12_Specialtechniques_Photoacoustics

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Imaging Modalities for Tumor Investigation Using the Vevo 2100 System

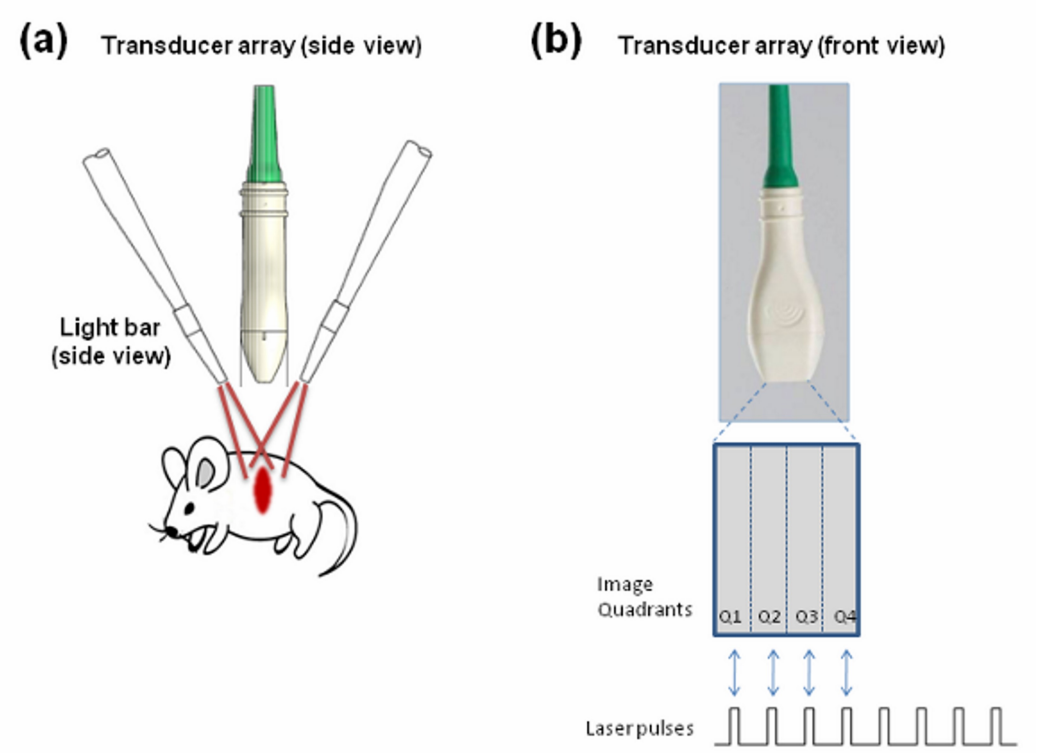
- **Vevo 2100 System Overview**
  - The **VisualSonics Vevo 2100** linear array system now includes **photoacoustic (PA) capabilities** in addition to traditional imaging modalities.
- **Imaging Techniques Used**
  - A **preclinical model of subcutaneous tumors** was investigated using multiple imaging techniques:
    - **B-Mode ultrasound**: Provides structural imaging.
    - **Power Doppler (PD) ultrasound**: Identifies **blood flow** within soft tissues.
    - **Photoacoustics (PA)**: Measures **hemoglobin concentration** and **oxygen saturation**.
- **Image Comparison and Integration**
  - **B-Mode and PD images** are compared with **PA parametric images** to assess **hemoglobin concentration** and **oxygen saturation**.
  - These imaging modes can be **co-registered** to the same image plane, facilitating the comparison of measurements across modalities.
  - The fusion of these complementary imaging techniques provides both **structural** and **physiological** information.
- **Benefits for Researchers**
  - The system allows researchers to gain a **better understanding** of biological models by offering **combined structural and physiological data** for more comprehensive analysis.

Photoacoustic (PA) Imaging for Biomedical Applications

- **Principle of PA Imaging**
  - **PA Imaging** combines **optical contrast** from biological tissues with the **spatial resolution** and **tissue penetration** of ultrasound.
  - The technique involves illuminating tissue with light, causing a **thermoelastic expansion** (dependent on optical absorption). This expansion generates an **ultrasound wave**, which is detected by an ultrasound transducer (Oraevsky and Karabutov, 2003).
- **Advantages of PA Imaging**
  - PA imaging provides **superior resolution** compared to pure optical methods, especially for **deep tissue visualization**.
  - It allows for the detection of **light-absorbing structures** deep inside tissues, which is especially useful in **cancer research** and **brain imaging** (Siphanto et al., 2005; Zhang et al., 2006; Wang and Wu, 2007).
  - Since light is a **non-ionizing electromagnetic wave**, PA imaging is considered **safe**, as long as the **optical power** is maintained within safety limits to prevent tissue damage.
- **PA Imaging Techniques**
  - The **modern PA effect** is realized by **laser pulse irradiation** and the **ultrasound detection** of PA waves at multiple viewing angles outside the tissue.
  - **Two main PA scanning methods**:
    Tomographic Geometry (PAT): Similar to **x-ray CT**, where scanning occurs around the subject (Xu and Wang, 2006).
    Planar Geometry: Uses a **linear transducer array** (Kruger et al., 2003; Zeng et al., 2004), offering higher frame rates with fewer laser pulses per 2D frame.
  - **Tomographic geometry** provides a large effective aperture for data collection but has low frame rates (>10 minutes per 2D frame). The **linear array** method, as used by **VisualSonics**, allows faster acquisition of 2D frames.
- **PA Imaging for Small Animal Imaging**
  - PA imaging is well-suited for **small animal imaging**, as the light penetration and image resolution align well with the size and scale of small animals.
  - The **image resolution** is scalable with the **ultrasound frequency** detected,

offering high-resolution images of tissues.

- **VisualSonics Vevo 2100 System**
  - **VisualSonics** launched the **Vevo 2100** system, a **high-frequency micro-ultrasound** imaging system with a **64-channel beamformer** capable of operating in the **12-70 MHz range** (Foster et al., 2009).
  - **PA imaging** was integrated into this system using linear array technology, enabling the collection of both **ultrasound** and **PA images** from the same plane. These images can be **co-registered and overlaid**, which is advantageous over PAT systems, which often lack detailed anatomical information.
- **Application in Tumor Imaging**
  - The **VisualSonics PA system** was tested for imaging **subcutaneous murine tumors**, focusing on imaging the **vasculature and microvasculature** of the tumor.
  - **Parametric maps** of **hemoglobin concentration** and **oxygen saturation** were generated to evaluate the tumor's physiological significance.
  - **Comparison** of **PA images** with **B-Mode** and **Power Doppler ultrasound** demonstrated the potential of **PA imaging** for **pre-clinical research** in oncology.



PA Imaging System Implementation and Image Acquisition

- **PA System Setup** (Fig. 1)
  - **Laser Source**:
    - The laser used in the system is an **OPO (optical parametric oscillator)** pumped by a **doubled Nd:YAG** laser, tunable from **680–950 nm**, with a **20 Hz repetition rate**, **5 ns pulse width**, and **50 mJ pulse energy**.
  - **Light Delivery**:
    - Light from the laser is delivered to the tissue through a **fiber bundle**, split into **two rectangular light bars**, mounted on either side of the **transducer array** (MS-250 with **21 MHz center frequency**).
  - **Ultrasound Detection**:
    - **PA waves** generated by light absorption propagate back to the transducer, coupled through **ultrasound gel**, and are acquired by the **transducer array**.
- **Parallel Acquisition for Image Formation**
  - For each **laser pulse**, the **PA signals** are captured on one **quadrant** of the transducer array (64 elements).
  - Once all **quadrants** are acquired (4 pulses total), the **full dataset** is used to **beamform an image** using a **delay and sum algorithm** on dedicated PC software.
  - The frame rate is **one fourth of the laser repetition rate** (5 Hz) due to the need for **four pulses** per image.

- **3D PA Imaging**
  - **3D imaging** was achieved by scanning the transducer with a **linear stepper motor** while capturing **2D images**.
  - **Multiple wavelengths** were used for acquisition, and **parametric maps** of **hemoglobin concentration** and **oxygen saturation** were calculated using a **two-wavelength approach** (Wang et al., 2006).
- **Figure 1 Details**
  - **(a)** The **layout** of the light delivery system and **ultrasound detection**:
    - Laser light is delivered via rectangular light bars mounted on either side of the transducer array.
    - **Ultrasound gel** is used to couple the PA waves from tissue to the transducer.
  - **(b) Image acquisition sequence in Parallel mode**:
    - For each laser pulse, one **quadrant** of the transducer array is read out.
    - Once all quadrants are acquired, the data is beamformed to create an image.

$$\mu_a^{\lambda 1}(r) = \frac{P^{\lambda 1}(r)}{\Gamma \cdot F^{\lambda 1}(r)}$$

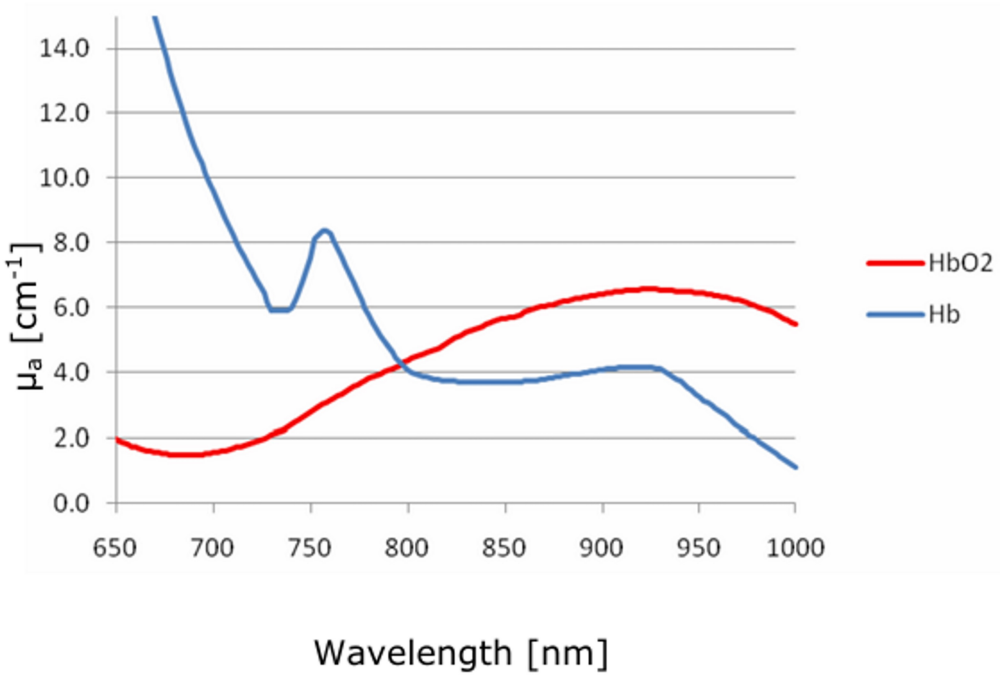
$$\mu_a^{\lambda 2}(r) = \frac{P^{\lambda 2}(r)}{\Gamma \cdot F^{\lambda 2}(r)}$$

$$HbT(r) = \frac{a_1 \mu_a^{\lambda 1}(r) - a_2 \mu_a^{\lambda 2}(r)}{a_3}$$

$$StO_2(r) = \frac{a_4 \mu_a^{\lambda 2}(r) - a_5 \mu_a^{\lambda 1}(r)}{a_6 \mu_a^{\lambda 1}(r) - a_7 \mu_a^{\lambda 2}(r)}$$

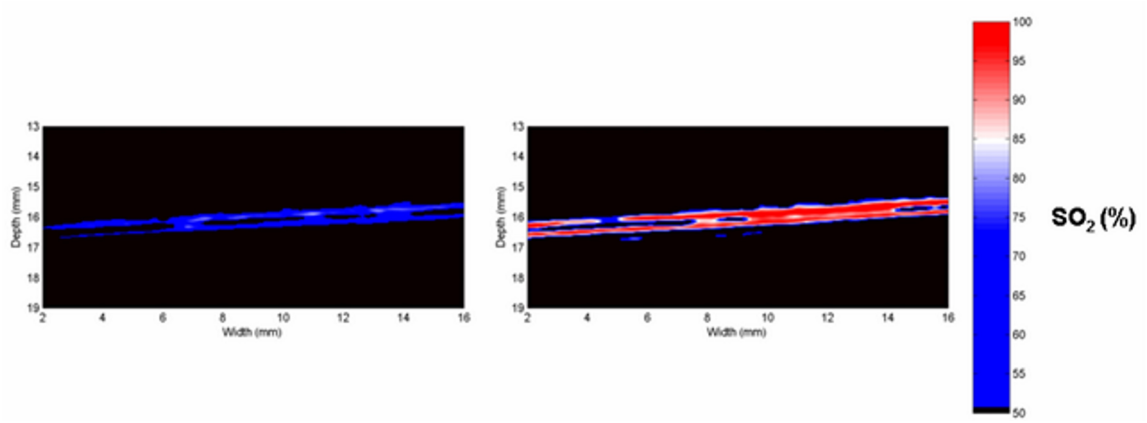
Calculation of Hemoglobin Concentration and Oxygen Saturation

- **Key Parameters**:
  - **P(r)**: Detected pressure at point **r**.
  - **Gruneisen parameter**: Typically between **0.1–0.2** in soft tissue.
  - **F(r)**: Estimated fluence of the excitation light at point **r**.
  - **a(r)**: Calculated absorption coefficient at point **r**.
  - The superscripts **1** or **2** refer to the first or second wavelengths of light used for measurement.
- **Absorption Coefficients**
  - Once the **absorption coefficients** are determined, the **total hemoglobin concentration (HbT)** and **oxygen saturation (SO2)** can be calculated.
- **Hemoglobin Extinction Coefficients**
  - The parameters **a1 to a7** are functions of the **extinction coefficients** for **oxygenated** and **deoxygenated hemoglobin**.
  - Detailed expressions for these coefficients can be found in **Wang et al. (2006)**.
- **Literature Values**
  - **Fig. 2** presents the **literature values** for the **absorption coefficients** of hemoglobin at **physiological concentrations**.

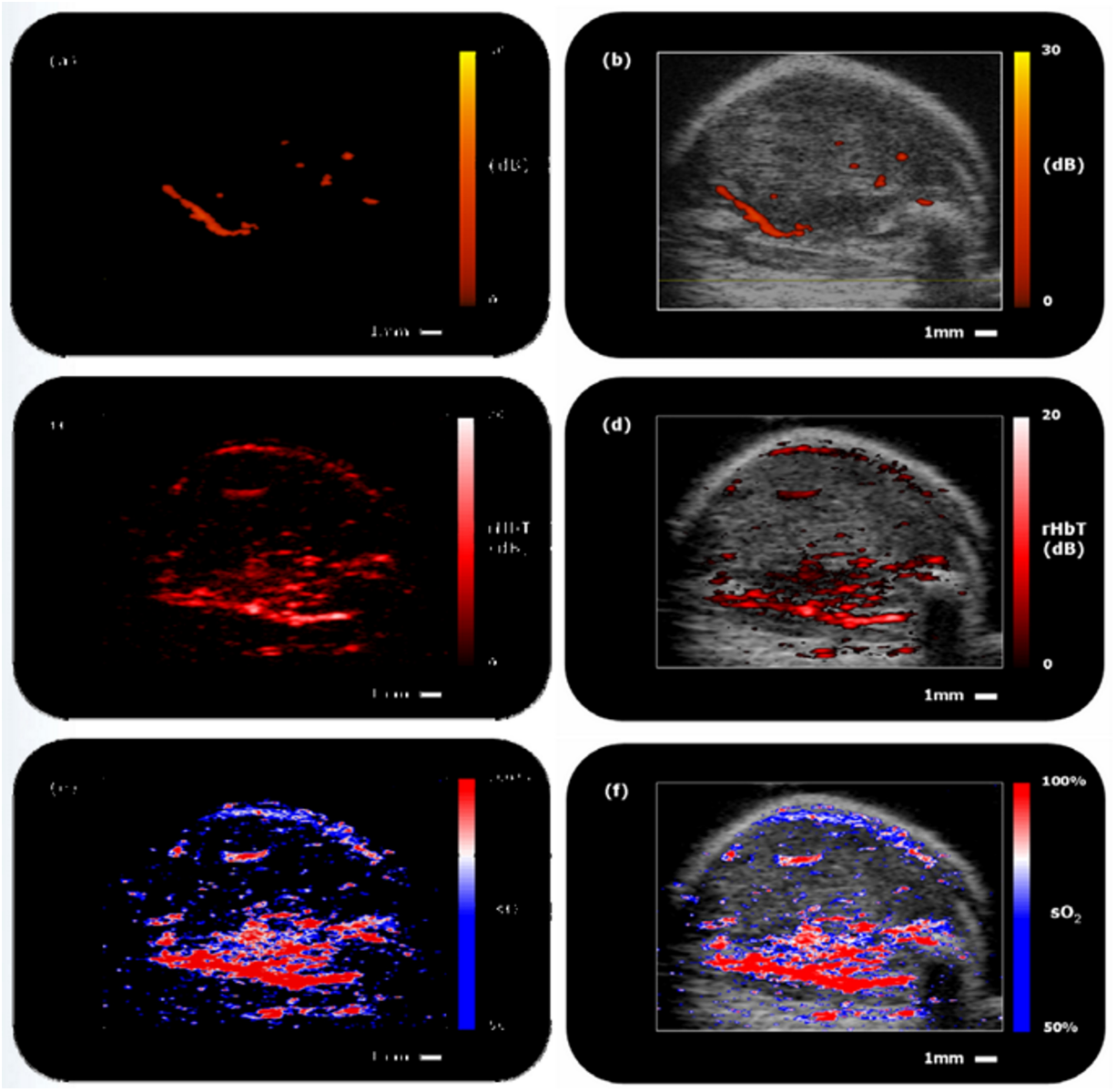


Oxygenation Validation and In Vivo Imaging of Murine Tumors

- **Oxygenation Validation**



  - **Fig. 3** shows two maps of **oxygenation saturation (SO2)** in a tube filled with venous blood from a mouse's tail vein.
    - **Left**: Blood drawn after the mouse inhaled anesthetics mixed with **room air**, resulting in **SO2** in the **50-75% range**.
    - **Right**: Blood drawn after the mouse inhaled anesthetics mixed with **100% oxygen** for 10 minutes, leading to an increase in **SO2** to the **95% range**.
  - This experiment demonstrates the **Vevo 2100 system's ability** to track changes in **oxygenation saturation** of blood using **PA imaging**.
- **In Vivo Imaging of Murine Tumors**
  - **Subcutaneous tumors** on the hind limb of **nude mice** were imaged using different imaging modes on the **Vevo 2100 system**:
    - **B-Mode**: Provides **structural information** about the tumor.
    - **PD Mode**: Maps the **spatial distribution of blood flow**.
    - **PA Mode**: Provides functional information, presented as **Total Hemoglobin Concentration (HbT)** or **Percentage Oxygen Saturation (SO2)**.
  - **PA Imaging**:
    - **HbT and SO2 maps** can be overlaid on B-Mode and PD images to correlate **blood flow regions** with **functional PA signals**.



- **Fig. 4** shows various images from a representative **image plane** within a subcutaneous tumor:
  - **Fig. 4a**: **PD image** showing regions of **blood flow** within the tumor, overlaid on the **B-Mode image** (Fig. 4b).
  - **Fig. 4c**: **PA image of total hemoglobin concentration (rHbT)**, generated from a stack of **20 2D images** (spanning **2 mm**). Each pixel in **Fig. 4c** reflects the **maximum rHbT** at the given location over the image stack and can be overlaid with the **B-Mode image** (Fig. 4d).
  - **Fig. 4e**: **Spatial map of SO2**, showing oxygen saturation, overlaid with the **B-Mode image** (Fig. 4f). The **SO2 image** has a threshold applied based on the **rHbT map**.
- **Dual-wavelength measurement (700 nm and 800 nm)** was used to generate all images of **rHbT** and **SO2**.

- **Comparison of Imaging Modes**
  - **PD Mode** and **PA images** show differences, likely due to:
    - **PD Mode** being more sensitive to **vessels angled** with respect to the transducer face.
    - **PA Mode** being more sensitive to **structures parallel** to the transducer face.
  - These differences suggest that the two imaging techniques are **complementary**.
- **Fusion of Functional and Structural Information**
  - **Fig. 4** illustrates how the **Vevo 2100 system** can effectively **fuse functional information** related to the **distribution** and **oxygenation** of blood with **structural information** of **cancerous tissue**, enhancing tumor analysis.

Optimizations and Use of Contrast Agents in PA Imaging

- **Preliminary Results and Future Optimizations**
  - Preliminary tumor imaging results show promise, but there are areas for optimization in **signal** and **image processing** to improve the quality of **PA images**.
  - Further **validation** will involve correlating **oxygen saturation measurements** with conventional methods, such as **blood-gas analysis** or **histological staining**.
- **Use of Contrast Agents to Enhance PA Imaging**
  - **Contrast agents** are recommended to improve the **sensitivity** and **specificity** of **PA imaging**.
  - There are two main types of contrast agents: **Dye-based** and **Nanoparticle-based**.
    - **Dye-based Contrast Agents**:
      - **Indocyanine Green (ICG)** and **Methylene blue** are commonly used dyes with strong absorption in the **near infrared**.
      - **ICG** can be introduced into the bloodstream to enhance the contrast of **blood**, improving **vasculature visibility** in deep tissues (Wang et al., 2004).
      - **Methylene blue** can be injected under the skin and drains into the **lymphatic system**, accumulating in the **Sentinel Lymph Node (SLN)**, where its strong contrast allows accurate **3D identification** of the SLN using PA (Song et al., 2008).
        - The scanning protocol used by Song et al. requires **tens of minutes**, but with the **Vevo 2100 system**, the process takes **less than 1 minute**.
    - **Nanoparticle-based Contrast Agents**:
      - **Nanoparticles (NPs)** exhibit stronger light absorption than dyes and can be **conjugated with antibodies** to specifically target **disease-associated cells** (e.g., cancer).
      - **Photothermal therapy** is another application of NPs, where they absorb light and generate heat to destroy **tumor cells**.
      - Imaging NP concentration in tumors before treatment initiation is highly advantageous.
      - Examples of NP contrast agents:
        - **Carbon nanotubes** conjugated with peptides can **enhance PA signal** from tumors by **eight times** (De La Zerda, 2008).
        - **Gold NPs** have been delivered to tumors using either **targeting** or the **enhanced permeability** of tumor vasculature, exploiting the **leaky vasculature** phenomenon (Yang, 2009).

PA Imaging for Preclinical Research Using the Vevo 2100 System

- **Integration of PA Imaging**
  - Recent developments on the **Vevo 2100 micro-ultrasound platform** have integrated **PA imaging**, allowing it to be **fused with B-Mode ultrasound**.
  - This fusion enables the imaging of **blood flow regions** and provides **functional information** on **hemoglobin concentration** and **oxygen saturation**.
  - **Functional information** such as this cannot be obtained with **micro-ultrasound alone**.
- **Advantages of PA Imaging**
  - PA imaging leverages the **optical absorption properties of blood** while maintaining the **image resolution** and **imaging depth** of micro-ultrasound.
  - This combination allows PA imaging to provide deeper and higher resolution images than purely optical methods, which cannot penetrate tissue as effectively.
- **PA Contrast Agents**
  - A variety of **PA contrast agents** are available to enhance the PA signal from **blood**, **tissues**, or **cell types**, providing improved imaging capabilities.
- **Unique Potential for Tumor Studies**
  - The addition of **PA imaging** to the **Vevo 2100 system** offers **unique potential** to add **functional oxygenation information** to tumor studies using **micro-ultrasound**, improving the assessment of tumor physiology.