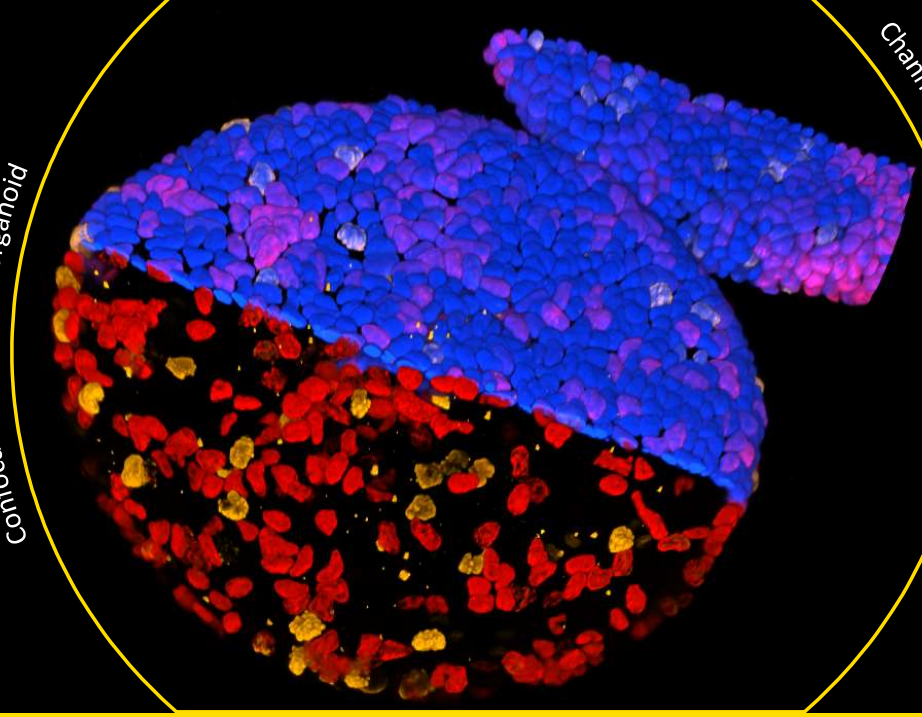
APPLICATION CARD

Nikon BioImaging Lab
Leiden
Netherlands



3D imaging and segmentation of organoids

Organoids are three-dimensional (3D) cell cultures with the ability to mimic organs and tissues more accurately than traditional cell cultures. As such, they are gaining popularity in advanced biological research and drug discovery. However, their size and density pose a significant challenge in imaging and, more critically, in image analysis.

Challenges

One key challenge stems from their thickness and dense cellular packaging, which causes significant light scattering and absorption, leading to image distortions, signal reduction and less resolution deeper into the organoid. Due to these imaging hurdles, accurate segmentation of individual cells becomes increasingly challenging. Especially in the inner regions of the organoid, where cell boundaries are obscured by the surrounding high-density cellular environment.

Resolving these challenges…

A fast resonant scanner of the Nikon AX-R confocal was used to accurately image a complex organoid structure consisting of four different cell populations. Using a combination of sophisticated analysis tools, we were able to segment 1900 nuclei in 3D and accurately quantify the number of cells that were positive for each marker.

Talk to an expert

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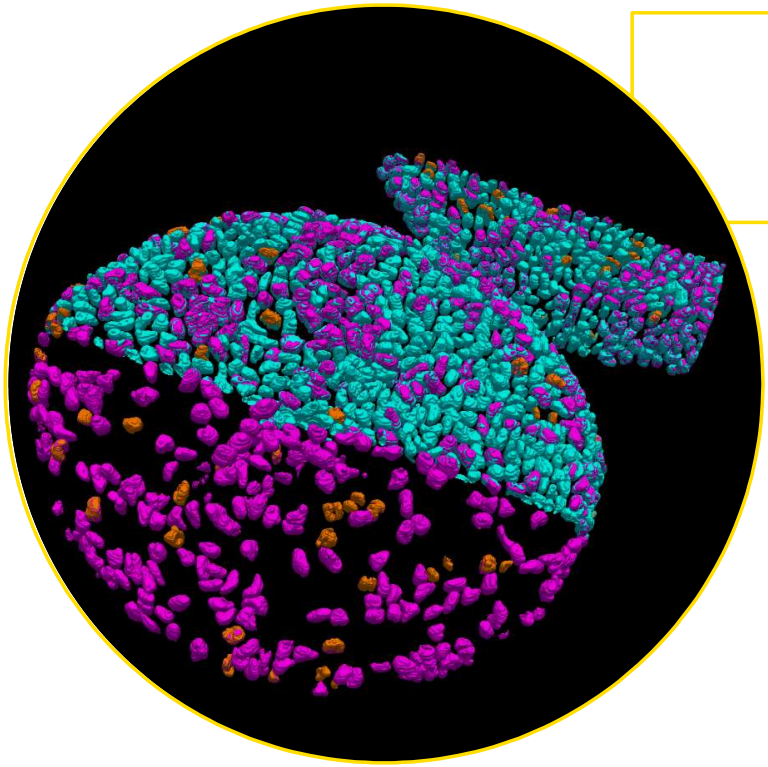
3D imaging and segmentation of organoids

Leveraging the rapid resonant scanning capabilities of the Nikon AX confocal microscope, we acquired images of a 300-micrometer organoid at 20X magnification. The spheroid specimen was fixed and stained with DAPI to visualize cell nuclei, along with Alexa Fluor 647 and mCherry fluorescent markers to distinguish multiple cell populations within the organoid. The sample was provided by the Suijkerbuijk lab, University Utrecht.



ANALYSIS

DAPI channel was segmented in 3D (cyan). Then, intensity in mCherry and AF647 channels was measured to find positive cells (orange and magenta, respectively)

RESULTS

1,900 nuclei in total were segmented. 113 nuclei were mCherry-positive, 764 nuclei Alexa Fluor 647-positive, 44 double positive and 979 negative for all markers.

Advanced analysis workflow allows for accurate quantification in 3D organoid structures

To mitigate the effects of scattering and absorption inherent in dense specimens, we employed intensity equalization along the Z-axis and AI denoising techniques. The segmentation process involved analyzing 2D slices from all three orthogonal planes (XY, XZ, and YZ) and subsequently merging the results. These comprehensive measures were crucial for achieving accurate results. Without this preprocessing, dimmer nuclei from deeper layers would have been overlooked, and proper segmentation of adjacent cells would have been compromised without considering their size and shape in three dimensions.

This workflow allowed for accurate organoid imaging and analysis by employing advanced analysis techniques and sophisticated image processing methods. Overcoming challenges posed by organoid complexity, this approach opens new possibilities for organoid research and drug discovery endeavors.

Nikon BioImaging Lab's services:

01 Assay development › **02** Imaging › **03** Analysis › **04** Reporting