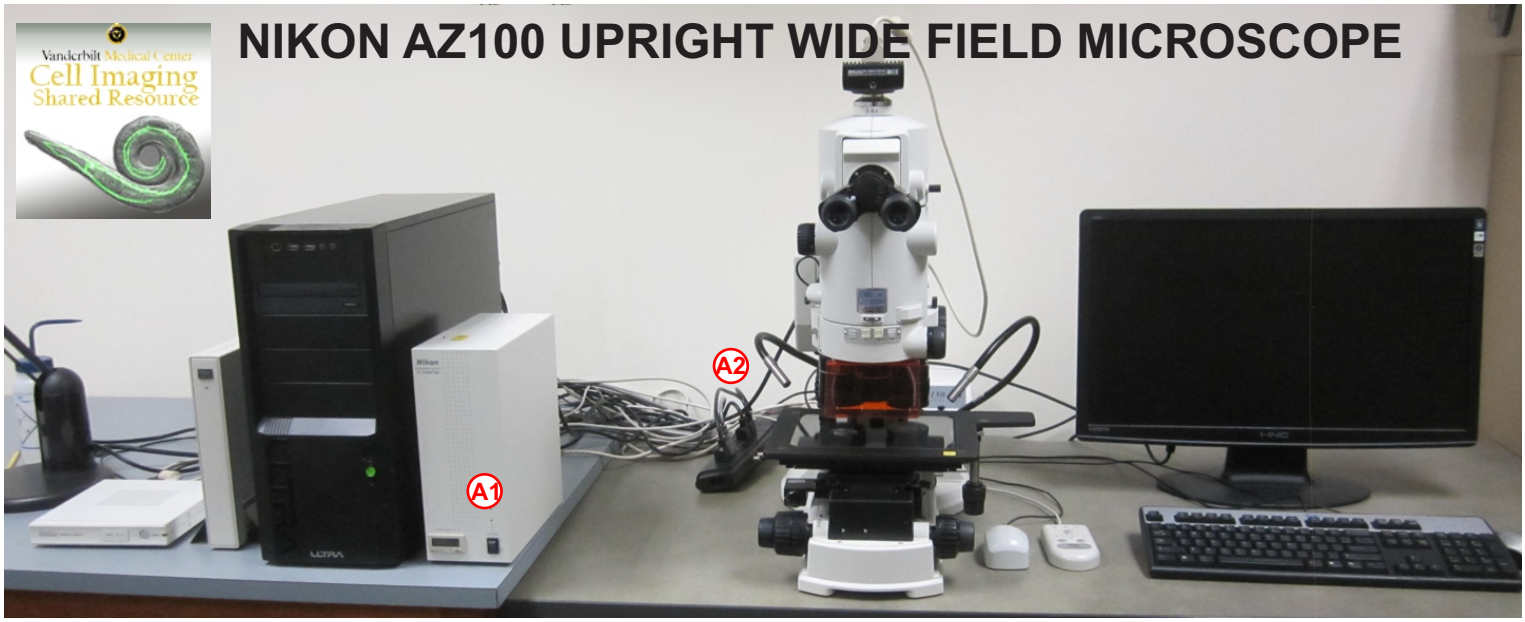
NIKON AZ100 UPRIGHT WIDE FIELD MICROSCOPE



A. GETTING STARTED

1. Record your name, your PI's name and your start time in the log book.

2. For viewing with transmitted light only, skip to step 5.

3. If the Hg lamp is not already on, check the logbook to confirm that the lamp has been off for at least 15 minutes; otherwise wait until the lamp is cool, at least 15 minutes since it was shut off.

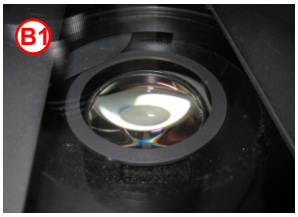
4. Turn on the Hg lamp using the switch on the "**Intensilight**" A1 box to the **left** of the microscope. This should be turned on BEFORE the microscope to avoid damage to the electronics.

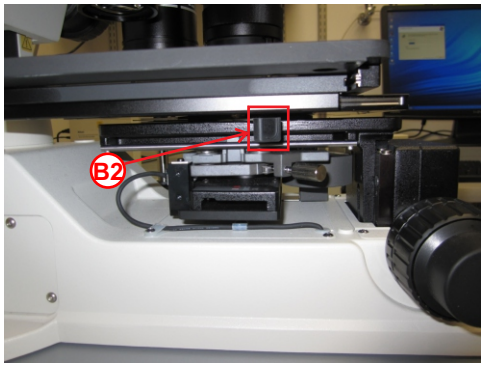
5. Switch on the **power strip** A2 on the wall to the left of the microscope. This turns on the halogen lamp, the microscope, the camera controller, and the monitor.

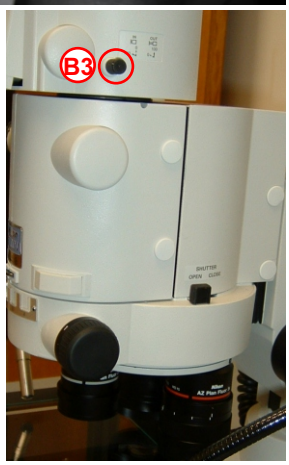
7. Turn on the computer.

8.Logon to Windows using your VUnetID and your password.

The Hg lamp should not be cycled quickly; let it equilibrate for at least 15 minutes before turning it off again, and let it cool for at least 15 minutes before turning on again. It is essential for the Hg lamp to COOL DOWN before it is fired again or it may be damaged.







B. USING THE TRANSMITTED LIGHT MODE

1. Open the **transmitted light shutter** B1 using the **slider** B2 below the stage on the left side of the microscope.

2. Push the **side beam splitter** B3 **IN** to direct light to the oculars.

3. Select the appropriate objective. The objective choices are:

Plan Apo	**0.5x**	**.05 NA**
Plan FL	**2x**	**.2 NA**
Plan FL	**5x**	**.5 NA**

Please note that while the 2x and 5x are *roughly* parfocal, the 0.5x is not.

Change the objective by gently rotating the nosepiece B4 in either direction.

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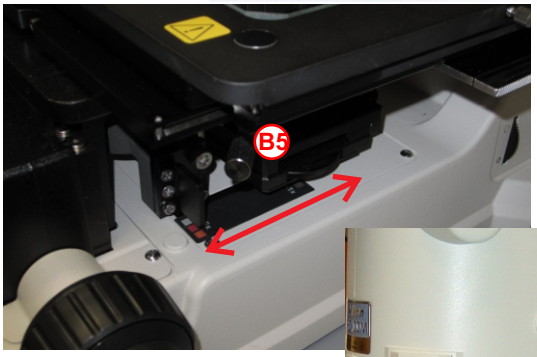
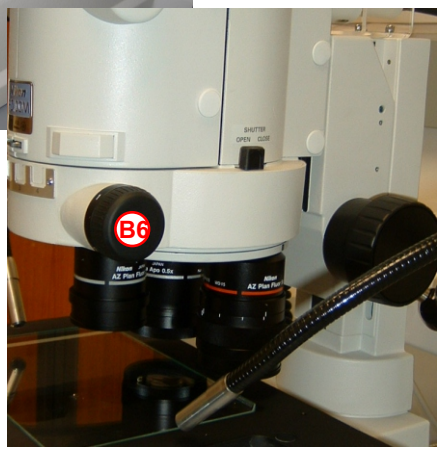
4. Slide the **condensor** B5 into the correct position for the chosen lens: forward for the 2x and 5x, back for the 0.5x.



5. Move the **filter wheel** B6 to position **1** for transmitted light viewing.

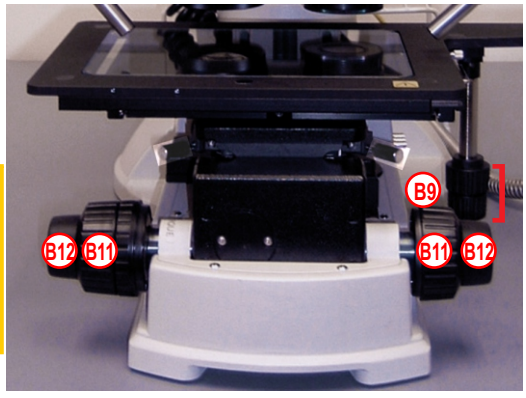


6. The light intensity on the **Fiber Illuminator** B7 has been set to produce the whitest light. Do not adjust it. Instead, use **Neutral Density Filters** B8 on the right rear of the microscope singly or in combination to adjust the brightness.

8. Move the specimen field of interest into view using the **stage movement knobs** B9.

B9

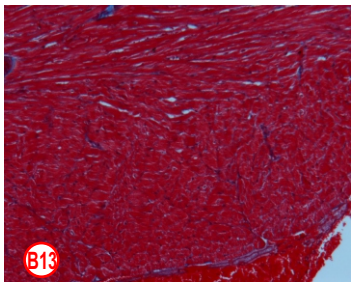
9. For focusing when Nikon Elements software is NOT controlling the microscope, first set the appropriate working distance for the chosen lens using the focus/photo button while simultaneously holding in the coarse button on the **hand controller** B10. Continue focusing using either the focus/photo button without the coarse button or the coarse B11 or fine B12 adjustment knobs below the stage. After the software is open, the hand controller will no longer work.



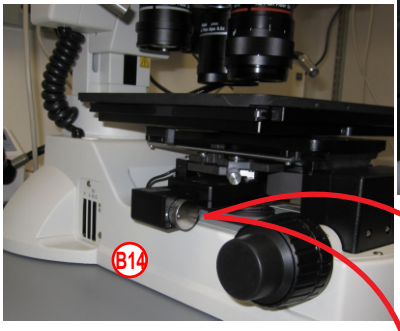
You may find it useful to begin focusing with the stage in the middle position along the z-axis. That will allow you to manually fine-focus after the software is open.

10. Köhler illuminate. The purpose of Köhler Illumination (also called “alignment”) is to create the brightest and most even light in the focal plane for optimal imaging with transmitted light.

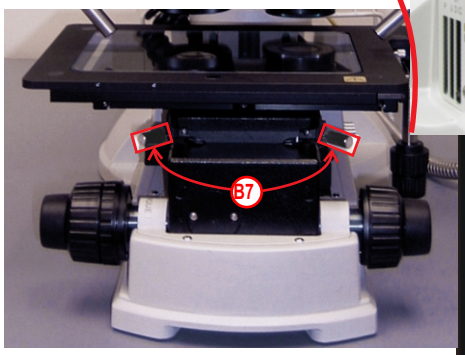
a) Using transmitted light and either the 2x or 5x lens, bring the specimen into focus B13 . (Köhler alignment is not necessary with the 0.5x lens.)

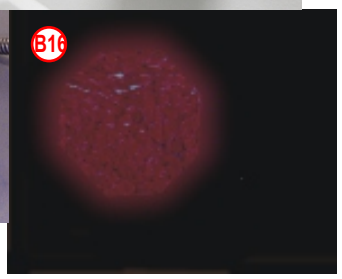


B) Make sure the **Oblique Illumination Controller (OIC)** B14 is in the open position (white dot to white dot.)

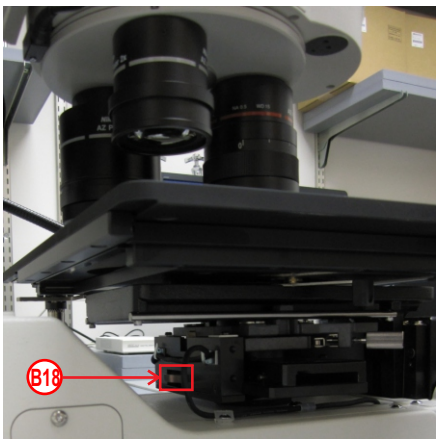


b) Close the field iris B15 on the right rear of the microscope until only a small spot of light B16 remains. (Depending on how out-of-focus the condensor is, the spot may not be very small.) If you can’t see a small spot of light, move the condensor using the centration knobs B17 .

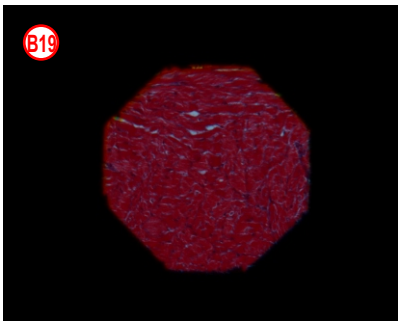




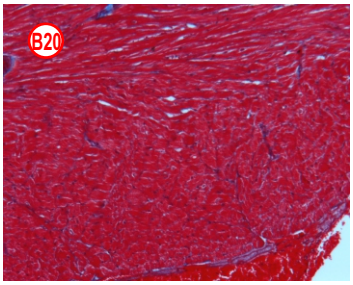
c) Adjust the condenser wheel B18 below and to the back of the stage until the polygon is in focus (edges as sharp as possible.)



d) Adjust the centration knobs to center the polygon B19 .

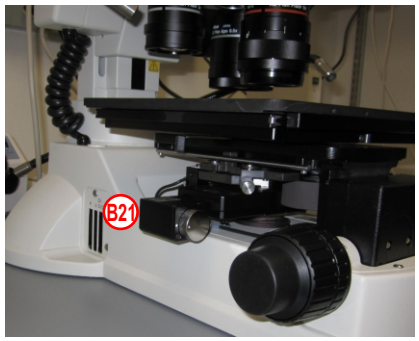


e) Open the iris just until the field of view is filled with light B20 .



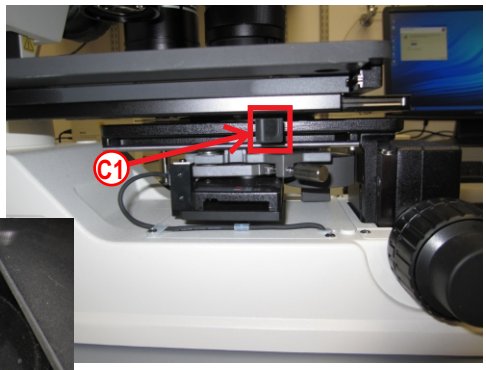
11. The Oblique Illumination Controller (OIC) B21 can be used to increase contrast in samples such as cells in culture that have too little contrast to be seen easily. Turn the knob on the OIC either direction to angle the transmitted light to enhance contrast.

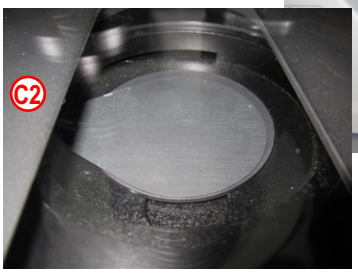
12. For brightfield image acquisition, skip to section D.



C. USING THE FLUORESCENCE MODE

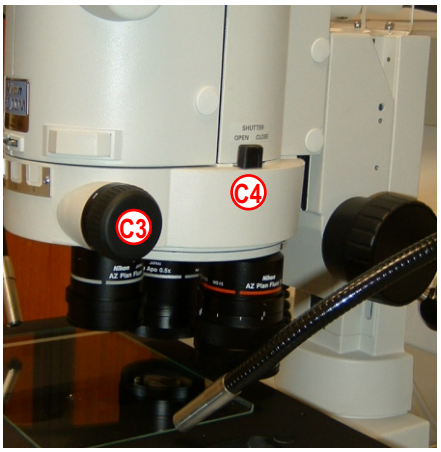
1. Using the **slider** C1 below the stage on the left side of the microscope, close the **transmitted light shutter** C2**.**





2. Select the appropriate fluorescence filter C3. The positions are:

1 = Transmitted/white light
2 = UV excited, Blue emission - DAPI.
3 = Blue excited, Green emission - GFP, FITC, Alexafluor 488.
4 = Green excited, Red emission - Cy3, rhodamine.
Also available: Near infrared excitation and emission: Cy5, TOPRO3.

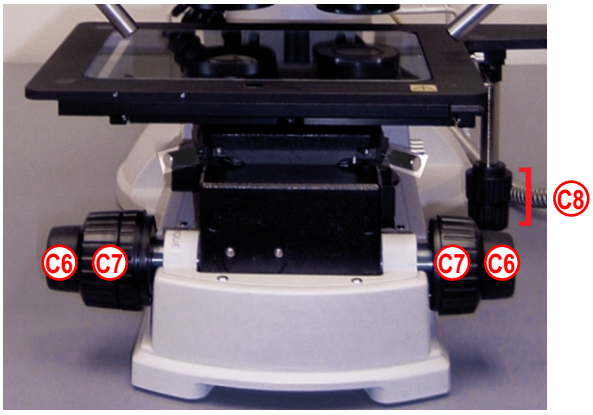


3.Make sure the manual fluorescence shutter C4 is in the open position.

4. Open and close the electronic mercury lamp shutter by pressing the **shutter** button on the **HG Controller** C5. For brightest excitation, the LED under "1" should be lit.

5. For focusing, use the **fine** C6 **or coarse** C7 **focus knobs** on either side of the scope.

6. Move the specimen field of interest into view using the **stage movement knobs** C8.



Remember to close the shutter as soon as possible to prevent fluorophor bleaching.

D. CAPTURING SINGLE BRIGHTFIELD IMAGES WITH NIS ELEMENTS

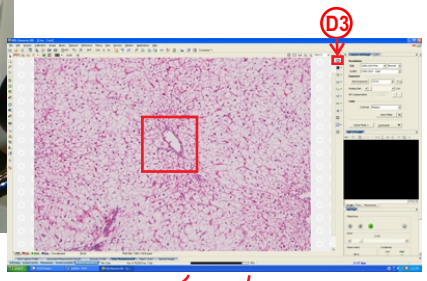
1. Double click on the NIS-Elements icon on the computer desktop.

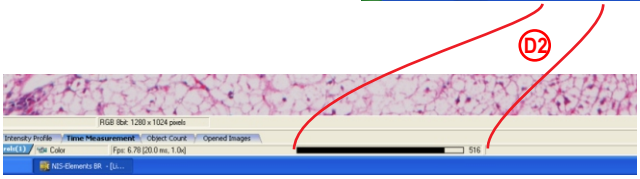
2. Choose the correct camera (usually the color camera, DS-Ri1.) The microscope head will then automatically move up and down to calibrate.

3. Right-click in the gray docking pane on the right of the screen to open any menus of tools you may need that are not displayed.

4. Pull the **side beam splitter rod** D1 OUT to direct the image to the camera.

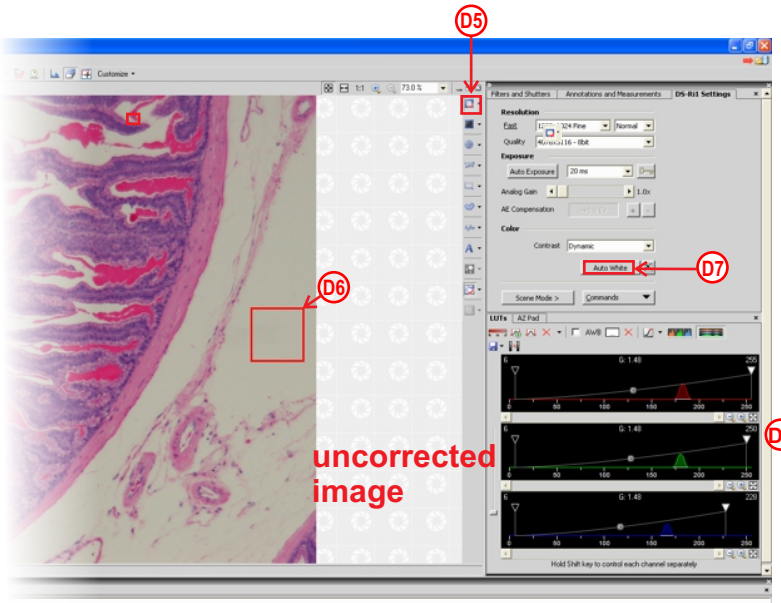
5. Click on the green arrow on the Elements main toolbar to see a live image. Adjust the focus if necessary. The black line D2 at the bottom of the image window indicates the sharpness of focus; the higher the number, the sharper the focus. Use the probe tool D3 to select the area where focus is most critical; with the probe tool active, the black bar refers to the focus within that square. Deselect the probe tool before continuing.





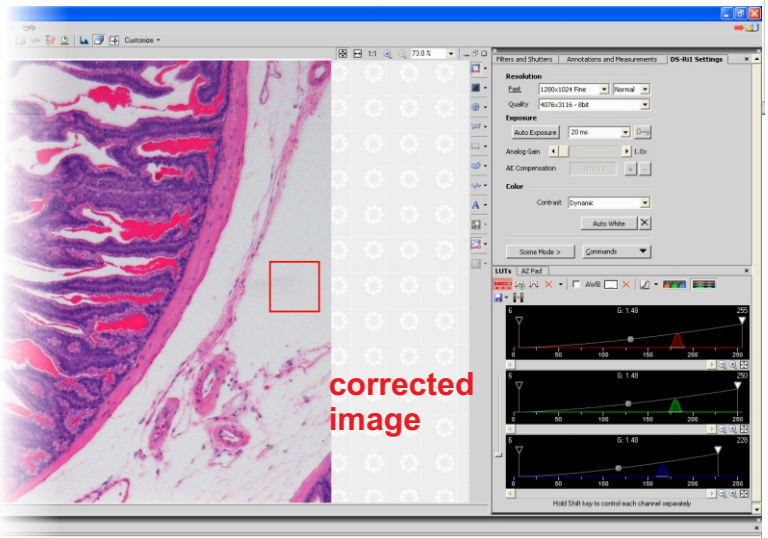
6. Adjust the exposure time to achieve the desired intensity.

For a gray-scale image, balance the ND filters and exposure length so that the histogram D4 peaks around 50%. (However, if you will be overlaying with a fluorescent image, you may prefer a slightly darker gray-scale channel.)



7. Correct the color balance in a bright field image by clicking on the probe D5. Move the probe D6 to an empty area on your dish or slide.

8. Select **Auto White** D7, wait for the background to change to gray, and capture the image.



9. If this image is satisfactory, skip to step 11.

10. To correct uneven illumination (e.g. vignetting due to a low zoom factor), capture a shading image which will be subtracted from the image of your sample. Move to an area of your slide or dish where there is no sample, or, if that is impossible, remove the dish or sample.

11. Click **Acquire**,
Background Correction,
Capture Correction Image,
Multiplicative.

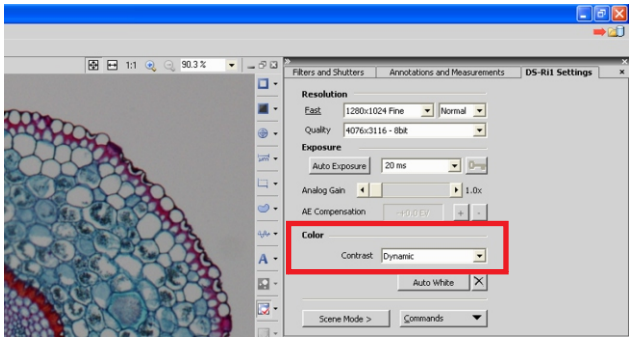
12. Capture the correction image by clicking **Next**, then **Finish**.

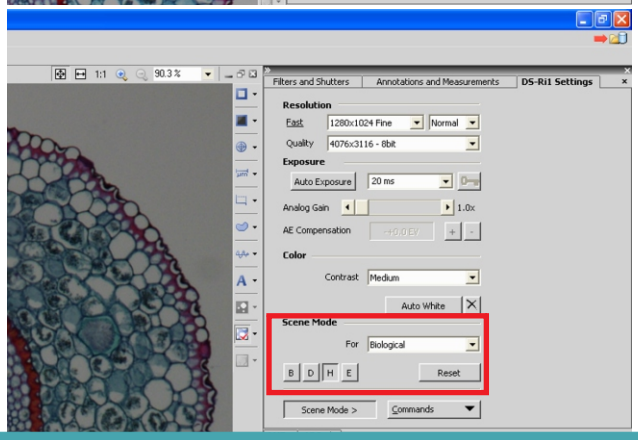
13. Return to your sample and capture a corrected image by clicking on the plain camera icon near the top of the window.

14. Save your image as a JPEG2000 or TIFF to preserve the original data and settings.

15. There are several other options for optimizing a bright field image in NIS-Elements. Under **Color** there is a drop-down menu of different contrast modes. In combination with exposure times, one of these modes may improve the image.

16. Scene Mode offers four color balance options designed to enhance the color difference in stains like H & E or HRP.





E. SINGLE CHANNEL FLUORESCENT IMAGING

1. Follow the instructions in Section C. for viewing your sample in fluorescent mode.

2. Pull the beam splitter rod out to direct light to the camera.

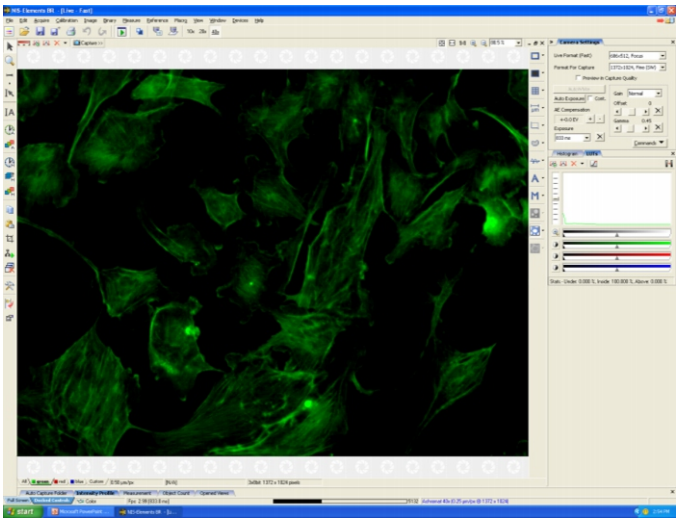
3. Click the Live button to view your sample on the monitor.

4. Adjust the exposure time and fine-tune the focus.

5. Click the stop button to end Live viewing.

6. Click on the plain camera icon to capture the image.

7. Save as a TIFF or JPEG2000.



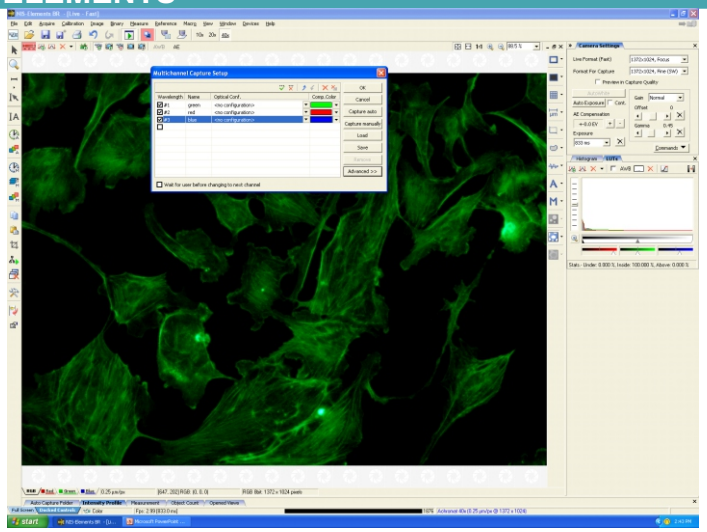
F. CAPTURING MULTICHANNEL IMAGES WITH NIS ELEMENTS

1. Before capturing multichannel images, switch the color camera to “emulate mono.” Click on:

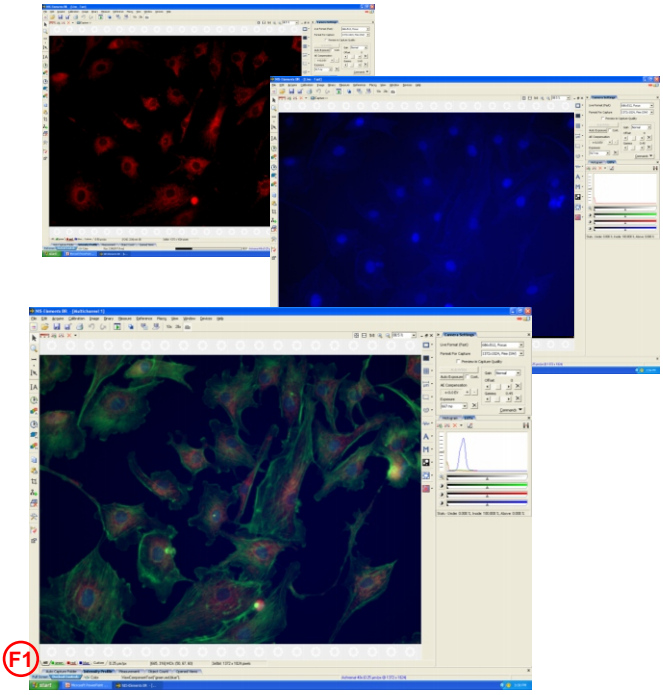
Acquire
Select Nikon DS-U2/ LS Ri1
emulated mono
Close

2. For capturing multichannel images (e.g., OIC or brightfield plus fluorescence, samples with multiple fluorescent labels,) click on:

Acquire (on the main tool bar)
Capture Multichannel Image
Multichannel Setup.



3. In the **Multichannel Capture Setup** window identify the desired channels *in the order you want to capture them*, and select the preferred color for display. Choose “no configuration” under “Optical conf.” If OIC will be one of your channels, select white (not brightfield) as the display color*. Click the button **Capture Manually**. You will only have to setup once unless you change your conditions.



4. Select the appropriate filter, open the fluorescent shutter, adjust exposure time for the desired intensity, then select **Capture**. (The default for multichannel viewing is gray-scale; if you prefer to see channels in color, right-click the channel’s tab F1 and select “View channel in color.”)

*If your purpose is to image a visible stain *and* a fluorescent dye, it may be appropriate to choose “brightfield” as the display color for the transmitted light image.

5. After a delay for capture, the image will change color to the next channel. Change the filter appropriately, adjust the focus and exposure time, and capture the second channel.

6. Repeat as necessary. When all channels have been acquired, select **Finish**. You will have separate images of each channel and a composite image of the overlay. Save as an Nd2 file to preserve the acquisition settings as well as the image. The file will include each channel as well as the overlay.

7. To acquire another multichannel image using the same channels, click the Acquire Multichannel Manually icon  on the left toolbar.

8.The Nd2 file format maintains multiple layers in multichannel images, stores the acquisition settings, and can be opened by some analysis programs such as ImageJ. However, to save files that are compatible with PowerPoint or other general use programs, you may want to export Nd2 files. To use other features of the Nikon AZ100M microscope or Nikon Elements software such as time lapse or image tiling, please ask a CISR staff member.

G. SHUTTING DOWN

1. Check the online calendar to see whether another user is expected within an hour.

IF YES:

2. Simply exit the software, log out of the computer, and sign out in the log book.

IF NO:

3. Exit Nikon Elements software.

4. Shut down the computer.

5. Remove your samples.

6. Turn off the Intensilight G1.

7. Turn off the power strip G2.

8. Record your time in the log book.

To download the free NIH Elements Viewer, go to http://www.nikoninstruments.com/Products/Software/NIS-Elements-Advanced-Research/NIS-Elements-Viewer

Acknowledgment:

Our funding depends upon your citing the Cell Imaging Shared Resource when you publish data obtained with equipment or services from CISR. This would include images from the microscopes, training in the use of software, prints from the Codonics, etc. The minimum acceptable acknowledgment should read:

"Experiments/Data analysis/presentation [include what you use]" were performed in part through the use of the VUMC Cell Imaging Shared Resource, (supported by NIH grants CA68485, DK20593, DK58404, HD15052, and Dk59637).

Please let us know when you publish and, if possible, send us a reprint of the paper. This is how we justify our existence.