Development of a Laser Scanning Confocal Microscope with Programmably Switchable Vector Beam Illumination

Synopsis Report

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1 Introduction

Laser scanning confocal microscopy (LSCM) is a powerful imaging tool for obtaining high contrast optically sectioned images. The optical sectioning property is achieved by keeping a spatial filter (a pinhole) at a plane conjugate to the illumination spot in the specimen plane to obstruct the out of focus light from the volume of a thick specimen [1–3]. One may also capture a large number of thin optical sections out of a thick specimen to build an impressive three-dimensional view of the same using a confocal microscope. The ability of the confocal microscope in the fluorescence mode to image deep inside the tissue in a noninvasive manner has made the microscope extremely popular for biological applications, such as fluorescence imaging of living cells and tissues, [4, 5]. There are reports on scanning laser ophthalmoscopy in animal models for in vivo confocal imaging of the retina. [6]. Owing to enhanced imaging capabilities, LSCM also finds application in the studies of crystal morphology [7], material deformation [8], food products [9, 10], and so on.

The polarization property of the illumination beam is often exploited in optical microscopy to extract additional information on molecular organization in the specimen. This relies on the fact that, upon illuminating the specimen, the excitation or absorption efficiency of the molecules depends on the angle between the molecular dipole moment and the excitation electric field. Thus amount of fluorescence from a molecule varies based on this angle, and the same gives an indication of the molecular orientation relative to the excitation field. On the other hand, when a light beam is focused by a lens, the electric field orientation at the focus directly depends on the polarization profile of the beam at the entrance pupil of the objective lens [11, 12]. Thus, the electric field orientation at the focus to excite the molecules in the specimen can be modified by controlling the polarization profile at the entrance pupil. The polarization based optical microscope has found applications in the studies of molecular structure [13–18], protein function and tissues [19–23], exciton fine structure of nanocrystals [24], properties of fluorescent nanoemitters [25], topological defects in liquid crystal materials [26], and so on. The differential polarization laser scanning microscope (DP-LSM) is the popular polarization based microscopy technique for study of molecular orientation of the specimen [16, 27–29]. It involves imaging of the specimen using two orthogonal linearly or circularly polarized beams. The difference of the images corresponding to the two polarizations of the illumination beam is the measure of the linear dichroism (LD), that yields a two-dimensional mapping of the anisotropic optical organization of molecules in the specimen. Therefore, for complete exploration of the three dimensional spatial orientation of the molecules, one need to image the specimen with three mutually orthogonally polarized illumination beam.

In the last few decades, several techniques have been proposed to modulate the polarization of the illumination beam in an optical microscope. Many of these techniques use an electro-optic modulator such as Pockels cell [27, 30, 31], or magneto-optic modulator such as Faraday rotator [32] to modulate the polarization of the illumination beam using different classical polarization states. However, the accuracy of producing a particular plane of polarization of light beam by Pockels cell or Faraday rotator depend on the stability of the

externally applied field; that is, the stability of the modulation voltage applied across the Pockels cell or that of the longitudinal component of the magnetic intensity applied across the Faraday rotator. A combination of two liquid crystal spatial light modulators (LCSLM) [11], or a universal compensator made up of liquid crystal variable retarders [33–36] can be used to produce two dimensional spatial distribution of polarization of the illumination beam in a microscope. However, the methods discussed till now only employ the inter-frame polarization switching; that is, the polarization of the illumination beam is switched after acquiring the entire image frame of the specimen area. This technique leads to a delay equal to at least the acquisition time of one complete image frame, between the imaging with two different polarizations. For extraction of detail information on molecular organization in a specimen, which is in a dynamic environment, one has to reduce the above delay. There are a few techniques that enable to switch the polarization of the illumination beam after every line scanned [27, 29, 37], where polarization modulation is achieved by using Pockels cell [27] or photoelastic modulators (PEM) [29, 37]. But the methods can generate and alter the polarization of the illumination beam among the classical polarization states only, which are plane polarized, circularly polarized, and elliptically polarized. Also, for real-time monitoring of molecular order in a dynamic environment, a high-speed spinning disk can be utilized to improve the image acquisition rate of a polarization based optical microscope [31]. However, the performance of the spinning disk suffers from the pinhole-cross talk, as unwanted scattered or fluorescence light from the specimen can still enter the detector through adjacent pinholes.

The programmability of a liquid crystal spatial light modulator can be exploited to generate a user-defined two dimensional spatial polarization profile of a light beam, called arbitrary vector beam. A combination of two [38] or three LCSLMs [39], or one LCSLM along with a Wollaston prism [40, 41], or double passing of the beam through a single LCSLM [42], one can produce a two-dimensional spatial distribution of polarization in a light beam. However, until now, in a microscope that uses LCSLM to modulate the polarization of the illumination beam employs the switching of polarization of the illumination beam after every image frame only.

The LCSLM based vector beam forming schemes usually involve two successive incidence on one or two display panels [38, 39, 42]. Thus the final beam undergoes two diffraction losses after each incidence on the LCSLM. The energy in the final vector beam is decided by the maximum power of the beam that corresponds to the first incidence. However all commercial LCSLMs have a fixed damage threshold in so far as the incident power is concerned [43]. Therefore the maximum power of the vector beam generated using an LCSLM based scheme is limited by the damage threshold of the device. Such scheme if employed in a confocal microscope will not be suitable for applications needing high power vector beam to illuminate the specimen.

In general, to capture the full-frame image of the specimen, the confocal microscope uses a galvanometer mirror scanner to scan the laser beam all over the imaging area in a raster fashion [44]. However, high-speed operation of such scanners can lead to temperature variations [45], thereby affecting the stability and accuracy of the scanner [46]. These is-

sues may affect applications, especially those involving weak signals from the specimen [47], thereby requiring long time acquisition [48] or those involving a time-lapsed study of the specimen [49].

Considering the above issues, the present thesis discusses the development of an LC-SLM based division of wavefront based assembly for the generation of an arbitrary vector beam. The generation of the desired arbitrary vector beam is a result of the coherent addition of two orthogonally polarized beams. The two said beams are generated using computer generated holography (CGH) technique in such a way that the complex amplitude profile of the beams represent the two orthogonally polarized pupil planes corresponding to the desired vector beam. The proposed setup has an additional advantage in terms of the generation of higher average-power vector beams.

We propose a laser scanning confocal microscope using the LCSLM based arbitrary vector beam forming unit in conjunction with a galvanometer based scanning unit, a detector unit, and a microprocessor baser control unit. The LCSLM assembly, along with other units facilitate intraframe polarization switching; that is, the illumination beam polarization profile is switched at the end of each line scanned, thereby considerably reducing the time in illuminating a particular portion of the specimen with two different polarizations. We propose a few schemes depending on the number of different illumination beam polarizations generated and the switching manner of the polarization profile to scan the specimen. A proof of principle experiment is carried out using epi-illumination reflectance mode to demonstrate the working of the proposed microscope utilizing two of the suggested schemes for switching between different polarizations of the illumination beam.

We then further develop the proposed microscope enabling the quick polarization switching of the illumination beam among different polarizations to achieve imaging using fluorescence light. A hologram written on a liquid crystal spatial light modulator can act as a diffraction grating [50–52] to achieve beam steering with superior stability and repeatability. In this thesis such a computer generated holography based beam scanning scheme is exploited to implement a scanning microscope that works in both the reflection and fluorescence light imaging, besides providing superior beam repeatability relative to galvo mirror based scanning.

2 Thesis overview

Below, the proposed chapter-wise overview of the thesis is provided.

Chapter 1: This Chapter will provide a general introduction of the research problem and an overview of the thesis.

Chapter 2: This Chapter will start with a brief description of the principle and operation of the laser scanning confocal microscope, followed by a literature review in the

area relevant to the research work related to the thesis. The chapter will be concluded with a detail description of the important hardware components required to build an LSCM for the purpose the experimental work related to the thesis.

Chapter 3: In this chapter, we will introduce computer generated holography and discuss the liquid crystal spatial light modulator used to implement the CGH technique. This will be followed by a description of the use of CGH to generate an arbitrary scalar beam, a beam with user-defined complex amplitude profile. Finally, the chapter will describe the implementation of CGH in an optical arrangement to generate an arbitrary vector beam.

Chapter 4: This chapter will provide a discussion on the existing techniques followed by the introduction of our proposed LCSLM based setup for the generation of an arbitrary vector beam. We will also provide a description of the displacement theorem to perform an axial shift of the three-dimensional focal volume intensity distribution. This chapter will be concluded by demonstrating the experimental results to validate the working of the proposed vector beam forming setup.

Chapter 5: This chapter will introduce a laser scanning confocal microscope facilitating quick switching of the polarization profile of the illumination beam. Here, we will briefly explain three schemes for intraframe polarization switching of the illumination beam among a number of different polarizations. We will conclude the chapter by presenting a proof of principle experiment to demonstrate the working of the proposed microscope in the reflection mode utilizing two of the schemes to switch the polarization of the illumination beam after each line scanned.

Chapter 6: This chapter will present the implementation of the proposed intraframe polarization switching in the confocal microscope working in the fluorescence mode. A proof of principle experiment will be presented to demonstrate the capability of the microscope for quick switching of the illumination beam among different scalar as well as vector beams. Implementation of computer generated holography based beam scanning in a laser scanning microscope will also be presented.

Chapter 7: In this chapter, we will provide an overall discussion on the research work carried out which leads to the thesis with some future prospects that can be explored in the near future.

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Publication

Patent

1. "A system and method for laser beam scanning with periodic switching of polarization of the beam", **Ranjan Kalita**, S. S. Goutam Buddha, and Bosanta R. Boruah, *Indian Patent Application No.*: **201831006652** dated 21 Feb 2018, *Publication* dated 23 Aug 2019.

Journals and Conference Proceedings

1. **Ranjan Kalita**, A. Saikia, A. C. Bhuyan, and Bosanta R. Boruah, "Holographic scanning microscopy for both reflected and fluorescence light imaging", *Rev. Sci. Instrum.* **90** (10) 106103 (2019)

2. **Ranjan Kalita**, S. S. Goutam Buddha, and Bosanta R. Boruah, "A laser scanning microscope executing intraframe polarization switching of the illumination beam", *Rev. Sci. Instrum.* **89** (9) 093705 (2018)

3. **Ranjan Kalita**, S. S. Goutam Buddha, and Bosanta R. Boruah, " Laser scanning confocal microscopy using illumination beams with different polarization's in quick succession", *Proc. SPIE*, **10772**, 107720I (2018)

4. **Ranjan Kalita**, S. S. Goutam Buddha, and Bosanta R. Boruah, " Suitability of holographic beam scanning in high resolution applications", *Proc. SPIE*, **10499**, 104991P (2018)

5. **Ranjan Kalita**, Md. Gaffar, and Bosanta R. Boruah, "Generation of arbitrary vector beams using a division of wavefront based setup", *J. Opt. (IOP)*, **18** (7) 075604 (2016)

6. **Ranjan Kalita**, and Bosanta R. Boruah, "Confocal imaging with orthogonally polarized illumination beams", *Proc. SPIE*, **9713**, 971316 (2016)

Publications not directly related to the PhD work

1. **Ranjan Kalita**, and Bosanta R. Boruah, "Effect of aberration on the electric field orientation around the focus of a polarized light beam", *Proc. SPIE*, **10772**, 107720Y (2018)

2. 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)

3. Md. Gaffar, **Ranjan Kalita**, and Bosanta R. Boruah, "Experimental observation of the aberration effects on a radially polarized beam", *J. Opt. Soc. Am. A* **33** (11) 2178 2187 (2016)

4. Md. Gaffar, **Ranjan Kalita**, and Bosanta R. Boruah, "Experimental demonstration of a light beam with superior aberration resilience", *Opt. Lett.* **41** (19) 4425 4428 (2016)

Academic achievements

1. Received "DST-SERB International Travel Support" to attend "SPIE Optics + Photonics 2018, held at San Diego, California, USA (August 19 23, 2018).
2. Received "SPIE Student Author Travel Grant (USD 750)" to attend "SPIE Optics + Photonics 2018, held at San Diego, California, USA ((August 19 23, 2018).
3. Presented the second prize in the poster presentation at the Research Conclave - 2018, organized by Students' Academic Board, IIT Guwahati. (March 8 11, 2018)
4. Certificate of merit in the poster competition organized by IIT Patna OSA Student Chapter (during the DST-SERB School on "Modern Optics & Its Applications"), held at the Department of Physics, IIT Patna. (December 16, 2015).
5. Presented best poster award (3rd position, IITG category) in the TEQIP Symposium to celebrate the 2015 International Year of Light held at the Department of Physics, IIT Guwahati. (October 31, 2015).

Conference attended

1. Participated and presented two papers in the "SPIE Optics+Photonics 2018" held at San Diego, California, USA (August 19 23, 2018).
2. Participated and presented a paper 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. (November 23 26, 2017).
3. Participated and presented a paper in the "International conference on light- and light-based technologies (ICLLT 2016)" organized jointly by Tezpur University and Optical Society of India. (November 26 28, 2016) .
4. Participated and presented a paper in the "International conference in optics and photonics (ICOP 2015)" organized jointly by University of Calcutta and Optical Society of India. (February 20 22, 2015)

School/Workshop/Training program 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. (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. (November 30 December 18, 2015).

3. Participated and presented a paper in the "South Asian Workshop on Optics & Photonics (SAWOP 2015)" organized by Indian Institute of Technology Guwahati. (November 17 18, 2015).

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