

## **Laboratory for Advanced Electron and Light Optical Methods**

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### **Operation of Spot RT Slider Camera with Vanox Microscope**

**Version 4[1].6.4.0**

Installed 6/13/06 MJD

**Revised 5/7/08 M.J. Dykstra**

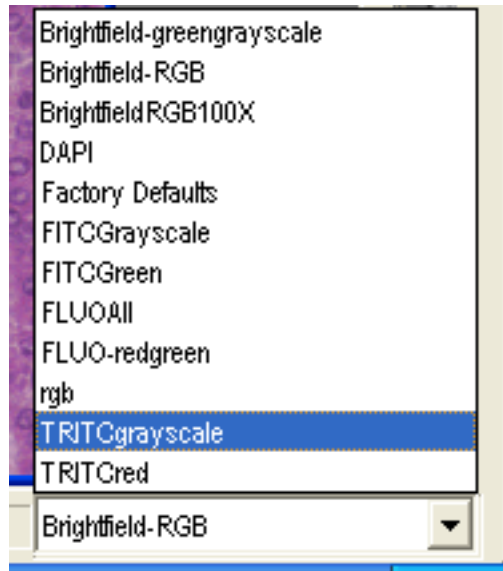
#### I. Set Up

1. Remove Sony CCD camera by loosening 2 large silver knurled knobs. Find the ocular in the left top desk drawer and remove the plastic cap and put it onto the end of the ocular attached to the Sony camera.
2. Insert the ocular into the top of the TV/DO port on the Vanox. Slip the Spot RT Slider camera assembly over the TV/DO port and tighten the two silver knurled knobs.
3. Turn on Vanox. Turn on the **RT Power Supply** to the right of the Vanox.
4. Select TV/DO on Vanox control pad.
5. Turn on computer and click on **SPOT Advanced** icon.



II. Click on the **SPOT Advanced** Software Icon:

1. Select **Image Type** from pull-down menu on lower right of screen



<b>Pull-Down Selection</b>	<b>Description</b>	<b>Use</b>
Brightfield-greenyscale	B & W Bright Field Through Green Filter	For B & W images of H & E sections
Bright Field-RGB	Bright Field Color	For full color images
DAPI	Blue Colorized Grayscale Image	For use with DAPI stain and UV filter cube (U)
Factory Defaults	<b>LAELOM USE ONLY</b>	
FITCGrayscale	B&W image of FITC	B&W image of FITC from B cube
<b>Pull-Down Selection</b>	<b>Description</b>	<b>Use</b>
FITCGreen	FITC in Green	Grayscale image of FITC colorized to green from B cube
FLUOall	RGB Fluorescent Image	Full color capture of all fluorochromes
FLUO-redgreen	FITC/TRITC Image	Grayscale image of FITC and TRITC colorized green and red, respectively (IB filter cube)
TRITCgrayscale	B&W Image of TRITC	Grayscale image of TRITC from G cube
TRITCred	TRITC in Red	Grayscale image of TRITC colorized to red from G cube

2. For **Brightfield Work**, do a white/color balance (do not do this for Fluorescence Work). Click on **Balance Icon** on right side of screen.



Follow instructions (move off of the specimen to a clear area of the slide) click “begin”, when prompted, click “OK”

3. Move back onto the area of interest of the slide, focus, click on the **Live** button found towards the upper right of the screen. Select the **Image Type** from the pull-down menu on the lower right of the screen.

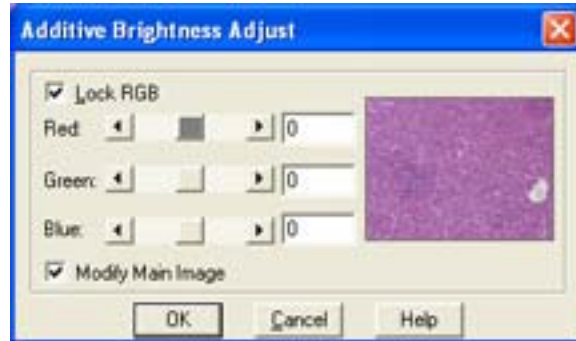


- a. Click on **Controls** to bring up the menu. All types of images are easiest to focus and refresh most quickly if a **Clear Filter** is selected. To focus even faster, select **Binning 4 X 4**, but make sure you go back to **Binning None** for capture to get highest resolution.
  - b. If the **Connection Icon** on the right side of the **Control Screen** has a red line across the middle, the settings for the **Viewing Screen** are not necessarily close to the **Captured Image** setting. If the red line is missing, the **Viewing Screen** settings are linked to the **Captured Image** settings, though not identical. The latter setting is preferred.
4. To take a picture, click on the **Camera Icon**



Clicking on **Previous Exposure Icon** sets up the next picture with the same parameters. The captured still image appears behind the Live Image window. Close the **Live Image** window to edit/save captured image. This feature allows subsequent images to be captured in less time (important for fading fluorochromes). It also allows you to document different intensity levels found in subsequent images.

5. To manipulate the image use features located under the **Edit** menu. For example, if the image is too dark, use: Edit -> adjust RGB -> Adjust Brightness (additive) to lighten the image.



If the image is grainy or blurry, use: Edit -> Filters -> Smooth/Sharpen/Unsharp Mask/Filter Noise.



**Smooth** improves grainy images.

**Sharpen** improves the appearance of soft or blurry looking images.

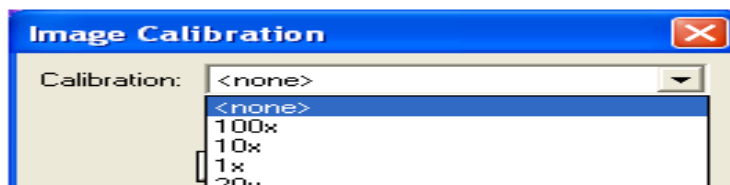
**Unsharp Mask** improves the appearance of an image's high frequency detail (it sharpens the image by subtracting brightness).

**Filter Noise** corrects the effects of electrical or thermal noise.

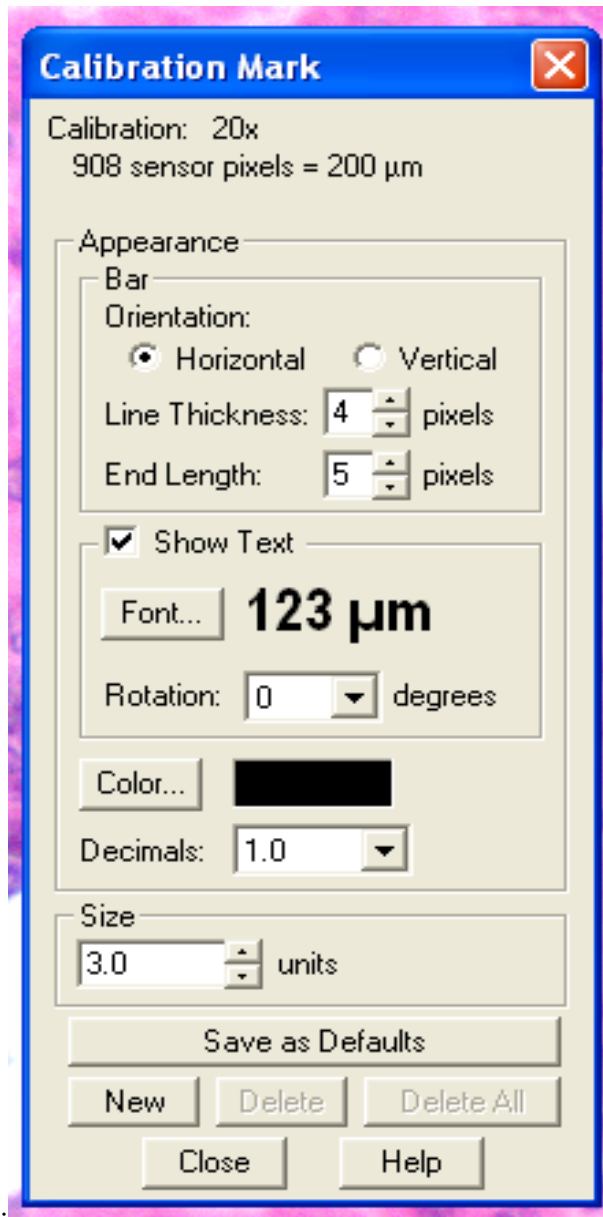
6. Calibrations are already set up for all the objectives on the Vanox (see under Edit).

a. **To add a Micron Bar**

1. Go to Edit->Set Calibration
2. Select the **Objective lens** in use (e.g., 10X)

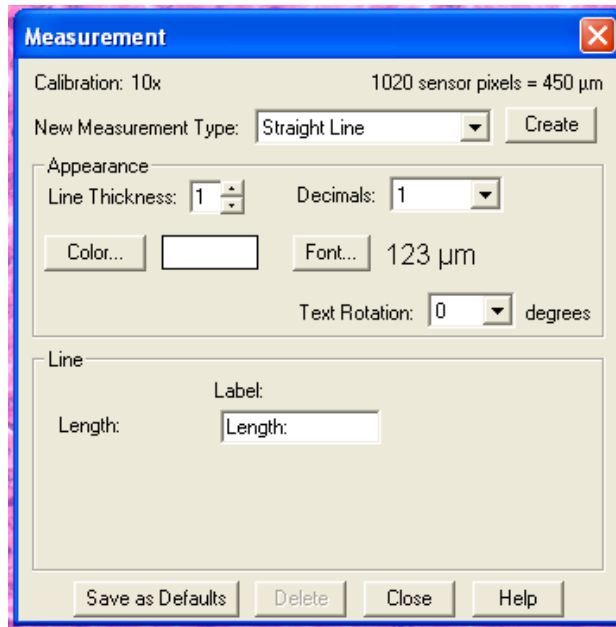


3. Click on Edit, **Add/Edit Calibration Marks**, which brings up the **Calibration Mark** screen:



4. Move cursor to point on screen where you want size bar (typically near the edge of the image, where it can be trimmed off of a printed image without losing significant image)
5. If the **Size bar** is not the size you want, grab one of the white boxes at the end of the bar and lengthen or shorten the bar, or enter a number of units in the window under **Size**
6. When the **Calibration Mark** window is closed, the mark is permanently associated with the image
7. If you click on **Delete** in the **Calibration mark** window before closing, the mark is removed
8. You may also select:
  - a. Vertical or horizontal orientation
  - b. Line thickness
  - c. Number of decimal places

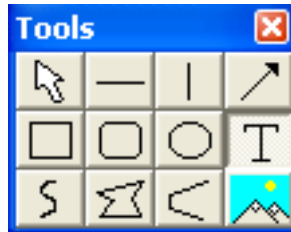
- d. Color for the both the line and number
- e. Font style and size
9. To keep **Calibration Mark** associated with image so that it can travel into other programs like Photoshop, choose **Edit/Merge/Merge Calibration Marks**, which will merge the marks into the image permanently.
- b. To add **Measurements**
  1. Go to Edit -> Set Calibration
  2. Select the **Objective** in use (e.g., 10X)
  3. Click on Edit, **Add/Edit Measurements**, which brings up the **Measurement** screen:



4. Make sure **Calibration** at upper left of **Measurement** window agrees with the **objective lens** in use
5. You may change:
  - a) Line thickness
  - b) Number of decimal places
  - c) Color for the lines and numbers
  - d) Font style and size
6. There are a variety of **Measurement Types** available on the **New Measurement Type** pull-down menu
7. Click on **Create** button to begin. Click on first point. Click on second point to end measurement
8. You may reposition any line, angle, or shape by clicking and dragging the object
9. Once the **Measurement** window is closed, the measurements remain permanently attached to the image
10. To keep **Measurement** associated with image so that it can travel into other programs like Photoshop, choose **Edit/Merge/Merge Measurement**, which will merge the marks into the image permanently.

7. **Custom Image Annotation**

To add text, arrows, lines, rectangles, ellipses, freehand lines, or graphic stamps: click on Edit -> Annotation. **Notice the menu bar changes.** All graphic elements may be repositioned (click and drag the object), resized (click and drag a handle) or edited (right click on the object) while in this mode. **It is necessary to click on Done to exit this mode and return to the SPOT Image capture/edit mode.** Once you have exited the annotation mode, you can no longer edit the graphic elements.



To insert another image into your working image select the **Image Icon**.



Then click at the insertion point on the working image, you will be prompted to select an image, locate the desired image, click OK, resize and position.

To add additional information to the image:

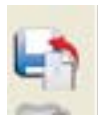
Click on Object -> **Image Date** to stamp the date on the file. Click on Object -> **Image Time** to stamp the time on the file. Click on Object -> **Image Title** to title the image. Click on Object -> **Image Memo** to create a memo associated with the image. Left click on the image to place any of these options. Click and drag to reposition. Right click to change font size. Click **Done** when finished. This will incorporate all annotations into the image file (see note below).

*If the TOOL BOX is closed, it will not reappear until you go to VIEW and click on SHOW TOOLS.*

To keep **Annotation** associated with image so that it can travel into other programs like Photoshop, choose **Edit/Merge/Merge Annotation**, which will merge the marks into the image permanently.

**Note:** Any **Stamps** or **Annotations** (e.g., micrometer bars, measurements, lines, arrows) put on images, stay with images. You may want to save your image before adding calibration marks, measurements or objects and then **Save As** a different file name with the additions. Stamps or annotations cannot be modified after the image is saved.

8. To save an image, click on **Fast Save Image** icon at right side of screen (the top one)



- a. Saving an image in the TIF format is preferred for best resolution.
- b. When saving JPEG files, a lower number indicates higher compression and poorer image quality, but smaller files. **A JPEG setting of 60 produces a 178 kb image suitable for on-screen viewing or PowerPoint presentations.**
- c. When saving the first image during your session, make sure that it is saving the images in your preferred format to your preferred drive (Zip disk). Once you save the first image, the rest should get put in the same place.

12. To delete images, right click on thumbnail and choose delete

13. **Edit**

- a. Adjust RGB for B & W
- b. Adjust HSL for Brightfield
- c. Adjust HSV for Fluorescence  
-adjust gamma for fluorescence
- d. Leave at 24 bit

14. Make sure that **Size** under the **Resize** bar is set at 1600 X 1200.

15. To Use the Spot Camera with Image Pro Plus or Pax-it:

- a. **For Image Pro Plus:**  
Go into **Select Scanner** and select **Spot Camera**
- b. **For Pax-it:**  
Go to tool bar and select **Twain** icon.

## **Trouble Shooting the SPOT Camera while using Fluorescence Optics (For LAELOM Staff ONLY)**

1. Live Image Control settings:
  - a. Increase speed
  - b. Increase brightness
  - c. Decrease gamma (gamma default is 1.5)
  
2.
  - a. Delete Fluorescence setting(s)
  - b. Recreate setting(s):
    - FLU ALL = RGB (3 passes)
    - DAPI = blue filter
    - FITC = green filter
    - TRITC = red filterby using the all the software defaults, EXCEPT **auto-gain limit**. Change auto-gain limit to **8**.
  
3. To edit gamma independently of settings dialog box, go to:  
**Edit -> Adj -> HSV -> gamma**
  
4. Additional things to check:
  - a. Make sure the neutral density filter is out.
  - b. 40X is best objective for fluorescence.
  - c. The oculars should be set to 0 because the camera is parfocal.

## **Using Adobe Photoshop to output images captured with the SPOT RT Slider camera and Advanced SPOT Software**

While in the **Advanced SPOT software** program:

Click on **Edit -> Resize** -> increase to the file's resolution **200%**.

Save the file (click on **File -> Save As**) as an **uncompressed TIF** file.

Launch the **Adobe Photoshop** software. Click on **File -> Open** and locate the desired image.

Click on **Image -> Image Size** (make sure **Resample Image** box is NOT checked) and change the image's width to 8" (height and resolution will follow, giving you an 8" X 6", 400 dpi image. The original file size doesn't change because the pixel size has not been resampled.

You may now print the image as is, add additional images, additional layers, text, arrows, etc.

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Michael J. Dykstra

**Settings for the Live Screen for Fluorescence Imaging and Capture**

Auto

Use filter-checked

Clear

Binning 4 X 4 (for focusing); none for capture

Fluorescence

Brightness somewhere between 1.0 and 1.5

Gain Limit 16

Auto Brightness-checked

Imaging area-Full Chip

Gamma 1.0

Indicate Saturated Pixels-checked for brightness adjustments; unchecked for capture

**Settings for Live Screen for Brightfield-RGB Imaging and Capture**

Auto

Use Filter-checked

RGB

Binning- none

Brightfield transmitted light

Brightness 0.75-1.0 (use intensity filters on microscope to get into range)

Gain limit at 16

Autobrightness checked

Imaging Area-Full Chip

Correct color technology-checked

Neutral

Gamma 1.00