中国激光

共聚焦内窥显微成像技术及其应用

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摘要 在过去的几十年中，内窥镜已被用于以微创或无创的方式观察人体内空腔内部或人体内部器官表面，以进行诊断或治疗。然而，临床上常用的普通白光内窥镜和放大内窥镜的分辨率低、对比度差，需要通过病理活检来确诊。近年来，新应用于临床的窄谱技术通过光学或数字滤波的方式利用蓝光照射组织，以强化黏膜表面的细微结构和微血管形态，提高成像对比度，但仍未解决成像分辨率低的问题。因此，白光和窄带光内窥镜无法实现真正的光学活检，严重降低了诊断的准确性。共聚焦内窥镜由于分辨率可达亚微米量级并且具有光学切片的能力，可以呈现出与病理活检高度一致的细胞形态。共聚焦内窥显微成像技术在消化道、皮肤、眼部等疾病的诊断方面具有重要作用。本文对共聚焦内窥显微成像技术进行了简述，主要对荧光共聚焦显微成像和反射式共聚焦显微成像、探头式共聚焦内窥成像技术和整合式共聚焦内窥成像技术进行介绍，讨论了共聚焦内窥显微成像技术在生物医学领域的应用。

关键词 显微；内窥镜；共聚焦显微成像；分辨率；光学活检

中图分类号 O439 **文献标志码** A **DOI**：10.3788/CJL202249.1907002

1 引 言

在光学显微镜中，照明光在整个视野中应尽可能均匀地穿过样品。对于较厚的样品，如果物镜没有足够的焦深，来自焦平面上方和下方的样品平面的光就会被检测到[1]。失焦的光线会增加图像的模糊度，从而降低分辨率。在荧光显微镜中，视野中的任何染料分子都会受到刺激，包括离焦平面中的染料分子[2]。共聚焦显微技术利用共聚焦系统有效地排除了焦面以外光信号的干扰，提高了分辨率，实现了光学切片。目前，共聚焦显微成像技术是生物医学领域非常重要的分析工具，借助该技术，研究人员能够对细胞中的特定成分进行光学切片和三维(3D)重建。

自 20 世纪 60 年代引入柔性胃肠(GI)内窥镜检查以来，内窥镜成像技术不断取得进步。在过去的几十年中，内窥镜已被用于以微创或无创的方式观察空腔内部或人体内部器官的表面，以进行诊断或手术[3-4]。但目前临床上常用的白光和窄带光内窥镜无法达到细胞水平的分辨率，因此无法实现真正的光学活检，严重降低了诊断的准确性。共聚焦显微成像技术的分辨率可以达到亚微米级别并且具有光学切片的能力，它可以呈现与病理活检高度一致的细胞形态。自 2004 年问世以来，共聚焦激光内窥镜(CLE)技术已成为胃肠成像的重要工具。基于该技术，内窥镜医师可以在焦平面处进行细胞成像和组织结构评估，获得实时的体内组织学信息，从而实现“光学活检”[5-6]。这一技术为体内组织学研究提供了快速、可靠的诊断工具，使内窥镜的临床应用前景更为广阔。

2 共聚焦显微成像的基本原理

共聚焦显微成像技术[7]是 Minsky 于 1957 年首次提出的，通过在同一共轭图像平面上使用照明侧和检测侧针孔来实现“共聚焦”。1967 年，Egger 和 Petrán[8]首次成功地将共聚焦显微成像技术用于神经组织的无标记成像。在共聚焦显微成像技术中，单色激光经过一个照明针孔后形成点光源，一个物镜将点光源聚焦到样品上，在探测端由另一个物镜将来自样品的信号聚焦到探测针孔处，如图 1 所示。在这个过程中，使用单光束扫描的方式使点光源在样品上进行逐点扫描，实现二维成像，这就是激光扫描共聚焦显微镜(LSCM)的工作原理[9]。共聚焦显微成像技术的关键在于两个针孔的“双聚焦”可以屏蔽所有来自非焦面的信号，在探测针孔后的光电倍增管只能探测到来自焦平面的信号。因此，该技术具有光学切片的能力[10-11]。

在光学显微镜中，分辨率由物镜的数值孔径、样品的特性(折射率)和光的波长决定。当针孔接近最小尺寸时，共聚焦显微镜的横向分辨率比传统的宽视场荧光显微镜有所提高，可以达到衍射极限。在宽视场荧光显微镜中，用于确定横向分辨率的公式[10,12]为

收稿日期：2022-05-23；**修回日期**：2022-07-12；**录用日期**：2022-07-14

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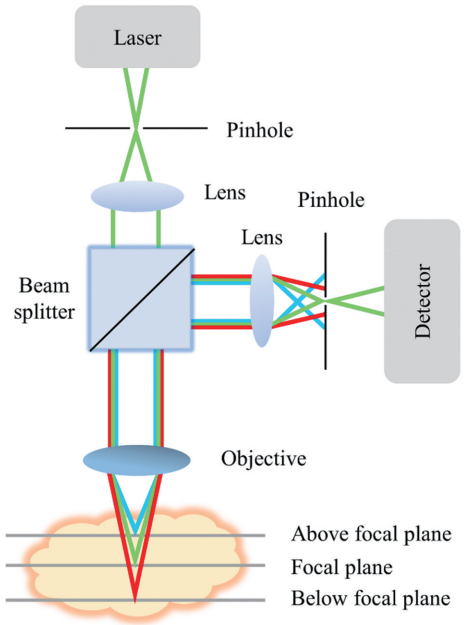


图1 共聚焦显微技术的原理

Fig. 1 Schematic of confocal microscopic technology

$$R_{\text{Lateral}}=\frac{0.51\lambda}{NA},\tag{1}$$

而共聚焦显微镜横向分辨率的计算公式为

$$R_{\text{Lateral}}=\frac{0.37\lambda}{NA},\tag{2}$$

式中：R 是分辨率；λ 是发射光波长；NA 是物镜的数值孔径。这表明，在横向平面上，宽视场荧光显微镜的分辨率约为共聚焦显微镜的$\sqrt{2}$倍[12]。

尽管存在针孔，但共聚焦显微镜的轴向分辨率仍比横向分辨率差。在纵向平面上，宽视场显微镜纵向分辨率的计算公式[10,12]为

$$R_{\text{Axial}}=\frac{0.89\lambda}{n-\sqrt{n^2-(NA)^2}},\tag{3}$$

而共聚焦显微镜纵向分辨率的计算公式为

$$R_{\text{Axial}}=\frac{0.64\lambda}{n-\sqrt{n^2-(NA)^2}},\tag{4}$$

式中：n 为介质的折射率。可见，宽视场显微镜在纵向平面上的分辨率约为共聚焦显微镜的1.4倍[2,10]。

根据图像对比度的来源，激光扫描共聚焦显微镜可以在荧光或者反射模式下工作。荧光共聚焦显微镜需要使用荧光造影剂来产生对比，可以得到有关内源性自发荧光以及外源性标记分子和结构的空间和功能信息[10-11,13]；而反射式共聚焦显微镜则依赖于细胞结构的折射率差异产生自然对比度[14-16]。接下来本文将对荧光共聚焦显微成像技术和反射式共聚焦显微成像技术分别进行概述。

2.1 荧光共聚焦显微成像

在荧光共聚焦显微镜中，光照射并激发样本中的荧光团，被激发的荧光团发射较低能量的荧光，用滤光片将发射光滤除，只探测荧光信号[17-19]。对应不同的激光波长，每个荧光团都有一定的激发和发射最大值。由于发射的荧光光谱相对于吸收光谱具有更长的波长，因此荧光光谱通常会发生红移[18]。

荧光显微镜的一个限制是在光照时荧光团会由于光漂白而失去发射荧光的能力[18,20]。此外，虽然使用荧光共聚焦显微镜可以对活细胞进行分析，但容易产生光毒性[21]，尤其是使用短波长光进行照射时。并且，荧光分子在被照射时往往会产生反应性化学物质，这会增大光毒性效应[19]。荧光共聚焦的另一个限制是对已被荧光标记的特定结构进行成像，然而，在许多生物样本中，自发荧光会增强背景，并且无法完全利用滤光片来解决这一问题[9]。

2.2 反射式共聚焦显微成像

荧光共聚焦显微成像技术需要使用荧光染料才可以实现胃肠、皮肤和眼科等疾病的快速诊断。与荧光共聚焦显微成像不同，反射式共聚焦显微成像无须对组织进行固定、染色等特殊处理，可以直接在完整的活体内进行成像，也可以在体外对新鲜组织进行无标记成像[5,16]。反射式共聚焦显微成像的机理：组织微结构和单个细胞有不同的光学反射指数，会产生强度不同的反射光，这些反射光可以被连续地转换成不同灰度级的数字图像。在反射式共聚焦显微成像中，具有高折射率的结构的亮度较大，而具有低折射率的结构较暗，这使得该技术无需任何标记就可以使图像具有自然对比度，从而得到组织结构的信息[15]。

反射式共聚焦显微成像用于生物医学成像的最大优势是其能够对未标记的活体组织进行成像。该技术在早期就已被用于人体和动物组织的在体成像，也已被证明有望成为临床诊断的有效工具。日本Hiroshima大学的Nakao等[22]采用单波长反射式共聚焦内窥镜发现癌组织中的细胞膜和细胞核等结构的异常变化具有应用于胃和食管癌早期诊断的潜力。在眼科疾病的诊断方面，反射式共聚焦显微成像已被用于对眼角膜和视网膜血管的成像[23]。在皮肤科，反射式共聚焦显微成像可用于对皮肤病患者的不同皮肤状况进行表征，并且可以在体进行皮肤病经激光治疗后的临床监测[24]。在耳鼻喉科，反射式共聚焦显微内窥镜已被证明有望为口腔疾病的恶性转化提供筛查依据[25]。

3 共聚焦内窥显微成像技术

根据扫描方式的不同，共聚焦内窥镜主要分为两种模态：整合式共聚焦内窥镜和探头式共聚焦内窥镜。整合式共聚焦内窥镜采用远端扫描模式[26]，如图2(b)所示，探头式共聚焦内窥镜采用近端扫描模式[26]，如图2(a)所示。

3.1 整合式共聚焦内窥镜(eCLE)

eCLE采用的是远端扫描模式，即扫描装置位于

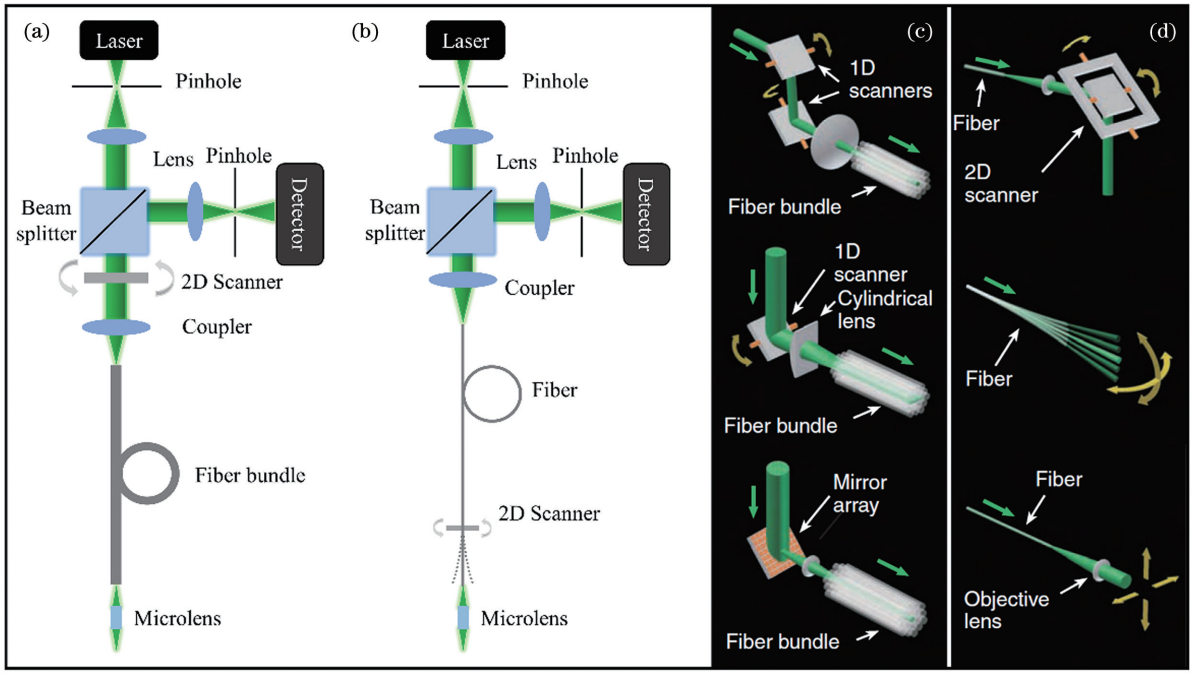


图2 不同模式的共聚焦内窥镜的原理及其对应的扫描机制[25-26]。(a)(c)在光纤束近端扫描的探头式共聚焦内窥镜及其近端扫描机制;(b)(d)在单根光纤远端扫描的整合式共聚焦内窥镜及其远端扫描机制

Fig. 2 Principle of confocal endoscope with different modes and their scanning mechanisms[25-26]. (a)(c) Probe-based confocal endoscope with scanning at proximal end of fiber bundle and its proximal scanning mechanism; (b)(d) integrated confocal endoscope with scanning at distal end of single fiber and its distal scanning mechanism

光纤末端。eCLE系统由单根光纤、产生机械振动的器件、套管和反向散射信号的检测器组成。目前,该系统的机械扫描方式主要有两种:1)利用微机电系统(MEMS)进行二维扫描[27-28];2)压电材料驱动器驱动光纤悬臂偏折或振动[29]。

3.1.1 MEMS扫描装置

MEMS扫描镜是一种基于MEMS技术制成的可驱动反射镜,其镜面直径通常只有几毫米。与传统的光学扫描镜相比,MEMS扫描镜具有重量轻、体积小、易于大批量生产和生产成本较低等优点。根据扫描镜的运动方式,MEMS扫描镜可以分为谐振式和准静态两种。谐振式MEMS扫描镜在机械谐振状态下工作,具有扫描角度大、驱动功耗和扫描电压低等特点,主要应用于图像化激光扫描和激光成像。准静态MEMS扫描镜工作于非谐振状态,可以在扫描角度范围内的任意扫描角度下暂停,主要应用于激光指向、激光矢量化图形扫描,但其扫描角度范围相对较小。1994年,Dickensheets和Kino[30]使用共振悬臂对与菲涅耳波带片微型物镜相连的单根光纤进行扫描,开创了基于MEMS的共焦内窥镜扫描仪领域。后来,他们在单光纤内窥镜中首次使用了微机械硅扫描镜[31]。MEMS反射镜在扫描速度和批量制造方面具有很高的灵活性,但是内窥镜内的成像光路通常为折叠路径,导致探头直径在4~5.8 mm范围内,这使得MEMS反射镜在前视应用的紧凑封装方面仍然存在一定局限性[32]。2016年,Seo等[33]利用电热硅微致动器驱动MEMS光纤扫描仪实现了二维Lissajous图案扫描,热臂和冷臂微结构之间的热膨胀差异导致光纤表现出耦合的双向运动。在此基础上,Seo等使用倒装芯片键合电热MEMS光纤扫描仪,使其进一步小型化,完全封装在1.65 mm直径的共聚焦内窥镜导管内,并在16 V工作电压范围内实现了378 μm×439 μm视场(FOV)的Lissajous图案扫描[34]。

3.1.2 压电材料驱动光纤扫描

远端扫描方式通过压电材料驱动光纤进行扫描。在这种扫描方式中,光纤端部的任何移动都会转化为聚焦点的横向位移,通过压电材料使光纤尖端进行偏转,即可实现聚焦平面上的二维扫描[35-38],如图3所示。压电陶瓷管的外表面有两对正交电极,当给两对电极输入电压时,压电陶瓷管可以通过压电形变分别在纵向和横向产生机械位移。因此,若将光纤与压电陶瓷管连接,光纤悬臂会因压电陶瓷管的机械位移而产生偏折。该扫描方式的主要优势是可以实现快速扫描,而且可以通过振幅调制谐振光纤来产生高质量的图像[38]。

近年来,多个研究团队都开发了由压电陶瓷驱动器驱动光纤扫描的内窥镜技术。2002年,Seibel教授团队[39]使用压电陶瓷管驱动单模光纤悬臂产生共振,实现了螺旋扫描。他们在实验中使用的压电陶瓷套管长2.3 mm,单模光纤内芯直径为3.8 μm,共振频率为500 Hz,系统分辨率为10~20 μm。2004年,Liu教授团队[40]开发了一种实时、快速的光学相干断层扫

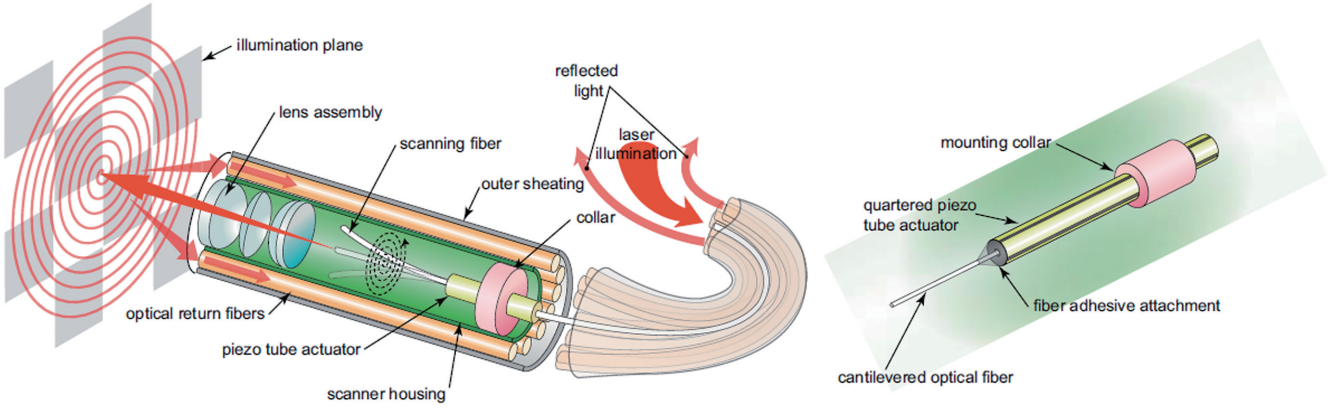


图3 以螺旋扫描模式对光纤进行扫描的示意图[38]（同轴扫描仪由同轴固定的单模光纤和压电陶瓷管组成，该光纤伸出压电陶瓷管的部分作为尖端悬臂）

Fig. 3 Schematic of fiber scanning in spiral scan pattern[38] (co-axial scanner consists of single-mode optical fiber and tubular piezoelectric actuator which are fixed coaxially, and the part of the fiber that sticks out of the tubular piezoelectric actuator acts as cantilever)

描显微内窥镜，其中的压电陶瓷套管长7.2 mm，光纤悬臂的长度和共振频率分别为8.5 mm和1.4 kHz。随后，Li教授团队[41]开发了基于光纤扫描的双光子荧光显微内窥镜，光纤悬臂的长度和共振频率分别为8.2 mm和1.3 kHz，内窥镜分辨率为2 μm。2008年，Seibel教授团队[29]开发了一种基于光纤扫描内窥镜探头的紧凑型双光子荧光成像显微镜，光纤悬臂的长度和共振频率分别为4.5 mm和5 kHz。2011年，Xu团队[42]开发了一种紧凑型光栅扫描多光子内窥镜，并将其用于对未染色组织成像，光纤悬臂的长度和共振频率分别为9 mm和1.05 kHz，内窥镜的成像速度为41 frame/s。Fu团队[43]为不同应用开发了多种谐振式光纤压电扫描仪（RFPS），其共振频率和扫描范围取决于压电贴片和悬臂的几何尺寸以及施加于它们之上的驱动信号。

压电元件以其灵敏度高、响应速度快、成本低等优点逐渐成为偏转光纤尖端的一种很有前景的选择；但是由于扫描速度沿扫描半径增加，视场（FOV）上的照明密度分布不均匀，中心区域的高光照密度容易导致组织光损伤或光漂白。此外，由于正交扫描轴之间的串扰，压电管和单根光纤之间一旦出现微小的离轴偏差，就会导致螺旋扫描图案严重失真[38]。

综上所述，eCLE使用点扫描的方式通过扫描装置驱动单根光纤进行扫描，进而实现高分辨率的共聚焦内窥成像。由于eCLE采用的是远端扫描方式，机械扫描装置包含在成像探头内，因此需要对机械扫描装置进行微型化。然而，eCLE所需的机械扫描装置的小型化在技术上还存在挑战，并且扫描装置价格昂贵，因此在临床应用上受到一定限制。

3.2 探头式共聚焦内窥镜（pCLE）

与eCLE不同，pCLE采用近端扫描模式，即扫描装置位于光纤束前端。如图2（d）所示，扫描装置不包含在远端探头内，而是在光纤束的近端使用扫描装置实现二维扫描，因此其尺寸不受限制[44]。光纤束通常由30000～100000根独立的阶跃折射率光纤组成，光纤束的总直径在数百微米到几毫米之间，纤芯直径为2.0～4.0 μm，纤芯间距为3.2～6.0 μm。在光纤束中，较高折射率的纤芯都嵌在一个具有较低折射率的包层中[3]。pCLE有两种成像模式，一种是光纤束直接接触式[45]，另一种是在光纤束远端配微型探头[46]。在光纤束直接接触式成像模式中，探头内部无镜头，焦面位于光纤端面，成像时光纤束与样本直接接触。为了提高横向分辨率同时增加探头的工作距离，需要在光纤束的远端配备微透镜[46]，如图4所示[47]。

1993年，Aziz和Gmitro[48]使用标准Zeiss LSM10共聚焦显微镜在荧光模式下对10000 pixel的相干光纤束进行光栅扫描，并在此基础上首次提出了使用光纤束进行共聚焦成像的方法。2004年，Mauna Kea Technologies公司开发了光纤共聚焦荧光显微镜系统（FCFM），并基于该显微镜系统实现了在细胞水平上实时观察活体和原位生物组织，视野为160 μm×120 μm，实现了横向分辨率为2.5 μm和轴向分辨率为20 μm的成像，组织成像深度为80 μm[49]。2010年，Sun等[50]开发了低成本、小型化针式反射共聚焦探头（通过偏振成像来抑制光纤束镜面反射的背景噪声），该探头的外径仅为450 μm，横向分辨率约为3.5 μm。2018年，Wang等[51]开发了一种基于近红外探头的共聚焦显微内窥镜，该内窥镜的直径为2.6 mm，由780 nm波长激发，可以实现1.55 μm的横向分辨率以及330 μm×330 μm的成像视野，并且可在细胞分辨率下实现300 μm深度组织的成像。

光纤束的主要缺点是纤芯之间的非成像空间以及光纤之间的芯到芯距离会造成像素化伪影[3]，因此，光纤束在样品平面上成像的横向分辨率有限。此外，由于相邻纤芯之间包层的光学串扰，成像对比度会降

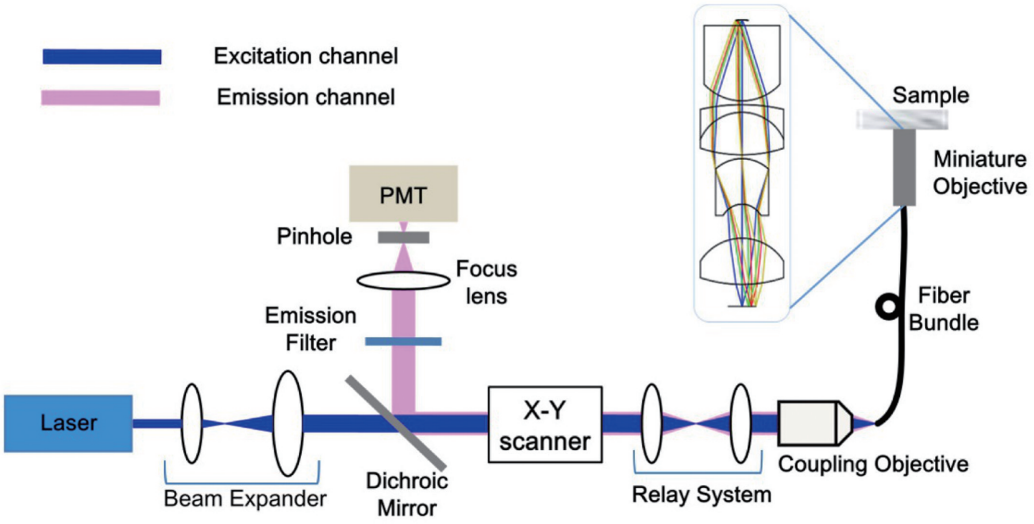


图4 光纤束扫描共聚焦内窥镜示意图[47]

Fig. 4 Schematic of fiber bundle scanning confocal endoscopy[47]

低[26]。为了减少像素化伪影，目前已有多种图像处理方法被开发出来。Kyrish等[52]使用定制的机电致动器获取样本的位移模式，然后使用算法组合重新对齐有位移的图像，以提高横向分辨率。Han等[53]提出了另一种方法，即，通过直方图均衡化和高斯空间平滑滤波器的组合对图像进行后处理，以去除光纤束结构的像素化伪影。

eCLE和pCLE两种模式的共聚焦内窥镜的优劣势如表1所示。eCLE通过点扫描可以实现较高的分辨率，但由于其扫描装置位于探头内，探头尺寸会受到扫描装置的限制。pCLE探头中不包含扫描装置，不受扫描装置尺寸的限制，但其分辨率受限于纤芯之间的距离且成像质量受光纤束蜂窝状结构的影响。eCLE和pCLE利用的都是传统的共聚焦显微成像技术，且均利用单波长进行激发，而传统的单波长共聚焦内窥镜需要通过机械扫描完成三维成像，成像速度慢。因此，传统的共聚焦内窥显微成像方案均无法实现快速的三维深层组织成像，不能满足临床上实时光学诊断的需求。

表1 不同类型的共聚焦内窥镜

Table 1 Different types of confocal endoscopes

Type	Fiber type	Advantage	Disadvantage	Stage
eCLE	Single-core fiber[29,38-42]	Point scanning to achieve high resolution	Distal scanning: scanning device is contained in the probe, the scanning device needs to be miniaturized	Commercial EC3870CILK, Pentax (no sale)
pCLE	Fiber bundle[47-51]	1) Proximal scanning: no scanning device is included in the probe, no size limitation of the scanning device; 2) Easy to operate	1) The resolution is limited by the distance between the cores; 2) The pixelation of the fiber bundle affects the image quality; 3) The background reflection on the end face of the fiber bundle limits the image contrast	Commercial Cellvizio, Mauna Kea Technologies
Chromatic confocal endoscope	Fiber bundle & single-core fiber[54-56]	1) Multi-wavelength excitation, three-dimensional imaging without mechanical scanning; 2) Simultaneous acquisition of multi-depth information to improve imaging speed	Hard to guarantee large chromatic aberration and small spherical aberration at the same time in the case of lens miniaturization, and the axial resolution is poor due to the spherical aberration of the lens	In research

3.3 光谱编码共聚焦内窥镜

由于共聚焦显微内窥镜的视野一般小于0.5 mm×0.5 mm，因此难以对可疑病变区域进行大范围成像，无法在短时间内检查整个病变区域。光谱编码共聚焦显微技术是一种通过光谱编码横向位置的反射式共聚焦显微技术。在光谱编码共聚焦显微镜中，照明光通过单模光纤传输至光栅，然后进行衍射分光，不同波长的光通过高数值孔径的物镜聚焦在样品的不同横向位置。因此，相比传统的激光共聚焦显微镜，光谱编码共聚焦显微镜可以显著提高成像速度，实现短时间内大

面积成像。

1998年，Tearney等[57]首次开发出了光谱编码共聚焦显微镜系统。在该系统中，中心波长为940 nm、带宽为75 nm的光源通过单模光纤耦合器后分别进入样品臂和干涉臂，样品臂为包含光栅和物镜的共聚焦探头，而参考臂中放置有线性平移镜，用于进行傅里叶变换，进而解码波长与空间位置的对应关系。该系统的视野仅为130 μm×130 μm，横向分辨率为0.78 μm，扫描单幅图像需要60 min。在此基础上，Tearney团队[58-59]分别于2005年和2013年针对系统的成像速度进行两次改进：使用100 kHz扫描速率、80 mW输出功率的快速扫频光源，对样品进行快速扫描；将波长与样本横向位置的函数转化为时间与空间位置的函数，省去了快速傅里叶变换的时间，对离体组织成像的速率达到了6.6 mm^2/s，成像的横向分辨率为1.6 μm，成像深度为350 μm。2010年，Tao等[60]利用超发光二极管和最大线成像速率可达52 kHz的高速光谱仪开发了用于活体眼底成像的光谱编码共聚焦显微镜系统。2017年，Tao团队[61]又将光谱编码共聚焦系统和频域OCT结合，以200 frame/s的速度同时采集OCT和光谱编码共聚焦图像，以提供视网膜横向和轴向的运动信息。2021年，Rashtchian团队[62]将光谱仪作为探测器搭建光谱编码共聚焦系统，利用中心波长为1311 nm的近红外光实现了147 kHz的成像频率，横向和轴向分辨率分别为2 μm和10 μm。

光谱编码共聚焦显微镜的高成像速率可在一定程度上解决共聚焦视野小的问题，但成像深度仍限制在200 μm以内。在较大的成像深度下，由于光散射和光学像差，光谱编码共聚焦显微镜的有效分辨率会显著降低。此外，在光谱编码共聚焦显微镜系统中，光束经过了衍射元件分光，光束离轴传播，因此该系统物镜的设计既要保证垂轴色差，又要消除其他像差，以保证图像质量。

3.4 色散共聚焦内窥镜

色散共聚焦技术在实现高分辨率快速多深度成像方面具有一定潜力[63-67]。与传统的共聚焦显微技术相比，色散共聚焦显微技术可以利用镜头的色焦位移来实现多深度同步成像，无需任何轴向的机械扫描。色散共聚焦技术依据光学元件色散的原理，即不同波长的光在光学器件内的传播速度不同，将多光谱光源聚焦在样品的不同深度处，实现多深度信息的同时探测。Olsovsky等[63]开发了一种色散共聚焦显微镜，该显微镜使用4个非球面透镜和一个数值孔径为0.8的水浸透镜作为物镜，在590～775 nm波长范围内实现了150 μm的成像深度和约3 μm的轴向分辨率。Liang团队[68]开发了一种基于数字反射镜设备的色散共聚焦显微镜，其成像深度为45 μm，波长为505～650 nm，轴向分辨率约为12 μm。上述工作已经证明了色散共聚焦系统在医疗应用方面的潜力，但上述系统并未小型化为内窥镜。Lane等[54]提出了一种基于光纤的色散共聚焦显微内窥镜，并通过直径为1 mm的梯度渐变折射率透镜(GRIN)在200 nm波段范围内实现了40 μm的色散焦移范围；此外，Lane等通过微处理器芯片的成像实验验证了其光学切片能力。Kang等[56]设计了一种光谱编码的共聚焦内窥镜探头，该探头通过使用以倾斜角排列的物镜来生成不平行于组织表面的焦线，以增大成像深度。

为了进一步增加色散共聚焦内窥镜的成像深度，满足临床中对肿瘤侵犯深度的判断，本团队自制了一种同时具备高分辨率和大深度的基于光纤的大深度3D色散共聚焦显微内窥镜[55]。在色散共聚焦技术中，物镜是实现大成像深度和高分辨率的关键。物镜的轴向色差范围决定着成像深度的范围，具有大的轴向色差是大深度成像的前提。本团队设计了一种既有大轴向色散又有球差校正功能的微透镜，可在保证大轴向色差的同时实现高分辨率成像。同时，本团队使用了一根包含50000个单模纤芯的光纤束来采集共焦反射信号。最后，利用高速多像素光谱仪实现了多深度信号的同时检测，以更快的速度获取沿组织轴向的3D信息。最终实现了2.3 μm的横向分辨率和12.2 μm的轴向分辨率，对三维仿体的成像深度可达570 μm。

以上研究验证了色散共聚焦内窥镜可以改善传统共聚焦内窥镜成像深度不足的缺陷，同时也彰显了其在胃癌诊断中的巨大潜力。但由于镜头加工工艺的限制，相比传统的共聚焦内窥镜，色散共聚焦内窥镜无法在镜头微型化的情况下同时保证大色差和小球差，从而导致轴向分辨率受限。

4 生物医学应用

4.1 消化道疾病诊断

作为一种“光学活检”工具，共聚焦显微内窥镜以其卓越的亚细胞分辨率被应用于消化道的早期诊断[69-70]。近年来，新应用于临床的窄谱技术，如窄带成像技术(NBI)、可变光谱影像增强技术(FICE)、I-SCAN技术和蓝激光技术(BLI)，通过光学或数字滤波的方式利用蓝光照射组织，以强化黏膜表面的细微结构和微血管形态，进而提高成像对比度，但仍然没有解决成像分辨率低的问题[70]。共聚焦内窥镜通过探测组织中反射的荧光进行成像，分辨率可达亚微米量级，能够观察到普通内镜观察不到的黏膜细微结构，而且其成像结果呈现出与病理活检高度一致的细胞形态。共聚焦内窥镜具有光学切片的能力，有助于诊断黏膜病变[71-72]。

4.1.1 食管疾病诊断

已经有研究表明共聚焦内窥镜在诊断食管癌前病变和肿瘤方面具有潜在优势，可以指导活检并可以提高诊断准确性，同时可以减少随机活检的次数[73]。

非发育异常的巴雷特食管(BE)上皮在共聚焦成像中很容易识别。共聚焦内窥镜将杯状细胞中包含的黏蛋白显示为柱状细胞内的特征性黑色圆形结构,这些结构很容易通过明亮的轮廓来识别[74]。此外,腺体具有线性轮廓,腺间隙大小规则,如图5所示。Gaddam等[75]在一项研究中定义了用于诊断高度不典型增生(HGD)和黏膜内腺癌的pCLE标准。他们选择了预测BE瘤准确度最高(81.5%)的6个标准:1)锯齿状上皮表面;2)难以识别的杯状细胞;3)非等距腺体;4)大小和形状不等的腺体;5)扩大的细胞;6)非等距和不规则的细胞。近期,低度不典型增生(LGD)的pCLE标准也被建立[76]。低度不典型增生在共聚焦内窥镜下的特征与更晚期的异型增生相似,但其可以反映更细微的组织学异常。低度不典型增生的pCLE标准包括:1)深色非圆形腺体;2)不规则的腺体形状;3)杯状细胞缺失;4)不同程度的暗度,具有明显的截止;5)可变单元大小;6)细胞分层。最近,一项荟萃分析论文回顾了共聚焦内窥镜在诊断BE相关瘤形成方面的总体准确性,该荟萃分析包括来自709名患者的8项研究[77]。BE相关肿瘤诊断的总体敏感性和特异性分别为89%(95%置信区间,0.80~0.95)和75%(95%置信区间,0.69~0.81)。

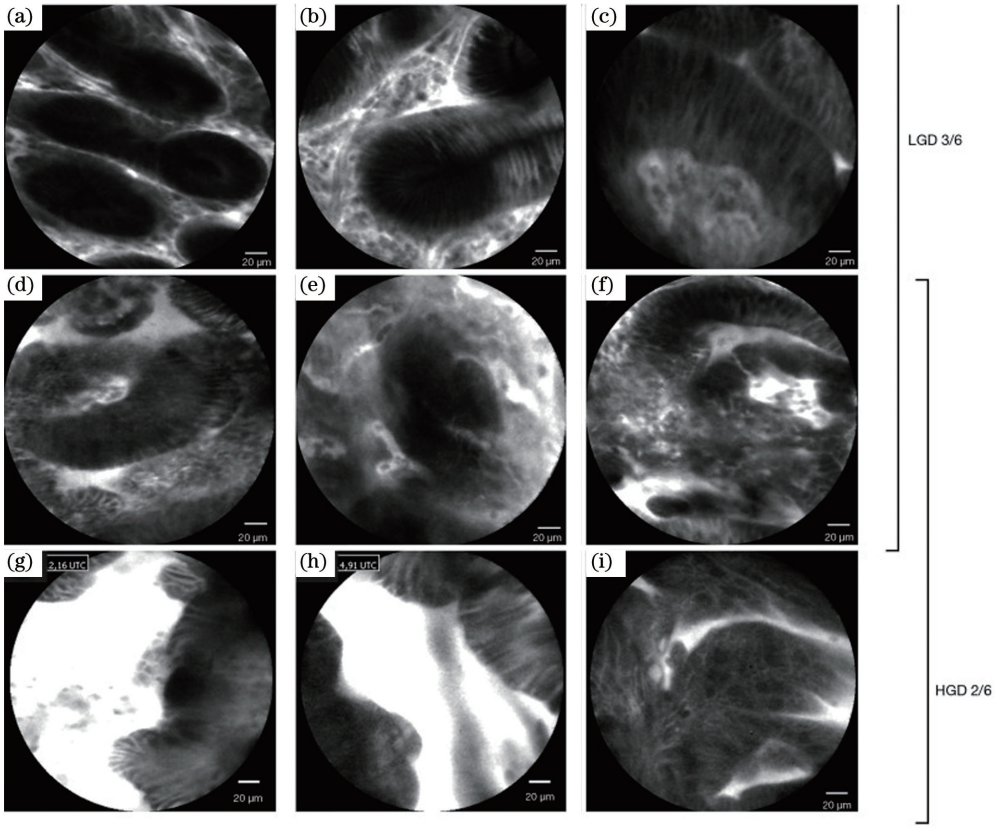


图5 巴雷特食管低度不典型增生和高度不典型增生的pCLE标准[73]。(a)深色非圆形腺体;(b)不同程度的黑暗与尖锐的截止边缘;(c)细胞分层;(d)难以识别的杯状细胞;(e)大小和形状不等的腺体;(f)非等距不规则细胞;(g)锯齿状上皮表面;(h)非等距腺体;(i)扩大的细胞

Fig. 5 Probe-based confocal laser endomicroscopy (pCLE) criteria for low-grade dysplasia (LGD) and high-grade dysplasia (HGD) in Barrett's oesophagus[73]. (a) Dark non-round glands; (b) variable degree of darkness with sharp cut-off; (c) cellular stratification; (d) poorly identifiable goblet cells; (e) glands unequal in size and shape; (f) non-equidistant and irregular cells; (g) "saw-toothed" epithelial surface; (h) non-equidistant glands; (i) enlarged cells

已有研究[78]报道了CLE对早期食管鳞状细胞癌(OSCC)的诊断。正常鳞状上皮的CLE特征为:深色均质的上皮细胞,结构规则,边界和毛细血管清晰可见,造影剂没有渗透到周围组织中。肿瘤组织的特征是具有不规则结构的暗细胞,大小不一,缺乏清晰可见的边界,有新血管(扭曲、不规则和细长的肿瘤血管,直径较大)生成的迹象,并且荧光素通过毛细血管壁渗漏[78]。在对21名疑似早期OSCC患者进行的共聚焦成像小型前瞻性队列研究中,研究人员先进行0.5% Lugol溶液染色内镜检查,而后对43个未染色区域进行了共聚焦内窥成像。最终,OSCC诊断的总体准确率为95%,敏感性和特异性分别为100%和87%。

4.1.2 对胃成像

共聚焦激光内窥镜已被广泛应用于检测胃癌(GC)的肿瘤性病变和癌前病变,例如萎缩性胃炎(AG)或肠化生(IM)。2008年,第一个应用于胃组织成像的共聚焦激光内窥镜图像分类方法被提出,它描述了与疾病谱相关的7种类型的胃凹模式,从

正常黏膜到AG/IM，最终到早期GC[79]。近期，这种分类被改进为包含胃凹模式和血管结构[80]。其中：Ⅰ型胃小凹模式呈现为具有圆形/宽/狭缝状开口的规则小凹，分别对应于位于贲门/胃体/胃窦的正常黏膜；Ⅱ型胃小凹模式被细分为呈开口细长且凹坑规则的Ⅱa型、呈开口扩张且凹坑缩小的Ⅱb型以及呈杯状细胞外观且带有深色黏蛋白的Ⅱc型，它们分别代表炎症性黏膜、萎缩黏膜以及IM；Ⅲ型细分为呈现轻度至中度不规则凹坑且衬里上皮宽度可变的Ⅲa型、呈现具有不规则衬里上皮且凹坑明显扭曲的Ⅲb型以及呈现非典型腺体/不规则暗细胞分散的Ⅲc型，它们分别代表低度上皮内瘤变、高级别上皮内瘤变和分化/低分化腺癌[80]。血管结构也分为3种类型，其中Ⅰ型为正常黏膜（毛细血管正常，管径正常，贲门/胃体/胃窦分别呈杵状/蜂窝状/盘绕状），Ⅱ型为炎性胃黏膜（毛细血管增多，渗漏增多），Ⅲ型以肿瘤性胃黏膜为主（不规则毛细血管，不均匀渗漏/扩张口径）[80]。该pCLE标准对AG的敏感性和特异性分别为89%和99%，对IM的敏感性和特异性分别为92%和99%，对GC的敏感性和特异性分别为90%和99%。肿瘤性病变与非肿瘤性病变区分的观察者间一致性较好（Kappa值为0.70）[80]。共聚焦激光内窥镜下正常和病理性胃黏膜的外观如图6所示[73]。

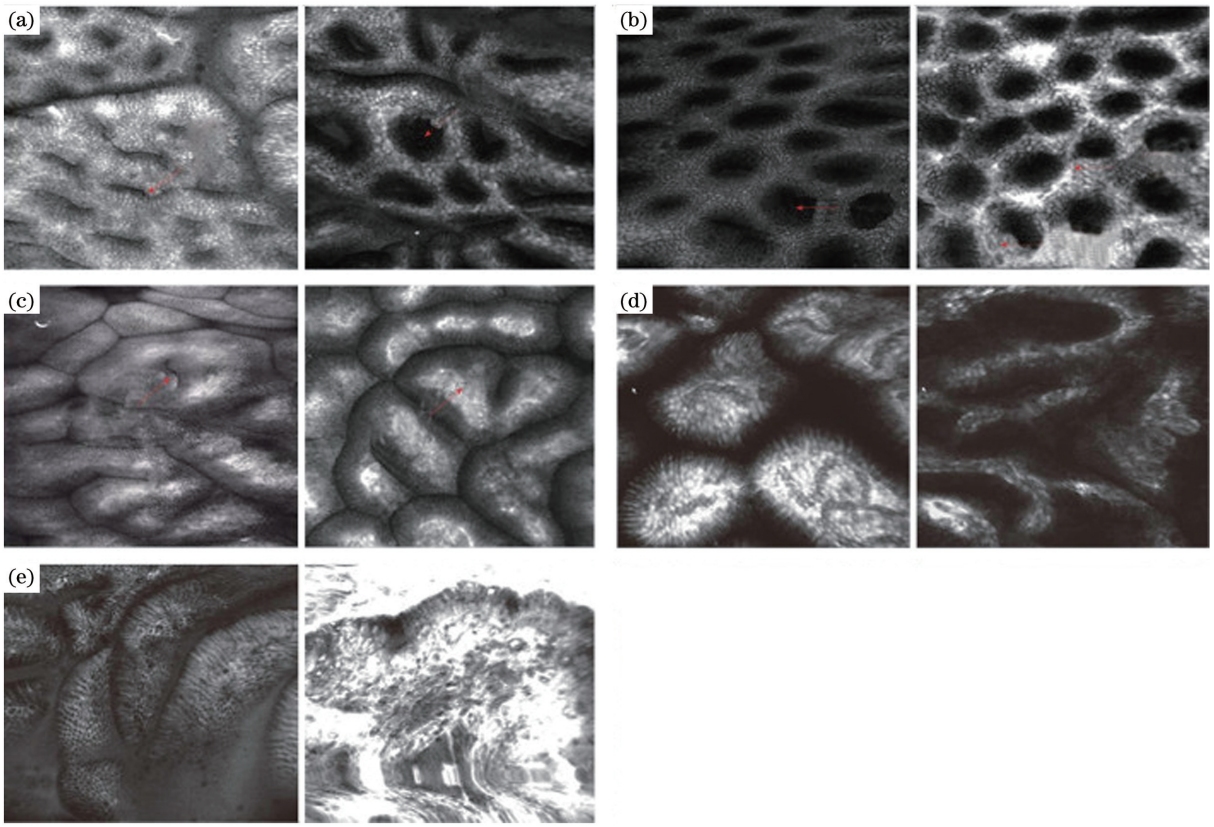


图6 正常和病理性胃黏膜的eCLE图像[73]（放大倍率：1000倍）。(a)胃底处的正常胃黏膜，其胃小凹是圆形（实线箭头），网状上皮下毛细血管网络围绕胃小凹（虚线箭头）；(b)胃体部的正常胃黏膜，其胃小凹是圆形（实线箭头），蜂窝状上皮下毛细血管网络围绕胃小凹（虚线箭头）；(c)胃窦部的正常胃黏膜，其胃小凹为线形（实线箭头），螺旋形上皮下毛细血管网络围绕胃小凹（虚线箭头）；(d)低级别胃上皮内癌变，其胃小凹大小不一，毛细血管网增厚迂回；(e)高级别胃上皮内癌变，其胃小凹排列异常，增厚的毛细血管网络和增加的分支呈团状

Fig. 6 eCLE appearance of normal and pathological gastric mucosa[73] (magnification: 1000×). (a) Normal gastric mucosa in the fundus, where gastric pits are round (solid arrow) and net-like subepithelial capillary network patterns surround the gastric pits (dash arrow); (b) normal gastric mucosa in the body, where gastric pits are round (solid arrow), and honeycomb-like subepithelial capillary network patterns surround gastric pits (dash arrows); (c) normal gastric mucosa in the antrum, where gastric pits are the line type (solid arrow) and coil-shaped subepithelial capillary network patterns surround gastric pits (dash arrow); (d) low grade gastric intraepithelial neoplasia, where gastric pits is different in sizes, capillary network is thickened and circuitous; (e) high grade gastric intraepithelial neoplasia, where gastric pits exhibit abnormal arrangement, and the thickened capillary network and increasing branch present a mass shape

4.2 对皮肤成像

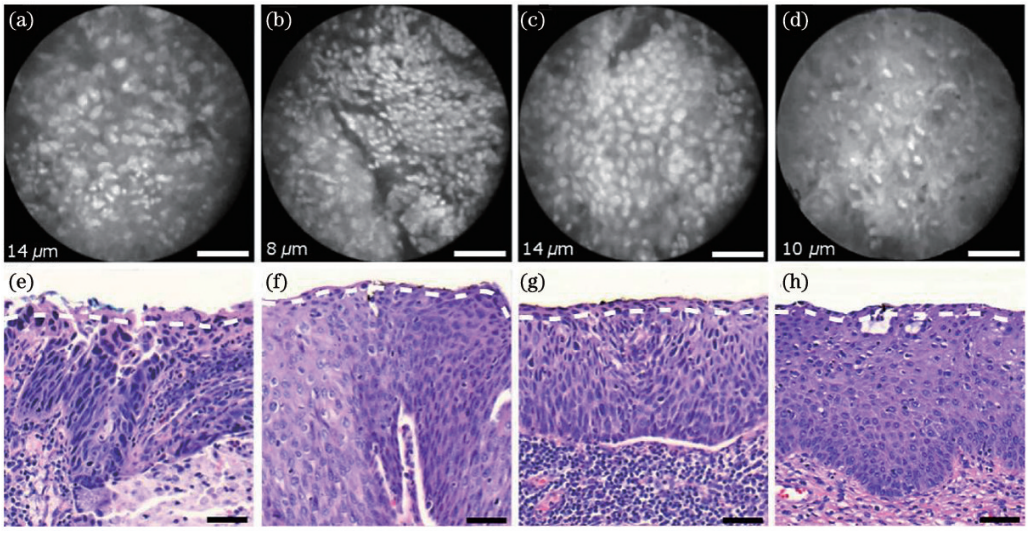
共聚焦激光扫描显微镜可以在不同深度下对皮肤进行无创虚拟切片，在水平平面中获得的灰度图像平行于皮肤表面，不需要进行组织处理或着色[81-82]。它允许在不同时间间隔下对同一皮肤区域进行实时重复成像，是监测疾病进展和治疗效果以及研究皮肤动态

行为的绝佳工具[83-90]。根据图像对比度的来源，共聚焦激光扫描显微镜可以工作于荧光或反射模式下。荧光共聚焦显微镜(FCM)需要使用荧光剂[91]，而且主要用于实验研究，在损伤和非损伤皮肤中的应用潜力巨大[92-93]。基于细胞结构折射率差异[94]设计的反射共聚焦显微镜(RCM)已被广泛应用于黑色素细胞[95-98]和非黑色素细胞皮肤肿瘤[96,99]的无创评估，评估结果与皮肤镜检查和组织学检查结果具有良好的相关性。此外，这种新型成像技术已被证明可用于诊断各种炎症性皮肤病[100]以及具有皮肤病学表现的病症[101]，并可用于研究伤口愈合等动态过程[102-103]，实时评估血流以响应各种局部刺激[90,104]或白细胞迁移[85-86]。

体内共聚焦显微镜具有多种功能，可以非侵入性地获取3D高分辨率图像[105]。在皮肤病学中，这种非侵入性技术可以用于对人体皮肤进行渐进深度的非破坏性光学切片[105-106]。目前，体内反射共聚焦显微镜被认为是最有前途的非侵入性成像技术，可用于浅表皮肤肿瘤的准显微形态学观察和动态表征[99]。反射共聚焦显微镜成像与皮肤镜检查相结合不仅可以提高皮肤癌诊断的准确性，还可以减少良性皮肤病变的活检次数。此外，它还有助于在手术切除或其他侵入性治疗之前确定肿瘤边缘[99]。目前，已有研究团队开发了将反射模式下的共聚焦激光扫描显微镜与荧光技术相结合的新型多激光设备，与单独使用一种共聚焦显微镜模式相比，该设备可以提供更多附加信息[16]。目前，许多皮肤病学研究已经证实了体内共聚焦成像的诊断潜力[92]。

4.3 宫颈成像

宫颈癌被认为是从宫颈上皮化生、不典型增生和随后逐渐变化为癌症的多步骤病变发展而来的[107]。体内共聚焦成像可以提供有关上皮细胞亚细胞形态和生化变化的信息，有助于识别和监测宫颈上皮癌前病变[107-110]。目前，很多研究已经证明了宫颈的共聚焦内窥成像可以提供核和粗细胞形态的相关信息，并且可以结合组织学和癌前状态进行定量评估[111]或定性评估[112]。有研究证明，在无须切除组织的情况下，采用体内反射共聚焦显微镜可以看到与宫颈上皮内瘤变进展相关的核质比和核密度的增加[109-111]，如图7所示。因此，共聚焦内窥镜检查可以在阴道镜检查时评估和诊断宫颈上皮内瘤变。将荧光共聚焦显微镜与癌症功能基因组学结合可以增加早期癌症检测的特异性，例如可以通过检测代谢指标NADH和FAD来检测伴随发育不良进展的细胞代谢变化[107,110]。



Notes: Figs. (d) and (h) are classified as CIN 2, while all other images contain CIN 3 tissue.

图7 高度鳞状上皮内病变表层的内窥镜成像以及对应于相同成像位置的染色组织切片(比例尺尺寸为50 μm)[109]。(a)～(d)内窥镜成像；(e)～(h)染色组织切片

Fig. 7 Endomicroscopy images of high-grade squamous intraepithelial lesion (HSIL) surface and stained histology sections corresponding to the same imaging locations (scale bar: 50 μm)[109]. (a)-(d) Endomicroscopy images; (e)-(h) stained histology sections

宫颈活体共聚焦内窥显微镜可以为临床医生提供实时细胞水平图像，以评估形态和结构特征。因此，它可以用作阴道镜检查的辅助手段。如果体内共聚焦内窥显微镜的使用可以从单纯的活检指导扩展到即时和准确诊断的独立工具，那么它的临床价值将会显著增加[109]。

4.4 眼部成像

1985年，Lemp等[113]发表了第一张全层人类角膜的共焦图像。在随后的20年中，随着共聚焦显微镜在体内的进一步发展和临床应用，人们对正常、患病和术后人类角膜的了解进一步提高[114-115]。体内共聚焦显微镜通过对活体角膜的全层进行成像来提供正面光学切片的连续图像[116]。共聚焦显微镜的非侵入性特性允许对角膜的生理状态进行快速检查，并允许对角膜的同一部位进行重复分析，这对于纵向评估以识别

疾病进展和评估治疗效果非常重要。在过去的10年中，活体共聚焦显微镜（IVCM）在临床中逐渐获得了应用，有助于诊断、治疗和研究多种疾病，例如治疗由防腐剂引起的毒性[117-118]、不同的眼疾[119-120]、医源性损伤[121]，研究感染[122]和营养不良[123]、结膜[124-126]和角膜缘[127]病理学，诊断眼表肿瘤[128]和角膜沉积物[129-130]。除了提供定性信息外，共聚焦显微镜还允许对活角膜的层、神经和细胞进行定量分析，并可以确定角膜的光散射和可见结构的深度[114]。

综上，共聚焦内窥成像技术已被大量研究证明可用于诊断食管、胃、皮肤、眼睛和宫颈等疾病，如表2所示。

表2 共聚焦显微内窥技术在生物医学中的应用

Table 2 Application of confocal endomicroscopy techniques in biomedicine

Position	Disease	Modality
Esophagus	1) Early esophageal squamous cell carcinoma[78] 2) High-grade dysplasia and intramucosal adenocarcinoma[75] 3) Barrett's esophagus and related neoplasia[74,77] 4) Mild and moderate dysplasia[76]	Fluorescence confocal
Stomach	1) Atrophic gastritis, intestinal metaplasia, gastric cancer[79] 2) Low- and high-grade intraepithelial neoplasia and differentiated/poorly differentiated adenocarcinoma[80] 3) Low- and high-grade intraepithelial neoplasia[73]	Fluorescence confocal
Skin	1) Psoriasis[87-88] 2) Malignant pigmented, hyperpigmented and non-pigmented macules on the face[95] 3) Melanoma[96-99] 4) Skin inflammation[89-90,106]	Reflectance confocal
Eye	1) Keratitis[114-116] 2) Eye toxicity of preservatives[117-118] 3) Glaucoma[119,124] 4) Cataract[120-121] 5) Corneal dystrophy[116,123] 6) Corneal intraepithelial neoplasia[128] 7) Corneal pathology in Crohn's disease[129]	Fluorescence confocal
Cervix	1) Epithelial precancerous lesions of the cervix[107-110,112-113,132-134] 2) Cellular metabolic changes in dysplastic progression[107,117,120]	Fluorescence confocal & reflectance confocal

5 结 论

光学切片显微镜是生物学研究的有力工具，而共聚焦显微镜通常是首选工具。由于共聚焦内窥镜比传统内窥镜具有更高的放大率和分辨率，带有微型探头的共聚焦内窥镜能够对不同的组织、细胞、分子甚至是细菌进行体内和实时成像[135-136]。大量研究证实，共聚焦内窥镜具有很高的诊断准确性，共聚焦内窥技术已成为临床和研究应用的重要工具。

虽然共聚焦内窥镜有望实现光学病理诊断，但其在临床应用上仍存在一些局限性。在早期胃癌诊断的临床应用中，一旦肿瘤深入黏膜下层，就存在淋巴结转移的风险。因此，有必要从组织黏膜的下层获取信息[3,22]。临床上亟需能够提供足够成像深度的工具来评估早期胃部肿瘤的侵袭深度，而传统的共聚焦显微技术都是由单波长激发的，其成像深度与波长相关，这使得共聚焦显微技术的成像深度有限（限于200～300 μm），阻碍了对组织表面的观察[137]。除此之外，单波长激发下只能对特定的组织深度进行成像，而且一次只能进行一个深度平面的成像。因此，传统共聚焦显微技术需要通过机械扫描的形式完成3D成像，成像速度慢，不能满足实时3D成像的临床需求。色差共聚焦技术有可能实现高分辨率的快速多深度成像[63-68,138]。在彩色共聚焦成像技术中，当白光通过镜头时，由于传统镜头对不同波长光的折射率不同，每个单色光分量在光轴上的焦点不同。因此，可以基于色散元件用波长对光轴进行编码。与传统的共聚焦显微镜相比，色差共聚焦显微镜无需任何机械轴向扫描即可实现多波长成像。但受镜头加工工艺的限制，同时满足小尺寸、大色差及小球差是一个巨大的技术挑战。近年来，一些新兴的扫描技术也有应用于内窥成像的潜力。例如，Deguchi等[139]将超声波驱动的液态透镜（TAG透镜）集成到具有共振振镜的共聚焦显微镜中进行连续轴向移焦，实现了30 Hz的3D成像。未来，

为了促进临床应用，应致力于开发集高分辨率、大成像深度及高速成像于一体的内窥镜显微技术。

参 考 文 献

[1] Elliott A D. Confocal microscopy: principles and modern practices[J]. Current Protocols in Cytometry, 2020, 92(1): e68.

[2] Bayguinov P O, Oakley D M, Shih C C, et al. Modern laser scanning confocal microscopy [J]. Current Protocols in Cytometry, 2018, 85(1): e39.

[3] Oh G, Chung E, Yun S H. Optical fibers for high-resolution *in vivo* microendoscopic fluorescence imaging[J]. Optical Fiber Technology, 2013, 19(6): 760-771.

[4] 张伟, 牛春阳, 游兴海, 等. 高倍率大视场细胞内镜成像系统研究[J]. 光学学报, 2021, 41(17): 1717001.
Zhang W, Niu C Y, You X H, et al. Endocytoscopic imaging system with high magnification and large field of view[J]. Acta Optica Sinica, 2021, 41(17): 1717001.

[5] Neumann H, Kiesslich R, Wallace M B, et al. Confocal laser endomicroscopy: technical advances and clinical applications [J]. Gastroenterology, 2010, 139(2): 388-392.

[6] Aisenberg J. Gastrointestinal endoscopy nears the molecular era[J]. Gastrointestinal Endoscopy, 2008, 68(3): 528-530.

[7] Fine A, Amos W B, Durbin R M, et al. Confocal microscopy: applications in neurobiology [J]. Trends in Neurosciences, 1988, 11(8): 346-351.

[8] Egger M D, Petrăn M. New reflected-light microscope for viewing unstained brain and ganglion cells[J]. Science, 1967, 157(3786): 305-307.

[9] Paddock S W, Eliceiri K W. Laser scanning confocal microscopy: history, applications, and related optical sectioning techniques[J]. Methods in Molecular Biology, 2014, 1075: 9-47.

[10] Conchello J A, Lichtman J W. Optical sectioning microscopy [J]. Nature Methods, 2005, 2(12): 920-931.

[11] Paddock S W. Principles and practices of laser scanning confocal microscopy [J]. Molecular Biotechnology, 2000, 16 (2): 127-149.

[12] Wilson T. Optical sectioning in fluorescence microscopy [J]. Journal of Microscopy, 2011, 242(2): 111-116.

[13] Kiesslich R, Goetz M, Hoffman A, et al. New imaging techniques and opportunities in endoscopy[J]. Nature Reviews Gastroenterology & Hepatology, 2011, 8(10): 547-553.

[14] Ilie M A, Caruntu C, Lupu M H, et al. Current and future applications of confocal laser scanning microscopy imaging in skin oncology[J]. Oncology Letters, 2019, 17(5): 4102-4111.

[15] Unglert C I, Namati E, Warger W C, et al. Evaluation of optical reflectance techniques for imaging of alveolar structure [J]. Journal of Biomedical Optics, 2012, 17(7): 071303.

[16] Ghita M A, Caruntu C, Rosca A E, et al. Reflectance confocal microscopy and dermoscopy for *in vivo*, non-invasive skin imaging of superficial basal cell carcinoma [J]. Oncology Letters, 2016, 11(5): 3019-3024.

[17] Ragazzi M, Piana S, Longo C, et al. Fluorescence confocal microscopy for pathologists[J]. Modern Pathology, 2014, 27 (3): 460-471.

[18] Welzel J, Kästle R, Sattler E C. Fluorescence (multiwave) confocal microscopy[J]. Dermatologic Clinics, 2016, 34(4): 527-533.

[19] Sahl S J, Hell S W, Jakobs S. Fluorescence nanoscopy in cell biology[J]. Nature Reviews Molecular Cell Biology, 2017, 18 (11): 685-701.

[20] 曹怡涛, 王雪, 路鑫超, 等. 无标记光学显微成像技术及其在生物医学的应用[J]. 激光与光电子学进展, 2022, 59(6): 0617012.
Cao Y T, Wang X, Lu X C, et al. Label-free optical microscopy technique and its biomedical applications[J]. Laser & Optoelectronics Progress, 2022, 59(6): 0617012.

[21] St Croix C M, Shand S H, Watkins S C. Confocal microscopy: comparisons, applications, and problems[J]. BioTechniques, 2005, 39(6): S2-S5.

[22] Nakao M, Yoshida S, Tanaka S, et al. Optical biopsy of early gastroesophageal cancer by catheter-based reflectance-type laser-scanning confocal microscopy[J]. Journal of Biomedical Optics, 2008, 13(5): 1204-1208.

[23] Webb R H, Hughes G W, Delori F C. Confocal scanning laser ophthalmoscope[J]. Applied Optics, 1987, 26(8): 1492-1499.

[24] González S, White W M, Rajadhyaksha M, et al. Confocal imaging of sebaceous gland hyperplasia *in vivo* to assess efficacy and mechanism of pulsed dye laser treatment [J]. Lasers in Surgery and Medicine, 1999, 25(1): 8-12.

[25] White W M, Rajadhyaksha M, González S, et al. Noninvasive imaging of human oral mucosa *in vivo* by confocal reflectance microscopy[J]. The Laryngoscope, 1999, 109(10): 1709-1717.

[26] Flusberg B A, Cocker E D, Piyawattanametha W, et al. Fiber-optic fluorescence imaging[J]. Nature Methods, 2005, 2(12): 941-950.

[27] Piyawattanametha W, Barretto R P J, Ko T H, et al. Fast-scanning two-photon fluorescence imaging based on a microelectromechanical systems two- dimensional scanning mirror[J]. Optics Letters, 2006, 31(13): 2018-2020.

[28] Fu L, Jain A, Xie H K, et al. Nonlinear optical endoscopy based on a double-clad photonic crystal fiber and a MEMS mirror[J]. Optics Express, 2006, 14(3): 1027-1032.

[29] Engelbrecht C J, Johnston R S, Seibel E J, et al. Ultra-compact fiber-optic two-photon microscope for functional fluorescence imaging *in vivo* [J]. Optics Express, 2008, 16 (8): 5556-5564.

[30] Dickensheets D L, Kino G S. Scanned optical fiber confocal microscope[J]. Proceedings of SPIE, 1994, 2184: 39-47.

[31] Dickensheets D L, Kino G S. Micromachined scanning confocal optical microscope [J]. Optics Letters, 1996, 21(10): 764-766.

[32] Tekpinar M, Khayatzadeh R, Ferhanoglu O. Multiple-pattern generating piezoelectric fiber scanner toward endoscopic applications[J]. Optical Engineering, 2019, 58(2): 023101.

[33] Seo Y H, Hwang K, Park H C, et al. Electrothermal MEMS fiber scanner for optical endomicroscopy[J]. Optics Express, 2016, 24(4): 3903-3909.

[34] Seo Y H, Hwang K, Jeong K H. 1.65 mm diameter forward-viewing confocal endomicroscopic catheter using a flip-chip bonded electrothermal MEMS fiber scanner[J]. Optics Express, 2018, 26(4): 4780-4785.

[35] Sawinski J, Denk W. Miniature random-access fiber scanner for *in vivo* multiphoton imaging [J]. Journal of Applied Physics, 2007, 102(3): 034701.

[36] Wang J F, Yang M, Yang L, et al. A confocal endoscope for cellular imaging[J]. Engineering, 2015, 1(3): 351-360.

[37] Zhang Y, Akins M L, Murari K, et al. A compact fiber-optic SHG scanning endomicroscope and its application to visualize cervical remodeling during pregnancy[J]. PNAS, 2012, 109 (32): 12878-12883.

[38] Lee C M, Engelbrecht C J, Soper T D, et al. Scanning fiber endoscopy with highly flexible, 1 mm catheterscopes for wide-field, full-color imaging[J]. Journal of Biophotonics, 2010, 3 (5/6): 385-407.

[39] Seibel E J, Smithwick Q Y J. Unique features of optical scanning, single fiber endoscopy [J]. Lasers in Surgery and Medicine, 2002, 30(3): 177-183.

[40] Liu X M, Cobb M J, Chen Y C, et al. Rapid-scanning forward-imaging miniature endoscope for real-time optical coherence tomography[J]. Optics Letters, 2004, 29(15): 1763-1765.

[41] Myaing M T, MacDonald D J, Li X D. Fiber-optic scanning

two-photon fluorescence endoscope[J]. Optics Letters, 2006, 31(8): 1076-1078.
[42] Rivera D R, Brown C M, Ouzounov D G, et al. Compact and flexible raster scanning multiphoton endoscope capable of imaging unstained tissue[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(43): 17598-17603.
[43] Li Z, Fu L. Note: a resonant fiber-optic piezoelectric scanner achieves a raster pattern by combining two distinct resonances[J]. Review of Scientific Instruments, 2012, 83(8): 086102.
[44] 贺正权, 任立勇, 庄斌, 等. 单光纤成像技术[J]. 激光与光电子学进展, 2017, 54(3): 030005.
He Z Q, Ren L Y, Zhuang B, et al. Single optical fiber imaging technology[J]. Laser & Optoelectronics Progress, 2017, 54(3): 030005.
[45] Lane P M, Dlugan A L, Richards-Kortum R, et al. Fiber-optic confocal microscopy using a spatial light modulator[J]. Optics Letters, 2000, 25(24): 1780-1782.
[46] Rouse A R, Gmitro A F. Multispectral imaging with a confocal microendoscope[J]. Optics Letters, 2000, 25(23): 1708-1710.
[47] Yang L, Wang J F, Tian G, et al. Five-lens, easy-to-implement miniature objective for a fluorescence confocal microendoscope[J]. Optics Express, 2016, 24(1): 473-484.
[48] Aziz D J, Gmitro A F. Confocal microscopy via a fiber optic imaging bundle[J]. Proceedings of SPIE, 1993, 1893: 53-61.
[49] Laemmel E, Genet M, Le Goualher G, et al. Fibered confocal fluorescence microscopy (Cell-viZio^{TM}) facilitates extended imaging in the field of microcirculation. A comparison with intravital microscopy[J]. Journal of Vascular Research, 2004, 41(5): 400-411.
[50] Sun J T, Shu C H, Appiah B, et al. Needle-compatible single fiber bundle image guide reflectance endoscope[J]. Journal of Biomedical Optics, 2010, 15(4): 040502.
[51] Wang J F, Li H, Tian G, et al. Near-infrared probe-based confocal microendoscope for deep-tissue imaging[J]. Biomedical Optics Express, 2018, 9(10): 5011-5025.
[52] Kyrish M, Kester R, Richards-Kortum R, et al. Improving spatial resolution of a fiber bundle optical biopsy system[J]. Proceedings of SPIE, 2010, 7558: 17-25.
[53] Han J H, Lee J, Kang J U. Pixelation effect removal from fiber bundle probe based optical coherence tomography imaging[J]. Optics Express, 2010, 18(7): 7427-7439.
[54] Lane P M, Elliott R P, MacAulay C E. Confocal microendoscopy with chromatic sectioning[J]. Proceedings of SPIE, 2003, 4959: 23-26.
[55] Yang X F, Wang Y, Zhang H J, et al. Fiber-optic large-depth 3D chromatic confocal endomicroscopy[J]. Biomedical Optics Express, 2022, 13(1): 300-313.
[56] Kang D, Carruth R W, Kim M, et al. Endoscopic probe optics for spectrally encoded confocal microscopy[J]. Biomedical Optics Express, 2013, 4(10): 1925-1936.
[57] Tearney G J, Webb R H, Bouma B E. Spectrally encoded confocal microscopy[J]. Optics Letters, 1998, 23(15): 1152-1154.
[58] Boudoux C, Yun S, Oh W, et al. Rapid wavelength-swept spectrally encoded confocal microscopy[J]. Optics Express, 2005, 13(20): 8214-8221.
[59] Schlachter S C, Kang D, Gora M J, et al. Spectrally encoded confocal microscopy of esophageal tissues at 100 kHz line rate[J]. Biomedical Optics Express, 2013, 4(9): 1636-1645.
[60] Tao Y K, Izatt J A. Spectrally encoded confocal scanning laser ophthalmoscopy[J]. Optics Letters, 2010, 35(4): 574-576.
[61] Malone J D, El-Haddad M T, Bozic I, et al. Simultaneous multimodal ophthalmic imaging using swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography[J]. Biomedical Optics Express, 2016, 8(1): 193-206.
[62] Rashtchian S, Youssef K, Rezai P Y, et al. High-speed label-free confocal microscopy of *Caenorhabditis elegans* with near infrared spectrally encoded confocal microscopy[J]. Biomedical Optics Express, 2021, 12(6): 3607-3618.
[63] Olsovsky C, Shelton R, Carrasco-Zevallos O, et al. Chromatic confocal microscopy for multi-depth imaging of epithelial tissue[J]. Biomedical Optics Express, 2013, 4(5): 732-740.
[64] Tadayon M A, Chaitanya S, Martyniuk K M, et al. 3D microphotonic probe for high resolution deep tissue imaging[J]. Optics Express, 2019, 27(16): 22352-22362.
[65] Shi K B, Li P, Yin S Z, et al. Chromatic confocal microscopy using supercontinuum light[J]. Optics Express, 2004, 12(10): 2096-2101.
[66] Rayer M, Mansfield D. Chromatic confocal microscopy using staircase diffractive surface[J]. Applied Optics, 2014, 53(23): 5123-5130.
[67] Garzón J, Gharbi T, Meneses J. Real time determination of the optical thickness and topography of tissues by chromatic confocal microscopy[J]. Journal of Optics A: Pure and Applied Optics, 2008, 10(10): 104028.
[68] Li S B, Liang R G. DMD-based three-dimensional chromatic confocal microscopy[J]. Applied Optics, 2020, 59(14): 4349-4356.
[69] Kakeji Y, Yamaguchi S, Yoshida D, et al. Development and assessment of morphologic criteria for diagnosing gastric cancer using confocal endomicroscopy: an *ex vivo* and *in vivo* study[J]. Endoscopy, 2006, 38(9): 886-890.
[70] East J E, Vleugels J L, Roelandt P, et al. Advanced endoscopic imaging: european society of gastrointestinal endoscopy (ESGE) technology review[J]. Endoscopy, 2016, 48(11): 1029-1045.
[71] Committee A S G E T. Confocal laser endomicroscopy[J]. Gastrointestinal Endoscopy, 2014, 80(6): 928-938.
[72] Li Z, Yu T, Zuo X L, et al. Confocal laser endomicroscopy for *in vivo* diagnosis of gastric intraepithelial neoplasia: a feasibility study[J]. Gastrointestinal Endoscopy, 2010, 72(6): 1146-1153.
[73] Pilonis N D, Januszewicz W, di Pietro M. Confocal laser endomicroscopy in gastro-intestinal endoscopy: technical aspects and clinical applications[J]. Translational Gastroenterology and Hepatology, 2022, 7: 7.
[74] Neumann H, Langner C, Neurath M F, et al. Confocal laser endomicroscopy for diagnosis of barrett's esophagus[J]. Frontiers in Oncology, 2012, 2: 42.
[75] Gaddam S, Mathur S C, Singh M, et al. Novel probe-based confocal laser endomicroscopy criteria and interobserver agreement for the detection of dysplasia in Barrett's esophagus[J]. The American Journal of Gastroenterology, 2011, 106(11): 1961-1969.
[76] di Pietro M, Bertani H, O'Donovan M, et al. Development and validation of confocal endomicroscopy diagnostic criteria for low-grade dysplasia in barrett's esophagus[J]. Clinical and Translational Gastroenterology, 2019, 10(4): e00014.
[77] Wu J, Pan Y M, Wang T T, et al. Confocal laser endomicroscopy for detection of neoplasia in Barrett's esophagus: a meta-analysis[J]. Diseases of the Esophagus, 2014, 27(3): 248-254.
[78] Pech O, Rabenstein T, Manner H, et al. Confocal laser endomicroscopy for *in vivo* diagnosis of early squamous cell carcinoma in the esophagus[J]. Clinical Gastroenterology and Hepatology, 2008, 6(1): 89-94.
[79] Zhang J N, Li Y Q, Zhao Y A, et al. Classification of gastric pit patterns by confocal endomicroscopy[J]. Gastrointestinal Endoscopy, 2008, 67(6): 843-853.
[80] Li Z, Zuo X L, Li C Q, et al. New classification of gastric pit patterns and vessel architecture using probe-based confocal laser endomicroscopy[J]. Journal of Clinical Gastroenterology, 2016, 50(1): 23-32.

[81] González S, Swindells K, Rajadhyaksha M, et al. Changing paradigms in dermatology: confocal microscopy in clinical and surgical dermatology[J]. Clinics in Dermatology, 2003, 21(5): 359-369.

[82] Aghassi D, González E, Anderson R R, et al. Elucidating the pulsed-dye laser treatment of sebaceous hyperplasia *in vivo* with real-time confocal scanning laser microscopy[J]. Journal of the American Academy of Dermatology, 2000, 43(1): 49-53.

[83] Diaconeasa A, Boda D, Neagu M, et al. The role of confocal microscopy in the dermato-oncology practice[J]. Journal of Medicine and Life, 2011, 4(1): 63-74.

[84] Rajadhyaksha M, González S, Zavislan J M, et al. *In vivo* confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology[J]. Journal of Investigative Dermatology, 1999, 113(3): 293-303.

[85] González S, Sackstein R, Anderson R R, et al. Real-time evidence of *in vivo* leukocyte trafficking in human skin by reflectance confocal microscopy[J]. The Journal of Investigative Dermatology, 2001, 117(2): 384-386.

[86] Peppelman M, Wolberink E A W, Gerritsen M J P, et al. Application of leukotriene B4 and reflectance confocal microscopy as a noninvasive *in vivo* model to study the dynamics of skin inflammation[J]. Skin Research and Technology, 2015, 21(2): 232-240.

[87] Batani A, Branisteanu D E, Ilie M A, et al. Assessment of dermal papillary and microvascular parameters in psoriasis vulgaris using *in vivo* reflectance confocal microscopy[J]. Experimental and Therapeutic Medicine, 2018, 15(2): 1241-1246.

[88] Căruntu C, Boda D, Căruntu A, et al. *In vivo* imaging techniques for psoriatic lesions[J]. Romanian Journal of Morphology and Embryology, 2014, 55(3): 1191-1196.

[89] Ghiță M A, Căruntu C, Rosca A E, et al. Real-time investigation of skin blood flow changes induced by topical capsaicin[J]. Acta Dermatovenerologica Croatica: ADC, 2017, 25(3): 223-227.

[90] Caruntu C, Boda D. Evaluation through *in vivo* reflectance confocal microscopy of the cutaneous neurogenic inflammatory reaction induced by capsaicin in human subjects[J]. Journal of Biomedical Optics, 2012, 17(8): 085003.

[91] Okabe M, Ikawa M, Kominami K, et al. 'Green mice' as a source of ubiquitous green cells[J]. FEBS Letters, 1997, 407(3): 313-319.

[92] Meyer L E, Otberg N, Sterry W, et al. *In vivo* confocal scanning laser microscopy: comparison of the reflectance and fluorescence mode by imaging human skin[J]. Journal of biomedical optics, 2006, 11(4): 044012.

[93] Skvara H, Plut U, Schmid J A, et al. Combining *in vivo* reflectance with fluorescence confocal microscopy provides additive information on skin morphology[J]. Dermatology Practical & Conceptual, 2012, 2(1): 3-12.

[94] Rajadhyaksha M, Grossman M, Esterowitz D, et al. *In vivo* confocal scanning laser microscopy of human skin: melanin provides strong contrast[J]. Journal of Investigative Dermatology, 1995, 104(6): 946-952.

[95] Guitera P, Pellacani G, Crotty K A, et al. The impact of *in vivo* reflectance confocal microscopy on the diagnostic accuracy of lentigo maligna and equivocal pigmented and nonpigmented macules of the face[J]. Journal of Investigative Dermatology, 2010, 130(8): 2080-2091.

[96] Guida S, Longo C, Casari A, et al. Update on the use of confocal microscopy in melanoma and non-melanoma skin cancer[J]. Giornale Italiano Di Dermatologia e Venereologia: Organo Ufficiale, Societa Italiana Di Dermatologia e Sifilografia, 2015, 150(5): 547-563.

[97] Pellacani G, De Pace B, Reggiani C, et al. Distinct melanoma types based on reflectance confocal microscopy[J]. Experimental Dermatology, 2014, 23(6): 414-418.

[98] Serban E D, Farnetani F, Pellacani G, et al. Role of *in vivo* reflectance confocal microscopy in the analysis of melanocytic lesions[J]. Acta Dermatovenerologica Croatica: ADC, 2018, 26(1): 64-67.

[99] González S, Sánchez V, González-Rodríguez A, et al. Confocal microscopy patterns in nonmelanoma skin cancer and clinical applications[J]. Actas Dermo-Sifiliográficas (English Edition), 2014, 105(5): 446-458.

[100] Białek-Galas K, Wielowieyska-Szybińska D, Dyduch G, et al. The use of reflectance confocal microscopy in selected inflammatory skin diseases[J]. Polish Journal of Pathology, 2015, 66(2): 103-108.

[101] Constantin M M, Bucur S, Serban D E, et al. Dermoscopic and laser confocal features of an exogenous ochronosis case[J]. Giornale Italiano Di Dermatologia e Venereologia: Organo Ufficiale, Societa Italiana Di Dermatologia e Sifilografia, 2020, 155(3): 367-368.

[102] Lange-Asschenfeldt S, Bob A, Terhorst D, et al. Applicability of confocal laser scanning microscopy for evaluation and monitoring of cutaneous wound healing[J]. Journal of Biomedical Optics, 2012, 17(7): 076016.

[103] Altintas A A, Altintas M A, Ipaktchi K, et al. Assessment of microcirculatory influence on cellular morphology in human burn wound healing using reflectance-mode-confocal microscopy[J]. Wound Repair and Regeneration, 2009, 17(4): 498-504.

[104] Altintas A A, Guggenheim M, Oezcelik A, et al. Local burn versus local cold induced acute effects on *in vivo* microcirculation and histomorphology of the human skin[J]. Microscopy Research and Technique, 2011, 74(10): 963-969.

[105] Malvehy J, Pérez-Anker J, Toll A, et al. *Ex vivo* confocal microscopy: revolution in fast pathology in dermatology[J]. The British Journal of Dermatology, 2020, 183(6): 1011-1025.

[106] Lu Q S, Jiang G. Progress in the application of reflectance confocal microscopy in dermatology[J]. Postepy Dermatologii i Alergologii, 2021, 38(5): 709-715.

[107] Tan J, Delaney P, McLaren W J. Confocal endomicroscopy: a novel imaging technique for *in vivo* histology of cervical intraepithelial neoplasia[J]. Expert Review of Medical Devices, 2007, 4(6): 863-871.

[108] Drezek R A, Collier T, Brookner C K, et al. Laser scanning confocal microscopy of cervical tissue before and after application of acetic acid[J]. American Journal of Obstetrics and Gynecology, 2000, 182(5): 1135-1139.

[109] Schlosser C L, Bodenschatz N, Lam S F, et al. Fluorescence confocal endomicroscopy of the cervix: pilot study on the potential and limitations for clinical implementation[J]. Journal of Biomedical Optics, 2016, 21(12): 126011.

[110] Carlson K, Pavlova I, Collier T, et al. Confocal microscopy: imaging cervical precancerous lesions[J]. Gynecologic Oncology, 2005, 99(3): S84-S88.

[111] Tan J, Quinn M A, Pyman J M, et al. Detection of cervical intraepithelial neoplasia *in vivo* using confocal endomicroscopy[J]. BJOG: an International Journal of Obstetrics and Gynaecology, 2009, 116(12): 1663-1670.

[112] Collier T, Lacy A, Richards-Kortum R, et al. Near real-time confocal microscopy of amelanotic tissue: detection of dysplasia in *ex vivo* cervical tissue[J]. Academic Radiology, 2002, 9(5): 504-512.

[113] Lemp M A, Dilly P N, Boyde A. Tandem-scanning (confocal) microscopy of the full-thickness cornea[J]. Cornea, 1985, 4(4): 205-209.

[114] Erie J C, McLaren J W, Patel S V. Confocal microscopy in ophthalmology[J]. American Journal of Ophthalmology, 2009, 148(5): 639-646.

[115] Guthoff R F, Zhivov A, Stachs O. *In vivo* confocal microscopy, an inner vision of the cornea: a major review[J]. Clinical & Experimental Ophthalmology, 2009, 37(1): 100-117.

[116] Tavakoli M, Hossain P, Malik R A. Clinical applications of corneal confocal microscopy[J]. Clinical Ophthalmology, 2008, 2(2): 435-445.
[117] Iester M, Oddone F, Fogagnolo P, et al. Changes in the morphological and functional patterns of the ocular surface in patients treated with prostaglandin analogues after the use of TSP 0.5%® preservative-free eyedrops: a prospective, multicenter study[J]. Ophthalmic Research, 2014, 51(3): 146-152.
[118] Iester M, Telani S, Frezzotti P, et al. Ocular surface changes in glaucomatous patients treated with and without preservatives beta-blockers[J]. Journal of Ocular Pharmacology and Therapeutics: the Official Journal of the Association for Ocular Pharmacology and Therapeutics, 2014, 30(6): 476-481.
[119] Fogagnolo P, Dipinto A, Vanzulli E, et al. A 1-year randomized study of the clinical and confocal effects of tafluprost and latanoprost in newly diagnosed glaucoma patients[J]. Advances in Therapy, 2015, 32(4): 356-369.
[120] Fogagnolo P, Sacchi M, Ceresara G, et al. The effects of topical coenzyme Q_{10} and vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate after cataract surgery: a clinical and *in vivo* confocal study[J]. Ophthalmologica, 2013, 229(1): 26-31.
[121] De Cillà S, Fogagnolo P, Sacchi M, et al. Corneal involvement in uneventful cataract surgery: an *in vivo* confocal microscopy study[J]. Ophthalmologica, 2014, 231(2): 103-110.
[122] Randon M, Liang H, El Hamdaoui M, et al. *In vivo* confocal microscopy as a novel and reliable tool for the diagnosis of Demodex eyelid infestation[J]. The British Journal of Ophthalmology, 2015, 99(3): 336-341.
[123] Tuft S, Bron A J. Imaging the microstructural abnormalities of meesmann corneal dystrophy by *in vivo* confocal microscopy[J]. Cornea, 2006, 25(7): 868.
[124] Frezzotti P, Fogagnolo P, Haka G, et al. *In vivo* confocal microscopy of conjunctiva in preservative-free timolol 0.1% gel formulation therapy for glaucoma[J]. Acta Ophthalmologica, 2014, 92(2): e133-e140.
[125] Labbé A, Dupas B, Hamard P, et al. *In vivo* confocal microscopy study of blebs after filtering surgery[J]. Ophthalmology, 2005, 112(11): 1979
[126] Labbé A, Gheck L, Iordanidou V, et al. An *in vivo* confocal microscopy and impression cytology evaluation of pterygium activity[J]. Cornea, 2010, 29(4): 392-399.
[127] Patel D V, Sherwin T, McGhee C N J. Laser scanning *in vivo* confocal microscopy of the normal human corneoscleral limbus[J]. Investigative Ophthalmology & Visual Science, 2006, 47(7): 2823-2827.
[128] Gabison E E, Labbé A, Brignole-Baudouin F, et al. Confocal biomicroscopy of corneal intraepithelial neoplasia regression following interferon alpha 2b treatment[J]. The British Journal of Ophthalmology, 2010, 94(1): 134-135.
[129] Ceresara G, Fogagnolo P, de Cillà S, et al. Corneal involvement in Crohn's disease: an *in vivo* confocal microscopy study[J]. Cornea, 2011, 30(2): 136-142.
[130] Ceresara G, Fogagnolo P, Zuin M, et al. Study of corneal copper deposits in Wilson's disease by *in vivo* confocal microscopy[J]. Ophthalmologica, 2014, 231(3): 147-152.
[131] Sung K B, Richards-Kortum R, Follen M, et al. Fiber optic confocal reflectance microscopy: a new real-time technique to view nuclear morphology in cervical squamous epithelium *in vivo*[J]. Optics Express, 2003, 11(24): 3171-3181.
[132] Collier T G, Guillaud M, Anais Malpica M D, et al. Real-time reflectance confocal microscopy: comparison of two-dimensional images and three-dimensional image stacks for detection of cervical precancer[J]. Journal of Biomedical Optics, 2007, 12(2): 024021.
[133] Sheikhzadeh F, Ward R K, Carraro A, et al. Quantification of confocal fluorescence microscopy for the detection of cervical intraepithelial neoplasia[J]. Biomedical Engineering Online, 2015, 14: 96.
[134] Goetz M, Ziebart A, Foersch S, et al. *In vivo* molecular imaging of colorectal cancer with confocal endomicroscopy by targeting epidermal growth factor receptor[J]. Gastroenterology, 2010, 138(2): 435-446.
[135] Moussata D, Goetz M, Gloeckner A, et al. Confocal laser endomicroscopy is a new imaging modality for recognition of intramucosal bacteria in inflammatory bowel disease *in vivo*[J]. Gut, 2011, 60(1): 26-33.
[136] Maher N G, Solinas A, Scolyer R A, et al. *In vivo* reflectance confocal microscopy for evaluating melanoma of the lip and its differential diagnoses[J]. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 2017, 123(1): 84-94.
[137] Cui Q, Liang R G. Chromatic confocal microscopy using liquid crystal display panels[J]. Applied Optics, 2019, 58(8): 2085-2090.
[138] Deguchi T, Bianchini P, Palazzolo G, et al. Volumetric lissajous confocal microscopy with tunable spatiotemporal resolution[J]. Biomedical Optics Express, 2020, 11(11): 6293-6310.

Confocal Endoscopic Microscopy and Its Applications

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Abstract

Significance Over the past few decades, endoscopes have been used to view the interior of cavities in the human body or the surfaces of internal human organs noninvasively for diagnosis or treatment. However, white light endoscopy and magnifying endoscopy widely used in clinical practice have poor resolution and contrast and require pathological biopsy examination to confirm the diagnosis. In recent years, narrow-spectrum technology has used blue light via optical or digital filtering to irradiate tissues and enhance the microstructural and microvascular morphology of the mucosal surface, improving the imaging contrast. However, it still exhibits poor resolution. White light and narrow-spectrum endoscopy cannot achieve cellular-level resolution; therefore, a purely optical biopsy cannot be performed, significantly reducing diagnosis accuracy. Confocal endoscopy has emerged owing to its submicron resolution and optical sectioning capability.

Cell morphology observed using confocal endoscopy is highly consistent with the biopsy pathology. Since its introduction in 2004, confocal laser endomicroscopy (CLE) has become a vital technique in gastrointestinal endoscopic imaging. Confocal laser endoscopy enables endoscopists to perform cellular imaging and tissue structure assessments at the focal plane during endoscopic testing. Thus, real-time *in vivo* histological information can be obtained, enabling "optical biopsy."

Progress Confocal microscopy was first developed in 1957 by Minsky, who used pinholes on the illumination and detection sides in the same conjugate image plane to achieve "confocal." In 1967, Egger and Petrăn successfully used confocal microscopy for label-free imaging of neural tissues. The key to confocal microscopy imaging technology is that the "double focus" of the two pinholes can shield all signals from the nonfocal plane, and the photomultiplier tube behind the detection pinhole can detect only the signal from the focal plane to achieve optical sectioning. Depending on the source of the image contrast, laser scanning confocal microscopy can be performed in the fluorescence or reflectance mode. Fluorescence confocal microscopy requires fluorescent contrast agents to generate contrast, yielding spatial and functional information about endogenous autofluorescence and exogenously labeled molecules and structures. Reflection confocal microscopy relies on differences in the refractive indices of cellular structures to generate natural contrast.

Based on the scanning method, confocal endoscopy in confocal endoscopic imaging technology is divided into endoscopy-integrated and probe-based confocal endoscopy. As shown in Figure 2, the endoscopy-integrated confocal endoscope adopts the distal scanning mode [Fig. 2(b)], whereas the probe-based confocal endoscope adopts the proximal scanning mode [Fig. 2(a)]. The eCLE uses a point-scanning method to drive a single optical fiber to scan through a scanning device, achieving high-resolution confocal endoscopic imaging. Because the eCLE adopts a distal scanning method and the mechanical scanning device is included in the imaging probe, it is necessary to miniaturize the mechanical scanning device. However, the miniaturization of this device required for confocal endoscopy is technically challenging and expensive. Therefore, eCLE is limited to clinical applications because of the limited size of the mechanical scanning device. The pCLE probe does not contain a scanning device, and the scanning device does not have size limitation. However, its resolution is limited by the distance between the cores, and the imaging quality is affected by the honeycomb structure of the fiber bundle.

As both eCLE and pCLE are based on traditional confocal microscopy imaging techniques, they use a single excitation wavelength. However, the traditional confocal endoscope requires mechanical scanning to complete three-dimensional imaging, and the imaging speed is low. Therefore, traditional confocal endoscopic microscopy-imaging solutions cannot achieve rapid three-dimensional deep tissue imaging or real-time optical diagnosis in clinical practice. Spectral-encoded confocal microscopy (SECM) is a reflection confocal microscopy technique. It can be used to determine the spatial position of a sample by measuring the spectrum of light reflected from the sample. It can significantly increase the confocal imaging speed, enabling large-area imaging within a short time. The high imaging rate of SECM can potentially increase the confocal field of view, but the imaging depth of the focus is still limited to 200 μm. At more significant imaging depths, the effective resolution of SECM is significantly reduced owing to light scattering and optical aberrations.

In recent years, chromatic confocal technology has been used to achieve high-resolution, fast, and multi-depth imaging. Chromatic confocal endoscopy solves the problem of insufficient imaging depth in traditional confocal endoscopy, and shows significant potential in gastric cancer diagnosis. However, it cannot guarantee large chromatic and small spherical aberration simultaneously in miniaturization, owing to the limitations of lens-manufacturing technology, resulting in limited axial resolution.

Confocal endoscopes enable *in vivo* and real-time imaging of different tissues, cells, molecules, and even bacteria owing to the higher magnification and resolution of confocal endoscopes than those of conventional endoscopes. In particular, confocal endoscopic imaging technology has promising applications in diagnosing diseases in the human body, such as the gastrointestinal tract, skin, cervix, and eye.

Conclusions and Prospects In this paper, confocal endoscopic imaging technology is briefly described. A comparative introduction is presented for fluorescence confocal imaging, reflectance confocal imaging, and probe-based and endoscope-integrated confocal endoscopic imaging. Furthermore, the application of confocal endomicroscopy in biomedical science is discussed.

Key words microscopy; endoscopy; confocal microscopic imaging; resolution; optical biopsy