Quick Start
Axiovision

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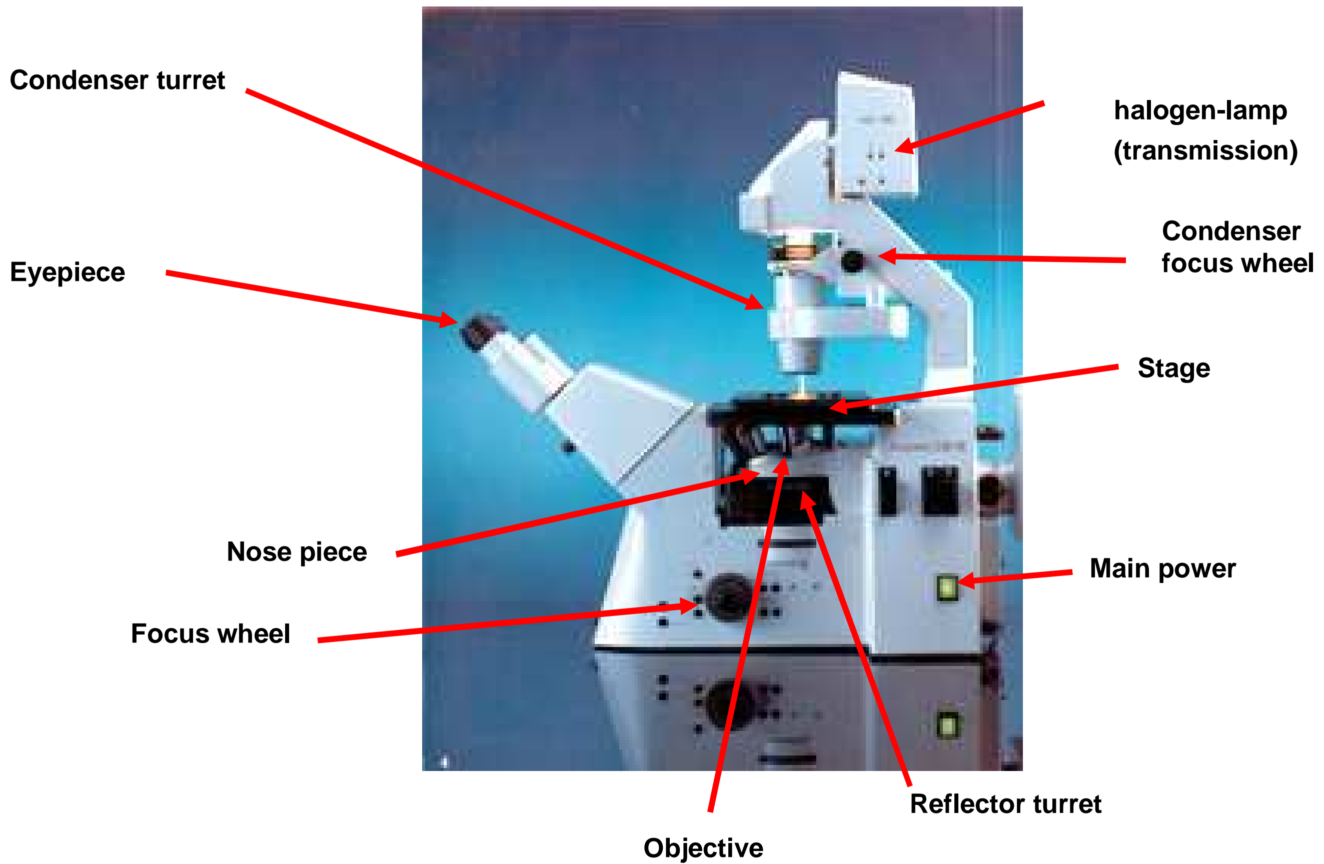
1. Switch on Hardware

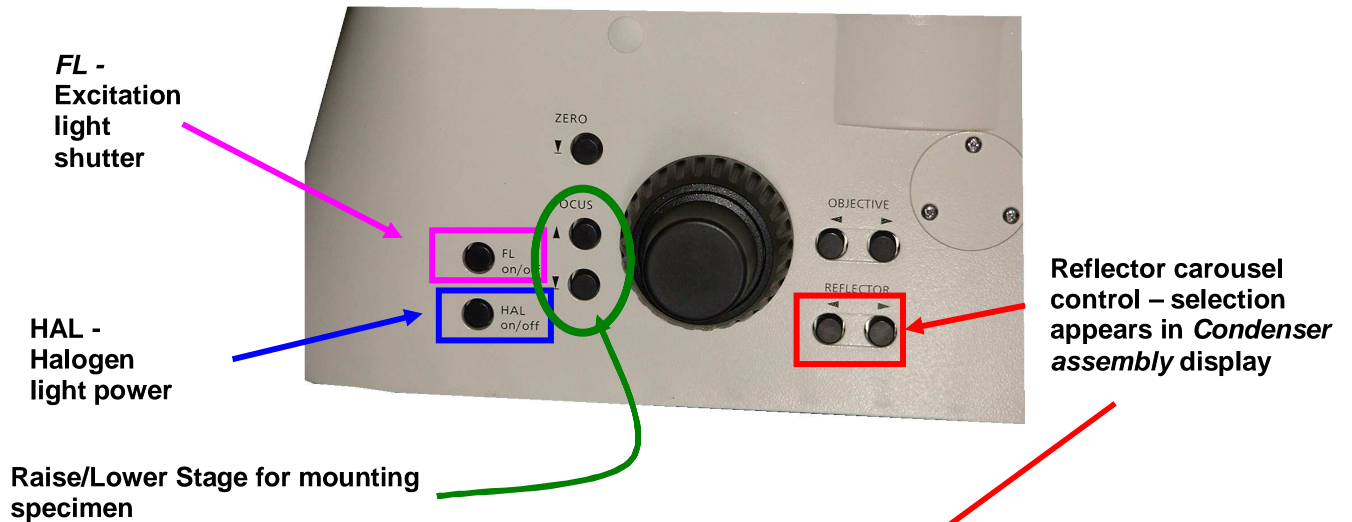
1. Arc-lamp power supply (under table)
2. Switch on ‘scope.
3. Switch on Uniblitz shutter
4. Switch on camera.
5. Start PC.

2. Microscope parts

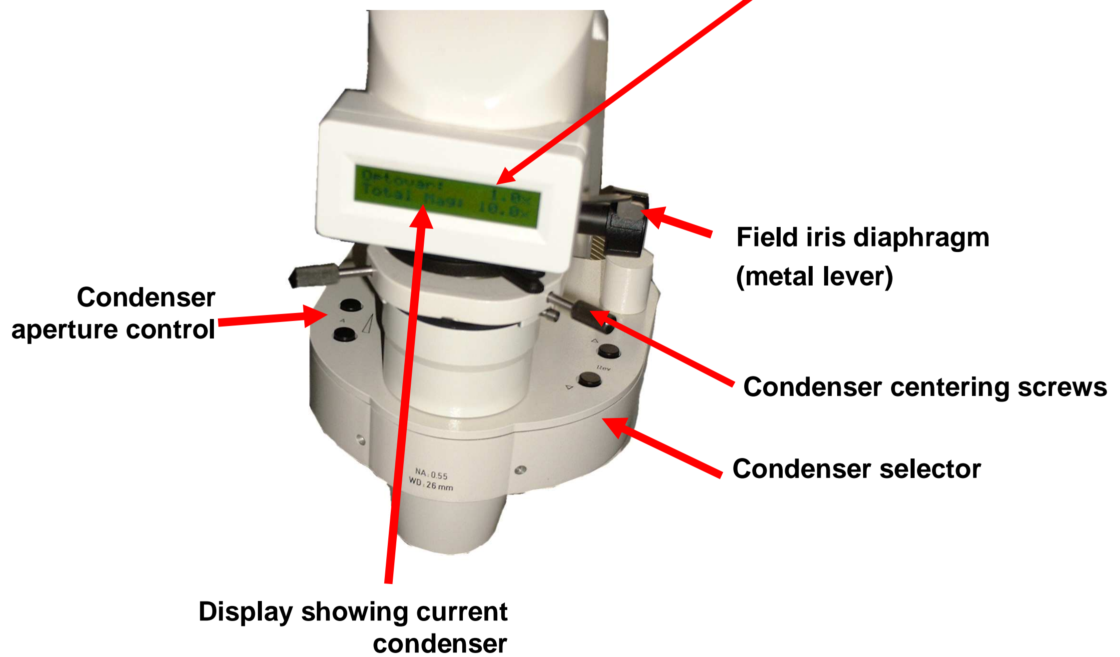


2.1. Uniblitz shutter control box

2.2. Right hand side of microscope

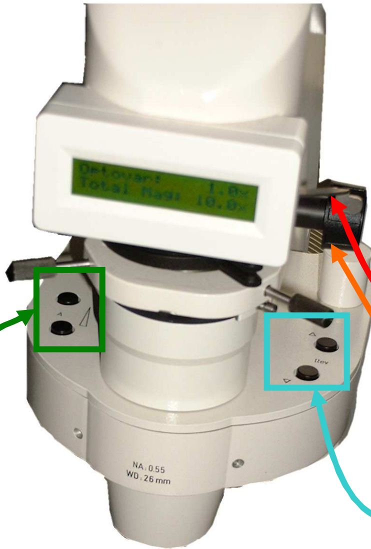


2.3. Condenser assembly

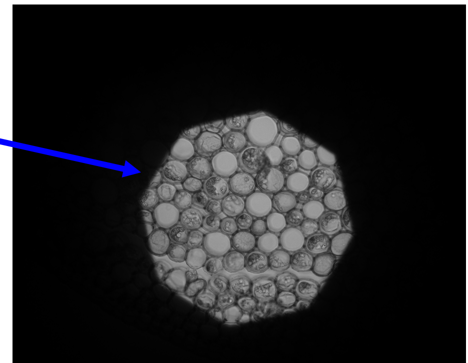


3. Set up Köhler Illumination (if required)

Köhler illumination was first introduced in 1893 by August Köhler of the Carl Zeiss corporation as a method of providing the optimum specimen illumination. Köhler illumination is not important for fluorescence or confocal, however if a transmitted light image is required, the microscope should be set up with Kohler illumination.



1. Mount slide.
2. Switch to 10× objective.
3. Focus on sample (maybe use fluorescence channel if possible then turn to brightfield).
4. Close silver lever ***Field Iris Diaphragm***.
5. Raise condenser close to sample using ***Condenser Focus Wheel***
6. Turn condenser to **I/H** for bright field using the ***Condenser Selector*** buttons on the condenser turret.
7. Focus condenser using ***Condenser Focus Wheel*** until ***iris-diaphragm*** (NOT THE SPECIMEN!) is in sharp focus.



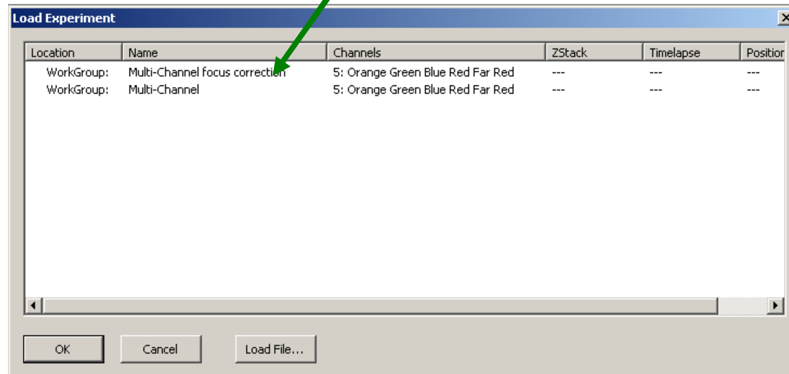
8. Re-centre condenser if necessary using silver, ***Condenser centring screws***.
9. Open the ***Field Iris Diaphragm*** until it just fills the field of view as observed through the eye pieces
10. Remove one eyepiece and close/open the ***Condenser Aperture*** until it just disappears from the ocular. Put eyepiece back!

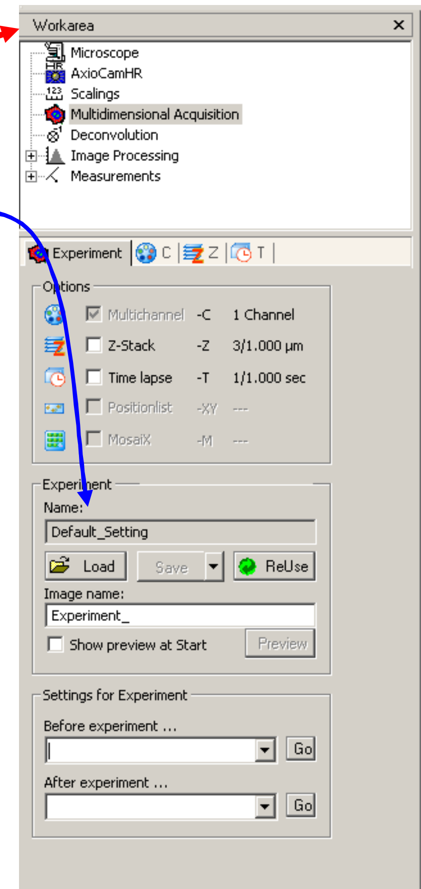
4. Start Software

1. Log on to WCIF intranet.
2. Start *Axiovision*.

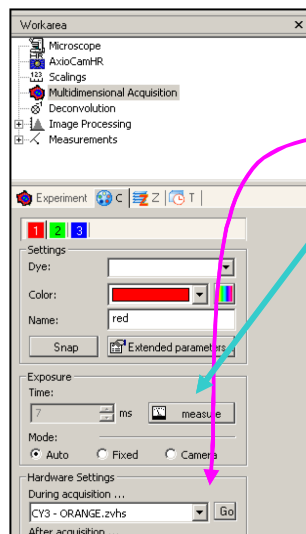
5. Start new experiment

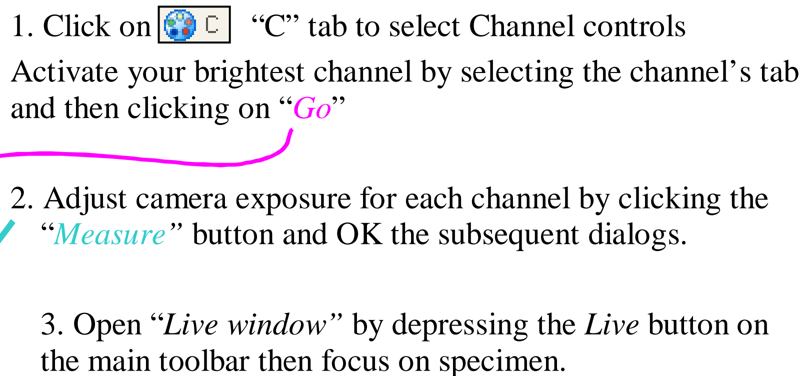
1. In the "Work Area" dialog, *Load* a new experiment template.
2. Select an experiment.





6. Focus on specimen





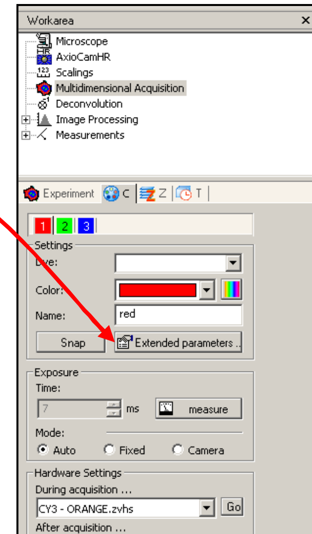
1. Click on C "C" tab to select Channel controls
Activate your brightest channel by selecting the channel's tab and then clicking on "*Go*"

2. Adjust camera exposure for each channel by clicking the "*Measure*" button and OK the subsequent dialogs.

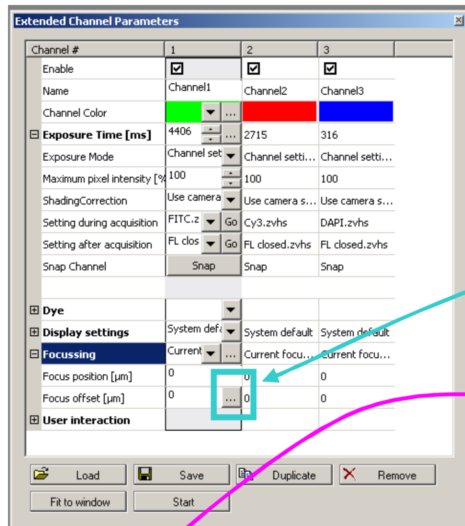
3. Open "*Live window*" by depressing the *Live* button on the main toolbar then focus on specimen.

7. Set focus-correction (if required)

Each wavelength has a slightly different focal plane due to poor chromatic correction of the objectives and filter block effects. This may be negligible for your specimen and you may not need to correct for it. If you do…



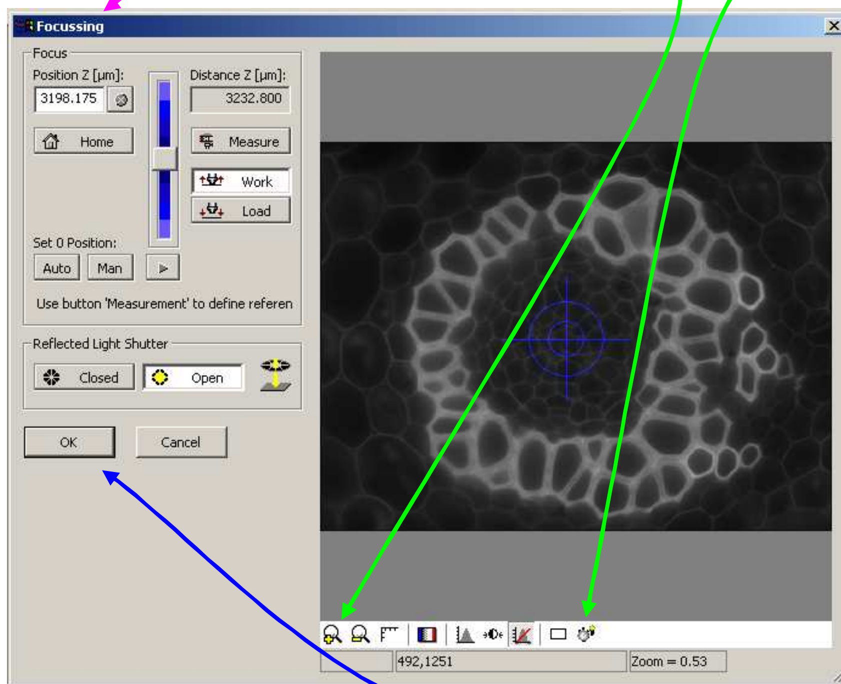
A. Click on the "***Extended Parameters***" button in the Multi-Channel Acquisition dialog to open the "Extended Parameters" dialog.



B. Click on the "***Focus offset (µm)***" button to open the "***Focussing***" window.

C. Focus the specimen using the microscope focus control.

Use the ***zoom*** and ***auto-exposure*** buttons to help you focus.



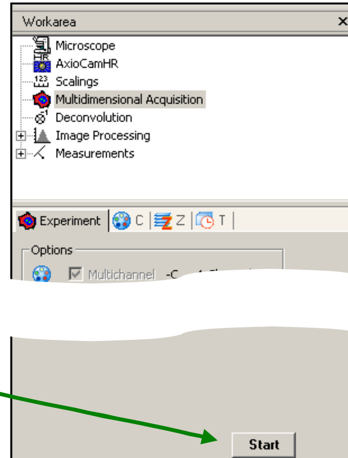
D. When the specimen is in focus, click ***OK*** and this will enter the focus offset value in to the channel's properties in the "*Extended Parameters*" dialog.

E. Repeat for all channels.

You may need to change these values when you change objectives or specimen.

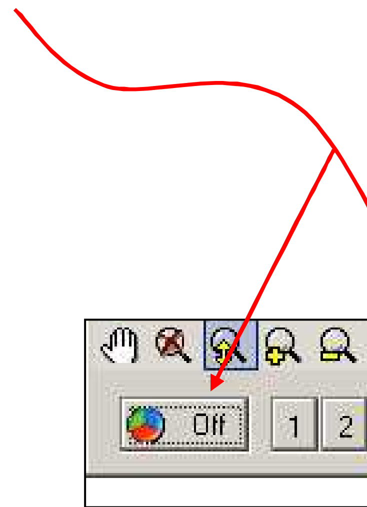
8. Acquiring

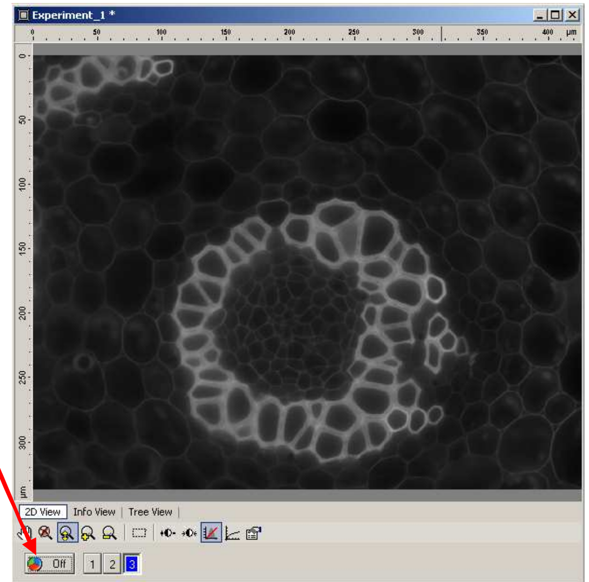
Click the “Start” button to start acquisition.



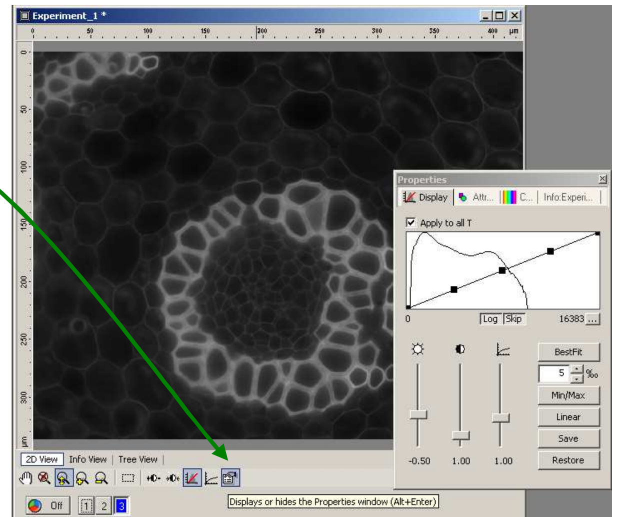
9. Adjust Brightness/Contrast

1. Turn off pseudocolouring.





2. Open properties dialog.



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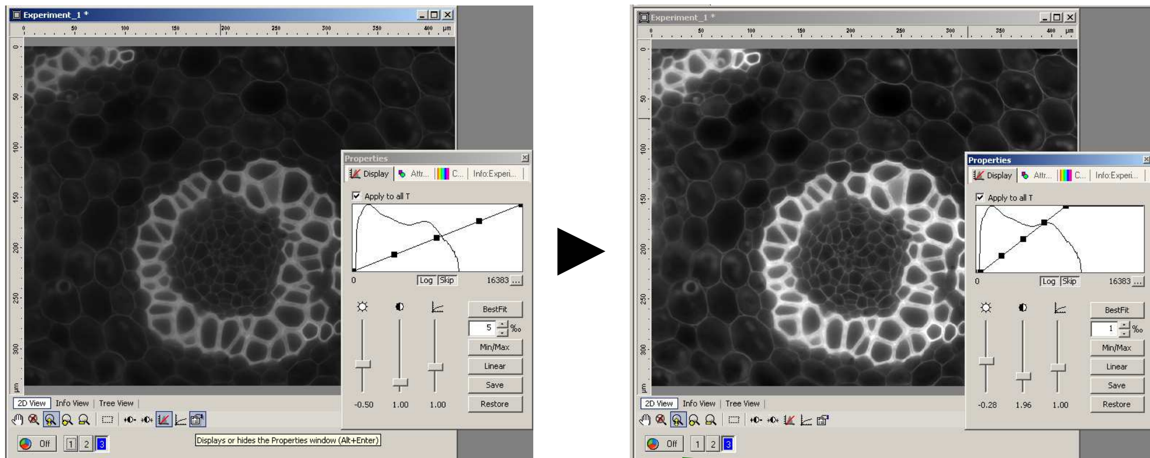
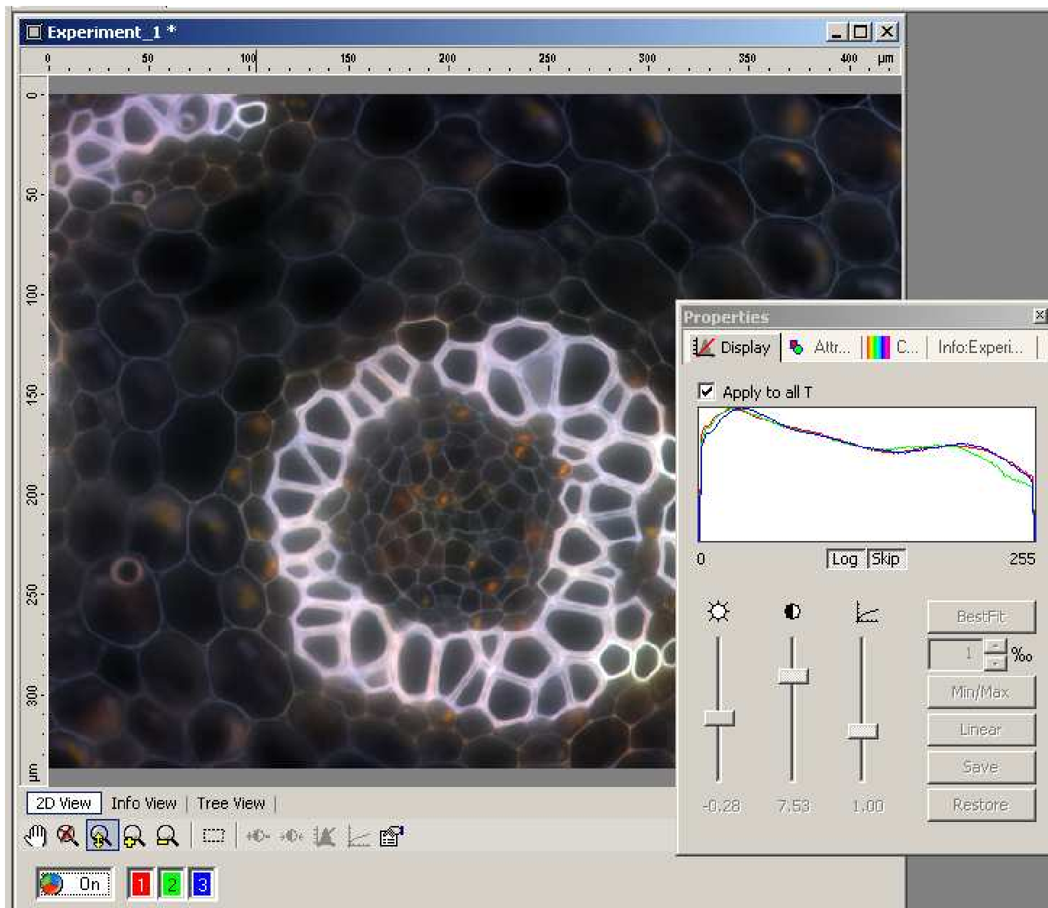
3. Change percentage to 1% and click "Best Fit".

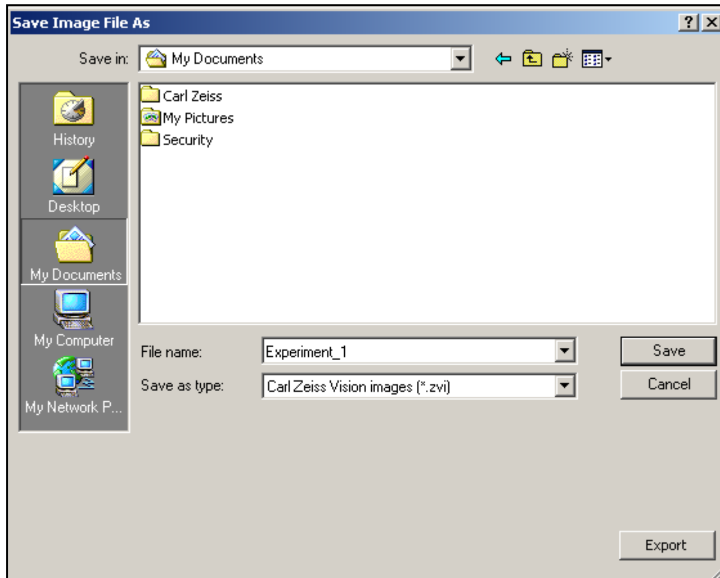


4. Repeat for each channel the turn back on colour.



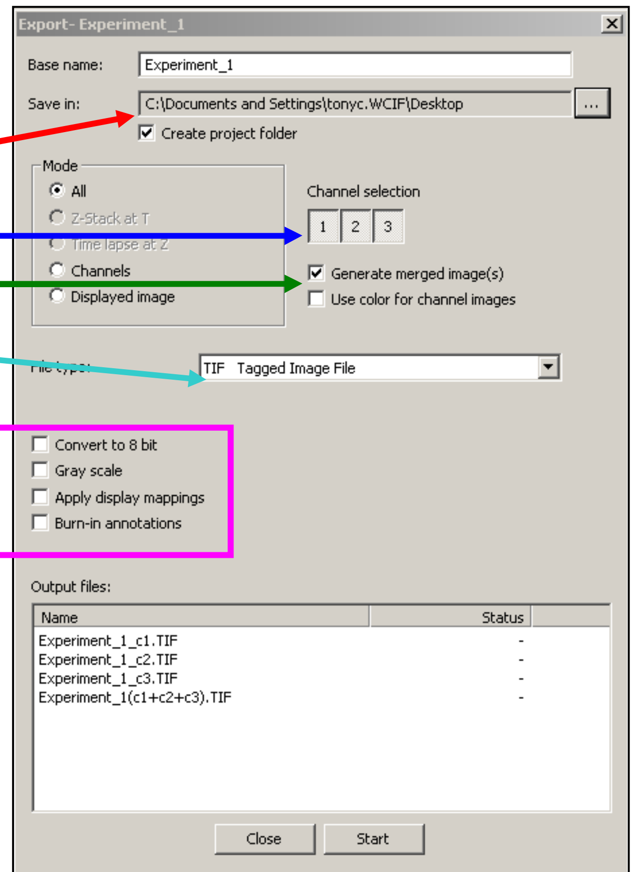
10. Save as *ZVI* file

Save as ZVI as this will save many of you imaging parameters. Exporting your data without saving as a ZVI can result in data loss. ***DON'T DO IT!***



11. Export

1. Select location for files.
2. Select all channels.
3. Select "Generate Merged Image".
4. Select TIFF.
5. Unselect all other boxes.
6. Click Start.



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Quick Reference Zeiss Axiovision 4

1. Start System

a. Arc Lamp
b. Camera
c. Microscope
d. Shutter
e. PC
f. Software

2. Load Multidimensional Acquisition

a. Focus using red channel
b. Set exposures

3. Click "*Start*" to acquire

4. Adjust Brightness & Contrast

a. Turn off pseudocolour
b. Select 1% "Best Fit" for each channel
c. Turn pseudocolour back on

5. Save as *ZVI*

6. Export as *TIFF*

7. Shutdown

a. Remove sample and clean objective
b. Move to a low power objective
c. Turn off arc-lamp
d. Exit software
e. Remove data
f. Power down system