Electronic Supplementary Information for

Multi-color Polymer Pen Lithography for Oligonucleotide Arrays

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1. Experimental Details

Stamp fabrication

The silicon master and PDMS stamp were fabricated according to previously published work.[1] In short, the silicon master was prepared by a photolithography and wet chemical etching process with a modified design for the photomask to fabricate different sections. The PPL stamp was fabricated by pouring the hard-PDMS on the silicon master and curing overnight at 72°C. The stamp was plasma cleaned by oxygen plasma (0.2 mbar, 100 W, 10 sccm O_2, 2 min., ATTO system, Diener electronics, Germany) prior to the inking process to make it more hydrophilic, which induces more homogeneous inking and further helps the ink molecules to transfer from the pens during printing.

Substrates

Alkyne terminated glass substrates for the CuAAC patterning were prepared using a procedure similar to previously reports.[2] In brief, glass cover slips (VWR, Germany) were subsequently sonicated in chloroform, ethanol and DI water (18.2 ΩM cm) for 5 min in each solvent for cleaning. Then, the cover slips were dried with a N_2 stream and activated in oxygen plasma (0.2 mbar, 100 W, 10 sccm O_2, 5 min., ATTO system, Diener electronics, Germany). After activation, the cover slips were submersed in a 1 vol-% GPTMS [(3-Glycidyloxypropyl) trimethoxysilane] solution in toluene for 8-12 h for silanization. To remove excess silane, glass slides were sonicated in acetone for 1 min before being submerged into a solution of 2 vol-% propargylamine in acetonitrile for 8 h at 50°C for alkynization. Finally, the cover slips were sonicated in ethanol for 30 s, dried with N_2 and stored in a glass petri dish until use. All chemicals for this process were purchased from Sigma Aldrich, Germany.

[1] D. J. Eichelsdoerfer, X. Liao, M. D. Cabezas, W. Morris, B. Radha, K. a Brown, L. R. Giam, A. B. Braunschweig and C. A. Mirkin, *Nat. Protoc.*, 2013, **8**, 2548–60.

[2] S. Oberhansl, M. Hirtz, A. Lagunas, R. Eritja, E. Martinez, H. Fuchs and J. Samitier, *Small*, 2012, **8**, 541–5.

PBAG [Poly(Bisphenol A-co-epichlorohydrin)] slides were prepared similar as previously described.[3] Microscopy glass slides (18 × 18 mm, VWR) were cleaned with an aqueous solution of 14.3% NH_3, 14.3% H_2O_2 in DI water at 60°C for 20 minutes. Subsequently the slides were washed with water, ethanol and were dried under a N_2 stream. Following, the slides were silanized by incubation in a 1% APTES (3-aminopropyltriethoxysilane, Sigma Aldrich, Germany) solution (95% methanol, Merck, 5% H_2O, 1 mM acetic acid, Sigma Aldrich, Germany) for 20 minutes. The slides were then washed with ethanol (Merck, Germany) and DI water and dried under a N_2 stream. Finally, the slides were incubated in an acetone solution containing 5% PBAG [Poly(Bisphenol A-co-epichlorohydrin)] (Sigma Aldrich, Germany) over night at room temperature. The slides were washed with acetone, dried under nitrogen stream and stored at -20°C.

Ink preparation

Ink solutions for CuAAC were based on copper sulfate (10 mM) and sodium ascorbate (20 mM) solutions in DI water that are mixed with fluorescently labeled azide compounds (labels used are TAMRA, cyanine-3 (cy3) (Jena Bioscience, Germany), cyanine-5 (cy5) (Lumiprobe, Germany) Alexa 647, and Alexa 488-azides (Thermo Fisher Scientific, Germany), conc. 50-100 µM). An amount of 20 % (v/v) of glycerol was added to the ink solutions as an ink carrier and to avoid premature drying of the ink.

Inks for the multi-color DNA arrays contained single-stranded oligonucleotides (aF1, aF5, aF6, aF9, aF10, aF1_12, and aF10_12, purchased from Sigma Aldrich, Germany, sequences are below). The oligonucleotide concentrations were 35-100 µM in a Trehalose buffer (200 mM K_2HPO_4, 200 mM KH_2PO_4, 0.5% v\v Trehalose-Dihydrate and 0.1% v\v Tween 20) which contained 20 % (v/v) glycerol. These ink solutions were used for the coating of different sections of the stamp by spin coating, as described below.

[3] A. Angelin, S. Weigel, R. Garrecht, R. Meyer, J. Bauer, R. K. Kumar, M. Hirtz and C. M. Niemeyer, *Angew. Chemie Int. Ed.*, 2015, **54**, 15813–15817.

Printing

For the printing of the arrays, a commercial DPN setup (NLP2000, NanoInk, USA) was used which was equipped with a custom made holder to allow attachment of large PPL stamps. The printing was done under controlled humidity (60-70 % RH) at ambient temperatures. Details on the leveling process for the stamp and the offset corrections for multi-color printing are given further below in the ESI. Dwell times and pressure were varied for different patterns as described in later. After lithography, the samples were generally left at rest to allow binding of the ink components to the substrates. In case of the azide ink/alkyne substrate, typically 1-4 h were permitted for binding before washing of the sample. In the case of the oligonucleotides/PBAG substrates, the patterned slides were incubated for ~ 16 h (overnight). The oligonucleotide arrays were stored dry and cool until further use.

Oligonucleotide hybridization

Arrays containing capture oligonucleotides prepared by PPL were washed with TETBS buffer (20 mM Tris-Cl, 5 mM EDTA, 150 mM NaCl, 0.05 vol-% Tween20, and pH 7.5) and blocked with 50 µl CAS block (Thermo Fisher scientific, Germany) for 30 min to reduce non-specific binding. 50 µl of complementary, fluorophore labeled oligomers (10 nM) were allowed to hybridize for 1-1.5 h and the slides were then washed with TETBS and DI water. Regeneration of DNA arrays was achieved by incubating previously hybridized chips with 50 µl NaOH (50 mM) for 5 to 10 min, followed by washing with TETBS buffer and DI water. For the next hybridization, the above described method was used. In the case of the regeneration of multicolor arrays, 50 µl of each different complementary oligomer solution were mixed and the mixture was allowed to hybridize on the glass chips.

Preparation and hybridization of DNA-STV conjugates

The synthesis and purification of covalent DNA-STV conjugates (aF9 and aF10) was carried out using the corresponding thiolated oligonucleotides (tcF9 and tcF10, Sigma Aldrich, Table S1) and

streptavidin, as previously described.[4] Briefly, 10 nmol of STV was derivatized with maleimido-groups using a heterobispecific crosslinker (sulfo-SMCC, Pierce), reacted with the thiolated oligonucleotide (10 nmol) and subsequently purified by anion-exchange chromatography. The one-to-one molar ratio of oligonucleotide to protein and the conjugate concentration was determined by gel-electrophoresis and spectrophotometry.

Coupling of the above described covalent DNA-STV conjugates with biotinylated dye BioAtto647 was achieved by mixing 1µl of DNA-STV conjugate (10 pmol) with 5 µl PBS buffer (3.5 mM NaH_2PO_4, 8 mM Na_2HPO_4, 150 mM NaCl, pH 7.3) and 4 µl of the biotinylated BioAtto647 (30 pmol). The mixture was incubated for 20 min. For the coupling of DNA-STV conjugates with the epidermal growth factor, 1 µl of DNA-STV conjugate (10 pmol), 5 µl of biotinylated EGF (30 pmol) and 4 µl of PBS buffer were mixed and incubated for 30 min. In a second step, 10 µl (30 pmol) BioAtto550 dye were added and the mixture was again incubated for 20 min to yield fluorophore-labeled DNA-STV-bEGF conjugate. After the binding of the biotinylated compounds, 150 µl of Buffer A (20 mM Tris buffer, 150 mM NaCl, 5 mM EDTA, containing 0.05 % (w/v) Tween-20, pH 7.5 and 800 µM biotin) were added in both cases and the mixture was incubated for 30 minutes. The various DNA-STV conjugates were mixed to a final concentration of 200 nM each in buffer A and allowed to bind on DNA capture arrays.

Cell experiments

Human MCF-7 breast cancer cells stably transfected to express the eGFP-labeled EGF receptor were a gift from Pedro Roda-Navarro, Max-Planck Institute for Molecular Physiology, Dortmund. The cells were cultured in MCF-7 medium, composed by Eagle's Minimum Essential Medium (ATCC 30-2003), with addition of 1% Penicillin/Streptomycin (Life Technology), 10% Fetal Bovine serum (FBS, Biochrome) and 0.6% G418 disulfate salt solution (Sigma Aldrich). The cells were washed twice with PBS (-/-) (Dulbecco's Phosphate Buffer Saline, without calcium and magnesium, Life Technologies, Germany) and treated with trypsin by adding 500 µl 0.25% Trypsin solution in PBS-EDTA (PBS with 0.02% EDTA) for 3 minutes. The trypsin activity was blocked by adding 9.5 ml of fresh MCF7 medium. The cell concentration of the resulting suspension was determined by hemocytometer analysis and a total number of 40000 cells in 100

[4] R. Wacker and C. M. Niemeyer, *Curr. Protoc. Nucleic Acid Chem.*, 2005, **Supplement 21**, 12.7.1.

µl of MCF7 medium was pipetted onto the slides and allowed to sediment for 10 minutes before adding 3 ml of fresh MCF7 medium. The cells were incubated on the EGF functionalized array overnight and then fixed by washing the slide three times with PBS (+/+) and cooling on ice. Subsequently, 1 ml of fixing solution (70% cold MeOH and 30% cold Acetone) was pipetted onto the cooled slide and incubated for a maximum of 15 minutes. After removal of the fixing solution the slide was washed twice with PBS (+/+) and it was blocked again with 150 µl of CAS-Block for 20 minutes at room temperature. After removal of the blocking solution, 150 µl of a 1:300 solution of primary antibody αP-EGFR (anti phospho Y1068 EGFR, Abcam ab32430) in PBS (+/+) were added and incubated overnight at 4°C in a humidity chamber. Subsequently, the slides were washed several times in PBS and incubated with 150 µl of a 1:300 solution of fluorophore conjugated secondary antibody (Goat anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488 conjugate, Life technology) in PBS (+/+). Finally, the slides were washed several times in PBS and incubated with 150 µl of a 1:1000 solution of DAPI staining stock solution (14.3 mM) in PBS (+/+).

2. Oligonucleotide Sequences

All amino modified single strand oligonucleotide (aF1, aF5, aF6, aF9, aF10, aF1_12, and aF10_12) were purchased from Sigma Aldrich, Germany. The sequences of all used capture oligonucleotides are shown in Table S1.

Table S1 Sequence of oligonucleotides.

Name	Sequence (5' - 3')	Modification
aF1	CCT GCG TCG TTT AAG GAA GTA C	5’ amino link (C12)
aF5	GGT CCG GTC ATA AAG CGA TAA G	5’ amino link (C12)
aF6	GTC CTC GCC TAG TGT TTC ATT G	5’ amino link (C12)
aF9	GTG GAA AGT GGC AAT CGT GAA G	5’ amino link (C12)
aF10	GGA CGA ATA CAA AGG CTA CAC G	5’ amino link (C12)
aF1_12	dT8 CCT GCG TCG TTT	5’ amino link (C12)
aF10_12	dT8 GGA CGA ATA CAA	5’ amino link (C12)
tcF9	CTT CAC GAT TGC CAC TTT CCA C	5’ thio link (C12)
tcF10	CGT GTA GCC TTT GTA TTC GTC C	5’ thio link (C12)

3. Optical Levelling Method

The pen array with a glass support is first glued to a microscopic glass slide with two component epoxy resin adhesive (UHU, Germany). Then, the whole glass slide is glued to the bar of the custom made holder mounted into the instrument (Fig. S1). As the pen array is glued manually, a small misalignment of the plane of the pen array in respect to the surface of the substrate is expected. To compensate this misalignment, the levelling of the stamp is performed by an optical leveling method (Fig. S2), controlling the elastic tip deformation of the pen array at the point of pen array-surface contact on a clean sacrificial silicon slip. The silicon surface also gives a very good contrast to see the square formation at the point of contact.

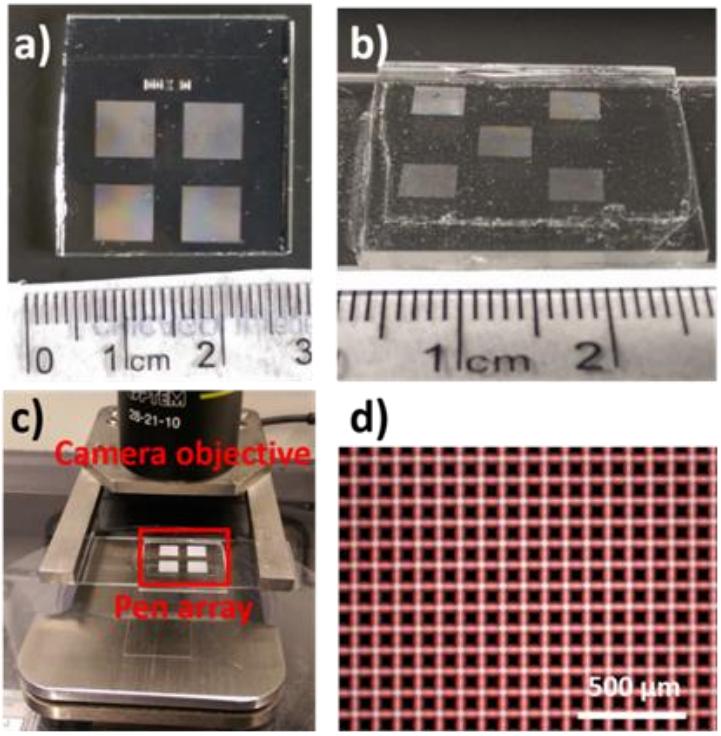

Fig. S1 A PPL pen array with sections of pens and experimental set-up. a) A pen array having 10^4 pens in total, with four sections with 2500 pens each. b) Pen array with five sections, each having 2000 pens. c) The custom made holder holding the pen array in a NLP 2000 system. The pen array is hold in a fixed position and the piezo-controlled xyz-stage (also capable of tilting) controls the lithography process. The camera objective is mounted above the holder, enabling to monitor the writing and optical levelling process. d) One section of a pen array after spin coating. The ink (here TAMRA-azide) homogenously spreads over the pen array, additionally building ink reservoirs around the pens.

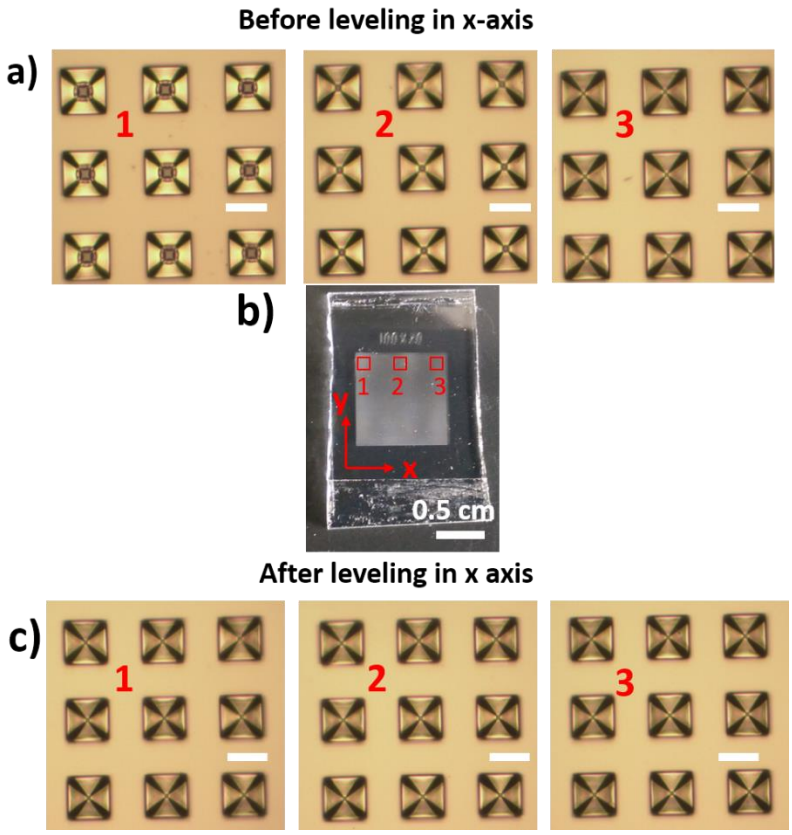

Fig. S2 Optical levelling method. a) All three position before levelling the pen array to the surface of the substrate. Position 1, 2, 3 are shown by red boxes on the pen array in (b). c) The same positions after the levelling. All square formed at the centre of the pen are equal in size. Scale bars equal 50 µm.

First, the NLP 2000 sample stage is initialized and set into origin position (T_x and T_y = 0). The sample stage is approached towards the pen array until a square forms by (elastic) pen deformation at the contact point on the silicon slip. When the stage is about ~100-200 µm away from pen array, shadows around the pens will appear due to the reflective silicon surface, guiding to approach more carefully until contact is achieved. Initially, some pens of the pen array might touch harder than the others in x-axis or y-axis, because the plane of the pen array is not parallel to the surface of the substrate. The square formation at the contact points is compared along the stamp by optically observation through the camera while moving the objective in different positions (*e.g.* position 1, 2 and 3, Fig. S2). The substrate stage is tilted in angular direction step by step until the plane of the substrate stage is parallel to the plane of the pen array by using the goniometer stage

(T_x, T_y) of the NLP 2000 system. For example, at position 1 (before leveling in x-axis) the square formation is bigger (meaning the pens touch harder) than at position 2 and 3. Therefore, the stage has to be tilted in x-axis so that position 3, 2 and 1 exhibit equally sized squares.

To ensure the 2D pen arrays are parallel to the surface, the above described process is repeated in both x- and y-axis. The crucial point in the levelling process is comparing the squares sequentially after each tilting step of the pen array because even a small amount of angular misalignment can make a huge difference in the features produced by pens that are 1 cm away. The stage should not touch the pen array during the tilting process of the substrate stage; otherwise it is more likely to destroy the apex of pens. The levelling method of the pen array having five or four sections is similar to the single section pen array, but instead of levelling the whole pen array (2×2 cm^2) only one section is levelled and conformity is assumed for the other sections.

4. Offset Correction

To calculate the offset values, a sacrificial sample is used to print a test pattern of a single dot feature per pen, as described in the main text (Fig. 1 b). After these "approach dots" of all five section are stamped, the offset correction values in x- and y-axis can be measured and calculated for each section from the resulting merged fluorescence image (Fig. S3). By taking ink 1's approach dots (red dots) as reference, parallel lines are drawn through the reference dots and all other inks' approach dots in x and y direction. The offset correction values are determined by measuring the distance between parallel lines in both x- and y-direction. The set of parallel lines and measured distances are shown in different colors according to the different ink numbers, all with respect to ink 1. A negative distance denotes that the respective approach dots are positioned lower or behind the reference dots in direction of the x- or y-axis. In case of a perfectly aligned pen array, all five approach dots would overlay each over, though this is usually not tested to prevent intermixing of inks on the stamp. A fresh substrate is introduced after calculating the offset correction value and the desired multi-color pattern is generated taking into account all measured offset values and the gap value between sections.

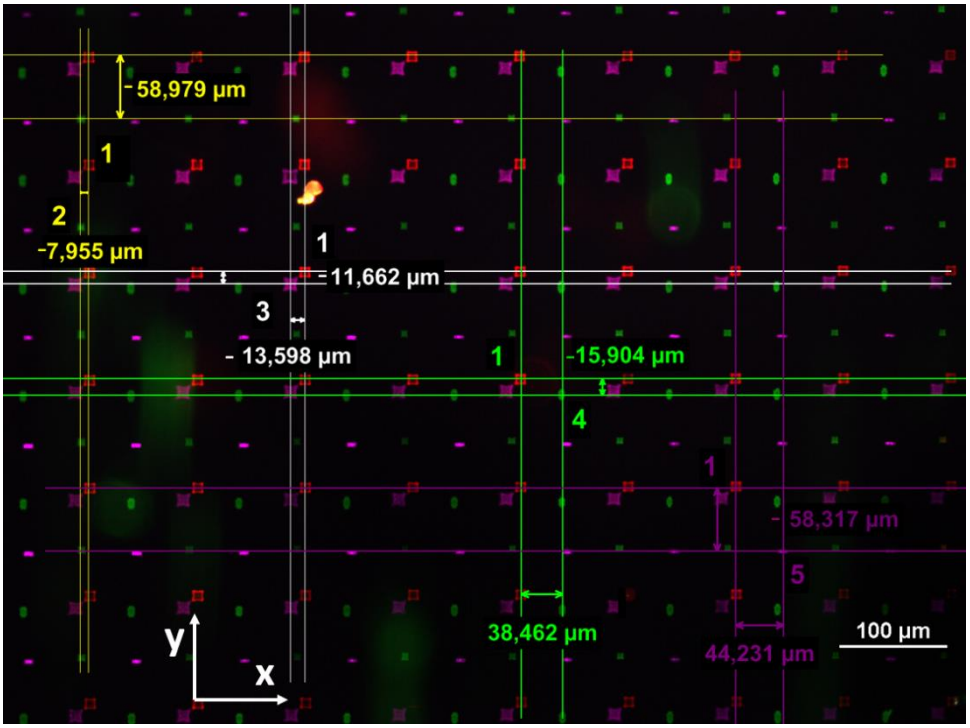

Fig. S3 Offset correction. Merged fluorescence images of all approach dots from a five ink section stamp. Here, a five sections pen array is used with five coatings, ink 1 to 5. Ink 1 is TAMRA-azide (red), ink 2 and 4 are Alexa 488-azide (green), Ink 3 is cy5-azide and ink 5 is Alexa 647-azide (both in magenta color). Alexa 488 azide ink is used in two different section because of the lack of different fluorescently labelled inks. The emission wavelength of cy5- and Alexa 647-azide are overlapping in the same microscope filter (cy5 channel). To differentiate between inks with the same fluorescent appearance, the approach features are patterned in shapes of lines (here ink 2 and 4, ink 3 and 5, respectively).

5. CuAAC Control Experiments

In order to ensure that CuAAC is taking place as expected, control experiments via microchannel cantilever spotting (µCS).[5] Fluorescent ink with and without copper catalyst was spotted on alkyne terminated glass and on non-functionalized bare glass. After washing, only ink containing copper catalyst and only on alkyne terminated glass showed a stable binding to the surface. The negative controls (no copper catalyst on alkyne terminated glass and ink with copper catalyst on bare glass) resulted in only negligible binding, most likely by unspecific adhesion (Fig. S4).

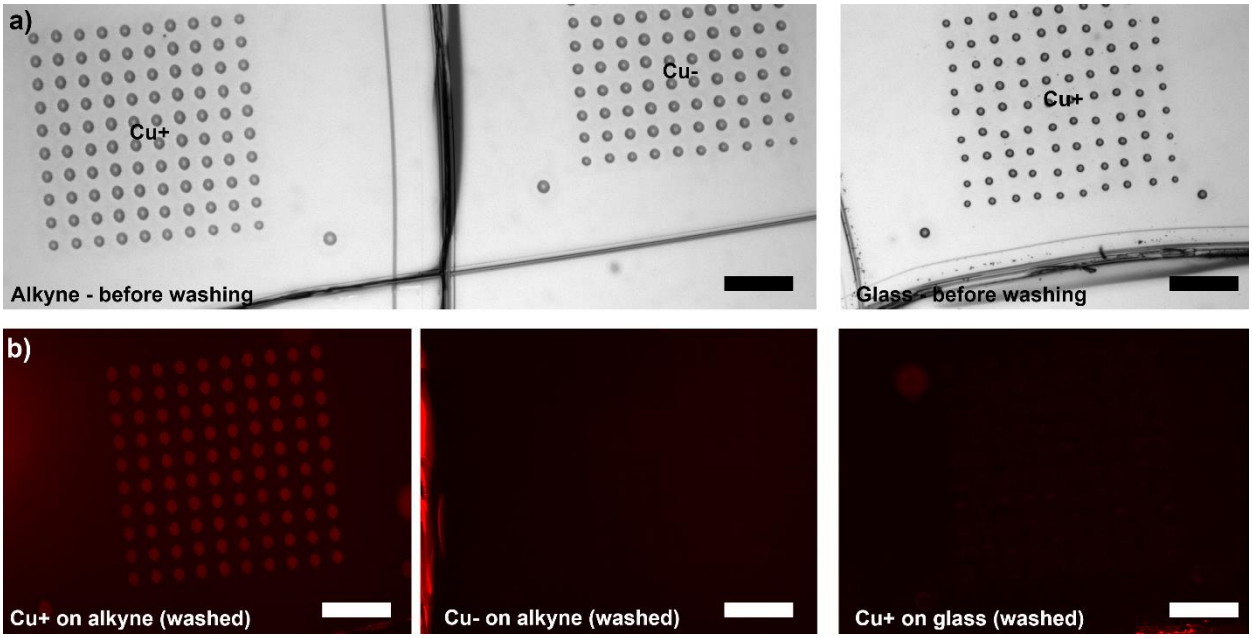

Fig. S4 CuAAC control experiments. a) shows bright field images of an alkyne terminated glass (left) and a bare glass (right) directly after µCS. All samples are marked by scratches to reliably identify positions after washing. Ink droplet arrays of copper containing (Cu+) and inks without copper (Cu-) are easily discernible in bright field. (b) shows the same areas of the samples after washing. Only the array with Cu+ ink on alkynized glass is visible in fluorescence after washing, indicating that binding takes place (as expected) only on alkynized glass and in the presence of copper catalyst. Scale bars equal 150 µm.

[5] M. Hirtz, A. M. Greiner, T. Landmann, M. Bastmeyer, and H. Fuchs, *Adv. Mater. Interfaces*, 2014, **1**, 1300129.

6. DNA Microarray Characterization

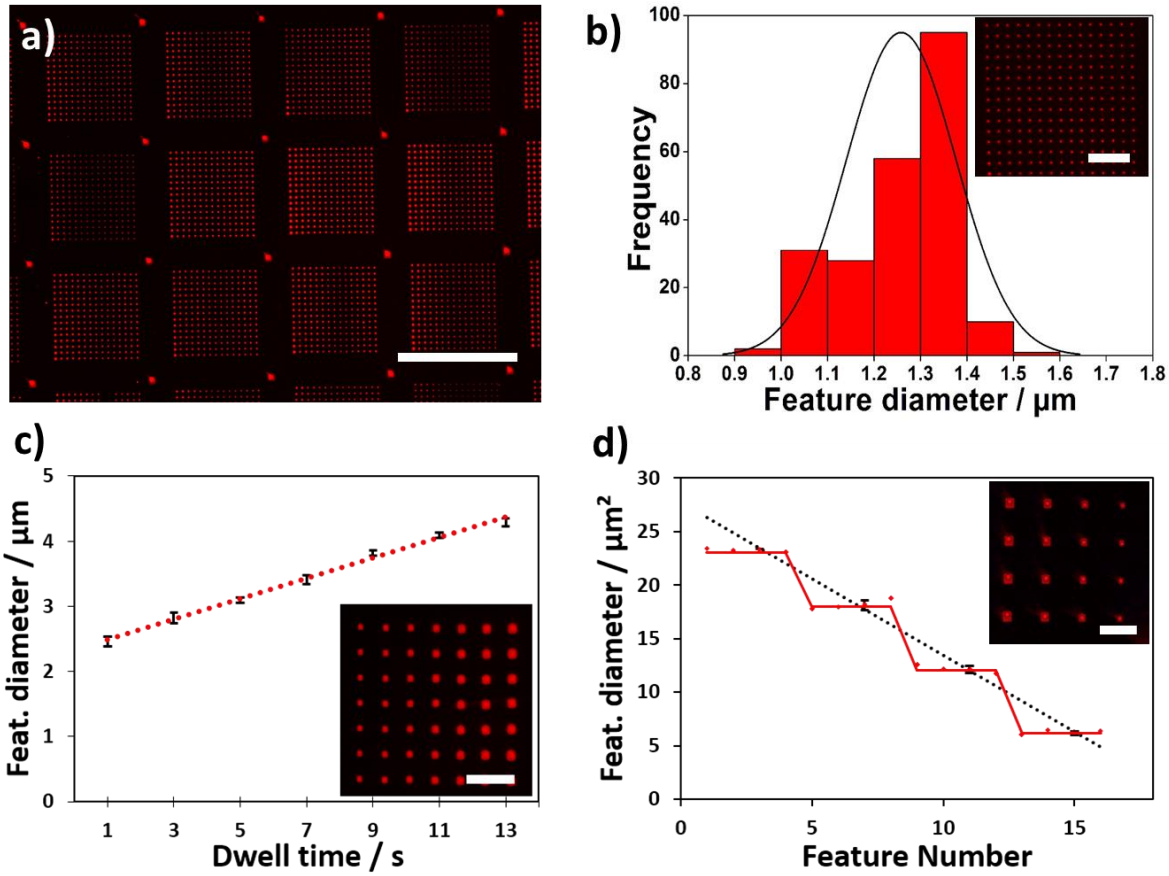

Fig. S5 Fluorescence microscopy image of oligo (acF10) array on functionalized glass. a) A fluorescence image of large area dot pattern by PPL pen array after hybridization with c_oligo (acF10_cy3). The scale bar equals 100 µm. b) Histogram of dot feature diameters in a close up of the 15 x 15 dot array (inset, scale bar 20 µm) in (a), average diameter of the dot features is (1.26 ± 0.12) µm. c) Graph of the dot feature diameter versus dwell time for the microarray (inset, scale bar 20 µm). The dashed line is a linear fit to the average feature diameter for each dwell time. d) Feature area of a 4 × 4 array with features written by applying varying pressures within each column (inset, scale bar equals 20 µm). Solid line shows the average feature area in each column, the dashed line is a linear fit to the average feature area.

To optimize the writing conditions and controlled feature size, experiments were done with varying dwell time and pressure on the PPL pen array (Fig. S5). For hybridization, slides were

washed with TETBS buffer (20 mM Tris-Cl, 5mM EDTA, 150 mM NaCl, 0.05 vol-% Tween20, and pH 7.5) and surface was blocked by CAS-block to avoid nonspecific binding. The complementary oligo (c_oligo) were incubated then for 1-1.5 hour at ambient conditions, and washed with TETBS buffer and ultrapure water. Fig. S5 shows the fluorescence microscopy image of microarrays patterned with oligonucleotide probe (aF10) and hybridized with the respective fluorophore labeled complementary oligo (cF10_cy3). A large area microarray fabricated by PPL (1×1 cm^2) is shown in Fig. S5a (20x low magnification overview, exposure time 5 s). A histogram of average dot feature diameter is shown in Fig. S5b with a close-up of a 15×15 dot pattern written by single pen at 65% RH and with 5 s dwell time in the inset (exposure time 1 s). The distribution of dot size in this array shows an average dot diameter of (1.26 ± 0.12) µm.

By varying the contact time of the PPL stamp with the sample surface, the amount of ink delivered can be controlled. Fig. S5c shows the dependency between dot diameters and dwell time. In the inset image (exposure time 850 ms), a 7×7 oligo pattern was written with different dwell times of 1 s, 3 s, 5 s, 7 s, 9 s, 11 s and 13 s, respectively, in each column. The linear fit in the graph shows the linear dependence of dot diameter on dwell time. The average dot diameter of the dot for 1 s dwell time was (2.46 ± 0.08) µm, (2.82 ± 0.08) µm for 3 s, (3.09 ± 0.04) µm for 5 s, (3.41 ± 0.06) µm for 7 s, (3.82 ± 0.04) for 9 s, (4.09 ± 0.04) for 11 s and (4.29 ± 0.06) µm for 13 s.

Square features can be generated by exploiting the elastic pen deformation on contact with the sample surface with higher forces (Fig. S5d inset, exposure time 780 ms). Here, a 4×4 square array of an aF10 oligo ink was written with different amount of pressure applied to the stamp in each column: each feature within a column is written with the same pressure on the pen array. For each column, the stamp was raised by an additional 2 µm, resulting in reduced pressure on the stamp. The average area of the dot in the first column was (23.26 ± 0.14) µm², in the second column (18.17 ± 0.45) µm², in the third column (12.18 ± 0.34) µm², and in the fourth column (6.30 ± 0.18) µm². The linear fit (black dashed line) shows the feature area depends linearly on the z-extension. The solid red line shows the linear fit of average feature area in each column with different z-extension.

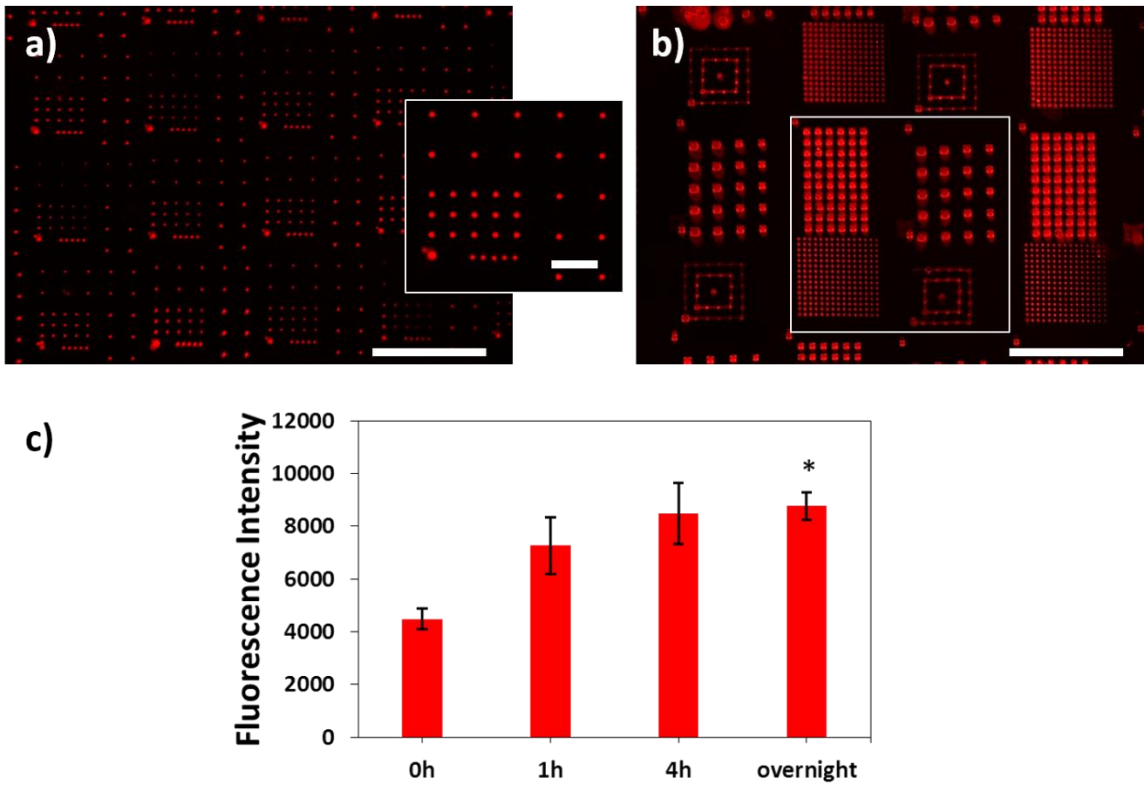

Fig. S6 Effect of time between lithography and sample washing on the amount of surface-immobilized oligonucleotide. a) Fluorescence microscopy image of an oligonucleotide (acF10_cy3) array on functionalized glass with differently pitched arrays (20, 10, 5 µm), the inset image is the dot array patterned by a single pen. b) A square and (20 µm, 10 µm, 5 µm) pitched dot arrays (the subpattern marked by the white box is patterned by a single pen). The scale bars equal 100 µm in the main images and 20 µm in the inset. c) Graph showing the comparison of fluorescence intensity of microarrays (acF10_cy3) washed and hybridized directly after writing and with varying incubation time (1h, 4h and overnight) between lithography and washing and hybridization. The obtained intensity after hybridization increases with the time between lithography and washing and saturates at about 4h. *The overnight comparison was done on a different batch of microarrays.

Fig. S6 shows some more patterns that were used to estimate the impact of incubation time between patterning and washing to remove excess ink for the immobilization of DNA. Up to 4h waiting time before washing after lithography took place increases the signal strength after hybridization. This implicates, that more and more of the printed oligonucleotides binds to the array during this

time. After 4h the substrate saturates and even incubation overnight before washing the samples does not lead to higher intensity when subsequently doing the hybridization steps.

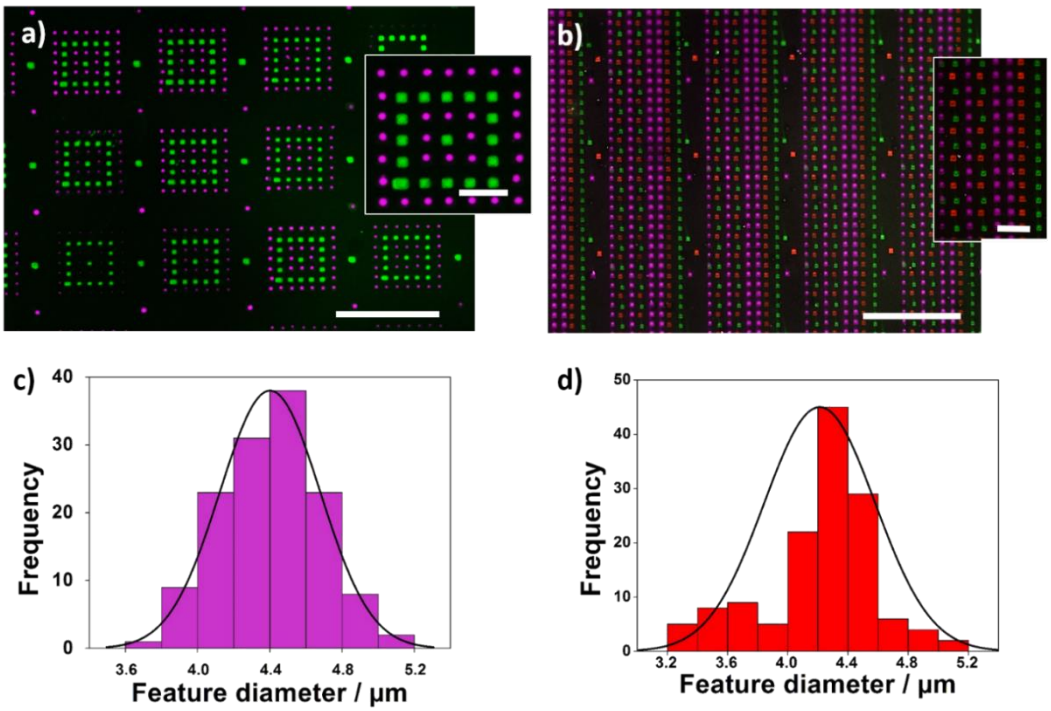

Fig. S7 Multi-color pattern examples with fluorescent labelled oligonucleotides. a) Fluorescence micrograph of oligonucleotide arrays hybridized with fluorophore-tagged complementary sequences containing two (acF9-cy5 and acF10-FITC), and b) three different oligonucleotides (acF9_cy5, acF10_cy3, and acF1_FITC), respectively. Scale bars equal 100 µm in main images and 20 µm in inset images. c) and d) show histograms of the dot size distribution in the multicolor microarray shown in Fig. 2a (main text). The feature diameter for both inks is similar, suggesting that control over the size is retained even for different inks.

Some additional examples of multicolor PPL with more complex design are shown in Fig. S7. The histograms (Fig. S7c, d) show the distribution of dot sizes in the multi-color array with two oligomers acF9_cy5 and acF10_cy3. The average dot diameter was determined to (4.4 ± 0.28) µm and (4.2 ± 0.38) µm, respectively. It clearly suggests that control over the feature size is retained even during multiplexing.

7. Regeneration of Microarray

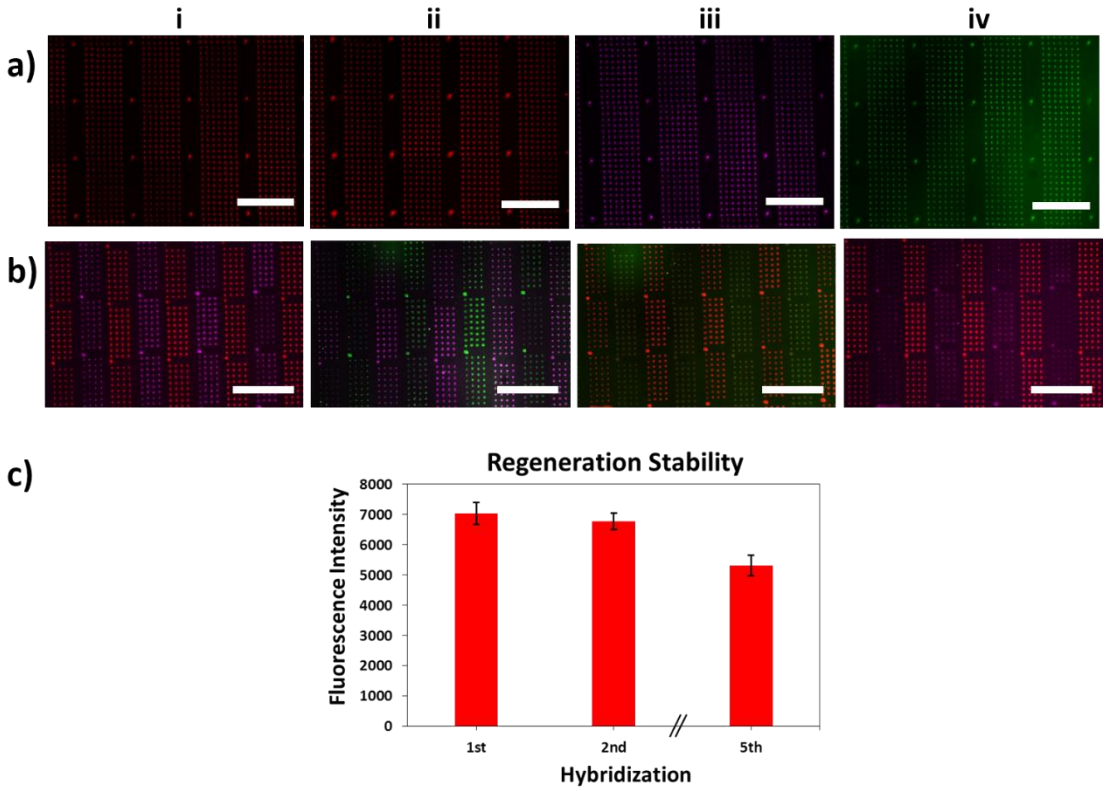

Fig. S8 Regeneration of DNA microarrays. Fluorescence microscopy images of a microarray of aF10 oligo (a), and two different oligos aF10, aF9 (b) after hybridization with complementary fluorophore tagged target oligomers. The microarray in (a) is shown after 1st and 2nd hybridization with cF10_cy3 (red), 3rd with cF10_cy5 (magenta) and 4th with cF10_FITC (green) in (i), (ii), (iii) and (iv), respectively. The multiplexed microarray in (b) is shown after 1st hybridization with cF9_cy5 (magenta) and cF10_cy3 (red), 2nd with cF9_FITC (green) and cF10_cy5 (magenta), 3rd with cF9_cy3 and cF10_FITC (green), 4th with cF9_cy5 (magenta), cF10_cy3 (red). The scale bars equal 100 µm in all images. c) The bar chart shows the fluorescence intensity of an oligo microarray after 5 times hybridization (only hybridizations with the same fluorophore were included in the chart for better comparability). The overall intensity of the arrays decreases by 24 % after 5 times regeneration.

The regeneration of a PPL printed DNA microarray is shown in Fig. S8 to demonstrate the stability and reusability of the microarrays. After the first hybridization, the microarray was washed with 50 mM sodium hydroxide (NaOH), which separates the target complementary oligo from the

capture oligo. The re-hybridization of the microarray was performed either with the same fluorophore labelled complementary oligo or different fluorophore labelled complementary oligo. For the multiplexed microarray, the oligo complementary to the capture oligomers were hybridized with different fluorescent dyes (Fig. S8b). The arrays can clearly be reconfigured by regeneration and subsequent re-hybridization without any deterioration on pattern fidelity. The graph in Fig. S8c shows the regained fluorescence intensity of the microarray after several regeneration cycles (only cycles with the same fluorophore were tabulated to ensure comparability of the intensities). The overall intensity (thus binding efficiency) of the arrays decreases by 24 % after 5 times regeneration (Fig. S8c).