Fluorescent Speckle Microscopy

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Whereas too much of a good thing can be bad, too little of a good thing can be good. In this case, the accidental dilution of X-rhodamine tubulin injected into living cells resulted in a heterogeneous labeling of microtubules, rather than visualization of continuous structures. This heterogeneous labeling rendered a speckled image, hence the term fluorescent speckle microscopy (FSM) was introduced. It turns out that FSM yields particularly useful information on dynamic events within living cells, for example the assembly and disassembly of microtubules.

There are two recent reviews of FSM by Clare Waterman-Storer and Gaudenz Danuser, one emphasizing the biologic applications of the technique[2], the other emphasizing the quantitative aspects.[3] For their purposes, they defined a "speckle" as a diffraction-limited region of the image that is significantly brighter than its immediate environment. It can be calculated from the point-spread function (which is determined by the numerical aperture of the lens) that optimally a diffraction-limited region of about 250 nm can be imaged. Within such a region, about 450 tubulin dimers (components of a microtubule) are present. If more than 20% of the dimers along the length of a microtubule are tagged with a fluorescent dye, the microtubule appears to be continuously labeled. If a smaller percentage (1-2%) is labeled, the microtubule is speckled. It was demonstrated that the appearance of speckles over time is indicative of microtubule assembly, whereas the disappearance of speckles is evidence of disassembly. These dynamic events can be observed quantitatively with good spatial and temporal resolution by FSM. Even assembly at one end and simultaneous disassembly at the other (referred to as "treadmilling") can examined to determine if the underlying mechanism is mediated by microtubule motor proteins or tubulin exchange at the ends of the microtubules.

In addition to studies of microtubule assembly and motion, Danuser and Waterman-Storer reviewed several other applications of FSM. These include, but are not limited to, dynamic studies of microtubules in mitosis and in neurons, f-actin in migrating cells, and analyzing microtubule-associated proteins. Conjugating target proteins with Green Fluorescent Protein (GFP) also is useful in FSM. It was suggested that binding and dissociation dynamics or single molecule fluorescence analysis of many proteins might be studied *in vivo* and *in vitro*.

Methods of analysis of FSM currently include the use of kymographs to determine the velocity of speckle movement and laborious hand tracking of a small subset of speckles. Although these methods had provided us with a deeper understanding of the dynamics of polymer kinetics, their use is limited and does not fully exploit the information inherent in time lapse FSM. Waterman-Storer and Danuser suggested that the full potential of FSM would become available once the specialized computational tools are developed for the statistical analysis of speckle translocation and intensity fluctuation. The task of tracking over half a million moving speckles is daunting, but not out of reach for the technology in the future.

Future prospects for FSM are bright, particularly because it can be applied to other fluorescence microscopy technology. For example, FSM can be combined with spinning disk confocal microscopy, total internal reflection fluorescent microscopy, and multi-spectral microscopy. Waterman-Storer and Danuser envision the use of FSM technology to analyze the kinetics of important molecules in many biologic processes, as well as material science applications. It will be exciting to see the information that can be gleaned with this technology that offers so much spatial and kinetic information! ■

References:

1 The authors gratefully acknowledge Dr. Clare Waterman-Storer for reviewing this article.

2 Waterman-Storer, C.C., and G. Danuser, New directions for fluorescent speckle microscopy, *Current Biology* **12**:R633-R640, 2002.

3 Danuser, G., and C.M. Waterman-Storer, Quantitative fluorescent speckle microscopy: Where it came from and where it is going, *J. Microscopy* **211**:191-207, 2003.

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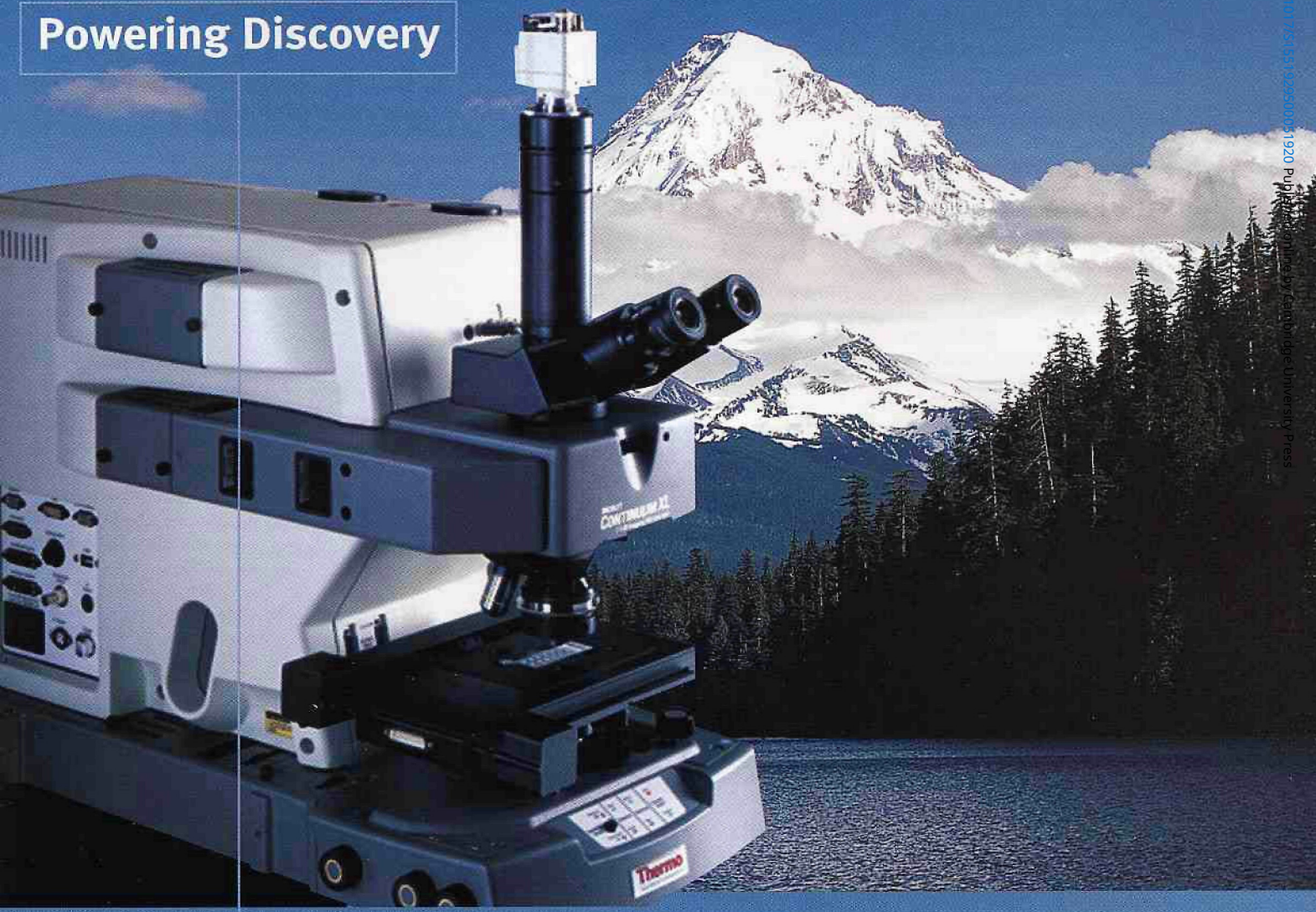

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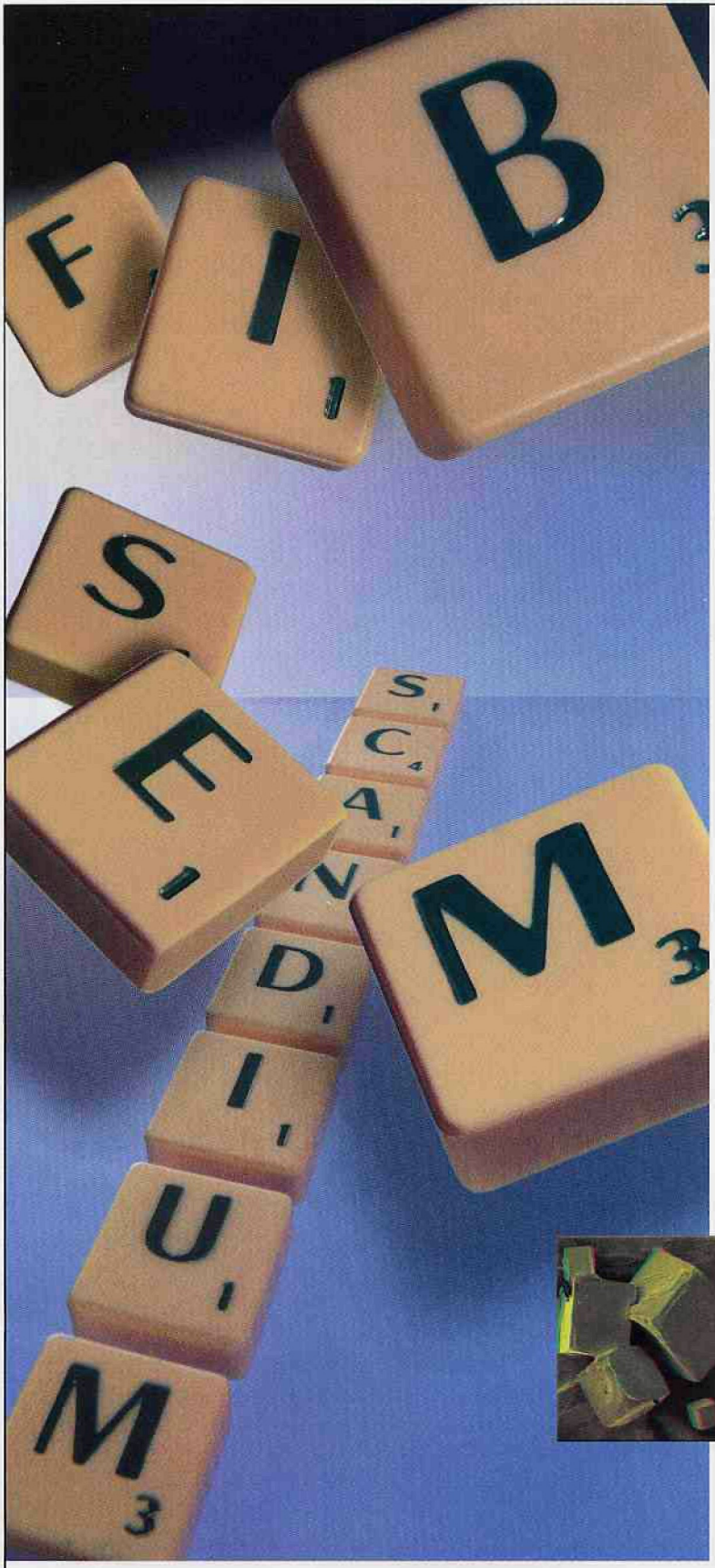

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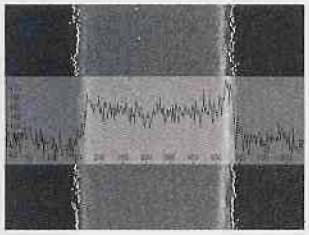

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