TomoWave Laboratories, Inc.

High-resolution optoacoustic microscopy for *in vivo* imaging

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Introduction. Optoacoustic (OA) microscopy is a high-resolution variant of optoacoustic imaging technology which requires higher ultrasound (US) frequencies and large numerical aperture (NA) to generate *in vivo* images with resolution below 100 µm at depths of several millimeters. Lateral R_L and axial R_a resolution of an imaging system is defined as:

$$R_L \approx 0.5\frac{c}{f\cdot NA} \qquad R_A \approx \frac{3\cdot c}{f} \qquad (1)$$

where, where $c \approx 1500$ m/s is the speed of US in water, f is the upper frequency limit, and NA defines the numerical aperture of the system. Earlier OA microscopy systems which utilize either focused transducers or acoustic lenses for US focusing have inherent limitations that impair their performance [1,2]. For example, since apertures of available focused transducers are relatively small, only ~ 0.1 - 0.3, higher frequencies are required to achieve the desired resolution. However, strong frequency dependent attenuation in tissues severely limits imaging depth. Refractive elements (e.g. quart lens [1]) introduce large (> 90%) insertion losses and severely limit bandwidth of an imaging system, thereby affecting its imaging depth and resolution.

We propose a novel experimental design based on an off-axis parabolic reflector which will achieve diffraction-limited US focusing. Parabolic mirror will perform an ideal and lossless conversion of a spherical wavefront into a plane wave to allow the use of arbitrary flat-surface transducer for detecting US signals. Large NA, up to 0.7, can be achieved with these types of reflectors. System with high NA will require lower frequencies and thus will achieve better imaging depth.

Principles of experimental design. By definition, a parabola is the family of points equidistant from a focal point (focus) and a line (directrix). It follows from the definition that the incoming flat wavefront will be focused by a reflecting parabolic surface to a point. Parabolic mirrors can be manufactured with a micrometer scale precision, which exceeds requirements for ultrasound focusing by more than an order of magnitude. NA of an off-axis parabolic reflector defined by parameters *a*, z, and *F* (see Fig. 1a) is given as:

$$s\ \beta = \frac{z}{z+2a} \qquad s\ \alpha = \frac{a - F^2/4a}{a+F^2/4a} \qquad N\ (a,z,F) = s\left(\frac{\alpha+\beta}{2}\right) \quad (2)$$

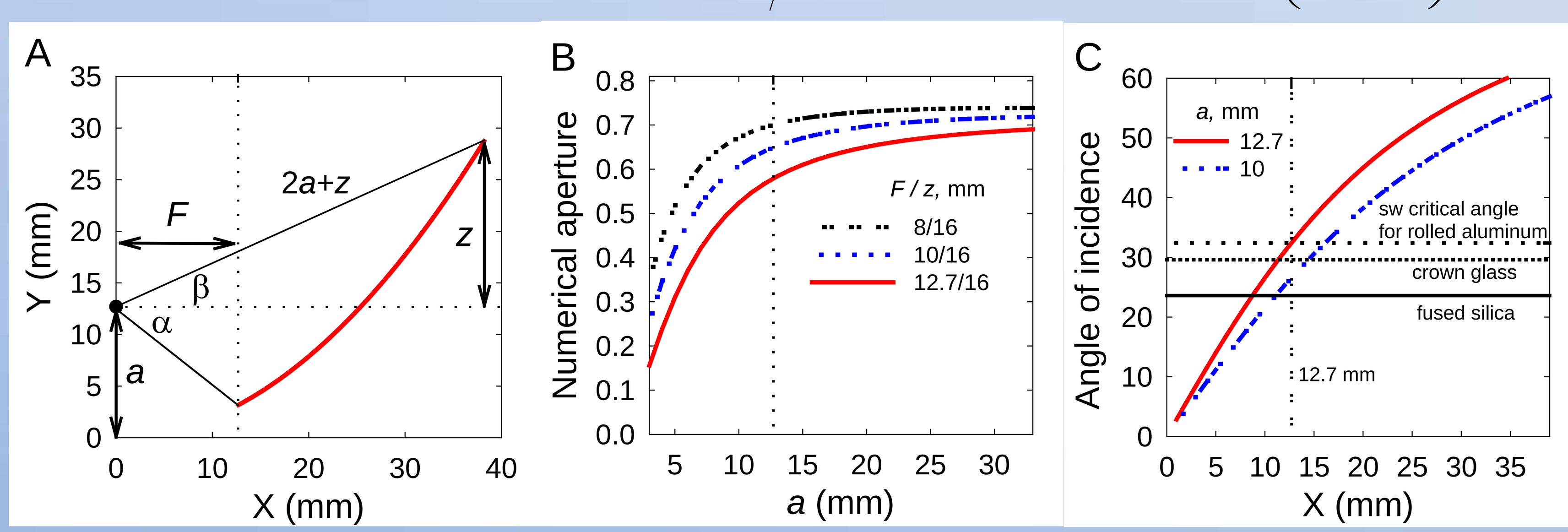

Figure 1. (A) Cross-section of a parabolic mirror defined by parameters a= F = 12.7, and z = 16 mm. (B) NA of a parabolic reflectors as a function of *a* at selected *F* and *z* values. Vertical line is plotted at *a* = 12.7 mm. (C) Variation in the angle of incidence at the surface of a reflector as a function of X-coordinate.

Red lines in Fig. 1 show the actual reflector in our experimental setup. As follows from Fig. 1b,c, large NA values of 0.6 – 0.7 can be readily achieved. Reflector made from materials with sufficiently high shear wave velocity will satisfy conditions for total internal reflection in the large range of apertures. Hence, **an off-axis parabolic reflector will perform an ideal and lossless conversion of a spherical US wave into a flat wavefront**. Absence of insertion losses ensures that the US will be focused to a smallest possible spot defined by diffraction.

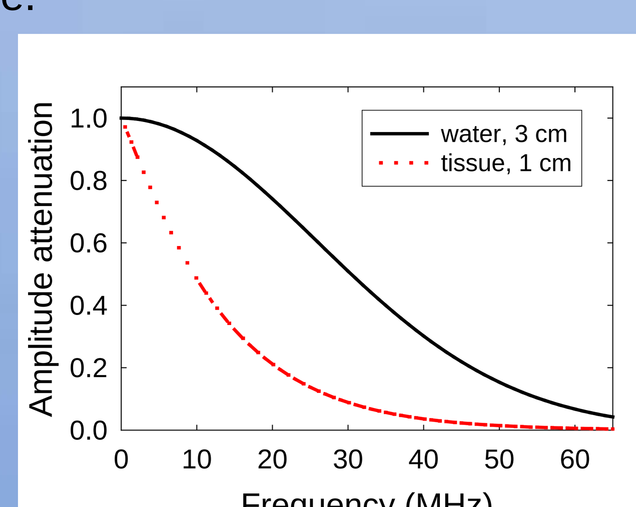

Figure 2. US attenuation in water and tissues.

Advantages of a proposed design. **(1)** large NA; **(2)** absence of insertion losses, except those for US attenuation in water; **(3)** imaging at lower frequencies to achieve larger depths; **(4)** allows using inexpensive flat surface transducers of any frequency; **(5)** broadband system, its bandwidth is defined by the transducer only.

Performance of OA microscopy system. Figure 3 shows a schematics of the OA microscope. It utilizes and off-axis aluminum parabolic mirror with a = 12.7 mm, and 1" and NA of 0.54. To allow to allow straight downward sample illumination, an orifice ~ 5 mm in diameter was drilled through the mirror. We imaged an US wavefront created by a spherical point source after reflection from the parabolic mirror by scanning a 3×3 cm area with a point ultra-broadband transducer (see Fig. 3). The reflected wavefront is flat and has a uniform intensity across its area. Diffraction effects at the edges and the central part corresponding to a drilled hole are visible. A sample of crossed human hairs with diameter ~ 100 µm was imaged to determine resolution of the microscopy system. The measured FWHM of match the theoretical diffraction-limited resolution of 0.28 µm with 5 MHz upper frequency limit and 0.54 NA (Fig. 5).

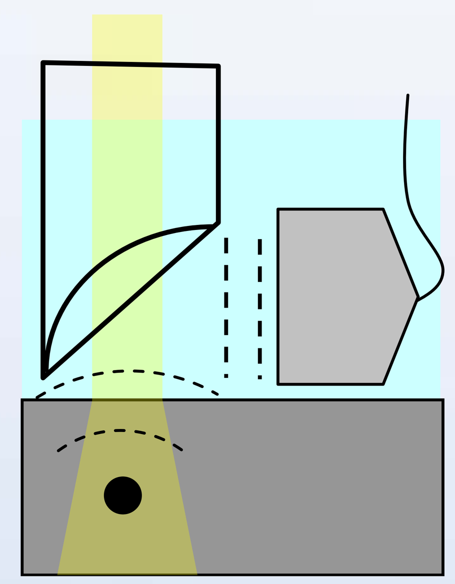

Figure 3. Schematic diagram of the OA microscopy system.

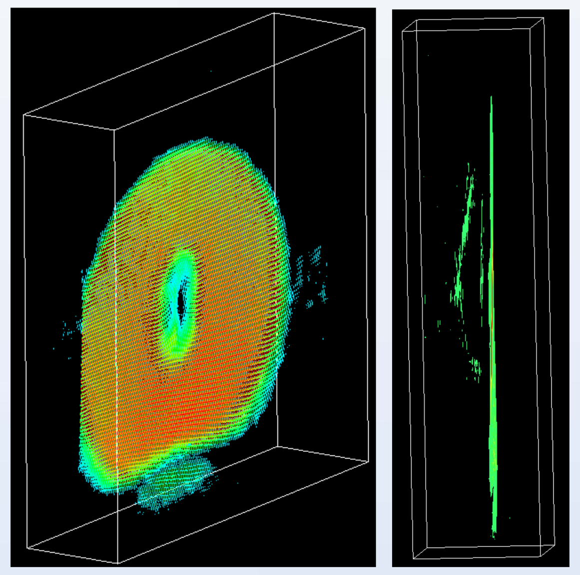

Figure 4. Conversion of a spherical wavefront into a plane wave by an off-axis parabolic reflector.

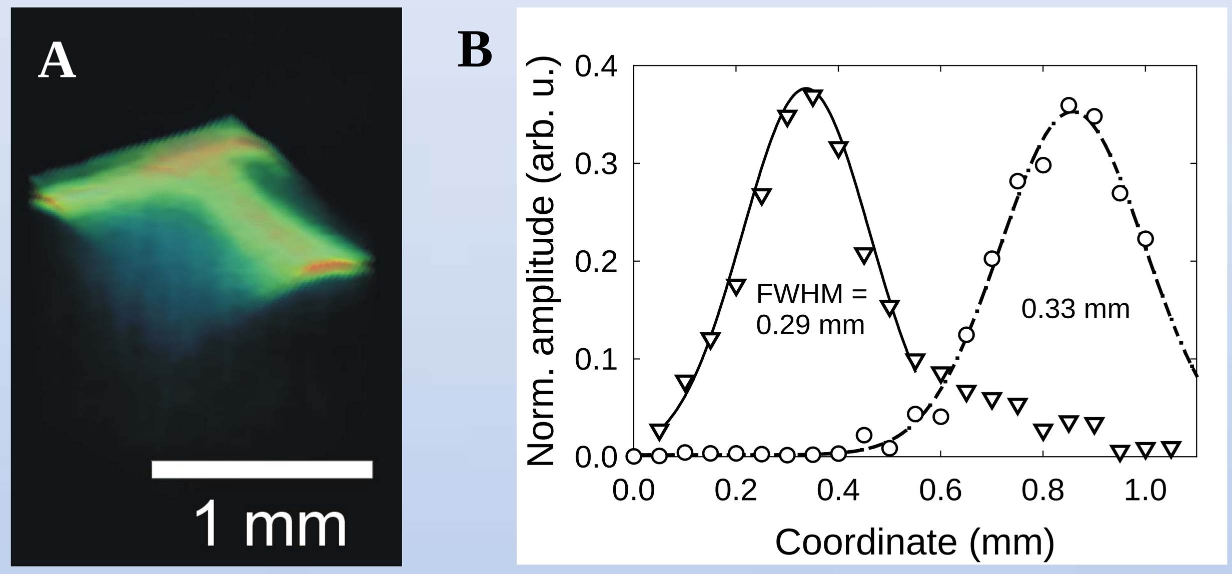

Figure 5. (A) Volumetric maximum intensity projection image of a pair of crossed hairs. (B) Intensity profiles across both hairs measured at the position of maximum intensity.

Further modifications and improvements.

(1) Improving transducer sensitivity and bandwidth. Transducers with higher upper frequency limits are needed to increase resolution. We have designed and built a new transducer that has ×2 better sensitivity and ×2.6 larger bandwidth (Fig. 6).

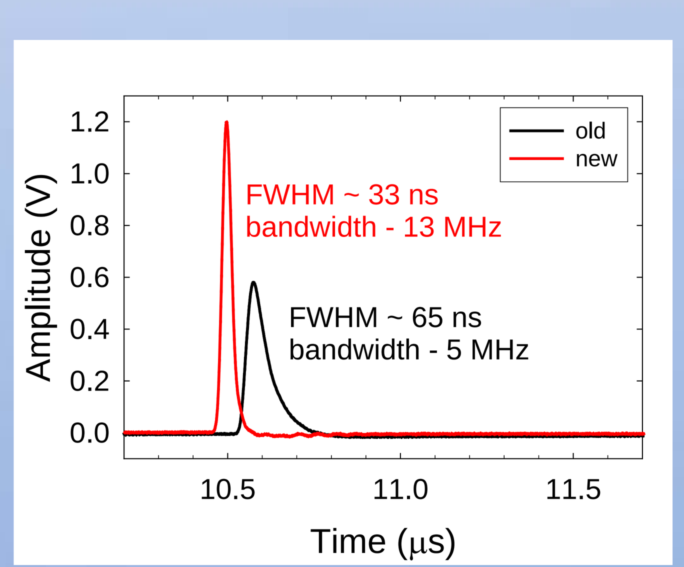

Figure 6. Impulse responses of the old and the new transducer designs.

(2) Increasing the rate of scanning. Point-by point scanning at high resolution is relatively slow. At present, the imaging volume in a single acquisition is defined by lateral resolution and depth of field. To improve the rate of data acquisition, we propose the use of an imaging array with small number of elements instead of a single-element transducer. This will allow limited image reconstruction in volume which is ~ a factor of 10 larger than diffraction-limited volume.

(3) Design efficient-light delivery. Optically transparent reflector is desired to allow most efficient light delivery (as shown at Fig. 3). These can not be easily manufactured with a required precision. However, with the transducer array exact parabolic profile is not required, since array allows wavefront correction. Hence, Pyrex glass parabolic reflectors found in arc-lamp illuminators, which are manufactured within 0.5 mm tolerance, will be suitable for US focusing.

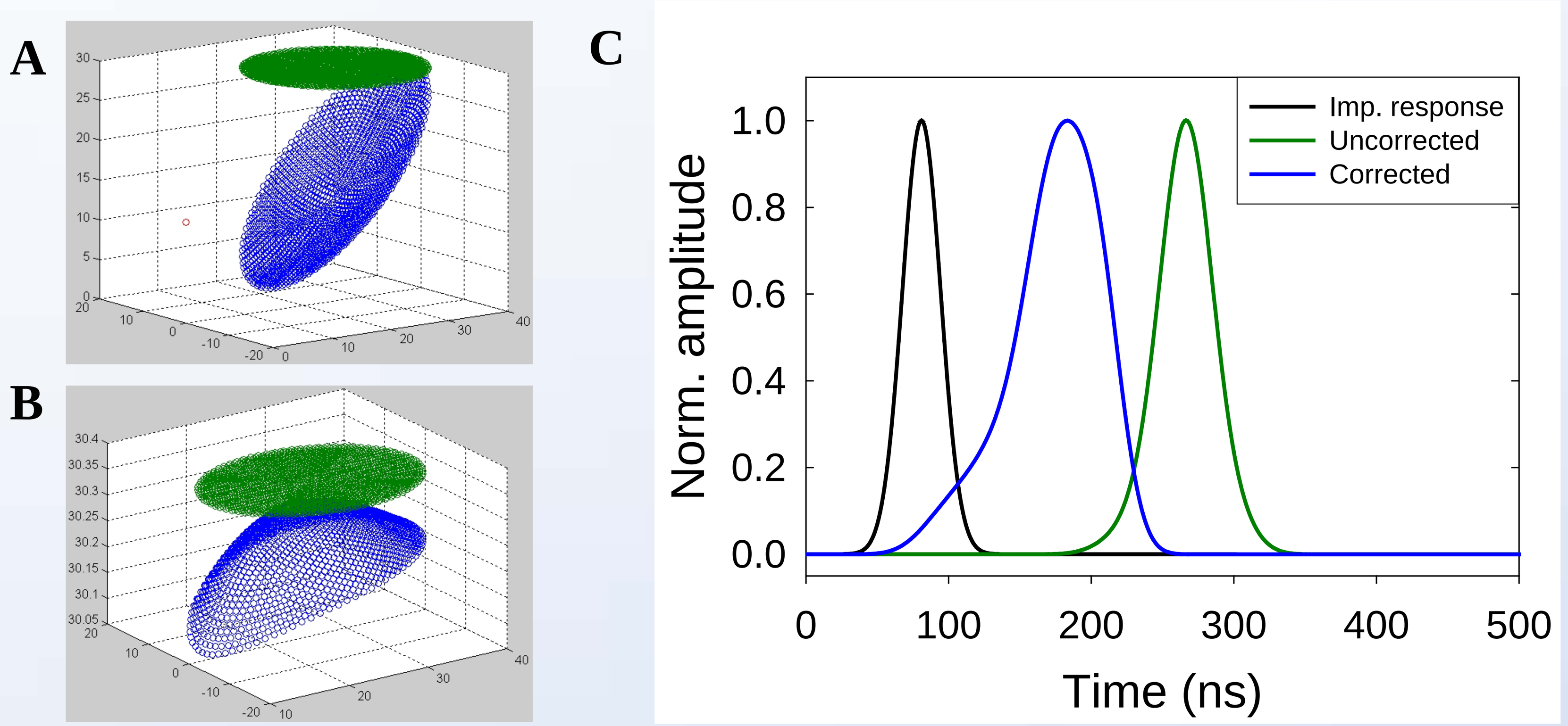

Figure 7. (A) Numerical simulations of a reflected wavefront from a point source (red circle) positioned in a focal point of the reflector. The reflector surface is shown in blue, and the reflector wavefront is green. (B) Reflected wavefronts from a point in the focus (green) and a point at 1 mm distance from the focal point (blue) The image is significantly expanded along the Z-axis. (C) Simulated impulse response of the system from point source in a focal point (black line), and a point source offset by 1 mm from the focus (blue line). Green line shows the corrected impulse response with 4x4 transducer array.

Potential commercial applications.

(1) Optoacoustic microscope for preclinical applications. Non-invasive high-resolution imaging of blood vasculature in small animals is needed to study models of various diseases. We expect to achieve imaging depth up to 1 cm with a resolution below 100 µm with our novel design. Applications of optoacoustic microscopy include:

a) Visualizing tumor growth and angiogenesis
b) Monitoring nanoparticle accumulation in tumors
c) Imaging brain vasculature and brain pathology (stroke, ischemia).

(2) Optoacoustic endoscopy. Miniaturized version of the proposed design will be translated into clinical endoscopic applications for early detection and staging of esophageal and colon cancers. Current experimental designs of OA endoscopes have limited imaging depth and resolution due to narrow bandwidth detection as in conventional US imaging [3,4]. US imaging generates bandwidth-limited signal and detects almost the same signal reflected from the boundaries with acoustic impedance mismatch. In optoacoustics, tumor size primarily defines signal bandwidth.

For comparison, a tumor with a size of 1.5 mm will generate ~ 1 MHz broadband OA signal and will typically not be imaged by resonance transducers operating at central frequencies of 5 MHz and above.

To achieve the best performance, our proposed design will include a rotating parabolic which includes a rotating off-axis parabolic reflector and a broadband transducer array to allow dynamic focusing.. Central element of an array will image tissue structures close to the wall of the transducer, while more distant structures will be imaged by a whole array.

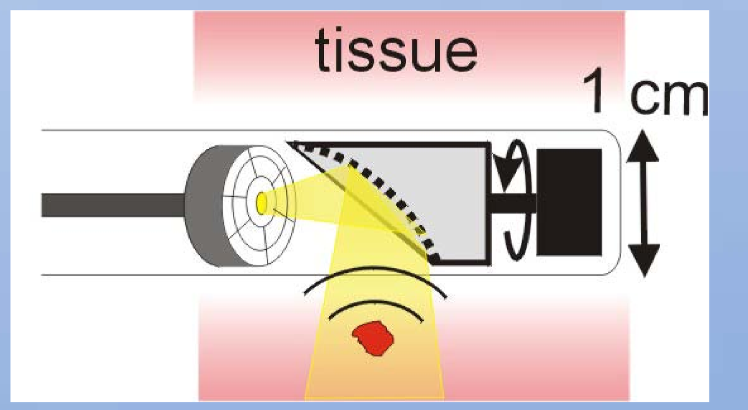

Figure 8. A proposed design of optoacoustic endoscope, which includes a rotating off-axis parabolic reflector and a broadband transducer array.

References.

[1] Maslov K., Stoica G., Wang L.H.V. "In vivo dark-field reflection-mode photoacoustic microscopy", Optics Letters 30(6), 625-627 (2005).
[2] Zhang H.F., Maslov K., Wang L.H.V. "In vivo imaging of subcutaneous structures using functional photoacoustic microscopy", Nature Protocols 2(4), 797-804 (2007).
[3] Yang J.M., Maslov K., Yang H.C., Zhou Q.F., Shung K.K., Wang L.H.V. "Photoacoustic endoscopy", Optics Letters 34(10), 1591-3 (2009).
[4] Yuan Y., Yang S.H., Xing D. "Preclinical photoacoustic imaging endoscope based on acousto-optic coaxial system using ring transducer array", Optics Letters 35(13), 2266-2268 (2010).