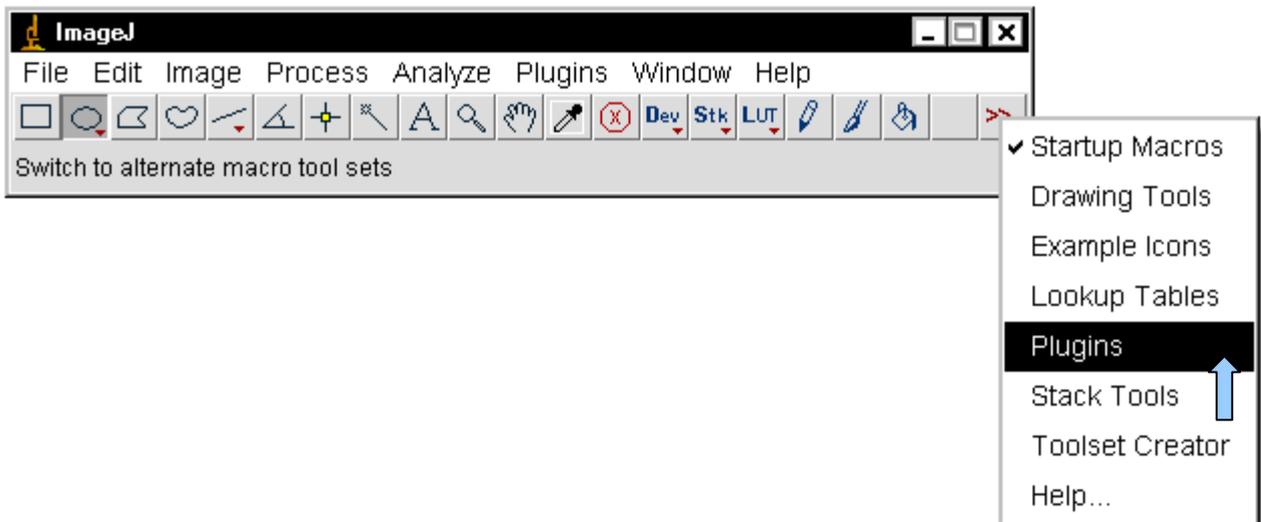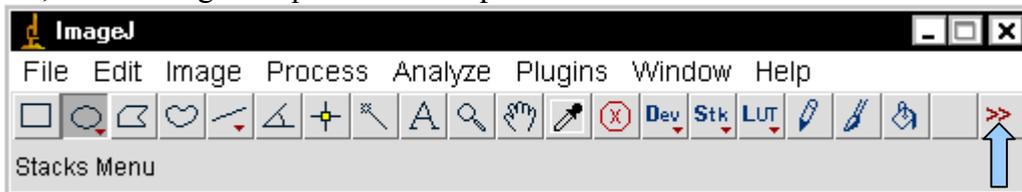# AutoFRAP macro description and instruction.

About.

This little macro is a wrapper for FRAP Profiler plug-in from MBF-ImageJ collection (http://www.macbiophotonics.ca/imagej/intensity_vs_time_ana.htm). It facilitate mass analysis of FRAP movies. It requires ImageJ 1.39 or higher, FRAP_Profiler plugin (it can be downloaded either from our site http://cellbiol.genebee.msu.ru/research.html or directly from http://www.macbiophotonics.ca/imagej/intensity_vs_time_ana.htm) and at least two time-series images of same dimensions, one of which containing FRAP data and the other with background data. FRAP data MUST contain FOUR prebleach images.

Installation and Launch.

Being downloaded from our site (http://cellbiol.genebee.msu.ru), the zip-folder contains the following: this pdf-document, macro file (autofrap_.txt), source code of FRAP Profiler plug-in (FRAP_Profiler.java) by MBF and the compliled FRAP_Profiler plug-in file (FRAP_Profiler.class).
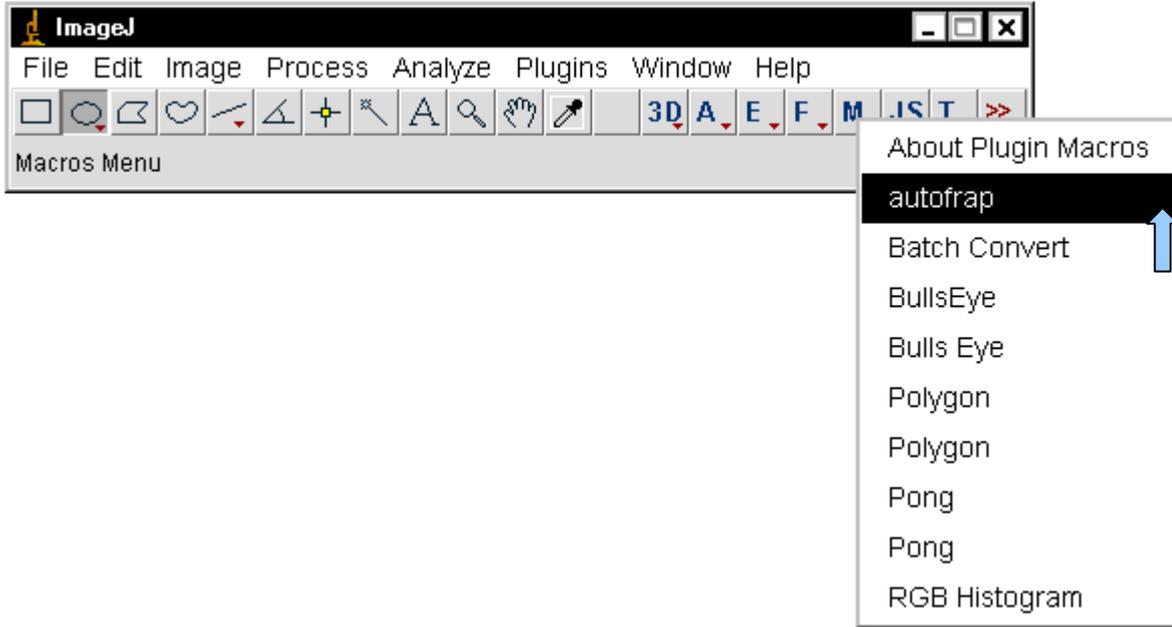
Copy *FRAP_Profiler.class* to your *ImageJ/plugins* folder, copy *autofrap_.txt* to your *ImageJ/plugins/macros* folder. Restart ImageJ. To find autoFRAP macro click at «>>» icon in icon bar, select «Plugins» option from drop-down menu.

Icon bar will switch to the following appearance.
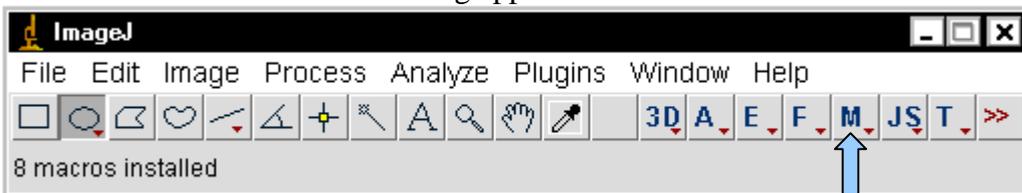
AutoFRAP macro hides in drop-down menu under «M» button. Run it by selecting it.

Outline and instruction.

Being launched, macro displays a «Guide» dialogue. Read it.

After clicking «Ok» you would be prompted to select image containing BACKGROUND data, then to open CELL data image. After files are opened, macro do the following:

1) Subtract background data from cell data frame by frame.

2) Extract first four images into separate stack.

3) Make average image of extracted frames.

4) Substitute prebleach images with their average.

At this point macro displays the frame containing bleached cell and a message that encourages you to manually select bleached area. By default the oval selection tool is picked. By clicking «ok» button you are confirming selection and the selected area is added to ROI manager. Second message appears calling to select the entire object (cell, nucleus, etc.). And again, by clicking «ok», you send new area to ROI manager and macro automatically calls for FRAP Profiler plug-in and displays results. At this point you can examine results, copy numerical data to another program, and by clicking «ok» continue to run the macro. Steps 1-4 are called «a measurement round».

5) Dialogue will appear. Read it. If you click «ok» macro would close all windows except ROI Manager and Log an go for next measurement round. You would be prompted to select another file with cell data that shares the same background properties. Clicking «cancel» quits macro.

Known issues:

1) Don't close windows at the end of measurement round. Doing so will abort macro. Macro will close them automatically if you choose to continue running it.