SPREAD ANALYSIS IN VIDEO-CONFOCAL MICROSCOPY RESOLVES BETTER, FASTER AND CHEAPER

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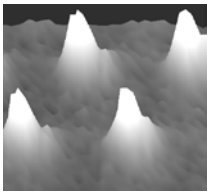
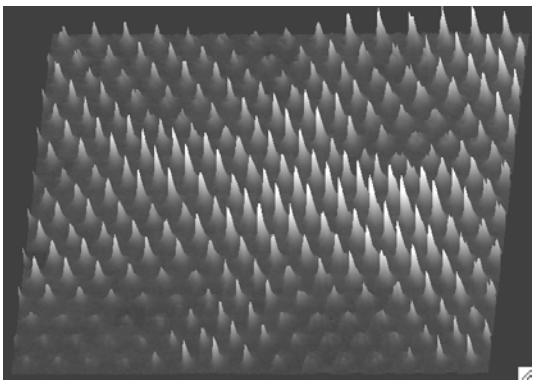
Recent advances in confocal microscopy newly stimulate the interests of users offering improved performance to emulate existing structured illumination designs aimed to diffractive superresolution. However, closer to the latter class, Video-Confocal Microscopy (VCM) based on multi-spot illumination and wide-field detection, yields higher parallelism warranting sub-diffraction resolution, lower photo-damage, higher speeds and substantial economies.

Being u and v the illumination scanning coordinates, the VCM information resides in emission peaks I(u,v) called “pixelets”, that describe in some detail the off-axis emission surrounding on-axis one, for each illumination spot.



4 enlarged central pixelets



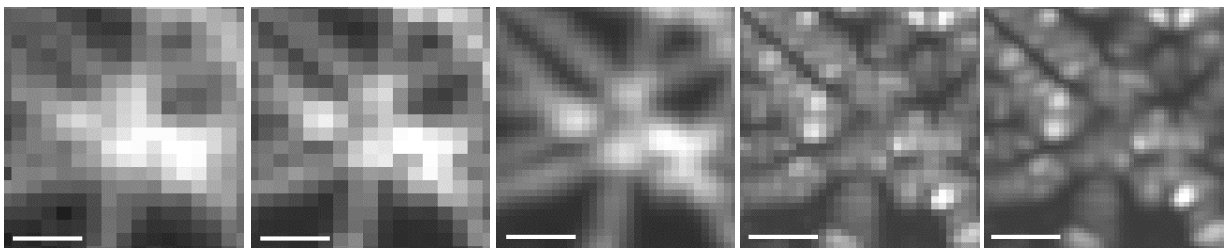
Pixelet array (U2OS fluorescent microtubules)

Being x and y the coordinates of specimen section, I(x,y;u,v) is the multidimensional function corresponding to an array of “pixelets”, collected during VCM raw images acquisition,

Depending on the specific algorithm adopted to process the set of raw images, the pixelet information contained in them permits to evaluate pixel intensities in the optical sections.

Some analogies exist in VCM with methods adopting an image sensor placed in the pinhole position. Data processing methods can recall and include pixel reassignment* strategies.

The following figures illustrate some results obtained from above pixelet array (bar = 500 nm)



WideField MaxProjection Confocal Super M9 Super M9*

During progresses of VCM in the last two decades, the so called super-confocal algorithms [1] were firstly introduced with simplifications and improved performance with respect to confocal microscopy especially regarding axial resolution down to better than 300 nm. Additional progresses were further achieved based on non-linear algorithms [2] exploiting the value of pixelet statistics and reaching almost isotropic lateral and axial resolutions, down to better than 100 nm. Practical instrumentation based on both principles is available in VCM systems offered in the international market [3]. Recent improvements to above mentioned detection strategies and related results will be briefly presented based on spread analysis tending to fully exploit the information contained in multidimensional data collected.

[1] P.A. Benedetti et al. “Method for the acquisition of confocal images” US Pat. 6,016,367 (filed 1997)

[2] P.A. Benedetti ”Improved confocal microscopy methods and devices” US Pat. *2015/0097942* Al (filed 2013)

[3] e.g. see: “X-Light VCS by CrestOptics” <www.crestopt.com/html/x-light_v2_vcs.html>