

# Semi-Tracker Tutorial

## Table of Contents

<b>1. Intruduction.....</b>	<b>2</b>
<b>2. Environment Setup .....</b>	<b>2</b>
<b>3. Software Architecture.....</b>	<b>3</b>
3.1 Main Window.....	3
3.2 View panel.....	4
3.2 Function panel.....	4
3.3 Cell management panel .....	5
<b>4. Analysis workflow.....</b>	<b>6</b>
4.1 Start up Semi-Tracker.....	6
4.2 Load input images and define project folder .....	6
4.3 Normalization.....	7
4.4 Segmentation and Tracking.....	7
4.5 Result output.....	7
<b>5. Annotation tools.....</b>	<b>8</b>
<b>6. Training module of Deep Neural network.....</b>	<b>8</b>

## 1. Intruduction

Semi-Tracker is a highly integrated graph user interface software developed for the cell segmentation and tracking of time-lapse microscopy image. Semi-Tracker covers essential steps for full analyzing procedure of microscopy images with the following features:

- ✓ A GUI for cell image normalization
- ✓ A GUI for cell image segmentation
- ✓ A GUI for cell image tracking
- ✓ A GUI for manual correction
- ✓ A GUI for cell property profiling and intensity quantification
- ✓ A GUI for training data annotation
- ✓ A GUI for deep CNN model training
- ✓ A Python API for deep CNN model training

Semi-tracker is an open-source software under the GPL-3.0 license. It is implemented in pure Python. The source code, instruction manual, and demos can be found at <https://gitlab.com/ecart18/semitracker/>.

## 2. Environment Setup

Semi-Tracker supports popular operating systems including Windows, Linux, and MacOS. To eliminate the unexpected environment dependency errors caused by the operating system or customized environment of users, the python virtual environment is strongly recommended for users. The environment setup and Semi-Tracker installation procedure from scratch are also available on the project homepage in GitHub.

Semi-Tracker software runs on Python 3.5 or higher version and requires some dependency packages as shown in Table S1. In principle, all these packages can have dependencies that must be fulfilled.

package	version
numpy	1.17.2
pandas	1.0.4
matplotlib	3.1.2
opencv-python	4.1.1.26
PyQt5	5.14.1
PyQt5-sip	12.7.1
pyqtgraph	0.10.0
scipy	1.4.1
six	1.13.0
sklearn	0.22.1
Jinja2	2.11.1
torch	1.4.0
torchvision	0.5.0
imageio	2.6.1

### 3. Software Architecture

Semi-Tracker is designed for cell segmentation and tracking analysis of time-lapse microscopy image. The overall architecture of Semi-Tracker is decoupled as four separate modules according to its function, including file explorer, algorithm analysis pipeline (normalization, segmentation, tracking, and visualization output), annotation tools, and retraining module for deep CNN model.

#### 3.1 Main Window

The users can start Semi-Tracker by running the file main.py in the terminal after that Semi-Tracker will launch the initial GUI main window as shown in Figure 1. The main window of Semi-Tracker is divided into four parts, which consist of a function panel, main view panel, cell management panel, and image preview panel.

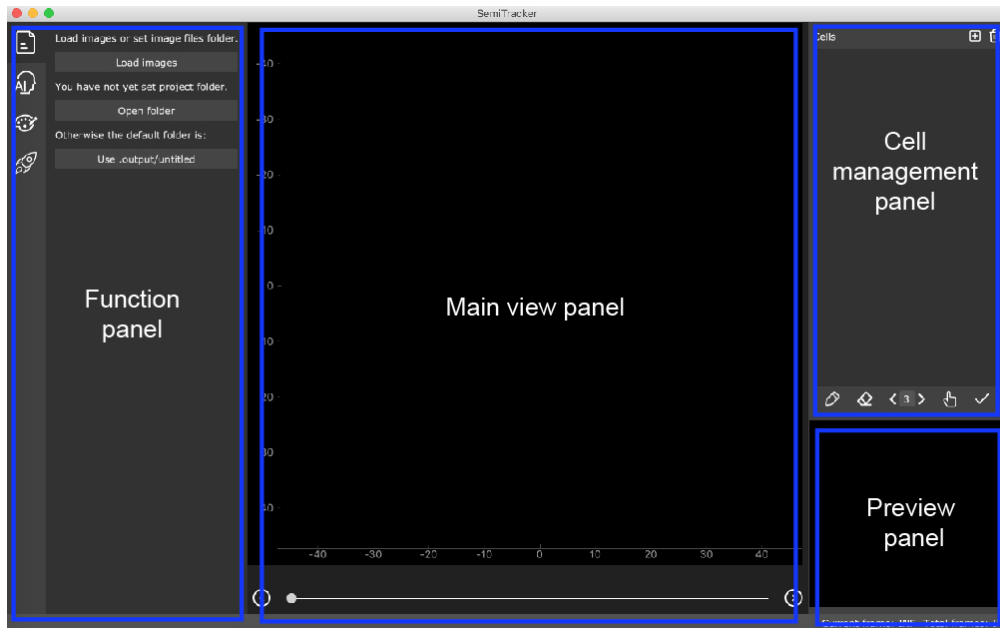


Figure 1: Main window of Semi-Tracker with its four image subpanels, function panel (left blue rectangle), main view panel (central blue rectangle), cell management panel (top right blue rectangle), image preview panel (bottom right blue rectangle).

### 3.2 View panel

In this section, we simply describe the two view panel of Semi-Tracker including the main view panel and the preview panel. The main view panel is used to show the resulting image of each process step such as normalization, segmentation, and so on. The slider at the bottom of the main view is the indicator of the frame index of the image sequence. Browsing through the time series can be achieved by moving the slider, which leads to synchronous updating of the images in the main view. The main view panel supports the zoom function. Users can scroll up or down the mouse wheel to adjust the focal zone of the image. Meanwhile, the coordinate axis in the main view provides the absolute location information of the focal zone. The preview panel is a small window to display the origin image..

### 3.2 Function panel

The function panel provides access to the file explorer, algorithm analysis pipeline (normalization, segmentation, tracking, and visualization output), annotation tools, and retraining module as shown in Figure 2. Users can expand each subpanel by clicking the corresponding icon.

The algorithm analysis pipeline defines the workflow for the general task of microscopy image analysis, which is composed of four modules, normalization, segmentation, track, and output. The third function panel is annotation tools, which are used to generate supervised labels for customized datasets. This tool also can be used to correct the results of automatic algorithms at pixel resolution. The last function panel, retraining module, provides a GUI for deep CNN model training.

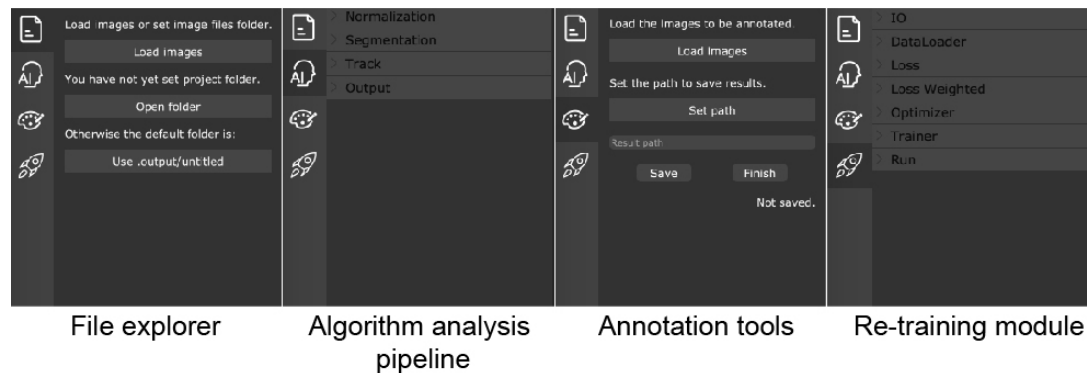


Figure 2: Function panel of Semi-Tracker with its four subpanels, From left to right are file explorer, algorithm analysis pipeline, annotation tools, and re-training module.

### 3.3 Cell management panel

The cell management panel has two functions: one is to manage all the cell instances such as add and delete, and the other one is to edit the cell segmentation mask.

Firstly, the cell management panel organizes all the segmented cells for the corresponding image displayed in the main view. Double-clicking the cell name will bring up a menu showing the cell properties, such as cell size, cell intensity, cell centroid coordinates and other attribution as shown in Figure 3.

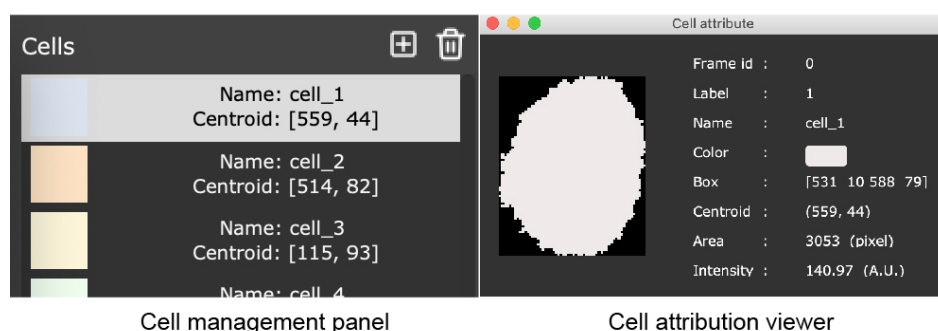


Figure 3: The cell management panel. Double-clicking the cell name pop up the cell attributions.

Second, as shown in Figure 4, the cell edit toolbar at the bottom of the cell management panel is used to edit the cell mask manually. The cell edit toolbar is composed of five sub-buttons, a brush button for adding a new mask, an eraser button to delete history mask, a drag button to move the image in the main view, a size modify button to change the size of brush or eraser, and an update buttons to ensure and save user changes. By leaving the mouse arrow on buttons for a while, a help text will appear with a short description.



Figure 4: The cell edit tool bar. The cell mask can be edit and delete as you want.

## 4. Analysis workflow

### 4.1 Start up Semi-Tracker

Create a new folder and drag inside all the files of the GUI. To run Semi-Tracker, open a terminal (terminal in macOS or Linux, or cmd on Windows), and create a new virtual environment as <https://gitlab.com/ecart18/semitracker/>. Move into the directory where the files are and run: python main.py and then press Enter. The GUI of Semi-Tracker will pop-up. It runs on Linux, Windows, and Mac.

### 4.2 Load input images and define project folder

Loaded images are required before the analysis process. The user should define the project folder which is used to save the output results of the analysis. The image size of a given microscope must be the same. If the user loads a set of images, the displayed images will follow the order of the file names. After the time-series image is loaded, you can browse the time series by moving the slider at the bottom of the main view panel.

The supported formats for input image including static image format 'png', 'jpg', 'jpeg', 'tif' and video format 'mp4', 'avi', 'mpg'.

### 4.3 Normalization

After loading images, the algorithm analysis pipeline is divided into four major steps, which consist of normalization, segmentation, tracking, and results output. Users can click on the options to choose the corresponding function.

Semi-Tracker provides four optional normalization methods, including Equalize normalization, Min-Max normalization, Retinex algorithm MSRCR, and Retinex algorithm MSRCR. Normalization allows user to increase the amount of visible detail by enhancing contrast.

### 4.4 Segmentation and Tracking

To improve the processing capability of the multi-modal image in real-world datasets, four candidate segmentation algorithms are provided for selection in Semi-Tracker. One of them, GradCut, is interactive algorithms. The remaining three methods are the baseline algorithm Otsu threshold, Watershed algorithm, and the deep model-based convolution network. After segmenting, the tracking algorithm is used to track cells across frames in temporal and keep the lineage information for each cell. For each algorithm, the default parameters are recommended for the common case. When needed, Semi-Tracker allows users to modify the hyper-parameters.

Note that all algorithms are automatic, but users can correct the result of segmentation and tracking with the cell management panel manually. Please see the details in Section 3.3.

### 4.5 Result output

For user convenience, Semi-tracker provides a comprehensive analysis reports with HTML and CSV, which includes tables of cell property profiling and figures of long-term single-cell fluorescence intensity quantification. Moreover, exporting images and videos for the visualization of cell segmentation and tracking are also supported. By select the attribution name with the checkmark, the user can define the output attribution in the visualization image including color mask, bounding box, cell edge, trajectories, and cell label. Finally, click on the push button “Output” to output the results, and the output file will be saved in the project folder.

## 5. Annotation tools

Before annotation, the users should load the initial set of original images and define the output folder of annotation images. Semi-tracker can label images based on automated segmentation results to simplify the annotation process. Hence this hand-drawing work which consumes most of the time in annotation could be mitigated. Besides, Semi-Tracker supports annotating the image with interruption resuming capability to prevent annotation file missing caused by possible software corrupting.

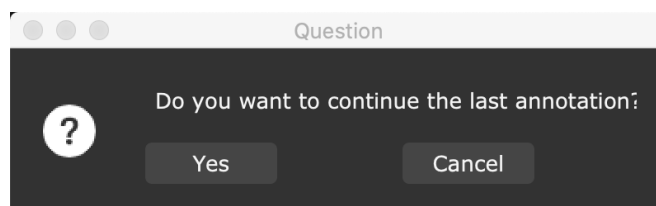


Figure 5: Semi-Tracker supports annotating the image with interruption resuming capability. Users can continue the last annotation based on the annotation cache.

## 6. Training module of Deep Neural network

Semi-Tracker provides a GUI and Python API for training the deep U-Net model of customized datasets. Before training U-Net, the users must set the following parameters in the GUI or python scripts (the demo code is released in GitHub).

- ***Set source folder [source\_img\_root in Python API, default= None]***

The folder path of source image used to train the model. The supported formats for source image including static image format 'png', 'jpg', 'jpeg', 'tif'.

- ***Set label folder [label\_img\_root in Python API, default= None]***

The folder path of label image used to train model. The supported formats for label image including 'png' and 'tif'. The numbers of source image and label image should be equal, and the order of the source image and label image should be consistent.

- ***Set log folder [log\_root in Python API, default= None]***

The log file save path for training.

- ***Batch size [batch\_size in Python API, default= 4]***



The batch size for one forward and backward iteration in training.

- **Validation ratio [validation\_ratio in Python API, default= 0.3]**

The random split ratio of validation set.

- **Scale image [scale\_img in Python API, default= 1]**

The scale ratio of the training image compared to the original image. If the size of the original image is too large or too small, this parameter could be used to reduce computing costs or enhance image quality.

- **Parallel works [workers in Python API, default= 0]**

The number of parallel threads in the training stage. It can be used to lower the training time.

- **Augmentation [aug\_list in Python API, default= None]**

The optional augmentation methods for training, including Flip, Rotate, GaussainNoise, and GaussainBlur. Parameter aug\_list should be list type in Python API.

- **Loss [loss\_type in Python API, default= DiceLoss]**

The loss function used to training U-Net, the valid option includes DiceLoss, WeightedSoftDiceLoss, BCELoss, WBCELoss, MSELoss, and MAELoss.

- **Weight [weighted\_type in Python API, default= None]**

The weight option used to balance the positive and negative samples in training. Can be edge\_weighted, sample\_balance and None.

- **Weight decay [weight\_decay in Python API, default= 0.0005]**

The weight decay for Adam optimizer.

- **Learning rate [lr in Python API, default= 0.001]**

The initial learning rate for Adam optimizer.

- **epoch [epochs in Python API, default= 100]**

The epoch number of training.

- **GPU number [gpu\_num in Python API, default= 0]**

The GPU number in the training stage.

- **Load model [resume in Python API, default= None]**

The string of model path. If there is a valid pre-train model, the users can finetune U-Net based on the pre-train model.