

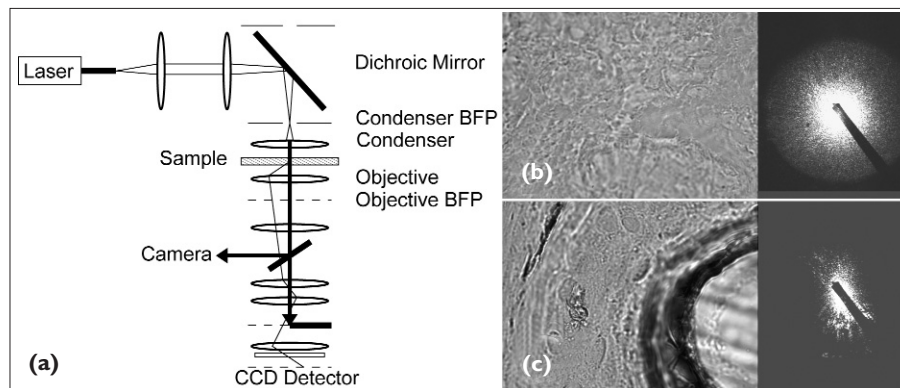
Microscope-Based Static Light-Scattering Instrument Enables Precise Measurements of Heterogeneous Materials

M. T. Valentine, A. K. Popp, P. D. Kaplan and D. A. Weitz

A broad range of materials, including colloids, ceramics, and biological samples, are characterized by structures that are from 10 nm to 100 μm in size. Ideally, one would like to investigate these complex materials noninvasively on all the relevant length scales; however, this cannot be accomplished easily with the well-established techniques. Optical microscopy has diffraction-limited resolving power, which precludes the study of fine detail. In contrast, static light scattering (SLS) provides high-resolution measurements of structure and organization through measurements of the intensity of scattered light as a function of scattering angle. Although length scales as small as 30 nm can be probed, each measurement averages over the entire illumination volume, making the interpretation of scattering from heterogeneous samples, which contain many different structures and orientations, particularly challenging.

A new combination of these two methods, the static light-scattering microscope, simultaneously collects high-resolution scattering data that were obtained with a beam of the size of relevant structures, and images that are used for identification and interpretation of SLS experiments.¹ Key features include a well-defined scattering geometry and volume as well as controlled size and placement of the illuminating beam. For our design [Fig. 1(a)] we focused a fiber-launched laser beam to a point in the back-focal-plane (BFP) of an oil-immersion condenser, producing a collimated beam at the sample. The scattered light is collected through a wide range of scattering angles by a high-numerical-aperture, oil-immersion objective lens. A camera provides an image of the sample; the scattering data were obtained by a separate optical train that imaged the BFP of the objective with a beam block onto a cooled CCD detector.

This instrument is particularly well suited for the study of biological samples such as tissue that include many microscopic structures with a wide range of shapes and orientations. Although light propagation through tissue has important



implications for laser surgery, optical biopsy, and photodynamic therapy, the microscopic origins of light scattering in tissue are poorly understood.^{2,3} For example, although considerable effort has been focused on the measurement of average absorption and scattering coefficients, little attention has been given to the effects of small yet abundant heterogeneities on bulk scattering properties. Simultaneous optical imaging and light scattering can provide new insight into the nature of light propagation through heterogeneous tissue.

To illustrate this we show data from thin slices of porcine skin tissue. In acellular derma, hairs or hair follicles and pigments can cause dramatic changes to the scattering patterns, as seen in Figs. 1(b) and (c). Figure 1(b) shows a region devoid of any large heterogeneities; the resulting scattering pattern is isotropic. Figure 1(c) shows a region from the same slice of tissue near the vicinity of a hair follicle. The scattering pattern is anisotropic and is more structured than the homogeneous case. These results suggest that models that do not incorporate details of local tissue microstructure could fail to give a correct description of light propagation on short length scales. Additionally, the sensitivity of the instrument to variations in structure and organization in thin tissue samples makes it a potentially useful tool for pathohistological studies.

References

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M.T.Valentine, A.K. Popp, D.A. Weitz, Department of Physics and the Division of Engineering and Applied Science, Harvard University, Cambridge, MA and P.D. Kaplan, Unilever Research, Edgewater, NJ.

Figure 1. (a) Schematic of the optical arrangement of the SLS microscope. (b) Real space image of porcine skin tissue of a region devoid of any large heterogeneities; the resultant scattering pattern is isotropic. (c) Porcine skin tissue region from the same slice in the vicinity of a hair follicle, resulting in an anisotropic scattering pattern.