Generation of miniaturized planar ecombinant antibody arrays using a microcantilever-based printer

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Abstract

Miniaturized (Ø 10 μm), multiplexed (>5-plex), and high-density (>100 000 spots cm\textsuperscript{−2}) antibody arrays will play a key role in generating protein expression profiles in health and disease. However, producing such antibody arrays is challenging, and it is the type and range of available spotters which set the stage. This pilot study explored the use of a novel microspotting tool, Bioplume\textsuperscript{TM}—consisting of an array of micromachined silicon cantilevers with integrated microfluidic channels—to produce miniaturized, multiplexed, and high-density planar recombinant antibody arrays for protein expression profiling which targets crude, directly labelled serum. The results demonstrated that 16-plex recombinant antibody arrays could be produced—based on miniaturized spot features (78.5 um\textsuperscript{2}, Ø 10 μm) at a 7–125-times increased spot density (250 000 spots cm\textsuperscript{−2}), interfaced with a fluorescent-based read-out. This prototype platform was found to display adequate reproducibility (spot-to-spot) and an assay sensitivity in the pM range. The feasibility of the array platform for serum protein profiling was outlined.

Keywords: antibody arrays, miniaturization, printing, microcantilever, recombinant antibodies

(Some figures may appear in colour only in the online journal)

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| CV           | coefficient of variation |
| DPN          | dip-pen nanolithography |
| LOD          | limit of detection |
| scFv         | single-chain fragment variable |

Introduction

Technology platforms which provide rapid, semi-/quantitative, multiplex, and high-resolution protein expression profile maps of crude samples, such as serum, in the microliter scale, are still, \textit{de facto}, a rate-limiting step for biomarker discovery and the subsequent clinical development of personalized medicine [1, 2]. Adopting parallelized and miniaturized microspot assays might resolve, as postulated by the ambient analyte theory [3, 4], these technical limitations. To this end, high-performing antibody arrays, predominantly in microscale, have been successfully developed [5–8] and applied to various biomarker discovery endeavours [9–12]. However, producing antibody arrays is challenging, and it is the type
and range of available spotters which set the stage [5]. Using predominantly non-contact printers based on ink-jet technology, antibody microarrays have been produced and applied having an overall foot print of <1 cm², based on \(18 \times 10^3 \mu m^2\) (Ø ~ 150 μm)-sized spots at a density of ≤2000 spots cm\(^{-2}\); and a multiplicity of <850 different antibodies/array [5–7, 13]. In an effort to evolve the array platform even further with respect to both spot density (>100,000 spots cm\(^{-2}\)) and spot size (<0.8 μm\(^2\), Ø < 1 μm), the first conceptual antibody (protein) nanoarrays have been produced [14–23]; for review see [24]. A set of technologies are available for producing such nanoarrays, e.g. atomic force microscopy (AFM) [25], dip-pen nanolithography (DPN) [26], nano-fountain probe [27, 28], nanoimprint lithography [16], and various nanodispensers [20, 22, 29]. Albeit successful, these nanoarray layouts have been found to be associated with a set of key methodological shortcomings. First, reducing the spot size to <1 μm might result in an impaired rather than improved assay performance [4]. Second, the nanoarray production technologies are, so far, compatible with generating mainly 1-plex arrays [14–24, 26, 29]. Third, set-ups for sensitive (fluorescent-based) read-out of high-density nanoarrays remain to be established [24].

Recently, it was highlighted that these three technological bottlenecks might be bypassed by aiming for miniaturized antibody arrays based on submicron-sized (Ø 10 μm) rather than nanosized (Ø < 1 μm) spot features [25, 30–34]. Using a DPN-based desktop printer (NL3000TM), we have produced the first 12- and 48-plex planar recombinant antibody arrays, based on 78.5 μm\(^2\) (Ø 10 μm)-sized spots at a density of 38,000 spots cm\(^{-2}\), interfaced with a fluorescent-based read-out [33, 34]. To the best of our knowledge, only one other complete microprinter system is currently at hand, the Nano eNablerTM, relying on a microcantilever-based surface patterning tool for printing [25, 32]. Noteworthy, a novel microspotting tool, BioplaneTM—consisting of an array of micromachined silicon cantilevers with integrated microfluidics channels—was recently introduced [34–37]. This spotting tool thus represents a novel opportunity for producing multiplex, miniaturized antibody arrays spots sized 5–20 μm in diameter.

Our pilot study has explored for the first time the use of BioplaneTM to produce miniaturized, high-density planar recombinant antibody arrays. The results have shown that 16-plex recombinant antibody arrays could be produced based on miniaturized spot features (78.5 μm\(^2\), Ø 10 μm) at 7–125-times increased spot density (250 000 spots cm\(^{-2}\) versus 38 000 spots cm\(^{-2}\) [33] or 2000 spots cm\(^{-2}\) [5]), interfaced with a fluorescent-based read-out. The feasibility of this conceptual array platform for serum protein profiling was outlined.

### Materials and methods

#### Antibodies and antigens

Ten human recombinant single-chain Fragment variable (scFv) antibodies directed against six high-abundant human serum proteins, including C1q, C3, C4, C5, factor B (FB), and properdin (FP), and four low-abundant human serum proteins, including interleukin (IL)-6, IL-8, IL-12, and vascular endothelial growth factor (VEGF), were included in this study (table 1). The scFv antibodies were stringently selected from a phage display library designed in-house. The specificity, affinity (normally in the 1–10 nM range), and on-chip functionality of scFv antibodies derived from phage display were ensured by using: (i) stringent phage-display selection protocols [38], and (ii) a molecular design, adapted for microarray applications [5, 39]. The specificity of these antibodies has previously been validated by using pure analytes; mixtures of pure analytes; well-characterized, standardized crude serum; and orthogonal methods, such as mass spectrometry (serum/tissue extract pull-down assays), ELISA, MesoScaleDiscovery (MSD) assay, immunohistochemistry, and/or cytometric bead assay; as well as by spiking and blocking experiments in crude sample formats (e.g. serum) [9, 40–47].

Purified C1q was purchased from ElectraBox Diagnostica (Tyresö, Sweden) and anonymized human serum samples were obtained from Skåne University Hospital (Lund, Sweden).

#### Production and purification of scFv antibodies

All scFv antibodies were produced in *E. coli*. Briefly, soluble scFvs, all carrying a C-terminal his\(_{6}\)-tag, were purified from expression supernatants or periplasmic preparations by affinity chromatography on Ni\(^{2+}\)-NTA (Qiagen, Hilden, Germany). Bound molecules were eluted with 250 mM imidazole (pH = 8), dialyzed against phosphate buffered saline (PBS) (pH = 7.4), and stored at 4 °C until further use. The integrity and degree of purity of the produced scFvs were evaluated by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Invitrogen, Carlsbad, CA, USA). The protein concentrations were determined by measuring the absorbance at 280 nm.

#### Labeling of samples

The serum samples were biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (Pierce Protein Biology Products, Thermo Scientific, Inc., Rockford, IL, USA) based on a previously

### Table 1. The different scFv specificities used for the miniaturized recombinant antibody arrays.

| Antigens |  |
|---|---|
| IL-6 | IL-8 |
| IL-12 | VEGF |
| C1q | C3 |
| C4 | C5 |
| Factor B | Properdin |

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optimized labeling protocol for serum proteomes [9, 41, 44]. Briefly, the serum samples were diluted 1:45 in PBS, resulting in a final concentration of ca. 2 mg mL⁻¹, and biotinylated at a molar ratio of 15 biotin:1 protein. Unreacted biotin was removed by dialysis against PBS for 72 h at 4 °C using a Slide-A-Lyzer (MWCO 3.5 kDa) (Pierce). The samples were aliquoted and stored at −20 °C until further use.

Pure C1q (ElectraBox Diagnostica, Tyresö, Sweden) was labelled with Alexa-647 (Molecular Probes, Eugene, OR, USA), at a protein:dye ratio of 10:1 mg, following the protocol provided by the supplier, except for using 40% (v v⁻¹) glycerol in PBS (pH 7.4) as a labelling buffer, and for forming the labelling on ice for 5 h due to the impaired stability of C1q in pure PBS.

**Production of miniaturized antibody arrays**

The microcantilever-based spotted, the Bioplume™ arrayer (Patent EP1509324) (NanoSen, Vichères, France) [34], was used to produce miniaturized antibody arrays. The spotting principle relies on microcantilever-based contact deposition. The microfabricated silicon cantilevers are 1500 μm long, 120 μm wide, and 5 μm thick, so the diameters of the obtained spots are in the 5–20 μm range (depending on contact time and surface hydrophobicity). The microcantilevers were filled by immersion in a 1 μL droplet of spotting reagent, thanks to capillary forces. The washing of the microcantilevers was achieved by dipping them into water for 30 s, and then allowing water evaporation to occur within a few seconds. The instrument was equipped with 3 cantilevers for printing.

The Bioplume™ was equipped with an automated xyz motion-control system, enabling arrays of spotted scFv antibodies (60–560 attomole antibody per spot) to be produced on black Polymer Maxisorp slides (NUNC A/S, Roskilde Denmark). The contact time was set to 300 min, resulting in 10 μm sized spots. The pitch-to-pitch distance was 20 (or 30) μm, giving a spot density of about 250 000 (or 111 100) spots per cm⁻². Three array layouts were printed. First, 1-plex arrays (labelled streptavidin) were printed in 15 × 3 subarrays in four separate printing areas. Second, 3-plex arrays (anti-C1q, anti-C3, and anti-VEGF) were printed in 5 30 × 30 subarrays (each antibody was printed in 27 replicates) in four separate printing areas. Third, 10 identical slides were printed, where each slide contained six identical arrays, composed of 16 spotting reagents (i.e. 16-plex), each spotted in 27 replicates (9 spots/cantilever × 3 cantilevers). The spotted reagents consisted of 10 different scFv, two of them (anti-C3 and anti-C5) being printed at 3 dilutions, as well as positive control (labelled streptavidin) and negative control (PBS). All samples were prepared in PBS containing 10% glycerol in order to prevent evaporation during the spotting procedure.

**Processing of miniaturized antibody arrays**

The quality of the miniaturized antibody arrays was first examined by evaluating the pattern of the deposited positive control, Alexa-647 labelled streptavidin, at 5 μm resolution, using a confocal microarray scanner, ScanArray Express (PerkinElmer Life & Analytical Sciences, Wellesley, MA, USA). A hydrophobic pen (DakoCytomation Pen, DakoCytomation, Glostrup, Denmark) was then used to draw a hydrophobic barrier around the arrays. Next, the arrays were blocked with 90 μl 5% (v v⁻¹) fat-free milk powder (Semper AB, Sundbyberg, Sweden) in PBS for 1 h. All incubations were performed in a humidity chamber at room temperature. The arrays were washed three times with 90 μl 0.05% (v v⁻¹) Tween-20 in PBS (PBS-T) and then incubated with 90 μl biotinylated serum sample, diluted from 1:2 to 1:75 (resulting in a final dilution of from 1.90 to 1.3375) or directly labelled C1q (0.01 nM to 100 nM) for 1 h. All samples were diluted in 1% (w v⁻¹) fat free milk powder and 1% Tween-20 in PBS (PBS-MT). The arrays were then washed three times with 90 μl 0.05% (v v⁻¹) PBS-T. Any subarrays incubated with biotinylated serum samples were also incubated with 90 μl 1 μg mL⁻¹ Alexa-647 conjugated streptavidin diluted in PBS-MT for 1 h. Finally, the arrays were washed three times with 90 μl 0.05% (v v⁻¹) PBS-T and one time with 90 μl PBS, directly dried under a stream of nitrogen gas, and scanned.

**Analysis of scFv Nanoarrays**

The arrays were scanned using a confocal microarray scanner (ScanArray Express, Perkin-Elmer Life & Analytical Science) with 5 μm resolution. The signal intensity of each spot was quantified using ScanArray Express software V4.0 (Perkin Elmer), using a spot diameter of 10 μm. The mean value of each set of nine replicate spots is reported (negative control subtracted). The limit of detection was defined two standard deviations above the negative control.

**Results**

In this conceptual study, we have explored the use of a novel microspotting tool, the Bioplume™ arrayer equipped with three printing needles, for producing miniaturized, high-density planar recombinant antibody arrays for protein expression profiling (figure 1).

First, we tested the feasibility of the printing approach by producing 1-plex (labelled streptavidin) (figure 2(A)) and 3-plex arrays (anti-C1q, anti-C3, and anti-VEGF) on black polymer Maxisorb slides (figure 2(B)). The nozzle-surface contact time was set to 300 min, resulting in 10 μm-sized spots. The pitch-to-pitch distance was set to 20 μm, giving a spot density of about 250 000 spots per cm⁻². The data showed that 1-plex protein arrays readily could be produced (figure 2(A)). The miniaturization was highlighted by the fact that about 57 spots could be printed within the same footprint as that of a conventional spot (≈ 150 μm) from an ordinary antibody microarray. The 3-plex antibody array showed that two of three antigens could be detected in a crude, biotinylated human serum sample (diluted 450 times) (figure 2(B)). The fact that the low-abundant serum protein VEGF could not be detected might be sample-dependent (the serum concentration varies from serum to serum) and/or assay-dependent (below limit of detection). Still, the experiments outlined...
the feasibility of the printing approach, clearly justifying further work. To evaluate the printing approach in more detail, 16-plex arrays were generated (figure 3).

Each of the 16 spotting reagents (10 scFv antibodies, 2 of them printed at 3 dilutions, and positive and negative controls) were dispensed in 27 replicates (9 spots/cantilever x 3 cantilevers) (figures 3(A) and (B)). The 10 μm sized spots

Figure 1. A schematic of the experimental set-up.

Figure 2. Scanned images of miniaturized protein arrays on black polymer Maxisorb slides. (A) shows 1-plex arrays (labelled streptavidin). The footprint of a conventional spot (Ø 150 μm) from an ordinary antibody microarray is indicated. (B) shows 3-plex arrays (anti-C1q, anti-C3, and anti-VEGF) hybridized with a crude, human serum sample (diluted 450 times).
(300 min contact time) were printed at a pitch-to-pitch distance of 30 μm, giving a spot density of about 111 100 spots per cm² (figure 3(C)).

At first, we examined the amount of antibody needed for printing to yield detectable array signals (figure 4). The 10 scFv antibodies were printed at 0.5 mg ml⁻¹ (560 attomole) (6 antibodies) or at the maximum concentration at hand, 0.38 mg ml⁻¹ (430 attomole) (1 antibody) and 0.14 mg ml⁻¹ (160 attomole) (3 antibodies) (figure 4(A)). Targeting a crude, biotinylated serum sample (diluted 450 times), 7 of 10 antibodies gave detectable signals, including antibodies against both high- (C3, C4, C5, and C1q) and low-abundant (IL-8, IL-12, and VEGF) serum analytes (figure 4(A)). Of note, targeting another serum samples, the antibodies directed against Factor B and IL-6 were also found to give detectable signals (figure 4(A)), indicating that printing ≥ 160 attomole scFv might be sufficient. Next, we titrated the amount of printed antibodies (560/220/60 attomole) for two of the clones, anti-C3 (figures 4(A) and (B)) and anti-C5 (figure 4(A)). In both cases, the data showed that 220 attomole of antibodies were required to give a detectable signal, indicating an appropriate amount of scFv antibody per spot required to produce functional arrays.

Next, we determined the printing efficiency of functional scFv by evaluating the spot-to-spot (figure 5), array-to-array (figure 6), and slide-to-slide (figure 7) reproducibility for three representative scFv antibodies after capturing both high- (C1q and C3) and low-abundant (IL-12) biotinylated targets in crude serum samples (diluted 450 and 1125 times). Being a pilot study, we chose to use the raw compiled array data without filtration (for e.g. spot imperfections due to e.g. dust particles) and normalization, in order to display the underlying reproducibility. The spot-to-spot reproducibility was based on nine replicate spots/needle, and expressed in terms of coefficient of variation (CV) (figure 5). The results showed that the CV values varied in a needle-, antibody-, and/or analyte-dependent manner, and were in the range of 1–36% (mean CV 17%) (serum diluted 450 times) and 15–50% (mean CV 31%) (serum diluted 1125 times). It should be noted that the preferred serum dilution is 450 times [41, 44], for which acceptable mean CV value was observed, especially considering that this is the 1st generation of array platform produced with the Bioplume™ microdispenser.

| scFv specificity | Amount of printed antibody (attomole) | Detectable signal (serum 1) | Detectable signal (serum 2) |
|------------------|---------------------------------------|-----------------------------|-----------------------------|
| C4, IL-8, IL-12, VEGF | 560                                   | +                           | +                           |
| C1q             | 430                                   | +                           | +                           |
| Factor-B, IL-6  | 160                                   | -                           | +                           |
| Properdin       | 160                                   | -                           | -                           |
| C3              | 560/220/60                            | +/+/+                       | +/+/+                       |
| C5              | 560/220/60                            | +/+/+                       | +/+/+                       |

Figure 4. Evaluation of the amount of antibody needed for printing to yield detectable array signals. (A) the 10 scFv antibodies were printed at (i) 0.5 mg ml⁻¹ (560 attomole) (6 antibodies) or at the maximum production concentration at hand, 0.38 mg ml⁻¹ and 0.14 mg ml⁻¹ (160 attomole), and/or (ii) titrated. Crude, biotinylated human serum (diluted 450 times) was analyzed to determine whether a detectable signal was observed. (B) array signal intensities for titration of the anti-C3 scFv antibodies.
The array-to-array reproducibility, i.e. arrays analyzed on the same slide, was based on 27 spot replicates per subarray (using three needles) targeting serum diluted 450 times, and expressed in terms of CV values (figure 6). Of note, the CV value within each set of 27 replicate spots was 13–61%, further supporting the spot-to-spot reproducibility observed above. The data showed that the array-to-array reproducibility, expressed as CV-values, was found to be in the range of 16–51% (mean CV 34%) (figure 6). This range of variability was expected, and should be handled (minimized) in future efforts by adopting slide-to-slide normalization.

The slide-to-slide reproducibility, i.e. arrays analyzed on the same slide, was based on 27 spot replicates per subarray (using three needles) targeting serum diluted 450 times, and expressed in terms of CV-values (figure 6). Of note, the CV value within each set of 27 replicate spots was 13–61%, further supporting the spot-to-spot reproducibility observed above. The data showed that the array-to-array reproducibility, expressed as CV-values, was found to be in the range of 16–51% (mean CV 34%) (figure 6). This range of variability was expected, and should be handled (minimized) in future efforts by adopting slide-to-slide normalization.

Next, we determined the limit of detection (LOD) by titrating pure, directly labelled analyte (C1q) and/or well-characterized crude, biotinylated serum targeting 4 high (C1q, C3, C4, and Factor B) and 1 low-abundant (IL-12) analytes (figure 8). In the case of C1q, the LOD was found to be 10 pM for pure C1q (figure 7(A)), while the LOD was reduced 12.5 times (125 pM/1:2250 dilution) when targeting crude, biotinylated serum (figure 8(B)). In comparison, the OD was also found to be represented by a 2250- times dilution of crude, biotinylated serum for both C3 (3100 pm) (figure 8(C)) and IL-12 (70 fg ml⁻¹) (figure 8(D)). Further, the LOD was found to be 330 pM for C5, 880 pM for C4, and 6600 pM for Factor B. Hence, the set-up was found to display adequate LODs in the pM range when targeting various analytes in crude, directly biotinylated serum samples.
Finally, the feasibility of the 16-plex set up for serum protein profiling was evaluated by analyzing a well-characterized crude, biotinylated serum sample (figure 9). The results showed that low non-specific background binding and dynamic specific (spot) signals were observed (figure 9(A)). In more detail, the results showed that 7 of 10 antibodies, including both high- (C1q, C3, C4, and C5) and low-abundant (IL-8, IL-12, and VEGF) analytes, gave detectable signals when analyzing this particular serum sample. Hence, the data supported the feasibility of the printing process for producing miniaturized, planar recombinant antibody microarrays for protein expression profiling.

Discussion

In this pilot study, we explored the use of the new micro-spotting tool, the Bioplume™ arrayer, equipped with three printing needles, for producing antibody arrays. We demonstrated the feasibility of the printer for producing miniaturized, high-density planar recombinant antibody arrays for protein expression profiling. Hence, the early design of this printing device should be added to the short list of currently available instruments capable of generating such miniaturized arrays [25, 30, 32, 33, 34, 48].

Compared to previous efforts in generating miniaturized (78.5 um², Ø 10 μm) recombinant antibody arrays using DPN-technology (NLP3000) [33, 34], the spot density was increased ~7-times, 250 000 versus 38 000 spots cm⁻². In addition, the array density was increased 125-times compared to conventional antibody microarrays (250 000 versus 2000 spots cm⁻²) [5]. Moreover, the Bioplume™ is able to dispense down to 5 μm sized spots, leading to an even further increased spot density if required.

In the current configuration, the Bioplume™ instrument was equipped with three needles, so that multiplexity was achieved in incremental steps of 3, including a washing step of the needles in between the printing of new reagents. In comparison, using the DPN-technology (NLP3000™), the multiplexity is extended in incremental steps of 6, 12, 24, or 48 by loading the printer with a new 6-, 12-, 24-, or 48-pen and a matching inkwell prepared with the next set of reagents. While possible, in practice it might be challenging to succeed with this due to the reorientation, etc that will be required after mounting a new pen. Hence, Bioplume™ works more like standard ink-jet printers used to produce conventional antibody microarrays (250 000 versus 2000 spots cm⁻²)[ 5]. Moreover, the Bioplume™ instrument is able to disperse down to 5 μm sized spots, leading to an even further increased spot density if required.

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Figure 8. LOD for the miniaturized recombinant antibody arrays: (A) titration of pure, directly labelled C1q; (B) titration of a well-characterized crude, biotinylated serum targeting C1q; (C) titration of a well-characterized crude, biotinylated serum targeting C3; (D) titration of a well-characterized crude, biotinylated serum targeting IL-12; (E) LOD for six complement proteins (based on titration of a well-characterized, crude, biotinylated human serum sample with known levels of the complement proteins).

| Analyte          | LOD (pM) |
|------------------|----------|
| Pure C1q         | 10       |
| Serum C1q        | 125      |
| C3               | 3100     |
| C4               | 880      |
| C5               | 330      |
| Factor B         | 6600     |

Figure 9. Profiling of a crude, biotinylated human serum sample using a 16-plex miniaturized recombinant scFv antibody array. (A) scanned array image; (B) array signal intensities.
The array-to-array and slide-to-slide reproducibility were found to be higher, which could be expected in evaluating non-normalized data [5, 33, 34]. Being a pilot study, we identified the efforts to apply such normalization strategies [5, 33, 34] to be more suited to follow-up studies, where the aim would be to evolve the platform from a proof-of-concept set-up to an optimized (established) methodology aiming to run (clinical) applications. In this context, it might still be of interest to note that CV values in the range of 5% was observed for the second generation of miniaturized antibody arrays (DPN technology) [33, 34], while CV values in the same range [5–7, 13] or better (1.6%) (Wingren et al unpublished data) regularly have been observed for established conventional antibody microarray set-ups. In comparison, the inter- and intra-assay CV values for well-optimized conventional ELISAs are frequently ≤15%.

The set-up interfaced with a confocal fluorescent-based scanner for sensitive sensing, also compatible for reading high-density arrays. At the time when the wet-lab experiments were performed, we only had access to a scanner with 5.0 μm resolution. This explains the somewhat rough view of the array images; however, this should not impair our evaluation and conclusion in a significant manner. Of note, we have recently acquired a high-resolution (0.5 μm resolution) scanner, to be adopted for future work. The LOD was found to be in the pM range, which is highly adequate for tentative proteomic applications [5, 52], and in the same range as that observed for the miniaturized recombinant antibody arrays produced using DPN technology [33, 34]. Hence, most of the targeted analytes could also be successfully detected in directly labelled, crude serum samples, representing one of the most common clinical sample format. In comparison, LODs in the pM to fM range have been observed for high-end conventional antibody microarrays [5, 11, 41, 44, 53] (Wingren et al unpublished observations), but these set-ups have then been subjected to significant optimization and method refinement.

Taken together, in this pilot study, we have demonstrated for the first time the feasibility of the novel microdispensing tool Bioplume™ for producing miniaturized, multiplexed, high-density planar recombinant antibody arrays—displaying adequate performance—for protein expression profiling of crude, directly labelled proteomes. In this, we have successfully extended the range of candidate printers that can be used to produce miniaturized array. Further, the Bioplume™ printer provides a key edge towards manufacture of more high-density antibody array designs. Fully automated platforms based on printers such as Bioplume™ could represent the next generation of printers, setting the stage for high-throughput screening efforts. Miniaturized (recombinant) antibody microarrays will be an essential tool for large-scale, multiplexed profiling of crude proteomes, in both health and disease research, opening up novel opportunities for biomarker discovery, while consuming minimal amounts of both reagents and samples.

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