Application Note

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Making spatial transcriptomics accessible: Cost-effective manufacturing of DNA arrays using double-barcoded probes and the sciFLEXARRAYER S3 precision microdispenser

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Abstract

Background: Spatially resolved transcriptomics (SrT) is a cutting-edge technique that enables scientists to measure all gene activity in a tissue sample and map where the activity is occurring.

Challenge: Up to now, current methods can be cost-prohibitive in their use limiting their accessibility to innovative research institutions.

Solution: Here a low-cost strategy for manufacturing molecularly double-barcoded DNA arrays is presented using the sciFLEXARRAYER S3 precision microdispenser. This strategy has been successfully applied to perform large-scale SrT studies of human brain organoids, allowing a three-dimensional molecular cartography of this complex tissues.

Introduction

Many new biological research fields are not yet accessible to scientific institutions due to the high cost of current techniques. For instance, array manufacturing for SrT at large scale is challenging due to high instrument and reagent expenses, particularly due to the high number of unique DNA probes needed [1].

In this case study, an improved and less costly strategy for manufacturing molecularly barcoded DNA arrays for SrT, based on the sciFLEXARRAYER S3 picoliter microdispenser, is presented. Instead of using just one molecular barcode per region, the method, described in more detail in the article by Lozachmeur et al., 2023[2], combines two probes with molecular barcodes for the x and y coordinates. To distribute the double-barcoded DNA probes most efficiently on the arrays the sciFLEXARRAYER S3 picoliter microdispenser was used. With this method, large-scale spatially resolved transcriptomics studies were enabled, and verified by analyzing consecutive sections of human cerebral organoids, producing a three-dimensional molecular tissue reconstitution.

Materials and Methods

In this application note the deposition of DNA probes using the precision microdispenser sciFLEXARRAYER S3 is described as part of the overall method for manufacturing double-barcoded poly T-DNA arrays for SrT. Method details for the entire workflow can be found in the article by Lozachmeur et al., 2023[2].

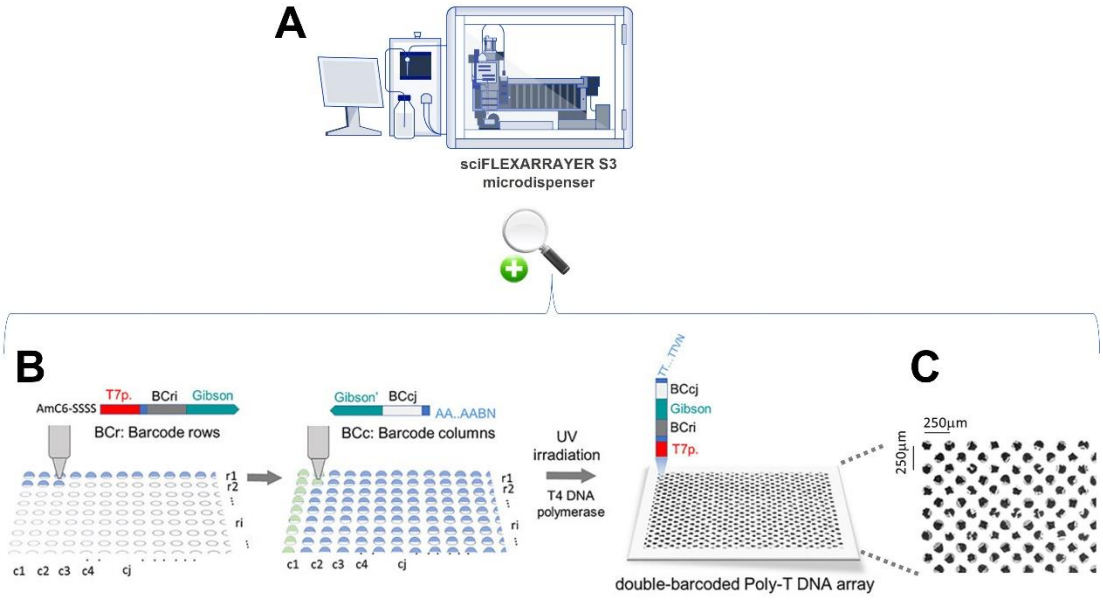

Figure 1: Method to manufacture double-barcoded DNA arrays for spatially resolved transcriptomics (SrT). **(A)**The sciFLEXARRAYER S3 microdispenser was used to print the double-barcoded DNA arrays. **(B)** Strategy for printing DNA arrays based on the deposition of two types of oligonucleotides harboring distinct molecular barcodes (per row: BCri; per column: BCci). Both oligonucleotides share a complementary sequence (Gibson). The 5′ end of the BCri oligonucleotide contains an amino C6 linker modification, followed by 4 “S” (cytosine or guanine) nucleotides, for UV crosslinking. Once BCcj primers are deposited on top of the printed BCri oligonucleotides, the glass slide is UV irradiated for covalent crosslinking, followed by probes’ elongation thanks to their complementary Gibson region. This elongation process (T4 DNA polymerase) generates a long probe composed by a T7 promoter (T7p), two unique molecular identifiers (UMIs), two molecular barcodes flanking the complementary Gibson sequence, and a poly(T). **(C)** Micrograph illustrating a part of a manufactured DNA array composed by 32 × 32 printed spots in an interstitial manner, leading to a density of 2,048 different probes. Figure taken from Figure 1, Lozachmeur, Gwendoline, et al. and adapted.

Manufacturing double-barcoded poly T-DNA arrays

Figure 1 summarized the strategy to manufacture double-barcoded DNA arrays for spatially resolved transcriptomics (SrT). In brief, DNA arrays containing 2,048 probes (177 mm pitch distance; 100 mm printed spot) were generated by depositing first 250 pL of each of the 32 "Barcode for rows"(BCr) oligonucleotides per row (250 mm between contiguous spots) following 250 pL of 32 "Barcode for columns" (BCc) oligonucleotides on top using the precision microdispenser (SCIENION sciFLEXARRAYER S3). Then, the same 32 BCr oligonucleotides were printed per row with a shifted position of 125 mm in both axis (i.e., interstitial printing, Figure 1 C), followed by the deposition of other 32 different BCc oligonucleotides printed per column. In total, 32 unique BCr (printed twice) and 64 unique BCc oligonucleotides were needed.

Precision microdispensing using the sciFLEXARRAYER S3

The sciFLEXARRAYER S3 is an automated piezo driven, non-contact micro dispensing system specifically designed as an economical entry unit for academia and R&D labs. It contains Piezo Dispense Capillaries (Figure 2A) to aspirate and dispense liquid at the picoliter scale and is suitable to produce miniaturized DNA, protein and glycan arrays, cell transfection arrays, and for loading MALDI-MS targets or biosensor surfaces.

Results and discussion

With the previously introduced approach, large-scale DNA arrays for spatial transcriptomics composed of 2,048 different probes were manufactured. The precise depositing of this high number of probes, partly on top of each other, was possible due to the unique features of the sciFLEXARRAYER S3 precision microdispenser. Using that technique, ultra-low volumes of DNA probe were printed on the target surface, in this study 250 pL per probe. The dispenser also allows you to deposit precisely along the x and y printing axis with minimal distances between spots to cover the target glass slide most efficiently with probes. This was shown by printing in an interstitial manner, depositing 32 X 32 spots twice with a minimal shifted position of 125 µm in both axis (Figure 1 G). Due to its high accuracy in depositing ultra-low volumes, the sciFLEXARRAYER S3 is ideal for miniaturizing assays and dispensing on customized targets.

As a result of the dual high-resolution cameras (Figure 2, Drop and Head cameras), the sciFLEXARRAYER system provides full control over drop generation and target positioning, allowing to verify the quality of the printout. With the drop camera, the generation of droplets, as well as the drop volume, quality, and deviation from the printing axis were monitored, revealing stable droplets at the picoliter scale (Figure 2 B). At the end of the printing process, the head camera allowed inspection of the target, revealing spots that failed quality control, such as fused spots (Figure 2 C).

The use of significantly fewer probes results in significant cost savings

Due to the combinatorial deposition of two independent barcodes, only 96 unique oligonucleotides are required for manufacturing a grid composed of 2048 unique molecules. Considering that a synthetic oligonucleotide cost ~60 euros, a total of ~6000 euros is necessary for covering these reagents; while more than 120 000 euros might be required if purchasing 2048 unique oligonucleotides; i.e. a cost-saving of ~20-fold.

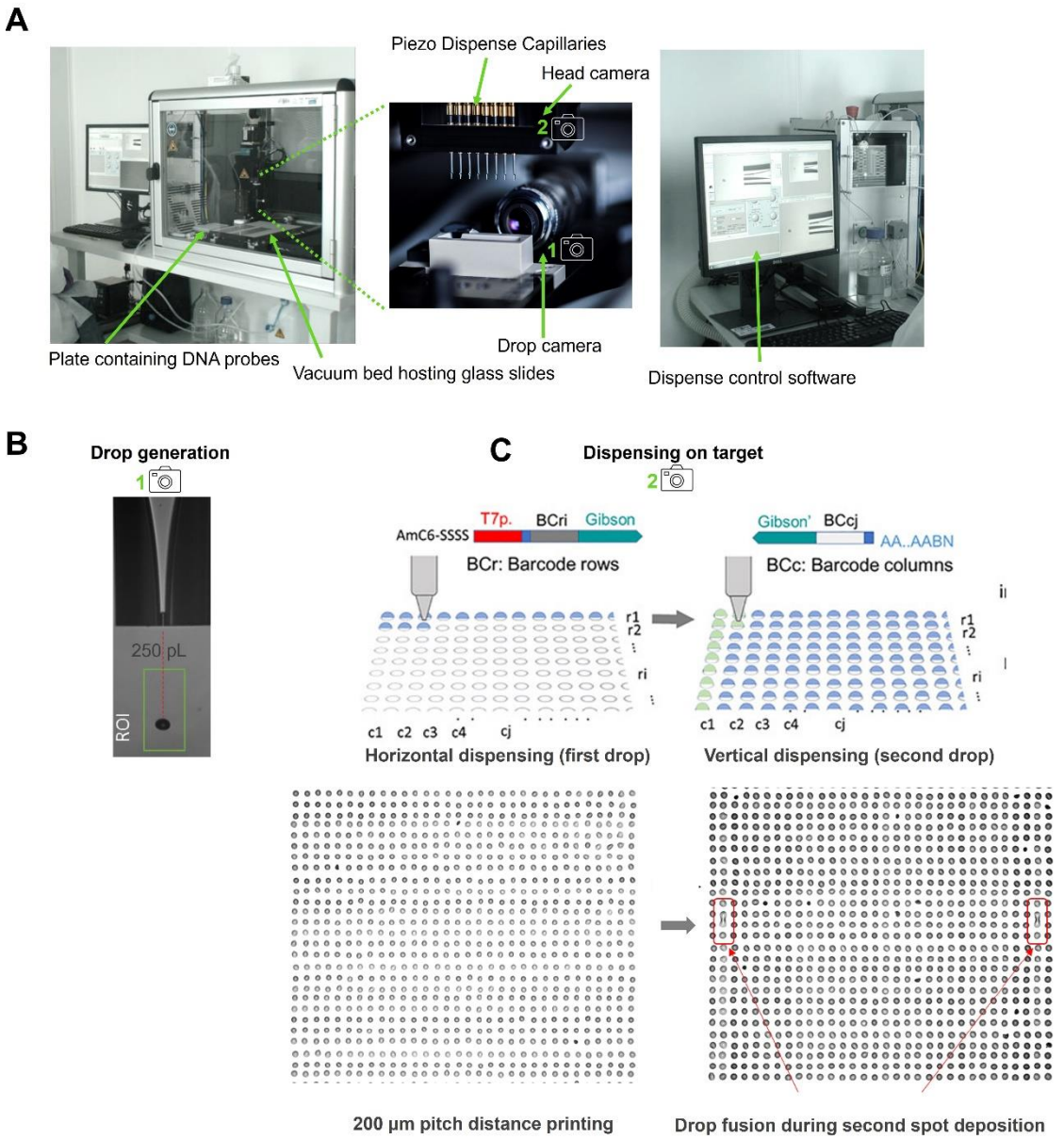

Figure 2. Printing probes with the microdispenser sciFLEXARRAYER S3 (SCIENION), related to Figure 1 (A) Overview photographies of Genoscope's sciFLEXARRAYER S3 instrument. Arrows and numbers indicate the presence of (i) the Piezo Dispense Capillaries; (ii) the head camera used for imaging glass slides after printing; (iii) the drop high resolution camera allowing to visualize and quantify drop parameters; (iv) the vacuum bed in which glass slides are immobilized; (v) and the location of a 96-well plate containing DNA probes to be printed (vi) the computer screen with the software controlling the sciFLEXARRAYER S3 microdispenser. In the screen the drop visualization by the high-resolution camera is shown. (**B) Drop generation control.** Image captured by the high-resolution drop camera allowing to visualize the tip of the needle as well as the drop that is ejected. The ROI (region of interested) allows to quantify the deviation of the central axis, the drop volume as well as the distance between the tip of the needle and the position of the drop. **(C) Drop dispense control** of the target surface using the head camera. Top: Scheme illustrating the strategy for depositing probes harboring the molecular barcode for the horizontal axis, then those associated to the vertical axis. Bottom: Example of a printed slide, first in the horizontal axis (left image), then in the vertical axis on top of the previous printing (right image). Note that two fused drops are visible in the second printout. As part of the manufacturing process, all printed glass slides are evaluated in this manner to evaluate the potential fusion errors and discard glass slides in case of major problems. Figure taken from Figure S1, Lozachmeur, Gwendoline, et al. and adapted.

Conclusion and future direction

This study presents a strategy that brings SrT within reach of researchers who have previously been unable to access it due to the high cost of manufacturing the assays. By using fewer probes and dispensing them on the target efficiently, scientists can perform molecular biology strategies that were previously too costly.

References

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