Application Note
#2007

Webinar Recap: Imaging Breast Cancer Evolution in 3D Organoid Cultures with Luxendo Light-Sheet Microscopy

Bruker's Luxendo light-sheet technology can image a wide range of live, fixed, and cleared biological samples from organoids and embryos to large whole organisms. Overcoming many of the barriers that conventional light microscopy faces, light-sheet fluorescence microscopy, also called selective plane illumination microscopy (SPIM), supports advanced research in the life sciences. SPIM works by de-coupling the fluorescence excitation and detection beams and together with a sheet of light for excitation, selectively illuminates a focal plane for high-resolution images without the danger of phototoxicity. In this webinar recap, Dr. Martin Jechlinger, the Senior Scientist and Head of VISION Laboratory at the MOLIT Institute, talks about how his lab utilizes light-sheet microscopy to investigate 3D organoid cultures. Specifically, the molecular mechanisms underlying mammary tumor development during breast cancer, as well as what is happening when treatments are failing.

Why Use Light-Sheet Technology

SPIM generates a thin sheet of light that images the entire focal plane at once using a full-frame camera for fast, but gentle, imaging of a wide variety of samples. Furthermore, Bruker's Luxendo selection of microscopes has a variety of mounting techniques and benefits depending on the scientific research needs (see Figure 1).

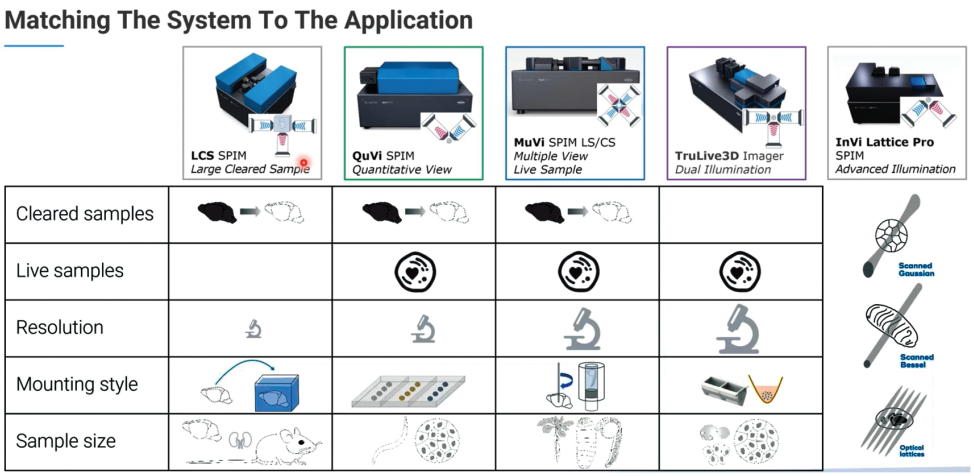

FIGURE 1

Bruker's Luxendo light-sheet microscopes have a variety of unique benefits, including fast imaging speed, excellent signal-to-noise ratio, and minimal phototoxicity.

Understanding the Underlying Mechanisms of Mammary Tumors and Treatments

Dr. Jechlinger and his lab utilized Bruker's **InVi SPIM** and single-cell tracking to understand how the behavior of oncogenes, active in a few individual cells, contribute to the formation and relapsing of mammary tumors. 30% of patients who are treated for breast cancer will have a relapse, but earlier studies lacked the methodologies needed to follow single cells throughout therapeutic intervention. Therefore, the research team wanted to explore single cells at different stages of cancer (early, carcinomic, and relapsed) to establish a model mouse system with many important future implications for cancer treatment in humans.

Single-Cell Manipulation and Observation

To study the formation and regression of mammary tumors, Dr. Jechlinger and his team created organoid cultures with cells from mammary glands. The team developed a stochastic model of tumorigenesis, where only a subset of cells was expressing oncogenes. Using a viral transfection system, the team induced oncogenes in a cell subset by the addition of doxycycline. The resulting in-depth and real-time visualization of cancerous cells and their behavior in otherwise healthy 3D tissue is critical for understanding cell behavior in tumors.

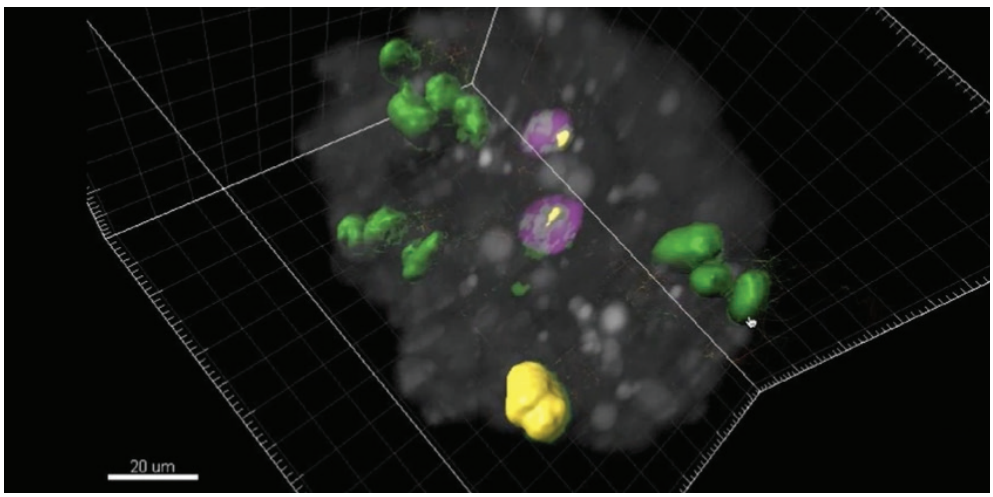

FIGURE 2

By marking single cells with different colors based on behavior, it was found that cancerous cells were proliferating at different speeds. This single-cell tracking during oncogene induction suggests establishment of intra-tumoral heterogeneity. Cells were marked with H2B-GFP and mitotic events in purple.

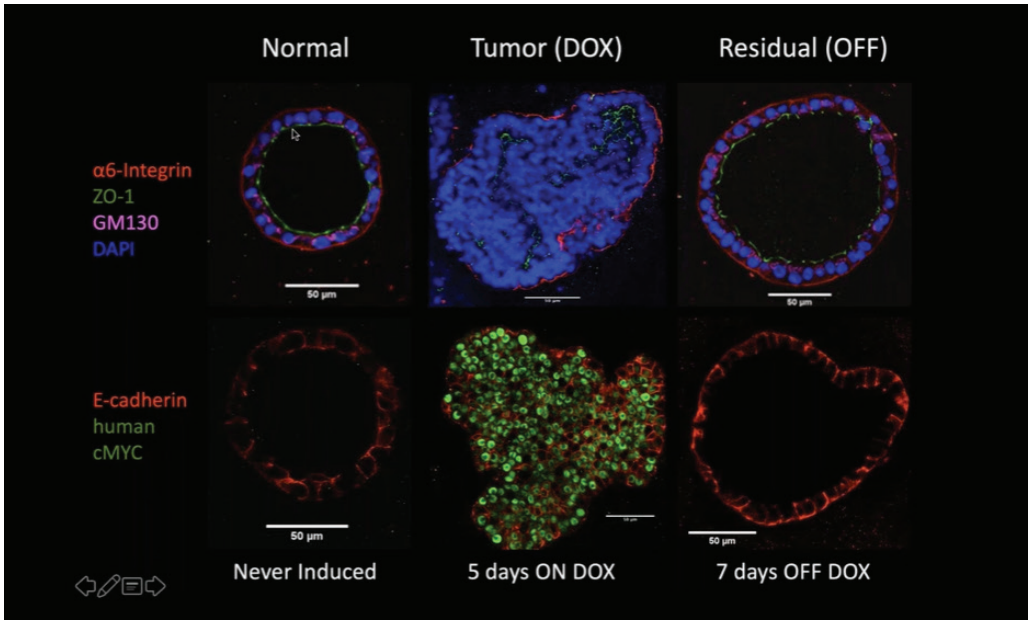

FIGURE 3

To get a better understanding of cell survival, cells were marked and observed before, during, and after turning on the oncogenes. While observing residual cells, they found small differences in the transcriptional profile in the control and residual profiles. This suggests that the cells responsible for relapses are normal phenotypically but have an "oncogenic memory" that predisposes them to relapse.

Light-Sheet Microscopy for Compelling Research

An ultimate goal for Dr. Jechlinger and his lab is to design an improved stochastic model of breast cancer. Turning on oncogenes, specifically in mammary glands, is an effective method to study tumor formation/relapse in the lab but is not what really happens in patients, as whole tissue isn't commonly transformed. Therefore, additional single-cell tracking was needed to further understand these processes, so the team employed long-term imaging of virally transfected oncogenic cells. Using long-term light-sheet imaging with a dedicated image analysis pipeline for single-cell tracking, single-cell dynamics could be studied with the main goal to establish cellular behaviors and calculate the rates at which they proliferate into tumors within an organoid.

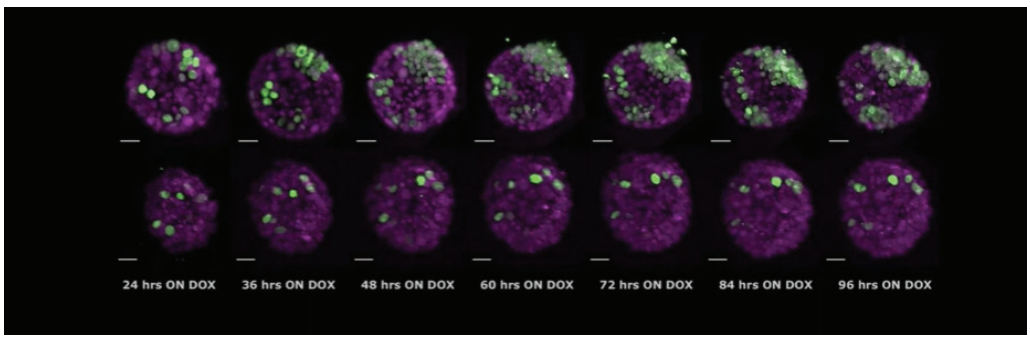

FIGURE 4

Single-cell tracking was employed over a duration of 96 hours to create 3D visualization of nuclear segmentation and better understand the processes underlying relapsed tumor formation.

They determined that the number of single infected cells and their closeness within a cluster volume determines the successful establishment of tumor growth. This means that isolated oncogenic cells would neither proliferate nor migrate and ultimately, not develop an established tumor. However, future studies are still needed to determine the underlying molecular mechanisms of this finding such as how these cells interact with the direct normal environment (do they stiffen, produce secretions, etc.).

Main Takeaways

The in-depth understanding of mammary tumor formation provided by single-cell tracking and explored in Dr. Martin Jechlinger's research has many important implications for our understanding of breast cancer evolution and tumor biology. Bruker's Luxendo light-sheet microscopes provide the speed, resolution and sensitivity needed to successfully image important processes over a long period of time and continue to make advances in the life sciences.

Resources

To view the entire on-demand webinar "Imaging Breast Cancer Evolution in 3D Organoid Cultures with Luxendo Light-Sheet Microscopy" visit
https://www.bruker.com/en/products-and-solutions/fluorescence-microscopy/light-sheet-microscopes/what-is-light-sheet-microscopy.html
To learn more about Bruker's impressive suit of light-sheet microscopy solutions visit
www.bruker.com/light-sheet

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