



# Focal Points



Application Note FP-165

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## Colony Counting Non-Fluorescing Samples With the ColonyDoc-It™ Imaging Station

### Introduction

Colony counting, once a tedious and time-consuming task, has been greatly simplified with tools such as UVP's ColonyDoc-It™ Imaging Station (Fig. 1) and Doc-It® Colony Counter software.

Once the colony plate is imaged, the Doc-It Colony Counter software is capable of creating numerous reports regarding the attributes of the sample. Such results include the number of colonies, the area of each colony, the perimeter of each colony, colony circularity, and so forth. This Application Note will describe the process of using the ColonyDoc-It and Doc-It Colony Counter software to perform colony counting tasks quickly and easily.



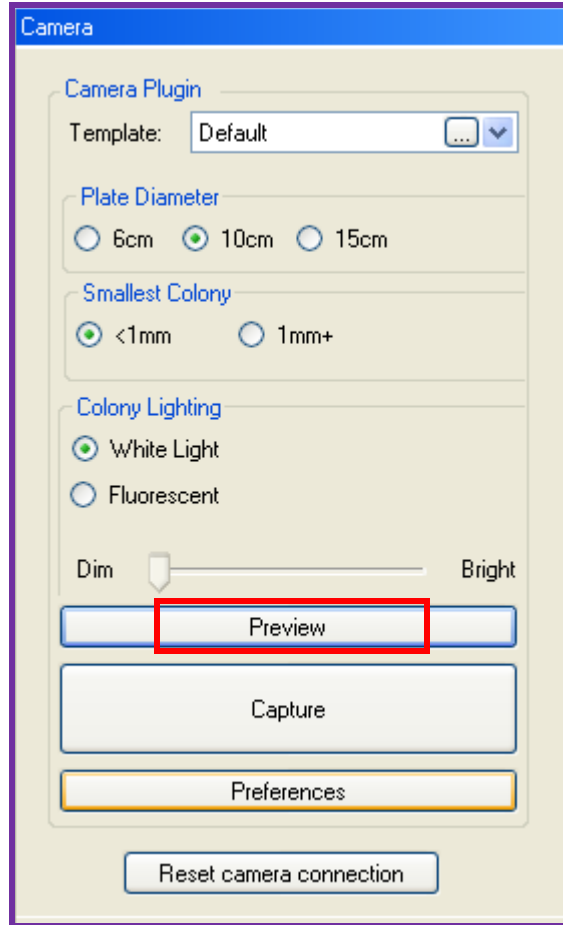
**Fig. 1 – ColonyDoc-It Imaging Station**

### Procedure

1. Set up and make all connections for the ColonyDoc-It:
  - a. Plug in the computer, monitor and ColonyDoc-It to wall power.
  - b. Plug in the mouse, keyboard and monitor to the back of the computer.
  - c. Connect the ColonyDoc-It to the computer via the supplied USB cable.
2. Once logged in to the computer, double-click the “Doc-It Colony Counter” icon located on the desktop screen.



3. Once the software opens, click “Login” (if prompted for a password, enter “12345” in both fields and click “OK”).
4. Click the “Preview” button in the “Camera” module on the right side of the screen.

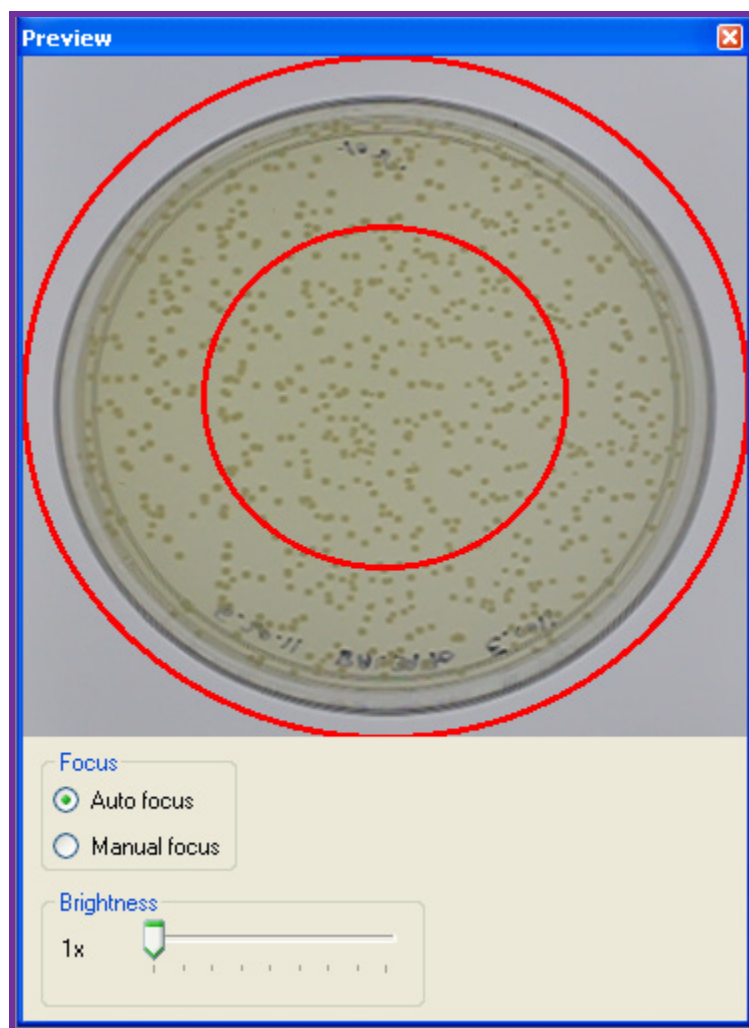


5. Make sure the lighting selectors on the front of the ColonyDoc-It are in the correct positions:
  - a. For non-GFP samples:
    - Select “Off” under Epi Lighting and “Trans” under Base Lighting.
    - With the system’s doors open, make sure the metal filter selection tab is in the left position, indicating “Filter 1”.
  - b. For GFP/fluorescent samples:
    - Select “Blue” under Epi Lighting and “Off” under Base Lighting.
    - Place the black metal circular plate over the system’s built-in transilluminator to avoid light reflection.
    - With the system’s doors open, make sure the metal filter selection tab is in the right position, indicating “Filter 2”.



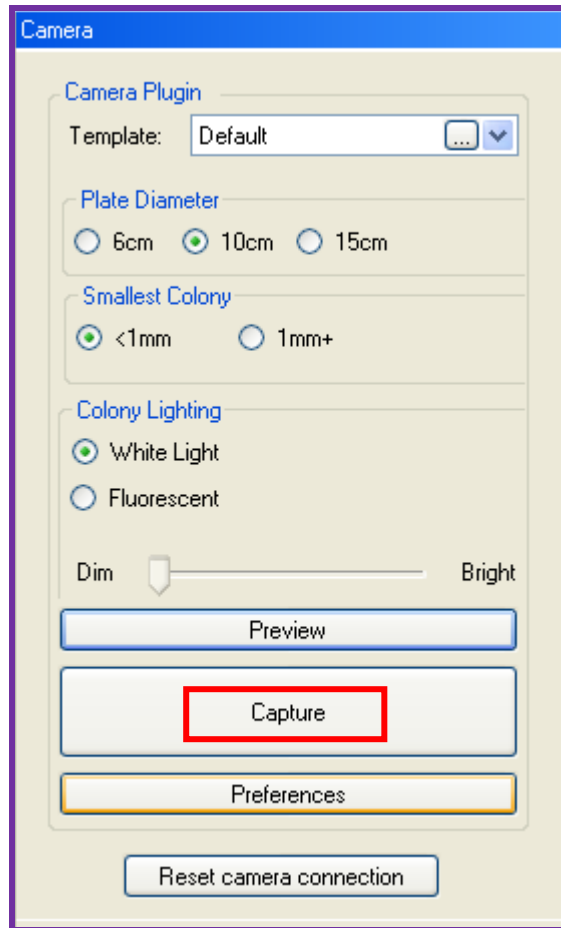
- Open the doors of the ColonyDoc-It and place the sample on the transilluminator surface (or black plate for fluorescent colony counting). Visualizing the sample on the computer screen, align the sample with the red circles. You may use the black adapter ring plate to assist in lining up the plate.

*Note: If condensation has built up inside the sample, either remove the sample lid or flip the sample upside down before placing it inside the ColonyDoc-It.*

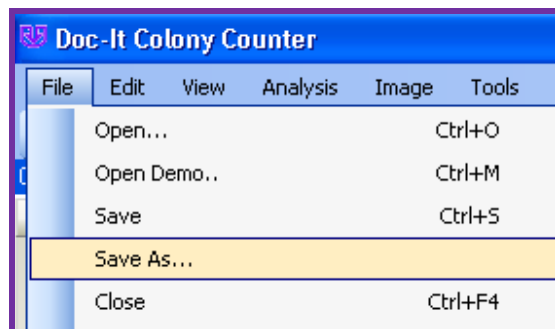


- Close the ColonyDoc-It doors.

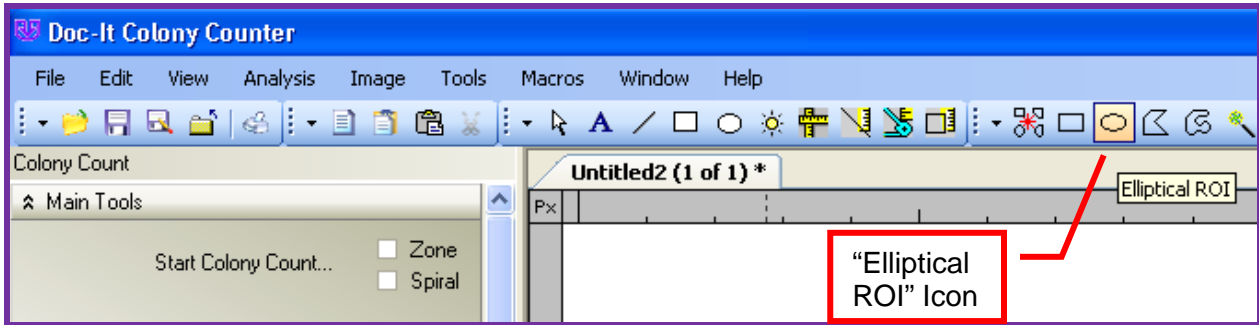
- Click the “Capture” button in the “Camera” module on the right side of the screen. The actual image capture will take approximately 15 to 20 seconds.



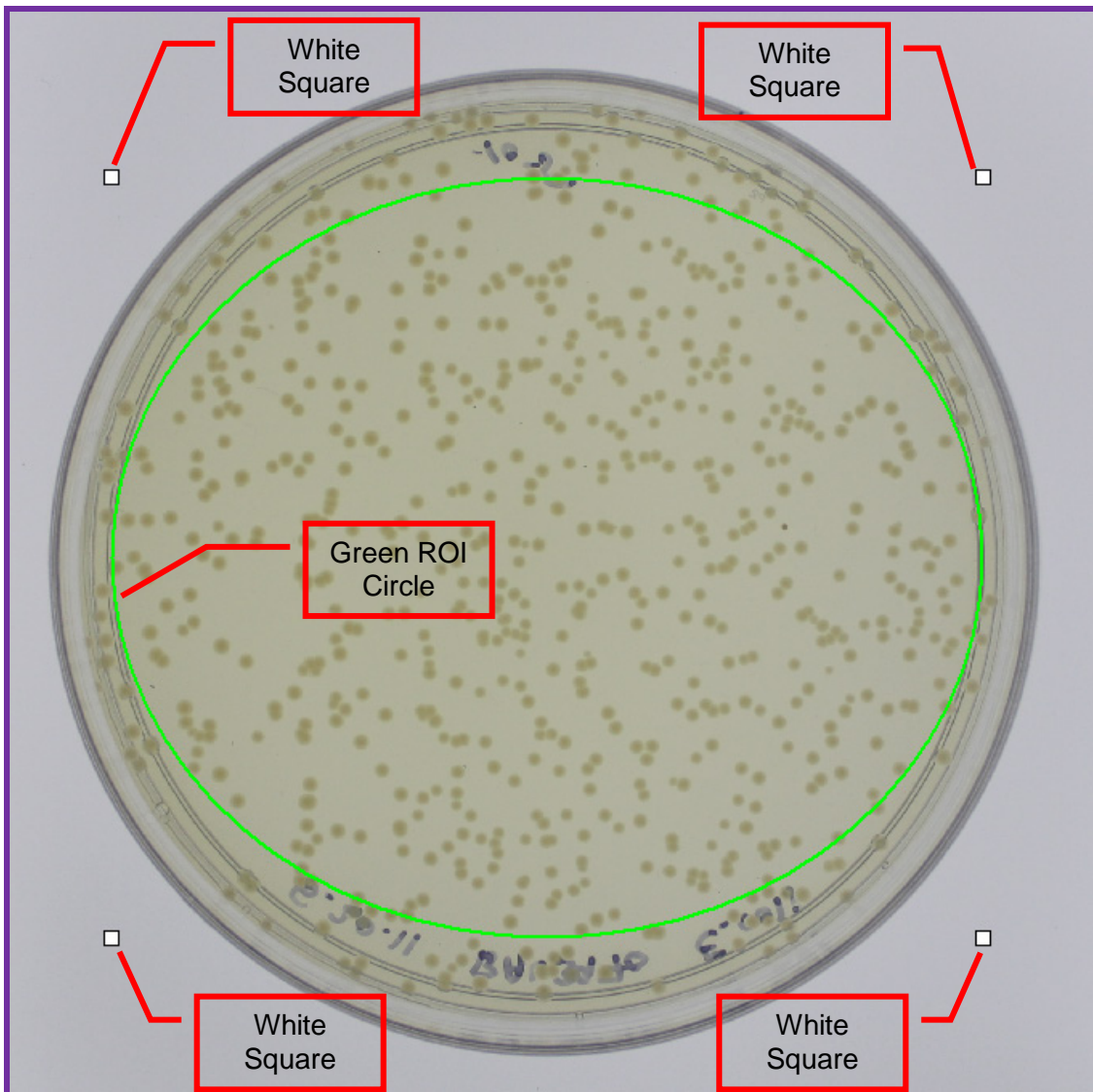
- After the image has been captured, click on “File” → “Save As” to save a copy of the image.



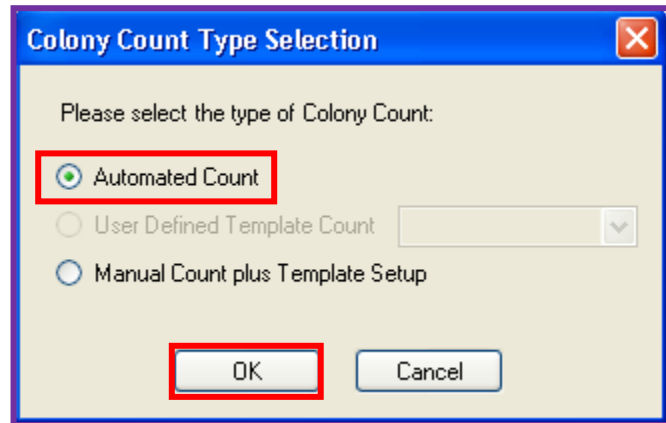
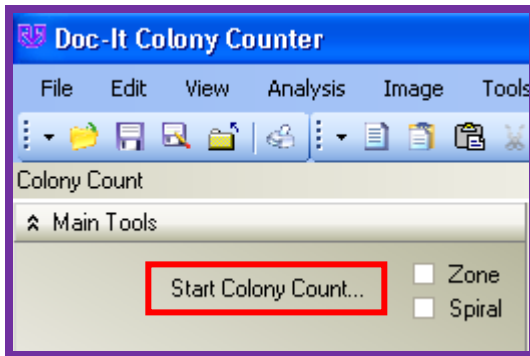
- Once the capture is complete, adjust for the desired Region of Interest (ROI) by selecting the "Elliptical ROI" icon at the top of the software.



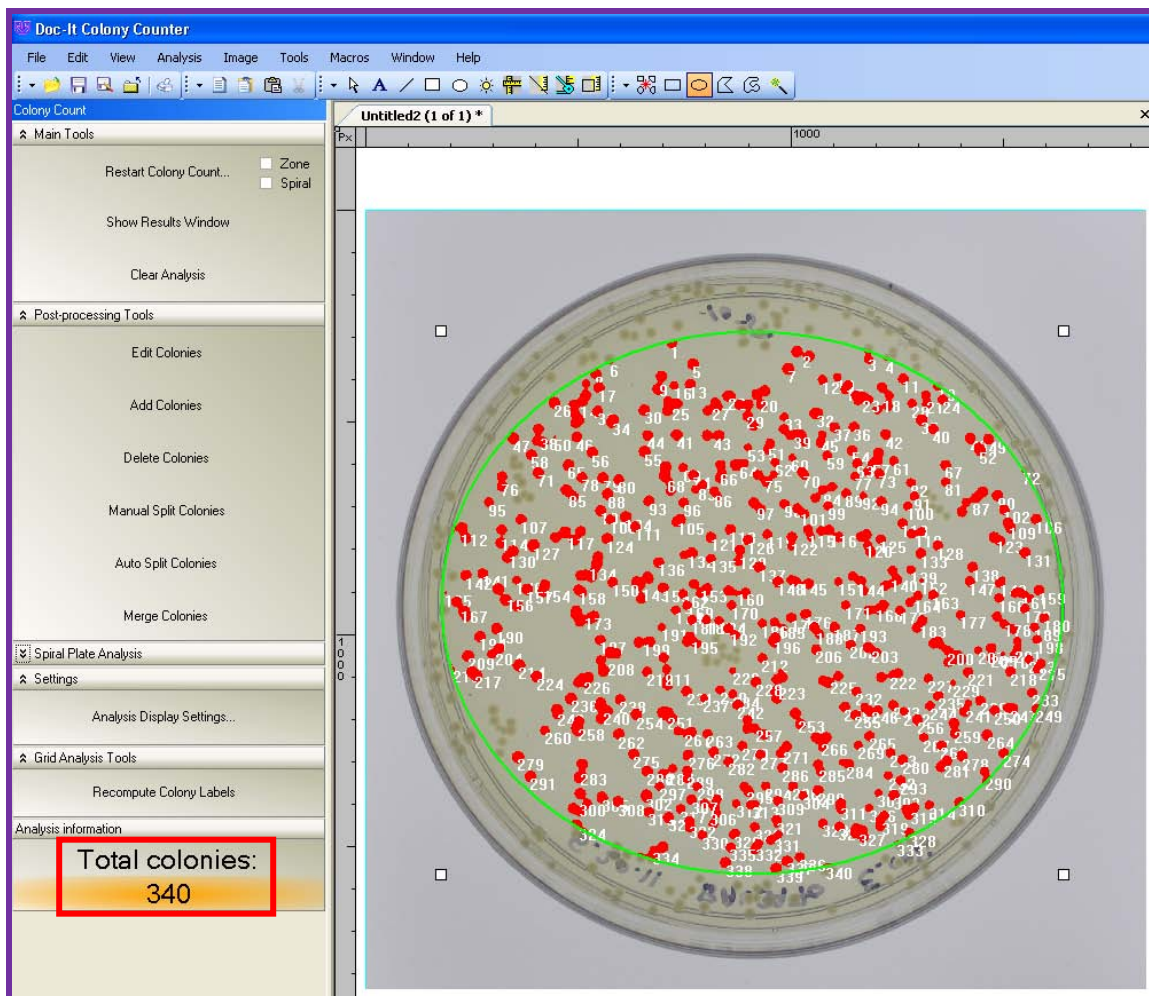
- Use the four white squares that appear to adjust the green ROI circle to count only the area of interest, avoiding the edges of the sample or any areas where writing appears.



- On the left side of the screen under the “Colony Count” module, click “Start Colony Count”. Select “Automated Count” and click “OK”.

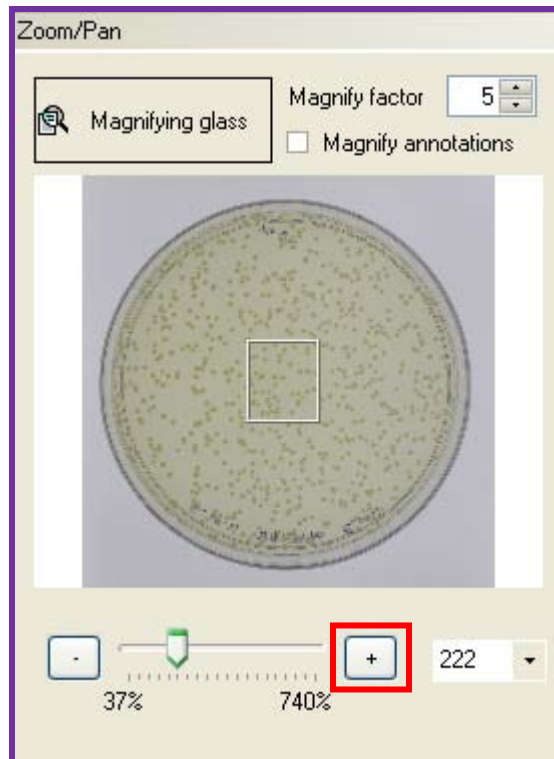


- Wait approximately 15 seconds for the system to process the total colony count. In the sample below, the system found 340 colonies as shown in the “Analysis Information” section of the “Colony Count” module on the left side of the screen.



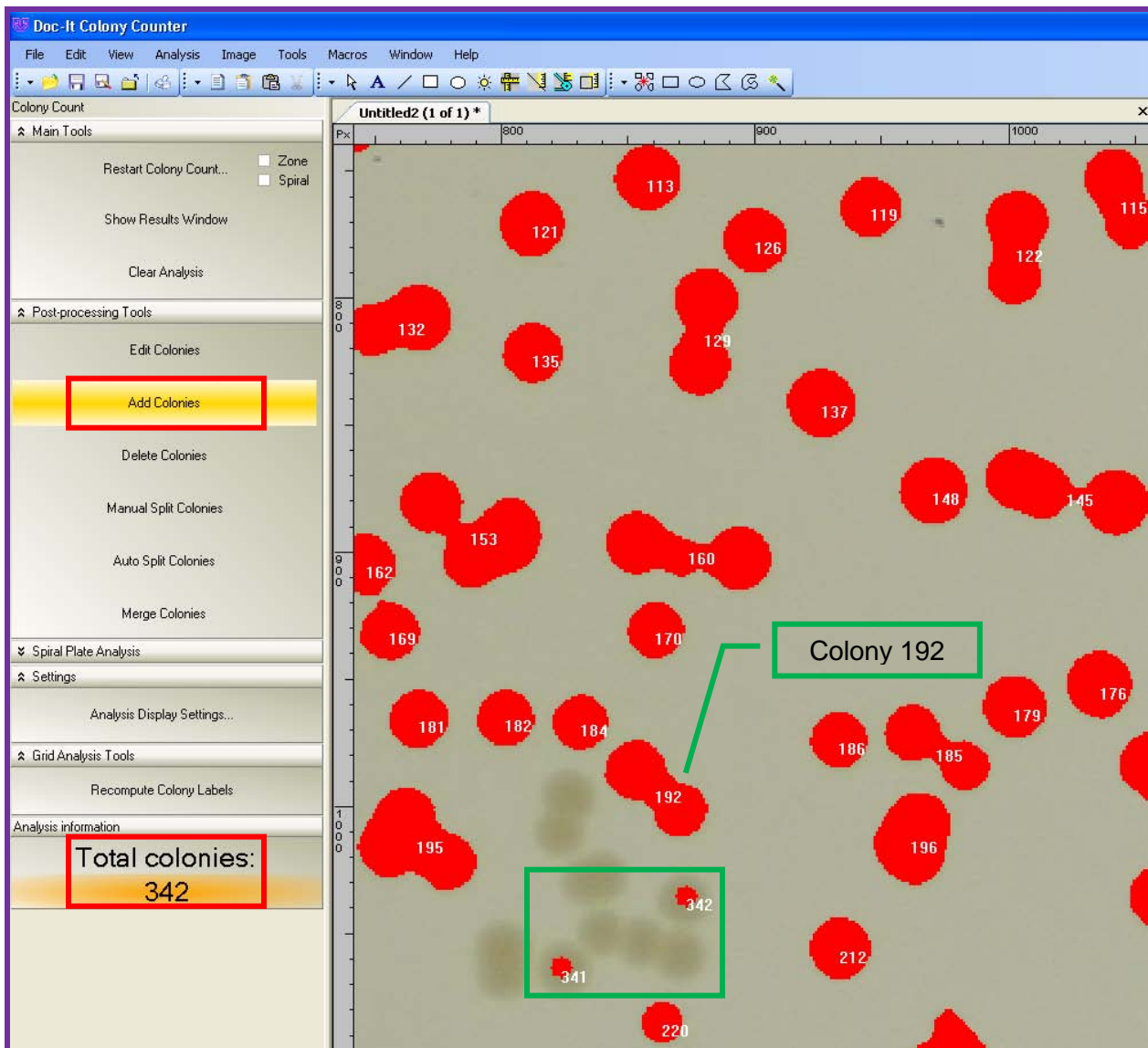
14. At this point, you may opt to add new or uncounted colonies, to split colonies, or to delete colonies.

*Note: In the sample image, a certain area of the sample was zoomed in on by clicking the “+” sign under the “Zoom/Pan” menu in the lower-right corner of the screen.*



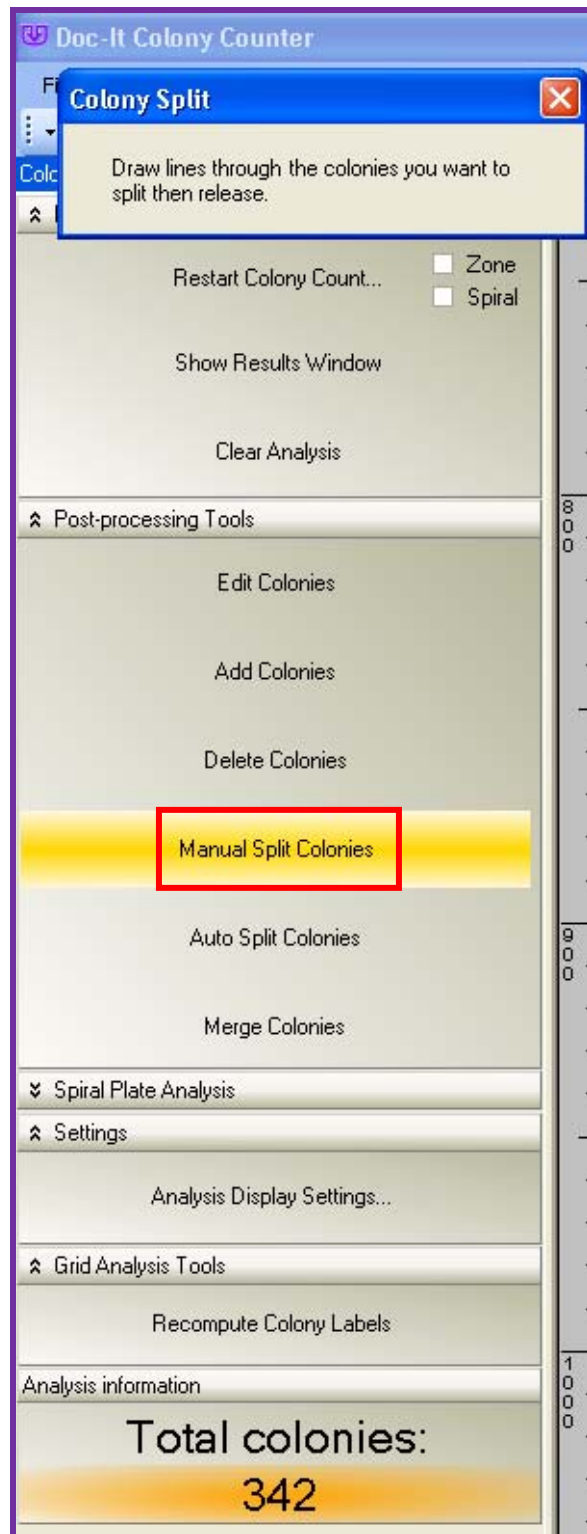
- a. **To Add Colonies:** In this example, let's assume you'd like to add a few colonies that were not found automatically by the system below Colony 192. To do so, click "Add Colonies" under the "Colony Count" module on the left side of the screen. Then, click on the colony you'd like to add.

In this example, you can see we successfully added Colonies 341 and 342 in the lower portion of the screen. Note that the system has now updated the total number of colonies to 342 in the lower left portion of the software.



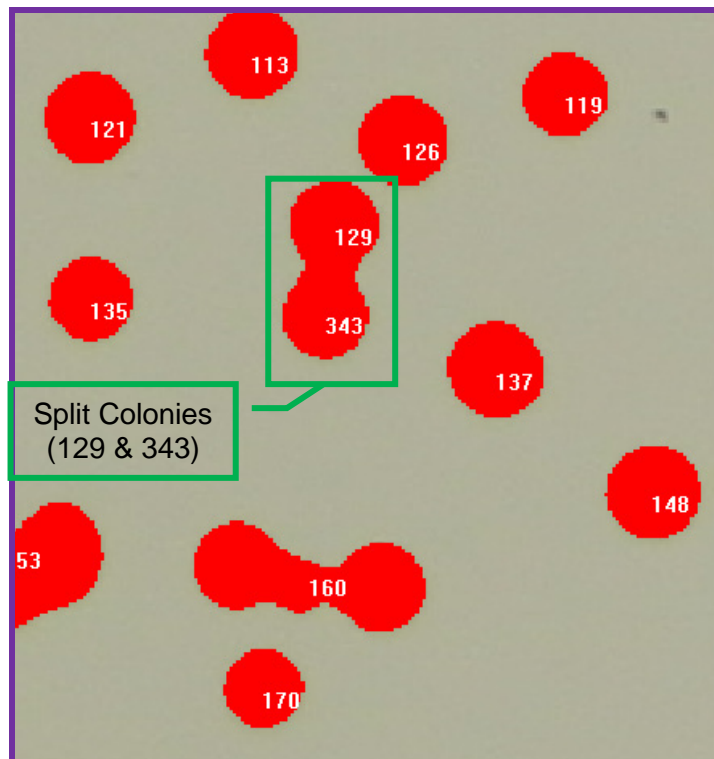
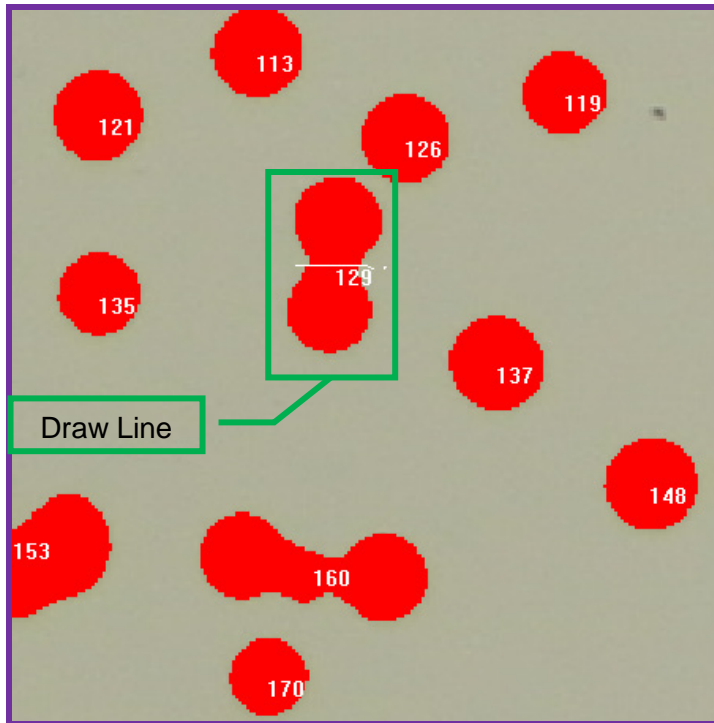


- b. **To Split Colonies:** Let's assume you'd like to split Colony 129 (which the system automatically recognized as one colony) into two separate colonies. To do so, click "Manual Split Colonies" under the "Colony Count" module on the left side of the screen.

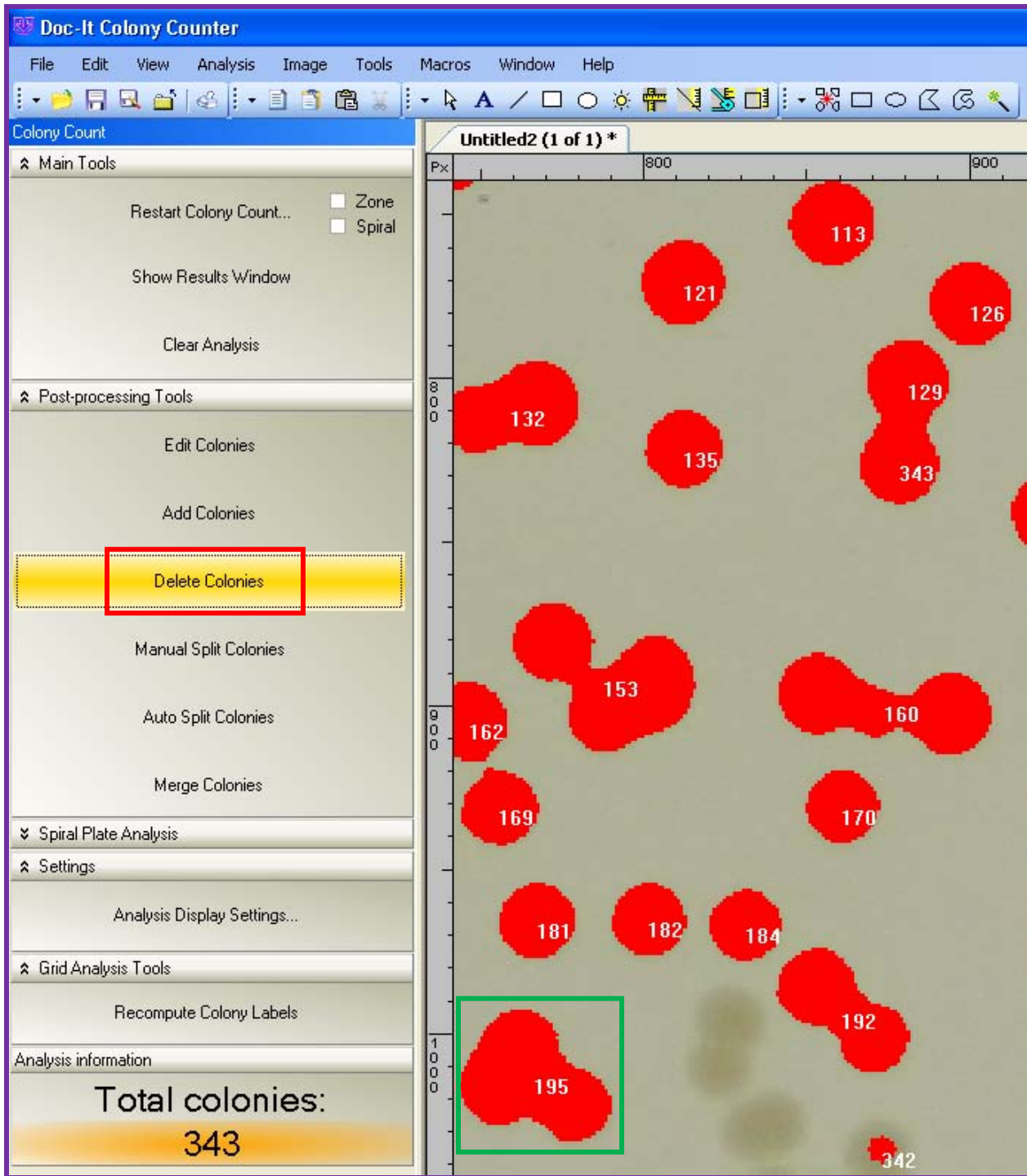


While holding the left mouse button, draw a line through the colonies you'd like to split and then release the mouse button.

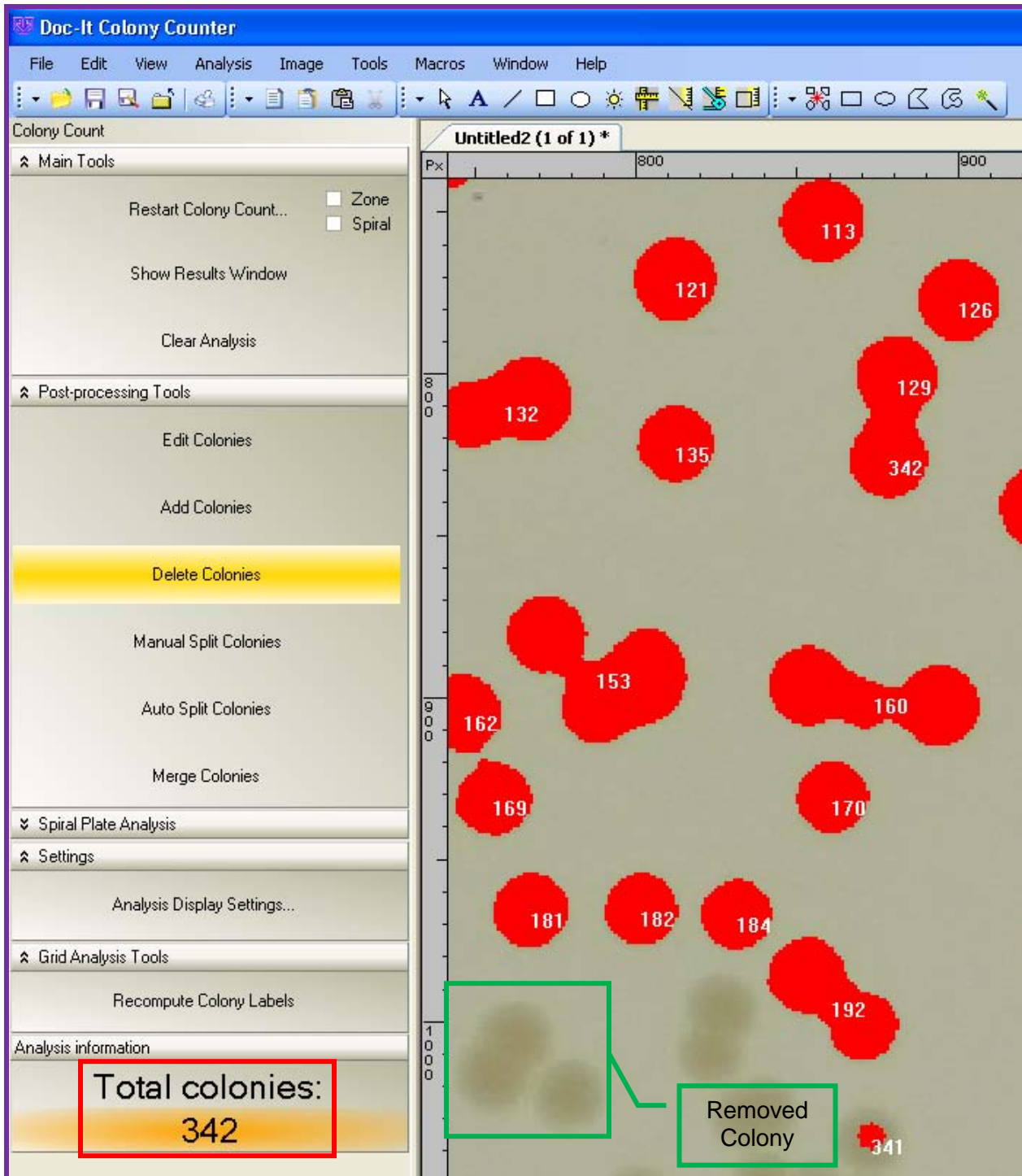
In this example, you can see we successfully split Colony 129 into Colonies 129 and 343, and the system has now updated the total number of colonies to 343 in the lower left portion of the software.



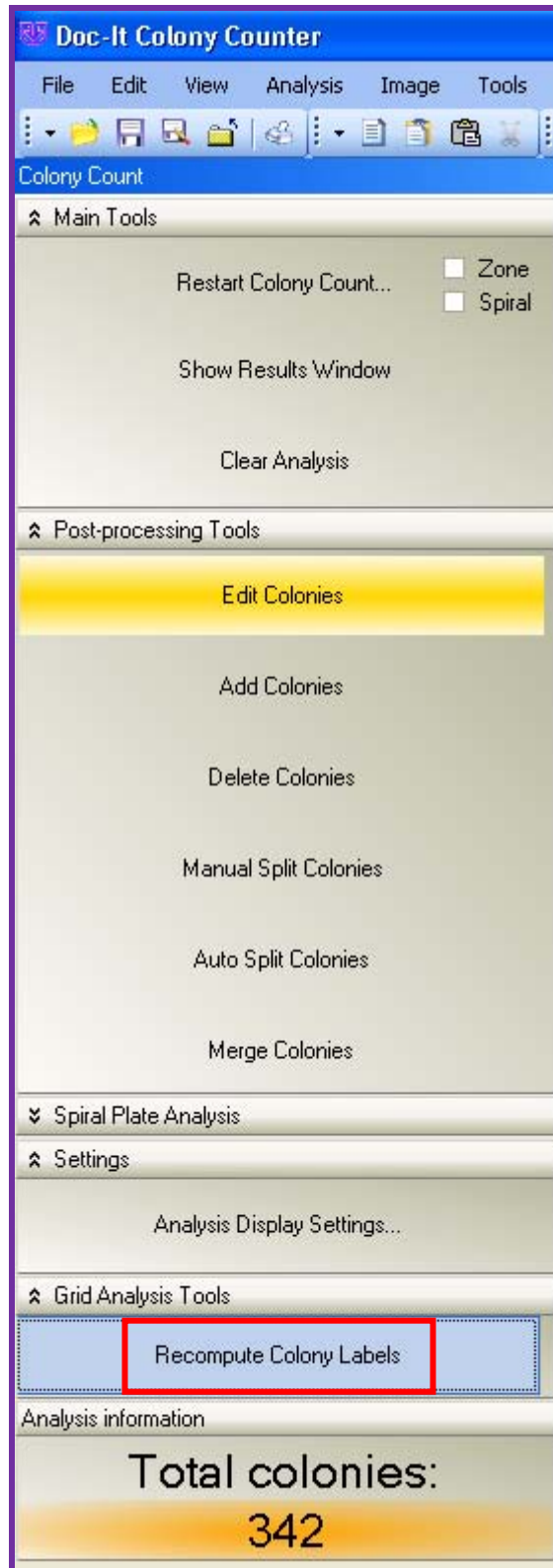
- c. **To Delete Colonies:** Let's assume you'd like to delete Colony 195 in the lower left portion of the image. To do so, click "Delete Colonies" under the "Colony Count" module on the left side of the screen. Then, click on the colony you'd like deleted (in this example, Colony 195).



In this example, you can see that Colony 195 has been removed, and the system has updated the total number of colonies to 342 under the “Colony Count” module.

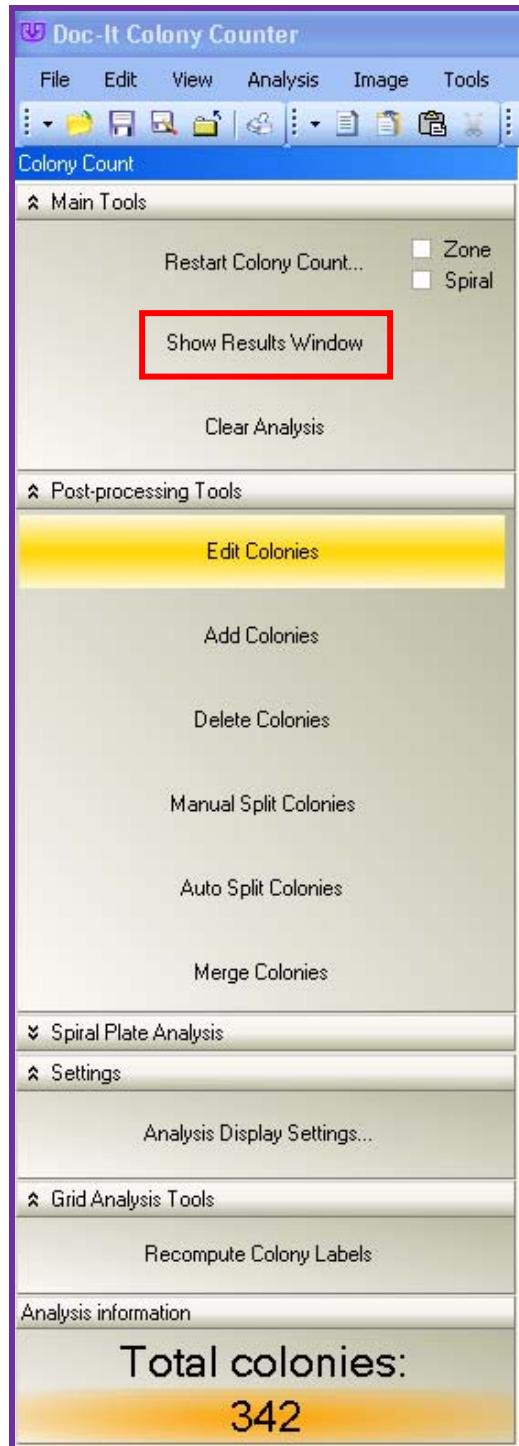


15. After all modifications have been made, click “Recompute Colony Labels” under the “Colony Count” module.



- To view the results of the analysis, click “Show Results Window” under the “Colony Count” module on the left side of the screen. From here, the software creates a detailed report of each colony counted.

Results of the colony count are automatically entered into a tabular format and can be exported into Excel for further analysis. The reported parameters include classes, colonies, statistics and distribution of colony area.



- a. The “Classes” parameter of the report details what number and type of classes were detected in the count.

Class Number	No. of colonies	% colonies	Total Area(px)	% Area	Mean Area(px)	Std. dev. Area
1	342	100	236565	100	691.71	452.56

- b. The “Colonies” parameter lists each colony by number and provides information related to the class, area, perimeter, average diameter, and circularity of each colony counted.

Colony Number	Class	Area(px)	Perimeter(px)	Avg Diameter(px)	Circularity
1	1	336	70.7	20.75	1.18
2	1	1139	164.68	31.92	1.89
3	1	76	43.63	8.24	1.99
4	1	606	90.84	28.04	1.08
5	1	647	93.08	29.14	1.07
6	1	493	101.33	24.86	1.66
7	1	75	41.63	8.71	1.84
8	1	2854	326.71	37.59	2.98
9	1	500	82.6	25.59	1.09
10	1	1241	168.34	32.47	1.82
11	1	511	82.84	25.84	1.07

- c. The “Statistics” parameter of the report presents the numerical value of each value in a statistical format.

Property	Area(px)
Min.	24
Obj. No.	165
Max.	2854
Obj. No.	14
Range	2830
Mean	691.71
Std. dev.	452.56
Sum	236565
Samples	342

d. The “Distribution” parameter provides a bar graph of each colony area.

