

## ProMoIJ Quick User's Guide

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### 1. Installation.

- 1.1. Download required plugins from <http://www.achucarro.org/downloads>, unzip, and save the corresponding files to the Fiji plugins folder.
- 1.2. Download ProMoIJ from <http://www.achucarro.org/downloads> (ProMoIJ), unzip, and save the ProMoIJ folder to the Fiji plugins folder.

### 2. Folders

Folders where temporary analysis files will be saved may be downloaded from <http://www.achucarro.org/downloads> (Folders Example). Save original images to be analyzed in the "Original" folder.

### 3. Open ProMoIJN

Open Fiji and access ProMoIJ by clicking on the Plugins menu and then on the ProMoIJ submenu (Plugins ▷ ProMoIJ ▷ Menu ProMoIJ ↓).

### 4. Macro 1: Cropping.

The use of this cropping step is mandatory to select the channel to be analyzed.

- 4.1. Select input and output folders.
- 4.2. Select parameters (crop cells, channel, Z-slices and time-frames) and click OK.
- 4.3. If the option Crop cells was selected:
  - 4.3.1. Select cells in the green and red color-coded image.
  - 4.3.2. Add selections to the ROI manager by pressing the letter "t".

### 5. Macro 2: Registration.

- 5.1. Select input and output folders.
- 5.2. Select parameters.
  - 5.2.1. Use optimization mode to optimize registration. An appropriate registration is achieved when the soma of microglial cells remains almost static through all time-frames.
  - 5.2.2. Register images using optimized parameters ("Register all images with the current settings" option in the "Optimization mode" window).

### 6. Macro 3: Image corrections

- 6.1. Select input and output folders.
- 6.2. Select parameters.
  - 6.2.1. Use optimization mode to optimize images correction. It is recommended to use the smallest minimum sigma (under 3) and the highest maximum sigma that adequately subtract noise and background.
  - 6.2.2. Correct images using optimized parameters ("Correct all images with the current settings" option in the "Optimization mode" window).

**7. Macro 4: Process selection**

7.1. Select input and output folders.

7.2. Select parameters.

7.2.1. Use Optimization mode to select the threshold level used to identify static regions (red pixels in the image after clicking OK). This threshold should be higher than the threshold used for analysis in Macro 5.

7.3. Select processes using optimized Threshold ("Select processes using the current Threshold" option in the "Optimization mode" window) and annotate the Threshold used.

7.3.1. Select a process by drawing a ROI around it. It is recommended to draw the ROI as small as possible. Selected process should be confined within the XYZT stack of images and include a constant region (highlighted in red).

7.3.2. Add selection to the ROI manager by pressing the letter "t" and then click OK.

7.3.3. Indicate the orientation of the process by drawing an arrow pointing from a constant basal region of the process to its tip (orientation vector).

7.3.4. Add orientation vector to the ROI manager by pressing the letter "t".

7.3.5. Repeat steps from 7.3.1 to 7.3.5 to select more processes.

**8. Macro 5: Motility analysis.**

8.1. Select input and output folders.

8.2. Select parameters.

8.2.1. Main menu (basic parameters):

ID	Name	Possible values	Recommended value	Description
M5.1*	Time interval	0 - ∞	N/A	Time interval between frames, in minutes
M5.2*	<b>3D Gaussian sigma</b>	0 - ∞	1	3D smoothing (higher values = smoother image)
M5.3*	<b>Skeleton smoothing range</b>	0 - ∞	2	Range (in pixels) for 3D skeleton smoothing, (higher values = smoother skeleton)
M5.4*	<b>Maxima threshold</b>	0 - ∞	N/A	Intensity threshold used to identify a process
M5.5	Advanced menu	<input type="checkbox"/> /√	<input type="checkbox"/>	Advanced parameters (Suppl. Table 1)
M5.6	Optimization mode	<input type="checkbox"/> /√	√	Optimization and parameters saving

8.2.1.1. Use Optimization mode to check the match between the original process and its reconstructed 3D skeleton in several processes (5-10) from different images. If there is not adequate match, first try changing parameters from M5.2 to M5.4 (M5.2 and M5.3 parameters should never be above 2 and 4, respectively). If the problem persists (no match between reconstructed skeleton and process) try changing advanced parameters.

8.3. Analyze motility using optimized parameters ("Analyze all processes using current parameters" option in the "Optimization mode" window).