Three\_tracker(k, cropped\_mask, boxes, raw\_path):

Three tracker inputs k (the number of detected signal locations), the cropped\_mask (image stacks), and the output file path (raw\_path) and returns a python dictionary indexed by the number of detected signal transients and including measured parameter information.

First, a binarized version of the set of cropped images is generated, then the z-axis sum of these images are computed individually to define the locations of signal locale within the cropped images which are a subset of the original image stack.

Next, particle analysis is performed using the label command from scikit-image library to generate a label image. Next, the centroid, region coordinates, bounding box, and time lapse image accumulate are computed to determine the number of signal sites.

Then, each signal site is split using the computed bounding box. This lets all bounding boxes be processed in parallel rather than iteratively.

Next, each bounding box is processed by shuffling blank frames into every other frame of the input image stack, and particle analysis is rerun in order to compute slice-wise region of interest information within the identified signal volume stack. For each slice-wise region, mean signal intensity (p\_amp), area encompassed by the ROI (p\_area), centroid position (p\_cent), polygonal ROI definition (p\_region), signal frequency (area\_freq) are computed.

Additionally, divergence (or signal splitting) binary value is defined by the overlap between multiple identified signal regions (np.diff(are\_freq[1]).any()>0: divergence = TRUE) or signal merging (np.diff(area\_freq[1].any()<0:convergence = TRUE) are defined. Wave behavior binary value is also defined by a change in the centroid position greater than 5 pixels.

Lastly, a python dictionary indexed to each signal locale is generated, where the dictionary key is the index of each signal and the values are as follows:

0: an array of cross-sectional area values for each signal

1: the maximum of the value 0 array (the maximum spatial spread)

2: the centroid of all cross-sectional regions for each signal

3: The signal duration

4. The array of cross-sectional area mean amplitude values

5. The change in the mean amplitude from 4 (max - min)

6. The frame number of the origination of the signal

7. The cross-sectional polygonal ROI at the first frame

8. The cross-sectional polygonal ROI for all frames

9. The boolean value for divergence or convergence

10. The Fourier frequency elements of the signal

11. The primary fourier component of the signal

12. The boolean value for waves

13. The location of the extracted bounding boxes