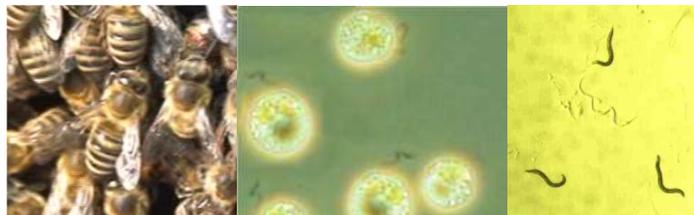
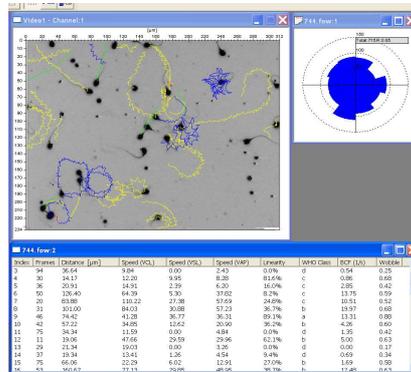




medeaLAB Tracking Image Analysis System *Tutorial*





is a product of



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Thank you for your interest in our medeaLAB system!

This tutorial will help you to get a first impression of the practical use and easy handling of the medeaLAB imaging system.

1. Program installation

Important:

Administrator rights are essential to install the software.

To install the medeaLAB Software put the installation CD into your drive. The medeaLAB setup starts automatically, otherwise start the installation by double clicking on the file **setup.msi** in the CD root directory. Choose "Typical" setup and follow the instructions.

2. Basic information

2.1. Registration

When starting medeaLAB for the first time a message "medeaLAB is not registered" appears. You may continue to work without registration for a period of 30 days (button "Continue unregistered") or purchase and register your copy of medeaLAB Tracking (choose the "Register" button).

For online registration you will need a valid email address and internet access. Follow the four steps in the "Register medeaLAB" dialog.

2.2. Image sources

medeaLAB supports the following video sources:

- Analog video cameras or VCR (using MATROX frame grabbers)
- FireWire (IEEE1394) cameras (if WDM driver is provided)
- USB cameras (if WDM driver is provided)
- Prosilica Gigabit Ethernet cameras
- Video files (AVI or MPEG)

3. First steps

In this section we want to show you how easy it is to achieve good measurement results with the medeaLAB system.

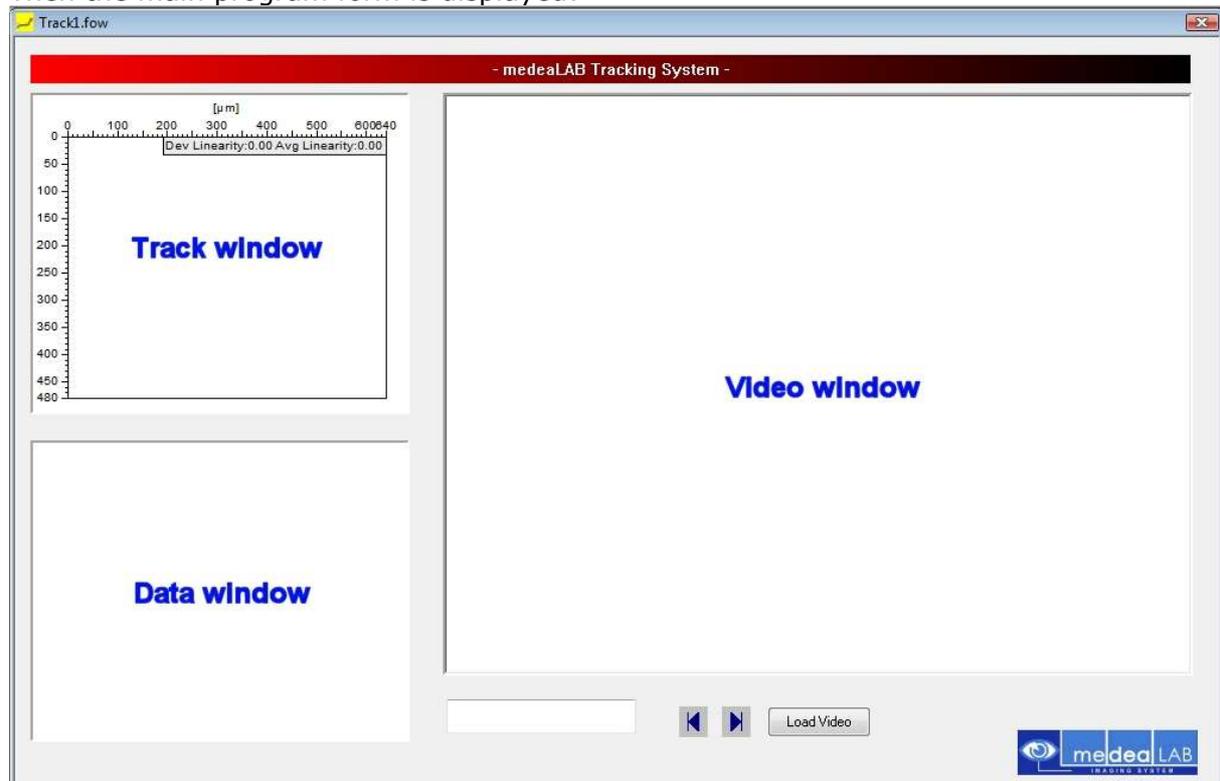
3.1. Create a new measurement document

Click the "Start Measurement" button on the toolbar:



A File dialog appears. Accept the default filename for the new Track File (extension .fow) or choose another one. Confirm the creation of the new file.

Then the main program form is displayed:



3.2. Initialize video source

After installation the system is configured to use video files as input and a sample video file (medealabtracking.avi) will be used. You simply have to initialize (load) the video file with the menu command **Video – Init Video** or the  button on the toolbar. With the **Video – Options** menu command you are able to change the video input source later (camera or other video file).

If video files are used a video player window appears upon initialization allowing you to navigate through the video sequence (play, stop, step, rewind):



3.3. Start measurement

Click the "Start Measurement" button  on the toolbar again.

The system will perform automatic object detection first. In most cases this step will succeed and tracking of the objects will start. If no or not enough tracks are recorded try to switch off "Automatic Threshold" and adjust settings manually or change the tracking method (see the Configuration section below).

After the measurement runtime (default configuration is 8 s) the measurement is stopped with a message. You may interrupt the measurement with the <ESC> key on the keyboard.

If you want to repeat a measurement with the same video file you have to rewind to the beginning before starting measurement again. Use the |< button in the video player window or at the bottom of the main form to step to the first video frame.

3.4. Measurement data

Numerical data of the tracks are displayed in the data window on the lower left side of the main form. To analyse single tracks you may switch to the single track mode by unchecking the menu item **Evaluate - All Tracks** or by clicking on the Σ button. In the single track mode navigation through the tracks is possible by menu command **Evaluate / Next Track** and **Previous Track**. A single track may be deleted by menu command **Evaluate / Delete Track**.

Toolbar: 

The segments (interval between image frames) of all or a single track may be evaluated for speed distribution (**View - Show Speed Histogram**), speed progress (**View - Show Speed Chart**) and directional distribution (2D circular histogram, menu command **View - Circular**).

All table data may be exported using the menu command **File - Export** which is active if the data window is activated by clicking inside. For direct export to MS-Excel the program MS-Excel has to be installed.

4. Configuration

In this section only the most important settings of the tracking system are described. For a complete overview please refer to the User Manual.

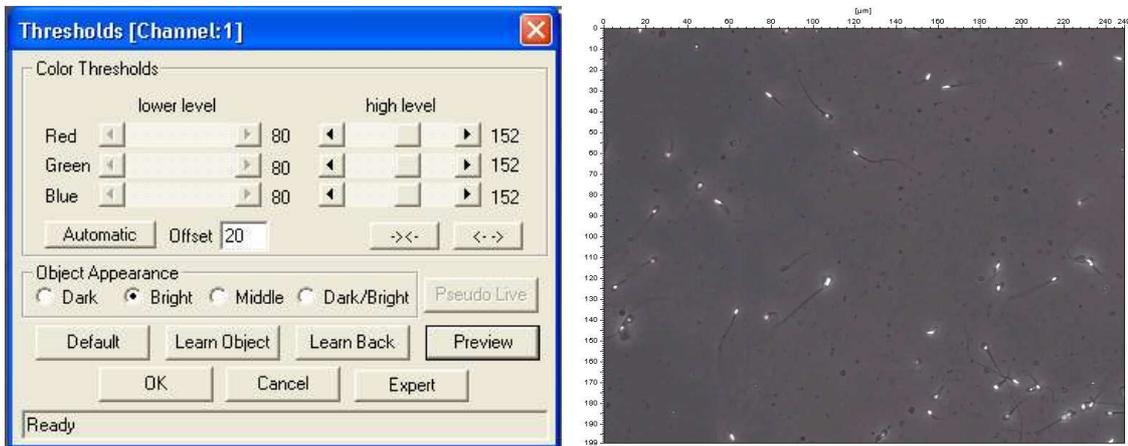
4.1. Thresholds

Normally medeaLAB Tracking uses an automatic threshold function to distinguish between objects and image background. The threshold adaptation is automatically done before each measurement. So the measurements are not sensitive for image brightness changes. You can activate or deactivate the Autothreshold function with the menu command **System - Options** (main form must be closed).

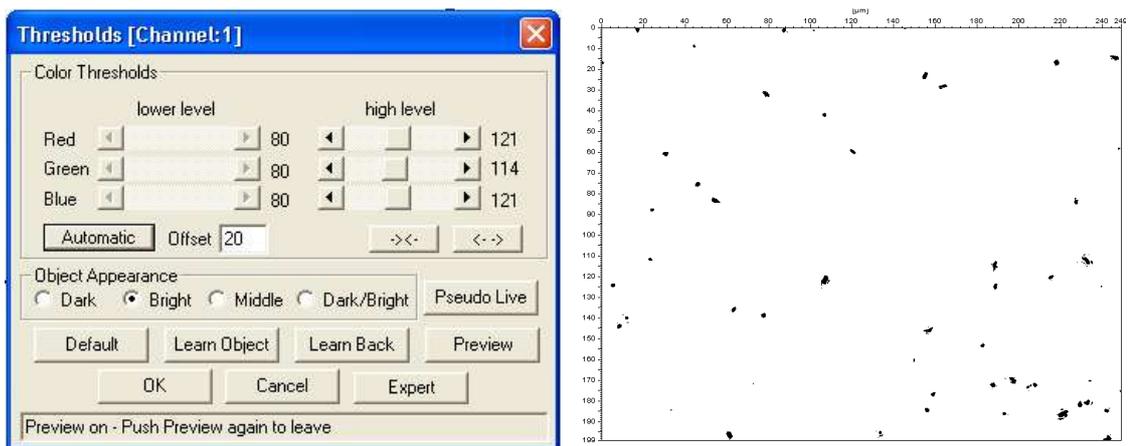
In special cases you should adjust thresholds manually:

1. Choose the **Options - Thresholds** menu command and indicate whether the **Object Appearance** is Dark (against a bright background), Bright (against a dark background), Middle (against a dark and bright background) or Dark/Bright (background is in the middle). The correct setting is important because it is the only hint for the system what objects it should search for.
2. Push the **Automatic** button to set the thresholds automatically (with the parameter Offset you may adjust the automatic threshold function (e.g. given below)
3. Push the **Preview** button to check the binarized image in the camera window. Your investigation objects should appear black on white background in this

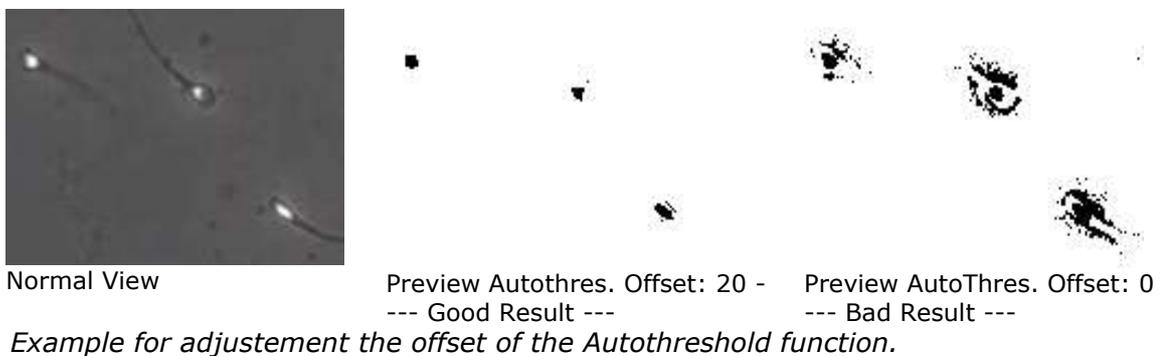
- preview mode. If necessary modify the offset value and press the **Automatic** button again.
- If Auto Threshold is not activated under **System - Options** you have to adapt the thresholds by using the scroll bars or by using the buttons for increasing and decreasing the object area.



Dialog for setting the grey and colour thresholds. The image on the right shows cells in the current grey image.



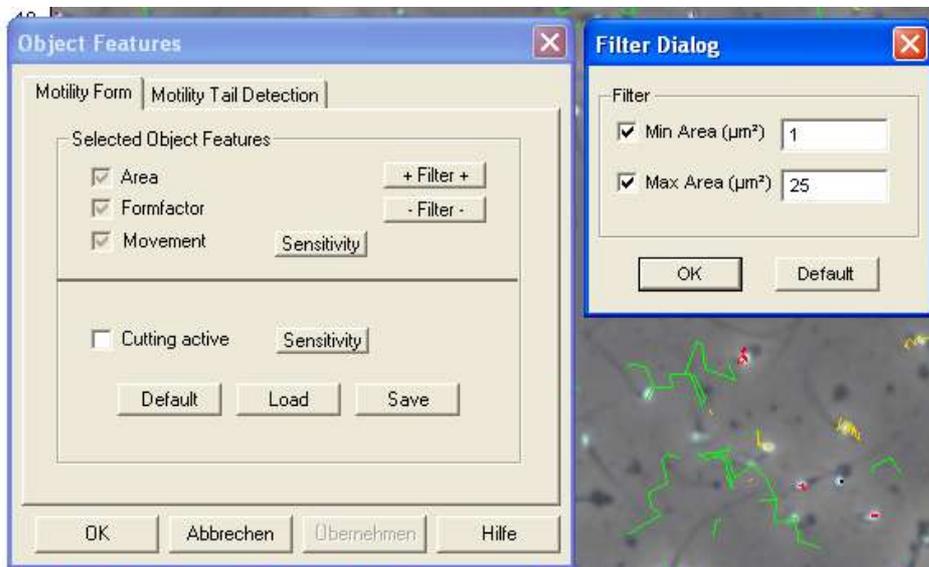
Same dialog and image in the "Preview" mode after correct setting of thresholds



Pushing the button **Pseudo Live** in the "Preview" mode shows the binary image in a live simulation.

4.2. Object Features

Object features are describing area, shape and size of investigation objects. They are calculated continuously during image processing. By this, unwanted objects can be excluded from the analysis by setting the upper and lower limits for these filters appropriately. Choose **Options - Object Features** from the menu.



Dialog for setting the Object features for a motility measurement

Description of the parameters characterizing object shape, area and size:

- Area** Object area calculated on the base of its contour. Due to the current calibration the value is converted into metrical units.
- Formfactor** Ratio calculated from object circumference and area, expressing the shape
- Movement** To distinguish a active motile object from debris the movement sensitivity is a another feature used in the system. The standard sensitivity for movement detection is 2.

By use of the **"Filter"** buttons () the upper and lower filter limit of the respective parameter can be set (Filter Dialog in the above image). Please be aware that upper and lower filter limits have to be activated separately by clicking the check boxes.

In order to get an impression of object area and shape for the correct setting of filters, you may select **Image - Count** from the menu.

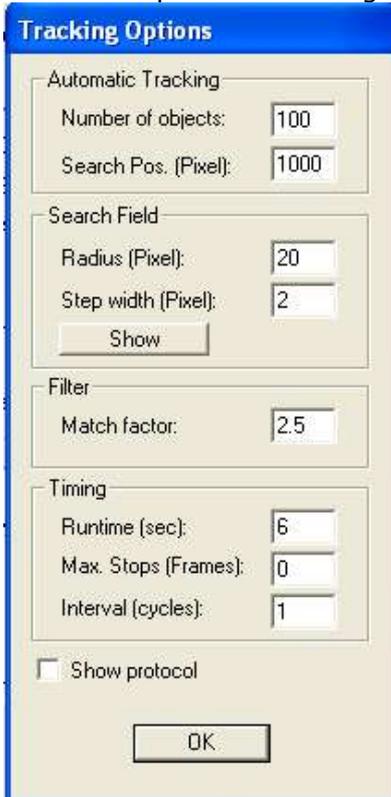
Object area and form will be listed in a table. By clicking on a table row the corresponding object in the image will be marked by a black box. Vice versa, if clicking on an object in the image the corresponding table row will be highlighted.

Check whether all objects have been identified correctly and adjust the maximum and minimum area and of the "Form" parameter filter limits if necessary.

4.3. Tracking options

Some of these settings are only used for processing speed optimization in the realtime tracking mode (Contour Tracking) when analyzing video frames directly. As a default the Contour (Scan) Tracking mode is activated i.e. video files are analyzed in a more time consuming but also more precise mode.

Choose **Options - Tracking Options** from the menu. The duration (in seconds) of the tracking measurement can be set by **Timing - Runtime (sec)**. The settings shown below have proven to be a good default:



Dialog for setting the system parameters needed for tracking.

Number of objects: to be tracked simultaneously. This value depends on the performance of your computer and should be set between 100 and 200.

Search Pos.: Number of search positions in the image where the system searches for objects using random generated positions (not used in Contour Scan Tracking mode).

Radius: of the search area used to find the new object position during tracking (not used in Contour Scan Tracking mode).

Step width: of search positions within the search area (should be smaller than object sizes). Not used in Contour Scan Tracking mode.

Button Show: makes the search field visible in the video image (not used in Contour Scan Tracking mode).

Match factor: A factor calculated during tracking to check the object parameters, e.g. to identify collisions of objects. A large match factor makes the system less sensible.

Runtime: duration of motion analysis run in seconds.

Max. Stops: The number of frames, during which an object does not move, before it is excluded from further evaluation.

Interval cycles: Allows reduction of temporal resolution by skipping of frames. Default is 1 for maximal resolution.

Show protocol: A summary of analysis is displayed after each measurement.

CAUTION: if choosing long runtime values, the motile / non-motile proportion in your sample will be biased towards motile objects which are entering the field of view while the analysis is running. A runtime of about 4 sec is recommended to minimize this effect.

4.4. Profiles

All parameters are automatically stored if you leave the program and restored on program launch.

You may also store your settings as named profiles in order to switch easily between various kinds of samples. The profile management is available under **System - Options** if the main form is closed.

The methods described here are only a small subset of the possibilities of the medeaLAB system. For more information please refer to the medeaLAB Tracking User Manual.

If you have any questions or problems with your application don't hesitate to send us images together with a short description (email address info@medelab.de or visit <http://www.medealab.de>).