Analysis 1: Correcting for unevenness of illumination.

1.1.1. Prepare solution of any fluorophore, e.g. 1 µM Fluorescein isothiocyanate (FITC) in BRB-80. Such solution can be prepared in advance, aliquoted and stored at -20°C.

1.1.2. Assemble a chamber with a regular coverslip. Add fluorophore solution and seal the chamber using VALAP.

1.1.3. Collect > 50 images of the entire microscope field: move the stage to a new unbleached area while the illumination shutter is closed, and acquire the images immediately after opening the shutter.

1.1.4. Create average projection of this stack and filter with Gaussian blur with 5 pixel radius using ImageJ or other software. The resulting image represents the distribution of the illumination intensity of the field (*Illum(x,y)*, where *x* and *y* correspond to pixel's coordinates.

1.1.5. Determine the maximum pixel brightness of this image (*Max(Illum)*).

1.1.6. With the closed illumination shutter and using same camera settings, acquire one image, determine the average pixel intensity of this image; this value corresponds to CN, camera noise.

1.1.7. Use the above values and image (*Illum(x,y)* to normalize the experimental image *Img(x,y)* using the following expression:

$$img^{norm}(x,y) = \frac{Max(Illum) - CN}{(Illum((x,y) - CN)}(img(x,y) - CN)$$

Use the resulting image $Img^{norm}(x,y)$ for the quantitative analysis of the brightness of the stationary fluorescent complexes, and also to normalize the images with tip-tacking complexes.