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# Breaking the acoustic diffraction barrier with localization optoacoustic tomography

X. Luís Deán-Ben[a] and Daniel Razansky[a,b,*]

[a] Institute for Biological and Medical Imaging, Technical University of Munich and Helmholtz Center Munich, Neuherberg, Germany.

[b] School of Medicine and School of Bioengineering, Technical University of Munich, Munich, Germany.

## ABSTRACT

Diffraction causes blurring of high-resolution features in images and has been traditionally associated to the resolution limit in light microscopy and other imaging modalities. The resolution of an imaging system can be generally assessed via its point spread function, corresponding to the image acquired from a point source. However, the precision in determining the position of an isolated source can greatly exceed the diffraction limit. By combining the estimated positions of multiple sources, localization-based imaging has resulted in ground-breaking methods such as super-resolution fluorescence optical microscopy and has also enabled ultrasound imaging of microvascular structures with unprecedented spatial resolution in deep tissues. Herein, we introduce localization optoacoustic tomography (LOT) and discuss on the prospects of using localization imaging principles in optoacoustic imaging. LOT was experimentally implemented by real-time imaging of flowing particles in 3D with a recently-developed volumetric optoacoustic tomography system. Provided the particles were separated by a distance larger than the diffraction-limited resolution, their individual locations could be accurately determined in each frame of the acquired image sequence and the localization image was formed by superimposing a set of points corresponding to the localized positions of the absorbers. The presented results demonstrate that LOT can significantly enhance the well-established advantages of optoacoustic imaging by breaking the acoustic diffraction barrier in deep tissues and mitigating artifacts due to limited-view tomographic acquisitions.

**Keywords:** Optoacoustic imaging, photoacoustic imaging, acoustic diffraction, super-resolution, localization.

## 1. INTRODUCTION

Diffraction of waves leads to blurring of sharp edges and is associated to the resolution limit in light microscopy and other imaging techniques.[1] The resolution of an imaging system is quantified via its point spread function (PSF), corresponding to the image of a point source. Indeed, it is typically considered that only points separated by a distance larger than the full width at half maximum (FWHM) of the PSF can be can be unambiguously differentiated. However, the FWHM of the PSF does not affect all dimensional measurements in the images. For example, the position of an isolated source can be measured with a much higher accuracy than the diffraction limit. An image can then be formed by superimposing the estimated positions of individual sources, provided these can be isolated. Localization microscopy is based on this principle, where an image is formed as a set of dots corresponding to the locations of individual fluorophores.[2–4] The same approach has also been used for breaking through the acoustic diffraction barrier in ultrasound imaging. In ultrasound, individual microbubbles flowing in blood are imaged.[5,6] Due to their motion, these particles can be localized in different positions in a sequence of B-mode images, in a way that an image of blood vessels can then be built by superimposing all localized positions.

Optoacoustic imaging is based on light excitation and ultrasound detection and hence is affected by both optical and acoustic diffraction, being the depth-to-resolution ratio typically $\sim 200$.[7] Light focusing is enabled for $\sim 1$ mm within biological tissues. In this range, it has been shown that the optical diffraction barrier can be overcome.[8,9] At deeper locations, OA imaging relies on acoustic inversion methods and a resolution barrier

*E-mail: xl.deanben@helmholtz-muenchen.de

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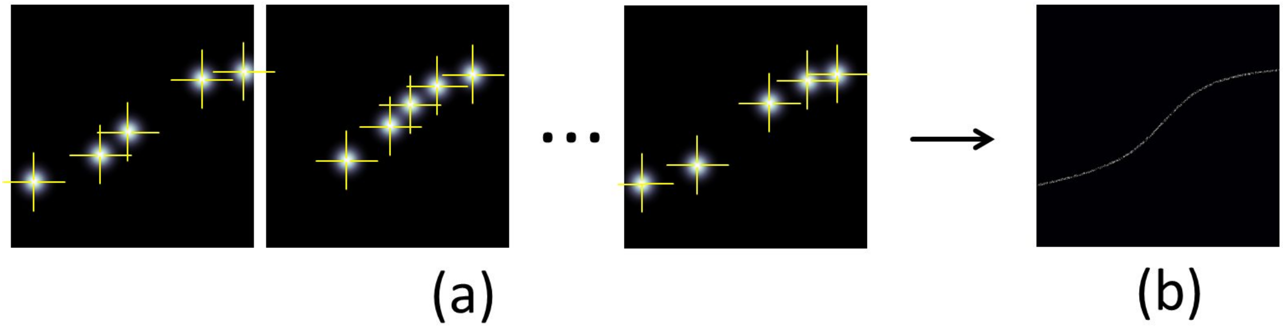


Figure 1. Imaging principle of localization optoacoustic tomography (a) Sequence of images of sparsely distributed absorbers, where the positions of individual sources are determined. (b) Localization image formed by combining all measured positions.

is dictated by acoustic diffraction. Tomographic optoacoustic systems based on ultrasound arrays can acquire a large amount of signals for each laser pulse, so that two- and three-dimensional imaging at rates higher than those achieved in standard ultrasound has been enabled.[10–14] The resolution of such systems has been enhanced via processing a sequence of images acquired by slightly shifting the detection array.[15] Also, dynamic imaging of flowing absorbers has been showcased,[16] which was also exploited for super-resolution imaging.[17] Herein, we describe localization optoacoustic tomography (LOT)[18] as an approach to break through the acoustic diffraction barrier in optoacoustic imaging.

## 2. MATERIALS AND METHODS

### 2.1 Localization optoacoustic tomography

LOT has been recently introduced as an approach to break through the acoustic diffraction barrier in optoacoustic imaging.[18] The imaging principle of LOT is depicted in Fig. 1. The starting point is a sequence of images of sparsely distributed absorbers. In each image of the sequence, the positions of the individual sources are measured. Provided the sources are sufficiently separated to be distinguished individually, the accuracy in the measured positions can be very high. The localization image (Fig. 1b) is then formed by superimposing the lcoalized positions of individual sources. In practice, a two-dimensional ultrasound transducer array is required for the acquisition of a sequence of three-dimensional images corresponding to flowing particles that change position with time. Any structure enabling the flow of particles is defined as the coordinates of the points in the encapsulated volume, so that it can be accurately mapped with the positions occupied by multiple absorbers flowing through it.

Herein, LOT was experimentally implemented with a previously-developed spherical array.[19] The array has a radius of 40 mm and consists of 256 piezocomposite elements with 4 MHz central frequency and $\sim 100\%$ detection bandwidth. The array surface provides an angular coverage of 90°. The enclosed spherical volume was filled with agar, which served as acoustic coupler and was further used to fixed the imaged samples. A short-pulsed ($< 10$ ns) optical parametric oscillator (OPO) laser (Innolas GmbH, Germany) tuned to a wavelength of 720 nm was used for optoacoustic excitation. The light beam was guided via a custom-made fiber bundle (CeramOptec GmbH, Germany) and delivered through a cavity of the array. As absorbers, polyethylene microspheres with a diameter of $\sim 30\mu$m (Cospheric BKPMS 27-32) were used. Image reconstruction was performed with a three-dimensional model-based algorithm implemented on a graphics processing unit (GPU).[20] Prior to reconstruction, the acquired optoacoustic signals were deconvolved with the impulse response of the transducer and band-pass filtered with cut-off frequencies 0.1 and 3 MHz to deliberately reduce the effective image resolution. Reconstruction was performed in a Cartesian grid of $320 \times 320 \times 320$ voxels ($8 \times 8 \times 4$ mm$^3$).

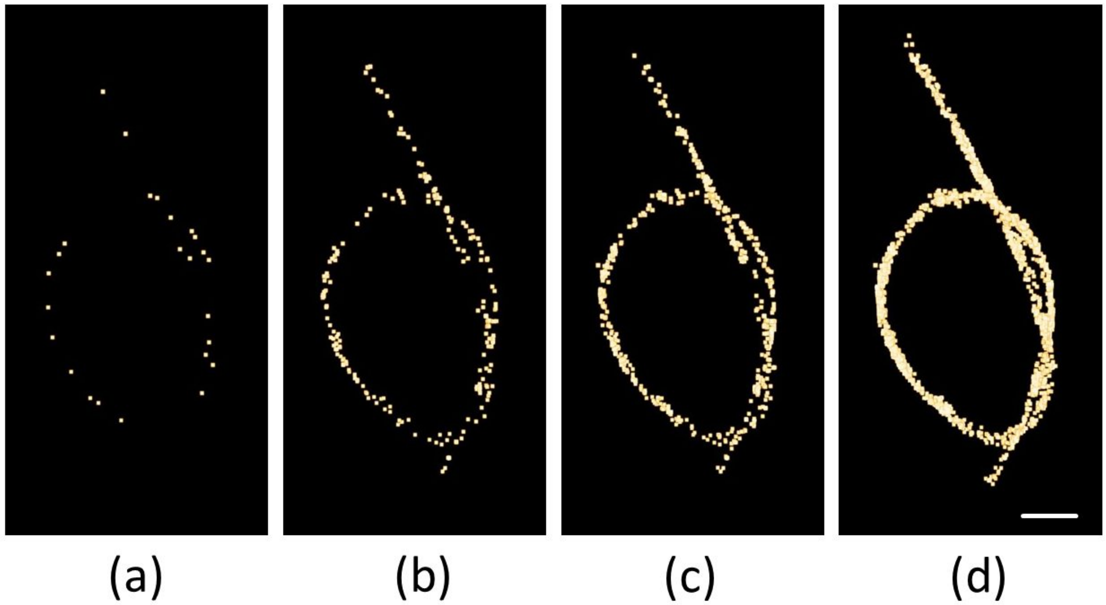

Figure 2. Localization optoacoustic tomography image of the tubing taking 25 (a), 200 (b), 500 (c) and 1500 (d) localized positions, respectively. Scalebar - 1 mm.

### 2.2 Imaging experiment

In order to test the performance of LOT, the absorbing particles were suspended in ethanol and circulated through a 20 $\mu$l Eppendorf microloader pipette tip bent to form a knot. A sequence of 1000 images was acquired, where the pulse repetition frequency of the laser was set to 10 Hz. The absorbing particles were sufficiently separated to be individually detected. Typically, $3-4$ particles per frame were localized on average. As a reference, the optoacoustic image of the tubing filled with India ink (Higgins, Chartpak, optical density 20) was also taken.

## 3. RESULTS

Fig. 2 illustrates the process of building a localization optoacoustic tomography image. Specifically, the lateral maximum intensity projections (MIPs) of the three dimensional images resulting from the combination of different number of localized positions are displayed. It is clearly shown that LOT allows resolving the shape of the knot once a sufficient number of points are localized. On the other hand, the tubing is equally visible at any point regardless of its orientation, even though the transducer array provides a rather limited angular coverage.

A comparison of the images obtained with localization optoacoustic tomography and with standard optoacoustic tomography is shown in Fig. 3. Specifically, the lateral MIPs are displayed. As opposed to standard optoacoustic tomography, LOT enables clearly resolving the shape of the knot, which indicates a clear enhancement in spatial resolution. On the other hand, the LOT image clearly shows and enhanced visibility of the lateral sides of the tubing with respect to the standard optaoacoustic image, regardless of the high absorption of ink. This is due to the fact that small individual sources are always visible in the image, as opposed to elongated structures affected by limited-view effects.[21]

## 4. DISCUSSION AND CONCLUSIONS

The presented results demonstrate that LOT can overcome the acoustic diffraction barrier and provide super-resolution three-dimensional images of structures supporting the flow of absorbing particles. In biological samples, LOT can be used for imaging vascular structures, where extrinsically-administered absorbers can circulate.

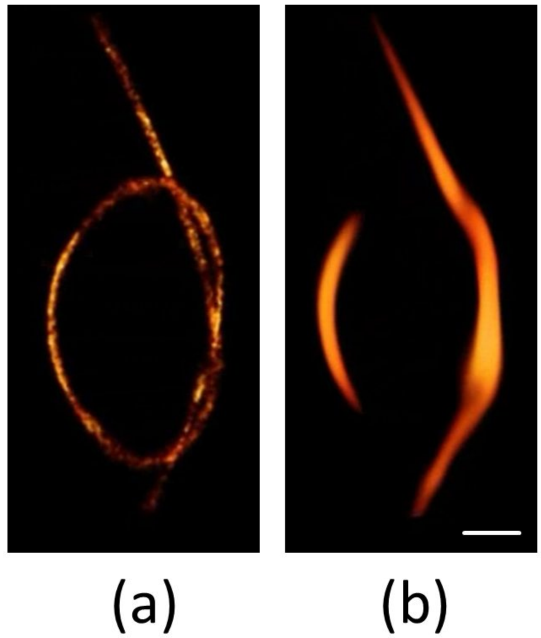


Figure 3. Comparison of the images obtained with localization optoacoustic tomography (a) and the image obtained with standard optoacoustic tomography (b). Maximum intensity projections along the lateral direction are shown. Scalebar - 1 mm.

The resolution in LOT for angiographic imaging is ultimately limited by the separation between the smallest capillaries. In practice, the achievable resolution in living organisms is expected to be conditioned by the local light fluence, the number of localized points and the amplitude of the signals generated with an individual absorber. Indeed, the applicability of LOT *in vivo* depends on the availability of biocompatible particles that generate a sufficiently strong signal to be detected individually. The dynamic range of the acquisition system must then cover these signals along with those generated by strongly-absorbing blood background, where the signals of interest can potentially be differentiated e.g. via spectral or temporal unmixing.[22,23]

The growing interest in optoacoustic imaging is strongly driven by its capability to provide optical absorption contrast at centimeter-scale depths with a spatial resolution at least an order of magnitude higher than diffuse optical imaging modalities.[7,24] LOT may serve to further increase the spatial resolution, thus opening new capabilities for deep-tissue imaging. Cancer research, neuroscience and many other biological fields may greatly benefit from a better characterization of the microcirculation beyond the acoustic diffraction limit. Also important is the fact that the rendered images are not affected limited-view effects for acquisitions with $< 180°$ angular coverage and vascular structures in arbitrary directions are accurately visualized. Localization in the time domain may also be of interest e.g. for a better understanding of neuronal connectivity. Recently, optoacoustics has been shown to be sensitive to genetically encoded calcium indicators, which enabled direct monitoring of neuronal activation.[25] In conclusion, LOT can substantially enhance the capabilities of optoacoustic imaging by breaking through the acoustic diffraction barrier at depths within the diffusive regime of light and by further providing an enhanbced visibility of vascular structures under limited-view conditions. This anticipate new insights in many physiological and pathological processes, especially those involving microcirculation changes.

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