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Review article

# Bond-selective photoacoustic imaging by converting molecular vibration into acoustic waves

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ABSTRACT

The quantized vibration of chemical bonds provides a way of detecting specific molecules in a complex tissue environment. Unlike pure optical methods, for which imaging depth is limited to a few hundred micrometers by significant optical scattering, photoacoustic detection of vibrational absorption breaks through the optical diffusion limit by taking advantage of diffused photons and weak acoustic scattering. Key features of this method include both high scalability of imaging depth from a few millimeters to a few centimeters and chemical bond selectivity as a novel contrast mechanism for photoacoustic imaging. Its biomedical applications spans detection of white matter loss and regeneration, assessment of breast tumor margins, and diagnosis of vulnerable atherosclerotic plaques. This review provides an overview of the recent advances made in vibration-based photoacoustic imaging and various biomedical applications enabled by this new technology.


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## 1. Introduction

Molecular vibration is the basis of numerous microscopy approaches and enables the detection of specific molecules within cells and tissues. These approaches include Raman scattering, infrared absorption, and near-infrared (NIR) absorption, which have been widely used for chemical imaging in biomedicine [1–3]. Similarly, nonlinear vibrational methods, such as coherent anti-Stokes Raman scattering [4,5] and stimulated Raman scattering [6] microscopies, have enabled new discoveries in biology [7] on account of their high sensitivity and 3D spatial resolution. However, all these approaches have limited imaging depth on the order of a few hundred micrometers due to significant optical scattering in biological tissue. Thus, their potential applications at the organ level *in vivo* and in clinical settings are restricted.

A deep-tissue imaging modality able to maintain both high chemical selectivity and spatial resolution would certainly satisfy the functional requirements for many diagnostic applications in biomedicine. A promising approach is the development of photoacoustic (PA) imaging platforms, which combine optical excitation with acoustic detection. With this approach, the imaging depth is significantly improved, as acoustic scattering by biological tissue ($\sim 1.2 \times 10^{-3}\,mm^{-1}$ in human skin at 5 MHz) [8] is more than three orders of magnitude weaker than optical scattering ($\sim 10\,mm^{-1}$ in human skin at 700 nm) [9]. Unlike nonlinear optical microscopy that relies on tightly focused ballistic photons, the diffused photons contribute equally to the generation of PA signal and thus further enhance the penetration depth. Over the past decade, researchers have developed various PA imaging platforms, including photoacoustic microscopy (PAM) [10,11], photoacoustic tomography (PAT) [10,12,13], photoacoustic endoscopy (PAE) [14,15], and intravascular photoacoustic (IVPA) imaging [16]. Many excellent review articles provide comprehensive insight into different aspects of the imaging technology [17–19], applicable contrast agents [20–22], and a variety of biomedical applications [23–26]. In most of the aforementioned applications, the PA signal comes from the electronic absorption of endogenous tissue pigments, such as hemoglobin and melanin, or from exogenous contrast agents, such as nanoparticles and dyes.

Molecular vibrational transitions in biological tissue have recently been demonstrated as a novel contrast mechanism for PA imaging. It describes the periodic motion of atoms in a molecule with typical frequencies ranging from $10^{12}$ to $10^{14}$ Hz. The molecular population in the $i$th vibrationally excited state relative to the ground state follows the Boltzmann's distribution law as $N_i/N_0 = \exp(-\Delta E/kT)$, where $\Delta E$ is the energy gap, $T$ is the temperature, and $k$ is the Boltzmann constant. Thus, the Boltzmann distribution describes how the thermal energy is stored in molecules. When the incident photon energy matches the transition frequency between the ground state and a vibrationally excited state, the molecule absorbs the photon and jumps to the excited state. During subsequent relaxation of the excited molecule to the ground state, the thermal energy is converted into acoustic waves detectable by an ultrasound transducer.

The fundamental vibrational transitions in the mid-infrared wavelength region have been previously exploited for PA detection of glucose in tissues [27]. Nevertheless, this approach is limited in detecting molecules only tens of micrometers under the skin, where strong water absorption in the mid-infrared region predominates. Vibrational absorption with minimal water absorption can occur in two ways. One is through the stimulated Raman process and the other is through overtone transition. In stimulated Raman scattering, the energy difference between the visible or NIR pump and Stokes fields is transferred to the molecule to induce a fundamental vibrational transition. The concept of stimulated Raman-based PA imaging has been previously demonstrated [28,29]. However, because stimulated Raman scattering is a nonlinear optical process relying on ballistic photons under a tight focusing condition, this approach is not suitable for deep-tissue imaging. The overtone transition is based on the anharmonicity of chemical bond vibrations. Taking the C—H bond as an example, the first, second, and third overtone transitions occur at around 1.7 μm, 1.2 μm, and 920 nm, respectively, where water absorption is locally minimized. Since C—H bonds are one of the most abundant chemical bonds in biological molecules including lipids and proteins, photoacoustic detection of C—H bond overtone absorption offers an elegant platform for mapping the chemical content of tissue with penetration depths up to a few centimeters.

In the following sections, we introduce the mechanism for vibration-based PA signal generation. Then, applications of vibration-based PA imaging in forms of microscopy, tomography, and intravascular catheter will be reviewed, followed by a discussion of the improvements needed to overcome technical challenges that limit translation of these imaging modalities to the clinic.

## 2. Vibrational absorption as a photoacoustic contrast mechanism

### 2.1. Photoacoustic signal generation based on molecular overtone absorption

Vibration-based PA signals arise from the molecular overtone transitions and combinational band absorptions, which are allowed by anharmonicity of chemical bond vibration. According to the anharmonicity theory, the transition frequency for an overtone band has the following relation with the fundamental frequency, $\Omega_n = \Omega_0 n - \chi\Omega_0(n+n^2)$, where $\Omega_0$ is the transition frequency of fundamental vibration, $\chi$ is the anharmonicity constant, and $n = 2,3\ldots$ representing the first, second, and subsequent overtones. When the frequency of an incident pulsed laser matches the transition frequency of an overtone, the energy of the incident photons is absorbed and then induces a local rise in temperature. When both thermal and stress confinements are satisfied [30], the accumulated heat is subsequently released through a thermal-elastic expansion in tissue, which generates acoustic waves detectable by an ultrasound transducer. Fig. 1 depicts this process for PA signal generation based on first and second overtone transitions. The generated signal contains depth-resolved information of absorbers on which the image

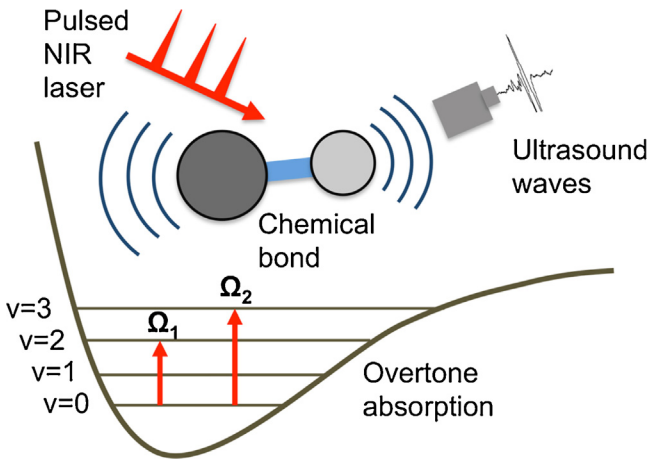


**Fig. 1.** Schematic of vibration-based PA signal generation and the 1st and 2nd overtone absorption of a molecule. $v$ denotes the vibrational energy level; NIR demotes near infrared.