High-Throughput Parallel Digital Light Processing 3D Bioprinting on the Droplet Microarray

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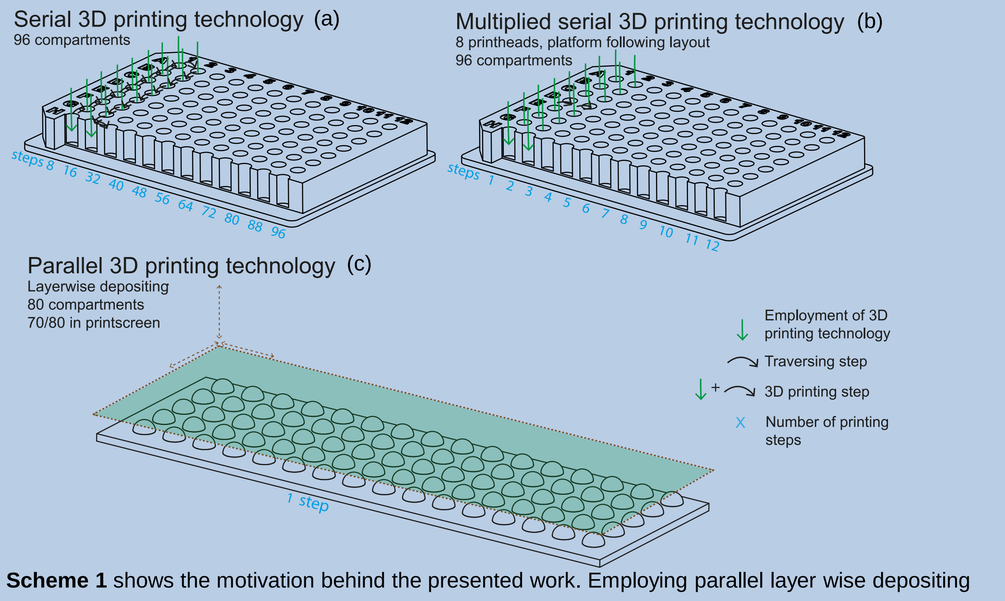
3D MATTER MADE TO ORDER

Karlsruher Institut für Technologie

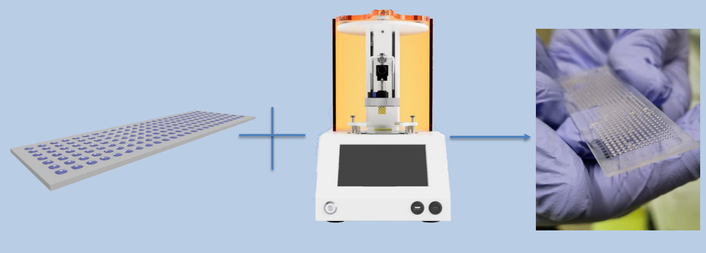
Motivation



Scheme 1 shows the motivation behind the presented work. Employing parallel layer wise depositing 3D printing technology on a platform capable of compartmentalizing liquids into small volumes (c) allows a reduction of the printing time into a fraction compared to singular serial line wise depositing (a) or multiplied serial 3D printing technology (b). The serial technology was needed because of the physical walls required for compartmentalization. The Droplet Microarray (DMA) compartmentalizes liquids into stable droplets based on its functionalized surface with locally different wettability without needing physical walls. These droplets can then fully immerse the printed hydrogel structures.

Goal

Enable parallel Digital light processing (DLP)-technology on the DMA

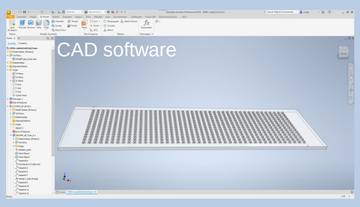


Scheme 2: The chosen Droplet Microarray is type 201 with 80 spots from Aquarray. A system was developed making parallel DLP 3D printing with the Cellink LumenX+ employable on the DMA.

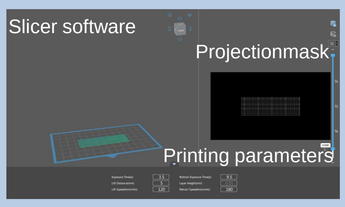
Methods / Approach

Pre-processing

1. CAD-model creation

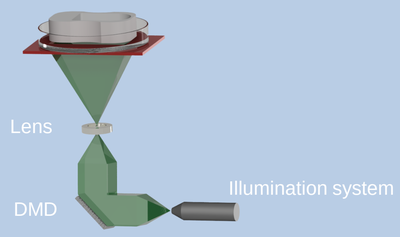


2. Printing parameter definition



3D-printing

3. Projection lithography with 405 nm light



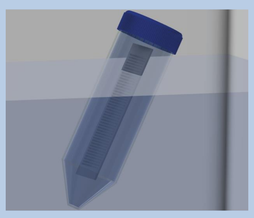
Digital Light Processing (DLP) System:

Projection system:
Digital micromirror device (DMD)
(De-) Magnification lens

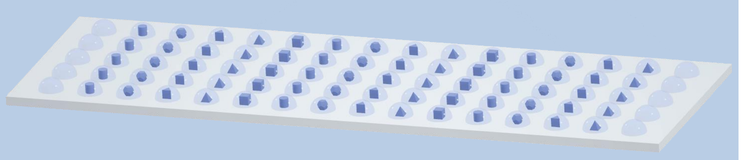
Illumination system:
Light source
Reflector
Collimator

Post-processing

4. Washing



5. Finished 3D hydrogel structures in compartmentalized liquids



Scheme 3: The Workflow of 3D (bio-) printing starts with the pre-processing. This includes the creation of the 3D models under consideration of the imposed constraints. Next the printing parameters such as exposure time and layer height need to be defined. Then the printing process can commence. The dynamic photomask generated based on the 3D data of the structures to be printed exposes the hydrogel ink and the crosslinking photopolymerization is performed in all hydrophilic spots in the projection plane at once, layer by layer. After printing, the uncrosslinked ink is washed away, revealing the structures printed on the hydrophilic spots, which can still compartmentalize liquids.

Results

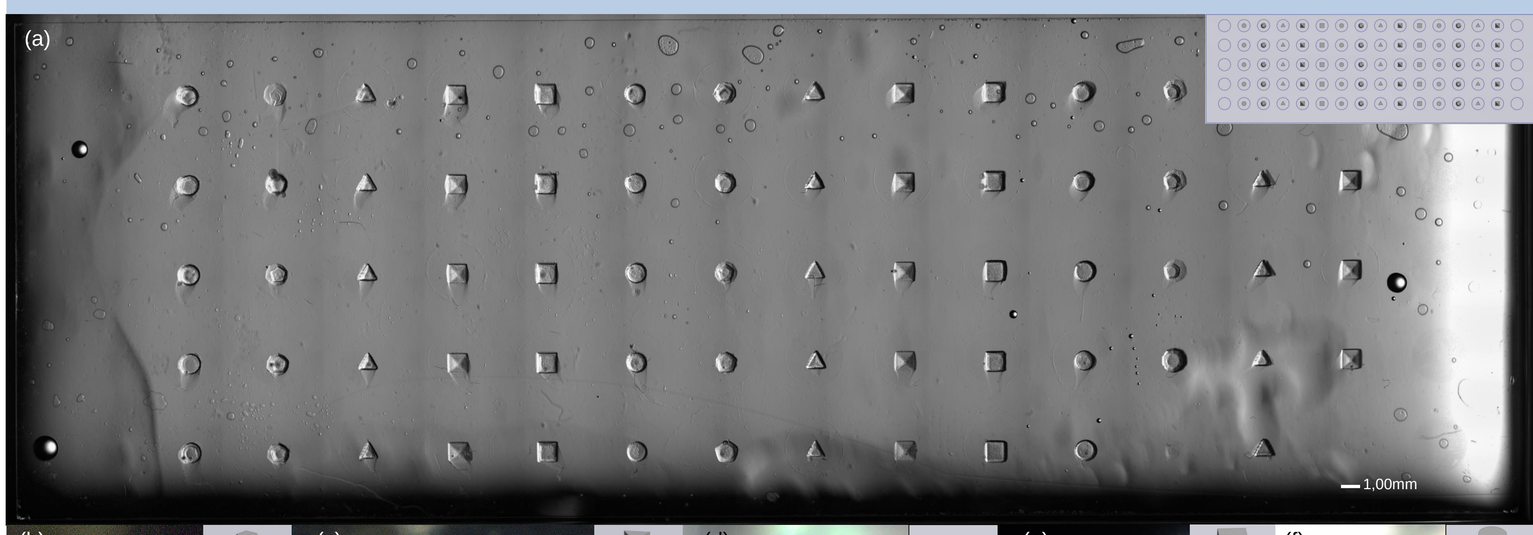


Figure 1: 70 3D structures of 5 different geometries printed on a DMA with 80 hydrophilic spots. The hydrogel ink is composed of gelatin-methacryloyl (GelMA) with a concentration of 10 % (w/v). As photoinitiator lithium-phenyl-2,4,6-trimethylbenzoylphosphinat (LAP) was used at a concentration of 0,25% (w/v). The array of 70 3D structures of 1mm height with a layer height of 50 µm is printed in 6 min 10 sec. The 5 different 3D geometries are dodecahedrons (b), triangular prisms (c), pyramids (d), cubes (e) and cylinders (f) each with an edge length of 1 mm and a height of 1 mm printed with layer height of 50 µm.

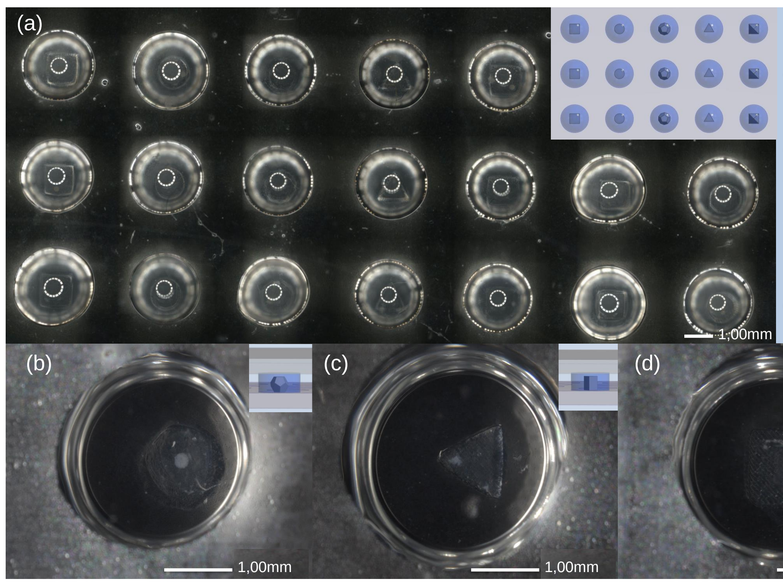


Figure 2: GelMA hydrogel structures in compartmentalized liquid on a DMA after printing. PBS was dispensed with the Gyger Certus Flex in each hydrophilic spot. The printed structures are completely immersed in the liquid (a). The menisci of the droplets reflect the incident light from the microscope. To get a better view of the structures inside, another slide was placed over the printed structures touching the droplets (b-f), showing the 3D structures immersed within the liquid volumes of the droplets. The compartmentalization capabilities of the DMA are unaffected by the 3D printing process.

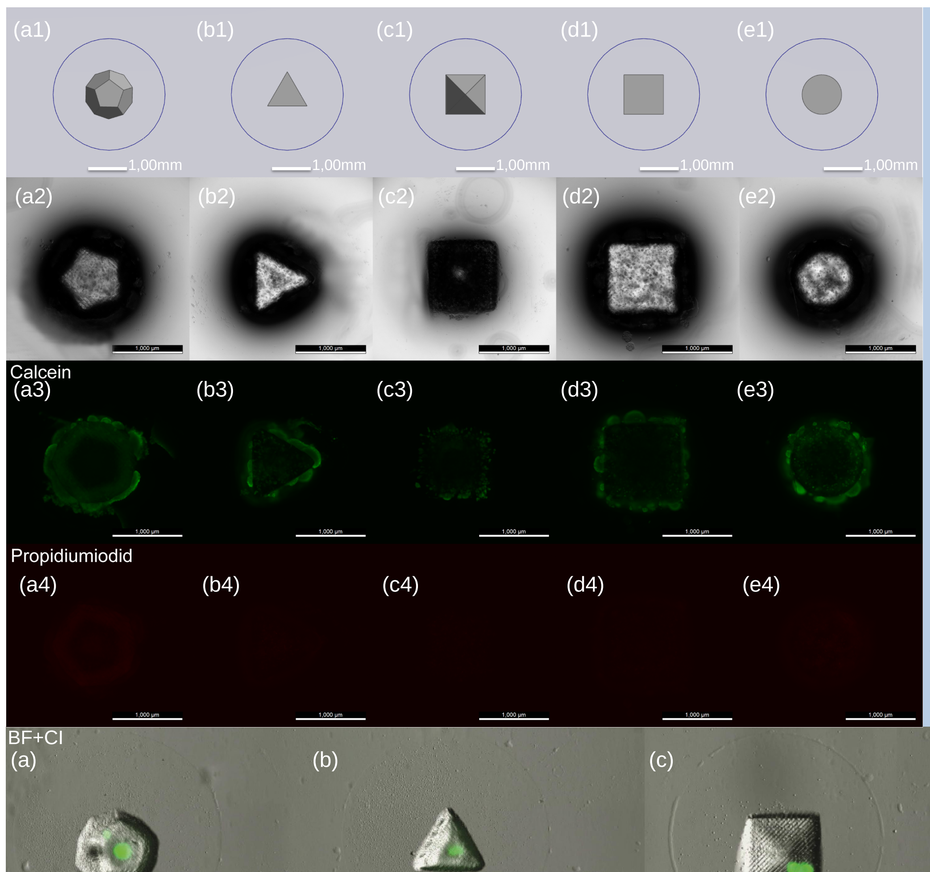


Figure 3 Combining the GelMA photoink with dispersed HEK 293 cells for 3D bioprinting of the 5 different geometries (a1-e1) did not affect the printability of the structures (a2-e2).The use of fluorescent markers in the droplets around the structures such as calcein (CI) (a3-e3) and propidium iodid (PI) (a4-e4), showed not only that the cells survive the process, but also that diffusion from the liquid in the droplet into the cells in the hydrogel is possible.

→ This allows for the **high throughput formation** of an **on-chip library** of **hydrogel structures** of **varying** controlled and **complex geometries** containing **living cells** immersed in **different liquid microcompartments**.

→ This **platform** can be used to perform **resource-efficient spatially** and **temporally separated experiments** or **high-throughput screenings** of **3D hydrogel structures** / **3D cell cultures**.

Figure 4: Brightfield (BF) image of the 3D bioprinted hydrogel structures containing spheroids of HEK 293 cells overlayed with an fluorescence microscope image using calcein (CI) as a marker. The spheroids are trapped within the 3D printed structures and the staining indicates that the spheroids contain live cells whose viability has not been affected by the printing process.

Current State of Work

Figure 5: Current work is focussed on printing a greater number of smaller structures in a single process for higher throughput using less material. The printed cubes have an edge length of 500 µm and are printed on a DMA with 672 hydrophilic spots of 1 mm x 1 mm dimensions.

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