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Single Image Fourier Ring Correlation

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Abstract: We address (super)resolution assessment of light microscopy via Fourier Ring Correlation (FRC), based on a single camera image. Based on Poisson statistics we can split an image into two noise independent halves, and use this to compute the FRC. The technique is demonstrated on widefield, STED, ISM, and RCM modalities.

Abbe's diffraction limit, restricting the resolution of an imaging system to lengths scales on the order of λ/NA, with λ the wavelength and NA the Numerical Aperture of the imaging system, has been the cornerstone of optical imaging systems for over a century. The advent of super-resolution microscopy has resulted in numerous ways to circumvent the diffraction [1-4]. This has raised the question how the resolution should be assessed if the diffraction limit is no longer appropriate. Several years ago we have proposed the concept of Fourier Ring Correlation (FRC) for optical imaging and in particular for single molecule localization microscopy (SMLM) [5], which checks the internal consistency of the image across all length scales. The correlation approach needs two noise independent image of the same object, and is computed from the correlation between these two images, averaged over rings in Fourier space. This correlation is high at a certain spatial frequency when signal dominates and low when noise dominates. The image resolution is defined as the length scale where the correlation level drops below a suitably defined threshold value, usually 1/7. This approach works well with SMLM, as then there is a natural way to generate two independent images, i.e. splitting the time series dataset by simply grouping the total set of localization events in two sub-groups. The FRC has also been applied to conventional widefield fluorescence microscopy by us [5], and to STED in [6], but has the obvious drawback for those modalities that two images must be taken. Here, we propose a procedure that makes use of a single image acquisition, works for any sampling density, and is straightforward to apply. The method relies on the fact that modern day sCMOS or EMCCD cameras are shot noise limited. It turns out that there is a simple way to split the image signal in two noise independent halves for each pixel independently by random binomial splitting. This approach was suggested by York [7], and traces back to Fried [8]. Consider a single Poisson random variable n with rate μ, e.g. the photon count of a single pixel of an image. The observed photon count is split in two parts $n = n_1 + n_2$ according to binomial statistics with success probability $p = 1/2$. This procedure is repeated for all pixels of the image. The FRC computed from these two image halves we denote as "1FRC". We have compared the method on widefield and STED images to conventional FRC based on two acquired images, and found the two to be in good agreement. We further used 1FRC to compare ISM [9] and RCM [10] super-resolution techniques to conventional confocal scanning microscopy (see Figure 1), quantifying the resolution improvement of ISM and RCM over confocal scanning microscopy, and showing that this resolution improvement of ISM and RCM is similar.

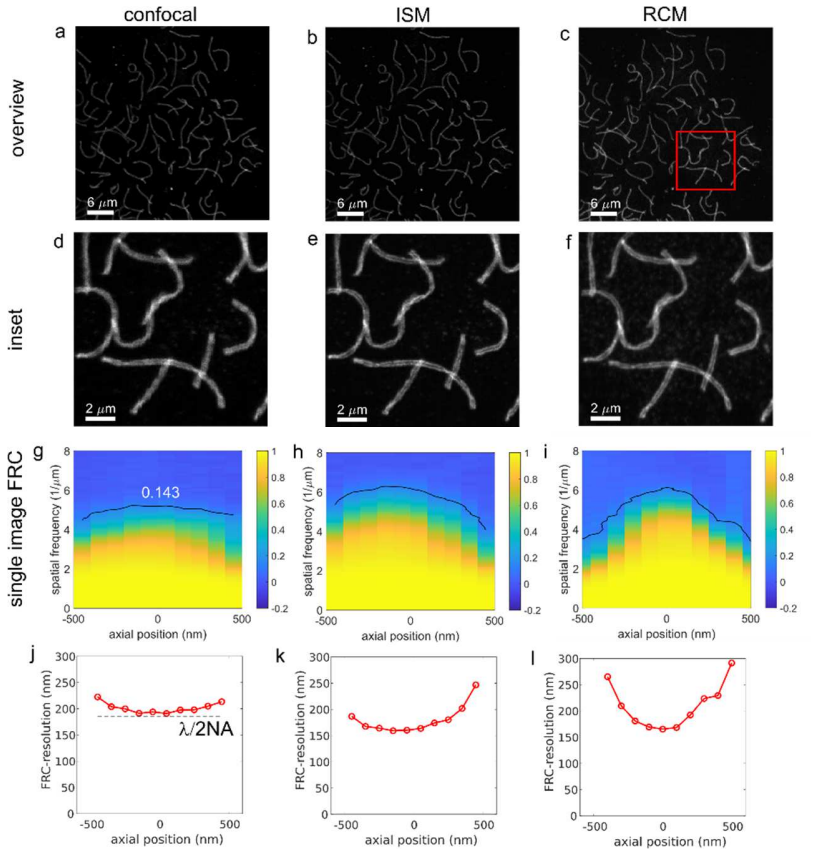

Fig. 1. Single image FRC for confocal, ISM and RCM technqiques. (a-c) Images of synaptonemal complex with conventional confocal, ISM and RCM. (d-f) Insets showing the similarly improved resolution of ISM and RCM compared to confocal. (g-i) Quantization of resolution by single image FRC for a through-focus stack for confocal, ISM, and RCM. (j-l) Single image FRC resolution values as a function of focus position for confocal, ISM, and RCM..

References

[1] T.A. Klar, S. Jakobs, M. Dyba, A. Egner, and S.W. Hell, Proc. Natl Acad. Sci. USA 97, 8206–8210 (2000).
[2] E. Betzig, et al., Science 313, 1642–1645 (2006).
[3] M.J. Rust, M. Bates, and X. Zhuang, Nat. Methods 3, 793–795 (2006).
[4] A. Sharonov, and R.M. Hochstrasser, Proc. Natl Acad. Sci. USA 103, 18911–18916 (2006).
[5] R.P.J. Nieuwenhuizen, K.A. Lidke, M. Bates, D. Leyton Puig, D. Grünwald, S. Stallinga, and B. Rieger, Nat. Methods 10, 557-562 (2013).
[6] G. Tortarolo, M. Castello, A. Diaspro, S. Koho, and G. Vicidomini, Optica 5, 32–35 (2018).
[7] A.G. York, https://twitter.com/AndrewGYork/status/974311139956875265, (2018).
[8] D.L. Fried, Appl. Optics 4, 79-80 (1965).
[9] C.B. Müller and J. Enderlein, Phys. Rev. Lett., 104, 198101 (2010).
[10] G.M.R. De Luca, R.M.P. Breedijk, R.A.J. Brandt, C.H.C. Zeelenberg, B.E. de Jong, W. Timmermans, L.N. Azar, R.A. Hoebe, S. Stallinga, and E.M.M. Manders, Biomed. Opt. Express 4, 2644-2656 (2013).