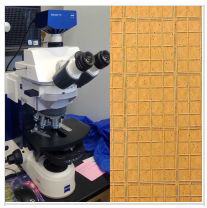

Feb 08, 2022

Visualization and Enumeration of Ostreococcus via Kohler Transmitted Light/Dark-Field on the Axio A1 Imager Microscope and AxioCam HrC

Lynn Doran[1]

[1]Realizing Increased Photosynthetic Efficiency (RIPE)

dx.doi.org/10.17504/protocols.io.b4ktquwn

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Lynn Doran
Realizing Increased Photosynthetic Efficiency (RIPE)

Basic setup and usage instructions for visualization of Ostreococcus species on the Axio A1 Imager Microscope using the AxioCam HrC and AxioVision for live and static imaging on the desktop using the Kohler Transmitted Light/Dark-field Method. Captured static images using the AxioCam HrC can then be exported for organism enumeration manually or using ImageJ Cell Counter or equivalent programs.

DOI

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https://dx.doi.org/10.17504/protocols.io.b4ktquwn

Ostreococcus, microscope, Axio, camera, microscopy, O. tauri, hemacytometer, photo

protocol ,

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Review Axio A1 Imager manual included in materials section for instructions on basic usage of microscope.

Axio A1 Imager from Zeiss is customizable. The settings and hardware may differ between microscopes. This protocol is written for the Axio A1 Imager with AxioCam HrC using AxioVision SE64 in the 1200 lab of the Institute for Genomic Biology at UIUC.

The microscope settings presented here are guidelines for basic introductory imaging and microscope use and to reproduce images comparable to what is presented in this protocol. All microscope settings may be adjusted based on differences in microscope hardware, experimental design needs, or personal preference.

Materials

- Disposable Hemacytometers, SKC, Inc. C-Chip™,Fisher Scientific 22-600-100
  C-ChipDHC-N01 ManualN.20.21-0525.pdf
- 1-10 ul pipette tips, sterile

Equipment

- 10 ul single-channel pipette
- Laminar flow hood or biological safety cabinet
- Axio A1 Imager Microscope and AxioCam HrC
  ZEISS-AxioScope-A1.pdf
- Axiovision SE64 Software
  AxioVision Users Guide.pdf

- Plastic hemacytometers can be disposed of in biohazard.
- Glass microscope slides can be disposed of in the appropriate sharps container.

Reserve the Axio A1 Imager in the UIUC IGB (IGB login required).

Prepare organisms

1 Sterilize a laminar flow hood or biological safety cabinet, pipettes, and gloves. Autoclave pipette tips. Sterilize the outside of the culture vial, especially the lid.

2 Gently rock culture vial to evenly suspend organisms. Using aseptic technique, pipette **10 µL** of culture into the hemacytometer. Depending on cell density, the culture may need to be diluted with the appropriate culture media before being transferred to hemacytometer.

Ostreococcus sp. are a non-motile organism. They can be transferred directly to the hemacytometer. If using this protocol for enumeration of a flagellated or motile organism, add an appropriate chemical fixative to a small aliquot of culture before

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transferring to the hemacytometer to impede organism movement during visualization.

Setup Microscope Controls

3 Turn the Axio A1 Microscope on using the power switch on the left side near the base of the instrument.

Power Switch for Axio A1 Imager

4 Both the TL and 3200k shutters on the microscope should be open (LEDs on). These switches are on the right side at the back of the scope.

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TL and 3200k shutters open.

5 Adjust the transmitted light brightness to high using the dial on the right side near the base of the instrument.

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Transmitted light brightness control knob.

6 For Kohler Dark-Field imaging, adjust the condenser below the stage to "D".

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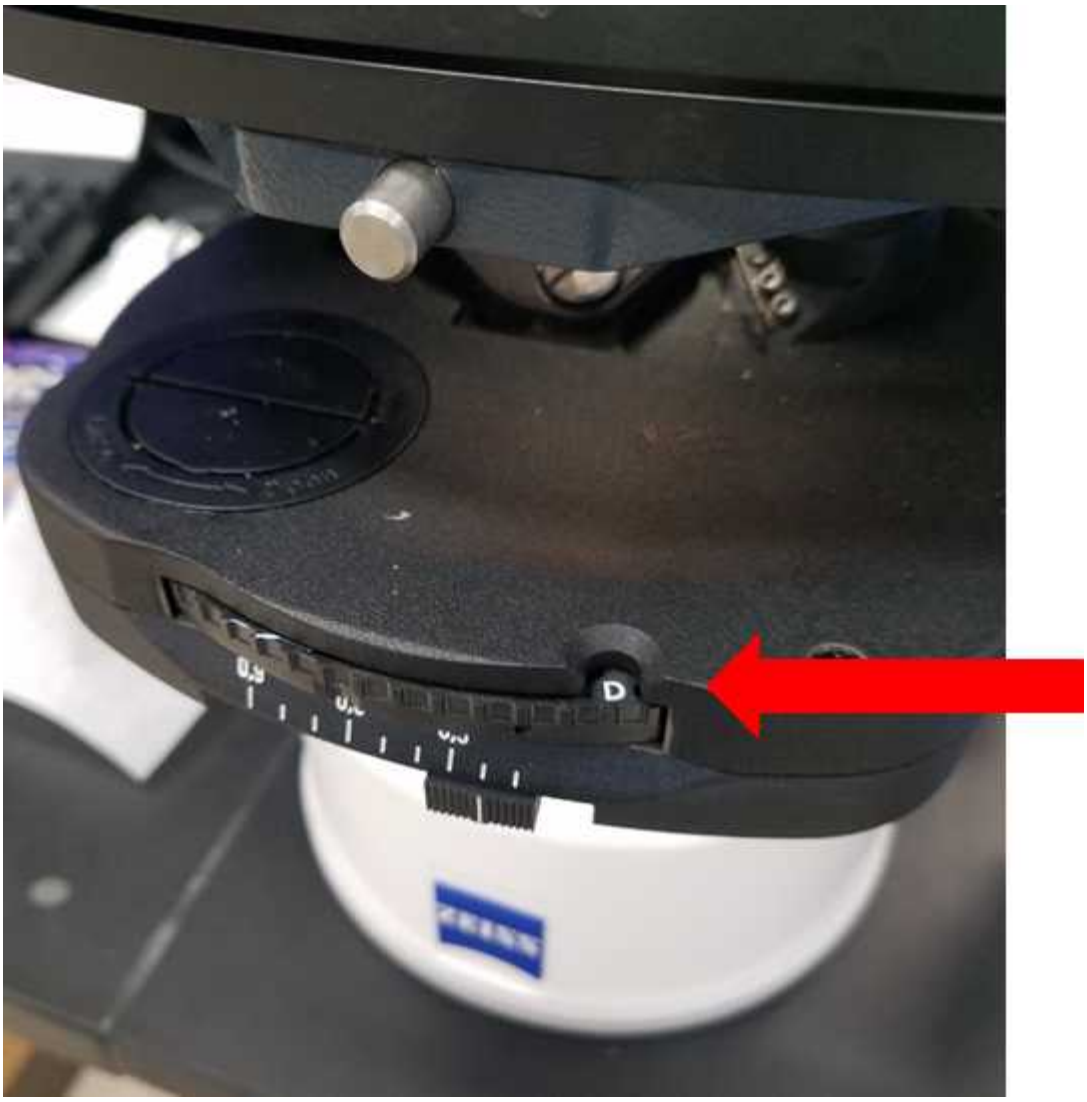

Condenser set to D for dark-field mode.

Dark field, works best with low numerical aperture (NA) objectives (10x, 20x and 40x).

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20X NA Objective in use.

7 Adjust the condenser light intensity using the slider below the condenser selection wheel. Smaller organisms are easier to see under lower light intensities (~0.3).

Condenser light intensity slider location.

8 The lever below the condenser light intensity slider controls an iris diaphragm that is used to focus the light beam. Ensure that this lever is pushed towards the back of the microscope and the full light field is visible.

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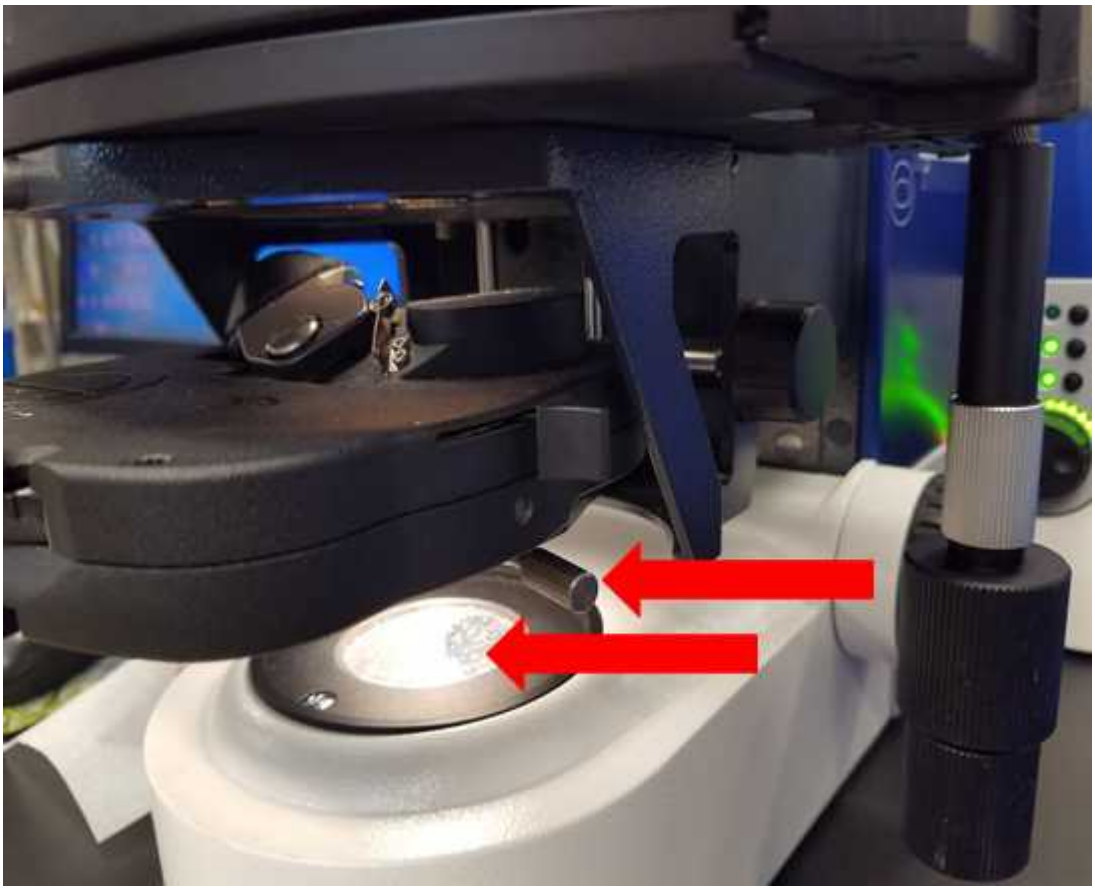

Iris diaphragm control lever and light field that is affected by iris diaphragm control.

9 Remove all items from the stage to avoid the possibility of scratching the objective when switching to a higher magnification if the stage is too close for the larger objective. Select the desired objective. Use the wheel to change objectives. Do not use the objective to turn the wheel or you could damage it.

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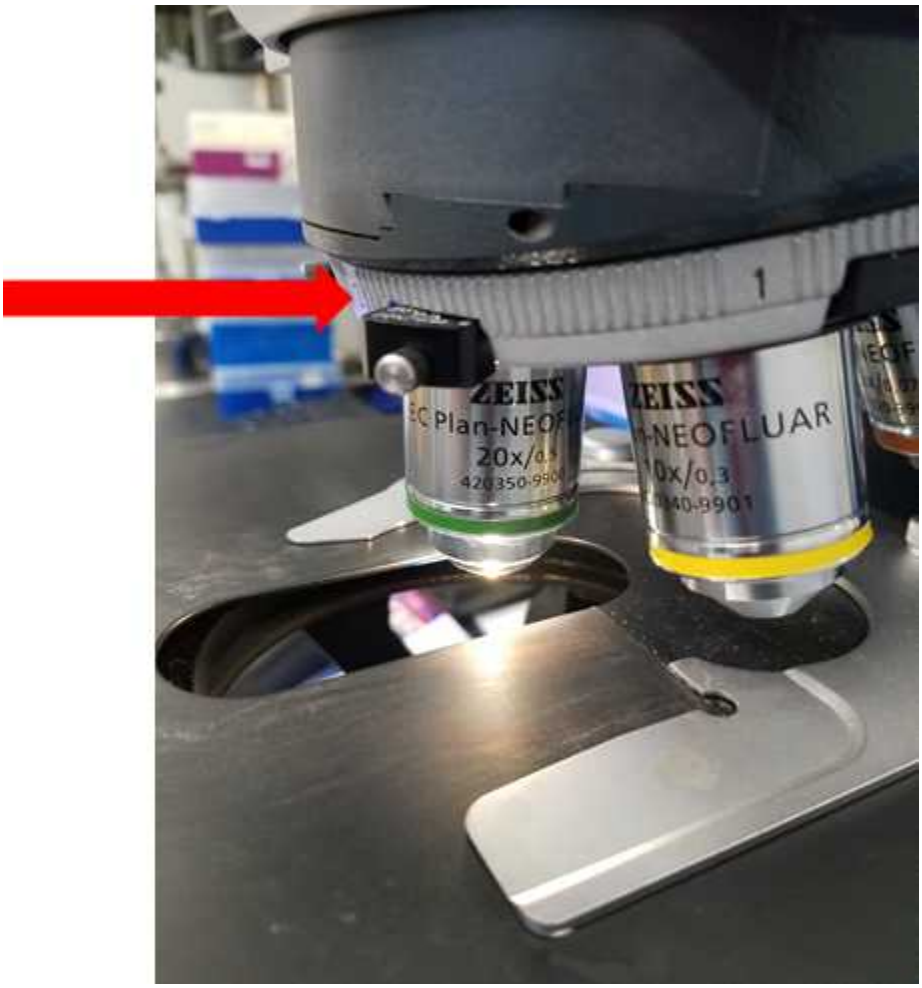

Wheel used to change objectives.

Ostreococcus can be visualized under 10X but appear as black dots. Under 20X the green nucleus surrounded by a clear membrane is visible. Under 40X both nucleus and outer cell membrane is clearer but enumeration requires more images for accuracy. Under 63X or higher magnification oil should be used on the slide.

Information about each objective can be found on the outside of the objective and as indicated by the color-coded ring on the objective.

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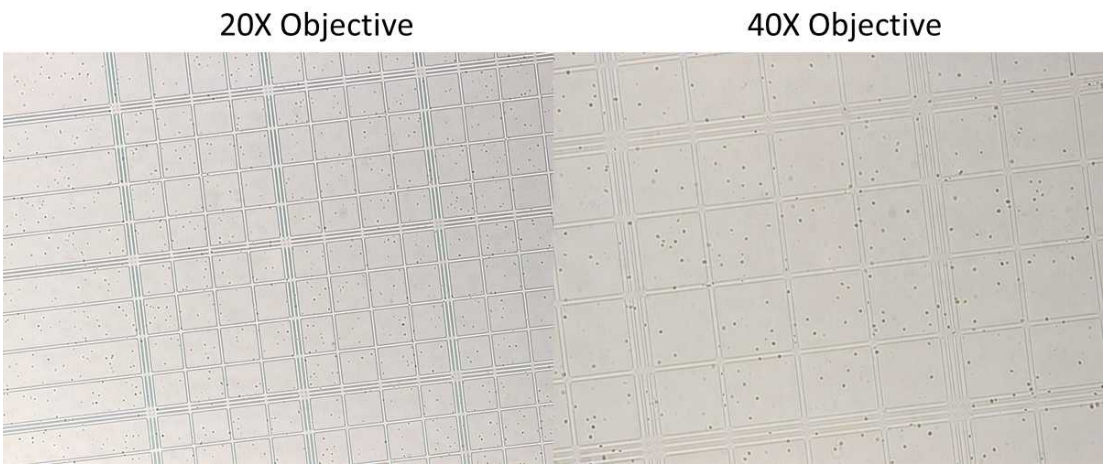

Ostreococcus tauri under 20X NA Objective vs 40X NA Objective using automatic gray-scale settings.

10 To visualize the live image on the computer screen using the AxioCam HrC the eyepiece control knob at the top of the microscope should be pulled to half or all the way out as indicated by the pictogram.

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Eyepiece or camera control knob in the camera only position.

11 Ensure the filter slider is pulled out to the second hole and is providing maximum visibility through the eyepiece or camera.

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Filter slider pulled out to second hole.

Activate AxioCam HrC Software

12 On the attached computer, double click on the AxioVision SE64 icon.

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Desktop icon for Axiovision SE64.

13 Click on the "Live" button to initiate live display of the AxioCam HrC of the stage of the microscope.

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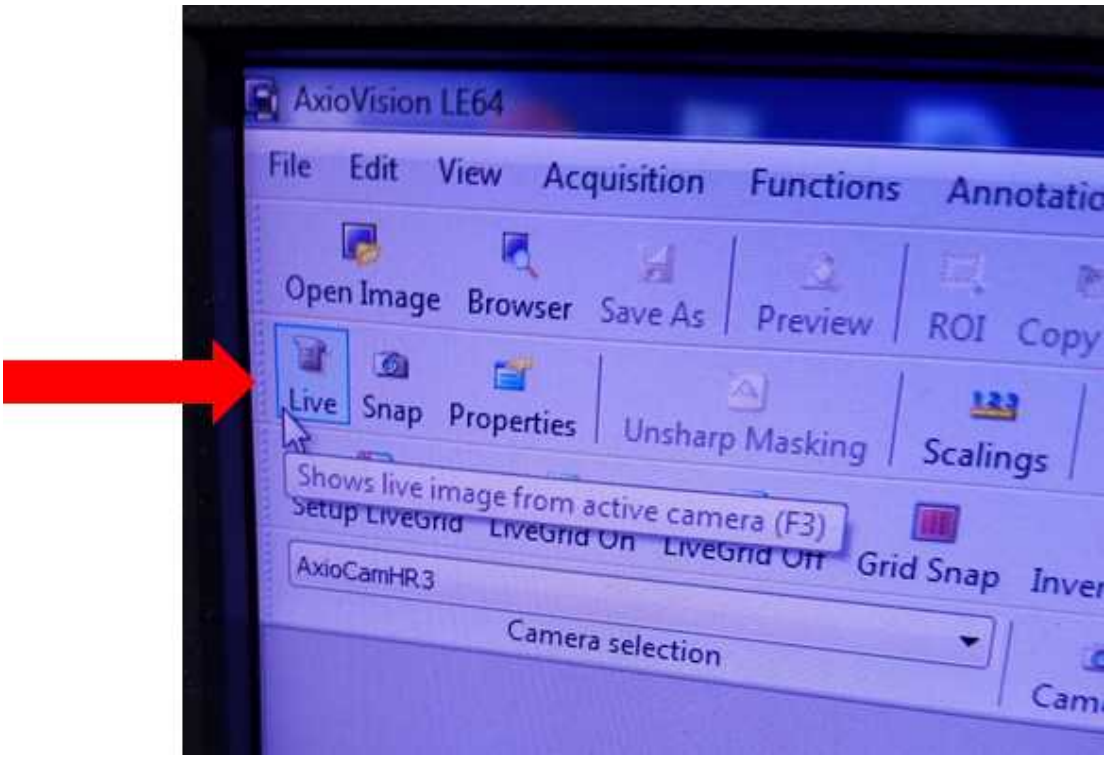

Camera live display activation button.

Focus Sample

14 Place the hemacytometer squarely in the stage clips.

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Stage clip location.

15 Use the knob on the left side just under the stage to raise and lower the stage until the stage is within a few centimeters of the objective.

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Stage height knob.

16 Use the knobs on the 90 degree handle on the right side of the stage to adjust the stage left, right, forward, and back until the center of your hemacytometer is located under the objective.

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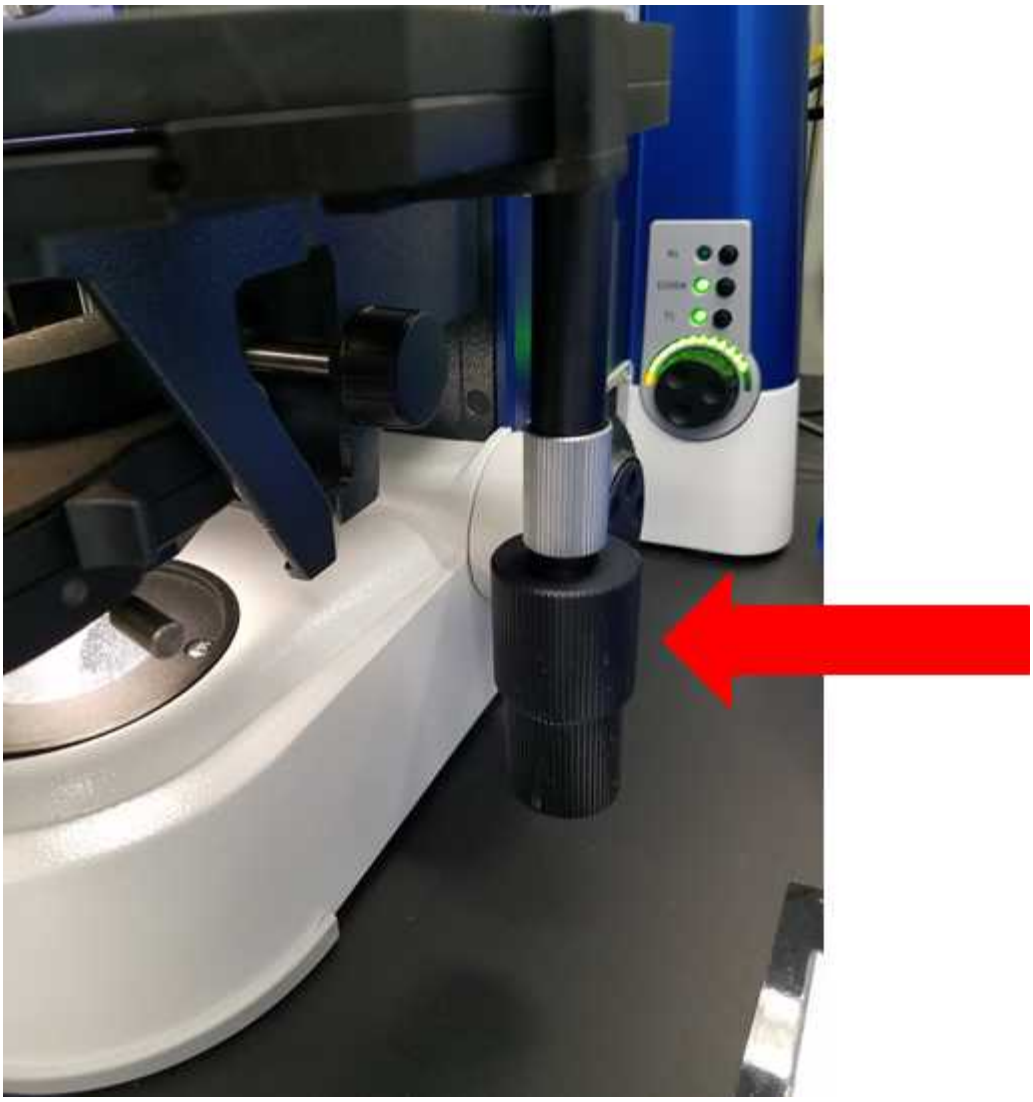

Stage directional control knobs.

17 If needed, adjust the intensity of the light using the control wheels on the right side of the microscope. These settings can also be affected through the image properties tab in the Axiovision software.

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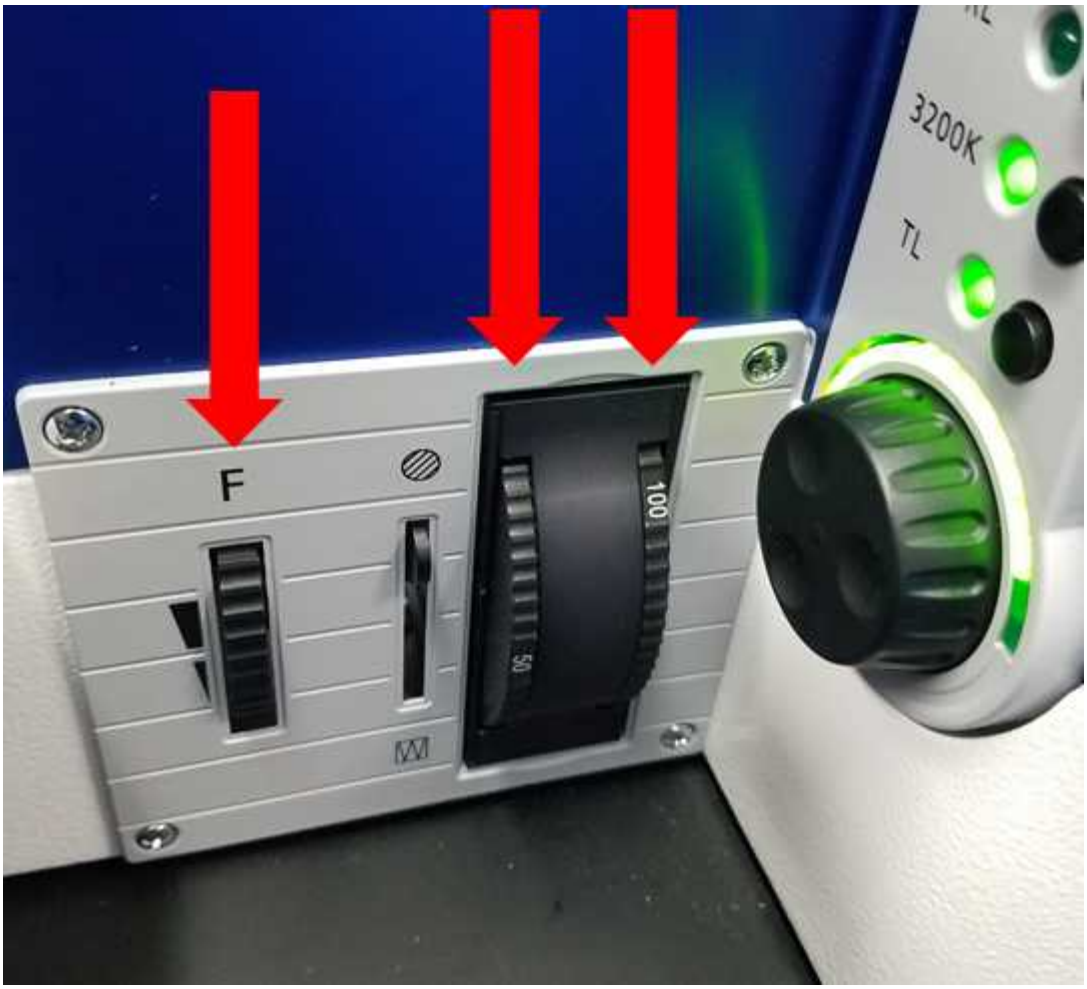

Light intensity control knobs.

18 Adjust the coarse focus using the larger outer ring on the control knob on the left side of the microscope.

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Coarse focus knob.

19 When the hemacytometer grid lines are visible, use the fine focus knob inside the coarse focus knob on the left side of the microscope until the organisms are visible.

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Fine focus knob.

20 Once the image on the desktop is in focus, color adjustments can be made in the software.

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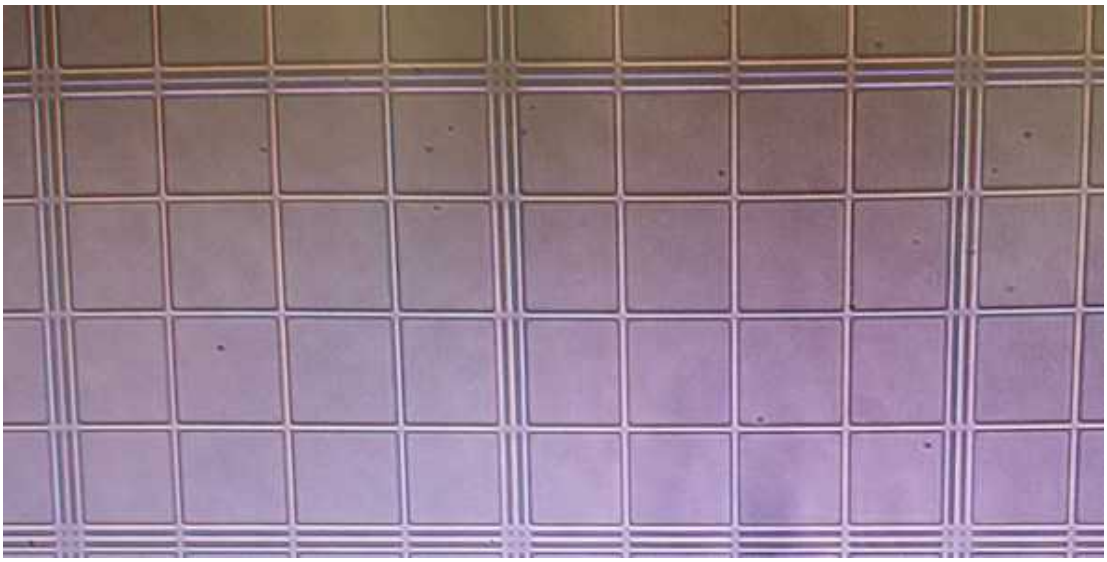

In focus hemacytometer with several individual O. tauri visible (20X).

Software image adjustments

21 If the hemacytometer is squarely in the stage and the image on the computer is still crooked, gently adjust the Axiocam HrC on top of the microscope until the image is square in the imaging frame.

Camera adjustment to straighten image.

22 If an image appears on the live screen that is not representative of your sample and does not move when you move the platform, an overlay may have been previously saved on the software. To remove the overlay image, right click in the live view and a new window of options will open, select “load factory defaults”.

23 For basic automatic grayscale and camera light exposure adjustments, click the black and white circle icon on the bottom toolbar on the software display.

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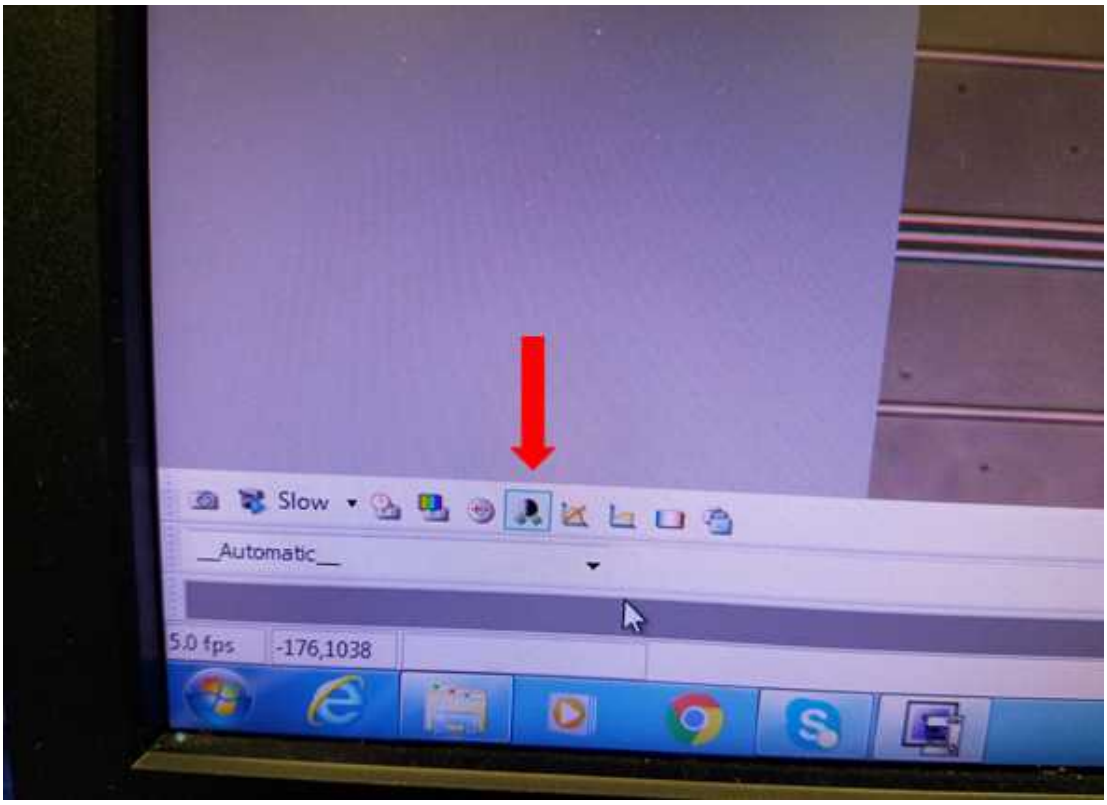

Automatic grayscale image adjustments.

24 For basic automatic color adjustments, click the graph icon on the bottom toolbar on the software display.

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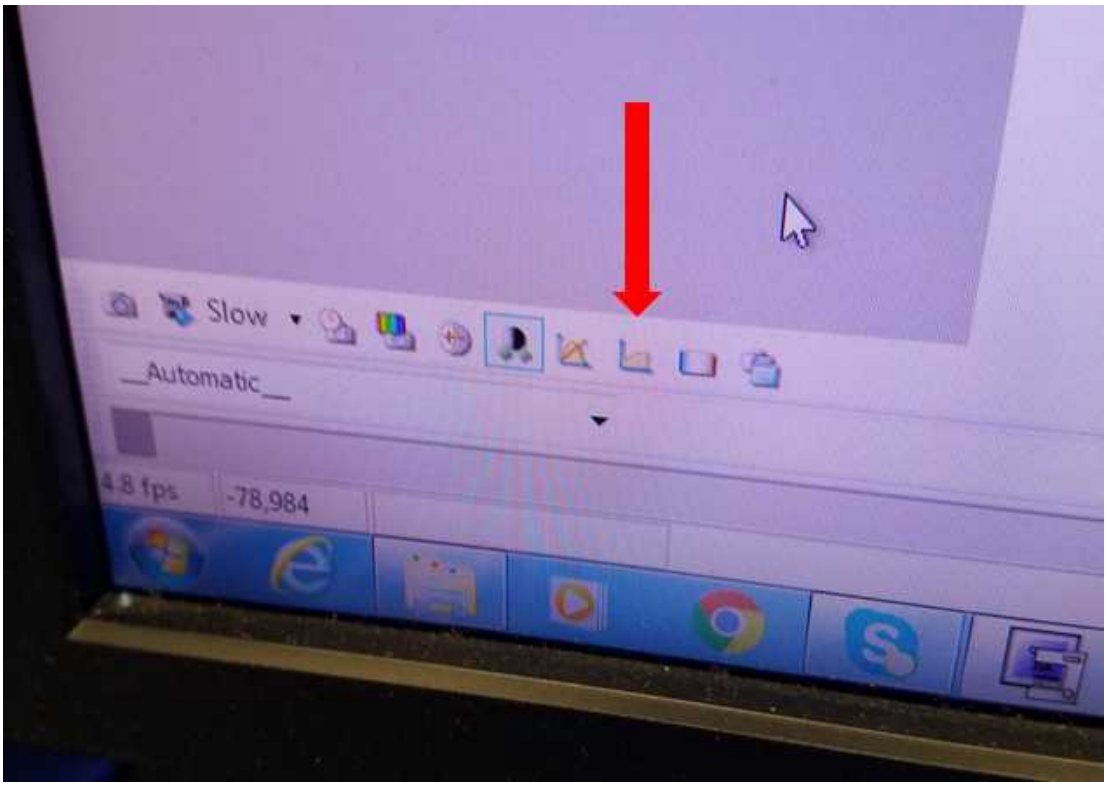
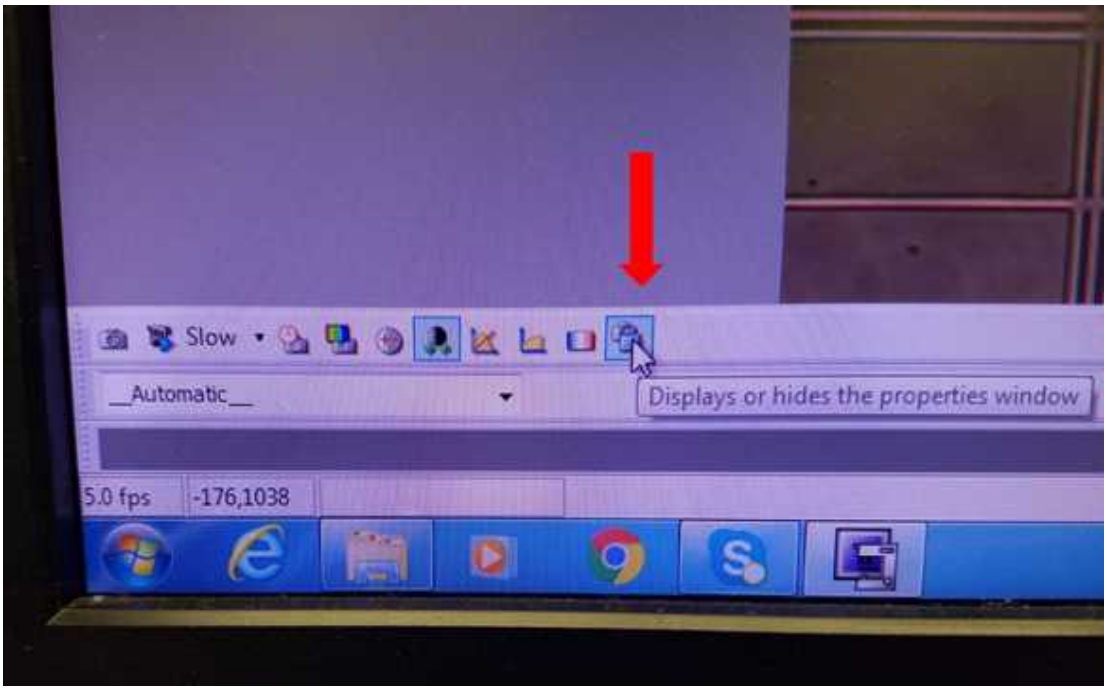


Automatic color image adjustments.

25 For customized camera color and light exposure adjustments, click the clipboard icon on the far right of the bottom toolbar on the software display. A new options menu with several tabs will appear.



Opens camera properties window.

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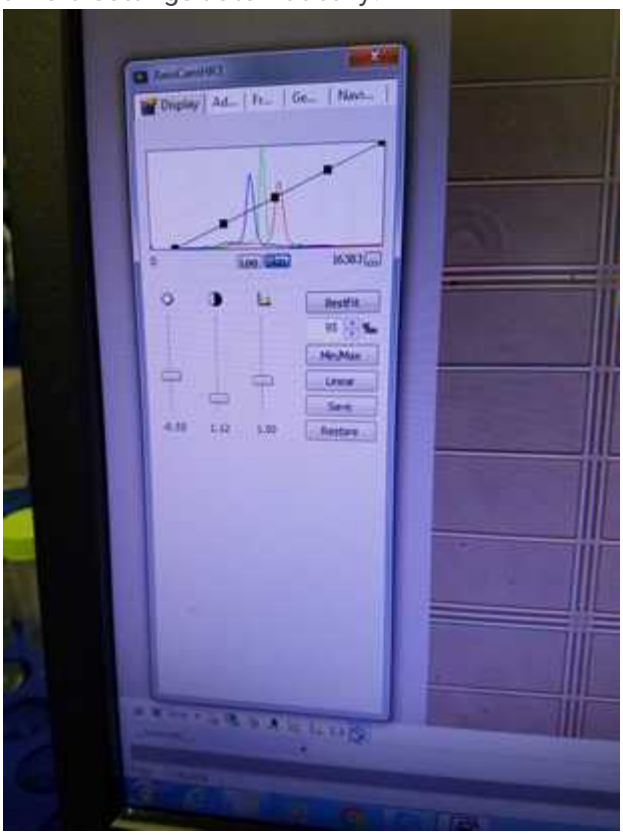
26 Adjusting the bars on the "Display" tab will immediately generate visible changes to the live image screen. Selecting "restore" button on the first settings tab, "Display", will reset all camera settings automatically.



27 On the second tab, "Adjust", sliding the tone slider to warmer or cooler changes the color hue of the image and may make the organisms easier to identify. Images presented in this protocol have Ostreococcus appearing as a black/green nucleus on an orange hued background with white hemacytometer grid lines. To recreate this color scheme, move the slider to "warmer".

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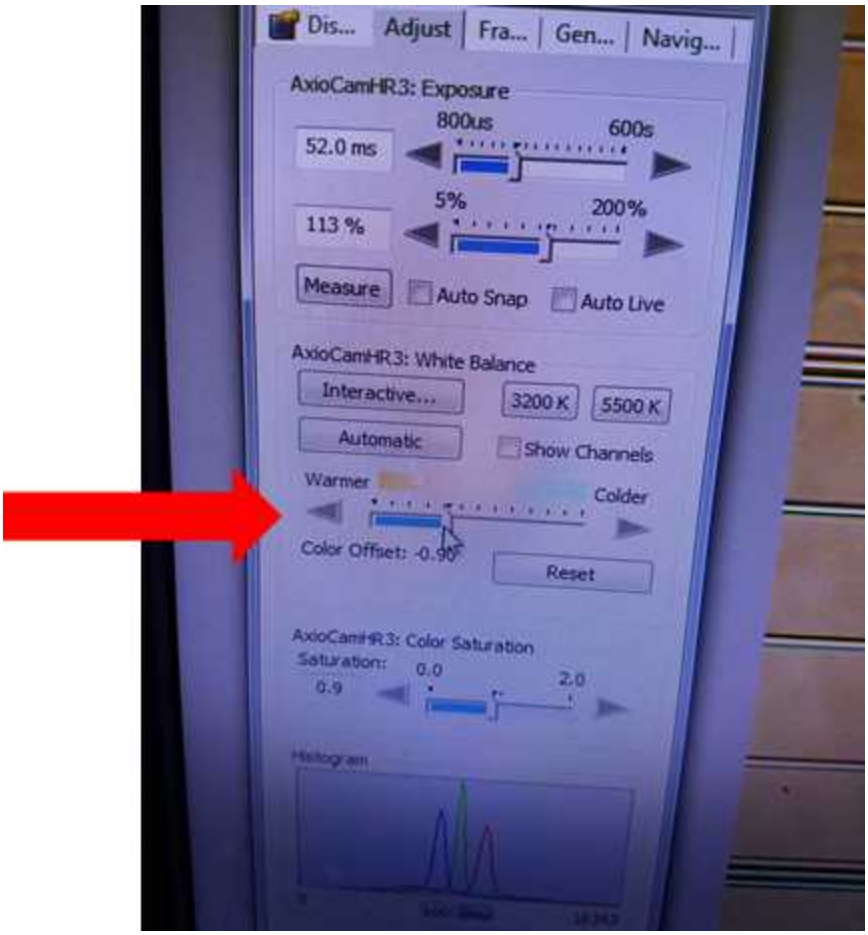

Image hue adjustment slider bar.

This color adjustment is not true to the sample and will not appear if the sample is visualized via the microscope eyepiece. It is forced by the camera to allow easier visualization in the visible light spectrum by the naked eye of the observer. The warmer or cooler color scheme is selected based solely on what is easiest for the individual researcher to see and is a personal preference.

28 Additional desired image adjustments can be made in the properties menu. For additional image options, please refer to the AxioVision manual provided in the Materials tab.

Image Capture

29 Capture a time-point image from the live image feed using either the "Snap" button with a camera icon in the second from the top toolbar or using the camera icon "acquisition" button in the bottom toolbar of the software.

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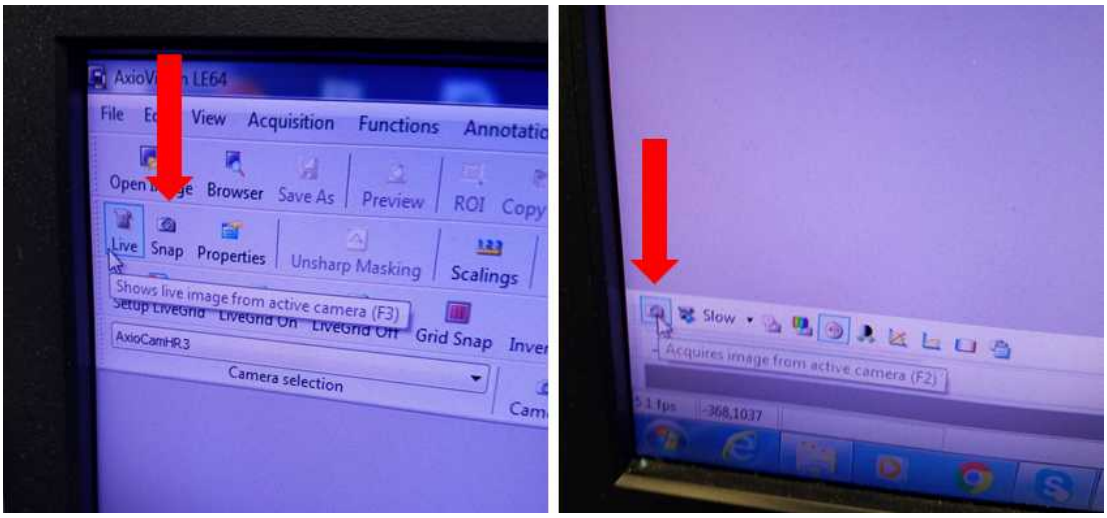
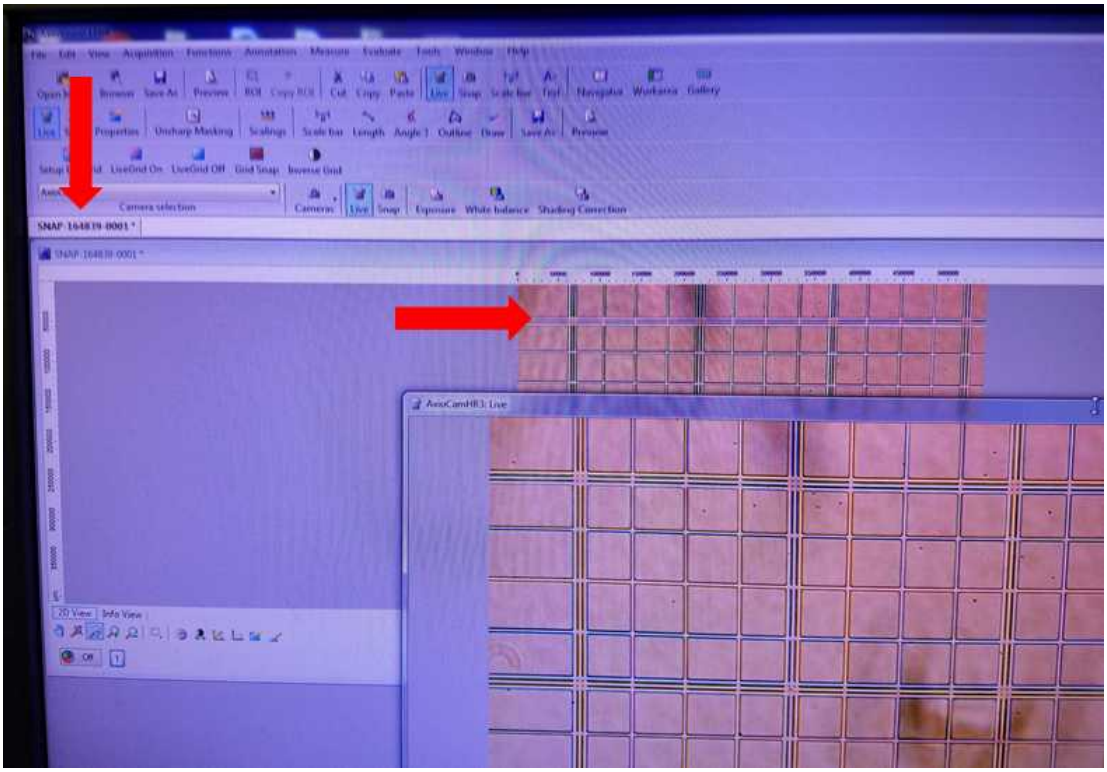


Time-point image capture command buttons.

30 Multiple static time-point images can be captured or static time-point images can be captured after moving the hemacytometer. They will appear as sequential numerated snapshot tabs on the display behind the live image display.



Captured time-point static images behind live image display.

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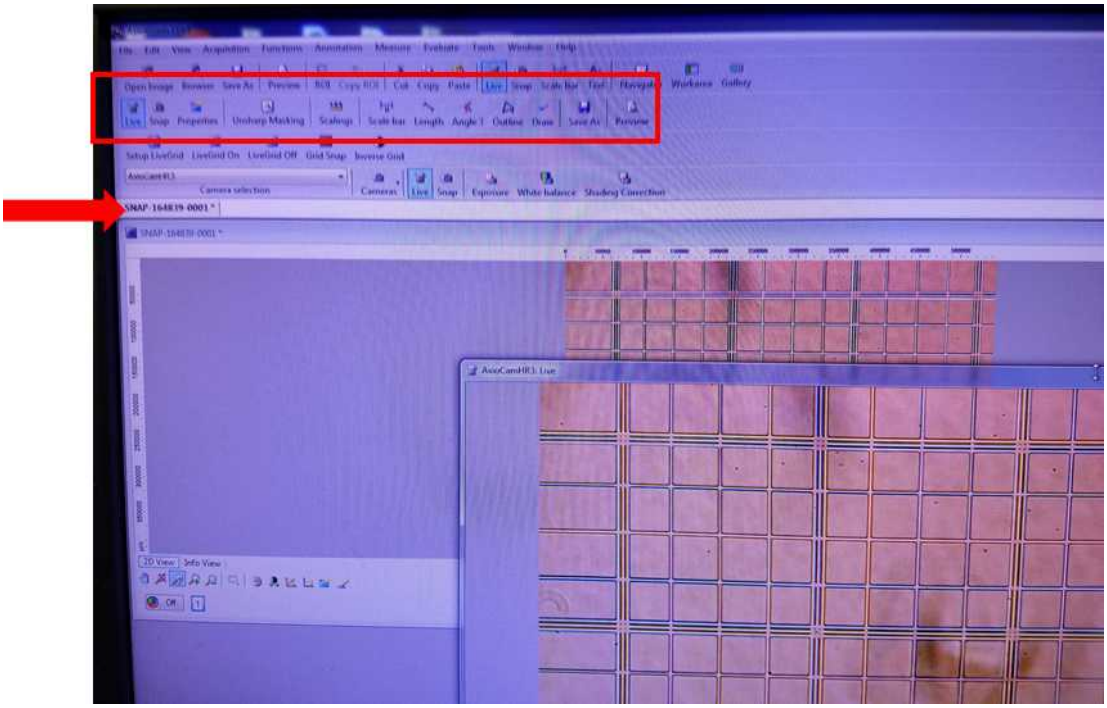
31 Click on the desired snapshot to perform image analysis in the software such as scaling, angle measurement, or freehand drawing. Image analysis can not be performed on live image. Additional information about post-processing image analysis can be found in the AxioVision manual.



Snapshot image post-processing options.

32 Click on the desired snapshot and then highlight the "Save As" disc icon in the second from the top toolbar in the software to save the individual snapshot as an individual photo file.

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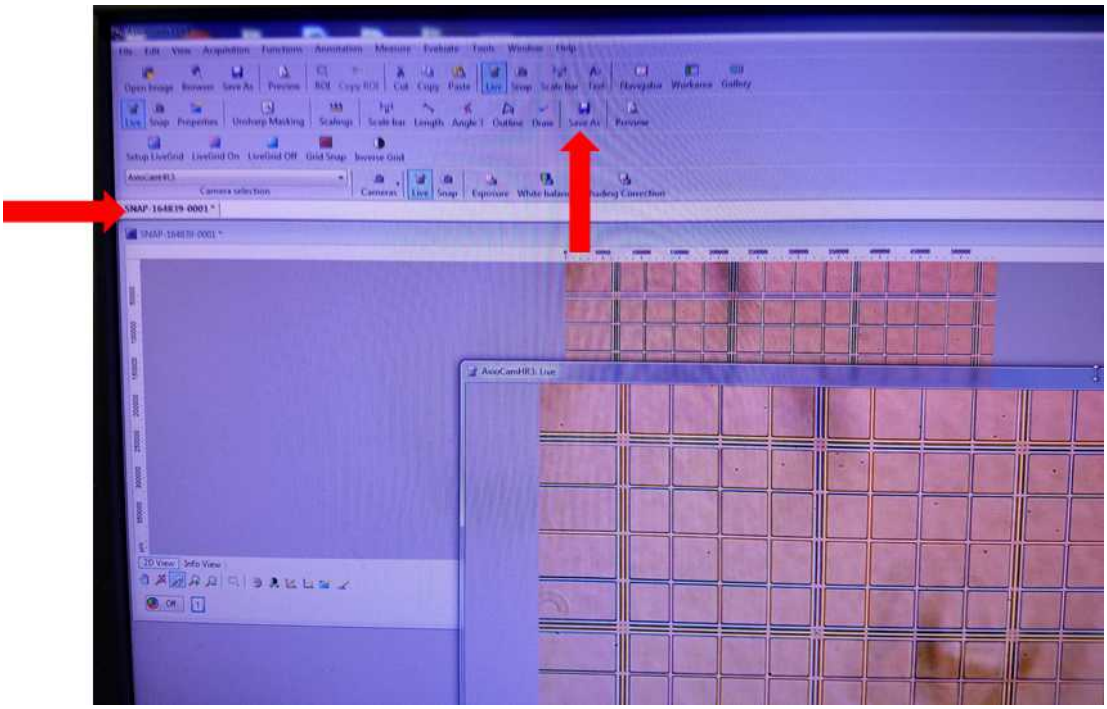

Save as icon for snapshot image.

33 The individual photo files are now able to be transferred to other analysis software such as ImageJ with Cell Counter Add-In for enumeration.

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