

# A Nikon Eclipse TE300 inverted epi-fluorescence microscope with a Retiga-SRV CCD Digital Camera System

## I. Basic Microscope Operation:

1. **Turn ON** the TE300 power box switch on the shelf (**Figure 1**) and **depress** the halogen lamp switch (left front) to the **On** position (**Figure 2**). Adjust the brightness of the lamp with the intensity control dial on the base of the microscope (**Figure 2**). Insure the  **Eyepiece Turret** is on **O** for Bright Field, the **Light Path selector knob = A** (**Figure 3**)for the Eyepieces (right front), the **Annular Diaphragm = A** and that the **NCB filter** is inserted (above the condenser).



Figure 1



Figure 2



Figure 3



Figure 4



Figure 5



Figure 6



Figure 7



Figure 8



Figure 9

2. If you will be using **fluorescence filter cubes**, **Turn ON** the power switch to the **Mercury Lamp Burner**. **Depress** the **Ignition Switch** until you **hear a click** and then **release the switch**. Wait at least **5 minutes** for the mercury-arc lamp to stabilize before using a fluorescence filter cube and **leave it ON** for at least **1 hour**! In addition, it is necessary for the lamp to **cool** for at least **1 hour** before it is turned on again.

3. **Place a sample (dishes/ plates/ slides)** on the microscope stage and **Select the 10x objective**. With the **Filter Block Lever** on "**O**" for Bright Field, **focus** the section using the **Left or Right Fine Focus Knob (Figure 5)**. Adjust **Light Intensity (Figure 2)** using the halogen lamp knob (left front). Never turn the left and right-hand knobs in opposite directions at the same time!

4. **Eyeiece Diopter Focus.** **Close/cover** your **Left eye** and use the **Focus Knob** to view a sharp image with your Right eye. **Close/cover** your **Right eye** and move the **Left Ocular Diopter Adjustment Ring (Figure 6)** until you visualize a sharp image. This is essential to **insure both Oculars** are **correctly focused** for your eyes so the **Objectives** will be **parfocal!** You can insert the photomask into the optical path (front lever) and use the double crosshairs of the photomask to obtain sharp focus.

5. **Objective Focus.** The **20x** and **40x Objectives** have “**Correction Rings**” to compensate for different thicknesses of the bottom of cell culture dishes.

- a. Adjust the scale of the correction ring to the thickness of the bottom plate according to the value provided by the manufacturer of the dishes.
- b. Focus the specimen with the coaxial coarse/fine focusing knob.
- c. If the image has poor resolution and/or contrast, rotate the correction ring slightly clockwise or counterclockwise.

Since the specimen image will become slightly out of focus, adjust the focus with the fine focusing knob.

- d. If the resolution and contrast are improved, rotate the correction ring further in the same direction and re-adjust the focus. If not, rotate the correction ring in the opposite direction. Repeat this operation until the best focus is obtained. Record the scale number for future use.

6. Check **Alignment** of the microscope via **Koehler Illumination.**

- a. **Close** the **Field Aperture (Iris)** located above the condenser until only 1/4<sup>th</sup> of the field of view is illuminated.
- b. **Focus** the edge of the image of the **Field Aperture** using the Condenser Focus Control knob.
- c. If the Field Aperture is not centered, gently move the image to the center of the field of view with the 2 Condenser Centering Screws.
- d. **Open** the **Field Aperture** so that it is slightly larger than the field of view. If you subsequently select a higher objective, such as 40X, it may be necessary to increase the opening of the Field Aperture.

## II. Phase Contrast Imaging:

1. **Replace** the **NCB filter** with the **GIF Filter.**
2. **Select** a **Phase Contrast Objective** and the appropriate **Annular Diaphragm** as follows:

Objective	Diaphragm
10X	Ph1
20X	Ph1
40X	Ph2

3. It may be necessary to **Open** the **Condenser Aperture Diaphragm.**
4. **Return** the **Annular Diaphragm** to **A** when you are finished.

Filters available:

Filter	Application
GIF	↑ Contrast for phase contrast microscopy and monochrome

	<b>imaging</b>
<b>NCB</b>	<b>For normal microscopy and color imaging</b>
<b>ND</b>	<b>For normal microscopy to reduce brightness by 50%</b>

### III. Fluorescence Imaging:

1. Once the **Mercury Arc Lamp** has warmed-up for at least **5 minutes** (according to I-2), **Turn OFF** the **Halogen Lamp** (left front) and **gently Move** the **Filter Cube Lever** (right) to the **desired filter cube**.

<b>Filter Cube</b>	<b>Excitation</b>	<b>Emission</b>
Bright Field (Left)	380 – high pass	420 - High pass
HQ:FITC 41001 606 (Middle)	Blue 465-495	Green 515-555
HQ:TRITC 41002B 609 (Right)		

2. **Remove** the **Shutter** for the **Mercury Arc Lamp** (left side).
3. **Replace** the **Mercury Arc Lamp Shutter** and **move the Filter Cube Lever** to **O** before you **leave**. If the Hg lamp has been **on for 1 hour**, and no one has signed-up to use the mercury lamp right after you, **turn off the power to the mercury arc lamp**.

### IV. The Qimaging Retiga-SRV Digital CCD Camera:

1. Once the Microscope Workstation PC has booted-up, **Turn ON** the **Power** to the **camera** (Button on the left side if the camera)
2. Turn the microscope **Light-Path Selector** (right, front) to **C (100% of signal to the camera) or D (80% to camera, 20% to eye-piece)**, **Double-click** the **Image Pro 6.2 Icon** and **Select camera icon** on the toolbar.
  2. When the imaging window appears, press **“Preview”** button on the top left panel. Press **“Calc Auto Exp”** on the middle left panel for the images to show up in the preview display.
  3. **Focus** the images using **Left or Right Fine Focus Knob**.
  4. Select **“Image” tab** on the middle right panel of the menu. **Select “New Image”**.
  5. Take a picture and save by clicking on the **“Snap”** on the top left panel.
  6. Save and close all the files, then go to **File -> Batch conversion**. Select the files you have created, then convert them to 8 bit TIFF or JPEG to view them with any imaging software.

### V. Before you leave:

1. **Close all Image Files and Close the Image Pro Menu.**
2. **Turn OFF** the **CCD Camera**.
3. **Turn OFF** the **Mercury Lamp (See III-3)**.

4. Turn OFF the Halogen Lamp (left, front) and the TE-300 Power Supply.
5. Insure all the TE-300 settings are for the "Bright Field" Mode.
6. Use lens tissue to clean off the oil-immersion condenser (if used) and any objectives which have contacted the immersion oil. NEVER USE KIM WIPES ON LENS SURFACES!!!
7. Leave the Microscope Workstation in the condition you would like to find it.  
Thank you!