VoltView On-site analysis

1. System requirements:

Software dependencies:

Code writing and testing was done in Windows 10:
- Matlab 2021b
- Anaconda3 2021.11 (Python 3.9.7 64-bit)
- Cellpose 1.0

Hardware requirement:

- minimum 8 GB memory but since files are large, 16 GB is advisory, 32 GB is optimal

2. Installation Guide

Instructions:

1: Install Anaconda3 - installer version below is provided in the "Voltage_Seq_VoltView_DEMO" folder
"Anaconda3-2021.11-Windows-x86_64_3.9.exe"

2: Install Cellpose 1.0 (minimal version without GUI) from:
(https://github.com/MouseLand/cellpose)
steps from the link:
- Open an anaconda prompt / command prompt which has conda for python 3 in the path
- Create a new environment with "conda create --name cellpose python=3.8"
(We recommend python 3.8, but python 3.9 and 3.10 will likely work as well.)
- To activate this new environment, run "conda activate cellpose"
- To install the minimal version of cellpose, run "python -m pip install cellpose"

(Typical install time on a "normal" desktop computer < 2 min)

3: Install Matlab 2021b
(Typical install time on a "normal" desktop computer ~30 min)

4: Download "bfmatlab" folder from "Voltage_Seq_VoltView_DEMO" folder and add it to Matlab path
(in Matlab: right click on folder/Add to path/Selected folders and subfolders)

5: Download "VoltView_On_site" folder from the "Voltage_Seq_VoltView_DEMO" folder
on the provided link (no install time)

Instructions to run VoltView on Demo data

1: in the folder "VoltView_On_site", open "Start_Python_Setup_Cellpose.m" add the path of Cellpose installation instead of the given example, and save:

"python_installation = 'C:\Users\Janos\.conda\envs\cellpose\python.exe';"

2: "Start_Python_Setup_Cellpose.m" has to be ran only once per Matlab session (lack of running this, or double running can be fixed with restarting Matlab), when running well the output will be:

```
>> Start_Python_Setup_Cellpose

ans =
PythonEnvironment with properties:

Version: "3.8"
Executable: "C:\Users\Janos\.conda\envs\cellpose\python.exe"
Library: "C:\Users\Janos\.conda\envs\cellpose\python38.dll"
Home: "C:\Users\Janos\.conda\envs\cellpose"
Status: NotLoaded
ExecutionMode: InProcess
```

3: from the "Voltage_Seq_VoltView_DEMO" folder download demo data (".CXD" files): (Data00042.cxd, Data00047.cxd, Data00057.cxd, Data00063.cxd, Data00089.cxd)
Download into the "VoltView_On_site" folder!

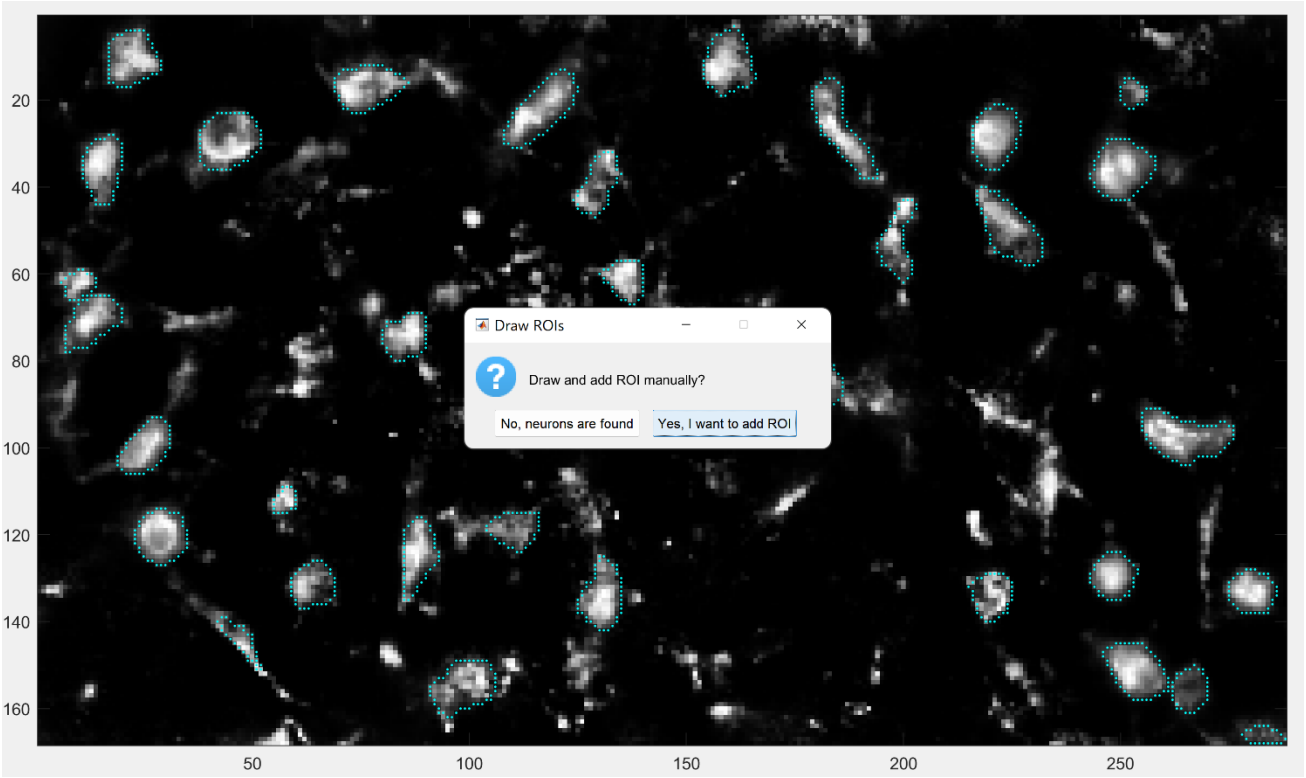
4: in the folder "VoltView_On_site", Open "Analyze_ONSITE_First.m" and set up the parameters:

For "c" set to 42 or 47 or 89

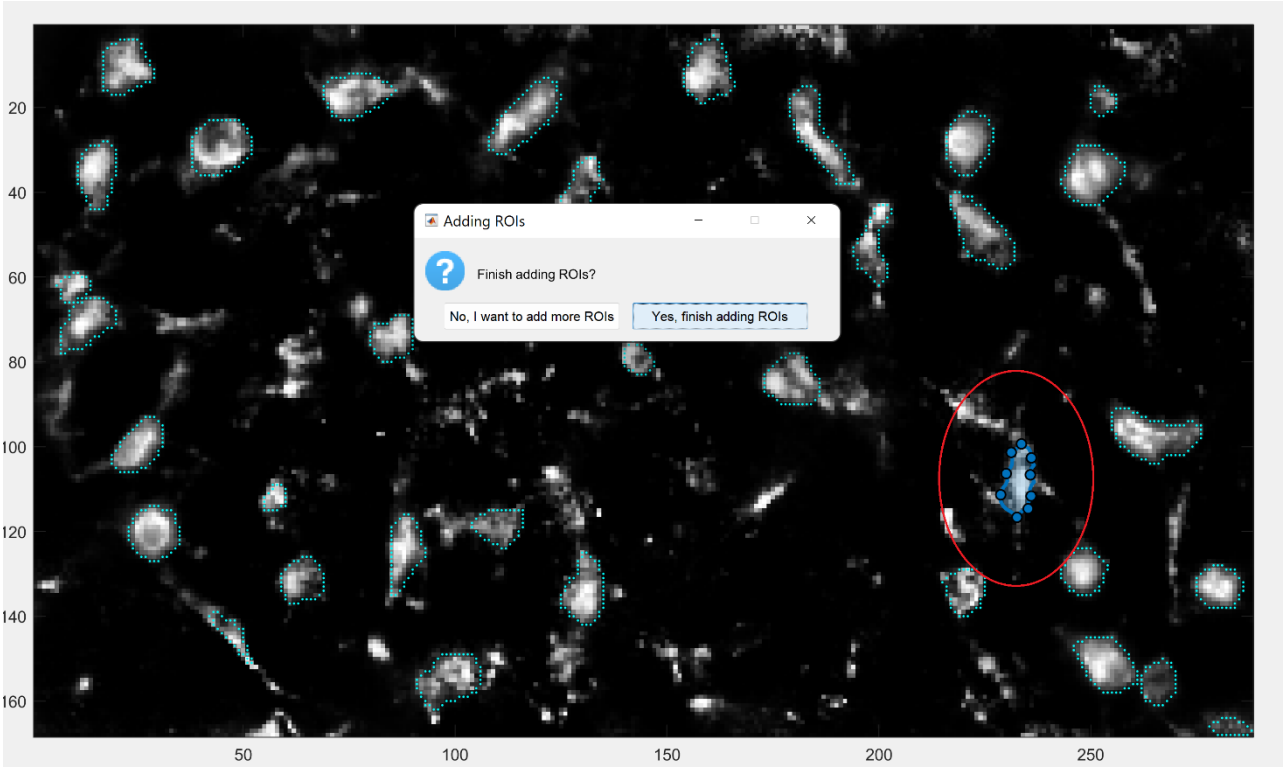
```
c = 42;
% choose file, "Control" (Rec1, to optionally compare with Rec2)
RE = 1;
% if RE=1 ROI Explorer opens, if 0 ROI Explorer is off
SomaDiam = 12;
% Set the size of soma to detect (diameter in pixels)
manual_ROI = 0;
% if manual_ROI = 1, user gets the option to manually draw additional ROIs
% if manual_ROI = 0, only detected ROIs will be used
Suggest_ROI = [99];
% clusters to suggest:
% leave empty = no clusters suggested (all ROIs white)
% add cluster numbers to label, e.g. [1 2 3]
% type [99] to label every ROI with its classified cluster
Discard_sweep = 0;
% Choose 1 (only 1!) sweep to discard (gets deleted from all ROIs)
```

Run:

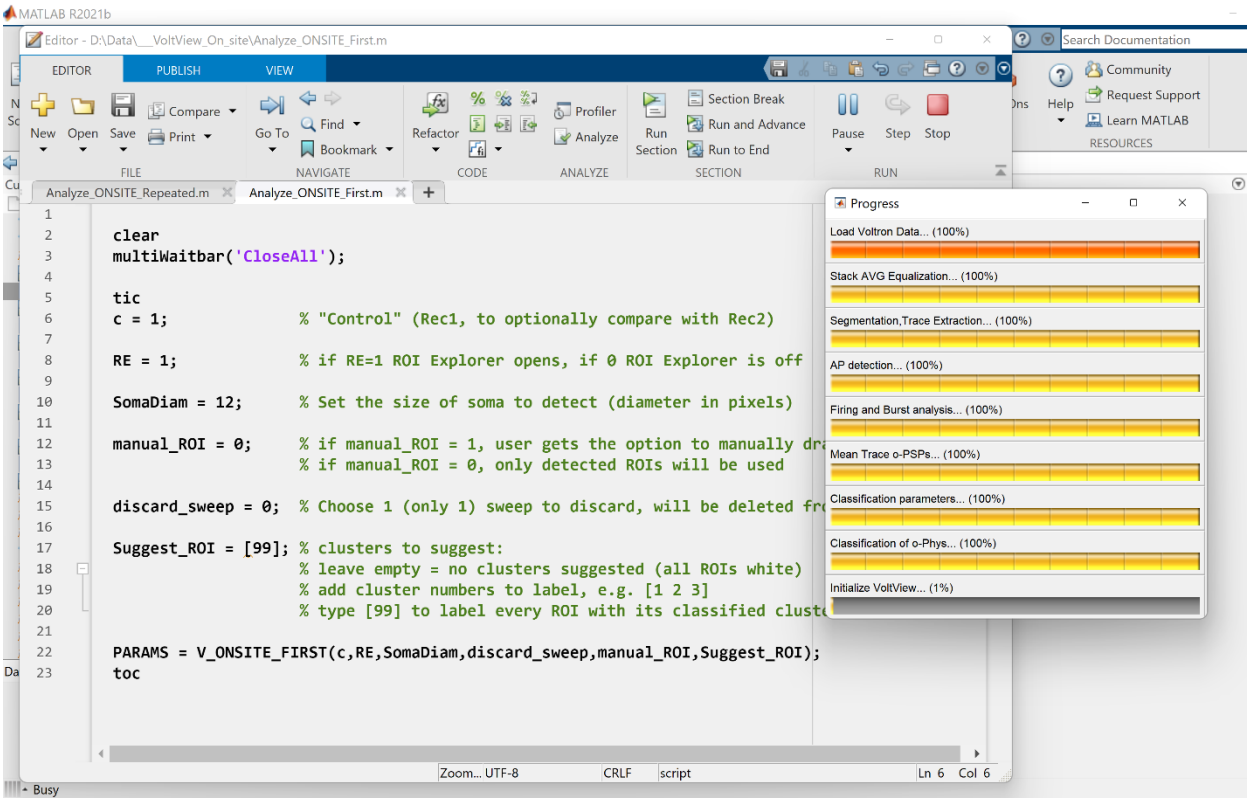
- after ROI segmentation VoltView analysis offers the option to have a quick overview and add ROIs manually if necessary:



- we draw a ROI, and can stop adding ROIs, or add more:

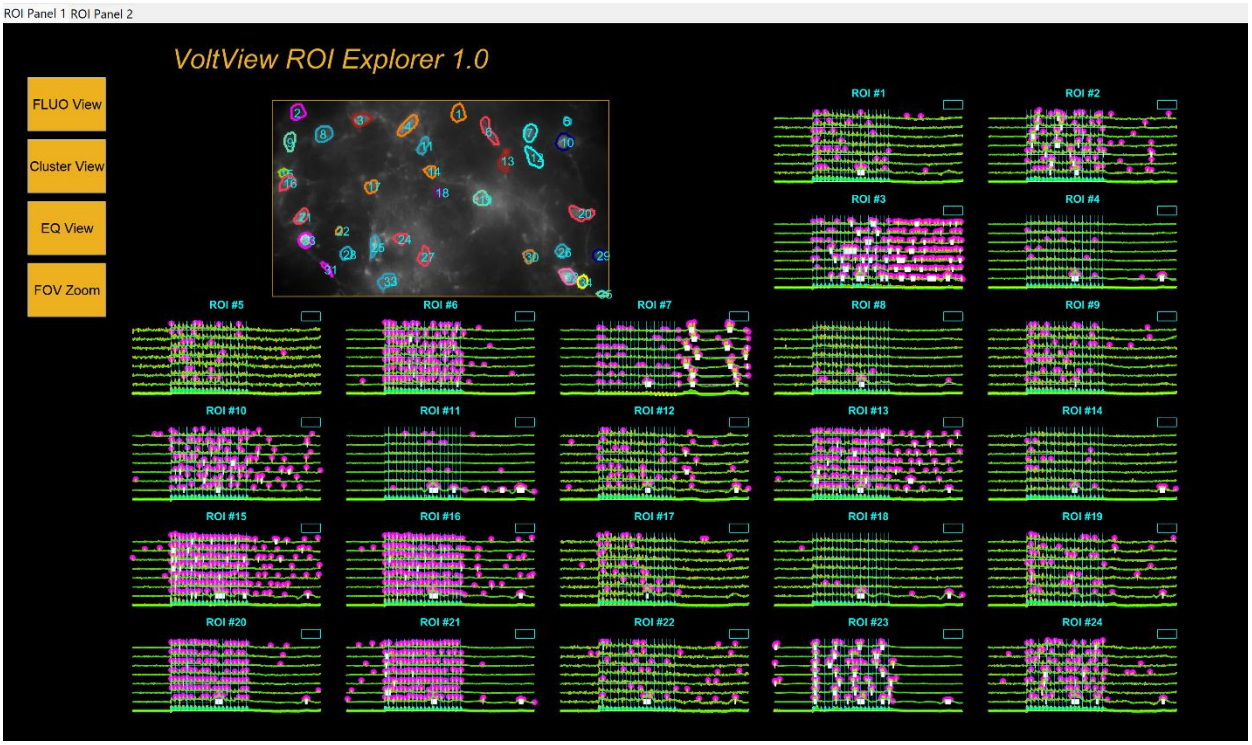


- during run the analysis modules are all completed (progress bars):



VoltView starts in less than a minute:

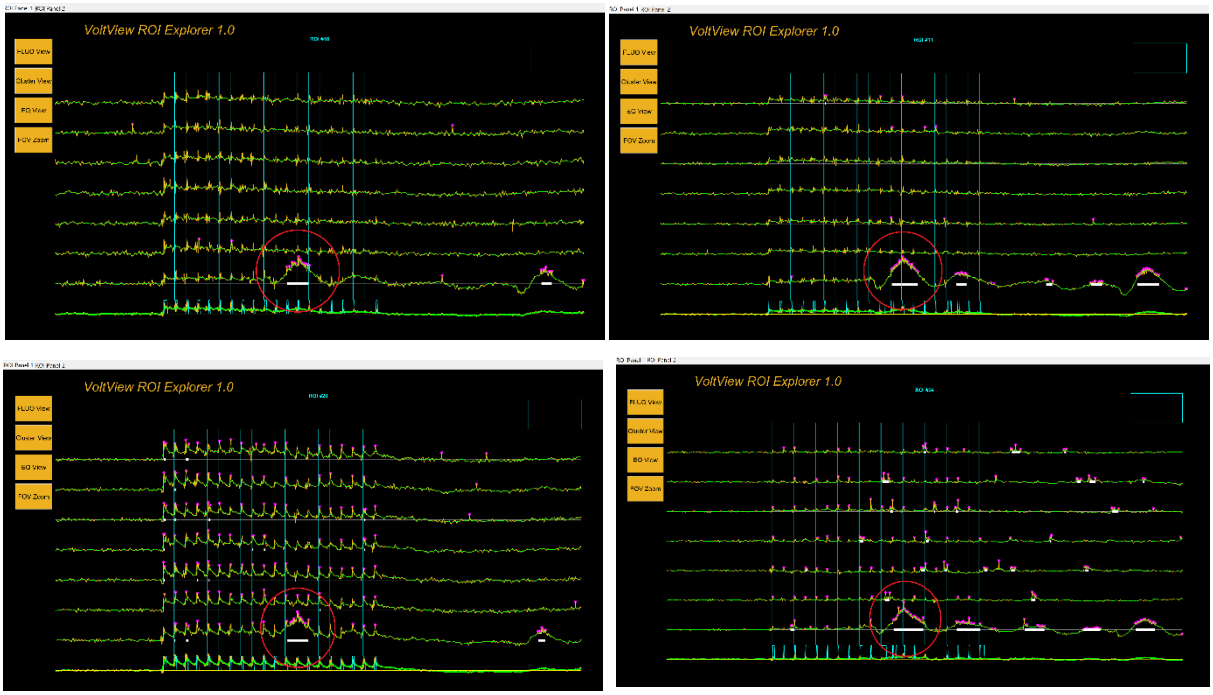
In this example “Suggest ROI” was set to [99], thus every ROI has a cluster ID color code from the VMH-PAG connectome classification done by the built-in classifier in VoltView.



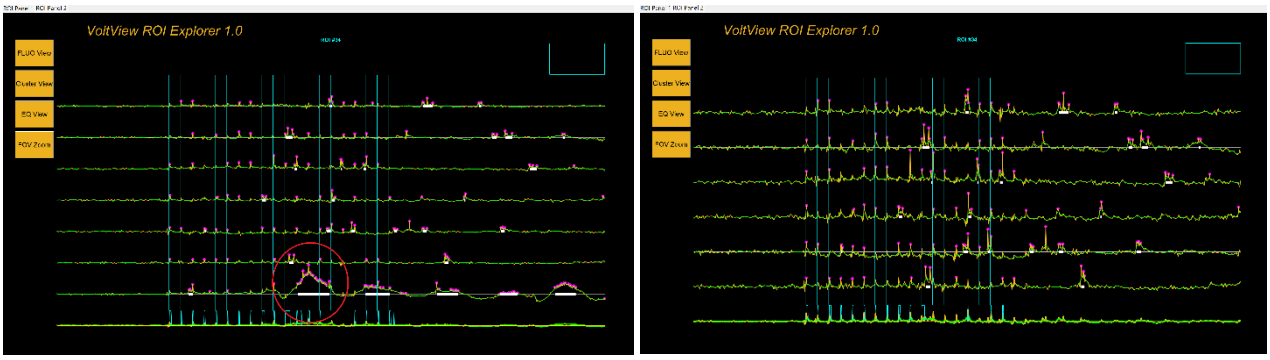
- ROIs are on multiple tabs (“**ROI Panel 1, ROI Panel 2**”), switch tabs to see them all in the top left corner

- **Cluster View button**: switches the ROI numbers to the cluster IDs, color coded by the cluster ID
- **EQ View button**: changes the default “Fluo View” to the local equalized and contrast enhanced Field of View (FOV) image with showing the ROI contours
- **FOV Zoom button**: at any View it is going to enlarge the FOV image so that Soma positions can be seen better
- Every ROI has a cyan rectangle in the top right corner, clicking there is going to enlarge the all-optical sweeps of the ROI with the o-APs lapeled magenta, o-Sub in green, Bursts underlined white

It may occur that one sweep has a large noise caused by a movement of sample, that is visible in many or all the ROIs, here are 4 example ROIs zoomed in with the cyan rectangle:

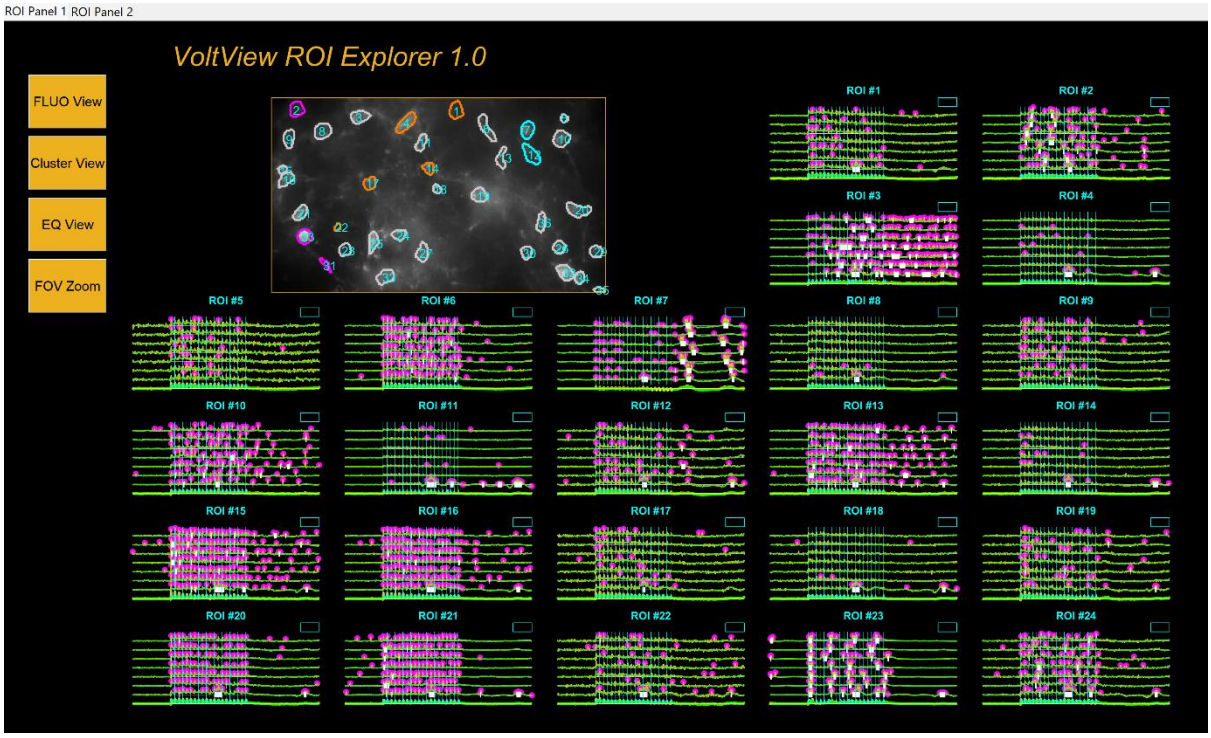


The user has the option to remove a sweep with setting “discard_sweep” to the number of the sweep to discard, this may prevent the distortion of scales and the averaging of parameters across sweeps; we discarded the 1st sweep and comparing the last ROI from just above we can observe the signal without the large artifact:



IMPORTANT: if we compare multiple videos, the number of sweeps should be equal, thus if we discard a sweep from the “First” analysis, we should discard one from the “Repeated” analysis as well.

If “Suggest ROI” is set to e.g. [3 8 15] we will only see the ROIs classified into these three clusters

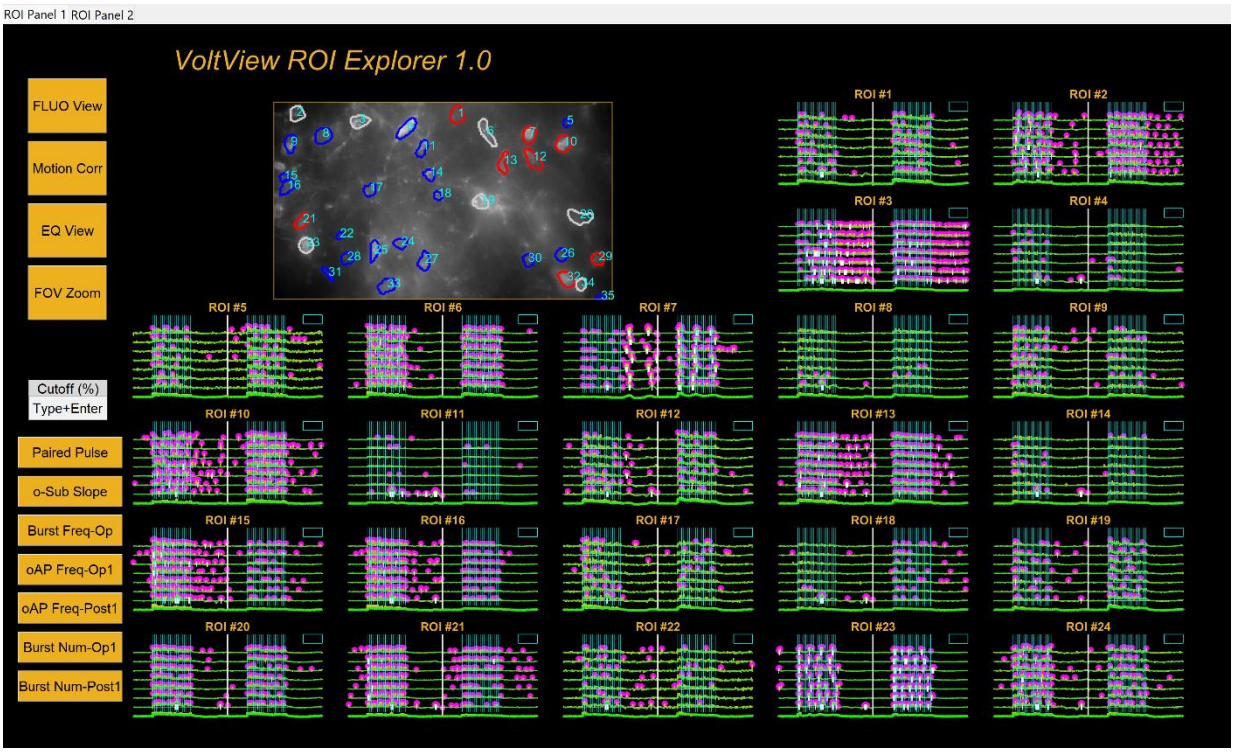


5: in the folder "VoltView_On_site", Open "Analyze_ONSITE_Repeated.m" and set up the parameters:
This analysis is for multiple video comparisons on-site where a video had to be first analyzed with "Analyze_ONSITE_First.m" (e.g. 42) and here we compare it to 57 by running "Analyze_ONSITE_Repeated.m"

- For “c” and “s” pairs set to c = 42 and s=57 OR c=47 and s=63

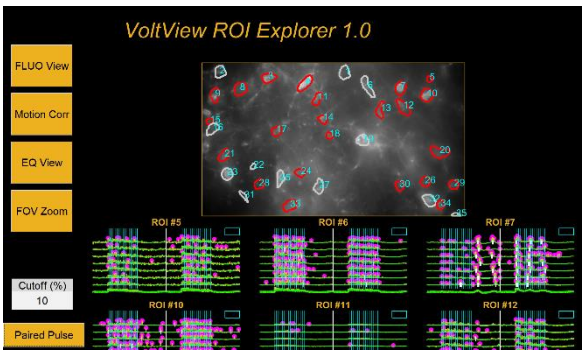
```
c = 42;
% "Control" (Rec1, to compare with Rec2)
s = 57;
 % "Shifted"/repeated/pharmacology (Rec2)
RE = 1;
% if RE=1 ROI Explorer opens, if 0 ROI Explorer is off
Param_Comp = 9;
% leave empty "[]": no parameter user-defined for Rec2 vs. Rec1
% OR set a parameter from the list below  e.g. [9] for Rec2 vs. Rec1
% IMPORTANT: value can be adjusted in the ROI Explorer as well
cutoff = 20;
% set "cutoff" e.g. [20] will cut at 20% increase and decrease of
"Param_Comp" for Rec2 vs. Rec1 comparison
% IMPORTANT: value can be adjusted in the ROI Explorer as well
discard_sweep = 0;
% Choose 1 (only 1!) sweep to discard (deleted from every ROI) if we
discard a sweep from the “First” analysis, we should discard one from
the “Repeated” analysis as well.
```

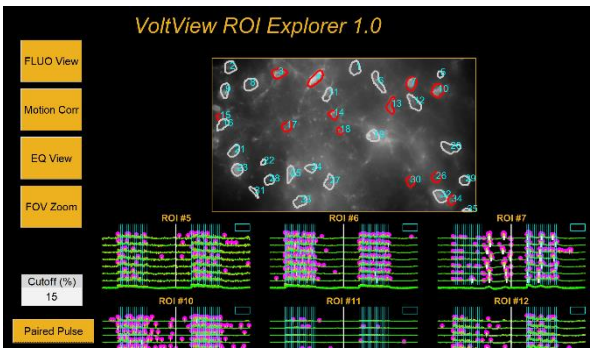
In this example of video comparisons, parameter 9: “Burst_Freqs_Op” was user-defined (**Param_Comp = [9];**) to compare the same neurons across the two videos. Cutoff was ±20, thus larger than 120% was labeled with red, smaller than 80% was labeled blue, change smaller than ±cutoff is white.
Instead of re-running the whole analysis for choosing another parameter to compare across the videos,



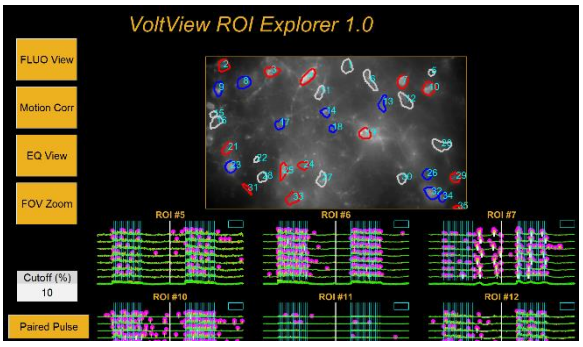
the user can choose from the buttons on the bottom left. For any chosen parameter the “cutoff” of comparison is the same. In case we would change the “cutoff” we can type in a number to the “cutoff (%)” edit field, **and hit Enter**. Running the comparison by the same parameter button at different “cutoff” levels will show the increase (red) and decrease (blue) with different proportions. First example shows Paired pulse with 10% and 15% cutoff, second is the o-Sub Slope with 10% and 30% cutoff levels.

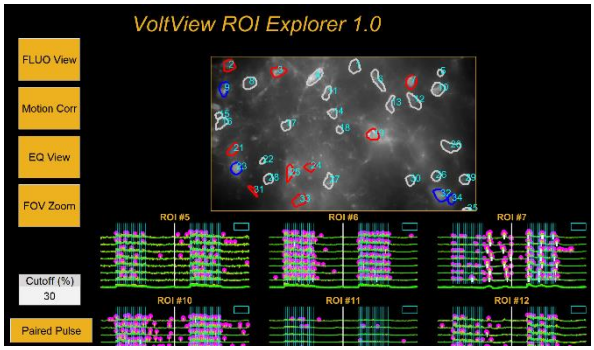
Paired pulse





o-Sub Slope





Between compared videos, the ROI shift is also corrigated, here between video 42 (cyan contours) and video 57 (yellow contours)
- **Motion Corr button**: overlays the ROI contours from Rec1 (cyan) and Rec2 (yellow).
Other main buttons (3/4 top left buttons) function as described above.

Here the motion correction is enlarged with the “FOV Zoom” button