Additive Laser Nanofabrication and 3D Printing

Boris N. Chichkov
Leibniz University Hannover

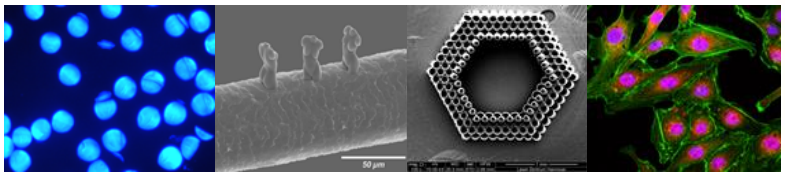
Laser Zentrum Hannover, Germany

13.05.2016

b.chichkov@lzh.de

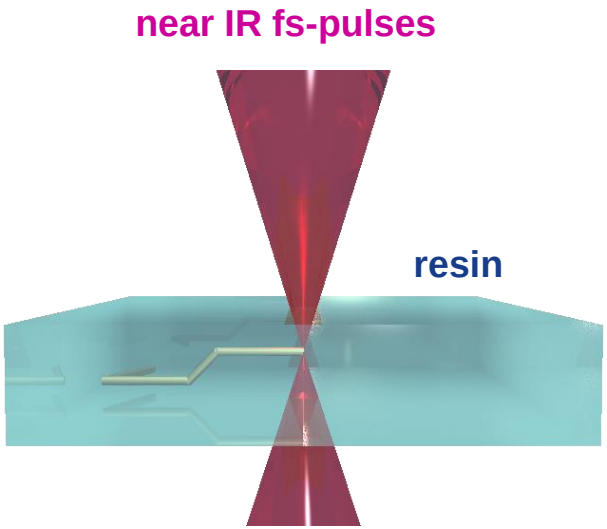
rebirth

BIOFAB BIOFABRICATION

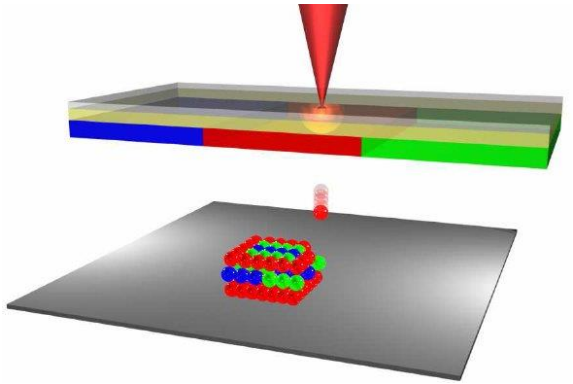


LZH LASER ZENTRUM HANNOVER e.V.

Laser Printing of…

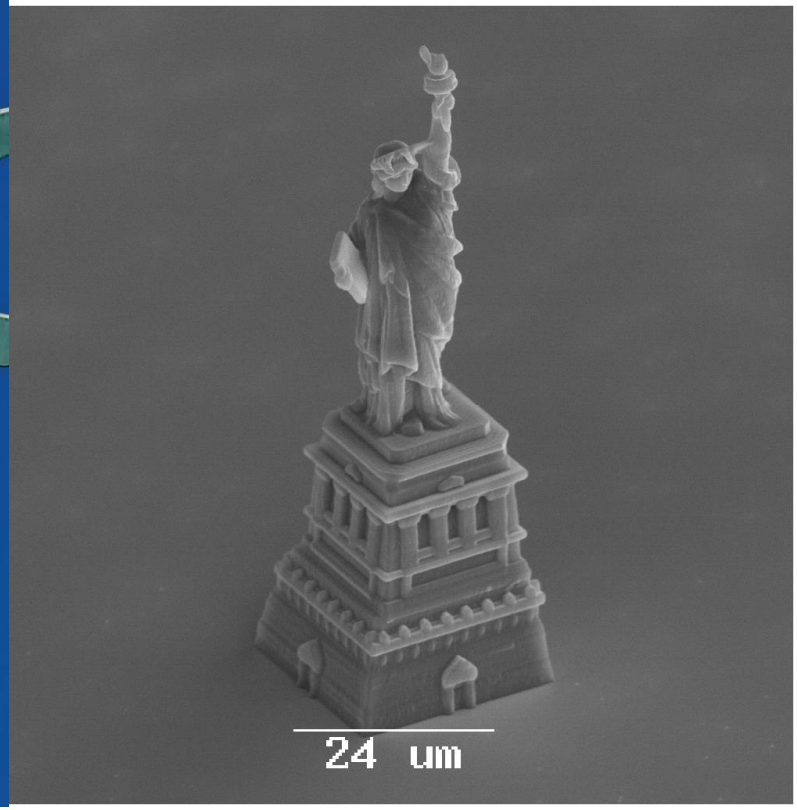


Micro-structures by two-photon polymerization



Nanoparticles and living cells

Two-photon polymerization



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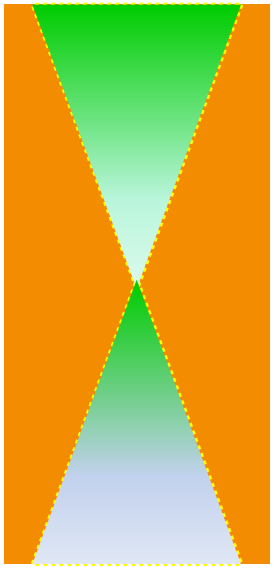
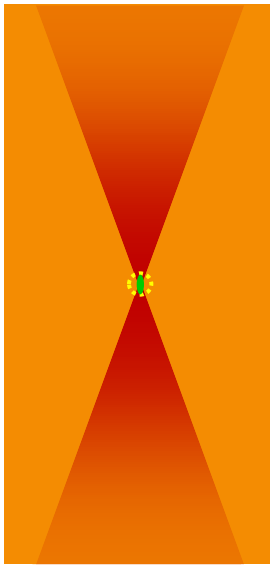
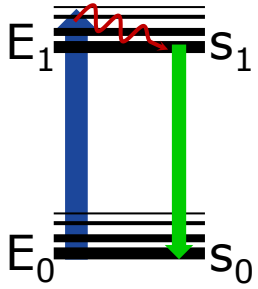
Nonlinear microscopy with fs-pulses

Linear excitation

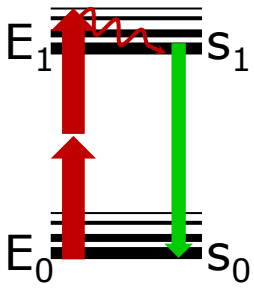


Non-linear excitation





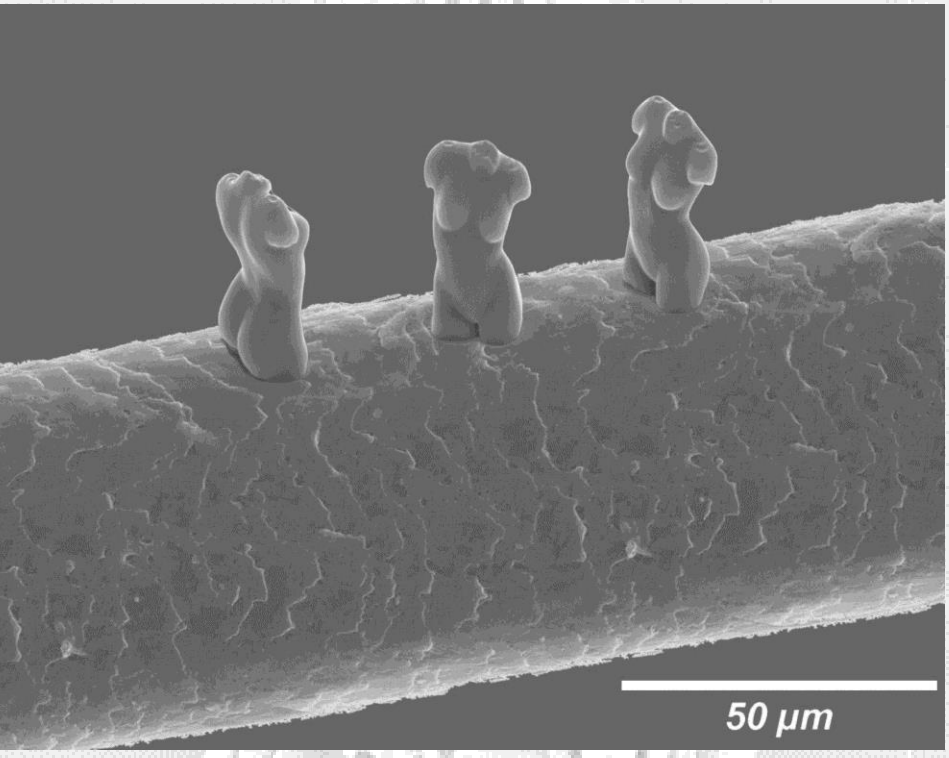
1P induced Fluorescence



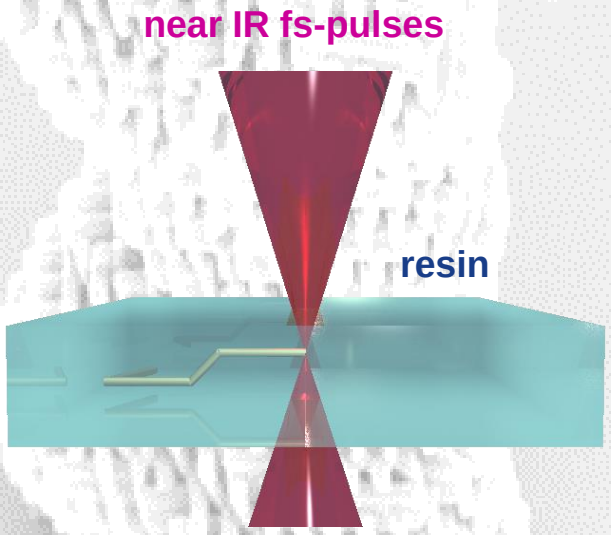
2P induced Fluorescence

Fluorescein

3D nanostructuring by two-photon polymerization

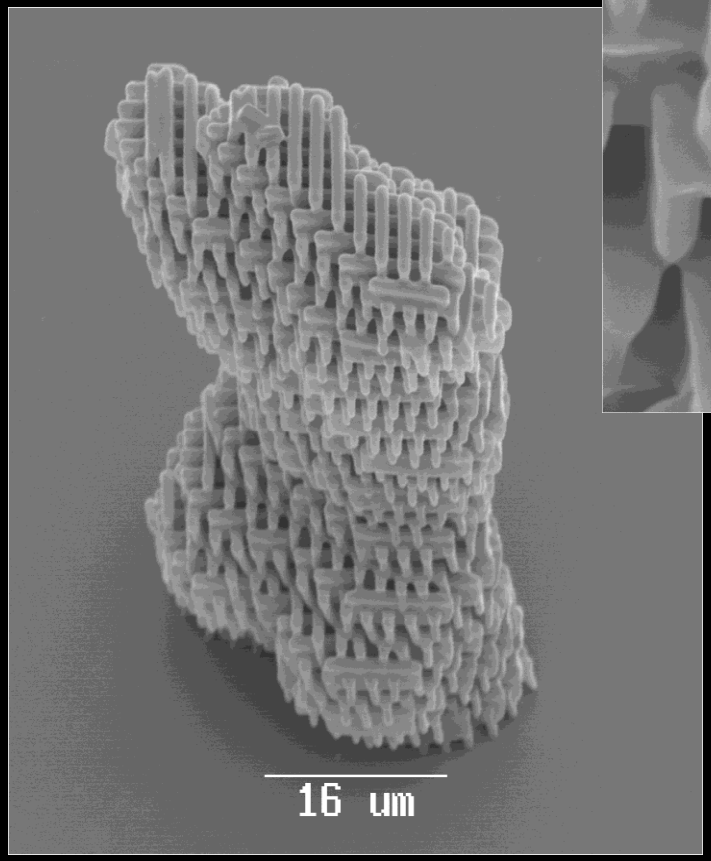


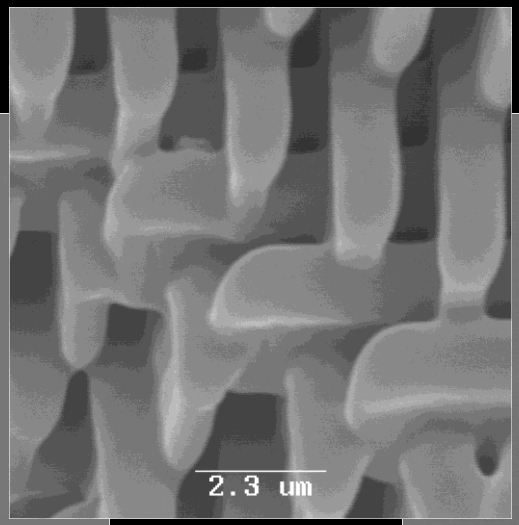
Ormocer

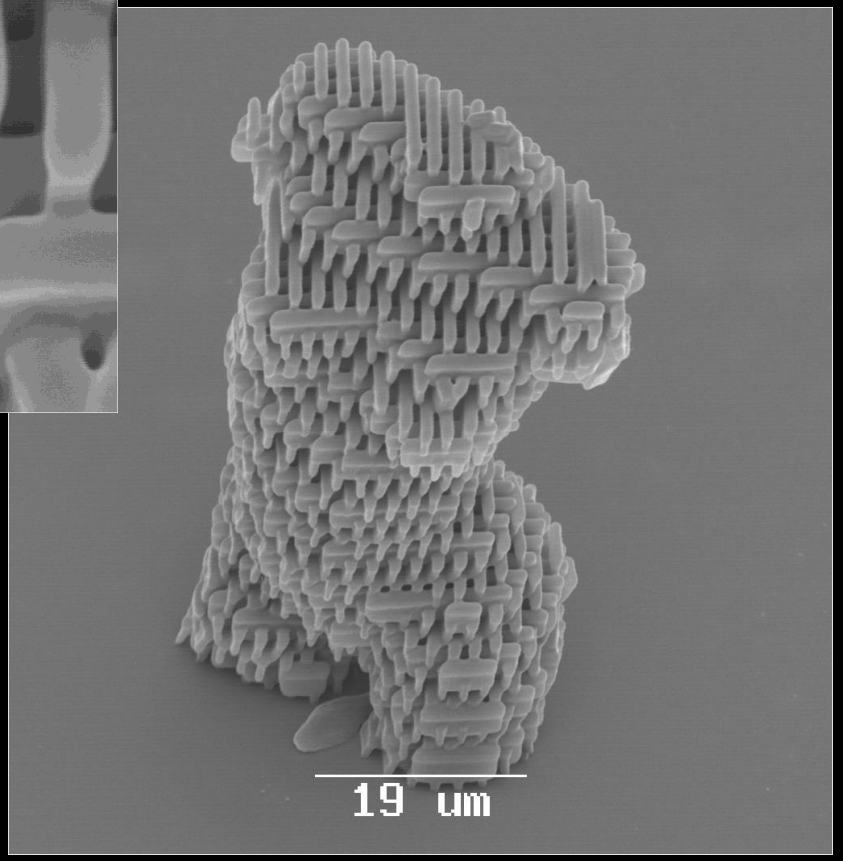


Opt. Lett. **28**, 301, (2003)
Adv. Eng. Mat. **5**, 551, (2003)

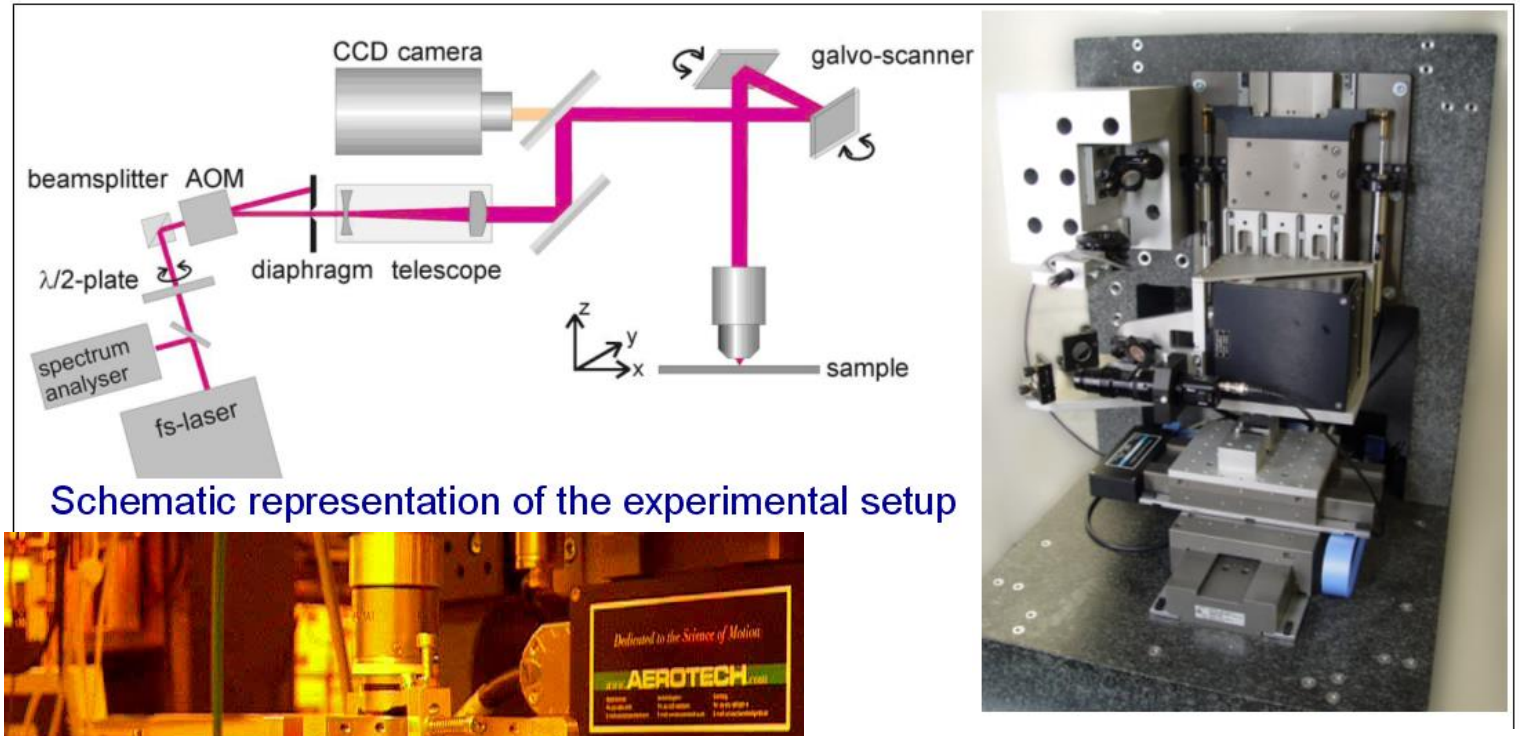
3D microstructured µm-Venus (Ormocer)



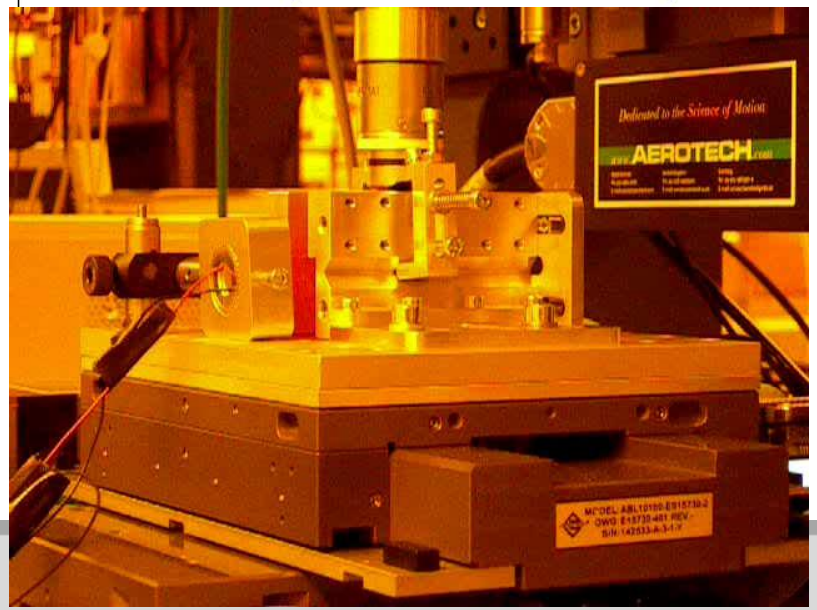




Two-photon polymerization (5cm/s – 5m/s)



Schematic representation of the experimental setup



Commercially available 2PP system from LZH: b.chichkov@lzh.de

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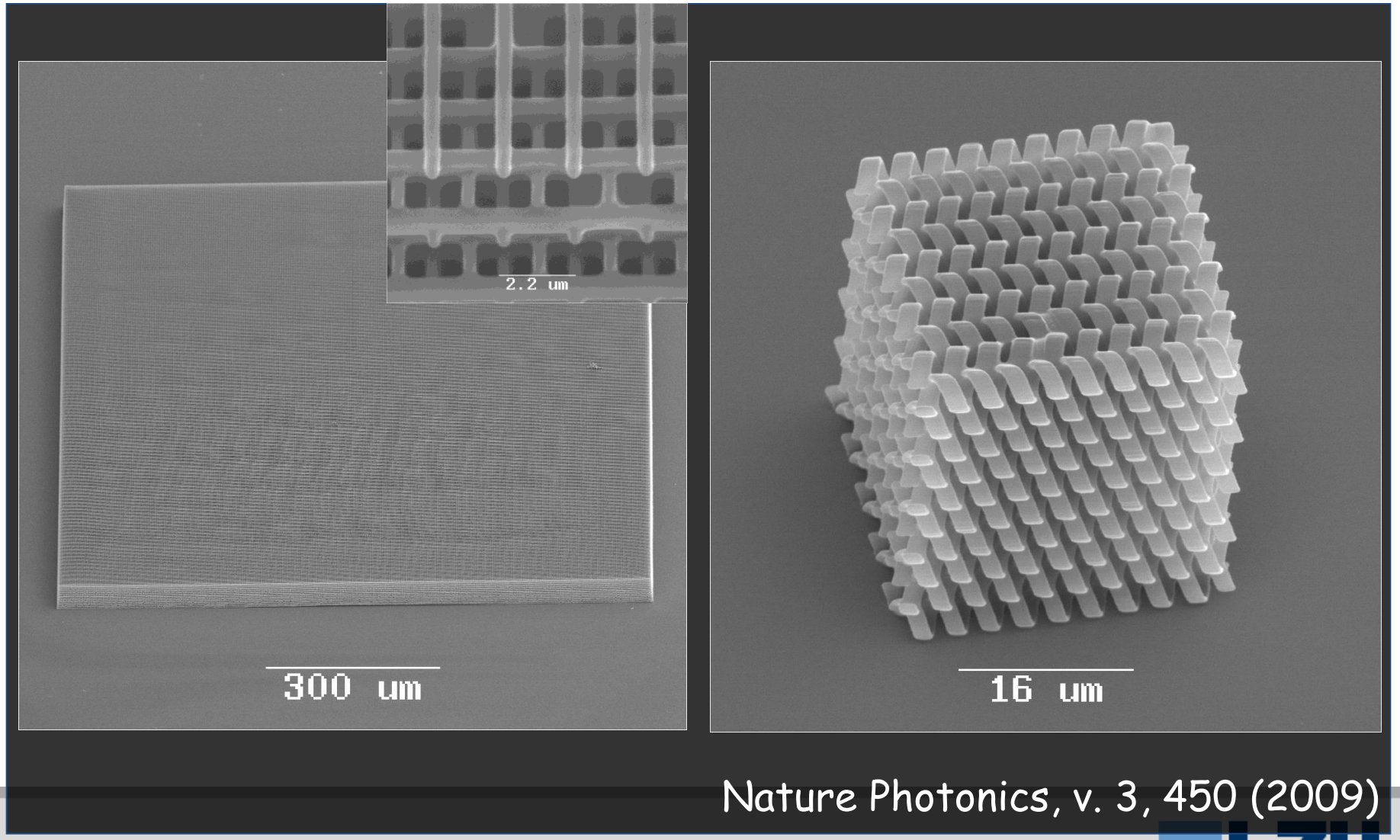
PhCs fabricated in Zr-hybrid polymers



Nature Photonics, v. 3, 450 (2009)

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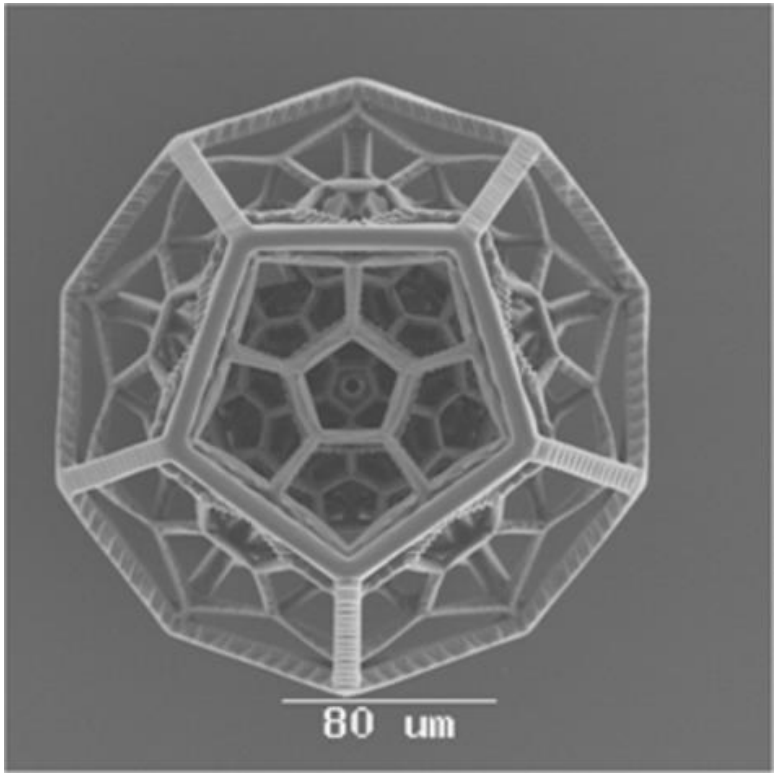
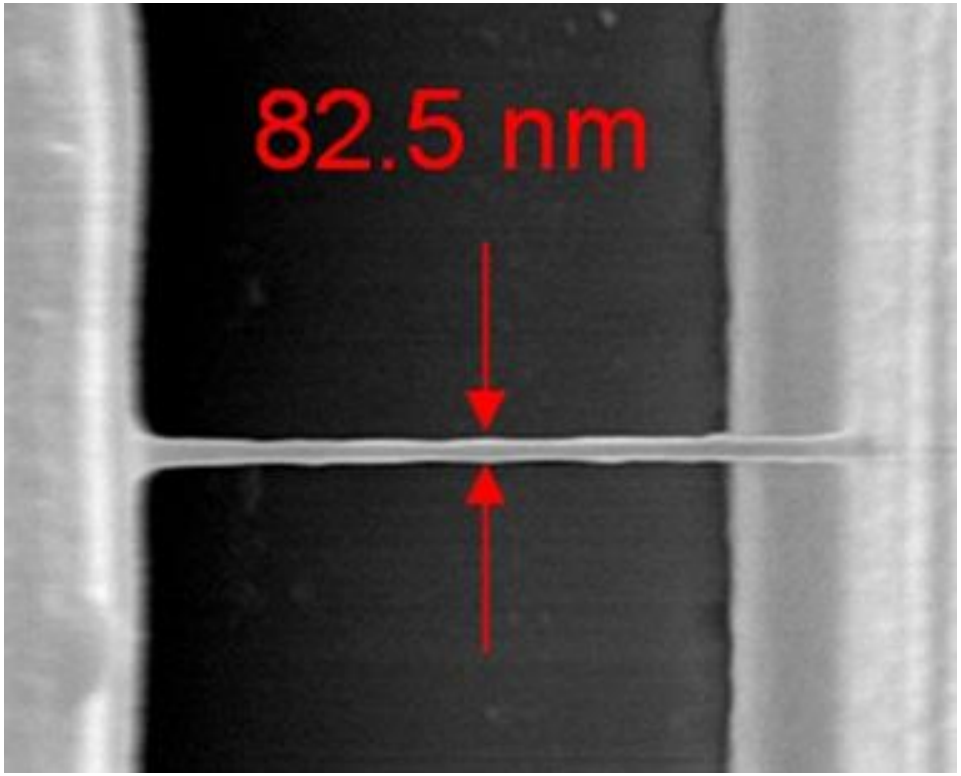
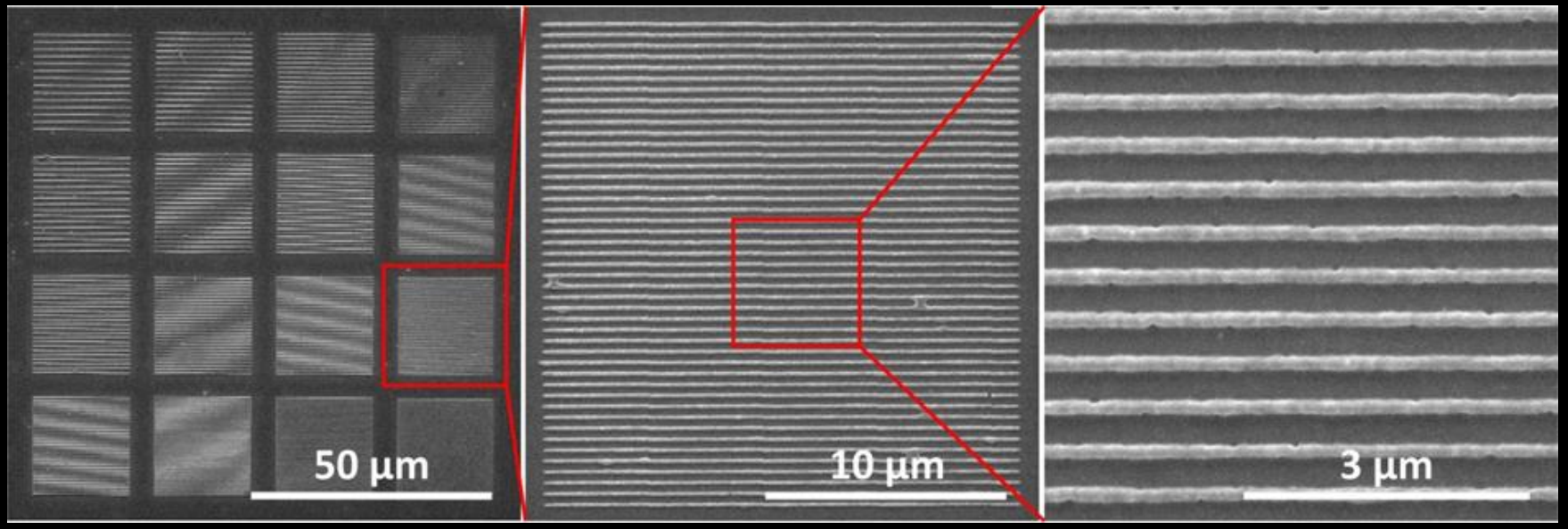
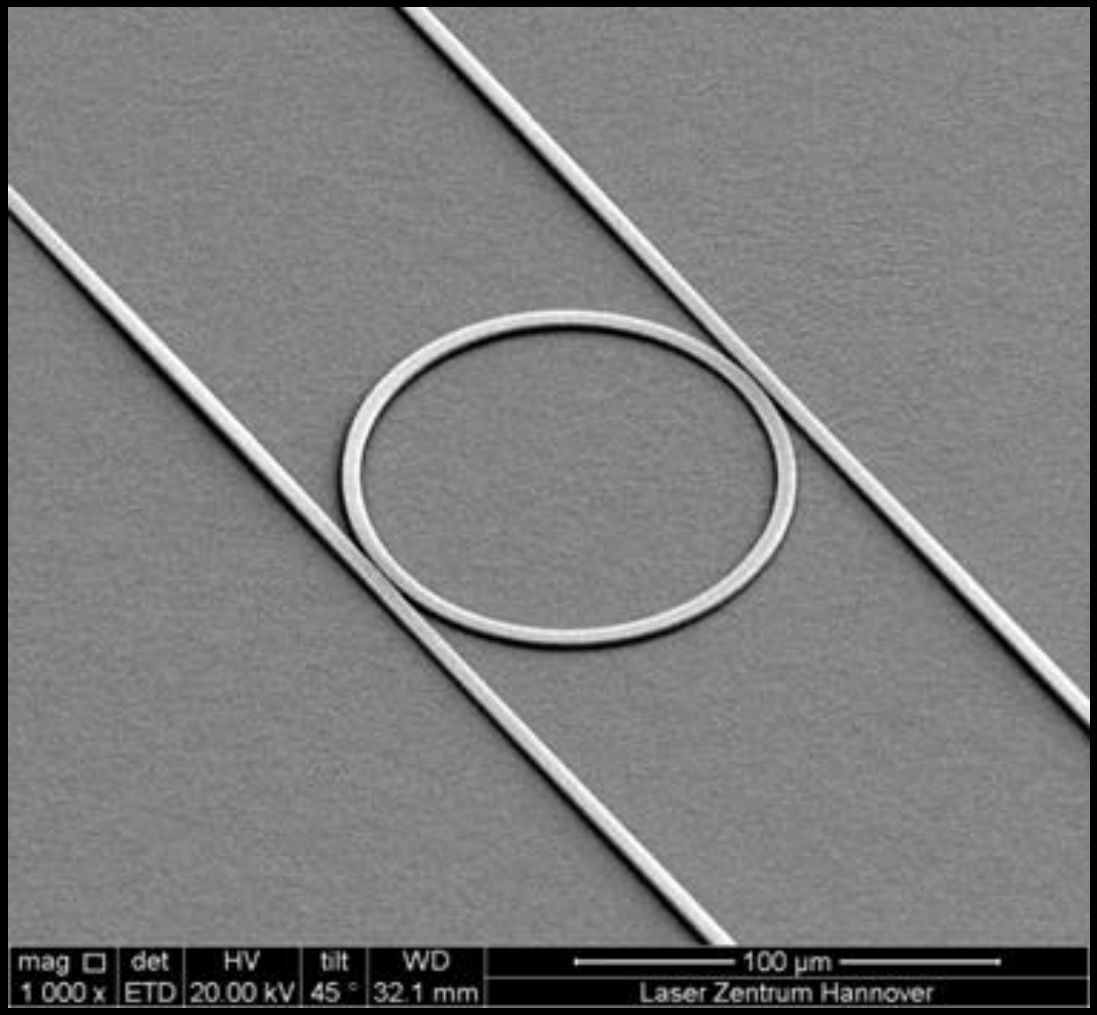
3D-STRUCTURES

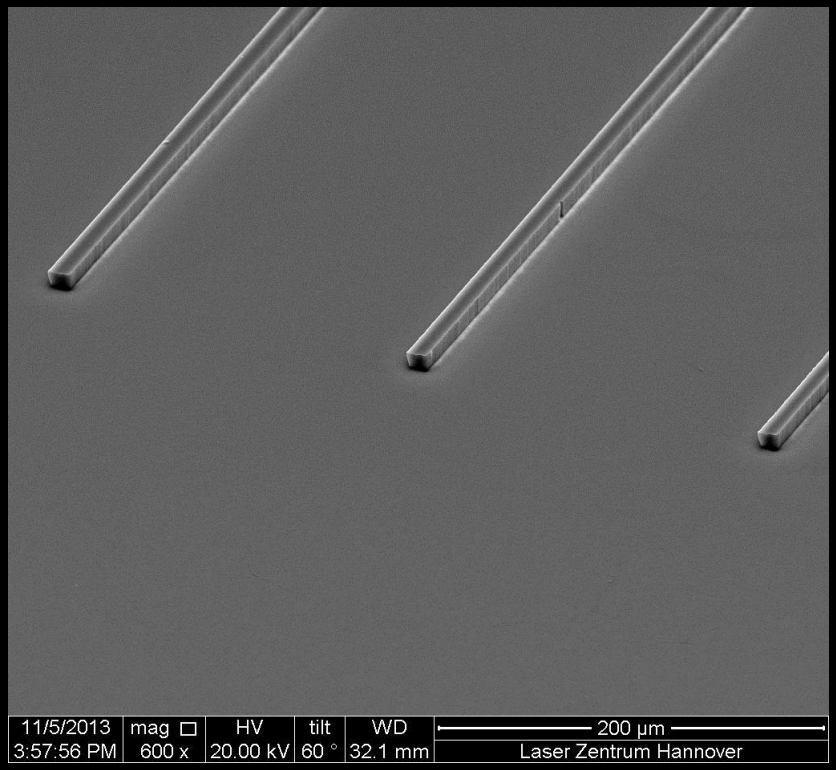


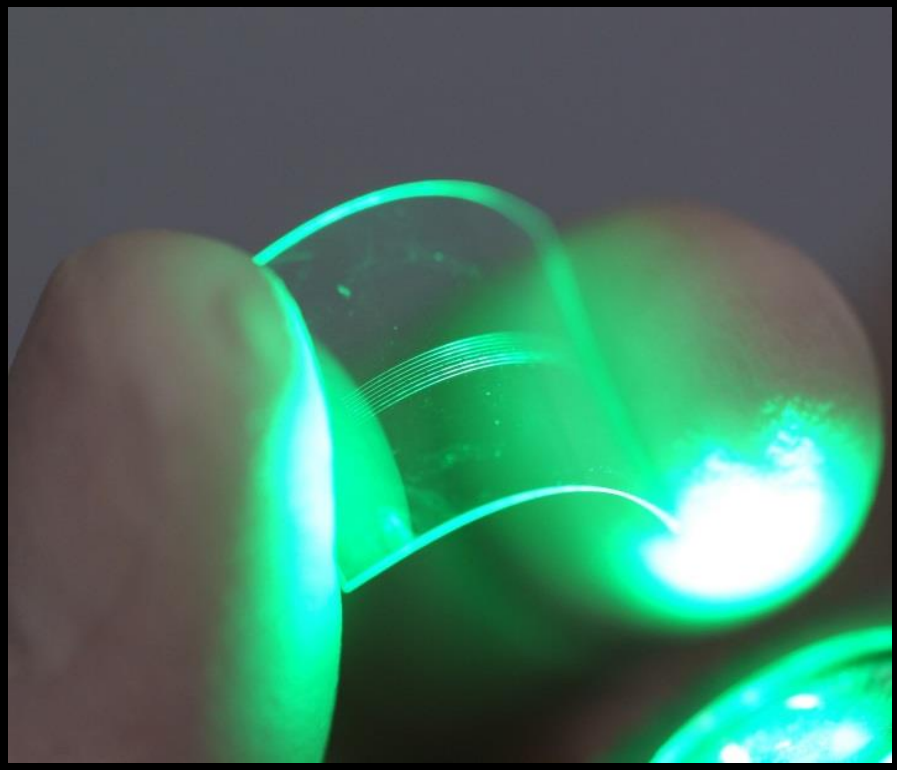


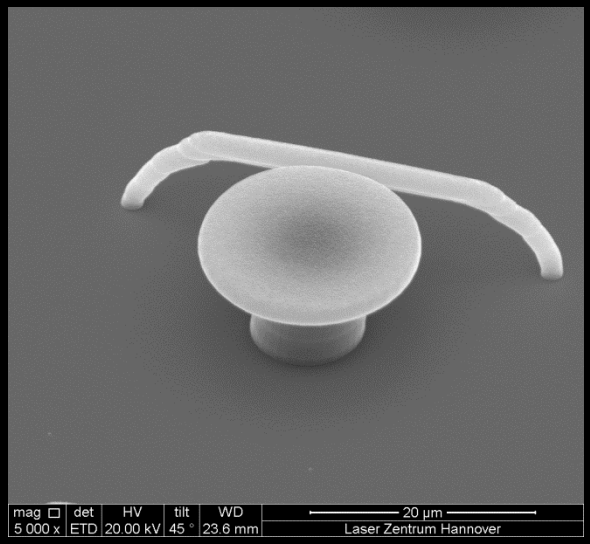
- With the resolution down to 100nm

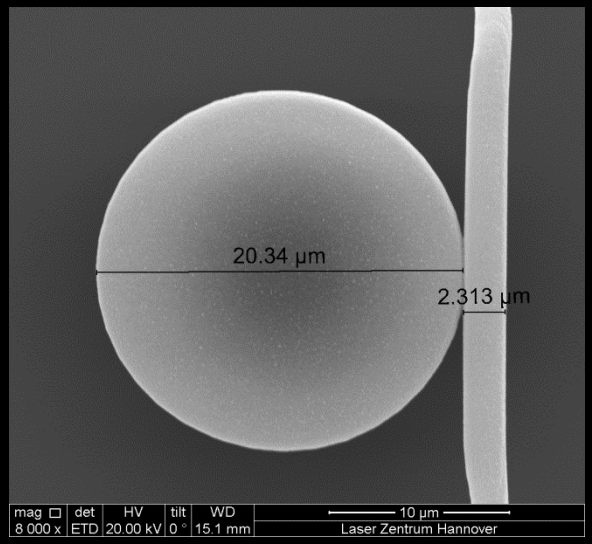

50 µm
10 µm
3 µm

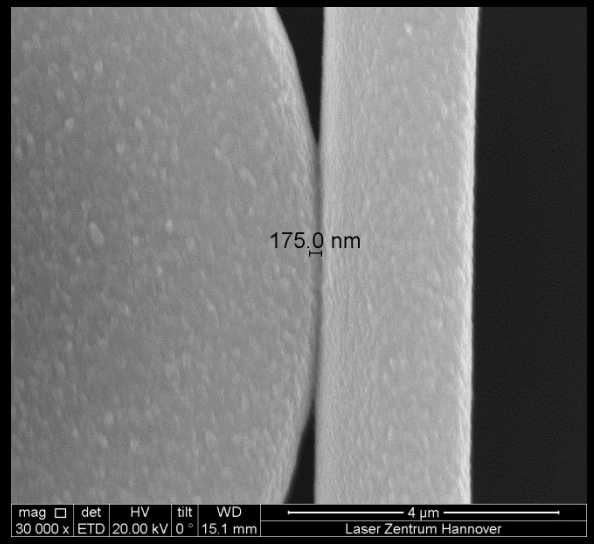

mag
1 000 x
det
ETD
HV
20.00 kV
tilt
45 °
WD
32.1 mm
100 µm
Laser Zentrum Hannover


11/5/2013
3:57:56 PM
mag
600 x
HV
20.00 kV
tilt
60 °
WD
32.1 mm
200 µm
Laser Zentrum Hannover




mag □ det HV tilt WD 20 µm
5 000 x ETD 20.00 kV 45 ° 23.6 mm Laser Zentrum Hannover


20.34 µm
2.313 µm
mag □ det HV tilt WD 10 µm
8 000 x ETD 20.00 kV 0 ° 15.1 mm Laser Zentrum Hannover

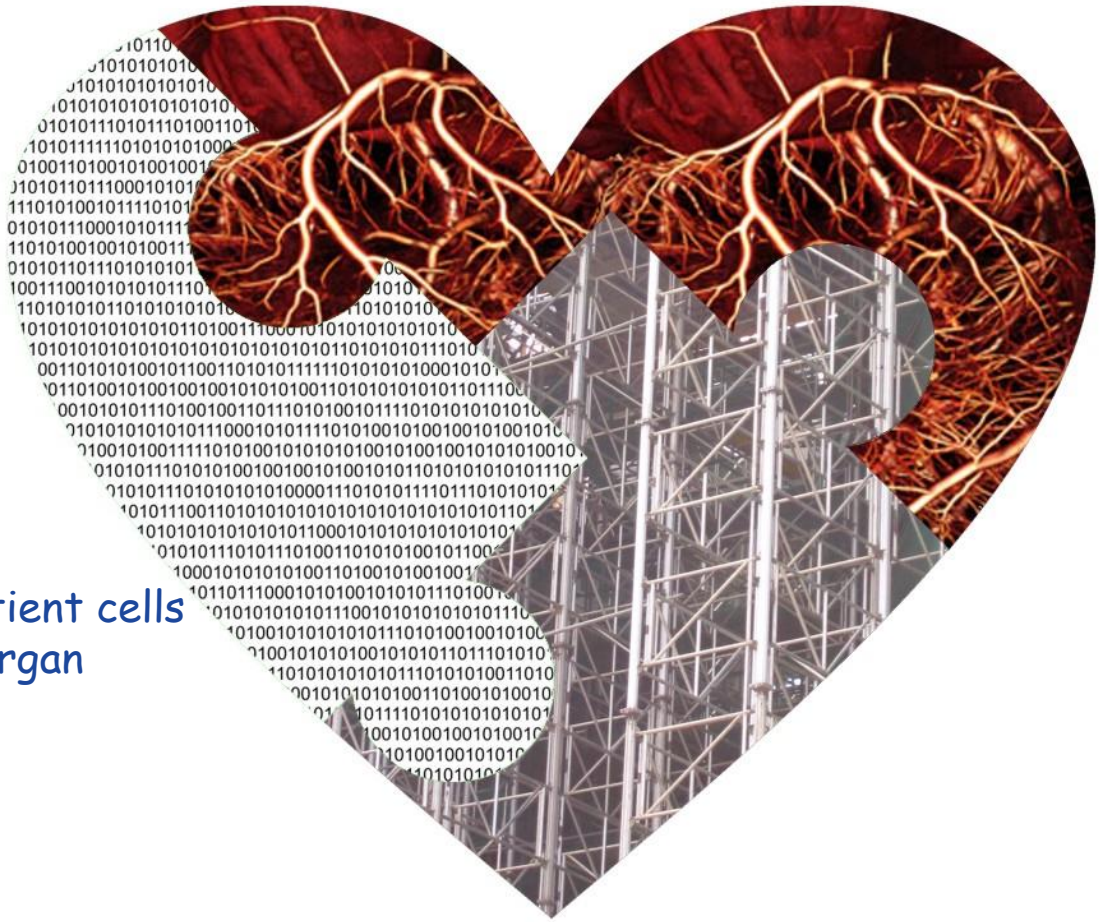

175.0 nm
mag □ det HV tilt WD 4 µm
30 000 x ETD 20.00 kV 0 ° 15.1 mm Laser Zentrum Hannover

Regenerative medicine and tissue engineering

Interdisciplinary Research
(Mathematics, Physics, Material Science, Engineering, Biology, Medicine)

Regenerative Medicine:

Replacement or repair of ill organs, which body cannot restore itself



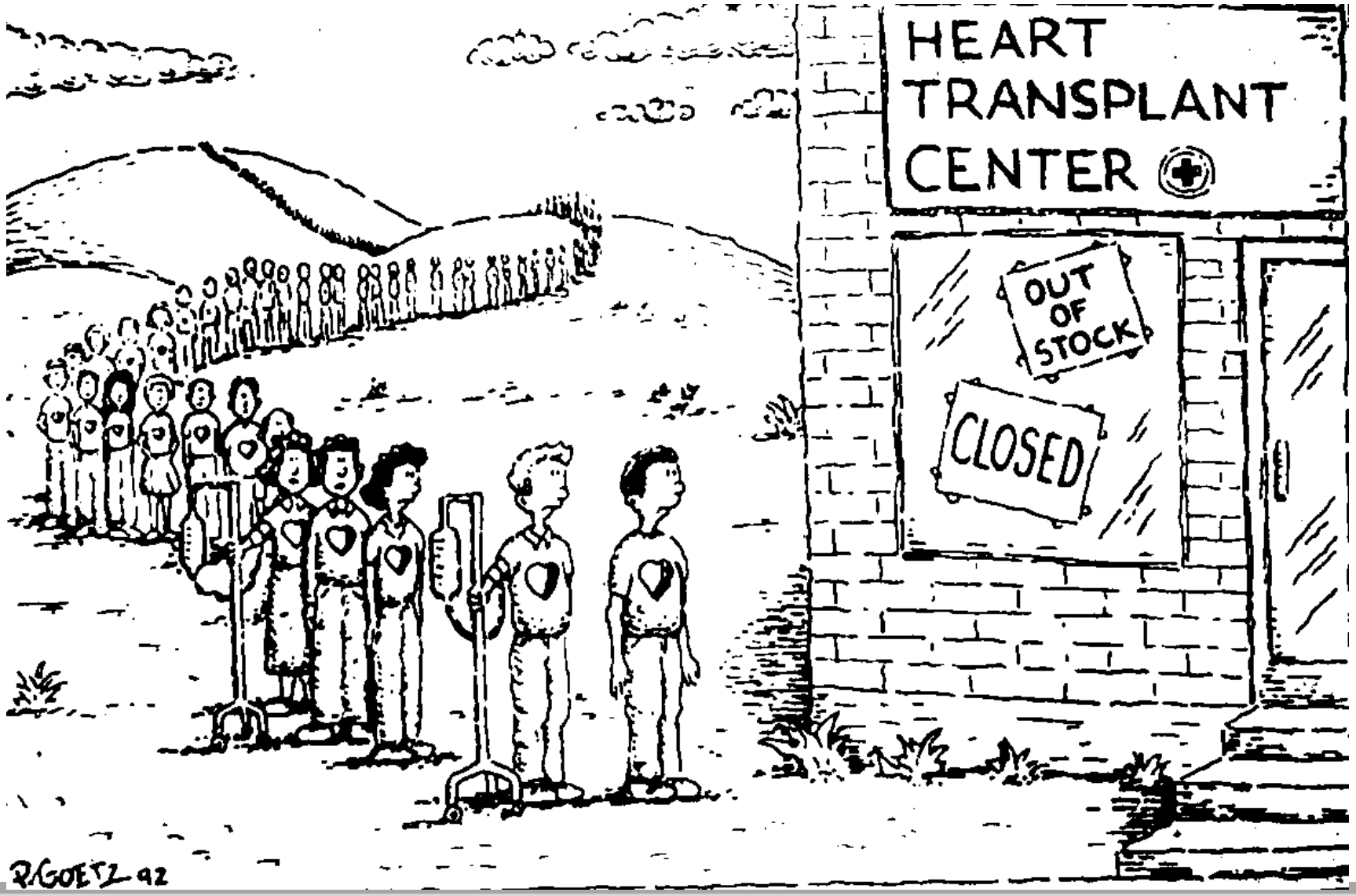
Tissue Engineering:

Fabrication of living tissue from patient cells
Transplantation inside a damaged Organ

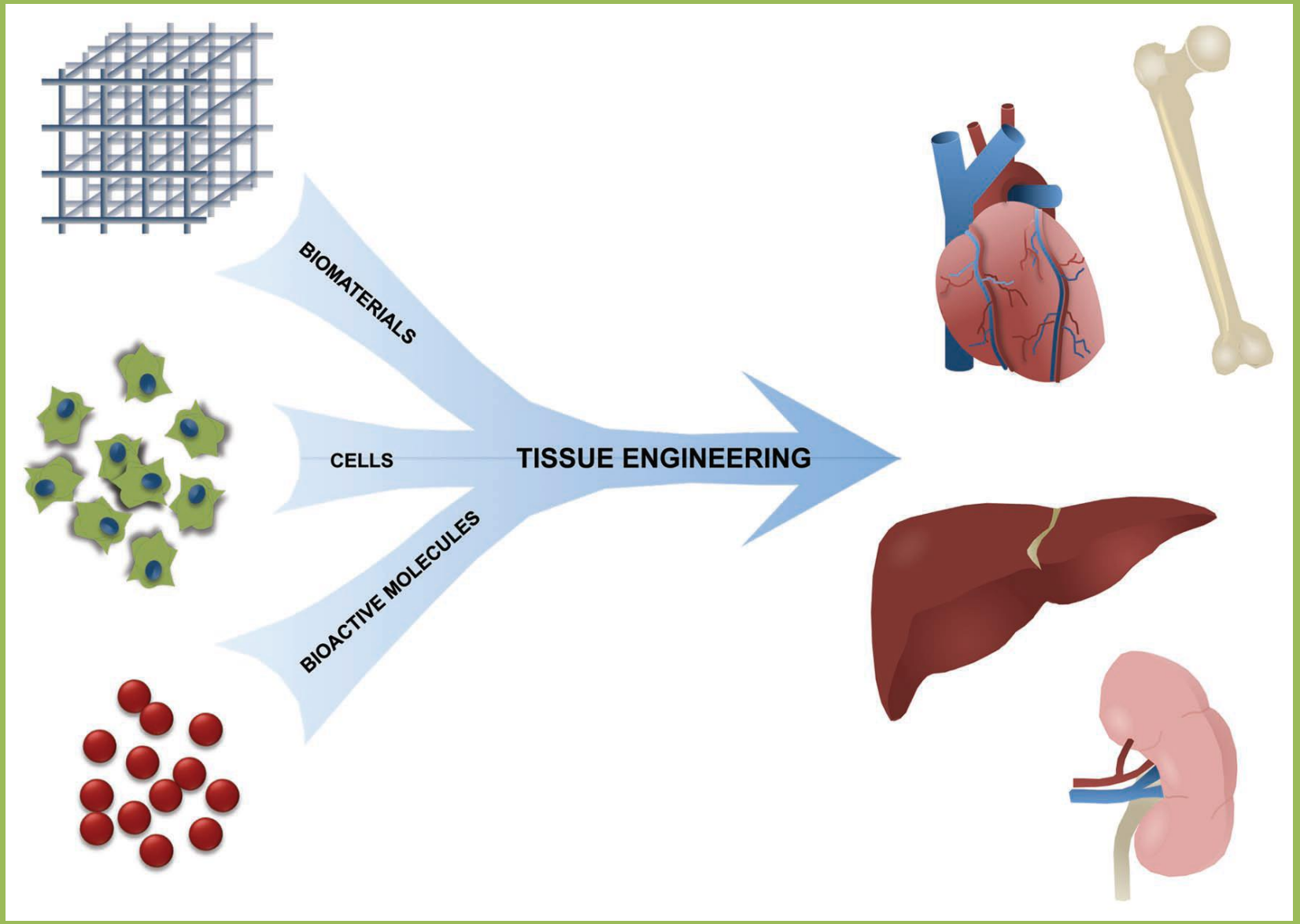
Organ on Chip Constructs:

Development of 3D cell models

Organ transplantation demand



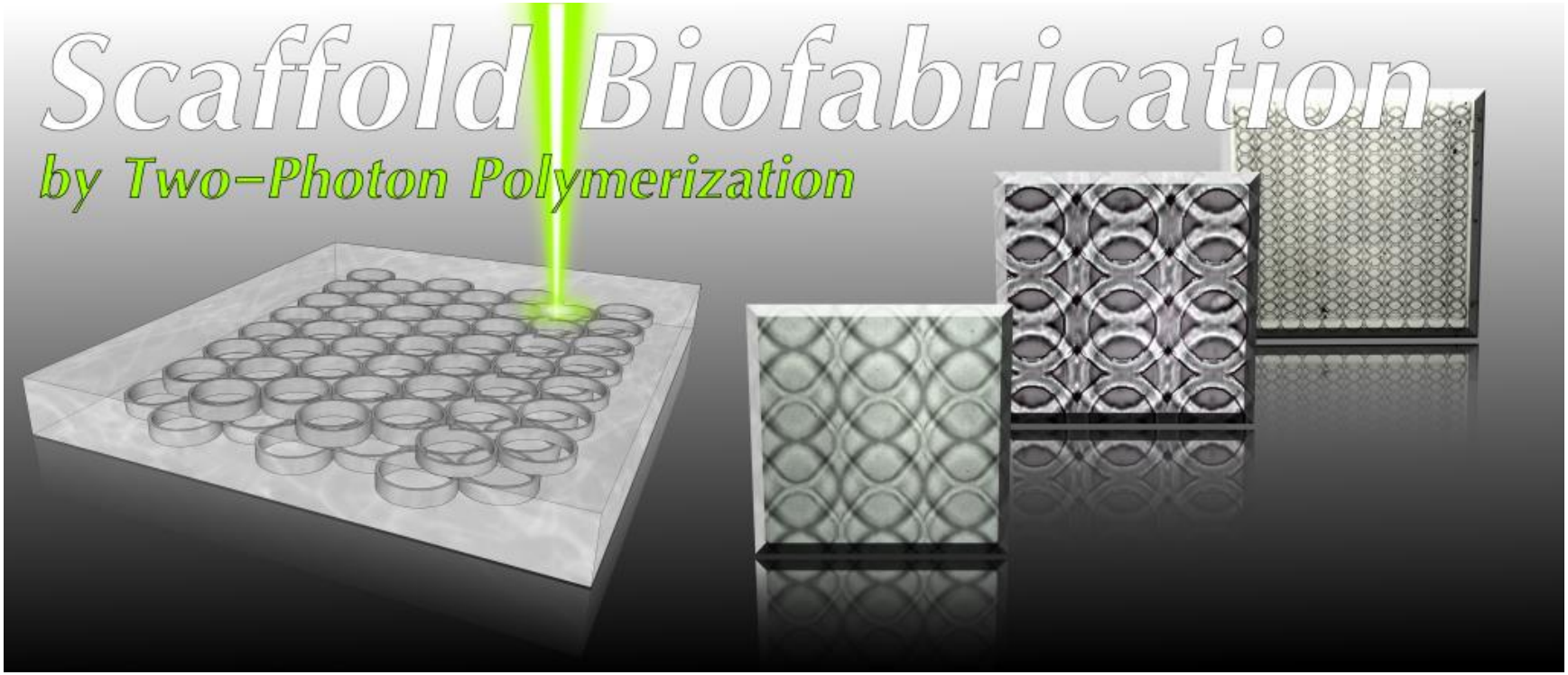
Basic idea of tissue engineering



Kristine C Rustad, *et.al.* Organogenesis 2010 Jul-Sep; 6(3): 151–157.

2PP EXAMPLES

Materials: Ormocers®, PEG-DA, Organic-Inorganic Zr-hybrids, PLA; Gelatin…



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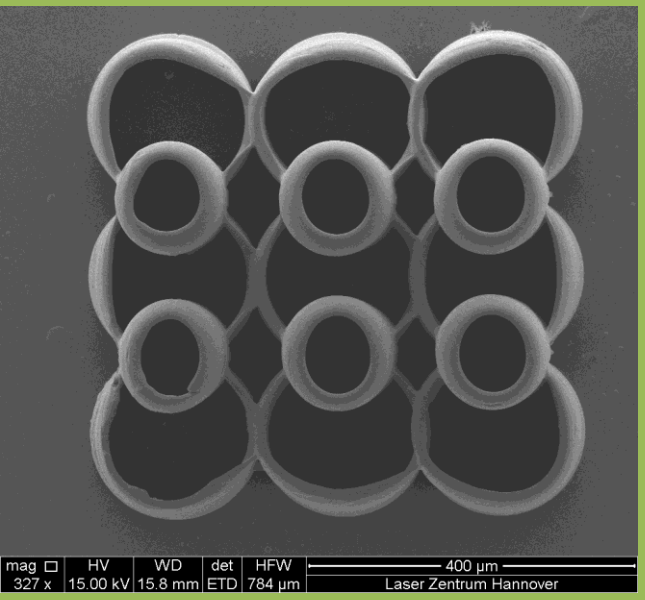
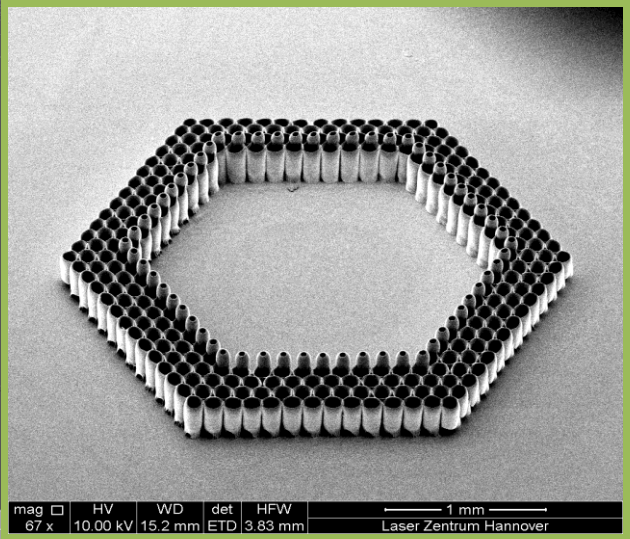
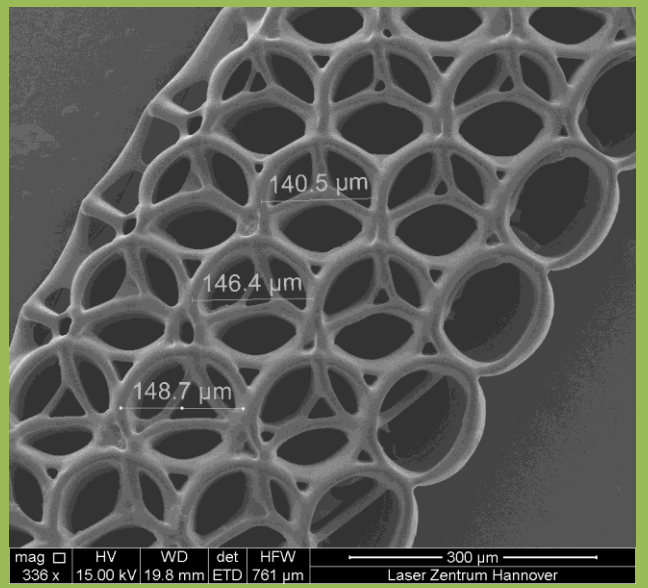
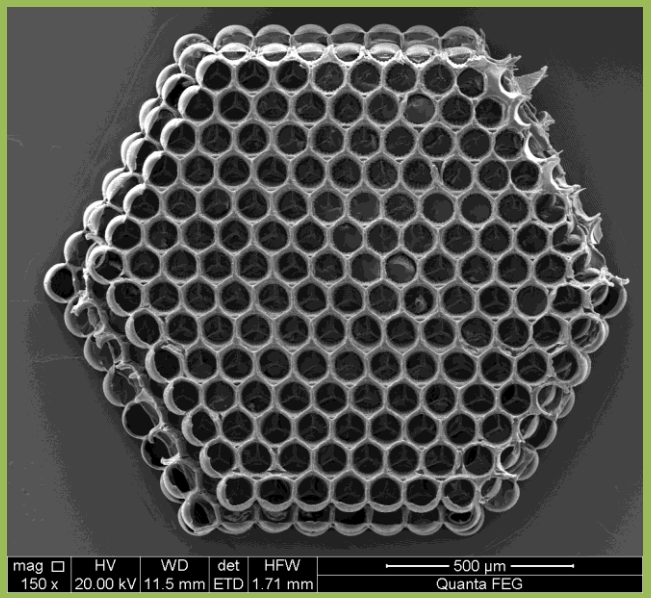
3D Scaffolds for TE / Examples

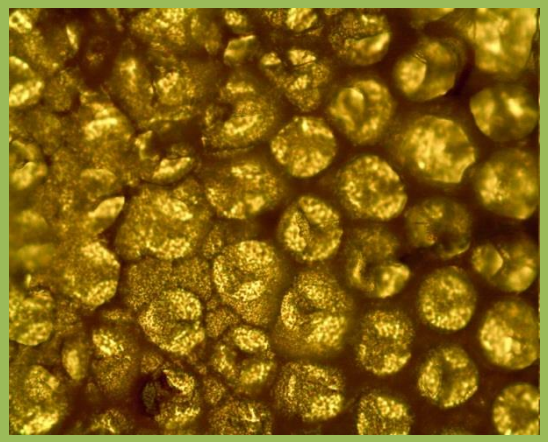




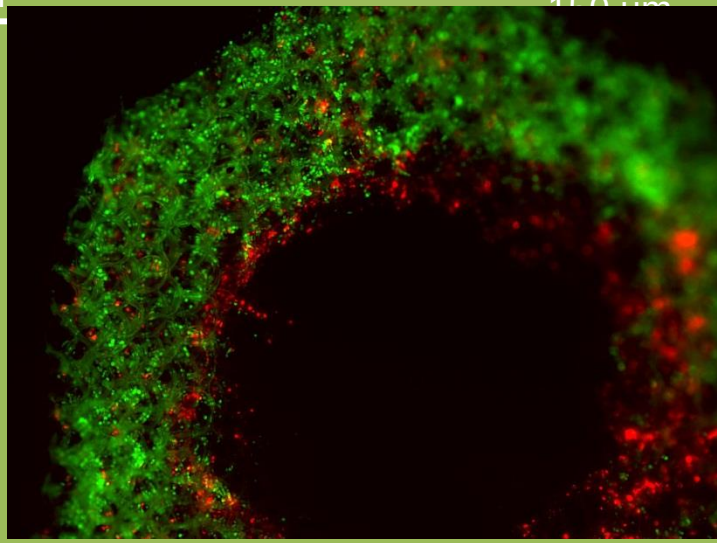




1 mm

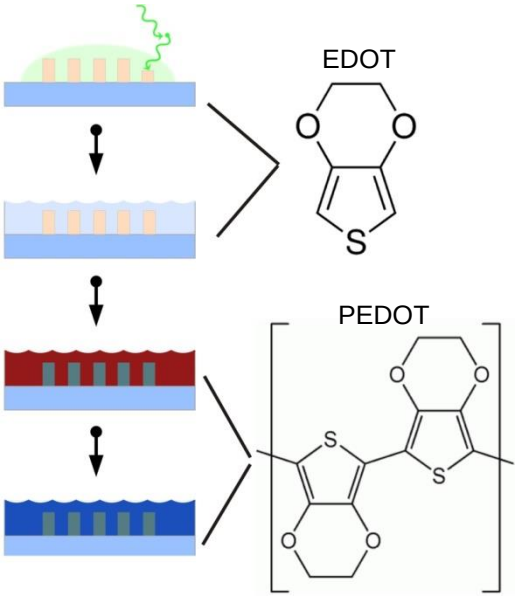


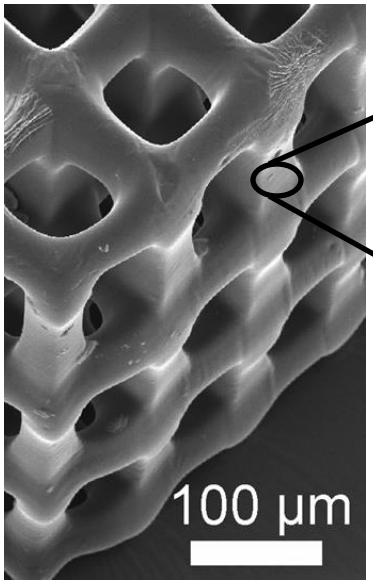
Fibrin scaffold

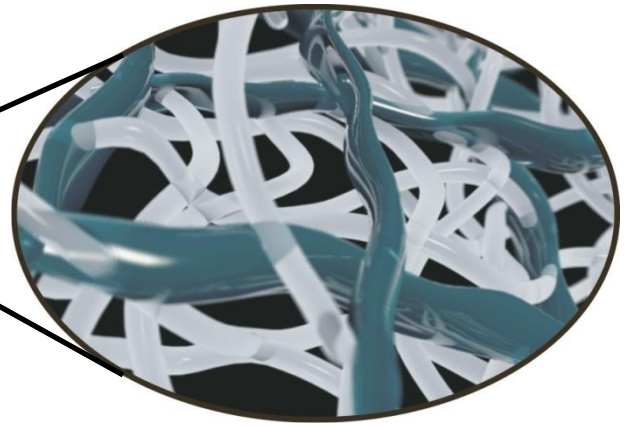


3D conductive polymer structures

- PEG-DA and EDOT blends are used for 2PP and sequential *in-situ* oxidative polymerization;
- Real-3D, physically stable and biocompatible microstructures are produced;
- Interpenetrating polymer network of PEG-DA and PEDOT leads to conductivities of up to 0.04 S/cm.







PEG-DA : poly(ethylene glycol) diacrylate
EDOT : 3,4-ethylenedioxythiophene
PEDOT : poly(3,4-ethylenedioxythiophene)

Opt. Express, **21**, 31029 (2013)

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BioMACROMOLECULES

Article

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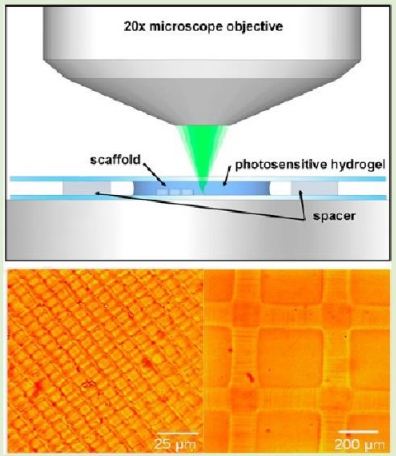
Hyaluronic Acid Based Materials for Scaffolding via Two-Photon Polymerization

Olga Kufelt,*[,†] Ayman El-Tamer,[†] Camilla Sehring,[†] Sabrina Schlie-Wolter,[†,‡] and Boris N. Chichkov[†,‡]

[†]Laser Zentrum Hannover e.V., Nanotechnology Department, Hollerithallee 8, 30419 Hannover, Germany

[‡]Institute of Quantum Optics, Leibniz University Hannover, Welfengarten 1, 30167 Hannover, Germany

ABSTRACT: Hydrogels are able to mimic the basic three-dimensional (3D) biological, chemical, and mechanical properties of native tissues. Since hyaluronic acid (HA) is a chief component of human extracellular matrix (ECM), it represents an extremely attractive starting material for the fabrication of scaffolds for tissue engineering. Due to poor mechanical properties of hydrogels, structure fabrication of this material class remains a major challenge. Two-photon polymerization (2PP) is a promising technique for biomedical applications, which allows the fabrication of complex 3D microstructures by moving the laser focus in the volume of a photosensitive material. Chemical modification of hyaluronan allows application of the 2PP technique to this natural material and, thus, precise fabrication of 3D hydrogel constructs. To create materials with tailor-made mechanochemical properties, HA was combined and covalently cross-linked with poly(ethylene glycol) diacrylate (PEGDA) *in situ*. 2PP was applied for the fabrication of well elaborated 3D HA and HA−PEGDA microstructures. For enhanced biological adaption, HA was functionalized with human epidermal growth factor.



dx.doi.org/10.1021/bm401712q | *Biomacromolecules* 2014, 15, 650−659

Acta Biomaterialia 18 (2015) 186–195

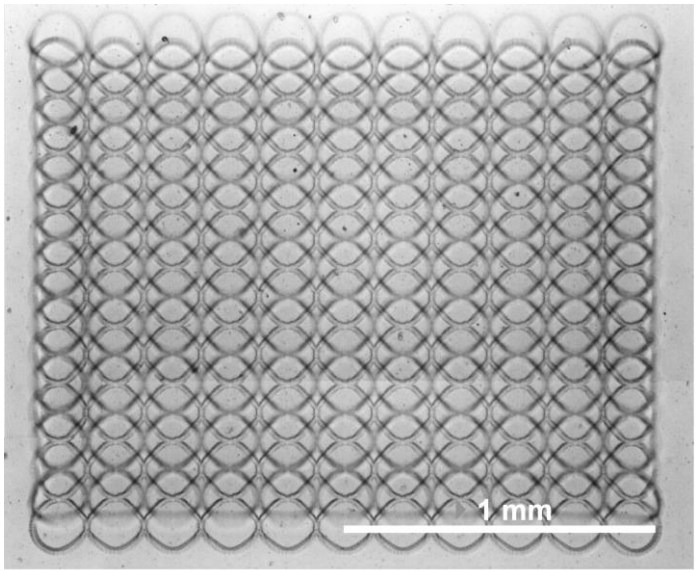
Contents lists available at ScienceDirect

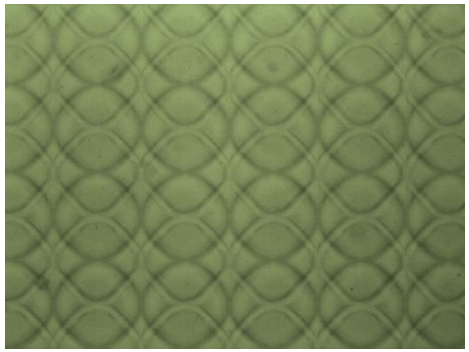
Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Water-soluble photopolymerizable chitosan hydrogels for biofabrication via two-photon polymerization

Olga Kufelt [a,*], Ayman El-Tamer [a], Camilla Sehring [a], Marita Meißner [a], Sabrina Schlie-Wolter [a,b], Boris N. Chichkov [a,b]

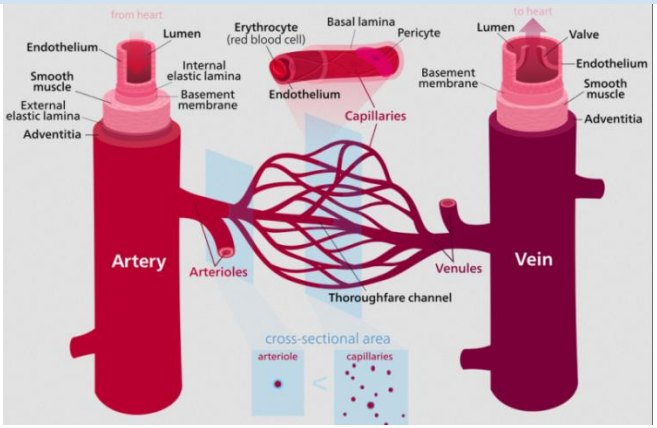


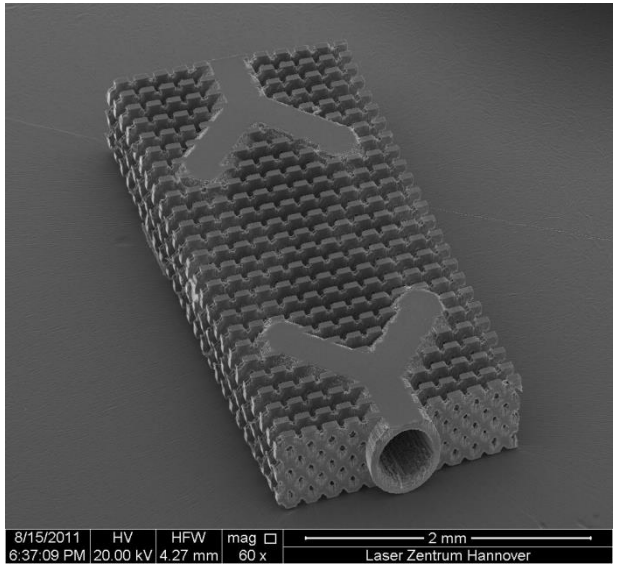


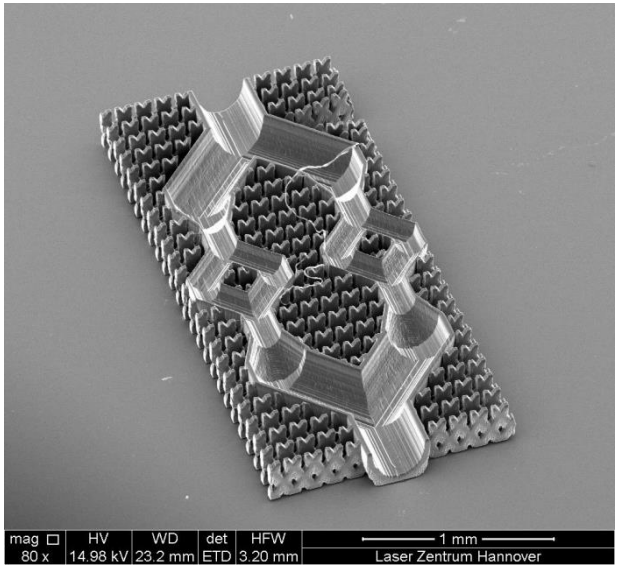
10x

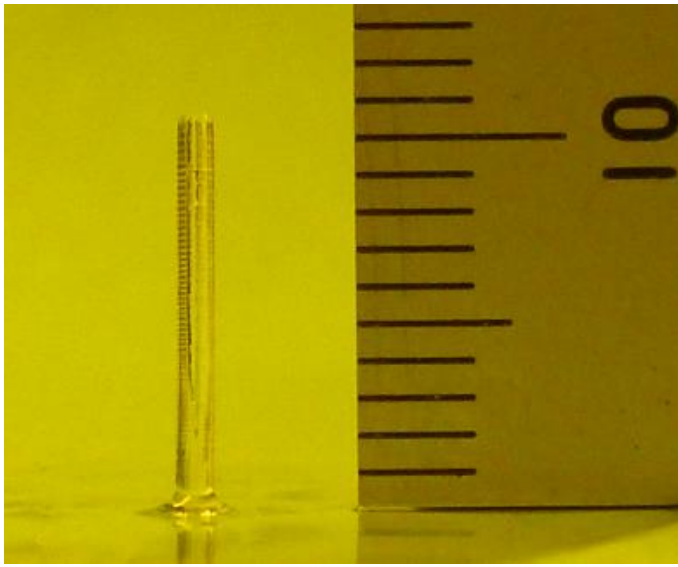
SCAFFOLDS WITH MICROFLUIDIC CHANNELS

- Use 2PP to generate scaffolds with capillary network
- Seed and culture to form vasculature
- Second round of seeding to make tissue of interest surrounding vasculature
- Make vasculature reducing from 1 mm diameter to 10 microns diameter









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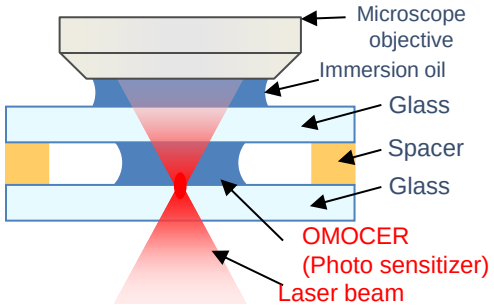
ACCELERATION OF 2PP PROCESSING BY MULTI-FOCUS SPOTS PRODUCED BY SLM

Two-photon polymerization (2PP)



Two-photon polymerization (2PP) of photosensitive polymer materiales using femtosecond laser is an attractive technique for true three-dimensional micro- and nano-scale structuring.

Recently, the increasing of process resolution leading to much slower processing speeds becomes a problem for practical use.

Two-photon polymerization by multi-focus beam process using Spatial light modulator(SLM)

SLM can modulate the phase of incident laser beam, resulting in generation of multi-focus spots on the sample surface. These multi-focus beams can induce 2PP at each focus position. In addition, each focus spot can be individually controlled in position and laser intensity by refresh of CGH in PC control.

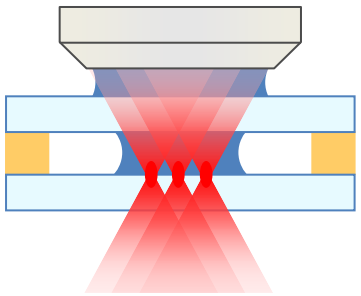
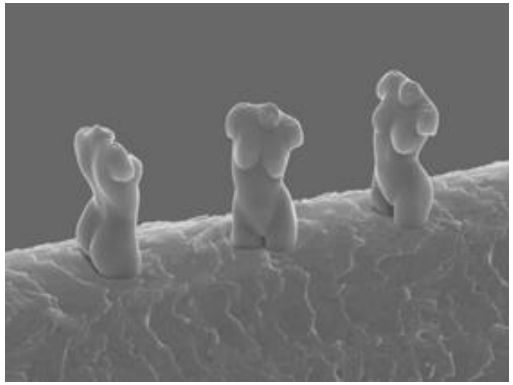
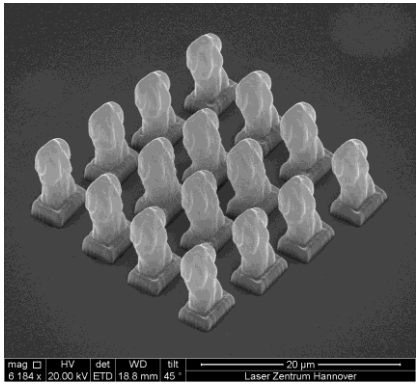
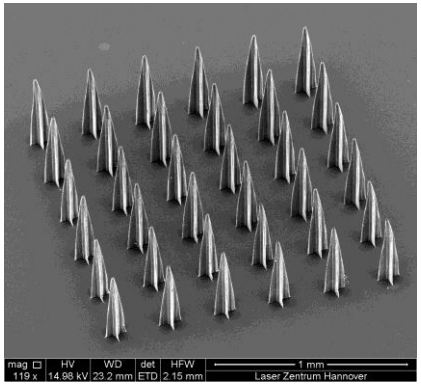


Image of multi-focus spots 2PP

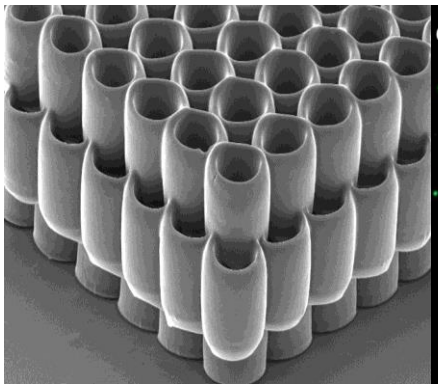
3D STRUCTURING WITH MULTIFOCUS SPOT (16-FOCI)







Marie Curie Actions
Nano²fun
Initial Training Network



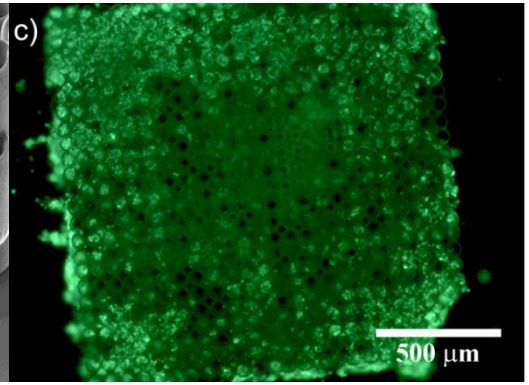
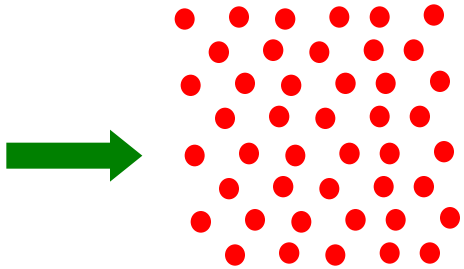
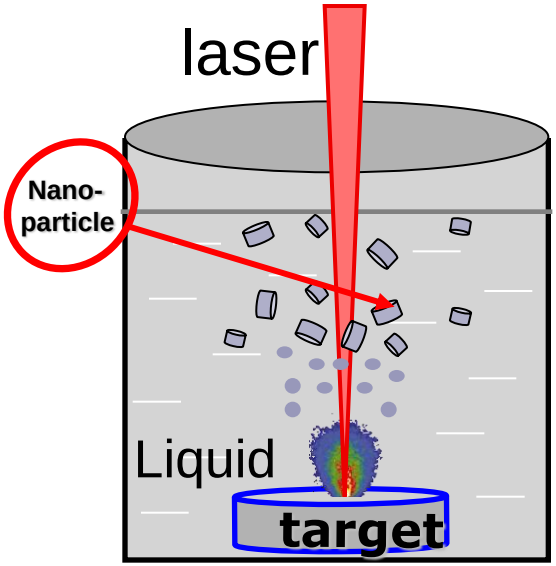


Image of bovine endothelial cells growing on a scaffold

BIOMEDICAL OPTICS EXPRESS, v. 2 3168 (2011)

Laser generation of nanoparticles



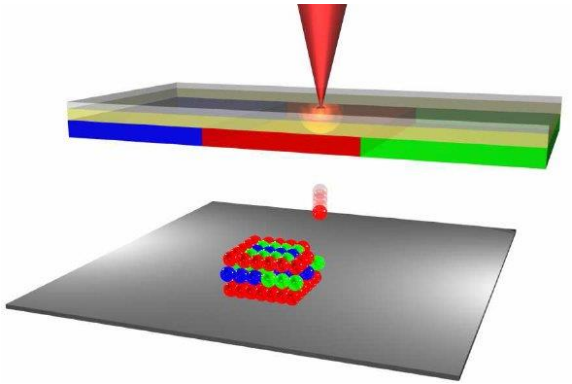


- High purity and stability
- Monoatomic materials
- Alloy nanoparticles
- Particle surface-functionalization
- Polymer-embedded nanoparticle
- Coatings with nanosized particles
- Controlled drug-release
- Stoichiometric nanoparticles
- Novel methods → better control

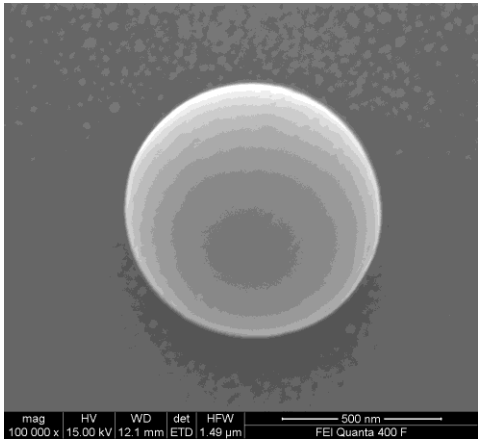
J. Phys. Chem. C, 2010
Appl. Phys. A, 2010

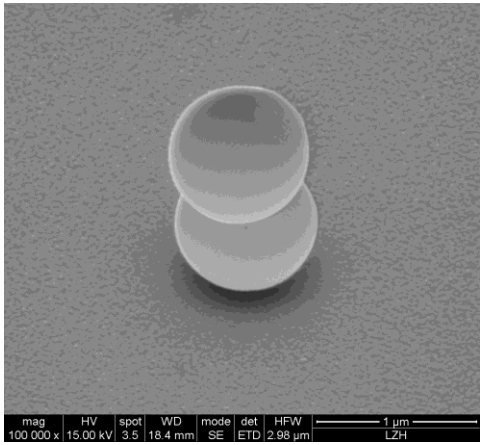
LASER ZENTRUM HANNOVER e.V.

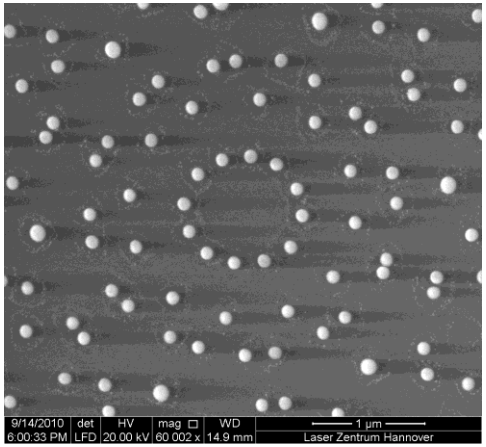
Laser Printing of Spherical Gold and Silicon Nanoparticles



Laser printing





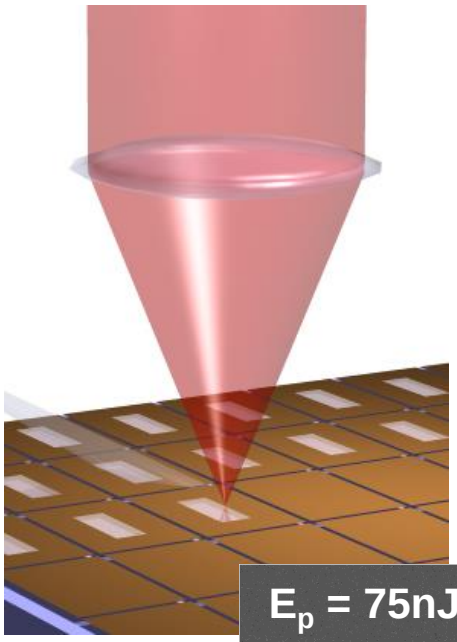


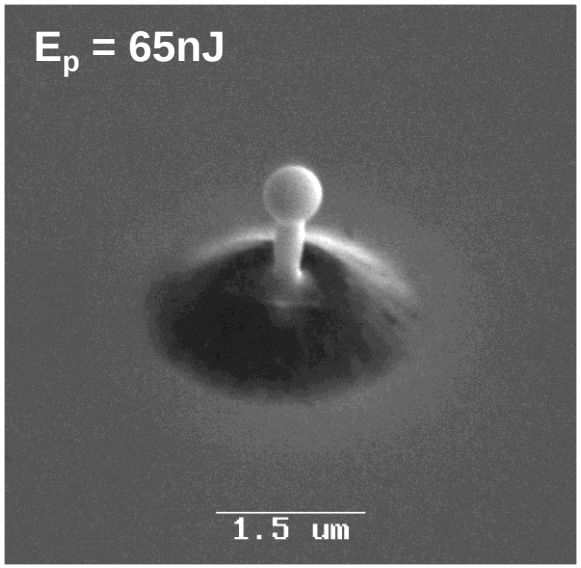
Opt. Express 17, 18820 (2009)

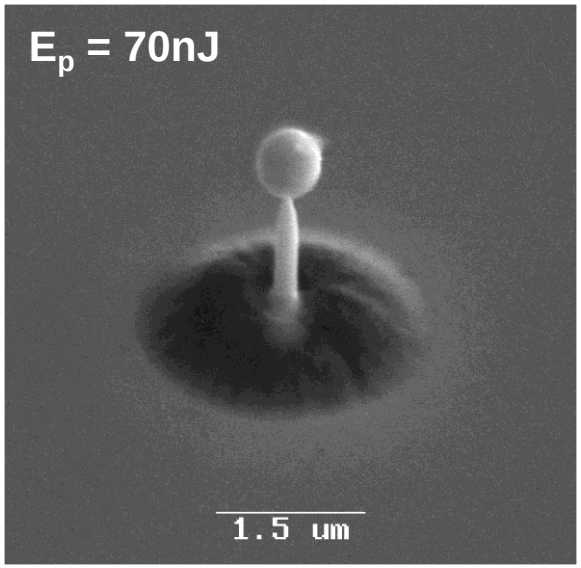
J. Opt. Soc. Am. B, 26, 130 (2009)

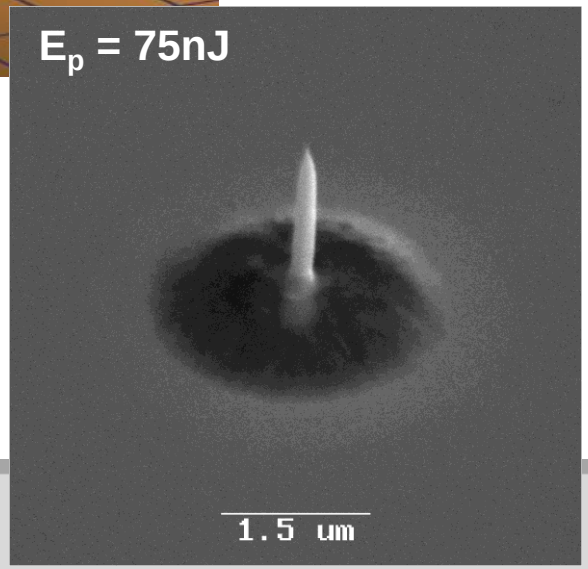
***Opt. Express* 18, 21198 (2010).**

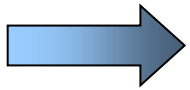
Jet and droplet formation







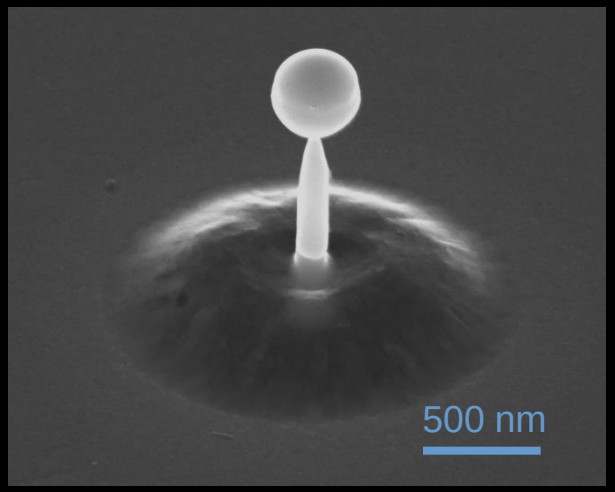




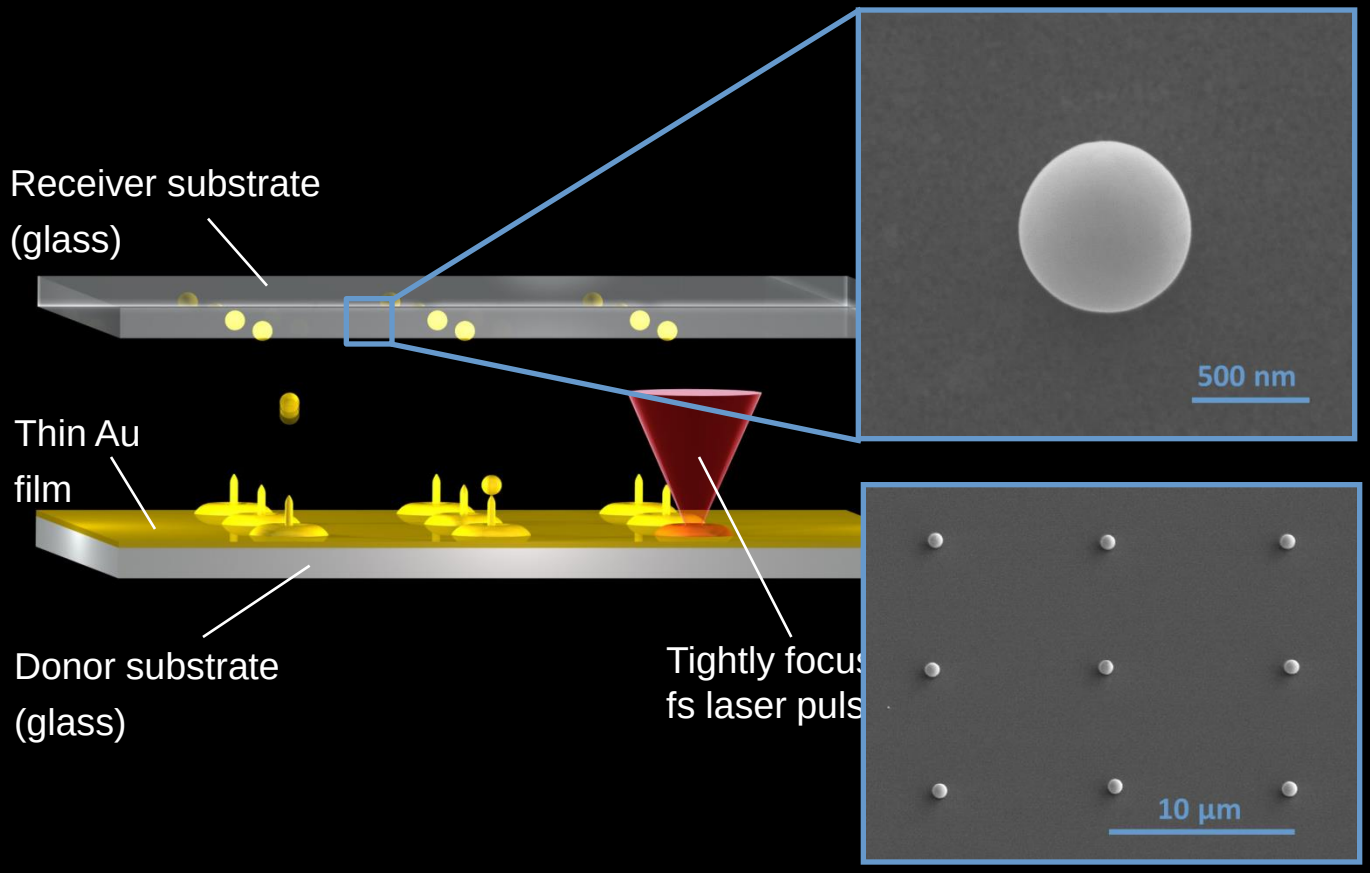
Appl. Phys. A **79**, 879 (2004)

Fabrication of spherical nanoparticles

10 000 x
Reduction



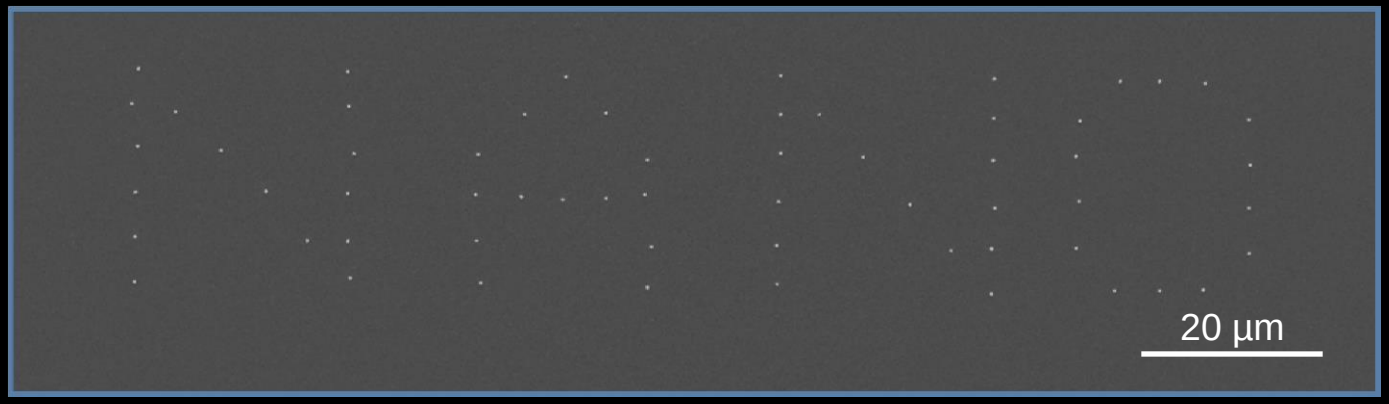
Fabrication of spherical nanoparticles by laser printing



„Laser-induced transfer of metallic nanodroplets for plasmonics and metamaterial application“
JOSA B, Vol. 26, No. 12, B130, 2009

„Nano“-letters from Au

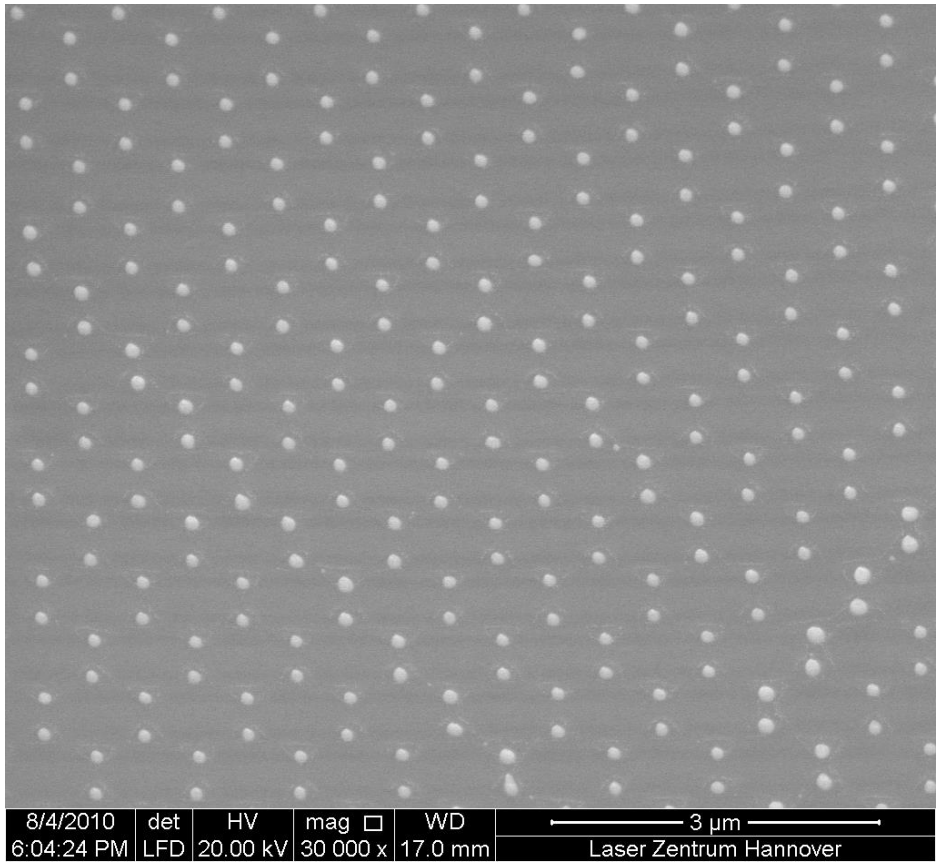
- SEM-image of nanoparticles

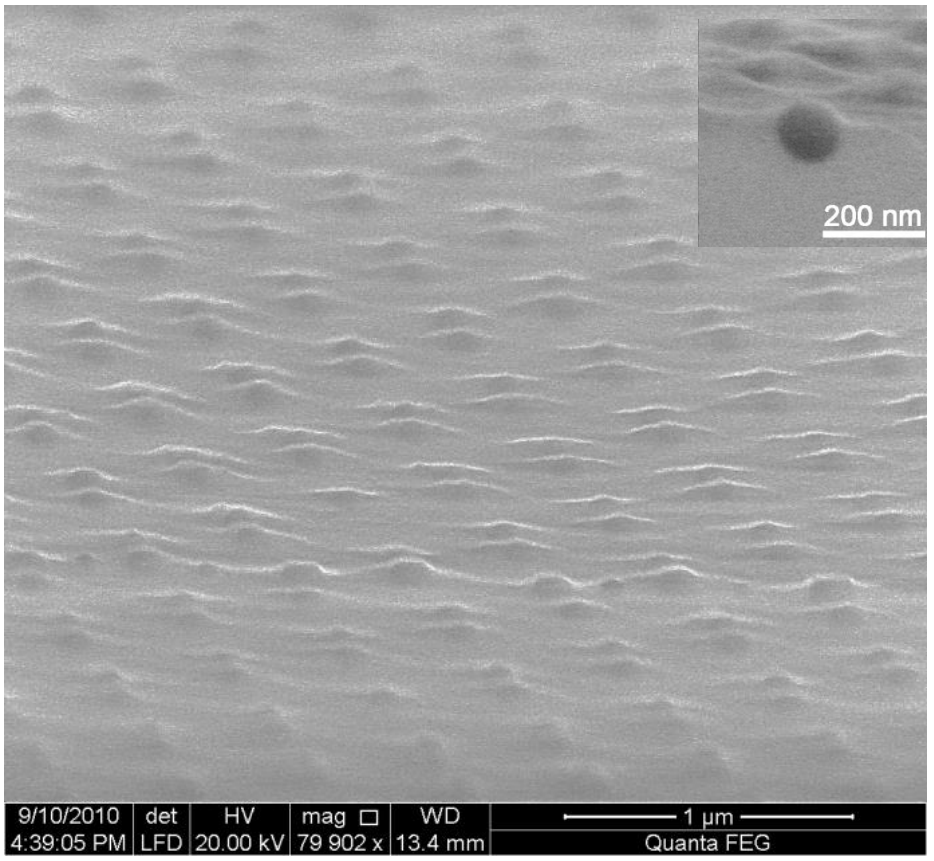


- The same image made by darkfield microscopy

Laser fabrication of periodic nanoparticle arrays

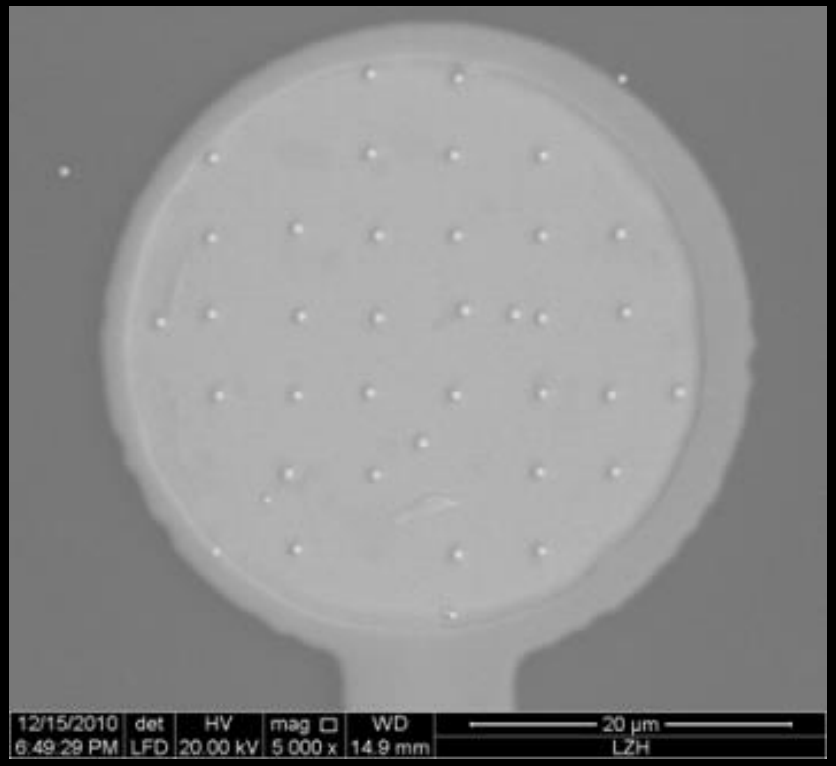
nanoparticle sizes: 40 – 200 nm

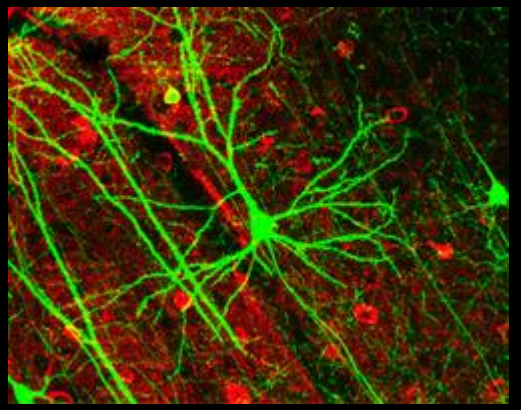


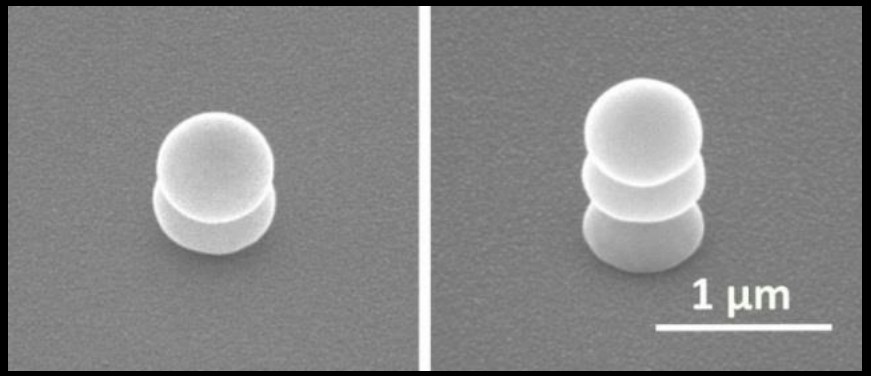


LASER ZENTRUM HANNOVER e.V.

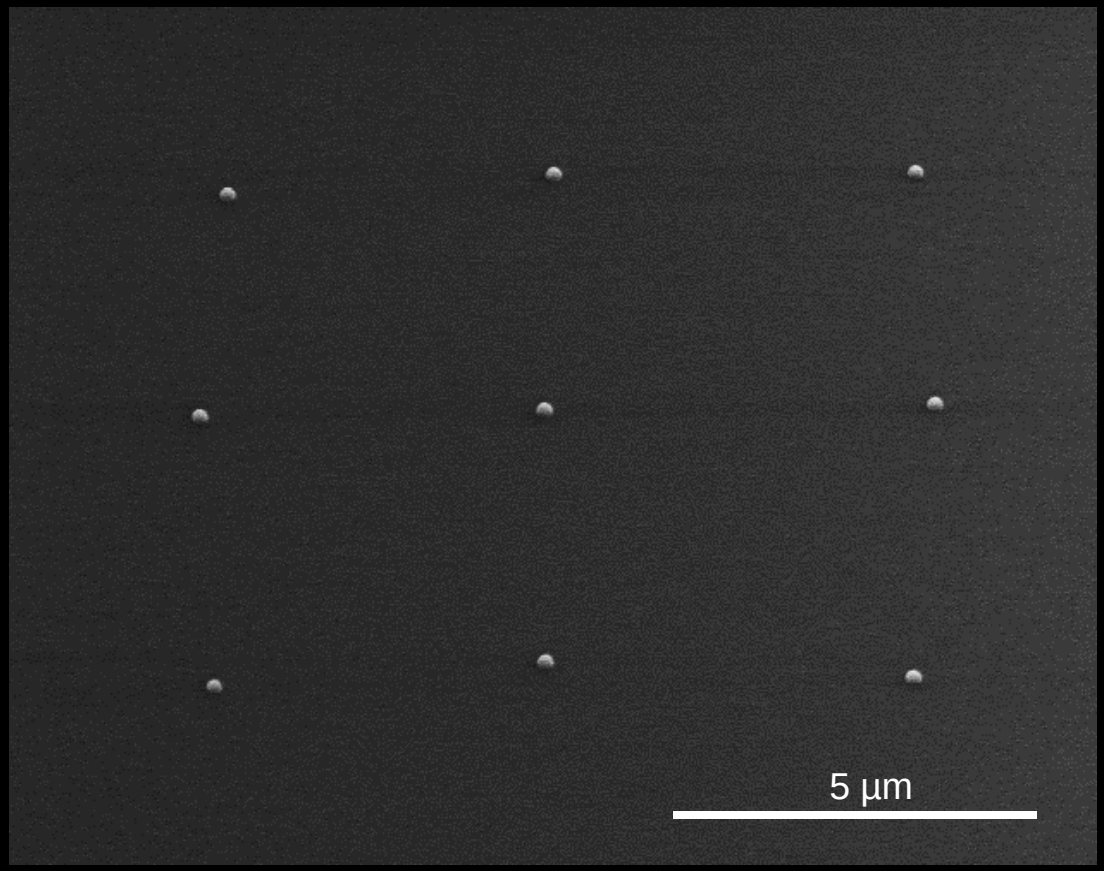
LASER PRINTING OF NANOPARTICLES FOR IMPROVING OF ADHERENCE AND ELECTRICAL COUPLING OF NEURONS

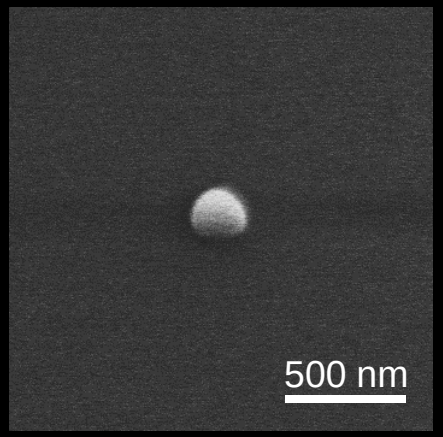




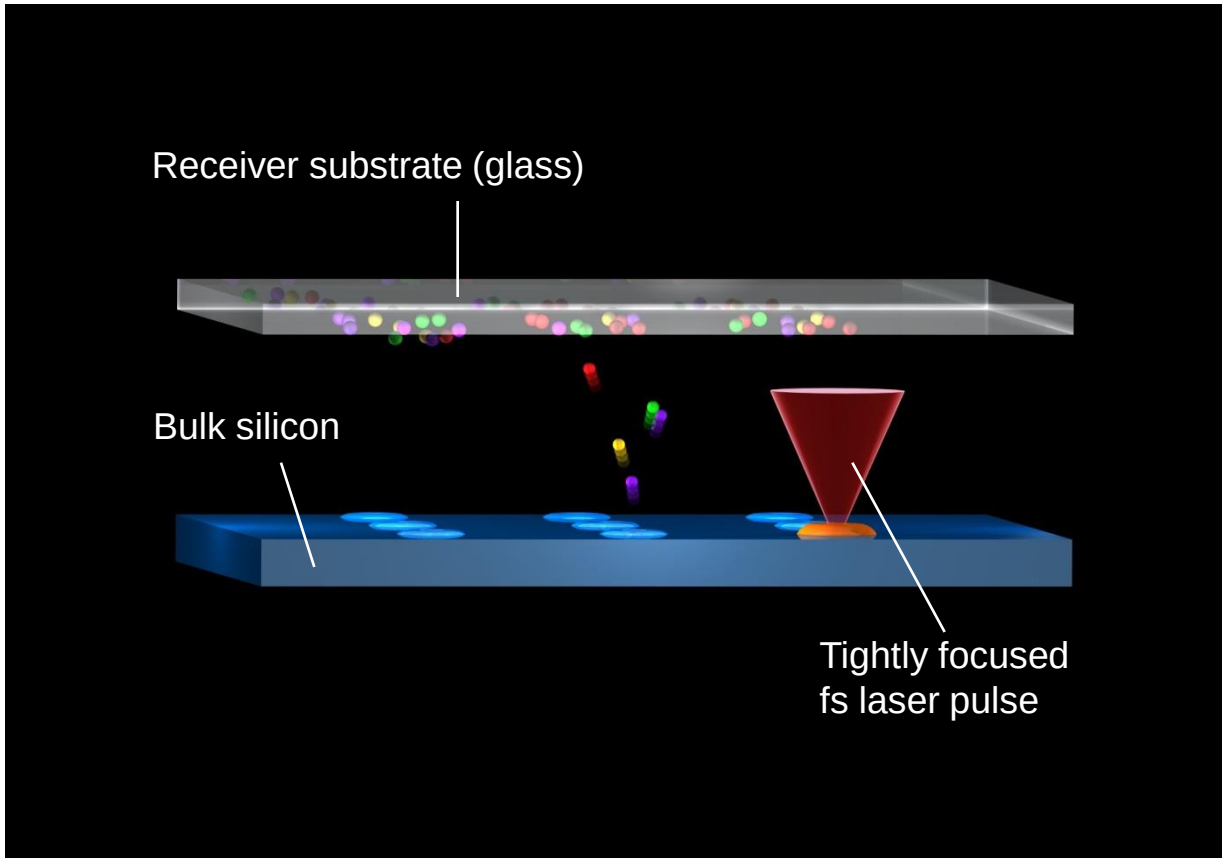


Laser printing of Fe nanoparticles



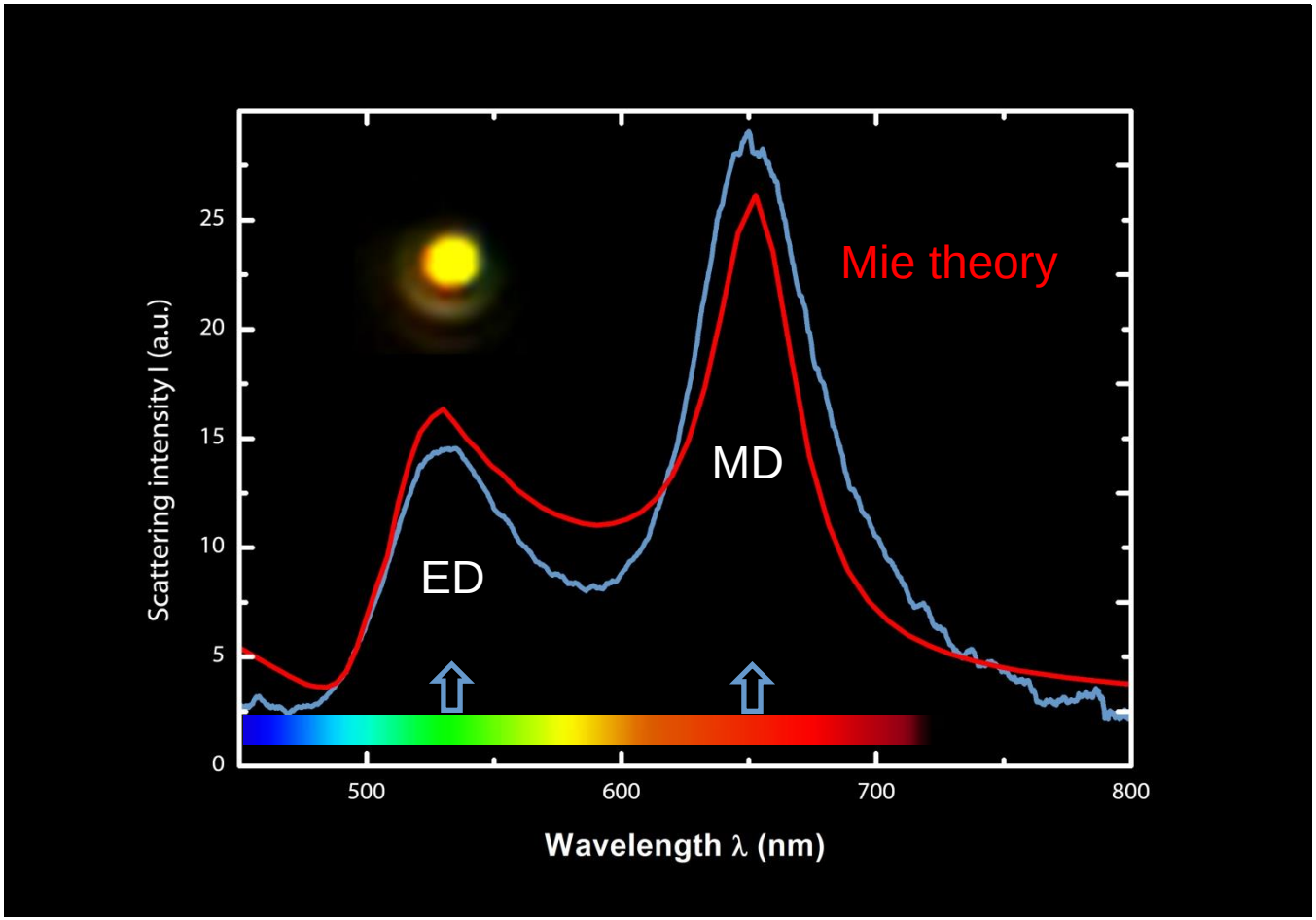


Laser printing of silicon nanoparticles from bulk silicon



Evlyukhin et al. Nano Lett. 12, 3749 (2012)

Resonant response of silicon nanoparticles



Evlyukhin et al. Nano Lett. 12, 3749 (2012)

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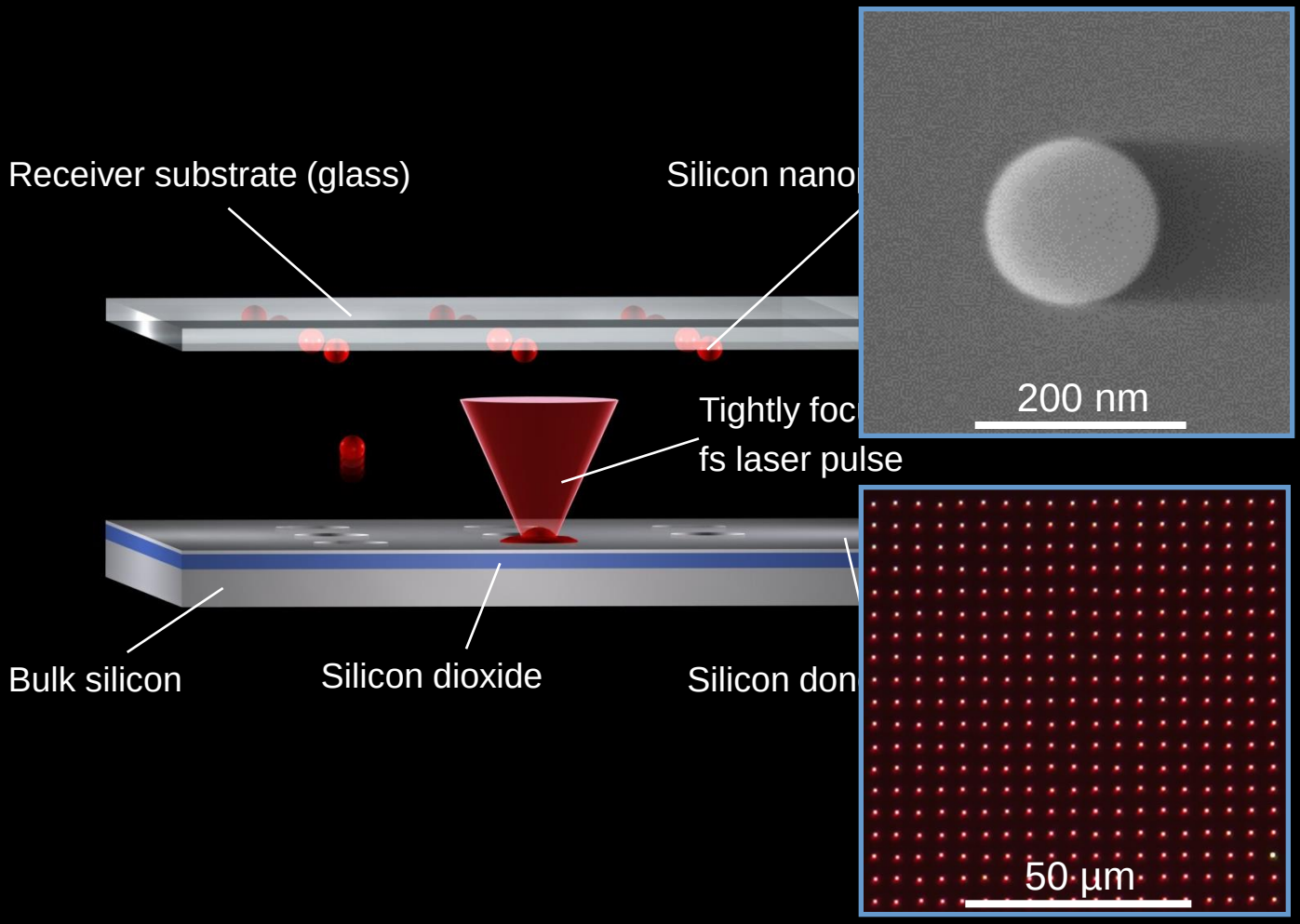
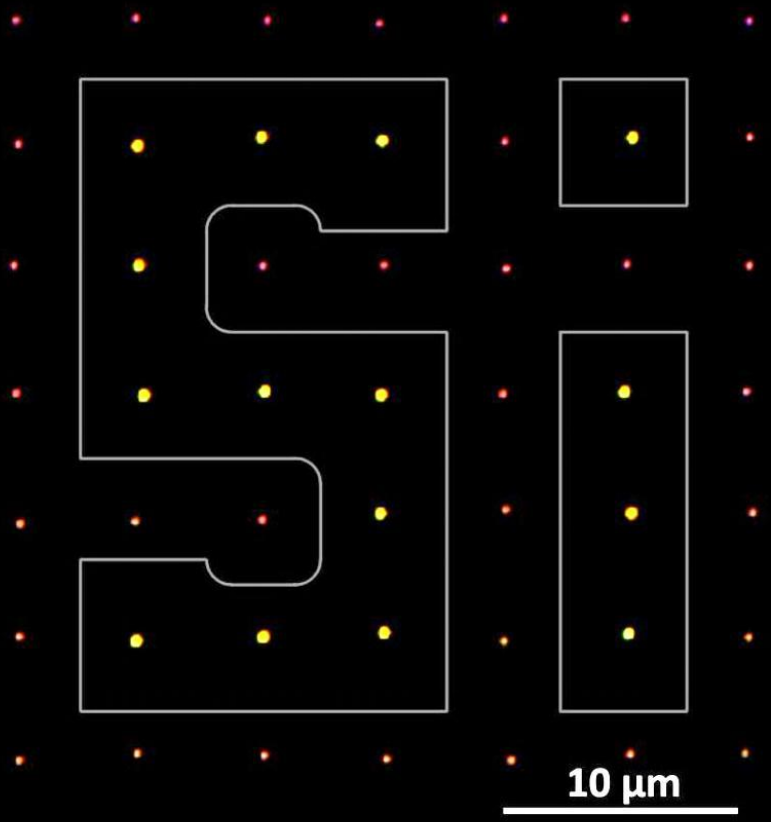
Laser printing of silicon nanoparticles



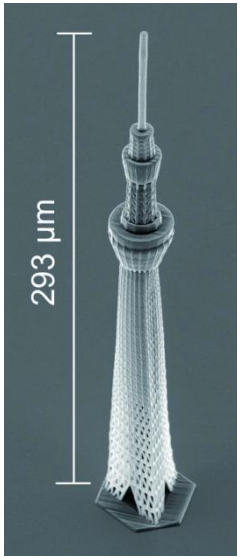
U. Zywietz, C. Reinhardt, A.B. Evlyukhin, B.N. Chichkov, „Laser printing of silicon nanoparticles with resonant optical electric and magnetic responses“, Nature Communications, 5, No. 3402, (2014).

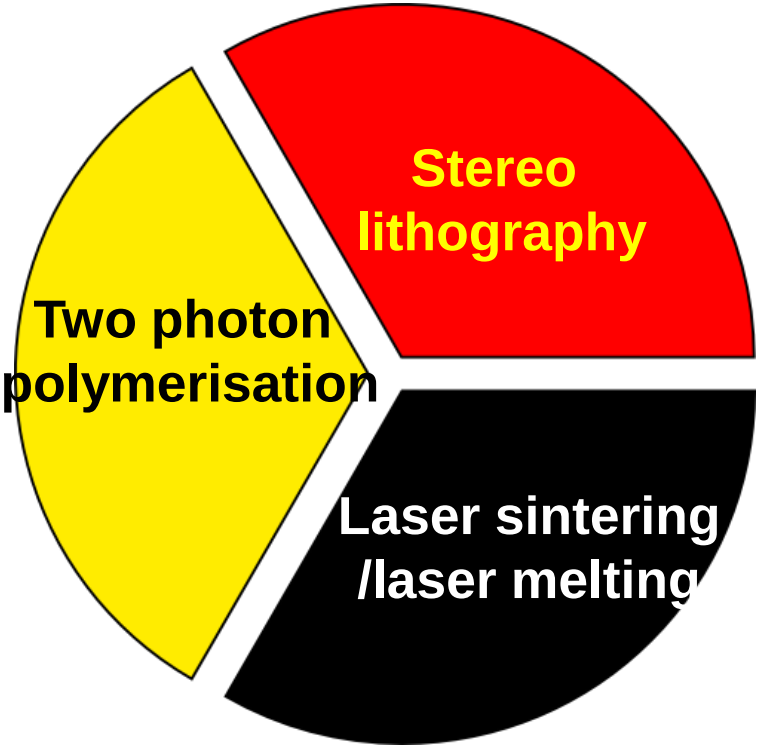
Selective phase change of Si nanoparticles

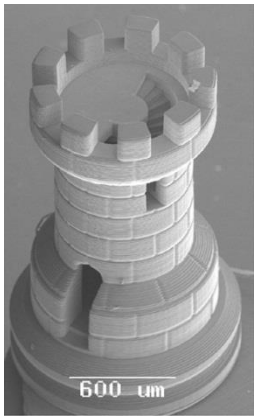


U. Zywietz, C. Reinhardt, A.B. Evlyukhin, B.N. Chichkov, „Laser printing of silicon nanoparticles with resonant optical electric and magnetic responses“, Nature Communications, 5, No. 3402, (2014).

Additive Manufacturing at the LZH

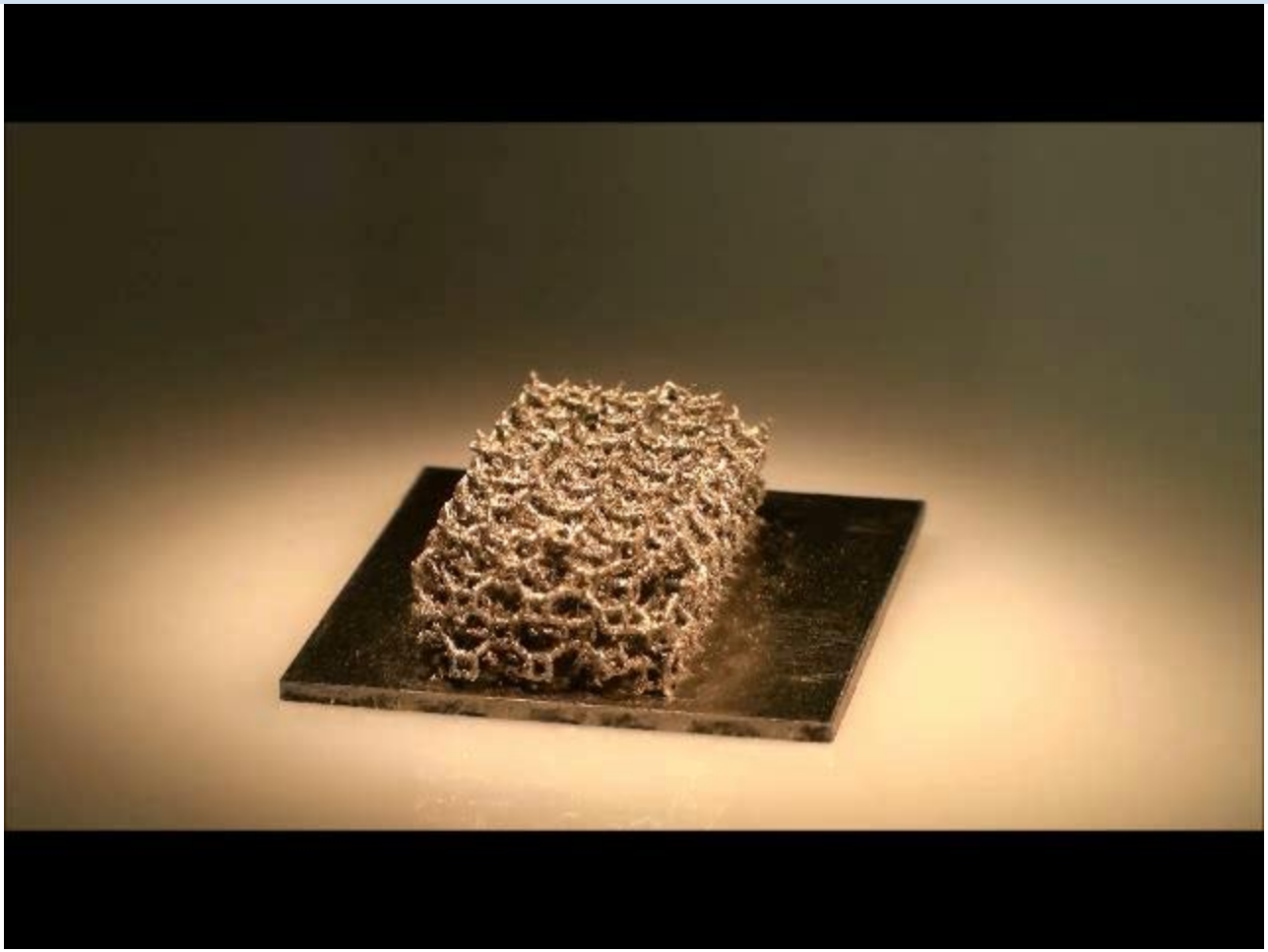






For Selective Laser Melting (SLM) one needs metal powder …

FUNCTIONAL NITI 3D STRUCTURES



Dr. Stefan Kaierle, LZH

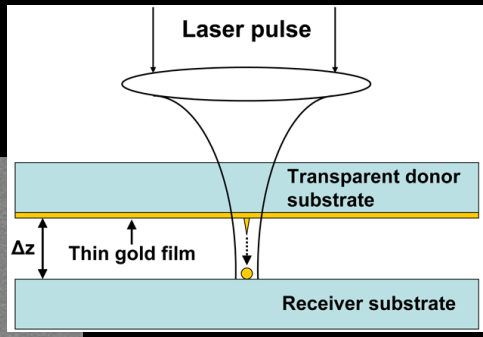
LATEST RESULTS OF MG-SLM

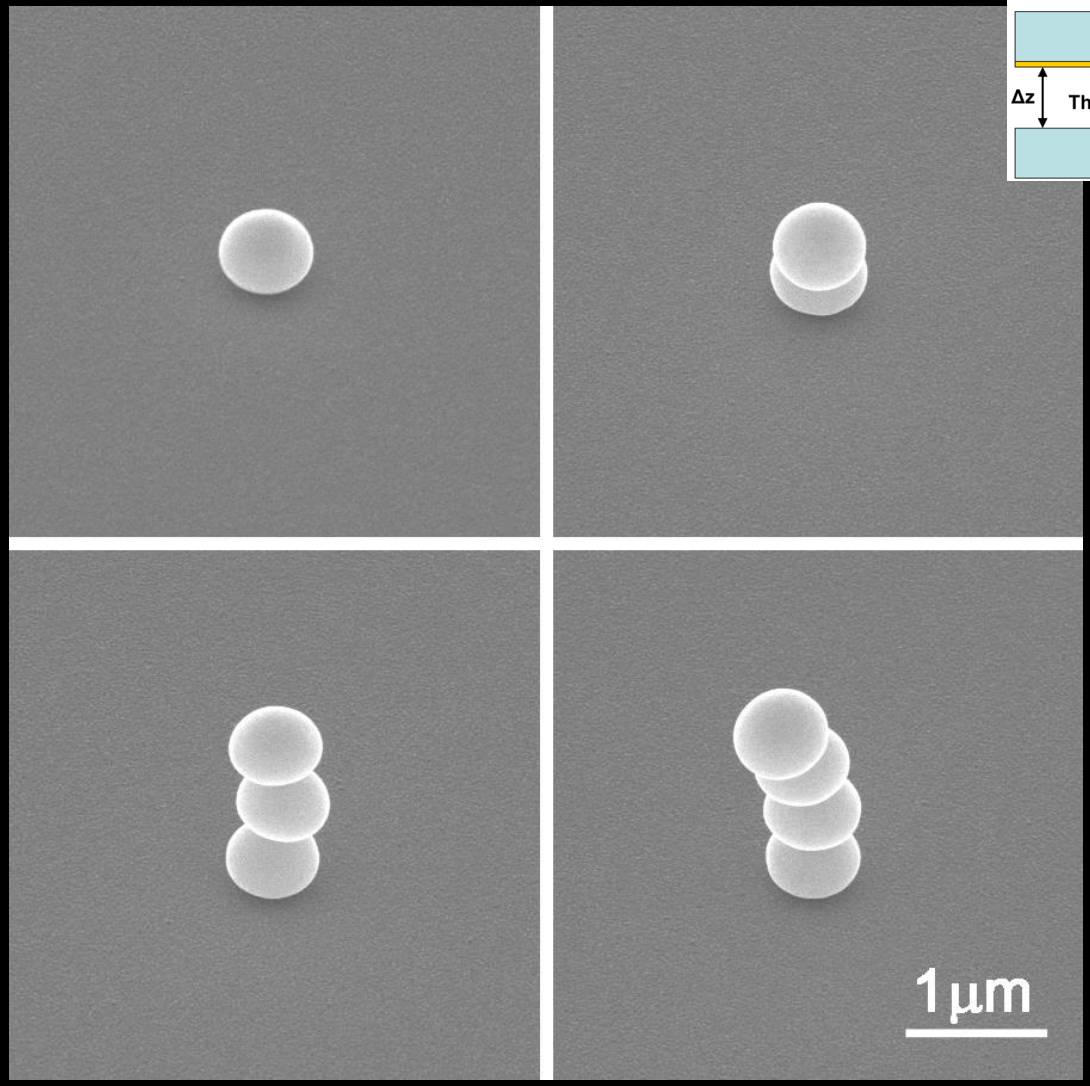
Excellent results:
resolution 100 μm

Can we improve resolution by a factor of 100? down to 1 μm?

Dr. Stefan Kaierle, LZH

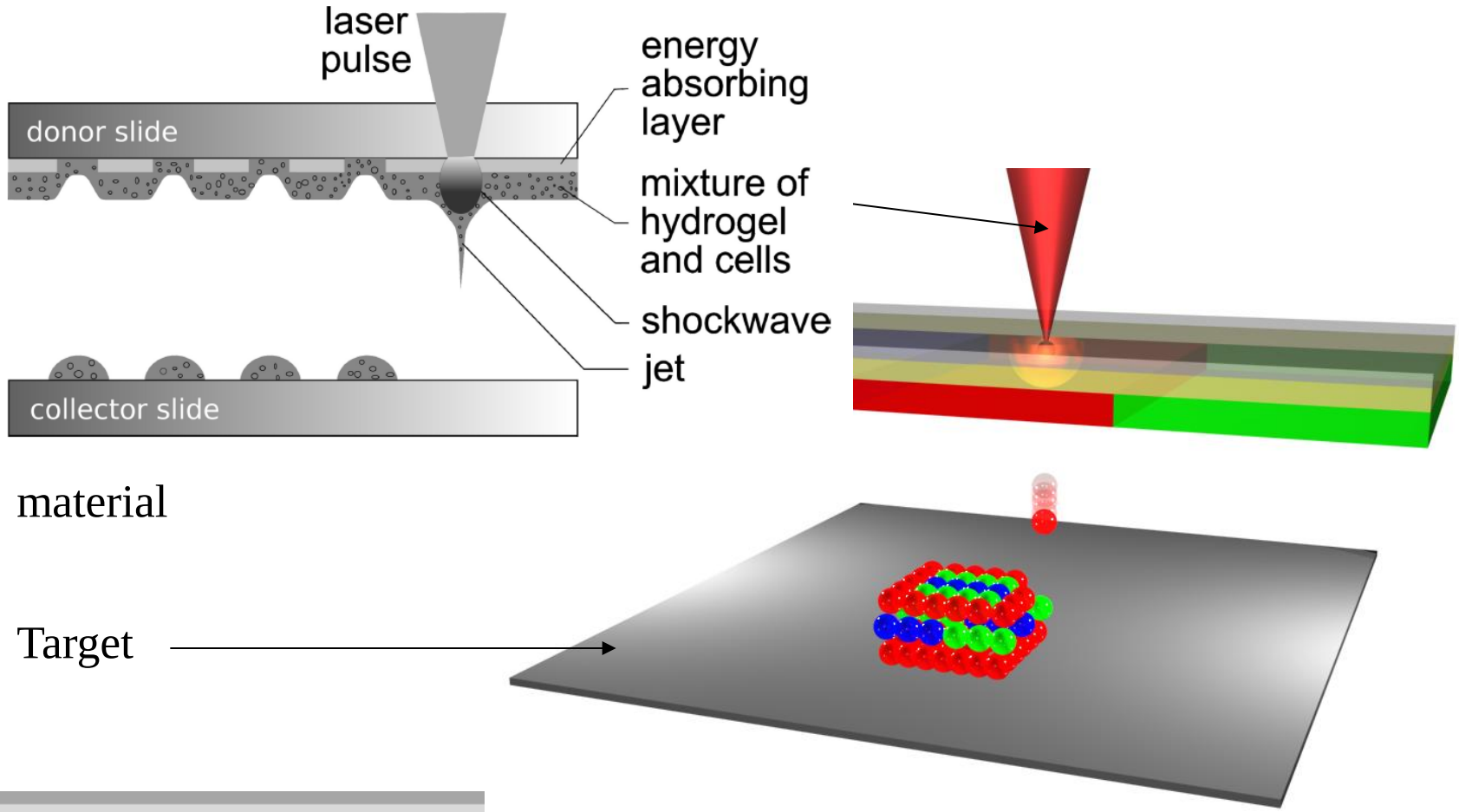
Additive Manufacturing by Laser Printing



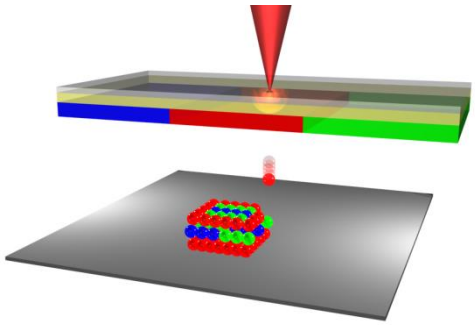


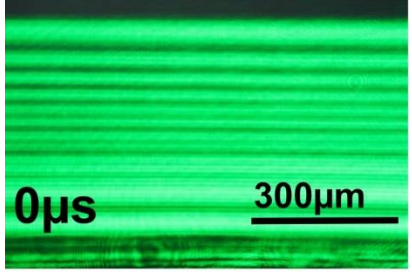
Appl. Phys. A **106**, 479 (2012)

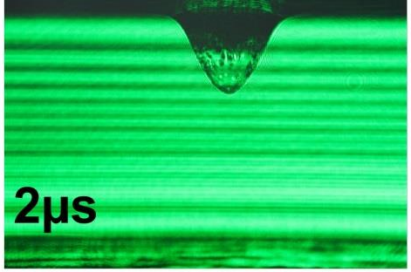
Biological laser printing

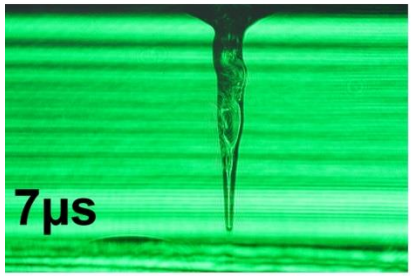


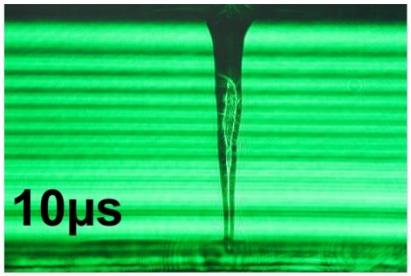
LIFT dynamics

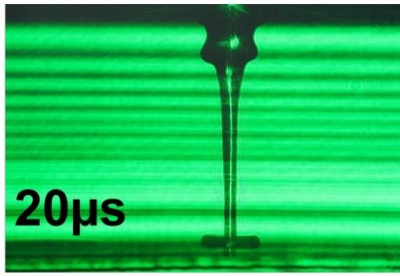


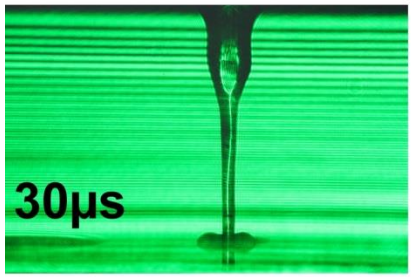


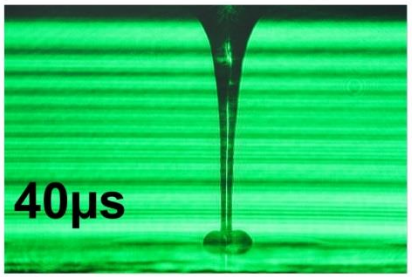


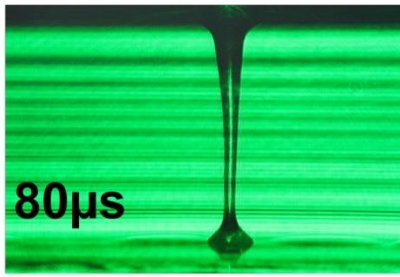


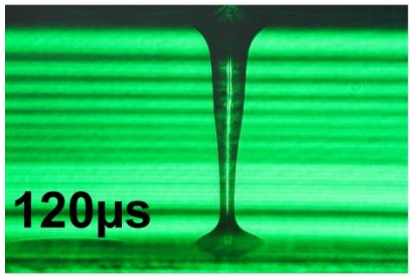


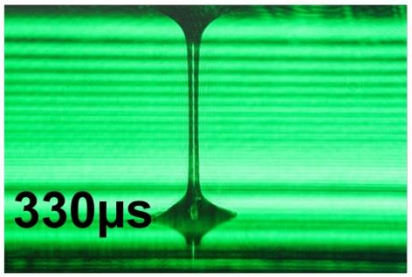


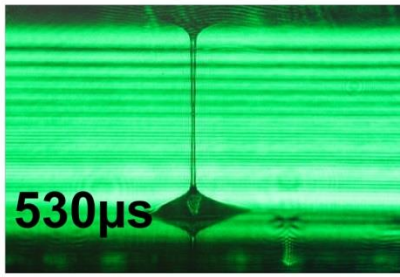


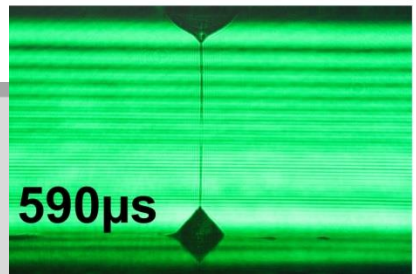


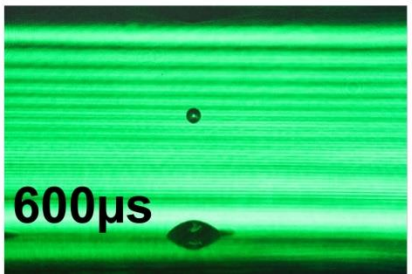


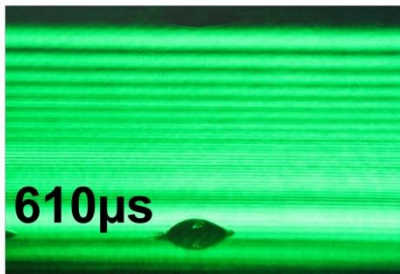




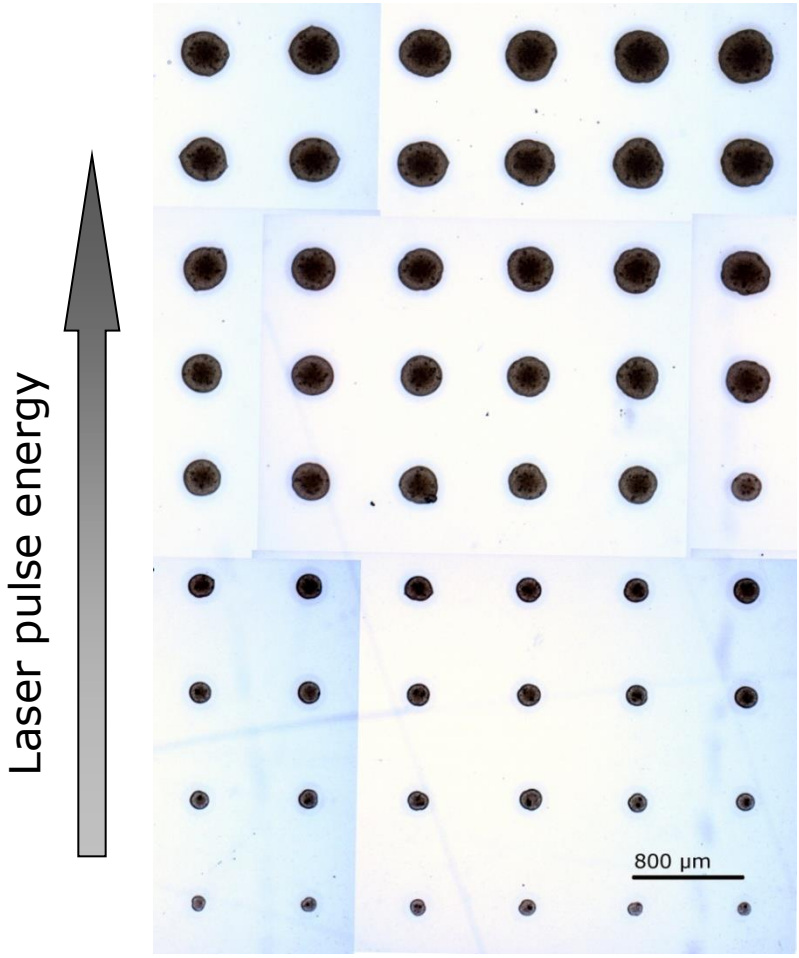








LASER PRINTING OF DROPLETS



Influence on the printed droplet volume

Controlling the droplet volume from a few picoliters to a few nanoliters by varying

- layer thickness
- viscosity
- laser pulse energy

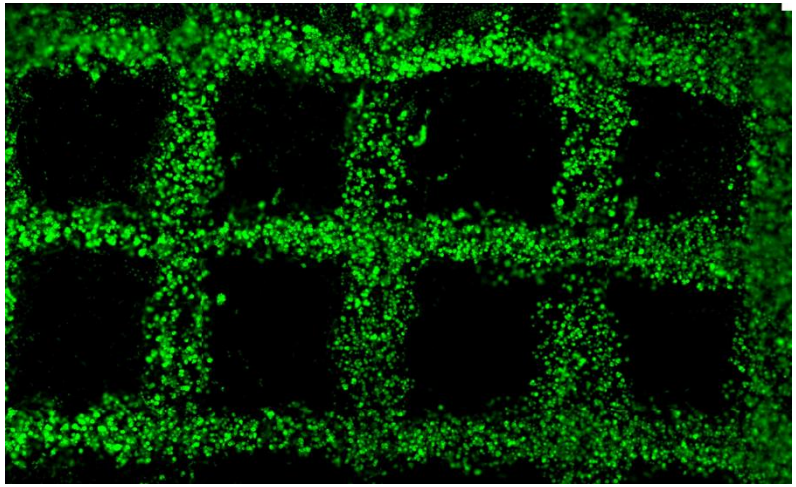
Unger et al., Time-resolved imaging of hydrogel printing via laser-induced forward transfer, Appl Phys A (2011) 103:271–277

LASER ZENTRUM HANNOVER e.V.

EFFECT OF LASER PRINTING ON CELLS – SURVIVAL RATE

Survival rate:

Fibroblasts (NIH3T3)	98% ± 1%
Keratinocytes (HaCaT)	98% ± 1%
human adipose-derived stem cells (ASC)	99% ± 1%
cord blood derived endothelial colony forming cells (ECFC)	98% ± 3%



Live/Dead-staining with
Calcein AM (green; vital cells) and
Ethidium Homodimer 1 (red; dead cells)

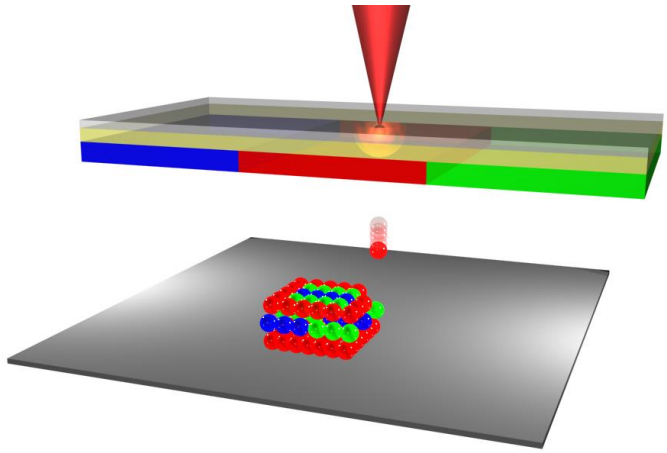
Nearly all cells survive the printing process

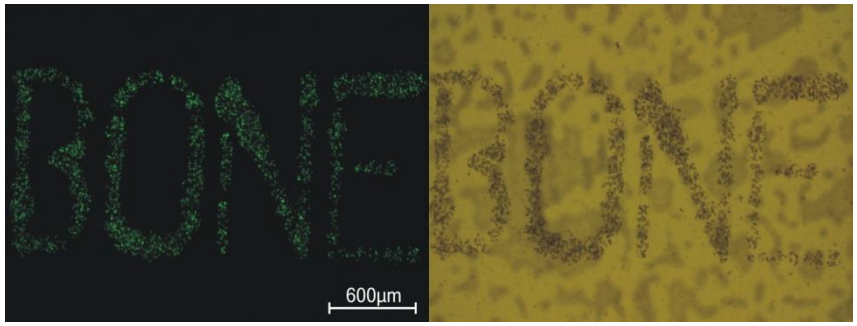
Koch et al., Laser Printing of Skin Cells and Human Stem Cells,
Tissue Eng Part C: Methods **2010**, 16(5): 847-854

Fundamental studies

Advantages of *Laser assisted Bioprinting*:

1. Printing of single to dozen of cells with a micrometer precision
2. No observable damage to the pheno- and genotype of the cells
3. Utilization of cross linkable hydrogels (e.g. Fibrin) enables 3D free form fabrication





Examined cells	***Assessments***
hBMSCs	Survival rates
hASCs	Proliferation
ECFCs	Apoptose
Cardiomyocytes	Comet assay
Fibroblasts	RT-PCR
Keratinocytes	Immunohisto-
Chondrocytes	chemistry

Laser printing has no influence on the cell behaviour

Koch et al. ***Tissue Eng Part C*** 16(5): 847-854 (2010)

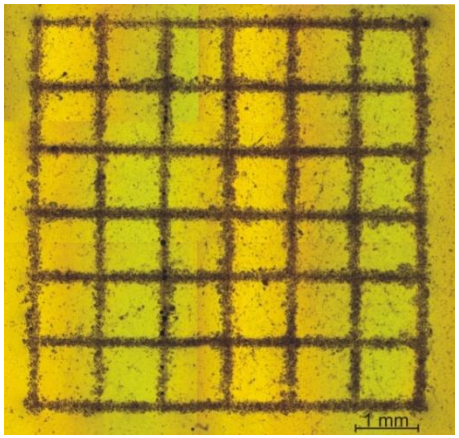
BENEFITS OF LASER PRINTING TECHNOLOGY

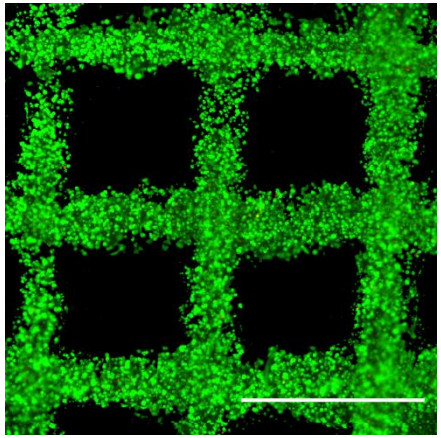
Benefits compared with other printing techniques (e.g. ink-jet or extrusion techniques / robotic dispensing):

Capability to print …

- small volumes – down to ~ 1pL
- material with high or low viscosities
- high cell densities
- high cell survival

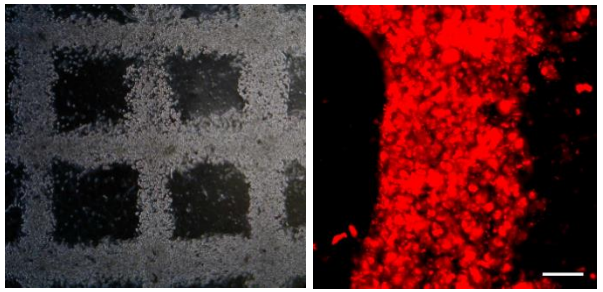
DIFFERENTIATION OF 3D MSC GRAFTS





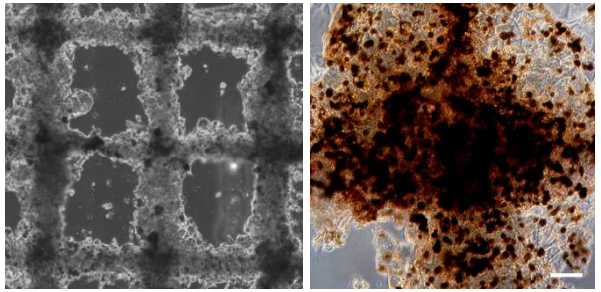
Live/dead-staining after 6 days under chondrogenic culture conditions

Cartilage



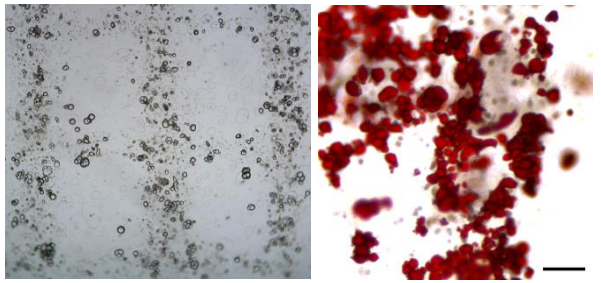
Collagen type II

Bone



Calcium accumulation

Fat



Oil-Red-O staining

Stems cells can be differentiated within the printed pattern

Gruene et al., Adipogenic differentiation of laser-printed 3D tissue grafts consisting of human adipose-derived stem cells, *Biofabrication* **2011** 3(1): 015005

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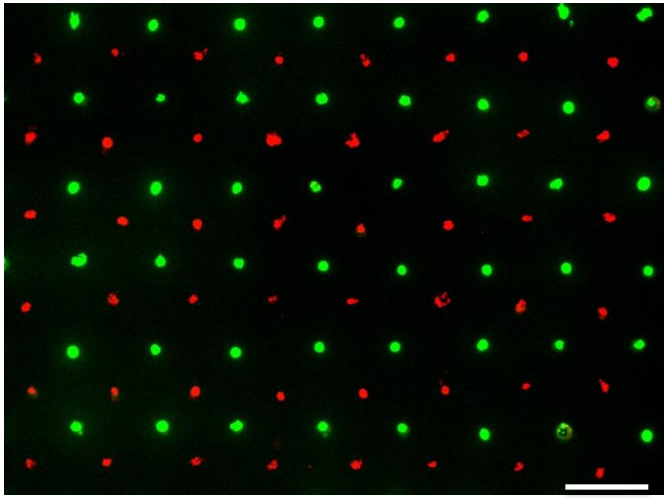
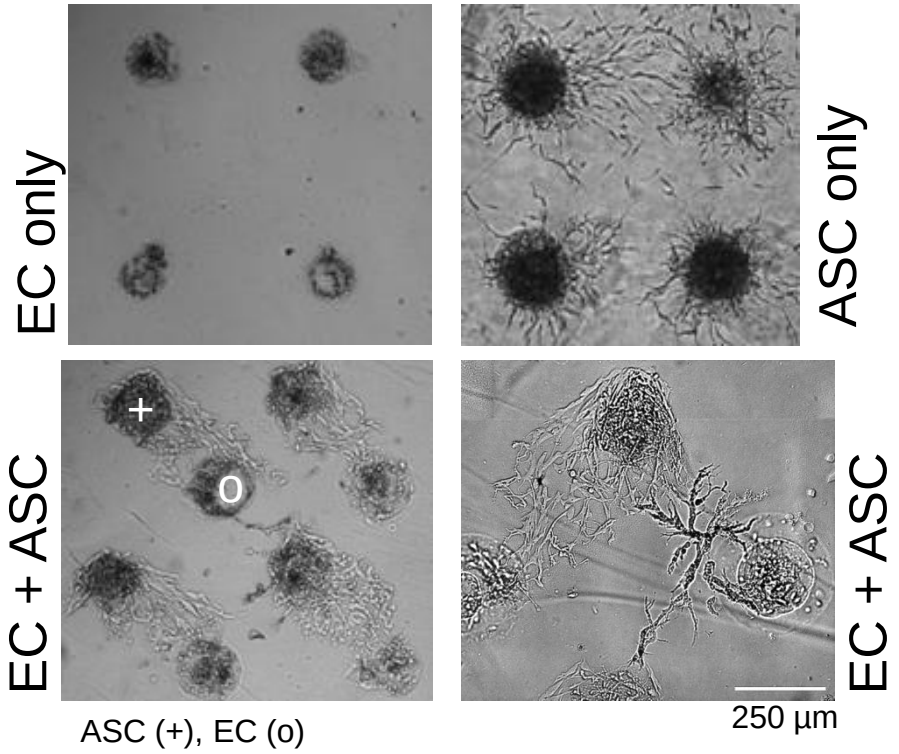
CELL-CELL INTERACTIONS IN MULTICELLULAR ARRAYS

Printed droplets containing endothelial cells (EC, red) or adipose derived stem cells (ASC, green)



After 5 days cultured in VEGF-free media



ASC (+), EC (o)

A printed predefined pattern of cell containing droplets allows to study cell-cell or cell-environment interactions

Gruene et al., Laser printing of three-dimensional multicellular arrays for studies of cell-cell and cell-environment interactions. *Tissue Eng Part C Methods* 17(10): 973-82 (2011)

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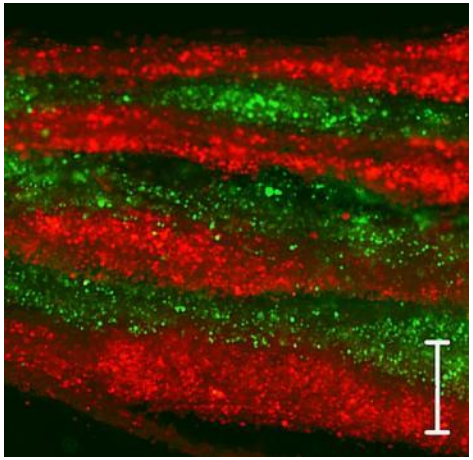
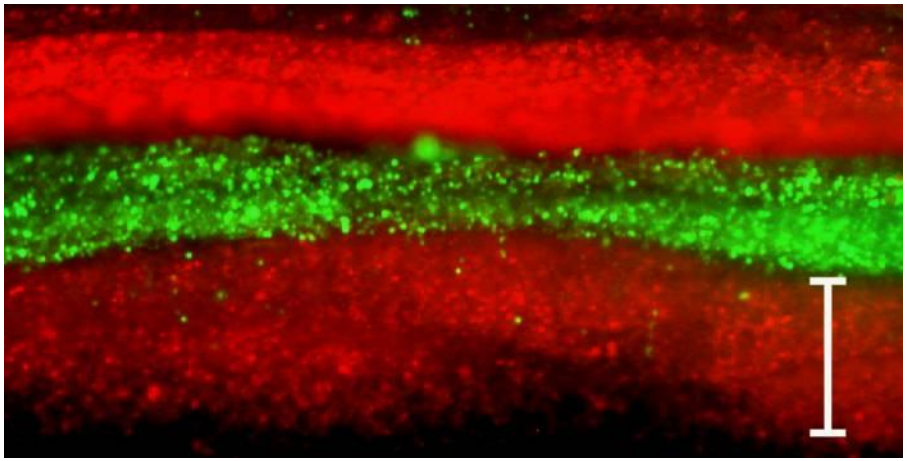
GENERATION OF 3D SKIN TISSUE

Layers of fibroblasts (NIH3T3) and keratinocytes (HaCaT), in collagen I on Matriderm™

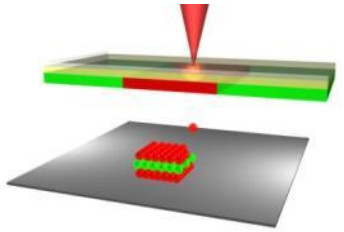


Layered arrangement of red and green HaCaT (eGFP, mCherry), 18 h after printing



Each color layer consists of four printed sub-layer. The whole construct is about 2 mm high.

scale bars
500 µm

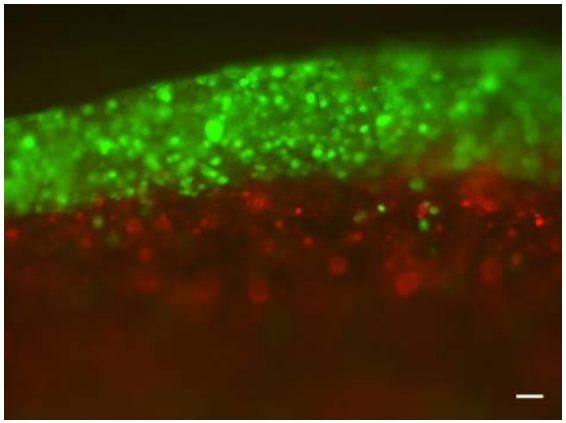


The cells have been embedded in collagen directly before printing.
The layers do not intermix during or after printing.

Together with Prof. Vogt, MHH

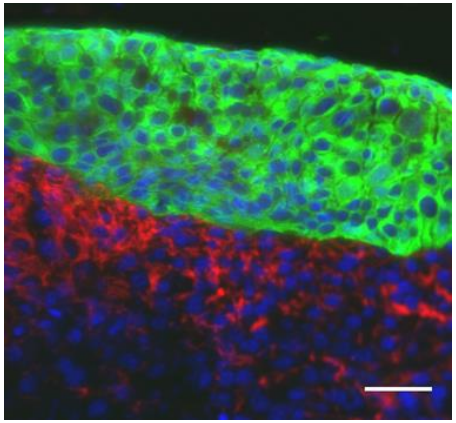
Koch et al., Skin Tissue Generation by Laser Cell Printing.
Biotechnol Bioeng. 109(7): 1855-1863 (2012)

GENERATION OF 3D SKIN TISSUE

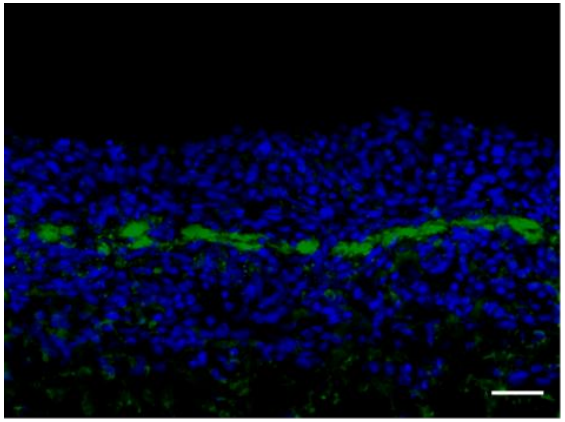


Directly after Printing: NIH3T3 (eGFP), HaCaT (mCherry)

- Laser printing on Matriderm™
  - cells embedded in a collagen type I matrix
  - 20 layers of fibroblasts (NIH3T3) (red)
  - 20 layers of keratinocytes (HaCaT) (green)
- Cryostat sections were prepared 10 days after printing



HaCaT green (Cytokeratin14),
NIH3T3 red (Panreticular),
cell nuclei in blue (Hoechst 33342)



Anti-laminin staining in green, cell
nuclei staining in blue (Hoechst 33342)

Formation of a
basement membrane
/ basal lamina

Scale bar 50 µm

Koch et al., Skin Tissue Generation by Laser Cell Printing.
Biotechnol Bioeng. 109(7): 1855-1863 (2012)

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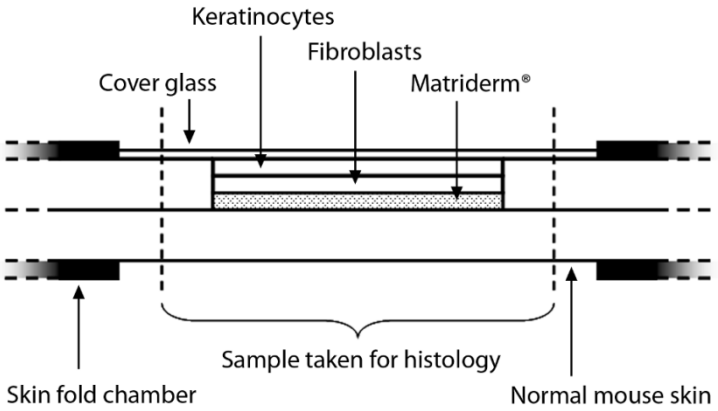
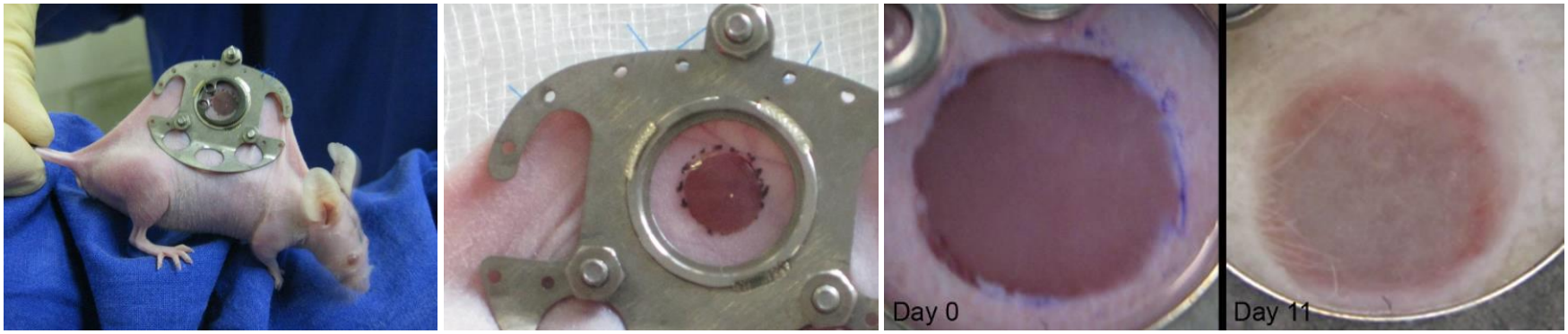
GENERATION OF 3D SKIN TISSUE *IN VIVO*

- Fibroblasts and keratinocytes in collagen I printed layer-by-layer on Matriderm©
- implanted into mice (dorsal skinfold chamber) and explanted after 11 days.
- Implanted Matriderm without cells as control
- Cryo- and paraffin sections were analysed by histology





A part of the dorsal skin is stamped out and replaced by the printed structure

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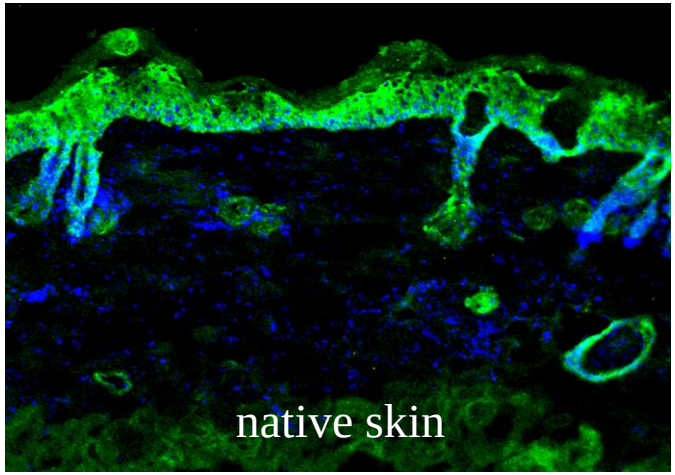
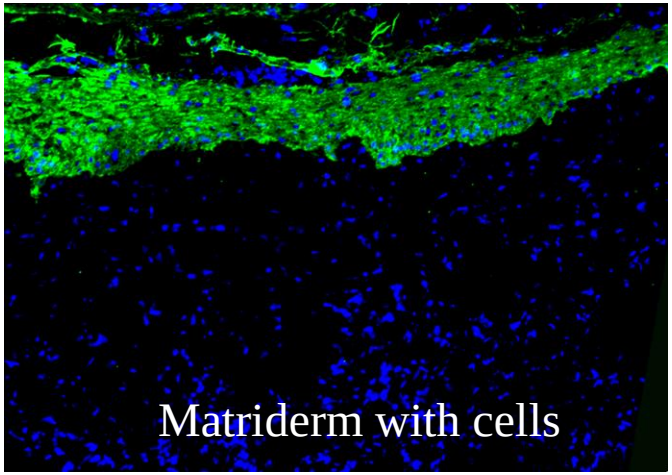
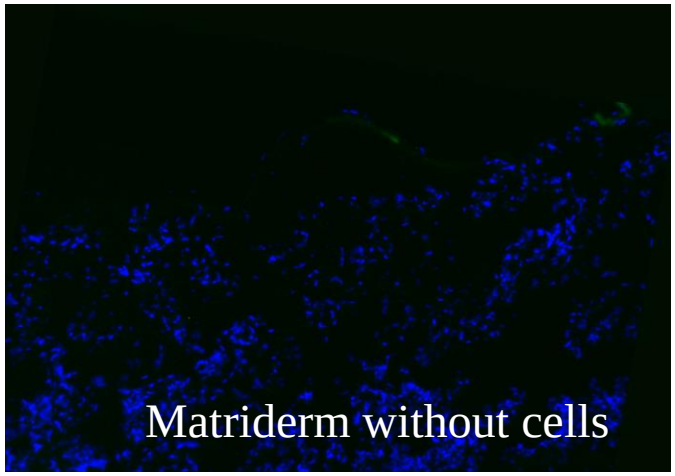
Generation of skin equivalents by laser printing



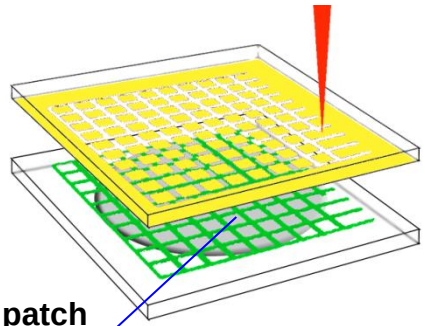




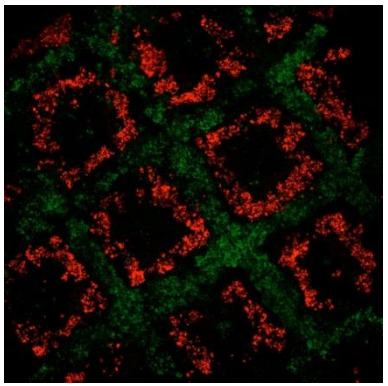
Immunofluorescence staining of cytokeratin 14 (green) for keratinocytes cell nuclei were stained with Hoechst 33342 (blue)

Together with Prof. Vogt, MHH

MICROVASCULARIZATION

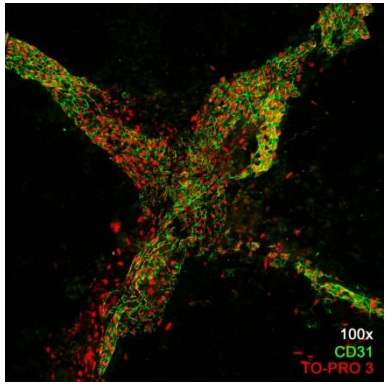


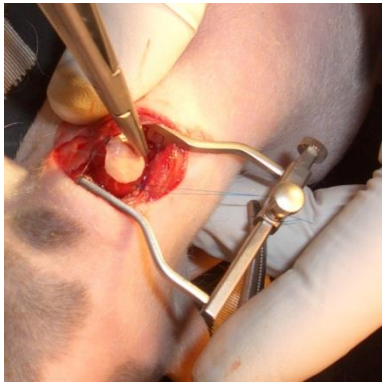
PEUU **cardiac patch** immersed in Matrigel

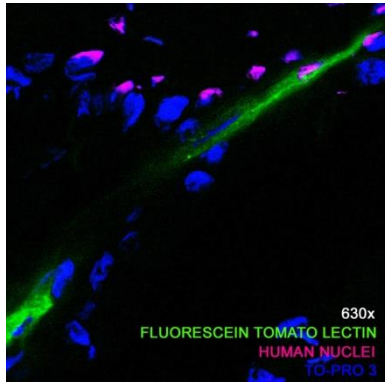


Printed human endothelial cells (green) and human mesenchymal stem cells (hMSC, red) on a cardiac patch

Vessel formation in the printed structure after 8 days (HUVEC/hMSC-co-culture on Matrigel-coated cardiac patch)







Human cells, integrated in the murine vascular network at the border of the cardiac infarct zone, 8 weeks post-infarct

Accelerated vessel formation in printed structure (EC/MSC co-culture) *in vitro*

Improved heart function after myocard infarction and implantation of printed cardiac patch

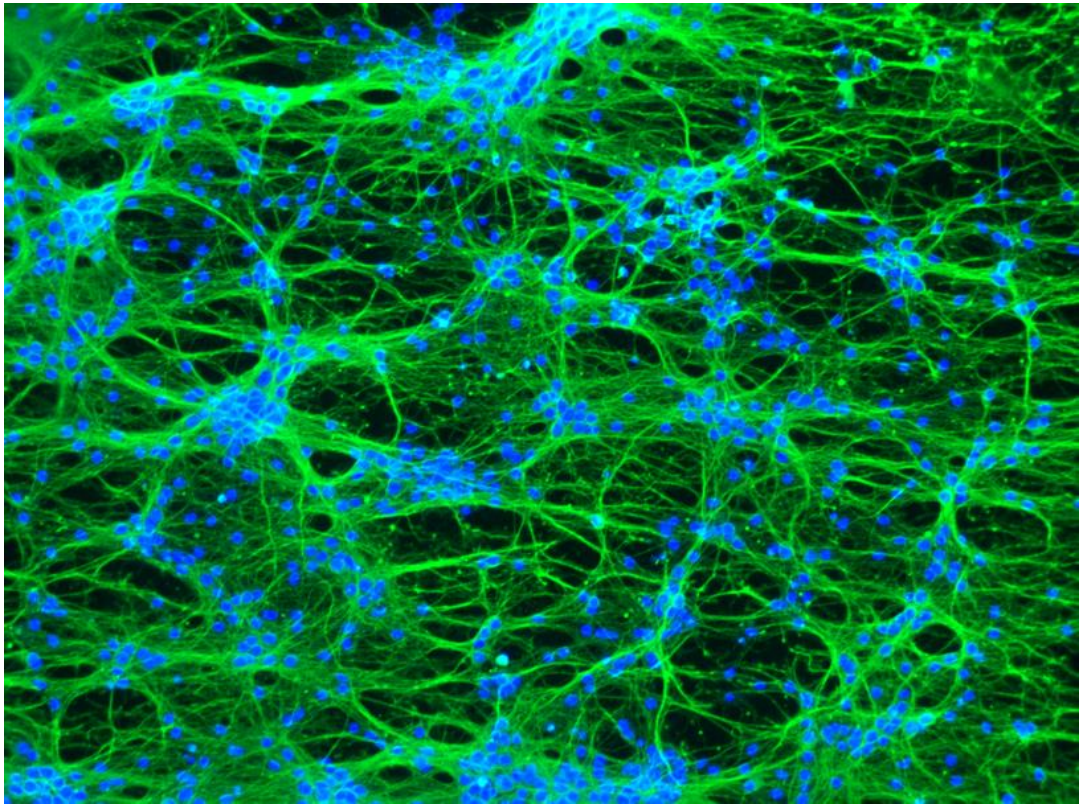
Gaebel et al., Biomaterials 32: 9218-9230 (2011).
C. Klopsch et al. J. Tisssue Eng. Regen. Med. 9, E177 (2015).

LASER ZENTRUM HANNOVER e.V.

Team acknowledgement

LZH NT Department

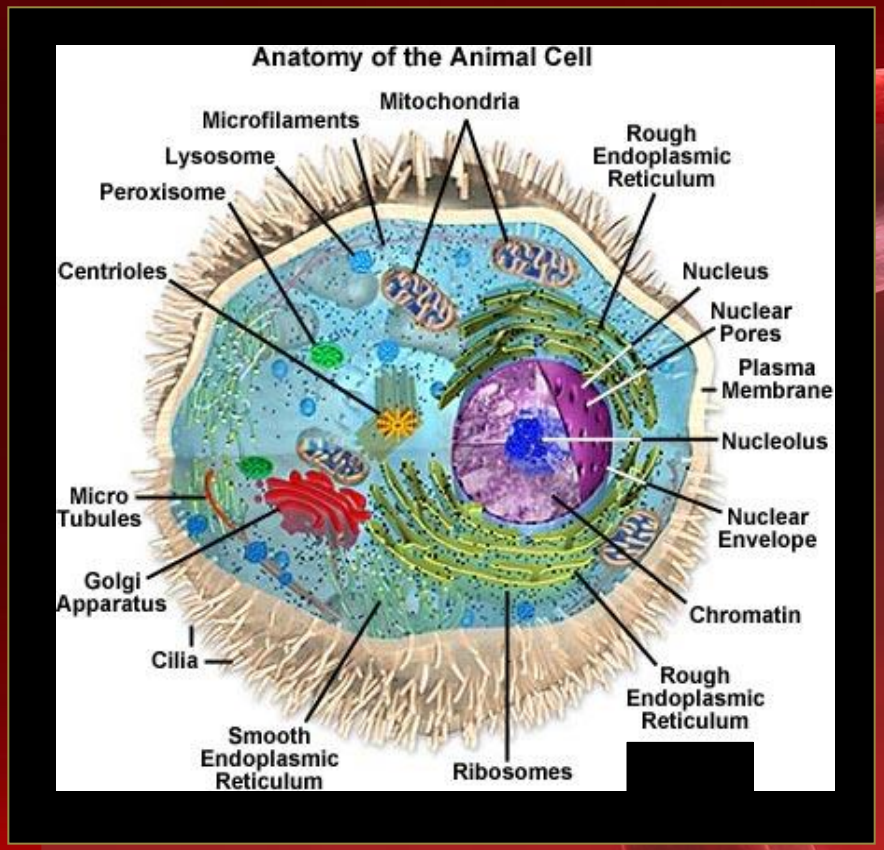
Annette Barchanski,
Tobias Birr,
Andrea Deiwick,
Ayman El-Tamer,
Andrey Evlyukhin,
Elena Fadeeva,
Ulf Hinze,
Roman Kiyan,
Lothar Koch,
Anastasia Koroleva,
Olga Kufelt,
Kestutis Kurselis,
Carsten Reinhardt,
Laszlo Sajti,
Sabrina Schlie-Wolter,
Urs Zywietz



Thank you for your attention

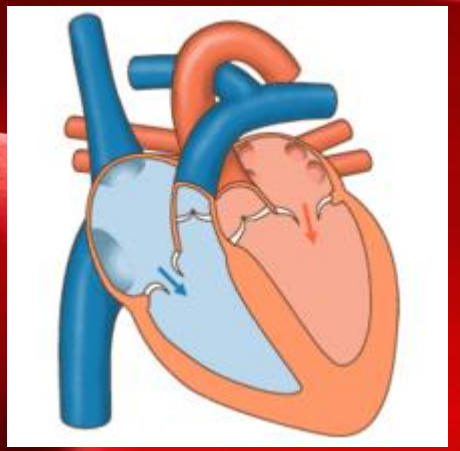

Is it possible to print a MAN?

How many cells has a Man?



An ordinary man (100 kg) consists of **10^{14} or 100 Trillion**s of single cells.

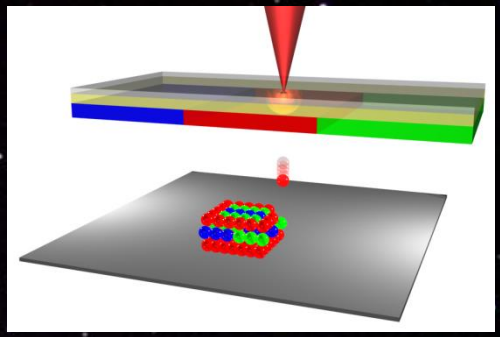
An adult human heart



has 3x**10^{11}** cells

In an ordinary man 50 Millions of cells die every second and approximately the same ammount of cells is produced

Biological laser printing

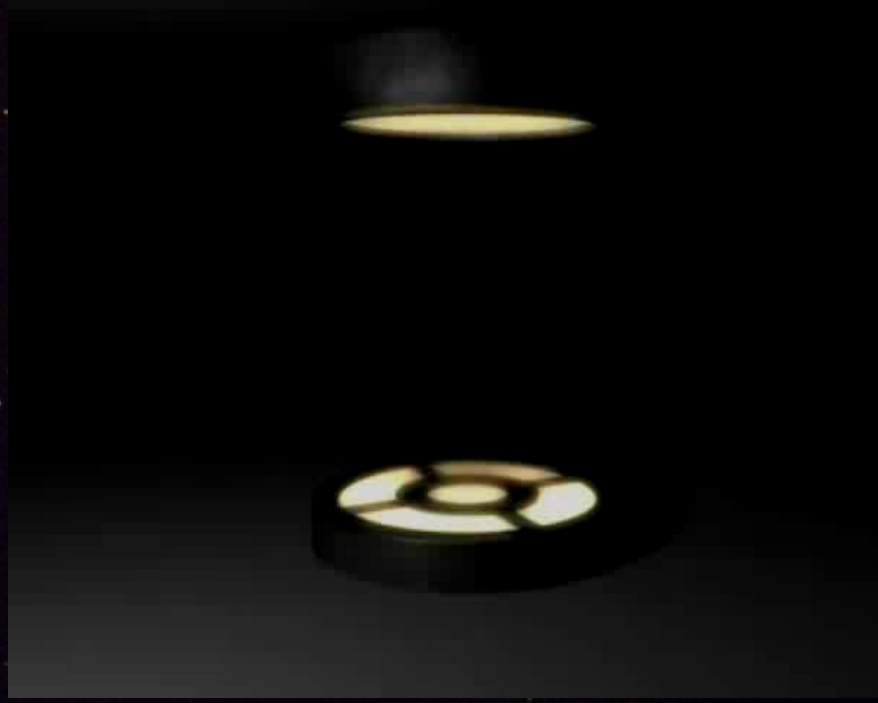


1. Fs-Laser 10^8 pulses per second

2. Transfer of 100 cells per pulse

3. All together 10^{10} cells per second

After a solution of some technical problems



A Man (10^{14} cells) can be printed in 2 h 47 min

An adult human heart ($3x10^{11}$ cells) can be printed in 30 seconds