

1 Photoacoustic Tomography Shows the Branching Pattern of Anterolateral Thigh Perforators in Vivo

2

3 Abstract

4

5 **Background**

6 The distal branching pattern of perforators is associated with thin anterolateral thigh (ALT) flap failure. The  
7 purpose of this study is to investigate the feasibility of using photoacoustic tomography (PAT) as a diagnostic  
8 imaging modality to identify ALT perforators and their branching patterns in the subcutaneous layer.

9 **Methods**

10 Ten thighs in five healthy males were studied. The anterolateral aspect of the mid-thigh was examined using  
11 PAT. The correlation between PAT and ultrasound (US) findings was evaluated. To determine the  
12 detectability of PAT by depth, the depth of vessels in the stem portion was compared to the depth of the  
13 deep fascia measured by US. Branching patterns of vessels in the adipose and suprafascial layers were  
14 evaluated by three-dimensional observation.

15 **Results**

16 A total of 18 perforators were visualized by PAT. PAT and US had comparable diagnostic potential for the  
17 detection of perforators. PAT visualized microvessels in the subcutaneous layer, especially those in oblique  
18 or horizontal orientations. The estimated mean depth of visualized vessels was 9 mm; the maximum was 13

19 mm. There was a strong correlation between the depth of visualized vessels in the stem portion and the  
20 depth of the deep fascia. Three-dimensional observation of PAT images showed the branching morphology  
21 of perforators.

## 22 **Conclusions**

23 This study showed the applicability of PAT to identification of the branching patterns of ALT perforators in  
24 vivo although limited visualization of subfascial vessels is a technical issue. We believe PAT has the potential  
25 to be a new imaging modality for thin ALT flap surgery.

26

27 Key words

28 Anterolateral thigh, imaging, perforator, photoacoustic tomography, thin flap

29

30 **Introduction**

31 Flap thinning<sup>1</sup> is technically challenging. In particular, a high rate of failure has been reported for the thin  
32 anterolateral thigh (ALT) flap.<sup>2</sup> Previous experimental studies have suggested that skin perfusion is changed  
33 during thinning as a result of injuries to vessels in the suprafascial layer.<sup>3</sup> Thus, an appreciation of the  
34 individualized vascular anatomy of the thigh is essential for thin flap planning; however, suitable imaging  
35 methods that can be used safely and easily *in vivo* are lacking. Photoacoustic tomography (PAT) is an  
36 emerging vascular imaging modality that has been used in basic research and clinical practice, which allows  
37 for visualization of subcutaneous vessels on the basis of the photoacoustic mechanism. Energy from a near-  
38 infrared pulse laser is introduced into hemoglobin. As a result, red blood cells become swollen and release  
39 ultrasonic waves, which allows vessels to be visualized without contrast agents.<sup>4</sup> The purpose of this study  
40 is to investigate the feasibility of using PAT as an imaging tool for identifying branching vessels in the  
41 subcutaneous layer associated with the thin ALT flap.

42

43 **Materials and methods**

44 After approval by our institutional ethics committee and clinical trial registration, we recruited five healthy  
45 male adults (mean age, 41 years; mean body mass index, 23.1 kg/m<sup>2</sup>) for this study. PAT was performed  
46 bilaterally in all subjects.

47 The PAT we employed was the same system that was used for a previous study on breast cancer

48 angiogenesis. In that study, the estimated maximum depth of vessels visualized was 27 mm.<sup>5</sup> The system  
49 comprised a Ti:sapphire laser transmitter and a tabletop scanning platform (Supplemental Digital Content  
50 Figure 1). The wavelength of the laser was 797 nm. The maximum laser power was set to less than the half  
51 the maximum permissible exposure recommended by the American National Standards Institute. Multiple  
52 ultrasonic transducers were placed into a hemispherical cup built into the platform. Each subject was placed  
53 on the platform in the semi-prone position with the anterolateral surface of the mid-thigh facing the cup. The  
54 space between the skin and the cup was filled with de-aired water for smooth transmission of ultrasonic  
55 waves. Each scan took approximately 2 minutes for a 14 × 14 cm<sup>2</sup> region. Acquired data were processed  
56 three-dimensionally using laboratory-made imaging software. Visualization of vessels in the adipose or  
57 suprafascial layer was performed using the following steps. First, the surface of the skin was identified  
58 automatically using signals from the dermis. Next, color gradation was applied to the images according to  
59 the depth from the skin surface. Subdermal venous networks in the most superficial layer were visualized in  
60 blue. Finally, vessels in the adipose layer or deeper were visualized independent of the venous networks by  
61 semi-automatically cropping out the superficial layer that included the networks (Fig. 1).

62 To study the diagnostic potential of PAT, the correlation between the locations of perforators identified on  
63 PAT versus conventional ultrasound (US) was evaluated. To determine the detectability of PAT by depth, the  
64 depth of vessels in the stem portion was measured and compared to the depth of the deep fascia measured  
65 using US. The orientation of perforators in the stem portion was also evaluated. The branching morphology

66 of vessels in the adipose and suprafascial layers was evaluated using three-dimensional observation.

67

## 68 **Results**

69 PAT visualized the branching vessels of perforators in the subcutaneous layer in all subjects. PAT showed

70 18 perforators and US showed 15 perforators, all of which were visualized by PAT. However, discrepancies

71 in location of less than 10 mm were frequently observed. The estimated mean depth of vessels visualized

72 by PAT was 9 mm; the maximum was 13 mm. Unlike US, PAT does not visualize the soft tissue components.

73 Comparison of the depth of a vessel visualized by PAT with the depth of the deep fascia visualized by US at

74 the corresponding site revealed a strong correlation (Pearson's correlation = 0.8). Among the 18 perforators,

75 nine had an orientation of approximately 30 degrees relative to the horizontal plane and the remaining nine

76 had an orientation of 30–60 degrees.

77 Three-dimensional observation of PAT images showed the branching pattern of perforators. Fourteen Type

78 I and four Type II perforators according to a classification system proposed by Schaverien et al.<sup>6</sup> were

79 observed. Figure 2 is a representative image showing a Type II perforator with horizontal branches visualized

80 in the deepest layer and oblique branches more superficially (see also Supplemental Digital Content Video

81 1). Such branching vessels in the suprafascial layer were almost always undetectable by Doppler mode of

82 US (Supplemental Digital Content Figure 2).

83

84 **Discussion**

85 This is the first report to show the feasibility of using PAT imaging to visualize the branching morphology of  
86 perforators in the subcutaneous layer in the anterolateral thigh. The three-dimensional nature of PAT imaging  
87 contributed to identification of the branching morphology of perforators.

88 Compared with conventional US, PAT visualized vessels to the level of the deep fascia. By contrast, vessels  
89 deeper than the fascia were undetectable, suggesting that the deep fascia could be a factor limiting the  
90 depth performance of PAT. The lack of anatomical references of PAT imaging is a barrier to its intraoperative  
91 use. Cooperation of US with PAT would resolve these issues.

92 PAT has two other technical limitations. First, there are currently no established methods to distinguish  
93 arteries from veins using PAT, thus a triple bundle appearance was common, especially in vessels near the  
94 stem portion (Fig. 2). The subdermal venous networks were distinguishable owing to their superficial location  
95 and polygonal morphology.<sup>7</sup> Second, the orientation of a vessel affects its visualization by PAT. This is known  
96 as the limited view problem.<sup>8</sup> More vertically distributed vessels are reportedly less likely to be visualized.  
97 Indeed, no vertical vessels were observed in this study, although vessels at an angle of approximately 60  
98 degrees were visualized. This might be the reason why no Type III perforators were found in this study.

99 Although further technical refinements are needed for PAT to establish its clinical utility, we believe that PAT  
100 would be a promising new imaging modality for thin ALT flap surgery.

101

102 Authors' role/participation in the authorship of the manuscript

103 SS conducted the study and IT performed the experiments. HS developed imaging software. IT, AY and SS

104 analyzed the data. All the authors contributed to writing of the manuscript.

105

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136 **Figure legends**

137

138 Fig. 1

139 Anteroposterior photoacoustic tomography images in the all-layer (left) and deep-layer views (right). The  
140 color corresponds to the depth from the skin surface. Note that the blue superficial venous networks in the  
141 all-layer image are not shown in the deep-layer image; deep branching vessels in red are visualized  
142 separately. Scale bar = 20 mm.

143

144 Fig. 2.

145 Photoacoustic tomography projection images showing a Type II branching pattern in a perforator. Insets are  
146 lateral views for the regions indicated in the anteroposterior image. In the inset A–B, a perforator bifurcates  
147 into vessels coursing horizontally and oblique to the skin surface. In the inset C–D, a branch coursing  
148 obliquely from the bifurcation changes its direction, becoming more horizontal in the distal portion. Note the  
149 triple bundle appearance of the branches (arrowhead). Scale bars = 10 mm. See also Supplementary video

150 1.

151

152 Supplemental Digital Content Figure 1

153 Schematic of the photoacoustic tomography system.

154

155 Supplemental Digital Content figure 2

156 Images show a difference in visual performance between PAT and Doppler ultrasound. Note that the oblique

157 portion of the perforator system in the subcutaneous layer shown by PAT is not visualized by Doppler US

158 (arrowhead).

159

160 Supplemental Digital Content Video 1

161 The video highlights three-dimensional visualization of the branching vessels in Fig. 2. The color of the

162 vessels corresponds to the depth from the skin. X, Y, and Z coordinates indicate the medial, distal, and

163 posterior directions, respectively.

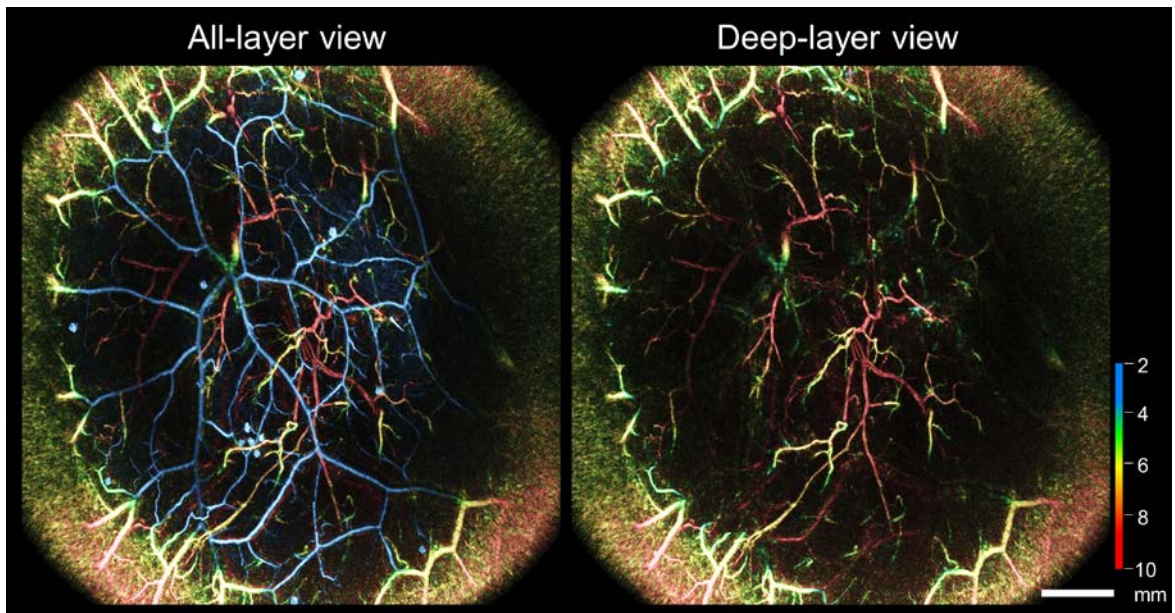


Fig.1

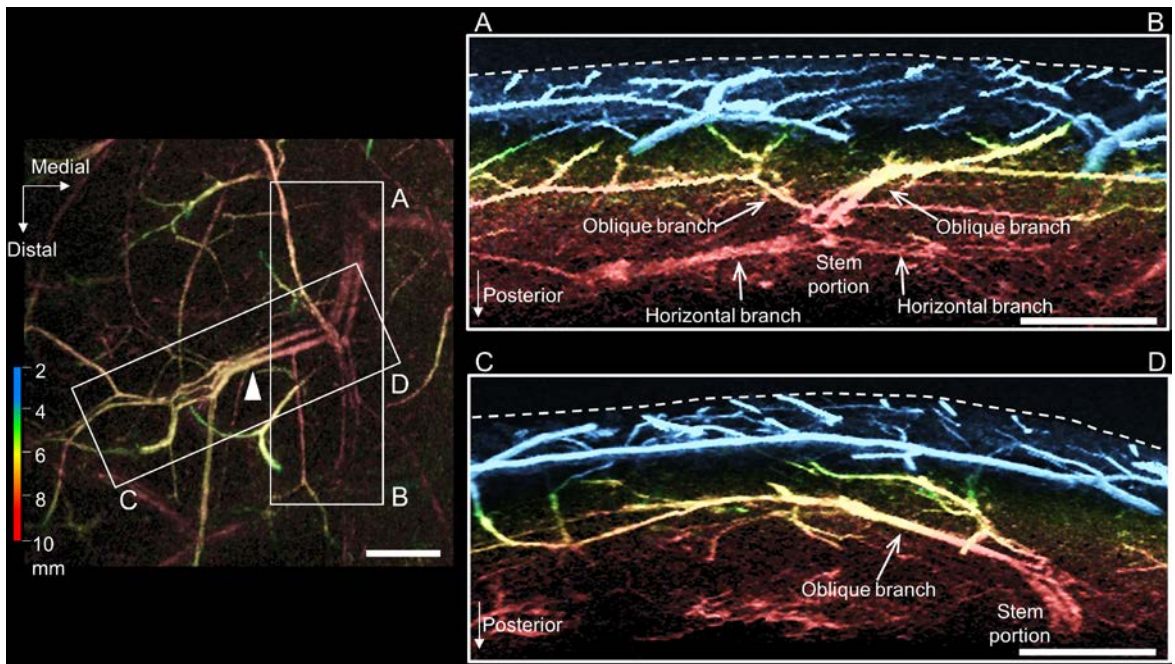
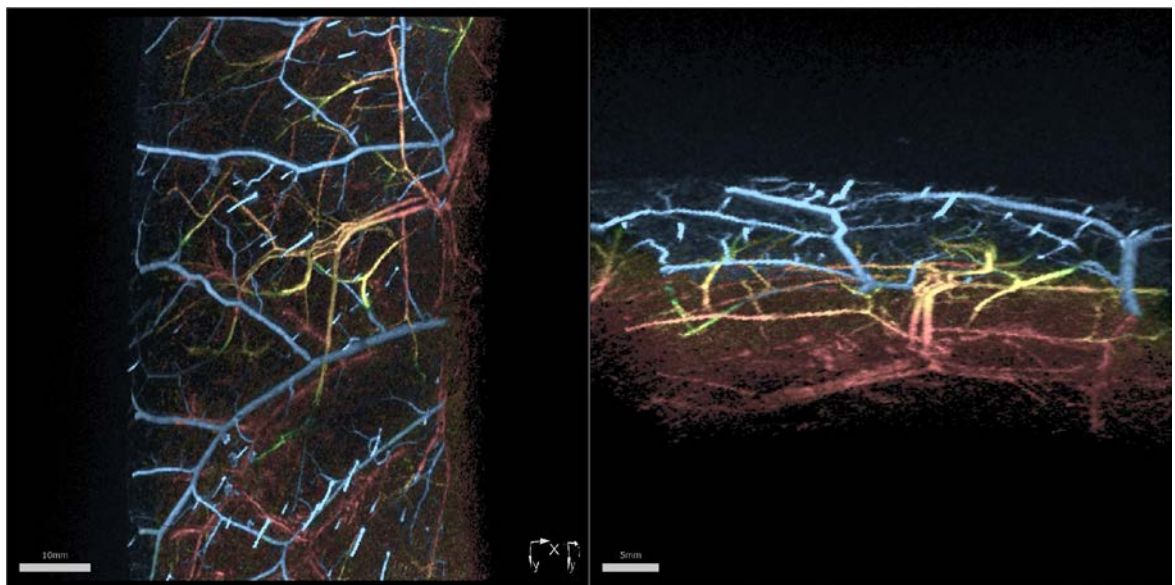
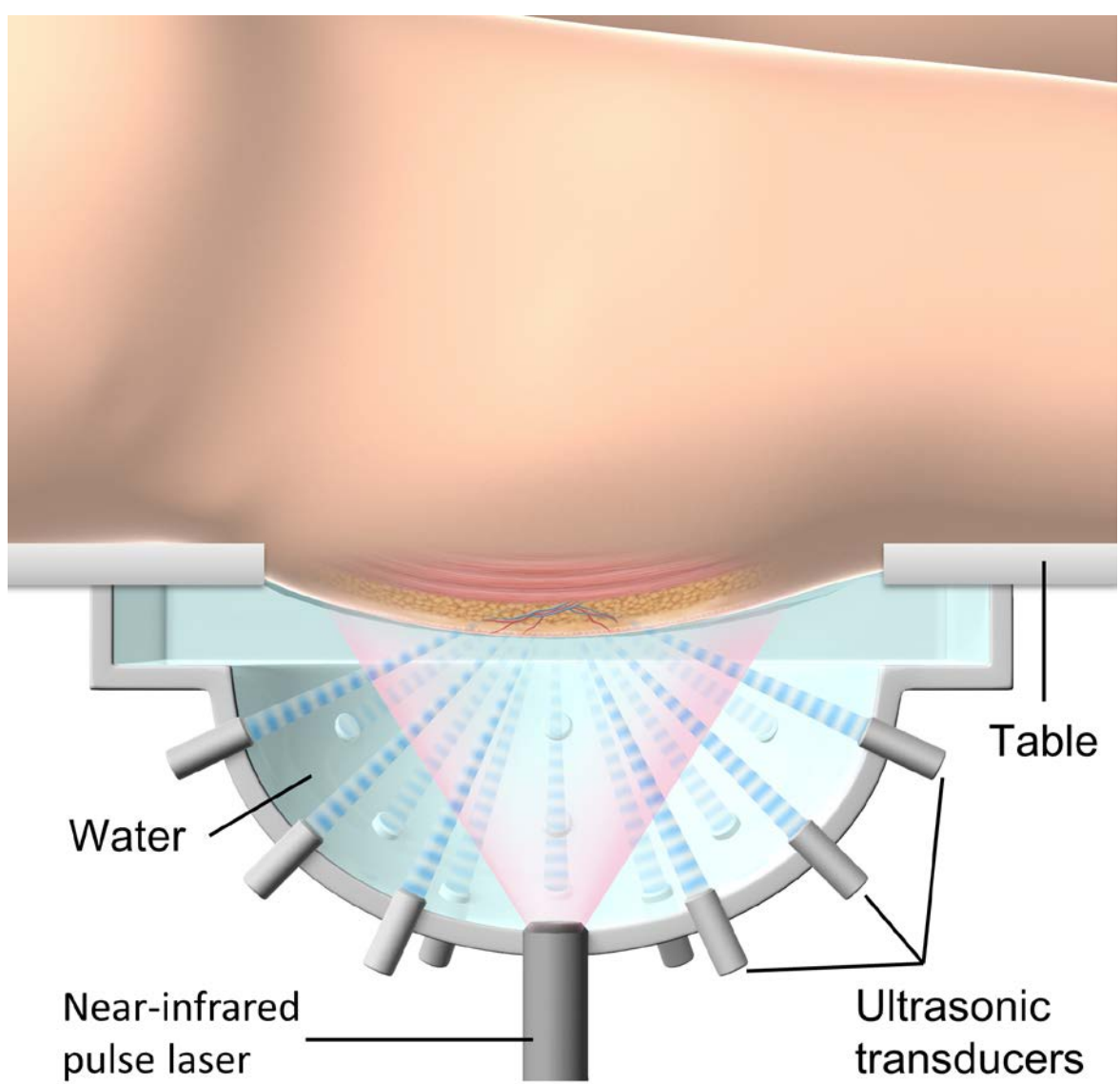


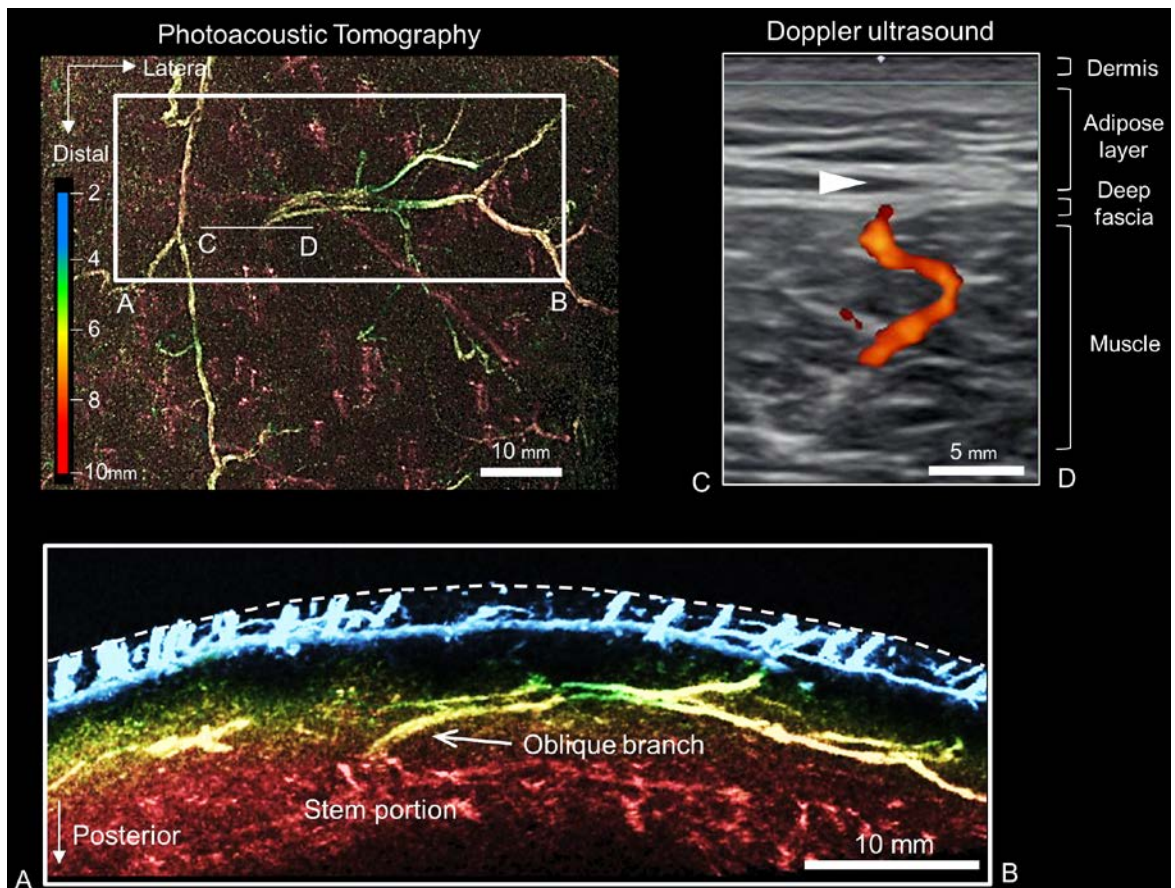
Fig.2



Supplementary video capture



Supplemental Digital Content Figure 1



Supplemental Digital Content Figure 2