

Project #17: Philip Santangelo and Peng Xi: *Subdiffraction limited imaging of single RNA sensitive probes using STED microscopy*

One of the primary limitations of conventional widefield or confocal fluorescence microscopy is the diffraction limit; light of a given wavelength focused by a lens, cannot discern objects any closer together than a fixed distance, because diffraction blends their images into a single blur. In the same way, light cannot be focused more sharply than to a spot of $d \approx 200$ nm in diameter and 400–700 nm in axial length, which corresponds to the resolution limit of basically all far-field light microscopes. Fortunately, in the last 20 years, and especially in the last 10, a number of solutions to the problem of diffraction for fluorescence microscopy have emerged. One of the leading methods is commonly referred to as STED, stimulated emission depletion optical nanoscopy. It has advantages over other subdiffraction methods in that is primarily optical in nature, therefore no image processing is needed, and can be used in thick samples. To date STED has not been applied to RNA imaging due to a lack of compatible probes; STED requires probes to be exceptionally bright and utilize fluorophores compatible with the optical processes it utilizes. The probes and strategy recently developed in the Santangelo lab (multiply-labeled tetravalent RNA imaging probes, MTRIPs), given their brightness and use of compatible fluorophores, should be ideal candidates for STED. In this grant application the expertise in optical microscopy of the Xi lab and in RNA probe engineering and delivery of the Santangelo lab will be brought together in the application of STED to the imaging of MTRIP tagged RNA with subdiffraction resolution.