Differential Interference Contrast Object Tracker (DICOT): GUI User Guide

DICOT is a MATLAB-based software developed for 2D particle tracking in DIC images.

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Download source code

The source code can be downloaded from GitHub https://github.com/Self-OrganizationLab/DICOT_GUI

Prerequisites

In order to use DICOT via source code, the user needs to install MATLAB supplemented with the **Image processing** Toolbox, the **Curve fitting** toolbox and the **Statistics** Toolbox.

The graphical interface of DICOT requires **MATLAB version R2019b** or higher (Figure 1).

The command line interface requires MATLAB version R2017b or higher.

Note: Please close other figure tabs if open in MATLAB, before running DICOT.

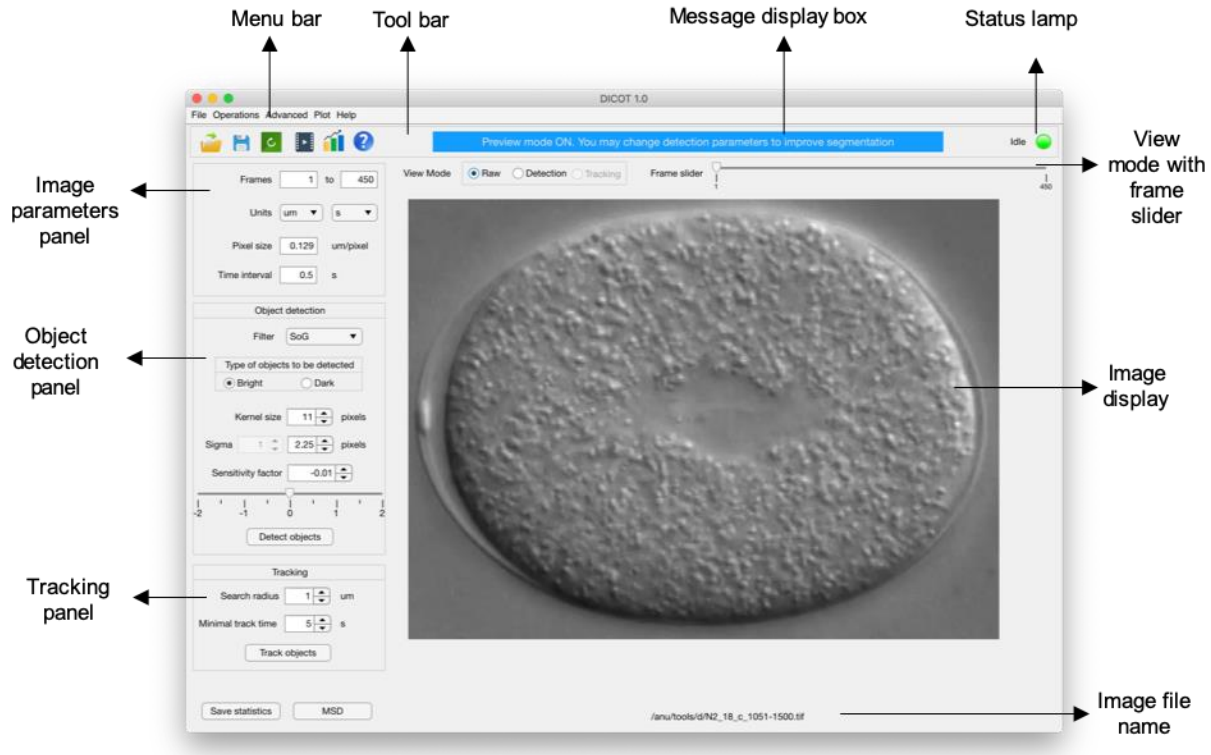


Figure 1. Graphical interface of the DICOT app.

Run the file '**dicot.mlapp**' from the DICOT folder MATLAB. The graphical interface (Figure 1) will pop up.

If the interface appears cramped or distorted on your screen, please **resize** the window. Resizing will restore the original form of the interface.

The '**message display**' box flashes messages during the various operations performed.

The colours of the '**status lamp**' indicate the status of operations in the GUI. The lamp is green when idle and red when busy.

1. Workflow

1.1. Opening an image

DICOT accepts input in the form of **TIFF** image-series i.e. movies. To open an image, either select

File > Open Image Series from the menu bar or click the  button in the toolbar. The user will be prompted to choose the image time-series on which particle tracking is to be performed. The first image in the series will be displayed in the GUI. The path and name of the opened file will be displayed below the image in the GUI.

The accepted format for the image time-series is **grayscale TIFF (.tif)**.

Output from DICOT will be stored in a subfolder named as 'DICOT-<image-name>' in this parent directory.

1.2. Image parameters

Frames: The number of images (frames) in the input movie gets displayed as soon as the image file is opened. The user can modify the start frame and end frame number in order to analyse a subset of the frames.

Units: The user should choose physical distance and time units in which the quantitation will be performed.

Pixel size: Dimension of each pixel in terms of (selected) distance units. If the image has an implicit pixel size, it will be identified and displayed automatically when the image is opened. The user can manually input and alter the pixel size value.

Time interval: The time interval between two consecutive image-frames, in terms of (selected) time units needs to be provided.

1.3. Object detection

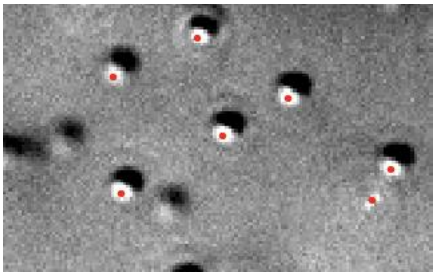


Figure 2**. Detection output**. Red dots mark the centroid of detected objects

Parameters

Type of objects to be detected: The user can choose to detect bright or dark objects in the image.

Kernel size: The average size of the SoG filter in pixel units. Typically, a kernel size slightly higher than object's major axis works optimally.

Sigma: The standard deviation of filter size in pixel units.

Sensitivity factor: It determines the scaling of the Gaussian filter. The user needs to vary the sensitivity factor **slowly** using the slider (for coarse adjustment) and spinner textbox (for fine adjustment) in order to refine segmentation.

Note: The user may weak parameters to refine segmentation.

Detecting objects

In order to detect objects, the user can click the '**Detect Objects**' button in the panel or can choose ***Operations > Detect Objects*** from the menu bar. The user may choose any of the three segmentation methods, viz. Scaling of Gaussian (**SoG**), Laplacian of Gaussian (**LoG**) or Difference of Gaussian (**DoG**).

The output will be displayed in the GUI in the form of the original image overlaid with red dots marking the centroids of objects (Figure 2).

The coordinates of centroids of detected objects are stored in the file 'untracked.txt'.

1.4. Tracking

Parameters

Search radius: The user needs to provide the approximate distance moved by objects (in selected units) in between two frames. The algorithm uses it as a threshold distance for linking centroids to make a track.

Note: The user may weak the search radius to improve tracking.

Minimum track time: Only tracks spanning a total time (in selected units) equal to or above this cut-off will be saved, displayed and analysed further.

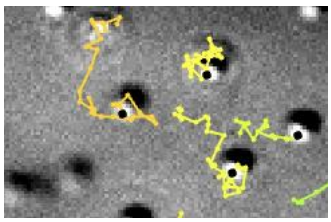


Figure 3. **Tracking output**. Coloured lines indicate tracks of particles.

Tracking objects

Tracking is commenced by clicking the 'Track Objects' button in the panel or by selecting ***Operations > Track Objects*** from the menu bar.

The tracking output will be displayed in the GUI in the form of the original image overlaid with coloured lines that indicate tracks (Figure 3).

The coordinates of tracks are stored in the file 'trajectories.txt'.

1.5. Quantification

Statistics files

On pressing the 'Statistics' button, two files get saved in the output folder. The 'InstStats.txt' file stores instantaneous (step-wise) quantification of motion of objects. The 'StatsPerTrack.txt' file stores track-wise quantification output.

An alternate way to save these files is by selecting ***Operations > Statistics*** from the menu bar.

Mean Squared Displacement (MSD)

MSD can be calculated either by clicking the 'MSD' button or by choosing ***Plot > Time profiles > MSD*** from the menu bar. Track-wise MSD is saved automatically in the file 'ID_Time_MSD.txt'.

MSD ($< r^2 >$) of particles was calculated as described in (Khetan and Athale, 2016) using the Cartesian coordinates (x,y) obtained from tracking.

$$< r^2(\ \delta t) >= [x(t + \delta t)\ - x(t)]^2 + \ [y(t + \delta t)\ - y(t)]^2 \qquad \text{(Equation 1)}$$

In the above equation, *r* is the displacement of the particle at two time-points separated by a time-step δt that ranges from the minimal time-step in experiment to $3/4^{th}$ of the length of the trajectory.

D_{eff} was calculated by fitting the average MSD profile as a whole or its linear portion, based on user input.

The user can choose from the diffusion model to which the average MSD curve can be fit by selecting ***Advanced > Set Diffusion Model*** in the menu bar.

The anomalous diffusion model is given by:

$$< r^2 > = 4Dt^{\alpha}\ + c \qquad \text{(Equation 2)}$$

Here, *t* is the time-step, *c* is the error, *D* is the effective diffusion coefficient and α is the anomaly parameter that indicates the nature of diffusion. The motion is said to be purely diffusive if $\alpha = 1$, sub-diffusive or 'restricted' when $\alpha < 1$ and super-diffusive or 'transported-like' when $\alpha > 1$.

The diffusion-with-drift model is given by:

$$< r^2 > = 4Dt + (vt)^2 + c \qquad \text{(Equation 3)}$$

Here, *t* is the time-step, *c* is the error, *D* is the effective diffusion coefficient and *v* is the drift velocity.

Plots

Note: Please ensure the statistics files have been saved prior to generating plots from the menu and tool bar.

In the toolbar, clicking the  button results in a combined plot of histograms of track-wise pathlength, speed and tortuosity. It also includes a histogram of instantaneous displacement of detected objects.

Histograms can also be generated by selecting ***Plot > Histograms*** from the menu bar.

The ***Plot > Time Profiles*** menu provides profiles of MSD, cumulative displacement and instantaneous speed over time.

1.6. Saving movie, images and files

The plots, static images and tracking movie need to be saved manually by the user.

The output from detection and tracking can be saved in the following two ways:

a. Using the ***File > Save Image*** menu: The user is prompted to enter a suitable name for the saved image that gets saved as a .tif file in the output folder.
b. Using the **interactive** option in the GUI: In order to interactively save an image from the interface, the user needs to hover on the top right corner of the image in the display panel. A menu of icons pops up, which contains options to save, zoom, pan and restore the image in the axes.

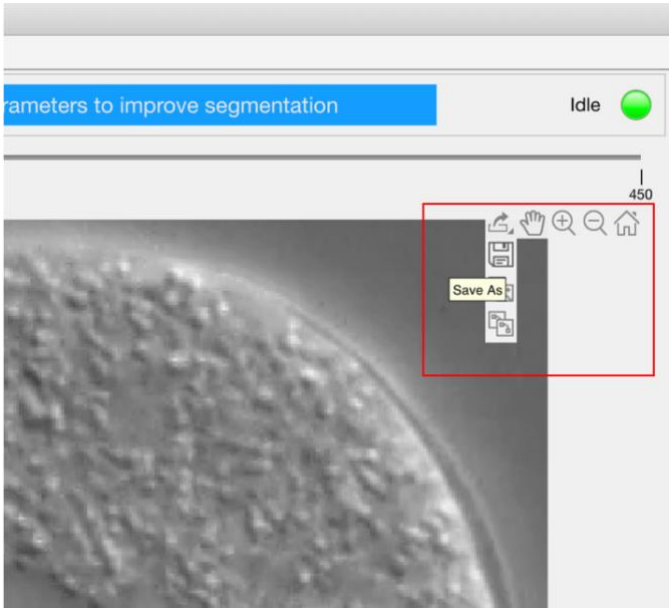


Figure 4**. Interactive saving of output images from GUI**. A menu of icons pops up (red box) on hovering on the top right corner of the image in the GUI.

The tracking movie of objects can be saved either by clicking the 'Save movie'  button or be selecting ***Operations > Make movie*** from the menu bar. The process may take a few minutes depending on the number of frames in the input movie.

The parameters chosen by the user for object detection and tracking can be saved as a text file or Excel sheet. The 'Save parameters' button  in the toolbar helps save the 'Parameters.txt' file. Alternatively, the ***File > Save Parameters As*** menu helps the user to save parameters.

1.7. Reset parameters

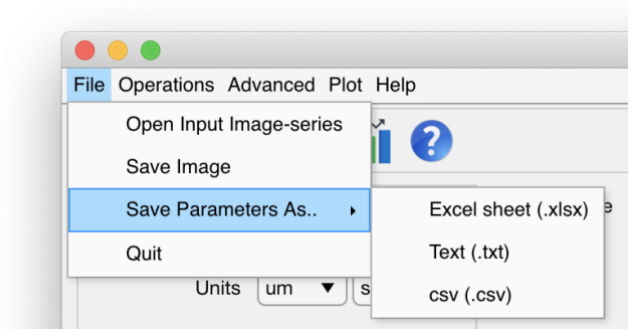
As the name indicates, the 'Reset' button in the toolbar sets all parameters in the GUI to default and clears the image display.

1.8. Help

The User Guide for DICOT can be opened by pressing 'Help' button in the toolbar or by selecting ***Help > User Guide*** from the menu bar.

2. Menu Bar Options

2.1. File



2.1.1. Open Input Image-series

The user is prompted to open an image-series (movie). DICOT accepts input in the form of **TIFF** files.

2.1.2. Save Image

The image currently displayed in the GUI axes can be saved as a **TIFF** file using this option.

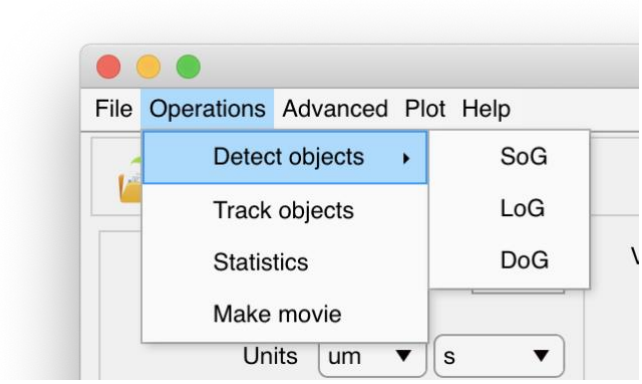
2.1.3. Save Parameters As

The parameters currently displayed in the GUI panels can be saved using this option. The user may choose to save parameters in any of the three forms viz. **.txt, .csv or .xlsx.**

2.1.4. Quit

Allows the user to exit the application.

2.2. Operations



2.2.1. Detect Objects

This option allows the user to detect objects once the image parameters and segmentation parameters are fed into the GUI panels. The user may choose any of the three segmentation methods, viz. Scaling of Gaussian (**SoG**), Laplacian of Gaussian (**LoG**) or Difference of Gaussian (**DoG**).

2.2.2. Track Objects

Once the detection is completed and tracking parameters are fed in, the user can choose this option to track the detected objects in time.

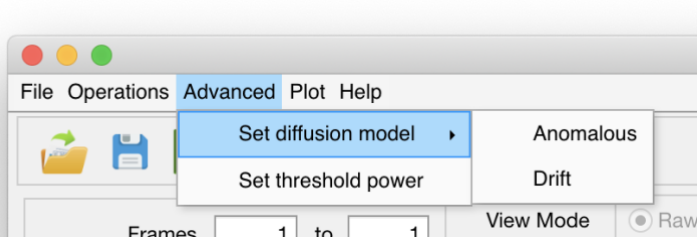
2.2.3. Statistics

This option performs quantification post tracking and saves the output statistics (instantaneous and average) files in the output folder.

2.2.4. Make Movie

This option needs to be selected once tracking has been completed if the user would like to save the tracking output as a TIFF file.

2.3. Advanced



2.3.1. Set diffusion model

In order to fit the mean squared displacement profiles of tracked particles, the user could select any of the two models viz. anomalous diffusion (Equation 2) or diffusion with drift (Equation 3).

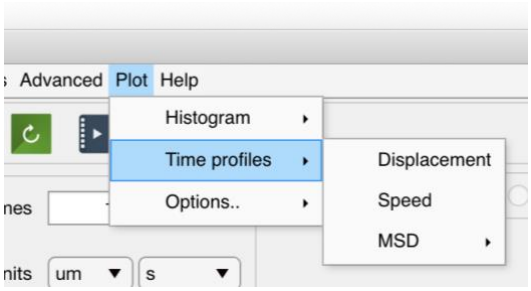
2.3.2. Set threshold power

The power (p) of the threshold used by the segmentation algorithm can be set by the user using this option, such that:

Threshold = (Otsu's threshold)$^{1/p}$

2.4. Plot

Plots can be generated post tracking and quantification.



2.4.1. Histogram

Histograms of pathlength, displacement, speed and tortuosity can be plotted using this option.

2.4.2. Time profiles

Time profiles of displacement, speed and MSD (average with fit) can be plotted.

2.4.3. Options

These options allow the user to choose the marker size and line width of the plots overlaid on the detection and tracking output image in the GUI axes.

2.5. Help

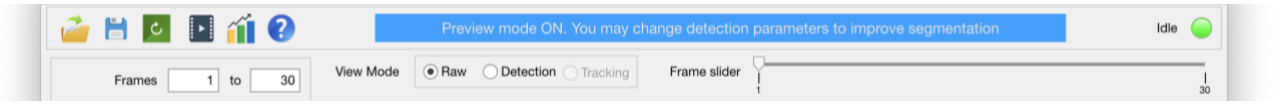
2.5.1. About

Brief information about DICOT pops up.

2.5.2. Help

This option provides access to the DICOT user guide.

3. Toolbar Options



Symbol/ Feature	Function
	Open input image-series
	Save parameters as .txt
	Reset parameters
	Make and save tracking movie as .tif
	Plot histograms

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	Help, access user guide
Message display	Displays the activity currently undertaken or completed by the GUI
Status lamp	Indicates the busy/idle status of the app
View mode and Frame slider	Can be used to view the detection and tracking output across all image frames in the input movie