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TAILORED ULTRASOUND FIELDS FOR ACOUSTO-OPTIC LIGHT CONTROL DIRECTLY WITHIN A SAMPLE

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ABSTRACT

One of the main challenges in optics is to rapidly deflect, focus or guide light directly inside a sample. As of today, the only widely accepted method to achieve this is using endoscopes, that is, optical fibers that are inserted into the sample. However, this approach is intrinsically invasive and highly sample dependent. A recently developed strategy consists of using ultrasound for light control. The idea is to exploit the acousto-optic effect to generate virtual ultrasonic endoscopes, allowing fast and targeted deep light delivery while remaining non-invasive. Nonetheless, realistic applications of this technique still require further research on the optimal acoustic generation process and the subsequent light-coupling. Here, we discuss different hybrid ultrasound-light implementations designed for light control inside samples, highlighting optimal design features and current challenges. In particular, we present the development of a piezoelectric transducer that obviates the need for acoustic cavities and provides micrometric light guiding at MHz frequencies. We also show how ultrasound can be used to enhance imaging depth in a two-photon microscope.

Keywords: *acousto-optics, ultrasound transducer, remote scanning, scattering mitigation, two-photon microscopy*

1. INTRODUCTION

Light control technologies are a key tool for our society, enabling a wide range of medical and industrial applications such as confocal microscopy [1], optical topography [2] or laser surgery [3]. A paramount challenge in modern optics is to non-invasively extend current light-based techniques to deeper within scattering samples, such as biological tissue, as this would allow a paradigm change in light-based biomedical methods. One of the main ways to tackle this challenge is by controlling light directly within the samples.

Traditionally, light control within samples is achieved by inserting optical fibers [4] into the sample, as in traditional endoscopies [5]. Despite allowing operation at arbitrary depths, these methods are invasive and can produce relevant damage to the studied samples or tissues, and thus are not possible or not optimal for many applications. A promising non-invasive alternative is to generate acoustic lenses or waveguides within these samples [6], allowing fast light control while re-directing scattered photons to target locations. Previous studies show the applicability of this method to enhance a range of optical techniques. Despite these promising results, further research is still needed to circumvent geometrical constraints of current ultrasound generation implementations, and to prove the applicability of these methods in real world scenarios.

Here, we propose the use of acousto-optic scattering mitigation for Two-photon excitation fluorescence microscopy (TPEF). We also present the development of an ultrasound transducer geometry that allows the generation of an acoustic lens without the need of an acoustic cavity, circumventing a key restriction of most current implementations. We expect these advances to be a step forward in acousto-optic light control within samples and scattering-mitigation within biological tissues.

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2. ACOUSTO-OPTIC DEEP LIGHT CONTROL

Light control within a sample is usually restricted by its absorption and scattering. While in deep light techniques light absorption is usually minimized by selecting a proper wavelength, scattering remains the main challenge that prevents controlling and focusing deep within samples. This scattering is caused by micrometric inhomogeneities within samples, that cause it to lose its initial phase and direction. Intensity I of not-scattered light (ballistic photons) within a sample can be expressed as: [7]

$$I = I_0(z)\cdot e^{-\mu z} = I_0(z)\cdot e^{-\tau}, \quad (1)$$

where $I_0(z)$ is the intensity expected in free propagation, z is the propagation distance within the sample and μ is the scattering coefficient of the sample. The total amount of scattering can be measured through the optical thickness τ, a dimensionless magnitude that can be interpreted as the average number of scattering events that photons have experienced to reach depth z.

The acousto-optic effect allows us to control light through pressure waves [8]. This is because a local change in pressure $\Delta P(\mathbf{r},t)$ leads to a local change in density, causing a local change in refractive index $\Delta n(\mathbf{r},t)$. For water-based materials, at pressure differences below 100 MPa, the acousto-optic effect can be expressed as: [9]

$$\Delta n(\mathbf{r},t) = C_{PO}\cdot \Delta P(\mathbf{r},t), \quad (2)$$

where C_{PO} is the piezo-optic constant of water, at room temperature $C_{PO} = 1.51\cdot 10^{-4}$ MPa^{-1}. This change in refractive index creates a change in light phase, allowing us to remotely focus or redirect light. Thus, exploiting this effect we can generate an acoustic lens within a sample, redirecting scattered photons to desired locations and thus enhancing light-based methods that require light to reach deep within scattering tissues or materials. Using this same principle, we can redirect light to focus it on target locations, allowing fast and dynamic light delivery within samples. [10]

3. ACOUSTIC CAVITIES FOR ACOUSTO-OPTIC SCATTERING MITIGATION

Recent advances in acousto-optics have described how to create acoustic lenses within samples, enabling scattering mitigation by redirecting scattered photons [11]. The usual method to create these acoustic lenses inside a sample is by placing the sample inside a cylindrical piezoelectric transducer, which acts as an acoustic cavity. Using this implementation, past work has shown applications in direct point-scanning imaging [6], light-pattern formation for imaging [12] and virtual waveguide creation for direct imaging [10].

Here, we propose that acousto-optic scattering mitigation can be used to enhance Two-photon excitation fluorescence microscopy (TPEF), synergically combining two state-of-the-art techniques for deep imaging within samples. In TPEF microscopy, the obtained image signal depends on the focal intensity squared. Thus, signal enhancement obtained using acousto-optic scattering mitigation becomes quadratically more relevant when combining it with TPEF microscopy. We exemplify this synergy in Fig. 1, using a waveguide generated with an acoustic cavity at 2.2 MHz frequency and 2 MPa peak pressure. Furthermore, TPEF microscopy usually relies on a near-infrared wavelength for excitation light, minimizing absorption and scattering in biological tissues and thus enhancing imaging depth.

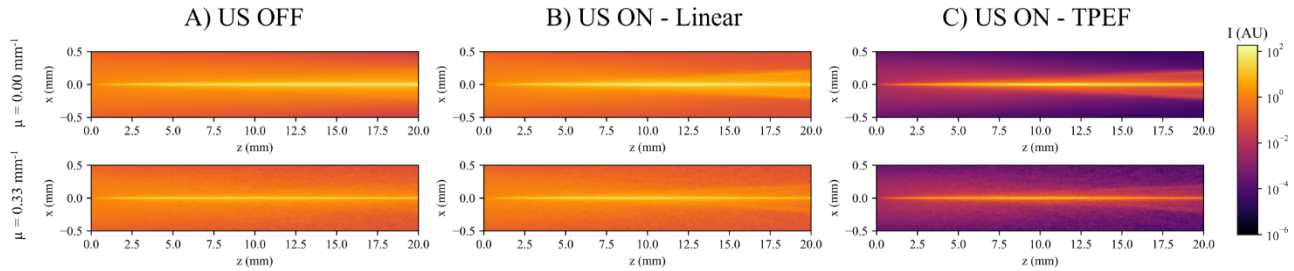

Figure 1. Signal intensity when using a Bessel Beam in different scenarios. (A) Simulated intensity of a Bessel beam, in a homogeneous or scattering medium. (B) Simulated intensity when using the same beam in conjunction with an ultrasound (US) waveguide within a cylindrical acoustic cavity. (C) Simulated intensity when using two photon excitation fluorescence microscopy (TPEF) combined with the same ultrasound waveguide, normalized to show the same peak intensity as (B). As TPEF signal depends on the intensity squared, ultrasound-induced intensity enhancements become quadratically more relevant in the proposed experiment. Note that thresholding effects have not been included in (C), despite potentially offering a substantial increase in resolution.

4. BEYOND ACOUSTIC CAVITIES

Despite generating an acoustic pressure profile optimal for light focusing and scattering mitigation, cylindrical piezoelectric transducers present the great disadvantage that the sample must be confined inside of them for direct deep light control. This means that big samples, including many relevant biological tissues, should be cut for the method to be applied, compromising the non-invasiveness of these techniques. Thus, this restriction often limits the applicability of these methods to phantom samples, far from real-world problems. For this reason, recent research is focusing on finding cavity-free ultrasound generation methods that allow direct deep light control. To this end, past studies have proposed using the photoacoustic-effect [13], two flat transducers [10], or acoustic holograms [14]. However, these implementations can be quite complex and often produce acoustic pressure profiles not optimal for direct light control within samples.

There are two main features that make the acoustic field generated by cylindrical transducers optimal for internal light control. First, it features highly focalized peak pressures, which maximize the change in refractive index. Second, it allows phase differences suffered by light to accumulate over the transducer's length, thus maximizing light focusing (see Fig. 2 A-C). In our recent research [15], we propose to use the angular section of a cylindrical transducer instead (see Fig. 2 D-E). This geometry maintains the key features needed for direct deep light control while allowing the generation of a cavity-free acoustic lens, obviating the need of confining the sample inside an acoustic cavity and thus expanding the applicability of acousto-optic lenses for direct light control within samples.

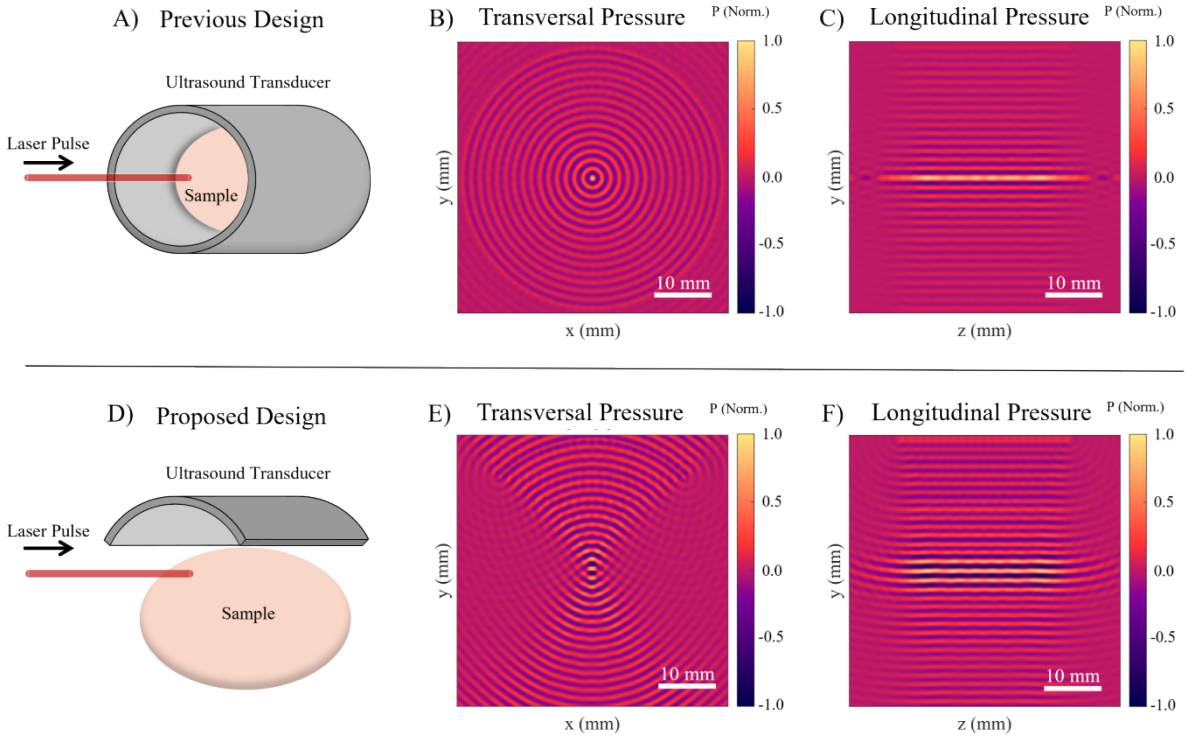

Figure 2. Comparison between acoustic field generation with a cylindrical piezoelectric transducer and our proposed geometry. (A) Scheme of a cylindrical transducer and corresponding simulated transversal (B) and longitudinal (C) normalized instantaneous pressure profiles at 1 MHz (transducer radius 23 mm). (D) Scheme of our proposed 90° cylindrical angular section transducer and simulated transversal (E) and longitudinal (F) instantaneous pressure profiles at 1 MHz. Our design is much less restrictive regarding sample geometry, while maintaining a highly intense elongated acoustic focus.

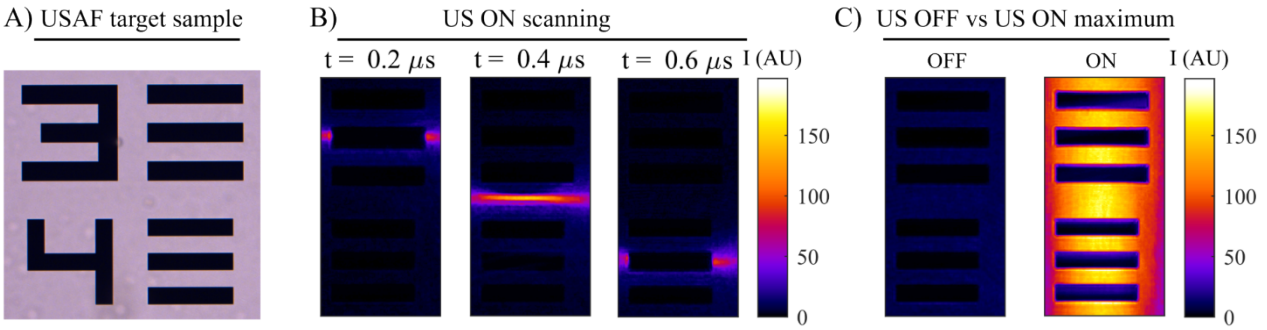

Figure 1. Fast light scanning using an ultrasound-induced lens. (A) Optical microscopy image of the USAF target elements imaged. (B) Captured image when ultrasound is applied, at different time delays between the ultrasound and the light pulse. (C) Maximum intensity detected at each pixel when scanning the light pulse across the whole sample.

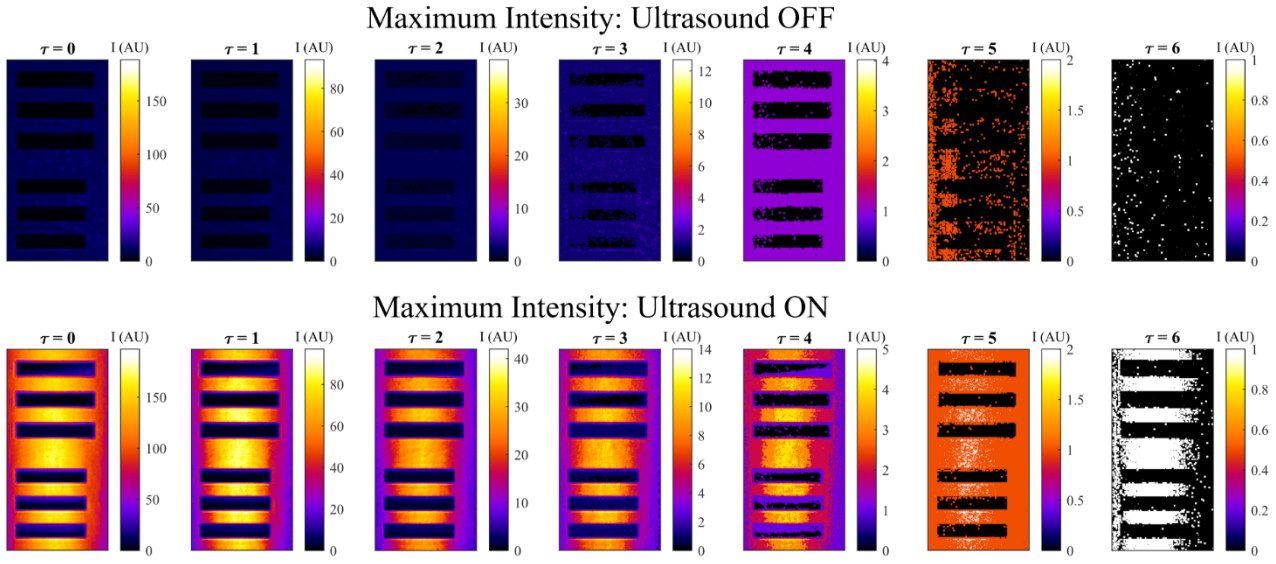

Figure 4. Maximum intensity detected at each pixel with and without ultrasound. We show the maximum intensity detected at each pixel in a 2000 frames video with and without scanning ultrasound. We can observe a huge increase in maximum intensity when using the ultrasound.

By using the proposed transducer geometry and a pulsed laser light source, a focal light line could be rapidly scanned through the sample, allowing fast targeted light delivery at MHz frequency rates over a millimetric region (See Fig. 3). By exploiting the stroboscopic effect, a sample was scanned for different scattering conditions, showing a factor 10 enhancement in light intensity when the ultrasound lens was used. Note that this allowed to reconstruct the sample structure even at scattering conditions where without the acoustic lens, only noise was detected (see Fig. 4). Furthermore, a single photon detector could be used to measure the transmitted intensity profiles, allowing to obtain a 1D profile of the 1.2 mm long sample in just 0.8 μs.

5. CONCLUSIONS

The acousto-optic effect can be used to control light directly within samples, enabling scattering mitigation for deep microscopy and fast light delivery to target locations. As our simulations demonstrate, the combination of Two-photon microscopy and acousto-optic scattering mitigation shows great potential for deep microscopy within scattering samples. Meanwhile, using a piezoelectric transducer consisting in the angular section of a cylinder allows to generate an acoustic profile substantially optimal for internal light control without the need of an acoustic cavity. The proposed geometry can remotely generate an acoustic lens that can be embedded inside a sample non-invasively, allowing targeted light delivery even in the case of large samples. We anticipate that these results will be a step towards non-invasive acousto-optic light control within a variety of materials, including biological tissues.

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