Microarray-to-Microarray Transfer of Reagents by Snapping of Two Chips for Cross-Reactivity-Free Multiplex Immunoassays

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ABSTRACT: Whereas microarray and microfluidic technologies have progressed on many fronts, servicing microchips with minute amounts of reagents still constitutes an important challenge for many applications. Recently, chip-to-chip reagent transfer methods were introduced that simplify the delivery of reagents but required manual, visual alignment, custom-built microwells, and only showed the reaction of a single sample with multiple chemicals. Here, we present the snap chip, which uses common glass slides for transfer, back-side alignment for achieving precise alignment in spite of mirroring, and a snap-apparatus for facile transfer of arrays of chemicals at once by snapping the two slides together. We recently established that cross-reactivity was a significant problem in multiplex assays both theoretically and experimentally and found that it can be eliminated by avoiding mixing, but which necessitates delivering each detection antibody to a single spot with the cognate capture antibody. Using the snap chip, multiplexed sandwich immunoassays without mixing were performed: a slide with multiple arrays of 10 different capture antibodies was incubated with a sample, and then all detection antibodies transferred at once by snapping, each to the single cognate spot. All binding curves were established and limits of detection in the pg/mL range were obtained. Snap chips were stored up to 3 months prior to usage. The snap chip, by dissociating microarray production, which requires expensive equipment, from assay execution, which can be achieved using a hand-held alignment apparatus, will allow for multiplex reactions to be performed using a user-friendly kit. This new liquid handling format can be easily adapted to other applications that require transfer of minute amounts of different reagents in parallel.

Microarray technologies have been developed in the past years. Fabrication of microarrays depends on the transfer of minute amounts of reagents. An important challenge is to transfer and pattern chemicals stored in macroscopic containers as microarrays on slides. The macro-to-micro challenge was first addressed using pin spotters to transfer minute amount of liquids from microtiter plates to chips by repeatedly printing the pins onto multiple chips. The upload and transfer are controlled by capillary effects that need to be precisely engineered. Inkjet spotters for biological applications with front-loading of reagents through the nozzles have subsequently been developed and used for microarraying. The number of nozzles is typically much larger than that of pin spotters, however the programmability and rapid dispensing of droplets compensates for the limited parallelism. A highly parallel system named the top spot comprises a spotting head that is filled using capillary forces and that dispenses reagents by compressing the air above the nozzles. This system is simpler than inkjet spotters but lacks individual addressing of the nozzles and requires larger volumes of reagents to load the head. However, all of these systems rely on robotics, are quite complex, expensive to acquire and operate, and there is no provision for removing a substrate from the spotter and placing it back subsequently for repeatedly addressing the same spot.

One option to circumvent the need for expensive spotters is to prespot reagents into miniaturized storage plates that may then be used at a different time and place and thus dissociate production of the array from the execution of a multiplex reaction. Hunter pioneered this concept using preloaded microwell arrays. Whereas no peer review papers were apparently published to our knowledge, this technology was developed into a commercial product called the OpenArray that is used for DNA analysis. This technology is based on custom chips made of 300 μm thick steel sheets with 3072 holes, each 300 μm in diameter. The sheets are hydrophobic, but the inside wall of the holes is hydrophilic. Samples are typically applied to a block of wells using pipet tips.

More recently, several groups proposed novel approaches to transfer minute amounts of reagents en bloc. Ismagilov and colleagues developed an elegant approach called the slipchip. Nanoliter droplets of reagents are first trapped in channels and recesses on one chip that serve as reaction chambers. A sample is loaded in a chip comprising microchannel that runs parallel to the recesses. Next, the channels and the recesses are overlaid by sliding the two microstructured chips. To date, slipchips have been used to deliver a single sample to an array of reagents such as 48 crystallization wells or to different chambers for
sandwich immunoassays,10,11 which are examples of reacting one chemical with a number of N reagents, (a 1-to-N transfer).

Chip-to-chip transfer methods have also been performed by clamping two chips together. The first demonstration was carried out using sol–gel droplets immobilized on a glass slide and loaded with drugs or metabolites. The chemicals were then transferred to cell monolayers by diffusion ensuring that only cells close to each droplet were exposed to significant concentration of chemicals.12 Subsequently, similar approaches were extended to alginate gel droplets and to cells encapsulated in collagen.13,14 More recently, Khademhosseini and colleagues adopted an approach to transfer drugs from ~200 μm wide posts made of either PDMS15 or a hydrogel16 that were coated or loaded respectively with a drug library by inkjet spotting. An array of 400 μm wide microwells containing cells (from the same cell line) was perfused with a drug library by clamping the chips and letting the drugs diffuse into the buffer contained in each well. The alignment between the two slides was performed manually with the aid of a stereomicroscope, which is cumbersome while at the same time a small number of wells were typically misaligned, all of which limits the versatility of this approach. In summary, for the chip transfer methods described above, manual alignment based on visible structures on the chip was used, and the transfer followed an N-to-1 or a 1-to-N arrangement with a number of N different reagents being reacted or mixed with one other reagent.

Multiplexed sandwich immunoassays are a chemical reaction where N analytes are sandwiched between (bound by) N capture and N detection antibodies.17 In conventional multiplexed sandwich assays in either array or bead formats, the detection antibodies are applied as a mixture over the whole array, and the pairwise interaction between nonmatched reagents each constitutes a liability for cross-reactivity, which in practice can lead to significant levels of cross-reactivity for an only 14-plex assay.18 Cross-reactivity can be minimized by careful optimization and selection of reagents for some classes of analytes, but notwithstanding best efforts, cross-reactivity cannot be suppressed entirely in practice. Moreover, the vulnerability to cross-reactivity scales as 4N(N − 1) irrespective of optimization efforts18 and cross-reactivity events can occur during a test owing to the uniqueness of each sample. These characteristics limit the sensitivity and reliability of multiplexed sandwich assay. Recently, we proposed the antibody colocalization microarray (ACM)19 that overcomes these limitations by avoiding mixing and using two spotting rounds, one to array the capture antibodies and a second one to deliver each detection antibody to a single capture antibody spot. The assay configuration of the ACM reproduces the one of the enzyme linked immunoabsorbent assays (ELISA) – the gold standard for immunoassays – where only a single pair of antibodies is used in each microwell and the dual binding of capture and detection antibodies discriminates against nonspecific binding and cross-reactivity. The execution of an ACM entails the following key steps: Aligned spotting of capture antibodies, removing the slide from the spotted, whole-slide blocking and incubating with samples, washing and rinsing as needed, placing it back on the deck for aligned spotting of the detection antibody, and finally whole-slide incubation of labels and secondary antibodies. The ACM thus depends on the transfer of N different detection antibodies to N different capture antibody spots, representing an N-to-N transfer. This was achieved using a custom built microarrayer with alignment mechanisms for precise overlay of the capture and detection antibody spots.18 The ACM protocol thus entails spotting during the assay execution, which is cumbersome and requires the use of a complex and expensive microarrayer.

Here, we present the snap chip for (i) N-to-N transfer of a microarray of reagents stored as semispherical liquid droplets on a transfer slide to a target microarray on an assay slide by snapping two microscope slides together. (ii) Visualization-free transfer thanks to a protocol with back-side alignment and a hand-held snap apparatus used to mechanically align the two slides without the need of a microscope enabling simple and reliable transfer of reagents. (iii) Dissociation of the assay execution from the time-consuming and costly microarray fabrication by establishing long-term storage of both assay and transfer slides that can be spotted ahead of time and stored. Using the snap chip, we performed multiplexed sandwich immunoassays with colocalization of capture and detection antibodies with detection limits in the pg/mL. Ten targets were assayed simultaneously in both buffer and serum.

## EXPERIMENTAL SECTION

**Materials.** Rabbit antigoat IgG (H+L) labeled with the fluorescent dye Alexa Fluor 488 and goat antimouse IgG (H + L) labeled with Alexa Fluor 647 were purchased from Invitrogen. Matched antibody pairs (capture and biotinylated detection antibodies) and antigens used in this study include human epidermal growth factor receptor 2 (HER 2), Endoglin (ENG), Leptin (LEP), fibroblast growth factor (FGF), osteopontin (OPN), tumor necrosis factor receptor-II (TNF RII), granulocyte macrophage colony-stimulating factor (GM-CSF), chemokine (C–C motif) ligand 2 (CCL 2), chemokine (C–C motif) ligand 3 (CCL 3), and interleukin-1 beta (IL 1β) are from R&D Systems. Streptavidin-conjugated Cy 5 was purchased from Rockland. Phosphate buffered saline (PBS) tablets were purchased from Fisher Scientific. Tween-20 and glycerol were obtained from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Jackson ImmunoResearch Laboratories, Inc. Normal human female serum from a single donor was purchased from Golden West Biologicals. BSA-free StabilGuard Choice Microarray Stabilizer was obtained from SurModics, Inc. Nitrocellulose coated slides were obtained from Grace Bio-Laboratories, Inc. Aminosilane coated slides were purchased from Schott North America.

**Preparation of Microarrays on Slides.** Capture antibody solutions containing 400 μg/mL antibodies and 10% glycerol in PBS were spotted on a nitrocellulose slide (assay slide) at a relative humidity of 60%, 1.2 nl were delivered to each spot. Detection antibody solutions containing 20 μg/mL antibodies, 20% glycerol, and 1% BSA were spotted on an aminosilane slides (transfer slide) at a relative humidity of 80% to prevent evaporation; 8 nl were delivered to each spot. Spotting was performed using an inkjet spotter (Nanoplotter 2.0, GeSiM). The center-to-center spacing between spots was 800 μm for the 1024-spot array (Figure 4), and 1 mm for the immunoassays. After spotting, the assay slide was incubated for 1 h with spotted capture antibodies at room temperature with a relative humidity of 60%. A slide module gasket with 16 compartments (Grace Bio-Laboratories, Inc.) was then clamped on the slide dividing it into 16 wells for immunoassays. After incubation the assay slide was rinsed twice with PBS containing 0.1% Tween-20 (PBST) for 5 min on the shaker at 450 rpm and once with PBS, also for 5 min at 450 rpm.

**Alignment and Snapping of Slides.** A back-side alignment approach was used to achieve mirror symmetric
alignment between the assay and the transfer slides. Both slides were placed on 500 μm thick rubber sheets (McMaster-Carr) in a recess on two vacuum chucks. The vacuum chucks featured alignment rods and holes, and were used to hold the slides in place during snapping. Two 25 μm thick Kapton sheets (McMaster-Carr) were inserted between the slides at both extremities. To snap the slides, the two vacuum chucks were pushed together by hand, and clamped tightly using two C-Type clamps (Canadian Tire) applied at the extremities. After 1 min, the clamps were removed, the vacuum chucks were separated, and the slides were removed from the snap apparatus. Additional explanations are given in the Results and Discussion section.

10-plex Sandwich Immunoassays with Snap Chips. Ten proteins including HER 2, ENG, LEP, FGF, OPN, TNF RII, GM-CSF, CCL 2, CCL 3, and IL 1β were measured in spiked buffer or spiked, diluted serum (10% in PBS buffer). The assay slide onto which capture antibodies for these ten proteins were spotted was blocked for 1 h on the shaker at 320 rpm with StabilGuard. Five-folds, seven-points dilution series of ten proteins in PBS or 10% human serum diluted in PBS were applied to the slide and incubated for 1 h at 320 rpm (starting concentrations: 200 ng/mL for HER 2, ENG, LEP, FGF, OPN and 50 ng/mL for TNF RII, GM-CSF, CCL 2, CCL 3, IL 1β). Blank samples of PBS and 10% serum in PBS containing no spiked proteins were also incubated on the assay slide at the same time. The slide was rinsed twice with PBST and once with PBS on the shaker at 450 rpm for 5 min, the slide module gasket was removed, and the slide was dried under compressed nitrogen gas. Next, the assay and the transfer slides were clamped on the snap apparatus, snapped together for 1 min, separated, and the assay slide was incubated in a Petri dish saturated with humidity for 1 h. Then a slide module gasket was clamped on the assay slide, and the slide was rinsed three times with PBST and once with PBS on the shaker at 450 rpm for 5 min, and incubated with 2.5 μg/mL of streptavidin conjugated Cy 5 for 20 min on the shaker at 320 rpm. The slide was then rinsed twice with PBST, once with PBS and once with deionized water on the shaker at 450 rpm for 5 min, and dried with a stream of nitrogen gas.

Storage of Snap Chip Assay and Transfer Slides. We spotted both assay and transfer slides, stored them for 3 months, performed the immunoassays and compared them with freshly spotted slides. The assay slide was blocked with StabilGuard after incubation with capture antibodies and both assay and transfer slides were immediately stored in an airtight bag with desiccant and placed in a −20 °C freezer. Sealed bags were removed from the freezer and kept for 30 min at room temperature prior to opening and removing the slides to prevent condensation on the surface. The transfer slides were then incubated in a Petri dish saturated with humidity for 30 min to rehydrate the glycerol droplets before the antibody transfer process.

Scanning and Analysis. A microarray laser scanner (LS ReloadedTM, Tecan) was used to scan slides. For the large scale arrays, a 488 and 633 nm lasers were used to image capture antibody spots and the transferred proteins, respectively. For sandwich immunoassays, only the 633 nm laser was used. The fluorescence intensity was computed by subtracting the background signal in the vicinity of each spot. All experiments were performed in triplicate, and the data was analyzed using Array-Pro Analyzer (MediaCybernetics). Y-intercept of the fit curves incremented by two or three times the standard deviation of the independent assays was calculated as the limit of detection (LOD) using GraphPad Prism (GraphPad Software).

RESULTS AND DISCUSSION

Microarray Fabrication. The procedure for the microarray-to-microarray transfer of detection antibodies to slides with immobilized capture antibody spots is shown in Figure 1.

Figure 1. Process flow for microarray-to-microarray transfer of reagents for multiplexed sandwich immunoassays. (a) Capture antibody spots are formed by spotting 1.2 nl onto a nitrocellulose assay slide, and transfer droplets by spotting 8 nl of biotinylated detection antibodies on a transfer aminosilane slide with precise alignment. (b) Incubation of the nitrocellulose assay slide with the sample solution. (c) Microarray-to-microarray transfer of detection antibodies to assay slide with snap apparatus. (d) Incubation of the assay slide with streptavidin-Cy 5. (e) After drying, the fluorescence was acquired with a microarray scanner.

Corresponding detection and capture antibodies are colocalized thus following the format of an ACM. A slide with a 12 μm thick nitrocellulose coating was used as assay slide because the porous nitrocellulose provides a high protein binding capacity, and absorbs the solution from the transfer slide thus promoting rapid binding of detection antibodies. For the transfer slides, we first tested uncoated glass slides, but because of the low contact angle between glass and the antibody solutions, the resulting droplets were too thin to permit a reliable transfer to the assay slide. Slides with an aminosilane coating (contact angle with water ~65°)19 were chosen because they yielded a compromise between having a slightly enlarged diameter for relaxed alignment between the two microarrays and a droplet thickness of tens of micrometers to ensure fluidic contact to the assay slide and reliable transfer. 1.2 nl of solution was delivered to each capture spot yielding a 300 μm spot on the nitrocellulose slide, whereas 8 nl of detection antibody solution was applied to the aminosilane-coated transfer slide and produced a droplet that was 700 μm in diameter on the nitrocellulose slide upon transfer. Following snapping and separation, all droplets were transferred entirely and reliably to the assay slide.
Mirror Alignment of Slides. Our goal was to establish a microarray-to-microarray transfer without visual adjustment using purely mechanical alignment. This was motivated by the fact that the spots on the nitrocellulose slide are invisible when dried and that it is in any case cumbersome to do visual alignment under a microscope. When using mechanical alignment, one however needs to consider the mirror symmetry between spotting and transfer (part c of Figure 1). During spotting, the bottom right corner of each slide was pushed against a ridge on the slide deck of the microarrayer. However, during the transfer the two slides face one another and when seen from the top, the bottom-right corner of the transfer slide becomes the bottom-left corner. In conventional microarrays, there is no need for accurate positioning of the spots and all of the spot positions can be defined relatively to the position of the first spot. However, for the microarray-to-microarray transfer protocol proposed here this would lead to misalignment between the two arrays because of the mirroring. Mechanical alignment following mirroring is further complicated by the fact that the size of typical glass slides can vary and that it would thus not be possible to align the two slides by aligning relative to a single corner corresponding to two opposing edges on the transfer and assay slide, respectively (i.e., when looking at the assay slide through the transfer slide, the bottom right corner in fact corresponds to the bottom-left corner of the mirrored transfer slide).

We considered two options for achieving exact overlay during the transfer. The first was to spot at exact coordinates in a mirror pattern on both slides and to align each slide relative to the bottom-left edge on each half of the snap apparatus. The second option was to first spot an alignment mark on the back-side of the assay slide (at the same coordinate than the rightmost spot of the top row of the assay slide) while aligning both slides relative to the bottom right corner, flip the transfer slide, align it again relative to the bottom right corner (as seen from the top), use the image recognition system of the inkjet spotter to extract the coordinate of the alignment mark, and use it as the coordinate of the first spot of the array, as seen in parts a–c of Figure 2. In this manner, both arrays will be aligned when they are aligned in the transfer apparatus (Figure 3) and the alignment accuracy is independent of the size of the slides. Given the availability of an image recognition system, and of the difficulty in correcting for size variation in the first option, we used the second option for our experiments.

Snapping of Slides. The assay and the transfer slides were placed in a custom-built snap apparatus comprising two precision milled vacuum chuck, a steel plate, and four steel rods, as shown in parts a and b of Figure 3. The vacuum chucks contain a recess for mechanical slide alignment, steel rods, and a precision-machined support plate (width: 12.5 cm, length: 15 cm) used for supporting the slides during snapping.
during snapping to support the two chucks while they were being manually clamped together. Small pieces cut out of a 25 μm thick Kapton sheet were placed between the two slides at the edges to keep a constant separation over the entire area and thus avoid excessive squeezing of the droplets during snapping. 500 μm thick rubber cushions were inserted between the slides and the vacuum chucks to accommodate small imperfection and distribute the pressure evenly across the two slides. Following snapping of the two slides, a liquid bridge formed atop each of the spots connected the slides across the gap, and upon separation, the droplets and reagents were transferred to the assay slide, as seen in part a of Figure 3.

Accuracy of Microarray-to-Microarray Transfer. We characterized the alignment accuracy using an assay slide with 16 nitrocellulose pads each spotted with 16 droplets. IgGs labeled with green and red fluorescent dyes were spotted on both the assay and transfer slide, respectively, snapped together, and the nitrocellulose slide was scanned immediately without any washing. The average center-to-center distance between the spotted and transferred droplet was 147 μm, whereas the largest distance was 216 μm. The misalignment increased from the left to the right side of the slide, and in fact doubled following mirrored transfer, indicating that there was an angular misalignment between the slides and the motorized inkjet stage. The different droplet sizes of capture and detection antibody spots however relaxed the alignment constraints and ensured complete overlap in spite of some misalignment.

Microarray-to-Microarray Transfer of Antibodies. We first evaluated the use of the snap chip for carrying out simple one-step immunoassays. An array of 256 fluorescently labeled goat IgGs (the analyte) were transferred to an assay slide patterned with an array of 1024 fluorescently labeled rabbit anti-goat IgGs (the capture antibody), incubated and washed, as seen in Figure 4. 20% glycerol was added to the detection buffer to prevent drying of the antibodies being transferred while the assay slide was dried under a stream of nitrogen prior to the transfer to promote the absorption of the droplets in the nitrocellulose and minimize lateral spreading. Visual inspection reveals a selective and homogeneous transfer of proteins across the entire slide, as seen in part a of Figure 4. The fluorescence intensity profiles of the two proteins shows excellent overlap, as seen in part b of Figure 4.

10-plex Sandwich Immunoassays in Buffer and Serum. To evaluate the use of microarray-to-microarray transfer for multiplexed sandwich immunoassays, we selected 10 proteins, including one breast cancer biomarker (HER 2), 4 cancer related proteins (ENG, LEP, FGF, OPN), and 5 cytokines (TNF RII, GM-CSF, CCL 2, CCL 3, IL 1β). HER2 is a plasma membrane-bound receptor tyrosine kinase, and it has been used for the typing of breast cancer by measuring its amount in biopsy samples by immunohistochemistry. When HER2 is overexpressed breast cancer patients can be treated with trastuzumab, a monoclonal antibody against the HER2 receptor that could inhibit cell proliferation. ENG is a cell membrane glycoprotein that is overexpressed in tumor blood vessels but not in most normal tissue and therefore has prognostic significance. Lepin is a protein hormone and overexpressed in obese individuals and also in breast cancer cells. FGF is important in tumor angiogenesis and has been investigated for cancer therapeutics. OPN is correlated with malignant transformation and its elevated level was found to be associated with shorter survival in breast cancer patients. TNF RII is one of the TNF α receptors and has been reported to be increased in the blood of breast cancer patients. GM-CSF is a cytokine that may activate immune cells and has been investigated in breast cancer treatment. CCL2 and CCL3 are chemokine ligands that have been found to be increased in tumor. IL 1β is a cytokine that has been reported to interact with estrogen receptors in breast cancer cells and may modulate hormonal activity in human breast tumors. The experiment flow is shown in parts b–e of Figure 1. The spotting solution containing detection antibody was supplemented with 1% BSA to block the aminosilane-coated slide surface and prevent the adsorption of the detection antibodies, which helped increase the transfer efficiency.

We established binding curves for all proteins spiked in PBS, as seen in parts b and c of Figure 5. A four-parameter logistic equation was used for curve fitting and the LOD obtained were in the pg/mL range for all of the 10 proteins, as seen in Table 1. The LODs were calculated as background signal incremented by 2 SD for the comparison with ELISAs from R&D Systems, and 3 SD corresponding to the scientific convention. Most proteins achieved comparable LODs with commercial ELISAs, for CCL 3 the LOD obtained using snap chip exceeds that of ELISAs, and for FGF and OPN further optimization is needed. The LOD values were compared with the physiological range of these proteins in the serum of healthy persons as found in the scientific literature, and our assays were found to exceed this limit for all of the 10 targets. Moreover, by selecting antibodies from other suppliers, and by thorough optimization...
using design of experiment approaches such as the Taguchi method, significant improvement of the LOD of an order of magnitude or more can be achieved.\textsuperscript{43}

To test the applicability of the snap chip for immunoassays with complex biological samples, we performed a multiplexed immunoassay for the same 10 proteins spiked in 10% serum, as seen in parts d and e of Figure 5. Serum was diluted to minimize matrix interference and background signals, as it is commonly done in ELISA assays.\textsuperscript{33,44} For most proteins, the background signals at zero concentration of spiked proteins are

![Figure 5. Fluorescent image and binding curves for sandwich immunoassays for 10 proteins in buffer solutions and 10% serum.](image)

Table 1. LOD Values Obtained from 10-plex Immunoassays in PBS; the Units Are pg/mL

<table>
<thead>
<tr>
<th>protein</th>
<th>LOD (3 SD)</th>
<th>LOD (2 SD)</th>
<th>LOD from R&amp;D Systems\textsuperscript{a} (2 SD)</th>
<th>average concentration in serum of healthy controls\textsuperscript{b}</th>
<th>refs</th>
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<tr>
<td>HER 2</td>
<td>155</td>
<td>81</td>
<td>n/a</td>
<td>≤15 000</td>
<td>Kong et al.\textsuperscript{38}</td>
</tr>
<tr>
<td>ENG</td>
<td>138</td>
<td>74</td>
<td>30</td>
<td>150 000</td>
<td>Takahashi et al.\textsuperscript{41}</td>
</tr>
<tr>
<td>LEP</td>
<td>52</td>
<td>28</td>
<td>8</td>
<td>26 430 ± 19 400</td>
<td>Aliustaoglu et al.\textsuperscript{34}</td>
</tr>
<tr>
<td>FGF</td>
<td>85</td>
<td>51</td>
<td>3</td>
<td>123 000</td>
<td>Bramwell et al.\textsuperscript{35}</td>
</tr>
<tr>
<td>OPN</td>
<td>263</td>
<td>171</td>
<td>24</td>
<td>3180 ± 600</td>
<td>Rutkowski et al.\textsuperscript{39}</td>
</tr>
<tr>
<td>TNF RII</td>
<td>36</td>
<td>21</td>
<td>2</td>
<td>900 ± 90</td>
<td>Scholl et al.\textsuperscript{40}</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>173</td>
<td>Kim et al.\textsuperscript{37}</td>
</tr>
<tr>
<td>CCL 2</td>
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<td>10</td>
<td>5</td>
<td>88.3</td>
<td>Kim et al.\textsuperscript{37}</td>
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<td>CCL 3</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>40</td>
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</table>

\textsuperscript{a}Standard deviation indicated when available.
higher than those in PBS, presumably due to the presence of endogenous proteins given that the typical physiological concentrations are well beyond the LOD, as well as because of matrix effects.\textsuperscript{45} Whereas further investigations are needed beyond the scope of this manuscript, the binding curves indicate that it will be possible to quantify proteins in blood samples using the snap chip. These results indicate that high sensitivity can be achieved using snap chips that already rivals the one obtained with ELISA for some assays.

**Storage of Snap Chips.** To dissociate the production of the slides—which requires advanced equipment such as a pin or an inkjet spotter—from the execution of the assay, which can be done at low cost without need for peripheral equipment, slides must be stored prior to usage. Here, using TNF RII as a model protein, we developed a protocol for storing transfer and assay slides in a freezer at \(-20\) °C. After spotting, slides were blocked, dried, and stored at \(-20\) °C and thawed prior to usage. We noticed that when the prespotted slides were thawed in the open, water condensation would form on the surface, and as a result the droplets were disrupted. To prevent condensation, we sealed the slide together with a desiccant in a plastic bag and stored it in a freezer. Prior to an experiment, the bag was taken out of the freezer and equilibrated to room temperature before being opened. Using this protocol, no loss of signal was observed between freshly prepared slides and 1 month storage, and a factor 4 of increased LOD for 3 months storage, as seen in Figure 6. After 1 month storage, there was a higher background signal (Y-intercept of the fit curve), but also a stronger signal, and for 3 months storage a higher LOD may be due to loss of activity of the antibodies. The LODs for all three conditions was still well below the average physiological concentration in healthy patients for this protein. These results confirm the possibility for storing the snap chips and set the stage for future optimizations to establish longer term storage while also developing protocols for storage at \(-20\) °C, or maybe even at room temperature.

**CONCLUSIONS**

In this work we developed a snap chip for the collective transfer of reagents from microarray-to-microarray in an N-to-N configuration with a density of 130 spots/cm\(^2\). The alignment method enables end users to perform visualization-free transfer by simply snapping two slides using a hand-held snap apparatus without the need of a microscope. The snap chip was used for multiplexed immunoassays, and LODs in the pg/mL range were obtained. The LODs obtained with the snap chip were similar to the ones obtained with commercial ELISA. By prespotting on the slides and storage, snap chip overcomes the need for spotting during the experiments and the need for spotting equipment for the end users.

The snap chip transfer may be expanded to collectively transferring chemicals to a micro-structured microfluidic chip and help address the so-called “world-to-chip” interface.\textsuperscript{36–48} Next steps in the development of the snap chip will be to improve the alignment accuracy between transfer and assay chip to increase spot density, and optimize the conditions for the intended application, such as immunoassays while tackling important practical issues such as slide storage. Finally, it will be possible to build portable and convenient-to-use snap chip for the use by unskilled end users in research and eventually in clinical settings as point-of-care diagnostics.

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**Notes**

The authors declare the following competing financial interest(s): McGill has filed a patent application on some aspects of the work reported here with H.L. and D.J. as inventors.

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**REFERENCES**
