

Simultaneous Polychromatic Staining and Consecutive Acquisition of Lymphoid Tissue Sections

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Confocal microscopy has been an important imaging tool for life scientists for over 20 years. Early techniques focused on indirect staining processes that involved staining with an unconjugated primary antibody, followed by incubation with a secondary fluorescent antibody to reveal and amplify the signal of the primary antibody. With more and more directly-conjugated fluorescent primary monoclonal antibodies becoming commercially available, staining with multiple fluorescent primary antibodies is now more frequent. Currently, staining with up to three primary antibodies and a nuclear dye is widely practiced.

We describe an important improvement to the standard immunofluorescent staining protocol that allows the detection of multiple markers simultaneously. By incorporating recently available tandem dyes that emit in the blue, green and red regions of the visible light spectrum (Brilliant Blue™ and Brilliant Violet™), we were able to differentiate up to 10 fluorochromes simultaneously. Furthermore, we have developed a simple methodology to optimize antibody concentrations and straightforward guidelines regarding how to identify and correct non-specific staining signals. Following these simple steps it is possible to identify multiple cell populations in the same tissue section and describe their spatial relationships to better understand the role these cells play in health and disease.

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