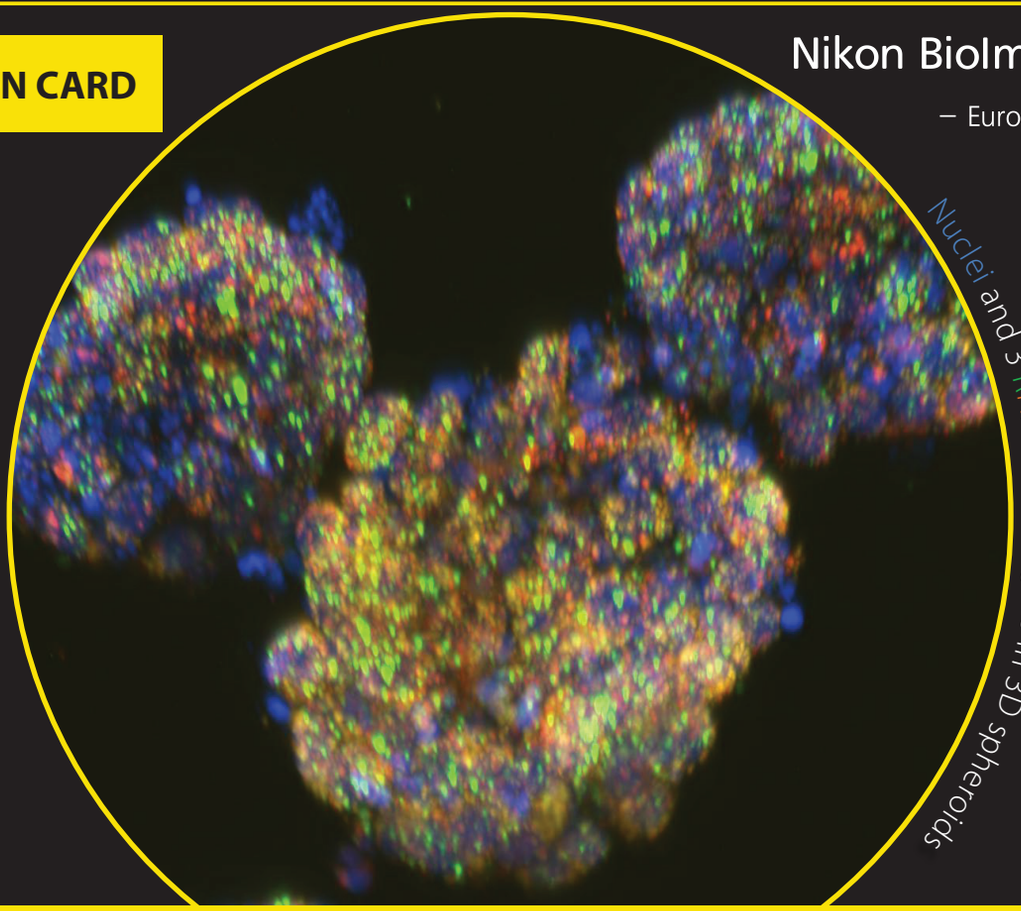
APPLICATION CARD

Nikon BioImaging Lab

– Europe –



3D Multiplex Single-Molecule FISH

Fluorescence **I**n **S**itu **H**ybridization (FISH) is a technique for visualizing individual nucleotides. RNAscope takes this further by detecting multiple single RNA transcripts within cells and tissues at single-cell resolution. While RNAscope has been traditionally used on 2D cell cultures or thin tissue sections, this limits its ability to capture full gene expression profiles. By adapting RNAscope for thicker, 3D tissues, we improve gene expression analysis, target identification, and spatial biology research, offering a more comprehensive view of cellular heterogeneity.

CHALLENGES

RNAscope captures individual RNA molecules by quantifying small, bright spots, and consistent segmentation of these spots is essential for reliable data analysis. However, imaging thicker tissues or 3D cultures is often challenging due to weak fluorescence signals, scattering, high background (auto)fluorescence, and the close proximity of adjacent spots. To overcome these hurdles, optimizing sample pretreatment and target hybridization is key to improving signal clarity and ensuring consistent, high-quality results.

APPLICATIONS

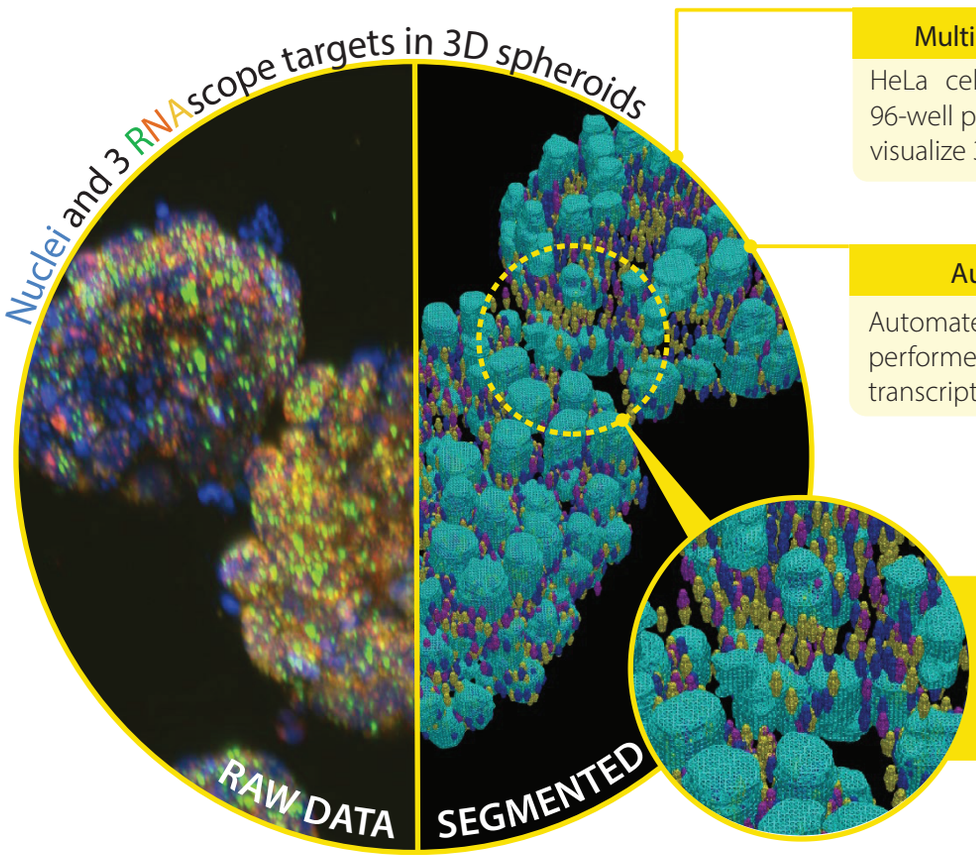
- ✓ Highly sensitive gene expression analysis
- ✓ Compatible with complex, 3D culture or tissue systems
- ✓ Optimized for automated analysis and data presentation

Talk to an expert

nbil.eu@nikon.com

Multiplex RNAscope in 3D Spheroids

In this assay, HeLa cervical cancer cells were suspended as single cells within a 3D extracellular matrix and seeded into 96-well plates, growing into 50- to 150 µm-thick spheroids over time. The thin, high-clarity foil plate bottom allows direct processing of RNAscope within the well and ensures seamless compatibility with high-resolution confocal imaging. Probes targeting three housekeeping genes (POL2RA, PPIB, and UBC) were used. Probes targeting genes absent in mammalian cells (not shown) served as a negative control to validate the specificity of the RNAscope workflow.



Multiplex RNAscope in 3D spheroids

HeLa cells were grown as spheroids in 96-well plates. RNAscope was performed to visualize 3 housekeeping genes.

Automated analysis workflow

Automated segmentation and analysis were performed to visualize and quantify RNA transcripts.

NBIL can perform, image, and analyze multiple RNAscope targets in 3D

OUR APPROACH

To improve gene expression analysis in 3D cultures, we have integrated advanced imaging techniques with AI-driven analysis.

- ✓ **Minimized Scattering and Absorption**: Intensity equalization along the Z-axis and AI-based denoising are applied to overcome challenges in dense spheroid specimens.
- ✓ **Advanced Imaging**: The fast, high-sensitivity imaging capabilities of the **Nikon AX confocal microscope** allow capture of 3D spheroid images at 40X magnification.
- ✓ **AI-Assisted Segmentation**: Automatically, accurately, and consistently segments cell nuclei and RNA transcripts in 3D cultures at single-cell resolution.

By combining our advanced RNAscope assay with precise, automated 3D tissue analysis, we can uncover novel insights into gene expression.

Nikon BioImaging Lab's services:

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