UPRIGHT FLUORESCENT MICROSCOPE: Olympus BX60

Operating Instructions:

1. **Turn on PC:**
    a. Press power switch on PC (if not already on)
    b. Login with **user= smic; pswd = B!01ogy**
2. **Turn on the microscope:**
    a. Switch on the back right corner to turn on transmitted light.
3. **For fluorescence: turn on the Mercury lamp.**
    a. The power supply is located to the side of the microscope. Allow the bulb to warm up for approx. 2 min before beginning imaging.

****Note:** it is helpful to first find and focus your specimen using transmitted light, then switch to fluorescence. Use this method (see below) until you are proficient with using fluorescence.

4. **Load your slide onto the stage:**
    a. Lower the stage with the coarse focus knob, and then clip your slide onto the stage.
5. **Select the desired objective lens (usually start with a lower objective 4, 10, or 20x):**
    a. Rotate the objective turret.
    b. Then raise the stage with coarse focus knob (make sure you don't get too close to the lens!!)
6. **Remove any filters/shutters (**See Figure 1):**
    a. Rotate the black 'wheel' above the objective turret until it says 0
    b. Make sure slider is in the 'eye and camera' position (middle stop)
    c. Make sure the polarizer and analyzer are in the 'out' position.
7. **Find and focus your specimen:**
    a. Look through the ocular lenses, adjust the interpapillary distance of the eyepieces, light intensity, and find your specimen using XY stage control on the right hand side.
    b. Focus your specimen using the **coarse focus knob first,** then the **fine focus knob.**
    c. Go up to a higher magnification (20 or 40x) if necessary.
8. **For OIL OBJECTIVE: 100x**
    a. First focus your specimen at 40x. Then swing the 40x objective out of the way, but only a partial turn.
    b. Place a drop of immersion oil on the slide, and then swing the 100x objective into place, to immerse it in the drop of oil.
    c. **Use only **FINE FOCUS** from here on.
9. **Capturing an image:**
    a. Once you have located a desired area of your slide, you can either

take an image (see Imaging Instructions below) or switch to fluorescence.

Fluorescence Instructions:

1. Turn off transmitted light.
2. Rotate the filter wheel to your desired filter for your specific sample. (Green for CY3, TRITC; blue of Alexa488, FITC; UV for DAPI, etc.)
    a. You should see a colored light coming through the objective lens (i.e. blue, green)
3. Find an area of your specimen that you want to image using the XY stage control and fine focus knob.

Imaging Instructions:

1. Pull out slider to block the light to the objective lens (right side level with the oculars)
    a. Third stop, camera icon
2. Open the software on the computer: **cellSens Entry**
3. Click the **live** button to turn on the camera
4. Adjust the exposure by moving the slider to right until your image appears properly exposed
    a. The exposure normally can be set as **auto** in bright field image mode and as **manual** in fluorescent image mode
5. Normalize background:
    a. For Bright field images: on the top right toolbar, click the white dropper icon
    b. For fluorescent images: on the top right toolbar, click the black dropper icon
    c. Then select an area of 'background' on your image slide by drawing a small box encompassing only the background. This sets the write or black balance for your image and reduces background noise.
6. Focus your image to the camera using the fine focus if needed
7. Scale Bar:
    a. Make sure you have the correct value for the reducer/magnifier lens tab
    b. Select scale bar by going to **view** drop down on the top menu and click on **Scale Bar**
8. Click the **Snapshot** icon button to capture an image (next to the live button)
    a. Save your image under the **user** folder on the desktop (if you have scale bar on your images, when you save the images as .tiff, the images will have two layers; when you save the images as .jpeg, the images will only have one layer)
9. When finished, close the software and turn off the microscope and mercury lamp.

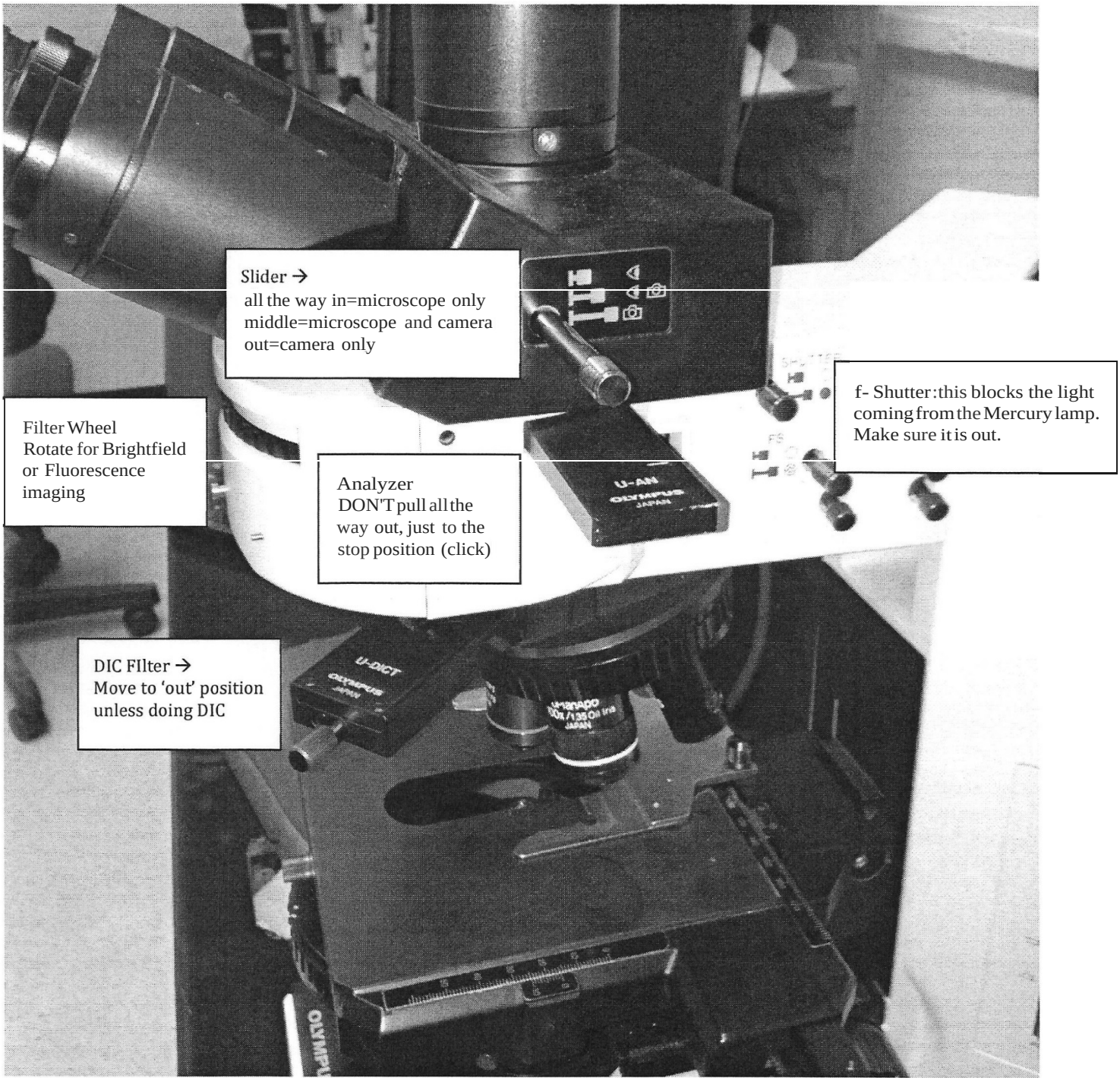

Fig. 1: Slider, Filter Wheel, Analyzer, Shutter and DIC