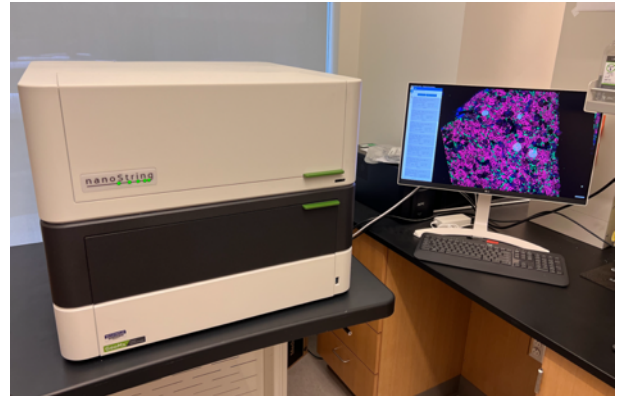


EQUIPMENT

Spatial Biology Core facility

The Spatial Biology Core facility is available for use by investigators at the University of Washington and other institutions. The Spatial Biology Core facility is hosted by the UW Department of Laboratory Medicine and Pathology. This is physically located on the first floor of the E building in the UW Medicine South Lake Union Campus, adjacent to the Pathology Research Services Laboratory (*see below*). Slides can be prepared in the Pathology Research Services Laboratory or provided to the core's scientists from another source.



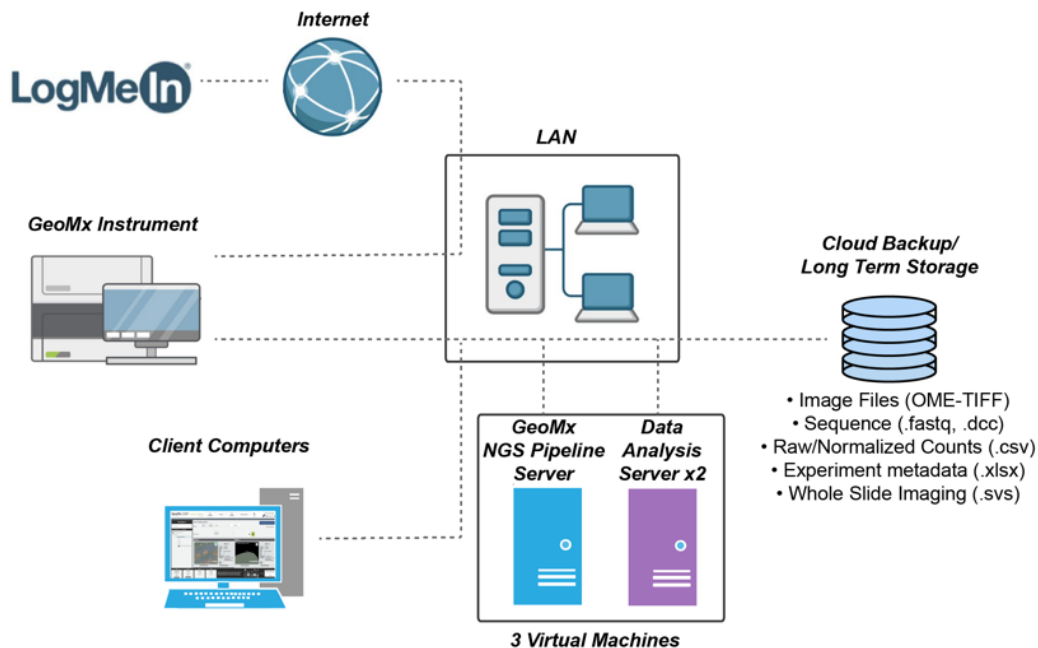
NanoString GeoMx DSP instrument and workstation

Spatial Biology Core **GeoMx-related Equipment**

To reduce cross-contamination, the core has separate bays with dedicated equipment for **1**) Slide Hybridization and antigen retrieval (Equipment: 2 hybridization ovens: Boekel Scientific and ACDBio Slide Hybridizer, water baths, steamer, and digital thermometers), **2**) Staining and washing of slides (Equipment: humidity chamber, water baths, dedicated Coplin jars), **3**) ROI collection (Equipment: NanoString GeoMx DSP, CosMx SMI and client computers, *see photos*), and **4**) Next generation sequencing (NGS) library preparation (Equipment: AirClean 600 PCR workstation, centrifuge with plate adapters, vortexes, heat blocks, picofuges, BioRad T100 thermocycler, and Qubit 3.0 fluorometer). The core has also acquired a Leica Bond RX which can be used for automated hybridizations for GeoMx, CosMx and RNA-ISH (e.g., RNAScope) workflows. The Department of Laboratory Medicine and Pathology owns a second GeoMx DSP instrument at Harborview Medical Center that is available for use in case of main instrument downtime. The instruments undergo annual preventative maintenance and are protected under a service contract from NanoString.

GeoMx Digital Spatial Profiler (GeoMx DSP)

For the GeoMx DSP, informatics support is provided by the Department of Laboratory Medicine and Pathology Division of Informatics (Director Noah Hoffman). An overview of the DSP informatics topology is shown in the figure below:



Briefly, the GeoMx DSP workstation runs on Windows 10 and updates are installed remotely. Remote assistance and desktop control is provided via LogMeIn. Windows Defender is installed for virus protection and AppLocker ensures only NanoString white-listed programs are allowed to run on the system.

Any computer (Mac or PC) on the same internal network can connect to the GeoMx DSP instrument via Google Chrome browser. Access is controlled by the UW's single sign-on (SSO) authentication. This allows for remote ROI selection/segmentation during sample collection as well as post-hoc data analysis and visualization of slide images and ROI.

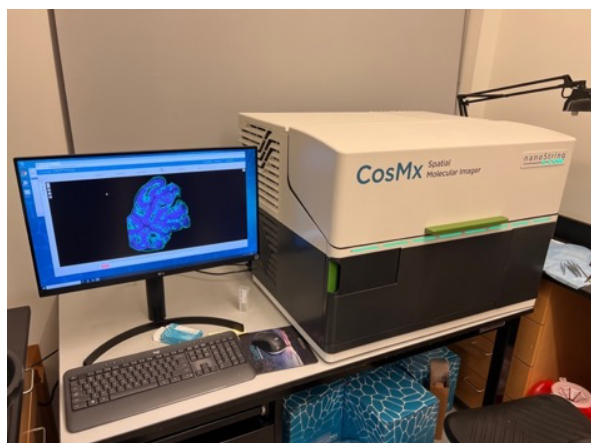
As outlined in the topology diagram above, two separate server types are required for large scale data analysis. First, the **NGS Pipeline Server** converts raw NGS data (FASTQ files) to the digital count conversion (DCC) files (i.e., the data matrix with counts per target per segment). We have implemented an Amazon Web Services (AWS) instance of the NGS Pipeline Server eliminating the requirement for an on-site server.

To enable the computation during analysis of larger datasets (e.g., coming from the Cancer Transcriptome or Whole Transcriptome Atlas panels), an **Auxiliary Data Analysis server** is required. This server frees up GeoMx DSP internal resources allowing uninterrupted running of the system while performing remote data analysis. Each GeoMx DSP instrument requires a 1:1 mapping with an Auxiliary Data Analysis Server. However, two GeoMx DSP instruments can utilize the same NGS Pipeline Server. The NGS Pipeline Server and 2 Auxiliary Data Analysis Servers can also reside in one physical host as three virtual machines (VM).

The configuration of this physical host is: 3.4 GHz CPU with 16 cores, 256 Gb RAM, Server 2019 (Standard or Enterprise), Boot OS Disk (256 Gb) for VM Host (server 2019), 1.5 Tb+ of SSD storage for 2 VM Auxiliary Data Analysis Servers and 1 VM NGS Server (OS Partition) and 8 Tb HDD storage for 3 VMs.

CosMx Spatial Molecular Imager (CosMx SMI)

On November 15, 2023, a NanoString CosMx Spatial Molecular Imager was installed at the core (see photo below). The CosMx SMI platform is an integrated system with cyclic *in situ* hybridization chemistry coupled to an ultra-high-resolution imaging readout. The resulting image data are pushed to a cloud portal for interactive data analysis and visualization using the AtoMx software suite. Key specifications of the CosMx SMI system are listed below (source NanoString, Inc.). The CosMx SMI will enable fully FFPE-native analysis of highly multiplexed analytes (1000-plex for RNA, 100-plex for protein) at single cell resolution. The core's staff have undergone training and are now accepting end-user experiments to be run on the platform.

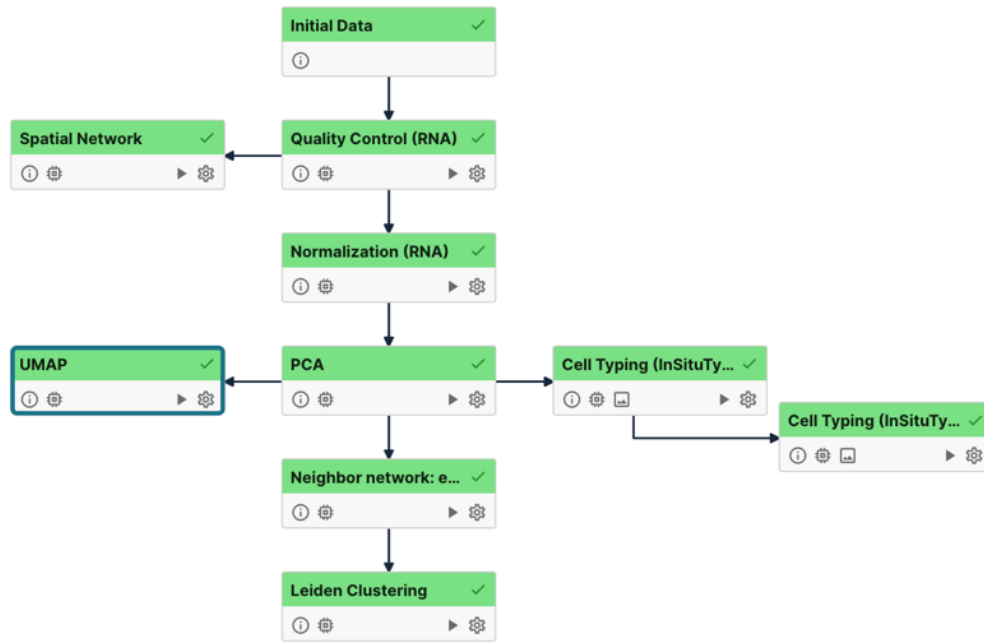


NanoString CosMx SMI instrument and workstation

System Specifications for CosMx™ Spatial Molecular Imager	
Resolution	Subcellular resolution (<100 nm)
Throughput	Up to 1 million cells/sample, 4 samples/run Up to 16 slides/week
Tissue compatibility	FFPE, Fresh Frozen, organoids, cultured cells
Multiplexing capability (option to customize)	• Up to 1,000-plex RNA panels • Up to 100-plex Protein panels
Flexible scan area	Up to 100mm ²
Flexible scan area	Includes instrumentation, validated panels and reagents, cloud data storage and analysis

Cloud-based Analysis Portal (AtoMx)

Data generated by the CosMX SMI are pushed in real-time to the AtoMx cloud-based portal. Once acquisition is completed, the data can be processed using established analytic pipelines and cloud-based servers (see *analysis pipeline schematic below*). Raw and processed data (e.g., images, cell IDs, gene counts) can be exported to an Amazon Web Services S3 bucket in standard formats (e.g., TIFF, TileDB arrays, Seurat objects) for further analysis.



Within the AtoMx environment, it is possible to visualize output data and metrics for each of the steps displayed in the processing pipeline. For example, clicking on the UMAP tile in the pipeline brings up the cellular typing projection. When visualized side-by-side with the tissue section, the color-coded cell-type assignments can be mapped back onto the tissue structure, thereby achieving spatial localization of cell-types and their expression signatures (see image below derived from our training experiment on human tonsil). Additional analyses that can be performed within AtoMx include receptor-ligand interaction analysis and spatial neighborhood analysis.



Left, UMAP projection of human tonsil CosMx SMI experiment color-coded by cell-type. Right, the cell type color map is overlaid onto the histologic image of the tonsil tissue.

Sequencing

Multiple sequencing services and equipment are available locally. Within Dr. Akilesh's host Department of Laboratory Medicine and Pathology, the following next generation sequencing instruments and associated instrumentation are available, including Illumina HiSeq2500 (x2), Illumina MiSeq (x2), Illumina NextSeq 500 (x3), Illumina NextSeq 2000 (x1), Agilent Bravo automated liquid handler, Cyclone liquid handler (x2), Agilent 2200 TapeStation, Perkin Elmer LabChip DS, ThermoScientific Nanodrop 1000, Qiasymphony nucleic acid extraction

platform (x3), Life Technologies ViiA7 Real-time PCR instrument, Siemens Purity Light water filtration system, and a Covaris E220 96-well Ultrasonicator.

Sequencing for the Spatial Biology Core facility (i.e. readout for GeoMx DSP experiments) is typically performed by the core's scientists at one of two locations. For smaller experiments requiring <1 billion paired end reads, the sequencing core facility at the UW's Institute for Stem Cell and Regenerative Medicine (ISCRM) is utilized. This is located adjacent to the Spatial Biology Core facility. This core facility has an Agilent 2200 TapeStation, Qubit fluorometer and an Illumina NextSeq 2000. For larger experiments requiring >1 billion paired end reads, the library is loaded onto a NovaSeq 6000 in the Virology Division of the Department of Laboratory Medicine and Pathology. Library quality control and quantification are performed by Virology Division technical staff and coordinated by the Department's Research Sequencing Services as a benefit to departmental faculty.

Finally, sequencing can also be accessed locally at the Fred Hutchinson Cancer Research Center's Genomics Core (Seattle, WA). Dr. Akilesh is a Seattle Cancer Consortium member enabling discounted sequencing and service rates. The core has multiple Illumina sequencers including a NovaSeq 6000, a NextSeq 2000, a HiSeq 2500, and two MiSeqs. Library quantification and QC is performed prior to sequencing on an Agilent 4200 TapeStation. The genomics team, in partnership with bioinformatics colleagues, provides end-to-end support of Illumina next-generation sequencing projects. Their integrated resources support experimental design, library prep and downstream analysis. Other services provided by the core include 10X single cell sequencing, 10X Visium spatial transcriptomics, NanoString nCounter, PacBio and Sanger sequencing.

Pathology Research Services Laboratory

The Pathology Research Services Laboratory is located within the Kidney Pathobiology Laboratory at the South Lake Union Research campus (Building 3) of the University of Washington. This laboratory is directly adjacent to the Spatial Biology Core Facility which will facilitate rapid sectioning and processing of specimens for GeoMX DSP and CosMX SMI experiments. The primary morphologic techniques offered by the Pathology Research Services Laboratory, include paraffin, frozen and electron microscopy tissue processing and embedding, routine histology including special histologic stains (PAS, Jones, Trichrome), tissue enzyme histochemistry, immunohistochemistry (IHC), RNA-ISH, immunofluorescence, TUNEL staining, digital photomicrography, computerized quantitative morphometric analysis, and ancillary techniques (such as competitive inhibition assays and Western blotting necessary to ensure the specificity and sensitivity of reagents). More complex tasks such as antibody optimization for IHC can also be performed. The 1,500sf lab space includes all resources needed for these morphologic investigations: fume hoods, wet bench space, an RNase free area for RNA isolation and in situ hybridization, freezers, computer terminals, centrifuges, a Sakura VIP tissue processor and embedding center for processing paraffin blocks, Leica microtomes for sectioning paraffin blocks, Leica cryostat, and Nikon and Olympus brightfield microscopes equipped with digital cameras. Shared spaces in the Kidney Pathobiology Laboratory include a microscopy suite which includes a confocal microscope and two Evos fluorescent microscopes. The laboratory also uses portions of the electron microscopy lab of the University of Washington Medical Center, which includes working space for three AO/Reichert ultramicrotomes, bench space for tissue processing for electron microscopy, with an electron microscopy suite containing two FEI electron microscopes with digital cameras.