

**Extended Field Laser Confocal Microscopy (EFLCM): Combining automated Gigapixel image capture with in silico virtual microscopy**

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**Abstract**

**Background:** Confocal laser scanning microscopy has revolutionized cell biology. However, the technique has major limitations in speed and sensitivity due to the fact that a single laser beam scans the sample, allowing only a few microseconds signal collection for each pixel. This limitation has been overcome by the introduction of parallel beam illumination techniques in combination with cold CCD camera based image capture.

**Methods:** Using the combination of microlens enhanced Nipkow spinning disc confocal illumination together with fully automated image capture and large scale in silico image processing we have developed a system allowing the acquisition, presentation and analysis of maximum resolution confocal panorama images of several Gigapixel size. We call the method Extended Field Laser Confocal Microscopy (EFLCM).

**Results:** We show using the EFLCM technique that it is possible to create a continuous confocal multi-colour mosaic from thousands of individually captured images. EFLCM can digitize and analyze histological slides, sections of entire rodent organ and full size embryos. It can also record hundreds of thousands cultured cells at multiple wavelength in single event or time-lapse fashion on fixed slides, in live cell imaging chambers or microtiter plates.

**Conclusion:** The observer independent image capture of EFLCM allows quantitative measurements of fluorescence intensities and morphological parameters on a large number of cells. EFLCM therefore bridges the gap between the mainly illustrative fluorescence microscopy and purely quantitative flow cytometry. EFLCM can also be used as high content analysis (HCA) instrument for automated screening processes.

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