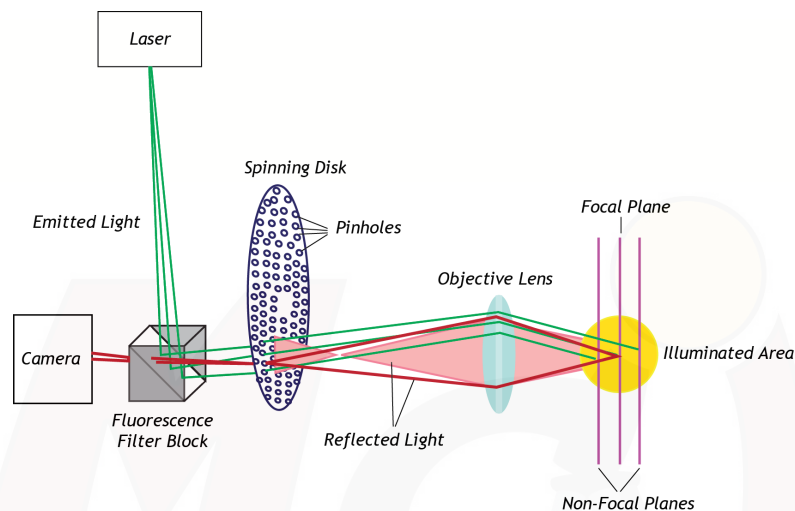


Confocal Basics

Basic principle of a confocal microscope

The principle of confocal imaging was advanced by Marvin Minsky and patented in 1957, and is employed in all modern confocal microscopes.

In a conventional widefield microscope, the entire specimen is bathed in light from a mercury or xenon source, and the image can be viewed directly by eye or an image can be taken. The significant amount of fluorescence emission that occurs at points above and below the objective focal plane is not confocal with the pinhole and forms extended Airy disks in the aperture plane, usually experienced as a blurry image.



In a spinning disk confocal microscope (SD), the laser beam also illuminates the entire sample. Only one laser can be activated at the time. The emitted light of the sample passes through a spinning disk before it is captured by a high-resolution monochrome camera. The spinning disk has thousands of small pinholes in a specific arrangement, thus creating the confocal image of a thin optical section. The depth of the focal plane is determined by the excitation wavelength, the numerical aperture of the objective, and the diameter of the pinholes.

Although unstained specimens can be viewed using light reflected back from the specimen, samples are usually labeled with one or more fluorescent probes/proteins.

Major improvements of a confocal microscope over a conventional microscope

1. Optical sectioning: Signal not originating from the focal plane will not be captured
2. Change of the focal plane does not create blurring, but gradually cuts out parts of the object as they move away from the focal plane
3. Three-dimensional data sets can be recorded
4. Scanning the object in x/y-direction as well as in z-direction (along the optical axis) allows viewing the objects from all sides
5. Image processing allows superimposition of many optical slices, giving an extended focus image which can only be achieved in conventional microscopy by reduction of the aperture and thus sacrificing resolution

Leica DMI8 Microscope

General Information & Care

Objectives

Lenses	Immersion	Refractive Index	Numerical Aperture (NA)	Coverslip μm (#)	Recommended Step Size for Image Stacks (μm)
10 x	dry	1	0.3	0.17 (1.5)	2 - 4
40 x	dry, LWD	1	0.6	0-2	0.6 - 0.7
40 x	Oil	1.52	1.8	0.17 (1.5)	0.4 - 0.5
63 x	Oil	1.52	1.4 - 0.6	0.17 (1.5)	0.3 - 0.4
63 x TIRF	Oil	1.52	1.47	0.17 (1.5)	0.3 - 0.4
100 x	Oil	1.52	1.4 - 0.7	0.17 (1.5)	0.3 - 0.4

Immersion oil

Use only the immersion oil (Type F) provided by the facility. Do not mix the immersion oil with other substances including immersion oil from another source. If you used any other immersion oil during previous observations with a different microscope, remove all traces with Windex.

Carefully apply the immersion oil to the lens.

Cover slip

Sealing the coverslip completely with nail polish or other sealing materials is highly recommended. This prevents mixing of immersion oil and embedding materials, movement of the coverslip when moving the stage, contamination of the objectives, and moving embedded materials during z-sectioning. Be sure that any nail polish is completely dried before you place the slide onto the stage.

Lens care

After use, clean the lenses with lens paper only. Fold the paper and hold both sides of the paper, keeping the folded edge straight. Draw the folded edge back and forth over the lens surface. Repeat with another piece of lens paper and continue until there are no more traces of immersion oil on the paper. Use Kim Wipes to remove excess oil on the objective (around the lens).

Spinning Disk Confocal Microscope Startup and Sample Setup

System Startup

1. X-Cite light bulb (Fig. 1, only if needed)
2. Main Switch (Fig. 2, starts microscope, camera, scan head and all other accessory equipment on the top shelf); wait 1 minute before continue with startup!
3. Laser emission key (Fig. 3, arrow), make sure at least one of the red bulbs is on
4. Computer
5. Login: .\Imaging, Password: MCIF
6. Volocity

Accessory Equipment

Only the most important ones are listed here.

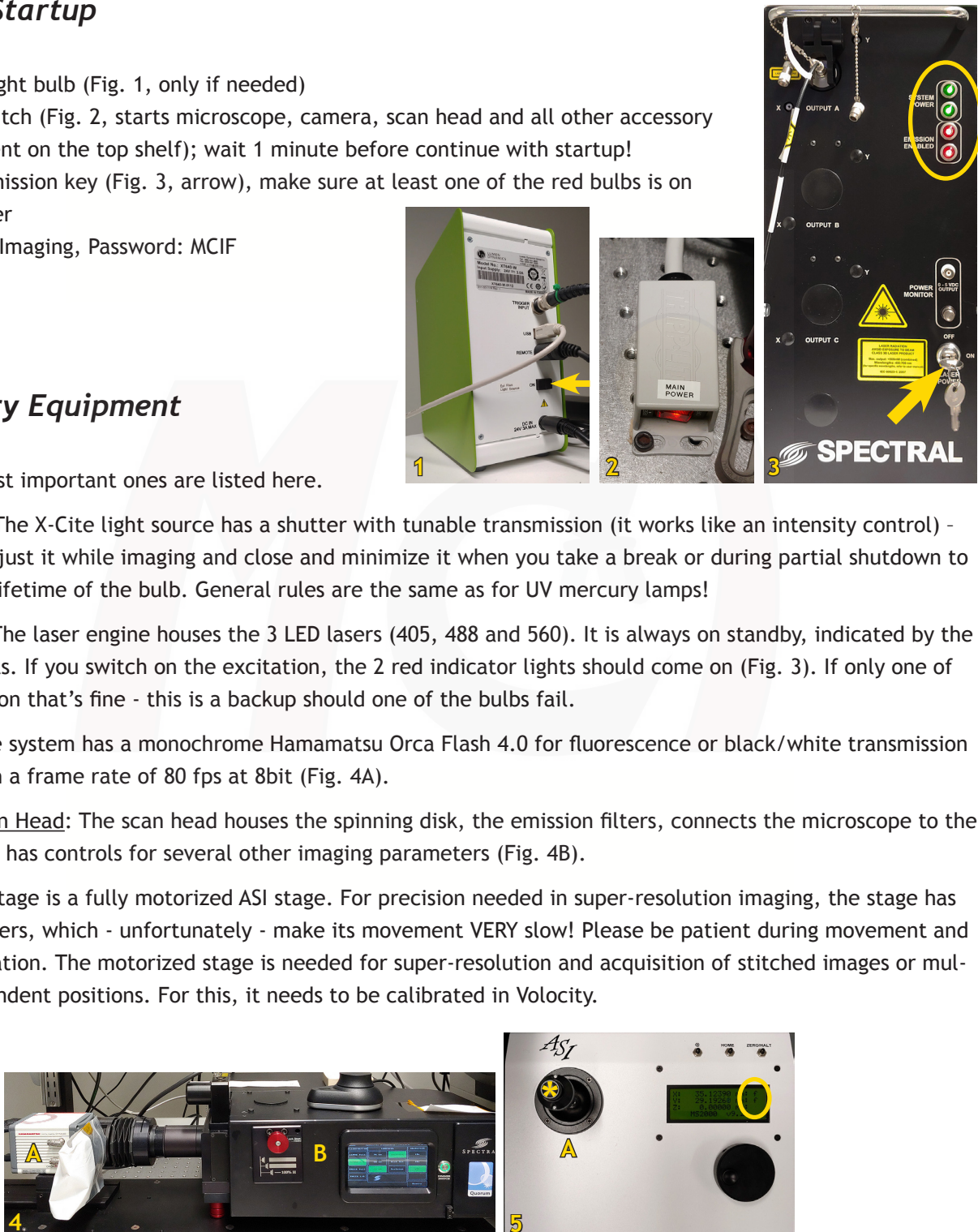
X-Cite 200: The X-Cite light source has a shutter with tunable transmission (it works like an intensity control) - open and adjust it while imaging and close and minimize it when you take a break or during partial shutdown to extend the lifetime of the bulb. General rules are the same as for UV mercury lamps!

LED lasers: The laser engine houses the 3 LED lasers (405, 488 and 560). It is always on standby, indicated by the 2 green lights. If you switch on the excitation, the 2 red indicator lights should come on (Fig. 3). If only one of the lights is on that's fine - this is a backup should one of the bulbs fail.

Camera: The system has a monochrome Hamamatsu Orca Flash 4.0 for fluorescence or black/white transmission imaging with a frame rate of 80 fps at 8bit (Fig. 4A).

Spectral Scan Head: The scan head houses the spinning disk, the emission filters, connects the microscope to the camera, and has controls for several other imaging parameters (Fig. 4B).

Stage: The stage is a fully motorized ASI stage. For precision needed in super-resolution imaging, the stage has linear encoders, which - unfortunately - make its movement VERY slow! Please be patient during movement and stage calibration. The motorized stage is needed for super-resolution and acquisition of stitched images or multiple independent positions. For this, it needs to be calibrated in Volocity.

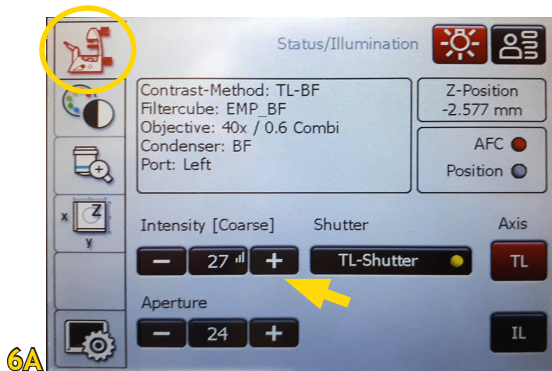


Sample Setup and Observation With the Light Microscope

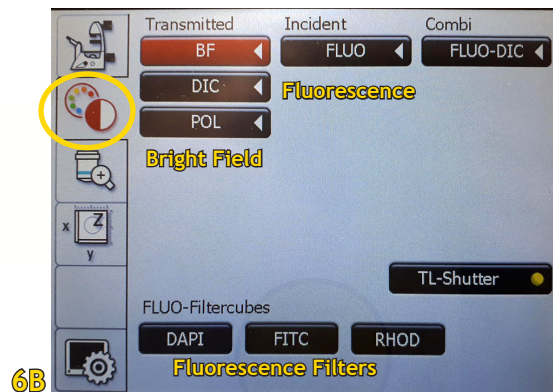
Most features can be controlled either via Volocity or the touchscreen dialog.

On the microscope display (Fig. 6):

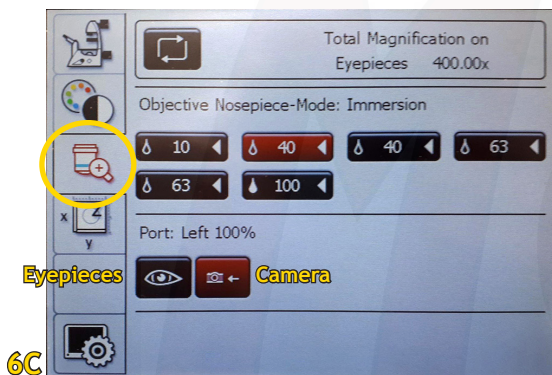
- Select the 'Light paths' icon and select BF (Fig. 6B); BF intensity is adjusted on the Status screen (Fig. 6A, arrow)
- Select the 'Objectives' icon and select the objective you want to use (Fig. 6C)



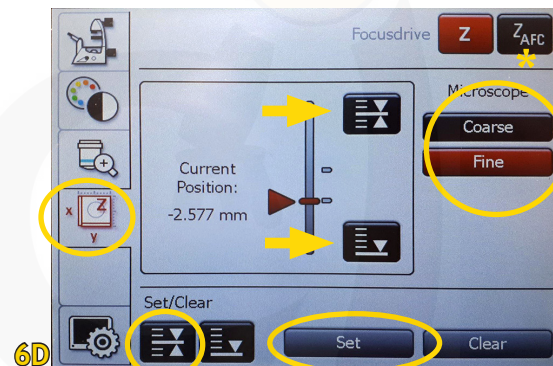
6A



6B



6C

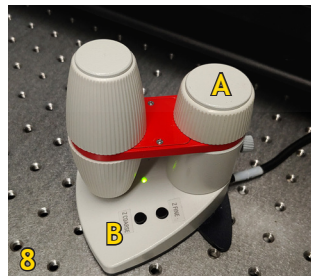


6D

Place your slide, petri dish, or well plate on the stage and - if you use any of the oil immersion objectives - add the immersion oil (carefully place a drop of oil on the lens).



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If you use the 40x LWD (Long Working Distance) objective (Fig. 7), you can not only image through a glass coverslip but also through relatively thin plastic (e.g., specific well plates). To achieve this, the 40x LWD objective has a correction collar (Fig. 7A) that needs to be adjusted for your sample (glass - black dot closer to '0'; plastic - black dot closer to '2').

The X/Y of the stage drive is the joystick on the ASI controller box (Fig. 5A). Switching between fast and precise movement is done by a click on the top of the joystick (Fig. 5,*) - the speed is indicated by the letters 'f' (fast) and 's' (slow) on the ASI display (Fig. 5).

Using the Smart Move focus control (Fig. 8A), move the objective nosepiece up until your sample is in focus (clockwise = up, counterclockwise = down). Switch to 'Fine Focus' on the Smart Move (Fig. 8B) or the display (Fig. 6D).

Observe through the oculars, find your ROI (region of interest) and focus your sample; change to the objective you will use for imaging.

Once your sample is in focus, select the 'Stage' icon on the display and set this position as your reference focal plane by selecting the focal plane icon and pressing 'Set' (Fig. 6D).

You now have saved your focal plane as 'upper threshold' and can move the stage quickly between the limits in the 'Stage' dialog window (Fig. 6D, arrows).

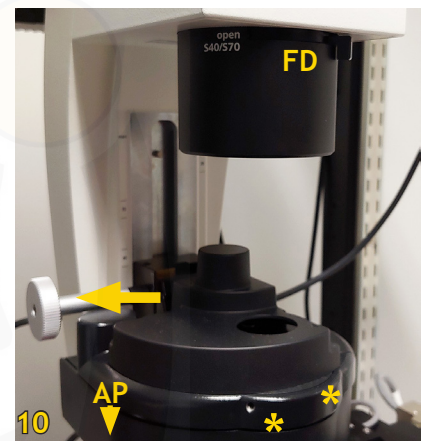
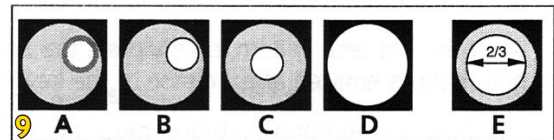
Köhler Illumination

Köhler illumination provides homogeneous illumination of your specimen without stray light. You get images with optimum contrast and resolution. Please do this in the BF setting!

These are the steps (Figs. 9, 10):

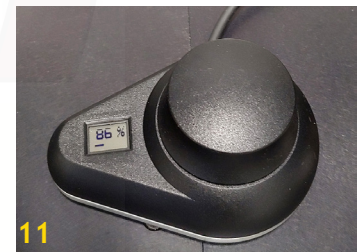
Focus the image

- A Close the field diaphragm (FD), adjust the aperture diaphragm (AP) if necessary
- B Adjust condenser height with focus wheel (Fig. 10, arrow) until the edge of the field diaphragm is in focus
- C Centre the field diaphragm with the screws on the condenser (Fig. 10*)
- D Open the field diaphragm (FD) until the edge just disappears from the field of view
- E Adjust the aperture diaphragm (AP) so that it is two-thirds open



Once you have adjusted these settings, switch to epifluorescence (Fig. 6B), select the filter for your dye/fluorescent protein and open the FluoShutter by pressing the large dial button (Fig. 11). Adjust the intensity and check if signal strength is sufficient. Find your region of interest and adjust the focal plane if necessary.

Close the FluoShutter and switch to camera view on the 'Objective' screen (Fig. 6C), then continue with Volocity.



Objectives - will be changed on the microscope touchscreen (Fig. 6C):

- Dry objectives: 10x - 40x LWD (long working distance)
- Oil immersion objectives: 40x - 63x - 63xTIRF - 100x

Fluorescence filters - will be changed on the microscope touchscreen (Fig. 6B):

Chroma: ET-DAPI (UV) / ET-GFP (blue) / ET-Rhodamine (green)

AFC (Adaptive Focus Control):

The AFC continuously measures the distance to the coverslip and keeps this distance constant. You can find more details on AFC on page 15.

Volocity Acquisition Setup

Volocity Startup

Double-click the Volocity icon. At this point, you do not need to sign in.

Volocity files are called libraries. What is a Library?

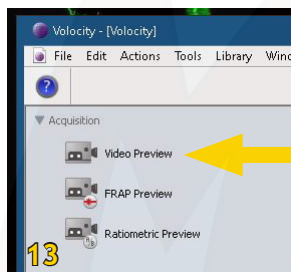
A library is the term given to the data storage format used by Volocity. You have to create a library for Volocity to acquire data. If you forget this, you will end your session and no images will be saved!!!

Libraries are based on a database structure and will store changes as they are made.



Creating libraries

You can select 'Create a new library' or 'Open an existing library' from the prompt shown when Volocity starts (Fig. 12). Or, once you are running the application, select 'New Library' from the File menu. You can only have one library open at a time. If you already have a library open, you will be prompted to close it first. Use the dialog to name the library and choose a location to store it.

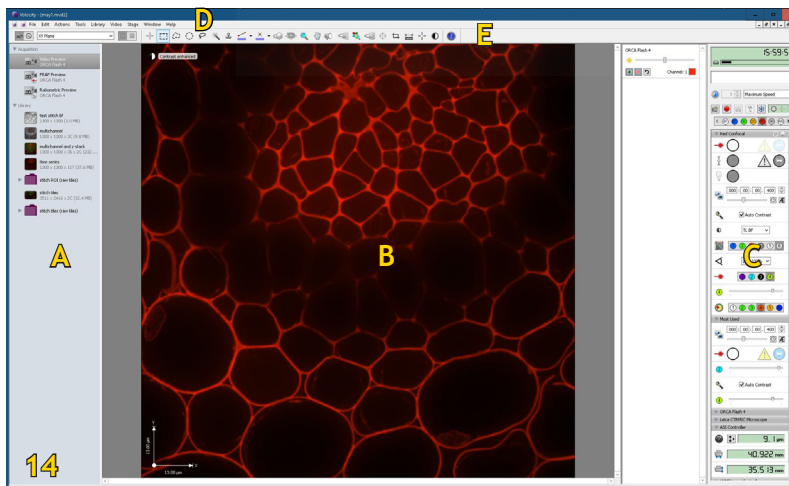


Next, select the icon 'Video Preview' to call up the control interface (Fig. 13).

Software Interface

The Volocity program interface is divided into 3 main areas.

The left panel shows the 'Video Preview' icon that you need to select for live streaming and the current library with all images and image series (Fig. 14A).



The centre screen area (Fig. 14B) is used to display your live image or selected images/ image series.

The right panel (Fig. 14C) provides you with all imaging parameter controls like light paths, shutters, exposure times etc.

- A - Left Panel
- B - Centre Screen Area
- C - Right Panel (Control Panel)
- D - Menu Bar
- E - Tool Bar

Basic Image Acquisition

Velocity has the following setting short cuts (if it's not labeled, you won't need it!)

Light Paths

All light paths you will need are arranged in the 'Light Path Manager' (Fig. 15A):

- BF - Bright Field
- BC - Blue Confocal (405 laser)
- GC - Green Confocal (488 laser)
- OC - Orange Confocal (560 laser, emission 600 ± 20)
- RC - Red Confocal (560 laser, emission 620 ± 20)
- DIC - Differential Interference Contrast

Whenever you make changes to a light path setting (e.g., exposure time!), save these changes by clicking on the small disk symbol beside the light path header (Fig. 15B)!

Image Acquisition

To start the live stream, select one of the light paths. Adjust the exposure time (Fig. 15C) if necessary. 200-500 ms are a good starting point. You may also need to adjust the focus on the screen.

The right panel seems to have a large number of controls, but most of them are duplicated or automatically changed by the software. In practice, you will only need the 'Light path', 'Most used', and 'ASI Controller' submenus open (see Fig. 15).

The shutters (Fig. 15D) are automatically opened and closed when you change light paths and after image acquisition. To see your live image after acquisition, you will need to open the shutter again. Click directly on the shutter icon to change its state. Gray indicates shutter closed. White indicates shutter open.

The laser strength can be adjusted - start with 15% for the 405 and 488 line and 30% for the 561 line (Fig. 15, arrows).

If you select any of the confocal light paths, make sure the checkbox for 'Auto Contrast' is marked active (Fig. 15E) - otherwise you will not see any signal.

Once you have adjusted all parameters, click on the 'Camera' icon (Fig. 15F) or tap on the space bar to take a single-channel image.



Advanced Image Acquisition

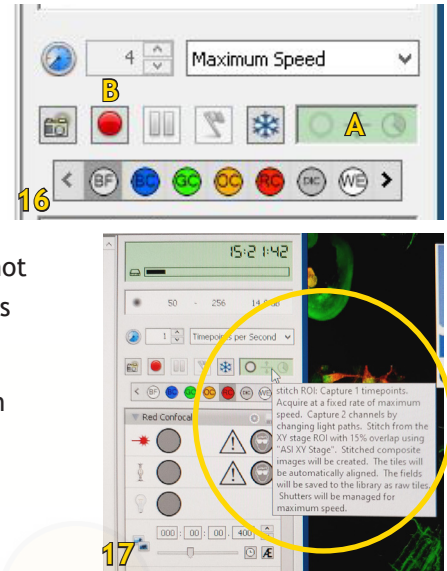
If you want to take a multichannel image or an image series like a Z-stack, time series, or stitched image, you need to define these actions in the ‘Acquisition Setup Window’ (Fig. 16A). Double-click on the acquisition panel to open the ‘Acquisition Setup Window’.

The dialog is divided into tabs according to the type of experiment being performed (details see below). Configure only the tabs needed and you may ignore the rest. Check the summary to make sure a previous user has not configured a step you do not want! You can rename your image/image series under the Channels/Z tab (Fig. 18E).

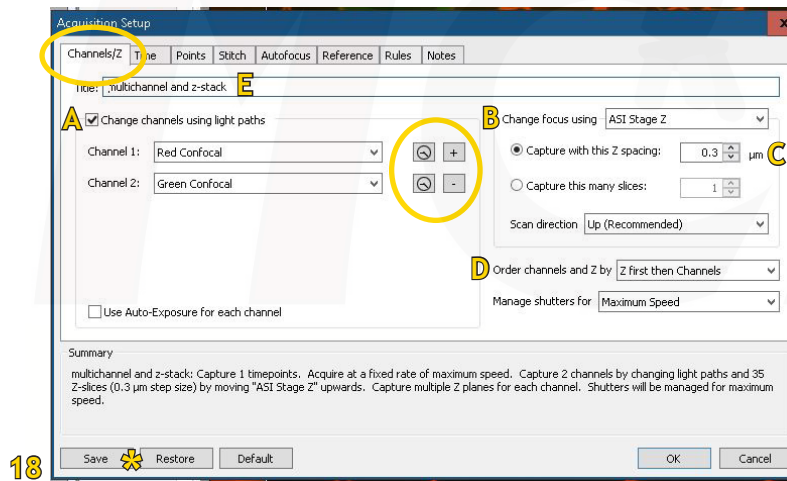
You can see the summary if you hover the mouse cursor over the acquisition setup panel (Fig. 17).

Image acquisition is started by clicking on the red button (Fig. 16B).

The acquisition protocols most often used are: Channel/Z - Time - Stitch. The stage needs to be calibrated for ‘Stitch’ and ‘Points’ (see page 14).



Channels /Z (Fig. 18)



If you wish to acquire a multichannel sequence check the ‘Change channels using light paths’ box (Fig. 18A). Add those channels you want to acquire (+’ and drop-down menu). Select the fluorescence channels in the order from longest to shortest wavelengths to minimize interferences!

If you wish to acquire a stack of images, select ‘ASI stage’ under ‘Change focus using’ (Fig. 18B).

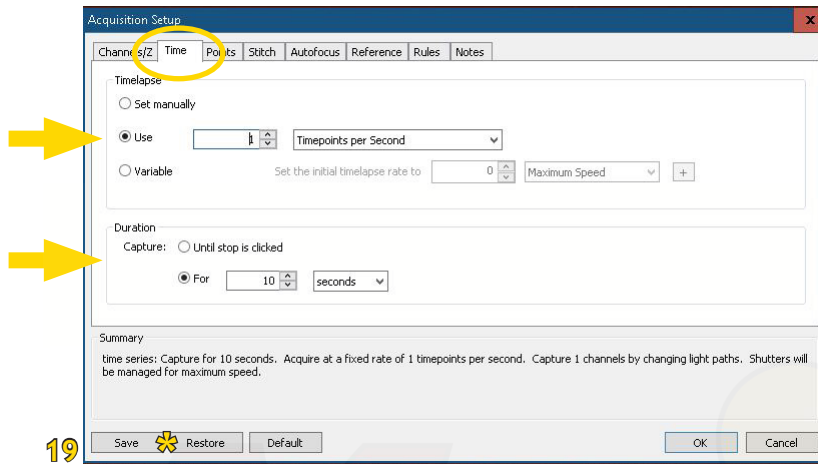
The recommended step size (Fig. 18C) depends on the objective. Recommended step sizes are listed on page 5. More details on Z-Stack acquisition can be found on page 13.

You can choose (in multichannel images) if you want to acquire first the stack and then switch channels, or if you want to first acquire each channel and then change planes (Fig. 18D).

Time (Fig. 19)

To define a time series, select 'Time'. Under 'Timelapse' you define the interval - maximum speed, x timepoints per ms, per second, per hour, etc.

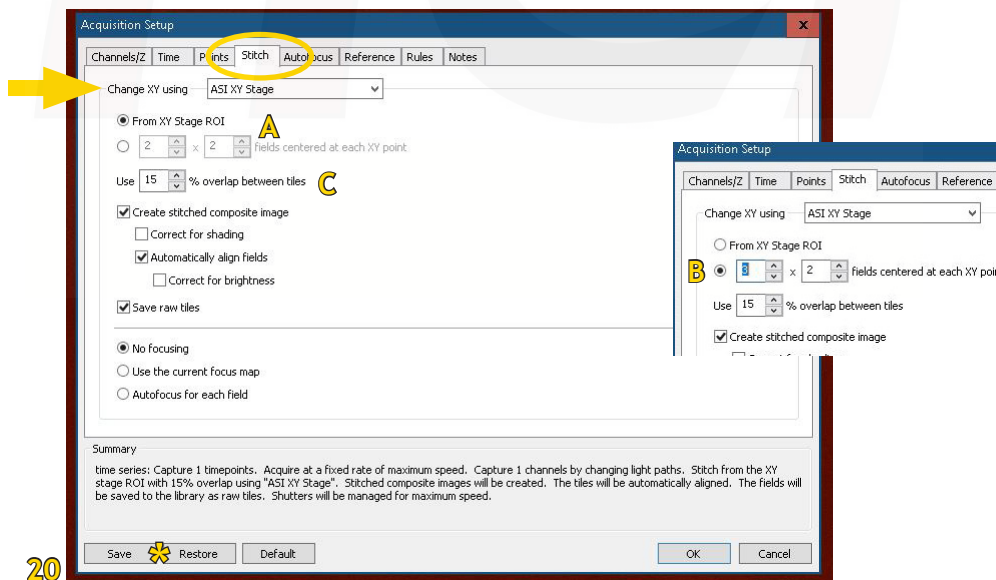
Under 'Duration' you define the length of your time series - 'until stop is clicked' or xx seconds, minutes, hours, etc.



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Stitch (Fig. 20)

To compose a large image from several regular images, select the 'Stitch' tab. Select 'ASI XY Stage' under 'Change XY using'. Then choose either 'From XY Stage ROI' (Fig. 20A) or define the number of fields in X and Y around your current position (Fig. 20B, inset). 10-15% overlap between the tiles is recommended (Fig. 20C). See also page 14 for more details on Stitching.



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Points: This is usually used in combination with a time series. Select the 'Points' tab, select 'ASI XY Stage' under 'Change XY using'. Find the points you want to image and mark them on the stage (see page 14).

Restoring Acquisition Protocol Settings

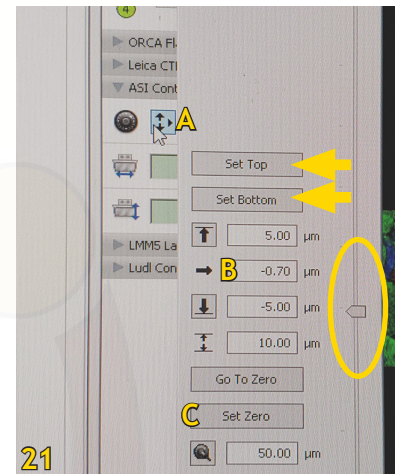
If you always use the same acquisition protocol, you can save it and easily recall it in your next session. In the 'Acquisition Setup Window', click on 'Save' to save your protocol in a designated folder and on 'Restore', to display a list of saved acquisition protocols (Figs. 18-20, *). Select the protocol that you wish to restore and click 'Restore'.

Z-Stacks Details

Define the range of your Z-stack

Once you have set the parameters for Z-stack imaging in the 'Acquisition Setup Window' (Fig. 18B-D), you need to define the beginning and end points of your stack.

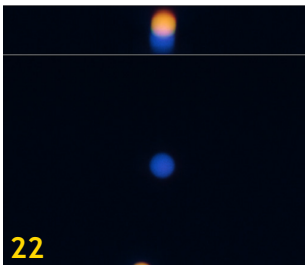
- To do this, close the 'Acquisition Setup Window' and open the 'ASI Controller' submenu in the right control panel.
- Click on the icon with the 3 arrows (Fig. 21A)
- Use the slider on the right side to focus through your sample (Do not use the Leica Smart Move to focus!!!)
- Set the bottom limit of your stack by clicking 'Set Bottom' and the top by clicking 'Set Top' (Fig. 21, arrows).



Given the recommended step size, the software will automatically calculate the number of images in your stack. It is important to remember that the positions here refer to settings of the ASI controller and not the Leica DMI8 microscope! Therefore, ensure that your centre focal plane is close to '0' before you define beginning and end of your stack. If the stage position value is much different from zero (Fig. 21B), select 'Set Zero' to define your focal plane (Fig. 21C).

Registration correction

If you take Z-stacks of multiple confocal channels including the 'Blue Confocal' (405 laser) path, you need to perform a 'Registration Correction' on your Z-stack. The blue confocal channel has a mismatch in the Z-plane (chromatic shift) from the visible light paths (488, 560 lasers), see Fig. 22.



Volocity can generate a 'Registration Correction' that can be applied for 3D data to shift pixels in all channels to their correct positions. This is done routinely twice a year and the current Registration corrections are saved on the SD computer desktop. However, it may be necessary that you repeat this procedure.

Please contact Michaela and she will show you how to do the correction.

Working With the Motorized Stage (Stitch and Points)

Stage calibration

Before you can define ROIs and points on the stage, it needs to be calibrated in Velocity.

Due to the linear encoders, this process takes about 5 minutes!!!

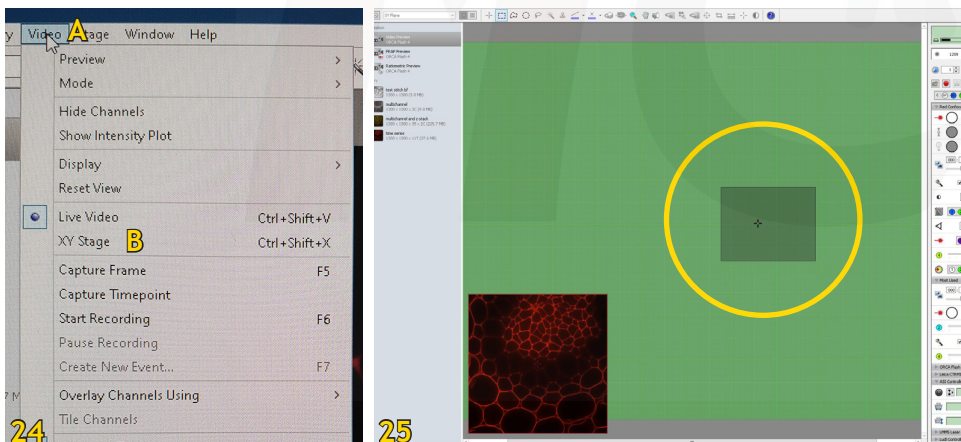
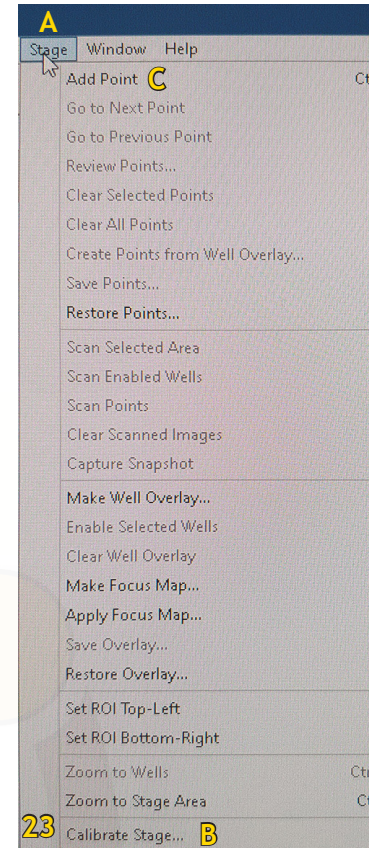
- In the main menu, select 'Stage' (Fig. 23A)
- Select 'Calibrate Stage ...' (Fig. 23B)
- The software will prompt you to remove your sample and lower the objective nosepiece - so this is best done at the very start of your session!
- Wait until the calibration process is finished. Then place your sample on the stage.

Define ROIs for stitching on the stage screen

- In the main menu, select 'Video' (Fig. 24A)
- Select 'XY Stage' (Fig. 24B)

You will now see the stage area in green on your screen and a small live image in the corner. Your current position is marked with a crosshair.

You can define the ROI by drawing a rectangle around your current position (Fig. 25).



To define the ROI more precisely, you can move the stage and add position markers by selecting 'Add point' under the 'Stage' menu (Fig. 23C). Then create the ROI limited by those reference points.

Define multiple points on the stage screen

This is done when 'Points' is selected in the 'Acquisition Setup Window'. Move the stage to the position you want to image and add a marker by selecting 'Add point' under the 'Stage' menu. Proceed to the next position and repeat.

Adaptive Focus Control (AFC)

The AFC is a focus maintenance system that automatically corrects focus shift of the specimen or the stage due to temperature. It is most useful in long-term imaging sessions.

The AFC uses a mismatch in the refractive index (e.g., dry objectives, or oil immersion objectives with water-based samples) to measure, track, and maintain the distance between the objective and the sample surface (i.e., cover glass, well plate bottom, etc). It has an optical offset function, which allows for manual adjustment of the focal plane position within the specific range for each objective.

With the exception of the 40x oil objective, all objectives are compatible with the AFC. The system will give an error message on the stage display if the objective can't be used with AFC.

The AFC should always be activated but does not need to be set to 'hold'. Activation puts a prism in the light path and all alignment and calibration of the system has been done with this prism. Inactivation of AFC results in a focus shift!

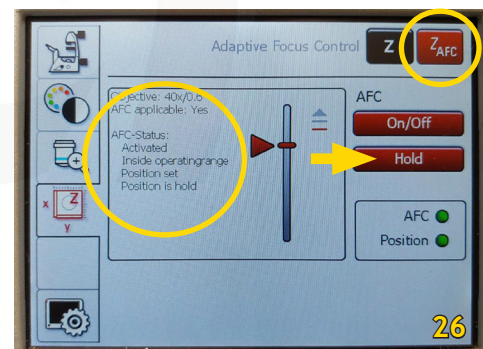
The AFC can be set through the microscope controls or in Volocity.

The Adaptive Focus Control is not very useful with ...

- Fixed, mounted specimen and oil immersion objectives, since the mounting medium mostly has the same refractive index as glass and immersion oil
- Thick samples or samples that generally strongly scatter light
- Samples in plastic dishes other than the ones recommended
- Samples which have the cover glass or well plate bottom contaminated with dust, oil, etc.

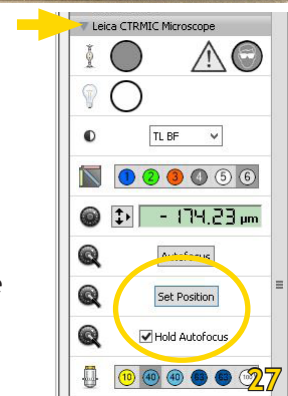
Setting the AFC via the microscope (Fig. 26)

- On the touch screen, select the 'Stage' display, and select 'Z_{AFC}' (Fig. 6D, *)
- Make sure the AFC is shown as 'Activated'
- Start focusing your sample with the Smart Move. When the objective comes into the AFC range, it will be indicated as 'Inside operating range'. Continue to focus until you have the correct focal plane.
- Press the 'Hold' button. The display should now show: 'activated - Inside operating range - Position set - Position is hold'.



Setting the AFC in Volocity (Fig. 27)

- In Volocity, open 'Leica CTRMIC Microscope' in the right control panel
- Make sure the AFC is shown as 'Activated' on the microscope touchpad screen
- Start focusing your sample with the Smart Move. When the objective comes into the AFC range, it will be indicated as 'Inside operating range' on the microscope display. Continue to focus until you have the correct focal plane.
- Click on the 'Set Position' icon, then select 'Hold Autofocus'

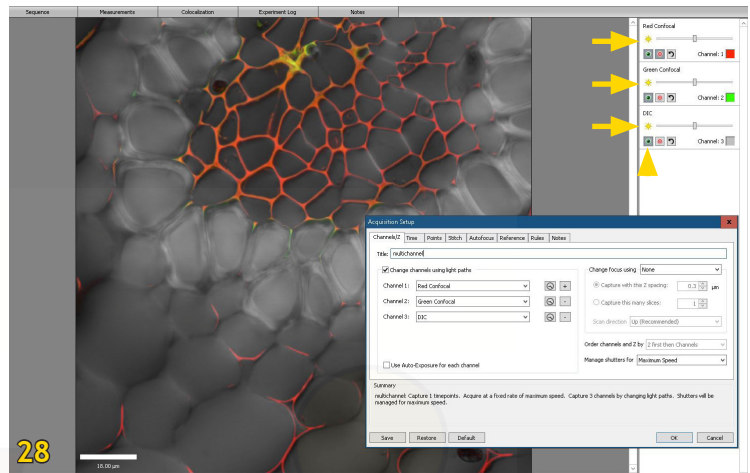


Viewing Options

Once the image is acquired, a thumbnail will appear in the left panel. To view it, simply click once on the thumbnail (double-click will open it in a new window).

Overlay Image

Multichannel images will automatically be displayed as overlay. To hide one channel, you can click on the eye icon in the right viewing pane (Fig. 28, arrowhead) - here, you can also change the intensity of your channels (Fig. 28, arrows).

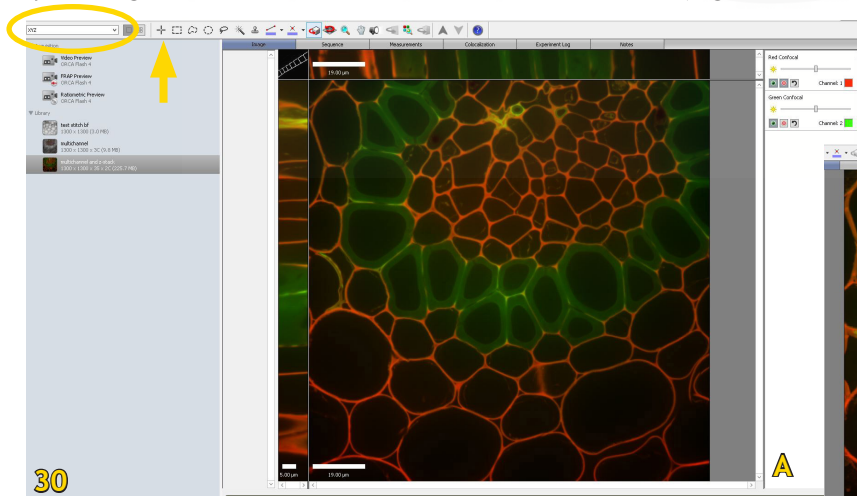
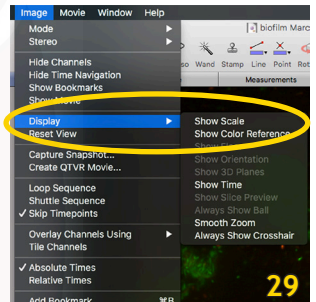


Scale Bars

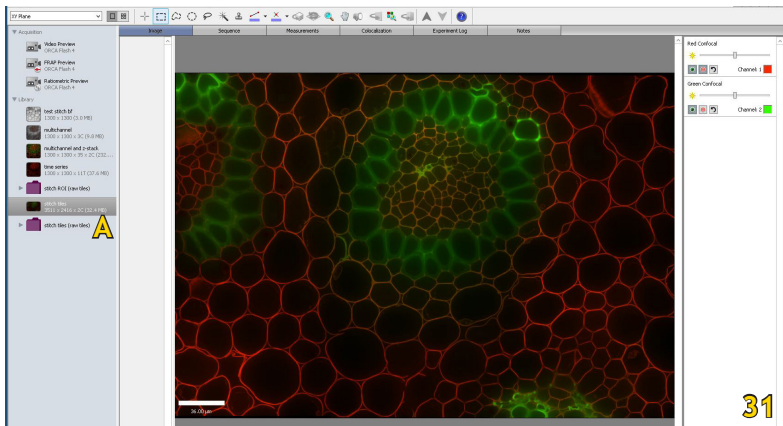
The scale bars have been calibrated and you can select to see the scale bar under 'Image' - 'Display' - 'Show Scale' (Fig. 29).

Z-stack Image

Multichannel Z-stack images will automatically be displayed as overlay in 'XYZ' view (Fig. 30A). The XY plane is the large centre image and the XZ and YZ planes along 2 crosshair lines are shown top and left of it. To navigate within you image, make sure the 'Crosshair' icon is selected (Fig. 30A, arrow).



You can switch to 'Extended Focus' view (Fig. 30B) in the top left drop-down menu (Fig. 30A). This will merge all Z-planes into one 2D image.



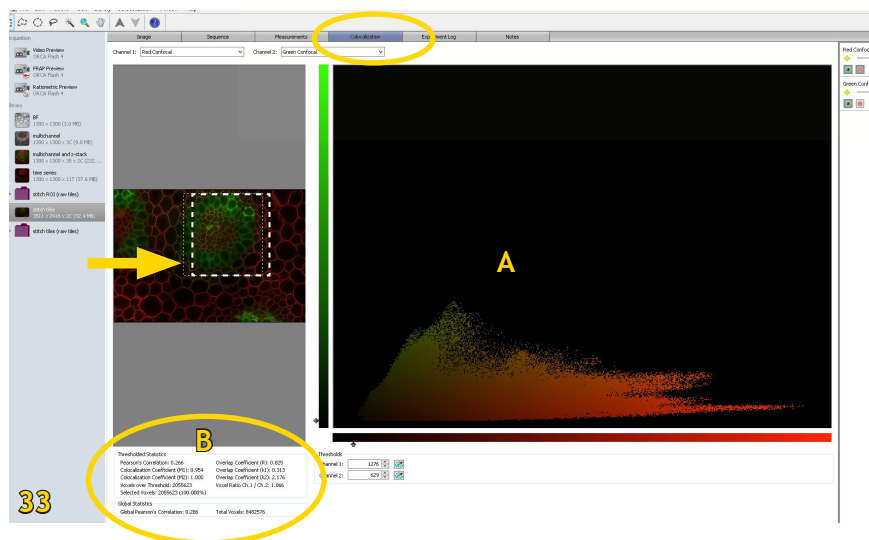
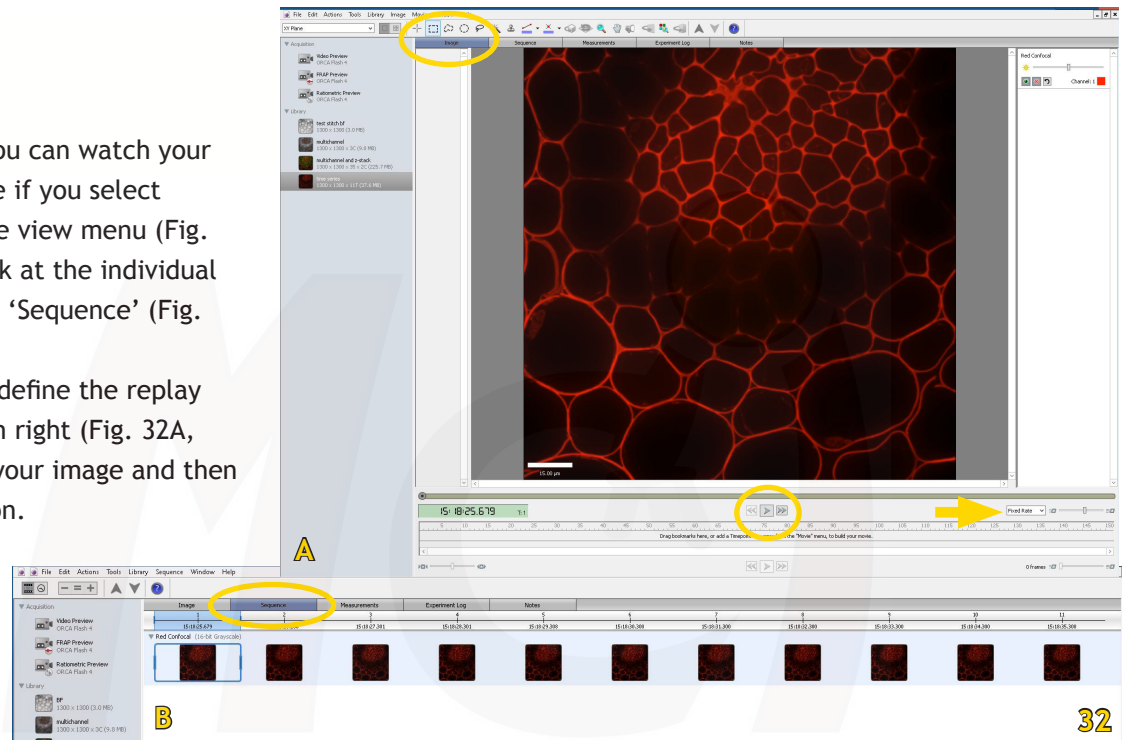
Stitched Image

If you use the default setting in the 'Acquisition Setup Window', the tiles will automatically be stitched to a large image and this image is shown (Fig. 31) - a folder with your individual tile images is also saved (Fig. 31A).

Time Series

After acquisition, you can watch your time series as movie if you select 'Image' in the image view menu (Fig. 32A) or, you can look at the individual images if you select 'Sequence' (Fig. 32B).

To view the movie, define the replay speed at the bottom right (Fig. 32A, arrow), just below your image and then press the play button.



Colocalization

The Colocalization tab automatically shows the scatter plot (Fig. 33A) and Pearson Correlation Coefficients (Fig. 33B) for 2 channels in the entire image or for a selected ROI (Fig. 33, arrow).

If you acquired more than 2 channels, you have to deselect all but the 2 channels you want displayed.

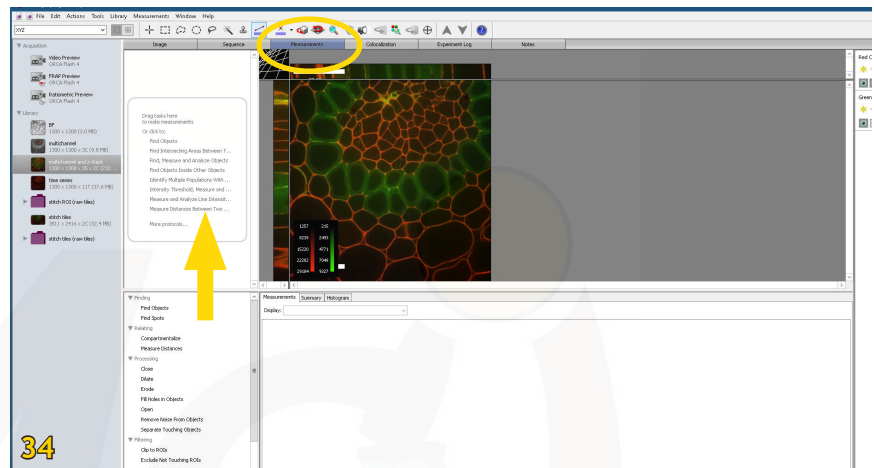
Data Processing

You will do the processing likely not on the imaging library Measurements computer but with the offline modules of Volocity that are available. The three modules are:

- Measurements (Quantitation)
- 3D (Visualization)
- Decon (Restoration)

Measurements

When you select the 'Measurements' tab in the centre viewing area (Fig. 34), you will see a list of options for quantitation. You will need to select the function you want to use (i.e., 'Measure distances') and drag it into the top area (Fig. 34, arrow). The Volocity 'Help' function will guide you through setup and processing.



3D Reconstruction and Visualization

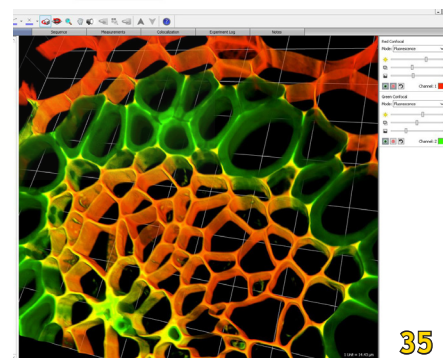
There are several options to reconstruct and view your Z-Stack images as 3D data.

3D Opacity (Fig. 35) is an interactive high resolution renderer with options for how the data is displayed. Resolution is managed to maintain interactivity. If Volocity Visualization is used in conjunction with Volocity Quantitation measurements, feedback can be shown in the 3D Opacity mode. Control over brightness, density and black level is given on a per channel basis. You can also create and export a video of your rendering.

3D Ray tracing is a processor intensive rendering that requires more time to calculate the image and is therefore not interactive. Different effects may be generated with the ray tracer but it does not support measurements feedback or scale shown on the view.

3D Slice allows 3D or 4D data to be viewed at a different orientation of slicing and, if required, the data may be re-sliced to create another version of the data at the new orientation.

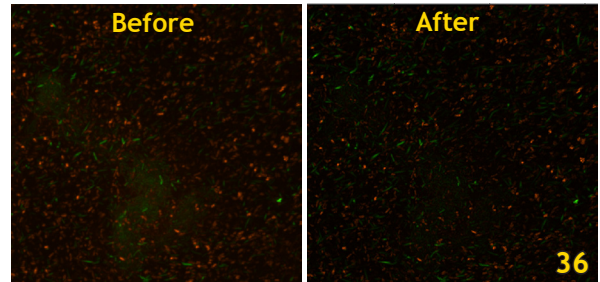
Please refer to the Volocity User Manual or Online Help for more detailed instructions and examples.



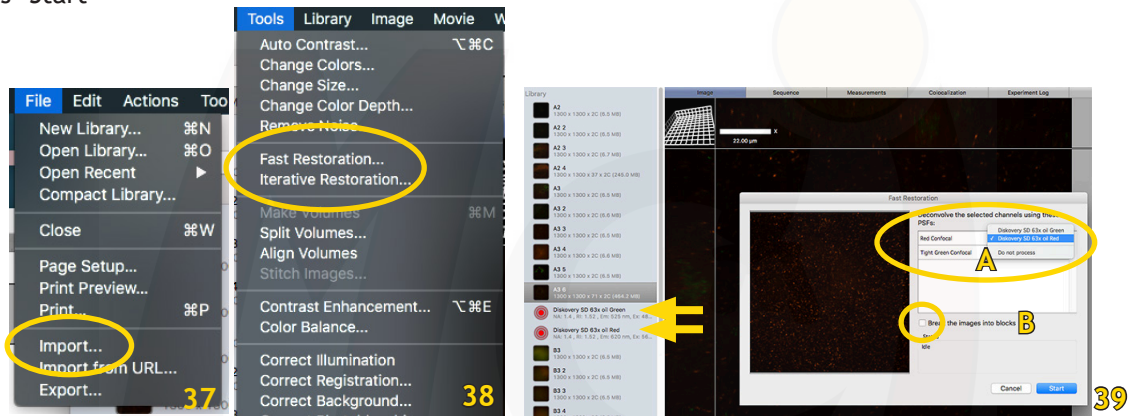
Deconvolution

Deconvolution might be necessary for some image stacks (see Fig. 36).

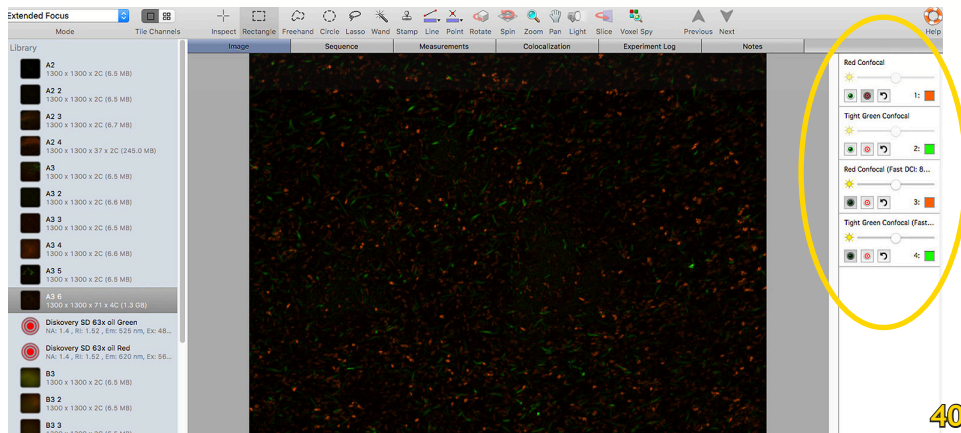
To be able to do this, you need to import the PSFs (Point Spread Functions) for the corresponding objectives (Fig. 39, arrows). Each objective has its own PSF for each channel. They are available on the SD computer (folder on desktop) and on the MCIF group page under 'Documents' - 'Diskovery Spinning Disk'.



- Import the PSFs you need via 'File' - 'Import' (Fig. 37)
- Select 'Tools' - 'Fast Restoration' (Fig. 38)
- In the new window, verify that the correct PSF is assigned to each channel (Fig. 39A).
- If your image is very large, select 'Break the images into blocks' (Fig. 39B)
- Press 'Start'



Once the deconvolution is finished, all 4 channels (original and deconvolved) will be shown as overlay. Deselect (hide) the original channels with the eye icon to see the deconvolved image only (Fig. 40). Create a snapshot of the deconvolved image and export the snapshot (see page 20, Fig. 44).



Data management

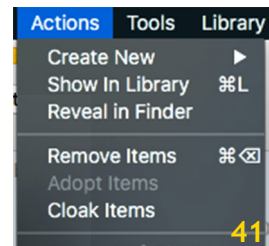
File format

Your saved data will appear in a folder with the experiment name. Within this folder you find another folder called 'Data' and a small file with the extension .mvd2.

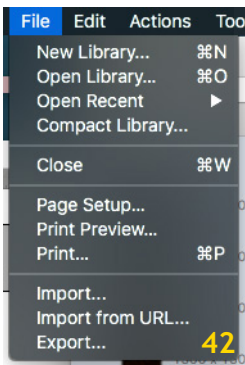
Both items need to stay together in this folder! Volocity or other imaging programs are not able to open your images only from the .mvd2 file.

Delete images from your library (Fig. 41)

- Select the image/image series in the left panel
- In the 'Actions' menu select 'Remove Items'



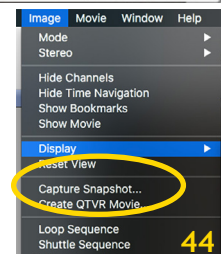
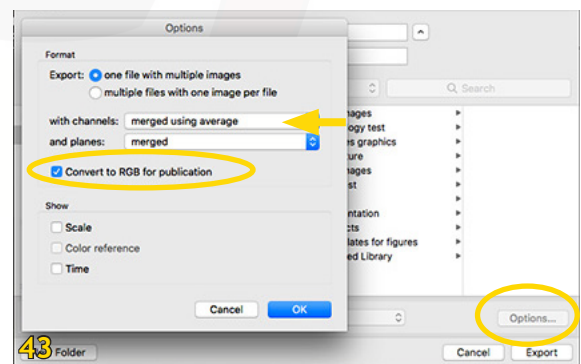
Export images



- Select the image/image series in the left panel
- In the 'File' menu select 'Export ...' (Fig. 42)
- Define/find the folder where you want to save your image
- In the drop-down menu select the format in which you want to save your image. You can choose between 'Item as...' and 'View as ...' - for regular image export without annotations this doesn't make a difference. If you have a multichannel image and you want to export the overlay, select 'Options' (Fig. 43) and under 'with channels' select 'merged using average' (Fig. 43, arrow). You can also select to add a scale bar to your image.

Export annotated images

- Select the image/image series in the left panel
- In the 'File' menu select 'Export'
- Define/find the folder where you want to save your image
- In the drop-down menu select the format in which you want to save your image. You can choose between 'Item as ...' and 'View as ...' - if you want to export your image with annotations, you need to select 'View as ...'
- Make sure in the options window the box 'Convert to RGB for publication' is checked.



Sometimes you also have to create a snapshot of your annotated image first:

- Select 'Image' - 'Capture Snapshot' (Fig. 44)
- This snapshot can then be exported as described above

Shutdown

1. Lower the nosepiece, remove your sample, clean objective lenses with lens paper
2. Close Volocity and transfer your files to OneDrive (preferred) or the server
3. **Switch off laser emission key**
4. Switch off main power switch
5. Minimize intensity of X-Cite Light Source (at remote control) **and then switch off**
6. Shut down the computer
7. Log your usage into the log sheet and **cover the microscope**

2-Hour Rule

If someone else has booked the microscope within the next 2 hours, please leave the X-Cite and the laser key switched on, just minimize the intensity of the X-Cite! You can also leave the computer on. Don't cover the microscope.