

# **Doing Low-Level processing of MIBI Data**

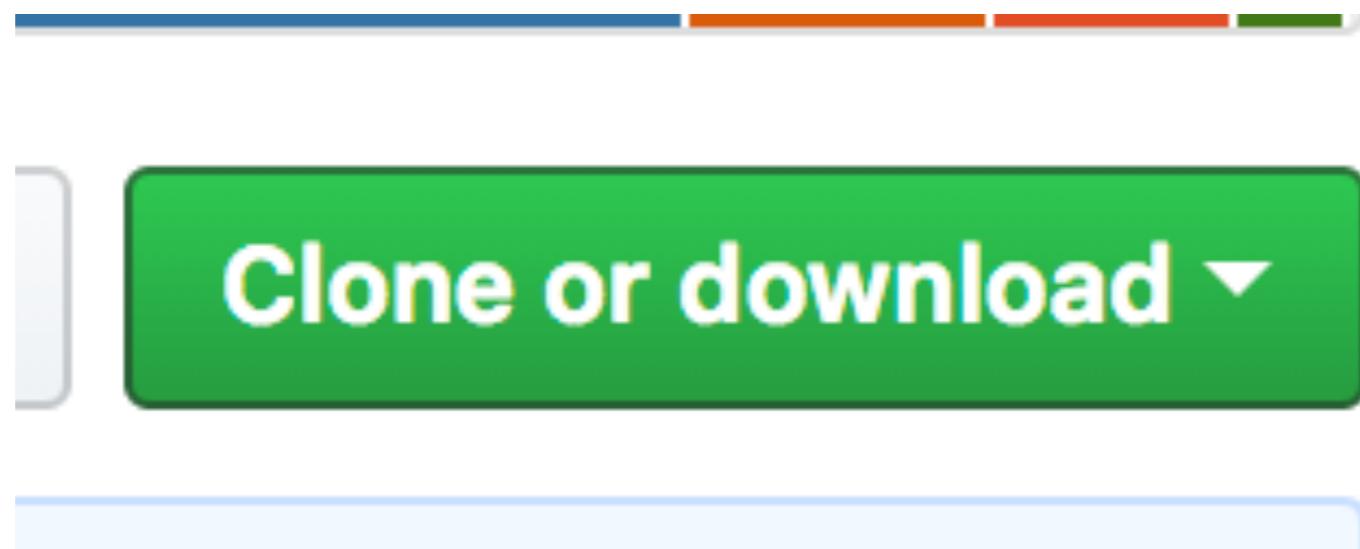
## **Using the MIBI GUI**

# Setting up MATLAB

- Download MATLAB
- Install the ‘Image Processing Toolbox’
- Install the ‘Statistics and Machine Learning Toolbox’

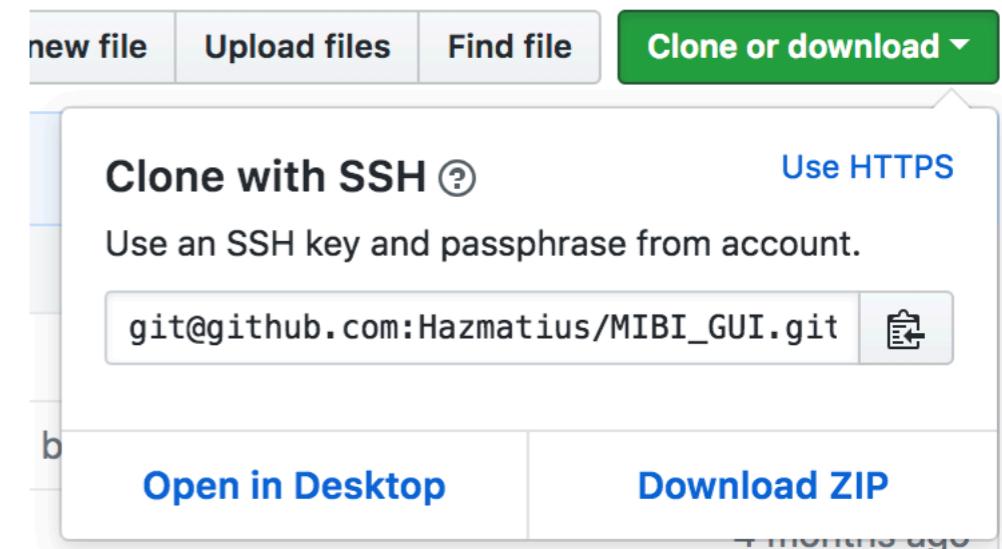
# Downloading the MIBI GUI from GitHub

- Go to [https://github.com/Hazmatius/MIBI GUI](https://github.com/Hazmatius/MIBI_GUI)
- Click the green “Clone or download” button



# Downloading the MIBI GUI from GitHub

- If you clone, copy the link and them open your Terminal
- Navigate where you want the GUI to go, then type "git clone [https://github.com/Hazmatius/MIBI\\_GUI](https://github.com/Hazmatius/MIBI_GUI)"

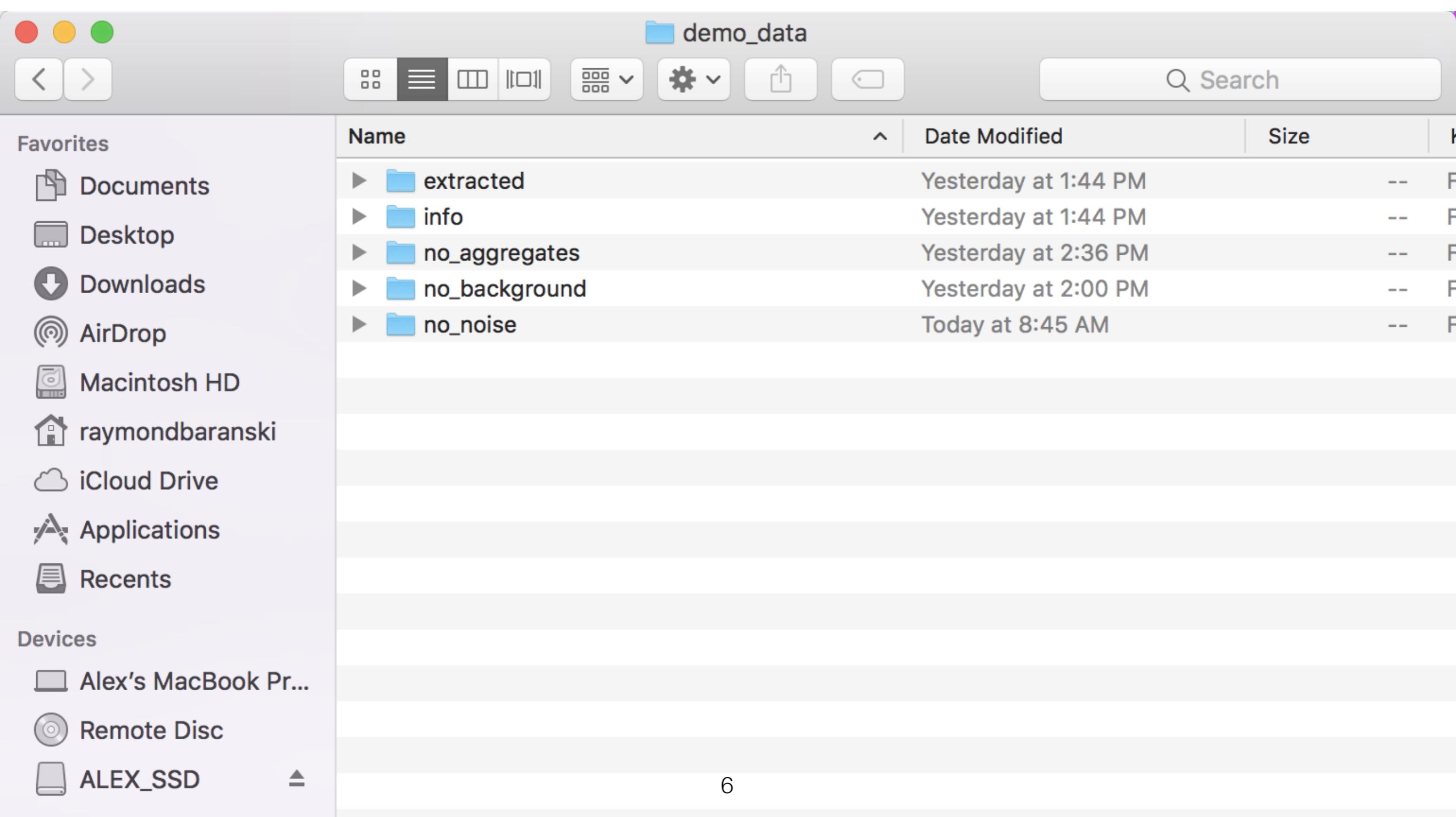


```
1. bash
Last login: Wed Feb 13 08:57:17 on ttys002
Alexs-MacBook-Pro-3:~ raymondbaranski$ cd Documents
Alexs-MacBook-Pro-3:Documents raymondbaranski$ git clone git@github.com:Hazmatiu
s/MIBI_GUI.git
```

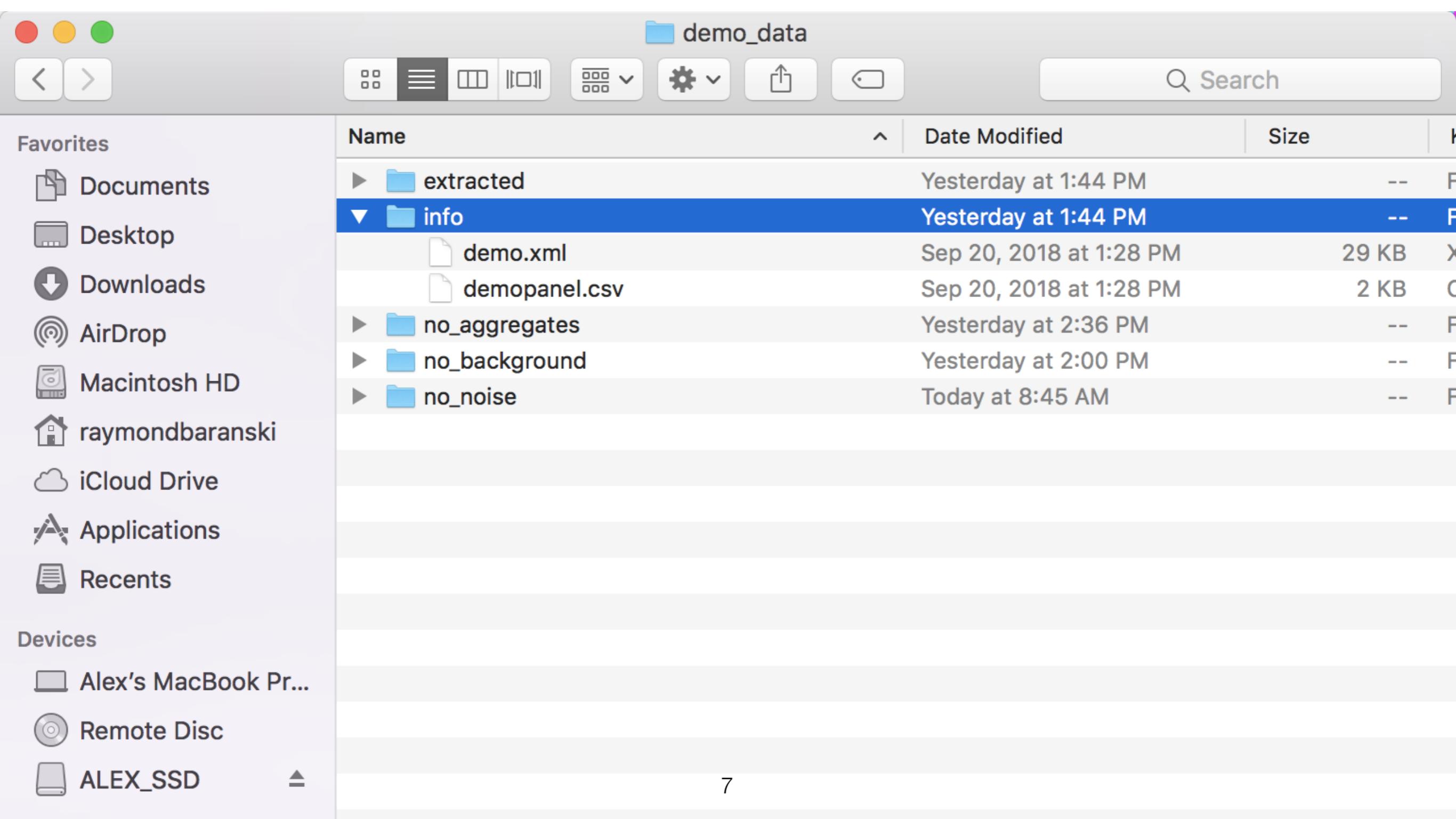
# The Structure of your Data Folder

- Everything should be inside of a folder named for your run
- The tiff files for each point should be inside of a “TIFs” folder inside of the appropriate “Point#” folder
- Raw Points should be inside of the “extracted” folder
- The panel .csv file and run .xml file should be placed inside of a folder called “info” underneath the main run folder

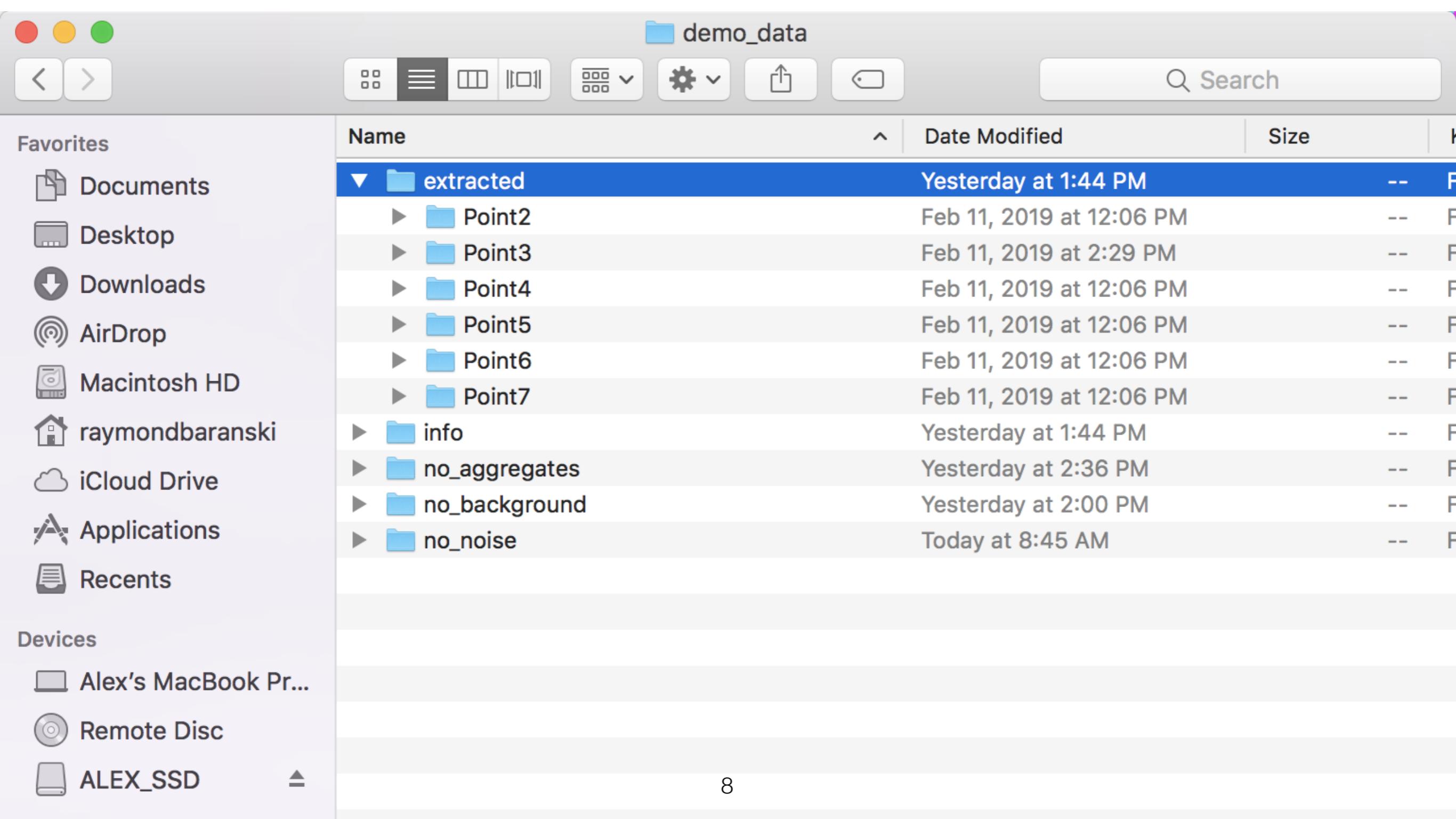
# The Structure of your Data Folder



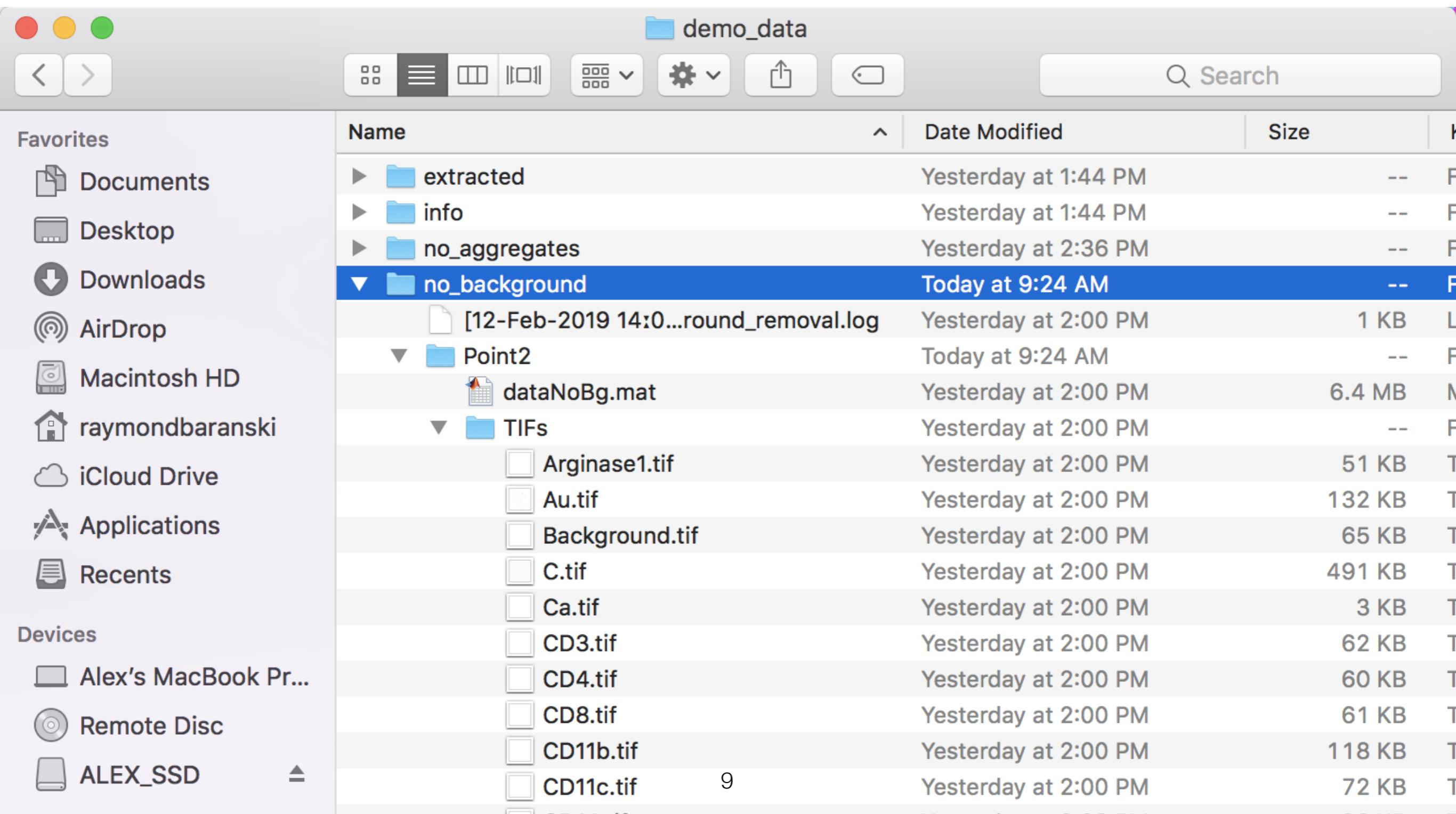
# The Structure of your Data Folder



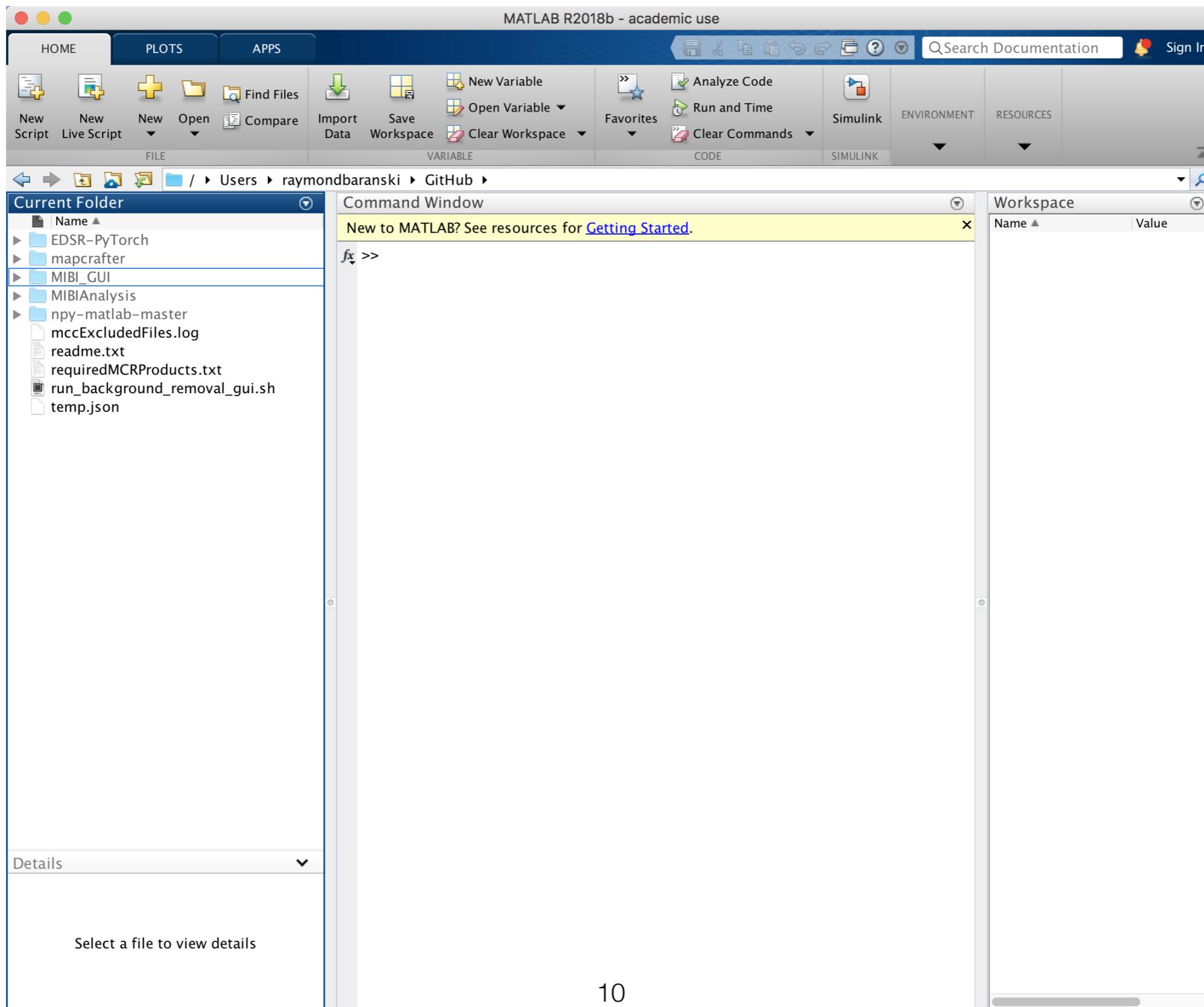
# The Structure of your Data Folder



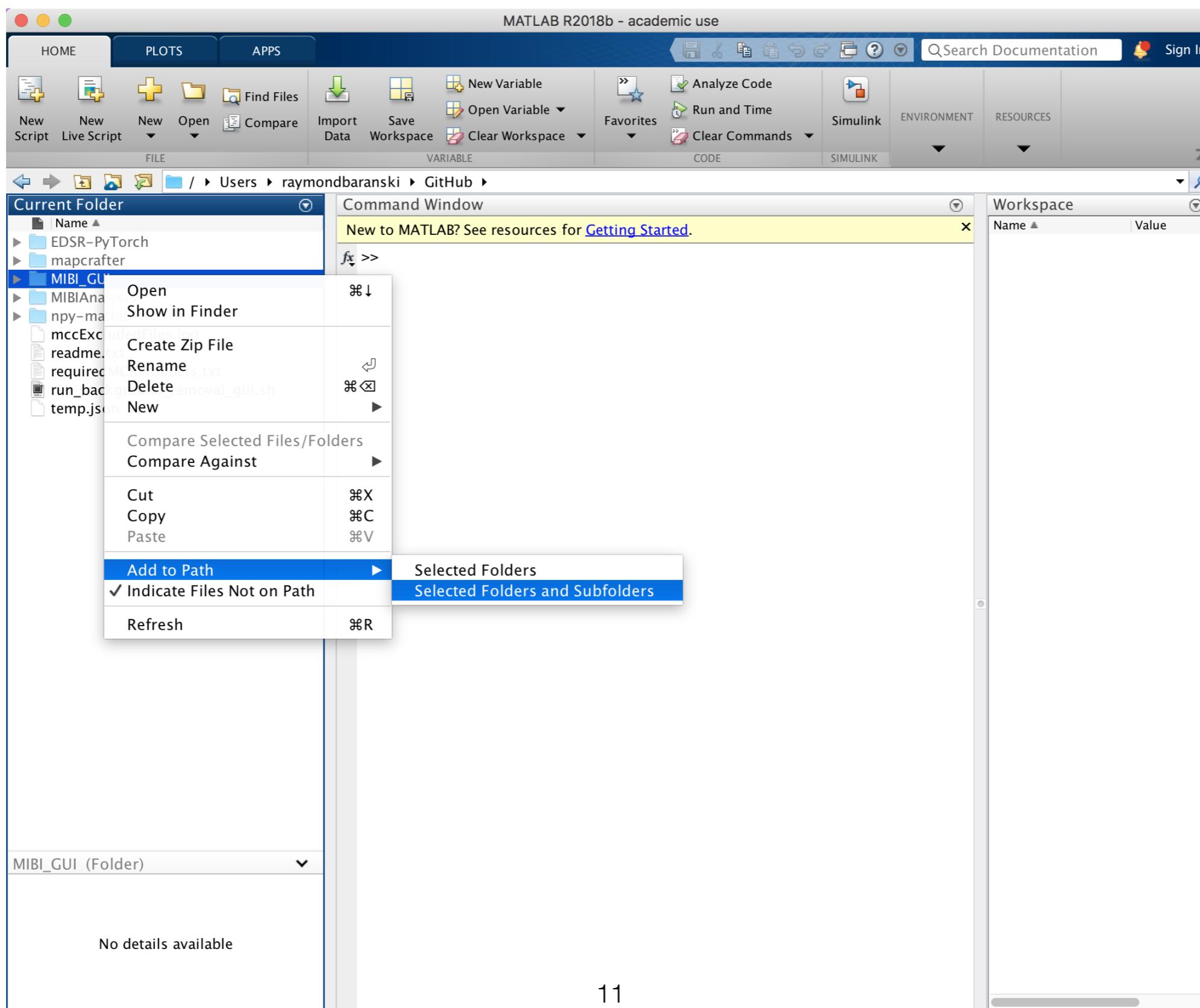
# The Structure of your Data Folder



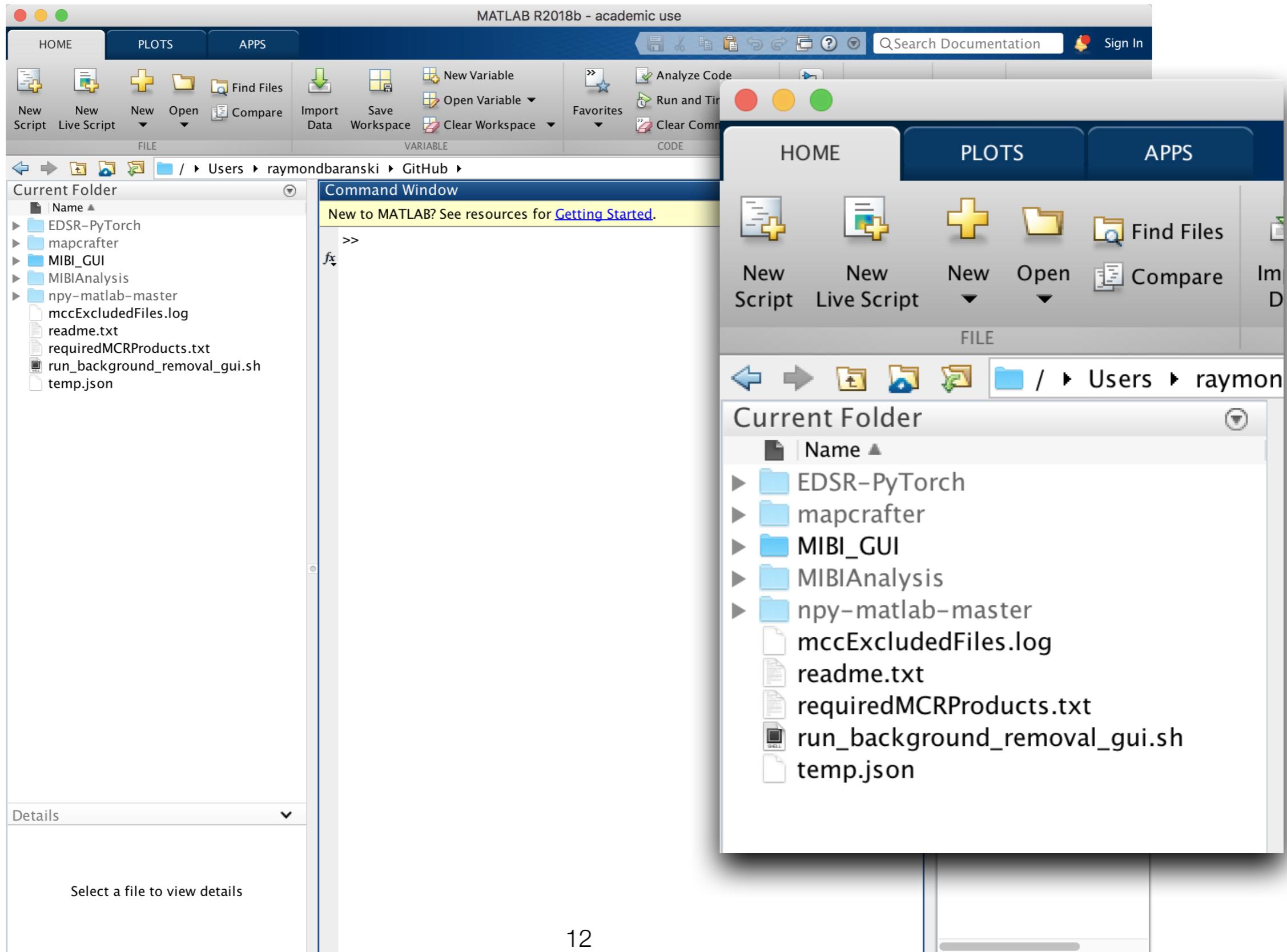
# Add the MIBI GUI to the MATLAB path by right clicking on the MIBI\_GUI folder



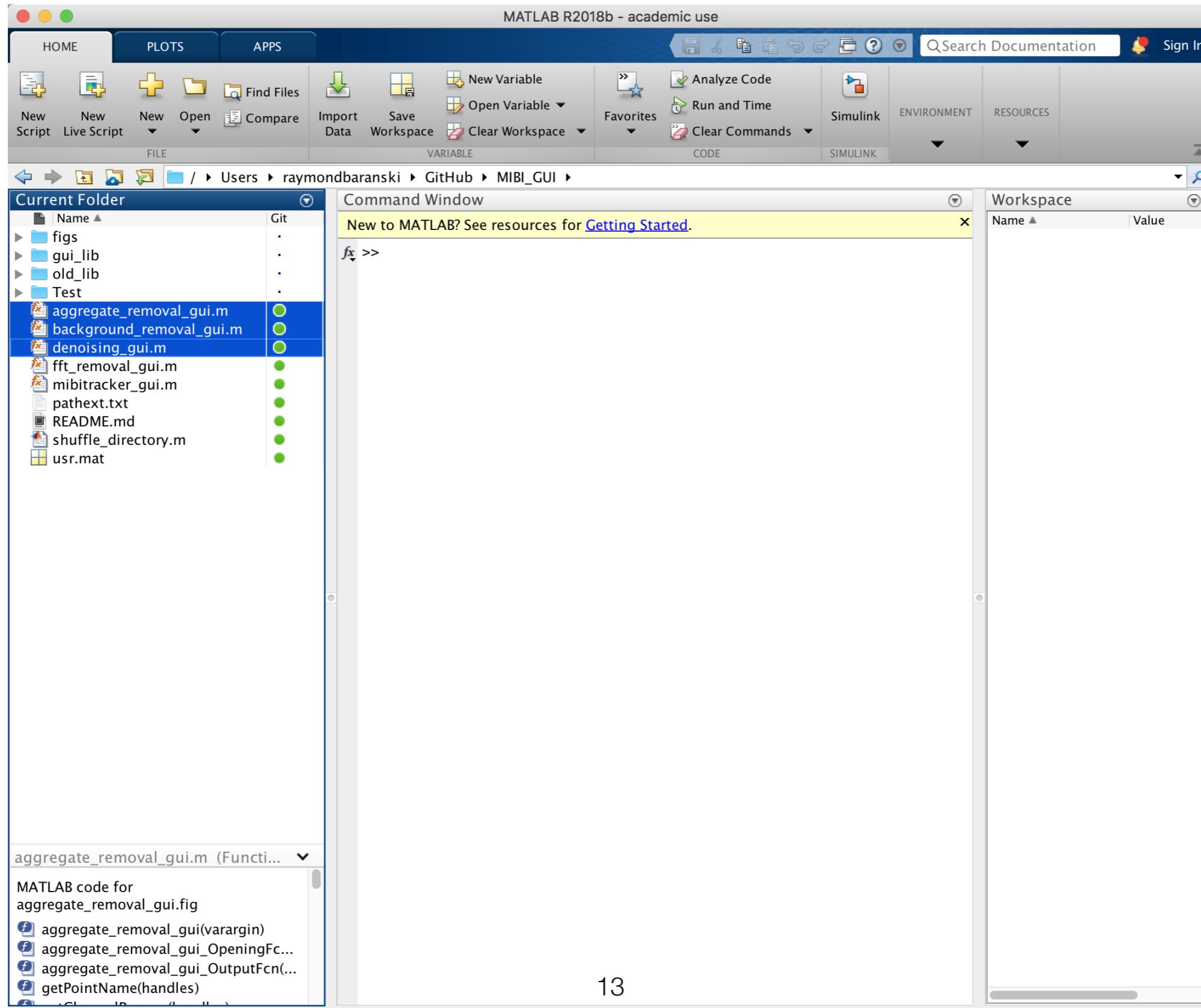
# Then navigate to “Add to Path” and click “Selected Folders and Subfolders”



# Now the folder has been added to the path



# Now MATLAB can access all of the scripts and functions in MIBI\_GUI



# Saving the path variable

- Type “`savepath`” in the Matlab command line and you won’t have to repeat the previous steps next time you open MATLAB

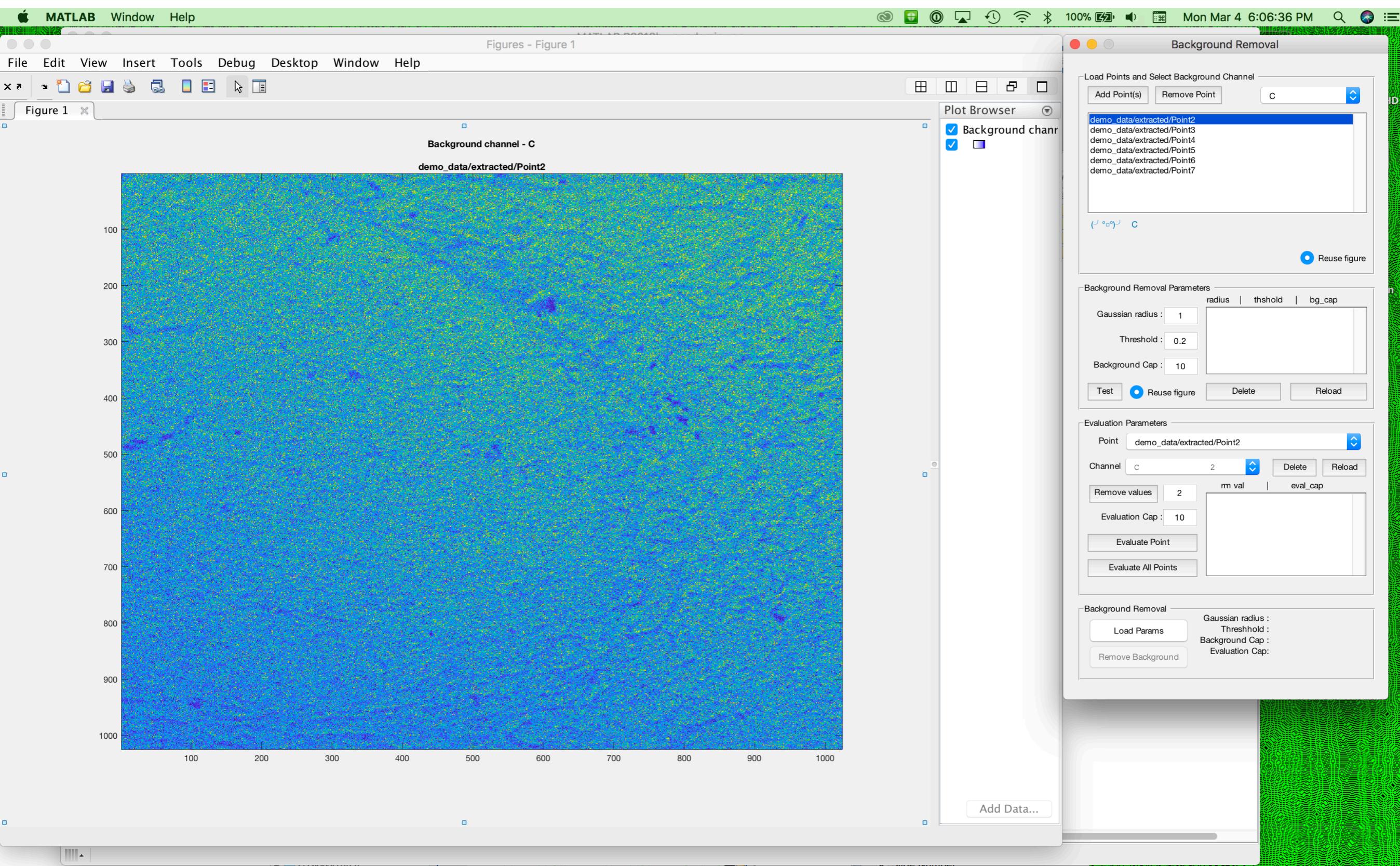
# Low-level processing steps

- Background Removal - removes slide background
- Noise Removal - removes low-intensity signal
- Aggregates Removal - removes antibody clumps

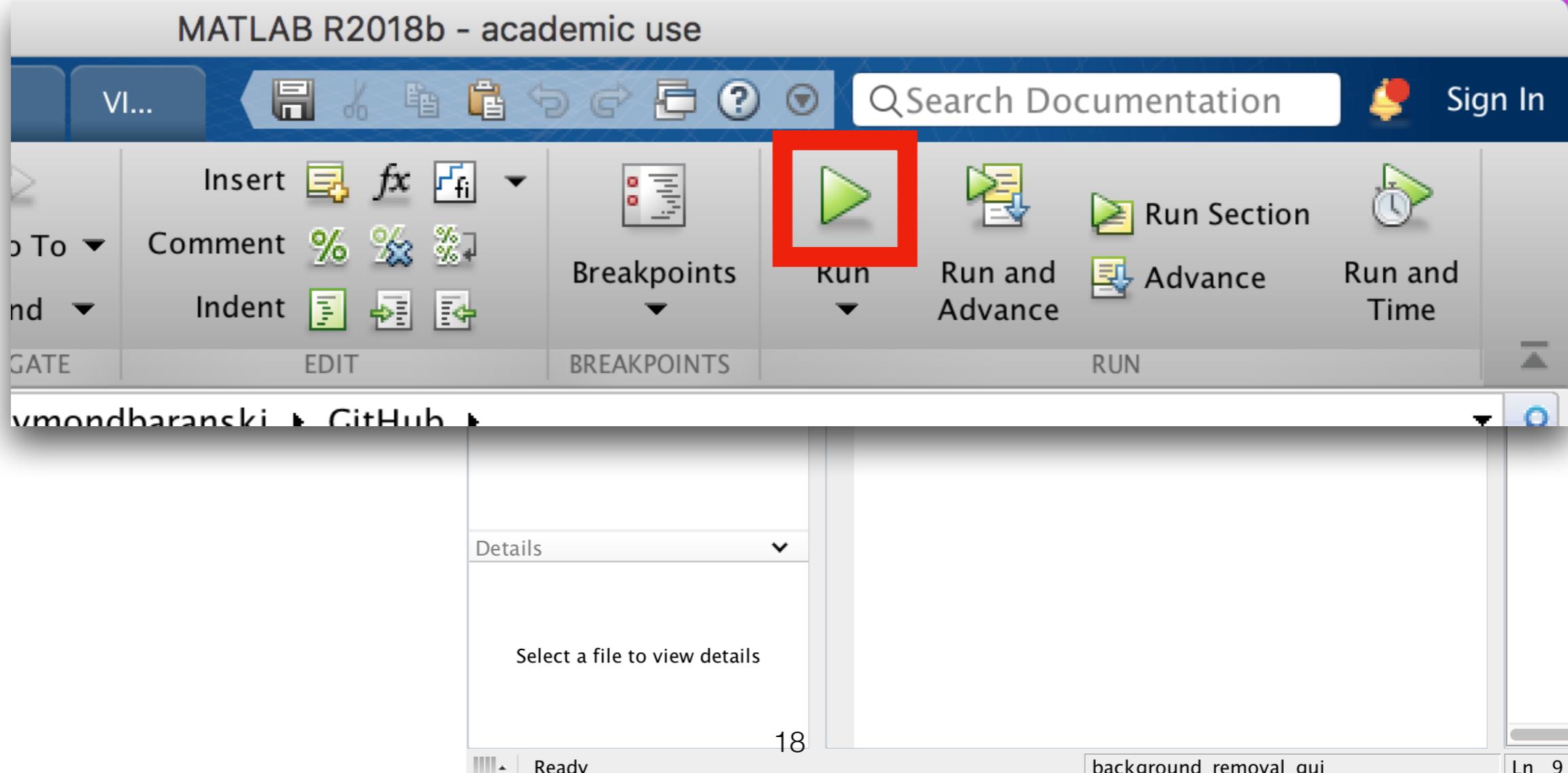
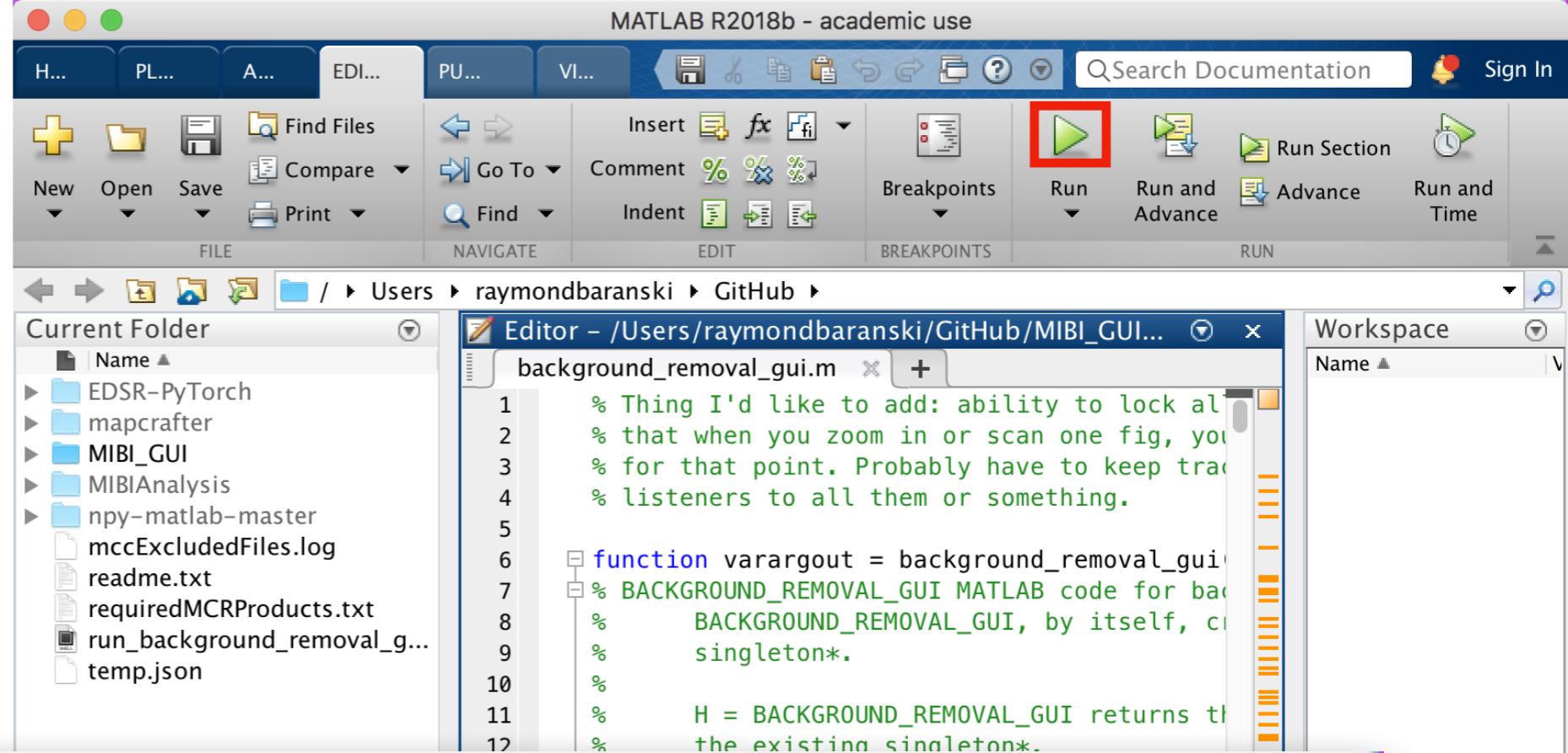
# Background Removal

- Background is a machine artifact that occurs where there is exposed slide
- We create a binary mask (an image that only takes values of 0 or 1) that corresponds to the location of background
- Then we remove a certain number of counts from each channel at the pixels that have a value of 1 in the mask

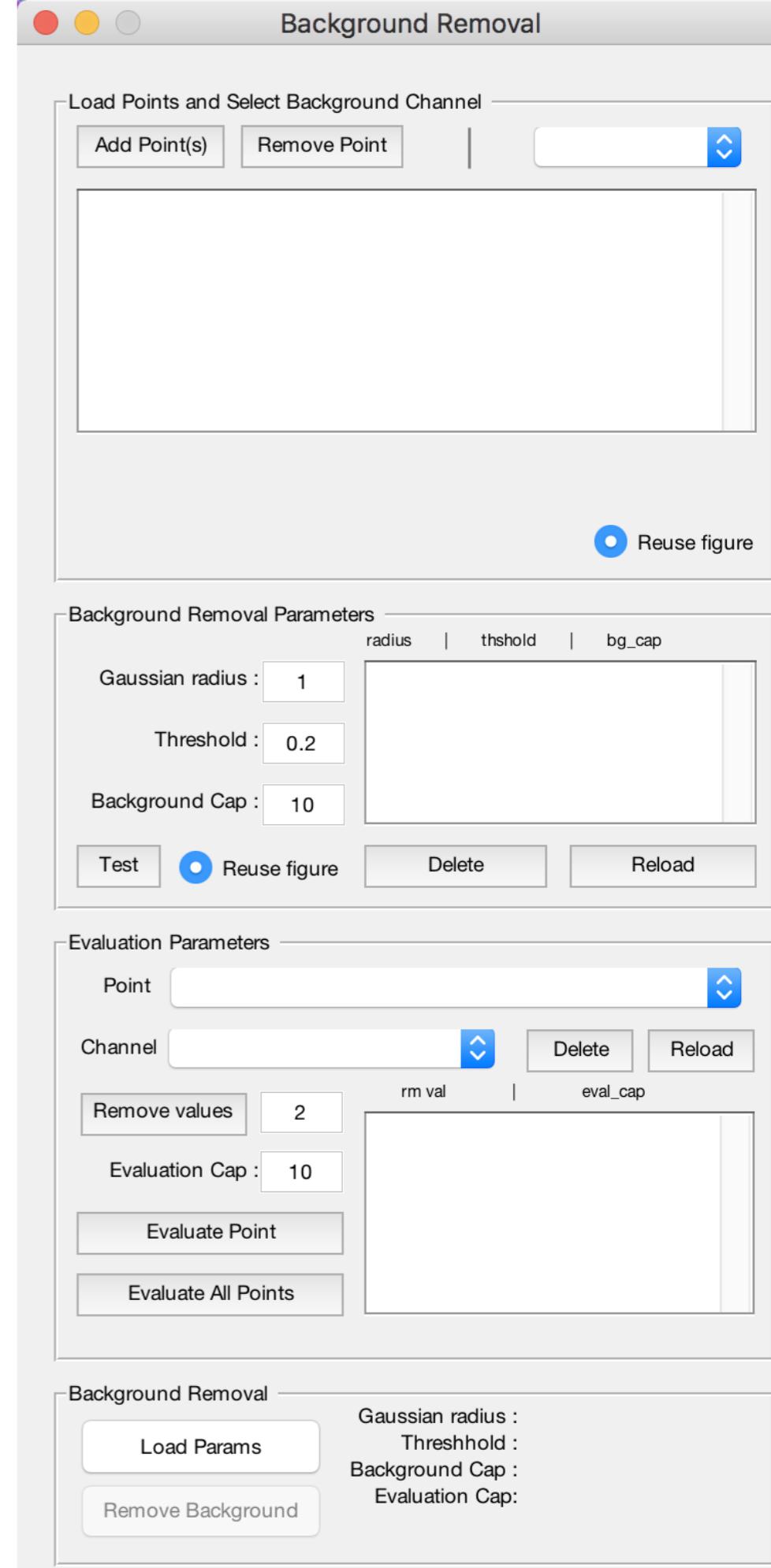
# Background removal GUI



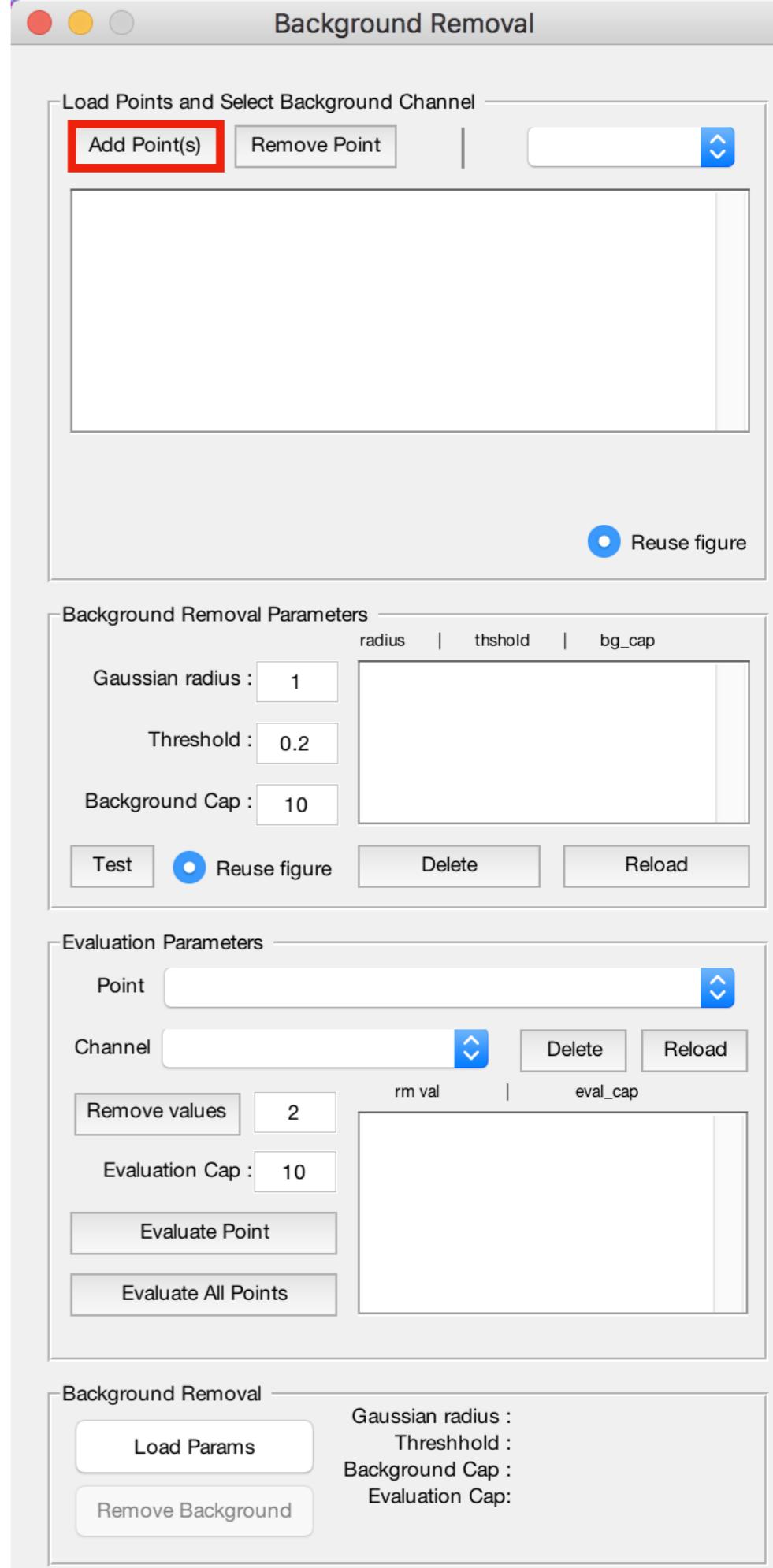
We open the  
background\_removal\_gui.m script and  
run it



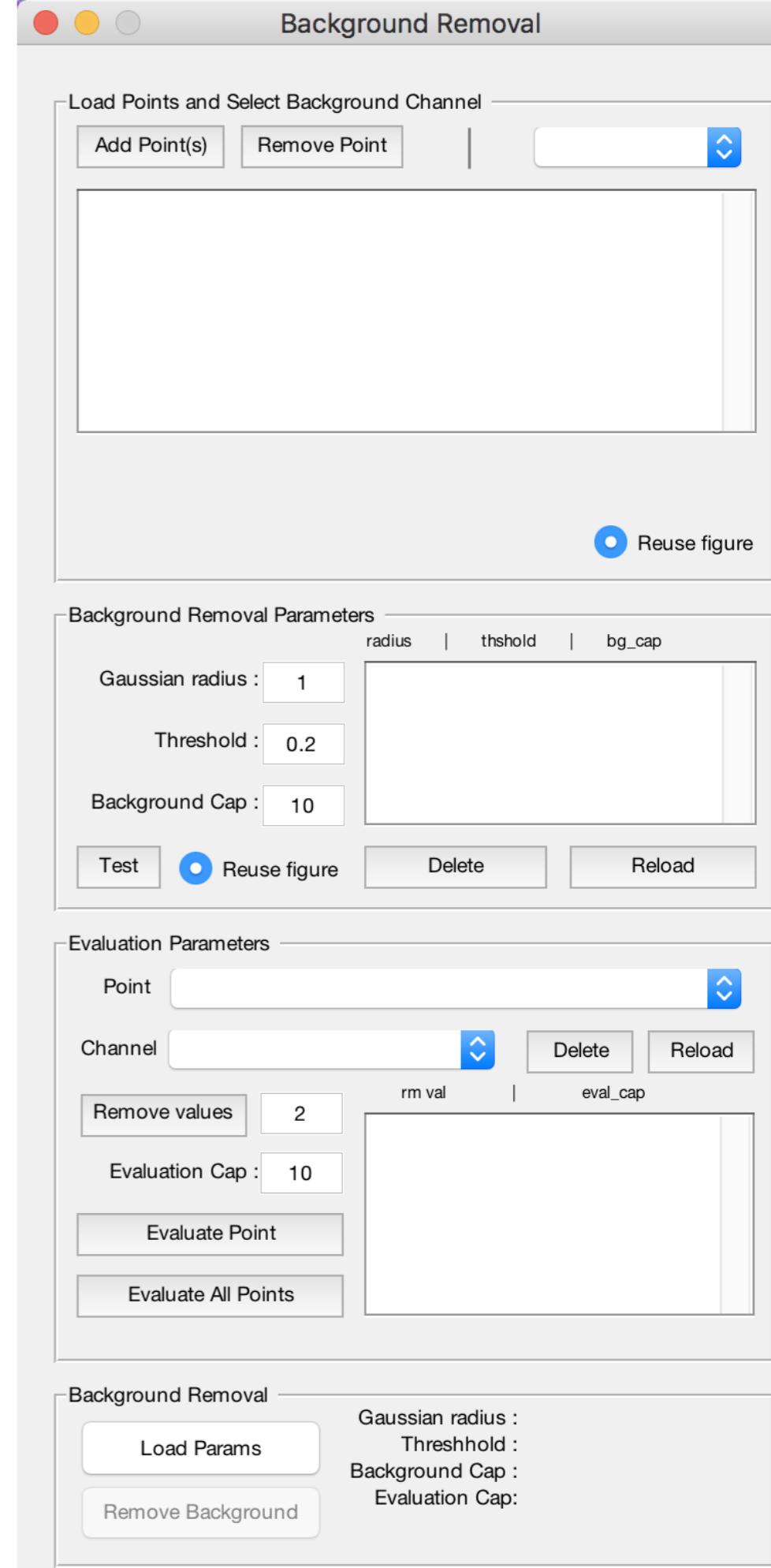
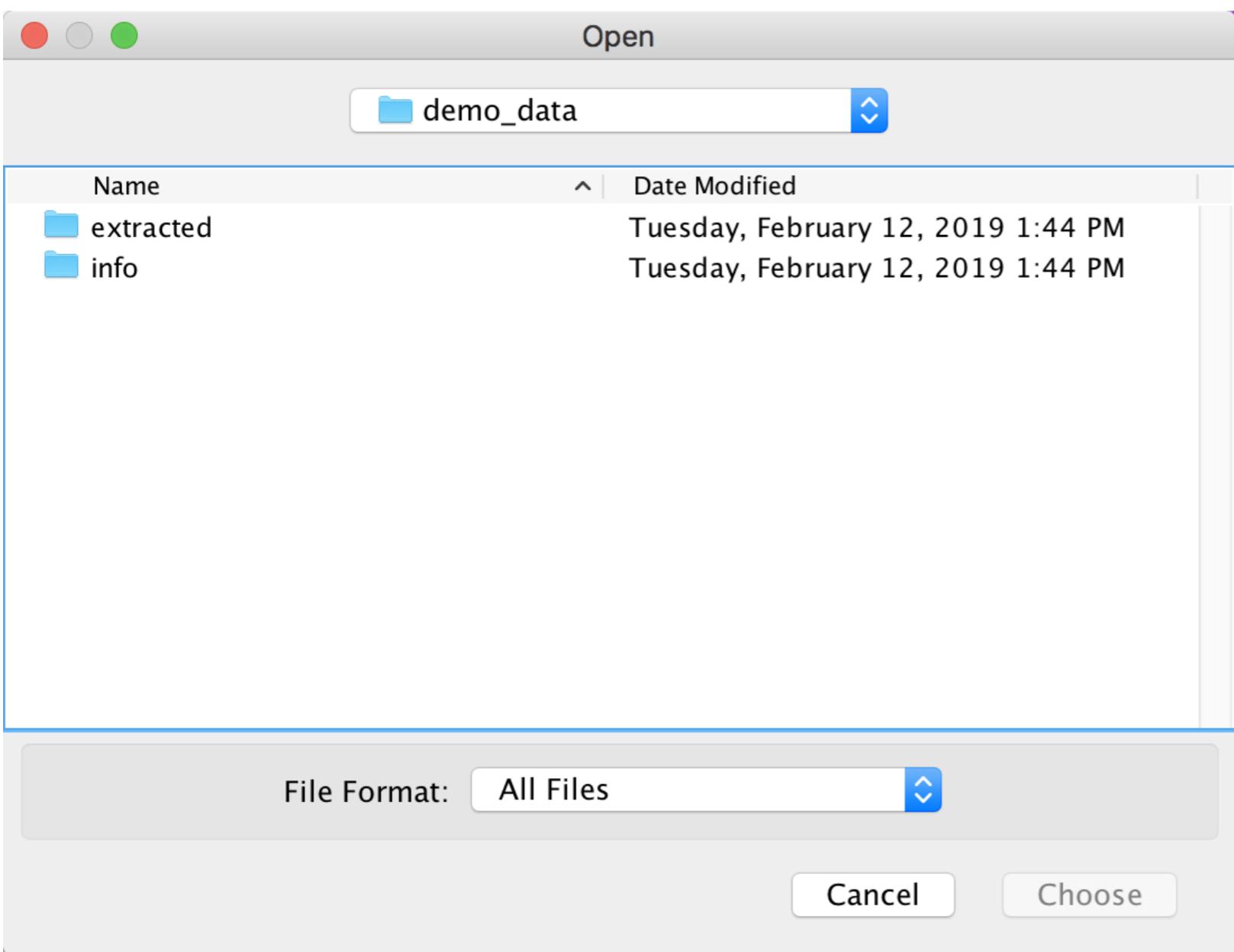
# This opens the background removal GUI



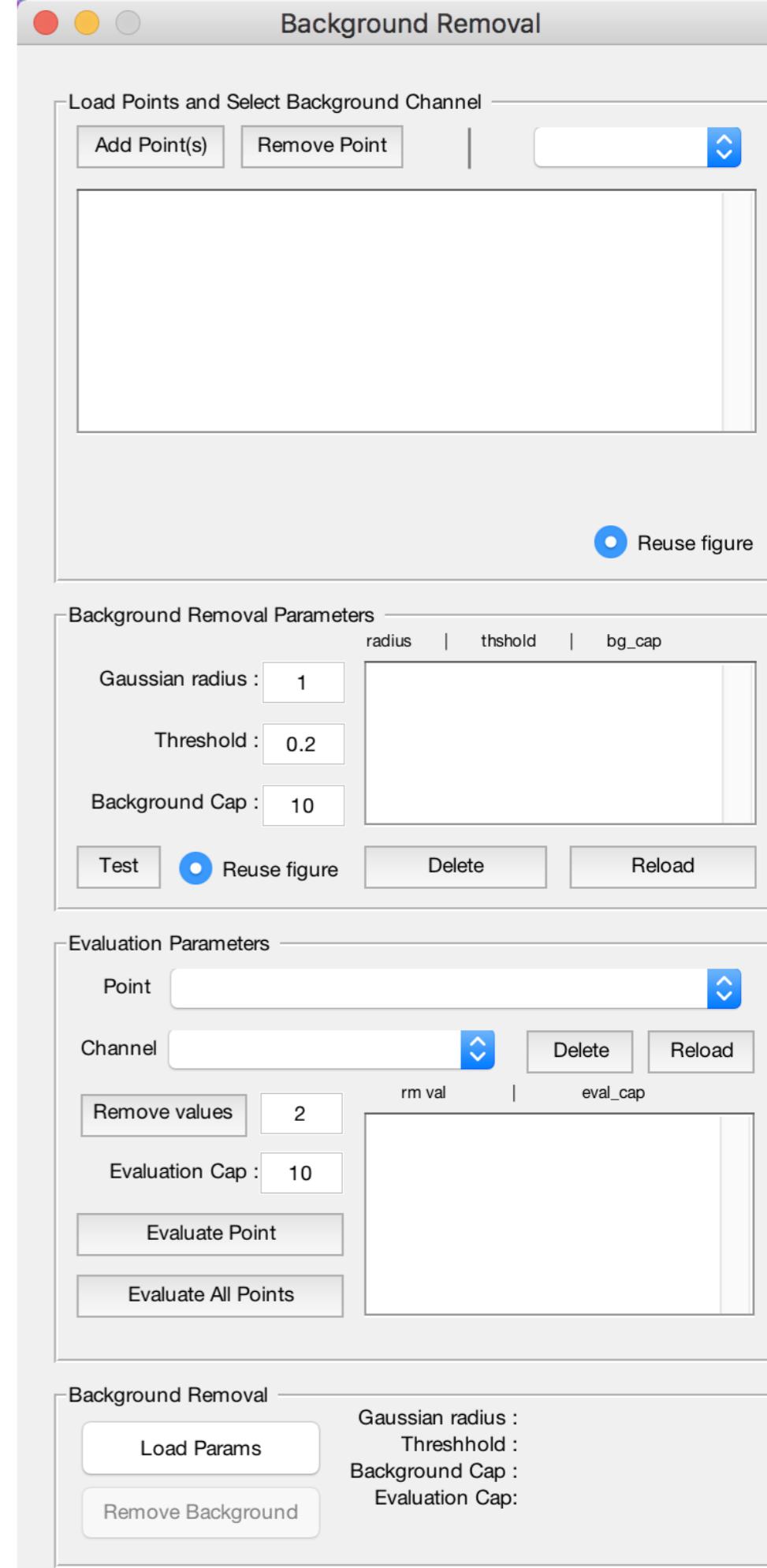
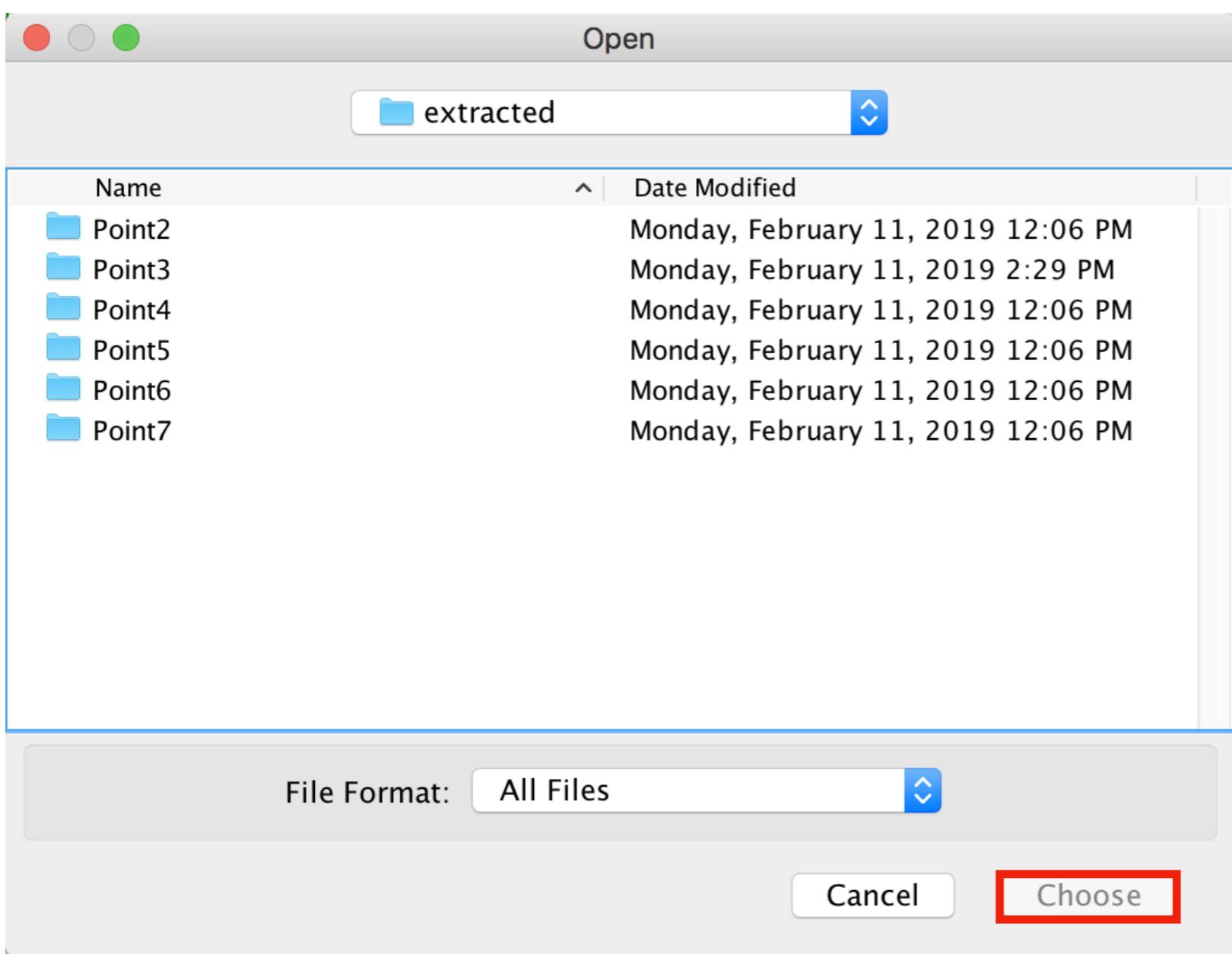
# Load points for analysis by clicking on the “Add Point(s)” button



# This opens a window for navigating to your data



“Choose” the points you want to analyze. You have to select them in the finder browser first.



# Now we have loaded Point2 into the background removal GUI

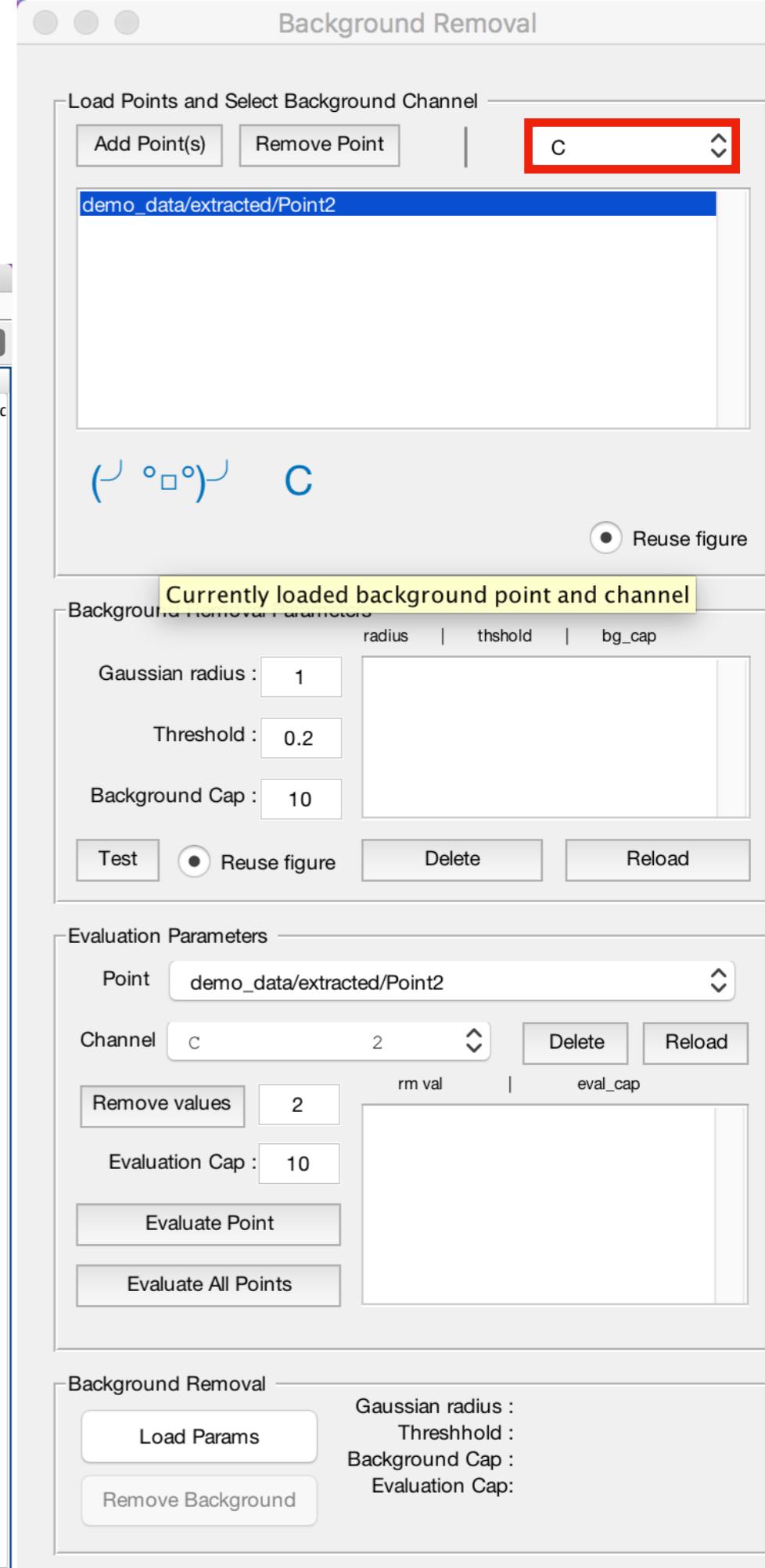
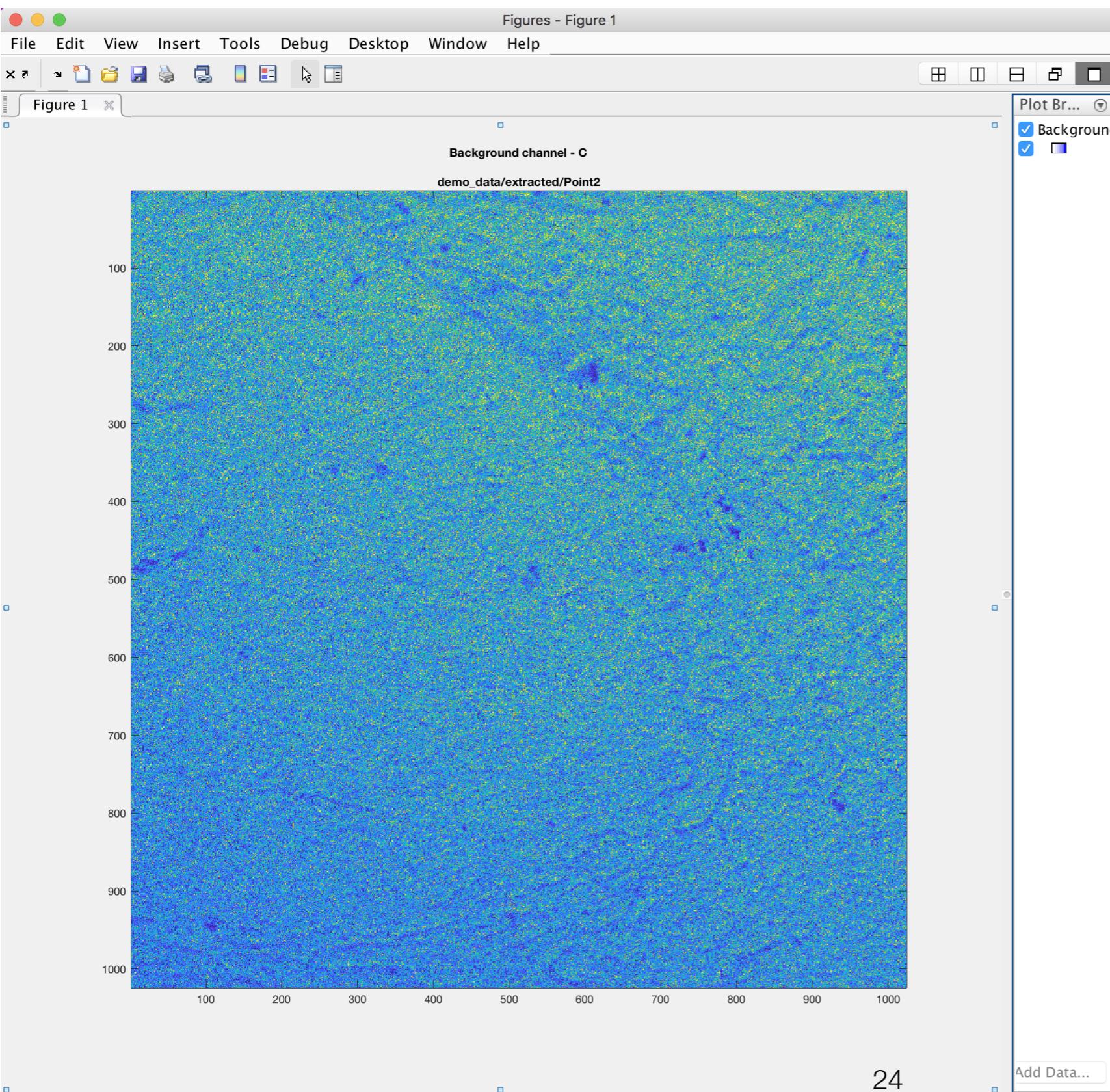
The screenshot shows the 'Background Removal' application interface. At the top, there are two windows side-by-side.

**Left Window:** The title bar says 'Background Removal'. Below it is a section titled 'Load Points and Select Background Channel'. It contains two buttons: 'Add Point(s)' and 'Remove Point'. To the right is a dropdown menu currently set to 'C'. Below this is a list box containing the text 'demo\_data/extracted/Point2', which is highlighted with a blue selection bar.

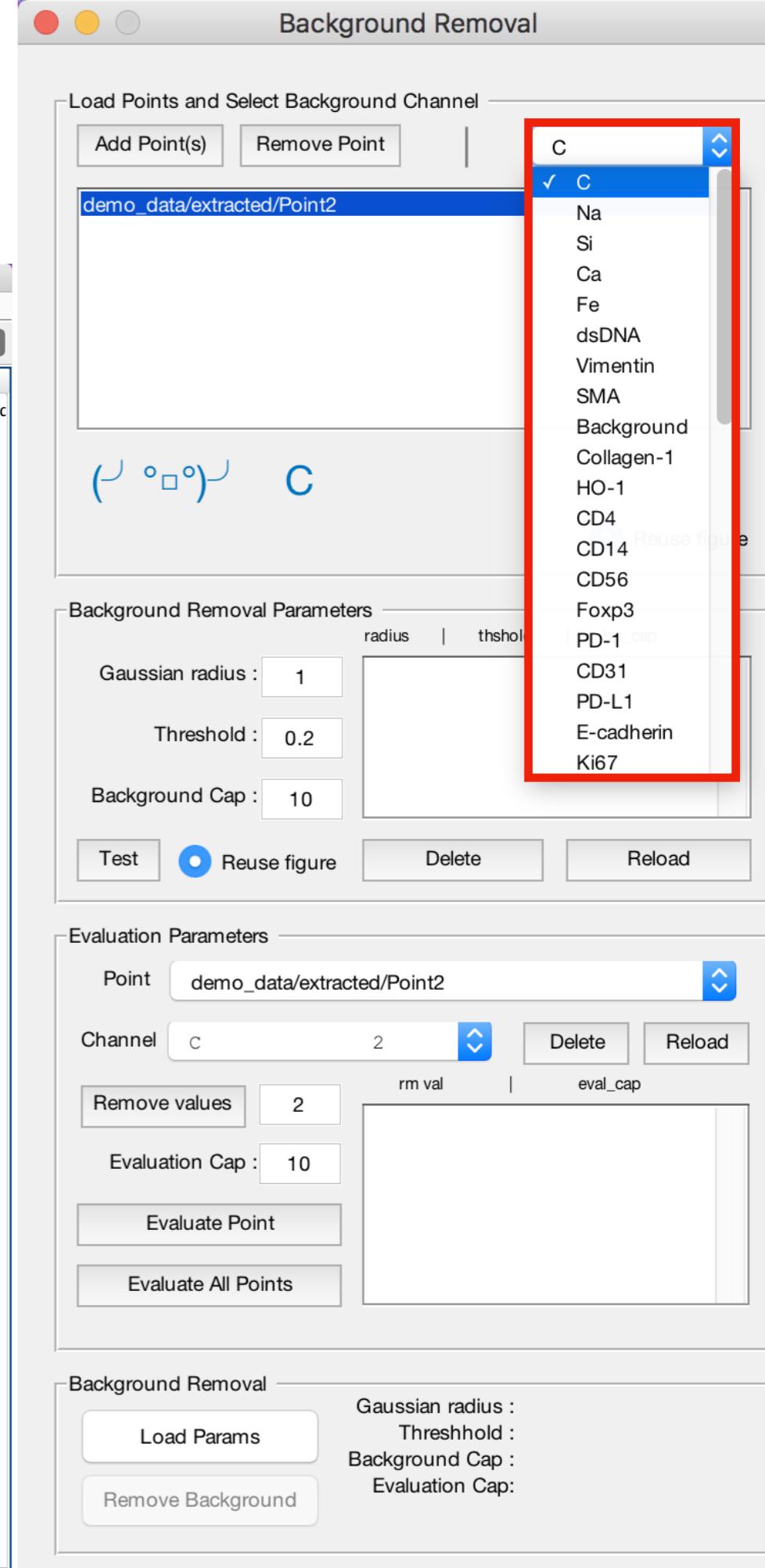
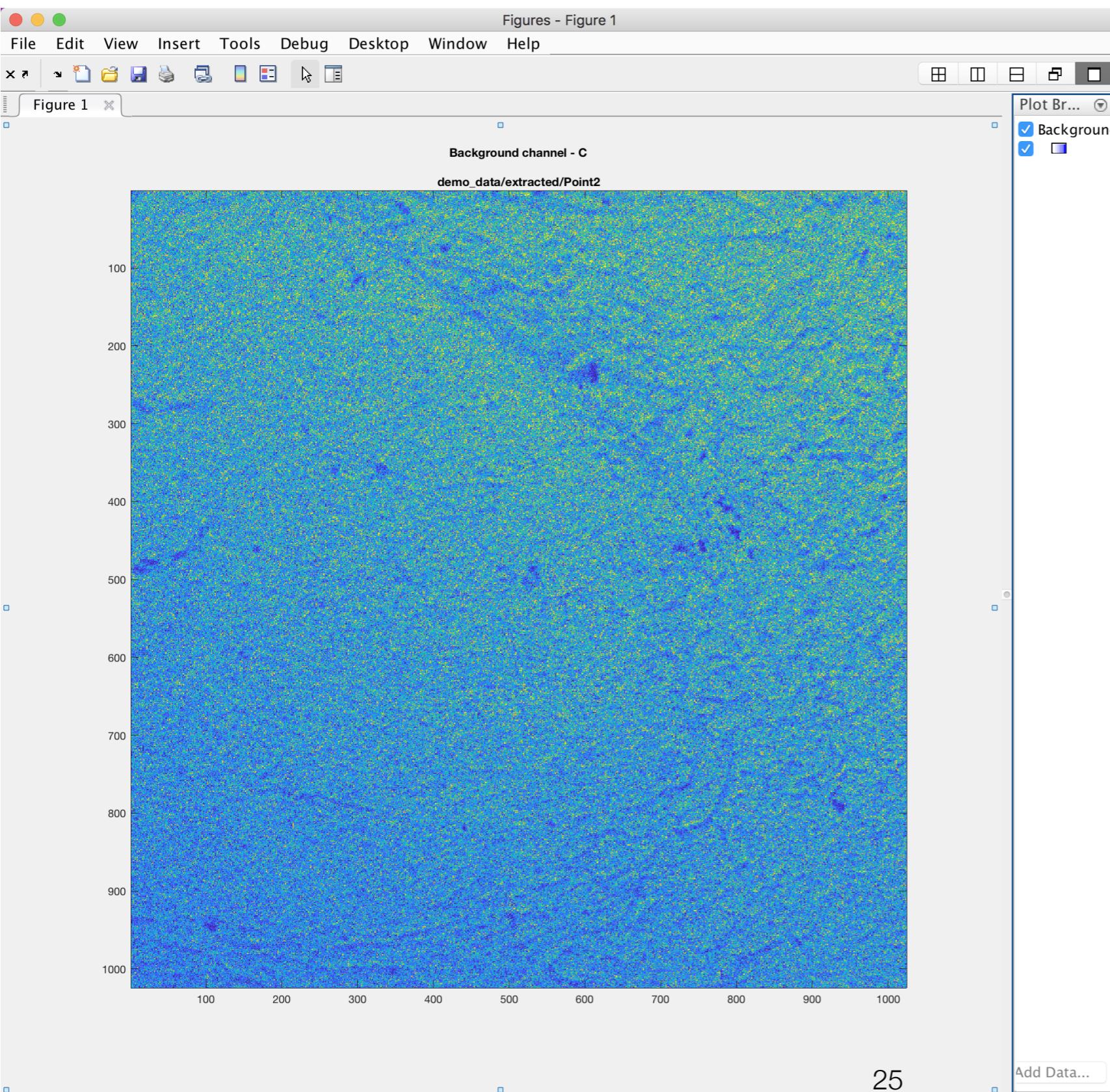
**Right Window:** The title bar says 'Background Removal'. This window is more detailed and includes the following sections:

- Currently loaded background point and channel:** Shows 'C' as the selected channel.
- Remove Parameters:** Includes fields for 'radius' (1), 'thshold' (0.2), and 'bg\_cap' (10). There are also 'Reuse figure' and 'Delete' buttons.
- Parameters:** Shows 'demo\_data/extracted/Point2' as the current file. It has a dropdown menu with the value '2'. It includes 'Delete' and 'Reload' buttons.
- Background Removal:** Contains buttons for 'Load Params' and 'Remove Background'. To its right, there are labels for 'Gaussian radius', 'Threshold', 'Background Cap', and 'Evaluation Cap' with their respective input fields.

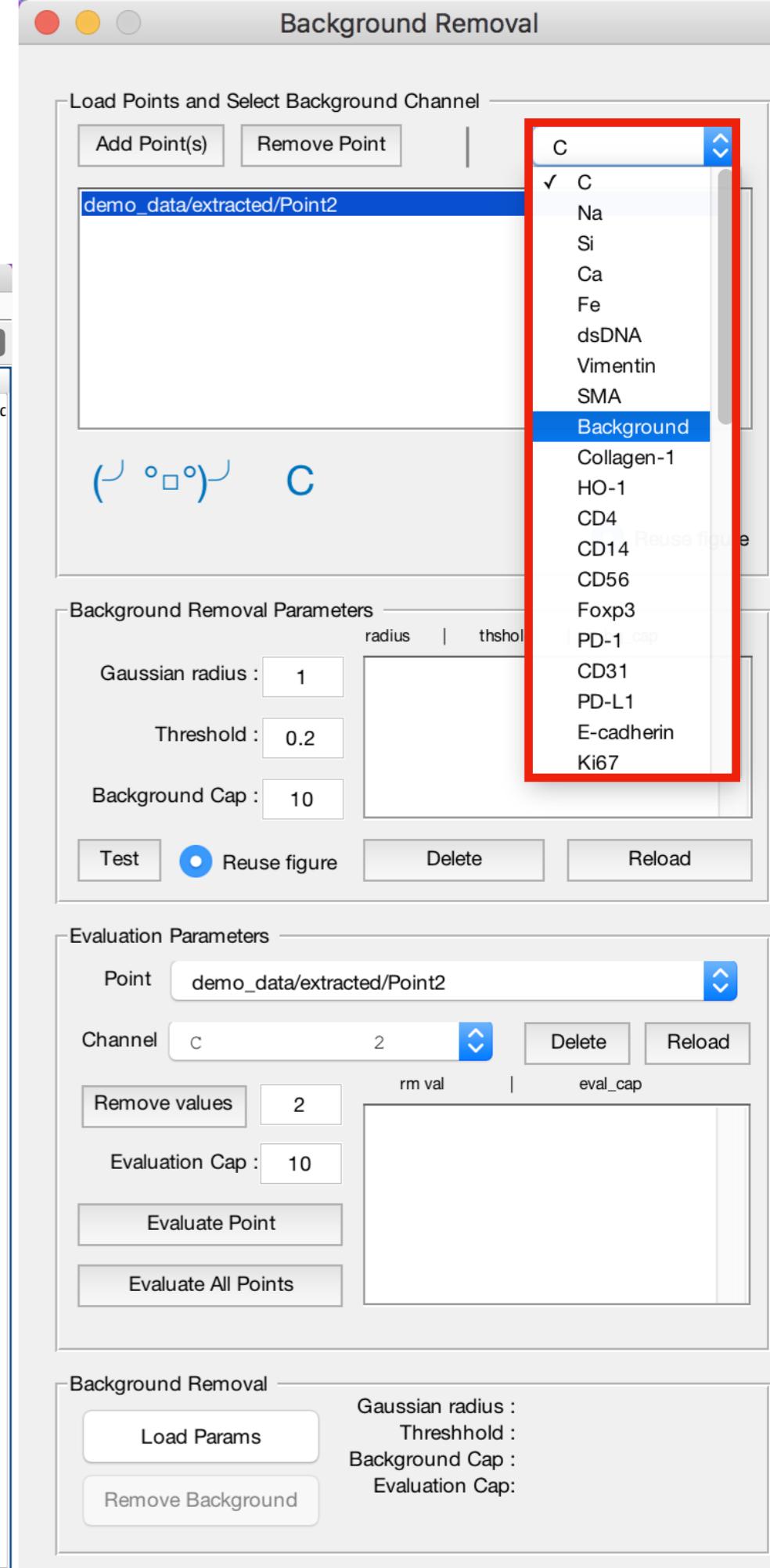
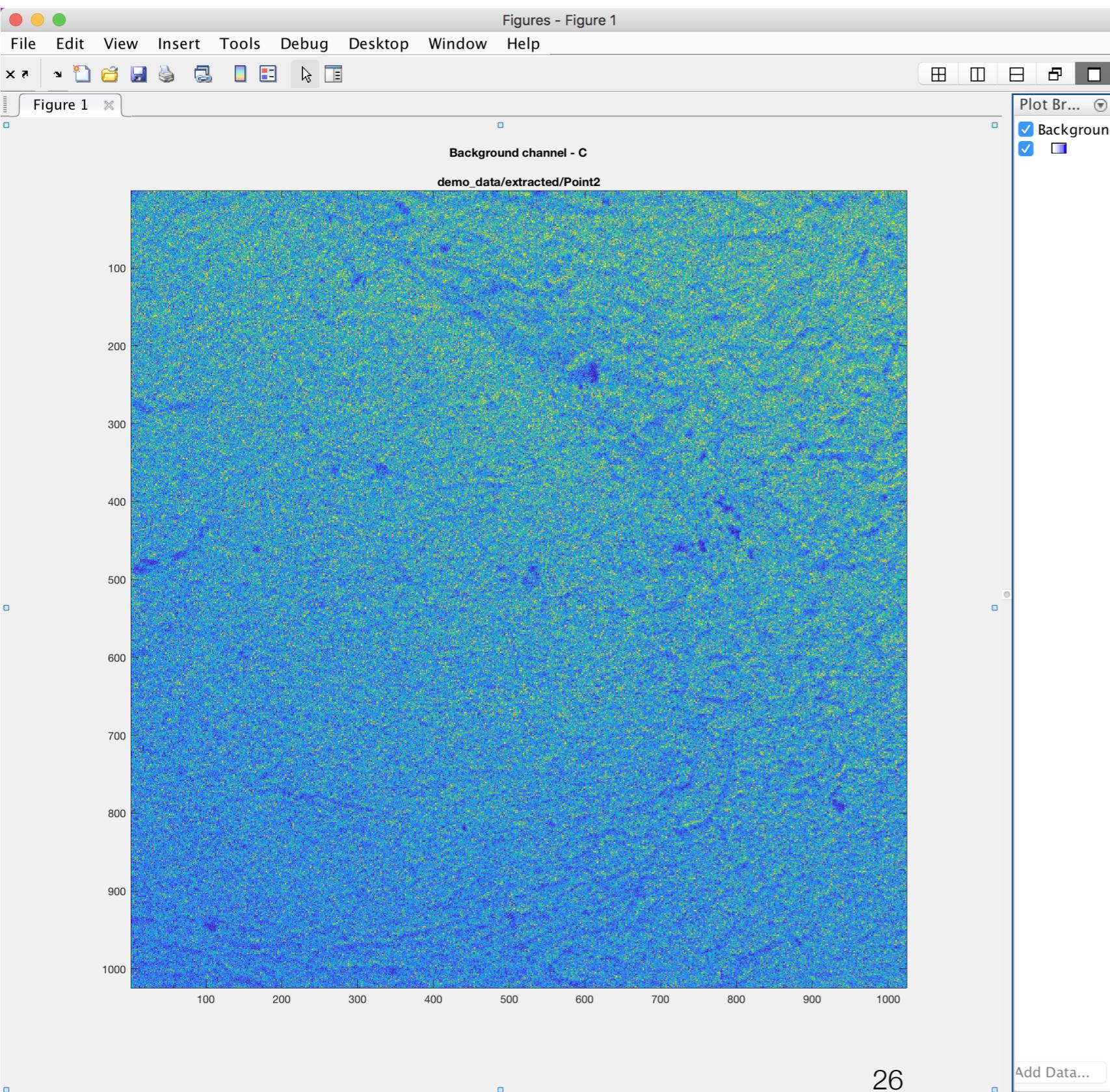
# We can view different channels for creating the background removal mask



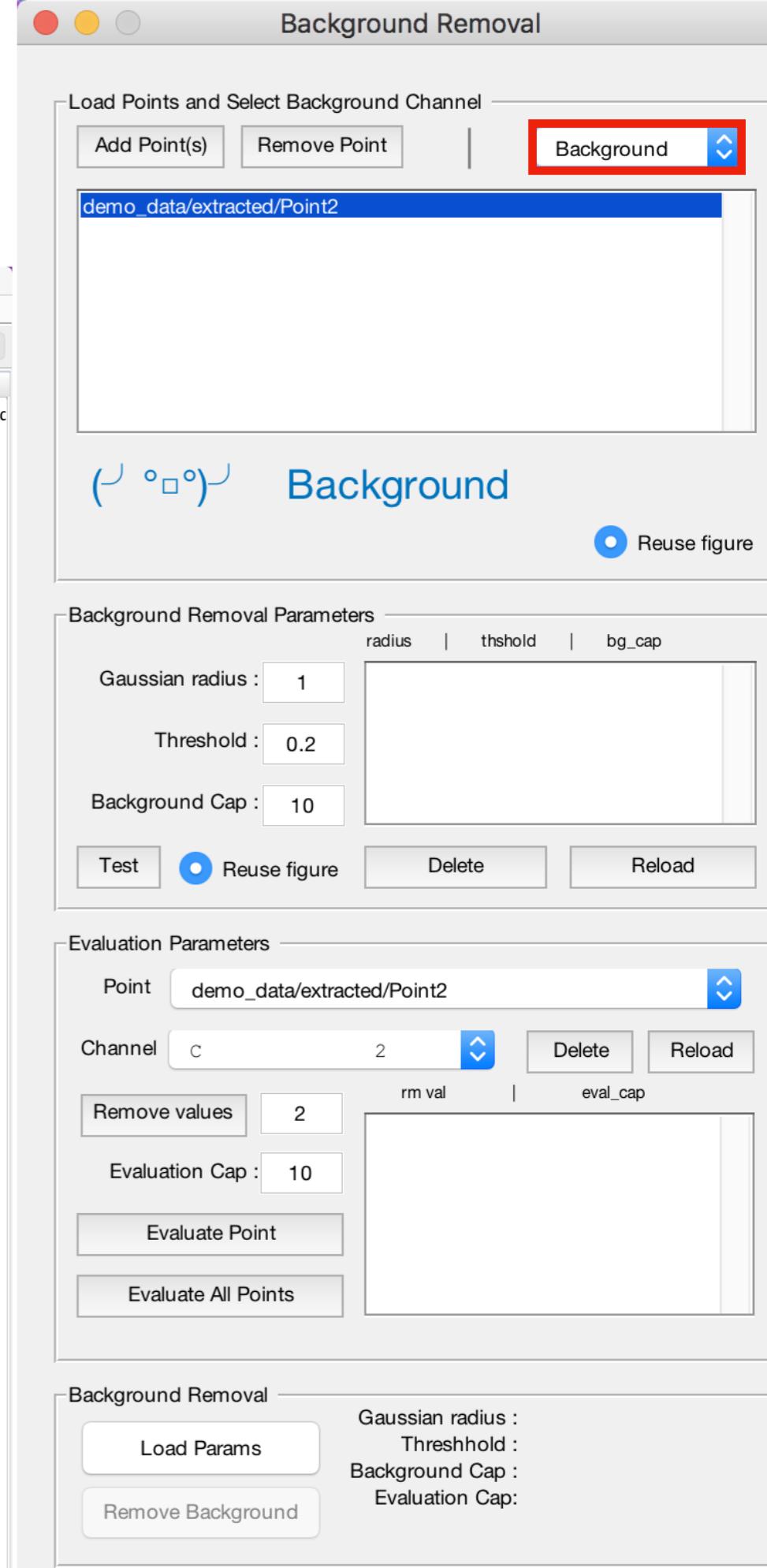
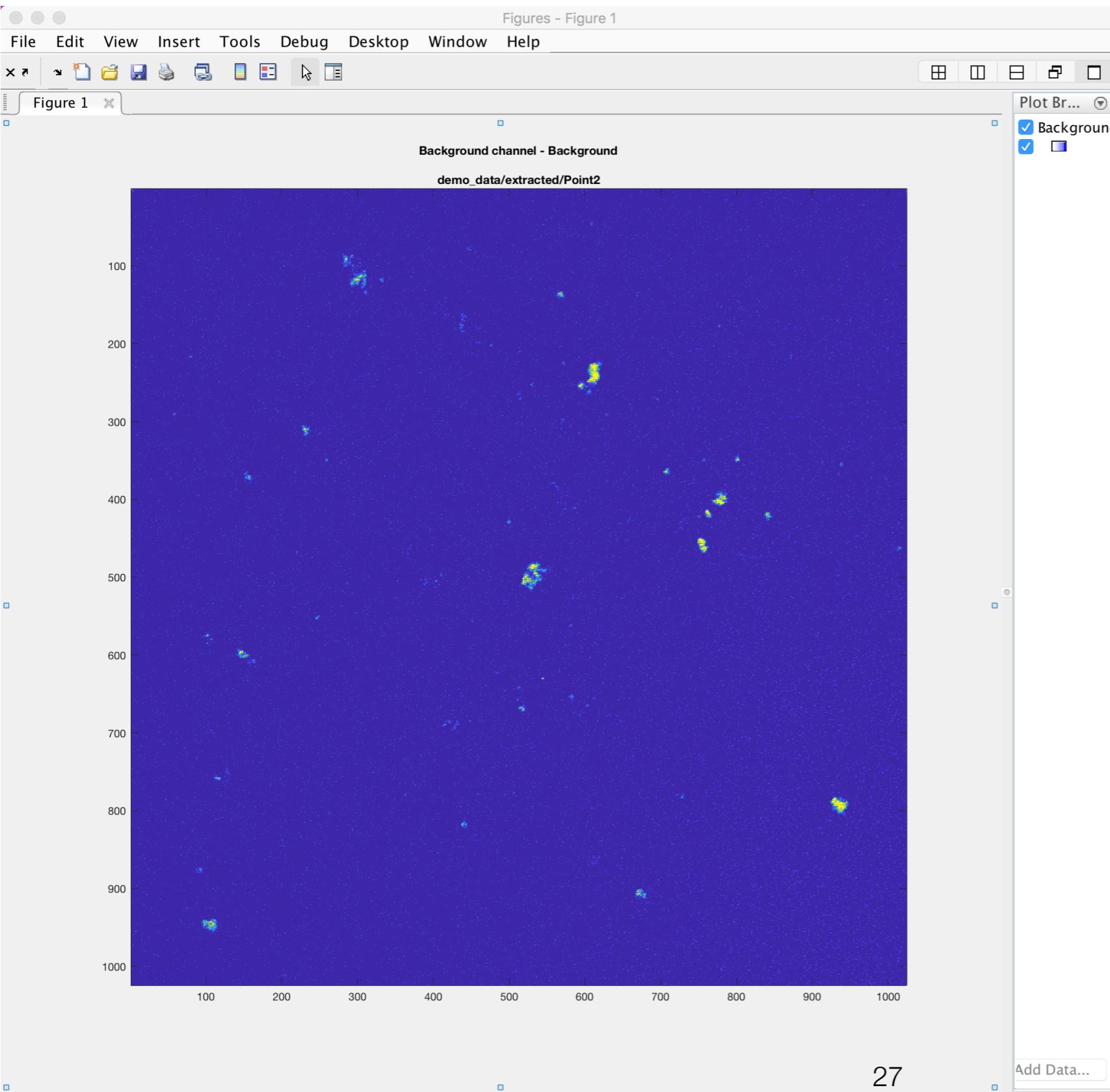
# We can view different channels for creating the background removal mask



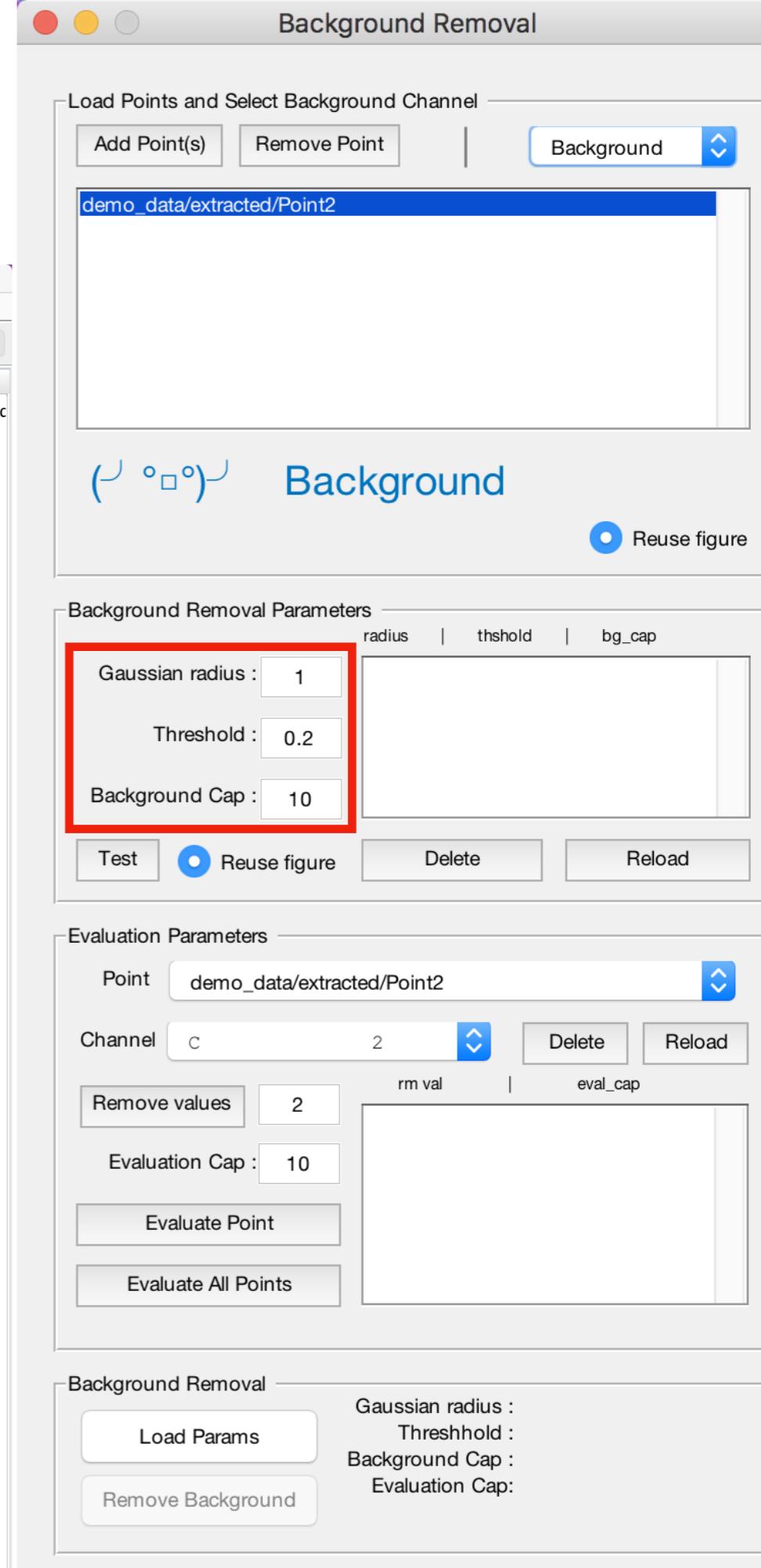
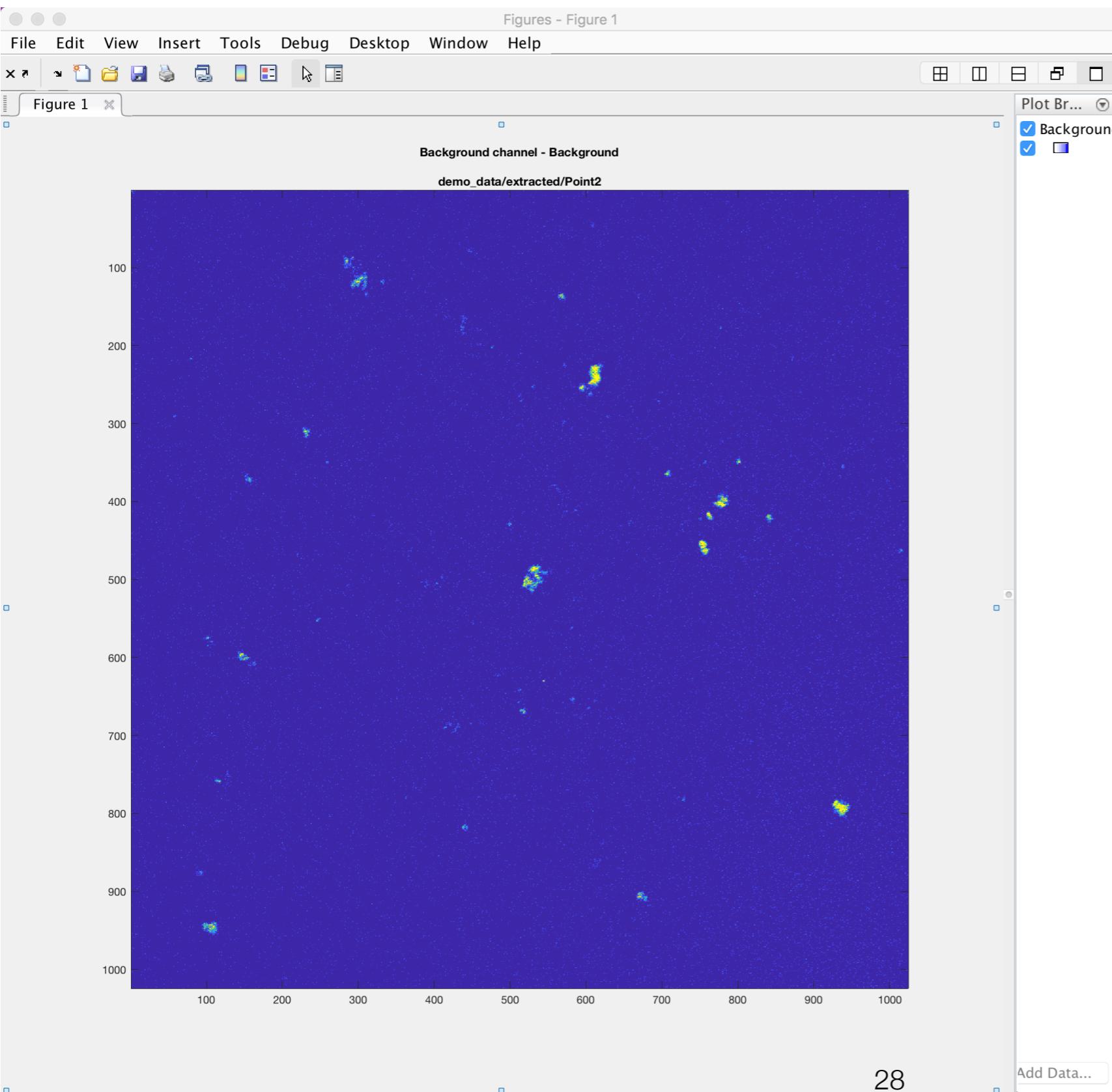
# We can view different channels for creating the background removal mask



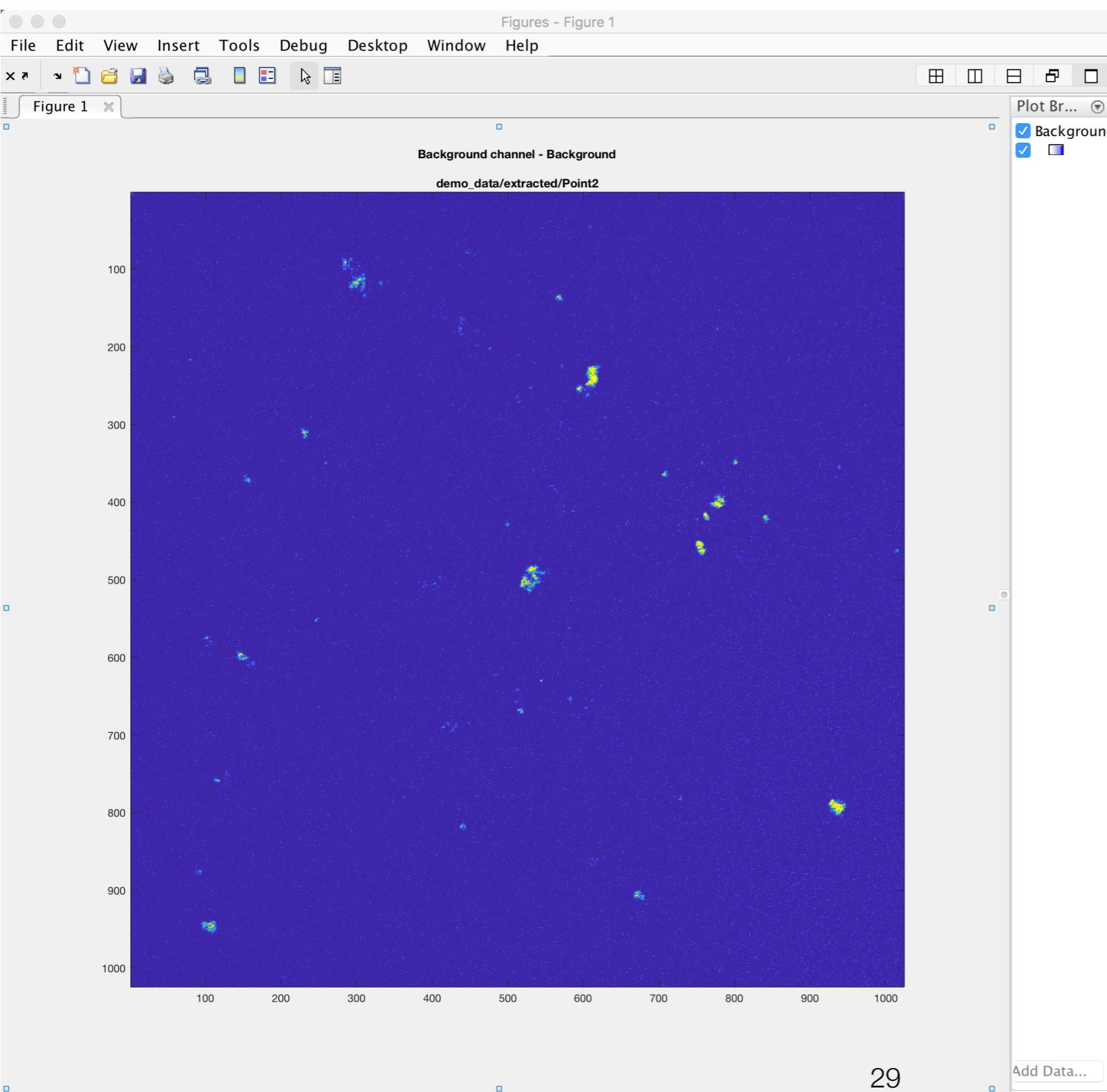
# We can view different channels for creating the background removal mask



# We can view the mask generated by these parameters...



# On this channel...



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point  Background

demo\_data/extracted/Point2

(° ° °) **Background**  Reuse figure

Background Removal Parameters

Gaussian radius : 1

Threshold : 0.2

Background Cap : 10

Test  Reuse figure Delete Reload

Evaluation Parameters

Point demo\_data/extracted/Point2

Channel C 2

Remove values 2 rm val eval\_cap

Evaluation Cap : 10

Evaluate Point

Evaluate All Points

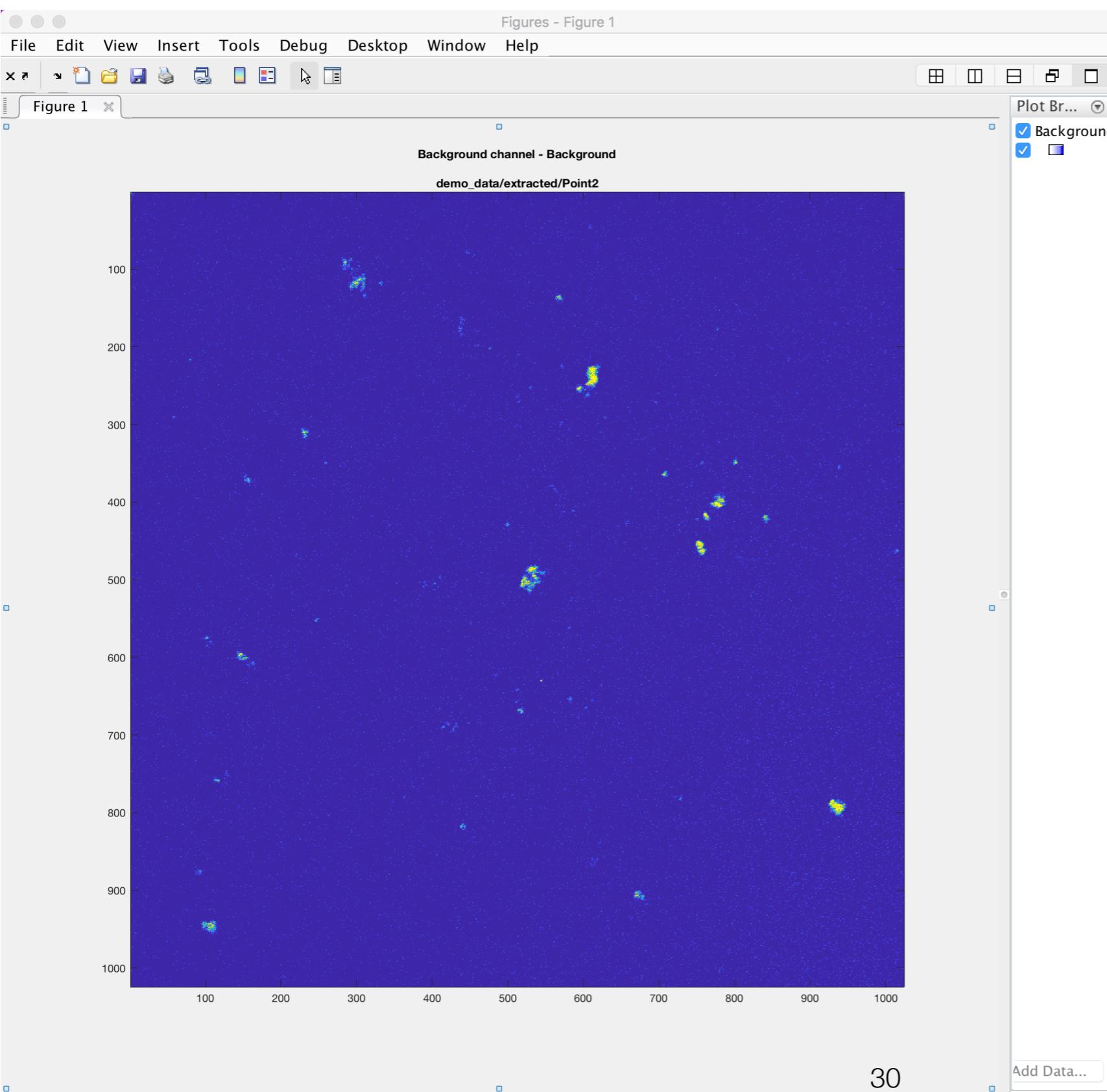
Background Removal

Load Params Gaussian radius :

Remove Background Threshold :

Background Cap : Evaluation Cap:

# For this point...



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point

demo\_data/extracted/Point2

(° ° °) Background  Reuse figure

Background Removal Parameters

radius | thshold | bg\_cap

Gaussian radius : 1

Threshold : 0.2

Background Cap : 10

Reuse figure

Evaluation Parameters

Point demo\_data/extracted/Point2

Channel C 2

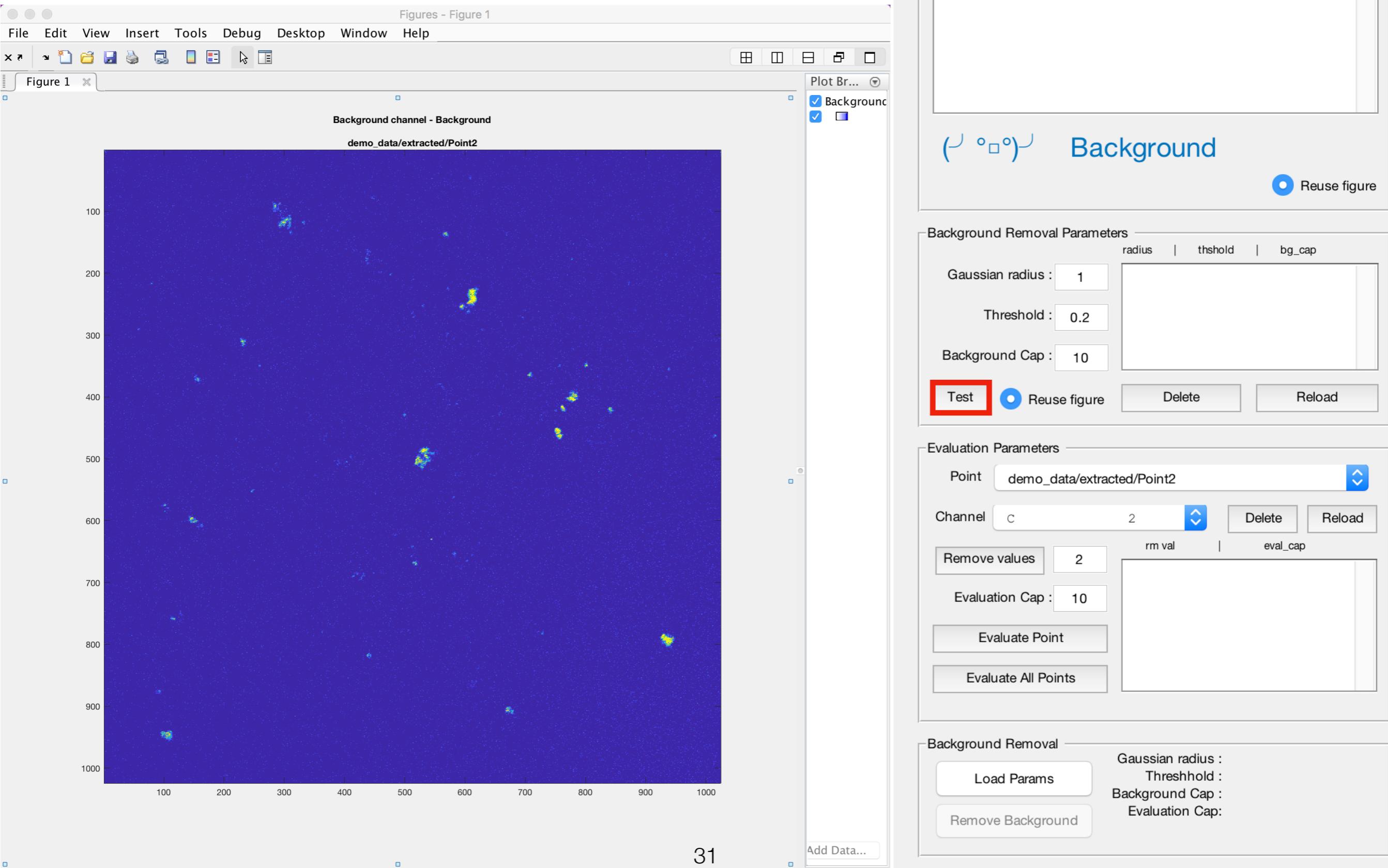
Remove values 2

Evaluation Cap : 10

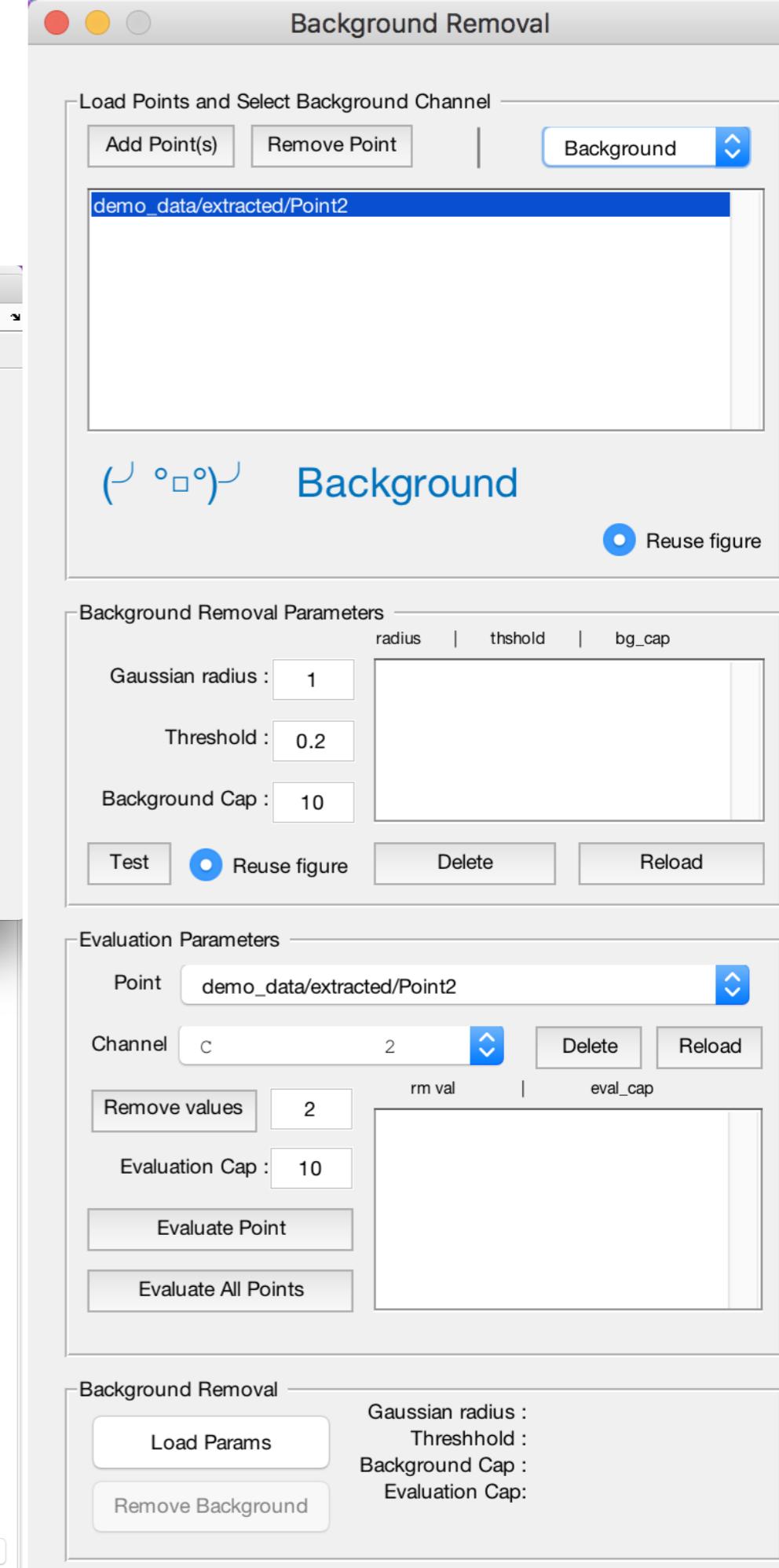
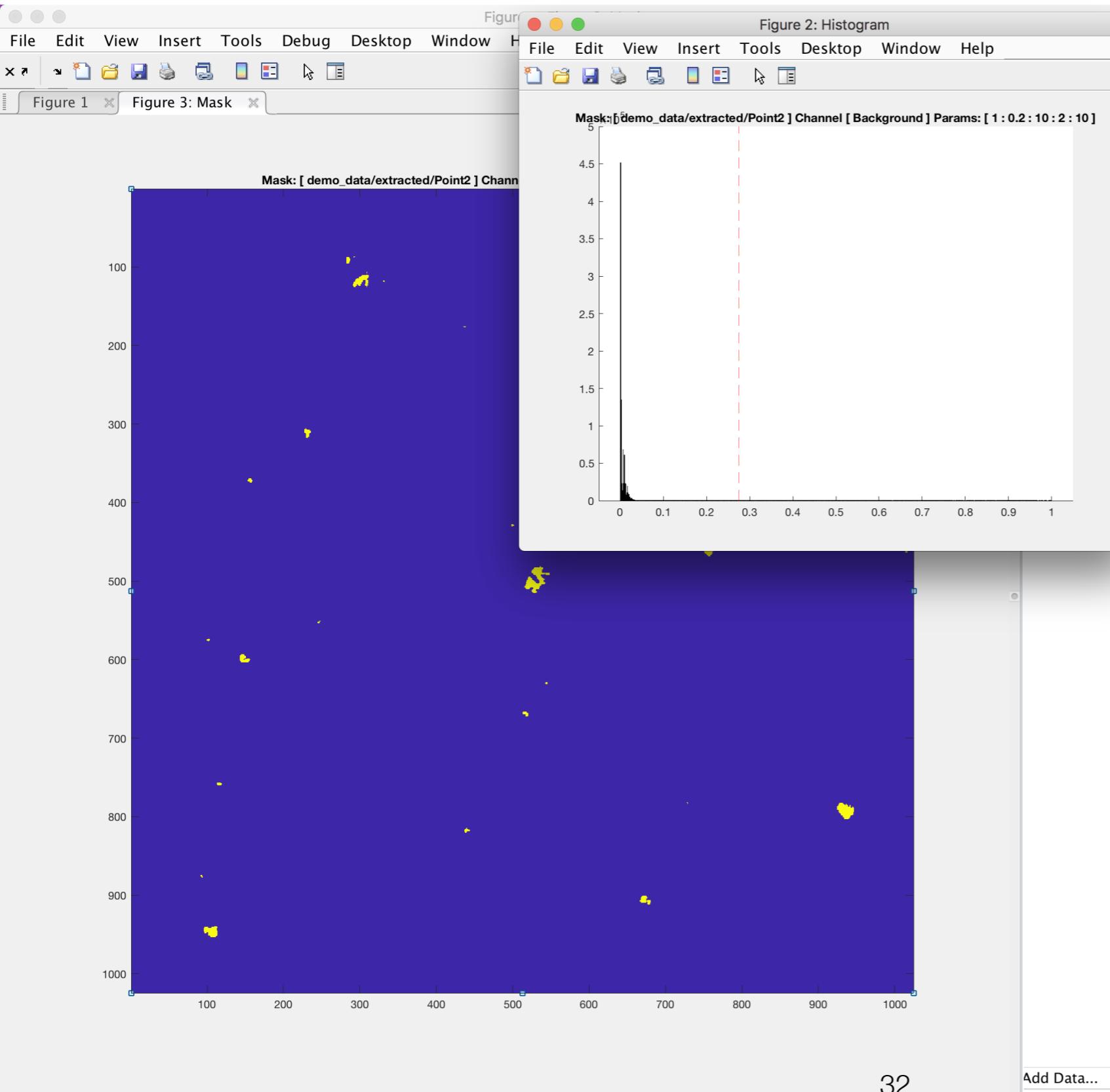
Background Removal

Gaussian radius :   
Threshold :   
Background Cap :   
Evaluation Cap:

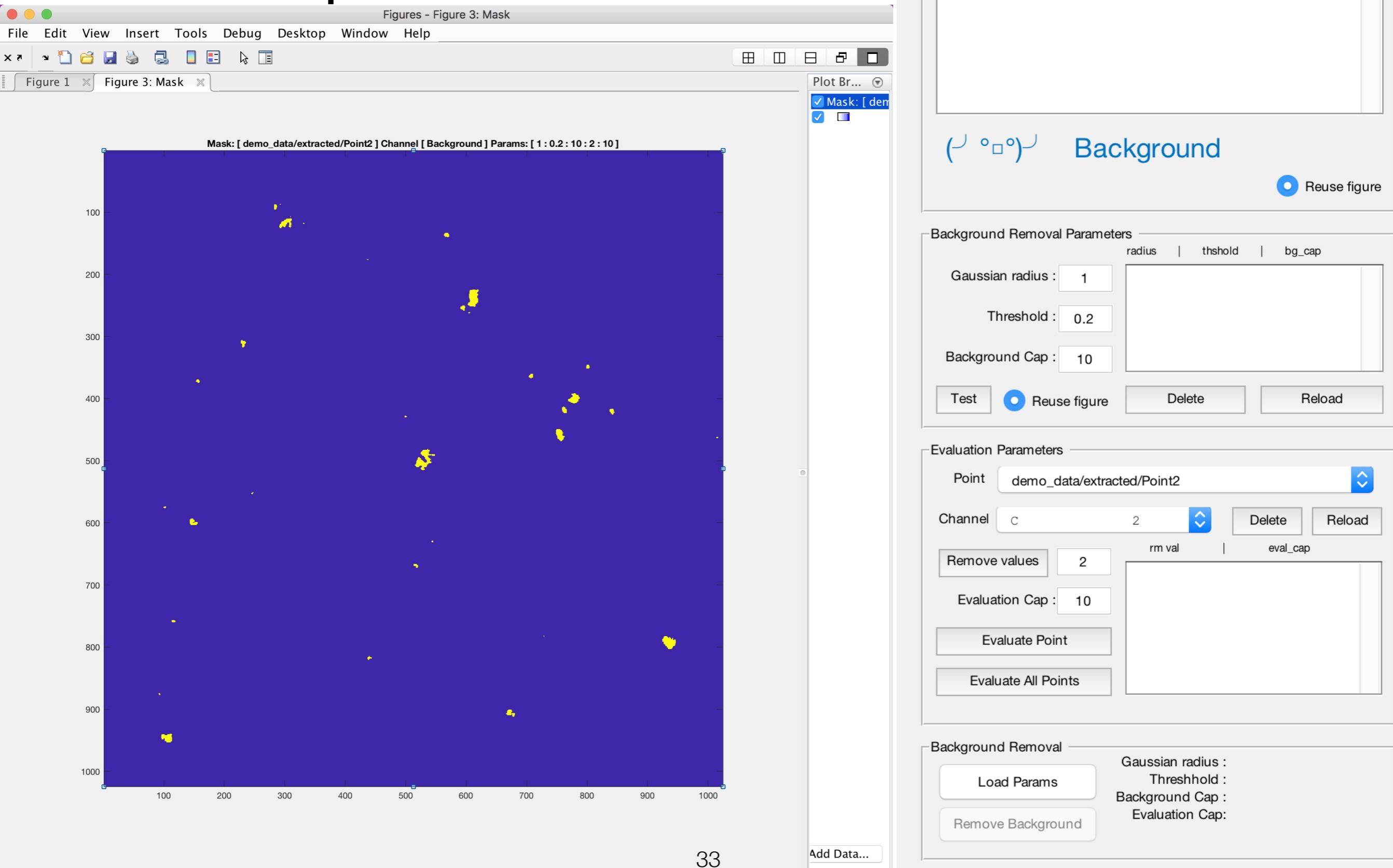
# By clicking the “Test” button



This displays an image of the generated mask, as well as a histogram of counts for the raw channel



# The mask is a binary image which can be modified by choosing different parameters



We'll load another point to look at how each parameter effects the mask

The screenshot shows the 'Background Removal' software interface with three main panels:

- Load Points and Select Background Channel:** A panel at the top left with buttons for 'Add Point(s)', 'Remove Point', and a dropdown menu set to 'Background'. It lists loaded points: 'demo\_data/extracted/Point2' and 'demo\_data/extracted/Point4'.
- Background Removal Parameters:** A panel in the center containing three sets of sliders:
  - Gaussian radius : 1
  - Threshold : 0.2
  - Background Cap : 10
 To the right is a table of parameters:
 

	radius	thshold	bg_cap
1	0.2	10	
1	0.09	10	
.1	0.2	10	
.1	0.09	10	
1	0.2	10	
.1	0.2	10	
.1	0.09	10	

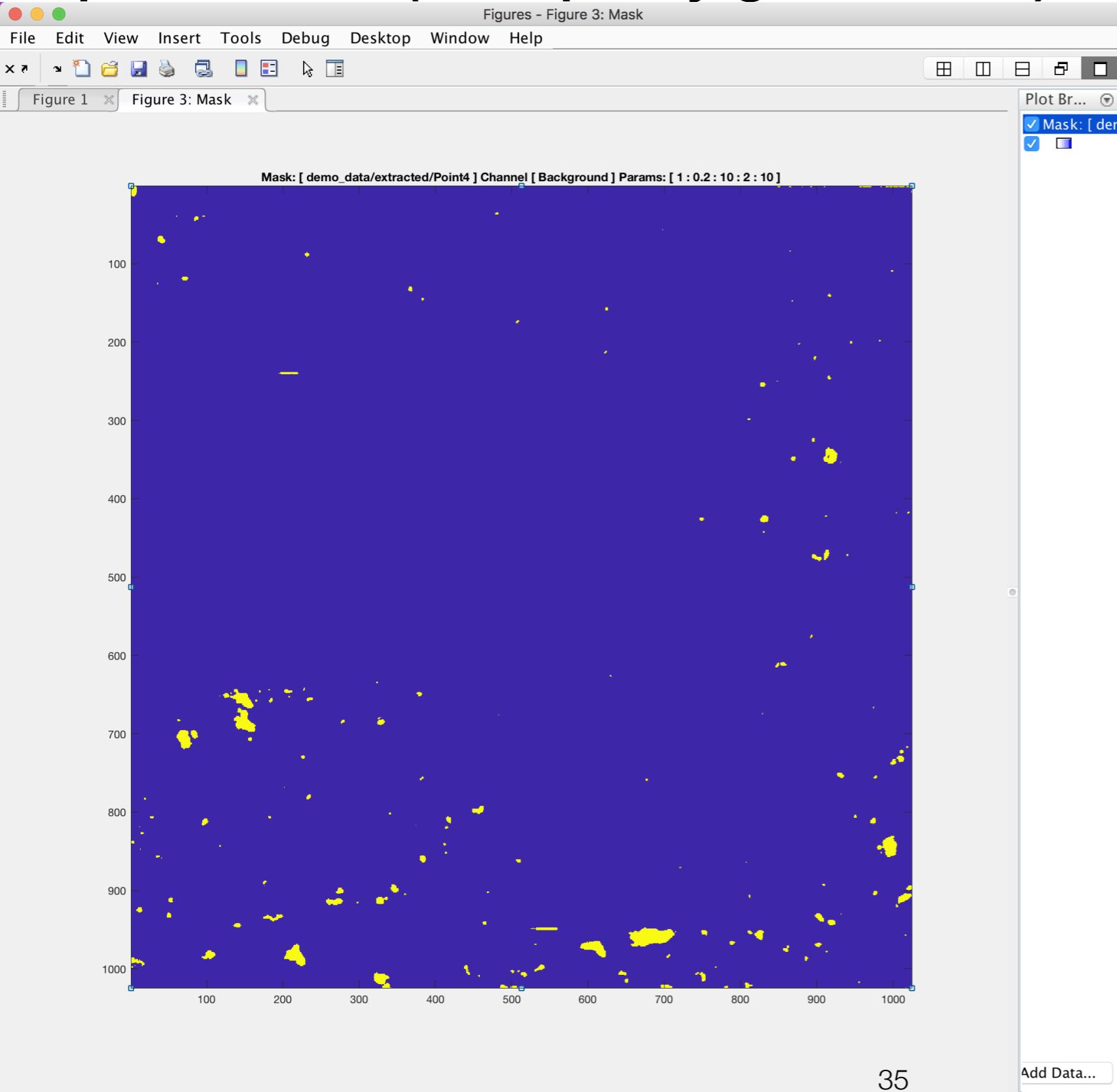
 Buttons at the bottom include 'Test', 'Reuse figure' (which is checked), 'Delete', and 'Reload'.
- Evaluation Parameters:** A panel at the bottom left with a dropdown for 'Point' set to 'demo\_data/extracted/Point4', a dropdown for 'Channel' set to 'CD45', and a table of evaluation values:
 

rm val	eval_cap
2	5
2	8
2	10

 Buttons include 'Evaluate Point' and 'Evaluate All Points'.
- Background Removal:** A panel at the bottom right with buttons for 'Load Params' and 'Remove Background'. To its right, the current parameter settings are listed:
 

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

# Here's a mask generated using the Background channel and the default parameters (it's a pretty good mask)



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point

demo\_data/extracted/Point2  
demo\_data/extracted/Point4

(° ° °) Background  Reuse figure

Background Removal Parameters

radius | thshold | bg\_cap

Gaussian radius : 1  
1 0.2 10  
1 0.09 10  
.1 0.2 10  
.1 0.09 10  
1 0.2 10  
.1 0.2 10  
.1 0.09 10

Threshold : 0.2

Background Cap : 10

Reuse figure

Evaluation Parameters

Point demo\_data/extracted/Point4

Channel CD45 2

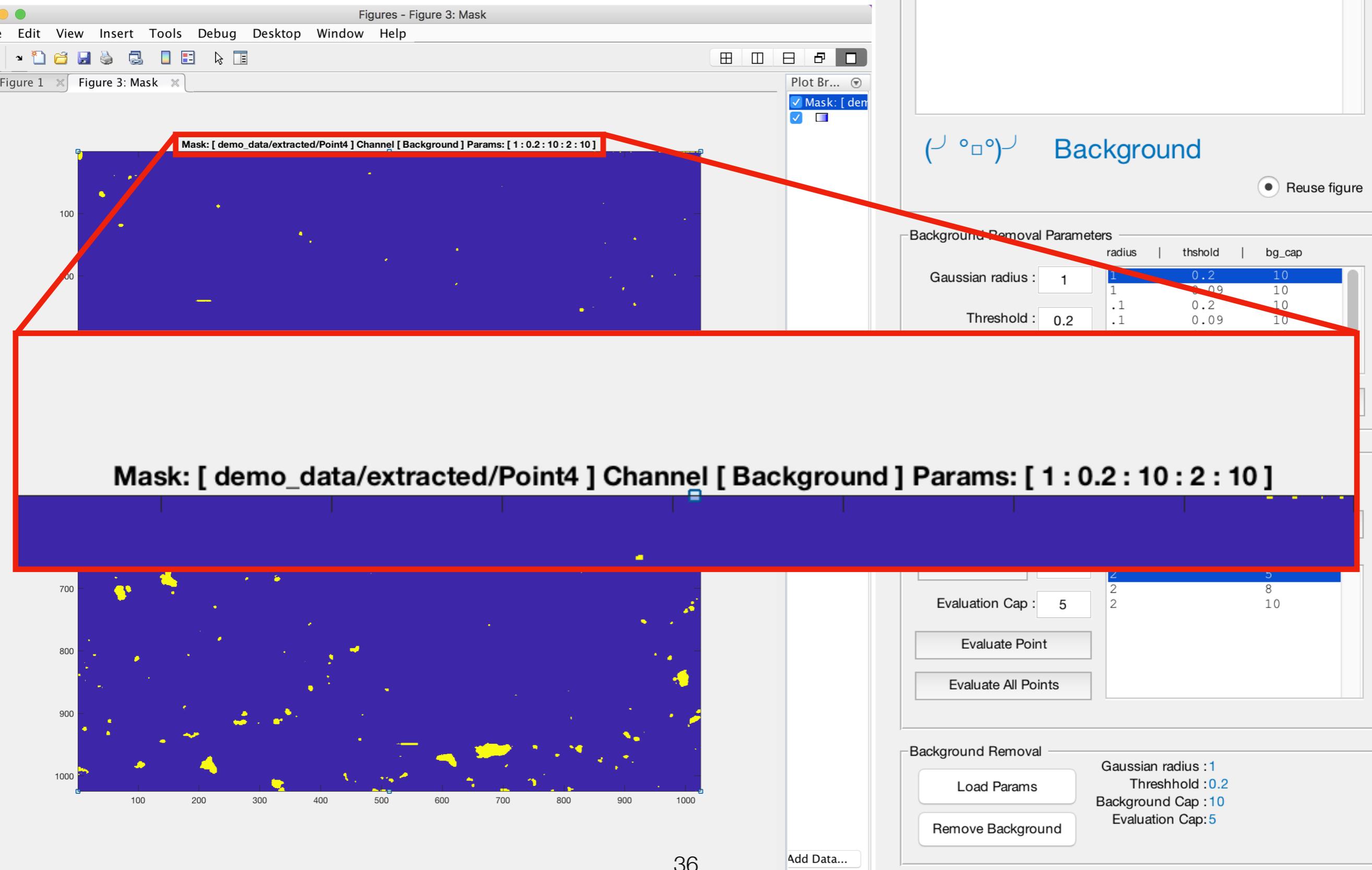
Remove values 2

Evaluation Cap : 5

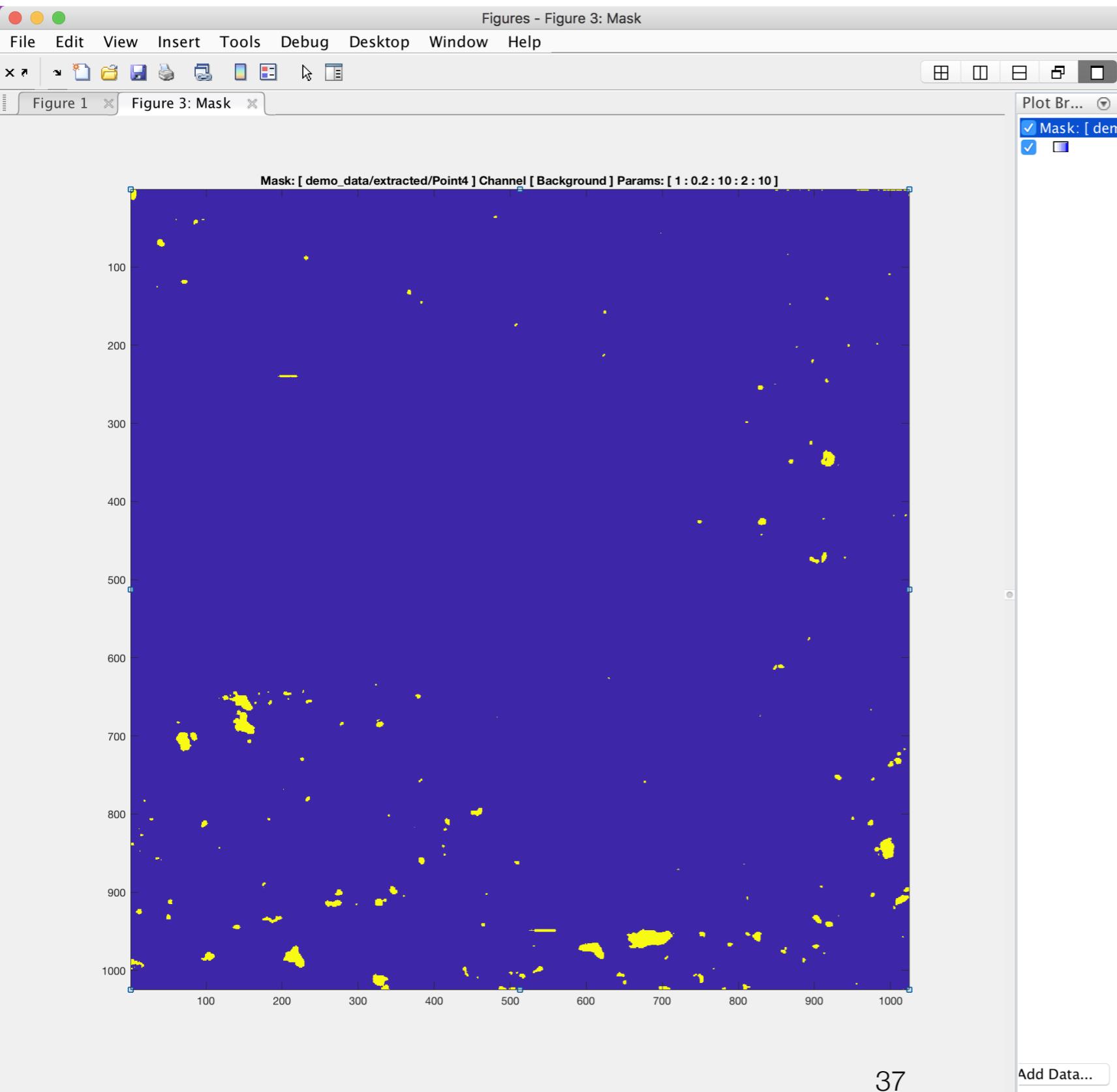
Background Removal

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

Notice that the parameters used to generate the mask are in the image title



# The default mask



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point

demo\_data/extracted/Point2  
demo\_data/extracted/Point4

( ) **Background**

Reuse figure

Background Removal Parameters

radius	thshold	bg_cap
1	0.2	10
1	0.09	10
.1	0.2	10
.1	0.09	10
1	0.2	10
.1	0.2	10
.1	0.09	10

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10

Reuse figure

Evaluation Parameters

Point: demo\_data/extracted/Point4

Channel: CD45

rm val	eval_cap
2	5
2	8
2	10

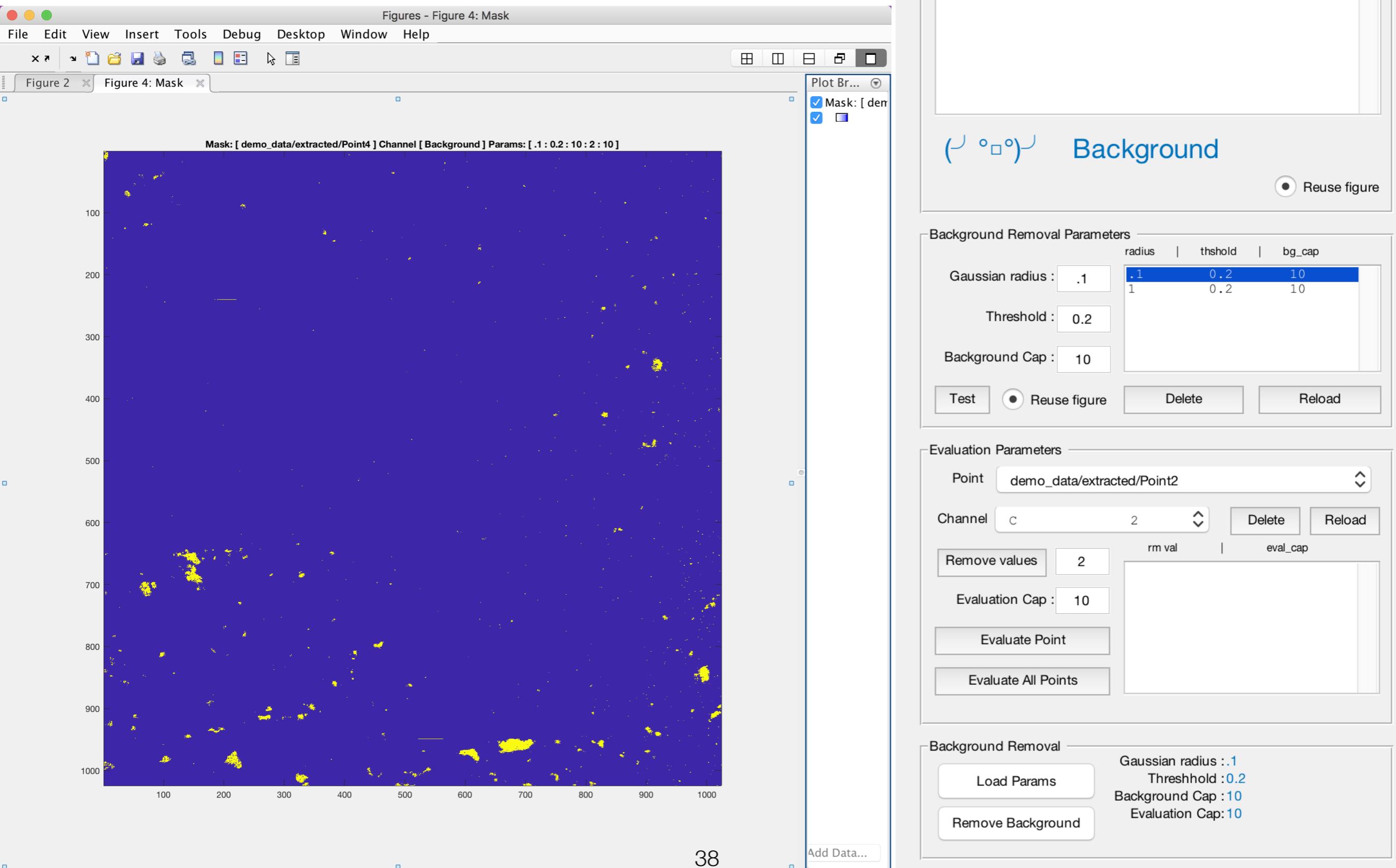
Remove values : 2  
Evaluation Cap : 5

Background Removal

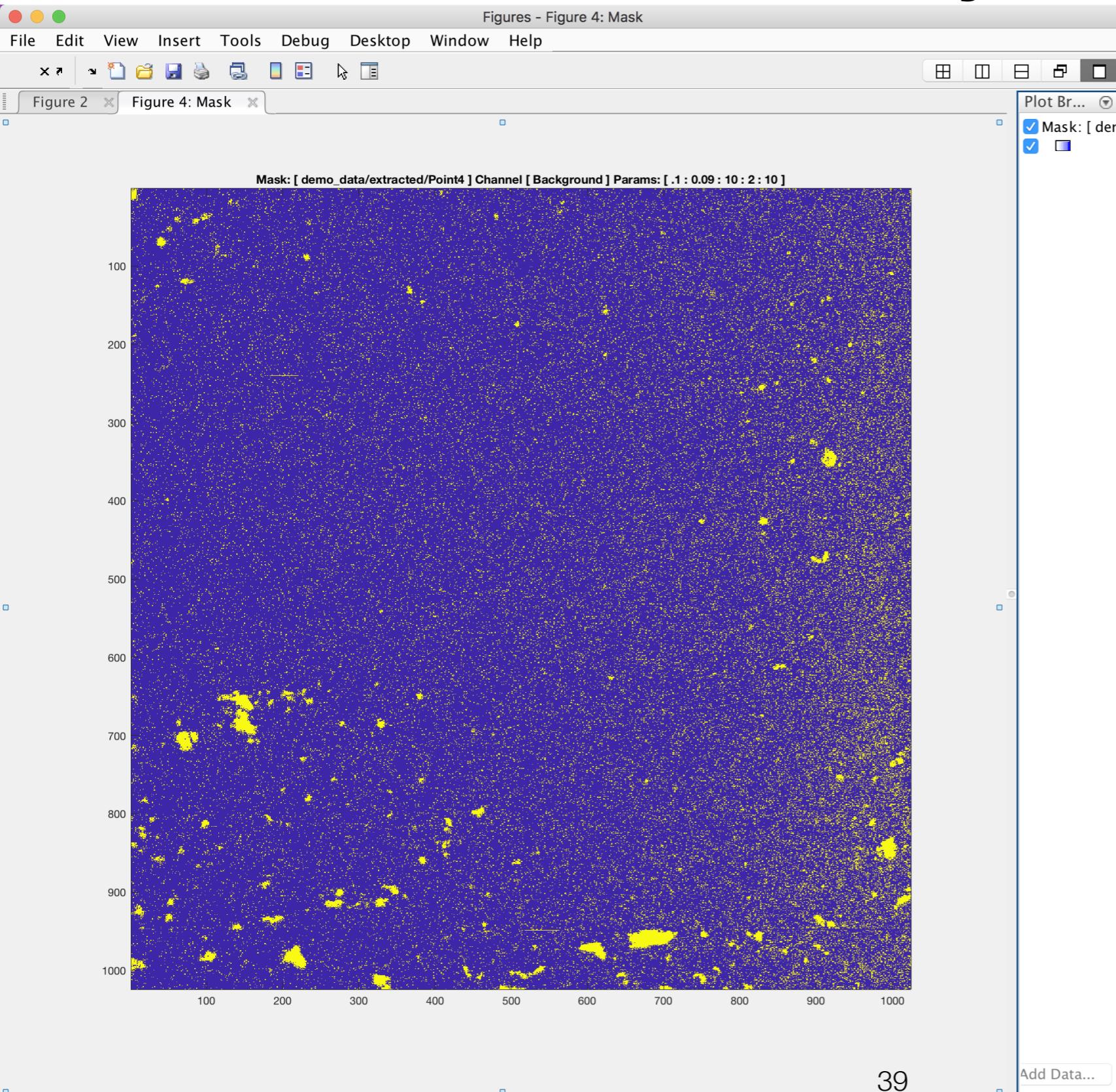
Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

Add Data...

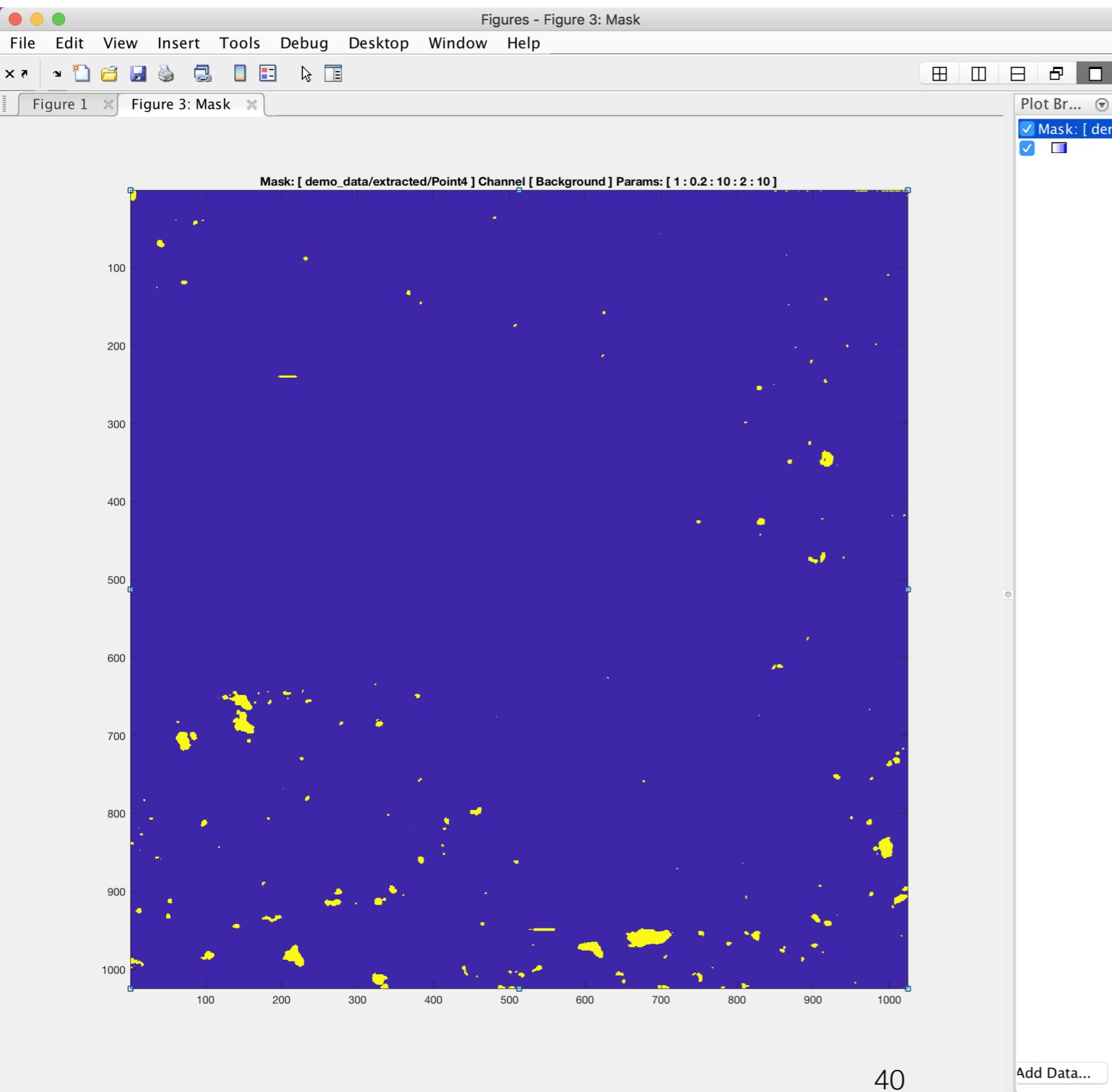
# Setting the Gaussian radius lower makes the mask less smooth



# Making the threshold lower makes the mask noisy



# We can evaluate the parameters we've chosen on other points and channels



Load Points and Select Background Channel

Add Point(s) Remove Point Background

demo\_data/extracted/Point2 demo\_data/extracted/Point4

(° ° °) Background  Reuse figure

Background Removal Parameters

radius	thshold	bg_cap
1	0.2	10
1	0.09	10
.1	0.2	10
.1	0.09	10
1	0.2	10
.1	0.2	10
.1	0.09	10

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10

Test  Reuse figure Delete Reload

Evaluation Parameters

Point demo\_data/extracted/Point4

Channel CD45 2 Delete Reload

rm val	eval_cap
2	5
2	8
2	10

Remove values Evaluation Cap : 5 Evaluate Point Evaluate All Points

Background Removal

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

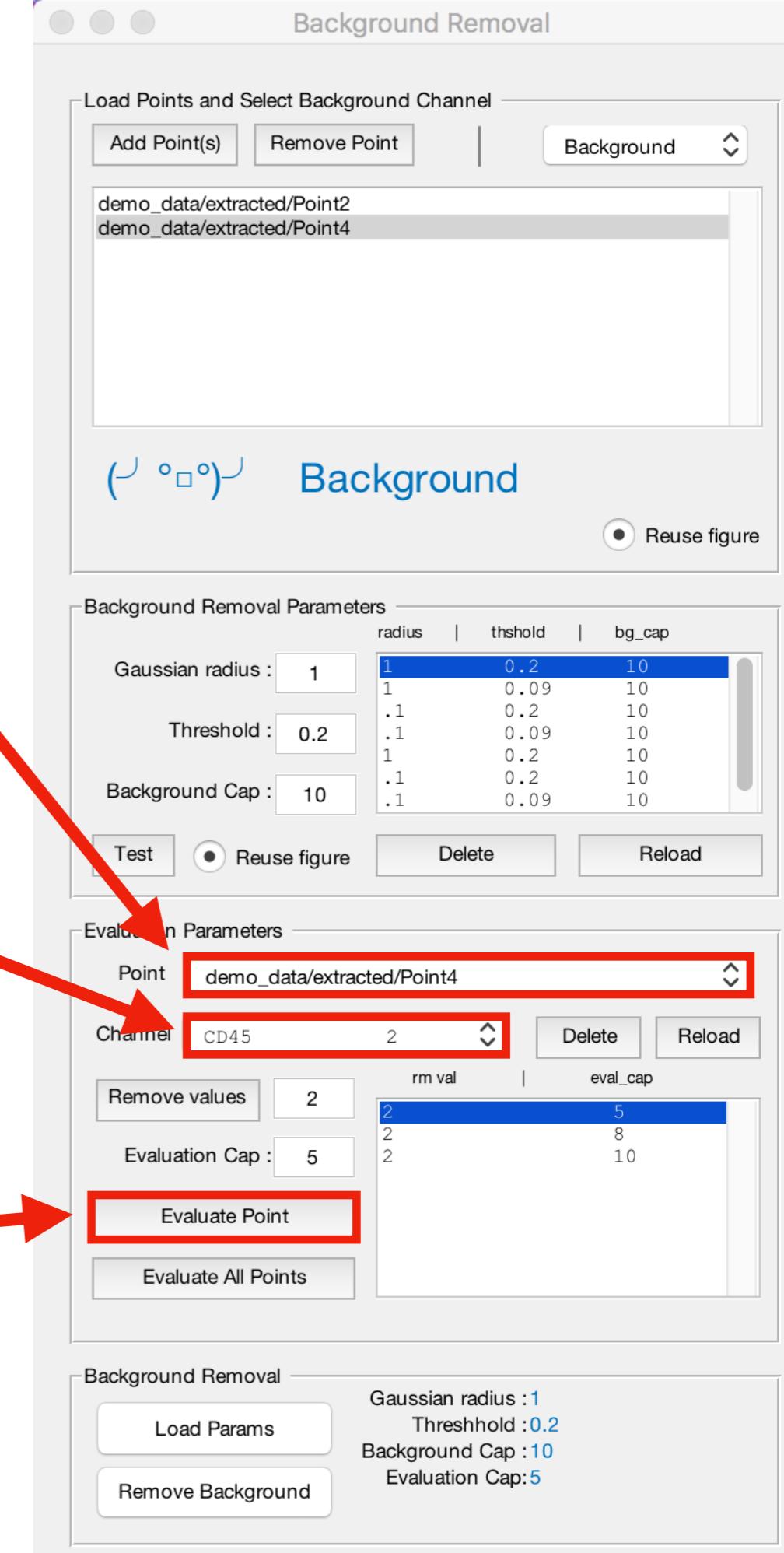
Load Params Remove Background Add Data...

We do this by selecting a Point and Channel, then clicking Evaluate Point

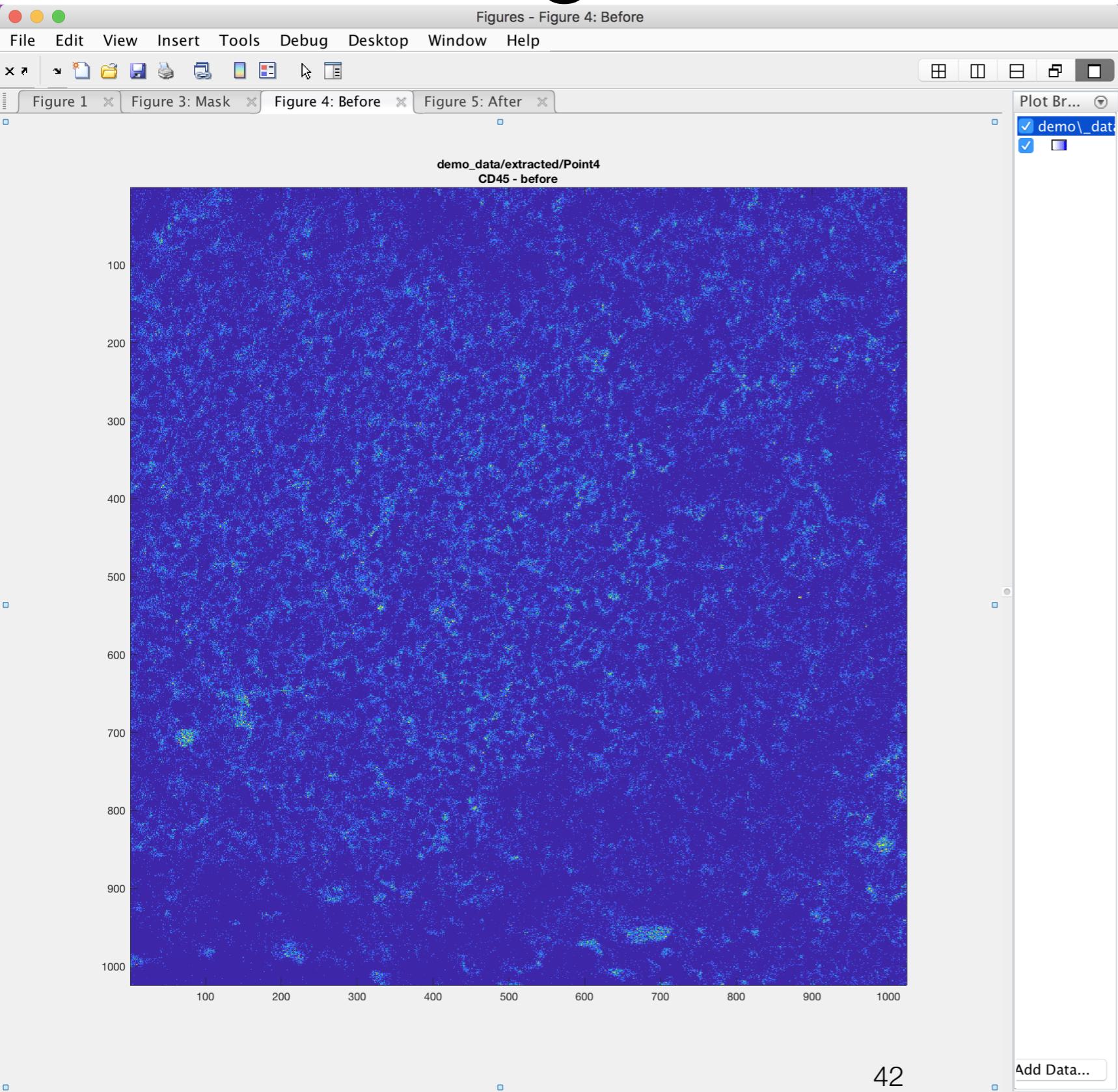
**Point to be evaluated**

**Channel to be evaluated**

**“Evaluate Point” button**



# This generates a “Before” image...



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point Background

demo\_data/extracted/Point2  
demo\_data/extracted/Point4

(<sup>o</sup> <sup>o</sup> <sup>o</sup>) **Background**

Reuse figure

Background Removal Parameters

	radius	thshold	bg_cap
Gaussian radius :	1	0.2	10
	1	0.09	10
	.1	0.2	10
	.1	0.09	10
	1	0.2	10
	.1	0.2	10
	.1	0.09	10

Threshold : 0.2

Background Cap : 10

Test Reuse figure Delete Reload

Evaluation Parameters

Point demo\_data/extracted/Point4

Channel CD45 2

Remove values 2

Evaluation Cap : 5

rm val eval\_cap

2	5
2	8
2	10

Evaluate Point

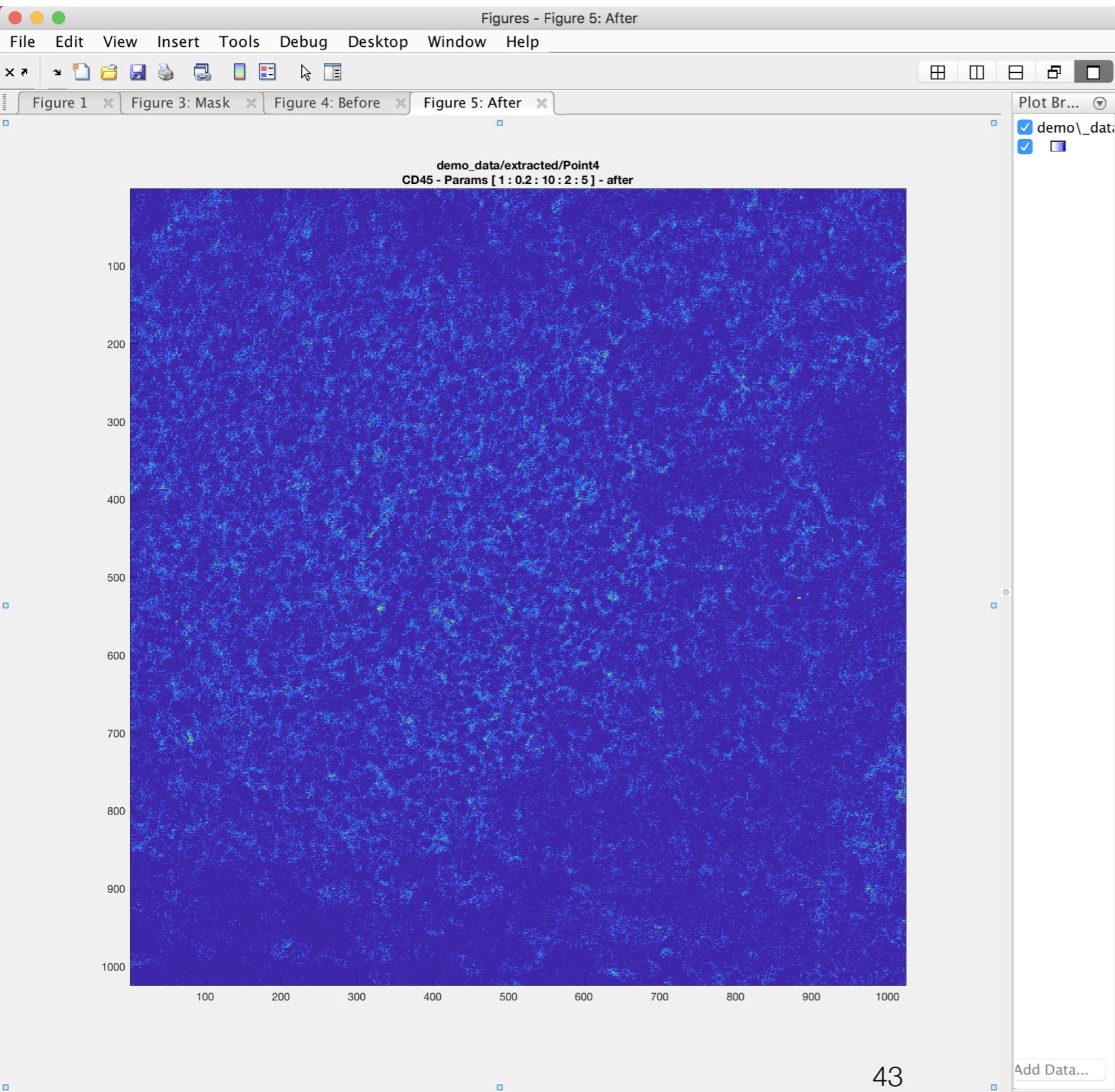
Evaluate All Points

Background Removal

Gaussian radius : 1  
Threshold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

Load Params Remove Background

# ... and an “After” image



Background Removal

Load Points and Select Background Channel

Add Point(s) Remove Point

demo\_data/extracted/Point2  
demo\_data/extracted/Point4

( ) **Background**  Reuse figure

Background Removal Parameters

	radius	thshold	bg_cap
Gaussian radius :	1	0.2	10
	1	0.09	10
Threshold :	.1	0.2	10
	.1	0.09	10
Background Cap :	1	0.2	10
	.1	0.2	10
	.1	0.09	10

Reuse figure

Evaluation Parameters

Point

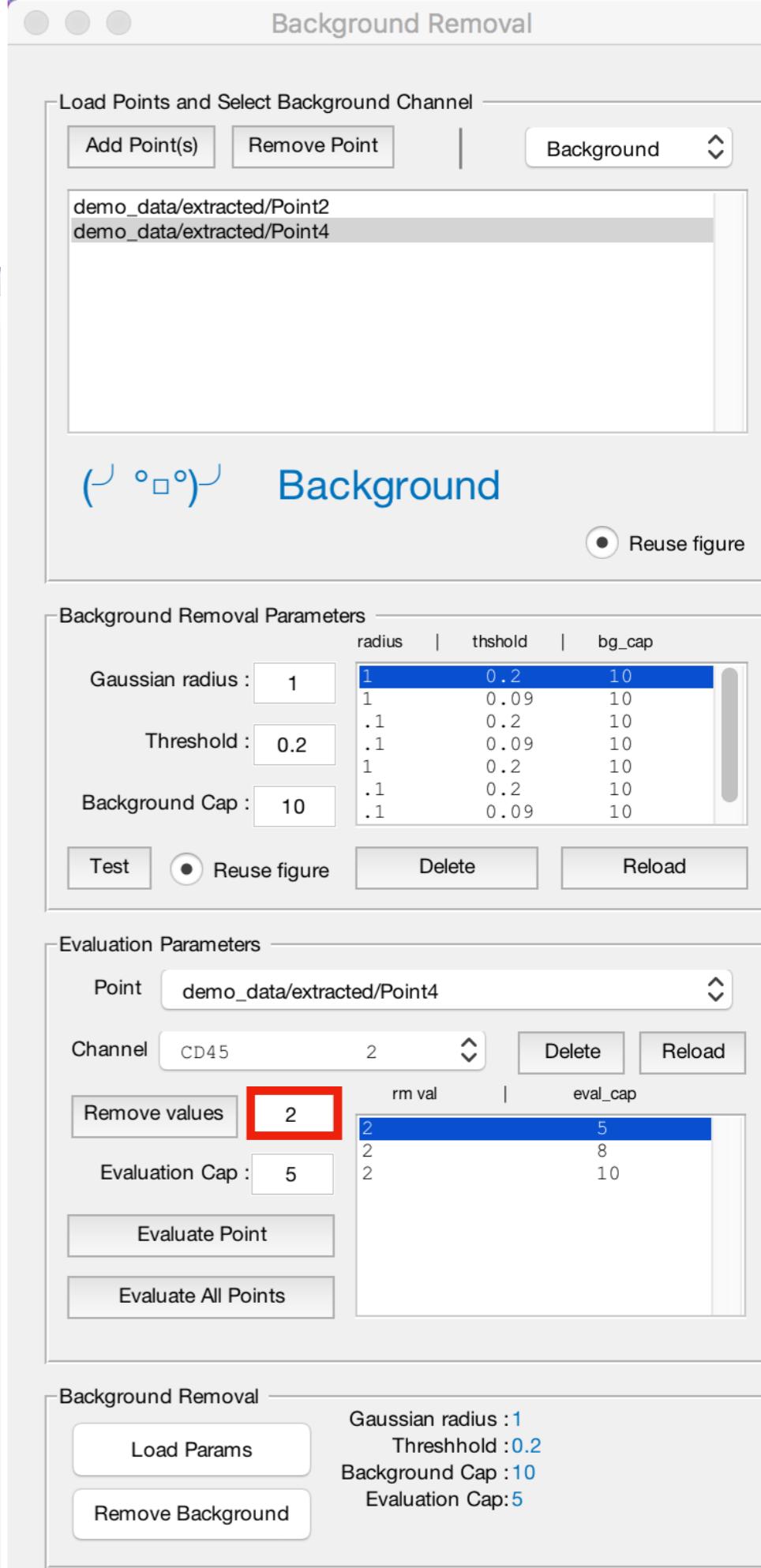
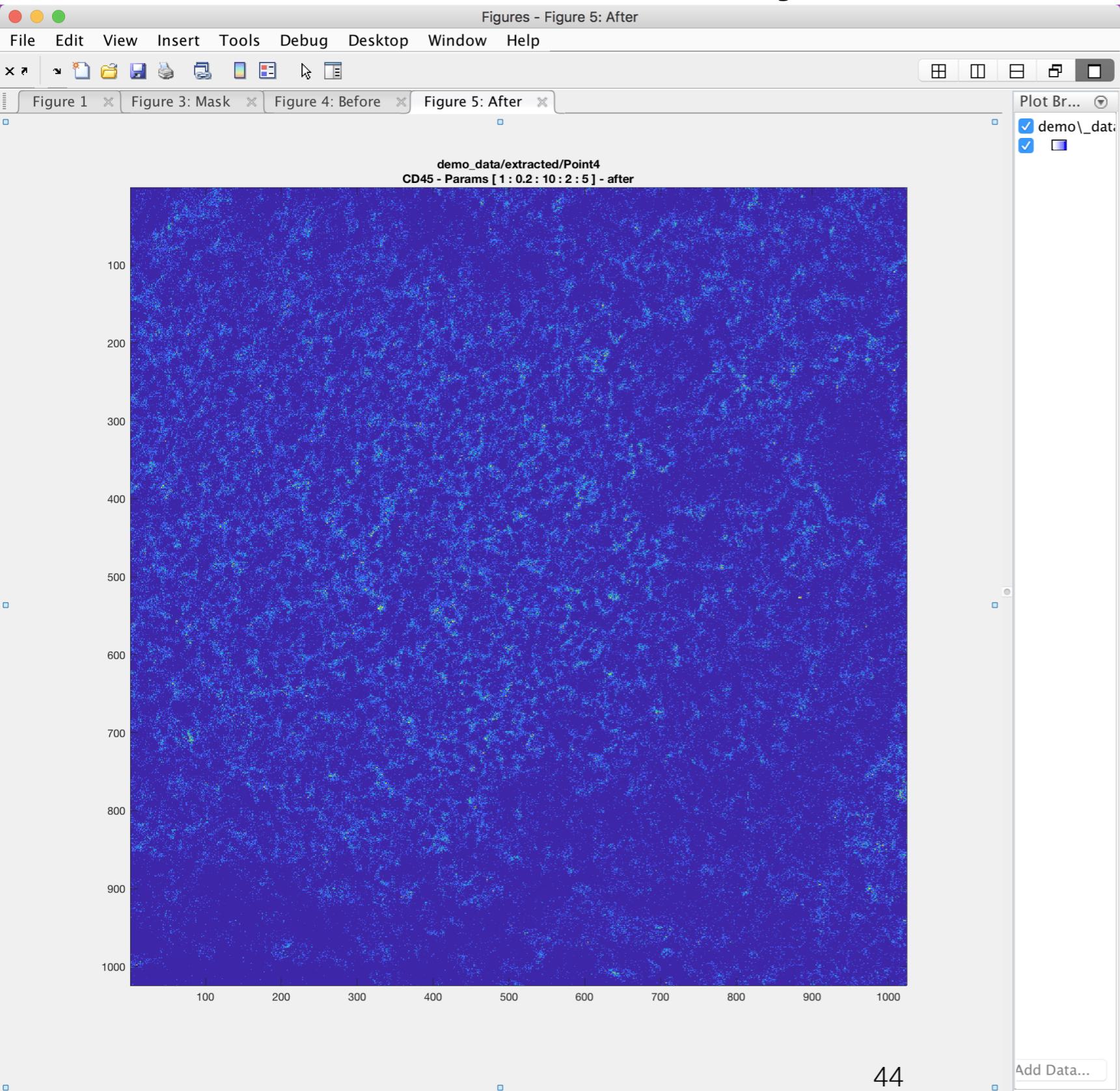
Channel CD45

rm val	eval_cap
2	5
2	8
2	10

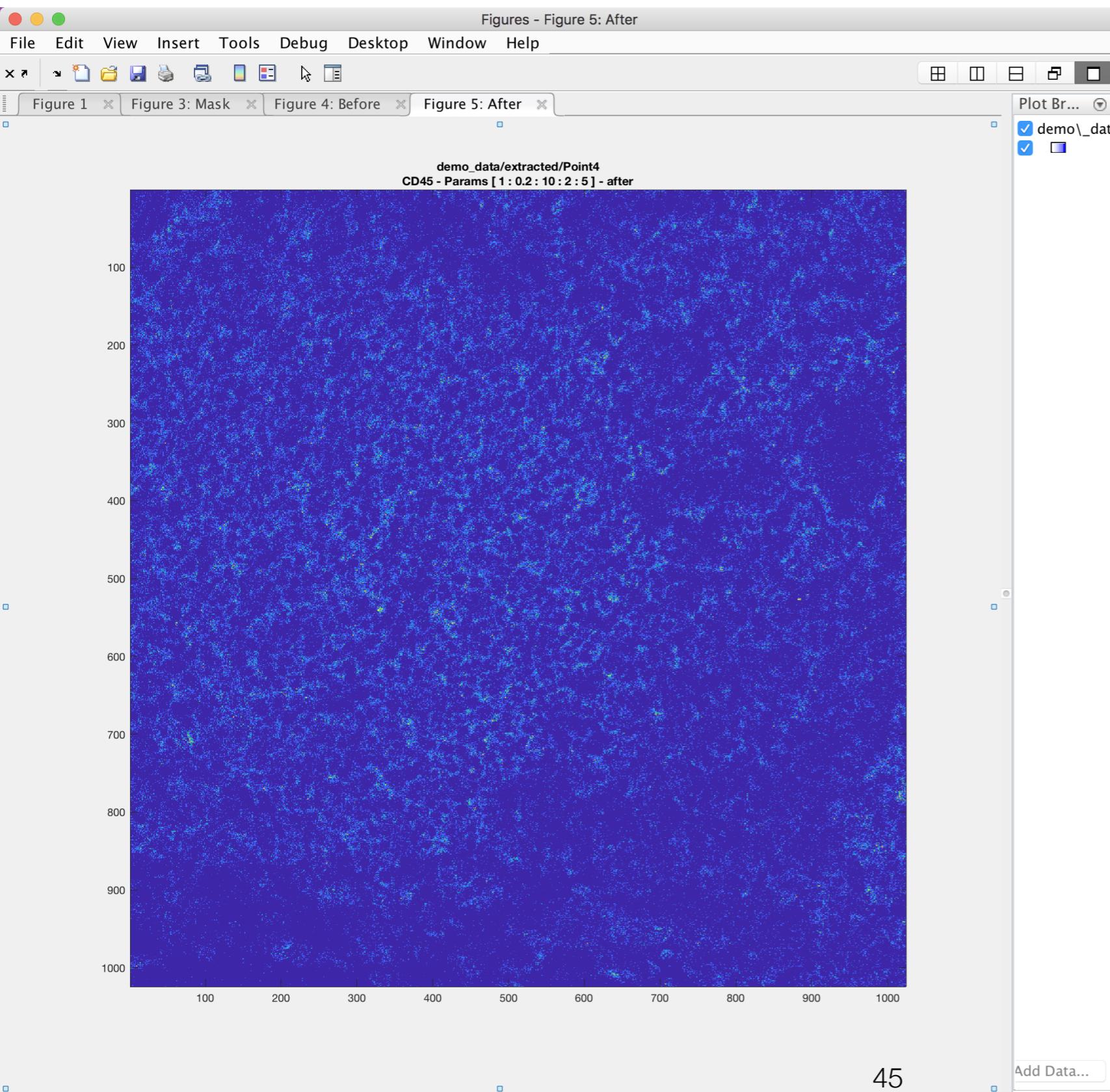
Background Removal

Gaussian radius : 1  
Threshhold : 0.2  
Background Cap : 10  
Evaluation Cap: 5

# The “Remove values” value is how many counts are removed from each channel at the locations indicated by the mask



# We can also remove too much. Here's the after image with remove value 2



Load Points and Select Background Channel

Add Point(s) Remove Point C

demo\_data/extracted/Point2  
demo\_data/extracted/Point4

( C

Reuse figure

Background Removal Parameters

Gaussian radius :	1	radius		thshold		bg_cap
Threshold :	0.2					
Background Cap :	10					

Reuse figure

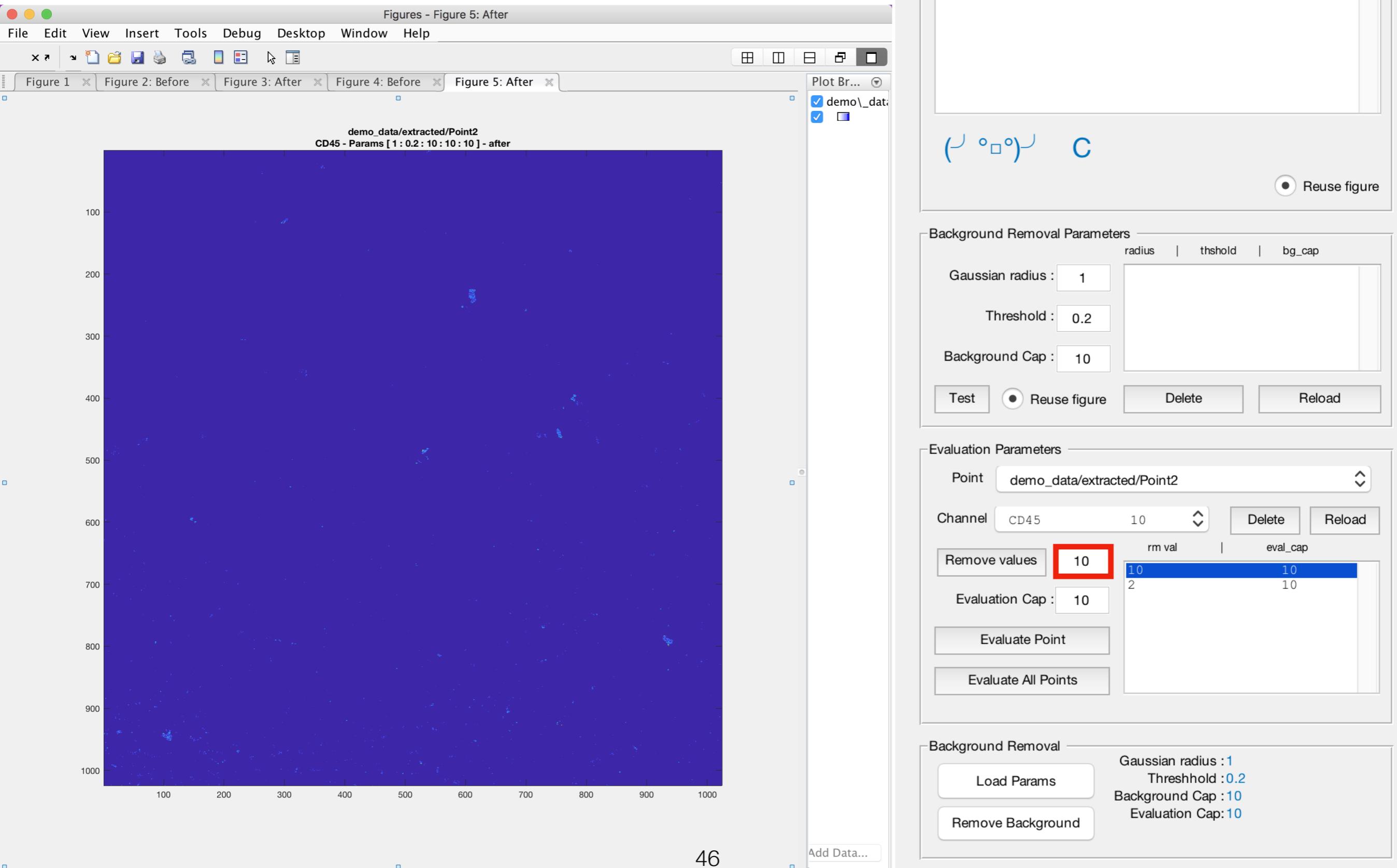
Evaluation Parameters

Point	demo_data/extracted/Point2	rm val		eval_cap
Channel	CD45	2	<input type="button" value="Delete"/>	<input type="button" value="Reload"/>
Remove values	2	2	10	
Evaluation Cap :	10			

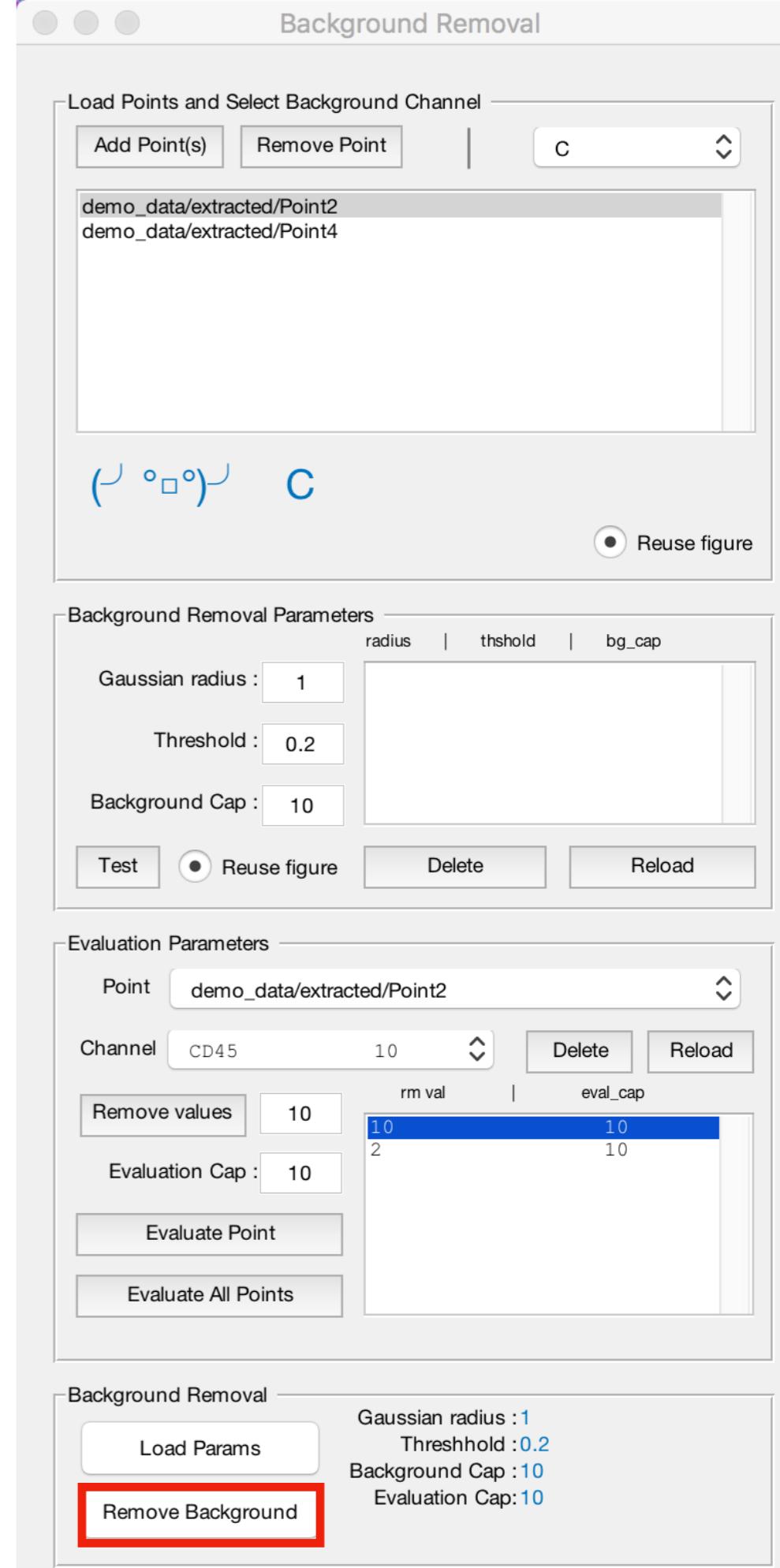
Background Removal

Load Params	Gaussian radius : 1
Remove Background	Threshold : 0.2
	Background Cap : 10
	Evaluation Cap: 10

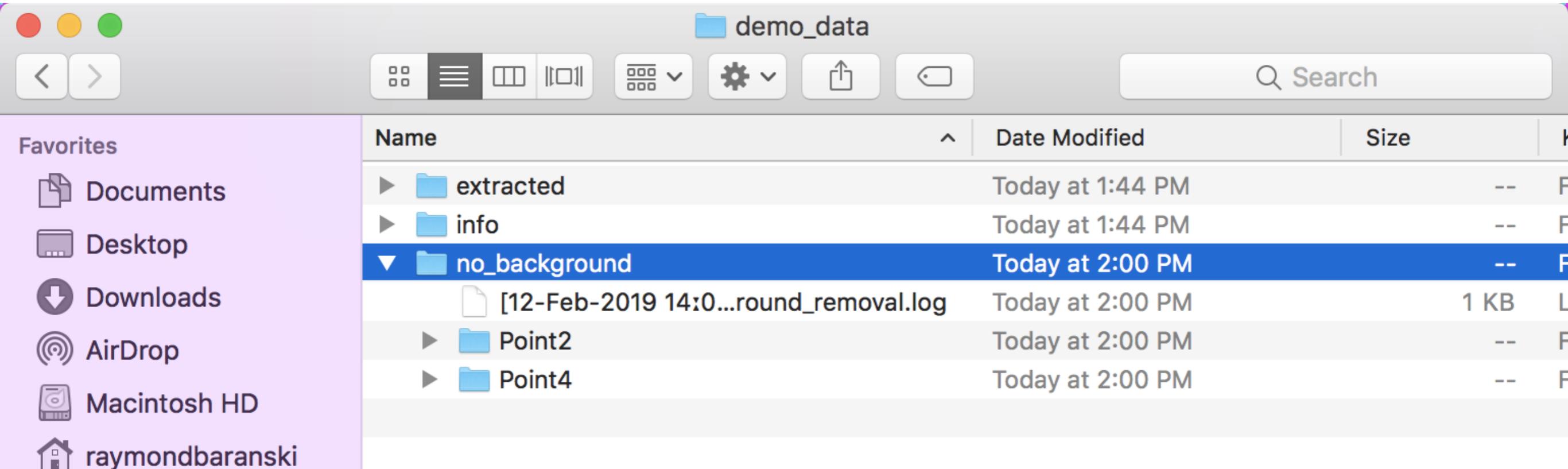
# And here's the same channel on the same point using the same mask, but with a remove value of 10



Once you've found some parameters that work and pass the evaluation step, simply click the “Remove Background” button



Now the data is saved in a folder called “no\_background” inside of the run folder



**A log file is created which saves the parameters you used on each channel, as well as the location of the data you removed the background from**

```
background channel: Background
background cap: 10.000000
evaluation cap: 5.000000
gaussian radius: 1.000000
threshold: 0.200000

C {rm_val: 10.000000}
Na {rm_val: 10.000000}
Si {rm_val: 10.000000}
Ca {rm_val: 10.000000}
Fe {rm_val: 10.000000}
dsDNA {rm_val: 10.000000}
Vimentin {rm_val: 10.000000}
SMA {rm_val: 10.000000}
Background {rm_val: 10.000000}
Collagen-1 {rm_val: 10.000000}
HO-1 {rm_val: 10.000000}
CD4 {rm_val: 10.000000}
CD14 {rm_val: 10.000000}
CD56 {rm_val: 10.000000}
Foxp3 {rm_val: 10.000000}
PD-1 {rm_val: 10.000000}
CD31 {rm_val: 10.000000}
PD-L1 {rm_val: 10.000000}
E-cadherin {rm_val: 10.000000}
Ki67 {rm_val: 10.000000}
CD209 {rm_val: 10.000000}
CD206 {rm_val: 10.000000}
gdTCR {rm_val: 10.000000}
iNOS {rm_val: 10.000000}
CD68 {rm_val: 10.000000}
CD36 {rm_val: 10.000000}
CD8 {rm_val: 10.000000}
CD3 {rm_val: 10.000000}
IDO {rm_val: 10.000000}
CD11c {rm_val: 10.000000}
Arginase1 {rm_val: 10.000000}
CD163 {rm_val: 10.000000}
CD20 {rm_val: 10.000000}
CD16 {rm_val: 10.000000}
IFNg {rm_val: 10.000000}
HLA-DR {rm_val: 10.000000}
CD11b {rm_val: 10.000000}
CD45 {rm_val: 10.000000}
H3K9Ac {rm_val: 10.000000}
Keratin-pan {rm_val: 10.000000}
CD103 {rm_val: 10.000000}
MastChyTry {rm_val: 10.000000}
MPO {rm_val: 10.000000}
NaKATPase {rm_val: 10.000000}
HLA-Class-1 {rm_val: 10.000000}
Ta {rm_val: 10.000000}
Au {rm_val: 10.000000}

/Volumes/ALEX_SSD/demo_data/extracted/Point2
/Volumes/ALEX_SSD/demo_data/extracted/Point4
```

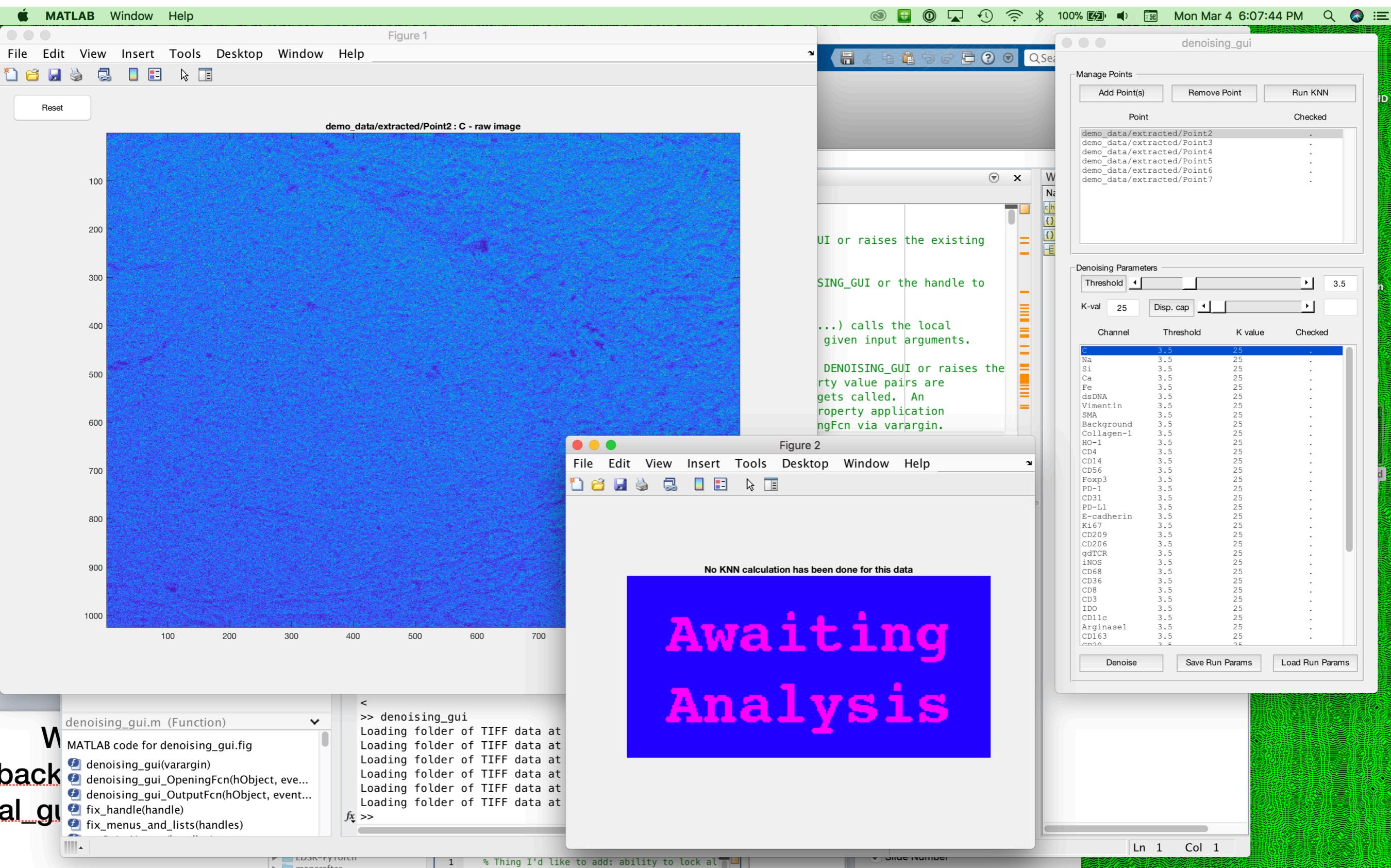
# Noise Removal

- We remove noise from images by first looking at a single channel on a single pixel, and calculating the average distance to the k-nearest counts of the same channel
- We do this for every pixel in the image
- We pick a threshold on average distance, and we throw away pixels that are above that threshold, because this indicates the signal is sparse

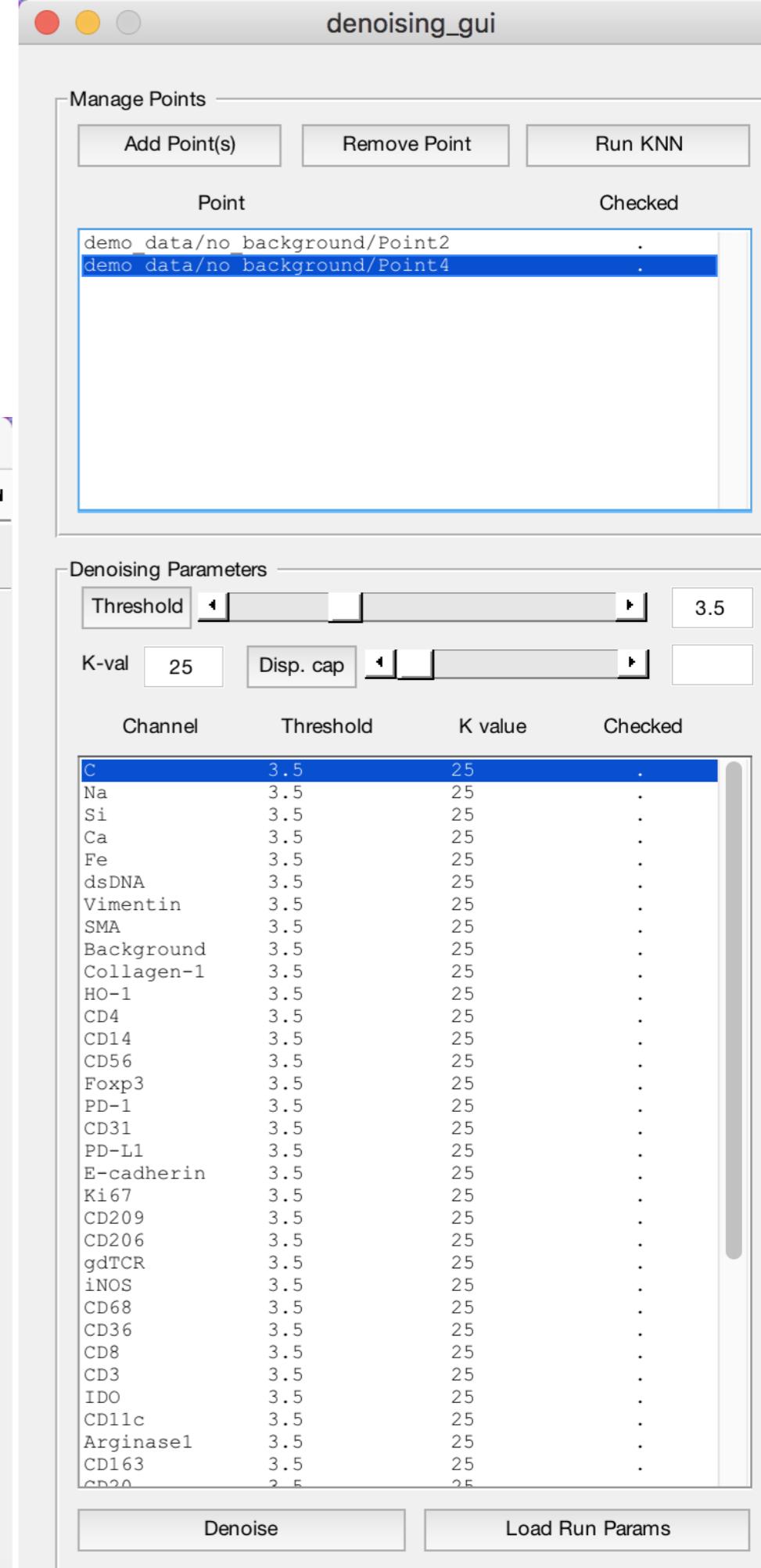
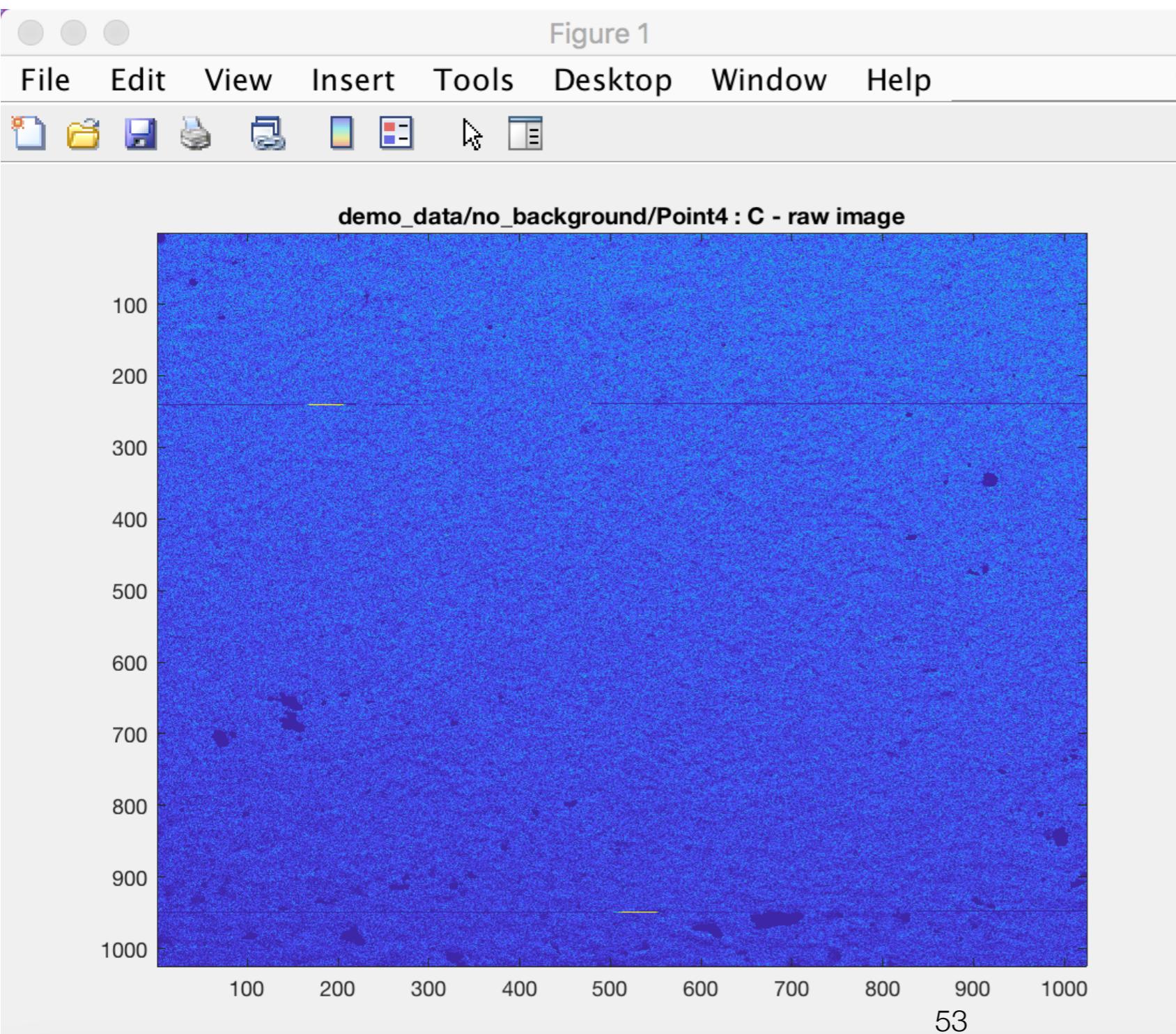
# Noise Removal

- First open the script “denoising\_gui.m” and run it
- Add the points you want to denoise (probably you should use points inside of the no\_background folder, if you’ve already done background removal)

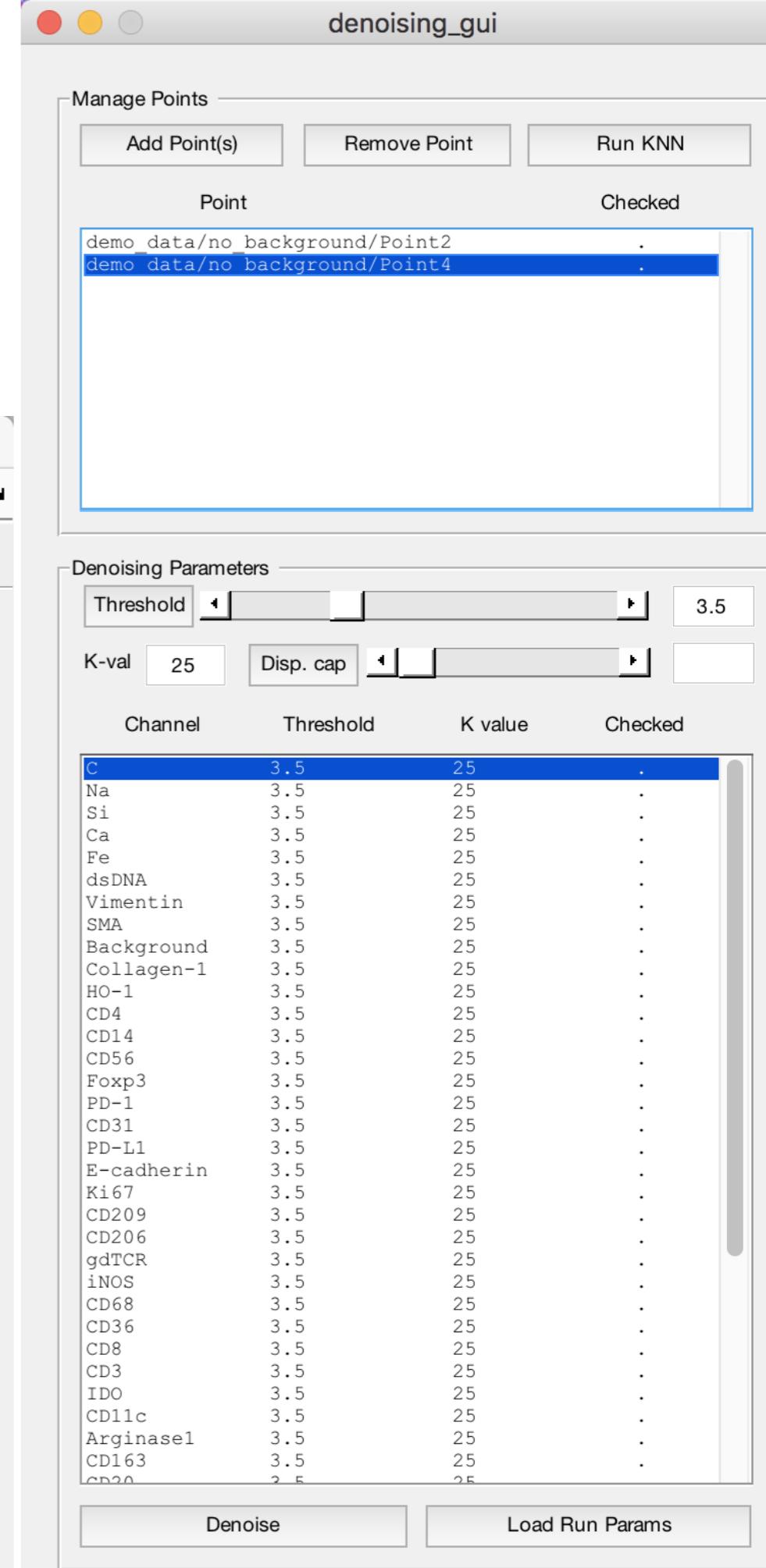
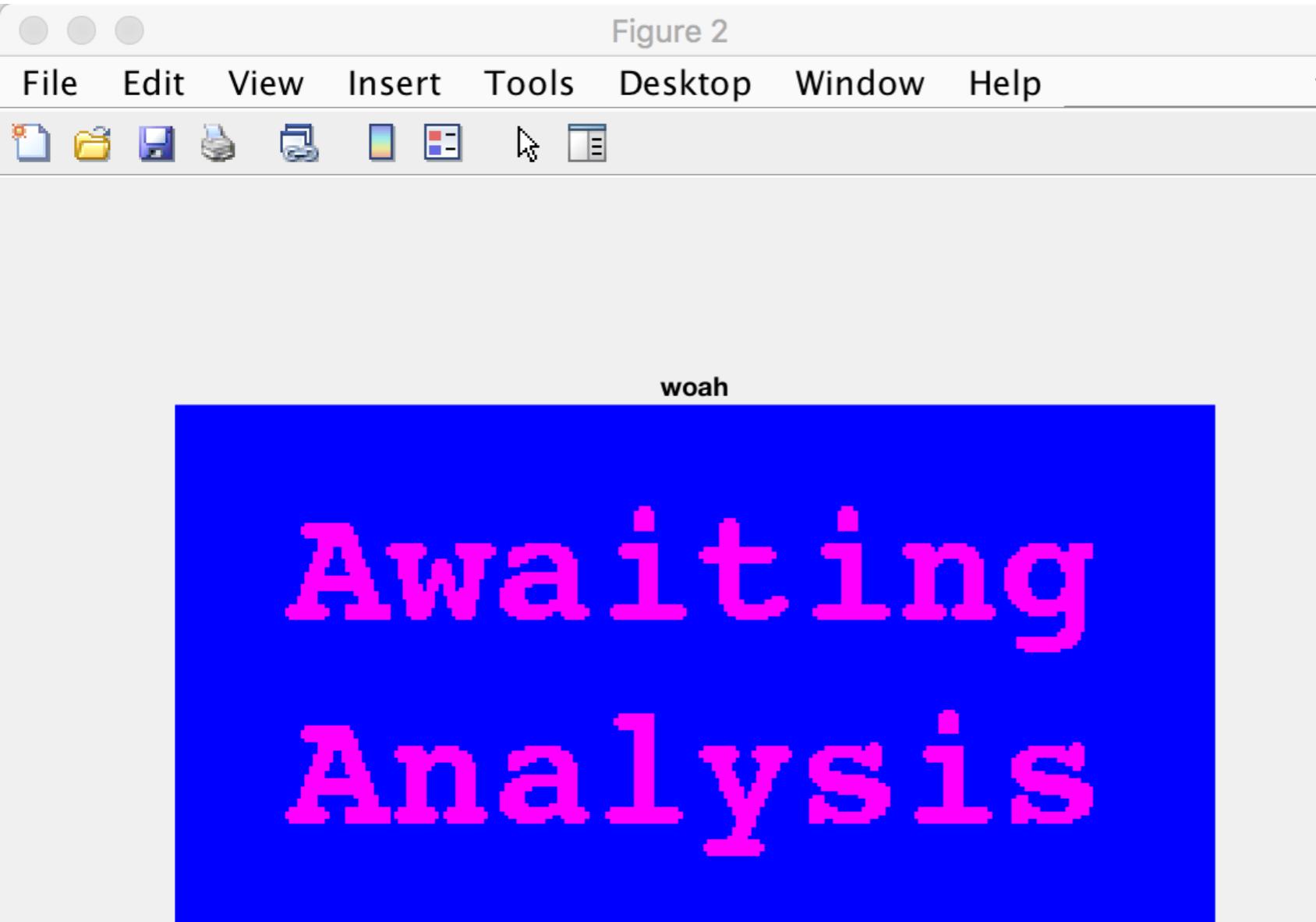
# Noise removal GUI



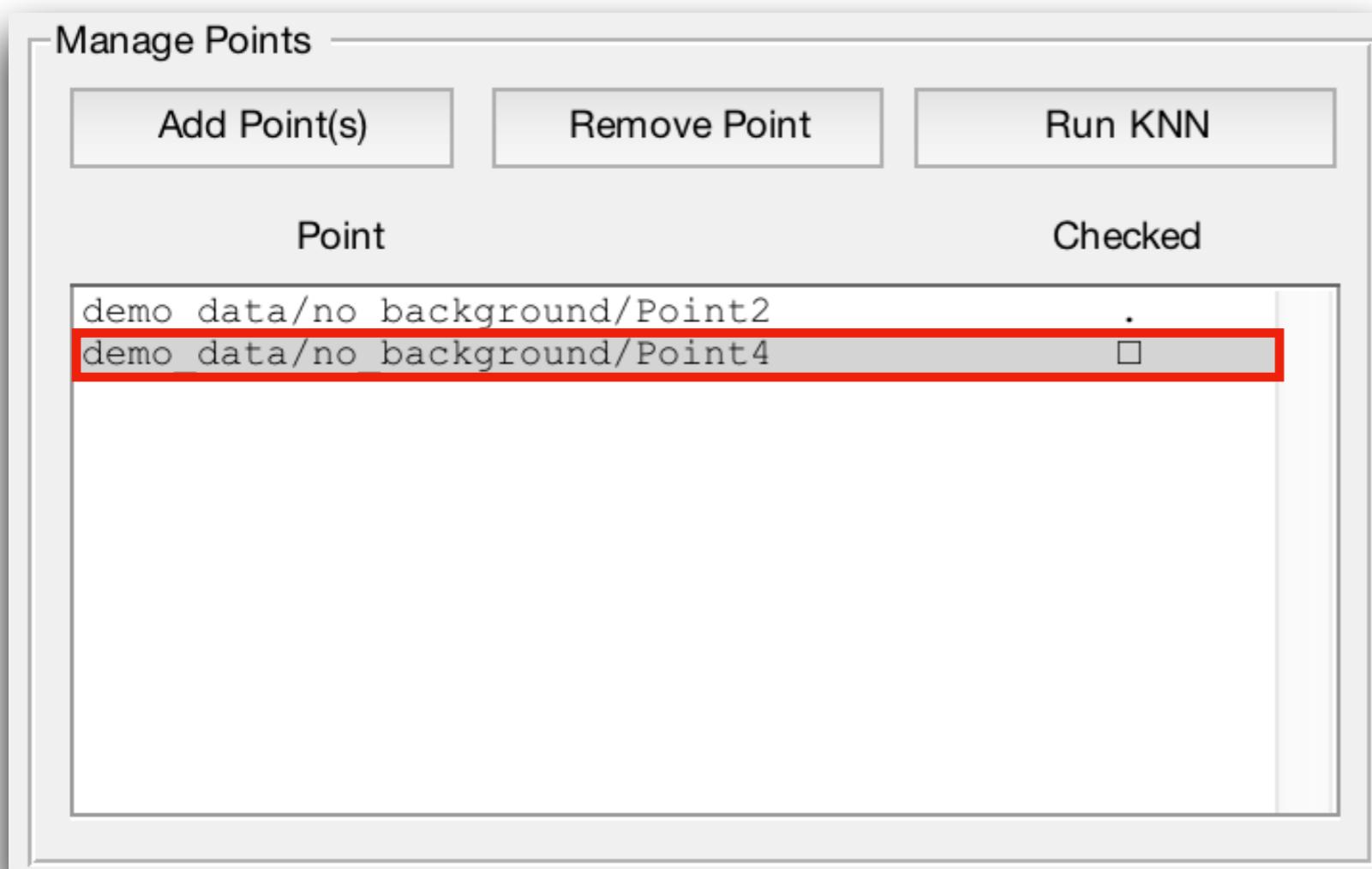
# View different points and channels by selecting them



Until we actually do the KNN calculation, we aren't able to see the KNN histogram



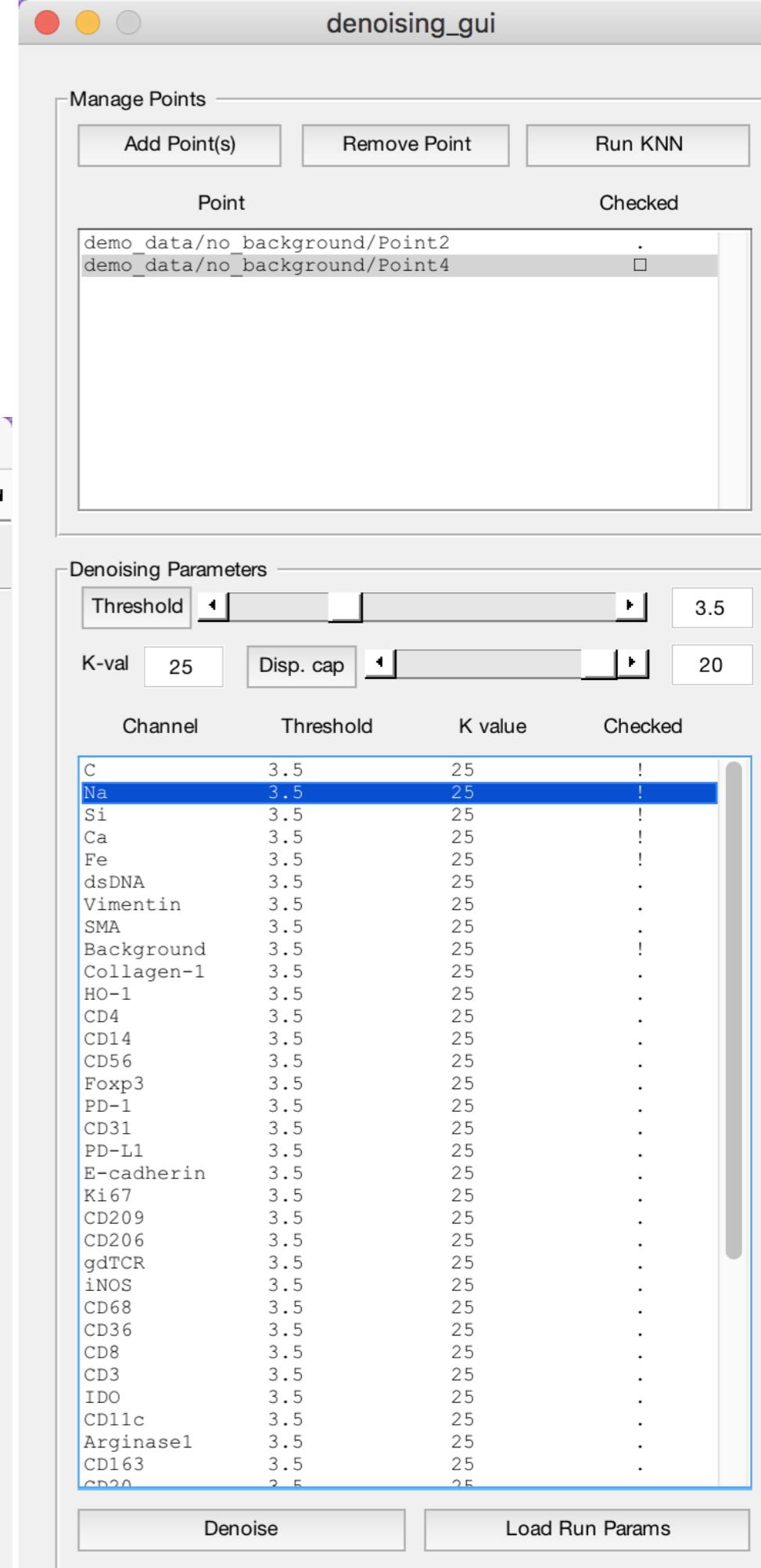
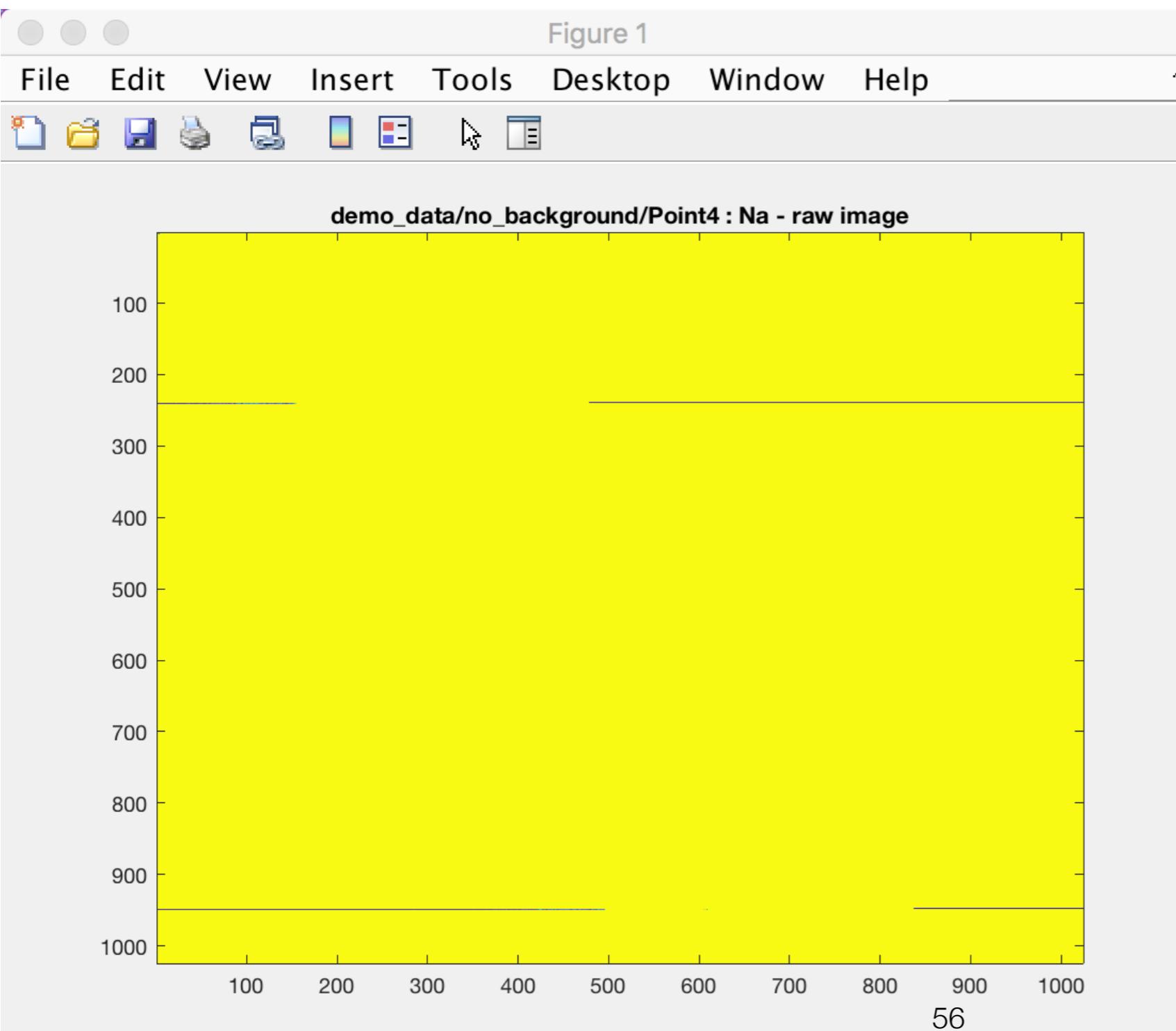
We have to “toggle” a point or channel so that the GUI knows to run the KNN algorithm on it



Channel	Threshold	K value	Checked
C	3.5	25	.
Na	3.5	25	.
Si	3.5	25	.
Ca	3.5	25	.
Fe	3.5	25	.
dsDNA	3.5	25	.
Vimentin	3.5	25	.
SMA	3.5	25	.
Background	3.5	25	.
Collagen-1	3.5	25	.
HO-1	3.5	25	.
CD4	3.5	25	.
CD14	3.5	25	.
CD56	3.5	25	.
Foxp3	3.5	25	.
PD-1	3.5	25	.
CD31	3.5	25	.
PD-L1	3.5	25	.
E-cadherin	3.5	25	.
Ki67	3.5	25	.
CD209	3.5	25	.
CD206	3.5	25	.
gdTCR	3.5	25	.
iNOS	3.5	25	.
CD68	3.5	25	.
CD36	3.5	25	.
CD8	3.5	25	.
CD3	3.5	25	.
IDO	3.5	25	.
CD11c	3.5	25	.
Arginase1	3.5	25	.
CD163	3.5	25	.
CD20	3.5	25	.

Denoise      Load Run Params

# Some channels we don't actually want to do the KNN calculation for, like Gold and Background



Select these and press “Delete”. This tells the GUI to ignore them during denoising.

Denoising Parameters

Threshold	<input type="text" value="1"/>	<input type="button" value="3.5"/>		
K-val	25	Disp. cap	<input type="text" value="1"/>	<input type="button" value="20"/>

Channel	Threshold	K value	Checked
C	3.5	25	!
Na	3.5	25	!
Si	3.5	25	.
Ca	3.5	25	.
Fe	3.5	25	!
dsDNA	3.5	25	.
Vimentin	3.5	25	.
SMA	3.5	25	.
Background	3.5	25	!
Collagen-1	3.5	25	.
HO-1	3.5	25	.
CD4	3.5	25	.
CD14	3.5	25	.
CD56	3.5	25	.
Foxp3	3.5	25	.
PD-1	3.5	25	.
CD31	3.5	25	.
PD-L1	3.5	25	.
E-cadherin	3.5	25	.
Ki67	3.5	25	.
CD209	3.5	25	.
CD206	3.5	25	.
gdTCR	3.5	25	.
iNOS	3.5	25	.
CD68	3.5	25	.
CD36	3.5	25	.
CD8	3.5	25	.
CD3	3.5	25	.
IDO	3.5	25	.
CD11c	3.5	25	.
Arginase1	3.5	25	.
CD163	3.5	25	.
CD20	3.5	25	.

denoising\_gui

Manage Points

Add Point(s)	Remove Point	Run KNN
--------------	--------------	---------

Point	Checked
demo_data/no_background/Point2	.
demo_data/no_background/Point4	<input type="checkbox"/>

Denoising Parameters

Threshold	<input type="text" value="1"/>	<input type="button" value="3.5"/>		
K-val	25	Disp. cap	<input type="text" value="1"/>	<input type="button" value="20"/>

Channel	Threshold	K value	Checked
C	3.5	25	!
Na	3.5	25	!
Si	3.5	25	.
Ca	3.5	25	.
Fe	3.5	25	.
dsDNA	3.5	25	.
Vimentin	3.5	25	.
SMA	3.5	25	.
Background	3.5	25	.
Collagen-1	3.5	25	.
HO-1	3.5	25	.
CD4	3.5	25	.
CD14	3.5	25	.
CD56	3.5	25	.
Foxp3	3.5	25	.
PD-1	3.5	25	.
CD31	3.5	25	.
PD-L1	3.5	25	.
E-cadherin	3.5	25	.
Ki67	3.5	25	.
CD209	3.5	25	.
CD206	3.5	25	.
gdTCR	3.5	25	.
iNOS	3.5	25	.
CD68	3.5	25	.
CD36	3.5	25	.
CD8	3.5	25	.
CD3	3.5	25	.
IDO	3.5	25	.
CD11c	3.5	25	.
Arginase1	3.5	25	.
CD163	3.5	25	.
CD20	3.5	25	.

<input type="button" value="Denoise"/>	<input type="button" value="Load Run Params"/>
----------------------------------------	------------------------------------------------

# Select the rest of the channels you want to look at

Manage Points

Point	Checked
demo_data/no_background/Point2	.
demo_data/no_background/Point4	<input type="checkbox"/>

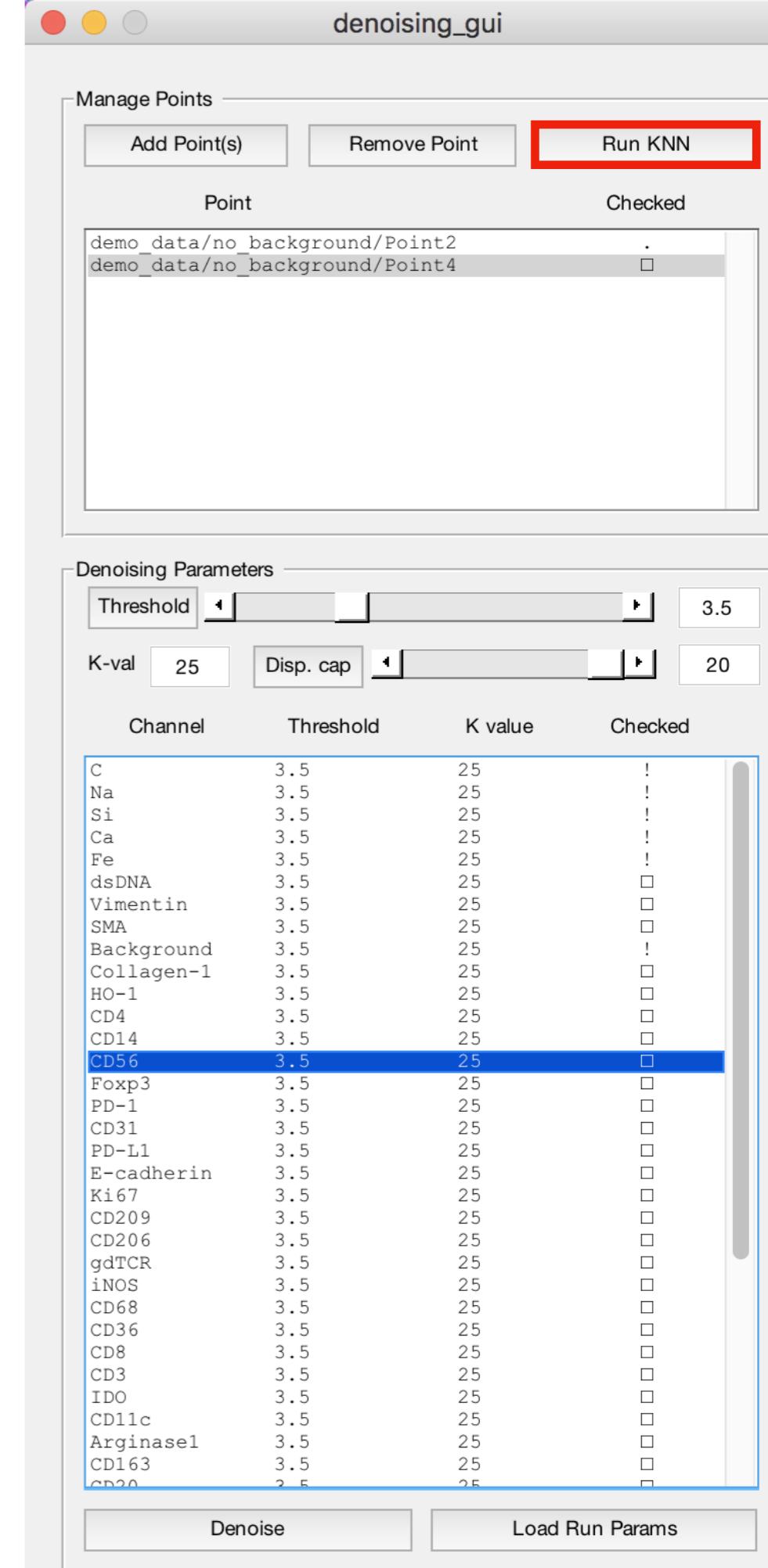
Denoising Parameters

Threshold	3.5
K-val	25
Disp. cap	20

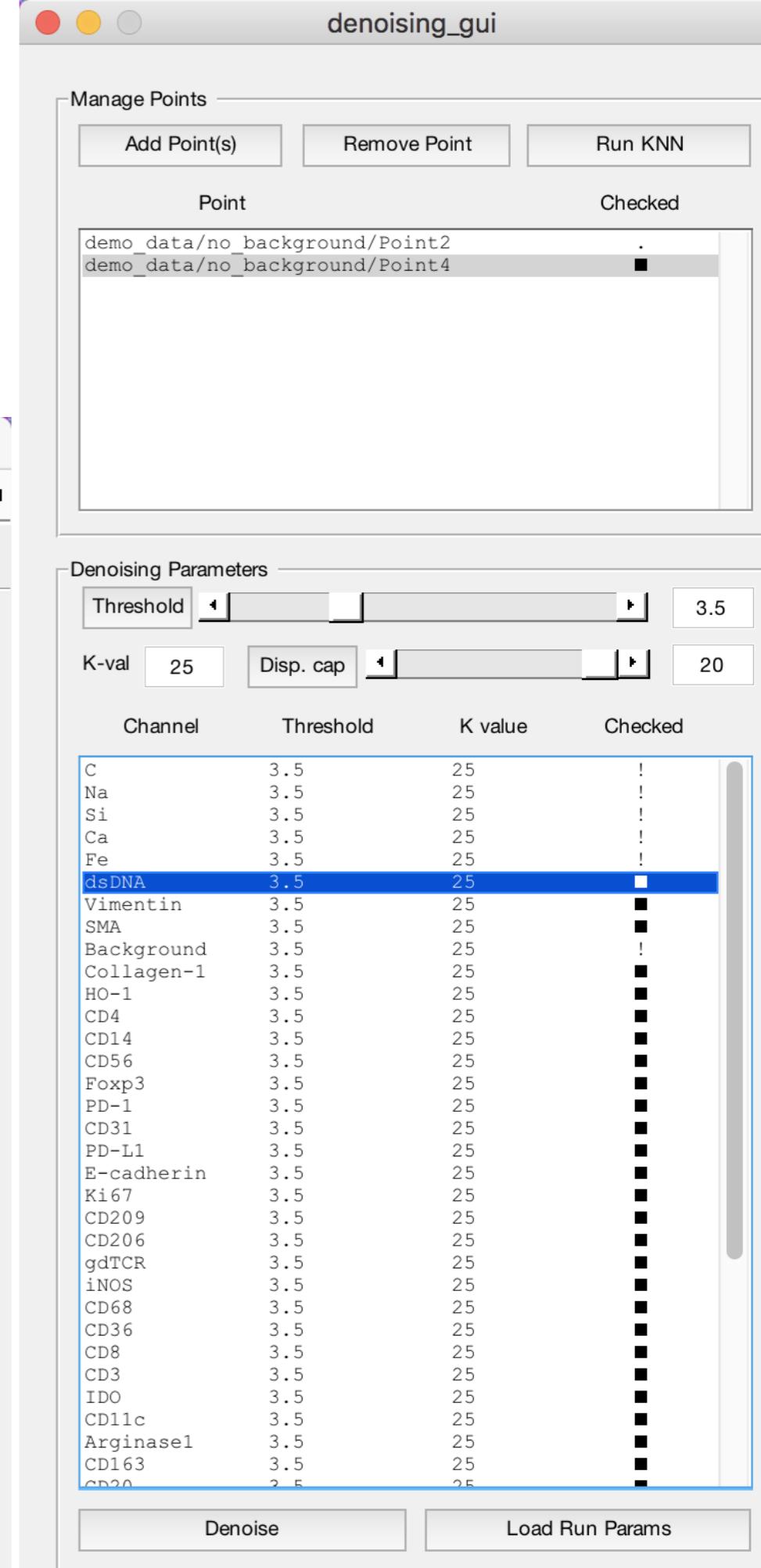
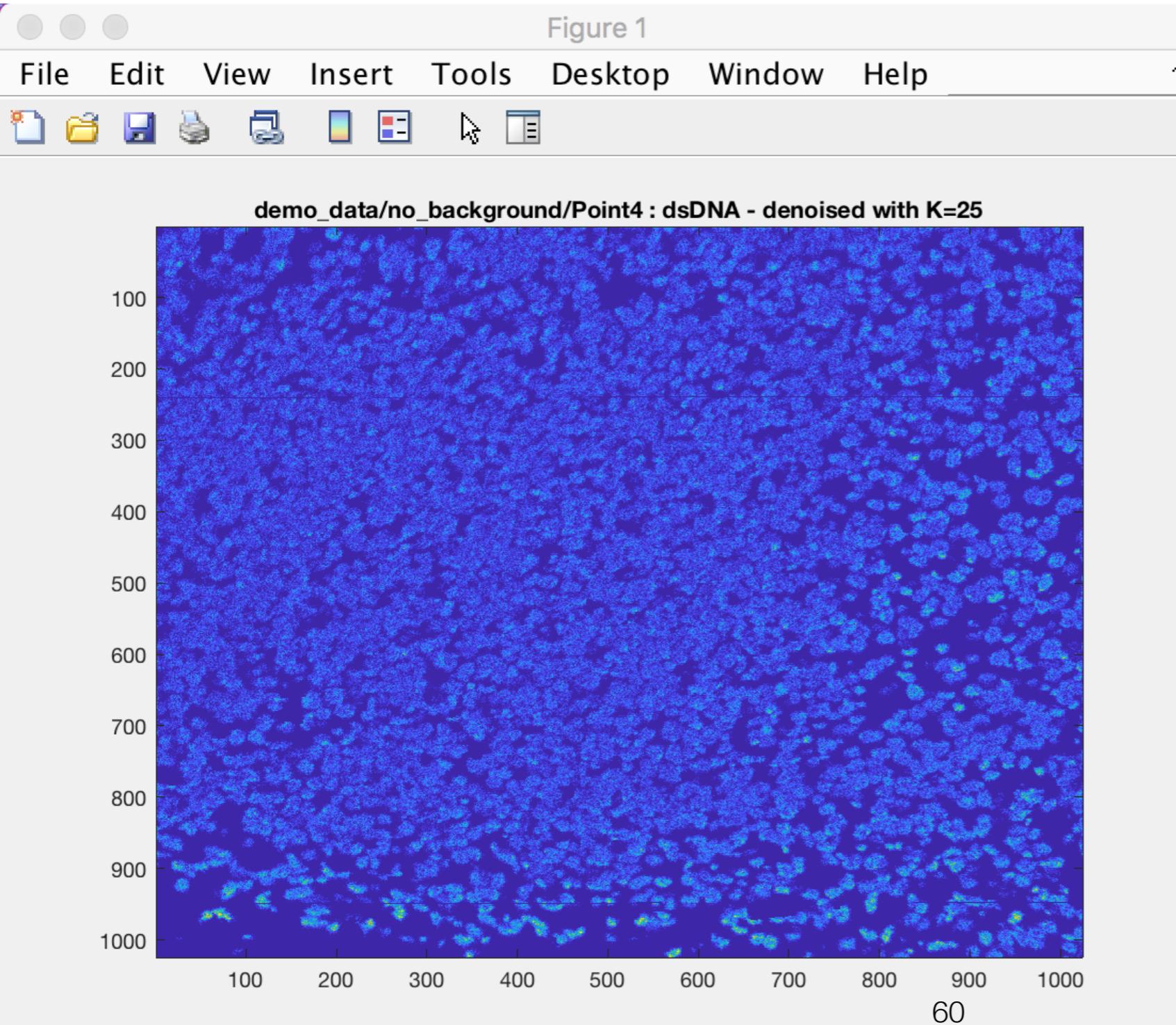
Channel	Threshold	K value	Checked
C	3.5	25	!
Na	3.5	25	!
Si	3.5	25	!
Ca	3.5	25	!
Fe	3.5	25	!
dsDNA	3.5	25	!
Vimentin	3.5	25	!
SMA	3.5	25	!
Background	3.5	25	!
Collagen-1	3.5	25	!
HO-1	3.5	25	!
CD4	3.5	25	!
CD14	3.5	25	!
CD56	3.5	25	<input type="checkbox"/>
Foxp3	3.5	25	!
PD-1	3.5	25	!
CD31	3.5	25	!
PD-L1	3.5	25	!
E-cadherin	3.5	25	!
Ki67	3.5	25	!
CD209	3.5	25	!
CD206	3.5	25	!
gdTCR	3.5	25	!
iNOS	3.5	25	!
CD68	3.5	25	!
CD36	3.5	25	!
CD8	3.5	25	!
CD3	3.5	25	!
IDO	3.5	25	!
CD11c	3.5	25	!
Arginase1	3.5	25	!
CD163	3.5	25	!
CD20	3.5	25	!

Denoise      Load Run Params

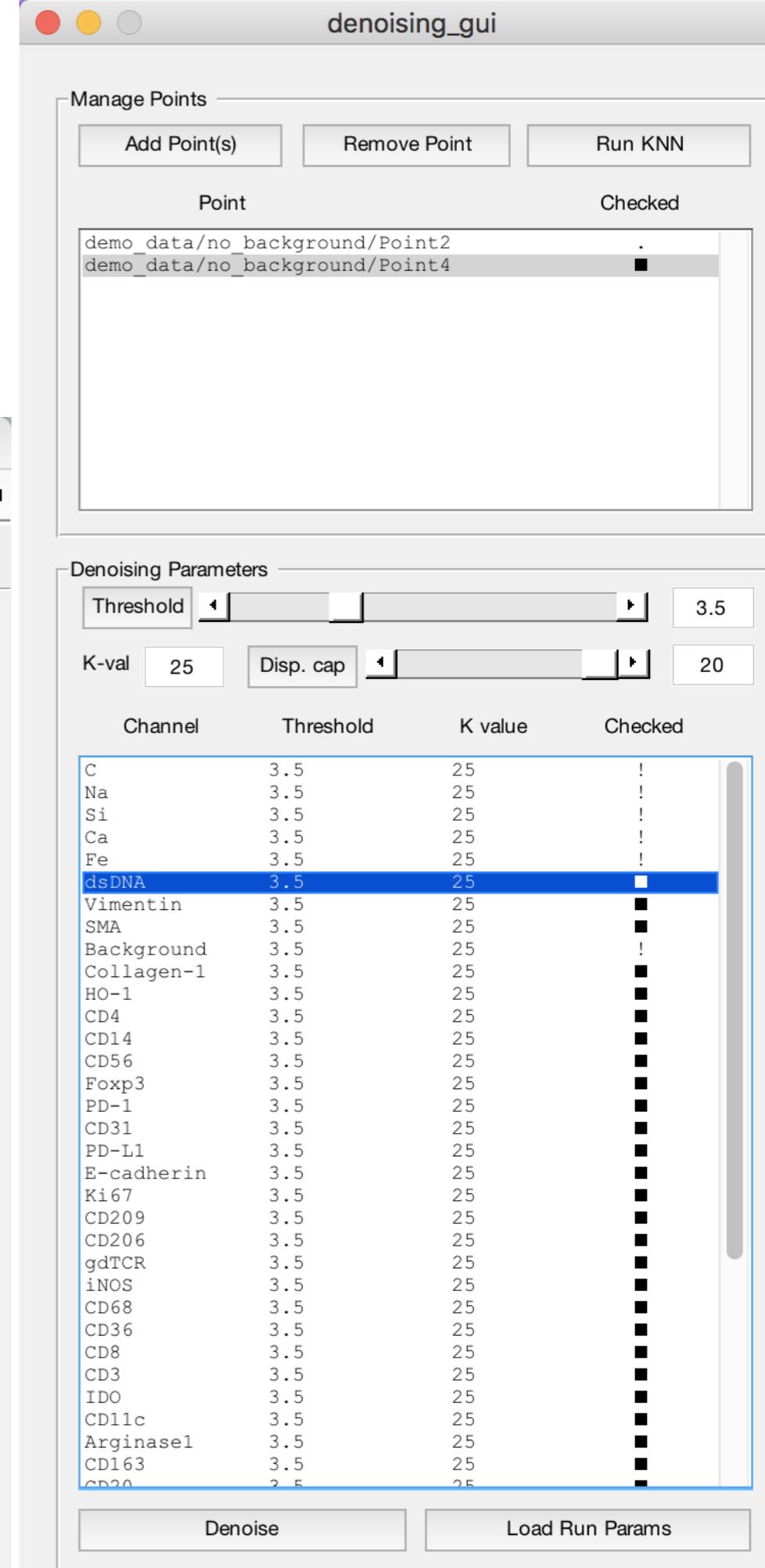
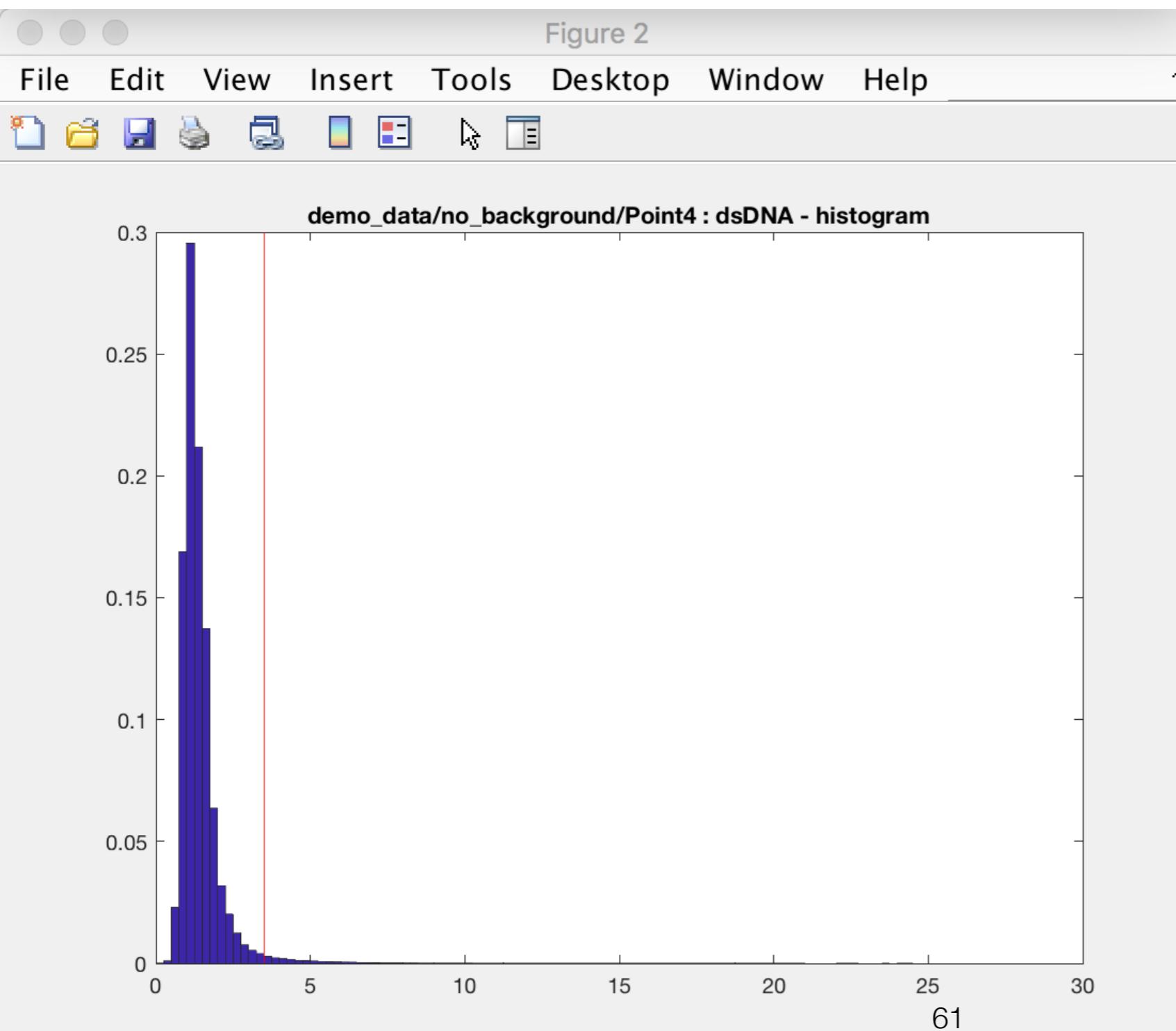
Now click the “Run KNN” button. This executes the calculation of the KNN distributions for all selected points/channels



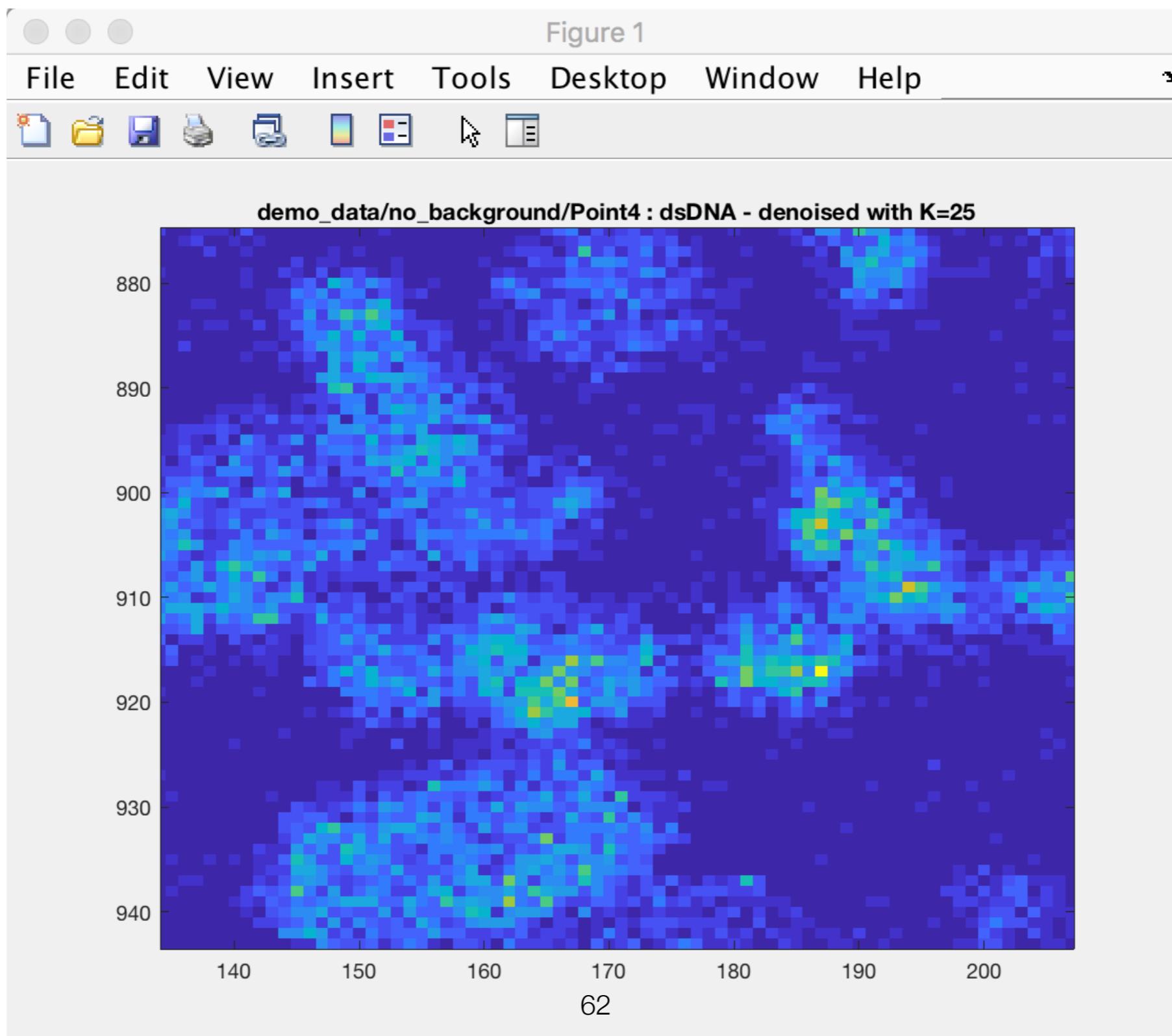
Once the calculation is done, the boxes you marked will become solid, indicating that there is a calculated KNN distribution for that point or channel



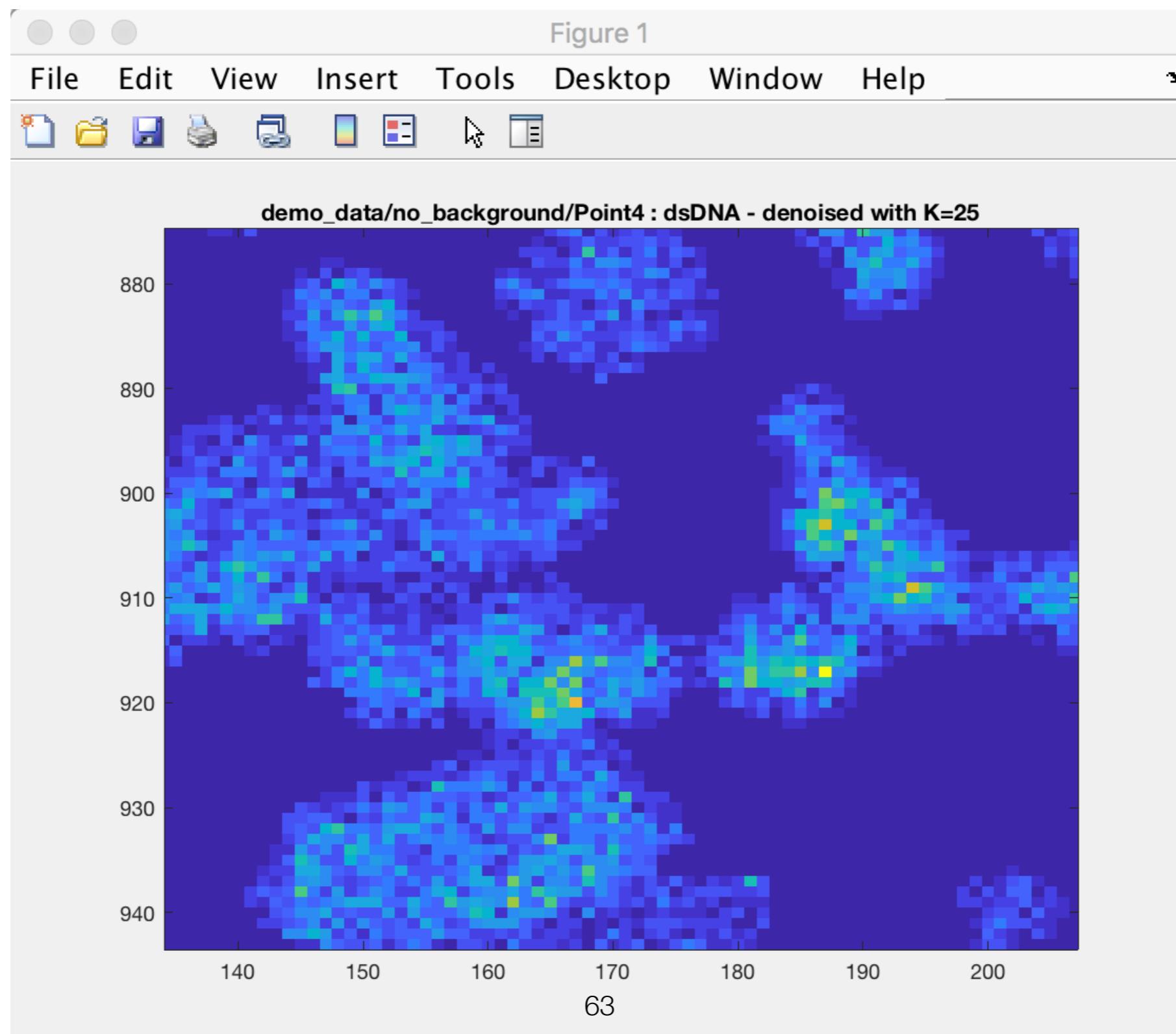
# dsDNA is an example of a very clean channel, meaning there's almost no noise



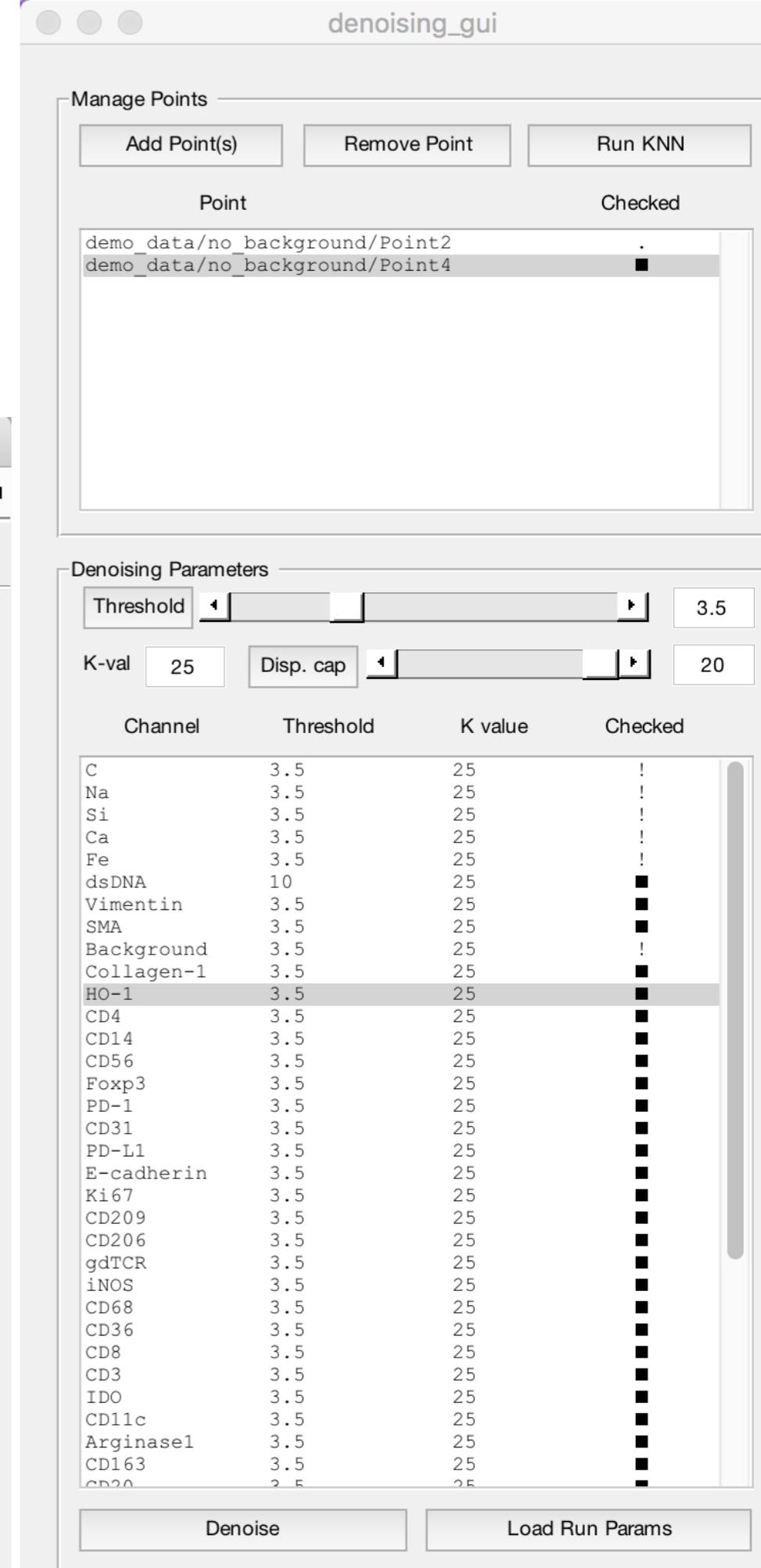
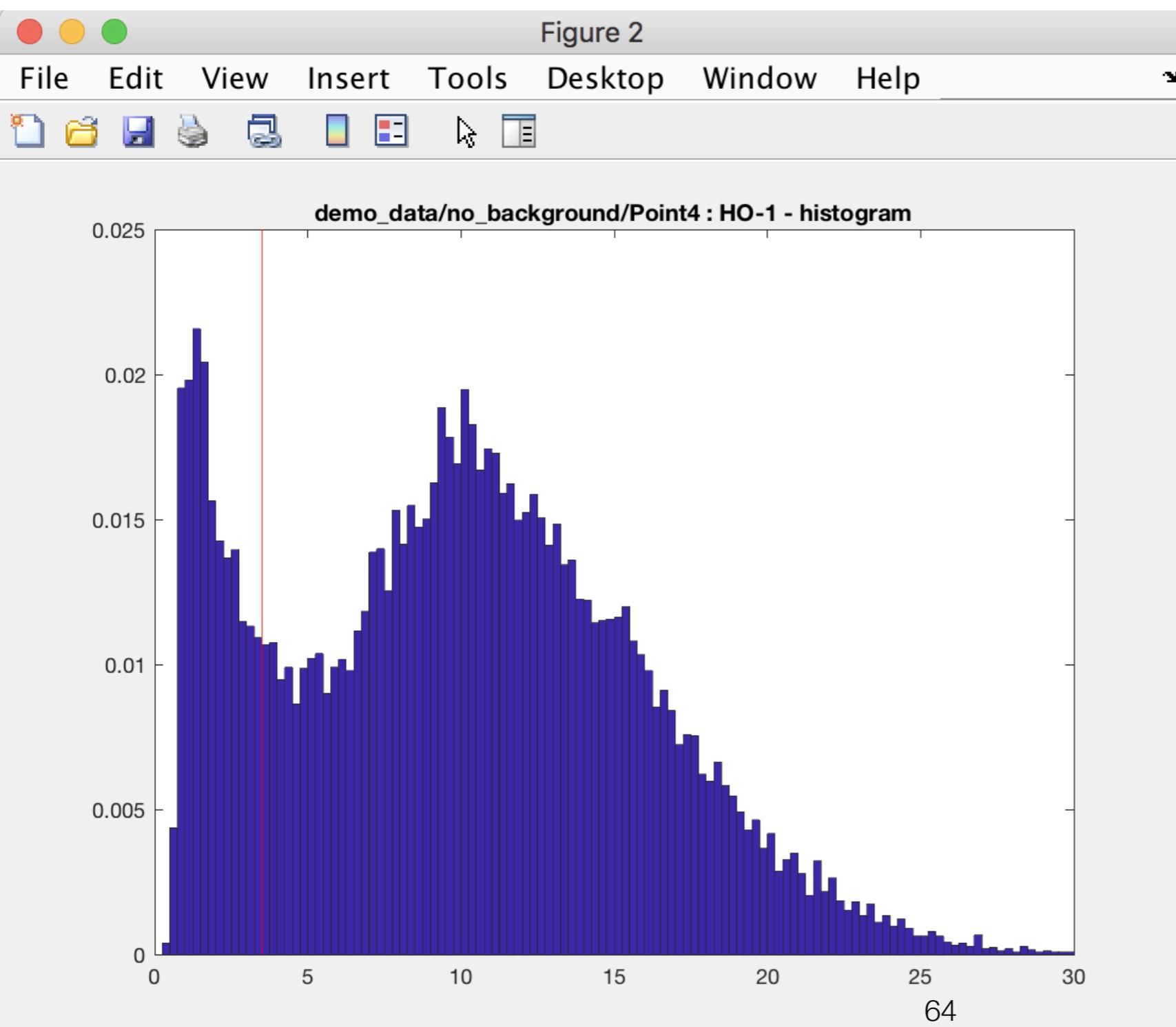
# dsDNA, unprocessed



# dsDNA, noise removed



**HO-1 is an example of a channel with a very clear bimodal distribution. The threshold should go in the middle**



# We adjust the threshold (the red line) with this slide-bar

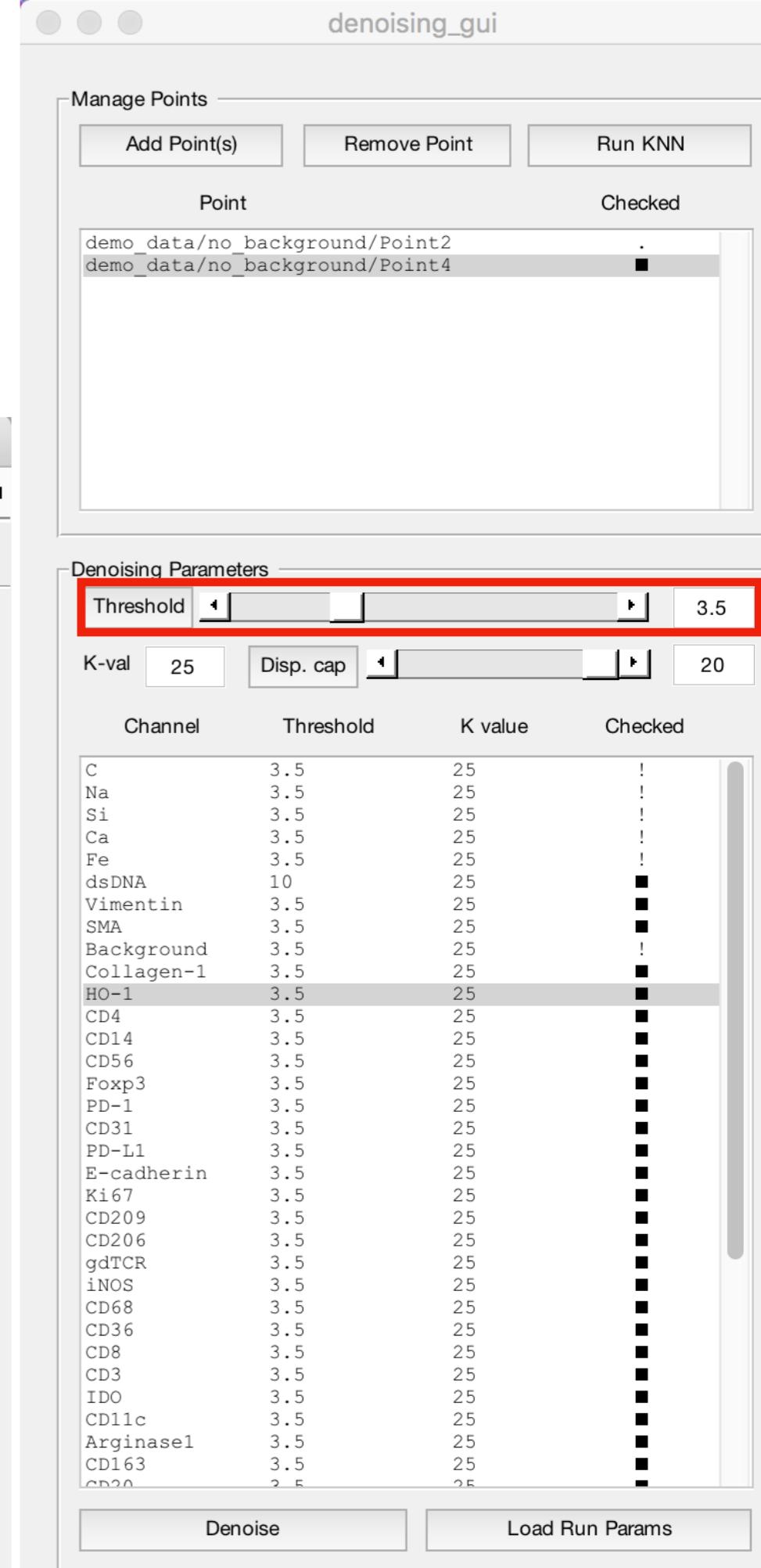
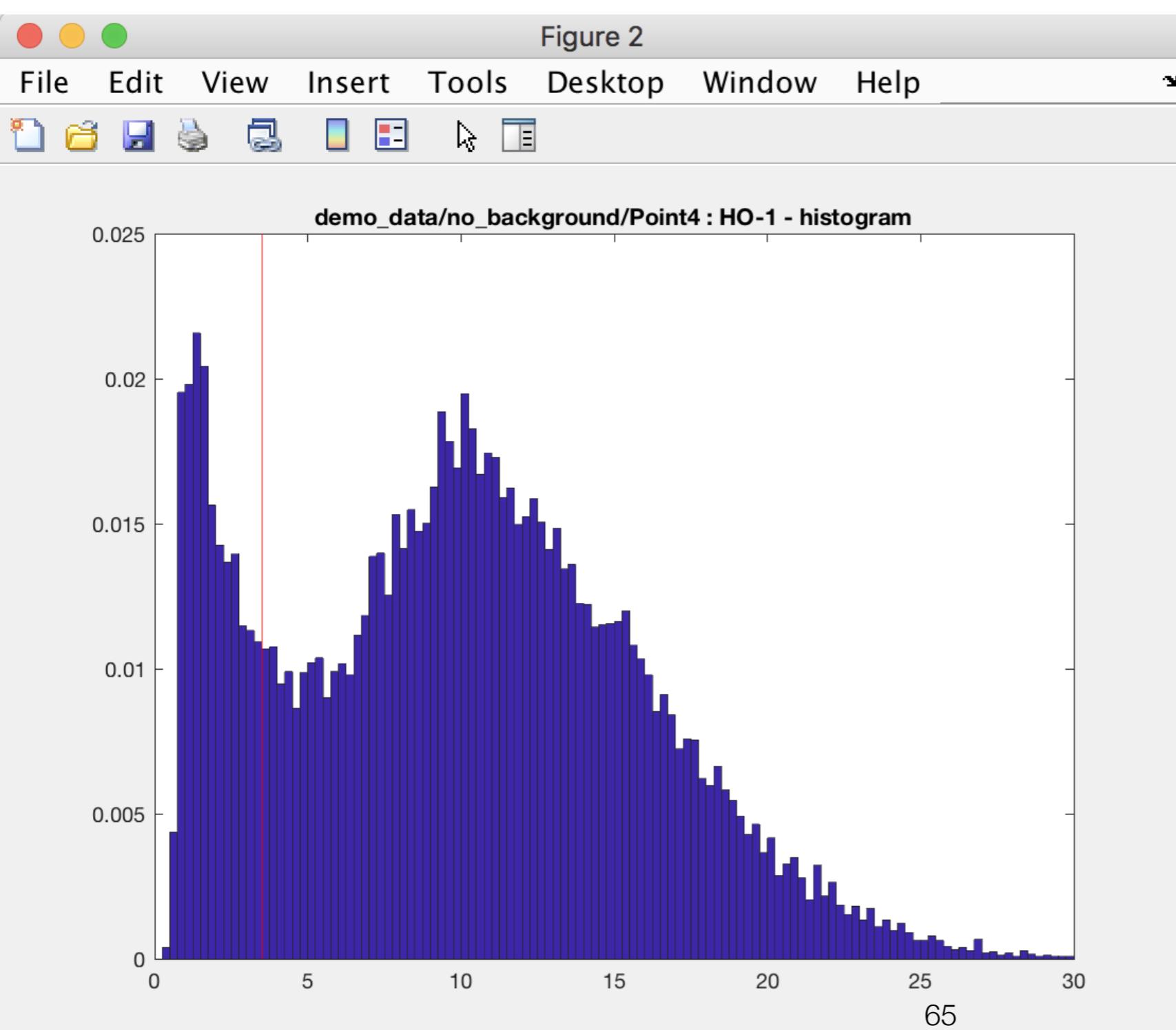


Figure 2

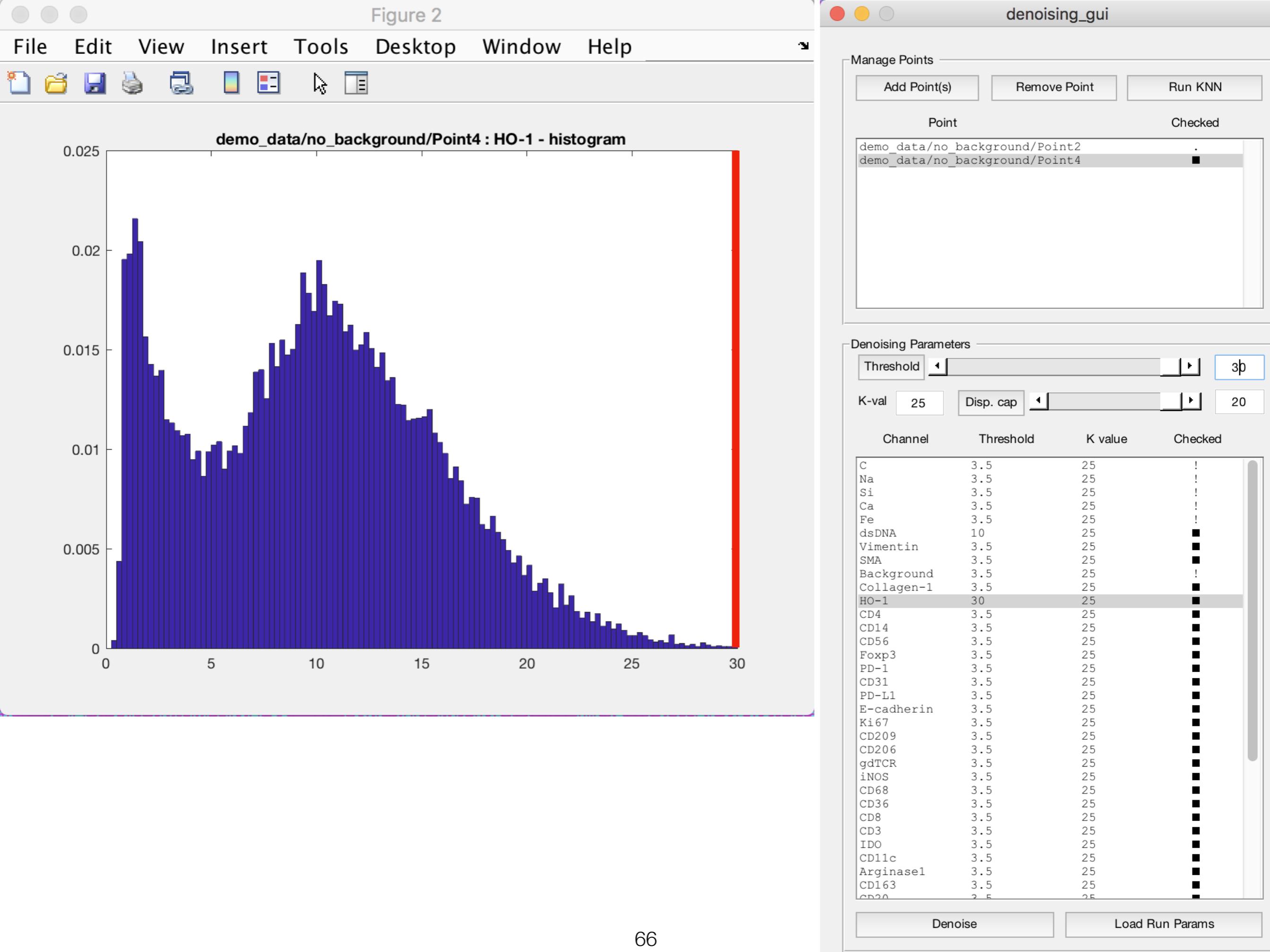


Figure 2

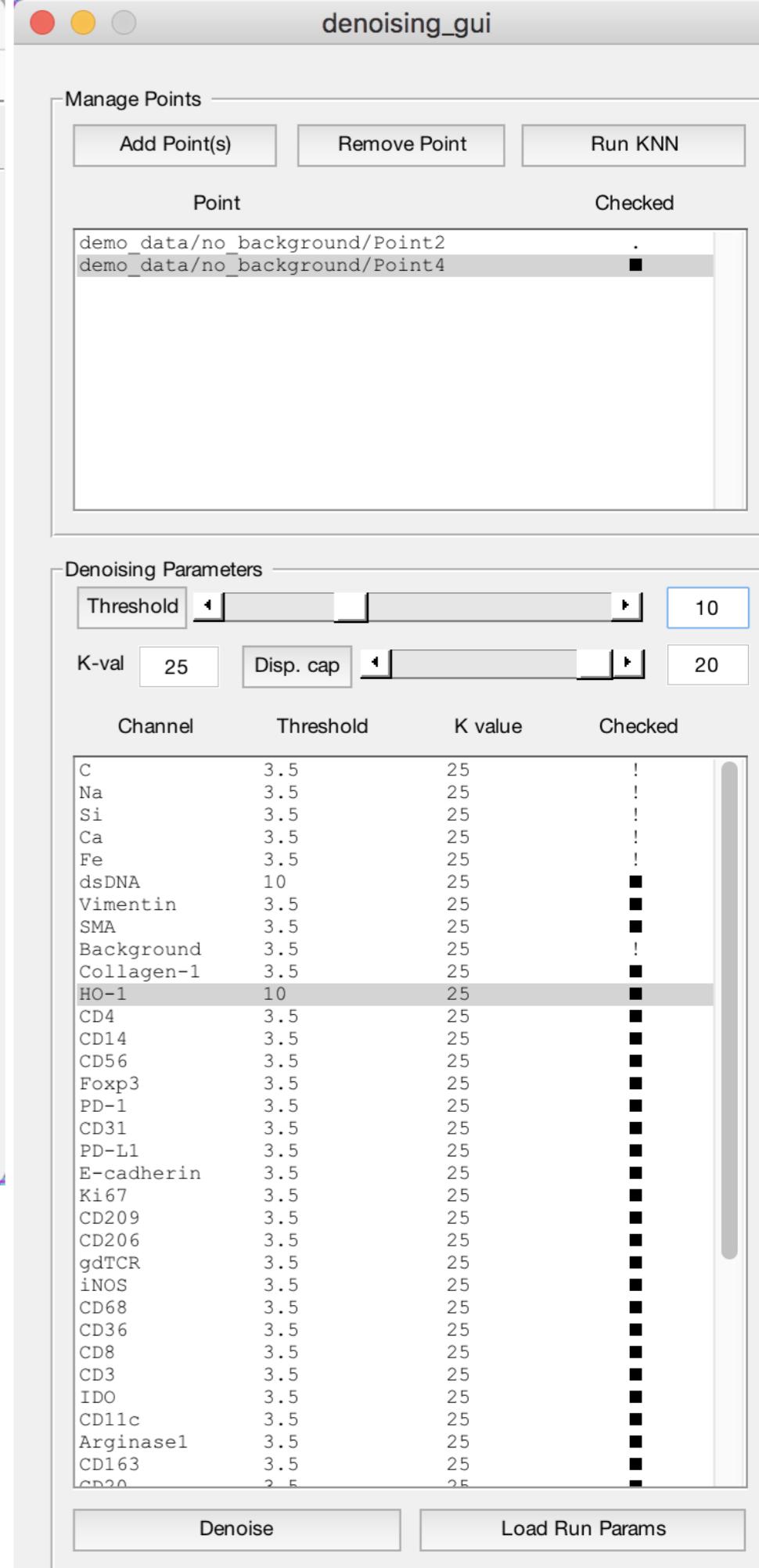
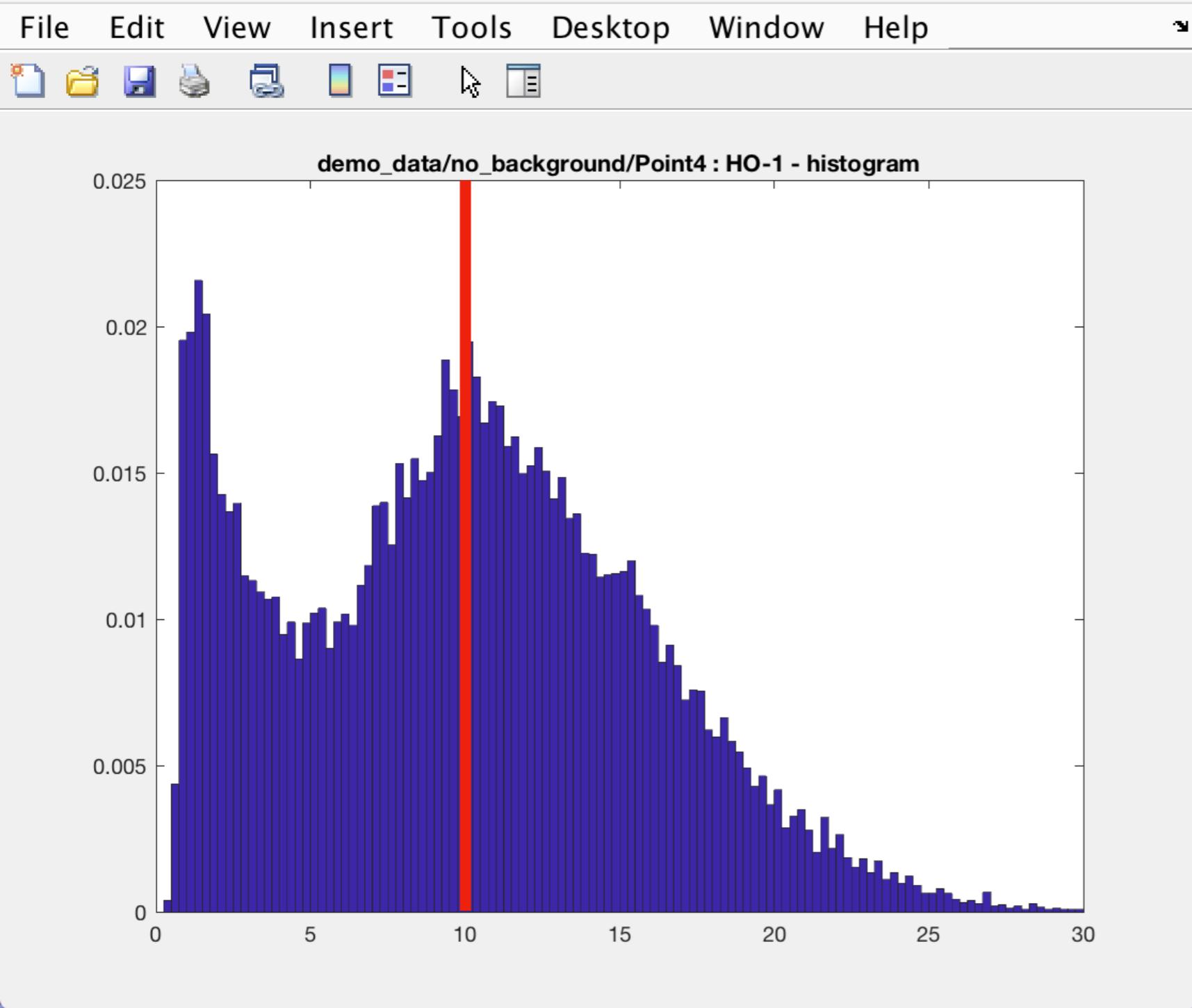


Figure 2

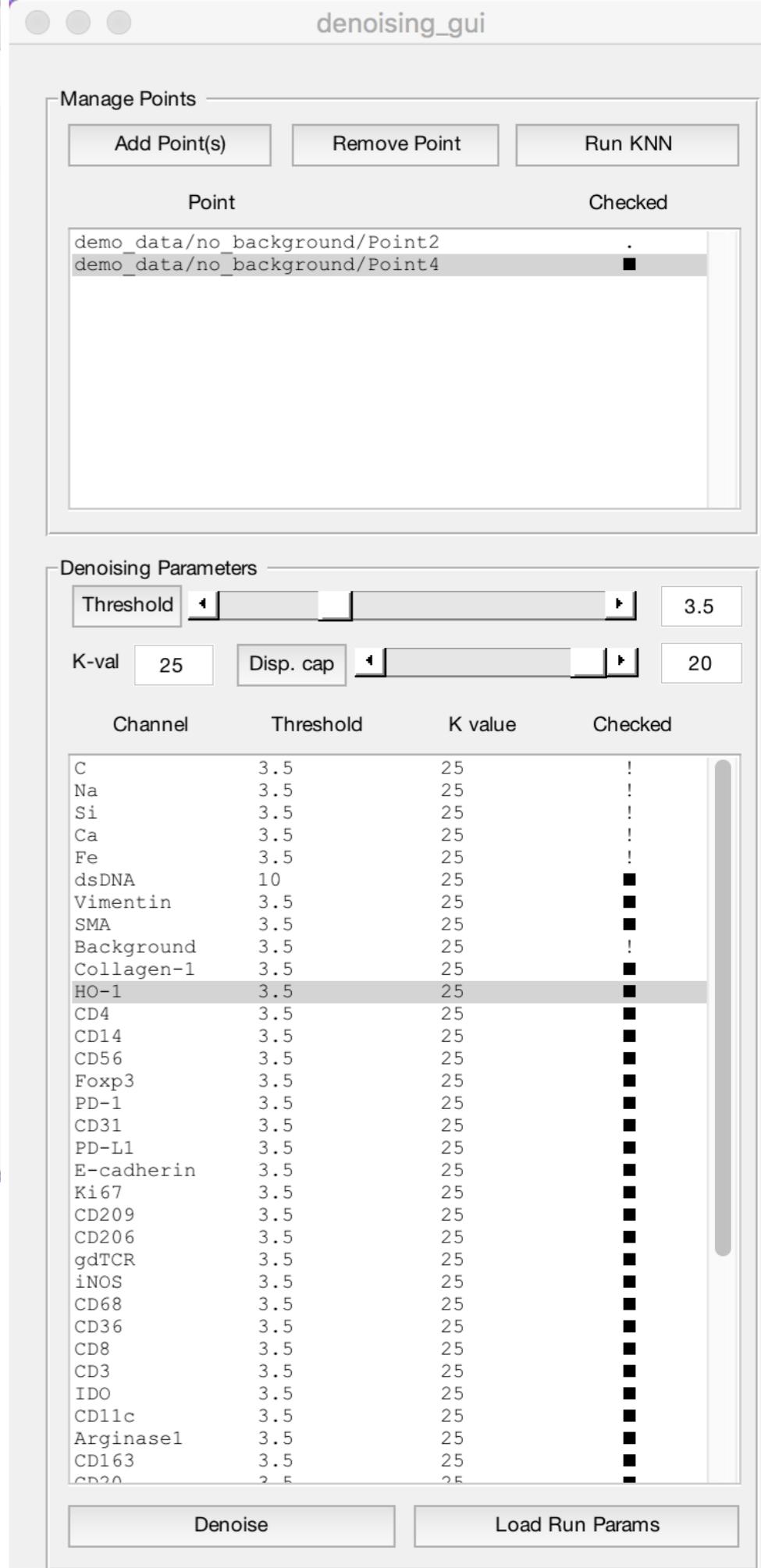
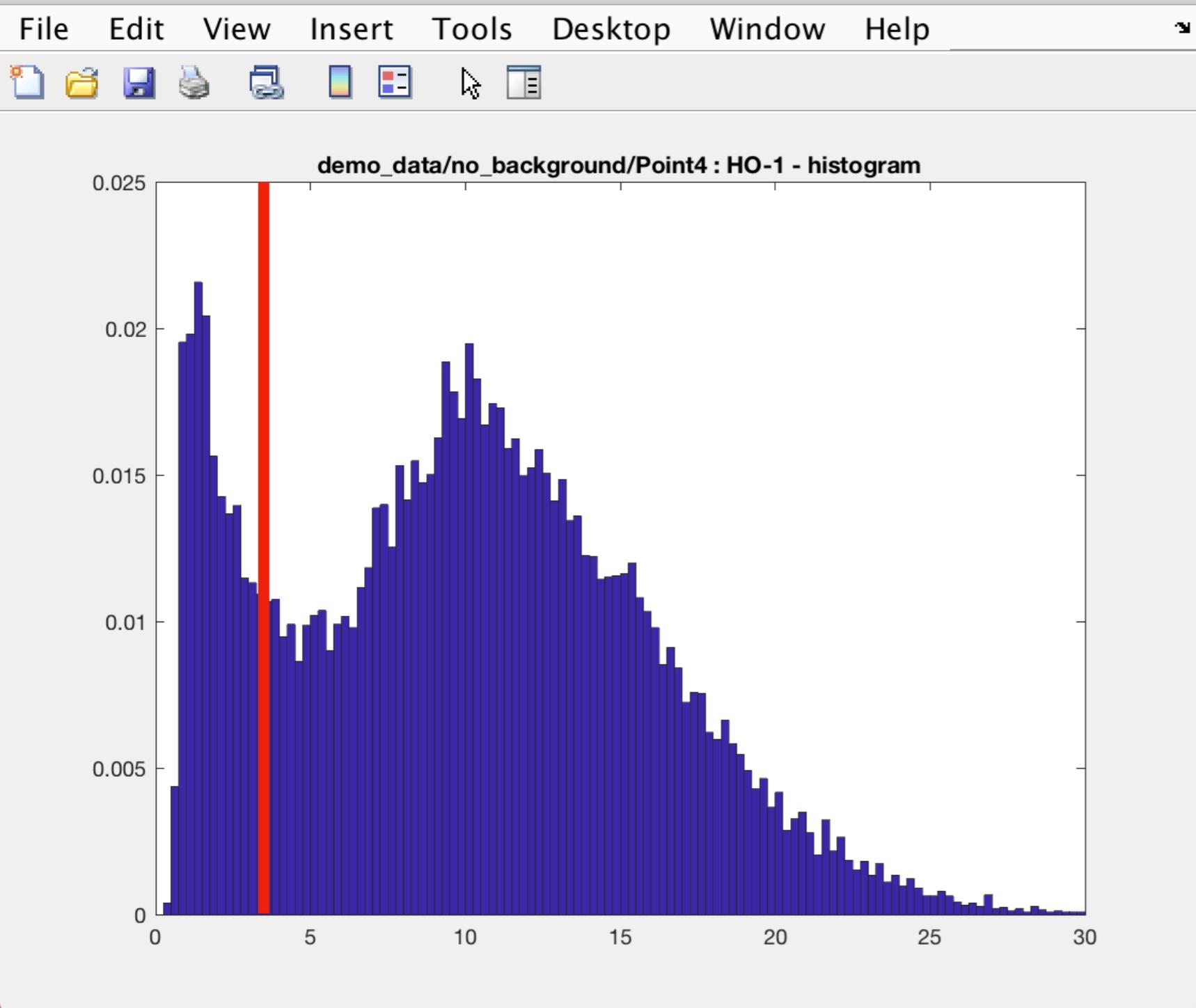
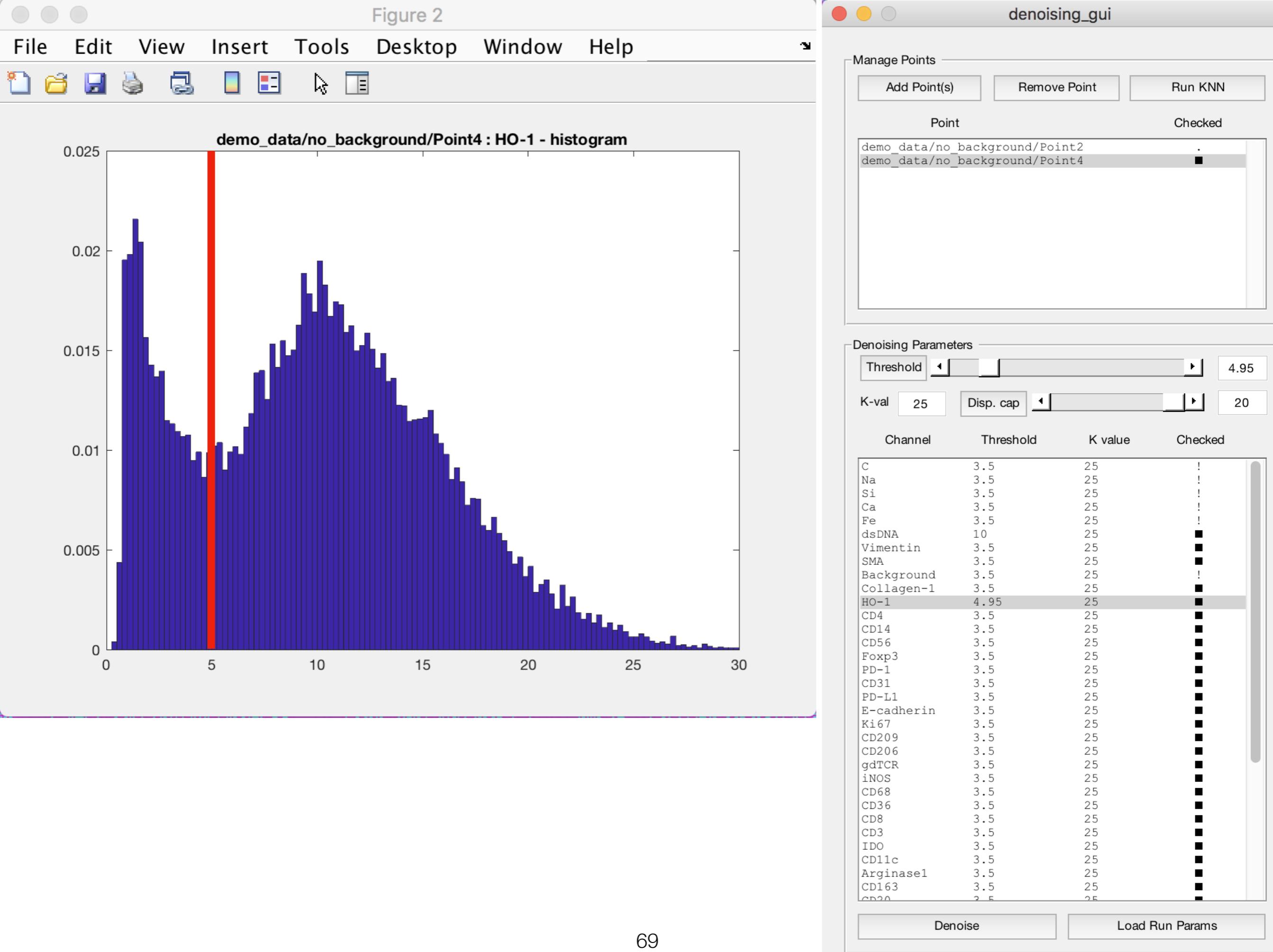
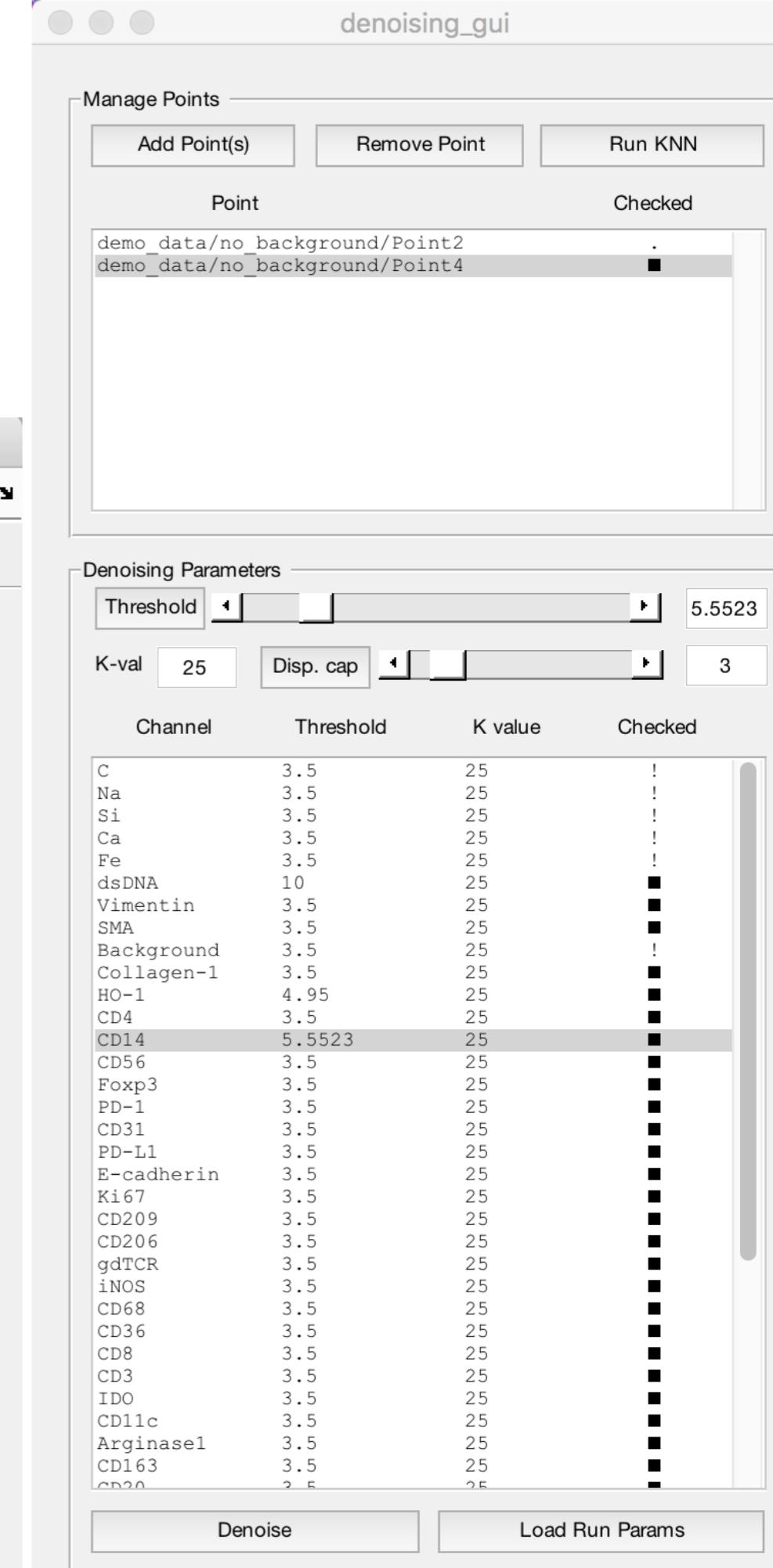
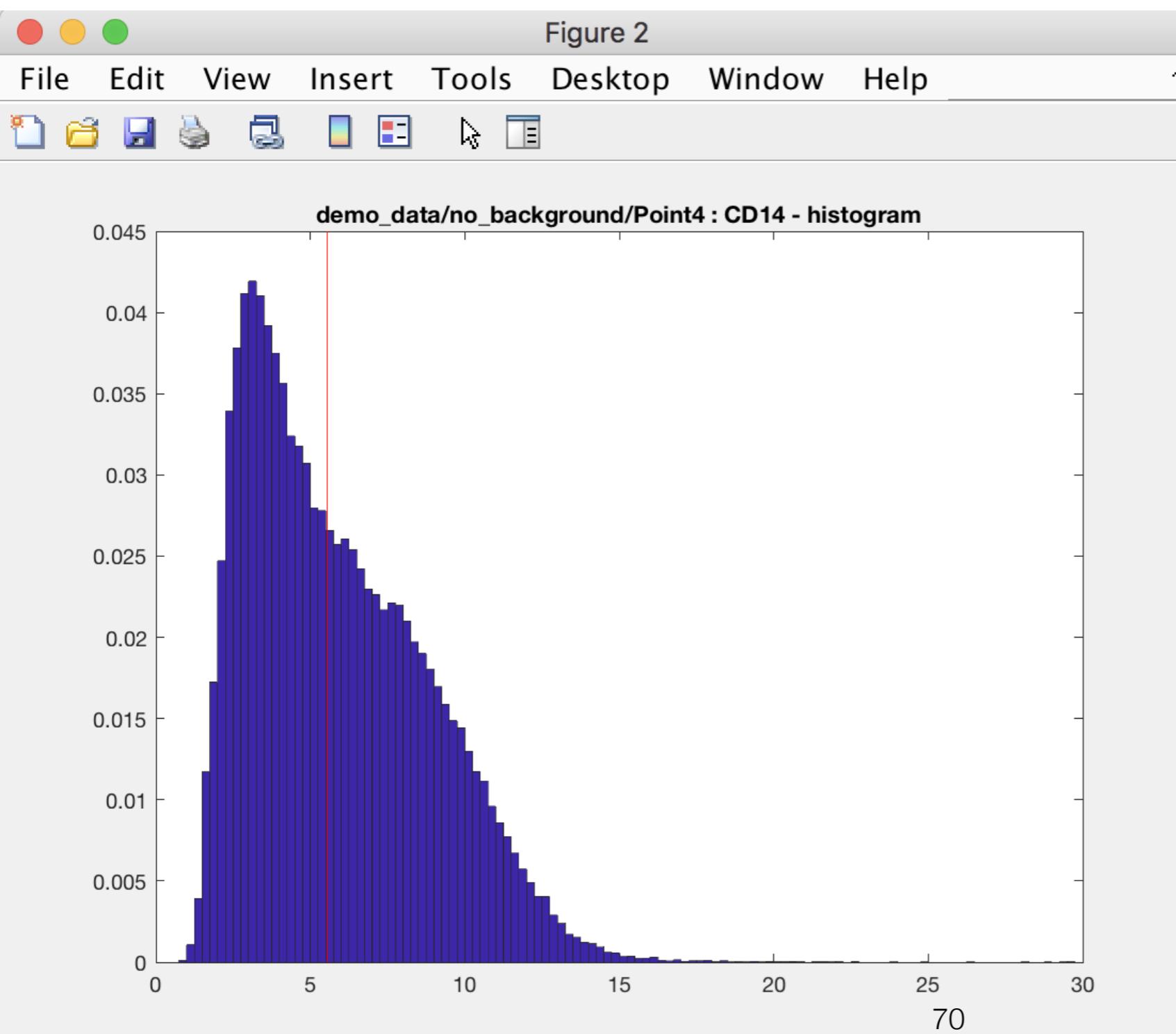


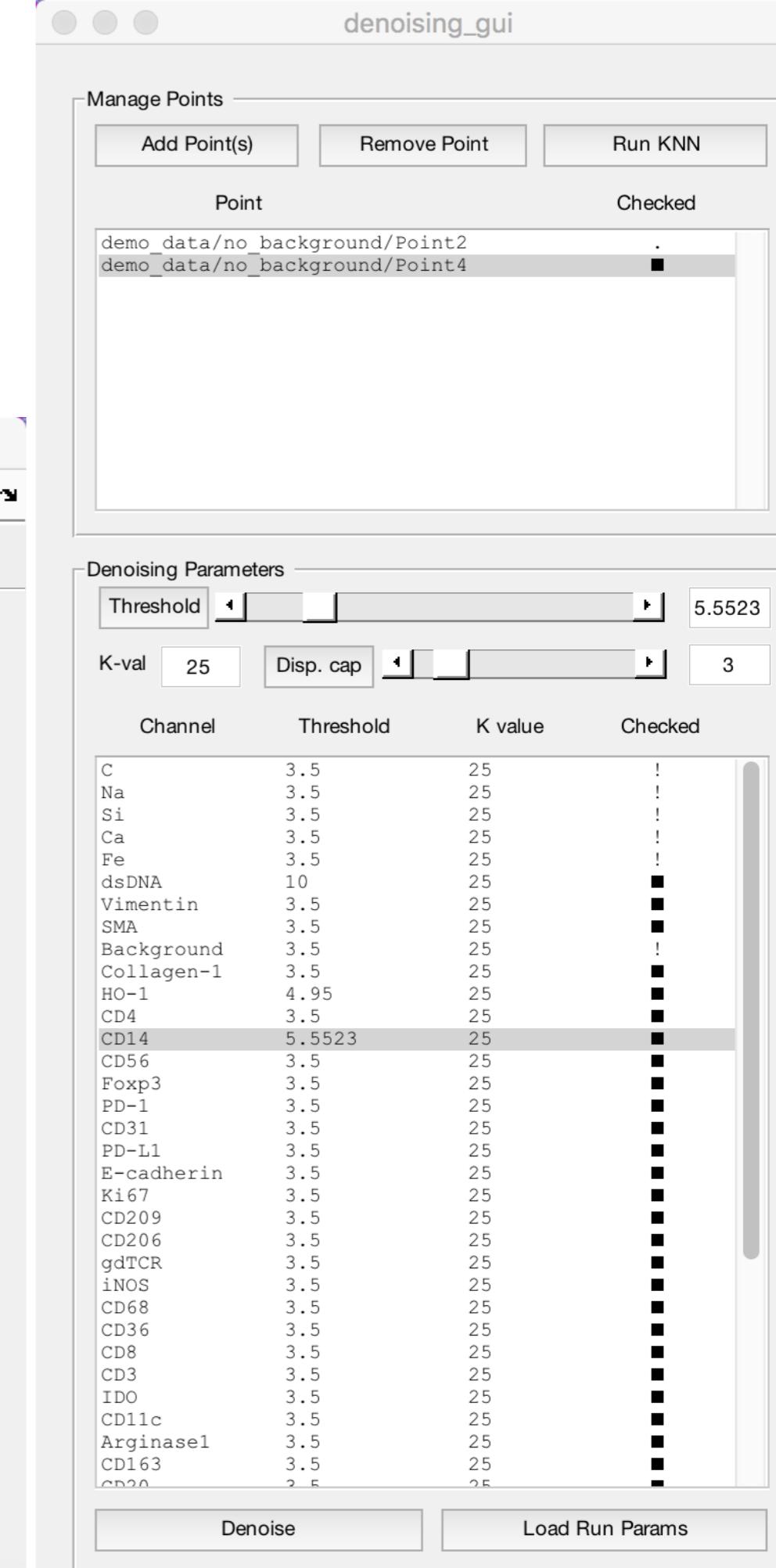
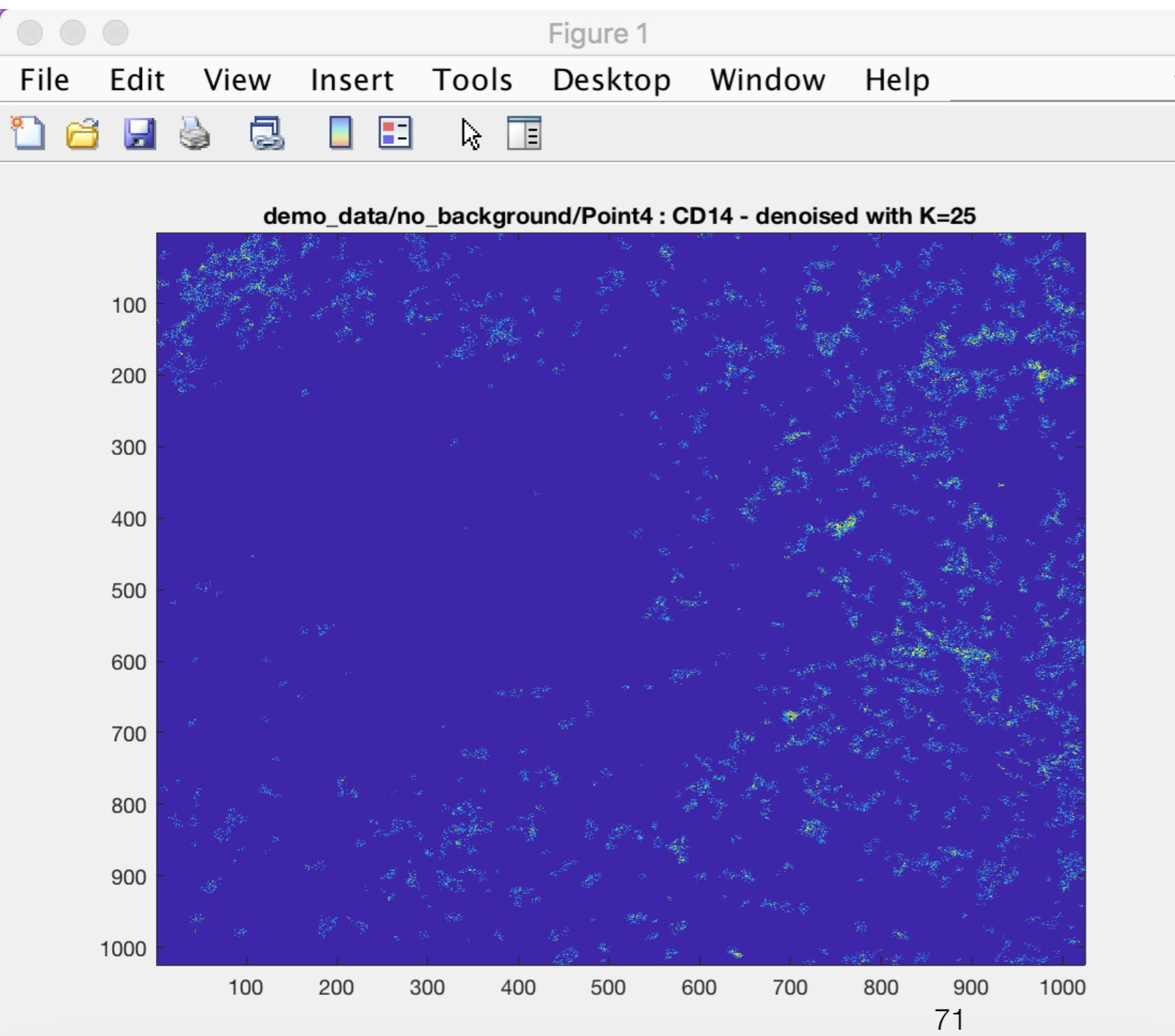
Figure 2



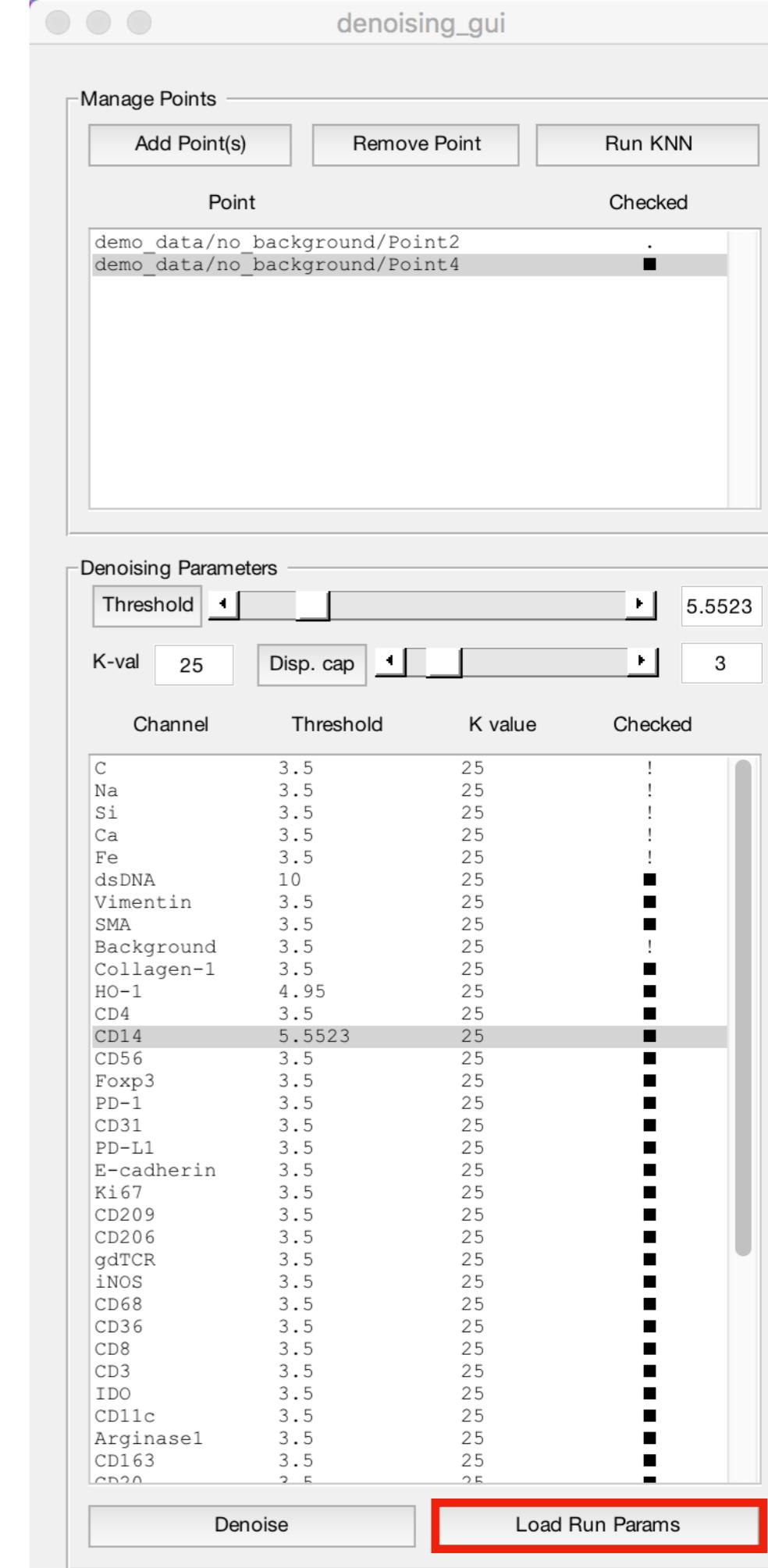
CD14 is an example where there's a lot of noise compared to the signal, so you need to use more biological expertise



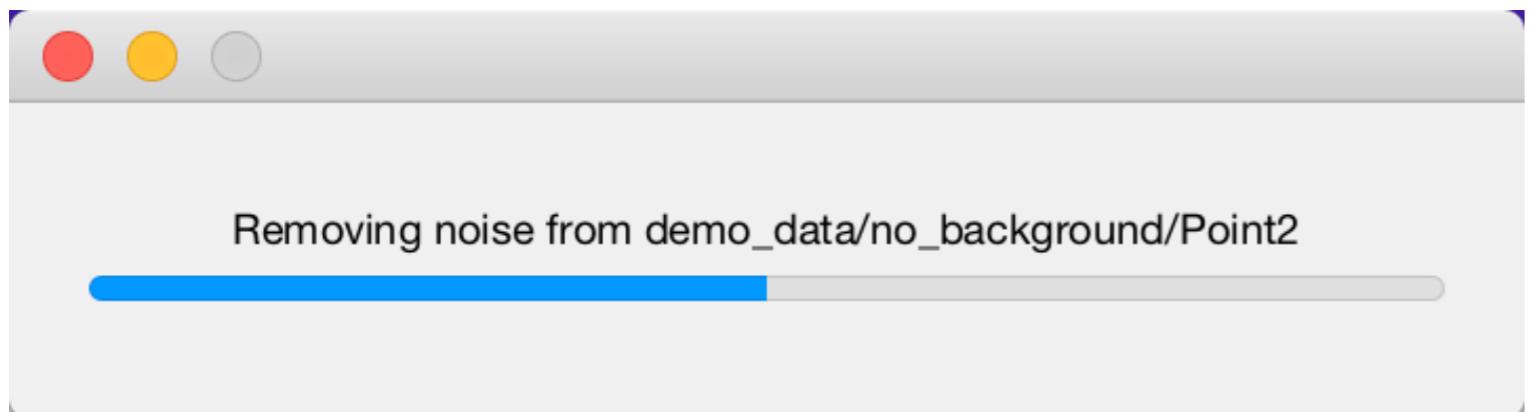
CD14 is an example where there's a lot of noise compared to the signal, so you need to use more biological expertise



If you've already done some denoising and want to reuse the parameters, click the “Load Run Params” button and navigate to a denoising log file. Selecting it will load the parameters saved in the log file



Once you've found denoising parameters you like, click the "Denoise" button. This may take a while, especially if you didn't ignore the high-density channels like Gold

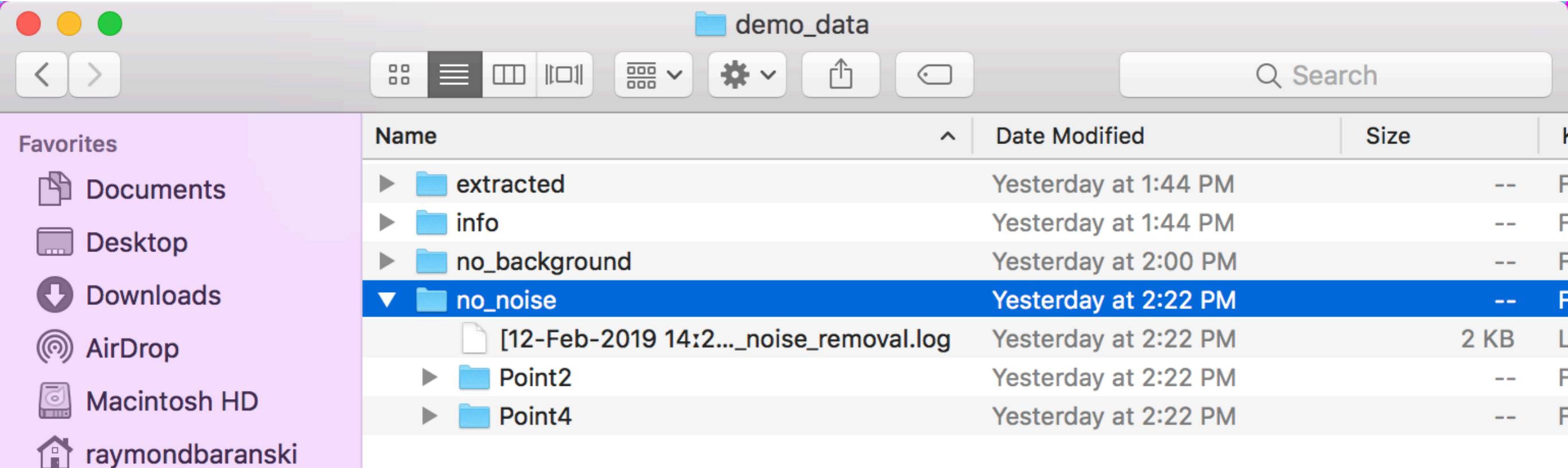


Note: The GUI automatically does the KNN calculation and noise removal for all points and channels you have explicitly ignored

A screenshot of the "denoising\_gui" application. The top section, "Manage Points", contains buttons for "Add Point(s)", "Remove Point", and "Run KNN". Below this is a table with columns "Point" and "Checked". Two rows are visible: "demo data/no background/Point2" and "demo data/no background/Point4", with the latter being checked. The bottom section, "Denoising Parameters", includes input fields for "Threshold" (set to 3.5), "K-val" (set to 25), and "Disp. cap" (set to 20). A large table below lists "Channel", "Threshold", "K value", and "Checked" status for various channels. The "SMA" channel is highlighted with a gray background and has its "Checked" status set to true. At the bottom are buttons for "Denoise" (which is redboxed) and "Load Run Params".

Channel	Threshold	K value	Checked
C	3.5	25	!
Na	3.5	25	!
Si	3.5	25	!
Ca	3.5	25	!
Fe	3.5	25	!
dsDNA	10	25	!
Vimentin	3.5	25	!
SMA	3.5	25	■
Background	3.5	25	!
Collagen-1	3.5	25	■
HO-1	4.95	25	■
CD4	3.5	25	■
CD14	5.5523	25	■
CD56	3.5	25	■
Foxp3	3.5	25	■
PD-1	3.5	25	■
CD31	3.5	25	■
PD-L1	3.5	25	■
E-cadherin	3.5	25	■
Ki67	3.5	25	■
CD209	3.5	25	■
CD206	3.5	25	■
gdTCR	3.5	25	■
iNOS	3.5	25	■
CD68	3.5	25	■
CD36	3.5	25	■
CD8	3.5	25	■
CD3	3.5	25	■
IDO	3.5	25	■
CD11c	3.5	25	■
Arginase1	3.5	25	■
CD163	3.5	25	■
cn20	2.5	25	■

# The denoised data will be saved to a new folder called no\_noise



# This folder will contain a denoising log file listing the parameters used for denoising

```
C: { not denoised }
Na: { not denoised }
Si: { not denoised }
Ca: { not denoised }
Fe: { not denoised }
dsDNA: {
    K-value: 25
    threshold: 10 }
Vimentin: {
    K-value: 25
    threshold: 3.5 }
SMA: {
    K-value: 25
    threshold: 3.5 }
Background: { not denoised
Collagen-1: {
    K-value: 25
    threshold: 3.5 }
H0-1: {
    K-value: 25
    threshold: 4.95 }
CD4: {
    K-value: 25
    threshold: 3.5 }
CD14: {
    K-value: 25
    threshold: 5.5523 }
CD56: {
    K-value: 25
    threshold: 3.5 }
```

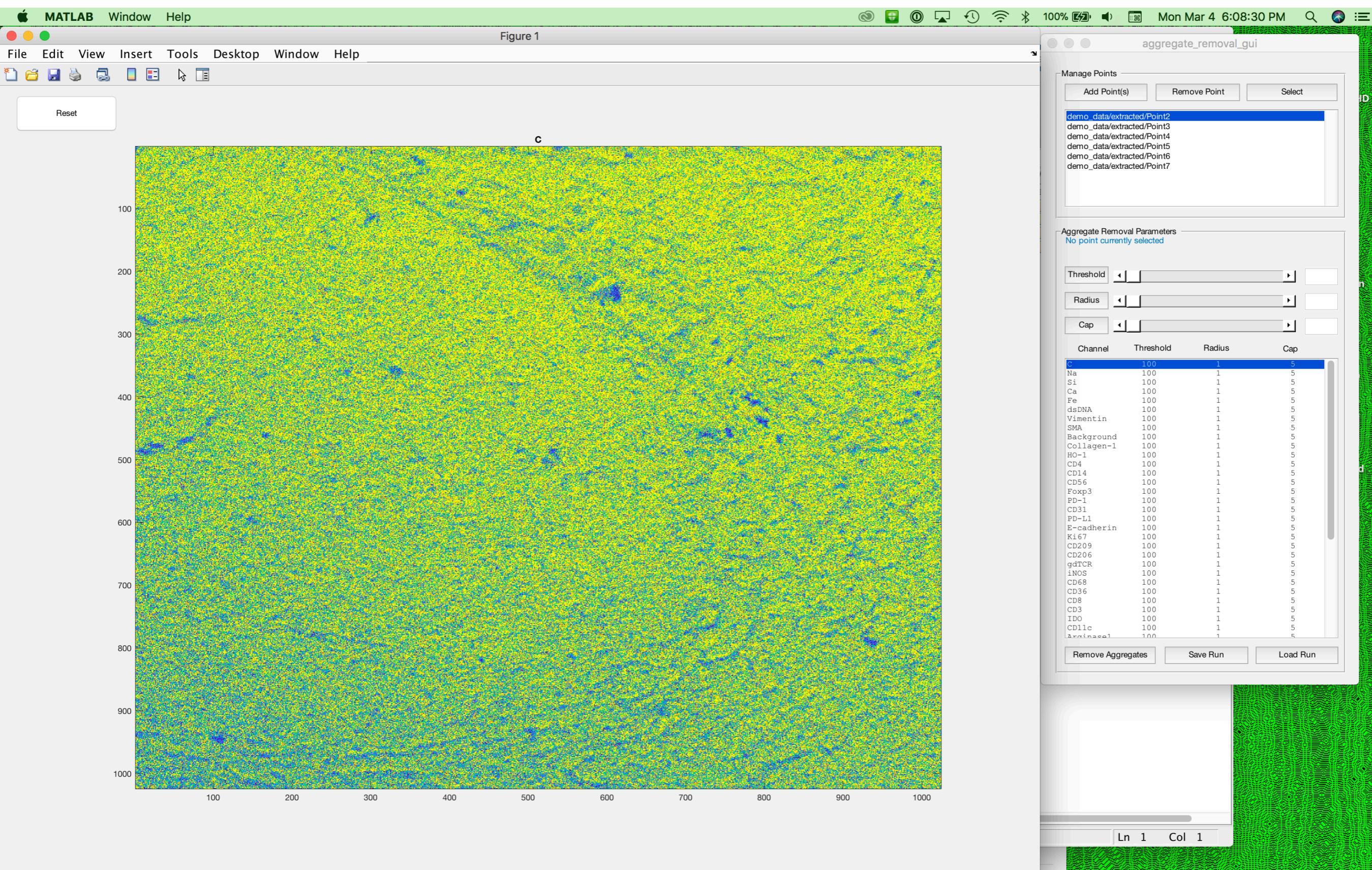
# Aggregate Removal

- Aggregates are an experimental artifact resulting from antibodies clumping together
- If you know the biology of the marker in question, they can be relatively easy to identify and remove
- Each channel is blurred with a gaussian (controlled by the Gaussian Radius parameter)
- Then contiguous areas are found. Areas with fewer than Threshold pixels are removed from the image

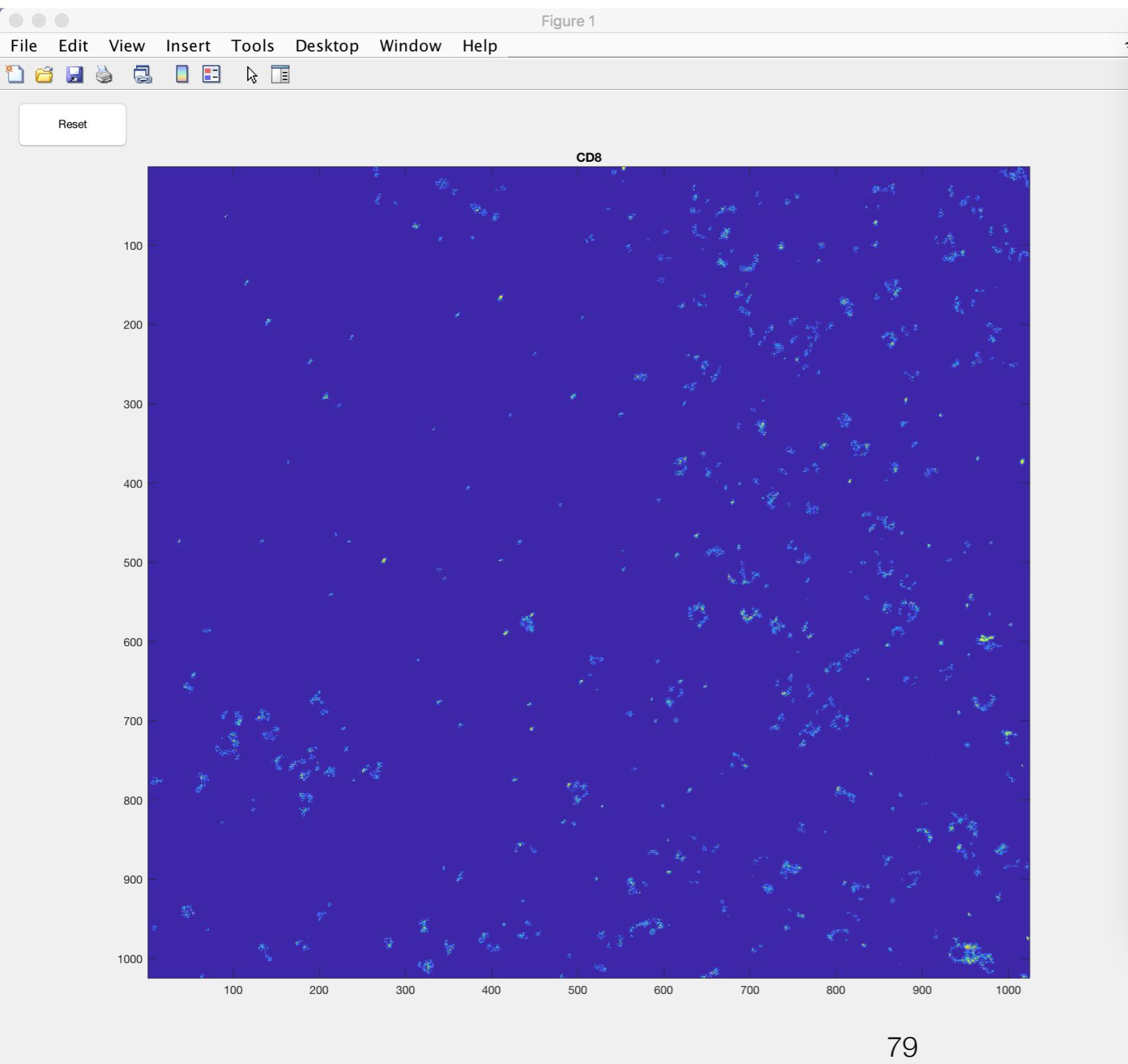
# Aggregate Removal

- Open the script “aggregate\_removal.m” and run it
- Load the points you want to remove aggregates from (probably from the folder called “no\_noise”)

# Aggregate removal GUI



The Cap slider just change the dynamic range of the displayed image (this image has no aggregates removed)



aggregate\_removal\_gui

Manage Points

Add Point(s) Remove Point Select

demo\_data/no\_noise/Point2  
demo\_data/no\_noise/Point4

Aggregate Removal Parameters  
No point currently selected

Threshold	<input type="text" value="1"/>	<input type="button" value="▼"/>	<input type="button" value="▼"/>	0
Radius	<input type="text" value="1"/>	<input type="button" value="▼"/>	<input type="button" value="▼"/>	10
Cap	<input type="text" value="1"/>	<input type="button" value="▼"/>	<input type="button" value="▼"/>	5

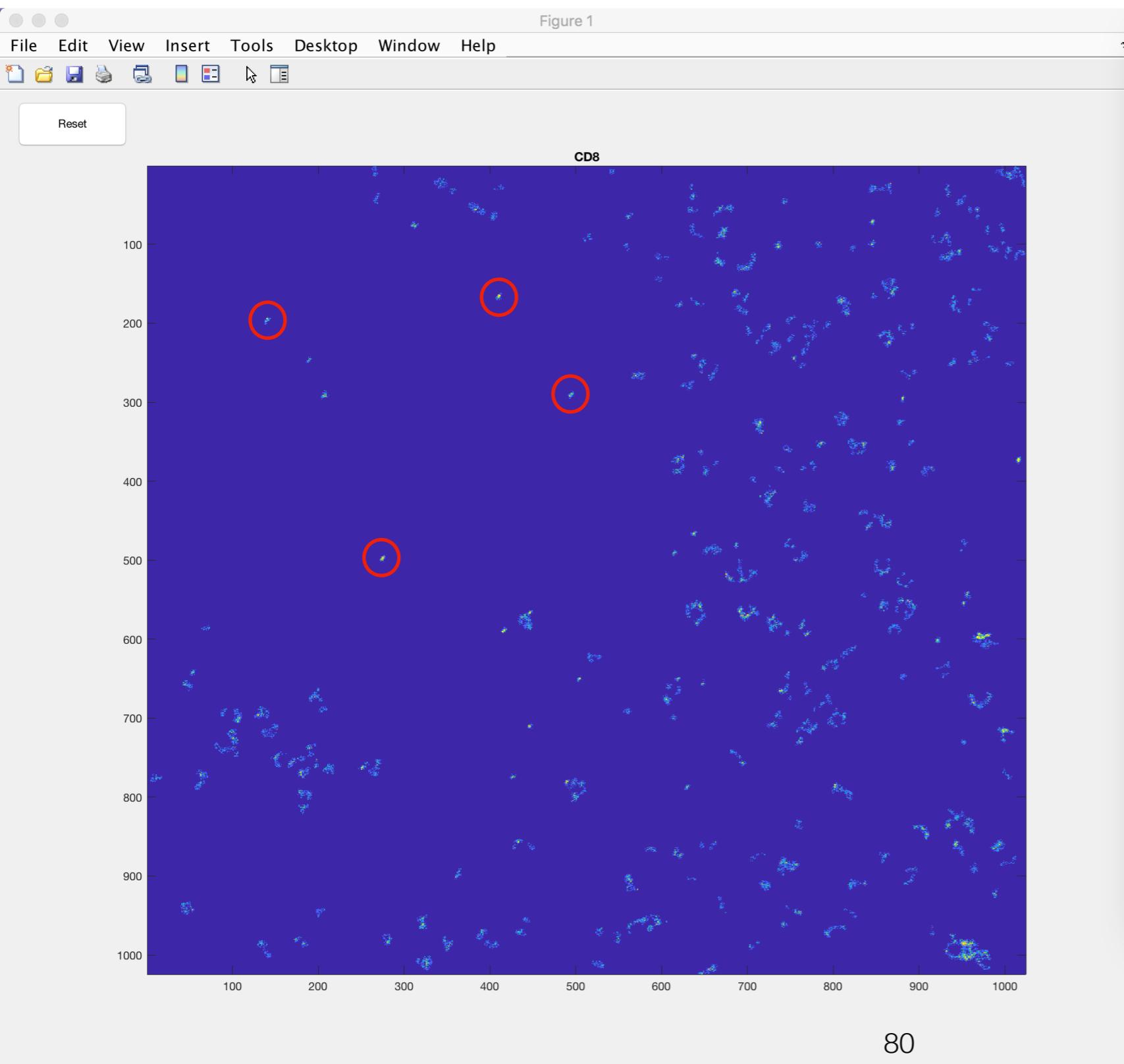
Channel Threshold Radius Cap

Channel	Threshold	Radius	Cap
C	100	1	5
Na	100	1	5
Si	100	1	5
Ca	100	1	5
Fe	100	1	5
dsDNA	100	1	5
Vimentin	100	1	5
SMA	100	1	5
Background	100	1	5
Collagen-1	100	1	5
HO-1	100	1	5
CD4	100	1	5
CD14	100	1	5
CD56	100	1	5
Foxp3	100	1	5
PD-1	100	1	5
CD31	100	1	5
PD-L1	100	1	5
E-cadherin	100	1	5
Ki67	100	1	5
CD209	100	1	5
CD206	100	1	5
gdTCR	100	1	5
iNOS	100	1	5
CD68	100	1	5
CD36	100	1	5
CD8	0	10	5
CD3	100	1	5
IDO	100	1	5
CD11c	100	1	5
Arabinosol	100	1	5

Remove Aggregates Save Run Load Run

# Threshold = 100, Radius = 1

## There are still many aggregates



Manage Points

Add Point(s) Remove Point Select

demo\_data/no\_noise/Point2  
demo\_data/no\_noise/Point4

Aggregate Removal Parameters  
No point currently selected

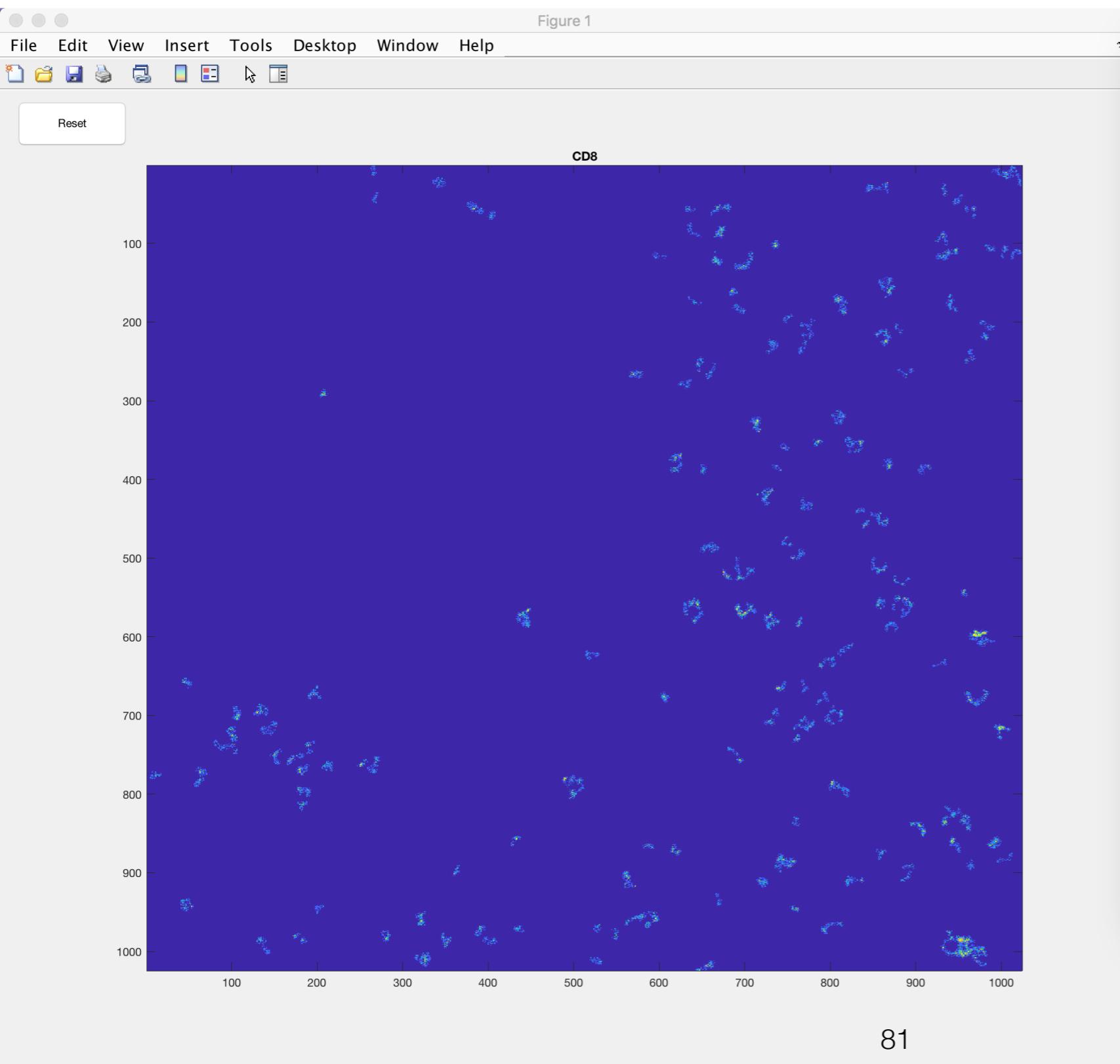
Threshold	<input type="text" value="100"/>
Radius	<input type="text" value="1"/>
Cap	<input type="text" value="5"/>

Channel	Threshold	Radius	Cap
C	100	1	5
Na	100	1	5
Si	100	1	5
Ca	100	1	5
Fe	100	1	5
dsDNA	100	1	5
Vimentin	100	1	5
SMA	100	1	5
Background	100	1	5
Collagen-1	100	1	5
HO-1	100	1	5
CD4	100	1	5
CD14	100	1	5
CD56	100	1	5
Foxp3	100	1	5
PD-1	100	1	5
CD31	100	1	5
PD-L1	100	1	5
E-cadherin	100	1	5
Ki67	100	1	5
CD209	100	1	5
CD206	100	1	5
gdTCR	100	1	5
iNOS	100	1	5
CD68	100	1	5
CD36	100	1	5
CD8	100	1	5
CD3	100	1	5
IDO	100	1	5
CD11c	100	1	5
Arainaco1	100	1	5

Remove Aggregates Save Run Load Run

This panel contains the 'aggregate\_removal\_gui'. It includes a 'Manage Points' section with buttons for 'Add Point(s)', 'Remove Point', and 'Select'. A list box shows 'demo\_data/no\_noise/Point2' and 'demo\_data/no\_noise/Point4'. Below this is an 'Aggregate Removal Parameters' section with sliders for 'Threshold' (set to 100), 'Radius' (set to 1), and 'Cap' (set to 5). A table lists parameters for various channels, with 'CD8' highlighted. At the bottom are buttons for 'Remove Aggregates', 'Save Run', and 'Load Run'.

**Threshold = 100, Radius = 0.5**  
**There are still some aggregates,  
 but looks a lot better**



aggregate\_removal\_gui

Manage Points

Add Point(s) Remove Point Select

demo\_data/no\_noise/Point2  
demo\_data/no\_noise/Point4

Aggregate Removal Parameters

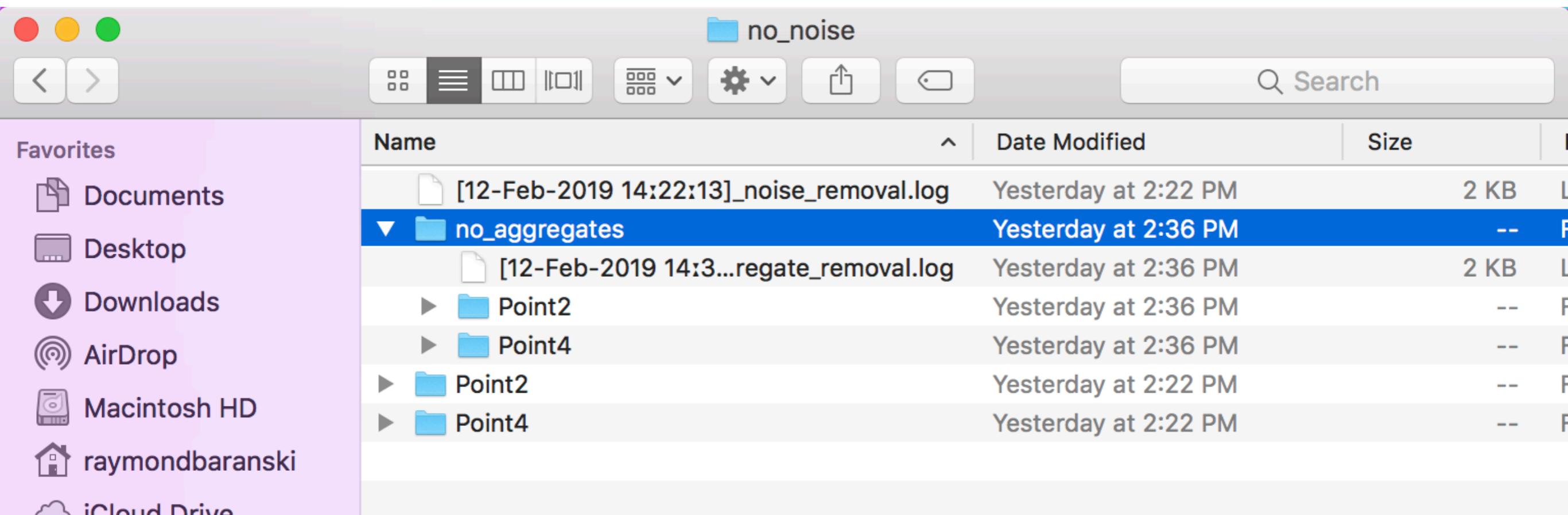
No point currently selected

Threshold	<input type="range"/>	100
Radius	<input type="range"/>	0.5
Cap	<input type="range"/>	5

Channel	Threshold	Radius	Cap
C	100	1	5
Na	100	1	5
Si	100	1	5
Ca	100	1	5
Fe	100	1	5
dsDNA	100	1	5
Vimentin	100	1	5
SMA	100	1	5
Background	100	1	5
Collagen-1	100	1	5
HO-1	100	1	5
CD4	100	1	5
CD14	100	1	5
CD56	100	1	5
Foxp3	100	1	5
PD-1	100	1	5
CD31	100	1	5
PD-L1	100	1	5
E-cadherin	100	1	5
Ki67	100	1	5
CD209	100	1	5
CD206	100	1	5
gdTCR	100	1	5
iNOS	100	1	5
CD68	100	1	5
CD36	100	1	5
<b>CD8</b>	<b>100</b>	<b>0.5</b>	<b>5</b>
CD3	100	1	5
IDO	100	1	5
CD11c	100	1	5
Arginase1	100	1	5

Remove Aggregates Save Run Load Run

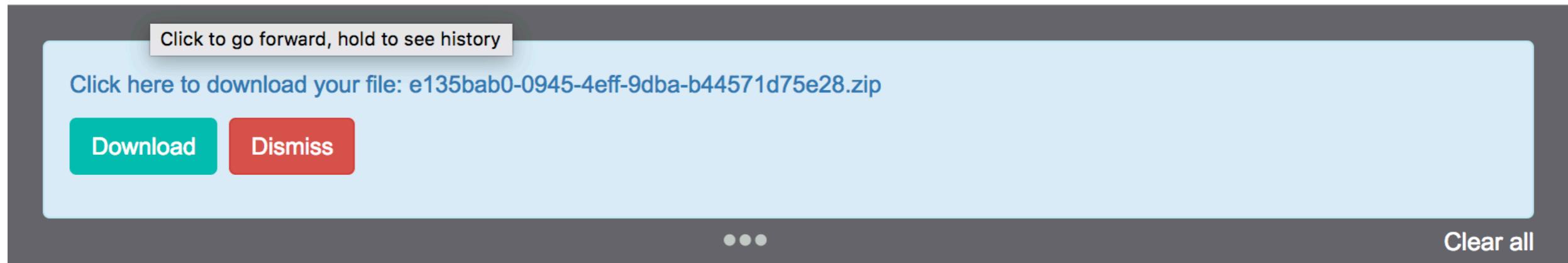
# Now the aggregate-removed data is saved into a folder called no\_aggregates



# no\_aggregates will contain an aggregate removal log recording the parameters you used for aggregate removal

```
CD206: {  
    threshold: 100 }  
    radius: 1 }  
gdTCR: {  
    threshold: 100 }  
    radius: 1 }  
iNOS: {  
    threshold: 100 }  
    radius: 1 }  
CD68: {  
    threshold: 100 }  
    radius: 1 }  
CD36: {  
    threshold: 100 }  
    radius: 1 }  
CD8: {  
    threshold: 100 }  
    radius: 0.5 }  
CD3: {  
    threshold: 100 }  
    radius: 1 }  
IDO: {  
    threshold: 100 }  
    radius: 1 }  
CD11c: {  
    threshold: 100 }  
    radius: 1 }  
Arginase1: {  
    threshold: 100 }  
    radius: 1 }  
CD163: {  
    threshold: 100 }  
    radius: 1 }  
CD20: {  
    threshold: 100 }  
    radius: 1 }  
CD16: {  
    threshold: 100 }  
    radius: 1 }
```

# Downloading data from IonPath



Run History					
	Name	Description	View Images	Download Images	Share
▶	TissuetilingMediumResFields	Run TissuetilingMediumResFields i...	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
▶	s30_ROI1_ROI2	Run s30_ROI1_ROI2 image set	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
▶	181019_TA239_DCIS_Outcome_M...	Run 181019_TA239_DCIS_Outco...	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
▶	181011_Ta239_DCIS_Outcome	Run 181011_Ta239_DCIS_Outcom...	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
▶	decidua181105test	Run decidua181105test image set	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
▶	decidua181107	Run decidua181107 image set	<button>View</button>	<button>Download</button>	<input type="checkbox"/>
...					

The basics of low-level MIBI  
data processing are complete