Quantitative Analysis of 3D Refractive Index Maps

Abstract

Nanolive's 3D Cell Explorer allows for the creation of high-content 3D images of living cells with very high spatio-temporal resolution (x, y:180nm; z:400nm; t:1.7sec). Being able to extract quantitative data describing the objects (population of cells, cells, organelles) contained in such images is at the core of leading-edge cell biology research. This application note will start where application note 2 (AppNote2017-2) stopped by looking at how meaningful numbers can be extracted from segmented objects using FIJI. We will then bring some cellular features in perspective to help you to start elegant quantitative studies with the 3D Cell Explorer.
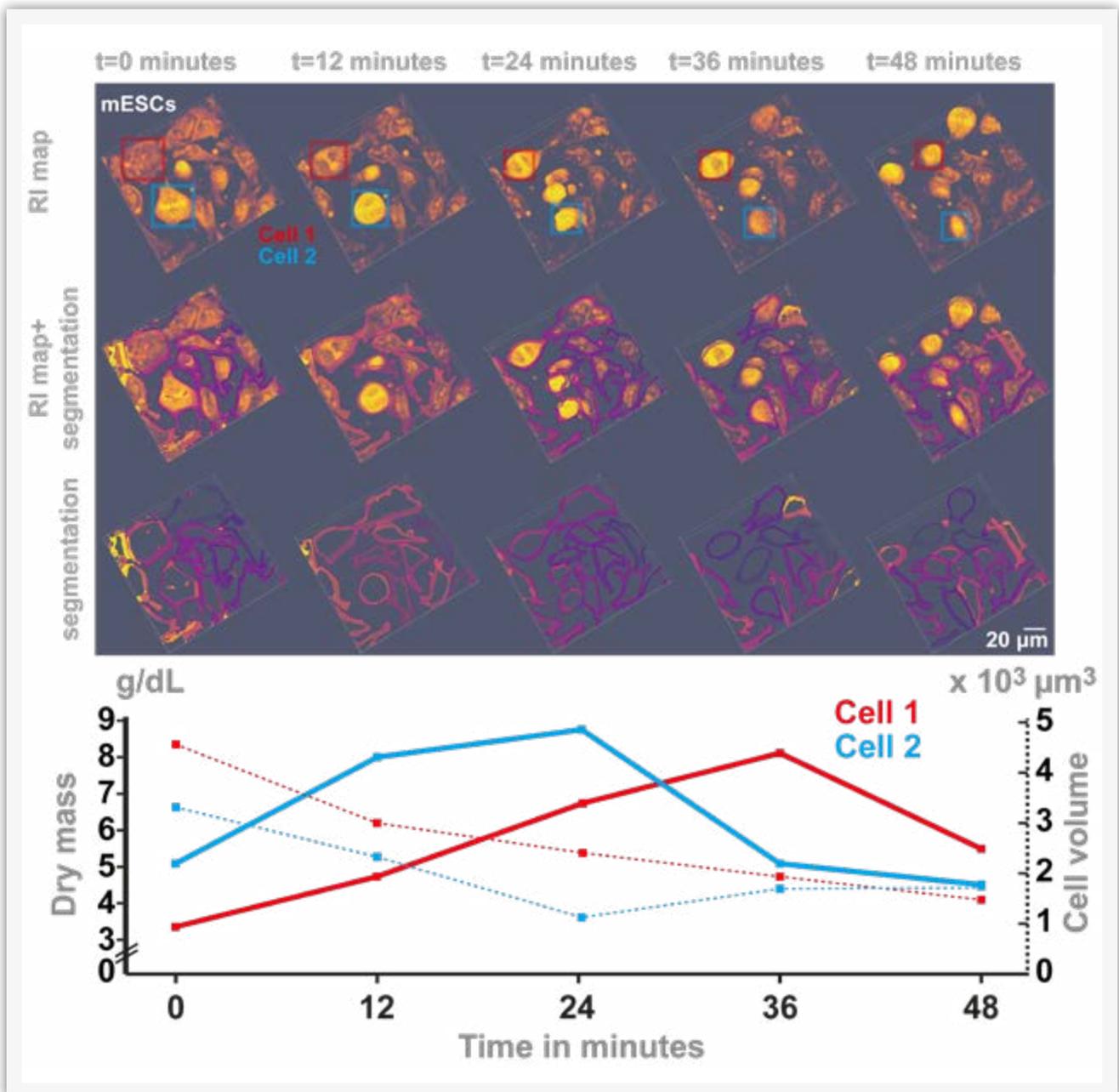
1. Introduction

The 3D Cell Explorer allows to acquire a three-dimensional map of the refractive index (RI) composing your objects of interest (Cotte et al., 2013). The finesse of the device allows to observe differences of RI as small as 0.001, by far the best sensitivity on the market, thus providing a fine view of the different elements composing a sample, such as the different compartments of a cell for example. Consequently, even a non-expert would be able to identify by eye the nucleus of a mammalian cell in an RI map. However, at the age of quantitative cell biology (Langen et al., 2015; Li et al., 2007) where seeing, feeling and manual image analysis is no more the norm, it is crucial that the computer crunches your image to extract meaningful object features organized in space, time and object identity (Frechin et al., 2015). The goal of this application note is to demonstrate how to obtain a set of numerical features that will describe your objects of interest once they have been segmented (See AppNote2017-2). We will use two examples to illustrate the use of FIJI for extracting quantitative data based on sets of 3D segmented objects. The two examples will concern i) HeLa cell nuclei and ii) a time series of mouse embryonic stem cell images. This will require the software FIJI (Schindelin et al., 2012; Schmid, Schindelin, Cardona, Longair, & Heisenberg, 2010; Schneider, Rasband, & Eliceiri, 2012) and the software STEVE (http://nanolive.ch/software/#trySteve). We will see how to extract a large panel of features from an object as well as simple visualization cases, more advanced 3D object segmentation and feature plotting with a focus on how to calculate dry mass out of refractive index values. Please keep in mind that the parameters and procedures shown here are no rule of thumb and should be carefully evaluated before applying them to your specific problem.

2. Prerequisites

First, you will need the latest version of STEVE (http://nanolive.ch/software/#trySteve), the software that allows to control your 3D Cell Explorer, and second, the **latest version of FIJI** (http://fiji.sc/#download) with the **3D ImageJ suite** (See AppNote2017 1 or 2).
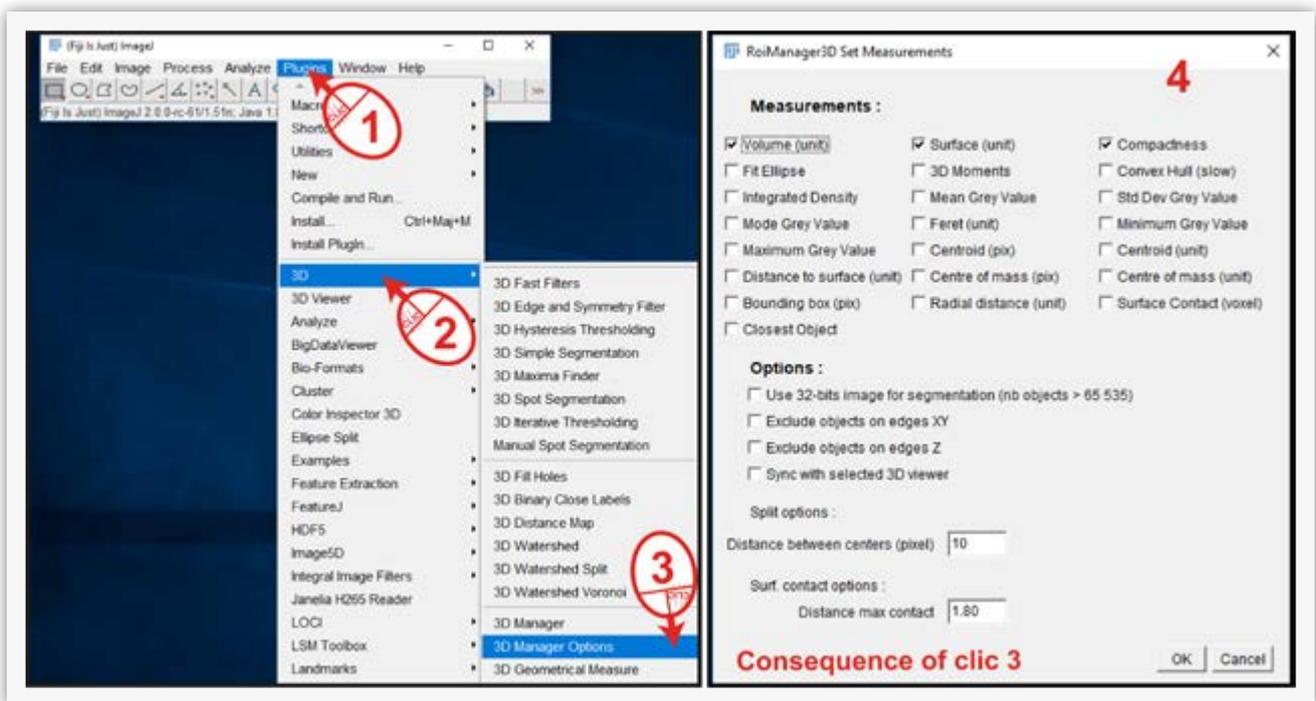
Figure 1: Setup of the 3D manager options

This application note starts exactly where AppNote2017-2 stopped. Thus, you should launch the latest version of FIJI and load the stack of 3D segmented HeLa nuclei. Follow the procedure described in **Figure 1** to set up the **3D manager options**. This set of options will ensure that your first calculations are processed quickly. Only a few measurements will be requested later such as the volume and surface of each object, expressed in voxels, as well as the compactness that gives information on how spherical an object is (from 0 to 1).

3. Using the 3D manager with segmented objects
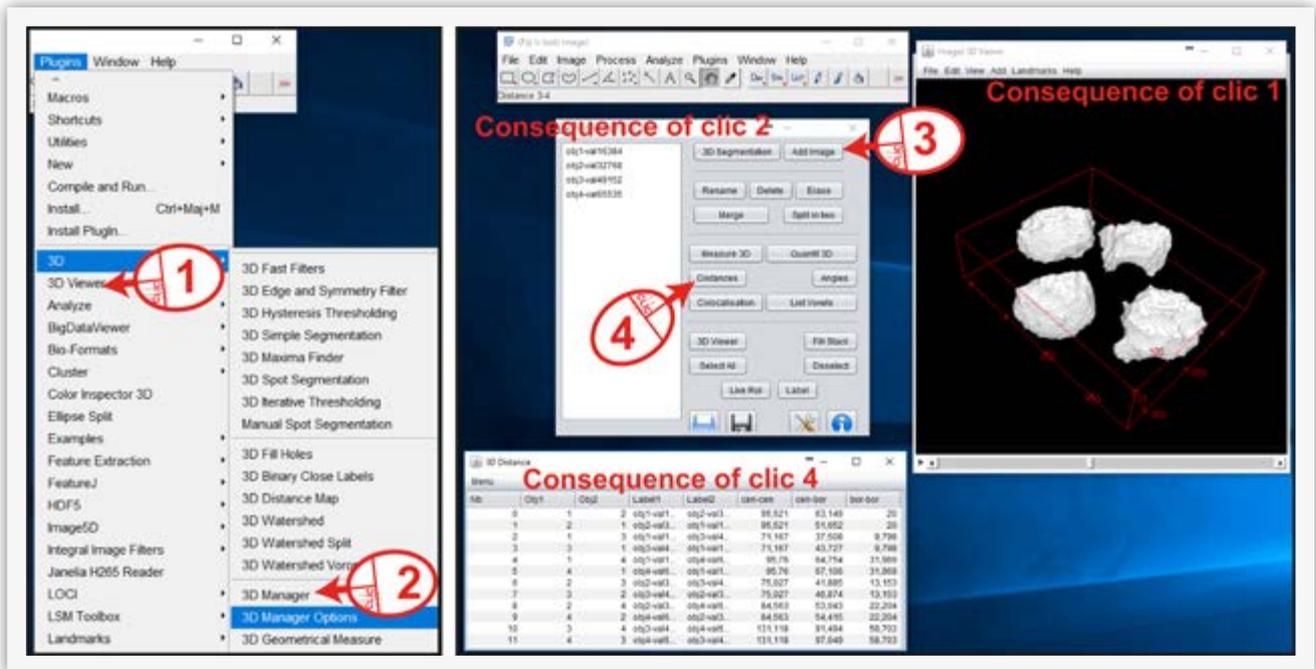
Figure 2: Using the 3D manager

The 3D manager is certainly the best free 3D object analysis tool, and it is very easy to use (for a complete manual of the 3D analysis suite of ImageJ see: https://goo.gl/syLkaG). To visualize the stack of segmented nuclei in 3D, click on the stack that you want to see in 3D, then go to Plugins > 3D Viewer (**Figure 2, step 1**). To analyze these objects, go to Plugins > 3D > 3D manager (**Figure 2, step 2**). A window opens, click on the segmentation stack and then click on the **AddImage** button (**Figure 2, step 3**). This action will load the objects contained in the segmented image. Click now on the **Distances** button. This action creates a table that lists various distances between each object such as centroid to centroid or edge to edge distances (**Figure 2, step 4**). **Figure 3** shows a selection of features given by the **Distances and Measure 3D** functionalities of the 3D manager plugin. The tool provides measurements in voxels and conversion to µm2 or µm3 is done by considering the dimensions of a single voxel in x,y,z which are 0.186, 0.186 and 0.372 µm.

You can increase the number of features that are returned by the **3D manager** by changing the **3D manager options** (**Figure 1**).

Object	Volume µm^3	Surface µm^2	Compactness Sphere = 1
1	902	553	0.4
2	951	493	0.6
3	1380	633	0.63
4	1070	560	0.55
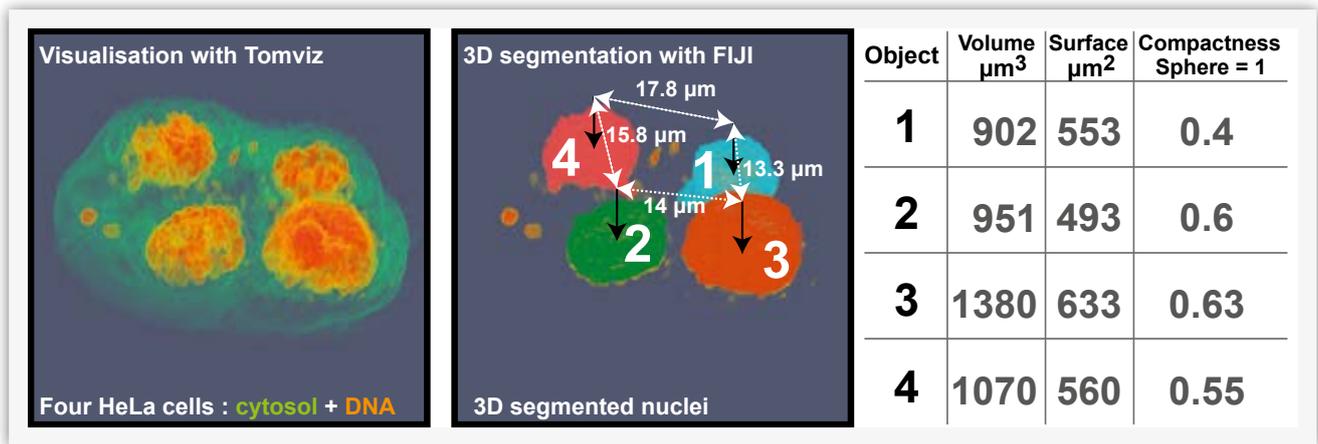
Figure 3: Extraction of HeLa nuclei features

4. Advanced segmentation using MorphoLibJ

We will now look at a slightly more advanced segmentation of mouse embryonic stem cells. This procedure follows the same procedure as exposed in the A p p N o t e 2 0 1 7 – 2. Instead of segmenting nuclei, we segment entire cells here. While nuclei could be segmented using a simple segmentation based on thresholding (**Plugins > 3D > 3D Simple Segmentation**, see AppNote2017-2), we use here the **MorphoLibJ** plugin. Follow the installation procedure of the plugin: **Help > Update > Manage update sites** and tick **IJPB-plugins**, close and apply changes.
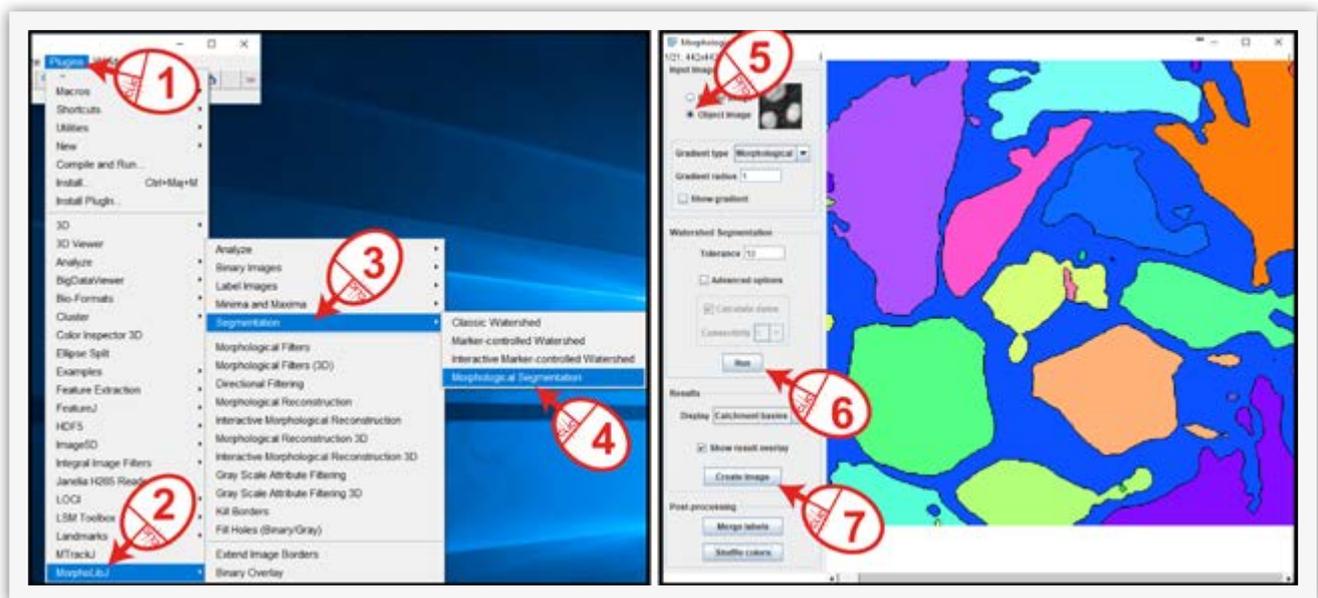
Figure 4: MorphoLibJ: morphological segmentation allows for a more advanced segmentation

Follow the procedure shown in Figure 4 to open the morphological segmentation tool (**Figure 4, step 1-4**). As a starting point for your specific case, we recommend clicking **object image** (unless you have a border image you already derived from your original image) to leave the **gradient radius on 1** (parameter used to detect object borders) and the tolerance on 10 (minimal distance to detect successive maxima in the image). Click **Run** and see what happens. This turns out to be an excellent starting point for 3D Cell Explorer images in general (**Figure 4, step 5 and 6**). You can then create

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an image out of **Catchment bassins** (**Figure 4, step 7**). You will then need to transform it into grayscale to be able to use it back as a normal segmented image. To do so, click on **Image > Type > 8bit** after selecting the colored image you just produced. The usage of such a tool certainly requires time to find the proper parameters to obtain the desired results.

5. Example of quantitative analysis of mouse embryonic stem cells division
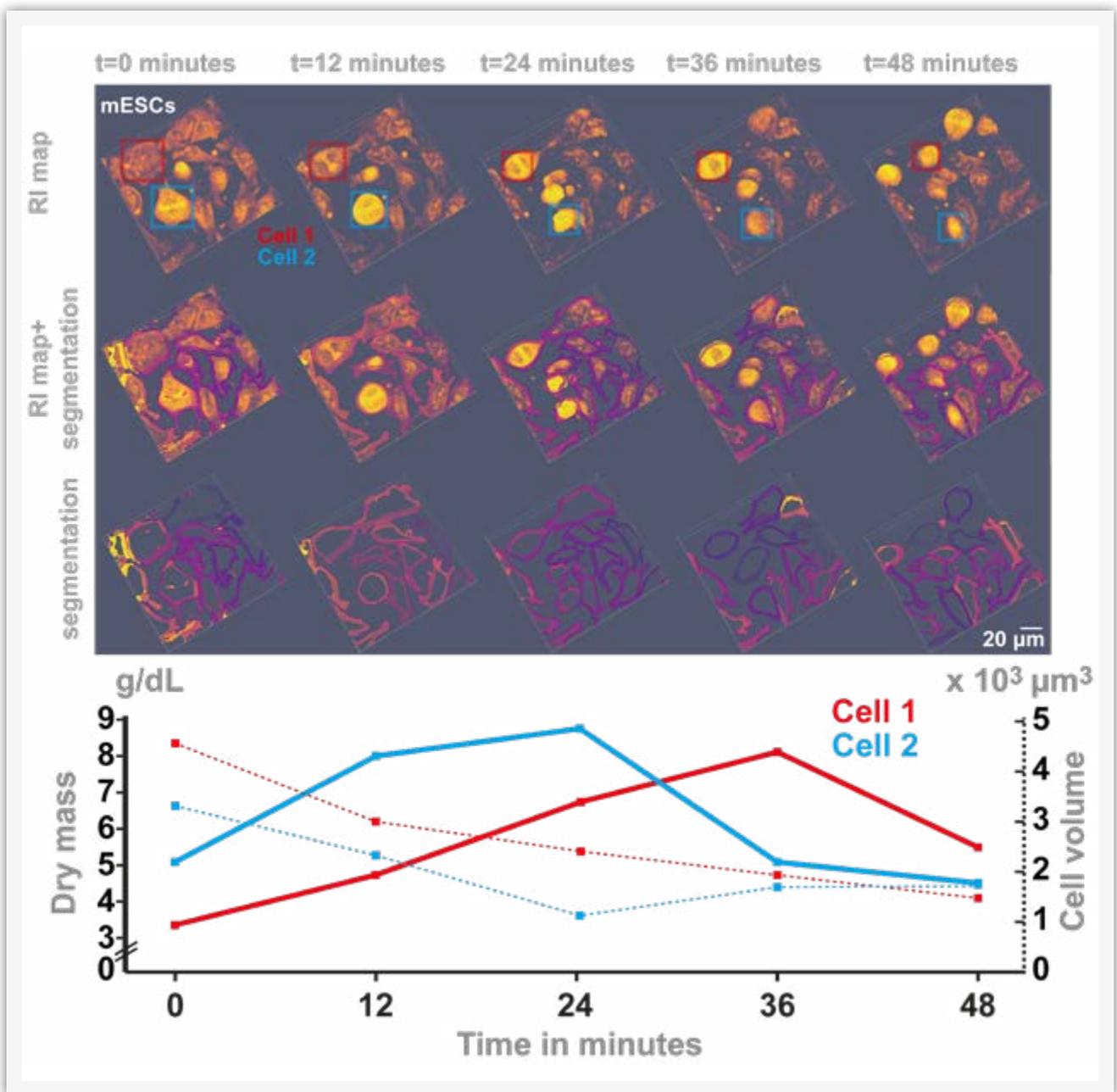
Figure 5: Analysis of cell volume and dry mass of two dividing mESCs

It is very easy to extract information from another image such as a 3D refractive index map if a specific 3D segmentation has been made. Load your 3D segmentation in the **3D manager** as described in **Figure 2 in paragraph 3**. Then, open the image from which you want to extract information (the 3D refractive index map). This image must have the same size as the segmented image. Select it as the active window by clicking on it, then go on the **3D manager** window and click on **Quantif 3D**. The table that opens lists all the required measures, one line per object contained in the segmentation image you loaded first. The features that can be returned by **Quantif 3D** are those related to gray values in the **3D manager options** panel. By making adjustments, you will get a feel of it and will certainly obtain the results you are looking for.

The analysis displayed in **Figure 5** illustrates mouse Embryonic Stem Cells (mESCs) imaged over 48 minutes with the 3D Cell Explorer. The cells in the recorded images have been segmented following previously described procedures (see AppNote2017-2 **and paragraph above**) to be able to extract not only the volume but also the refractive index values of each object. The dry mass of each object has then been calculated using the following formula (Friebel & Meinke, 2006; Phillips, Jacques, & McCarty, 2012):

$$[DryMass] = \left\{ \frac{RI}{RI_{H2O}} - 1 \right\} \times \frac{1}{k}$$

where

$k = 0.002$

for material that has no specific light absorbance characteristic.

Those values are then plotted as a function of time, for two cells that undergo mitosis one after the other. One can observe that the concentration of cellular dry mass increases during mitosis, and decreases afterwards. Yet interestingly, the volume of those cells is still relatively small when the dry mass concentration starts decreasing, suggesting an active loss of cellular material during the post cytokinesis times.

6. General Hardware & Software Requirements

3D Cell Explorer models:
      3D Cell Explorer
      3D Cell Explorer-*fluo*

Software:
      STEVE – all versions
      FIJI with the **3D ImageJ suite**

7. References

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