A method employing the specificity of antibody labeled with fluorescein for the localization of antigen under the fluorescence microscope is presented. Included in the paper are a description of the synthesis of fluorescein isocyanate, the labeling material, and a method for removing over labeled proteins which bind indiscriminately to tissue elements and obscure specific reactions. [The SCI™ indicates that this paper has been cited over 1,465 times since 1961.]

Albert H. Coons
Department of Pathology
Harvard Medical School
Boston, MA 02115

January 14, 1981
(revised)

"This paper described improvements in the method published earlier for the specific localization of foreign antigenic materials in tissue cells. It was a general method for the histological localization of any antigen because it utilized specific antibody labeled with fluorescein as a histochemical reagent. Diluted specific antibody solutions so labeled were flooded over tissue sections. Any antigen present bound the antibody and fixed it in place. Excess reagent could be washed away leaving the bound antibody in place and it in turn could be localized by bombardment by light of appropriate wavelength and visualized under the fluorescence microscope. Naturally, the critical step was the binding of the fluorochrome-labeled antibody by the antigen and the ability to wash away any excess fluorescent reagent. This principle joined the specificity of the antibody molecule to the resolving power of the light microscope; such a union provided a general method now called immunohistochemistry for the investigation of native and foreign antigenic molecules in many locations and under many circumstances. Since then the same principle has been extended for use with the electron microscope by using antibody labeled with ferritin or with enzymes like horse radish peroxidase.

"Of course such reagents localizing and identifying antigens rapidly came to be used for the specific identification of various infectious agents: bacteria, rickettsiae, and viruses. It has also been applied to the study of autoimmune disease, e.g., nephritis, and in the detection of autoantibodies against tissue components. Immunofluorescence so-called therefore became a feature of the diagnostic, as well as the research, laboratory.

"Immunohistochemistry has gradually become useful in many areas of biology. Till now its weakness as a scientific method has been the difficulty of quantitating it.

"Recently however, computer activated light microscopes have made possible the rapid measurement of µ2 areas of fluorescent cells. Such microscopes, attached to a computer, print out measurements of fluorescence intensity, allowing rapid comparison of the amount of antigen per unit area in various cells and parts of them. So far this ability is only beginning to be exploited.

"Surprisingly enough, and to the good fortune of anyone who wants to apply such a method, it has turned out that the antibody molecule is quite stable to many chemical manipulations and does not lose its specificity unless the label attaches itself close to the actual combining site.

"Addendum. Albert H. Coons died suddenly September 30, 1978. His commentary above is remarkably understated. The impact of this work on research in many biological disciplines, and, in particular, on studies of immunobiologic and pathologic processes is universally recognized. We remember him for the charm of his company, his penetrating wisdom, and his admonition: 'Good research work stands on its own legs' " —Melvin H. Kaplan

1. Coons A H, Creech H J, Jones R N & Berliner G. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. J. Immunology 45:159-70, 1942. [The SCI™ indicates that this paper has been cited over 285 times since 1961.]