3D spatial quantification of lymphocyte infiltration and collagen features in the tumor microenvironment using a novel assay: 3D I/O Pro™

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Background
The composition of the tumor microenvironment (TME) is a major determinant of response to therapy in many solid tumors. To-date, characterization of the TME has been based on limited analysis of thin tissue sections. Here we demonstrate the utility of an end-to-end 3D spatial biology workflow, the 3D I/O Pro™, based on whole tissue imaging, to identify and quantify tumor cells, lymphocytes, and collagen features in human FFPE tissue samples.

Methods
Five human colorectal cancer FFPE blocks were deparaffinized, stained with nuclear (TO-PRO-3) and general protein (eosin) dyes, and cleared using a modified iDISCO protocol. Entire samples were imaged at 2 µm/pixel resolution with a hybrid open-top light-sheet microscope, the 3D*i*™. Regions of interest (ROIs) with a volume of 0.5 mm^3 were re-imaged at 0.33 µm/pixel. Cell nuclei and collagen were segmented and 3D spatial relationships between tumor cells, lymphocytes, and collagen fibers were quantified. Analyses were performed within select ROIs using 3D*ai*™ tools including U-Net, CytoMAP, CT-FIRE, CurveAlign, and custom Python scripts.

Results
The ratio of stromal to tumor parenchymal lymphocytes (lymphocyte infiltration ratio) varied from 1.4 up to 9.1 in 3D volumes and 1 to 25 in 2D virtual sections taken throughout all 5 CRC samples. The ratio of the collagen fibers within 100 µm of the tumor border-oriented perpendicular to the tumor-stromal boundary compared to those oriented parallel to the tumor-stromal boundary was 0.37 in a proof-of-concept evaluation of a 2D virtual section with a lymphocyte infiltration ratio of 12. Qualitatively, areas with perpendicular collagen had more lymphocyte infiltration into the tumor parenchyma than areas with parallel collagen orientation.

Conclusions
We demonstrated that the 3D I/O Pro™ pipeline can quantify lymphocyte density in tumor parenchyma and stroma and analyze collagen features, including orientation, within 3D ROIs in the TME. This workflow allows us to characterize tumors based on many complex spatial relationships and could have broad applicability in research and development of novel cancer therapies that target tumor fibrosis or other features of the TME. In the future, we plan to correlate features from the 3D TME with response to immunotherapy and use these features to refine histologic definitions of immune spatial phenotypes.