CellTrack: An Open-Source Software for Cell Tracking and Motility Analysis

Motivation: Cell motility is a critical part of many important biological processes. Automated and sensitive cell tracking is essential to cell motility studies where the tracking results can be used for diagnostic or curative decisions and where mathematical models can be developed to deepen the understanding mechanisms underlying the cell motility.

Results: We propose a novel edge-based method for sensitive tracking of cells, and propose a scaffold of methods that achieves refined tracking results even under large displacements or deformations of the cells. The proposed methods along with other general purpose image enhancement methods are implemented in CellTrack, a self-contained, extensible, and cross-platform software package.

Availability: CellTrack is an Open Source project and is freely available at http://db.cse.ohio-state.edu/CellTrack

Contact: sacan@cse.ohio-state.edu

Publication: If you use CellTrack for you own research, please cite the following paper:

> Ahmet Sacan, Hakan Ferhatosmanoglu, and Huseyin Coskun. *CellTrack: An Open-Source Software for Cell Tracking and Motility Analysis.* Bioinformatics (2008, submitted)

Download

Depending on your operating system, you need to download one of the following files:

- Windows (98,2000,XP,Vista) x86 binary installer: celltrack_x86.exe
- For other platforms, please download and compile the source: celltrack.tgz

Installation Instructions

- **Binary Distribution for MS Windows:** If you are using Microsoft Windows, the binary installer should be sufficient to have CellTrack installed and running. Just follow the on-screen instructions of the installer. If you wish to extend CellTrack, you need to install the required libraries and recompile CellTrack with your changes. Please follow the instructions below for compiling from source.
- **Compiling from Source:** You need to first install the prerequisite libraries. Download and install wxWidgets and OpenCV libraries. Before you start compiling CellTrack, please make sure to test your wxWidgets and OpenCV installations using the test samples that come with these packages.

On a linux-based system, you can download, uncompress, and compile CellTrack using the following commands:

```
wget http://db.cse.ohio-state.edu/CellTrack/celltrack.tgz
tar -xzf celltrack.tgz
cd CellTrack
# you may need to edit the Makefile to reflect the library paths
make
make install
```

There is also a Visual Studio project file provided under "msvc" folder if you wish to develop using MS Visual Studio. You still need to update the library paths for the project: Right-click on the project in Solution Explorer and select Properties. Go to C-C++/General and update the entries for OpenCV and wxWidgets header paths in `Additional Include Directories`. Go to Linker/Input page and update the OpenCV and wxWidgets library paths under

`Additional Library Directories`.

Please feel free to contact sacan@cse.ohio-state.edu if you have any compiling or installation questions.

Quick Start Guide

The following are the common steps to be followed in CellTrack to perform a cell tracking:

1. Load your movie
2. Crop the image to contain only the cells of interest
3. Smooth the image to remove noise.
4. removing_frames remove frames that are very low quality (caused especially by a moving camera capture.
5. Go to the first frame where you want to start tracking and remove all the previous frames
6. Use Automated Cell Detection to automatically outline cells. You can also manually_editing_cell_boundaries manually outline cells if you wish.
7. Apply Filtering to remove too small or too big cells (or such boundaries that are incorrectly detected), or to remove cells that are too close to the frame edges.
8. Manually remove cells you are not interested in tracking.
9. Improve boundaries to obtain a smoother structure and a better fit to the underlying image plane.
10. Resample boundaries to achieve a uniform sampling of the boundaries.
11. Apply Combined tracking to track the cells as they shift and deform.
12. Save the resulting movie or frames, or export the tracking/trajectory image or data file.

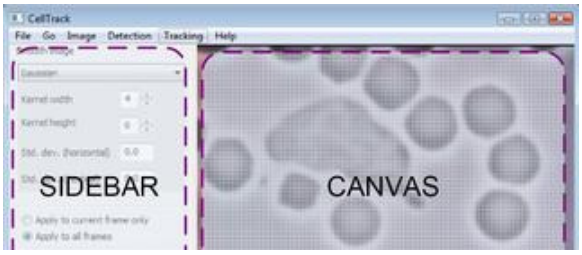
Limitations

Note that CellTrack is by no means a final solution to all cell tracking problems. Depending on the movie you are processing, you might not get satisfactory results. We recommend that you go through each method and preview the effect of the method parameters on tracking results. The current version of CellTrack provides you with default parameters, but does not provide an automatic mechanism that can adjust the parameters to suit best the nature of the environment the cells are being tracked in or the conditions that your movie is captured. Furthermore, the current version of CellTrack does not handle occlusions. The occlusions are planned to be handled using modelling of the trajectory of individual snaxels using Kalman Filter and/or Condensation algorithm.

Detailed User Guide

Here we describe the available operations you can perform in CellTrack software. For some of the image processing operations, we have used the description of the parameters from the OpenCV documentation. The movie used for creating sample snapshots is copyright to the Cell Biology and Cytoskeleton Group at Harvard.

Below, you see a snapshot of the graphical user interface components. The current frame of the movie is shown in `Canvas` area. For the tracking operations, the canvas is split into two, showing *the current frame on the **right*** and the *previous frame on the **left*** Any contours or feature points of the current frame are drawn on top of the image. The image processing, cell detection and tracking modules are implemented as ***plugins***. For most plugins, you are able to change the default parameters using the `sidebar` shown for the current plugin. A navigation bar at the bottom of the window is also provided for easy navigation through the movie and playback.



Common Plugin Sidebar Options

- **Show Preview:** Whenever possible, the plugins will allow you to preview the result of applying the plugin with the given parameters. The changes are not committed unless you press one of the `Apply` or `OK` buttons in the sidebar.
- **Application Scope:** You can specify the scope of applying a plugin by selecting one of the `Apply to current frame only` (only the current frame is modified), `Apply to all frames` (all frames are modified), or `Apply to current frame on` (all frames after and including the current frame are affected).

Menus and Shortcuts

- File
  - Open movie file (Ctrl+0)
  - Open image files (Ctrl+Shift+0)
  - Save movie as… (Ctrl+S)
  - Save current frame as… (Ctrl+Shift+S)
  - Export tracking data
  - Export tracking image
  - Export trajectory data
  - Export trajectory image
  - Preference (Ctrl+K)
- Go
  - Next frame (Alt+Right)
  - Previous frame (Alt+Left)
  - First frame (Alt+Up)
  - Last frame (Alt+Down)
  - Play/pause movie (Ctrl+Enter)
- Image
  - Resize
  - Crop
  - Smooth (Ctrl+M)
  - Delete current frame (Ctrl+Backspace)
  - Delete preceeding frames
  - Delete proceeding frames
- Detection
  - Detect cell boundaries (Ctrl+D)
  - Filter cells (Ctrl+F)
  - Edit cells (Ctrl+E)
  - Improve boundaries (Ctrl+I)
  - Resample boundaries
  - Find good features (Ctrl+G)
  - Background subtraction (Ctrl+B)
- Tracking
  - Template-matching (Ctrl+M)
  - CAM-Shift tracking (Ctrl+C)
  - Extended Active Snake tracking (Ctrl+A)
  - Combined tracking (Ctrl+T)
- Help
  - Help contents (F1)
  - About CellTrack

Loading A Cell Movie

Commands: `File → Open movie file (Ctrl+O)`, `File → Open image files (Ctrl+Shift+O)`

In CellTrack, your cell movies can either be an *.avi movie file or a set image files. CellTrack can read avi files using platform-specific video libraries. If you are having difficulty loading your movie file, please use a conversion tool to convert your movie into DIB/I420/IYUV format or uncompressed format. You can use one of the following freely available tools for this conversion: FFmpeg, mencoder, or RAD Video Tools.

Instead of a movie file, you can also use a set of image files. Make sure the alphabetical order of the image file names are the same order of the real motion capture. The following image formats are supported:

- Windows bitmaps - BMP, DIB;
- JPEG files - JPEG, JPG, JPE;
- Portable Network Graphics - PNG;
- Portable image format - PBM, PGM, PPM;
- Sun rasters - SR, RAS;
- TIFF files - TIFF, TIF;
- OpenEXR HDR images - EXR;
- JPEG 2000 images - jp2.

Saving Movie or Frames

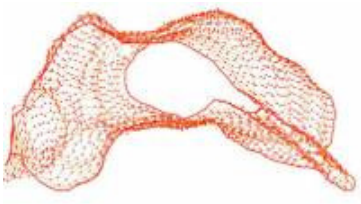
Commands: `File → Save movie as… (Ctrl+S)`, `File → Save current frame as… (Ctrl+Shift+S)`

You can save the whole movie (with object boundaries and intracellular components drawn) or individual frames. For saving individual frames, the following image formats are supported: bmp, png, jpeg, giv, pcx, pnm, xpm, ico, and cur. For saving the whole movie, you will only be able to save as an .avi movie file, but you can select the compression codec to be applied. The preferences dialog (File→Preferences Ctrl+K) has a list of available codecs you can choose from on the `Saving` tab. You can also enter the FOURCC code of the codec you want to use in the preferences dialog. Windows users can select to be prompted for the codec while saving (use the dropdown menu under `Video codec`. The preferences dialog also lets you overwrite the size and frame-rate of the movie to be saved.

Exporting Tracking Results

Commands: `File → Export tracking data`, `File → Export tracking image`, `File → Export trajectory data`, `File → Export trajectory image`

You can either export tracking of all boundary points (and intracellular features), or export only mean trajectory of the whole cell. The export for each of these can be either in data text file or image file.



Tracking gives the movement of each of the control points (whether it be on the borders or intracellular features) throughout the whole movie frames. After you obtain the trackin information using the detection and tracking steps, you can save the tracking image (sample shown on the left). You can also export the tracking information as a data text file. The following shows a sample exported tracking data. The first line shows the width and height of the movie (in pixels), frame count, and number of cells being tracked. The tracking data of a cell is included only if this cell is outlined in the first frame of the movie. In the following lines, for each of the cells, the object identifier and the number of control points on this cell are printed, followed by the x and y coordinates of each control point across all frames. For instance, the first control point of the first cell below moves from <117,43> to <110,44> to <117,44>.

```
width: 320, height: 240, frameCount: 3, cellCount: 2
cell: 1, pointCount: 167
117 43 110 44 117 44
114 43 112 43 121 44
112 44 109 44 119 44
```

The *trajectory*, on the other hand, gives only the tracking of the center of each cell, providing information about the overall movement of the cell. For reference, the starting and ending configurations of the cell are also shown on the trajectory image. The velocity of the whole cell can then be calculated using the amount of displacement between frames. The data format for the trajectory is similar to that of tracking, except that only the coordinates of the center of mass is given for each cell. The data sample below shows that the cell-1 (its center of mass) moves from <118,42> to <115,43> to <117,43>.

```
width: 320, height: 240, frameCount: 3, cellCount: 2
cell: 1, pointCount: 167
118 42 115 43 117 43
```

Navigation and Playback

Commands: `Go → Next frame (Alt+Right)`, `Go → Previous frame (Alt+Left)`, `Go → First frame (Alt+Up)`, `Go → Last frame (Alt+Down)`, `Go → Play/pause movie (Ctrl+Enter)`

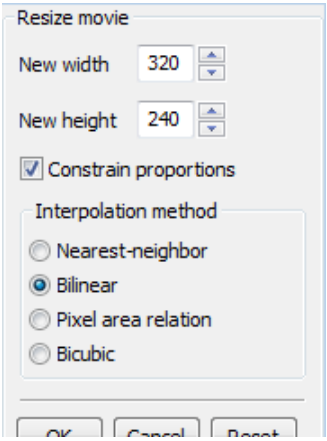
You can navigate through the loaded cell movie using the menu commands or using the navigation bar at the bottom of the window. When you are playing a movie file, the frame rate of the movie file is used for playback. When you are playing a set of image files, the default frame rate (5 f.p.s.) is used; the default frame rate can be changed in `Preferences → Saving → Default fps`. Note that the time to compute the display of a new frame may cause the playback rate to differ from the frame rate, especially if you have the preview option of an active plugin `on`.

Basic Image Operations

If you are working with large movies or images, we recommend that you reduce the size and frame count during the test phase. Once you have determined the best set of operations and parameters suited to your movie, you can apply them to the original data.

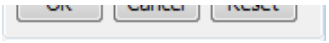
Resize

Command: `Image → Resize`



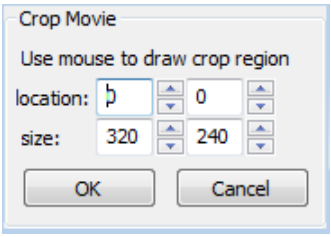
Performs a resize of the underlying image frames. Note that reducing the image size would make the application of detection and tracking operations faster, but would also degrade the resolution and image quality. Uncheck the `Constrain proportions` option if you wish to change the aspect ration of the frames. You can use `Reset` button to recall the original image size. The following interpolation methods can be performed for resizing:

- nearest-neigbor interpolation,
- bilinear interpolation (used by default)
- resampling using pixel area relation. It is preferred method for image decimation that gives moire-free results. In case of zooming it is similar to nearest-neighbor method.
- bicubic interpolation.



Crop

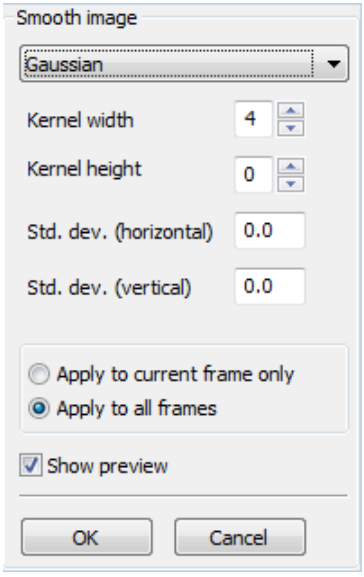
Command: `Image → Crop`

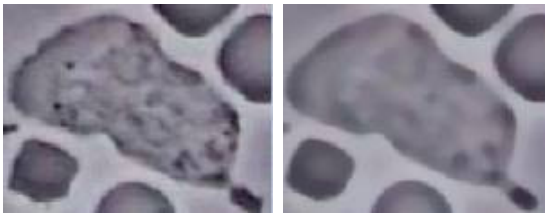


If you are concerned with only a limited subregion of the movie, you can crop the movie to use only this subregion. Cropping will remove the rest of the area and will save computational time for subsequent detection or tracking operations. You can use your mouse to draw the crop region on the canvas, or use the location and size input controls in the sidebar to define the region.

Smooth

Command: `Image → Smooth (Ctrl+M)`





You can perform smoothing to remove the noise in your images. The following smoothing methods are available:

- Simple Blur: Summation over a pixel neighborhood is performed with subsequent scaling. If `neigbhorhood scaling` parameter is zero, it is set to the `initial neighborhood size`.
- Gaussian: The image is convolved with a Gaussian kernel of the given size and deviation. If `kernel height` is zero, it is set to be the same as kernel-width. If the horizontal or vertical standard deviation is zero, it is calculated from the kernel size: `sigma = (n/2 -1)*0.3+0.8`, where n is the kernel width or height, respectively.
- Median: The median pixel value over a square neighborhood is used for smoothing.
- Bilateral: Bilateral 3×3 filtering is applied with the given color and space values. See Bilateral Filtering for more information.

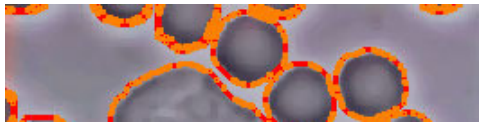
Removing frames

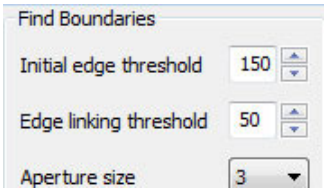
Commands: `Image → Delete current frame (Ctrl+Backspace)`, `Image → Delete preceeding frames`, `Image → Delete proceeding frames`

If you are only concerned with a specific section of the movie, you can remove the rest of the frames using the commands above. The tracking methods in CellTrack assume that the first frame includes the boundaries of the object you wish to track. If you define boundaries of interest in another frame, you need to delete the preceeding frames to force CellTrack to track these boundaries. You can also remove low-quality frames where the image is too noizy or blurry. If the movie capture is from a nonstable, moving camera, you might get some fuzzy transition frames which might need to be removed to obtain higher-quality tracking results.

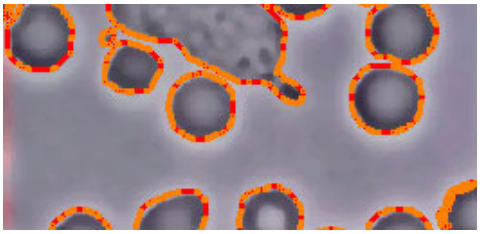
Automated Cell Detection

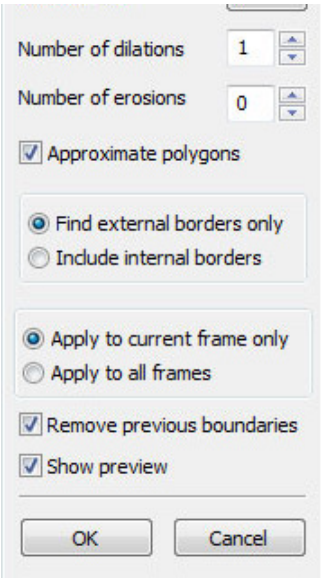
Command: `Detection → Detect cell boundaries (Ctrl+D)`





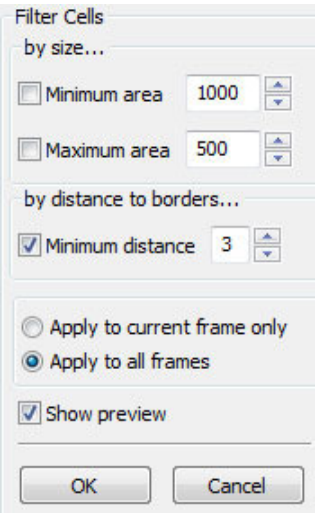
The automated cell detection first identifies the edges using Canny edge detection algorithm with the given initial edge and





linking intensity threshold values. The aperture parameter is used for calculation of image derivatives. The detected edges are then dilated and optionally eroded to achieve connection of discontinuous edge segments. The connected components are then computed from this edge information to obtain the object contours. The object contours can be represented as approximated polygons (only list of vertices are kept) or as a list of all points along the contour, which can be controlled via `Approximate polygons` option. You also have the option of detecting only the external borders of the cells or including the intracellular components (such as the nucleus).

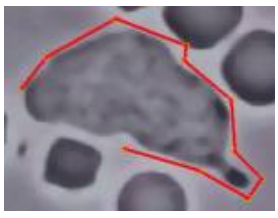
Filtering Cells

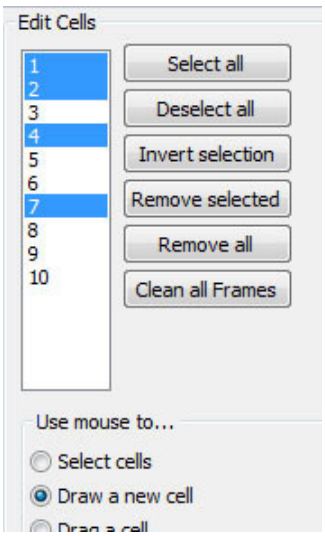


Command: `Detection → Filter cells (Ctrl+F)`

The automated cell detection may detect contours which may or may not be cells and may or may not be of interest to you. Fitering Cells is a quick way of removing unwanted objects. You can restrict the size of objects that are to be considered as cells, or specify a minimum distance to frame borders to filter out the cells that are not completely captured within the frame.

Manually Editing Cell Boundaries



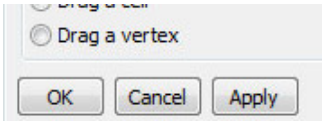


Command: `Detection → Edit cells (Ctrl+E)`

Use this plugin to manually outline a cell using the mouse. Click to start, move mouse to next vertex and click again. You can complete the drawing by either double-clicking at the next vertex, or clicking on the starting point. The sidebar will show the list of contours in the current frame. You can click on the cell id numbers to make selection. Use Shift, or Control keys in combination with mouse clicks to select multiple entries. You can then remove these contours from the current frame using `Remove Selected` button. `Select all` selects all the contours in the list. `Deselect all` clears all the selections. `Remove all` will remove all the contours from the current frame, whereas `Clean all frames` will remove all contours from all of the frames.

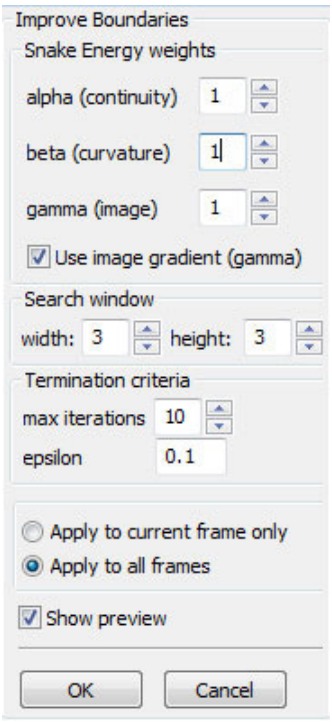
Choose from the `Use mouse to...` radio box to determine the mouse operation. `Select`

`cells` will let you click within the contours in the canvas area to select/deselect contours. `Draw a new cell` lets you outline a new cell. `Drag a cell` lets you click and drag a whole contour of a cell. `Drag a vertex` lets you drag the vertices of the contours.



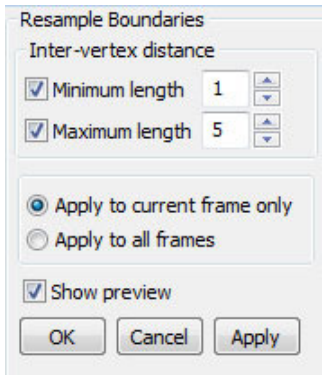
Improving boundaries

Command: `Detection → Improve boundaries (Ctrl+I)`



Once you automatically or manually outline cells, it is a good idea to improve these boundaries to obtain a smoother shape and at the same time obtain a good fit on the underlying image region. In CellTrack, this improvement is achieved Active contours. Active contours (or snakes) are curves that move on the image plane to minimize an energy functional. The energy functional is composed of internal and external energy terms. The internal energy is a weighted sum of continuity (stretching, weight parameter: `alpha`) and curvature (bending, weight parameter: `beta`). The external energy is the image gradient (weight parameter: `gamma`). The search window specifies the neighborhood searched for the next local minimal location of each snaxel. Maximum number of iterations and the minimum ratio of the changed snaxels can be specified as termination criteria for the iterative energy minimization procedure.

Resampling boundaries

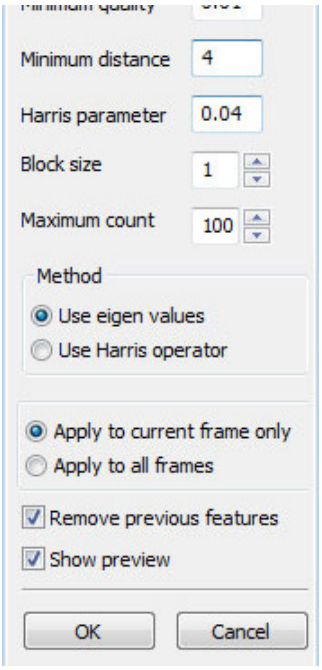


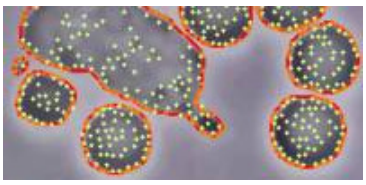
Command: `Detection → Resample boundaries` Automated or manual contours may not contain uniformly distributed vertices along the border of the cell. Improving boundaries or tracking operations may also cause the vertex distribution to change. For sensitive tracking results, we recommend resampling of the snaxels on the polygon curve before any tracking operation is performed. The resampling provided in CellTrack removes the points that are closer than a user-specified threshold (`minimum length`) and introduces new points on the edge if the edge is longer than `maximum length` threshold. Note that if you have already performed tracking, resampling will disrupt the association of the border vertices made with the boundary of the previous or next frame. So, if you desire the keep the current association of the points, you should not resample boundaries (Perform resampling only before tracking). On the other hand, if you are only concerned with tracking the whole cell and not concerned about asscociation of the border points, then resampling boundaries on an already tracked cell will give better results on further tracking.

Finding Intracellular Points

Command: `Detection → Find intracellular features (Ctrl+G)`

This plugin finds corners (*features*) with big eigen





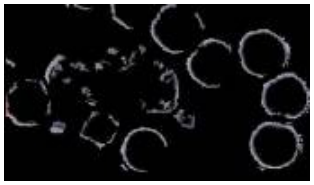
values in the image. The eigenvalue of each pixel is calculated and local maxima in 3×3 neighborhood is selected over the image. Corners with eigenvalue less than `minimum quality` of the maximum eigen value in the image are rejected. The features that are too close to stronger features by the `minimum distance` are also eliminated. `Maximum count` determines the maximum number of features to be detected within each cell. `Block size` is size of the averaging block used in eigen value calculation (or Harris edge detection). The `Harris parameter` is used in calculation of gradient covariation matrix if Harris `method` is used.

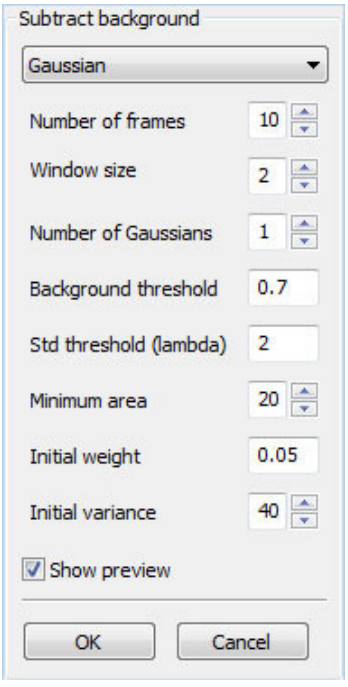
Tracking intracellular points would be useful if you are interested in the reorganization of the cell interior as it moves. CellTrack includes limited functionality for tracking intracellular points (*features*). The tracking methods that only shift/rotate the cells will perform tracking on the cell border and interior features equivalently and result in no conflicts. However, the optical-flow based tracking method is not *interior-aware*. Each of the border points and intracellular points are tracked independently, which may result in intracellular feeatures *leaking* out of the cell (The outlier detection and local interpolation may help avoid this conflict). The active snake methods work only on the cell boundaries and do not modify or track intracellular points.

Note that, if you have high-resolution images where intracellular components (organelles or nucleus) are distinguishable, you can represent and track these components using internal boundaries instead of using intracellular points described in this plugin. Use `Include internal borders` option in Automated Cell Detection, or manually draw the boundaries of these components.

Background Subtraction

Command: `Detection → Background subtraction (Ctrl+B)`





If your application includes moving cells on a stable background, you can improve detection and tracking results by performing background subtraction. CellTrack provides two methods of background subtraction (sample results shown above). The first one (left) simply subtracts from each frame, the average of all frames. The second one (right) builds gaussian models for background and foreground, and replaces each frame with the foreground model.

The Gaussian method is based on “An Improved Adaptive Background Mixture Model for Real-time Tracking and Shadow Detection” by P. KaewTraKulPong and R. Bowden (*Proceedings of the 2nd European Workshop on Advanced Video-Based Surveillance Systems*, Sept. 2001). `Number of frames` is used to determine the how many of the previous frames to use in building the model. For the frames that are at the beginning of the movie (where not enough previous frames are available), the model is built on the following frames.

Tracking methods

Below are some of the common properties and parameters you need to be aware of when running the tracking plugins:

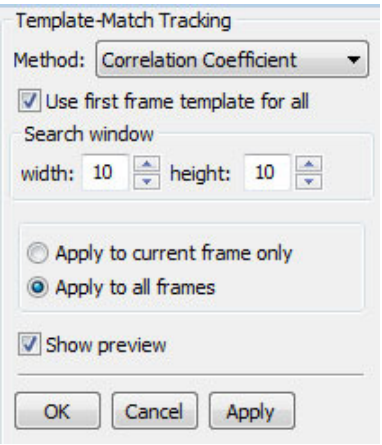
Side-by-side display: You will see two canvas areas when you activate a tracking plugin. The canvas on the left will display previous frame, and the canvas on the right will display the next frame. If you are on the first frame currently, no frame will be shown on the left canvas; you will have navigate one frame forward.

Search window: parameter limits the neighborhood that is searched in the new frame (around the location of the bounding rectangle in the previous frame). You need to use bigger search windows if the cells move at a very high speed (or if there is a shift in camera location – or equivalently the media is moved). A very low frame-rate would also result in having a larger shift in the location of the cells, requiring a bigger search window.

Use avilable tracking, if any: This option, if selected, will initialize the tracking method with any boundaries or feature points already available in the current frame (The number of points must be the same as that of the previous frame, otherwise this option is ignored). Otherwise, an exact copy of the previous features is used as the initial guess.

Template-matching based tracking

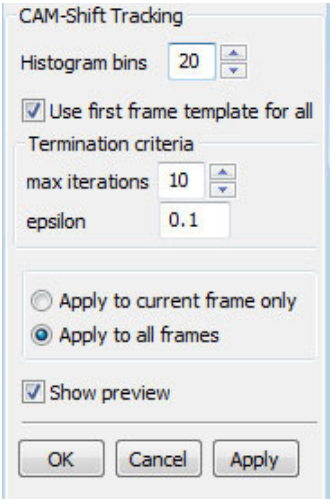
Command: `Tracking → Template-matching (Ctrl+M)`



In order to track each cell, *template matching* method first finds the minimal rectangular area that covers the cell in the previous frame. This rectangle (pattern) is then searched in overlapped patches of the next frame using the specified comparison function. The cell is then considered to move to the best-scoring patch in the next frame. If you select `Use first frame template for all` option, then the cells in the first frame are taken as templates when searching them in the rest of the frames. Otherwise, each frame serves as template for only its immediately proceeding frame.

CAM-Shift tracking

Command: `Tracking → CAM-Shift tracking (Ctrl+C)`

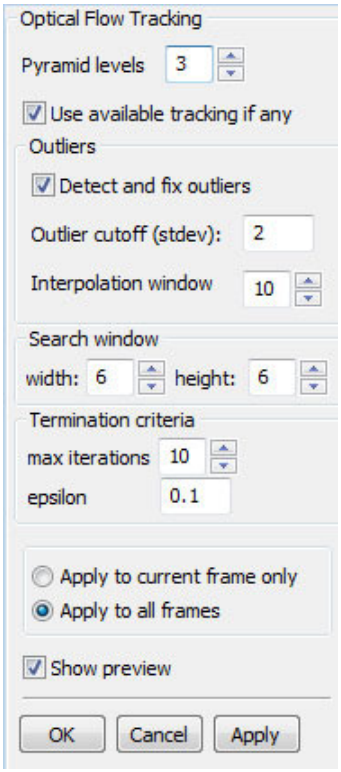


Continuously Adaptive Mean SHIFT (CAM-Shift) tracking algorithm is an implementation of "Real Time Face and Object Tracking as a Component of a Perceptual User Interface" by Gary R. Bradski (1998, *Fourth IEEE Workshop on Applications of Computer Vision (WACV'98)* p. 214). CAM-Shift is based on the mean shift algorithm ("Mean shift: a robust approach toward feature space analysis" by Comaniciu and Meer, *IEEE Trans. on Pattern Analysis and Machine Intelligence,* 1997), a robust non-parametric iterative technique for finding the mode of probability distributions.

CAM-Shift first finds the center of the cell in the new frame using the back projection of the histogram generated from the previous frame. Then it calculates the new size and orientation for the cell. `Histogram bins` specify number of bins to store the histogram of the cell in the previous frame. The search termination criteria is specified as the maximum number of iterations and the minimum amount of change (`epsilon`) between search iterations.

Optical Flow tracking

Command: `Tracking → Optical flow tracking (Ctrl+L)`

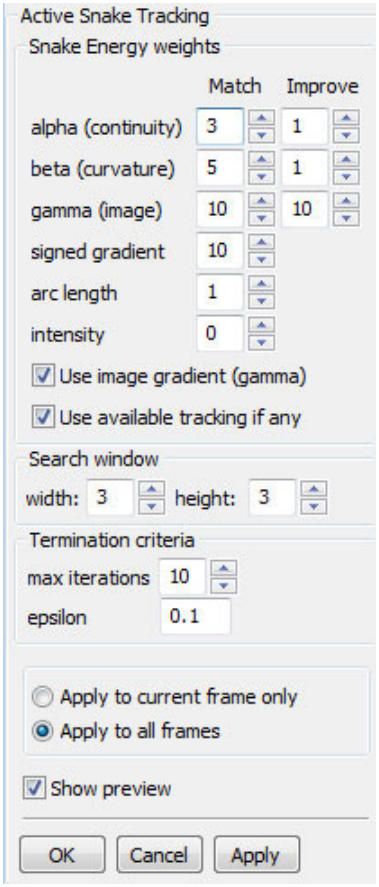


This plugin uses sparse iterative version of Lucas-Kanade optical flow in pyramids ("Pyramidal Implementation of the Lucas Kanade Feature Tracker" by Jean-Yves Bouguet, Intel Corporation, Microprocessor Research Labs, 2000. OpenCV Documents). The vertices of the cell boundaries and any intra-cellular feature points are tracked from the previous frame to the next frame.

Note that the optical flow method itself does not guarantee that the relative locations of the points will be preserved during tracking. This may result in inconsistent tracking results along the boundaries, or leakage of intra-cellular features to outside the cell. Moreover, the optical flow method may even loose some of the features (i.e., these features are not found in the new frame). To remedy these problems, we have implemented an outlier detection and update mechanism. The points that move a distance that is above a certain threshold, which is specified as a user defined coefficient of the standard deviation of all the shifts (`Outlier cutoff - stdev`), are considered as outliers. These outliers are then updated based on the mean-shift of the points around them that are not themselves outliers. The extend of this local window can be specified as the `Interpolation window`, i.e., the number of neighboring windows to consider while fixing lost or outlier points.

Extended Active Snake tracking

Command: `Tracking → Extended Active Snake tracking (Ctrl+A)`

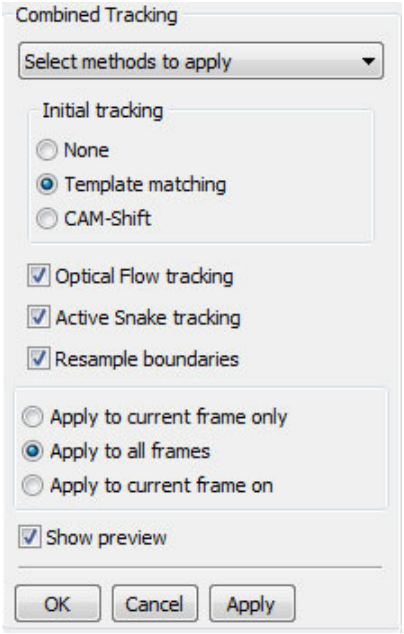


Extended Active Snake tracking implements a new energy functional we have developed for sensitive tracking of cells. The additional energy terms help to match up the new snake with the old snake (that is, the one from the previous frame) both internally (in relation to itself) and externally (in relation to the image plane). See the section on Improving Boundaries for the explanation of the original snake parameters (alpha, beta, and gamma) used for *improving* a boundary; these parameters are used without any changes here. The `Match` parameters, on the other hand are the weights of the energy terms that measure how well the new snake matches the old snake.

To illustrate the `Match` energy terms, let us consider the signed gradient energy term. Let us further consider a snaxel (point on the snake) p from the old snake (snake of the previous frame). Let p' be the current new location of this snaxel on the new frame. The energy minimization algorithm will search the neighboring pixels of p' to see if p' can be moved to a lower energy location. Let p^* be such a possible neighboring location. The matching signed gradient energy of p^* is calculated as follows: $|E^*_{signed_gradient} - E_{signed_gradient}|$, where $E^*_{signed_gradient}$ is the siged gradient value at location p* and $E_{signed_gradient}$ is the signed gradient value of the old snaxel p. A similar calculation is performed for the other energy terms. The cumulative energy at p* is the weighted sum of all energy terms (These weights can be adjusted by the user in the plugin sidebar). If p* has the minimal energy in the neighborhood, then p' is updated to p*. A similar update is performed for all of the snaxels at each search iteration.

Combined tracking

Command: `Tracking → Combined tracking (Ctrl+T)`

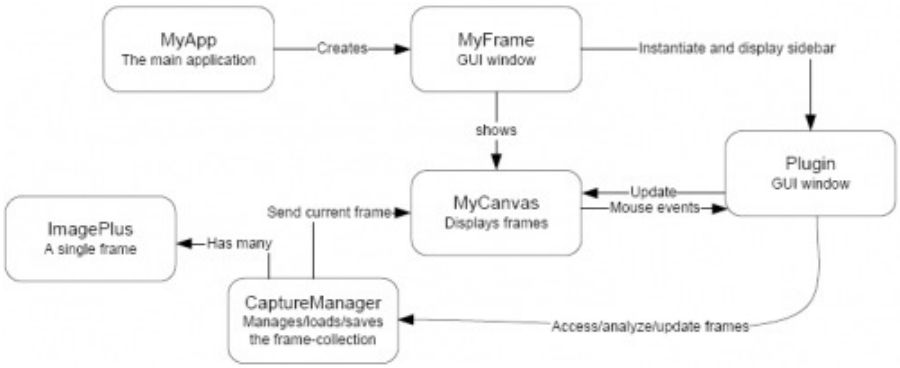


Combined tracking provides an ensemble of the other tracking methods, one applied after the other, for each frame. Note that this is different than applying the methods separately for all frames (i.e., in combined tracking, method A and then method B is applied to track from frame 1 to frame 2, and method A and then method B is applied to track from frame 2 to frame 3; whereas applying the plugins separately would be equivalent to applying method A to track 1→2 and 2→3, and then applying method B to track 1→2 and 2→3).

You can select which methods to apply. An initial tracking can be calculated using either Template-matching or CAM-Shift methods. This initial method can then be improved by Optical Flow and/or Extended Active Snake tracking. The parameters for each of these methods can be adjusted within this plugin. After tracking at each new frame, you can select to `Resample boundaries` which will eliminate the snaxels that come too close to each other, or introduce new points in between snaxels that grow too far apart. This resampling will help obtain a sensitive tracking of the cell. Note that if your application requires the association of snaxels as output, you should not use this resampling which will disrupt the association obtained from tracking.

Notes for Developers

This section is geared toward researchers or developers wishing to extend CellTrack by implementing new plugins. A general overview of the source architecture is given followed by instructions to get you started in writing a new plugin. The figure below shows a simplified outline of the classes and modules used in CellTrack.



`MyFrame` implements the main graphical interface and manages user-interaction. The frames are loaded from a movie file or image files into the `CaptureManager` which manages the collection (`book`) of frames. Each frame is an instantiation of `ImagePlus` class. The `ImagePlus` encapsulates the original loaded image, the boundary polygons, and the intracellular features. Each plugin is instantiated by the `MyFrame` upon a user request. The plugins can access the movie frames through CaptureManager, change the underlying image or the boundaries, and request an update of the canvas after the processing. The CaptureManager includes a frame separate from the frame book so that the plugin preview can be performed on this working frame without committing changes into the book.

If you want to develop a new plugin, you will need to first design a sidebar to accept the parameters (if any) from the user. See `src/gui/Gui.h` and `src/gui/Gui.cpp` files for the definition of the available plugin sidebars. The processing logic is separated from the user interface; you need to create a new class inheriting the sidebar design class. For example, the `FindContoursSidebar` (in `src/plugins/` folder) processing class inherits the `FindContoursSidebar_` user interface design class. The `src/plugins/PluginDefinitions.h` file includes macros to automate this inheritance.

The plugin module itself needs to be a subclass of `PluginBase` and override the relavant functions important to your plugin. If your plugin analyzes and operates on the current frame only, you need to override `void ProcessImage( ImagePlus *img )` function where `img` parameter will be given to be a pointer to the current frame. In this case, you don't need to access the CaptureManager object (`cm`), you can simply work on the `img` object. On the other hand, if you are implementing a tracking plugin where you need to analyze more than one frame, you need to override `void ProcessImage( ImagePlus *img, int pos )` function where `img` is again a pointer to the current frame, and `pos` is the position of the current frame. You can access other frames through the `CaptureManager` object `cm`.

The previewing and committing of changes to all frames is automatically performed by the base class. You may want to change the default processing by overriding the relavant functions (such as `DoPreview`, `OnOK`).

In most cases, you won't need to access the Canvas directly. However, if you are implementing a plugin where you need to gather information through user's actions on the canvas (such as dragging a cell using the mouse), you need to register the plugin as a listener of the relavant events. Please see the `src/plugins/EditContoursPlugin.cpp` plugin for a sample of this functionality.