LOW-COST, HIGH LIQUID VOLUME SILICON QUILL PINS FOR ROBUST AND REPRODUCIBLE PRINTING OF ANTIBODY MICROARRAYS

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Abstract

Microarray printing can be achieved using a number of printing methods, including inkjet printing and contact printing using quill pins. Contact printing using pins is preferred in applications requiring great position precision.

Metal or silicon quill pins available commercially are expensive and have a single channel used for the delivery of liquid. In order to improve on the liquid capacity of pins and reduce production costs, three-pronged pins were previously developed. These pins performed well when printing on a 3D nitrocellulose surface, but not on 2D functionalized glass surfaces. Moreover, the three-pronged tips were not robust under normal operation due to its pointy design.

We present two variants of an improved two-pronged, blunt pin which are also low-cost, have a high loading capacity, are more robust, can print on nitrocellulose as well as on flat 2D surfaces, and lead to consistent and reproducible spots.

Introduction

Silicon quill pins are used to produce microarrays with high precision of the spots' positions. This precision is required to align spots in consecutive spotting rounds as in the Antibody Colocalization Microarray, developed in our lab [1].

Previously designed three-pronged pins had two channels for high liquid capacity, and were produced in-house to reduce cost. They were used to print on 3D nitrocellulose surfaces. Two-pronged blunt pins were designed to further improve robustness and the ability to print on 2D functionalized glass surfaces.

Three-pronged pins do not print reproducibly on glass surfaces

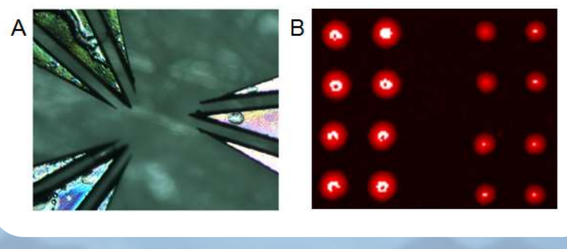

Figure 1. Tips of three-pronged Si quill pins with double channel (A) and printed fluorescent spots (B) from four pins (four groups of four) printed simultaneously on a nitrocellulose slide. Tips of the three-pronged pins are sensitive to variation in the microfabrication processes which lead to different spot sizes by different pins.

Design and Fabricated Si Pins

Two improved pin designs

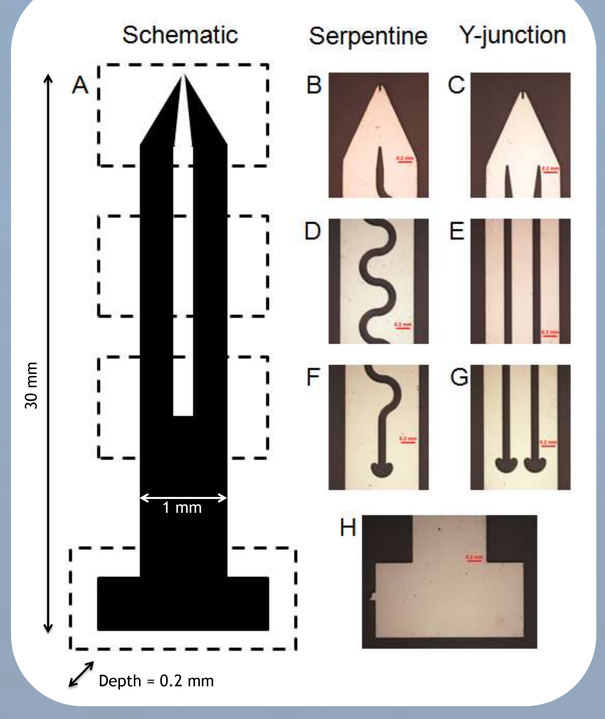

Figure 2. Schematic (A) of pin design and pictures of back side of tip (B, C), channel (D, E), top of channel (F, G) of serpentine and y-junction two-pronged blunt pin designs, respectively. Pins are attached to the wafer (H) by the side. Top of channels feature a stop valve [2] which allows accurate liquid metering by stopping liquid at the top of the microchannel. Scale bars in B through H are 200 microns.

3-sided pin tip

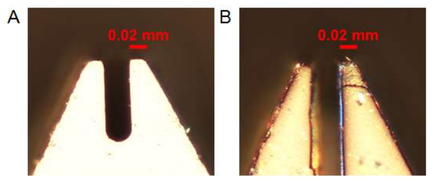

Figure 3. Closed back side (A) and open front side (B) of y-junction pin makes a 3-sided channel at the tip only. This is due to incomplete DRIE etch at the thinnest part of the channel which is the tip. Remaining parts of channels are 2-sided.

Improved pin designs:

- **High-volume** is achieved with double-channel (figure 2E) or serpentine channel (figure 2D).
- 3-sided tip channel (figure 2B, 2C and 3) leads to **a robust**, flat tip that does not break under normal printing conditions.
- **Flat tip** leads to ~100 microns spots on surfaces and easy transfer of liquid on any hydrophilic surface.

Experimental

Fabrication:

- Pins were fabricated at the McGill Nanotools Facility, using a 200 micron thick silicon-on-insulator wafer and etched by deep-reactive ion etching (DRIE).
- Pins are then detached in an HF bath overnight, and manually separated from the carrier structure.

Printing experiments:

- Four pins are loaded into a printing head mounted on a customized GeSIM nanoplotter. Those four pins print in parallel, simultaneously.
- Spots of AlexaFluor 647-conjugated chicken anti-goat IgG are spotted on 3D nitrocellulose surface (Avid Oncyte) or 2D functionalized glass surface (Xenobind).
- Slides are incubated overnight to allow binding, washed, and imaged using a fluorescent scanner.

Results and discussion

Characterization of spot morphology

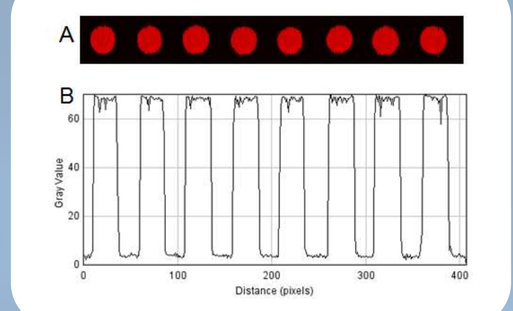

Figure 4. Spot intensity profile printed with y-junction pin. Analyzed using ImageJ.

Improved spot morphology:

- Spots are round and signal intensity is constant throughout the spot (< 5% variation locally).
- **Decreased variation** between technical replicate spots directly leads to **greater sensitivity** of assay.

Print results on different surfaces

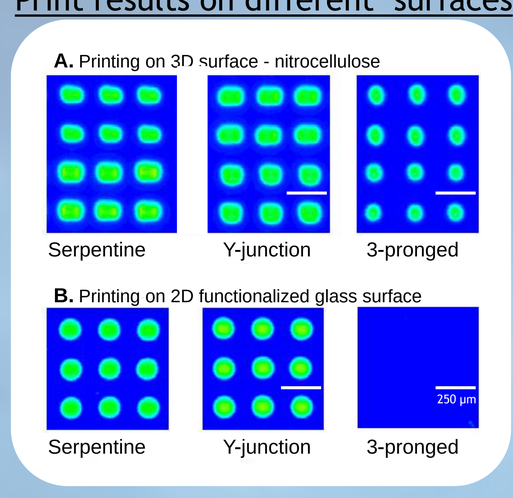

Figure 5. Spot morphology printed on 3D nitrocellulose (A) and 2D functionalized glass surface (B) shows that the 2-pronged serpentine and y-junction pins successfully print on all surfaces including glass. Scale bars are 250 microns.

Printing on glass:

- New pins can print on **2D glass surfaces** because of the flat tip.
- There is a much **greater variety** of 2D functionalized glass surfaces to choose from, which can be printed on with the two-pronged blunt pins.

Number of spots that can be printed on glass

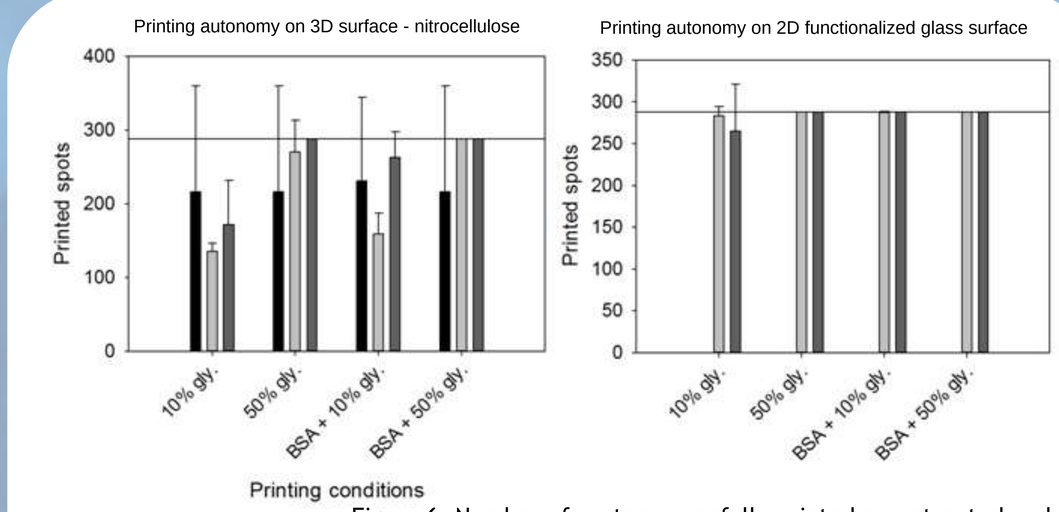

Figure 6. Number of spots successfully printed on untreated and treated slides (1% bovine serum albumin) in 10% or 50% glycerol, on 3D nitrocellulose (Avid Oncyte) and 2D functionalized glass slide (Xenobind). Serpentine and y-junction two-pronged blunt pins print for the full run (reference line, 288 spots) while the three-pronged pins are emptied before printing run is finished.

Improved printing autonomy:

- High-capacity two-pronged blunt pins can print over 700 droplets of < 1 pL each with a single dip.
- Greater printing autonomy (number of spots printed in a single run) leads to decreased reagent consumption and decreased printing time.

Conclusion

New designs:

- Common flat tip with thin single channel leads to robust printing of reproducible spots.
- Serpentine or double-channel more than doubles loading capacity, allowing spotting many more slides using a single dip.

Printing:

- Spots display consistent morphology, which facilitates analysis of images.
- Intensity of spots have a whole-slide coefficient of variation (CV) of ~30%, which can be further improved by data normalization.
- **Assay performance is expected to improve as lower CVs improve the limit of detection and detect analytes at lower concentrations.**

References

1. M. Pla-Roca et al., "Antibody Colocalization Microarray: A Scalable Technology for Multiplex Protein Analysis in Complex Samples", *Mol. Cell. Proteomics*, vol. 11, no. 4, pp. M111.011460-1-12 (2012).
2. R. Safavieh et al., "Straight SU-8 pins", *J. of Micromech. Microeng.*, vol. 20, pp. 055001-055009 (2010).

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