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Towards multifocal ultrasonic neural stimulation II: design considerations for an acoustic retinal prosthesis

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Abstract

Ultrasound waves, widely used as a non-invasive diagnostic modality, were recently shown to stimulate neuronal activity. Functionally meaningful stimulation, as is required in order to form a unified percept, requires the dynamic generation of simultaneous stimulation patterns. In this paper, we examine the general feasibility and properties of an acoustic retinal prosthesis, a new vision restoration strategy that will combine ultrasonic neuro-stimulation and ultrasonic field sculpting technology towards non-invasive artificial stimulation of surviving neurons in a degenerating retina. We explain the conceptual framework for such a device, study its feasibility in an *in vivo* ultrasonic retinal stimulation study and discuss the associated design considerations and tradeoffs. Finally, we simulate and experimentally validate a new holographic method—the angular spectrum-GSW—for efficient generation of uniform and accurate continuous ultrasound patterns. This method provides a powerful, flexible solution to the problem of projecting complex acoustic images onto structures like the retina.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Some of the most common causes of blindness are neurodegenerative diseases of the outer retina, characterized by gradual photoreceptor loss while the inner retinal neurons are largely maintained functional. Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) globally affect approximately 25–30 million and 1.5 million individuals, respectively, 10% of them considered legally blind [1]. Artificial stimulation of the relatively well-preserved retinal nerve cells is one of the primary strategies currently being pursued towards vision restoration for the blind.

Current approaches towards artificial retinal stimulation largely rely on the development of microelectrode array or optoelectronic photo-diode array implants [2–5]. While early electronic implants are already being used by RP patients, attempts to use this framework to approach highly functional vision still has inherent challenges associated with the longterm stability of the interface between electrode arrays and the delicate retinal tissue, with current spread and with creating interfaces with thousands of independent channels. An alternative, more recent effort is based on direct optical stimulation of retinal neurons using 'optogenetic' probes—

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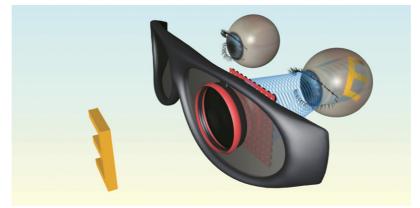


Figure 1. Simultaneous excitation pattern of an ARP. A world image is recorded by an external camera, processed and transmitted to the retina from the US-phased array.

light-gated ion channels or pumps [6–10]. Optogenetic stimulation offers cell-type specificity and potentially single cell resolution, which, when combined with dynamically patterned illumination [9, 11, 12], could allow the controlled rapid generation of arbitrary excitation patterns. However, stable expression of optogenetic probes can currently only be obtained through viral transfection, and this powerful approach could thus face a number of substantial consistency, safety and regulatory challenges. Photo-thermal stimulation, based on transient heating of exogenous photo-absorbers [13], is a potential high-resolution optical stimulation approach that will not require genetic transduction, but is still in the early stages of biophysical characterization.

In this paper we explore the feasibility of acoustic excitation as a fully non-invasive alternative strategy for artificial stimulation of the retina. The use of ultrasound (US) waves is popular in diverse imaging and therapeutic applications including routine ophthalmic retinal imaging [14]. Applying focused ultrasonic energy to modulate neural activity dates back to early works by Harvey, Fry, Gavrilov and others [15–18], and recent studies confirmed that focused ultrasonic energy pulses could be used for the stimulation of neural structures within the mammalian central nervous system both in vitro [19, 20] and in vivo [21, 22]. The mechanism behind this excitation effect is not fully understood (like other low-intensity ultrasound-induced bio-effects), and could rely on intramembrane cavitation between the bilayer membrane leaflets [23] or the mechanical effects of radiation pressure, shear stresses and micro-bubble cavitation [18, 24].

Meeting the fundamental requirements of a sensory substitution interface, i.e. the induction of complex percepts in response to varying sensory inputs, requires the ability to mimic the parallel sensory inputs by simultaneously stimulating in multiple loci. One possible approach is to have a hopping single-point US excitation focus, however, the long millisecond-scale dwell times used for *in vivo* neural excitation (longer than 25 ms in [21]), place a strong limit on the possible complexity of 'simultaneous' patterns that can be generated. This limitation can be circumvented by taking a holographic multifocal approach, in which the required pattern is created simultaneously when the waves emanating from multiple sources on a phased array interfere appropriately. Indeed,

several multifocal US algorithms were developed in the framework of hyperthermia research [25–27]. In a companion paper [28] we began exploring the adaptation of the multifocal acoustics framework to neural pattern stimulation applications, introducing efficient patterning algorithms with superior performance for generating sparse patterns of *discontinuous ultrasonic spots* by adapting ideas from optical computer generated holography (CGH).

In the following sections, we introduce, conceptually validate and discuss fundamental design considerations for a new visual restoration approach: the acoustic retinal prosthesis (ARP), in which ultrasonic waves are transmitted from a multi-element phased array into the eye and interfere to create a projected pattern for exciting surviving retinal neurons. A non-invasive ARP could potentially operate many more information channels than current implanted devices, without involving genetic transfection procedures required by optogenetics. Section 2 presents an overview of the ARP and section 3 presents an experimental validation of this concept using visually evoked potentials measured in response to pulsed ultrasonic stimulation of the eye, as well as a shortterm safety assessment of this approach. In section 4 we address the continuous pattern generation problem and report, for the first time, an algorithm for generating uniform and accurate continuous ultrasound patterns using the weighted Gerchberg-Saxton (GSW) algorithm and angular spectrum field calculations, validating its performance using results from simulations and magnetic resonance (MR) thermometry In section 5 we discuss general design experiments. considerations for an ARP and the tradeoffs between spatial and temporal resolution, efficacy and safety, and discuss the arising conclusions in section 6.

2. ARP overview

The ARP device is required to efficiently and accurately generate acoustic patterns on the retina, which is an almost spherically curved surface at the back of the eyeball (figure 1). The phased array, located externally to the cornea, emits waves into an acoustic coupling component (e.g. a bag containing water or US coupling gel), aimed at minimizing the energy reflected from boundaries between the media. After penetrating the eye through the cornea, the waves traverse approximately 25 mm of ocular structures, including the cornea, the aqueous humor (and the more peripheral ciliary bodies), the lens, the vitreous humor and finally, the retina [29]. The fundamental acoustic properties of these structures are characterized in the literature [30–32], and involve a minor attenuation of less than 0.5 dB and reflection of ~1% of the incident energy at 1 MHz, which increase gradually at higher frequencies until their effect becomes major (see section 5.3).

The density of retinal ganglion cells (RGCs) in the primate retina falls exponentially with the distance from the fovea, from 250 RGCs per 100 \times 100 μ m² to ~10 in the most external areas [33, 34]. Considering an ARP operating at 2.5 MHz with 600 \times 600 μ m² pixels, the total human retinal area of $\sim 1000 \text{ mm}^2$ could potentially support as many as 2500 excitable pixels. For comparison, the first electronic retinal prosthesis approved for human use has 60 electrodes with millimeter-scale spacing (Argus II, Second Sight device, [5]). The joint thickness of the ganglion cell layer (GCL), the inner plexiform layer (IPL) and the inner nuclear layer (INL) in humans is on the order of 200 μ m [35], similar to the best axial resolution expected when transmitting US at a frequency as high as 80 MHz [36]. Thus, layer-specific stimulation is unfeasible and the RGCs will experience direct US modulation whenever the retina is targeted. As in other retinal prostheses, input images will require extensive online processing for optimizing the perception.

3. Experimental validation of acoustic retinal stimulation

3.1. Visual and ultrasound evoked potentials

3.1.1. Methods. To examine whether the retina is responsive to US stimuli, we measured visual evoked potentials (VEPs) from anesthetized Sprague Dawley rats, in response to full-field light flashes and to full-field ultrasonic pulses at acoustic frequencies of 0.5 MHz (n = 6 animals) and 1 MHz (n = 3). The animals were anesthetized using a ketamine:xylazine:acepromazine cocktail (induction with 50:6.25:1.25 mg kg⁻¹ body weight; anesthetic maintenance with ketamine:diazepam 50:2.5 mg kg⁻¹ body weight). VEPs were recorded using a pair of needle electrodes (Axon systems, DSN1260, 13 mm 27 G monopolar), inserted subcutaneously in a caudal-rostral orientation, ~ 1 mm medial of each ear. Another subcutaneous electrode on the animal's trunk served as ground. The recorded VEP signal was amplified, filtered at 0.1-500 Hz and digitized by a single device (Psychlab, 4-channel EEG). Additional digital processing included bandpass filtering (7.5-37 Hz, -3 dB cutoff frequencies) and averaging of the traces triggered by the stimulus onset (at least 150 repeats), performed using Matlab.

First, light flashes were projected from a bright blue light emitting diode directed to the rat's eye. Subsequently, an US transducer was coupled to the eye using a custombuilt conical coupler and ophthalmic gel (Viscotears). For each animal, we used either an Olympus V301 (0.5 MHz, **Table 1.** Range of US stimulation parameters used in animal experiments. The pressures and intensities denote the estimated values incident on the cornea. See section 5.3 for definitions and further discussion of ophthalmological US safety indices and guidelines.

	US frequency	
	0.5 MHz	1 MHz
Burst train duration (ms)	5-20	10–20
Single burst duration (μ s)	50-100	100
Repetition frequency (Hz)	1900-2000	1667
Peak acoustic pressure (kPa)	86-160	564-725
Mechanical index (MI) (MPa MHz ⁻¹)	0.12-0.23	0.56-0.72
Peak instantaneous intensity ($W \text{ cm}^{-2}$)	0.24-0.84	10.3-17.0
$I_{\text{SPPA}} (\text{W cm}^{-2})$	0.12-0.42	5.15-8.52
$I_{\rm SPTA} \ ({\rm W \ cm^{-2}})$	0.012-0.083	0.86-1.42

solid Teflon coupling cone) or an Imasonic 3034 (1 MHz, degassed water filled Plexiglas cone) US transducer, excited every 1 or 2 s by burst trains generated by a function generator (Tabor 8024) and amplified by 50 dB (Amplifier Research model AR75 or ENI model 550L). The animals' ears were sealed with dental elastomer to avoid auditory artifacts. Based on calibrations performed beforehand in degassed water, the peak pressures incident on the cornea were estimated, from which the instantaneous intensities were estimated based on the corneal acoustic impedance quoted from [30]. Based on these and the duty cycles in each experiment, the mechanical index (MI), the spatial peak pulse average intensity (I_{SPPA}) and the spatial peak temporal average intensity (I_{SPTA}) safety indices were readily calculated, all of which appear in table 1. To estimate how these incident pressures translate to retinal pressures we measured the pressure decrease during propagation of US waves thorough an isolated rat eyeball (0.5 MHz US), and found a 12% decrease in the pressure.

Injection of tetrodotoxin (TTX) was performed by gently piercing the sclera-cornea boundary with a needle (30 G), inserting a micro-liter syringe (Hamilton, 32 G) and injecting 1–3 μ l of TTX at 500 μ M. These TTX injections were performed to evaluate the role of RGCs' activity in the US-driven VEP signals by blocking (at least partially) their voltage-gated sodium channels. Assuming a vitreous humor volume of approximately 50 μ l the final average TTX concentration was 10–30 μ M. In several cases TTX had no visible effect and an additional dose of TTX was administered. Care was taken to minimize electrode movement during the experiment. At the end of the experiments the anesthetized animals were sacrificed by decapitation. The animal experiments were approved by the Technion's animal use ethics committee.

The experiments utilizing acoustic frequencies of 0.5 and 1 MHz (and their respective controls) were performed on different animals and using different equipment, and were analyzed as two separate experimental sets. In each experiment, an equal number of traces was averaged for each condition, and the mean values subtracted from each average signal to discard dc components. We defined the *response power* as the difference between the average signal power in the 200 μ s after stimulus onset and the average power in

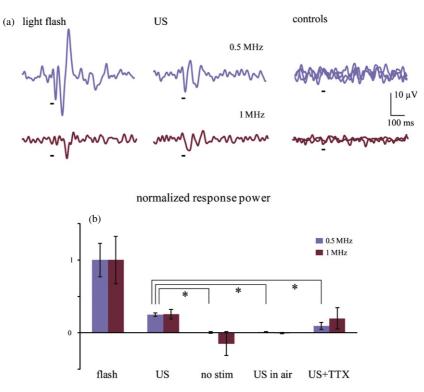


Figure 2. *In vivo* retinal acousto-stimulation. (a) Examples of average traces from two different experiments with 0.5 MHz (blue) and 1 MHz (red) US stimulation frequencies, showing averaged responses to light flashes (left), US stimuli (middle) and control conditions (right). Bars indicate the stimulation interval. (b) Group statistics for all conditions across all animals. Blue and red bars denote group averages of response powers for each condition at 0.5 and 1 MHz respectively, normalized to the group average of the flash condition. Error bars indicate standard error of the mean (SE), stars indicate statistically significant differences (p < 0.05).

the 200 μ s before onset. The results are presented below as grouped averages, normalized to the mean responses in either the light-flash or the US condition.

3.1.2. Results. Examples of traces from two experiments, obtained by averaging the responses to all the presented stimuli in each condition (figure 2(a)), show significant evoked potentials to the US stimuli at both stimulation frequencies. Lumping together response powers from all the animals, US stimulation resulted in responses that were $24.9\% \pm 2.3\%$ and $25.4\% \pm 6.4\%$ of average flash responses (mean \pm SE response power at 0.5 and 1 MHz respectively, figure 2(b)). The light flash evoked responses were both larger and had a larger response variability (SE = 23%).

Several control conditions were recorded for each animal. Recorded potentials when no light or US stimulus was present (no stim) showed negligible average responses of $0.5\% \pm$ 1.3% and $-14\% \pm 16.4\%$ of the flash responses, for the two stimulation frequencies (0.5 and 1 MHz respectively). In a second control, US stimuli were present but the US–eye coupling was eliminated by moving the cone a few mm away. This condition (US in air) also led to negligible, statistically insignificant (p < 0.05) responses of $1.2\% \pm 0.6\%$ and $-0.5\% \pm 1\%$. In a third control, both stimuli and coupling were present, and responses were recorded following the injection of TTX into the vitreal space (US + TTX). TTX injection led to attenuation of the US responses which was complete only in a subset of the experiments (probably due to variability in the injection efficiency). On average, these responses to the US stimulation were $37.3\% \pm 19.2\%$ and $77.4\% \pm 49.4\%$ of the average US responses in the absence of TTX. In the 0.5 MHz stimulation group (which was larger) the differences between the responses to US stimulation and the three controls were statistically significant with p < 0.05. All the statistical hypotheses were tested using the nonparametric multiple comparison Friedmann test followed by the Schaich–Hamerle *post-hoc* test [37].

3.2. Short-term damage assessment

3.2.1. Methods. In order to test for possible US-induced damage to the retina, we studied the effect of the exposure to the US stimulation on treated eye electroretinograms (ERGs), and on the morphology of histological sections [38].

Electroretinograms: The ERG responses to light flashes were recorded from a separate group of three animals. The animals were anesthetized and prepared as before, after which pulsed US was transmitted to one eye continuously for a period of 90 min and consisting of burst trains spaced 1 s apart, with burst train durations of 20 ms, single burst durations of 100 μ s, repetition frequency of 2000 Hz and peak acoustic pressure of 160 kPa (MI: 0.23, *I*_{SPPA}: 0.42 W cm⁻², *I*_{SPTA}: 0.105 W cm⁻²; this stimulation regime was selected to represent an upper limit of the dosages used in the 0.5 MHz study). During the stimulation and at least 90 min afterward the animals were maintained in dim red illumination, after which the

Table 2. Retinal function assessment by	ERG: average \pm standard error of fit	parameters.

		Treated eyes	Untreated eyes	Control
3 h				
a-wave	$V_{\rm max}$ (μV)	510 ± 86	483 ± 18	
	$Log(\sigma)$ (cd·s m ⁻²)	-0.277 ± 0.335	-0.218 ± 0.39	
	n	0.71 ± 0.06	0.54 ± 0.04	
b-wave	$V_{\rm max}$ (μV)	1083 ± 176	1020 ± 109	
	$Log(\sigma)$ (cd·s m ⁻²)	-1.056 ± 1.056	-0.031 ± 0.332	
	n	0.35 ± 0.04	0.27 ± 0.04	
24 h				
a-wave	$V_{\rm max}$ (μV)	435 ± 38	505 ± 35	303
	$Log(\sigma)$ (cd·s m ⁻²)	0.068 ± 0.327	0.031 ± 0.332	-0.037
	n	0.58 ± 0.01	0.48 ± 0.04	0.59
b-wave	$V_{\rm max}$ (μV)	841 ± 208	844 ± 92	780
	$Log(\sigma)$ (cd·s m ⁻²)	-1.193 ± 1.125	-0.736 ± 0.192	-0.629
	n	0.29 ± 0.05	0.29 ± 0.01	0.24

animals were prepared for measuring ERG responses to flashes. The pupils were fully dilated with cyclopentolate hydrochloride 1% and topical anesthesia (benoxinate HCl 0.4%) was administered.

Scotopic ERG responses were recorded from one eye using corneal electrodes (Medical Workshop, Gröningen, The Netherlands), while the second eye was covered with tape. Reference and ground electrodes were inserted into the ears and ERG signals were amplified ($\times 20000$) and filtered (0.3-300 Hz) by differential amplifiers (Grass, West Warwick, RI). Light stimuli (generated by UTAS 3000, LKC Technologies, Gaithersburg, MD) began at intensities of 3 \times 10^{-4} cd·s m⁻² and increased gradually to a maximum of 665 cd·s m⁻². Several responses were averaged for each intensity level, with the number of flashes presented decreasing (from 10 to 3) with the intensity and the time interval between them increasing (from 2 to 60 s). After another 2 h of dark adaptation, the responses to the same stimulation sequence were measured from the second, untreated eye. The process of measuring responses from both eyes was repeated after an overnight dark adaptation period, approximately 24 h after the US stimulation has ended. A fourth additional animal had not received any US exposure and had its ERG measured from a single eye after an overnight adaptation, as a control.

The ERG analysis consisted of amplitude measurements of the a-wave, measured from the baseline to the trough of the a-wave, and the amplitude of the b-wave, determined from the a-wave trough to the peak of the b-wave. The relationships between the amplitudes of the a- and b-waves and stimulus intensity were fitted to the following relationship:

$$V = V_{\max} \frac{I^n}{I^n + \sigma^n}.$$
 (1)

In this equation, V is the measured a-wave or b-wave amplitude, V_{max} is the amplitude of the ERG wave elicited by a stimulus of super-saturating intensity, σ denotes the stimulus intensity eliciting a response of half-maximal amplitude and n is a positive real number.

Histological examination: After the second ERG examination (24 h after US exposure) one rat was directly taken for histological examination. Under the continued ketamine,

xylazine and acepromazine anesthesia both eyes were enucleated and the animal was sacrificed with an overdose (80 mg kg⁻¹ body weight) of sodium pentobarbital. The enucleated eyes were soaked for 10 min in a solution of 4% paraformaldehyde in 0.1 M of phosphate buffer solution (PBS, pH 7.4). The eyeball was then opened posterior to the limbus (pars plana) and fixed in the same solution for 1 h, after which the lens and vitreous humor were removed and the posterior eyecup was left for another 24 h of fixation. It was then rinsed in 0.1 M PBS and dehydrated twice in 70% alcohol for three h and twice in 96% alcohol for three h, and embedded in JB-4 resin (Bio-Rad, Watford, UK) overnight. After cutting the tissue with a microtome (Reichert-Jung, Nussloch, Germany) into 2 μ m sections it was placed on slides, stained with Richardson's solution and examined with a light microscope.

3.2.2. Results. The ERG traces from all three US-exposed eyes exhibited normal morphology consisting of a-waves, b-waves and oscillatory potentials, exemplified in figure 3(a) (gray/upper traces). The shapes, amplitudes and latencies appear similar to those of the ERGs recorded from their control counterparts (figure 3(a), green/lower traces), both 3 h and 24 h after the US stimulation. The group results (using curve fits of each recording session to equation (1)) appear in table 2 as mean values \pm standard error. In general, the average values of V_{max} and σ for treated and untreated eyes do not differ appreciably, considering the variability within each group. Only an increase in the exponent, *n*, associated with the a-waves 3 h after exposure was found to be different with statistical significance (paired Student's *t*-test, $\alpha = 0.05$).

In line with the apparently unaffected functionality seen in the ERGs, the histological sections revealed no visible damage to the retina (example sections in figure 3(b)). The different retinal layers appeared normal and undisrupted in these sections, without signs of retinal detachment from the pigment epithelium.

4. Pattern generation

CGH iterative algorithms are based on the Fourier relations between the two focal planes on either side of a converging

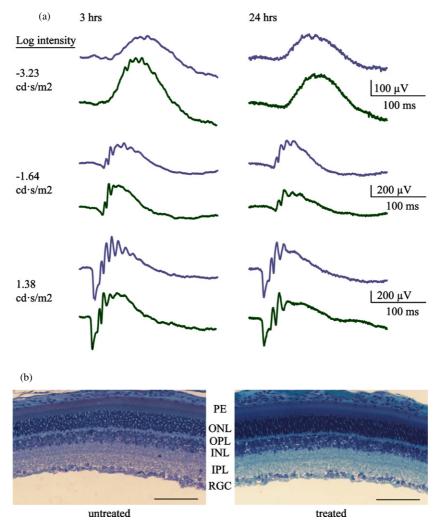


Figure 3. Assessment of retinal function and morphology after US stimulation. (a) Examples of averaged ERG traces recorded in one animal in response to flashes with log intensities of -3.23, -1.64 and 1.38 cd·s m⁻² (top, middle and bottom panels respectively). The traces from the US-stimulated eye are shown directly above traces from its unexposed counterpart, 3 h after US stimulation (left) and 24 h after stimulation (right). (b) Examples of stained 2 μ m thick sections of an untreated retina (left) and an ultrasonically stimulated retina (right). The following retinal layers are marked: the retinal ganglion layer (RGL), inner plexiform layer (IPL), inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL) and pigment epithelium (PE). Scale bars indicate 100 μ m.

lens. The iterative Gerchberg-Saxton (GS) and related algorithms [39] project the (optical) field from the source at the back focal plane to the target at the front focal plane, perform an optimization step and then project back to the source and impose the relevant constraints (typically, flat magnitude). The ARP design we suggest is lensless; instead, the algorithm we apply here is based on an exact computation of the projected fields via the angular spectrum method [40], implemented using a fast Fourier transform (FFT). This method allows an accurate, high-resolution and rapid field computation over a large area, as required for the generation of continuous patterns. Our implementation below is based on the Insightec planar US phased array, a 2×4 cm² device with 987 elements transmitting at 2.3 MHz with phase-only control (imposing a unity amplitude constraint). We used simulations and MRthermometry measurements to map the US fields at a plane 25 mm from the phased array (an eye's diameter).

4.1. Simulations

Continuous two-dimensional ultrasound holograms were calculated in Matlab using the iterative GSW algorithm [28, 39] coupled with the angular-spectrum forward and backward field calculations [40]. Each one of the iterations begins with forward projection of the planar transducer acoustic field to the target plane using an FFT-based calculation. The target acoustic field amplitude is then modified according to requested hologram intensities, and the iteration ends by sampling the transducer elements' phases from the acoustic field back-projected to the transducer plane and imposing the unity amplitude constraint. Following the GSW algorithm weightings, the target hologram magnitudes are modified according to the relative magnitude derived in the previous iteration:

$$w_m^t \equiv w_m^{t-1} \frac{\langle |p^{t-1}| \rangle}{|p_m^{t-1}|},$$
(2)

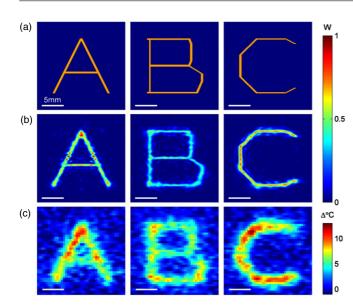


Figure 4. Acoustic pattern generation. (a) Two-dimensional 1.5 cm targets used as input commands to the continuous ultrasonic hologram algorithm. (b) Acoustic intensity normalized-power simulations generated using the proposed angular spectrum-GSW algorithm. (c) MR temperature elevation measurements induced by transmission of US to a target plane 25 mm away from the transducer plane, using the computer generated acoustic phase maps.

where $\langle | p^t | \rangle$ is the mean magnitude in the required target region in iteration number t, p_m^t is the acoustic field complex amplitude in the *m*th pixel and w_m^t the weight assigned to that pixel before back-projection to the source. The algorithm was initialized with random phases for the transducer elements and $w_m^0 = 1$ for all *m*, and eight iterations were performed before the final pattern was achieved. Simulations were performed using 2048 × 2048 pixels to span an acoustic field plane of $10.2 \times 10.2 \text{ cm}^2$ at $50 \times 50 \ \mu\text{m}^2$ resolution. The three letter patterns, 'A', 'B' and 'C', were manually built into $1.5 \times$ 1.5 cm^2 masks (figure 4(a)) to represent the requested target fields 25 mm from the transducer plane. The simulation results are generally smooth, uniform and show that most of the power is indeed directed at the required pattern (figure 4(b)).

The computational complexity of a single projection in the holographic algorithm is equal to the complexity of the FFT algorithm, $O(Nlog_2(N))$. This resulted in a measured runtime of ~100 ms per iteration (with forward and backward projections), using a megapixel pressure matrix on an Intel[®] CoreTM i5-2410M processor and MATLAB version 7.10.

4.2. Measurements

The acoustic holograms were projected and measured using an experimental setup with a gel-based phantom similar to that described in [28]. Sonication of 49.4 W acoustic power was performed to a target plane 25 mm away from the source and MR temperature elevation images were acquired on a GE 1.5 T scanner (FSPGR sequence, TR/TE = 35.8/22.8 ms, FOV = 12.8×12.8 cm², slice thickness = 5 mm, scan time = 4.6 s, in plane resolution of 0.5×0.5 mm²) equipped with the phased array described above. A reference scan that was taken before sonication was subtracted from a scan taken 5 s after the beginning of sonication (mid-scan time) to measure the temperature elevation. The results resemble the simulation results, showing generally smooth temperature elevations. The measured patterns have wider lines than the simulated ones; this can be due primarily to thermal diffusion: a point heat source produces a temperature profile with about 2 mm full width at half the maximum (FWHM) after 7.5 s of diffusion, the average diffusion time in the thermal acquisition.

5. Design considerations

5.1. Efficient coupling

The ARP–cornea coupling quality may be quantified by the energy reflection coefficient, equal to the ratio of reflected to incident energy at an interface between two media:

$$R = \left[(Z_2 \cos \theta_i - Z_1 \cos \theta_t) / (Z_2 \cos \theta_i + Z_1 \cos \theta_t) \right]^2, \quad (3)$$

where Z_1 and Z_2 are the characteristic acoustic impedances of the pre- and post-interface media, respectively, and θ_i and θ_t the incident and transmitted angles, respectively. *R* is negligible at small angles (expectedly R < 5% for $\theta_i < 63^\circ$), and can be further reduced using a curved transducer (or lens) that follows the corneal geometry so that at each point on the interface $\theta_i \approx 0^\circ$. An optimal coupling medium has impedance Z_1 as close as possible to the corneal impedance Z_2 , which is approximately 1.54×10^6 kg m⁻² s. Commercial ultrasound gels with similar impedances are available today and can be used as an efficient and bio-compatible coupling material, or alternatively, one can use a liquid solution as is also used for ultrasonic bio-microscopy [41]. Either liquid or gel will be kept either in a water-tight flexible bag or a solid-walled bath.

5.2. Frequency-resolution tradeoffs

The system's spatiotemporal resolution is critical for its success. In the temporal domain, the switching of the array's transmission pattern may be carried out practically instantaneously compared to the required stimulation dwell time, which may remain as the primary limiting factor of the ARP's temporal resolution. Another potential factor here could be the maximal duty cycle for a given average intensity allowed by safety considerations (see section 5.3).

The spatial resolution of ultrasonic stimulation also requires careful consideration. This resolution is determined by two major factors: the acoustic wavelength λ and the system's *F*-number (*F*_#), via

$$D_l = K\lambda F_{\#} = K\frac{c}{f}F_{\#},\tag{4}$$

where D_l is the lateral (in-plane) FWHM of intensity, *K* is a constant (equal to 1 in most cases, especially if $\theta_i < 50^\circ$) [36], *f* the acoustic frequency and *c* is the speed of sound in the medium (ranging between 1500 and 1630 m s⁻¹ in ocular structures [30]). The $F_{\#}$ denotes the ratio between the distance from the array to the focal plane *L* and the effective aperture; the $F_{\#}$ of a circular transducer of diameter *D* is $F_{\#} = \frac{L}{D}$. Placing the phased array as near as possible to the cornea, and maximizing the effective aperture by utilizing the entire space

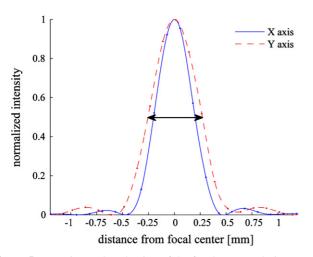


Figure 5. Experimental evaluation of the focal spot resolution at a plane 20 mm distant from the 2.3 MHz phased array described in section 4 (dots: normalized intensity measured in a water bath using a hydrophone; solid lines: cubic spline interpolations). The array transducer is not square, resulting in different resolutions in the respective axes.

between the zygomatic and frontal bones will decrease the $F_{\#}$ until it approaches 1, and improve resolution. As a convenient rule of thumb, in the case of US directed at the retina one may assume that K is close to 1, and that $c \approx 1.5 \text{ mm } \mu \text{s}^{-1}$, so that the resolution is roughly D_l [mm] $\approx 1.5/f$ [MHz].

Using sub-MHz acoustic frequencies, similar to those used for neural stimulation in [19, 21] (who chose acoustic frequencies suitable for penetrating the skull), will result in a wavelength of 3-6 mm and a spatial resolution of a similar order. Sub-mm resolution is readily achieved at higher frequencies: the measured intensity distribution at a focal plane situated 20 mm distant from the 2.3 MHz phased array used in our patterning experiments (section 4) reveals a FWHM of ~400 μ m and ~525 μ m in the X and Y axes respectively (figure 5). Additional studies reported neural stimulation at higher frequencies of 4.04 MHz [20] and even 40 MHz [42], which would correspond to significantly sharper spatial resolutions. However, higher frequencies also need to be considered in light of their associated (higher) intensity requirements, as discussed in the next section.

5.3. Frequency-intensity tradeoffs and safety considerations

The dependence of neuro-stimulation efficacy on acoustic frequency has received little systematic treatment so far (e.g., [21]). The 'bilayer sonophore' model [23] provides a possible framework for predicting this dependence. According to the model, a central measure of the acoustic effect, the maximal areal strain of the bilayer membrane leaflets $\varepsilon_{A,max}$, is frequency dependent:

$$\varepsilon_{A,\max} \propto P_A^\beta f^{-0.5},$$
 (5)

where P_A is the peak negative pressure and β is ~0.8–0.9 (see [23] for details). Assuming that $\varepsilon_{A,\text{max}}$ predicts the stimulation efficacy, induction of a similar effect at varying frequencies requires applying ultrasonic pressures that vary according to

$$P_A \propto f^{1/2\beta}.$$
 (6)

Thus, the acoustic intensity is expected to increase proportionally to $f^{1/\beta}$ (power of 1–1.25) in order to maintain the same stimulatory effect. We note that the relationship in equation (4) has an interesting similarity to the acoustic mechanical index, $MI \equiv P_A f^{-0.5}$, and for $\beta = 1$ the scaling of intensity will also maintain a constant *MI*.

In addition, the US attenuation coefficient is dependent on frequency according to

$$\alpha = \alpha_0 f^{\gamma},\tag{7}$$

where α_0 is the attenuation coefficient at 1 MHz and γ is reportedly between 1.0 and 1.9 for ocular structures [31, 32]. Using reported values of attenuation in the cornea, aqueous and vitreous humors and the lens [30–32] the total expected attenuation at 1, 10, 20 and 30 MHz is approximately 0.4, 6, 14 and 25 dB, respectively, translating to intensity losses of ~9%, 75%, 96% and 99.7%, respectively. These numbers may further increase if the patient also has an implanted intra-ocular lens (IOL). For example, a common IOL material—PMMA [43]—is expected to have increased attenuation by 0.4 dB at 1 MHz and roughly 3 dB at 10–20 MHz (calculated using values from [44]) in addition to much stronger reflections (24% versus 1%).

The combination of the known frequency-dependent attenuation and the predicted frequency-dependent efficacy indicates that increasing the frequency necessitates a strongly super-linear corresponding increase in the incident intensity. Apart from being unfavorable in terms of the device's power consumption, the standards for safe use of ultrasound in ophthalmology limit the intensity which can be applied. The safety guidelines published by the American Institute for Ultrasound in Medicine (AIUM) and the American Food and Drugs Association (FDA) are based on temperature, intensity and pressure indices [36], and are aimed at protecting the tissue from possible damage due to the effects of heating, cavitation, radiation pressure and other potentially harmful phenomena. The guidelines for ophthalmology applications, generally more restrictive than for other applications, are given in table 3. Studies on US neuro-stimulation indicate that ultrasonic neural stimulation is associated with a negligible increase in temperature [19-22], so in practice it appears that the pressure and intensity guidelines will be the limiting factors in this case.

In previous studies of *in vivo* US neuro-stimulation, excitation of mouse motor cortex and hippocampus was reported at frequencies between 0.2 and 0.5 MHz using excitation waveforms with $I_{\text{SPPA}} \leq 0.23$ W cm⁻² and $MI \leq 0.2$ [21], while rabbit motor cortex was stimulated *in vivo* at 0.67 MHz, with $I_{\text{SPPA}} = 12.6$ W cm⁻² and MI = 0.66 [22]. The retinal stimulation regimes we described in section 3 are similar to these, and when transmitting at 0.5 MHz responses were recorded at intensities that generally conform to these guidelines (with the exception of an experiment on a single animal in which the I_{SPTA} was 83 mW cm⁻²). When utilizing focused, rather than diffuse, US stimulation, the maximum intensity will be incident on the retina, resulting in higher efficacy and allowing further reduction of transmitted power.

Index	Definition	Safety limit	Source
Spatial peak time average intensity	Intensity at the spatial peak, averaged over the sonication time	$I_{\rm SPTA}\leqslant 50~{ m mW/cm^2}$	FDA
Spatial peak pulse average intensity	Intensity at the spatial peak averaged over the time of a burst of pulses	$I_{\rm SPPA}\leqslant 150~{ m W/cm^2}$	FDA
Mechanical index	$MI = \frac{P_{\text{neg}}[\text{MPa}]}{\sqrt{f[\text{MHz}]}}$, where P_{neg} is the negative peak pressure	$MI \leqslant 0.23$	FDA
Temperature rise Thermal index	Increase in temperature due to sonication $T_i = \frac{\text{transmitted acoustic power}}{\text{power predicted for}\Delta T = 1^{\circ}\text{C}}$ The power is predicted from the bio-heat equation assuming a constant attenuation coefficient	$\Delta T \leqslant 6 - rac{\log_{10}(ext{exposure time})}{0.6}$ $T_i \leqslant 1$	AIUM FDA

Table 3. Safety guidelines for ophthalmological US applications, according to [36].

6. Conclusions

In summary, we have presented, validated and analyzed the basic properties of an ARP, a device that is aimed at non-invasive patterned excitation of populations of retinal neurons using acoustic interference patterns projected from a multi-element phased ultrasonic array. Although many questions about this technological framework remain open, our *in vivo* experiments and the analysis in section 5 suggest that a low-acuity ARP with sub-mm resolution and intensities that comply with international ophthalmic safety guidelines appears to be feasible using frequencies in the low MHz range. Moreover, a preliminary assessment showed no short-term damage to the retina, which appeared to remain functionally and morphologically intact.

A prosthesis operating in the 2-10 MHz range could potentially become an external, implant-less, alternative to implantable systems like the Argus II with a similar spatial resolution. Such a device could also have a larger field of stimulation, and the added advantage of having a naturally shifting retinal stimulation field during eye movements, as in opto-electronic devices [3]. Transmitting at very high US frequencies of 30-100 MHz, as in current ultrasound biomicroscopy applications, is expected to lead to the superior resolution required for high-acuity prostheses. However, our retinal stimulation study and model-based initial analysis both indicate that an increase in US frequency requires a corresponding super-linear increase in intensity, suggesting that safety considerations could become prohibitive at high frequencies. 'Optimum' frequencies (and duty cycle) will probably have to be selected as a tradeoff between the effective resolution, safety considerations, power requirements and image refresh rate. Further research is clearly required to expand our understanding of the mechanisms behind US neuro-stimulation and explore the excitation requirements of retinal neurons at higher frequencies (see [42] for a preliminary report).

The algorithm introduced and experimentally validated in section 4 allows efficient projection of *continuous* stimulation patterns, which should enhance the likelihood for inducing integrative (*gestalt*) percepts. Using optimized software and/or hardware implementations of this algorithm, combined with the short stimulation times that appear to effectively excite retinal neurons (~ 10 ms), it may be possible to achieve refresh rates as high as tens of images per second.

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