The pilot feasibility study to assess PAT-US imaging for SLN detection included women who were being treated for pathologically proven breast cancer but had not yet undergone definitive surgical interventions. The eligibility criteria included newly diagnosed breast cancer patients (clinical Stages I, II, or III) with negative axillae based on clinical examination and US that was scheduled for SLNB as part of the standard clinical care. After obtaining informed consent, PAT-US imaging of participants was performed on the same day as, and just prior to, SLNB. Study participants had a single subcutaneous injection of MB dye (5 mL, 2 mg/mL) with a 25-gauge needle near the areola in the same breast quadrant as the primary tumor. This technique was comparable to current methods utilized during routine SLNB. The breast was manually massaged for approximately five minutes to facilitate lymphatic flow to the axilla. PAT-US imaging of the axilla was performed following MB administration to identify the accumulation of MB in suspected SLNs. A small tissue marking clip and/or guide wire was placed under PAT-US guidance into suspected SLNs. Participants underwent conventional SLNB using MB dye and radiocolloids for SLN detection immediately following the experimental protocol. The surgically removed SLNs were pathologically and radiographically examined to determine the presence of the tissue-marking clip, which served to validate SLN identification by PAT-US imaging.

PAT-US studies were performed on the same day as the standard-of-care SLNB and the definitive surgery of the primary lesion, avoiding the need for an extra visit for study participants. However, the clinical workflow on the day of surgery for breast cancer patients is busy, often requiring visits to the nuclear medicine department and radiology department prior to surgery. Research studies had to be scheduled in a way that did not disrupt the clinical workflow, and administrative commitment was essential for smooth operation. Typically, PAT-US was performed following radioisotope injection and lymphoscintigraphy, and before guide-wire needle localization of the primary breast lesion. Research studies needed to be flexible in order to accommodate delays or advances in the planned clinical schedule.

5.4 Clinical Translation

5.4.1 PAT-US system

A prototype system (Fig. 5.2) capable of performing PAT-US imaging was developed around a modified clinical US scanner (iU22, Philips Healthcare, Andover, Massachusetts). The iU22 US platform offered a wide variety of imaging probes and capabilities, a modern data-flow architecture, and fully parallelized data acquisition. More importantly, the channel data (the received acoustic signals from each element recorded as voltage–time traces) from the system can be accessed via the modified channel board architecture.
All per-channel data from the US transducer and the beam-summed radiofrequency (RF) data from the processing block of the iU22 were stored in a large bank of memory in the custom-built data acquisition (DAQ) computer. The DAQ system performed image reconstruction and display, and a field-programmable gate array (FPGA)-based electronic board synchronized iU22 data acquisition with the laser firings used for PAT.

A wavelength-tunable dye laser (PrecisionScan-P, Sirah, Kaarst, Germany), pumped by a Q-switched Nd:YAG laser (QuantaRay PRO-350-10, Spectra-Physics, Santa Clara, California) served as the light source for PAT. The laser pulse was coupled to a fused-end, bifurcated fiber bundle (Light Guide Optics, Rheinbach, Germany) that flanked both sides of an US array transducer (L12-5, L8-4, L15-7io, S5-1, Philips Healthcare). The laser emitted pulses at a repetition rate of 10 Hz and pulse duration of 6.5 ns. An optical wavelength near the peak absorption wavelength of MB dye (667 nm) was chosen, and light fluence on the skin was less than 10 mJ/cm², which is within the ANSI safety limits by a factor of 2. The blue dye absorption peak is within the optical spectral window that allows deep light penetration in tissue due to reduced blood absorption.

5.4.2 Preclinical SLN mapping using PAT-US

The prototype PAT-US system was initially evaluated for SLN mapping using MB dye in a preclinical animal model. Animal protocols were approved by the Animal Studies Committee, and animal handling was performed according to the guidelines on the care and use of laboratory animals. Healthy Sprague Dawley® rats (n = 7, 250–390 g) were initially anesthetized using a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg). The hair in the left axillary region was gently depilated before imaging. PAT and US images were collected before and after 0.1 mL intradermal injection of 1%
MB dye (10 mg/mL, American Regent, Inc., Shirley, New York) into the left forepaw pad. Animals stayed under full anesthesia throughout the experiment using isofluorane gas (1 L/min oxygen and 0.75% isofluorane, Euthanex Corp., Palmer, Pennsylvania). Simultaneously, both heart rate and saturation of peripheral oxygen (S\textsubscript{p}O\textsubscript{2}) were monitored using a pulse oximeter (8600V, Nonin Medical, Inc., Plymouth, Minnesota). After imaging, animals were euthanized with an overdose of pentobarbital, and SLNs were dissected.

PAT-US images were collected before and after MB injection in seven healthy Sprague Dawley rats. Photoacoustic monitoring of the SLN following MB injection continued for an average of 77 ± 30 min (range: 33–113 min). Pre-injection PAT B-mode images feature photoacoustic signals from superficial blood vessels and skin surfaces. Soon after injection, MB accumulates in SLNs, as detected photoacoustically (Fig. 5.3). Pre- and post-injection images for each probe displayed with the same dynamic range demonstrate strong photoacoustic signal from SLNs following accumulation of MB. Co-registered PAT-US images demonstrate the ability to combine functional (photoacoustic) and structural (ultrasonic) features for SLN mapping.

**Figure 5.3** Noninvasive photoacoustic (PA) B-mode images of SLNs from three different rats acquired in vivo with the S5-1 probe [(a)–(c)], L8-4 probe [(d)–(f)], and L15-7io probe [(g)–(i)]. (a), (d), (g) Control photoacoustic images acquired before MB injection. (b), (e), (h) Photoacoustic images acquired 20 min following MB injection. (c), (f), (i) Co-registered photoacoustic and US images acquired 20 min following MB injection. (Reprinted from Ref. 10 with permission.)
Spectroscopic PAT can be used to determine the presence of MB dye without the need for a pre-injection baseline image. Spectroscopic PAT was performed using eight optical wavelengths between 607 and 678 nm to confirm that measured photoacoustic spectra of SLNs matched the optical absorption spectrum of MB. Thirty PAT images were collected at each optical wavelength to determine the effect of shot-to-shot variations in laser pulse energy. The imaging depth was increased by adding biological tissue (turkey breast tissue) on top of the rat skin surface, and lymph nodes were imaged 2.4 cm below the top surface of the overlying tissue with the S5-1 probe (Fig. 5.4). Optical spectra based on photoacoustic signals from SLNs closely match the optical absorption spectrum of MB, confirming the presence of MB in detected lymph nodes ($R = 0.995$).

Photoacoustic signal amplitudes from SLNs increase significantly following MB injection ($P$ value $= 0.0078$). In five rats measured without overlying biological tissue, the average photoacoustic signal enhancement of SLNs at 20 min post injection was $32.7 \pm 7.2$ (range: $25.3 - 41.8$) from pre-injection baseline signals, and the average contrast of SLNs relative to tissue background was $76.0 \pm 23.7$ (range: $46.1 - 112.2$). In two rats measured with overlaid biological tissue, the photoacoustic signal enhancements were 5.4 (S5-1 probe) and 3.7 (L8-4 probe), and the contrasts were 8.3 (S5-1 probe) and 4.6 (L8-4 probe).

5.4.3 Clinical SLN mapping using PAT-US

PAT-US was performed immediately prior to SLNB to determine the feasibility of in vivo SLN detection. Initially, axillary lymph nodes were
located by using US alone. Then, following the injection of MB, real-time PAT-US was used to identify the suspected SLN and guide the deployment of a commercially available breast-tissue marker clip. The tissue marker clip and/or guide-wire were used to validate the PAT-US technology. If the clip was found radiographically in the surgically excised SLN immediately after the conventional SLNB, then the PAT-US procedure was deemed successful. Figure 5.5(a) shows an US image of an axillary lymph node, while Fig. 5.5(b) shows the corresponding PAT image. The co-registered PAT and US image shown in Fig. 5.5(c) indicates that the axillary lymph node is indeed a sentinel node based on the strong optical absorption and image contrast from MB dye within the node. The same node was defined as a sentinel node during the standard surgical procedure; this was confirmed by the presence of the titanium clip inserted under PAT-US guidance.

PAT-US is a promising technology for guiding needle-based medical interventions. For SLNB, PAT-US can serve as a real-time modality to first

**Figure 5.5** Representative images from a breast cancer patient: (a) In vivo US B-mode image of axillary lymph node. (b) In vivo PAT image represented by optical absorption of MB. (c) Corresponding overlaid PAT-US B-mode image of the SLN (color: PAT, gray: US). (d) Radiograph of an ex vivo SLN showing the presence of the tissue marking clip.
locate the SLN and then guide a needle (fine or core needle) for lymph node sampling. Figures 5.6(a) and (b) show the corresponding US and PAT images, respectively, revealing the SLN and the needle used to insert the titanium marking clip. The photoacoustic contrast from the needle is much higher than that in the corresponding US image because the US array receives more emitted photoacoustic energy than it does reflected ultrasonic energy. While the photoacoustic wave propagation is centered along the needle normal toward the array, the vertically incident ultrasonic wave is reflected by the needle toward a nearly horizontal direction. The co-registered PAT and US image [Fig. 5.6(c)] shows the capability of the technology to locate lymph nodes via anatomical features in US and to verify the lymph node as sentinel using PAT. In addition, these results demonstrate the feasibility of PAT-US to guide needles to the SLN in breast cancer patients.

**Figure 5.6** In vivo images of the SLN and needle acquired using PAT-US in a representative breast cancer patient: (a) In vivo US B-mode image showing the lymph node and needle. (b) In vivo PAT B-mode image of the SLN represented by optical absorption of MB and needle. (c) Overlaid PAT-US B-mode image of the SLN and needle (color: PAT, gray: US).