RIM and extension for subcellular dynamics on tissue or medium content screening.

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We describe variance-based stochastic super-resolution imaging for live cell imaging with super-resolution comparable to the best 3D SIM. The method consists of processing multiple images of the sample under different illumination conditions. The autocorrelation function of these images is controlled. We show that in the case of speckle illumination, where the speckle correlation length coincides with the width of the observation point spread function, there is clearly a twofold increase in resolution [2]. Using a variance-matching algorithm called AlgoRIM [2-3-4-5-6], the super-resolved reconstruction is obtained numerically from the variance of the speckle images and the autocorrelation function of the speckles. This method allows the combination of strong optical sectioning and super-resolution for biological samples with out-of-focus fluorescence. In addition, the method is not affected by optical aberrations on the excitation side, is linear with respect to brightness, and is compatible with multi-colour live cell imaging over long time periods [2-3-6-9]. We will show a practical implementation from the TIRF configuration [2] to projected RIM imaging of large volumes in a single setup [6]. Finally, we will show that the scope of variance-based fluorescence imaging can be significantly extended by extending the concept to illumination other than speckle. Live imaging from cell culture to tissue will be presented. The methods improve 3D imaging and the reproducibility of media content analysis in abnormal conditions such as expansion microscopy or organoids [7], or the study of focal adhesion dynamics of human osteoclasts on bone [8]. Finally, the extension of long-term imaging allowed us to identify for the first time a novel chromatin domain D that regulates the response to DNA double-strand breaks [9].

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