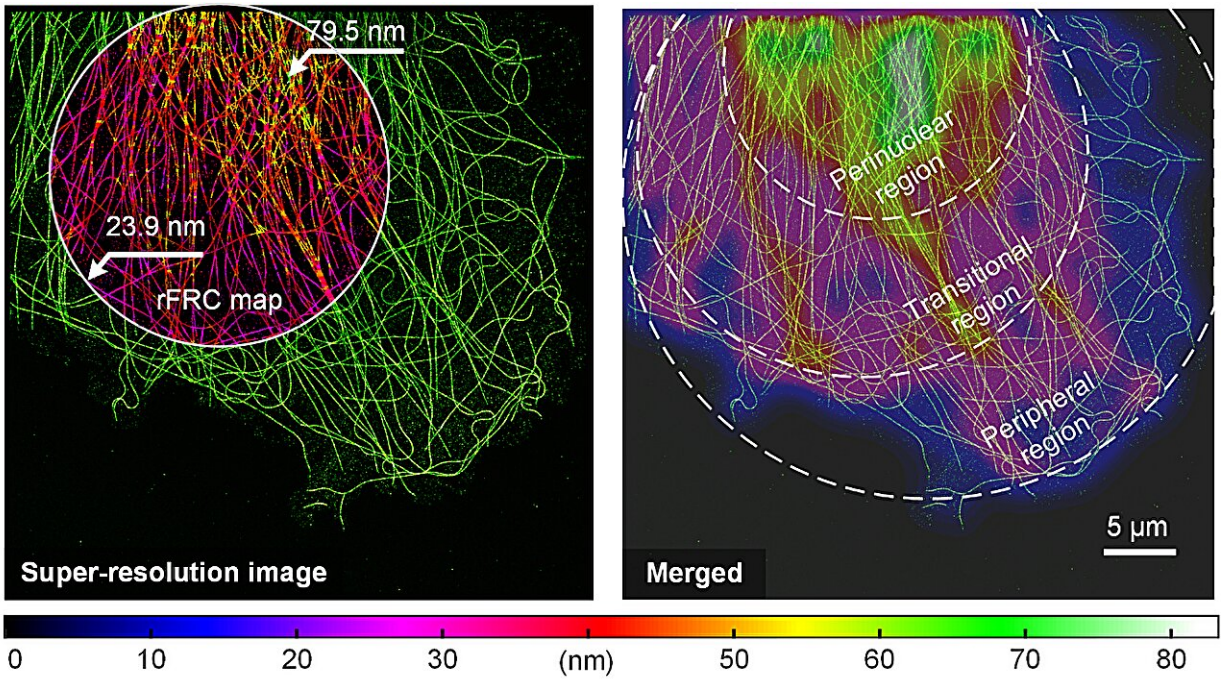
# Rolling Fourier ring correlation method maps local quality at super-resolution scale

January 23 2024




Detectingthe spatial resolution heterogeneity of super-resolution microscopy by using rFRC map. Credit: Weisong Zhao, Xiaoshuai Huang, Jianyu Yang, Liying Qu, Guohua Qiu, Yue Zhao, Xinwei Wang, Deer Su, Xumin Ding, Heng Mao, Yaming Jiu, Ying Hu, Jiubin Tan, Shiqun Zhao, Leiting Pan, Liangyi Chen and Haoyu Li

Super-resolution (SR) fluorescence microscopy, through the use of fluorescent probes and specific excitation and emission procedures,

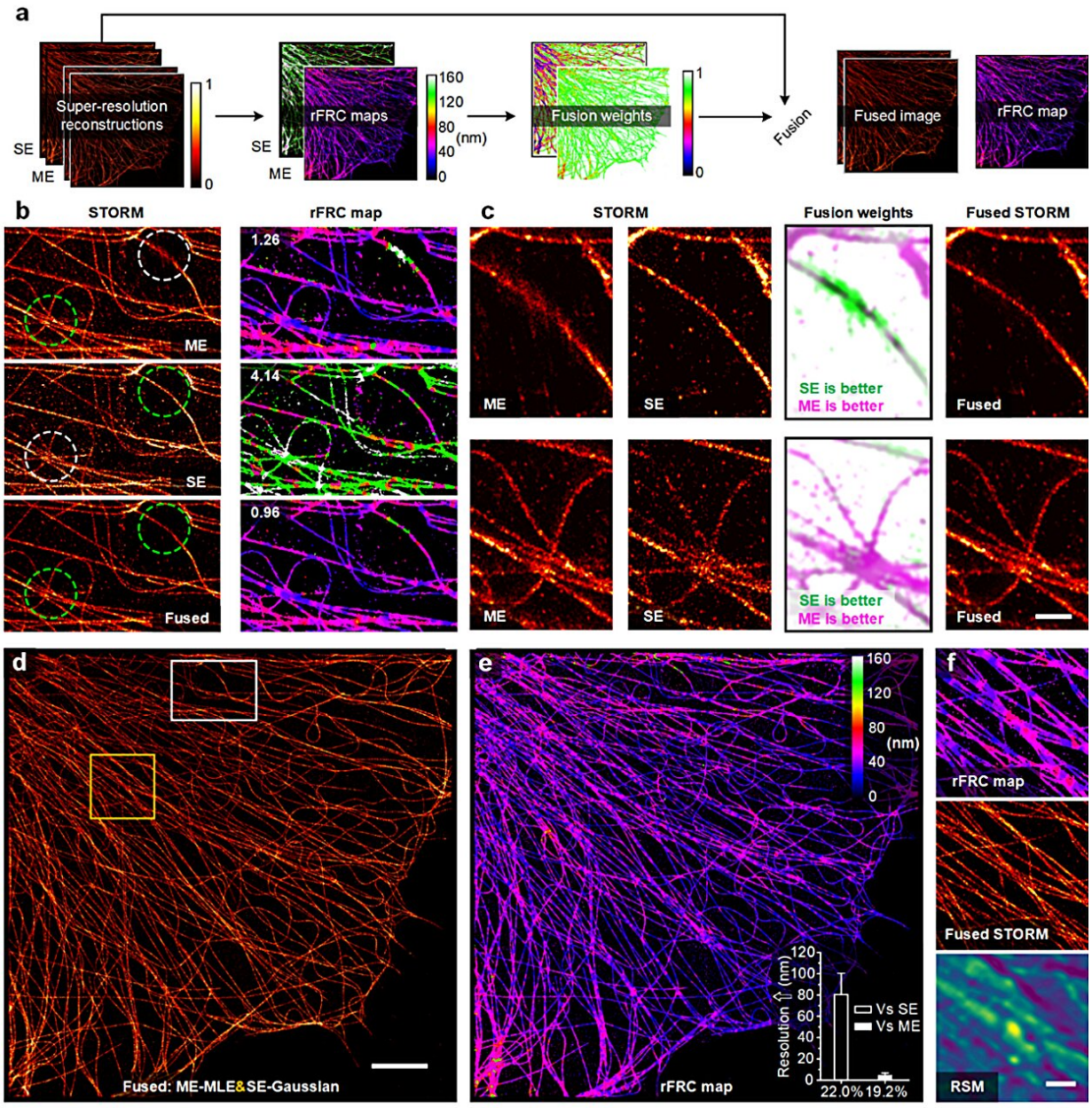
surpasses the diffraction limit of resolution (200~300 nm) that was once a barrier.

Most SR techniques are heavily reliant on image calculations and processing to retrieve SR information. However, factors such as fluorophores photophysics, sample's chemical environment, and optical setup situations can cause noise and distortions in raw images, potentially impacting the final SR images' quality. This makes it crucial for SR microscopy developers and users to have a reliable method for quantifying reconstruction quality.

Due to the increased resolvability of SR imaging, a thorough evaluation is necessary, yet existing tools often fall short when the local resolution varies within the field of view.

In a study published in *Light: Science & Applications*, a team of scientists have introduced a novel method known as the rolling Fourier ring correlation (rFRC). This method facilitates the representation of resolution heterogeneity directly in the Super Resolution (SR) domain, thereby enabling mapping at an unparalleled SR scale and an effortless correlation of the resolution map with the SR content.

In addition, the team developed an improvement on the resolution scaled error map (RSM), resulting in more accurate systematic error estimation. This was used in tandem with the rFRC, creating a combined technique referred to as PANEL (Pixel-level Analysis of Error Locations), which focuses on pinpointing low-reliability regions from SR images.




(a) Schematic of the STORM fusion. 'ME': Multi-emitter MLE result; 'SE': single-emitter Gaussian fitting result. (b) STORM results (COS-7 cells, α-tubulin labeled with Alexa Fluor 647, left) and their rFRC maps (right) are shown from top to bottom, which are magnified views of the white box in (d). From top to bottom: 'ME' result; 'SE' result; the fused result from the 'ME' and 'SE' reconstructions. The corresponding rFRC values are marked on the top left of the rFRC maps. (c) Magnified views of the dashed circles in (b). From left to right: ME results, SE results, fusion weights (inverted rFRC maps of ME results

and SE results merged as green and magenta channels, respectively), and fused STORM results. (d) The entire view of the fused STORM result (COS-7 cells, α-tubulin labeled with Alexa Fluor 647). (e) rFRC map of (d). The inset shows the improved resolution achieved by fusion compared with the SE (80.55 ± 1.52 nm at 22.0% region, hollow) and ME (4.28 ± 0.14 nm at 19.2% region, white solid) results. (f) Enlarged regions enclosed by the yellow box in (d). The results of the rFRC map, fused STORM, and RSM are shown from top to bottom. scale bars: (b, c) 500 nm; (d) 5 μm; (f) 1 μm. Credit: Weisong Zhao, Xiaoshuai Huang, Jianyu Yang, Liying Qu, Guohua Qiu, Yue Zhao, Xinwei Wang, Deer Su, Xumin Ding, Heng Mao, Yaming Jiu, Ying Hu, Jiubin Tan, Shiqun Zhao, Leiting Pan, Liangyi Chen & Haoyu Li

The scientists successfully applied PANEL in a variety of imaging approaches, including Single-Molecule Localization Microscopy (SMLM), Super Resolution Radial Fluctuations (SRRF), Structured Illumination Microscopy (SIM), and deconvolution methods, verifying the effectiveness and stability of their quantitative map.

PANEL can be used to improve SR images. For instance, it has been effectively used to fuse SMLM images reconstructed by various algorithms, providing superior quality SR images.

In anticipation of their method becoming a staple tool for local quality evaluation, the team has made PANEL accessible as an open-source framework. Related libraries for MATLAB and Python are available, as well as a ready-to-use Fiji/ImageJ plugin on GitHub.

Further details about this promising technique can be found in a behind-the-scenes post written by the core team member Weisong Zhao, accessible here.

**More information:** Weisong Zhao et al, Quantitatively mapping local

quality of super-resolution microscopy by rolling Fourier ring correlation, *Light: Science & Applications* (2023). DOI: 10.1038/s41377-023-01321-0

Provided by Chinese Academy of Sciences