

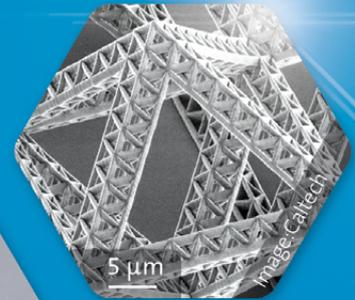
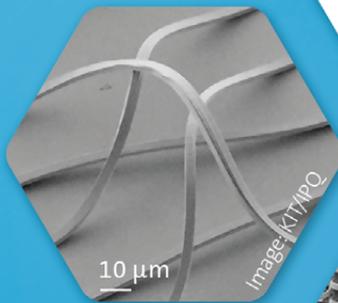
# Spectrum of Applications

New Trends in Microfabrication

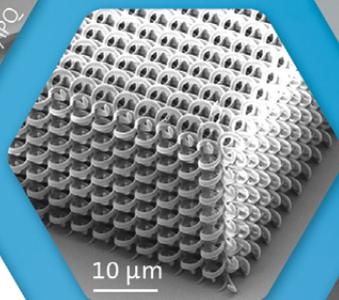
## Photonic Professional $\equiv$ GT

Highest Resolution 3D Printing

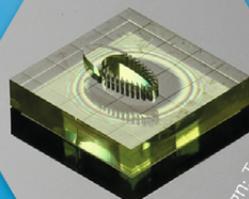
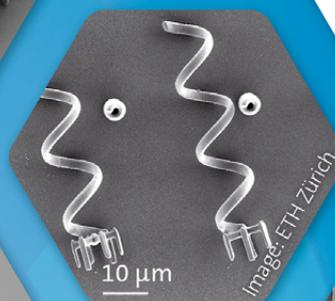
3D Design  
Freedom



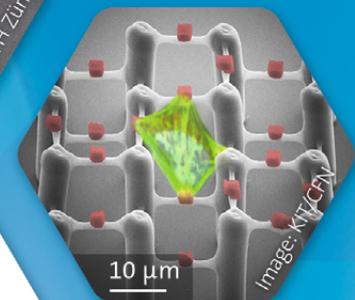
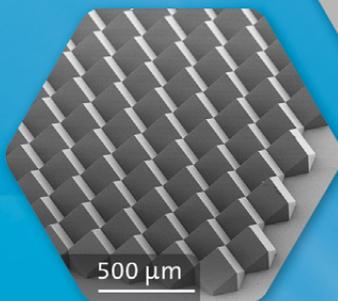
Diversity of  
Materials



Easy 3D Printing  
Workflow



Submicrometer  
Feature Sizes



## Additive Manufacturing & Maskless Lithography in One Device

The 3D laser lithography system, Photonic Professional *GT*, sets new standards in 3D microprinting and maskless lithography. This highest-resolution 3D printer enables the rapid fabrication of nano-, micro- and meso-structures with feature sizes starting from about hundred nanometers and heights up to several millimeters with layer thicknesses well below 1 µm with optical quality surfaces.

In combination with tailor-made photoresists, hardware- and software packages, the turn-key system is embedded best along the 3D printing workflow and allows for highest resolution with a previously unavailable freedom of design.

Subsequent independent processes enable the transfer and/or replication of polymeric 3D printed templates into a large choice of materials, including metals and semiconductors.

The additive manufacturing of 2D, 2.5D and 3D objects paves the way for a wide field of novel applications, including:

- Micro-optics and photonics
- Integrated wafer-level optics
- Micro-fluidics
- MOEMS / MEMS
- Cell scaffolds / tissue engineering
- Biomimetics
- Rapid Prototyping
- Maskless Lithography

In 2014, the outstanding performance of the Photonic Professional *GT* systems was underlined by the receipt of Prism Award in the category “Advanced Manufacturing”. In 2015 Nanoscribe was recognized as winner of the World Technology Award (WTN) for its outstanding achievements in “materials”.

### Photonic Professional *GT*



In this issue	Page
Additive Manufacturing & Maskless Lithography in One Device	2
Maskless Lithography	3
Rapid Prototyping of Micro- and Mesoparts	4
3D Photonics	6
Casting	6
Photonic Wire Bonds	7
Unfeelability Cloak Protects the Princess from the Pea	8
3D Printed Micro-Trusses	9
Additive Manufacturing for Microfluidics	10
Magnetic Helical Micromachines	11
2.5 D Surfaces	12
Simple Transmission Phase Gratings Make Colour Filters in the Visible	12
Application Perspective for Maskless Lithography	13
Cells Conquer the Third Dimension	14
Bio-Inspired Osteoprints for the Enhancement of the Osteogenic Differentiation	15

Imprint:

Nanoscribe GmbH  
 CEO: Martin Hermatschweiler  
 Registered office of the association:  
 76344 Eggenstein-Leopoldshafen (DE)  
 District court: Mannheim  
 HRB 703637, VAT-No. DE258161584  
 Tax-No. 34415/77104

Editor: Martin Hermatschweiler  
 Assistant Editor: Anke Werner  
 Contributions: Nicole Lindenmann (Karlsruhe Institute of Technology, KIT-IPQ), Tiemo Bückmann (KIT-APH), Jens Bauer (KIT-IAM), Michael Röhrig (KIT-IMT), Piotr Wasylczyk (University of Warsaw), Li Zhang (ETH Zürich), Attilio Marino (IIT Pontedera), Yann Tanguy and Fabian Niesler (Nanoscribe)  
 Layout & Design: Katja Thieme

## Maskless Lithography

### 2D manufacturing by direct laser writing

(Yann Tanguy, Fabian Niesler) While known for its outstanding 3D printing capabilities, the Photonic Professional GT also enables high-resolution 2D patterning of thin films, called maskless lithography.

The technology of direct laser writing (DLW) complements 2D manufacturing technologies available on the market and represents an alternative to traditional electron-beam lithography (EBL) and photolithography technologies offering similar performance levels. In terms of spatial resolution, a recent publication [1] positions DLW between these two technologies, while the patterning speed is now approaching EBL standards thanks to the implementation of galvanometric

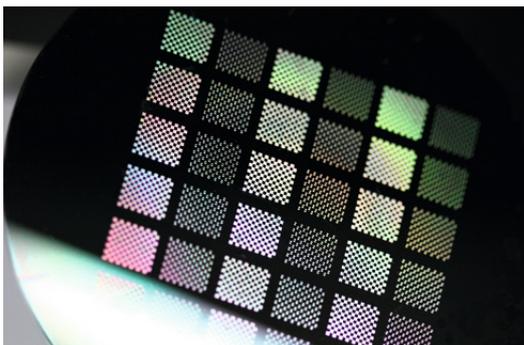
mirror scanners. 2D photoresist structuring using a DLW approach does not require expensive masks which makes it an ideal tool for all aspects of prototyping applications as well as fabrication of masters.

The underlying principle of two-photon absorption combines high resolution patterning together with a broad range of compatible UV sensitive photoresists. Structures with high-aspect ratios can be achieved without limitations from Beer's absorption law or electron scattering effects. Along the list of compatible negative-tone photoresists are SU-8 as well as Nanoscribe's IP resins that provide robust and reliable process results.

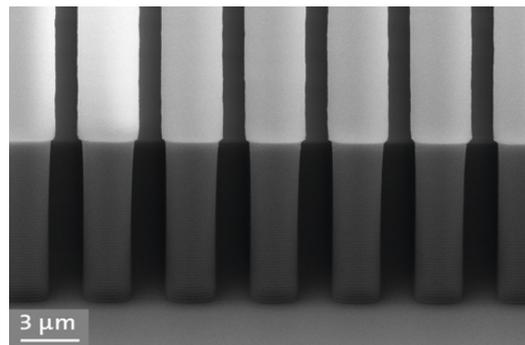
Nanoscribe has recently evaluated the performance of the Photonic Professional GT for 2D patterning of various thin- and thick-film positive tone resists of the AZ® series [2] (AZ® 9260, AZ® 5214E, AZ® MIR 701, AZ® 40XT) on glass and silicon substrates. These resists and substrates cover a broad range of application fields, e.g. etch masks, sputter masks, high-aspect ratio structures and electro-plating templates.

[1] D.S. Engstrom et al., „Additive nanomanufacturing – A review“, *J. Mater. Res.*, 2014.

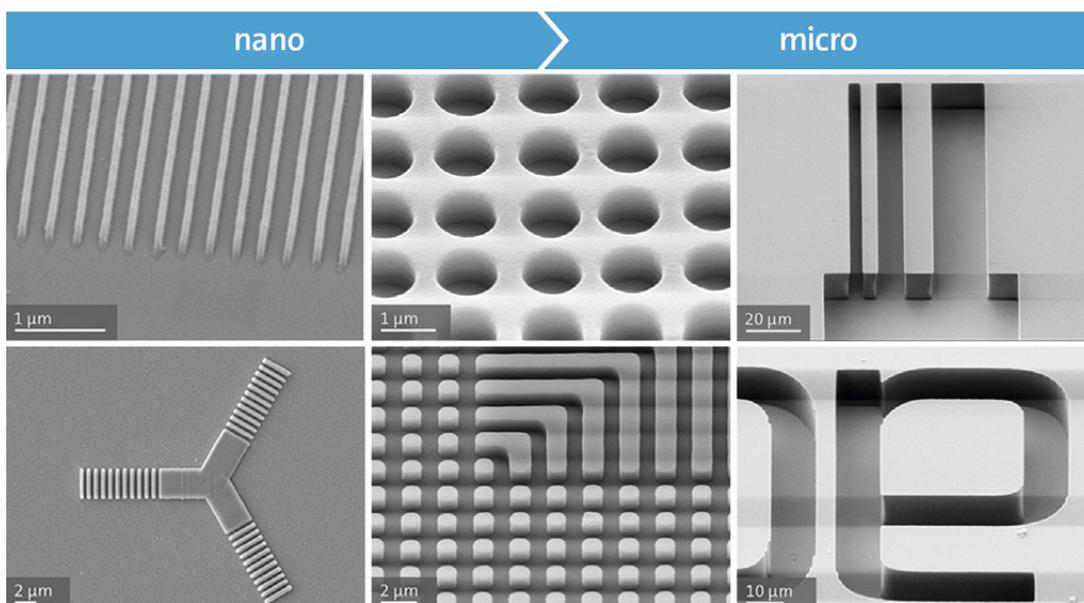
[2] These resists are available off the shelf from [www.microchemicals.com](http://www.microchemicals.com).



Checkerboard arrangement of test patterns fabricated in positive-tone resist AZ® 5214 on 4 inch silicon wafer.



Zoom-in on high-aspect-ratio grating fabricated in thick positive-tone resist AZ® 9260.



SEM micrographs of various 2D patterns in positive and negative tone photoresists fabricated with a Photonic Professional GT.

## Rapid Prototyping of Micro- and Mesoparts

Two-photon polymerization transfers the benefits of 3D printing to the microscale.

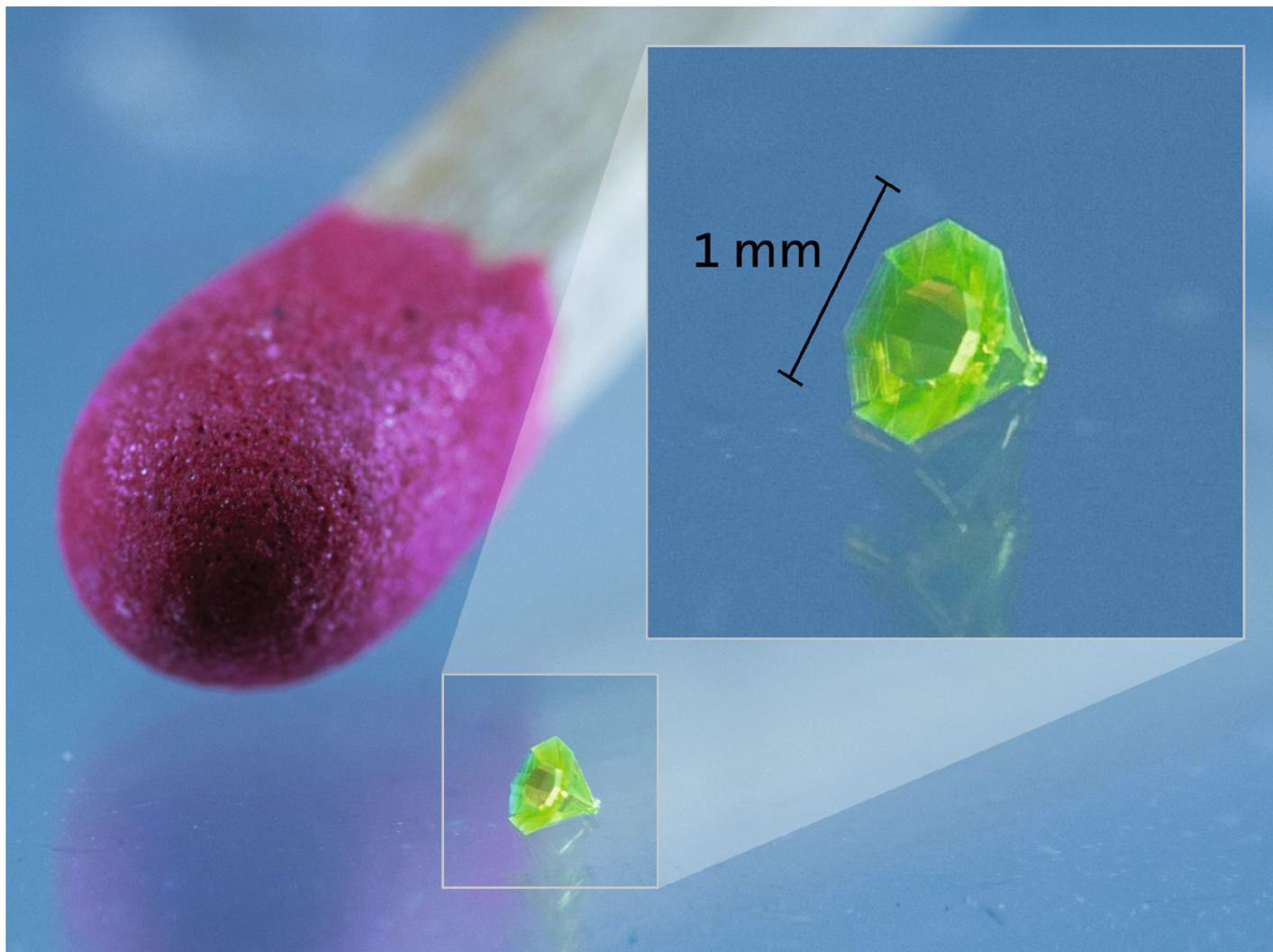


Figure (a): A prototype of a small nozzle for microfluidic applications.

Following the trend of miniaturization in a lot of application areas, 3D printing technologies for the microscale need to provide excellent resolution to allow for the fabrication of finest features.

Micro-sized parts have a great potential in a wide variety of applications like optics, photonics, biotechnology, life-sciences, microfluidics and many more. For applications where components and devices with feature-sizes on the micro/meso-scale ( $10^2 - 10^4 \mu\text{m}$ ) are needed, additive manufacturing based on two-photon polymerization provides the necessary resolution to transfer the benefits of 3D printing to this scale.

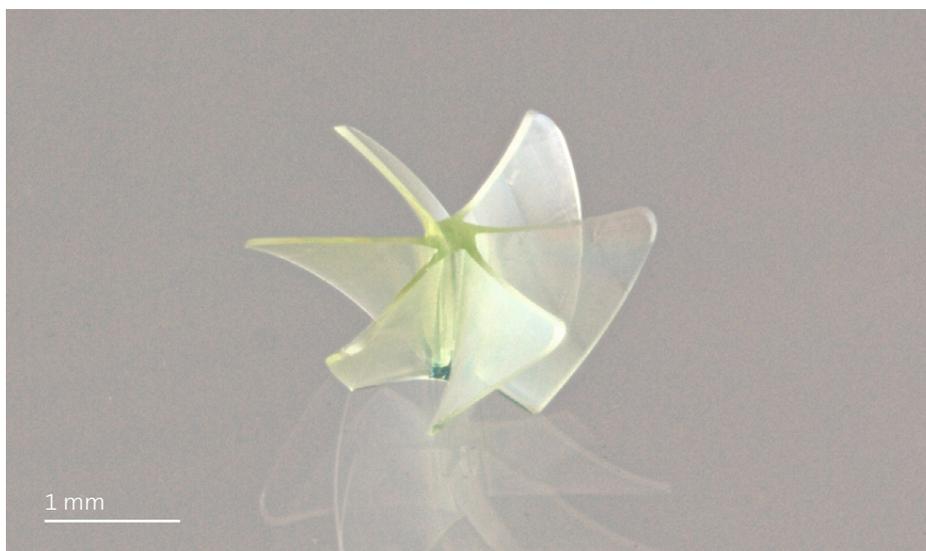


Figure (c): A mechanical mixer structure with filigree mixer blades.

In this image gallery, various micro-parts with a high shape- and functional complexity are shown. For microfluidics, the high-resolution additive manufacturing technology enables the fabrication of small nozzles (a, b) or mechanical microparts like stirrer elements (c).

Functional integration on the micro-scale can be used to build a gear with rotating elements in one production process available at your finger tips (d).

Even large structures can be realized in highest resolution within reasonable time. The miniature Eiffel Tower (img. 2) serves as a perfect demonstration of the superior quality. A total height of one millimeter allows to see this structure with the bare eye, as the photograph taken with a consumer DSLR and a macro objective documents. Only the SEM image of the very same structure (img. 2 (b)) reveals the fine details and proves the precision of the process.



Figure (b): This nozzle is mounted into a metallic syringe tip.

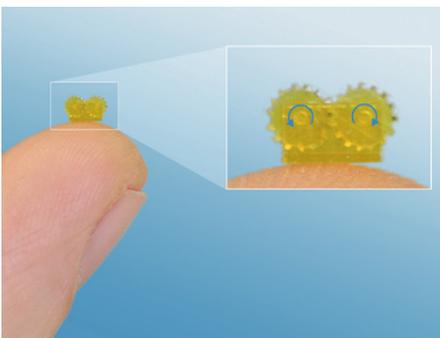
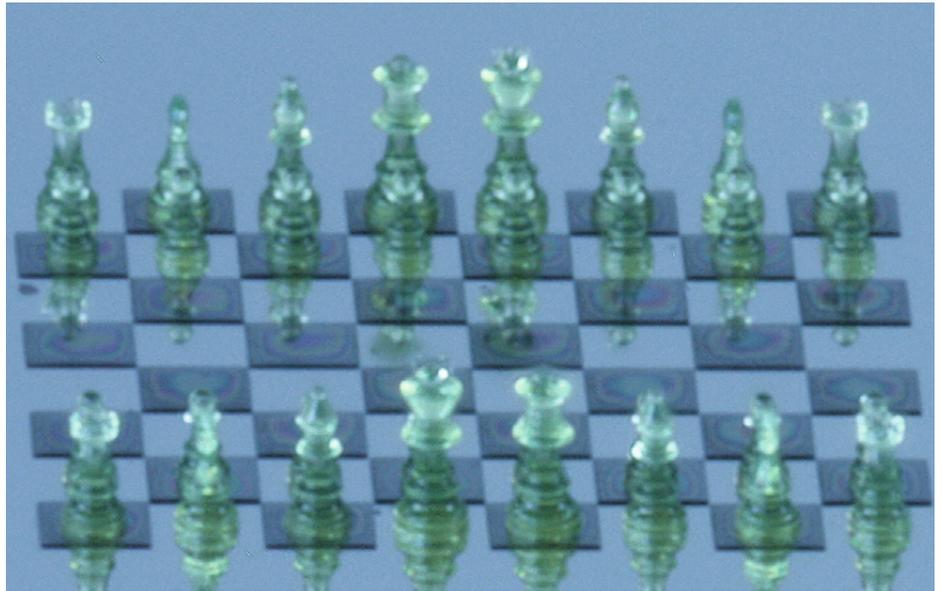
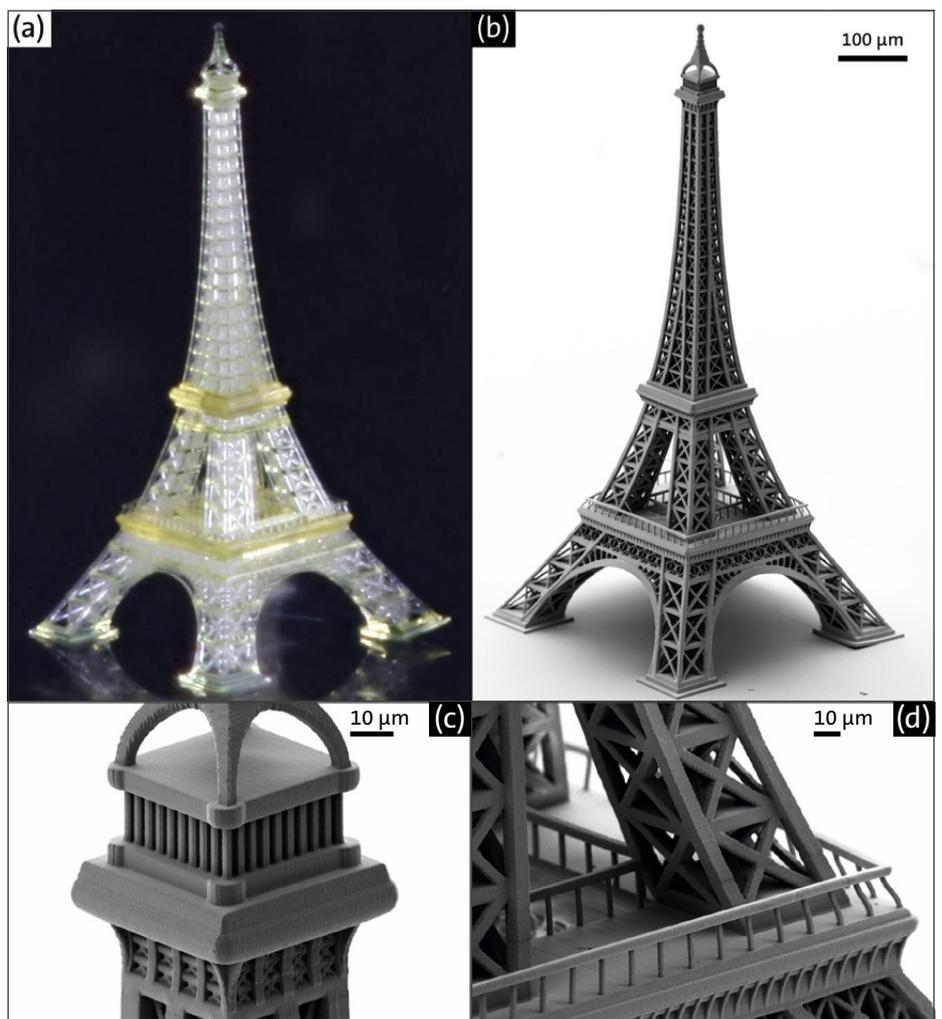


Figure (d): This micro-gear demonstrates functional integration enabled by the 3D printing process: The gears are freely movable.



Img. 1: The novel Photonic Professional GT system also enables printing of macro-objects, e.g., a chess board.



Img. 2: Did you know that there are more than 30 Eiffel Tower replicas around the world with different heights? Well, this one is definitely unique: Our miniature Eiffel Tower with a total height of 1 mm was 3D printed using the Photonic Professional GT combined with DiLL technology.

## 3D Photonics

### Semiconductors & metamaterials for light

After the invention of the laser in 1960, optics turned into photonics. In today's telecommunication technology, photons have already become the main carrier of information.

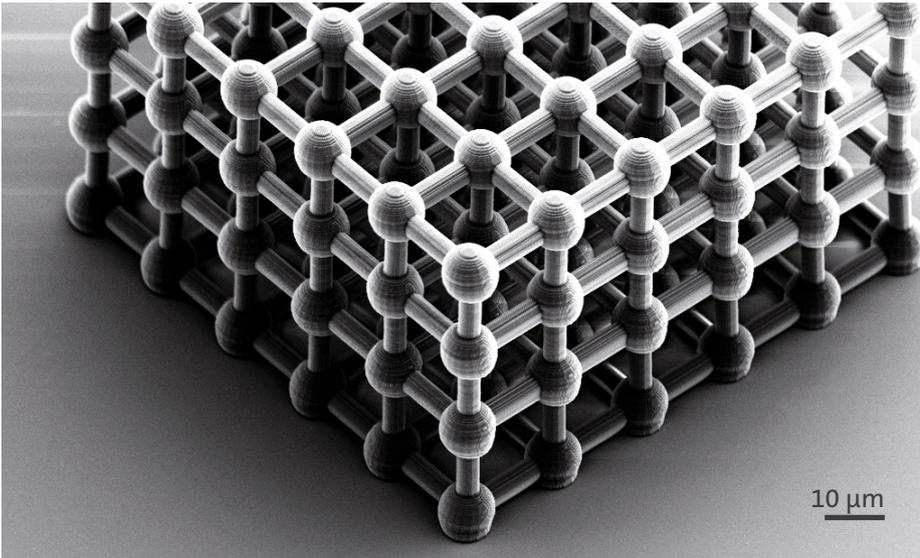
During the last decades, a number of pioneering developments such as, *e.g.*,

the concepts of photonic crystals and metamaterials have opened the door to a completely new class of materials.

Molding the flow of light as well as controlling the dynamics of photons are two key issues. The properties of

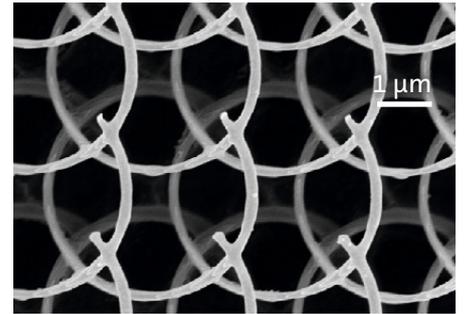
such nanostructured materials are subject of current research activities.

Shown is a series of structures fabricated by means of the direct laser writing process achieved with Photonic Professional GT systems.

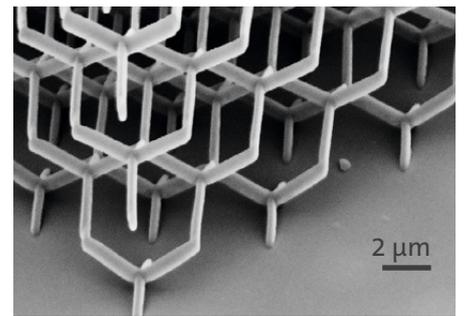


Above: Three-dimensional photonic crystal of cubic symmetry.

Right: 3D photonic crystalline diamond structure. With permission of Prof. Dr. Keiichi Edagawa, University of Tokyo.



Circular spirals seen from above.



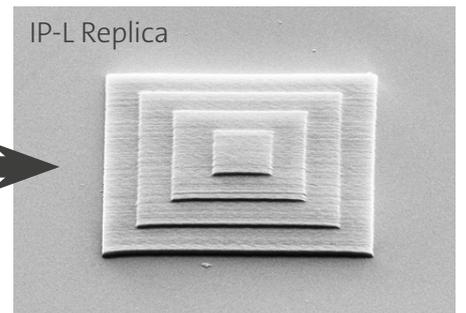
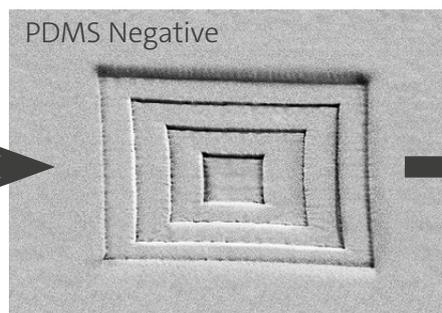
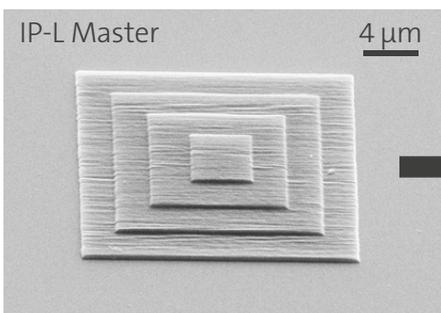
## Casting

### PDMS stamps for structure replication

Nanoscribe's Photonic Professional GT printers are the ideal tool for the fabrication of high-precision masters with a replicable surface topology. Once design requirements for replication are fulfilled, large-area 2.5D printed polymer structures can be reproduced

manifold times in different materials. The SEM micrographs below show an exemplary workflow: A master made of IP-L resist (left image) is casted into polydimethylsiloxane resist, Sylgard® 184 Silicone Elastomer (center image). Next, this negative copy is used as

a stamp to replicate the original structure (right image). Imprinting is the simplest technique that secures successful replication results for low quantity fabrication. Once it comes to mass production at low costs, injection molding is an ideal alternative.



A variety of casting techniques allow to transfer complex photoresist structures into materials such as metals, semiconductors, silica, silicon or PDMS.

## Photonic Wire Bonds

### A novel concept for chip-to-chip interconnects

(Nicole Lindenmann) The demand for ever higher data rates poses an increasing challenge for electrical interconnects. Fundamental limitations such as size, speed and crosstalk call for a radically new approach, especially with regard to interchip connections.

In this context, optical interconnects are considered a promising candidate to overcome communication bottlenecks in data centers and high-performance computers. However, while tremendous progress has been made in integrating optical transmitters and receivers on semiconductor chips, there is currently no technology at hand that can cope with these challenges beyond chip edges.

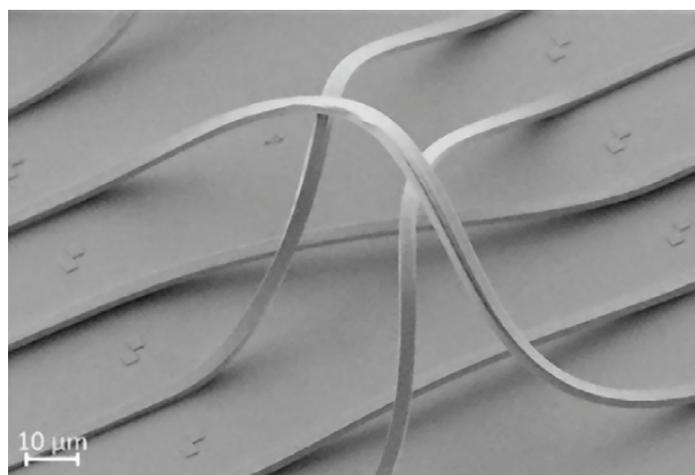
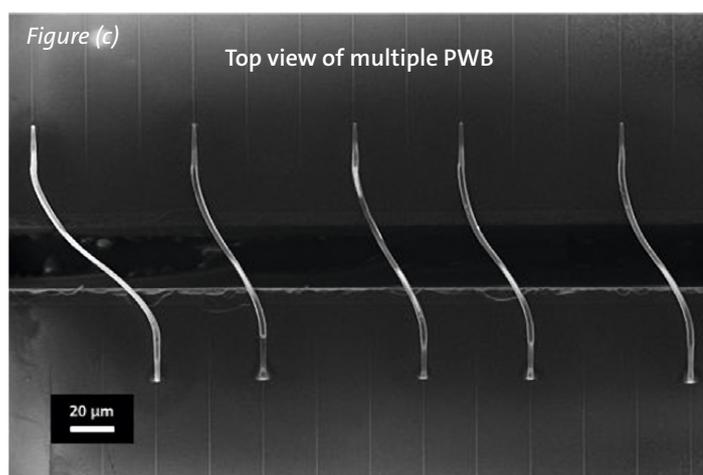
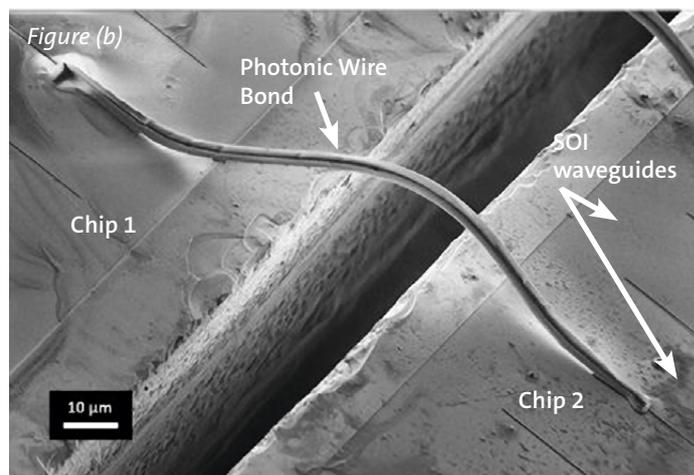
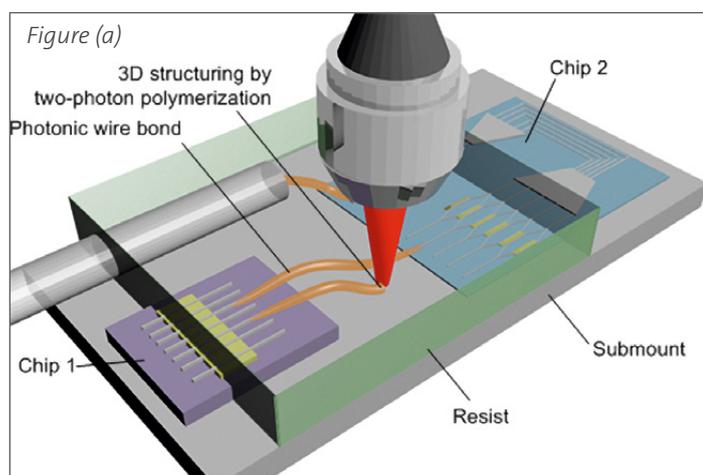
A group of researchers led by Prof. Christian Koos from Karlsruhe Institute

of Technology (KIT), Germany, has now demonstrated a photonic chip-to-chip interconnect, a Photonic Wire Bond (PWB), as they named it (1). This concept is illustrated in figure (a).

Taking advantage of live imaging and three-dimensional writing capability of the Photonic Professional, silicon-on-insulator waveguides on separate chips are connected by a freeform polymer PWB, figure (b). The structure is formed by tightly focused femtosecond laser pulses that expose the photoresist precisely along the computed trajectory. The shape of the wire bond is adapted to the position and orientation of the chips, rendering high-precision mechanical alignment unnecessary and bringing industrialscale application of PWBs into reach, see figure (c).

Proof-of-principle devices exhibit low losses at infrared telecommunication wavelengths around  $1.55\ \mu\text{m}$  and permit transmission of data rates exceeding 5 Tbit/s. This technological approach is considered a breakthrough in optical interconnects.

Nanoscribe is partnering with the group of Prof. Christian Koos in the government-funded project "Phoibos" (BMBF) together with - among others - IBM and Alcatel-Lucent. A first prototype of a photonic wire bonder machine has been developed and delivered by Nanoscribe.



All pictures: Courtesy of Prof. Christian Koos, Karlsruhe Institute of Technology (KIT/IPQ), Germany  
 Reference (1): N. Lindenmann, G. Balthasar, D. Hillerkuss, R. Schmogrow, M. Jordan, J. Leuthold, W. Freude, and C. Koos, "Photonic wire bonding: a novel concept for chip-scale interconnects," *Opt. Express* 20, 17667-17677 (2012).

## Unfeelability Cloak Protects the Princess from the Pea

Mechanical metamaterials open new dimensions

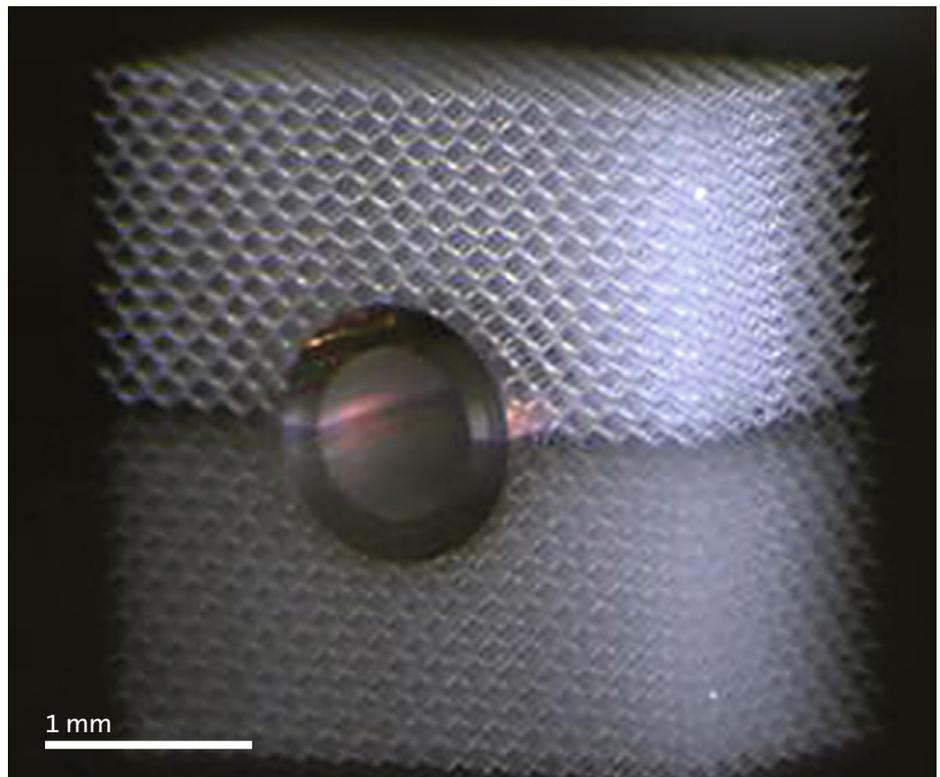
(Timo Bückmann) 3D galvo-scanner dip-in direct-laser-writing optical lithography using Nanoscribés Photonic Professional GT system allowed for the fabrication of mechanical metamaterial architectures with deep submicron features yet cubic millimeter overall volumes at the same time. Using these we have designed, fabricated and characterized polymeric core-shell elastostatic unfeelability cloaks composed of as many as 1024 extended face-centered cubic unit cells. The high precision of the fabrication was needed to adjust the mechanical properties of the surrounding and the cloaking shell to guide the forces around and let the solid cylinder vanish from being felt.

On the other hand the millimeter scale overall volume was essential to characterize the cloak optically and get in reach of possible applications.

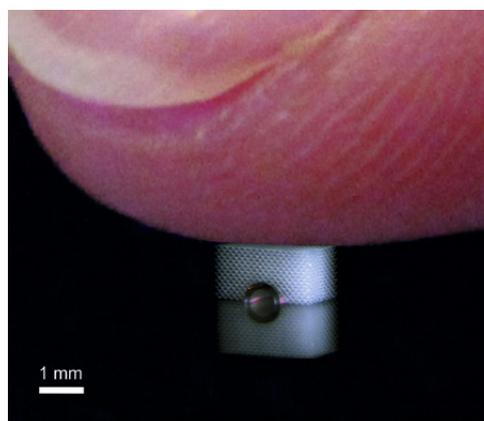
It is like in Hans-Christian Andersen's fairy tale about the princess and the pea. The princess feels the pea in spite of the mattresses. When using the cloak, however, one mattress would be sufficient for the princess to sleep well. The cloak shows that mechanical metamaterials are a growing field using direct laser writing as only here the true three dimensionality and interesting size scales can be covered opening the door for interesting applications.

To date, cloaking has been demonstrated experimentally in many fields of research, including electrodynamics at microwave frequencies, optics, static electric conduction, acoustics, fluid dynamics, thermodynamics and quasi two-dimensional solid mechanics.

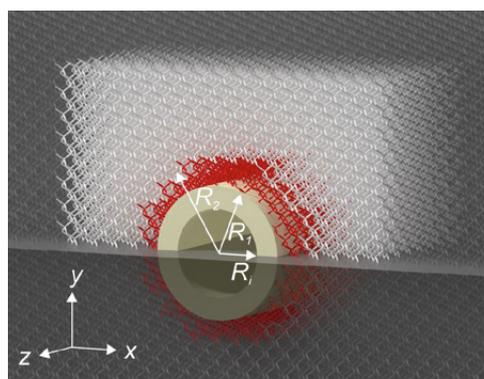
The results are now presented in the Nature Communications journal. Nat. Commun. 5, 4130 (2014)



Mechanical invisibility cloak. Metamaterials protect objects on the lower side from being felt. (Photo: T. Bückmann / KIT)



With the finger or a force measurement instrument, no information is obtained about the bottom side of the material. (Photo: T. Bückmann / KIT)



Design of the mechanical core shell cloak showing the red cloaking shell and the fabricated surrounding in white. (Image: T. Bückmann / KIT)

## 3D Printed Micro-Trusses

Strong as steel, but lighter than water

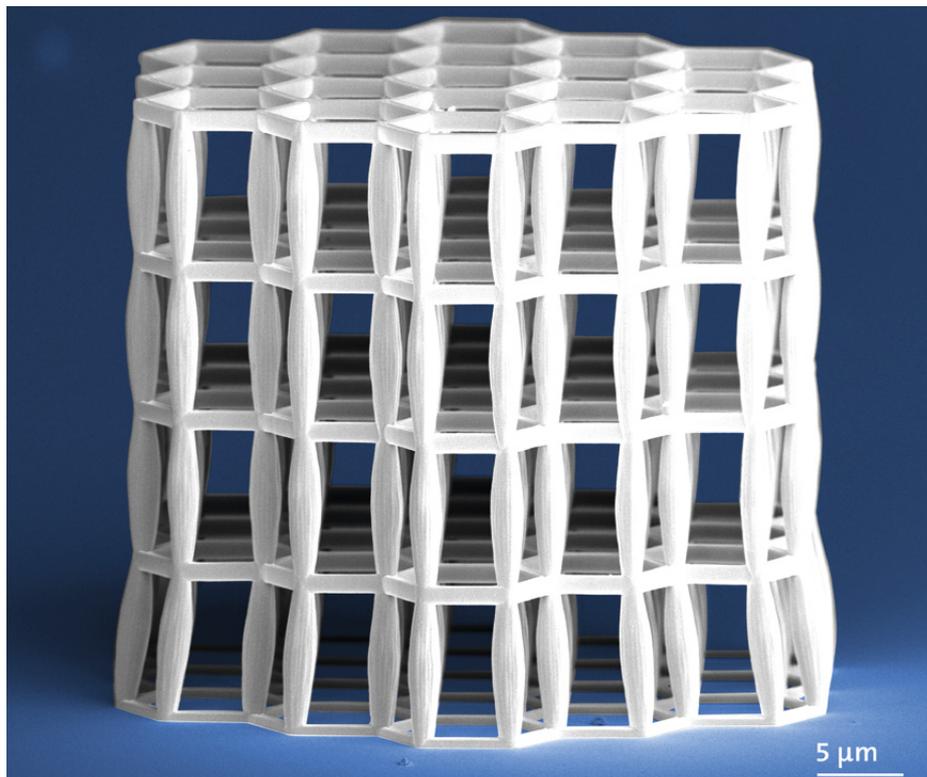
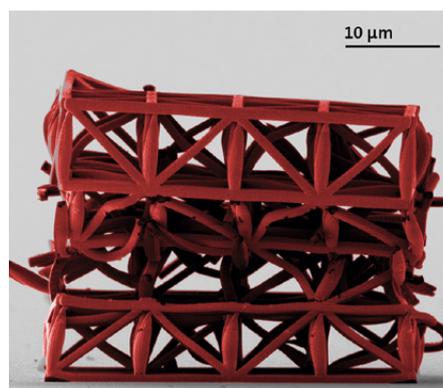
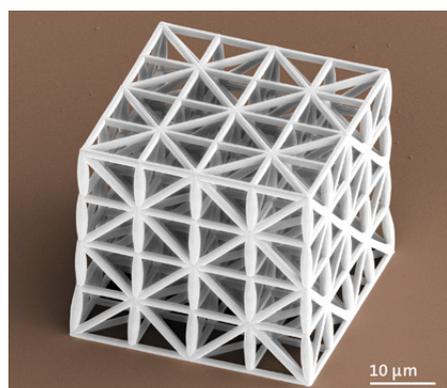


Fig.1 Hexagonal micro-truss structure (colorized SEM image). Compressive loads corresponding to  $550 \text{ kg/cm}^2$  may be carried, at a density less than the half of liquid water.

(Jens Bauer) Strong materials are heavy and light materials are weak. Strength and density, two materials properties of central relevance for engineering, are generally considered as strongly coupled. However, nature shows us how we may overcome long standing barriers on the search for light yet strong materials.

Containing several levels of hierarchical structuring from the macro- to

the nano-scale, certain porous biological materials such as bone and wood remain strong despite being extremely light, even though their basic material of which they are composed is generally considered anything but strong. Light man-made materials such as technical foams on the other hand, attain only limited mechanical properties compared with corresponding bulk materials. Foams are structured



randomly which is not weight efficient, with respect to strength. Cancellous bone and other natural cellular solids have an optimized architecture, designed adaptively to the loading situation.

On the lowest level of hierarchy bone consists of nanometer-size building blocks, additionally providing strongly enhanced material strength because of mechanical size effects.

Designing cellular materials with a specific microarchitecture allows one to exploit both structural advantageous mechanisms and size-dependent strengthening effects.

Applying 3D direct laser writing microarchitected lightweight materials from ceramic-polymer composites have been fabricated (Fig.2) and mechanically characterized (Fig.3). Exceeding all technical and natural materials with a density below  $1000 \text{ kg/m}^3$  as well as most metallic alloys, ratios of strength-to-weight comparable to high-performance steels and technical ceramics are reached (Fig.1).

Publication: PNAS (2014), doi: 10.1073/pnas.1315147111

Fig.2 left: Micro-truss structure from ceramic-polymer composite (colorized SEM image), fabricated using 3D-DLW and atomic layer deposition. The miniaturized, specifically designed architecture allows benefiting from both structural advantages and size-dependent material strengthening effects.

Fig.3 right: Deformed structure after uniaxial compression (colorized SEM image). Initial failure leads to a stackwise collapse. (Images: Jens Bauer / KIT)

## Additive Manufacturing for Microfluidics

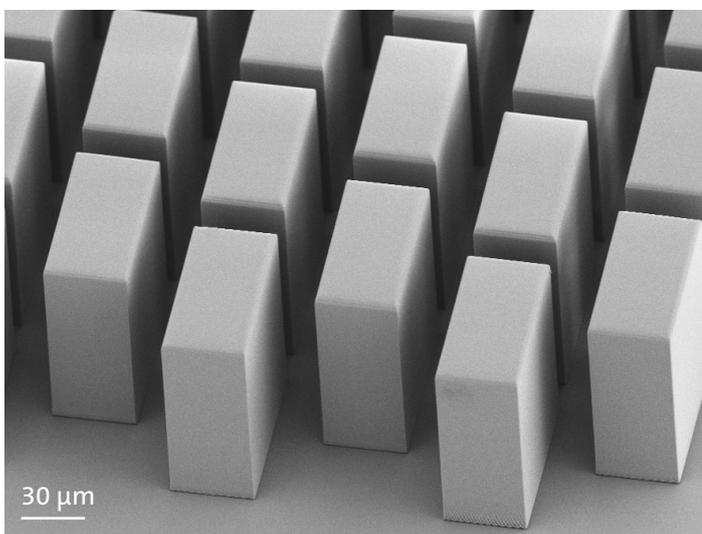
### Microfluidic applications benefit from highest resolution 3D printing

The precise control and manipulation of liquids in very small volumes is the essence of the multidisciplinary field of microfluidics. Typical devices rely on components like channel systems, connectors and 3D mixer elements to passively guide the flow of liquids. Active microfluidic components in contrast aim to actively manipulate the movement of the fluids by means of micro pumps or valves. To fabricate these components, a broad range of micromanufacturing technologies is

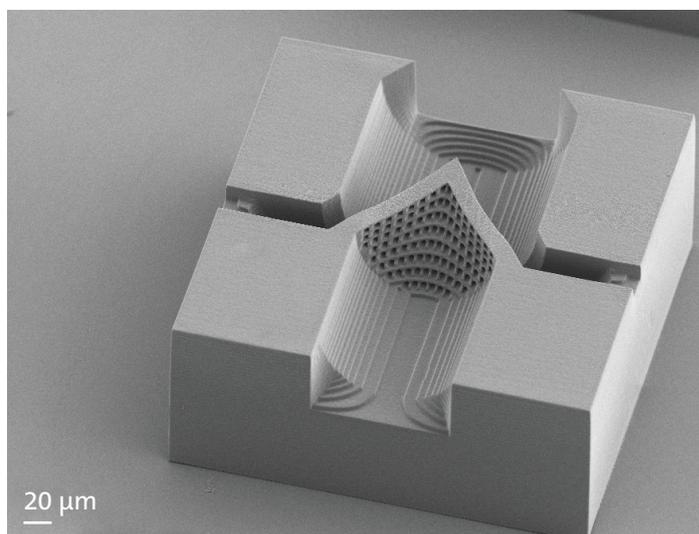
available today. However, the majority of these technologies are based on 2D and 2.5D manufacturing capabilities. Recently, 3D printing technologies attract an ever increasing interest for micromanufacturing of microfluidic applications.

Two-photon polymerization is the 3D printing technology with the highest resolution available today and is compatible with a broad range of polymers well suited for microfluidic applications. It transfers the benefits

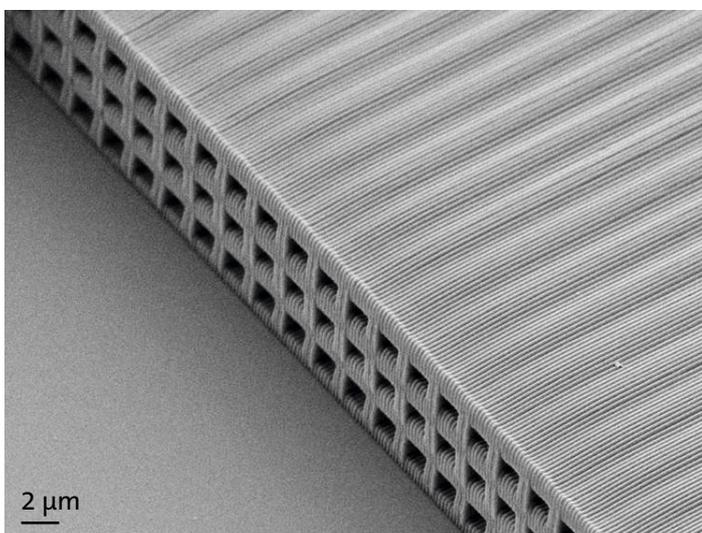
of additive manufacturing to the microscale and allows the fabrication of complex, arbitrarily shaped 3D structures in a single step. In addition, due to its process compatibility with a broad range of substrates, two-photon polymerization is well suited to be combined with other conventional silicon-based micromanufacturing technologies thereby leveraging the advantages or different technologies.



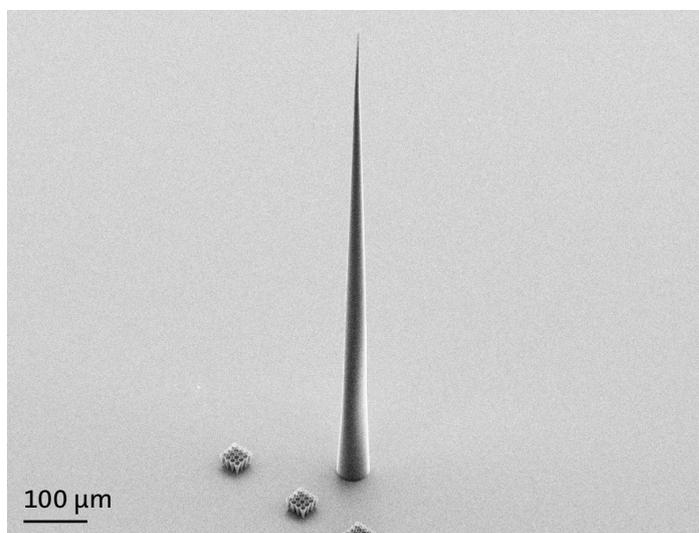
High-aspect-ratio blocks for the implementation of a capillary pump system.



Microfluidic filter element structured in SU-8 (Design provided by IMSAS).



Close-up of a microfluidic filter element



970 μm tall micro-needle polymerized by two-photon absorption

# Magnetic Helical Micromachines

## Fabrication, controlled swimming, and cargo transport

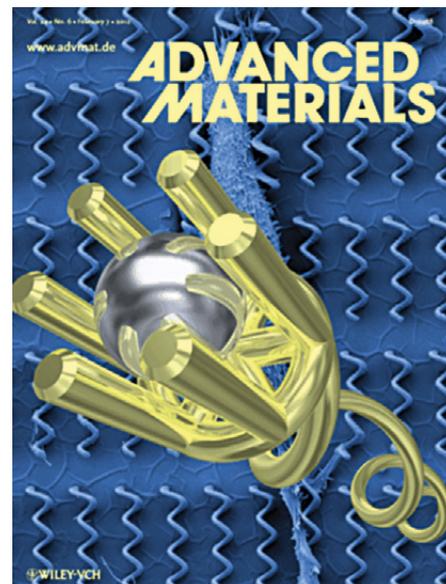
(Li Zhang) Helical micro-swimmers inspired by bacterial flagella have been successfully realized by researchers surrounding the group of Brad Nelson at the ETH Zürich.

Making use of the flexibility of the Photonic Professional 3D direct laser writing system micron-sized helices with and without microholders were written and subsequently coated with ferromagnetic nickel (figure 1, 2). In order to enable the use in biological applications, the surface was furthermore coated with a thin titanium layer in order to reduce potential cytotoxic effects. Thus, corkscrew-like motions in liquids were driven by a rotating

magnetic field at rotating frequencies of several 10 Hz at a few mT, enabling accurately steerable movement in 3D with velocities up to several 100  $\mu\text{m/s}$ .

This additionally was topped by cargo transport where microspheres were loaded, transported and unloaded from a basket (figure 3, 4).

No doubt: The researchers obviously enjoy that topic spending late hours in the lab – as seen in the time stamp of the recorded videos! Who would prefer going home to your playstation if you have such a research-tool in your lab?



Advanced Materials, Volume 24, Issue 6, 811–816 (2012), © Wiley-VCH Verlag GmbH & Co. KGaA, reproduced with permission.

Figure (1)

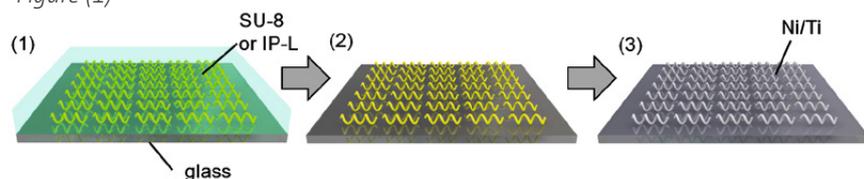
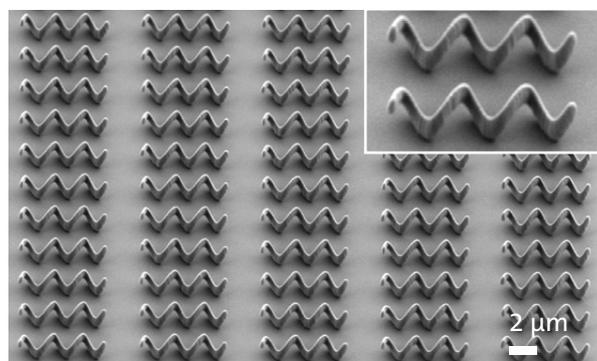


Figure (2)



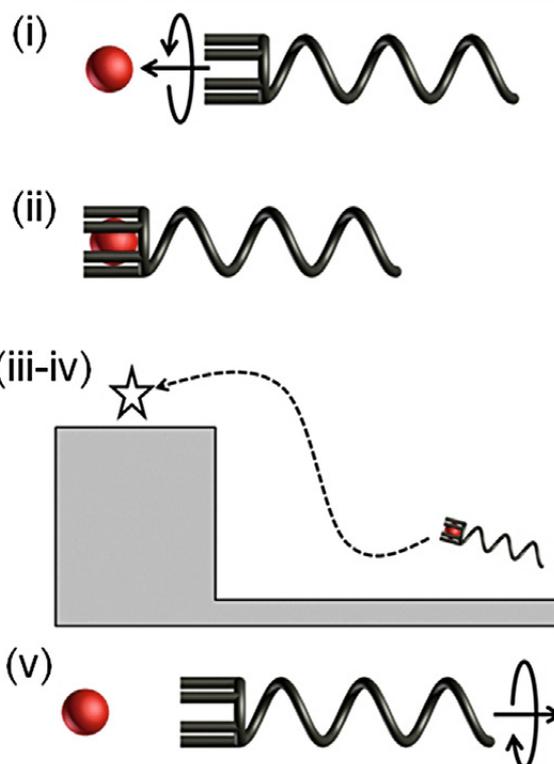
Images by courtesy of ETH Zürich

Figure (4)



[www.youtube.com/watch?v=sJP4rL57Dq8](http://www.youtube.com/watch?v=sJP4rL57Dq8)

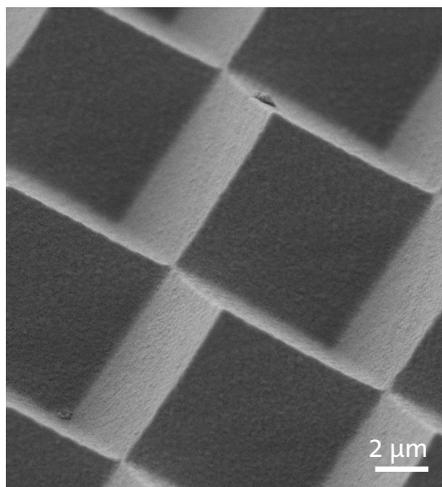
Figure (3)



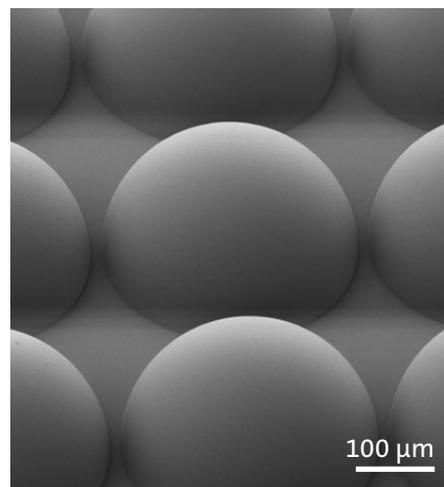
## 2.5 D Surfaces

### Flexibility and freedom in design

Closed surfaces of complex shapes are desired for many optical applications where the steering of light is achieved either by refractive, diffractive or combinations of both - hybrid optics. 3D laser lithography provides the full design freedom for closed, replicable surfaces - either as prototypes or as masters for mass fabrication via imprinting or injection molding. In contrast to diamond milling and gray scale lithography, the design freedom of two-photon polymerization goes beyond their limitations while providing optical quality surfaces: Patterns, slopes or shapes can be freely chosen. Even freeform surfaces are possible along a simple and reliable workflow.



Corner-cube retroreflector.



This array of micro-optics semispheres was directly fabricated with a Photonic Professional GT.

## Simple Transmission Phase Gratings Make Colour Filters in the Visible

### Current research of a Nanoscribe customer

(Piotr Wasylczyk) Optical transmission color filters can generally be realized using absorption, interference or diffraction.

In a series of experiments based on this last approach, researchers from Photonic Nanostructure Facility (PNaF), led by Piotr Wasylczyk, at the Faculty of Physics, University of Warsaw, fabricated and characterized diffraction structures in the form of a regular grid of pillars.

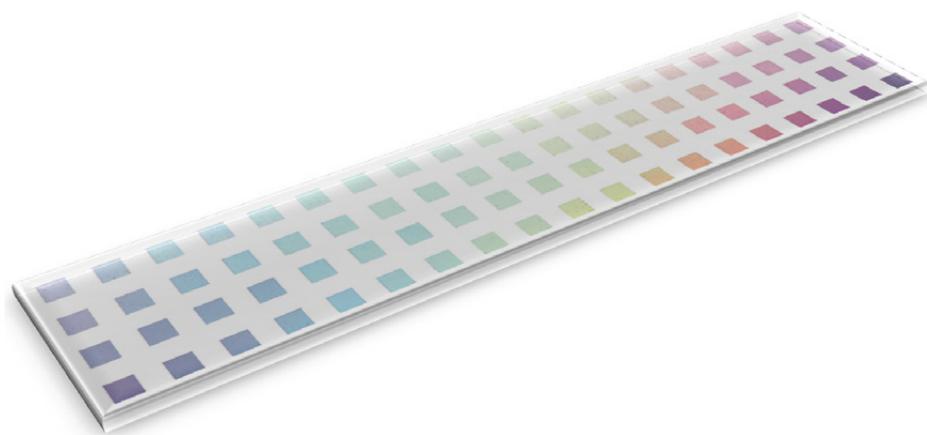
Such “forest of columns” as it has been nicknamed, can generate a significant

range of hues in white light transmission due to colour-dependent diffraction. By designing the structure topography, the wavelength-dependent diffraction efficiency can be tuned, resulting in the desired light transmission profiles. Red, green and blue filters have been demonstrated and the experimental results compared with numerical simulations of light propagation in the dielectric microstructures.

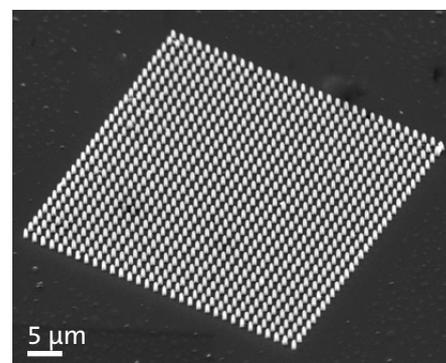
The simple phase diffraction gratings offer several advantages compared to other light filtering technologies: Not

only can they be mass-produced using replication, but also high resolution spatial patterning can be achieved as well as straightforward integration into micro-optical devices.

This research has been supported by National Science Center (Poland) within the DEC-2012/05/E/ST3/03281 grant funds and by ERDF within the POIG.02.01.00-14-122/09-00.



Computer graphic based on a white light microscope showing a range of colours available for the pillartype gratings.



SEM image of the pillar grating. More than thousand of 1.5 micron high polymer micropillars cover the  $38 \times 38 \mu\text{m}^2$  area. Publication: *Optics Express*, 21, 31919 (2013).

## Application Perspective for Maskless Lithography

### Anti-counterfeit features

Counterfeiting of goods has huge economic consequences with an estimated global loss of \$654 billion per year. This negative economic impact hits many different product categories ranging from medicine, electronics and watches to counterfeit documents like money and IDs. [1]

To fight product counterfeiting, manufactures nowadays have many anti-counterfeit technologies available. A whole industry is dedicated to provide security features that enable the authentication of products and deter product imitators based on difficulty or costs to replicate these features.

Most common features today belong to the class of security level 1. These overt features can be authenticated by naked eye or tactile sense therefore being user verifiable without the need of additional tools. [2] Most prominent examples known to the public from many banknotes found around the world are holograms, color-flipping or fine-line prints. Recent developments incorporate transparent windows into polymer banknotes that contain a diffractive optical element. [3]

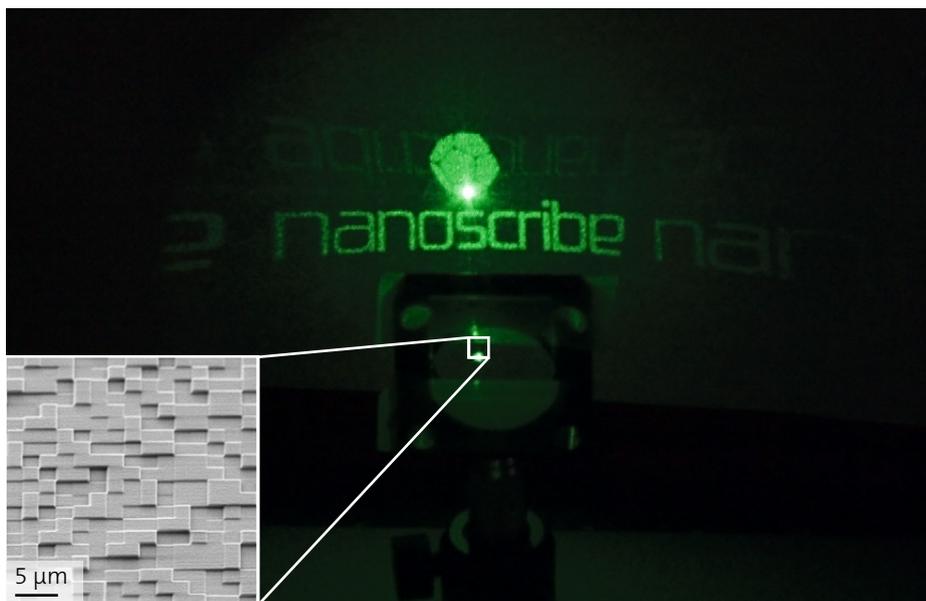


Fig. 2 Diffraction pattern after passing through a polymer diffractive optical element directly fabricated with a Photonic Professional GT.

When illuminated with coherent light from a laser pointer, a previously hidden image can be projected on a flat surface for authentication.

Fabrication of these features requires constructing a master using high-resolution lithography methods as a first step. This master is finally mass-replicated using stamping

or roll-to-roll fabrication to get the security labels. Nanoscribe's high-resolution 2D and 2.5D patterning capabilities enable the mastering of high-resolution overt features like fine-line prints, diffractive color prints or diffractive optical elements in one lithography step.

The examples of the photonic color image [fig. 1] and the diffractive optical element [fig. 2] projecting our company logo were fabricated using a Photonic Professional GT to demonstrate the feasibility for these applications. For further questions on this topic, please contact us via [info@nanoscribe.com](mailto:info@nanoscribe.com).



Example of a colorful QR code consisting of diffractive dot arrays.

[1] <http://www.havocscope.com/category/counterfeit-goods/>

[2] Anti-counterfeit Technologies for the Protection of Medicines <http://www.who.int/impact/events/IMPACT-ACTechnologiesv3LIS.pdf>

[3] WinDOE™, [http://www.polymernotes.de/files/WinDOE\\_Flyer\\_secure.pdf](http://www.polymernotes.de/files/WinDOE_Flyer_secure.pdf)

## Cells Conquer the Third Dimension

### Cell cages for tissue engineering

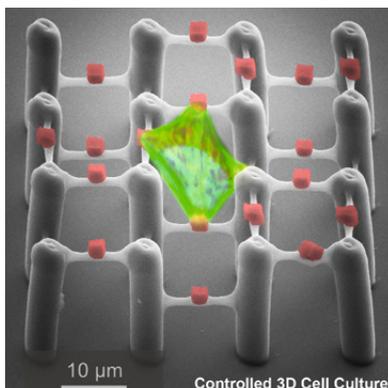
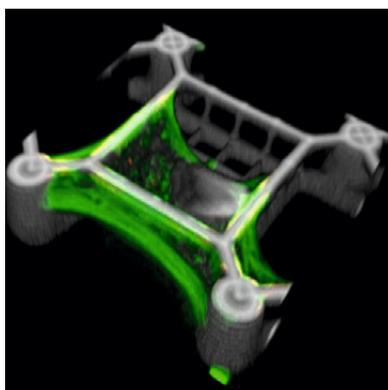
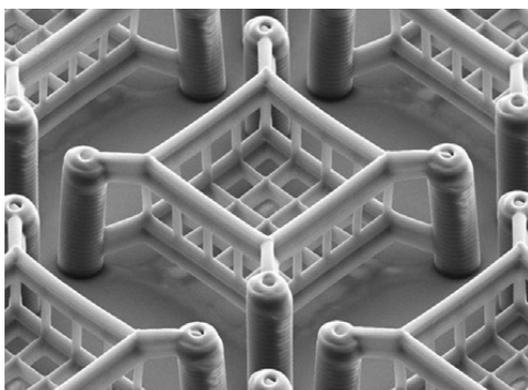
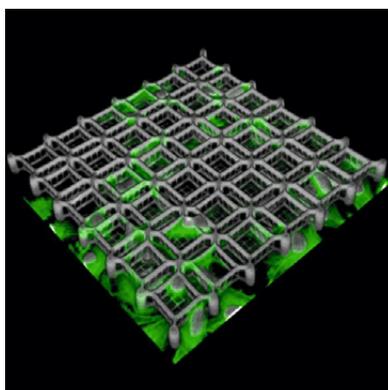
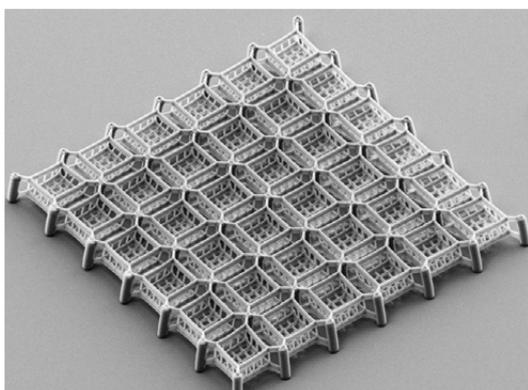
In the last decades cells have been intensively studied by biologists in artificially structured two-dimensional environments. In order to shine even more light onto their behavior in a rather natural environment 3D scaffolds were created by means of Nanoscribe's 3D printers.

In one of Nanoscribe's news letters chicken heart cells beating in a 3D scaffold were presented as an achievement of the group of Prof. Martin Bastmeyer at the Karlsruhe Institute of

Technology (KIT), Germany, revealing the force of individual cells exerted in a 3D topology.

In the same group different photoresists were combined to create a fully functional 3D environment to control the growth of single primary fibroblasts. First a mixture of PEG-DA and PETA was used as a photoresist to create a stable core scaffold which is non protein binding. After development and refilling with the photoresist Ormocomp®, protein binding islands/

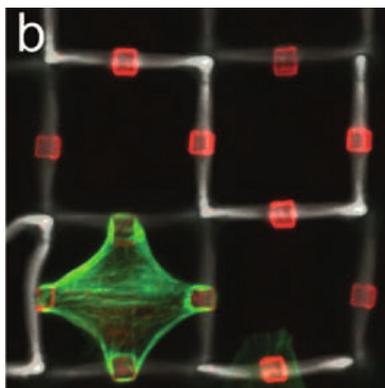
satellites were created by polymerization. After coating these islands with a protein that promotes cell adhesion, cells make contacts at these special sites only. Thus, for the first time a two-component functional scaffold for cells was 3D printed.



SEM images of cell scaffolds. White: structure/cell core; green: actin cytoskeleton. F. Klein et al., *Advanced Materials* 23, 1341 (2011), "Two-Component Polymer Scaffolds for Controlled Three-Dimensional Cell Culture".

By courtesy of Prof. Dr. Martin Bastmeyer, Benjamin Richter, Karlsruhe Institute of Technology (KIT), Germany

Primary chicken fibroblasts adhering to protein-binding Ormocomp cubes. KIT/CFN, Prof. Dr. Martin Bastmeyer, F. Klein et al., *Adv. Mater.* 23, 1341 (2011)



## Bio-Inspired Osteoprints for the Enhancement of the Osteogenic Differentiation

### Tissue engineering via two-photon polymerization

(Attilio Marino) Several biophysical investigations suggest that a peculiar cell behavior can be in vitro resembled by mimicking the corresponding in vivo conditions. [1] In order to in vitro mimic the 3D natural microenvironment of the bone tissue we prepared, thanks to the slice-by-slice two-photon polymerization (2PP) approach, trabecula-like structures (named “Osteoprints”) that resemble the typical microenvironment of trabecular bone cells. [2]

Particularly, the 3D model of the Osteoprint was obtained from X-ray micro-computed tomography ( $\mu$ -CT) scans of a human trabecular bone biopsy, taken from the femoral neck (Fig. 1). Osteoprints were subsequently fabricated through 2PP of the biocompatible ceramic photopolymer Ormocomp<sup>®</sup>, the mechanical properties of which are similar to that of the natural bone. SEM imaging (Fig. 2) of the obtained Osteoprints reveals a shape and size exactly resembling those of the corresponding 3D  $\mu$ -CT model, thus demonstrating the high resolution and reliability of the 2PP technique.

After an extensive Osteoprint characterization, the adhesion, proliferation and differentiation of SaOS-2 osteoblast-like cells were studied on the obtained scaffolds.

The presence of the Osteoprints was able to deeply affect cell behavior, promoting the cell cycle exit and the osteogenic differentiation. An

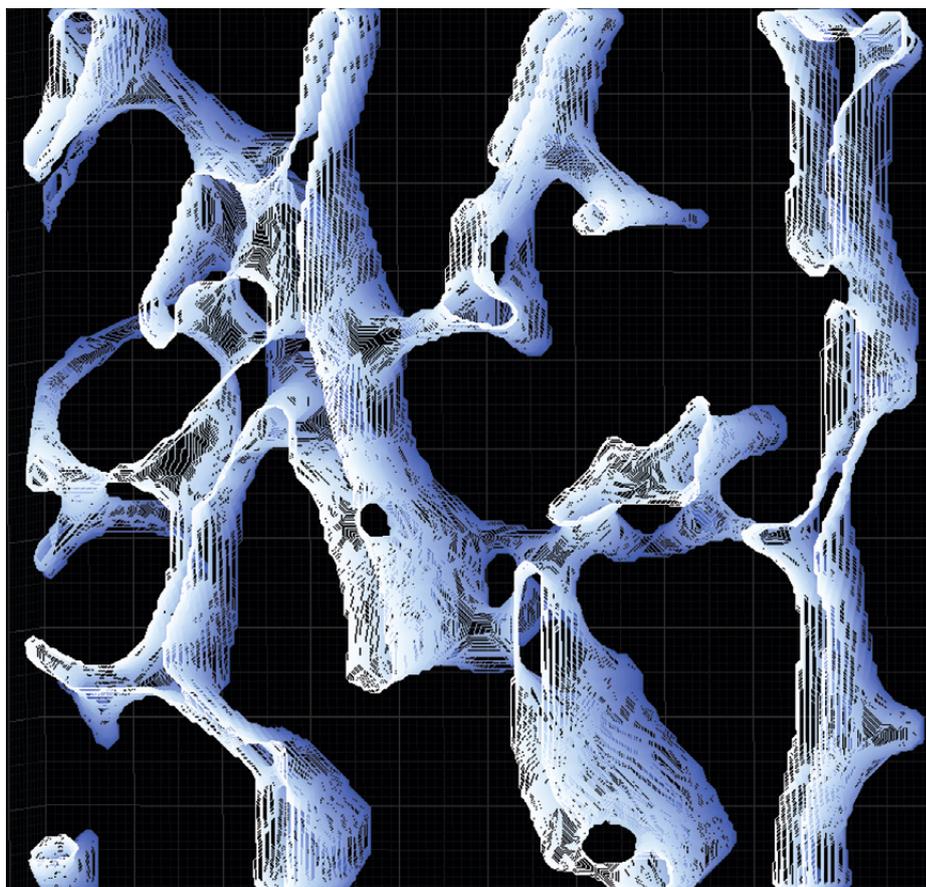


Fig. 1: 3D reconstruction of bone trabeculae obtained by  $\mu$ -CT scans of a biopsy from the human femoral neck.

up-regulation of the genes involved on osteogenesis and an enhancement of the hydroxyapatite nodule production were the major outcomes (Fig. 3).

These results encourage the exploitation of the 2PP for obtaining 3D biomimetic structures, useful for a wide

range of in vitro application and even in tissue engineering and regenerative medicine. [3]

*This article was provided by Nanoscribe's customer, the Italian Institute of Technology, Center for MicroBioRobotics @SSSA.*



Fig. 2: SEM imaging of an Osteoprint prepared by slice-by-slice 2PP of the hybrid ceramic Ormocomp<sup>®</sup> photoresist.

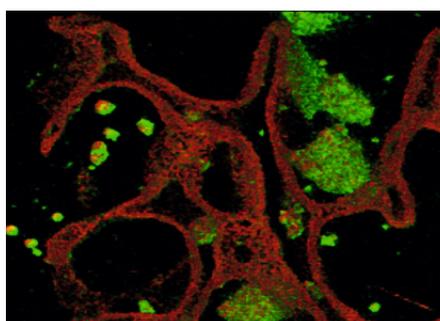


Fig. 3: 3D reconstruction of a bone cell culture on the Osteoprint (in red), highlighting the deposition of several hydroxyapatite nodules (in green).

#### References:

- [1] A. Marino, C. Filippeschi, V. Mattoli, B. Mazzolai and G. Ciofani, *Nanoscale*, 2015, 07, 2815–3320.
- [2] A. Marino, C. Filippeschi, G. G. Genchi, V. Mattoli, B. Mazzolai and G. Ciofani, *Acta Biomater.*, 2014, 10, 4303–4313.
- [3] S. D. Gittard, A. Koroleva, A. K. Nguyen, E. Fadeeva, A. Gaidukeviciute, S. Schlie-Wolter, R. J. Narayan and B. Chichkov, *Front. Biosci. Elite Ed.*, 2013, 5, 602–609.



**nanoscribe**

High Precision 3D Printing Solutions

**Nanoscribe GmbH**

Hermann-von-Helmholtz-Platz 1  
76344 Eggenstein-Leopoldshafen  
Germany

Phone +49 721 981 980 0  
Fax +49 721 981 980 130  
E-Mail [info@nanoscribe.com](mailto:info@nanoscribe.com)  
Web [www.nanoscribe.com](http://www.nanoscribe.com)



Founded in 2007 as a spin-off from the Karlsruhe Institute of Technology, Germany, and as a pioneer in the field of two-photon polymerization, Nanoscribe has established itself globally as market and technology leader in 3D printing on the nano-, micro- and mesoscale. Today it is ranked among the most successful young medium-sized companies in Germany.

Top institutions in academia as well as pioneers in industry in more than 30 countries worldwide already successfully use this new, award-winning standard for microfabrication.

On our website, you can find a multitude of examples for the broad range of applications as well as a long list of scientific papers published by our users. The close contact to our customers is supported by a worldwide sales and service network. Rapid and first-class customer service is a matter of course for us.