Nikon ECLIPSE TE300 INVERTED MICROSCOPE (CMC)

A. Transmitted Light Operation

1. Turn on power (right side of the MICROSCOPE).

2. Rotate the objective turret to the 10x lens making sure that the stage is lowered enough for the lens to easily clear a slide.

3. Place a microscope slide on the stage with specific holder.

4. Adjust the condenser.

5. Move the specimen using the stage controls until the portion to be viewed is in the optical path.

6. Adjust the interpupillary distance of eyepieces.

7. Locate sample and focus.

8. Rotate condenser to other settings (phase, DIC) as needed.

Align the condenser:

- Rotate condenser to brightfield.
- Close the field diaphragm until visible.
- Focus and center the condenser.
- Adjust the field diaphragm so that it is just outside the field of view.
- Set the condenser aperture diaphragm to ~75% of the NA printed on the objective lens (or remove an eyepiece and adjust the aperture diaphragm until it blocks the outer 25% of the view
- Switch to another lens if desired
- Refocus sample.
- Check and adjust the field diaphragm if needed.

* Be careful when using the 60x and 100x oil lenses. Do not use too much oil. Remove oil with a cotton swab or lens tissue when done. DO NOT GET OIL ON THE 4X, 10X, 20X OR 40 LENSES! If you have, please let a CMRF staff member know immediately.

Olympus DP-71 Digital Camera Operation:

- Then double click [DP controller] (DPController.lnk)

- To get a live image click on the eye icon (or click F7 function key on the keyboard). If the image color on the screen does not match the color visible through the eyepiece, select the color tab and set white balance to [ON]. Then select [One touch]. The curser will appear as an eyedropper over the image. Move the eyedropper to an area of the slide where there is no tissue. Click at this spot.
- Choose the [microscope] tab. Set the magnification to match the objective you are using.

- Choose the [scale] tab. Activate [show scale].

- Choose the [capture] tab. Adjust [image size] to set desired resolution.

- Freeze the image by clicking the camera icon.

- The image is frozen in the DP manager software automatically. Save the image to your online account, flash drive, or *temporarily* to the desktop, then to CD or DVD. Please remove images from the hard

drive when you finish your session. All images saved on the hard drive are subject to removal at any time without notice.

B. Epifluorescence Operation
1. Turn on X-Cite® 120 lamp power supply (120 W High Pressure Metal Halide ARC Lamp.
2. Rotate the objective turret to the desired lens.
3. Move epifluorescence filter slider to desired setting;
   1) P (BF and UV/DAPI)
   2) GFP
   3) Cy3
   4) Cy5
4. Open the shutter (behind the filter box)
5. Adjust the brightness with two ND4 and ND8 filter sliders and observe the sample*.

* When collecting images with the Spot camera exposure times may be several seconds. The time can be reduced by binning (in the **Image Setup** menu) at the cost of resolution

Spot Camera Operation
Align condenser to Köehler illumination as usual and center the region of interest Try to have the light set to at least 6V, 9V being optimum.

1. Turn on computer.

2. Turn on Spot Camera power supply.

3. Start the **Spot Advanced** software (icon on desktop or in start menu). Make sure the lever on the right side of the trinocular is pulled out (light to camera and eyepieces.)

4. Under **Setup** click on **Image Setup** and select the proper image mode (brightfield, fluorescence, DIC, etc.)

   -Select **Modify** to customize the settings if needed (usually not.) These settings will affect the acquired image, not the live image.

5. Click on **Live.** After a few seconds you should see an image. Focus as needed.

6. Move to an empty area and perform a white balance. Click on the **White Balance** icon on the left toolbar and follow instructions.

To acquire and image it is best to use the camera Icon on the toolbar. This will acquire an image using the settings from the **Image Setup** menu. You can also use the **Snap** command from the live window but it will not take advantage of the **Image Setup** process settings, the images will not be optimal and the resolution will be reduced.

Once the image is grabbed it can be annotated and/or adjusted. These choices can be found in the **Edit** menu. Measurements and scale bars can be added and burned into the image. Adjustments such as brightness and contrast are available, but it is probably best to do these later in Photoshop or different imaging software.