IVEA IMAGEJ PLUGIN SOFTWARE MANUAL

IVEA Version 2.0

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Note: IVEA generates a log file for each analysis. To replicate the analysis, please open the log file in the output folder of your previous analysis and verify the parameters used.

I. IVEA plugin location:

Fiji → Plugins → IVEA

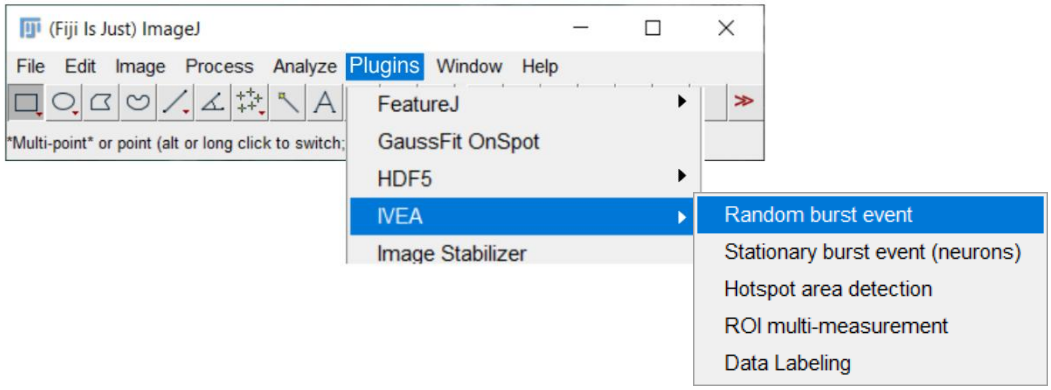


Figure 1 Fiji-IVEA integration

II. Random burst event:

This module is utilized for the analysis of mobile granules, including, but not limited to, T cells, chromaffin cells, INS1 cells, β cells, and others. This module facilitates event detection, classification, and measurement by leveraging neural network-based analysis techniques.

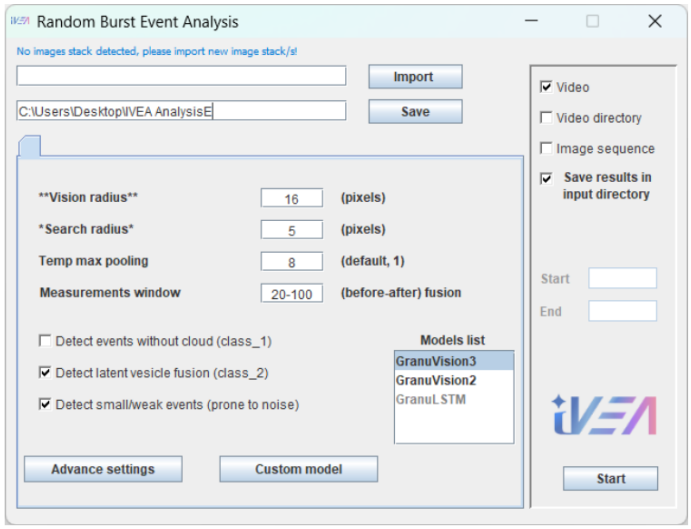


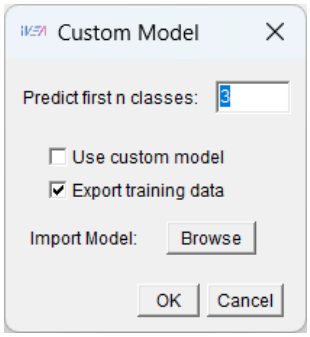
Figure 2 Random Burst Events GUI.

Key Parameters and Features

Each of the following settings is available as an input field or button within the GUI:

- **Vision Radius** *(Default: 14, Minimum: 6)*
  - Defines the radius of the neural network visualization area (patch size) surrounding an event.
  - This parameter is crucial for event classification.
  - At each ROI center, IVEA extracts the surrounding area over a time interval, enabling the Transformer neural network to analyze this region effectively.
- **Search Radius**
  - Determines the ROI radius surrounding an event.
  - Affects measurement accuracy and the Non-Maximum Suppression (NMS) algorithm.

- **Temporal Max Pooling**
  - Reduces video frames using the temporal max pooling technique (moving maximum intensity projection).
  - Useful when:
    - Event activity exceeds the neural network's temporal vision limit (26–28 frames).
    - Image acquisition frequency is particularly high (e.g., 50 Hz).
- **Measurement Window (a-b)**
  - Defines the time interval for event measurement:
    - a-b represents the frame range used for measurement calculations.
- **Model List**
  - Allows users to select pretrained models available for the Random Burst Events module:
    - **GranuVision3** *(Default Model)* – Encoder vision transformer trained on three exocytosis types:
      - Fusion with a cloud
      - Fusion without a cloud
      - Latent vesicle fusion
    - **GranuVision2** – Encoder vision transformer trained on two exocytosis types:
      - Fusion with a cloud
      - Fusion without a cloud
    - **GranuLSTM** – LSTM-based model (no longer supported).
      - While outdated, users can still experiment with it as a general classifier.
- **Detect Events Without Cloud** *(Category Label: 1)*
  - Enables detection of exocytosis events that do not exhibit an observable cloud.
  - These events are classified as abrupt disappearances.
- **Detect Latent Vesicle Fusion** *(Category Label: 2)*
  - Detects exocytosis events that occur suddenly without prior vesicle presence.
- **Include Small/Weak Events** *(Default: Enabled)*
  - Allows detection of low signal-to-noise ratio (SNR) events.
- **Advanced Settings Button**
  - Opens a dialog window for configuring default parameters for non-fixed burst event (FBE) analysis.
- **Custom Model Button**
  - Opens a dialog window that allows users to:
    - **Export training data** for model customization.
    - **Integrate custom neural networks** into IVEA.



Advance settings dialog

The Advanced Settings dialog provides additional configuration options for Random Burst Event analysis. These settings allow users to fine-tune event detection sensitivity, noise reduction, and fluorescence intensity tracking.

---

1. Key Parameters and Descriptions

- **Prominence** *(Default: Auto, User-Defined Option: p = 30)*
  - Defines the highest minimum value surrounding a local maximum.
  - Automatically determined, but can be overridden when the signal-to-noise ratio (SNR) is very low.
- **Variation Time Window**
  - Defines the frame subtraction interval: subtracts frame n+4 from frame n to detect intensity changes.
- **Event Spread** *(See Paper for Details)*
  - Directly influences the Non-Maximum Suppression (NMS) algorithm, which helps filter overlapping detections.
- **Neural Network Confidence** *(Default: 0.5)*
  - Sets the probability threshold for neural network classification.
  - Higher confidence values reduce false positives, but may also cause some true events to be missed.

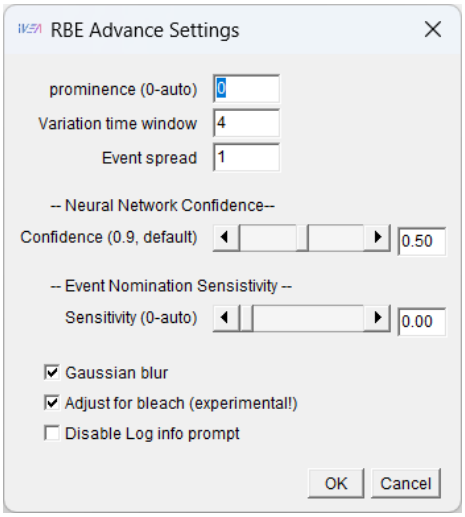


Figure 3 Random Burst Event (RBE) Advance Settings window.

- **Nomination Sensitivity** *(User-Defined Option)*
  - Adjusts the manual detection threshold sensitivity.
  - Recommended for:
    - Low SNR videos
    - Videos with non-uniform fluorescence intensity (e.g., bright cell regions vs. faded cell regions).
- **Gaussian Blur** *(Default: Enabled)*
  - Applies a Gaussian blur filter to reduce noise and smooth intensity fluctuations.
- **Adjust for Bleach Correction** *(Default: Enabled)*
  - Tracks fluorescence intensity variations over time to correct for photobleaching effects.
- **Disable Log Info Prompt** *(Default: Disabled)*
  - Prevents IVEA from displaying informational messages in the ImageJ Log window.

Output results:

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)

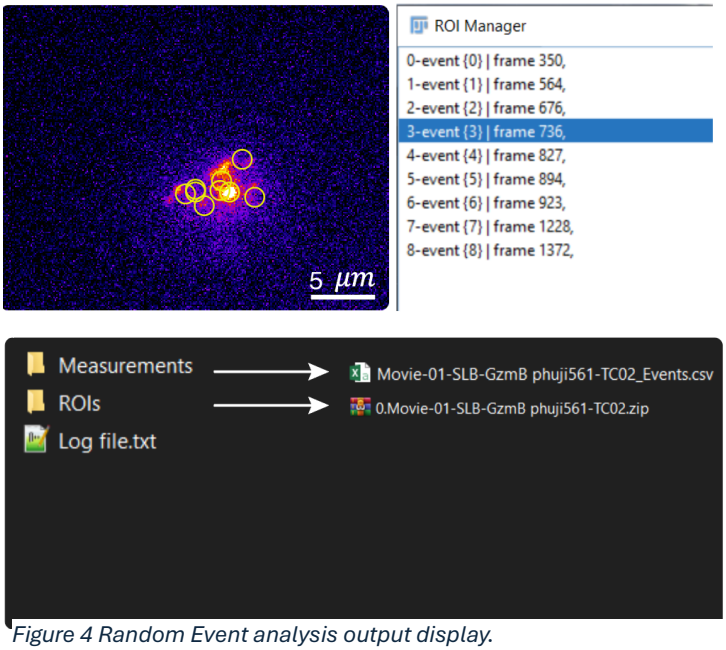


Figure 4 Random Event analysis output display.

III. Stationary Event Tab:

The Stationary Event module is designed for the analysis of videos with stationary events occurs at the neurons synapses, such as Dorsal Root Ganglion (DRG) neurons. This module enables precise event detection and processing for stationary burst events.

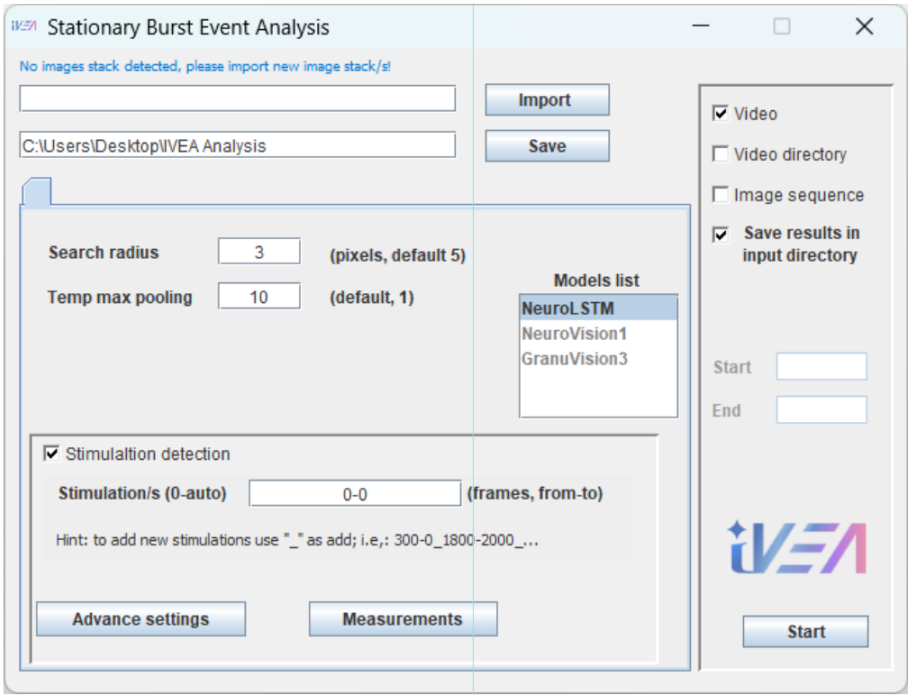


Figure 5 Stationary Burst Events module GUI.

Key Parameters and Features

Each of the following settings is available as an input field or button within the GUI:

- **ROI Radius**
  - Defines the size of the Region of Interest (ROI) surrounding an event.
  - This radius directly influences the event measurement accuracy.
- **Temporal Max Pooling**
  - Applies a temporal max pooling technique (moving maximum intensity projection) to reduce the number of video frames.
  - Useful in cases where:
    - Event activity slows down and exceeds the neural network's temporal vision limit (40 frames).
    - The image acquisition frequency is high (e.g., 50 Hz).
- **Model List**
  - Allows users to select from pretrained models for stationary burst event analysis:
    - **NeuroLSTM** – An LSTM model specialized for synaptic neurons (e.g., exocytosis events). *This is the default model for this analysis.*
    - **NeuroVision1** – An encoder-based vision transformer model trained on three synaptic neuron exocytosis types:
      - Short exocytosis
      - Long-stay exocytosis
      - Slow exocytosis

        - **GranuVision3** – Another encoder-based vision transformer model, trained on granules, similar to the model used in the Random Burst Events module.
    - **Important:** The encoder vision transformer networks in this module are experimental. They have been tested on a limited dataset, and their performance may vary. These models are still under continuous development and improvement.
- **Stimulation Detection**
    - A special feature designed to detect strong stimulation events, such as those occurring in DRG neurons.
- **Stimulation (a-b)**
    - Defines the stimulation timing window:
        - a-b represents the time range of stimulation.
        - If a or b is set to 0, they are determined automatically.
        - Use "_" to indicate multiple stimulation events.
- **Advanced Settings Button**
    - Opens a dialog window for configuring default parameters for Fixed Burst Event (FBE) analysis.
- **Measurements Button**
    - Opens a dialog window allowing users to customize measurement settings for event analysis.

Advance settings dialog

The Advanced Settings dialog provides additional configuration options for Stationary Burst Event (SBE) analysis. These parameters allow users to fine-tune event detection, background noise filtering, and neural network sensitivity settings.

Key Parameters and Descriptions

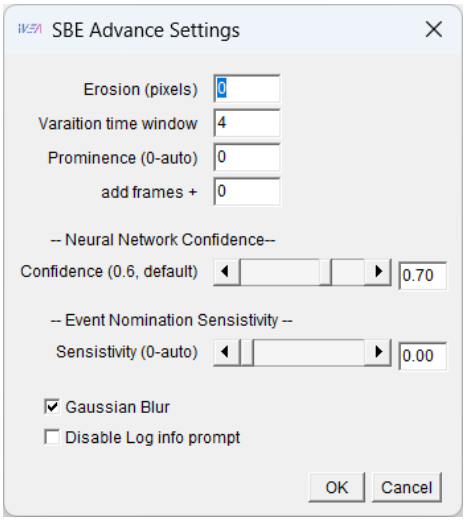


Figure 6 Stationary SBE advance settings window

- **Erosion** *(Default: 0)*
    - Applies an erosion filter to refine detected regions and minimize noise.
- **Variation Time Window**
    - Defines the frame subtraction interval: subtracts frame n+4 from frame n to detect changes over time.
- **Prominence** *(Default: Auto, User-Defined Option: p = 30)*
    - Represents the highest minimum value surrounding a local maximum.
    - Auto-detected, but can be manually adjusted when the signal-to-noise ratio (SNR) is very low.
- **Add Frames** *(LSTM Model Only)*
    - Extends the analysis window time, increasing the number of frames considered for event detection. *(Refer to the associated research paper for details.)*
- **Neural Network Confidence** *(Default: 0.5)*
    - Defines the probability threshold for neural network classification.

  - Higher confidence reduces false positives but may lead to missed true events.
- **Nomination Sensitivity** *(User-Defined Option)*
  - Adjusts the detection threshold sensitivity manually.
  - Useful when:
    - The signal-to-noise ratio (SNR) is low.
    - The video has regions of non-uniform fluorescence intensity (e.g., high-intensity and low-intensity regions within the same frame).
- **Gaussian Blur** *(Default: Enabled)*
  - Applies a Gaussian blur filter to reduce noise and smooth image intensity variations.
- **Disable Log Info Prompt** *(Default: Disabled)*
  - Disables IVEA's informational message prompts in the ImageJ Log window.

Output results:

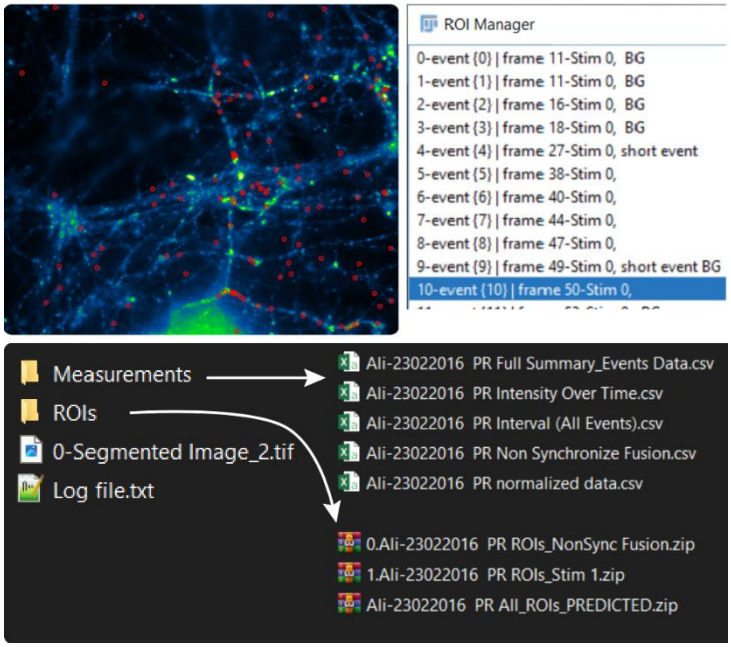


Figure 7 Stationary Burst Event analysis output display.

IV. Hotspot Area Extraction:

The Hotspot Area Extraction module is designed for analyzing videos with fixed image acquisitions, such as AndromeDA nanosensor paint experiments. This module enables precise event detection and hotspot localization by applying advanced filtering, selection, and brightness correction techniques.

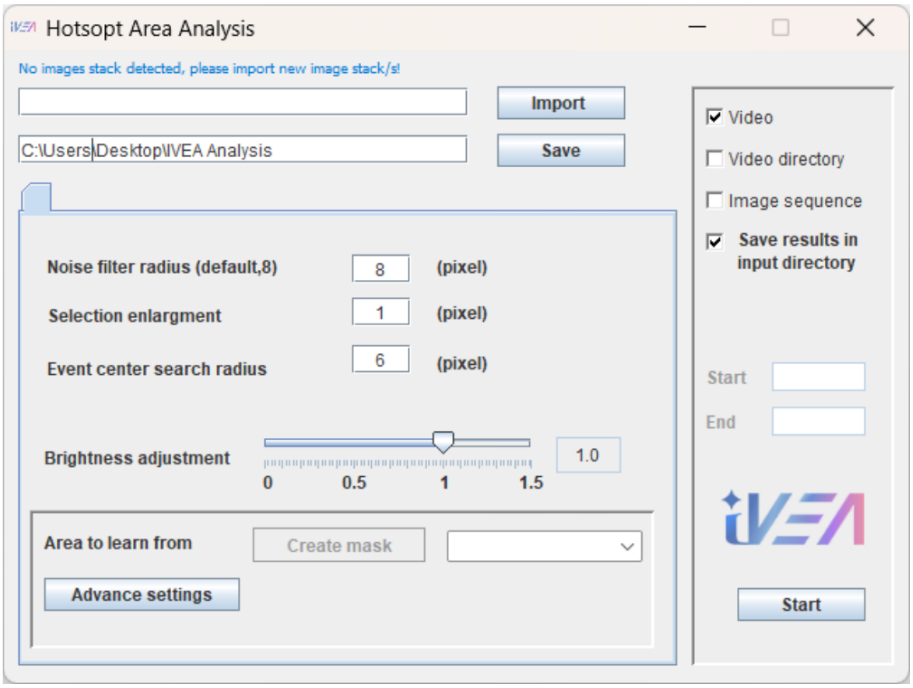


Figure 8 Hotspot Area Extraction module.

Key Parameters and Controls

Each of the following settings is available as either an input parameter field or a button within the GUI**:**

- **Noise Filter Radius**
  - Uses a median filter to eliminate noise before applying the iterative global threshold.
  - *(Default: 8 pixels)*
- **Selection Enlargement**
  - Expands the ROI (Region of Interest) by *n* pixels to include surrounding areas.
- **Event Center Search Radius**
  - Defines the spatial tracking radius for identifying the center of detected events.
- **Brightness Adjustment**
  - Controls fluorescence intensity fluctuations using the Multilayer Intensity Correction (MIC) algorithm.
  - Set this value to 0 if no fluctuations are present.
- **Create Mask Button**
  - Enables the user to manually select a region within the video(s) for noise identification.
  - Important: Do not select areas where events may occur, as this may affect detection accuracy.
- **Advanced Settings Button**
  - Opens a dialog window containing default parameters for Hotspot Area Extraction.

Advance settings dialog

Key Parameters and Descriptions

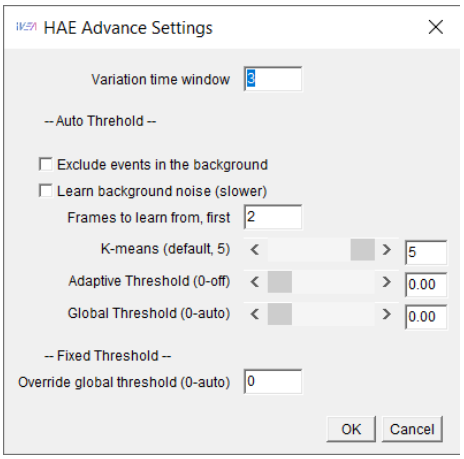


Figure 9 Hotspot Area Extraction advance window.

Each setting in the Advanced Settings dialog is designed to improve event detection accuracy.

- **Variation Time Window**
  - Determines the frame subtraction interval: subtracts frame n+3 from frame n to detect variations.
- **Exclude Events in the Background**
  - Prevents the detection of events occurring in the background.
- **Learn Background Noise** *(Disabled by default)*
  - Enables the software to analyze and learn the background noise level.
  - **Note:** If an event occurs in the background, this may increase the sensitivity threshold, potentially bypassing weaker events.
- **Frames to Learn From**
  - Defines the number of initial frames to analyze for background noise detection.
  - *(Default: first 2 frames, but can be adjusted for more frames as needed.)*
- **K-Means Clustering Algorithm**
  - Allows users to set the number of clusters (layers) for image segmentation.
  - If k = 1, the Multilayer Intensity Correction (MIC) functions as a simple ratio equation.
  - Users can monitor the segmentation results in the results folder and adjust the k value accordingly.
- **Adaptive Threshold** *(Default: 0 - Off)*
  - Enables adaptive thresholding to extract the maximum number of detected regions.
- **Global Threshold** *(Default: 0 - Auto)*
  - Uses an iterative thresholding method for detecting event regions.
- **Override Global Threshold** *(Not Recommended)*
  - Allows the user to apply a static threshold instead of the automatic iterative threshold.

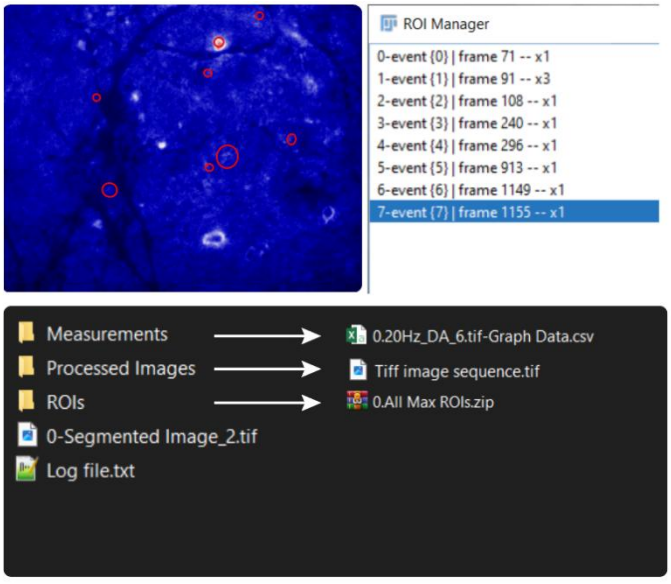


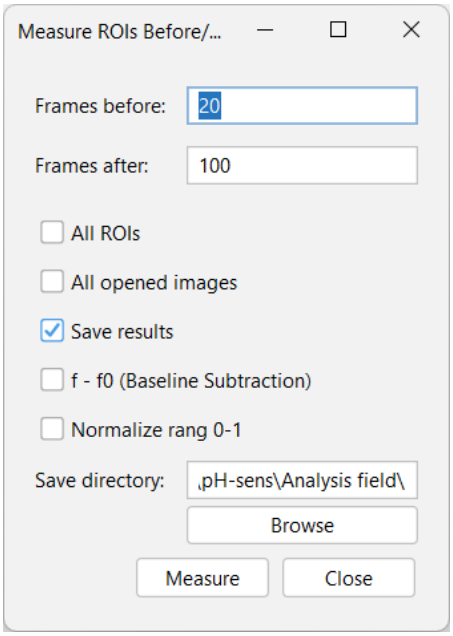
Figure 10 Hotspot Area Extraction analysis output display.

V. ROI multi-measurement plugin:

The IVEA Multi-Measurement ROI Plugin is designed to measure fluorescence intensity profiles over time within selected Regions of Interest (ROIs). This plugin enables users to analyze intensity fluctuations in single-channel or multi-channel opened videos. It serves as a helper plugin for studying IVEA-detected events or analyzing any ROI in ImageJ.

Key Parameters and Options

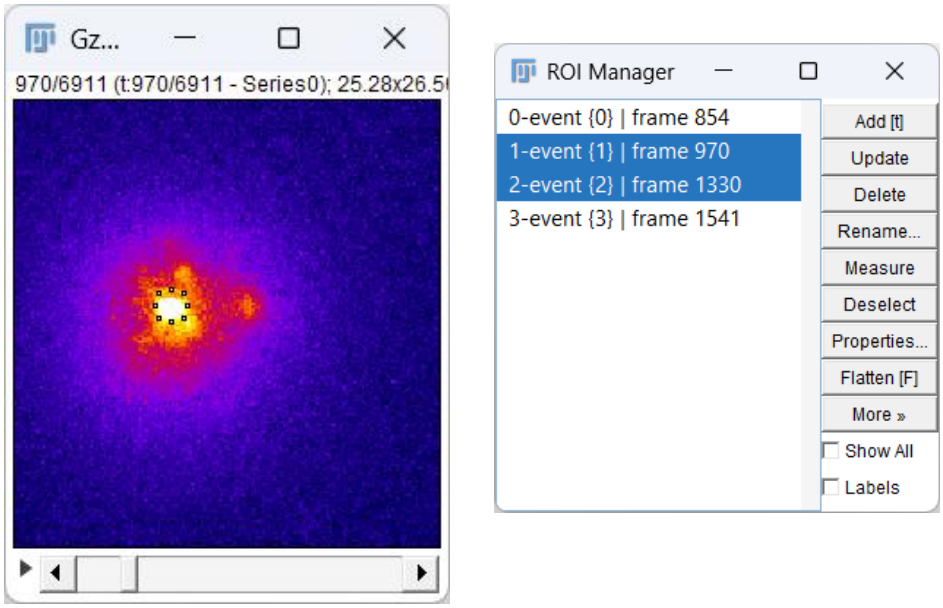
The plugin provides several options for customized intensity measurement, as shown in the interface:

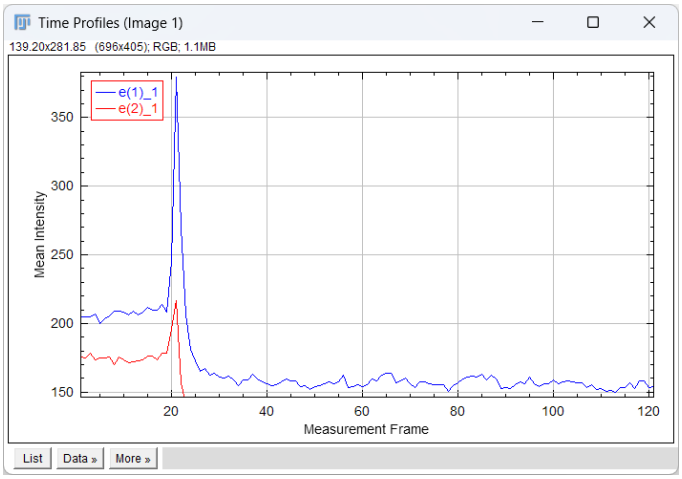


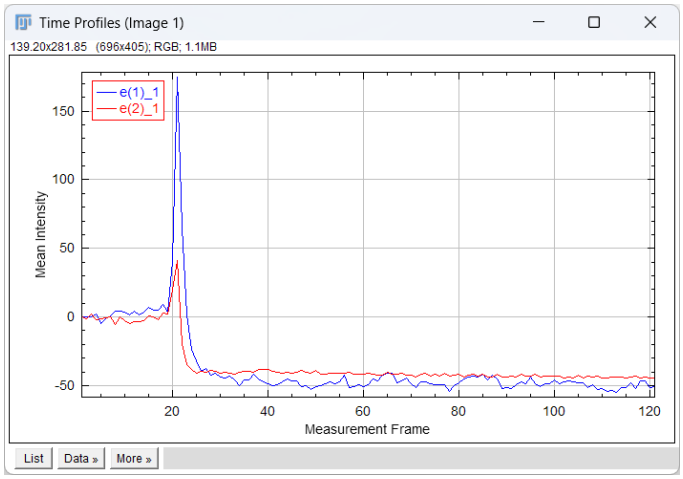
- **Frames Before** *(User-Defined Input, Default: 20)*
    - Specifies the number of frames before an event to include in the measurement window.
- **Frames After** *(User-Defined Input, Default: 100)*
    - Specifies the number of frames after an event to include in the measurement window.
- **All ROIs** *(Checkbox, Default: Unchecked)*
    - If enabled, the plugin measures intensity across all ROIs in the ROI Manager.
- **All Opened Images** *(Checkbox, Default: Unchecked)*
    - If enabled, the measurement is performed across all opened video frames.
- **Save Results** *(Checkbox, Default: Checked)*
    - If enabled, the measured intensity values are saved to a specified directory for further analysis.
- **f - f_0 (Baseline Subtraction)** *(Checkbox, Default: Unchecked)*
    - If enabled, baseline subtraction is applied to the intensity measurements using the equation f - f_0, where:
        - **f** = current intensity
        - $\mathbf{f_0}$ = baseline intensity (pre-event intensity)
- **Normalize Range 0-1** *(Checkbox, Default: Unchecked)*
    - If enabled, intensity values are normalized between 0 and 1, ensuring uniform scaling for comparative analysis.
- **Save Directory** *(Text Field & Browse Button)*
    - Specifies the directory where measurement results will be saved.

- Users can manually enter a path or use the Browse button to select a location.

- **Measure Button** *(Action Button)*
  - Initiates the intensity measurement process based on the selected parameters.







VI. Labeling New Data with the IVEA Data Labeling Plugin:

The IVEA Data Labeling Plugin is designed for labeling new data intended for training or refining models. This plugin enables users to efficiently categorize events and artifacts to enhance the performance of IVEA's machine learning models.

---

1. Labeling Categories

Exocytosis Labels (Positive Integers)

The following labels are assigned to exocytosis events:

- **0** – Fusion with a cloud
- **1** – Fusion without a cloud
- **2** – Latent vesicle fusion

Noise and Artifact Categories (Negative Integers)

Negative integer values are used to classify noise and artifacts:

- **-1** – Random noise
- **-2** – Vesicle intensity fluctuation
- **-3** – Moving vesicle
- **-4** – Random noise with intensity fluctuations
- **-5** – Intensity flickering and out-of-focus artifact
- **-6** – Intensity rise (vesicle docking)
- **-7** – Intensity decrease (vesicle undocking)
- **-8** – Light movement (e.g., passing light, cloud spreading as a wave, etc.)

---

2. Customizing Labels

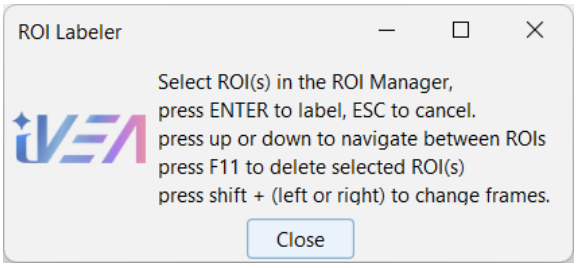
The available labels are **directly controlled** by the **IVEA JSON configuration file**, included with the **Python training project**. Users have the ability to:

- **Modify existing labels** to refine classification criteria.
- **Add new label categories** to expand the dataset's classification capabilities.
- **Adjust dataset labeling** for new datasets or manipulate existing datasets to fit specific research needs.

Expert users can further customize and optimize labeling parameters to improve model training and performance.

Labeling ROIs in IVEA:

IVEA provides an efficient keyboard-driven workflow for labeling Regions of Interest (ROIs) using the ROI Manager. Follow the steps below to label your data quickly and accurately.



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1. Navigating the ROI Manager

- Use the Up (↑) and Down (↓) arrow keys to navigate through the list of ROIs.
- Select the desired ROI(s) and press Enter to open the labeling dialog box.
- To navigate through frames while selecting ROIs, hold Shift and press Left (←) or Right (→) arrow keys to move between frames.

---

2. Assigning Labels

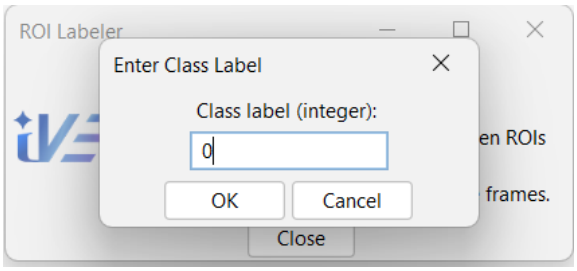
1. In the labeling dialog box, enter the class number for the selected ROI.
2. Press Enter again to confirm the label.
3. Press Esc to close the dialog if needed.

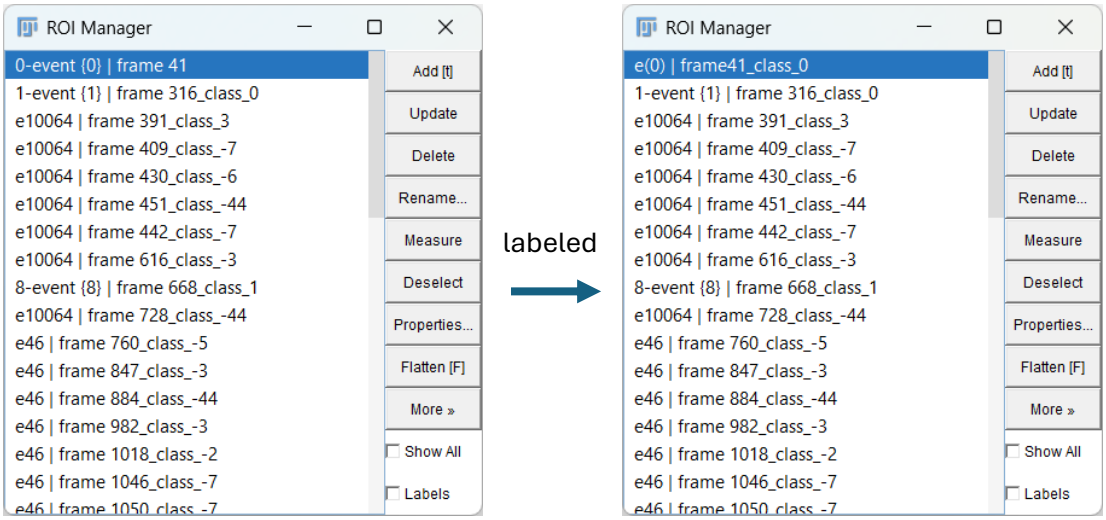
---

3. Labeling Categories

- **Fusion Events**: Assign positive integer labels (0, 1, 2, etc.) to indicate fusion event classifications.
- **Noise/Artifacts**: Assign negative integer labels (-1, -2, etc.) to classify unwanted artifacts or noise.

The keyboard shortcut-based workflow eliminates the need for excessive mouse interactions, ensuring a fast and streamlined labeling process.

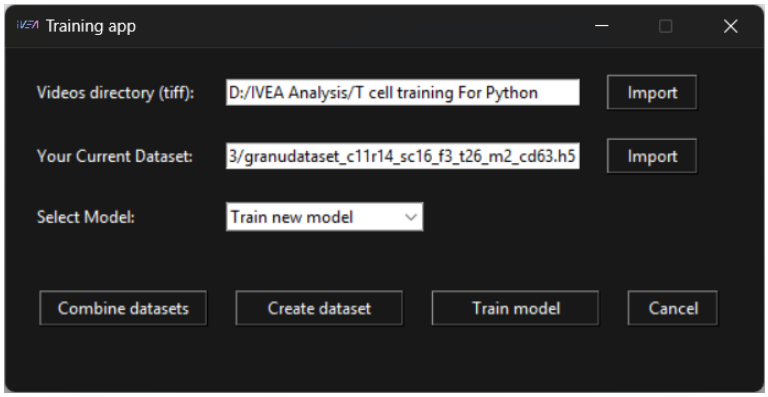

ROI Labeler
Enter Class Label
Class label (integer):
0
OK
Cancel
Close
en ROIs
frames.

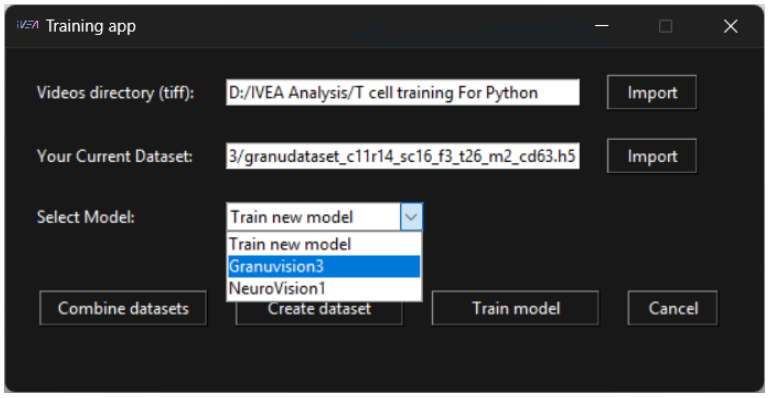

ROI Manager
0-event {0} | frame 41
1-event {1} | frame 316_class_0
e10064 | frame 391_class_3
e10064 | frame 409_class_-7
e10064 | frame 430_class_-6
e10064 | frame 451_class_-44
e10064 | frame 442_class_-7
e10064 | frame 616_class_-3
8-event {8} | frame 668_class_1
e10064 | frame 728_class_-44
e46 | frame 760_class_-5
e46 | frame 847_class_-3
e46 | frame 884_class_-44
e46 | frame 982_class_-3
e46 | frame 1018_class_-2
e46 | frame 1046_class_-7
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
labeled
ROI Manager
e(0) | frame41_class_0
1-event {1} | frame 316_class_0
e10064 | frame 391_class_3
e10064 | frame 409_class_-7
e10064 | frame 430_class_-6
e10064 | frame 451_class_-44
e10064 | frame 442_class_-7
e10064 | frame 616_class_-3
8-event {8} | frame 668_class_1
e10064 | frame 728_class_-44
e46 | frame 760_class_-5
e46 | frame 847_class_-3
e46 | frame 884_class_-44
e46 | frame 982_class_-3
e46 | frame 1018_class_-2
e46 | frame 1046_class_-7
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels

VII. Training using IVEA Python:

The **IVEA_main** script serves as the central interface for running the IVEA Python GUI. This section provides detailed instructions on how to train a new model, create datasets, and refine existing models within IVEA.

Main script GUI





1. Training a New Model

To train a new model using IVEA, follow these steps:

1. **Import Your Dataset**
    - Open the IVEA_main script.
    - Locate the Your Current Dataset field.
    - Import the dataset by specifying the path to your HDF5 (.h5) file.
2. **Select the Training Option**
    - In the Select Model dropdown menu, choose Train new model.
3. **Initiate Training**

- Click on the Train Model button to start training.

---

2. Creating a New Dataset

If you do not have an existing dataset, you can generate one from your labeled video files:

1. **Specify the Video Directory**
   - Locate the Videos Directory (TIFF) field.
   - Import the directory containing your video files.
2. **Generate the Dataset**
   - Click on the Create Dataset button.
   - The system will automatically process the videos and generate an HDF5 dataset.
   - Once completed, the new dataset location is loaded automatically.
3. **Train the Model**
   - Click on the Train Model button to start training using the newly created dataset.

---

3. Combining Datasets

To merge new data with an existing dataset, follow these steps:

1. **Import Data Sources**
   - Import the directory containing the new video files.
   - Import the directory of your current dataset.
2. **Combine the Datasets**
   - Click on the Combine Dataset button.
   - The datasets will be merged into a larger dataset.
   - The software will automatically update its reference to the new dataset.
3. **Start Training**
   - Click on Train Model to train using the newly combined dataset.

---

4. Refining an Existing Model

IVEA supports model refinement using your custom dataset. Currently, two models are available for refinement:

- **GranuVision3**

- **NeuroVision1**

To refine a model:

1. **Select the Model to Refine**
    - In the Select Model dropdown menu, choose the model you wish to refine.
2. **Provide Training Data**
    - Import the directory containing your labeled video files (ROI files, ZIP, or ImageJ ROI files).
3. **Prepare the Dataset**
    - Click on the Create Dataset button to process the videos and generate a new dataset.
    - If you already have a saved dataset, simply specify its directory path.
    - Once imported, the software will remember your last working directory for future sessions.
4. **Train the Model**
    - Click on Train Model to refine the selected model.

---

5. Managing Output Files

All outputs, including trained models and datasets, are stored in the project directory under IVEA_main.

To customize the output directory:

- Modify the JSON configuration files in the settings directory.
- There are three JSON files, each corresponding to a specific model available in the Select Model list.

Refer to Figure X (located below) for a visual guide to the IVEA interface and its key functionalities.

Configuration file

ivea_NeuroData_config.json | ivea_GranuData_config.json | ivea_default_config.json

settings > ivea_GranuData_config.json > ...

```
{
    "classes_map": [
        {"class_label": [0, 1, 11, 22], "class_id": 0, "radius": null},
        {"class_label": [6, 7], "class_id": 0, "radius": 10},
        {"class_label": [8], "class_id": 2, "radius": 12},
        {"class_label": [9], "class_id": 2, "radius": 10},
        {"class_label": [3], "class_id": 1, "radius": 10},
        {"class_label": [33], "class_id": 1, "radius": null},
        {"class_label": [-1], "class_id": -1, "radius": 12},
        {"class_label": [-2], "class_id": -2, "radius": null},
        {"class_label": [-3], "class_id": -3, "radius": null},
        {"class_label": [-4, -44], "class_id": -4, "radius": null},
        {"class_label": [-5], "class_id": -5, "radius": null},
        {"class_label": [-9], "class_id": -5, "radius": 11},
        {"class_label": [-6], "class_id": -6, "radius": null},
        {"class_label": [-7], "class_id": -7, "radius": null},
        {"class_label": [-8], "class_id": -8, "radius": 10}
    ],
    "_note_": " -------- Reading parameters for videos -------- ",
    "class_tag": "_class_",
    "roi_file_tag": "_training_rois",
    "file_extension": "tif",
    "outfile_dataset_name": "CTL-CC-INS_dataset",
    "model_name":"Granuvision3",
    "model_type":"random",
    "timeseries": 26,
    "more_frames":2,
    "default_radius": 14,
    "class_nolabel": 3,
    "radius_nolabel": 8,
    "_comment1": "**Turn off class_nolabel by setting it to (null) if you want to skip unlabeled ROIs**",
    "_note_1": " -------- Data handeling parameters -------- ",
    "num_augmentation": 2,
    "augment_data": true,
    "save_data": true,
    "output_dir":"E:/IVEA Analysis/T-Cells/Training_outputv3",
    "dataset_path": "E:/IVEA Analysis/T-Cells/Training_outputv3/granudataset_c11r14_sc16_f3_t26_m2_cd63.h5",
    "_comment2": "** Training new model with existing dataset provide the .h5 file, i.e, D:/project/CTL-CC-INS_dataset_c11r14_sc16_f3_t
    "_note_2": " -------- Neural network parameters -------- ",
    "epoch_num": 100,
    "batch_size": 100,
    "dropout_rate": 0,
    "call_patience": 5,
    "learning_rate": 3e-4,
    "scale_radius_to": 16,
    "_notes": "**label 5 skipped, is same as label 1 but very weak, **"
}
```