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Supplementary Materials for

Fast label-free multilayered histology-like imaging of human breast cancer by photoacoustic microscopy

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The PDF file includes:

- fig. S1. Illustrations of negative and positive margins.
- fig. S2. Experimentally measured spatial and axial resolutions of the UV-PAM system.
- fig. S3. Procedure for obtaining thin breast tissue slices without and with H&E staining.
- fig. S4. Imaging of a breast tumor from the third patient.
- fig. S5. Representative *xz*-projected human breast tumor image acquired over a 10 mm × 4.2 mm area from the first patient specimen.
- fig. S6. Photographs of the breast tissue specimens.
- fig. S7. Reproducibility of the UV-PAM system for breast tumor tissue imaging.
- Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/5/e1602168/DC1)

- movie S1 (.mp4 format). Closeup images of a row in the ROI of the first patient's breast tumor specimen imaged by UV-PAM.
- movie S2 (.mp4 format). Series of depth-resolved images of the first patient's breast tumor specimen imaged by UV-PAM.

SUPPLEMENTARY MATERIALS

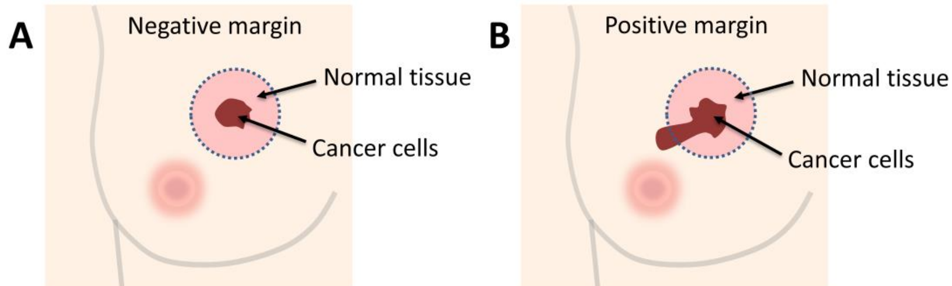

fig. S1. Illustrations of negative and positive margins. The circles (blue dashed lines) outline the region of excised breast tissue. (**A**) In the negative margin, all the cancer cells are encapsulated by normal tissue. (**B**) In the positive margin, cancer cells are present at the margin of the excised cancer. There are potentially residual cancer cells left in a patient.

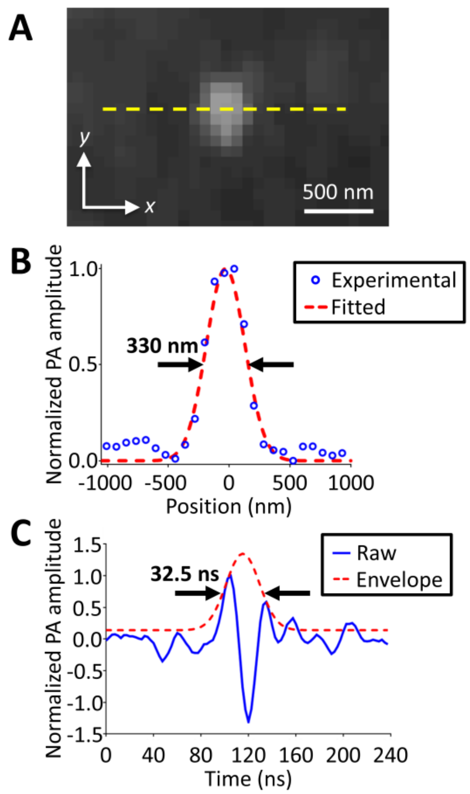

fig. S2. Experimentally measured spatial and axial resolutions of the UV-PAM system. (**A**) UV-PAM image of a gold nanoparticle 50 nm in diameter. The profile along the yellow dashed line is extracted for averaging. (**B**) Averaged line profile of five gold nanoparticles (blue circles). With Gaussian fitting (red dashed line), the lateral resolution is estimated to be 330 nm. (**C**) A-line signal (blue solid line) of the center position of the gold nanoparticle shown in (A). The axial resolution was determined by analyzing the FWHM of the amplitude of the Hilbert-transformed A-line signal (red dashed line). The axial resolution is estimated to be 32.5 ns, which corresponds to 48 μm.

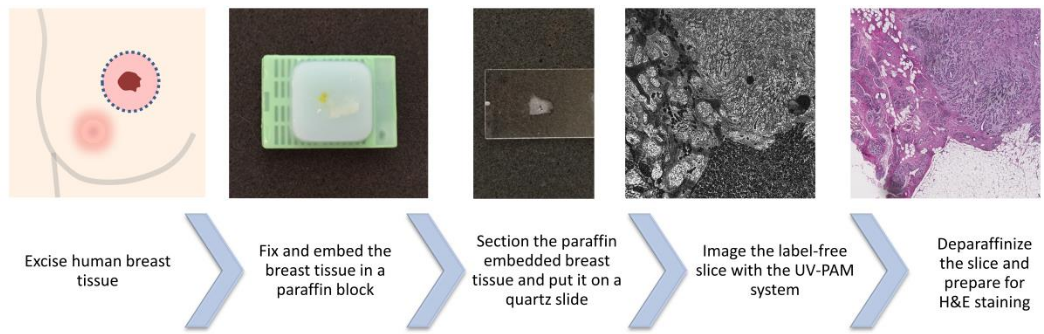

fig. S3. Procedure for obtaining thin breast tissue slices without and with H&E staining. A breast tumor was first excised by our surgeon, then fixed and embedded in a paraffin block. After that, the paraffin block was sectioned and put on a quartz slide, which was then imaged by our UV-PAM system. Afterwards, the tissue slice was deparaffinized and H&E stained to obtain a corresponding histology image.

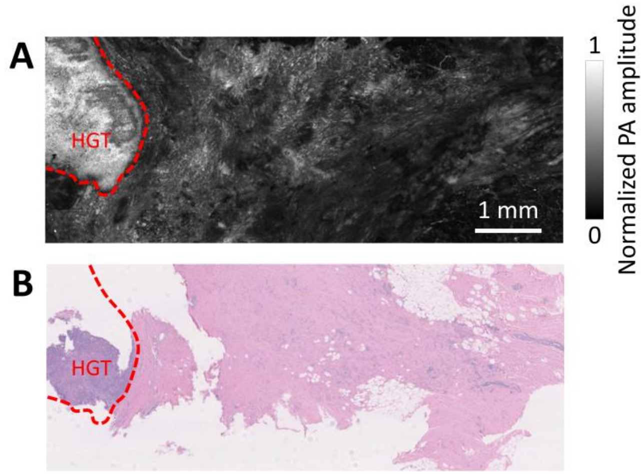

fig. S4. Imaging of a breast tumor from the third patient. (**A**) UV-PAM image of the fixed, unprocessed breast tumor. (**B**) H&E-stained histologic image of the same area shown in (A) acquired after processing, sectioning, and staining the excised breast tissue. The red dashed lines in (A) and (B) outline the boundaries of the high grade tumor (HGT).

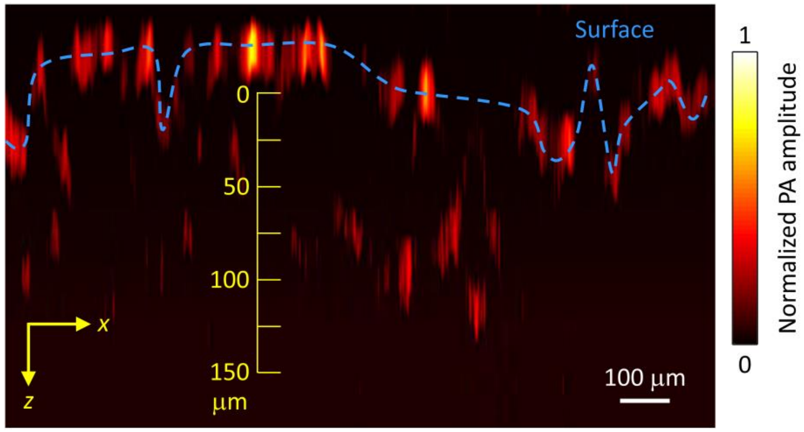

fig. S5. Representative *xz*-projected human breast tumor image acquired over a 10 mm × 4.2 mm area from the first patient specimen. The blue dashed line outlines the surface of the breast tissue. With UV light illumination, the deepest cellular structures are ~100 μm in depth measured from the tissue surface.

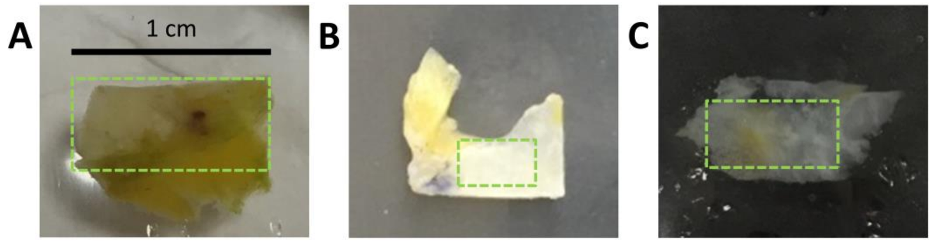

fig. S6. Photographs of the breast tissue specimens. (**A**) First patient's specimen with a thickness of ~2 mm. (**B**) Second patient's specimen with a thickness of ~3 mm. (**C**) Third patient's specimen with a thickness of ~1 mm. The green dashed regions are the imaging FOV for both UV-PAM and standard histology.

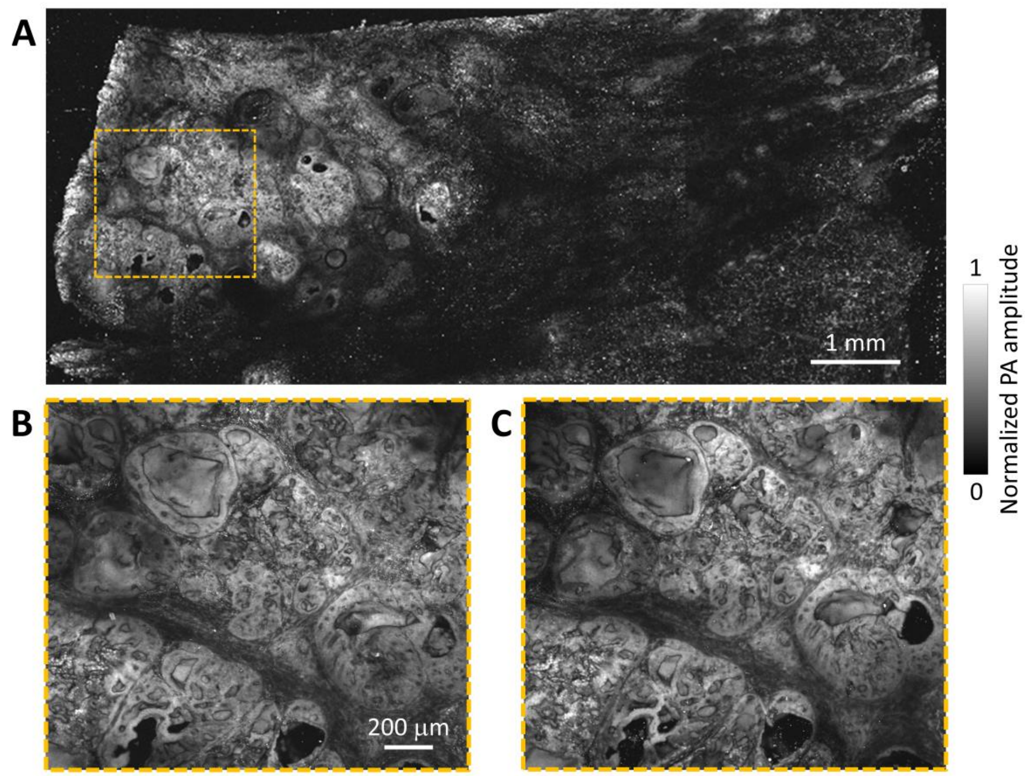

fig. S7. Reproducibility of the UV-PAM system for breast tumor tissue imaging. (**A**) UV-PAM image of the first patient's specimen, which is shown in Fig. 3A. (**B**) First re-imaging of the zoomed-in portion in (A) (orange dashed region). (**C**) Second re-imaging of the same zoomed-in area. Note that between images (B) and (C), the specimen was removed from the water tank and sample holder, and then put back in.

movie S1. Closeup images of a row in the ROI of the first patient's breast tumor specimen imaged by UV-PAM. The 6X close-up scanning window with a size of ~500 x 500 μm^2. With the identification of fundamental characteristics of the breast tissue structures in the close-up scanning window, tumor, tumor-to-normal, and normal regions are shown with tags colored in red, yellow, and green, respectively.

movie S2. Series of depth-resolved images of the first patient's breast tumor specimen imaged by UV-PAM. Two 5X close-up depth-resolved UV-PAM images of the cancer regions are also shown. All depth-resolved images are shown at 9 depths with an axial step size of 22 μm.