Femtosecond Laser Direct Write Integration of Multi-Protein Pattern and 3D Microcomponents into 3D Glass Microfluidic Devices
Supplementary Material

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1. Laser Properties

Table S 1. Laser properties of both femtosecond lasers used in this study.

	FCPA µJewel D-400 (IMRA America)	Mikan (Amplitude Systemes)
documented		
frequency [kHz]	200	54,000
pulse duration [fs]	360	400
duty cycle	7.34E-08	0.0000216
center wavelength	1045 nm	1000-1100 nm
Center wavelength of second harmonic generated by BBO*1	522 nm	525 nm
Polarization	Linear	Linear
Beam profile	Gaussian	Gaussian
20x CF Plan (Nikon) N.A.=0.46, W.D.=3.1, EPI, lens transmission for 525 nm light	0.935	0.935
measured		
average power loss from static power meter to before the lens	0.85	0.7241
spot area [cm^2]	2.636E-08	2.112E-08
spot diameter [cm]	1.832E-04	1.744E-04
spot radius [cm]	9.160E-05	8.719E-05
Fabrication ranges (for details see Table S2)		
Laser power range, corrected for losses of beam path and lens [mW]	0.0935-6.4	3.385-6.093
Scanning velocity [µm/s]	0.2-10	0.05-0.5

*1 BBO = beta barium borate crystal

Table S 2. All used fabrication parameter pairs of laser power and scanning velocity. Laser powers are corrected for loss during beam path and by objective lens transmission.

FCPA μJewel D-400 (IMRA America)	
Laser power [mW]	Scanning velocity [μm/s]
6.400	2
6.400	0.2
3.974	5
2.805	5
1.987	5
1.870	5
0.993	5
0.795	10
0.795	5
0.374	1
0.094	5
0.187	5

Mikan (Amplitude Systemes)	
Laser power [mW]	Scanning velocity [μm/s]
6.093	0.5
6.093	0.2
6.093	0.1
5.958	0.2
5.958	0.1
5.958	0.05
5.416	0.5
5.416	0.2
5.416	0.1
4.739	0.2
4.739	0.1
3.385	0.1

2. Fluence and Total Accumulated Fluence Calculation

Fluence Φ [J/cm^2] is calculated by considering the following equations.

$$\Phi = \frac{E_{Pulse}}{A_{focal\ spot}}, \tag{1}$$

$$P_{Peak} = \frac{E_{Pulse}}{\tau}, \tag{2}$$

$$P_{Peak} = \frac{P_{Ave}}{D}, \tag{3}$$

$$D = \frac{\tau}{T} = f\tau, \tag{4}$$

$$\Phi = \frac{P_{Ave}}{f\ A_{focal\ spot}}, \tag{5}$$

where E_{Pulse} is laser pulse energy [J], $A_{focal\ spot}$ is the effective focal spot area [cm^2], τ is pulse duration [s], f is pulse frequency [Hz], D is duty cycle of a pulse, T is the pulse period [s], and P_{Peak} and P_{Ave} are the peak power [W] and average power [W] of the laser pulse, respectively.

Total accumulated fluence (TAF, Φ_{NET} [J/cm^2]) or net fluence is calculated by applying an effective number of pulses, N_{eff}. The effective number of pulses considers the geometrical overlap between individual pulses while scanning a line. It could also consider geometrical overlap between different lines, but we keep lines purposefully separate and therefore only need to consider the focal spot overlap by Gaussian focal spot radius ω_0 [cm] and pulse frequency f versus scanning velocity v [cm/s]:

$$N_{eff} = \sqrt{\frac{\pi}{2}}\frac{\omega_0 f}{v}, \quad (6)$$

$$\Phi_{NET} = N_{eff}\Phi, \quad (7)$$

where the factor $\sqrt{\pi/2}$ is a result of integration over the so-called Gaussian integral. This result was mentioned by another work about effective pulse numbers of Gaussian polymerization [S1]. In our experiments, we change laser power and scanning velocity, while pulse frequency is constant. For comparing measurements with two or three quantities changed, we consider total accumulated fluence most reasonable.

3. Two-Photon Polymerization Fitting Process

Line width has been reported previously in dependence of laser power and processing time t_p [S2]. Laser power is introduced via photon flux $N_{flux,0}$ and power intensity I_0, where the 0 indicates the center of the Gaussian beam. Both quantities are related to fluence. Processing time is correlated with N_{eff}. In the following, we will search for an equation in dependence of fluence and scanning velocity instead of previously introduced dependence of laser power and processing time.

$$w(N_{flux,0}, t) = a_R\omega_0\sqrt{\ln(\frac{\eta\sigma_2 N_{flux,0}^2 f\tau t_p}{C})}, \quad (8)$$

$$\mathrm{t}_p = \frac{t_{irradiation}}{f\tau}, \quad (9)$$

$$t_{irradiation} = N_{eff}\tau, \quad (10)$$

$$N_{flux,0} = \frac{I_0}{h\nu_r}, \quad (11)$$

$$I_0 = \frac{\Phi D}{\tau}, \quad (12)$$

where a_R is a scaling factor, σ_2 is the absorption cross section of the photoinitiator for two-photon absorption, η is the efficiency of radical initiation, t_p is the processing time including dark phases of laser pulses, $t_{irradiation}$ is the irradiation time of accumulated laser pulses, h is the Planck constant, ν_r is the resonance frequency of light, w is diameter of the polymerized volume element that we measure as the line width, and C is the logarithm of the ratio of initial and threshold concentration.

Summarizing the constants into one variable, where possible, we find:

$$w(\Phi\Phi_{NET}) = a\sqrt{\ln(b\Phi\Phi_{NET})}, \quad (13)$$

As in the previous reporting, feature size is proportional to a complex expression reflecting the two-photon aspect as quadratic influence, note $\Phi\Phi_{NET} \propto \Phi^2 \frac{1}{v}$. For practical reasons, it is our goal to show all data as $d(\Phi_{NET})$, but a and b are obtained by fitting $d(\Phi\Phi_{NET})$.

For fitting, we utilize excel logarithmic function fitting. However, to match the complexity of Eqn. 13 we actually fit the square in order to abolish the square-root:

$$w^2(\Phi\Phi_{NET}) = a^2 \ln(b\Phi\Phi_{NET}) = a^2 \ln(\Phi\Phi_{NET}) + a^2 lnb$$

$$d^2(\Phi\Phi_{NET}) = A \ln(\Phi\Phi_{NET}) + B \quad (14)$$

$$\text{With } a = \sqrt{A} \text{ and } b = e^{B/A}$$

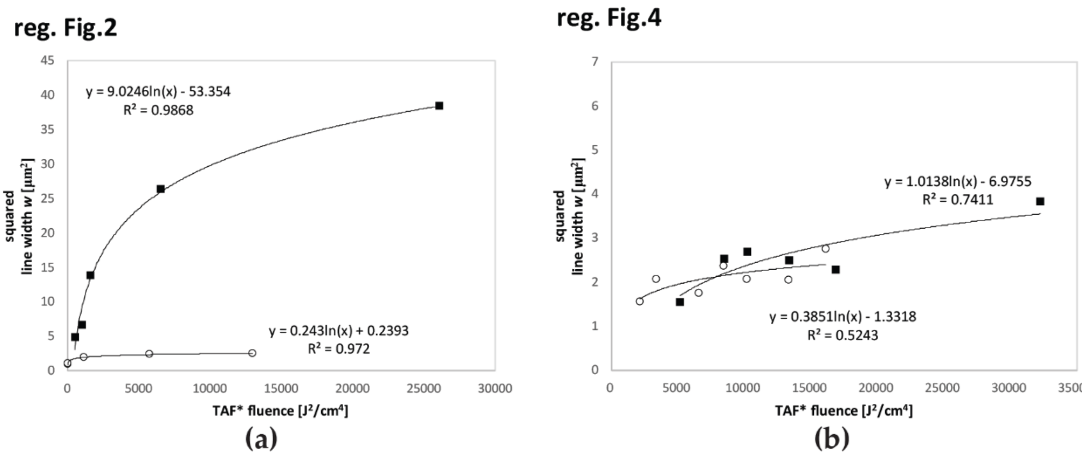

Figure S 1. Actual fitting results obtained as described. Square symbols are data for in-channel fabrication, Circles symbolize data for on-surface fabrication. Note that despite laser changes, fabrication range is overlapping.

4. Absorption Spectra and Penetration Depth

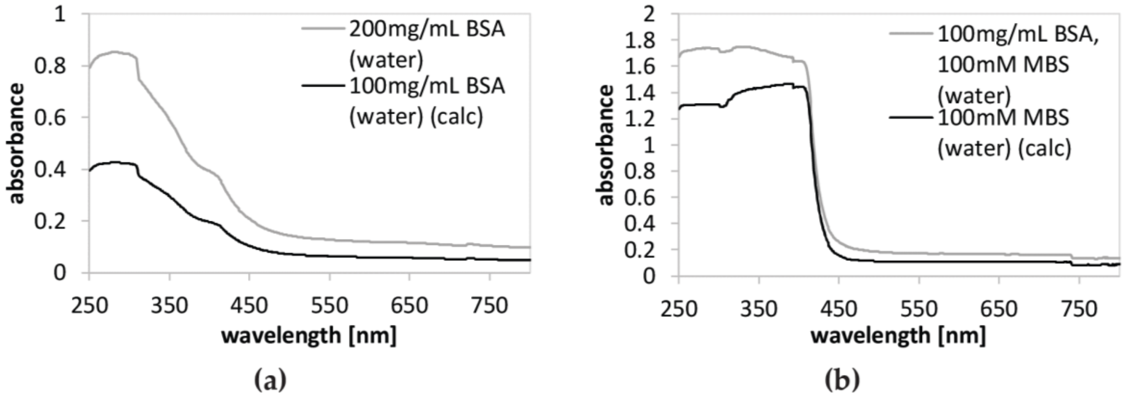

Figure S 2. Absorption spectra of (a) BSA in water and (b) BSA with MBS in water. Assuming linearity between absorbance and concentration, we scale 200 mg/mL BSA to 100 mg/mL BSA in water. These values are substracted from 100 mg/mL BSA, 100 mM MBS measurement to calculate 100 mM MBS in water. These spectra were measured by a UV-3600 Plus UV-VIS-NIR spectrophotometer (Shimadzu).

$$A_{\lambda,d} = log_{10}\frac{I_0}{I}, \quad (14)$$

$$A_{\lambda,d} = \varepsilon_\lambda dc \quad (15)$$

$$\varepsilon_\lambda = A_{\lambda,1cm}/(1cm\ \ c) \quad (16)$$

$$d_{\lambda,I=I_0/10} = \frac{1}{\varepsilon_\lambda c} \quad (17)$$

$$d_{\lambda,I=I_0/10} = \frac{1\ \ cm}{A_{\lambda,1cm}} \quad (18)$$

Table S 3. Estimate of penetration depths.

	$A_{\lambda=525nm}$ from Fig.S2	Estimate molar absorption coefficient $\varepsilon_{\lambda=525nm}$ [$cm^{-1}mM^{-1}$]	Penetration depth d, where $I = I_0/10$ [cm]
BSA, 1.5 mM	0.0665	0.0443	15.03
MBS, 100 mM	0.1095	0.001095	9.13

References

[S1] Tan, B., Venkatakrishnan, K., Makaronets, A. Effects of pulsewidth on two-photon polymerization. *Des. Monomers Polym*. **2013**, *16*, 145-150, doi:10.1080/15685551.2012.705502

[S2] Serbin, J., Egbert, A. , Ostendorf, A., Chichkov, B. N., Houbertz, R., Domann, G. , Schulz, J., Cronauer, C., Fröhlich, L., Popall March, M. Femtosecond laser-induced two-photon polymerization of inorganic–organic hybrid materials for applications in photonics. *Opt. Lett*. **2003**, *28*, 301–303, 10.1364/OL.28.000301.