



Investigating cellular metabolism with the 3D Cell Explorer

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Abstract

Nanolive's 3D Cell Explorer together with Nanolive's top stage incubator allows for the creation of powerful 3D images and 4D time-lapses of living cells up to weeks with high spatio-temporal resolution (x,y:180nm; z:400nm; t:1.7sec). These properties allow for unprecedented live imaging of subcellular organelles such as the nuclear membrane, nucleus, nucleoli, chromosomes, plasma membrane, lysosomes, mitochondria, lipid droplets, etc. These last two - mitochondria and lipid droplets - are two key structures known to be the cellular metabolic hubs. In this application note we will discuss new research possibilities offered by the 3D Cell Explorer for the study of organelle biology with a focus on mitochondria and lipid droplets biology in both qualitative and quantitative approaches in perturbed or unperturbed conditions.

1. Introduction

Long-term imaging of fine dynamics of cellular organelles is today's biggest challenge in cell biology (Frechin et al., 2015; Kruse & Jülicher, 2005; Kueh, Champhekar, Nutt, Elowitz, & Rothenberg, 2013; Skylaki, Hilsenbeck, & Schroeder, 2016). The goal is to acquire not only snapshots of dynamic biological systems, but to actually see processes unfolding over time in term of spatial and morphological changes and biological outcome (Muzzey, Gómez-Urbe, Mettetal, & van Oudenaarden, 2009). Imaging over time is of utmost importance in the study of key organelles implicated in cellular metabolism: mitochondria and lipid droplets. The current method of choice in high-content live imaging approaches is fluorescence microscopy. However, fluorescence microscopy induces phototoxicity when the sample is stimulated at various wavelengths. This stress induces cellular damages via radical-induced cellular structure alterations, which limits live imaging possibilities. Therefore, with the current live cell imaging strategies a tradeoff must be found between short live cell imaging with high-frequency acquisition or long-term live cell imaging with low-frequency acquisition.

On one hand, high-frequency acquisition induces a lot of phototoxic stress and, if successful, a researcher might observe fine dynamics but cannot be sure that they have not been perturbed by the imaging process. On the other hand, low-frequency acquisition might be more sustainable, however, fine dynamics are lost, while the observed phenomenon, to a lesser extent, could likewise be perturbed by the imaging process.

2. Prerequisites

First, you will need glass bottom dishes compatible with the 3D Cell Explorer (<http://nanolive.ch/wp-content/uploads/nanolive-ibidi-labware.pdf>) for performing typical cell culture. All animal cells grown in our recommended dishes, preferentially in monolayers, can be observed live with the 3D Cell Explorer. For proper mammalian cell culture compatible with the 3D Cell Explorer, please read our application note number 4 (Best Experimental Practices for Live Cell Imaging with the 3D Cell Explorer, <https://nanolive.ch/wp-content/uploads/nanolive-application-note-live-cell-imaging-08-web.pdf>) that can also be adapted to any other cell culture (e.g. bacteria, yeasts).

Second, 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 CO₂ mixer and air pump that will ensure a proper control of CO₂ proportions and will help you save some money on compressed air.

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

You will finally need a 3D Cell Explorer microscope and its controlling software STEVE installed on the controlling computer.

3. Observing the fundamental interplay between mitochondria and lipid droplets

The 3D Cell Explorer allows to observe a vast range of cellular structures that can vary as a function of the cell type. A non-exhaustive list includes the nucleus, the nuclear membrane, nucleoli, vacuoles, the plasma membrane, chromosomes, mitochondria and lipid droplets that interest us more particularly for this application note. Mitochondria, which are made of an external and internal lipid bilayer or lipid droplets that are mostly composed of lipids, possess a very specific refractive index signature, as you can observe in Figure 1.

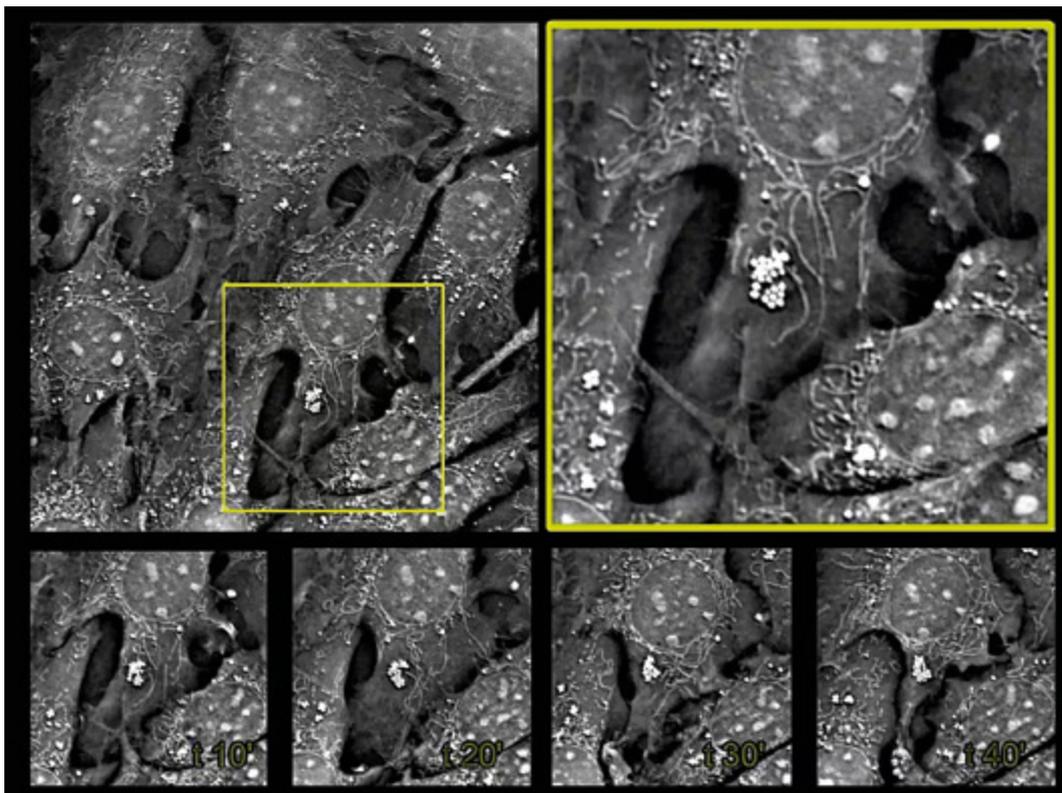


Figure 1: The 3D Cell Explorer allows unique imaging of mitochondrial networks shape and dynamics (watch full movie here: <https://vimeo.com/279995094>)

This signal is finely recorded by the 3D Cell Explorer, to such extent that it opens a new set of possibilities for biological investigations. The objects are observed with great contrast and resolution both in space and time. The conditions for capturing unique biological events such as fine cellular movements, rapid fusions and fissions, appearance and growth, are met like never before. Moreover, the absence of phototoxic stress allows to observe unperturbed cells for long periods of time. To fully appreciate the live cell imaging capabilities proper to the 3D Cell Explorer applied on mouse pre-adipocytes, please observe this movie.

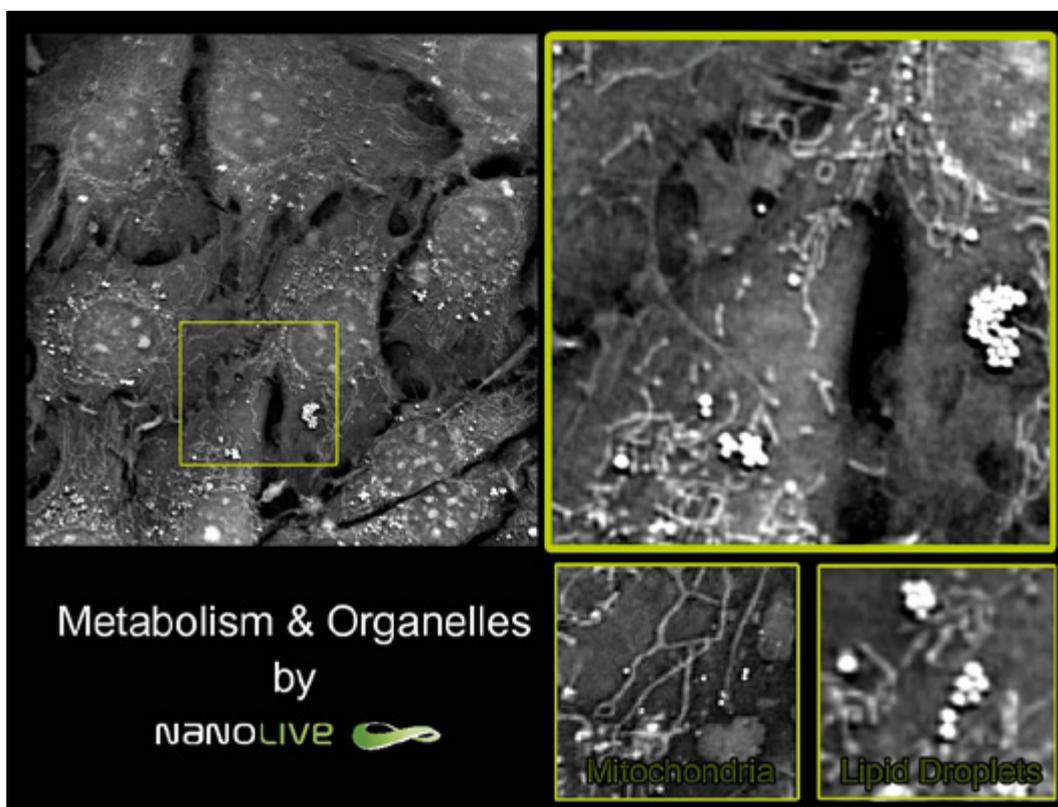


Figure 2: Unique imaging of the interaction between mitochondria and lipid droplets
(watch full movie here: <https://vimeo.com/279995694>)

Contacts between lipid droplets and mitochondria are more than ever in the spotlight (Ulman et al., 2017), since this elusive relationship is thought to be key for both organelle growth and function, to store, process and release lipids at the heart of lipid metabolism and by extension at the heart of cell life and death.

Figure 2 and its related movie illustrate the impact of the 3D Cell Explorer in the field of organelles and metabolism. The movie shows how contacts between lipid droplets and mitochondria and their relative dynamics can be observed.

The capacity of observing multiple biological structures, fine contrast and high temporal resolution over very long periods of time cannot be achieved by any other microscope, giving access to a new domain of subcellular dynamics that were inaccessible before because of the necessity of labeling and phototoxicity.

4. Perturbing the mitochondrial network

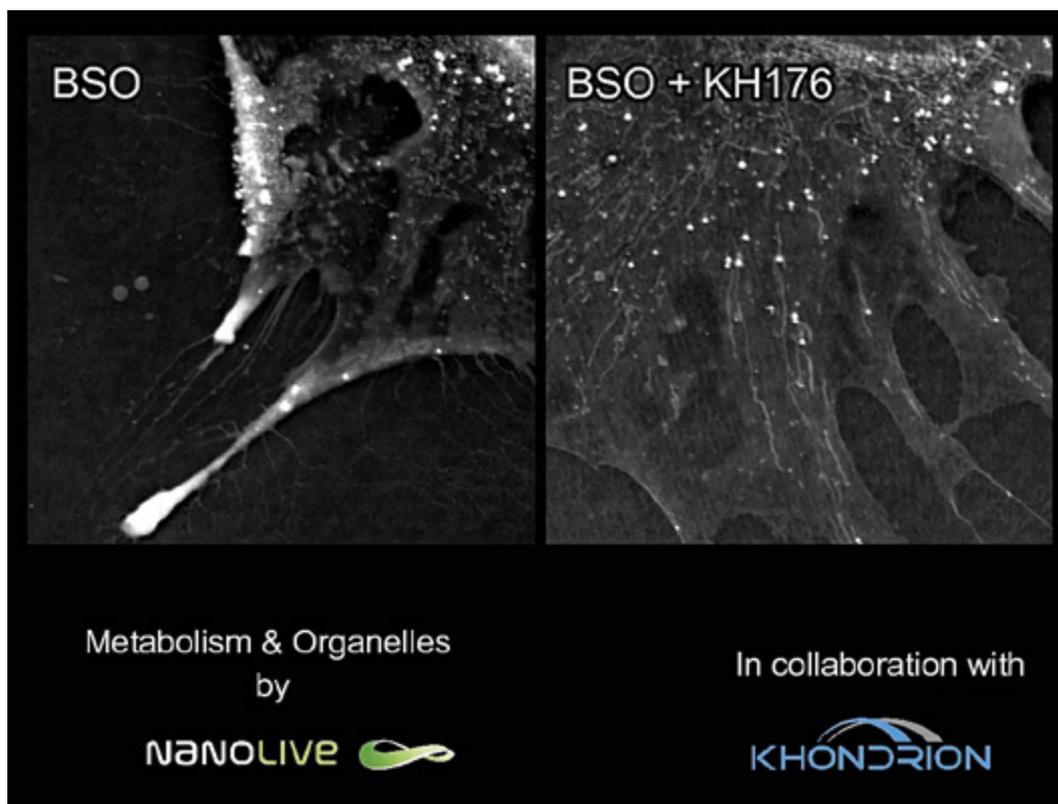


Figure 3: Perturbing and rescuing the mitochondrial network structure and function of mouse pre-adipocytes with buthionine sulfoximine and Khondrion's lead drug candidate KH176 (watch full movie here: <https://vimeo.com/288348604>)

The 3D Cell Explorer allows to observe and compare mitochondrial network perturbation with a normal healthy state: pre-adipocyte cells were treated with buthionine sulfoximine (BSO) that leads to cell death due to the failure of mitochondrial oxidative stress management. On the left BSO + vehicle was applied while on the right the rescuing drug KH176 was added to counter the effect of BSO and maintain perfect mitochondrial function. This experiment shows how the 3D Cell Explorer can efficiently help monitoring drug effects at the sub cellular scale.

5. Tracking lipid droplets

Besides morphological monitoring of intracellular organelles, the 3D Cell Explorer also allows for quantitative analysis of the dynamics of biological objects, such as lipid droplets. To do so, our software STEVE allows for image segmentation through digital staining. In the illustrated case in Figure 4, we performed a digital stain of the lipid droplets that are nicely distributed within pre-adipocyte cells. A guide on how to achieve a good digital stain can be found in our second

application note (3D object detection and segmentation inside an RI map, <https://nanolive.ch/wp-content/uploads/nanolive-application-note-3D-object-detection-web-2.pdf>). Finally, you will need an external software such as Cell Profiler or FIJI, or published algorithms that are freely available in order to perform particle tracking (Ulman et al., 2017) on exported objects mask.



Figure 4: The digital stain of lipid droplets (LD) can be exported for further object tracking with external solutions

Within this application note we developed our own tracking tool for demonstration purposes.

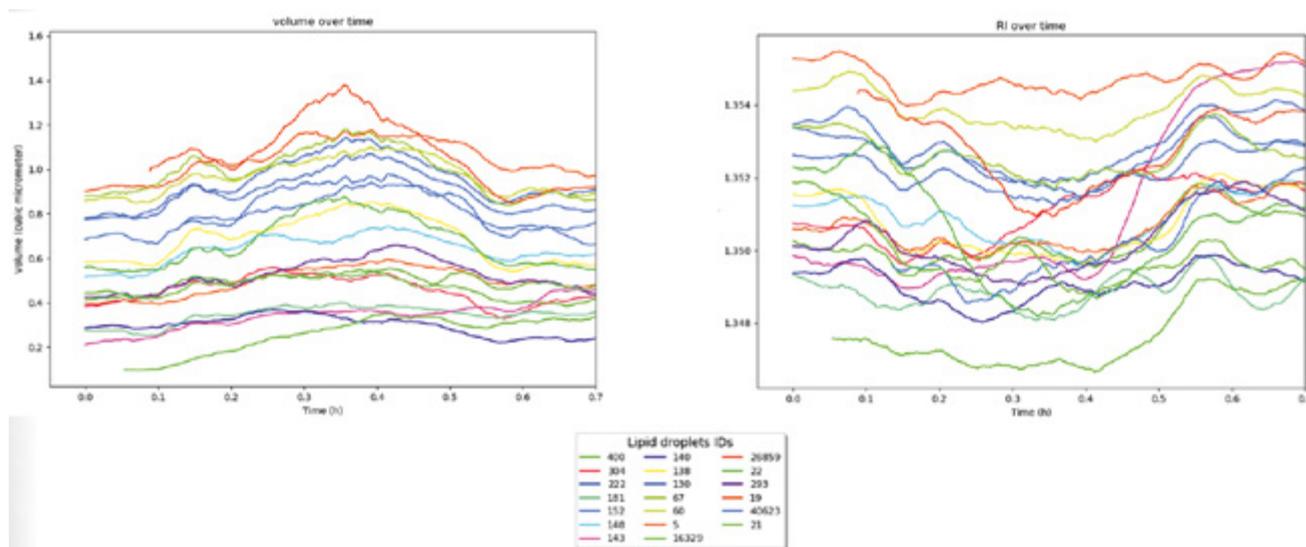


Figure 5: Example of features plotting as a function of time after tracking of many lipid droplets

Lipid droplets are very sensitive to the invasive processes of chemical labelling and to phototoxicity (Nan, Potma, & Xie, 2006). Despite the great number of researchers studying metabolism, lipid storage, and diseases, no satisfying movies of lipid droplets exist (Please find a list of the best time lapse imaging of mammalian lipid droplets recently published: (Dou, Zhang, Jung, Cheng, & Umulis, 2012; Gong et al., 2011; Jüngst, Klein, & Zumbusch, 2013; Jüngst, Winterhalder, & Zumbusch, 2011; Nan et al., 2006; Pfisterer et al., 2017)). To mitigate the labelling and phototoxicity problems, such movies are either relatively long but with very low frequency (Jüngst et al., 2013, 2011) (typically one image per hour), or taken at high frequency, but for a very short period (few seconds) and with very low image quality (Gong et al., 2011; Nan et al., 2006; Pfisterer et al., 2017). Consequently, a whole

scale of lipid droplet dynamics is simply missing. For the purpose of this application note we produced a unique lipid droplet movie in terms of length, temporal and spatial resolution and image quality, that shows yet unknown dynamics. Such movies could generate new research in the boiling field of metabolism. In Figure 5 the evolution over time of volume and mean refractive index of 20 different tracked lipid droplets is represented. This analysis is just a small example of the large possibilities offered by the 3D Cell Explorer label-free imaging, we hope that you will find in it a good source of inspiration for your own research.

6. General Hardware & Software Requirements

3D Cell Explorer models:

- 3D Cell Explorer

Incubation system:

- Nanolive Top Stage Incubator

Microscope stage:

- Normal 3D Cell Explorer stage

- High grade 3D Cell Explorer stage

Software:

- STEVE – version 1.6 and higher

- FIJI

- Cell Profiler 3

7. References

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