**Calibration**

1. Determine the background threshold

2. Estimate the brightest value of the focus spot (0-255)

3. Estimate the 1/e radius of the focus spot (sigma)

4. Use artificial\_gaussian.m to Generate artificial images of similar spots, set sigma based on step 3, and I0 based on step 2

5. In spotsize\_integral.m, set threshold based on step 1, sigma0 based on step 3

6. In spotsize\_integral.m, set calibration to 1, and select artificial images to calibrate, replace percent0 with the value returned in command line, then set calibration to 0

**Measure Spotsize**

1. Run spotsize\_integral.m, make sure calibration is set to 0