

CellBuster 1.0.3

User Manual

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REVISION HISTORY

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1 Introduction

1.1 What is cellbuster?

Cellbuster is a software created by Andrea Giacosi to analyse cell movement. Chemotaxis experiments are performed with a Dunn's chamber and migrating cells are observed using a time-lapse microscope. This program written for Microsoft Windows calculates the exact position of a cell through a stack of images. It also calculates distance direction and speed of a cell from the start position.

Cell tracking can be done manually or automatically. In the first case you have to follow a cell with your mousepointer and the position that is recorded is the one indicated through the mouse. In the second case, you have to select a filter and click on the first frame on the cells you want to track. The software will automatically calculate the pathways of the cells you have indicated.

1.2 CellBusterWizard

Starting from this version the CellBuster is distributed with an additional utility that helps the user in the creation of the CellBuster project: this utility is called CellBuster Wizard. This application has been developed using the .Net framework and so it is required to install it in order to use.

1.3 Technical support

For any problem regarding the use of this software contact Andrea Giacosi ([gjacos@libero.it](mailto:gjacosi@libero.it)).

2 Procedures

2.1 Using CellBuster Wizard

In order to create from scratch a new CellBuster project you can launch the CellBusterWizard from your StartMenu/JOK/CellBuster.

The wizard is composed of 5 steps:

- Welcome
- General Info
- Select Images
- Order images and select size
- Generate the project

The output of the Wizard is a folder containing all the images and the additional information required by the CellBuster in order to properly work.

The user can jump from one wizard step to the next/previous using the Prev and Next button on the bottom of each step.

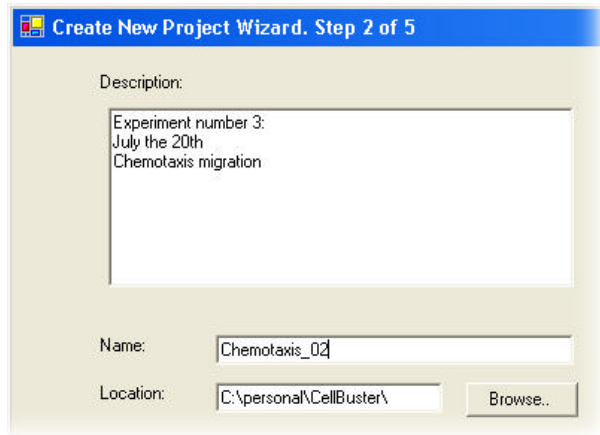


2.1.1 General Info Step

In the Description area you can write any information helps you to describe your experiment.

The name text field will be the name of the folder that will contain all the data describing the CellBuster project we are making.

The location is the parent folder that will contain the project folder we are creating: the Browse button can be used to easily select the folder without typing it.



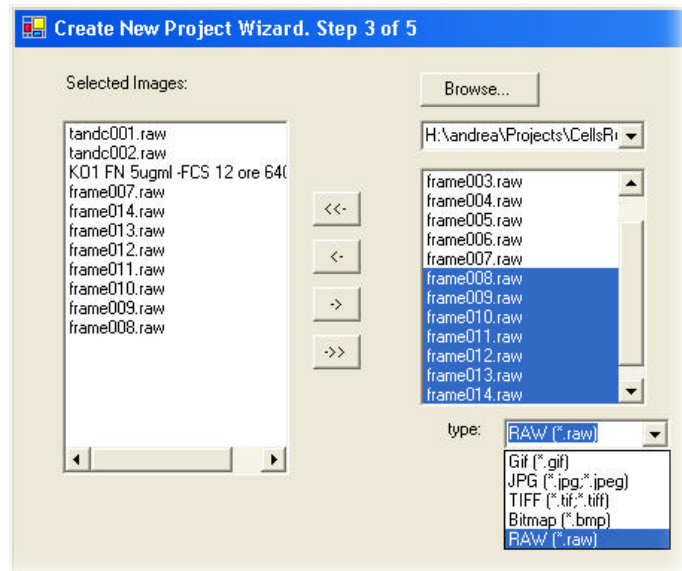
2.1.2 Select Image step

In this step you select all the images that you want to analyze with CellBuster.

On the left panel you will see the file you have selected and are currently part of the project. On the right panel you see the files available in the folder you have selected using the Browse button.

On the right panel you select one or more images and you add to the selected images using the arrow buttons in the middle.

To add to the selected images list use the first two buttons. To remove images from the selected images list you have to use the other two buttons.



You can browse for your image anywhere, the selected images are automatically copied to the project folder.

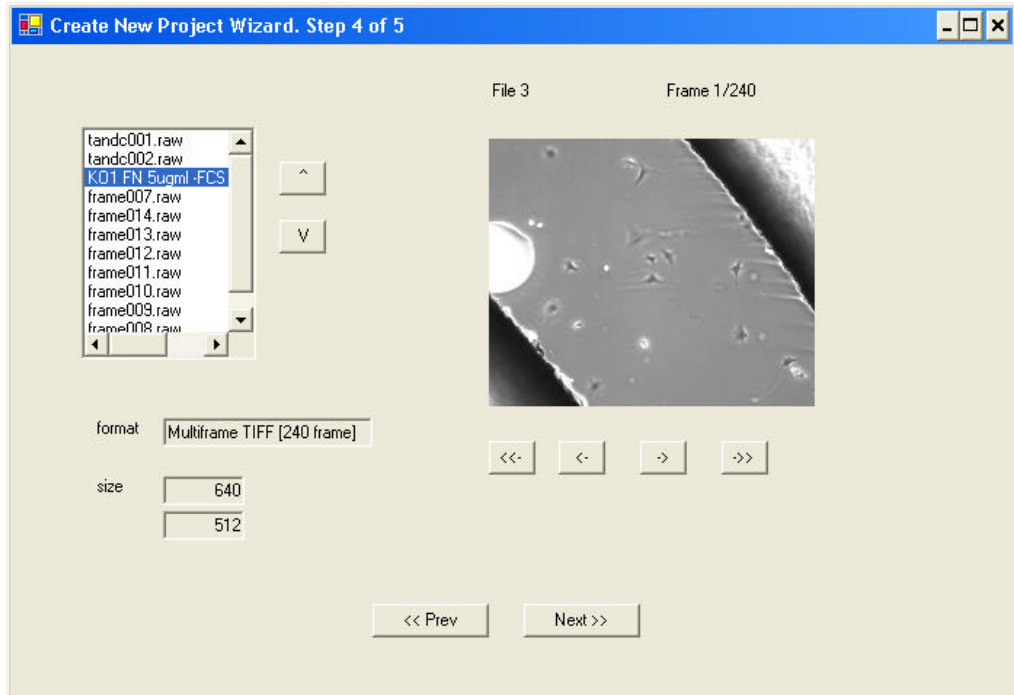
The supported image formats are GIF/JPEG/TIFF(also multiframe)/Bitmap/RAW. A project can contain images of different formats.

2.1.3 Order Images and select Size Step

In this step you can perform several actions:

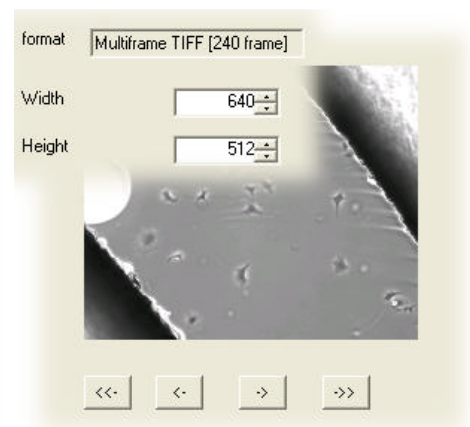
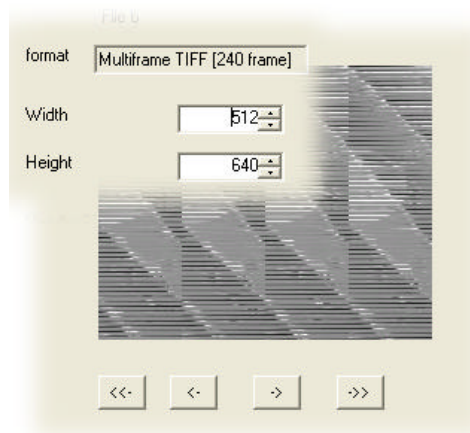
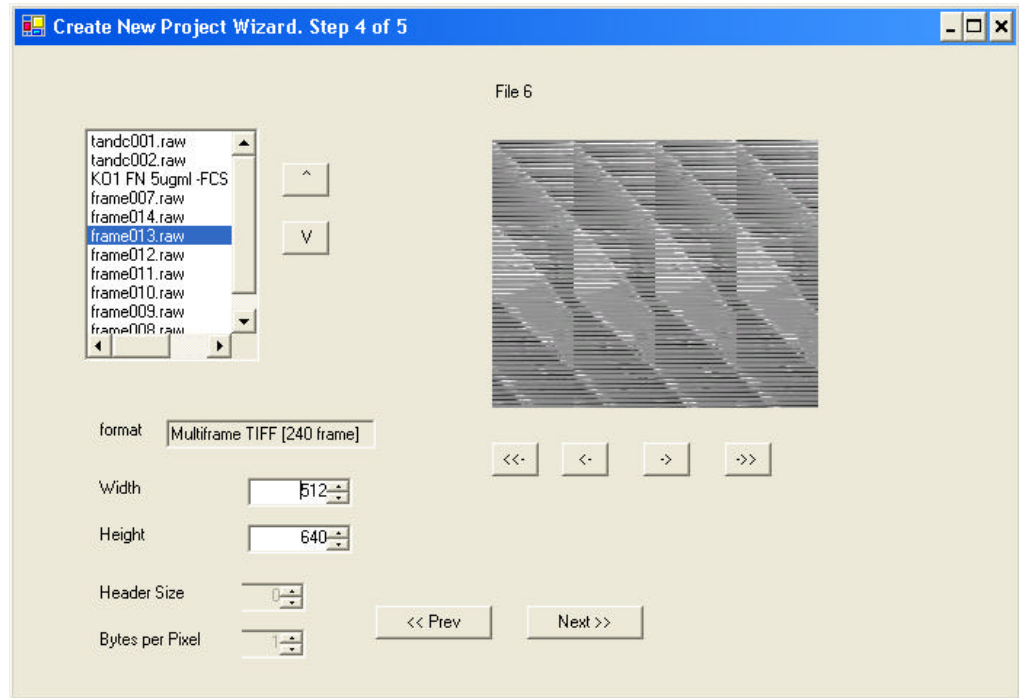
- Preview your images
- Select the size of the RAW images
- You can re-order the images.

The image reordering is performed selecting one image and pressing the up/down(^V) button until you reach the desired position (if in the previous step you select the images in the desired order you can save this step). If a file is a multiframe image, like a tiff image, you cannot change the internal order of the frames (to reorder frames in a multiframe image you have to use an external tool).



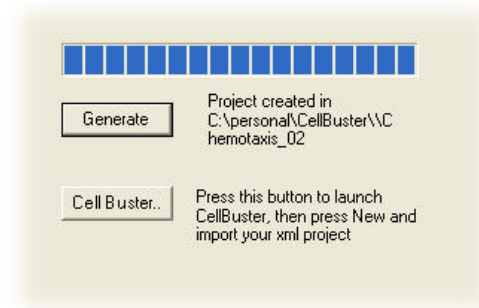
When you click on an image a preview appears on the right side of the form. If the file is a multiframe image you can use the two (<- and ->) button to move between the frame. The two <<- and ->> button can be used to move to the next or previous file.

In the bottom/left area of the form there are information about the format and the size of the image file. This area is important especially for RAW image files where the guessed size could not be the right one: you can change the width and the height of the image until you see in the preview area an image that looks to you familiar. Regarding the RAW format this version of CellBuster Wizard can handle only RAW files that have 0-header size and 1 byte per pixel (256 gray-level)

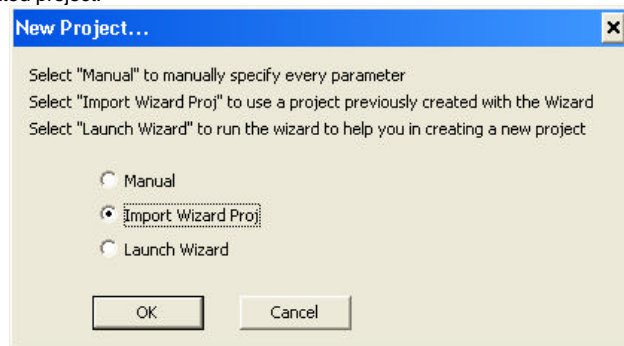


2.1.4 Generate the project

In this last step pressing the Generate button you generate the project. The Wizard verifies that all the images have the same frame, that all the images are readable and copy everything in the project folder, it also create a raw copy of the images selected and a file named "proj.cbw".



If some problem occurs you can step back and try to fix it, when everything is OK you can launch CellBuster and open the "proj.cbw": pressing CellBuster button the wizard is closed and CellBuster is launched, then you have to go to File/New menu and select *Import Proj Wizard*. See next section to see how to open the created project.



2.2 Using CellBuster application

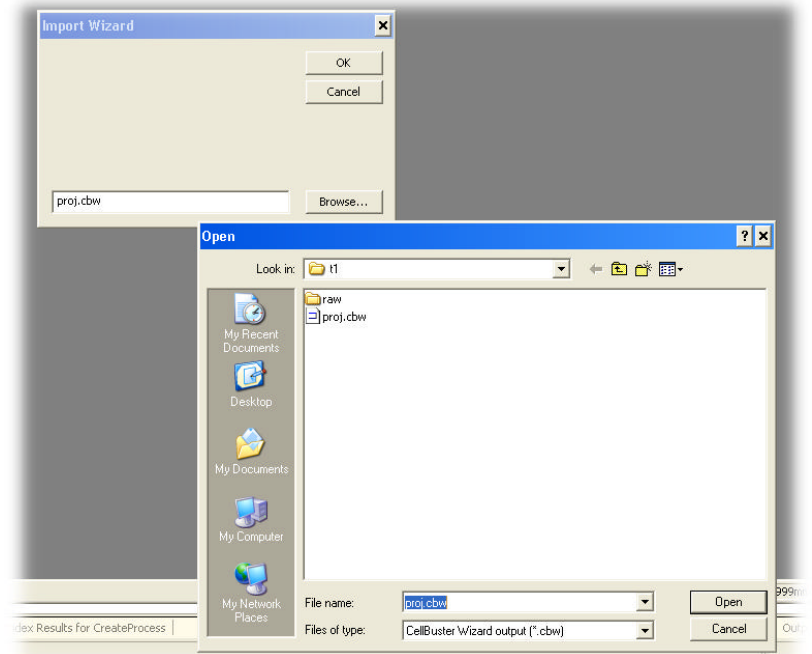
The application CellBuster can be used to open or create a CellBuster project, find cell movements and save result in terms of tracks on the screen and/or data to be analyzed with Excel.

CellBuster is not very user friendly (there is no help) and also the only available image format is RAW one: this is why I developed the CellBusterWizard.

To create a new CellBuster project you use the new button on the toolbar or you go to the File/New menu, then you have to select between three possible modes:

- **Manual** to manually create the project
- **Import Wizard Proj** to import a project created with the CellBusterWizard
- **Launch Wizard** to launch the CellBuster wizard and create an empty project

The **Import Wizard Proj** mode is the easiest way to start a new project: you have only to browse for the file proj.cbw you previously created and press OK.



After you have imported the Wizard created project you can work on it creating cell tracks as explained in the remaining part of this document.

When you have finished with your work you can save all your work in a CellBuster project (extension is TRK): you should save your project in the same folder you created with the Wizard.

Remember that is you want to restart later your work you have to open the CellBuster project (using File/Open) and not import again the proj.cbw: the proj.cbw does not contain tracks info and everything you changed after the import. You can have more than one cellbuster file (analysis1.trk, analysis2.trk, ...) in the same project folder that they can differ from tracks and analysis performed on the original "empty" project (only images) imported. You can also restart from the "empty" project re-importing the proj.cbw file.

How to create manually a project is described in the next chapter.

2.3 Create a new CellBuster project

To create a new file, choose the *file/new* command from the main menu. A dialog box will be displayed. You have to fill this box indicating:

- the length in pixel of the bridge where you can see the cells
- width and height in pixel of your frames
- time in minutes between two frames
- animation speed
- number of the last frame of the stack
- number of digits
- the folder containing your stack.

At this point press the command *create* and you will visualize your movie. You can move through the sequence with the arrows commands or using the arrows on your keyboard. Otherwise you can see the entire movie pressing the *film icon* on the toolbar. To change the animation speed press the *As* command on the toolbar.

2.4 File format

The movie should be composed of a stack of images saved as separate files. Each file should be any size but it needs to be grayscale 8 bit per pixel color depth (256 gray level). The files should be saved in raw format. You can use Paint Shop Pro to convert your stack of images into the raw format.

2.5 Analyse a new file

2.5.1 Create axis

If your images are not in the correct orientation you can insert axes so that the source of chemoattractant is in the direction of one of the two axis. The software calculates the positions of your cells respect to the axes you have drawn. To rotate the axes press on the *blue* button on the toolbar and adjust axes orientation with the mouse. Otherwise select the *axes/edit* command on the main menu.

2.5.2 Create cell tracks

To follow the track of a cell you have two options: a manual acquisition mode and an automatic acquisition mode.

2.5.3 Manual acquisition mode

Press on the *noose* command on the toolbar: a fore-sight will appear. Point it on the cell you are going to follow and click on the left button of your mouse. Keep the button pressed through all the sequence until the end. When you release the mouse button the movie will go on, but acquisition will stop. You can visualize the cell track by pressing on the *single line icon* on the toolbar. A number is assigned to this track and you can visualize it by pressing on the *1, 2, , icon*.

This kind of acquisition let you to know the position of your mouse pointer in each frame. If a cell is moving very fast and you can not follow it in this way even changing the animation speed, you can use the *click by click* mode. In this case you visualize just one frame at a time and you can go to the next frame once you have clicked on the cell you want to track.

2.5.4 Automatic acquisition mode

For this kind of acquisition you have to select a filter to distinguish a cell from the background. At the moment the filter that best approximates a cell is the threshold. Once you have set the threshold select the cells you are going to follow and the software will calculate their pathways through all the sequence.

2.5.5 Select a filter

Select the *threshold* filter from the *tool/select filter* command. To visualize the image with the threshold you have created, press on the *cell icon* on the toolbar. You can adjust the threshold through the *tool/filter option* on the main menu. Choose a threshold as stringent as possible. On the dialog box you will see just a part of the image but using the mouse you can move on different part of it. Press the arrows button to see how the cell looks like through all the sequence.

2.5.6 Create an automatic track

Press on the *1pt icon* on the toolbar and click on the cell you are going to follow just on the first frame. Select the *tool/refine track* command from the main menu. At this point the software will follow automatically the cell and a track will be created. The position that is recorded represents the center of the cell area that is over the threshold in each frame. You can also set some parameters in order to help the software to recognize and follow a cell. Press the *tools/refiner option* command from the main menu. A dialog box will be displayed. You can indicate the minimum and maximum area of the cell in pixel and the maximum shift of the cell center between two following frames (search zone radius).

You can analyse one cell at a time or many cells simultaneously. In this case after pressing the *1 pt icon* select all the cells you are going to follow.

2.5.7 Visualize a cell track

To visualize a cell track, click on one cell and press the *single line* icon on the toolbar. Otherwise press the right button of the mouse and select the *show track* command. To visualize all tracks on the movie select the *three lines* icon from the toolbar. Automatically created tracks are in blue while the others are in red. Selected track appears in green. Cell tracks are also recorded on the tracks editor. Select the *tool/tracks editor* command from the main menu to show it.

2.5.8 Visualize cell numbers

There is a number for each cell track. You can show them by pressing the *1, 2, 3 icon* on the toolbar. Automatically created tracks are numbered as P-1, P-2, P-3. Otherwise you can show cell numbers pressing the right button of the mouse and selecting *show numbers*.

2.5.9 Export data file

At the end of your analysis you can export the x, y coordinates of a cell in each frame into an excel file. Select the *file/export* command from the main menu and a dialog box will be displayed. Select the data you want to export:

- Cell position relative to the axes you have drawn
- Cell position relative to the origin, setting the starting point as (0, 0) coordinates
- Cell speed calculated on the shift of the cell center between two following frames.
- Vector distance, module and direction of the displacement of a cell from the starting position
- Straightened distance, total length of the pathway of a cell
- Average speed (module and direction) calculated on the vector distance

Indicate a file name and four different file will be created.