NANOLIVE

Growing and Filming Stem Cells with the 3D Cell Explorer

Abstract

Nanolive's 3D Cell Explorer allows for the creation of very powerful 3D images and 4D time-lapses of living cells with very high spatio-temporal resolution (x,y:180nm; z:400nm; t:1.7sec). However, to take full advantage of the microscope's live imaging capabilities, a proper setup of Nanolive's top stage incubator is necessary, especially when dealing with stem cells that are known to be sensitive to experiment-induced stresses.

In this application note we will provide some suggestions to perform long-time imaging (up to weeks) of mammalian stem cells, at high spatio-temporal resolution and in 3D. We will explain how to record unique stem cell movies and export them from the 3D Cell Explorer's software STEVE. Furthermore, we will cover the following key topics: perfect humidity maintenance, CO_2 and temperature control as well as correct imaging regime.

Please follow this link for great stem cells movies: https://vimeo.com/262374462.

1. Introduction

Long-term imaging of fine cellular dynamics is today's biggest challenge in cell biology (Frechin et al., 2015; Kruse & Jülicher, 2005; Kueh, Champhekhar, Nutt, Elowitz, & Rothenberg, 2013; Skylaki, Hilsenbeck, & Schroeder, 2016). The goal is not only to acquire snapshots of dynamic biological systems, but to actually follow such active processes (Muzzey, Gómez-Uribe, Mettetal, & van Oudenaarden, 2009). However, fluorescence microscopy, which is the current method of choice in high-content live imaging approaches, induces phototoxicity when the sample is stimulated at various wavelengths. This stress induces cellular damages via radical-induced cellular structure alteration, which limits live imaging possibilities. Therefore, with relatively robust cell types such as cancer cell lines, a tradeoff must be found between short live cell imaging with high-frequency acquisition or long-term live cell imaging with low-frequency acquisition. With sensitive cell lines, such as mammalian embryonic stem cells, there are simply no proper long-term movies produced yet.

Because the 3D Cell Explorer's laser injects 100 times less energy into the sample than the gentlest of the current fluorescent imaging approaches, given that proper data management and environment control are set up correctly, it is technically possible to perform endless live imaging at the maximal acquisition speed (1 image per 1.7 seconds). This certainly exceeds any live cell imaging capabilities of any other microscope and has revolutionized live cell imaging of stem cells.
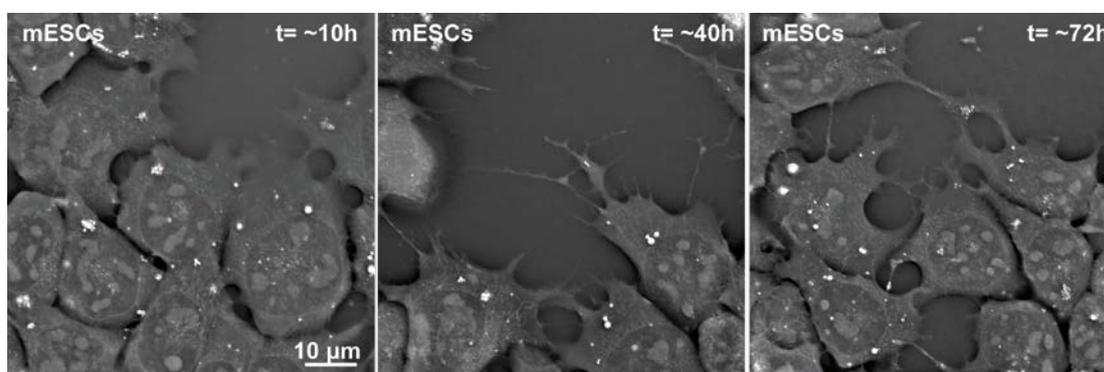

Figure 1: Live imaging of mouse embryonic stem cells for ~3 days. One image per minute. The 3D Cell Explorer generates no phototoxicity.

2. Prerequisites

First, you will need mouse Embryonic Stem Cells (mESCs), growing in a glass bottom dish compatible with the 3D Cell Explorer (http://nanolive.ch/wp-content/uploads/nanolive-ibidi-labware.pdf) inside a typical cell culture incubator. Note that this document is not about mESCs cell culture which is a research field on its own. If you wish to start such cell culture in your laboratory, please approach a specialist that will explain the necessary dish coating and medium composition for maintaining this very specific cell type.

Secondly, you will need Nanolive's top stage incubator equipment. This includes the top stage incubation chamber, a controller pad, and a humidity system. We recommend using our CO_2 mixer and air pump that will ensure a proper control of CO_2 proportions and will help you save some money on compressed air. Learn how to set up your Nanolive top stage incubator here: http://nanolive.ch/supporting-material/.

Finally, you will need the latest version of STEVE (Version 1.5 at least) to benefit from the powerful data export tool.

Figure 2: 3D Cell Explorer set up with its top stage incubator

Important note: the use of phenol-red free medium is preferred for best live cell imaging performances. Your favorite supplier certainly makes buffers optimized for live cell imaging.

3. Preparation of the environment control

a. Temperature

Letting the system equilibrate before use will ensure that the parameters will not drift out of your acceptable ranges. Our strongest recommendation is to first see how your top stage incubator reacts with your 3D Cell Explorer and with your imaging room, considering that each room has its own temperature and humidity. **We recommend limiting entry and exit from the room while the live cell imaging experiment is ongoing, and to be aware of automatic air conditioning programs in your room.**

Start the microscope and the computer, place the chamber on the microscope stage (if you just got it, remove the blue stickers within the removable part of the chamber that might impact the flatness of your field of view), connect it properly (Please follow this link for the setup video: http://nanolive.ch/supporting-material/#setup-incubator) and place the lid. Switch the controller on, and set the temperature to 38 degrees C°.

Figure 3: Top Stage Incubator Temperature Controller

Next, fill the humidity bottle with distilled water and start the injection of humid air at 1 liter per minute, which is when the small metal ball reaches the top of the mixer column. Leave the CO_2 off, it is not required yet. You can now wait ~2h so that the system equilibrates in temperature, particularly the microscope's stage.

You might think it is wrong to set the temperature to 38 degrees because mammalian cells grow at 37 degrees. The reason is that there is a difference between the temperature in the chamber, and the effective temperature in the well. We observed that in our conditions: a 38 degrees setting is suitable to obtain 37 degrees in the well. You can check this for your local conditions: after the two hours wait and without changing any of the parameters (gas, temperature), prepare a test well with culture medium in it and place it in the chamber with the green probe in the liquid, then close the chamber. You can immobilize the probe with tape if you cannot manage to keep it in. After 30 minutes, observe the temperature of the growth medium, it should be close to 37 degrees. If not, play with the temperature of the chamber and let it equilibrate. Be aware that the temperature you will set to generate 37 degrees in the well is in function of many parameters such as the temperature and humidity of the room (be especially careful with air conditioning cycles), but also the flux of gas sent into the chamber. Therefore, we recommend to always leave the flux at 1 liter per minute.

Finally, do not get too preoccupied: few tenths of degrees away from the gold 37 degrees standard won't hurt (Watanabe & Okada, 1967).

b. Humidity

Humidity control is very important for long term live cell imaging, especially for stem cells. The air humidity saturation is almost 100% when arriving in the chamber, however, almost is not enough. We always observe a tiny loss of medium in the well because of evaporation and that can be extremely perturbing for stem cells. To counterbalance this loss, the incubator manufacturer, Okolab, recommends placing a 1 cm thick stack of Whatman paper all around the inner profiles of the chamber and soak it with water.

To make such a stack simply cut enough Whatman paper sheets according to Figure 4. Because the absorbed water increases the thickness of the sheets, you will only need 2 to 6 sheets depending on your paper thickness.
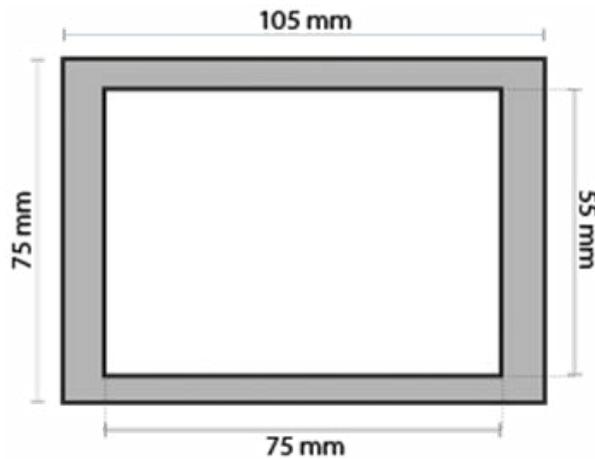

Figure 4: Dimensions for cutting a Whatman paper sponge

It is important to keep an eye on the amount of water contained within the paper. If necessary, you can open one access on the side of the chamber (remove a tiny screw) to easily re-humidify the Whatman paper without opening the chamber. We tested this solution and it ensures no medium loss for at least a week.

c. pH control

In the case of stem cells, we recommend setting the CO2 to 4-5%, which works well for the carbonate buffers.

Figure 5: Manual Air / CO_2 Mixer

4. Starting the live imaging experiment

Now that your temperature and humidity are under control, as well as your CO_2 mix, proceed with your experiment as quickly as possible to reduce the time cells are out of a controlled environment as well as the time the chamber lid is open. Once the cells are in the chamber, we recommend waiting for at least 30 minutes to let them adapt and to verify that everything is in order.

You are now ready to start a time-lapse.

5. Tips during acquisition

If you followed the procedure described above, you should observe very little drift in the Z position of your sample. This drift is usually due to incorrect equilibration of the system's temperature. It can also be due to sudden changes in room temperature. Keep people out of the room and ask for stable air conditioning during the experiment.

If you plan to do more than two days of acquisition, you will have to refill the water bottle. Do not touch anything else, just open the bottle and fill it while the gas is on. This is a fast procedure and will have no impact on your experiment. However, if the bottle is on the same table as the microscope, you must avoid bumping into it or even leaning on the table. Any movement or vibration can alter the positioning of your sample.

6. Acquisition setup

The automatic calibration of the microscope optical path can be made at any position of your well. Do not worry if you need to move to another field of view after calibration. We then recommend that you start with one acquisition of refractive index map every minute, for as long as you want, to see how your cell type reacts. With such regime, we successfully made a three-days movie of growing mESCs (see figure 1) and only stopped by the fact that the well became too crowded with cells. We also experienced perfect results with 4 images per minute. Do not hesitate to record at higher frequencies since the 3D Cell Explorer injects virtually no phototoxic stress to the cells.

7. Exporting timepoint images and movies with STEVE

Once the acquisition is complete, you will be able to export the time point images under different formats as well as the entire movie or subparts of your movie as .avi file. To do so, open the export tool integrated in STEVE, that will guide you step by step in the process of exporting your data in the appropriate format (Figure 6, part 1 and 3). You will be able to select which part of the movie you want to export (Figure 6, part 2), which z-slices you are interested in (Figure 6, part 4), the file format of the export (Figure 6, part 5), and the format of file naming (Figure 6, part 6). Thanks to that versatility, you will be able to export your data into any desired format, especially if you want to incorporate STEVE in an image analysis pipeline.
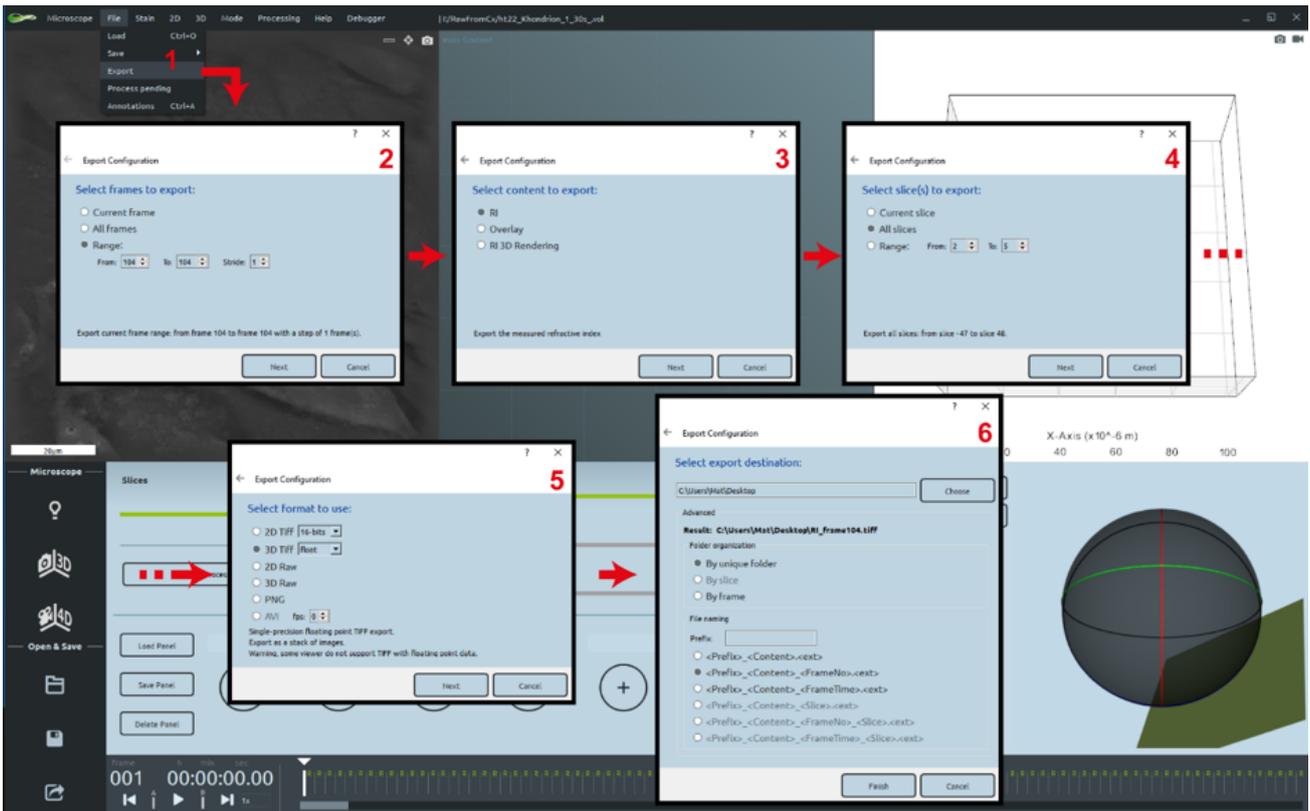
Figure 6: Exporting movies with the STEVE export tool

8. General Hardware & Software Requirements

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

Incubation system:
  Nanolive Top Stage Incubator

Microscope stage:
  Normal 3D Cell Explorer stage
  High grade 3D Cell Explorer stage

Software:
  STEVE – version 1.5 and and higher.

9. References

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