Improved Spatial Biology Analysis of the Tumor Microenvironment with the Next Generation of the MultiOmyx™ Platform

Michael Lazare, Harry Nunns, Fatemeh Jelvhei Moghaddam, Xiaowei Jin, Courtney Todorov, Eric Leones, Marianne Thio, Flora Sahafi, Erinn Parnell, Qingyan Au

NeoGenomics Laboratories

AACR 2024 #5487

Background: Multiplexed Immunofluorescence (IF) is a powerful tool for spatially characterizing and phenotyping cells within the tumor microenvironment. MultiOmyx has been one of the leading platforms for generating multiplexed immunofluorescence data to support translational and clinical research for more than 10 years. However, MultiOmyx and other similar platforms are often hampered by a limited imaging area due to either restricted staining areas or being cost prohibitive due to excessive imaging times. Here, we demonstrate the capabilities of an improved MultiOmyx platform which can be used to generate whole-tissue data from the iterative MultiOmyx multiplexing process using the CyteFinder® II (CFII) microscope and a customized software package developed by RareCyte, Inc. The new NeoLYTX image analysis pipeline in conjunction with the whole-tissue image output allows for improved interaction with pathologists, better histological context, and unbiased spatial analysis.

Methods: In this study we utilized a multiplexing panel to generate immune profiles for non-Small cell lung cancer. We compared the outputs of the CFII platform to the current MultiOmyx imager - the IN Cell 2200 (IC) - for each of the markers in the panel by imaging the slides on both the IC and CFII for each imaging round. The updated NeoLYTX image analysis pipeline was used to classify cells as positive or negative for each biomarker and the results were compared between the two platforms in order to assess concordance within matched regions of interest. We also compared the CFII outputs to traditional immunohistochemistry (IHC).

Results: The Pearson's correlation values for each of the biomarkers were assessed for concordance between the IC and CFII platforms. The Pearson's correlation values were all > 0.9. Each of the 6 biomarkers assessed yielded comparable staining on the MultiOmyx platform as compared to IHC.

MultiOmyx Imaging Workflows

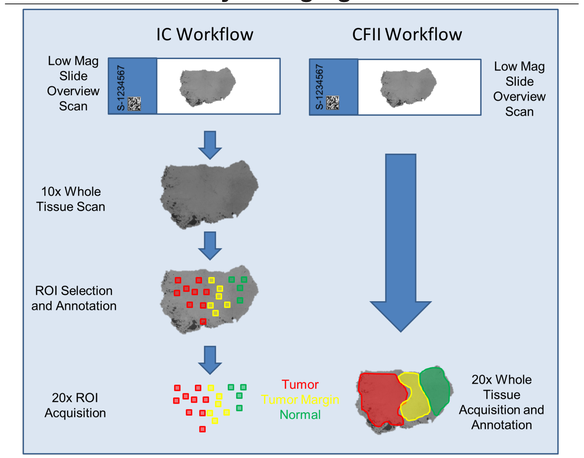

Figure 1: Comparison of IC and CFII Imaging Workflows for MultiOmyx. The CFII workflows allows for whole tissue acquisition and greater precision for annotations.

IHC-IF Concordance

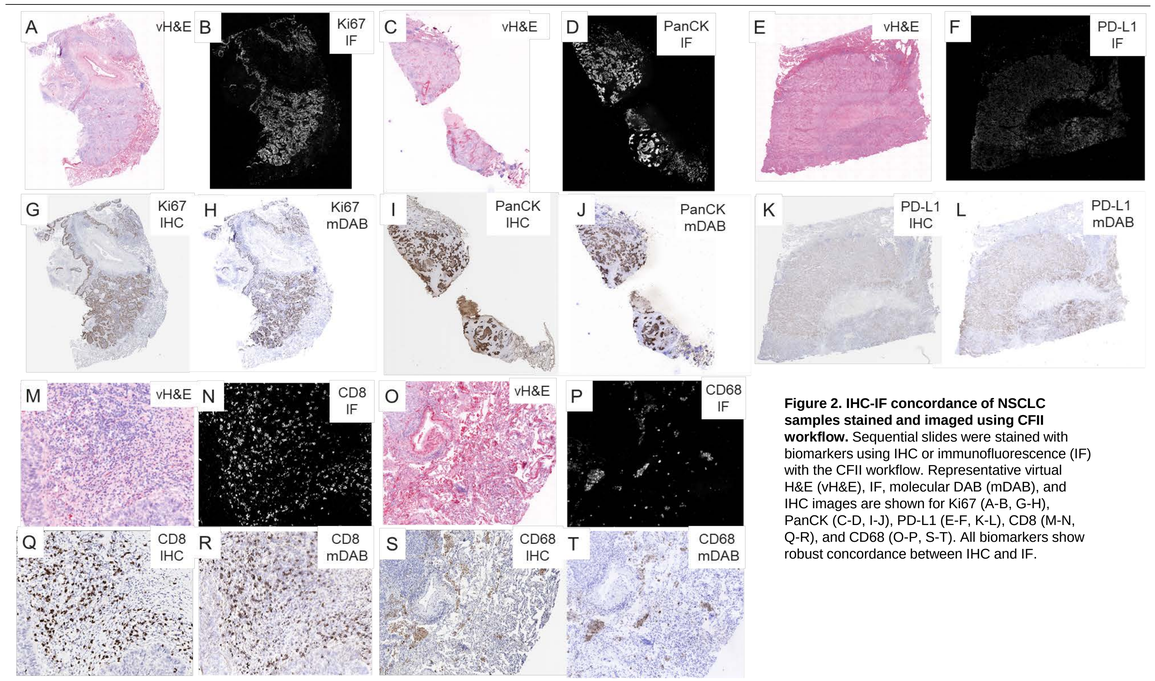

Figure 2. IHC-IF concordance of NSCLC samples stained and imaged using CFII workflow. Sequential slides were stained with biomarkers using IHC or immunofluorescence (IF) with the CFII workflow. Representative virtual H&E (vH&E), IF, molecular DAB (mDAB), and IHC images are shown for Ki67 (A-B, G-H), PanCK (C-D, I-J), PD-L1 (E-F, K-L), CD8 (M-N, Q-R), and CD68 (O-P, S-T). All biomarkers show robust concordance between IHC and IF.

Characterization of TME in NCSLC samples Using Whole Slide Scan Workflow

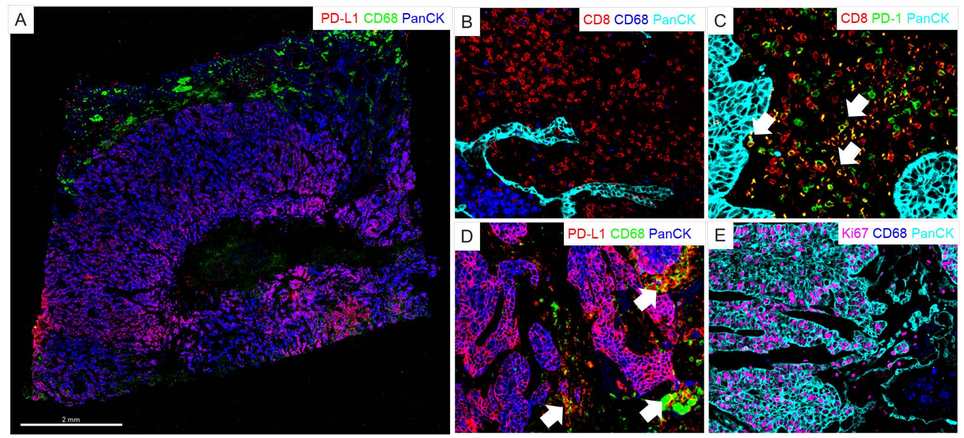

Figure 3. Biomarker co-expression and characterization in the tumor microenvironment of NSCLC samples. Representative color overlay images acquired using CFII in NSCLC slides (A-E). (A) PD-L1+ tumor appears as magenta. (B) Macrophages in blue, T cytotoxic cells in red, and tumor in cyan. (C) PD-1+ T cytotoxic cells appear as yellow (white arrows). (D) PD-L1+ macrophages appear as green (white arrows) and PD-L1+ tumor in magenta. (E) Proliferating tumor cells have pink nuclear stain and cyan membrane stain.

Highly Concordant Results between the Workflows

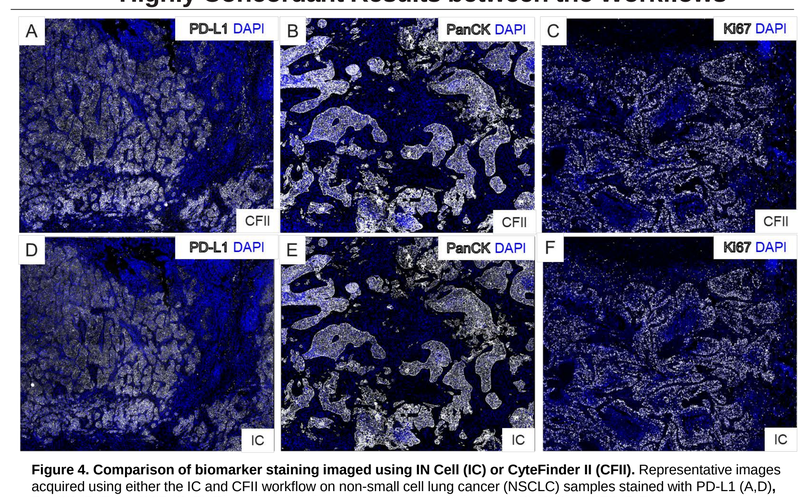

Figure 4. Comparison of biomarker staining imaged using IN Cell (IC) or CyteFinder II (CFII). Representative images acquired using either the IC and CFII workflow on non-small cell lung cancer (NSCLC) samples stained with PD-L1 (A,D), PanCK (B, E), or Ki67 (C, F). Biomarker stain images are comparable between IC (A-C) or CFII (D-E) workflows.

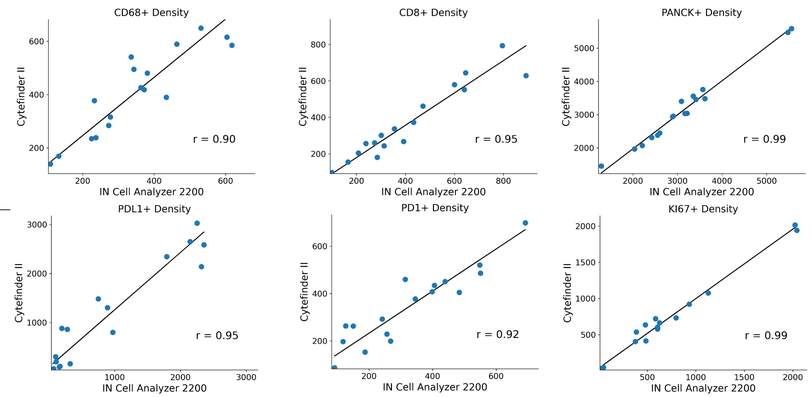

Figure 5. Concordance of cell classifications results obtained using IN Cell (IC) vs. CyteFinder II (CFII). Cell classification results for CD68, CD8, PanCK, PDL-1, PD1, and Ki67 were analyzed and Pearson's correlation values (r) were measured to be between 0.9 and 0.99, indicating strong concordance between the two workflows.

Key Take-Aways

- We demonstrated the capabilities of an improved MultiOmyx platform which can be used to generate whole-tissue data from the iterative MultiOmyx multiplexing process.
- The new platform maintains equivalence to both traditional IHC as well as the original MultiOmyx platform while offering greater histological context and unbiased spatial analysis due to the new whole-tissue acquisition workflow.

Michael.Lazare@neogenomics.com