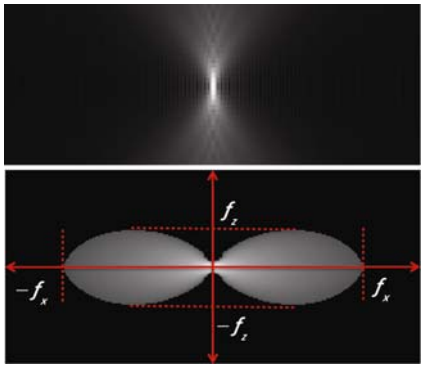
In this issue

Microscopy in biology

The importance of an image-processing step in live cell microscopy, known as image deconvolution, is discussed in the article by Arigovindan (**page 1501**). First, the author explains the reason why the maximum imaging duration tolerable by the imaging specimen and the obtainable image resolution conflict with each other. Then he explains how the efficiency of deconvolution plays an important role in maximizing the tolerable imaging duration for a given required resolution. Next, he elaborates on recently developed deconvolution method and demonstrates its significantly improved efficiency in comparison with other modern methods. He then explains how this improved efficiency will make possible newer types of live cell imaging experiments on light-sensitive cells with durations longer than ever. The author describes some approaches for further improving the



efficiency of deconvolution, and emphasizes how such improvements will lead to enabling technologies for the reliable study of elaborate live processes using live imaging experiments with much longer durations.

Cells communicate with each other mostly through chemical cues which trigger a series of biochemical changes inside a cell culminating into generation of appropriate adaptive response to the cue. Classical approaches for studying the underlying events in these processes have been based on end-point analysis, where the end response is used as readout of the presence of signalling events. Mitogen activated protein kinase (MAPK) signalling is one such pathway, where a cell responds to a growth trigger and in turn induces cell proliferation. It is one of the most well-studied signalling pathways owing to the function it regulates and has been majorly studied using conventional biochemical approaches. Though these classical techniques have been valuable in providing mechanistic insights into this cascade, they have failed to explain the dynamics of the signalling process. Standard signalling events are depicted as constrained to defined cellular locales, but now it is well appreciated that signalling is highly dynamic and the spatio-temporal analysis in living cells is the right way to study signaling events. The article by Ruchi Jain and Deepak Kumar Saini (**page 1512**) provides recent advancements in high-speed microscopy techniques and how these techniques have facilitated unmasking of spatio-temporal dynamics of various signalling pathways and specifically MAPK signalling in living cells. It will serve both as a technical primer and a template for utilization of modern microscopy techniques in studying cellular signalling in living cells.

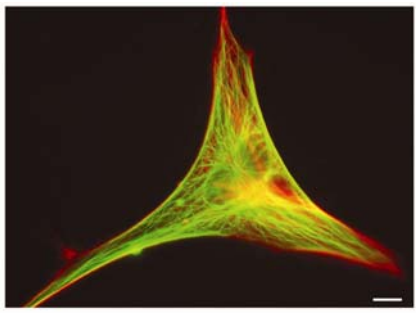
Roy (**page 1524**) discusses optical microscopy resolution and new approaches that are allowing us to beat the diffraction limit. Starting from an historical perspective, a new paradigm in optical microscopy is discussed that relies on controlling or limiting the signal generation during image formation and using the prior knowledge of the point spread function of the optical system to achieve super-resolution imaging. Up to tenfold enhanced resolution in 3D than conventional microscopy is bringing optical microscopy close to molecular scales. The two major super-resolution family of techniques, namely reversible saturable optical fluorescence transitions and stochastic reconstruction microscopy are discussed in detail. Furthermore, using key examples in cell biology, neurobiology, microbiology and virology, an attempt is made to highlight the successful application of these nascent technologies to biological questions. Further development of the super-resolution concept will find use in the development of quantitative imaging of biological function and not just spatial distribution.

Aditya Singh *et al.* (**page 1537**) discuss the principles of multi-photon microscopy technique to study the neuronal mechanisms of learning and memory. The article highlights the key principles of multi-photon microscope, specifically in reference to deep imaging of scattering tissues. Then there is a brief discussion of basic components of confocal microscope and its comparison with multi-photon microscope. The article elaborates on difference between PSFs of 1p- and 2p-excitation of fluorophores. These differences are caused by longer excitation wavelength and quadratic dependence of excitation probability on the intensity of incident radiation. Further, there is a general schematic of a multi-photon microscope set-up along with provisions for measuring the pulse width at the sample using Michelson interferometer and correcting the chirped pulse by prism pair compensator. Finally the article lists some of the key studies to demonstrate the enhanced range of questions that can be addressed after successful implementation of multi-photon microscope in studying learning and memory.

The tiny picolitre volume of a human cell interior serves as the space for exciting biological events that occur during its lifetime. To fully

understand and appreciate the inner workings of a living cell, it is necessary to know the physical nature of confined spaces like cytoplasm, mitichondrial matrix and so on. As an example, such spaces are known to be densely crowded with macromolecules like proteins, nucleic acids and membranes. It would be worthwhile to know how fast a molecule like glucose can diffuse in such a space? what factors impede this diffusion? and how is diffusion affected across different spatial locations inside the cell? Answers to these questions demand experimental approaches to observe the diffusion of small molecules inside living cells. Fluorescence microscopy has emerged as a powerful non-invasive technique to provide key insights into the spatial and temporal features of fluorescently tagged molecules inside living cells. Prasad and Swaminathan (**page 1549**) demonstrate how the translational and rotational diffusion of fluorescent probes like fluorescein or green fluorescent protein can be observed inside the cytoplasm or mitochondrial matrix of living cells using techniques based on fluorescence microscopy.

The actin and microtubule cytoskeletons regulate a host of cellular processes, including cell shape and motility, cell division, cellular trafficking and transport, cell–cell interactions and synaptic plasticity. Directed remodelling of these polymer systems is central to their functional diversity and occurs at length and timescales that are accessible to epifluorescence microscopy. Sahasrabudhe *et al.* (**page 1562**) review accessible microscopy techniques and their use in studying cytoskeleton dynamics. The authors provide a comparative summary of available labelling strategies and suggest that the method of choice depends very much on the information intended to be extracted. The discussion of standard epifluorescence methods compares widefield, widefield-deconvolution, laser scanning confocal



microscopy and spinning disc confocal microscopy, and includes a primer on selecting the method of choice using the haziness index of the sample under observation. A discussion of super-resolution technologies, especially those employing PSF engineering provides an entry point to these emerging technologies. The convenient tables listing resolution ranges and comparative advantages of various epifluorescence techniques should aid in selecting the relevant imaging modalities. Finally, the discussion on fluorescence speckle microscopy and kymograph analysis allows appreciation of techniques that enable quantitative analysis. This review should help interested biologists to navigate through the maze of emergent technologies in order to find imaging strategies best suited to their questions.

Mapping of water-ice deposits in the lunar polar regions

The confirmation of presence of water-ice in the lunar polar regions was one of the most significant results of India's Chandrayaan-1 mission. Even though the presence of H_2O-bearing minerals in the lunar polar regions was spectroscopically detected through the Moon Mineralogy Mapper (M^3) sensor on-board Chandrayaan-1, it was the Mini-SAR imaging radar that helped in identifying precise locations for the existence of water-ice in the lunar surface. Radar is regarded as one of the most effective tools for detecting water ice, because as a low dielectric loss medium, water ice could produce coherent backscatter opposition effect (CBOE) under certain conditions. The key parameter to address this is the radar circular polarization ratio (CPR) and previous studies indicated that if ice in the permanently shadowed areas of the lunar poles has radar characteristics similar to the ice on Mercury, Mars and the Galilean satellites, then they will have substantial radar enhancements characterized by a CPR greater than unity. As a result of CBOE, CPR can reach a maximum value of 2 at zero-phase backscattering condition. However, it should be noted that CPR greater than unity is not a unique signature for water-ice deposits. Geologic targets, such as lunar crater deposits, lava flows and rocky surfaces, can have CPR values as high as 4. In order to identify a unique signature of water-ice deposits, analysis of radar polarimetric scattering mechanisms in elevated CPR regions was carried out. Based on the signatures of diffuse scatterers and radar backscattering coefficient, a scattering mechanism-based algorithm was developed and then tested using Chandrayaan-1 Mini-SAR data. The algorithm shows promising results for identifying craters with water-ice deposits by separating them from young, fresh craters with elevated CPR values. See **page 1579**.