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Design of high-resolution wide field of view confocal line scanning laser microscopy

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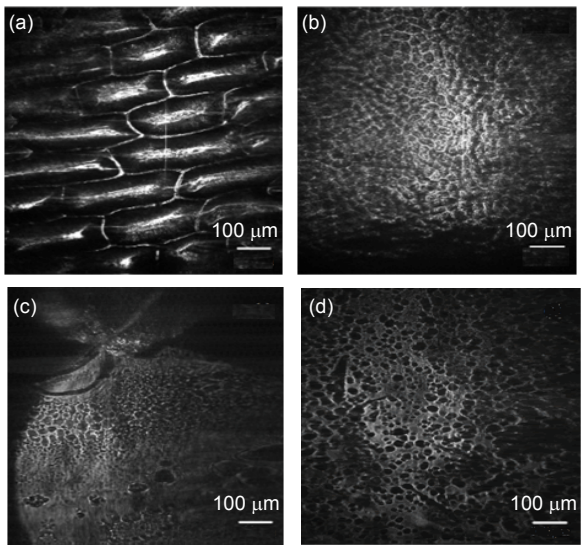

Images of (a) onion epidermal cells, (b) plant mesophyll cells, (c) human oral epithelial cells and (d) human blood cells.

Abstract: In traditional commercial confocal image system, detecting light emitted from the light source through a pinhole into a point light, the reflected light from specimen go through the pinhole into the detector. In this case, only the reflected light from the focusing plane could reach the detector. Non-focused light cannot pass through the pinhole and therefore cannot be imaged in the detector. However, traditional confocal imaging system use point by point scanning to image the sample, so the field of view is small (200 μm×200 μm), the imaging speed is slow (10 fps typical), and the image speed is much slower for larger field of view.

In order to improve the imaging speed and increase the field of view, the line scanning confocal systems use one-dimensional-focused line beam to scan the sample, and use the slit to filter light strayed from non-focused plane. Non-focused light cannot pass through the slit filter and be imaged by the detector. Compared with the point by point scanning system, the line scanning microscope system can image the sample by only one dimension scanning, which improves the image speed and field of view greatly.

The line scanning confocal microscopes uses high speed galvanometer scanning mirror and 28 kHz line array camera to get high resolution (512 pixels ×2048 pixels) image, and the frame frequency of the system could reach 50 fps (frame per second), which is much higher than the point scanning confocal microscope. And the higher line frequency the camera has, the higher imaging speed could the system reaches. Theoretical analysis and experimental result show that the optical magnification of the line scanning confocal microscope is 55, and the field of view is 713 μm×713 μm. We use resolution test target to find that the system could distinguish at least 288 line pairs in the target, which means that the lateral resolution of the line scanning confocal microscope is higher than 2.2 μm. The axial resolution of the microscope is defined as the FWHM (full width at half maximum) of the detected light intensity. The ground glass flat is placed as sample and the axial resolution is about 9 μm in this system. Finally, images of plants and human cells is got by the confocal line scanning microscope, and cells could clearly distinguish from the images, which proves that the system could be used in cells biological cell imaging.

Keywords: imaging system; line scanning; confocal; high-frame-frequency imaging

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