

Cone Counting Software Manual – v0.2

This program uses a match filter based algorithm to mark the presence of cones and determine the cone density of the image. There are two parts to using the software- the setup, and running the software.

While on average the algorithm finds ~95% of cells, an external observer will still need to review these images in order to find cones that the program may have missed, and to correct cones that may have been incorrectly marked. Scan each image carefully, paying close attention to dim cells.

Setting up the program:

The software relies on two types of input:

- 1) A scaling file (LUT.csv)
- 2) Images to count! (in .tif format)

Both of these should be placed in the “etc” folder within the base folder (Cone_Counting_Batch_Random).

The scaling file (LUT.csv) consists of three columns: An “ID” column, which should **always** be the first two underscore-delimited words in the image file, an “axial length” column, which contains the axial length of the associated ID in mm, and a “pixels per degree” column, which contains the pixels per degree scale of the associated ID. Each row of LUT.csv contains a separate ID, axial length, and scale. Feel free to add as many subject IDs to the list as you have, but remember, **the software only allows you to put in 1 row of scaling values per ID**. Also, LUT.csv **MUST** be a comma-delimited file. If you don’t know what that means, just make sure to save LUT.csv as a “.csv” and not a “.xlsx” or “.xls” in Excel.

An example GOOD image filename: RC_0009_pictures_of_stuff.tif

An example BAD image filename: RC_0009pictures_of_stuff.tif

An example BAD image filename: pictures_of_stuff_RC_0009.tif

An example BAD image filename: RC0009pictures_of_stuff.tif

An example table row for LUT.csv:

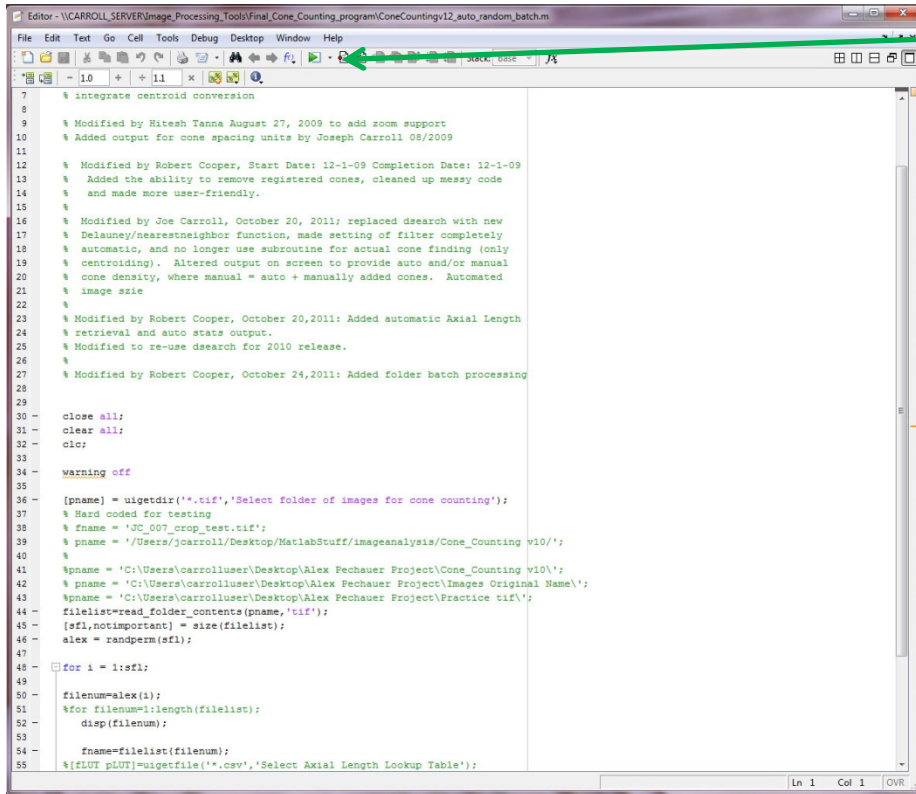
RC_0009	24	600
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An example LUT.csv is provided for you within the “etc” folder!

Opening the program:

- Double-click cone_counting.m within Cone_Counting_Batch_Random

At this point you should see this on your screen:



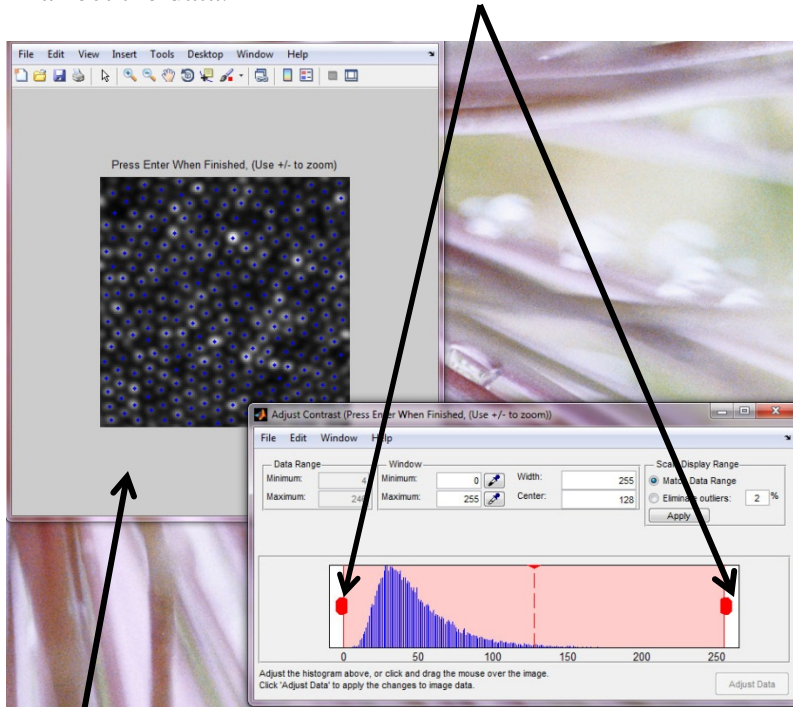
```
7 % integrate centroid conversion
8
9 % Modified by Hitesh Tanna August 27, 2009 to add zoom support
10 % Added output for cone spacing units by Joseph Carroll 08/2009
11
12 % Modified by Robert Cooper, Start Date: 12-1-09 Completion Date: 12-1-09
13 % Added the ability to remove registered cones, cleaned up messy code
14 % and made more user-friendly.
15
16 % Modified by Joe Carroll, October 20, 2011: replaced dsearch with new
17 % Delaunay/nearestneighbor function, made setting of filter completely
18 % automatic, and no longer use subroutine for actual cone finding (only
19 % centroiding). Altered output on screen to provide auto and/or manual
20 % cone density, where manual = auto + manually added cones. Automated
21 % image size
22
23 % Modified by Robert Cooper, October 20, 2011: Added automatic Axial Length
24 % retrieval and auto stats output.
25 % Modified to re-use dsearch for 2010 release.
26
27 % Modified by Robert Cooper, October 24, 2011: Added folder batch processing
28
29
30 - close all;
31 - clear all;
32 - clc;
33
34 - warning off
35
36 [pname] = uigetdir('*.tif','Select folder of images for cone counting');
37 % Hard coded for testing
38 % fname = 'JC_007_crop_test.tif';
39 % pname = '/Users/jcarroll/Desktop/MatlabStuff/imageanalysis/Cone_Counting v10/';
40
41 % pname = 'C:\Users\carrolluser\Desktop\Alex Fechauer Project\Cone_Counting v10\';
42 % pname = 'C:\Users\carrolluser\Desktop\Alex Fechauer Project\Images Original Name\';
43 % pname = 'C:\Users\carrolluser\Desktop\Alex Fechauer Project\Practice.tif\';
44 filelist=read_folder_contents(pname,'tif');
45 [sfl,notimportant] = size(filelist);
46 alex = randperm(sfl);
47
48 for i = 1:sfl;
49
50     filename=alex(i);
51     %for filename=1:length(filelist);
52     disp(filename);
53
54     fname=filelist(filename);
55     %[fLOT pLOT]=uigetfile('*.csv','Select Axial Length Lookup Table');
```

Press the play button to start the software

By default, the software analyzes the central 80, 55, and 35 micron regions. If you wish to change these sizes, line 26 contains a “sizes” vector. Adding/removing a size is as simple as adding/removing a number from the list.

Running the program:

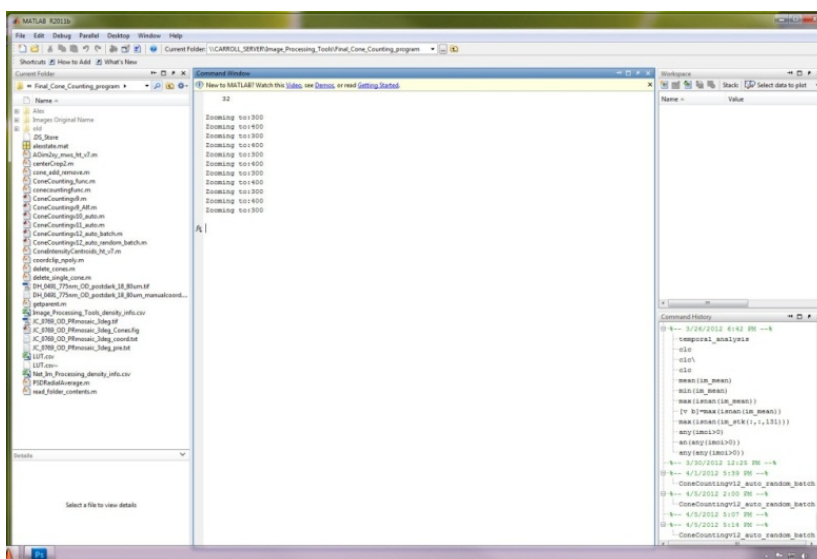
Move the red slider bar to adjust the brightness and contrast of the image. This will make the cones more visible and easier to distinguish. Feel free to adjust the slider as needed; it will not affect the data.



Select figure window, and begin scanning for mistakes

- If you click on the image a cone will be added or removed. Click on the gray border around the image to select window and not add or remove a cone.
- Use (+/-) keys to zoom in and out.
 - *Remember cones on the edges
- Left click on unmarked cones to add
- Right click on **blue dot** to delete
- **Press Tilde (~) to remove ALL found cones**

Scan the image several times. When finished, click on figure window then hit enter.



*If this screen pops up after pressing enter, minimize, then select figure window and press enter again. **DO NOT** exit out of window.

Be sure when selecting figure window not to add or delete a cone.

There is a progress bar provided that will show how many images remain. Once the software has completed, the analyses (Density, NND, etc) will be run on the coordinate files, and output to the coordinate_analysis folder.

Once you have finished running the software, the coordinate analysis folder will contain:

- **Cone_Counting_Batch_Random_density_info.csv**
 - This file contains a summary of all files run in the software. It also contains the density as calculated over the entire image.
- **coordinate_analysis_coordstats_XXum_box.csv**
 - XX will be the micron sizes that are in the “sizes” vector on line 26 of cone_counting.m
 - This file contains the filename, number of cells, density within the filename’s micron region, mean NND, cropped region in microns, mean Voronoi area, number of “bounded” Voronoi cells, and the total “bounded” Voronoi area.
- **The coordinates of each file run through the software**
 - Each coordinate file contains all coordinates found using the software.

**If you have any questions, please feel free to contact me at
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