Elongated Focus Optoacoustic Microscopy with Matched Bessel Beam Illumination and Ultrabroadband Axicon Detection

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Current optoacoustic (OA) microscopy configurations often have narrow focal ranges that limit their use for fast volumetric imaging applications. Herein, the focal range of OA microscopes is extended by matching the elongated optical illumination profile of a Bessel beam with the pencil beam acoustic sensitivity profile of a broadband axicon detector. An inverted OA microscope is developed with interchangeable optical illumination and acoustic detection units to assess the working distance and resolutions retained with several combinations of illumination and detection profiles. Matching Bessel illumination with axicon detection extends the depth of focus 17-fold over traditional configurations. Imaging a tilted mouse ear with the matched Bessel—axicon configuration reveals vasculature over a working distance exceeding 4.2 mm with optical resolution, while affording a sixfold increase in imaging volume over the same scanning duration compared with configurations using standard Gaussian illumination, demonstrating this approach’s promise for increasing applications for OA microscopy in preclinical research.

1. Introduction

Optoacoustic (OA) microscopy is a label-free hybrid approach that uses optical pulses to induce the emission of broadband sound waves in biological tissues, which are subsequently detected by an ultrasound transducer.[1] Common OA microscopy methods apply Gaussian beam illumination, which is diffraction limited and thus has a confined focal range (Rayleigh range). Spherically focused transducers are often used for detection in OA microscopy because their spatial acoustic sensitivity profile complements the optical sensitivity profile of Gaussian illumination, which maximizes the overall OA sensitivity.[2,3] Despite the excellent sensitivity afforded by the combination of Gaussian beam illumination and spherically focused transducers, such OA microscopy configurations only provide high-resolution imaging at the Gaussian beam focal point.[1,3–5] This narrow focus necessitates scanning the sample in the axial direction over the same focal plane to produce 3D images. Hence, current OA microscopy configurations are suboptimal for volumetric imaging applications that are time sensitive or involve sample motion, such as full-body zebra fish imaging, measuring thick optically cleared samples, or cell imaging and counting.

Unlike Gaussian beams, which have point foci irradiance distributions along the axial direction, the so-called ideal Bessel beams are not diffraction limited and therefore have line-shaped foci. However, ideal Bessel beams are not realizable because they exhibit an infinite number of concentric rings surrounding a main lobe in the plane orthogonal to the axial direction, thereby requiring infinite energy. Hence, physically realizable Bessel beams are truncated by an aperture, which results in a limited axial propagation distance over which the beam is diffraction free. In practice, Bessel beams are approximated by directing collimated Gaussian illumination through a conical (axicon) lens, which yields a line-shape optical sensitivity profile that enables imaging over extended depths without sacrificing lateral resolution.[6,7] As such, Bessel beam illumination has been investigated as a means of increasing working distance in several optical imaging modalities, including light sheet microscopy, optical coherence tomography, and second-harmonic generation.[8–12]

Attempts to increase working distance with Bessel beam illumination have also been reported in OA microscopy. For example, an OA microscope using Bessel beam illumination was used to image red blood cells with 7 μm lateral resolution over a depth of 1 mm.[13] Similarly, two imaging studies of mouse ears demonstrated a sevenfold increase in working distance when imaging with Bessel beams in place of Gaussian beams. The lateral...
resolution/depths of focus of these systems was reported to be 1.6 μm/483 μm and 300 nm/229 μm. However, these microscopes used either unfocussed or spherical transducers with acoustic sensitivity profiles mismatched to the Bessel beams’ line foci, thereby limiting the OA sensitivity in the axial direction. This problem has been circumvented using optical axicon interferometric ultrasound detection paired with Bessel beam illumination to image zebra fish with a lateral resolution of 2.4 and a 635 μm depth of focus. However, optical interferometric detection requires a thermally stabilized continuous-wave light source and a high-speed balanced photodetector with high bandwidth, which increases the system’s cost. In addition, optical ultrasound detection requires strict beam path alignment and environmental control, as the beam paths are sensitive to temperature fluctuations and microvibrations. Furthermore, trigger synchronization is required between the balanced photodetector driven by a secondary laser and the OA laser, thereby increasing the complexity of data acquisition. Finally, the interferometric signals measured by the photodetector require calibration and reconstruction to recover the original OA signal.

Our group recently demonstrated that a high-frequency ultrawideband ultrasound transducer integrating an axicon lens to focus the incoming ultrasound waves along the axial direction offers extended acoustic depth of focus with retained high lateral resolution. We postulated that the thin cylindrical axial sensitivity field (i.e., pencil beam sensitivity) of the axicon transducer would complement the optical profile of a Bessel beam, thus enabling a simple low-cost approach to sense acoustic waves with improved sensitivity along the axial direction, potentially maximizing the available working distance in OA microscopy and enabling fast volumetric imaging.

We therefore set out to establish whether the depth of focus in OA microscopy could indeed be maximized by matching the ultrasound detector sensitivity to Bessel beam illumination using an ultrabroadband axicon transducer, while also evaluating the effects of mismatches in illumination and detection geometry. Accordingly, we devised a custom OA microscope that allows modular exchange of illumination and detection units, while retaining the same imaging field of view. We perform a robust and direct comparison of working distance and lateral resolutions achieved by four combinations of illumination (Gaussian or Bessel) and detection (spherical or axicon) geometries. In a controlled experiment, we demonstrate that our proposed configuration, with matched Bessel illumination and axicon detection, extends the OA microscopy depth of focus by up to 17 times compared with configurations that combined Gaussian illumination and spherically focused transducers. We highlight the expanded depth of focus, long working distance, and high resolution of our OA microscope by imaging the vasculature of a tilted mouse ear over depths exceeding 4.2 mm. This extended depth of focus increased the imaging volume sixfold over the same scanning duration compared with the Gaussian spherical combination.

2. Results

2.1. Broadband Spherical and Axicon OA Transducer Characteristics

Two broadband single-element side-looking OA transducers of similar dimensions were manufactured and are depicted in Figure 1. Both sensors housed a planar lithium niobate (LiNbO3) active element. The acoustic beam is focused by mounting either a spherical (HFM36, Sonaxis, France) or an axicon (HFM37, Sonaxis, France) lens onto the active element. Both transducers also feature a 2 mm central aperture suitable for central illumination. Both transducers also feature a 2 mm central aperture suitable for central illumination. The spherical transducers have an 8 mm-diameter...
active element (central frequency = 60.5 MHz) and an acoustic lens with a curvature of 6 mm. The axicon transducer has a 9.6 mm-diameter active element (central frequency: 61 MHz) and an acoustic lens with an apex angle of 114.4° (for further transducer information, please refer to the study by Ali et al.[17]). To assess the OA response for all four possible combinations of Gaussian and Bessel illumination with spherical and axicon detection, we designed an inverted OA microscope with interchangeable illumination optics and broadband transducers (Figure 1c).

2.2. Gaussian and Bessel Beam Profile Characteristics

The transverse illumination profile in OA microscopy determines the lateral resolution of the imaging system. Commonly, Gaussian beams are produced with tight foci to increase the resolution at the expense of light divergence away from the focal point. In contrast, Bessel beams are produced to create a transverse illumination profile concentrated in a central lobe, which can propagate long distances without diverging. To create the Gaussian or Bessel illumination patterns, we focused a collimated laser beam through either a spherical or an axicon lens, respectively. We performed optical beam profile measurements to calculate the beams' transverse intensity distributions along different points in the propagation direction. Figure 2a–b shows the resulting full width at half maximum (FWHM) illumination beam diameters of ≈5 μm for both the Gaussian beam and the central lobe of the Bessel beam. As expected, the Gaussian beam displays light confinement within the 5 μm-spot, whereas the Bessel beam shows a transversal pattern with lower-amplitude concentric rings around the 5 μm central lobe. These concentric rings are an inherent property of Bessel beams, with each ring (including the central lobe) containing the same of energy. Hence the concentric rings generate secondary unwanted OA signals, which interfere with the signal generated from the central lobe and thereby degrade imaging lateral resolution. The Gruneisen relaxation effect[14] and blind deconvolution[15,16] have been exploited in OA microscopy to suppress Bessel beam side lobes and their associated artifacts. Here we also apply the blind deconvolution algorithm, as this method offers a computationally efficient and cost-effective solution (see Figure 5). The beam profile and relative peak irradiance were measured along the optical axis for both illumination patterns. Figure 2c shows the FWHM and relative peak irradiance of the Gaussian beam at 50 μm optical plane spacing intervals acquired along the optical axis over an axial distance of ±300 μm around the focal point. The peak irradiance is normalized to the maximum irradiance measured at focus. For Gaussian illumination, we confirmed a minimum beam diameter of ≈5 μm at focus and calculated a depth of focus of 108 μm. Figure 2d shows the FWHM and relative peak irradiance of the Bessel beam central lobe at 1 mm optical plane spacing intervals acquired along the optical axis over an axial distance of 12 mm moving away from the axicon lens. The arbitrary origin during the course of the Bessel beam measurement was set to ≈6 mm from the surface of the axicon lens. The Bessel beam diameter decreases with distance, reaching an average value of 5 μm over an 8 mm range. The relative irradiance for the Bessel beam's central lobe increases with distance, reaching a plateau where the Bessel beam is fully formed at a relative position of 8 mm.

Figure 2. Illumination beam profiles for OA microscopy. a) Gaussian and b) Bessel beam profiles measured at the lateral plane passing through the point of maximum irradiance. Relative peak irradiances and diameters of c) a Gaussian beam measured at 50 μm optical planes through the optical focus. d) A Bessel beam central lobe at 1 mm optical planes along the optical axis moving away from the axicon lens. Note: The arbitrary origin for the Bessel beam case is set to be 6 mm away from the surface of the axicon lens.
Matched Bessel beam-axicon detection extends depth of focus over the standard Gaussian beam spherical detection combination by an order of magnitude.

To study the effect of matching illumination and detection profiles on imaging performance, we developed a customized OA microscope in transmission mode with exchangeable illumination and detection units, which enable imaging of a test target with the same field of view. We studied four possible combinations of Gaussian and Bessel illumination and spherical and axicon transducer detectors (Figure 3a). Figure 3b shows the intensity-normalized maximum intensity projection (MIP) images of a tilted 7 μm carbon fiber for all four illumination-detection configurations. Both spherical and axicon detection, when combined with Gaussian illumination (Figure 3b(i–iii)), resolved the carbon fiber at the illumination focal point with an FWHM of 7 μm, while detecting the fiber over a total axial distance (depth of focus) of ≈z = 225 μm. The Bessel illumination combined with spherical detection (Figure 3b(iii)) resolved the carbon fiber at the point of highest OA intensity with an FWHM of 7 μm, while detecting the tilted fiber over the full field of view of 6 mm × 0.128 mm. However, the normalized OA sensitivity for the Bessel–spherical combination decreased significantly between y = 0 mm and y = 1 mm and between y = 4 mm and y = 6 mm, after which the carbon fiber is barely resolved.

Figure 3. OA microscopy of a tilted 7 μm carbon fiber using different acoustic and illumination configurations. a) Illustrations of the four OA microscopy configurations: (i) Gaussian beam, spherical transducer, (ii) Gaussian beam, axicon transducer, (iii) Bessel beam, spherical transducer, and (iv) Bessel beam, axicon transducer. b) Depictions of the normalized maximum intensity OA images. c) Corresponding false-color depth maps displayed within the depth of focus and lateral resolution for each imaging configuration.
distinguishable. The normalized intensity for the Bessel–spherical combination was ≈13% of the normalized maximum value at $y = 0.65\, \mu m$ and $y = 4.4\, \mu m$, while the corresponding working distance in that range was $z = 1.98\, \mu m$. This drastic decrease in intensity away from the center of the image is a function of the spherical shape of the detector, which concentrates sensitivity at the focal point. Finally, the combination of Bessel beam illumination with the axicon transducer (Figure 3b(iv)) reveals the tilted carbon fiber over the complete depth of $3\, \mu m$ with improved signal-to-noise ratio (SNR). Combining Bessel illumination with axicon detection results in retaining OA intensity above 30% of the normalized maximum intensity at $y = 0$ and above 23% of the normalized maximum intensity at $y = 6\, \mu m$. Notably, the FWHM was $\approx 7\, \mu m$ over almost the entire field of view (see supplementary Figure S4).

To visualize the depth of focus and corresponding lateral resolutions for the four imaging configurations, we generated false-color depth maps from the MIP images in Figure 3b, bounded by the depth of focus and lateral resolution (Figure 3c). We computed the depth of focus by extracting the axial range at which the normalized MIP is above 50% and we calculate the lateral resolution as the FWHM across the carbon fiber for each working distance within the depth of focus. Irrespective of the transducer used, either spherical (Figure 3c(i)) or axicon (Figure 3c(ii)), the Gaussian illumination resulted in a depth of focus of 75 $\mu m$. Bessel illumination used in combination with the spherical transducer (Figure 3c(iii)) extends the depth of focus by ninefold to 662 $\mu m$. As expected, the Bessel illumination combined with the axicon detection (Figure 3c(iv)) maximizes the depth of focus to 1275 $\mu m$. Hence, the matched Bessel illumination and axicon detection result in a doubling of the depth of focus when compared with Bessel illumination with spherical detection and a 17-fold increase in the depth of focus when compared with the Gaussian illumination combined with either of the transducers.

Matched Bessel beam-axicon detection can extend working distance over several millimeters while retaining optical resolution in biological tissue.

To evaluate the performance of our proposed OA microscope configuration in biological samples with irregular surfaces, we imaged a freshly excised mouse ear placed at a tilted surface (Figure 1d–e). Figure 4a–c shows the 3D projection and 2D top- and side-view MIPs attained by imaging the tilted mouse ear with the combination of Gaussian illumination and spherical detection. The vasculature is visible over a depth of about 700 $\mu m$ and a cubic volume of 28 $mm^3$ ($8\, mm \times 5\, mm \times 0.7\, mm$). Similar results are obtained for Gaussian illumination with the axicon detection case (Figure 4d–f), with vessels visible over a depth of 700 $\mu m$ and a cubic volume of 28 $mm^3$ ($8\, mm \times 5\, mm \times 0.7\, mm$). In contrast, Bessel illumination and spherical detection enable the visualization of vasculature over a depth of 2.8 $mm$ and a cubic volume of 112 $mm^3$ ($8\, mm \times 5\, mm \times 2.8\, mm$; Figure 4g–i). Finally, Bessel illumination matched with the axicon detection reveals the entirety of the mouse ear vasculature, over a depth of 4.2 $mm$ and a cubic volume of 168 $mm^3$ ($8\, mm \times 5\, mm \times 4.2\, mm$; Figure 4j–l). The retention of high resolution over the full depth of the ear allows visualization of small vessels in the upper region of the scan, which are otherwise invisible with any of the other configurations. In addition, the Bessel–axicon combination visualizes six times the volume in the same timeframe compared with the Gaussian illumination combinations and 1.5 times the volume compared with the Bessel–spherical combination. We note that the OA image quality was affected by the typical lack of blood perfusion in excised samples and, in our case, the sample manipulation required to mount the ear on a tilted surface and prolonged exposure to water (used as the coupling medium) during scanning.

Lateral resolution of OA images acquired with Bessel beams is improved by deconvolving with the optical beams’ point spread function (PSF).

To reduce image artifacts arising from the secondary side lobes of the Bessel illumination beam, MATLAB blind deconvolution algorithm was used to enhance resolution and reduce background noise (Figure 5). The raw MIP of the mouse ear acquired with the combination of Bessel illumination and axicon detection is shown in Figure 5a. The corresponding deconvolved projection presented in Figure 5b was attained by deconvolving the original image (Figure 5a) with the Bessel beam profile measurement (Figure 2b). The dotted white lines in both Figure 5a–b indicate the location of the line profile measurement for the raw and deconvolved data, as shown in Figure 5c. The line profiles show a 10% reduction in the background signal, resulting in an SNR improvement of 2.1 dB and improving the vessel width definition from 132 to 75 $\mu m$ after deconvolution.

3. Discussion

Current OA microscopy configurations are suboptimal for volumetric imaging or time-sensitive applications because they use Gaussian illumination restricting working distance within the Gaussian beam’s Rayleigh range, which can extend up to a few tens of micrometers to ensure high lateral resolution at the focal plane. We present a new OA microscope configuration that allows detection over ultralong depths of focus while retaining high lateral resolution over several millimeters. We show that the combination of Bessel illumination and OA axicon detection enables one-order-of-magnitude higher depth of focus compared with Gaussian illumination-based OA microscopy and doubles the depth of focus when compared with an unmatched combination of Bessel illumination and focused detection with a spherical transducer. This expanded depth of focus increased the cubic volume sixfold when compared with Gaussian illumination OA microscopy over the same scanning duration and without adjusting the imaging plane to produce image stacks. Such capability could significantly accelerate image acquisition for time-sensitive measurements.

Although for purely optical-based techniques the illumination and detection paths use a single optical beam profile to expand depth of focus,[18] in OA, the acoustic sensitivity field of the detection path requires matching and coalignment with the optical illumination field. The combination of Bessel illumination with matched axicon detection maximizes the depth of focus of an OA microscope without sacrificing lateral resolution. Previous attempts to increase depths of focus in OA microscopy using Bessel illumination[13–15] have been hampered by the use of unfocused or spherical transducers for detection, which limit SNR and sensitivity along the axial direction. Alternatively, OA signal
Figure 4. OA microscopy of a tilted mouse ear imaged with all four combinations of Gaussian or Bessel illumination and spherical or axicon transducers for detection. The sample was scanned using the same experimental configurations depicted in Figure 3a(i–iv). 3D projections, 2D (top), and lateral (MIPs) of the mouse ear scanned with the following illumination/transducer combinations: a–c) Gaussian–spherical, d–f) Gaussian–axicon, g–i) Bessel–spherical, and j–l) Bessel–axicon.
Figure 5. Deconvolution of an OA microscopy MIP image of the vasculature of a mouse ear from the combination of Bessel illumination and axicon detection. a) The raw and b) deconvolved MIP images. c) A comparison of FWHM measurements between the raw and deconvolved images, showing an improvement in image resolution and reduction in background noise.

detection via optical interferometry with Bessel beam illumination falls victim to the stringent beam path alignment and environment control as the differences between optical paths are sensitive to vibrations and ambient temperature. Furthermore, additional light sources and high-speed photodetectors are required to increase system complexity and cost. Here we demonstrate a simple low-cost approach to increase depth of focus, and thereby the accessible imaging volume, by matching the Bessel beams’ optical sensitivity profile with our axicon transducer’s thin cylindrical acoustic sensitivity response, affording a 17-fold larger depth of focus compared with combinations of Gaussian illumination and either spherical or axicon detection (Figure 3c). When imaging an excised tilted mouse ear, the Bessel beam–axicon transducer configuration allowed images of the entire vasculature network over a depth of 4.2 mm and cubic volume of 168 mm$^3$ (Figure 4j–l). In contrast, combining Bessel beam with a spherical transducer reduced the measured depth to 2.8 mm and cubic volume of the ear vasculature to 112 mm$^3$ (Figure 4g–i), while using Gaussian illumination with either detector limited the overall working distance to 700 μm and the cubic volume to 28 mm$^3$ of the ear vasculature (Figure 4a–f). Hence, the Bessel–axicon combination imaged sixfold the cubic volume of the ear vasculature over the same scanning duration when compared with the combinations that used Gaussian illumination. Our findings are further supported by 3D k-wave simulations, which show that preservation of the lateral resolution over extended depth of focus with single-element axicon detectors can only occur when matched with Bessel illumination (see Section 1, Supplementary Information).

While Bessel beam illumination enables extension of the focal depth without compromising the inner-lobe spot size, the secondary lobes surrounding the inner lobe generate undesired OA signals deteriorating the overall lateral resolution of the OA image. The lateral resolution in the Bessel beam–axicon transducer-matched OA microscope configuration can be improved by deconvolving the OA image with the PSF of the Bessel illumination. We found that the side lobes of the Bessel beam (Figure 2b) indeed result in secondary OA signals, which interfere with the signal generated by the inner lobe, degrading the lateral resolution and contrast. Recent endeavors to suppress side-lobe artifacts when using Bessel illumination include exploitation of the nonlinear Gruneisen relaxation effect, blind deconvolution, or interferometric detection. However, the nonlinear Gruneisen process increases system cost and interferometric detection increases experimental complexity. Hence, we follow a similar approach to Jiang et al. and performed postprocessing suppression of the side lobes via blind deconvolution, which is computationally efficient and cost effective. We deconvolved the acquired OA images with the optical beam profile measurement of the Bessel beam (Figure 2b) and improved the resolution measured across a blood vessel (Figure 5) by a factor of 1.7 and also suppressed the background signal by an SNR improvement of 2.1 dB.

Using axicon acoustic detection for microscopy offers several advantages. For instance, even in combination with Gaussian illumination, axicon detection enables elongated acoustic sensitivity fields where the illumination could be focused by means of a spatial light modulator (SLM) at different positions along the axial direction. As a result, high-resolution volumetric imaging could be obtained without mechanically moving the sample or imaging unit. Matching axicon ultrasound detection with Bessel illumination provides three main advantages. First, the configuration is robust to axial misalignment of the illumination, detector, and sample. Given the extended overlapping range of the matched illumination and detection sensitivity, the relative axial alignment can be coarse and does not affect image lateral resolution. Second, the expanded depth of focus enables high-resolution imaging of irregular surfaces, where structures would otherwise be only partially imaged by Gaussian illumination and spherically focused detectors. Hence, Bessel illumination used with axicon transducers are ideal for volumetric imaging in life-sciences applications such as OA imaging of full-body zebra fish, OA endomicroscopy, and OA flow cytometry. Finally, fast OA microscopy in volumetric and uneven surfaces is possible as axial adjustment is not required, and fast dynamic processes occurring in and out of plane could be imaged.
We have previously shown that the spherical transducer used in this study is 2.8 times more sensitive than its axicon counterpart but that the axicon transducer offers 4.2 times the depth of focus.\(^{[25]}\) Moreover, Bessel beams possess a cylindrically symmetric intensity profile that does not change in free space, with a central lobe that is remarkably resistant to diffusive spreading compared with that of Gaussian beams of similar beam diameter.\(^{[26]}\) Hence, our proposed OA microscopy configuration distributes both the excitation energy density and the acoustic detection sensitivity along an extended axial direction. The elongated sensitivity profile results in weaker OA signal generation and reduced detection sensitivity compared with Gaussian illumination and spherical ultrasound detection. Nevertheless, the tradeoff is most severe at the illumination and detection focal points and rapidly drops outside the focal plane, limiting the possibility to resolve out-of-plane structures without needing to adjust the foci to the plane of interest. Our configuration can uniquely resolve microscopic OA sources distributed over large working distances.

OA microscopy working distance could be further extended by increasing the size of the axicon detector or removing the central transducer aperture, while the lateral resolution could be increased by reducing the size of the Bessel illumination central lobe by adopting an axicon lens with a larger physical angle. However, reducing the Bessel beam central lobe diameter would also reduce the Bessel beam optical depth of focus.\(^{[25,26]}\) Although the central aperture of the axicon transducer was not used in the current investigation, we envisage placing miniaturized lenses inside the aperture in future studies to enable reflection-mode Bessel beam OA microscopy with axicon detection for in vivo applications. In addition, further investigations will follow to develop a thin optical pencil beam without secondary side lobes to avoid the postprocessing deconvolution process. Moreover, a built-in preamplifier would increase the sensitivity of the axicon transducer and improve SNR. Finally, model-based reconstruction approaches, which discretize the transducer surface shape into subelements,\(^{[26]}\) could enable acoustic resolution OA imaging with axicon detectors.

4. Conclusion

In conclusion, by carefully matching Bessel illumination with a broadband axicon OA transducer, we achieve unprecedented depths of focus for OA microscopy while retaining high lateral resolution. Although lateral resolution remains a function of the illumination spot size, we demonstrate that the depth of focus is limited by illumination when using focused Gaussian beams but otherwise limited by the ultrasound detection sensitivity field when Bessel beams are used. Hence, our proposed configuration significantly reduces the compromise between depth of focus and resolution, achieving lateral resolutions of 7 \(\mu m\) with working distance greater than 4.2 \(mm\). The expanded depth of focus with high lateral resolution opens exciting opportunities for future biological and clinical applications, including deep microscopic molecular images of full samples and tissues with irregular surfaces.

5. Experimental Section

**Inverted OA Microscope Configuration:** Our inverted microscope illustrated in Figure 1c consisted of a light source with 532 nm wavelength and a 1 ns pulse-width (Wedge HB532, Bright Solutions, Italy). The energy per pulse was regulated by means of a polarizing beam splitter and a beam dump. A 90:10 beam splitter was used to divert 10% of the light toward a photodetector for triggering. The remaining 90% of light was spatially filtered to clean and expand the beam prior to OA excitation. Gaussian illumination with a spot size of \(=5 \mu m\) was achieved using a 0.25 NA 10X plan achromat (RMS10X, Olympus, Japan). Alternatively, axicon illumination with \(=5 \mu m\) FWHM was realized by interchanging the objective lens with an axicon lens (AX255 UVFS, Thorlabs, USA).

The Gaussian and Bessel illumination profiles were characterized by measuring the beam profiles along the optical axis moving away from the illumination lens surface using a beam profiler (SP620U, Ophir, USA) with a secondary 10× plan achromat lens (RMS10X, Olympus, Japan) and an 80 mm optical spacer. Figure 2a,b shows the Gaussian and Bessel beam profiles at maximum irradiance along the optical axis, respectively. The FWHM and relative peak irradiances were measured along the optical axis to assess the illumination spatial confinement for each configuration. Figure 2c shows the Gaussian beam confinement measured at 50 \(\mu m\) optical plane intervals over an axial distance of \(=300 \mu m\) around the focal point. Figure 2d illustrates the Bessel beam confinement measured at 1 \(mm\) optical plane intervals over an axial distance of 12 \(mm\) moving away from the axicon lens surface. For the Bessel beam experiment, the arbitrary origin was set to \(=6 \mu m\) from the surface of the axicon lens.

**Tilted Carbon Fiber’s Experimental Arrangement and Parameters:** For OA imaging, each axicon transducer was mounted to face the illumination source and aligned with the optical beam using a manual XYZ translation stage (Figure 1c). All four possible illumination and acoustic detection combinations (Gaussian—spherical, Gaussian—axicon, Bessel—spherical, and Bessel—axicon) were evaluated by imaging a 7 \(\mu m\)-wide carbon fiber immersed in deionized water and fixed on a tilt with a height of 3 \(mm\) and an inclination angle of 23.2° inside a custom 3D-printed tank with a planar optical window for transmission mode illumination OA microscopy (Figure 1c). The tank was attached to a motorized XYZ scanner for precise adjustment of the carbon fiber along the illumination—detection axis with a linear stage for the X-scanning direction (M-511, Physik Instrumente, Germany) and two compact precision linear stages for the Y- and Z-scanning directions (MT550-Z8, Thorlabs, USA). Upon alignment of the optical beam, transducer, and carbon fiber, a raster scan was performed for each illumination and detection combinations (Figure 3a(i–iv); scan speed: 2 \(mm\) s\(^{-1}\), scan resolution: 500 \(nm\) \(\times\) 800 \(nm\), field of view: 6 \(mm\) \(\times\) 128 \(mm\)). The cross section of the Bessel beams consisted of concentric rings, each containing the exact same amount of energy. Therefore, only a small fraction of the total energy in Bessel central beams remained within the central lobe, as confirmed in simulations (Optics Studio, Zemax Europe Ltd., UK), with the axicon lens model provided by the manufacturer (AX255, Thorlabs), which yielded \(\approx0.025\%\) of the total energy within the 1\(\sigma\) value of the central lobe. Furthermore, the axicon transducer was 2.8 times less sensitive than the spherical transducer.\(^{[26]}\) Hence, in our experiments, pulse illumination energies of 10 \(nJ\) and 2 \(\mu J\) were used for the Gaussian and Bessel beams, respectively, to ensure SNRs of \(\approx28 dB\) when using the Gaussian—spherical and Bessel—axicon configurations.

The temporal OA signals were externally preamplified with a 30 dB amplifier (Sonaxis SA, France) and connected to a T-bias (ZF8BT-4RCGW+, Mini-Circuits, USA) prior to data acquisition. OA signals were acquired using a DAQ card (ATS9373, AlazarTech, Canada) synchronized to the external trigger port via the photodetector signal. Data acquisition was controlled by LabVIEW (National Instruments, USA) and data processing to generate 2D projections was performed in MATLAB (MathWorks, USA). 3D volumetric projections were generated with Amira-Avizo (Thermo fisher scientific, USA).

**Bessel Beam Side-Lobe Suppression by PSF Deconvolution:** Importantly, Bessel beam optical profiles also exhibited secondary concentric rings surrounding the inner lobe due to the nondiffacting nature, which generated unwanted secondary OA signals interfering with the signal generated from the central lobe, thereby degrading lateral image resolution. To suppress image artifacts and background signals arising from the secondary lobes of the Bessel beam, MATLAB’s built-in blind-deconvolution algorithm (deconvblind) was applied to the image projection acquired for the
Bessel–axicon and Bessel–spherical configurations. A maximum likelihood algorithm was used to approximate the optimal system PSF to improve contrast and resolution through Lucy–Richardson (L–R) iteration processes. To accelerate the number of iterations required, and thereby the blind deconvolution process to determine the optimal PSF, we input the measured Bessel beam profile (see Figure 2b) for initial estimation of the PSF. The blind deconvolution technique was previously validated in the field of OA microscopy to suppress side-lobe artifacts.15,16

Data Availability Statement
The authors declare no conflict of interest.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Keywords
axicon, Bessel beams, depth of focus, microscopy, photoacoustic imaging

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