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FIELD OF VISION

Recent advances in photoacoustic endoscopy

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Abstract

Imaging based on photoacoustic effect relies on illuminating with short light pulses absorbed by tissue absorbers, resulting in thermoelastic expansion, giving rise to ultrasonic waves. The ultrasonic waves are then detected by detectors placed around the sample. Photoacoustic endoscopy (PAE) is one of four major implementations of photoacoustic tomography that have been developed recently. The prototype PAE was based on scanning mirror system that deflected both the light and the ultrasound. A recently developed mini-probe was further miniaturized, and enabled simultaneous photoacoustic and ultrasound imaging. This PAE-endoscopic ultrasound (EUS) system can offer high-resolution vasculature information in the gastrointestinal (GI) tract and display differences between optical and mechanical contrast compared with single-mode EUS. However, PAE for endoscopic GI imaging is still at the preclinical stage. In this commentary, we describe the technological improvements in PAE for possible clinical application in endoscopic GI imaging. In addition, we discuss the technical details of the ultrasonic transducer incorporated into the photoacoustic endoscopic probe.

Key words: Photoacoustic techniques; Tomography; Endoscopy; Endosonography; Gastrointestinal neoplasm

Core tip: Photoacoustic imaging is an emerging modality, and provides image information of optical contrast or functional properties by detecting ultrasonic waves. The major advantage of photoacoustic imaging is the greater penetration depth, of millimeters to centimeters, in tissue. The aim of this article is to introduce the technological improvements in photoacoustic endoscopy (PAE) for possible clinical application in endoscopic gastrointestinal imaging. In addition, the technical details of an integrated PAE and endoscopic ultrasound imaging system are discussed.

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COMMENTARY ON HOT TOPICS

Photoacoustics is described as laser induced ultrasound[1]. Imaging based on photoacoustics uses short light pulses (nanosecond range) as the source. As pulsed light is absorbed by tissue absorbers, such as hemoglobin or melanin, a transient temperature increase is generated, resulting in local thermoelastic expansion, giving rise to ultrasonic waves[2]. These ultrasonic waves are then detected by ultrasonic detectors placed around the sample (Figure 1). An important advantage of photoacoustic imaging is that the method can overcome the high degree of scattering of optical photons in biological tissue, resulting in high spatial resolution deep within tis-

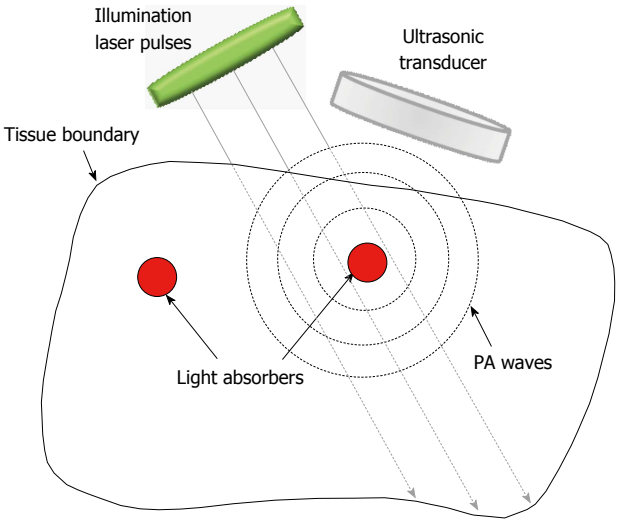

Figure 1 Illustration of the photoacoustic effect and photoacoustic imaging. Reproduced with permission from Yao *et al*[6]. PA: Photoacoustic.

sue[3]. Although photoacoustic spectroscopy and simple imaging was developed in the 1970s, only recently has photoacoustic imaging become important in biomedical research[4]. A major photoacoustic imaging for biomedical applications is photoacoustic tomography (PAT). PAT is similar to conventional ultrasound imaging, because image information is provided by capturing the ultrasonic waves using mechanical scanning or by detection arrays[2]. However, while conventional ultrasound imaging measures only mechanical contrasts, PAT detects optical and thermoelastic contrasts[5]. Currently, PAT has four major implementations: raster-scan based photoacoustic microscopy (PAM), inverse-reconstruction based photoacoustic computed tomography (PACT), rotation-scan based photoacoustic endoscopy (PAE), and hybrid PAT systems with other imaging methods (Figure 2)[6].

Recently, Yang *et al*[7] showed photoacoustic images of the rat gastrointestinal tract *ex vivo* using a novel photoacoustic endoscope with a miniaturized imaging probe, which integrated a light-guiding optical fiber, ultrasonic sensor, and mechanical scanning unit for circumferential sector scanning. More recently, the same group[8] developed an integrated PAE and endoscopic ultrasound (EUS) imaging system for simultaneous photoacoustic and ultrasonic imaging of internal organs *in vivo*. In this commentary, we describe the technological improvements in PAE for possible clinical application in endoscopic gastrointestinal (GI) imaging. We also discuss the technical details of the ultrasonic transducer incorporated into the photoacoustic endoscopic probe.

PAT

PAT is cross-sectional or three-dimensional imaging using photoacoustic effect, an emerging optical imaging modality that can offer volumetric images of biological tissues *in vivo* with high spatial resolution and deep tissue optical contrast[5]. PAT is similar to ultrasound imaging in that both use detected ultrasonic waves to produce images[8]. However, PAT uses optical absorption-based contrast of tissue. PAT can provide high spatial resolution because ultrasonic scattering coefficients in tissue are two to three orders of magnitude less than optical scattering coefficients[5]. Additionally, unlike ultrasonography or optical coherence tomography, PAT produces speckle-free images. As mentioned above, "PAT" includes PAM, PAE, PACT (Table 1). While PAM and PAE can image millimeters deep at microscopic resolution, PACT is available for microscopic and macroscopic imaging. In addition, PAT has been integrated into other imaging modalities, including ultrasound imaging[9], optical coherence tomography (OCT)[10], confocal microscopy[11], two-photon microscopy[6], and magnetic resonance imaging[12].

Single-wavelength photoacoustic measurements of hemoglobin, a prominent light absorber in tissue, can provide images of blood vessels without exogenous contrasts. Deeper-seated vascular structures can be detected using a red or near infrared wavelength shift[2]. In addition, the technique can evaluate oxygen saturation inside blood vessels because oxyhemoglobin and deoxyhemoglobin have significantly different optical absorption spectra[13]. Other endogenous optical absorbers, such as melanin and other tissue chromophores, can contribute to photoacoustic signals. Sound reflectors such as calcification are useful in images of some tumors, including leiomyomas, leiomyosarcomas, or mucinous adenocarcinomas[2].

Multispectral optoacoustic tomography (MSOT) with multiple illumination wavelengths can help differentiate extrinsic contrast agents (such as common fluorochromes, or photoabsorbing nanoparticles) from intrinsic contrasts (such as hemoglobin or melanin) by their unique spectral signatures[14]. This imaging modality can offer differentiation of physiological conditions with the combination of each image of different absorbers[2]. Using this method, Oh *et al*[15] reported three-dimensional images of subcutaneous melanomas and their surrounding vasculature in nude mice by dual-wavelength reflection-mode PAM, in which melanin distribution was imaged with a near-infrared light source and vascular system surrounding the melanoma with visible light. Extrinsically administered contrast agents for MSOT should have a sufficiently high optical absorption to be detected in tissues[3]. Such agents include near-infrared cyanine dyes, such as indocyanine green[16], reporter gene products[17], and light-absorbing nanoparticles, such as gold nanoparticles[18] and carbon nanotubes[19]. Several nanoparticles produce significantly stronger photoacoustic signals than organic dyes[2]. However, they also have limitations, including their larger size and safety concerns. MSOT can also detect activatable contrast agents, such as "smart probes" or molecular beacons, that are dark in their base state but produce fluorescence after target interaction[20]. MSOT can provide functional, genetic, and molecular imaging using these extrinsic contrast agents[5].

In recent years, PAT has been used in a number of preclinical applications, including imaging of angiogenesis, the microcirculation, drug responses, brain func-

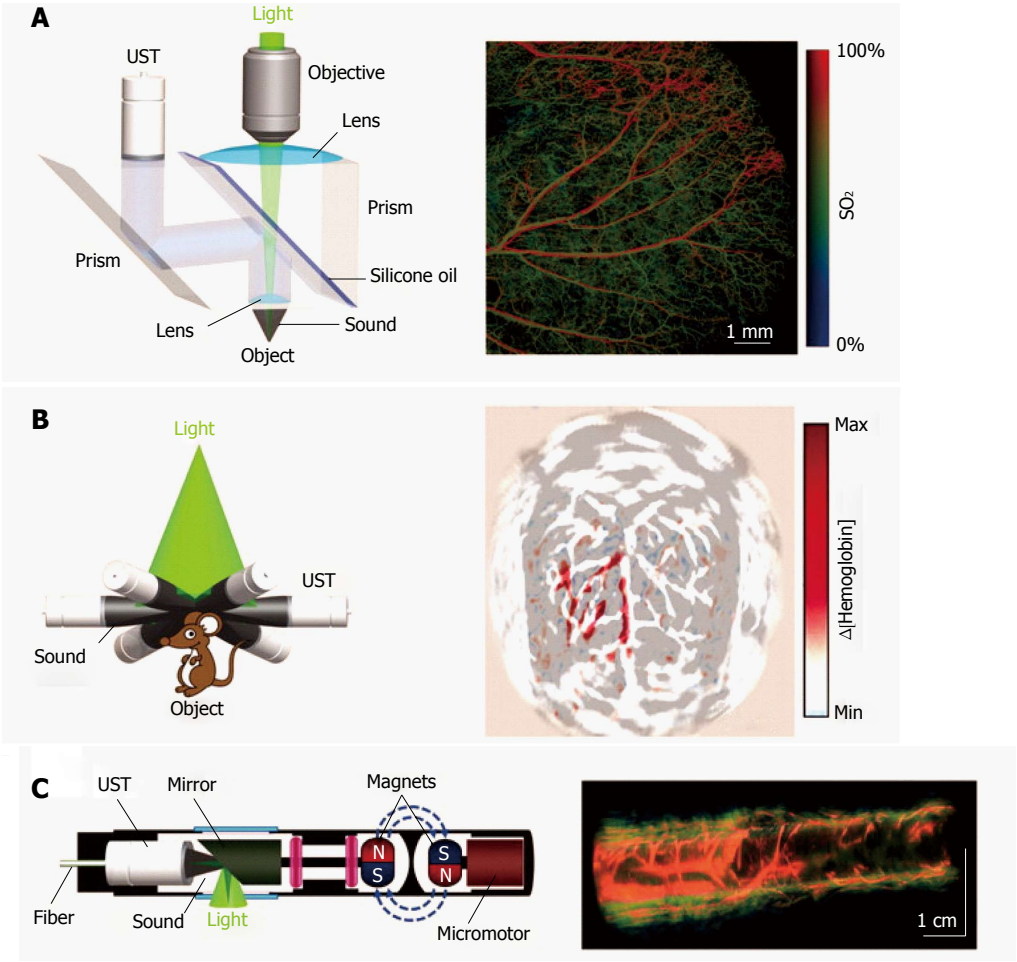

Figure 2 Three major implementations of photoacoustic tomography, with representative *in vivo* images. A: Optical-resolution photoacoustic microscopy and an image of hemoglobin oxygen saturation (SO_2) in a mouse ear; B: Circular-array photoacoustic computed tomography and an image of cerebral hemodynamic changes, Δ[hemoglobin], in response to one-sided whisker stimulation in a rat; C: Photoacoustic endoscopy and an image of a rabbit esophagus and adjacent internal organs, including the trachea and lung. Reproduced with permission from Wang *et al*[5]. SO_2: Oxygen saturation; UST: Ultrasonic transducer.

tion, tumor microenvironments, biomarkers, and gene expression[5]. PAT is also in the early stages of clinical application including breast cancer diagnosis[21], melanoma imaging[22], prostate cancer treatment[23], and non-invasive sentinel lymph node imaging[24]. Further developments in photoacoustic imaging techniques may provide better diagnosis of diseases and patient-management strategies.

PAE

Conventional white light endoscopic imaging of GI tract allows direct visualization of morphological changes and lesions, and subsequent histological analysis of tissue is the gold standard for final diagnosis. However, this method is limited by human vision and the lack of sensitivity to subsurface activity[2]. Recent advances in optics and digital imaging techniques have been introduced in GI endoscopy. Several methods, including narrow-band imaging, autofluorescence imaging, confocal endomicroscopy, OCT, and two-photon microscopy, have been developed and are under investigation. Some of these methods have been used in clinical practice; however, their diagnostic accuracy and efficacy need to be confirmed in large-scale clinical trials. Additionally, these imaging methods cannot achieve greater penetration depth[25]. EUS-based imaging can penetrate for several millimeters to centimeters in tissue. However, its limitations include poor contrast and difficult interpretation of data[2]. In addition, the mechanical contrast in EUS images often does not provide the required sensitivity and specificity[26].

PAE may be useful as a new, minimally invasive diagnostic imaging tool because it provides functional optical contrast with high spatial resolution and maintains the benefits of traditional ultrasound endoscopy[7]. Although the penetration depth of PAT can provide images that are centimeters deep, internal organs, such as the gastrointestinal tract and cardiovascular system, are not reachable[6]. The photoacoustic probe must be positioned close to the area of interest by means of endoscopy in hollow organs[7]. Viator *et al*[1] first developed a photoacoustic endoscopic probe for 1D sensing. Sethuraman *et al*[27] demonstrated photoacoustic images of rabbit blood vessels *ex vivo* using a high-frequency intravascular ultrasound imaging catheter. However, the system was not truly endoscopic because it used external illumination.

PAE has been investigated intensively as a tool of GI tract imaging. A prototype PAE system with a miniatur-

Table 1 Overview of currently available photoacoustic imaging technologies

Technology	Full name	Brief physics	Current applications	Future applications	Additional value to standard endoscopy
PAT	Photoacoustic tomography	Optical excitation of light absorbers in tissues by a pulsed laser and ultrasonic detection using mechanical scanning or detector arrays	Three major implementations include PAM, PACT, PAE	Functional information with the aid of an exogenous contrast	-
MSOT	Multispectral optoacoustic tomography	Utilization of multiple illumination wavelengths, spectral separation of optical reporter of interest from background absorption	Functional imaging of blood vessels, melanoma imaging of primary tumors and metastasis, characterization of atherosclerotic plaques, *etc.*	Tissue anatomy, function, molecular biomarkers, and gene expression	-
PAM	Photoacoustic microscopy	Based on a scanning focused ultrasonic transducer	Anatomical images of cutaneous microvasculature	Noninvasive imaging of individual cell nuclei	-
PACT	Photoacoustic computed tomography	Based on an array of unfocused ultrasonic transducers, use of an inverse algorhithm to reconstruct a tomographic image	Tumor boundaries and connections with surrounding blood and lymphatic vessels	Same as PAT	-
PAE	Photoacoustic endoscopy	Probe that combines light delivery, acoustic sensing, and mechanical scanning in one small unit placed at the distal end of the endoscope	Gastrointestinal tract imaging	Improve the accuracy of cancer staging	Optical absorption-based contrast with high spatial resolution at depths
PAE-EUS	Photoacoustic endoscopy and Endoscopic ultrasound	Integrated system for ultrasonic images produced with conventional pulse-echo imaging and photoacoustic images formed through detection of acoustic waves	Gastrointestinal tract and lymphovascular imaging	Early-stage tumor detection or *in situ* characterization of diseased tissues	Angiographic and spectral imaging function would enhance EUS's role

PAT: Photoacoustic tomography; MSOT: Multispectral optoacoustic tomography; PAM: Photoacoustic microscopy; PACT: Photoacoustic computed tomography; PAE: Photoacoustic endoscopy; EUS: Endoscopic ultrasound.

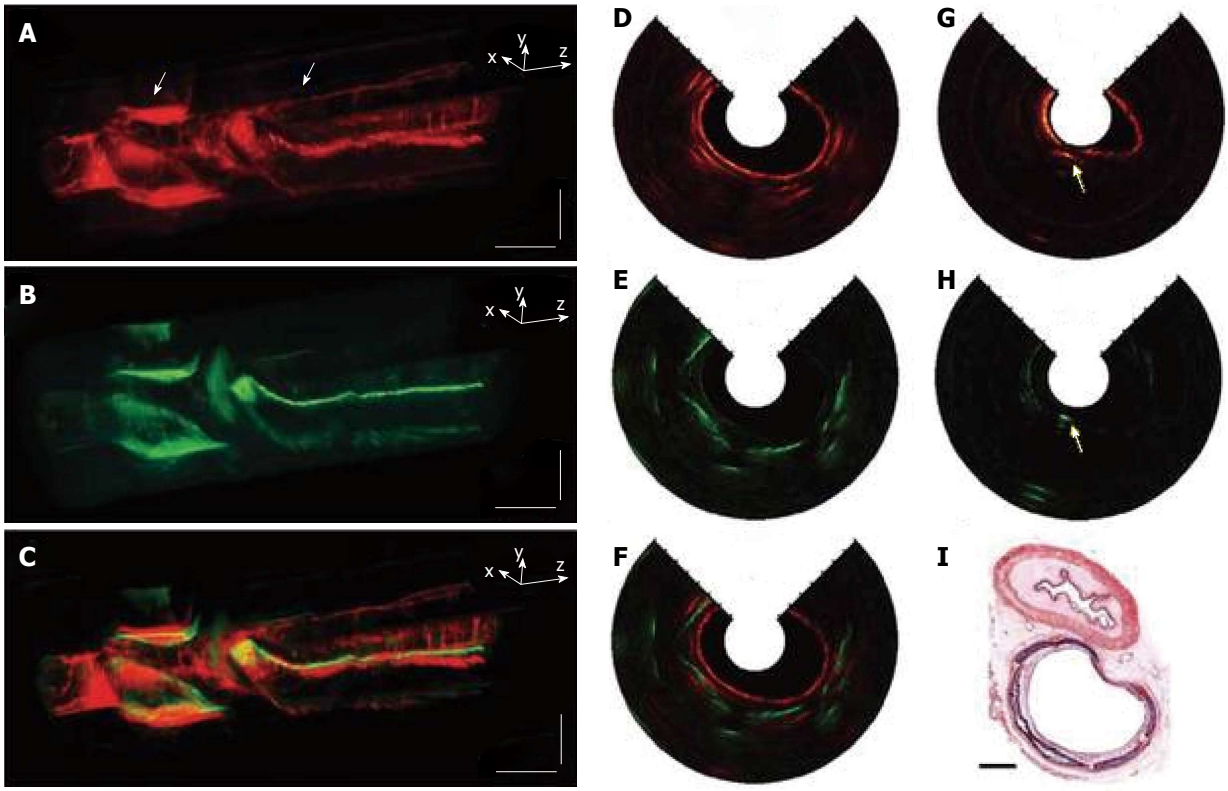

Figure 3 Simultaneous, co-registered, photoacoustic endoscopy and endoscopic ultrasound images of rabbit esophagus. A: Three-dimensionally rendered photoacoustic structural image. The left- and right-hand sides of this image correspond to the lower and upper esohagus, respectively, and the lower portion (-y axis) to the ventral side of the rabbit; B: Co-registered ultrasonic structural image for the same volume of A; C: An overlaid images of A and B. In A-C, horizontal and vertical scale bars represent 2 cm and 5 mm, respectively; D: A representative photoacoustic x-y cross-sectional image (18 mm in diameter) near the lung, as indicated by the left arow in A; E: Corresponding ultrasonic cross-sectional image of D; F: A combine image of D and E; G: A photoacoustic x-y cross-sectional image near the trachea, as indicated by the right arow in A; H: Corresponding ultrasonic cross-sectional image of G. In G and H, the dotted arrows indicate the contact point between the trachea and the esophagus; I: Histology of the esophagus (top) and the trachea (bottom) (HE stain). Scale bar, 1 mm. Reproduced with permission from Yang *et al*[8].

ized imaging probe integrates a light-guiding optical fiber, an ultrasonic sensor, and a mechanical scanning unit into one small unit placed at the distal end of the endoscope[7]. This probe used a scanning mirror system instead of conventional flexible shaft-based mechanical scanning, enabling circumferential sector scanning without moving other illumination optics or the ultrasonic detector. The large intestinal tract of a rat was imaged *ex vivo* with this probe. However, probe diameter was 4.2 mm due to the larger transducer size. One recently developed probe is 3.8 mm in diameter and approximate 38 mm in length, enabling simultaneous photoacoustic and ultrasound imaging using a single device[8]. In this endoscopic system, a focused ultrasonic transducer detects one-dimensional, depth-resolved signals (or the A-line). Additionally, cross-sectional images (or B-scan) can be achieved by constant rotation of a scanning mirror that directs both optical and acoustic waves. This system records and shows a set of dual wavelength photoacoustic to differentiate oxy- and deoxyhemoglobin, two of the dominant absorbers of visible light in soft biological tissues, and ultrasonic B-scan images in real time. It provides anatomical information about a rabbit esophagus and organs surrounding the esophagus, covering an approximately 14-cm long and 18-mm diameter volume (Figure 3). Volume rendering enabled three-dimensional visualization of the morphology and configuration of tissues and proximal organs surrounding the esophagus. Also, simultaneous, co-registered PAE-EUS colonoscopic pseudo-color images of the rat colon *in vivo*, and images of the lymphovascular system near the rat colon, could be achieved using the same scanning parameters as imaging of the esophagus. Thus, PAE-EUS system can provide high-resolution information on the GI tract vasculature and display differences between optical and mechanical contrast compared with single-mode EUS. However, the probe was too large to fit in the working channel (usually approximate 2.8- or 3.7-mm diameter) of a standard endoscope. More recently, a newer generation probe was further miniaturized, with probe diameter of 2.5 mm and a approximate 35 mm rigid length[28]. This mini-probe may be inserted into the working channel of a standard endoscope and be used with endoscopic guidance.

In conclusion, PAE is an emerging modality, and provides image information of optical contrast or functional properties by detecting ultrasonic waves. The major advantage of PAE is the greater pentration depth, of millimeters to centimeters, in tissue. It has great potential for *in vivo* endoscopic applications, such as early-stage tumor detection, accurate diagnosis of submucosal lesions, and *in situ* characterization of diseased tissues. Targeted contrast agents may improve the capabilities of endoscopic imaging, resulting in the earlier and more accurate detection of malignant and premalignant lesions, and further extend PAE to molecular imaging. Several technical challenges regarding the use of PAE in biomedical applications must be overcome. High-repetition lasers with fast wavelength tuning at each scan position are required for high-speed multicontrast PAE. Additionally, further miniaturization of the PAE probe is essential so that it can be inserted into the working channel of a standard endoscope. Although PAE for GI endoscopic imaging is at the preclinical stage, it would become an important imaging modality with further technological improvements.

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