Color Image Capture - AZCC Image Analysis Workstation

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SPECIFIC FOR: The Compix SimplePCI workstation located in the Arizona Cancer Center, room 3964. The installed software on this system is currently at SimplePCI version 5.3 (C Imaging, by Compix). The microscope is a Nikon E400 and the camera is a Diagnostic Instruments SPOT Jr color CCD camera.

APPLICATION: This handout is to assist users in setting up the microscope for color brightfield digital image capture.

Accessing and scheduling the workstation:

- This system does not have an on-line calendar. It is a "first come, first served" instrument.
- The Cancer Center is a controlled access building. For after-hours use of this workstation you will need CatCard access to the building (with a PIN number) and for the door to the room. Contact Elik Essif (626-6516) regarding access.
- Currently there are no fees are associated with the use of this workstation. We do ask that users sign in on the log book that is available on the table. The log book will allow us to demonstrate that the system has been used regularly when we need to find money for upgrades.
- Limited technical support is provided to the general University community by the author of this document. Please note, the author's primary responsibility is to the investigators of the Southwest Environmental Health Sciences Center.

Starting things up:

1. Turn on the computer, the two LCD monitors (button located in the lower right corner of both monitors), the microscope (right side, green switch near the back) and the SPOT camera controller (black & white box located on the shelf above the system).
2. When the computer has finished booting up, select the SimplePCI user login and use the password (posted on a piece of paper on site).
3. Once Windows XP has finished loading, launch the SimplePCI program from the desktop.

Setting up the Nikon microscope for brightfield (transmitted light) imaging:

NOTE: this microscope has the optics for Phase contrast imaging. Phase contrast can be useful for looking at unstained sections or cells that have only specific areas/cells stained (for example, using immuno-histochemical stains like DAB), but do not have a strong counterstain (e.g., methyl green, haematoxylin). The phase contrast images don't always photograph very nicely, but phase contrast is useful for determining where you are in a lightly stained or unstained sample. The advantage being that, for image analysis, you can capture and analyze the specifically stained areas without working so hard to exclude the non-specific counterstain from your analysis. Contact Doug Cromey for assistance with setting up this microscope for phase contrast imaging.

1. The Nikon microscope has the following objective lenses:

Objective	NA	Color Ring	Notes
4X PlanFluor	0.13	RED	
10X PlanFluor	0.30	YELLOW	
20X PlanFluor	0.50	GREEN	
40X PlanFluor	0.75	BLUE	Dry lens!
100X PlanFluor	1.30	WHITE	Oil immersion

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2. There is a slider up near the eyepieces (red arrow) that sends the light either to the camera or the eyepieces. There are three positions:

   - Pushed all the way in sends all the light to the eyepieces.
   - The middle setting sends some light to both the camera and the eyepieces. This setting should not be used for capturing images.
   - Pulled all the way out sends all the light to the camera.

   Please be gentle moving this slider into position, it controls optical elements that can become misaligned.

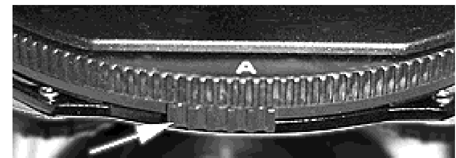
3. You can adjust the tungsten lamp intensity with the black rheostat knob that is located on the front left side of the microscope, near the base of the scope. To ensure the best colors for your captured images, press the white PHOTO button located next to the lamp rheostat. This should make the light in the eyepieces uncomfortably bright. The purpose of this button is to avoid the yellowish tint that comes from using lower lamp settings. On other microscopes this setting may be referred to as 3200°K.

4. To reduce the intensity of the lamp, there are three filters located on the right, behind the stage XY control.

   - The filters are labeled ND8, ND32 and NCB11.
   - The first two are neutral density filters, which can be used singularly or in combination to reduce the intensity of the light without changing the color (Note: ND32 reduces the light more than ND8).
   - The NCB11 filter is slightly blue in color and is useful when photographing H&E sections.
   - To use a filter, press inward on the appropriate silver colored rod, to remove the filter, press down on the black lever located directly above the silver rods.

5. For the highest quality images, be sure to align the microscope for Köeller illumination. The alignment should be quickly checked every time you change lenses

   - Note: the alignment for the 20x objective lens on this microscope is noticeably different than the other lenses.



   - Please note that for image analysis, it may be better to not have the condenser aperture set at quite as far to the right as you might set it for just observing by eye.
   - A handout entitled **Basic Microscope Alignment** is available at: http://swehsc.pharmacy.arizona.edu/exppath/resources/handouts.html

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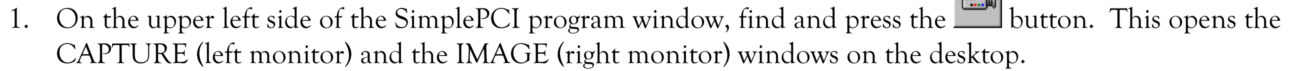
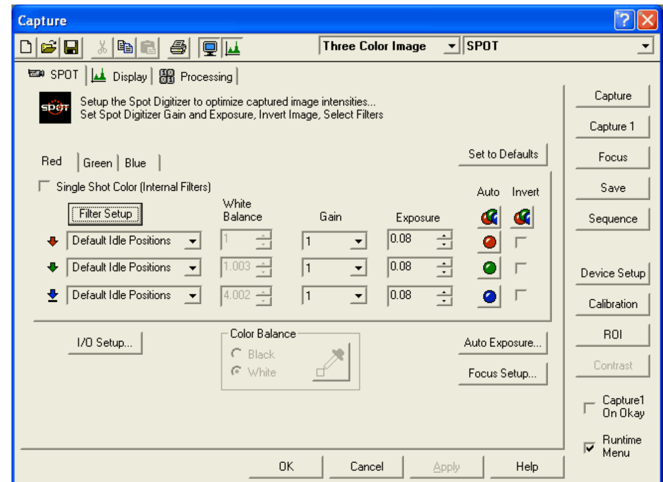
Working with SimplePCI:

1. On the upper left side of the SimplePCI program window, find and press the  button. This opens the CAPTURE (left monitor) and the IMAGE (right monitor) windows on the desktop.

2. Before you can acquire images, please check these important CAPTURE window software settings:

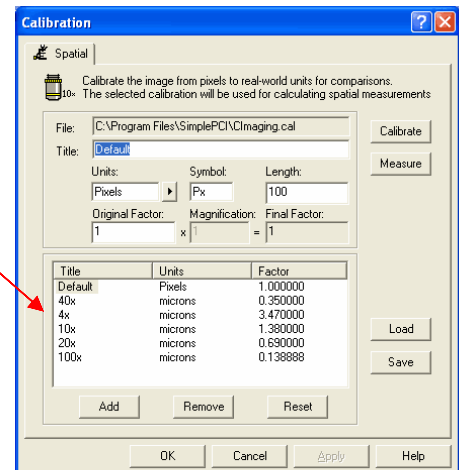


   A. Note: there should be no need to change the device settings on this system. The camera resolution is 766 by 510 pixels and this should not be changed.

   B. From the CAPTURE window (left monitor), press the CALIBRATION button (right side of the window). Each objective lens has its own calibration setting. Click on the calibration factor you want to use (red arrow), then click the OK button.

   Please DO NOT change any of these measured values.

   DO NOT use the **add**, **remove**, or **reset** buttons!



3. To begin capturing images from the microscope:

   A. Find your area of interest with the microscope eyepieces, focus, and then use the slider to switch the light path from the eyepieces to the camera (pull the slider all the way out).

   B. Choose AUTOEXPOSURE to roughly set the light levels. Another window will open, simply click OK.

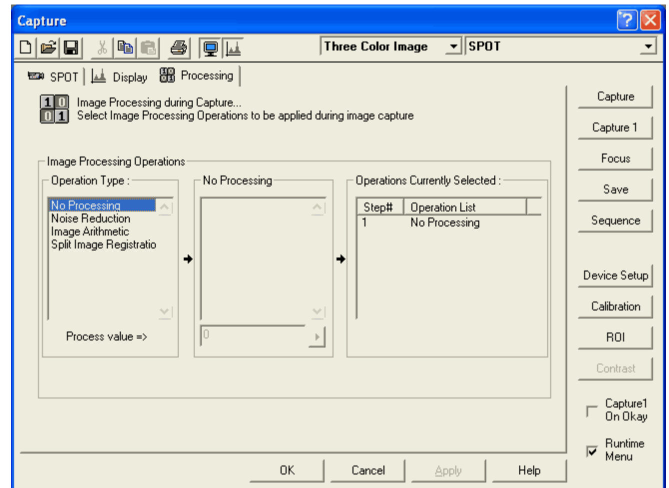
      - Do not change anything on the microscope while the system is calculating the exposure settings. If you forgot to pull the slider out, click the autoexposeure window's cancel button. Pull out the slider and restart the autoexposure.
      - If the autoexposure fails (ends very quickly) it may be that the light to the camera is too bright, use the neutral density filters to reduce the light and re-do the autoexposure.
      - If the exposure is too long, the camera may not be getting enough light, make appropriate adjustments to the filters and re-do the autoexposure.
      - Note: because the camera is mounted high above the microscope, it is sensitive to vibrations. To avoid blurring the image, users should not touch the microscope table while the camera is working.

C. To have an image show in the image window (right monitor), click on the CAPTURE1 button in the CAPTURE window (left monitor).

D. Next, click FOCUS (left monitor).
   - The camera will look at just the red channel (image becomes grey).
   - Adjust the microscope so that the image is in focus (the camera is not exactly parfocal with the eyepieces). NOTE: if your light levels are low, the camera responds very slowly to changes in focus.

E. Once your image is in focus, rerun AUTOEXPOSURE and then click CAPTURE1.

F. In some instances it may be necessary to perform a background subtraction to remove the effects of uneven illumination and/or dirt in the optical path. If you think this is necessary, do the following:



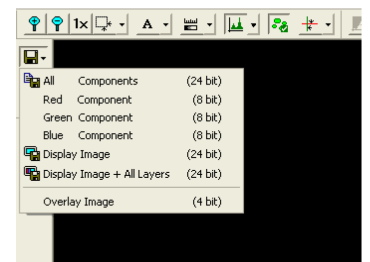
   - If possible, find a nearby place on the slide that has no cells/section and no obvious dirt. Be sure that this area is still under the coverslip so that all the optical parameters will be equal. Slightly defocus the image on the microscope. Click on CAPTURE1.
   - Save this image in your directory on the user disk (F:) as **background.tif**, overwriting the existing image if needed. Note: see instructions for saving files in section H. and I. below.
   - In the CAPTURE window (left monitor) click on the processing tab (see picture), under operation type choose IMAGE ARITHMETIC, the software will then ask you if you want to use the current image or a disk image, choose disk. Browse to the location of the **background.tif** image you just saved. Now the software will increase the contrast each time you acquire an image by subtracting the background image file. Since each microscope slide is slightly different, it may be necessary to capture a new background for each slide. See what works best for you.

G. If your image is still low contrast, move to the "image display" window (right monitor), and click the Contrast properties button. The dialog box that opens up (usually in the left monitor) has several ways to increase the image contrast. Try AUTO and see if you like what the image looks like. Click OK when you are finished adjusting the contrast. This only changes the image display, not the actual image. The contrast changes can be turned on and off by using the On/Off button. Always make sure that you maximize the contrast in the microscope optics before using software.

H. To save the image to disk, look for the floppy disk icon in the upper left of the image display window (right monitor). Click on the icon and select all components to save the captured image.

   To save the image with the contrast adjustments shown in the display window, choose display image.

   Make sure to save your files as 24bit tiff images, <u>not</u> JPEG.



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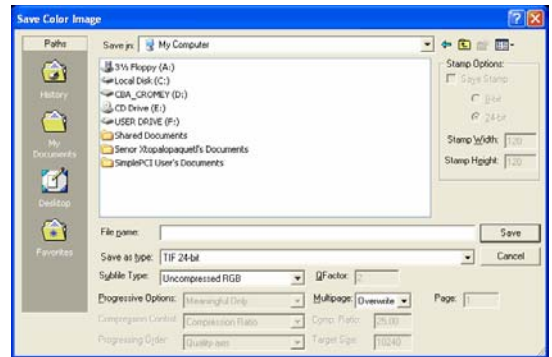
I. Save all your files on the F: drive. If you don't already have one, to create a directory on that drive using your name or faculty member's name.

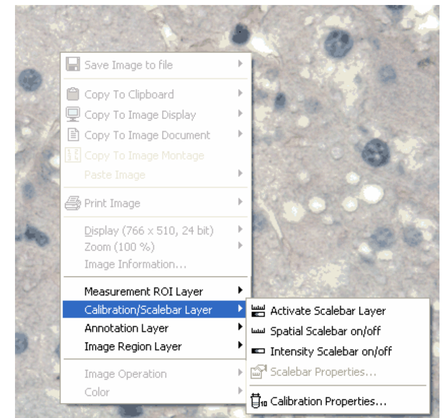
Alternatively, you can save your files to a ZIP disk (100MB or 250MB) or a USB "thumb drive".



J. When you are done with the images on this system move them to a ZIP disk or write a CD-R. **Old data left on this system is subject to deletion without prior notification.**

K. To add a scalebar to the image, right click on the image field and select activate scalebar layer. The scale bar can be repositioned and font characteristics (color, size) can be changed by right clicking on the object.

To save the image with the scalebar, use the Disk icon and choose "Display Image + All Layers" (see section H.).



Cleanup

1. To shut down the computer: go to the Windows START button and select SHUT DOWN.
2. Turn off both of the monitors (lighted button in lower right corner), the microscope and the SPOT camera controller.
3. If you used the 100X oil lens, clean the excess oil off with lens paper.
4. Cover the microscope.
5. Sign out in the log book.

Images credit:

Several of the images in this handout came from the following Nikon brochure: http://www.nikonusa.com/bluelink/brochures/E400brochure.pdf

About the author:

Mr. Cromey is the manager of the Cellular Imaging Core, a service that provides training & technical expertise to SWEHSC investigators interested in using microscopy and scientific imaging in their research. The SWEHSC is funded by the NIEHS, grant # ES06694. The Cellular Imaging Core is also host to **Microscopy & Imaging Resources on the WWW**, located at: http://swehsc.pharmacy.arizona.edu/exppath/

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