Development of a custom near single-cell spatial transcriptomics platform using photolithography to study cellular heterogeneity

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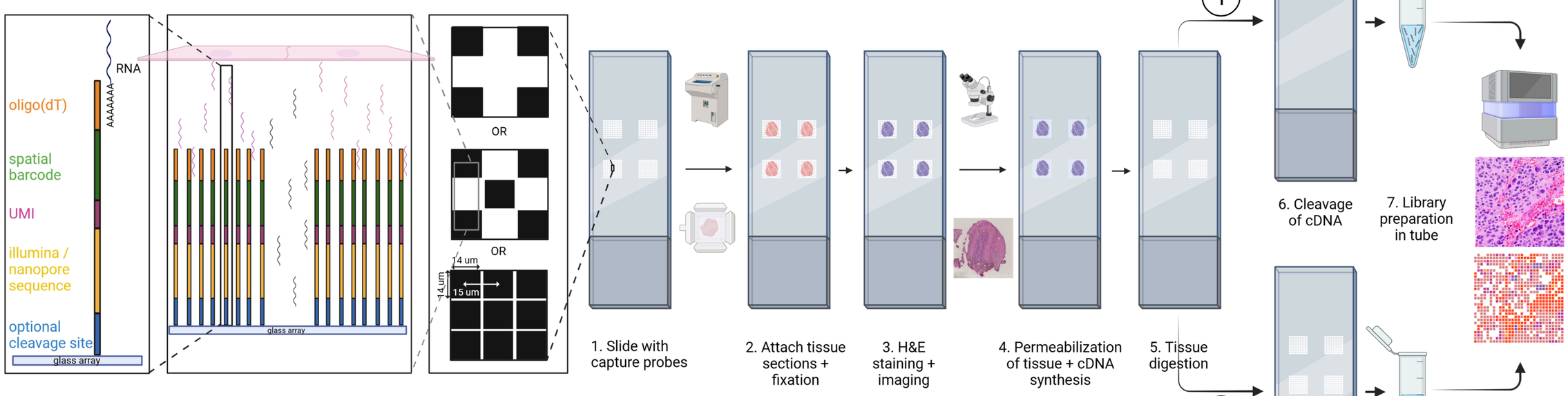
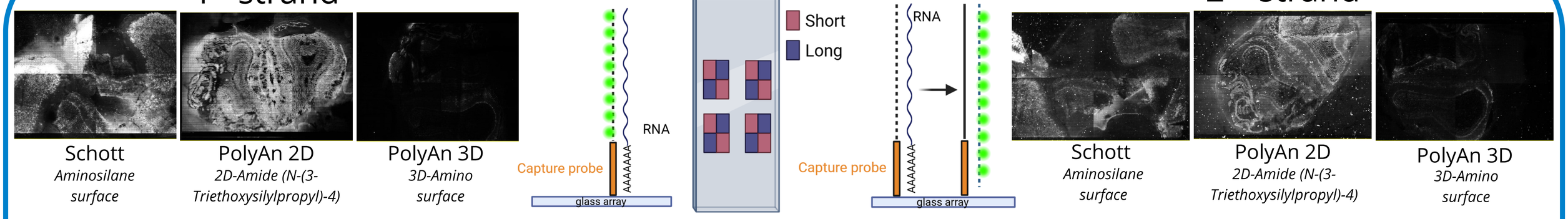
Platform design



Platform specifications: The spatial transcriptomics (ST) platform features slides with a 14 mm × 10 mm printable area, containing a maximum of 786 432 spots (14 µm × 14 µm) with 1 µm spacing. Each spot can hold 2 – 5 million capture probes consisting of an optional cleavage site, illumina or nanopore sequence handle, a 10 bp unique molecular identifier, a 36 bp spatial barcode, and 20 bp oligo(dT). Printing can be done in full density, 1/2 density, or 1/4 density, but also in other custom designs. For ST applications, we currently make use of a design with 4 subarrays, each allowing a separate ST reaction.

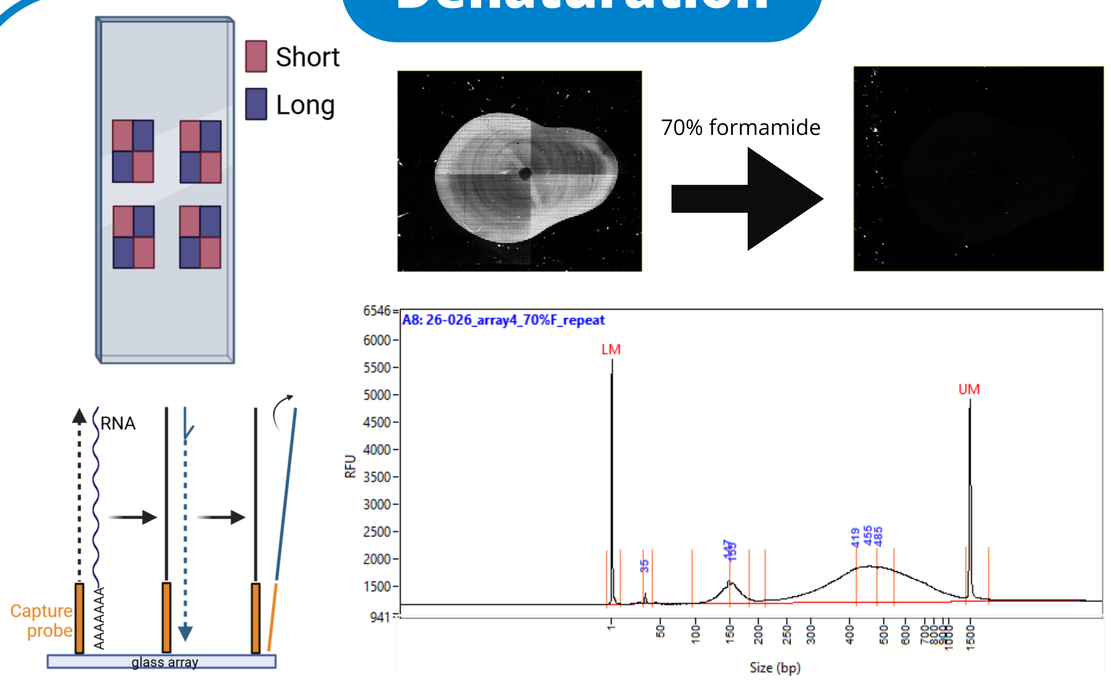
Workflow: First, 10 µm tissue cryosections are cut and methanol fixed on the arrays. Subsequently, H&E staining and imaging is performed, followed by tissue permeabilization and first strand cDNA-synthesis. Next, an exonuclease and RNA-removal step are performed, followed by tissue digestion. From here on, we can either (1) cleave the first strand cDNA or perform (2) 2nd strand on-slide and denature this product.

cDNA footprinting



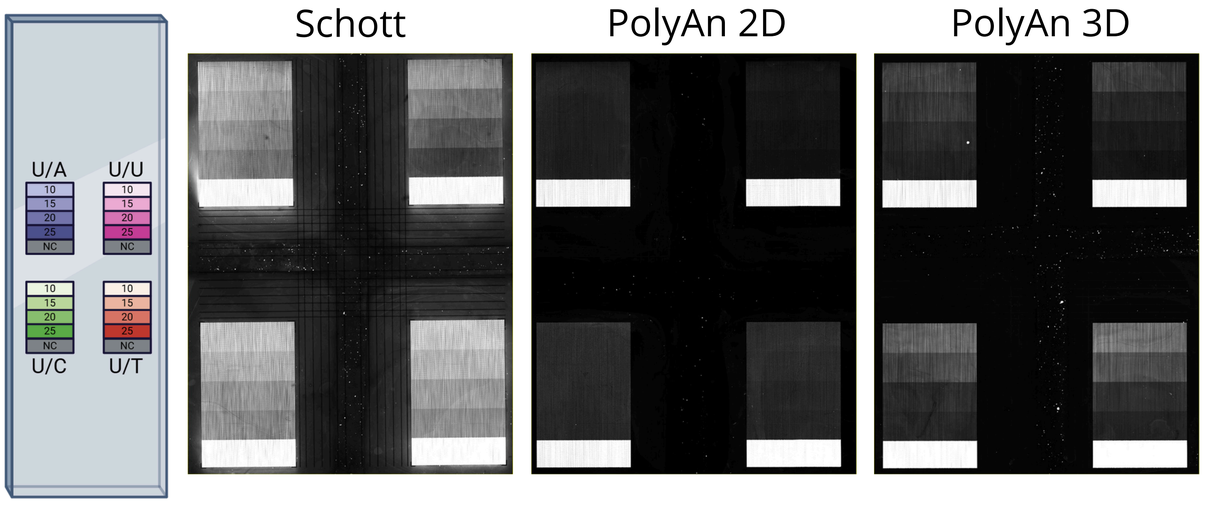
First & second strand cDNA synthesis: Generating a first strand cDNA copy from captured mRNA is the first crucial step in generating spatial libraries. By complementing the mastermix with fluorescently labeled dCTP, this process can be visualized and should yield images that resemble tissue architecture. By using slides from different suppliers on which subarrays contain both short oligodT (20 nt) probes or full length spatial capture probes (99 nt), we test both the effect of probe length as well as slide type. Also during the second strand synthesis step, this approach of fluorescent footprinting is followed. Resulting images show that both first strand and second strand cDNA is successfully generated on-slide from mouse olfactory bulb tissue. Additionally, it is clear that different types of slides with different types of functionalization have a big impact on the synthesis of both first and second strand cDNA.

Denaturation



Denaturation: After generating on-slide second strand cDNA, this product must be extracted from the slide. Extraction of second strand cDNA can be performed using different denaturing agents (NaOH, DMSO, formamide, heat…). 70% formamide proved to be the most succesful agent, effectively removing fluorescent second strand as well as yielding a succesful library. Sequencing of the latter is now the next step.

Alternative: cleavage



Cleavage: An alternative method under investigation is the use of a cleavage site incorporated in the capture probe. This would allow the use of the USER enzyme mix to cleave generated first strand cDNA from the slide. Additionally, this would enable us to print specific DNA sequences for a variety of other applications. Cleavage requires dUTPs that are recognized by the USER enzyme mix. Experiments exploring this approach have indicated some sequence dependent cleavage efficiency, but more importantly a big effect of the type of glass slide functionalization.

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Other interesting developments:
- Targeted spatial transcriptomics
- Long-read spatial transcriptomics

Future perspectives:
- Generate libraries from cleaved (c)DNA
- Generate libraries from denatured cDNA