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Evaluation of quantitativeness of a multi-wavelength photoacoustic tomography system

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1. Introduction

Recent advances in photoacoustic tomography (PAT) have enabled high-contrast, volumetric imaging of chromophores with improved depth penetration. When multi-wavelength laser sources are employed, PAT can further provide quantitative information, such as the spatial distributions of oxygen saturation and specific chromophore concentrations. To evaluate the feasibility of quantitative PAT (qPAT), it is essential to assess the sensitivity and precision of qPAT in detecting subtle changes in the optical absorption spectrum of target substances.

To design an experimental setup to evaluate quantitativeness of a multi-wavelength PAT system, we employed Arsenazo III as a model chromophore. Arsenazo III is a well-known calcium indicator that exhibits predictable and controllable spectral shifts upon binding with calcium ions. In this study, we utilized its calcium-dependent absorption characteristics to validate the system's ability to detect chemically induced spectral changes.

Using tissue-equivalent phantoms containing various Ca^{2+} concentrations, we performed multispectral photoacoustic imaging at selected wavelengths to assess the system's sensitivity to molecular interactions. This approach provides a foundational step toward developing and validating molecular contrast mechanisms for functional imaging using qPAT.

2. Material and methods

2.1 Preparation of Reagents

The reference chromophore phantom solution was prepared using EGTA, KCl, MOPS, $CaCl_2$, and Arsenazo III. EGTA was added to control free calcium ion concentrations; however, it lowered the pH of the solution. The pH was adjusted to physiological levels (7.0–7.4) using NaOH or HCl and stabilized with MOPS buffer. KCl was used to maintain ionic strength, and $CaCl_2$ was added to achieve desired free Ca^{2+} concentrations (0–10,000 nM). After mixing, the solution was gently heated to 36°C to facilitate binding between EGTA and Ca^{2+}. Arsenazo III was then added to change the optical absorption characteristics of the reagent that correspond to the concentration of calcium ion.

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2.2 Measurement of absorption coefficient

Prior to the photoacoustic measurements, the optical absorption spectra of Arsenazo III coupled with varying Ca^{2+} concentrations were measured using a spectrophotometer. Transmittance was measured across the 400–700 nm wavelength range, and the absorbance $A_{(\lambda)}$ was calculated using the Beer–Lambert law:

$$A_{(\lambda)} = \log_{10}\left(\frac{I_{ref(\lambda)} - I_{dark(\lambda)}}{I_{sample(\lambda)} - I_{dark(\lambda)}}\right)$$

where $I_{dark(\lambda)}$ is the background signal measured by the detector in the absence of light, $I_{ref(\lambda)}$ is the light intensity measured without the sample, and $I_{sample(\lambda)}$ is the light intensity measured after passing through the sample. These spectroscopic measurements enabled quantitative evaluation of the relationship between Ca^{2+} concentration and optical absorption, providing a basis for comparison with photoacoustic signal intensities in subsequent imaging experiments.

2.3 Experimental setup

To mimic in vivo conditions, a tissue-equivalent liquid phantom was prepared by dissolving Arsenazo III in a buffer solution.

Changes in photoacoustic signal intensity were measured, increasing Ca^{2+} concentration from 0nM to 10,000nM. Photoacoustic imaging was

Table 1 Composition of Arsenazo III solution

Solution name	Concentration in solution [nM]
EGTA	1×10^7
KCl	1×10^8
Arsenazo III	3×10^6
MOPS	3×10^8

performed using a hemispherical array-based PAT system equipped with an optical parametric oscillator laser (Opolette 410–2400nm, 20Hz, pulse width 7 ns, Opotek Inc.) [1]. The sample solution was injected into a PFA tube (Outer and inner diameters: 0.4 mm and 0.2 mm) to simulate a blood vessel. The phantom was irradiated with laser pulses at three wavelengths (560 nm, 600 nm, and 650 nm) with a pulse width of 100 ns and a repetition rate of 20 Hz. These wavelengths were selected based on the optical absorption characteristics measured in the previous section.

Resulting photoacoustic waves were detected with a hemispherical array transducer (256 elements, element size: 2.2 mm × 2.2 mm, aperture: 42.4 mm, focal length: 30 mm, aperture angle: 90°, numerical aperture: 0.8, center frequency: 11.5 MHz, bandwidth: 8–15.5 MHz). Images were reconstructed using delay-multiply-and-sum (DMAS) beamforming.

3. Results and discussion

Figure 1 shows the absorption spectra of Arsenazo III at different Ca^{2+} concentrations. At 0 nM Ca^{2+} (red line), a distinct peak appeared near 560 nm, whereas as Ca^{2+} concentration increased, the absorption peak shifted toward 600 nm and near 650 nm, indicating red-shifting due to complex formation between Ca^{2+} and Arsenazo III. These results demonstrated that both the presence and concentration of Ca^{2+} significantly affect the optical properties of the dye.

Figure 2 shows multispectral PAT results of the tube phantom filled with Arsenazo III solution with and without Ca^{2+}. Consistent with the absorbance results, no photoacoustic signal was observed at 650 nm in the absence of Ca^{2+} (Figure 2a-3). In contrast, in the presence of Ca^{2+}, the strong signal was observed at 600 nm (Figure 2 b-2), and a detectable signal was also observed at 650 nm (Figure 2 b-3). Those results validate the system's capability to detect chemically induced spectral changes in the photoacoustic response.

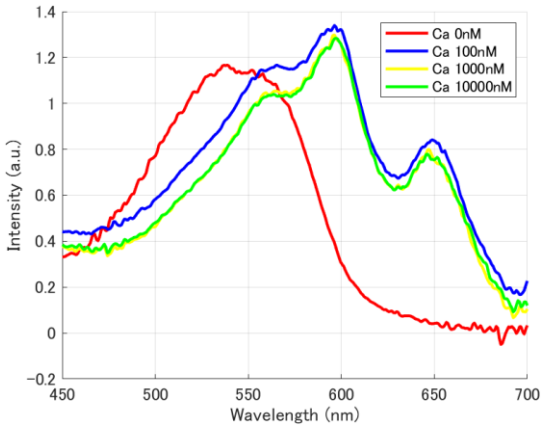

Figure 1 Absorption spectra of Arsenazo III at various Ca^{2+} concentrations.

4. Conclusion

We demonstrated that Arsenazo III enables detection of calcium-induced chemical changes via multispectral PAT images, showing its potential as a functional contrast agent for visualizing calcium dynamics. Future studies will focus on capturing dynamic changes in calcium ion concentration to further validate its applicability in physiological studies.

References

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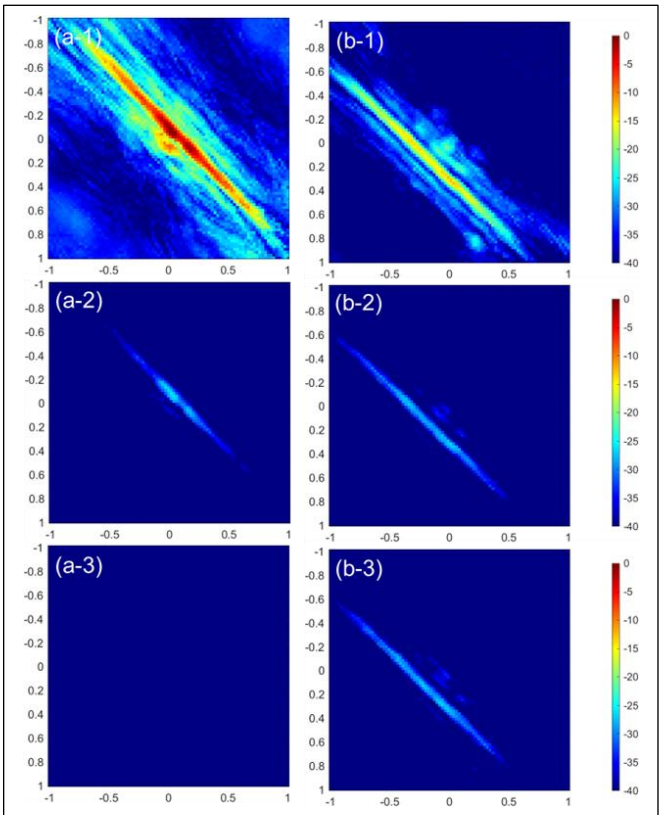

Figure 2. Photoacoustic signals at different Ca^{2+} concentrations and wavelengths.
(a-1, a-2, a-3): 0 nM Ca^{2+} at 560, 600, and 650 nm, respectively.
(b-1, b-2, b-3): 10,000 nM Ca^{2+} at 560, 600, and