# When holotomography meets immuno-oncology

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Application Note by Nanolive SA

## Abstract

Nanolive's 3D Cell Explorer together with Nanolive's top-stage incubator allows to create very powerful 3D images and 4D time-lapses of living cells with high spatio-temporal resolution (x,y:180nm; z:400nm; t:1.7sec) and for time periods up to weeks. Such properties allow for unprecedented live cell imaging of immune system cells, which are exteremely sensitive to stress. In this application note we will discuss the new research possibilities offered by the 3D Cell Explorer to study cell-cell interactions within the immune system with a focus on T-cell interactions with self and non-self cells.

## 1. Introduction

Long-term imaging 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 to acquire not only snapshots of dynamic biological systems, but to actually see processes unfolding over time in terms of spatial and morphological changes and biological outcome (Muzzey, Gómez-Uribe, Mettetal, & van Oudenaarden, 2009). This is of utmost importance in the study of inter-cellular dynamics occurring at the heart of the immune system (R. N. Germain, Robey, & Cahalan, 2012; Ronald N. Germain, Miller, Dustin, & Nussenzweig, 2006; Nitschke, Garin, Kosco-Vilbois, & Gunzer, 2008; Sarris & Betz, 2009). However, fluorescence microscopy induces phototoxicity when the sample is stimulated at various wavelengths. This stress induces cellular damages via radical-induced cellular alterations and potentially artificial observations that occur only in reaction to phototoxic stress ("Phototoxicity revisited," 2018). Therefore, the current live cell imaging techniques are limited and result from a trade-off between short live cell imaging with high-frequency acquisition and long-term live cell imaging with low-frequency acquisition.

In this application note we will discuss new research possibilities that arise from combining holotomography and immuno-oncology, which is more than ever in the spotlight. In fact, the 2018 Nobel Prize for Medicine went to Allison and Honjo for the discovery of a new strategy using antibodies to release the natural breaks that keeps the immune system from fighting cancer cells with spectacular results in melanoma and lymphoma for example, and brings significant hopes to people suffering from metastatic cancers (Allison, 2018). Uncovering such fundamental details of the immune system functions and dysfunctions is crucial. In this context, holotomography opens new possibilities for research.

This application note will focus on the content of unique time-lapse imaging of immune system cells that have been produced with our technology and that will serve as a support to provide knowledge and inspiration for research.

## 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 your typical culture of immune cells. 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 that can also be adapted to any other cell culture (e.g. bacteria, yeasts). You can read the application note here: https://nanolive.ch/wp-content/uploads/nanolive-application-note-live-cell-imaging-08-web.pdf.
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 $CO^2$ mixer and air pump that will ensure a proper control of $CO^2$ proportions and will help you save some money on compressed air.

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. Activation and homeostasis of the immune response, T-cells and macrophages interactions

Figure 1 and 2 feature key moments captured in our first movie. The movie has been acquired at a frequency of 1 image every 6 seconds for approximately 16h and has been edited to keep the focus on a set of rare events. We strongly encourage you to watch it since the dynamic aspects of the mechanisms we discuss here are important. In particular, this material features a T-cell mediated macrophage death that has never been filmed to date to the best of our knowledge. Please find the video here: https://vimeo.com/305783292.

You can observe two cell types, firstly macrophages which are the large cells with broad membrane ruffles. The macrophages have been isolated from a C57BL/6 mouse and pre-stimulated with ovalbumin peptide, interferon gamma and lipopolysaccharide to become antigen-presenting cells. The main role of the macrophages is to recognize and digest non-self material within the body and to present it to the immune system, they are long living and mostly located to favorite points of entry of non-self-material (Perdiguero & Geissmann, 2015).

The second type of cells are T-cells which are smaller and denser. The "T" stands for thymus, the tissue in which these cells mature. T-cells are central to the cell-mediated, adaptive immune system and are able to kill non-self cells such as cancerous or infected cells. They also have an assisting role by supporting the immune response or by keeping the memory of previous antigens (Kumar, Connors, & Farber, 2018).

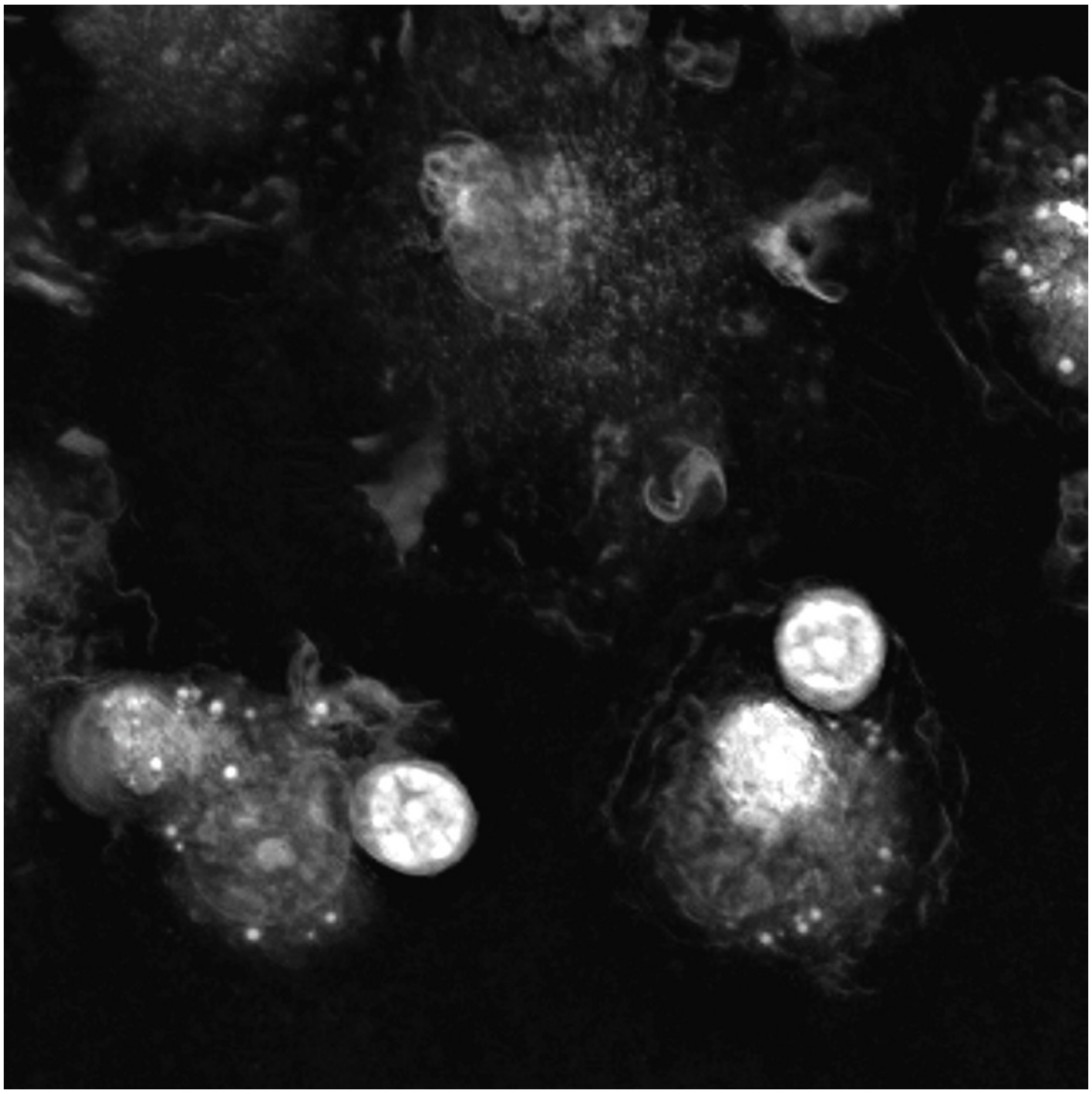

**Figure 1:** Macrophages present antigens to T-cells (1 image every 6 seconds).
Video link: https://vimeo.com/305783292

These T-cells have been isolated from an OT-1 mouse and are naïve at first but get activated by interacting with the macrophages that are presenting them the antigen to recognize, this activation event teaches the T-cells what to kill.

In the lower right corner of Figure 1, you can see two T-cells interacting with a macrophage that suddenly stops moving (https://vimeo.com/305783292) and eventually dies, which can be seen in Figure 2. This is a very rare and interesting type of event that we can observe thanks to the non-invasiveness of the technique. It is called T-cell killing of antigen-presenting macrophage and is mediated by receptor-ligand recognition. For more details please read the work of Kaplan et al. published in 2000 in the journal of immunology (Kaplan, Ray, Mo, Yung, & Richardson, 2000). While the details of this type of interaction are still elusive, its rational is clear: the human bone marrow can produce $10^{10}$ monocytes/macrophages per day, and a similar rate of destruction is required to maintain homeostasis.

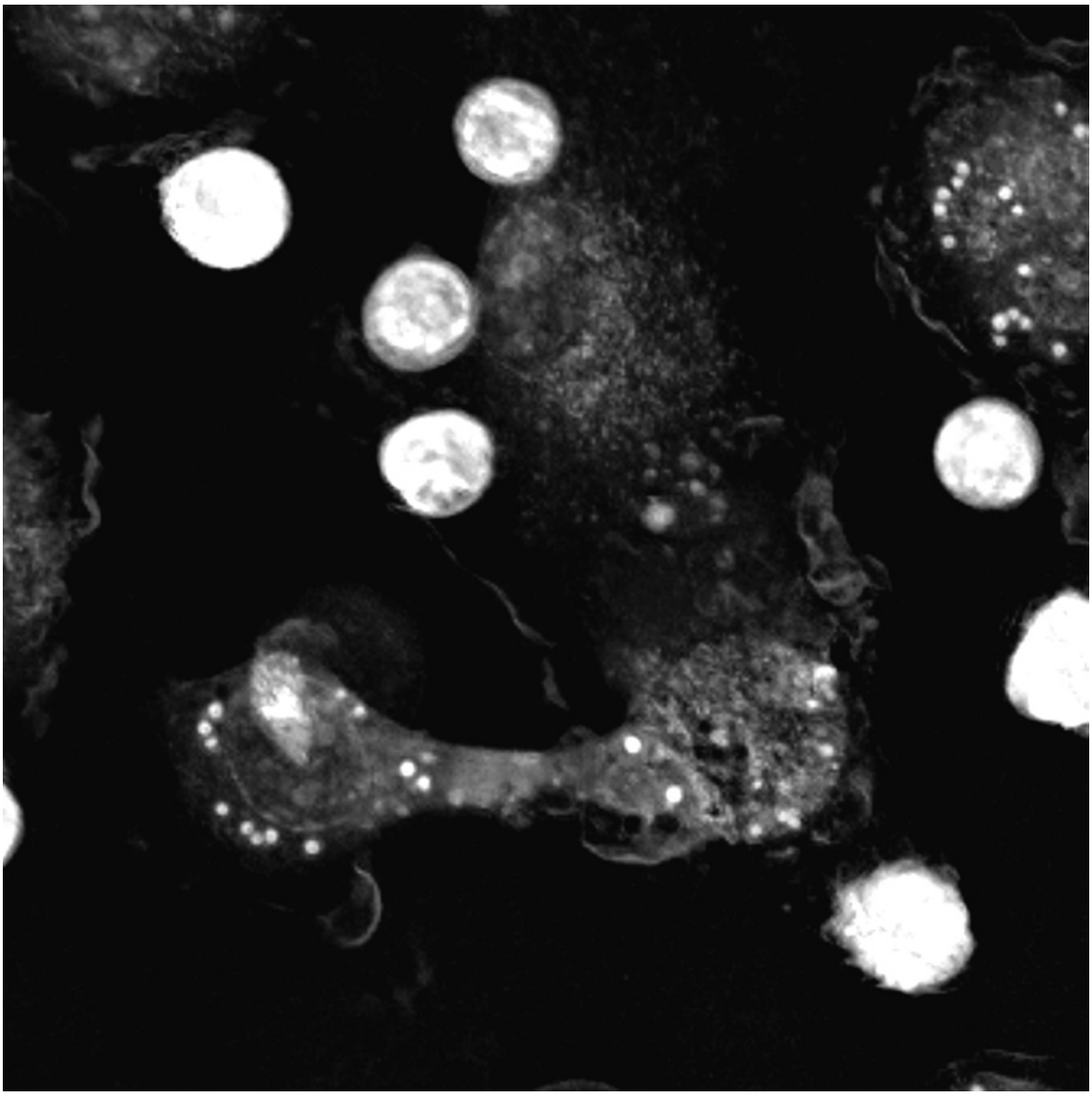

**Figure 2:** The dead macrophage is recycled by its fellow macrophages (1 image every 6 seconds).
Video link: https://vimeo.com/305783292

Finally, the dead macrophage is removed and digested by its two fellow macrophages. Here macrophages do their main job which is digesting and recycling dead material. What is really striking is the capacity of Nanolive's technology to catch the fine details of the membrane extensions while the cleaning macrophages are looking for dead material. Strikingly, they eventually recognize the dead cell which triggers phagocytosis, the process of engulfment and digestion of dead and foreign material. If you pay close attention it is possible to observe details of the dead macrophage digestion within the active macrophage.

Such cell-cell interplays rely on complex machineries, and their inner dynamics were so far inaccessible. Nanolive's technology gives access to them with great contrast and resolution in space, and most importantly in time. As an example, immunotherapies impact the fundamental dynamics of T-cell/APC or cancer cell interactions and has been hard to assess so far because of image quality, the necessity of labeling, or phototoxicity. The 3D Cell Explorer offers an elegant solution to these problems.

## 4. Membrane dynamics of dendritic cells: a bridge between innate and adaptive immune systems

Figure 3 represents a shorter movie that has been acquired at a frequency of 1 image every 6 seconds for approximately 11 minutes. You can watch the movie here: https://vimeo.com/304817232. It is nonetheless remarkable as it features another fundamental interaction between T-cells and dendritic cells, which are slightly larger and less dense than T-cells with very active membrane protrusions. Dendritic cells are fully specialized antigen-presenting cells that bridge the innate and adaptive immune system and are specific to mammals (Eisenbarth, 2019). Much like macrophages, dendritic cells are present where non-self might show up such as the skin or the inner linings of mucosa of the nose, lungs, stomach and gut and they constantly probe for bits of foreign material. You can observe unique dynamic details of this probing function in the movie where large and rapid membrane protrusions are created from the dendritic cell bodies in an apparent random fashion.

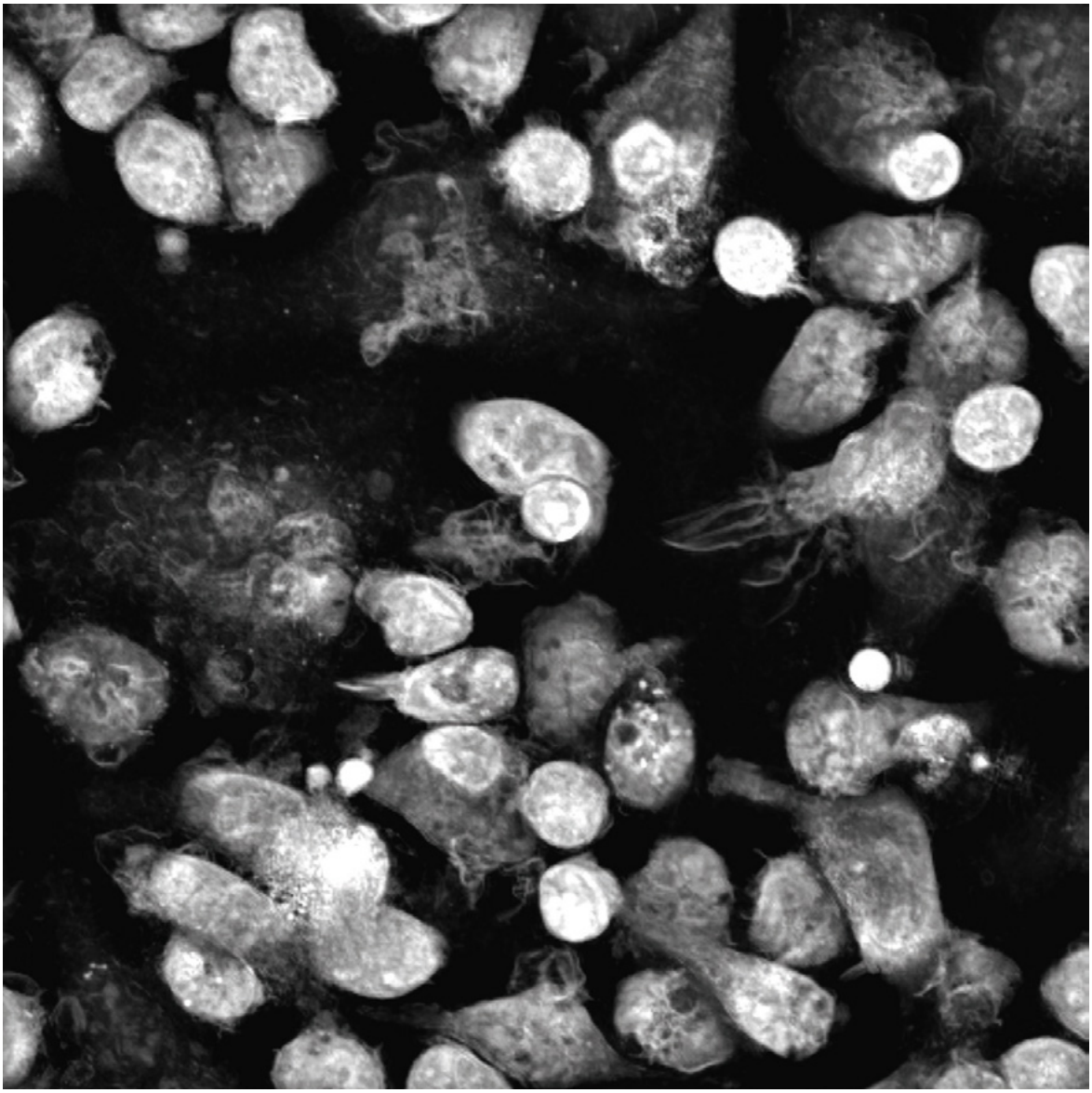

**Figure 3:** Dendritic cells are fully specialized antigen-presenting cells with large membrane protrusions made for probing antigens in the environment (1 image every 6 seconds). Video link: https://vimeo.com/304817232.

## 5. At the heart of immuno-oncology: T-cells recognize and kill (or not) cancer cells

Figure 4 features activated T-cells targeting and killing colon cancer cells. You can watch the movie here: https://vimeo.com/304818512). These T-cells are the same T-cells presented in the previous two movies, which were activated to target ovalbumin antigen. The cancer cells are the MC38-OVA cell line which makes them recognizable by our activated T-cells. You can observe nice killings of cancer cells through T-Cell-MHCI recognition (Wang, Yin, Wang, & Wang, 2014). However, T-cells sometimes recognize cancer cells but do not kill them. What is the difference between a killing and non-killing meeting? This is a fundamental question that time-lapse imaging made with Nanolive's technology, coupled to accurate perturbations, will allow to address.

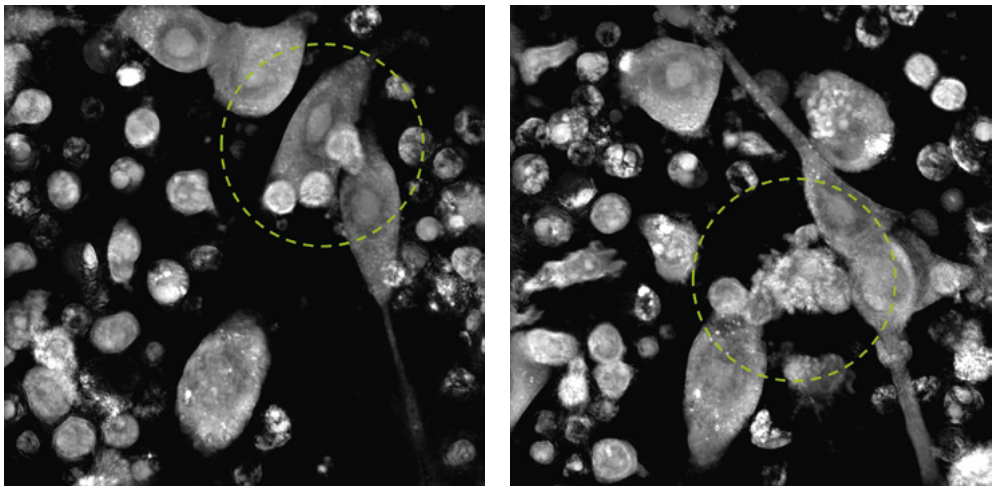

**Figure 4:** T-cells detect and kill colon cancer cells (1 image every 6 seconds). Video link: https://vimeo.com/304818512.

It is such a fundamental question that the first bit of understanding of this complex system was recently awarded of the Nobel Prize. Tasuku Honjo discovered the PD1 protein at the surface of T-cells in 1992 (Ishida, Agata, Shibahara, & Honjo, 1992) and demonstrated over two decades that PD1 is a break that slows down the capacity of T-cells to kill their target. By inhibiting PD1 with a monoclonal antibody, Honjo and coworkers demonstrated that it is possible to stimulate the immune system of patients for killing cancer cells, with stunning results especially against melanoma and more generally against metastatic cancers. There is no doubt that Nanolive's technology could help advancements in such research fields.

## 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 over.
- FIJI
- Cell Profiler 3

## 7. References

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