This exercise uses the small Zeiss microscope and the EO camera connected to micromanager.

1. Start Micromanager and choose the MMconfig_demo.cfg configuration file.

2. Obtain an “image” with two different acquisition times. Explain the differences in the two images. (Note this can be done from home).

3. Perform a Kohler alignment on the microscope.

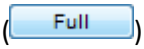
4. Change configuration file to MMconfig_uEye.cfg.

5. Take an image of the micro ruler, for the 10x objective. Based on the image, calculate the size of each pixel. Change the calibration in the ImageJ settings for the MM Snap/Live preview window (Image/Properties).

6. Set MM to run in “Live” mode.

7. Using the 10x objective, adjust the light intensity and locate the microruler on the glass slide.

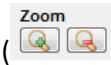
8. Choose an exposure time that is between 10-40 ms.

9. Select the LUT on MM to saturation mode (), select “Full” scale bit resolution (

) and eliminate red (over saturated) and blue (undersaturated) pixels.

10. Make sure that you have no undersaturation or oversaturation with exposure times of 10 or 40ms, respectively.

11. Using ImageJ, draw a line perpendicular to all 100 divisions on the microruler and do a line profile. Determine the number of pixels for each 1mm (the total size of the microruler) and then set size calibration in ImageJ (Image/Properties). Note: it should be around 0.45 um/pixel

12. Use the “Zoom” functions (

) in MM to see approximately 5-10 lines on the microruler.

13. Using ImageJ, draw a line perpendicular to at least 5 divisions on the microruler and do a line profile (with the live setting to see the results in real-time).

14. Very carefully adjust the fine focus knob on the microscope to have the steepest slope and the highest contrast between the bright and dark fringes of the microruler.

15. Click “copy” and paste the data in an excel spread sheet.

16. Plot the data using Excel, and find the maxima and minima for the peaks (I_{max}, I_{min})

17. Calculate the “Modulation” $M=(I_{max}-I_{min})/(I_{max}-I_{min})$. Read more about modulation here.

18. Defocus the microscope and copy the data for the plot profile again. Calculate M.

19. Refocus the microscope

20. Make the same measurement after changing the exposure time to 10, 15, 20, 25, 30, and 40ms.

21. Plot:

a. M vs. exposure time

b. I_{max} vs. exposure time

c. I_{min} vs. exposure time

22. Plot b and c on the same graph. Add a trend line, show the equation and show the R^2 value.

23. Using the equations from b and c, make a plot for predicted M over the range of exposure times from 0-100ms. Plot this predicted curve on the same curve as a (data for M vs. exposure time).