

**Supplementary Table 1: Primer Sequences.** List of all primers used for cloning. Primer sequences are given (5' to 3').

Primer name	Sequence
HindIII-GFP for	GACCCAAGCTTGATGGTGAGCAAGGGCGAG
PstI-GFP rev	CGACCTGCAGGATCATTATCACTTGTCCAGCTCGTCCATG
Sall-HTTex1-for	TGCAGGTGCACAGCGACCCTGGAAAAGCTG
BamHI-HTTex1rev	GTTCGGATCCGGTCCGGTGCAGCGGCTCC
KpnI-RFP-for	ATTGCGGCCGCATGGCCTCCTCCGAGGACGTC
NotI-RFP-rev	CTATAAGGTACCGGCCCGGTGGAGTG GCG GCC
Sall HTTex1 D2f	TCGGTTCGACATGGCGACCCTGGAAAAGCTGATGAAGG
BamHTTex1 D2r	AGCGGATCCGGTCCGGTGCAGCGGCTCCTCAGC

**Supplementary data: FRAP Macro for ImageJ: Region detection and mean intensity measurements in time series data.**

The **DZNE FRAP Macro** for Fiji [13] measures the mean fluorescence intensity of a moving object over time. The region of the object is computed for each time frame by dynamic intensity thresholding.

The user defines a masking channel for automatic cell region detection. This channel should contain a rather stable and homogeneous signal of the cell region. Cytoplasmic markers independent from the FRAP reporter that are not exposed to bleaching are an ideal choice. However, the FRAP reporter channel itself can also be used as masking channel under certain conditions.

To detect the cell region, a median filter is applied to the masking channel. The median filter smoothens the image in space and time. Smoothing over time is especially important when using the FRAP reporter channel as masking channel, because it performs a simple bleaching correction.

After smoothing, an intensity threshold  $t_0$  for each frame is computed, by using ImageJ's default auto threshold method. The threshold can be adjusted globally by the weight  $w$ :

$$t = t_0 \times w$$

Pixels with intensity values above  $w$  (in the mask channel) are defined as cell region. All other pixels are defined as background. To validate automatic cell region detection, cell region is outlined in blue on the mask channel. Finally, mean fluorescence intensity of cell region pixels is calculated for each time frame in the user defined measurement channel.

Some hints for parameter adjustments:

- **If possible, keep parameters stable! Try to find one “best compromise” setting for the whole dataset.**
- Increase the “**threshold weight**” to obtain smaller cell regions.
- Decrease the “**threshold weight**” to obtain larger cell regions.
- Increase the “**median filter radius time**” if there are unwanted “jumps” of the detected cell region from frame to frame.
- Decrease the “**median filter radius time**” if the detected cell region seems to “run after” the cell.
- Increase the “**median filter radius xy**” if the detected cell region is not smooth.
- Decrease the “**median filter radius xy**” if the cell outline is not precise enough.

### How to use it

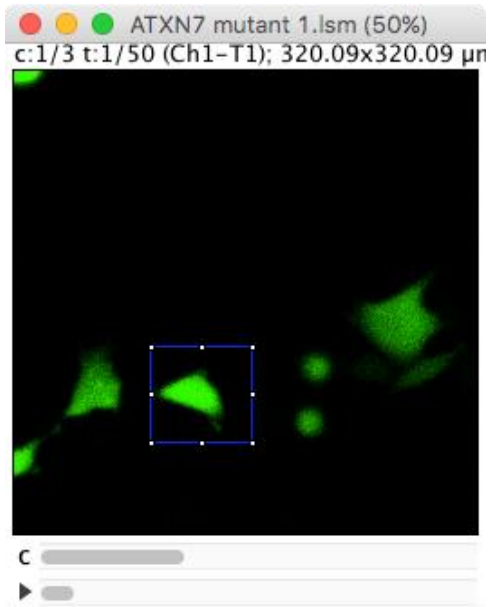
- Open Fiji
- Load the “**DZNE FRAP Macro**” by opening **frap.ijm**: *File >> open*.

```

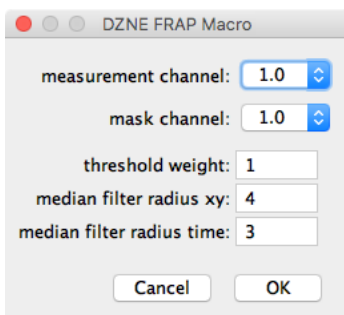
1 //+++++
2 //+++++
3 //DZNE FRAP Macro
4 //Image and Data Analysis Facility, Core Facilities, DZNE Bonn
5 //Author: Christoph Möhl
6 //Date: 27/06/2016
7 //+++++
8
9 macro "DZNE FRAP Macro"{
10
11
12 //+++++
13 //initialization
14 //+++++
15
16 resetThreshold():

```

- Open a multichannel time series in Fiji
- Define a region of interest with the rectangle selection tool



- Press “Run” in the macro editor window to start the macro
- Select the channel for masking and the channel for fluorescence intensity measurement. Adjust parameters for smoothing and thresholding.



- Inspect results: The macro produces 3 result windows.
- **Overlay\_masking\_channel:** displays the region that was measured with a blue outline on the channel that was used for defining that region. Check if the region was detected with sufficient precision. If not, run the macro again and adjust *threshold weight* and/or *median filter radius*.
- **Overlay\_measurement\_channel:** displays the channel where fluorescence intensity was measured. Please ensure, that the right channel for measurement was selected.
- **Results:** Displays the mean fluorescence intensity for each frame inside the analysis region (blue outline) on the measurement channel.