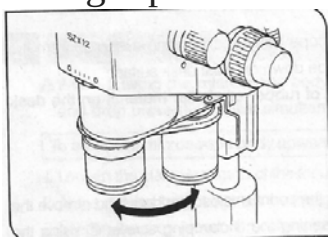
RESEARCH SETEOMICROSCOPE: Olympus SZX-12

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. Turn on transmitted light (NCL 150 unite).
3. **For fluorescence: turn on the Mercury lamp.**
    a. Turn on the power supply of the mercury lamp (Olympus U-RFL-T). Allow the bulb to warm up for approx. 2 min before beginning imaging.

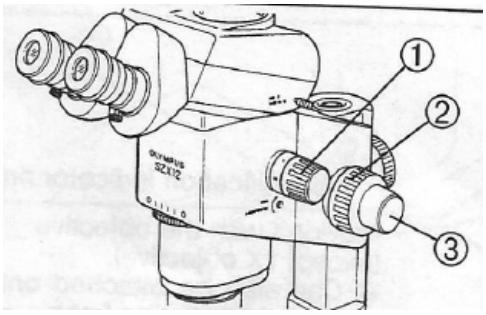
****Note:** it is helpful to first find and focus your specimen using transmitted light, then switch to fluorescence.

4. **Place your sample on the stage**
5. **Select the desired objective lens**
    a. Hold the objective and gently rotate it until a click position where the objective to be used is engaged in the light path



6. **Remove any filters/shutters**
    a. Rotate the black 'wheel' above the objective turret to open position
    b. Make sure the slider on the right side of microscope level with oculars is on the 'eye and camera' position
    c. Make sure the slider on the right side of the microscope below the oculars is on “O” position
7. **Find and focus your specimen:**
    a. Look through the ocular lenses, adjust the interpapillary distance of the eyepieces.
    b. Set the zooming knob 1 to the lowest zoom magnification and bring the microscope into focus by rotating the coarse focus adjustment knob2
    c. Rotate the zooming knob1 1to the desired magnification and precisely focus the microscope on the specimen with the coarse focus adjustment knob 2

and fine focus adjustment knob 3.



8. **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. Move the slider on the right side of the microscope below the oculars to choose your desired filter for your specific sample. (Green for CY3, TRITC; blue of Alexa488, FITC; 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 and focus the sample

Imaging Instructions:

1. Open the software on the computer: **cellSens Entry**
2. Click the **live** button to turn on the camera
3. 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
4. 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.

5. Focus your image to the camera using the fine focus if needed
6. Scale Bar:
    a. Make sure you have the correct value for the reducer/magnifier lens
    b. Select scale bar by going to **view** drop down on the top menu and click on **Scale Bar** tab
7. 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)
8. When finished, close the software, remove your sample, turn off transmitted light and mercury lamp