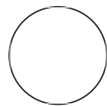




JUN 22, 2023

FIVTools Thresholding and Measures

d.grigore^{1,2}¹Washington University School of Medicine; ²Buchser Lab

d.grigore

ABSTRACT

[YouTube: FIVTools Thresholding and Measures Walkthrough](#)

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.e6nvwd6mdlmk/v1

Protocol Citation: d.grigore 2023. FIVTools Thresholding and Measures. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwd6mdlmk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development

We are still developing and optimizing this protocol

Created: Jun 21, 2023

Last Modified: Jun 22, 2023

PROTOCOL integer ID:
83838

Navigating FIVTools Image GUI

1 Open the FIVTools application.

- 2 Access recent scans by navigating to the correct directory under which the images are saved under "InCell Images dB (Root)". Click the "Refresh" button to update the directory of images.
- 3 Search for your scan in the "Plate ID Contains" filter box. Double click on the scan of interest. A pop-up with the GUI of the scan will appear.

Establishing Thresholding Parameters for Segmentation

- 4 Update the color scheme such that the nuclear channel is blue and mitochondria, red. This is necessary for the segmentation protocol to run correctly. The colors can be updated by clicking "Color" [#1 in Figure 1] until the desired color scheme appears, or by inputting hex RGB codes into the corresponding text boxes.

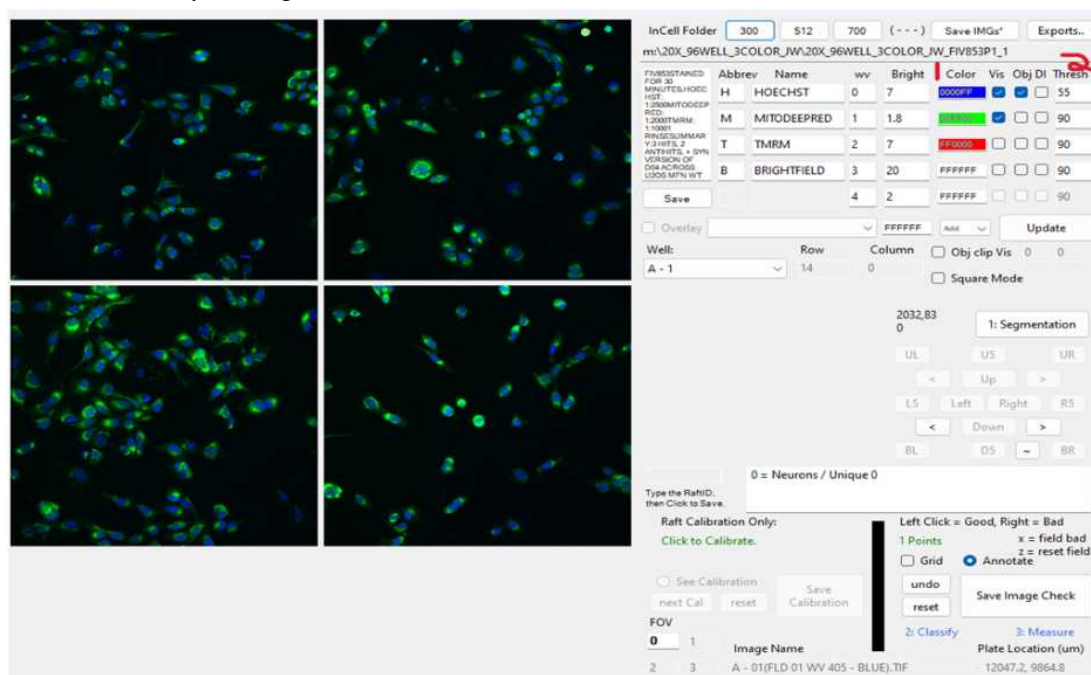


Figure 1: GUI with scanned images.

- 5 Select the blue nuclear and red mitochondrial channels to be active by ticking the checkboxes under "Vis". Press "Update" to apply these changes to the visualized images.

- 6 Under the Tresh column [#2 in Figure 1], double click the number associated with the nuclear channel. This will update the image visualization such that the image that was in the upper-left quadrant is represented as 1) the original image, 2) the threshold mask, 3) filtered objects, and 4) deoverlapped regions. Categories and explanations of these are as follows:
1. **Original** (top left quadrant). Original image as it appears from the scan.
 2. **Threshold** (bottom left quadrant). The point of this thresholding category is to separate out background vs nuclei. (Increased thresholding value = more black. Decreased value = less black.)
 3. **Filtered objects** (top right quadrant). For now, we are using the defaults under the hood. If you want to fine tune you can right-click "1: Segmentation". A GUI with crop width, min area, max area will pop up. This box is further culling down the originally thresholded nuclei in the previous threshold category based on set metrics.
 4. **Deoverlapped regions** (bottom right quadrant) final thresholding category that is further refining the filtered objects category. Anything in the box is considered single cell. These images can be exported.

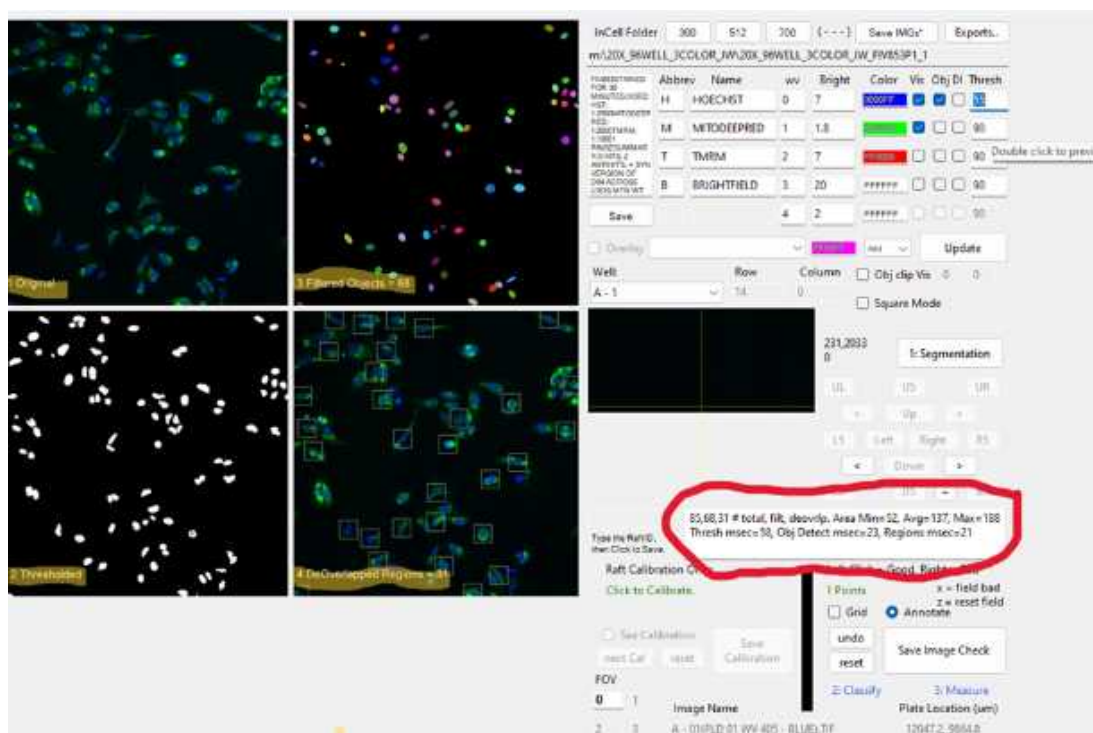


Figure 2: Thresholding visualization

- 7 Once satisfied with thresholding, click "Save" and exit out of the GUI.

Running "Classify and Measure"

- 8 Return to the main FIVTools interface. Set the pathway of the output "nMeasures.txt" file under "Destination Folder".
- 9 Navigate to the scans of interest. If more than one scan was selected, ensure that scan parameters are consistent before continuing. Click "mRaft" [B, Figure 3] and select "Classify and Measure" [C, Figure 3]. The "nMeasures.txt" file will be outputted in the desired directory. Follow the status by looking in the region to the right of "Layouts...", "Metadata...", "p Values...", buttons.

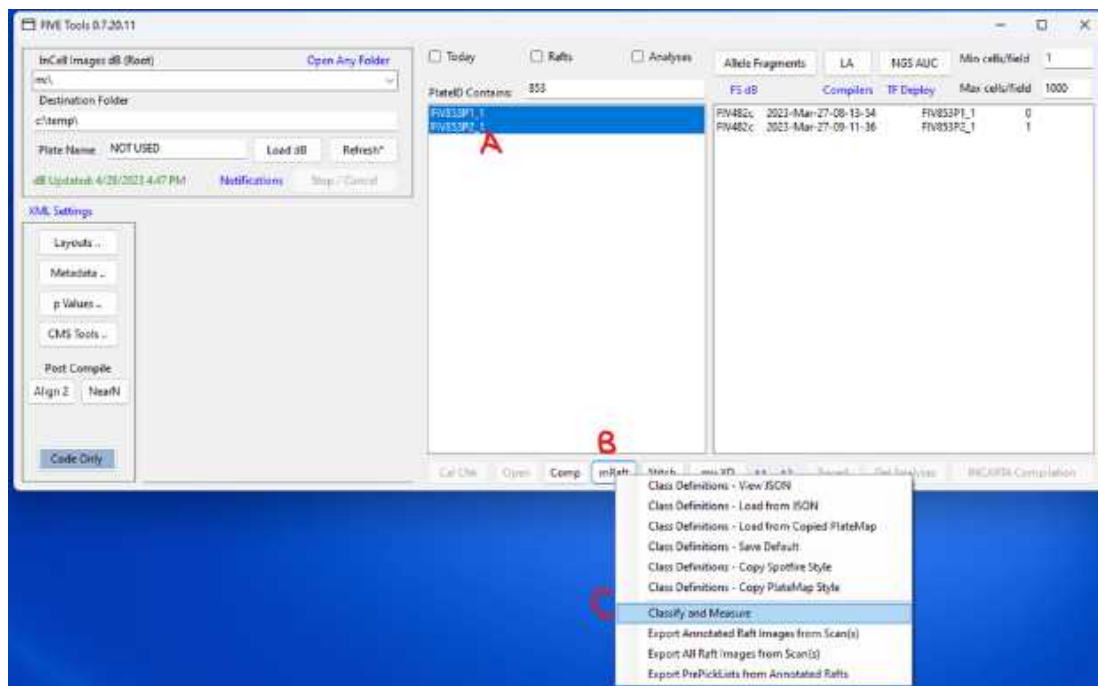


Figure 3: Main page of FIVTools. Running "Classify and Measure"

- 10 The resulting "nMeasures.txt" file can be visualized and analyzed in programs such as Excel and Spotfire.

Current measurement parameters include nuclear radius, nuclear area, cell rotation, mito size for each isolated single-cell, as determined from the Deoverlapped cells in Step 6. Each row in the data table represents a cell or model [Figure 4]. Multiple models can be run with the thresholded settings at the same time (currently under the hood only). Data table can be taken downstream into analytical tools such as Spotfire for further analysis, just like IN Carta data.

1	COLUMN	FOV	RaftID	OBJECT ID	miClass	RunDate	Row Time	Model Na	Model Na	Model Pat	Annotatio	A Score	A Full	Anno Sou	Load ms	i Crop	ms	i Pred	ms	Nuc Radiu	Nuc UWR	Nuc Rotat	Cell Radiu	Cell UWR	Cell Rotat	Cell Offse	n Mito	Mito Size	Mito Avg	
2	1	0	123.2.24.6	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	40.78412	0.199759	45.21363	0.656125	0.258082	18.91907	170.0572	0.545529	6.308910	
3	1	0	213.1.103.3	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	38.87322	0.690234	-0.2039	41.39948	0.504946	0.256265	16.362	295.8392	0.404832	5.14518
4	1	0	165.4.72.0	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	41.27801	0.601129	-0.30109	44.8065	0.575256	0.099958	9.88906	755.8452	0.429604	3.30351
5	1	0	63.3.71.1	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	35.34051	0.732832	0.182302	40.17976	0.605459	0.282478	25.01847	311.1246	0.483612	4.93926
6	1	0	145.1.108.	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	46.53817	0.611097	0.70137	51.30208	0.483524	0.491614	29.33169	384.0186	0.55208	6.67965
7	1	0	158.6.158.	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	38.46122	0.715237	0.052275	42.10944	0.6059	0.042461	15.71777	371.3851	0.483611	4.92485
8	1	0	389.5.164.	0	UNK	*****	*****	acc_0.159	acc_0.159	S/Phys/FIV833	Mito NaH								12.41392	0.1256587	37.53871	0.752277	0.209154	41.759	0.622178	20.54208	317.2132	0.562409	6.59105	

Figure 4: Example "nMeasures.txt" in Excel