CytoViva – Setting up Scale Bars in Ocular

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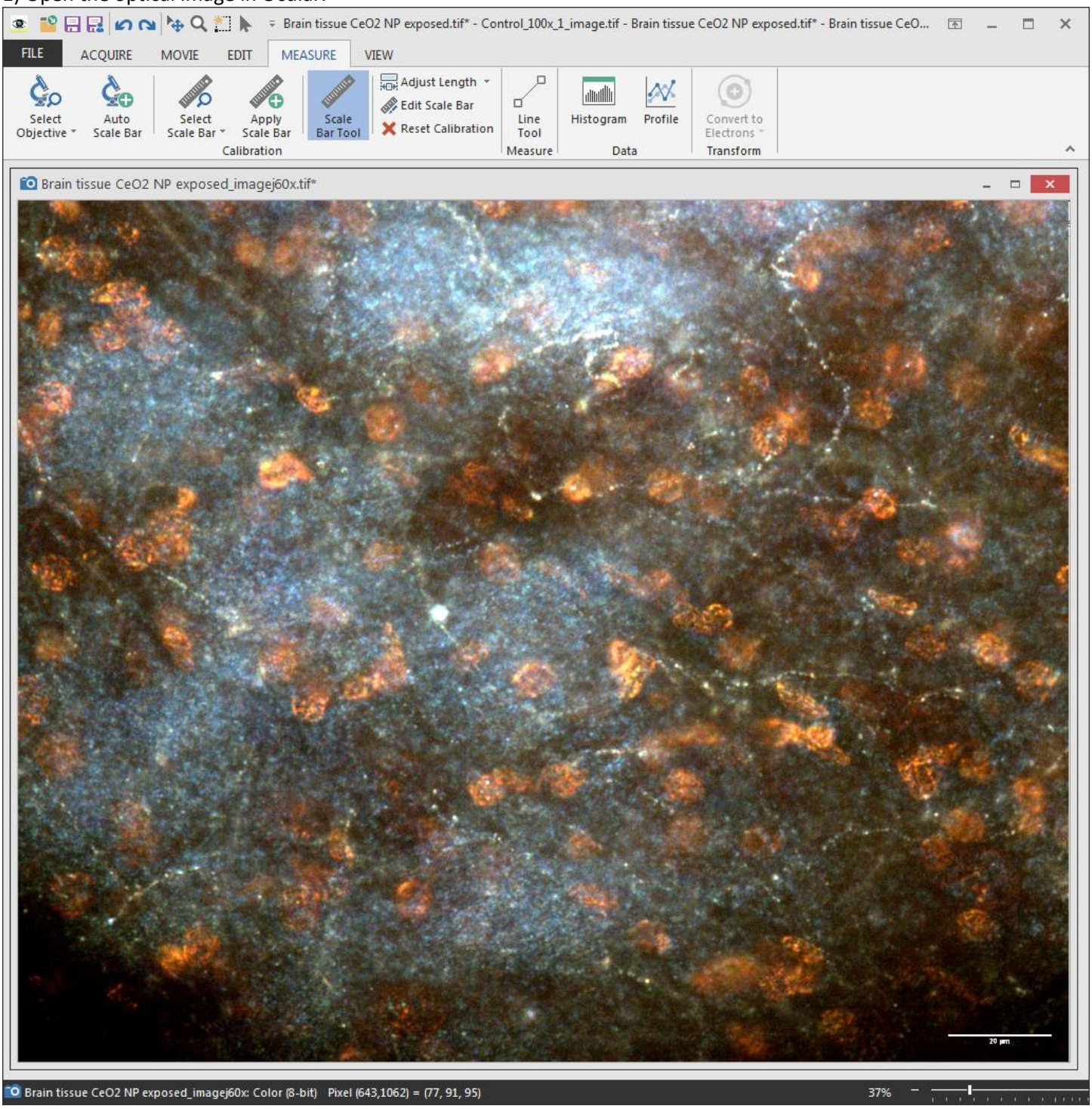
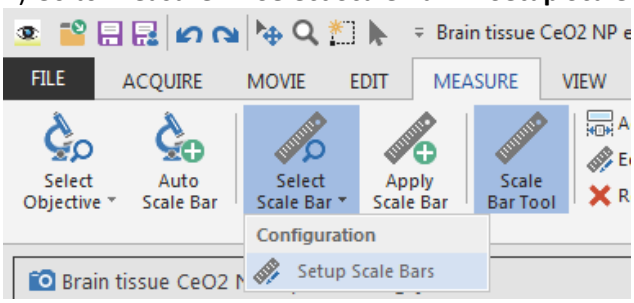
The following instructions illustrate setting up scale bars for images captured using a Retiga R6 optical camera at 60x magnification.

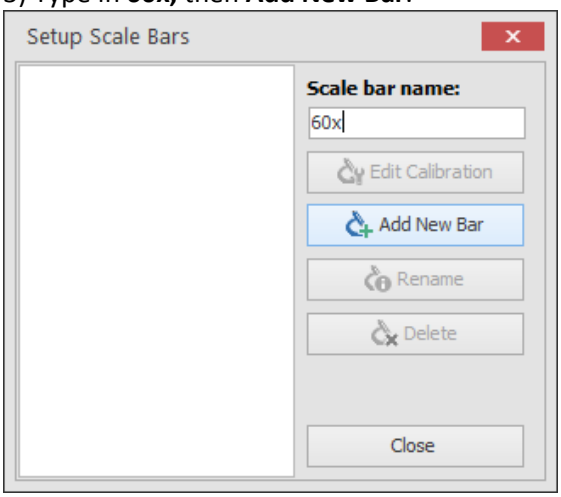
1) Open the optical image in Ocular.



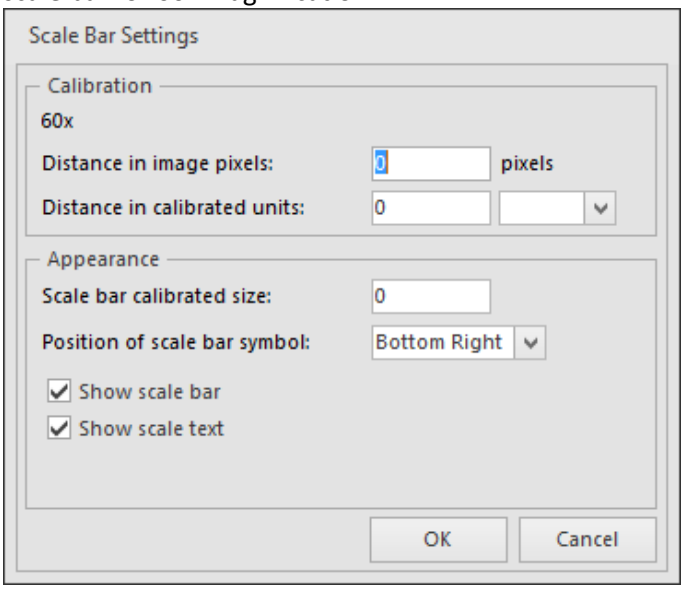
2) Go to **Measure** >> **Select Scale Bar** >> **Setup Scale Bars**.



3) Type in **60x,** then **Add New Bar**.



4) Next, we need to enter the calibration values for the scale bar we are setting up. In this example we are setting up a scale bar for 60x magnification.

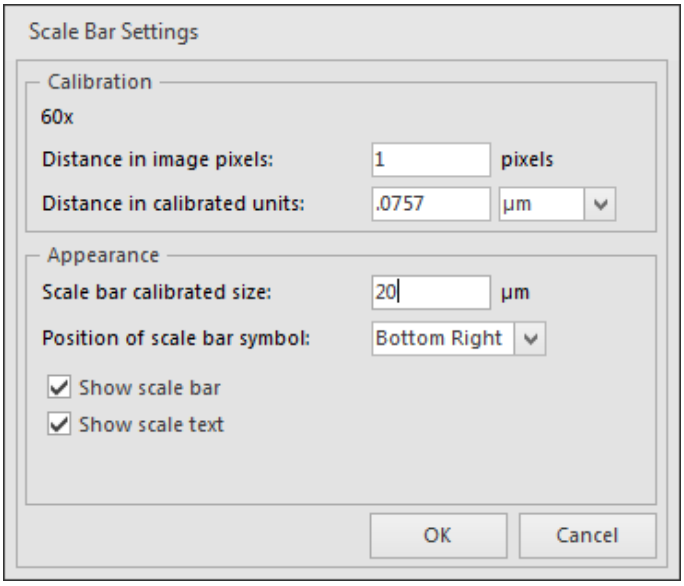


5) Reference the pixel resolution chart and select the proper value for 60x. Note that if you want to set your scale bar in nm, use the nm value and select nm from the drop-down menu. In this example, we will use um (micron). From this chart, we will need value .0757.

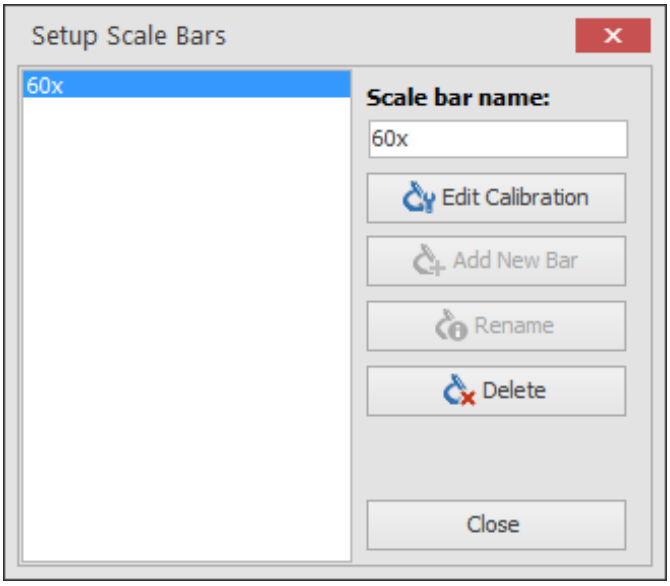
	Retiga R6 QI695 CCD 4.54 um Pixel Size No Binning		
Magnification	nm/pixel	um/pixel	nm Distance
1x	4540	4.54	0.220
4x	1135	1.135	0.88
10x	454	0.454	2.20
20x	227	0.227	4.41
40x	113.50	0.1135	8.81
50x	90.80	0.0908	11.01
60x	75.67	0.0757	13.22
100x	45.40	0.0454	22.03

6) Here we have entered the following values:
Distance in image pixels = 1 (*this means the next value is the distance for one single pixel*)
Distance in calibrates units = .0757 um (*this means that one pixel at 60x magnification is .0757 um, or 75.67 nm*)
Scale bar calibrated size = 20 (*this means that the scale bar displayed will be 20 um in length*)

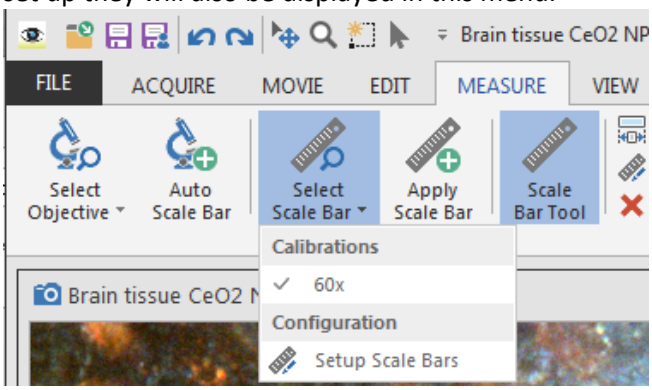
Once all values are entered, select **OK.**



7) Select **Close**.



8) Go to Measure, Select Scale Bar, then select the desired scale bar calibration from the list. In this example we have only established one scale bat for 60x magnification so this is selected by default. If additional scale bar calibrations are set up they will also be displayed in this menu.



9) Select **Apply Scale Bar** and the scale bar will be displayed. Note that in this example we included a previous scale bar applied in imageJ for reference and verification of the scale bar calibration.