WS4-1

Zenon technology: a novel, extremely rapid method to label primary antibodies on a microgram scale
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Simultaneous multicolor immunofluorescence detection has been limited by the necessity of using primary antibodies from unrelated species that are either directly labeled or used in conjunction with labeled secondary antibodies. Direct labeling of primary antibodies is frequently impractical because of the often very limited amounts of expensive antibody available. We have developed a method to directly and selectively complex sub-microgram quantities of mouse monoclonal IgG, antibodies with fluorescent dye- or enzyme-labeled anti-Fc Fab fragments of goat anti-mouse IgG antibody. This method is extremely easy, versatile and economic because it allows the rapid (<10 min) and quantitative formation of antibody-Fab constructs that are functionally equivalent to directly labeled antibodies. As an added advantage, using the Zenon technique, the label is located on the Fc portion, removed from the antigen-binding site of the antibody. Furthermore, the Zenon labeling method can be performed in the presence of other proteins in mixtures, such as hybridoma supernatant, BSA or gelatin-stabilized antibodies. Using this labeling method, we were able to detect by immunofluorescence three cell targets by adding simultaneously to the cells three mouse monoclonal antibodies labeled with Fab fragments that had been conjugated to Alexa Fluor dyes of separate spectral properties. Zenon labeling with phycobiliproteins, tandem conjugates of phycobiliproteins and enzymes is equally practical. Only the filters and the optical detection tools available limit the labeling possible. The Zenon labeling reagents may eventually be the only reagents needed for high-throughput experiments, replacing both direct conjugates of antibodies and dye- and enzyme-labeled secondary antibodies.

WS4-2

Adapting ultra-sensitive fluorescence methodologies for histological applications
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The ability to detect and measure macromolecules in tissue is a central component of target validation in animal models and diagnostic approaches to the study of human disease. Recent improvements in fluorescent and enzymatic detection chemistries and parallel advances in microscopic imaging allow highly sensitive visualization of protein and mRNA distribution in situ. Furthermore, new amplification schemes can be developed that enable fluorescent signals to be further enhanced and/or converted to permanent chromogenic reaction products. Tyramide Signal Amplification® (TSA) utilizes horseradish peroxidase to catalyze the deposition of fluorescent dyes or haptens, which can then be detected and further amplified via antibody or streptavidin-based methods. Such "double amplification" can lead to dramatic enhancement of staining sensitivity, as demonstrated in cultured cells pulsed with low doses of 5-bromo-2'-deoxyuridine (BrdU). This approach can also be applied to detection of low abundance targets in mouse brain sections yielding improved visualization of fine neuronal structure. Multi-target labeling can be achieved by sequential deposition of dyes with different spectral characteristics, or use of anti-dye/hapten antibodies and enzyme conjugates to produce distinct chromogenic reaction products. TSA technology can also be used to identify functionally relevant cellular markers in tissue microarrays, in which multiple tissue samples from normal and/or disease tissue are analyzed simultaneously. Essentially any tissue staining method that employs antibody or avidin-biotin reagents can be significantly improved by incorporating TSA technology.

WS4-3

Enzyme cytochemical techniques for metabolic mapping in living cells, with special reference to proteolytically
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Specific enzymes play key roles in many pathophysiological processes and therefore are targets for therapeutic strategies. The activity of most enzymes is largely determined by many factors at the post-translational level. Therefore, it is essential to study the activity of target enzymes in living cells and tissues in a quantitative manner in relation to pathophysiological processes to understand its relevance and the potential impact of its modulation. Proteinases are crucial in every aspect of life and death of an organism and are therefore important targets. Enzyme activity in living cells can be studied with various tools. These can be endogenous fluorescent metabolites or synthetic or fluorogenic substrates. The use of endogenous metabolites is rather limited and nonspecific because they are involved in many biological processes, but novel fluorogenic and fluorescent substrates have been developed to monitor activity of enzymes, and particularly proteases, in living cells and tissues. These substrates and methods in which they are applied will be discussed, as well as their advantages and disadvantages for metabolic mapping in living cells.

WS4-4

Strategies in nucleic acid detection: amplified FISH and ultrasensitive microarray methods
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Gene localization and expression studies rely ever more heavily on fluorescence-based nucleic acid hybridization assays, and new technology continues to put high demands on the sensitivity and utility of hybridization probes. Although nucleic acid probes are typically generated by enzymatic incorporation of modified nucleotides, most polymerases incorporate these nucleotides with low and variable efficiency, limiting the sensitivity of the probe and making it difficult to compare signals from probes labeled with different dyes or haptens. These and several other factors can contribute to poor signals from probes labeled by conventional methods. New methods for labeling probes as well as novel signal amplification techniques can enhance the sensitivity of the probe and the strength of the assay. We describe three technologies for increasing the signal in hybridization assays. EFLP 97 phosphate is a unique fluorogenic alkaline phosphatase substrate that gives rise to a bright, yellow-green precipitate at the site of enzymatic activity. We have found this substrate to be useful for amplifying signals from biotin- or hapten-labeled probes, and due to a large Stokes shift, the EFLP 97 precipitate is particularly useful with autofluorescent samples. In addition, we have found that Alexa Fluor tyramides, in combination with horseradish peroxidase-conjugates, provide high signal to noise ratios and several-fold higher signals from biotin- and hapten-labeled probes than fluorescently-labeled streptavidin. Recently, we found that DNA and RNA labeling using enzymatic incorporation of 5-O-aminosulfonyl-modified dUTP or dUTP, followed by reaction with amine-reactive dye, generates uniformly labeled, highly sensitive nucleic acid probes, regardless of fluorescent dye chosen, that are difficult to obtain by conventional approaches. The technique is intrinsically consistent and flexible by virtue of the efficient incorporation of primary amines and the reliable chemical labeling reaction, which can be performed with a variety of amine-reactive dyes.