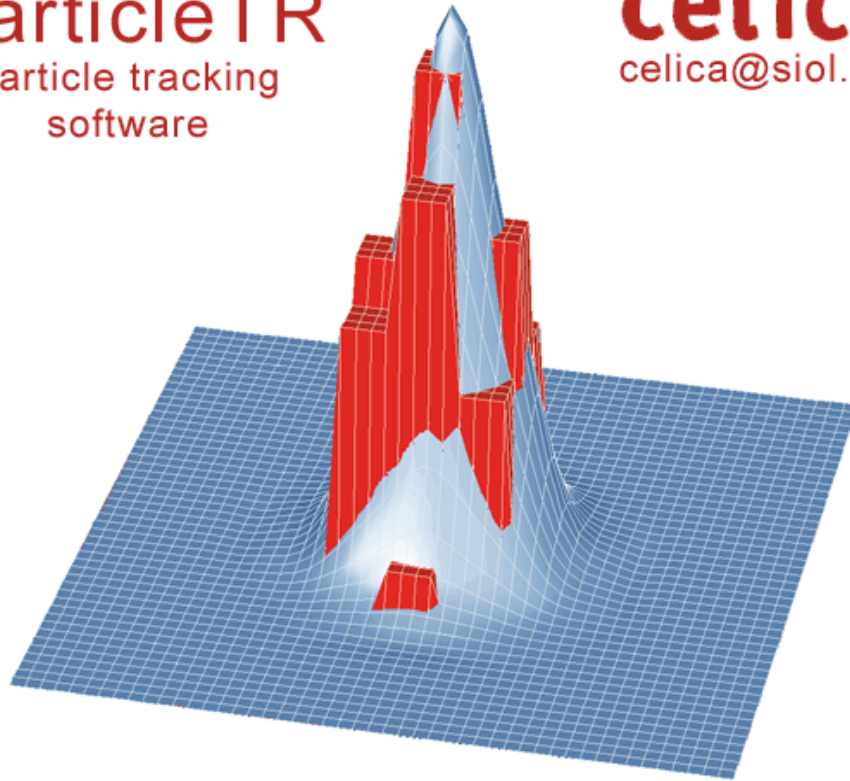


ParticleTR
particle tracking
software

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ParticleTR – Particle Tracking & Analysis Software

User's Guide

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Conditions of use

ParticleTR analysis program was developed to track moving particles in microscopy applications. However, it can also be used in any other imaging application in which the movement of any object in the image is of interest.

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1. Introduction and software installation

1.1 Introduction

ParticleTR is a particle tracking and analysis software which can be used to track moving objects in time lapse images. ParticleTR was developed primarily for tracking of moving fluorescent particles in microscopic time lapse images.

The tracking algorithm is based on fitting of a two-dimensional Gaussian curve to a specified region of the image. The peak of the Gaussian two-dimensional function is recorded as the coordinates of the particle in each frame.

ParticleTR is a menu driven program. It represents a powerful tool for fast automatic tracking of particles in time lapse images. The maximal number of particles in one image sequence is set to 50. The degree of user interaction during analysis depends on preferences of the user and on the type of images being analyzed. In images in which the same particles are present in all analyzed images and in which the paths of particles do not intercept, automatic tracking and analysis is straightforward.

ParticleTR provides tools to analyze data and visualize results in 2D and 3D line charts. The measured coordinates of a particle position as a function of time represents a data source which can be further analyzed. Software calculates several parameters for each particle, such as: the total length of the trajectory, the total motion time, the average speed, the displacement and the index of displacement/total length of the trajectory. Software also calculates parameters of each frame for all particles analyzed: current time, step length, cumulative length, squared displacement and speed.

1.1.1 Major features

- Automatic tracking of particles in a series of time lapse images.
- Verification of tracked coordinates and interactive determination of coordinates.
- Calibration of time and spatial dimensions.
- Visualization of trajectories in 2D and 3D line charts.
- Analysis of trajectories and line chart visualization of calculated parameters.
- Zoom of image series to a specified region.
- Intuitive user interface.
- Export of trajectories and calculated parameters to an ASCII file.
- Export of images together with trajectories to an AVI file.

1.2 Installing the ParticleTR software

Once you have closed all other Windows applications, you are ready to install ParticleTR.

- Insert the ParticleTR installation CD into your CD drive or open the folder with the downloaded ParticleTR software.

- Double click the "Install.exe" icon.
- Installation procedure will start and all ParticleTR program files will be copied to your hard disk. The setup program will create the C:\ ParticleTR folder. A ParticleTR program group containing the ParticleTR program icon will be created.
- Start ParticleTR by clicking the icon.

1.3 Hardware requirements

ParticleTR is designed to run on Windows NT[®], Windows 2000[®] and Windows XP[®].

To run ParticleTR you will require an IBM-PC compatible computer with at least Intel[®] Pentium III[®] class processor (higher recommended), minimum 128 MB RAM (256 MB RAM or more recommended) and an adequate hard disk capacity.

A VGA or higher resolution video interface card and monitor with at least 800x600 resolution is recommended.

2 Using ParticleTR

Quick reference

Import images

To start working with ParticleTR, first import a series of images. Click **Import images** on the **File** menu. Input the number of images to import and input the path and name of the folder where the series of images is stored. To browse for the location of the folder, click the **Browse** button. When ready, press the **Load** button. If you do not input the number of images to import, all images in the folder will be imported. The images should be in the 8-bit grayscale tagged image file format (TIFF).

Manual determination of particle position

To start tracking particles on images, you should first determine which particle you wish to track. Select the particle number in the list box in the Toolbar. Select **Manual determination of particle position** in the **Detection** menu. A crosshair will appear on the screen. With the left-mouse-click determine the position of the vesicle which you would like to track. Coordinates will be stored and the next image appears on the screen, unless this option is cleared in the **Options** window under the **Settings** menu. You can proceed manually by determining the coordinates of particles. For quicker access to **Manual determination of particle position** you can use the **Enter** shortcut key. Please note that shortcut keys are available only if the image window is active (click on the image to activate it).

Automatic detection of particle position

After you have manually determined the coordinates of the particle, you can use the algorithm for automatic detection of coordinates on the next image. Select **Automatic detection of particle position** in the **Detection** menu or use the **Right arrow** shortcut key. After coordinates have been successfully determined and stored, you can proceed to the next image by the **Up arrow** shortcut key or any other navigation tool (see the Toolbar section).

Continuous particle tracking

To automatically track and to proceed to the next image, select **Continuous particle tracking** in the **Detection** menu or use the **Double arrow** button in Toolbar. The procedure will stop at the last image in the series. If you want to stop the procedure before it finishes the tracking, release the **Double arrow** button.

Note that the speed of automatic detection depends on the type of computer processor and on the pause settings set in the **Options** window under the **Settings** menu.

Saving trajectories

When you have finished with or have discontinued the particle tracking, you can save coordinates of trajectories in an ASCII text file. This file is used for further tracking in the same set of images, for analysis by the ParticleTR software and for analysis by

any other software supporting the import of text files. To save trajectories use the **Save trajectories file** command in the **File** menu.

Analysis

ParticleTR provides tools to visualize and analyze trajectories. By selecting the **Visualize** checkbox in the Toolbar, trajectories will be superposed in the image. By pressing the **Visualize 3D** button, a 3D line chart will be opened. In addition to data with coordinates, other calculated parameters can be observed in a 2D line chart. ParticleTR estimates and displays any combination of time, step length in one frame interval, displacement from the origin and speed relative to the X- or Y-axis. Calculated data can also be saved to an ASCII text file. ParticleTR saves two files separately, one for detailed analysis for every time interval and the other for summary analysis for each particle. Please see chapter **2.2.6 Analysis Menu** for details.

2.1 Toolbar

The slider is used to select an individual image from a series of images. By clicking the Up or Down scroll arrow, the next image in series will be displayed. By clicking above or below the scroll box, software will skip nine images and display the tenth image. To scroll to a specific image, drag the scroll box, or type the number of the image in the upper text box with white background and press Enter.

Select particle No. is the list menu where one of 50 particle number can be selected. The label of the current particle corresponds to the selected particle number.

By pressing the **Assign color** button, the default color can be replaced by any other color. To apply a new color, click the color in the palette, or click **Define custom colors** for more colors. Note that the corresponding color of the current particle is also displayed in the small square in the top left corner of the image.

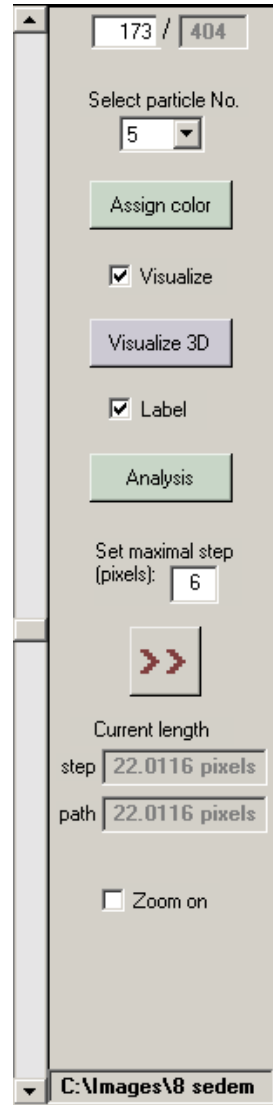
To turn the display of trajectories on the image on or off, select or clear the **Visualize** check box.

By pressing the **Visualize 3D** button, a three-dimensional line chart with trajectories will open. In the **Tools** menu click **Rotate 3D** or **Move camera** to position the chart to an appropriate perspective.

To turn the display of particle numbers in the image on or off, select or clear the **Label** check box. Note that particle numbers are displayed only for particles which are detected and have their position determined on the current image (also encircled with a corresponding color).

By pressing the **Analysis** button, a dialog box will open: "Analysis is started. Calibration has not been done. Would you like to do the calibration first?" Click "Yes" and type in the pixel dimension in micrometers and time interval of consecutive images in ms. When you press the **Calibrate** button, calibration factors will be stored and implemented. If you do not calibrate images, the unit of analysis results will be pixel instead of micrometer. A line chart window will open. Please see chapter 2.2.6 Analysis Menu for details.

In the **Maximal step** box enter the maximal expected dislocation of the particle in one frame interval. Pixels are units for this value. Eligible values are from 1 to 30, but in most cases values between 3 to 9 will allow software to successfully track individual particles. Select a lower value if many particles are in close proximity and a higher value if the expected maximal dislocation in one time interval is large. It is not possible to automatically track particles with large dislocation steps in one time interval if other particles are closer than the step size. To resolve this, one should acquire images at a higher frame rate.





Pressing the double arrowhead button starts continuous particle tracking. The position of the particle is automatically detected, marked by the circular sight and the tracking is paused for a defined period (see the Settings chapter). The procedure is repeated continuously for all consecutive images until the end of the record or until the procedure is interrupted by releasing the button. Note that the continuous particle tracking procedure can operate only when the position of a particle in the preceding image is determined. The procedure is stopped in case the particle gets close to the border of the image (closer than the maximal step setting).

Current length of step or path in specified units is displayed in two separate text boxes. Step length is the distance of particle movement in one time interval. Path length is the cumulative length of the particle's travel from the start to the time point of the current image.

If the **Zoom on** check box is checked, dragging with the left mouse button pressed will zoom the image to the specified rectangle. Uncheck the box to proceed with the particle tracking and analysis.

2.2 Menus

Menus are also accessible via keyboard commands as follows:

- File (alt-F)
- Edit (alt-E)
- View (alt-V)
- Track (alt-T)
- Detection (alt-D)
- Analysis (alt-A)
- Settings (alt-S)
- Help (alt-H)

2.2.1 File Menu

Import images

Click **Import images** in the **File** menu to import a series of images. Input the number of images to import and input the path and name of the folder where the series of images is stored. To browse for the location of the folder, click the **Browse** button. When ready, press the **Load** button. If you entered the number of images which is lower than the number of all available images, you will be prompted: "Number of images in this directory is larger than the number of selected images. Do you want to input all images?" If you do not input the number of images to import, all images in the folder will be imported.

The images should be in the 8-bit grayscale tagged image file format (TIFF). Only one series of images should be stored in one directory.

Load trajectories

If you previously analyzed the same set of images and saved the results to a trajectory file, you can open the saved trajectories and continue to track particles or to analyze results. To successfully load trajectories, you should not modify the

trajectories file by using any other software.

Save trajectories file

After tracking particles, you can save coordinates of all particles in all images in the ASCII text file by using the **Save trajectories file** option in the **File** menu. Data is organized in column pairs for x and y positions of the particle in the image. The origin of the coordinates is the top left corner of the image. First column of the pair in the file represents the horizontal distance of the particle and the second column of the pair represents the vertical distance of the particle. Units are pixels. All coordinates of separate images are organized in separate rows.

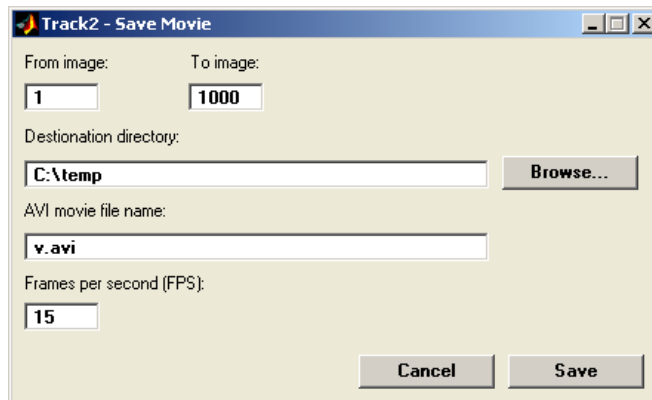
Data can be imported in any numerical or text editing software which supports import of ASCII text files.

Save trajectories file as

If you want to save trajectories file under a different file name or to save them to a different location on your storage device, you can use the **Save trajectories file as** option in the **File** menu.

Export AVI

A sequence of images together with their trajectories can be exported into the AVI video file format. After selecting the **Export AVI** option, a new window is opened in which you can set the time interval of the movie (the first and last images of the movie), destination directory and filename under which the AVI file will be stored, and the frame rate (speed) of playback of the movie.



Exit

Closes the ParticleTR program.

2.2.2 Edit Menu

Copy all trajectories

Copies all coordinates of all particles in all images in Windows clipboard as ASCII text. Data is organized in column pairs for x and y positions of the particle in the

image. The origin of the coordinates is the upper left corner of the image. The first column of the pair in the file represents the horizontal distance of the particle and the second column of the pair represents the vertical distance of the particle. Units are pixels. All coordinates of separate images are organized in separate rows. Data can be pasted into any numerical or text editing software. In frame positions where coordinates of a certain particle are not determined, the “NaN” sign is inserted.

Copy current trajectory

Copies coordinates of the current particle. See “**Copy all trajectories**” chapter for details.

2.2.3 View Menu

View/hide labels

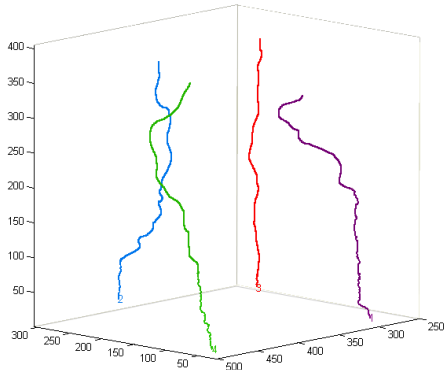
Toggles display of numerical labels of tracked particles. Labels appear next to the center of the particle.

View/hide trajectories

Toggles the 2D display of all trajectories (path) on/off in all frames analyzed.

View 3D trajectories

Opens a separate window with 3D display of all trajectories.



Show/hide step length

Toggles display of the current step length in one frame interval on/off. In noncalibrated data the unit is pixel, in calibrated data the unit is μm . The step length is calculated as the displacement of the particle from its position in the preceding frame.

Show/hide path length

Toggles display of cumulative length of the trajectory in the current frame on/off. In noncalibrated data the unit is pixel, in calibrated data the unit is micrometer. The path length is calculated as the cumulative trajectory length of the particle in all preceding frames.

2.2.4 Track Menu

Clear current position

Use this option to clear coordinates of the particle in the current time frame. You can also use the **Left arrow** shortcut key.

Clear current trajectory

Use this option to clear coordinates of the current particle in all time frames. This will entirely remove the trajectory of the current particle.

Clear all

Use this option to clear coordinates of all particles in all time frames.

Next frame

Use this option to proceed to the next time frame. You can also use the **Up arrow** shortcut key or click up the scroll arrow on the slider.

Previous frame

Use this option to go to the previous time frame. You can also use the **Down arrow** shortcut key or click the down scroll arrow on the slider.

Play

Use this option to present all time frames as a movie. Press **Esc** key to stop the play.

2.2.5 Detection Menu

Manual determination of particle position

When **Manual determination of particle position** option is selected, or **Enter** shortcut key is pressed, a crosshair will appear on the screen. With the left-mouse-click manually point to the position of the vesicle which you would like to track. Coordinates of the particle in the current frame are stored. Please note that the **Enter** shortcut key is available only if the image window is active (click on the image to activate it).

Automatic detection of particle position

Select the **Automatic detection of particle position** function or use the **Right arrow** shortcut key to activate the algorithm for automatic detection of coordinates. Please note that the shortcut keys are available only if the image window is active (click on the image to activate it). The algorithm for automatic detection is functional only if coordinates of the current particle are determined in the preceding image. If not, a dialog box will open: "Please, manually determine the particle position in the preceding image."

Continuous particle tracking

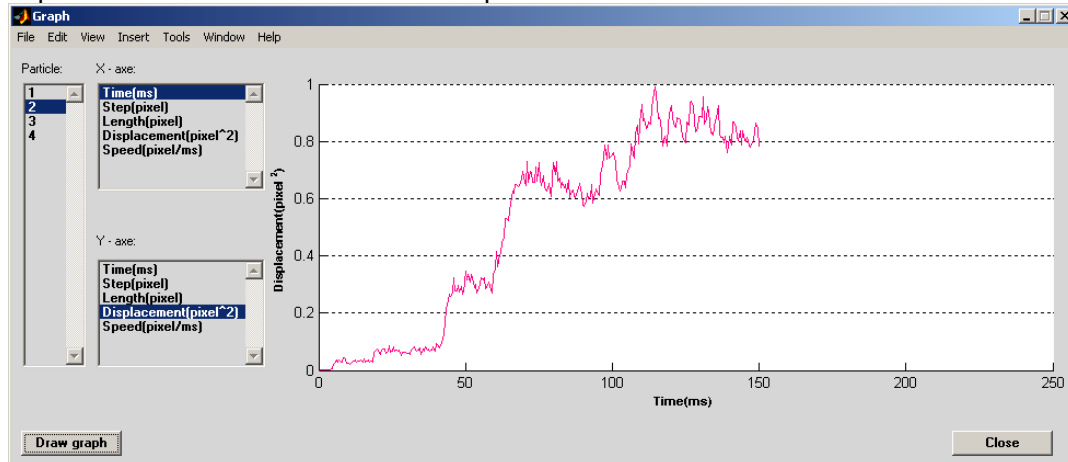
To automatically track and proceed to the next image select the **Continuous particle tracking** function or use the **Double arrow** button in Toolbar. The procedure will stop at the last image in the series. If you want to stop the procedure before it finishes the tracking, release the **Double arrow** button. The algorithm for automatic detection is functional only if coordinates of the current particle are determined in the preceding image. If not, a dialog box will open: "Please, manually determine the particle position on the preceding image."

Note that the speed of automatic detection depends on the type of computer processor and on the pause settings set in the **Options** window under the **Settings** menu.

2.2.6 Analysis Menu

Show analysis results

When the **Show analysis results** option in the **Analysis Menu** is selected, a separate window with a line chart will open:



You can choose any combination of variables to be displayed on x and y axes for any particle (time, step length, total length, squared displacement or speed). If trajectories are calibrated, results are displayed in micrometers, if trajectories are not calibrated, results are displayed in pixels.

Save all analysis results

Use this option to save all analysis results in two separate ASCII text files. One file contains the summary of analysis for each particle. Data is organized in six columns as follows: number of the particle, total length of the trajectory, total motion time, average speed, displacement and index of displacement/total length of the trajectory. The second file with a prefix "detail_" contains analysis of each frame for all particles analyzed. Data is organized in columns where the first column is frame number, followed by 50 clusters of five columns. Each cluster represents one particle. Columns in a cluster represent time, step length, cumulative length, squared displacement and speed. If trajectories are calibrated, results are saved in micrometers, if trajectories are not calibrated, results are saved in pixels.

Save detailed analysis results

Use this option to save the file which contains analysis of each frame for all particles analyzed. Data is organized in columns where the first column is the frame number, followed by 50 clusters of five columns. Each cluster represents one particle. Columns in a cluster represent time, step length, cumulative length, squared displacement and speed, respectively.

Save analysis summary

Use this option to save the file which contains the summary of analysis for each particle. Data is organized in six columns as follows: number of the particle, total length of the trajectory, total motion time, average speed, displacement and index of displacement/total length of the trajectory.

2.2.7 Settings Menu

Calibrate (manual)

Use this option to manually calibrate the time interval and pixel dimensions, and enter appropriate scale factors.

Calibrate (automatic)

If images contain a scale bar or objects of known dimensions, you can use the automatic calibration procedure. After selecting the **Calibrate (automatic)** option, first click with the left mouse button to one side of the object and then to the other. Input the size and the unit of the object and click the **Calibrate** button. Pixel dimensions are calculated and saved.

Clear calibration

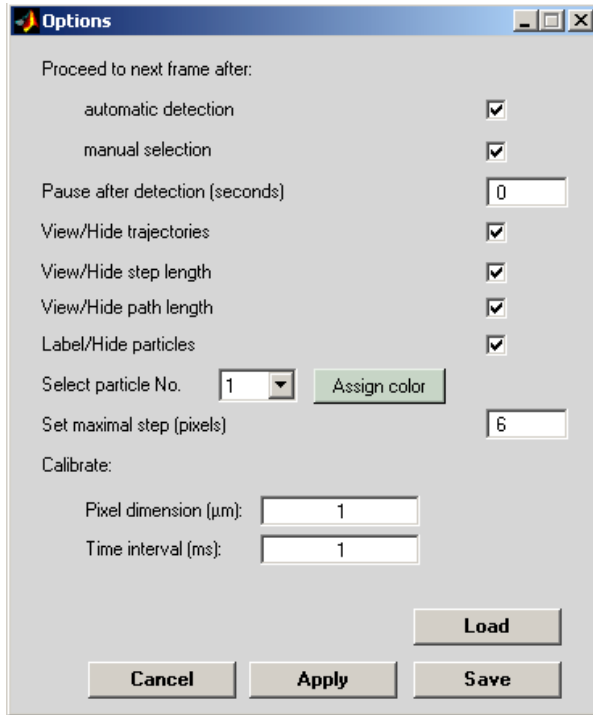
Use this option to clear calibration factors and to reset pixel dimensions units from micrometers to pixels.

Assign color

Use this option to assign a color to the current particle. Numerical particle label, the circular sight, trajectory and the corresponding line chart will all be displayed in the selected color. If no color is selected, a default color is assigned for each particle.

Options

In the Options control panel you can set, save or load several custom settings for particle detection and analysis:



Check the checkbox **Proceed to next frame after automatic detection** or **Proceed to next frame after manual selection** if you do not need to verify coordinates after every detection or selection. If these checkboxes are not checked, you can proceed to the next frame manually by pressing the **Up arrow** key. When you track particles by using the Continuous particle tracking, you may need extra time to verify coordinates in each frame. To increase the frame interval during continuous particle tracking, enter any value in the textbox **Pause after detection** (in seconds). You can also set to view or hide trajectories on the image, view or hide step and path length. Step length is a distance of particle movement in one time interval. Path length is the cumulative length travelled by the particle from the start to the time point of the current image.

The **View/hide labels** checkbox toggles display of numerical labels of tracked particles. Labels appear next to the center of the particle.

Use the **Assign color** button to assign color to any particle. Numerical particle label, the circular sight, trajectory and corresponding line chart will all be displayed in the selected color.

In the **Set maximal step** box enter the maximal expected dislocation of the particle in one frame interval. The unit for this value is pixel. Eligible values are from 1 to 30, but in most cases values between 3 to 9 will allow software to successfully track individual particles. Select a lower value if many particles are in close proximity and a higher value if the expected maximal dislocation in one time interval is large. To calibrate the time interval and pixel dimensions, use this option and enter appropriate scale factors.

2.2.8 Help Menu

The Help menu provides access to the ParticleTR Help files.

Frequently asked questions

When I press the double arrowhead button, it gets released without particle tracking. Why?

The continuous particle tracking procedure can operate only when the position of the particle in the preceding image is determined. Check if the position of the particle is determined in the preceding image. If it is, than the position may be too close to the margin of the image (closer than the maximal step setting).

The Enter shortcut key does not enable manual selection of particle coordinates.

The Enter, Right arrow and Left arrow shortcut keys are active only if an image window is selected (active). To select an image window, click with the left mouse button into the image. If the cursor is in any other window, slider or textbox, shortcut keys are not functional.

When I use the Play option, I am unable to stop playback of the time series.

To stop playback, use the Esc key on keyboard.

Why does the small square at the top left of the image window change color when I change the particle number?

The square is an aid for easier identification of an active particle. Each particle is characterized by a unique number and color. The color of the square matches the color of the numerical label and the circular sight.