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Adapting Cyclic Immunofluorescence for Human Skin and Melanoma

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Keywords

Cyclic immunofluorescence, melanoma, cancer, staining,

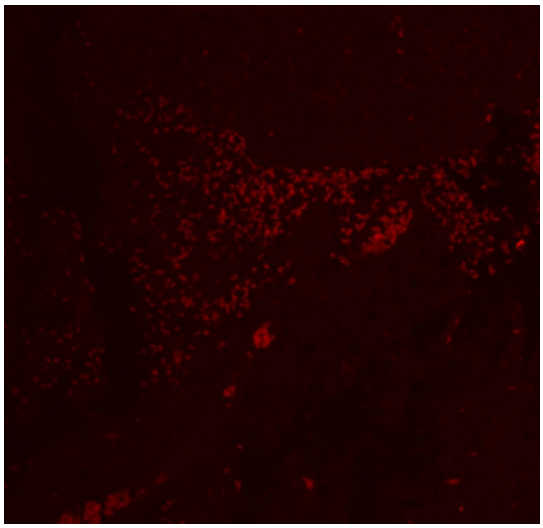
Abstract

CycIF as an alternative to mIHC

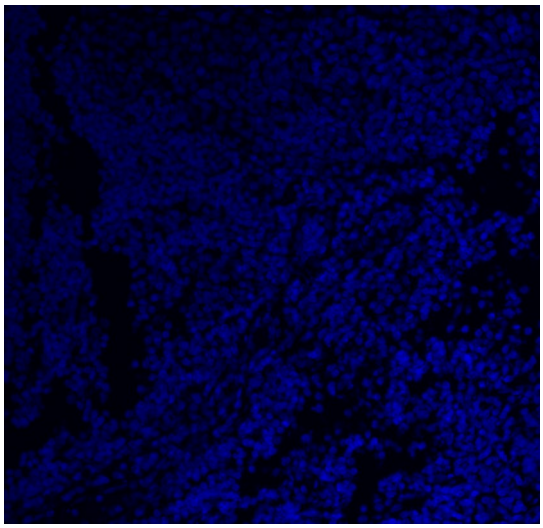
One of the most important tools for diagnosis, analysis, and study of cancer tissues is immunolabeling. Simultaneously analyzing both expression and spatial data of key surface markers provides important context when understanding the state of cancer tissue. While the standard approaches for labeling do allow for multiple proteins to be identified simultaneously, the number of markers is limited by spectral restrictions. Cyclic immunofluorescence has been developed as an alternative to standard multiplexed imaging strategies to allow for stains of large number of markers on the same section of tissue. Using a system of single stranded oligonucleotides, primary antibodies are labeled and then conjugated to a fluorophore via complementary imaging and docking strands which can later be cleaved to allow for additional markers to be imaged. This approach, developed by the Summer Gibbs lab, was first validated against human breast cancer tissue.

Immune labeling of human skin tissue

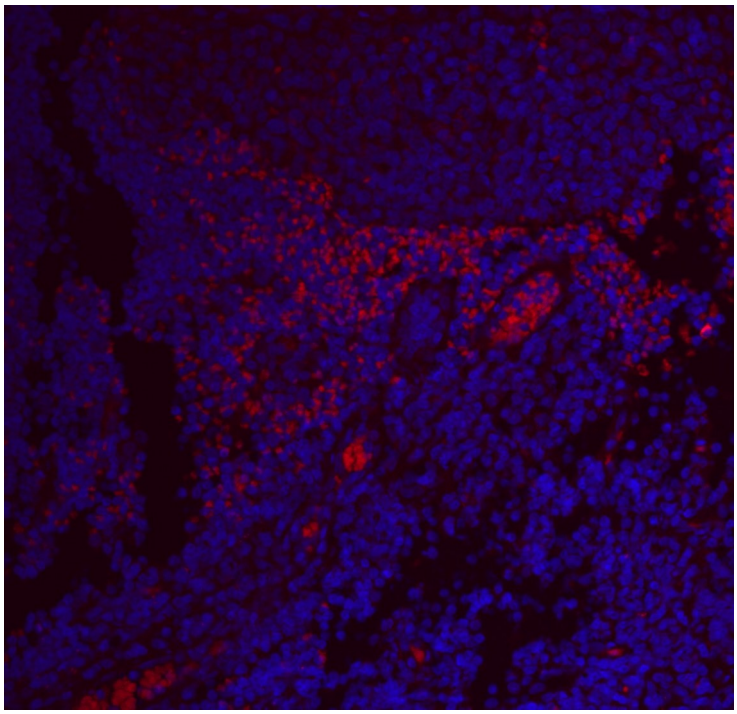
In an attempt to expand its applicability to other tissue types, I have worked on adapting the protocol and marker panels for immune cells found in human skin tissue. In hopes of being able to use this technique to image and analyze melanoma samples, I have modified and tested cycIF staining to work with human skin tissue. Using human tonsil tissue, a panel of immune antibodies were prepared and validated before staining human skin tissue as shown in **Figure 1**. I have prepared a panel of immune markers including but not limited to: CD45, CD68, CD3, and CD4.



(A) CD68 Stain in Human Tonsil Tissue



(B) DAPI Nuclear Stain



(C) CD68 and Nuclear Combined Channel

Figure 1. Validation of anti-CD68 cycIF antibody using human tonsil tissue

Successes with this panel demonstrates the promise of cycIF as a multiplexed imaging approach for the study of melanoma and other skin diseases.