

SUPERRESOLUTION 3D IMAGING IN VIDEO-CONFOCAL MICROSCOPY

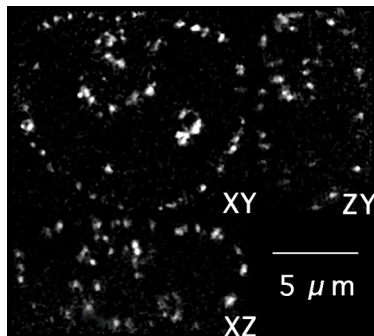
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In Video-Confocal Microscopy (VCM) an array of light spots is stepwise scanned across the specimen to collect a set of narrow-field illuminated images covering the whole specimen area. Non-linear [1] and linear [2] algorithms serve to extract optical sections from the above set of raw images. Important analogies exist in VCM with spatial filtering and detection processes being opto-mechanically performed in traditional confocal microscopes.

However, in VCM, the wide spectrum of spatial frequencies present in the illumination pattern and the highly selective extraction strategies adopted are able to reveal the details that correspond to higher spatial frequency components in specimen structure.

The detection characteristics of previously described VCM algorithms already permitted, also in instrumentation setup by some manufacturers, several imaging forms. These include: Conventional (similar to wide-field), Semi-Confocal (similar to spinning-disk confocal), Confocal (similar to single-point-scanning confocal) and Super-Confocal (exhibiting superior resolution, contrast and sectioning ability). Moreover, new processing approaches, both to improve noise performance and spatial resolution especially along the optical axis, are being introduced and will be demonstrated with practical examples.



Although recent progresses in some structured illumination microscopies exhibit excellent lateral resolution in the study of sparse specimens, VCM seems particularly attractive even in the presence of relatively thick and closely packed specimens in which sufficient spatial discrimination and contrast can not be obtained using current optical microscopy techniques. Superresolution is achieved in VCM in which 3D imaging performance in fluorescence currently permits a virtually isotropic (xyz) spatial resolution, down to 200 nm.

Some results will be shown in multicolor cytogenetic imaging [3] of nuclear chromatine distribution, in synthetic bead preparations and in other advanced application fields.

[1] P. A. Benedetti, V. Evangelista, D. Guidarini, S. Vestri, "Method for the acquisition of images by confocal microscopy," United States Patent **6,016,367** (priority: Sept. 25, 1996)

[2] R. Heintzmann and P.A. Benedetti "High-resolution image reconstruction in fluorescence microscopy with patterned excitation," *Appl. Opt.* **45**, No. 20, 5037-5045 (2006)

[3] J. Walter, B. Joffer, A. Bolzer, H. Albiez, P.A. Benedetti, S. Müller, M. Speicher, T. Cremer, M. Cremer, I. Slovei "Towards many colors in fish on 3D-preserved interphase nuclei," *Cytogenet. Genome Res.* 114: 367–378 (2006)