Improved method and means for high-resolution spatial nucleic acid detection in-situ

KEYWORDS

- ❑ RNA
- ❑ SPATIA-TRANSCRIPTOMICS
- ❑ SPATIAL RESOLUTIONS
- ❑ NUCLEIC ACID SEQUENCING
- ❑ SINGLE CELL RESOLUTION

AREA

- ❑ NANO-TECHNOLOGIES & MATERIALS

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Patent Type
Patent for invention.

Ownership / Co-Ownership
Sapienza University of Rome 13%, Max-Delbrück-Centrum für Molekulare 87%.

Inventors
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Industrial & Commercial Reference
Pharmaceutical and biotechnology. Can be used to map out drug target in space.

Time to Market
TRL is 4

Availability
Research,Development, Experimentation, Collaboration, Start-up business

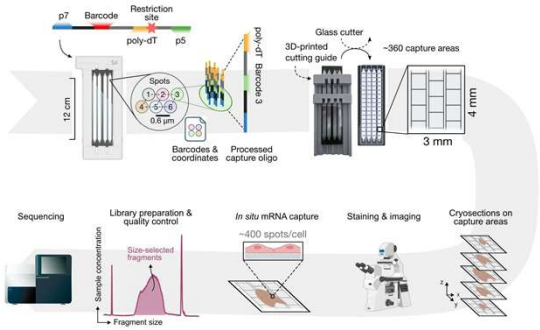

Fig.1 Open-ST is a sequencing-based method that captures mRNA transcripts at subcellular resolution across tissues and offers integrated H&E staining from the same tissue section..

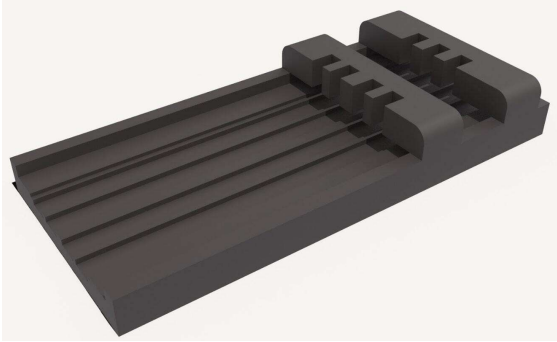

Fig.2 Representation of the 3D printable cutting guide that facilitates the subdivision of the flow-cell into regular capture areas. The size of the capture area can be adjusted within the limits of the flow-cell size..

Abstract
Spatial transcriptomics allows investigation of the molecular mechanisms underlying tissue or disease development. Current commercially available tools are found to be difficult to use and very expensive. Open-ST is an intuitive, high-resolution, inexpensive and scalable 3D method. Open-ST allows capturing transcripts at subcellular resolution, diversity of cell populations in space, organization and localization of cellular states and cell-cell communication hotspots. In addition, 3D reconstruction of the tissue allows for revealing spatially contiguous structures and potential biomarkers.

Technical Description

The spatial resolution achieved is down to 0.6 µm by using a capture area with regular structure generated through Illumina sequencing technology. Our 3D-printable tool facilitates cutting the flow-cell into small capture areas while preventing to surface scratches. Pepsin and hybridization buffer were combined in one solution to promote simultaneous tissue permeabilization and RNA capture. Single-step library amplification prevents PCR amplification errors or sample losses due to purifications between PCR reactions. Data can be analyzed at the single cell level and integrated with histological staining data for 3D spatial reconstruction of any tissue.

Technologies & Advantages

Open-ST is a spatial transcriptomics method for fresh frozen tissue samples to capture messenger RNA filaments at subcellular resolution. The 3D printable tool facilitates cutting the flow-cell into distinct capture areas. Methods for the preparation of RNAs libraries currently on the market turn out to be extremely difficult to use in the laboratory routine and, more importantly, extremely expensive. In fact, a standard analysis costs about €150 for a total capture area of 12 mm2. Compared with other sequencing-based technologies, Open-ST allows RNAs libraries to be obtained in a simplified manner and at a more advantageous cost. In addition, Open-ST produces high-resolution histological images used for cell segmentation and integrates them with transcriptomics data from the same section.

Applications

The method can be used by pharmaceutical and biotechnology companies. It can be used for molecular profiling of therapeutic targets in the tissue space, which is crucial for the evaluation of complex diseases and normal tissues, to delve into cellular function and organization.

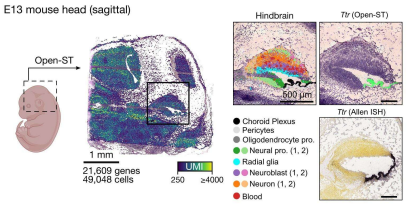

Fig.3. Use of Open-ST in an E13 mouse embryo. In a single experiment, 21,609 genes can be obtained from 49,048 cells (~3x4 mm tissue).

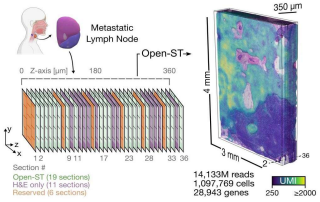

Fig.4. 3D-scale representation of a virtual tissue block of a metastatic lymph node (3x4x0.35 mm).

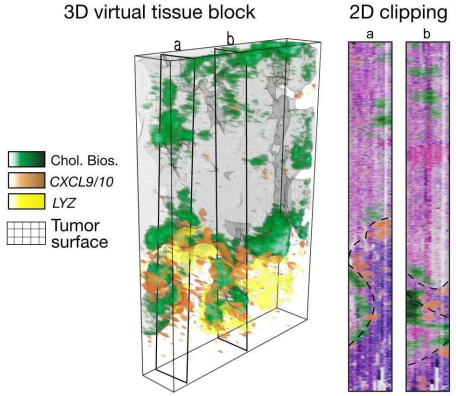

Fig.5. With our technology, for example, we were able to find a unique 3D boundary between the tumor and the lymph node in the metastatic sample. This consisted of cholesterol biosynthesis activity on the tumor side and LYZ/CXCL9/CXCL10 macrophages on the peri-tumor side.

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