Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for:

Photo-induced structural modification of silk gels containing azobenzene side groups

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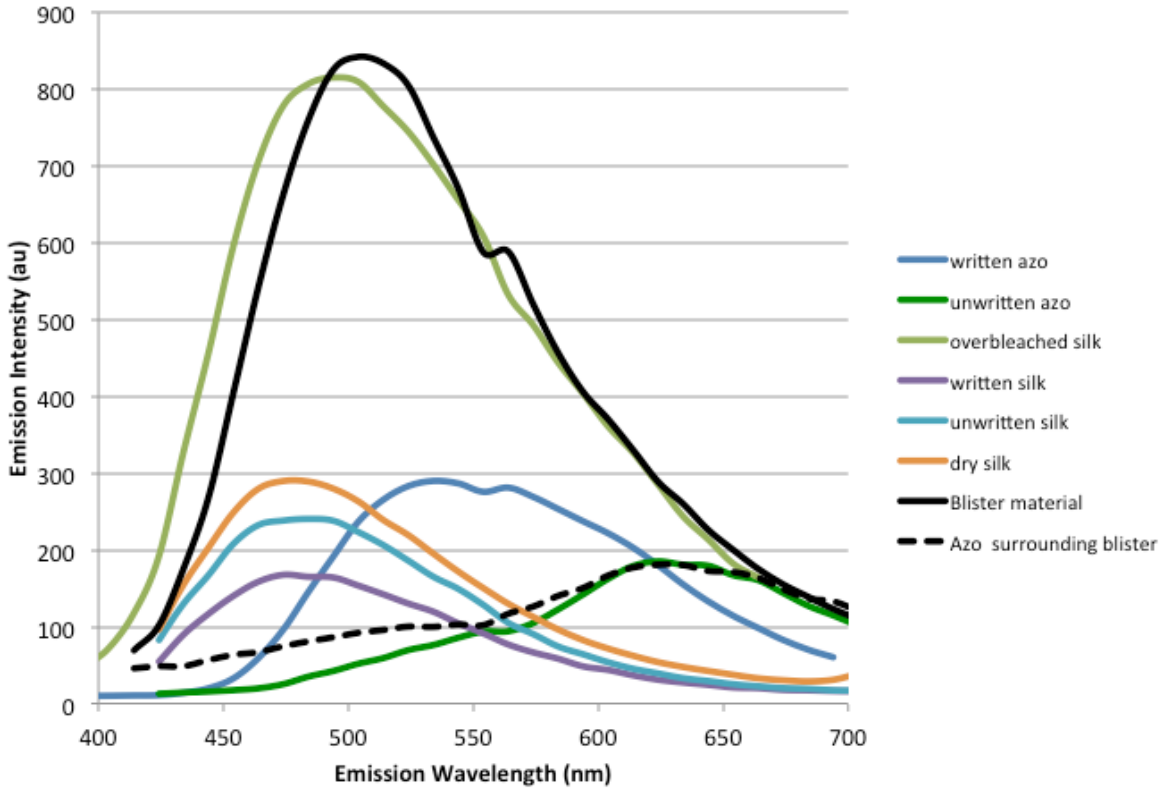

Figure S1: Emission spectra variously of: written azosilk, unwritten azosilk, over-bleached (photo-damaged) silk, written slow-dried silk, unwritten slow-dried silk, and liquid material captured from within the blisters, depicting the spectral shift of azosilk with increasing exposure to mode locked 800 nm light. Unexposed hydrated azosilk shows weak fluorescence peaking at 625 nm, then upon illumination with the 800 nm femtosecond beam, the fluorescence peak strengthens and blueshifts to 520 nm. This fluorescence shift is restricted to the illuminated region: silk regions above and below the irradiated areas retain their original fluorescence spectrum, thereby allowing the material to be used as a three-dimensional patterning medium, as indicated in **Figure 1a** (main manuscript) in which the 'McGill' and 'Tufts' logos are written in different depth planes.

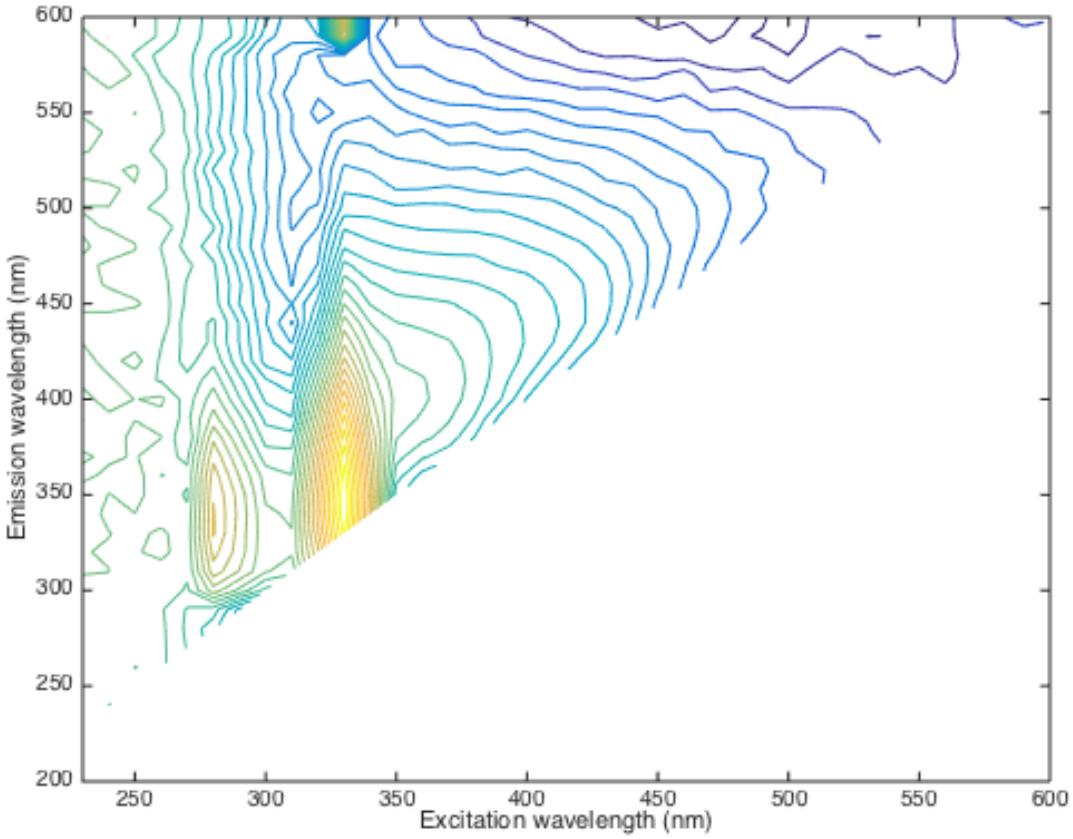

Figure S2: Excitation emission spectrum of 5% aqueous silk solution (logarithmic scale). Note the principal peak is centered at excitation wavelength 325 nm and emission at 340 nm, while the low intensity ridge is shifted by 50 nm from the excitation wavelength.

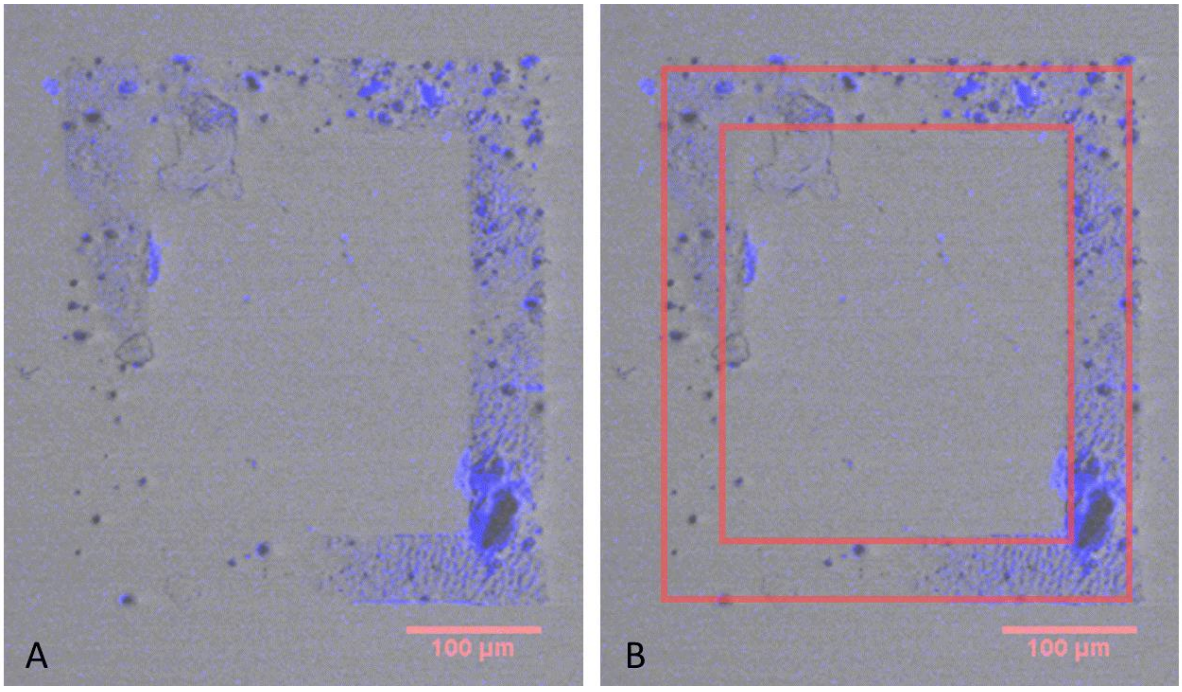

Figure S3: Image of structural damage on silk caused by laser irradiation at 900 µJ/µm^2. **(a)** shows the region of interest where irradiation occurred, and **(b)** shows the resulting fluorescence pattern from irradiation. Structural damage is observed extending outside the lithographic regions of interest from the higher power used.

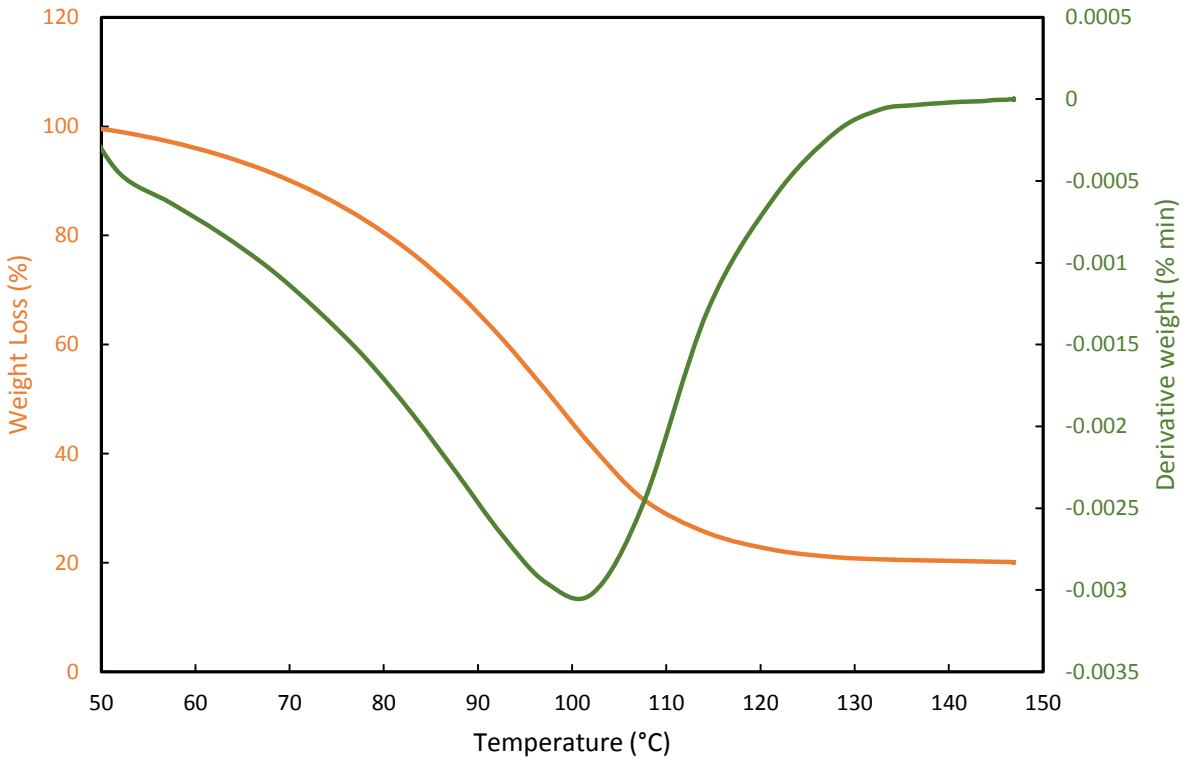

Figure S4: TGA experimental weight loss vs. Temperature curve (orange) to estimate the water content of the azosilk (left y axis). The sample analyzed was cut from a film that was hydrated in water for 30 minutes. Total water content was found to be 77% by heating 21.31 mg of sample to 150 °C and held isothermally at 150 °C for 45 minutes. The first derivative of weight loss is plotted in green to show the weight loss rate vs temperature (right y axis).

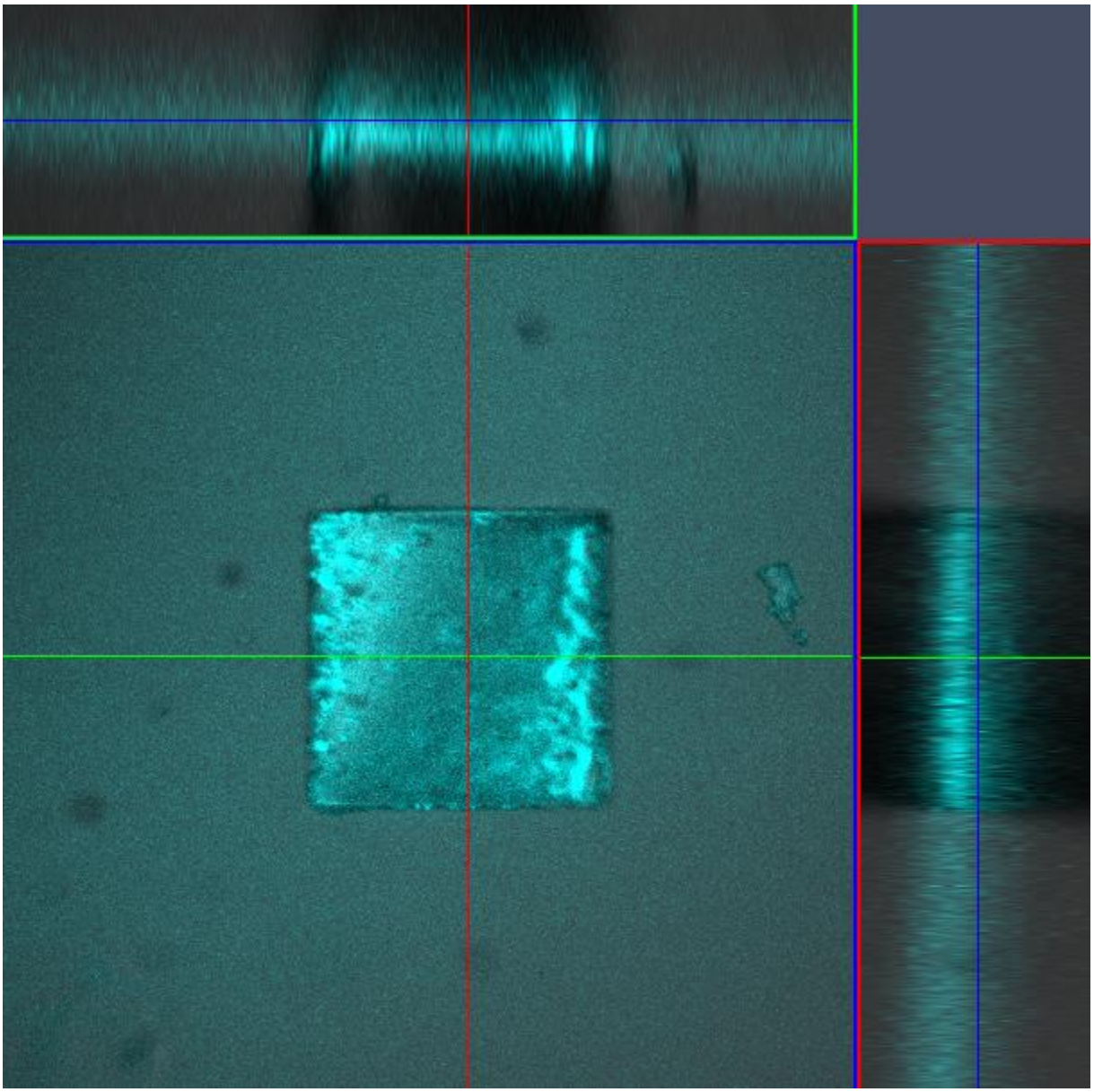

Figure S5: Written areas of the dry azosilk film (blue square) show increased fluorescence compared to the unwritten areas. The darkening shown in the writing planes is an artifact of the software. Vertical and horizontal projections of three-dimensional photolithography image are shown on the sides and demonstrate the absence of a microbubble compared to **Figure 1** in the main text.

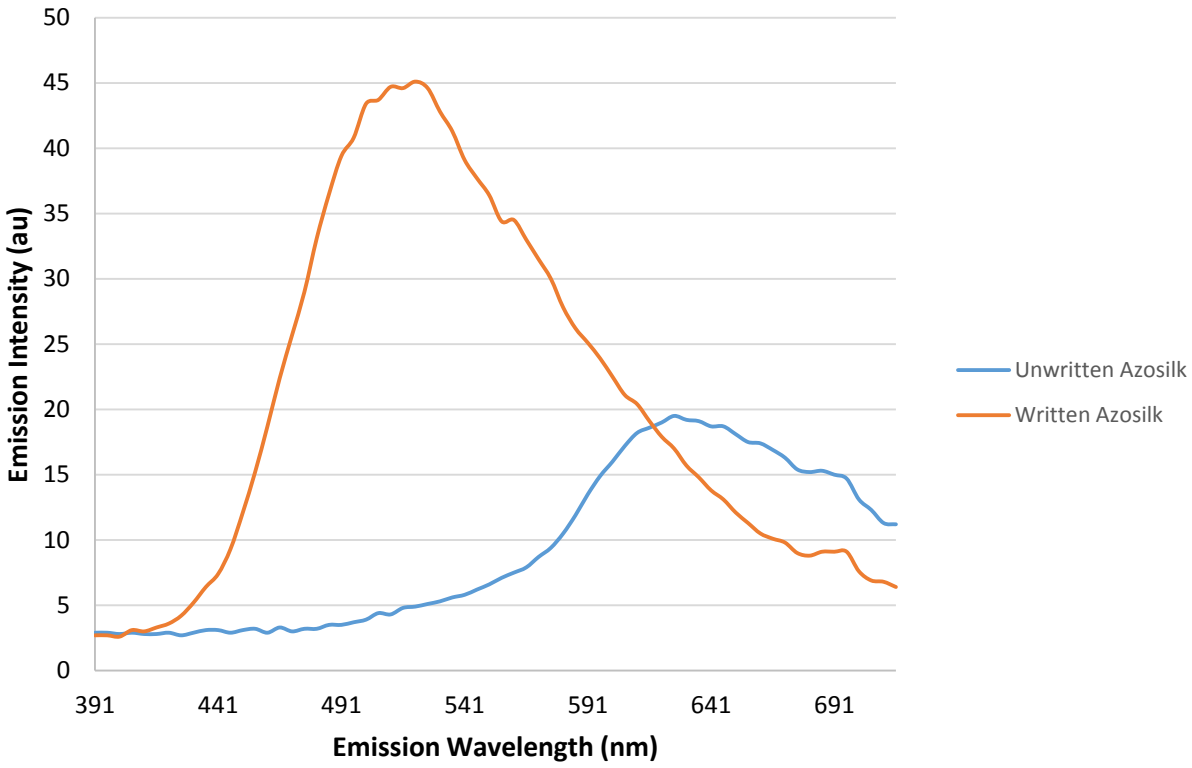

Figure S6: Emission spectra of written azosilk, unwritten azosilk in the dry conditions and taken from the image depicted in **Figure S5**. These spectra are achieved by following the same parameters used in creation of **Figure 1d**; however, under dry conditions.

Experimental Section

Methods and Materials

All chemicals were purchased from Sigma-Aldrich and used without further purification. The Bombyx mori cocoons were purchased from Tajima Shoji Co., LTD. Cell culture plates were purchased from Falcon (60 x 15 mm Style, treated by vacuum gas plasma) and used without further sterilization. Water used within the experiments was provided by a MilliQ water purification system. Imaging was performed on a Multi-Photon LSM: Zeiss Axioexaminer upright microscope using 800 nm as the writing and reading beam during experiments.

Preparation of silk solutions

Methods for the preparation of silk solutions from *Bombyx mori* silkworm cocoons were based on protocols from Rockwood *et al*.[1]

Preparation of azosilk solutions

The general procedure for making azosilk solutions was adapted from Murphy et al. with some modifications. The procedure used here instead was for the fabrication of azosilk-SO_4Na however, but can be generalized made by replacing the sulfanilic sodium salt with an alternate aniline in an appropriate solvent that is miscible with water (e.g. acetonitrile, acetone, or ethanol). Three solutions were prepared: 0.2 M aqueous solution of sulfanilic sodium salt, 1.6 M aqueous solution of p-toluenesulfonic acid, and an aqueous solution of 0.8 M $NaNO_2$. The 0.8 M $NaNO_2$ was cooled in an ice bath until a temperature of 5°C was achieved. Subsequently, 625 µL of the 1.6 M p-toluenesulfonic acid solution and 1.25 mL of 0.2 M sulfanilic sodium salt were briefly mixed by vortexing to produce a homogeneous solution. Then 625 µL of 0.8 M $NaNO_2$ solution was added and then submerged into an ice bath. The solution was let to react for 15 min or until a drop of the solution reacted positively on a piece of starch iodide paper. While this solution was reacting, 2 mL of silk solution was added to a vial and its pH was adjusted to a pH = 10. Once the diazonium solution was ready, 0.5 mL was added to the silk solution dropwise over 60 seconds duration, and vortexed quickly before adding it back to an ice bath. This solution could be used without further purification for optical experiments; however, it was further purified for biological experiments. For this process, 1-2 mL aliquot of the aqueous

solution was added to Sephadex size exclusion columns (NAP-25, GE HealthCare), pre-equilibrated with pH 8 boric acid/borate buffer solution at 0.1 M.

Preparation of azosilk films

Water-stable films were prepared in by adding 1–3 mL of the prepared azosilk solution to a 30 mm petri dish and allowing drying over 5 days. Nine small holes were drilled into the cover to prevent quick drying, since films that dry slowly (over 5 days) are found to be insoluble in water due to more complete self-assembly of the β-sheets within the silk material.[2] Films that are dried in less than 5 days are found to dissolve in water.

Writing in azosilk films

Once the films were dried in the petri dish, they were hydrated by adding 5–10 mL of Milli-Q water to a depth of about 5 mm and mounted onto the microscope. Either 10 or 40x water dipping lenses (Zeiss W N-Achroplan 10x/0.3 M27, W Plan Apochromat 40X/1.0 DIC M27) were used in conjunction with a mode-locked Ti-sapphire laser (Coherent Chameleon, 140 fs, 80 MHz repetition rate) tuned to 800 nm as a two-photon writing/reading beam. After 10000 fs^2 dispersion by the microscope's acousto-optic modulator and 5000 fs^2 dispersion by the objective lens the pulses were broadened to approximately 250 fs at the sample. Single photon fluorescence images were also taken at 488 nm. The laser powers measured before the objective lens used for writing and reading are typically 100 mW and 10 mW respectively, with pixel dwell times in the 3 to 5 microsecond range. Patterning was carried out using the method described by Culver et al.[3] Approximately 2500 pulses, each with 4.3 $nJ/\mu m^2$ were used to form blistering for a total energy of 100 $\mu J/\mu m^2$. Fluorescence emission spectra of various areas of

the observed images were taken using the microscope's spectroscopic lambda mode at 10 mW excitation power.

Characterization of fluorescent liquid

To characterize the fluorescent liquid present in the written bubbles, a series of 100 disc shaped blisters were created and their liquid was extracted using a needle. A few drops of the liquid were added to D_2O and ^1H NMR spectroscopy was performed. The presence of 4 amino acids were determined (alanine, valine, tyrosine and glycine) in trace amounts along with the presence of a large amount of water. ^1H NMR (D_2O, 400 MHz): δ 1.39 (br, 1H), 2.13 (s, 1H), 3.38 (m, 1H), 4.72 (H_2O, s, 30H), 6.88 (d, 1H, J=8 Hz), 7.15 (d, 1H, J= 8 Hz), 8.39 (br, 1H).

In this case, alanine corresponds to the NH peak found at 8.39 ppm, and the methyl peak at 1.39 ppm, valine's CH is found at 2.13 ppm, tyrosine's aromatic peaks are found at 6.88 and 7.15 ppm and finally glycine's CH_2 is found at 3.38 ppm. The peptide's backbone peaks were not found due to the high rate of exchange between D_2O.

References

1 D. N. Rockwood, R. C. Preda, T. Yucel, X. Q. Wang, M. L. Lovett, D. L. Kaplan, *Nat. Protoc.,* 2011, ***6***, 1612-1631.

2 Q. Lu, X. Hu, X. Q. Wang, J. A. Kluge, S. Z. Lu, P. Cebe, D. L. Kaplan, *Acta Biomater.,* 2010, ***6***, 1380-1387.

3 J. C. Culver, J. C. Hoffmann, R. A. Poche, J. H. Slater, J. L. West, M. E. Dickinson, *Adv. Mater.,* 2012, ***24***, 2344-2348.