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A Low-Cost Laser-Based Nano-3D Polymer Printer for Rapid Surface Patterning and Chemical Synthesis of Peptide and Glycan Microarrays

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A low-cost laser-based printing setup is presented, which allows for the spot-wise patterning of surfaces with defined polymer nanolayers. These nanolayer spots serve as a "solid solvent," embedding different chemicals, chemical building blocks, materials, or precursors and can be stacked on top of each other. By melting the spot pattern, the polymer-embedded molecules are released for chemical reaction. This enables researchers to quickly pattern a surface with different molecules and materials, mixing them directly on the surface for high-throughput chemical synthesis to generate and screen diverse microarray libraries. In contrast to expensive ink-jet or contact printing, this approach does not require premixing of inks, which enables in situ combinatorial mixing. Easy access and versatility of this patterning approach are shown by generating microarrays of various biomolecules, such as glycans for the first time, to screen interactions of antibodies and lectins. In addition, a layer-by-layer solid-phase synthesis of peptides directly on the microarray is presented. Amino acid–containing nanolayers are repeatedly laser-transferred and reacted with the functionalized acceptor surface in defined patterns. This simple system enables a reproducible array production, down to spot-to-spot distances of 100 µm, and offers a flexible and cheap alternative to expensive spotting robot technology.

1. Introduction

Microarrays allow for parallelized high-throughput screenings, which are important for the analysis of interactions of biopolymers,[1] such as proteins,[2] peptides,[3] oligonucleotides,[4] and glycans.[5] Meanwhile, microarrays have become a standard tool for basic research, as well as for diagnostics, drug, and vaccine development. However, to become as omnipresent as microtiter plates in medical diagnostics, they have to overcome some obstacles; biomolecule arrays still lack cost efficiency and ready access. Therefore, we recently developed a novel laser-based method for the cost-efficient combinatorial synthesis of high-density microarrays with natural and synthetic monomers.[6] In this laser-induced forward transfer (LIFT) approach, we laser-transfer tiny polymer nanolayer spots from donor slides (**Figure 1**A), which were spin-coated with a mixture of polymer and activated biomolecules (Figure 1B), to a functionalized acceptor slide. By heating the transferred spot pattern, the polymer nanolayers, which can also be stacked (Figure 1C), melt and couple to the functionalized acceptor surface, without losing their spatial resolution.[6,7]

Yet, this setup[6] can only be assembled and handled by specialists and is limited to peptides and peptoides. Therefore, we have developed a low-budget microarray synthesizer system and also showcase a first application in carbohydrate research. Employing a low-cost commercial laser engraving system (<$100), which is based on standard Blu-ray drive components, we upgraded the system with an Arduino microcontroller, to have full control over pattern, laser power, and laser pulse duration. Such open source microcontrollers[8,9] have recently enabled a vast variety of research applications, for example, in measurement and analysis methods,[10,11] educational robotics,[12] cost-efficient laboratory solutions,[13,14] and RNA screening.[15]

Here, we show a versatile, easily available, and low-cost method to flexibly print microarrays for many different applications in carbohydrate and proteomics research.

2. Results

2.1. Low-Cost LIFT Setup

Based on a commercially available low-cost laser engraving system, we built a laser-transfer system by upgrading the

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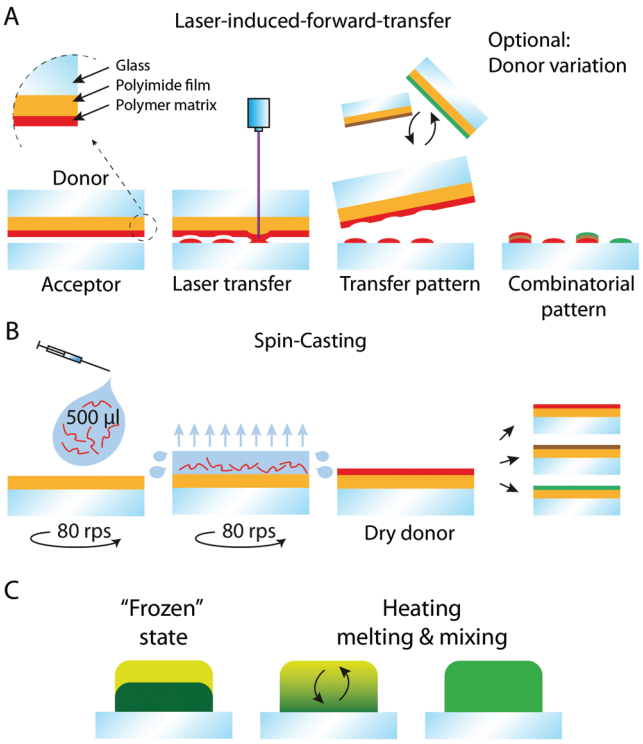

Figure 1. General procedure. A) Microarray generation with laser-induced forward transfer. B) Spin-coating of donor slides (glass slide ≈1 mm, covered with self-adhesive polyimide foil, ≈75 µm) with different polymer mixtures (e.g., polystyrene mixed with amino acid building block or fluorescent dye). C) Melting and mixing of transferred polymer spots (not to scale).

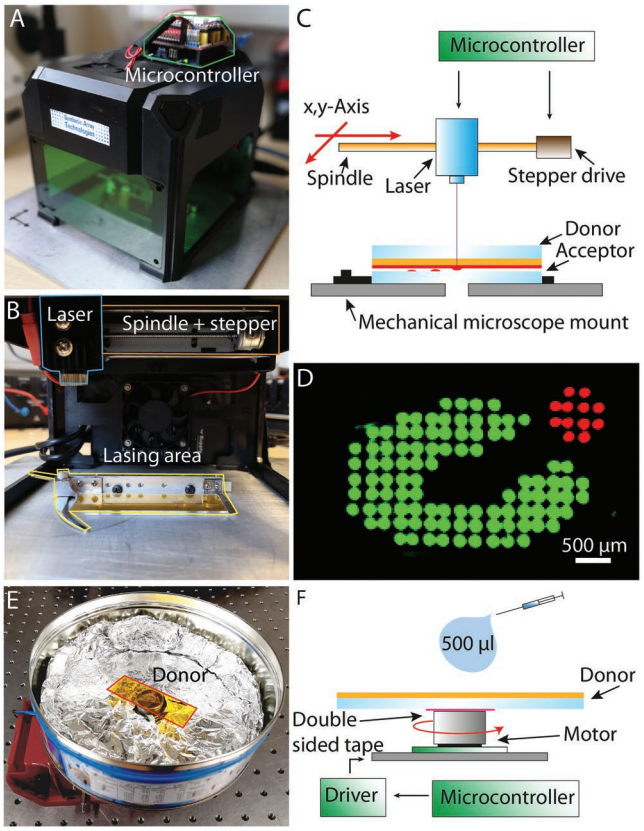

Figure 2. Low-cost LIFT system. A) Low-cost laser engraving system, upgraded with a microcontroller. B) Functional setup with components and (C) schematic overview. D) Microarray of the 9-mer peptides Flag- and HA-peptides, generated with the low-cost LIFT system (scale bar, 500 µm). E) Low-cost spin-coating device and (F) schematic overview.

electronics with an open source microcontroller including a stepper driver (**Figure 2**A–C).

Figure 2C shows a scheme of our low-cost LIFT setup. We use a typical clamp mechanism from a microscope sample mount to reproducibly position the acceptor surface (see the Supporting Information).

2.1.1. Low-Cost Spin-Coating Setup

A spin-coating (spin-casting) device is a versatile and easy-to-use setup to create thin and homogeneous films on planar supports.[16,17] In our case, a donor slide consists of a glass slide, covered with polyimide tape, which is spin-coated with a polymer matrix material, embedding the building blocks. We used different polymers, for example, polystyrene or copolymers thereof (see the Supporting Information). To build a low-cost spin-coater device (Figure 2E,F), we used the motor of a DVD drive in combination with a driving circuit. With a microcontroller, the speed of the motor can be controlled between optimal parameters of 60–80 rps. All electronic parts are fixed in a plastic box and the motor is fixed on top of this box. To avoid corrosion and short circuits, we covered all visible electronics with solvent-resistant tape and surrounded the spinning area with a metal box to make the process safe and collect excess solution. We use normal double-sided tape to fix a sample onto the motor axis.

The resulting coatings are indistinguishable from coatings done with an expensive commercial spin-coating machine, since the rotational speed is stable during the process (see Figure S3 in the Supporting Information).

2.2. General Procedure

Figure 1 shows the basic procedure for the production of microarrays. First, we prepare the donor slides by spin-coating a polymer mixture dissolved in an organic solvent, e.g., dichloromethane (DCM; Figure 1B). Next, we fix an acceptor slide in the microscope sample mount in the lasing area and put one donor slide on the acceptor slide (Figure 1A; Figure S2a, Supporting Information). Then, we load our desired pattern into the microcontroller[12] and start the process. The laser sequentially irradiates the donor for a defined time and power (typically 20 ms and 70 mW), which transfers parts of the polymer material from the donor to the acceptor slide. The typical material spot diameter d_{spot} is ≈100 µm, and the spot thickness h_{spot} is ≈10 nm.

When the transfer of the pattern is finished, we remove the donor slide and continue the patterning with other donors, incorporating other building blocks, until the desired pattern of different building blocks is finished. Finally, we remove

the acceptor and perform the actual synthesis, which involves heating, washing, and coupling steps (see the "Experimental Section").

2.3. Optimum Parameters and Resolution

We transferred a polymer incorporating a fluorescent dye with varying laser power (**Figure 3**A). The fluorescence signal and the spot size increase with increasing laser power, as we have shown before.[6]

Furthermore, we generated patterns (Figures S1 and S3b, Supporting Information) of various spot-to-spot distances (500, 250, 200, and 100 µm) for two overlapping differently dyed polymer mixtures (red and green). With optimum parameters, we reached a minimum pitch of 100 µm, where individual spots are still distinguishable.

To investigate the reproducibility of the LIFT system, we transferred these two polymer mixtures sequentially on top of each other in the same pattern shown before. The typical spot diameter is ≈100 µm. After the first transfers, we also removed the acceptor slide from the lasing area and, then, manually repositioned it in the lasing area for the next transfer. In Figure 3B, we show that we can reproducibly pattern two polymer mixtures each comprising a different fluorescent dye, on top of each other; the yellow color is a result of almost perfectly stacking red and green fluorescently dyed polymer spots (Figure 3C). The signals are systematically shifted for about 20 µm.

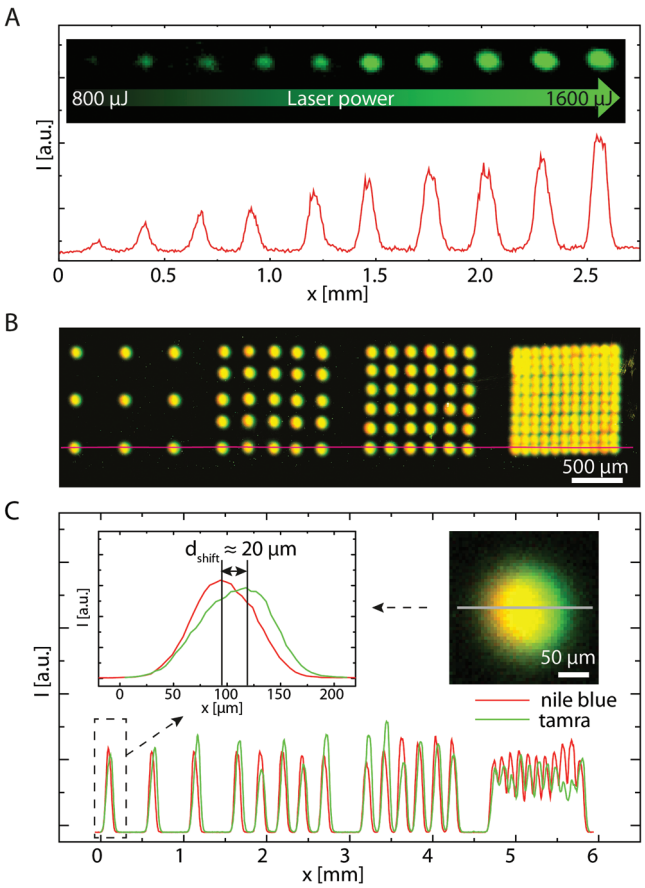

Figure 3. Transfer parameters and reproducibility. A) Transferred spots with laser energies from 800 µJ (40% power) to 1600 µJ (80%), line profile of the spot fluorescence intensity. B) Various spot-to-spot distances (500, 250, 200, and 100 µm), containing green (tetramethylrhodamine, TAMRA) and red (Nile blue) fluorescent dyes (green + red = yellow). Both dyes were consecutively transferred to the same locations on the surface. C) The line profile shows a process tolerance of ≈20 µm.

2.4. Mannose and Biotin Microarray

An α-D-mannose building block with an ethylene glycol spacer and an activated carboxyl terminus, attached to the anomeric center, was embedded in polymer and transferred in a spot pattern of 500 µm spot-to-spot distance. A complementary pattern of an activated biotin derivative embedded in polymer (shifted by 250 µm in *x*- and *y*-directions to the mannose pattern) was transferred to create an interlaced pattern.

Both building blocks were coupled to the surface in one heating step, followed by chemical washing to remove the polymer matrix. Then, the array was selectively stained with fluorescently labeled concanavalin A (ConA) lectin (red channel, 635 nm) and streptavidin (green channel, 532 nm), shown in **Figure 4**.

2.5. Low-Budget Peptide Microarray

Finally, we have performed a peptide microarray synthesis, comparing the here-presented low-cost LIFT system with our expensive and high-resolution LIFT system.[6] We synthesized an array of two different 9-mer peptides (**Figure 5**A,B; Figure S4, Supporting Information, Flag epitope amino acid sequence YDYKDDDDK, HA epitope sequence, derived from the human influenza hemagglutinin glycoprotein, YPYDVPDYA)

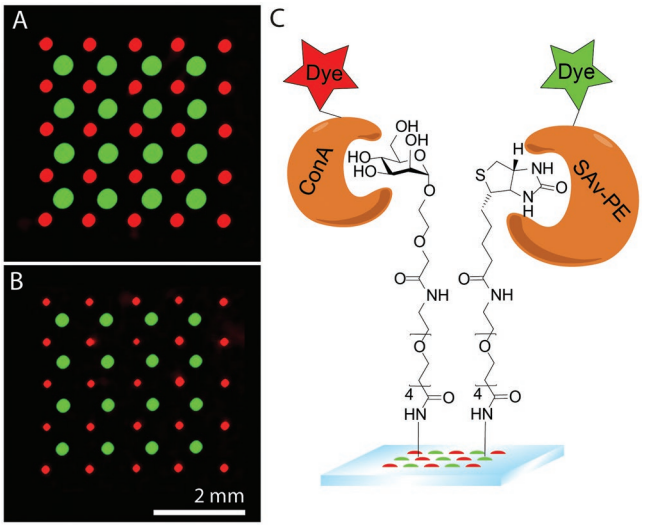

Figure 4. Mannose and biotin microarrays. Fluorescence scan of mannose stained with ConA (red) and biotin stained with streptavidin (SAv–PE, green) at (A) 100% and (B) 50% lasing power. C) Corresponding cartoon of the stained and coupled biomolecules.

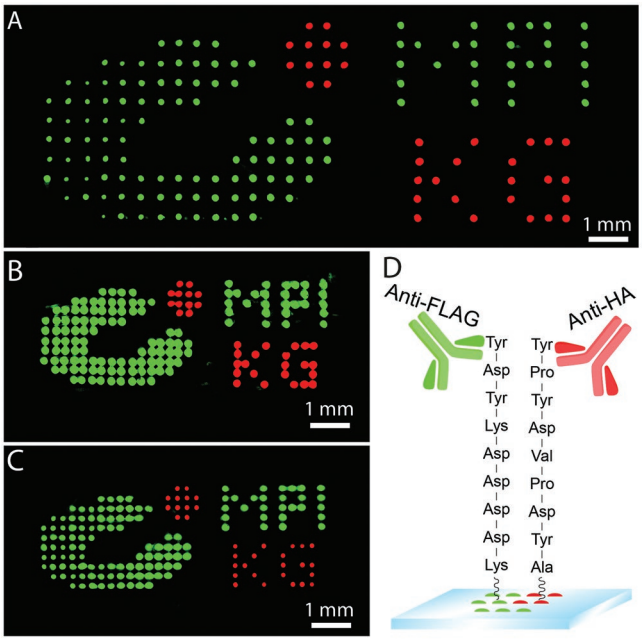

Figure 5. Fluorescence scan of synthesized peptide microarray. Synthesis was done with the low-cost LIFT system (laser power 80%) with spot distances of (A) 500 µm and (B) 250 µm. C) The same pattern was generated with the high-end LIFT setup. Flag (green) and HA (red) peptides were stained with their corresponding fluorescently labeled antibodies.

with pitches of 500 and 250 µm. This involved the transfer of specific patterns of amino acid building blocks for each layer, a coupling step in the oven (coupling and transfer were repeated once to increase the yield), followed by chemical washing steps. Thus, each spot represents 18 individual transfer processes on the same location of the acceptor slide, including manual repositioning in the sample area, coupling, and chemical washing, after each step. Figure 5C shows the same peptide microarray generated with the high-resolution LIFT setup. The peptide microarrays were stained with the corresponding antibodies to visualize the pattern. Figure S5 (Supporting Information) displays a topographical scan of such a microarray, where the binding of antibodies to individual peptide spots is visualized.

3. Conclusion

Our low-budget method allows for the rapid production of high-density microarrays of diverse biomolecules, comprising carbohydrates, peptides, and other biomolecules, to screen multiple different protein interactions, such as antibodies and lectins. The system is based on a readily available laser engraver, modified with common electronic components. Furthermore, we have also built a spin-coater from parts of an optical drive (DVD/Blu-ray). All components are easily available, and the total cost of this LIFT setup, including the spin-coating device, is <$200. In comparison to a conventional scientific grade device (≈$40 000), this is 200 times less expensive.

The relatively precise positioning of the laser system allows for the combinatorial patterning of surfaces with almost any polymer material. We show that it is possible to produce 9-mer peptides with this simple setup, which is comparable to the high-resolution LIFT method, reproducibly enabling a resolution of 250 µm (1600 spots cm^{-2}). Peptide arrays are becoming increasingly important in research, and several applications in immunology and diagnostics have been shown recently, for example, the development of therapeutic antibodies or diagnostic biomarkers.[18–24]

Although the spindle axes of this low-cost system are not highly precise (DVD drives also use optical calibration), which is visible by the slight distortion of the spot patterns (relative spot position in Figure 3), the accuracy of the absolute laser spot position is sufficient (<30 µm) for LIFT-generated arrays. Finally, we also show that together with more sophisticated mechanical positioning setup, a spacing of 100 µm should be still reproducible and precise, allowing for spot densities of up to 10 000 spots cm^{-2} in the future.

Furthermore, in comparison to typical spotting technologies, such as ink-jet, contact, or dip-pen lithography printing, this approach does not require premixing of the desired compounds or building blocks in one ink. Thus, it is possible to position multiple buildings blocks on top of each other, each embedded in polymer nanolayers in a "frozen" state. Thereby, the reaction or synthesis of complex molecular structures can be controlled and started by melting the polymer nanolayers in an oven, which results in mixing of the building blocks. Since the polymer spots are only a few nanometers thin, diffusion is efficient, which results in quasiinstantaneous mixing.[25] This should allow for many new applications in the future generation of microarrays.

Concluding, together with low-cost high-quality fluorescence scanning using smart phones,[10,11] our low-budget method allows for the synthesis and analysis of microarrays in any laboratory around the world, which also lends itself to academic education. This can quickly enable applied and fundamental high-throughput research in the fields of biotechnology, immunology, chemistry, and materials sciences.

4. Experimental Section

Donor Slide Preparation: 18 mg of SLEC PLT 7552 (Sekisui Chemical GmbH, Germany) was dissolved in 450 µL of DCM and 2 mg of *N*-[(9H-fluoren-9-ylmethoxy)carbonyl] (Fmoc)-protected- and pentafluorophenyl ester (OPfp)-activated L-amino acid in 50 µL of *N*,*N*-dimethylformamide (DMF) were added. The mixture was spin-coated (spin-casted) at 80 rps onto a microscope glass slide covered with self-adhesive polyimide foil (Kapton, DuPont, USA; cmc Klebetechnik GmbH, Germany; thickness of polyimide layer ≈ 25 µm, thickness of glue layer ≈ 45 µm). The materials and the spin-coating parameters were chosen according to ref. [16] to achieve donor film thicknesses between 200 and 500 nm.

Transfer of Nonactivated Mannose and Biotin Building Blocks: An amino-terminated poly(ethylene glycol) methacrylate/methyl methacrylate copolymer (PEGMA-*co*-MMA) coated and Fmoc-*β*-alanine functionalized glass slide (PEPperPRINT GmbH, Germany) was immersed in 10 mL of DMF for swelling. After 1 h (shaking 300 rpm) at room temperature, DMF was removed. The Fmoc-protected acceptor was deprotected with 20% piperidine in DMF (10 mL) for 20 min (shaking 300 rpm). After completion of the deprotection, the glass slides were washed 3× for 5 min with DMF (10 mL), 1× for 2 min MeOH (10 mL), 1× for 1 min DCM (10 mL), and then dried in a jet of air. Then, the Fmoc-protected diamino-trioxatridecan-succinamic acid (Fmoc-TTDS-OH, Iris Biotech GmbH, Germany) spacer was attached. The Fmoc-TTDS-OH spacer (50 µmol, 27 mg) was dissolved in DMF (250 mL) followed by addition of *N*,*N*′-diisopropylcarbodiimide

(DIC) (150 µmol, 18.9 mg, 23.2 µL) and hydroxybenzotriazole (HOBt) (50 µmol, 6.76 mg). The resulting solution was deposited on top of the acceptor slide under ambient atmosphere and incubated overnight. Fmoc-deprotection of the acceptor was attained with 20% piperidine in DMF (10 mL) for 20 min (shaking 300 rpm). After completion of deprotection, the glass slides were again washed 3× with DMF, 1× with MeOH, 1× with DCM for 1 min, and then dried in a jet of air. The donor glass slides were prepared in the following manner: nonactivated substances (biotin and mannose building blocks) were activated in situ (18 mg of SLEC PLT 7552, Sekisui Chemical GmbH, Düsseldorf, Germany, polymer matrix, 450 µL of dry DCM, 50 µL of dry DMF, and 6 and 4 µmol in case of mannose and biotin, respectively, 6 µmol of DIC, 6 µmol of PfpOH). The mixtures were spin-casted onto donor slides.

After the patterning of an acceptor slide with different monomers, the coupling reaction was initiated by heating the acceptor slide in an oven to 90 °C for 1 h under argon atmosphere. Afterward, the slide was washed twice with acetone (10 mL) for 2 min to remove the remaining matrix and dried in a jet of air.

Mannose and Biotin Staining: The acceptor was incubated with Rockland blocking buffer (1 mL, at 150 rpm) to reduce unspecific binding. After a short wash in "standard buffer" (0.05% (v/v) Tween 20 (Sigma-Aldrich, USA) in phosphate-buffered saline (PBS)), the slides were incubated for 1 h in the dark with a mixture of streptavidin–phycoerythrin (SAv–PE, 1 µg mL^{-1}) and ConA lectin (2 µg mL^{-1}) in "staining buffer," containing 10% (v/v) Rockland Blocking buffer and 0.05% (v/v) Tween 20 (Sigma-Aldrich, USA) in PBS. Finally, the slides were washed with standard buffer three times for 5 min, briefly dipped in 1×10^{-3} M tris(hydroxymethyl)aminomethane (Tris) buffer to remove the remaining salts, and dried in a jet of air.

Peptide Array Synthesis: For the peptide array synthesis a PEGMA-*co*-MMA Fmoc-*β*-alanine (PEPperPRINT GmbH, Germany) functionalized surface served as the acceptor slide. First, the slide was preswelled 20 min in DMF and deprotected 20 min using 20% (v/v) piperidine in DMF followed by washing. The standard washing procedure was performed as described: the slide was immersed three times in DMF for 5 min, one time in methanol for 2 min and one time in DCM for 1 min and finally dried. The laser-assisted transfer of the amino acid pattern from different donor slides to the acceptor was performed, followed by coupling at 90 °C for 60 min under inert gas atmosphere in an oven. Then, a short washing step in acetone was performed, and the transfer and coupling steps were repeated once again with the same pattern. Afterward, remaining free amino groups were capped, immersing the slide in a solution containing 10% acetic anhydride, 20% *N*,*N*-diisopropylethylamine (DIPEA), and 70% DMF (v/v/v), first for 1 min in an ultrasonic bath and then for 30 min with a fresh capping solution. Then the standard washing was performed. The Fmoc-protected amino groups on the surface were deprotected using 20% (v/v) piperidine in DMF. The steps were repeated with the respective amino acid patterns to synthesize the desired peptides HA (YPYDVPDYA) and Flag (YDYKDDDDK). After the peptide synthesis, the side chains of the amino acids were deprotected by washing the acceptor slide three times for 30 min in a solution containing 51% trifluoroacetic acid (TFA), 44% DCM, 3% triisobutylsilane, and 2% water (v/v/v/v). Then, a 5 min wash in DCM was performed, followed by 30 min immersion in 5% (v/v) DIPEA in DMF. Finally, the standard washing procedure was performed and slides were dried in a jet of air.

Peptide Staining: After the synthesis, the peptide array was stained with the antibodies anti-HA (conjugated with a Cy5 fluorescent dye) and anti-Flag (monoclonal Anti-Flag M2-Cy3 Sigma-Aldrich, USA). First, the acceptor slide was incubated with Rockland blocking buffer (Rockland Immunochemicals, USA) to reduce the unspecific binding of the antibodies. Then the slide was incubated with 1:1000 diluted anti-HA and anti-Flag in staining buffer containing 10% (v/v) Rockland Blocking buffer and 0.05% (v/v) Tween 20 (Sigma-Aldrich, USA) in PBS for 60 min. Finally, the slide was washed three times for 2 min with 0.05% (v/v) Tween 20 in PBS and dipped in 1×10^{-3} M Tris buffer with pH 7.4 and dried in a jet of air.

Fluorescence Scanning: Fluorescent image acquisition was performed with the fluorescent scanner Genepix 4000B (Molecular Devices, USA) at the wavelengths 532 and 635 nm with a laser power of 10%, a resolution of 5 µm, and a photo multiplier (PMT) gain (PMT) of 600.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

F.F.L. is named on pending patent applications related to laser-based microarray synthesis.

Keywords

combinatorial synthesis, laser-induced forward transfer, microarray, open source, solid phase synthesis

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