INVERTED FLUORESCENT MICROSCOPE: Olympus IX70

Operation Instructions:

1. **Turn on PC:**
   a. Press power switch on PC (if not already on)
   b. Login with user= smic; pswd = BIOlogy

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 on the shelf to the right 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, Petri dish, or other specimen holder onto the stage:**
   a. Place the area you want to image directly over the hole where the objective lens is.

5. **Select the desired objective lens (usually start with a lower objective 4, 10, or iOx):**
   a. Rotate the objective, turret by turning the wheel on the lower LEFT (see Fig. 1).

**Note: it is under the stage!

6. **Remove any filters/shutters**:
   a. Rotate the black 'wheel' under the shutter on the lower right until it says 0 (see Fig. 2).
   b. Make sure slider is in the 'eye and SP' position (see Fig. 3).

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 wand 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 60x objective:**
   a. Make sure you don't ram the objective lens into the bottom of your specimen (slide, petri dish, etc.). Lower the Objective turret with the coarse focus knob if you think it's going to touch the sample.

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 Imaging 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.) (See Fig. 2 for filter wheel)
   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. Select scale bar by going to view drop down on the top menu and click on scal bar tab.
   b. Make sure you have the correct value for the object lens

8. Click the Snapshot icon to capture 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 images as .tiff file, the images will have two layers; when you save your images as .jpeg file, the images will only have one layer)

9. When finished, close the software

10. Turn off microscope and mercury lamp.

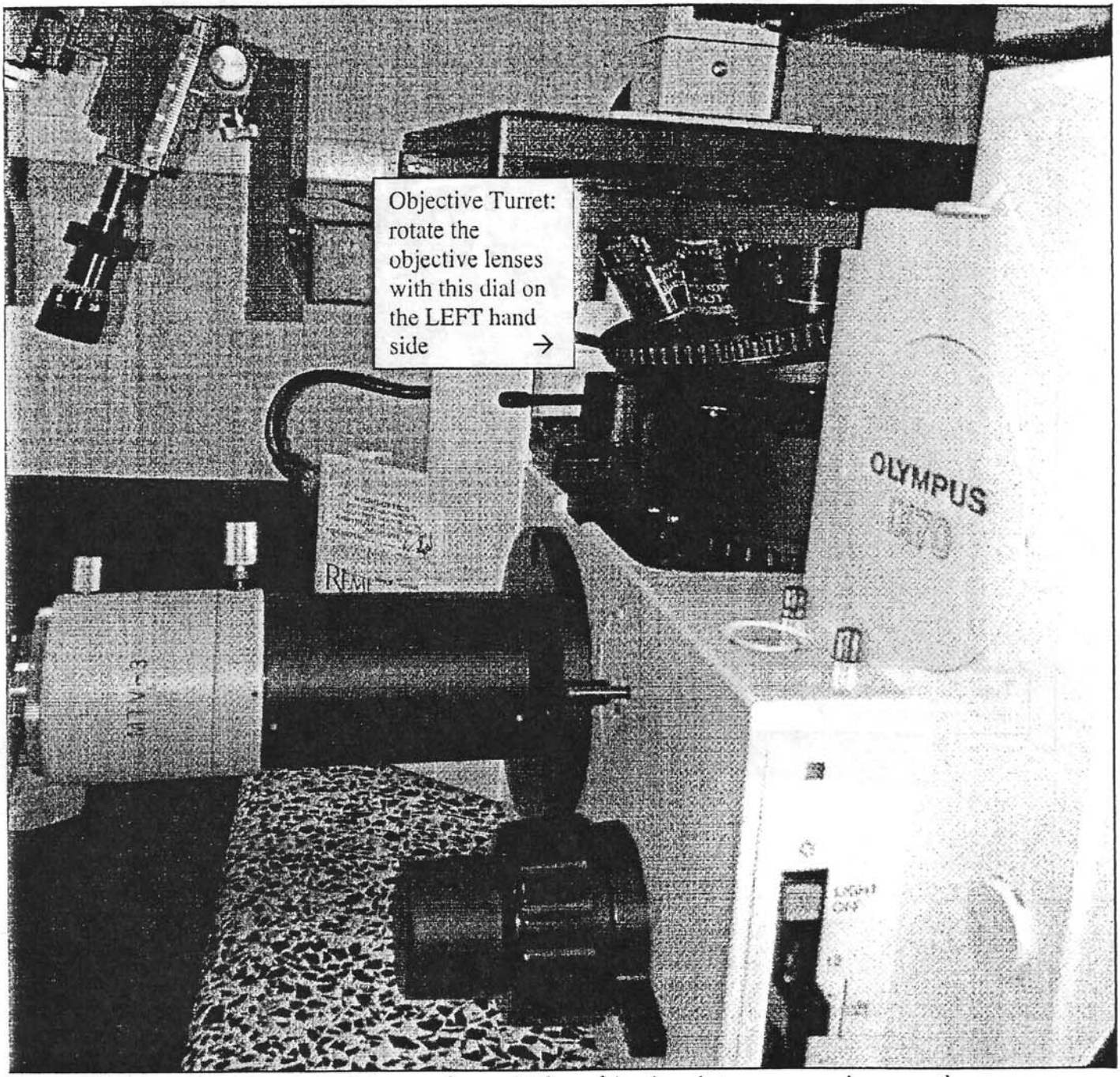

Fig. 1: Objective Turret. Be careful not to bump higher objectives into your specimen on the stage. Lower the objective turret (coarse focus knob) when going to higher objectives!

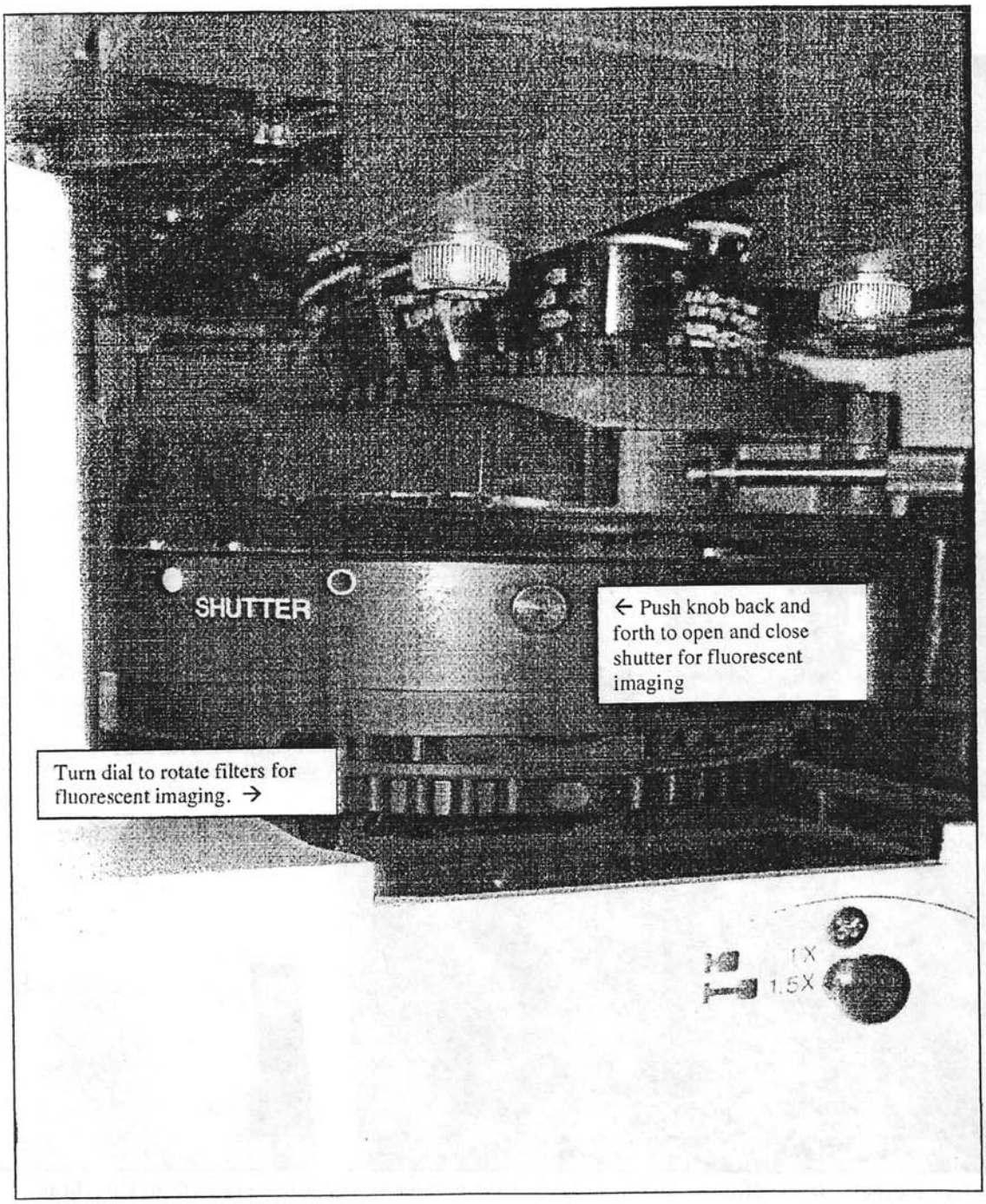

Fig. 2: Filter wheel for fluorescent imaging. Turn to 0 for normal brightfield imaging, and a colored filter for fluorescence. Make sure shutter is in the 'Open' position.

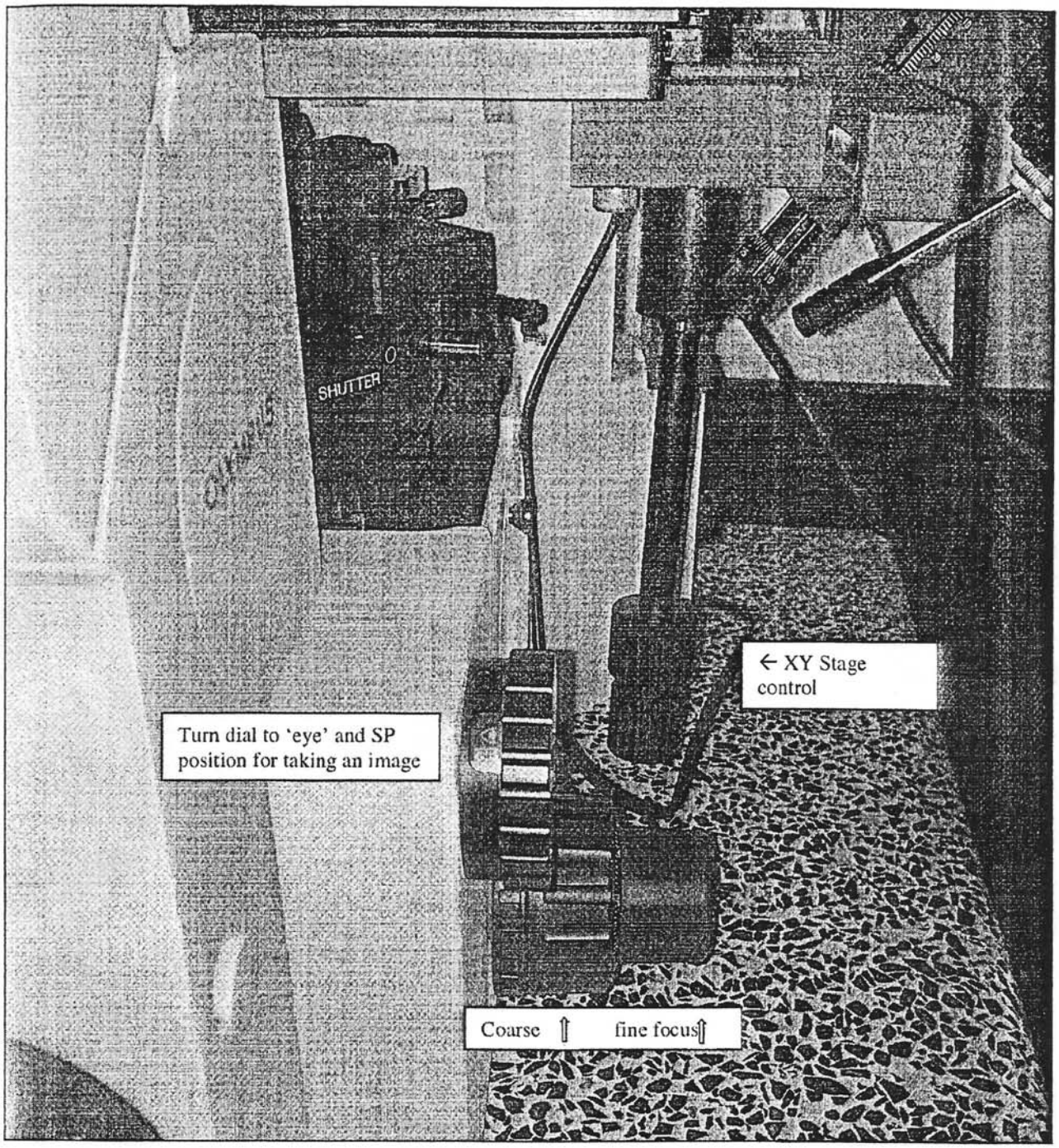

Fig 3: Turn Dial to 'eye' and SP for imaging with DP Camera (the 'camera' icon referring to the 35mm camera (on the front port) is obsolete). Also, don't confuse the microinjector arms with the XY stage control wand.