

LAELOM **Laboratory for Advanced Electron and Light Optical Methods**
North Carolina State University College of Veterinary Medicine
1060 William Moore Drive, Raleigh, North Carolina 27607
STANDARD OPERATING PROCEDURE

Subject: An Operations Manual for the Vanox Workstation Peripherals		
SOP No: VanoxPeriph	Authorized By: _____	Effective Date: March 20, 2009
Version No: 5.3	Signature _____ Date _____	Page: 1 of 11

An Operations Manual for the Vanox Workstation Peripherals

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I. Set Up for Image Pro Plus, version 5.1 with Nikon Digital Sight Camera

A. Start up for Using Nikon Digital Sight Camera

1. Remove the **SPOT RT Slider** camera by loosening 2 large silver knurled knobs and lay it gently upon the camera power supplies. Remove the **Ocular** in the camera port, put the plastic cap from the **Nikon Digital Sight** camera ocular over the tip of the ocular and put into the drawer to the right of the Vanox microscope.
2. Take the **Nikon Digital Sight** camera from the drawer to the right of the Vanox microscope and insert the **Nikon Digital Sight** camera into Vanox camera port. Tighten the 2 knurled knobs. Connect computer cable coiled underneath monitor to **Nikon Digital Sight** camera.
3. Turn on **Nikon Digital Sight Power Supply**.
4. Turn on **Vanox Microscope**.

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5. Select **TV/DO** on Vanox control pad.
6. Turn on computer and monitor.

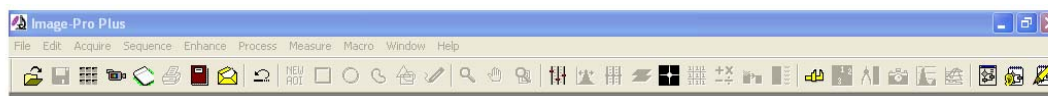
B. Shutdown


1. Turn off **Nikon Digital Sight camera** power supply.
2. Turn off **Vanox microscope**.
3. Shut down the computer and turn off the monitor.
Always exit Windows programs before shutting down the computer.

Description of capabilities: This is a morphometry program that allows image acquisition, manipulation of captured images (e.g., brightness, contrast, hue, conversion to gray scale, shifts in color balance, etc.), and data acquisition from the images (e.g., length, area, density, etc.). Images may be exported as TIFF, BMP, or PCT files (for use in other software programs) to formatted writeable CDs or ZIP disks (100 images/disk). Image data is typically exported directly into Microsoft EXCEL spreadsheets.

C. Operation

1. Open **Image Pro Plus 5.1** by double-clicking on the icon on the Windows XP desktop. To acquire an image, look on the tool bar



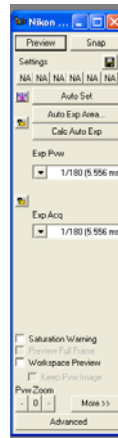
and click on the **Video** icon:  This will launch the **Nikon DS-U2 image capture menu screen:**

If a menu different than the two shown below appears, click on **Basic Dialog** to bring up the normal working screens.

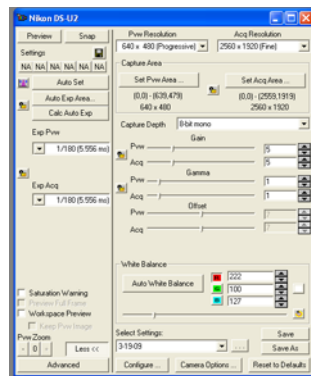
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Clicking on the **More>>** button will bring up the expanded **Nikon DS-U2 image capture menu**:



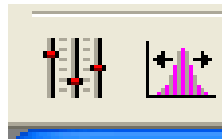
2. Click on the **Preview** button on either screen to view image.
3. Click on the **Snap** button on either screen to capture the image.
4. If the **Preview** image is too small, click on the **More>>** button on the small **Nikon DS-U2 menu**. Then, click on **Set Pwv Area** button and then click on **Reset area to full frame** button.
5. If the **Snapped image** is too small, click on **More>>** button on the small **Nikon DS-U2 menu**. Then, click on **Set Acq Area** button and then click on **Reset area to full frame** button.
6. To adjust for proper exposure, click on **Calc Auto Exp** button.

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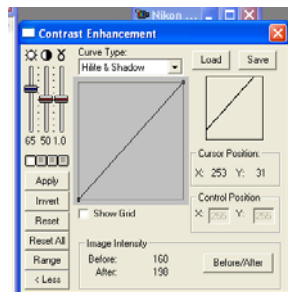
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- To Save an image**, click **File** -> **Save As**. A dialog box will prompt you to **Name** the file, assign a file name with your initials and some number of meaning to you, select a file **Type** (format), then select the location where you want the file to be save and click **Save**. **Any files saved to the C Drive are in danger of being erased.**
- To Manipulate captured images**, click on contrast enhancement icon on the tool bar (left icon below):



This will bring up the following controls:



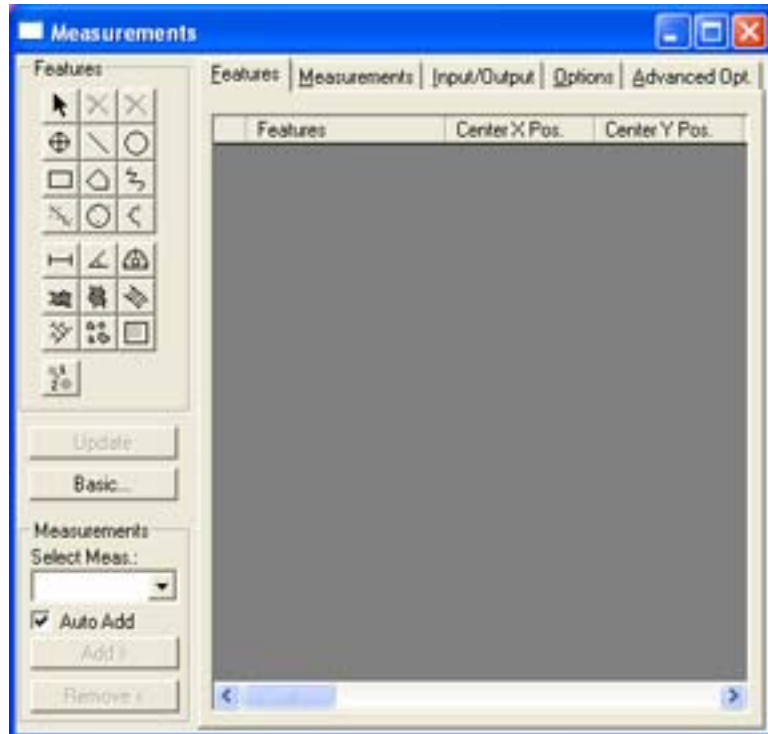
The slider bars adjust (from left to right) **brightness** (the overall amount of light in an image), **contrast** (the degree of difference between the brightest and the darkest components in an image) and **gamma** (contrast enhancement in the very dark or the very light areas of an image). Adjust the slider bars to the desired result and click **Apply**. The manipulation(s) are applied to the current active window. Therefore, if the previous version(s) of the image are needed, you may want to save the original captured still image and then do a **Save As** after each adjustment.

- To Calibrate for measurements**, click on **Measure, Calibration, Select Spatial...**, and then select the appropriate file for the objective being used (e.g., **nikon 1X** for 1X objective capturing images for the **Nikon DS-U2 camera**). Click **ok**.

Note that all calibrations are based on using the Nikon DS-U2 camera. They will not be accurate with the Spot RT Slider camera, which is calibrated within the Image Pro Plus 6.1 program below.

- Next click on **Measure**, then on **Measurements** and select the measurement icon for the task you wish to perform:

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Each feature prompts you will a dialog box of directions for using that tool. Features include (from left to right) **selection** (arrow), **delete** (X), **create point** (crosshairs), **line**, **circle**, **rectangle**, **polygon** (you must right click to finish the shape), **trace** (you must right click to finish the shape), **best fit line**, **best fit circle**, **best fit arc**, **measure distance between 2 features**, **measure angle between 2 intersecting lines**, **add an angle measurement between 2 existing lines**, **thickness measurement between horizontal, vertical, or any 2 lines**. Each feature also specifies whether it measures **length** or **area**. **All measurements are in μm** . Left click on the image to specify the area(s) to be measured. All measurements will remain on the image file. If you do not want to keep them, **Close** the file and do not save changes. If you would like to keep the measurements, you may **Save** the image, which updates your file, or you can do a **Save As** (recommended), which allows you to save the changes with a different name, leaving the original image intact.

After measuring features, clicking on **Features** in the **Measurement** window will display the measurements made.

To measure an AREA:

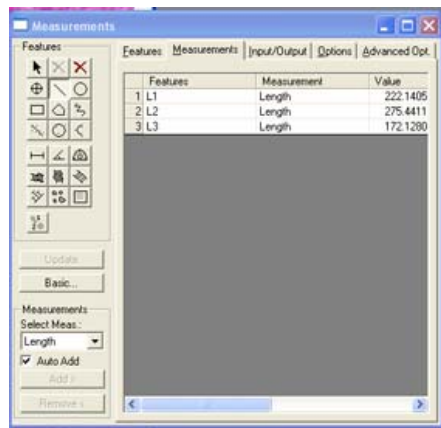
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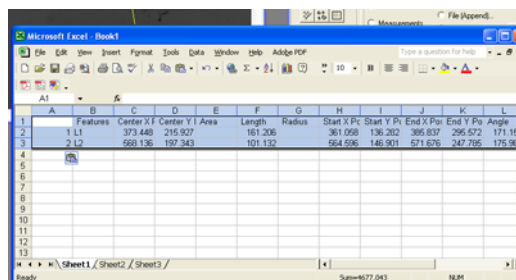
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- a. **Calibrate** the program for the objective you are using.
- b. Click on the **Polygon Icon**, hold down the left mouse button while describing the area, then click on the right mouse button when the perimeter line is closed.
- c. The **area measurement** will be displayed in the **Measurements Window** from which you selected the **Polygon Icon**.

11. **To Export your measurement data to a Microsoft Excel spreadsheet**, perform the measurements desired and click on the **Input/Output** tab in the **Measurements** window



Select **DDE to Excel** and click **Export Now**.



To **save data** in **Excel spreadsheet** click on **File -> Save As**, name the file, select the desired drive.

12. **To add a μm bar to a captured image**
 - a. Click on **Measure, Calibration, Select Spatial...**, then select the objective in use with the **Nikon DS-U2 camera** (e.g., nikon 4X).

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- b. Click on **Marker** button and select **Non-destructive** under **Style** pull-down menu. Select parameters desired and click **ok**.
- c. Click on **Remove** button to erase bar.

II. **Set Up for Image Pro Plus, version 6.1 with Spot RT Slider Camera**

A. **Start up for Using Spot RT Slider Camera (this has much slower acquisition speed than the Sony CCD camera, but higher resolution)**

1. Remove the **Nikon Digital Sight (DS-U2) camera** by loosening 2 large silver knurled knobs and lay it gently upon the camera power supplies. Remove the **Ocular** in the drawer, remove the plastic cap on it, and insert it into the ocular port at the top of the Vanox. Put the plastic cap on the ocular attached to the **Nikon Digital Sight** camera and place it in the drawer to the right of the Vanox microscope.
2. Position the **Spot RT Slider** camera over the Vanox camera port with the previously inserted ocular. Tighten 2 knurled knobs.
3. Turn on the **Spot Camera** power supply.
4. Turn on **Vanox** Microscope.
5. Select **TV/DO** on **Vanox** control pad.
6. Turn on computer and monitor.

B. **Shutdown**

1. Turn off **the Spot RT Slider** camera power supply.
2. Turn off **Vanox** microscope.
3. Shut down the computer and turn off the monitor.
Always exit Windows programs before shutting down the computer.

C. **Operation**

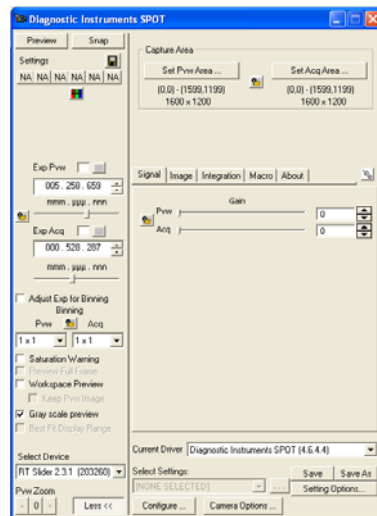
1. Click on **Image Pro Plus 6.1** icon.
2. Select **Biological**.

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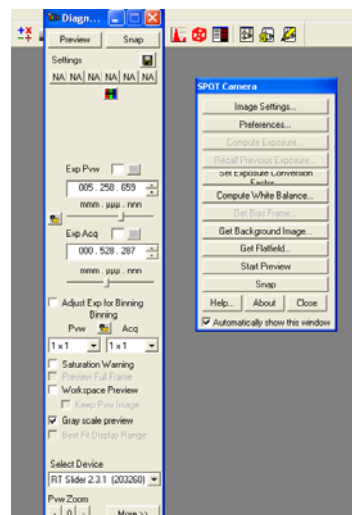
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- Click on **ok**.
- Click on **Camera** icon. This brings up either the **Diagnostic Instruments** full menu:



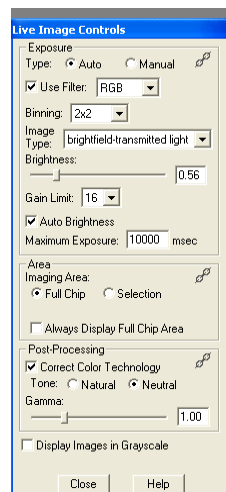
or the smaller basic menu (a smaller **Spot Camera** menu can also be seen to the right below):



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5. Click on **Preview** on the **Diagnostic Instruments** screen. If the image consists of large pixels, click on the **Reset** button on the **Spot Live Preview-Running** screen.
6. Click on **Controls** button to bring up the **Live Image Controls** menu:



The settings should be set as shown. The only one that can be changed usefully is the **Brightness** control.

7. Click on the **Focus Gauge** box to help focus the image.
8. If the image color needs adjusting, click on **Match Color**, then select a clear area on the image.
 - a. Click on **Make White/Gray**
 - b. Click on **ok**
9. Click on **Snap** to capture image. Right click on the captured image and then select **Zoom**. Click on **50%** and then grab the image frame and stretch it to full size.
10. **The other features of the program are similar to those described above for the Image Pro Plus 5.1 program.**

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11. **To calibrate images for different objectives**, select **Measure, Calibration, Spatial** and choose the appropriate standard from the pull-down menu (e.g., Spot 20X). Click on **ok**. Do measurements as previously described (C.2, ff).

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Instructions for Setting up Sony CCD Video Camera with the VHS Recorder

1. Plug in **Sony CCD Video Camera** power supply and **VHS Recorder**.
2. Connect **VHS Output Cable** from **Sony CCD Video Camera** to **VHS in** on **VHS Recorder**.
3. Connect **VHS Cable** between **VHS out** terminal on **VHS Recorder** and **VHS in** terminal on **Sony Monitor**.
4. Install **Sony CCD Video Camera** onto **Vanox Microscope**
 - a. Remove whatever camera is currently on the **Vanox** by loosening the 2 silver **Knurled Screws**. Gently place the removed camera onto the counter top near the **Vanox**.
 - b. Remove **Ocular** (if present) from **TV Port** at the top of the **Vanox**. Place the plastic ocular cap from the end of the **Sony CCD Camera Coupler** over the removed **Ocular** and place it in the drawer to the right of the **Vanox**.
 - c. Slip the **Sony CCD Camera Coupler** into the **TV Port** at the top of the **Vanox** and tighten the 2 silver **Knurled Screws**.
5. Turn on the **VHS Recorder**, **Sony Monitor**, and **Sony CCD Camera Power Supply**. The **Sony Monitor** should be set for the **A Channel**. The **Sony Monitor** should show a blue screen and **L-1**.
6. The **Remote Control** for the **VHS Recorder** is in the drawer to the right of the **Vanox**. Install the batteries found in the drawer and turn the **VHS Recorder** on.
 - a. Click on **Menu**.
 - b. Select **Function Set** and click **ok**.
 - c. **S-VHS ET** should be set **on**.
 - d. Page down to **Rear Aux Input** and set to **S-Video**.
 - e. Click on **Menu** to end.

When all of these steps are completed, you should have live video on the Sony Monitor and be capable of recording the live image by using the Remote Control for the VHS.