All algorithms are implemented in Python and were from the ndparse algorithms.py source code, or as outlined from ndod. Following the pipeline as outlined in ndod, I started with a 2D TIFF image screencap conversion of Fear200 from ndviz (coordinates: 2264, 991, 0).

To generate the initial classifier, I used the heads-up display (GUI interface) for llastik to manually select bright points and their respective backgrounds. By playing with the functions, I managed to get an h5 probability output. I've preliminarily outlined this part of the code, but this part mostly is user-input driven.

For our input, we need to take the .nii NIFTI images and convert them into a useable file format for Ilastik. After downsampling the image using a Docker with ndreg/ndio, I took the downsampled .nii and made TIFFs of each individual plane. The code is in a Jupyter Notebook linked here, I was able to convert each individual plane into a TIFF because by default the .nii files have three dimensions. The first dimension is the plane, while the second is the row/the third is the column. Thus, in order to get (x,y,b) arrays with x,y being coordinates in 2D space (like we have from CSV outputs) and b referring to the grey-scale brightness value, I've outlined the code below.

## Algorithm 1 Convert .nii file to TIFF images

Require: Raw 3D greyscale .nii file

**Ensure:** .nii file conforms to (pln, row, col), where the first element is the planes, the second is the rows, the third is the columns. The top left rows/columns are thus the darkest, while the bottom right are the brightest (greyscale).

function NIIToTIFF( $(p_i, x_i, y_i)$ , for  $i \in [n]$ )

- (1) Load file using nibabel
- (2) Use getdata command from nibabel to store nii data as memmap array
- (3) Find range of planes (from 0 to k)
- (4) Convert each plane into a TIFF

for  $j := 0, \dots, k$  do

Convert data at j plane (eg: data[j]) into a numpy array using np.asarray(). Use scipy to image on the converted numpy array and save the output as a TIFF.

end for

end function

Now, we have TIFF's of each individual plane. We now can pass it into llastik to generate a classifier using the GUI.

## Algorithm 2 Generate Ilastik Classifier on Subset of Input Data

**Require:** 1 representative sample subset of original image data (one representative TIFF plane). **Ensure:** The user manually selects a subset of the data (some representative region) and uploads the data to llastik by using the gui interface.

**function** CLASSIFIER(raw TIFF of representative plane)

- (1) Select at least two distinct training labels (eg: background, bright point)
- (2) Check to see if results are up to par.
- (3) Repeat until reasonable output is shown.
- (4) Save as classifier .ilp for later use with headless display

end function

## Algorithm 3 Run Pre-Trained Ilastik Classifier on Input Data

Require: .ilp classifier trained on previous data, from above.

function Running Pre-Trained ILASTIK CLASSIFIER(new data)

- (1) Load Ilastik Headless Display
- (2) Run .ilp on all new images

for  $k, l := 0, \dots, n$ , where n is the total number of planes generated do Run headless ilastik on predefined .ilp on all k TIFFs.

end for end function