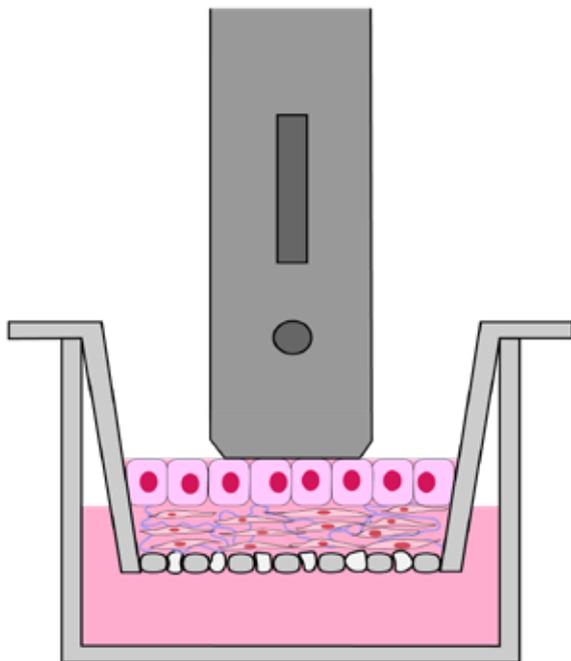


# Real-time imaging of in vitro 3D tissue culture

Tissue cultures are used in research for modelling normal tissue in vitro. Scaffolds are used to grow cultures in 3D to mimic the in vivo tissue environment. 3D environments enable specific exposure to signalling molecules and supplementary cues that are needed at specific growth phases so cells can differentiate into desired cell types. As a result, 3D tissue culture can resemble the cellular arrangement and tissue morphology of an in vivo environment.

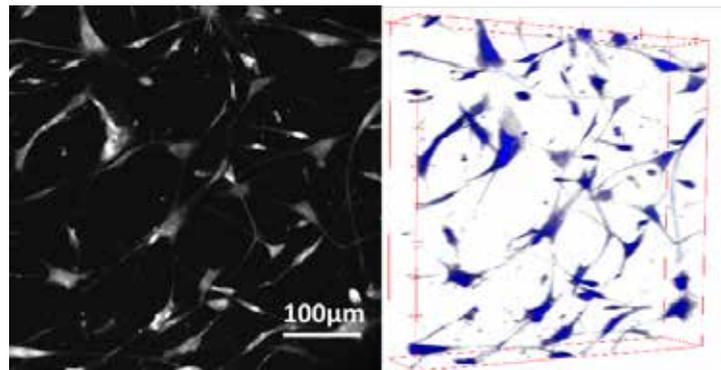
Tissue cultures are usually grown in culture wells or transwell inserts. High-resolution imaging of tissue cultures is challenging since conventional microscope objective lenses cannot access inside the wells and have limited working distance. Another approach is to image the tissue culture from underneath using an inverted microscope. However, this does not work well when using transwell inserts, as they require long working distances and contain devices that block light from entering the sample from below. As a consequence, high-resolution imaging of tissue culture is commonly performed by removing it from the well and fixing it prior to imaging.

This application note provides details on how endomicroscopy can be used for confocal imaging of live tissue cultures over time in incubators and biosafety cabinets.



A schematic representation of Optiscan's FIVE2 (ViewnVivo) endomicroscope scanner being used inside a tissue culture well.

Optiscan's small diameter confocal endomicroscopes can access the inside of culture wells to provide high-resolution 3D imaging of tissue cultures. In addition, Optiscan's endomicroscope system can be easily combined with a biosafety cabinet or an incubator allowing live imaging of laboratory grown tissue samples. The addition of endomicroscopy techniques in tissue culture has the potential to provide new information on cellular proliferation, death, rearrangement, and morphology, by enabling imaging of culture in platforms (e.g. scaffolds, perfusion systems and/or fluidic devices).



Maximum brightness projection (left) and 3D modelling (right) of human dermal fibroblasts. Imaged directly from tissue culture wells using the FIVE2 endomicroscope.

## Live Imaging of Tissue Culture Samples

### 1. Tissue culture

Grow cell culture in an appropriate media in a transwell insert or cell culture well plate.

### 2. Selection of appropriate imaging agents

Depending on specific structures/cells of interest, various stains can be used. Blue excited fluorophores like rhodamine, fluorescein, acriflavine, and 4-Di-2-Asp can be used for viewing the overall morphology of the culture. Antibody targeted stains and transgenic cells producing blue excitable fluorescent proteins can also be imaged.

### 3. Prepare sample for imaging inside the biosafety cabinet

In order to maintain sterility of live samples, live tissues may need to be imaged inside a biosafety cabinet or incubator. In this case, place a vibration isolated probe holding platform inside the cabinet and install the disinfected/sterilised probe. Place the sample well plate on the probe holding platform. Use the platform x, y, and z controls to align the probe to the imaging sample. The 4 mm diameter probe will easily reach the sample surface from the top.

With the endomicroscope focal plane set to its maximum depth (400µm from the tip of the probe) the sample surface can be detected without contact. A webcam can also be placed near the probe holder stage to visualise the gap between the sample and probe to assist with positioning the probe. The tip of the probe can be submerged in liquid or used with an air gap between the probe and the sample.

### 4. Live imaging

Once the probe is positioned correctly, the z-position of the focal plane can be adjusted with the Imager software application using the inbuilt z-controls within the probe. Use imaging parameters as needed to capture image. As the cells remain untouched, this process can be used to capture tissue growth over time by imaging at regular intervals.

## Worked example:

### 1. 3D Tissue culture

Human fibroblasts were grown in rat tail collagen type I for 7 days in a cell culture well [1,2,3].



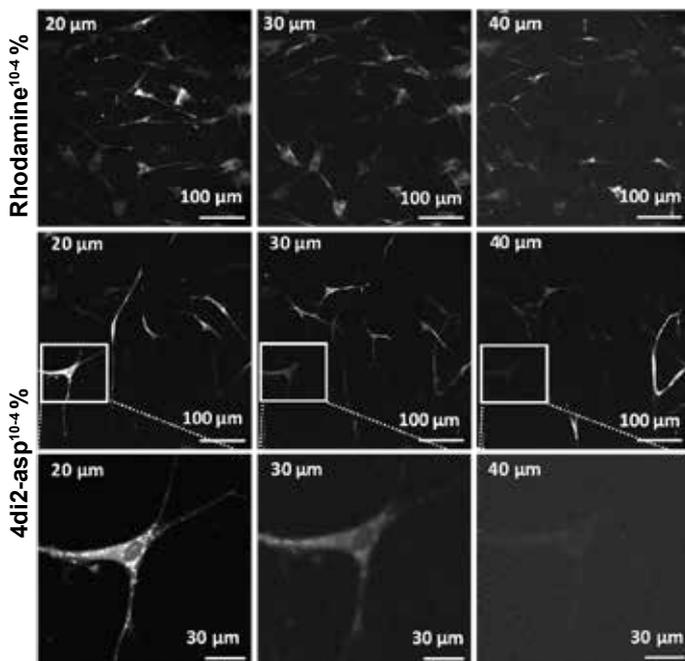
## 2. Prepare sample for imaging inside the biosafety cabinet



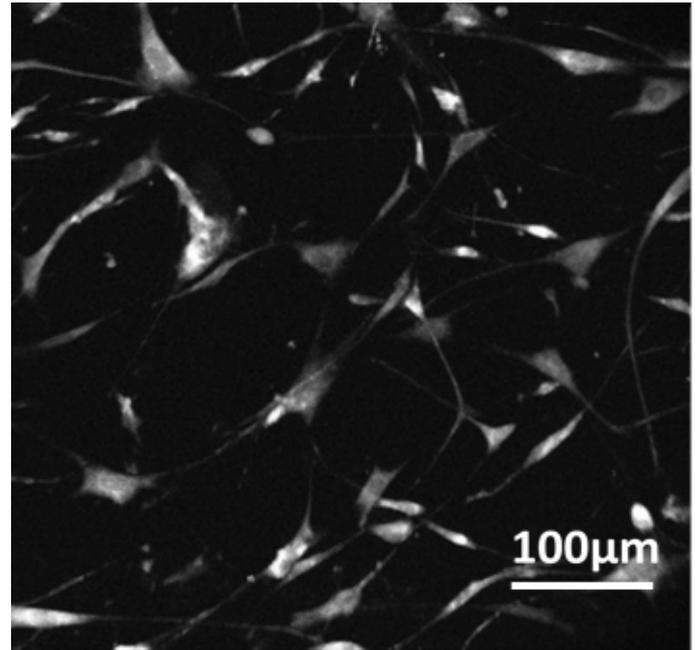
A prototype set up: Optiscan's FIVE2 confocal endomicroscope probe set up inside the biosafety cabinet for imaging tissue culture.

## 3. Selection of appropriate imaging agent

Acriflavine, 4di2-asp and Rhodamine-b were tested in concentrations of 10-2%, 10-3% and 10-4% to stain human fibroblasts (data not shown). 4di2-asp10-4% produced the best signal to noise ratio and was selected for staining the fibroblasts in this experiment (z-axis depth is indicated in top left).

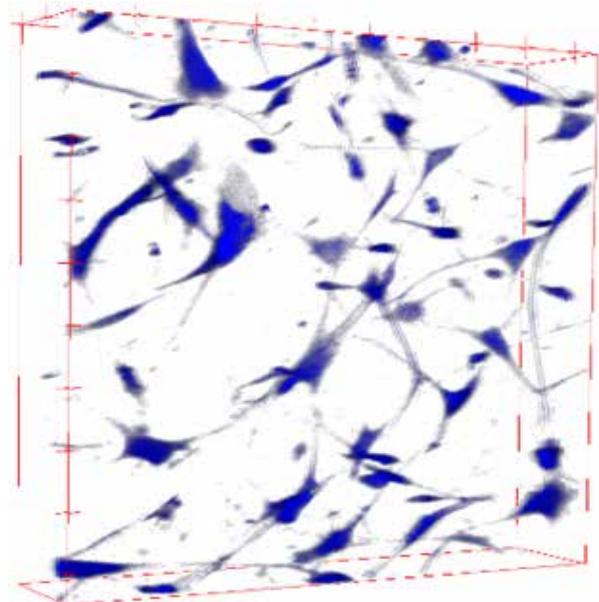


## 4. Image capture



Maximum Brightness projection of 100 $\mu$ m Z-stack with 3 $\mu$ m spacing between slices. Sample of human fibroblast culture cells stained with 4di2-asp10<sup>-4</sup>%.

## 5. Post-processing and 3D reconstruction in Image J



Special thanks to Dr Geva Hilzenrat<sup>‡</sup> and Prof. Sally McArthur<sup>\*</sup> for the application note.

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