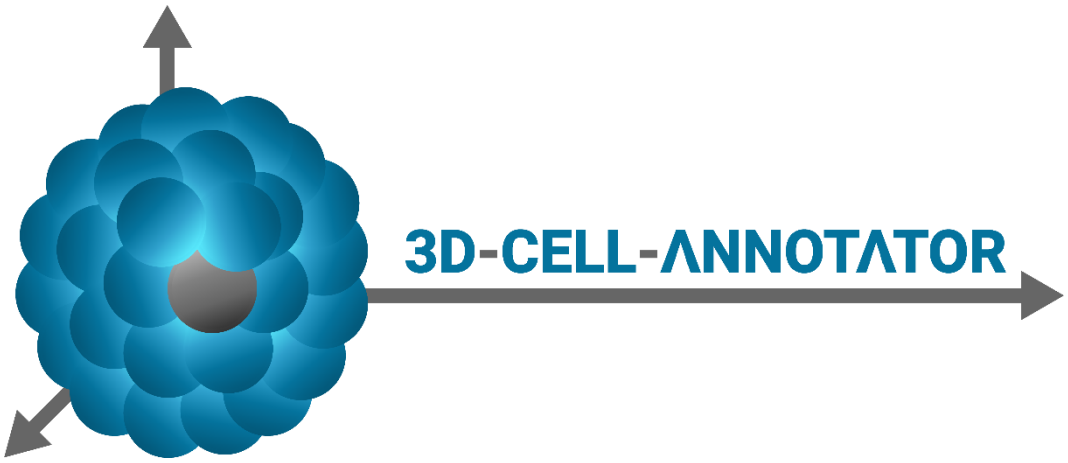
3D-Cell-Annotator

USER MANUAL

www.3d-cell-annotator.org



v1.0 - May 2019

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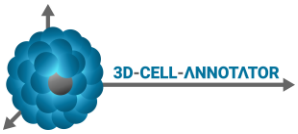

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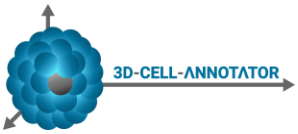

1. BRIEF DESCRIPTION

3D-Cell-Annotator is a freely available software tool for segmenting single cells and nuclei in 3D, starting from a 3D dataset typically acquired with confocal, multi-photon or light-sheet fluorescent microscopes. It uses 3D active contours with shape descriptors as prior information for true single cell annotation in a fully and semi-automatic fashion.

3D-Cell-Annotator is mainly developed at the Biological Research Centre of the Hungarian Academy of Sciences in Szeged, Hungary. Results show that running the software both in fully- and semi-automatic mode on single cells, the segmentation precision reaches the level of a human expert.

This document is a short help tutorial to describe the main functions of 3D-Cell-Annotator. It is written for non-experts. Additional information, video tutorials, source code, and literature references are available at: www.3D-cell-annotator.org

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3. SYSTEM REQUIREMENTS

3D-Cell-Annotator is written in C++/CUDA and It is distributed as a patch for the segmentation module of the popular Medical Interaction Toolkit (MITK, http://docs.mitk.org/nightly/). It works on Linux and Windows 64-bit operating systems and it requires a recent version of the NVidia drivers and a CUDA-enabled GPU.

4. INSTALLATION

You can download the 3D-Cell-Annotator enabled MITK distribution at: www.3D-cell-annotator.org.

1. Download the 3D-Cell-Annotator and MITK compiled version for your operating system (*i.e*. Windows, Linux) at: www.3D-cell-annotator.org
2. Extract the files and click on the "..\bin\MitkWorkbench.exe" launcher.
3. Enjoy!

Upon starting the “MitkWorkbench.exe” file, the main window of MITK appears:

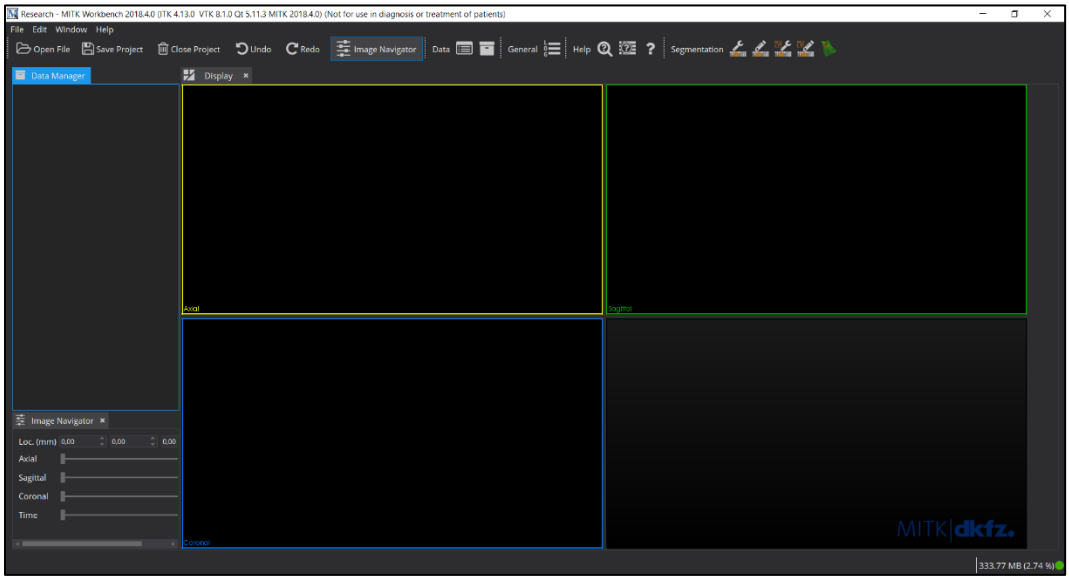


Fig. 1: MITK main window (before a project is opened/loaded).

5. GETTING STARTED

As a start, follow the steps described under "Installation" in Section 4 to install 3D-Cell-Annotator to your computer. Then, proceed as follows:

1. Download a test dataset available at the website: www.3D-cell-annotator.org. In the examples of this manual we are using the blue channel of the dataset called as “Embryo”. Note, the blue channel is the first channel in the dataset, it is the one related to the nuclear staining.
2. Open the MITK main window and click on: "File" -> "Open file" to load the dataset.

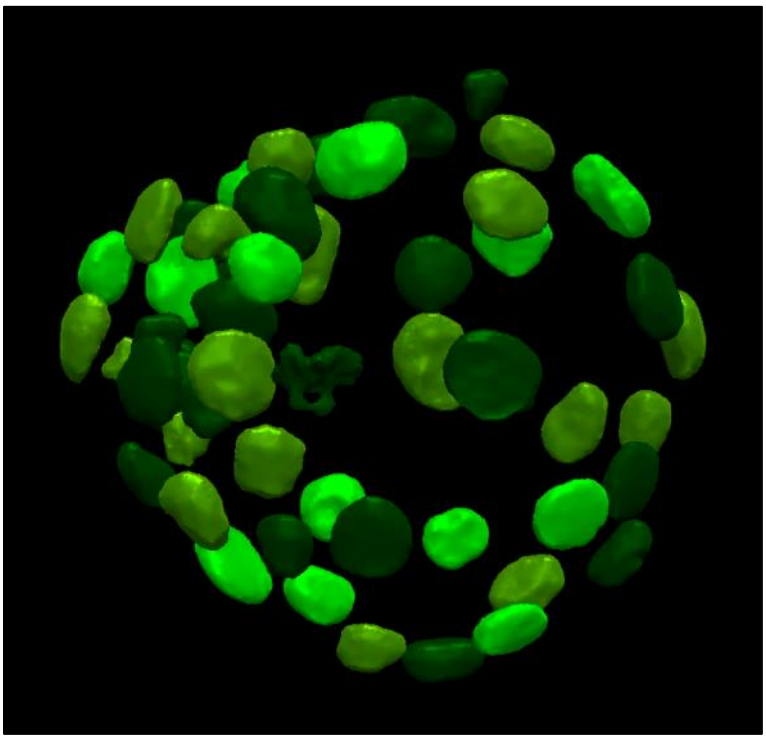


Fig. 2: Embryo dataset: single cells, all segmented.

Now you can explore the 3D data just using the simple intuitive commands of MITK:

- Click on the mouse's left button on a point in a view (*i.e*. axial, sagittal, coronal) to centre all the other views on that point.
- Roll the mouse's wheel to scroll the different sections in one selected view.
- Press the mouse's wheel and move the mouse to drag the object in one selected view.
- Press the mouse's right button and move the mouse left and right to zoom-in and zoom-out, respectively.

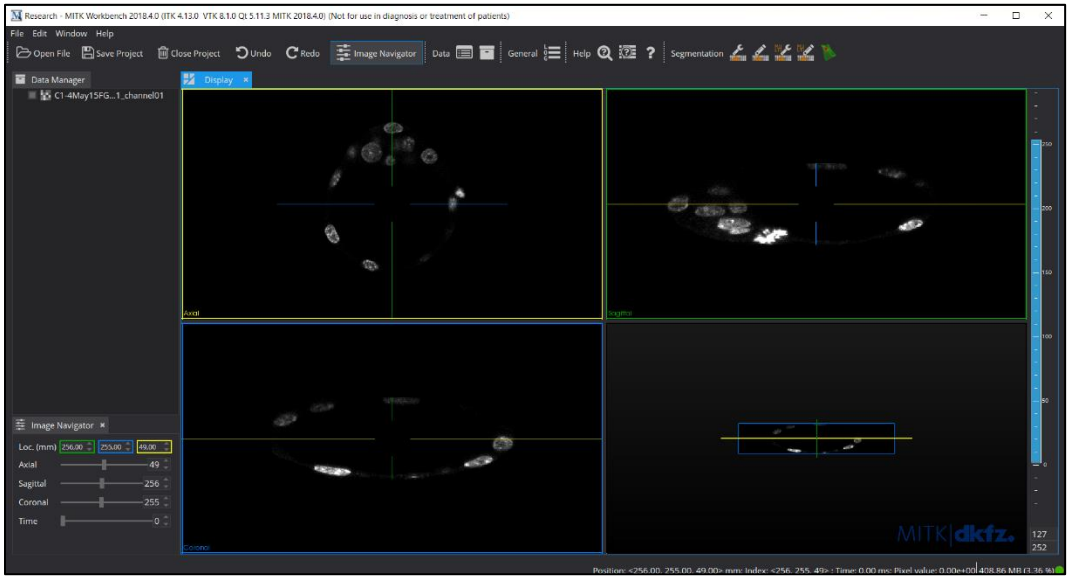


Fig. 3: MITK main window (when a dataset is opened/loaded).

When a dataset is loaded, MITK is ready for the cellular analysis. The following flow-chart summarises the main steps when using 3D-Cell-Annotator:

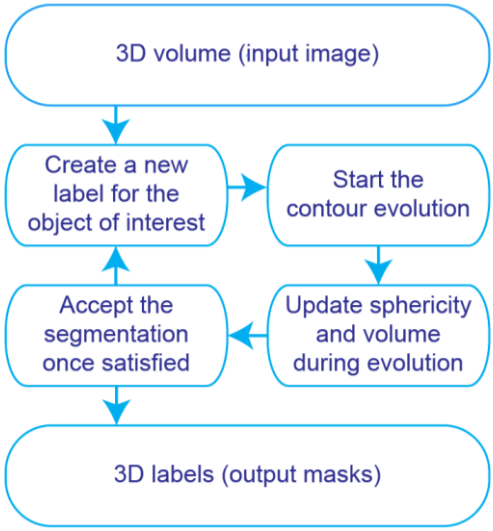
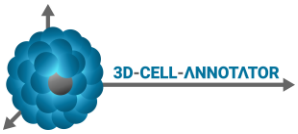


Fig. 4: 3D-Cell-Annotator flow-chart.

Briefly, the main steps for segmenting an object of interest include:



1. Open the “Segmentation” tab (the shortcut icon is on the right-upper part of the MITK GUI).

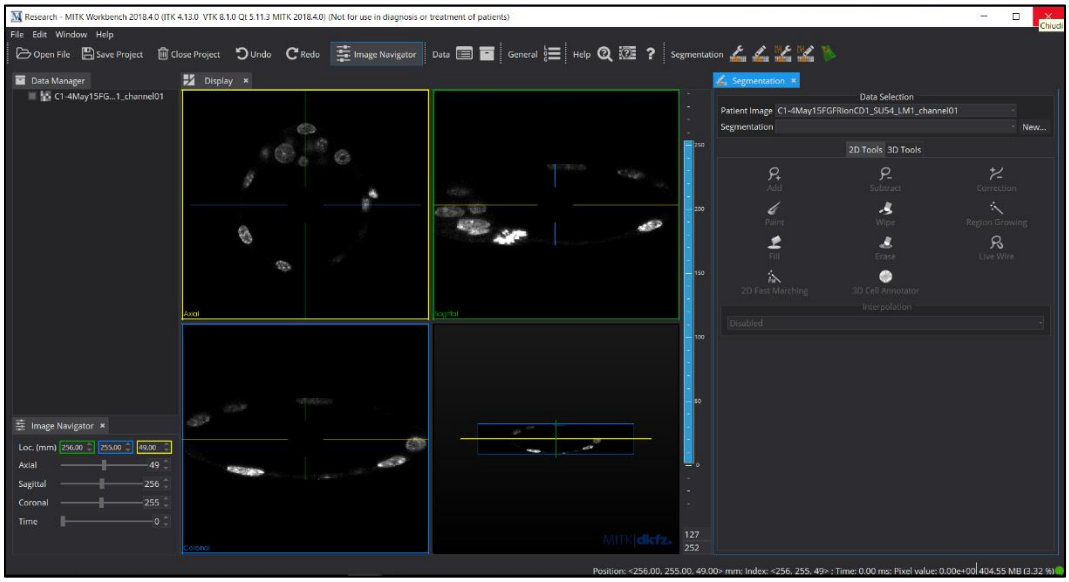


Fig. 5: MITK Segmentation tab.

2. Create a label for each object of interest. For instance, call as “cell01” the label for the first object and assign a colour to it (in our example we are assigning the red colour).

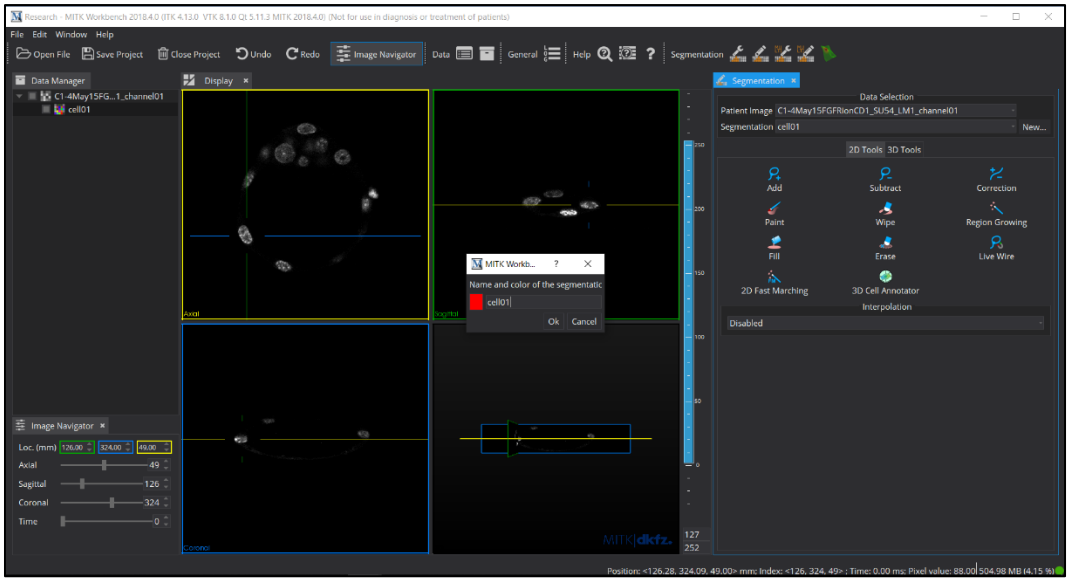


Fig. 6: Create a label for each object of interest.

3. Using the “Add” button of the “Segmentation” tab to create an initial rough 3D contour by manually segmenting just a slice in each of the 3 orthogonal planes. A few seconds are need for this operation.

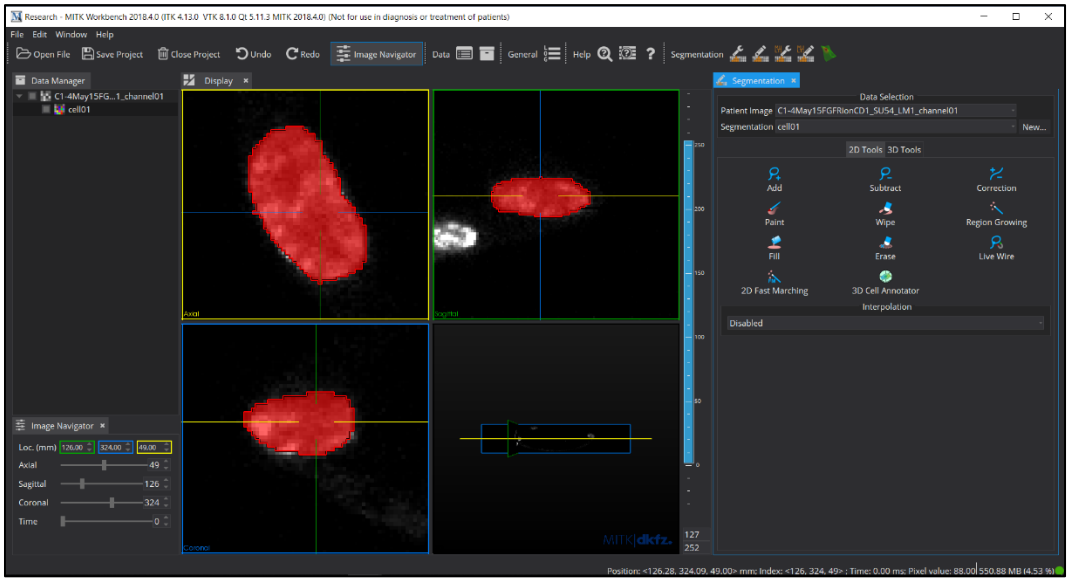


Fig. 7: Initial 3D contour.

4. Open the 3D-Cell-Annotator tool using its specific icon in the “Segmentation” tab.

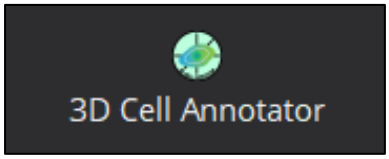


Fig.8: 3D-Cell-Annotator icon.

5. Play with the parameters of 3D-Cell-Annotator to obtain a nice segmentation.

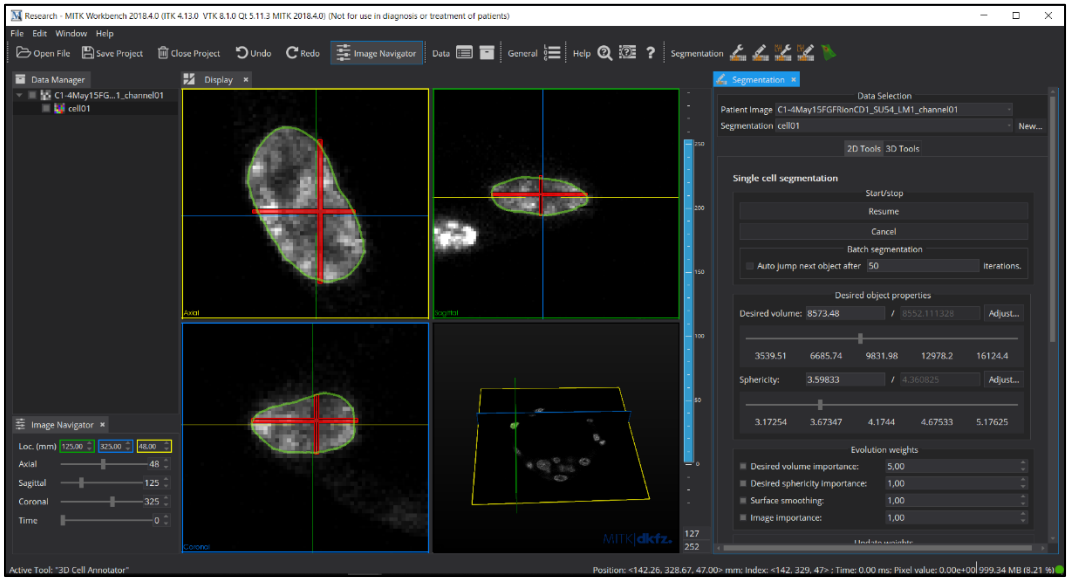


Fig.9: Final 3D segmentation (green line around the object of interest)

6. When you are satisfied with the segmentation, click on the “Accept surface as segmentation” button.
7. Finally, you can export the 3D binary mask obtained as a “.nii” file for further analysis. Note, the “.nii” file can be easily read with ImageJ/Fiji and similar tools. To export a 3D segmentation as a “.nii” file, just right-click on the segmentation and select “Save” as a “.nii” file

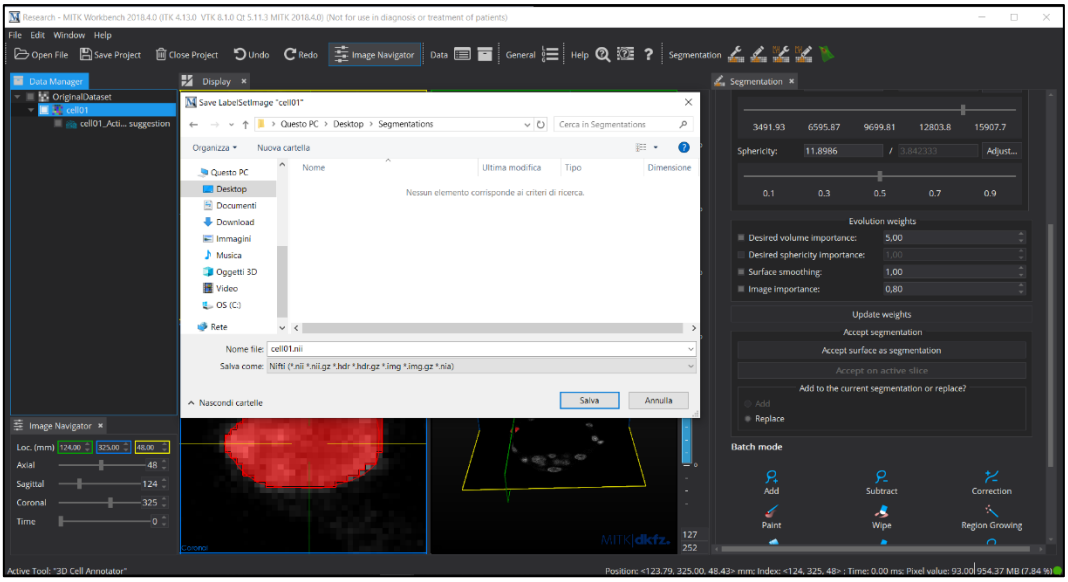


Fig.10: Export the 3D segmentations obtained as 3D binary masks.

All these steps, as well as the parameters that you can modify to obtain a better segmentation, are described in detail in the following sections. However, this “brief introduction” should give you a short overview about the basic feature of 3D-Cell-Annotator.

6. GRAPHICAL USER INTERFACE (GUI)

3D-Cell-Annotator appears as a plugin of the “Segmentation” tab of MITK. This is the Graphical User Interface (GUI) of 3D-Cell-Annotator:

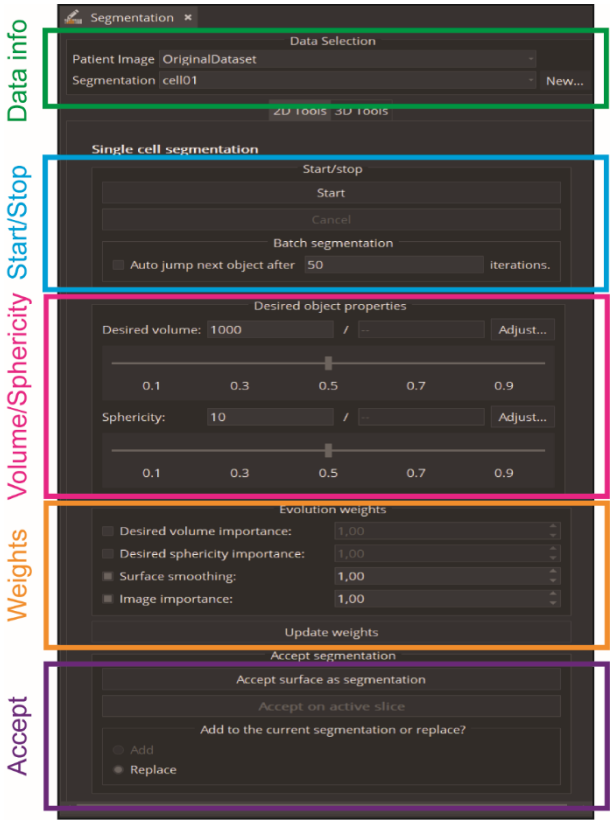


Fig. 11: 3D-Cell-Annotator GUI with main sections.

The GUI of 3D-Cell-Annotator can be subdivided into 5 different sections. Let us see more in detail all of them!

“DATA INFO” SECTION:

In this section the user can find information about the dataset currently analysed and the current selected label. To work with a different dataset/label, just select the correct one using the pop-up menu appearing by clicking on the arrows on the right part of the text-field.

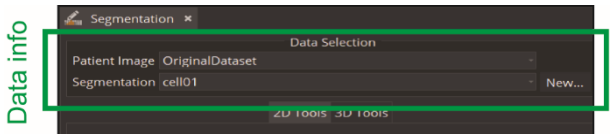


Fig. 12: 3D-Cell-Annotator GUI: “data info” section.

“START/STOP” SECTION:

Through the buttons in this section the user can “start”, “cancel”, “resume” the surface evolution. At the beginning, the buttons report the “start” and “cancel” labels. During the surface evolution, the labels change in “resume” and “cancel”, that means that you can start, stop, re-start as many times you want the surface evolution. This section also provides commands to run 3D-Cell-Annotator in a batch mode. This allows to segment a series of objects of interest in an automatic way. To better understand how to use 3D-Cell-Annotator to segment objects in a batch mode, read the “Usage tricks” section of this manual.

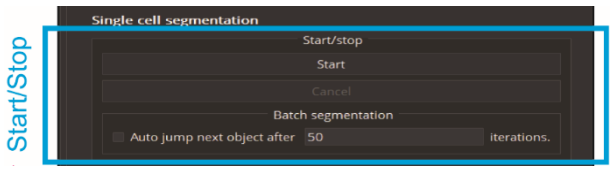


Fig. 13: 3D-Cell-Annotator GUI: “start/stop” section.

“VOLUME/SPHERICITY” SECTION:

This section is the core of 3D-Cell-Annotator. It has two symmetrical sub-sections to allow the user to adjust two main different parameters, volume and sphericity.

Fig. 14: 3D-Cell-Annotator GUI: "volume/sphericity" section.

For each subsection, the first text-field reports the "desired value", the second text-field the "current value", the "Adjust" button on the right part of the second text-field sets the central value of the slider to the current value. The slider below the text-fields allows a quick modification, in real-time during surface evolution, of the "desired value" reported in the first text-field. The user can also directly edit into the first text-field to change the "desired value". The parameters are active just if the corresponding enabling flag in the "weights section" is set on.

Fig. 15: 3D-Cell-Annotator GUI: flag for enabling the "volume/sphericity" section.

"WEIGHTS" SECTION:

This is the controlling section of the GUI. There are four flags, enabling the modification of four different parameters. "Desired volume importance" enables the commands regarding the "Desired volume"; "Desired sphericity importance" those for the desired sphericity. All the weights go from 0 to 10. Value 1 is set for all the parameters as default weight. To assign a value of 9 or 10 to a parameter means to give a strong importance to that parameter in the surface evolution. To make effective the modification in real time with the surface evolution, remember to press the "Update weights" button once you change some values in this section.

Fig. 16: 3D-Cell-Annotator GUI: "weights" section.

"ACCEPT" SECTION:

When the user is satisfied of the obtained surface, he/she must click on the "Accept surface as segmentation" button to accept it. From that time, the 3D surface can be also exported as a ".nii" file.

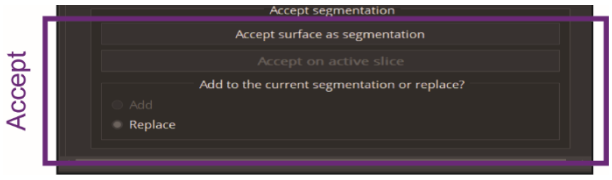


Fig. 17: 3D-Cell-Annotator GUI: "accept" section.

7. PARAMETER EFFECTS

In this section we describe the effects of all the parameters the user can modify for controlling the surface evolution.

VOLUME:

To obtain a nice approximation of the 3D surface of the object of interest, the volume of it would be a very important prior. In order to have an idea about the volume of the object of interest, we suggest the user to run a few times the segmentation of the same object, enabling the controls for the "Desired volume" and playing with the sliders without a pre-defined strategy. Once he/she will understand the approximate value of the volume, we suggest to start one more time the segmentation of the object from scratch, manually writing in the text-field of the "Desired volume" the rough value previously determined and slowly increasing the weight of the "Desired volume importance" from 1 to 10 during surface evolution.

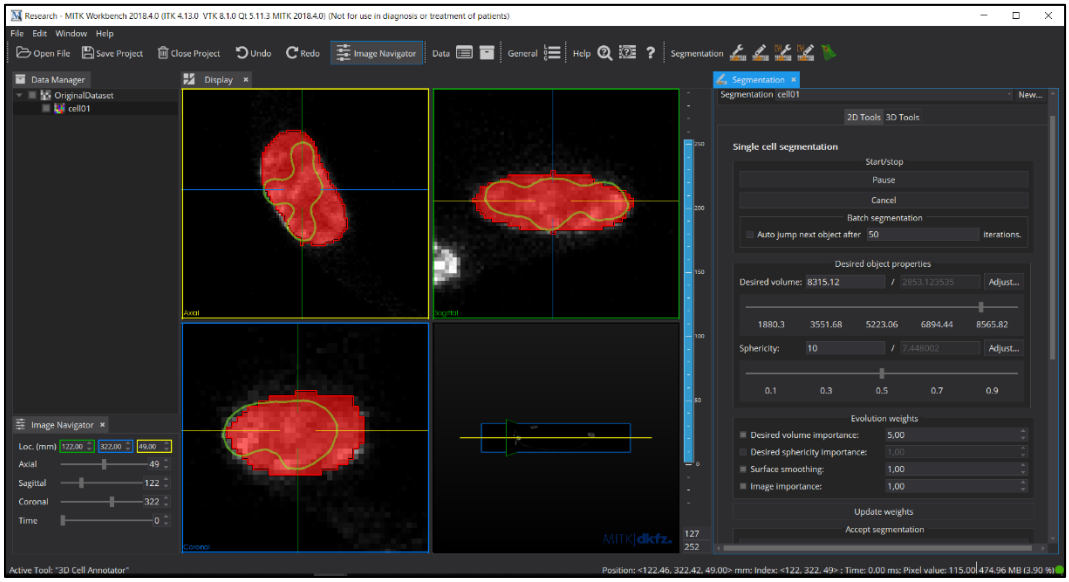


Fig. 18: Surface evolution controlled by the “Desired volume” parameter.

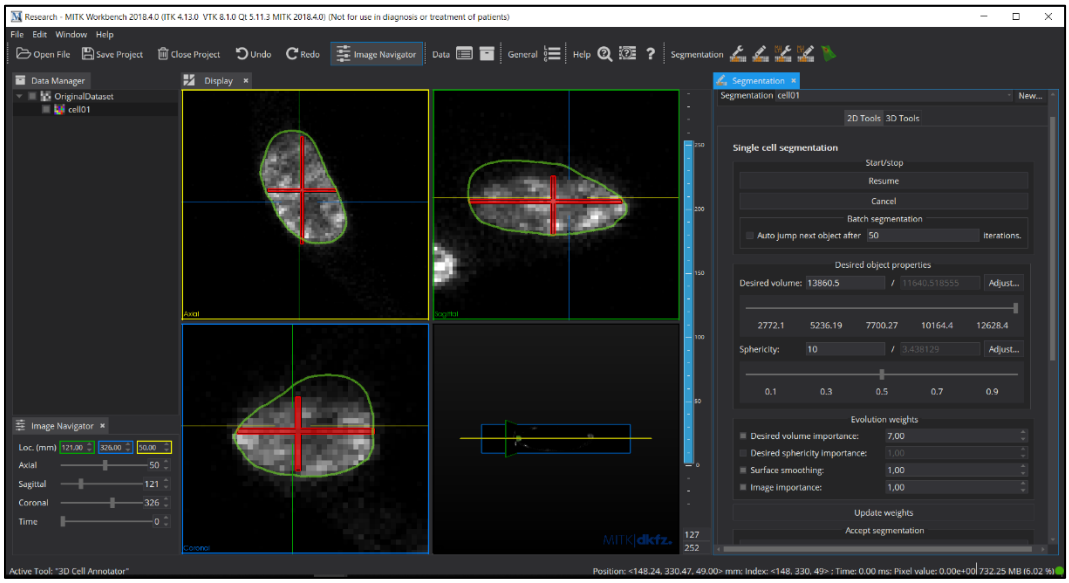


Fig. 19: Over-segmented object.

SPHERICITY:

Sometimes, also for expert microscopists, it is not easy to see the border of the object of interest such as a cell/nucleus, especially in blurry datasets. In this case, it is useful to control the sphericity of the surface

evolution, allowing the creation of indentations or imposing a spherical approximation. The “Desired sphericity” controls are enabled by the “Desired sphericity importance” flag. A “Desired sphericity” value close to 0 will impose a 3D sphere. A desired sphericity value very high, for instance 20, will force the 3D surface to have long indentations, like an “amoeba”. If you see that the surface evolution is block due to a group of black pixels in the inner part of the object of interest, just increase the sphericity to overcome that region, then go back to the initial value to proceed with the surface evolution without imposing an unnatural rounded shape.

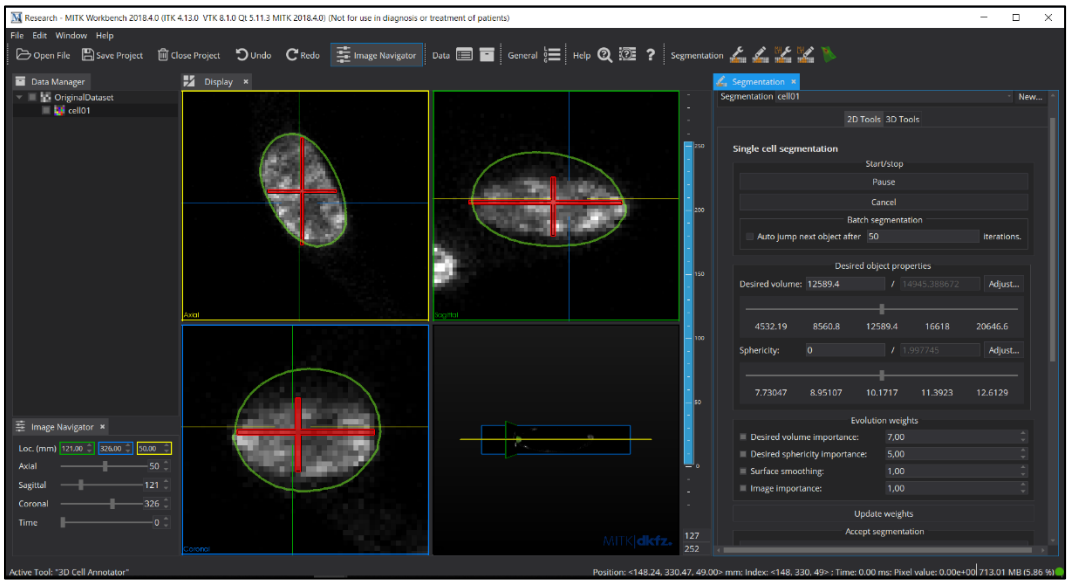


Fig. 20: Too low value set for the desired sphericity parameter.

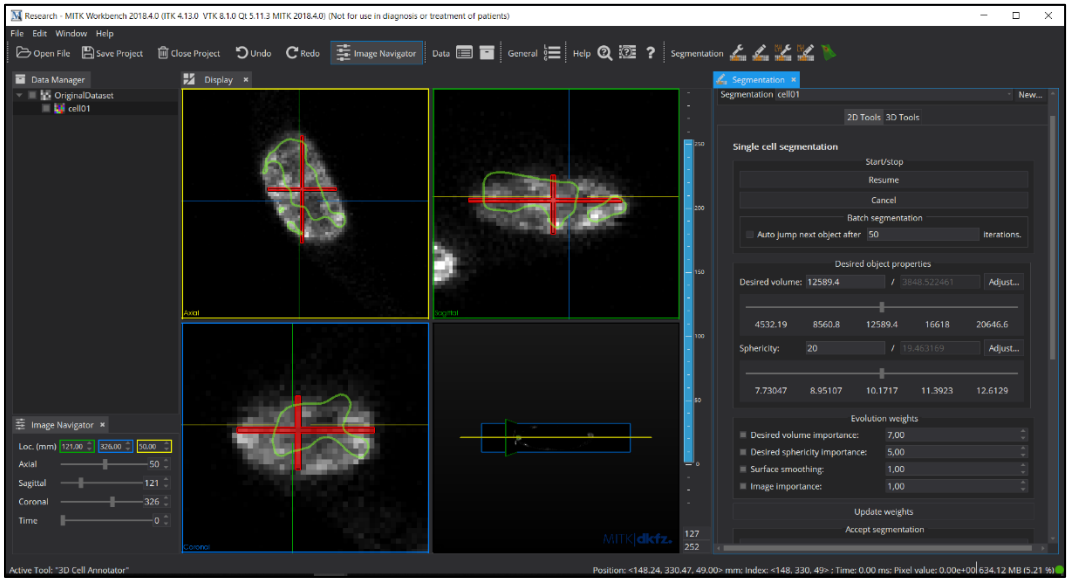


Fig. 21: Too high value set for the desired sphericity parameter.

SURFACE SMOOTHING:

This parameter controls the jagging degree of the surface. Wrongly, it can be considered a copy of the sphericity parameter. It is not: "sphericity" affects the global shape of the 3D surface, whilst "surface smoothing" just controls the jagging degree of the surface, independently from the global shape.

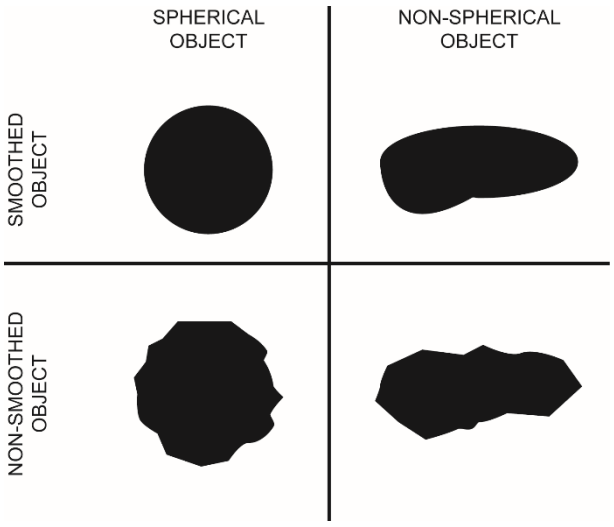


Fig. 22: Difference between spherical and smoothed objects.

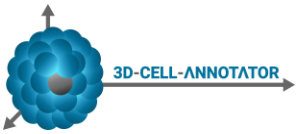


IMAGE IMPORTANCE:

Image importance parameter strongly connects the surface evolution with the image intensity gradients. A high value for this parameter will force the surface evolution to stay close to the voxels with an high intensity gradient.

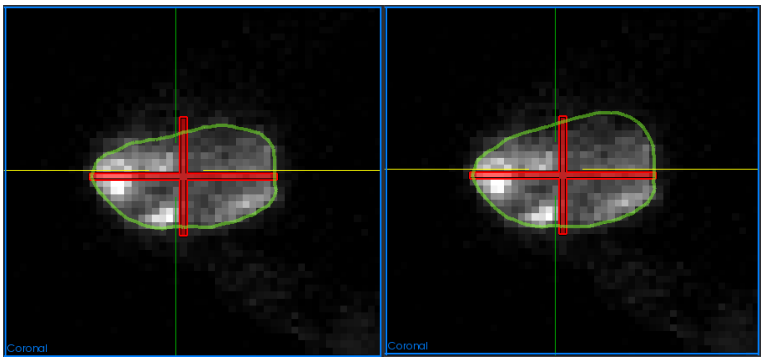


Fig. 23: Segmentation with fix Desired Volume but Image Importance 5 (left panel) versus 1 (right panel).

8. USAGE TRICKS

There is not a fixed predefined strategy to use 3D-Cell-Annotator. You will need a little bit of time to get into it. However, there are a few tricks that can help you to speed up the learning process.

MAKE EXPERIENCE WITH THE SAMPLE DATASETS:

First of all, we suggest you spending a while in segmenting single cells using one of the sample datasets provided at: www.3d-cell-annotator.org. Meanwhile you are a beginner, concentrate on an isolated cell not touching other objects. Then, manually create nice initial contours by manually segmenting three orthogonal sections crossing the centre of the cell. When you will click on the “start” button on the “start/stop” section of the GUI of 3D-Cell-Annotator, you will see that generally the initial contour shrinks. “Delete” the segmentation and try again, but this time playing with the “Desired Volume” value to impose the contours growing. Initially, you can also set a very low “Desired Sphericity” value, just to make the contour grooving and allowing you to understand the approximate volume of the object you are segmenting. Once you have an idea of the volume of the object, start gain from scratch the segmentation, imposing that volume ad desired one.

“SURFACE SMOOTHING” AND “IMAGE IMPORTANCE” PARAMETERS:

In the first tests, do not use these parameters. Just set the weights for them as 1 in the “Weights” section of the GUI of 3D-Cell-Annotator. Generally, these parameters help you only once you will have a rough segmentation with volume and shape well approximating the one of the object of interest. When you will be in that situation, start to play with the weights of the “surface smoothing” and “image importance” to improve the details of the segmentation, imposing that it nicely follows the contour of the object of interest.

REAL-WORLD BLURRY DATASETS:

Remember that 3D-Cell-Annotator is not a human: it is a tool that use an algorithm to compute 3D segmentation of objects of interest. Precisely, it uses 3D active contours with shape descriptors as prior information. Despite we proved that the segmentation precision generally reaches the level of a human expert, it can not do miracles! If the dataset is so blurry that is not possible also for a human to see the border of the cell, 3D-Cell-Annotator will not help you in segmenting that cell. Let us clarify this concept: 3D-Cell-Annotator will always provide you a segmentation, but it is up to you to confirm that it faithfully represents the 3D shape of the cell! Remember that real-world datasets are often blurry, especially for the limited *z* resolution of most of the microscopes. Please, always check the results obtained with 3D-Cell-Annotator, especially when you are analysing blurry datasets!

SEGMENT A SERIES OF OBJECTS OF INTEREST:

3D-Cell-Annotator works also in a batch mode. This allows to segment a series of objects of interest in an automatic way. In order to run 3D-Cell-Annotor in batch, the user must first exploit the “MultiLabel Segmentation” Tab of MITK to create a label and an initial contour for each object of interest.

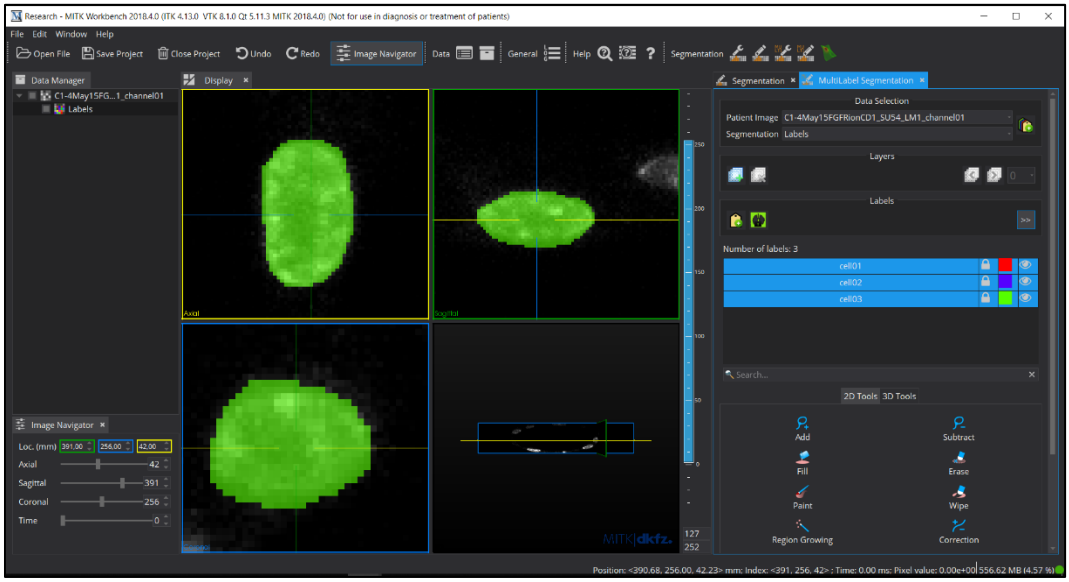


Fig. 24: 3D-Cell-Annotator run in a batch mode for segmenting a series of objects of interest.

Then, using the "Batch segmentation" options in the "Start/Stop" section of the 3D-Cell-Annotator GUI, he/she must set the number of iterations allowed for the segmentation of each single object of interest.

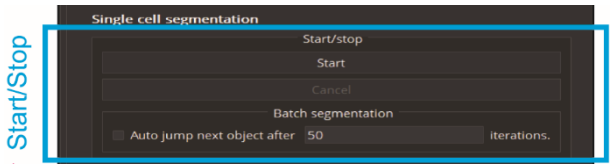


Fig. 25: 3D-Cell-Annotator GUI: "Batch segmentation" options.

Finally, the segmentation process starts by just enabling the "Auto jump next object after" flag and clicking on the "Start" button.

9. CONCLUSIONS

3D-Cell-Annotator allows the user to easily segment single cells/nuclei starting from 3D datasets acquired with confocal, multi-photon and light-sheet fluorescent microscope. Being distributed as a patch of the well-known MITK suite, it permits the user to exploit all the classical MITK functionalities that make analysis and

rendering of 3D data very easy. For instance, once segmented a group of cells, the user can render in 3D their surface, estimating morphological parameters, and also show with different colours cells classified in different groups. Finally, all the 3D surfaces can be exported as 3D binary masks to proceed in further analysis with external tools, for instance the popular ImageJ/Fiji.

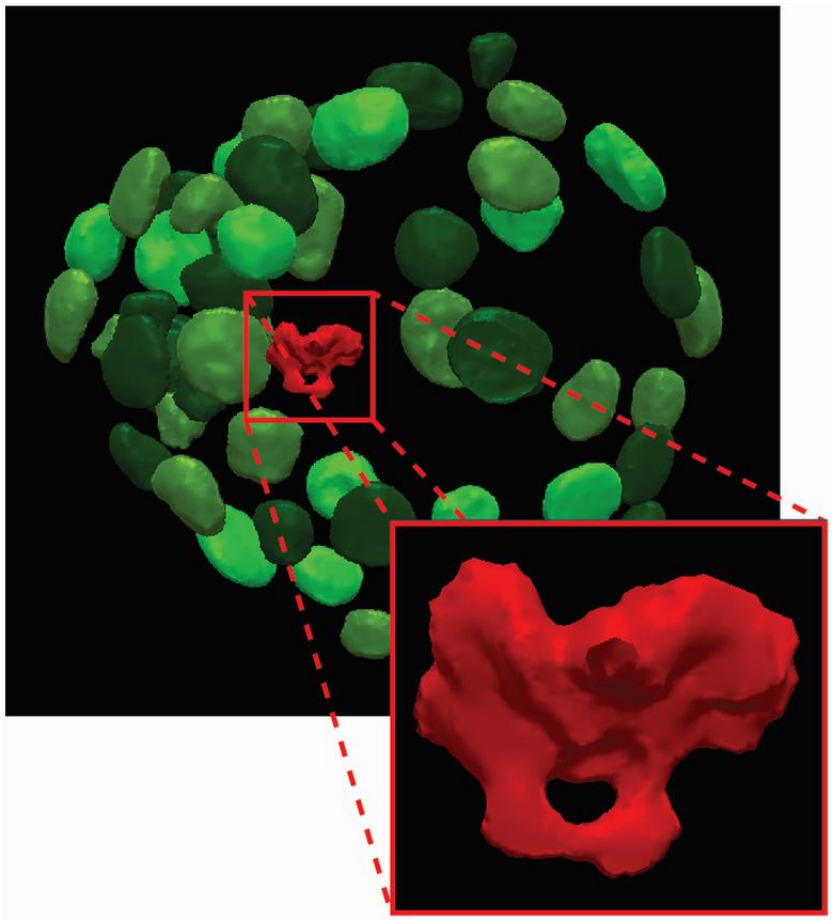


Fig. 26: Embryo dataset: cell with an interesting phenotype.

10. CONTACTS

By reading this help documentation manual you got a more complete overview of the various analysis options offered by 3D-Cell-Annotator. Please, visit also the 3D-Cell-Annotator website: www.3d-cell-

annotator.org, and If you need further information or you have special requests, remember that we are open to collaborations! In case, contact: horvath.peter@brc.mta.hu

Fig. 27: 3D-Cell-Annotator main contacts.

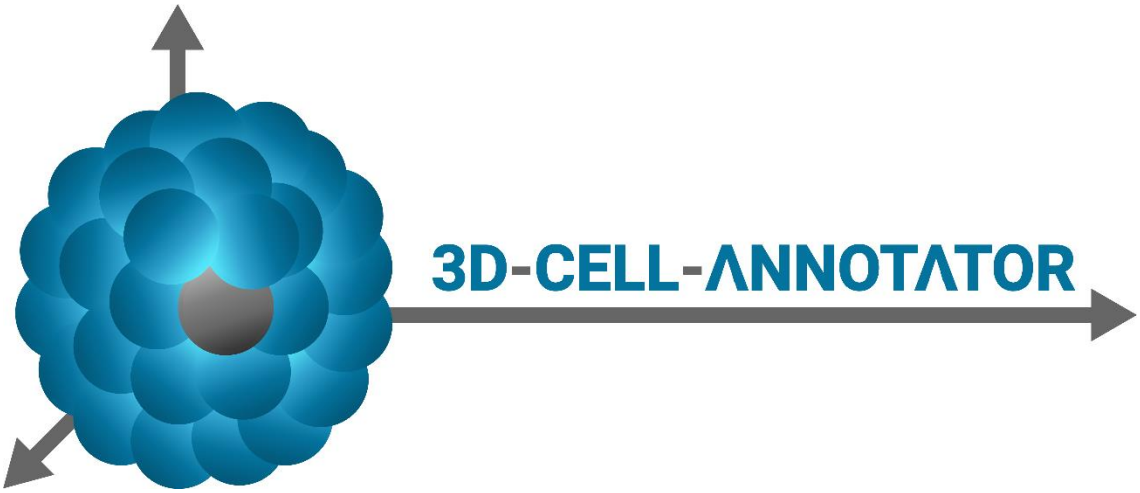


Fig. 28: 3D-Cell-Annotator logo.