

FLUORESCENCE IMAGING

I. Fluorescence-imaging with diffraction limited spots

The resolution in optical microscopy has been hampered by the smallest spot possible ($\sim \lambda/2$) that can be achieved by conventional methods.

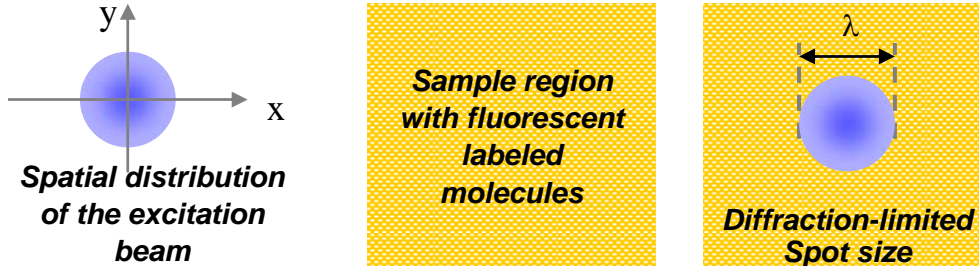


Fig.1. Excitation with a diffraction limited beam. The minimum area that can be excited is determined by the ability to create a spot with the minimum size.

II. High resolution fluorescence-imaging

A key aspect is to find a way to reduce the number of fluorescently labeled molecules that are excited simultaneously. Here we mentioned a couple of methods that have been successfully applied.

II.1 Stimulated Emission depletion (STED)

In this approach, the effective size of the exciting beam is reduced by quenching (reducing) the fluorescence emission of the fluorophores located in the periphery of the excitation beam with a doughnut-shaped beam.

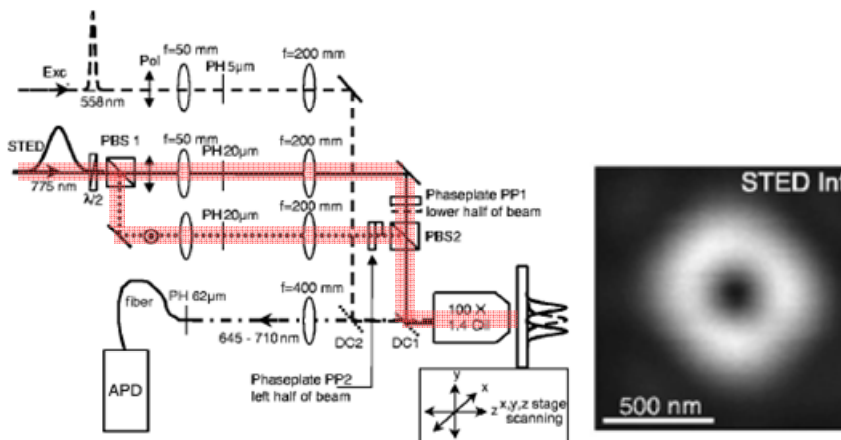


Fig.2 Experimental setup (left) for creating a doughnut shape beam Right) for quenching the fluorophore emission.¹

Although the application of a laser to quench the fluorescence may appear counterintuitive, this is indeed what happens (as will be explained below.) As a result, the effective area of excitation is reduced. “The result is akin to sharpening a pencil to draw finer lines. By scanning the ‘sharpened

spot over the sample, an image is built pixel by pixel, with a resolution currently down to 20 nm.” [Ref. F. Pinaud and M. Dahan, 2008]

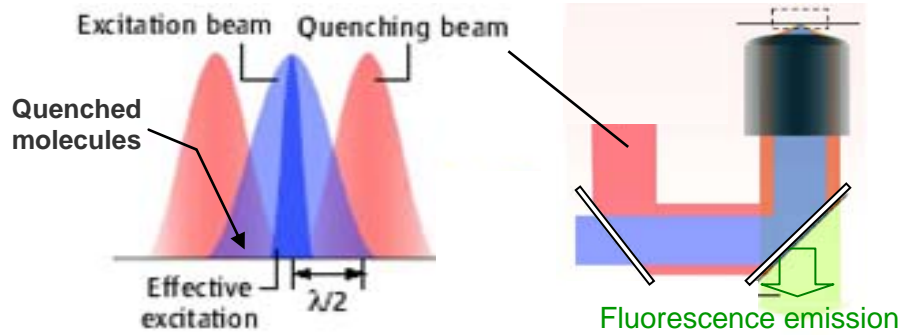


Fig.3 At the focal point, the effective excitation beam is much narrower than λ .² A key feature in STED is that, it turns out, the effective quenching process depends non-linearly with the intensity of the quenching beam.

¹ Thomas A. Klar, Egbert Engel, and Stefan W. Hell, “Breaking Abbe’s diffraction resolution limit in fluorescence microscopy with stimulated emission depletion beams of various shapes,” *Phys. Rev.* **64**, 066613 (2001).

² Fabien Pinaud and Maxime Dahan, “Zooming Into Live Cells,” *Science* **320**, 187 (2008).