Two-photon polymerization of polymeric and hydrogel scaffolds for 3D neural cell culture

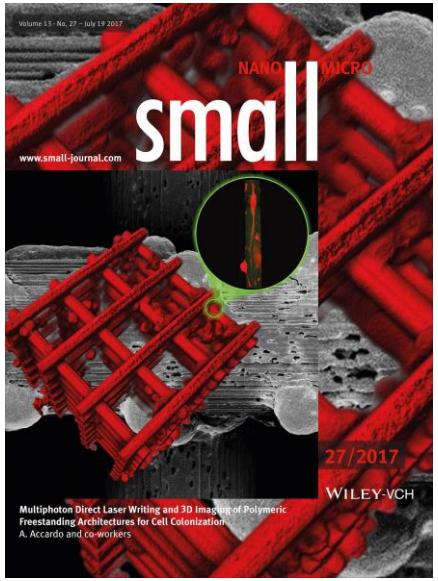
Dr. Angelo Accardo

Assistant Professor Soft Micro- Nanosystem Technology for Life Sciences and Biology
Department of Precision and Microsystems Engineering (PME)
Faculty of Mechanical, Maritime and Materials Engineering (3mE)
A.Accardo@tudelft.nl

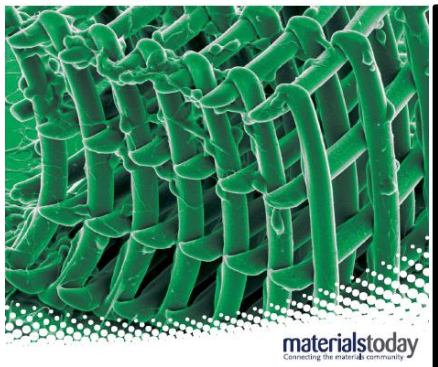
TU Delft

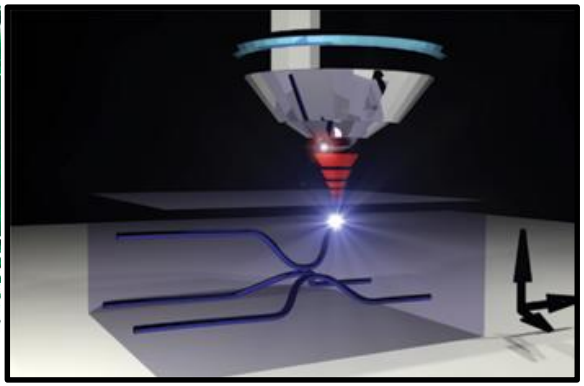
Nano 2020 Innovation
Rome, 15-18 September
Conference & Exhibition

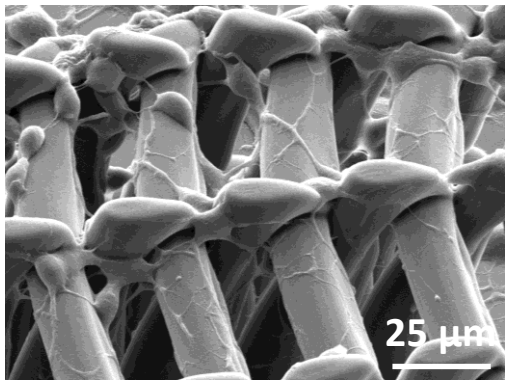
Outline

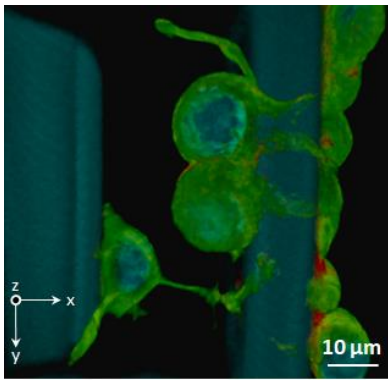


- **3D fabrication** of free-standing architectures by 2-photon polymerization (2PP)
  - Conventional 2P-DLW polymeric materials
  - Biocompatible hydrogels
- **3D Imaging** of neuronal cells colonization of the 3D scaffolds
  - ✓Scanning electron microscopy (SEM)
  - ✓Light sheet fluorescence microscopy (LSFM)
  - ✓Two-photon confocal imaging (2PI)
- Brief outlook on **ongoing projects related to neuronal microenvironments**



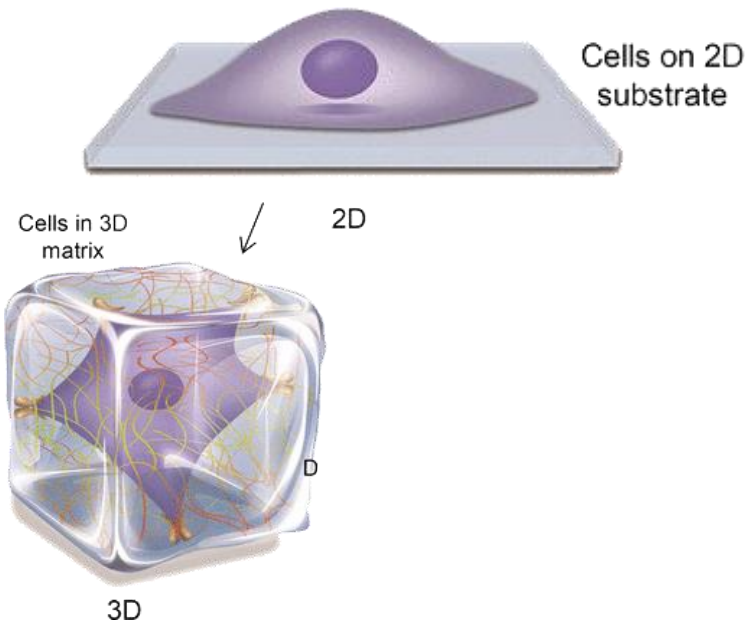


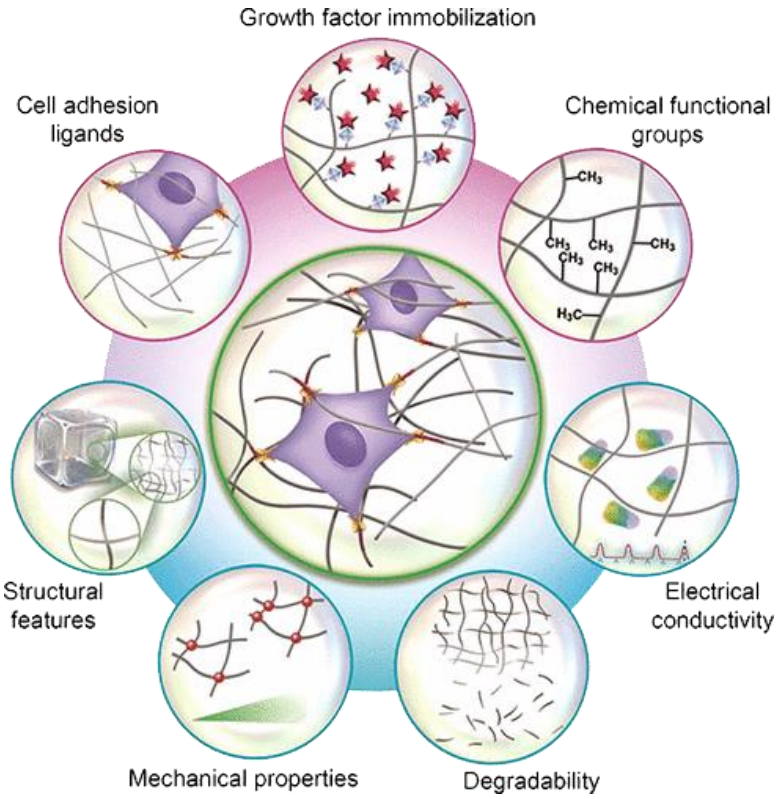




TU Delft

Evolution of cell culture models: from 2D to 3D (…to 4D)





Huang et al., *Chem. Rev.* **2017**, 117, 20, 12764-12850

Fan, Staufer, Accardo, *Bioengineering*, **2019**, 6 (4), 113

Mission

Engineer multi-scale 3D & 4D biomimetic microenvironments addressing cell biology applications

- ✓**Mechanobiology**
- ✓**In-vitro disease models**
- ✓**Tissue Engineering**

TU Delft

Two-photon polymerization
of 3D neuronal microenvironments

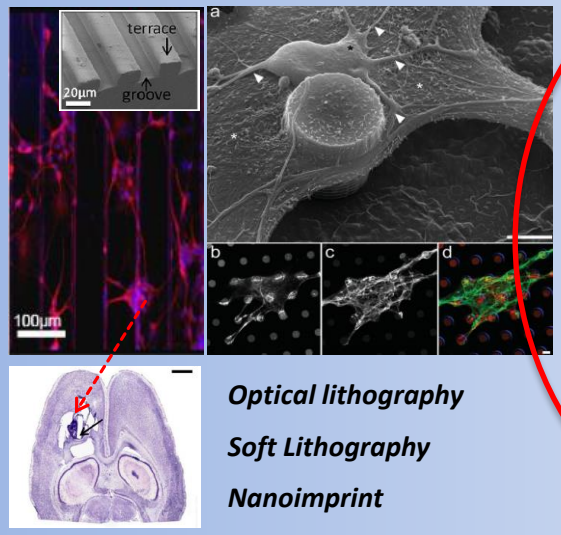
Neuronal Microenvironemnts

2D Scaffolds

3D Scaffolds

Tissue-like Constructs

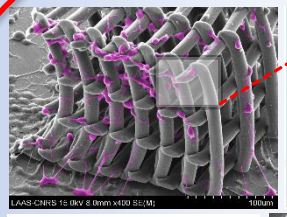
Top-down

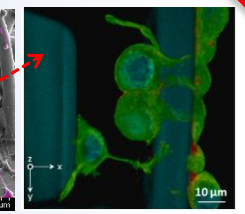


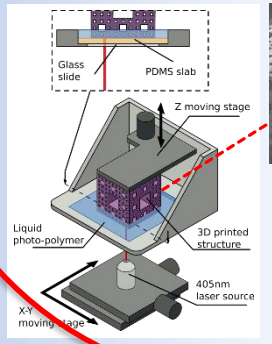
Optical lithography

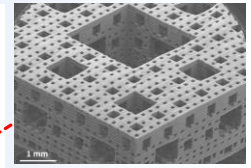
Soft Lithography

Nanoimprint





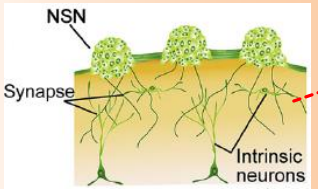


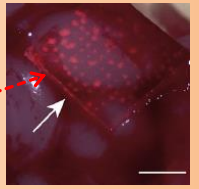


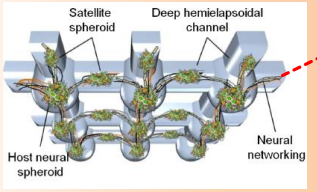
Two-photon polymerization

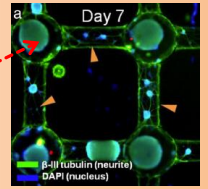
Stereolithography

Bioprinting

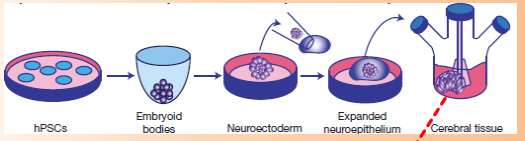






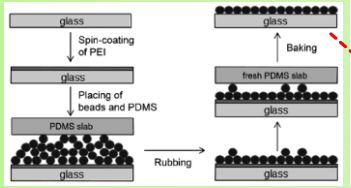


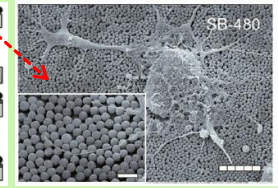
Neurospheroids

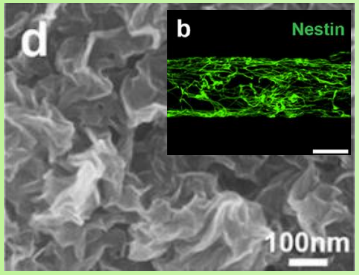


Cell-biology based strategies

Bottom-up

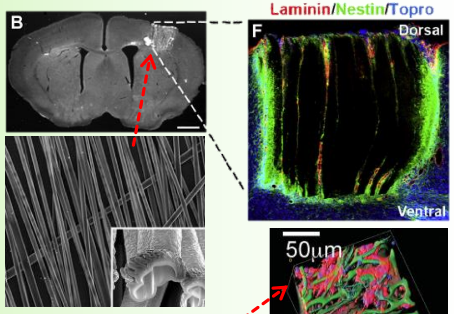


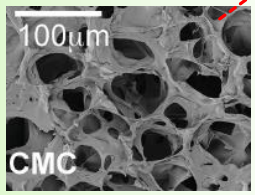




Microbeads self-assembly

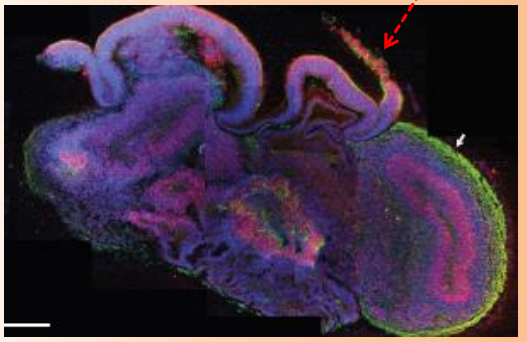
Graphene sheets/fibers

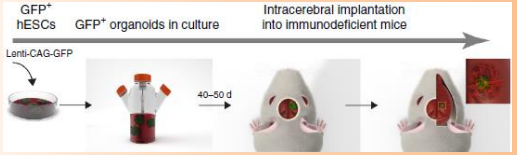


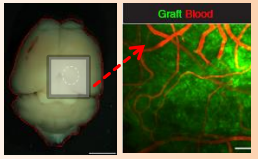


Electrospinning

Hydrogel self-assembly







Neural Organoids

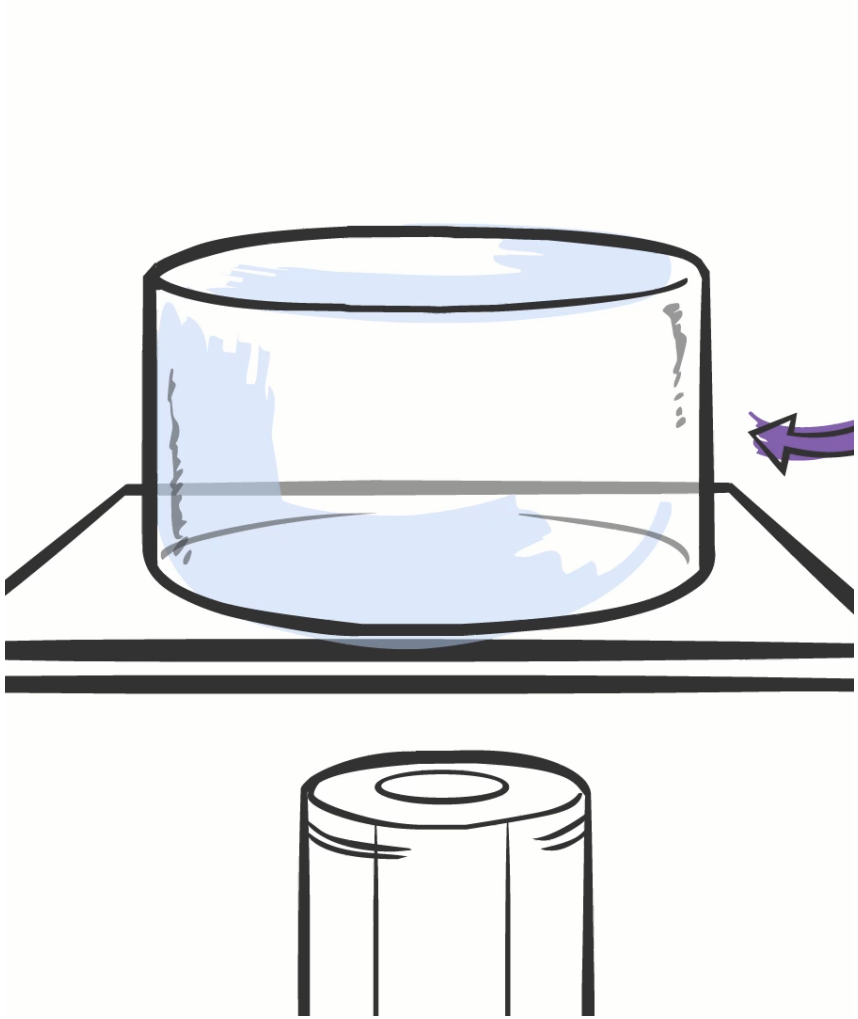
Matrigel

Accardo et al. *Brain Res. Bull.*, **2019**, 152, 202-211

Fan, Staufer, Accardo, *Bioengineering*, **2019**, 6 (4), 113

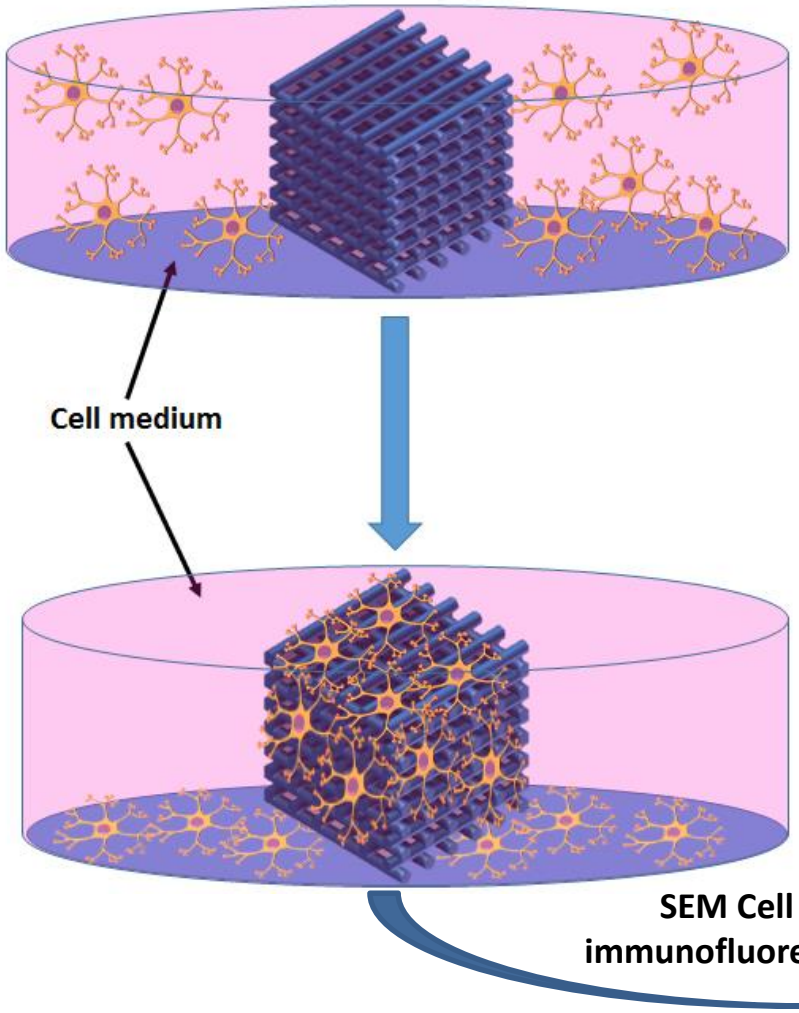
3D printing of cellular microenvironments

- **2-Photon polymerization (2PP) approach:**
  - ✓**Femto-second pulsed IR laser source**
  - ✓**The photosensitive material is «transparent» to IR radiation while absorptive in the UV one**
  - ✓**High resolution (≈200 nm) compared to other 3D fabrication approaches (fused deposition modeling, ink-jet, selective laser sintering, polyjet technology...)**

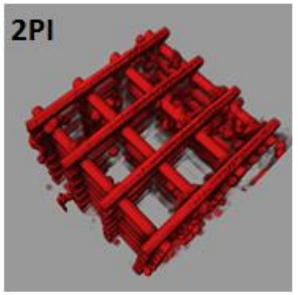


TU Delft

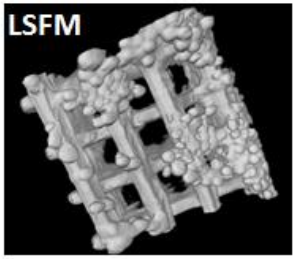
Overview of the 3D cell culture/3D imaging experimental protocol



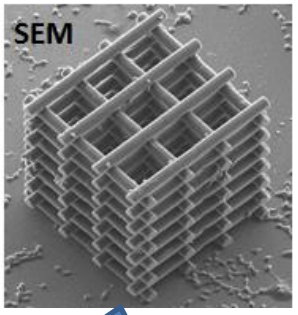
Multiphoton confocal imaging (Quantitative Cell localization within the scaffold)



Light Sheet Fluorescence Microscopy (Qualitative Cell localization within the scaffold)

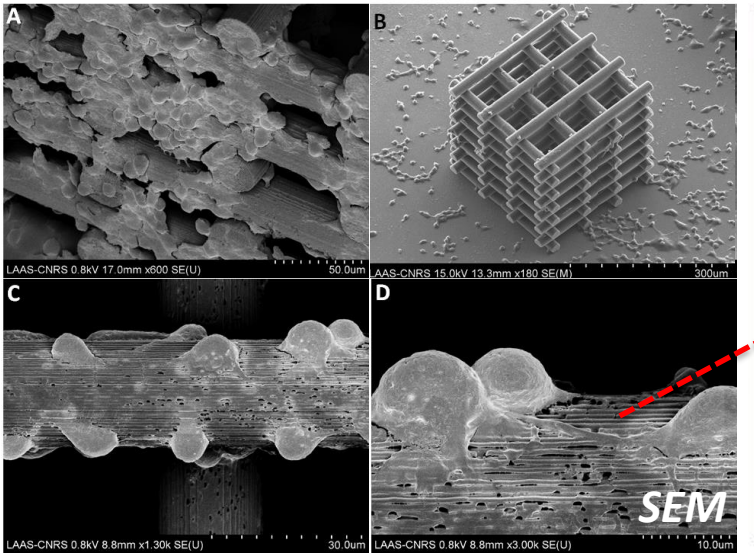


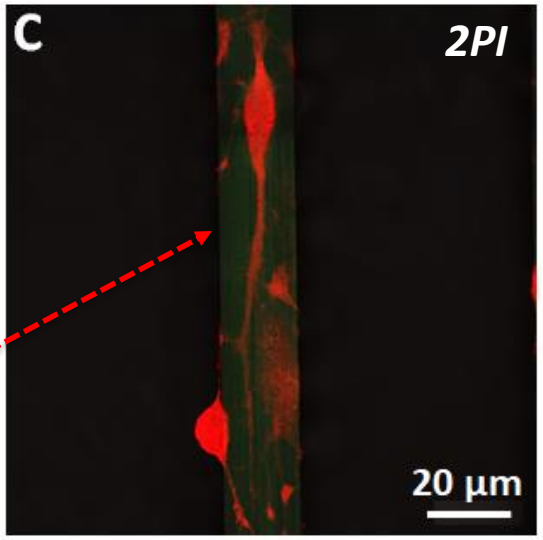
Scanning Electron Microscopy (Cell Morphology)

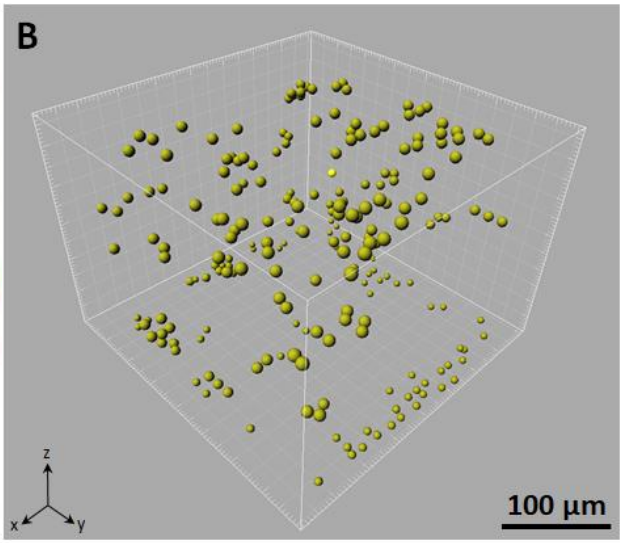


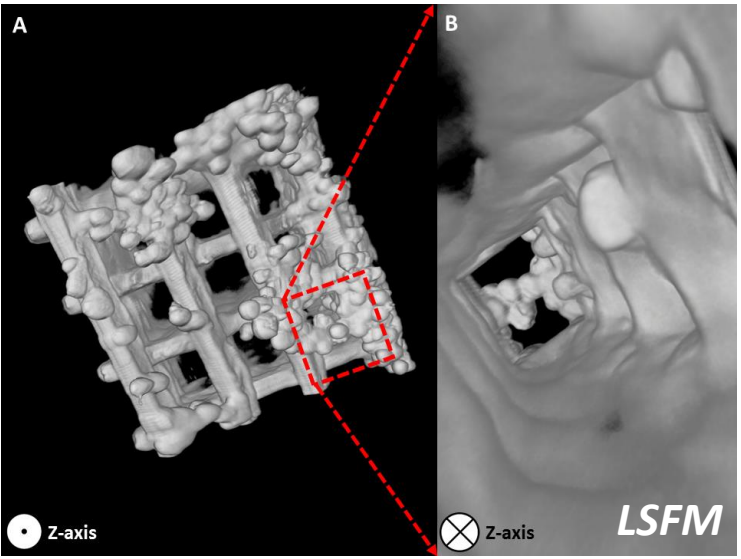
Accardo et al. *Small*, **2017**, 13 (27), 1700621
Accardo et al. *Biomed. Phys. Eng. Express*, **2018**, 4, 027009
Onesto et al. *Neural Regen. Res.*, **2020**, 15 (4), 759
Accardo et al. *Materials Today*, **2018**, 21 (3), 315-316
Accardo et al. *Brain Res. Bull.*, **2019**, 152, 202-211
Fan et al. *Bioengineering*, **2019**, 6 (4), 113

3D “wood-pile” neuronal scaffolds made of IP-Dip material









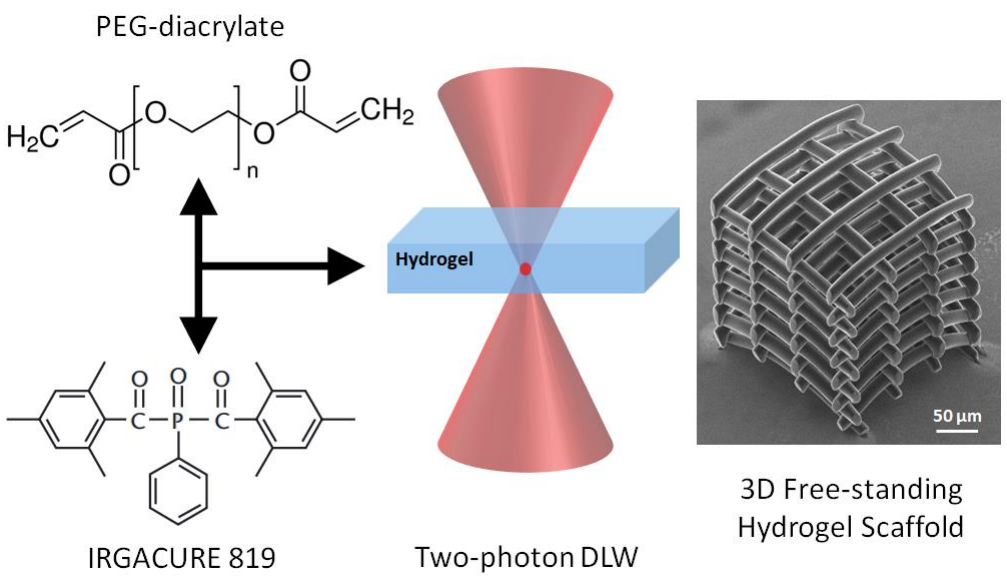
- **Efficient cell colonization in 3 dimensions, high cell adhesion,** formation of **freestanding cellular bridges** linking cells proliferating on different cylindrical subunits
- **Advanced 3D immunofluorescence imaging protocol** for **accurate detection** of the cells within the 3D scaffold and of **aligned, long (60 µm) neuritic connections**
- ***Following step:*** *integration of more biocompatible and less autofluorescent biomaterials*

TU Delft

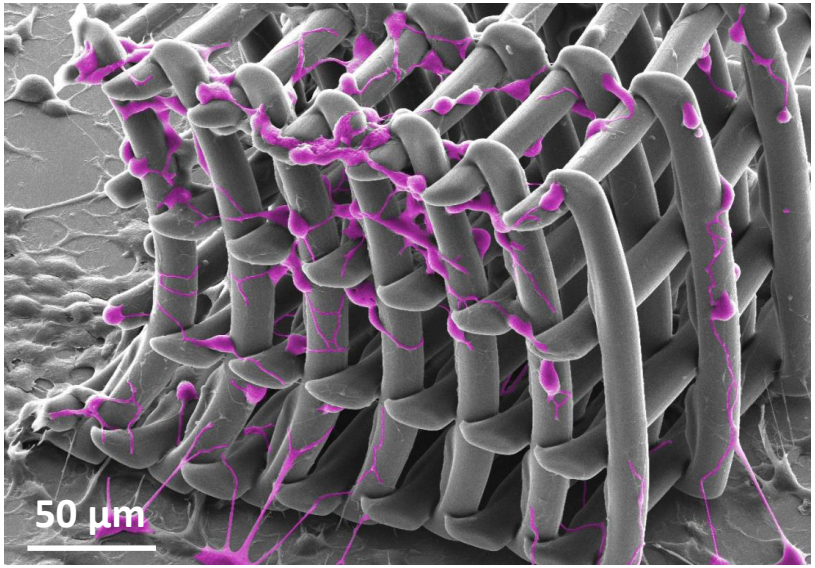
Accardo et al. *Small*, **2017**, 13 (27), 1700621

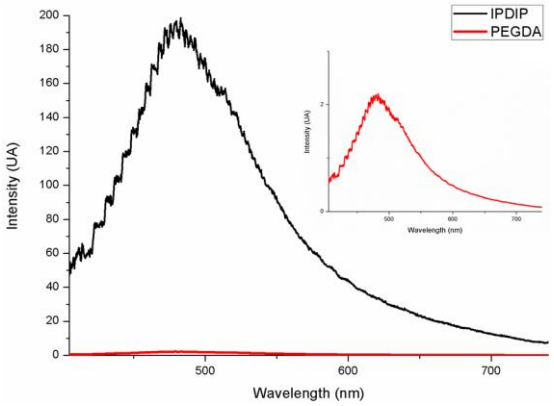
Towards more biocompatible (and transparent) biomaterials

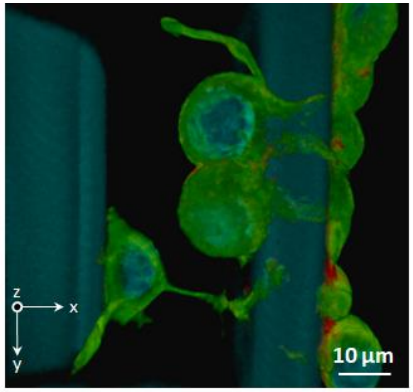
Two-photon lithography of free-standing PEGDA-hydrogel scaffolds for neuronal cell growth



3D Free-standing Hydrogel Scaffold







- Poly(ethylene glycol) diacrylate (PEGDA), a biocompatible hydrogel approved by FDA
- Lower stiffness compared to conventional 2PL materials (kPa VS GPa)
- Intrinsic fluorescence emission of the PEGDA hydrogel 100 times lower than IP-DiP
- 3D immunofluorescence multi-staining

TU Delft

Accardo et al. *Biomed. Phys. Eng. Express*, **2018**, 4, 027009; Accardo et al. *Materials Today*, **2018**, 21 (3), 315-316

Acknowledgements

TU Delft

Precision and Microsystems Engineering

Ahmed Sharaf
Daniel Fan
Beatriz Costa
Dian Veldkamp
Brian Roos
Qais Akolawala

Bionanoscience Department
Think big about life at the smallest scale

Dimphna Meijer
Catia Freias

nano scribe

Health TU Delft 10 YEARS

3mE

Biomedical Primate Research Centre

Jeffrey Bajramovic
Raissa Timmermann

LAAS CNRS

Christophe Vieu
Laurent Malaquin
Charline Blatche

European Commission

TU Delft

Thank you for your attention

¿Questions?