Nikon Eclipse L200N Microscope, Camera and Imaging Software

Sign up for training on the CNF users web site using the Education and Training link

Start the NIS Elements D Software

NIS Elements D can control the microscope in addition to capturing images.

Get the simple controls layout by clicking the **Docked Controls** tab at the bottom of the screen to . The simple layout is adequate for almost all work at CNF.

Set White Balance with a White Object

Show quickly-updated images using the menu item **Acquire** → **Live - Fast** (or type **+** or click ). Image a white object such as the frosted part of a microscope slide. Perform the Auto White Balance operation by clicking the **AWB button** just above the image.

Image Your Sample

Setup

Show quickly-updated images in the NIS Elements D software using the menu item **Acquire** → **Live - Fast** (or type **+** or click ).

Set the lowest magnification by pressing the **L button** repeatedly on the microscope or on the **L200 pad** in the software. Position the sample and raise the stage.

Use Focusing Target for Polished Surfaces

When you have a sample with a polished surface, use of the focusing target will facilitate focusing.

Pull out the focusing target lever on upper left side of microscope to put the focusing target in the optical path. Looking through the eyepieces, focus until edges of the

crosshair target look sharp. Remove the focusing target by pushing in the target lever, and the sample will also be in focus.

Stage Motion

Move the stage by hand with the handle and clutch on the right, then use the stage motion knobs for fine motion control. Set higher magnification by Pressing the **H button**.

Bright Field Microscopy

- Press the **NCB** (neutral color balancing) and **ND** (neutral density) filters **IN**.
- Slide the **Analyzer and Polarizer OUT**.
- Press the **BF/DF rod IN** for bright field microscopy.
- Slide the **DIC slider OUT**.
- Adjust the light level with the **LAMP** dial and adjust the aperture stop with the **A. S.** buttons.

Dark Field Microscopy

- Observe the sample using bright field microscopy.
- Pull the **BF/DF rod OUT** for dark field microsopy.
- Adjust brightness with the **LAMP** dial and **ND** (neutral density) filters.

Differential Interference Contrast (Nomarski) Microscopy

Differential Interference Contrast (DIC) microscopy is used to enhance the contrast in non-pigmented, transparent samples. DIC works on the principle of interferometry to gain information about the optical path length of the sample, to see otherwise invisible features.

- Observe the sample with bright-field microscopy.
- Adjust brightness with the **ND (neutral density) filters**.
- Press the **Polarizer and Analyzer IN**.
- Press the **DIC slider IN**.
- Rotate the **DIC slider knob to position A** (which is indicated on the objective).
- **Rotate the DIC slider knob** to change the background color.
- Adjust brightness with the brightness control (**LAMP**) dial.

Preview and Capture image

In the NIS Elements D software, show higher quality images by running **Acquire** → **Live - Quality** (or type **`Ctrl-+`** or click ).

Focus while watching the image. **To adjust fine focus, you can display a profile through the image**: in the **Intensity Profile** window, click on the → button to display a profile along a horizontal line; profile position can be moved up and down on the image with the mouse. Adjust the fine focus knob to get the sharpest peaks and valleys in the profile.

You can clamp the stage after focus is obtained with the large, thin knob on the left side of the microscope.

Acquire → **Capture** (or type **`Ctrl--`** or click ).

Add scale bar and annotation

Display the image scale bar by clicking the **Show Scale button**  to the right of the image.

Adjust scale bar thickness, size, orientation or color by **right clicking on the scale bar** and pick **Scale Properties** to .

Insert the scale bar permanently into the image by **right clicking** on the scale bar and pick **Burn Scale**.

Add annotation on the annotation layer, which contains annotation objects and measurement objects, by clicking the **A button** to the right of the image. Then use **Edit** → **Insert Text...**, press the **Insert** button, then **click on the image** to position the text.

To add an arrow, click the **A button** to the right of the image to turn on the **annotation layer** which contains annotation objects and measurement objects. Then use **Edit** → **Insert Arrow...,** press the **Insert** button, then **click on the image** to position the text.

Use the same method to add other annotation objects if you wish.

Measurements

Open the manual measurement control window by running the **View** → **Analysis Controls** → **Annotations and Measurements** command. Select a tool corresponding to the feature you are going to measure. There are several tools for measuring each feature. Measure the objects in the image using the mouse.

Save file in an uncompressed format on your USB drive

Save the file as bitmap (.bmp), TIFF (.tif), PNG (.png) or Lossless JPEG2000 (.jp2) by running **File** → **Save As...**. *Avoid lossy compressed formats such as JPEG (.jpg).* Save the

files on a USB drive. Check that images include annotation and scale bars by opening the saved file with NIS Elements or another image viewing program.

Delete image files on the PC to save disk space and prevent other people from viewing them.

Postprocessing

For image processing beyond adding a scale bar and measurement arrows to the image, we suggest that you use software such as ImageJ, the MATLAB image processing toolbox, or the Python Imaging Library (PIL) on another computer. It is also possible to use PhotoShop with more effort (see J. Sedgewick., *Scientific imaging with Photoshop – methods, measurement, and output*. Berkeley, CA: New Riders, 2008.
http://www.imagingandanalysis.com/).