

# RECONSTRUCTION OF IMAGE SCANNING MICROSCOPY DATA USING THE THICK SLICE METHOD AND LINEAR UNMIXING

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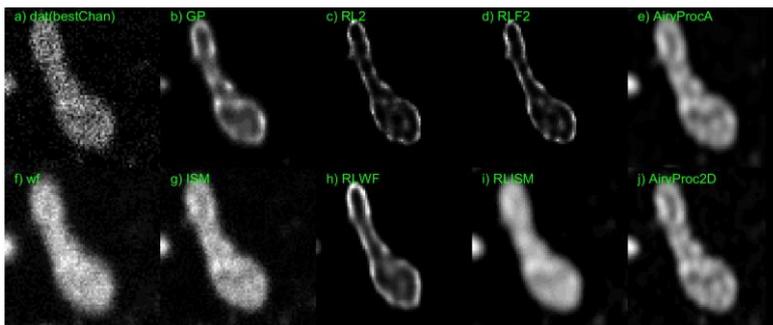
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**KEY WORDS:** super-resolution (SR), image reconstruction, fluorescence, imaging theory Image Scanning Microscopy (ISM), as suggested by Sheppard et Al [1] and first realized by Müller & Enderlein [2], changed the paradigm for Confocal Laser Scanning Microscopy (CLSM) image acquisition by achieving super-concentration of light with supreme Signal-to-Noise (SNR)[3]. ISM is superior in resolution when compared to CLSM at equivalent SNR-levels. By introducing a 32-channel GaAsP-PMT array and sampling 1.25AU of the system-PSF the ISM principle can be used for fast-live cell-imaging [4]. The commonly described reconstruction by photon reassignment does not possess any optical sectioning capability, even though each of the raw data channels clearly does.

An algorithm description of 2D scan data from 3D samples which achieves good resolution and section is still missing to our knowledge. Here we compare several reconstruction approaches to ISM-datasets including those of the commercial AiryScan reconstruction based on the same raw data. Even though multi-view iterative Maximum likelihood deconvolution yields a superior reconstruction, the additional quality gain by using more than 5-7 views in the reconstruction becomes negligible and computationally overly expensive. Finally, we describe a method which reconstructs a 3D-stack („thick slice“) – even though only a 2D-slice was acquired – with the aim to present only the in-focus slice of this thick slice reconstruction to the user. [5]



**Figure 1 - Mitochondria imaged with AiryScan system at 63x oil-immersion 1.4NA objective. Graphs are normalized to individual maximum for visibility. Different reconstruction methods are compared: a),f),g) Deconvolution free methods, b)-e), h)-j) deconvolved objects.**

## References:

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