**User Guide**

System Requirement

1. Requires MATLAB (R2015a) or newer to run.
2. Computer memory requirement. The RAM has to be bigger than the size of the movie being analyzed + 4 times the size of a single z stack in the movie
3. 1920x1080 screen is recommended to display UI.

UI vs Script

GUI or matlab script can be used to interact with the package. To start the GUI, open the program ‘main.m’ and the UI will pop up. To work with the script, please check out sample\_script\_\*.m first. A detail description of class is available at the end of this file. Use of script is strongly encouraged if you want to process a batch of files and speed things up by parallel computing.

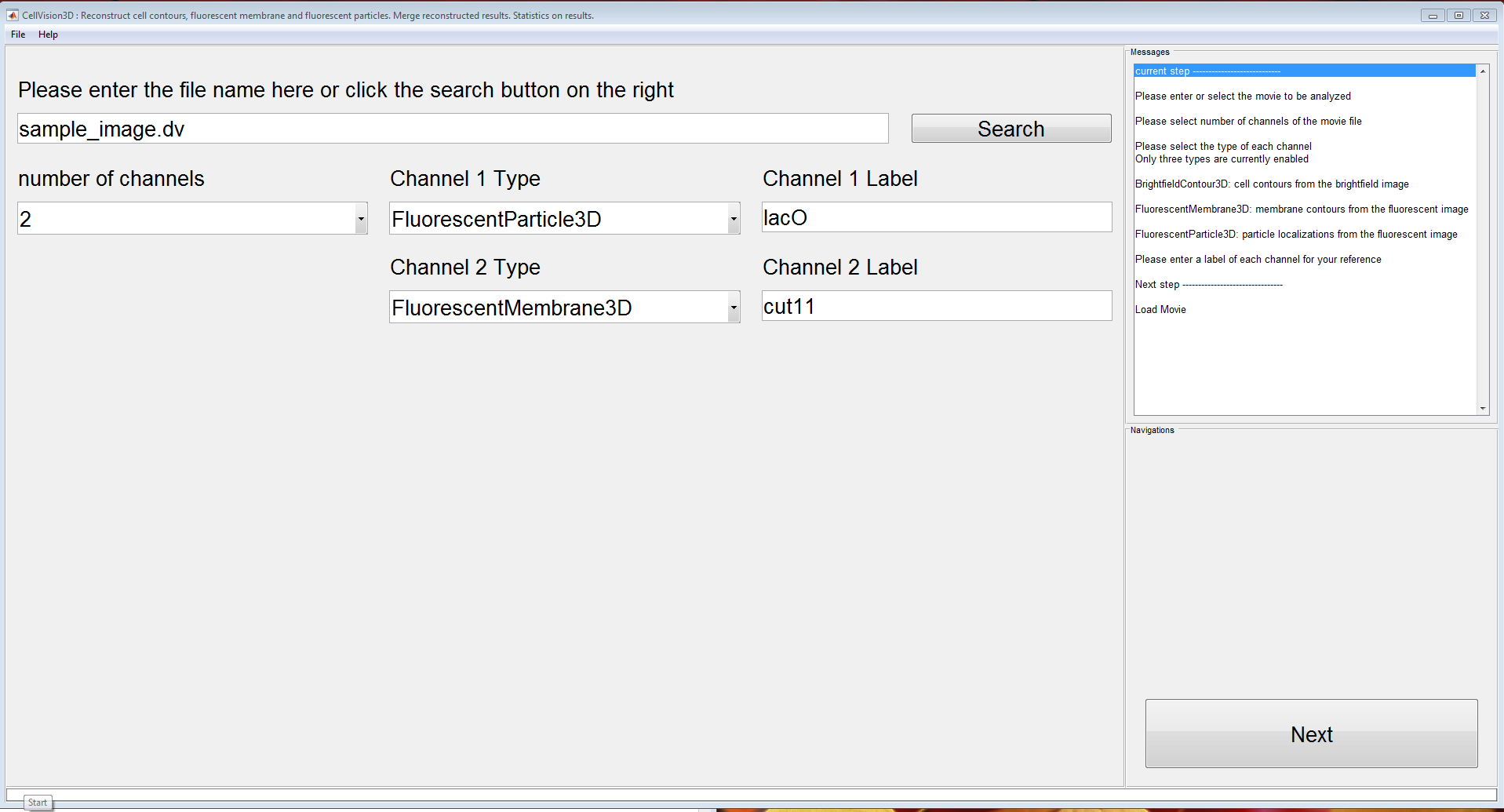
Sample Images

There are several different types of sample images

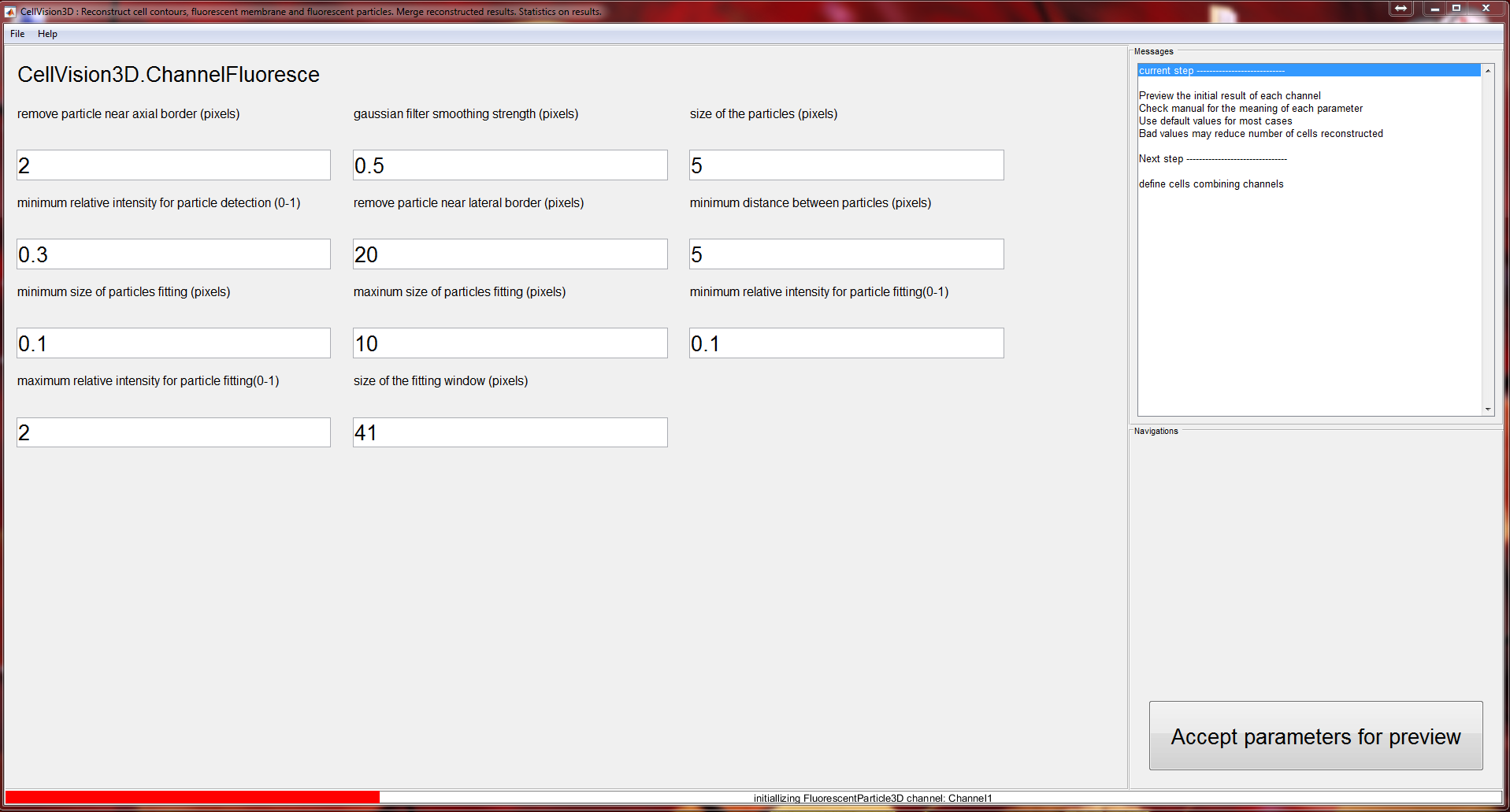
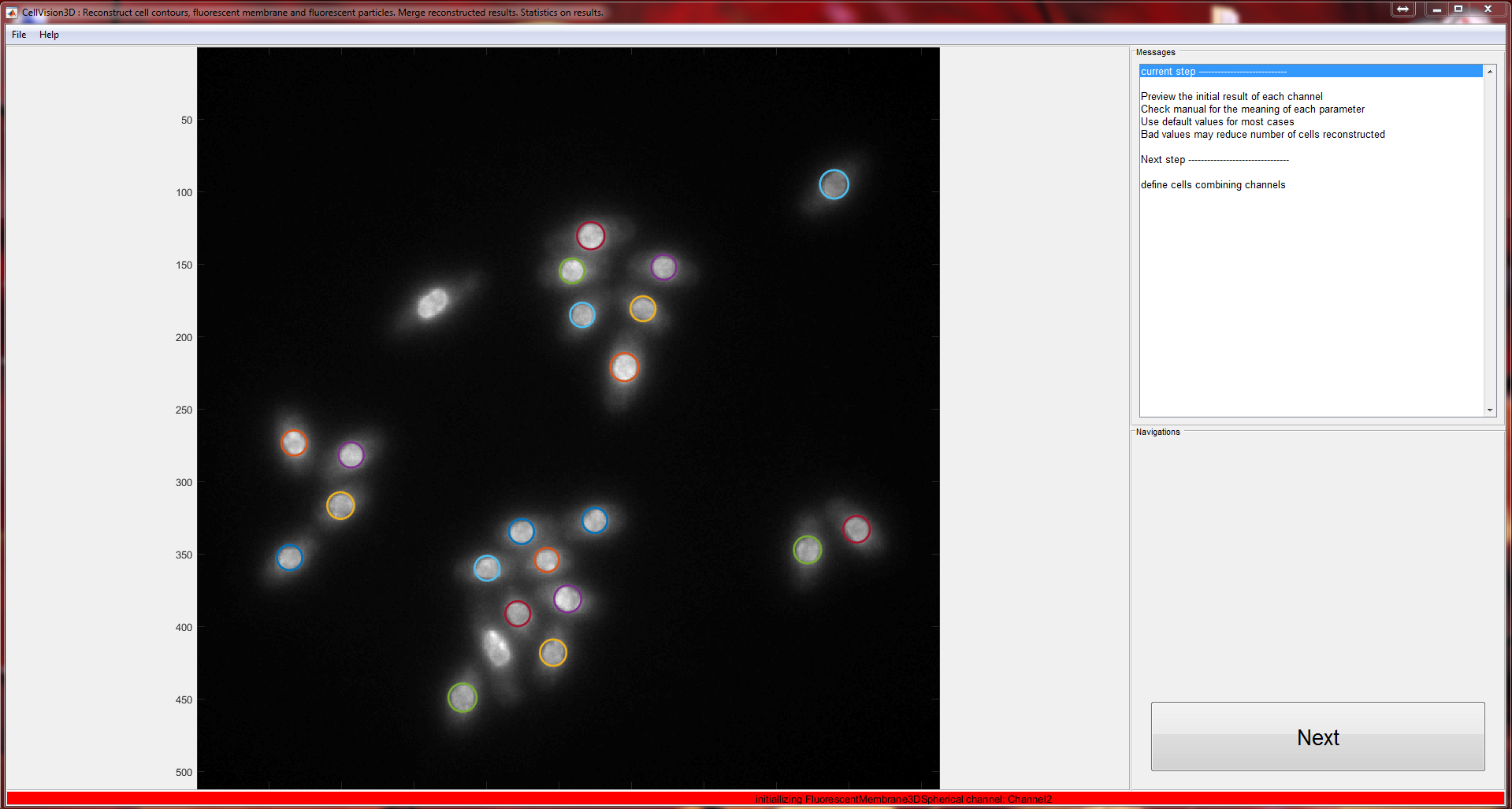
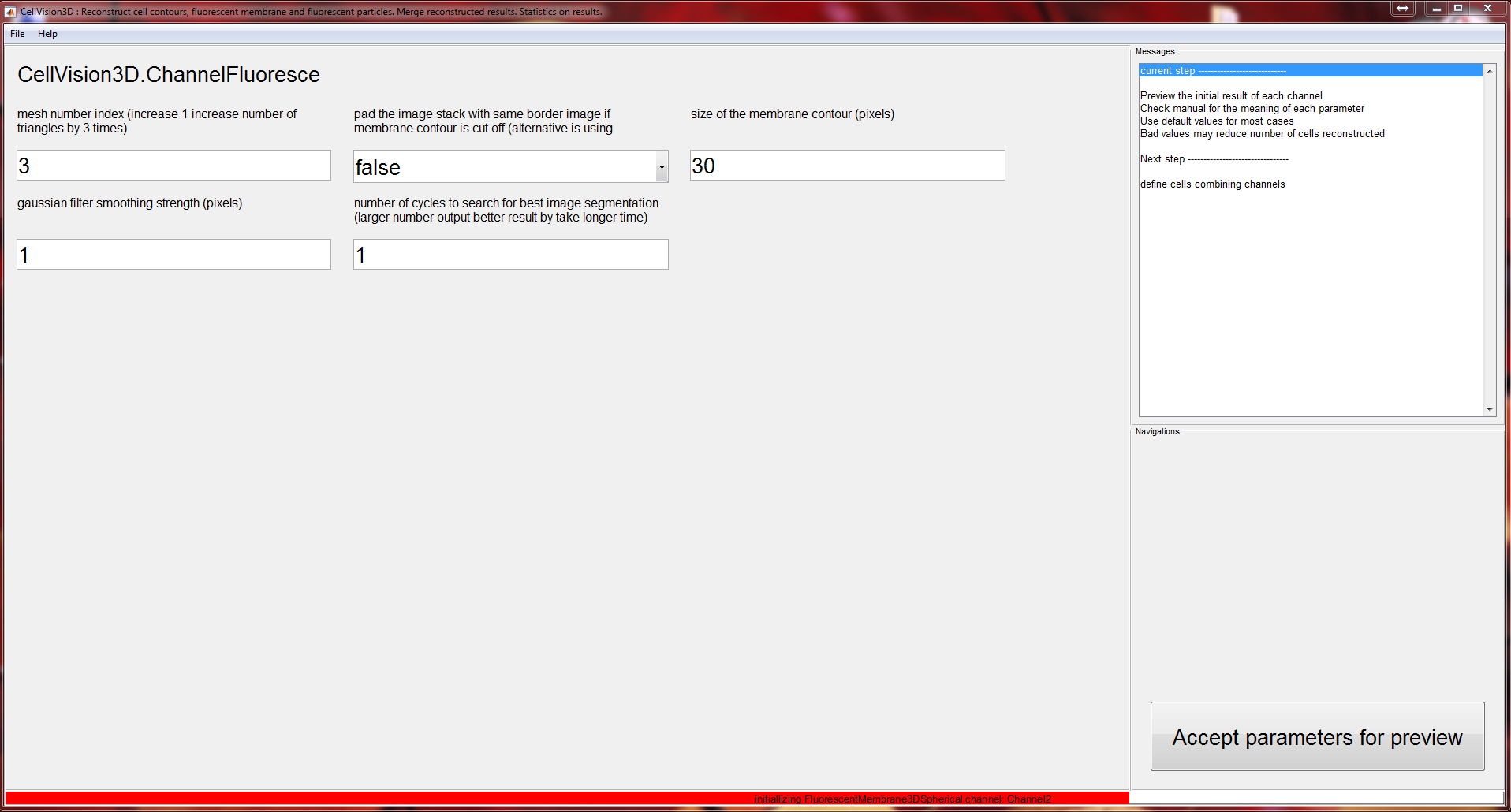
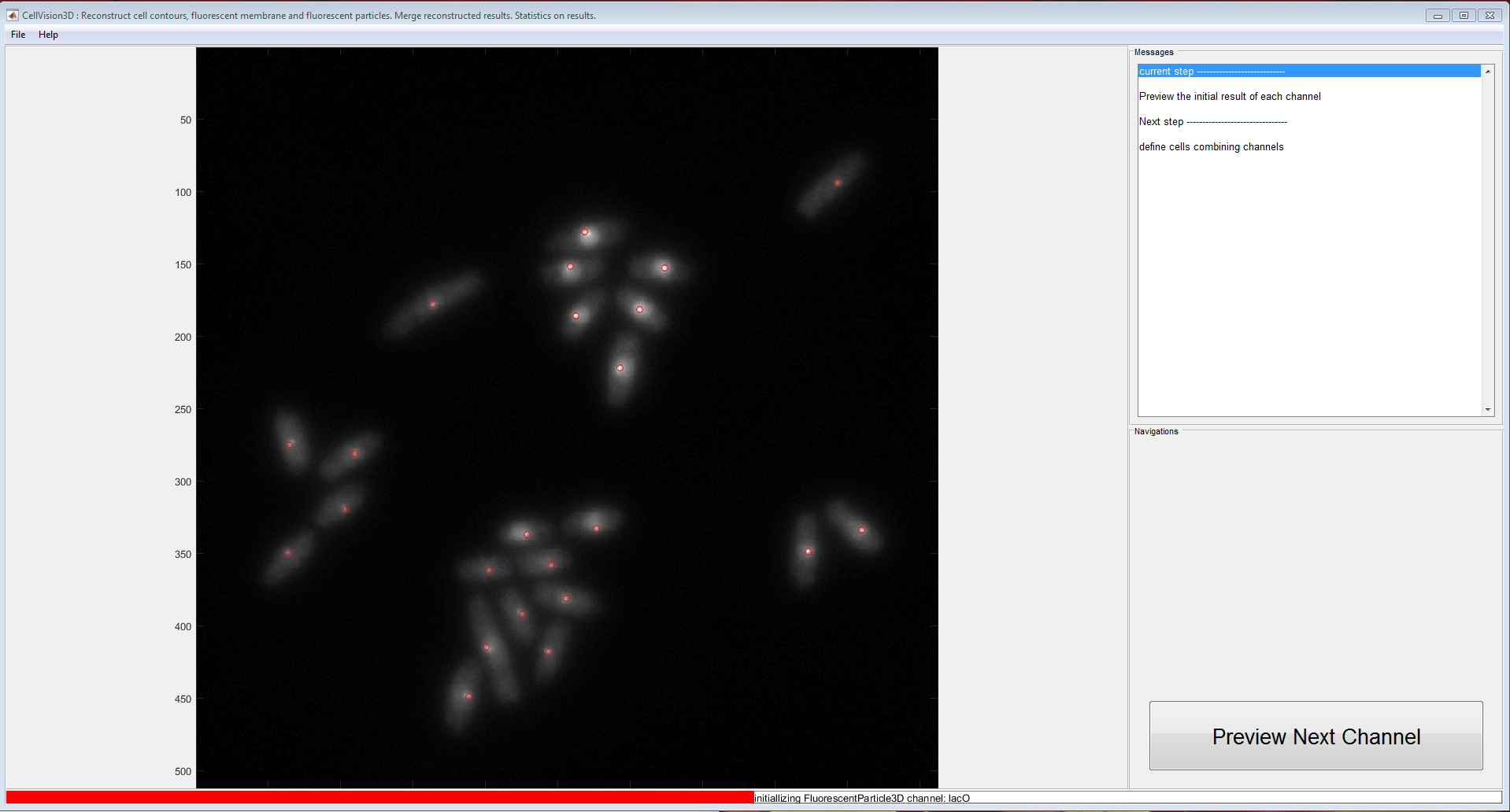
1. sample\_image.dv ---- fission yeast nuclei labeled with cut11-gfp
2. sample\_image\_er.dv ---- fission yeast, er labled
3. sample\_image\_mamalian.dv –---
4. sample\_image\_doubleparticle.TIF ----

UI Guide

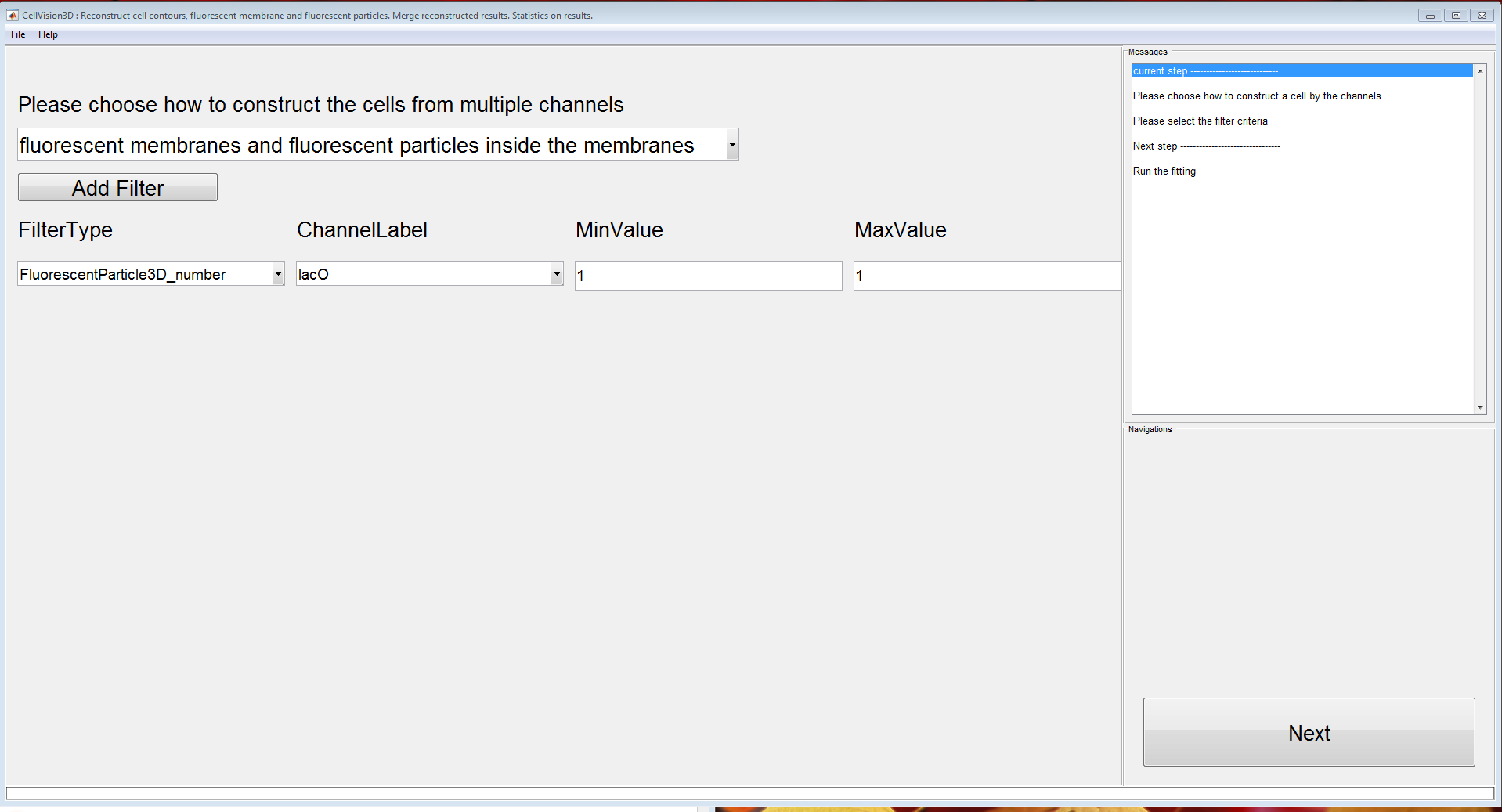
1. Run the program ‘main.m’ and a simple UI will pop up.
2. A sample image is provided with the example. Choose ‘sample\_image.dv’ from where you downloaded it. It is an image of fission yeast with fluorescently labeled nuclear membrane and lac operators. Select ‘FluorescentParticle3D’ as the type of channel 1 and select ‘FluorescentMembrane3D’ as the type of channel 2. Labels of channels are optional. Click next to go to the next view. Make sure the number of channels and their input orders are correct. For ER sample, it is the same as above. For double particle sample, choose only ‘FluorescentParticle3D’. For mammalian, choose only ‘FluorescentMembrane3D’.

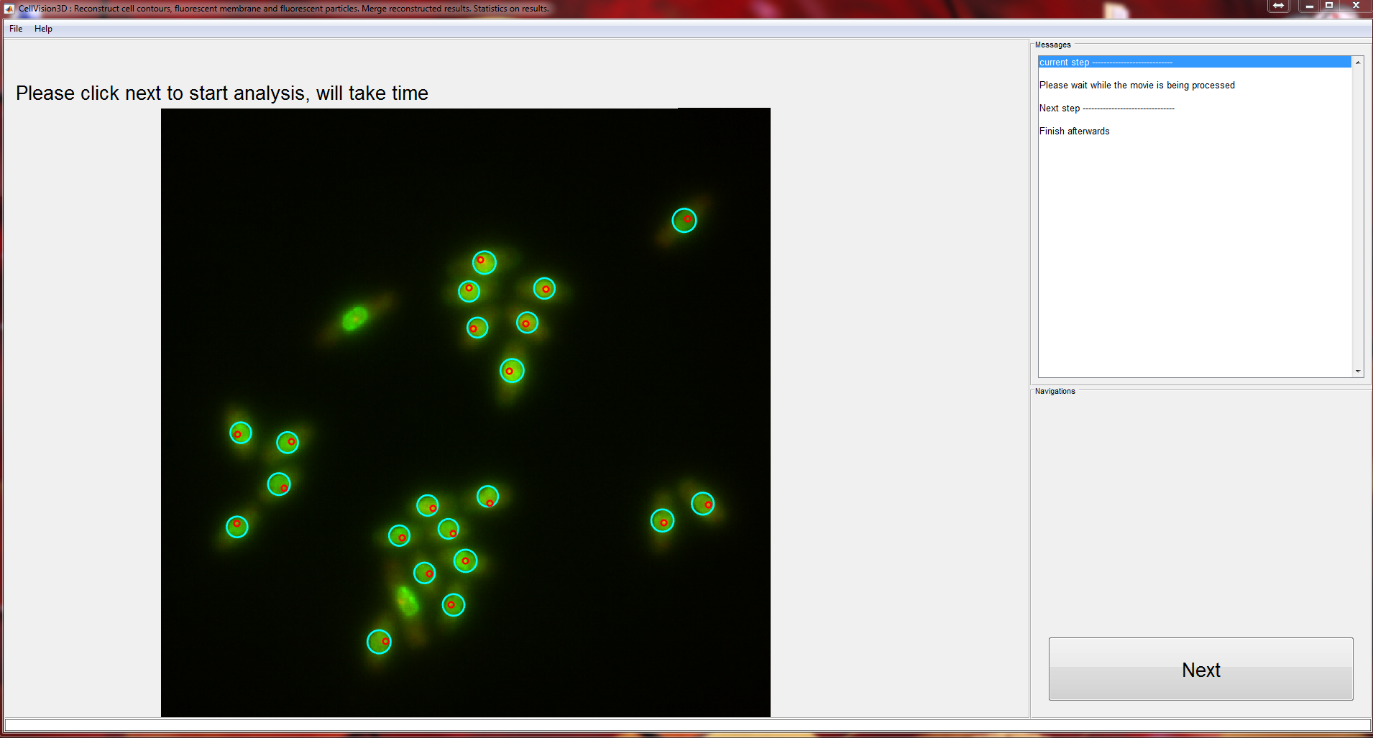


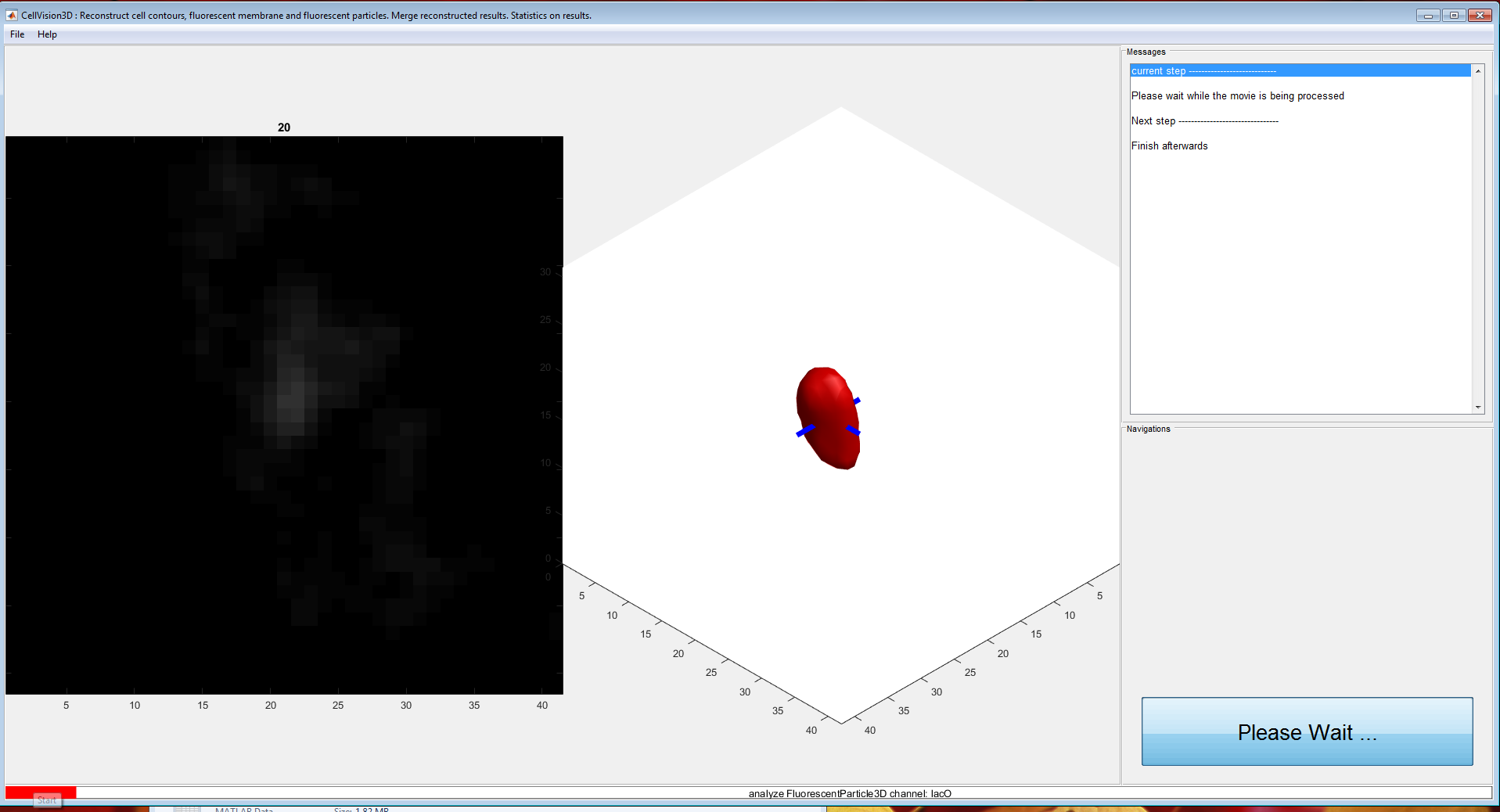
1. The program will ask for parameters of initial analysis of each channel and show you the results. For particle types of data, one important parameter is the minimum relative intensity for particle detection (for double particle sample, 0.1), it will directly influence the ability to include/not include dim particles. For fluorescent membrane types of data, one important parameter is the size of the membrane contour, set it larger than the actual size equal or large (for mammalian sample, 100) than the contour size. Mesh number index is not recommended to be changed. Image padding method only influences the reconstruction if the 3D object is being cropped by the zstack.

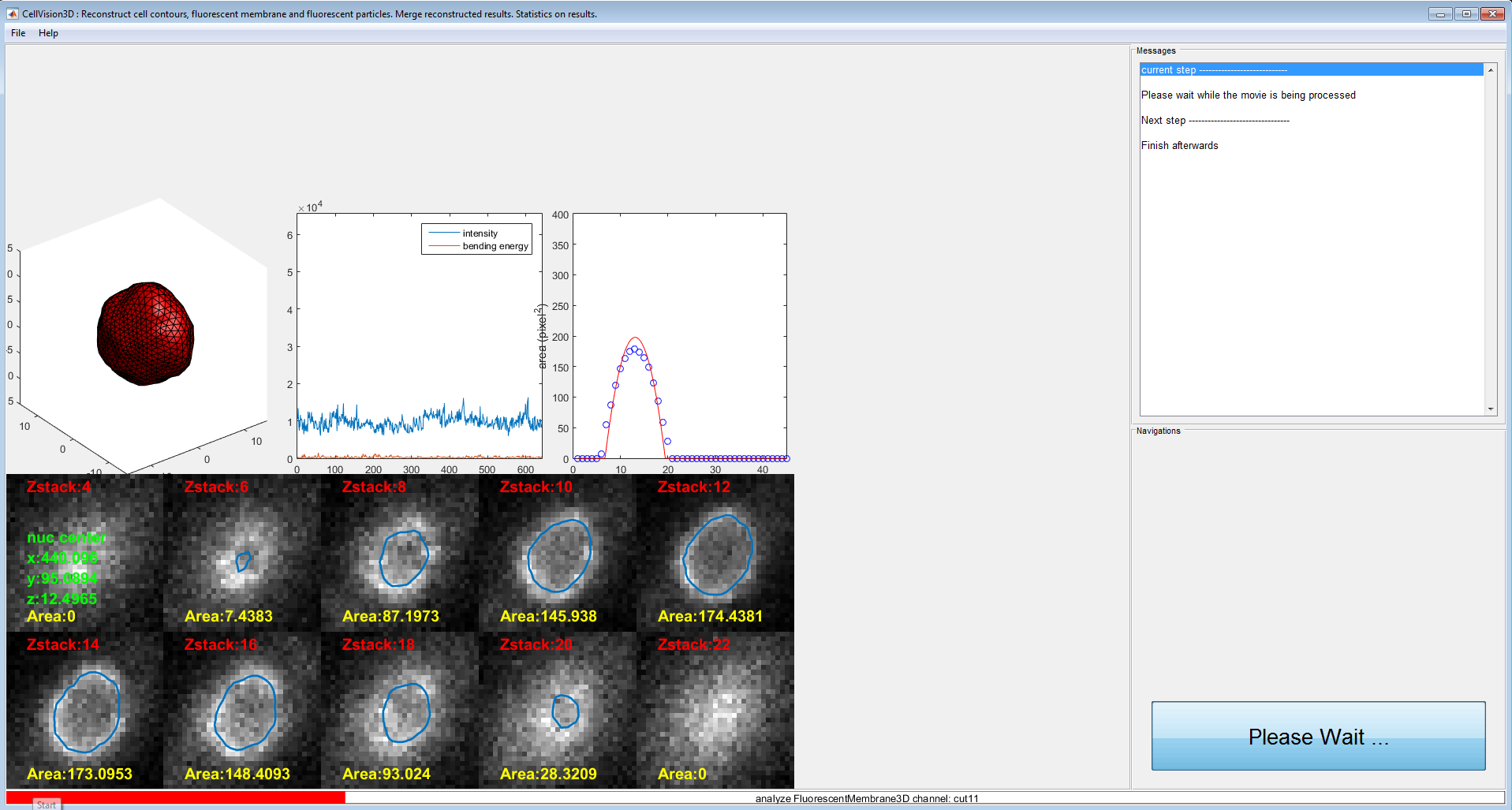
 

1. This view decides the relation between different channels. Select ‘fluorescent membrane and fluorescent particles inside the membrane’, it will figure out which particles belongs to nuclei. Currently only a basic cell filter is implemented. More filters can be added programmably. Choose FilterType to be ‘FluorescentParticle\_number’, and set the min value and max value to 1. This will filter out cells which has multiple particles or has no particles. The result will be shown in the next panel.

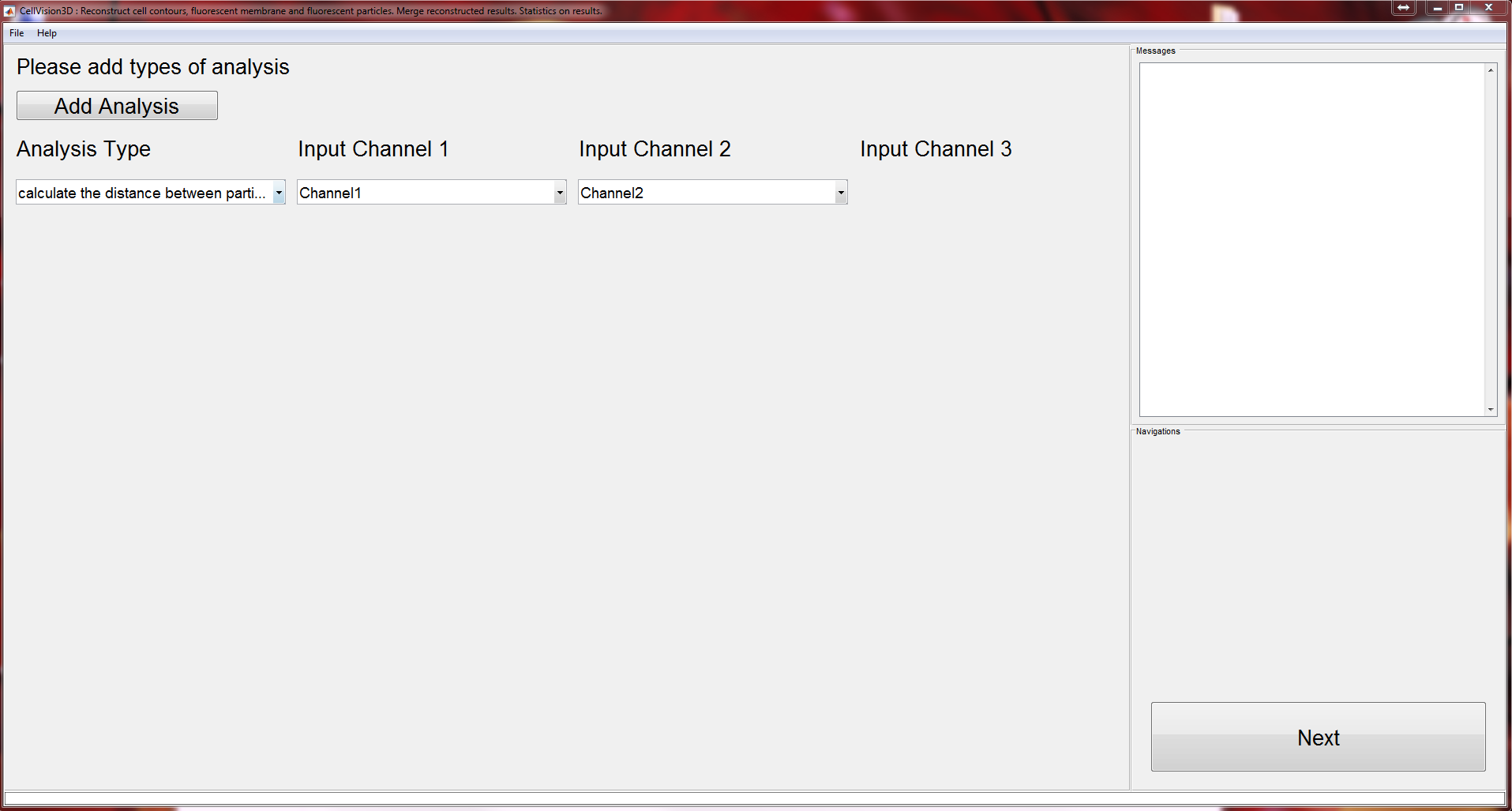




1. The program will analyze each channel. Intermediary results will be shown. 



1. After image reconstruction, choose analysis to be done. Pick the analysis type and all possible channels will be shown.



1. Click ‘Save and Close’ button. The program saves the tracks and shapes of the analyzed cells to a designated file. To analyze the result, please check the data structure of particles and contours to use the data . 