

## 生物医学影像中的光声成像技术

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**摘要** 光声成像结合了光学成像的高对比度和超声成像的深穿透性优势,能够利用内源性、外源性造影剂对比显示组织的结构、功能、代谢特征和分子、动力学信息等,同时可以实现从细胞器、细胞、组织到器官的多尺度成像,在生物医学研究中发挥着越来越重要的作用。简要回顾了光声成像的基本原理,重点总结了光声计算断层成像(PACT)、光声显微成像(PAM)、光声内窥成像(PAE)和光声分子成像近年来的研究热点及技术进展,主要涉及成像探测方式的选择与改进、低成本激发光源的替代方案、图像重建算法的进步、系统成像速度和分辨率的提高以及分子探针的新兴设计策略等,最后展望了光声成像的应用前景。

**关键词** 生物光学; 成像系统; 光声成像; 光声计算断层成像; 光声显微成像; 光声内窥成像; 光声分子成像

中图分类号 O436

文献标志码 A

DOI: 10.3788/CJL202249.2007208

## 1 引言

生物医学影像是生命科学研究的重要手段,已经有许多种成像方法帮助我们了解生物体内的各种微观/宏观现象,如核磁共振成像(MRI)、X射线计算断层成像(CT)、正电子发射断层成像(PET)、超声成像(US)、光学相干断层成像(OCT)、荧光成像(FI)等。但MRI成本高且安全限制多,不适合携带心脏起搏器患者、幽闭恐惧症患者等;CT会产生电离辐射,不适用于孕妇、儿童等;PET在生命体代谢和肿瘤等研究领域有大量应用,但其需要放射性元素标志并且成本高、难以大规模推广;US的特异性和空间分辨率差,对于疾病早期的发展缺乏精细的生物结构成像能力;光学成像技术由于受到光学扩散极限的限制,一般成像深度在1 mm以内。光声(PA)成像作为一种无损医学影像方法,结合了光学成像的高对比度和超声成像的深穿透性优势,其关键原理是光声效应,即将吸收的光能转换为声能的现象。光声效应于1880年由Bell首次报道,并早在20世纪70年代即开始应用于材料检测领域<sup>[1]</sup>。而在20世纪90年代,随着激光器、超声检测技术、数据采集系统和计算能力的进一步发展,光声技术开始应用于生物医学领域<sup>[2]</sup>。目前光声成像已经在生物医学影像中发挥了重要作用。

PA成像具备诸多优点。(1)无损成像:非电离激发过程,实现无损成像。(2)高对比度:PA成像的对比度机制来源于检测目标的光吸收差异,继承了光学

成像的高对比度优势。(3)成像深度:声波在生物组织中的衰减比光的衰减小两到三个数量级,可以提供比光学成像更深的成像深度。(4)无标记成像:生物体中有很多天然的生色团,如血红蛋白、黑色素、脂质、DNA/RNA、胶原蛋白等,可以作为内源性造影剂提供结构、功能信息。(5)分子成像:通过引入外源性造影剂(PA探针),可以特异性识别目标区域,实现高灵敏的特异性分子成像。(6)兼容多模态:PA成像可以和其他成像模式互补,同时提供更多的生物信息。(7)多尺度:由于光激发和声检测的方法可以实现高度扩展,因此PA成像可以灵活地实现从亚细胞到人体器官水平的多尺度成像。

近年来PA成像在技术方法和应用领域方面都取得了巨大进步,但还存在一系列的临床应用问题。针对不同的光声技术与应用,已经有较多文章发表。在这里,我们简要回顾近几年PA成像技术的发展和待解决的问题,并展望了未来应用前景。

## 2 光声成像基本原理和主要模式

当脉冲激光照射到目标组织时,组织中的生色团吸收脉冲光能量并发生非辐射跃迁,组织瞬间升温产生热弹性膨胀,进而以超声波(即PA信号)的形式向外传播,超声换能器(UT)获取PA信号后再经过数据处理,重建组织内的初始声压分布,进而反演出光吸收系数、热膨胀特性参数等的空间分布,从而获得组织的结构、功能等信息,如图1(a)所示<sup>[3]</sup>。初始声压的计

收稿日期: 2022-06-08; 修回日期: 2022-07-08; 录用日期: 2022-07-19

基金项目: 国家自然科学基金面上项目(12174125)、广东省自然科学基金面上项目(2021A1515011874)

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算公式为

$$p_0 = \Gamma \eta_{th} \mu_a F, \quad (1)$$

式中:  $p_0$  为初始压强;  $\Gamma$  为 Grueneisen 参数;  $\eta_{th}$  为光热转换效率,  $\mu_a$  为光吸收系数;  $F$  为光通量。

根据图像重建方式, PA 成像技术主要分为两大类<sup>[4]</sup>: 光声计算断层成像 (PACT) 和光声显微成像 (PAM)。PACT 可以实现厘米级别的穿透深度, 快速

大范围成像的优势使其在小动物成像、乳腺成像、脑功能成像等领域得到了广泛应用。PAM 适用于毫米深度的高分辨率成像, 在亚细胞、细胞等微观组织结构研究具有重要意义。光声内窥成像 (PAE) 则是在 PACT/PAM 的基础上开发出来的一种内窥成像技术, 适用于体内消化系统和血管内成像等。PACT、PAM 和 PAE 分别如图 1(b)~图 1(d) 所示。

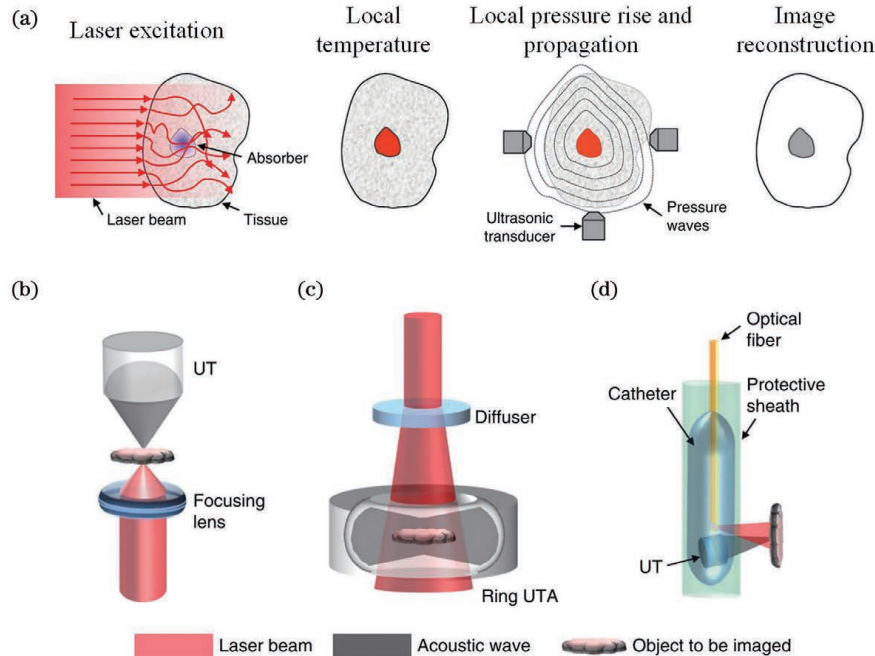


图 1 PA 成像原理和主要模式<sup>[3]</sup>。(a) PA 成像原理示意图; (b) PAM 示意图; (c) PACT 示意图; (d) PAE 示意图

Fig. 1 Principle and main modes of PA imaging<sup>[3]</sup>. (a) Principle of PA imaging; (b) schematic diagram of PAM; (c) schematic diagram of PACT; (d) schematic diagram of PAE

### 3 光声计算断层成像主要技术进展

PACT 采用均匀大光斑照射目标物体, 产生的光声信号被阵列式超声换能器获取后, 利用重建算法实现大范围成像。PACT 作为发展最早的 PA 成像技术之一, 经过十几年的发展, 从基础到应用研究的各个方面都取得了巨大的进步<sup>[3-4]</sup>。目前的 PACT 系统在成像速度和成像深度方面都有了较大改善, 但在临床转化过程中, 与超声成像等传统成像手段相比, 仍处于发展阶段, 经济性和便携性是两个主要的限制因素。PACT 的成本主要花费在阵列式超声换能器、激发光源和数据采集系统上, 显然大多数的 PACT 系统在这三个方面的花费仍是相当昂贵的。另外在临床应用中往往需要设备是紧凑甚至是便携的, 目前常用的激光器和阵列式换能器的尺寸较大。若在成本和尺寸上妥协, 又会影响成像质量。这些问题阻碍了 PACT 在临床转化阶段的进一步发展。近年来相关领域的研究者提出了众多方案以攻克上述难题, 主要涉及 3 个方面: (1) 超声换能器的选择; (2) 低成本激发光源的替代方案; (3) 图像重建算法的进步。

#### 3.1 超声换能器的选择

PA 成像产生的声波强度大约为  $800 \text{ Pa/mK}$ <sup>[5]</sup>, 需要考虑超声检测方式对成像质量的影响。目前 PACT 系统中主要采用阵列式超声换能器检测光声信号, 根据超声换能器形状可将其分为平面、圆柱形和球形阵列式超声换能器。超声成像中使用的超声换能器是一种平面阵列式超声换能器, 可以与光源集成, 成本低且便于搭建, 已广泛用于 PACT 中<sup>[6-7]</sup>, 如图 2(a) 所示。圆柱形和球形阵列式超声换能器需要定制, 成本高且体积庞大, 但可实现特定的成像要求<sup>[8]</sup>, 如小动物成像<sup>[9]</sup>、乳房成像<sup>[10-11]</sup>、人体四肢成像和脑结构功能成像<sup>[12]</sup>等, 如图 2(b) 和图 2(c) 所示。此外超声换能器的灵敏度、主频、带宽、尺寸等都会影响成像质量<sup>[13]</sup>: 当超声换能器的频谱与光声信号频谱匹配时, 会极大地改善成像质量; 更高的主频意味着更高的成像分辨率, 但需要考虑高频声衰减造成的成像深度问题; 带宽更宽, 就能更多地提取目标的组织结构信息, 但目前市场上的换能器带宽存在诸多技术限制。因此, 需要根据实际要求综合考虑, 选择合适的超声换能器以及检测方式, 以实现高效的 PA 成像。

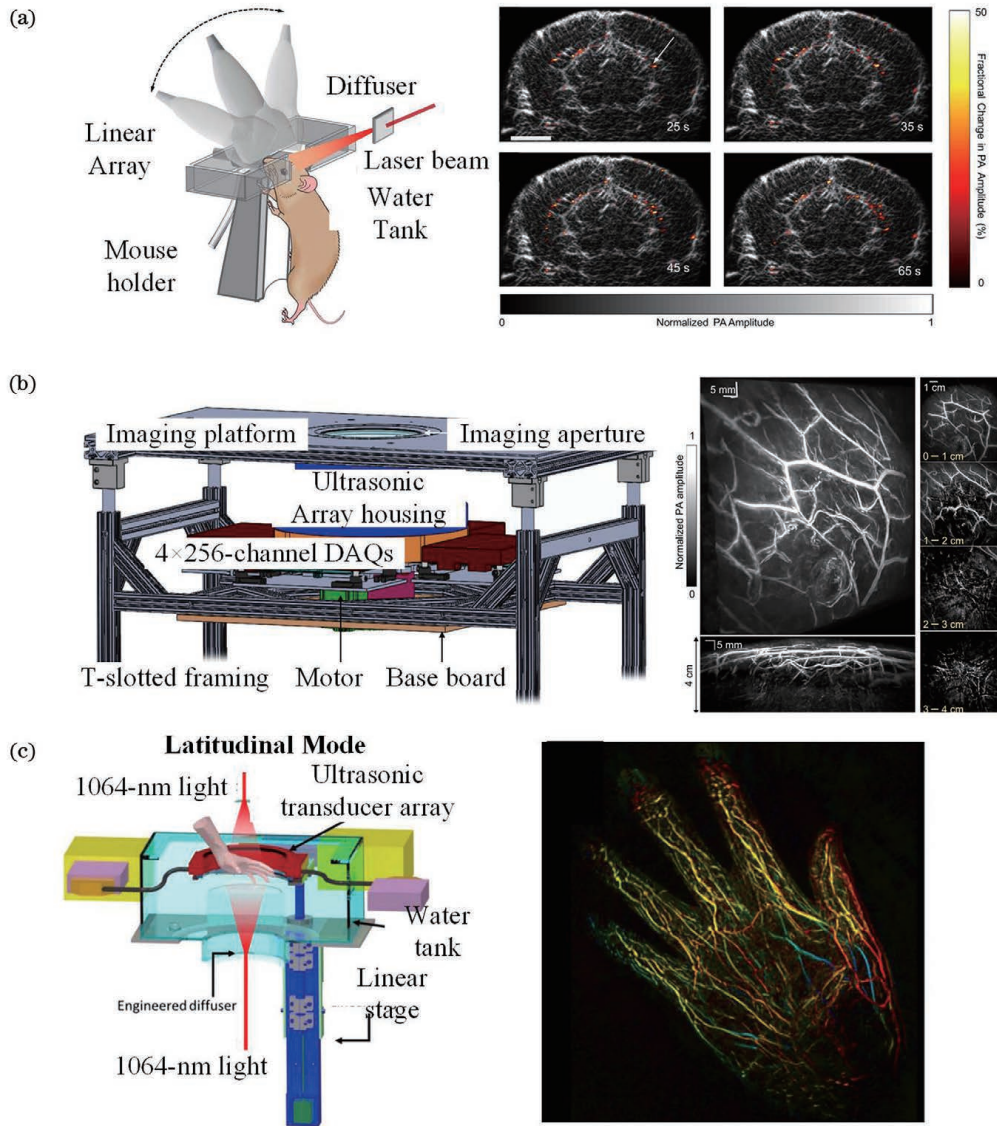


图 2 用于 PACT 的不同阵列式超声换能器实施例。(a) 基于平面阵列式超声换能器的 PACT 系统,用于小鼠脑 PA 成像<sup>[6]</sup>; (b) 基于圆柱形阵列式超声换能器的 PACT 系统,用于乳房 PA 成像<sup>[11]</sup>; (c) 基于球形阵列式超声换能器的 PACT 系统,用于手部 PA 成像<sup>[12]</sup>

Fig. 2 Embodiments of different arrayed ultrasound transducers for PACT. (a) PACT system based on planar array ultrasound transducer for PA imaging of mouse brain<sup>[6]</sup>; (b) PACT system based on cylindrical array ultrasound transducer for PA imaging of breast<sup>[11]</sup>; (c) PACT system based on spherical array ultrasonic transducer for PA imaging of hand<sup>[12]</sup>

### 3.2 低成本激光光源的替代方案

目前 PACT 系统常用的激光光源有 Nd:YAG 脉冲激光器和光学参量振荡器(OPO)等,但均体积较大且成本高昂,限制了更广泛的应用。PACT 系统对光源的光束发散度和光束质量要求可适当降低,低成本的闪光灯<sup>[14]</sup>、激光二极管<sup>[15]</sup>、发光二极管(LED)<sup>[16-17]</sup>与光纤激光器<sup>[18]</sup>等引起了人们的注意,其中发光二极管和光纤激光器的替代方案是主要的研究方向。发光二极管带宽较宽,空间相干性低,传统意义上不符合激光光源的条件,但在对带宽要求不高的 PA 成像应用中,仍可作为 PACT 的激光光源。Hariri 等<sup>[16]</sup>在 2018 年设计了一种经济便携的基于 LED 的 PA 成像系统,将两个 LED 阵列分别固定在医用超声

换能器两侧,可提供 0.55~0.59 mm 的横向分辨率,并验证了具备在 2 cm 深度检测视网膜血管的能力,如图 3 所示。光纤激光器采用光纤作为增益介质,在提供高质量光源的同时,还具有较高的可靠性和紧凑的结构设计,适于 PA 成像尤其是 PAE 的发展。除激发光源外,超声检测设备和数据采集系统也至关重要,可以进一步降低成本。关于降低 PACT 成本方案的更详细讨论可以参考文献<sup>[19]</sup>。当然,用低成本的光源作为替代方案,还需要具体考虑实际要求,包括激光能量、光束质量、光谱分辨率以及系统的时空分辨率、信噪比等。

### 3.3 图像重建算法的进步

除了硬件方面的支持外,图像重建也是 PA 成像

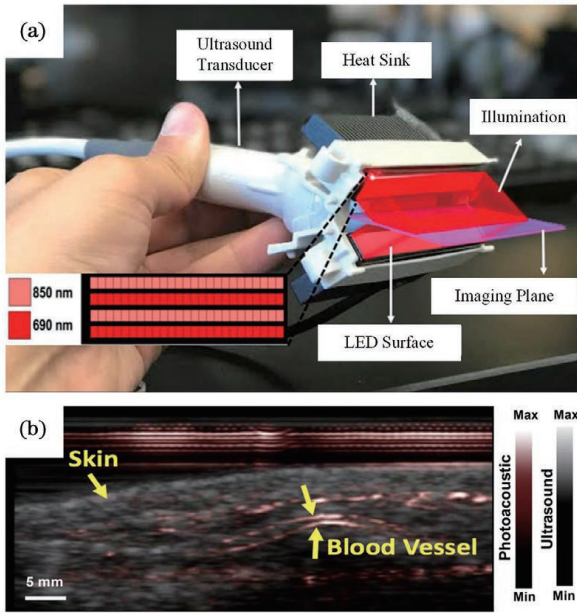


图 3 基于 LED 的光声计算断层成像实施例<sup>[16]</sup>。(a)具有成像平面和照明源的探头示意图;(b)皮肤和脉管系统的 PA/US 成像

Fig. 3 Embodiment of PACT based on LED<sup>[16]</sup>. (a) Schematic of probe with imaging plane and illumination source; (b) PA and US imaging of skin and vasculature

十分重要的过程。在有限的硬件条件支持下,若要获得更高的图像质量,需要借助先进的图像重建算法。传统的重建算法包括滤波反投影<sup>[20-21]</sup>、时域和频域重建<sup>[22-24]</sup>等。但上述算法采取了较多近似条件且通常要求全角度扫描以及连续采样。而在实际应用中,受到应用场景和超声换能器形状的限制,存在角度有限和欠采样的问题<sup>[25]</sup>。同步迭代法<sup>[26]</sup>和压缩感知<sup>[27-28]</sup>等重建方法可以在一定程度上解决上述问题,但也增加了计算成本。

尽管传统方案已经取得很多进展,但是一些长期挑战依然存在,包括有限角度扫描、图像伪影、噪声、稀疏采样、较慢的成像速度等。近年来,深度学习尤其是神经网络在医学图像重建领域快速发展,已被证明有希望解决上述困难<sup>[29-30]</sup>。深度学习是一种基于数据的自动重建方法,不仅可以提高重建图像质量,还能够降低对信号采集设备的要求,促进 PA 成像临床转化,已成为 PA 成像中图像处理的热门方法之一<sup>[31-35]</sup>。Lan 等<sup>[34]</sup>在 2020 年报道了一种 Y-NET 结构,如图 4(a)所示,同时将原始 PA 数据和常规算法得到的图像作为两个输入,分别对应编码器 I 和编码器 II,并共用一个解码器输出结果。图 4(b)所展示的鸡胸肉仿体实验体现了 Y-NET 结构在处理伪影等问题上的优势,填补了直接处理和后处理方法之间的空白。

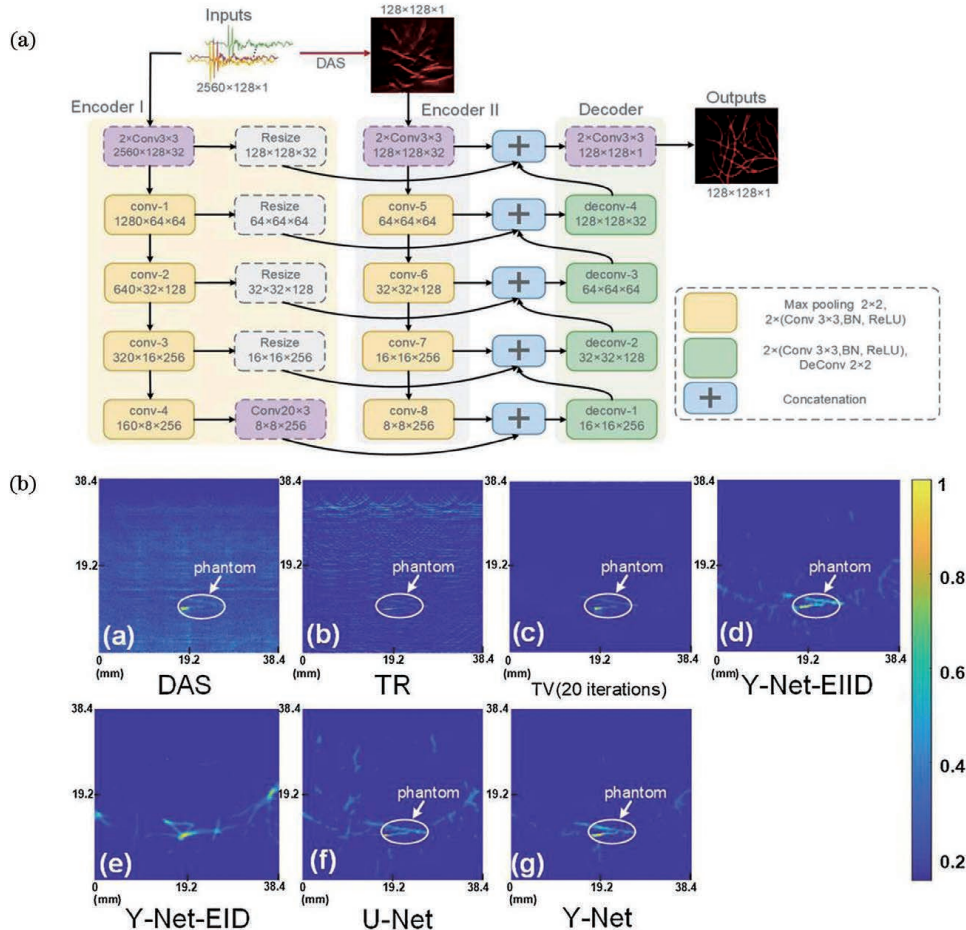


图 4 PA 成像深度学习的实施例<sup>[34]</sup>。(a)Y-NET 结构;(b)体外鸡胸肉模型实验结果

Fig. 4 Embodiment of deep learning for PA imaging<sup>[34]</sup>. (a) Architecture of Y-NET; (b) *in-vitro* results of chicken breast phantom

## 4 光声显微成像主要技术进展

与 PACT 大光斑激发的成像模式不同, PAM 采用光/声聚焦技术以获得高空间分辨率 PA 成像, 根据光/声聚焦方式的不同可分为光学分辨光声显微镜 (OR-PAM) 和声学分辨光声显微镜 (AR-PAM)<sup>[4]</sup>。得益于在分辨率和对比度方面的技术进展, PAM 在基础研究方面的能力已经被广泛认可<sup>[36-38]</sup>, 但仍需要克服一些关键挑战: (1) PAM 在体积成像应用中存在散焦问题, 严重影响了非焦点区的成像质量; (2) 临床对成像的一个必然要求是快速成像, 而传统 PAM 的机械扫描方式限制了其扫描速度, 阻碍了临床应用的进一步转化; (3) 传统的 PA 成像均需要耦合介质传递超声信号, 不适于烧伤组织成像等应用场景, 需要开发非接触式 PAM 系统。近年来针对上述挑战所报道的相关技术突破主要涉及 4 个方面: (1) 横向分辨率的提高; (2) 扩展景深技术; (3) 成像速度的提高; (4) 光学探测光声信号的方式。

### 4.1 横向分辨率的提高

OR-PAM 的横向分辨率 ( $R_{L-OR-PAM}$ ) 取决于聚焦光斑大小, 与光波长  $\lambda_0$  和物镜的数值孔径  $A_{NA-O}$  相关<sup>[38]</sup>:

$$R_{L-OR-PAM} = 0.51 \frac{\lambda_0}{A_{NA-O}} \quad (2)$$

AR-PAM 的横向分辨率 ( $R_{L-AR-PAM}$ ) 则取决于组织中的声速  $v$ 、超声换能器的数值孔径  $A_{NA-a}$  和探测的光声

信号的中心频率  $f_c$ <sup>[38]</sup>:

$$R_{L-AR-PAM} = 0.71 \frac{\lambda_a}{A_{NA-a}} = 0.71 \frac{v}{A_{NA-a} \times f_c} \quad (3)$$

二者的轴向分辨率 ( $R_{A-OR(AR)-PAM}$ ) 都与超声换能器的带宽  $\Delta f_c$  相关<sup>[38]</sup>:

$$R_{A-OR(AR)-PAM} = 0.88 \frac{v}{\Delta f_c} \quad (4)$$

因此, 使用高数值孔径的物镜是提升系统横向分辨率的简便方案<sup>[39-40]</sup>, 但该方案不仅受到光学衍射极限的限制, 还会牺牲成像深度。目前非线性效应是提高 PAM 空间分辨率的主要方案之一<sup>[41-44]</sup>。汪立宏课题组的 Wang 等<sup>[41]</sup> 在 2014 年提出了基于 Grueneisen 弛豫效应的热弛豫光声显微镜 (GR-PAM), Grueneisen 参数  $\Gamma$  具有温度依赖性, 第一束激发光对吸收体进行热标记, 初始声压为  $p_{01}$ :

$$p_{01} = \Gamma_0 \eta_{th} \mu_a F, \quad (5)$$

式中:  $\Gamma_0$  是初始温度时的 Grueneisen 参数。

经过第一发脉冲加热后组织温升, 导致 Grueneisen 参数增加, 因此在热弛豫时间内的第二束激发光将会产生更强的声压  $p_{02}$ :

$$p_{02} = (\Gamma_0 + b \eta_{th} \mu_a F) \eta_{th} \mu_a F, \quad (6)$$

式中:  $b$  是反映 Grueneisen 参数与温度相关性的系数。

通过检测两个光声信号幅值的差异, 将 OR-PAM 的横向分辨率从  $0.65 \mu m$  提高到  $0.41 \mu m$ , 同时将轴向分辨率从  $45 \mu m$  提高到  $2.3 \mu m$ , 如图 5(a) 所示。随后在 2019 年, 汪立宏课题组又利用该原理, 设计了

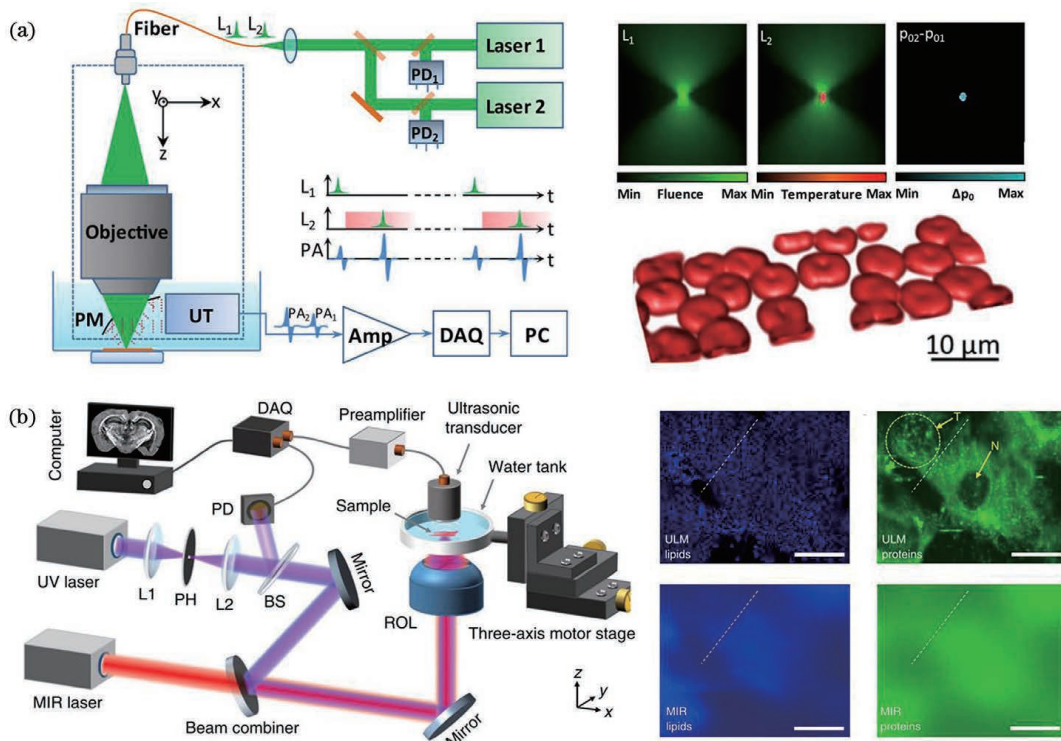


图 5 高/超分辨 PAM 实施例。(a) GR-PAM 系统示意图及红细胞的 GR-PAM 成像<sup>[41]</sup>; (b) ULM-PAM 系统示意图及脂质和蛋白质的 ULM-PAM 成像<sup>[42]</sup>

Fig. 5 Embodiments of high/super-resolution PAM. (a) Schematic diagram of GR-PAM and GR-PAM imaging of red blood cells<sup>[41]</sup>; (b) schematic diagram of ULM-PAM and ULM-PAM imaging of lipids and proteins<sup>[42]</sup>

紫外标定的中红外光声显微镜(ULM-PAM),采用中红外(MIR)脉冲光激发和紫外(UV)脉冲光探测的方法,在紫外分辨率的前提下,实现了对脂质、蛋白质等的高分辨率中红外光声成像<sup>[42]</sup>,如图 5(b)所示。

#### 4.2 扩展景深技术

虽然 PAM 可以提供高分辨率,但在非光学/声学焦点区域,分辨率将会显著降低。在保证横向分辨率基本不变的前提下,研究者们开发了一系列提高离焦区域横向分辨率的方案,如合成孔径聚焦技术(SAFT)<sup>[45-46]</sup>、贝塞尔光束照明<sup>[47]</sup>、变焦镜头<sup>[48]</sup>等。此外,还可以通过 AR/OR-PAM 相结合的多尺度成像方式,平衡分辨率和成像深度<sup>[49]</sup>。AR-PAM 中的散焦问题是由超声换能器的高数值孔径导致

的,SAFT 可以通过合成相关信号改善离焦区域的图像质量。Jeon 等<sup>[45]</sup>在 2019 年提出了一种用于 AR-PAM 的二维(2D)SAFT,他们在傅里叶域累积增强的频率分量并执行傅里叶逆变换以重建 2D SAFT 图像,可在各个方向上提高横向分辨率,如图 6(a)所示。OR-PAM 中光聚焦同样会导致散焦问题,贝塞尔光束照明是常见的解决办法之一。贝塞尔光束相比高斯光束具有更长的景深,可以在更广的深度范围内提供高分辨率。Hu 等<sup>[47]</sup>在 2019 年开发了一种具有双非衍射贝塞尔光束的扩展景深全光学光声显微镜,能够提高深度分辨能力,焦深可达 635  $\mu\text{m}$ ,最佳的横向分辨率为 2.4  $\mu\text{m}$ ,如图 6(b)所示。

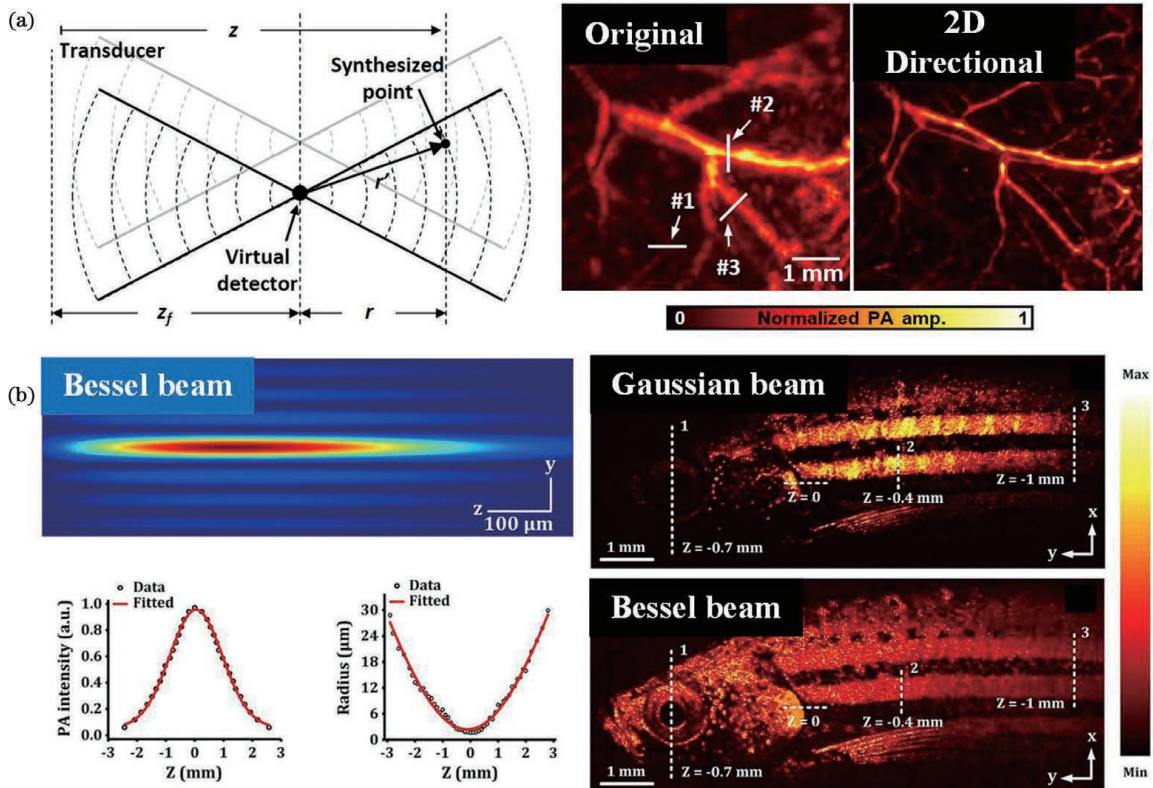


图 6 PAM 中扩展景深技术的实施例。(a)SAFT 中的虚拟探测概念及叶脉的原始图像和 2D SAFT PA 成像<sup>[45]</sup>; (b)贝塞尔光束参数的数值模拟和实验测量及基于高斯光束和贝塞尔光束照明的斑马鱼 PA 成像<sup>[47]</sup>

Fig. 6 Embodiments of extended depth-of-field technology in PAM. (a) Diagram of virtual detector concept in SAFT and original and 2D SAFT PA imaging of leaf vein<sup>[45]</sup>; (b) numerical simulation and experimental measurement of parameter of Bessel beam and PA imaging of zebrafish based on Gaussian beam and Bessel beam illumination<sup>[47]</sup>

#### 4.3 成像速度的提高

快速成像一直是临床应用的必然要求,PAM 系统的成像速度与激光重复频率、系统扫描机制和 PA 信号采集方案有关<sup>[50-52]</sup>。大多数 PAM 系统采取光声共聚焦的逐点机械扫描方式,无法满足实时成像要求。早年发展的音圈扫描方式<sup>[53]</sup>,虽然能实现快速扫描,但其扫描速度仍会受到驱动力和扫描探头质量的影响,且不适于小型化。近年来开发的水浸微机电系统(MEMS)扫描<sup>[54-55]</sup>和振镜(GM)扫描<sup>[56-57]</sup>既实现了快速扫描,又满足了小型化和稳定性的需求,如图 7(a)

和图 7(b)所示。不过 MEMS 扫描方式需要考虑长时间扫描带来的稳定性问题,GM 虽然相较于 MEMS 表现出了更稳定的性能,但成像视野受限。多边形扫描<sup>[58-59]</sup>以恒定速度旋转多边形反射镜,避免了上述扫描方式中重复的加速/减速过程,扫描过程中激光束可能会被传送到无法检测的区域,更多边数的多边形扫描仪有效解决了该问题。Zhu 等<sup>[59]</sup>在 2022 年开发了一种基于有 12 个面的多边形扫描仪的超快功能性 PAM,能够以大视场实时监测小鼠全脑血流动力和氧饱和度,如图 7(c)所示。此外,微透镜阵列<sup>[60-61]</sup>(多焦

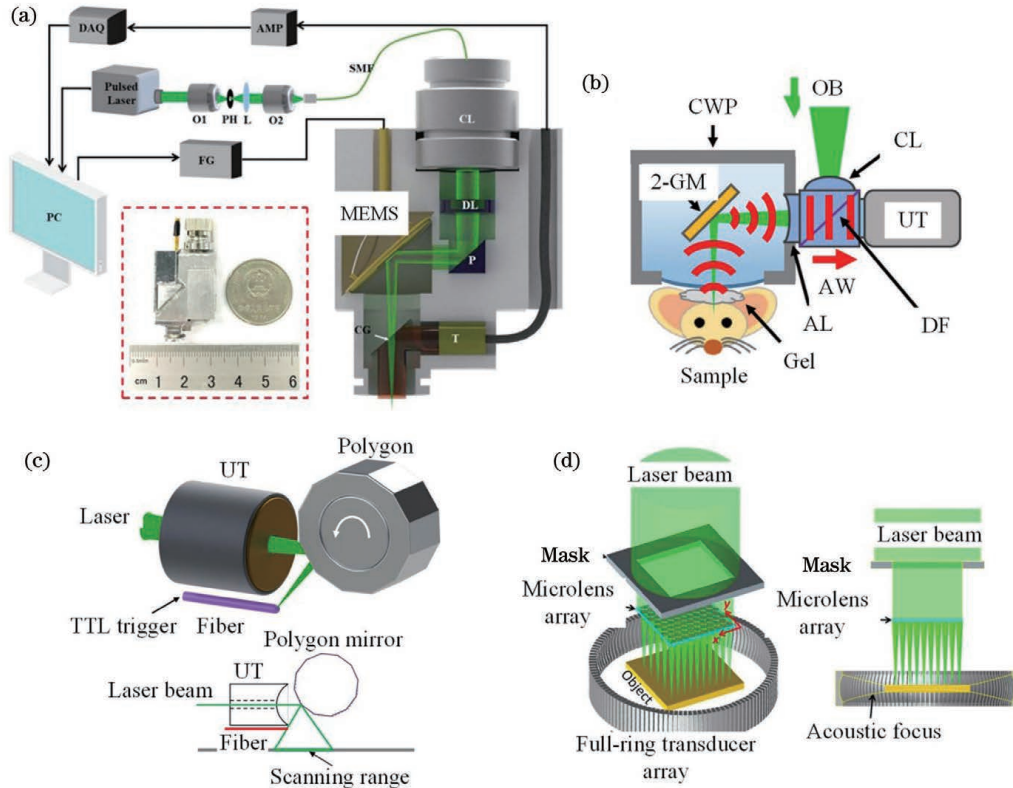


图 7 PAM 快速成像技术的实施例。(a) 基于 MEMS 的快速 PAM<sup>[54]</sup>; (b) 基于 GM 的快速 PAM<sup>[56]</sup>; (c) 基于多边形扫描仪的快速 PAM<sup>[59]</sup>; (d) 基于微透镜阵列的快速 PAM<sup>[60]</sup>

Fig. 7 Embodiments of high-speed PAM. (a) High-speed PAM based on MEMS<sup>[54]</sup>; (b) high-speed PAM based on GM<sup>[56]</sup>; (c) high-speed PAM based on polygon-mirror scanner<sup>[59]</sup>; (d) high-speed PAM based on microlens array<sup>[60]</sup>

点扫描)、光机混合扫描<sup>[62]</sup>等也是实现快速成像的策略。Xia 等<sup>[60]</sup>设计了一种宽视场二维多焦点的光学分辨率光声计算显微镜(OR-PACM), 摆脱了传统 PAM

基于单聚焦光激发和超声检测的扫描方式限制, 实现了宽视场的快速成像, 如图 7(d)所示。表 1 列出了近年 PAM 快速扫描技术方面的一些成果。

表 1 快速 PAM 扫描系统的表现

Table 1 Performance of scanning systems for high-speed PAM

Scanner type	Scanning speed	Imaging range	Lateral resolution / $\mu\text{m}$	Portable	Ref.
Voice coil	20 Hz/B-scan	9 mm	3.4	No	[53]
MEMS scanner	5 s/volumetric scan	2 mm $\times$ 2 mm	3.8	Yes	[55]
MEMS scanner	35 Hz/B-scan	2.8 mm	12.0	Yes	[54]
Galvanometer scanner	140 Hz $\cdot$ mm <sup>-1</sup> /B-scan	15 mm	4.9	No	[56]
Galvanometer scanner	500 Hz/B-scan	2.4 mm	7.5	Yes	[57]
Polygon-mirror scanner	900 Hz/B-scan	9 mm	10.0	No	[58]
Polygon-mirror scanner	2 Hz/volumetric scan	11 mm $\times$ 7.5 mm $\times$ 1.5 mm	10.0	No	[59]
Microlens array	36 s/cross-sectional scan	10 mm $\times$ 10 mm	29.4	No	[60]
Microlens array	10 s/volumetric scan	10 mm $\times$ 10 mm	13.0	No	[61]
MEMS+ motor	15 min/volumetric scan	5 mm $\times$ 5 mm	1.0	No	[62]

#### 4.4 光学探测光声信号的方式

超声信号检测方式是 PAM 临床应用中另一个需要考虑的问题。大多数 PAM 使用超声换能器接收光声信号, 需要配合耦合剂使用, 限制了应用范围。全光学 PAM 是利用光学的方法检测光声信号<sup>[63-64]</sup>, 优势在于非接触成像、高灵敏度和更宽的检测带宽。根据

信号检测原理, 全光学 PAM 主要分为干涉式和非干涉式。皮肤下方吸收体产生的光声信号传递到皮肤表面时, 局部声压会引起表面位移, 通过干涉式传感器测量表面位移可以重建光吸收分布图像。常见的干涉式传感器有法布里-珀罗(FP)干涉仪<sup>[65]</sup>、微环谐振器<sup>[66]</sup>、光纤干涉仪<sup>[67-68]</sup>等。但不必要的环境扰动会

影响测量效果,Chen 等<sup>[69]</sup>在 2015 年提出了一种基于低相干干涉仪的非接触宽带全光学光声显微镜(BD-AO-PAM),如图 8(a)所示,其中压电致动器驱动参考镜产生更快速、更规律的附加光路长度变化,以锁定最高灵敏度点,从而排除环境干扰以提高成像质量。Hajireza 等<sup>[70]</sup>于 2017 年提出了一种非干涉的光声遥感(PARS)显微镜,如图 8(b)所示,初始光

声压力会调制吸收界面的折射率,检测光声信号初始位置处的反射光强度变化,避免了不必要的相位调制问题,可以提供横向分辨率为 $(2.7 \pm 0.5) \mu\text{m}$ 的 PA 图像,但该技术丧失了单次扫描实现三维体成像的能力。全光学检测的方式对系统光路要求较高,必须考虑不必要的扰动、粗糙的表面等影响因素,特别是深度信息问题。

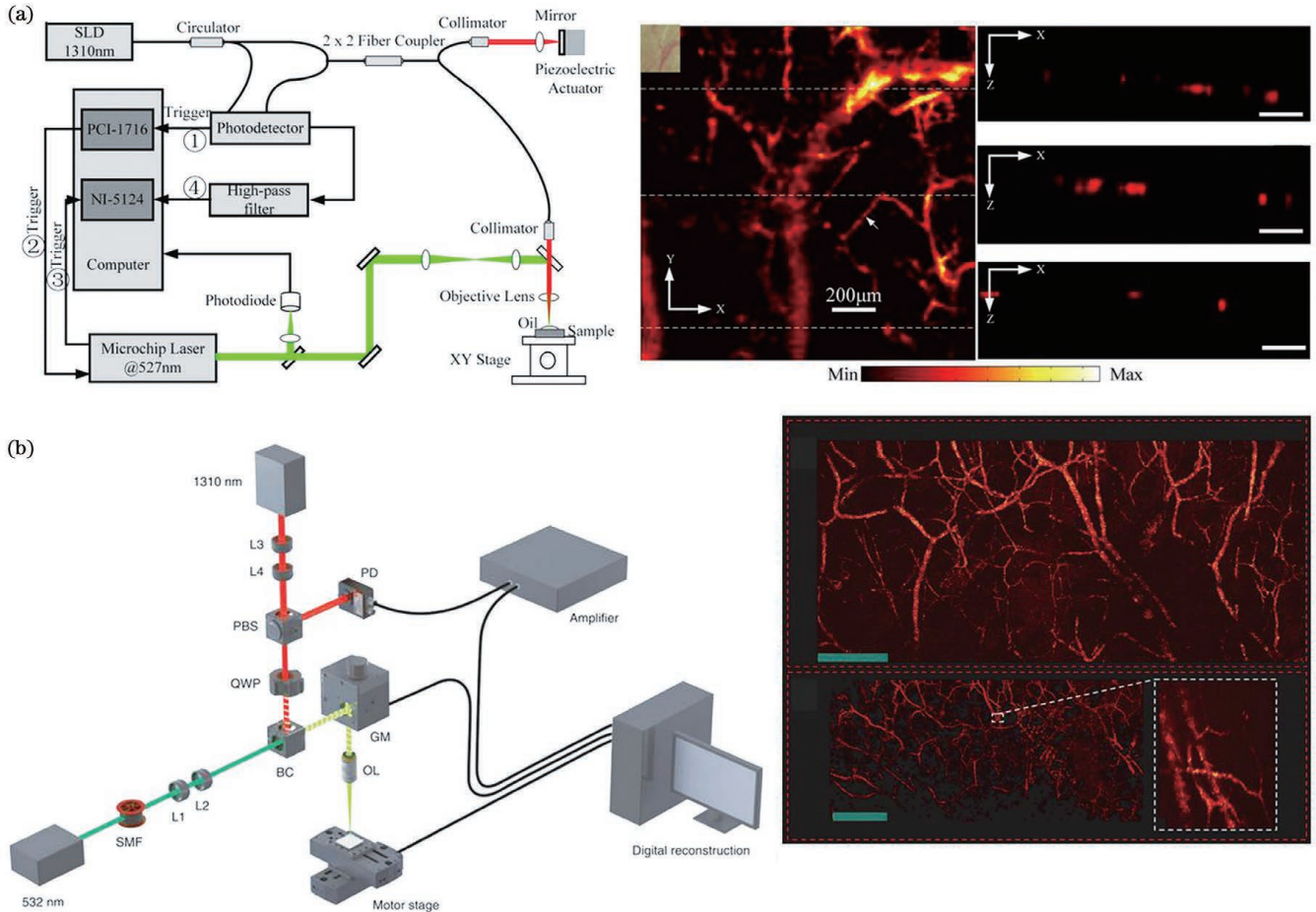


图 8 全光学 PAM 的实施例。(a)BD-AO-PAM 系统示意图及小鼠耳朵微血管系统的体内 PA 成像<sup>[69]</sup>; (b)PARS 显微镜系统示意图及鸡胚的绒毛尿囊膜的体内 PA 成像<sup>[70]</sup>

Fig. 8 Embodiments of all-optical PAM. (a) Schematic diagram of BD-AO-PAM system and *in vivo* PA imaging of microvasculature of mouse ear<sup>[69]</sup>; (b) schematic diagram of PARS microscope system and *in vivo* PA imaging of chorioallantoic membrane from a chicken embryo<sup>[70]</sup>

## 5 光声内窥成像主要技术进展

PAE 将 PA 成像技术和内窥技术结合在一起,可以对生物体内部组织/器官(如消化道、血管、泌尿生殖器官等)进行成像,是 PA 成像技术的主要研究方向之一<sup>[71]</sup>。与 PAT 的分类相似,根据光声聚焦及扫描方式的不同将其划分为光声计算内窥镜(PCE)<sup>[72]</sup>、光学分辨光声内窥显微镜(OR-PAEM)<sup>[73-74]</sup>以及声学分辨光声内窥显微镜(AR-PAEM)<sup>[75]</sup>;根据应用场景主要分为胃肠道内窥镜和血管内窥镜。虽然近几年 PAE 在技术和应用方面取得了很大进展,但临床应用方面还未成熟,这是由于与

PACT 和 PAM 相比,PAE 的应用场景要求更高。首先,内窥手段既严格限制了探头尺寸,又对扫描过程的稳定性有较高要求,而目前大多数 PAE 采用的外部机械扫描方式难以满足稳定性和紧凑性要求;此外消化道等组织/器官的内部复杂环境突出了散焦问题,对成像质量有较大影响;其次,内窥成像往往需要获取除光吸收外的更多信息,这对探头设计提出了更大挑战。主要围绕上述几个方面的解决方案介绍 PAE 的技术进展,具体包括:(1)扫描与探测方式的改进;(2)光声内窥成像中的扩展景深技术;(3)多模态内窥成像。

### 5.1 扫描与探测方式的改进

在内窥探头设计方面,直径、刚性长度及其成像视



角等是需要特别注意的。此外,探头设计也要考虑扫描方式的需求,采取光声同轴配置的方式可以使 PAE 系统变得紧凑。大多数 PAE 系统采用旋转光声内窥探头与轴向拉动探头的方式实现圆周或螺旋激发与采集,这种机械扫描方式不仅需要线性平台,还需要笨重的外部旋转电机,难以满足系统的稳定性和小型化需求,且扫描速度较慢。Guo 等<sup>[76]</sup>在 2017 年报道了一种基于 MEMS 扫描镜的光声内窥显微镜 (PAEM) 系统,通过控制探头前端的 MEMS 扫描镜,仅需利用线性平台轴向拉动探头即可以  $10.6 \mu\text{m}$  的横向分辨率对  $400 \mu\text{m} \times 400 \mu\text{m}$  的区域进行快速成像,如图 9(a) 所示。这种光学扫描方式的探头设计

既省略了外部电机旋转光声内窥探头的过程,简单易操作,又避免了机电噪声。此外大多数 PAE 系统以侧视方式进行成像,但术中引导等一些临床应用需要采取“向前看”的方式,这对超声换能器配置提出了更大挑战。利用全光学前视光声探头,可以满足术中引导的需求<sup>[77-79]</sup>。Ansari 等<sup>[77]</sup>在 2018 年设计了一种用于高分辨率内窥镜检查的全光学前视光声探头,探头前端采用 FP 传感器检测光声信号,获取了小鼠皮肤的微血管解剖结构,如图 9(b) 所示。该方案既避免了讨论耦合问题,又提供了与光学成像集成的可能,可实现光学-光声双模态内窥成像,便于医生直观操作。

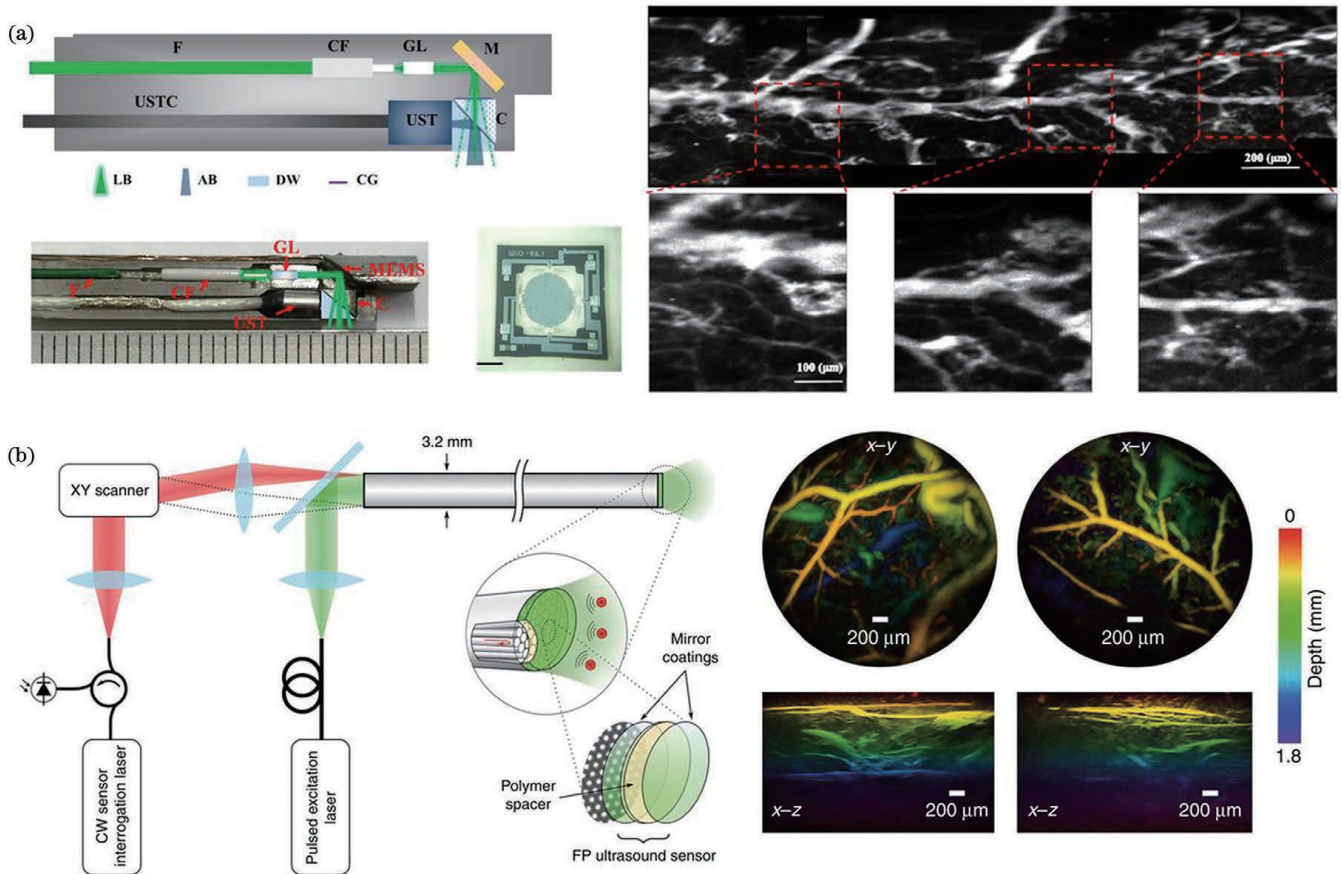


图 9 基于光学扫描的 PAE 实施例。(a) 基于 MEMS 扫描的 PAE 探头示意图及切除的小鼠结肠的 PA 成像<sup>[76]</sup>; (b) 全光学前视光声内窥镜探头示意图及小鼠腹部皮肤微血管的 PA 成像<sup>[77]</sup>

Fig. 9 Embodiments of PAE based on optical scanning. (a) Schematic diagram of PAE probe based on MEMS scanning and PA imaging of resected mouse colon tissue<sup>[76]</sup>; (b) schematic diagram of all-optical forward-viewing PAE probe and PA imaging of mouse abdominal skin microvasculature<sup>[77]</sup>

## 5.2 光声内窥成像中的扩展景深技术

PAM 中存在的散焦问题在 PAE 中同样会造成离焦区域的分辨率明显下降,可借鉴 PAM 中的处理方案,如合成孔径聚焦技术<sup>[80-81]</sup>、贝塞尔光束照明<sup>[74]</sup>等。Xiong 等<sup>[82]</sup>在 2018 年提出了自动聚焦的 PAE (AF-PAE),通过折射率可变的透镜将光聚焦到不同深度处以解决散焦问题,如图 10(a) 所示。但是自动聚焦技术增加了系统扫描时间与数据处理难度。因

此,他们在 2019 年又开发了一种大景深的 OR-PAE,以相对恒定的横向分辨率对不同深度区域进行成像<sup>[74]</sup>,如图 10(b) 所示。

## 5.3 多模态内窥成像

血管、脂质等具有光吸收差异的生物组分允许 PAE 的多场景应用,但内窥成像往往需要同时获取光学影像等信息,为此,将 PAE 与其他影像内窥手段结合,可以同时获取更多的信息,具有更大的临床应用价

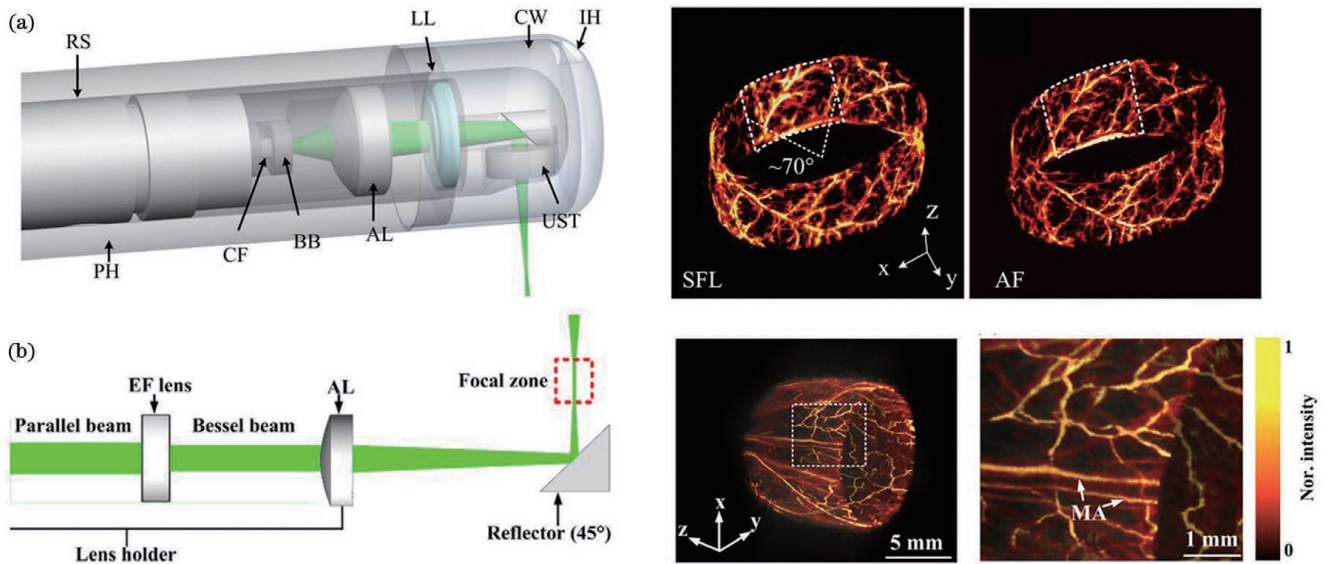


图 10 PAE 中扩展景深技术的实施例。(a) AF-PAE 的成像探头示意图及兔子直肠的 PA 成像<sup>[82]</sup>; (b) 大景深 OR-PAE 的成像探头示意图及兔子直肠的 PA 成像<sup>[74]</sup>

Fig. 10 Embodiments of extended depth-of-field technology in PAE. (a) Schematic diagram of imaging probe of AF-PAE and PA imaging of rabbit rectum<sup>[82]</sup>; (b) schematic diagram of imaging probe of large-depth-of-field OR-PAE and PA imaging of rabbit rectum<sup>[74]</sup>

值。例如:光声-超声(PA-US)多模态内窥成像<sup>[83-85]</sup>能够实现超声内窥成像的深度优势和 PA 成像的对比度优势的互补;光声-光学相干断层成像(PA-OCT)多模态内窥成像<sup>[86-87]</sup>和光声-荧光(PA-FI)多模态内窥成像<sup>[88-89]</sup>可以提供多种光学信息辅助临床检查;光声-高光谱(PA-HIS)多模态内窥成像能同时提供表层光学成像、深层断层成像以及氧饱和信息<sup>[90]</sup>;光声-光声黏弹(PA-PE)多模态内窥成像进一步提供组织的弹性信息,实现早期易损斑块等疾病的检测<sup>[91-92]</sup>。图 11 给出了多模态内窥成像的实施例。当然,这类多模态策略,在 PA 成像的其他模式下依然适用<sup>[93]</sup>。

## 6 光声分子成像及新兴的探针设计策略

生物体内的血红蛋白、黑色素、脂质等具有高识别度的吸收光谱,天然地可以作为内源性造影剂,实现高对比度的结构、功能成像<sup>[94]</sup>,如血管成像、血氧功能成像和脑部功能成像等。然而,大多数疾病的特征分子光吸收能力较弱,难以产生足够强的光声信号,需要借助外源性造影剂实现高特异性、高灵敏度的 PA 成像<sup>[94-95]</sup>。PA 探针的设计需要考虑特征吸收光谱、生物相容性、光热稳定性、细胞毒性等<sup>[96]</sup>,目前采用的主要有贵金属纳米粒子<sup>[97-99]</sup>、过渡金属硫化物/MXene 纳米材料<sup>[100-101]</sup>、碳纳米材料<sup>[102-103]</sup>、有机小分子<sup>[104-106]</sup>、半导体聚合物纳米粒子<sup>[107-108]</sup>等。下面介绍目前光声探针的主要设计策略:(1)近红外区吸收;(2)光声信号对比度动态增强策略;(3)诊疗一体化设计。

### 6.1 近红外区吸收

相比于可见光波段,近红外(NIR)窗口<sup>[109]</sup>的优势

在于:此波段下组织衰减和散射减少,可以实现对深层组织的 PA 成像。目前,绝大多数外源性造影剂的设计都以近红外吸收为前提以最大限度提高成像深度。近红外 II 区(1000~1700 nm)光声造影剂的研究占比逐渐增加,与近红外 I 区(700~1000 nm)相比其具有更深的穿透深度和更高的最大曝光量(MPE)<sup>[110]</sup>,如图 12(a)~图 12(c)所示。图 12(d)~图 12(f)列举了几个近红外 PA 分子成像的实例<sup>[111-113]</sup>。此外,随着激光技术的发展,太赫兹也开始应用于 PA 成像领域,将促进葡萄糖、蛋白质、DNA 和 RNA 等生物分子研究,具有广阔的发展空间<sup>[114]</sup>。

### 6.2 光声信号对比度动态增强策略

虽然光吸收对比机制是 PA 成像的优势之一,但同时可能会受到不必要的背景信号干扰(血红蛋白、黑色素等)。通过外部条件响应(温度<sup>[112]</sup>、超声<sup>[115]</sup>、光照<sup>[116]</sup>等)或非线性机制<sup>[117]</sup>等调制光声信号的振幅,利用调制前后信号的变化抑制背景信号,从而实现高对比度的“无背景”PA 成像。Cui 等<sup>[116]</sup>在 2021 年提出了一种基于光写入和氧化还原响应显色定影的超高灵敏度和肿瘤特异性 PA 成像技术,在外部光照条件触发后,AgBr@PLGA 纳米晶体(NC)被肿瘤区的谷胱甘肽(GSH)还原为强吸收的银纳米颗粒(NP),可以通过动态调节曝光时间有效抑制背景信号,进而特异性识别肿瘤区域,如图 13 所示。

检测内部条件改变引起的 PA 信号动态变化,可以特异性识别目标区域并定性分析对应的生物过程变化,但难以提取定量信息。一般地,用于定量研究的比率探针的设计点在于,针对同一变量,探针在两个波长处的 PA 信号变化趋势不同,而二者的比值随变量改

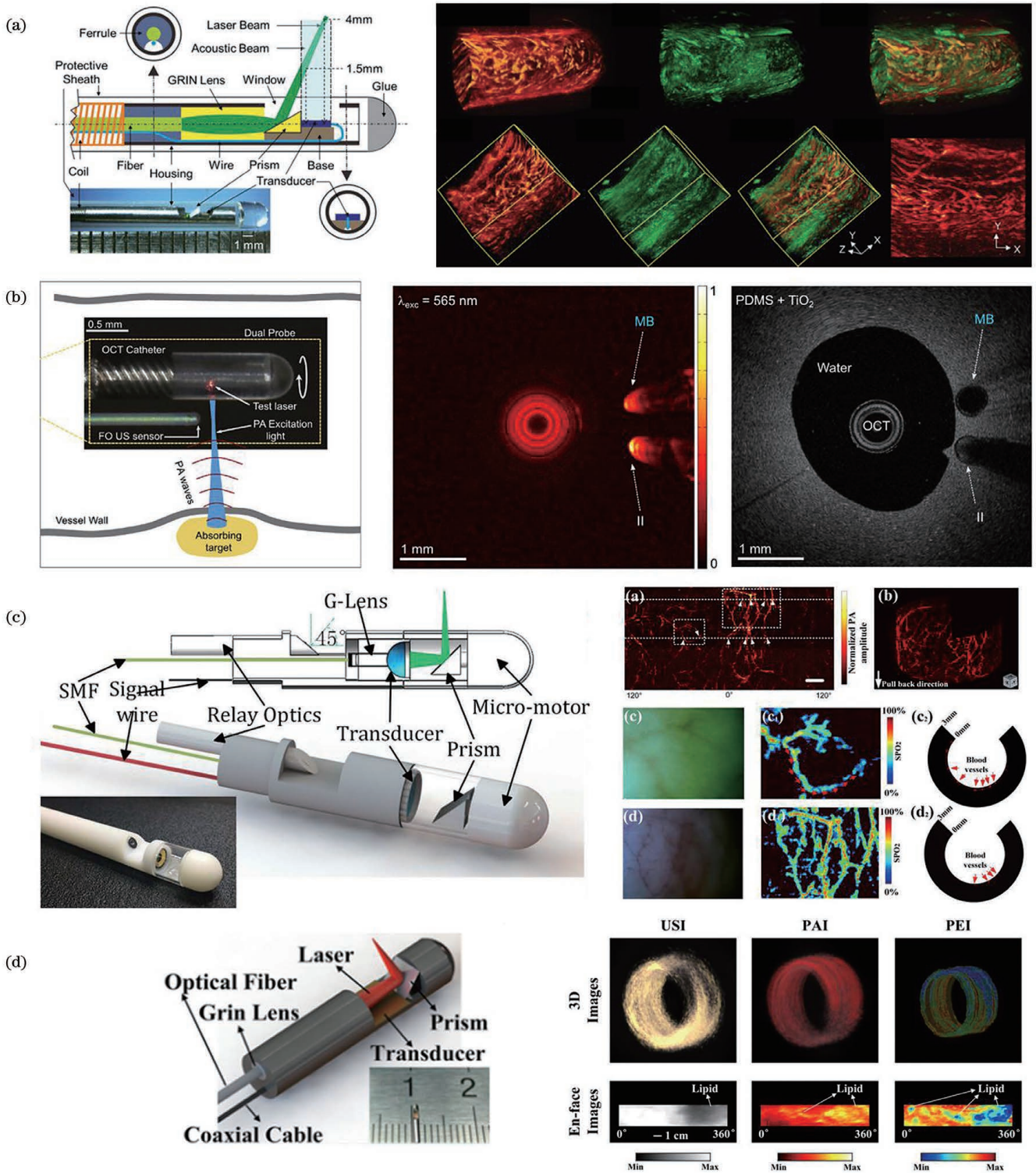


图 11 多模态 PAE 的实施例。(a) PA-US 内窥成像探头示意图及大鼠直肠的 PA-US 成像<sup>[84]</sup>；(b) 全光学 PA-OCT 血管内探头示意图及血管模型的 PA-OCT 内窥成像<sup>[87]</sup>；(c) PA-HSI 内窥成像探头示意图及兔子体内直肠 PA-HSI 成像<sup>[90]</sup>；(d) PA-PE 内窥成像探头示意图及主动脉 US-PA-PE 成像<sup>[91]</sup>

Fig. 11 Embodiments of dual-modality PAE. (a) Schematic diagram of imaging probe of PA-US endoscope and PA-US imaging of rat rectum<sup>[84]</sup>; (b) schematic diagram of all-optical PA-OCT intravascular probe and PA-OCT imaging of vascular phantom<sup>[87]</sup>; (c) schematic diagram of imaging probe of PA-HSI endoscope and PA-HSI imaging of rabbit rectum *in vivo*<sup>[90]</sup>; (d) schematic diagram of imaging probe of PA-PE endoscope and US-PA-PE imaging of aorta<sup>[91]</sup>

变呈线性变化,从而提供了定量信息<sup>[118-119]</sup>。Huang 等<sup>[118]</sup>在 2019 年提出了一种对胃和肠道功能障碍进行体内定量 PA 诊断的 pH 响应传感器,在 790 nm 处的

PA 信号对 pH 敏感,而在 1200 nm 处的 PA 信号与 pH 值无关,二者的比值随 pH 线性下降,从而实现胃肠道中 pH 差异的定量检测,如图 14 所示。

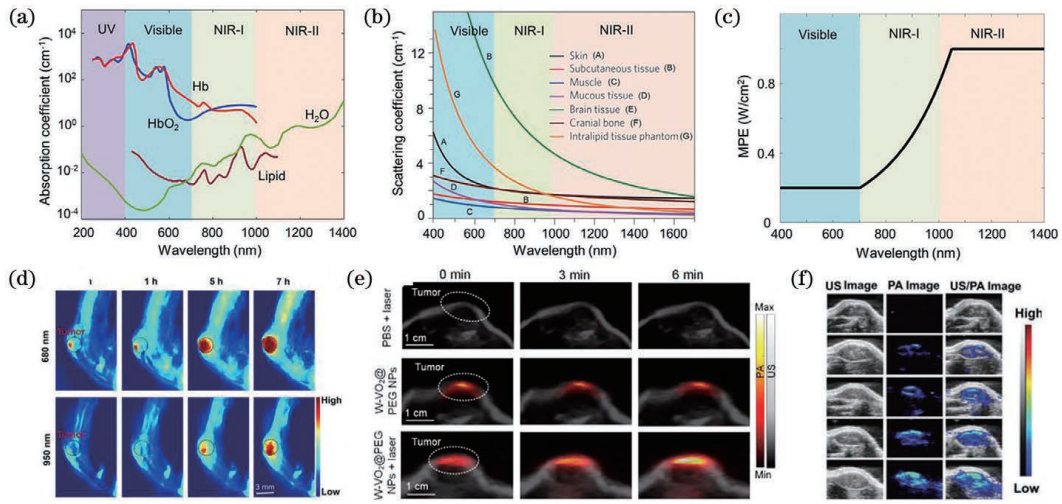


图 12 近红外 PA 分子成像。(a)内源性组织发色团在人体典型浓度下的吸收系数谱<sup>[110]</sup>；(b)在 400~1700 nm 区域内,不同生物组织和脂内散射组织模型的散射系数随着波长的变化而降低,涵盖可见光、NIR-I 和 NIR-II 窗口<sup>[110]</sup>；(c)MPE 作为激发波长的函数<sup>[110]</sup>；(d)680 nm 和 950 nm 波长下小鼠肿瘤 PA 成像<sup>[113]</sup>；(e)1064 nm 波长下的小鼠肿瘤 PA-US 成像<sup>[112]</sup>；(f)1280 nm 波长下的小鼠肿瘤 PA-US 成像<sup>[111]</sup>

Fig. 12 PA molecular imaging in NIR region. (a) Absorption coefficient spectra of endogenous tissue chromophores at their typical concentrations in human body<sup>[110]</sup> ; (b) reduced scattering coefficients of different biological tissues and of intralipid scattering tissue phantom as function of wavelength in 400–1700 nm region, which covers visible, NIR-I, and NIR-II windows<sup>[110]</sup> ; (c) MPE as function of excitation wavelength<sup>[110]</sup> ; (d) PA imaging of mice tumor with 680 nm and 950 nm<sup>[113]</sup> ; (e) PA-US imaging of mice tumor with 1064 nm<sup>[112]</sup> ; (f) PA-US imaging of mice tumor with 1280 nm<sup>[111]</sup>

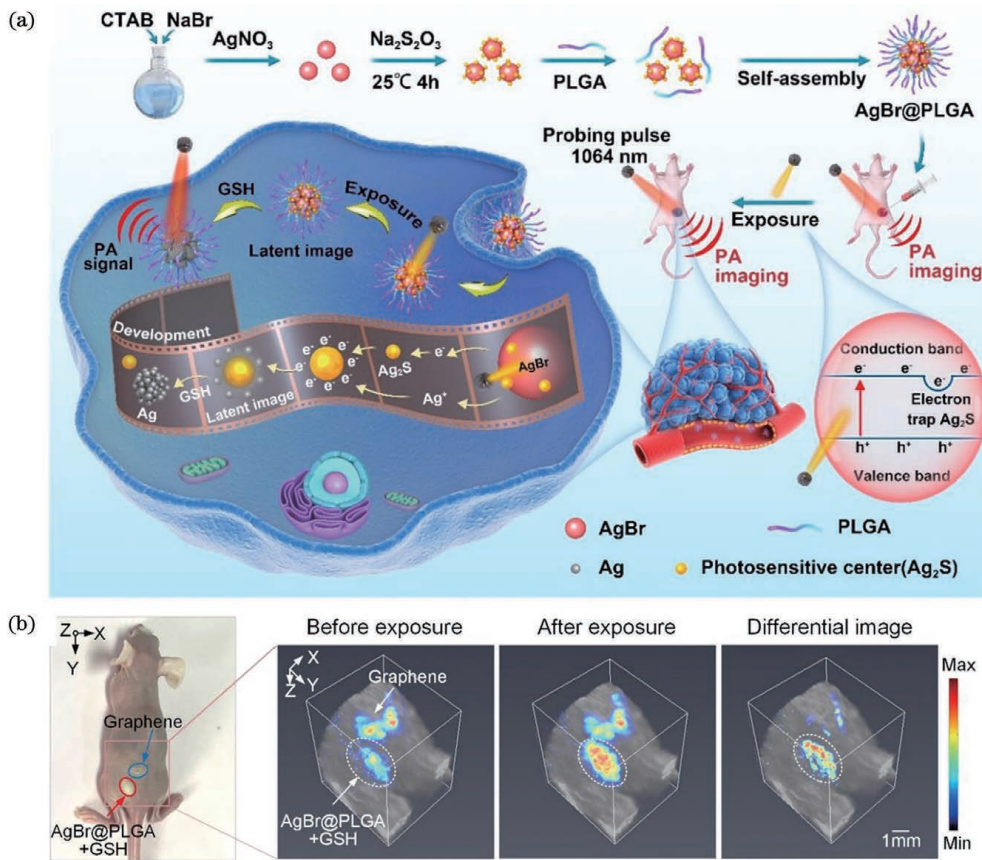


图 13 对比度增强的光声分子成像实施例<sup>[116]</sup>。(a)制备的 AgBr@PLGA 纳米晶体的合成过程和通过氧化还原反应以图形方式固定肿瘤区域的示意图；(b)注射 AgBr@PLGA+GSH 和石墨烯的小鼠 PA 成像

Fig. 13 Embodiment of contrast-enhanced PA molecular imaging<sup>[116]</sup> . (a) Synthesis process of prepared AgBr@PLGA NCs and schematic illustration that tumor area is graphically fixed via redox reaction; (b) PA imaging of mice injected AgBr@PLGA+GSH and graphene

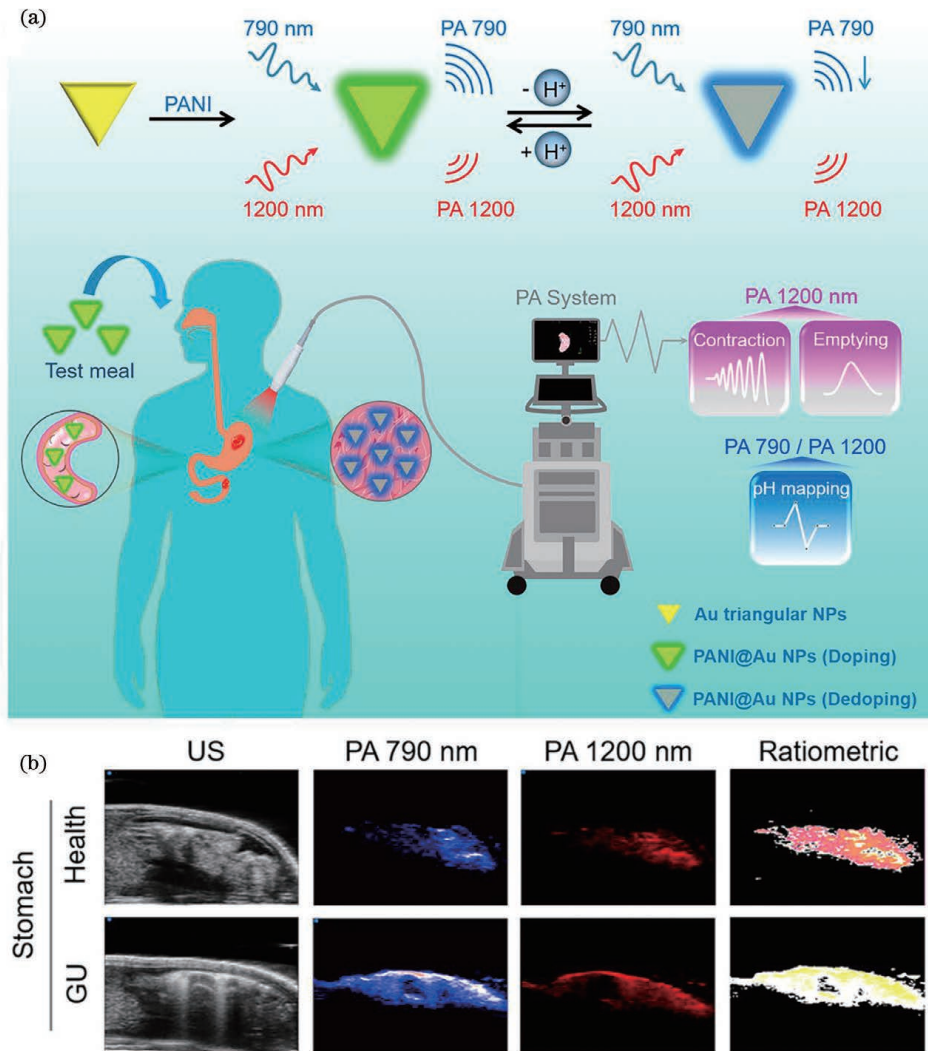


图 14 比率光声分子成像的实施例<sup>[118]</sup>。(a)胃和肠道疾病定量 PA 诊断的设计与合成示意图;(b)US 成像、790 nm 的 PA 成像、1200 nm 的 PA 成像和胃的比率 PA 分子成像

Fig. 14 Embodiment of ratiometric PA molecular imaging<sup>[118]</sup>. (a) Design and synthesis of quantitative PA diagnosis of gastric and intestinal diseases; (b) US imaging and PA imaging at 790 nm, PA imaging at 1200 nm, and ratiometric PA molecular imaging of stomach

此外,与基于实体瘤的增强渗透滞留(EPR)效应或简单靶向识别的探针相比,只有在特定的组织微环境或高水平的活性物质表达中(如酸度值、缺氧环境、GSH、过氧化氢等)才能被激活或光声信号幅值明显变化的可激活探针<sup>[111,113,120-121]</sup>,不仅可以用来特异性识别目标区域,提高特异性和灵敏度,还能够实时提供病理发展过程变化信息并降低对正常组织的毒副作用。Gao 等<sup>[120]</sup>设计了一种基于光敏色素的报告蛋白,在 780 nm 和 630 nm 光照射下具备高光声信号对比度,可以实现背景抑制成像,同时利用大肠杆菌作为载体,只有在肿瘤缺氧的微环境中才会大量繁殖并表达出强 PA 信号,而大肠杆菌后期可通过注射抗生素清除,实现了高特异性、高对比度的肿瘤 PA 成像,如图 15 所示。

### 6.3 诊疗一体化设计

与单一成像功能的探针相比,集成像、诊断与治疗

功能于一体的探针设计成为 PA 分子成像的热门研究方向之一。利用 PA 分子成像技术,能够高灵敏度、高特异性地实时识别目标区域并结合光热治疗<sup>[111,122-123]</sup>、光动力治疗<sup>[124]</sup>、药物治疗<sup>[125]</sup>、声动力治疗<sup>[126]</sup>等手段实现高效率的治疗诊断功能。其中,光声成像和光热治疗均要求探针具有较高的光热转换效率,光声成像引导的光热治疗成为主要研究方向之一。但考虑到小部分癌细胞获得耐热特性的可能性,联合其他方案有助于增强抗肿瘤的效果。Wang 等<sup>[127]</sup>在 2019 年设计了一种基于二酮吡咯并吡咯(DPP)的小分子染料 DPP-BT,既能够作为近红外波段的 FI 和 PA 造影剂,通过肿瘤靶向的方式为治疗提供精准影像引导、实时监测治疗效果,降低正常组织损害的风险,又兼备光热治疗和光动力治疗的功能,如图 16 所示。诊疗一体化探针展示出了精准高效的癌症治疗优势。

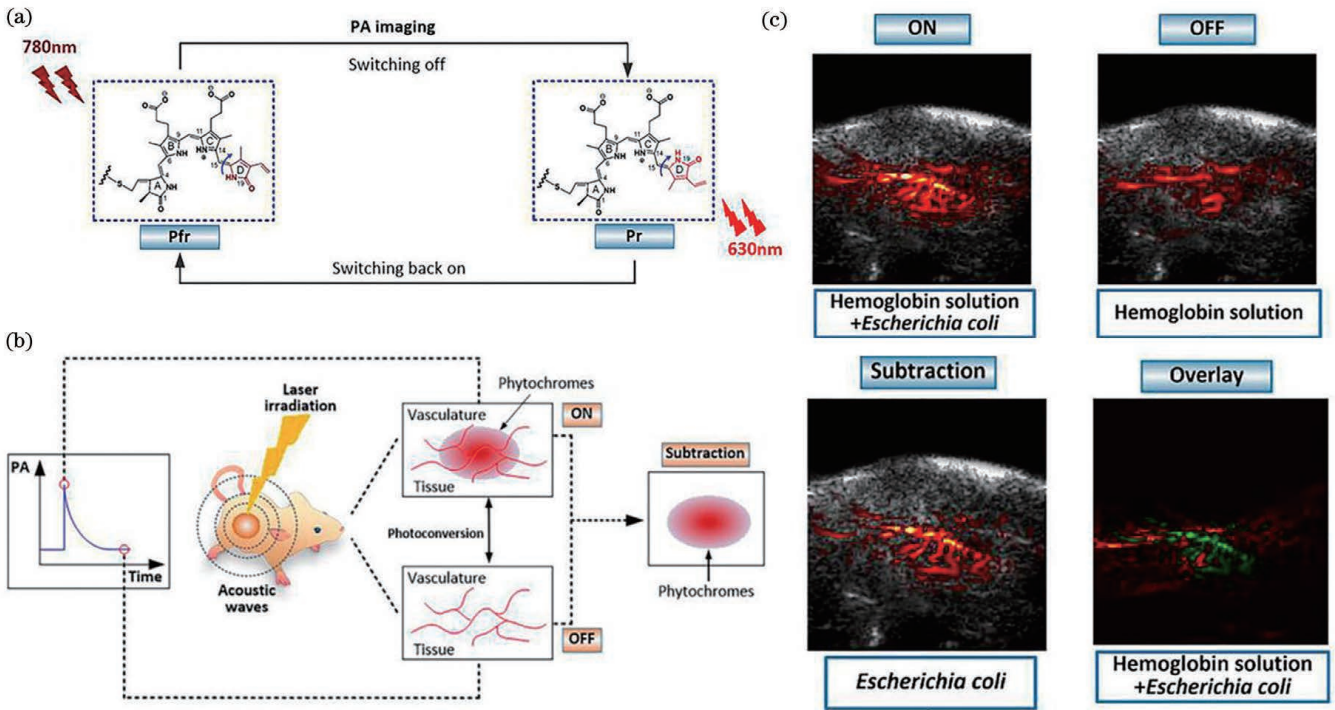


图 15 高特异性的可激活探针实施例<sup>[120]</sup>。(a)光转换过程示意图;(b)背景抑制成像的卡通示意图;(c)鸡胸组织的光声分子成像  
Fig. 15 Embodiment of highly specific activatable probes<sup>[120]</sup>. (a) Schematic diagram of photoconversion process; (b) cartoon illustration of background-suppressed PA molecular imaging; (c) PA molecular imaging of chicken breast tissue

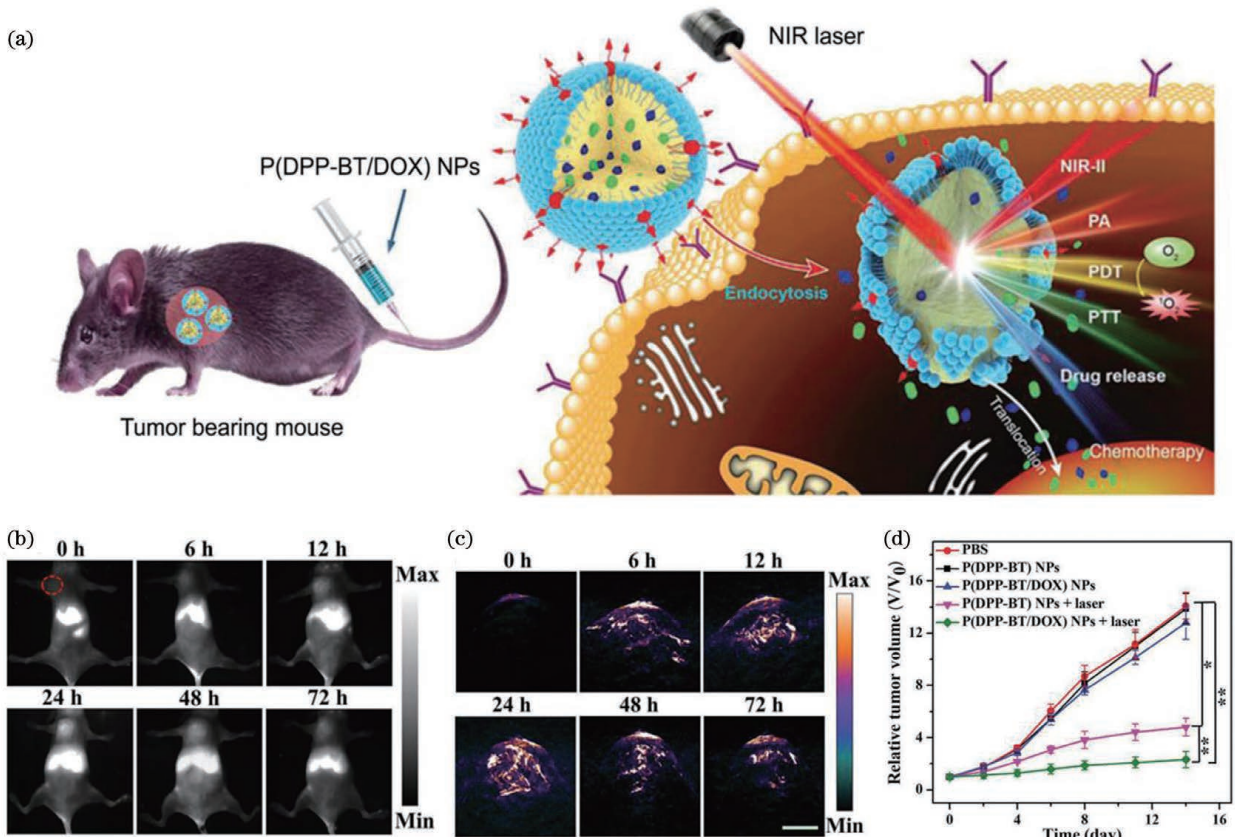


图 16 诊疗一体化探针的实施例<sup>[127]</sup>。(a)NIR-II FI/PA 双模态成像引导的肿瘤靶向联合治疗示意图;(b)移植 HeLa 肿瘤小鼠的 FI 成像;(c)移植 HeLa 肿瘤小鼠的 PA 成像;(d)不同组的小鼠肿瘤生长曲线

Fig. 16 Embodiment of probe for integration of diagnosis and treatment<sup>[127]</sup>. (a) Schematic illustration of NIR-II fluorescence/PA dual-modality imaging guiding tumor targeted combination therapy; (b) FI of HeLa-tumor-bearing mice; (c) PA imaging of HeLa-tumor-bearing mice; (d) tumor growth profiles of different groups of mice

## 7 结束语

本文介绍了 PA 成像中的 PACT、PAM、PAE 和分子成像及其主要技术进展。PACT 和 PAM 作为 PA 成像的两个主要技术手段, 分别在大范围的器官成像和高分辨率的细胞、组织微结构成像中发挥了重要作用。PAE 的发展拓展了 PA 成像在血管、消化系统等体内场景的应用。结合分子技术, PA 分子成像可以提供更准确、更丰富的病理信息。

PA 成像的侧重点仍会是技术进步和临床应用。光声系统的分辨率、对比度、灵敏度、成像深度、成像速度、成像视野等技术突破, 仍将是 PA 成像的重点研究内容。此外, 高昂的成本和高度定制的复杂系统限制了 PA 成像的广泛应用, 而低成本的开发策略如低成本光源、商业化探测器等, 将会促进 PA 成像系统的普及, 也将推动手持式、穿戴式 PA 成像设备的发展。人工智能和机器学习的发展, 也推动了 PA 成像的图像重建进展。光声系统将会更多地与其他成像手段相结合, 满足更多的临床需求。

尽管仍存在诸多挑战, 但 PA 成像已经在生物医学领域占据了一席之地。我们相信随着相关技术的发展, PA 成像将在实际临床应用中发挥更重要的作用, 让更多的患者受益。

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## Photoacoustic Imaging Technology in Biomedical Imaging

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### Abstract

**Significance** Medical imaging is a key tool for life science research, diagnosis and treatment. Traditional medical imaging techniques include magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), ultrasonic imaging (US) and optical coherence tomography (OCT), etc. Each of them plays an important role in medical imaging, with each showing its own advantages and limitations. MRI is not only expensive but also has many safety restrictions, which means it is not suitable for the patients with pacemakers or claustrophobic. CT is not suitable for children and pregnant women because of ionizing radiation. PET has a large number of applications in the study of life metabolism and tumor research. However, it requires radioactive element markers and is difficult to be widely used in clinical medical imaging due to its high cost. US has poor specificity and spatial resolution, and lacks fine biological structure imaging capabilities for the early development of the disease. OCT and other optical imaging technologies are limited by the optical diffusion limit, which results in the general imaging depth within about 1 mm.

Photoacoustic (PA) imaging is a hybrid biomedical imaging modality combining the advantages of high contrast of optical imaging and deep penetration of ultrasound imaging. The spatial scale of PA imaging covers from subcellular

structures to organs. In addition, it has many other advantages such as non-invasive imaging, label-free imaging, molecular imaging and compatible with multi-modality. Although PA imaging has encountered many challenges in the process of clinical translation, PA imaging has overcome a series of difficulties and will have broader application prospects in the field of biomedical imaging thanks to the development of related technologies. The purpose of this article is to help readers in related fields of biomedical imaging to form a more comprehensive understanding of PA imaging, and to quickly understand the main progress of PA imaging research in recent years.

**Progress** This review article provides a brief introduction to the basic principles and main modes of PA imaging. Photoacoustic computed tomography (PACT) and photoacoustic microscopy imaging (PAM) are the two main modes of PA imaging. Photoacoustic endoscopy (PAE) is the application of PA in endoscopy, and photoacoustic molecular imaging expands the capabilities of PA imaging in molecular-level detection.

PACT is suitable for large-scale imaging of the human brain, limbs, breast and other large-size targets. Over the past decade, PACT has made lots of advances in high-speed and deep imaging. However, the issues about economy and portability still hinder the further clinical translation of PACT. Fortunately, the technological development of ultrasonic transducers and low-cost laser sources and the advancement of advanced reconstruction algorithms have provided solutions to the above problems. PAM has broad application prospects from subcellular structure to organ level. High/super-resolution, fast imaging, and higher imaging quality have always been the common pursuit of researchers. The nonlinear effect greatly contributes to the improvement of the resolution of PA imaging. The extended depth-of-field technology can solve the defocus problem encountered by PAM in volume imaging. Advanced scanning methods are one of the main solutions to high-speed PA imaging. In addition, non-contact PA imaging is another important direction in clinical applications, and corresponding technological breakthroughs have also been made in recent years. As the expansion of PA imaging, PAE is a promising technology for endoscopic imaging by drawing on the related progress of PACT and PAM, such as extended depth-of-field technology, optical scanning methods and multi-modality imaging. Aiming at the problem that some tumors lack characteristic absorption peaks, PA molecular imaging holds great promise in the diagnosis and treatment of diseases. In recent years, the development of PA molecular imaging has focused on the near-infrared window to improve the depth of imaging. The PA signal enhancement mechanism can improve the sensitivity and specificity of imaging. Furthermore, the design of integrated diagnosis and treatment PA contrast agents is also a hot topic in PA molecular imaging.

**Conclusions and Prospects** Although PA imaging has made a lot of breakthroughs in recent years, it has not yet achieved a large-scale clinical application. In the next few years, the resolution, depth, speed and sensitivity of PA imaging will still be the research focus of researchers in related fields. Costs will be further reduced by using low-cost laser sources, while imaging speed and quality will meet the needs of most clinical applications thanks to technological advances in related fields. In addition, by the design of the PA contrast agent in the second near-infrared window (NIR-II), the depth of PA imaging will further increase. We believe that through the joint efforts of researchers in related fields, PA imaging will play a more important role in the clinic practices.

**Key words** bio-optics; imaging systems; photoacoustic imaging; photoacoustic computed tomography; photoacoustic microscopy; photoacoustic endoscopy; photoacoustic molecular imaging