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

3 **Whole-body tomographic system for small animals**

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8  
9 **Abstract**

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

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

21 Results: The resolution of the optoacoustic tomography system was measured to be better than 130µm in-plane and  
22 330µm in elevation (full width half maximum), and to be homogenous along a 15 mm diameter cross-section due to  
23 the translate-rotate scanning geometry. Whole-body volumetric optoacoustic images of mice were performed ex-  
24 vivo, and imaged organs and blood vessels through the intact abdominal and head regions were correlated to the  
25 mouse anatomy.

26 Conclusions: Overall, the feasibility of three-dimensional and high-resolution whole-body optoacoustic imaging of  
27 small animal using a conventional linear array was demonstrated. Furthermore, the scanning geometry may be used  
28 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.

31

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

33

## 34 I. INTRODUCTION

35 Optoacoustic (photoacoustic) imaging is a noninvasive imaging modality for high-resolution  
36 mapping of optical absorption in biological tissues<sup>1,2</sup>. The method resolves optical absorption by detecting  
37 broadband ultrasonic waves generated within the tissue following the absorption of pulsed  
38 illumination. The conversion from optical energy to mechanical waves occurs through the thermo-elastic  
39 expansion of transiently heated absorbers, and its efficiency is strongly sensitive to the optical absorption  
40 of the tissue structures. Similarly to optical imaging, the penetration of optoacoustic imaging, for  
41 ultrasonic frequencies below 20-30 MHz, is primarily limited by the light attenuation in tissues<sup>3</sup>. However,  
42 optoacoustic imaging offers at mesoscopic and macroscopic scale a higher resolution than optical  
43 imaging, even when compared to pulsed illumination time-domain optical imaging<sup>4</sup>, thanks to the weak  
44 scattering of ultrasound waves in soft tissues. Thereby, optoacoustic computed tomography (OAT) can  
45 provide optical contrast images with ultrasound diffraction-limited resolution<sup>5</sup>. Additionally, light  
46 scattering in biological tissue, and three-dimensional (3D) propagation of optoacoustic waves make  
47 macroscopic optoacoustic imaging an inherently three-dimensional modality.

48 Volumetric images of the organ<sup>6-10</sup> and vascular<sup>11-15</sup> anatomies of healthy small animals, as well  
49 as human breast<sup>13</sup> and arm<sup>16</sup>, have been obtained non-invasively using the endogenous optical absorption  
50 contrast in tissue, and were found to provide 3D visualization of tissue and vascular architecture.  
51 Localization of molecular biomarkers<sup>10, 17, 18</sup>, induced thermal lesions<sup>19</sup> or tumor vascularization<sup>20</sup> have  
52 further shown great potential and application versatility of optoacoustic imaging.

53 Different experimental tomographic configurations have been proposed and implemented to obtain  
54 3D macroscopic optoacoustic images<sup>21</sup>. In these configurations, ultrasonic detectors were arranged along  
55 spherical<sup>6-9, 13</sup>, cylindrical, circular<sup>5, 22-28</sup>, or planar<sup>11, 15-17, 29, 30</sup> surfaces. The choice of the detection  
56 geometry is usually dictated experimentally by practical constraints such as the size of the imaged animal  
57 or tissue, and the positioning of the illumination system. Overall, the geometrical arrangement of the  
58 detectors influences the spatial resolution achieved in optoacoustic tomography and the visibility of tissue  
59 structures, since limited view issues may arise from partial enclosure of the sample<sup>31, 32</sup>. Besides the  
60 detection geometry, detector properties such as spatial and electrical (bandwidth) responses also influence  
61 the achieved resolution and image quality. For example, the spatial directivity of a detector, due to its  
62 finite size and focusing properties, defines the angular aperture and the dimension of the volume covered  
63 by its sensitivity field, and may lead to distortions of the collected optoacoustic signal<sup>5, 33</sup>. Moreover, as

64 optoacoustic waves exhibit a broadband spectrum, with peak frequencies corresponding to different object  
65 sizes<sup>34</sup>, the central frequency and bandwidth of the ultrasound detector defines the range of structure  
66 sizes that a given detector is able to resolve.

67 Many optoacoustic imaging implementations rely on piezoelectric detectors and benefit from  
68 technologies developed for ultrasonic measurements. Arrays of detectors allow parallel acquisition and  
69 therefore reduce the acquisition time required for data collection. A number of systems have been  
70 implemented employing custom-made concave arrays<sup>6, 7, 9, 13, 26, 27</sup>, while conventional linear arrays  
71 developed for medical ultrasound are commercially available and attractive for optoacoustic imaging and  
72 tomography<sup>16, 28, 30, 35, 36</sup>. Medical ultrasound linear arrays are transducer assemblies with rectangular  
73 elements arranged in a line with a fine spatial sampling, and are typically available for central  
74 frequencies ranging from 1 to 20 MHz with bandwidth as high as 60–80%<sup>37</sup>. Such detectors are  
75 typically built with a cylindrical acoustic lens which enhances signal detection around the median plane of  
76 the array<sup>33</sup> and provides a large depth of focus<sup>38</sup>. For optoacoustic tomography, the in-  
77 plane spatial resolution perpendicular to an ultrasound array (axial resolution) is primarily defined by the  
78 bandwidth of the detectors, whereas the lateral resolution (along the array) is limited by the effective  
79 angular aperture of the array. Due to the length of a typical linear ultrasound array and the typically small  
80 size of an element along this length<sup>39</sup> -that provide a large angle of acceptance-, conventional linear arrays  
81 offer lateral resolutions in the order of a few tens to hundreds of microns, depending on the operating  
82 frequency. Conversely, the detector focus along the perpendicular (transverse) dimension is not sufficient  
83 to achieve similar resolution; instead the resolution in the transverse dimension is at least an order of  
84 magnitude worse than in the lateral dimension. Linear scanning of the array perpendicular to the  
85 imaging plane has been used to cover a planar detection surface, and volumetric images have been formed  
86 by stacking 2D images<sup>16, 30, 35</sup>. However, because of the small angular aperture due to the fixed cylindrical  
87 focus, the resulting volumetric images have poor resolution along the scanning axis. The lateral resolution  
88 was shown to improve by rotating the array<sup>28, 36</sup>, the rotation axis being perpendicular to the imaging  
89 plane. However, the limited view issues along the rotation axis remained unsolved.

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

96 and achieve high resolution imaging along the other two geometrical dimensions. This scanning geometry  
97 resembles the translate-rotate scanning method for the first generation of computed tomography x-ray  
98 imaging<sup>40</sup>. The capability of this geometry to provide high resolution three-dimensional optical images of  
99 large volumes is demonstrated here with a commercial 128 element linear array, which is used in  
100 ultrasonography at 5.0/7.0 MHz. The system is first characterized with phantoms, and its performance is  
101 then shown on *ex vivo* mice.

## 102 II. MATERIALS AND METHODS

### 103 A. EXPERIMENTAL SETUP

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

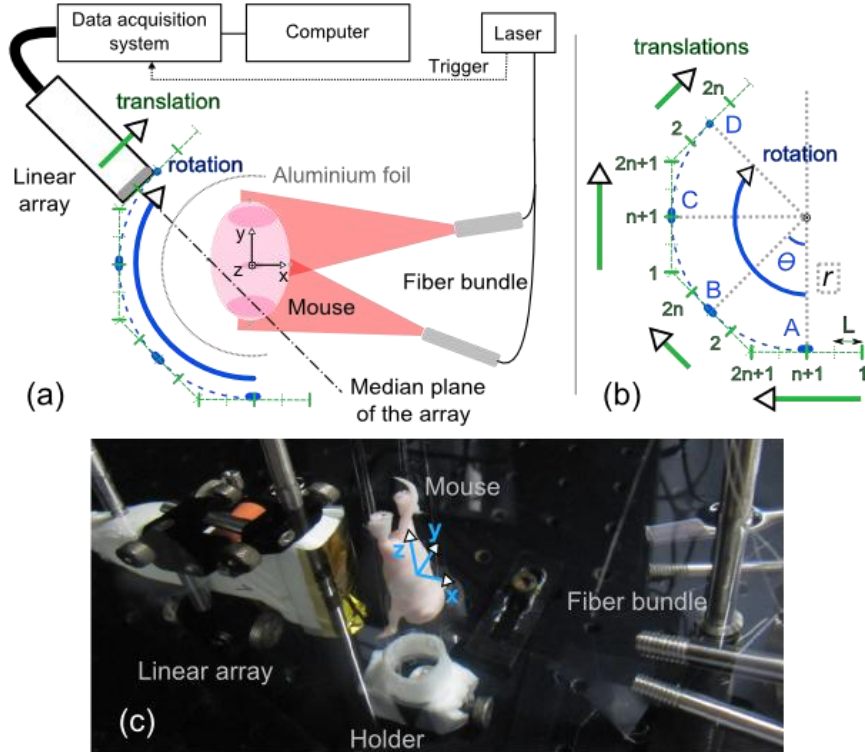
112 The excitation light originated from a tunable (690–900 nm) optical parametric oscillator laser  
113 (Phocus II, Opotek Inc., Carlsbad, California), delivering <10 ns duration pulses with a repetition  
114 frequency of 10 Hz. The beam was guided into a silica fused-end fiber bundle (CeramOptec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

124 pulse energy at the laser output of  $\sim 70$  mJ. This wavelength ensures a good penetration in biological tissue  
125 and a contrast to deoxygenated hemoglobin<sup>41</sup>.

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

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

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



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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.

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## B. SCAN GEOMETRY AND PARAMETERS

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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  $178.5^\circ$ , corresponding to



163  $180^\circ - \theta$ . This angle coverage had two main advantages over the full angle coverage ( $360^\circ - \theta$ ). First, the  
164 opening in the contour allowed setting a fixed and broad illumination of the object for all the positions of  
165 the array, i.e. an identical optoacoustic source. Second, it enabled to divide the total number of positions of  
166 the array by a factor of two, while fulfilling the ‘visibility’ condition in the xy plane<sup>31</sup>. On the condition  
167 that the lengths of the linear segments exceed the size of the imaged sample, the contour entirely contained  
168 the sample in the xy plane. The length of the polygon sides was chosen here to be a multiple of  $L = 750$   
169  $\mu\text{m}$  (Figure 1 (b)). This elementary length corresponds to the full width half maximum (FWHM) of the  
170 sensitivity field at 9.5 MHz calculated for the fixed cylindrical focus of the array<sup>38</sup>. The maximum length  
171 of the polygon sides was chosen to be 13.5 mm, and therefore comprised 18 elementary segments of length  
172  $L$  (corresponding to  $n=9$  on Figure 1(b)). The endpoints of these elementary segments defined in this case  
173 19 positions along a polygon side ( $2n+1$  on Figure 1(b)). To limit the total number of measurements and  
174 then reduce the acquisition time, the detector positions were actually spaced by  $2.L$  for each polygon side,  
175 and complementary patterns were chosen for two consecutive polygon sides. With the chosen angular  
176 step, this approach was determined to have no noticeable influence on the image resolution when  
177 compared to detector positions spaced by  $L$  for every linear segment.

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

184

## 185 C. SAMPLE PREPARATION

### 186 I. PHANTOMS FOR CHARACTERIZATION AND CALIBRATION

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

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

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

## 201 2. ANIMALS AND HANDLING

202  
203 Two CD1® mice (Charles River Laboratories, Research Models and Services, Germany GmbH),  
204 four days old and seven days old respectively, were euthanized and imaged ex-vivo. Mouse husbandry,  
205 handling and euthanasia were performed according to the institutional and Bavarian government  
206 regulations in frame of approved animal protocol. Hair was removed with a hair removing lotion(Veet,  
207 Germany), and the mice were placed on acustom-made holder made of nylon wires tightened between  
208 two hollow cylinders of 16mm in diameter. Nylon wires were chosen to limitthe interference of the holder  
209 withultrasound wave propagation. The mice were positioned so that their anteroposterior axis follows the  
210  $z$  axis with their head towardsthe negative  $z$ -direction, and with the illumination on their dorsal side  
211 (Figure 1). Different body parts were centered on the  $xy$  plane:the head for the younger mouse, and the  
212 abdomenfor the older one.

213

## 214 D. IMAGE RECONSTRUCTION

215

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

223 Volumetric images were reconstructed on a rectangular cuboid Cartesian grid centered on the  
 224 measurement coordinate system (Figure 1). The grid dimensions were adapted to the sample size, and the  
 225 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  
 226 with regard to the expected in-plane (xy plane) and elevation (along z-axis) resolutions. A filtered  
 227 backprojection algorithm was used in this study to reconstruct the volumetric images. Several  
 228 modifications were performed to the backprojection algorithm proposed by Xu and Wang<sup>43</sup> for  
 229 cylindrical geometries. The phenomenological description of the algorithm given in ref<sup>2</sup> was  
 230 considered to adapt the algorithm to the specificity of the detector. First, only the first time-derivative of the  
 231 processed signal  $:-\frac{\partial V}{\partial t}$  was backpropagated onto the image grid. The non-derivative part of Xu and  
 232 Wang's formula  $:\frac{V}{t}$  was found negligible with respect to the derivative term, most likely because of the  
 233 distance between the source and the detectors<sup>2</sup>. With the backprojection algorithm, the value of each point  
 234 of the image can be assigned independently. The reconstruction of the images was then performed using all  
 235 the detection signals, but for 2D grids at constant z independently. The 2D images were then stacked to  
 236 form the volumetric image. This approach has the advantage that a dynamic aperture reconstruction could  
 237 be implemented along the z axis in order to keep the elevation angle of acceptance constant for all the  
 238 points of the 3D image. Dynamic aperture reconstruction is a standard feature for conventional ultrasound  
 239 linear detector arrays<sup>44</sup>. It was performed for each position of the array independently by adapting the  
 240 receiving aperture to the depth until the maximum physical aperture of the array was reached. For each 2D  
 241 grid, the growing apertures were centered on its z coordinate. The ratio of the depth to the aperture  
 242 length (i.e. the f-number in receive) was set to 0.5 here. Dynamic aperture also compensates the geometrical  
 243 spreading (or diffraction attenuation) of the ultrasonic wave. In addition of being dynamic, the aperture  
 244 was also apodized to avoid strong side lobes. A window function with a half-sine window on each side (for  
 245 20% of the aperture on each side) and flat otherwise was implemented. This implementation aimed at  
 246 apodizing the aperture sides since other physical apodization of the signals also occurs during the  
 247 detection process, particularly due to the finite size of the detectors.

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

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

258 The images obtained with the filtered backprojection algorithm comprised noise and non-physical  
259 negative values<sup>45</sup> and were processed before 3D visualization. Image processing was based on the  
260 assumption that the majority of the reconstructed voxels were comprised only by noise, since the actual  
261 absorbing targets occupy only a small part of the total volume in the region of interest. With this  
262 assumption, the following postprocessing routine was developed. First, a histogram of the image values  
263 was computed. Voxels with values below the one corresponding to the maximum of the histogram were  
264 disregarded. This step allows removing the voxels with nonphysical values, mostly negative ones. For the  
265 remaining voxels, the first 0.02% was disregarded to limit the noise from being displayed, and the last  
266 0.02% to 0.1% was saturated to improve the image visualization. The 3D images were visualized using  
267 maximum amplitude projections and a rotation around one axis. This visualization was performed with the  
268 3D project option of ImageJ.

## 269 III. RESULTS

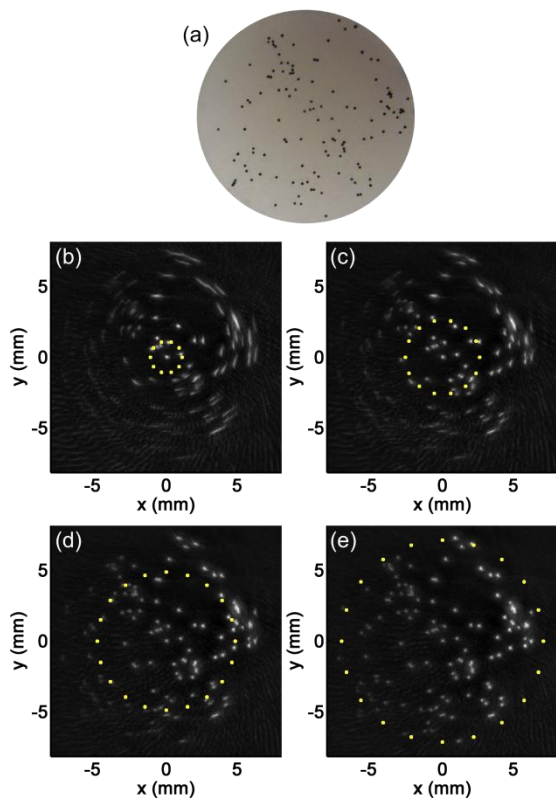
### 270 A. INFLUENCE OF THE NUMBER OF TRANSLATION POSITIONS

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

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

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

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



294  
 295 **Figure 2 Reconstructions with different translation ranges of the second phantom (comprising  $\text{Ø } 200\mu\text{m}$**   
 296 **microspheres randomly spread over a cross section). (a) Optical picture showing the spatial distribution of the absorbers.**  
 297 **(b)-(e) Maximum-amplitude projection optoacoustic images along the z-axis for translation ranges of: 1.5 mm, 4.5 mm, 9.0**

298 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  
299 account for the thickness of the focal spot.

## 300 B. RESOLUTION IN 3D

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

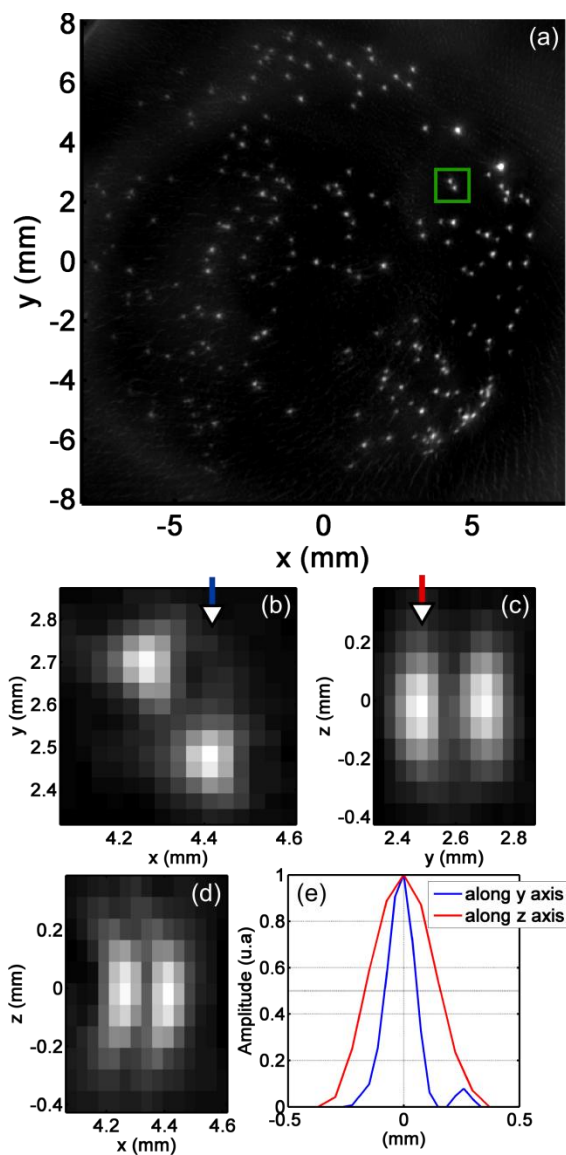
### 304 I. RESOLUTION OF ISOTROPIC OBJECTS

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

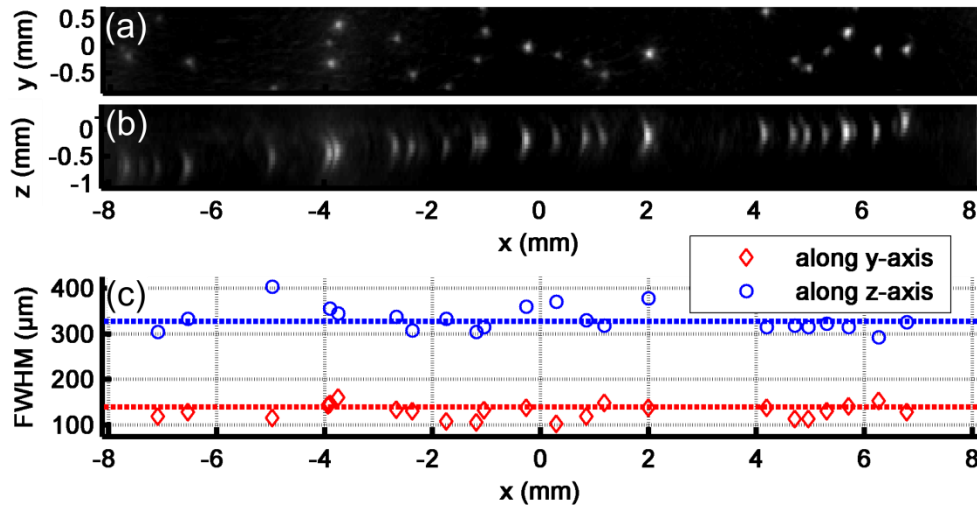
311 The 3D resolution of two microspheres located at a distance of  $\sim 5\text{mm}$  from the rotation axis and  
312 towards the open bound of the scan trajectory was analyzed. A cuboid containing the two microspheres  
313 ( $15 \cdot 15 \cdot 11$  voxels) was extracted from the 3D image (green square, Figure 3(a)). The maximum amplitude  
314 projections of this cuboid in the three orthogonal planes are displayed in Figure 3 (b)-(d), and the  
315 normalized and centered profiles of two of these MAP images are plotted in Figure 3 (e). Only the  
316 nonphysical negative values were disregarded to represent these images. The MAP images show  
317 that, along the three directions, the two microspheres are resolved identically. The FWHM dimensions of  
318 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  
319 discrepancy between the two resolutions could be attributed to the limited view in elevation as opposed to  
320 the full enclosure in-plane.

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

329 (mean +/- 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  $\text{Ø } 50 \mu\text{m}$  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 :  $327 \mu\text{m}$ .



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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.

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## 2. RESOLUTION IN TERMS OF SEPARATION BETWEEN 2 OBJECTS

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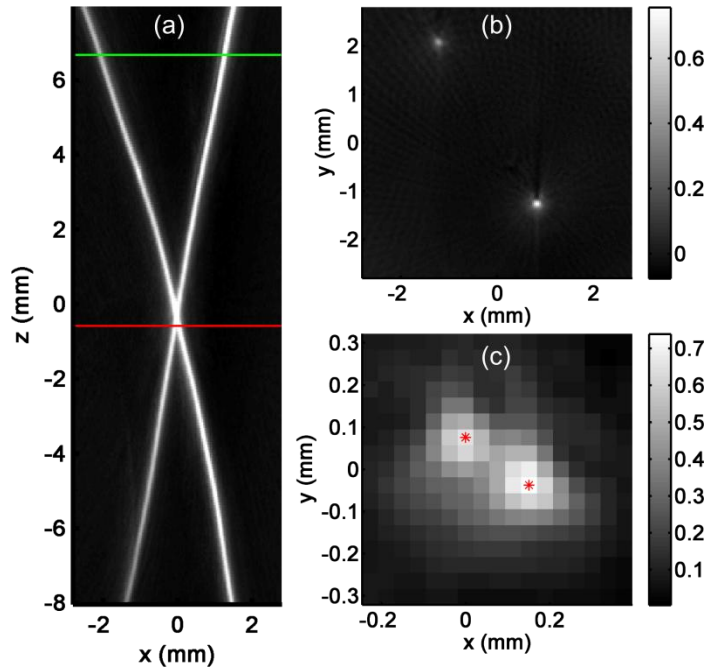
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The fourth phantom, comprised of a cross from a  $\text{\O} 50\mu\text{m}$  thread, was imaged to study the resolution in terms of ability to separate two objects. The MAP image along the y-axis is shown in Figure 5(a). Two slices orthogonal to the 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  $189\mu\text{m}$ , which is similar to their 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.





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Figure 5 Reconstructions of the fourth phantom (comprised of a cross from a  $\text{\O} 50\mu\text{m}$  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.

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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  $2^\circ$  angle between the projections, and at a frame rate of 15 images per second

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## C. EX-VIVO ANATOMIC IMAGES OF MICE

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### I. ABDOMEN

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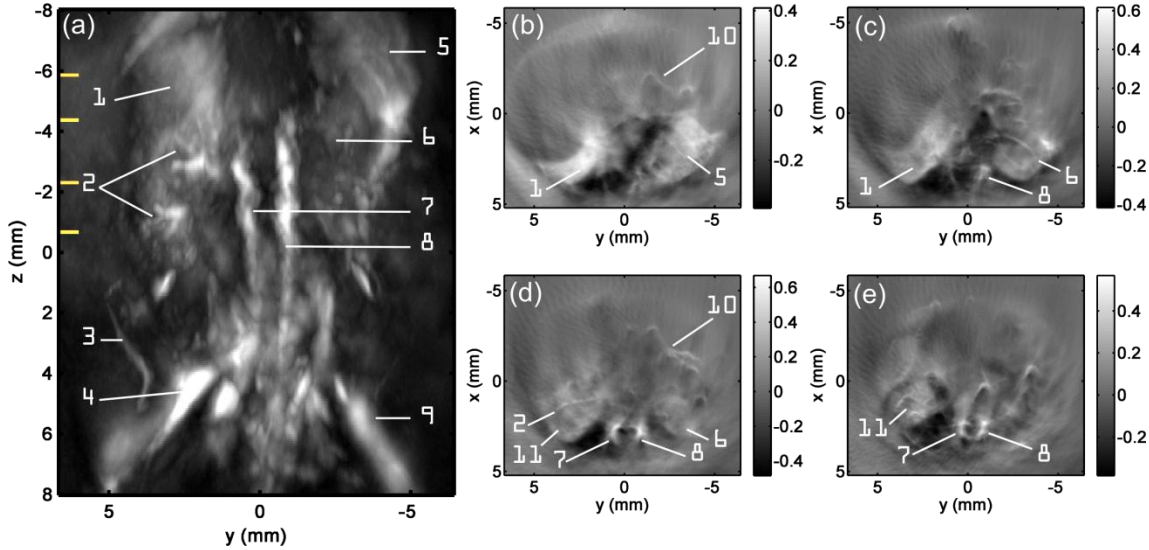
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The abdomen of the 7 day old mouse was imaged ex-vivo. The MAP image along the x-axis is presented in Figure 6 (a). This image corresponds to the frontal projection. It is displayed here so that the mouse legs are located at the bottom of the image, 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

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



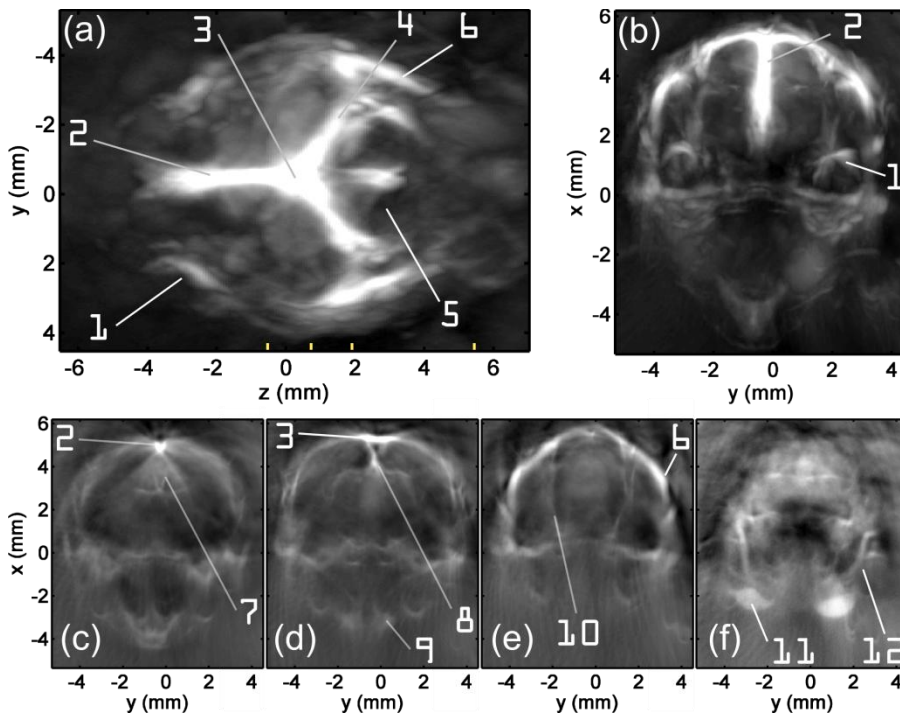
379  
 380 **Figure 6** Reconstructions of the abdomen of the 7-day-old mouse. (a) MAP image of the entire region-of-interest  
 381 along the x-axis. The slices (b)-(e) correspond to the yellow marks on the side of (a), and are ordered by increasing z. For  
 382 each slice the images were not post-processed. The voxel values are normalized by the maximum value of the 3D image.  
 383 Legend: 1. Spleen, 2. Interlobar vessels of the left kidney, 3. Ischiatic vein, 4. Saphenous vein, 5. Partial lobe of the liver,  
 384 6. Right kidney, 7. Abdominal aorta, 8. Vena cava, 9. Femur, 10. Intestine vessels, 11. Left kidney. The anatomical features  
 385 were correlated with published mouse anatomy<sup>48</sup>.

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

389 **2. HEAD REGION**

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

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



399  
 400 **Figure 7** Reconstructions of the head of the 4-day-old mouse. MAP image of the entire region-of-interest (a)  
 401 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  
 402 ordered by increasing z. Legend: 1. Supraorbital vein, 2. Superior sagittal sinus, 3. Confluence of sinuses, 4. Right  
 403 transverse sinus, 5. Cerebellum, 6. Temporal vein, 7. Inferior sagittal sinus, 8. Straight sinus, 9. Facial vein, 10. Sigmoid  
 404 sinus, 11. Jugular vein, 12. Maxillary vein. The anatomical features were correlated with published mouse anatomy<sup>48</sup>.

405 **Video 3:** This video corresponds to Figure 7(b), and shows the 3D optoacoustic volume image of the head of the  
 406 4-day-old mouse. Rotating maximum amplitude projection images around the x-axis are displayed with 2° angle between  
 407 the projections, and at a frame rate of 15 images per second.

#### 408 IV. DISCUSSION

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

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

421 With a translation range corresponding to the radial size of the sample, the in-plane resolution was  
422 found to have two-dimensional isotropy and homogeneity over the width of the sample even for absorbers  
423 emitting with peak frequencies higher than the low-pass cut-off frequency of the system<sup>46</sup> (Figure 3 and  
424 Figure 4). The isotropy and homogeneity of the in-plane resolution are a consequence of the scanning  
425 geometry, in which the sample is entirely contained and probed with a high density of sensor positions. In  
426 this manner, optoacoustic waves arising from the entire sample were captured at each rotation angle, and  
427 the full-view criterion was fulfilled in-plane. Indeed, for objects inside the circle defined by  
428 the translation range, normal vectors drawn onto all their in-plane boundary points pass in one direction the  
429 detection angular aperture. In-plane boundaries could therefore be recovered stably<sup>31</sup>. Conversely, because  
430 of the limited angle of acceptance of the focused detector, in-plane limited-view issues occur for objects  
431 outside the circle, and the azimuthal resolution was therefore degraded (Figure 2). The in-plane  
432 FWHM dimensions for 50  $\mu\text{m}$  diameter microspheres were found to be approximately 130  $\mu\text{m}$ , for the  
433 detection geometry and reconstruction algorithm selected in this study. The acoustic wavelength in the  
434 medium corresponding to the low-pass cutoff frequency of the system (12 MHz) is:  $\lambda_c \approx 123 \mu\text{m}$ , which  
435 implies that the reconstructed in-plane FWHM dimension of the sphere and the optimal resolution  
436 theoretically achievable ( $0.8\lambda_c \approx 99 \mu\text{m}$ ) are on the same order of magnitude. The discrepancy between the  
437 two values could first be attributed to the finite dimension of the microsphere. However, the sphere is a  
438 solid which longitudinal speed of sound is larger than the one of the surrounding aqueous medium. As a  
439 consequence, the sphere dimension cannot be simply subtracted to obtain the effective resolution of the  
440 system. The finite size of detectors may also have an effect on the resolution, as the spatial averaging on  
441 the detector surface distorts the signals detected for optoacoustic sources outside of the focal zone, and  
442 shifts their frequency spectrum towards lower frequencies<sup>33</sup>. This effect, termed spatial impulse response  
443 of the detector<sup>49</sup>, is not taken into account by the filtered backprojection reconstruction algorithm, which  
444 assumes point-like detectors and non-distorted signals. The finite size of the detectors, their focusing  
445 properties, and the discrete positions of the detector array could also partially explain the in-plane  
446 amplitude variation between the reconstructed microspheres (Figure 3(a) and Figure 4(a)). The spatial

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

453         Due to using a dynamic aperture adjustment, the elevation resolution was shown to be kept  
454 constant and uniform over a width of 15 mm (Figure 4), unlike circular tomography with cylindrically  
455 fixed focused detectors, which focal line is perpendicular to the axis of rotation<sup>23</sup>. The elevation FWHM  
456 dimension of microspheres of 50 $\mu$ m in diameter was found to be about 330 $\mu$ m, i.e. more than twice the in-  
457 plane FWHM value. Since the microspheres are isotropic absorbers, it can be concluded that the  
458 discrepancy between the in-plane and elevation resolution is due to the detection geometry. The main  
459 limitation of the proposed geometry in terms of angular aperture is the finite aperture of the array along  
460 the elevation direction. The sample is indeed only partially enclosed in terms of the elevation angle. The  
461 angle of acceptance was further reduced in order to obtain a uniform resolution in elevation. Finally, this  
462 resolution is degraded by the spatial averaging on the transducer surface, which effectively creates an  
463 apodization function, stronger at high frequencies. The elements of the array are indeed not infinitely thin  
464 but have a width of 270 $\mu$ m, that is to say around  $2\lambda_c$ , which results in non-negligible spatial averaging for  
465 sources located at a steep elevation angle with respect to the sensor. Taking into account the spatial  
466 impulse response of the detector in the reconstruction algorithm would reduce this apodization and should  
467 then improve the elevation resolution as well.

468         Despite the limitations due to the reconstruction algorithm and the limited view, the obtained  
469 images can be qualified as high resolution. The three-dimensional reconstruction of the cross (Video 1)  
470 and mice (Video 2 and Video 3) show that small structures can be visualized and that their shape  
471 correspond to the implanted absorbers or the expected anatomy<sup>48</sup>. Moreover, no complicated image post-  
472 processing was performed to obtain the 3D volume rendering, as opposed to the routines developed in  
473 other studies<sup>6</sup>. The negative and non-physical values that appear on the images (Figure 6) and that create  
474 some shadowing effects are most probably due to the non-optimal reconstruction algorithm, which is not  
475 adapted for finite-sized focused detector or limited solid-angle coverage.

476         For the implementation of the detection geometry used in this study, one full acquisition took  
477 1.5 hours with 10 time-averages on the signals, that is to say an average 4.8 seconds per position of the

478 array. Since the acquisition of the signals took around 1 second per position (due to the 10 Hz pulse-  
479 repetition rate of the laser), the time-consuming part was therefore the motion of the array. The current  
480 scanning is quite long for *in-vivo* experiments, however, several solutions can be considered to reduce  
481 significantly this scan duration. First, since the motion of the array is the most time consuming part of the  
482 acquisition, faster stepper motors could be used. Second, a continuous motion of the detector has been  
483 shown to reduce dramatically the acquisition time<sup>23</sup> and could also be implemented for this detection  
484 geometry. On the other hand, the large number of positions used in this study could probably be  
485 significantly reduced. Using a reconstruction algorithm that takes into account the spatial impulse  
486 response of the transducers, better quality images are expected to be obtained with less sensor positions.  
487 With the filtered backprojection algorithm, the spatial averaging of the optoacoustic waves on the detector  
488 surface for optoacoustic sources outside of the focal zone of the array is indeed compensated by the large  
489 number of positions, the signals originating from the focal zone being stronger than the distorted ones.  
490 Additionally, the fine translation step implemented here aimed at capturing the high-frequency  
491 components arising from the entire sample by translating the focal spot, so as to demonstrate the high  
492 resolution capability of the detection geometry. If the required resolution is lower than in the present  
493 study, the translation step-size could be accordingly adapted, which would result in shorter scanning  
494 duration. A systematic study of the influence of each scan parameters on the image quality is beyond the  
495 scope of this paper, but will be considered in the future to improve the acquisition time. Lastly, a higher  
496 repetition rate laser could also be used to accelerate the acquisition of the signals, and the number of time  
497 averages per position optimized in regards to the high number of individual measurements projections.

498 Finally, one major advantage of the novel detection geometry is its versatility in terms of the  
499 interchangeability of the transducer array. No dedicated prototype was built, instead a conventional  
500 ultrasound array was used. A large range of linear arrays have already been developed for  
501 ultrasonography, and are available commercially. Among the wide variety of length, number of elements,  
502 and central frequencies available, the high central frequency detectors, and the large aperture ones are of  
503 special interest for the proposed detection geometry. The combination of a large number of elements and  
504 a small pitch would indeed allow increasing the angular aperture in elevation. Commercially available  
505 linear arrays for medical ultrasound have as many as 512 elements, with a pitch ranging from  $\lambda/2$  to  $3\lambda/2$   
506 where  $\lambda$  is the wavelength corresponding to the central frequency of the elements<sup>39</sup>. The pitch of the linear  
507 array used in this study was on the higher pitch range considering a central frequency between 5MHz and  
508 7MHz. A smaller pitch would reduce the spatial averaging on the elements at frequencies higher than the  
509 central one and improve the image quality. On the other hand, linear arrays with central

510 frequencies ranging from 30MHz to 65MHz<sup>50-54</sup> have been developed recently, and some are already  
511 available commercially. Currently, to our knowledge, only linear and annular<sup>55, 56</sup> arrays have been  
512 developed for such high frequencies. Array geometries where the elements are arranged on a concave  
513 curved line or surface are a technical challenge at such frequencies. The high-frequency linear arrays  
514 developed so far are moreover cylindrically focused to enhance their sensitivity, and to limit spatial  
515 averaging on the detector surface within the focal region. The novel detection geometry proposed here is  
516 therefore adapted for these arrays, provided that the radial distance to the axis of rotation and the  
517 translation steps are adapted to the array characteristics and the sample under study. Such a high  
518 frequency system is expected to lead to volumetric images at mesoscopic scale of better quality than the  
519 optoacoustic methods proposed so far, and will be investigated in the near future. Alternatively, a multi-  
520 sector scanning scheme, as developed for two-dimensional imaging in Ref<sup>57</sup>, could be investigated with  
521 an array. Such scanning scheme resembles the fan beam scanning method for the third generation of  
522 computed tomography x-ray imaging<sup>40</sup>.

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

## 528 V. CONCLUSION

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

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

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

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