中国激光

深层生物组织光学技术发展及其应用(特邀)

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摘要 光学技术在生物医学中扮演着越来越重要的角色，其非电离辐射、高分辨率、高对比度和对生物组织异变高度灵敏等特性使其非常适用于生物组织的研究，包括成像、传感、治疗、刺激以及控制等。然而由于光折射因子在生物组织中的分布是不均匀的，光在生物组织中的传播会受到很强的散射影响，故纯光学技术的穿透深度和空间分辨率是“鱼和熊掌不可兼得”；高分辨率光学成像应用仅限于样品浅表层，当成像深度增加时分辨率急剧下降。实现光在深层生物组织里的高分辨率成像或应用是人们期盼已久的目标。近年来，为解决这一问题，研究者提出了不同的方法，例如切换到更长的光波长以减小组织散射系数，在信号检测时将漫射光转换为散射不明显的超声信号，逆转或者预先补偿由光的多次散射所带来的相位畸变，或借助光纤等微创光学通道实现深层生物组织的高分辨率光学成像、刺激等。基于团队在深层生物组织光学相关领域多年的耕耘，从光在生物组织中的传播特性出发，梳理和总结了近年来研究人员在光-声结合和光学波前整形技术等方面展开的诸多探索，以及在生物组织操控、成像、光学计算以及人工智能等领域中的应用尝试。虽然尚有诸多不足，但随着硬件设备的更新和计算技术的发展，在不远的将来有望实现活体深层生物组织光学高分辨率应用。在这一求索过程中，新方法和新能力将不断激发新的应用灵感，为光学尤其是生物医学光子学带来全新的理念和机遇。

关键词 生物光学；光学成像；生物医学光子学；深层组织；光学波前整形；光声成像

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1 引 言

光学作为物理学中一个重要的分支，其主要研究光的产生、传播、性质与应用。随着数学和物理学的发展，目前光学已经发展成为一门系统的、独立的学科，其揭示了光的本质和规律。在光学研究中，光学成像是一个重要的研究领域，其基于各种光的现象、性质，利用光学器件记录物体的图像[1]。随着生物医学研究的不断深入，光学技术正逐渐展现出其独特的优势：首先，光学成像利用非电离辐射(如可见光和红外线等)与生物组织之间的相互作用，通过光的散射、吸收和荧光等特性来获取高分辨的影像信息，因此相比于X射线、γ射线等传统医学成像方法[2]，光学成像的安全性更高，在安全阈值下，无放射性致癌风险[3]。其次，光学方法可以通过灵活配置光的幅值、相位、波长、偏振等特性来获取生物组织的不同信息，从而实现光学成像在不同的生物医学场景中的应用[4-5]。此外，高灵敏度也是光学成像技术在生物医学研究中的优势之一，光与组织成分之间的相互作用可以被精确地探测，且特异性造影剂的应用可以增强图像对比度，从而提升目标组织或分子的可视化能力[6]，并为疾病诊断和治疗提供了更多的可能性[7]。目前广泛应用于生物医学领域的光学成像技术除了基础的宽场光学显微镜外，还包括共聚焦显微镜[8]、多光子显微镜[9]、光声显微镜[10]、光学相干层析成像(OCT)[11]，以及基于以上技术的内窥镜[12]等。

尽管光学成像具有许多优点，如非电离辐射、高灵敏度和特异性以及实时成像等，但其在深层生物

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组织成像中的应用仍存在一个主要的局限：由于光折射因子在生物组织中的分布高度不均匀，光在生物组织中的传播受到很强的散射影响，故纯光学技术的穿透深度和空间分辨率难以两全，高分辨率光学成像应用仅限于样品浅表层，当深度增加时成像分辨率急剧下降[13-15]。目前光学成像在生物组织中的应用研究已经取得了极大进展，可以在较浅的生物组织中实现无创高分辨率成像，并实时提供组织结构和功能的可视化信息[16]；但由于深层组织对光的散射特性，目前的光学成像技术在深层生物组织中的分辨率和响应速度还无法很好地满足生物医学研究的需求[17]。

近些年来，许多科研人员致力于攻克这一难题，努力提高深层组织中的光学成像能力[18-19]，这些研究涉及物理光学、计算光学和深度学习等多个领域[20-21]。基于团队在深层生物组织光学相关领域多年的耕耘，本文梳理和总结了近年来研究人员在相关领域中展开的诸多探索，包括传统光学方法、计算光学方法、深度学习方法和光纤介入方法等。传统光学方法从物理原理出发，基于传统光学理论分析影响光学成像深度的诸多因素，并给出对应策略，致力于在深层生物组织中实现清晰的光学成像；计算光学方法将数值计算方法与光学系统结合，通过联合设计并优化光学系统和算法，实现一些传统光学系统无法实现的性能和应用；深度学习方法通过构建深层神经网络来学习并提取大量散斑光场数据中输入与输出关系的特征，为解决散射介质成像中的复杂问题提供了一个全新且强大的工具；光纤介入方法基于光纤直径小、可弯折、双向光传输等特性，可以实现微创的深层生物组织成像等应用。除了介绍这四种方法的研究进展之外，本文还将探讨深层生物组织光学相关领域未来的发展方向，包括新的光学成像系统和相关算法，并探索更多光学在深层生物组织中的应用。

2 传统光学方法

为了实现深层生物组织清晰的光学成像，可以首先从物理原理出发，基于传统光学理论，对光学成像系统中影响成像深度的因素进行分析并给出对应策略。目前的研究主要集中于波长转换、能量转换和相位补偿这三个方面，下面我们将从这三个方面分别介绍相关研究成果。

2.1 波长转换

一般而言，可以通过使用长波长的光来增加生物组织中的成像深度。波长转换通过改变激发光的波长，利用波长更长的激发光增加成像深度。目前广泛应用的波长转换技术集中于多光子荧光、上转换过程以及近红外II区成像应用。多光子荧光[原理如图 1(a)、(b)所示]于 1930 年被提出[22]，并于 1961 年首次得到实验验证[23]，其特点是目标分子在激发前同时吸收两个或更多的光子，而传统的荧光在激发前只吸收一个光子，因而多光子荧光的激发光波长更长、频率更低(一般为红外激光，波长为 700～1300 nm)[24-25]。在深层生物组织研究中，一些荧光剂所需的激发光波长在紫外波段，但直接使用高能紫外光激发对生物组织有害，且相关光学系统更复杂；改为多光子激发，由于红外激发光能量较低，可以避免对样品的损伤，且红外光的穿透能力更强，可以增加成像深度[26-27]。此外，双光子的激发概率与光子强度的平方成正比，而脉冲激光束强度与到焦平面的距离的平方成反比，焦点附近荧光团的激发概率与其到焦平面距离的四次方成反比，因此只有在入射激光的焦点处才会激发明显的荧光信号。实际应用中，则可以通过焦点扫描实现高分辨率、高穿透深度的二维或三维成像[图 1(c)、(d)为拟南芥花粉管和根尖细胞的双光子高分辨率成像结果][28]。目前获得广泛应用的是双光子显微镜或三光子显微镜[29-31]，其主要的应用场景在神经元活动成像、脑功能映射和肿瘤研究等领域[32]。

上转换是一种反斯托克斯发光过程(即发射的光子波长比吸收的光子波长短)，其与多光子荧光类似，吸收多个低能量的光子后转换为一个高能量的光子，但是在上转换过程中，多个低能量光子的吸收过程是有顺序的，而多光子荧光过程是同时吸收多个低能量光子[33]。上转换过程中所使用的纳米粒子(UCNPs)是一种掺杂稀土离子的无机纳米材料，其由吸收剂和发射剂组成[34]。吸收剂中的稀土离子具有丰富的电子能级和长寿命的亚稳态，吸收剂可以吸收两个或多个光子；发射剂分子能够从吸收剂的激发态跃迁到一个更高的能级，并发射出高能量的光子[35]。

上述多光子荧光和上转换过程中的激发光源主要采用近红外Ⅰ区(NIR Ⅰ)的激光。在近红外Ⅰ区范围内(700～900 nm)，血液和水对激光的吸收和散射较少，因而该范围的激光具有一定的穿透能力，能够较好地穿透生物组织[36-37]。2009 年，Welsher 等[38-40]提出近红外Ⅱ区(1000～1700 nm，NIR Ⅱ)成像，在该波长范围内，光在生物组织中有更低的散射，在生物组织内部的穿透能力更强，能够更深入地对生物组织内部进行成像，可以探索深至 2 cm 处的深层组织信息。2022 年，李怡霏等[41]提出，生物组织中适度的光吸收可以优先耗尽传播路径更长的散射背景光，增大弹道光与散射光之比，从而提高近红外II区成像的信噪比。据此，波长范围被拓宽至 900～1880 nm。目前，近红外Ⅱ区成像中的荧光探针已成为研究热点，已经报道的探针包括碳纳米管(CNT)、量子点(QD)、聚集诱导发光(AIE)分子探针和有机小分子染料等[42-43]。2020 年，Hu 等[44]设计了多光谱荧光(包括可见光、NIR-I 和 NIR-II)成像系统，并首次在人体

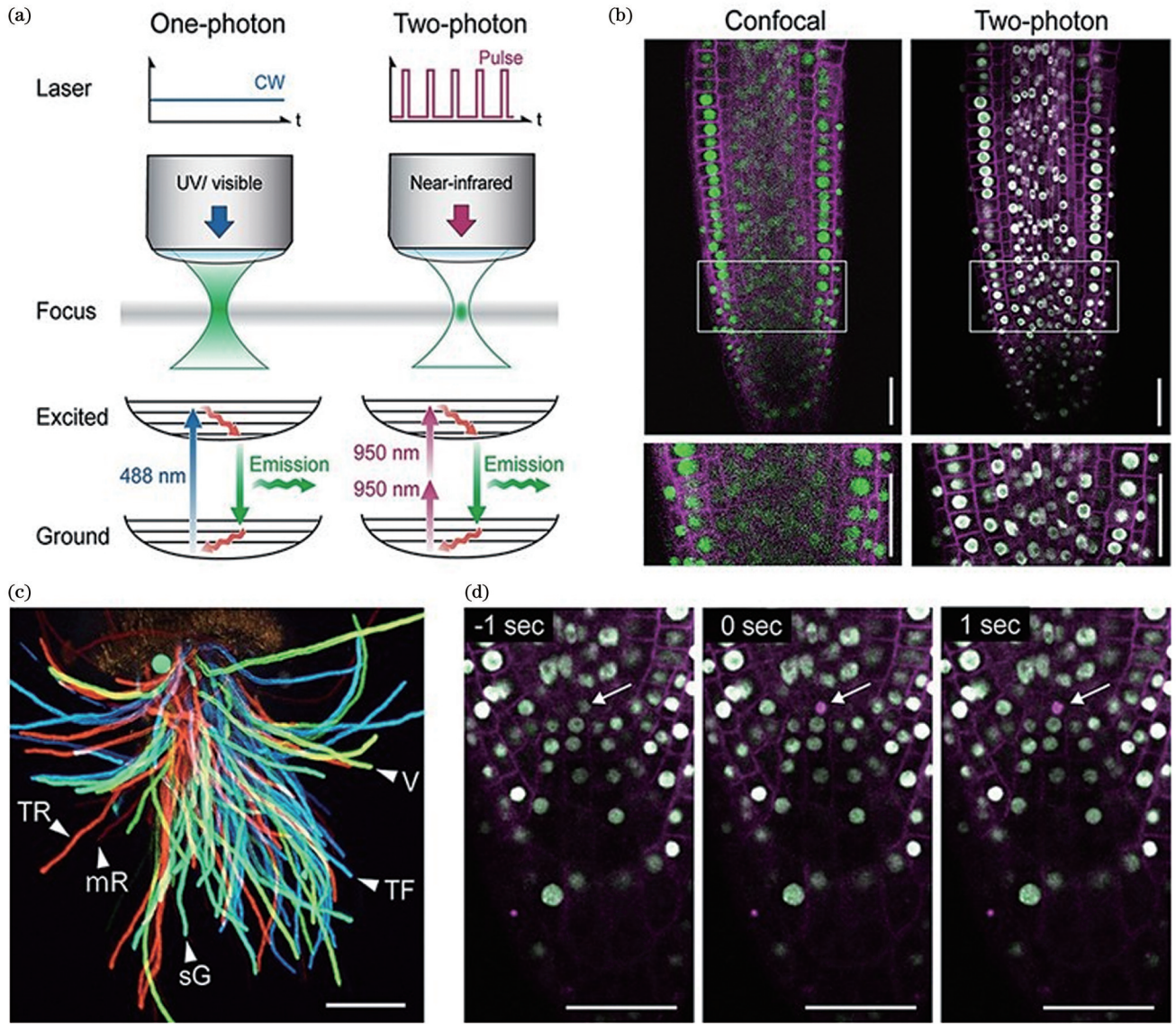


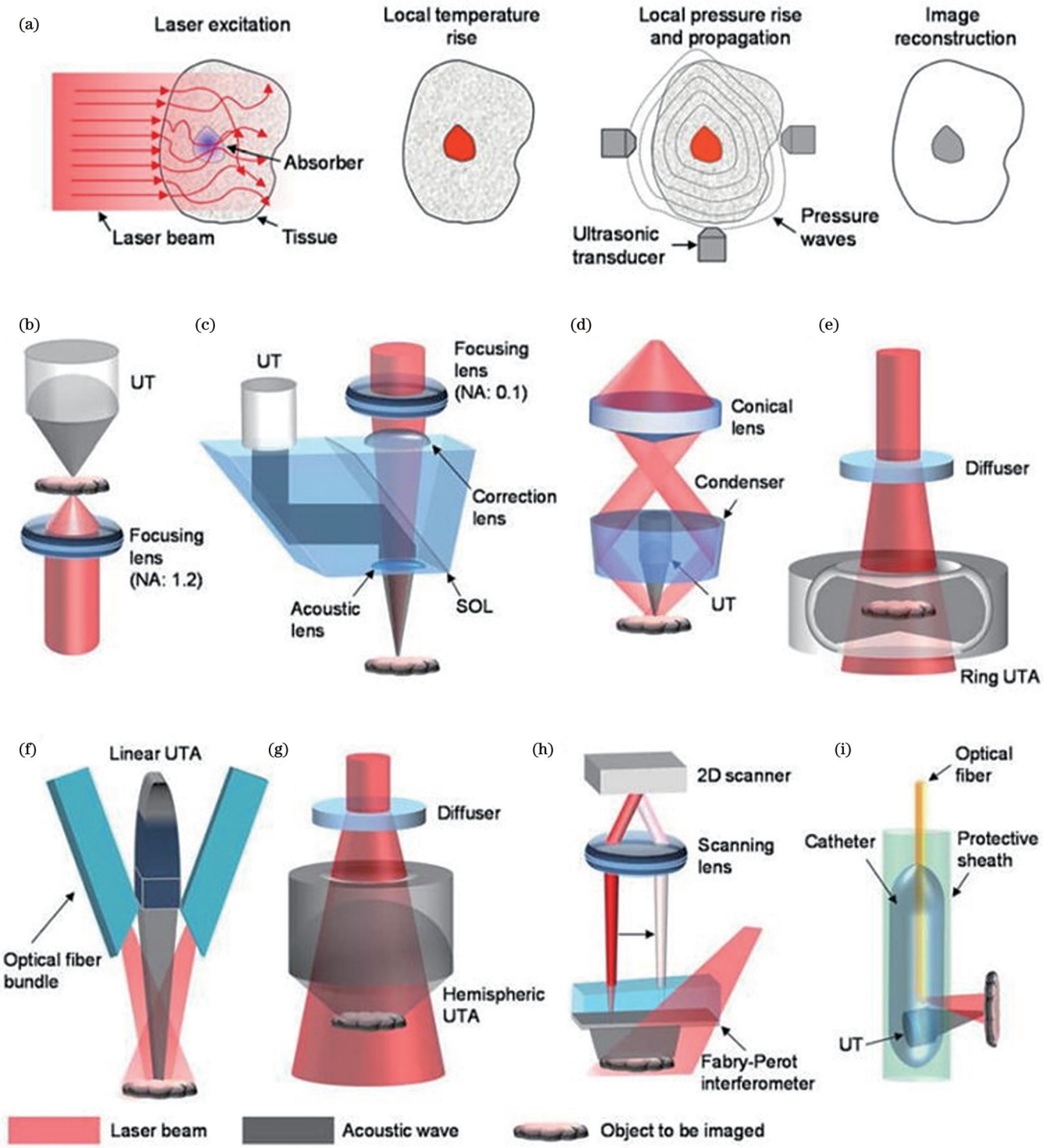
图 1 单光子和双光子显微成像的对比[46]。(a)单光子和双光子显微镜的原理示意图,单光子显微镜使用可见光连续波激光器作为光源,双光子显微镜使用飞秒近红外脉冲激光器作为光源;(b)拟南芥根尖的单光子(激发光波长为 488 nm 和 561 nm)和双光子(激发光波长为 980 nm)成像结果对比,图中比例尺为 50 μm;(c)拟南芥花粉管的多色双光子成像,从花柱末端萌发的花粉管,图中比例尺为 100 μm;(d)通过双光子激发同时进行多色成像和激光烧蚀,对拟南芥根尖的根干细胞进行激光烧蚀,图中比例尺为 50 μm

Fig. 1 Comparison between single-photon and two-photon microscopic imaging[46]. (a) Principles of single-photon and two-photon microscopes, single-photon microscope utilizes a visible continuous wave laser as a light source, and two-photon microscope utilizes a femtosecond near-infrared pulsed laser as a light source; (b) comparison of single-photon (excitation light wavelengths of 488 nm and 561 nm) and two-photon (excitation light wavelength of 980 nm) imaging results of Arabidopsis thaliana root tips with scale bar of 50 μm; (c) multicolor two-photon imaging of Arabidopsis thaliana pollen tubes, pollen tubes emerging from end of cut style with scale bar of 100 μm; (d) simultaneous multicolor imaging and laser ablation by two-photon excitation, laser ablation of root stem cells on Arabidopsis thaliana root tips with scale bar of 50 μm

内进行了多光谱荧光成像引导的肝脏肿瘤手术。研究结果表明,术中 NIR-II 成像的肿瘤检测灵敏度比 NIR-I 更高。2022 年,Zhang 等[45]设计了 AIE 探针,使用 1300 nm 的近红外Ⅱ区激光进行了三光子激发,并在实验中实现了猕猴大脑约 1 mm 的大深度血管成像。

2.2 能量转换

能量转换通过将输入的光能量转换成另一种形式的能量,减弱被探测信号的散射程度,从而提升成像的深度。本文将介绍基于光声(PA)效应的能量转换原理[图 2(a)]及光声成像技术[图 2(b)~(n)]。1880 年,Bell 首先提出了光声效应,当使用特定波长的脉冲激光束照射目标时,该目标物会吸收激光束的能量,局部温度升高并产生热膨胀,周围的介质也会发生热胀冷缩,从而产生机械振动,并以超声波的形式进行传播[47]。超声波信号被目标物外侧的超声换能器接收,


(a)
Laser excitation
Local temperature rise
Local pressure rise and propagation
Image reconstruction
Absorber
Laser beam
Tissue
Ultrasonic transducer
Pressure waves
(b)
UT
Focusing lens (NA: 1.2)
(c)
UT
Focusing lens (NA: 0.1)
Correction lens
Acoustic lens
SOL
(d)
Conical lens
Condenser
UT
(e)
Diffuser
Ring UTA
(f)
Linear UTA
Optical fiber bundle
(g)
Diffuser
Hemispheric UTA
(h)
2D scanner
Scanning lens
Fabry-Perot interferometer
(i)
Optical fiber
Catheter
Protective sheath
UT
Laser beam
Acoustic wave
Object to be imaged

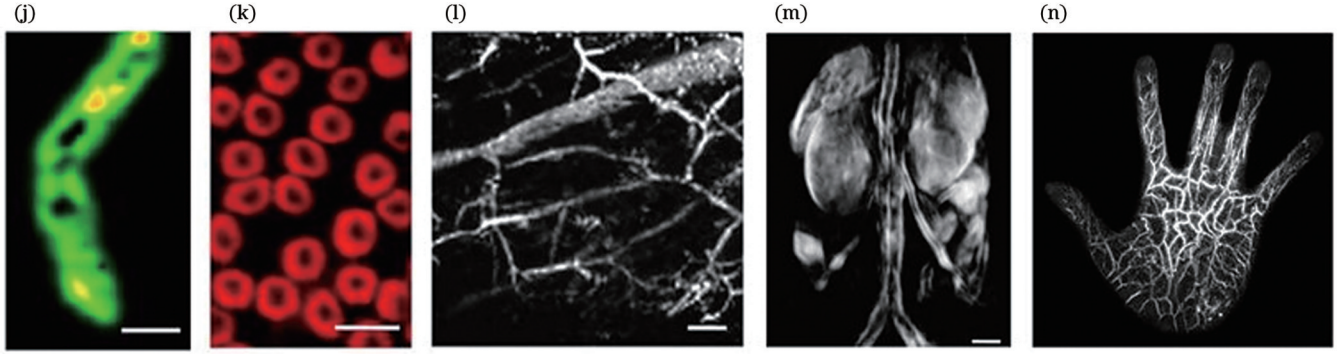

(j)
(k)
(l)
(m)
(n)

图 2 光声成像原理及其代表实现形态，譬如光声断层扫描成像、光声显微镜以及光声内窥镜的示意图[53]。(a)光声成像原理，利用光热的吸收产生超声信号，通过探测超声信号来重建目标处的结构和功能信息；(b)透射式 OR-PAM，超声波换能器(UT)和水浸聚焦透镜位于物体的相对两侧；(c)反射式 OR-PAM，光声耦合器传输光但反射声，SOL 是夹在两个棱镜之间的硅油层；(d)声学分辨率(AR)-PAM，激光束弱聚焦；(e)使用环形超声换能器阵列(ring UTA)的 PACT；(f)使用线性超声换能器阵列(liner UTA)的 PACT；(g)使用半球形超声换能器阵列(hemispherically UTA)的 PACT；(h)使用二维法布里-珀罗干涉仪作为声传感器的 PACT；(i)血管内 PAE，外径为 1.25 mm；(j)线粒体的超分辨 PAM 图像，比例尺为 300 nm；(k)红细胞的 OR-PAM 图像，比例尺为 7 μm；(l) 人手掌皮下血管的 AR-PAM 图像，比例尺为 1 mm；(m)小鼠全身 PACT 图像，比例尺为 5 mm；(n)人手掌皮下血管的 PACT 图像

Fig. 2 Principle of photoacoustic imaging and exampled diagrams of representative implementations of photoacoustic imaging, such as photoacoustic computed tomography, photoacoustic microscope, and photoacoustic endoscope[53]. (a) Principle of photoacoustic imaging, absorption of light and heat leads to ultrasound signals, and structural and functional information of the target is reconstructed by detecting ultrasonic signals; (b) transmission-mode OR-PAM, where object opposing sides are each equipped with an ultrasonic transducer (UT) and a water-immersion focusing lens; (c) reflection-mode OR-PAM, where an optical-acoustic combiner reflects sound but transmits light, and SOL is a silicone oil layer sandwiched between two prisms; (d) acoustic resolution (AR)-PAM, where laser is weakly focused; (e) PACT with ring-shaped ultrasonic transducer array (ring UTA); (f) PACT with linear ultrasonic transducer array (liner UTA); (g) PACT with hemispherically shaped ultrasonic transducer array (hemispherically UTA); (h) PACT with 2D Fabry-Perot interferometer as acoustic sensor; (i) intravascular PAE with outer diameter of 1.25 mm; (j) PAM image of mitochondrion with scale bar of 300 nm; (k) OR-PAM image of red blood cells with scale bar of 7 μm; (l) AR-PAM image of vasculature under skin of human palm with scale bar of 1 mm; (m) whole-body PACT image of mouse with scale bar of 5 mm; (n) PACT image of vasculature under skin of human palm

并转化为电信号以进行信号处理与分析，最终可得到目标组织光学吸收信息的图像信息。光声成像既可以得到超声分辨率的光学信息，也可以使激光照射区域小于声学换能器的接收敏感区域，从而进一步提升光声成像的空间分辨率。因此，光声成像技术不仅具有光学成像的高对比度，还兼具超声成像的深穿透特点[48-52]。此外，基于光声效应，不仅可以通过内源性造影剂进行组织的结构和功能(如新陈代谢、血氧活动等)成像，还可以通过外源性造影剂进行分子和细胞成像[53-59]。2000 年后，光声成像得到了快速发展，目前主要可以分为光声断层扫描成像(PACT)、光声显微镜(PAM)以及结合光学内窥镜技术的光声内窥镜(PAE)[60-63]。

PACT 的工作原理类似于 X 射线断层扫描(CT)[64]，短脉冲的激光束照射到待测组织中，生物组织中吸收光能的物质(如血液、黑色素、外源性造影剂等)产生光声信号，这些光声信号被超声探测器接收并记录下来，然后通过断层扫描的方式，旋转移动激光源与超声探测器，获取多张图像以进行三维重建[65-66]。PACT 的成像深度取决于超声探测器的探测深度，因而成像深度较深，但由于光学信号在组织中的散射，其分辨率只能达到超声的分辨率，无法达到光学的分辨率[67-68]。PAM 成像技术依托于光学焦点扫描，使单脉冲照明区域远小于超声接收的敏感区域，从而实现光学分辨率(OR)的光声成像。近十年，光学分辨率的光声成像的成像分辨率、成像速度、成像深度都有了进一步的提高[69]，但是 PAM 是借助光学聚焦来提升分辨率的特性，故目前还无法在深层组织中获取细胞级别的结构和功能信息。此外，为了解决上文提到的深层组织散射问题，研究者将光声成像技术与光学内窥镜技术相结合，研发出 PAE，在实现微创深入生物组织的同时，利用微型压电换能器检测光声信号，对深层组织进行原位光声成像。目前相关设计已经被用于食道、直肠、泌尿道等成像[70-71]。

2.3 相位补偿

除了改变波长或能量转换的方法外，还可以通过对光场进行调控的方法来补偿被散射的光，增加穿透深度：光在透过生物组织等浑浊介质时，会发生波前畸变，导致成像质量下降，因此，可预先施加波前补偿，以校正生物组织光散射引起的畸变，从而提升成像质量。在本节中，相位补偿的含义主要指直接通过光学器件来测量和补偿光学波前畸变，主要包括光学相位共轭(OPC)和直接的自适应光学(AO)。

OPC 是一种通过相位共轭镜实现时间反演的技术。OPC 通过干涉记录散射介质内部引导星位置的散射光的相位分布，并利用相位共轭镜(即相位共轭晶体)对反方向传播的入射光的波前进行共轭补偿，从而实现等效时间反演，重新将光聚焦到引导星位置[72]。OPC 所使用的引导星可以有多种形式。2008 年，Yaqoob 等[73]采用内部参考光在 0.69 mm 厚的鸡胸肉组织中实现了光学聚焦；2015 年，Liu 等[74-75]基于时间反演超声编码(TRUE)，采用聚焦超声调制的光信号作为引导星，在穿过小鼠耳朵后实现了光学聚焦(图 3)；2023 年，Cheng 等[76]通过结合 OPC 和受激辐射光放大，实现了超过传统 OPC 1000 倍的能量增益。

AO 是一种用于实时校正光学系统中波前畸变的技术，通过测量并补偿成像过程中的相位畸变，实现对光学系统的动态校正[77]。1953 年，Babcock 提出可以通过 AO 补偿地基天文望远镜中由大气湍流等造成的光学波前畸变[78]。随着生物医学应用中光学的不断发展，

目前AO也被用于生物组织成像，校正薄生物组织中由光折射率不匹配带来的畸变[79-80]。AO有直接（使用波前传感器测量相位畸变）和间接（使用软件算法代替波前传感器）两种实现系统。在本节我们探讨使用波前传感器探测波前畸变的直接AO：直接AO通常利用Shack-Hartmann波前传感器来测量相位畸变信息，然后根据相位畸变计算出校正信号，并调整变形镜（DM）的表面形状以补偿波前畸变[81-83]。2019年，Burns等[84]设计了一个自适应光学扫描激光检眼镜（AOSLO），展示了AO用于人体视网膜的成像结果和潜力。

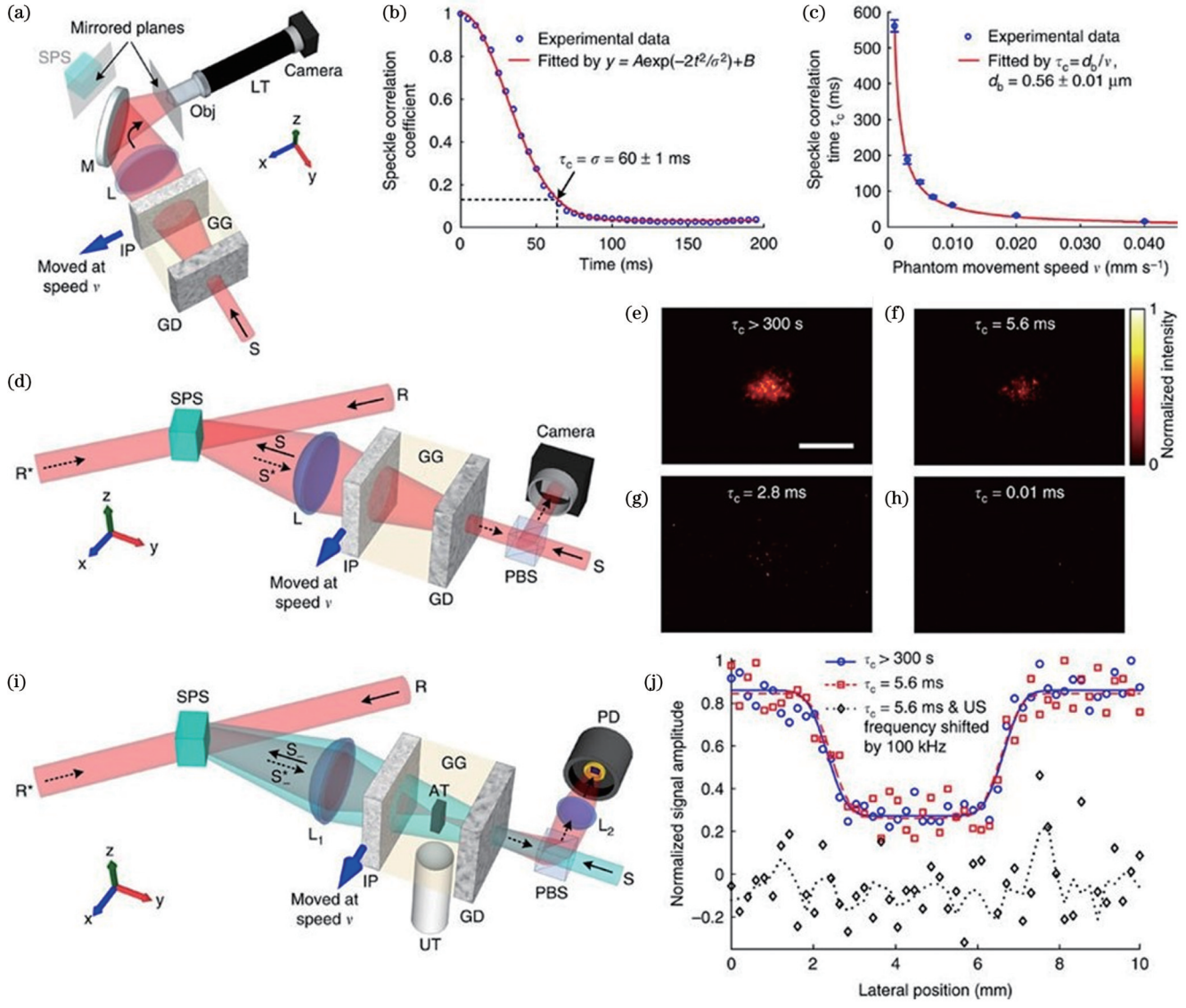


图3 时间反演超声编码（TRUE）光学聚焦实验结果[74]。（a）测量散斑相关时间（τ_c）与仿生模型（脂质-明胶）运动速度之间关系的光路图；（b）仿生模型以0.010 mm/s速度移动时（τ_c=60 ms）的散斑相关系数；（c）τ_c与仿生模型运动速度之间的关系；（d）TRUE的实验装置示意图；（e）仿生模型静止（τ_c>300 s）时的实验结果，图中比例尺为1 mm；（f）仿生模型以0.100 mm/s移动（τ_c=5.6 ms）时的实验结果；（g）仿生模型以0.200 mm/s移动（τ_c=2.8 ms）时的实验结果；（h）信号光频率移动100 kHz（τ_c=0.01 ms）后，没有观察到时间反演光信号；（i）TRUE对吸收性目标成像的实验装置；（j）吸收性目标成像的实验结果

Fig. 3 Experiment results of TRUE optical focusing[74]. (a) Optical path diagram measuring relationship between speckle correlation time (τ_c) and moving speed of phantom model (intralipid-gelatin); (b) speckle correlation coefficient when phantom model moves at 0.010 mm/s (τ_c=60 ms); (c) relationship between speckle correlation time and phantom model movement speed; (d) experimental setup of TRUE; (e) result when phantom model is static (τ_c>300 s) with scale bar of 1 mm; (f) result when phantom model moves at 0.100 mm/s (τ_c=5.6 ms); (g) result when phantom model moves at 0.200 mm/s (τ_c=2.8 ms); (h) no time-inversion light signal is observed after signal frequency is shifted by 100 kHz (τ_c=0.01 ms); (i) experimental setup of imaging an absorptive target with TRUE; (j) experimental result of imaging absorptive target

3 计算光学方法

虽然物理光学的方法取得了一些进展，目前仍存在传统物理模型无法解决的问题，如部分模型无解析解、实际应用需要设计特殊光场等。随着计算机科学的发展，传统物理光学与计算机科学紧密结合，有望突

破传统物理模型的限制，实现一些新的应用。计算光学将计算机算法与光学系统结合，通过联合设计并优化光学系统和算法，可提升光学系统的性能，实现一些传统光学系统无法实现的性能和应用。随着计算机性能的不断增强以及算法的不断发展，计算光学在光学系统设计、成像、通信和信息处理等方面发挥了重要作用。本节我们探讨目前一些应用于深层生物组织光学成像方面的计算光学方法，包括数字相位共轭、迭代波前整形、传输矩阵方法、反射矩阵方法、自相关成像等[85-86]。

3.1 数字相位共轭

前述的光学相位共轭 OPC 依赖于相位共轭材料，譬如光折射晶体，但该器件可以通过计算光学的方法进行替代，从而实现更多应用场景。数字相位共轭（DOPC）是利用计算机算法及空间光调制器（SLM）来实现相位共轭的技术，其基本物理原理与光学相位共轭相同，但不需要光折射材料作为相位共轭镜[87-89]。在 DOPC 中，首先利用全息技术对引导星位置的散射光进行测量并使用相机记录，然后使用计算机基于数字全息方法提取散射光相位并对其进行共轭操作，最后将计算得到的共轭相位加载到 SLM 上，即可对入射光的波前进行调制，以补偿散射介质带来的相位畸变，在散射介质后面或散射介质内部实现聚焦[90-91]。2014 年，Ma 等[92]通过时间反演可控扰动（TRAP）的 DOPC 实现了血红细胞上的光聚焦［图 4(a)～(d)］。2018 年，Yu 等[93-94]通过时间反演磁控扰动（TRMCP）实现了模拟血管中的光聚焦。2021 年，Cheng 等[95]改进了 TRUE 技术，实现了基于超声诱导场扰动的光学聚焦。DOPC 还可以用于聚焦以外的场景，如 2020 年，Li 等[96]利用 DOPC 实现了图像边缘增强。

3.2 迭代优化的波前整形

数字相位共轭 DOPC 需要干涉光路，光学装置较为复杂，这限制了其与常见光学系统的融合与一体化集成。相比之下，通过迭代优化算法，基于较简单的光学系统，即可实现散射介质后面或者内部的光学聚焦及进一步操控。2007 年，Vellekoop 等[98]基于迭代优化的波前整形（iWFS），首次实现了透过强散射介质的光学聚焦，为深层组织中的光学聚焦与成像带来了全新的思路。iWFS 根据反馈信号调整入射光波前的相位，并经过多次迭代不断提升焦点的亮度。iWFS 的光路设计简单，无需参考光路[99-100]，反馈信号形式通常有焦点处光强、聚焦对比度（PBR）、光声信号［图 4(e)、(f)］等[97-101]。在 iWFS 中，迭代优化算法对聚焦的效果有很大影响。由于复杂介质的波前优化是一个非凸优化问题，合适的校正波前不止有一个解，需要算法能够避免局部最优，且能够快速收敛并对环境噪声有较强的抵抗能力[102-105]。

iWFS 领域目前已有很多算法。2021 年，Li 等[106]提出动态变异算法（DMA），增强了散射介质不稳定情况下的聚焦效果。同年，Zhao 等[107]提出无参数优化算法（PFA），实现了对算法所需参数的自动调整。2022 年，Woo 等[108]通过结合遗传算法与蚁群优化（GA-ACO），实现了最优的聚焦效率。然而，这些算法往往需要数十秒甚至数分钟的优化时间，其主要瓶颈在于空间光调制器和相机等硬件的速度过慢，以及计算单元与各硬件之间的通信和数据传输所带来的延迟。因此，基于迭代优化的波前整形目前还无法在生物组织中实现实时聚焦[109]，亟须开发出具有更快响应速度的硬件，通过可编程门控阵列（FPGA）等减少系统延时，实现实时或者活体环境下的聚焦。

3.3 传输矩阵方法

上述迭代优化算法在每次聚焦前都要进行多次迭代，耗时长且无法实现实时成像。然而，如果我们通过数学模型建立散射介质输入和输出之间的关系，即可预先推导出在空间不同位置实现聚焦所需的对应波前补偿图案，从而实现快速光栅化扫描。传输矩阵（TM，$\boldsymbol{M}_{\mathrm{TM}}$）是一个用于描述复杂散射介质的入射与出射光场之间关系的线性数学模型[110-114]。在 TM 模型中，散斑被视为不同入射光场分量的相干叠加。如果输入光场的复振幅矢量为 $\boldsymbol{e}_{\mathrm{in}}$，输出光场的复振幅矢量为 $\boldsymbol{e}_{\mathrm{out}}$，那么 TM 可以表示为 $\boldsymbol{e}_{\mathrm{out}}=\boldsymbol{M}_{\mathrm{TM}}\cdot\boldsymbol{e}_{\mathrm{in}}$[115]。在测量 TM 的过程中，向散射介质投射大量不同的调制光（如哈达玛基）并记录相应的输出，即可根据这些输入和输出光场之间的关系推导计算出 TM，再通过矩阵逆运算推导出实现不同位置聚焦或图案投射所需的补偿波前[116-117]。2020 年，Boniface 等[118]通过对荧光散斑去混叠，获得了散射介质部分 TM，实现了非侵入式聚焦并基于记忆效应实现了成像。2023 年，Cheng 等[119]基于 TM 的交替投影方法进行了相位优化，实现了基于 TM 的图案投影和强光区域抑制。

基于 TM 还可以计算出特征值和特征向量。其中特征向量在散射介质中对应的通道称为特征通道，特征值表示光在散射介质中的放大或衰减程度（较大的特征值对应较高的能量透过率以及较弱的散射或吸收）[120]。根据特征值和特征向量我们可找到具有高能量透过率的光通道，从而提升光在深层生物组织中的穿透深度[121]。2012 年，Kim 等[122]利用散射介质的特征通道，实现了 4 倍的光能量传输。2022 年，Devaud 等[123]通过对时间门限的传输矩阵进行奇异值分解，实现了在不同时间增强或削弱焦点能量。此外，使用对应最小特征值的低传输特征通道，还可以实现透过散射介质的目标区域的强光抑制[124]。

3.4 反射矩阵方法

传输矩阵 TM 的测量需要获得透过散射介质的光反馈信号。然而，在很多生物医学成像场景下，生物组

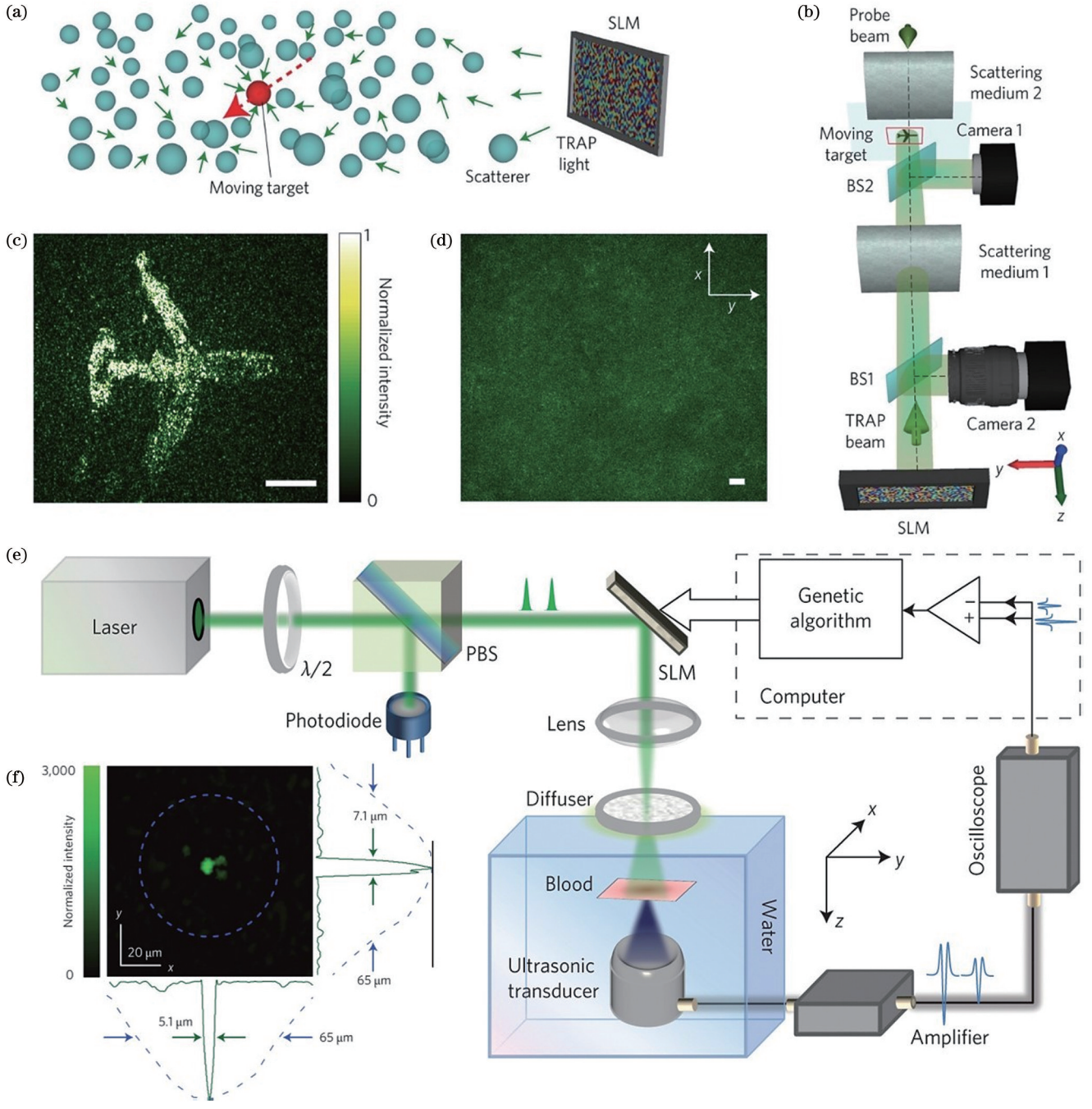


图 4 时间反演可控扰动与光声引导波前整形(PAWS)实验结果。(a)TRAP 的概念图,SLM 调制后的光被聚焦在散射介质内部的运动目标上[92];(b)TRAP 的实验装置[92];(c)TRAP 的实验结果,图中比例尺为 500 μm[92];(d)TRAP 优化前的散斑,图中比例尺为 500 μm[92];(e)PAWS 的实验装置[97];(f)PAWS 的聚焦结果,焦点大小为 5.1 μm×7.1 μm,图中比例尺为 20 μm[97]

Fig. 4 Experiment results of TRAP and photo-acoustically guided wavefront shaping (PAWS). (a) Concept diagram of TRAP, in which SLM-modulated laser is focused onto a moving target inside a scattering medium[92]; (b) experimental setup of TRAP[92]; (c) experimental result of TRAP with scale bar of 500 μm[92]; (d) speckle before TRAP optimization with scale bar of 500 μm[92]; (e) experimental setup of PAWS[97]; (f) focusing result of PAWS in which focus size is 5.1 μm×7.1 μm with scale bar of 20 μm[97]

织深处透射的光信号难以获取,因而目前很难实现基于 TM 的无创深层生物组织成像。然而,在实际应用中,我们可以通过无创方式获得反射或者背向散射的部分光信号,并根据反射光信号预测透射光信号,从而实现无创深层生物组织成像。反射矩阵(RM,$\boldsymbol{M}_{\mathrm{RM}}$)与传输矩阵类似,也是描述散射介质的线性数学模型。当光入射到散射介质时,散射介质不仅会散射入射光,还会将一部分入射光反射,RM 则描述了入射光场与被散射介质反射的光场之间的关系[125-126]。在活体内部的深层生物组织成像中,一般无法直接放置引导星或使用传感器获得反馈信号,因而 DOPC 或 TM 方法的应用受到限制。RM 方法使用反射光替代透射光来构建散射介质成像模型,由于入射光和反射光均在散射介质的同侧,从而避免了侵入式测量[127]。RM 的形

式也与TM类似：如果输入光场的复振幅矢量为$\boldsymbol{e}_{\text{in}}$，输出光场的复振幅矢量为$\boldsymbol{e}_{\text{inreflection}}$，那么光学反射矩阵可以表示为$\boldsymbol{e}_{\text{inreflection}}=\boldsymbol{M}_{\text{RM}}\cdot\boldsymbol{e}_{\text{in}}$[128]。测量RM后，一般不能直接通过矩阵运算实现散射介质后聚焦。现有RM方法集中在深层组织反射成像领域，如光学相干层析成像，通过测量时间选通的反射矩阵，将单次散射光和多次散射光分离，以高分辨重建深层组织反射率分布图像[129-130]。此外，对于透过散射介质成像，研究者也在致力于找到RM与TM之间的关系，利用反射式测量结果推导出TM，从而实现非侵入式的散射介质后聚焦等应用[131]。

2015年，Yu等[126]对散射介质的RM进行了测量，并根据RM的特征值分布提出了增强入射光能量的方法。2022年，Cao等[127]提出了基于RM的OCT（RM-OCT），并实现了光束在9.6倍散射平均自由程（SMFP）深度的聚焦（图5）。但是，现有的基于RM的方法由于依旧需要多次测量，并进行运算求解，无法在速度上匹配生物组织的去相关，故难以实现实际应用。

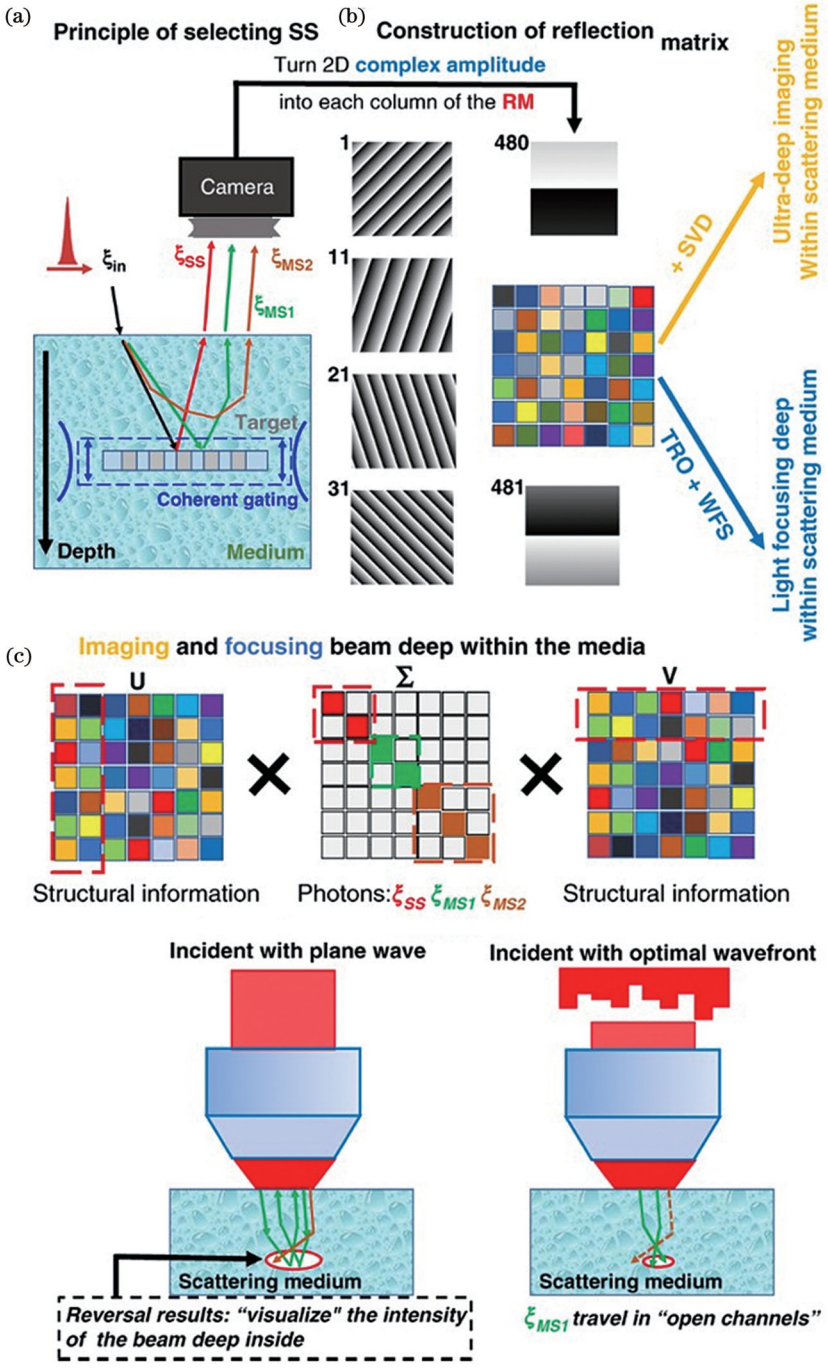


图5 基于RM的光学聚焦[127]。(a)三种类型的背向散射光子；(b)测量反射矩阵；(c)通过时间反演算子分离三种光子，然后利用波前整形在介质内聚焦光线

Fig. 5 Optical focusing based on RM[127]. (a) Three types of back-scattered photons; (b) measuring reflection matrix; (c) separating three types of photons using time-inversion operator, and then focusing light within medium using wavefront shaping

3.5 自相关成像

上述数字相位共轭DOPC和波前整形WFS等方法都是针对相干光进行相位补偿。在非相干光情况下，可以直接利用光学散斑的自相关提取物体信息。自相关成像利用散斑自相关与物体自相关之间的关系，基于散斑恢复输入物体信息。研究表明，在散射介质中存在一个狭窄的范围，输出场随着输入场的平移/倾斜发生相应的平移/倾斜，即光学记忆效应(ME)[132-133]。在ME范围内，由于散射介质的光学传递函数自相关近似于一个二维脉冲函数，因此物体所对应的散斑($\boldsymbol{S}$)自相关($\boldsymbol{S}*\boldsymbol{S}$)可近似视作物体($\boldsymbol{I}$)自相关($\boldsymbol{I}*\boldsymbol{I}$)与噪声项($\boldsymbol{C}$)之和，即$\boldsymbol{S}*\boldsymbol{S}=\boldsymbol{I}*\boldsymbol{I}+\boldsymbol{C}$；而噪声项的贡献较小，一般可忽略，因此可以认为图像自相关与散斑自相关近似相等，即$\boldsymbol{S}*\boldsymbol{S}\approx\boldsymbol{I}*\boldsymbol{I}$[134]。通过计算散斑的自相关，再使用相位恢复(PR)算法，即可重建原始图像信息[135-137]。但由于此方法被限制在ME范围内，且忽略了散射介质带来的自相关结果的变化，其应用目前只限制于一些简单物体图案的重建。2014年，Katz等[138]利用散斑自相关原理实现了非视域(NLOS)成像。2016年，Porat等[139]利用散斑自相关与物体自相关之间的关系，基于不同位置的散斑重建了入射数字图案。2022年，Liu等[140]提出可以将散射介质建模为大量随机排列的小孔，而相应的散斑图案则是这些小孔产生的图像的叠加。基于此模型，他们通过自相关成像，解释了非相干光照下散射介质内部的信息传输机制。

4 深度学习方法

上述基于计算光学的方法通常依赖于物理模型和大量的数值计算，通过对光在散射介质中的传播过程的模拟仿真来实现光场调控，或简单目标(如字母、数字等简单图案)的成像。但当散射介质不稳定，或成像目标太复杂时，计算光学方法的速度无法满足要求，无法有效地恢复图像或进行光场调控。近年来人工智能技术尤其是深度学习(DL)算法的突破性发展，为解决散射介质成像中的复杂问题提供了一个全新且强大的工具。DL通过构建深层神经网络(DNN)来学习并提取大量数据中的输入与输出关系之间的特征，在深层生物组织成像中，可以通过训练DNN来构建输入波前与输出波前之间的映射关系，从而完成更复杂的成像任务[141]。下面将介绍DL在聚焦、成像、染色等方面的应用。

4.1 深度学习用于聚焦

传输矩阵等物理模型对于理解散射介质的输入输出关系有很大帮助，其可以被神经网络模型代替。DNN用于聚焦时，其所依赖的物理模型基于迭代优化的波前整形或TM、RM。传统的iWFS需要进行大量的计算和迭代优化，而DL可以通过训练DNN，学习输入波前与输出波前之间的关系，直接给出所需的波前相位补偿，从而实现更高效的准确聚焦；或将DNN与迭代优化算法结合，可提升抗噪声能力，当散射介质不稳定时，算法仍能够跳出局部最优解，寻找散射介质变化后所需的波前以进行聚焦[142]。2020年，Luo等[143]提出GeneNN网络，可以直接根据散斑给出所需的波前补偿，再利用遗传算法进一步优化焦点。2021年，Luo等[144]提出了时间聚焦光学转换网络(TFOTNet)，通过对网络参数进行自适应调整，实现了在动态散射介质中的快速聚焦。此外，基于TM与RM之间的物理关系，可以通过DNN构建输入散射介质的波前调控信息，以及散射介质透射、反射的散斑信息之间的关系[145]。2022年，Fan等[146]提出了用于深度成像的生成对抗网络(DI-GAN)，DI-GAN可以根据输入到数字微镜器件(DMD)上的调制图案和反射的散斑，预测透射的散斑，再根据此结果进一步预测需要的波前补偿，实现透过散射介质的聚焦(图6)。

4.2 深度学习用于成像

基于iWFS和TM的方法首先实现散射介质后的聚焦，再通过光栅化扫描进行成像，虽可以实现较大的扫描和成像视野，但是迭代优化或者TM测量的耗时较长。随着DNN的发展，我们可以通过预先构建和训练DNN，直接利用散射介质散射的散斑图案恢复原始图像，不再需要进行聚焦和光栅化扫描，可有力简化成像过程，提升成像速度[147-150]。虽然基于TM的方法也可以直接利用散斑恢复原始图像，但是无参考光路的TM方法，由于模型简化，只能实现简单图案的恢复，如果要实现复杂图案的恢复，则需要设计复杂的参考光路记录散射后的波前相位[151]。相比之下，基于DL的散斑成像，利用DNN强大的学习能力和数据驱动的特点，仅需要基于已知的基准真值图像及对应的散斑图案数据训练DNN，即可学习散斑图案与原始图像之间的映射关系，实现无干涉恢复复杂图像[152-155]。

2018年，Li等[156]提出基于U-Net深度神经网络的Deep speckle correlation方法，利用散斑恢复数字图案，且在散射介质变化后，神经网络仍能够利用新的散斑恢复数字，展示了神经网络优异的泛化性能。2022年，Zhao等[157]将光学散斑作为信息加密载体，设计了基于U-Net和全连接层的DNN，利用光学散斑恢复人脸，所恢复的人脸保真度高，可用于人脸识别等(图7)，展示了神经网络在无参考光路时恢复复杂人脸图案的能力。2023年，Li等[158]设计了散斑超分网络，可以对远低于奈奎斯特判据的散斑进行插值，且插值后的散斑仍然能够用于人脸图像的恢复。2023年，Zhang等[159]根据DNN恢复的图像结果，提出散射介质中存在大量信息传输通道，散射介质可被建模成大量传输信息的小孔通道，进一步揭示了波前信息在散射介质中传播的物理通道模型。

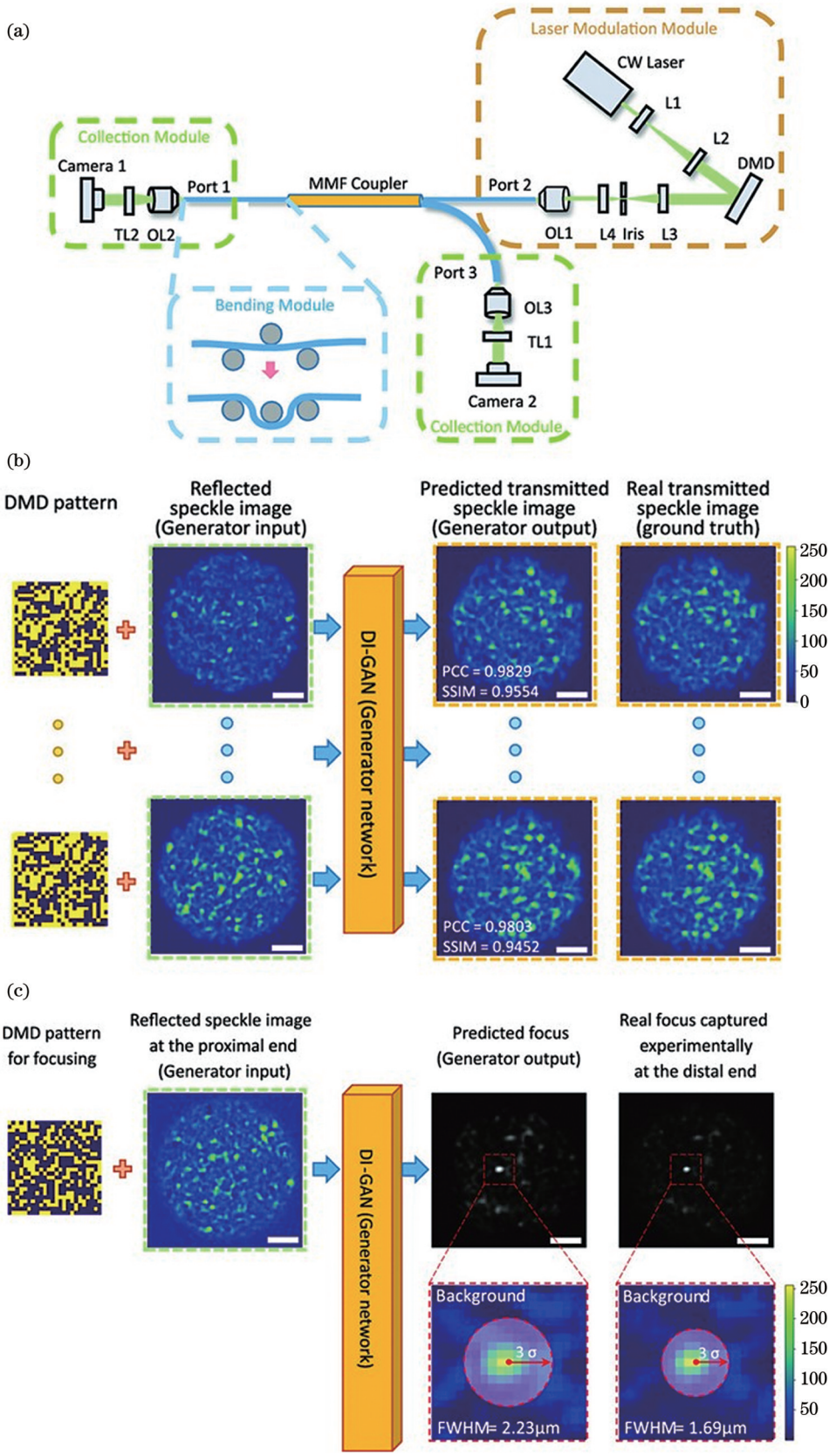


图 6 用于深层成像的生成对抗网络在远端聚焦的实验结果[146]。(a)获取多模光纤透射散斑与反射散斑的实验装置;(b)基于 DI-GAN 使用反射散斑预测透射散斑,图中比例尺为 10 μm;(c)使用 DI-GAN 预测远端散斑后得到的聚焦结果,图中比例尺为 10 μm

Fig. 6 Experimental results of distal focusing of DI-GAN[146]. (a) Experimental configuration for obtaining transmitted and reflected speckle patterns of multimode fiber; (b) predicting transmitted speckle patterns using reflected speckle patterns based on DI-GAN with scale bar of 10 μm; (c) result obtained by using DI-GAN to predict speckle patterns at distal end with scale bar of 10 μm

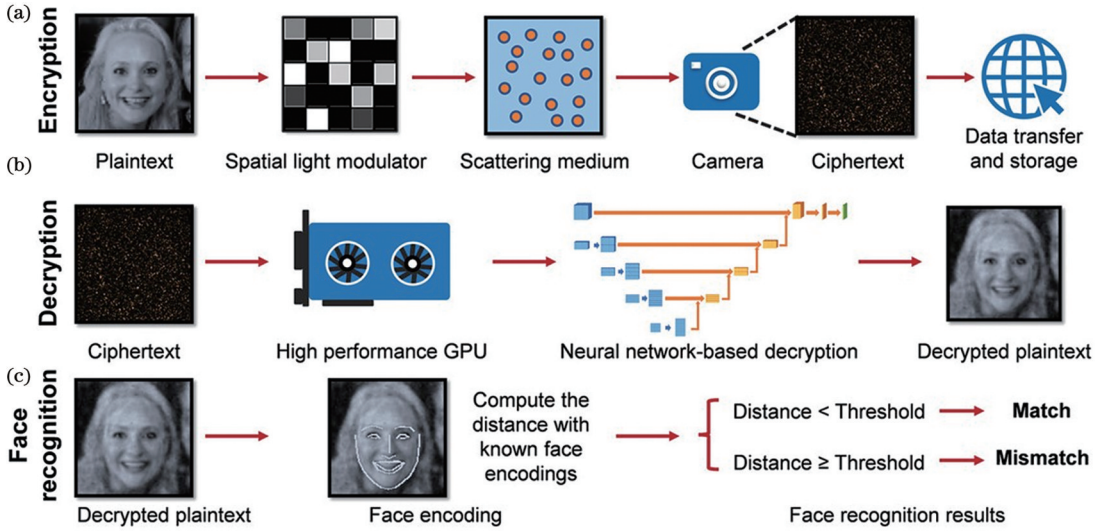


图 7 基于光学散斑的人脸识别加密系统流程图[157]。(a)加密,将人脸图像(明文)加载到空间光调制器上,在激光穿过散射介质后,生成对应的散斑(密文);(b)解密,将散斑输入到预训练的神经网络中进行解密;(c)人脸识别,将解密图像与已知人脸编码进行对比

Fig. 7 Flowchart of speckle-based cryptosystem for face recognition[157]. (a) Encryption, face images (plaintext) are loaded onto SLM to generate corresponding speckles (ciphertext) when light transmits through scattering medium; (b) decryption, speckles are fed into pre-trained neural network; (c) face recognition, comparing decrypted images with known face encodings

除了替代 TM 等方法在散斑成像研究中的应用外,DNN 还进一步推动了散斑成像在散射介质不稳定等情况下的应用。DNN 可以提取不同层次的散斑图案特征和结构,因而有很强的泛化能力。即使散射介质受到扰动,其状态发生改变(散射介质的输入、输出波前的关系发生变化),深度神经网络也能够适应这些变化,并利用散斑图案恢复原始图像。或者通过对散射介质受到扰动后不同状态的散斑进行学习,进一步增强深度神经网络在散射介质变化情况下的泛化能力,故 DL 方法在处理受到干扰的散射介质时,比传统的传输矩阵方法具有更强的鲁棒性[160]。

4.3 深度学习用于染色

基于深度学习模型,还可以对生物组织图像进行虚拟染色。传统的染色方法如苏木精-曙红染色和苏木精-伊红染色(H-E staining)等需要使用特定的染色剂来标记生物组织中的细胞或分子,染色过程涉及多个步骤,染色时间长,且需要昂贵的染色试剂。而基于 DL 的虚拟染色方法通过训练 DNN 来学习图像的染色映射关系,输入未染色的生物组织图像,网络输出标记后的目标区域,实现对生物组织图像的虚拟染色。因此深度学习模型可以实现更快、更低成本的染色。2019 年,Rivenson 等[161]提出了 PhaseStain 数字染色方法,使用 DNN 对无标记的组织切片图像进行染色,实现了与 H-E staining 接近的效果。2022 年,Kang 等[162]将紫外光声显微镜与 DL 相结合,提出了基于深度学习的 Deep-PAM 组织学成像方法,实现了对完整新鲜生物组织的快速染色。由于基于 DL 的虚拟染色过程并不需要昂贵的染色试剂,其染色速度更快,且成本远低于传统染色。此外,虚拟染色方法可以根据需要,灵活调整染色效果,如同时对多种目标区域进行染色,或对传统染色剂无法着色的区域进行染色。但是,与其他基于深度学习的方法一样,用于染色的神经网络仍然需要大量数据进行训练,且对数据质量要求高,需要大量前期工作对数据进行标记等预处理。

5 光纤介入方法

在前面的讨论中,我们在不同维度上探索了克服或抑制深层组织散射的思路。然而,活体生物组织的高度散射以及极小的去相关时间(毫秒级别),限制了这些技术在活体深处实现高分辨率聚焦和成像等应用。传统内窥镜采用内窥的方式,避开生物组织散射所带来的干扰,有助于高分辨率光学技术在目标组织深处的应用。然而,目前广泛应用的内窥镜通常包含电荷耦合器件(CCD)、透镜等传统光学元件,这些元件尺寸较大,在实际应用中难免带来植入损伤。光纤具有直径小(100~200 μm,相当于成年人的头发丝直径)、可弯折、双向光传输等特性,随着光纤光学和光场调控技术的发展,目前已有许多基于多芯光纤(MCF)实现光学内窥的相关研究和商业产品。同时,利用波前整形技术调控多模光纤(MMF)的输出光场,使得基于单根多模光纤实现高分辨率内窥显微成像成为可能,并成为最近的研究热点。接下来我们将介绍光纤介入方法的研究进展。

5.1 光纤荧光内窥镜

光纤荧光内窥镜具有微创、可弯折和成本低等优势,通过将光纤荧光内窥镜插到生物体内部,可以实现对深层组织的高分辨率成像[163-164]。因此,光纤荧光内窥镜为疾病诊断和治疗提供了更准确和方便的手

段[165]。目前常用的光纤荧光内窥镜一般是基于MMF和MCF[166]。MMF包含一个纤芯，能够同时传输多个光模式，因此MMF内窥镜比单模光纤（SMF）内窥镜具有更宽的视野、更大的信息容量[167]。此外，MMF直径与头发丝接近，常见单根MMF直径多为50～200 μm，可以在植入过程中很好地避开血管，从而在进入狭窄的组织时尽可能地降低损伤，且MMF成本很低，因此适合深层组织成像或微创手术[168-169]。但是，由于MMF中同时存在许多光模式，当使用MMF进行图像传输时，不同模式之间的色散、耦合、干涉在输出端表现为类似散斑的图案，故无法直接使用MMF传输清晰图像[170]。如果将MMF也视作一种散射介质，前面提到的克服光学散射进行成像的方法（如传输矩阵等）同样适用于MMF成像[171]。MCF的内部包含数千个纤芯，通过特殊的设计和制造技术，每个纤芯都可以传输独立的光信号，在弯曲的情况下，无需校准即可实现扫描或宽场成像，光信号传输距离比MMF更长。但是MCF会遇到“像素化”问题[172]，且不同纤芯传播的光会在MCF输出端发生干涉，形成类似散斑的光场，因而需要利用上述技术重建图像。

基于计算光学方法，可以通过迭代波前整形，在无透镜情况下，将激光输入MMF，并在深层生物组织中进行聚焦[173]；或测量光纤的TM后，在光纤的视野范围内进行光栅化扫描以重建图像[174]；基于RM的方法也可以应用于无法从MMF远端获得反馈信号的情况，只利用MMF近端的反射散斑实现深层生物组织的光聚焦[146]。基于深度学习方法，可以预先训练神经网络，构建输入波前与输出散斑之间的关系，然后利用散斑恢复图像[175-176]。但是MMF很细且易变形，因此需要设计具有更强泛化能力的神经网络，使训练数据包含MMF的多种状态，进而DNN在MMF变形后仍然能够恢复图像[177]。

2010年，Popoff等[151]通过测定TM控制散射体光传播，实现了基于TM的单根多模光纤成像。2018年，Turtaev等[165]使用MMF内窥镜对小鼠大脑深处进行成像，测量TM后进行光栅化扫描成像，实现了亚细胞分辨率的荧光成像。2022年，Sun等[178]提出了远场散斑幅值转换（FAST）算法，并将其应用在定量相位成像（QPI）中，利用无透镜的超薄光纤束内窥镜实现了对凝胶微粒的三维成像（图8）。2023年，Wen等[179]

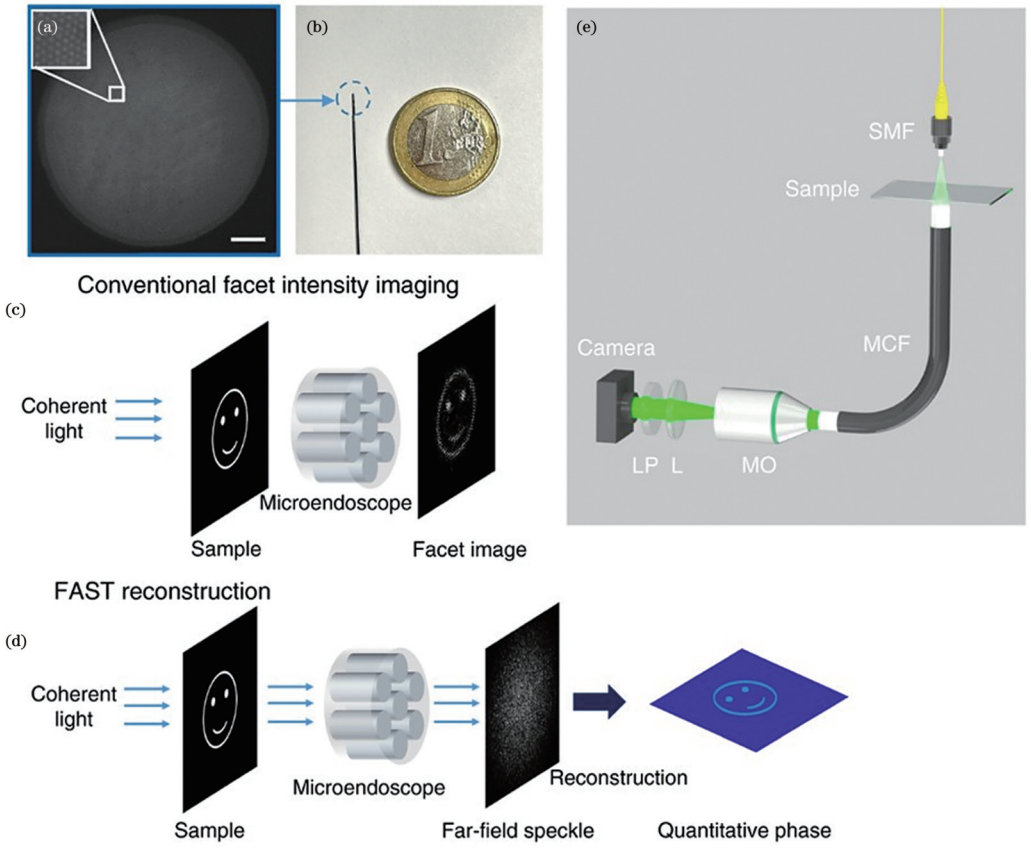


图8 超薄光纤束内窥镜[178]。(a)直径为350 μm的10000芯光纤束，比例尺为50 μm；(b)光纤束和一欧元硬币的对比；(c)传统微型内窥镜成像，样品需要靠近光纤端面；(d)利用FAST算法，样品无需靠近光纤束端面，即可基于远场散斑重建目标图像；(e)实验装置

Fig. 8 Ultra-thin fiber endoscope[178]. (a) Microscopic image of 10000-core fiber bundle with diameter of 350 μm and scale bar of 50 μm; (b) comparison between fiber bundle and one-euro coin; (c) for conventional micro-endoscopic imaging, sample needs to be close to fiber facet; (d) for FAST algorithm, sample does not need to be close to fiber bundle facet, and target images can be reconstructed based on far-field speckles; (e) experimental setup

提出了空间频率域编码追踪自适应信标光场编码(STABLE)方法,将其应用于单根MMF内窥镜,实现了MMF运动状态下的超分辨成像,并展示了小鼠肠道的体内成像结果。

5.2 光纤光声内窥镜

除了上述计算光学和深度学习方法外,基于光声现象对MMF内窥镜进行改进,将PAM技术与MMF相结合,开发出光纤光声内窥镜。2022年,Liang等[180]设计了一种基于光学外差超声探测的光纤光声内窥镜,使用光纤将激发光传导到成像部位,并采用光纤超声传感器将超声信号转换为激光频率变化信号,然后以光外差探测方式读取光声信号(图9)。该光纤超声传感器具有尺寸小(探头直径仅为2 mm)、灵敏度高、不受电磁干扰等优势,适用于深层生物组织中的超声信号检测。2023年,Wang等[181]提出了一种全光学血管内超声(AO-IVUS)内窥镜,利用亚纳秒激光脉冲对碳纳米管和聚甲基丙烯酸甲酯复合材料(MWCNT-PMMA)进行光声激发以产生带宽超声信号,并利用π相移光纤布拉格光栅传感器(π-FBG)探测超声信号,实现血管内成像(轴向分辨率为18 μm,横向分辨率为

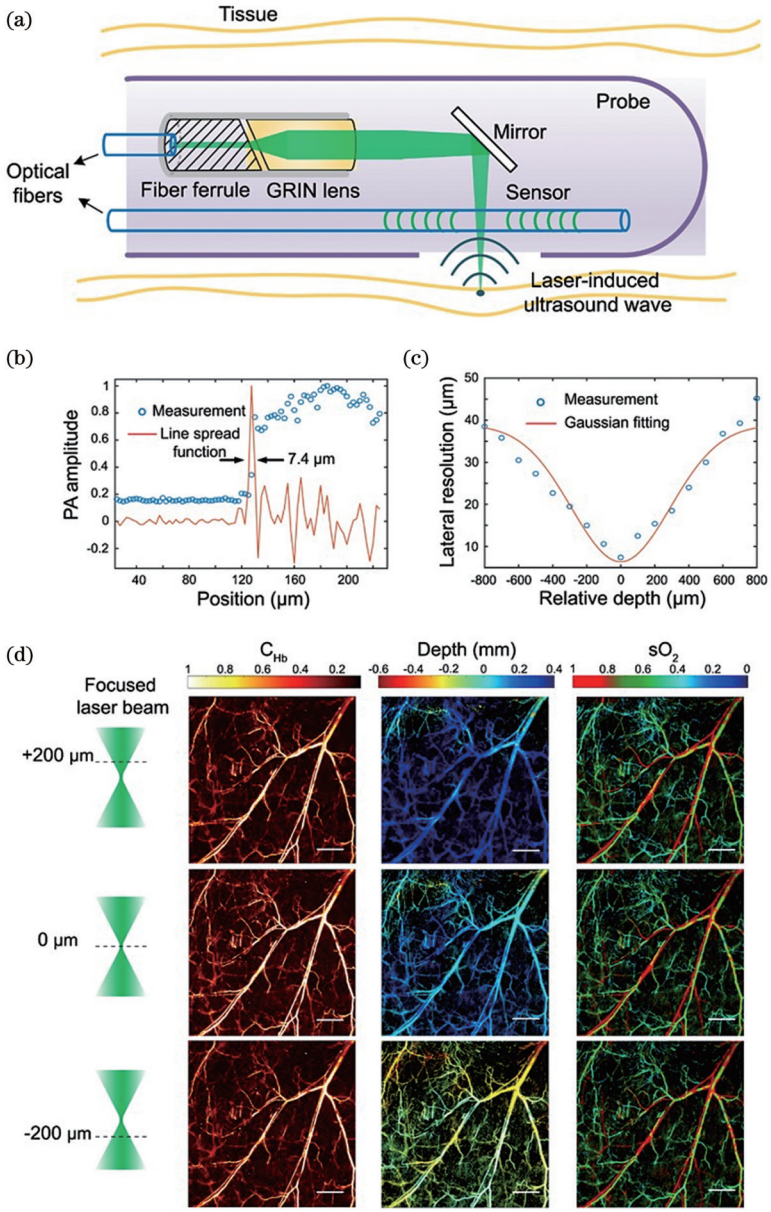


图9 光纤光声内窥镜及成像示意图[180]。(a)内窥镜探头结构图,包括用于激光传递和聚焦的梯度折射率(GRIN)光纤和用于超声检测的激光传感器;(b)焦点处的横向分辨率为7.4 μm;(c)不同深度的横向分辨率;(d)小鼠耳的血红蛋白浓度(CHb)、血氧饱和度(sO_2)以及深度信息标定的血管实验结果,图中比例尺为1 mm

Fig. 9 Fiber-based photoacoustic endoscope and imaging diagrams[180]. (a) Schematic of endoscopic probe, including graded-index (GRIN) fiber for laser delivery and focusing and laser sensor for ultrasound detection; (b) lateral resolution is 7.4 μm at focal point; (c) lateral resolutions at different depths; (d) experimental results of hemoglobin concentration (CHb), oxygen saturation (sO_2), and depth encoded blood vessels of mouse ear with scale bar of 1 mm

124 μm，成像深度为 7 mm）。

5.3 基于光纤的光遗传学研究

基于光纤的光遗传学研究是光纤介入方法在深层生物组织中应用的另一个热门研究方向：光遗传学利用细胞或蛋白质的光响应特性，通过光激发实现对生物体内特定细胞或组织的精准调控。利用光纤我们可以将激光传输到特定目标区域，激活光敏蛋白质并记录其产生的荧光信号，实现对特定细胞或组织的神经活动成像。传统的光遗传学研究采用开颅等方式对大脑进行研究，这意味着无法在大脑正常运行时对神经元进行调控与成像。由于 MMF 直径非常小，我们可以通过微创手术将其植入大脑，在大脑正常活动时对特定的功能区域进行控制和成像，找到大脑中不同的功能区域及其对应的神经环路[182]。借助于上面提到的计算光学方法或 DL 方法，可以使用 MMF 在整个视野内进行光栅化扫描聚焦，对大脑内的神经元进行精准调控或成像，进而揭示大脑的功能奥秘[183]。2017 年，Park 等[184]开发了基于 MMF 的多功能探针，实现了对小鼠前额叶皮层的成像和神经元的调控。2018 年，Ohayon 等[164]通过高速 DMD 研发了基于多模光纤的荧光内窥镜，用于小鼠深脑神经元的活动记录。2022 年，Zhong 等[185]通过 TM 方法操纵 MMF 的输出光场，并通过光栅化扫描实现了脑区深层应用场景下神经元的精准调控（图 10）。

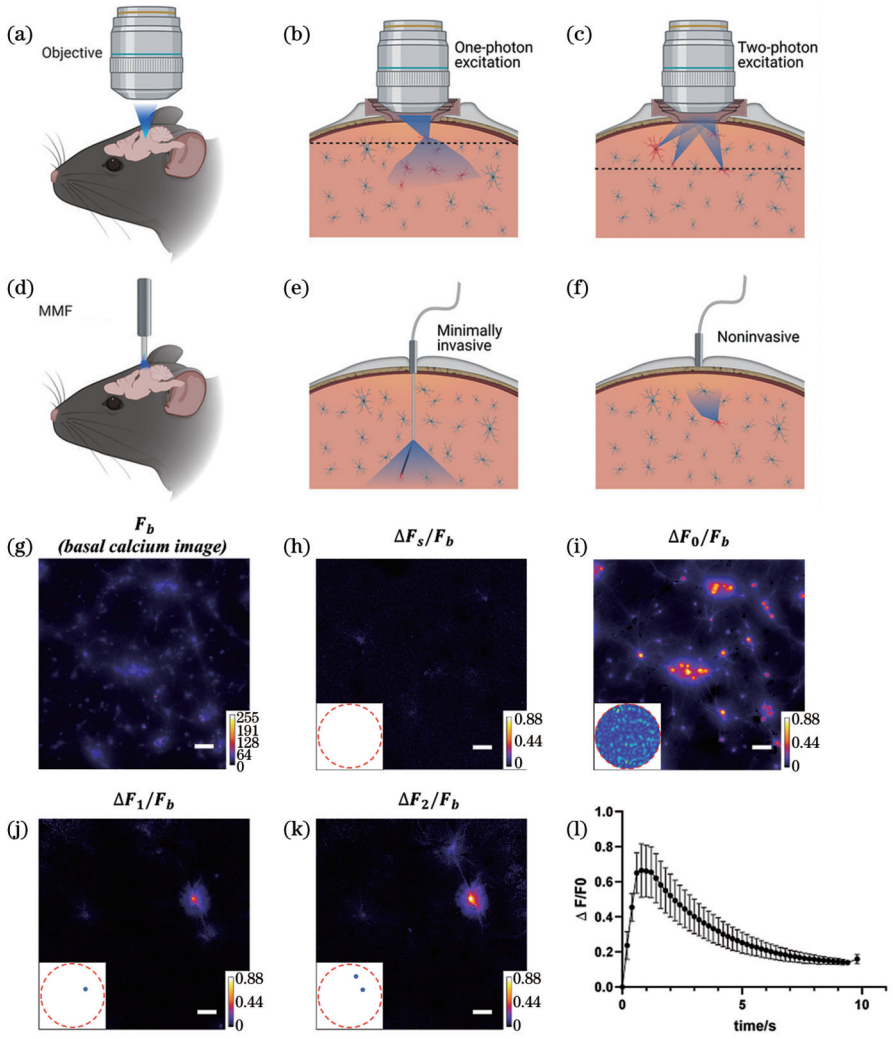


图 10 基于多模光纤的精准光遗传学调控[185]。(a)(b)生物组织的散射导致无法精确调控；(c)双光子激发可以刺激单个目标神经元并增加光子的穿透深度；(d)(e)通过微创多模光纤结合波前整形，可以在大视场中精确调控神经元；(f)结合多模光纤与波前整形，可以透过头骨进行聚焦，实现无创深层神经元调控；(g)～(l)利用波前整形增强的多模光纤，实现选择性光遗传学调控的体外钙离子成像的结果，刻度尺为 50 μm

Fig. 10 Precise optogenetic regulation based on MMF[185]. (a)(b) Scattering of biological tissue results in an inability to accurately regulate; (c) two-photon excitations can stimulate single-target neurons and increase penetration depth of photons; (d)(e) neurons can be precisely regulated in large field of view through minimally invasive MMF combined with wavefront shaping; (f) combined with MMF and wavefront shaping, it can be focused through skull to achieve non-invasive deep neuron regulation; (g)–(l) results of selective optogenetically-regulated calcium ion imaging *in vitro* using MMF enhanced by wavefront shaping with scale bar of 50 μm

6 发展趋势

光学成像因为其非电离辐射、高分辨率、高灵敏度的特性，目前已经在生物医学领域中得到广泛的应用。然而，在深层生物组织成像中，光在组织中的散射问题导致信息的损失和失真，使得光学成像往往面临分辨率与成像深度的抉择难题。本文总结了部分针对这一问题的研究，并从应用的角度，介绍了基于光纤的新型内窥镜工作。此外，本文还介绍了计算光学、深度学习等算法，并从不同的角度提出了针对深层组织散射问题的解决思路。不同方法的性能对比如表1所示。尽管取得了一些进步，深层生物组织光学在实际应用中还有很多的挑战：譬如目前的成像深度和成像速度仍无法很好满足小动物全脑成像的应用需求。与此同时，实现更大视场、降低系统成本等都是未来亟须攻克的技术难点。

表1 不同深层生物组织光学方法的对比

Table 1 Comparison of different optical methods for deep biological tissues

Optical method	Approach	Penetration depth	Resolution	Imaging mode	Real-time imaging
Physical optics	Multiphoton imaging	Millimeter scale	Optical resolution	Point scanning	Y
	Up-conversion imaging	Millimeter scale	Optical resolution	Wide field	N
	Photoacoustic imaging	Centimeter scale	Optical resolution/acoustic resolution	Point scanning/wide field	Y
	Optical phase conjugation	Centimeter scale	Optical resolution	Point scanning/wide field	–
	Direct adaptive optics	Millimeter scale	Optical resolution	Wide field	Y
Computational optics	Digital optical phase conjugation	Millimeter scale	Super-resolution	Point scanning	Y
	Iterative wavefront shaping	Millimeter scale	Super-resolution	Point scanning/wide field	N
	Transmission matrix	Millimeter scale	Super-resolution	Point scanning/wide field	N/Y
	Reflection matrix	Millimeter scale	Super-resolution	Point scanning/wide field	N/Y
	Autocorrelation imaging	Millimeter scale	Optical resolution	Wide field	Y
Learning optics	Deep learning for optical focusing	Millimeter scale	Super-resolution	Point scanning	N
	Deep learning for optical imaging	Millimeter scale	Super-resolution	Wide field	Y
	Deep learning for virtual staining	Millimeter scale	Optical resolution	Wide field	Y
Fiber optics	Fiber fluorescent endoscope	Fiber length	Optical resolution	Point scanning/wide field	N/Y
	Fiber photoacoustic endoscope	Fiber length	Optical resolution	Point scanning	N/Y
	Fiber-based optogenetics	Fiber length	Optical resolution	Point scanning	–

光声成像技术通过将光学高对比度与超声高穿透深度相结合，实现了更深的成像深度。但是PACT无法提供高分辨率的成像信息，且面临在安全激光功率阈值范围内，如何使激发光到达大型实验动物（如兔、猴等）组织深处的技术问题。同时，PAM成像虽然可以提供高分辨率的影像信息，但其依托于高分辨

率光学扫描，需要其他光学技术来辅助增大成像深度。

其次，成像速度也是深层组织成像的关键指标之一。低脉冲能量的PAM成像，往往需要使聚焦超声换能器与光学焦点对齐，并逐点扫描整个视场，这限制了PAM的成像速度。波前整形技术的发展使得控制复杂散射介质中的光传输过程成为可能。基于TM方法，通过一次测量，可以实现输出光场的聚焦与扫描，还可以进一步基于矩阵信息利用散斑提取信息。但无论是迭代波前整形还是TM、RM方法，依托现有空间光调制器，校正所需的时间较长（毫秒至秒量级），无法满足活体生物组织的临床应用。DOPC技术虽然避免了迭代或者TM测量的过程，但针对不同的应用往往需要寻找合适的生物体引导星，且整体调制效率还处于较低的水平，很难在安全阈值内进行活体的临床实践。

深度学习目前已经被广泛应用在深层生物组织光学中。深度神经网络模型具有强大的特征提取能力，可以利用大量数据构建图像与光学散斑之间的非线性模型，基于散斑直接恢复原始图像。深度学习方法还拓宽了散射成像的应用范围，从光场调控、克服光学散射进行成像，到利用散射进行光学加密（图7）[157]、构建光神经网络[186]等。未来相关的研究有望继续将深度学习与深层生物组织光学相结合，进一步推进深层生物组织光学的发展；深度学习模型与计算光学中的物理模型（如TM、RM等）相结合，有望进一步提升成像的质量和速度[187-188]。此外，模型的泛化性弱、训练时间长、训练数据难以获取等问题，有待进一步解决。

上述研究均致力于实现无创深层生物组织成像，在这些技术尚未成熟时，可以先行考虑基于光纤的微创深层生物组织成像。目前基于多芯光纤、多模光纤的深层生物组织成像是一个很有潜力的发展方向，将其与光遗传学调控相结合，并利用上述提到的计算光学及深度学习方法，可研究大脑中的神经环路，有望揭秘大脑的奥秘[189-191]。

7 总结与展望

深层生物组织光学的发展前景越来越广阔，基于物理模型的传统光学与基于计算、数据驱动的深度学习相结合，大大提升了光学系统的成像深度和成像速度。基于理论、模型、数据、应用相结合的光学系统设计，是未来深层生物组织光学的重要发展方向。随着算力的提升以及大模型等深度学习技术的发展，未来深层生物组织光学会出现突破性进展。此外，基于光纤的微创深层生物组织聚焦、成像已经取得了广泛应用，尤其是在光遗传学的神经元调控及成像中已经有多个研究成果，上述深层生物组织光学聚焦、成像的研究成果也可应用于基于光纤的微创深层生物组织研究。展望未来，不断发展的深度学习与传统光学、计算光学、光纤光学相结合，将进一步促进深层生物组织光学的发展，逐步推动深层生物组织光学在医学诊断和治疗中的应用。

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Deep-Tissue Optics: Technological Development and Applications (Invited)

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Abstract

Significance Optics, which is a significant sub-discipline of physics, focuses on the study of the phenomena, properties, and applications of light. Optics has evolved into an independent discipline over time. Optical imaging plays a crucial role in optical research by utilizing the phenomena and properties of light to record images of objects. Optical imaging has extensive applications in diverse fields, including astronomy, medicine, communication, and photography. For example, with the ongoing advancements in biomedical research, optical imaging has progressively showcased its distinctive advantages. First, optical imaging offers high resolution that is free from ionizing radiation, making it safer than X-rays or gamma rays that pose the potential risk of cancer. In addition, optical imaging can be flexibly configured to provide rich biomedical information based on the amplitude, phase, wavelength, polarization, and other characteristics of light. Another advantage of optics is their exceptional sensitivity, which enables the precise and sensitive detection of interactions between light and tissue components or molecules. Finally, the application of contrast agents further enhances the imaging specificity and contrast, thereby improving the visualization of desired targets and opening new avenues for disease diagnosis and treatment.

These have spurred the development of a vast range of high-resolution optical imaging technologies, such as confocal microscope, multiphoton microscope, and super-resolution imaging, which have been achieved by exciting fluorescence signals and/or utilizing gating or nonlinear optical effects in tissue samples. However, these implementations without exception have encountered fundamental challenges in thick biological tissues. This limitation stems from the strong scattering of light in tissue due to the inherent inhomogeneous spatial distribution of the refractive index of the medium encompassing diverse tissue constituents and functions. As a result, when light propagates within biological tissues, the light beam spreads quickly and is accompanied by the accumulated scattering of light (approximately one scattering event per 0.1-mm optical path length at visible wavelengths), which also rapidly weakens the intensity of non-scattered light *in situ*. In combination, these result in an intrinsic trade-off between spatial resolution and penetration depth for optics in biological tissues. This is also why optical techniques that utilize ballistic or quasi-ballistic photons typically have an effective penetration depth of less than or approximately 1 mm beneath the skin, which corresponds to 10 times the transport mean free path in the visible and near-infrared regimes. Excessive laser power may further enhance tissue penetration depths, but it also poses a risk of damaging biological tissues, particularly the skin and subsurface.

In the past two decades, numerous studies have been conducted to address these challenges, including switching to longer wavelengths to obtain lower tissue scattering coefficients, converting diffused light into non-scattered ultrasound at the signal detection side, and creating a minimally invasive optical path via ultrathin fibers to deep tissue regions. We believe that summarizing these advancements is not only worthwhile, but also critical for inspiring further research aimed at greater penetration depths and faster speeds toward wider applications.

Progress In this review, we summarize the recent efforts in deep-tissue optics from various perspectives based on the mechanism of operation, including physical, computational, learning, and fiber optics. Note that this is not a complete list but only an empirical one.

Regarding physical-optics-based efforts, relevant research has primarily focused on the three aspects of wavelength engineering, energy conversion, and phase compensation. Wavelength engineering, such as multiphoton imaging and up-conversion imaging, involves the transformation of the input light wavelength into a different output wavelength to enhance the penetration depth. In multiphoton fluorescence imaging, two or more photons with longer wavelengths but lower energies are absorbed almost simultaneously before exciting the target fluorescent molecules at depth, generating one photon with shorter wavelength but higher energy. The longer wavelength in excitation and elevated photon energy in emission both contribute positively to the increased

penetration depth for imaging. Up-conversion imaging entails the sequential absorption of multiple low-energy photons and their conversion into a single high-energy photon, thereby increasing the penetration depth.

Among approaches based on energy conversion, the photoacoustic (PA) effect, which converts input pulsed light into ultrasonic waves, has been extensively studied. When a biological tissue absorbs light energy, it undergoes thermal transformation, leading to localized expansion in the region of interest. Conversely, when the optical illumination is switched off, the local temperature decreases, causing the tissue region to contract. When the activation and deactivation of optical illumination (such as pulsed light) are manipulated, the expansion and contraction of tissues can be controlled, generating periodic mechanical waves in the ultrasonic frequency (MHz) range. These are usually referred to as photoacoustic or optoacoustic signals and are detected by one or an array of ultrasound transducers positioned outside the tissue sample. Because the generation of PA signals relies on the optical absorption of light, optical absorption contrast is obtained in PA imaging. However, the generation of signals does not distinguish between ballistic or diffused photons, and the detection of signals is based on ultrasound, which scatters much less (~1/1000) than light in the tissue. In combination, these features lead to a considerably boosted balance between imaging resolution and penetration depth and enable many exciting applications that are not possible with pure optical technologies.

In phase compensation, optical devices are utilized to measure and compensate for the optical phase distortion induced by light scattering. One representative example of phase compensation is optical phase conjugation, which captures the phase distortion of the wavefront emitted by a guide star within the scattering medium and compensates for it by conjugately adjusting the incident wavefronts and then refocusing light onto the position of the guide star. The phase-conjugation mirror, which is typically a photorefractive material, is responsible for recording the incident wavefront pattern and generating conjugated light that propagates along the optical path opposite the original transmission path.

Computational optics is an interdisciplinary field that merges optics and computers to leverage physics and algorithms, thereby enabling applications beyond those that can be achieved using traditional optical systems. The primary computational optics-based efforts in deep-tissue optics include digital optical phase conjugation (DOPC), iterative wavefront shaping, and transmission and reflection matrices. In DOPC, the phase-conjugation mirror previously discussed is replaced by the integration of a digital camera, computer, spatial light modulator, and algorithms for determining and generating the phase-conjugated wavefront. In iterative wavefront shaping, the phase of the incident light wavefront is adjusted based on feedback signals and the focusing performance is iteratively optimized. Feedback signals can take various forms, such as focal intensity, peak-to-background ratio (PBR) in the captured pattern, and photoacoustic signal strength. In the transmission matrix, a linear mathematical model is used to describe the relationship between the incident and scattered output wavefronts to characterize the scattering medium. If we denote the input wavefront as $\boldsymbol{e}_{\mathrm{in}}$ and the output wavefront as $\boldsymbol{e}_{\mathrm{out}}$, the transmission matrix ($\boldsymbol{M}_{\mathrm{TM}}$) can be characterized as $\boldsymbol{e}_{\mathrm{out}}=\boldsymbol{M}_{\mathrm{TM}}\cdot\boldsymbol{e}_{\mathrm{in}}$. By measuring the transmission matrix, we can focus the diffused light, project specific patterns through a scattering medium, or retrieve images from speckles. The reflection matrix establishes the relationship between the incident and reflected wavefronts from a scattering medium. In deep tissues, it is typically impractical to define or position guidestars or obtain guidestar signals within or on the opposite side of a tissue sample. Thus, applications of transmission matrices are limited. The introduction of a reflection matrix addresses this challenge by utilizing a reflected wavefront instead of a transmitted wavefront. In this scenario, both the incident and reflected light detectors are present on the same side of the scattering medium, thereby circumventing the need for guidestars to be placed on the opposite side of the scattering medium.

These computational optics-based efforts typically rely on intricate physical models to achieve the focusing or imaging of simple targets, such as letters, numbers, and other basic patterns, through scattering media. With recent advances in artificial intelligence, complicated problems involving speckles can now be addressed using deep learning. For example, deep-learning-based speckle imaging has powerful learning capabilities and data-driven characteristics. Deep neural networks can be trained using known data pairs, including ground-truth images and corresponding speckles, to extract various dimensions of information features. This can enable the high-fidelity reconstruction of target images, such as human face images. In addition, by training the speckle patterns obtained under different states of perturbed scattering media, the generalization capabilities of deep neural networks can be further improved, and the robustness of handling perturbed scattering media exceeds that of transmission-matrix-based methods.

In addition to these endeavors, which are all aimed at noninvasive deep-tissue optics, minimally invasive solutions that employ ultrathin optical multimode fibers as light guides into the tissue are also attractive and have seen promising advancements in recent years. Multimode fiber-based imaging is advantageous due to its minimally invasive nature, flexibility, and affordability. However, because of mode dispersion and coupling within multimode fibers, the optical field output from the fiber appears to be similar to a speckle pattern from tissue-like scattering media, making it infeasible to directly interpret the transmitted spatial information. Nevertheless, if multimode fibers are treated as scattering media, the aforementioned wavefront shaping approaches can be applied to multimode fibers. Thus, with the integration of wavefront shaping, the speckled output from a lensless multimode fiber can be focused onto a single optical mode, and then the raster can scan at a high speed within the field of view of the fiber. The excited or responding signals can also be detected and relayed using the same fiber for further use. This creates a scenario very similar to laser confocal microscope, except that the probe is inserted deep into the tissue. As a result, spatially and/or temporally resolving optical signals from deep tissues can be excited and detected with high resolution, which opens avenues for exciting new optical practices that require high resolution at depths in tissue. This capability can also be extended beyond imaging, such as for optogenetics, where wavefront

shaping-empowered multimode fibers can deliver light precisely to targeted neurons within deep tissues and pick up fluorescence signals reflecting neuronal activities, enabling precise activation or inhibition of neurons to study brain functions.

Conclusions and Prospects Optics have gained significant attention in the study of deep biological tissues due to their non-ionizing radiation, exceptional contrast, exquisite specificity, and heightened sensitivity. In addition, the integration of computational optics and deep learning with conventional optics has substantially enhanced penetration depths while preserving moderate resolution in deep biological tissues. Despite these remarkable advancements, the practical implementation of deep-tissue optics still encounters critical challenges that must be addressed before moving forward.

The first is the penetration depth. With photoacoustic efforts and wavefront shaping techniques, which are sometimes further aided by computational optics and deep learning, current practices have achieved high-resolution optical focusing and/or imaging far beyond the optical diffraction limit. While most experimental research efforts to date still concentrate on small animal models such as mice, future studies are anticipated to improve the depth capability and extend to large animal models such as rabbits and monkeys. This transition is necessary for assessing the practicality, safety, and reliability of clinical diagnostics and therapeutic applications before working with human patients.

Speed is another crucial factor in the operation of deep-tissue optics. To reverse or compensate for the scattering-induced wavefront distortion, the scattering medium or multimode fiber should theoretically remain stationary to maintain the medium status, equivalent to the transmission matrix, during the wavefront optimization process. However, in practical applications, this requirement is hardly met, particularly for living biological tissues, whose optical field decorrelates rapidly on the order of milliseconds or even faster due to factors such as blood flow and respiration. Although some operations based on physical optics, such as optical phase conjugation, can reach this time scale, the majority of wavefront shaping implementations to date, consume seconds or hundreds of milliseconds, which is mainly limited by the response rate of the hardware such as spatial light modulators.

Over the past few years, deep learning has significantly affected deep-tissue optics. By leveraging the power of deep neural network models, it excels in extracting features and establishing nonlinear relationships between the target information (the ground truth) and the corresponding speckles, enabling high-fidelity retrieval of the original information from speckles. In addition, the use of deep learning has expanded the scope of speckle imaging, enabling breakthroughs in scattering, virtual staining, optical encryption, optogenetic networks, *etc*. The integration of deep learning with deep-tissue optics is expected to improve the speed, penetration depth, and immunity to system and medium disturbances. In addition, the combination of deep learning with physics-based scattering models holds great potential for accurately understanding and modeling multiple scattering processes, which is essential for designing efficient computation algorithms.

Finally, noninvasive deep-tissue optics *in vivo* still remains limited in some respects and may require a few more years to achieve technical maturity. Accordingly, a temporary yet effective alternative is to integrate wavefront shaping with ultrathin multimode fibers. Because the diameter of the multimode fiber can be 100–200 μm, close to the typical hair diameter of adults, this integration can create a minimally invasive optical path into deep biological tissue, enabling high-resolution and fast-scanned optical focusing, imaging, stimulation, and manipulation at depths in tissue. Although it is not a perfect solution, it is practically useful in many studies, particularly for those at the early and preclinical stages, or when the insertion of a fiber-based probe is accompanied by invasive surgery , and the insertion of the probe does not considerably increase the degree of invasion or discomfort to the patient.

The developments to date in this field have demonstrated the feasibility and potential of deep-tissue optics. With continuing efforts and progress in related areas, technical barriers, such as the speed bottleneck associated with the response rate of spatial light modulators and the insufficient generalization capability of neural networks, can be overcome. It is strongly envisioned that in the near future, deep-tissue optics will reach practical maturity and be usable *in vivo*, which can extend many exciting optical applications to tissue regions that are currently optically inaccessible. This could reshape the landscape of light use in biomedicine and many other areas.

Key words bio-optics; optical imaging; biomedical optics; deep tissue; optical wavefront shaping; photoacoustic imaging