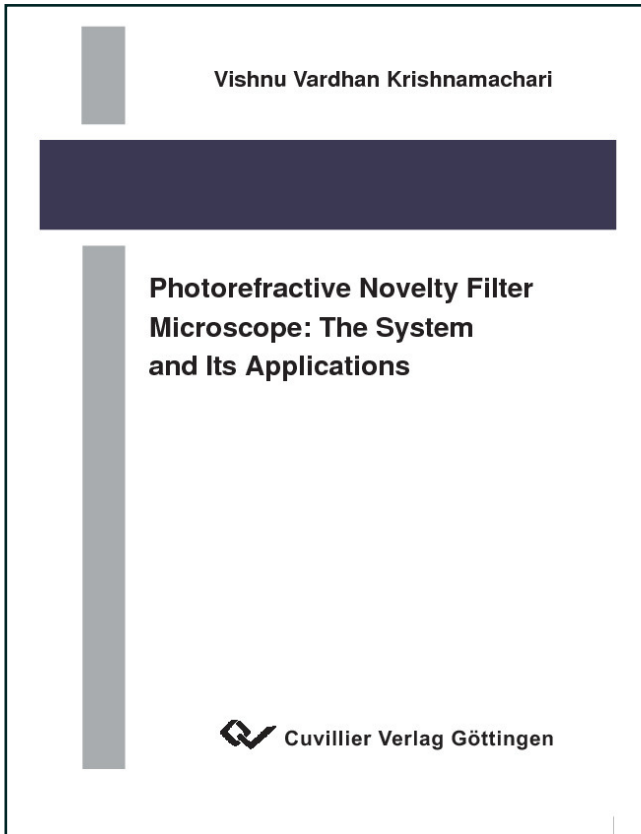




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Photorefractive novelty filter microscope: The system and its applications



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CHAPTER 1

SYNOPSIS

One of the two technological fields of research which has attracted the interest of the scientific community, the entrepreneurial firms and also the government agencies in the recent years is the field of biophotonics. This frontier of research is a fusion of photonics, one of the revolutionary technological breakthroughs of the past century, and biology. It deals with the interaction of light with biological matter. The key word, of late, has been *Harnessing the light* to achieve light-assisted diagnosis, light-guided therapy and light-activated surgery. Being an interdisciplinary subject, the task of an optical engineer in this area of research is clearly cut out: to develop *in vivo*-operable, non-invasive, compact imaging systems to examine and monitor in real-time the molecular and cellular dynamics for fast, efficient and infallible clinical diagnosis, therapy and surgery.

The second field of research which has witnessed a radical growth in the recent years is the field of nanotechnology. With the possibility of collaborative research among scientists from varied backgrounds of Chemistry, Physics, Engineering and Fluid dynamics, the amount of progress that has been achieved in this branch of knowledge is awesome. With the development of miniaturized micro-electro-mechanical systems (MEMS) and their nano-scale analogues, the accessibility and the availability of cost-effective and efficient chemical and biochemical reaction platforms has become a reality. Due to their minute detection volumes and extremely small analyte concentrations, these *lab-on-a-chip* devices present the optical engineers with a formidable challenge of building sophisticated imaging devices for diagnosing the fluid flows in the engraved or embedded micro-channels and conduits.

Thus, the need of the hour in either of these exciting fields of research is the availability of a compact microscope system which enables real-time non-invasive monitoring of dynamic processes. In this work, a microscope system is presented which (a) detects changes in its field of view at a rate limited only by

the speed of light (b) requires no preparation or fluorescent marking of biological cells or fluid flows (c) requires light power levels less than one micro-Watt on the biological probes and (d) exhibits extreme phase-sensitivity thus enhancing the contrast of transparent biological microorganisms or equally transparent fluids. This microscope is called a photorefractive novelty filter microscope.

Based on the nonlinear photorefractive effect, a compact novelty filter microscope and a portable novelty filter module for integration in commercial microscope systems are designed and realized. An interference model of the photorefractive effect is developed and it is used to explain the origin and the features of the trail formation exhibited by the novelty filter microscope. Two experimental techniques are developed to effectively suppress the trail formation and thus enable to perform reliable phase-change measurements using the novelty filter microscope. A phase triggering technique and a two-wavelength technique are presented to extend the phase measurement range of this microscope beyond 2π radians. Also, an expression for the point spread function of this novel microscope system is derived and its properties and predictions are discussed. Finally, exemplary application examples of this microscope in the fields of live cell imaging and micro-fluid flow diagnosis are demonstrated.

With the successful design, development, characterization and application of this microscope system, a significant contribution to the growth of the fields of biophotonics and nanotechnology has been made.

CHAPTER 2

INTRODUCTION

The concept of novelty filtering is introduced and the relevance of a photorefractive novelty filter microscope in different technological fields is discussed. The objectives of the current work are enumerated and the structure of the thesis is presented.

2.1 What is a novelty filter?

The term *novelty filter* was introduced by Anderson et al. [1] and it stands for a filter or a device which detects only the dynamics or changes and suppresses the non-moving or non-changing portions in an image sequence. To define the functionality of this filter more precisely, let me reproduce the words of Anderson et al. [2] from their pioneering article on optical novelty filters.

“Imagine a quiet lily pond viewed through a novelty filter. At first the filter displays the pond. In time, however, the filter adapts to its input and removes the image of the stationary pond from the output. On the other hand, a flying bug is a constant stream of newness, so the bug remains visible. The peculiar feature is that if a frog should leap from one lily pad to another, the frog would instantly appear in two places: where it is, because it was not there before, and where it was, because its sudden absence is as novel as its sudden presence.”

A novelty filter can be implemented both electronically and optically. The software- or hardware-based electronic novelty filters, known as *scene change detectors* [3–5], are in vogue and are mostly employed for compression of large video data or for transmission of satellite images. On the other hand, optical novelty filters, though not yet commercially implemented, have the advantage of possessing extreme speeds of processing (limited only by the speed of light) of high resolution images due to the inherent parallelism involved in optics.

The operation of a generic optical novelty filter can be roughly divided into two steps: the first step includes storing stationary (or non-moving) two-dimensional image information and the second step involves comparing successive images with the stored information and transmitting only those details which are different. Though, this interpretation of the functioning of a novelty filter is oversimplified and does not consider the temporal behavior of the stored information, the fact is that any optical novelty filter requires employment of a medium which can (a) temporarily store image information and (b) compare and subtract the incoming information from the stored image.

The nonlinear photorefractive materials [6, 7], which are available in good optical quality, can perform these two tasks efficiently and hence have been used to implement optical novelty filters [1, 2, 8–18]. A photorefractive material stores the stationary image information in the form of a phase hologram (or a Bragg grating) resulting due to light-induced refractive index modulation. The actual comparison between the stored information and the incoming image occurs due to the interference between the incoming image and the diffracted light beam from the hologram. In diffusion-dominated photorefractive crystals, the diffracted light beam is shifted in phase by π radians with respect to the incoming image. Hence, if the incoming image is same as the stored one, then the outgoing image is dark. But if certain portions in the incoming image are different, then only the modified (or *novel*) regions in the image appear bright at the output.

In the above discussion, we did not consider the temporal variation of the hologram stored in the photorefractive crystal. The time required for writing a hologram in a photorefractive material is determined by a *non-zero* grating formation or erasure time constant τ_g . However, the photorefractive crystals being dynamic holographic materials, the hologram stored in them gets continuously written and erased as new image information is presented to them. It is important to note that the time constant has to be non-zero for the functioning of a photorefractive novelty filter. If τ_g were to be zero, then two things happen simultaneously: (a) the current information gets recorded as a hologram instantaneously and (b) this recorded information gets compared with the same information resulting in a perpetual dark output. Another consequence of finite grating formation time is the appearance of a trail in the wake of moving objects, a feature, appropriately christened as *trail formation*. This feature of the novelty filter is extensively studied in this work.

2.2 Novelty filter microscope and its relevance

Unlike an electronically-implemented scene change detector, in a photorefractive novelty filter the complex amplitude (and not the absolute intensity) of the incoming image is compared with that of the stored hologram and hence it is sensitive to changes in both *amplitude* and *phase* of the incoming image. In other words, this filter detects input changes in phase (of an optical beam carrying the information) and converts them to output intensity variations in *real-time*. This real-time phase sensitive feature of the device makes it attractive for investigation of microscopic processes (involving optical phase changes) in the fields of biology and fluid dynamics.

The idea of using a photorefractive novelty filter for microscopic investigations was first proposed and demonstrated in 1988 by Cudney et al. [19]. In their experimental arrangement, the incoming information was the magnified image relayed from a microscope objective. Using this setup, they could detect mobile microorganisms in the field of view of the objective. Almost a decade later, Sedlatschek et al. [18] demonstrated the potential of the device to detect complex motions of different microorganisms. They also presented experimental results of visualization of gas flows from a cigarette lighter using a photorefractive novelty filter.

Though the potential of the novelty filter microscope (NFM) is enormous, at the time of commencing this PhD work, it was not yet sufficiently exploited. Known only to a group of specialists working in the field of photorefractives, this microscope had escaped the attention of other potential user groups. Thus, the effort in this work has been to demonstrate the applicability of this microscope in different fields of research and to develop a compact system whose handling is as simple as possible so that scientists from different research backgrounds can readily perform experiments using this device. Before I go on to describe in detail the objectives of this work, let me give a brief note on the current relevance of this microscope in the fields of biology and fluid dynamics.

Of late, the emphasis in bioscience research has shifted towards detecting and visualizing the dynamics of intra- and inter-cellular processes [20]. With the availability of numerous specialized fluorescent markers and with the increasing popularity of fluorescent microscopes, a lot of progress has been made in visualizing cell dynamics. But tagging the cells or its constituents with fluorescent particles still remains an invasive technique which undoubtedly influences the behavioral characteristics of sensitive biological probes. On the other hand, a novelty filter microscope due to its dual properties of suppressing the back-

ground intensity (and thus highlighting the dynamic portions) and converting optical phase changes to intensity variations can be efficiently used to detect motion of cells and microorganisms. Also, as this technique does not require use of foreign markers, it is completely non-invasive.

Another area of research which can gain a lot by employing a novelty filter microscope is the field of micro-fluid dynamics. In the recent years the innovative concepts of Lab-on-a-chip and μ -TAS (micro total analysis systems) [21] have become a reality [22]. Due to the large surface-to-volume ratio offered by these devices, the reactions take place efficiently requiring smaller processing times and providing higher yields. Thus these miniaturized devices have found a routine use in different chemical and biological laboratories.

However, due to the reduced analyte concentrations and small detection volumes, the diagnosis of flows in these devices is still a major challenge. Use of tracer particles to study flow properties is detrimental as this may not only lead to occlusion of micro-channels rendering the device unusable but also adversely impact the flow characteristics. Secondly, coloring the flowing fluids to visualize the process of mixing, an often used technique, may also not give correct results as the viscosity of the fluids is influenced by the usage of coloring dyes. On the other hand, a novelty filter microscope due to its phase sensitivity can detect optical path changes as small as 25 nm, thus helping to detect fluids whose density difference is of the order of 10^{-4} g/cm³. This aspect has been successfully investigated and clearly demonstrated in this work.

2.3 Objectives of this work

The broad objectives of this work have been to develop a compact three-dimensional novelty filter microscope system for investigation of probes which have to be laid horizontally and to exploit the potential of this microscope system for studying phase changes in the fields of biology and fluid dynamics. To achieve these objectives, various smaller but significant analytical and numerical calculations had to be performed and a few novel experimental techniques needed to be developed. In the following, a list of tasks undertaken during the course of this work are presented:

- To develop an improved interference model of the photorefractive effect to understand the formation of trail.
- To develop experimental techniques to suppress the trail formation for performing reliable phase measurement using a novelty filter microscope.

- To develop techniques to extend the phase measurement range of a novelty filter microscope beyond π radians and if possible beyond 2π radians.
- To design, realize and characterize a compact novelty filter microscope system which can eventually be integrated in a commercial microscope.
- To demonstrate the applicability of the novelty filter microscope for observing processes occurring in and around microorganisms and to study their characteristic movements.
- To demonstrate the applicability of the novelty filter microscope for studying the fluid flow properties in micro-channels.

2.4 Structure of the thesis

Though, at this stage of the thesis it may be difficult for the reader to perceive the importance and the uniqueness of a novelty filter microscope and its relevance in day-to-day research, the author has taken care to present the same in this thesis in a manner as comprehensible as possible by sticking to a lucid style of writing and introducing and building one concept at a time. In spite of this if the reader, at any stage of reading this thesis, feels himself or herself lost in the details, he or she may refer to Fig. 2.1 which sketches a *road map*, a *big picture* of this work.

In Fig. 2.1 each block represented by a smooth-edged (or rounded) rectangle corresponds to a chapter in the thesis. The contents contained in a solid rectangle are the topics which are discussed in complete detail. On the other hand, the topics enclosed in a dashed rectangle are the topics which were not dealt with in this work and are only briefly discussed. The direction of arrows in the sketch indicate the flow of ideas and concepts.

At the beginning of each chapter, the most important results and highlights of that particular chapter are summarized. Since the novelty filter microscope realized in this work is based on the nonlinear photorefractive effect, a short review of the same is presented chapter 3. Apart from providing a consistent mathematical description, this chapter also gives a *feel* for the complexity and the variety of this effect. Chapter 4 introduces an improved interference model of photorefractive two-beam coupling and discusses the model's merits and limitations. Chapter 5 deals with the direct application of the concepts developed in the previous chapters for implementing a novelty filter. Here, the concepts of amplitude contrast, phase contrast and phase transfer function are introduced.

In chapter 6, the design and the realization of three different novelty filter microscope systems are presented. In the succeeding four chapters, three important features of these microscope systems, namely trail formation (chapters 7 and 8), phase sensitivity (chapter 9) and point spread function (chapter 10), are discussed. Apart from mathematical description, these chapters also contain a detailed account of novel experimental configurations and exciting experimental results. In chapters 11 and 12, the results of the application of the novelty filter microscope in the fields of biology and fluid dynamics are presented.

The thesis contains three appendices. Most importantly, Appendix B contains experimental realization of phase-only modulation using a liquid crystal display. Though this has been included at the end of the thesis, its importance for characterizing the novelty filter microscope cannot be underestimated. The reason for its inclusion in the appendix rather than in one of the main chapters was to maintain the continuity of flow of ideas without interrupting it with details of a liquid crystal display and its phase characteristics (which at first sight may seem to be completely unrelated to this work).

Due to the varied application possibilities of this microscope system in the fields of biology and fluid dynamics, excerpts of this thesis have been published in various journals and conference proceedings. Appendix C contains a list of important publications and conference contributions presented during the course of this work.

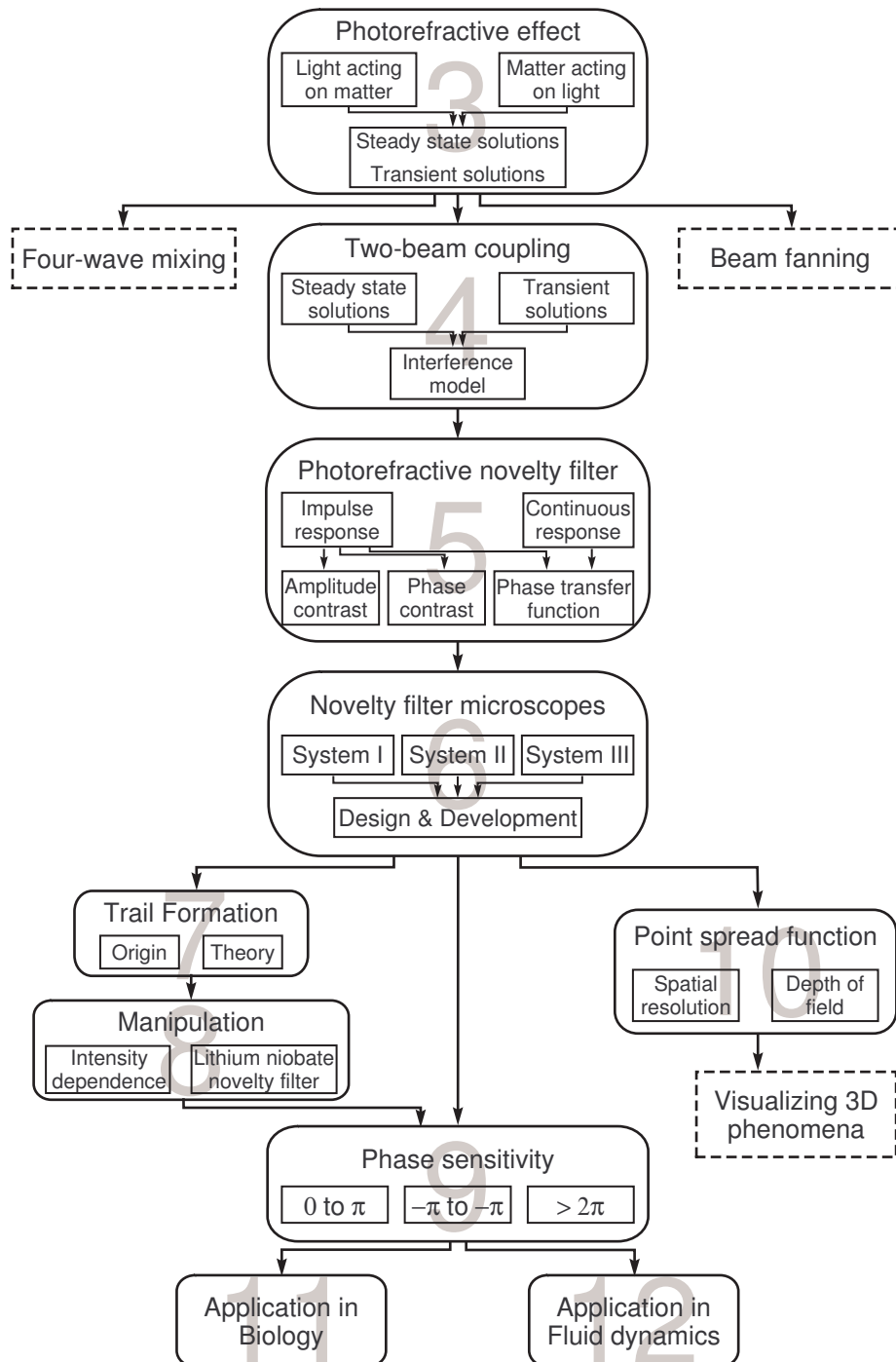


Figure 2.1: Big picture of the thesis.