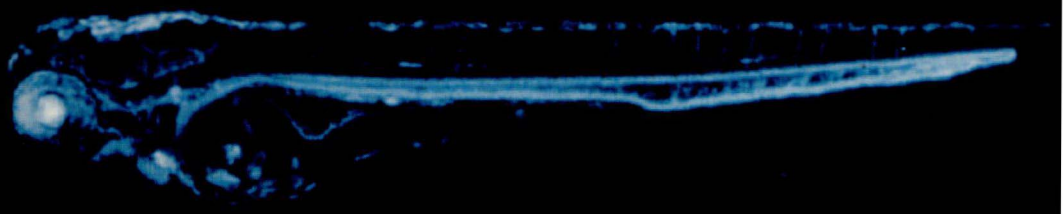
Optical Nanoscopy and Novel Microscopy Techniques



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Preface

Microscopy is at the forefront of multidisciplinary research. It was developed by physicists, made specific by chemists, and applied by biologists and doctors to better understand the function of the subunits of the human body. For this very reason, the field has been revolutionized constantly in past decades.

The objective of this book is to choose some of those revolutionary ideas and help a general audience from broad disciplines to achieve a fundamental understanding of these technologies and to better apply them in their daily research. It is organized as follows: Chapters 1–3 are on super-resolution optical microscopy, in which the targeted modulation, such as STED and SIM, or the localization methods, such as PALM, are discussed; Chapters 4–7 are on the novel development of fluorescent probes, such as organic small-molecule probes, fluorescent proteins, and inorganic labels such as quantum dots; Chapters 8–10 are about advanced optical microscopy, such as fluorescence lifetime imaging, fiber optic microscopy, scanning ion conductance microscopy, and the joining of optics and acoustics—photo-acoustic microscopy. Following each chapter is a detailed list of references so that the readers can use this book as a review of the relevant fields. In addition, to confirm understanding of the materials presented, we provide several problems after each chapter.

I express my deepest appreciation to the contributors of each chapter. Usually readers only remember the name of the editor, but the contributors are the real teachers and where the credit is due. I thank Li-Ming Leoung for her invitation to edit the book and for continuous support during the manuscript's preparation. I thank Amit Lal for proofreading the manuscript. I would like to thank all the CRC editorial team for their professional service, which is essential for the book to reach readers. Also, my sincere gratitude to my wife, Guanqin Chang, and our daughter, Yueming Xi, for their understanding and for sacrificing their quality time with me.

And thank you, my dear reader, for choosing this book. I guarantee that it will be a delightful and resourceful journey.

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chapter one

Optical nanoscopy with stimulated emission depletion

Peng Xi, Hao Xie, Yujia Liu, and Yichen Ding

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1.1 Introduction

Since its invention in the seventeenth century, optical microscopy has been applied to biological study. For the past 400 years it has been at the forefront of biological study, from the macroscopic level to the microscopic, cellular level. For "seeing is believing," optical microscopy has been widely extended to almost all disciplines of scientific research. Yet, due to the fact that optics has a wave nature, the diffraction limit becomes the resolution barrier. In the past decade, numerous super-resolution

techniques have been created. Stimulated emission depletion (STED) is one of the techniques that employs the feature of fluorescence to generate a smaller effective point spread function (PSF), or PSF engineering.

In this chapter, we first discuss the diffraction limit and its appearance as a PSF; then, the STED process in a single molecule energy level, as well as its behavior in a fluorescent medium. The combination of other techniques with STED, such as time-gating, iso-STED, STED and 4Pi, two-photon excitation, and self-aligned STED techniques are discussed. Next, the technical notes on the experimental instrumentation of STED are presented. Finally, the applications of STED to a variety of biological specimens are listed.

1.2 *Point spread function and diffraction limit*

1.2.1 *Diffraction limit criteria*

Before breaking it, we need to investigate how the barrier of optical diffraction limit is formed. We need to take a history tour, to a beautiful small town in Germany called Jena. We understand that the development of optics requires three key elements: theory, engineer, and material. The time was back in the 1870s, when three giants met magically here. Ernst Karl Abbe, an optical theory master; Carl Zeiss, a great founder of optical engineering; and Otto Schott, a pioneer in optical materials. They have made concerted efforts to lead optics to a higher level for the whole world. Nowadays, the enterprises founded by Schott and Zeiss, respectively, are still renowned around the world.

When light propagates through a barrier such as the aperture of objective, diffraction happens. With wave optics, one can describe the optical distribution of the focal plane as

$$I(0,\upsilon)=\left[\frac{2J_1(\upsilon)}{\upsilon}\right]^2 I_0, \tag{1.1}$$

where

$$\upsilon=\frac{2\pi}{\lambda}NA\cdot r,$$

$NA = nsin\alpha$ is the numerical aperture of the objective, and r is the radius of the point of interest. I_0 and λ are the intensity and wavelength of the incident beam, respectively. This gives us a Gaussian-like function as illustrated in Figure 1.1, called the point spread function (PSF), that is, the image (spread function) of a perfect point (or equivalently, a planar wave). It is known as Airy pattern, or Airy disk in the two-dimensional form.

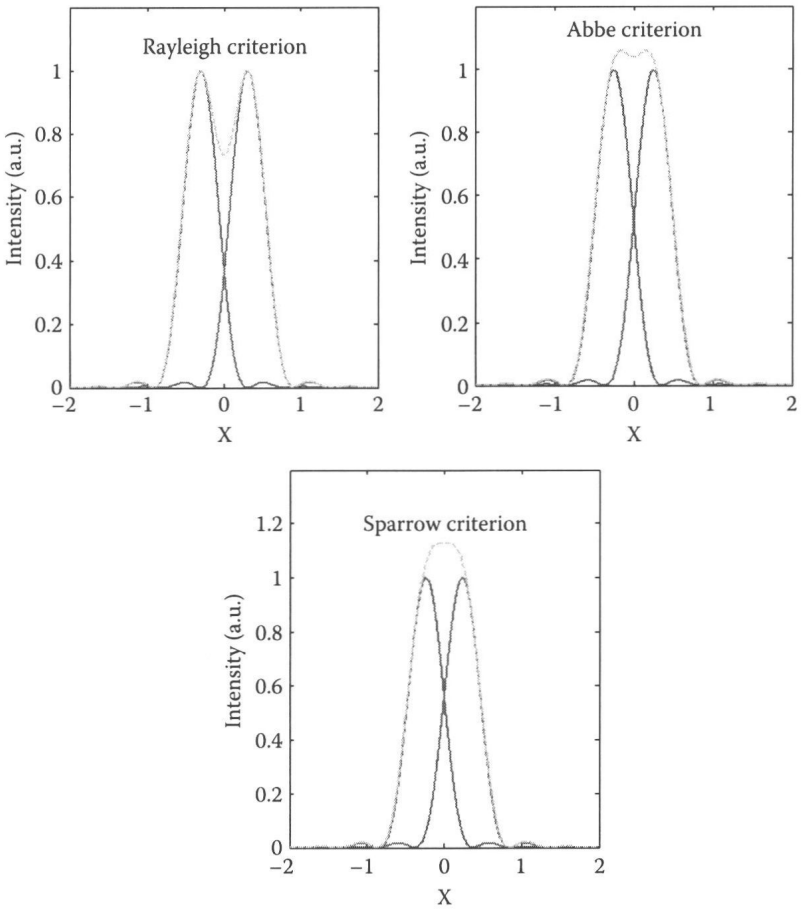

Figure 1.1 **(See color insert.)** Comparison of the various diffraction limit criteria.

As one can see immediately from the plot, the closer two PSFs are, the more difficult it is to separate them. Lord Rayleigh formulated the Rayleigh criterion (Lord Rayleigh 1896):

$$d_{\text{Rayleigh}} = 0.61\frac{\lambda}{NA} \tag{1.2}$$

This criterion is measured from the maximum of the PSF to the first minimum. More technically, the full width at half maximum (FWHM) is often used, which can be expressed as:

$$d_{\text{FWHM}} = 0.51\frac{\lambda}{NA} \tag{1.3}$$

By putting two PSFs at the distance of FWHM, one can notice that, there is a dip of ~3%. A more elegant expression is given by Abbe (Abbe 1873):

$$d_{\text{Abbe}} = \frac{\lambda}{2NA} \tag{1.4}$$

Clearly, to distinguish the two points, they cannot get too close to each other. However, there is still ambiguity as to how well these combined PSFs can be resolved. Alternative evaluations are like Sparrow's resolution limit (Sparrow 1919), which states that the resolution limit is where the dip disappears (becomes flat, or the second-order derivative equals zero).

$$d_{\text{Sparrow}} = 0.47\frac{\lambda}{NA} \tag{1.5}$$

In a confocal setup, a pinhole is placed before a point-detector (such as a photomultiplier tube, PMT, or an avalanche photodiode, APD). Under ideal conditions (the pinhole is infinitely small), the FWHM of such a system can be described as:

$$d_{\text{Confocal}} = \frac{0.51\lambda}{\sqrt{2}NA} \approx \frac{0.36\lambda}{NA} \tag{1.6}$$

Note that this indicates the detector cannot get any photon since its size equals to zero. Increasing the pinhole will get more signal, with a scarification of resolution.

1.2.2 PSF Measurement

The PSF of a confocal system is the excitation PSF modulated by the pinhole's modulation spread function (MSF):

$$\text{PSF}_{\text{Confocal}} = \text{PSF}_{\text{Excitation}} \cdot \text{MSF} \tag{1.7}$$

When the pinhole size is a point (delta function), the MSF equals the PSF at the fluorescent wavelength. Since practically the size of the confocal pinhole is not infinitely small, the MSF can be calculated with the two-dimensional convolution of the PSF, and the size of the pinhole is given by:

$$\text{MSF} = \text{PSF}_{\text{Detection}} \otimes \text{Pinhole} \tag{1.8}$$

where $\otimes$ denotes the convolution process.