An All-at-Once Factorial Method to Optimize Dip-Pen Deposition of Liquid Protein Inks

A. K. Henning*[a], S. Rozhok[b], J. Fragala[a], R. Shile[a], and K. Ouyang[b]
[a]NanoINK, Inc., 215 E. Hacienda Ave., Campbell, CA 95008 USA
[b]NanoINK, Inc., 8025 Lamon Ave., Skokie, IL 60077 USA

Abstract

An all-at-once factorial method is presented, which optimizes protein ink deposition using microfabricated pens by identifying the pen design which writes the greatest number of uniform-size spots or droplets without re-inking. Pen features associated with capillary ink transport are varied according to statistical design-of-experiment (SDOE) principles, and evaluated using a special 1D pen array of twelve pens. Variable parameter pens are bracketed by control pens. Each pen array element embodies one component of the SDOE matrix. All parameters are evaluated simultaneously with a single droplet writing pass. Results can also be evaluated simultaneously, leading to rapid choice of those pen parameters which deliver the greatest number of printed features having the smallest coefficient of variation.

Keywords: dip-pen nanolithography, protein microarrays, statistical design of experiments, biomolecular printing

1. INTRODUCTION

Protein arrays for disease assay, and pharmaceutical development, are a recent advancement taking off from genomic array successes.

Dip-pen nanolithography (DPN) methodologies[1,2] enable multiplexed droplet array printing with the small volumes needed for simultaneous preservation/conservation of high-value protein reagents, and quantitative assay of protein concentration[3,4]. Array evaluation demands small coefficients of variation (CV%). That is, the spot-to-spot variation must be small, in order to ensure maximum sensitivity of the assay measurement. Droplet diameters down to 0.5 um can be printed, depending upon the microfluidic parameters in play (e.g. fluid viscosity, fluid surface tension, contact angle between the protein-containing fluid and the solid surface, and evaporation rate). A wide range of protein inks may be printed. Each has unique fluid characteristics. Even with external condition control (specifically, control of temperature and relative humidity during deposition), liquid transfer from pen to surface can vary dramatically, depending upon the number of droplets already written, and which pen is being used to print the spot or droplet. Easy optimization of pen designs therefore becomes desirable, in order to make best/most efficient use of the high-value inks.

Here, an all-at-once factorial method is presented which optimizes protein ink deposition pens. Pen features associated with capillary ink transport are varied according to SDOE principles, and evaluated using a special 1D pen array of 12 pens. Pens with varying spatial dimensions of features important to capillary ink transport, are bracketed by control pens. Each pen array element embodies one component of the SDOE matrix. All parameters are evaluated simultaneously with a single droplet writing pass. Results can also be evaluated simultaneously, leading to rapid selection of those pen parameters which deliver the greatest number of printed features having the smallest coefficient of variation.

2. PEN PARAMETERS AND EXPERIMENT DESIGN

Figure 1 shows, first, a 1D array of 12 identical pens, used in current manufacture of protein assay arrays; and second, the experimental pen array, fabricated according to SDOE principles, used in optimized pen selection. Table 1 shows the specific pen parameters used in the SDOE array. “Throat width” is the narrowest width in the keyhole portion of the pen (see Figures 1 and 5). This structure provides wall constraints for passive ink flow (driven by capillary forces). “Tail width” is the widest part of the keyhole. “Tip-to-throat distance” defines the distance between the writing tip, and the throat. Additional variables were the thickness of the silicon nitride in the pen cantilevers (nominally 0.6 um), and the depth of the ink-holding cavity in the cantilevers (the ‘keyhole’, nominally 0.25 to 0.5 um deep).

*ahenning@NanoINK.net; phone 1-408-379-9069; fax 1-408-379-9072; http://www.NanoINK.net

The keyhole structure evident in all pens is formed during the casting of the silicon nitride thin film (approximately 0.6 um thick) onto a silicon mold wafer during pen fabrication. The keyhole is formed by means of an oxide layer between 0.25 and 0.5 um thick. The presence of the oxide, in the shape of the keyhole, forms sidewalls with a height equal to the oxide thickness. These sidewalls constrain the motion of the protein fluid ink, and help drive it from the cantilever to the tip of the pen, when the pen tip comes into contact with a hydrophilic surface (e.g. a conventional glass slide used commonly in biology studies). Details of the transport physics may be found in several references[5-7], but are not included in this work.

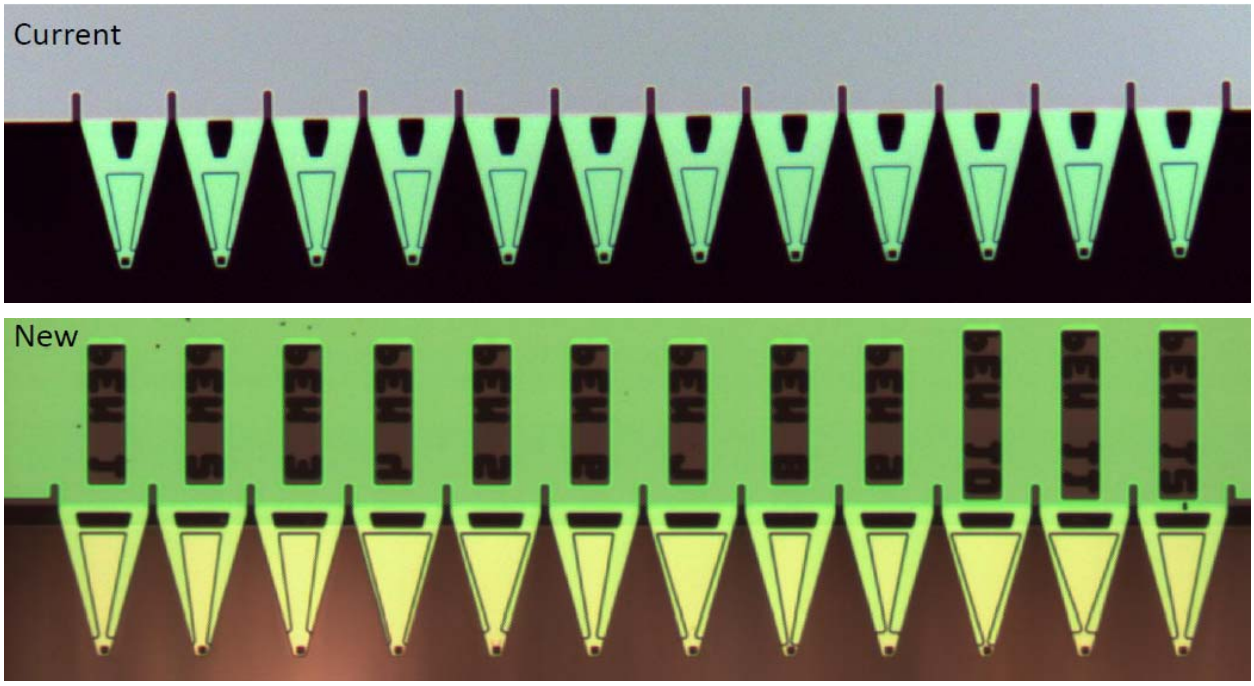

Figure 1: 1D array of pens for liquid deposition. Top: Current state of the art. Bottom: 1D array of pens with varying parameters, used in the experimental design.

Pen Number	**W_{Tail}**	**W_{Throat}**	**$L_{Tip\text{-}to\text{-}Throat}$**	**x**	**y**	**z**
1 (control)	33	6	6.7	0	0	0
2	29	9	2	-	+	-
3	29	9	11	-	+	+
4	49	9	2	+	+	-
5	49	9	11	+	+	+
6	29	6	6.7	-	0	0
7	49	6	6.7	+	0	0
8	29	3	2	-	-	-
9	29	3	11	-	-	+
10	49	3	2	+	-	-
11	49	3	11	+	-	+
12 (control)	33	6	6.7	0	0	0

Table 1: Pen Parameters in Experiment Design (in um). The last three columns show the relative value of each of the three variables against the nominal value. There are three independent variables, so the cube in Figure 2 depicts the variation of the parameters.

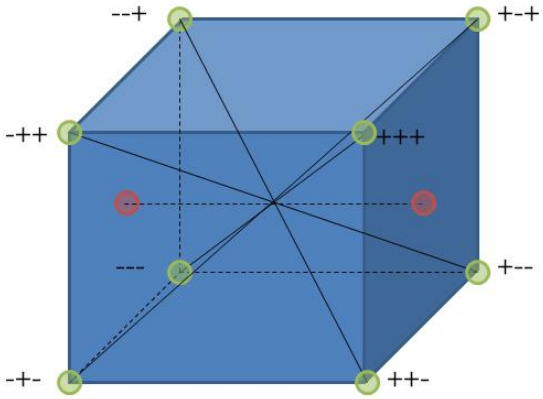

Figure 2: Schematic of the three-dimensional variable space used in the statistical experiment design (a partial factorial design). The x-axis corresponds to the 'tail' dimension; the y-axis corresponds to the 'throat' dimension; the z-axis corresponds to the 'tip-to-throat' distance. The green spheres represent the portions of the variable space explored by devices built according to Table 1. Additional devices (pens 6 and 7, red spheres) were added, because the usual 1D pen array constitutes 12 pens and allowed exploration of more of the full-factorial parameter space.

The transfer of ink to the pens prior to writing is a three-part process. Ink is first transferred from a micropipette to a silicon inkwell chip[8] (see Figure 3). Passive-force capillary action (consistent with the requirements of protein inks, which tolerate temperature, electric field, and high pressure only poorly) draws the ink from the large reservoirs to the microwells on the same silicon inkwell chip. From this position, the NLP-2000 system[9] positions the 12 pen array over the microwells, then drops the pen tips into the microwells. When the tips are in contact with the ink in the microwells, capillary forces again allow the ink to be drawn up onto the pens. The pens are then said to be 'loaded' with ink, which is, in effect stored directly on the silicon nitride cantilevers. In these experiments, the same ink was used for all 12 inkwells, although the system facilitates using a different ink for each inkwell.

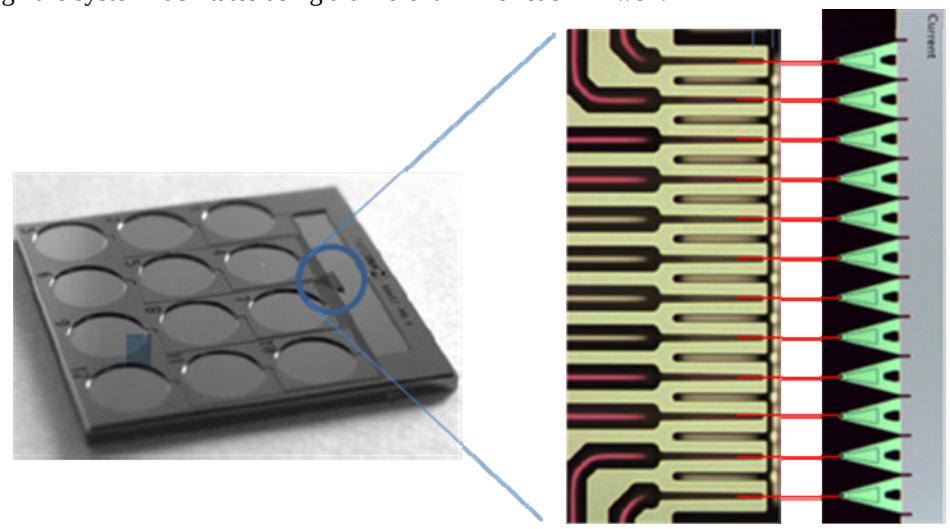

Figure 3: How ink is transferred to the writing pens. Left: Micropipettes transfer up to 12 different inks to the 12 reservoirs of a silicon inkwell chip. Center: Capillary flow transfers the inks from the reservoirs to the micro-wells (finger structures). Right: Pens pick up ink from the micro-wells by dipping pen tips into the ink-filled slots. Each pen aligns with a single slot (red lines), and there is no cross-talk between slots.

Following previously-established procedures[10], pens were plasma cleaned for 40 sec at low power in a plasma cleaner. NanoINK A2-type carrier inks, containing cytokine IL-5 capture Ab protein, and Alexa 555 tracking dye, were used for printing. Pen arrays were mounted into a NanoINK NLP-2000 system. Arrays of 100 spots were printed on Schott Slide E epoxy substrates. Relative humidity was 30%. Dwell time during each print interval was 0.2 sec; spot-to-spot time interval was 1.2 sec. Vertical clearance for spot-to-spot stepping was 200 um. Spot spacing was 30 um.

3. PRINTING RESULTS

Figure 4 shows qualitatively the pen writing process. After pens are loaded with ink, it is possible for the first spots to be large, resulting in the requirement for a 'blotting' step, prior to deposition of spots in the area targeted for array fabrication. Writing then proceeds, slowly drawing down fluid from the effective reservoir of fluid stored on the pen. Finally, the reservoir is depleted, so that any spots written further will be much smaller than desired, with a CV% outside of the specified range for array manufacture.

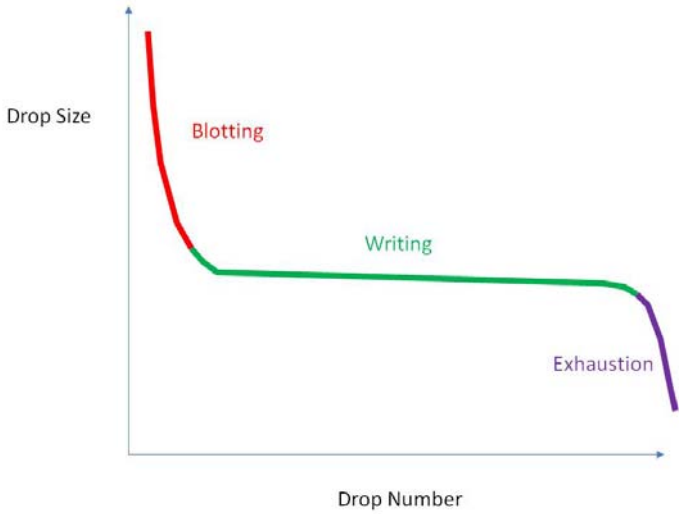

Figure 4: Schematic of the pen writing process for liquid inks. Overall goals are: minimize or eliminate blotting duration and ink depletion during blotting; maximize duration of writing; minimize coefficient of variation during writing.

Figure 5 shows raw results recorded using an Innopsys fluorescent scanner. Data were processed using Mapix software, to relate the measured fluorescence to a spot size. Figure 6 shows an example of the processed data, comparing the control pen, to the worst and best pen.

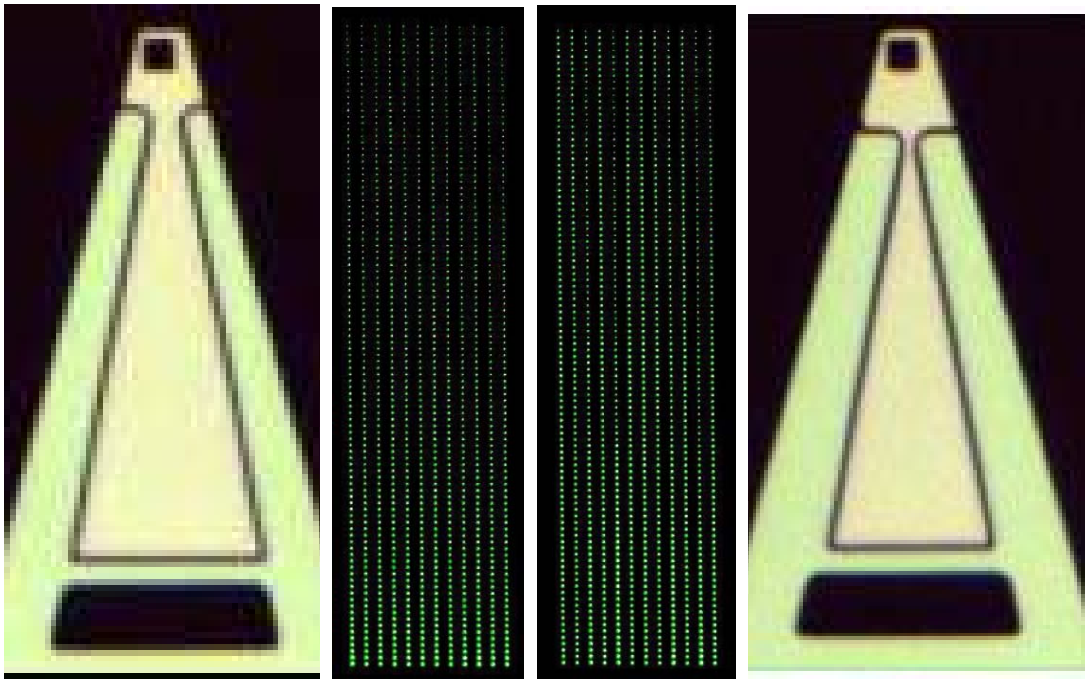

Figure 5. Left: Photo of Pen #1. Middle left: Results for 1D array of all-Pen #1. Middle right: Printing results for 1D array of SDOE pens. Right: Photo of Pen #11, having best results.

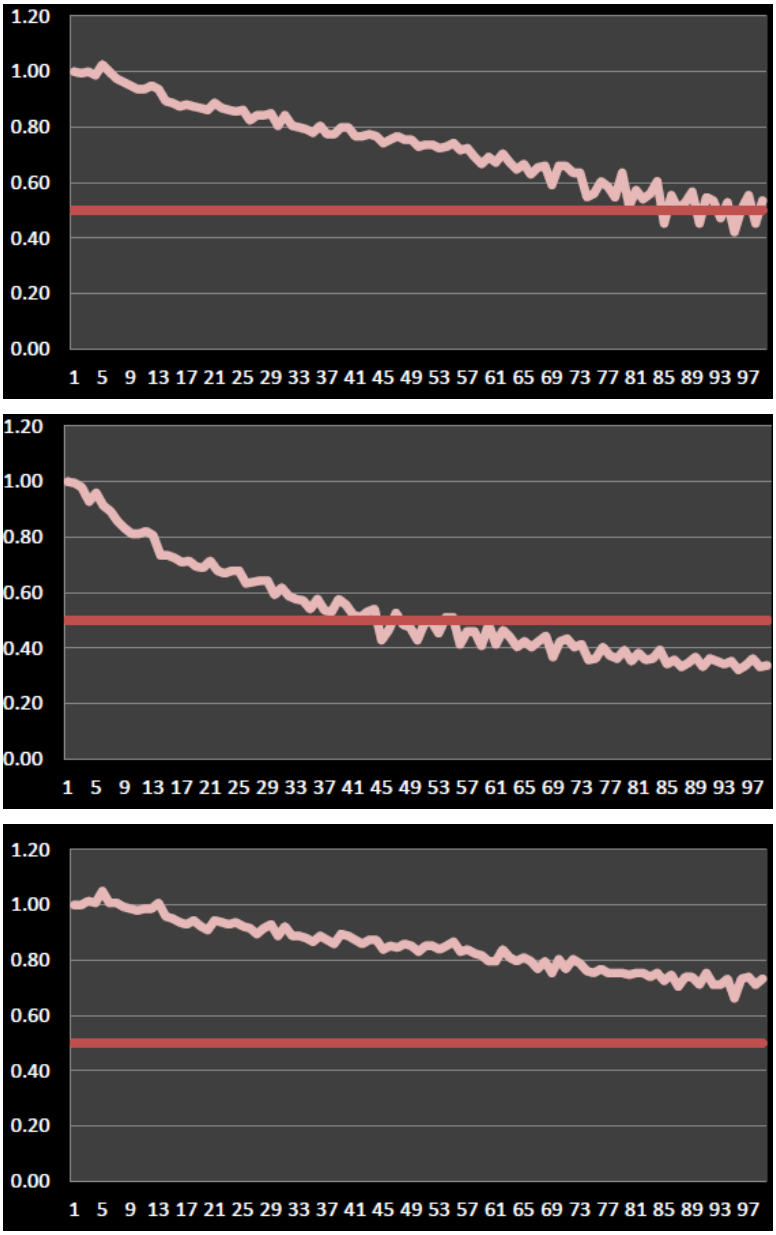

Figure 6: Normalized drop size vs. drop number. Top: Results for Pen 1 (control/center of SDOE cube). Middle: Results for Pen 2 (poorest). Bottom: Results for Pen 11 (best).

4. DISCUSSION

The best results for this ink matrix and protein combination are achieved for the narrowest throat width, and the largest tip-to-throat distance. Tail width was a less important contributor to overall performance. Tip-to-throat distance proved to be of greatest importance, confirmed by the performance of related pens.

Having identified the pen type best-suited to deliver ink, with the longest duration and best CV%, we then proceeded to refine the other deposition parameters, such as residence time (that is, the contact time between the pen tip and the deposition surface), pull-away velocity (the rate at which the pen is pulled up off the surface after ink deposition), and the time between deposited spots/droplets.

A 12-pen array comprising pen type 11 was used to repeat the experiments of the preceding section. The results are shown in Figure 7.

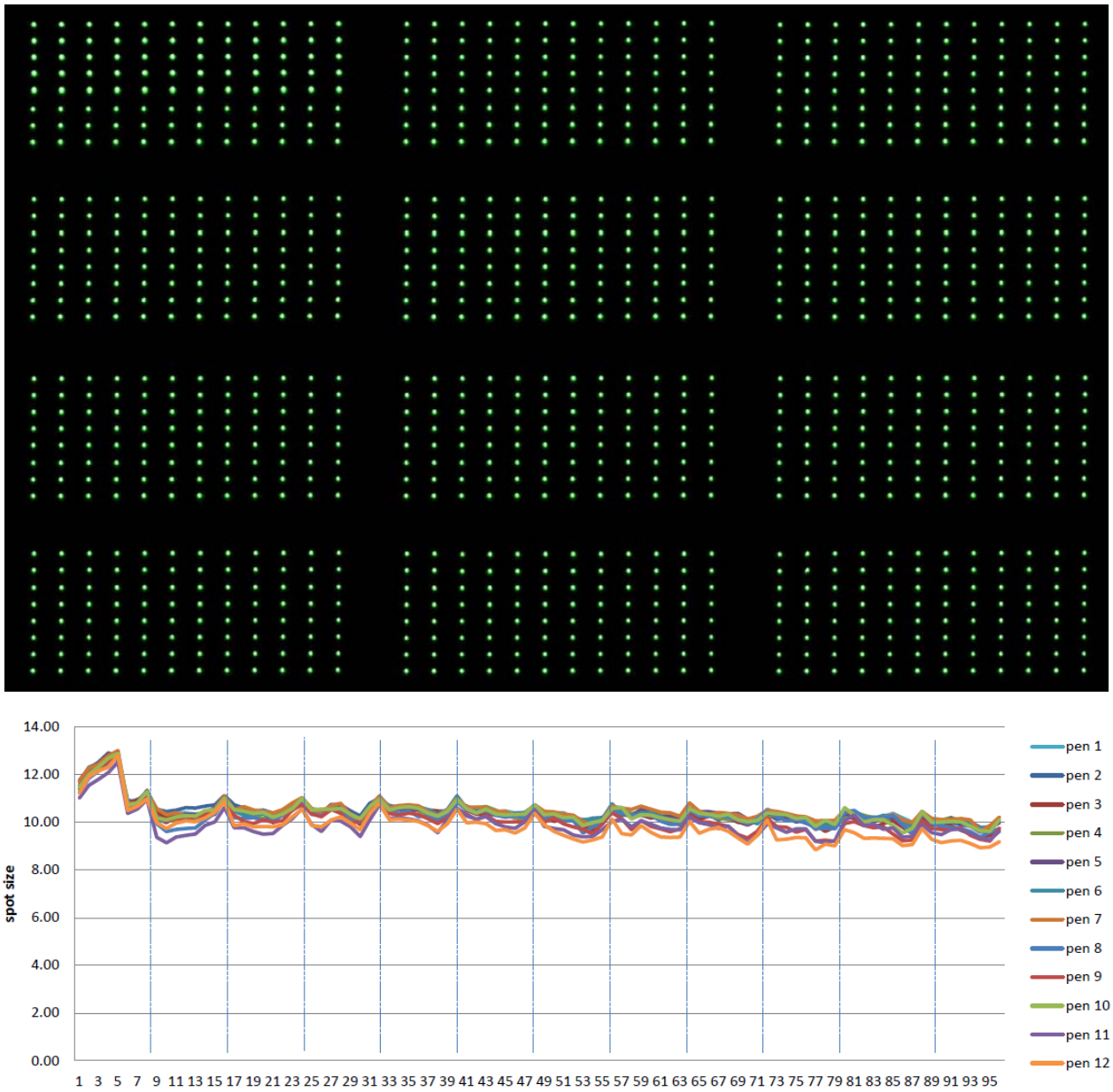

Figure 7: Top: Raw fluorescence images of printing from a 12-pen array, starting at upper left, moving top to bottom, then left to right. Bottom: Drop size vs. drop number.

5. CONCLUSIONS

Using an all-at-once factorial SDOE approach, we have demonstrated improvements in pen designs for liquid protein spot deposition. The SDOE approach uses a 1D array of twelve pens, so that printing of all parts of the experiment parameter space occur simultaneously. The results show an all-at-once approach can be used to optimize the pen design rapidly for best ink deposition, given a set of material and deposition/environmental parameters.

REFERENCES

[1] Piner, R. D., Zhu, J., Xu, F., Hong, S., and Mirkin, C. A., "Dip-Pen Nanolithography". Science 283 (5402), 661-663 (1999).

[2] Demers, L. M., Ginger, D. S., Park, S.-J., Li, Z., Chung, S.-W., and C. A. Mirkin, "Direct Patterning of Modified Oligonucleotides on Metals and Insulators by Dip-Pen Nanolithography," Science 296 (5574), 1836-1838 (2002).

[3] Collins, J. M. and Nettikadan, S., "Subcellular scaled multiplexed protein patterns for single cell co-cultures," Analytical Biochemistry 419 (2), 339-341 (2011).

[4] Irvine, E. J., Hernandez-Santana, A., et al., "Fabricating protein immunoassay arrays on nitrocellulose using dip-pen lithography techniques," Analyst 136 (14), 2925-2930 (2011).

[5] Berthier, J. and Brakke, K. A., [The Physics of Microdroplets], John Wiley & Sons, Hoboken, NJ (2012).

[6] Rosner, B., Duenas, T., Banerjee, D., Shile, R., Amro, N., and Rendlen, J., "Functional extensions of Dip Pen NanolithographyTM: active probes and microfluidic ink delivery," Smart Mater. Struct. 15, S124–S130 (2006).

[7] Cruchon-Dupeyrat, S., Nelson, M., Elghanian, R., Fragala, J. S., Touzov, I., and Banerjee, D., "Methods and apparatus for ink delivery to nanolithographic probe systems," U.S. Patent 7,762,638 (27 July 2010).

[8] -------, "NLP 2000 System," <http://www.nanoink.net/nlp-2000-system.html> (24 December 2012). http://www.nanoink.net/nlp-2000-system.html

[9] -------, "DPN Inkwell Arrays," <http://www.nanoink.net/consumables.html#inkwell> (24 December 2012). http://www.nanoink.net/consumables.html#inkwell

[10] Jang, J.-W., Smetana, A., and Stiles, P., "Multiplexed dip pen nanolithography patterning by simple desktop nanolithography platform", Scanning 31, 1-6 (2006).