AQuA

USER'S GUIDE

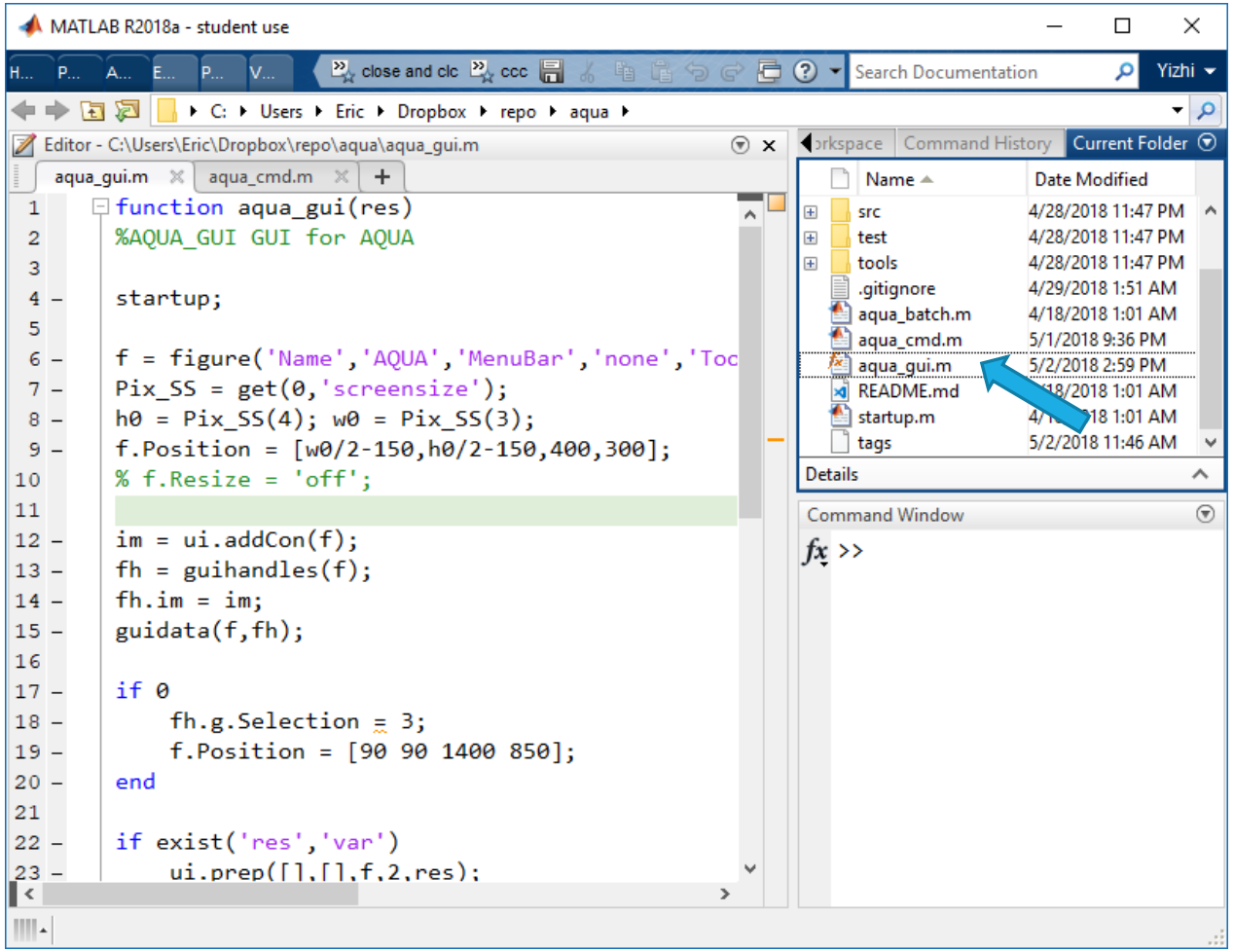
Before you start

- Use TIFF stack with one channel
  - 8, 16 or 32 bit supported
- Or use MAT file with only one variable (data matrix)
- Motion is (at least roughly) corrected
- Not too severe light noise or bleaching effects
- We recommend 32 GB of memory or more
- MATLAB GUI
  - MATLAB 2017a or later on Microsoft Windows.
  - Mac OS and Linux are not officially supported.
  - Required toolboxes: image processing, statistics and machine learning, curve fitting
  - Recommended toolbox: parallel computing.
- Fiji plugin
  - Requires latest version of Fiji. ImageJ is not supported.

Load data and navigate

Open the software (MATLAB)

1. Download AQuA
2. Start MATLAB
3. Switch to the folder of AQuA and add it along with its subfolders to the path
4. Open ‘aqua_gui.m’ and run



Open the software (Fiji plugin)

1. Download the plugin
2. Put the plugin to Fiji's 'plugins' folder
3. Start Fiji
4. Run the plugin

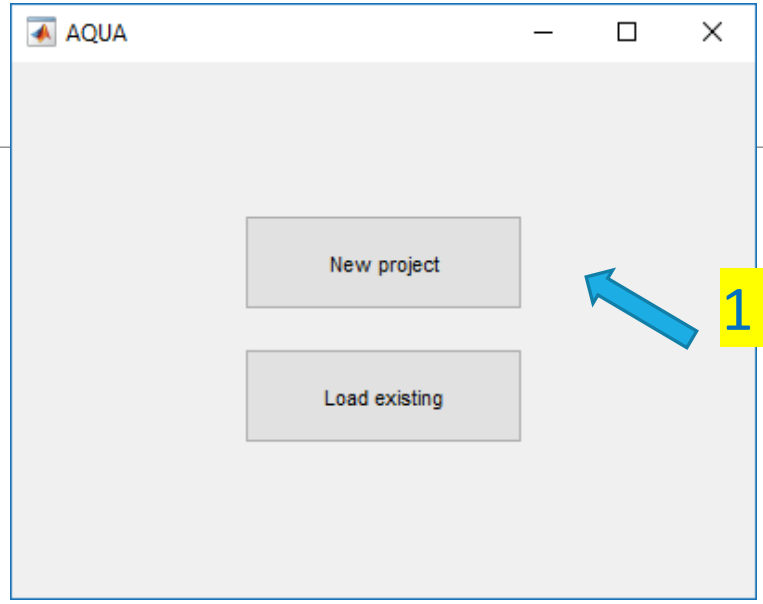
This user guide is based on the MATLB version, but Fiji plugin has almost the same function and interface.

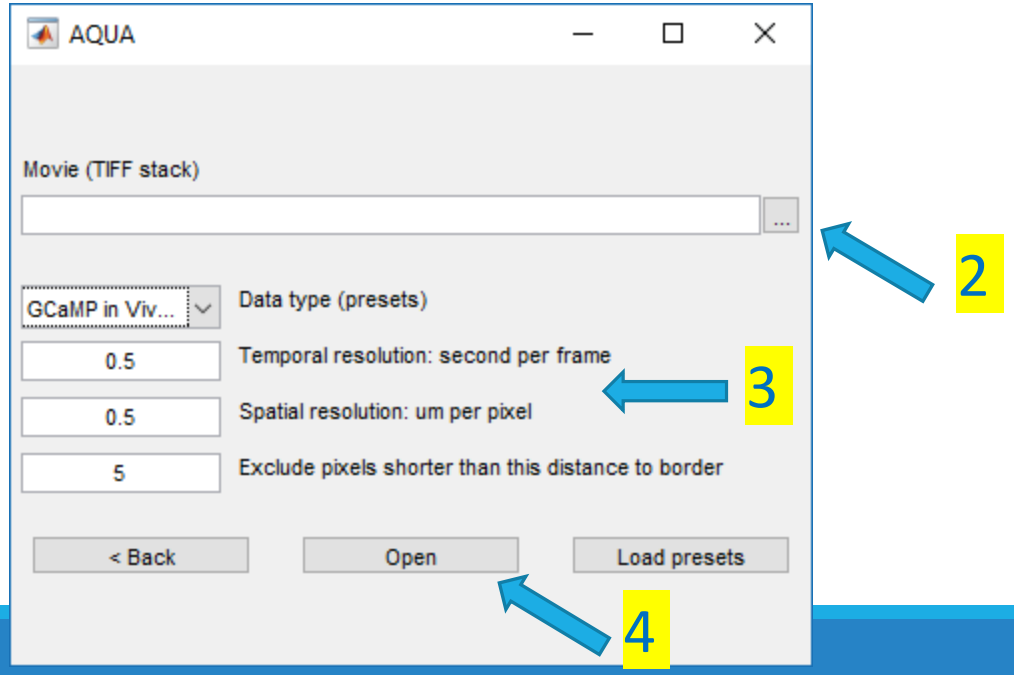
There could be some minor differences in interface in implementation.

Open data

1. Click new project.
   - If you saved an experiment before, click ‘Load existing’.
2. Select the data in the prompted dialogue.
3. Select data type and set imaging parameters.
   - Default values come from presets.
4. Click open to load the data.
   - It may take a while for large data sets.

You need to choose the working directory as well if you use AQuA Fiji plugin.





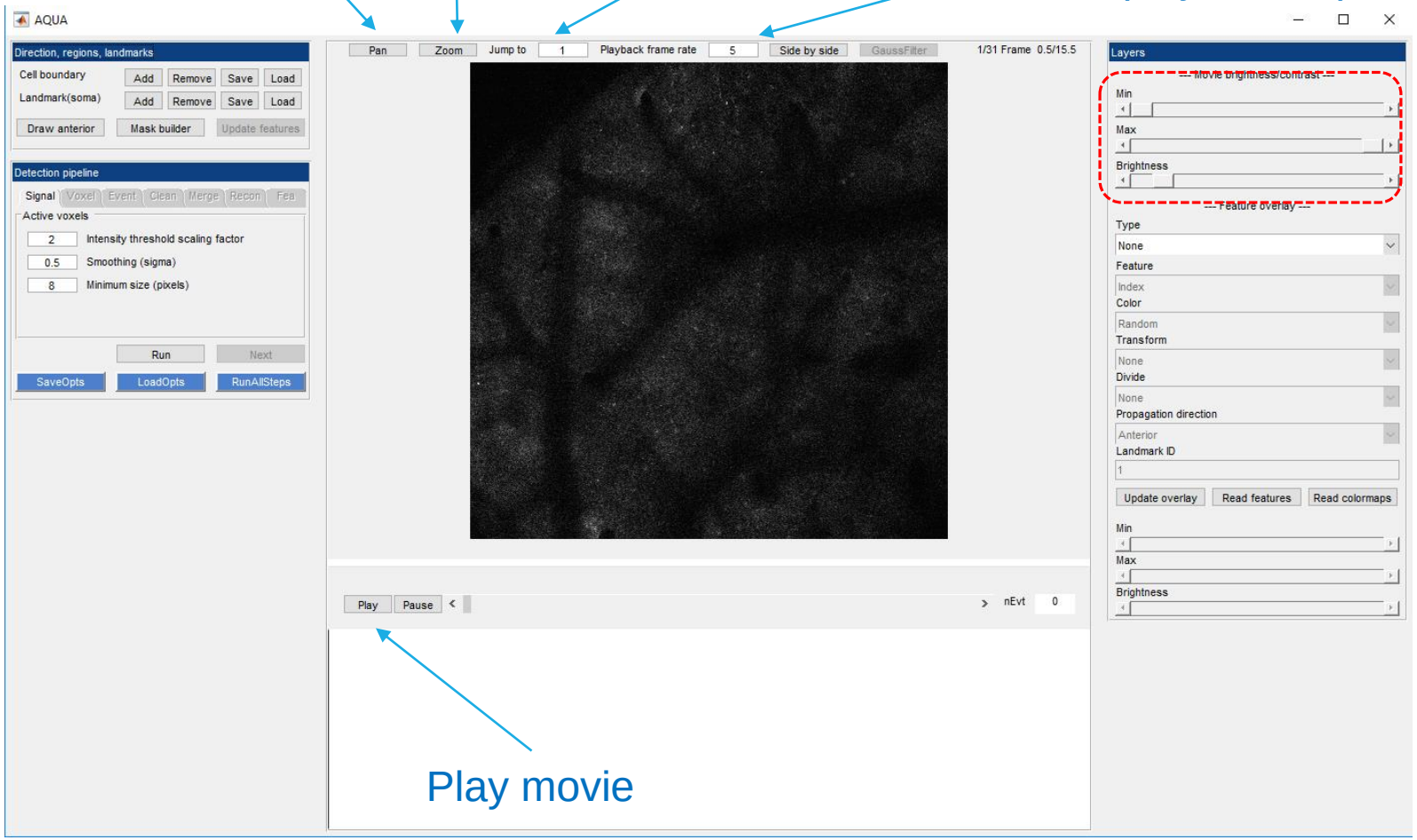
Navigate and adjust

Zoom into the movie. Left click the movie: zoom in. Right click the movie: zoom out.

Jump to specified frame

Pan the movie

Control play back speed



Play movie

Adjust contrast and brightness of the movie for better viewing

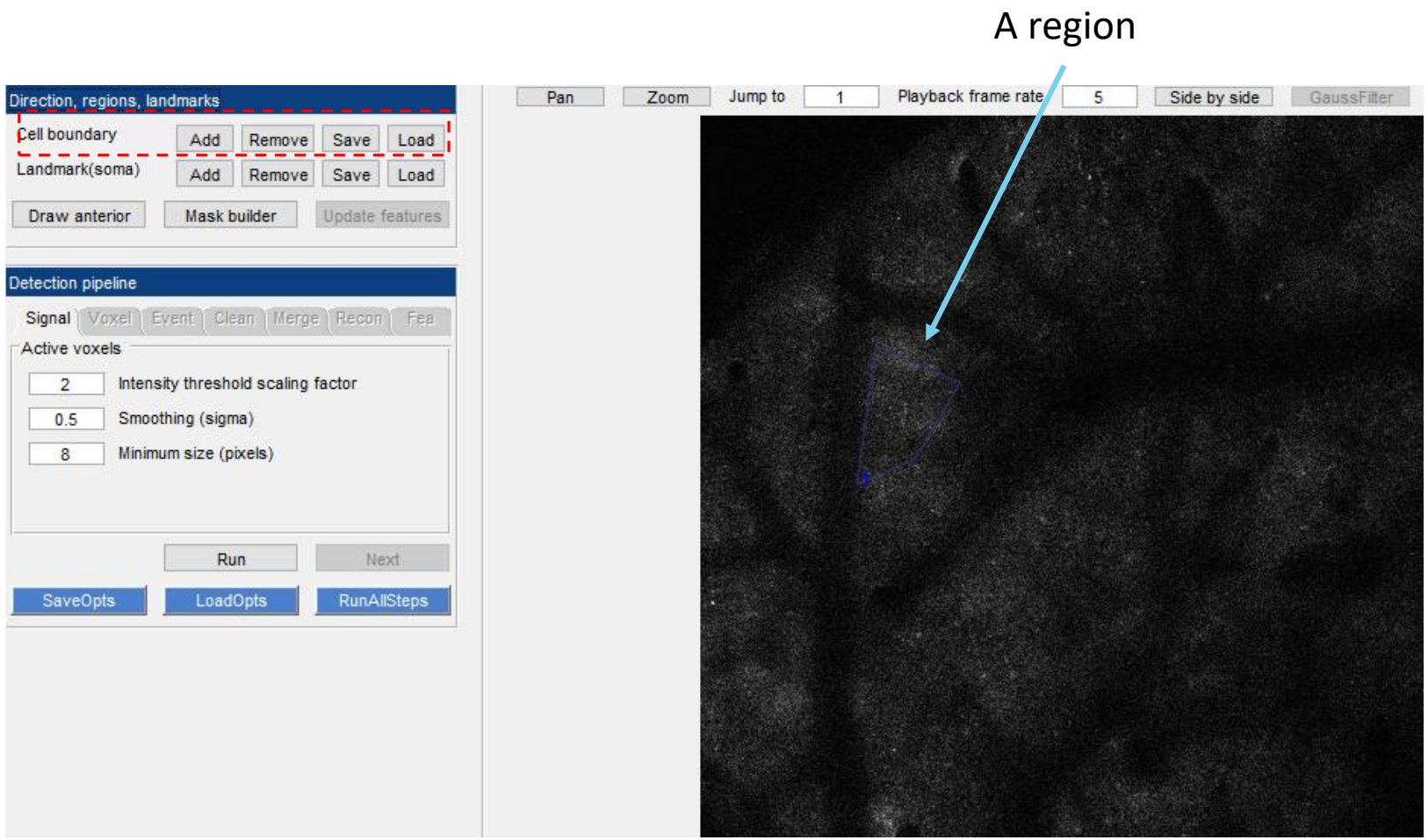
Values from ‘Min’ to ‘Max’ is mapped to [0,1] for viewing

Brightness is an extra multiplication factor

Region, landmark, mask and direction

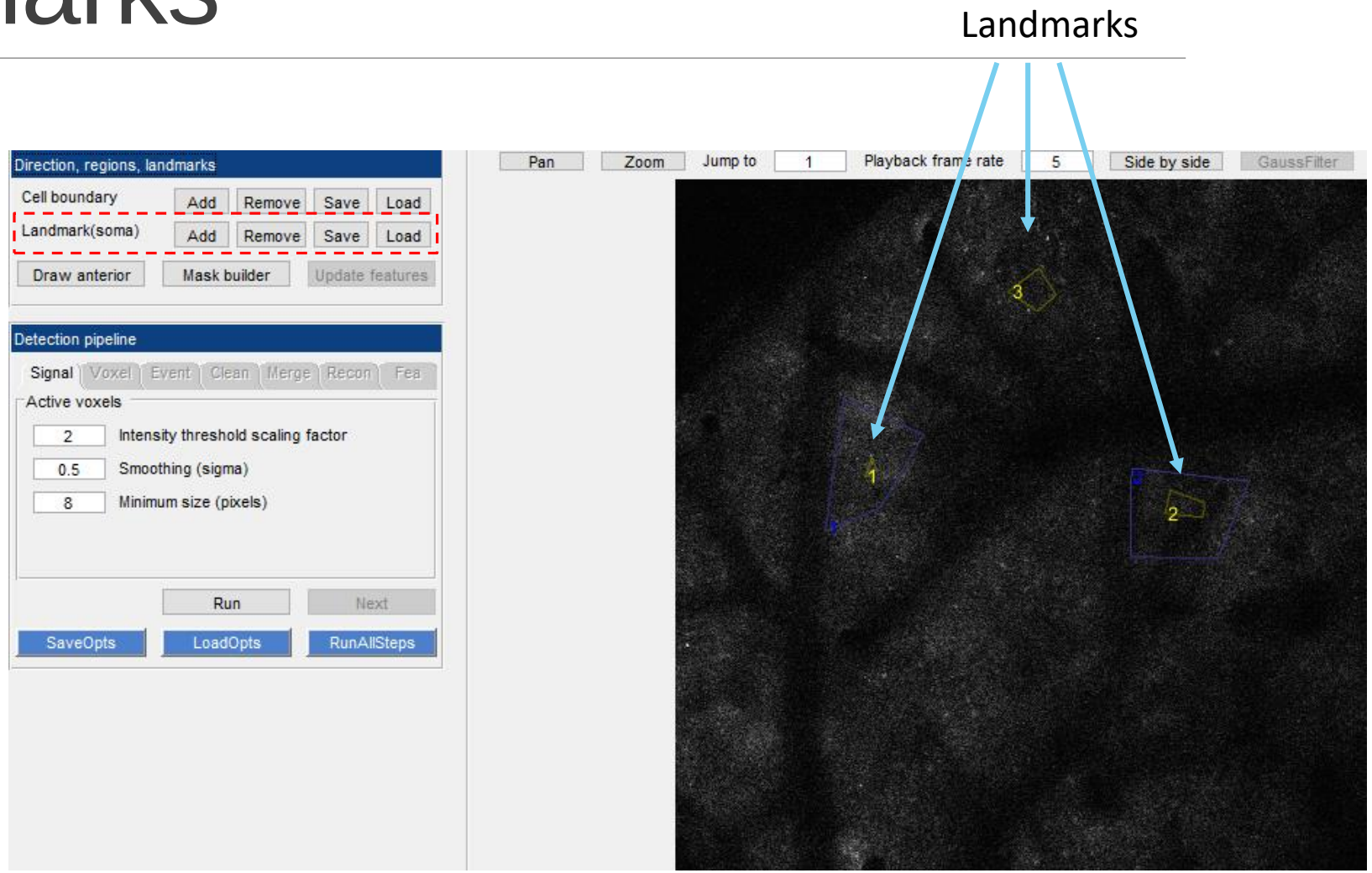
Define cell boundary/regions

- Draw cell boundaries, or regions, if you:
  - Do not want events outside drawn regions to be detected
  - To calculate region-related features after detection if regions are drawn
- Click ‘Add’ to draw a region. To draw another region, click ‘Add’ again.
- To remove a drawn region, click ‘Remove’ and click the region to delete.
- To save and load the region, click ‘Save’ and ‘Load’.



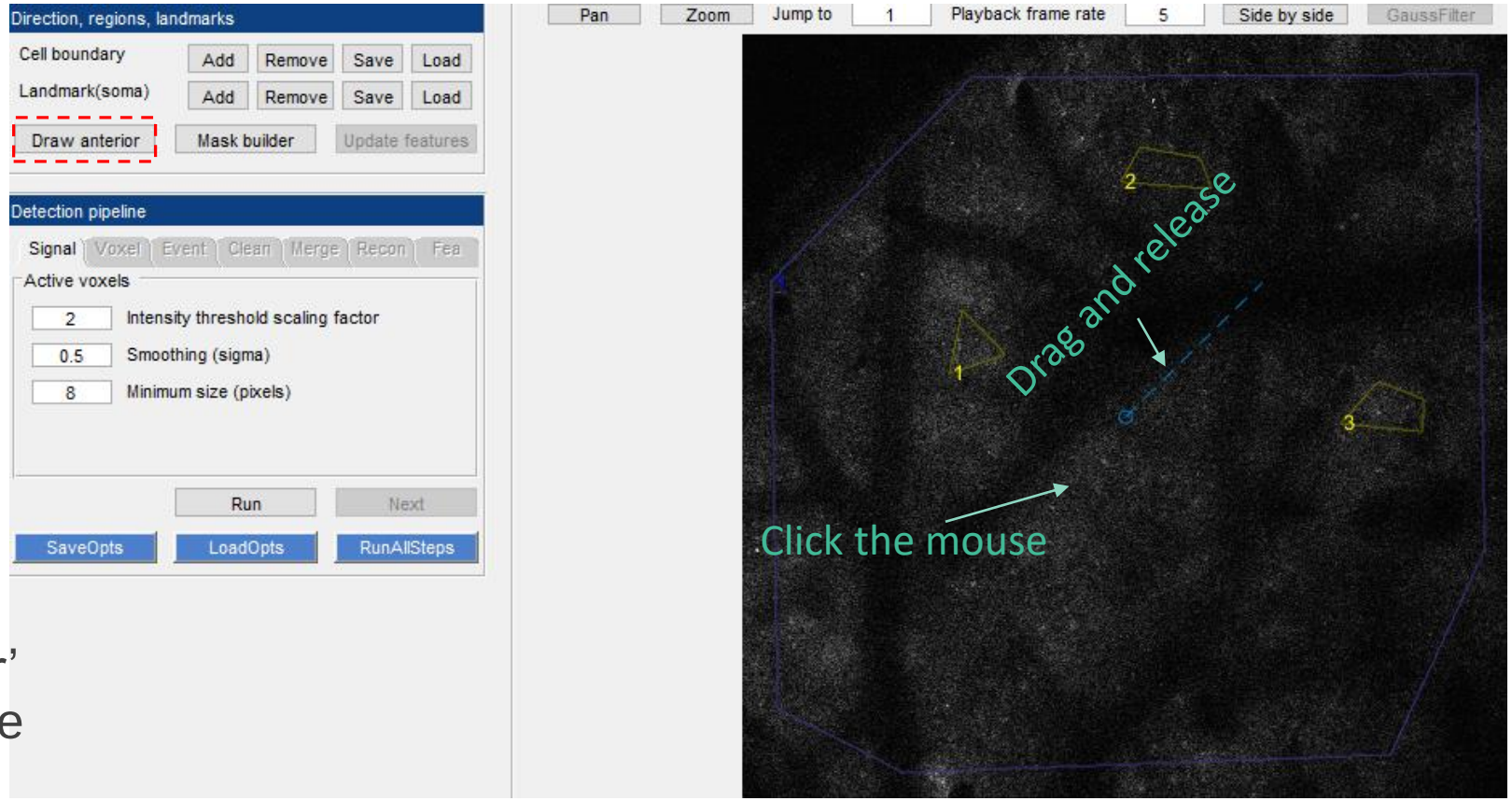
Define landmarks

- Draw landmarks, if you want landmark-related features
- Click ‘Add’ to draw a landmark. To draw another landmark, click ‘Add’ again.
- To remove a drawn landmark, click ‘Remove’ and click the landmark to delete.
- To save and load the landmark, click ‘Save’ and ‘Load’.
- You can draw multiple landmarks.
- Landmarks do not need to be within regions.



Draw anterior directions

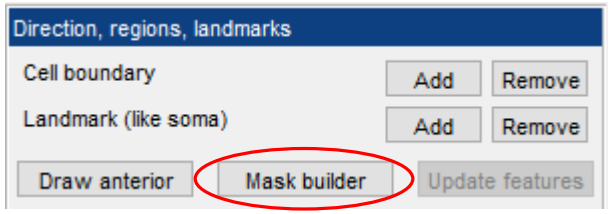
- Some propagation-related features are reported with respect to the anterior direction of the movie
- Click ‘Draw anterior’, Left click anywhere in the movie, do not release the button, drag the mouse toward the anterior direction. Release the button when done.
- If you want to re-draw the direction, click ‘Draw anterior’ and draw again. The new one will override the old one.



Anatomical Masks

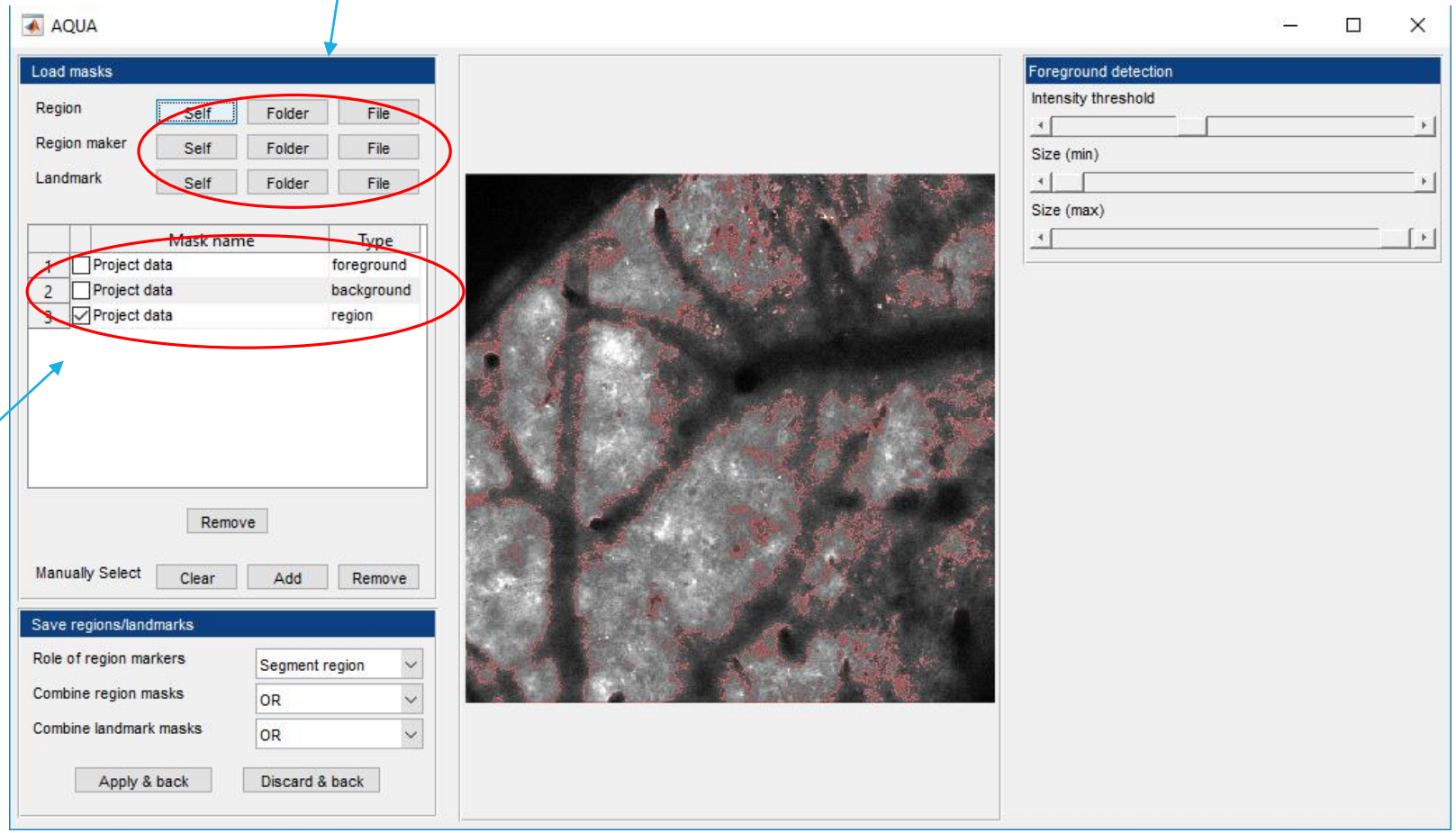
- With anatomical mask module, you can use any image (for example, the average of some movie in another channel) to obtain the regions and landmarks
  - We call these images masks
  - These images should share the same FOV as current movie
- You will be asked to set a threshold on the image to get the foreground regions, and apply a minimum and maximum size threshold to clean un-needed ones
- You can use multiple images and take some operations between them

Anatomical Masks



After loading movie, click ‘Mask builder’

Load mask from the opened movie itself, from a TIFF file, or from a folder.



Names and types of loaded masks will be shown here. Select to switch them. Click ‘remove’ to delete it.

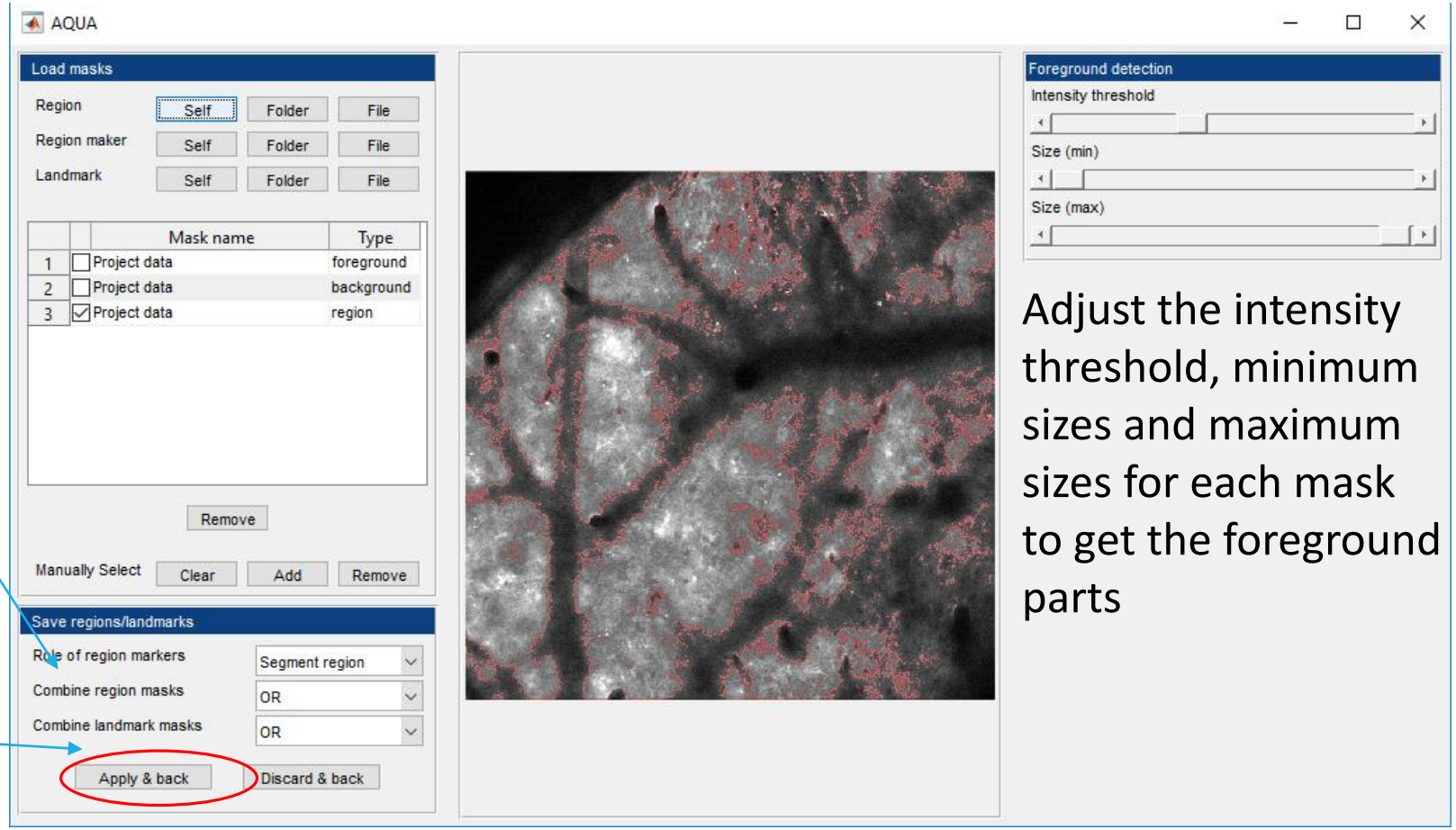
Anatomical Masks

- In the main window, you can edit the regions and landmarks from masks. You can also draw new ones.
- You can load zero, one, or more region masks.
- Each region obtained in the region mask might contain multiple regions of interest. If you have another mask that can further separate the regions, you can load it as the region marker mask.
  - For example, if we have a movie of two cells that are very close to one another, we can first use the region mask to only detect events in that region. However, because of their spatial proximity this might detect them as only one cell. If we want to extract features from each cell, then we can load another mask with more cellular markers, for example an image of the cells' two somas. Now the region is split to two cells as desired.
  - You can only load one region marker mask. And it will take effect only when at least one region mask is loaded
- You can load zero, one, or more landmark masks.

Anatomical Masks

In case multiple regions are loaded, you can choose to use OR or AND operations between them. The same applies to landmark masks.

Click ‘Apply & back’ to save the masks as region and landmarks

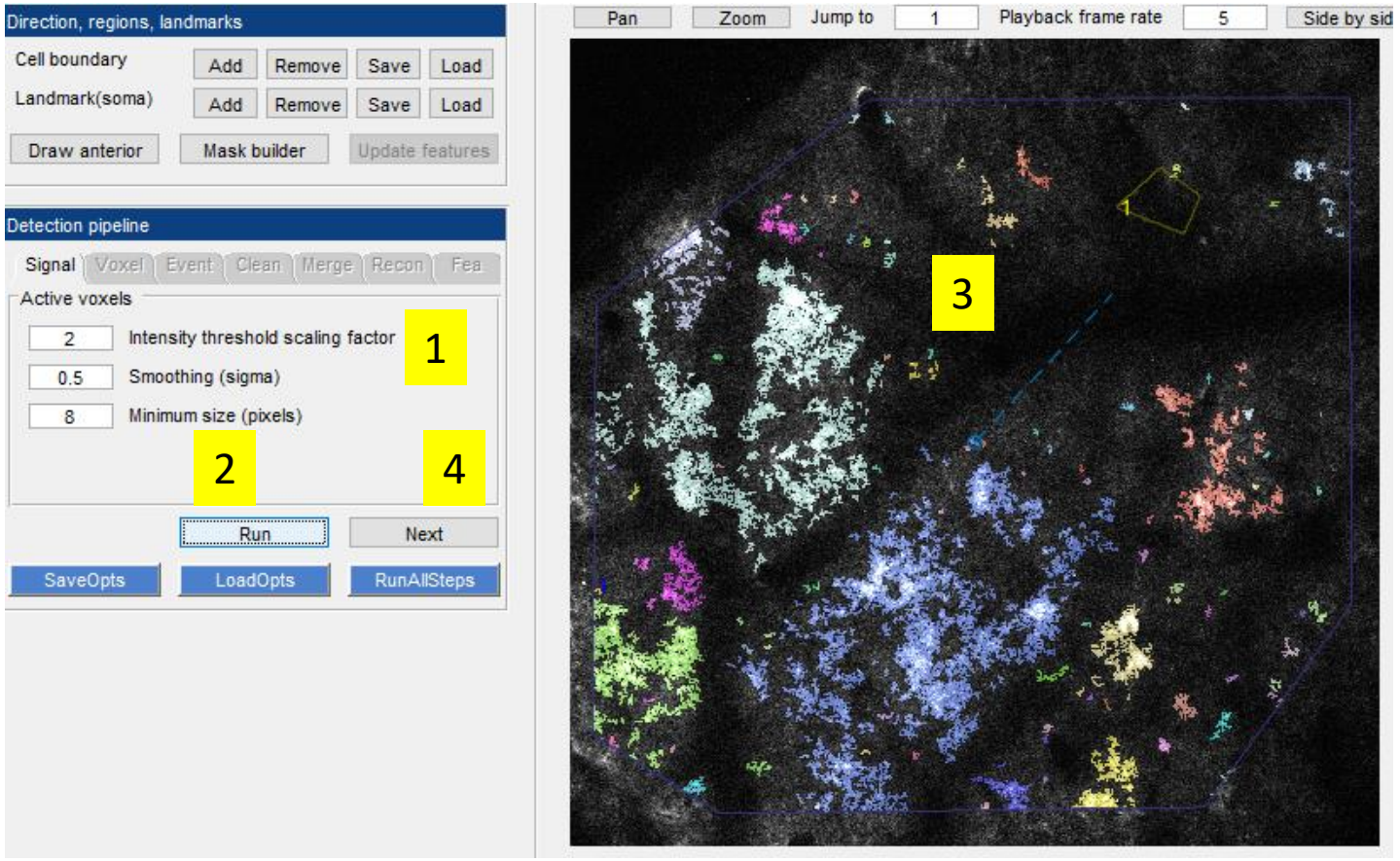


Adjust the intensity threshold, minimum sizes and maximum sizes for each mask to get the foreground parts

Detection pipeline

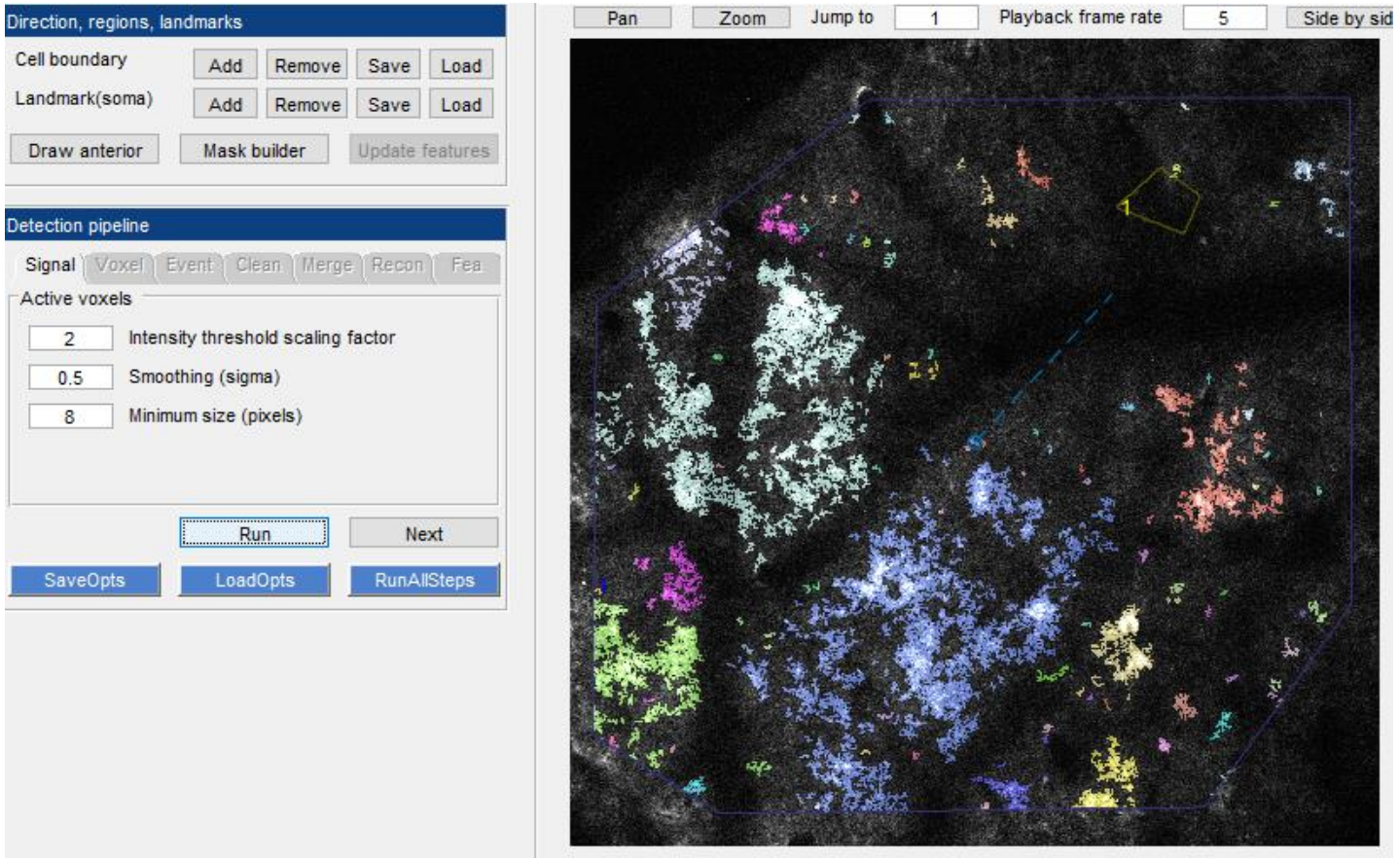
Event detection pipeline

- Event detection pipeline is the major part of the GUI. Event detection steps are organized in tabs.
- In any tab, the work flow is the same
  1. Adjust parameters if needed
  2. Click Run,
  3. Check the results (color overlay on the movie)
  4. If the results are good, click next
     - Otherwise adjust parameters
- Click back to go to previous steps. You can also click the tabs to go to any previous step.



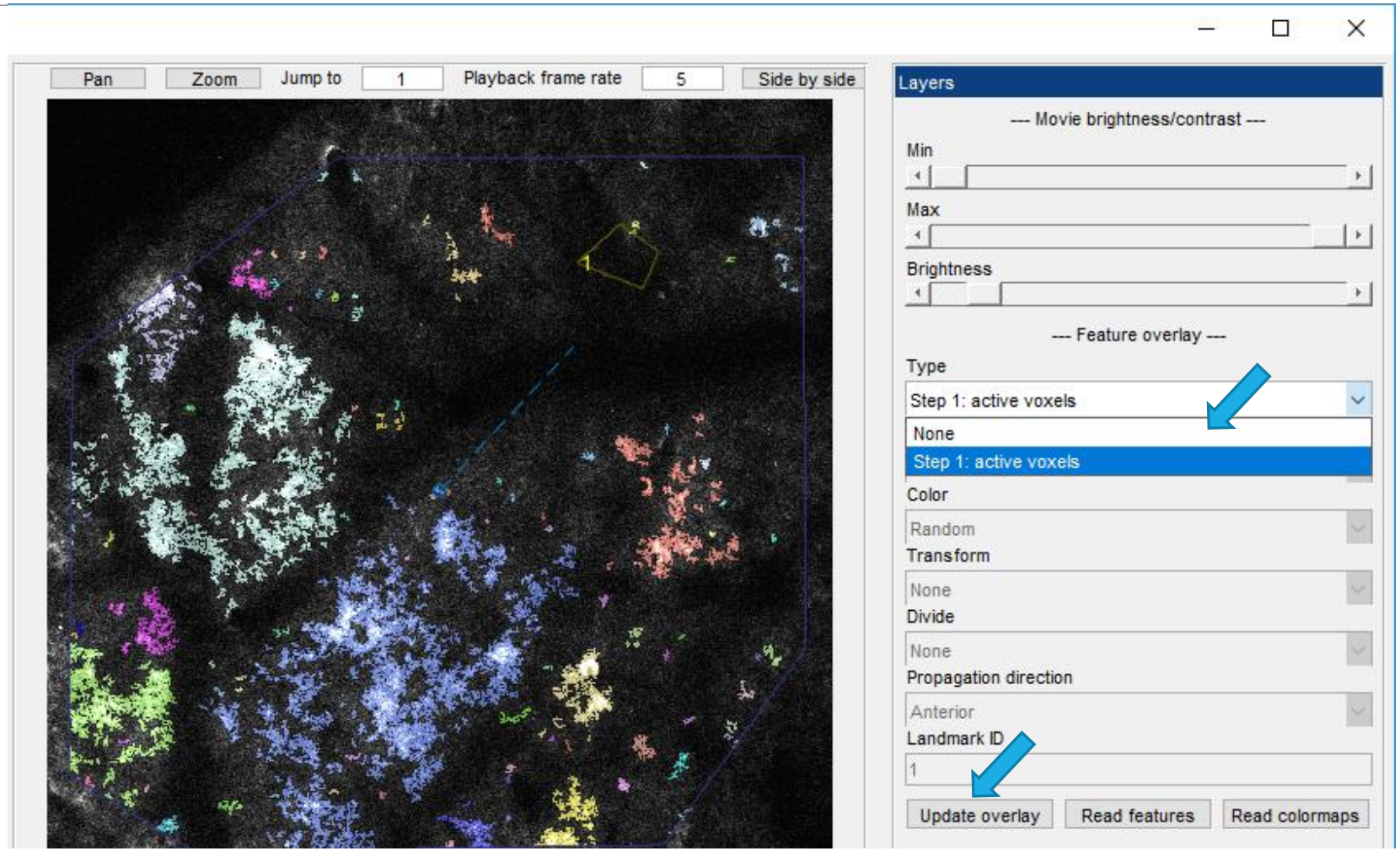
Run pipeline: active signal

- Signal step detects active signals, which are color coded.
  - The specific color used here is irrelevant.
  - If too many noises are detected as signal, increase ‘Intensity threshold’. If some signals are missed, decrease it.
  - For very noisy data, increase ‘Smoothing (sigma)’.
- Click ‘Run’ after adjusting parameters.
  - When it finishes, the ‘Next’ button will be enabled.



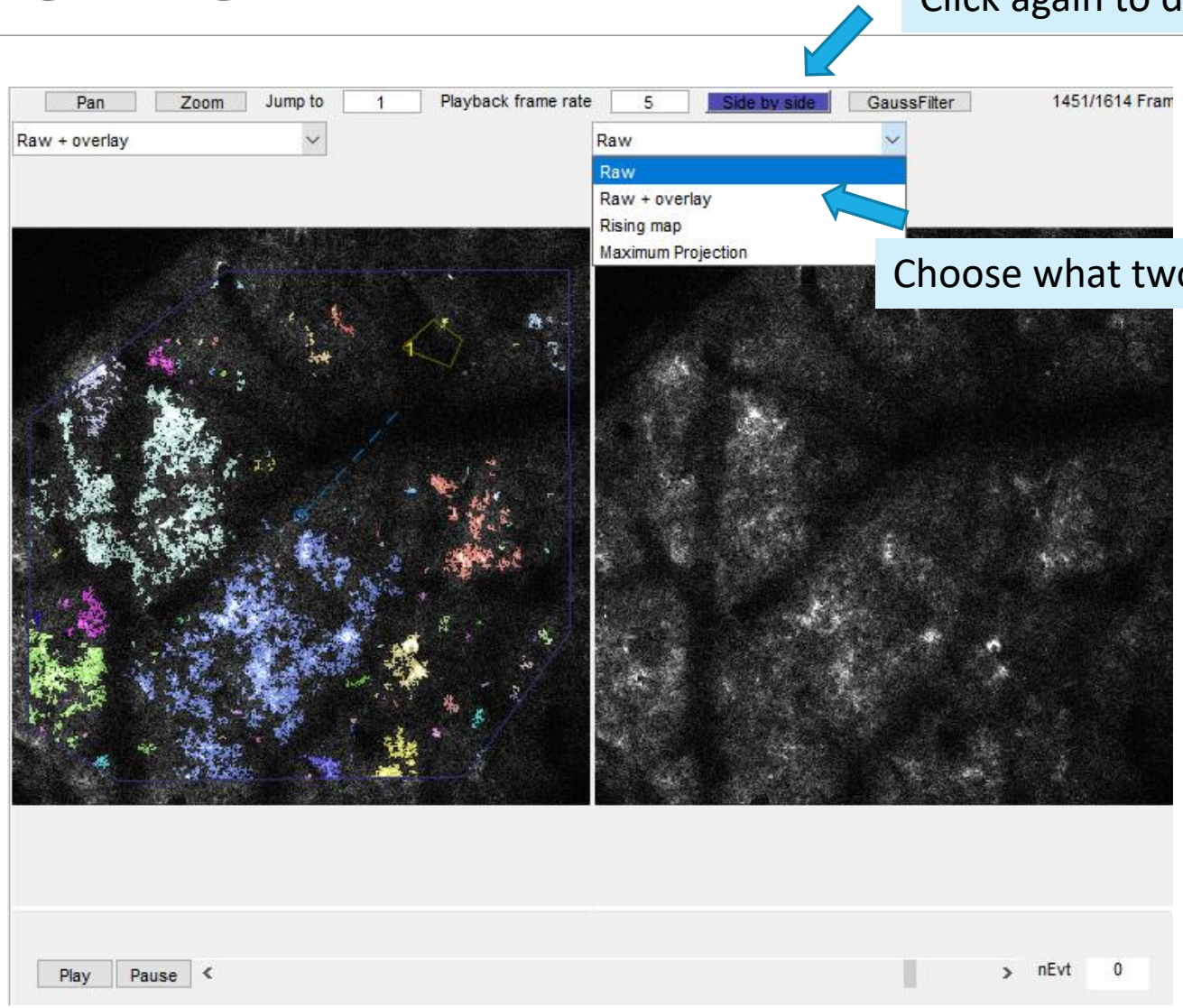
Switch views

- You can switch between the color overlay and raw movie to check whether the results are good or not.
- Select the overlay type, then click ‘Update overlay’.



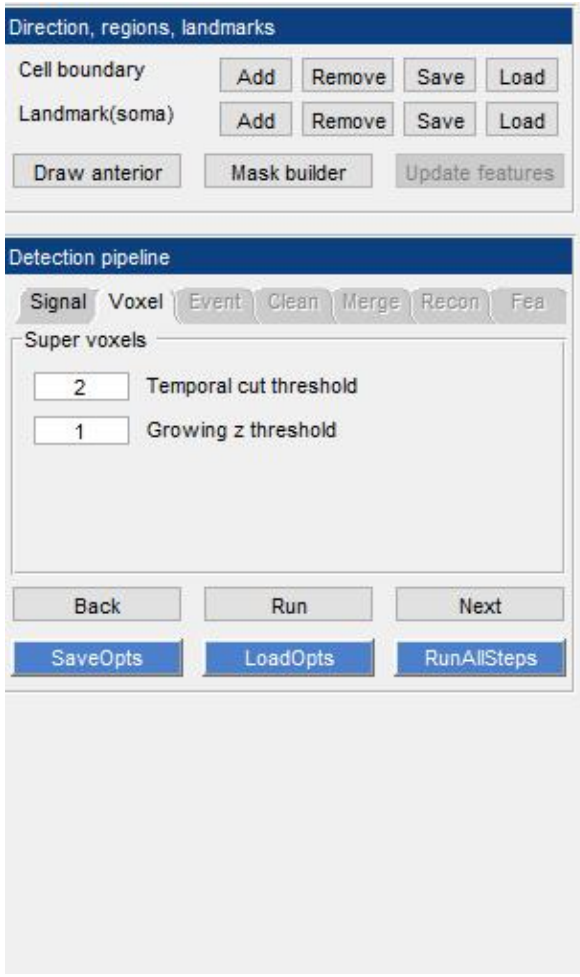
Side by side view

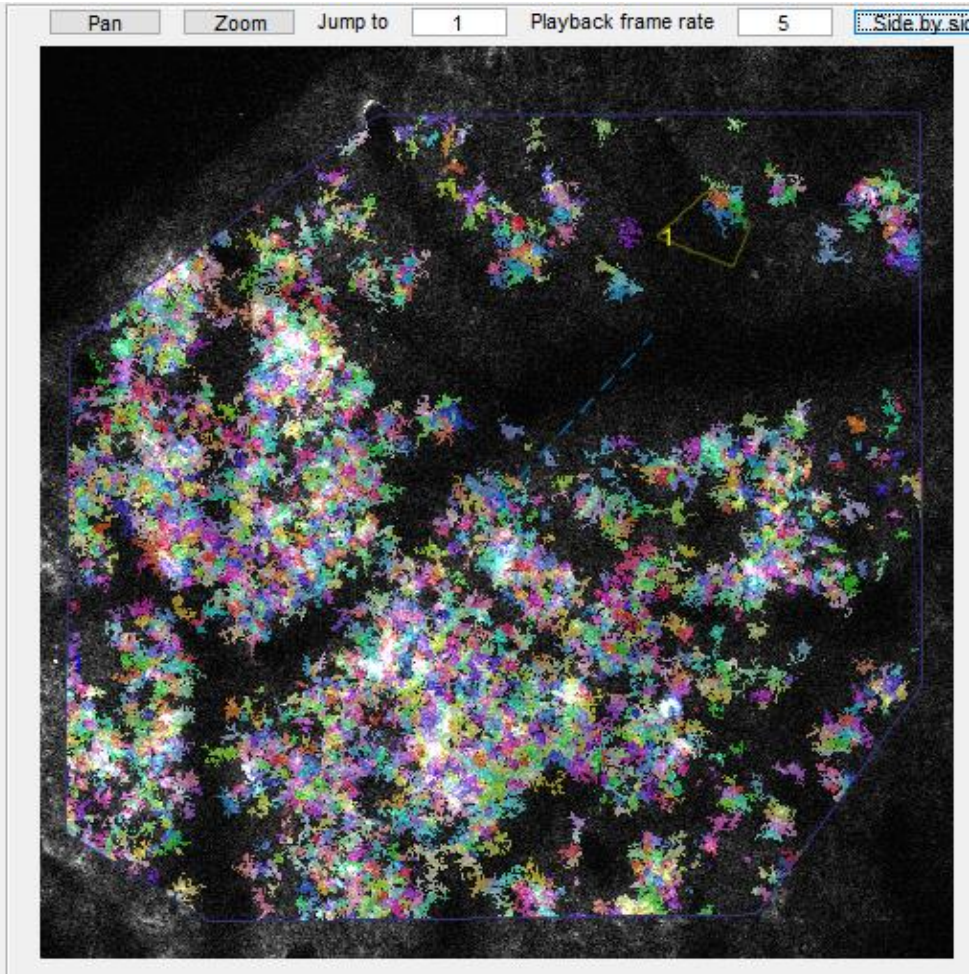
- You can also view the overlay and raw movie side by side



Run pipeline: super voxel

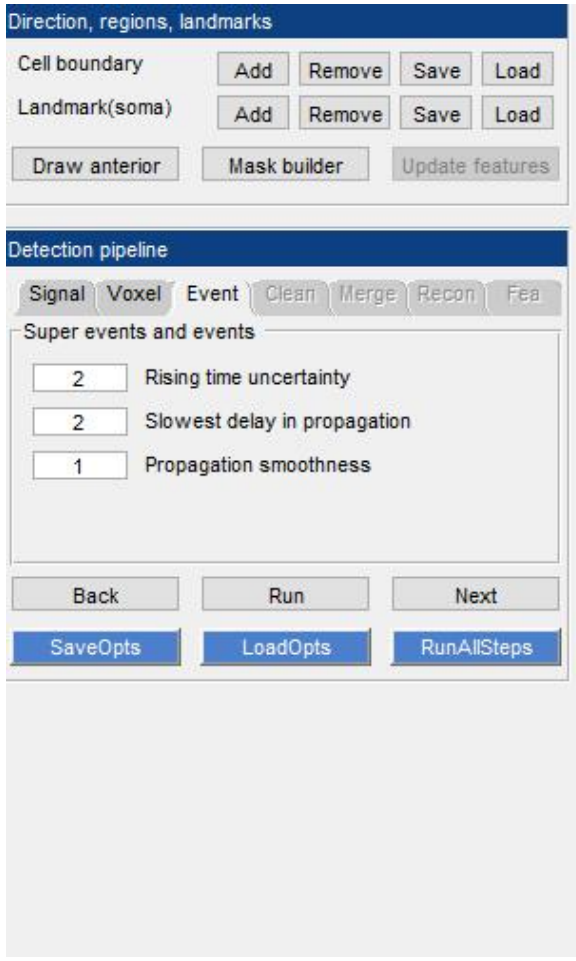
- Voxel step will detect super voxels. Different super voxels are represented by different colors.
  - The specific color used here is irrelevant.
  - If two voxels should be temporally separated, but they are not, decrease ‘temporal cut threshold’.
  - If some active pixels are missed, decrease ‘Growing z threshold’.

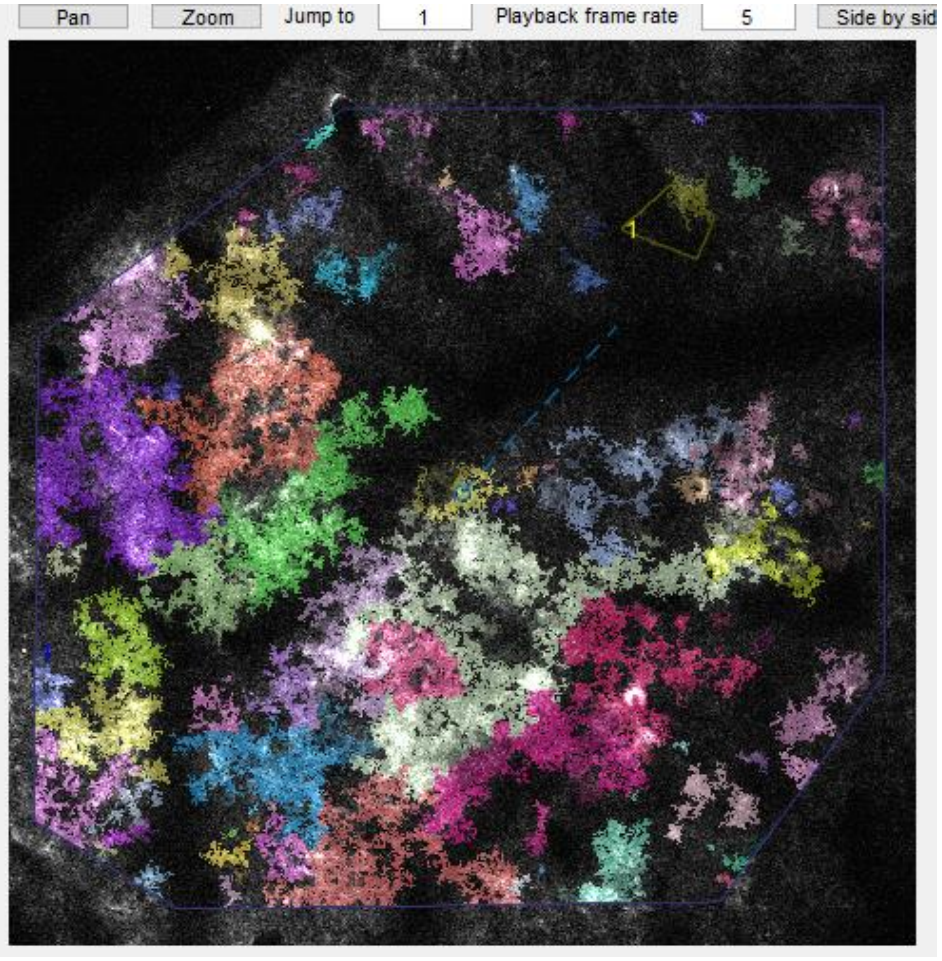




Run pipeline: event detection

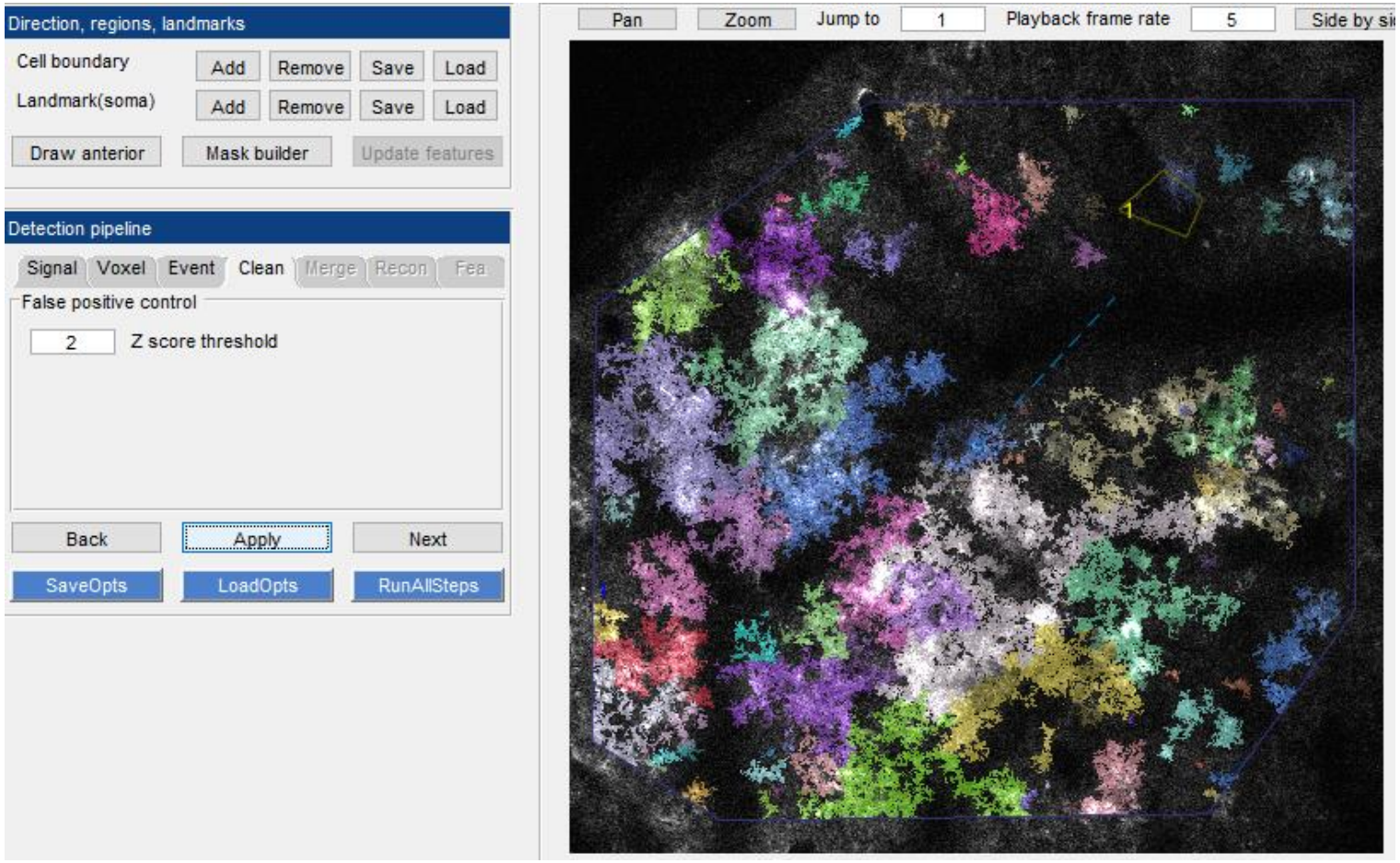
- Event step will detect super events and events.
  - After this step finishes, events will be labelled by different colors. The specific color used here is irrelevant.
  - To view super events instead, choose that in feature overlay.
  - If too many events detected, increase ‘Rising time uncertainty’.
  - If less continuous propagation is prefered, increase ‘Slowest delay in propagation’.
  - If there are lots of fine structures, consider reducing ‘Propagation smoothing’.





Run pipeline: clean events

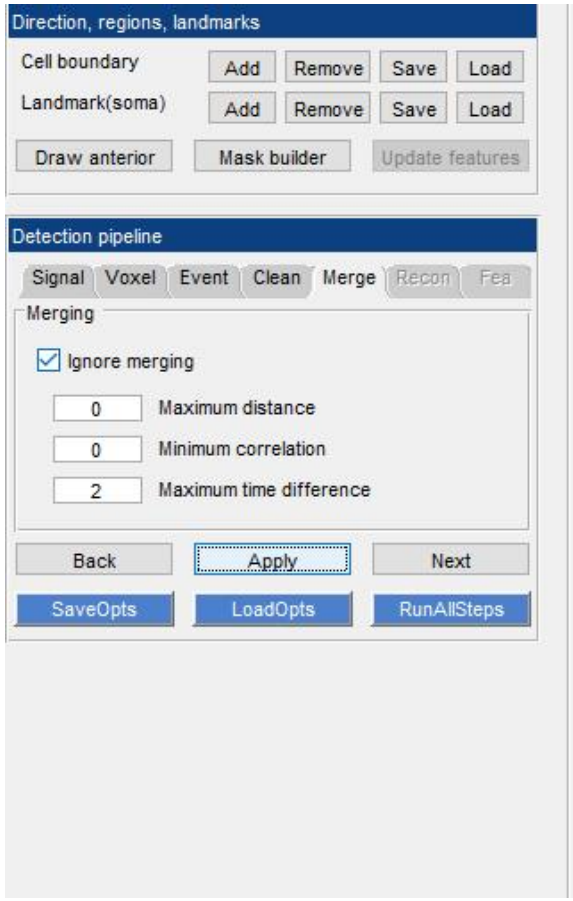
- Events with low SNR are removed in clean step
  - Each remaining event is color coded. The color chosen does not matter.
  - The SNR is represented as a Z-score. If too many events are removed, reduce this ‘Z-score threshold’.

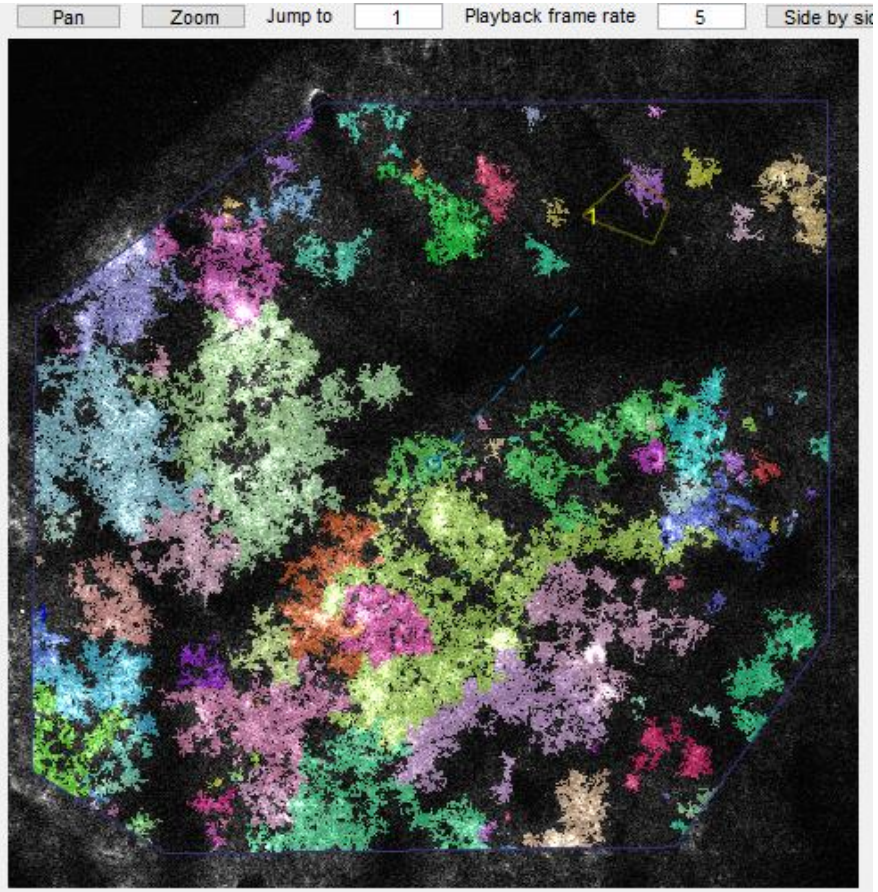


Note: the parameter is peak $\Delta F/F$ divided by noise level for the curve of that event. A threshold usually ranges from 2 to 6. The larger the parameter is, the fewer the events are retained.

Run pipeline: merge events

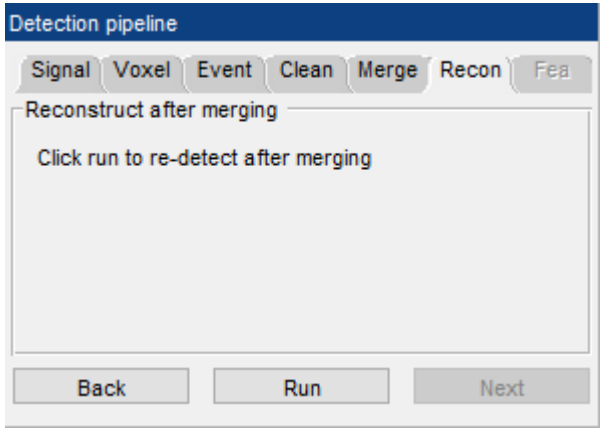
- Disconnected events can be combined in merge step.
  - This is mainly for Glutamate data.
  - If this step is not needed, choose ‘Ignore merging’.
- We have three criteria to control merging:
  1. Maximum distance
  2. Minimum correlation
  3. Onset time difference



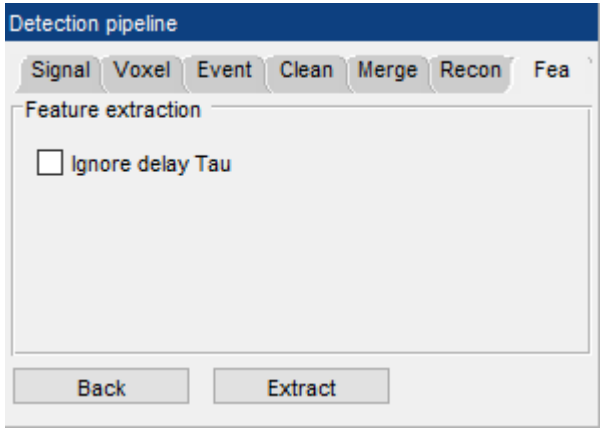


Run pipeline: re-construct and extract features

- If merging is performed, events are re-constructed.
  - Otherwise, no operation is performed in this step.
- Then we extract features for each event. Click ‘Extract’.
  - Decay tau () calculation takes some time. If it is not of interest, click the checkbox to ignore it.



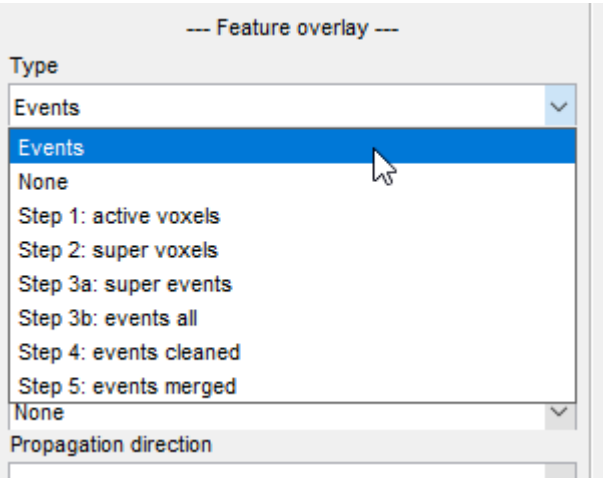
Update events



Extract features

View results of previous steps

- Click ‘Type’ in feature overlay.
- Choose the step to view results.
- ‘Events’ is the final step.

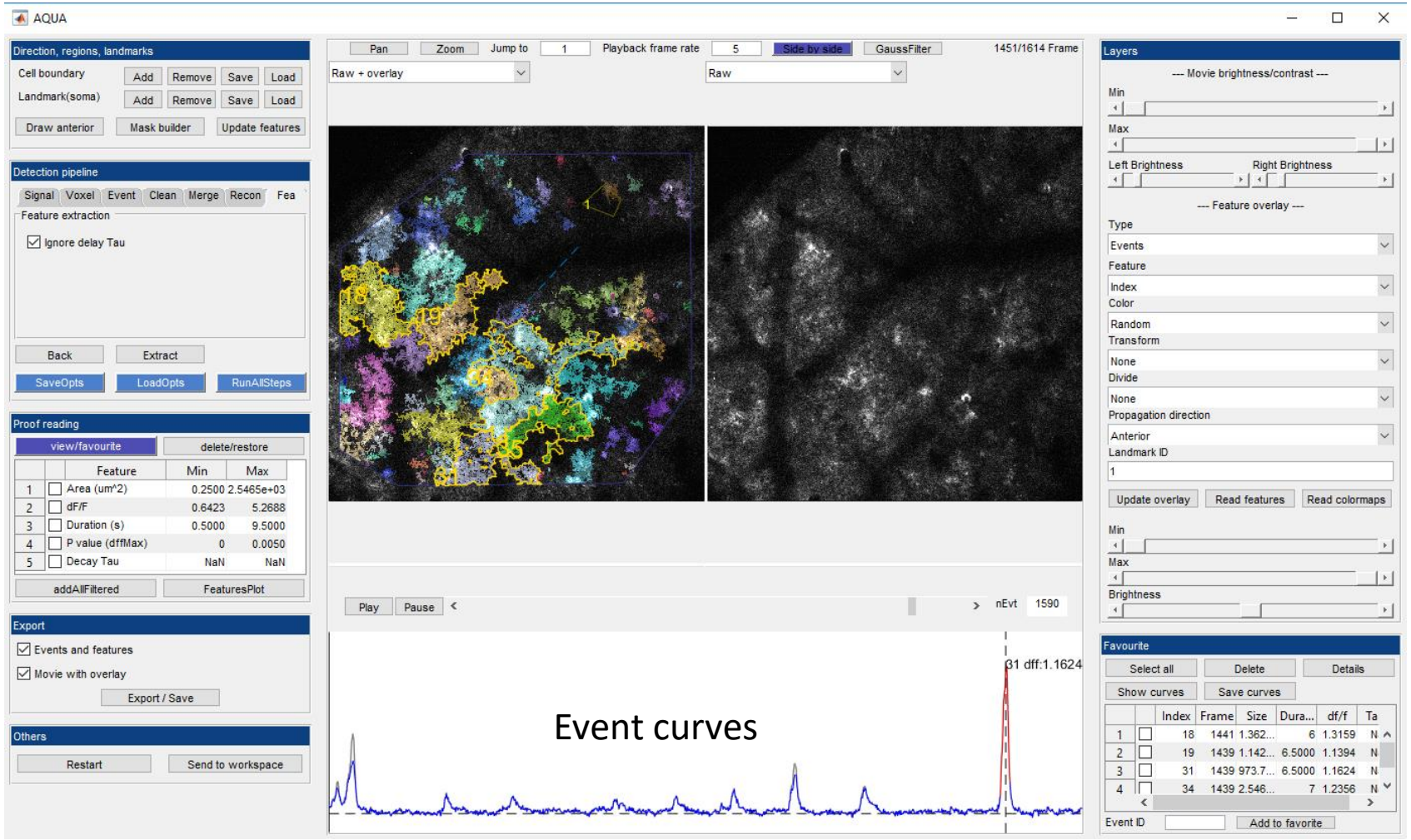


Proof read and view events

Post-detection GUI and tasks

Proof reading

Export

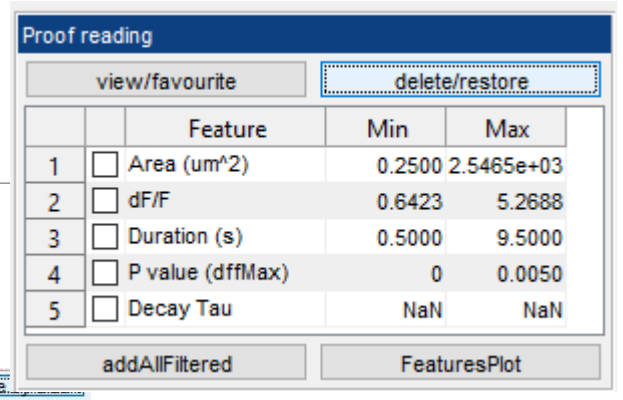


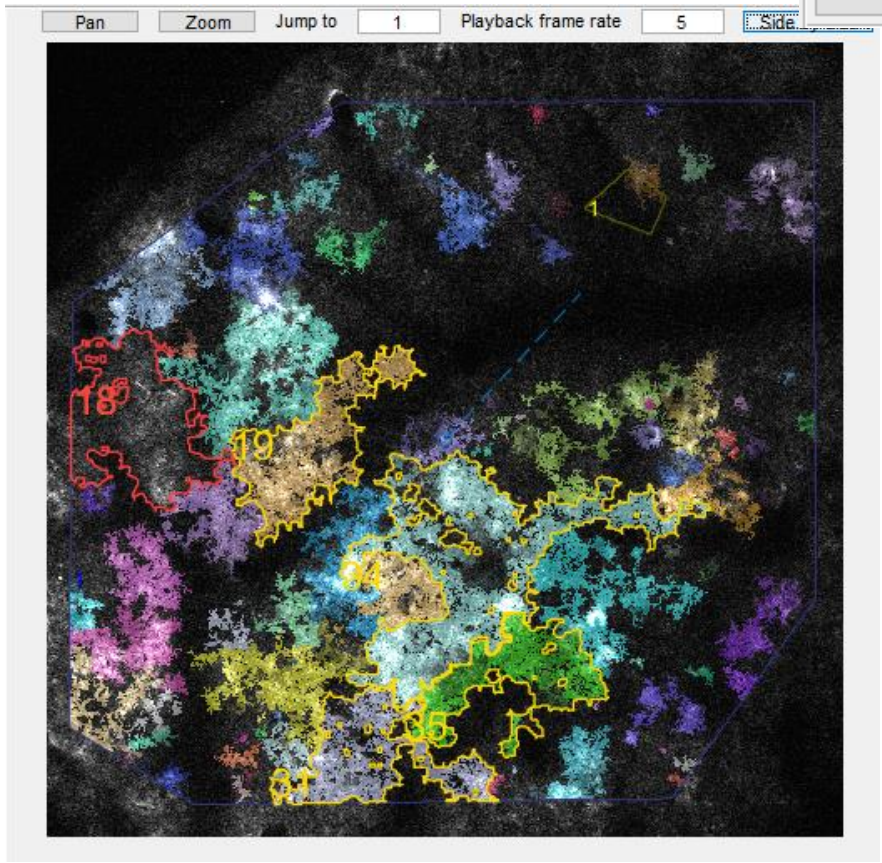
Feature overlay

Favorite list

Proofreading

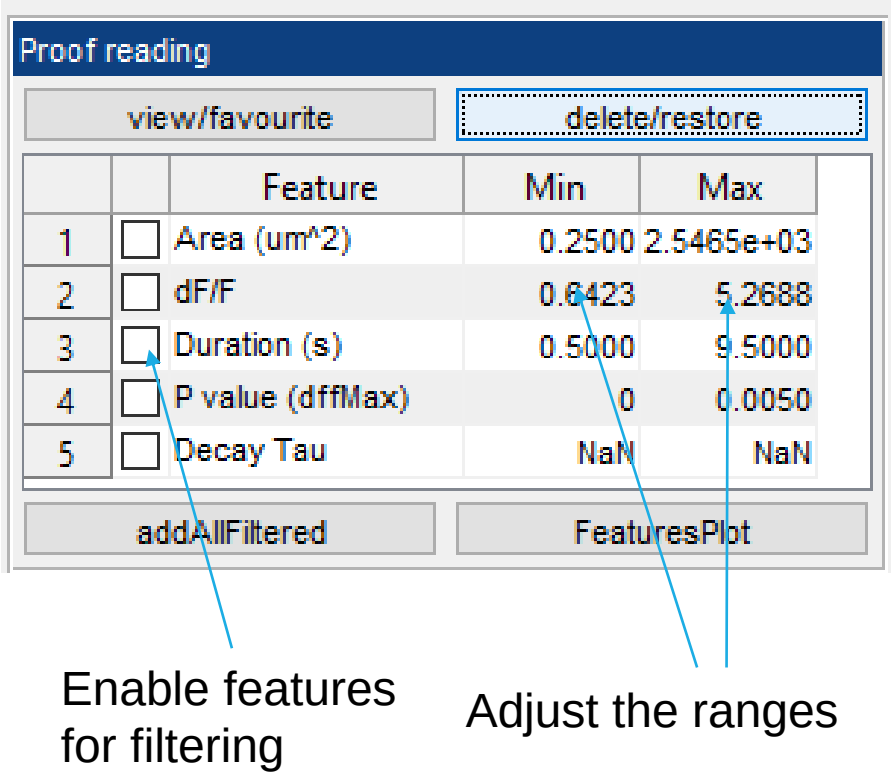
- Click view/favorite, then click an event in the movie to view its curve. This will also add it to the favorite list.
  - An event in favorite list has a green boundary. The event ID is also shown.
- Click ‘delete/restore’, then click an event in the movie to delete that event. If it is already deleted, it will be restored.
  - A removed event has a red boundary.





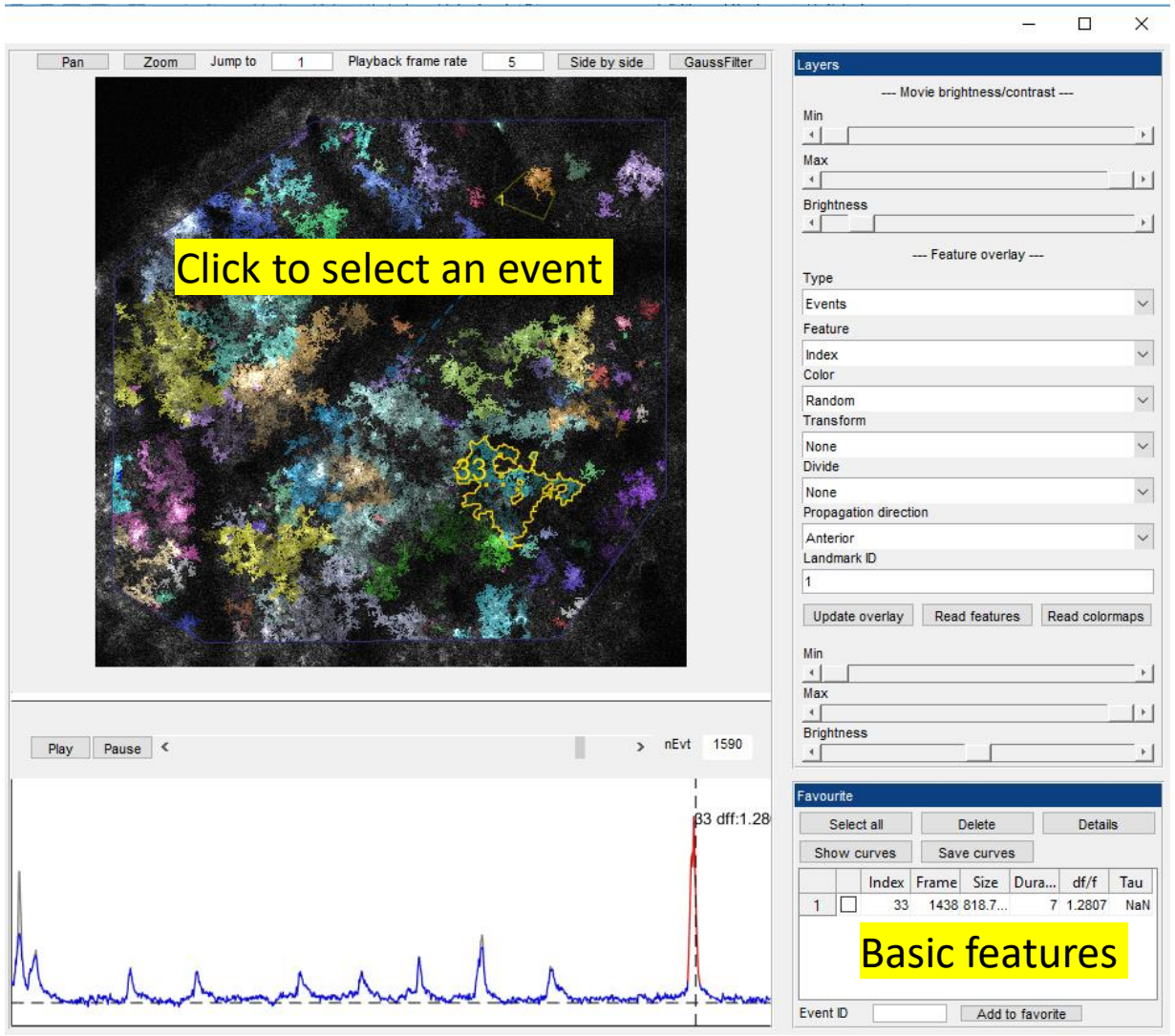
Proofreading

- We can also remove events based on some criteria.
- For each feature listed here, we can remove events that are outside the range specified.
- Features removed here will not be exported and not shown in movie.



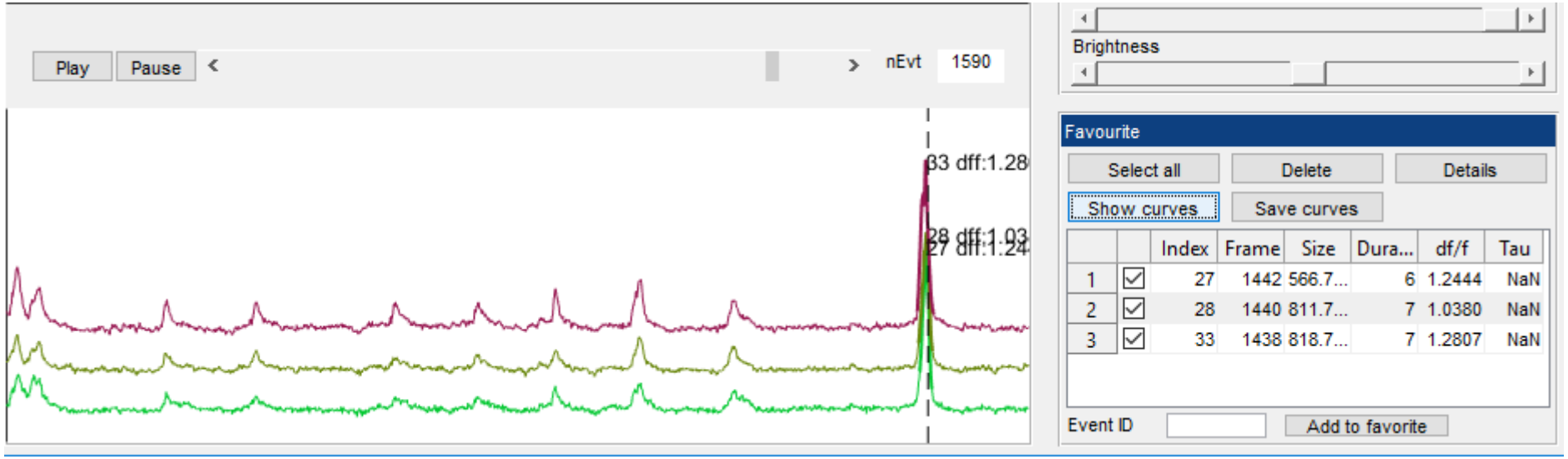
View events

- Click an event to view its $\Delta F/F$ curve.
  - For curve inside selected event: $\Delta F/F$ curves calculated with other events removed are shown in red. If other events are not removed, it is shown in gray.
  - Curve outside selected event are shown in blue.
- It will be added to event manager where its basic features are shown.
- Selected events will be surrounded by green boundaries.



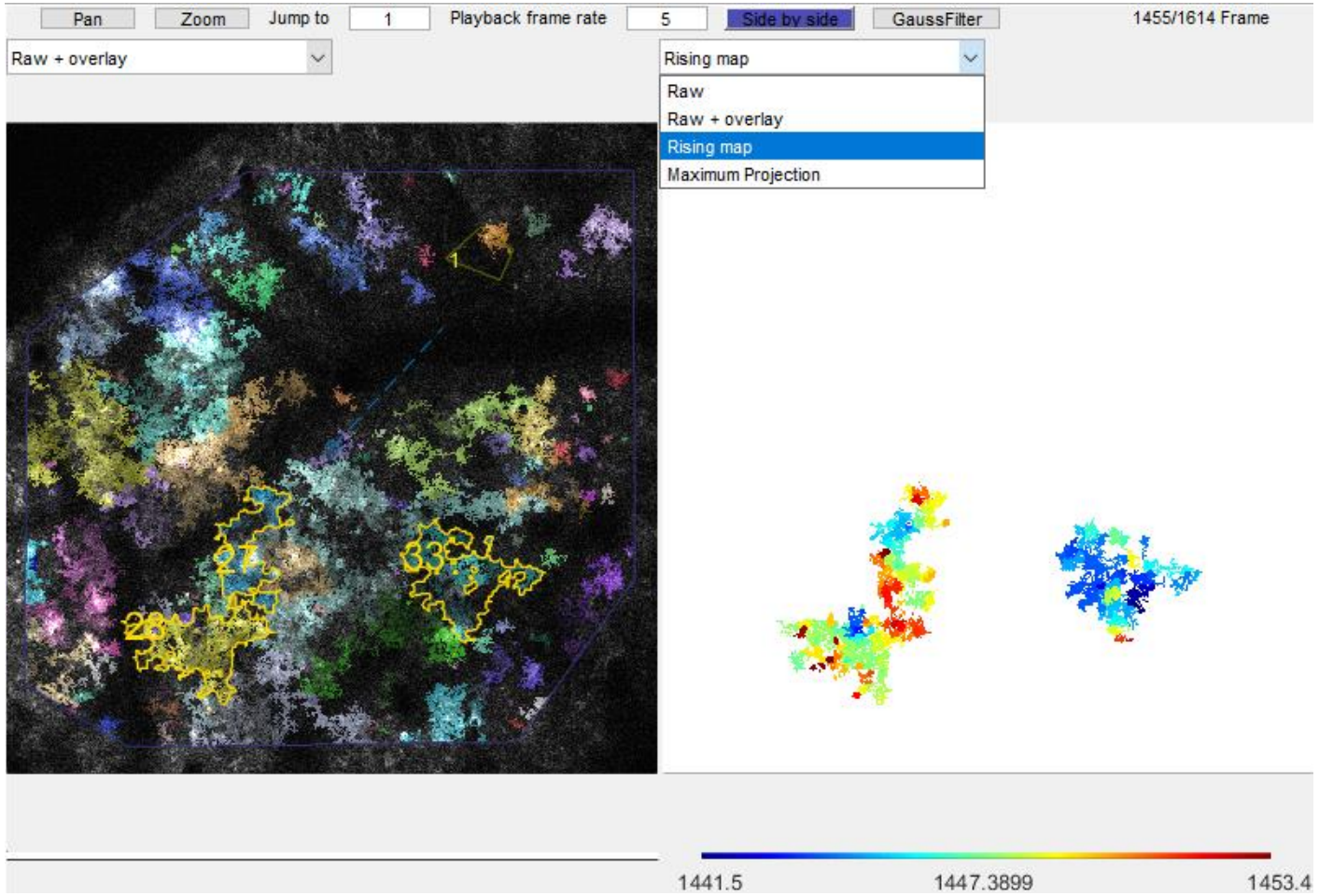
Event manager

- When multiple events are selected, we can view their curves by selecting them and click ‘show curves’
- To delete events, select them and click ‘Delete’



View onset time map

- To view the onset time map of each event:
  1. Enable side by side view.
  2. Choose ‘Rising map’ in the dropdown menu.
- The color bar below indicates the onset time.
  - When the movie frame slider changes, the range and color of onset time may also change accordingly.
- Only events already in the favorite list will have an onset map shown.

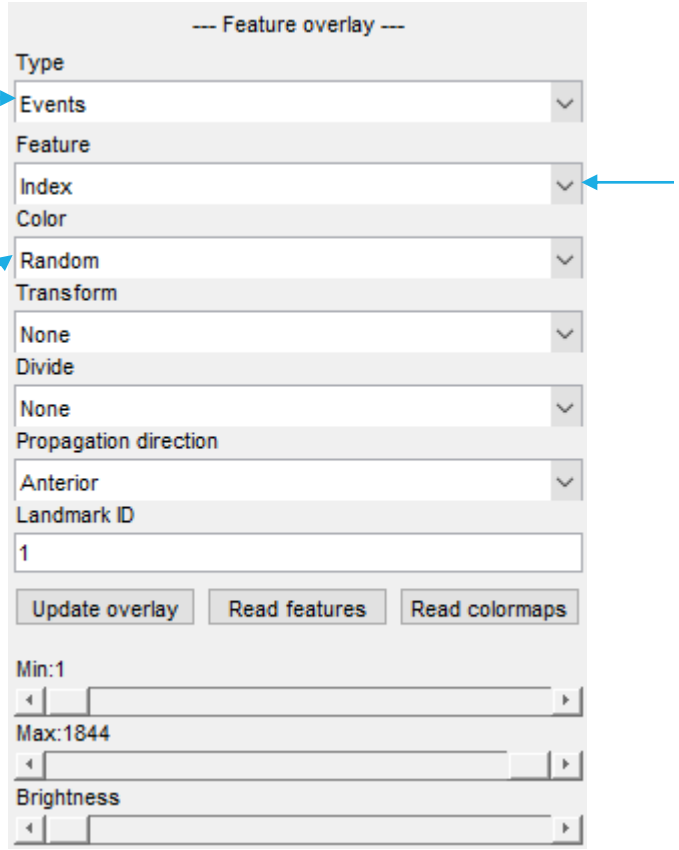


View features

Feature overlay

Choose ‘Events’ if you want to use a color code to represent feature values

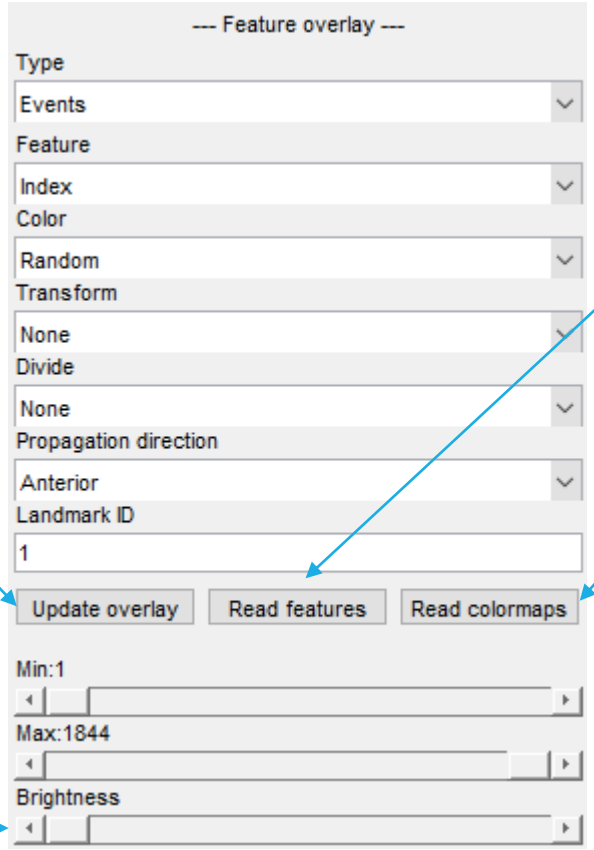
- Choose random to randomly assign colors to events.
- Choose ‘greenRed’ to assign green for lowest feature values and red for highest one. Values in between will be between green and red.
- You can also choose other color maps.



- Choose which feature to use.
- Choose ‘Index’ if you simply want to view individual events.
- For a complete list of features that can be used as well as their description, see online document. https://aqua-doc.readthedocs.io

Feature overlay

Once you changed the above settings, click ‘update overlay’ to take effect



You can define features by modifying ‘./cfg/userFeatures.csv’

Load user-defined color maps in ‘./cfg/userColors.csv’

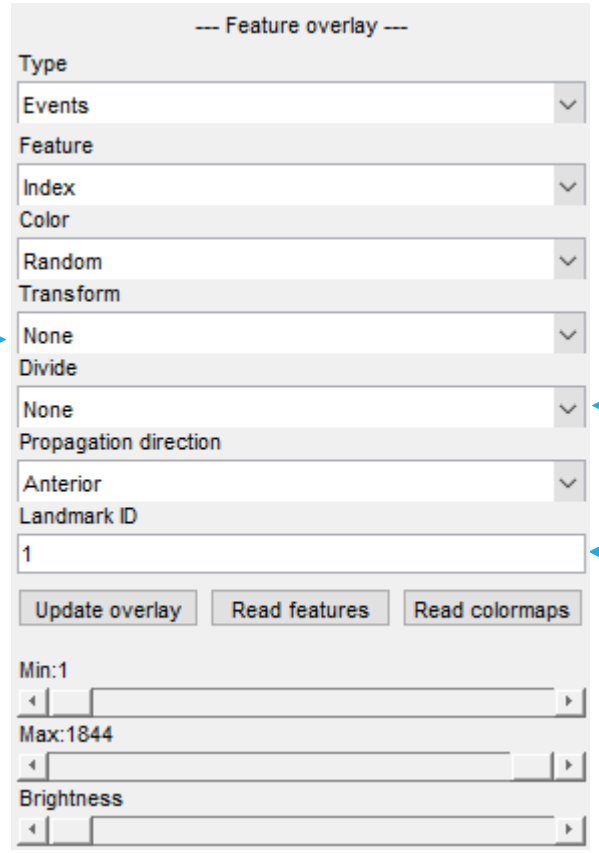
Choose the range of values of features to show

Adjust brightness of overlay

Feature overlay

Use original feature, or take some transform, like square root, because some features have very wide distributions making the color code less meaningful

Choose which propagation direction to show. Only relevant to propagation based features

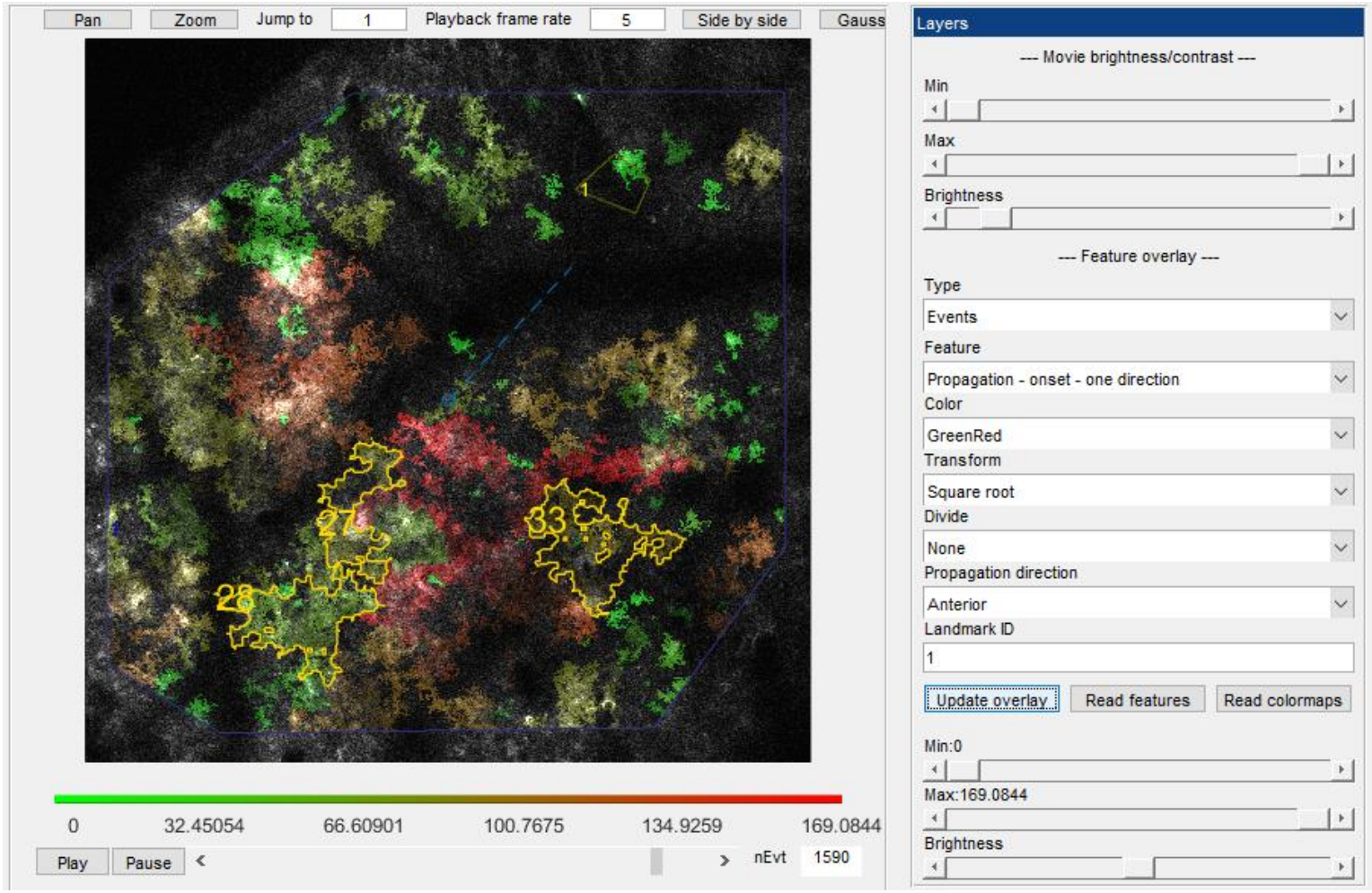


Use original feature, or divide it by other features, like size

Choose which landmark to use
Only relevant to landmark based features.

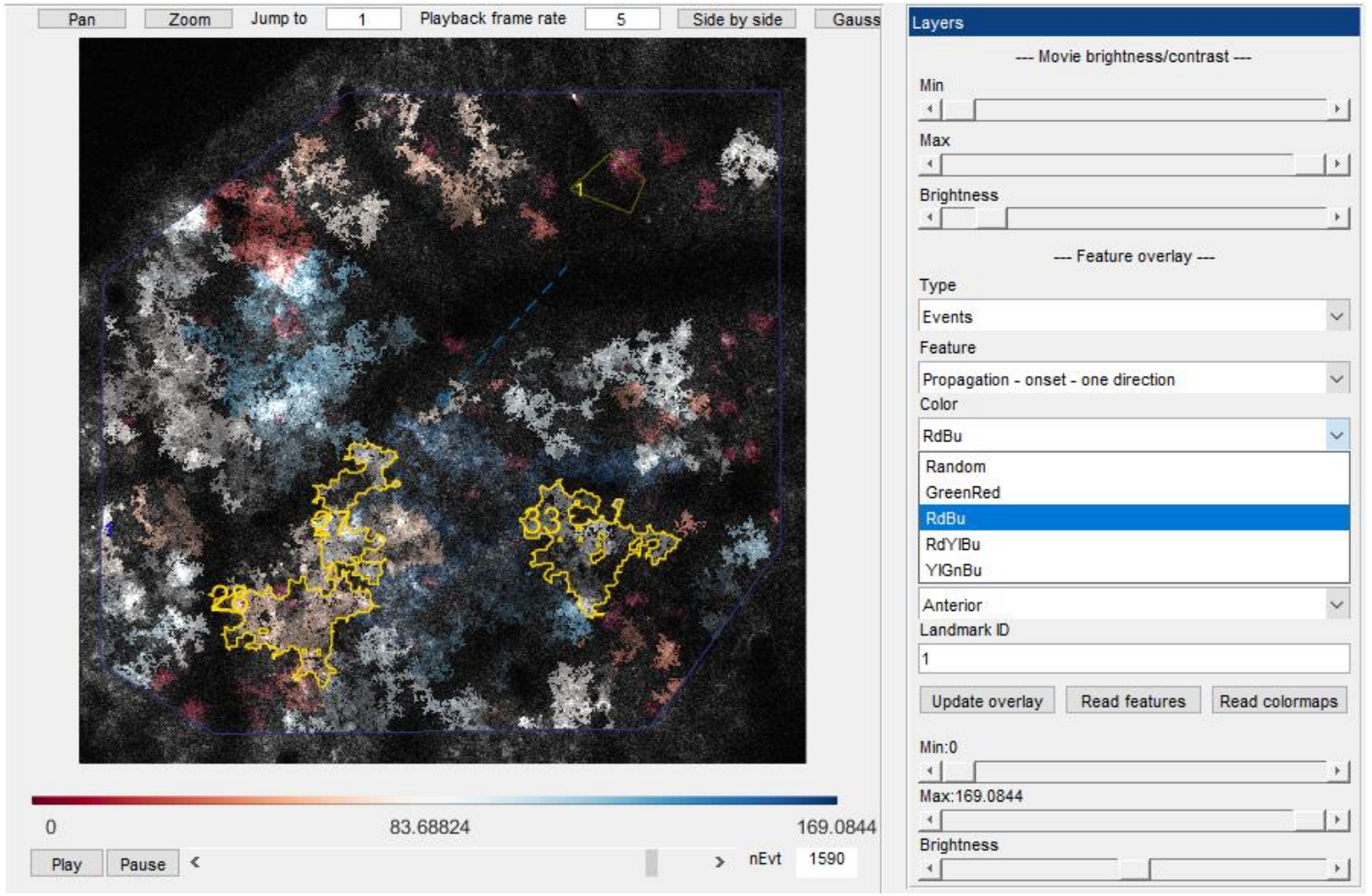
Example: propagation feature

- Onset propagation score.
- After square root transform.
- Anterior direction propagation.
- Use green-red color map. The color bar is shown in the bottom of the movie.



Example: color map

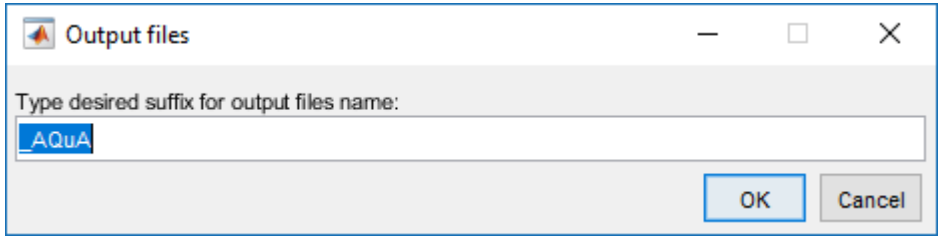
- We support several color maps. User can also define their own color map to view the features.
- Here we show the ‘RdBu’ colormap for the same feature as above.



Export and load experiments

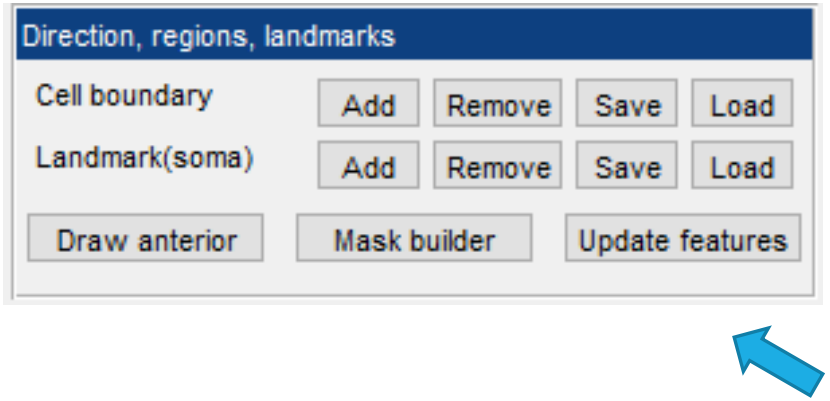
Export results

- To export results, click ‘Export/save’ and you will be asked to choose the place for exporting.
  - A dialogue will appear and ask you to give a postfix to the export file name. The movie name will be automatically included.
  - We will save the results in the ‘res’ data structure in the exported ‘mat’ file.
  - We will also export an Excel file listing features for filtered events.
- To export the movie overlay as well, check ‘Movie with overlay’.
  - Un-check ‘Events and features’ if you only need the movies.



Define/modify regions and landmarks

- After detection, you can still add/remove regions and landmarks, or draw the anterior direction
- After you make any changes, you need to click ‘Update features’ to re-extract the features



Load experiments

- You can load the results later. After opening the GUI, select ‘Load existing’ and select the saved ‘.mat’ experiment file
- Alternatively, you can load the ‘.mat’ to MATLAB, and launch the GUI with:
  - aqua_gui(res)