#8604 Spatial Transcriptomic Profiling of the Human and Mouse Retina Prepared with CryoJane Tape Transfer System using GeoMx DSP and CosMx SMI Spatial Analysis

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Abstract

The goal of this study is to identify key transcriptomic markers within layers of the retina by individually profiling layers using cellular and subcellular spatial transcriptomics, additionally, comparing the results between each level. Both human and mouse retina samples, prepared fresh frozen and fixed frozen, are analyzed using the GeoMx® Digital Spatial Profiler (DSP) using the whole mouse transcriptome atlas then compared to FFPE mouse retina on CosMx™ Spatial Molecular Imager (SMI) using the 1,000-plex mouse neuro panel.

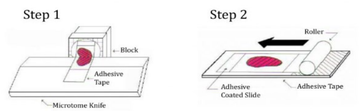
Samples are fixed using Cryo-Jane Taper Transfer system. Samples are mounted on to adhesive coated slides as well as adhesive tape to mount samples to glass slides. This method is used to secure fragile frozen tissue, such as the retina. Human and mouse samples were stained using immunofluorescent microscopy targeting neurofilament H (NF-H), glial fibrillary acidic protein (GFAP) and NeuN on DSP and 18s rRNA, amyloid-beta and GFAP on SMI. Staining allows for identification of structural layers in the retina. Simultaneously, regions of interest (ROI) for spatial profiling are selected based on immunofluorescent stains. On DSP, each sample had 3x ROIs in the photoreceptor layer, inner nuclear layer and ganglion cell layer, then, oligonucleotides were collected and sequenced. Finally, raw counts were Q3 normalized for analysis. For SMI data analysis, 6 field of views (FOVs) were put on each section to cover most regions with multiple layers.

~6000 genes were detected on human retina samples using DSP. Around 500 unique genes were detected between the photoreceptor and inner nuclear layer using DSP.
Preliminary SMI results show we were able to identify cell types (amacrine, horizontal cell, bipolar cell, ganglion cell, etc) and cell specific markers for outer nuclear layer, inner nuclear layer and ganglion cell layer. Data between DSP and SMI showed high concordance with one another, identifying multiple genes in each layer that are consistent with what is biologically relevant.

Our preliminary data demonstrate that DSP and SMI can be used to identify unique genes in the retina while specifically targeting different morphological structures. We show the viability of tape transfer system using RNA based assays

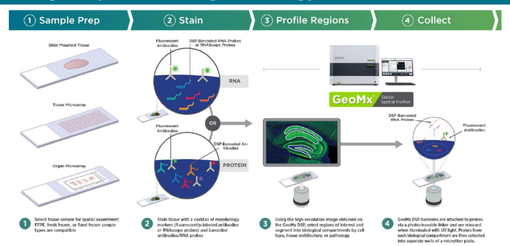
Methods

Donor Eyes were obtained from the Foundation Fighting Blindness Eye Donor Program. Samples were shipped to Cleveland Clinic in 4% paraformaldehyde and 0.5% glutaraldehyde made in Dulbeccos's phosphate buffered saline and subsequently cut ora serrata then sectioned using CryoJane Tape Transfer System.



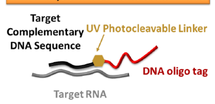
Picture Diagrams are provided by Electron Microscopy Sciences Instructional Manual

GeoMx Digital Spatial Profiling Technology Workflow With NGS Readout



The **GeoMx Digital Spatial Profiler (DSP)** contains a tunable micromirror array which enables profiling of distinct tissue compartments, complex morphological structures, and cell populations dispersed throughout a tissue.

RNA Analysis: Antisense Detection Probe

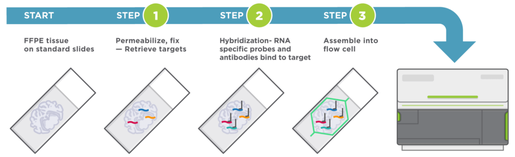


Tagged Oligonucleotide Chemistry

GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue

Subcellular profiling using CosMx™ Spatial Molecular Imager

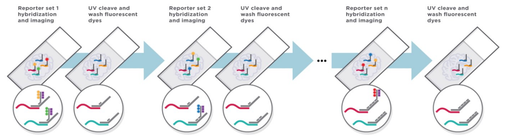
Easy Sample Preparation, Compatible with Any Sample Type



Streamlined and simple workflow that integrates with standard ISH protocol with no need for tissue expansion or clearing, cDNA synthesis or amplification. Go from sample to result faster.

Fixed Frozen Human Retina and ROI selection

Automated Cyclic *in situ* Hybridization Chemistry



Robust hybridization chemistry that provides higher sensitivity and supports high-plex assays in your tissue samples to uncover deeper biological insights.

Biology

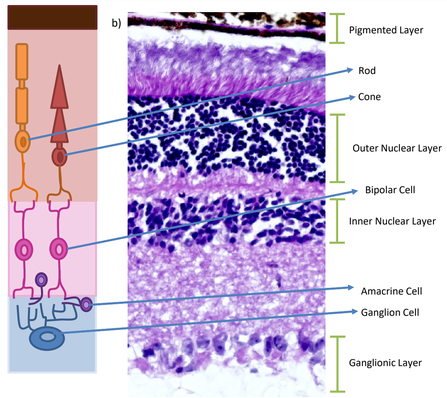


Figure 1

a) Representation of the layers of the retina. This highlights the distribution of the key cell types that are identified in this experiment. b) H&E image of fresh frozen mouse retina, mounted using CryoJane Tap Transfer System.

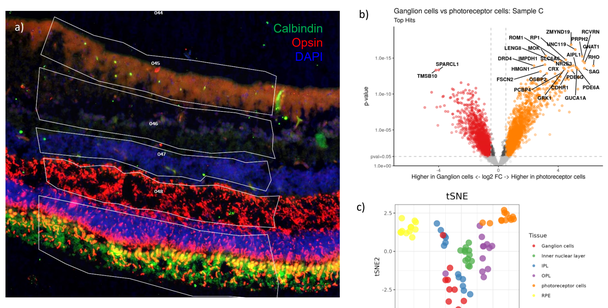
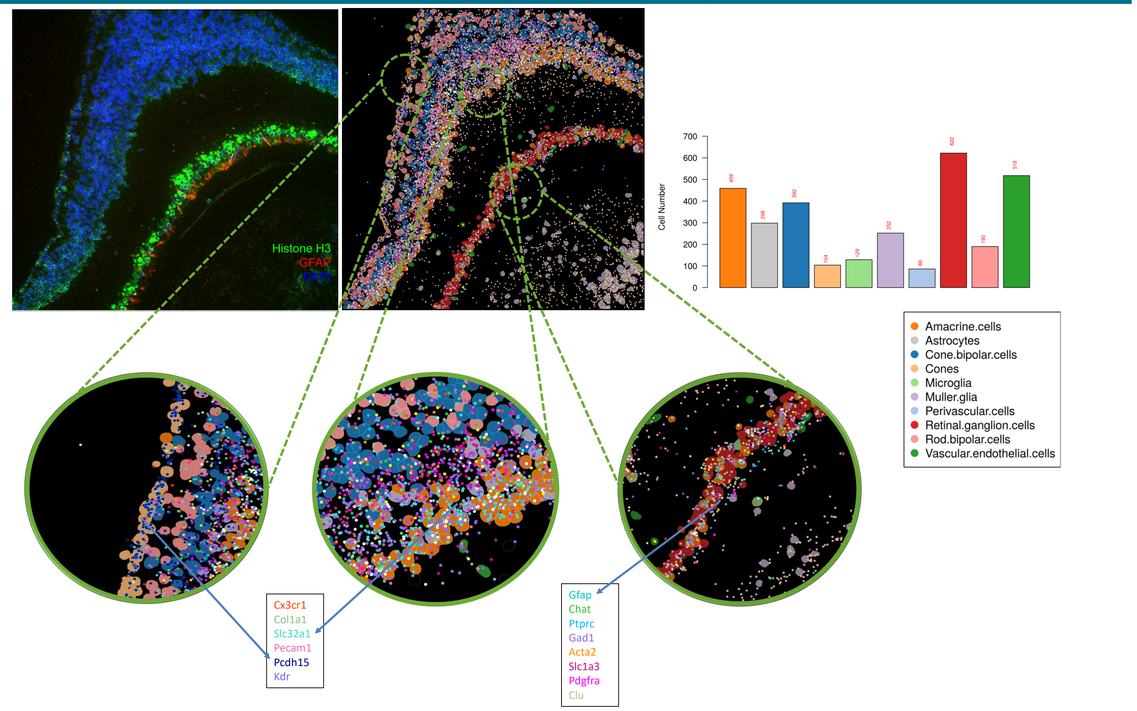


Figure 2

a) ROI (n=5) of the ganglion cell layer, IPL, inner nuclear layer, OPL and photoreceptor layer are collected (representative ROI shown). Morphology markers are used to identify cell types - Opsin (red), Calbindin (green) and DNA (blue). b) Volcano plot showing the different genes detected between the ganglion cell layer and the photoreceptor layer. Most notably, RHO is highly expressed in the photoreceptor layer, which is expected since RHO encodes for rhodopsin, a key protein in rod cells. c) t-Distributed Stochastic Neighbor Embedding (t-SNE) is a non-linear dimension reduction method that show the variability of each ROI in relation to each other.

Mouse Retina GeoMx DSP and CosMx SMI Comparison



Fixed Frozen Mouse Retina and ROI selection

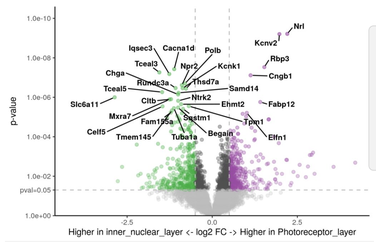


Figure 3

This Volcano plot shows the differential expression between the inner nuclear layer and the photoreceptor layer. Key genes identified in the photoreceptor layer Nrl and Rbp3 are consistent with what is expected in the biology.

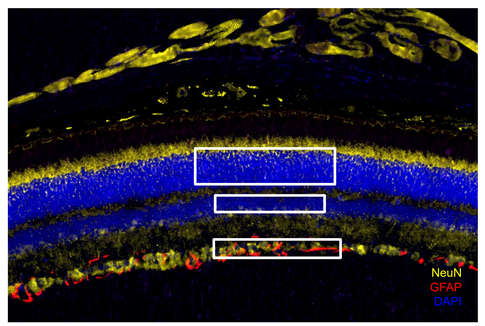
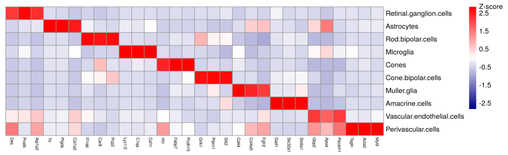


Figure 4

Representative ROIs (n=3) on Fixed frozen mouse tissue are selected in the ganglionic cell layer, inner nuclear layer and outer nuclear layer Morphology markers are used to identify cell types - GFAP (red), NeuN (yellow) and DNA (blue).

Cell Tying



- **Cell type specific markers:**
  - Amacrine: Pax6, Slc32a1, Chat, Gad1, Gad2
  - Horizontal cells: Pax6, Slc32a1, Lhx1
  - Bipolar: Vsx2
  - RGC: Slc17a6
  - Cone: Arr3
  - Muller cells: Slc1a3, Pax6
  - Astrocyte: Gfap, Slc1a3
  - Endothelial cells: Cdh5, Vwf, Kdr, Plvap, Pecam1
  - Pericytes: Pdlim1
  - Hematopoietic cells/microglia: Ptprc, Aif1, Cx3cr1
  - RPE: Rpe65, Ttr
  - Stromal cells: Pdgfra, Pdgfrb, Col1a1
  - Smooth muscle cells: Cspg4, Acta2, Pdgfrb
  - Melanocytes: Pmel

Conclusion

- Samples prepared using the CryoJane Tape transfer system is compatible with GeoMx DSP following the standard fixed-frozen preparation protocol
- GeoMx DSP and CosMx SMI can identify distinctive, biologically relevant genes in the retina as specifically targeting different morphological structures.
- Further optimization is possible through modifying fixation and preparation conditions.

The CosMx™ SMI and decoder probes are not offered and/or delivered to the following UPC member states* for use in these countries for the detection of RNA in a method used for the detection of a plurality of analytes in a cell or tissue sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the Unitary Patent EP 4 108 782 B1. The use for the detection of RNA is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation).
*Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Portugal, Slovenia, Sweden.
The CosMx™ SMI and decoder probes are not offered and/or delivered to the Federal Republic of Germany for use in the Federal Republic of Germany for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof in a method used in fluorescence in situ hybridization for detecting a plurality of analytes in a sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the German part of EP 2 794 928 B1. The use for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation).

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