ImaEdge 1.0

User Manual

Content

1. Preparation

1.1 Source File

Please download the software package and unzip it (Fig. 1). The unzipped folder includes the following two image stacks (each stack contains 19 frames) for testing.

(1) Channel1.tiff. Test image for the first channel.

(2) Channel2.tiff. Test image for the second channel.

** Default values are provided in the GUI for these two test images.*

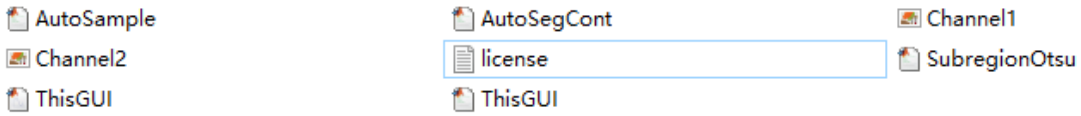

Figure 1. Files included with ImaEdge 1.0 package

1.2 Data Directory

While using ImaEdge, three new folders will be created containing all intermediate and ultimate data.

(1) data. This folder contains all intermediate and final data as .mat files.

(2) result. The final heatmaps are saved in this folder.

(3) verification. The materials that need verification are kept in this folder.

2. Data Processing

To start, run the script, ThisGUI.m to load the GUI (Fig. 2).

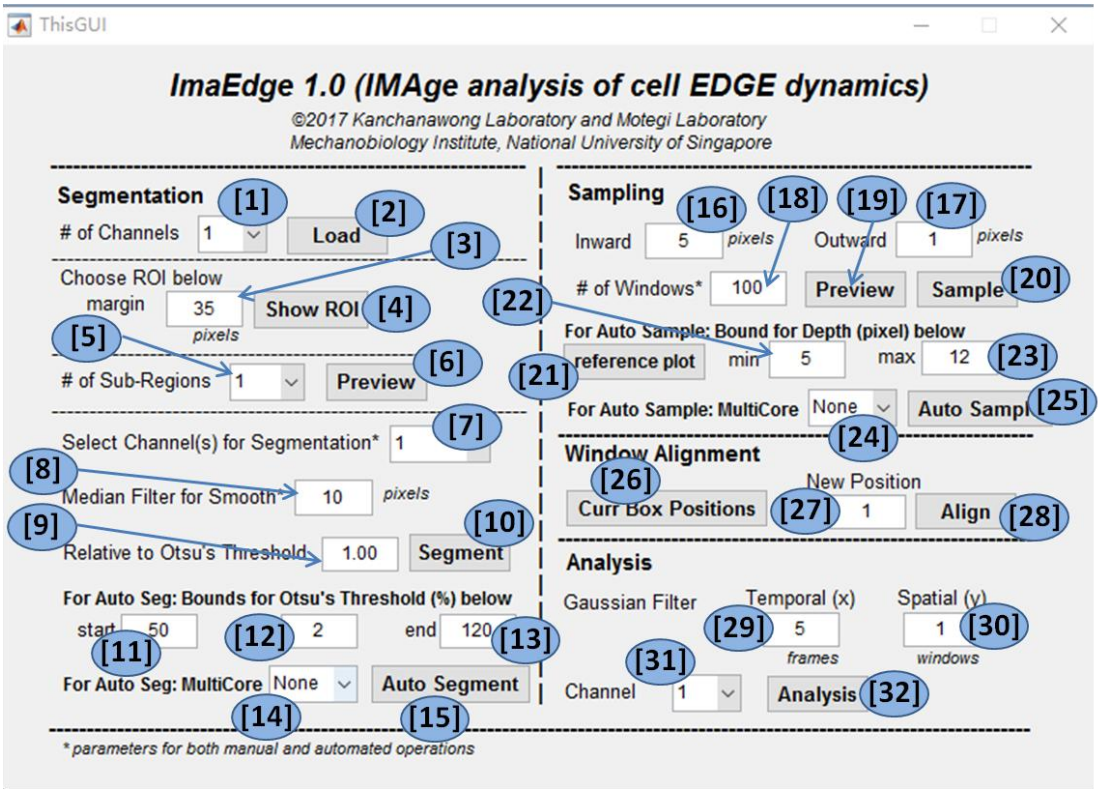

Figure 2. GUI of ImaEdge 1.0

2.1 Segmentation

2.1.1 Load Image

ImaEdge allows movie entries from up to 3 channels (Fig. 3A).

** If multi-channel images are acquired, it is recommended to use a combined multi-channel image for reliable segmentation (choose at [7] in Fig. 2). However, segmentation using a single channel is still available in ImaEdge as shown in Fig. 3C.*

Choose the number of channels to process at [1] (Fig. 2 and Fig. 3A) and click [2] (Fig. 2) to load image stacks sequentially.

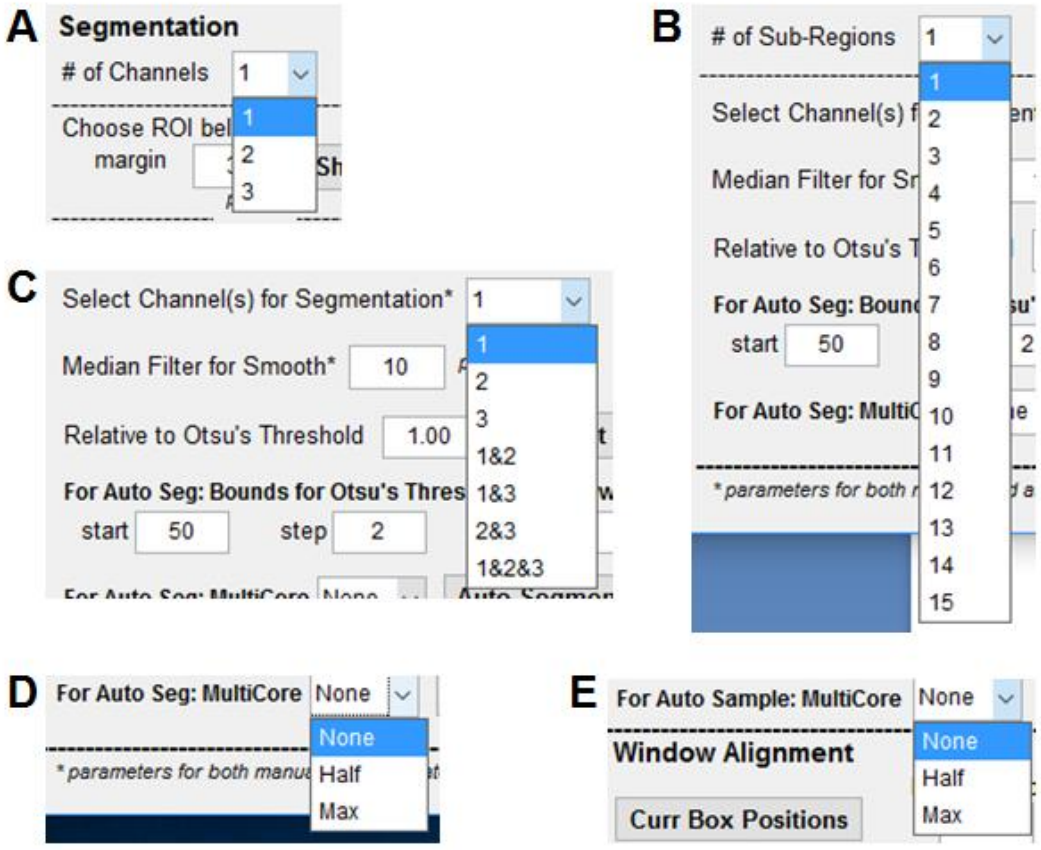

Figure 3. Pop-up menus in ImaEdge

2.1.2 Choose ROI

Choose ROI by setting the margin relative to the contour of the cell (automatically estimated by the program) for segmentation at [3] (Fig. 2).

Click [4] (Fig. 2) to check whether the ROI for radial segmentation is appropriate (Fig. 4).

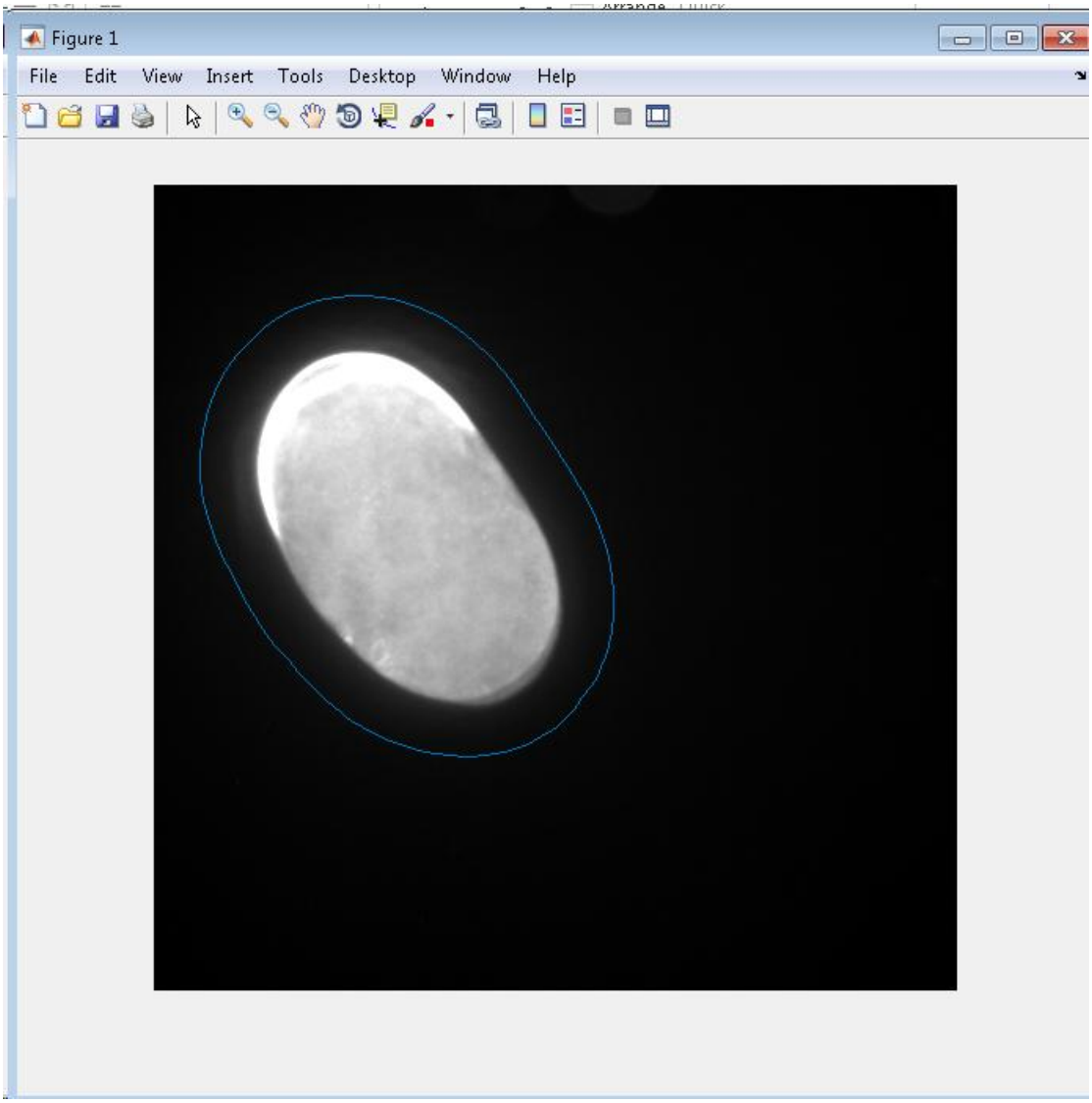

Figure 4. Mask (blue contour) for image segmentation

2.1.3 Choose Sub-regions for Radial Segmentation

Choose the number of sub-regions for radial segmentation at [5] (Fig.2 and Fig. 3B) and click [6] (Fig. 2) for preview (Fig. 5). Up to 15 sub-regions can be selected.

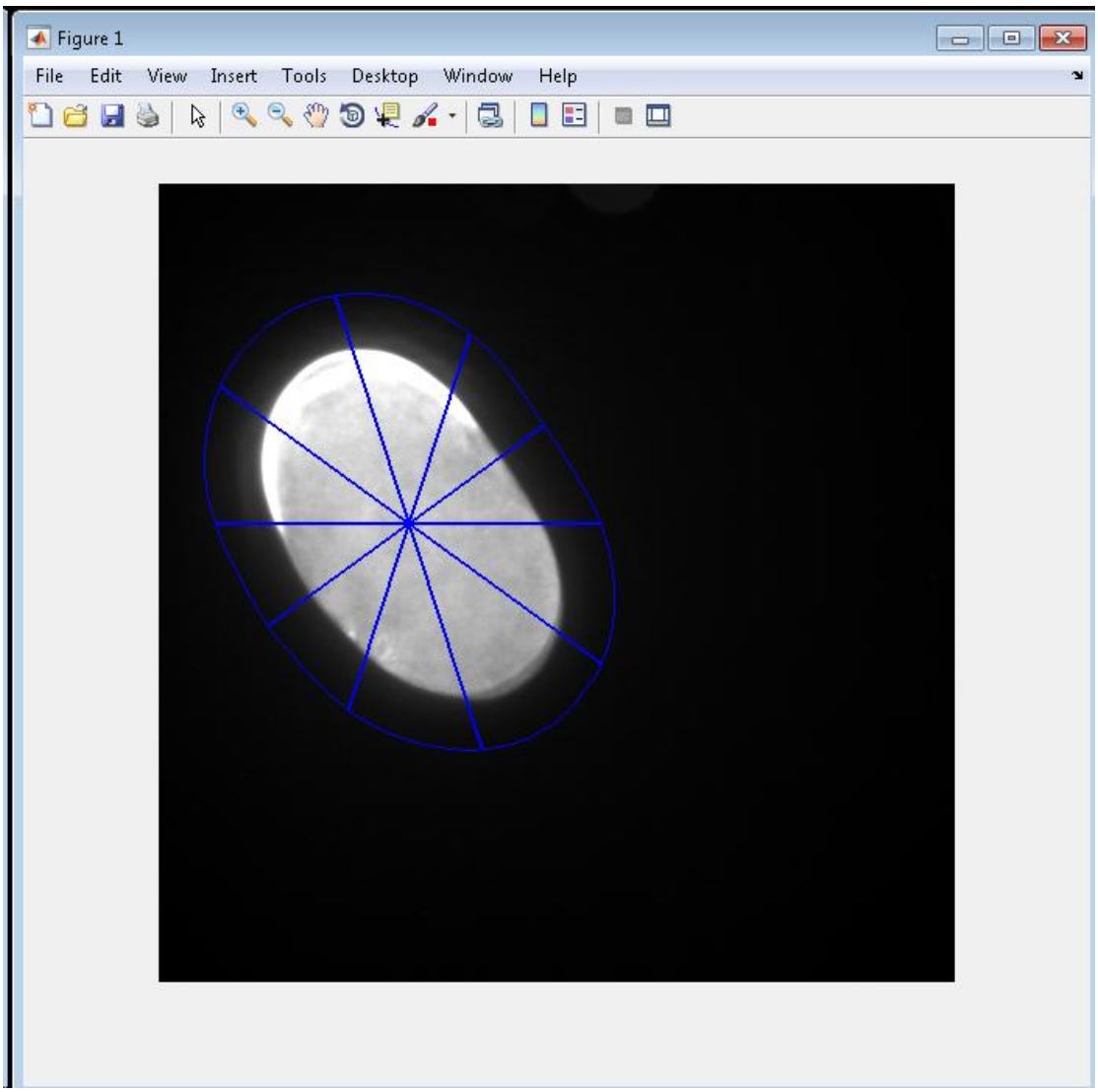

Figure 5. Radially divided subregions for adaptive segmentation

2.1.4 Simple Segmentation

** Users can choose to perform either simple or automated (see 2.1.5) segmentation.*

** Conditions for using Simple segmentation. For simple cases (e.g. with stained cell membrane or uniform distribution of cortical proteins where the cytoplasm signal is much higher than background), the radial adaptive segmentation approach we have proposed here may not be necessary. In such cases, the threshold directly calculated by Otsu's method (or multiplied with a universal scaling factor as shown at [9] in Fig. 2) is already robust enough to segment all frames. Hence, for simple cases, we suggest this simple option since it is faster than the radial adaptive segmentation approach which needs to scan a set o parameters specified by users.*

Choose a single channel or a composite of multiple channels at [7] (Fig. 2 and Fig. 3C) for segmentation.

Define the size of median filter at [8] (Fig. 2).

Define the scaling factor at [9] to the threshold value calculated using Otsu's method and click [10] to start segmentation.

** Each image frame has a unique threshold based its image information but the scaling factor is the same for all frames.*

After the previous step is done, there is a message box asking the user to verify the detected cell contours in the suggested directory.

2.1.5 Automated Segmentation

** Users can choose to perform either simple (see 2.1.4) or automated segmentation.*

Choose a single channel or a composite of multiple channels at [7] (Fig. 2 and Fig. 3C) for segmentation.

Define the size of median filter at [8] (Fig. 2).

Set the bounds at [11] and [13] (Fig. 2) and increment at [12] (Fig. 2) for threshold value. For example, the default values, 'start->50; step -> 2; end->120' means the algorithm scans the range from 50% to 120% with an increment of 2% to determine the cell edge for each frame.

Choose multicore processing at [14] (Fig. 2 and Fig. 3D) and click [15] to start automated segmentation.

After the previous step is done, there is a message box asking the user to verify the detected cell contours in the suggested directory.

2.2 Cortex Sampling

2.2.1 Simple Sampling

** Users can choose to perform either simple (see 2.2.2) or automated sampling.If the simple option is chosen, a fixed cortex width will be applied to all frames.*

** In our experiments, the second maximum spatial derivative (inner cortex bound) of an image gradient map is generally smaller than the first one, especially for discrete signals (e.g. MNY-2::mKate). In such cases, user can use either automated or simple option to detect the cortex width. In our experience, the quality difference is quite small as long as the observable cortical region is included in the user-defined width.*

Set inward depth and outward depth at [16] and [17] (Fig. 2) respectively.

Set the number of sampling windows along cell cortex at [18] (Fig. 2).

Click [19] (Fig. 2) for preview (Fig. 6).

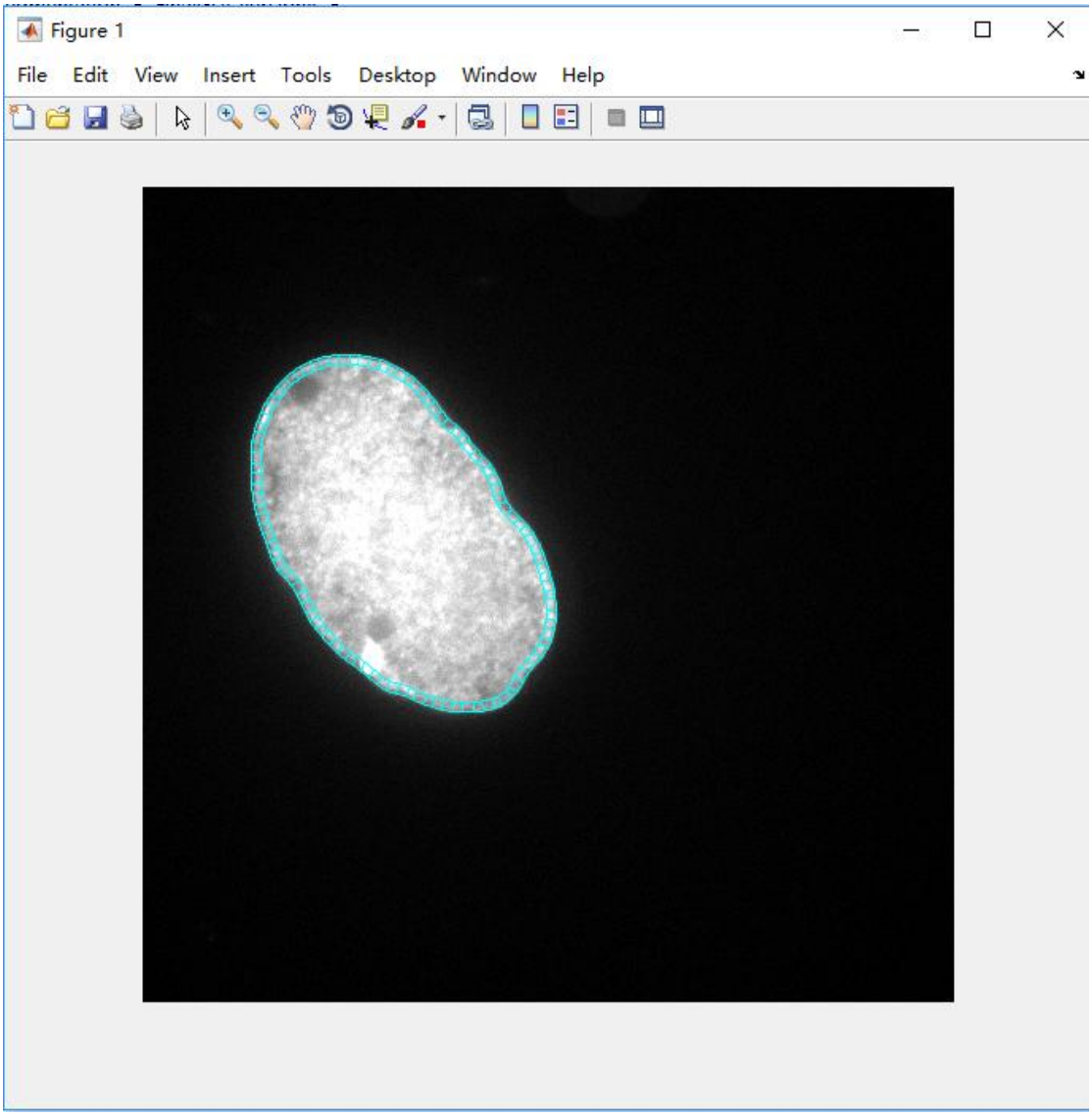

Figure 6. Preview of cortex sampling

Click [20] (Fig. 2) to start cortex sampling.
After the previous step is done, there is a message box asking the user to verify the sampled cell cortex in a suggested directory.

2.2.2 Automated Sampling

** Users can choose to perform either simple (see 2.2.1) or automated sampling.*

Click [21] (Fig. 2) to generate the plot of average image gradients as a function of inward depth (Fig. 7). Based on this plot, users can estimate the bounds for automated cortex sampling.

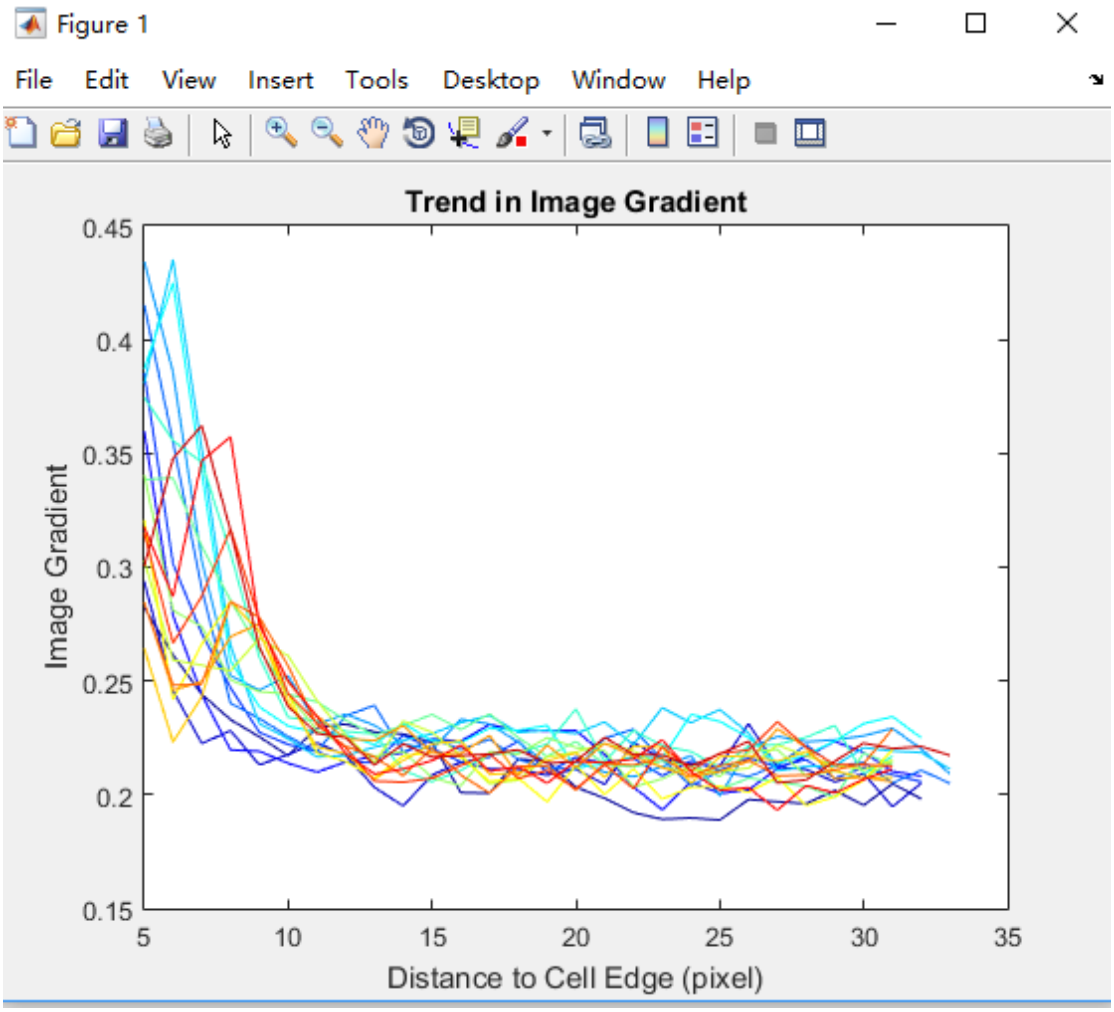

Figure 7. Preview of average image gradients along cell cortex

Set the bounds at [22] and [23] (Fig. 2) for minimum and maximum inward depth respectively.
Choose multicore processing at [24] (Fig. 2 and Fig. 3E) and click [25] (Fig. 2) to start automated sampling.
After the previous step is done, there is a message box asking the user to verify the sampled cell cortex in a suggested directory.

2.3 Window Alignment

To move the starting sampling box to a proper position (e.g. to better separate anterior and posterior cortical domains), click [26] (Fig. 2) to check the positions of all sampling box (Fig. 8) and indicate the new position at which the user wants to relocate the first sampling window at [27] (Fig. 2).
Click [28] (Fig. 2) to start the alignment.
** Leave the value at [27] as '1' if relocation is not needed.*

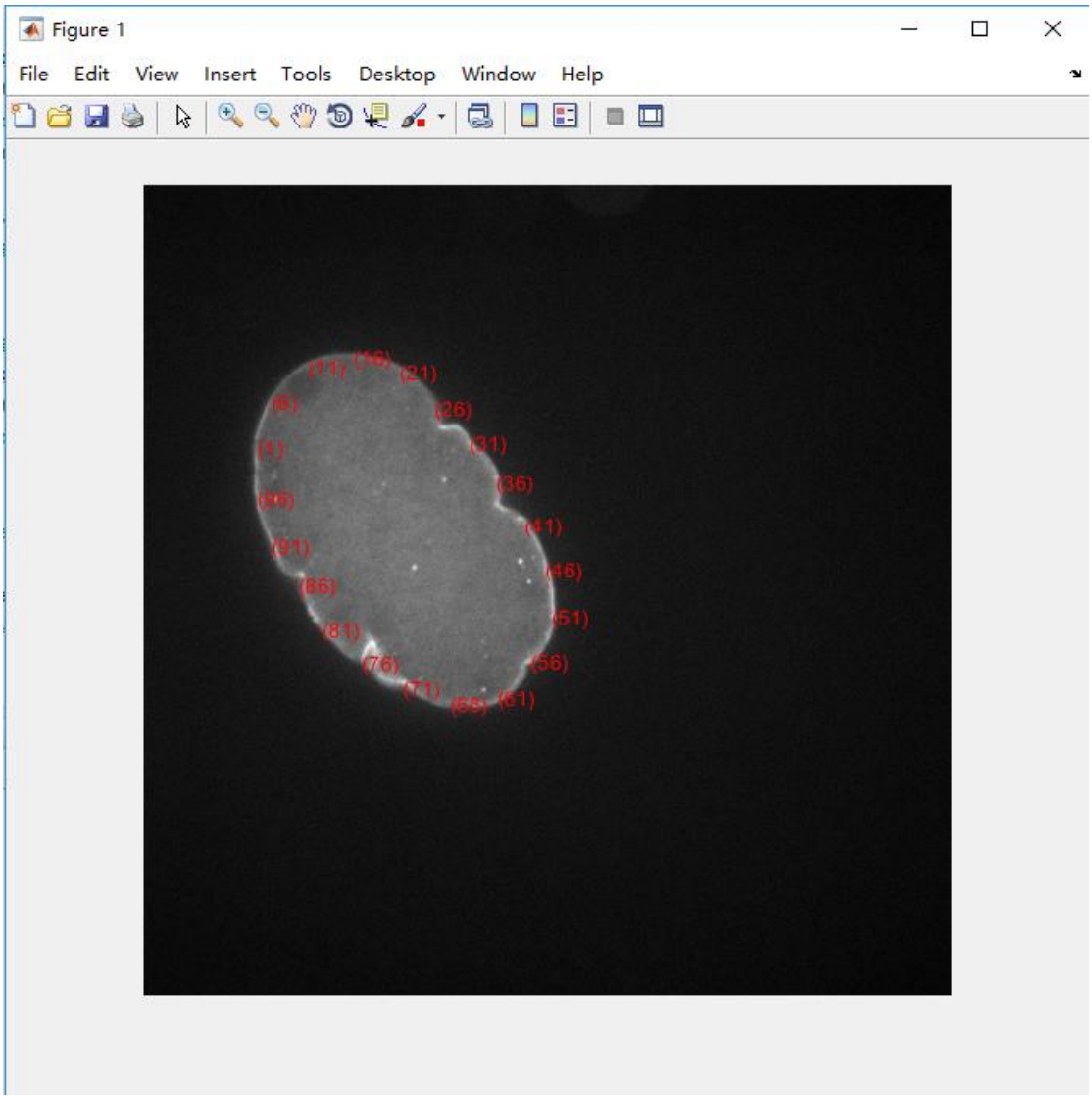

Figure 8. Preview of sampling box positions

2.4 Generate Integrated Cortical Information

ImaEdge performs discrete sampling at cell cortex. In order to smoothen the detected signal along both temporal (frames) and spatial (sampling windows) axes, a Gaussian filter convoluted with the discrete information is needed.

Define the sigma of Gaussian filter along temporal and spatial axes at [29] and [30] (Fig. 2).

Choose the channel for analysis at [31] (Fig. 2) and click [32] (Fig. 2) to generate heatmaps.