Multiplex data utilizing a single-step staining and imaging workflow for the investigation of multiple sample types

Selena Larkin[1], Tad George[1], Eric Kaldjian[1], [1]RareCyte, Inc. Seattle, WA USA

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Background

Tissue consists of heterogenous cell types with diverse functions and states, where spatial organization can inform – and impact - patient health status. Understanding the tumor microenvironment has proven particularly important for oncology and immune oncology studies, with fast-turnaround insights into immune response to tumors being critical for biomarker discovery, validation and the ultimate development of cancer therapeutics and diagnostic tests.

Resolving tissue complexity across a statistically relevant number of patient biopsies at a cellular level has historically been challenged by image resolution, the number of targets that can be simultaneously assessed and sample throughput. These barriers have recently been broken with generation of high plex, whole-slide immunofluorescence (IF) imaging data in a single cycle using the Orion™ platform to deliver multiplex biomarker quantitation in just hours.

Described here are some specific examples of how the established Orion one-step stain-and-scan approach has been used to rapidly generate high-quality, subcellular quantitative IF data for multiple biomarkers across whole tissue sections. Further, we describe how this, combined with traditional H&E on the same platform, can be used to provide accretive insights across the same cells and tissue microenvironments from the same biopsy sample.

Methods

A range of whole-slide healthy and diseased tissues were stained and scanned to quantitate a variety of 15- to 18-plex protein biomarker panels and generate same-section H&E images. In each case, 20 immunofluorescence channels were simultaneously collected and quantitated using the Orion spatial biology platform from RareCyte Inc.. This provided a measure of the expression level for each target, nuclear markers, and each tissues' inherent spatial autofluorescence; the latter which was specifically isolated to provide enhanced information about tissue morphology. Following immunofluorescence imaging, the slide sections were stripped of IF reagents, then subjected to H&E staining. Brightfield microscopy was performed using the same Orion spatial biology platform and the images aligned for same-section multimodal analysis.

The same-slide, multimodal imaging protocol is rapid and straightforward to execute using standard IF and H&E histology protocols:

- Mount sections on standard glass microscope slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with a panel of ArgoFluor™ conjugated antibodies & nuclear stain
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion in IF mode
- De-coverslip slides in aqueous solution
- H&E stain of the same slides (no serial sections required)
- Image slides at 20X magnification using Orion in brightfield mode

The multiplex IF and H&E images are collected as open-format ome.TIFF files which may be interpreted independently or overlaid to provide enhanced insights from combined same-section / same cell biomarker and morphology information.

Results

Orion instruments and reagents are used by RareCyte customers to apply over 50 panels across more than 20 unique tissue types for their studies, including whole tissue sections and tissue microarrays from different species. With an ability to rapidly and simultaneously quantify biomarkers across a broad dynamic range of expression, Orion applications encompass a broad range of therapeutic indications including Oncology, Autoimmunity and Infectious Diseases.

Examples shown here (figures 1-8) demonstrate a breadth of applications enabled by the Orion approach. The one-round staining and quantitative IF imaging process preserves sample integrity such that H&E can be carried out on the same tissue section and the images directly overlaid for same-cell spatial analysis.

The ability to align H&E imaging with same-cell quantitative spatial data allows traditional pathology analysis to be augmented by cellular phenotyping and molecular biomarker quantitation to reveal important biological insights across a variety of cellular microenvironments. With a scan speed of around 70 minutes per square centimeter for 20 IF channels, and subcellular resolution, the Orion approach has proven valuable for biomarker validation, comprehensive phenotypic profiling and characterization of tissue architecture, tumor heterogeneity, and the immune response across large studies of tens to hundreds of patient samples.

Conclusions

Spatial analysis of the tumor microenvironment has been established as key to validating new spatial biomarkers, testing therapeutic targets, and monitoring clinical studies. With unprecedented throughput for targeted biomarker quantitation across whole-slide patient cohorts, and with the added benefit of onboard, same-section H&E, the Orion platform delivers on the need for an in-depth understanding of each patient sample and overall improved spatial insights.

Summary of Orion Workflow

PREPARE
Standard microscope slide and tissue section

STAIN
Single staining process, standard IF protocols

IMAGE
Highly multiplexed imaging in one scan

ANALYZE
OME-TIFF output for viewing and quantification

Sample Information

Each of these tissue sections were stained with a multiplex immunofluorescence (IF) panel in one staining round followed by whole slide imaging with Orion in one imaging round. Tissue autofluorescence was simultaneously imaged and isolated as discrete fluorescence channels at subcellular resolution. H&E imaging of the same sections was carried out after the IF multiplex scan.

ORION BENCHTOP FOOTPRINT

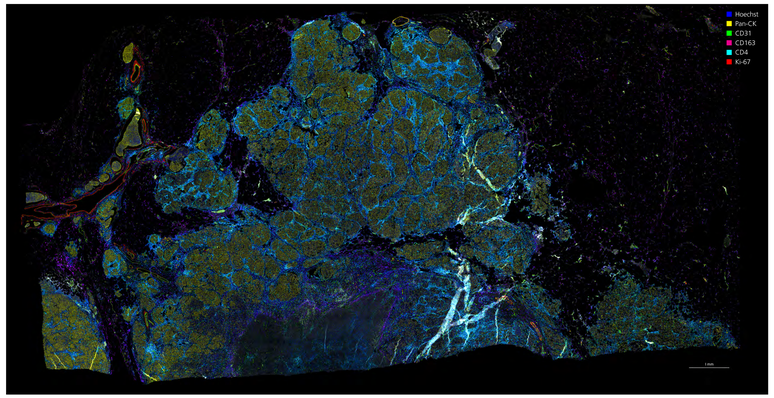


Fig 1. 15-plex whole slide image of breast ductal adenocarcinoma

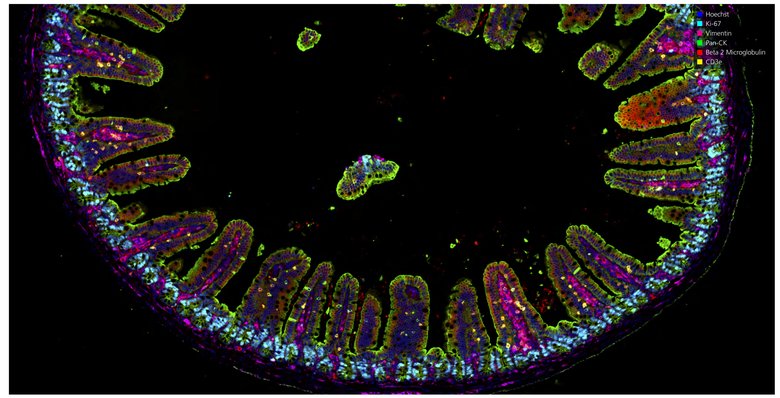
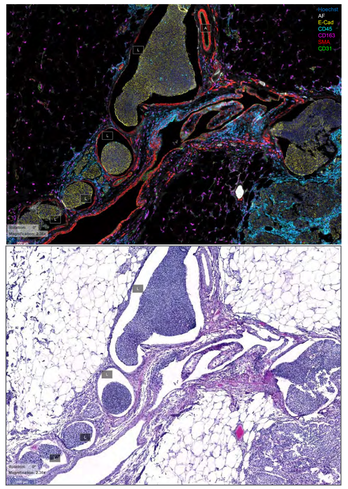
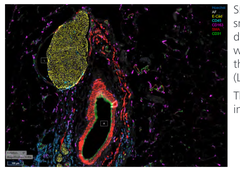


Fig 2. 15-plex whole slide image of mouse ileum

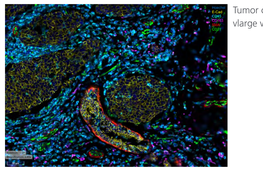


MARKER LIST
Hoechst
CD31
Ki-67
CD68
CD163
CD20
CD4
CD8a
PD-L1
FOXP3
E-cadherin
PD-1
CD45
Pan-CK
SMA

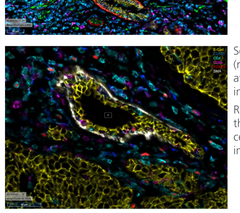


SMA (red) highlights smooth muscle & differentiates thick-walled small artery (A) vs thinwalled lymphatic vessel (L).

The lymphatic vessel is invaded by tumor (yellow).



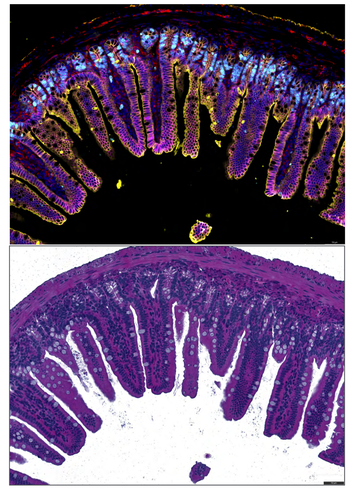
Tumor cells present within vlarge vessel (V).



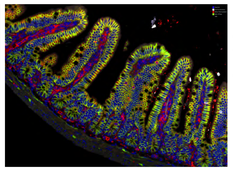
Several regulatory T cells (red nuclei) are present at the site of vascular invasion (V)

Regulatory T cells facilitate the spread of the tumor cells by suppressing the immune system.

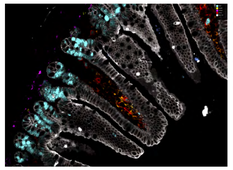
Extensive lymphatic invasion by tumor (L) The lymphatic vessels have a very thin wall in comparison to small arteries (A). Lymphocytes (cyan) and macrophages (magenta) surround the lymphatic vessels.



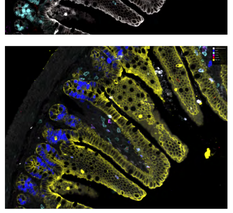
MARKER LIST
Hoechst
Beta 2
CD3e
CD8a
CD11c
CD45R
CD163
E-Cadherin
F4/80
FOXP3
KI67
NaK-ATPase
Pan-CK
PD-L1
Vimentin



Epithelial cells express Beta 2 Microglobulin and NaK-ATPase (green/orange/yellow), while cells in the lamina propria predominantly lack NaK-ATPase (red)



CD11c+ DC/macrophages (red) are largely confined to the lamina propria within the villi, while CD163+ macrophages (cyan) are confined to the lamina propria surround the proliferating crypt cells.



T cell subsets:

- CTL: CD3+CD8+ (lavender)
- T helper cells: CD3+CD8- (cyan)
- T reg cells: CD3+FOXP3+ (cyan with red nuclei)

Note epithelial cell at the top right with cytoplasmic FOXP3 (red).

Normal epithelium (yellow/magenta) with numerous proliferating crypt cells (cyan). Vimentin (red) expressed by cells in lamina propria (red) and submucosa.

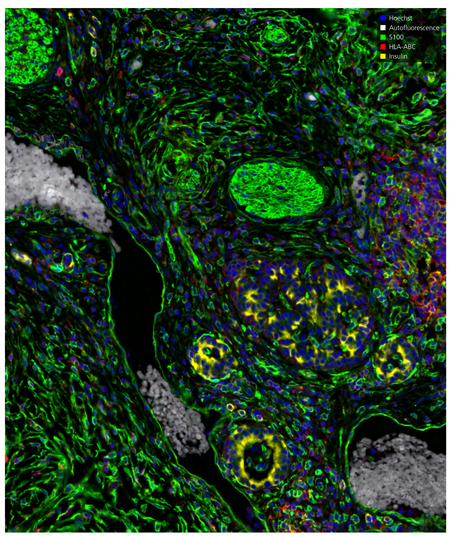


Fig 3. Tissue: Pancreas (insulin in yellow)

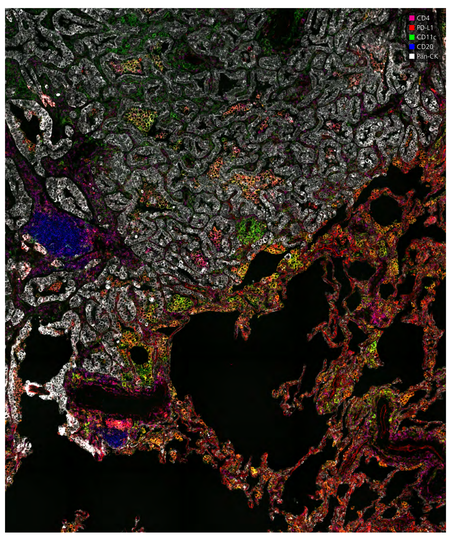


Fig 4. Tissue: Non-Small Cell Lung Cancer

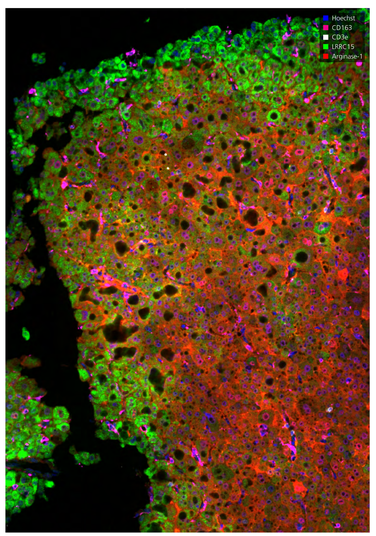


Fig 5. Tissue: Liver (Hepatocelluar Carcinoma)

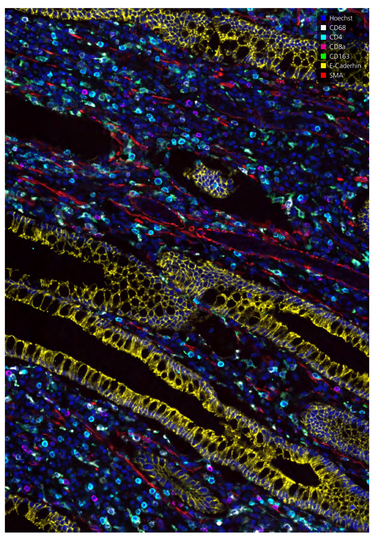


Fig 6. Tissue: Gastrointestinal (Inflammatory Bowel Disease)

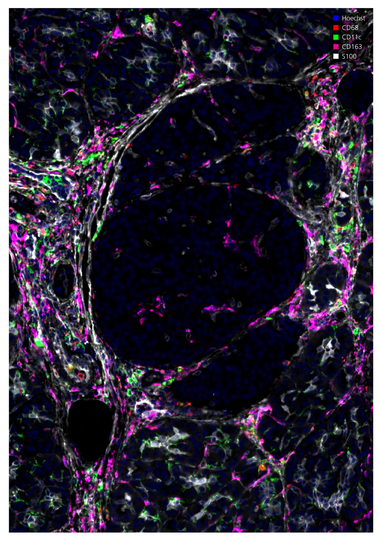


Fig 7. Tissue: Pancreas (Type 1 Diabetes)

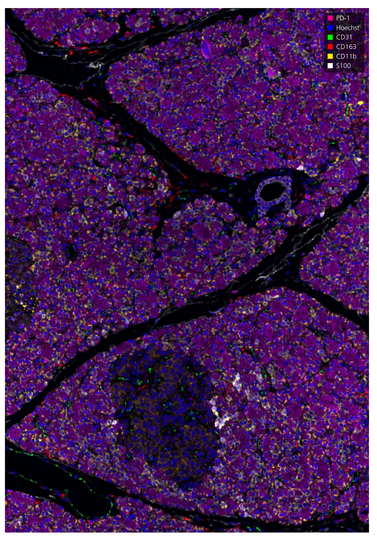


Fig 8. Tissue: Pancreas