

Hyperspectral Imaging: Converting Colors to Molecular Information

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Abstract

*We have developed several hyperspectral fluorescence microscopes. For each pixel (2D) or voxel (3D) within the specimen, a hyperspectral microscope will collect hundreds of wavelengths throughout the spectrum. Our new microscopes can record the entire emission spectrum from 500 nm to 800 nm for every location in the image. Both microscopes are being used in biological research projects such as genomic analyses, interactions of pathogens with the innate immune system, and carbon sequestration from the atmosphere by photosynthetic bacteria. One of our microscopes is a 3D confocal fluorescence microscope that can optically section live cells with submicron spatial resolution. When coupled with multivariate curve resolution (MCR) analysis methods, the new microscope can "discover," quantify, and resolve multiple spatially and spectrally overlapped emission components, thereby greatly increasing the number of fluorescent labels that can be monitored simultaneously. The design and operation of the microscope will be discussed along with the multivariate methods used to analyze the hyperspectral images. The utility of the new approach will be demonstrated with hyperspectral images of live *Synechocystis* cells. *Synechocystis* is a cyanobacterium, a class of organisms responsible for a large fraction of carbon sequestration from the atmosphere. In these experiments, cells from wild type and from mutants lacking a photosystem or a step in chlorophyll*

biosynthesis were imaged to better understand relative concentrations and spatial distributions of photosynthetic pigments in these bacteria. Hyperspectral images obtained from biological systems including microarrays for whole genome gene expression analyses, interaction of bacteria pathogens with macrophage cells, growth of biofilms on water purification membranes, and cell signaling in rat basophilic leukemia cells labeled with multiple-color quantum dots will also be presented. The new microscope and associated multivariate analyses constitute an enabling new technology for cell imaging and for understanding a variety of molecular and physical processes occurring in live cells.

Sandia is a multi-program laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-ACO4-94AL85000.

Author Biography

David Haaland received his BS in chemistry from the University of New Mexico (1968) and his PhD in physical chemistry from the University of Rochester (1972). Since 1972 he has worked at Sandia National Laboratories in Albuquerque, NM in materials characterization and biology related departments. His work has focused on the development of multivariate analysis of spectral data and hyperspectral imaging. He is recipient of the 2004 Bomem-Michelson Award and the 2005 EAS Award for Achievements in Chemometrics.