

detection. Submicroscopic spherical RLS gold and silver particles of uniform dimension (40–140 nm diameter range) scatter incident white light to generate highly intense colored light. The intensity of colored light signal generated by a single RLS Particle is 10^4 to 10^6 times greater than the signal obtained from commonly used fluorophores. The color and intensity of scattered light generated by RLS Particles™ does not photobleach and depends upon particle composition and size according to developed algorithms^{1,2}. The high level of signal intensity allows single RLS Particle labels to be detected under configured dark field illumination conditions using low power magnification. RLS Particles can be derivatized with a variety of biomolecules including antibodies to impart specific particle binding for detection of specific haptens or antigens. Microscope-detection systems consisting of an illumination source, configured dark field optics, CCD-camera and image analysis software have been developed for in situ hybridization and immunohistology applications. RLS Particle optical properties, RLS Detection systems and examples of IH, ISH and microarray applications are presented.

1 Yguerabide, J. and Yguerabide, E. E. (1998) *Anal. Biochem.* 262, 137–156.

2 Yguerabide, J. and Yguerabide, E. E. (1998) *Anal. Biochem.* 262, 157–176.

P1-09

Improved metaphase spreads for immortalized human ovarian cells

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Good chromosome preparations are essential for the success of molecular cytogenetic studies such as fluorescence in situ hybridization (FISH), spectral karyotyping (SKY) and comparative genomic hybridization (CGH). The quality of metaphase spreads is particularly critical for tumor cells and immortalized cells since they tend to show high frequencies of hyperdiploidy. Although there is no shortage of suggestions and tips in the literature on how to improve the cytogenetic slide-making procedures, the mechanisms behind them are not fully understood. In this study, we report our systematic characterization of the metaphase spreading process for immortalized human ovarian surface epithelial (HOSE) cells. The effects of different hypotonic treatments, fixatives, slide-drying time, slide surfaces, slide temperature, relative humidity, and the dropping height of the cell suspension, on the quality of metaphase spreads were studied quantitatively. We found that the choice of hypotonic solution and the humidity over the slide were the two most important factors affecting the quality of chromosome spreading. Our observations led us to develop a new and simple method using 0.8 M sodium citrate in hypotonic treatment and a controlled environment for chromosome spreading. The cells were dropped on a dry glass slide placed in a shallow metal tray, which was lowered into a covered 50°C water bath for slide-drying. Significantly larger metaphase area, decreased overlaps per metaphase, and reduced percentage of un-analyzable hyperdiploid metaphases were obtained, despite the high frequency of hyperdiploidy. We also propose that dynamic cell rehydration, which occurs as fixative absorbs atmospheric moisture during slide-drying, helps chromosome spreading, and should be coordinated with prompt immobilization of spread chromosomes to the slide.

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Phenotype analysis of tumor cells with eight color FISH

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High throughput gene expression profiling using cDNA microarrays generates a wealth of information and often demonstrates tumor-specific changes. These measurements, however, provide average values for tumor cell populations that may be rather heterogeneous. Our technical developments address the issue of heterogeneity in tumor research by developing an analytical system capable of performing semi-quantitative multi-gene expression profiling of single cells. Targeting cell-by-cell measurements of expression levels of multiple tumor markers, our approach uses RNA/cDNA fluorescent in situ hybridization (FISH) combined with Spectral Imaging and digital image analysis. While the system is capable of deconvoluting images of objects stained with up to nine fluorochromes, we performed initial tests of system resolution and reproducibility with commercially available beads fluorescing in seven different wavelength intervals. The system measured up to our expectation of being able to quantitate the seven different fluorescent reporter molecules with relative standard deviations ranging from 1% to 6.1%. Using eight different fluorochromes, we then analyzed the expression levels of 6 different tyrosine kinase gene and one genomic target in breast and thyroid cancer cells counterstained with DAPI. In artificial mixtures, the system was able to recognize the tumor cells based on the level of expression of one or two genes, and could identify cells present in only a few percent. Supported by NIH grants CA88258 and CA80792 and the United States Army Medical Research and Material Command, United States, Department of the Army (DAMD17-99-1-9250, DAMD17-00-1-0085).

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Multispectral imaging for multicolor immunohistochemical and transmission in-situ hybridization

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Molecular medicine now requires molecular pathology, that is, the imaged-based detection of specific genes, transcripts, proteins and other macromolecules. Because of the need to interpret molecular data in context, simultaneous assessment of more than single species is desirable. While fluorescence has traditionally been used for high-resolution multiplexed molecular imaging, clinical practitioners express strong preferences for non-fluorescent multicolor methods. However, in brightfield, typically, only one color is used at a time, and if more than one molecular target is to be analyzed, serial sections are made and a different probe applied to each slice. Such practice precludes assessment of co-expression on a cell-by-cell basis. Similar constraints apply to brightfield in-situ hybridization techniques. Double- and triple-staining procedures are rarely performed in non-research settings not only because the wet chemistry can be difficult, but also because it can be challenging or impossible to determine visually where and to what extent different chromogens may physically overlap. Spectral imaging, that is, the acquisition of partial or full spectra at each pixel of an image, can be used to examine such double- and triple-stained specimens. Two methods of acquiring spectral images are described, along with their application to multicolor