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EXPLOITING THE ACOUSTO-OPTIC EFFECT FOR ADVANCED OPTICAL MICROSCOPY

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ABSTRACT

Optical microscopy is a key tool for investigating the structure and dynamics of biological systems at sub-cellular resolution. However, most conventional microscopy setups are designed to capture two-dimensional (2D) data from moderately thin samples labeled with fluorophores. Due to limitations imposed by light scattering and the need for mechanical focus adjustments, these systems struggle with deep tissue penetration and label-free imaging, making them insufficient for non-invasively studying biological complexity in medium-sized organisms such as organoids. Here, we survey our efforts to address these challenges and achieve label-free sub-millisecond imaging at potential depths of up to millimeters. Our approach leverages the acousto-optic effect – specifically, ultrasound-induced refractive index gradients – to focus, modulate, and guide light. The unique interaction between light and ultrasound enables rapid 3D control of light, making it ideal for developing inertia-free microscopes with no moving parts while achieving label-free imaging at hundreds of frames per second. Additionally, shaped ultrasound waves in a medium can act as an instantaneous waveguide, redirecting light toward a focus deeper than conventional optics. The synergistic integration of acousto-optics with advanced microscopy paves the way for an unprecedented in-situ characterization of dynamic processes.

Keywords: *acousto-optics, optical microscopy, deep imaging, light scattering, illumination encoding*

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1. INTRODUCTION

A key frontier in optical microscopy is achieving non-invasive, real-time, label-free imaging of biological samples at sub-cellular resolution. However, current microscopes are primarily optimized for acquiring 2D images at tens of frames per second from fluorescently labeled samples that are only a few hundred microns thick [*1*]. Deeper imaging, which is central for studying complex biological specimens such as organoids, remains limited by light scattering to depths of less than 1 mm [*2*]. Similarly, enhancing image contrast and specificity typically requires fluorescent dyes, introducing additional complexity and potential invasiveness to microscopy systems.

Several strategies have been developed to address these issues and advance toward deeper, faster, and label-free microscopes. Among them, exploiting the acousto-optic effect has proved effective in achieving unprecedented control of light outside and inside biological samples [*3*]. In this short paper, we present an overview of our work in this area. Specifically, we highlight the use of acousto-optics in two important microscopy scenarios: rapid axial focus control in quantitative phase imaging [*4*], and light guiding to enhance deep imaging [*5*].

2. PRINCIPLE OF ACOUSTO-OPTICS

The essence of the acousto-optic effect is to induce refractive index gradients within a medium using ultrasound. By sending ultrasound waves (pressure waves) within a medium, it is possible to locally and rapidly modify its density – and consequently, its refractive index. This enables dynamic control of the medium's optical properties, allowing light to be split, focused, or even guided on microsecond time scales. While acousto-optics has long been used in traditional microscopy – particularly

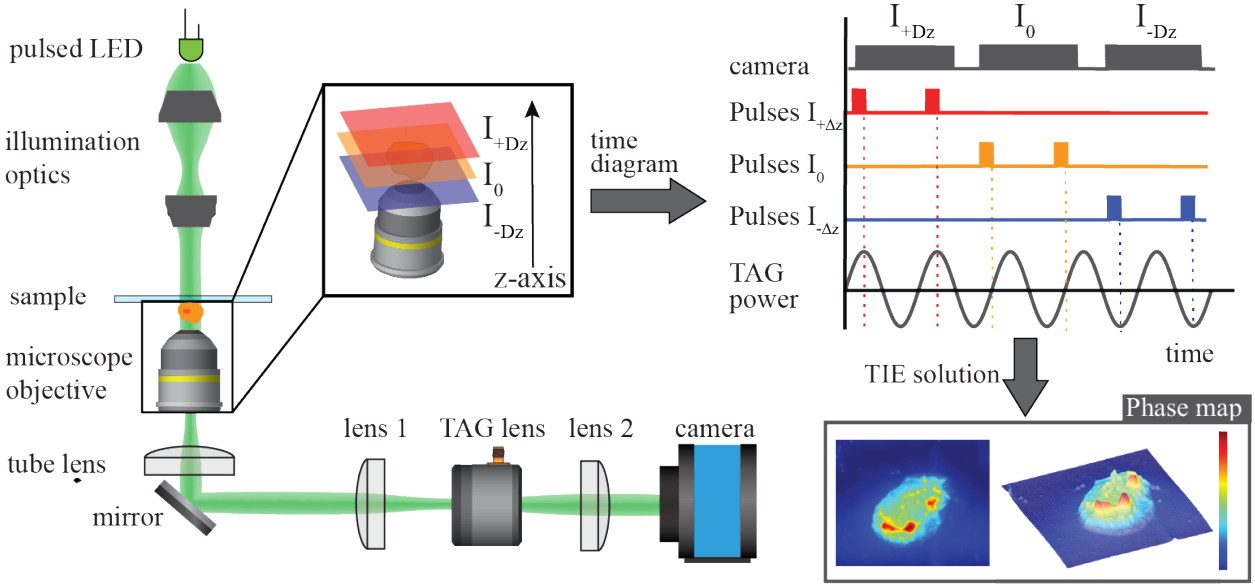

Figure 1. Schematic of the setup for rapid label-free imaging using acousto-optics. An acousto-optic liquid lens (TAG lens), synchronized with pulsed illumination, enables rapid selection of the three focal planes required to solve the Transport of Intensity Equation and retrieve a phase map of the sample. Quantitative phase imaging is thus be achieved at rates only limited by the camera frame rate.

through acousto-optic modulators and deflectors for beam conditioning – our focus here is on two more recent implementations. They have in common the use of non-traditional ultrasound geometries, which have opened new avenues for advancing microscopy.

3. QUANTITATIVE PHASE IMAGING WITH RAPID FOCUS CONROL

The use of fluorescent dyes for high-contrast microscopy can be obviated by measuring the phase shifts that light undergoes as it passes through a specimen – a technique known as phase imaging. Traditionally used as a qualitative method for contrast enhancement, phase imaging has evolved over recent decades into a quantitative approach. This advancement, known as Quantitative Phase Imaging (QPI), not only enhances contrast but also enables the extraction of physical properties such as the refractive index and thickness of the sample [*6*]. Among the various QPI techniques, the Transport of Intensity Equation (TIE) stands out for its simplicity and non-interferometric nature [*7*]. In fact, only two or three brightfield images acquired at known, distinct focal planes are sufficient to retrieve phase information. Unfortunately, precise axial movement of the sample relative to the microscope – often requiring fine adjustments for each specimen – is typically implemented with mechanical actuators, drastically reducing imaging speed in practice.

A promising solution toward real-time TIE imaging is the use of an acousto-optics liquid lens known as the TAG lens [*8*, *9*]. When synchronized with pulsed illumination, it enables remote focus control without any mechanically moving parts. This inertia-free approach allows for microsecond-scale focusing [*10*]. As shown in Figure 1, the sequential capture of three images – each at a different time delay relative to the TAG lens oscillation – allows for the retrieval of a phase map at a speed only limited by the camera's frame rate [*4*]. This high speed enables phase imaging of dynamic events. and, when combined with image stitching, facilitates large field-of-view imaging. This capability is particularly important for the label-free characterization of histopathology samples

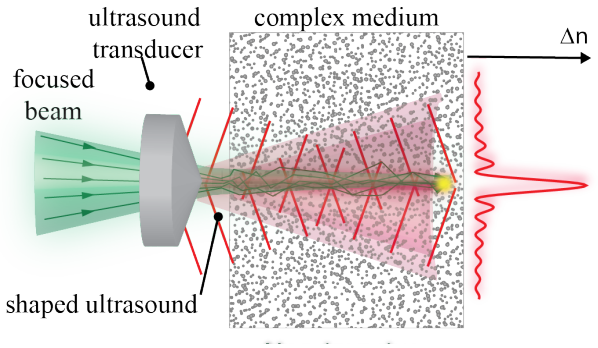

Figure 2. Principle of acousto-optic light guiding within scattering (complex) media. The refractive-index gradients induced by ultrasound act as an embedded waveguide directly inside the medium, helping light reach a deeper focus in analogy to an endoscope.

4. ULTRASONIC ENDOSCOPES

The acousto-optic effect has recently emerged as an effective strategy for mitigating light-scattering inside inhomogeneous media such as biological tissue [*5*, *11–13*]. In this case, shaped ultrasound – ultrasound waves with tailored spatial profiles – are directly sent into the medium of interest. The so-induced refractive index gradients act as effective lenses or waveguides embedded within the medium, helping light reach a deeper focus. In other words, ultrasound acts as a virtual endoscope, as shown in Figure 2. While promising, most implementations primarily rely on resonant acoustic cavities, which require the sample to be placed within the cavity – making the method inherently invasive.

Recent efforts to eliminate the need for resonant cavities include using specially designed ultrasound transducers [*14*, *15]* and specific geometries [*16*]. These methods have reached significant enhancements in the amount of light delivered within scattering media. For example, in samples with an optical thickness of 2.5 – where light is scattered an average of 2.5 times within the medium – the use of two parallel piezoelectric plates resulted in a 700% gain in delivered light [*16*]. This impressive result can be transformative for microscopy applications that rely on light delivery over large areas, such as photoacoustic microscopy.

5. CONCLUSIONS

Acousto-optics offers a promising path toward rapid, deep, and label-free microscopy. By combining innovative ultrasound geometries with new concepts – such as using ultrasonic endoscopes – there has been a recent surge in the potential applications of acousto-optics. We believe that ongoing progress in this area will significantly transform the microscopy landscape, bringing us closer to the coveted goal of a real-time, non-invasive, and deep optical tool.

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