Supporting Information

# Direct Creation of Biopatterns via a Combination of Laser-Based Techniques and Click Chemistry

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## Surface characterization

### Contact angle measurements

For the characterization of surfaces, a side view imaging setup was developed consisting of a 10× microscope objective lens and a CCD camera (Unibrain 810c) and the analysis of the recorded images was carried out by using the ImageJ software. Figure S1 depicts the side view images recorded after pipette deposited droplets of the binding buffer on alkene- and alkyne-terminated $Si_3N_4$ surfaces and unmodified $Si_3N_4$ surfaces. It was observed that after their modification, the $Si_3N_4$ surfaces became more hydrophobic due to the hydrocarbon (alkene/-yne) coating, as expected according to the literature.[S1]

**Table S1.** Contact angles values (º) for pipette-deposited droplets of 1 μL of binding buffer on alkene-terminated $Si_3N_4$, alkyne-terminated $Si_3N_4$ and unmodified $Si_3N_4$ surfaces. All measurements were performed at room temperature in ambient atmosphere.

| Sample | CA(º) |
|---|---|
| $Si_3N_4$-alkene | 89 ± 4 |
| $Si_3N_4$-alkyne | 83 ± 4 |
| $Si_3N_4$ unmodified | 47 ± 4 |

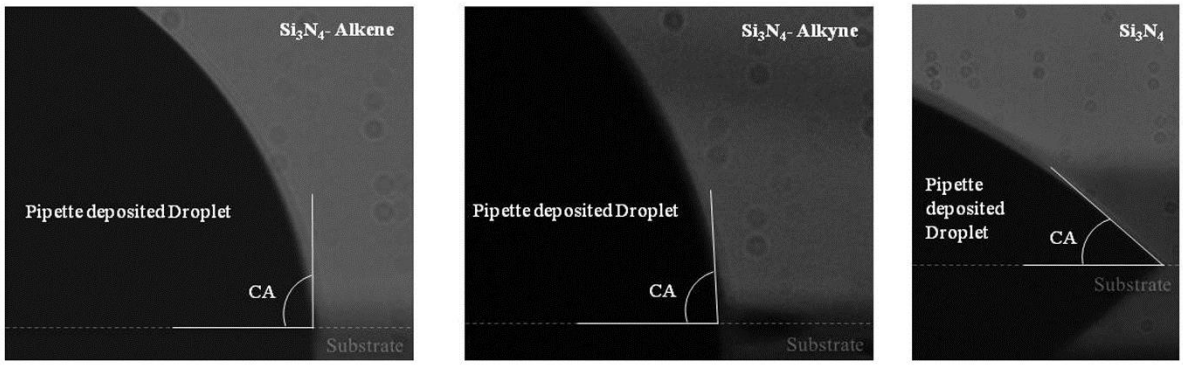


**Figure S1.** Binding buffer contact angle on the different modified surfaces.

**X-ray photoelectron spectroscopy**

The surfaces were analyzed by X-ray photoelectron spectroscopy (XPS) using mono-chromatic Al Kα X-ray radiation at 12 kV and 20 mA under ultrahigh vacuum conditions on a JPS-9200 photoelectron spectrometer (JEOL, Japan). The binding energies were calibrated at 285.0 eV for the C 1s peak. The spectra were processed using CasaXPS software (version 2.3.16 PR 1.6). The high resolution spectra were corrected with a Shirley background fitting. Wide scan spectra revealed the presence of fluorine, oxygen, nitrogen, carbon and silicon. The silicon and nitride signal corresponds to the $Si_3N_4$ substrate whereas the carbon signal is attributed to the prepared alkene or alkyne monolayer. The presence of fluorine and oxygen is in accordance with earlier observed results [S2] and is caused by the aqueous HF-etching step which results in surficial Si–F and Si–OH groups on the freshly-etched and oxide-free $Si_3N_4$ surface.

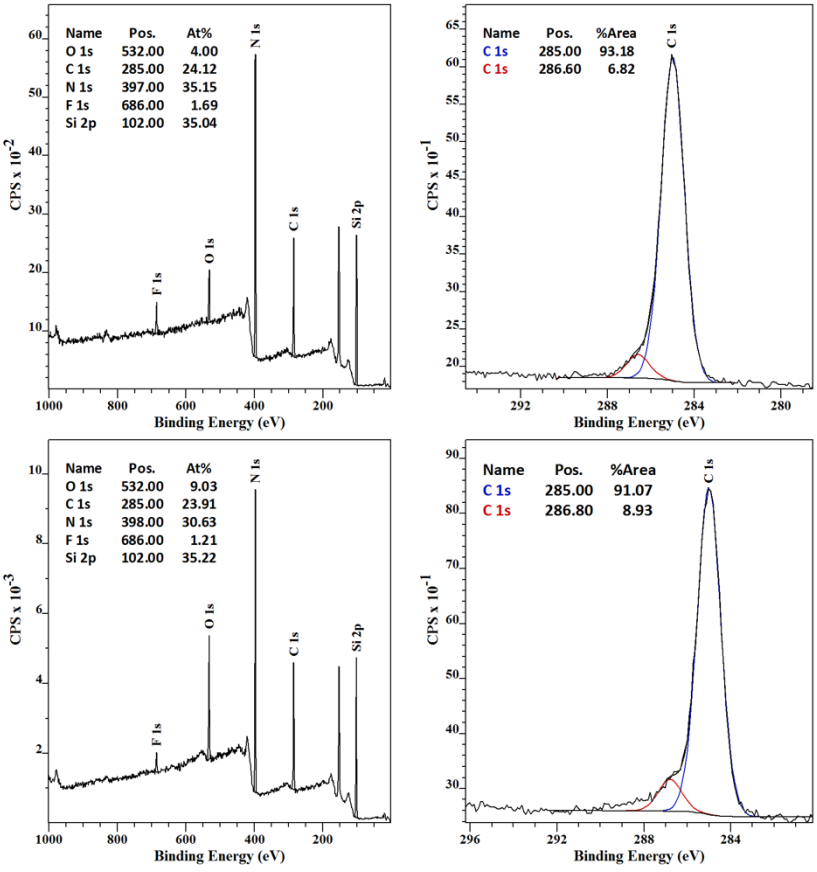

**Figure S2.** XPS spectra of $Si_3N_4$-alkene (top) and $Si_3N_4$-alkyne monolayer (bottom): left) wide scan and right) carbon 1s narrow scan.

## 1. Experimental setup

The experimental setup employed for the single-step and the two-step LIFT process for the immobilization of the thiol-modified aptamers onto alkene(/-yne)-terminated surfaces (receiver substrate), with spatial control, is shown in Figure S3. The setup comprises a solid-state lamp-pumped Nd:YAG (Litron Nano-L 200-10) nanosecond laser ($\tau$ = 10 ns) at 355 nm wavelength and a high power imaging micromachining system. In the single-step LIFT process, the experimental setup includes a donor substrate, which carries the biomaterial to be deposited, diluted in solution. The donor substrate is an 1 inch quartz plate coated with a 40-nm titanium laser-absorbing layer where 10 μL solution of the thiol-modified aptamers is dropcast, creating a liquid film of about 20 μm thickness. The imaging system of the setup comprises of a beam expander, an attenuator, a square aperture of variable size and a microscope objective lens (15×, NA 0.32), and is used to direct and project the laser-printing beam onto the Ti layer of the donor substrate. Upon projection of the laser-printing beam onto the donor substrate, a high pressure vapor pocket is created in the interface between the Ti layer and deposited liquid, due to the absorption of the laser pulse from the Ti layer of the donor (Ti layer, a= $1.0167 \cdot 10^6$ $cm^{-1}$ at 355 nm). This high-pressure vapor pocket, expands and provides flow in the remaining liquid film,[S3] creating a high speed jet which drives the liquid solution to the receiver substrate, resulting in a nL droplet of aptamers printed onto the alkene/yne-terminated surface for each laser beam pulse. Both the donor and the receiver substrates, are placed parallel and in close proximity (~200 μm) onto a holder which can be moved in relation to the laser beam by a micromachining $x-y$ translation stage (1 μm positioning resolution, 25 mm × 25mm max. movement). The energy of the projected laser

beam is controlled by the aid of the attenuator, while the size and shape of the beam can be modulated through the square aperture. In this work, the spot size of the laser-printing beam, at the donor substrate was fixed at 50 × 50 $\mu m^2$, while the LIFT of the aptamers solution was done at energy fluence of 300 $mJ/cm^2$. The microarrays of aptamers is formed by repeating the transfer process at different target and receiving substrate locations by means of the computer-controlled translation stages.

For UV irradiating the nL droplets (two-step LIFT process) of the LIFT printed aptamers onto the alkene (/-yne)-terminated surface, the donor substrate is moved away from the beam path and the laser beam used for LIFT printing the aptamers onto the surface is now projected at a specific site of each LIFT printed droplet, used as a secondary photo-activation laser beam. The size and shape of the photo-activation laser beam were fixed at 50 × 50 $\mu m^2$.

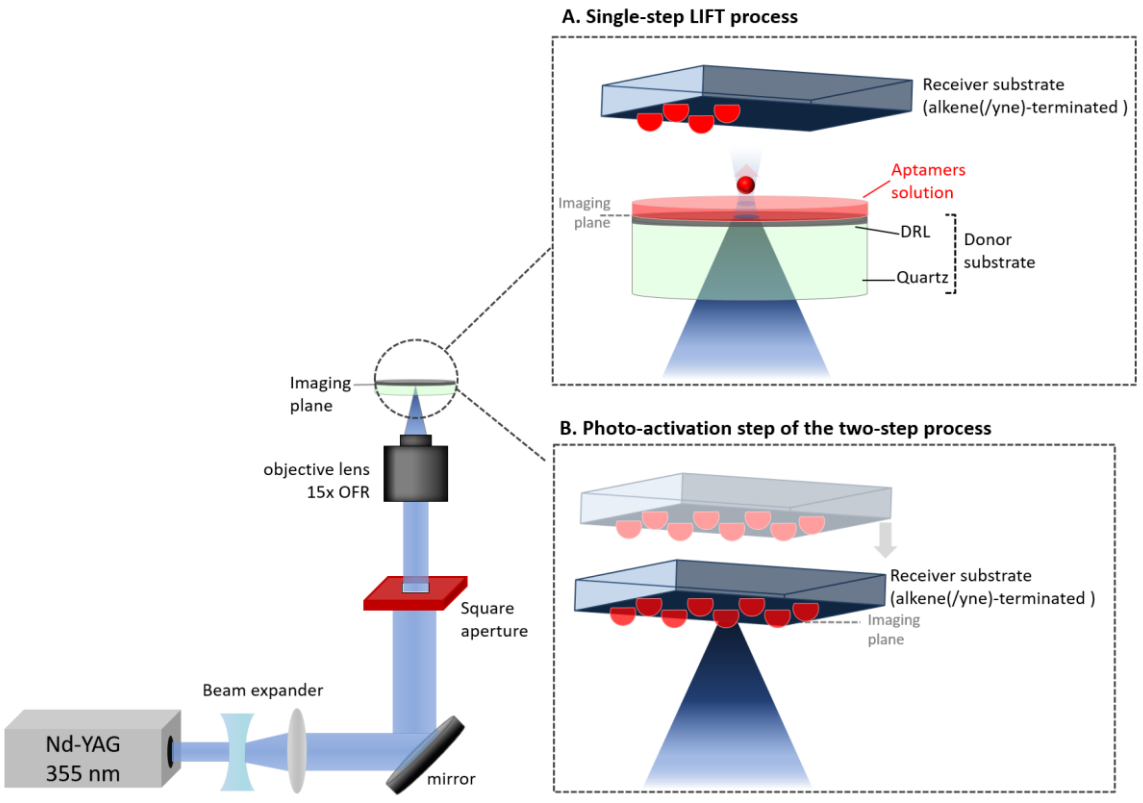


**Figure S3.** Schematic illustration of the experimental setup employed for creating biopatterns of aptamers on alkene (/-yne)-terminated surfaces in a) the single-step and (b) a two-step LIFT process.

**Photoactivation energy fluence measurements**

The enhancement of the immobilization efficiency of the thiol-modified aptamers on alkene-terminated surfaces by following the two step process has been also studied as function of the photo-activation energy fluence applied and the number of laser pulses. Figure S4 presents the normalized fluorescent signal for two different laser photo-activation energy fluences, 100 mJ/cm$^2$ and 150 mJ/cm$^2$.

We observed that at higher laser photo-activation energy fluence (at 150 mJ/cm$^2$) a 15±2 % increase of the fluorescent signal of the immobilized aptamers is recorded, while as we increase the number of the photo-activation laser pulses, the fluorescent signal is further increased up to 76% more (for twenty laser pulses). It is worth mentioning that for both energy fluences, the recorded fluorescent signal changes rapidly upon photo-activation with multiple secondary laser pulses, showing high increase rate up to 7-10 pulses.

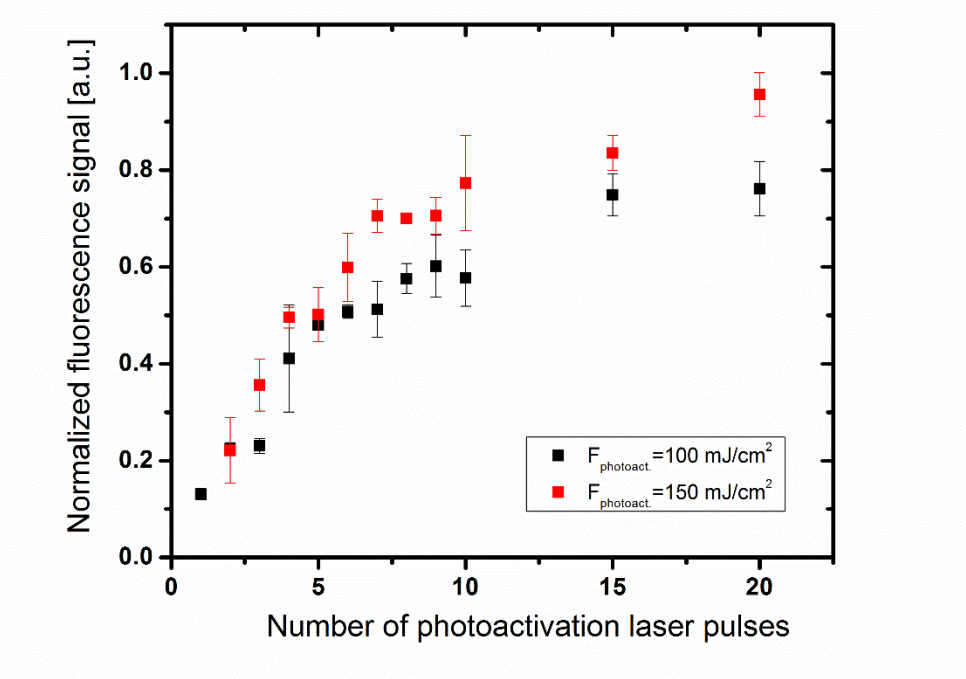


**Figure S4:** (a) Fluorescent signal of laser printed and photo-activated aptamers against OTA on alkene-terminated surface, for 100 and 150 mJ/cm$^2$ laser photo-activation energy fluences, as a function of the number of laser pulses.

**Recognition of OTA mycotoxin by the LIFT-printed and photoactivated aptamer microarrays.**

The activity of the immobilized aptamers was further evaluated by taking advantage of the natural fluorescence that Ochratoxin A (OTA) exhibits upon UV exposure (330-340 nm). The study was performed using functionalized surfaces with aptamers that had been LIFT-printed and photo-activated following the single-step LIFT process on alkene-terminated $Si_3N_4$ surfaces (see Figure S5a). After repeated washing of the functionalized surfaces with TETBS solution, the surfaces were exposed to a solution of 1 μM of OTA (from Petromyces albertensis, ≥98%, purchased by Sigma) in binding buffer, for 40 minutes, at room temperature. By recording the fluorescent signal in the wavelength range of 420-470 nm, where the OTA possesses natural fluorescence upon exposure, fluorescent patterns were observed at the specific locations of the surface where the aptamers had been LIFT-printed and photo-activated. The fluorescence microscopy image, shown in Figure S5b, was recorded after the binding of the mycotoxin on the immobilized aptamers and washing the sample with BB solution, proving the ability of the LIFT-printed and photo-activated aptamers, to bind specifically to their target analyte. Consequently, this confirms that the immobilized aptamers retain their biological activity during and after LIFT printing and photo-activation.

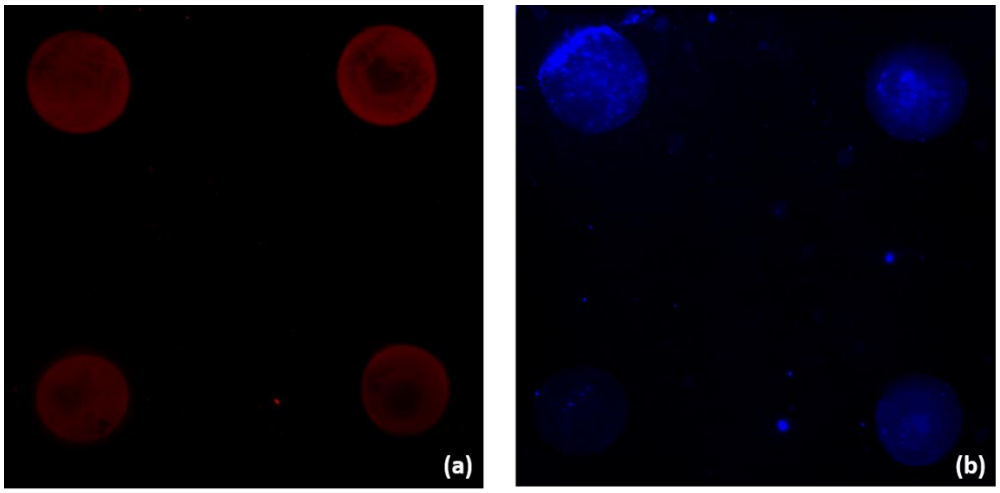


**Figure S5:** a) Single-step LIFT-printed microarray of Texas-Red tagged, thiol-modified aptamers against OTA on alkene-terminated $Si_3N_4$ surface and b) binding of the mycotoxin OTA by the LIFT-printed aptamers.

## REFERENCES

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