Development of a custom spatial -omics platform using a photolithographic DNA printer

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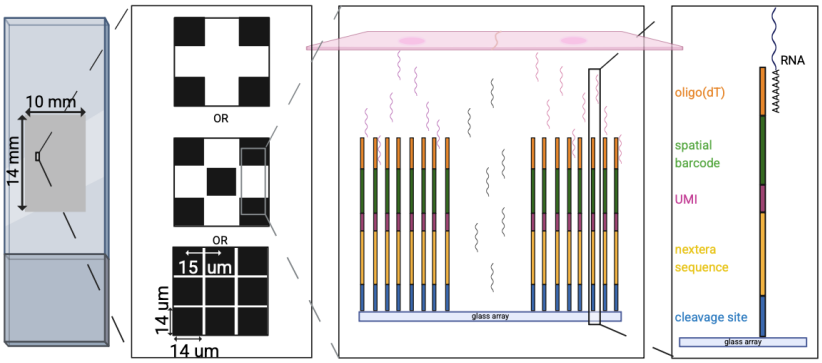
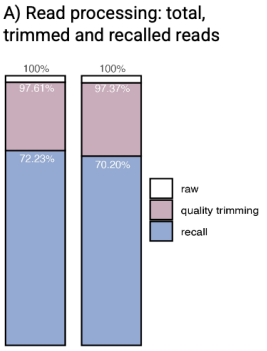
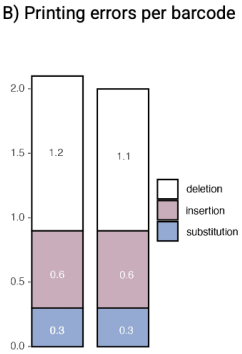
PLATFORM DESIGN



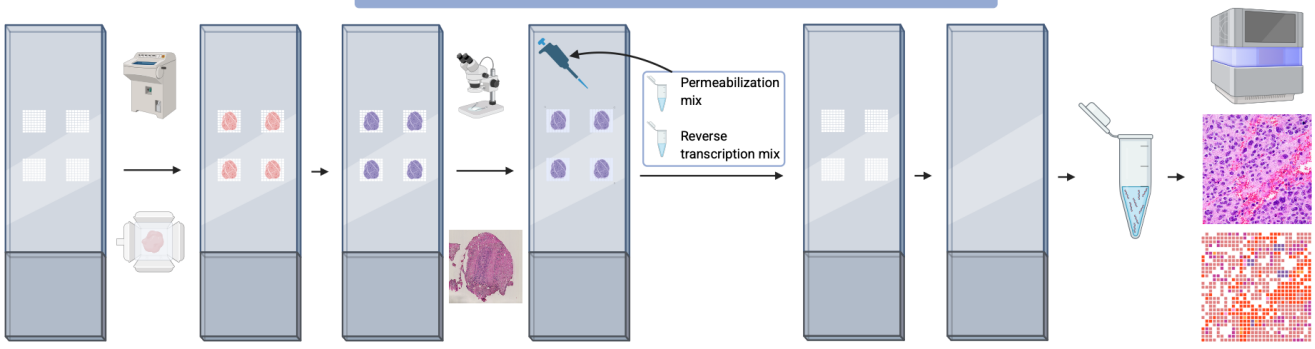




Platform specifications: The spatial transcriptomics (ST) platform features slides with a 14 mm × 10 mm printable area, containing 786 432 spots (14 µm × 14 µm) with 1 µm spacing. Each spot holds 2–5 million capture probes consisting of a cleavage site, nextera sequence, unique molecular identifier (UMI), spatial barcode, and oligo(dT). Printing can be done in full density, 1/2 density, or 1/4 density.

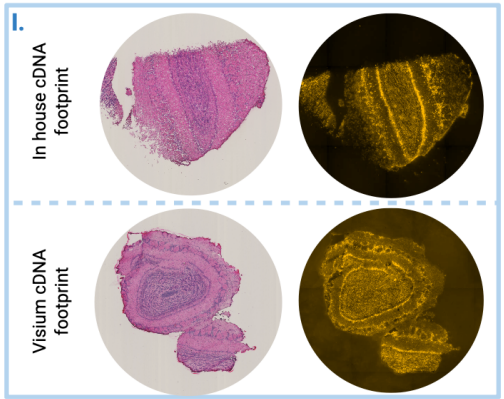
Barcode printing quality: The capture probes are printed onto the slides using photolithography, an error prone technology. **A)** shows the recall rate of probes, which is approximately 70% for the 1/4 design. **B)** presents the average number of errors per barcode (36 nt), with deletions being the most common errors.

OPTIMIZATION ST WORKFLOW

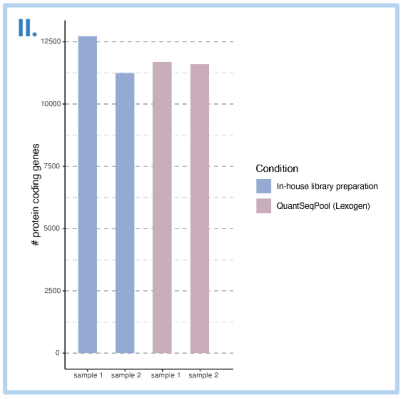


I.
1. Slide with capture probes
2. Attach tissue sections + fixation
3. H&E staining + imaging
4. Permeabilization of tissue + cDNA synthesis
5. Tissue digestion
6. Cleavage of cDNA

II.
7. Library preparation in tube
8. Sequencing and data analysis



Fluorescent cDNA footprints: Fluorescent cDNA footprints from olfactory bulb tissue were successfully generated by incorporating Cy3-dCTP into the reverse transcription (RT) reaction mix. After tissue digestion, the slide was scanned with a fluorescence scanner to visualize the footprints. The top image was obtained using the Visium 10X Genomics reagents, the bottom image with in-house reagents.



In-house library preparations: Comparison of protein-coding genes detected using our in-house library preparation method versus QuantSeqPool (Lexogen) with identical RNA input quantities. Both methods yield comparable results.

FUTURE GOALS

- Sequence libraries obtained with spatial transcriptomics workflow
- Make spatial transcriptomics workflow compatible with FFPE tissue
- Develop a targeted spatial transcriptomics protocol

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