TRACMIT User Guide

TRCACMIT 1.0

https://github.com/lacan/TRACMIT

Contents

Installation

Dependencies

As you complete the installation steps below, make sure that the following update sites are enabled as well:

- **IBMP-CNRS** Contains the ActionBar Plugin by Jerôme Mutterer.
- **PTBIOP** Contains the BIOPLib and attached plugins used for managing. TRCACMIT's settings and other internals.
- **Imagescience** Contains the FeatureJ Laplacian Plugin used by TRACMIT.

Using Fiji Update Sites

The simplest way to install TRACMIT is to use the TRACMIT Update site through Fiji:

1. From Fiji, go to **Help > Update...**
2. Select **Manage Update Sites.**
3. Click on **Add Update Site**, this will create a new line on the table.
4. Change the Name to "**TRACMIT**", for clarity's sake.
5. In the URL column, enter or paste **http://biop.epfl.ch/TRACMIT/**
6. Click on **Close.**
7. Finally click on **Apply Changes** and **restart Fiji.**
8. After these steps, you should find **TRACMIT under Plugins > ActionBar.**

Manual Installation

We do not recommend manual installation as TRACMIT depends on multiple packages that would become difficult to manage outside of the Fiji Update Site solution.

Sample Dataset

To test TRACMIT, you can download a sample dataset from ZENODO with the following DOI:

https://doi.org/10.5281/zenodo.232218

Interface

ActionBar User Interface

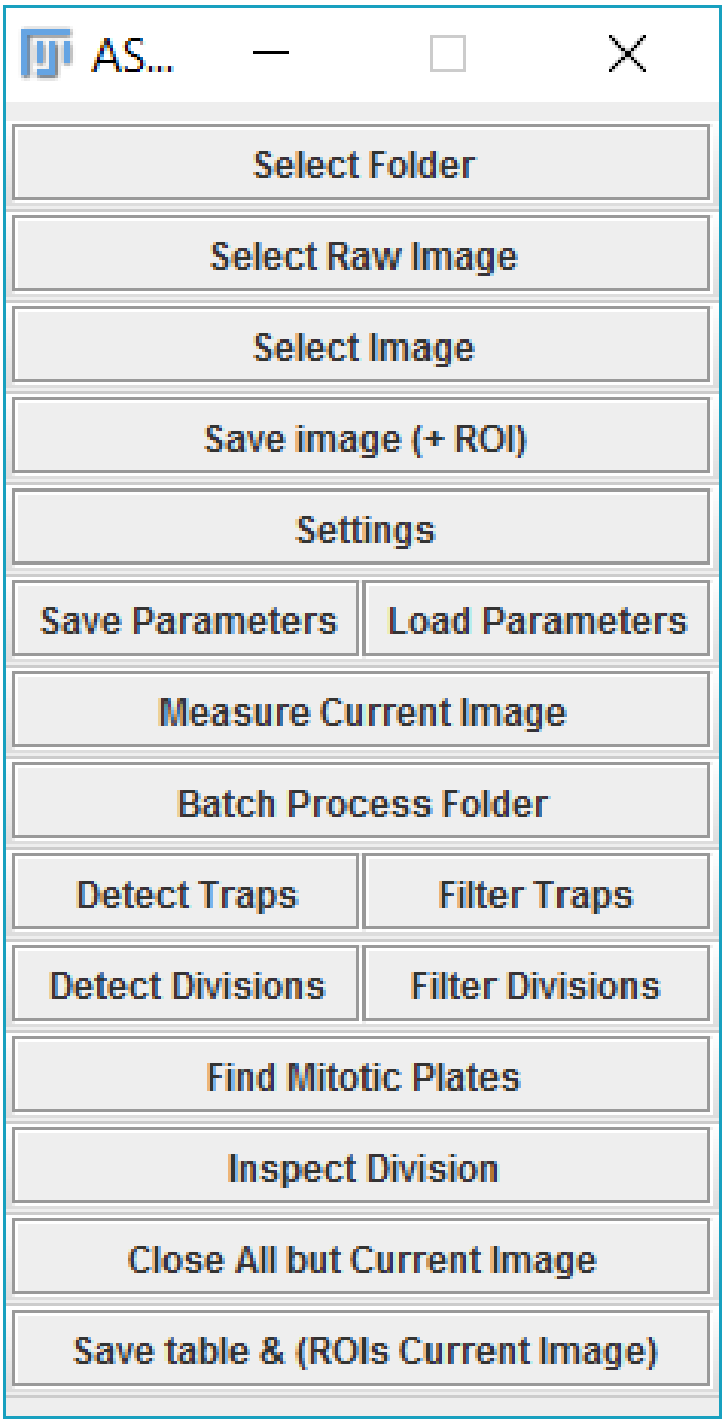


Image Handling

Access all ASMIT Settings

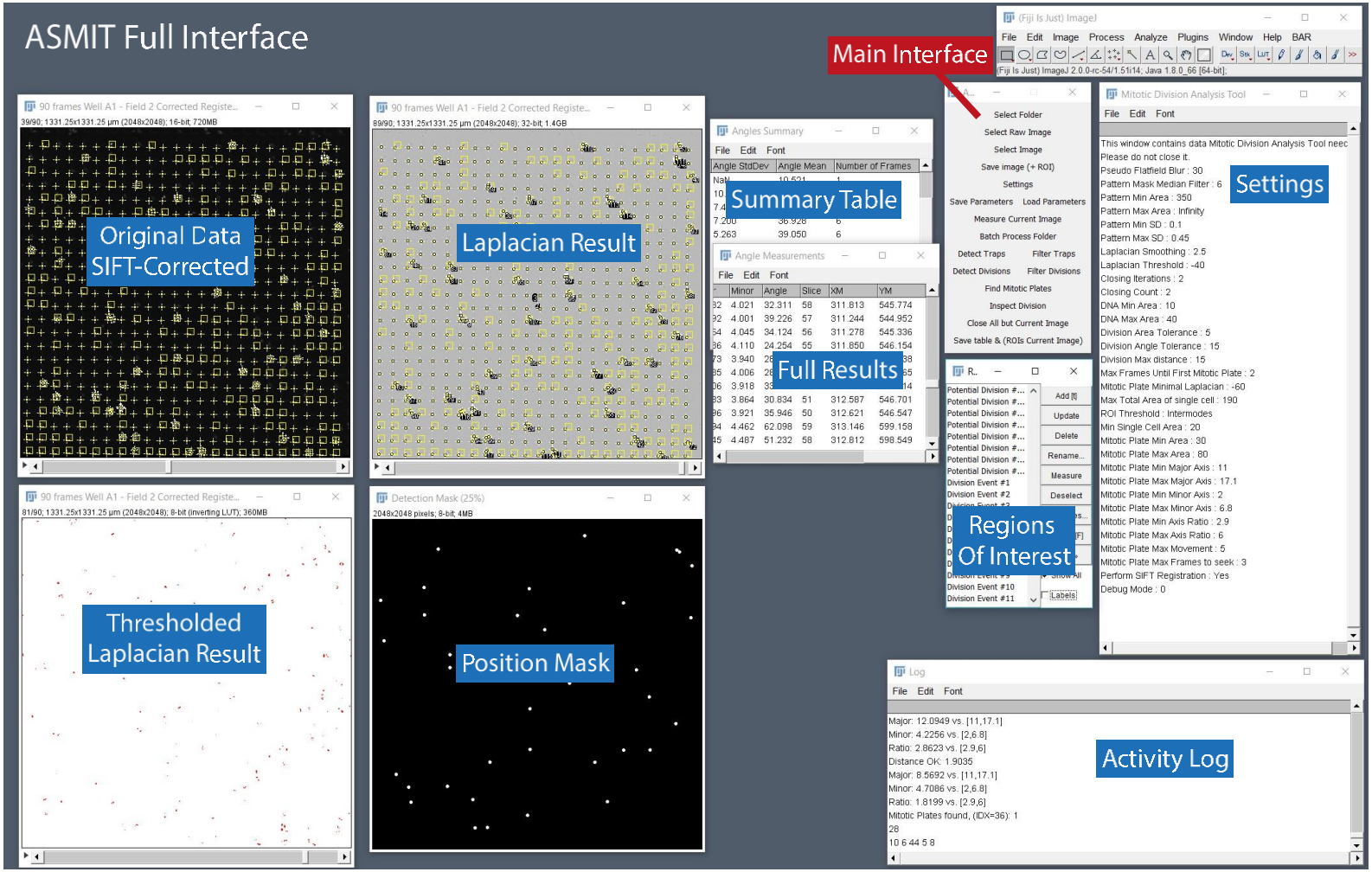
Full Analysis of Current Image Stack

Run Each Step Individually
(Useful for initial parameter setting)

Inspects Currently Selected ROI

Convenience Shortcuts

Full Interface when running



When TRACMIT is running, several Windows work together as shown above.

- **Original Data** contains the SIFT-Corrected original dataset loaded with Select Image or Select Raw Image.
- **Laplacian Result** is the 2D Laplacian of Gaussian timelapse used throughout TRACMIT.
- **Thresholded Laplacian Result** contains the binarized Laplacian Image used for extracting shape features.
- **Position Mask** helps TRACMIT check if a division event was already detected at a certain location, to avoid duplicate detections.
- **Full Results** contains all the data on the detections for each detection at each frame.
- **Summary Table** is also available, with one line per division event.
- **Regions Of Interest** is the ROI Manager of ImageJ with all detections named (See Regions of Interest Manager section).
- **Settings** contains a list of all the variables used by TRACMIT. Regions of Interest Manager
- **Activity Log** helps the user follow the progress of TRACMIT.

Regions of Interest Manager

At the end of processing, the ROI Manager contains 5 Types of ROIs:

- **Detected Traps**: a series of points that should match the bottom left of the patterns. (See main publication, Figure 2 Step 5).
    - **Parameters used**: Pattern Mask Median Filter, Pattern Min Area, Pattern Max Area.

- **Selected Candidates**: a series of points that represent the patterns kept after Standard Deviation Filtering. (See main publication Figure 2 Step 8).
    - **Parameters used:** Pattern Min SD, Pattern Max SD.

- **Position Masks**: an ROI showing where the divisions will be searched for within the stack. (See main publication, Figure 2 Step 8).

- **Potential Division #X**: Each ROI consists of two points that indicate the objects that were interpreted by TRACMIT as being anaphase figures. (See main publication, Figure 2 Step 12). You can visit them by clicking on the **Inspect Division** Button.
    - **Parameters used:** DNA Min Area, DNA Max Area, Division Area Tolerance, Division Angle Tolerance, Division Max Distance, ROI threshold, Max Total Area of Single Cell, Min Single Cell Area.

- **Division Event #X**: Is a point ROI showing the stack position of a division event where a metaphase plate was found and backtracked. You can visit each division event by seleting the desired ROI and clicking on the **Inspect Division** Button. (See main publication, Figure 2 end of step 16)
    - **Parameters used**: Max Frame until first mitotic plate, Mitotic Plate Minimal Laplacian, Mitotic Plate Min Area, Mitotic Plate Max Area, Mitotic Plate Min Major Axis, Mitotic Plate Max Major Axis, Mitotic Plate Min Minor Axis, Mitotic Place Max Minor Axis, Mitotic Plate Min Axis Ratio, Mitotic Plate Max Axis Ratio, Mitotic Plate Max Movement, Mitotic Plate Max Frames to seek.

Parameter Optimization

As the interface provides a way to perform each step individually, you can optimize parameter sets independently. Refer to the section above to see which parameters are used in which step.

In order to find the values that would best match the data, such as Min/Max Areas of mitotic plates, the simplest approach is as follows:

1. Locate mitotic plates manually within the **Thresholded Laplacian Result** image.
2. Use the ImageJ Magic Wand tool to select a mitotic plate.
3. Hit "M" which will measure the current selection and give you the values associated with that mitotic plate.
4. Repeat for multiple observations and at different timepoints, and you will obtain a table where you can infer the min and max values to use.

This works for most parameters.

Example Use

To run our example dataset, proceed as follows:

1. Download the default parameters (TRACMIT Default Settings.txt) for this dataset from https://github.com/lacan/TRACMIT
2. Download the sample dataset from https://doi.org/10.5281/zenodo.232218 .
3. Extract the dataset ZIP file.
4. Launch TRACMIT from Plugins > ActionBar > TRACMIT 1.0.
5. Click on **"Load Parameters"** and use the downloaded txt file.
6. Click on **"Select Raw Image".**
   You will be prompted to provide the location of the extracted folder.
7. It will offer you two fields, open whichever one you would like to analyze.
8. Click on **"Measure Current Image".**
9. The results table are located EXACTLY behind an empty results table. Make sure that you move it to find them.
10. You can inspect the detected divisions by highlighting the one you would like to see from the ROI Manager and clicking on **"Inspect Division".**

Troubleshooting

Feel free to report any issues and questions on TRACMIT's GitHub Page

https://github.com/lacan/TRACMIT/issues