CMED Image Analysis Documentation Manual

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1 Setup

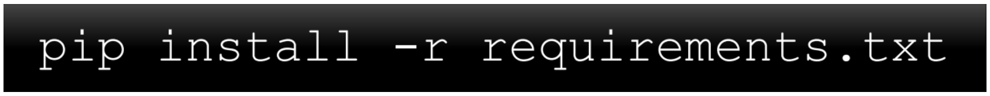
The program requires Python to run and knowledge of using a terminal/command line interface on the operating system of your choice. Python can be downloaded through the official website, and tutorials like these can be found on YouTube for basic terminal navigation and code running.

The code repository should look as follows:

- assets/
- Export.py
- Image.py
- ImageCollection.py
- ImageViewer.py
- qrangeslider.py
- main.py
- main.ui
- requirements.txt
- ACKNOWLEDGEMENTS.md
- LICENSE.md

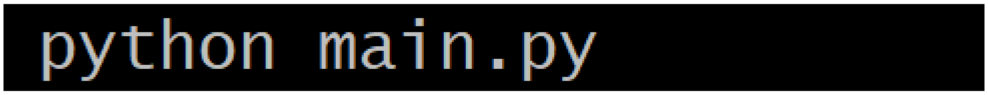
In order to execute the code, the packages in the requirements.txt must be installed along with Python 3.8. Place all files into the same directory and navigate there through your preferred terminal. The following commands can be used to execute and run the code, provided a valid version of Python is installed:

Installing requirements:



```
pip install -r requirements.txt
```

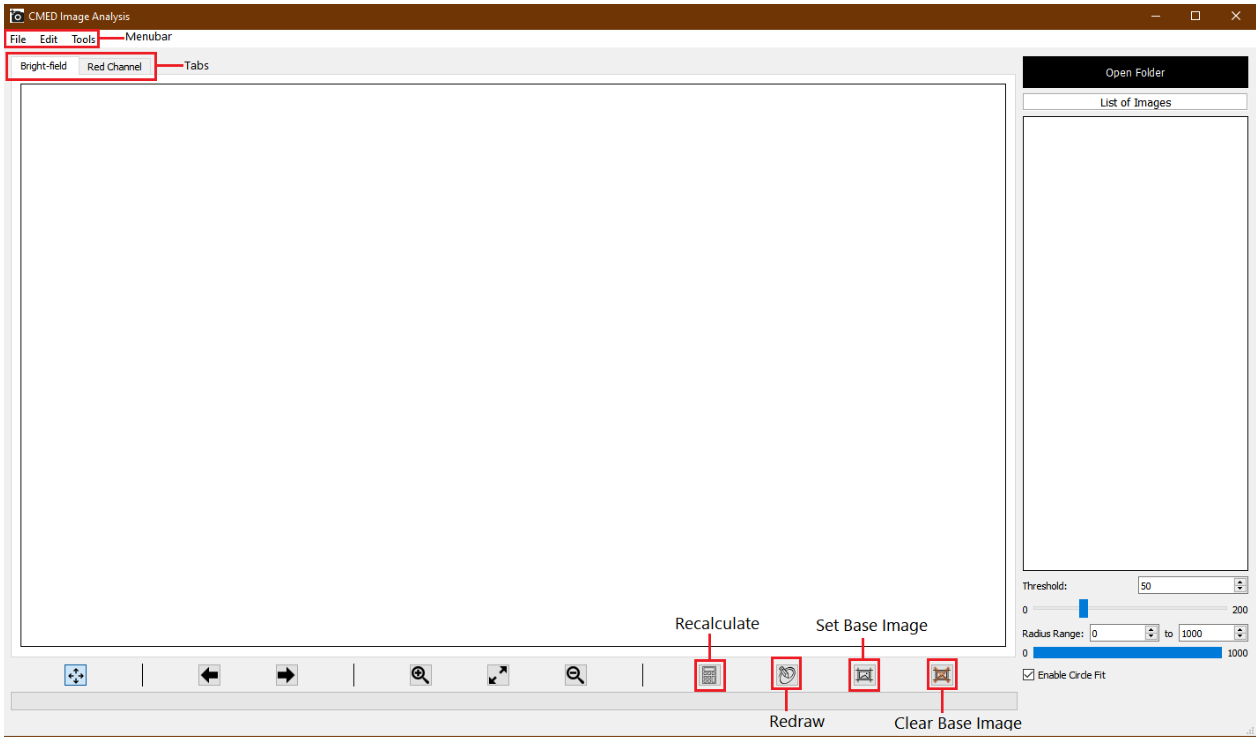
Executing code:



```
python main.py
```

2 USAGE

2.1 Initial GUI + opening and selecting a folder



Threshold: 50

Radius Range: 0 to 1000

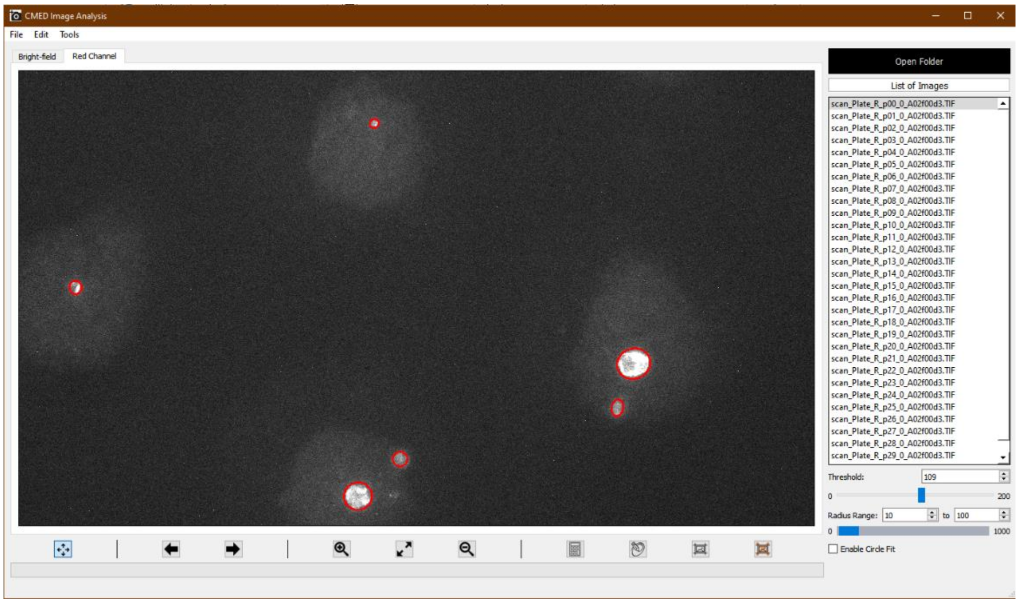
☑ Enable Circle Fit

A directory is first opened through the “Open Folder” button. Two different folder structures are supported for mapping shapes to images and extracting data from it. An additional folder structure is supported for the viewing and analysis of images termed “Z-Stack” images that measure the spheroids and sensors on the z-axis. The program supports this structure for the calculation of image sharpness and general viewing of the images. The full folder structure and regular expressions being used to parse text can be found in the Appendix.

After a folder has been selected, the program loads in the image sets and immediately displays the image and begins calculating fits and drawing shapes based on default parameters. The default thresholding parameter for red channel images is set to 120, with minimum and maximum radii of 10 and 100 pixels respectively. For the bright field images, the thresholding defaults at 120 as well, but with a minimum and maximum radii of 40 to 500 pixels respectively.

2.2 Fitting shapes to the images

Ensure that when using the program, bright field images are fit *after* fitting the red channel image for a given pair. The reason being is that the spheroids chosen to trace are based around which ellipses are traced. If not done, when setting the base image it's possible that no ellipses gets traced. In which case, simply clearing the base image, and recalculating the spheroid traces should resolve the issue.



scan_Plate_R_p00_0_A02f00d3.TIF
scan_Plate_R_p01_0_A02f00d3.TIF
scan_Plate_R_p02_0_A02f00d3.TIF
scan_Plate_R_p03_0_A02f00d3.TIF
scan_Plate_R_p04_0_A02f00d3.TIF
scan_Plate_R_p05_0_A02f00d3.TIF
scan_Plate_R_p06_0_A02f00d3.TIF
scan_Plate_R_p07_0_A02f00d3.TIF
scan_Plate_R_p08_0_A02f00d3.TIF
scan_Plate_R_p09_0_A02f00d3.TIF
scan_Plate_R_p10_0_A02f00d3.TIF
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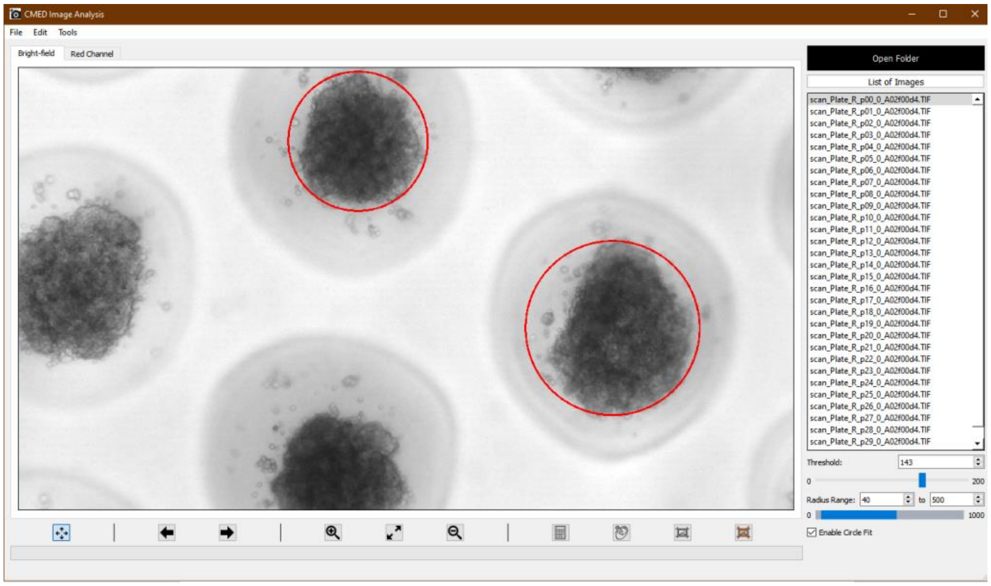
scan_Plate_R_p13_0_A02f00d3.TIF
scan_Plate_R_p14_0_A02f00d3.TIF
scan_Plate_R_p15_0_A02f00d3.TIF
scan_Plate_R_p16_0_A02f00d3.TIF
scan_Plate_R_p17_0_A02f00d3.TIF
scan_Plate_R_p18_0_A02f00d3.TIF
scan_Plate_R_p19_0_A02f00d3.TIF
scan_Plate_R_p20_0_A02f00d3.TIF
scan_Plate_R_p21_0_A02f00d3.TIF
scan_Plate_R_p22_0_A02f00d3.TIF
scan_Plate_R_p23_0_A02f00d3.TIF
scan_Plate_R_p24_0_A02f00d3.TIF
scan_Plate_R_p25_0_A02f00d3.TIF
scan_Plate_R_p26_0_A02f00d3.TIF
scan_Plate_R_p27_0_A02f00d3.TIF
scan_Plate_R_p28_0_A02f00d3.TIF
scan_Plate_R_p29_0_A02f00d3.TIF

Threshold: 109
0 200
Radius Range: 10 to 100
0 1000
☐ Enable Circle Fit



scan_Plate_R_p00_0_A02f00d4.TIF
scan_Plate_R_p01_0_A02f00d4.TIF
scan_Plate_R_p02_0_A02f00d4.TIF
scan_Plate_R_p03_0_A02f00d4.TIF
scan_Plate_R_p04_0_A02f00d4.TIF
scan_Plate_R_p05_0_A02f00d4.TIF
scan_Plate_R_p06_0_A02f00d4.TIF
scan_Plate_R_p07_0_A02f00d4.TIF
scan_Plate_R_p08_0_A02f00d4.TIF
scan_Plate_R_p09_0_A02f00d4.TIF
scan_Plate_R_p10_0_A02f00d4.TIF
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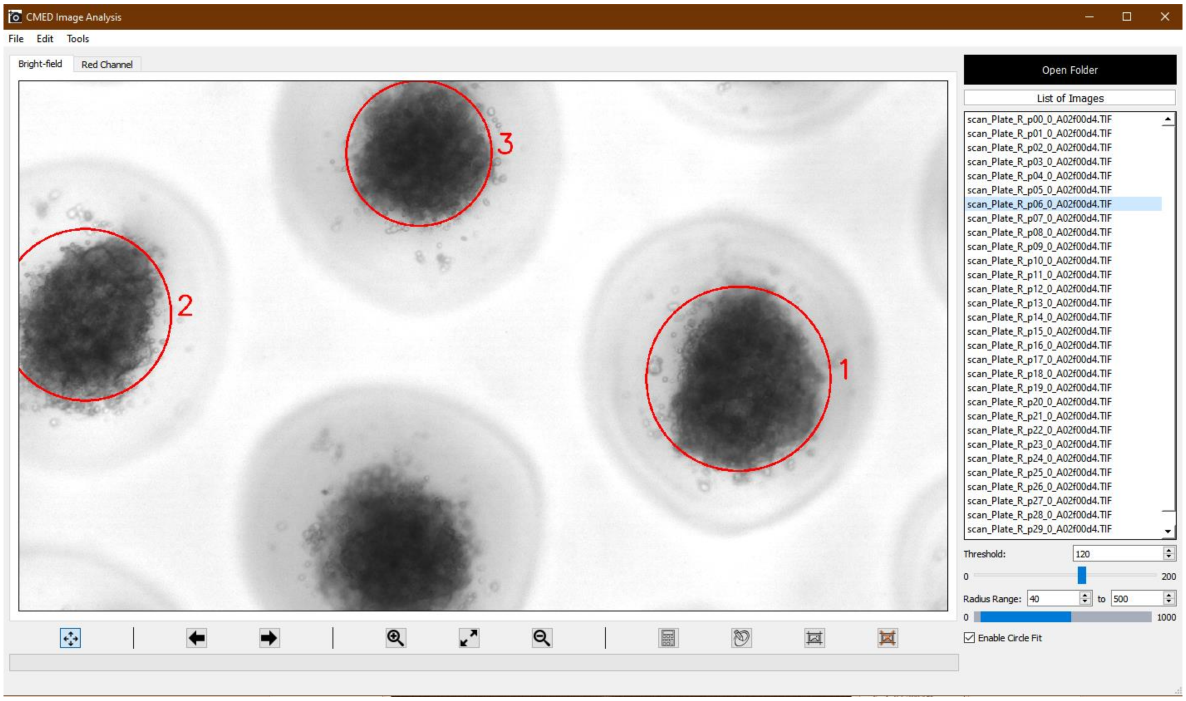
scan_Plate_R_p13_0_A02f00d4.TIF
scan_Plate_R_p14_0_A02f00d4.TIF
scan_Plate_R_p15_0_A02f00d4.TIF
scan_Plate_R_p16_0_A02f00d4.TIF
scan_Plate_R_p17_0_A02f00d4.TIF
scan_Plate_R_p18_0_A02f00d4.TIF
scan_Plate_R_p19_0_A02f00d4.TIF
scan_Plate_R_p20_0_A02f00d4.TIF
scan_Plate_R_p21_0_A02f00d4.TIF
scan_Plate_R_p22_0_A02f00d4.TIF
scan_Plate_R_p23_0_A02f00d4.TIF
scan_Plate_R_p24_0_A02f00d4.TIF
scan_Plate_R_p25_0_A02f00d4.TIF
scan_Plate_R_p26_0_A02f00d4.TIF
scan_Plate_R_p27_0_A02f00d4.TIF
scan_Plate_R_p28_0_A02f00d4.TIF
scan_Plate_R_p29_0_A02f00d4.TIF

Threshold: 143
0 200
Radius Range: 40 to 500
0 1000
☑ Enable Circle Fit

The list of images on the right allows for quick identification for which image in the set you are on and navigation between different points in the set with ease. You can swap between the bright field and red channel image sets using the tabs on the top left. All input parameters will be saved specific to the image being analyzed, and default parameters will adjust accordingly. By default, all spheroids and Day 0 sensors will be calculated and fit to circles, whereas any other spheroids will be calculated and fit to ellipses. However, the checkbox on the bottom right ensures that you can fit whichever shape you want.

The threshold slider and number box and radius double slider and number box allows you to select the specific parameters for the calculation and fit of shapes on the images. You should adjust the parameters until the shapes fit properly. Partially cutoff shapes may not be able to be detected.

After you have gone through the list of images, fitting shapes appropriately to the red channel images, and subsequently the bright field ones, you should set the base image. The base image can technically be set at any time, but as soon as it is set, the only shapes that will be detected from that point forward are shapes that can be related back to the shapes on the base image. As such, it is advisable to select the base image at the end when the user has context for which images would be best suited. As soon as the base image is set, identification numbers appear for the images as can be seen above and below.



scan_Plate_R_p00_0_A02f00d4.TIF
scan_Plate_R_p01_0_A02f00d4.TIF
scan_Plate_R_p02_0_A02f00d4.TIF
scan_Plate_R_p03_0_A02f00d4.TIF
scan_Plate_R_p04_0_A02f00d4.TIF
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scan_Plate_R_p08_0_A02f00d4.TIF
scan_Plate_R_p09_0_A02f00d4.TIF
scan_Plate_R_p10_0_A02f00d4.TIF
scan_Plate_R_p11_0_A02f00d4.TIF
scan_Plate_R_p12_0_A02f00d4.TIF
scan_Plate_R_p13_0_A02f00d4.TIF
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scan_Plate_R_p19_0_A02f00d4.TIF
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scan_Plate_R_p22_0_A02f00d4.TIF
scan_Plate_R_p23_0_A02f00d4.TIF
scan_Plate_R_p24_0_A02f00d4.TIF
scan_Plate_R_p25_0_A02f00d4.TIF
scan_Plate_R_p26_0_A02f00d4.TIF
scan_Plate_R_p27_0_A02f00d4.TIF
scan_Plate_R_p28_0_A02f00d4.TIF
scan_Plate_R_p29_0_A02f00d4.TIF

Threshold: 120
0 200

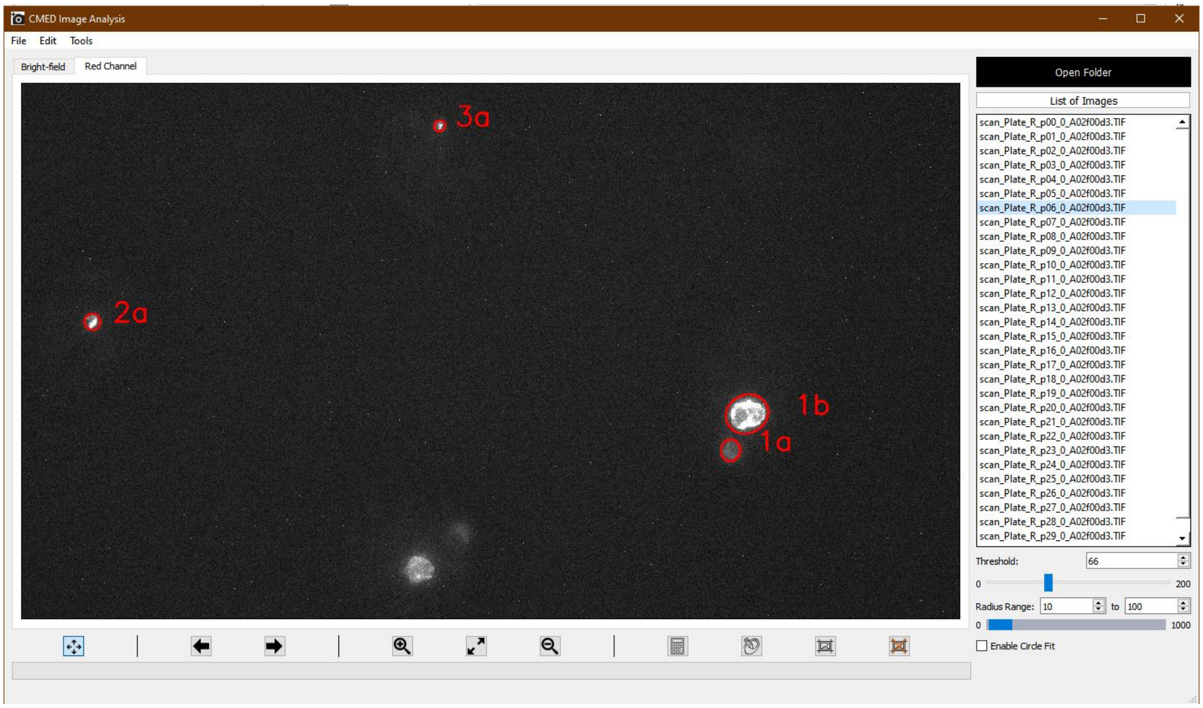
Radius Range: 40 to 500
0 1000

☑ Enable Circle Fit

2.3 Setting the base image

The concept of a “Base Image’ and “Base Shapes” are core to the way the shapes are tracked across images. The base image is an image for which the shapes are identified and labelled arbitrarily. The value in having this image, is that the shapes for all other images can now be identified and labelled according to the shapes in the base image. This is done by finding the closest shapes in an image to the shapes of the base image; these shapes are then known as the base shapes for that image. All shapes other than the base shapes found in the image are then ignored for the purposes of data collection and export. As such, it is favourable to choose a base image that successfully maps all of the spheroids and sensors that you are interested in.

The base image is set and cleared through the buttons on the bottom right below the image window. It should be noted that when setting or clearing a base image, it is applicable to the pair of images, one from the red channel, and one from the bright field. The recalculate button is used to run the thresholding, finding contours, and fitting of shapes functions again. The redraw button is used to recorrelate the image shapes with those of the base image, i.e. redraw the base shapes of the image.



scan_Plate_R_p00_0_A02f00d3.TIF
scan_Plate_R_p01_0_A02f00d3.TIF
scan_Plate_R_p02_0_A02f00d3.TIF
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scan_Plate_R_p08_0_A02f00d3.TIF
scan_Plate_R_p09_0_A02f00d3.TIF
scan_Plate_R_p10_0_A02f00d3.TIF
scan_Plate_R_p11_0_A02f00d3.TIF
scan_Plate_R_p12_0_A02f00d3.TIF
scan_Plate_R_p13_0_A02f00d3.TIF
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scan_Plate_R_p26_0_A02f00d3.TIF
scan_Plate_R_p27_0_A02f00d3.TIF
scan_Plate_R_p28_0_A02f00d3.TIF
scan_Plate_R_p29_0_A02f00d3.TIF

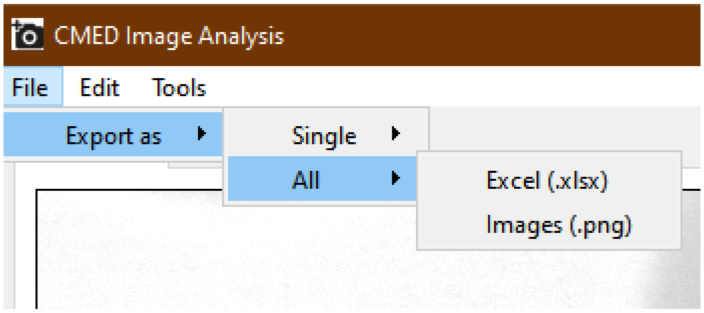
Threshold: 66

Radius Range: 10 to 100

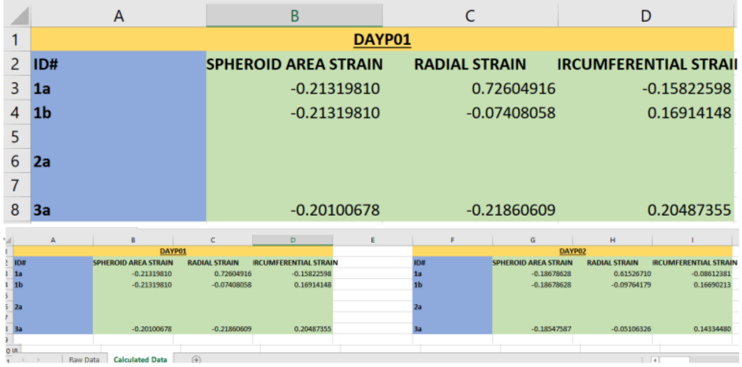
☐ Enable Circle Fit

After the base image is set, all of the calculated shapes will be identified and related based on the base image. Any shapes that could not be correlated to base image shapes will be discarded for drawing and data collection purposes. Data can then be exported using the options in the menu bar.

2.4 Exporting Excel data



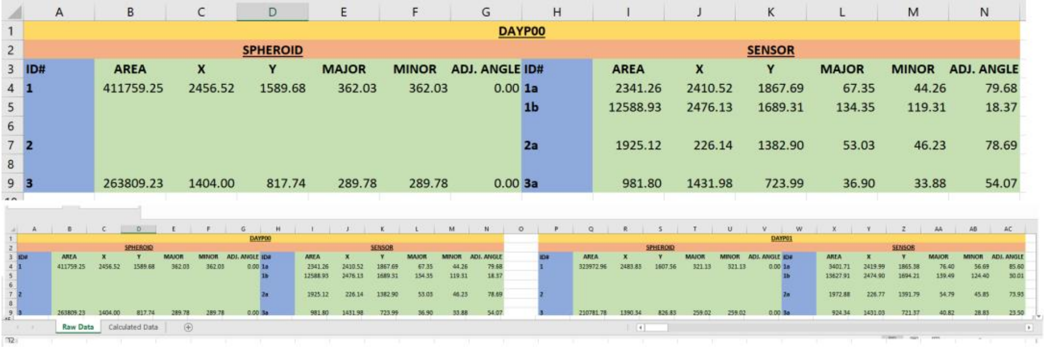
The single export option will set the current image being viewed as the base image and just export the raw shape parameters from that image. Exporting all images will produce the data for all images in the series, with shapes identified consistently through the base shape concept. This data has been converted from pixel values to μm using a scale value of $0.638\,\frac{pixels}{\mu m}$. A sample of the raw and calculated data sheets from an Excel file of a series of images can be seen here:



	A	B	C	D
1	DAYP01			
2	ID#	SPHEROID AREA STRAIN	RADIAL STRAIN	IRCUMFERENTIAL STRAI
3	1a	-0.21319810	0.72604916	-0.15822598
4	1b	-0.21319810	-0.07408058	0.16914148
5				
6	2a			
7				
8	3a	-0.20100678	-0.21860609	0.20487355

	A	B	C	D	E	F	G	H	I
1	DAYP01					DAYP02			
2	ID#	SPHEROID AREA STRAIN	RADIAL STRAIN	IRCUMFERENTIAL STRAIN		ID#	SPHEROID AREA STRAIN	RADIAL STRAIN	IRCUMFERENTIAL STRAIN
3	1a	-0.21319810	0.72604916	-0.15822598		1a	-0.18678628	0.61526710	-0.08612381
4	1b	-0.21319810	-0.07408058	0.16914148		1b	-0.18678628	-0.09764179	0.16690213
5									
6	2a					2a			
7									
8	3a	-0.20100678	-0.21860609	0.20487355		3a	-0.18547587	-0.05106326	0.14334480

Raw Data | Calculated Data



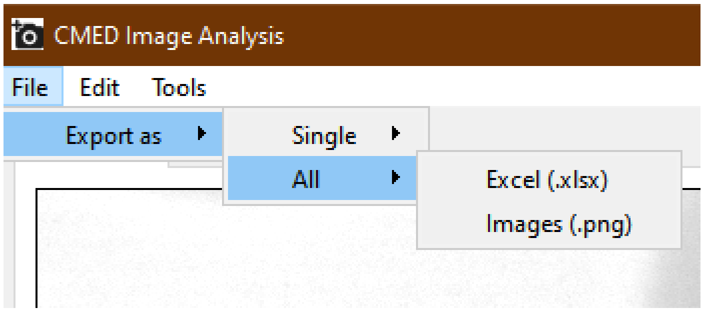
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	DAYP00													
2	SPHEROID							SENSOR						
3	ID#	AREA	X	Y	MAJOR	MINOR	ADJ. ANGLE	ID#	AREA	X	Y	MAJOR	MINOR	ADJ. ANGLE
4	1	411759.25	2456.52	1589.68	362.03	362.03	0.00	1a	2341.26	2410.52	1867.69	67.35	44.26	79.68
5								1b	12588.93	2476.13	1689.31	134.35	119.31	18.37
6														
7	2							2a	1925.12	226.14	1382.90	53.03	46.23	78.69
8														
9	3	263809.23	1404.00	817.74	289.78	289.78	0.00	3a	981.80	1431.98	723.99	36.90	33.88	54.07

	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1		DAYP01													
2		SPHEROID							SENSOR						
3		ID#	AREA	X	Y	MAJOR	MINOR	ADJ. ANGLE	ID#	AREA	X	Y	MAJOR	MINOR	ADJ. ANGLE
4		1	323972.96	2483.83	1607.56	321.13	321.13	0.00	1a	3401.71	2419.99	1865.38	76.40	56.69	85.60
5									1b	13627.91	2474.90	1694.21	139.49	124.40	30.01
6															
7		2							2a	1972.88	226.77	1391.79	54.79	45.85	73.93
8															
9		3	210781.78	1390.34	826.83	259.02	259.02	0.00	3a	924.34	1431.03	721.37	40.82	28.83	23.50

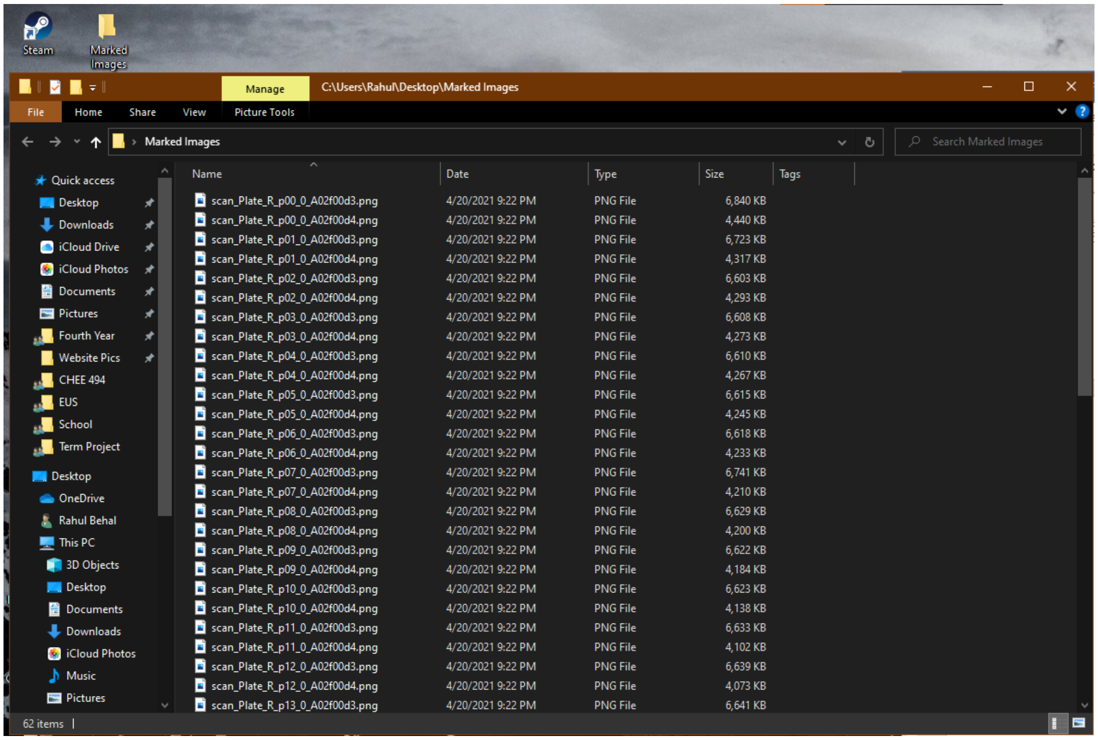
Raw Data | Calculated Data

2.5 Exporting images data

The marked down versions of the images with the circle and ellipse tracings and identification numbers can also be exported in the same menu bar.



This creates a folder "Marked Images"/ within the selected directory and outputs the images as .png files with their name as it was inputted to the program.



Name	Date	Type	Size	Tags
scan_Plate_R_p00_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,840 KB	
scan_Plate_R_p00_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,440 KB	
scan_Plate_R_p01_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,723 KB	
scan_Plate_R_p01_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,317 KB	
scan_Plate_R_p02_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,603 KB	
scan_Plate_R_p02_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,293 KB	
scan_Plate_R_p03_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,608 KB	
scan_Plate_R_p03_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,273 KB	
scan_Plate_R_p04_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,610 KB	
scan_Plate_R_p04_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,267 KB	
scan_Plate_R_p05_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,615 KB	
scan_Plate_R_p05_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,245 KB	
scan_Plate_R_p06_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,618 KB	
scan_Plate_R_p06_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,233 KB	
scan_Plate_R_p07_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,741 KB	
scan_Plate_R_p07_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,210 KB	
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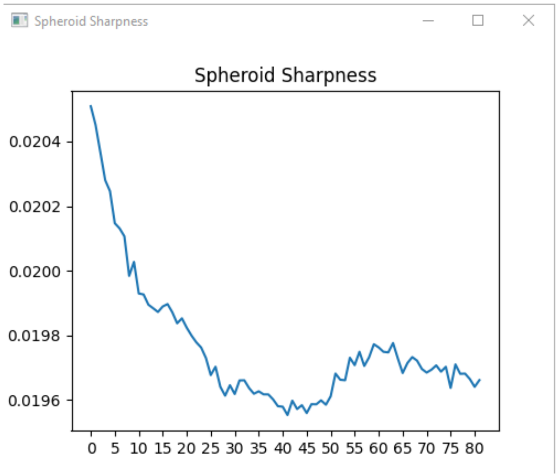
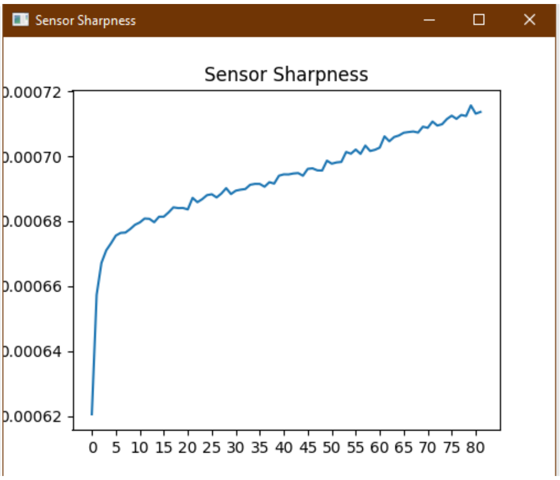
scan_Plate_R_p08_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,200 KB	
scan_Plate_R_p09_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,622 KB	
scan_Plate_R_p09_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,184 KB	
scan_Plate_R_p10_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,623 KB	
scan_Plate_R_p10_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,138 KB	
scan_Plate_R_p11_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,633 KB	
scan_Plate_R_p11_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,102 KB	
scan_Plate_R_p12_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,639 KB	
scan_Plate_R_p12_0_A02f00d4.png	4/20/2021 9:22 PM	PNG File	4,073 KB	
scan_Plate_R_p13_0_A02f00d3.png	4/20/2021 9:22 PM	PNG File	6,641 KB	

2.6 Analyzing Z-Stack images

When a Z-Stack folder is chosen, two graphs will pop up when the images are loaded that represent the sharpness as a function of the image number in the series. These graphs are generally give inconsistent results, but can sometimes be useful for finding regions of sharpness. Shapes cannot be traced on Z-Stack images, the program will just act as a viewer.





2.7 General image navigation

The image viewer has several standard features that can be used to assist in the processing. This includes a zoom and a pan. Zoom can be done through the respective buttons, but can also be done by holding CTRL + Scroll Bar, or by using a laptop trackpad two-finger zoom. The left and right arrow keys, along with the respective buttons, can be used to navigate between images in the sequence as well. These buttons can be held down for quick image navigation, which is useful for detecting the sharpness of the image.

3 Tips and Tricks

3.1 Basic Troubleshooting

- No shapes detected for sensors
    - The bright field base image shapes were likely before the red channel image shapes. Clear the base image and recalculate the sensor and then the spheroid shapes to fix.
- Program crashes → QThread: Destroyed while thread is still running
    - If the program crashes and this error appears, the program crashed as a result of trying to calculate things too quickly. A short term fix is reloading, and adjusting the slider more smoothly/slowly. A long term fix would be storing the threads differently in the code (perhaps adding threads to an array instead of replacing the reference, though this poses challenges of too many unnecessary calculations).
- Image shapes completely messed up/not mapped to base shapes properly
    - Hitting recalculate + redraw usually solves the issue. If persists, base shape may need to be cleared and reset.

3.2 Helpful Tips

- Sensor shapes in the red channel should always be fit before spheroid shapes in BF
- The progress bar is useful to tell the status of many operations including:
    - Loading images
    - Calculating and drawing circles + ellipses
    - Calculating Z-Stack sharpness values
- However, the progress bar does not include Export progress
    - Though it's usually quick, if the program goes not responding for a brief period of time it is advisable to wait it out
- It can often be easier to enter numbers in the number box instead of adjusting the slider
- If a change to the code breaks it or something irreparable happens to the code and original files cannot be found, they should always be available on my GitHub

3.3 Quick Changes

There are a few lines of codes it may be helpful to take note of as they can be very quickly adjusted to change the format or output. These are one-line, obvious code changes that can be found in the table as follows:

Purpose	Filename	Line number	Options to Change
Adjusting the scale value of $pixels/\mu m$	Export.py	20	Must be a number.
Default threshold value for all images	Image.py	21	Must be an integer.
Default radius range for red channel and bright field images	Image.py	22	Must be a tuple with integers. First number must be lower than the second.
Maximum distance in pixels for relating a shape to the base image shape	Image.py	320	Must be a number.
Valid image formats	ImageViewer.py	80	Must be a valid file extension with "." preceding.
Number beside "d" for determining BF/TR in Z-Stack images.	ImageViewer.py	133, 136	Must be a string representing a number that is after the "d" in the image file name.
Number beside "d" for determining BF/TR in Day images	ImageViewer.py	160, 163	Must be a string representing a number that is after the "d" in the image file name.
Delay between adjusting a slider and new shapes being calculated and drawn.	main.py	92	Must be an integer, in milliseconds.

4 Appendix

Folder/File Structure for mapping shapes:

- One of two folder structures:
    - Root Folder:
        - BF
            - ***p##***.[Valid Image Extension]
        - Texas Red
    - Root Folder
        - ***Day##***
            - ***p00***_[Any 6 Characters]d4.[Valid Image Extension]
            - ***p00***_[Any 6 Characters]d3.[Valid Image Extension]
        - ***Day##***
        - ***Day##***
        - ***Day##***
- Code being used for detection:
    - Checks if BF and Texas Red folders exist in selected directory
        - or checks if folder exists with Day Regex: DAY(\d{1,2})
    - Checks for id using ID Regex: (p\d{1,4})
    - For daily folders, BF and TR image sets are separated through the following Regex: _.{6}d(\d), checking for the digit at the end (“4” = BF, “3” = TR)

Folder/File Structure for z-stack:

- Root Folder
    - ***z##***d2.[Valid Image Extension]
    - ***z##***d4.[Valid Image Extension]
- Code being used for detection:
    - Regex in selected folder and to extract id and image type: z(\d{1,4}).*d(\d)