Caltracer 2.5

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Disclaimer

The creators of Caltracer 2.x, offer no guarantee that this product will work. Additionally, they take no responsibility if this product is misused, tampered with, or causes any damage of any sort. To put it simply, this product comes completely unsupported, so use it at your own risk.

What is Caltracer?

Caltracer is software written and designed to help analyze the changes in levels of fluorescence in cells over time. It was created with a focus on calcium based imaging of neurons in Rafael Yuste's laboratory at Columbia University.

What is this "Manual"?

This "manual" is an attempt to explain how to use the most basic features of Caltracer. Users should start by reading this simplified guide, then trying to use the software themselves. For detailed explanations regarding specific functionality of any particular subsection of this program, please see the comments within the .m file corresponding to that function.

System Requirements

1- Caltracer 2.2 has been tested on Windows xp/vista/7, OS X, and some versions of linux effectively. However, due to some driver issues, some features may not work properly in certain distributions of linux.
2- Matlab 2008a or newer is required to run Caltracer 2.2, if you have an older distribution of Matlab, proceed at your own risk.

Installing Caltracer 2.2

1- Download and unzip Caltracer into a local folder.
2- Set the Matlab path to include the Caltracer2 folder and all subfolders.
    a. In Matlab, under the "File" menu, select the "Set Path" option.
    b. Select the "Add with Subfolders" button, then browse to and select the Caltracer2 folder.
    c. Save the path and close the window.
3- To run, type "Caltracer2" in the Command Window.

Using Caltracer 2.5

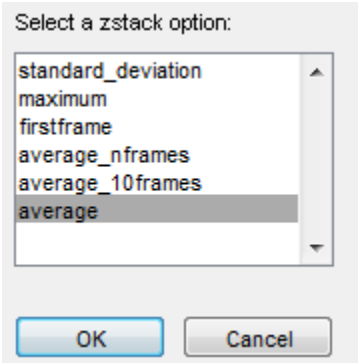
General image requirements:

1- Caltracer only load movies saved as a single multipage Tiff image file or sequential files saved in the same folder.
2- The image stack should be black and white and either 8-bit or 16-bit bits.

Opening an image for contour (cell) detection:

1- To load a movie into Caltracer simply click on the "Open" or "Open Sequence" buttons (depending on how your movie is saved) and select the first file of movie you desire to load.

2- When the "Select a zstack option" window pops up, choose an appropriate function to visualize all cells in your movie.



3- Once this is chosen, Caltracer will run the selected operation and your movie will be displayed as a single image.

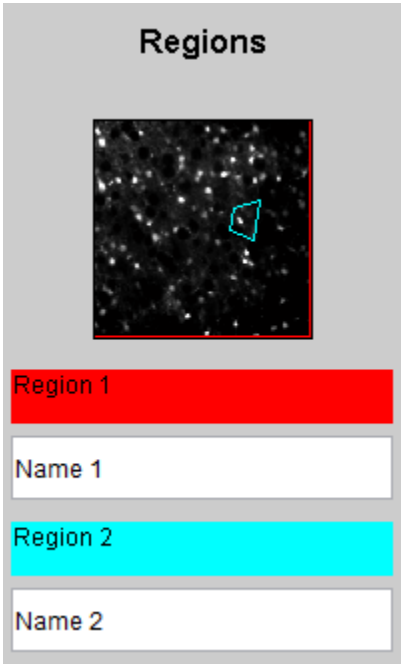
4- At this point, you may adjust the brightness and contrast as you see fit using the sliding bars to the right of your image.

5- In this window, you should also input your spatial and temporal resolution in the appropriate boxes on the right. These measurements are crucial and will be used throughout the program.

6- Once these values are entered, continue to region selection by clicking on "Next".

Regions

If you would like to specify different regions within your movie to analyze separately, you may do so on this screen. To add a region:

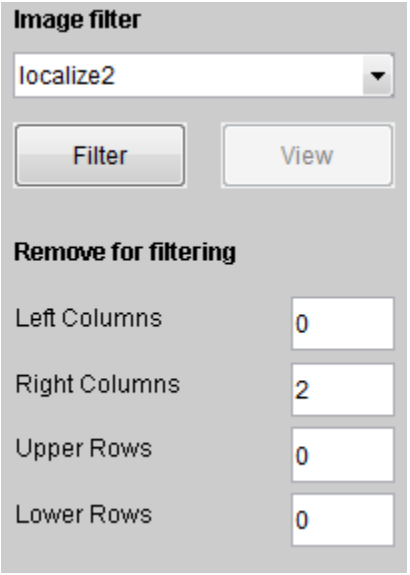


1- Select the “Add” button.
2- Using the crosshairs that appear, select points on your image to outline the chosen region. Ctrl-click or right-click when placing the last point to finish creating the polygon. The finished polygon will appear in your main image as well as the small image map on the right.
3- Repeat this process to add as many regions as you like, when finished click on “Next”.

In the next window, you will have the option to choose names for the individual regions, choose “Next” to continue to the filtering dialog.

NOTE: You will have the option to name a region even if you do not add any additional regions as the program considers your original image as one large region.

Filtering / Remove for filtering



Filtering will allow the program to detect cells more effectively.

Before filtering it is important to choose any rows and columns to be removed prior to filtering. This option is available in case of camera misalignment or and artifacts that may exist on the borders of your images. To remove columns or rows from being filtered (and therefore from being considered when detecting cells), simply specify the number of pixels to exclude in the corresponding text box below the filtering options.

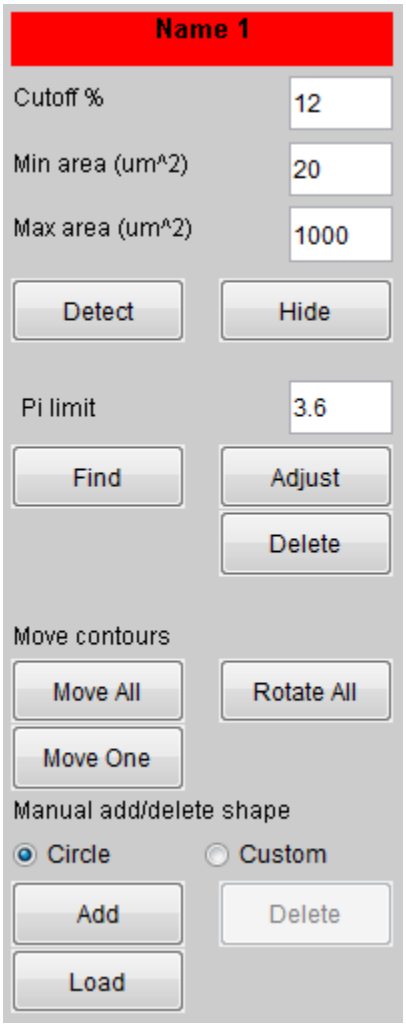
To filter the image, select the appropriate filter from the pull down menu, then choose “Filter”.

When the cell diameter dialog box appears, choose an approximate size for your cells and click “Ok” to proceed.

Detecting Cells

This next window allows you to create contours (outlines highlighting the location of given cells) around fluorescently labeled cells.

NOTE: To detect cells in different regions, select the region in the minimap and repeat the steps below.



There are a number of ways to create contours:

1- Automatically, by clicking the “Detect” button. This option will consider the cutoff % as well as the minimum and maximum allowable areas for contours when detecting your cells and will then draw contours around cells that meet these guidelines. The cutoff % is based on fluorescent signal.
    a. Once contours are detected, the user is given the option to separate any cells which may have been combined into one cluster. The way the program does this is using the Pi limit value to find contours which are not very round and split them. To do this, simply, set a Pi limit, click “Find” to find cells which are not “round enough” and then “Adjust” to split them.
2- Manually by using the add/delete shape tools.
    a. With the “Add” button, you can add either elliptical contours by using the “Circle” radio, or any polygon using the “Custom” radio button and simply drawing and adjusting the shapes on the image. To accept the shape created, simply double click on the image.
    b. To delete contours, use the “Delete” button. If “Circle” is selected a single click will delete the contour encasing the point selected. If “Custom” is selected than the user will be able to remove all contours within a polygon that the user can draw on the image.
3- Loading previously created contours from a saved analyzed movie. The “Load” button will allow you to load the contours from a previously saved experiment,

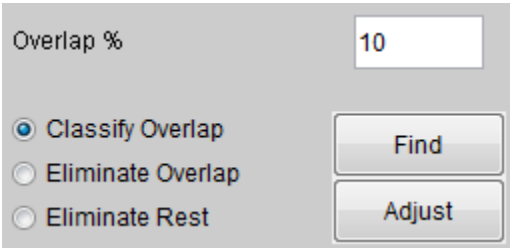
thus saving the time of recreating contours every time a movie of the same field of view is to be analyzed.

Additional adjustments can be made using the Move and Rotate Contours settings. Once you are happy with the contours, select “Next” to continue.

Load Mask

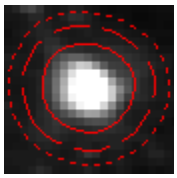
After the Contours are created, the program asks if you would like to load a mask. A mask is another image of the same field of view whose fluorescent cells you would like to compare to your original image. If you do not, select “No” and move on to the next section. If you would like to load a mask, select “Yes” and follow these steps:

1- Naming your mask – The program will ask you to name your mask, choose a name and select “OK”.
2- Choose a mask image; this is an image which you would like to compare your original movie to. Browse to your image, and select “Open”.
3- As when loading your original image stack, you will be given z-stack options. Select the option you would like and click “OK”.
4- Repeat the image filtering steps with your mask and choose your cell diameter.
5- Repeat the cell detection steps to create contours on your mask.
6- Repeat these steps for any additional masks you would like to load, when done, select “No” in the load mask dialog box.
7- The next window will layer all your masks on top of your original cell contours. Your original movie will have red contours. This window allows you to change your original movie’s detected cells based upon the overlap with your mask layers. All three options use the overlap % entered on the right to determine which contours overlap.



   a. Classify Overlap – This will find the overlap, but will not change your original movie. To use, simply select this radio button and click on “Find”.
   b. Eliminate Overlap – This will remove all overlapping contours from your original movie. To use, select this radio, then click on “Find”. Once the program has finished processing, click on “Adjust” to remove the overlapping contours.
   c. Eliminate Rest – This option will remove all contours which do not overlap. To use, select this radio, then click on “Find”. Once the program has finished processing, click on “Adjust” to remove the non-overlapping contours.
8- This window also gives the option to “Split”. This option will separate your movies (the original as well as any masks) into multiple instances of Caltracer.

9- When done adjusting the masks, select “Next”.



Halos

Halos are an extension of your contours which will add a decreasing amount of weight to the area just outside the contours depending on distance. The “Halo area” textbox determines the size of the halos. To create, simply click on “Update” or if the “Use halos” checkbox is selected, click on “Next”.

Flipping the signal

Once past the halo window, a question regarding flipping the signal appears. Some dyes produce a downward signal and as the program works based upon the assumption that a signal is of a higher value than baseline, the program wants to know if it should flip the signal to accommodate these specific dyes. Choose based on your dyes to continue.

Working Memory Estimate

The working memory estimate will allow you to select how many slices of your stack to load at once. This is important if you are working on an older machine which may not be able to open many images at once. Select an appropriate number and hit “OK” to continue.

Once the working memory is set, the program loads your actual movie and sets up the next user interface, in which the user can analyze the activity of the cells based upon changes in fluorescence over time.

Preprocessing

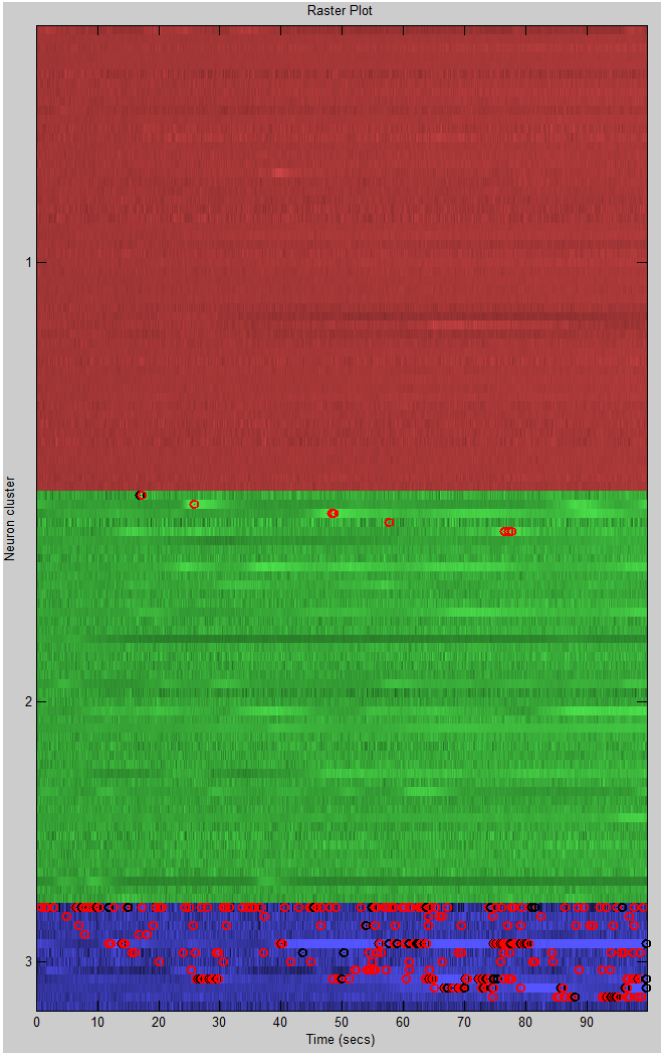
When Caltracer loads a movie, it runs the data through any chosen preprocessors. These preprocessors can help the user visualize and/or analyze the data in different ways, often making signal detection easier. For details regarding the functionality of any preprocessor, see the comments within the corresponding file in the “preprocessors” folder in Caltracer.

To change the preprocessor you are currently using to visualize and analyze the data, select “Preprocessing” from the toolbar at the top of the window, and then select, “Preprocessing Options”. The window that opens allows the user to change the preprocessors used as well as modify their options.

NOTE: To change the default preprocessor used, see Caltracer Preferences below.

Clustering

The clustering options available in Caltracer can be found to the right of the raster plot in a pull down bar. These cluster methods allow the user organize the raster plot in a way which separates cells based on the criteria of the specific cluster method chosen. To use, simply select a cluster method, set any options that may be applicable (such as the number of clusters or trials) and choose “Cluster”.



Contour Order

The contour order options allow the user to select one of a number of options to rearrange the order of the cells within the clusters created. To use, simply select the contour order method from the pull down bar, and then click on “Order”

NOTE: If no clusters have been previously created, the contour order treats the entire raster plot as a single large cluster.

Signal Detectors

The signal detector options allow the user to find signals based on the method of the chosen signal detector. Signals are depicted as red and black circles drawn on the raster plot and above the selected trace. To use, select the appropriate signal detector from the pull down bar, and then click the “Detect” button.

NOTE: Some signal detectors have options which the user must set in order for the signal detector to work. These options may differ for different signal detectors. A description of each of these values may be found in the comments section of the corresponding .m file within the “signaldetectors” folder in Caltracer.

Signal detector functions include:

Threshold – Finds the times in which the fluorescence of each contour is above a user defined threshold.

Single Max – Finds the time at which the fluorescence is at a maximum for each contour.

Risingfaces – Finds the times in which the fluorescence of each contour is rising dramatically (the specific amount required to find a signal is set by the user).

Find Upstates – Finds the times in which the fluorescence of each contour indicates that an upstate may be occurring.

Find Laser Pulses – Finds the times at which a laser pulse occurred based on dramatic changes in fluorescence.

Edit Signals

To add signals manually, the edit signals option is available. When selecting this checkbox, located on the lower right of the screen, the program starts operating in a single contour display mode. In this mode, only one cell can be selected at a time. To create a signal, select the appropriate cell by clicking on it either in the raster plot or in the contour map, then click on the plot of the fluorescence trace twice. The first click should signify the onset time of the signal, and the second should signify the offset. After the second click the program will draw a red circle with a line to a black circle to depict the chosen signal onset and offset times on both the raster plot and the fluorescence trace plot.

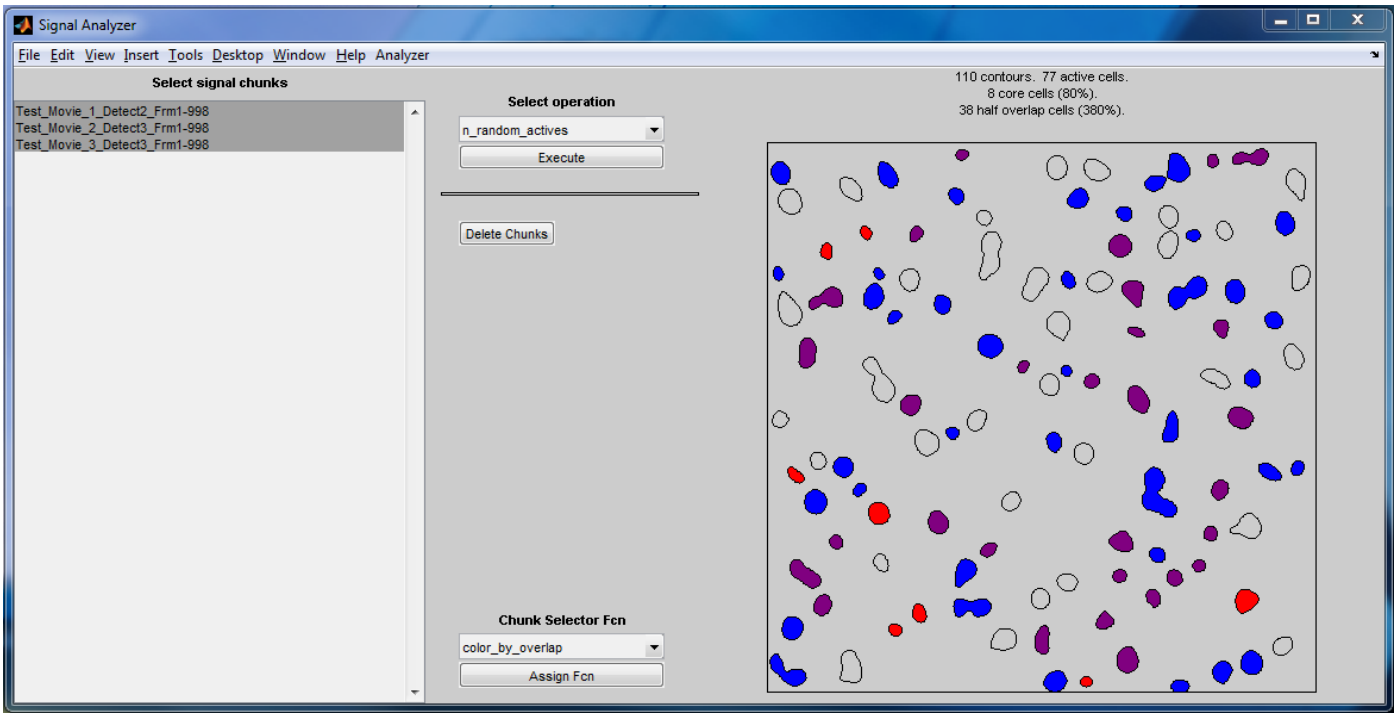
Analyzer

The “To Analyzer” button will allow the user to compare which cells had signals over a number of movies of the same field of view. To use, detect signals in the movies (numerous instances of Caltracer may be required), then click on the “To Analyzer” button in each. A new window will open, and with each click of “To Analyzer”, there will be another addition to the list on the left hand side. As each movie is selected, the cells with signals will appear in color on the right hand side. To compare which cells are common to more than one movie, select the chosen movies using the CTRL or SHIFT buttons and take notice of the colors on the right hand side. Cells common to more than one movie will be colored differently than those which only had signals in a single movie.

Caltracer Preferences

Caltracer preferences allow the user to set default values for many of the options within Caltracer. Additionally, it gives the user the ability to automate certain steps within Caltracer, allowing the program to be run faster, with fewer clicks. To use, select “Edit Caltracer Preferences” under the preferences menu at the top of the screen. Many of the questions/options detailed in the steps above can be set within the Caltracer Preferences window.

NOTE: Any changes made to the Caltracer preferences will not affect any currently loaded images/settings. To be sure that the preferences affect the movie you wish to analyze, change them prior to loading the movie into Caltracer.



Other Options

Exporting

The “Export” option in the toolbar at the top of the window contains a number of functions designed to export data from Caltracer to be used by the user in any way he or she sees fit. This functionality allows the user to export not only graphical data but also the actual fluorescence traces created by Caltracer.

Contours

The “Contours” option in the toolbar at the top of the window allows the user to adjust many settings regarding the contours created by Caltracer. These include highlighting and unhighlighting contours based on specific criteria, changing the order of contours, activating and deactivating contours, etc. These options are mostly used in the final user interface where the user has the option to activate contours.

Clustering

The “Clustering” option in the toolbar at the top of the window allows the user to visualize the clusters created in a number of different ways as well as allowing the user to combine or remove certain clusters entirely.

Functions

The “Functions” option in the toolbar at the top of the window contains a number of separate functions which do not fall under any of the other headings. To add a function to this menu, simply add your code to the “signalfunctions” folder within the main Caltracer folder.