INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

3D polymer structures for the identification of optimal dimensions for cellular growth for 3D lung alveolar models

Christian Maibohm [1], Alberto Saldaña Lopez [1], Oscar F. Silvestre[1] and Jana B. Nieder [1]

1. INL - International Iberian Nanotechnology Laboratory, Braga, Portugal

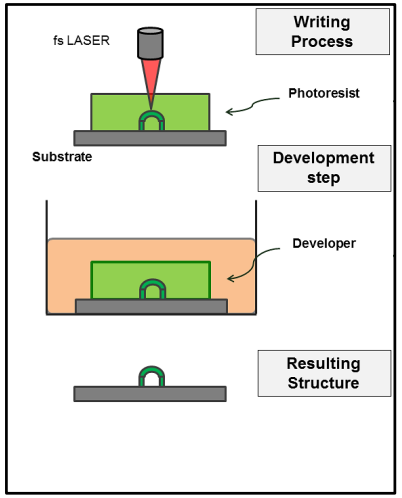
Christian Maibohm

Christian.maibohm@inl.int

Ultrafast Bio- and Nanophotonics group

Graphical abstract

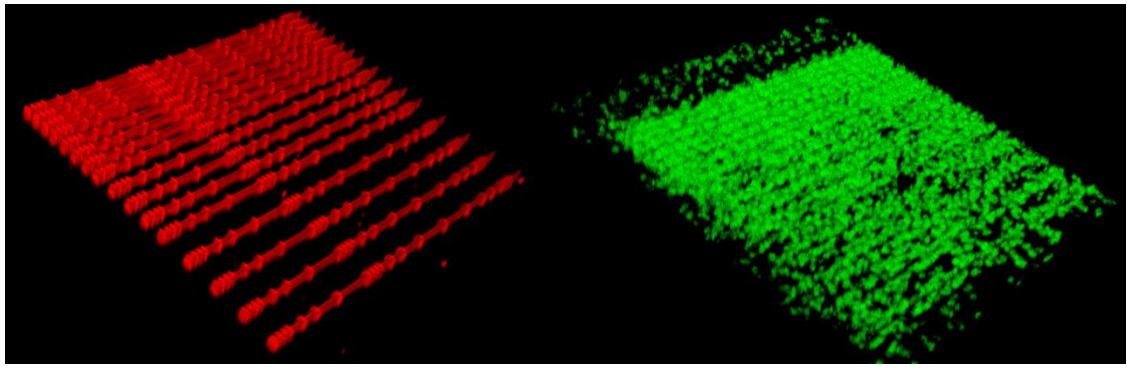
Two-photon polymerization fabrication of scaffold



Spectral separation

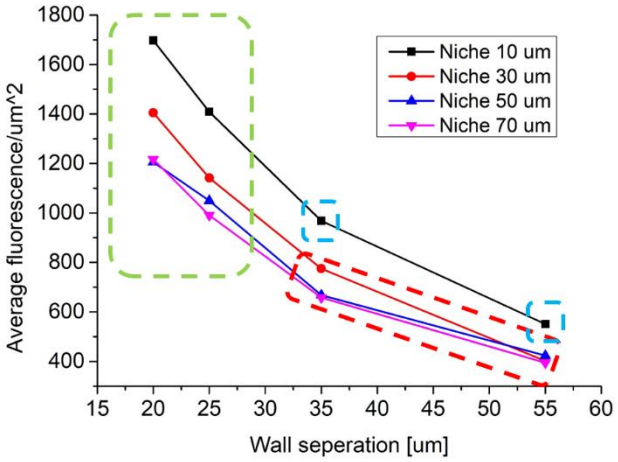
Scaffold

Cells



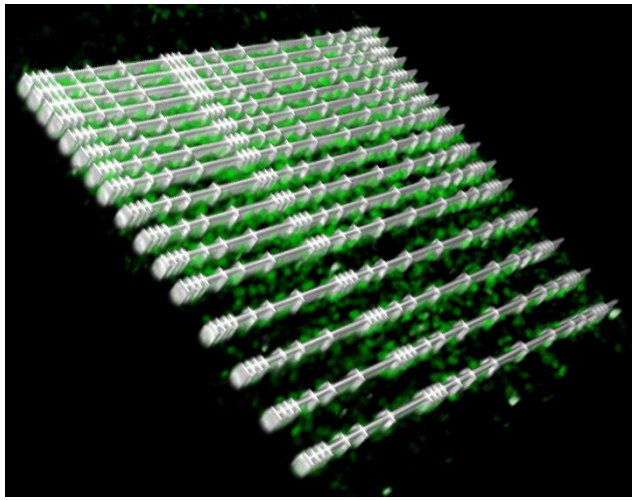
Analysis

Influence of scaffold dimensions on cell behaviour



A549 cell incubation on scaffold

Confocal imaging



Christian Maibohm
www.inl.int

Abstract and Keywords

Organ-on-chips and scaffolds for tissue engineering are vital assay tools for pre-clinical testing and prediction of human response to drugs and toxins, while providing an ethical sound replacement of animal testing and a low-cost alternative to expensive clinical studies. An important success criteria for these models is the ability to have structural parameters for optimized performance.

In this study we show that two-photon polymerization fabrication can be used to create 3D test platforms for analysing optimal scaffold parameters for cell growth. We design and fabricate a 3D grid structure, designed as a set of wall structures with niches of various dimensions for probing cell attachment. The 3D grid structures are fabricated from bio-compatible polymer SZ2080 and subsequently seeded with A549 lung epithelia cells. The seeded structures are imaged with confocal microscopy, were spectral imaging with linear unmixing is used to separate the auto-fluorescence contribution from the scaffold from the fluorescence of the cells and to determine the volume of cell material present different sections of the grid structure. The variation in structural parameters influences A549 cell distribution. In the future this kind of differentiated 3D growth platform, could be applied for optimized culture growth, cell differentiation and advanced cell therapies.

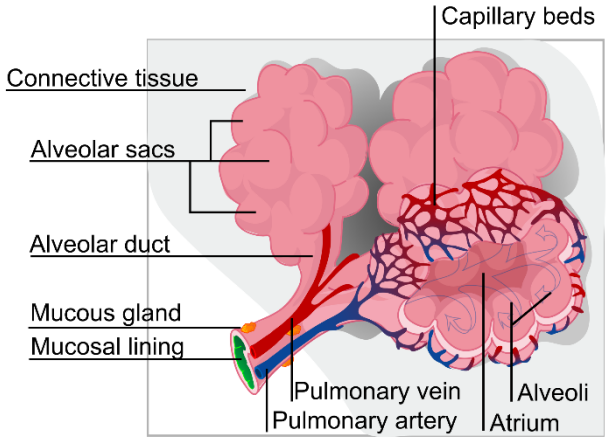
Keywords: Two-photon polymerization, 3D cell scaffolds, Spectral imaging, Linear unmixing

Introduction

Organ-on-chips

- Description:
  - Organs-on-chips are microchips lined by living cells used in drug development, disease modelling, toxicology studies and personalised medicine.
  - Organ-on-chips incorporate the 3 R´s: Reduce, Refine and/or Replace animal models.
- Sort after organ-on-chips qualities:
  - Mimicking full organ or specific parts, including 3D morphological hierarchy and functionality.
  - Optimized and realistic growth conditions.
  - Ease of measurement, e.g. imaging, etc..

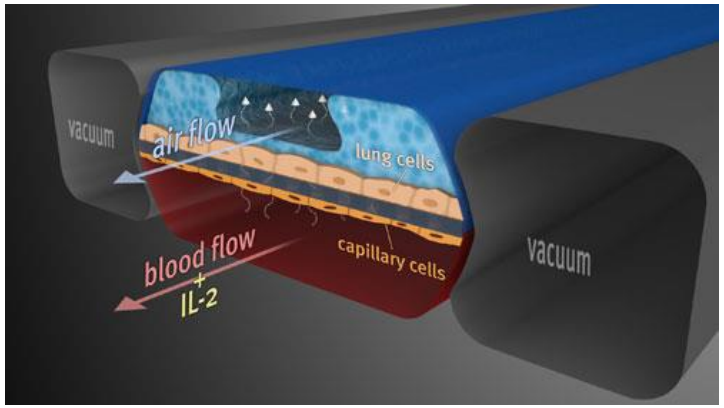
Representation of human alveolar model



https://en.wikipedia.org/wiki/Alveolar_duct

- Replication of the morphological hierarchy of organs requires:
  - Feature sizes from sub cellular (>10 µm) to large tissue and organ parts (cm, mm).
  - Encompass 3D architecture.

Organs-on-chips, alveolar model



https://wyss.harvard.edu/

- Here we show that two-photon polymerization (TPP) is a well suited fabrication technique to encompass the requirements for free form 3D architecture and spans the needed feature sizes within a single fabrication step.

- Functionality
- Ease of measurement

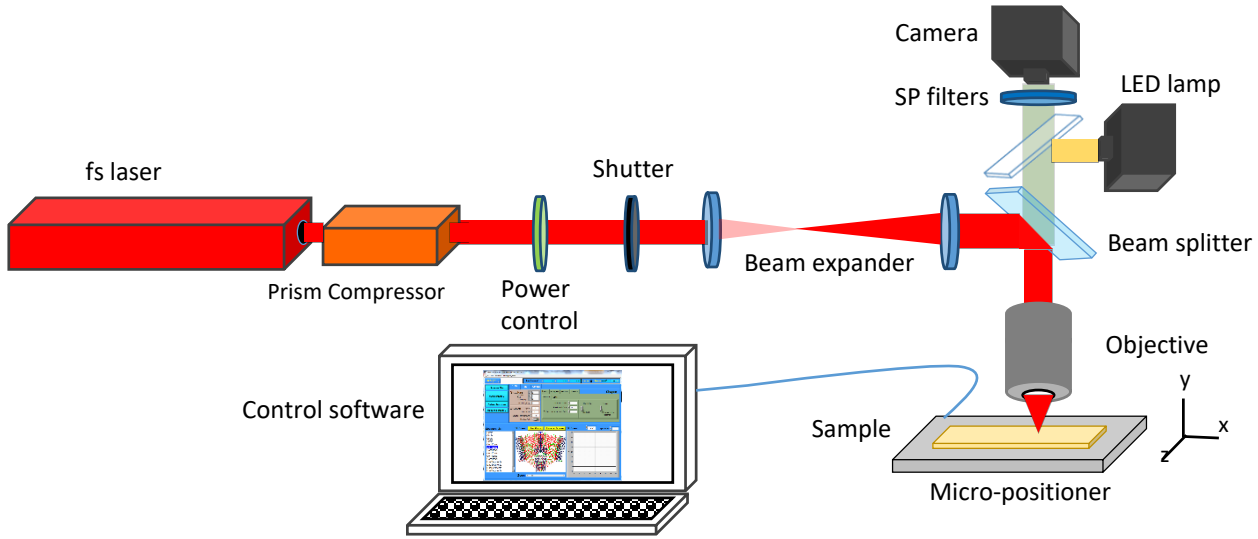
- 3D morphological hierarchy

Christian Maibohm
www.inl.int

Introduction

Two-photon polymerization fabrication

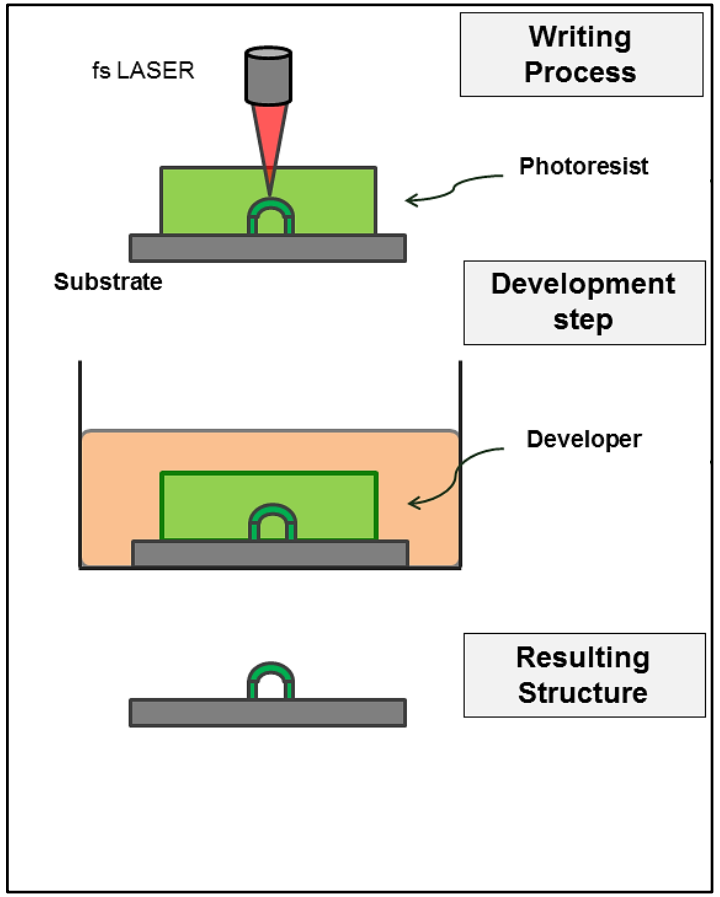
Setup



- Fabrication parameters:
  - Laser power 12 mW
  - Writing speed 25 µm/s
  - Objective x40 dry

For more information on the TPP process:
Maibohm. *et al. Sci Rep* 10, 8740, 2020).

Process



Negative photon resist (e.g. SZ2080)

Results

Design and optical image

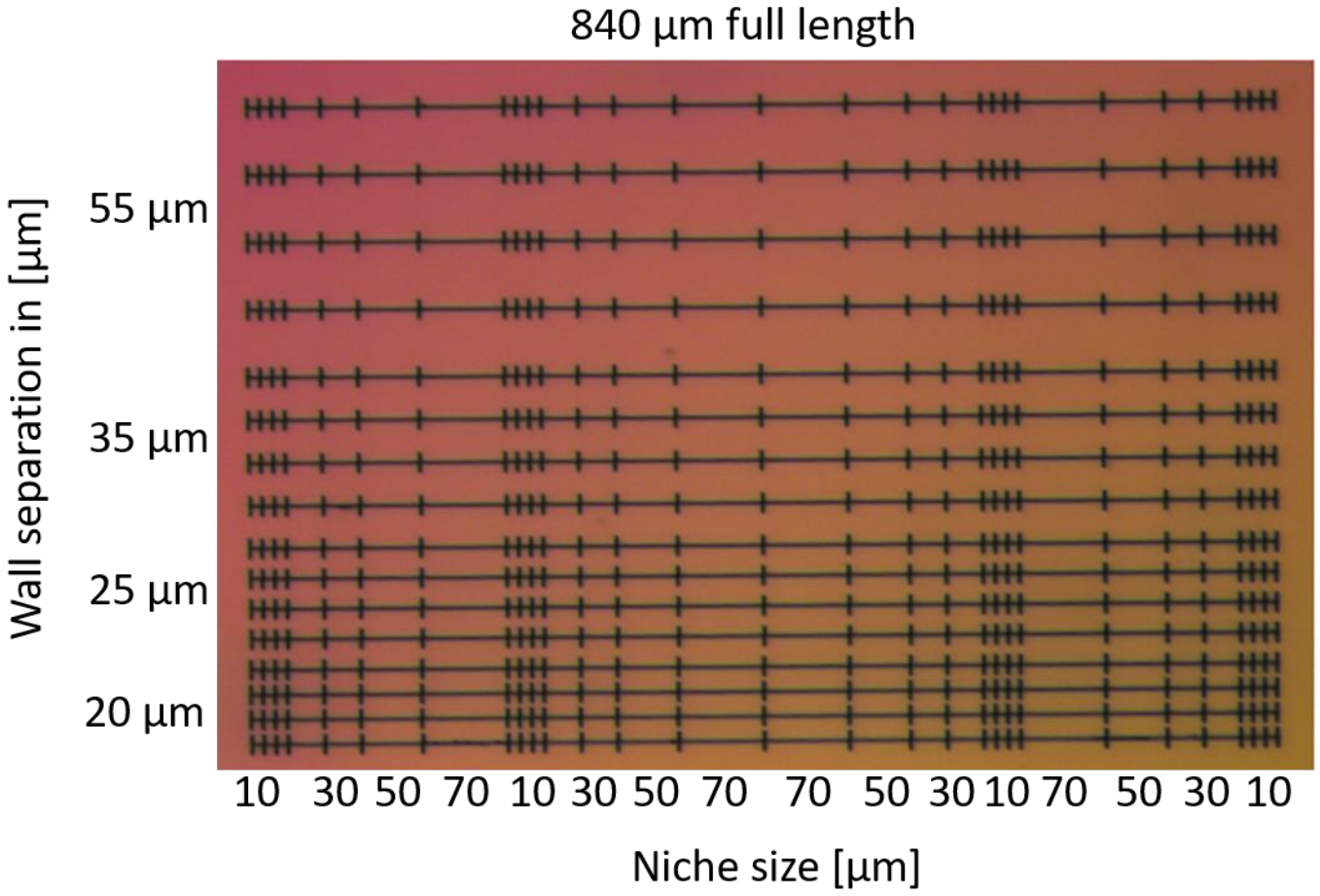
- Goals: To fabricate a structure that encompass morphological hierarchy and easy imaging

- Design:
  - An open vertical scaffold for easy imaging

  - Morphological hierarchy is created by varying two parameters throughout the structure:
    - Niche size is varied (10, 30, 50 and 70 µm) along the wall length and are protruding 7.5 µm from the wall.
    - Wall separation are varied (20, 25, 35 and 55 µm).
    - Height of the scaffold is 24 µm.

- Scaffold fabricated by two-photon polymerization in the bio-compatible negative resist SZ2080

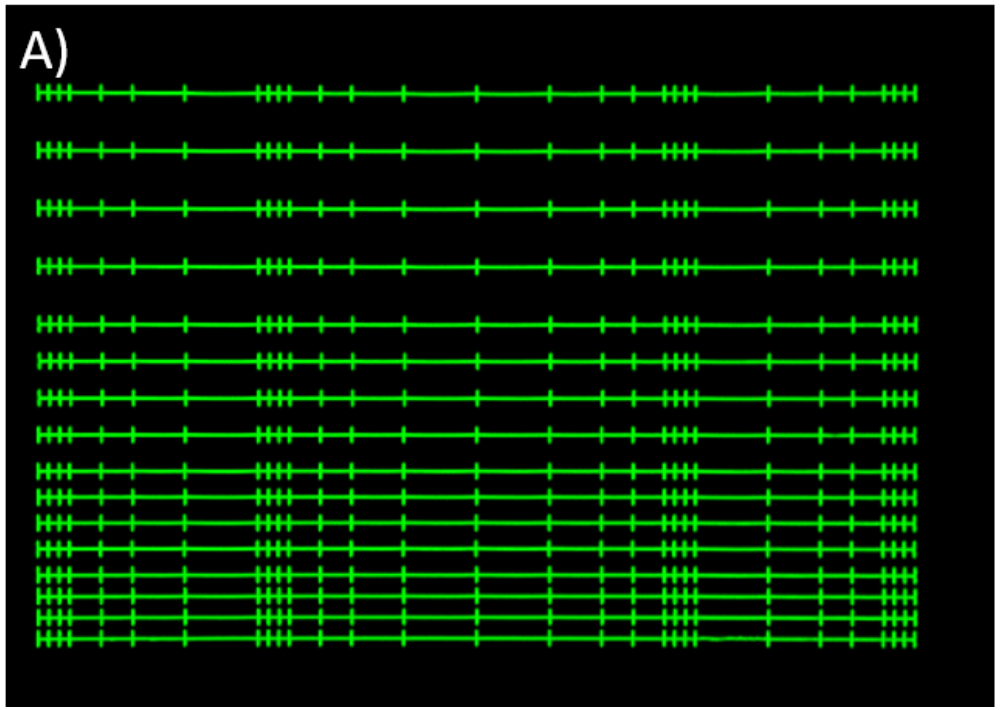
Optical image of full scaffold



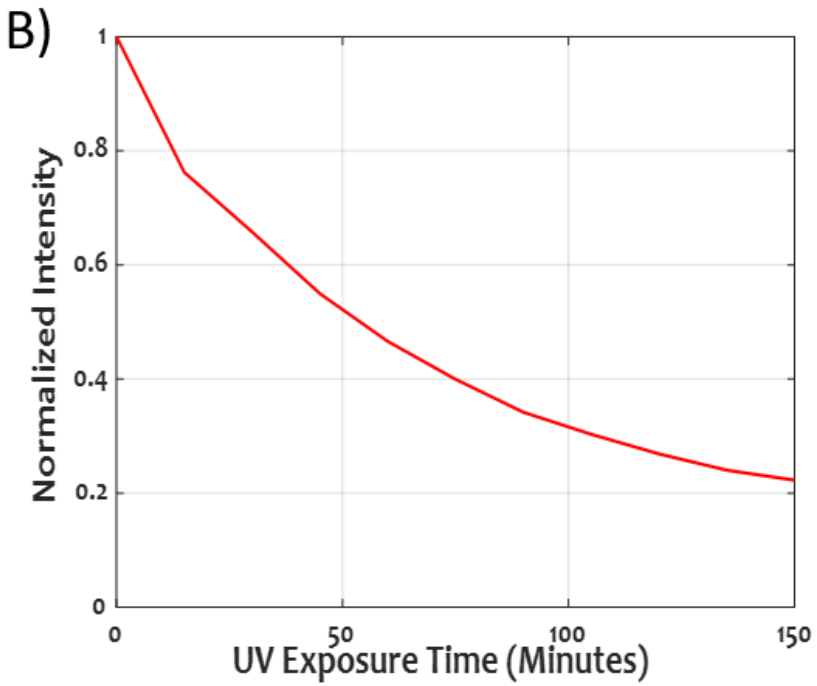
Results

Confocal imaging, Autofluorescence and photo bleaching

Confocal image, excitation@405 nm



Decrease of fluorescence intensity



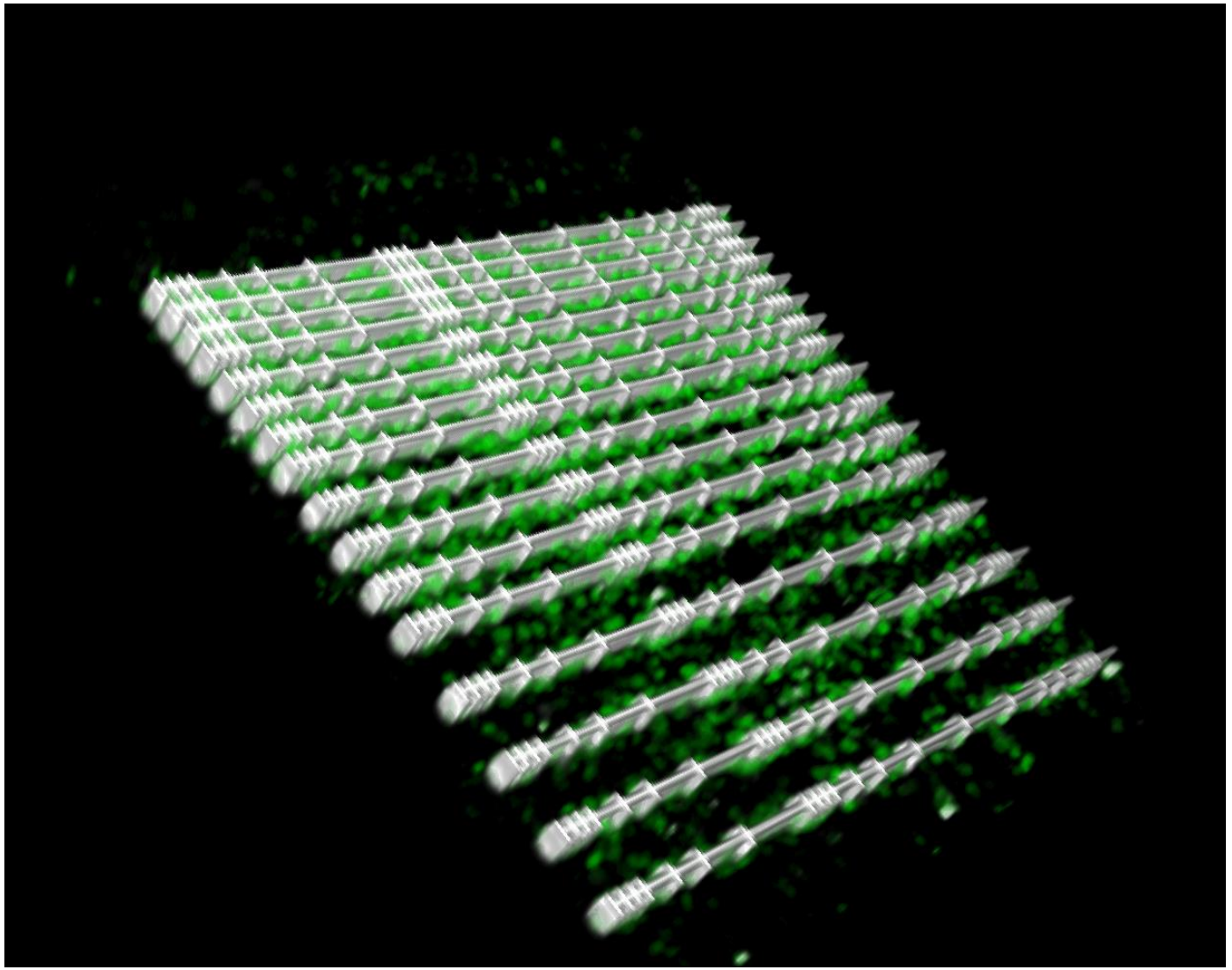
- The use of photo-initiators in the resist leaves the scaffold autofluorescence after development.
- The autofluorescence is reduced to 20% by UV photobleaching.

Results

Epithelia cells A549 on scaffold

- Seeding of scaffold with lung epithelia cell A549
- A549 cells stained with 3 dyes: Hoechst 33342 (Nuclei), CF488 (actin filaments), Mito tracker red (mitochondria)
- A549 cell size: diameter 10-14 µm
- Imaging: Confocal Z-stack (λ-mode)

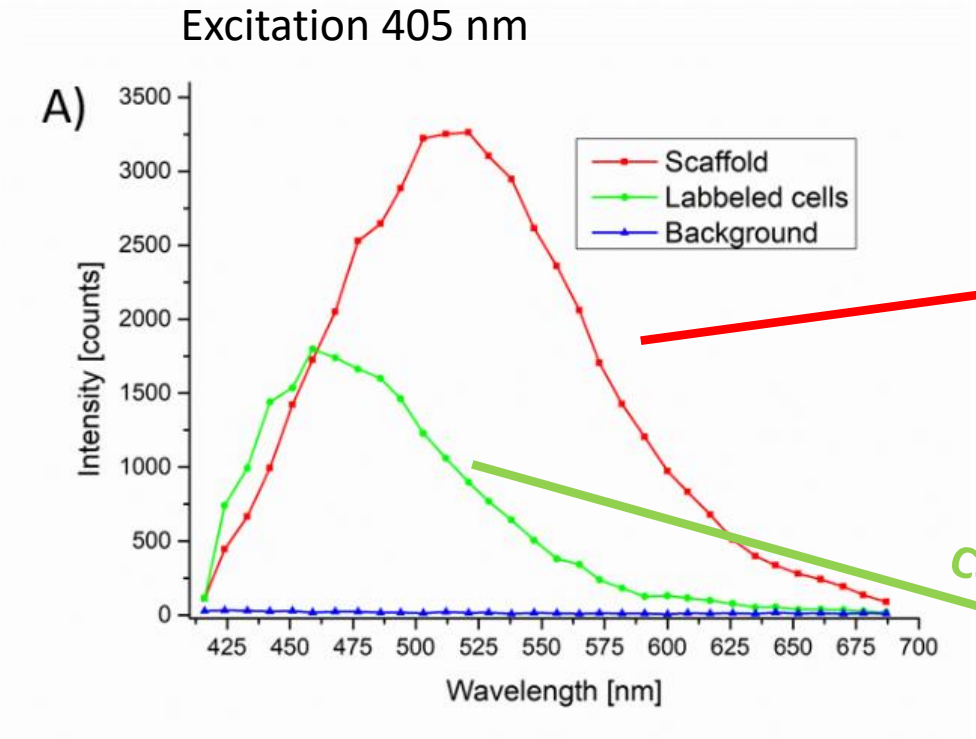
3D Confocal imaging

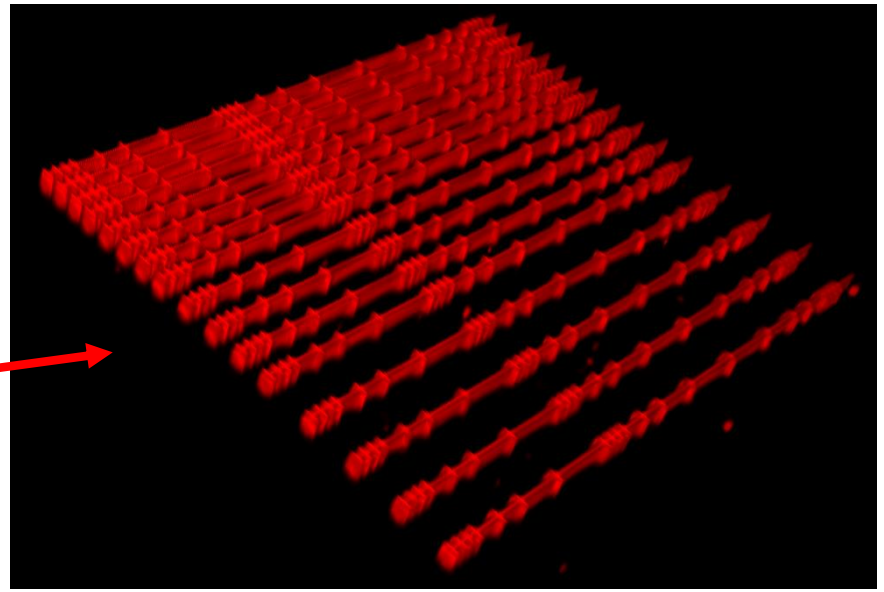


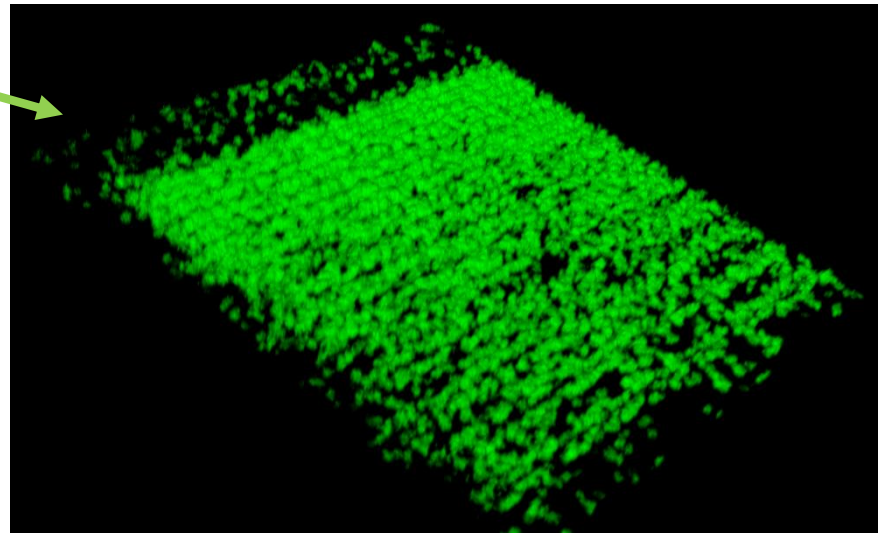
Excitation@405 nm, exciting scaffold (white) and cell nuclei (green)

Results

λ-mode, spectral separation





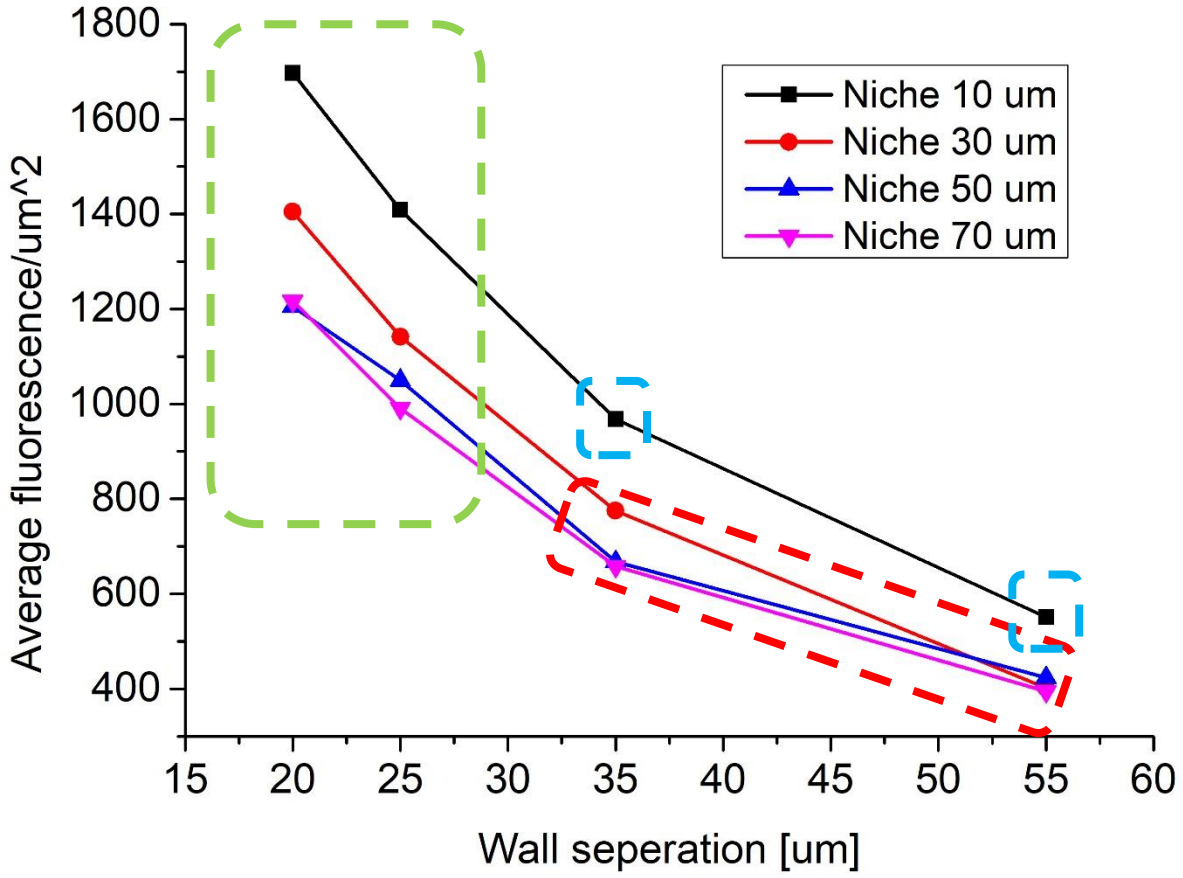


- 32 detector channels creates a spectral fingerprint enabling separation the different fluorescence components (LSM 780 Zeiss)

Discussion

Average cell nuclei material per unit volume

- Question: Does the morphological hierarchy cause any change in the cell material per unit area?



- Observation:
  - Increased number of fluorescence per unit area with deceasing available volume.

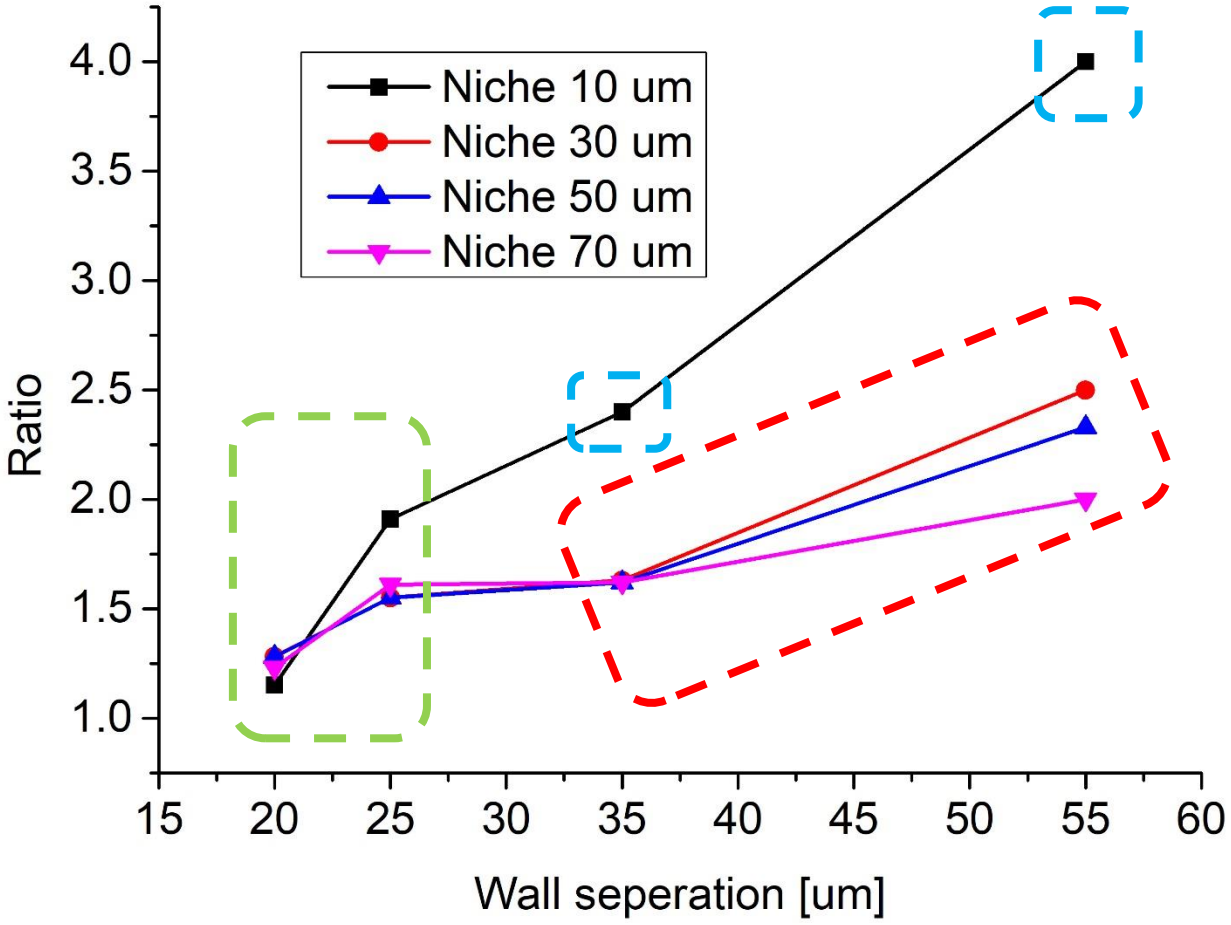
At least one morphological dimension is similar to the cell diameter

Both morphological dimensions are larger than the cell diameter

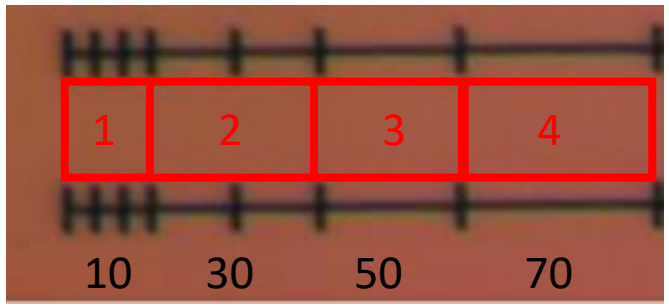
Sub cellular niche features, long distance to next wall

Discussion

- Question: Are the cells located on the scaffold or between the walls?



Example of defining the areas between walls



55 µm

Niche size [µm]

Wall separation similar to cellular size

Both morphological dimensions are larger than the cell size

Sub cellular niche features, long distance to next wall

Conclusion

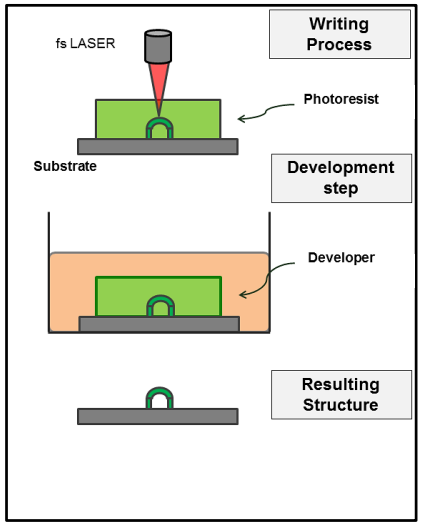
- TPP is a suited fabrication method for 3D organ-on-chips architecture.
  - Feature size range, single step fabrication.

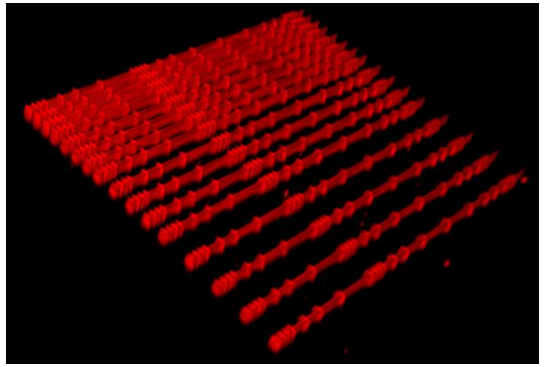
- Confocal λ-mode provides a way to spectrally separate fluorescence components including autofluorescence from the scaffold

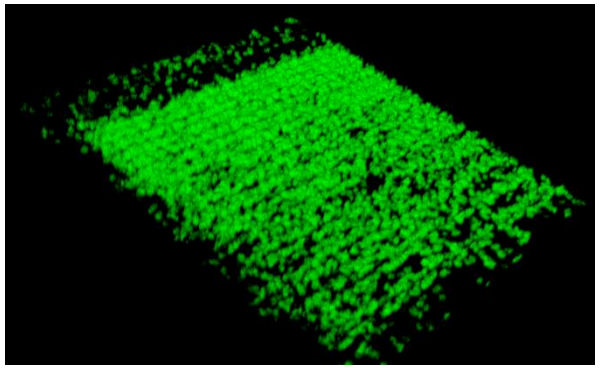
- 3D morphological hierarchy plays an important role in cell behaviour in organ-on-chips
  - We observe for the A549 cells:

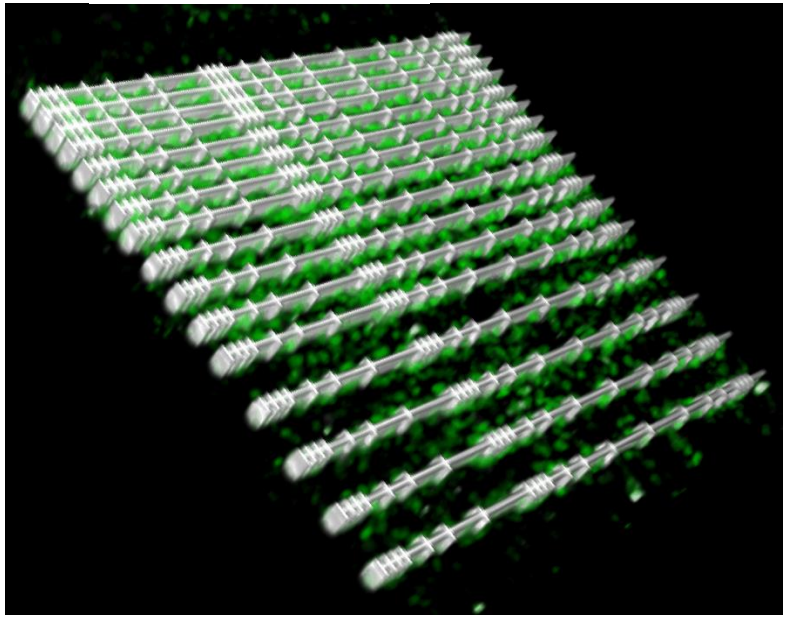
    - When one or both scaffold dimensions are larger than the cell size:
      - The cells prefer to stay close to the wall, also avoiding the flat 2D surface between the walls.

    - When both parameters are similar to the cell size, the cell can span either or both the niche and the wall separation:
      - The cells tend to be more uniformly distributed in the structure volume.









Acknowledgments

I would like to thank the following colleges for their large contribution to this work:

Jana B. Nieder
Research Group Leader
Head of NBI facility

Oscar F. Silvestre
Former Postdoc at INL
Now Postdoc at Center for Cooperative Research in Biomaterials (CIC biomaGUNE)

Alberto Saldaña Lopes
Former scientific visitor at INL
Now Ph.D. student at University of Geneva

Thank you for your attention!