

Resource Summary Report

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Tissue Cyclic Immunofluorescence (t-CyCIF)

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Proper Citation

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Protocol Information

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Summary: The architecture of normal and diseased tissues strongly influences the development and progression of disease as well as responsiveness and resistance to therapy. We describe a tissue-based cyclic immunofluorescence (t-CyCIF) method for highly multiplexed immunofluorescence imaging of specimens mounted on glass slides. t-CyCIF generates up to 60-plex images using an iterative process (a cycle) in which conventional low-plex fluorescence images are repeatedly collected from the same sample and then assembled into a high dimensional representation. t-CyCIF requires no specialized instruments or reagents and is compatible with super-resolution imaging; we demonstrate its application to quantifying signal transduction cascades, tumor antigens and immune markers in diverse tissues and tumors. The simplicity and adaptability of t-CyCIF makes it an effective method for pre-clinical and clinical research and a natural complement to single-cell genomics. Key resources, reagents, and software are listed at the HMS LINCS Center Publication Page <http://lincs.hms.harvard.edu/lin-elife-2018/> (RRID:SCR_016370). This page provides links to an OMERO image database from which individual images can be obtained; stitched and registered image panels can be obtained at www.cycif.org (RRID:SCR_016267) and a video illustrating the t-CyCIF method can be found at <https://vimeo.com/269885646>. This protocol is used in the following manuscripts: Lin J-R, Izar B, Wang S, Yapp C, Mei S, Shah P, Santagata S, Sorger PK. (2018). Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes. eLife. PMID: 29993362 Du Z, Lin JR, Rashid R, Maliga Z, Wang S, Aster J, Izar B, Sorger PK, Santagata S. (2019). Qualifying antibodies for image-based immune profiling and multiplexed tissue imaging. Nature Protocols. PMID: 31534232 The original t-CyCIF protocol can be found at

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