

Colony / Plaque Counter Manual (Hardware / Software Guide)

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I. Introduction

The Mark 1 Claytor Industries colony / plaque counter is a top of the line ad hoc laboratory instrument. This document details how to use and modify it. For additional help you may e-mail the author at claytor@rice.edu.

II. Quickstart

- 1) Make sure equipment is aligned and plugged in.
 - a) Ethernet for computer is plugged in.
 - b) Webcam is plugged in to right side USB port.
 - c) Webcam is clipped onto the top of the cardboard setup.
 - d) The lamp is plugged in.

- 2) Run MATLAB
 - a) Type “cd claytor” and press return.
 - b) Type Camera_Expt and press return.

- 3) Set parameters and launch CameraAq
 - a) Set the file path and file names. Both may be changed later.
 - b) Click on “Capture Images” to launch the capture window. Select “winvideo” and “RGB1600x1200.” After a few seconds you should see a preview of the image in the upper left quadrant of the window.

- 4) Acquire Images
 - a) Place a Petri dish, without the lid, face up in the box.
 - b) Close the door and wait for the camera to focus on the dish.
 - c) Click “Capture image only.” After a few seconds, you will see the captured image in the upper right hand quadrant, and in the Filepath box the letter will have changed from ‘a’ -> ‘b’, indicating the picture was taken.
 - c) Rotate the dish ~90° (this does not need to be exact)
 - d) Center the dish and click “Capture image only” again. A few seconds later, the right figure will again change to reflect the new image, the counter in the Filepath box will increase by 1 and the letter will change back to ‘a’.
 - e) Repeat steps a through d for the remaining plates.
 - f) When you have finished with all the plates, click “Exit”

- 5) Analyze Data
 - a) In the Camera_Expt window select the method of analysis from the pull down box, either “HR Watershed” (recommended) or “HR Local Max” (Slower) to run the respective method.
 - b) Click “Choose Experiment & Run”. Select one or multiple image files and click Ok.
 - d) Wait, and don’t touch anything, MATLAB won’t save the proper output image if you have another window running on top of it.
 - e) Type ‘global colonies’ to access the data as a variable

III. Software Operation Instructions

The software can either be run through the MATLAB command prompt by navigating to the appropriate directory, and running Camera_Expt, or by launching Camera.exe from the desktop. If MATLAB runs out of network licenses for the Image Processing Toolbox, or is not connected to the network, use Camera.exe.

Figure 1 shows the main Camera_Expt window. This gives you access to the three main subfunctions; image acquisition, image processing, and post-identification processing, which will be discussed in turn.

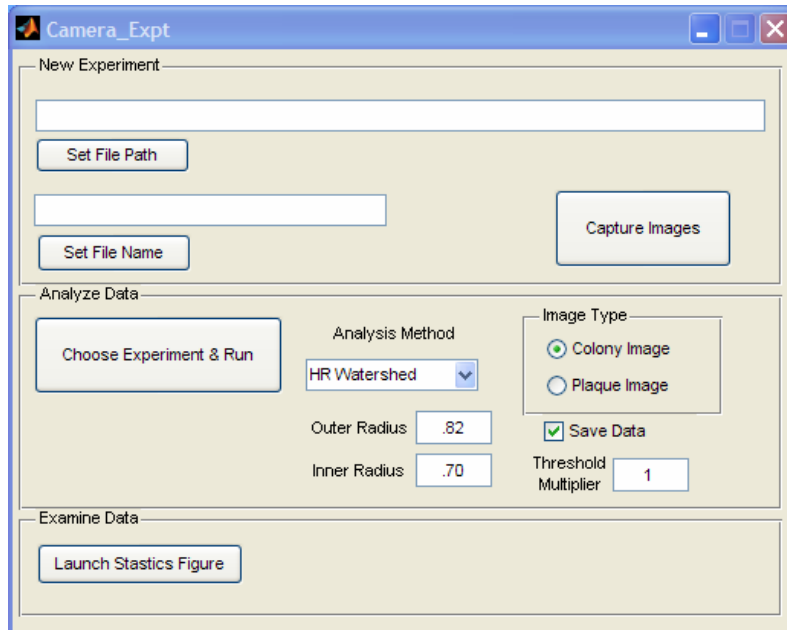


Figure 1: The main Camera_Expt window. This allows access to all the other functions.

Image Acquisition

The upper box of Camera_Expt is devoted to image acquisition. You can either set the file path and generic file name first, or you may click on “Capture Images” which will prompt you for a file path and name if they were not entered. Clicking “Capture Images” launches the capture window (Figure 2). The main window is initialized then a menu comes up asking for the adapter to use, typically “winvideo” is the correct adaptor. Another menu with options for supported resolutions appears. It is recommended to use the highest resolution (usually the native camera resolution), and RGB color. The device will be initialized, which may take a few seconds, and a preview will appear on the left (Figure 2).

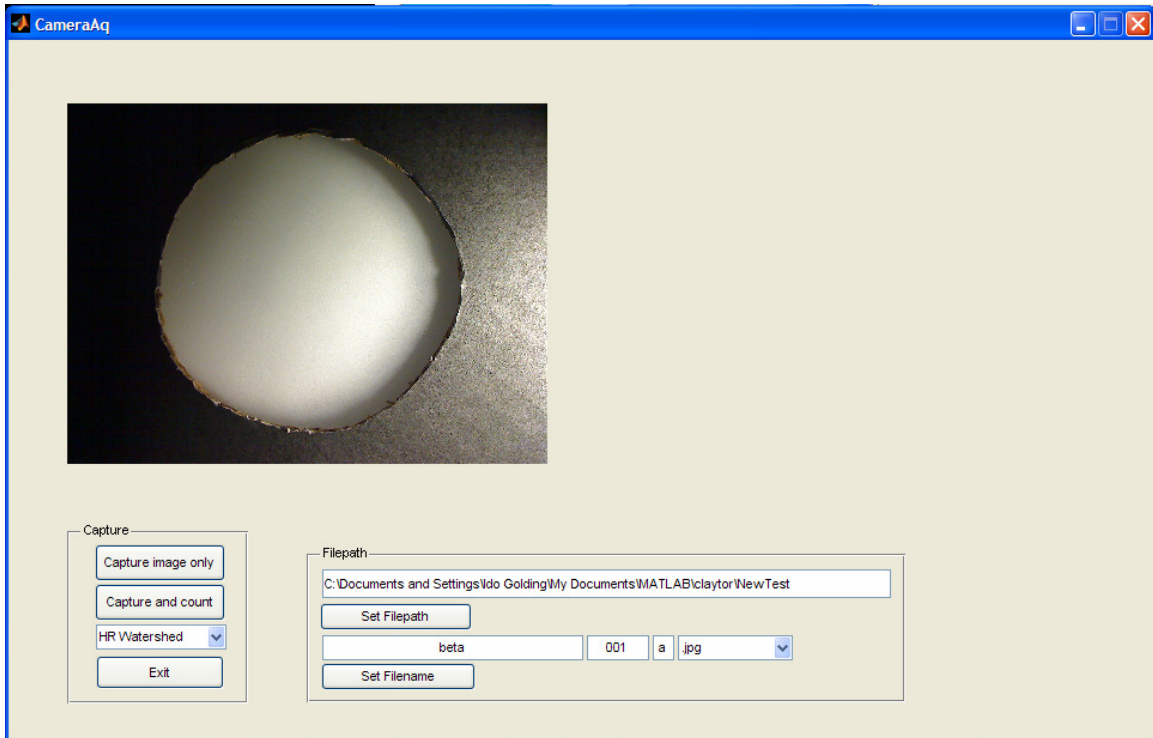


Figure 2: The CameraAq window upon being initialized with the camera info.

For best results, give the camera a few seconds to update and focus on the sample after it is placed in the holder. It is usually clear in the preview window when the sample is focused. If focusing does not occur within a few seconds, occlude the view with your hand momentarily.

You may change the directory where images are saved by clicking on “Set Filepath”. Likewise, the file name, number, and letter may be changed. To change the generic file name, clicking the “Set filename” button. To the right of that is a counter, which increments with every two images taken. The number may be changed by clicking in the box and entering another number. The image letter changes with every image from ‘a’ to ‘b’ and back to ‘a’. Clicking in this box allows you to change the value for the next image saved.

NOTE: You will NOT be prompted to overwrite a file if one already exists with the same filename. For this reason, it is recommended that you use a new folder whenever beginning a new sequence of images.

There are two options for capturing images. Clicking on “Capture image only” saves the image and readies the program to capture another image. This is the preferred method for capturing data, and analysis may be done later. Clicking on “Capture and count” captures the image and runs the counting method selected in the drop-down box below the button. Capturing an image takes 2-5 seconds to read the data from the camera and write it as a high-quality jpg image to the specified folder. During this time do not disturb the setup. Once the file is saved, the image will be displayed to the right of the preview window.

Click the “Exit”, button to close the window and turn off the video input.

Image Processing

The core of Camera_Expt is the routines that are used to count the cell colonies, or viral plaques. The parameters for analysis are set using the central set of options. First select a method of colony segmentation from the pull down menu. The default is HR Watershed. Second is HR Regional Max, followed by Morphological, Watershed, and Regional Max. The first two (HR) methods were optimized for 1600 x 1200 pixel images, while the final three were written with smaller 640 x 480 images in mind. Morphological processing is used to determine the first guess in any case. From here either watershed segmentation of a distance transform, or the regional maxima of the original image are located. The regional maxima method is only slightly more aggressive at segmenting colonies, however it usually takes twice the time, thus the watershed method is preferred.

To the right of the method is the Image type. Colony Images need to be inverted before analysis, thus the correct radio button for the data should be selected. The Save Data checkbox below this allows one to save, or disable saving output files. Disabling the data save speeds up the analysis, however, one cannot perform any detailed analysis later, and no images are saved.

The other text boxes are for more advanced options. Outer radius specifies the maximum area that will be used for counting. Usually this is slightly smaller than the full bright area identified as the dish, and as long as dishes are placed face up this does not need to be changed. Inner radius is used for removing text. For larger text one should select a smaller inner radius. If you find that text is being identified as colonies in the output, reduce this inner radius value. Both these values should be decimal values between 0 and 1.

The threshold box is for manually adjusting the threshold. Although the automatic threshold determination should work for most images, it will fail in some instances. For example, in very low density plates, the threshold will often be determined to be lower than it needs to be. This results in the background being identified as an object of interest and segmented and counted, overcounting the plate (Figure 3). To fix this problem, enter a value for threshold multiplier greater than one. For very high density plates the reverse may happen (not enough of the area is determined to be of interest and thus the sample is undercounted). To fix this problem, enter a value for the threshold multiplier that is less than one, but greater than zero.

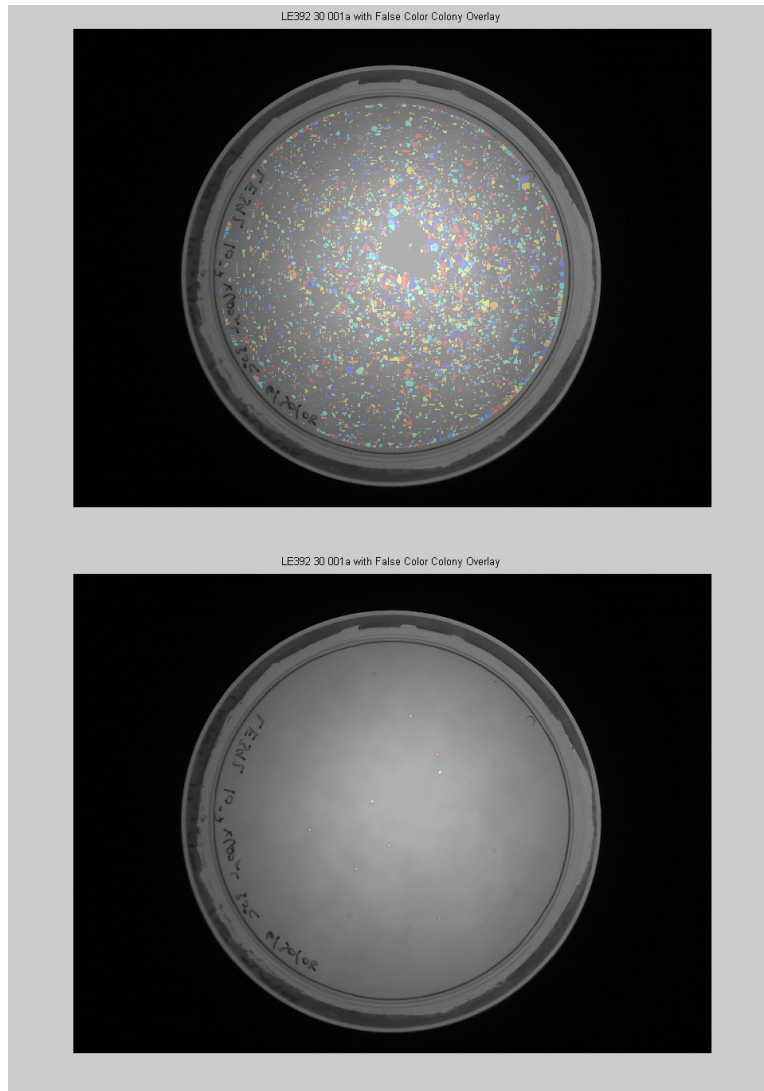


Figure 3: Top: A sample image with a threshold that was determined to be too low, resulting in false segmentation and identification. Bottom: The same sample with the threshold multiplied by 15, giving correct identification of the small (almost non-visible) colonies.

To get a rough idea of what value to enter for this multiplier, look at densmetric output from the unsatisfactory run. You should see a pattern establish itself as the density of the plates varies, usually with lower density plates having a higher value than the higher density plates. Densmetric is the threshold divided by the average intensity of the area of interest. The ratio should give you an idea of the value to enter for the multiplier.

With the parameters set (usually only the analysis method and Image type need to be specified), click Choose Experiment and Run. This brings up a dialog box asking for the images you wish to analyze. Find and select all the image files you wish to run and click “Ok.” You may select one or more image files, if you click “cancel” a warning will be displayed, however, you will be allowed to continue. Processing the images may take a few minutes. A rule of thumb to use for the time is adding 10 s per image to the watershed method, 20 seconds per image to the regional max, and an additional 5 s per

image if the data will be saved. As the program runs you will be kept updated on the status in the command window and figures showing the segmentation will appear if the data is to be saved.

NOTE: For these images to be saved properly, no other program can be running on top of the window while analysis is taking place.

Once the analysis is done, the count, time taken, and densmetric values for each image will be displayed in the command window (see Figure 4). If the function was run from MATLAB, you may access the variables using the global command. They will also be saved to a *temporary* text file called “diary” in the “MATLAB\work” or in the folder that the Count.exe program was run from.

```
C:\Documents and Settings\ldo Golding\Desktop\Counter.exe
Save successful!
colonies =
      2925      3428
      3116      3164
       236       195
       186       183
       624       615
       710       723
      1433      1339
      1437      1305

comptime =
  11.5729  11.8781
  11.7259  11.7118
  10.6504  10.7575
  10.5563  10.5821
  10.9699  10.8553
  10.8509  10.8917
  11.2960  11.2402
  11.3375  11.0984

densmetric =
   1.3467   1.3168
   1.2001   1.4474
   3.6894   5.1034
   5.0362   4.9959
   2.2124   2.1952
   1.9395   1.8853
   1.1833   1.3097
   1.1646   1.3387

Type:
global colonies
global comptime
global densmetric
To access any of the above variables.
```

Figure 4: The command window output of the program. If the program were run from MATLAB this would be displayed in the command window and typing “global colonies”, would place that variable (colonies) in the Workspace.

Post-Processing

An optional program that can be run after an image has been analyzed and data saved is Camera_Stats. This is run by clicking on the “Launch Statistics Figure” button. Figure 5 shows the window that will be launched.

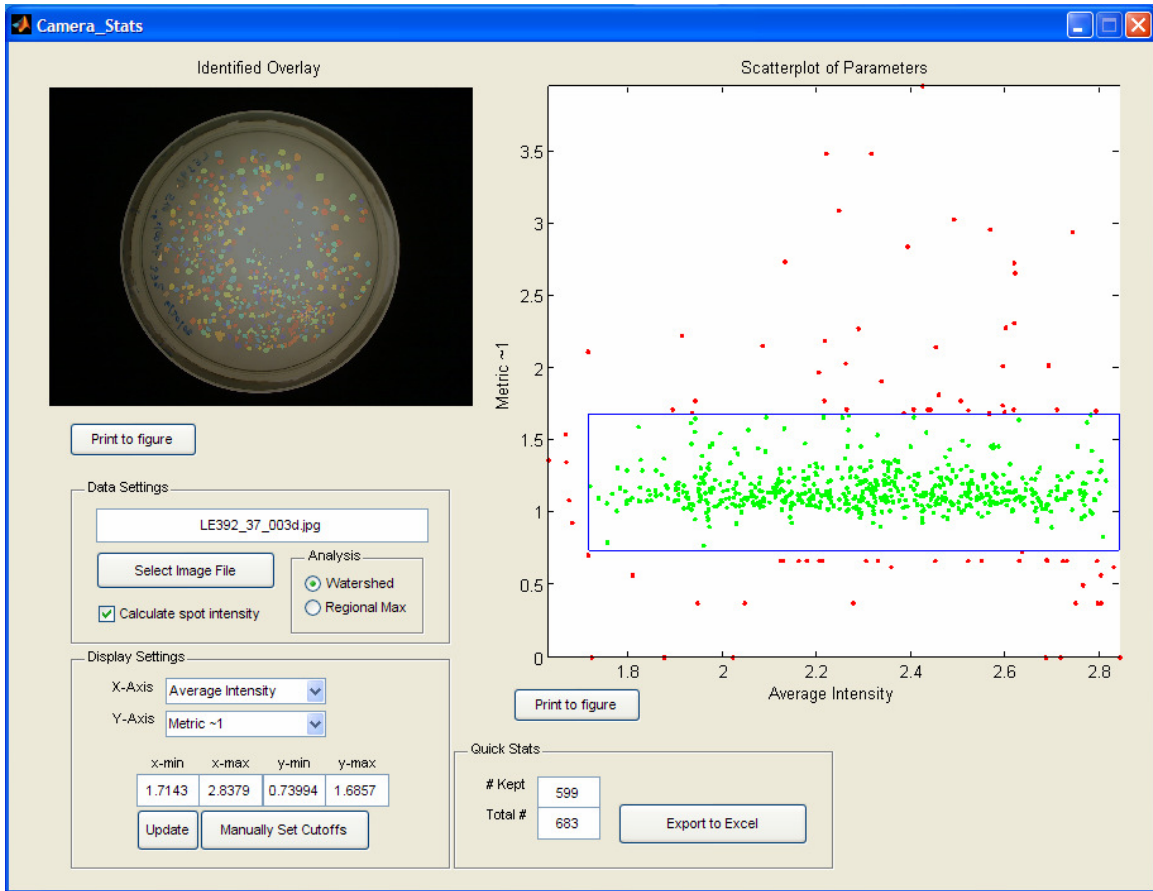


Figure 5: The Camera_Stats window. This window allows for easy selection and post-processing of data.

To load an image and corresponding output into Camera_Stats, click “Select Image File”. Navigate to an image file, *not* the data files. Since the names are automatically generated, the data file will be found automatically. If no data file exists, you will be prompted to perform analysis on the image using the default values for outer radius, inner radius, and threshold multiplier. Use the radio buttons to analyze the data saved by the watershed or regional maximum methods. Check the “Calculate spot intensity” box if you would like to calculate the intensity of the colonies. Since this may take a few minutes the default is unchecked.

Under display settings you will find drop down menus allowing you to select which variable will be displayed along the x and y axes of the scatterplot. The cutoff values may either be entered by hand into the boxes, and then clicking update, or by clicking on “Manually Set Cutoffs” and then clicking twice in the scatterplot where you would like the corners of the cutoff box to be. Points inside the box will be flagged as “good,” and those outside will be flagged as “bad.”

Clicking “Export to Excel” will export all data columns found under the pull-down menus, as well as another column corresponding to if the colony satisfies the cutoff (1 or good) or does not (0 or bad). Clicking either “Print to figure” button will open a new window with either the image or the scatterplot in it.

IV. Hardware Operation

Figure 6 shows the overall hardware setup. The webcam clips into a holder on the top of the box (Figure 7a) keeping it in position and motionless. The box itself isolates the sample from external sources of light. The sample sits in the middle of the box (Figure 7b) and is illuminated by a light from underneath (Figure 8a). A removable diffuser (Figure 8b) acts to even out the intensity from the light source. The diffuser is made from a sanded piece of plastic and several sheets of paper. Black ink printed on the paper further evens out the light intensity (Figure 8c). These sheets can be removed and replaced at will until the intensity is found to be level across the surface of the dish.



Figure 6: An overview of the setup.

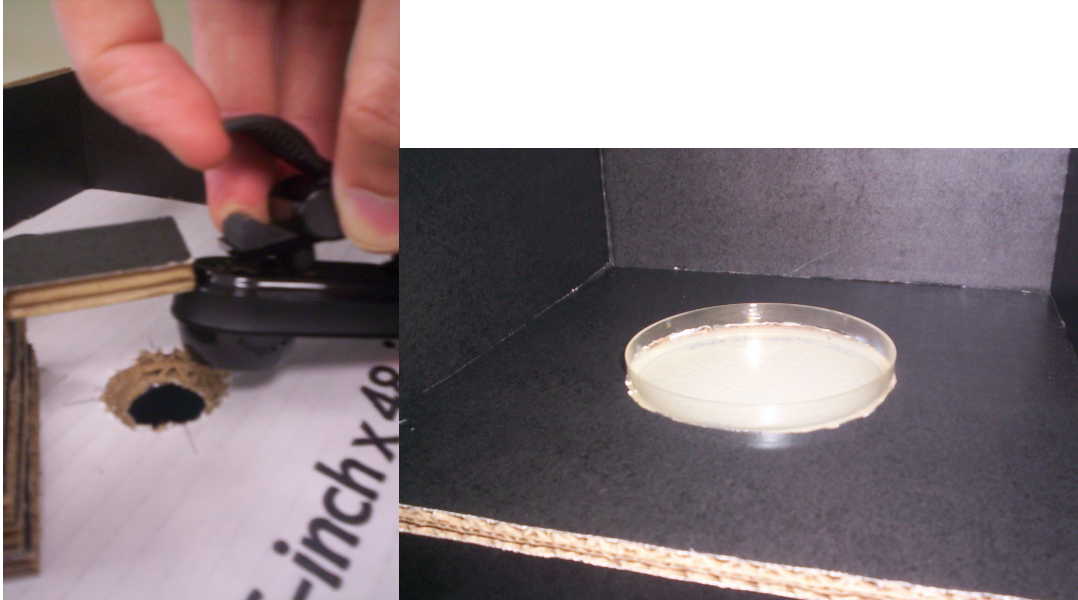


Figure 7: a) Left: A photo showing how the webcam clips to the holder on the top of the box. b) Right: The sample sitting on the diffuser in the center of the box.

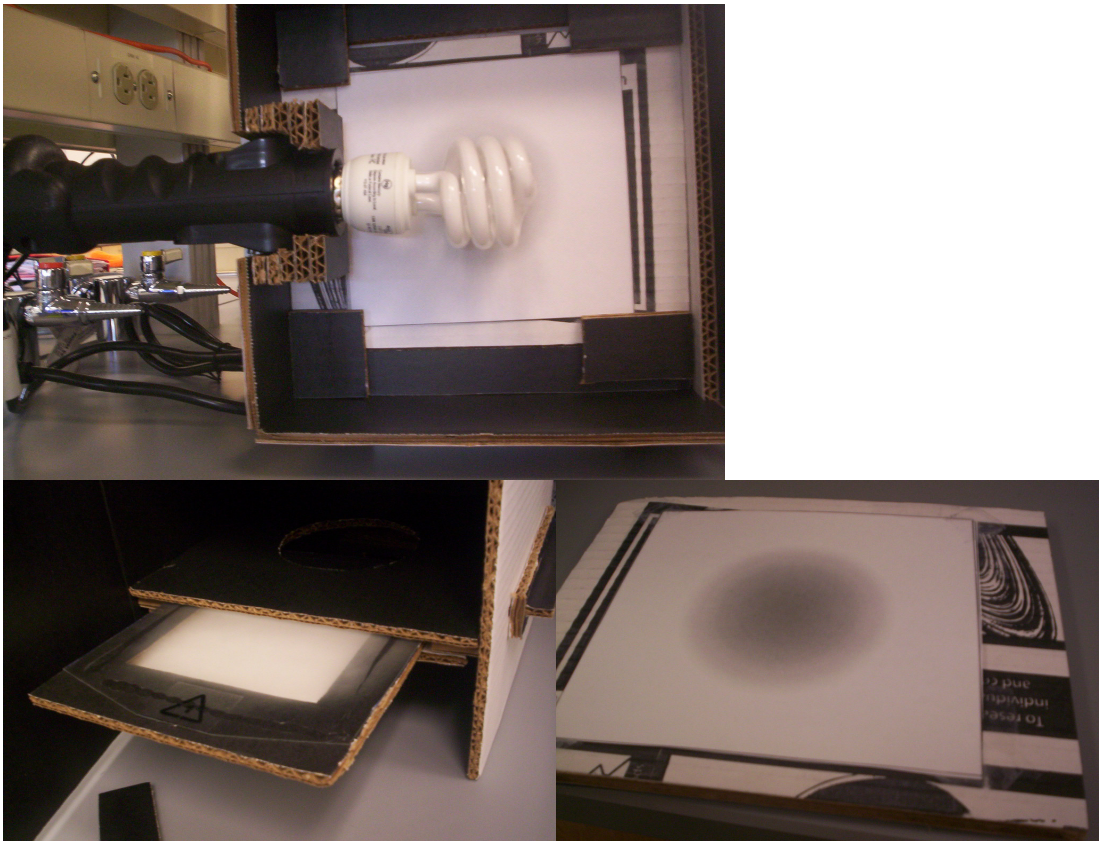


Figure 8: a) Top: A photo showing the underside of the box. The lamp is held so the bulb is directly below the sample. b) Lower left: The diffuser being pulled out of its place beneath the sample. c) Lower right: The reverse of the diffuser plate showing the paper print out used to equalize the light intensity across the sample.

V. Code Walkthrough

Figure 9 shows a flow-chart of how the code is organized, ignoring helper functions. Table 1 contains a list of all the functions used and a short description of each. For more detail on any of the functions here type into the MATLAB command window “help function_name” without the quotes. All the functions should be thoroughly documented for convenience.

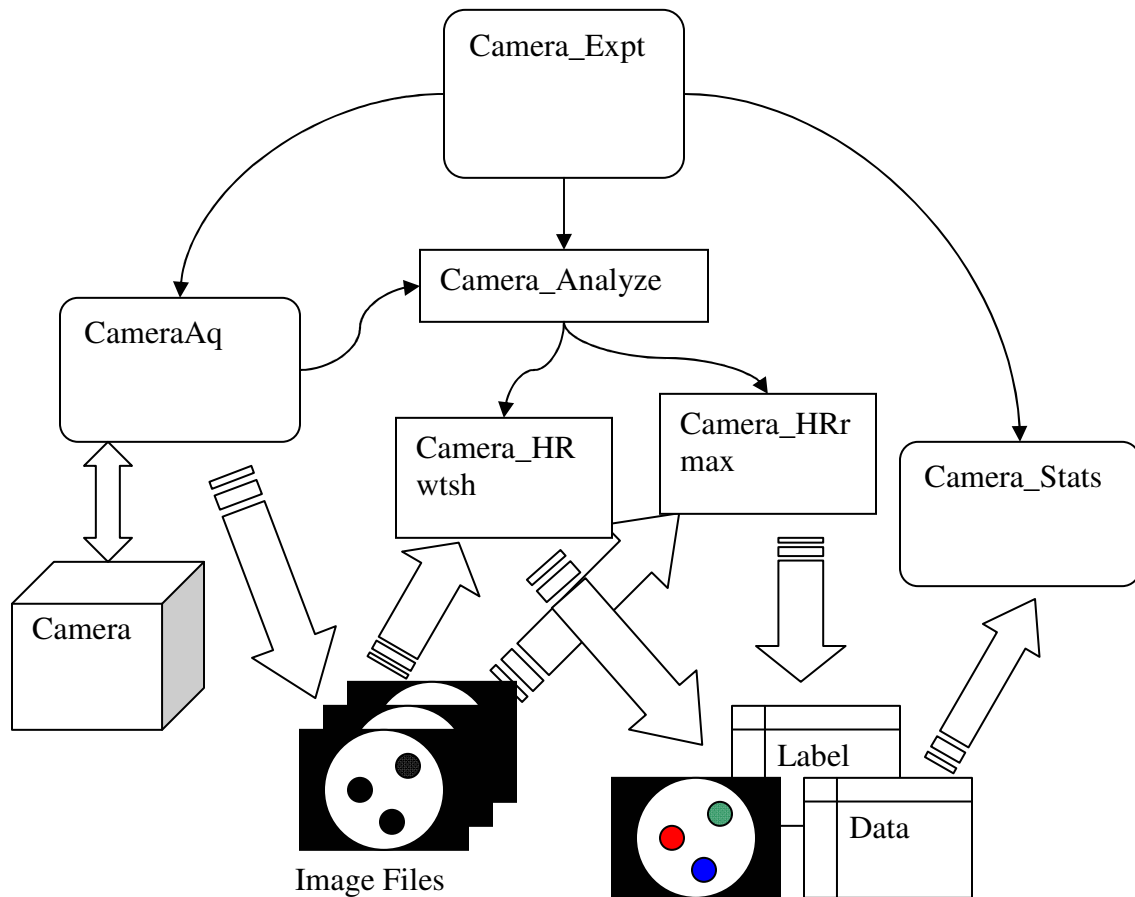


Figure 9. A flow chart of the high level organization of the code. `Camera_Expt` launches figures `CameraAq` and `Camera_Stats`, or it runs the function `Camera_Analyze`. Solid lines with arrows indicate function calls and data passing, block arrows indicate I/O transfer, and striped arrows indicate file reads or writes.

`Camera_Analyze` is the core of the analysis program. This loads up multiple files and prepares them for sequential processing. It also takes the output from the identification functions and displays it in an easy to view format.

The five segmentation functions are listed in Table 1. Only `Camera_HRrmax` and `Camera_rmax` use `myreconstruct` to restore colonies to their proper size after identification. `SaveCellImageData` is called on by all segmentation functions. Finally `getIntensityValues` is used only by `Camera_Stats`.

Function name	Purpose	Function name	Purpose
Camera_Expt.m	GUI for accessing all functions	myreconstruct.m	Reconstruction maintaining distinct markers
Camera_Expt.fig	Figure file for Camera_Expt	saveCellImageData.m	Saves the data
CameraAq.m	GUI for capturing images	getIntensityValues.m	Calculates spot intensity values
CameraAq.fig	Figure file for CameraAq	Camera_HRwtsh.m	See Table 2
Camera_Analyze.m	Function for batch processing of images	Camera_HRrmax.m	See Table 2
Camera_Stats.m	GUI for post-processing	Camera_morp	See Table 2
Camera_Stats.fig	Figure file for Camera_Stats	Camera_rmax	See Table 2
sizeMask.m	Calculates the center and radius of the dish.	Camera_wtsh	See Table 2
makeMask.m	Makes the mask		

Table 1: A list of all functions used and their purpose.

Function	Analysis Method Used	Comments
Camera_HRwtsh	High resolution watershed	High resolution threshold, followed by a light morphological smooth, then a watershed transform of the distance map formed from the smooth.
Camera_HRrmax	High resolution regional maximum	High resolution threshold, followed by a light morphological smooth, then a regional maximum search of the areas of the original image corresponding to the map of the smoothed regions.
Camera_morp	Low resolution morphological	Low resolution threshold, followed by a light morphological smooth.
Camera_wtsh	Low resolution watershed	Low resolution threshold, followed by a light morphological smooth, then a watershed transform of the distance map formed from the smooth.
Camera_rmax	Low resolution regional maximum	Low resolution threshold, followed by a light morphological smooth, then a regional maximum search of the areas of the original image corresponding to the map of the smoothed regions.

Table 2: Short descriptions of all the analysis methods used in the program.

VI. Modification Recommendations

Adding / modifying colony identification methods

To add another method of identifying colonies, it is recommended that you do not modify any of the existing tried and true methods. Rather, copy and paste an existing one, give it a new name (remember to re-name the function as well), make your changes to this version and save it.

Now incorporate it into the menu; launch GUIDE, open Camera_Expt.fig, double click on the drop down menu, then edit the drop down options (see Figure 10).

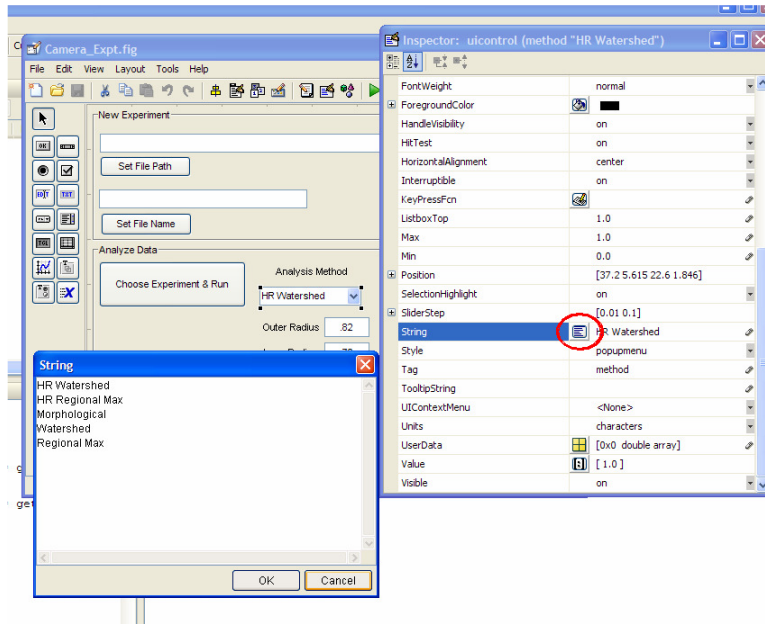


Figure 10. How to access the drop down options in GUIDE. After double-clicking on the drop-down box, click on the red circled button. This brings up a box with the options listed. Add yours to the end of the list.

Now open Camera_Analyze with a text editor and find the following lines;

```
% Run the analysis on the images based on the method we chose
switch method
    case 1
        [numcol,time,metric] = Camera_HRwtsh(fullPathName,...
    case 2
        [numcol,time,metric] = Camera_HRrmax(fullPathName,...
    case 3
        [numcol,time,metric] = Camera_morp(fullPathName,...
    case 4
        [numcol,time,metric] = Camera_wtsh(fullPathName,...
    case 5
        [numcol,time,metric] = Camera_rmax(fullPathName,...
end
```

simply add your method to the end of the list;

```

switch method
    case 1
        [numcol,time,metric] = Camera_HRwtsh(fullPathName,...
    case 2
        [numcol,time,metric] = Camera_HRrmax(fullPathName,...
    case 3
        [numcol,time,metric] = Camera_morp(fullPathName,...
    case 4
        [numcol,time,metric] = Camera_wtsh(fullPathName,...
    case 5
        [numcol,time,metric] = Camera_rmax(fullPathName,...
    case 6
        [numcol,time,metric] = Camera_newmethod(full...
end

```

and save. The next time you run the figure, you should see your option in the drop down box, and it will behave as any other analysis method.

Adding another plotting parameter to Camera_Stats

To add another plotting parameter, we'll need to write code to calculate the parameter, and then insert it in the matrix that contains the data read when plotting, as well as add a value to the variable that labels the plotting parameters. First, open Camera_Stats in an editor, around line 140 you will see the code;

```

r = (bbox(:,3)+bbox(:,4))./4;
metric = peri./area.*r/2;
% Get some extra info about the image if the user wants it

```

Insert the code for your new parameter after metric, for example;

```

r = (bbox(:,3)+bbox(:,4))./4;
metric = peri./area.*r/2;
myparam = <some expression here>
% Get some extra info about the image if the user wants it

```

In the next sections you will need to modify the variables colheaders, and data in every instance from;

```

colheaders = {'Area', 'Eccentricity', ...
data = [area, ecce, ...

```

Will become

```

colheaders = {'Myparam', 'Area', 'Eccentricity', ...
data = [myparam, area, ecce, ...

```

Make sure that the position that the variable name appears on the list is the same position it appears in the data. Otherwise, when you select “Myparam” you may be plotting “Area” instead! With this your modifications are done. Your variable should appear in the drop-down menus when you run Camera_Stats.

VII. Notes

