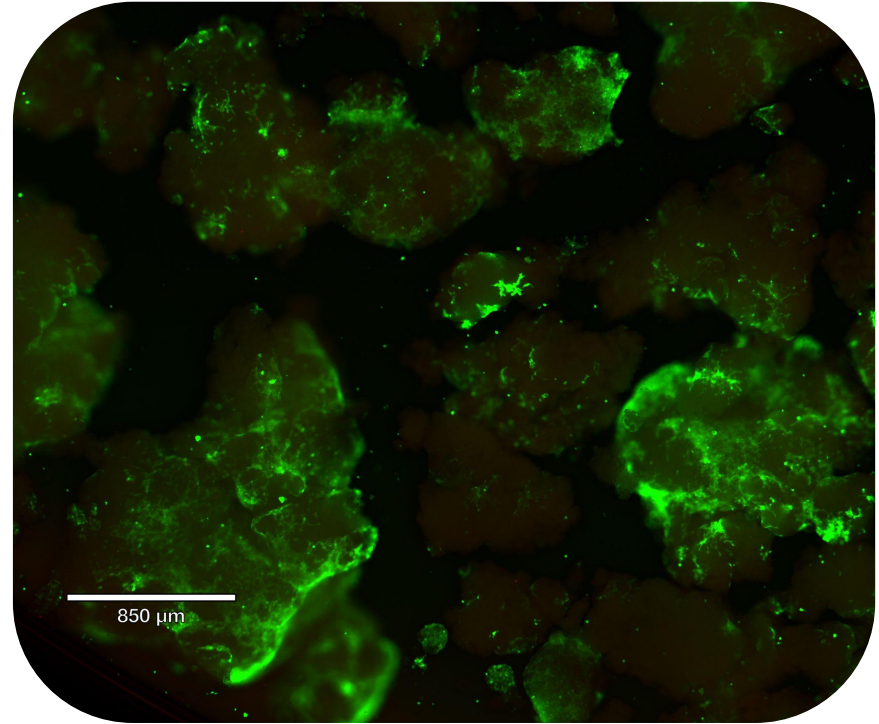
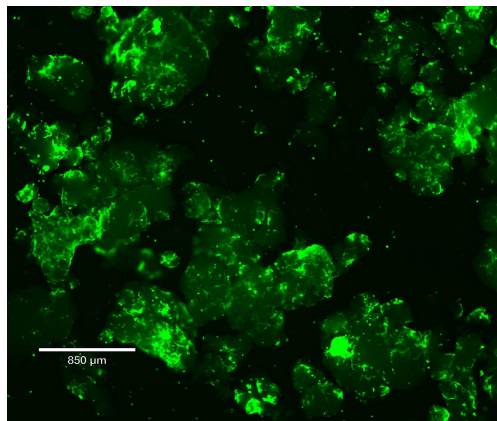


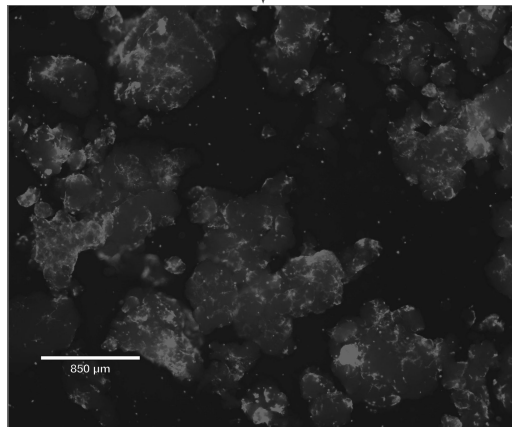
Precision Confluency Analysis in Live/Dead Stain Assays



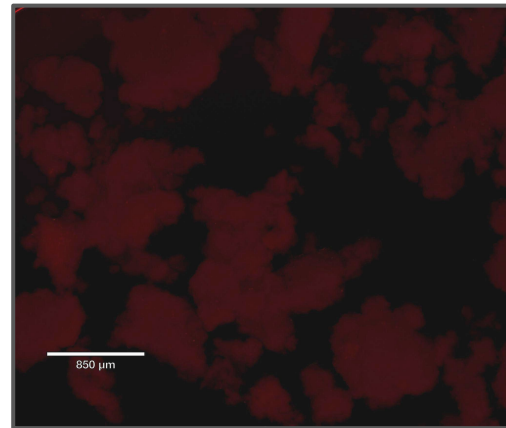
488 nm



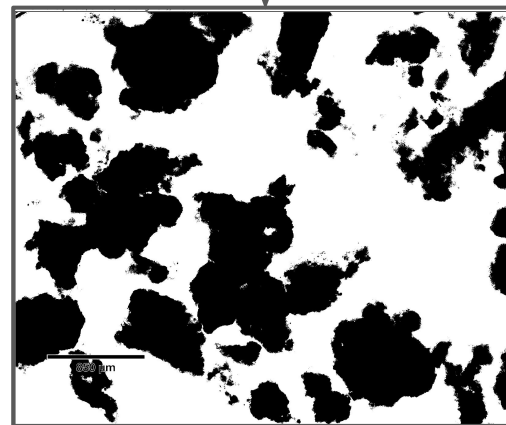
RGB to 16 Bit



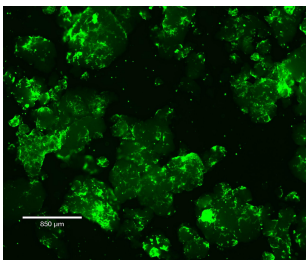
594 nm



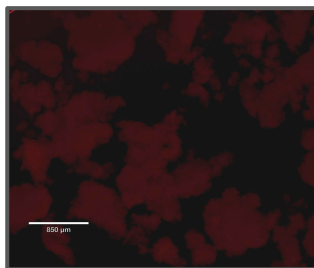
Thresholding



488 nm



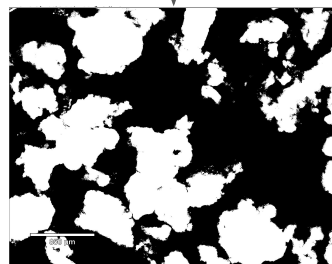
594 nm



Thresholding



Inverting



RGB to 16 Bit

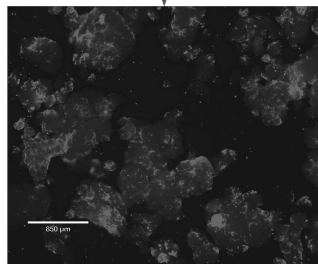
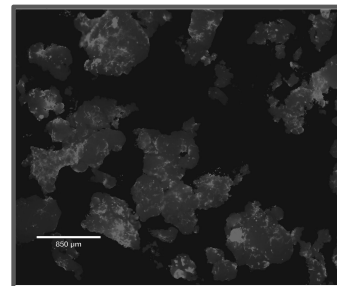
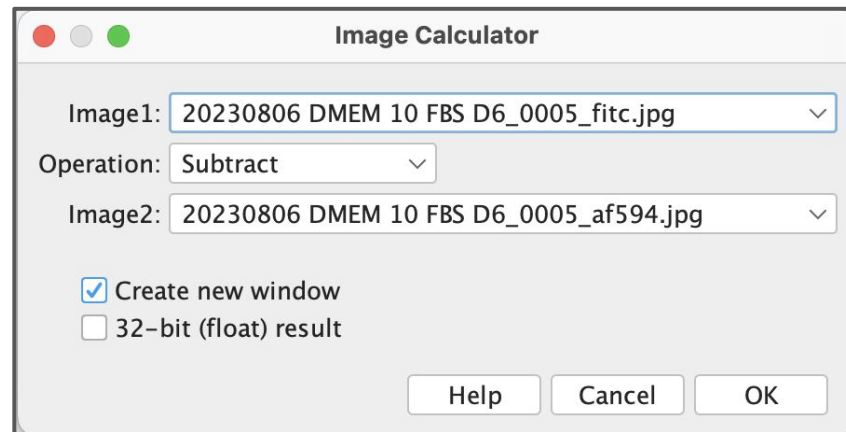
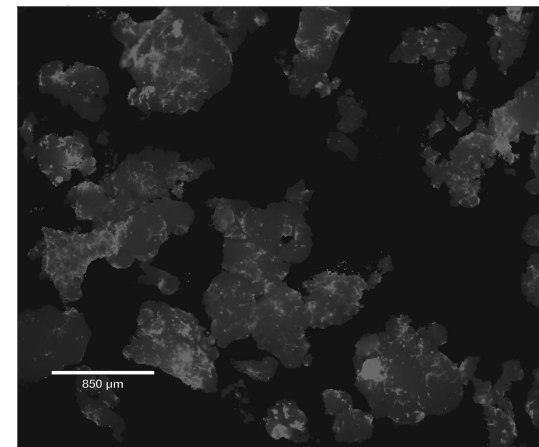
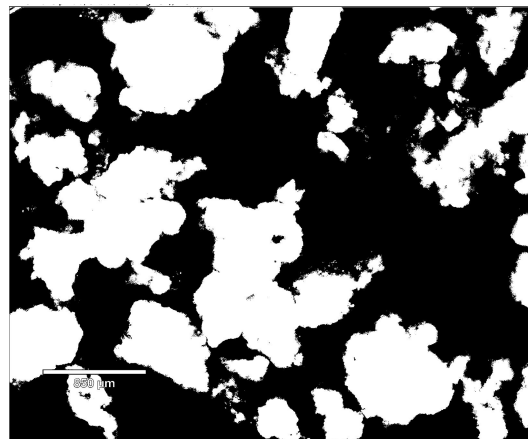
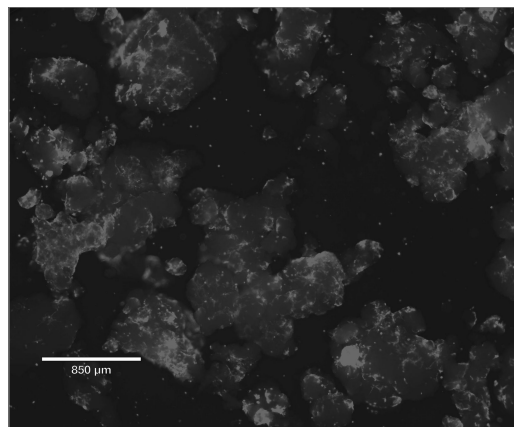


Image
Subtraction

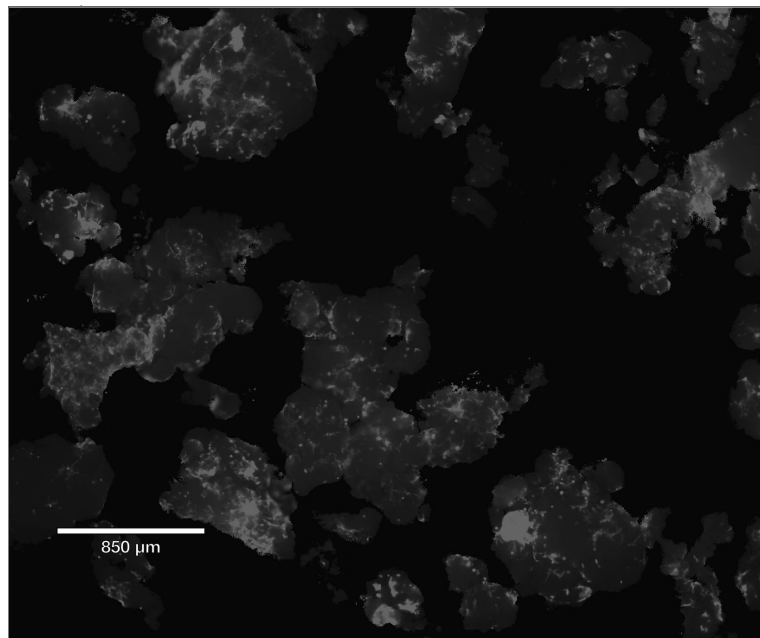


Segmented
Microcarriers with
no floating cells

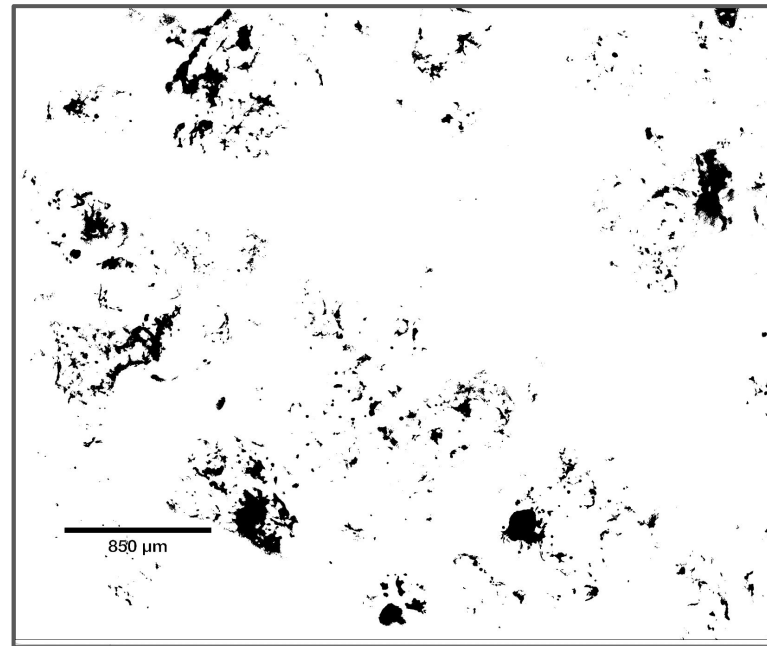
Removing Non-Adherent Cells Through Image Subtraction



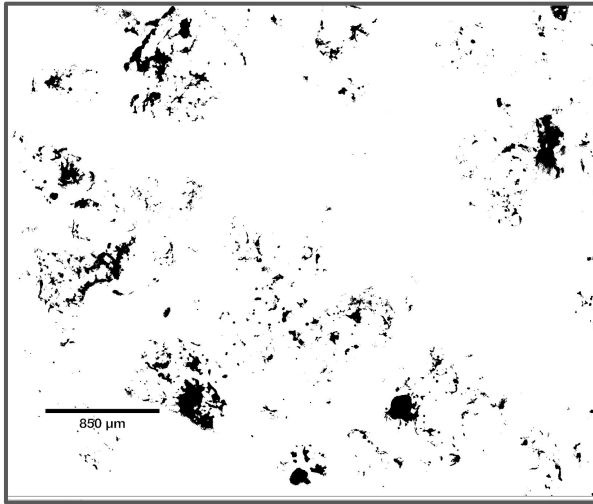
Isolating Adherent Cell Signaling Through Image Thresholding



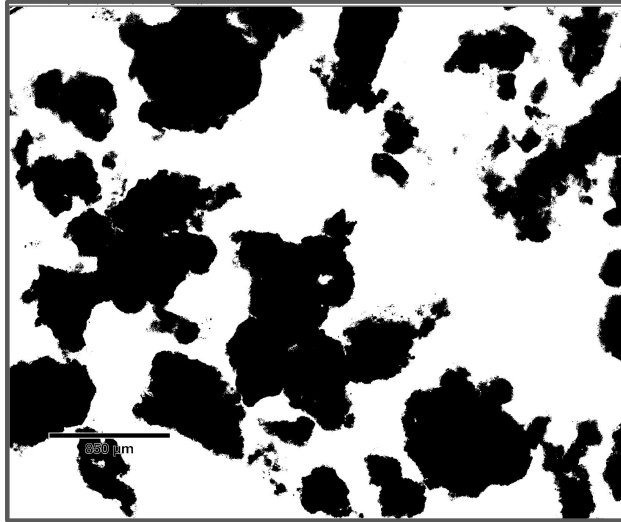
Thresholding



Adhered Cell Area



Microcarrier Area



Total
Adhered Cell Area

Total Microcarrier
Area

Slice	Count	Total Area	Average Size	%Area
20230806 DMEM 10 FBS D6_0005_af594.jpg	840	6676633.469	7948.373	41.419
Result of 20230806 DMEM 10 FBS D6_0005_fitc.jpg	1290	725007.877	562.022	4.498

$6676633 / 725007 = 10.8$ Percent Confluency

Project:

- A. Develop image analysis pipeline for 3D cell confluency measurements.
- B. Deploy an in-house web application that can be readily used by scientists to assess 3d cell growth



Cell Confluency Calculator

Note: Make sure the live/dead pairs have the same name other than differing by _fitc/ _af594

Example: test_file_0000_fitc.jpg / test_file_0000_af594.jpg

Live Images (FITC)

Upload Your 3d Live(s) Image Below:



Drag and drop files here

Limit 200MB per file

Browse files

Dead Images (594 nm)

Upload Your 3d Dead Image(s) Below:



Drag and drop files here

Limit 200MB per file

Browse files

- Link to application: <https://cellconfluencyappy-4xdscga9uxkzbsssqritkq.streamlit.app/>



Cell Confluency Calculator

Note: Make sure the live/dead pairs have the same name other than differing by _fitc/ _af594

Example: test_file_0000_fitc.jpg / test_file_0000_af594.jpg

Live Images (FITC)

Upload Your 3d Live(s) Image Below:



Drag and drop files here

Limit 200MB per file

Browse files

Dead Images (594 nm)

Upload Your 3d Dead Image(s) Below:



Drag and drop files here

Limit 200MB per file

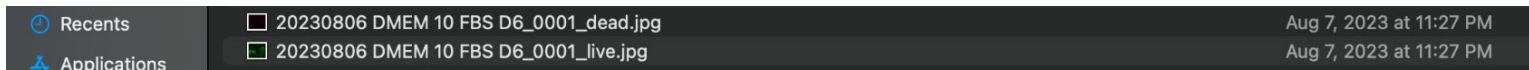
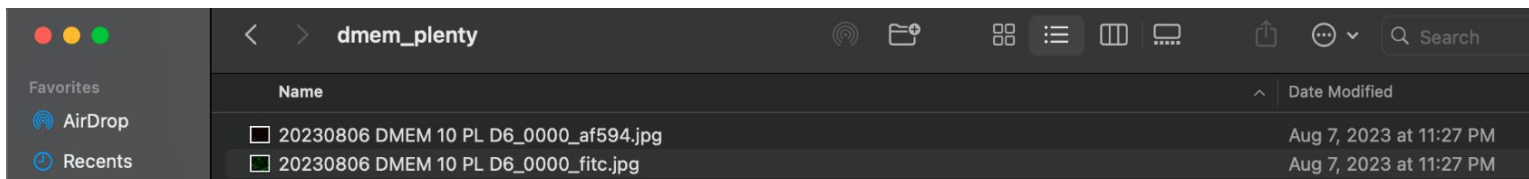
Browse files

Loading Images:

Favorites AirDrop Recents	dmem_plenty				Search	
	Name	Date Modified	Size	Kind		
	20230806 DMEM 10 PL D6_0000_af594.jpg	Aug 7, 2023 at 11:27 PM	338 KB	JPEG image		
	20230806 DMEM 10 PL D6_0000_fitc.jpg	Aug 7, 2023 at 11:27 PM	1 MB	JPEG image		

Important Rules:

1. Make sure the live/dead pairs have identical names but only differ in channel (e.g. **af594** , **fitc**)



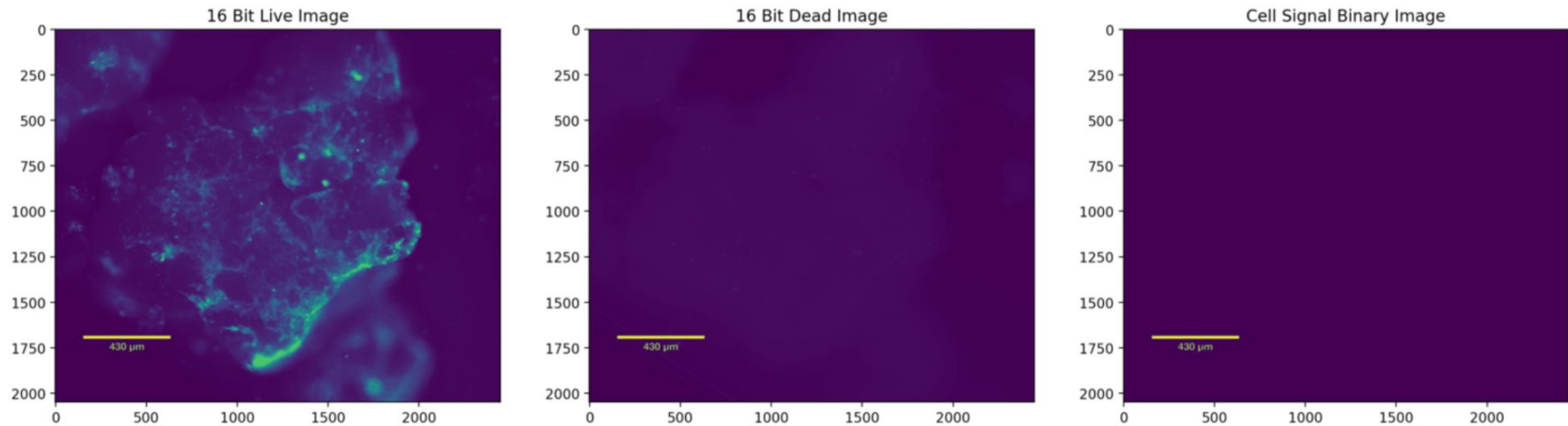
***on the Echo microscope, you can automate this process by choosing 'overlay' as the photo collection option. The program will save your designated name then tag on the af594/fitc options

Important Rules:

- 1. High magnification (greater than 2X/ 0.8 scale) resolves **less microcarriers** and introduces shallow depth of field artifacts.



Analyzing images: 20230806 DMEM 10 PL D6_0010_fitc.jpg ----- 20230806 DMEM 10 PL D6_0010_af594.jpg



Percent Confluence: 1.43%

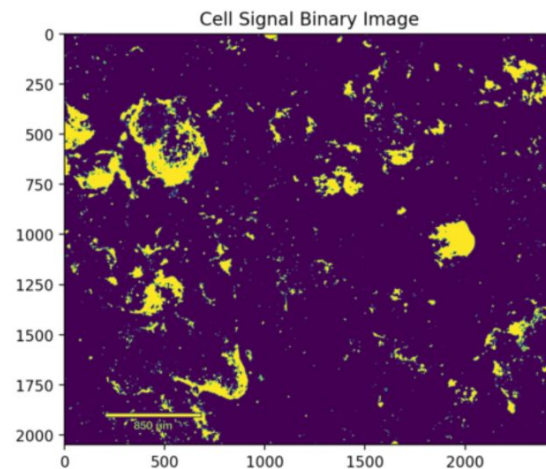
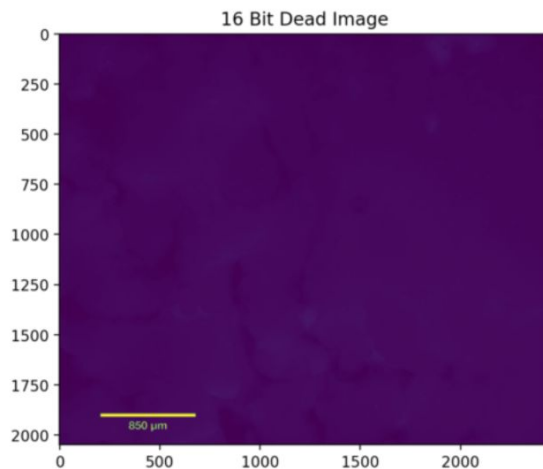
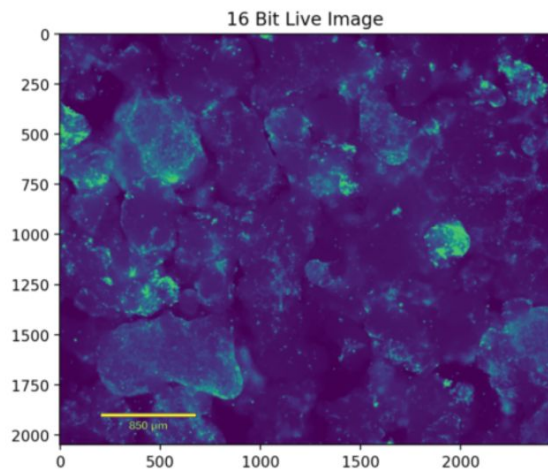
	Live File	Dead File	Percent Confluence
0	20230806 DMEM 10 PL D6_0010_fitc.jpg	20230806 DMEM 10 PL D6_0010_af594.jpg	1.43

Important Rules:

1. An in-focus image at (2x/0.08) resolves multiple microcarriers which are readily analyzed



Analyzing images: 20230808 dmem 10 PL d8_0007_fitc.jpg ----- 20230808 dmem 10 PL d8_0007_af594.jpg



Percent Confluence: 55.46%

	Live File	Dead File	Percent Confluence
0	20230808 dmem 10 PL d8_0007_fitc.jpg	20230808 dmem 10 PL d8_0007_af594.jpg	55.46




Cell Confluency Calculator

Note: Make sure the live/dead pairs have the same name other than differing by _fitc/ _af594









Example: test_file_0000_fitc.jpg / test_file_0000_af594.jpg

Live Images (FITC)

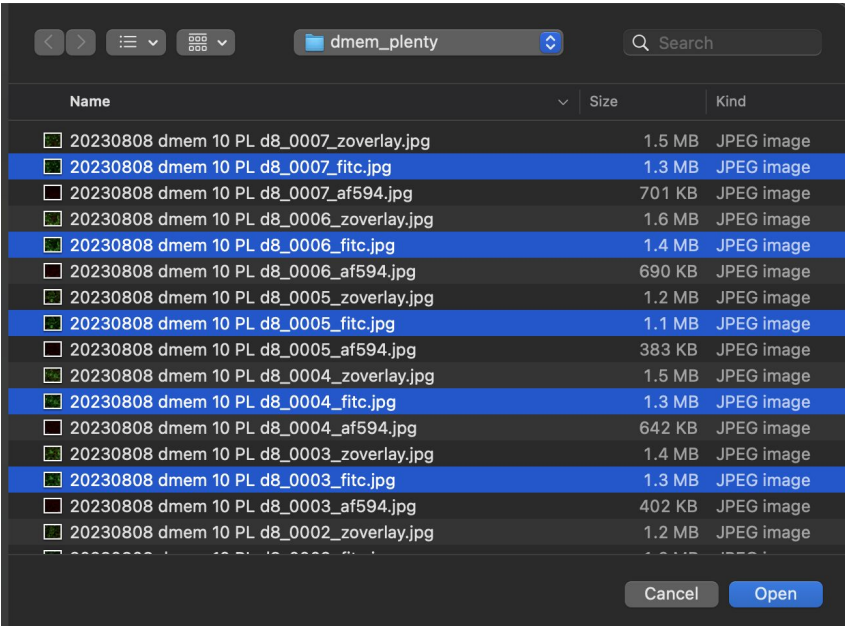
Upload Your 3d Live(s) Image Below:

 Drag and drop files here
Limit 200MB per file

Browse files

-  20230806 DMEM 10 PL D6_0005_fitc.jpg 1.5MB 
-  20230806 DMEM 10 PL D6_0004_fitc.jpg 1.0MB 
-  20230806 DMEM 10 PL D6_0003_fitc.jpg 1.1MB 
- Showing page 1 of 2  

Analyzing images: 20230806 DMEM 10 PL D6_0000_fitc.jpg ----- 20230806 DMEM 10 PL D6_0000_af594.jpg



- Feel free to drop replicates of each image type

	Live File	Dead File	Percent Confluence
0	20230806 DMEM 10 PL D6_0000_fitc.jpg	20230806 DMEM 10 PL D6_0000_af594.jpg	34.56
1	20230806 DMEM 10 PL D6_0002_fitc.jpg	20230806 DMEM 10 PL D6_0002_af594.jpg	34.66
2	20230806 DMEM 10 PL D6_0003_fitc.jpg	20230806 DMEM 10 PL D6_0003_af594.jpg	31.53
3	20230806 DMEM 10 PL D6_0004_fitc.jpg	20230806 DMEM 10 PL D6_0004_af594.jpg	32.94
4	20230806 DMEM 10 PL D6_0005_fitc.jpg	20230806 DMEM 10 PL D6_0005_af594.jpg	141.6

Download data as CSV

- Doing so will generate averages/ standard deviations for your replicates
- If you want to plot your own data, download your measurements to a csv file



Mean Percent Confluence: 55.06 Standard Deviation: 43.29