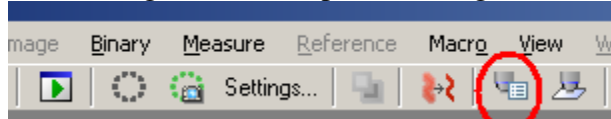


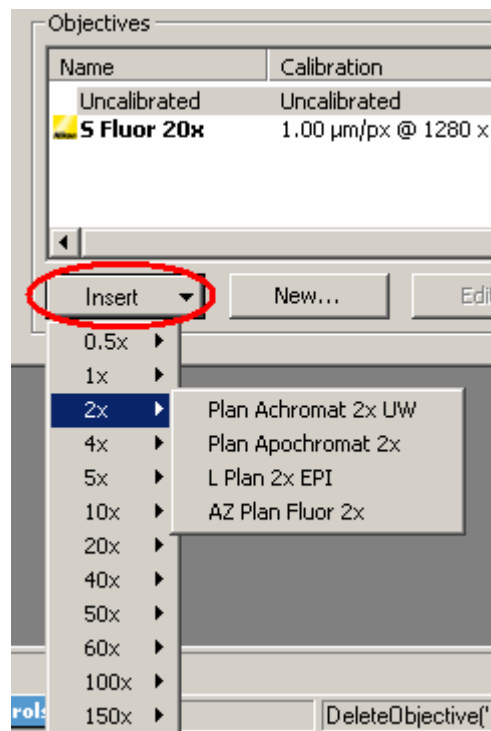
Calculated Objective Calibration for NIS Elements

To calibrate an objective in NIS elements using the calculation method you'll need to know the objective magnification, whether you are using a 1x or 1.5x tube lens setting, the adapter magnification for your camera and the camera pixel size.

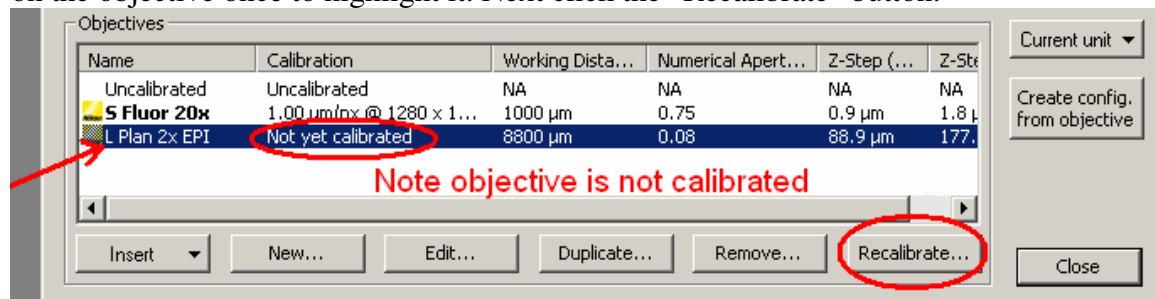
1. With elements running click the “Optical Configurations” icon:



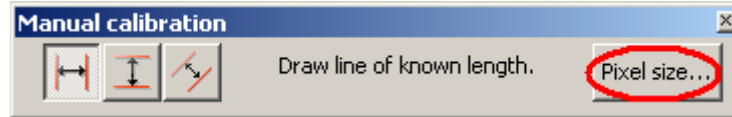
2. The optical Configurations window has an upper and lower portion. The lower “Objective” portion is where we will be adjusting settings. At the bottom of this area is an “insert” button. Click this and select the objective you want to calibrate from the list of available objectives.



3. The added objective will now appear on the list of available objectives. Click on the objective once to highlight it. Next click the “Recalibrate” button.



4. A live preview window will now appear and a new window will pop up. In the new window click the button that reads “Pixel Size...”



5. In the textbox provided, enter the calibrated value of a single pixel. (See “How to calculate Calibration” below.

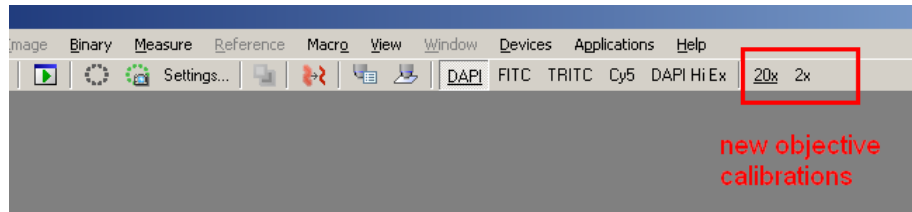
6. When finished press OK. The window will close and the objective calibration will now read showing a “Um/Pix” ratio.

7. Next we need to create an optical configuration for this objective. To do this click the button that reads “Create Config from Objective”.

8. In the new window that appears you can adjust the name of the configs and press OK when complete.

9. Repeat steps 2 through 8 as needed to add objectives to your system.

10. To use these new calibrated optical configurations when acquiring new images make sure the one you want is selected before acquiring an image. Any new objectives added will appear as buttons below the file menu to the right of the optical configuration button:



11. You can also calibrate old images. To do this open the image you wish to calibrate, right-click on the image and select “Image Properties” from the list that appears. Next select the drop-down button that says “Optics” and select your calibrated objective. Make sure to re-save the image!

How to calculate Calibration

In order to enter the pixel value requested in the routine above we need to find the effective pixel size as it is reduced through the microscope to the sample. To do this we need to find the total magnification of the microscope, and we need to know the pixel size.

To find the total magnification we need:

1. Objective Magnification
2. Tube Lens Magnification (Always 1x unless there is an adjustment for this on your microscope – most scopes don't have an adjustment.)
3. Camera Adapter magnification (ranges from 1x to .5x – check the mount between the camera and the microscope body for a magnification number.)

For this example the Objective will equal 10x, tube lens 1x and camera adapter .63x.

Total Magnification is then calculated by:

$$(\text{Objective Mag} * \text{Tube Lens}) * \text{Camera Adapter}$$

$$\text{Or in this example } (10x * 1x) * .63x = 6.3x$$

So the total magnification of the optical system is 6.3x.

Next we need the camera pixel size. In this example we will use 6.45um.

Take the camera pixel size / optical system magnification or:

$6.45\text{um} / 6.3 = 1.024\text{um}$. This means the size of one pixel at the sample is 1.024um, so **our calculated calibration is 1.024um per pixel.**

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