**Step 1:** Sort the images collected in the field to obtain a folder containing only the good images that you would like to process. For this step, I have been using the free photo sorting software [PhotoSift](https://www.rlvision.com/photosift/about.php). I like this method because you can load your collected images into PhotoSift and sort the images into folders by simply pressing any letter on the keyboard, directing the image to a specified folder. For example, I usually choose to press the “g” key for good images and “b” for bad images.

**Step 2:** With the images sorted into the “good” folder from step 1, process the images with the MATLAB code. You want the data from all of the collected images to be contained within a single text file. This can be accomplished by pasting the processing code to the directory immediately above the folder that contains all of the good images. Within the Renamer\_file\_name\_folder\_ehsan.m file, setting the input parameter “count” at the top of this file to 1 will process all of the images from the good images folder and store the output data in a single text file. The files that need to be in the directory above the folder with the good images include: (1) gaussgradient.m (2) Matlab\_code.m (3) Renamer\_filer\_name\_folder\_ehsan.m (4) ij.jar (5) ImageJ\_code.bat and (6) ImageJ\_code.txt. In the directory above where the aforementioned 6 files are located, the files (1) movefiles.m and (2) sort\_nat.m should be present.

You should then be ready to run the processing code by running the Renamer\_filer\_name\_folder\_ehsan.m file within MATLAB.

The outputted text file folder from processing will show up in the directory containing the images folder and the 6 files needed for processing. The folder will be named “Output\_text\_files\_subtracted”

I have been saving all particles identified by imageJ, then filtering particles within python based on “clarity” from the out of focus filter. However, this can result in an overwhelming number of particles in the outputted text file if you are processing a large number of image (> ~1000). The out of focus particles can be excluded from the outputted text file by modifying line 93 of the Matlab\_code.m file.