

**ParticleCO**  
**Particle Counting Software**  
User's Guide

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## Conditions of use

**ParticleCO** analysis program was developed to count particles in microscopy applications. However, it can also be used in any other imaging application in which the counting of particles in the stack of images is of interest.

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# 1. Introduction and software installation

## 1.1 Introduction

ParticleCO is a particle counting software which can be used to count objects in three dimensional stacks of images. ParticleCO program was developed to count particles in microscopy applications. However, it can also be used in any other imaging application in which the counting of particles in the stack of images is of interest.

ParticleCO is a menu driven program. It represents a powerful aid for rapid counting of particles in images.

ParticleCO provides tools to visualize results in 3D charts. The selected coordinates of a particle position can be visualized.

### 1.1.1 Major features

- Manual counting of up to three different particle types in a 3D stack of images.
- Verification of particle coordinates and interactive correction of coordinates.
- Visualization of positions in 2D and 3D charts.
- Zoom of image series to a specified region.
- Intuitive user interface.
- Export of coordinates to an ASCII file.
- Selected visualization of one, two or three channels of RGB color image.
- Export of images together with marked positions to an AVI file.

## 1.2 Installing the ParticleCO software

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

- Insert the ParticleCO installation CD into your CD drive or open the folder with the downloaded ParticleCO software.
- Double click the "Install.exe" icon.
- Installation procedure will start and all ParticleCO program files will be copied to your hard disk. The setup program will create the C:\ ParticleCO folder. A ParticleCO program group containing the ParticleCO program icon will be created.
- Start ParticleCO by clicking the icon.

## 1.3 Hardware requirements

ParticleCO is designed to run on Windows NT<sup>®</sup>, Windows 2000<sup>®</sup> and Windows XP<sup>®</sup>. To run ParticleCO you will require an IBM-PC compatible computer with at least Intel<sup>®</sup> Pentium III<sup>®</sup> 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 ParticleCO

### Quick reference

#### *Import images*

To start working with ParticleCO, 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 24-bit RGB color tagged image file format (TIFF).

#### *Manual determination of particle position*

To start counting particles on images, use the **Enter** key. A crosshair will appear on the screen. With the left-mouse-click determine the position of the particle. Coordinates will be stored and the circular mark will appear on the image. You can reposition the circular mark by pressing Enter key again. To mark next particle, press **Right arrow** key on the keyboard. The particle number on the display will be automatically increased by one and a crosshair will appear. Please note that key commands are available only if the image window is active (click on the image to activate it). **Up Arrow** will proceed to next image in the stack. Marks of earlier marked particle positions will remain on the screen, but will be dimmer.

#### *Saving trajectories*

When you have finished with or have discontinued the particle counting, you can save coordinates of particle positions in an ASCII text file. This file is used for further counting in the same set of images, and for analysis by any other software supporting the import of text files. To save trajectories use the **Save positions file** command in the **File** menu.

#### *Viewing 3D positions of particles*

ParticleCO provides tools to visualize particle positions. By pressing the **Visualize 3D** button, a 3D chart will be opened. Please see chapter **2.2.6 View 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 edit menu where any particle number can be selected. The label of the current particle corresponds to the entered particle number.

**Red particle**, **Green particle** and **Yellow particle** radio button will assign color to particles. This may be used to distinguish up to three different particle types.

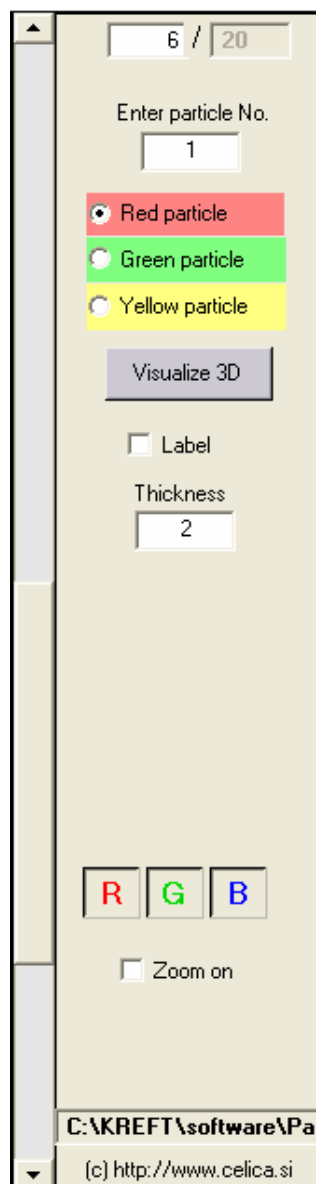
By pressing the **Visualize 3D** button, a three-dimensional chart with particle positions will open. In the **Tools** menu click **Rotate 3D** or **Move camera** to position the chart to an appropriate perspective.

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 for all particles which are selected and have their position determined on any image.

Use **Thickness** text box to enter the number of adjacent images, where dimmer circular marks will be visible. This tool prevents multiple markings of the same particle, which may be visible in adjacent images. In this text box enter the thickness of particles measured as the number of consecutive images in 3D stack.

**R**, **G** and **B** buttons enable turning on and off each color channel in the 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)
- Mark (alt-M)
- 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 24-bit RGB color tagged image file format (TIFF). Only one series of images should be stored in one directory.

#### *Load positions*

If you previously analyzed the same set of images and saved the results to a positions file, you can open the saved positions and continue to count particles or to analyze results. To successfully load positions, you should not modify the positions file by using any other software.

#### *Save positions file*

After counting particles, you can save coordinates of all particles in all images in the ASCII text file by using the **Save positions file** option in the **File** menu. The first column is the particle number. The coordinate data is organized in the next three columns as triplets for x, y and z positions of the particle in the image. The origin of the coordinates is the top left corner of the image. The second column in the file represents the horizontal distance (x) of the particle, the third column represents the vertical distance (y) of the particle and the fourth column represents the number of the image or the z position. Units are pixels.

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

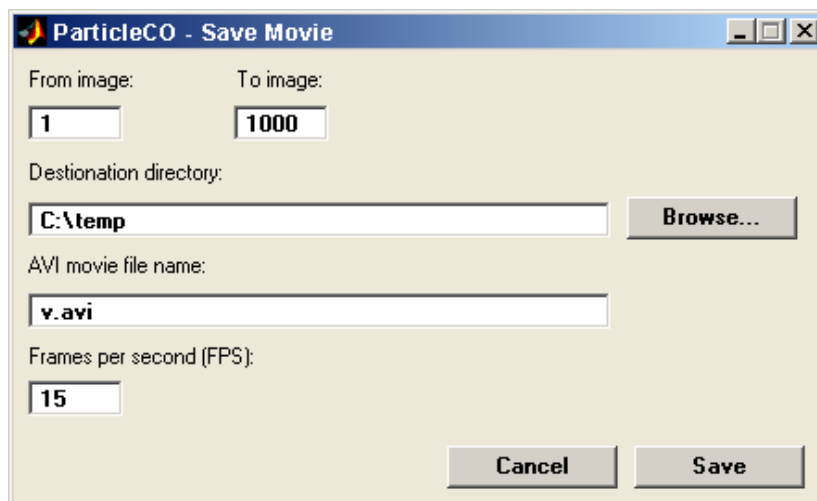
#### *Save positions file as*

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 positions 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 ParticleCO program.

## 2.2.2 Edit Menu

### Copy all positions

Copies all coordinates of all particles in all images in Windows clipboard as ASCII text. The first column is the particle number. The coordinate data is organized in the next three columns as triplets for x, y and z positions of the particle in the image. The origin of the coordinates is the top left corner of the image. The second column in the file represents the horizontal distance (x) of the particle, the third column represents the vertical distance (y) of the particle and the fourth column represents the number of the image or the z position. Units are pixels.

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.

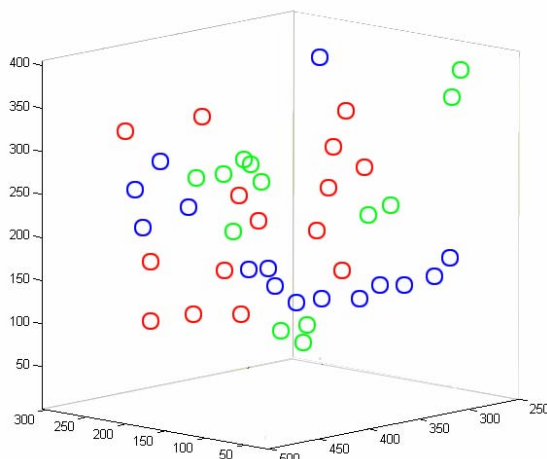
## 2.2.3 View Menu

### *View/hide labels*

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

### *View 3D positions*

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



## 2.2.4 Mark Menu

### *Clear current position*

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

### *Clear all*

Use this option to clear coordinates of all particles in all images.

### *Next frame*

Use this option to proceed to the next image 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 image 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 an on screen movie. Press **Esc** key to stop the play.

## 2.2.8 Help Menu

The Help menu provides access to the ParticleCO Help files.

## Frequently asked questions

*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 image display.*

To stop playback, use the Esc key on keyboard.

*How can I change the position of one individual particle?*

To change the position of an individual particle first check Label checkbox to identify the particle. Then enter the particle number into the Enter particle No. edit box, than press Enter and click with the left mouse button to the new position.