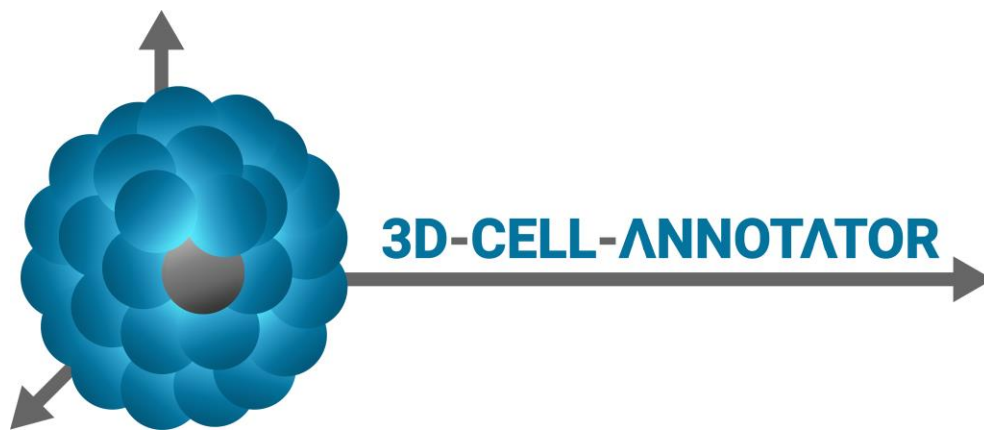


# 3D-Cell-Annotator

## USER MANUAL

[www.3d-cell-annotator.org](http://www.3d-cell-annotator.org)

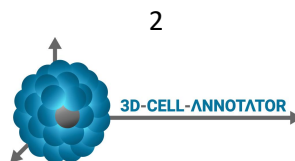


**v1.0 - December 2019**

**Prof. Peter Horvath, PhD**  
Synthetic and Systems Biology Unit  
Biological Research Centre, Szeged  
[horvath.peter@brc.hu](mailto:horvath.peter@brc.hu)

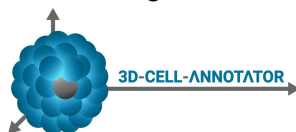
## INDEX

1	<b>Brief description</b>	p. 4
2	<b>License</b>	p. 4
3	<b>System requirements</b>	p. 5
4	<b>Installation</b>	p. 5
5	<b>Getting started</b>	p. 6
6	<b>Graphical user interface</b>	p. 12
7	<b>Parameter effects</b>	p. 15
8	<b>Usage tricks</b>	p. 19
9	<b>Conclusions</b>	p. 22
10	<b>Contacts</b>	p. 23



## LIST OF FIGURES

1	MITK main window (before a project is opened/loaded)	p. 6
2	Embryo dataset: single cells, all segmented	p. 7
3	MITK main window (when a dataset is opened/loaded)	p. 8
4	3D-Cell-Annotator flow-chart	p. 8
5	MITK Segmentation tab	p. 9
6	Create a label for each object of interest	p. 9
7	Initial 3D contour	p. 10
8	3D-Cell-Annotator icon	p. 10
9	Final 3D segmentation	p. 11
10	Export the 3D segmentations obtained as 3D binary masks	p. 11
11	3D-Cell-Annotator GUI with main sections	p. 12
12	3D-Cell-Annotator GUI: “data info” section	p. 13
13	3D-Cell-Annotator GUI: “start/stop” section	p. 13
14	3D-Cell-Annotator GUI: “volume/sphericity” section	p. 13
15	3D-Cell-Annotator GUI: “volume/sphericity” section flag	p. 14
16	3D-Cell-Annotator GUI: “weights” section	p. 14
17	3D-Cell-Annotator GUI: accept section	p. 15
18	Surface evolution controlled by “Desired volume”	p. 16
19	Over-segmented object	p. 16
20	Too low value set for the desired sphericity parameter	p. 17
21	Too high value set for the desired sphericity parameter	p. 18
22	Difference between spherical and smoothed objects	p. 18
23	Fix Desired Volume but Image Importance 5 versus 1	p. 19
24	3D-Cell-Annotator run in a batch mode	p. 21
25	3D-Cell-Annotator GUI: “Batch segmentation” options	p. 22
26	Embryo dataset: cell with an interesting phenotype	p. 23
27	3D-Cell-Annotator main contacts	p. 23
28	3D-Cell-Annotator logo	p. 24



## **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 semi-automatic fashion.

3D-Cell-Annotator is mainly developed at the Biological Research Centre (BRC) in Szeged, Hungary. Results show that, running the software on single cells, the precision of segmentation reaches the level of a human expert.

This document is a short 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](http://www.3D-cell-annotator.org)

## **2. LICENSE**

The software and all the materials available at [www.3D-cell-annotator.org](http://www.3D-cell-annotator.org) are copyright protected.

Copyright (©) 2019, Peter Horvath. All rights reserved.

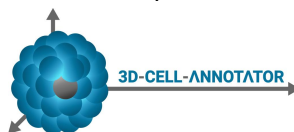
3D-Cell-Annotator is licensed under the:

3-clause BSD License

The exact license text is:

Redistribution and use in source and binary forms, with or without modification, are permitted provided that the following conditions are met:

- \* Redistributions of source code must retain the above copyright notice, this list of conditions and the following disclaimer.



- \* Redistributions in binary form must reproduce the above copyright notice, this list of conditions and the following disclaimer in the documentation and/or other materials provided with the distribution.
- \* Neither the name of the Biological Research Centre (BRC, Szeged) nor the names of its contributors may be used to endorse or promote products derived from this software without specific prior written permission.

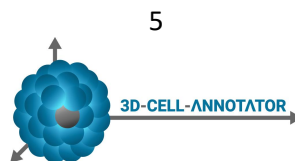
THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND CONTRIBUTORS "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL <COPYRIGHT HOLDER> BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.

### **3. SYSTEM REQUIREMENTS**

3D-Cell-Annotator is a patch for the segmentation plugin of the Medical Imaging Interaction Toolkit version 2018.04 (MITK, <http://docs.mitk.org/nightly/>) written in C++/CUDA and distributed as a full MITK distribution, however building from source is also possible. 3D-Cell-Annotator works on Windows and Linux operating systems. It requires a CUDA-enabled Graphic Processing Unit (GPU) and a recent version of NVidia driver (Linux: 410.104; Win: 411.31). For normal usage a mid-category GPU with several hundreds of processing threads (CUDA cores) is required, such as GeForce 850M or above. The software needs 9 bytes of memory per voxel on the GPU at peak load, therefore a ~750 by 750 by 750 image can be processed with a GPU with 4GB RAM.

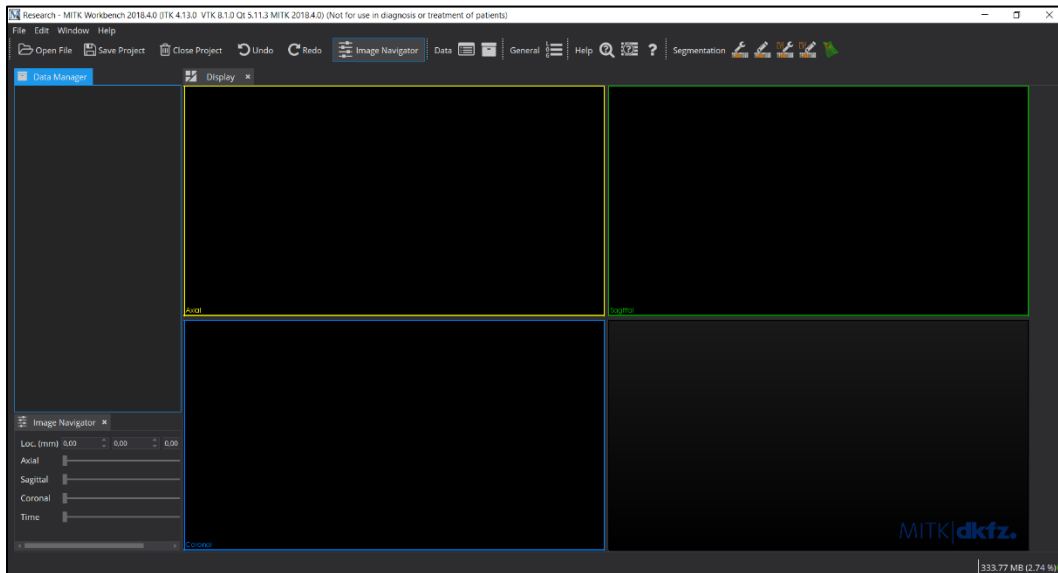
### **4. INSTALLATION**

You can download the 3D-Cell-Annotator enabled MITK distribution at: [www.3D-cell-annotator.org](http://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](http://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:

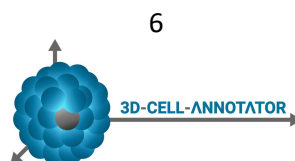


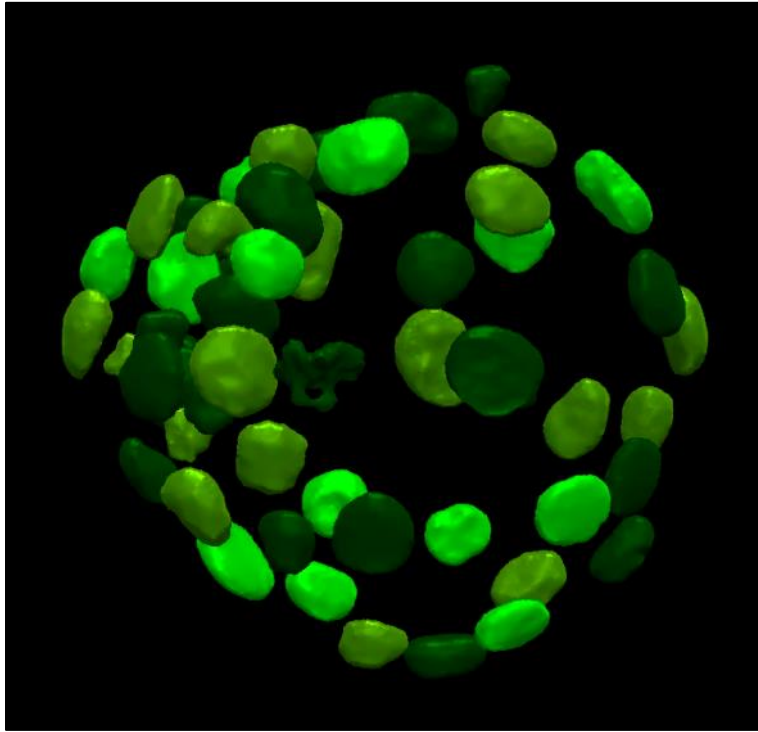
**Fig. 1:** MITK main window (before a project is opened/loaded).

## **5. GETTING STARTED**

To start with, 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](http://www.3D-cell-annotator.org). In the examples of this manual we are using the blue channel of the dataset called “Embryo”. Note that the blue channel is the first channel in the dataset, it is the one related to nuclear staining.
2. Open the MITK main window and click on: "File" -> "Open file" to load the dataset.

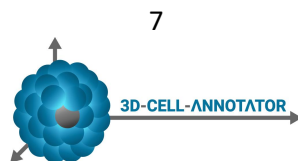


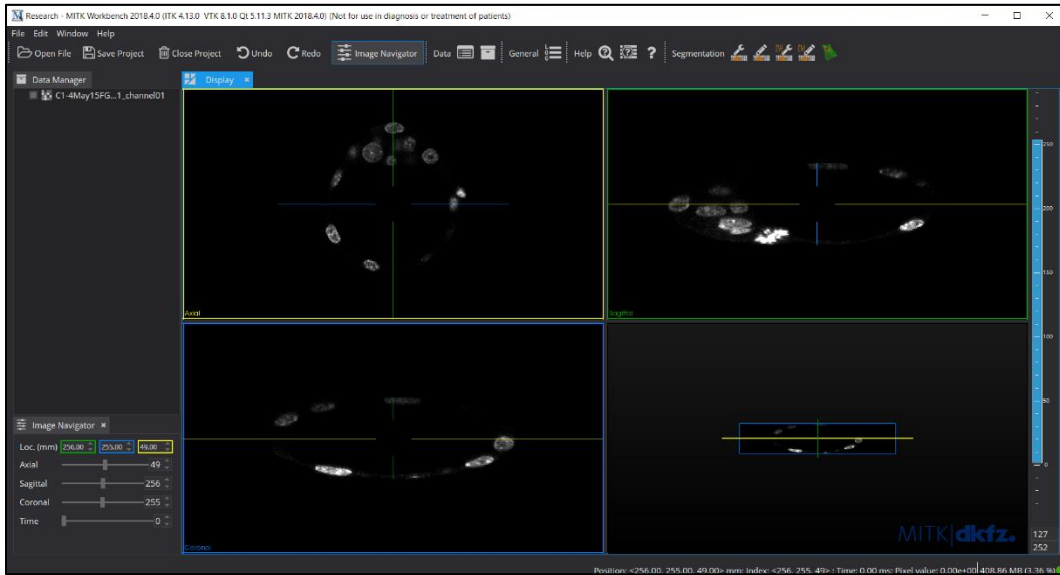


**Fig. 2:** Embryo dataset: single cells, all segmented.

Now you can explore the 3D data by using the simple and straightforward commands of MITK:

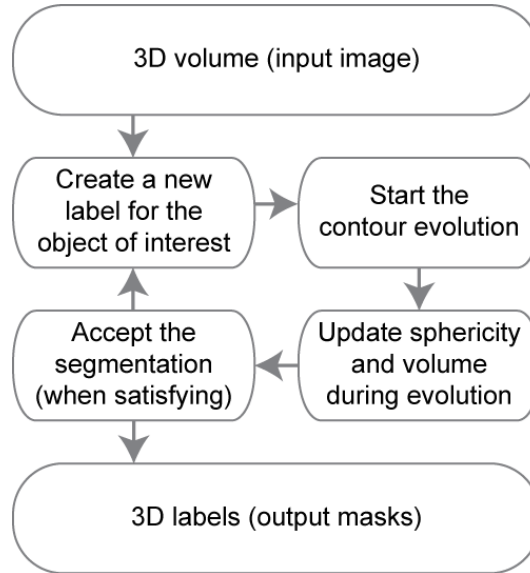
- Left-click on a point in a view (*i.e.* axial, sagittal, coronal) to focus all the other views on that point.
- Roll the mouse's wheel to scroll the different sections in the selected view.
- Press the mouse's wheel and move the mouse to drag the object in the selected view.
- Press the mouse's right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





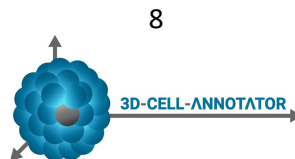
**Fig. 3:** MITK main window (when a dataset is opened/loaded).

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



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

The main steps of segmenting an object of interest are summarised briefly below:





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

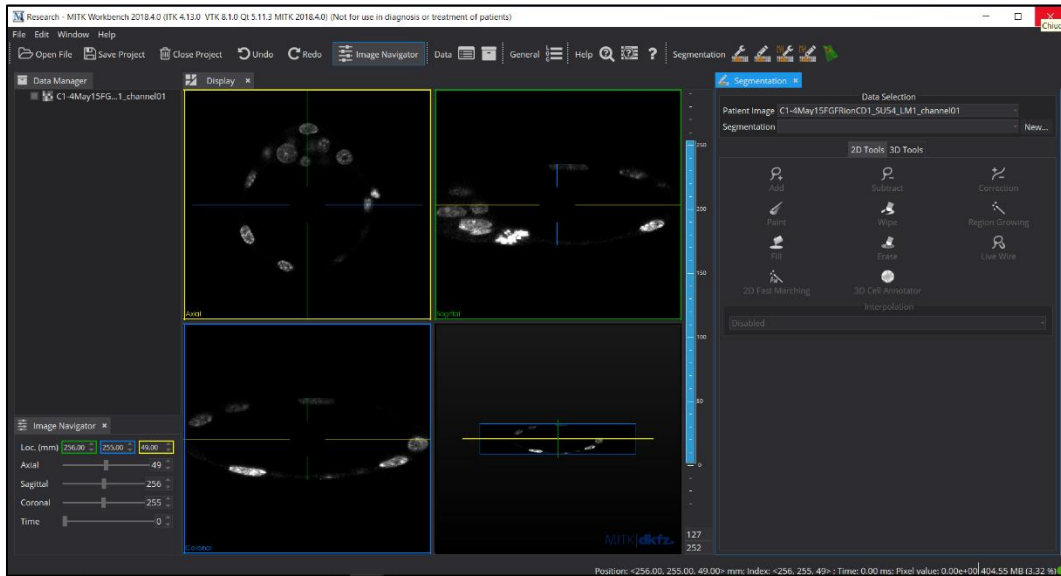


Fig. 5: MITK Segmentation tab.

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

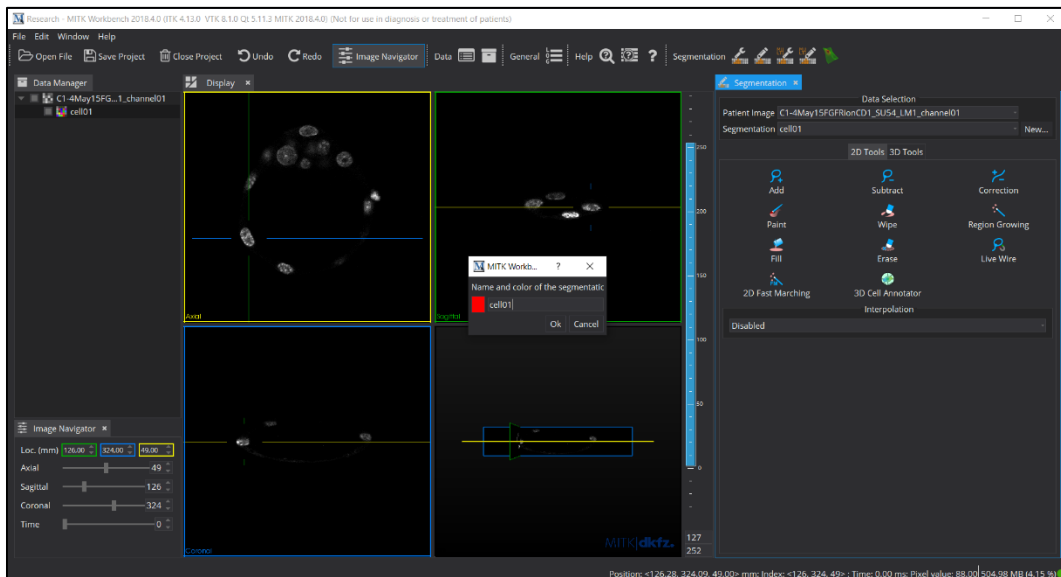
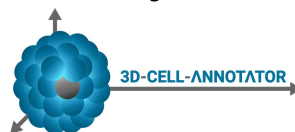
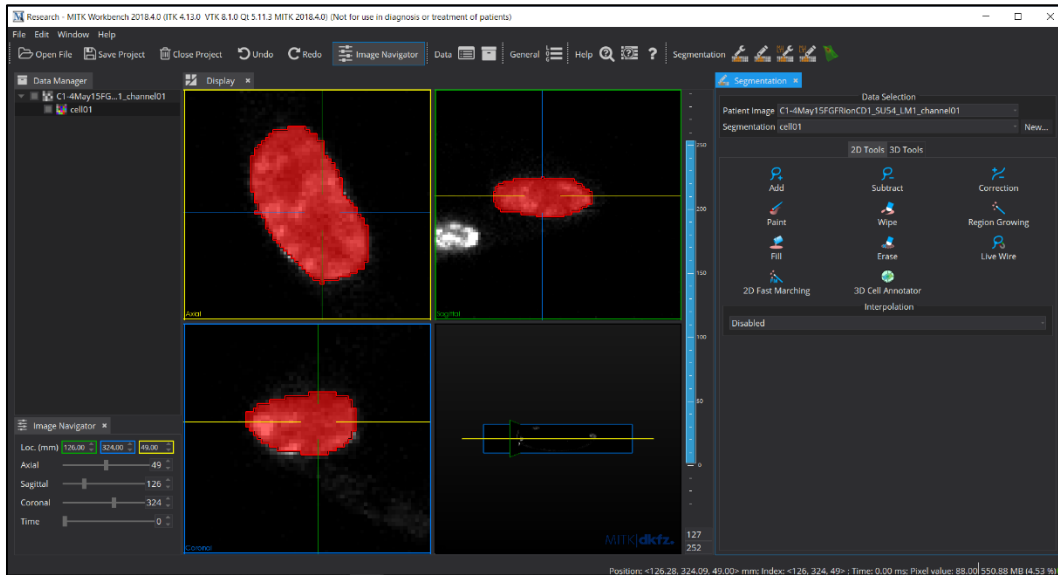


Fig. 6: Create a label for each object of interest.

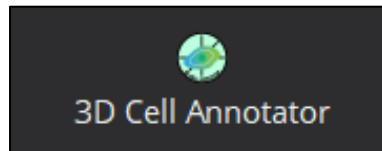


- Use the “Add” button of the “Segmentation” tab to create an initial, rough 3D contour by manually segmenting just a slice in each of the 3 orthogonal planes. This operation will take a few seconds.



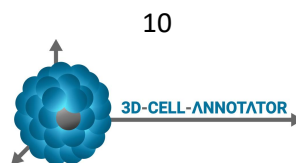
**Fig. 7:** Initial 3D contour.

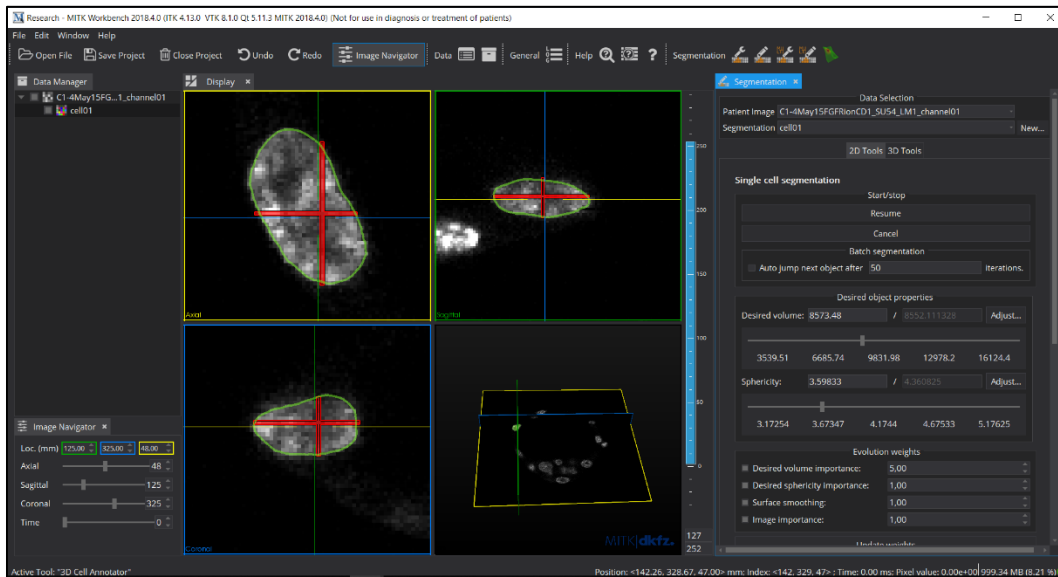
- Open the 3D-Cell-Annotator tool with its icon in the “Segmentation” tab.



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

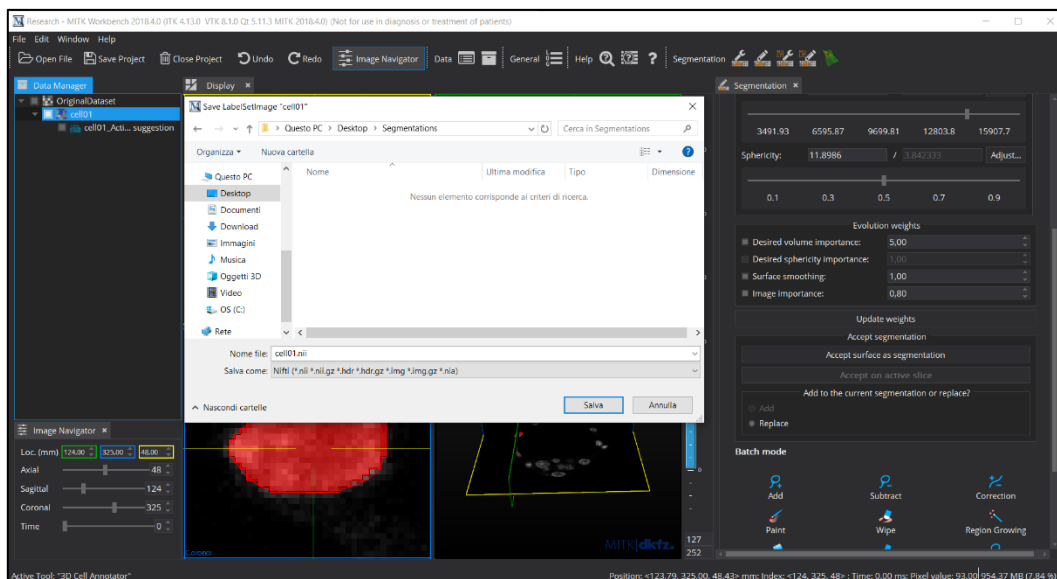
- Adjust the parameters of 3D-Cell-Annotator until you get a nice segmentation.



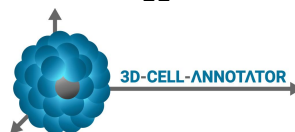


**Fig.9:** Final 3D segmentation (green line around the object of interest)

- When you are satisfied with the segmentation, click on the “Accept surface as segmentation” button.
- Finally, you can export the 3D binary mask obtained as a “.nii” file for further analysis. Note that 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



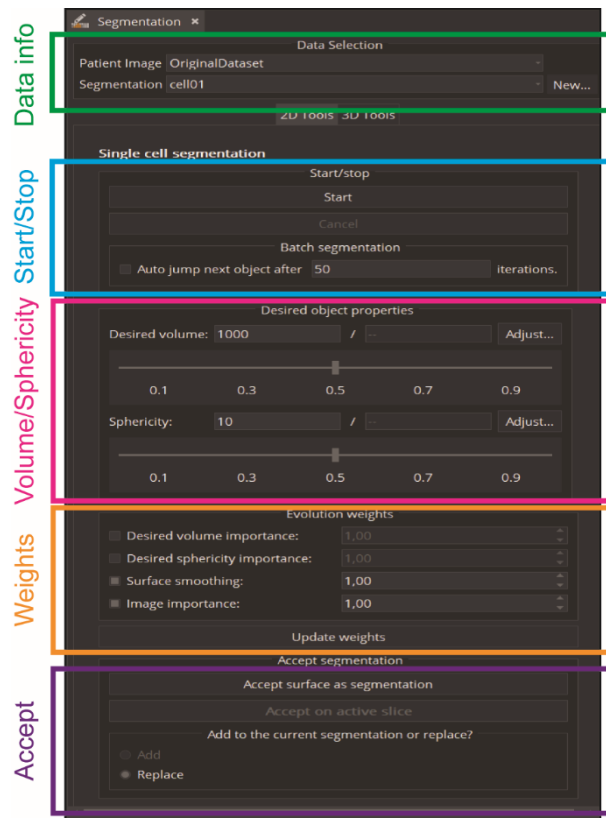
**Fig.10:** Export the 3D segmentations obtained as 3D binary masks.



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, the above “brief introduction” should provide a short overview about the basic features of 3D-Cell-Annotator.

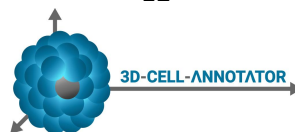
## 6. GRAPHICAL USER INTERFACE (GUI)

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 all of them in more detail!



### “DATA INFO” SECTION:

In this section you can find information about the dataset currently analysed and the label currently selected. To work with a different dataset/label, just select the desired one using the pop-up menu that appears by clicking on the arrows in the right side of the text-field.

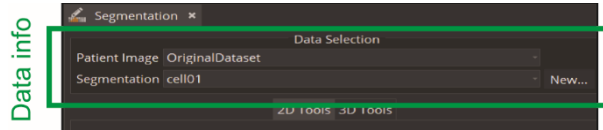


Fig. 12: 3D-Cell-Annotator GUI: “data info” section.

### “START/STOP” SECTION:

Using the buttons of this section you can “start”, “cancel”, “resume” the surface evolution. At the beginning the labels “start” and “cancel” appear on the buttons. During the surface evolution, the labels change to “resume” and “cancel”; that means that you can start, stop and re-start the surface evolution as many times as you want. This section also provides commands to run 3D-Cell-Annotator in batch mode. This function allows you 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 batch mode, read the “Usage tricks” section of this manual.

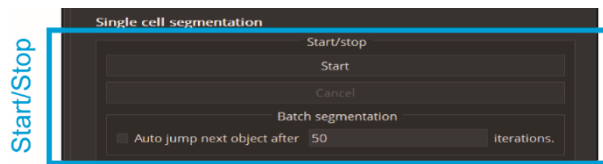


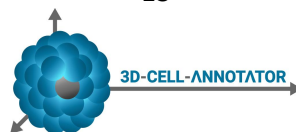
Fig. 13: 3D-Cell-Annotator GUI: “start/stop” section.

### “VOLUME/SPHERICITY” SECTION:

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



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



For each subsection, the first text-field shows the “desired value”, the second text-field the “current value”, and the “Adjust” button on the right side 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 of the “desired value” in the first text-field in real-time, during the surface evolution. You can also directly edit into the first text-field to change the “desired value”. The parameters are only active if the corresponding enabling flag in the “weights section” is turned on.

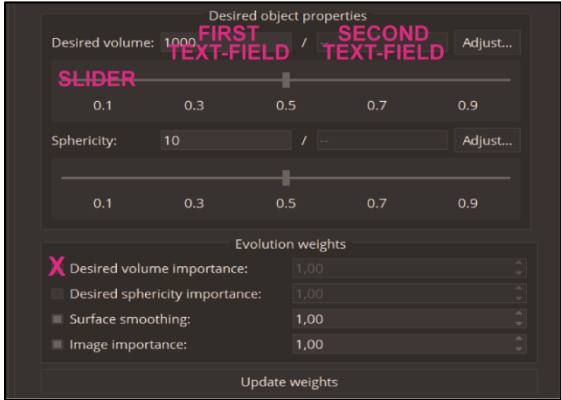


Fig. 15: 3D-Cell-Annotator GUI: flag for enabling the “volume/sphericity” section.

**“WEIGHTS” SECTION:**

The control section of the GUI is shown below. There are four flags, enabling the modification of four different parameters. “Desired volume importance” enables the commands regarding the “Desired volume” while “Desired sphericity importance” those for the desired sphericity. All the weights can take values from 0 to 10. Value 1 is set for all the parameters as a default weight. Assigning a value of 9 or 10 to a parameter means that it is of high importance in the surface evolution. To make the modification effective during the surface evolution in real time, remember to press the “Update weights” button after changing some of the values in this section.

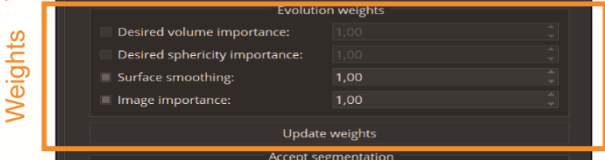
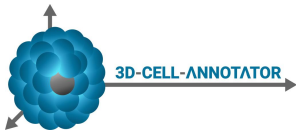


Fig. 16: 3D-Cell-Annotator GUI: “weights” section.



### “ACCEPT” SECTION:

When you are satisfied with the obtained surface, click on the “Accept surface as segmentation” button to accept it. After that, the 3D surface can also be exported as a “.nii” file.

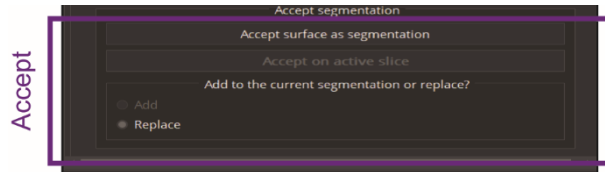


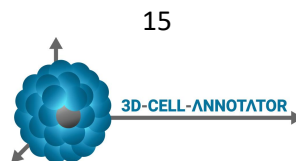
Fig. 17: 3D-Cell-Annotator GUI: “accept” section.

## 7. PARAMETER EFFECTS

In this section we describe the effects of all the parameters you can modify to control the surface evolution.

### VOLUME:

To obtain a nice approximation of the 3D surface of the object of interest, its volume should be given due attention. In order to have an idea about the volume of the object of interest, we advise you to run the segmentation of the same object a few times, enabling the controls for the “Desired volume” and to play with the sliders without a pre-defined strategy. Once you understand the approximate value of the volume, we suggest to start the segmentation of the object one more time, from scratch, manually writing the rough value previously determined into the text-field of the “Desired volume” and slowly increasing the weight of the “Desired volume importance” from 1 to 10 during the surface evolution.



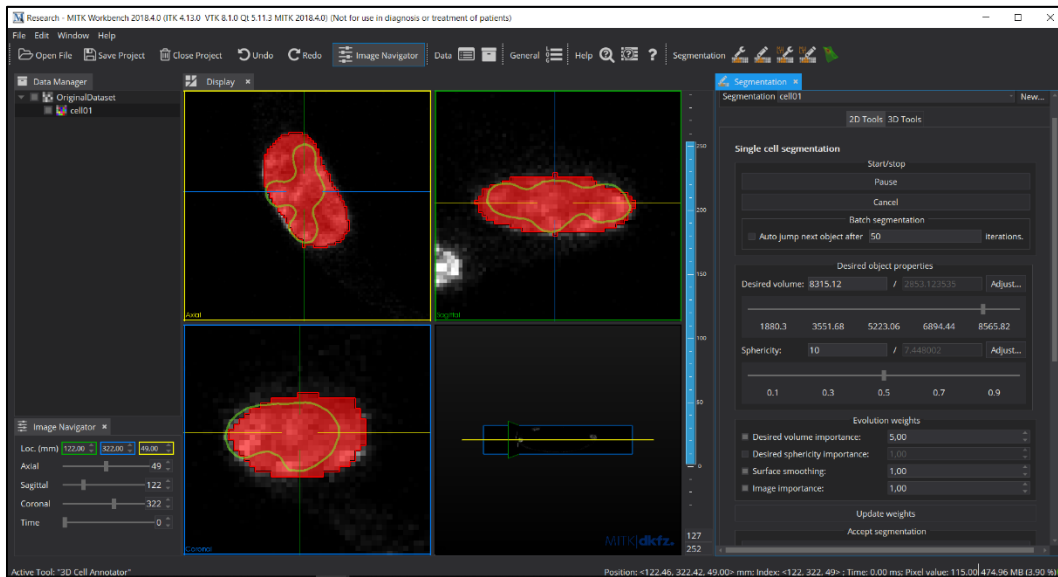


Fig. 18: Surface evolution controlled by the “Desired volume” parameter.

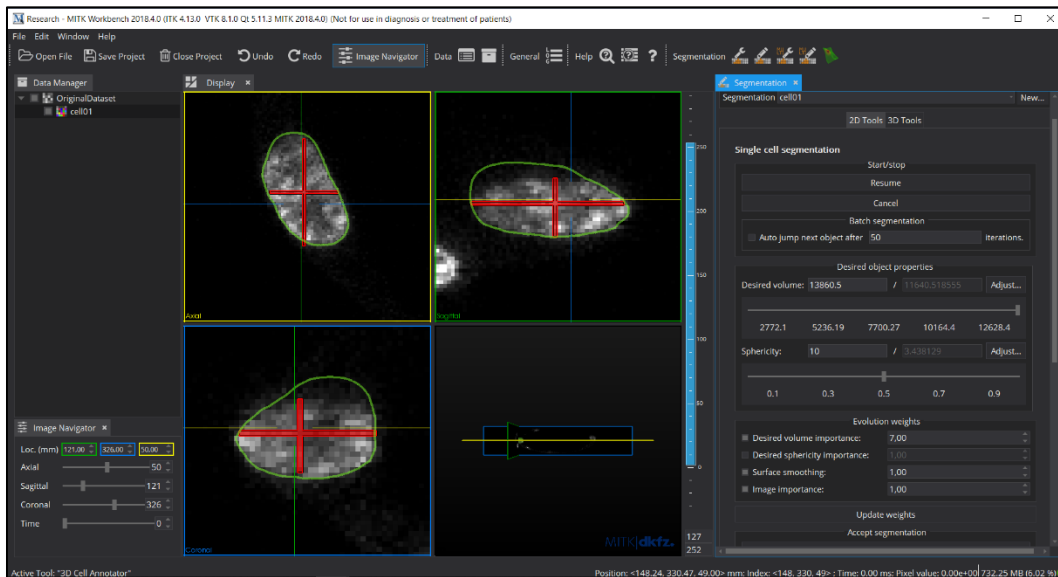
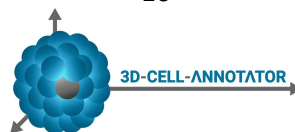


Fig. 19: Over-segmented object.

### SPHERICITY:

Sometimes, even 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. Note that while the “Desired sphericity importance” value ranges from 0 to 10, the “Desired sphericity” value ranges from 0 to 100. 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 the surface evolution is blocked 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.

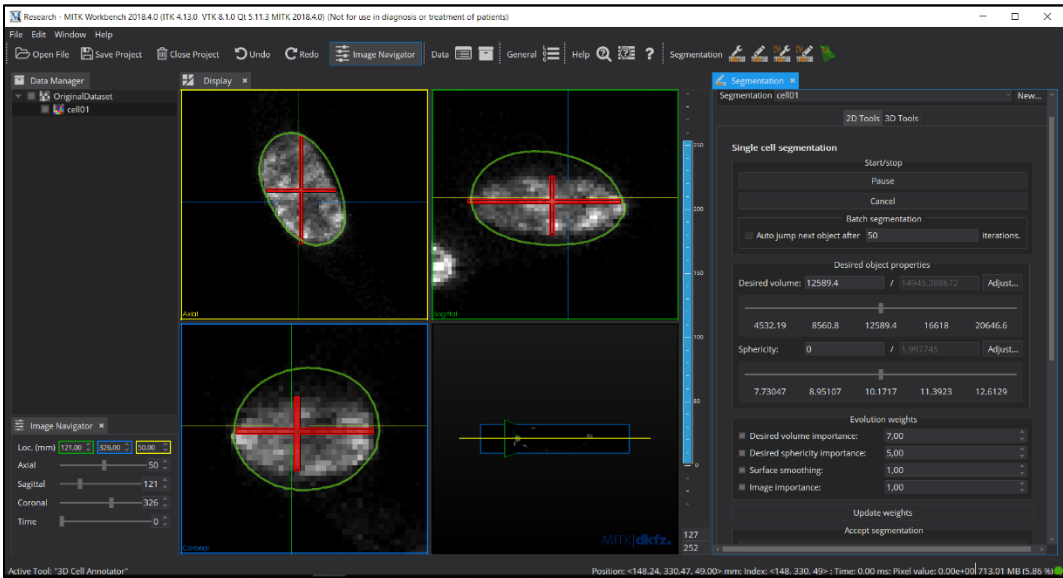
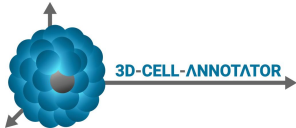
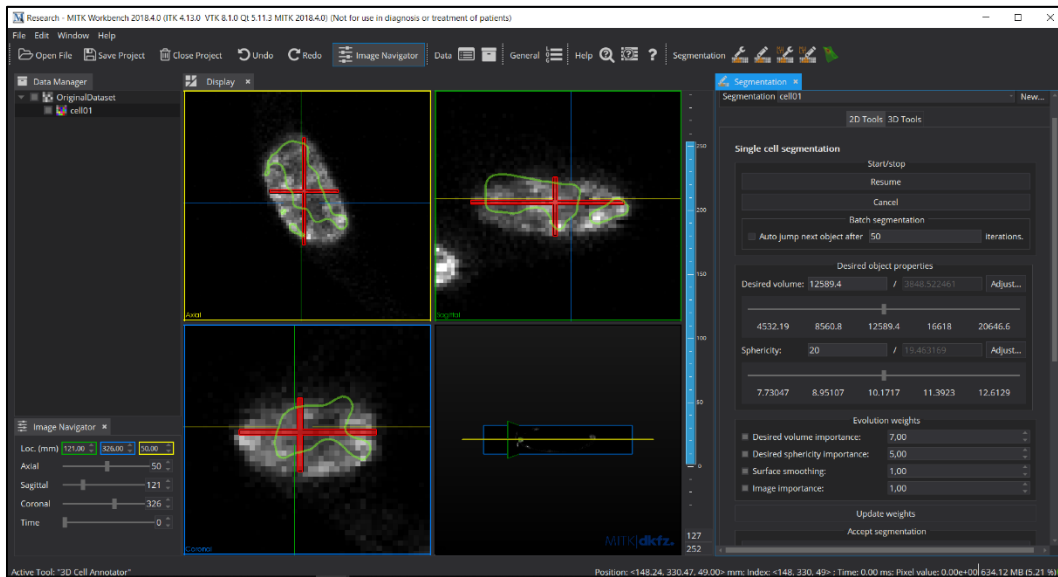


Fig. 20: Too low value set for the desired sphericity parameter.

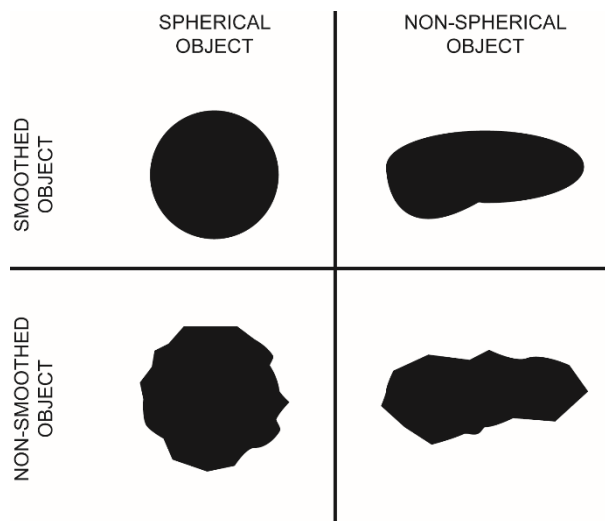




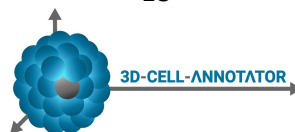
**Fig. 21:** Too high value set for the desired sphericity parameter.

**SURFACE SMOOTHING:**

This parameter controls the jagginess degree of the surface. It can wrongly be considered identical to the sphericity parameter. It is not: “sphericity” affects the global shape of the 3D surface, whilst “surface smoothing” only controls the jagginess degree of the surface, independently from the global shape.



**Fig. 22:** Difference between spherical and smoothed objects.



### IMAGE IMPORTANCE:

The 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 a high intensity gradient.

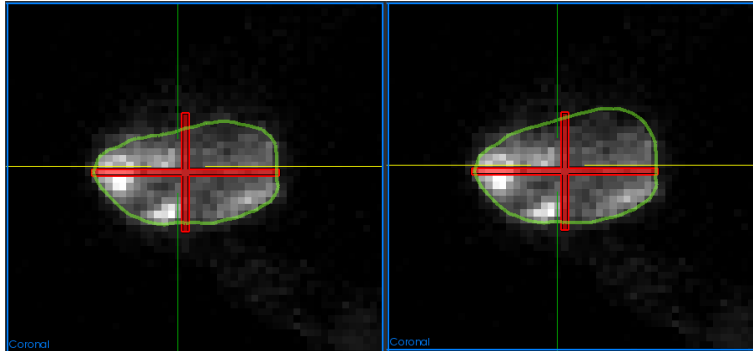


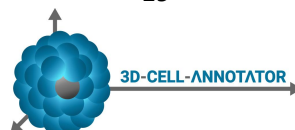
Fig. 23: Segmentation with fix Desired Volume and Image Importance value 5 (left panel) versus 1 (right panel).

## 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 to speed up the learning process.

### MAKE EXPERIENCE WITH THE SAMPLE DATASETS:

First of all, we advise you to spend some time with segmenting single cells using one of the sample datasets provided at: [www.3d-cell-annotator.org](http://www.3d-cell-annotator.org). As a beginner, concentrate on an isolated cell not touching other objects, and create nice initial contours by manually segmenting three orthogonal sections crossing the centre of the cell. By clicking on the “start” button in the “start/stop” section of the GUI of 3D-Cell-Annotator, you will see that the initial contour generally shrinks. “Delete” the segmentation and try again, but this time play with the “Desired Volume” value to force the contour to increase. Initially, you can also set a very low “Desired Sphericity” value, just to make the contour grow which allows you to understand the approximate volume of the object being segmented. Once you have an idea of the volume of the object, start the segmentation again from scratch by forcing that volume to increase to the desired value.



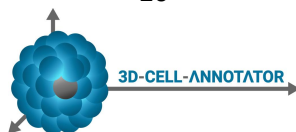
### “SURFACE SMOOTHING” AND “IMAGE IMPORTANCE” PARAMETERS:

In the first test, do not use these parameters. Just set the values to 1 in the “Weights” section of the GUI of 3D-Cell-Annotator. Generally, these parameters help you only after you have a rough segmentation with volume and shape, well approximating the object of interest. When you have reached that state, start to play with the weights of “surface smoothing” and “image importance” to improve the details of the segmentation, so that it nicely follows the contour of the object of interest.

### PARAMETERS MORE IN DETAIL:

In summary, 3D-Cell-Annotator has 4 main parameters, “Desired volume importance”, “Desired sphericity importance”, “Surface smoothing”, “Image importance”. When the flags in the “Evolution weights” Section are not active, these parameters assume the default value, *i.e.* 1. However, for all these parameters the range of values is  $[0, 10]$ . The following Table summarises the main effects of these parameters.

PARAMETER	MAIN EFFECT
Desired volume importance	It forces the 3D segmentation to reach the volume entered in the “Desired volume” field in the “Desired object properties” section. Typically, the segmentation starts from a volume lower than the real VOI. Accordingly, setting this parameter to a value higher than 1 (for instance, 3) in combination with a high “Desired volume” value, forces the 3D segmentation to expand.
Desired sphericity importance	Most of the cells/nuclei have a nearly spherical shape. Accordingly, setting this parameter to a value higher than 1 (for instance, 3) in combination with a high “Sphericity” value in the “Desired object properties” section, forces the 3D segmentation to grow as a sphere. This parameter can be very useful to avoid over-segmentation in case of touching objects.
Surface smoothing	Most of the time, the fluorescence signals of the cell/nucleus membranes are blurry. This causes the generation of a 3D surface with a high jaggig degree ( <i>i.e.</i> with well visible indentations). This parameter helps to obtain a sharp 3D surface, not following artefacts caused by the staining for instance.
Image importance	Surface evolution is based on the local characteristics of the cells/nuclei, represented as intensity gradients in the image. Setting this parameter to a value higher than 1 (for instance, 3) forces the curve evolution to strongly consider the local value of the voxels in the image. Using a too high value for this parameter, in combination with a high value of “Surface smoothing”, generally causes the tool not to reach an equilibrium point.



## REAL-WORLD BLURRY DATASETS:

Remember that 3D-Cell-Annotator is not a human: it is a tool that uses an algorithm to compute 3D segmentation of objects of interest. Precisely, it uses 3D active contours with shape descriptors as prior information. Although we proved that the segmentation precision generally reaches the level of a human expert, it cannot do miracles! If the dataset is so blurry that it is not possible 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 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 analysing blurry datasets!

## SEGMENTING A SERIES OF OBJECTS OF INTEREST:

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

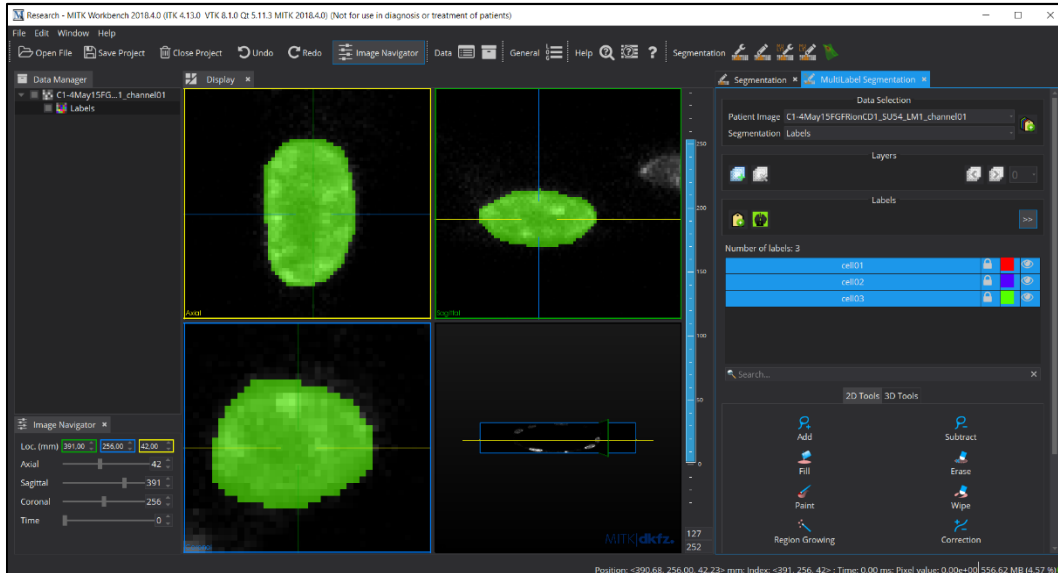
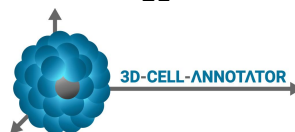
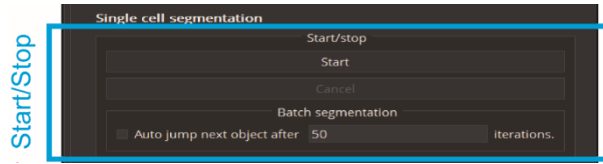


Fig. 24: 3D-Cell-Annotator run in batch mode to segment a series of objects of interest.



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

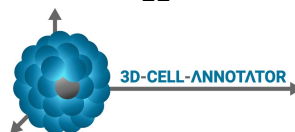


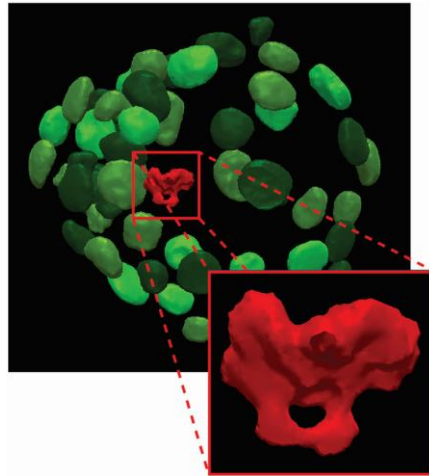
**Fig. 25:** 3D-Cell-Annotator GUI: “Batch segmentation” options.

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

## **9. CONCLUSIONS**

3D-Cell-Annotator is a tool for the easy segmentation of 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, you can exploit all the classical MITK functionalities that make analysis and rendering of 3D data very easy. For instance, once a group of cells is segmented, you can render their surface in 3D, estimate morphological parameters, and also use different colours to identify the cells classified in different groups. Finally, all the 3D surfaces can be exported as 3D binary masks, and so can be further analysed with external tools, for instance the popular ImageJ/Fiji.

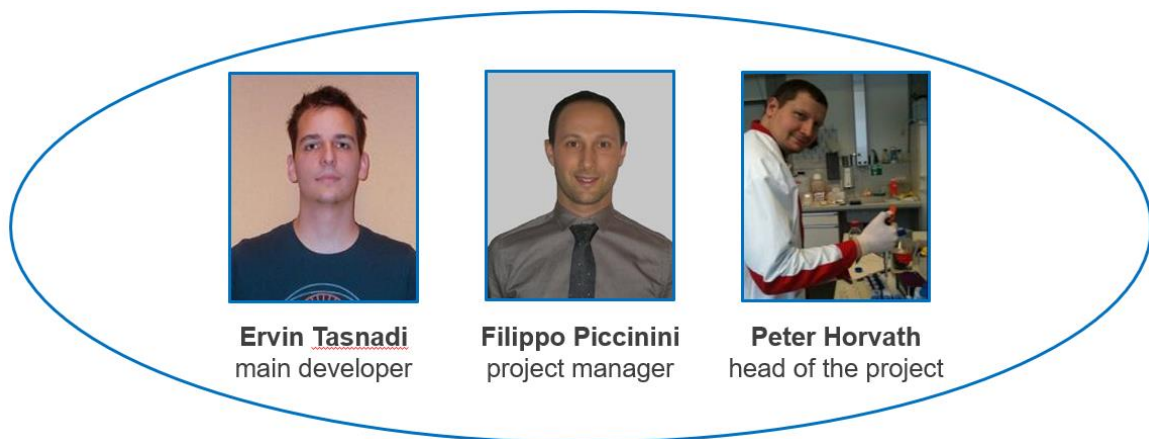




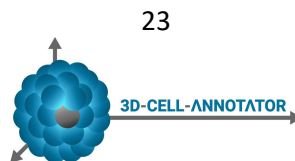
**Fig. 26:** Embryo dataset: cell with an interesting phenotype.

## **10. CONTACTS**

By reading this 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](http://www.3d-cell-annotator.org), and If you need further information or you have special requests, remember that we are open to collaborations! In that case contact: [horvath.peter@brc.hu](mailto:horvath.peter@brc.hu)



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



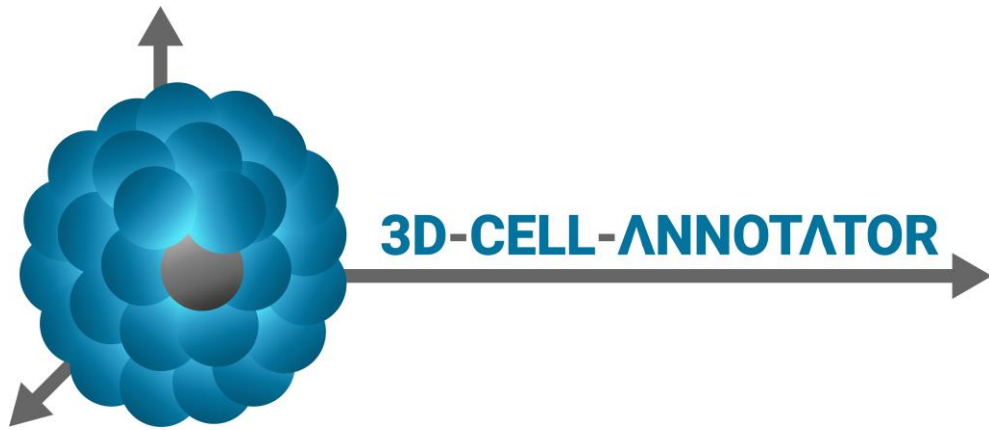


Fig. 28: 3D-Cell-Annotator logo.

