How to use the tracking program from the “multi-hipo” repository

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The link to the repository: https://github.com/ssklykov/multi-hypo

Introduction to the field of possible application

This project is intended to improve the quality of multiple particle tracking of fluorescently labelled objects transported in living cells (e.g. the case of organelles trafficking). Fluorescent objects usually have the appearance of bright structures (with round-like shape or approximately with Gaussian intensity profile or elongated shape (e.g. mitochondria) fused in the black (ideally) background. Objects with sizes comparable or less than the diffraction limit commonly possess the Gaussian spatial distribution of intensity. Therefore, particles are considered as moving bright objects. In general, they could be localized using different segmentation methods.

The developed tracking program include three main steps:

- Localization of objects of interest by applying different manually adjusted filters
- Localized particles linking and resulting tracks filtering
- Checking and selection for further analysis type of data

All of these points will be explained below.

Prerequisites for launching this project:

- At least LabVIEW 2017 SP1 version of LabVIEW Professional Development System.

Complementary Toolkits (all – not later than 2017 versions):

- Advanced Signal Processing Toolkit; - Digital Filter Design Toolkit; - Vision Development Module

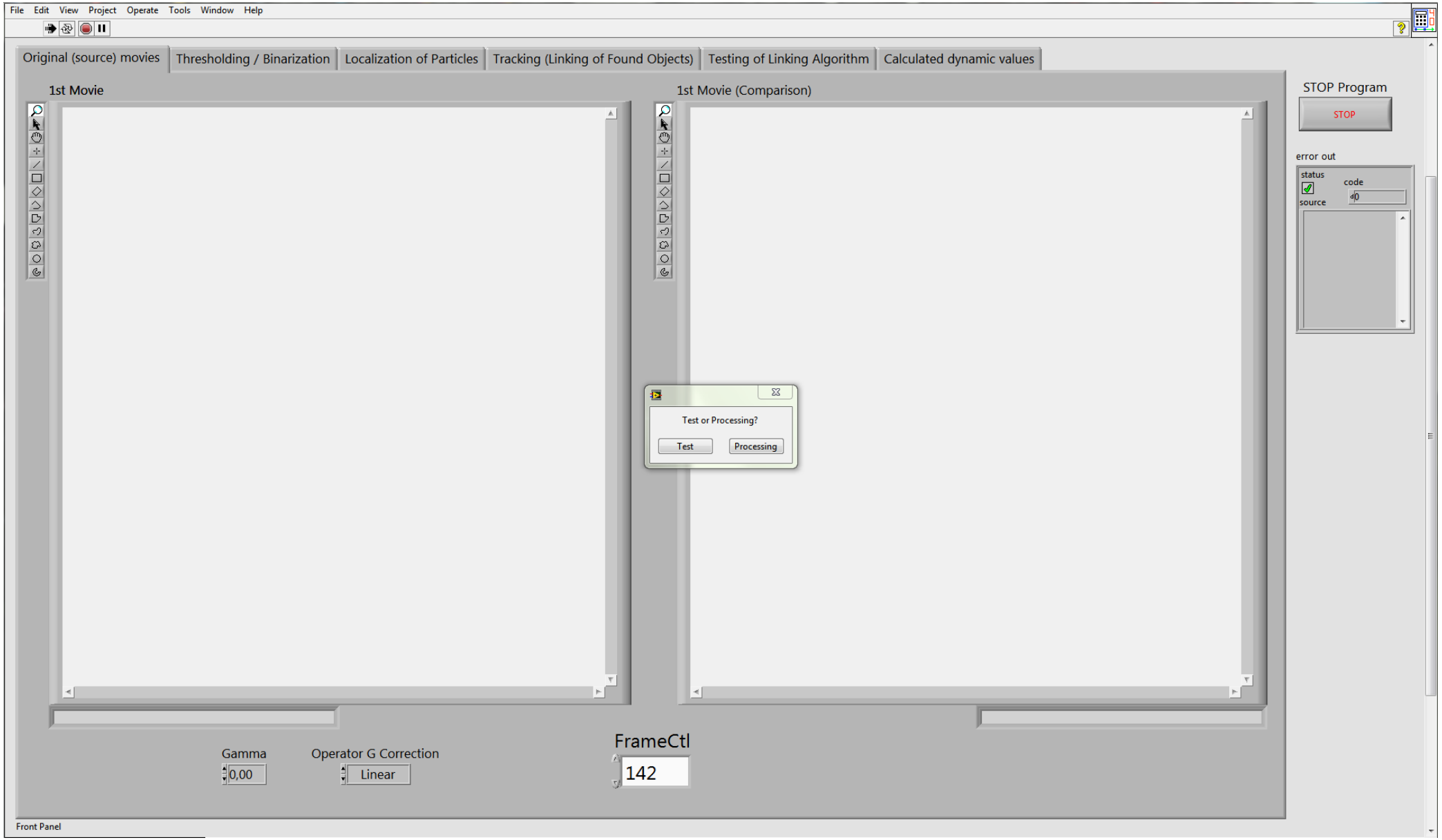
The project has been developed and tested in OS Windows 7. LabVIEW may be also installed in Linux and macOS.

Input data format: videos with ***avi*** format (they could be made in the Fiji/ImageJ)

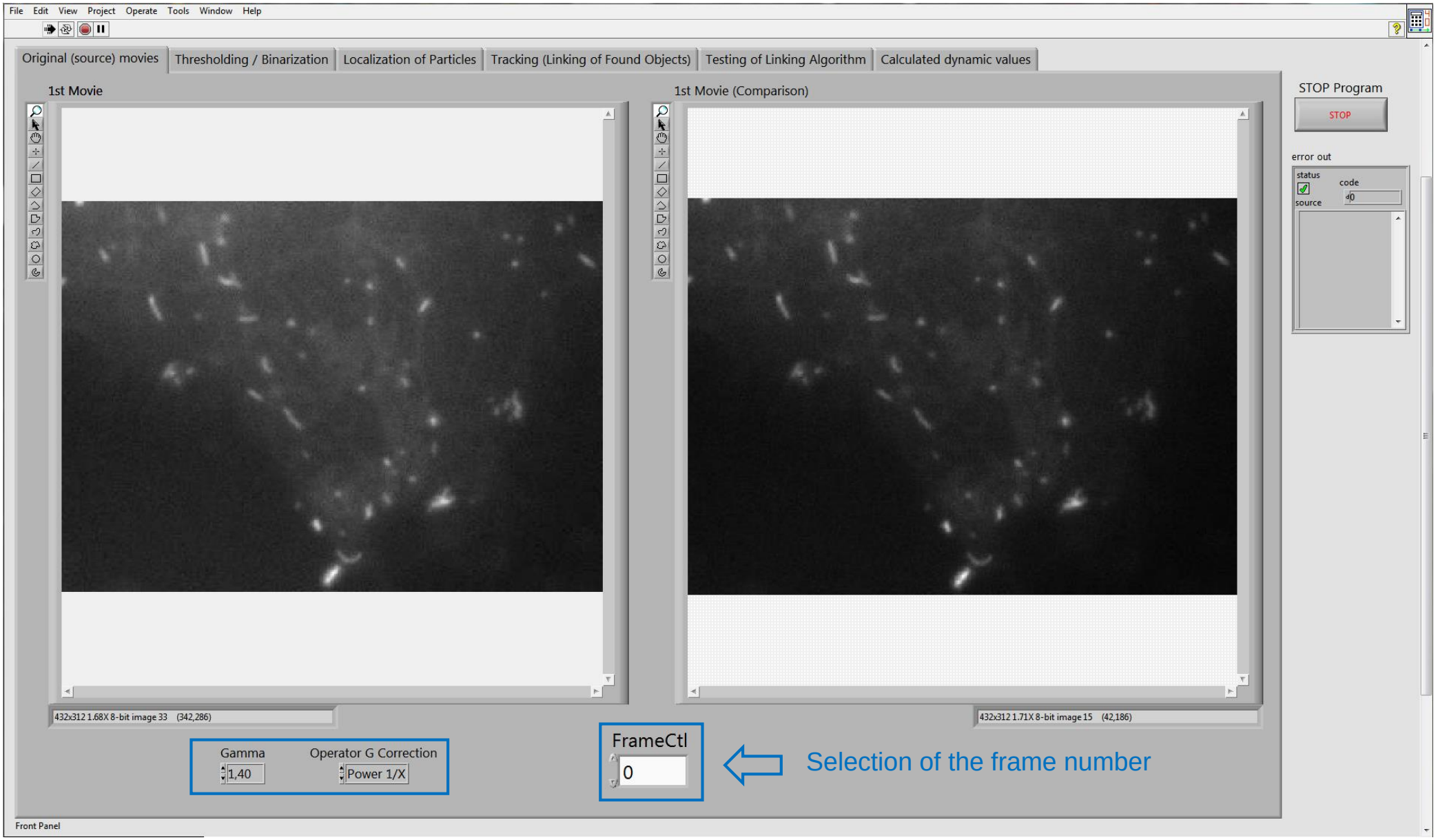
Default values of controlling input parameters (like a threshold value) are specified in the ***ini*** file, located in the main application folder.

1. After launching the program, now it starts with choice menu: explore the capabilities of program on the test examples (also it's helpful for testing improvements in tracking algorithm and this is why this feature is preserved till current moment) and processing of user's movies

The main GUI window after launching of program with dialogue window:

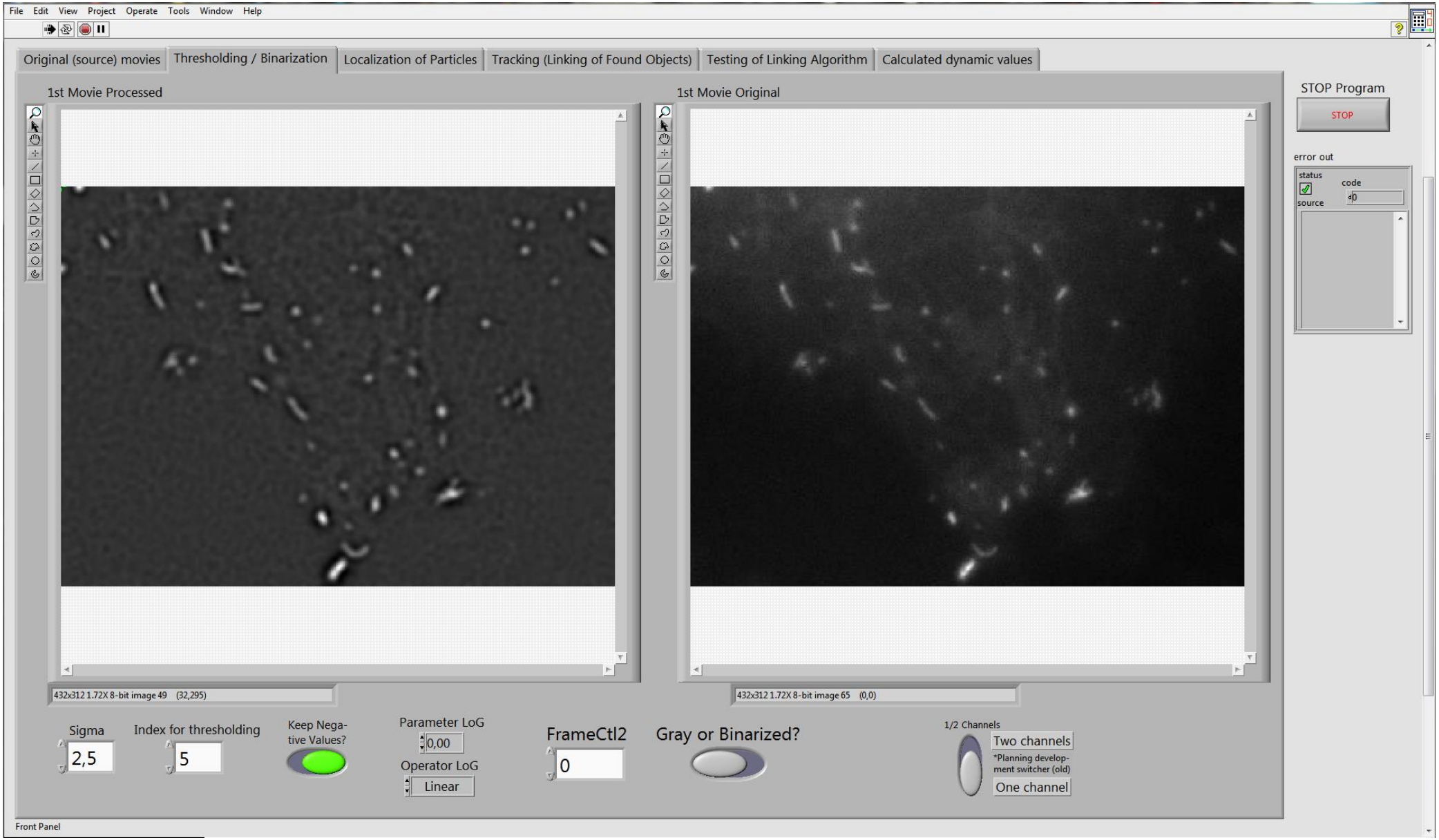


2. Initial step procedure (**“Original (source) movies”**): If you select “Testing” then the test video should be uploaded as the sample for tracking; otherwise you need to find the *avi file as the tracking sample (please, prepare your data before)



The main setting here are: “gamma” and “Operator G Correction” – they regard to gamma correction of the frames from the sample movie (point-like correction of an intensity value in each pixel). Here is the wiki-page about it: gamma_correction.

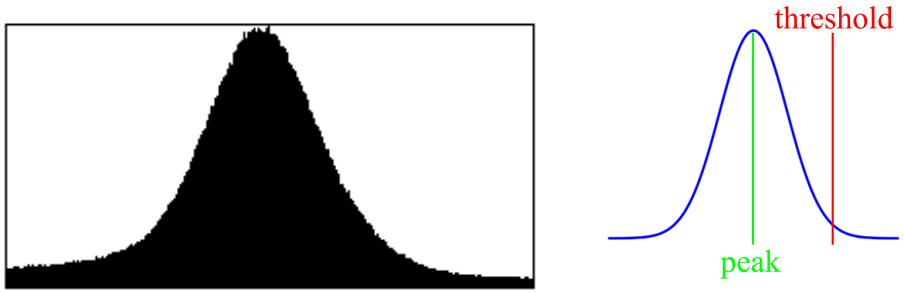
3. Next step – **“Thresholding / Binarization”** – this step is intended to separate objects in a frame to background (dark) and particles (green). Among others, the LoG (Laplacian-of-Gaussian) spatial filtering has been chosen as the main method to enhance signal-to-noise ratio and suppress always accompanying fluorescent microscopic images noise. It was stated in the work [D. Sage et al., 2005] that the LoG filter is optimal for the objects with Gaussian spatial profiles.



Here is the list of the available settings:

1) “Sigma” – this setting defines the size of the LoG kernel and is proportional to a size of object (in a case of round object the sigma will be proportional to a radius)

2) “Index for thresholding” – it defines the intensity threshold that separates objects and background. The intensity histogram after convolution of image with a LoG kernel commonly possess the bell-like shape with an easy findable peak:



The common histogram after LoG and defining of the threshold value

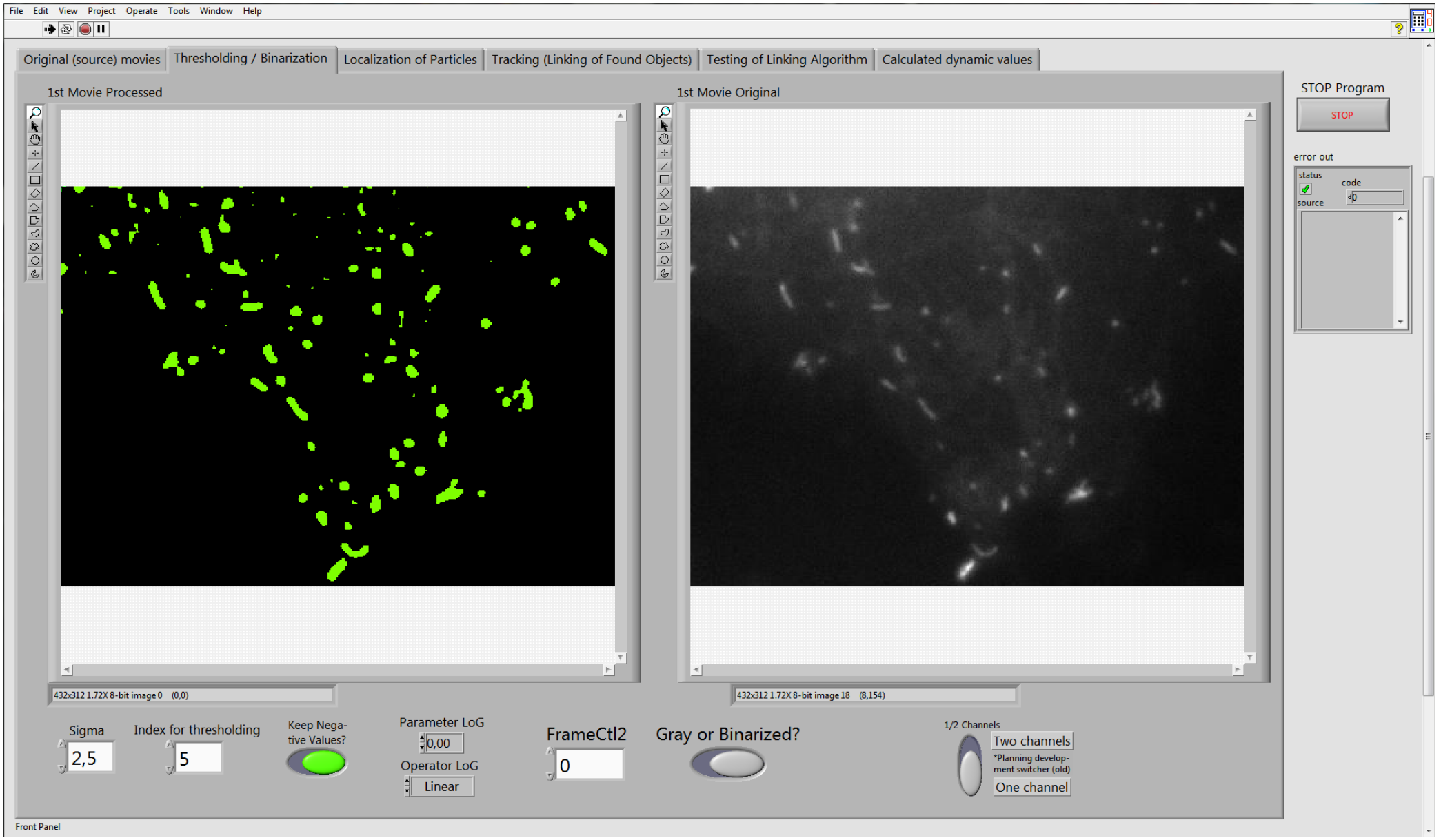
Therefore, “index for thresholding” is defined as the additional intensity value to calculated peak and could be changed by the user for achieving better localization of objects

3) “Keep Negative Values?” switcher is used for adjusting results of the LoG, this feature could be used for enhancing of visual quality of binarisation and, consequently, quality of particle localization

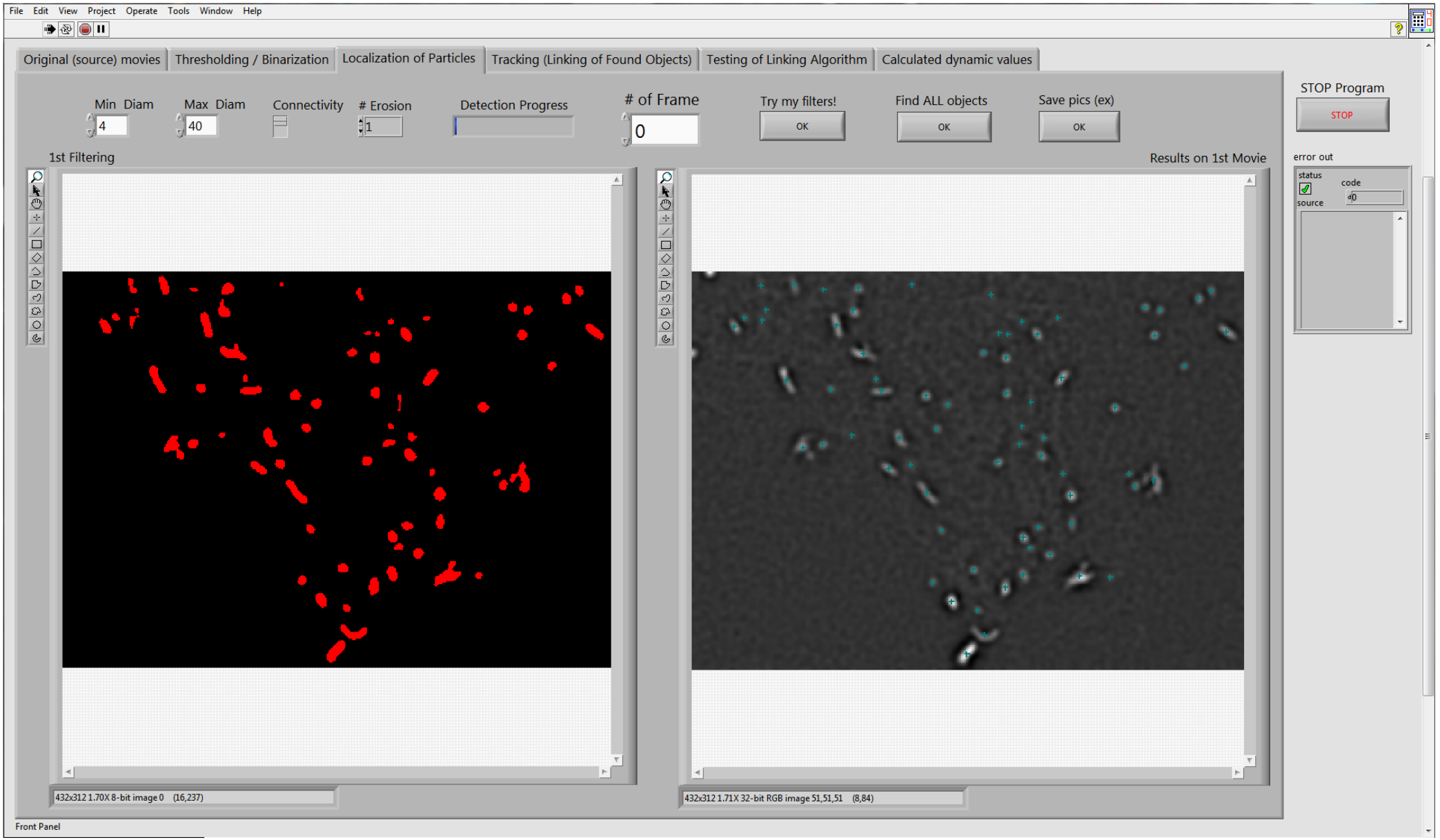
4) “Parameter LoG” and “Operator LoG” refer to gamma correction of obtained after LoG filtering pictures

5) “Gray or Binarized?” switcher refers to checking results of LoG processing on the gray (initial) picture (above) and the binarized picture (below)

Binarized picture – results of the LoG filtering:



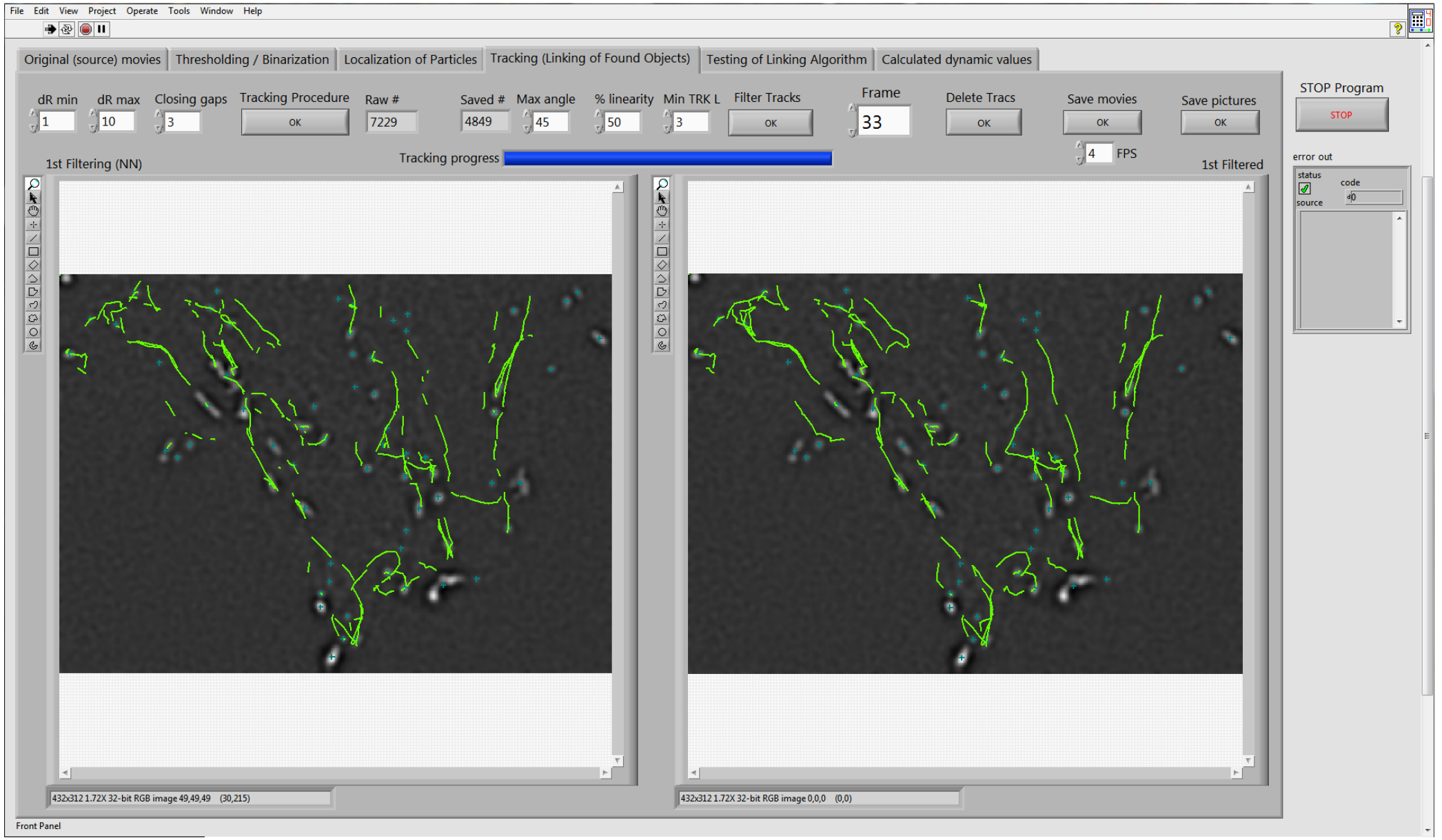
4. **“Localization of Particles”** – applying different particle filters for retrieving the objects:



Here is the list of the available settings:

1) “Min Diam” = “Minimal Diameter” – the numerical selector for specifying the minimal Feret diameter of binarized particle, it’s defined in pixels
2) “Max Diam” = “Maximal Diameter” – the numerical selector for specifying the maximal Feret diameter of binarized particle, again this parameter defined in pixels
3) “Connectivity” – this setting refers to the setting from the built-in Image Processing function. You can keep it with the default value
4) “Erosion” selector defines the number of consequent morphological erosion operations; it helps to cut out the fake particles (e.g. emerging from noisy background). You can keep it with the default value
5) “Try my filters!” button shows results of selected settings (binarized image on the left picture and dark-blue crosses marking centers of objects upon LoG filtered frame)
6) “Find ALL objects” button launches the localization of objects through all frames from the processed movie; the progress of localization is shown as the progress bar among other settings
7) “Save pics (ex)” buttons allow saving the results of object localization in the selected frame as a *png picture

5. **“Tracking (Linking of Objects)”** – connection of localized at previous steps objects



Here is the list of the available settings:

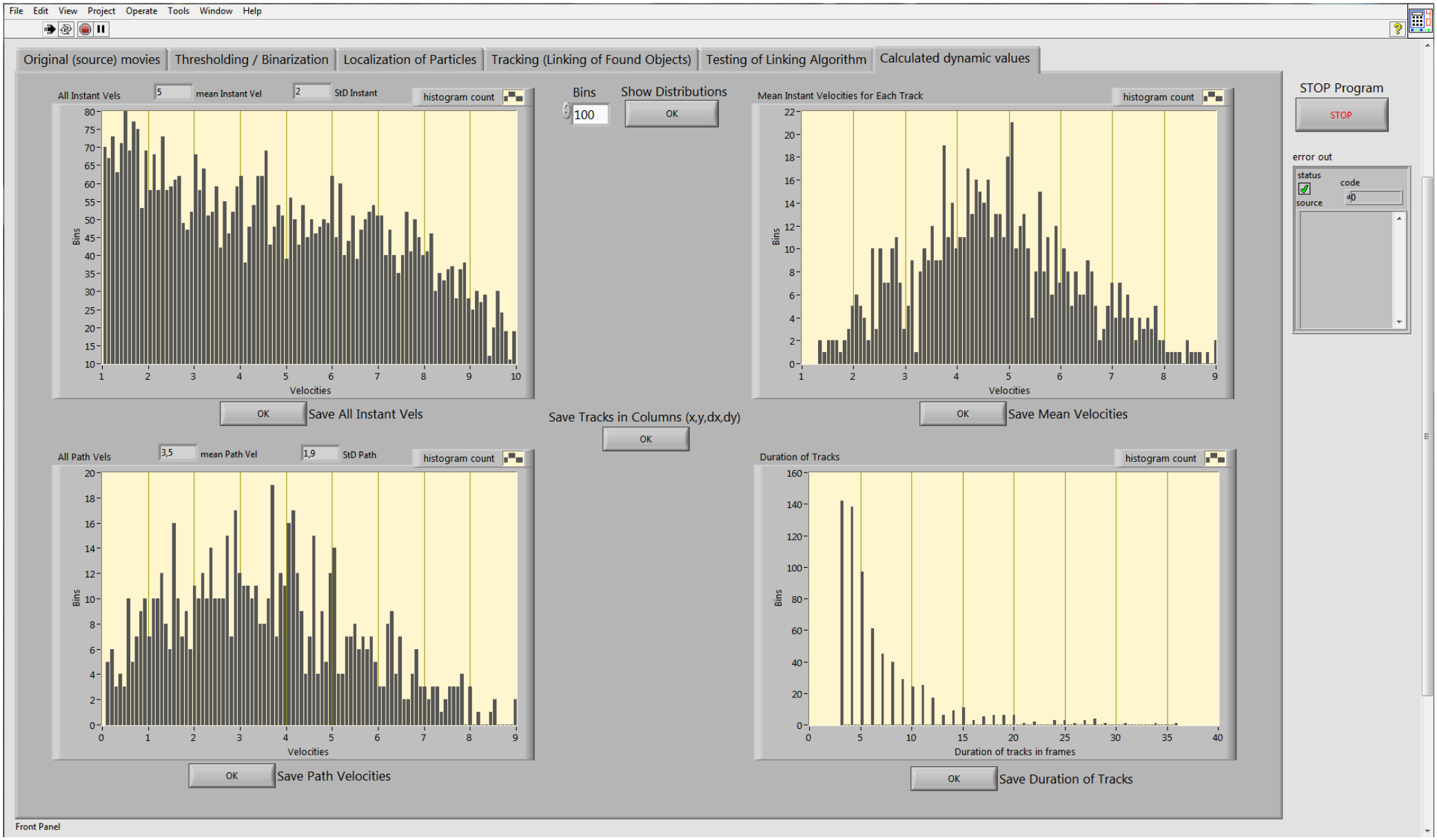
1) “dR min” – the numerical selector for specifying of minimal possible frame-to-frame displacements in pixels; it can’t be less than zero due to the fact that localization precision is finite and instant displacement less than this precision already should be treated as belonging to the stationary object;
2) “dR max” – for specifying of maximal displacement between frames in pixels;
3) “Closing gaps” – for defining the number of frames between which stationary objects (the objects with displacements less than defined minimal one) could be connected if such stationary objects disappear between frames. You can keep it with the default value
4) “Tracking Procedure” – this button launches the tracking procedure with selected parameters (1-3), its progress is shown as the progress bar;
5) “Raw #” displays the amount of found frame-to-frame displacements, represents, in general, that some tracks have been found *****
6) “Saved #” displays the amount of preserved after application of filters (explained below) frame-to-frame displacements *****
7) “Max angle” – the numerical selector for specifying of the maximal angle between two subsequent displacements (frame-to-frame, instant displacements) which is maximal for accounting such displacements as linear; this angle is specified in degrees;
8) “% linearity” – the numerical selector for specifying of a threshold for distinguishing between linear and nonlinear tracks; this parameter (linearity) is calculated as following: $l = \frac{linear\ displacements}{all\ displacements\ in\ a\ track} \cdot 100\%$

Therefore, selection of “Max angle” defines the number of linear displacements in a track and its linearity.
9) “Min TRK L” – for defining the minimal length of tracks counted in frames (too short tracks will be cut out from further calculations);
10) “Filter Tracks” - this button launches the filtering of tracks with selected parameters (7-9)
11) “Delete tracks” – clear drawn tracks from the pictures below
12) “Save Movies” – saving two movies which represent two tracking algorithms implemented in the program;
13) “Save Pictures” – saving the pictures with overlaid tracks in *png format

1st Filtering (NN) display and the connected movie are developed for comparison and display the result of implementation of simple nearest neighbor tracking algorithm. **1st Filtered display** and the connected movie display the tracking results obtained by applying developed Multi Hypothesis Tracking algorithm. The first one display and movie again have been retained for development purposes.

***** - these parameters are more intended for development purposes

6. **“Calculated dynamic values”** – this page shows (after clicking on button “Show Distributions”) the calculated dynamic values from refined tracks. All of them could be saved as a *csv files, except “tracks in columns”, which could be saved as *txt file



The list of calculated values:

1) “All instant Vels” = All Instant Velocities, that means the instant, frame-to-frame displacements saved in one big array from persisting after filtering step tracks;

2) “Mean Instant Velocities for Each Track” – the mean velocities which have been calculated for each track;

3) “All Path Vels” – these velocities represent the linearity of motion, because they are defined as the ratio between total displacement or “path” connecting starting and finishing point of each track divided by length of this track counted in frames;

4) “Duration of Tracks” – this display and related values equal to length of persisting tracks counted in frames;

5) “Save Tracks in Columns (x,y,dx,dy)” – this button allows to save preserved tracks in format there in each column is saved the starting point of displacement (x,y) and related displacement to the next point (Δx,Δy). Each column belongs to a separate track. It’s schematically shown below: $\begin{pmatrix} x_1 \\ y_1 \\ \Delta x_1 \\ \Delta y_1 \end{pmatrix}_1 \quad \begin{pmatrix} x_1 \\ y_1 \\ \Delta x_1 \\ \Delta y_1 \end{pmatrix}_2$, where the track is recorded as points and their displacements in columns (subscripts in columns)

$\begin{pmatrix} x_1 \\ y_1 \\ \Delta x_1 \\ \Delta y_1 \end{pmatrix}_1 \quad \begin{pmatrix} x_1 \\ y_1 \\ \Delta x_1 \\ \Delta y_1 \end{pmatrix}_2$

All values are available for saving after clicking to related buttons.