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Review

Listen to the chemical and histological information in biological tissue

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ABSTRACT

Photoacoustic imaging (PAI), as an emerging biomedicine diagnostic technique that has been developed quickly in the past decade, inherits the high spatial resolution of ultrasonography in imaging deep tissue and the high sensitivity of optical imaging in evaluating tissue chemical and physiological information. In this paper, after introducing the basic principles of PAI including both photoacoustic tomography and photoacoustic microscopy, we will review some recent progress of PAI in biomedicine and demonstrate the capability of PAI in detecting the chemical compositions and in evaluating the histological microstructures in biological tissue.

1. Introduction

The chemical compositions of tissue, such as DNA/RNA, hemoglobin, melanin, water and lipid, bring important information regarding the anatomical, functional, metabolic, and molecular/genetic processes in biological systems. Therefore, tissue chemical compositions can be employed as the reference standards for the diagnosis of many diseases [1,2]. Characterizing chemical information in biological tissue has always been the subject of intense research in chemistry as well as medicine.

Due to the unique optical spectra of atoms and molecules, optical spectroscopic techniques have been used to analyze the chemical properties of materials in analytical chemistry [3], material science [4] and astronomy [5] for a long time. With the advantages in detecting chemical information, optical modalities, such as optical coherence tomography [6], two-photon microscopy [7] and confocal laser scanning microscopy [8], have also shown great success in biomedical imaging [9–11]. Most biological tissues are strongly scattering for electromagnetic waves in the spectral range from ultraviolet to near-infrared. Within this range, the optical diffusion limit, *i.e.* optical mean free path, in most biological tissues is on the order of 1 mm. When beyond this depth, multiple light scattering would quickly randomize the trajectories of incident photons and prevent effective optical focusing in tissue [12–15]. Therefore, pure optical modalities either suffer from poor spatial resolution or have limited imaging depth [16].

In comparison to optical wave, acoustic wave is much less scattered in biological tissue. In soft biological tissues, the acoustic scattering coefficient is about three orders of magnitude less than the optical scattering coefficient [17]. Benefitted from the lower scattering, acoustic method can achieve much better spatial resolution in deep tissue, usually equivalent to 1/200 of the imaging depth [12]. Conventional acoustic imaging, such as B-mode ultrasonography, however, is purely relying on the detection of mechanical properties and structures of biological tissues without considering tissue chemical information (*i.e.* the contents and changes in chemical components). Therefore, B-mode ultrasonography is not sensitive to tissue physiology and function which are highly valuable for the diagnosis of many diseases.

PAI is a recently developed technique whereby non-ionizing, non-invasive laser pulses are used to generate ultrasonic signals from biological tissues followed by signal detection *via* ultrasonic transducers to form images. Benefited from the low scattering of ultrasonic signals, PAI is able to achieve high resolution in optically scattering biological tissues at unprecedented depths. Moreover, like other conventional optical imaging modalities, PAI can be used to differentiate tissues containing various chemical substances

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based on their different optical absorption spectra. The hybrid PAI technology, by combining the advantages of both light and sound, shows tremendous potential applications in medicine and biology, and has already been used in evaluating the anatomical, functional, metabolic, molecular and genetic information of biological samples [12–20].

In this mini-review article, we will first introduce the basic principles and two major implementations of PAI, photoacoustic tomography (PAT) and photoacoustic microscopy (PAM). The applications of PAI in detecting the chemical compositions and evaluating the microstructures in biological tissues will also be introduced.

2. Principles and implementations

When biological tissue is illuminated by laser light, the electrons transit from the ground state to an excited state as a result of photon absorption. The excitation is defined as thermally or stress confined if the laser pulse duration is much shorter than the thermal relaxation. The rapid deposition of laser energy causes an immediate increase in pressure within the radiated region. The release of this pressure transforms thermoelastic expansion to ultrasonic wave, named photoacoustic (PA) wave. The PA pressure $p(\mathbf{r}, t)$ at position $\mathbf{r}$ and time t can be described as [21,22]:

$$\nabla^2 p(\mathbf{r},t) - \frac{1}{c^2}\frac{\partial}{\partial t^2} p(\mathbf{r},t) = -p_0(\mathbf{r})\frac{\partial \tau(t)}{\partial t} \quad (1)$$

where c is the speed of sound, $\tau(t)$ is the waveform of the electromagnetic illumination, and $p_0(\mathbf{r})$ is the initial PA pressure excited by optical illumination. $p_0(\mathbf{r})$ is directly proportional to the Grüneisen parameter $\Gamma(\mathbf{r})$ and the optical absorption coefficient $A(\mathbf{r})$, *i.e.* $p_0(\mathbf{r}) = \Gamma(\mathbf{r})A(\mathbf{r})$. After PA signals are picked up by an ultrasound transducer(s), the information of the location and the density of the optical absorbers in tissue can be recovered by the following equation [23]:

$$p_0(\mathbf{r}) = \int_{\Omega_0}\left[2p(\mathbf{r}_0,\bar{t}) - 2\bar{t}\frac{\partial p(\mathbf{r}_0,\bar{t})}{\partial \bar{t}}\right]\frac{d\Omega_0}{\Omega_0}|_{\bar{t}=|\mathbf{r}-\mathbf{r}_0|}, \quad (2)$$

where $\bar{t} = ct$, Ω_0 is a solid angle of the whole surface S_0 with respect to the reconstruction point inside S_0, $\mathbf{r_0}$ is the measurement position, and $d\Omega_0$ is the solid angle for a detection element dS_0 with respect to a reconstruction point at $\mathbf{r}$.

Assuming $\Gamma(\mathbf{r})$ is homogenous, the spatially distributed optical absorption coefficient $A(\mathbf{r})$ in a 3-dimensional space or a 2-dimensional cross-section can be obtained. Because the optical absorption $A(\mathbf{r})$ of a biological tissue is closely related to its chemical composition, the spectroscopic form of PAI, *i.e.* imaging the target tissue with multiple optical wavelengths, allows quantitative measurements of the chemical and functional information in the tissue.

Based on the different methods for image acquisition as well as the differences in targeted spatial resolution and imaging depth, PAI can be divided into two large categories which are PAT and PAM. Fig. 1(a) [24] shows a typical PAT system where an unfocused laser beam covering a large area on the sample generates PA signals and an unfocused ultrasound transducer detects the PA signals. The tomographic images are generated from PA signals by using a reconstruction algorithm based on the inversely solving the photoacoustic wave equation. Several reconstruction algorithms, such as back projection [23], Radon transform [25], and Fourier-domain algorithms [26], have been developed for PAT. The spatial resolution and the penetration depth of a PAT system are scalable with the ultrasonic frequency. The lateral resolution of PAT can be estimated as $R_L = 0.71c/(NA_c \cdot f_0)$, where c is the speed of PA signal, f_0 is the center ultrasonic frequency, and NA_c is the numerical aperture of the transducer. The axial resolution of PAT can be estimated by $R_A = 0.88c/\Delta f$, where Δf is the bandwidth for photoacoustic signal detection and is proportional to f_0 [12]. The imaging depth is limited by not only the frequency-dependent acoustic attenuation but also the optical attenuation in the biological sample.

In contrast to PAT, Fig. 1(b) [27] shows a typical PAM system where a focused laser beam is used for signal generation and a spherically focused ultrasound transducer is used for signal detection. To generate an image, PAM scans along the sample surface point by point without involving tomographic image reconstruction. PAM is further divided into two categories including optical resolution PAM (OR-PAM), where the lateral resolution is determined by optical focus, and acoustic resolution PAM (AR-PAM), where the lateral resolution is determined by ultrasonic focus [12]. OR-PAM utilizing a strongly focused light beam for signal excitation results in a diffraction-limited optical resolution in the lateral direction which can be estimated by $0.51\lambda_{opt}/NA$, where NA is the numerical aperture of the optical lens, and λ_{opt} is the wavelength of the laser. PA signals could be picked up by an unfocused ultrasonic transducer, or a focused ultrasonic transducer [12] aligned confocally with the optical lens to maximize the detection sensitivity. The axial resolution of OR-PAM is still acoustically determined by the bandwidth of the ultrasonic transducer, Δf, following the equation of $0.88c/\Delta f$ [17]. The imaging depth of OR-PAM is determined by the optical mean free path which is usually within 1 mm for most biological tissues. Similar to OR-PAM or PAT, the axial resolution of AR-PAM is determined by the bandwidth of the ultrasonic transducer. Its lateral resolution, however, is determined by the acoustic focal diameter, following the equation of $0.71cl/(f_0 \cdot NA_c/2)$, where NA_c is the aperture of an ultrasound transducer and l is the focal length [17]. AR-PAM can provide spatial resolution on the order of micrometer and imaging depth of up to several millimeters [17]. Fig. 1(c) [28] shows a miniaturized PAM system for endoscopic imaging of internal organs. Compared to routinely used clinical

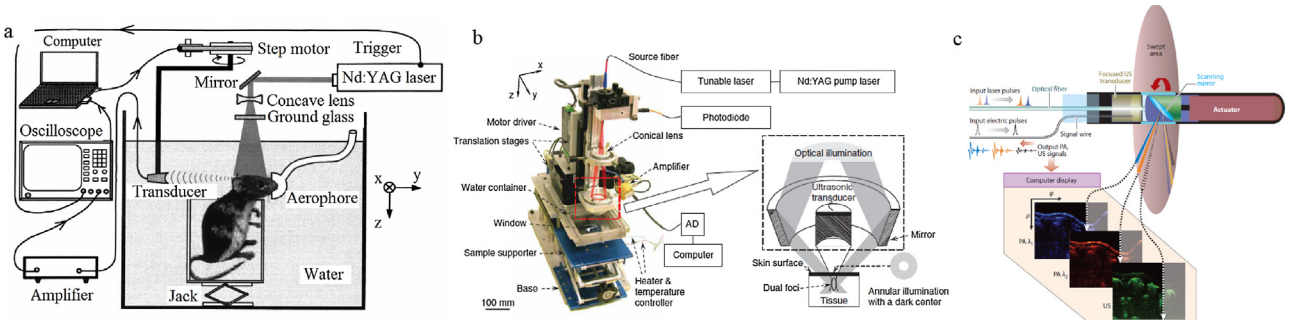

Fig. 1. Example implementations of PAI, including (a) PAT [24], (b) AR-PAM [27], and (c) PAE [28].