

## **POLYCHROMATIC IMMUNO FLUORESCENT STAINING USING NEW GENERATION FLUORESCENT DYES.**

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The identification of specific cell-subsets plays a critical part in every immunological study, helping to understand the dynamic interactions taking place between cells of the immune system.

The standard technique for labelling different cell types is to conjugate cell-specific antibodies with organic or non-organic fluorescent dyes. Some of the more conventional fluorescent dyes are not sufficiently bright or photo stable enough to be used in microscopy, resulting in a limited number of markers available to develop polychromatic panels.

However, after the revolutionary creation of conductive polymers in 2000 [1], a new generation of fluorescent dyes emerged that are very bright and extremely photo stable, making them the perfect complement for use in immunostaining protocols for microscopy and flow cytometry.

Using cryo-sections of the ear draining lymph node, conductive polymers conjugated to antibodies and a multi-detection scanning system; we have designed a polychromatic immuno-staining protocol that reveals the location of cellular subsets in the context of a tissue. This allows for the identification and study of the interaction between cells types.

We found the best results for polychromatic staining were obtained, when a mix of conductive polymer dyes were used in complement with chemical synthetic dyes, to cover the entire light spectrum. The crucial steps during staining are to ensure the blocking is performed correctly to reduce non-specific binding and to use the correct antibody concentration to reduce background and conserve the antibodies.

The use of this staining protocol, allows the researcher to observe and record cellular subsets of interest and their specific interactions in the context of a whole sectioned lymph node. We expect this technique to be highly beneficial for many immunological studies.

**Reference:** "The Nobel Prize in Chemistry 2000". *Nobelprize.org*. Nobel Media AB 2014. Web. 9 Jan 2017 <[http://www.nobelprize.org/nobel\\_prizes/chemistry/laureates/2000/](http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2000/)>