APPLICATION NOTE

AX/AX R Confocal Microscope

# Confocal imaging of vasculature network using the HUMIMIC Chip

In this application note, we introduce 3D imaging of a vascular network formed in TissUse's **HUMIMIC Chips**. By visualizing actin filaments (F-actin) and a CD31 marker of endothelial cells with fluorescence, and observing them with a confocal microscope, we confirmed that 3D tubular network structures were formed in the cell culture compartments. The vascularization will enable the physiological culture conditions for spheroids or organoids in the chips (e.g capillarization of spheroids/organoids).

## Experimental overview

An organ-on-a-chip is a device with fine flow paths and organ culture compartments. TissUse's **HUMIMIC Chip** is a "Multi-Organ-Chip", meaning it connects different organ models in a common circulating medium flow. It is possible to imitate the physiological environment of human organ models and simultaneously culture multiple organ models. Especially, vascularization in the chips is important for recreating physiologically relevant microenvironments and it is possible to create various vascular structures not only in the microfluidic channels, but also in the culture compartments using the **HUMIMIC Chip**. This application note shows 3D imaging of a vascular network formed in culture compartments using **HUMIMIC Chip2$^{vasc}$** (Fig. 1) .

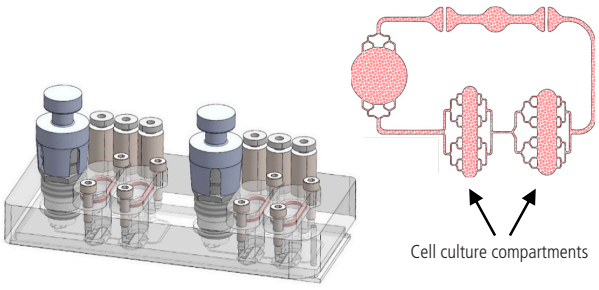


Figure 1: The **HUMIMIC Chip2$^{vasc}$** at a glance and an overview of the **Chip2$^{vasc}$** microfluidic channels

Endothelial cells were cultured on fibrin/collagen gel in the culture compartments.

## Formation of vascular network using the HUMIMIC Chip2$^{vasc}$

It is possible to create advanced vascularization structures in **HUMIMIC Chip2$^{vasc}$**. Channels were created by bioprinting endothelial cells in the cell compartments (Fig. 2). We can recognize from our 3D images, sprouting of endothelial cells into the hydrogel and endothelialization of the channels (Fig. 3), as well as formation of new vessels between the bioprinted channels with CD31 signals (Fig. 4).

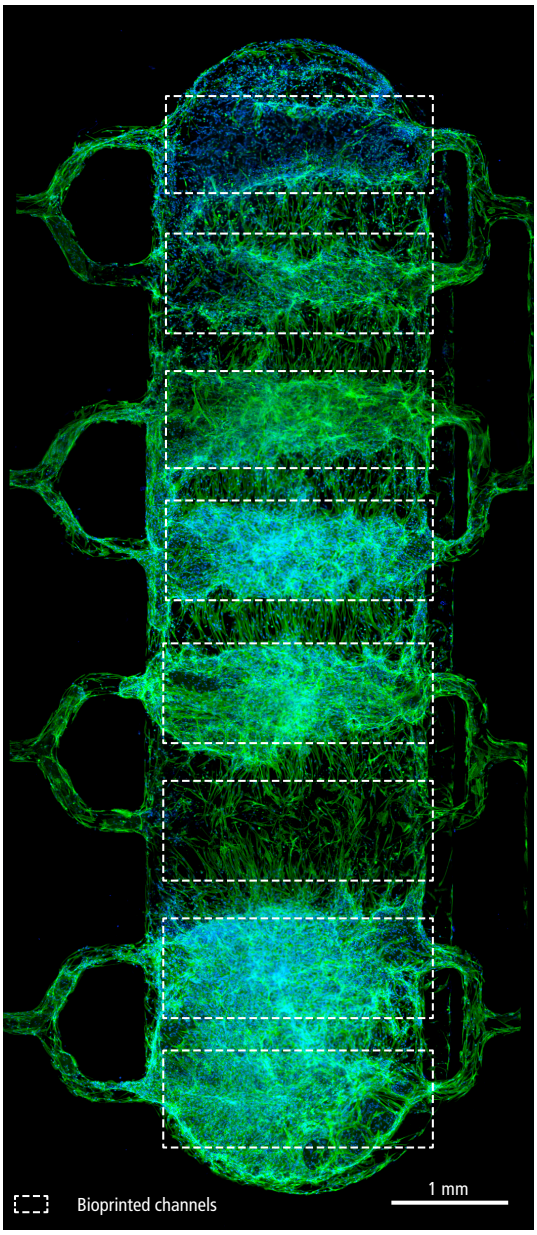


Figure 2: Bioprinted channels in **HUMIMIC Chip2$^{vasc}$**

Endothelial cells were cultivated in the bioprinted channels and co-cultured with mesenchymal stem/stromal cells (MSCs) in the hydrogel. Samples were stained with F-actin (green) and DAPI (blue). MIP image of the entire cell culture compartment, Scale bar indicates 1000 µm. Objective: CFI Plan Apochromat Lambda 4X (NA 0.20)

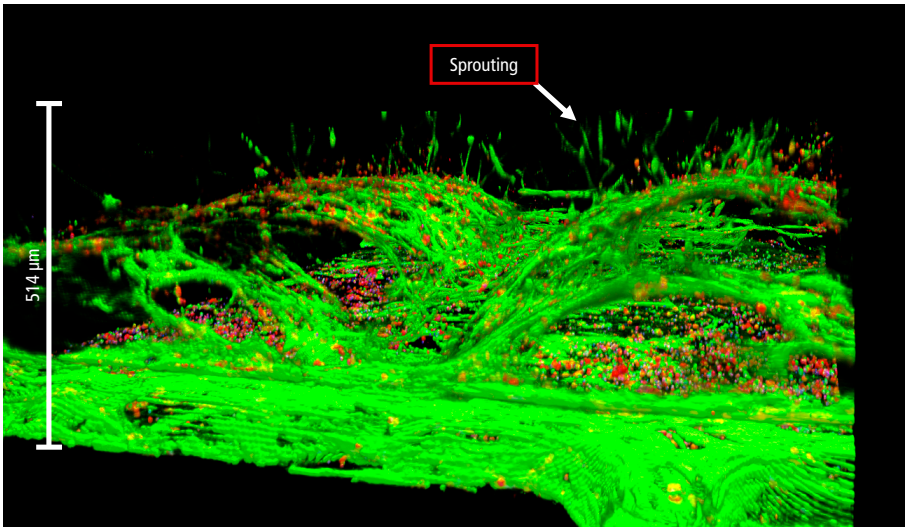


**Figure 3: Sprouting from the bioprinted channels**
Endothelial cells and immune cells (PBMCs – peripheral blood mononuclear cells) were co-cultured. PMA, FGF, and VEGF were used to promote angiogenesis.
Samples were stained with F-actin (green), DAPI (blue), and CellTracker Red CMPTX (Red).

Diagonal view of 3D image
Objective: CFI S Plan Fluor LWD 20XC (NA 0.70)

movie

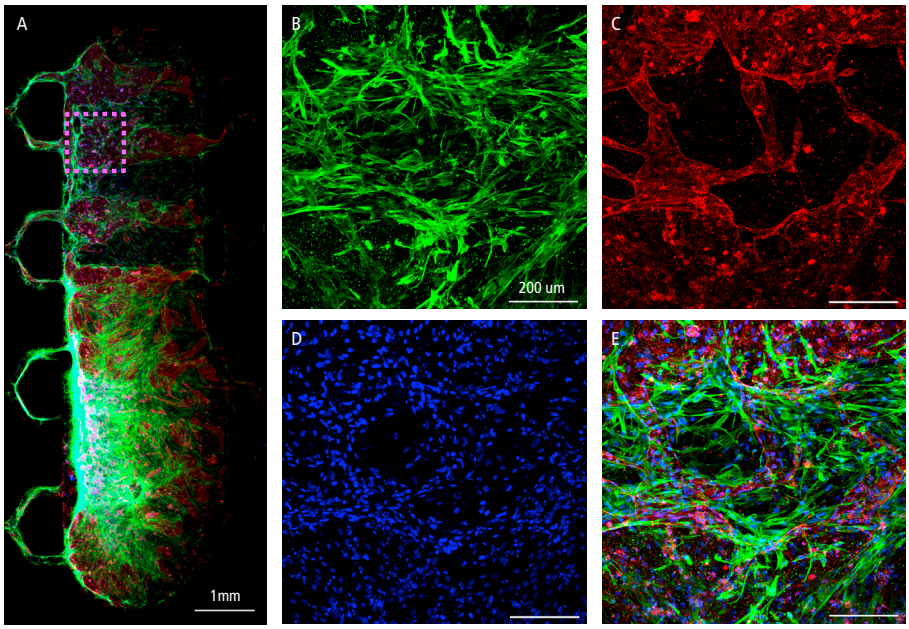


**Figure 4: Vessels between bioprinted channels**
Endothelial cells were cultured.
VEGF was used to promote angiogenesis.
Samples were stained with F-actin (green), DAPI (blue), and CD31 (Red).

A
Confocal image of the entire cell culture compartment
Scale bar indicates 1000 µm
Objective: CFI Plan Apochromat Lambda 4X (NA 0.20)

B, C, D, E
MIP images of the boxed region in A
Scale bar indicates 200 µm
Objective: CFI S Plan Fluor LWD 20XC (NA 0.70)

## Summary

In this application note, it was demonstrated that **HUMIMIC Chip2$^{vasc}$** can be used to create advanced vascularization structures. By selecting the appropriate image acquisition method, 3D structures and localization of markers could be clearly confirmed. The combination of **HUMIMIC Chip2$^{vasc}$** and Nikon confocal microscopes creates the future possibility of monitoring capillarization toward spheroids, such as kidney spheroids.

**Author Information**

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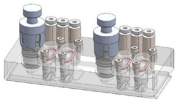
## Product information

### HUMIMIC Starter

Pumping pressure can be continuously adjusted then set automatically by the **HUMIMIC Starter**. This product is also equipped with a USB-port for easy management of pressure profiles and transfer of data. The **HUMIMIC Starter** is compatible with **HUMIMIC Chip2, Chip3, Chip3plus** and **Chip4**.

### HUMIMIC Chip2$^{vasc}$



The **HUMIMIC Chip2$^{vasc}$** is designed for endothelial cells to spread and cover all sides of the microfluidic channels. These channels are connected to a vascular bed where they can sprout to build the vascular connection to the organ models.

### AX/AX R Confocal Microscope

These microscopes achieve high resolution images of 8K x 8K pixels, which is four times that of conventional models. A large FOV with a diagonal of 25 mm allows acquisition of a large area of samples in a single scan, reducing phototoxicity. The AX R's resonant scanner achieves a high resolution of 2K x 2K pixels, allowing acquisition of live sample dynamics with high-speed imaging of up to 720 fps (2048 x 16 pixels).