

# ADVANCED MATERIALS TECHNOLOGIES

## Supporting Information

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Toward Printing the Brain: A Microstructural Ground Truth Phantom for MRI

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## Supporting Information

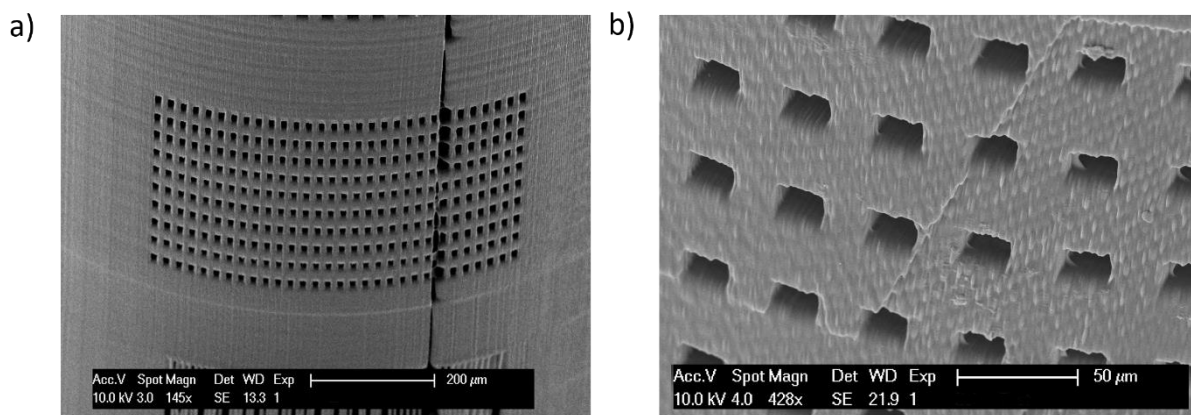
## Towards Printing the Brain: a microstructural ground truth phantom for MRI

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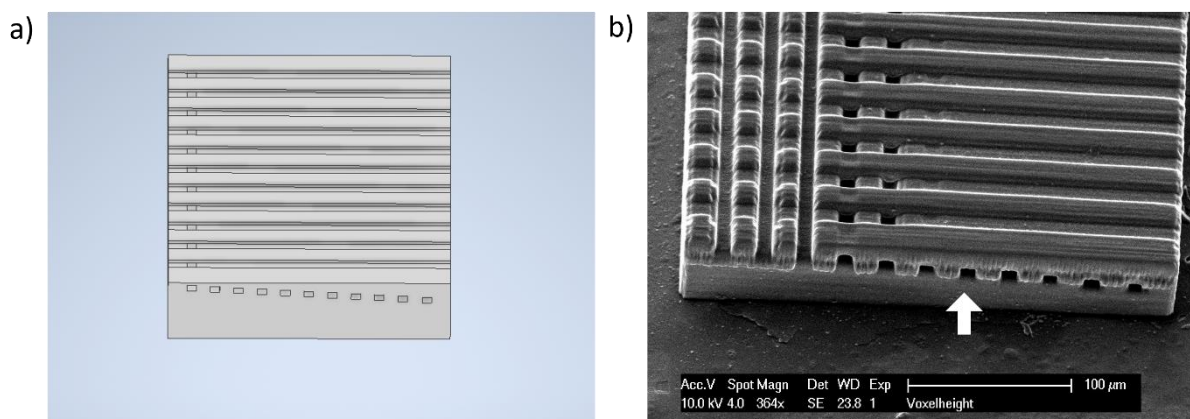
A variety of structures were fabricated to achieve, optimize and verify the design:

**Stitching:** a stitching angle was introduced to improve the structure quality. To avoid the laser being shaded by already polymerized material, a stitching angle was introduced between single structuring blocks. Structures that were fabricated with axial stitching showed insufficient stitching (Figure S1a). A stitching angle of  $12^\circ$  helped to improve the structure quality significantly.



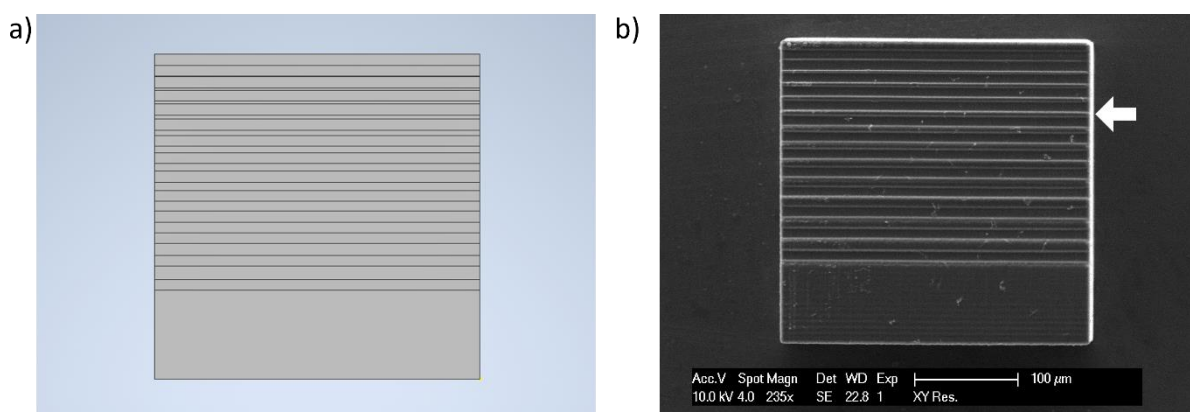
**Figure S1. Axial vs. angular stitching.** SEM image of a) axial stitching that resulted in detached structuring blocks and b) a stitching angle of  $12^\circ$  that improved the structure-quality.

**Channel-Density:** to determine the minimum achievable distance between the channels and subsequently the highest channel density, different distances between the channels were tested. Figure S2 shows the test structure that was prepared to determine the minimum feasible distance before the channels started to intersect. A distance between channels of 12 μm was found to be suitable.



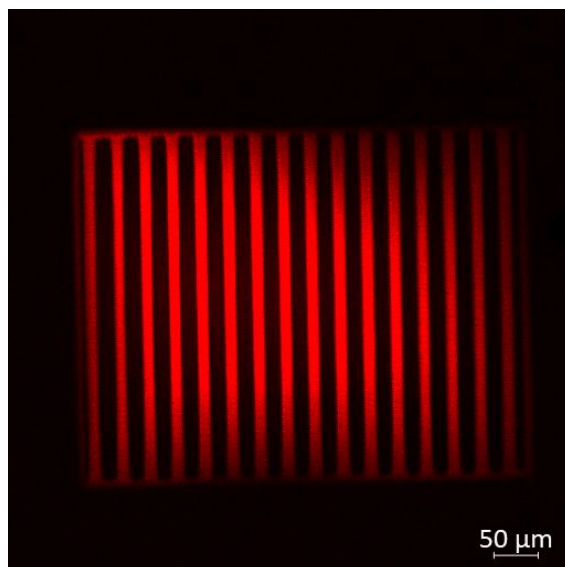
**Figure S2. Minimum channel depth-distance.** A test object was fabricated containing orthogonally oriented channels in two layers. The distance between the layers was varied between 0  $\mu\text{m}$  and 20  $\mu\text{m}$  (in steps of 2  $\mu\text{m}$  from left to right in the image), in order to determine the minimum suitable distance between the channels. a) Computer rendering of the structure design. With 0  $\mu\text{m}$  distance between the channel layers the orthogonal channels are connected, as can be seen at the left edge of the structure. b) Scanning electron microscopy of the printed structure. A distance of at least 10  $\mu\text{m}$  was necessary to separate the channels. To ensure stability a distance of 12  $\mu\text{m}$  (indicated with a white arrow) was used for the final, dimensionally upscaled phantoms.

The polymerization voxel has an ellipsoidal shape and is longer in depth therefore the in-plane resolution is better, allowing to increase the channel density. Fig. S3 shows the test structure that were specifically manufactured for the purpose of determining the optimal plane-distance of 5  $\mu\text{m}$ .



**Figure S3. Minimum in-plane channel distance.** A test object was fabricated with a single layer of channels. The channel sizes were constant while the distance between adjacent channels was varied from 1  $\mu\text{m}$  to 12  $\mu\text{m}$  (in steps of 1  $\mu\text{m}$  from top to bottom in the images). a) Computer rendering of the structure design, b) Scanning electron microscopy of the printed structure. Although a 1  $\mu\text{m}$  distance clearly separated the channels already, for the final design a 5  $\mu\text{m}$  (indicated with a white arrow) distance was used to provide higher structure stability within the dimensionally upscaled final phantoms.

Wall impermeability: additional test structures following the design of test\_structures#2 with a single layer of channels were immersed in fluorescent dye (Rhodamine B, 10mM dissolved in water) to investigate if the channels could be filled with water, and to prove that there was no liquid diffusing into the surrounding polymerized material. Figure S4 shows an image of the dye in the channels captured with laser scanning microscopy (LSM). The dye was only detectable within the channels.



**Figure S4| Demonstration of Wall Impermeability.** A test structure with a single layer of channels was filled with fluorescent dye and imaged with LSM. The channels were fully filled but no dye diffused into the walls between the channels.