Mitometer

USER'S MANUAL

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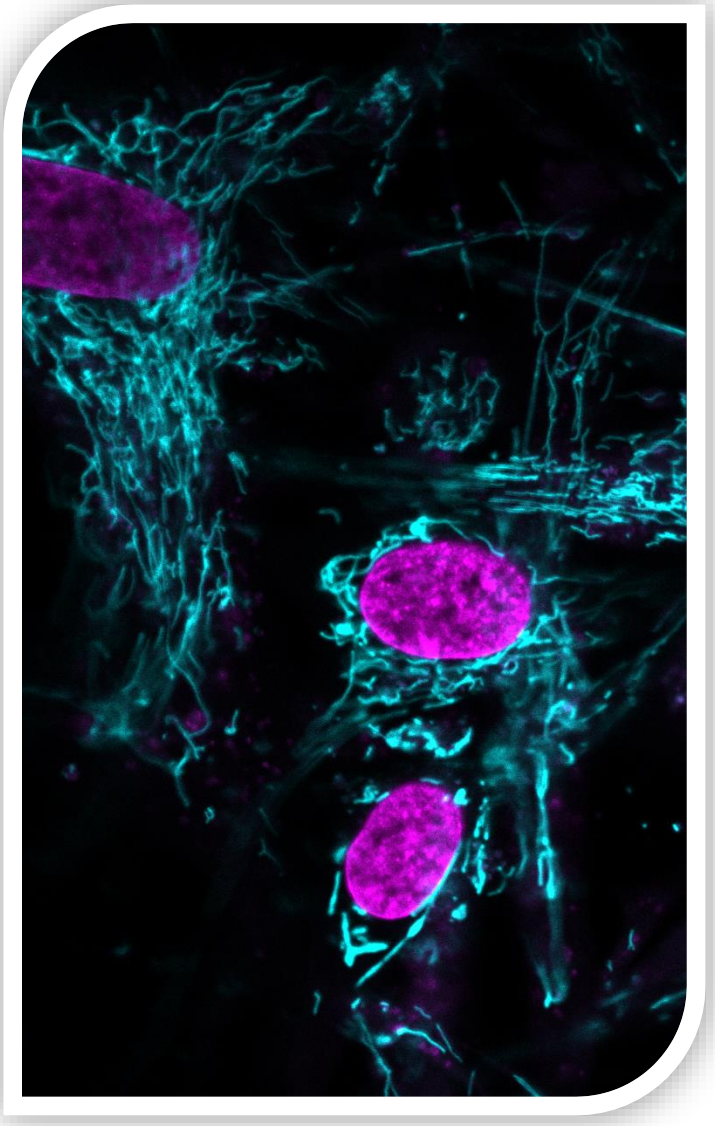


Table of Contents

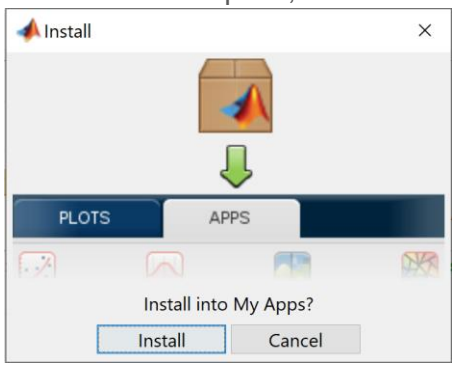
1. Installation

DOWNLOAD

The Mitometer program is written in MATLAB (MathWorks). The MATLAB GUI Mitometer app and corresponding source code is freely available online at https://github.com/aelefebv/Mitometer.

APP EXECUTION

1. Open the MATLAB App Installer file titled “Mitometer”.
2. Once MATLAB opens, click “Install”.



3. Click the “Mitometer” app in the Apps menu.

Note: Installation typically takes no more than 1 minute.

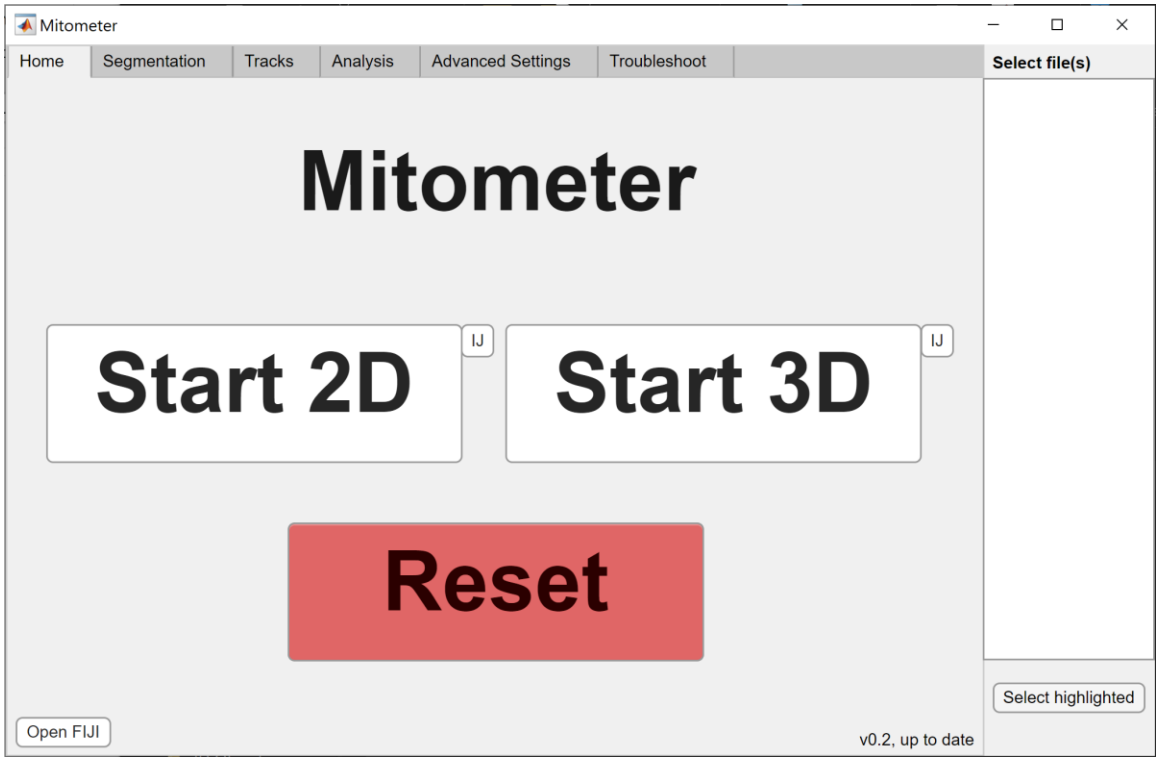
SOURCE CODE

All functions used in the Mitometer app are included in the Source Code folder. This includes the app’s App Designer GUI, titled “GUI”.

2. UI Tutorial

HOME TAB

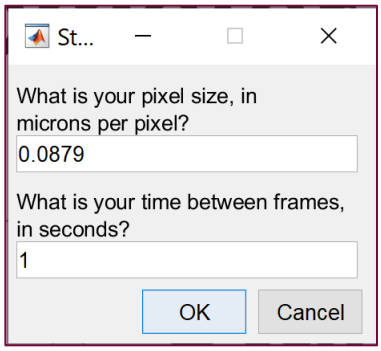
An instance of Mitometer can take as an input either 2D or 3D images:



Start 2D:

1. Input pixel size and time between each temporal frame.
2. Upload one or more Tif image stacks with temporal frames as the stack.

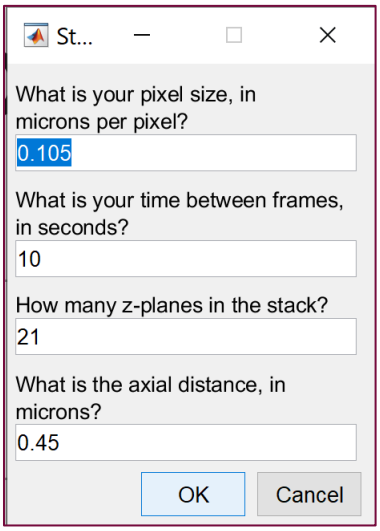
- Runs segmentation and tracking on each file in order of selection.
- Uploaded files will be shown in the right column under “Select file(s)”
- Note: You can analyze additional 2D files by pressing on **Add 2D** and following steps 1 and 2 again.



Start 3D:

1. Input pixel size, time between each temporal frame, number of spatial planes, and axial distance between the spatial planes.
2. Upload one or more Tif image stacks with the temporal and spatial frames as the stack. Order should follow z → t, i.e. all sequential (bottom to top) z-planes for time 1, followed by all sequential z-planes for time 2 (Note: This is the default order for LSM files).

- Runs segmentation and tracking on each file in order of selection.
- Uploaded files will be shown in the right column under “Select file(s)”
- Note: You can analyze additional 3D files by pressing on **Add 3D** and following steps 1 and 2 again.

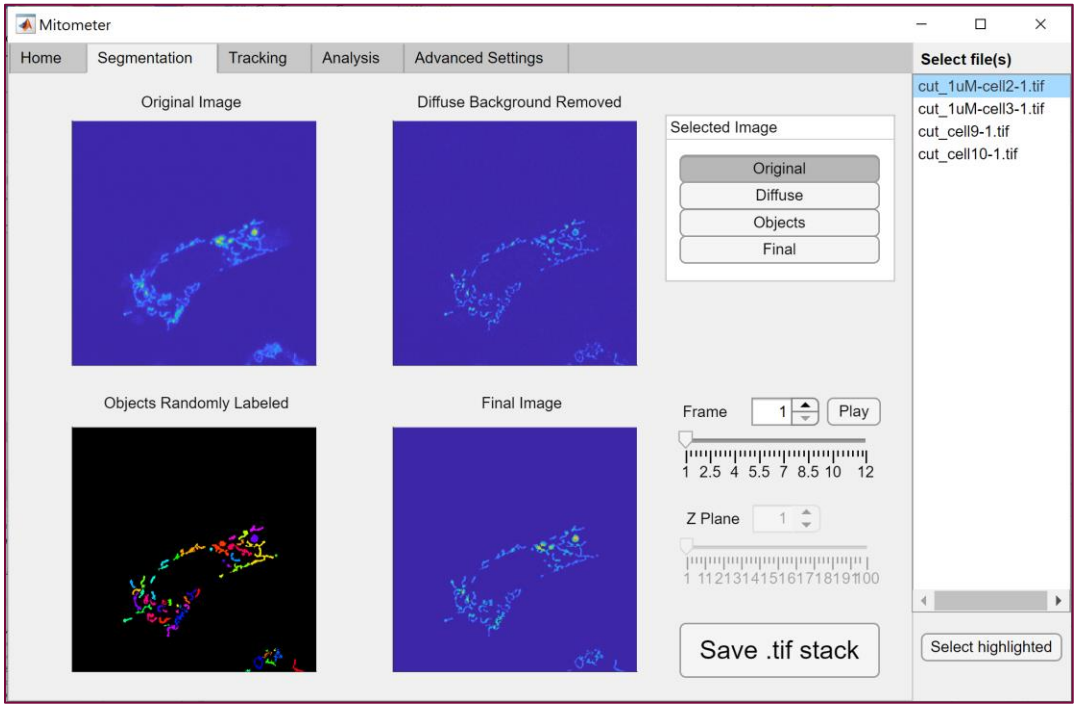


Reset:

- Reloads Mitometer.

SEGMENTATION TAB

After the processing from the home page is finished, visualization of mitochondria segmentation for each individual file will be available.



Visualize:

1. Select the file you wish to visualize under the “Select file(s)” menu.
2. Press the “Select highlighted” button.

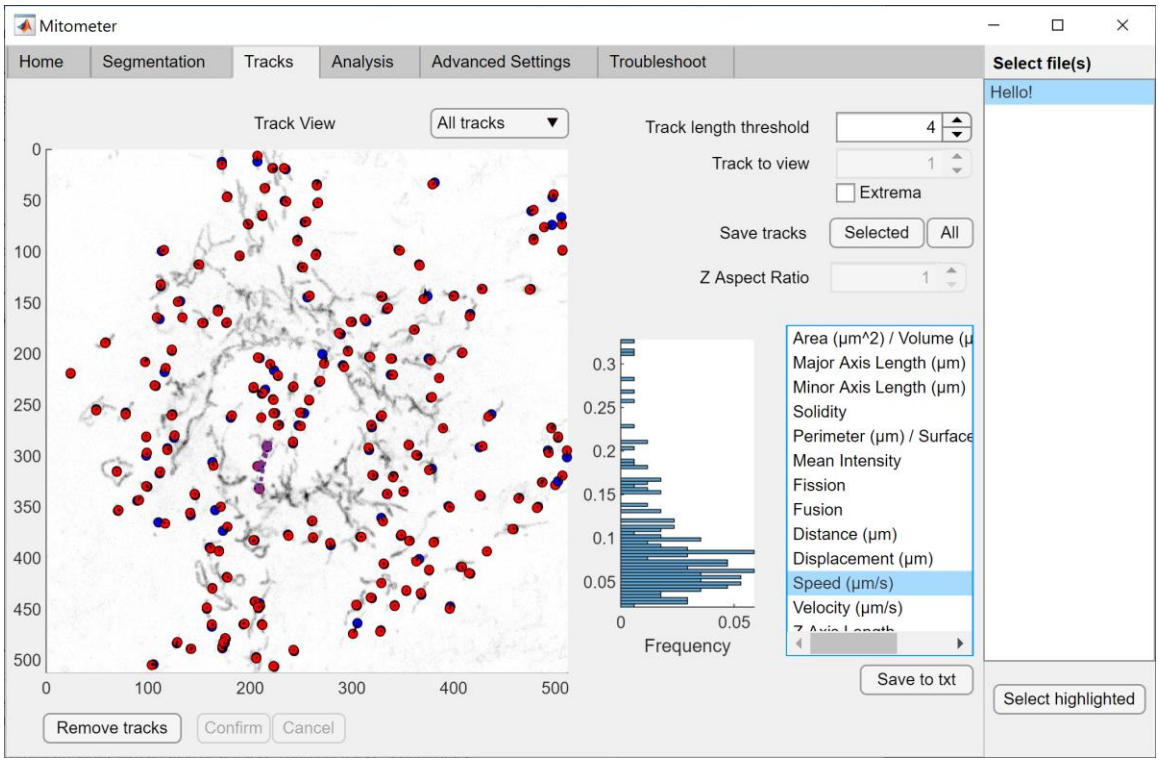
- The software allows visualization of the uploaded image, the image after diffuse background removal, randomly colored connected components (mitochondria), and the final segmented image.
- To scrub through frames of the images:
    1. Select the image you wish you view under “Selected Image”
    2. Use the frame spinner, slider, or play button to view temporal frames.
- In 3D, you can view different spatial frames by changing the spinner or slider next to “Z Plane”.

Save images:

- To save the image stack to a tif file:
    1. Select the image you wish to save under “Selected Image”.
    2. Press the “Save .tif stack” button.
- To save a single spatiotemporal frame:
    1. Hover over the image you wish to save.
    2. Use the MATLAB menu that appears above the image.

TRACKING TAB

After the processing from the home tab is finished, visualization of mitochondria tracks for each individual file will be available.



Visualize:

1. Select the file you wish to visualize under the “Select file(s)” menu.
2. Press the “Select highlighted” button.
3. Select the feature you want to visualize and it will appear as a histogram with the feature value as the y-axis, and the normalized frequency in the x-axis.

- The software allows visualization of all tracks, confident tracks, a single track (one track at a time), perinuclear tracks, and telenuclear tracks (as defined by below and above the Otsu’s threshold, respectively, of the histogram of mitochondrial distances from the mitochondrial aggregate center of mass).
- In 3D, you can change the aspect ratio of the Z plane in respect to the X-Y plane by changing the spinner next to “Z Aspect Ratio”.
- To remove tracks containing less than a specified number of points, change the spinner next to “Track length threshold”
- When “Single track” is selected, you can change the selected track by changing the spinner value next to “Track to view”.
- Track starting points are shown in blue, track ending points are shown in red, fission events are shown in purple, and fusion events are shown in green.

- Outlines of mitochondria at every frame can be visualized by enabling the “Extrema” checkbox

Remove tracks:

To remove specific tracks:

1. Press the “Remove tracks” button.
2. Click a track or draw a region around the tracks you wish to remove.
3. Press the “Confirm” button to remove the selected tracks.
4. Press the “Cancel” button to cancel.

Save feature data:

To save the feature data of an individual file (refer to Section 3. Output, for information on the saved data):

1. Select the type of track you wish to save under “Track View” (default is all tracks).
2. Select a track length threshold (default is 1).
3. Click on the feature you would like to save.
4. Press the “Save to txt” button.
5. Select where to save the data.

Feature data will be saved in a .txt file.

Save tracks:

To save the track data (refer to Section 3. Output, for information on the saved data):

1. Select the type of track you wish to save under “Track View” (default is all tracks).
2. Select a track length threshold (default is 1).

- To save specific file(s):
  1. Select one or more files you wish to save under the “Select file(s)” menu.
  2. Press the “Select highlighted” button.
  3. Press the “Selected” button next to “Save tracks”.
- To save all files:
  1. Press the “All” button next to “Save tracks”.

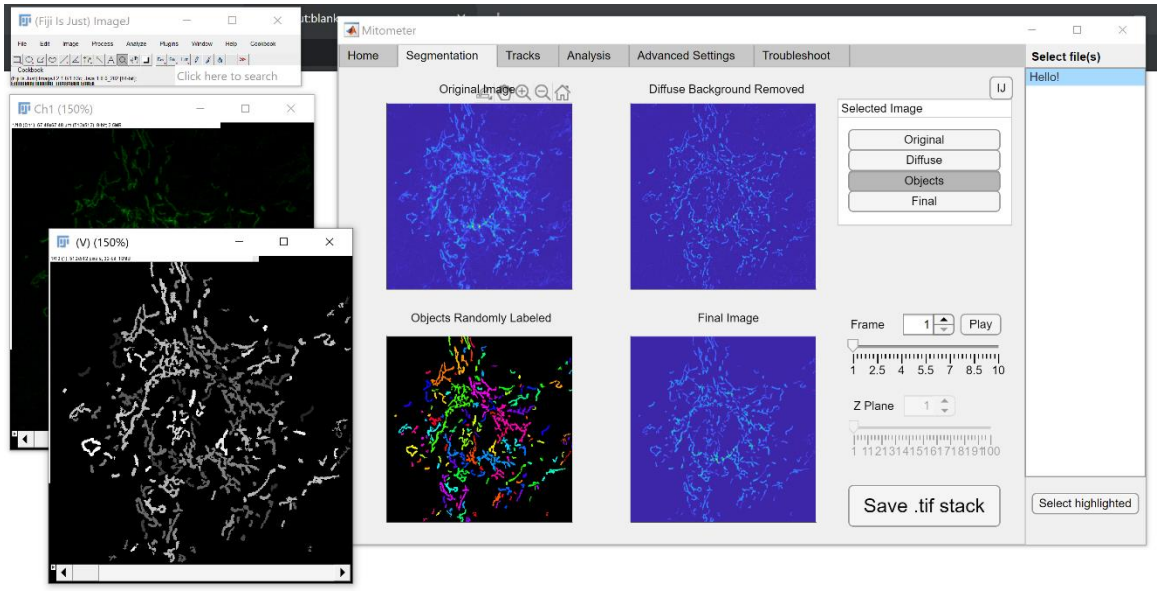
Track data will be saved in a .mat file.

Open in ImageJ

To open segmented files in ImageJ, first follow steps in section 4: ImageJ Guide.

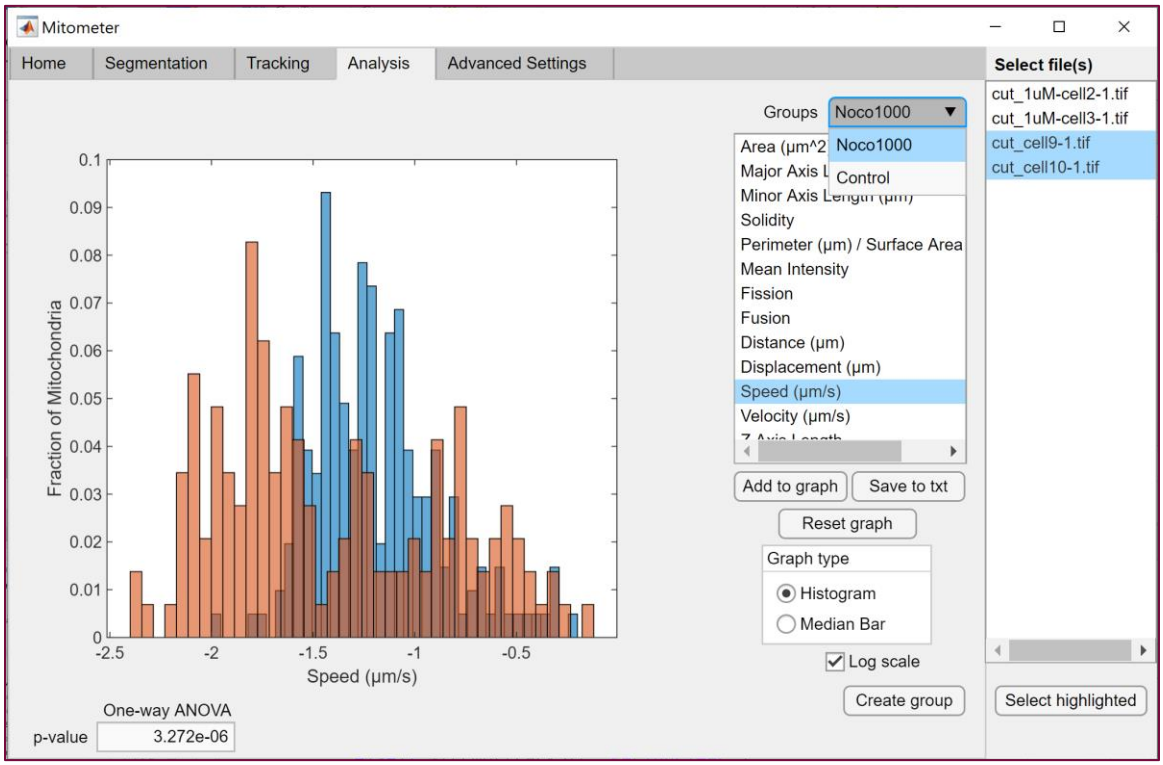
1. Open Fiji through Mitometer.
2. On the “Segmentation” tab, select the image to transfer to ImageJ under “Selected Image”

3. Click the IJ button.

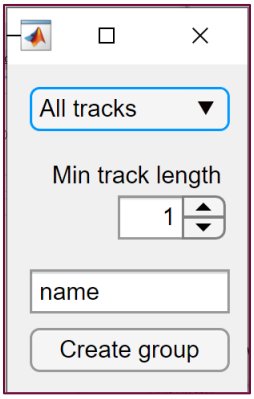


ANALYSIS TAB

After the processing from the home tab is finished, preliminary analysis of basic mitochondrial morphological, motility, and dynamic features between files and conditions will be available.



Visualize:



1. Select the file(s) whose features you wish to visualize under the “Select file(s)” menu.
   a. E.g. select all files of your control group.
2. Press the “Select highlighted” button.
3. Press the “Create group” button.
4. Select whether you wish to analyze all tracks, confident tracks, perinuclear tracks, or telenuclear tracks (default is all tracks).
5. Select a track length threshold (default is 1).
6. Name your group.
7. Repeat 1-6 for each condition/group you wish to compare.
8. Select the first group to compare under the “Groups” dropdown.
9. Select the feature you wish to analyze.
   a. Note: Z Axis Length is only available in 3D.
10. Press “Add to graph” to visualize the data.
11. Repeat steps 8-10 for each condition/group you wish to compare.

- The software allows visualization of either histogram distributions or bar graphs of median values of normal or logarithmically transformed data.
- Statistical comparison between groups is done via a One-way ANOVA.
- Clear off all groups from the graph by clicking the “Reset graph” button.

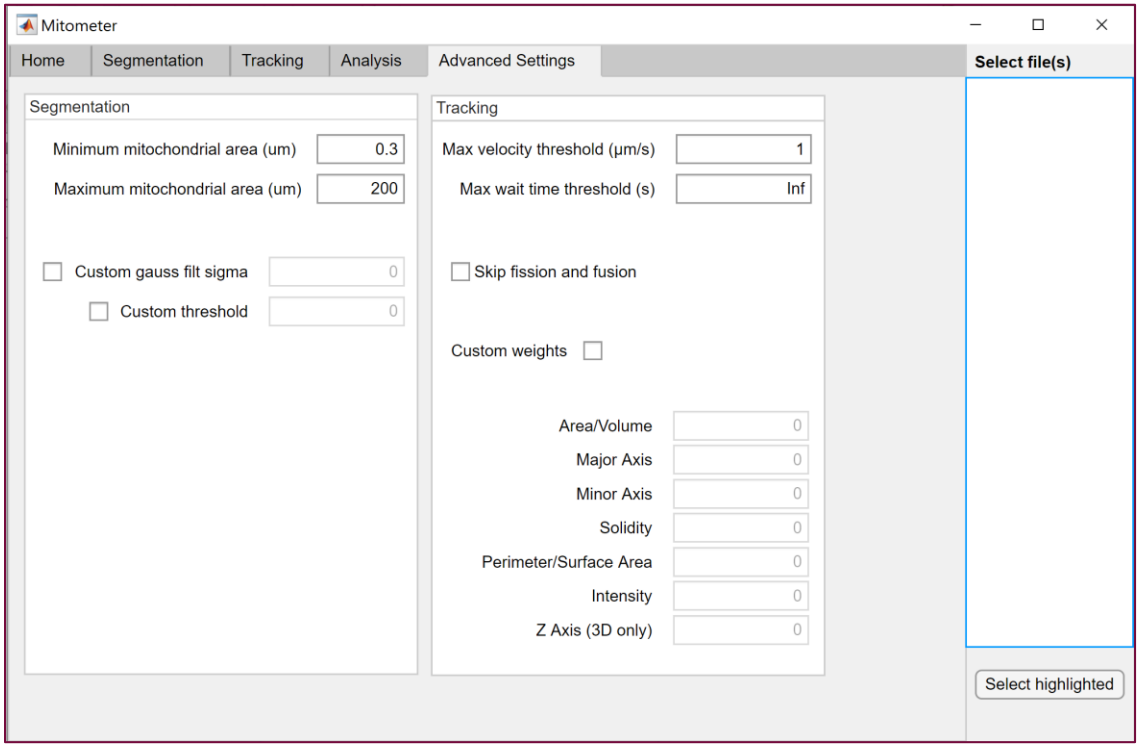
Save feature data:

To save the feature data (refer to Section 3. Output, for information on the saved data):

1. Select the file(s) whose features you wish to save under the “Select file(s)” menu.
   a. E.g. select all files of your control group.
2. Press the “Select highlighted” button.
3. Press the “Create group” button.
4. Select whether you wish to save all tracks, confident tracks, perinuclear tracks, or telenuclear tracks (default is all tracks).
5. Select a track length threshold (default is 1).
6. Name your group.
7. Select the group to save under the “Groups” dropdown.
8. Select the feature(s) you wish to save.
   a. Note: Z Axis Length is only available in 3D.
9. Press the “Save to txt” button
   a. Your data will be saved to a comma delimited text file where each row is a track, and each column is a frame.
10. Repeat 1-9 for each condition/group you wish to save.

ADVANCED SETTINGS TAB

Before processing files from the Home tab, it is possible to adjust both segmentation and tracking parameters, though the default parameters are recommended.



Segmentation settings

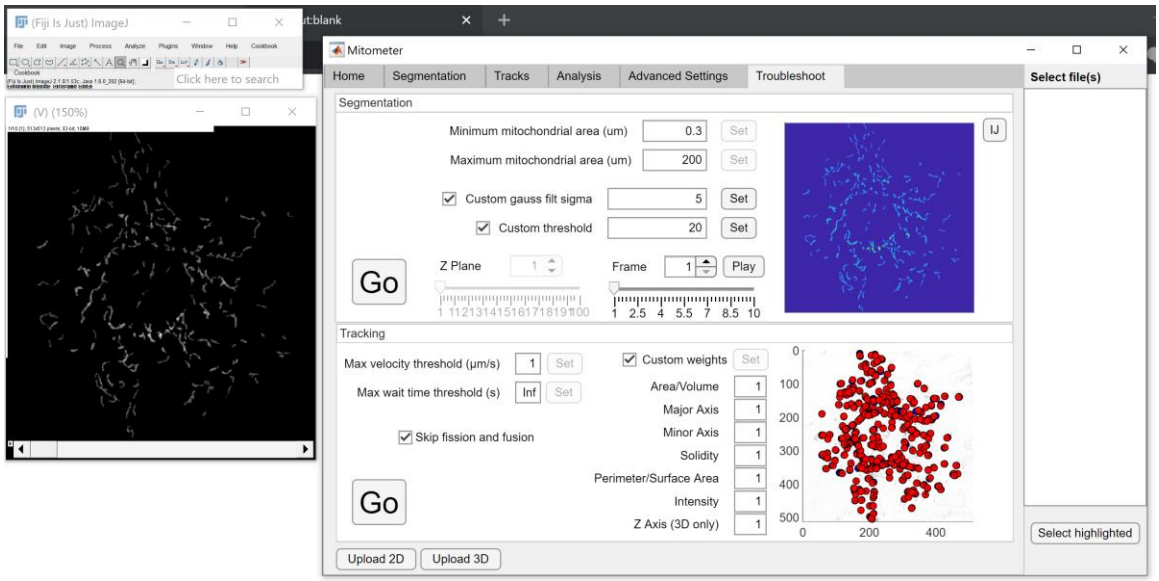
- **Minimum mitochondrial area** is the minimum area threshold for a connected component to be analyzed as a mitochondrion.
- **Maximum mitochondrial area** is the maximum area threshold for a connected component to be analyzed as a mitochondrion.
- **Custom gauss filt sigma** is the standard deviation used in the 3x3 gaussian filter after the diffuse background subtraction, but before the mask creation.
- **Custom threshold** is the threshold level (1-256) used for creating a mask from the gaussian filtered image.

Tracking settings

- **Max velocity threshold** is the maximum speed a mitochondrion can travel between frames.
- **Max wait time threshold** is the amount of time the algorithm will attempt to assign an unassigned track to a mitochondrion.
- **Skip fission and fusion** will skip fission and fusion assignments and speeds up processing if these features are not needed.
- **Custom weights** allows you to choose the cost matrix weighting for each mitochondrial morphological feature, and will speed up processing.

TROUBLESHOOT TAB

If a particular image does not segment or track correctly, you can use the troubleshooting tab to find parameters that work better.



Segmentation:

1. Manually enter any parameters you would like to test for troubleshooting.
2. If a custom gaussian filter sigma or threshold is desired, enable the checkbox next to the respective parameter.
3. Press the “Go” button in the segmentation section.
4. If the output image seems reasonable, click on the “Set” button next to the changed parameters. This will update Mitometer to use these parameter settings from the Home tab.

Note: You can open the segmented image through Fiji using the “IJ” button if Fiji has been opened as described.

Tracking:

1. Once you have a segmented image, you can troubleshoot tracking parameters.
2. Manually enter any parameters you would like to test for troubleshooting.
3. If custom morphology parameter weights are desired, enable the checkbox next to the respective parameter.
4. If you wish to skip fission and fusion events, enable the checkbox next to this option.
5. Press the “Go” button in the tracking section.
6. If the output image seems reasonable, click on the “Set” button next to the changed parameters. This will update Mitometer to use these parameter settings from the Home tab.

3. Output

TRACKING OUTPUT

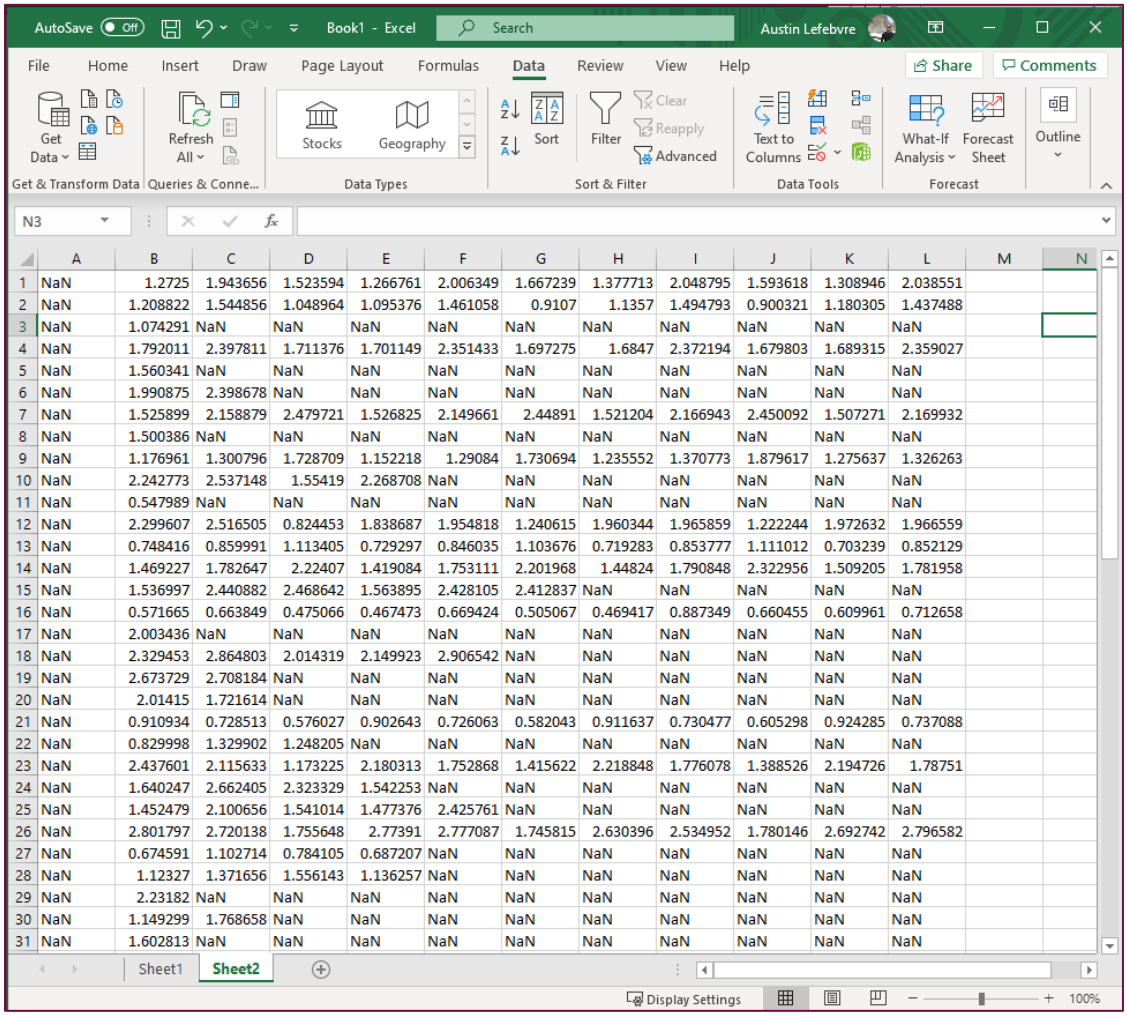
Saving a file's data from the tracking tab will result in a .mat file of many essential and extra data from that file's segmentation and tracking processes. Opening the .mat file in MATLAB will produce a variable named "trackList". This variable is a 1xN structure where N is the number of tracks in the file. Each track has the following 1xM fields, where M is the number of frames in the track:

2D	3D	Description
Extrema	ConvexHull	The coordinates of the extrema or convex hull points comprising the mitochondrion.
PixelIdxList	VoxelIdxList	The indices of every pixel comprising the mitochondrion.
WeightedCentroid	WeightedCentroid	The intensity weighted centroid of the mitochondrion.
NN	NN	The distance (in pixels) to the next nearest neighboring (NN) mitochondrion.
NPA	NPA	The number of potential track assignments (NPA) to which the mitochondrion can be assigned to.
label	label	The mitochondrion's corresponding label number in the object image.
OGid	OGid	The ID of the mitochondrion before thresholding based off of track length.
confident	confident	Whether the assignment was confident (1) or not (0) during that frame.
lost	lost	Whether the track was lost (1) or not (0) during that frame.
frame	frame	The frame number corresponding to vectors' column.
Area	Volume	The area or volume of the mitochondrion at that frame in microns.
Solidity	Solidity	The solidity of the mitochondrion, which is the ratio of the mitochondrion's area to the area of its convex hull.
Perimeter	SurfaceArea	The perimeter or surface area of the mitochondrion in microns.
MeanIntensity	MeanIntensity	The mean intensity (1-256) of the mitochondrion.
MajorAxisLength	MajorAxisLength	The major axis length of the mitochondrion in microns.
MinorAxisLength	MinorAxisLength	The minor axis length of the mitochondrion in microns.
	ZAxisLength	The z axis length of the mitochondrion in microns.
fission	fission	Gives the track number from which the mitochondrion fissioned or not (0).
fusion	fusion	Gives the track number with which the mitochondrion fused or not (0).

ANALYSIS OUTPUT

Saving a file's data from the analysis tab will result in a .txt file of the selected features. The file is comma delimited and is easily opened with programs such as excel for more complex analysis. Each row is a mitochondrion track, and each column is a frame.

Tip: When pasting data from the text file into excel, you can split up the columns by going to Data → Text to Columns → Delimited → Comma → Finish.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	NaN	1.2725	1.943656	1.523594	1.266761	2.006349	1.667239	1.377713	2.048795	1.593618	1.308946	2.038551		
2	NaN	1.208822	1.544856	1.048964	1.095376	1.461058	0.9107	1.1357	1.494793	0.900321	1.180305	1.437488		
3	NaN	1.074291	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
4	NaN	1.792011	2.397811	1.711376	1.701149	2.351433	1.697275	1.6847	2.372194	1.679803	1.689315	2.359027		
5	NaN	1.560341	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
6	NaN	1.990875	2.398678	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
7	NaN	1.525899	2.158879	2.479721	1.526825	2.149661	2.44891	1.521204	2.166943	2.450092	1.507271	2.169932		
8	NaN	1.500386	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
9	NaN	1.176961	1.300796	1.728709	1.152218	1.29084	1.730694	1.235552	1.370773	1.879617	1.275637	1.326263		
10	NaN	2.242773	2.537148	1.55419	2.268708	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
11	NaN	0.547989	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
12	NaN	2.299607	2.516505	0.824453	1.838687	1.954818	1.240615	1.960344	1.965859	1.222244	1.972632	1.966559		
13	NaN	0.748416	0.859991	1.113405	0.729297	0.846035	1.103676	0.719283	0.853777	1.111012	0.703239	0.852129		
14	NaN	1.469227	1.782647	2.22407	1.419084	1.753111	2.201968	1.44824	1.790848	2.322956	1.509205	1.781958		
15	NaN	1.536997	2.440882	2.468642	1.563895	2.428105	2.412837	NaN	NaN	NaN	NaN	NaN		
16	NaN	0.571665	0.663849	0.475066	0.467473	0.669424	0.505067	0.469417	0.887349	0.660455	0.609961	0.712658		
17	NaN	2.003436	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
18	NaN	2.329453	2.864803	2.014319	2.149923	2.906542	NaN	NaN	NaN	NaN	NaN	NaN		
19	NaN	2.673729	2.708184	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
20	NaN	2.01415	1.721614	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
21	NaN	0.910934	0.728513	0.576027	0.902643	0.726063	0.582043	0.911637	0.730477	0.605298	0.924285	0.737088		
22	NaN	0.829998	1.329902	1.248205	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
23	NaN	2.437601	2.115633	1.173225	2.180313	1.752868	1.415622	2.218848	1.776078	1.388526	2.194726	1.78751		
24	NaN	1.640247	2.662405	2.323329	1.542253	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
25	NaN	1.452479	2.100656	1.541014	1.477376	2.425761	NaN	NaN	NaN	NaN	NaN	NaN		
26	NaN	2.801797	2.720138	1.755648	2.77391	2.777087	1.745815	2.630396	2.534952	1.780146	2.692742	2.796582		
27	NaN	0.674591	1.102714	0.784105	0.687207	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
28	NaN	1.12327	1.371656	1.556143	1.136257	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
29	NaN	2.23182	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
30	NaN	1.149299	1.768658	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		
31	NaN	1.602813	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN	NaN		

4. ImageJ Guide

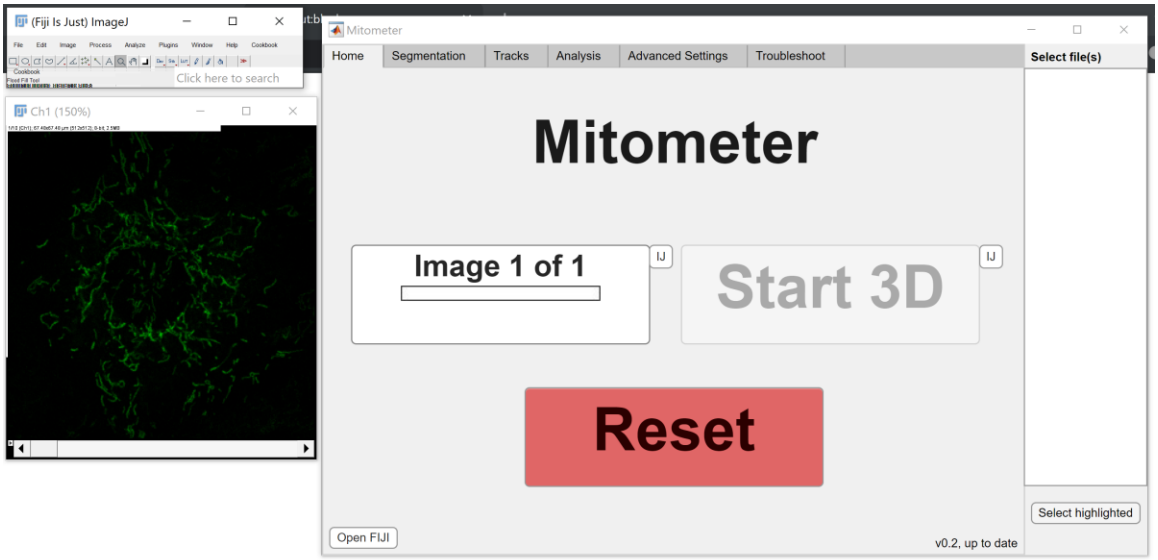
PREREQUISITES

1. Download FIJI (https://imagej.net/Fiji)
2. Open FIJI
3. Go to Help > Update
    a. Wait for the status check to finish
4. Open Manage update sites
5. Enable ImageJ-MATLAB
6. Click close
7. Click Apply changes on ImageJ Updater.
8. Close FIJI.

Note: You only need to do this once.

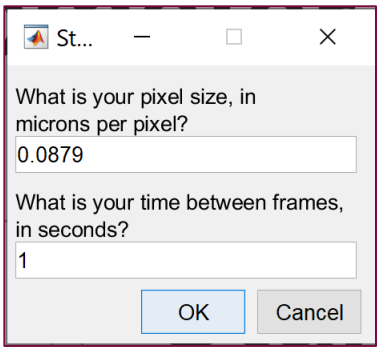
MITOMETER

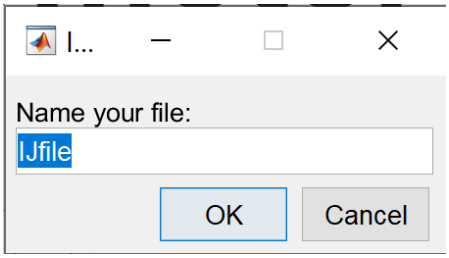
1. Click “Open FIJI” in the home tab.
2. Select your FIJI scripts folder.
    a. On Mac: Select the folder where the FIJI app is located (likely in the “Applications” folder).
    b. On PC: Select the FIJI scripts folder (default is Documents > Fiji.app > scripts).
    c. An ImageJ toolbar should open.
3. Open the image you wish to analyze as you would normally through ImageJ
4. On the Mitometer Home tab, click the IJ button next to Start 2D if you have opened a 2D image, and next to Start 3D if you have opened a 3D image.



Start 2D:

1. Input pixel size and time between each temporal frame.
2. Name your file.
   - Uploaded files will be shown in the right column under “Select file(s)”
   - Note: You can analyze additional 2D files by again pressing on **IJ** with a different file open.

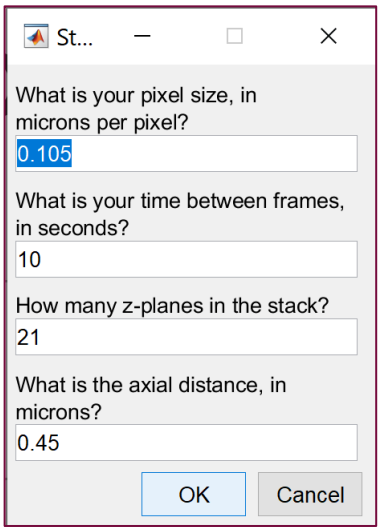




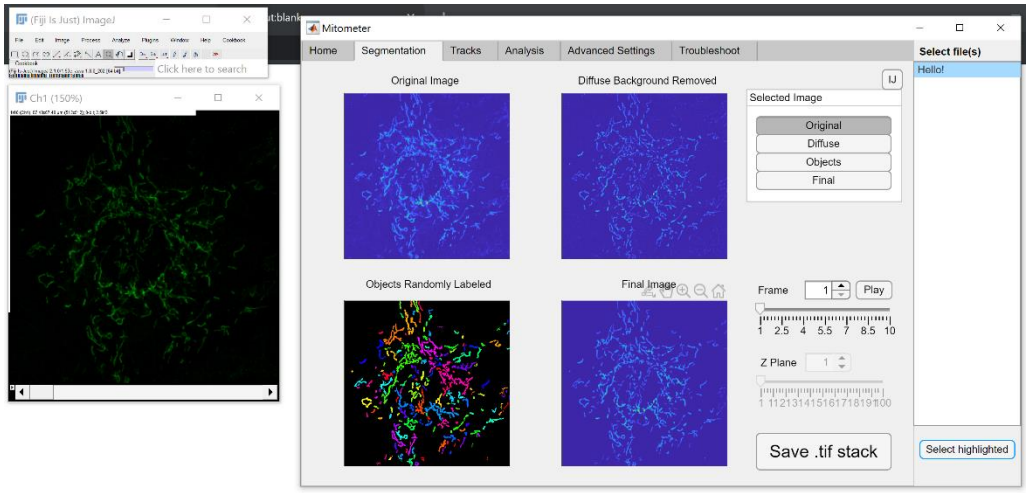
Start 3D:

1. Input pixel size, time between each temporal frame, number of spatial planes, and axial distance between the spatial planes.
2. Name your file

   - Uploaded files will be shown in the right column under “Select file(s)”
   - Note: You can analyze additional 3D files by pressing on **IJ** with a different file open.



Note: As long as you don’t quit out of Fiji, you can keep using it through Mitometer.



5. Requirements

WINDOWS

Operating systems

- Windows 10 (version 1803 or higher)
- Windows 7 Service Pack 1
- Windows Server 2019
- Windows Server 2016

Processors

- Minimum: Any Intel or AMD x86-64 processor
- Recommended: Any Intel or AMD x86-64 processor with four logical cores and AVX2 instruction set support

RAM

- Minimum: 4 GB
- Recommended: 8 GB

MAC

Operating systems

- macOS Catalina (10.15)
- macOS Mojave (10.14)

Processors

- Minimum: Any Intel x86-64 processor
- Recommended: Any Intel x86-64 processor with four logical cores and AVX2 instruction set support

RAM

- Minimum: 4 GB
- Recommended: 8 GB

LINUX

Operating systems

- Ubuntu 20.04 LTS
- Ubuntu 18.04 LTS
- Ubuntu 16.04 LTS
- Debian 10
- Debian 9

- Red Hat Enterprise Linux 8
- Red Hat Enterprise Linux 7 (minimum 7.5)
- SUSE Linux Enterprise Desktop 12 (minimum SP2)
- SUSE Linux Enterprise Desktop 15
- SUSE Linux Enterprise Server 12 (minimum SP2)
- SUSE Linux Enterprise Server 15

Processors

- Minimum: Any Intel or AMD x86-64 processor
- Recommended: Any Intel or AMD x86-64 processor with four logical cores and AVX2 instruction set support

RAM

- Minimum: 4 GB
- Recommended: 8 GB

ALL

Mitometer has been tested only on Windows 10 and macOS Catalina with MATLAB R2020a and R2020b.

6. License

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