LAELOM **Laboratory for Advanced Electron and Light Optical Methods**
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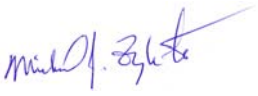
STANDARD OPERATING PROCEDURE

Subject: Operating Instructions for the VANOX AH-3 Photomicroscope		
SOP No: Vanox Microscope	Authorized By:  11/5/10	Effective Date: November 5, 2010
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Operating the VANOX AH-3 Photomicroscope (Installed April 5, 1994)

I. Start-up

A. Turn on the **Green Switch** at the right rear of the **Microscope**

B. Depress **Gray Button** labeled **Photo** on the right rear of the M**icroscope**

C. Push in the **Black Knob** on the right top of the **Microscope** for normal viewing. When it is in, 50% of the light is delivered to the oculars and 50% is delivered to one of the camera ports. When it is out, 100% of the light is delivered to the selected camera port.

D. Push the **Shutter Switch** on the upper right side of the **Microscope** down to the open position for normal viewing. When working with extremely low light levels (e.g.,fluorescence), you can push switch up to exclude ambient light from entering oculars during image capturing.

II. Using the Automatic Microscope Controls Located on the Control Pad (All of the buttons with a range of settings beep when the end of the range is reached)

A. The **Orange Buttons** are used **by the LAELOM management** to set the computer for the **Objectives** to be used and to make sure that the appropriate **Condenser Lens** configurations are in place. **These butttons should not have to be used**. If the **Objectives** are rearranged or changed:

1. Select one of the **Photo modes**. Put an **Objective** over a blank glass **Slide**
2. Use the **Ob. Mag Button** to match the **Objective** in use
3. Depress the **Type Button** to select the type of **Objective** in use (written on the **Objective Barrel**)
4. Depress the **Set Button** to enter the configuration into memory
5. Repeat, in turn, for each **Objective**

B. The **Image Port** buttons allow an image to be sent to different ports. When doing digital imaging and porting the image to **TV/DO**, the **Camera Control Panel** in the base of the microscope will not be operational and the various windows will not be lit. In addition, the **Projected Reticle** will not be visible in the oculars.

The **TV/DO** button sends the image to the port where **video or digital cameras** are attached, while still sending 50% of the light to the oculars.

C. **Objectives** are changed by depressing the **Blue Arrows Button** on the **Control Pad** or **Microscope Base**

D. The **Stage** is lowered for changing slides or for changing from an **oil** to a **non-oil objective** by depressing the **Stage Button**. A blinking **Orange LED** appears. Load a slide and depress the **Stage Button** again to bring up the **Stage.**

The Stage may be LOWERED with the 100X objective in place, but should only be RAISED with either the 1X or 4X lens in place to avoid damaging the higher power objectives.

E. Focusing is preferably done with the **Focus Button** (coarse and fine, up and down). Try **not** to use the **Fine Focus Knob** on the **Microscope**

F. The **Intensity Button** selects one of the 10 built-in **Neutral Density Filters**

G. The **A. Stop Button** (controlling the substage condenser aperture) can be used to increase the depth of field and contrast by depressing the right side of the **Button**. If too much image diffraction (graininess) is observed, the aperture is too small and needs to be opened by depressing the left side of the **Button**, which opens up the diaphragm

H. To effect **Kohler Illumination** and to focus the light source precisely on the **Specimen**:

1. Select the **10X Objective**

2. Adjust the interpupilary distance of the **Oculars**, focus each **Ocular** independently on the specimen.

3. Use the **F. Stop Button** to close down the **Field Diaphragm** until the sharp edges of the **Diaphragm Blades** are visible. By focusing the **Condenser Focusing Knob** (lower left side and behind the **Stage**), a red fringe will be seen on one side of true focus on the **Field Diaphragm** and a blue fringe will be seen on the other side of true focus. Try to focus in between these two chromatic aberrations

4. Open up the **Field Diaphragm (F. Stop Button)** until it almost fills the viewing area (not the reticle) visible through the **Oculars**

5. Center the **Image** of the **Field Diaphragm** with the two **Silver Knobs** below and to the back to the **Stage**. Open the **F-Stop (Field Diaphragm)** completely.

The VANOX now has its light source perfectly centered and focused on the specimen no matter what objective you are using.

I. When using **Oil Immersion Lenses (60X and 100X lenses)**, the **Objective Lens** must be oiled in order to achieve the highest possible resolution. The **Front Condenser Lens Element** does not need to be oiled (it does not appreciably improve resolution)

J. When using the **40X High Dry Objective** with the **Focusing Collar**, note that it has numbers from 0.11 to 0.23 inscribed on the moveable **Barrel**. These correspond to **Coverglass** thickness.

A standard #1.5 **Coverglass** (recommended, if you are in the position of purchasing any) should be used with the **Objective** set at 0.15-0.17. This means that the **Objective** is adjusted for a **Coverglass** that is about 0.15-0.17 mm thick (a #1.5 **Coverglass** is about 0.15 mm thick, a #1 is about 0.1 mm thick and a #2 is about 0.2 mm thick).

If you are using an immunocytochemistry or autoradiography type of slide, mounted in gelatine, it will probably be thicker than average, so it is useful to try around 0.2 as a starting point. Each time the **Barrel** is readjusted, the VANOX must be refocused.

III. Use of the Filters on the Right Bottom of the Microscope

The only filter that should be pushed in for digital imaging is the #1 Filter (LBD).

IV. Using the Polarizers:

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A. Slide the **Polarizing Filter** (in the top left desk drawer)into the slot on the left side below the **Stage**

B. Slide the **Polarizing Analyzer** (in the top left desk drawer) into the **Bright Field Nose Piece** after removing the protective **Plastic Guard**

C. Put a **Specimen** on the **Stage** and bring it into focus. Select area of interest

D. Move **Polarizing Analyzer Lever** until the background is darkest (the polarizers are crossed). Any polarized material should light up well at this point. The **Polarizing Analyzer** can be moved to increase background lighting for better visualization of non-polarized material

V. Epifluorescence Work

A. Cautionary Notes

1. When using this mode, be careful not to look directly at the **Excitation Light**. When handling **Slides**, look through the orange protective **UV Shade**. If you notice a reflection of UV light from the microscope base keypad, push the **Photo Button** (gray button) on the right side of the **Microscope.** After the condenser assembly has rotated the condenser lens into proper position, the UV light reflecting off the base should be gone. Turn off the transmitted light by depressing the **Black Button** at the bottom right rear of the **Microscope**.

3. **The UV Lamp must be changed at 400 hrs**

4. The **Automatic Focus** feature does not work with the **Epifluorescence Nose Piece**

5. **Do not** cover the **Microscope** until the **Excitation Light Housing** has cooled down

B. Start-up

1. Turn on the **VANOX Microscope Switch**

2. Push the **Blue Button** on the **Control Pad** to bring the 4X **Objective**

into place over the **Condenser Lens**

3. Push the **Stage Button** to lower the **Stage**

4. Remove the **Bright Field Nose Piece** and the **UV Port Cover** behind the **Nose Piece** after loosening the **Locking Knob** on the right side and install the **UV Nose Piece** found in the lower right desk drawer. Lock in place with the **Locking Knob**

5. Attach the orange **UV Shade** by sliding the metal tab into the **Slot** below the **G Button** on the front of the **UV Nose Piece**. **Please handle the UV Shade ONLY by the metal tab to keep from breaking the plastic.**

6. Turn on the **UV Power Supply** switch (green) on the large **Power Supply Unit** to the right of the **Vanox Microscope**. It may take up to 10 min for the **Power Supply** to stabilize. **Do Not** switch off the **UV Power Supply** less than 15 min after ignition (it greatly shortens bulb life)

7. **Field Iris Centration:**
   a. Remove the **ND** (neutral density) **Filter** by pulling it out to the first click stop (it is at the left rear of the **UV Nose Piece**)

   b. Rotate the 10X **Objective** into place and focus on the **Specimen**

   c. Stop down the **Field Iris Diaphragm (labeled Field)** on the right rear of the **UV Nose Piece** until it is at minimum diameter

   d. Center **Diaphragm Image** with the **F.S. Knobs** (silver knobs directly in front of the **ND Filter Slider** on the left side of the **UV Nose Piece**)

   e. Open the **Field Diaphragm** fully

C. **Fluorescence Observation:**

1. Turn on the **Microscope Power Switch**
2. Place a **Specimen** on **Stage**

3. Select **Objective** desired

4. Focus on the **Specimen** (the **Autofocus Mode** is inoperable with the **UV Nose Piece**)

5. Depress the **Black Off Button** on the right rear of the **Microscope** to shut off the **Bright Field Light Source**

6. Select the **Filter Cube** of choice (see **Section C.10** below)

7. Depress the **Shut Button** on the front of the **UV Nose Piece** to open the **Excitation Shutter**. A green **LED** will light up when the **Shutter** is **closed**

8. If the **Excitation Light** is too bright, push in the **ND Filter Slider** (on left side of **UV Nose Piece**)

9. Adjust the **Aperture Dial** at the right rear of the **UV Nose Piece** to set the signal-to-noise ratio. Closing down the aperture decreases both signal and noise, but tends to decrease background noise more quickly than signal.

10. Selection of appropriate **Filter Cubes**:

Filter Name	Excitation λ	Emission λ	Applications
IB (dual Red/Green emission)	490		FITC/TRITC, acridine orange
B (Blue)	435	490	FITC
G (Green)	546		TRITC, Feulgen,Texas Red, Cy3, Rhodamine
U (ultraviolet)	334	365	DAPI

Spectral Characteristics of Some Common Fluorochromes (from Sigma Chemical Co.) August 20, 1996

Fluorochrome	Excitation (nm)	Emission (nm)	Color
Fluorescein (FITC)	495	525	green
Hoechst 33258	360	470	blue
R-Phycoerythrin (PE)	488	578	orange-red
Rhodamine (TRITC)	552	570	red
Quantum Red	488	670	red
Texas Red	596	620	red
Cy3	552	570	red

D. **Fluorescence Image Capture:**

Note: When the Microscope Pad says the 1X Objective is in place, it is actually a 4X; when it says the 60X Objective is in place, it is actually a 100X oil Objective.

E. **Shut-down**

1. If the **Excitation Light** has been on for **more than 15 min,** turn off the **UV Power Supply**
2. Depress the **Gray Photo Button** on the right rear of the **Microscope**
3. Gently pull the **Orange UV Filter** from the **UV Nose Piece**
4. Remove the **UV Nose Piece** from the **Microscope** and replace it with **Bright Field Nose Piece**. Lock it in place with the **Locking Knob**
5. Turn off the **Microscope Power Switch** and log out in the **Log Book**
6. **Do not Cover the Microscope Until the Excitation Light Source is cool to the Touch**

F. **Anti-quenching Additive for Mounting Media Used for Fluorescence Microscopy** from Sigma Chemical Co. Several Choices are Available (August 20, 1996)

1. Sigma #P3130, n-Propyl gallate ($18.45/100g)

used as a 0.1 M solution in 90% glycerol in PBS (90 ml glycerol + 10 ml PBS into which 2.12 g of n-Propyl gallate is mixed)

2. Sigma #P6001, p-phenylenediamine, free base ($8.10/50 g); or Sigma #P1519, p-phenylenediamine, dihydrochloride ($10.80/25 g)

reference: **J. Immunol. Methods, 43:349-350 (1981)**

3. Sigma #D2522, 1,4-Diazabicyclo[2,2,2]octane (DABCO) ($12.70/25g)

added to mounting medium to 2.5% concentration

reference: ***Antibodies: A Laboratory Manual***, Harlow and Lane, 1988, Cold Spring Harbor
reference: **J. Histochem. Cytochem**. 33:755 (1985)

VI. Changing the 12V, 100W Lamp (*Done by the LAELOM Management)*:

A. Remove the **Lamp Holder** on the lower left rear of the **Microscope**
B. Remove blown **Lamp** by depressing gold **Levers**
C. Insert a new **Lamp** without touching the glass **Lamp** with bare fingers because body oils will damage the **Lamp**
D. Re-insert the **Lamp Holder** into the **Microscope**, lining up the **Locator Pin**

VII. Calibrating the VANOX for Individual Objectives (*Done by the LAELOM Management)*:

A. Place clear glass portion of glass slide under objective
B. Select the **Objective** to be used. Depress orange **Obj. Mag** button on the **Control Pad**. Select the magnification of the **Objective** in place
C. Depress the orange **Type Button** on the **Control Pad** and select the type of **Objective** in place
D. Depress the **Set** button on the **Control Pad** to save settings
E. Repeat this set of operations for each **Objective** located on the **Brightfield Nose Piece.** When finished, the **AF mode** should work