

## TraCoP: Image Processing Platform for Tracking Coral Polyps Solution

Our solution to tracking coral polyps involves specific software which we will be outlining below. The purpose of this article is to outline how to recreate results used in the TraCoP research.

In order to track and measure coral polyps, the following steps are needed.

*Assumes you have a sample TIF file or coral polyps over time, or adjacent data. The data used for TraCoP was a TIF file containing anywhere between 300 and 50,000 individual “frames”, which are samples taken at a given time interval.*

1. Load the desired TIF file into ImageJ (FIJI edition), download provided below
  - a. Ensure that MOSAIC Tool Suite is downloaded as an extension. To do this, in the ImageJ application, navigate to:  
Help > Update > Manage update sites - select MOSAIC ToolSuite, close, and apply changes. Restart ImageJ.
  - b. Ensure that Fast\_Filters plugin is also downloaded, link (with installation guide) provided below
2. From the GitHub repository, download LocatePolyp3.ijm. In ImageJ, with the TIF file open, navigate to:  
Plugins > Macros > Run - select LocatePolyp3.ijm
3. Now you should have a completely processed TIF file with clear indications of polyps (or other), so long as the data is appropriate. Note: the polyps, or objects, you intend to identify should be clearly filled black shapes at this point. We recommend you perform the following trajectory steps manually, but a ‘macro’ is provided on the repository (‘ParticleTrackerShortcut.ijm’):
  - a. Plugins > MOSAIC > ParticleTracker2D/3D- select ‘No’ to 3D data.
  - b. Enter the following parameters into the popup wizard:  
Radius: 30, Cutoff[0-1]: 0.05, Per/Abs: 0.500, Absolute: True  
Displacement: 15, Dynamics: Brownian
4. Once MOSAIC has completed the tracking, a popup will show statistics for the stack. Choose ‘Visualize All Trajectories’ if you wish to see a visual representation of particle linkage (or to see how you may want to adjust parameters, larger or smaller objects). When you are ready, select ‘All Trajectories to Table’, then File > Save As... and save the file as a CSV.
  - a. Note: With a large TIF file, it may take a long time for the Results window to populate. It is recommended to let it run unattended at this point. Visualize All Trajectories may take even longer.

5. At this point, we have everything we need from ImageJ, feel free to exit out. The remaining portion of this will be done using a Python script. Please have 'PolypDensityCalculator.py' downloaded and available to run. We ran this using PyCharm editor for ease of use.
  - a. We have provided the code, as well as an example CSV file for you to run, in your editor, ensure the EASY.csv file is in the root directory, then execute PolypDensityCalculator.py (additionally, make sure the correct packages are installed in Python - pandas, numpy, matplotlib).
  - b. This code will output a list of average polyp counts per interval, a visual error bar graph of the data, and information such as total polyps counted, average polyps, and standard deviation.
6. Lastly, we have included code for stress event removal (specific to coral polyps). Though this was not included in TraCoP, it may still be useful as a small snippet of code to iterate through the data, removing frames with less than a given threshold. This is present in the repository.

### **Downloads**

IMAGEJ (Fiji edition): <https://imagej.net/software/fiji/downloads>

Fast Filters: [https://imagejdocu.list.lu/plugin/filter/fast\\_filters/start](https://imagejdocu.list.lu/plugin/filter/fast_filters/start)

GitHub repository: <https://github.com/lukeklegrafe/Coral-Polyp-Research>