IVEA IMAGEJ PLUGIN SOFTWARE MANUAL

IVEA Version 1.1

Note: IVEA generates a log file for each analysis. In order to replicate the analysis, please open the log file in the output folder of your previous analysis and verify the parameters used.
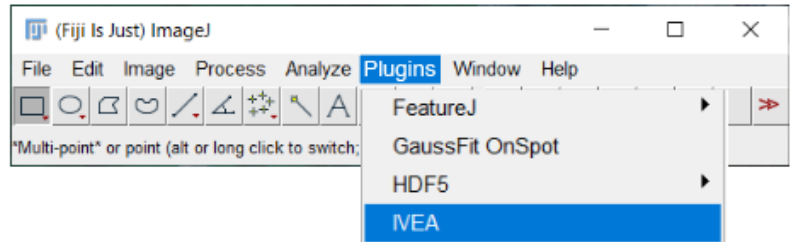
A. **IVEA location**: Fiji → Plugins → IVEA

Figure 1 Fiji-IVEA integration

B. **Non-Fixed Event Tab:** This tab is utilized for the analysis of mobile vesicles, including, but not limited to, T cells, chromaffin cells, INS1 cells, β cells, and others.
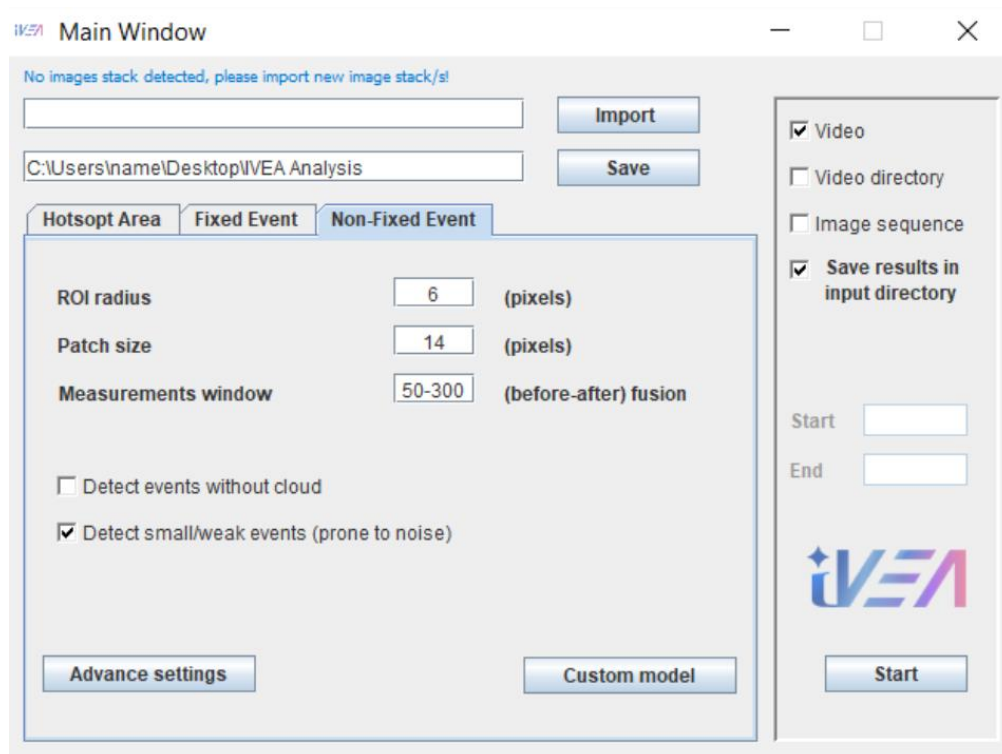

Figure 2 Non-Fixed Events Tab.

1- **ROI radius**: Size of the ROI radius surrounding an event. (This radius would impact the results measurements and the non-maximum suppression algorithm)
2- **Patch size**: Sequence image patch dimension. (This radius would affect the size of the patch surrounding the selected region, which is used by the neural network for visualization purposes)
3- **Measurements window**: Measurement time interval from frame a to frame b (a-b).

4- **Detect events without cloud**: Include events that undergo exocytosis without exhibiting any observable cloud. (Abrupt disappearance)

5- **Include small/weak events**: This option enables the software to accept low signal-to-noise events.

6- **Advanced settings button**: Opens a dialog for non-fixed FBE default parameters.

7- **Custom model button**: Opens a dialog that allows the user to control the export of training data and the use of custom neural networks.

1- **Prominence**: The highest minimum surrounding a local maximum. (This value is automatically determined, but users may choose to override it in cases where the signal-to-noise ratio is very low) (i.e., p = 30)

2- **Variation time window**: Subtract frame $n+4$ from frame n.

3- **Event spread**: This value can directly influence the non-maximum suppression algorithm. (see paper)

4- **Neural Network Confidence**: The degree of probability or certainty that a neural network assigns to its classification for a given input. (A higher confidence value indicates a lower probability of false positives, although it may result in the loss of some true events, default 0.5)

5- **Nomination sensitivity**: This option allows the user to adjust the detection threshold sensitivity manually. (User would change this value when, SNR is very low or when the video exhibits regions of non-uniform fluorescence intensity) (i.e., regions where cells are very bright and regions with faded cells)

6- **Gaussian blur**: Use gaussian blur to reduce noise. (default, true)

7- **Adjust for bleach correction**: Track fluorescence intensity over time. (default, true) (only for IVEA version 1.1 and up)

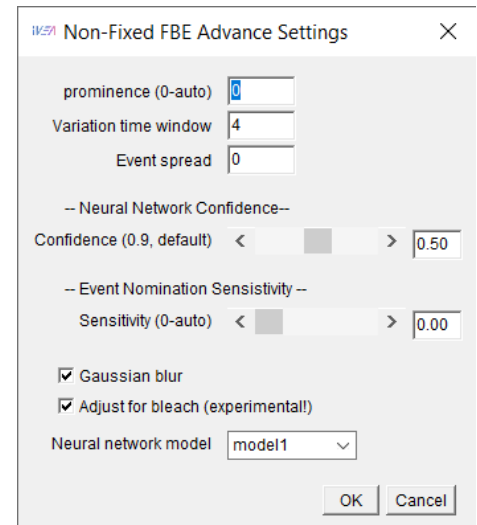8- **Neural network model**: Allow the user to switch between different pretrained models.

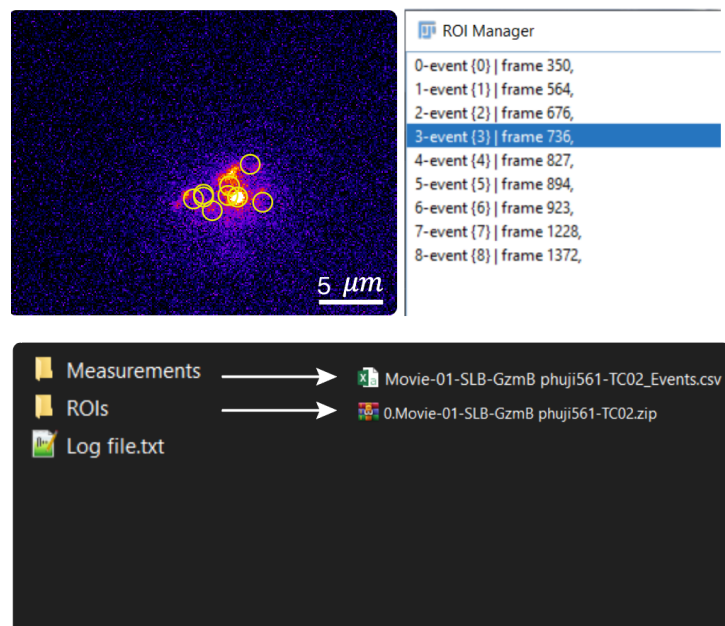*Figure 3 Non-Fixed Event Advance Settings window.*

Figure 4 Non-Fixed Event analysis output display.

Hint: To utilize the ROIs in Fiji with ease, simply click on the ROI manager tool and select the ROI you want. Then, press the Shift key + the arrow keys (left or right). From multi-channel mode Fiji allow the user to add Ctrl and Alt keys. (i.e., Shift + Ctrl + Alt + arrow keys)

C. **Fixed Event Tab:** This tab is utilized for the analysis of videos with fixed image acquisitions, such as DRG neurons.
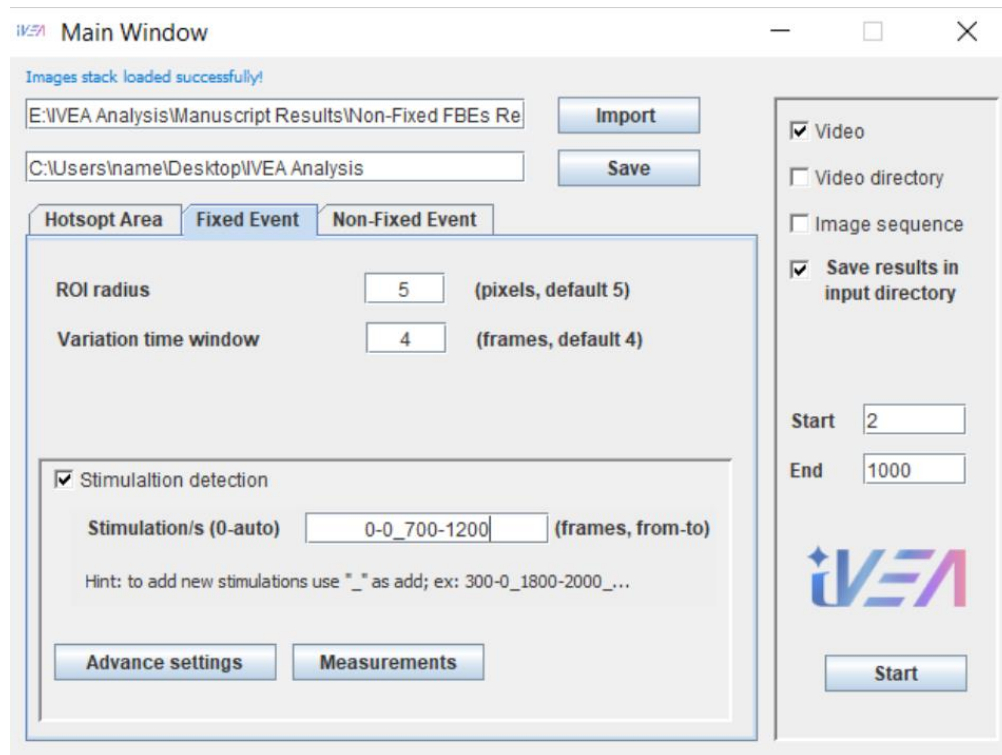

Figure 5 Fixed Event Tab.

1- **ROI radius**: Size of the ROI radius surrounding an event. (This radius would impact the results measurements)
2- **Variation time window**: Subtract frame *n+4* from frame *n*.
3- **Measurements window**: Measurement time interval from frame a to frame b (a-b).
4- **Stimulation detection**: Is a special feature for analysis with strong simulation to be detected like in DRG neurons.
5- **Stimulation/s**: Simulation timing from a to b (a-b). a or b are determined automatically if set to 0, "_" is used for more than one simulation. (Is used to detect strong simulation events)
6- **Advanced settings button**: Opens a dialog for fixed FBE default parameters.
7- **Measurements button**: Opens a dialog that allows the user to control the measurements to be conducted.

1- **Erosion**: Is the erosion filter. (default, 0)
2- **Prominence**: The highest minimum surrounding a local maximum. (This value is automatically determined, but users may choose to override it in cases where the signal-to-noise ratio is very low) (i.e., p = 30)
3- **Add frames**: This field allows the user to extend the analysis window time. (see paper)
4- **Neural Network Confidence**: The degree of probability or certainty that a neural network assigns to its classification for a given input. (A higher confidence value indicates a lower probability of false positives, although it may result in the loss of some true events, default 0.5)
5- **Nomination sensitivity**: This option allows the user to adjust the detection threshold sensitivity manually. (User would change this value when, SNR is very low or when the video exhibits regions of non-uniform fluorescence intensity) (i.e., regions where cells are very bright and regions with faded cells)
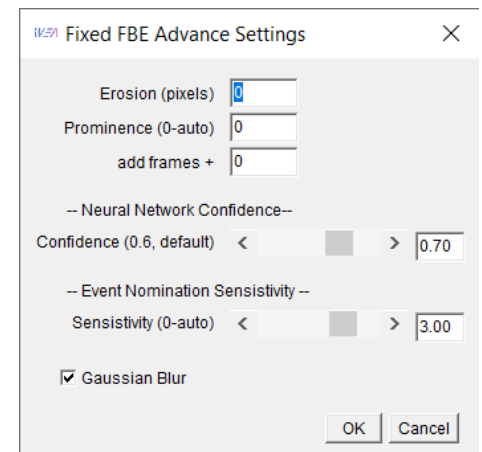6- **Gaussian blur**: Use gaussian blur to reduce noise. (default, true)
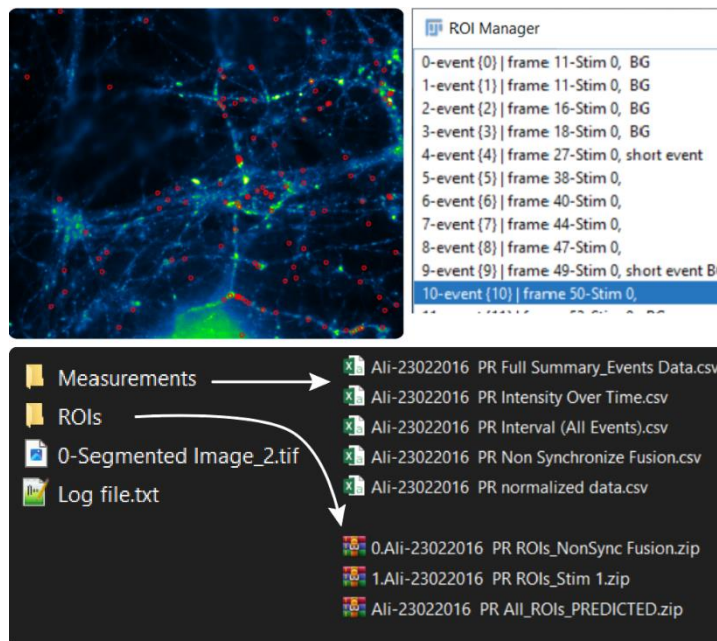
Figure 6 Fixed FBEs advance settings window

Figure 7 Fixed Event analysis output display.

D. **Hotspot Area Extraction:** This tab is utilized for the analysis of videos with fixed image acquisitions, such as AndromeDA nanosensor paint.
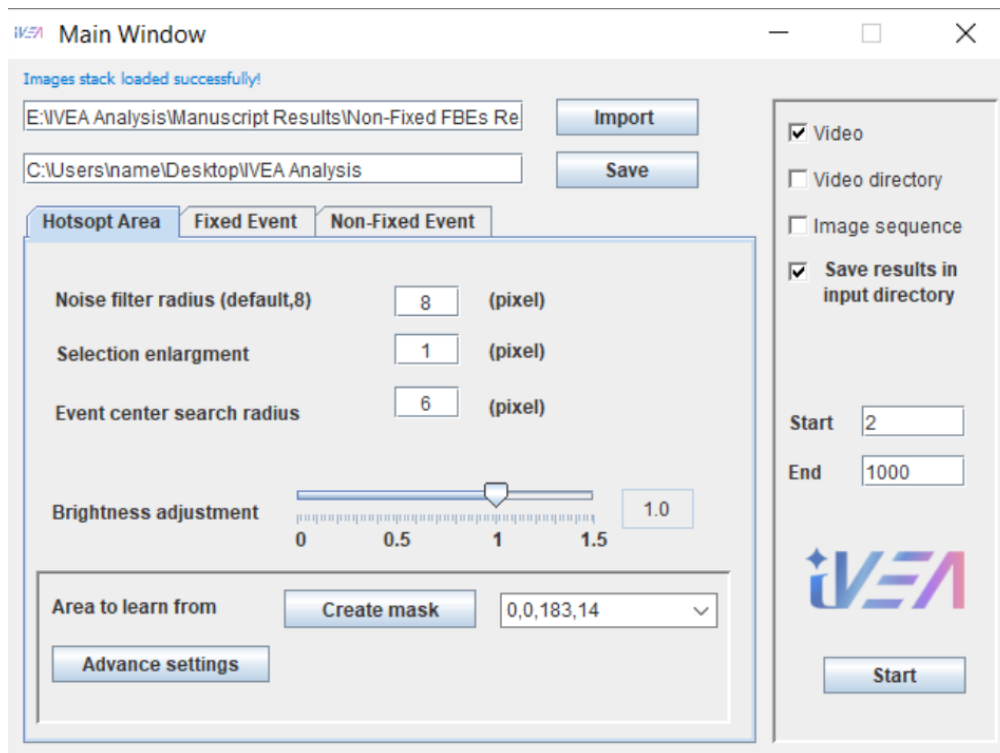

Figure 8 Hotspot Area Extraction Tab.

1- **Noise filter radius**: uses median filter to eliminate noise for the iterative global threshold. (default, 8)
2- **Selection enlargement**: enlarge the ROI by "n" pixels.
3- **Event center search radius**: spatial tracking radius.
4- **Brightness adjustment**: This value controls the fluorescence intensity fluctuations multilayer intensity correction (MIC) algorithm. (User would put this value to 0 if no fluctuations is taking place)
5- **Create mask button**: Enables the user to select a region on the video/s so that the software would try to learn noise from. (don't select area with events may occur in)
6- **Advanced settings button**: Opens a dialog for hotspot area extraction default parameters.

1- **Variation time window**: Subtract frame *n+3* from frame *n*.
2- **Exclude events in the background**: Don't detect events in the background.
3- **Learn background noise**: Allow the software to detect the background noise level and learn from it. (This option is disabled by default. In the event of an action occurring in the background, it may influence the sensitivity threshold by increasing it, thereby bypassing weaker events)
4- **Frames to learn from**: Study the first 2 or more frames.
5- **K-means clustering algorithm**: This option allows the user to adjust the number of clusters (layers) for image segmentation. (if k = 1, MIC will act like simple ratio equation) (User can monitor the result in the result folder for the image segmentation and adjust the k number)

Figure 9 Hotspot Area Extraction advance window.

6- **Adaptive Threshold**: Allow the user to extract the maximum regions. (default, 0-off)
7- **Global Threshold:** Is the iterative threshold. (default, 0-auto)
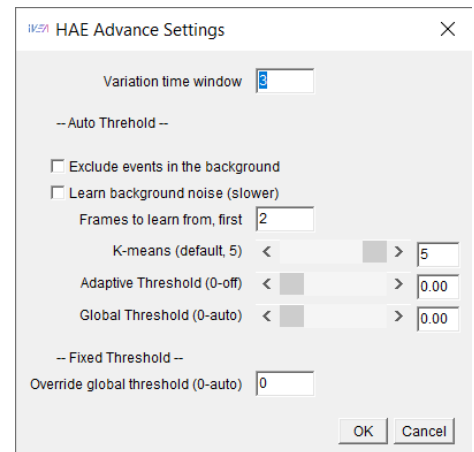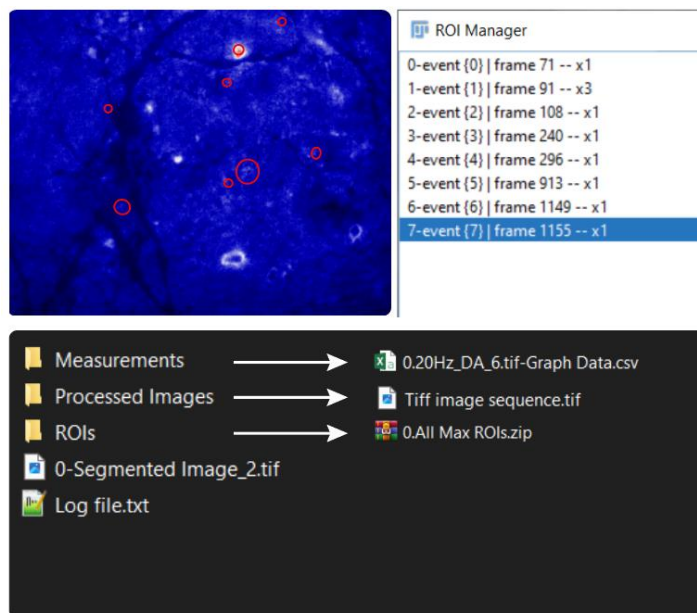8- **Override global threshold:** Allow the user to use static threshold. (not recommended)

Figure 10 Hotspot Area Extraction analysis output display.