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Three-dimensional opto-acoustic tomography using a conventional ultrasound linear detector array.

Whole-body tomographic system for small animals

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Abstract

Purpose: Optoacoustic imaging relies on the detection of ultrasonic waves induced by laser pulse excitations to map optical absorption in biological tissue. A tomographic geometry employing a conventional ultrasound linear detector array for volumetric optoacoustic imaging is reported. The geometry is based on a translate-rotate scanning motion of the detector array, and capitalizes on the geometrical characteristics of the transducer assembly to provide a large solid angular detection aperture. A system for three-dimensional whole-body optoacoustic tomography of small animals is implemented.

Methods: The detection geometry was tested using a 128-element linear array (5.0/7.0MHz, Acuson L7, Siemens), moved by steps with a rotation/translation stage assembly. Translation and rotation range of 13.5 mm and 180° respectively were implemented. Optoacoustic emissions were induced in tissue-mimicking phantoms and ex-vivo mice using a pulsed laser operating in the near-IR spectral range at 760nm. Volumetric images were formed using a filtered back-projection algorithm.

Results: The resolution of the optoacoustic tomography system was measured to be better than 130µm in-plane and 330µm in elevation (full width half maximum), and to be homogenous along a 15 mm diameter cross-section due to the translate-rotate scanning geometry. Whole-body volumetric optoacoustic images of mice were performed ex-vivo, and imaged organs and blood vessels through the intact abdominal and head regions were correlated to the mouse anatomy.

Conclusions: Overall, the feasibility of three-dimensional and high-resolution whole-body optoacoustic imaging of small animal using a conventional linear array was demonstrated. Furthermore, the scanning geometry may be used for other linear arrays and is therefore expected to be of great interest for optoacoustic tomography at macroscopic

29 and mesoscopic scale. Specifically, conventional detector arrays with higher central frequencies may be
30 investigated.

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32 **Keywords :** optoacoustic, ultrasound, linear array, computed tomography, small animal imaging

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I. INTRODUCTION

Optoacoustic (photoacoustic) imaging is a noninvasive imaging modality for high-resolution mapping of optical absorption in biological tissues^{1, 2}. The method resolves optical absorption by detecting broadband ultrasonic waves generated within the tissue following the absorption of pulsed illumination. The conversion from optical energy to mechanical waves occurs through the thermo-elastic expansion of transiently heated absorbers, and its efficiency is strongly sensitive to the optical absorption of the tissue structures. Similarly to optical imaging, the penetration of optoacoustic imaging, for ultrasonic frequencies below 20-30 MHz, is primarily limited by the light attenuation in tissues³. However, optoacoustic imaging offers at mesoscopic and macroscopic scale a higher resolution than optical imaging, even when compared to pulsed illumination time-domain optical imaging⁴, thanks to the weak scattering of ultrasound waves in soft tissues. Thereby, optoacoustic computed tomography (OAT) can provide optical contrast images with ultrasound diffraction-limited resolution⁵. Additionally, light scattering in biological tissue, and three-dimensional (3D) propagation of optoacoustic waves make macroscopic optoacoustic imaging an inherently three-dimensional modality.

Volumetric images of the organ⁶⁻¹⁰ and vascular¹¹⁻¹⁵ anatomies of healthy small animals, as well as human breast¹³ and arm¹⁶, have been obtained non-invasively using the endogenous optical absorption contrast in tissue, and were found to provide 3D visualization of tissue and vascular architecture. Localization of molecular biomarkers^{10, 17, 18}, induced thermal lesions¹⁹ or tumor vascularization²⁰ have further shown great potential and application versatility of optoacoustic imaging.

Different experimental tomographic configurations have been proposed and implemented to obtain 3D macroscopic optoacoustic images²¹. In these configurations, ultrasonic detectors were arranged along spherical^{6-9, 13}, cylindrical, circular^{5, 22-28}, or planar^{11, 15-17, 29, 30} surfaces. The choice of the detection geometry is usually dictated experimentally by practical constraints such as the size of the imaged animal or tissue, and the positioning of the illumination system. Overall, the geometrical arrangement of the detectors influences the spatial resolution achieved in optoacoustic tomography and the visibility of tissue structures, since limited view issues may arise from partial enclosure of the sample^{31, 32}. Besides the detection geometry, detector properties such as spatial and electrical (bandwidth) responses also influence the achieved resolution and image quality. For example, the spatial directivity of a detector, due to its finite size and focusing properties, defines the angular aperture and the dimension of the volume covered by its sensitivity field, and may lead to distortions of the collected optoacoustic signal^{5, 33}. Moreover, as

optoacoustic waves exhibit a broadband spectrum, with peak frequencies corresponding to different object sizes³⁴, the central frequency and bandwidth of the ultrasound detector defines the range of structure sizes that a given detector is able to resolve.

Many optoacoustic imaging implementations rely on piezoelectric detectors and benefit from technologies developed for ultrasonic measurements. Arrays of detectors allow parallel acquisition and therefore reduce the acquisition time required for data collection. A number of systems have been implemented employing custom-made concave arrays^{6, 7, 9, 13, 26, 27}, while conventional linear arrays developed for medical ultrasound are commercially available and attractive for optoacoustic imaging and tomography^{16, 28, 30, 35, 36}. Medical ultrasound linear arrays are transducer assemblies with rectangular elements arranged in a line with a fine spatial sampling, and are typically available for central frequencies ranging from 1 to 20 MHz with bandwidth as high as 60–80%³⁷. Such detectors are typically built with a cylindrical acoustic lens which enhances signal detection around the median plane of the array³³ and provides a large depth of focus³⁸. For optoacoustic tomography, the in-plane spatial resolution perpendicular to an ultrasound array (axial resolution) is primarily defined by the bandwidth of the detectors, whereas the lateral resolution (along the array) is limited by the effective angular aperture of the array. Due to the length of a typical linear ultrasound array and the typically small size of an element along this length³⁹ -that provide a large angle of acceptance-, conventional linear arrays offer lateral resolutions in the order of a few tens to hundreds of microns, depending on the operating frequency. Conversely, the detector focus along the perpendicular (transverse) dimension is not sufficient to achieve similar resolution; instead the resolution in the transverse dimension is at least an order of magnitude worse than in the lateral dimension. Linear scanning of the array perpendicular to the imaging plane has been used to cover a planar detection surface, and volumetric images have been formed by stacking 2D images^{16, 30, 35}. However, because of the small angular aperture due to the fixed cylindrical focus, the resulting volumetric images have poor resolution along the scanning axis. The lateral resolution was shown to improve by rotating the array^{28, 36}, the rotation axis being perpendicular to the imaging plane. However, the limited view issues along the rotation axis remained unsolved.

Despite the limitations in imaging performance achieved so far, commercially available ultrasound arrays are attractive detector assemblies for optoacoustic imaging. To extend the performance achieved by conventional linear arrays when employed in the context of three-dimensional optoacoustic tomography, we investigate in this paper a novel scanning geometry. The proposed geometry capitalizes on the large aperture of the array to achieve good resolution along the length of this array while it employs two movements of the detectors, i.e. translation and rotation, to enclose the imaged object

and achieve high resolution imaging along the other two geometrical dimensions. This scanning geometry resembles the translate-rotate scanning method for the first generation of computed tomography x-ray imaging⁴⁰. The capability of this geometry to provide high resolution three-dimensional optical images of large volumes is demonstrated here with a commercial 128 element linear array, which is used in ultrasonography at 5.0/7.0 MHz. The system is first characterized with phantoms, and its performance is then shown on *ex vivo* mice.

II. MATERIALS AND METHODS

A. EXPERIMENTAL SETUP

The experimental setup used in this study is shown in Figure 1. The optoacoustic system consists of three main components: the illumination part comprising a nanosecond pulsed laser and a fiber bundle, the ultrasound detector and its data acquisition system, and the array holder with motorized translation and rotation. The fiber bundle ends, the ultrasound transducer and the sample were immersed in a water tank to ensure acoustic coupling. The tank was filled with a home-made isotonic saline solution (0.90% w/v of NaCl in deionized water) to preserve the *ex-vivo* samples. The solution was stabilized at room temperature.

The excitation light originated from a tunable (690–900 nm) optical parametric oscillator laser (Phocus II, Opotek Inc., Carlsbad, California), delivering <10 ns duration pulses with a repetition frequency of 10 Hz. The beam was guided into a silica fused-end fiber bundle (CeramOptec GmbH, Bonn, Germany) consisting of 640 fibers partitioned into 4 legs. The legs were positioned 5 cm away from the sample to create an illumination pattern of ~15 mm height and ~20 mm width on the surface of the sample. The illumination was fixed and one-sided. A 12 μ m thick aluminum foil, shaped as a half hollow cylinder of radius ~ 15 mm and height 10 cm (figure 1(a), not represented on Figure 1(c)), was positioned around the sample opposite to the illumination, and acted as an optical mirror to reflect diffused and stray light back to the sample. The foil was determined experimentally to be transparent for the ultrasound frequencies considered here by comparing the broadband signals measured through water from an optoacoustic source with and without the foil (maximum one-way insertion loss of 2 dB at 12 MHz). The optical excitation was performed for all the experiments at a single wavelength: 760 nm, and with a per-

pulse energy at the laser output of $\sim 70\text{mJ}$. This wavelength ensures a good penetration in biological tissue and a contrast to deoxygenated hemoglobin⁴¹.

Ultrasound detection was performed with a 128-element linear array designed for small parts and vascular ultrasonography imaging: Acuson L7 (Siemens Healthcare). The width of one element is $270\text{ }\mu\text{m}$, with a kerf of $30\text{ }\mu\text{m}$ (i.e. a pitch of $300\text{ }\mu\text{m}$), and its height is 4.0 mm . The elements are cylindrically focused using an acoustic lens. The focal length was determined experimentally to be around 19mm . The fixed f-number of the probe is therefore ~ 4.75 , which can be considered as a weak focus. The probe has been designed to be used in ultrasonography at 7MHz and in Doppler mode at 5MHz . The array was covered with an acoustically-transparent metallized Mylar film to avoid direct illumination of the elements. The detected signals were digitized at 40MS/s and with a 12-bit resolution over a 16mV range, with a 128 channel custom built data acquisition system.

The linear array was mounted on two motorized stages: a rotary stepper motor (PRM1/MZ8, Thorlabs GmbH, Karlsfeld, Germany) and a linear translation stage (MTS50/M-Z8, Thorlabs GmbH, Karlsfeld, Germany). The stages were hanged from a platform above the water tank, and arranged in order that the translation stage could be rotated. The linear array was mounted on the linear translation stage so that its median plane was perpendicular to the translation axis of the stage.

The data acquisition system and the motors were computer-controlled. The following sequence was used for the acquisition. The laser was run continuously at 10Hz and triggered the acquisition system. For each detector position, ten acquisitions were averaged and the result stored in the computer before advancing to the next position. Averaging was employed to increase the signal-to-noise ratio by averaging noise and laser fluctuations.

Figure 1 Experimental set-up shown here for the acquisition performed *ex vivo* on a mouse. (a) Schematic top view of the set-up. A Cartesian coordinate system is specified. The origin of the system is set so that the plane $z=0$ correspond to the middle of the linear array, and the x- and y-axis so that the axis of the rotary stage corresponds to the z-axis. The z-axis corresponds to the elevation direction. (b) Schematic description of the scan geometry shown here for 4 rotary positions. The different positions of the array are presented. The positions of the rotary stage are indexed with capital letters while the positions of the translation stage are indexed with numbers from 1 to $(2n+1)$. (c) Annotated picture of the experimental set-up.

B. SCAN GEOMETRY AND PARAMETERS

The detector array was moved to discrete positions along the contour of a polygon (Figure 1 (b)). Each of the linear segments of the contour (polygon sides) was tangent to a circle centered on the axis of rotation. The radius r of this circle was set to be approximately equal to the focal length of the array ($r \approx 19$ mm), and was more accurately determined by imaging a calibrated phantom (see D). For the sake of simplicity, the linear segments were chosen symmetric with respect to the perpendicular radii of the circle, and the angles between two consecutive radii had all the same measure $\theta = 1.5^\circ$. The two extreme radii of the polygonal contour define the angle coverage in azimuth, and made an angle of 178.5° , corresponding to

180°- θ . This angle coverage had two main advantages over the full angle coverage (360°- θ). First, the opening in the contour allowed setting a fixed and broad illumination of the object for all the positions of the array, i.e. an identical optoacoustic source. Second, it enabled to divide the total number of positions of the array by a factor of two, while fulfilling the ‘visibility’ condition in the xy plane³¹. On the condition that the lengths of the linear segments exceed the size of the imaged sample, the contour entirely contained the sample in the xy plane. The length of the polygon sides was chosen here to be a multiple of $L = 750 \mu\text{m}$ (Figure 1 (b)). This elementary length corresponds to the full width half maximum (FWHM) of the sensitivity field at 9.5 MHz calculated for the fixed cylindrical focus of the array³⁸. The maximum length of the polygon sides was chosen to be 13.5 mm, and therefore comprised 18 elementary segments of length L (corresponding to $n=9$ on Figure 1(b)). The endpoints of these elementary segments defined in this case 19 positions along a polygon side ($2n+1$ on Figure 1(b)). To limit the total number of measurements and then reduce the acquisition time, the detector positions were actually spaced by $2.L$ for each polygon side, and complementary patterns were chosen for two consecutive polygon sides. With the chosen angular step, this approach was determined to have no noticeable influence on the image resolution when compared to detector positions spaced by L for every linear segment.

The scan geometry was practically implemented by using the following motion sequence. The rotary stage was moved to a total of 120 positions, with a $\theta=1.5^\circ$ angular step. Between each rotation step (named by a capital letter in the schematic of Figure 1), the array was scanned linearly along the tangent to the circle by moving the translation stage in $2.L = 1.5 \text{ mm}$ steps, respectively to $n=9$ or $n+1=10$ translation positions. A total of 1140 positions of the array were covered per scan, for a translation range of $2.n.L = 13.5 \text{ mm}$. The total acquisition time per scan was 1.5 hours with 10 time-averages on the signals.

C. SAMPLE PREPARATION

1. PHANTOMS FOR CHARACTERIZATION AND CALIBRATION

Turbid phantoms mimicking the optical scattering properties and the speed of sound of soft tissues were prepared by mixing 2% w/m agar gel (Agar for microbiology, Fluka analytical) with 6% v/v intralipid-20% (Sigma). The gel was poured in cylindrical molds: 16 mm in diameter and 5 cm in height. Different calibrated absorbers were inserted in the gel for the characterization of the system.

The first phantom contained a single black polyethylene microsphere of $200 \mu\text{m}$ in diameter (BKPMS 180-212 μm , Cospheric, Santa Barbara, CA). This phantom enabled us to determine an average

speed of sound (dependent on the temperature in the water tank), and the value of the radius r (Figure 1). A second phantom was prepared with 200 μm microspheres. The microspheres were randomly spread over a cross section of the phantom, around 1 cm away from the top. A similar and third phantom was prepared with black polyethylene microspheres of 50 μm in diameter (BKPMS 45-53 μm , Cospheric, Santa Barbara, CA). A fourth phantom contained a black nylon suture of size 7-0 (diameter $\sim 50 \mu\text{m}$, Ethilon Monofilament Polyamide 66, Ethicon, Inc.) arranged in a cross along the length of the cylinder. The phantoms were placed in the tank so that the axis of the cylinder corresponded approximately to the z -axis, and the middle of the absorbers to the plane $z=0$.

2. *ANIMALS AND HANDLING*

Two CD1® mice (Charles River Laboratories, Research Models and Services, Germany GmbH), four days old and seven days old respectively, were euthanized and imaged ex-vivo. Mouse husbandry, handling and euthanasia were performed according to the institutional and Bavarian government regulations in frame of approved animal protocol. Hair was removed with a hair removing lotion (Veet, Germany), and the mice were placed on a custom-made holder made of nylon wires tightened between two hollow cylinders of 16 mm in diameter. Nylon wires were chosen to limit the interference of the holder with ultrasound wave propagation. The mice were positioned so that their anteroposterior axis follows the z axis with their head towards the negative z -direction, and with the illumination on their dorsal side (Figure 1). Different body parts were centered on the xy plane: the head for the younger mouse, and the abdomen for the older one.

D. *IMAGE RECONSTRUCTION*

The recorded signals were first deconvolved from the electrical impulse response of the detection system using the Wiener filter algorithm. The impulse response was recorded for one element of the array as described by Rosenthal et al.⁴² using an optoacoustic point source (focused light on a thin absorbing layer) located along the focal line in front of the element. The deconvolution process compensated for the frequency-dependent response of the sensor and signals with frequencies up to 12 MHz could be recovered with good signal-to-noise ratio (SNR). Afterwards, the deconvolved signals were band-pass filtered (Butterworth, order 3) between 100 kHz and 12 MHz for noise removal.

Volumetric images were reconstructed on a rectangular cuboid Cartesian grid centered on the measurement coordinate system (Figure 1). The grid dimensions were adapted to the sample size, and the voxel dimensions were set to $\Delta x \cdot \Delta y \cdot \Delta z = 37\mu\text{m} \cdot 37\mu\text{m} \cdot 74\mu\text{m}$ to achieve sufficient spatial sampling with regard to the expected in-plane (xy plane) and elevation (along z-axis) resolutions. A filtered backprojection algorithm was used in this study to reconstruct the volumetric images. Several modifications were performed to the backprojection algorithm proposed by Xu and Wang⁴³ for cylindrical geometries. The phenomenological description of the algorithm given in ref² was considered to adapt the algorithm to the specificity of the detector. First, only the first time-derivative of the processed signal : $-\frac{\partial V}{\partial t}$ was backpropagated onto the image grid. The non-derivative part of Xu and Wang's formula : $\frac{V}{t}$ was found negligible with respect to the derivative term, most likely because of the distance between the source and the detectors². With the backprojection algorithm, the value of each point of the image can be assigned independently. The reconstruction of the images was then performed using all the detection signals, but for 2D grids at constant z independently. The 2D images were then stacked to form the volumetric image. This approach has the advantage that a dynamic aperture reconstruction could be implemented along the z axis in order to keep the elevation angle of acceptance constant for all the points of the 3D image. Dynamic aperture reconstruction is a standard feature for conventional ultrasound linear detector arrays⁴⁴. It was performed for each position of the array independently by adapting the receiving aperture to the depth until the maximum physical aperture of the array was reached. For each 2D grid, the growing apertures were centered on its z coordinate. The ratio of the depth to the aperture length (i.e. the f-number in receive) was set to 0.5 here. Dynamic aperture also compensates the geometrical spreading (or diffraction attenuation) of the ultrasonic wave. In addition of being dynamic, the aperture was also apodized to avoid strong side lobes. A window function with a half-sine window on each side (for 20% of the aperture on each side) and flat otherwise was implemented. This implementation aimed at apodizing the aperture sides since other physical apodization of the signals also occurs during the detection process, particularly due to the finite size of the detectors.

For accurate reconstructions, we experimentally determined the average speed of sound in the medium c and the radius r (Figure 1(b)). The speed of sound was determined by measuring the first phantom for a single position of the array. The microsphere was then placed in the median plane of the array. Using the positions of the array elements specified by the manufacturer, the first time derivative of the processed signals was backpropagated, on a 2D grid including the microsphere, assuming different

speeds of sound. The amplitude of the image was maximized for $c = 1480 \text{ m.s}^{-1}$, which was thus considered as the speed of sound in the medium. To determine the value of the radius r , 3D reconstructions with different r values were computed for a scan acquisition of the first phantom. The value that maximized the amplitude of the 3D image was determined to be $r = 19.25 \text{ mm}$ and was set as a parameter for subsequent reconstructions.

The images obtained with the filtered backprojection algorithm comprised noise and non-physical negative values⁴⁵ and were processed before 3D visualization. Image processing was based on the assumption that the majority of the reconstructed voxels were comprised only by noise, since the actual absorbing targets occupy only a small part of the total volume in the region of interest. With this assumption, the following postprocessing routine was developed. First, a histogram of the image values was computed. Voxels with values below the one corresponding to the maximum of the histogram were disregarded. This step allows removing the voxels with nonphysical values, mostly negative ones. For the remaining voxels, the first 0.02% was disregarded to limit the noise from being displayed, and the last 0.02% to 0.1% was saturated to improve the image visualization. The 3D images were visualized using maximum amplitude projections and a rotation around one axis. This visualization was performed with the 3D project option of ImageJ.

III. RESULTS

A. INFLUENCE OF THE NUMBER OF TRANSLATION POSITIONS

Four different reconstructions of the data acquired for the second phantom (sparse 200 μm microspheres) were performed. For each reconstruction, a different subset of the 1140 positions of the array was selected to artificially vary the translation range. The positions considered for each of the subsets respected the symmetry to the radii of the circle, and the number of rotary positions and the translation step between positions on a linear segment were kept constant and respectively equal to 120 and 1.5 mm. Only the number of translation positions was changed, and datasets corresponding to translation ranges equal to 1.5 mm, 4.5 mm, 9 mm and 13.5 mm were formed. The corresponding total numbers of translations positions for two successive linear segments were respectively: 3 (corresponding to $n=1$ on Figure 1(b)), 7 ($n=3$), 13 ($n=6$) or 19 ($n=9$).

An optical picture of the absorbers embedded in the phantom and the maximum-amplitude projection (MAP) optoacoustic images along the z-axis corresponding to the four reconstructions are

presented in Figure 2. For translation ranges smaller than the effective diameter of the phantom (Figure 2 (b)-(d)), the microspheres located in the vicinity of the rotation axis, within a circle of diameter slightly larger than the travel range plus $L=750\mu\text{m}$ (to account for the thickness of the focal spot), appear with a round shape on the MAP images. However, for the microspheres located further away from the rotation axis, the lateral resolution along the azimuthal direction reduces with the radial distance. This smearing effect can be interpreted as a limited view effect due to the directivity of the detectors. For the translation range matching the size of the phantom, all the microspheres of the phantom can be identified and were reconstructed with a round shape on the MAP image (Figure 2 (e)). An amplitude gradient can be seen along the x direction, due to the one sided illumination and optical scattering.

The reconstructions show that the translation range determines the extent of the region where their in-plane resolution has a two-dimensional isotropy and homogeneity. With the scan geometry proposed here, the translation range can therefore be adjusted to fully contain the imaged object.

Figure 2 Reconstructions with different translation ranges of the second phantom (comprising $\varnothing 200\mu\text{m}$ microspheres randomly spread over a cross section). (a) Optical picture showing the spatial distribution of the absorbers. (b)-(e) Maximum-amplitude projection optoacoustic images along the z-axis for translation ranges of: 1.5 mm, 4.5 mm, 9.0 mm.

mm and 13.5 mm respectively. The yellow dotted circles have a diameter equal to the translation range plus $L=750\mu\text{m}$ to account for the thickness of the focal spot.

B. RESOLUTION IN 3D

The three-dimensional resolution of the system was investigated by imaging absorbers which peak frequency is expected to be greater than the highest recorded frequency^{46, 47}: $\varnothing 50\mu\text{m}$ microspheres and threads.

1. RESOLUTION OF ISOTROPIC OBJECTS

The third phantom, comprised of $\varnothing 50\mu\text{m}$ microspheres randomly spread over a cross section, was imaged with the system. The MAP image along the z axis of the entire phantom is presented in Figure 3 (a), and shows that the microspheres could be reconstructed over the entire cross section. Besides amplitude variations that could partially be attributed to the inhomogeneity of the light fluence, and a thresholding of the brighter voxels, no loss of azimuthal resolution with the distance to the rotation axis is noticeable.

The 3D resolution of two microspheres located at a distance of $\sim 5\text{mm}$ from the rotation axis and towards the open bound of the scan trajectory was analyzed. A cuboid containing the two microspheres ($15 \cdot 15 \cdot 11$ voxels) was extracted from the 3D image (green square, Figure 3(a)). The maximum amplitude projections of this cuboid in the three orthogonal planes are displayed in Figure 3 (b)-(d), and the normalized and centered profiles of two of these MAP images are plotted in Figure 3 (e). Only the nonphysical negative values were disregarded to represent these images. The MAP images show that, along the three directions, the two microspheres are resolved identically. The FWHM dimensions of one of the spheres were determined to be: $140\mu\text{m}$ in-plane and $327\mu\text{m}$ in elevation (Figure 3(e)). The discrepancy between the two resolutions could be attributed to the limited view in elevation as opposed to the full enclosure in-plane.

In addition to the two microspheres, the resolution was analyzed along the width of the phantom. A volumetric slice perpendicular to the y-axis was selected. Its width was chosen so that microspheres are spread all over the width of the phantom but with distinct x coordinates. The MAP images of the slice along the z-axis and the y-axis are shown in Figure 4 (a) and (b). The FWHM values of the MAP amplitude profiles along the y axis and z axis were determined for 23 distinct microspheres, and compared to the values found previously (Figure 4 (c)). The results confirm that the in-plane and elevation resolution are approximately constant over the width of the phantom, and consistent with the values found for the two microspheres of Figure 3(b)-(e). The FWHM of the amplitude profiles along the y axis was

329 (mean $\pm$ standard deviation): $129 \pm 16 \mu\text{m}$, and along the z-axis : $337 \pm 39 \mu\text{m}$. The plane containing
 330 the microspheres was observed to have a small angle with the xy plane.

331
 332 **Figure 3** Reconstructions of the third phantom (comprising $\varnothing 50 \mu\text{m}$ microspheres randomly spread over a cross
 333 section). (a) MAP image of the entire region-of-interest along the z-axis. The green square indicates the limits of a sub-
 334 region of interest. MAP images of this sub region along (b) the z-axis, (c) the x-axis, and (d) the y-axis. (e) Amplitude
 335 profile of the lines marked with an arrow on (b) and (c). The amplitude profiles were normalized and the peak values were
 336 centered. The FWHM of the profile along the y axis is : $140 \mu\text{m}$ and along the z-axis : $327 \mu\text{m}$.

Figure 4 Reconstructions of a volumetric slice of the third phantom, perpendicular to the y axis (a) MAP image along the z-axis. (b) MAP image along the y-axis. (c) FWHM of the amplitude profile along the y axis and z axis of 23 distinct microspheres distributed along the length of the slice. The dashed lines mark the value found for the microsphere of Figure 3.

2. RESOLUTION IN TERMS OF SEPARATION BETWEEN 2 OBJECTS

The fourth phantom, comprised of a cross from a $\varnothing$ 50 μm thread, was imaged to study the resolution in terms of ability to separate two objects. The MAP image along the y-axis is shown in Figure 5(a). Two slices orthogonal to the z-axis were selected from the volume and are presented in Figure 5 (b) and (c) respectively. The slices were not post-processed. The first slice (Figure 5 (b)) was taken well above the cross intersection and shows the position of the threads in the xy plane. The threads appear with a round shape on this slice: no elongation in the direction given by the projection of the thread in the xy plane can be noticed. The second slice (Figure 5 (c)) was taken close to the location of the intersection of the threads, and corresponds to the slice where the saddle point between the individual reconstructions of the threads first develops. The distance between the two maxima corresponds to the resolution in the sense of the Sparrow resolution criterion. It is determined here to be 189 μm, which is similar to the in-plane resolution determined with the previous phantom. As opposed to spherical objects, however, the elevation resolution interferes due to the absorber extension along the z-axis. The 3D visualization of the thread cross is presented on Video 1.

Figure 5 Reconstructions of the fourth phantom (comprised of a cross from a $\varnothing 50\mu\text{m}$ thread). (a) MAP image of the entire region-of-interest along the y-axis. (b) slice corresponding to the plane marked with a green line on (a). (c) slice corresponding to the plane marked with a red line on (a). The star markers indicate the position of the maxima corresponding to the reconstruction of each thread. The distance between the two stars is $189\mu\text{m}$. For the two slices (b) and (c), the images were not postprocessed but the voxel values were normalized by the maximum value of the 3D image, the brightest voxel being situated at the intersection of the cross.

Video 1: This video corresponds to Figure 5(a), and shows the 3D optoacoustic volume image of the thread cross. Rotating maximum amplitude projection images around the z axis are displayed with 2° angle between the projections, and at a frame rate of 15 images per second

C. EX-VIVO ANATOMIC IMAGES OF MICE

I. ABDOMEN

The abdomen of the 7 day old mouse was imaged ex-vivo. The MAP image along the x-axis is presented in Figure 6 (a). This image corresponds to the frontal projection. It is displayed here so that the mouse legs are located at the bottom of the image, and the left side of the mouse is on the left of the image (as if the mouse was seen from its back). Anatomical structures can be visualized, in particular the kidneys, the spleen, a partial lobe of the liver, and the femurs (bone marrow). Several major vessels as the abdominal aorta and the vena cava could be identified, but multitude of smaller vessels can be seen as well, especially in the 3D visualization (Video 2) and in single slices (Figure 6(b)-(e)). Four different

slices on the upper region of the abdomen are displayed in Figure 6(b)-(e). The slices were not post-processed, and negative values can therefore be seen. The presence of these negative values can shadow some structures such as the abdominal aorta and the vena cava for $z < -4$ mm (Figure 6(a-c)).

Figure 6 Reconstructions of the abdomen of the 7-day-old mouse. (a) MAP image of the entire region-of-interest along the x-axis. The slices (b)-(e) correspond to the yellow marks on the side of (a), and are ordered by increasing z. For each slice the images were not post-processed. The voxel values are normalized by the maximum value of the 3D image. Legend: 1. Spleen, 2. Interlobar vessels of the left kidney, 3. Ischiatic vein, 4. Saphenous vein, 5. Partial lobe of the liver, 6. Right kidney, 7. Abdominal aorta, 8. Vena cava, 9. Femur, 10. Intestine vessels, 11. Left kidney. The anatomical features were correlated with published mouse anatomy⁴⁸.

Video 2: This video corresponds to Figure 6 (a), and shows the 3D optoacoustic volume image of the abdomen of the 7-day-old mouse. Rotating maximum amplitude projection images around the z axis are displayed with 2° angle between the projections, and at a frame rate of 15 images per second.

2. HEAD REGION

The head of the 4 day old mouse was imaged ex-vivo. The MAP images along the x-axis and the z-axis are shown respectively in Figure 7(a) and (b). The head is oriented so that the mouse snout points towards the negative z-direction. The vascular anatomy of the head can be visualized on these two projections, and in particular the dural venous sinuses and the supraorbital veins (Figure 7). The different parts of the head can be clearly identified and many fine structures can be visualized on Video 3. Three coronal slices were selected (Figure 7 (c)-(e)) to better show vascular structures hidden on the MAP projection because of the brightness of the superior sagittal sinus. This brightness can be explained by

theone-sided illumination. The slices were not post-processed. A fourth coronal slice (Figure 7 (e)) shows the structures of the neck including the jugular veins.

Figure 7 Reconstructions of the head of the 4-day-old mouse. MAP image of the entire region-of-interest (a) along the x-axis, (b) along the z-axis. The slices (c)-(f) correspond to the yellow marks on the bottom of (a), and are ordered by increasing z. Legend: 1. Supraorbital vein, 2. Superior sagittal sinus, 3. Confluence of sinuses, 4. Right transverse sinus, 5. Cerebellum, 6. Temporal vein, 7. Inferior sagittal sinus, 8. Straight sinus, 9. Facial vein, 10. Sigmoid sinus, 11. Jugular vein, 12. Maxillary vein. The anatomical features were correlated with published mouse anatomy⁴⁸.

Video 3: This video corresponds to Figure 7(b), and shows the 3D optoacoustic volume image of the head of the 4-day-old mouse. Rotating maximum amplitude projection images around the x-axis are displayed with 2° angle between the projections, and at a frame rate of 15 images per second.

IV. DISCUSSION

We examined in this study the performance of a novel detection geometry for volumetric optoacoustic tomography using a conventional linear array employed in ultrasound imaging. To make optimal use of the detection characteristics of such a transducer, we investigated the role that translation and rotation of the detector array in relation to the imaged object plays on the achieved image quality. The study demonstrated that the proposed geometrical configuration provides a versatile system adapted to perform whole-body small animal imaging. The arrangement allows theseamless adjustment of scan

parameters to fit the radial dimensions of the region-of-interest within the sample. The translation range along the polygon sides was shown to define the radial distance for which no loss of in-plane resolution along the azimuthal direction could be noticed (Figure 2). Conversely, the number of positions of the detector array, and therefore the scanning time, can be adapted depending on the dimensions of the sample. Two different mice and four phantoms were imaged in this study, which shows the capabilities of the imaging system.

With a translation range corresponding to the radial size of the sample, the in-plane resolution was found to have two-dimensional isotropy and homogeneity over the width of the sample even for absorbers emitting with peak frequencies higher than the low-pass cut-off frequency of the system⁴⁶ (Figure 3 and Figure 4). The isotropy and homogeneity of the in-plane resolution are a consequence of the scanning geometry, in which the sample is entirely contained and probed with a high density of sensor positions. In this manner, optoacoustic waves arising from the entire sample were captured at each rotation angle, and the full-view criterion was fulfilled in-plane. Indeed, for objects inside the circle defined by the translation range, normal vectors drawn onto all their in-plane boundary points pass in one direction the detection angular aperture. In-plane boundaries could therefore be recovered stably³¹. Conversely, because of the limited angle of acceptance of the focused detector, in-plane limited-view issues occur for objects outside the circle, and the azimuthal resolution was therefore degraded (Figure 2). The in-plane FWHM dimensions for 50 μm diameter microspheres were found to be approximately 130 μm , for the detection geometry and reconstruction algorithm selected in this study. The acoustic wavelength in the medium corresponding to the low-pass cutoff frequency of the system (12 MHz) is: $\lambda_c \approx 123 \mu\text{m}$, which implies that the reconstructed in-plane FWHM dimension of the sphere and the optimal resolution theoretically achievable ($0.8\lambda_c \approx 99 \mu\text{m}$ ⁵) are on the same order of magnitude. The discrepancy between the two values could first be attributed to the finite dimension of the microsphere. However, the sphere is a solid which longitudinal speed of sound is larger than the one of the surrounding aqueous medium. As a consequence, the sphere dimension cannot be simply subtracted to obtain the effective resolution of the system. The finite size of detectors may also have an effect on the resolution, as the spatial averaging on the detector surface distorts the signals detected for optoacoustic sources outside of the focal zone, and shifts their frequency spectrum towards lower frequencies³³. This effect, termed spatial impulse response of the detector⁴⁹, is not taken into account by the filtered backprojection reconstruction algorithm, which assumes point-like detectors and non-distorted signals. The finite size of the detectors, their focusing properties, and the discrete positions of the detector array could also partially explain the in-plane amplitude variation between the reconstructed microspheres (Figure 3(a) and Figure 4(a)). The spatial

averaging is indeed not equivalent for all the microspheres of the phantom when a translation scan is performed. This effect is however expected to be less significant for larger absorbers since their peak frequency is lower, which results in a corresponding focal zone with larger dimensions. A reconstruction algorithm able to take into account the spatial impulse response of the detector, such as in ref.³³, would be needed to improve the in-plane resolution and amplitude homogeneity. Such investigations are beyond the scope of this paper, but will be considered in future studies.

Due to using a dynamic aperture adjustment, the elevation resolution was shown to be kept constant and uniform over a width of 15 mm (Figure 4), unlike circular tomography with cylindrically fixed focused detectors, which focal line is perpendicular to the axis of rotation²³. The elevation FWHM dimension of microspheres of 50 μm in diameter was found to be about 330 μm , i.e. more than twice the in-plane FWHM value. Since the microspheres are isotropic absorbers, it can be concluded that the discrepancy between the in-plane and elevation resolution is due to the detection geometry. The main limitation of the proposed geometry in terms of angular aperture is the finite aperture of the array along the elevation direction. The sample is indeed only partially enclosed in terms of the elevation angle. The angle of acceptance was further reduced in order to obtain a uniform resolution in elevation. Finally, this resolution is degraded by the spatial averaging on the transducer surface, which effectively creates an apodization function, stronger at high frequencies. The elements of the array are indeed not infinitely thin but have a width of 270 μm , that is to say around $2\lambda_c$, which results in non-negligible spatial averaging for sources located at a steep elevation angle with respect to the sensor. Taking into account the spatial impulse response of the detector in the reconstruction algorithm would reduce this apodization and should then improve the elevation resolution as well.

Despite the limitations due to the reconstruction algorithm and the limited view, the obtained images can be qualified as high resolution. The three-dimensional reconstruction of the cross (Video 1) and mice (Video 2 and Video 3) show that small structures can be visualized and that their shape correspond to the implanted absorbers or the expected anatomy⁴⁸. Moreover, no complicated image post-processing was performed to obtain the 3D volume rendering, as opposed to the routines developed in other studies⁶. The negative and non-physical values that appear on the images (Figure 6) and that create some shadowing effects are most probably due to the non-optimal reconstruction algorithm, which is not adapted for finite-sized focused detector or limited solid-angle coverage.

For the implementation of the detection geometry used in this study, one full acquisition took 1.5 hours with 10 time-averages on the signals, that is to say an average 4.8 seconds per position of the

array. Since the acquisition of the signals took around 1 second per position (due to the 10 Hz pulse-repetition rate of the laser), the time-consuming part was therefore the motion of the array. The current scanning is quite long for *in-vivo* experiments, however, several solutions can be considered to reduce significantly this scan duration. First, since the motion of the array is the most time consuming part of the acquisition, faster stepper motors could be used. Second, a continuous motion of the detector has been shown to reduce dramatically the acquisition time²³ and could also be implemented for this detection geometry. On the other hand, the large number of positions used in this study could probably be significantly reduced. Using a reconstruction algorithm that takes into account the spatial impulse response of the transducers, better quality images are expected to be obtained with less sensor positions. With the filtered backprojection algorithm, the spatial averaging of the optoacoustic waves on the detector surface for optoacoustic sources outside of the focal zone of the array is indeed compensated by the large number of positions, the signals originating from the focal zone being stronger than the distorted ones. Additionally, the fine translation step implemented here aimed at capturing the high-frequency components arising from the entire sample by translating the focal spot, so as to demonstrate the high resolution capability of the detection geometry. If the required resolution is lower than in the present study, the translation step-size could be accordingly adapted, which would result in shorter scanning duration. A systematic study of the influence of each scan parameters on the image quality is beyond the scope of this paper, but will be considered in the future to improve the acquisition time. Lastly, a higher repetition rate laser could also be used to accelerate the acquisition of the signals, and the number of time averages per position optimized in regards to the high number of individual measurements projections.

Finally, one major advantage of the novel detection geometry is its versatility in terms of the interchangeability of the transducer array. No dedicated prototype was built, instead a conventional ultrasound array was used. A large range of linear arrays have already been developed for ultrasonography, and are available commercially. Among the wide variety of length, number of elements, and central frequencies available, the high central frequency detectors, and the large aperture ones are of special interest for the proposed detection geometry. The combination of a large number of elements and a small pitch would indeed allow increasing the angular aperture in elevation. Commercially available linear arrays for medical ultrasound have as many as 512 elements, with a pitch ranging from $\lambda/2$ to $3\lambda/2$ where λ is the wavelength corresponding to the central frequency of the elements³⁹. The pitch of the linear array used in this study was on the higher pitch range considering a central frequency between 5 MHz and 7 MHz. A smaller pitch would reduce the spatial averaging on the elements at frequencies higher than the central one and improve the image quality. On the other hand, linear arrays with central

frequencies ranging from 30 MHz to 65 MHz⁵⁰⁻⁵⁴ have been developed recently, and some are already available commercially. Currently, to our knowledge, only linear and annular^{55, 56} arrays have been developed for such high frequencies. Array geometries where the elements are arranged on a concave curved line or surface are a technical challenge at such frequencies. The high-frequency linear arrays developed so far are moreover cylindrically focused to enhance their sensitivity, and to limit spatial averaging on the detector surface within the focal region. The novel detection geometry proposed here is therefore adapted for these arrays, provided that the radial distance to the axis of rotation and the translation steps are adapted to the array characteristics and the sample under study. Such a high frequency system is expected to lead to volumetric images at mesoscopic scale of better quality than the optoacoustic methods proposed so far, and will be investigated in the near future. Alternatively, a multi-sector scanning scheme, as developed for two-dimensional imaging in Ref⁵⁷, could be investigated with an array. Such scanning scheme resembles the fan beam scanning method for the third generation of computed tomography x-ray imaging⁴⁰.

To go beyond the frequencies available for arrays, spherically focused transducers could be used in similar detection geometry by translating the sensor along the elevation direction as well, or by scanning the tangent planes of a sphere centered on the sample. As for the array, the focal zone (volume of high sensitivity and bandwidth) shall be translated and rotated in order to efficiently sample in the entire volume of the region of interest.

V. CONCLUSION

An original detection geometry for optoacoustic tomography, leading to high-resolution volumetric imaging, was presented in this work. The proposed geometry is based on the combination of a translate-rotate scanning arrangement and the use of a conventional ultrasound linear array. This combination provides a large solid angular detection aperture and versatility in terms of dimensions of the region-of-interest.

The implementation of the geometry with a conventional medical ultrasound linear array, designed to perform ultrasonography at 5.0/7.0 MHz, showed that whole-body volumetric optoacoustic images of mice could be performed, with imaged features correlating well to the mouse anatomy. The proposed geometry is therefore relevant for optoacoustic imaging of biological tissue.

With the possibility to use high frequency detector arrays, the method presented herein is expected to be of great interest for volumetric imaging of optical absorption contrast at mesoscopic scale. The additional use of a multispectral approach could moreover provide useful information on the three-dimensional location of specific and targeted chromophores in the anatomy.

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