Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting information –

Laser-assisted protein micropatterning in a thermoplastic device for multiplexed prostate cancer biomarker detection

André Kling[1], Lorin Dirscherl[1] and Petra S. Dittrich[1]*

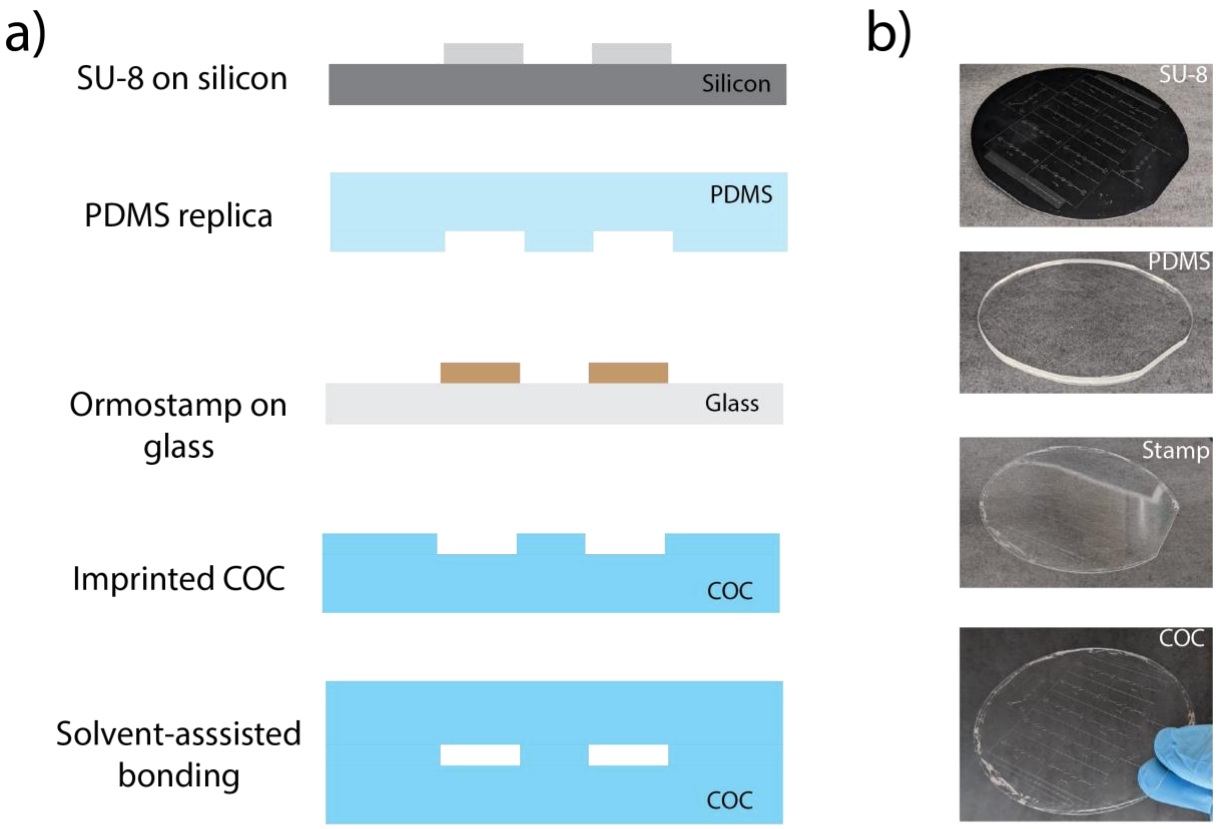
[1]ETH Zurich, Department of Biosystems Science and Engineering, 4058 Basel, Switzerland

Table 1. *List of biomolecules employed for the characterization of the LAPAP process.*

Name	Article number	Company
Bovine serum albumin	A7906	Sigma Aldrich
Biotin (5-fluorescein) conjugate	53608	Sigma Aldrich
Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, FITC	A16167	Thermo Fisher Scientific
Goat Anti-Rabbit IgG H&L (TRITC)	ab671	Abcam
Streptavidin PE Conjugate	12-4317-87	Thermo Fisher Scientific
Brilliant Violet 421 Streptavidin	405226	Biolegend
Alexa Fluor® 647 anti-human CD3 Antibody	317312	Biolegend
FITC rabbit anti 6xHis tag antibody	ab1206	Abcam
FITC rabbit anti GST tag antibody	ab3445	Abcam
FITC rabbit anti Flag tag antibody	ab2492	Abcam

Table 2. *List of biomolecules employed for the detection of prostate cancer biomarkers.*

recombinant protein		
Name	**Article number**	**Company**
Recombinant Human CEACAM-5/CD66e Protein	4128-CM-050	R&D Systems
Recombinant Human Prostate Specific Antigen protein (denatured)	ab126692	Abcam
Insulin-Like Growth Factor I, Recombinant, Human, aa49-118, GST-Tag (IGF1)	373766-20uG	USBio / LucernaChem
C-Reactive Protein (CRP) protein (Myc-DYKDDDDK Tag)	ABIN2715372	Antibodies online
native protein		
Name	**Article number**	**Company**
Native CEACAM5 protein	ab742	Abcam
Native Human Prostate Specific Antigen protein	ab78528	Abcam
Recombinant human IGF1 protein	ab155614	Abcam
Native Human C-Reactive Protein	1707-2029	BioRAD
biotinylated antibody		
Name	**Article number**	**Company**
Human Carcino Embryonic Antigen CEA Antibody (Biotin Conjugate)	33335-05121	Hölzel Biotech
Prostate Specific Antigen Antikörper (PSA) (Biotin)	ABIN1993673	Antibodies online
Biotin Anti-IGF1 antibody	ab83137	Abcam
Biotin Anti-C Reactive Protein antibody	ab271233	Abcam



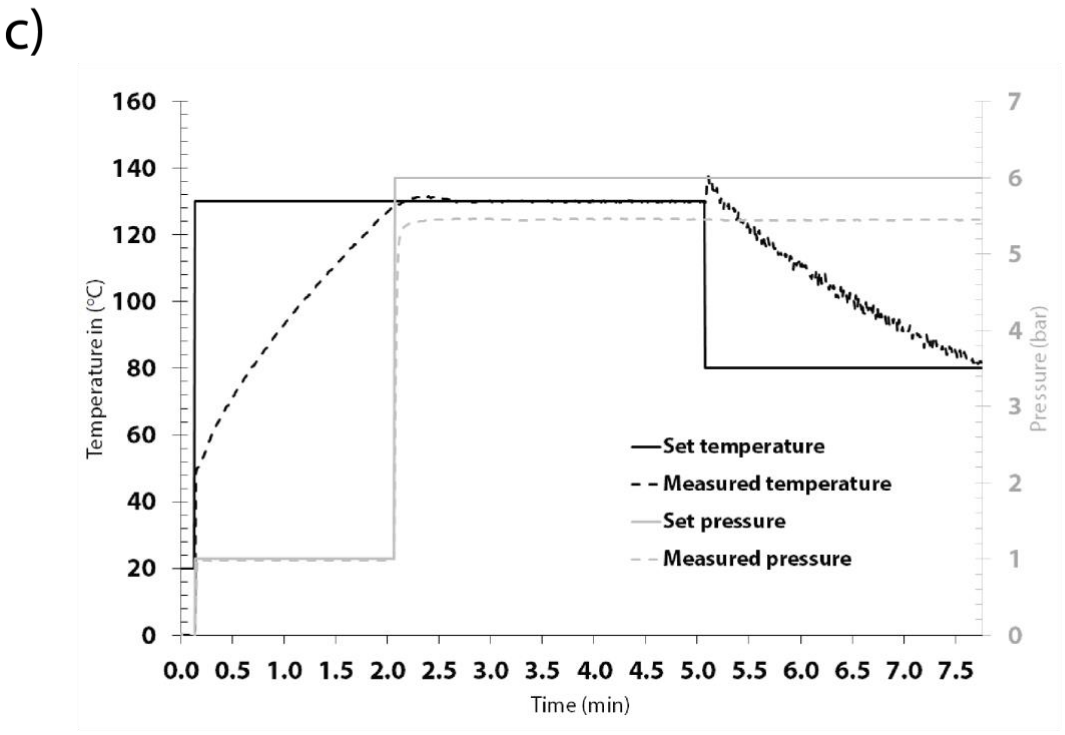


Figure S1. *Chip fabrication. a) Schematic and photographs of the different manufacturing steps. The initial negative SU-8 master mold is replicated into a positive PDMS mold. From that mold a temperature and pressure resistant stamp is replicated that is used for the imprinting of the channel structures into COC. b) Photographs of the SU-8 master mold, PDMS intermediate replica, stamp and the imprinted COC foil. c) Graph showing the set and measured profile of the temperature and pressure during the imprinting of the microchannels under a vacuum of 10 mbar.*

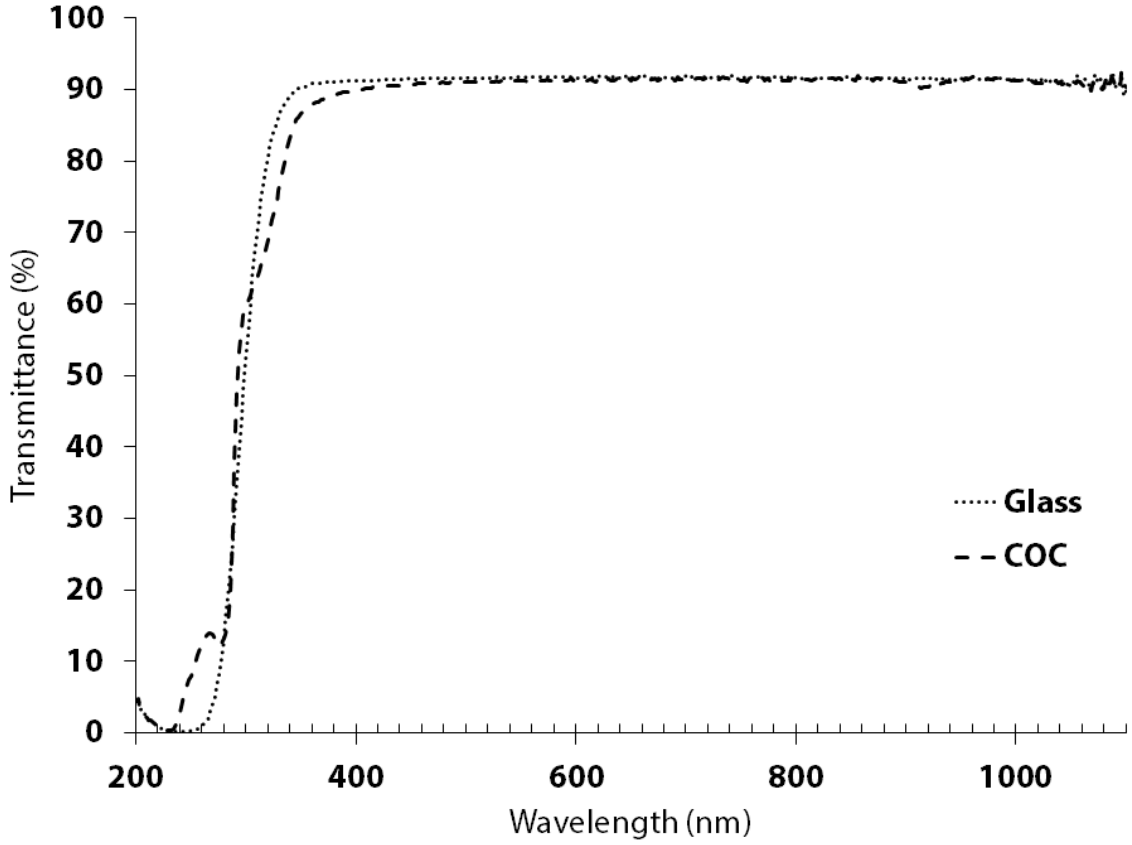


Figure S2. *Light transmittance through 1 mm thick COC and glass measured at different wavelengths using a UV-VIs spectrophotometer.*

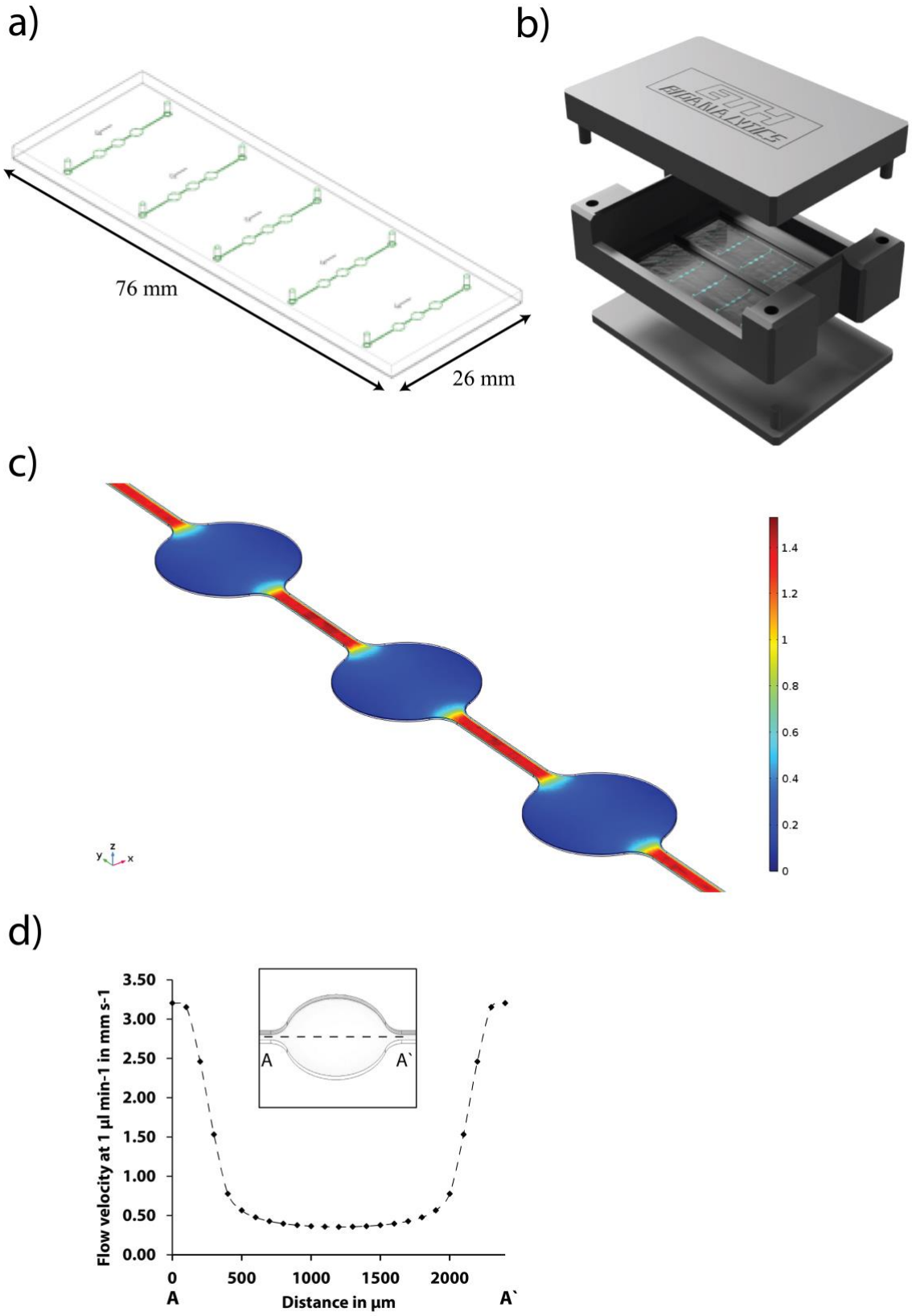


Figure S3. *a) CAD design of the microfluidic chip. b) CAD design of the chip holder in a 96-well plate format with a lid and bottom used for transport. c) Comsol simulation of the flow velocity (m s^{-1}) in different regions of the channel (COMSOL Multiphysics). d) Calculated wall shear rate at 1 µL min-1 across the chamber area that is used for the surface functionalization.*

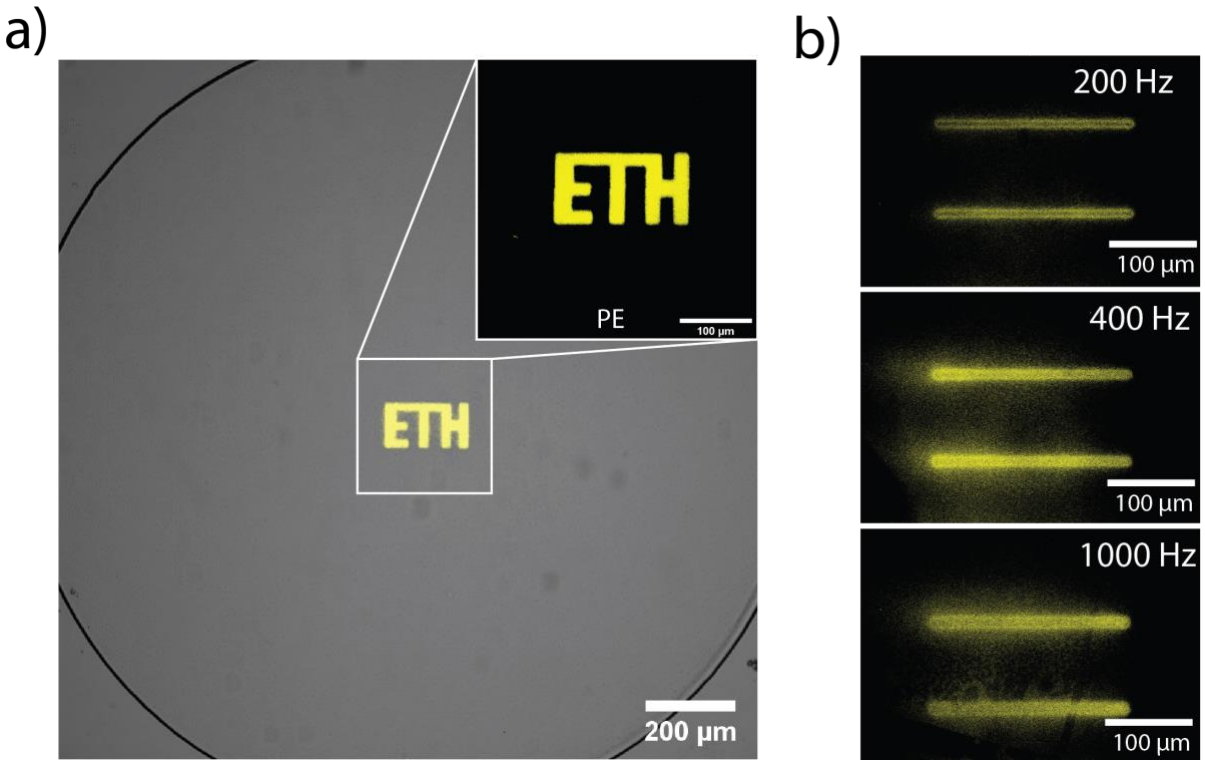


Figure S4. *a) Brightfield and fluorescent image (inset) of a biotin functionalized ROI. b) Influence of the laser scan rate applied for bleaching biotin-FITC with a line width of 16 pixels.*

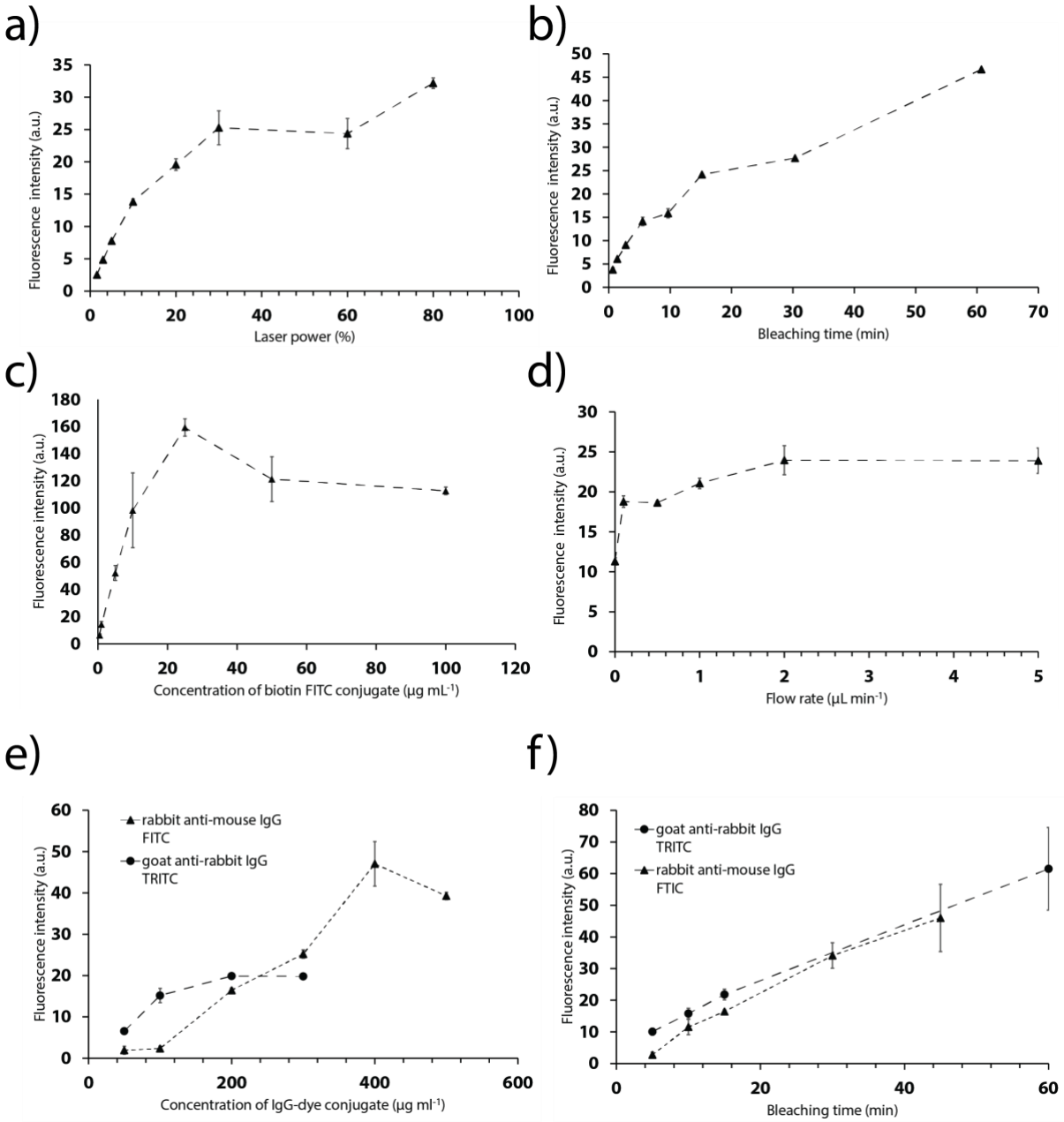


Figure S5. *Characterization of laser-induced adsorption of proteins. Influence of the laser power given in % of the maximum intensity (a), bleaching time (b), concentration (c) and flow rate (d) on the bleaching process of biotin-FITC. To visualize the resulting immobilization of biotin, we added in a subsequent step streptavidin-phycoerythrin (SAPE), which is the same combination as depicted in Figure 3a,b of the main manuscript. e,f) Dependency of the bleaching process from the supplied anchor-dye concentration and bleaching time, respectively, for goat anti-rabbit IgG TRITC and rabbit anti-mouse IgG FITC. Here, visualization was achieved by addition of rabbit anti-mouse IgG FITC and of mouse anti-CD3 Alexa Fluor 647, respectively. It should be noted that the intensive laser exposure leads to bleaching of TRITC and FITC, but we have no indication that the laser light has a harmful effect on the antibody or its binding site. The linear increase of the fluorescence intensity confirms (i) that antibodies are immobilized and (ii) that the epitopes are active and bind to the second antibody.*

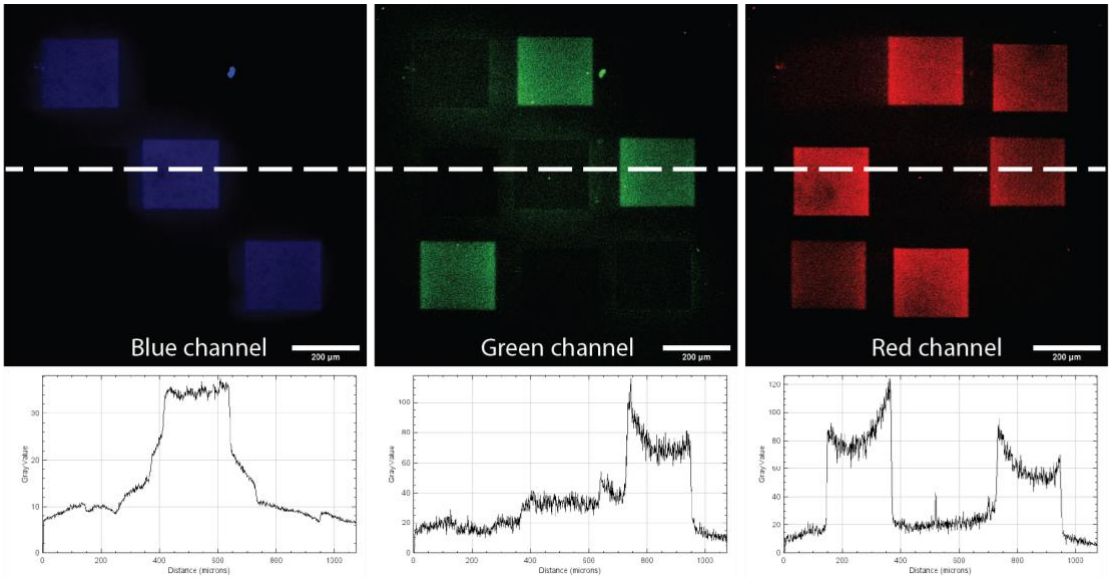


Figure S6. *Fluorescence images after sequential functionalization. These images are the same as in Figure 4 of the main manuscript. Below, the grey scale pixel intensity values are plotted along the dashed lines for the blue, green and red channel.*