HOW TO - Measure the experimental lateral resolution of an optical system by imaging fluorescent beads and extracting their full width half maximum (FWHM).

1. Calculate the theoretical lateral resolution with the Airy formula :

   $\Delta = \frac{1,22\lambda}{NA_{obj}}$

   Where Δ is the lateral resolution, λ is the wavelenght of the excitation light and NA_{obj} is the numerical aperture of the objective.

2. Make a slide with fluorescent beads smaller than the lateral resolution (step 1) to make sure the system images beads' point spread function.

3. Acquire in focus images of fluorescent beads with the system you want to characterize. If the focus is hard to find, acquire z-stacks. Make sure to have around 10-15 beads to measure.

4. Extract datas from images of step 3 in Fiji :

   1. Analyze > Set scale... Indicate the right pixel number by microns. It could be the pixel width of the camera found on the datasheet or an experimental measure made previously with known width beads larger than the lateral resolution.
   2. Open the in focus image of beads in Fiji.
   3. Zoom on a bead to measure.
   4. Use the line tool to make a line on the bead to cover its diameter and a bit more.

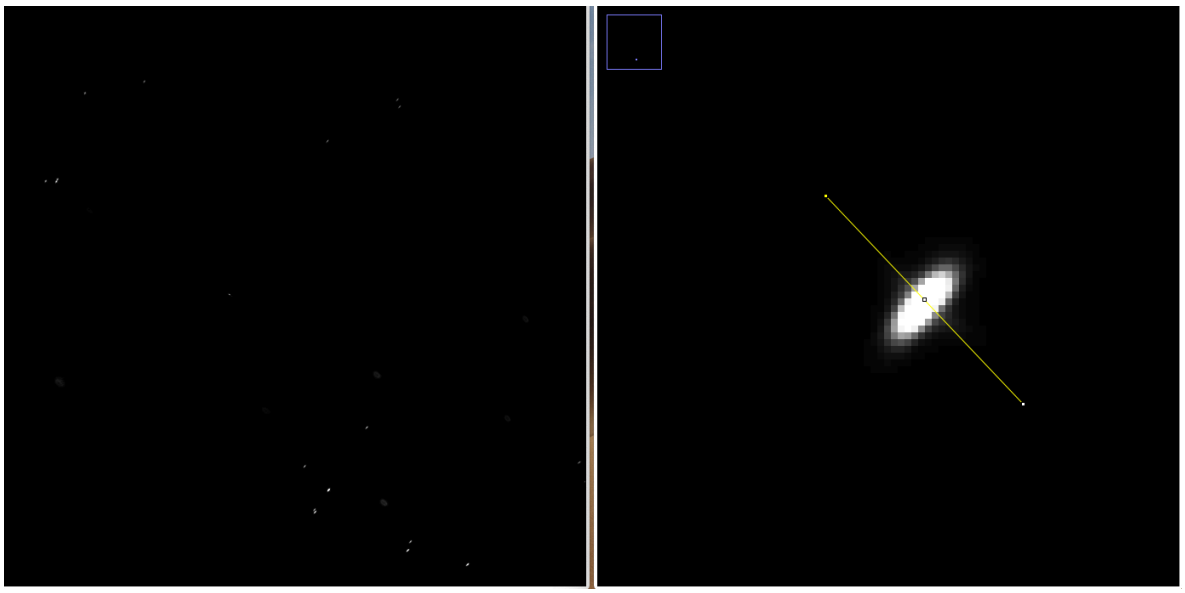

Figure 1 : In-focus image of 200 nm fluorescent beads (left) and the same image zoomed on a single beads (right). A line is drawn on the latter to produce the *plot profile* of step 4,6.

5. Ctrl+t can be used to save the position of the line in case z-stacks were acquired. This line is needed to avoid as much uncertainty as possible between each frame of the z-stack.
6. Ctrl+k. A *Plot profile* appears that shows the intensity according to the pixel number.
7. Click on the *List* button on the *Plot profile* window. Select and copy all the points in the list with the right click on the mouse and select *Select All* (see Figure 1).
8. Analyze > Tools > Curve Fitting... Paste the list in the box of the window named *Curve Fitter* (see Figure 2).
9. Choose *Gaussian* as the type of curve to fit the list of points. Make sure that the *Show Settings* box is checked before clicking on the *Fit* button (see Figure 2).

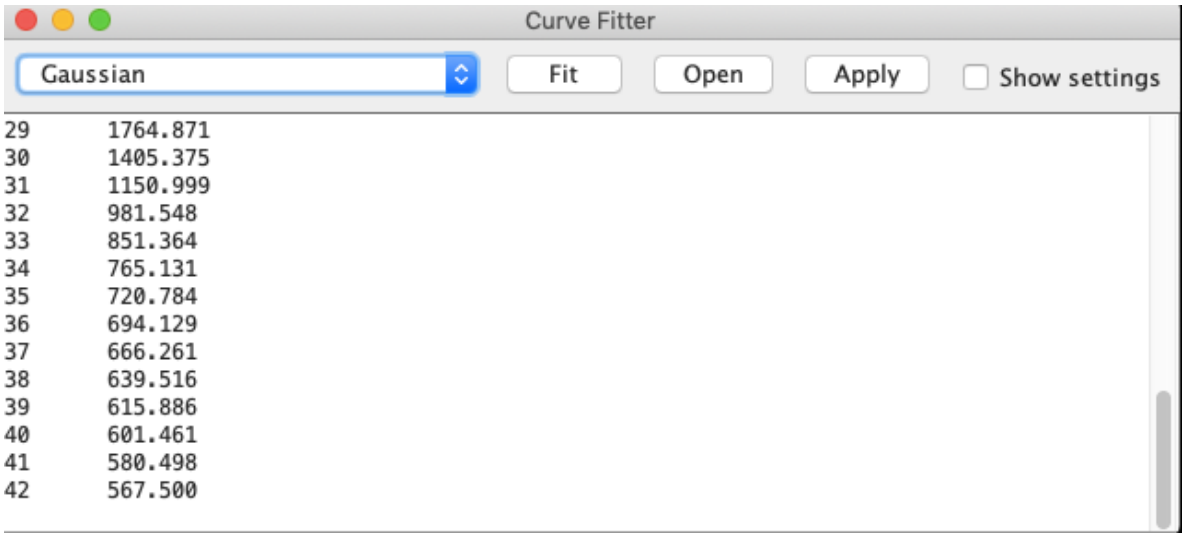

Figure 2 : Curve Fitter window on Fiji with points of a gaussian function in the principal box.

5. Calculate the FWHM with the python code *FWHM calculation with Gaussian Fitting.py* found on GitHub > DCC-Lab > HiLoZebrafish > FWHM_GaussianFittingCurve :
    1. In the *Log* window on Fiji, the values of the *a, b, c* and *d* parameters are written for the present curve fitting. Match those values to the appropriate variables in the python code (*a* in Fiji goes with *a* in the python code, etc.).
    2. Run the python code.
    3. Values of the *a, b, c* and *d* parameters, roots of the gaussian function and FWHM are printed in the terminal. Those datas can be saved in a excel file, for example.
    4. The mean FWHM of mutiple in focus beads smaller than the lateral resolution is the experimental lateral resolution.