

Resolution enhancement in a scanning optical microscope using array detection

SYNOPSIS SEMINAR REPORT

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S S Goutam Buddha
Roll no. : 146121020
Email: satya.buddha@iitg.ac.in

Supervisor
Prof. Bosanta R. Boruah

Doctoral Committee:
Prof. Pravat Kumar Giri (Chairman)
Dr. Gagan Kumar (Member)
Dr. A. S. Achalkumar (External Member)

Optical Imaging Laboratory
Department of Physics
Indian Institute of Technology Guwahati
Guwahati-781039, Assam



1 Introduction

An optical microscope is an imaging instrument that uses visible light illumination and a combination of lenses to obtain a high-resolution image of the object. Depending on the illumination type, optical microscopes are classified into two broad categories, namely, widefield microscope¹ and point scanning microscope². In the widefield microscope, usually an incoherent light source illuminates the specimen plane, and the entire specimen area is imaged simultaneously. Whereas in a point scanning microscope, each point of the specimen is illuminated and imaged in a sequential manner using some kind of a scanning mechanism. At the end of the scanning, image of each point is combined to construct the final image of the scanned area. The optical microscope has become an essential tool, owing to its far field imaging capability, in areas such as life science³, nanotechnology⁴, biotechnology⁵, pharmaceutical research⁶ and so on. It is especially very useful to view living biological samples for medical diagnosis, which is not possible with other powerful microscopes such as the electron microscope.

Resolution is a very important parameter of the microscope which represents the capability of the imaging system to distinguish two object points, placed close to each other, as two separate image points. When a point object is seen through a microscope, due to diffraction it no longer looks like a point rather like a diffraction pattern with finite width and is called point spread function (PSF). The minimum separation between two point objects in the specimen in the lateral direction, provided the microscope forms two distinguishable PSFs in the image plane, is called lateral resolution, while the same for two point objects in the specimen separated in the axial direction is called axial resolution. The lateral resolution of the microscope is primarily responsible for deciding the amount of finer details in the image, whereas the axial resolution decides the depth of field or amount of background light entering the image of a certain plane in the specimen. Higher axial resolution can thus lead to a property called optical sectioning in an optical microscope.

Therefore it is the PSF of the microscope that determines its resolution limit and the same is considered as a fundamental quantity to characterize the imaging performance of the microscope. The PSF of an optical microscope, hence, is vital in the calibration of the microscope in terms of quantifying the resolution. Besides it is useful in the post-acquisition image deconvolution to further enhance the quality of the image acquired. Deconvolution is a mathematical transformation of the image which reduces the out of focus information or blur present in the image data with the help of the PSF of the imaging system⁷.

Ernst Abbe suggested that the resolution of an optical imaging system cannot be more than the limit set by the diffraction phenomenon which is half of the illumination wavelength. However microscope users are finding many application areas where they need to go beyond the diffraction limit of resolution^{8,9,10}. In last few decades a number of indirect approaches have been developed which have attempted to push the resolution beyond the diffraction limit. Below we discuss a few such prominent approaches.

Confocal microscope is a special type of point scanning microscope which eliminates the out-of-focus information using a pinhole placed at the conjugate plane of the object plane and in front of the detector. The pinhole allows only the in-focus portion of the light from the specimen to reach the detector thus producing a high-resolution optically sectioned image of the specimen^{11,12}. However, the lateral resolution obtained from the confocal microscope is only marginally better than the conventional widefield microscope.

Although the use of array detector in a scanning optical microscope was proposed several decades back¹³, only recently the tremendous potential of array detection towards resolution enhancement has been realised. In image scanning microscope (ISM), the point detector of a conventional confocal microscope is replaced by an array detector (i.e. a digital camera) consisting of a two dimensional (2D) array of pixels. The array detector records an image of

the illumination beam for each scan position. At the end of the scanning, the microscope thus gathers a 2D array of image data for each pixel of the image. This provides the flexibility to perform a pixel re-assignment scheme to construct a final image with enhanced lateral resolution^{14,15}.

Resolution can be enhanced in a widefield microscope as well. One such example is structured illumination microscopy (SIM). Here the object is illuminated by a high spatial frequency grid pattern distribution instead of a uniform distribution of light. The high frequency content of the object can be overlapped with the grid pattern to produce Moiré pattern. By moving and rotating the grid pattern in different directions and taking the widefield images of the Moiré fringes, a final image can be constructed which has a two fold lateral resolution enhancement as compared to a widefield microscope^{16,17}.

Stimulated emission depletion (STED) microscopy is a technique that truly overcomes the diffraction limit in the fluorescence mode of optical microscopes by manipulating the effective emission volume in the illuminated region of the specimen. This is achieved by switching off the fluorescent molecules in the outer regions of the diffraction limited excitation volume via stimulated emission using an intense laser beam called STED beam. Fluorescence from only the narrow region is thus received by the detector to produce a super-resolution image of the specimen¹⁸.

Stochastic optical reconstruction microscopy (STORM) is yet another super resolved imaging technique applicable for a widefield microscope which relies on the stochastic switching of individual fluorophores. The random stochastic switching of fluorophores allows to record temporal separation of individual molecules. Then each fluorophore molecule can be precisely located by finding their position coordinates from the Gaussian fitting of each fluorophore image. The positions of all the fluorophores from a large number of image frames are combined to produce the final super-resolution image¹⁹.

In so far as applications are concerned scanning optical microscopes are particularly useful as they allow illumination with a specifically designed laser beam. However in order to achieve the optimal resolution the confocal pinhole needs to have a zero diameter, which then results in no signal received by the detector. Fortunately the use of array detection in a scanning optical microscope has provided much more flexibility to implement non conventional detection. One such approach namely the ISM, discussed already, provides for higher resolution without comprising the signal level. Therefore ISM is gaining popularity in the imaging of biological samples^{20,21} where signal level is usually less. In fact the four dimensional image data provided by such a microscope opens up the possibility of several other resolution enhancement techniques. Nevertheless scanning microscopes using array detection still face a number of challenges such as imaging speed²¹ which is limited by either the speed of the scanner or the frame rate of the camera, whichever is the lower. Precision and repeatability of the scanner, especially in case of a high speed operation, are another two important factors effecting performance of array detector based scanning optical microscope.

However, the array detection microscope still cannot provide true super-resolution images, which can be provided by a STED or a STORM microscope. On the other hand building a STED microscope is a tedious task as it involves a very complex experimental arrangement. In contrast the STORM microscope provides lateral resolution very close to that of the STED microscope while involving a much simpler and cost effective experimental arrangement. Therefore several research groups are using STORM as an alternative for super-resolution imaging technique²².

In this thesis, we are going to develop a scanning optical microscope with an array detector to achieve enhancement in lateral and axial resolutions in both the reflection and fluorescence mode of the microscope. We will use both a high speed CMOS camera and an ordinary camera, and both galvanometer based and holographic beam scanning mechanisms, to achieve an

optimal imaging speed without compromising on the other performance parameters. We will also develop a PSF estimation scheme to characterize the resolution enhancements achieved in the imaging systems. Usually, the STORM microscopes are not combined with beam scanning microscope, as these are two different microscopes. However if the two microscopes are combined it will offer the possibility of exploiting the benefits from both the versions for the same specimen being imaged. In this thesis we will attempt to build a combined form of a STORM and an array detector based scanning optical microscope.

Below, we provide a chapter-wise overview of the thesis.

1.1 Thesis overview:

- Chapter 1: This chapter provides a general introduction to the research problem and an overview of the thesis.
- Chapter 2: In this chapter we start with the principle of the optical microscope including the widefield and point scanning microscope. The theory of image formation in the widefield and confocal microscope is outlined, followed by a discussion on optical resolution and the optical transfer function. We then discuss the present state of the art in regards to resolution enhancement techniques in optical microscopy and motivation behind the present thesis.
- Chapter 2: This chapter aims to describe the important techniques and components associated with the beam scanning microscope developed in this thesis. We start with a discussion on the illumination beam shaping using the computer generated holography (CGH) technique. We explain how binary holograms can be implemented with a spatial light modulator to dynamically reconfigure the properties of a laser beam. This is followed by the descriptions of the important hardware components required to build the microscope. The chapter concludes with the illustration of the proof-of-principle experimental setup as an example of the proposed array detection microscope.
- Chapter 3: In this chapter, we introduce a PSF estimation scheme that can estimate the PSF of an optical microscope irrespective of the imaging technique involved. The chapter begins with the stepwise description of the proposed scheme along with a flowchart diagram. We then employ the proposed scheme on a one dimensional (1D) numerically constructed target followed by a two dimensional (2D) numerically constructed target. We also experimentally demonstrate the proposed scheme in a widefield and a point scanning microscope.
- Chapter 4: The chapter presents the implementation a beam scanning microscope with an array detector. We first develop an array detection microscope using galvanometer based beam scanner and a high speed camera, and then another array detection microscope exploiting the holographic beam scanning mechanism and a common low speed camera. We demonstrate implementation of image scanning microscopy for lateral resolution enhancement and image subtraction microscopy for axial resolution enhancement. We further introduce a novel intensity subtraction microscopy scheme for axial resolution enhancement. We present results from proof-of-principle experiments to show working of our imaging systems in both reflection and fluorescence modes.
- Chapter 5: In this chapter, we first describe the implementation of a STORM microscope to obtain super-resolution images of fluorescent specimens. We then combine the STORM with our array detection beam scanning microscope to enable super-resolved fluorescence imaging as well as reflected light imaging of the same specimen.

- Chapter 6: In this chapter, we provide a summary of the research work carried out. We also provide a discussion on the possibility of future works in connection with the imaging systems developed in this thesis.



References

- [1] J. Mertz, *Introduction to optical microscopy*. Cambridge University Press, 2019. [1](#)
- [2] T. Wilson and C. Sheppard, *Theory and practice of scanning optical microscopy*, vol. 180. Academic Press London, 1984. [1](#)
- [3] B. Matsumoto, *Cell biological applications of confocal microscopy*. Elsevier, 2003. [1](#)
- [4] N. Yao and Z. L. Wang, *Handbook of microscopy for nanotechnology*. Springer, 2005. [1](#)
- [5] K.-J. Halbhauer and K. König, “Modern laser scanning microscopy in biology, biotechnology and medicine,” *Annals of Anatomy-Anatomischer Anzeiger*, vol. 185, no. 1, pp. 1–20, 2003. [1](#)
- [6] R. A. Carlton, *Pharmaceutical microscopy*. Springer Science & Business Media, 2011. [1](#)
- [7] W. Wallace, L. H. Schaefer, and J. R. Swedlow, “A workingperson’s guide to deconvolution in light microscopy,” *Biotechniques*, vol. 31, no. 5, pp. 1076–1097, 2001. [1](#)
- [8] Y. Kawata and W. Inami, *High-Resolution Optical Microscopy for Biological Applications*, pp. 407–427. Dordrecht: Springer Netherlands, 2017. [1](#)
- [9] J. Vangindertael, R. Camacho, W. Sempels, H. Mizuno, P. Dedecker, and K. Janssen, “An introduction to optical super-resolution microscopy for the adventurous biologist,” *Methods and applications in fluorescence*, vol. 6, no. 2, p. 022003, 2018. [1](#)
- [10] Y. Garini, B. J. Vermolen, and I. T. Young, “From micro to nano: recent advances in high-resolution microscopy,” *Current opinion in biotechnology*, vol. 16, no. 1, pp. 3–12, 2005. [1](#)
- [11] G. S. Kino and T. R. Corle, *Confocal scanning optical microscopy and related imaging systems*. Academic Press, 1996. [1](#)
- [12] J. Pawley, *Handbook of biological confocal microscopy*, vol. 236. Springer Science & Business Media, 2006. [1](#)
- [13] C. R. SHEPPARD, “Super-resolution in confocal imaging,” *Optik (Stuttgart)*, vol. 80, no. 2, pp. 53–54, 1988. [1](#)
- [14] C. B. Müller and J. Enderlein, “Image scanning microscopy,” *Physical review letters*, vol. 104, no. 19, p. 198101, 2010. [2](#)
- [15] C. J. Sheppard, S. B. Mehta, and R. Heintzmann, “Superresolution by image scanning microscopy using pixel reassignment,” *Optics letters*, vol. 38, no. 15, pp. 2889–2892, 2013. [2](#)
- [16] M. A. Neil, R. Juškaitis, and T. Wilson, “Method of obtaining optical sectioning by using structured light in a conventional microscope,” *Optics letters*, vol. 22, no. 24, pp. 1905–1907, 1997. [2](#)
- [17] M. G. Gustafsson, “Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy,” *Journal of microscopy*, vol. 198, no. 2, pp. 82–87, 2000. [2](#)

- [18] S. W. Hell and J. Wichmann, “Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy,” *Optics letters*, vol. 19, no. 11, pp. 780–782, 1994. [2](#)
- [19] M. J. Rust, M. Bates, and X. Zhuang, “Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (storm),” *Nature methods*, vol. 3, no. 10, pp. 793–796, 2006. [2](#)
- [20] E. N. Ward and R. Pal, “Image scanning microscopy: an overview,” *Journal of microscopy*, vol. 266, no. 2, pp. 221–228, 2017. [2](#)
- [21] I. Gregor and J. Enderlein, “Image scanning microscopy,” *Current opinion in chemical biology*, vol. 51, pp. 74–83, 2019. [2](#)
- [22] J. Tam and D. Merino, “Stochastic optical reconstruction microscopy (storm) in comparison with stimulated emission depletion (sted) and other imaging methods,” *Journal of neurochemistry*, vol. 135, no. 4, pp. 643–658, 2015. [2](#)



Patents

1. * "A system and method for determining Point Spread Function of an imaging system ", S S Goutam Buddha and B R Boruah, Patent Application No.: 202031014419, 31 March 2020 ¹
2. "A system and method for laser beam scanning with periodic switching of polarization of the beam ", Ranjan Kalita, S S Goutam Buddha and B R Boruah, Patent Application No.: 201831006652, 21 Feb 2018

Journals

1. * S S Goutam Buddha, and Bosanta R. Boruah, " Estimation of point spread function of an optical microscope using stochastic minimization of least square errors ", Journal of Optics (IOP): 22 (5) , 055603 (2020) ¹
2. * S S Goutam Buddha, Ranjan Kalita and Bosanta R. Boruah, " Array detection in a holographic scanning microscope ", Optics Communications: 462 , 125339 (2020) ¹
3. Ranjan Kalita, S. S. Goutam Buddha and Bosanta R. Boruah, "A laser scanning microscope executing intraframe polarization switching of the illumination beam ", Review of Scientific Instruments: 89(9), 093705 (2018)

Proceedings

1. Karuna Sindhu Malik, S S Goutam Buddha, Ranjan Kalita, Bosanta R Boruah, " Estimation of maximum translation speed of a trapped particle in holographic optical tweezers " Proceedings of SPIE : 11297 112970Z, 2020
2. * S S Goutam Buddha, R Kalita, Bosanta R Boruah, " Optical sectioning microscopy with both mechanical and non-mechanical beam scanning mechanisms " IEEE Xplore, Advances in Photonics (WRAP) : 1-3, 2019 ¹
3. * S S Goutam Buddha, Bosanta R Boruah, " Signal to noise ratio enhancement in confocal microscope with an array detector " IEEE Xplore, Advances in Photonics (WRAP) : 1-3, 2019 ¹
4. * S. S. Goutam Buddha, Ranjan Kalita, and Bosanta R. Boruah, " Estimation of point spread function of an imaging system using a programmable target ", Proc. SPIE, 10499, 104991O (2018) ¹
5. Ranjan Kalita, S. S. Goutam Buddha, and Bosanta R. Boruah, " Suitability of holographic beam scanning in high resolution applications ", Proc. SPIE, 10499, 104991P (2018)
6. Ranjan Kalita, S. S. Goutam Buddha, Bosanta R Boruah, "Laser scanning confocal microscopy using illumination beams with different polarizations in quick succession " Proceedings of SPIE : 104772 107720I, 2018

¹* N.B. Publications are included in the thesis

Awards

1. Received a grant of GBP 2000 for visiting Imperial College, London as a part of the open Scope project funded by the Imperial College Global Challenge Research Fund for implementing a low-cost super resolution microscope.
2. Received second best poster for the paper entitled "Development of a standalone confocal imaging system with CGH based as well as galvanometer scanner based beam scanning Mechanism", at the Research Conclave 2018, held on 8-11 March 2018 organised by Students' Academic Board in association with IIT Guwahati.
3. Received "SPIE Student Officer Travel Grant" (USD 3100) to attend "SPIE Photonics West -2018" at San Francisco, California, USA from 27 January-01 February, 2018, organized by SPIE.
4. Received second best poster award for the paper entitled "Development of a Scanning Optical Microscope with a Holographically Designed Illumination Beam", at the International Conference on Advances in Optics and Photonics (ICAOP-17), held on 23-26 November, 2017, organized by Guru Jambheshwar University of Science and Technology, Hisar (Haryana), India in association with OSI (Optical Society of India).

Conference presentation

1. Participated and presented two paper entitled "Signal to noise ratio enhancement in confocal microscope with an array detector" and "Optical sectioning microscopy with both mechanical and non-mechanical beam scanning mechanisms", in the "IEEE Workshop on Recent Advances in Photonics (WRAP) 2019", organised by Department of Physics, IIT Guwahati at IIT Guwahati, Guwahati, during Dec 13 - 14, 2019.
2. Participated and presented a paper entitled, "Effect of Aberration on Resolution in an Optical Scanning Microscope", in the "Photonics 2018, The International Conference on Fiber Optics and Photonics", organized by Indian Institute of Technology Delhi, New Delhi at IIT Delhi, during December 12 - 15 2018.
3. Participated and presented two paper entitled "Suitability of holographic beam scanning in high resolution applications" and "Estimation of point spread function of an imaging system using a programmable target", in the "SPIE Photonics West 2018", organised by SPIE at San Francisco, California, USA, during Jan 27 - Feb 01, 2018.
4. Participated and presented a paper entitled "Development of a Scanning Optical Microscope with a Holographically Designed Illumination Beam " in the "International conference on advances in optics and photonics (ICAOP - 2017)", organized jointly by Guru Jambheshwar University of Science & Technology and Optical Society of India at Guru Jambheshwar University of Science and Technology, Hisar, during November 23 - 26, 2017.
5. Participated and presented a paper entitled "Investigation of Beam Stability in a Galvanometer Based Scanning Mirror System" in the "International conference on light and light based technologies (ICLLT - 2016)", organized jointly by Tezpur University and Optical Society of India at Tezpur University, Tezpur, during November 26 - 28, 2016.

School/ Workshop attended

1. Participated in the course on "Advanced microscopy and imaging techniques", jointly organized by DSS imagetech Pvt. Ltd., Olympus medical systems India Pvt. Ltd. and supported by Indian Institute of Technology Guwahati at IIT Guwahati, Guwahati, during, April 18 - 20, 2017.
2. Attended in the DST-SERB School on "Modern Optics & Its Applications", organized by Indian Institute of Technology Patna (IITP), Bihar at IIT Patna, Patna, during, November 30 - December 18, 2015.
3. Participated in the "IEEE Workshop on Power, Mechatronics, Robotics and Control" (PMRC 2015), organized by IEEE student branch IIT Guwahati and National Instruments at IIT Guwahati, Guwahati, during August 29 - 30, 2015.

