FACT SHEET

The Complexity of the Gastrointestinal Tract:

A MULTILAYERED CHALLENGE FOR DRUG AND BIOMARKER STUDIES

The gastrointestinal (GI) tract is a highly specialized and dynamic system, playing a central role in digestion, absorption, immune response, and microbiome interaction. From the stomach to the small intestine (duodenum, jejunum, ileum) and the large intestine (colon, rectum), each segment presents unique physiological and structural characteristics that influence drug delivery and disease progression.

ANATOMICAL REGIONS OF INTEREST

- **Small Intestine:** Primary site for nutrient absorption; rich in villi and microvilli, with high permeability and metabolic activity.
- **Large Intestine:** Dense microbial population, slower transit, and key site for inflammatory diseases such as ulcerative colitis and Crohn's disease.

SPATIAL IMAGING CAPABILITIES

Understanding drug behavior and biomarker expression requires precise analysis across the four main layers of the intestinal wall:

- **Mucosa:** The innermost layer, including the epithelium, lamina propria, and muscularis mucosae. It is the first barrier to drug absorption and the primary site of immune surveillance.
- **Submucosa:** A connective tissue layer containing blood vessels, lymphatics, and nerves, critical for systemic drug uptake and inflammatory signaling.
- **Muscularis externa:** Responsible for peristalsis; its integrity affects drug transit and local inflammation.
- **Serosa:** The outermost layer, providing structural support and separating the intestine from surrounding tissues.

This complex architecture demands advanced analytical tools to map drug penetration and track biomarker expression with spatial precision, making spatial bioanalysis and spatial biology indispensable for drug development programs dedicated to these organs.

SPATIAL BIOANALYSIS: MAPPING DRUG PENETRATION ACROSS INTESTINAL LAYERS

Spatial bioanalysis enables direct visualization and quantification of drug distribution within intestinal tissues, layer by layer: From the mucosa to submucosa, muscularis, and serosa, each compartment can be independently analyzed to assess penetration, retention, and diffusion of small molecules or complex formulations.

To fully understand drug behavior in the gastrointestinal tract, Aliri leverages two complementary tissue preparation methods in rodent models:

- Transverse sections allow for high-resolution analysis of drug penetration from the lumen to the muscular layers, capturing the vertical distribution across the intestinal wall.
- Swiss roll preparations offer a panoramic view of the intestine from proximal to distal regions, enabling longitudinal mapping of drug exposure along the entire tract.

These two dimensions, depth (lumen to muscle) and length (proximal to distal), provide a comprehensive view of the tissue complexity

The quantitative mass spectrometry imaging (qMSI) is then used to generate label-free distributions of small molecule drugs directly within frozen tissue sections. This technique preserves tissue architecture and avoids the need for chemical labeling or antibodies, offering a precise and unbiased visualization of drug localization at the histological level **(Figure 1).**

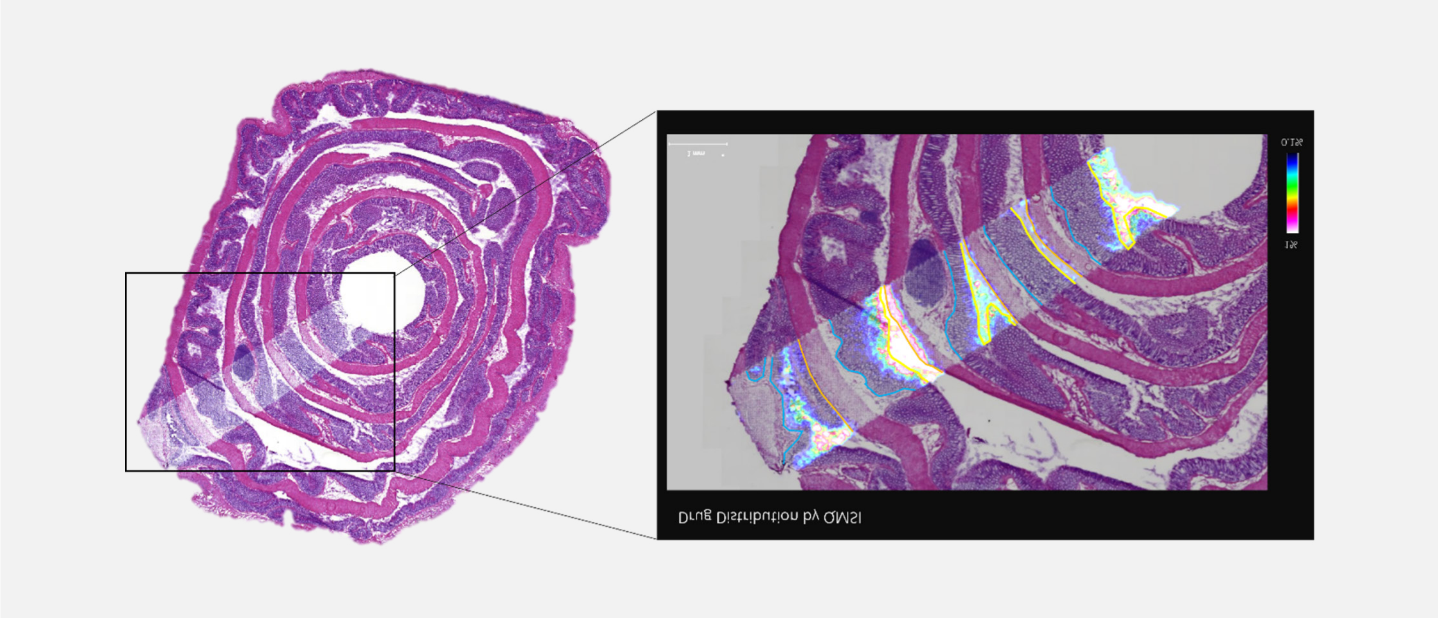

FIGURE 1. H&E staining picture obtained on a mouse swiss role GI preparation overlaid with a small drug distribution generated by Mass Spectrometry Imaging in the different layers of the intestines

From the high-resolution images generated via quantitative mass spectrometry imaging (qMSI), Aliri extracts penetration profiles that quantify drug concentration across the different layers of intestinal tissue. These profiles provide a precise, layer-by-layer view of how an active compound diffuses from the lumen through the mucosa, submucosa, and muscularis **(Figure 2)**.

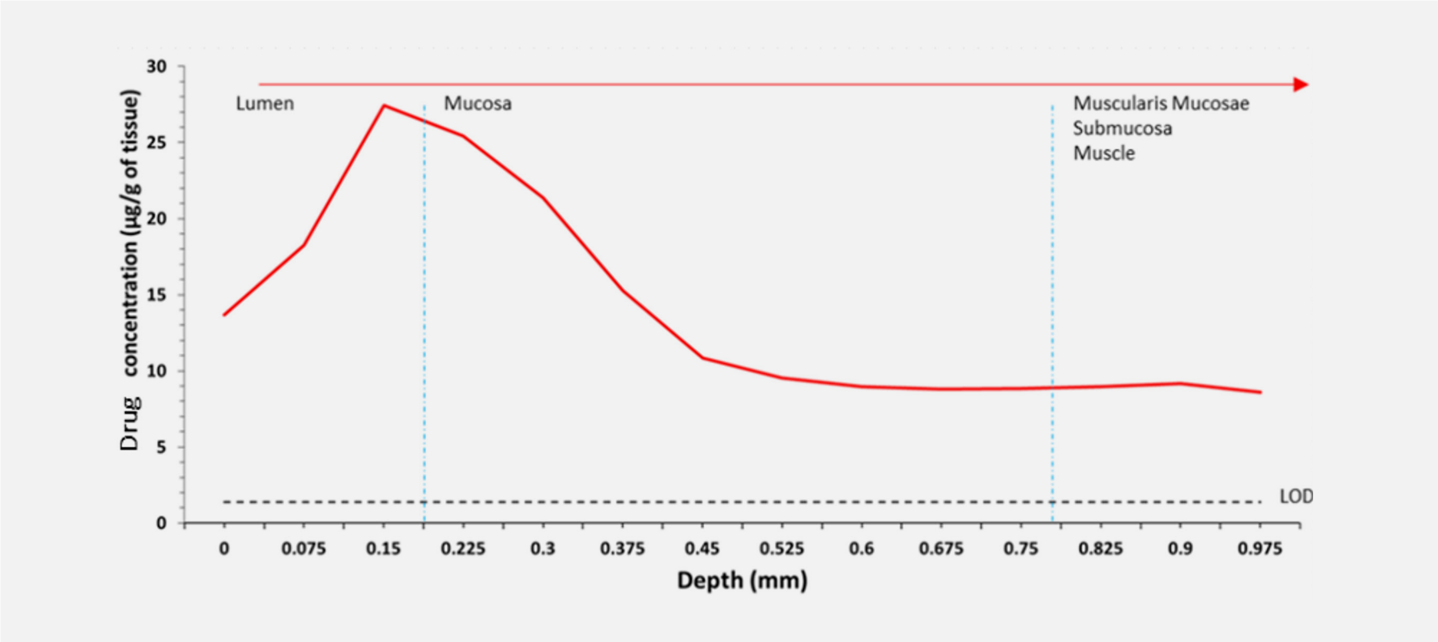

FIGURE 2. Illustration of a drug penetration profile in the intestine layers generated from the Mass Spectrometry Imagining results

This analytical capability enables:

- Fine-grained assessment of drug small molecule behavior at the tissue level.
- Comparative analysis across different formulations, time points, or experimental conditions.
- Identification of barriers to penetration or zones of accumulation, which can inform formulation optimization and therapeutic strategy.

This approach helps correlate drug presence with therapeutic targets or pathological zones, optimizing oral or local drug delivery strategies.

SPATIAL BIOLOGY: TRACKING AND IDENTIFYING INFLAMMATORY BIOMARKERS

While spatial bioanalysis defines where a therapeutic compound distributes, spatial biology reveals what the tissue does in response uncovering how cell types, signaling pathways, and microenvironmental structures organize across the intestinal wall.

In the gastrointestinal tract, inflammation and repair are highly compartmentalized processes. Each layer of the intestinal wall, epithelium, lamina propria, and submucosa contains distinct immune and stromal ecosystems that interact dynamically with both the microbiota and administered drugs. Mapping these interactions in situ is key to understanding why pathology and treatment responses vary across intestinal regions.

At Aliri, spatial transcriptomics and multiplex spatial proteomics are applied together to profile these immune niches within intact intestinal architecture. By preserving morphology and quantifying high-plex molecular signals, these technologies enable visualization of:

- Immune activation microdomains where macrophages (CD68$^+$), B cells (CD20$^+$), and epithelial cells (PanCK$^+$) form coordinated clusters within the mucosa and lamina propria **(Figure 3)**.
- Cytokine and chemokine gradients trace the spread of inflammation or epithelial barrier stress **(Figure 4)**.
- Transitions from regenerative mucosa to fibrotic muscular layers show how inflammation reshapes the intestinal wall.
- Epithelial–immune interfaces, where altered PanCK$^+$ structures coincide with macrophage infiltration or lymphoid follicle formation potential early predictors of chronic disease or therapeutic non-response.

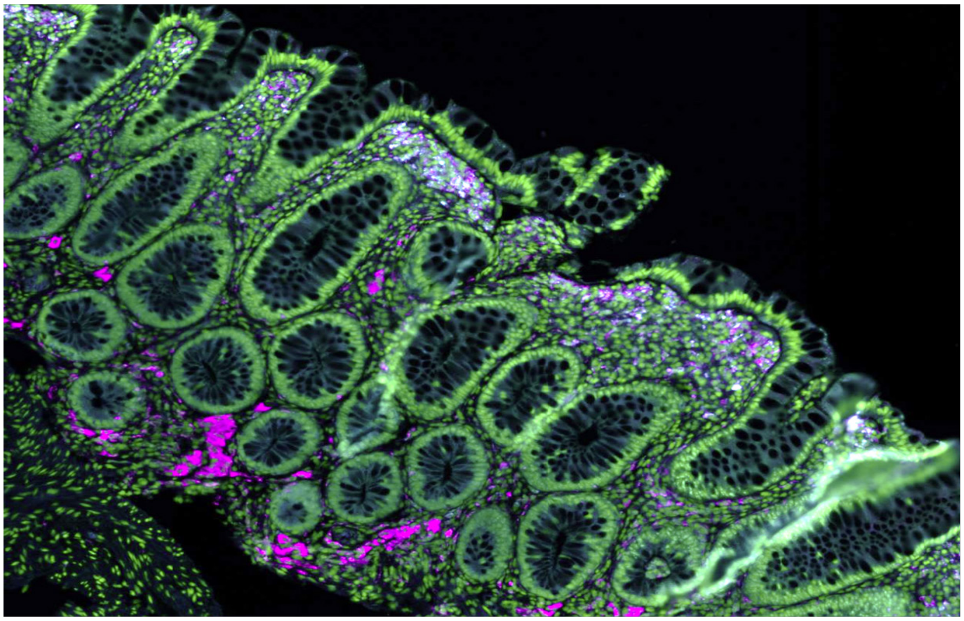

FIGURE 3. Spatial mapping of immune activation within the intestinal mucosa. Multiplex immunofluorescence image showing epithelial (PanCK, green), B cell (CD20, cyan), and macrophage (CD68, magenta) markers in a mouse intestinal section. The distribution highlights distinct immune microdomains along the mucosal folds, with macrophage infiltration and lymphoid clusters at the epithelial–stromal interface illustrating how spatial biology resolves layer-specific inflammatory organization within the gastrointestinal tract.

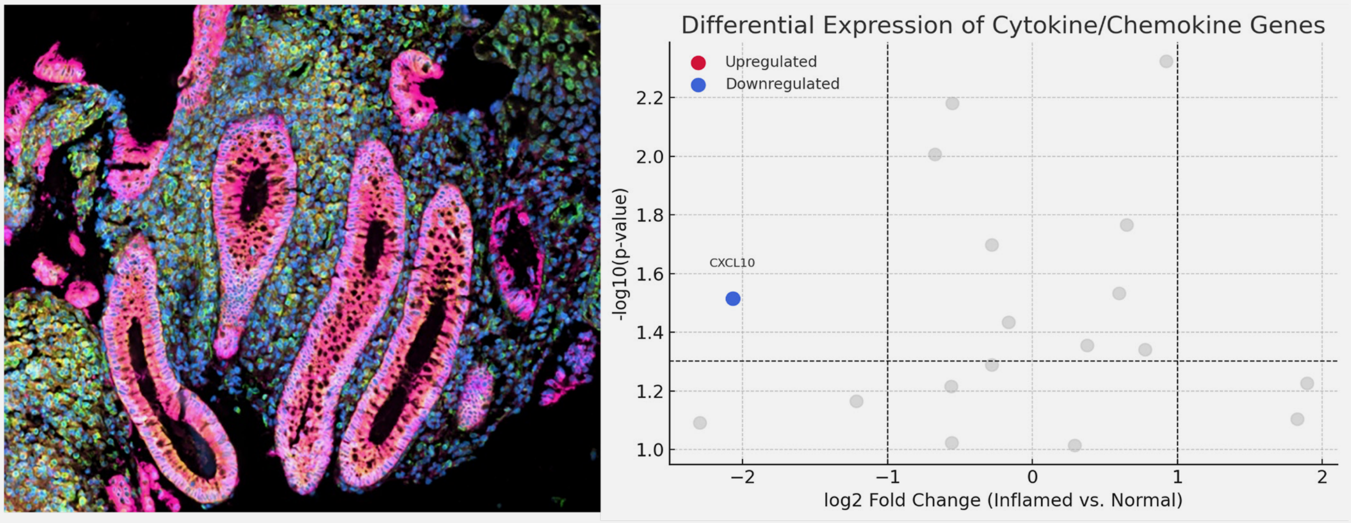

FIGURE 4. Visium HD spatial transcriptomics of intestinal tissue reveals localized epithelial activation and immune infiltration (left). Gene-level expression highlights differential expressions of cytokine and chemokine transcripts between inflamed and quiescent zones, illustrating how spatial gradients of inflammation align with epithelial barrier stress.

Using integrated platforms such as GeoMx DSP, Visium HD, and Imaging Mass Cytometry (IMC), Aliri reconstructs these spatial landscapes at multiple scales from broad transcriptomic gradients to single-cell immune interactions linking molecular pathways to precise tissue locations.

This spatially resolved view enables correlation between drug exposure maps (from qMSI) and biological response maps (from spatial biology). For example, a molecule may penetrate the submucosa efficiently but leave residual inflammatory signaling at the epithelial front, revealing incomplete pharmacodynamic engagement or limited immune modulation.

Through this integrative framework, Aliri provides:

- Layer-specific biomarker signatures that explain differential efficacy across intestinal compartments.
- Quantification of cellular crosstalk driving persistent inflammation or mucosal healing.
- Spatial biomarkers of response or resistance suitable for translational and clinical studies.
- A unified readout connecting drug localization, target engagement, and immune remodeling turning tissue architecture into actionable biological insight.