Quantitative assessment of the tumor microenvironment using the Aurora™ 3D Spatial Biology Platform

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Background

Analysis of the three-dimensional (3D) landscape of the tumor microenvironment (TME) has the potential to transform diagnosis and drug development. Current 2D histology techniques introduce sampling error, interobserver variability, and fail to capture the biology contained within the entire tissue. To-date, practical barriers have hampered widespread adoption of 3D imaging and quantification. We have developed a suite of technologies, the Alpenglow Biosciences Aurora™ 3D Spatial Biology platform, to stain, chemically clarify, image, and analyze entire, intact tissue samples, addressing these barriers. Herein, we present these methods for comprehensive 3D assessment of cancer tissues.

Methods

Five de-identified human colorectal cancer (CRC) FFPE blocks were deparaffinized and stained with nuclear (TO-PRO-3) and general protein (eosin) fluorescent dyes, and optically cleared using a modified iDISCO protocol. Samples with a volume of 200-500 mm^3 were imaged at 2 μm/pixel resolution with a hybrid open-top light-sheet microscope, the 3D***i*** ™. Smaller regions of interest (ROIs), 0.5 mm^3, were re-imaged at higher resolution, 0.33 μm/pixel. Nuclear segmentation and 3D spatial analysis were performed using our 3D***ai*** ™ tools consisting of a U-Net, CytoMAP, and custom python scripts capable of scaling to the 10-20 billion pixel image datasets the 3D***i*** ™ can produce. Tumor infiltrating lymphocytes (TILs) were identified and classified spatially into tumor associated stromal or parenchymal. The variation in stromal to parenchymal TILs was then quantified in 3D and compared to virtual 2D sections of the 3D images.

Results

All tissue samples were imaged in 3D, and computational approaches were successfully scaled to 3D and used to segment and classify tumor stroma, parenchyma, and TILs within the high-resolution ROIs. Across the 5 samples analyzed here, 641,800 TILs were identified and spatially characterized. We found significant variation in the ratio of stromal to parenchymal TILs across the 3D volumes (**Figure 1)** and showed as many as 85 traditional 4 μm slices would be needed to accurately quantify the spatial organization of the TILs within some samples.

Conclusions

We successfully implemented 3D machine learning analysis pipelines using images from intact CRC tissue samples to identify and quantify key histological features such as tumor regions and TILs within the TME. In addition, quantification of the 3D spatial heterogeneity of key biological metrics highlights that 3D imaging enables a more accurate assessment of disease state. Further investigation is ongoing to link these results to prediction of patient outcomes and treatment response.


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