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Large scale and high-throughput spatial-omics to deeply characterize cancer and normal tissue

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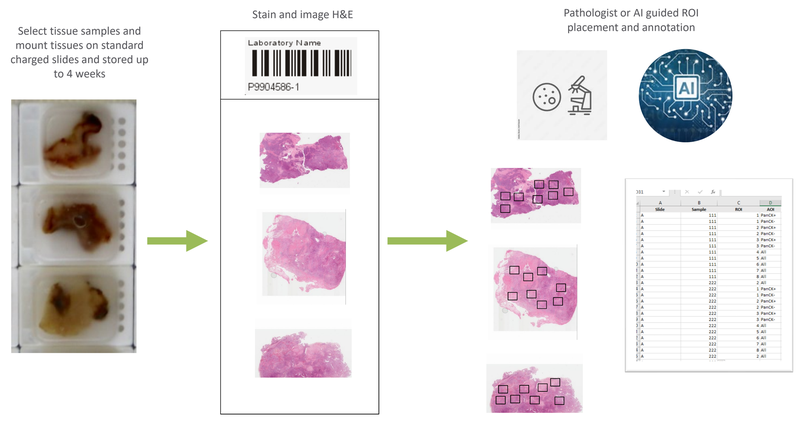
Abstract

To understand heterogeneity of human tissue and identify biomarkers for predicting response to therapies it is necessary to evaluate thousands of patient samples. Here we present a process for high-throughput spatial analysis of the whole transcriptome and over 100 proteins for thousands of clinically relevant cancer and normal samples using the GeoMx® digital spatial profiling (DSP) platform. From these analyses we are able to characterize the tumor microenvironment in separate compartments of the interepithelial, stromal and immune cell rich areas of multiple solid tumor indications and subtypes. Similarly, in normal tissue we are able to deeply characterize tissue specific histology such as glomeruli in kidney, hippocampus in brain or peyer's patches in colon tissue.

Here we present a method to spatially analyze 96 FFPE samples on a DSP instrument per week using established automated procedures for each step of the DSP workflow. This process incorporates region of interest (ROI) and area of illumination (AOI) selection, slide processing, the DSP instrument run, sequencing preparation, sequencing and data analysis. Pre-selection of up to 12 ROIs/AOIs per sample is performed on sequential H&E slides, which then can be rapidly overlayed onto the DSP immunofluorescent scans, easily placed and automatically segmented on specific tissue such at cytokeratin-positive tumor and cytokeratin-negative stroma. Slide processing is done automatically using the Leica BOND system for 32 slides with 3 samples per slide. All annotations chain of custody can be tracked during the process using a barcode reader. After DSP on-instrument processing, collection plates can then be stored until ready for automated pre-sequencing processing. Following sequencing, data is processed and uploaded to AtoMx™ Spatial Informatics Platform for data QC, analysis, collaboration and storage.

This high throughput workflow spatially analyzing both protein and whole transcriptome gene expression allows for the creations of databases and consortiums containing meta-data for large numbers of tissues. These databases can be used to train new AI methods, to deeply evaluate tissue neighborhoods, and in the case of clinical trials, to discover biomarkers to understand mechanism of disease and potentially help improve selection of therapies in the clinic.

Tissue Preparation: Slide Generation and Region of Interest (ROI) Preselection

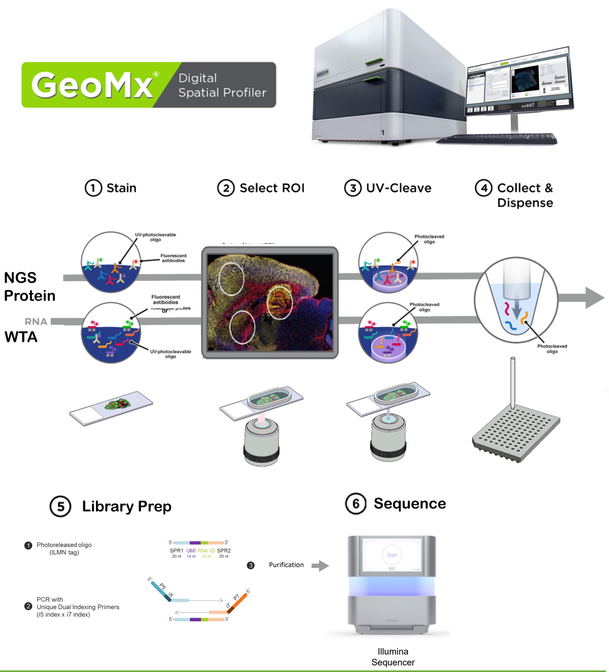


3 Tissues selected and mounted per slide
4 slides cut: H&E, 1 slide protein, 1 slide WTA, 1 slide backup
→ Cut slides can be stored for up to 4 weeks for RNA NGS profiling
→ Stain and scan sequential H&E slide
→ Pathologist/AI selection of ROIs and generation of annotations

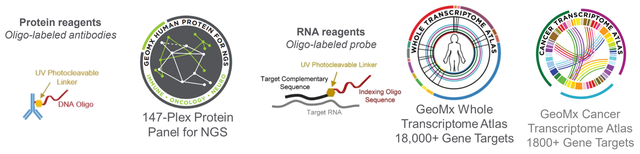
Day 1 Automated slide Processing

32 slides in two batch of 16 slides stained with protein or WTA
→ NGS RNA readout fully automated from start to finish including fluorescent morphology markers.
Semi-automated protein prep
→ Slides stored and stable for profiling for 2 weeks

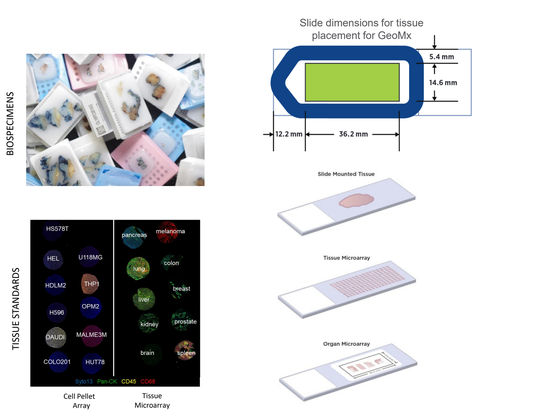
GeoMx Digital Spatial Profiling Platform



GeoMx Assays for High Throughput Studies

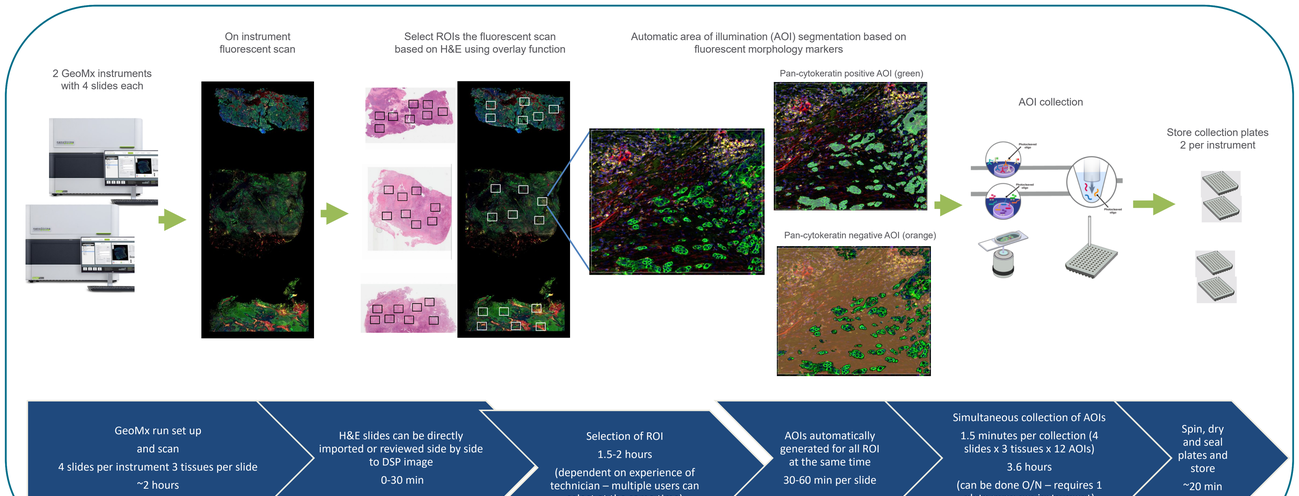


Multi-plexing of Tissue for Large Studies



Scan here to download or learn more

Day 2 DSP Instrument Automated Area of Illumination Selection Based on Tissue Morphology and Collection

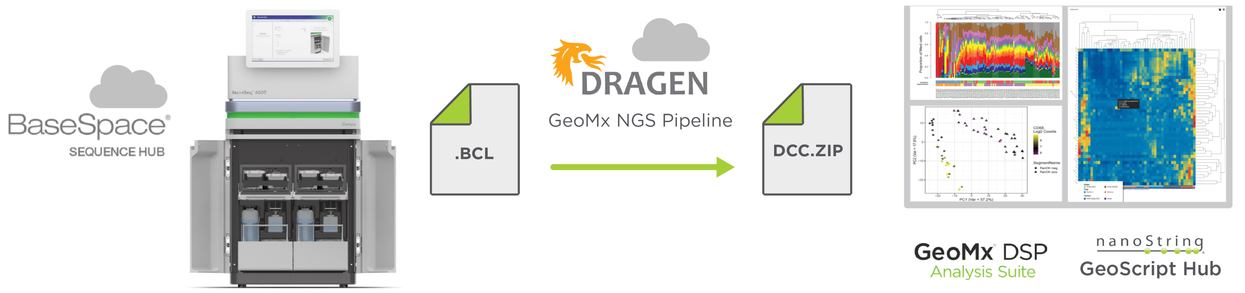


GeoMx run set up and scan
4 slides per instrument 3 tissues per slide
~2 hours
→ H&E slides can be directly imported or reviewed side by side to DSP image
0-30 min
→ Selection of ROI
1.5-2 hours
(dependent on experience of technician – multiple users can select at the same time)
→ AOIs automatically generated for all ROI at the same time
30-60 min per slide
→ Simultaneous collection of AOIs
1.5 minutes per collection (4 slides x 3 tissues x 12 AOIs)
3.6 hours
(can be done O/N – requires 1 plate swap per instrument)
→ Spin, dry and seal plates and store
~20 min

Day 3: Automated PCR and Purification

Weekly batched plate processing of 6 plates protein and 6 plates WTA with automated PCR set up on Nimbus
→ PCR on thermocycler
→ Post-PCR pooling on Nimbus 4
→ Purification and library prep on Kingfisher Apex

Day 4 & 5: Integrated Data Processing, Chain of Custody and Analysis

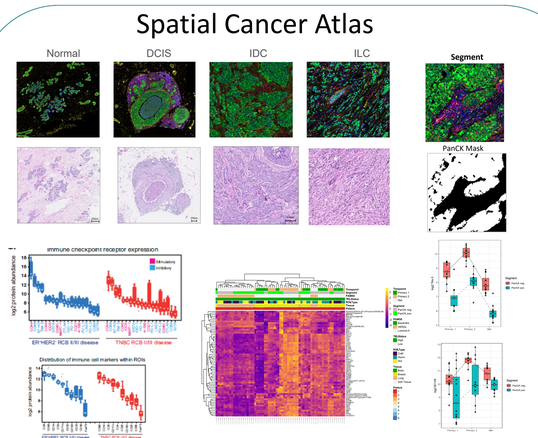


Weekly transfer of batched data and libraries for 12 plates from two GeoMx instruments to NovaSeq
→ Sequencing
1 day
→ Data processing pipeline to get DCC files
1 hour
→ Data upload to AtoMx
20min - 1 hr
→ Initial data QC (sequencing saturation, NTC, nuclei counts, normalization, tissue area, ROI/AOI drop out)
1-3 hours

This workflow with 3 samples per slide 4 DSP runs on 2 instrument per week allows QC and analysis of >4600 AOIs from 384 tissues per month

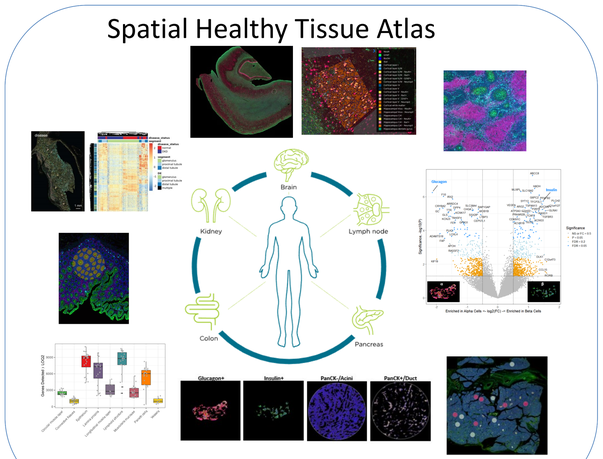
Spatial Data Utility for Large Scale Studies

Spatial Cancer Atlas



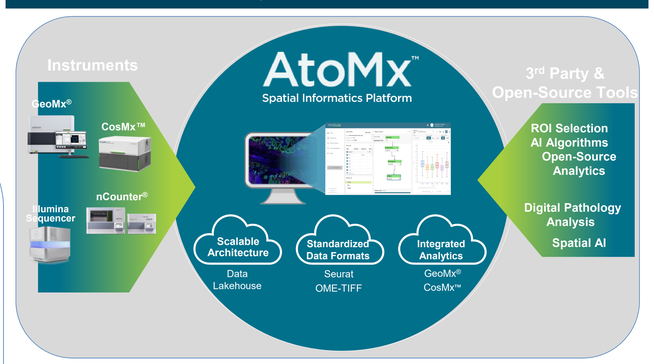
Spatial profiling of breast cancer subtypes from patients from different subtypes, metastatic sites and before and after treatment from the GeoMx Breast Cancer Consortium

Spatial Healthy Tissue Atlas



Normal tissue profiling from the GeoMx Spatial Organ Atlas

Meta Data Integration and Database Generation



Conclusions

This GeoMx DSP workflow with 3 samples per slide 4 DSP runs on 2 instrument per week allows QC and analysis of >4600 AOIs from 384 tissues per month of over 100+ proteins and 18000+ gene targets for both human and mouse. . Using similar workflows 100-1000s of samples can be profiled depending on number of tissues and AOIs per slide.
The reproducibility of the GeoMx platform makes it ideal for generating data for large cohorts of cancer and normal samples that is based on tissue architecture and can be easily combined across multiple instruments, institutions and users to generate spatial multi-omic databases to better understand mechanism of disease and discover novel biomarkers.