

Digital Spatial Profiling: Project Design & Preparation Guide

Protein, RNA & Multiomics
Projects

TRIP Lab
Translational Research Initiatives in Pathology Lab

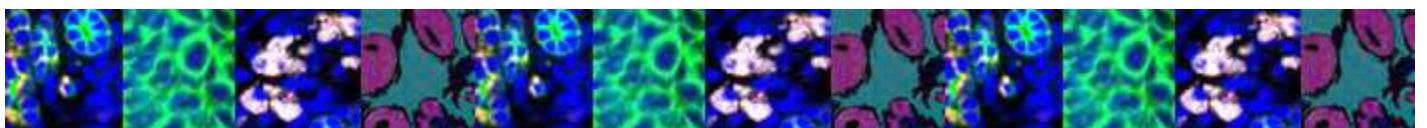


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Introduction

Technology Overview

Bruker Spatial Biology's GeoMx® Digital Spatial Profiling (**DSP**) platform is a high-plex spatial molecular profiling technology that enables quantitative analysis of RNA and protein expression while preserving the spatial and morphological context of intact tissue sections. The technology combines fluorescence-based tissue imaging with spatially resolved molecular profiling, allowing molecular measurements to be associated with defined anatomical regions, cellular populations, or tissue microenvironments. Unlike conventional bulk molecular assays, in which nucleic acids or proteins are extracted from an entire tissue specimen and spatial information is lost, GeoMx DSP maintains the relationship between molecular measurements and their original location within the tissue.

GeoMx DSP supports a range of spatial RNA and protein assays, including highly multiplexed whole-transcriptome and proteomic applications. Depending on the assay configuration, >18,000 **RNA** transcripts and hundreds to >1,200 **protein** targets can be evaluated from spatially defined regions. The platform can therefore be applied to characterize tissue architecture, cellular phenotypes, transcriptional states, signaling pathways, disease-associated molecular programs, and interactions between spatially distinct tissue compartments.

Molecular Profiling Options and High-Plex Assays

GeoMx DSP uses molecularly barcoded probes to enable spatially resolved detection of RNA and protein targets. In protein assays, antibodies are linked to unique DNA oligonucleotide tags, while RNA assays use sequence-specific probes containing associated oligonucleotide tags. These molecular tags provide the target identity required for digital counting or sequencing while maintaining the spatial association of each measurement with its originating tissue region.

The number and type of targets available depend on the selected GeoMx assay. **RNA profiling** can range from focused panels designed around specific biological questions to broad whole-transcriptome profiling. For example, the GeoMx Human Whole Transcriptome Atlas (**WTA**) provides spatial profiling of more than 18,000 protein-coding genes, while focused assays such as the Cancer Transcriptome Atlas (**CTA**) target more than 1,800 genes associated with cancer and immune biology.

Protein profiling is similarly available across multiple plex levels and biological applications. The GeoMx Discovery Proteome Atlas (**DPA**) includes more than 1,200 protein targets, including pathway and disease associated markers and more than 130 post-translational modification targets. The GeoMx IO Proteome Atlas (**IPA**) includes more than 570 protein targets focused on immuno-oncology biology. Additional focused protein panels are available for specific research applications.

Selecting RNA, Protein, or Multiomics

RNA profiling is most appropriate when the primary objective is to characterize transcriptional programs and gene-expression patterns, including cellular state, differentiation, pathway activity, immune responses, and disease-associated transcriptional signatures. Whole-transcriptome profiling is particularly useful when relevant pathways or biomarkers are not known in advance, as it provides broad transcript coverage rather than restricting analysis to a predefined target set. RNA profiling can therefore be used to identify spatially localized transcriptional signatures associated with disease state,

tissue compartment, treatment response, or cellular phenotype.

Protein profiling is most appropriate when the biological question depends on cellular phenotype, protein abundance, receptor expression, immune-cell characterization, signaling pathways, or functional protein states. Protein measurements can provide phenotypic information that may not be apparent from RNA expression alone. Assays containing post-translational modification targets can also provide information about protein activation or signaling state that cannot be directly inferred from transcript abundance.

Spatial Multiomics enables the simultaneous profiling of RNA and protein within the same tissue section and spatial regions, providing an integrated view of cellular state, phenotype, and function while preserving anatomical context. Combining RNA and protein measurements is particularly advantageous when both transcriptional regulation and protein-level biology are important for interpreting the tissue phenotype.

Tissue Quality Control and Assay Optimization

The GeoMx DSP workflow begins with tissue quality control (**QC**) to assess RNA quality/expression level and confirm that the submitted tissue is suitable for spatial profiling. Tissue QC is performed using ACD's RNAscope® Multiplex Fluorescent Reagent Kit in combination with a species-specific positive control probe. This initial assessment provides an indication of RNA quality and tissue suitability prior to proceeding with assay development and optimization.

Following successful tissue QC, antibody and probe **optimization** is performed for the selected GeoMx assay. Candidate antibodies and RNA probes are evaluated to determine appropriate reagent performance, specificity, signal intensity, and background under GeoMx assay conditions.

Importantly, antibodies that perform successfully in conventional immunohistochemistry (IHC) or immunofluorescence (IF) are not necessarily compatible with GeoMx DSP. Differences in sample preparation, staining conditions, assay chemistry, and detection methodology can affect antibody performance. Therefore, antibodies intended for GeoMx profiling will be evaluated and optimized specifically for the GeoMx platform by TRIP staff rather than relying solely on prior IHC/IF validation.

Optimization is an essential component of project preparation and may influence the final selection of antibodies, probes, assay conditions, and experimental samples used for spatial profiling.

Tissue Imaging and Region of Interest (ROI) Selection

The GeoMx workflow continues with the preparation and staining of a tissue section using the appropriate molecular assay and fluorescent **morphology markers**. Morphology markers provide the spatial framework required to identify tissue compartments and cellular populations. These markers may distinguish structures such as tumor versus non-tumor tissue, epithelial and stromal compartments, immune populations, vascular structures, or other biologically relevant features.

Following staining, the tissue is imaged on the GeoMx DSP instrument. The resulting fluorescence image is used to define regions of interest (ROIs) for molecular profiling. ROIs can be selected according to tissue morphology, cell phenotype, anatomical location, or experimental design. ROI geometries may include defined shapes, free-form contours, or other spatial configurations. Individual ROIs can also be subdivided into segments using morphology-based markers, allowing molecular measurements to be

generated from distinct cellular or tissue compartments within the same ROI.

ROI size should be selected according to tissue type, cellular density, target abundance, and the sensitivity requirements of the assay. A minimum of 20 nuclei per ROI is recommended for protein profiling and 100 nuclei per ROI for RNA profiling. These values represent minimum guidelines rather than absolute requirements; ROIs containing more cells are preferred whenever possible. RNA profiling generally benefits from larger numbers of nuclei because transcript abundance can be lower and more variable.

Digital Spatial Collection

Following ROI selection, the GeoMx DSP instrument uses targeted ultraviolet illumination to release the molecular oligonucleotide tags from the selected tissue region. The photocleavable barcode is selectively cleaved within the illuminated area, releasing the oligonucleotide tags associated with the RNA or protein targets present in that ROI.

A microcapillary collection system aspirates the released oligonucleotides from the illuminated region and dispenses them into a designated well of a collection plate. Each collection-plate well therefore corresponds to a specific spatial location or segment on the tissue. The spatial coordinates and molecular identity information are retained computationally so that the resulting molecular measurements can subsequently be mapped back to the original ROI.

The collection plate represents the transition point between the spatial profiling portion of the experiment and downstream molecular detection. Following completion of ROI collection, the plate is processed according to the selected readout method. The downstream workflow is either nCounter-based digital counting or next-generation sequencing (NGS).

nCounter Readout – Based Data Generation

For nCounter-based assays, the final output is a digital count dataset representing the abundance of targeted molecular analytes within each collected ROI or segment. Each collection well is associated with its corresponding spatial location, allowing molecular measurements to be directly linked to the anatomical or cellular region from which they were obtained.

The resulting data provides a quantitative, targeted measurement of RNA or protein targets across individual ROIs or segments. Following appropriate quality control and normalization, these measurements can be used to evaluate relative differences in target abundance across tissue regions and experimental conditions. The resulting dataset can support downstream analyses including differential expression, identification of molecular signatures, pathway and biological process analysis, clustering, and assessment of spatially associated molecular patterns.

Because each measurement retains its association with the originating ROI or segment, nCounter-derived data can be integrated with the spatial and histological context of the tissue. This enables researchers to investigate where molecular targets are detected within the tissue and how their abundance varies across defined spatial regions.

The resulting count data are generated as a targeted molecular profile rather than an untargeted transcriptome and therefore reflect only the genes or proteins represented in the selected assay panel.

The nCounter system generates Reporter Code Count (**RCC**) files, which are uploaded to the GeoMx analysis software for demultiplexing and calibration. Once processing is complete, the user can generate the initial spatially resolved dataset and export the processed data as an Excel (.xlsx) file for downstream analysis.

The exported Excel file contains the molecular count data associated with the corresponding ROIs or segments. The user then uploads this dataset to the **TRIP Lab GeoMx workstation** for downstream quality control, normalization, visualization, and statistical analysis.

NGS-Based Data Generation and Processing

For GeoMx assays utilizing an NGS readout, sequencing is performed in collaboration with the UWBC **Gene Expression Center (GEC)**, **DNA Sequencing Core**, and **Bioinformatics Resource Center (BRC)**. Together, these cores take the raw sequencing data generated from the Illumina and process it through the GeoMx NGS pipeline. The UWBC cores convert the resulting data (**FASTQ** files) into Digital Count Conversion (**DCC**) files containing the molecular count data associated with the individual ROIs/segments. These files are uploaded by the user to the **TRIP Lab GeoMx workstation** for downstream spatial analysis.

The primary output of the NGS workflow is a spatially resolved digital count dataset in which molecular measurements are assigned to their corresponding ROIs or segments. This data provides quantitative information on the abundance of targeted RNA or protein-associated molecular tags detected within each spatial region, allowing molecular expression profiles to be evaluated in relation to tissue location, morphology, and experimental condition.

Within the GeoMx analysis environment, the DCC data can be subjected to quality control, normalization, visualization, and statistical analysis. Depending on the assay and experimental design, resulting data may be used to evaluate differential molecular expression, identify spatially associated expression patterns, characterize molecular signatures, and perform pathway or biological process analyses.

Contacts

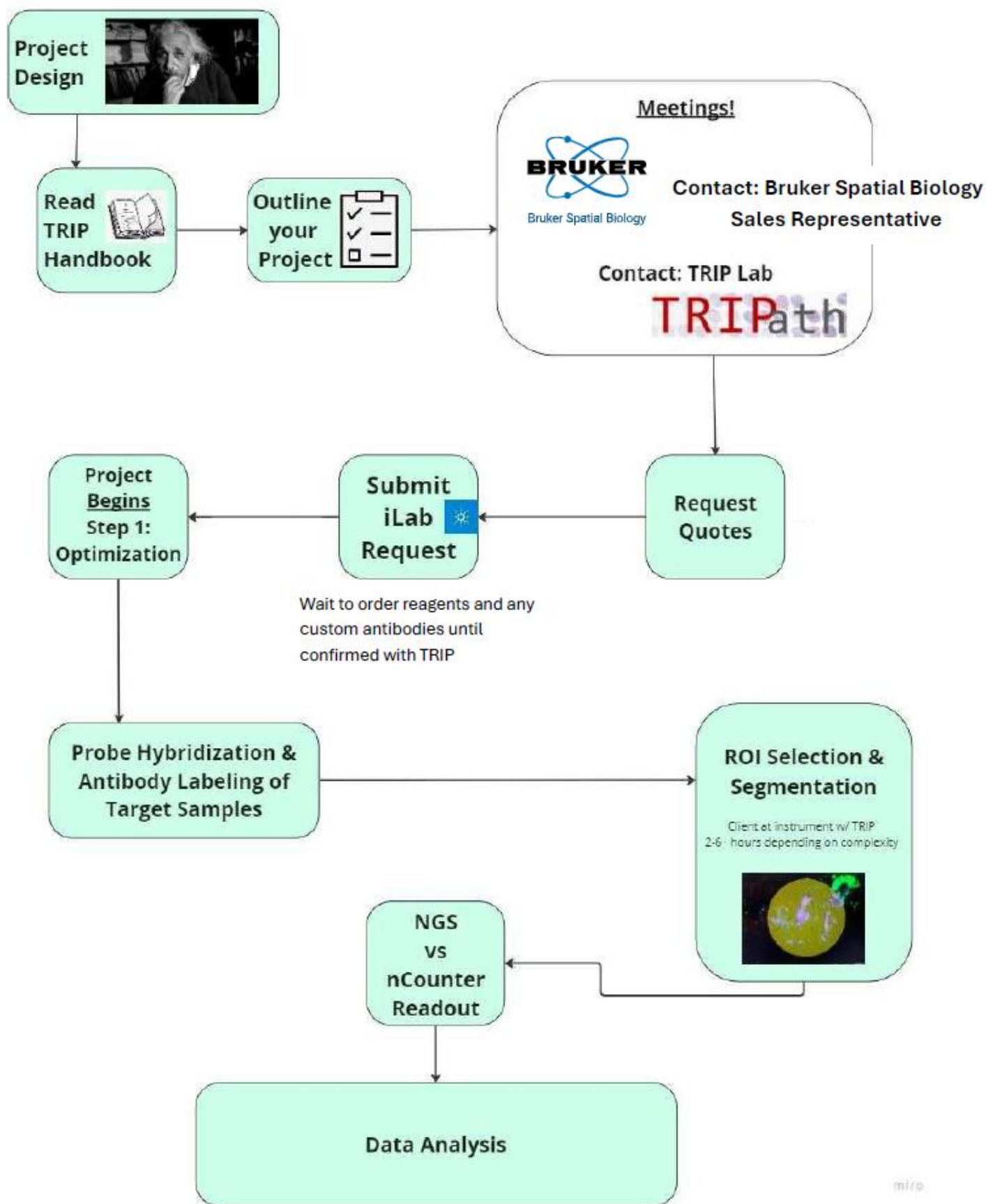
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mirp

Project Design

When reaching out to TRIP Lab to discuss a new spatial project, it is important to be prepared with the information needed to clearly communicate your experimental goals and project requirements. To help ensure a productive discussion and successful project planning, the sections below highlight the key topics you should familiarize yourself with and the information you should be prepared to provide before scheduling a meeting with TRIP Lab.

1. Know How Your Tissue Was Collected, Treated, and Stored

Understanding how your tissue was collected, processed, and stored prior to coming to TRIP Lab is essential for obtaining high-quality spatial data.

Tissue collection & formalin fixation

To minimize RNA degradation:

- Transfer harvested tissue to formalin within **5 minutes of collection**
- Wear gloves and clean the work area with **RNase Away®** or an equivalent RNase decontamination product.
- Cut tissue into appropriately sized pieces to allow complete formalin penetration, including the center of the tissue.
- Determine the appropriate fixation time based on the **tissue type** and **tissue size**. If you are unsure of the recommended fixation time of your tissue, contact TRIP lab before harvesting.

Proper fixation is critical for both RNA quality and tissue morphology. **Over-fixation can reduce antigen accessibility** and make optimization of morphology markers more difficult.

Following formalin fixation, transfer the tissue to **70% ethanol** (EtOH) and deliver it to TRIP Lab. Avoid prolonged storage in EtOH, as extended time in EtOH can effectively prolong the fixation process and contribute to over-fixation.

Decalcification

For bone or other calcified tissues, **avoid acid decalcification reagents**, which can negatively impact RNA and downstream spatial assays. EDTA-based decalcification methods should be used. If your tissue requires decalcification, contact TRIP Lab before processing to discuss the appropriate approach.

Archived FFPE tissue

Archived FFPE blocks may also be suitable for spatial analysis. In general, FFPE blocks <10 years old are expected to provide the best results, although tissue quality and storage conditions can vary.

TRIP will prepare fresh sections directly from the FFPE block for spatial analysis. Pre-cut slides will not be accepted.

2. What is your Biological Question?

Before beginning a spatial project, be prepared to describe **what you want to learn from your tissue using GeoMx Spatial Profiling**. Clearly defining your biological question will help TRIP Lab determine the most appropriate assay, tissue preparation, and experimental design for your project.

Some common biological questions include:

Discover biomarkers predictive of therapeutic response	Understand disease mechanisms and progression
Characterize the tumor microenvironment	Identify molecular subtypes of diseases
Investigate mechanisms of drug action	Evaluate preclinical models
Compare molecular profiles between tissue regions, cell populations, or experimental conditions	Identify spatially localized patterns of gene or protein expression

Consider what you ultimately want your spatial data to tell you. Having a clearly defined biological question will help TRIP Lab work with you to develop an experimental strategy that is aligned with your project goals.

3. What Morphology Markers Will Best Answer Your Questions?

Selecting morphology markers that are compatible with both **GeoMx Spatial Profiling** and your **tissue type** is one of the most important, and often most challenging, parts of a GeoMx project.

Morphology markers are used to identify and visualize specific cell populations or tissue structures and help guide the selections of regions for spatial profiling. Importantly, **not every antibody that works well in conventional IHC or IF will withstand the processing conditions used for GeoMx.**

When selecting morphology markers, consider:

- What cell populations or tissue structures do you need to identify?
- What markers will best distinguish the regions or cell populations relevant to your biological question?
- Has the antibody or probe been validated for GeoMx?
- Has it been tested in your specific tissue type and species?

1. **Bruker Off-the-Shelf Morphology Marker Kits**

Whenever possible, consider using one of Bruker's pre-designed Morphology Marker kits. These kits contain markers that have been validated for GeoMx and a diverse group of tissue types. Example: Solid Tumor Morphology Marker kit (PCK, CD45)

Using an off-the-shelf kit can reduce the amount of optimization required and will **save both time and project cost** compared with developing a custom morphology panel.

<https://brukerspatialbiology.com/products/geomx-digital-spatial-profiler/geomx-dsp-configuration-files/>

2. **Bruker Validated Morphology Marker Reference List**

If an off-the-shelf kit does not meet your experimental needs, Bruker's Validated Morphology Marker Reference List can be used to identify additional antibodies that have been tested for GeoMx.

The list includes antibodies from Bruker's partner vendors and provides information about the **species and tissue types in which each antibody has been tested.**

Contact your Bruker Representative for the most up-to-date version of the list.

Important: An antibody being included on the validated list does not mean it has been validated for every tissue type. Tissue-specific testing and optimization will still be required.

Selecting antibodies from this list may require testing multiple clones to identify the best-performing

antibody for your tissue. This additional optimization can **increase both project cost and turnaround time.**

3. Custom Antibodies

If your desired markers are not available through Bruker's off-the-shelf kits or validated reference list, custom antibodies may be considered.

See "Selecting a Custom Morphology Marker" for tips and considerations when selecting a custom morphology marker.

Important: An antibody that performs well in conventional IHC or IF is not necessarily compatible with GeoMx. Custom antibodies require additional optimization, which can **add significant time and cost** to a project.

4. RNA Morphology Targets

If RNA-based morphology markers are more appropriate for your project, Bruker partners with Advanced Cell Diagnostics (ACD) to provide RNAscope® probe combinations compatible with GeoMx. <https://brukerspatialbiology.com/about-us/partnerships/geomx-partners/acd-partnership/>

5. Additional Resources

Canopy Biosciences is an experienced spatial biology Contract Research Organization (CRO) that has developed validated marker resources covering areas such as immunology, oncology, and neuroscience. These resources may be useful as a final resort when selecting morphology markers. https://share.hsforms.com/1H4Mix0xkR0SdxtcbOoozsg49a3v?utm_campaign=NanoString%20Referral%20Traffic&utm_source=NS%20web

4. Selecting A Custom Morphology Marker

If your desired morphology marker is not available through Bruker's off-the-shelf kits or validated morphology marker list, a custom antibody may be considered. Selecting an appropriate antibody before purchase can prevent unnecessary optimization, delays, and additional project costs.

When evaluating a custom antibody, consider the following:

a) **Species compatibility**

The antibody should be raised in a species different from the species of the tissue being analyzed to minimize background staining from endogenous immunoglobulins.

b) **Antibody specificity**

Monoclonal antibodies are generally preferred when high target specificity is important.

c) **Recommended dilution**

Look for an antibody with recommended dilution of approximately **1:50 or greater**. TRIP Lab does not accept antibodies for spatial profiling when the manufacturer's recommended dilution is as concentrated as 1:10.

d) **Tissue-Specific Staining**

Whenever possible, select an antibody that has demonstrated reliable staining in the **specific tissue type being analyzed**. This may be supported by manufacturer data, published studies, or previous successful use in your laboratory with IHC-P. Prior successful IHC-P staining can provide valuable evidence of antibody performance, although optimization for GeoMx is still required.

e) **Previous GeoMx experience**

Antibodies from manufacturers such as **Abcam**, **Cell Signaling Technology**, and **Novus Biologicals** have been successfully used for GeoMx projects. However, previous success does not guarantee performance in every tissue type, and optimization is still required.

f) **IHC validation**

The antibody must be validated for **IHC on paraffin-embedded tissue** (IHC-P). Validation for ICC/IF (cultured cells) alone will not be accepted.

g) **Species Reactivity**

Confirm that the antibody demonstrates reactivity in the species being analyzed. Ideally, the manufacturer should provide evidence of reactivity in the specific species and tissue type of interest. The **Human Protein Atlas** can also be used as a resource when evaluating target expression: <https://www.proteinatlas.org/>

h) **Fluorophore compatibility**

The antibody must be available conjugated to an appropriate fluorophore, such as **Alexa Fluor® 532**, **Alexa Fluor® 594**, or **Alexa Fluor® 647**, depending on the morphology panel and available channels.

Fluorophore and Channel Considerations

GeoMx morphology imaging uses specific fluorescence channels. When selecting a custom antibody, consider the channels available for your project and ensure the antibody is conjugated to a compatible fluorophore. See the table below for available channels and fluorophores.

Fluorophore	Typical Use
Alexa Flour® 488	Reserved for use with the nuclear stain SYTO13
Alexa Flour® 532	Typically used for an antibody from a Bruker off-the-shelf Morphology kit (ex. Pan-Cytokeratin from the Solid Tumor Morphology Marker kit); this channel may be used for a custom antibody instead
Alexa Flour® 594	Typically used for an antibody from a Bruker off-the-shelf Morphology kit (ex. CD45 from the Solid Tumor Morphology Marker kit); this channel may be used for a custom antibody instead
Alexa Flour® 647	Available for a custom antibody or may be left empty

Because **Alexa Flour® 532** and **Alexa Flour® 594** may already be occupied by markers in an off-the-shelf Morphology Marker kit, the available channel should be confirmed with **TRIP Lab** before purchasing a custom antibody.

Antibody Conjugation

TRIP Lab **does not offer antibody conjugation as a service**, and we discourage investigators from attempting antibody conjugation themselves. Improper conjugation can affect antibody performance and introduce additional variables during assay optimization.

Before Purchasing Your Antibody

Do not purchase a custom antibody until it has been reviewed and approved by Becky.

Before making a purchase, send the manufacturer's datasheet and product information to Becky for review. This allows TRIP Lab to confirm that the antibody meets the project requirements and that the proposed fluorophore and channel are compatible with the planned morphology panel.

5. Using H&E or IHC-DAB to define Morphology

Using an **H&E or IHC-DAB stained slide to define morphology and select ROIs** is an approach that requires minimal optimization and can be particularly useful when ROI selection is based primarily on tissue morphology, histological features, or known anatomical regions.

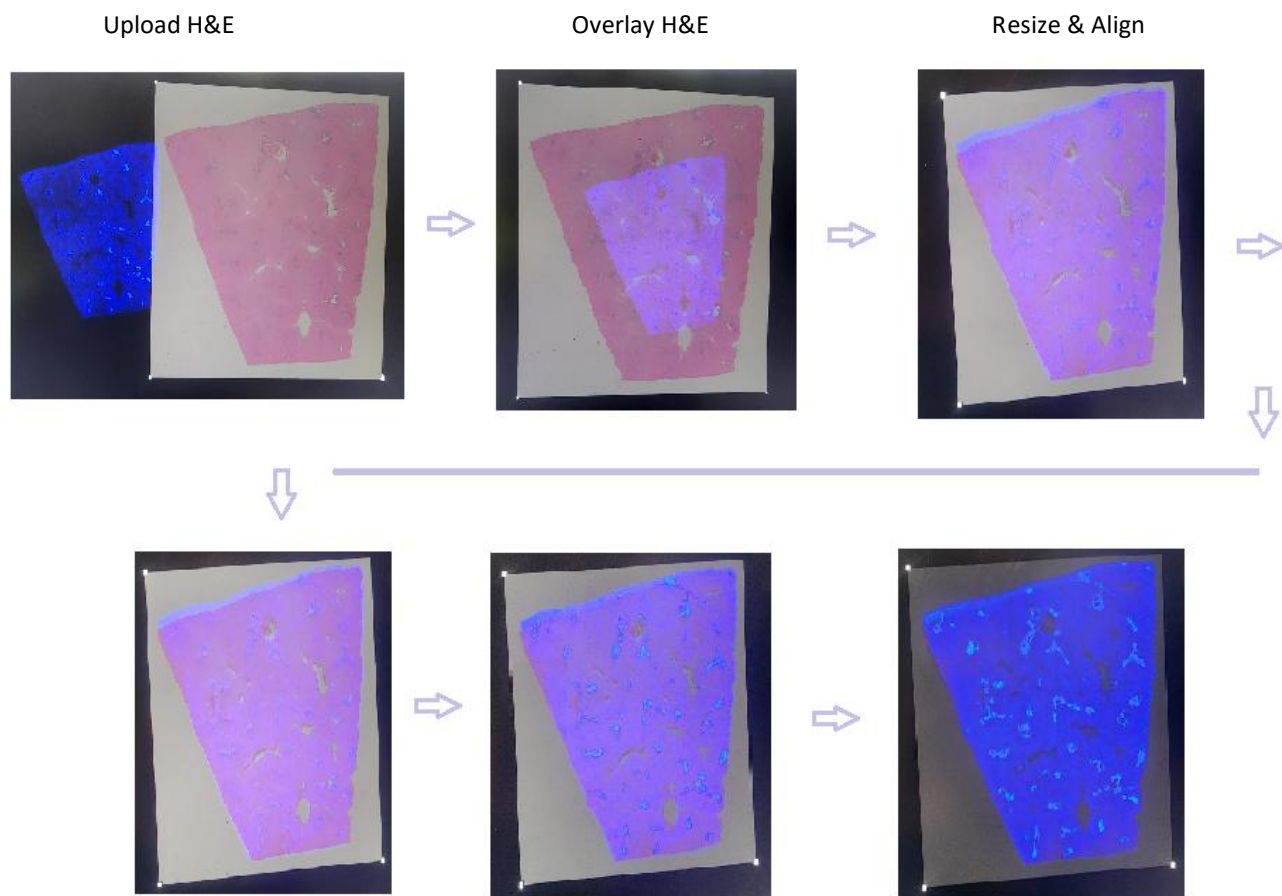
With this approach, a single test slide can be used to visualize the tissue using the **nuclear stain (SYTO13) with an H&E or IHC-DAB overlay**. This allows you to confirm that the tissue morphology is clearly represented and that the areas of interest can be identified before proceeding with the final tissue.

Once the morphology and tissue orientation have been confirmed, the information can be used to guide **ROI selection on the final tissue section**.

This approach has been effective for projects in which ROIs are selected based on:

- **Tissue morphology or anatomical features**
- **Known regions of interest**
- **Distinct histological structures**
- **Areas identified by H&E or IHC-DAB staining**

Using an H&E or IHC-DAB slide to guide ROI selection can simplify the morphology optimization process and may reduce the need for additional morphology marker development when specific cell populations do not need to be identified by immunofluorescence.



6. Tissue Size

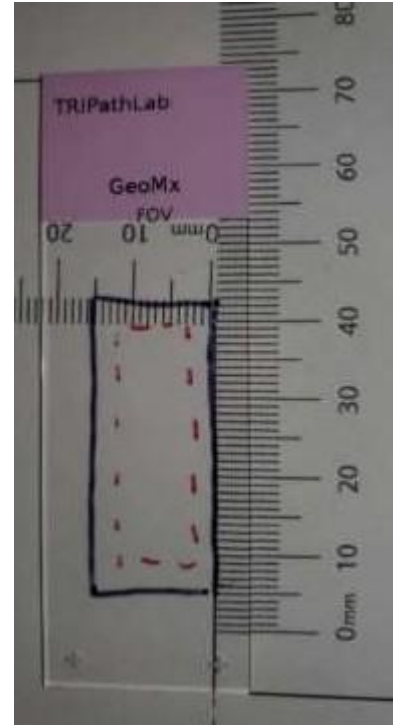
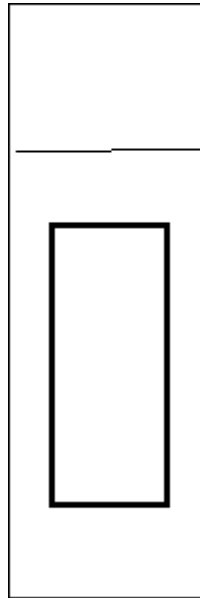
The GeoMx DSP instrument has a **field of view (FOV)** of ~33mm x 15mm (black rectangle).

To allow the instrument to clearly distinguish **the edge of the tissue from the edge of the gasket**, the tissue section should be no larger than ~30mm x 12mm (red rectangle).

Maintaining this size limit provides sufficient space between the tissue edge and the gasket edge for proper tissue recognition and imaging.

Image to Scale

**Maximum recommended tissue size:
30mm x 12mm**



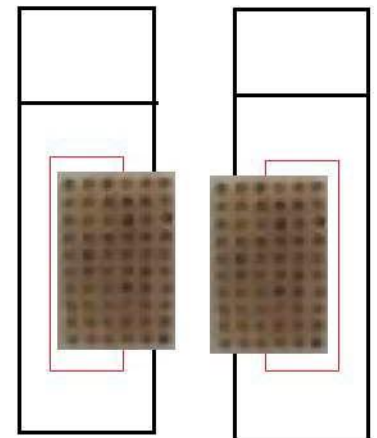
Tissue MicroArrays (TMAs)

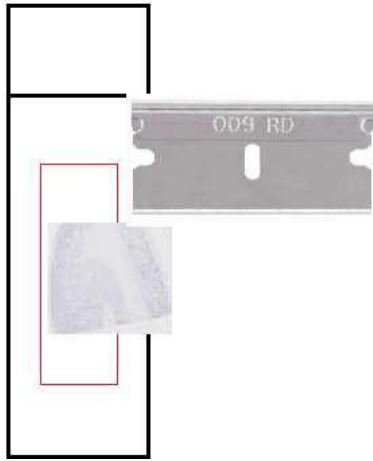
TRIP Lab can accommodate **tissue microarrays (TMAs)** by using **two slides** to achieve full coverage of the TMA.

Two sections are cut from the TMA block:

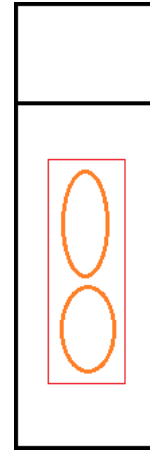
- One slide maintains coverage of the **left side** of the TMA
- The second slide maintains coverage of the **right side** of the TMA.
- The two sections will generally have some overlap, ensuring that all cores are represented and available for analysis.

This approach allows the full TMA to be captured while accommodating the GeoMx DSP field-of-view limitations.

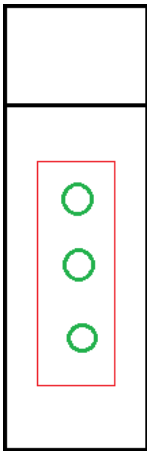




Tissue exceeding the FOV (red lines): Tissue will be positioned in the center and any tissue extending beyond the designated FOV will be carefully removed by scraping the excess tissue away with a razor blade to ensure proper imaging.

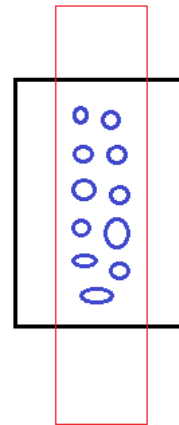


When working with smaller tissue, it may be possible to place **2 samples on a single slide**. This will depend on the **size & shape** of the tissue.



In instances where samples are very small (ex. **biopsies, eyes**) it may be possible to fit **three** samples on a single slide. This will depend on **size & shape** of tissue. It is recommended to perform QC on each sample prior to placing in a single block.

FFPE Block



Plan ahead and ask for your tissue to be embedded in FFPE blocks for GeoMx.

We will place the tissue in the block so that it all lands within the FOV of the slide.

FOV of GeoMx Slide

For very small tissue where you would like to analyze **>3 samples/slide**, the samples will need to be embedded into a **single block**. If needed, we can melt and re-embed existing samples or create a TMA. It is recommended to perform QC on each sample prior to placing in a single block.

Contact Bruker Spatial Biology

Working with Bruker and Ordering Reagents

A **Bruker Sales Representative** can answer many of your initial questions and is a valuable resource when planning a GeoMx project. Bruker representatives have extensive knowledge of the available **Morphology Marker kits and assay panels** and can provide the most up-to-date **Morphology Marker Reference List** (Validated Antibodies).

Before Ordering Reagents

You may request a **reagent quote** from Bruker during the initial planning stages; however, **do not place the order until you have:**

- **Met with Karla**
- **Obtained a reagent quote from Bruker and provided it to Becky. This quote is required for TRIP Lab to prepare the project quote and calculate the total project cost.**
- **Received a project quote from Becky**
- **Reviewed your project with Becky**

Not all the GeoMx reagents included in your order will necessarily have the same expiration date. Ordering reagents before your project is ready to proceed may result in reagents expiring before they can be used.

Before placing your order

- Discuss your project with Becky and provide the relevant project details
- Confirm that your project meets TRIP Lab requirements.
- Wait for confirmation from Becky before placing the reagent order. We may have some reagents in stock in the lab.
- Once the project and the reagent requirements have been confirmed, proceed with the order

Reagent Shipment & Labeling

*****Do not ship reagent orders directly to TRIP Lab*****

To prevent reagent mix-ups between projects and laboratories, **label every reagent box with your PI's name** before bringing them to TRIP Lab.

Important: Please do not purchase or ship GeoMx reagents until TRIP Lab has confirmed that your project is ready to proceed.

Contact TRIP Lab

Once you have defined your biological question, identified your tissue and morphology requirements, and familiarized yourself with GeoMx Spatial Profiling

Karla Esbona, TRIP's Operational Director, meets virtually with clients for an initial **TRIP Lab-Client GeoMx meeting**. During this meeting, you will have the opportunity to describe your project in detail, discuss your experimental goals and tissue requirements, and ask questions about the GeoMx workflow. Together, we can determine whether your project is feasible and identify the appropriate next steps.

Before Your Meeting

Prior to meeting with TRIP Lab, you should have a basic understanding of GeoMx Spatial Profiling technology and its capabilities. There are several resources available to help you become familiar with the technology, including:

- **Bruker Field Application Specialists (FASs)** – Reach out to a Bruker FAS for technical and application-specific questions.
- **Bruker Spatial Biology University** – Bruker's free educational platform provides introductory information, training materials, and videos on the GeoMx Digital Spatial Profiler.
<https://university.brukerspatialbiology.com/page/geomx-digital-spatial-profiler>
- **TRIP Lab-sponsored seminars** – Attend education seminars and presentations offered by TRIP Lab
- **Independent research** – Review available literature, application notes, and other educational resources relevant to your project.

Request Sequencing Quote

Sequencing costs are based on the **total area of all ROIs/AOIs across all plates** and are calculated using shared Illumina sequencing through the DNA Sequencing Facility.

In most projects, samples are sequenced using the facility's shared sequencing resources. In rare cases where the **total ROI/AOI area is very large**, it may be more cost-effective to purchase an entire lane of a **NovaSeq S4 flow cell**, which provides an average of **~2.25 billion reads per lane**.

Because sequencing requirements depend on the final ROI/AOI area, an accurate sequencing cost estimate cannot be determined until **ROI/AOI selection is complete and the samples have been collected**.

A final sequencing cost estimate will be provided once the total ROI/AOI area is known.

Submit iLab Request

When you submit your iLab Request, select “**Spatial & Molecular Profiling**.” You will then be presented with the following GeoMx service options:

1. GeoMx DSP Multiomics

Spatial: Protein + RNA Simultaneously

This option allows you to profile **both protein and RNA targets within the same tissue section and spatial regions**, providing complementary information about cellular phenotype and molecular activity while preserving tissue architecture.

2. GeoMx DSP + NGS Readout

Select the appropriate NGS readout based on your project:

- **RNA NGS Readout**
- **Protein NGS Readout**

With **NGS readout**, your samples are sent to the **Gene Expression Center (GEC)** for sequencing on the **Illumina platform**. The resulting sequencing data are processed by the **Bioinformatics Resource Center (BRC)** and converted to **.DCC** files for downstream GeoMx analysis on the GeoMx workstation.

GeoMx NGS RNA panels

Readout	Panel/Core	Species	Targets	Notes
NGS	Hu Whole Transcriptome Atlas (HuWTA)	Human	18,000+	Broad, unbiased profiling of protein-coding genes
NGS	Mu Whole Transcriptome Atlas (MuWTA)	Mouse	21,000+	Broad, unbiased profiling of protein-coding genes
NGS	Cancer Transcriptome Atlas (CTA)	Human	1,834	Cancer biology, tumor microenvironment, & immune response
NGS	Canine Cancer Atlas (CCA)	Canine	1,962	Canine cancer & immune-response profiling
NGS	TCR Profiling Add-On	Human	146 TCR segments	Add-on only; must be run with Hu WTA or CTA

GeoMx NGS Protein panels

Readout	Panel/Core	Species	Targets	Notes
NGS	GeoMx Human Protein Core for NGS	Human	4	CD45, Ki-67, β 2-microglobulin, CD31 + controls
NGS	Immune Cell Typing	Human	10	Module; requires NGS protein core
NGS	Immune Activation Status	Human	10	Module
NGS	IP Drug Target	Human	10	Module
NGS	Pan-Tumor	Human	10	Module
NGS	Myeloid	Human	9	Module
NGS	MAPK Signaling	Human	9	Module
NGS	PI3K/AKT Signaling	Human	9	Module
NGS	Neural Cell Typing	Human	10	Module
NGS	Alzheimer's Pathology	Human	10	Module
NGS	GeoMx Mouse Protein Core for NGS	Mouse	4	CD45, Ki-67, Cd31, GFP + controls
NGS	Immune Cell Typing	Mouse	10	Module
NGS	IO Drug Target	Mouse	7	Module
NGS	Immune Activation Status	Mouse	8	Module
NGS	Pan-Tumor	Mouse	9	Module
NGS	Cell Death	Mouse	10	Module

NGS	Myeloid	Mouse	10	Module
NGS	MAPK Signaling	Mouse	10	Module
NGS	PI3K/AKT Signaling	Mouse	8	Module
NGS	Neural Cell Typing	Mouse	9	Module
NGS	Alzheimer's Pathology	Mouse	8	Module
NGS	Alzheimer's Pathology Extended	Mouse	9	Module
NGS	Parkinson's Pathology	Mouse	9	Module
NGS	Glial Cell Subtyping	Mouse	10	Module
NGS	GeoMx IO Proteome Atlas (IPA)	Human	570+	Hi-plex protein assay; NGS/Illumina readout
NGS	GeoMx Discovery Proteome Atlas (DPA)	Human	1,200+	High-plex discovery protein assay; includes 130+ PTMs

3. GeoMx DSP + nCounter Readout

Select the appropriate nCounter Readout:

- **RNA with nCounter readout** (*CTA and WTA are not compatible with this option*)
- **Protein with nCounter readout**

With nCounter Readout, your samples remain within the TRIP Lab and are processed using the appropriate nCounter reagents for your GeoMx panel. The resulting data are processed and converted to .DCC files on the GeoMx workstation for downstream analysis.

nCounter RNA panels

Readout	Panel/Core	Species	Targets
nCounter only	Immune Pathway Panel	Human	84 + controls
nCounter only	Custom RNA panel	Custom	Up to 96

nCounter Protein panels

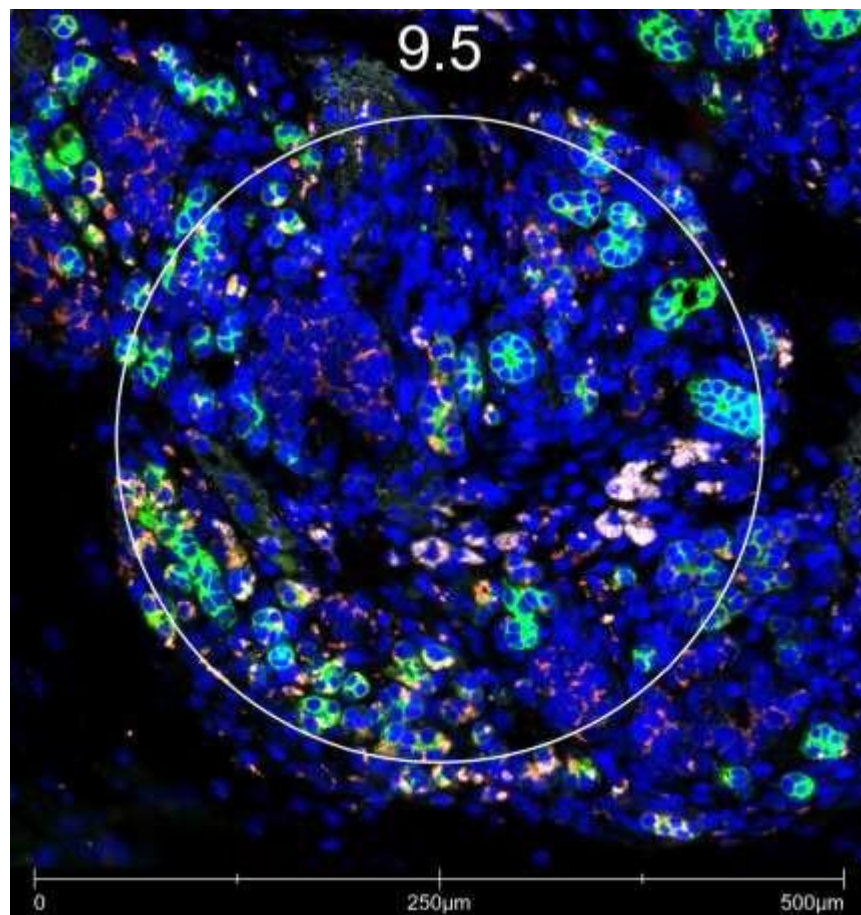
Readout	Panel/Core	Species	Targets	Notes
nCounter only	Immune Cell Profiling Core	Human	18	Core; can add modules
nCounter only	IO Drug Target	Human	10	Module
nCounter only	Immune Activation Status	Human	8	Module
nCounter only	Immune Cell Typing	Human	7	Module
nCounter only	Cell Death	Human	10	Module
nCounter only	Myeloid	Human	10	Module
nCounter only	MAPK Signaling	Human	10	Module

nCounter only	PI3K/AKT Signaling	Human	8	Module
nCounter only	IO Pan-Tumor	Human	10	Module
nCounter only	Neural Cell Profiling Core	Human	20	Neuroscience core
nCounter only	Alzheimer's Pathology	Human	10	Module
nCounter only	Alzheimer's Pathology Extended	Human	9	Module
nCounter only	Parkinson's Pathology	Human	9	Module
nCounter only	Autophagy	Human	10	Module
nCounter only	Glial Cell Subtyping	Human	10	Module
nCounter only	Immune Cell Profiling Core	Mouse	18	Core
nCounter only	IO Drug Target	Mouse	7	Module
nCounter only	Immune Activation Status	Mouse	8	Module
nCounter only	IO Cell Typing	Mouse	10	Module
nCounter only	Cell Death	Mouse	10	Module
nCounter only	IO Pan-Tumor	Mouse	9	Module
nCounter only	MAPK Signaling	Mouse	10	Module
nCounter only	PI3K/AKT Signaling	Mouse	8	Module
nCounter only	Neural Cell Profiling Core	Mouse	20	Neuroscience core
nCounter only	Alzheimer's Pathology	Mouse	8	Module
nCounter only	Alzheimer's Pathology Extended	Mouse	9	Module
nCounter only	Parkinson's Pathology	Mouse	9	Module
nCounter only	Autophagy	Mouse	10	Module
nCounter only	Glial Cell Subtyping	Mouse	10	Module

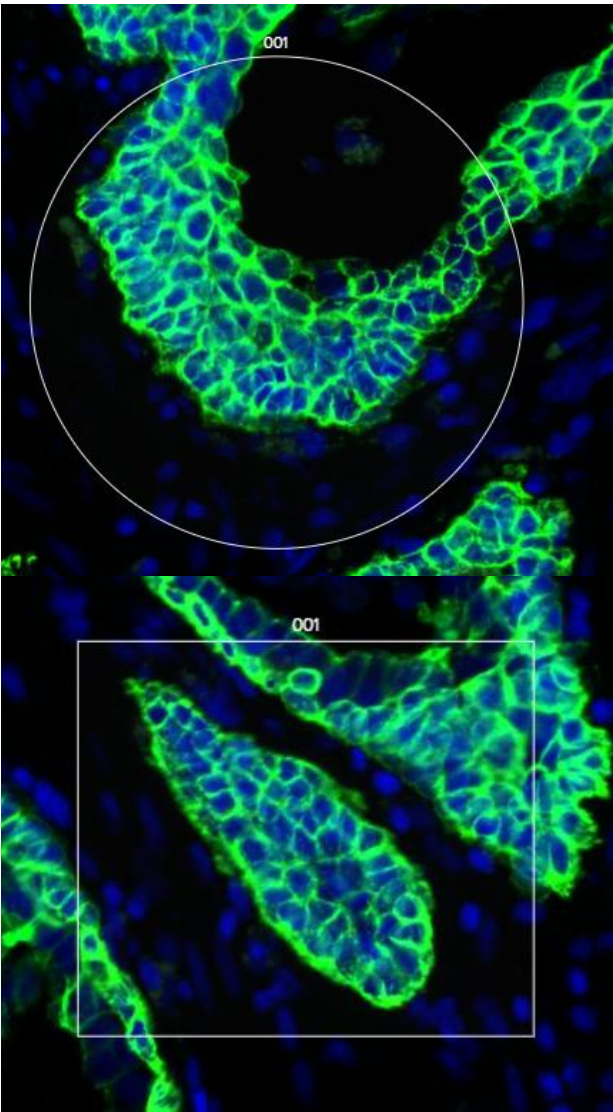
Understanding ROI Selection & Segmentation

Regions of Interest (ROIs) are specific areas within your tissue sample selected for spatial profiling and analysis.

During ROI selection, you will work with **TRIP Lab staff** to identify and define the areas of your sample that you would like to analyze. ROIs can be drawn using geometric shapes, such as **circles, squares, or rectangles** or can be **customized to follow specific tissue structures** or regions of interest.



Dinh, Huy and Fitzpatrick, Megan 220216



Example of a circular ROI.

ROI: [Delete](#)

Circle - Center: (,), Diameter: μm

Area: 31415.9 μm^2 , Nuclei Count: 201

Comment:

Created: 12:48:16 PM

[Export](#) [Import](#)

Test Image Rebecca Baus 2023

Example of a geometric ROI.

ROI: [Delete](#)

Rectangle - Center: (,) Size: (x μm)

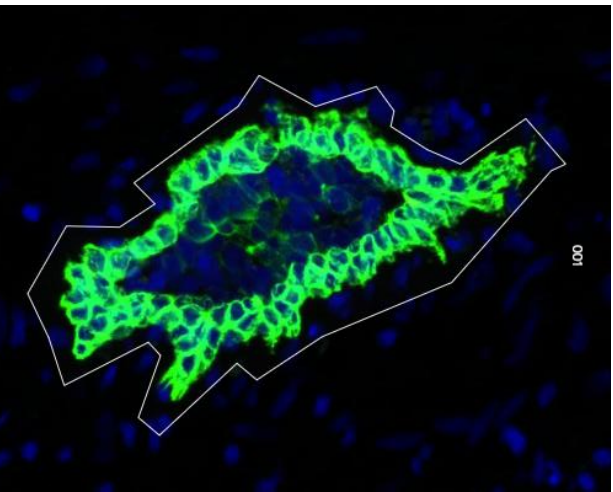
Area: 23070.9 μm^2 , Nuclei Count: 151

Comment:

Created: 12:51:13 PM

[Export](#) [Import](#)

Test image Rebecca Baus 2023



Example of a customized ROI.

ROI: [Delete](#)

Polygon - Center: (,)

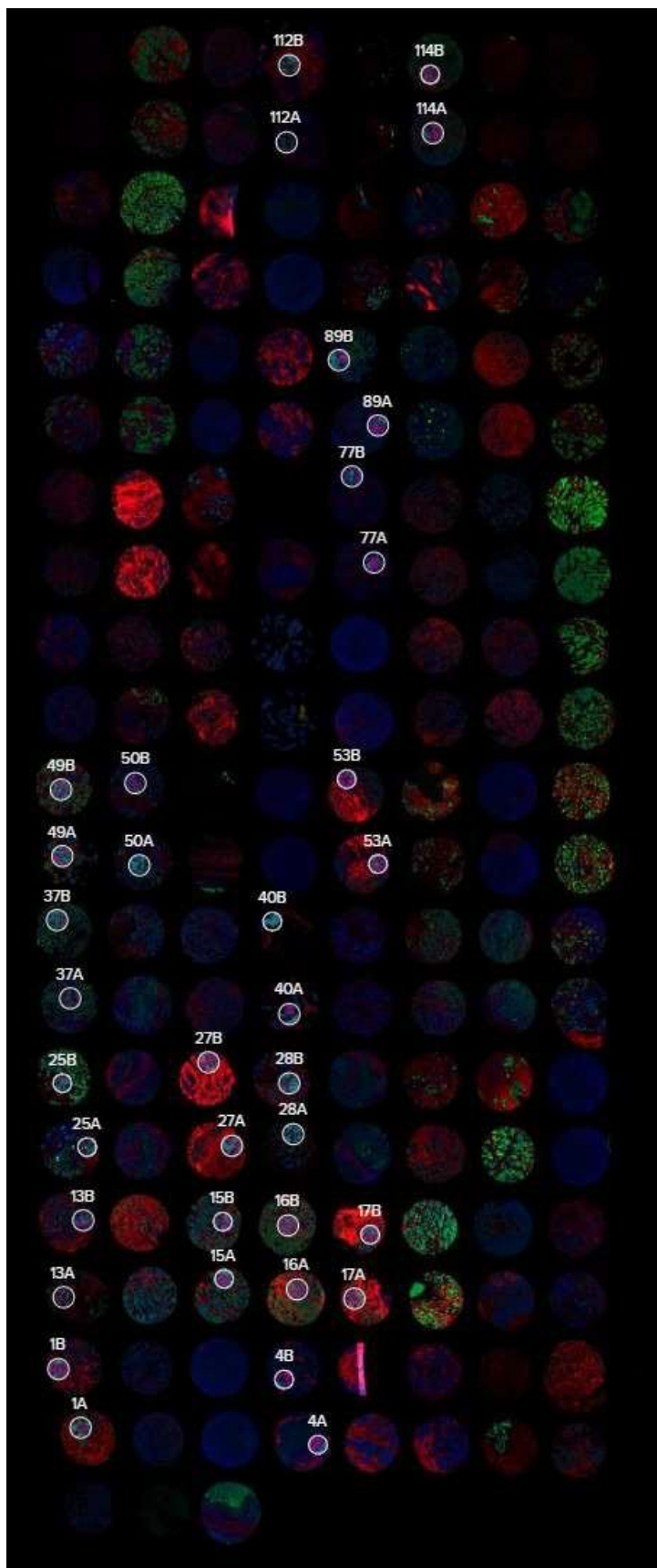
Area: 21071.6 μm^2 , Nuclei Count: 164

Comment:

Created: 12:49:57 PM

[Export](#) [Import](#)

Test image Rebecca Baus 2023



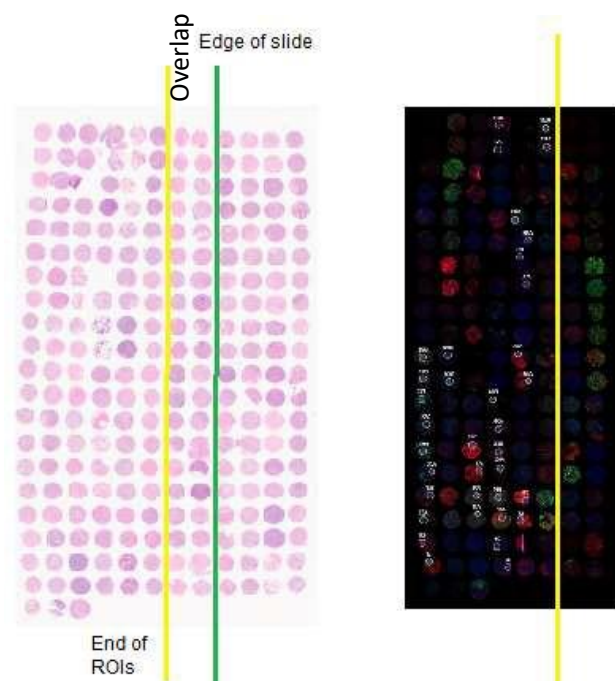
Archived FFPE Blocks and Tissue Microarrays

Many investigators use **archived FFPE tissue blocks** for GeoMx studies. **Blocks less than 10 years old are preferred** to help maximize tissue and RNA quality.

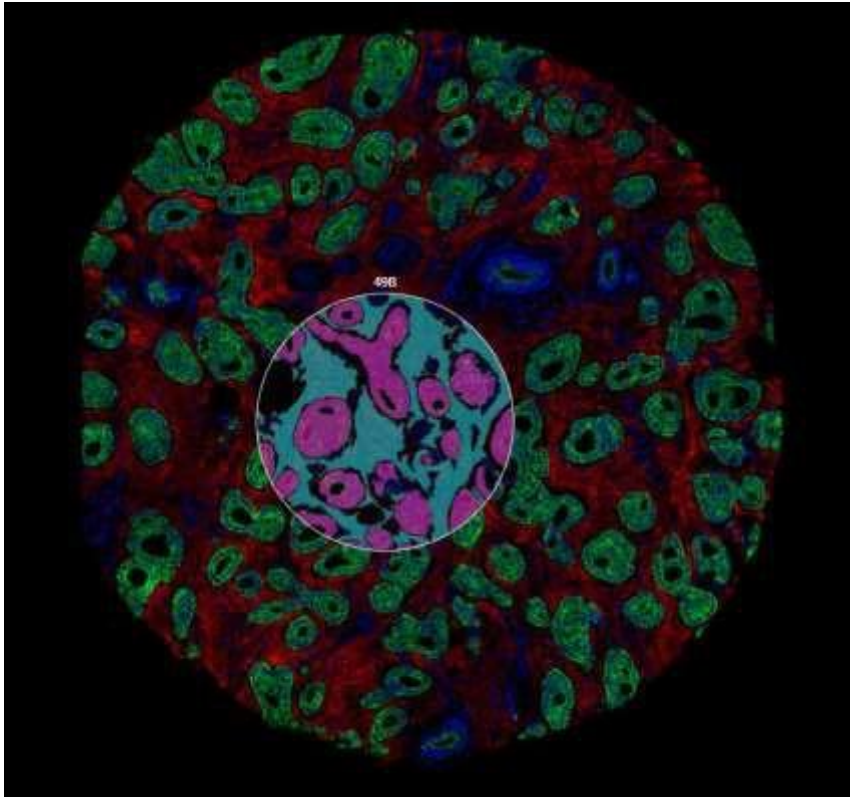
Tissue Microarrays

Tissue microarrays (TMAs) are useful when you want to analyze a large number of samples in a single experiment. Because a TMA contains multiple cores, it allows you to profile many samples while preserving their special context.

For a TMA with **12 rows of cores**, splitting the TMA across 2 slides with overlapping rows can provide complete coverage of all cores in the block. This approach ensures that every core is represented while allowing sufficient tissue for spatial profiling.



TMA for ROI selection used in GeoMx project.
Sheena Kerr, David Beebe, and H&N SPORE 2022



For perspective, this image shows a single 1mm TMA core with a 400µm ROI selected for spatial profiling.

Sheena Kerr, David Beebe, and H&N SPORE 2022

ROI Segmentation

Once you have selected your ROIs, you can analyze the **entire ROI as a whole** or further segment it into defined regions. Segmentation allows researchers to isolate and compare expression patterns within specific tissue compartments or cell populations while maintaining their spatial context.

Segmentation involves defining specific biological markers, or combinations of markers, to identify distinct sub-regions within an ROI. For example, with the **Solid Tumor Morphology kit**, and a nuclear stain, you can distinguish and compare tumor and stromal regions by defining segmentation parameters such as PCK⁺ for tumor cells and PCK⁻/CD45⁺ for immune/stromal regions.

The image on the following page provides an example of this tumor-vs-stromal comparison.

Segmentation identifies tumor regions (PCK⁺, purple) and stromal regions (PCK⁻/CD45⁺, yellow) within the selected ROI.

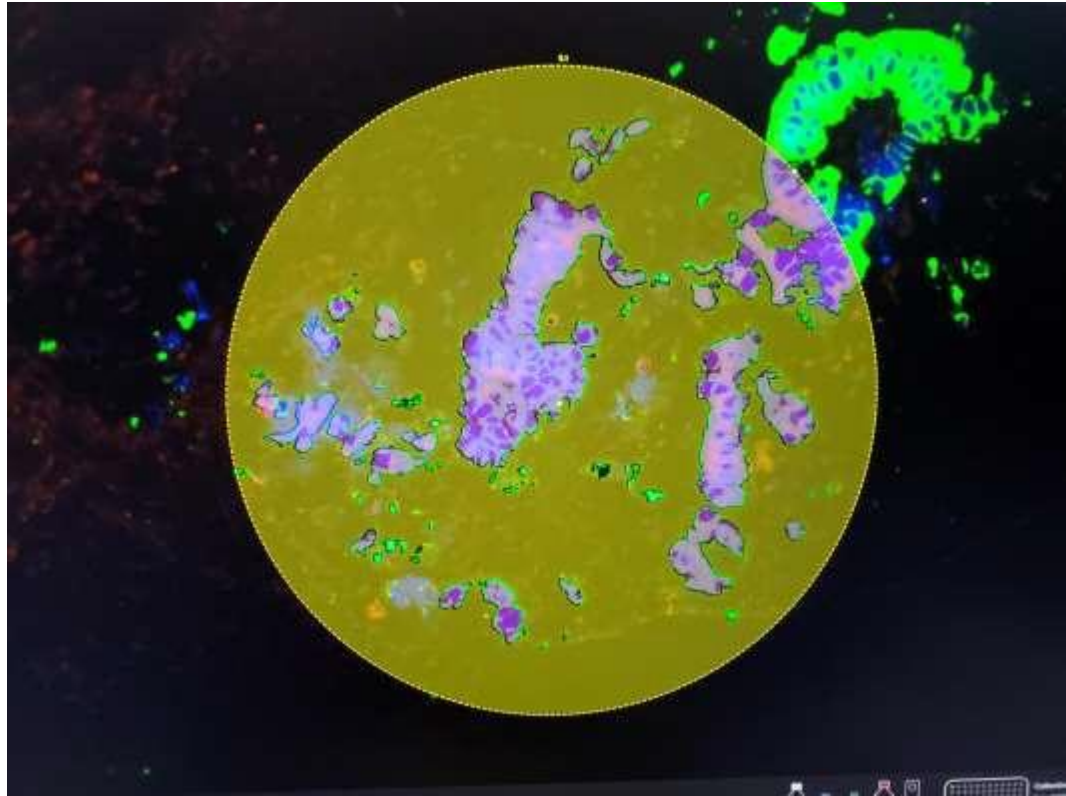


Image by Rebecca Baus 2023

Keep Segmentation Simple

Bruker recommends keeping segmentation rules as simple as possible. Using too many segmentation rules can reduce the effectiveness of automated segmentation and may result in segments containing too few nuclei to generate reliable data. In cases where segments contain very few nuclei, there may not be enough material to generate data at all.

Minimum Nuclei Requirements

Each segment should contain a minimum of **100 nuclei for RNA** and **20 nuclei for protein** to support reliable data generation. When segments contain fewer nuclei than recommended, **low-abundance transcripts or proteins may not be detected** or may be omitted from the resulting data. For this reason, selecting rare or low-expressing targets in segments with very few nuclei may not produce reliable results.

Published studies have reported successful data generated from segments containing **50 nuclei or fewer**; however, outcomes are highly dependent on factors such as **sample quality, tissue characterization, target abundance, and experimental objectives**. Successful data generation from segments below the recommended threshold cannot be guaranteed.

If you choose to proceed with segments containing fewer than **100 nuclei**, please be aware that **data quality, data yield, and overall assay performance may be compromised**. Results generated from segments from below the recommended threshold are outside our standard performance specifications. Limitations in data generation or quality resulting from selection below the recommended threshold do not qualify for reanalysis, reruns, or refunds.

Planning Your 96-Well Plates

To determine how many 96-well plates are needed for your project, first determine how many **Areas of Illumination (AOIs), or segments**, you plan to collect from each ROI. A maximum of **3 AOIs can be collected per ROI**.

Each 96-well plate includes **one well reserved for the negative control (NTC)**, leaving **95 wells available for sample collection**. The number of ROIs that can be collected per plate therefore depends on the number of AOIs collected from each ROI.

For example:

- **1 AOI per ROI**: up to 95 ROIs per plate
- **2 AOIs per ROI**: up to 47 ROIs per plate
- **3 AOIs per ROI**: up to 31 ROIs per plate

For robust experimental comparisons, we recommend selecting at least **8 ROIs per sample** when tissue size allows. For smaller tissue, such as biopsies, **3-4 ROIs per sample** may be sufficient.

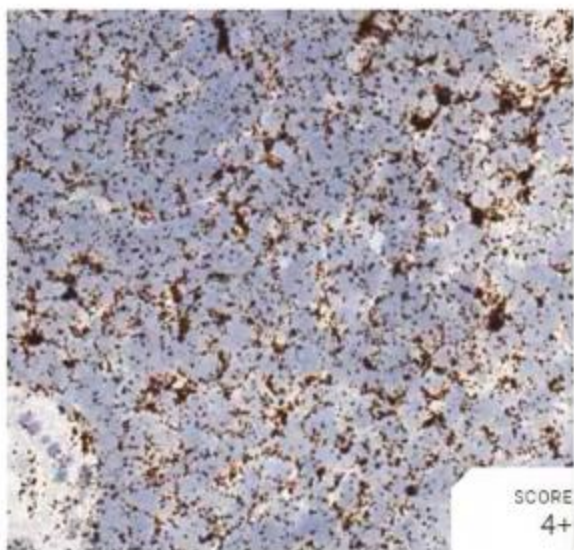
For statistical comparisons, **4-6 biological replicates per treatment group** are recommended.

Tissue Selection & RNA QC

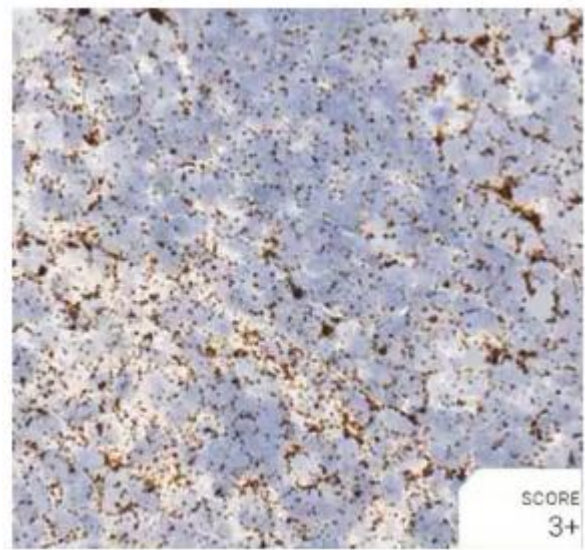
The GeoMx DSP workflow begins with tissue QC to assess RNA quality using ACD's RNAscope® Multiplex Fluorescent Reagent Kit in combination with a species-specific positive control probe. If the tissue does not express an adequate amount of quality RNA based on ACD's positive control scale, it is not suitable for GeoMx.

PPIB and UBC probes expression are quantified based on copies per cell. PPIB is expected to express 10-30 copies of RNA per cell, while UBC is expected to express >20. Below are examples of the expected staining pattern from PPIB and UBC probes in rat tissue.

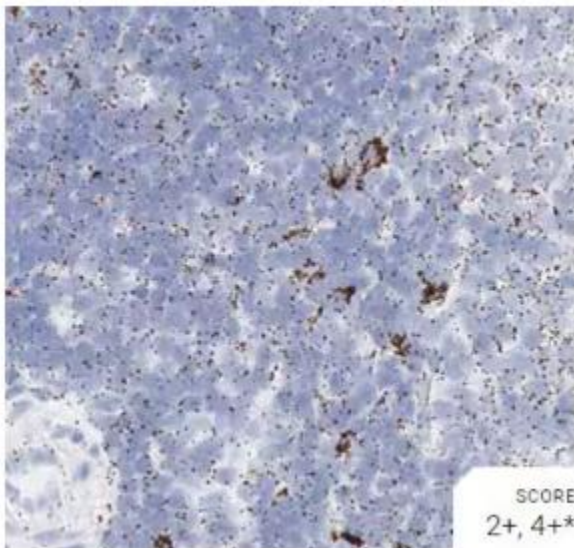
UBC – Rt Spleen, (med/high expression)



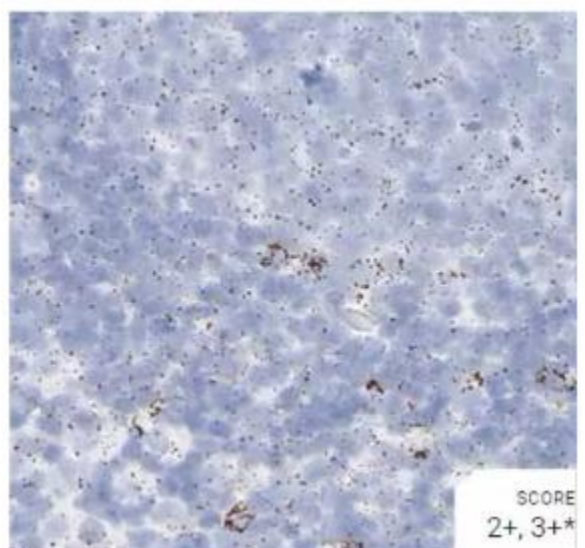
UBC – Rt Lymph node, (med/high expression)



PPIB – Rt Spleen (med expression)



PPIB – Rt Lymph Node, (med expression)



Images: <https://www.bio-techne.com/reagents/rnascope-ish-technology/rnascope/rnascope-ish-reference-guide>

Case Study: RNAscope® -Based Tissue QC for Block Selection

	TMA Block 1	TMA Block 2
Age range of FFPE cores included in the TMA (at time of staining)	12 – 23* years old	7 -13* years old
# of cores > 10 years old	100%	42%
# of cores > 15 years old	62%	0%
# cores > 20 years old	29%	0%

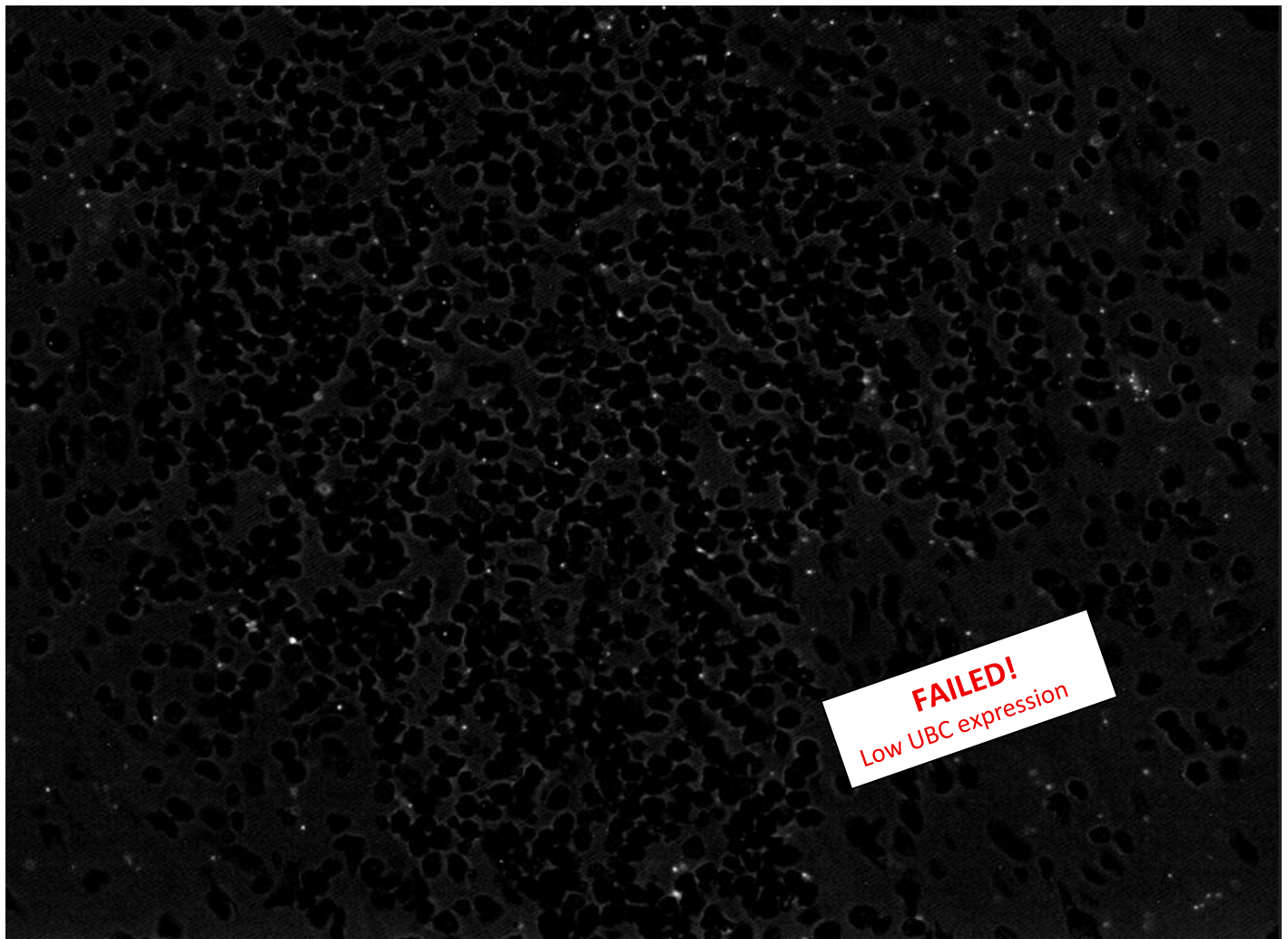
Two TMA blocks containing the same tissue type were evaluated using RNA QC to determine which block was better suited for GeoMx analysis.

*Note – Bruker recommends using FFPE blocks <10 years old for best results

RNAscope® results from Block 1:

TMA Block 1

UBC: expected med/high expression



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TMA Block 1

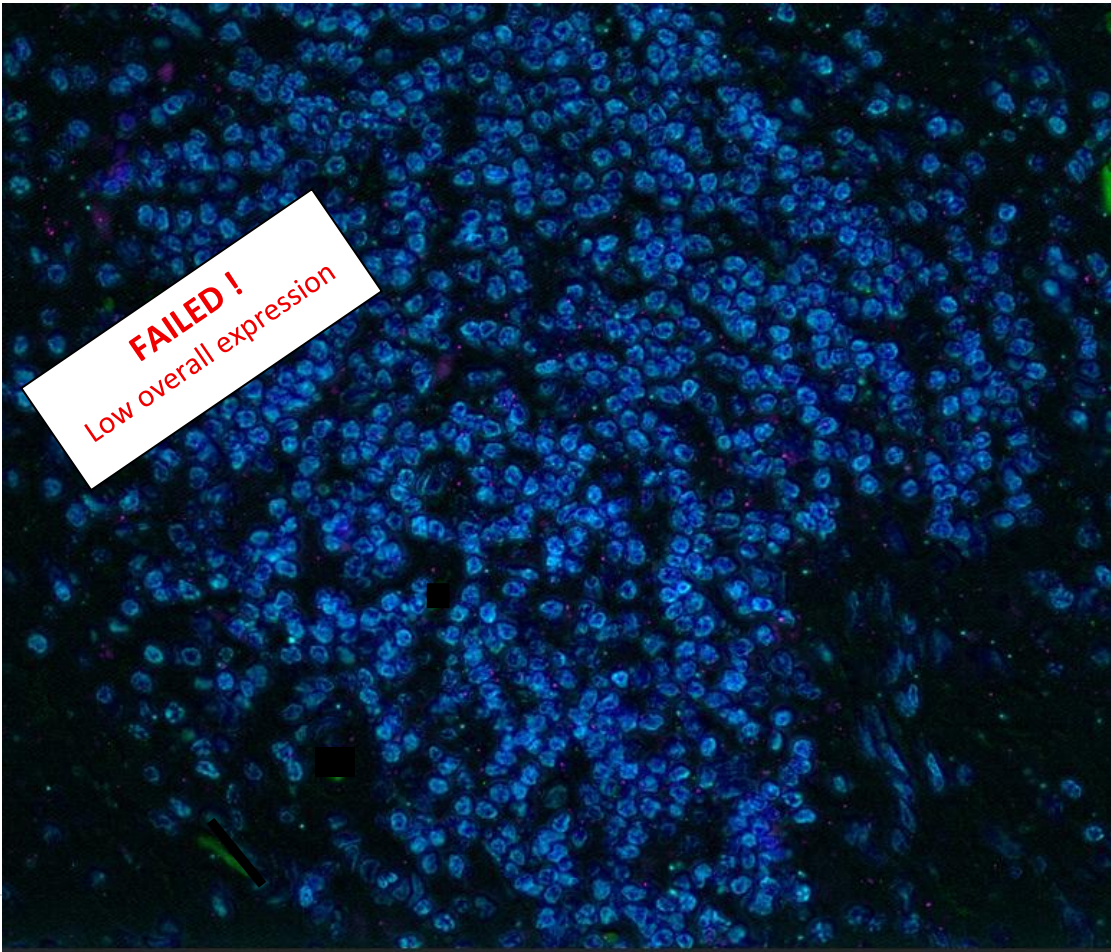
PPIB: expected medium
Expression



TMA Block 1

Composite:

UBC = Green
PPIB = Magenta



RNAscope® results from Block 2

TMA Block 2

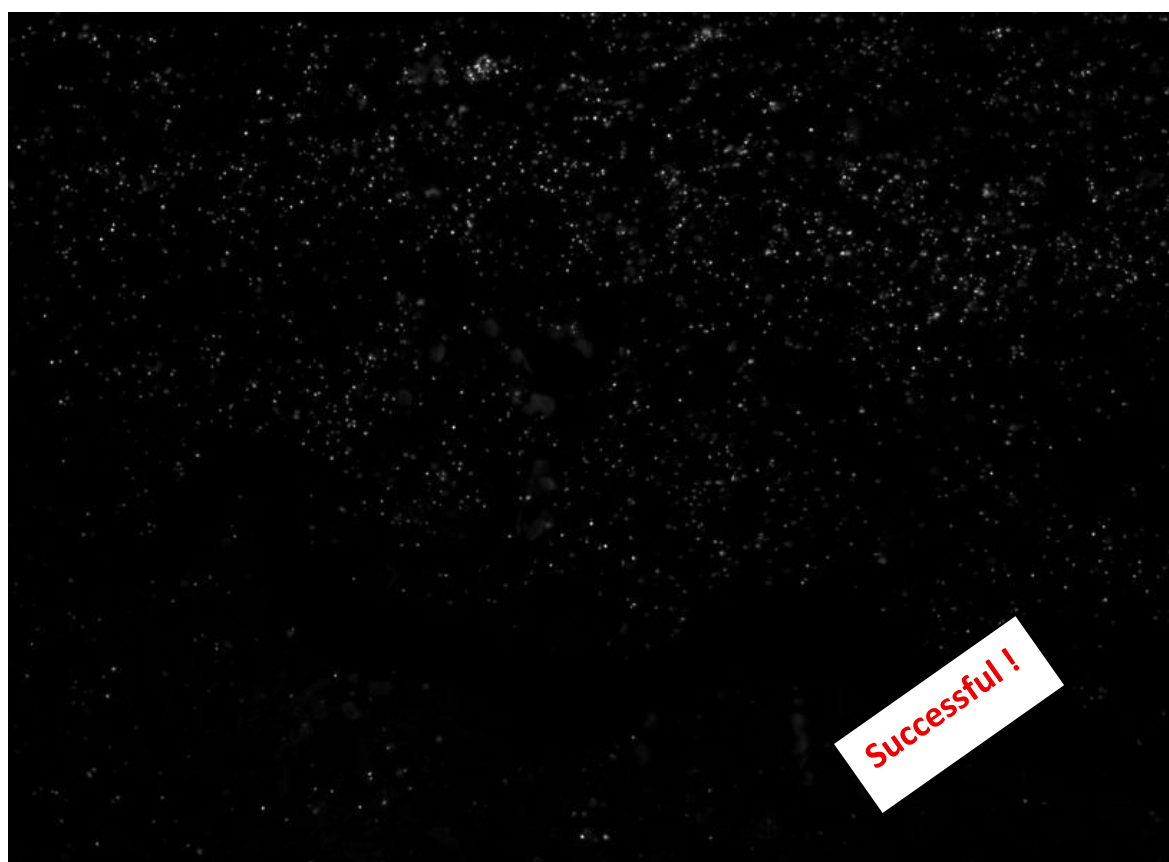
UBC:

med/high expression



TMA Block 2

PPIB: expected medium
expression



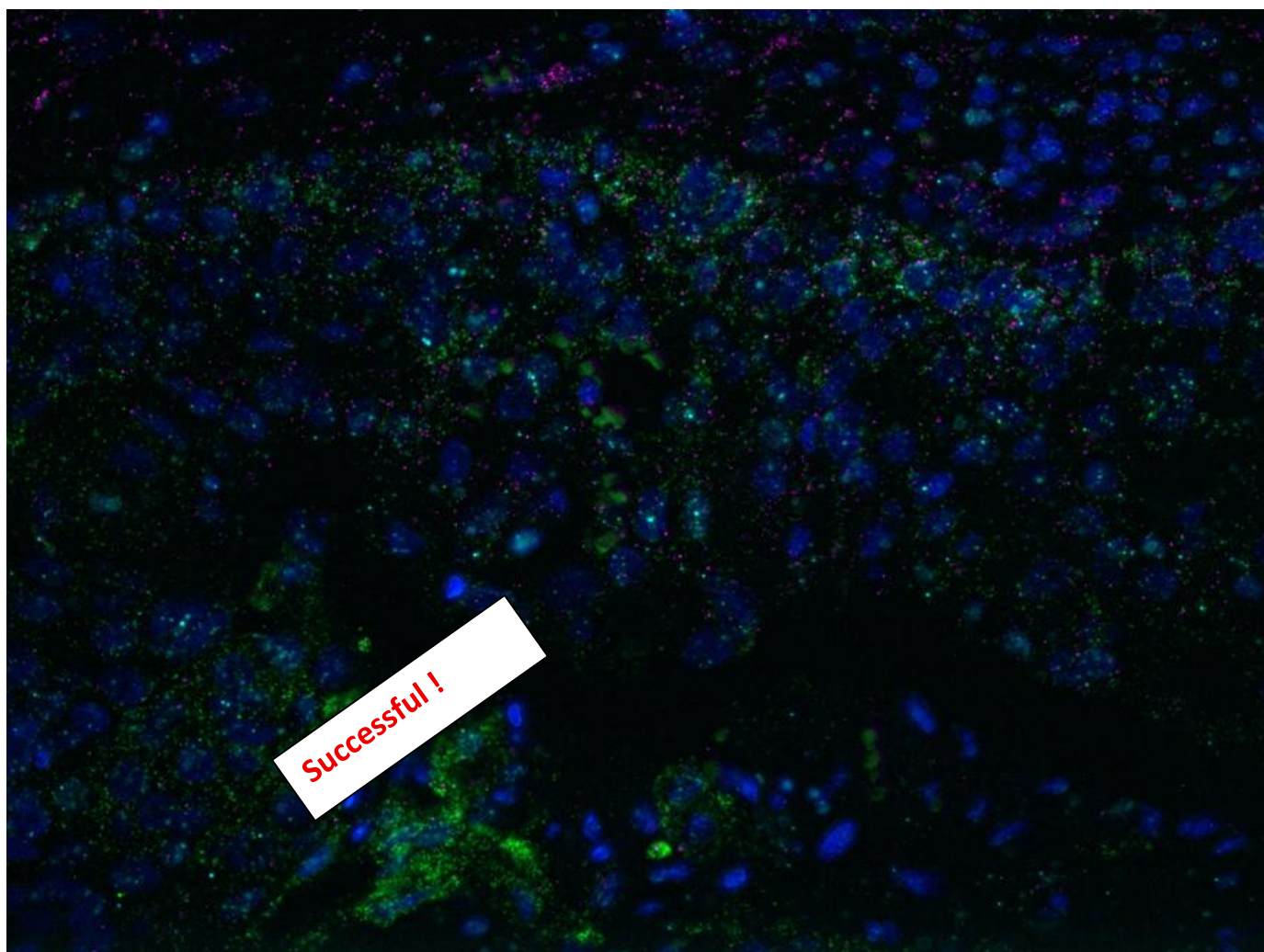
Sheena Kerr, David Beebe, and H&N SPORE 2022

TMA Block 2

Composite:

UBC = green

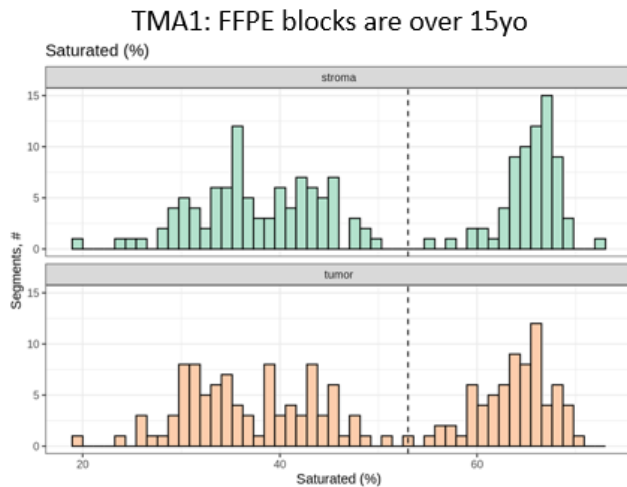
PPIB = magenta



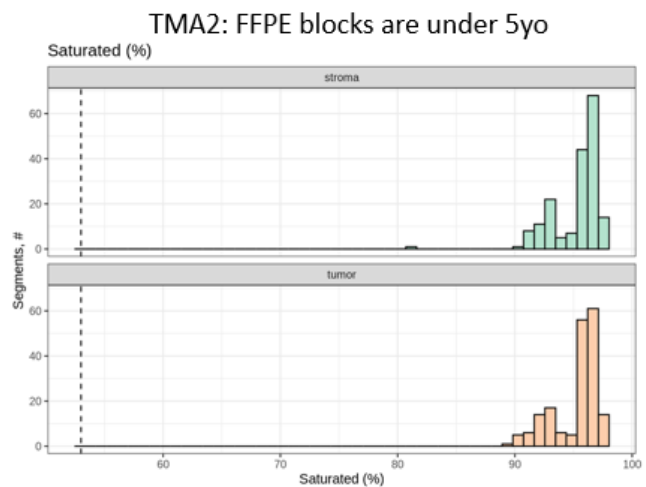
Sheena Kerr, David Beebe, and H&N SPORE 2022

Slides from both TMAs were processed using the same GeoMx DSP collection workflow and subsequently sequenced. The resulting data were compared for gene detection rates and sequencing saturation to determine which block was best suited for GeoMx analysis.

Data Quality: Sequencing saturation



TMA1 -poor saturation across the samples



TMA2 -good saturation across the samples

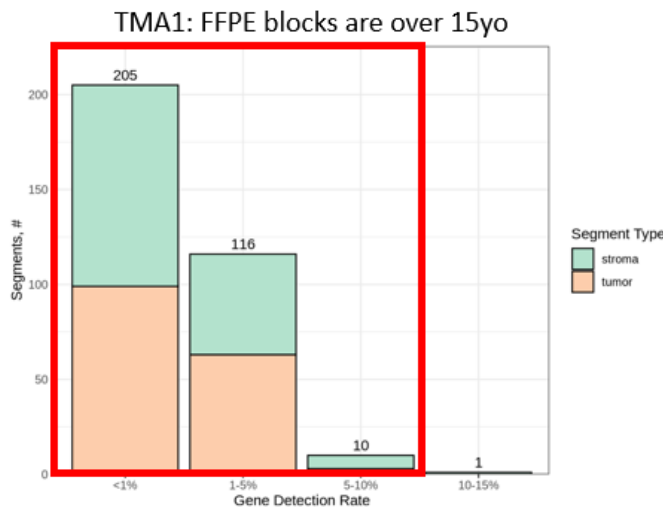
50% saturation = Resequencing necessary

TMA1: Half the reads fall below 50% saturation, 189 showed warning flag vs 143 that passed QC

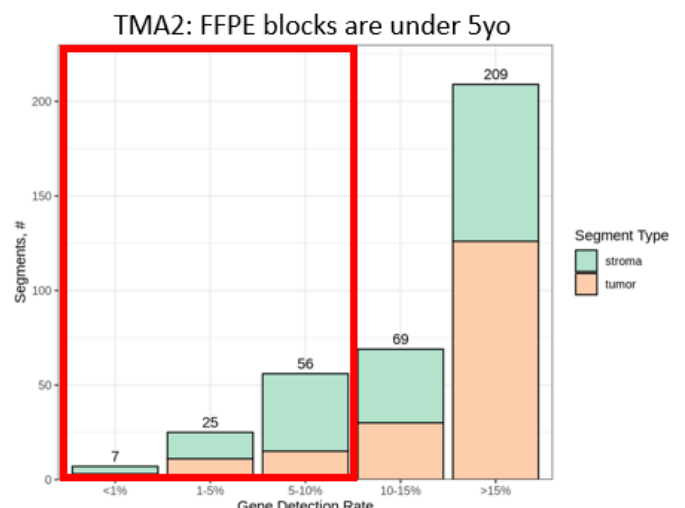
TMA2: 100% passed QC

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Data Quality: Gene detection rates



TMA1: Poor gene detection, little usable data



TMA2: Higher amount of genes fall into the desired categories

*Percentage of genes detected.

- Low gene detection rates suggest low numbers of reads available for that gene.
- Bruker suggests removing reads below 10%

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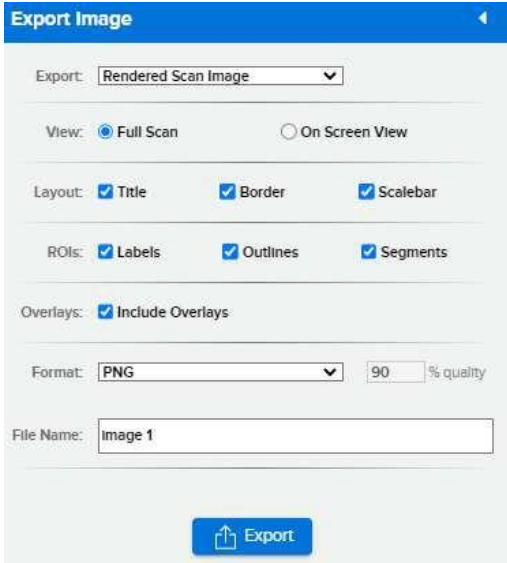
Conclusion:

Based on QC and Sequencing results, Block 2 is the preferred choice for GeoMx, demonstrating higher overall RNA expression and stronger correlation with the expected per-cell expression for each probe in RNAscope® staining. Additionally, Block 2 exhibited greater sequencing saturation, indicating more complete detection of the available gene expression signal.

NGS vs nCounter Readout

NGS Checklist:

- ☒ Set up an account with GEC and complete the required submission form for your samples.
- ☒ Transport your 96-well plate to GEC for NGS processing. *See page 34, “After your Samples Leave TRIP” for additional information.
- ☒ Send Karla the numbers from both sides of your WISCARD to request badge access to WIMR 2214A, where the analysis workstation is located.
- ☒ GEC staff will contact you to confirm the number of reads based on the lab worksheet. You will need to approve the libraries prepared by GEC before they are submitted to the DNA Sequencing Core.
- ☒ When the sequencing data is available, you will receive an email from the DNA Sequencing Core with instructions for accessing your data. The data will be provided as FASTQ files. Be sure to save a copy of your FASTQ files, as they are deleted after 60 days. For internal/UW-Madison users, a copy of the data is also saved to your lab’s Research Drive account. Note: FASTQ files are the raw sequencing data and are not the files used for downstream GeoMx analysis.
- ☒ The Bioinformatics Resource Center (BRC) will convert your sequencing data to DCC files. You will receive an email from the BRC when the DCC files are ready. Copy the DCC files to a 1-2TB external drive and bring the drive with you when analyzing data.
- ☒ Your username and password from Becky will allow you to access your images and the GeoMx software on the Analysis Workstation. You will upload your DCC files to the workstation for analysis. Reserve workstation time through iLab. Log your time on the clipboard when using the workstation. Access is required for each slide to be visible in your account. Keep all saved files within your Lab’s designated folder. Saving files outside your lab’s folder allows other labs access and potentially modify your files.
- ☒ Save your images using the Export Image  icon. Bring an external drive to store your exported images. Do not save images to the desktop. All files saved on the desktop will be deleted.



nCounter readout Checklist:



Send Karla the numbers on both sides of your WISCARD so you have badge access to WIMR 2214A where the analysis workstation is located.



Your username and password from Becky allows you to access your images and the GeoMx software on the Analysis Workstation. You will convert your files to DCC format and upload them to the Analysis Workstation. Reserve your time on the workstation through iLab. Log your time on the clipboard. Access is required for each slide to be visible in your account



Save your images with the Export Image  icon. Bring an external drive to store your images. All images saved on the desktop will be deleted.

After Your Samples Leave TRIP

NGS:

NGS requires you to transport your samples to GEC for library preparation. Once library preparation is complete (~**2 days**), you will receive an email from GEC to confirm the recommended number of sequencing reads and the expected cost.

Once approved, your libraries will be submitted for sequencing on the **DNA Sequencing Core's shared Illumina sequencing system**.

After sequencing is complete (~2 weeks), you will receive an email with instructions for accessing your sequencing data. These files are provided as **FASTQ files**. Be sure to save a copy of these files.

FASTQ files are not the files used for analysis on the TRIP Lab Analysis Workstation.

At the same time, the **Bioinformatics Resource Center (BRC)** will convert the sequencing data into DCC using the Bruker's NGS pipeline (<1 day). You will receive an email when the DCC files are ready. Be sure to save a copy of these files.

The DCC files are the files you will upload to the TRIP Lab Analysis Workstation for GeoMx analysis.

nCounter readout:

With nCounter readout, your samples remain at TRIP Lab throughout the workflow. TRIP staff will contact you when your FASTQ files are available and you can begin converting them to DCC format for analysis on the TRIP Lab Analysis Workstation.

Data Analysis

Data Analysis Resources:

<https://university.brukerspatialbiology.com/page/geomx-digital-spatial-profiler>

Analysis Manuals

GeoMx DSP Data Analysis Manual (v3.1.4 software), MAN-10154-07

<https://university.brukerspatialbiology.com/geomx-dsp-data-analysis-user-manual>

Unlocking the Power of GeoMx DSP: Best Practices for Protein Data Analysis

<https://university.brukerspatialbiology.com/geomx-best-practices-protein-data-analysis>

Quick Guide: GeoMx DSP – NGS Data Analysis, MAN-10134-03

<https://university.brukerspatialbiology.com/quick-guide-geomx-dsp-ngs-data-analysis>

Quick Guide GeoMx DSP - nCounter Data Analysis, MAN-10105-04

<https://university.brukerspatialbiology.com/quick-guide-geomx-dsp-ncounter-data-analysis>

How to create a GeoMx Data Analysis Report

<https://university.brukerspatialbiology.com/how-to-create-a-geomx-data-analysis-report>

Introduction to GeoMx CTA: Normalization

<https://university.brukerspatialbiology.com/introduction-to-geomx-cancer-transcriptome-atlas-normalization>

Training Videos

GeoMx® DSP: Data Analysis for RNA NGS Readout – 60 min

<https://university.brukerspatialbiology.com/geomx-data-analysis-for-ngs-readout>

GeoMx® DSP Data Analysis Workflow Session for RNA NGS Readout – 98 min

<https://university.brukerspatialbiology.com/geomx-academy-ngs-data-analysis-for-rna>

GeoMx® DSP: Data Analysis for Protein NGS Readout – 70 min

<https://university.brukerspatialbiology.com/geomx-dsp-data-analysis-for-protein-ngs-readout>

GeoMx® Data Analysis for nCounter Readout – 91 min

<https://university.brukerspatialbiology.com/geomx-data-analysis-for-ncounter-readout>

How to use the Spatial Decon Custom Script – 6 min

<https://university.brukerspatialbiology.com/how-to-use-the-spatial-decon-custom-script>

Normalization of GeoMx DSP Protein Data using a Custom Script – 16 min

<https://university.brukerspatialbiology.com/normalization-of-geomx-dsp-protein-data-using-a-custom-script>

Identifying and Troubleshooting Sequencing Data Flags – 20 min

<https://university.brukerspatialbiology.com/identifying-and-troubleshooting-sequencing-data-flags>

How to run GeoMx DSP Data Analysis Workflows – 4 min

<https://university.brukerspatialbiology.com/how-to-run-geomx-dsp-data-analysis-workflows>

How to create a GeoMx Data Analysis Report – 6 min

<https://university.brukerspatialbiology.com/how-to-create-a-geomx-data-analysis-report>

