

PROS AND CONS IN STRUCTURED ILLUMINATION MICROSCOPIES

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During the past decade, a class of methods has been emerging in optical microscopy, based on narrow-field specimen illumination and wide-field image detection. Different terminologies have been adopted in specific cases, but Structured Illumination Microscopy or SIM is now widely used to indicate the above class of instruments.

Motivations of proposed SIM methods are multiple. On the one hand, there is the interest in more simply and cheaply achieving higher spatial resolutions and obtaining optical sections of thick specimens, avoiding the higher costs of lasers-based confocal microscopes. Some of the more rudimentary approaches, also developed industrially, belong to the above group not pretending to achieve really confocal results. On the other hand, other methods proposed can achieve truly confocal performance, even offering distinguished advantages in a multitude of applications in which usual confocal microscopes exhibit important limitations.

Apparently, in SIM, simplifications and advantages both derive from the elimination of the detection pinhole(s). As a result, specimen illumination can be distributed across the whole field thus permitting the more efficient use of conventional sources and camera sensors, respectively in place of laser and photomultipliers. Including costs, several important aspects can be significantly improved such as spectral flexibility and specimen photo-invasivity.

Spectral flexibility is certainly important in many practical uses of fluorescence microscopy, while using lasers not only is expensive but also spectrally very inflexible. Many needing to use multiple or specific fluorochromes, still avoid confocal microscopes. Eventually, these users resort to conventional microscopy joined with computed deconvolution techniques.

Specimen photo-invasivity can be another important issue in single-point scanning confocal microscopy because fluorescence saturation, bleaching and photo-decomposition are effects typically occurring when specimens are exposed to the very intense laser beam.

In conclusion, it's worth underlining that in SIM techniques all the radiation emitted by the specimen can be collected and virtually no information has to be lost outside a pinhole. It has been shown that properly taking advantage of that information, can offer important improvements tending to superresolution. Video-Confocal Microscopy (VCM) practically demonstrate some of the above possibilities. In VCM part of the information contained in the radiation surrounding the original pinhole position is included into the detection and image formation process, improving sensitivity, noise rejection and spatial resolutions.