Protocol to measure brightness of the beads coated with GFP protein

This protocol was prepared by Katya Tarasovetc, Eirini Zhou and Ivan Gonchar. MATLAB program was written by Nikita Gudimchuk and Vadim Mustyatsa.

Current program was designed to measure brightness of fluorescent objects (e.g. microbeads). It can be useful to compare the amount of fluorescent protein immobilized on bead surface, as in (Gudimchuk et. al, 2018; Chakraborty et. al, 2018). User needs to collect images of investigated objects in two channels: fluorescent and non-fluorescent (e.g. DIC or Brightfield), then save tiff. images in a numbered file sequence (GFP_1, GFP_2,… and DIC_1, DIC_2, …). Imaging in two channels is needed so that the bead brightness is collected using unbiased approach: beads are selected in the non-fluorescent channel and their brightness is then measured in fluorescent channel.

Program function:

The program measures integral fluorescent intensity of a bead (selected with a rectangular region) and of a background area located near the bead. User first defines the size of a rectangular region to encompass the bead (e.g. 9x9 pixels), and then selects each bead by clicking on its center in non-fluorescent channel image. The program shows the region around the bead, then it automatically positions second rectangular region of the same size but on the right side near this bead. The program automatically saves results in an Excel file, which is located in the folder with input images.

User instructions:

1. Open MATLAB.

2. Open the folder containing MATLAB “beads_brigthness.m” program. Copy the folder path at the top of the window, e.g., “G:\Experiments\180601\GFP beads”.

3. Paste the path on top of the "Editor" window in MATLAB. To open the program, click on “beads_brigthness.m” that will appear on the left of the MATLAB window.

4. In the "Editor" window, go to line #20, delete DIC_ without deleting any other symbols or spacing, and write the **sequence** name of the image files you want to analyze in the non-fluorescent channel. Go to line #56 and replace GFP_ with the **sequence** name of images taken in the fluorescent channel. If only fluorescent images are used, enter the same file names in both places (lines #20 and #56).

6. To adjust contrast of the non-fluorescent images, go to line #25 in the "Editor" window and change numbers inside the brackets (minimum/maximum intensity). Adjust contrast of the fluorescent images analogously on line #75. Contrast values of images in Image J can be used as a guide.

7. Enter the input information for the program: the pathway to folder with images, which will be analyzed; and size of the region of interest by typing the “Command” window: beads_brigthness(‘pathway to images’, region size in pixels).

9. The first non-fluorescent image will open. Select bead one by one by left clicking on their images, and making sure that cursor is positioned at bead center. You should *only* select those beads that do not have any other beads or bead aggregates on the *right* side from the selected bead. This is important because the program will measure background intensity within the rectangular region located on the right side of the bead. When finished selections, right click anywhere on the field. This will open image of the same field but taken in the fluorescent channel. In this image, all selected beads

will be shown within the rectangular regions together with the background selections. Right click anywhere on the field to move to the next non-fluorescent image.

10. After the last image is analyzed, open folder with these images to find Excel file "e_table". This file contains the following information:

Column A – number of the analyzed bead; column B – number of analyzed image, containing this bead; columns C and D – X and Y coordinates of the bead; column E – integral brightness of the bead square; column F – integral brightness of the background square; and column H – background adjusted brightness of the analyzed bead.

11. Also, in MATLAB the average bead brightness (average of column H), SEM and N (number of beads) will be calculated automatically and provided in "Command" window.

References**:**

Gudimchuk, N., Tarasovetc, E. V., Mustyatsa, V., Drobyshev, A. L., Vitre, B., Cleveland, D. W., Ataullakhanov, F. I. & Grishchuk, E. L. Probing Mitotic CENP-E Kinesin with the Tethered Cargo Motion Assay and Laser Tweezers. *Biophys. J.* **114,** 2640–2652 (2018).

Chakraborty, M., Tarasovetc, E. V. & Grishchuk, E. L. In vitro reconstitution of lateral to end-on conversion of kinetochore–microtubule attachments. *Methods in Cell Biology* **144,** (Elsevier Inc., 2018).