

Instructions – HeartJ

HeartJ-Gomori_v5

Image requirements: Gomori silver staining for reticulum, standard image formats like BMP, GIF, JPEG, PNG, or TIFF.

This macro measures the size of cardiomyocytes (Group 1) in different parameters on slides stained with Gomori silver staining. The results are reported in an Excel spreadsheet on the desktop, which contains the individual results as well as an average value. An overlay image and color-coded instances will also be created. In addition, the size of the nuclei of the cardiomyocytes (Group 2) and the size of the capillaries (Group 3) are measured. Furthermore, the number of capillaries bordering each cardiomyocyte is measured (capillary contacts).

HeartJ FL WGA+DAPI+CD31_v8

Image requirements: Immunofluorescence staining (WGA (Channel: Green), Nuclei (e.g. DAPI) (Channel: blue), Capillaries (e.g. CD31) (Channel: red)), standard image formats like BMP, GIF, JPEG, PNG, or TIFF.

This macro measures the size of cardiomyocytes (Group 1) in different parameters on slides stained with WGA+CD31+DAPI. The results are reported in an Excel spreadsheet on the desktop, which contains the individual results as well as an average value. An overlay image and color-coded instances will also be created. In addition, the size of the nuclei of the cardiomyocytes (Group 2) and the size of the capillaries (Group 3) are measured. Furthermore, the number of capillaries bordering each cardiomyocyte is measured (capillary contacts).

HeartJ FL WGA+DAPI+intramyocyte-signal_v2

Image requirements: Immunofluorescence staining (WGA (Channel: Green), Nuclei (e.g. DAPI) (Channel: blue), intramyocyte or/and intramyocyte-nuclei signal (e.g. pro-ANP) (Channel: red)), standard image formats like BMP, GIF, JPEG, PNG, or TIFF.

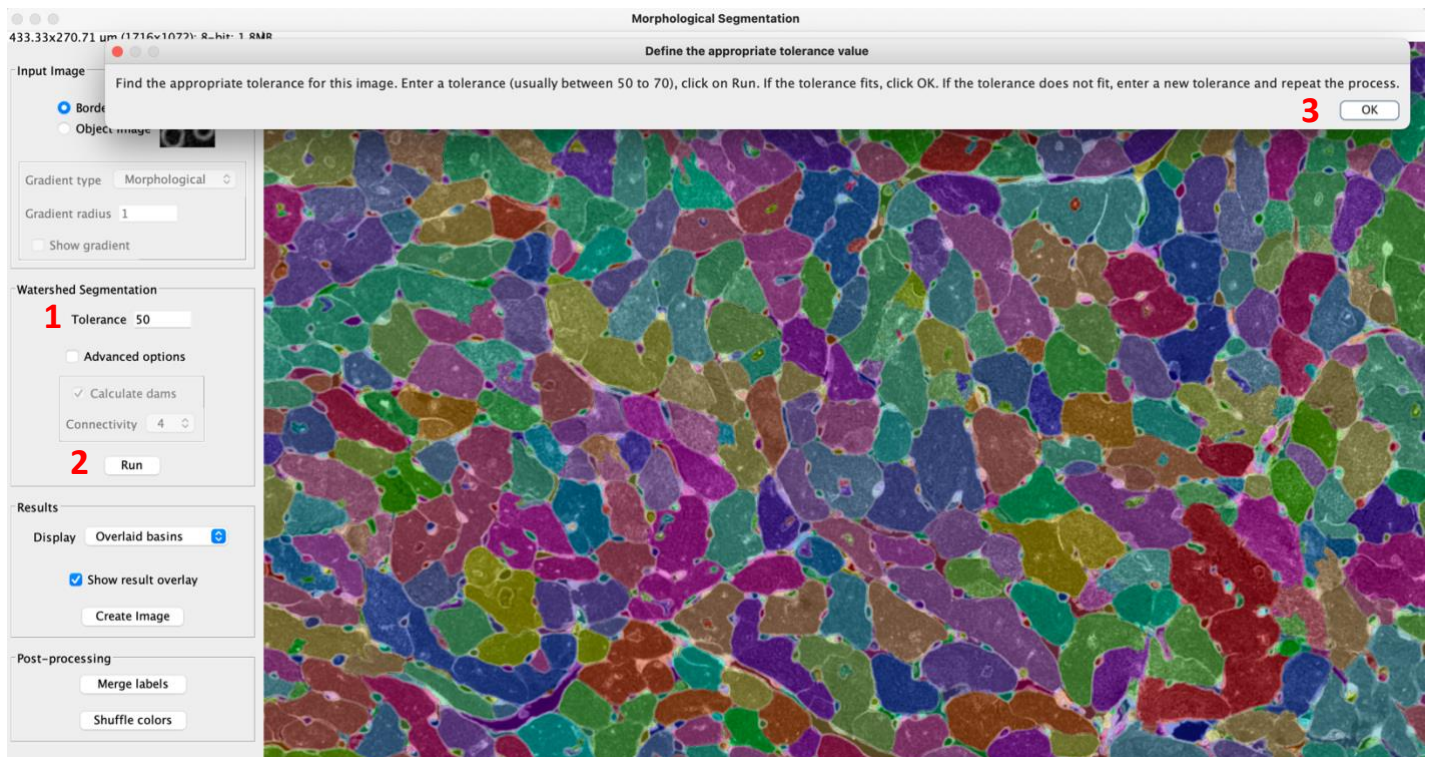
This macro measures the size of cardiomyocytes (Group 1) in different parameters on slides stained with WGA+CD31+intramyocyte and intramyocyte-nuclei signal. The results are reported in an Excel spreadsheet on the desktop, which contains the individual results as well as an average value. An overlay image and color-coded instances will also be created. In addition, the size of the nuclei of the cardiomyocytes (Group 2) are measured. Furthermore an intramyocyte and intramyocyte-nuclei signal in channel red is measured, the result is reported as %-value per instance.

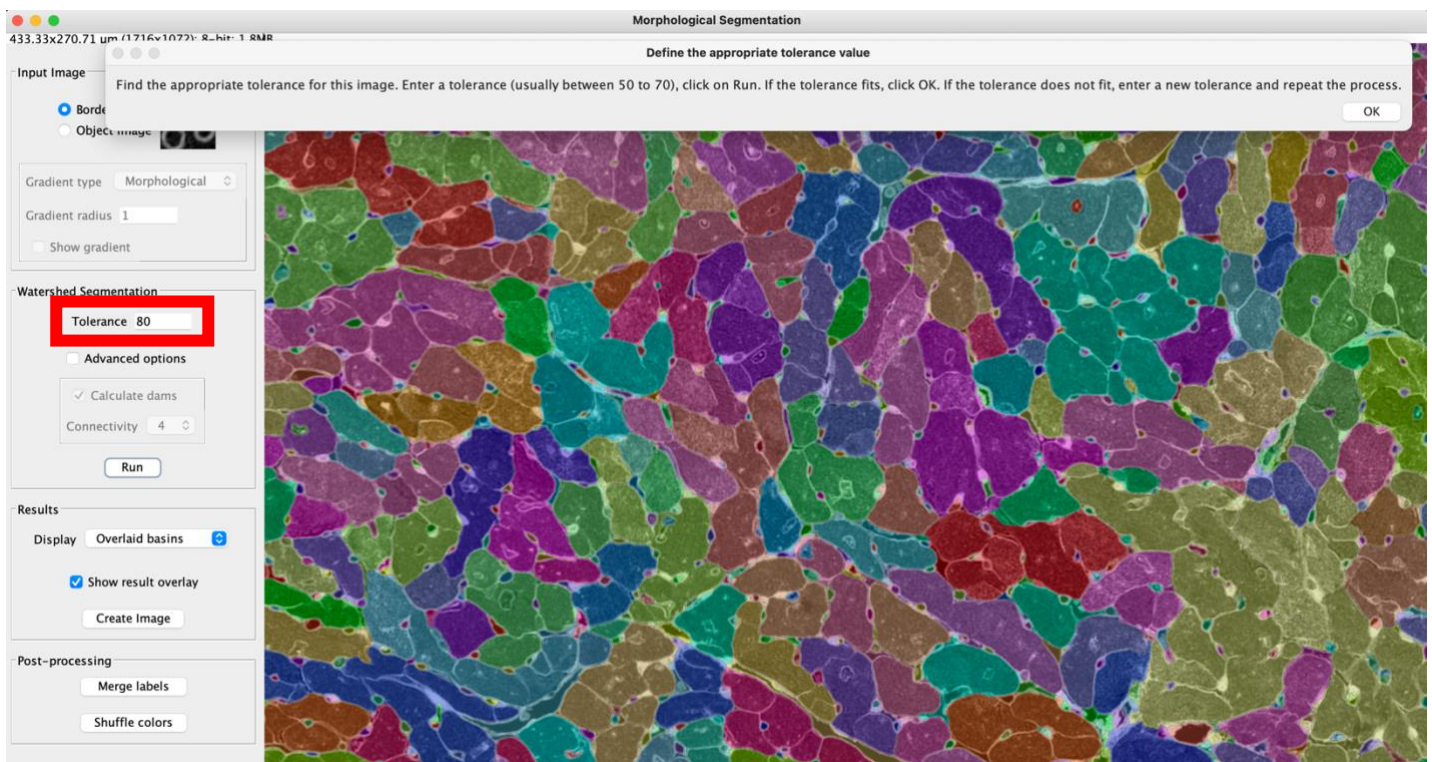
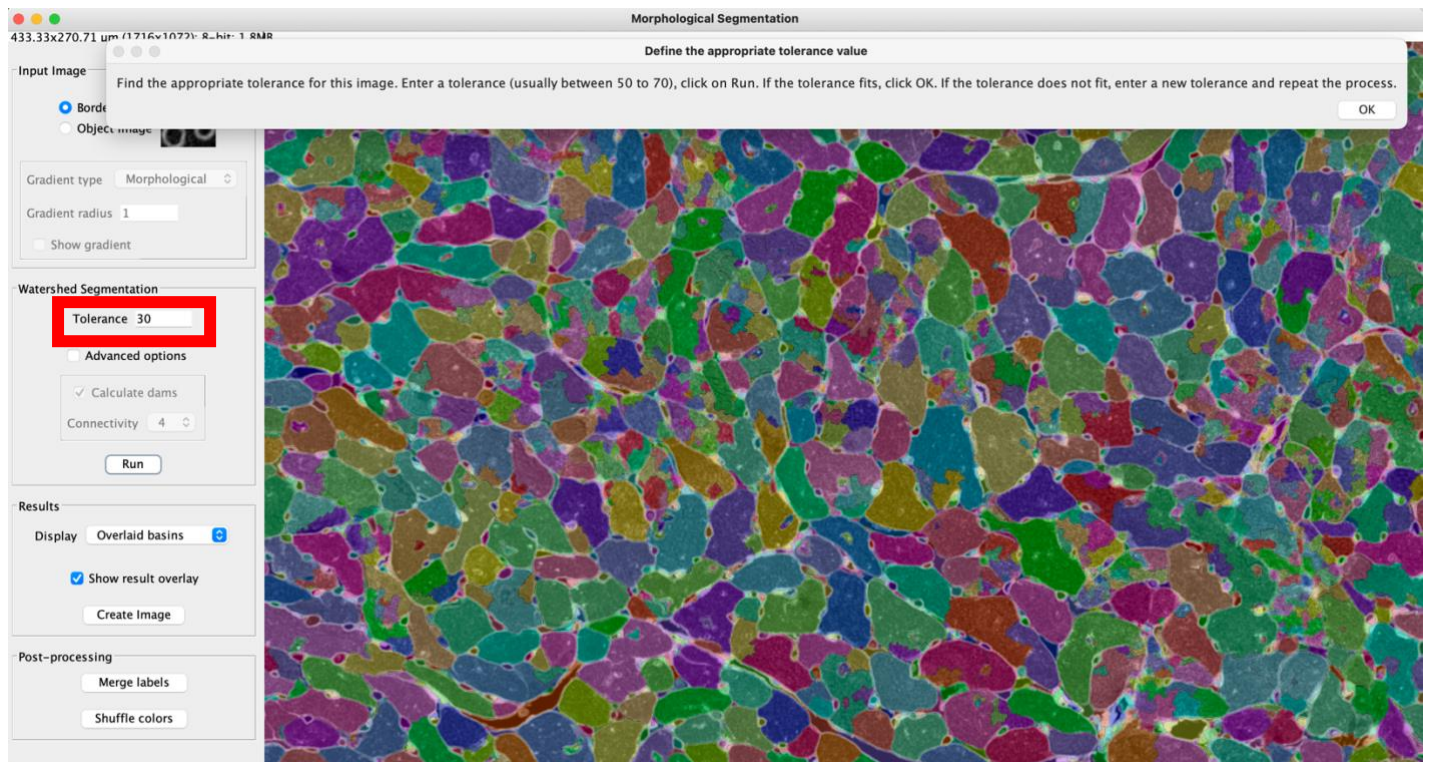
General Preset

1. Install ImageJ (<https://imagej.nih.gov/ij/download.html>)
2. Install the required plugins:
MorphoLibJ_-1.4.0 (<https://github.com/ijpb/MorphoLibJ>)
Read and Write Excel (https://github.com/antinos/Read_and_Write_Excel_Modified)
3. Open the macro itself in a text editor. Define within the macro the "scale": How many pixels are a micrometer ($1\mu\text{m} = \text{"scale" Pixel}$)? You can also adjust the thresholds for cardiomyocytes, nuclei, and capillaries.
4. Install the HeartJ macro, which you want to use: Plugins -> Macros -> Install (Permanent installation is also possible: See ImageJ instructions)

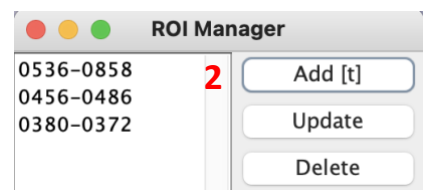
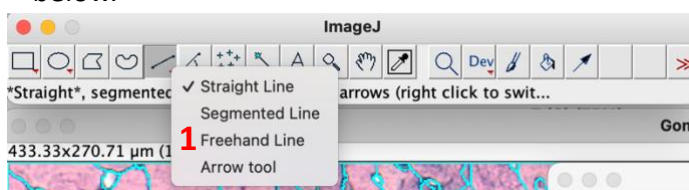
General Procedure (by using the example of Gomori)

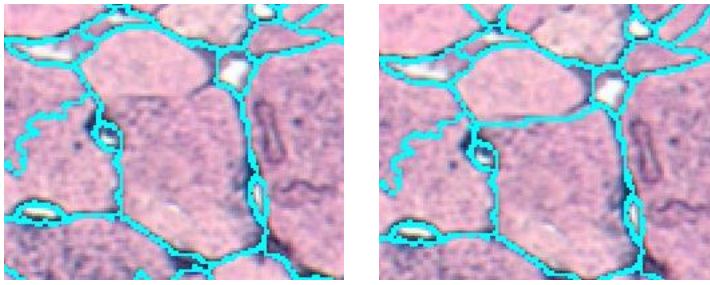
1. Open the image to be analyzed in ImageJ, also possible with drag and drop.
2. Start the Macro (also possible through shortcut "q")
3. Find a suitable tolerance. Enter a tolerance (1), which is usually between 50 to 70. Press Run (2), look at the image, and see if the segmentation fits well. If too many cardiomyocytes are divided into several segments, increase the tolerance and vice versa. In this example a tolerance of 50 fits, look at the example pictures of what mismatched tolerances look like. If you have found an appropriate tolerance, press "OK" (3).





- If necessary, complete missing lines of segmentation. Use "Freehand line" (1) to draw the line and add them to the ROI manager (2). You can find an example of missing segmentation below.

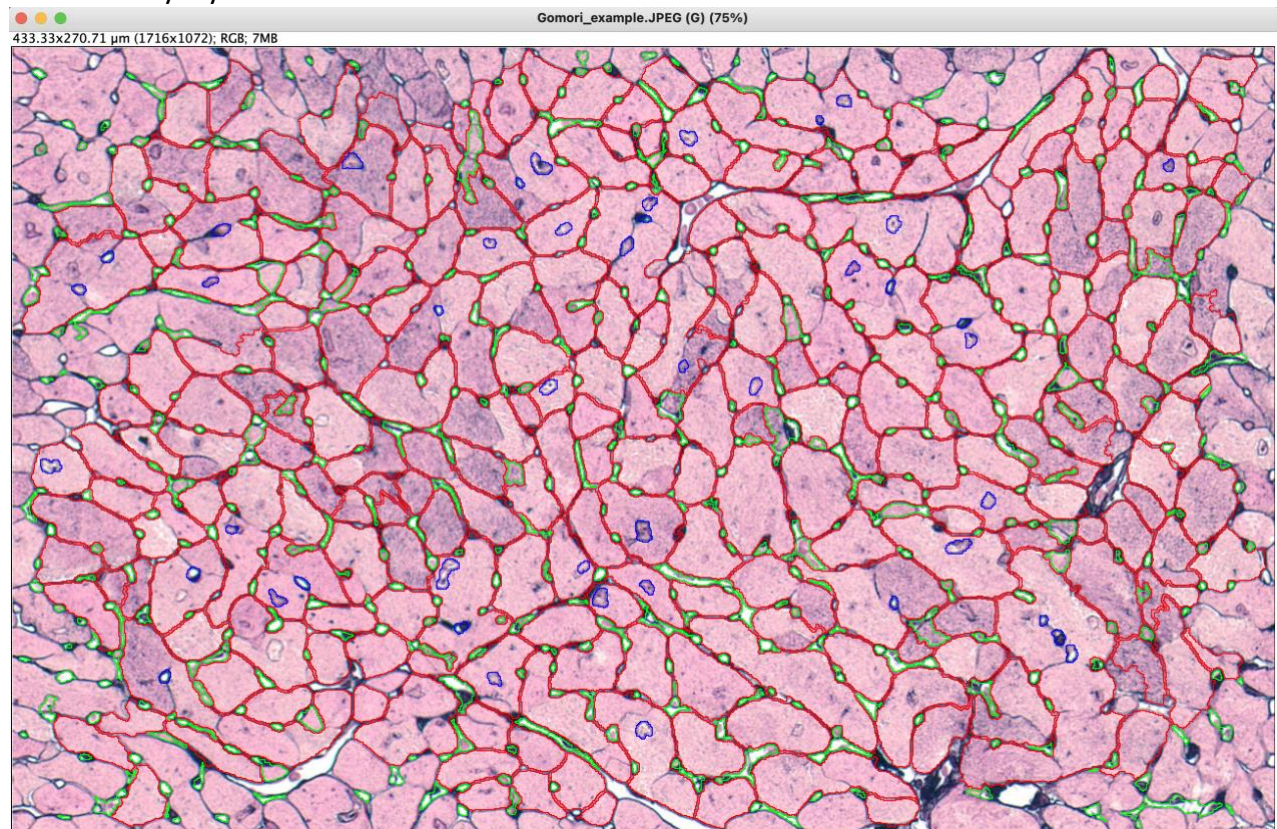


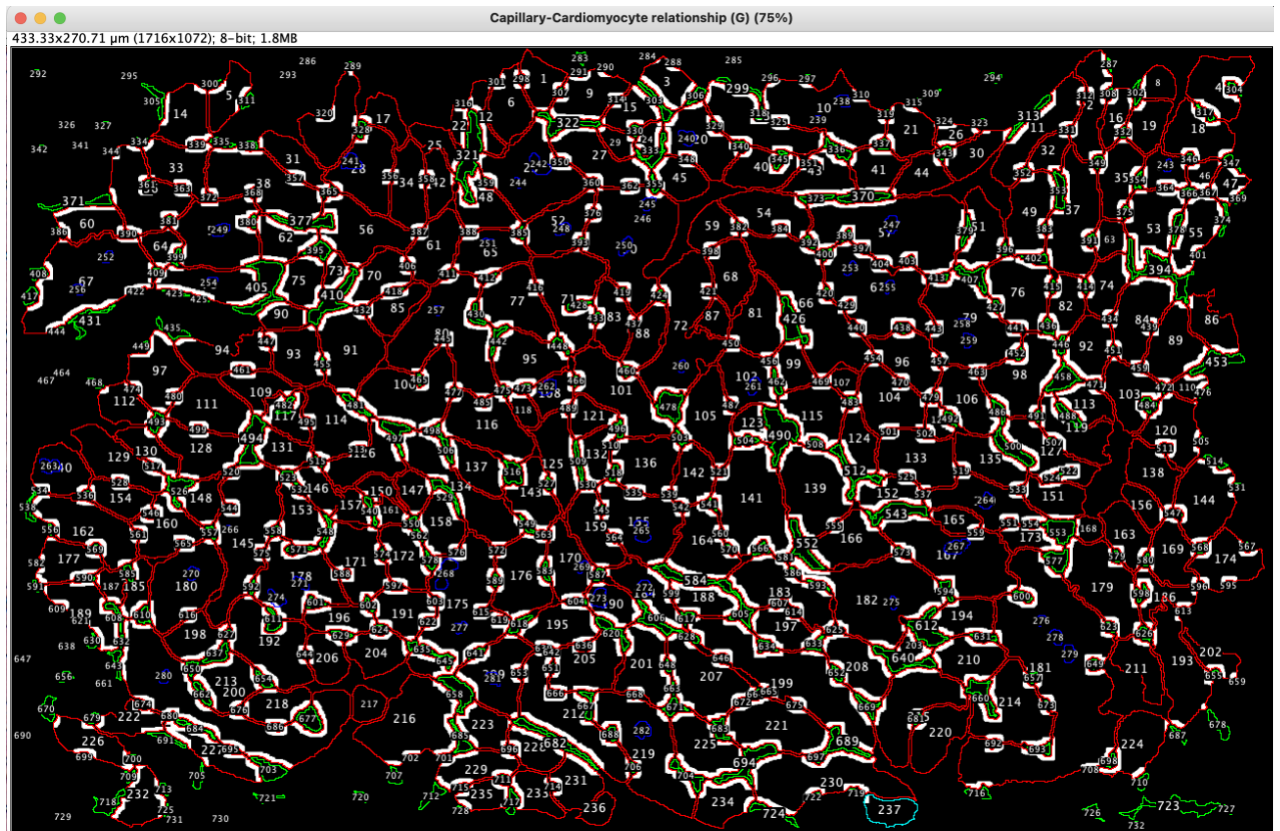


- Press "OK" when you have added all the lines, then the macro will calculate for a few seconds.
- Summarized results and individual results are plotted in an Excel spreadsheet on the desktop (File name: "Rename me after writing is done"). The first line contains the image name. The first columns (A to J) show the average values of individual structures (cardiomyocytes, nuclei of cardiomyocytes and capillaries). In the columns (L to S), each segmented structure is given. Column S indicates to which class the structure belongs: cardiomyocytes (1), nuclei (2) or capillaries (3). Column V shows only for the cardiomyocytes how many capillaries surround each of them. All lengths or areas are given in micrometers. Other average values must be calculated manually, such as capillary density.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	Gomori_example.JPEG																							
2	Count	Structure	Amount	Total Area	Average Si	Feret	FeretX	FeretY	FeretAngle	MinFeret		Count	Area	Feret	FeretX	FeretY	FeretAngle	MinFeret	Group		Capillary-Cardiomyocyte relationship			
3		1 Cardiomyo	237	75989,57	320,631	28,957	832,253	497,928	104,167	16,657		1	188,82	22,205	701	2	107,199	15,568	1		Count	Capillary contacts		
4		2 Cardiomyo	45	801,831	17,818	6,069	799,089	481	84,623	4,04		2	293,337	38,524	1470	155	85,112	13,227	1		2	5		
5		3 Capillaries	450	4641,554	10,315	6,297	815,653	531,131	98,363	2,757		3	194,113	19,642	855	41	171,87	12,856	1		3	4		
6												4	327,263	25,941	1599	65	31,059	20,508	1		4	2		
7												5	199,469	25,554	273	115	71,565	12,491	1		5	2		
8												6	263,43	28,108	673	131	81,215	16,162	1		6	3		
9												7	369,35	36,403	426	163	77,989	19,8	1		7	3		
10												8	112,233	13,592	1551	27	125,166	11,89	1		8	1		
11												9	300,097	29,498	733	91	21,073	17,545	1		9	5		
12												10	653,313	44,511	1022	59	159,406	24,715	1		10	7		
13												11	393,519	46,124	1350	188	58,66	13,013	1		11	4		
14												12	117,654	26,778	628	47	110,999	8,89	1		12	2		
15												13	454,099	44,742	949	47	151,699	17,374	1		13	6		
16												14	283,836	24,735	189	121	40,03	20,187	1		14	3		
17												15	173,26	20,58	802	101	6,24	14,227	1		15	4		

- Two control images are also created. One of them shows an overlay with all structures. The other shows only the segmented structures to better visualize the capillary contacts of the cardiomyocytes.





8. If necessary, the user can delete some segmentations in the Excel sheet that are not segmented correctly. To control all structures, it is recommended to deactivate "show all" in ROI Manager (1) and use the arrow keys to navigate through all ROIs. On the overlayer now only one ROI is displayed, in the ROI Manager the corresponding number can be read (Red box). This number can be found in column L of the Excel spreadsheet. The average values must then be recalculated.

