

High Density Peptide Array Printing and Synthesis

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I. Abstract

The ability to print high-density arrays has become an important tool in such applications as drug screening, molecular biology, and genetic analysis. Printing many samples onto a solid substrate allows researchers to efficiently screen thousands of conditions in a very small space saving time and money and increasing throughput.

We have developed a system capable of printing high-density arrays for antibodies and peptides using micro-solenoid valves. This printing technology can also be used to synthesize peptides on derivatized cellulose membranes. This allows researchers to produce thousands of variations of peptides in very small amounts and at high density.

Consistently printing small volumes on solid substrates involves many factors. Pulse length, pressure, solvent concentration and composition, substrate surface treatment, and robotic movement and speed were studied. Through careful optimization, we were able to consistently print a volume of 15nL onto epoxy coated glass slides. We will discuss the optimization procedure and present data using our printing technology and explore antibody array applications.

II. Introduction

Synthetic peptides play a useful role in the study of protein interactions with other biomolecules. This has led to a growth in the importance of peptide-based microarray printing. Improvements in the manufacturing of peptide arrays and advances in applications of them are opening new opportunities to probe the expression and function of the proteome (1). This technology allows for peptide interactions to be studied at ultra high-throughput. Thousands of conditions and/or experiments can be studied in a very small area. Advancements in peptide libraries, in particular, have accelerated the process (2).

There are several methods that are capable of producing large numbers of peptides in high purity, but these pose technical difficulties under full automation conditions. In such procedures, manual intervention is necessary between each automated synthesis cycle. Aurora Biomed has also researched the development of an instrument to carry out peptide synthesis on specialized membranes and such technologies could be used to produce peptides for such array printing.

Here we present our findings in printing volumes as low as 15nL onto glass slides. We were unable to present some of the peptide data at the time of this poster and so we will present here some of the conditions that we optimized in order to get stable array printing at these volumes.

III. Materials & Methods

A. Versa 1000:



Optimum Settings:

- Pressure → We used a pressure between 8-15psi, but found that stability is more important than the actual pressure. Using a feedback control loop we were able to obtain pressure values with 0.5% CV.
- Pulse length → 850 microseconds
- Volume → 15nL
- Instrument Layout:
 - stainless steel dispensing pins
 - xyz arm with optic ruler (25 micron resolution)
 - custom billet aluminum glass slide holder

The robotic movement also required optimization. For example a brief pause was required in the sequence before each sample dispensing step, otherwise dot spacing would not be accurate. The pause was presumably to cancel out any small vibrations in the arm or kinetic energy effects. This pause allowed the use of relatively high arm speeds, which more then outweighed the effect of the pause on total printing time.

B. Glass Treatment:

The glass slides generally used for array printing are very specialized and are relatively expensive. We chose to develop our own method for glass treatment to reduce our development costs. The two methods we found to be quite successful are:

- 1) **Sylgard Method:** Sylgard is a two part silicone elastomer made by DOW CORNING and is available from WPI (World Precision Instruments). The use of this epoxy has yet to be fully tested with peptide arrays, but it does provide for a very durable coating which is very hydrophobic.
- 2) **Sigmacote Method:** Sigmacote is available from Sigma. This has not been tested with any peptide diagnostic system, but it is a very economical and quick way to make very hydrophobic glass slides. This technique involves taking a clean glass slide and coating it in a thin layer of Sigmacote and allowing it to dry.

In both methods, it is important that before the coating process the glass slides be as dry as possible and free from any contaminants.

C. Solvents and Solution:

For our early testing and system debugging we chose to use colored dye solutions as replacement for peptide solutions. Various concentrations of glycerol were tried and a 30% glycerol solution gave the best results. Concentrations higher than this were difficult to aspirate and dispense, and concentrations lower than this led to evaporation occurring too quickly which resulted in unstable arrays.

IV. Results

D. What is the performance of the Versa 1000 for array printing?

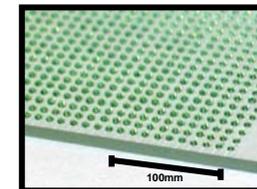


Figure 1: Magnified view of 30nL spots on a glass slide

The current minimum volume for array printing using the Versa 1000 is 15nL. The percent CV at this volume is 9.5%, which is acceptable since the use of a peptide array is generally in qualitative assays.

Further results will be published on the use of these arrays for diagnostic purposes.

V. Conclusion

Printing small volumes on glass requires the optimization of many factors, including glass surface treatment and solvent concentration and composition. We hope that with further optimization and testing that we can bring our minimum volume down to 1nL or less.

VI. Acknowledgements

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VII. References

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