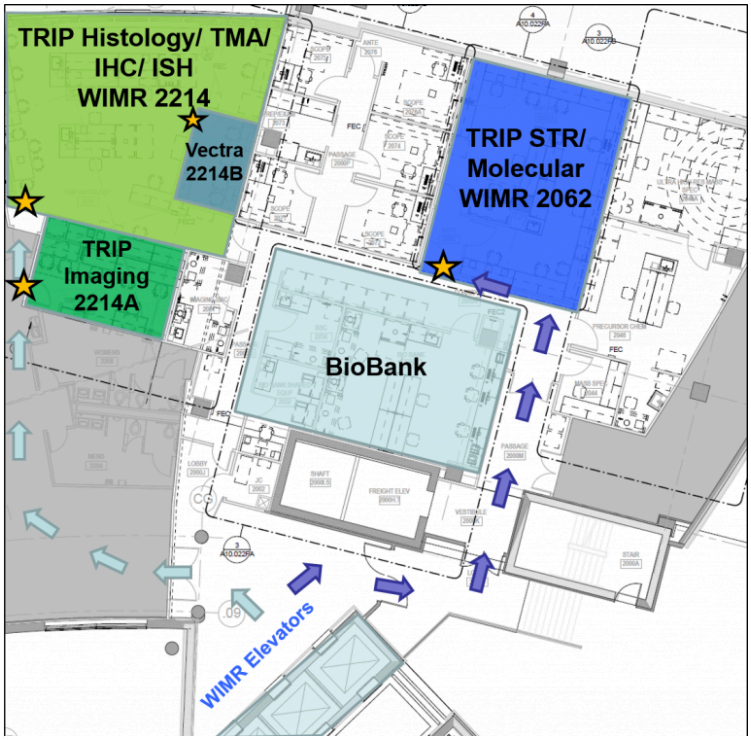
Facility description and TRIP support:

The TRIP Lab provides support to faculty and trainees working on translational and collaborative research projects and partners with the UW Carbone Cancer Center's BioBank as part of the Translational Science BioCore (TSB), and the UW Biotech Center's Gene Expression Center (GEC). The laboratory offers high-quality histological, molecular/morphometric analyses and consultative services such as creation of tissue microarrays (TMAs) for high-throughput molecular profiling of tumor specimens, and quantitative morphometric analyses via imaging on a fee-for-service basis available to the campus community and beyond.



TRIP Lab facility map.

Expertise in pathologic diagnoses, histology, molecular techniques, spatial transcriptomics/proteomics and digital/multispectral imaging are provided by board-certified pathologists and highly skilled scientists/technicians who assist with data acquisition and analysis. Technical expertise in the TRIP Lab is ample and will be fully utilized to support the shared instruments and its users.

The TRIP Lab is ~2,100 square feet of laboratory space within the Integrated Research Cores (IRC) on the second floor of the Wisconsin Institute for Medical Research Tower I (WIMR I). There are separate rooms for core imaging instruments, a separate area for molecular analyses and a large central lab space for histology and immunohistochemistry/ in situ hybridization procedures. Additional space has been provided in the L5 area of UW Hospital and Clinics (UWHC) Clinical Sciences Center (CSC) for one manager office and three workstations for lab personnel. The TRIP Laboratory provides services to over 175 investigators and it is directed by Karla Esbona, PhD, and Faculty Lead Eduard Matkovic, MD., an Assistant Professor in Pathology.

Our services encompass general and specialized histology, tissue microarray design and construction, customized immunohistochemistry and in situ hybridization, spatial transcriptomics and proteomics, molecular profiling, image acquisition and analysis, and customized molecular pathology. These services are carried out by a diverse and highly trained staff consisting of one Operational Director, four technicians and four TRIP faculty which includes our Faculty Lead. The TRIP laboratory owns many automated equipment to meet clinical and translational investigators' research demands.

We have recently acquired spatial and molecular profiling equipment to expand our services. The GeoMx Digital Spatial Profiler (DSP) from Bruker, is a technology that enables high-plex spatial and molecular analysis of RNA and protein in tissue specimens on slides. Tissue slides

are stained with large panels of pre-mixed biological probes, which each contains a unique, UV-cleavable DNA barcode; and fluorescently-labeled morphology markers to visually or computationally elucidate the morphology of the tissue. Using the morphology markers for guidance, then ROIs are selected and are illuminated with UV light. The cleaved barcodes from these ROIs are then counted on an nCounter platform (Bruker). The resulting counts constitute an expression or protein profile of key targets across that ROI and elucidate the biology specific to that region. The investigator then can achieve the analysis of up to 800 targets with nCounter readout or up to 18,000 targets with NGS readout. For NGS readout, the TRIP laboratory has partnered up with the UW Biotech Center.

The nCounter MAX system is a 2-machine system that allows molecular profiling of nucleic acids and protein extracted from a diverse array of samples. Gene and protein expression can be measured respectively from total RNA/DNA tissue samples, FFPE, plasma, serum and other biofluids, exosomes, whole cell lysates among others. After sample extraction and hybridization to nCounter barcodes according to the NanoString target kit of choice, samples are first setup in the nCounter Prep Station. The Prep Station is a multi-channel pipetting robot that cleans and processes samples to prepare them for data collection on the Digital Analyzer. Then, samples are transferred to the nCounter Digital Analyzer, a multi-channel epifluorescence scanner specifically configured for use with NanoString's nCounter Cartridges. Data acquired in the nCounter Digital Analyzer is transferred to Nanostring's open source nSolver and Rosalind software suite for QC and data analysis.

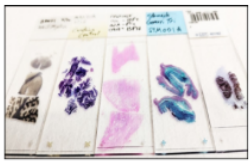
Moreover, we acquired in 2020 an Aperio AT2 Digital Pathology Slide Scanner system, which is capable of scanning 400 slides at a time. This is a whole slide, 20x or 40x brightfield imaging system that produces high resolution files compatible for viewing and analysis with the Aperio ImageScope software or other open source software like FIJI. The scanner is also capable of scanning large format slides (2×3).

In addition, we own a Vectra multispectral automated imaging system to aid with imaging and quantitation of multiplex IHC/IF ISH/FISH experiments. It has the capacity to scan 200 slides at a time at 4x, 10x, 20x and 40x in either an automated or semi-automated manner. This system uses machine learning algorithms to automatically scan region of interest on slides. Resulting images can either operator-selected or random sections and images can be analyzed via Nuance and inForm software available at the core. We also own a Nuance manual multispectral microscope available to trained investigators.

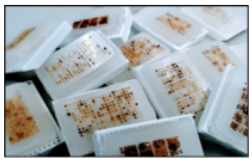
Finally, we acquired through a S10 grant, a 3D HISTECH TMA Grand Master tissue microarray system with modern software and downstream molecular capabilities. The 3DHISTECH TMA Grand Master with PCR Extraction Function is a next-generation tissue microarray instrument that merges traditional array layout planning and integrates digital pathology imaging with automated tissue microarray features. This system design allows for precise tissue localization and identification and tissue annotation and resulting reduced tissue waste from precious archived tissue samples to create a well-designed, detailed resource for use in a multitude of research experiments.

Histology, immunohistochemistry (IHC/IF) and *In situ* hybridization (ISH/FISH):

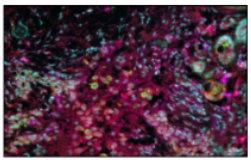
Histology



TMA Creation



IHC & ISH



Control Tissue & TMA Repository

Imaging and image analysis:

Imaging

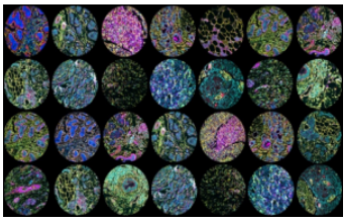
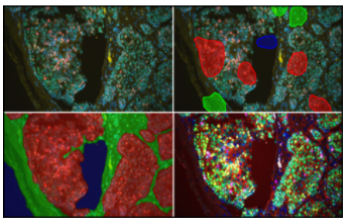
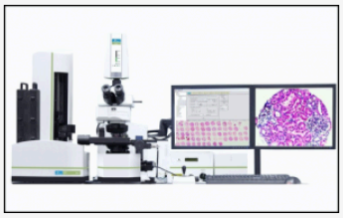


Image Analysis

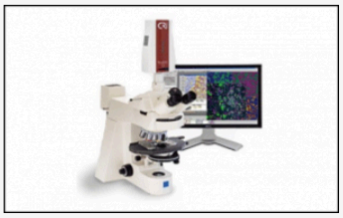


Aperio

Vectra

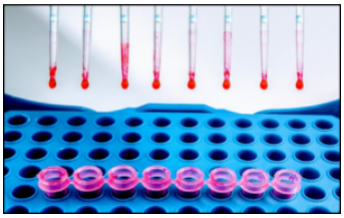


Nuance

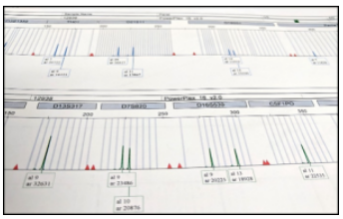


Molecular pathology:

Molecular

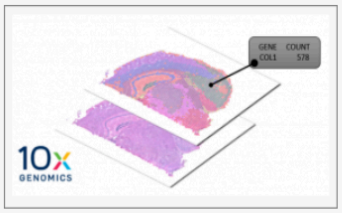


Cell Line Authentication

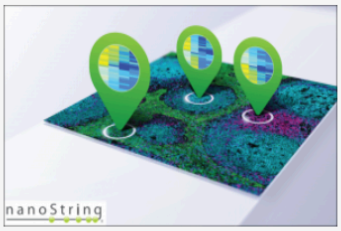


Spatial and molecular profiling:

Visium



GeoMx



nCounter

Translational Initiatives in Pathology (TRIP) Laboratory Equipment

- Sakura Tissue-Tek VIP 5 tissue processor
- Leica EG1160 embedder
- Leica RM2125RT microtome
- Olympus Cut 4060 microtome
- Reichert Jung 2030 microtome
- Leica CM1950 cryostat
- Leica CM1850 cryostat
- Sakura Tissue-Tek SCA film cover slipper
- ThermoScientific SlideMate AS slide printer
- ThermoScientific PrintMate AS cassette printer
- Water baths
- Histology staining stations
- Olympus BH-2 multi-head (5) scope
- Olympus BX-41 multi-head (2) scope
- Nikon SMZ 1500 dissecting scope
- Aperio AT2 Digital Slide Scanner
- Roche DISCOVERY ULTRA IHC/ISH system
- Olympus BX43 microscope with Nuance Multispectral Imaging System
- Vectra2 Multispectral Imaging System
- InForm image analysis software from Perkin Elmer
- ACD HybEZ hybidization oven
- Robbins Scientific Hybridization Incubator
- Promega Maxwell 16 nucleic acid purification system
- Savant DNA120 DNA Speed Vac
- Eppendorf Mastercycler
- Roche LightCycler
- NanoDrop ND-1000 Spectrophotometer
- 3D HISTECH Automated Tissue Microarrayer
- Bruker GeoMx Digital Spatial Profiler (DSP)
- Bruker nCounter MAX Prep Station and Digital Analyzer