The role of confocal laser endomicroscopy in pulmonary medicine

Sen Tian[1,3], Haidong Huang[1,3], Yifei Zhang[1,2,3], Hui Shi[1], Yuchao Dong[1], Wei Zhang[1] and Chong Bai[1,2]

[1]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Naval Medical University, Shanghai, China. [2]Department of Biomedical Engineering, University of Shanghai for Science and Technology, Shanghai, China. [3]These authors contributed equally to this work.

Corresponding author: Chong Bai (Chongbai@smmu.edu.cn)

Shareable abstract (@ERSpublications)
Confocal laser endomicroscopy (CLE) serves as an effective and minimally invasive technique in multiple pulmonary diseases. In the future, a combination of CLE and artificial intelligence has potential to become the main trend. https://bit.ly/3ic7eCK

Cite this article as: Tian S, Huang H, Zhang Y, *et al*. The role of confocal laser endomicroscopy in pulmonary medicine. *Eur Respir Rev* 2023; 32: 220185 [DOI: 10.1183/16000617.0185-2022].

Received: 29 Sept 2022
Accepted: 21 Nov 2022

Abstract

Accurate diagnosis and subsequent therapeutic options in pulmonary diseases mainly rely on imaging methods and histological assessment. However, imaging examinations are hampered by the limited spatial resolution of images and most procedures that are related to histological assessment are invasive with associated complications. As a result, a high-resolution imaging technology – confocal laser endomicroscopy (CLE), which is at the forefront and enables real-time microscopic visualisation of the morphologies and architectures of tissues or cells – has been developed to resolve the clinical dilemma pertaining to current techniques. The current evidence has shown that CLE has the potential to facilitate advanced diagnostic capabilities, to monitor and to aid the tailored treatment regime for patients with pulmonary diseases, as well as to expand the horizon for unravelling the mechanism and therapeutic targets of pulmonary diseases. In the future, if CLE can be combined with artificial intelligence, early, rapid and accurate diagnosis will be achieved through identifying the images automatically. As promising as this technique may be, further investigations are required before it can enter routine clinical practice.

Introduction

At present, imaging examinations and histological assessment are required for accurate diagnosis and planning of subsequent treatment of patients with pulmonary diseases. However, currently applied imaging techniques such as computed tomography (CT) scans are limited by the spatial resolution of images, do not provide real-time feedback on lesions and are associated with radiation exposure [1]. For histological assessment, even minimally invasive transbronchial biopsies are suboptimal due to false-negative results, inconsistent sample harvesting and a distinct complication rate [2].

A novel "optical biopsy" technique – confocal laser endomicroscopy (CLE), with a combination of high-resolution and real-time microscopic analysis of tissue architecture – has therefore been developed, aiming to bridge the gap between *ex vivo* conventional sampling procedures and *in vivo* imaging examinations. Whereas this technique is still its infancy, this review gives a comprehensive overview based on the current literature and presents promising clinical data for the CLE imaging of pulmonary diseases such as pulmonary malignancies, interstitial lung diseases (ILD), pneumonia, lung transplantation, COPD and other lung diseases (table 1).

Technical outline

CLE, also referred to as fibred confocal fluorescent microscopy, is an emerging endoscopic imaging technology that enables real-time and high-resolution imaging of the area of interest (*e.g.* alveolar structure, pleura, microvessels and lymph nodes). During CLE, a flexible miniprobe is guided using transbronchial procedures and transthoracic biopsy needles to the target area, where it hits the specimen

TABLE 1 Human clinical trials using confocal laser endomicroscopy (CLE) imaging in pulmonary diseases

Pulmonary diseases	Imaging area	pCLE	nCLE	Application
Malignancies	Airway wall	*Lung cancer* Differentiation of normal and malignant lesions [5, 9, 10,16]		Biopsy guidance Early, rapid, and accurate diagnosis of lung cancer Real-time surveillance and evaluation on surgical procedures
	Lung parenchyma	*Lung cancer* Detection of lung cancer nodules [11, 12,17–19, 26, 29–32]	*Lung cancer* Detection of peripheral pulmonary nodules [34–37, 39]	
	Mediastinal lymph nodes		*Lung cancer* Identification of malignant lymph nodes [42, 43]	Distinguish malignant from benign reactive lymph nodes
	Pleura	*Pleural lesions* Discrimination of normal and abnormal pleura [44, 45] Detection of malignant cells in pleural effusion [46] Detection of VPI [47]	*Pleural lesions* Discrimination of MPM and pleural fibrosis [45]	Biopsy guidance Improve cytological-aided diagnosis of malignant effusions Determine surgical procedures
ILD	Lung parenchyma	Specific pCLE patterns of ILD; manually [50–57] and automatically [59]		Biopsy guidance An increase of diagnostic yield and a decrease of the complication risk during TBCB
Pneumonia	Lung parenchyma	Identification of pCLE features in pneumonia [62,64–72]		Rapid *in situ* detection of pathogens Study host–pathogen interactions in response to therapies
Lung transplantation	Lung parenchyma	Diagnosis of ACR [75–79]		Early diagnosis of ACR after lung transplantation
COPD	Airway wall	Visualisation of elastin fibres in ECM [81, 82] Relation between pCLE imaging and lung function parameters [84, 85]		Study the regional microenvironment in COPD Evaluate the impact of therapeutic interventions
Other pulmonary diseases	Airway wall or lung parenchyma	pCLE characteristics of asthma [86], ARDS [87], TBOP [88], bronchial amyloidosis [89], PAM [90], endobronchial hamartoma [91] and metastatic pulmonary calcification [92]		Improvement of the (early) diagnosis of other pulmonary diseases

ACR: acute cellular rejection; ARDS: acute respiratory distress syndrome; ECM: extracellular matrix; ILD: interstitial lung disease; MPM: malignant pleural mesothelioma; nCLE: needle-based confocal laser endomicroscopy; PAM: pulmonary alveolar microlithiasis; pCLE: probe-based confocal laser endomicroscopy; TBCB: transbronchial cryobiopsy; TBOP: tracheobronchopathia osteochondroplastica; VPI: visceral pleural invasion.

with a low power laser light (most commonly 488 nm). Reflected light emitted from the specimen travels back up the pinhole to the detector. Meanwhile, out-of-focus light is blocked by the pinhole, resulting in microscopic imaging [3] (figure 1a).

Clinical evidence has indicated the utility and safety of CLE imaging in gastrointestinal and pancreatobiliary diseases [4]. In 2007, Thiberville *et al*. [5] first introduced this technique into the respiratory system and described the unique microscopic patterns of the normal areas from the trachea down to the respiratory bronchioles (figure 1b–d and video 1). They also demonstrated that the CLE images mainly relied upon the elastin component that constitutes the backbone of the alveolar ducts and the extra-alveolar microvessels [5, 6] (figure 1e). These findings formed the basis for future work to harness the potential utility of CLE and, to date, in respiratory diseases, there is a commercially available CLE system (Cellvizio®; Mauna Kea Technologies, Paris, France) with a depth of focus between 0 and 50 µm, a lateral resolution of up to 3.5 µm, a maximum field of view of 600 µm and a video frame rate of scanning between nine and 12 images per second.

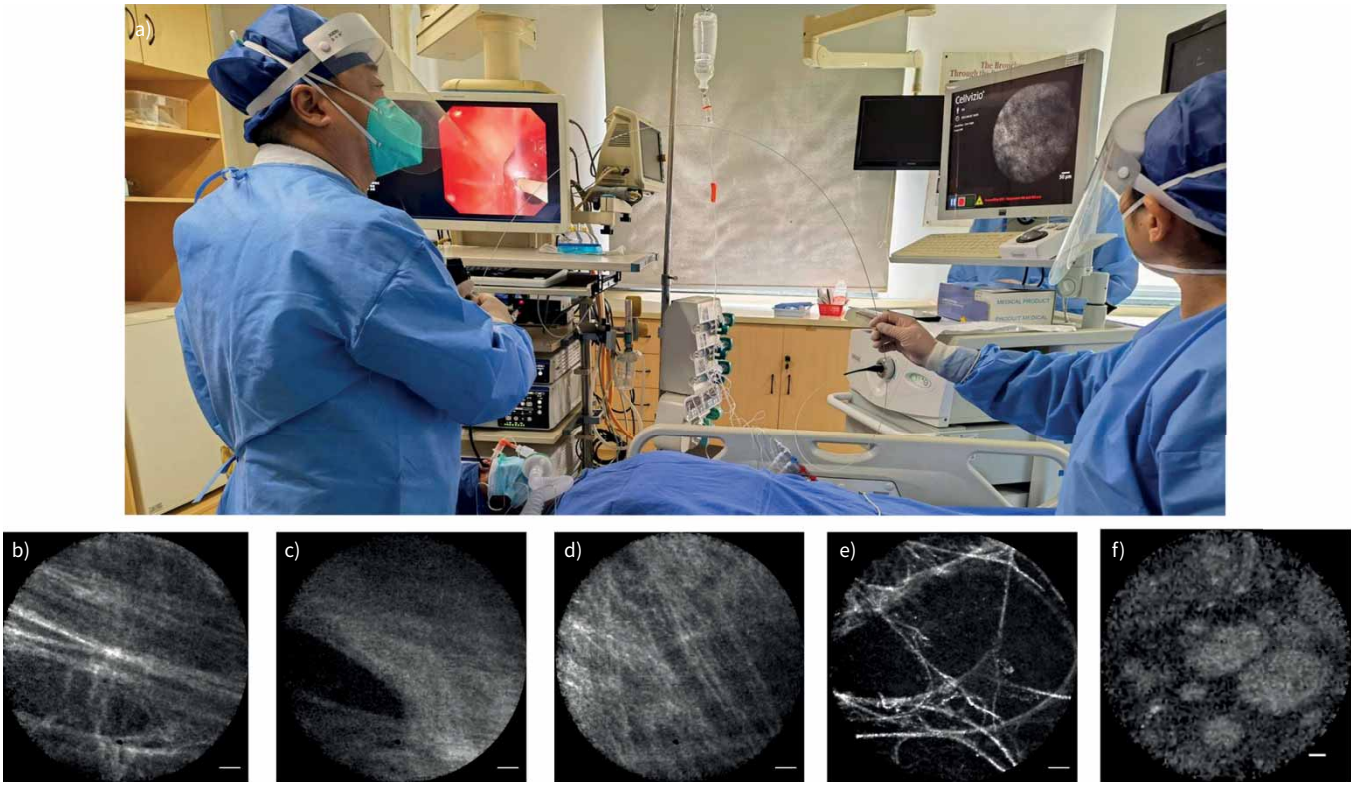

FIGURE 1 a) The operation of confocal laser endomicroscopy (CLE) in the clinical setting. Normal probe-based CLE (pCLE) microscopy images of: b) main carina; c) bronchial gland opening, right main bronchus carina; d) respiratory bronchiole, fibred network; e) alveolar wall, medial-basal segment of right lower lobe; f) capsular structures of a lymph node. Video 1 is available as supplementary data to display the autofluorescence microstructure obtained during pCLE imaging corresponding to normal bronchial areas (main carina to left main bronchus carina). Scale bars: b–e) 50 μm; f) 20 μm.

At present, CLE applications in clinical practice include probe-based CLE (pCLE) and needle-based CLE (nCLE). Although pCLE is more frequently used, nCLE with a smaller probe allows the visualisation of individual malignant cells and different anatomical structures of lymph nodes, and consequently it is particularly suitable for the assessment of lymph nodes (figure 1f).

Clinical applications

Malignancies

Lung cancer

Globally, lung cancer is the most frequent malignant tumour and the leading cause of cancer-related mortality in men [7]. Tissue biopsy remains the gold standard for accurate diagnosis of malignancies; however, there are associated time and cost complications. Even less-invasive techniques such as rapid on-site cytological evaluation have associated complications [8]. CLE, as a noninvasive and optical biopsy tool that can provide real-time histopathological information of the surface epithelium, is potentially attractive to resolve the limitations described above.

Central lung cancer

Thiberville *et al*. [5] reported the very first clinical application of pCLE imaging for pre-malignant bronchial lesions *in vivo*. Disorganisation of the fibred network was present in almost every pre-malignant lesion and it was significantly distinct from the normal airways. Filner *et al*. [9] showed that pCLE was able to differentiate between normal and malignant lesions with a high level of image reliability. In 2015, a comparative study between pCLE images and histopathological findings was conducted by Wellikoff *et al*. [10] on 25 nonsmall-cell lung cancer patients who underwent bronchoscopies; the results indicated that the septal studding, mottled elastin and disorganised and fragmented tissue with increased friability seen in pCLE images correlated well with the findings of histopathology. These findings were also observed in our centre. However, one study published by Luo *et al*. [11] investigated the efficacy of pCLE

imaging in 39 consecutive patients with lung cancer. Their study demonstrated that a fibred structure could not be found in 11 of 14 lesions and thus malignant lesions could not be identified. This could be also observed in the study performed by Seth *et al*. [12], who explored the utility of pCLE nodule imaging for assessing risk of malignancy. When using this technique, no significant advantage was found in discriminating malignant from benign nodules. The aforesaid differences may be attributed to the use of generic contrast agents that make the epithelial cells with low autofluorescence visible. Previous studies demonstrated the usefulness of fluorescein in further characterising the epithelium [13, 14]. Unfortunately, fluorescein administration does not permit staining of central airwave cellularity as this substance cannot penetrate into the epithelial layer of the bronchial mucosa, therefore restricting its application for detecting malignancy [15].

One pilot study performed by Fuchs *et al*. [16] on 32 patients with suspected malignancies compared 75 522 images obtained from pCLE after using topical acriflavine hydrochloride and pathological features collected by biopsy specimen. Their study concluded that acriflavine-aided pCLE allowed subsurface imaging and a meticulous analysis at cellular and subcellular levels; the results showcased an accuracy of 91% for diagnosing lung cancer, which was comparable to the diagnostic yield of biopsies. The fact that acriflavine can improve pCLE image interpretation has been validated in recent research as well [17]. Furthermore, methylene blue with the requisition of the 660 nm Cellvizio® system has been used in the clinical setting and proven to have a similar diagnostic efficacy to acriflavine [18]. Similarly, Sorokina *et al*. [19] conducted a comparative study between light microscopy and pCLE findings, where 18 lobectomy specimens from 18 different patients with lung cancer nodules were collected and subsequently stained by methylene blue. They found and described three specific patterns in all specimens, as follows: alveolar dystelectasis with thickening of alveolar walls, alveolar oedema and influx of macrophages. In addition, pCLE could distinguish different subtypes of lung cancer, including adenocarcinoma, squamous cell carcinoma and small-cell carcinoma, on the basis of alterations of stromal and parenchymal components. A comparative study between acriflavine and methylene blue genotoxicity performed by Obstoy *et al*. [20] on both NCI-H460 cells and cultured human lymphocytes using the comet assay showed that methylene blue could be applied more safely as compared to acriflavine when imaging pre-cancerous lesions by pCLE *in vivo*. Surprisingly, along with contrast agents, molecularly targeted agents have the potential to help identify the presence of malignancy [21–24]. Nevertheless, considering that this technology is still used preliminarily, careful assessment is warranted.

Apart from differentiating benign and malignant lesions, pCLE imaging might contribute to the real-time surveillance and evaluation of surgical procedures. In the field of gastrointestinal surgery, the value of pCLE in the margin delineation of the tumour-resected tissue has been investigated [25]. Recently, Terra *et al*. [26] reported a young man who was diagnosed with endobronchial carcinoid and underwent robot-assisted bronchoplasty. pCLE imaging was employed to precisely detect the neoplastic area during the bronchoplasty and finally avoided associated lung resection. It should be noted, however, that the experience of pCLE for margin assessment in lung cancer surgery is currently limited to case reports and, thus, larger validation studies are clearly needed.

Peripheral lung cancer

There has been a rising number of solitary pulmonary nodules (SPNs) being detected due to lung cancer screening initiatives and the growing use of CT. The accurate diagnosis of SPNs, especially peripheral lung nodules, is becoming a challenging task for interventional pulmonologists [27]. Currently, where multiple innovative technologies are clinically available for the diagnosis of peripheral lung cancer, although promising, the limitation of any single technique should be recognised [28]. Integrating and synergising CLE and other techniques may represent a new avenue.

A landmark study was done by Arenberg *et al*. [29], who proposed the first criteria of pCLE for peripheral lung nodules (the Columbus classification) based on the “compact alveoli” pattern. When applying this classification, cancer was detected with 70–80% sensitivity and 58–74% specificity; but this classification for predicting cancer lacked prospective validation. Shulimzon *et al*. [30] reported a series of five patients with a mediastinal mass and another five with a lung mass who underwent pCLE imaging with CT-guided transthoracic needle biopsy (TTNB) and the results showed that pCLE was able to augment the yield of CT-guided TTNB where vascularised tissue could be differentiated from fibrotic and necrotic areas before biopsy; however, cellular identification remained difficult. In 2015, one research group performed a study involving three cases with SPNs using pCLE imaging combined with radial endobronchial ultrasound (r-EBUS) and navigational bronchoscopy. Its main conclusion was that this novel method was safe and effective for *in vivo* imaging of SPNs [31]. It was noted that it was difficult for a 1 mm pCLE confocal probe (AlveoFlex®) to reach the upper lobes as a result of its stiffness. In view of

this, the same research group developed a thinner and more flexible probe (CholangioFlex®) with an external diameter of 0.6 mm designed for bile duct exploration and demonstrated that pCLE imaging with the miniprobe was achievable in all lobes of the lungs [32]. However, although the detection range could be expanded when using the CholangioFlex®, it came at the cost of spatial resolution and image quality.

From our own experience with *in vivo* pCLE analysis of peripheral pulmonary nodules, we could identify the associated characteristics that permit precise localisation and thereby significantly improve the diagnostic capabilities of bronchoscope biopsy. Tumour tissue displays a chaotic arrangement of fibrous tissues with heterogeneous densities, accompanied with large dark spots or black hole structures (figure 2a). We present here the case of a benign peripheral pulmonary nodule. By using chest CT, a 60-year woman with cough and expectoration was found to have a pulmonary nodule in the left lower lobe (figure 3a). pCLE imaging was employed to analyse the lesion after confirming the location of the nodule using r-EBUS (figure 3b). Homogeneous distribution of elastin fibres without black hole or spot structures could be observed at both ends of the lesion (figure 3c and d), implying a non-neoplastic nodule. Finally, a high rate of diagnostic consistency was found between pCLE changes and histopathological analysis (fibrin exudation and inflammatory tissues).

Further uptake of pCLE imaging has been hampered by the limited probe size, with the associated lack of visualisation of malignant lesions deep to the bronchial wall [32]. nCLE characterised by a smaller size of miniprobe in conformation with the anatomy of airway might present a prospective approach to resolving that dilemma. Although all the previous studies of nCLE are confined to pancreatic diseases [33], Wijmans *et al*. [34] examined for the first time the utility of nCLE in the case of a patient with a peripheral pulmonary nodule. The authors described consistent characteristics found both by nCLE imaging (dark aggregates within alveolar structures) as well as later histopathology (the malignant adenocarcinoma cells). Su *et al*. [35] explored the application of nCLE, in conjunction with X-ray and endoscopic-ultrasound (EUS), to assess peripheral lung cancer. They found that nCLE was conducive to positioning and characterising the extraluminal pulmonary nodules. Kramer *et al*. [36] used bronchoscopic nCLE imaging with the help of r-EBUS and fluoroscopy to identify suspected peripheral lung cancer; the accuracy of which was up to 95%. In addition to combining with EUS, r-EBUS and fluoroscopy, nCLE could integrate with robotic navigational bronchoscopy whose diagnostic yield is limited by the high near-miss rate of the target lesion and thereby optimises the analysis of peripheral lung nodules [37]. As prospective as this technique can be, nCLE does not deliver specific detection of individual tumour cells due to the principle of reverse-contrast imaging; in addition, imaging interpretation is highly dependent upon experienced observers [36, 38]. When taking into account these limitations, Kennedy *et al*. [39] proposed a novel

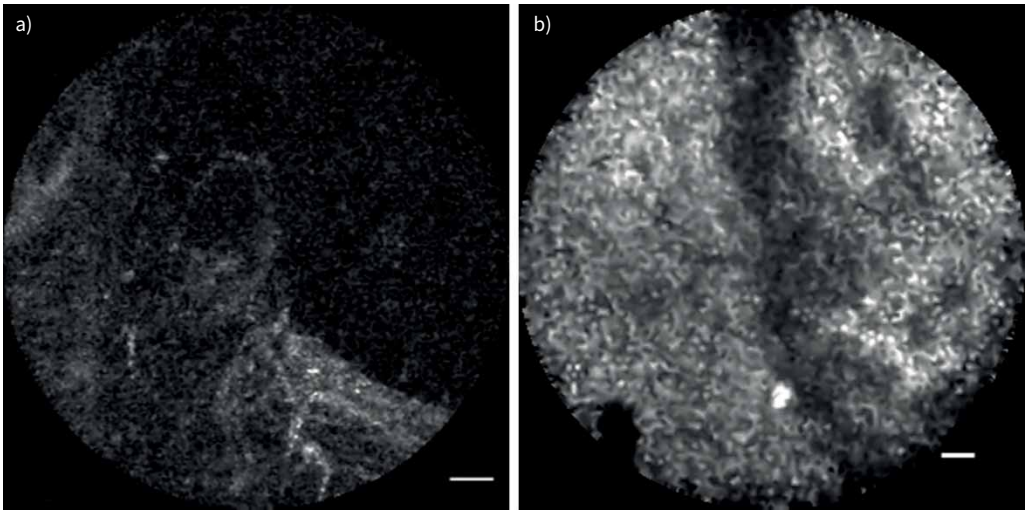

FIGURE 2 a) Probe-based confocal laser endomicroscopy images showing disordered fibrous tissues with deep degradation of the original structure, accompanied with a spot or black hole structure. b) Needle-based confocal laser endomicroscopy (nCLE) *in vivo* real-time images of lymphocytes in reactive lymph nodes showing dark clumps caused by a cluster of enlarged cells. Video 2 is available as supplementary data to display the autofluorescence microstructure obtained during nCLE imaging corresponding to the 11R-lymph node. Scale bars: a) 50 μm; b) 20 μm.

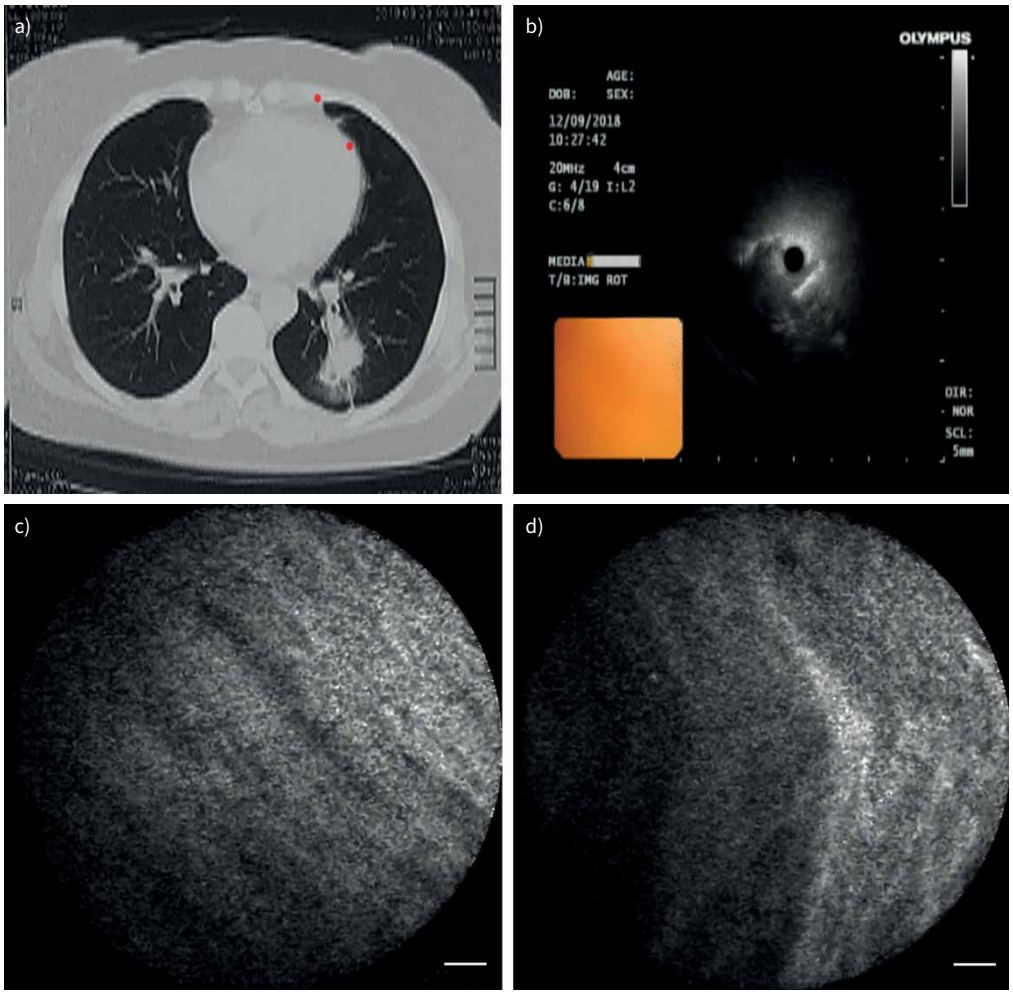

FIGURE 3 a) Computed tomography scan of the chest showing a pulmonary nodule located in the left lower lobe. b) Radial endobronchial ultrasound confirming the lesion location outside the bronchial lumen. Probe-based confocal laser endomicroscopy images showing the uniform distribution of arrangements of elastin fibres c) at the proximal and d) distal end of the lesion. Scale bars: c) and d) 50 μm.

method (NIR-nCLE), in which near-infrared (NIR) imaging using a cancer-targeted tracer (pafolacianine) was integrated with nCLE, aiming to achieve real-time cancer detection at the cellular level. The post-resection specimens of 20 patients with SPNs were imaged by NIR-nCLE. All imaging findings were identified by five blinded nonexperts. NIR-nCLE displayed excellent diagnostic accuracy (97%) and inter-observer agreement (κ=0.95) in the classification of SPNs. Furthermore, ground-glass opacity (GGO), which is a diagnostic challenge owing to the features of the early stage adenocarcinoma spectrum [40], could be accurately, rapidly and consistently identified using NIR-nCLE. Additional studies *in vivo* are required to better confirm these findings.

Mediastinal lymph nodes

nCLE marked by the imaging miniprobe exhibits a remarkable advantage in terms of the assessment of lymph nodes. In gastro-intestinal diseases like pancreatic carcinoma, CLE has been proven to be superior to conventional needle aspiration [41]. Benias *et al.* [42] evaluated the feasibility of nCLE at the time of EUS in 28 consecutive patients with enlarged lymph nodes. All patients successfully experienced the procedure without complications. In this study, benign, malignant and inflammatory lymph nodes could be discriminated according to different nCLE features; especially for malignancy, dark pleomorphic cells were easily identified. In an observational study performed by Wijmans *et al.* [43] on 22 patients who were scheduled for EUS-guided fine-needle aspiration (FNA), 21 mediastinal nodes were evaluated by nCLE before EUS-FNA, without adverse events. Three nCLE imaging features consisting of dark enlarged

pleomorphic cells, dark clumps and directional streaming were developed to distinguish malignant lesions from surrounding normal tissue. The criteria showed a high identification accuracy of 89%, substantial inter-observer agreement and excellent intra-observer reliability. Our centre analysed real-time nCLE images from mediastinal nodes and the results revealed that nCLE enabled quick identification of dark enlarged pleomorphic cells or dark clumps in most of the malignant lymph nodes (figure 2b and video 2).

Pleura

When it comes to pleural lesions, both pCLE and nCLE play a part in detecting different pleural lesions. Bonhomme *et al.* [44] presented a small series of three referred patients who underwent pCLE imaging during medical thoracoscopy. Three different pCLE images of correlative patients were precisely described, namely normal pleura, carcinomatous pleuritis and a malignant mesothelioma. A multicentre study undertaken by Wijmans *et al.* [45] showed that either pCLE or nCLE imaging, obtained by diagnostic pleural biopsy procedures, allowed real-time visualisation of pleural abnormalities at a microscopic level, which could be differentiated from pleural fibrosis. Therefore, CLE has the potential to be a guidance tool for pleural biopsy, thereby improving the accuracy and reducing the number of repeated traditional biopsy procedures required. In malignant pleural effusion, a pilot study demonstrated that pCLE was capable of detecting malignant cells of pleural effusion rapidly and simply [46]. Moreover, Sawada *et al.* [47] investigated the feasibility of intraoperative detection of visceral pleural invasion (VPI) using pCLE imaging in patients with primary lung cancer. They demonstrated that the accuracy rate in the diagnosis of VPI through pCLE was 86.7%. However, to corroborate the aforementioned findings, a prospective randomised study with a larger data accrual is necessary.

ILD

Rapid, accurate and minimally invasive diagnosis is paramount for both therapeutic intervention and prognostication in ILD patients. Whilst transbronchial cryobiopsy (TBCB) has been documented to be a promising alternative to surgical procedures in the diagnosis of ILD [48], there has been concern regarding the nondiagnostic rate and TBCB-related complications (*e.g.* bleeding, pneumothorax and acute exacerbation of ILD) [49], which has hampered its further development. To cope with the bottleneck of this technique, pCLE has been introduced as an emerging technology for providing real-time feedback of the elastin fibre network within the alveoli.

Salaün *et al.* [50] reported the first utility of pCLE examination in a patient diagnosed with pulmonary alveolar proteinosis (PAP), in which the globular lipoproteinaceous material caused by a fluorescent floating amorphous substance filling the alveolar areas was compatible with the bronchoalveolar lavage (BAL) findings. Similar results were reported in a larger series of six PAP patients [51]. Mamenko *et al.* [52] presented a case diagnosed with desquamative interstitial pneumonia (DIP) in nonsmoker and analysed this case using pCLE imaging. The thickened intra-alveolar septal and clusters of autofluorescent cells within the alveoli were observed *via* pCLE imaging, which could offer further assistance to pulmonologists in the differential diagnosis of DIP. Salaün *et al.* [53] evaluated the diagnostic yield of pCLE imaging in 36 nonsmoking patients with amiodarone-related pneumonia (AMR-IP). The manifestation of at least one alveolar area with constantly large fluorescent cells showed a sensitivity of 100% and specificity of 88% in the diagnosis of AMR-IP. A study included 27 suspected ILD patients who underwent transbronchial pCLE imaging in an outpatient setting; the authors identified six specific pCLE patterns, namely normal, increased fibres, densely packed fibres, hypercellular, thickened fibres and others/nonspecific. On the basis of these patterns, they could differentiate between chronic fibrosing ILD and other ILD and demonstrated that the hypercellular pattern on pCLE was associated with inflammatory patterns on CT [54]. Another study performed by Salaün *et al.* [55] described nine distinct patterns to identify different types of ILD. Wijmans *et al.* [56] retrospectively evaluated the pCLE images from 20 ILD patients who underwent BAL. In contrast to high-resolution CT (HRCT), pCLE provided additional valuable information such as alveolar cells with GGO and lung fibrosis with increased alveolar elastin fibres. In addition, pCLE has the potential to provide high-resolution guidance for TBCB. One study involving 14 ILD patients compared data obtained from *in* and *ex vivo* pCLE images and histological data collected from lung biopsies. Three different patterns identified by pCLE were non-, mild and dense fibrotic areas respectively [57], in which dense fibrotic areas should be avoided for TBCB due to limited diagnostic value [58].

Whereas all the studies reviewed above for the application of pCLE in the diagnosis of ILD have mainly focused on qualitative evaluation by the expert observer or the methods manually selected post-processing parts, Bondesson *et al.* [59] proposed a fully automatic workflow based on the connectivity and thickness statistics of alveoli elastin for the structural identification of alveoli elastin *via* pCLE imaging. They showed that this method appeared to be a valuable tool for the differentiation of normal and ILD alveoli.

It is important to note that pCLE in its current form cannot replace histology, because pCLE does not allow for the regional evaluation of tissue, which is crucial in the diagnosis of ILD. As a consequence, pCLE could be an accessory technique in flexible bronchoscopy together with BAL to differentiate inflammatory phenotypes from predominant fibrotic phenotypes.

Pneumonia

Multiple pioneering studies have been undertaken to identify unique features with the distal lung using pCLE imaging in different types of pneumonia. Morisse *et al*. [60] established a rat model of immunosuppressant-induced invasive pulmonary aspergillosis (IPA) using a fluorescent transformed Tag red fluorescent protein (Tag-RFP) *Aspergillus fumigatus* strain or a wild strain of *A. fumigatus* and assessed the utility of pCLE imaging in IPA. They demonstrated that pCLE could identify the hyphae in Tag-RFP *A. fumigatus* and the infiltration of fluorescent alveolar macrophages in both groups. When using these characteristics, pCLE imaging showcased appreciable sensitivity (83%) and specificity (100%) in detecting IPA. Later, the same research group explored the ability of a fluorescent peptide tracer of pCLE binding specifically to *A. fumigatus* (the c(CGGRLGPFC)-NH2([FITC]) peptide). Compared with other areas, the IPA foci presented higher fluorescence intensity ratio [61]. An optical Smartprobe labelled by the NBD-UBI_{dend} compound was also developed by Akram *et al*. [62], with potential clinical utility in the specific and quick detection of IPA using pCLE imaging. Vanherp *et al*. [63] demonstrated that transbronchial pCLE enabled real-time visualisation of the morphologies of fungal cells in mice with pulmonary *Aspergillus* or *Cryptococcus* infections, which could be differentiated from noninfected mice. Clinically relevant was a case report executed by Danilevskaya *et al*. [64] on pCLE images of an IPA patient. Fibrillar branching fluorescent structures could be observed in pCLE imaging, which correlated well with HRCT characterisation. Whether pCLE imaging is superior to HRCT in diagnostics of IPA remains to be determined; however, it can aid *in vivo* express diagnosis by providing additional highly detailed information. Furthermore, Shafiek *et al*. [65] described their experience with pCLE imaging in 32 patients with pneumocystis jirovecii pneumonia (PJP) suffering from HIV. pCLE images of alveoli that were performed with no procedure-related complications at the time of the bronchoscopy represented in real-time intra-alveolar exudates. Based on the characteristics, the study claimed high sensitivity and specificity for PJP diagnosis, especially in smokers.

In addition to fungi, the specific characteristics of bacteria and viruses can be recognised using pCLE imaging. Mills *et al.* [66] designed a bacteria-specific probe for the identification of bacteria *in vitro* and obtained excellent proof-of-concept outcomes that could increase clinical tractability confidence. Recently, there has been a rising interest in developing smartprobes with the required selectivity, sensitivity, compatibility and accuracy for detecting bacteria, showing promising results [62, 67–69]. To meet the needs of separating fluorescent targets during pCLE imaging, Parker *et al*. [70] designed a simple widefield ratiometric pCLE imaging platform with low cost in which the contrast between similar fluorescent signals could be enhanced and showed that this system was able to detect labelled Gram-negative bacteria even in the context of extremely autofluorescent lung tissue. Moreover, Eldaly *et al*. [71] presented an algorithm based on a hierarchical Bayesian model without the limitation of a specific spatial organisation of the fibre bundle. It represented a fully automatic approach that helps detect labelled bacteria in pCLE imaging and distinguish controls from bacterial loads of different concentrations. Surprisingly, the first application of pCLE imaging in a case of a patient with coronavirus disease 2019 was reported by Vasilev *et al*. [72] in 2021. In this case, the characteristics of pCLE imaging including alveolar thickened fibres with increased density, elastin network with a disordered arrangement and a large number of intra-alveolar secretions were described. While these are encouraging studies, it remains challenging to precisely classify the broad categories of pneumonia due to the relatively limited datasets explored.

Lung transplantation

Acute cellular rejection (ACR) is highly prevalent in the first year following lung transplantation [73]. The demonstration of ACR has relied mostly on the detection of alveolar tissue with perivascular lymphocytic infiltration, which requires the involvement of transbronchial biopsy (TBB) complemented with BAL, but TBB - carries inherent risks such as bleeding and pneumothorax, and have considerably variable interpretation and inevitable sampling error [74]. pCLE imaging allowing high-resolution vascular, alveolar and cellular visualisation has been explored for the diagnosis of ACR in lung transplant recipients. So far, a total of five studies in humans have been executed, with meaningful results. First *in vivo*, in humans, reports on pCLE in the diagnosis of acute lung rejection date back to 2010 [75]. In the earliest study, pCLE images of patients with diagnosis of ACR revealed moderate to severe alveolar cellularity, which prepared the ground for the following research. Yserbyt *et al*. [76] found an association between ACR and the number of autofluorescent cells at the level of the alveolar space (ACA) and their autofluorescence

intensity (ACA AF). Shortly thereafter, they established standardised descriptive criteria for reporting pCLE images in patients with ACR, including ACA, the number of frames with ACA and ACA AF. When applying these criteria, the sensitivity and specificity of diagnosis of ACR were 93% and 83%, respectively [77]. Also, KELLER *et al.* [78] disclosed vessels with perivascular cellular infiltration (PCI) on patients with ACR and vessels free from PCI in all other cases without ACR. This has also been verified in a recent publication undertaken by the same group [79]. In the prospective trail, they assessed two pCLE criteria (*i.e.* abundant alveolar cellularity (AAC) and PCI) by comparing with histopathologic criteria of ACR. PCI identified by pCLE imaging displayed a significantly higher diagnostic performance and inter-observer agreement than those of AAC. At the same time, a significant correlation between the number of blood vessels identified with PCI and histopathologic grading of ACR was observed, and PCI could contribute to the assessment of ACR, but requiring substantial training for image interpretation.

Our centre often utilises pCLE imaging to identify patterns ranging from minimal to absent alveolar cellularity (figure 4a) to AAC (figure 4b and video 3) and vessels with negative PCI (figure 4c) to positive PCI (figure 4d and video 4) for lung transplant surveillance, and has achieved good results. It is noteworthy that irrespective of the appearance of ACR, fluorescent cells can also be observed in active smokers because macrophages containing tobacco tar possess the characteristics of autofluorescence, which may interfere with the interpretation of pCLE imaging [77, 80].

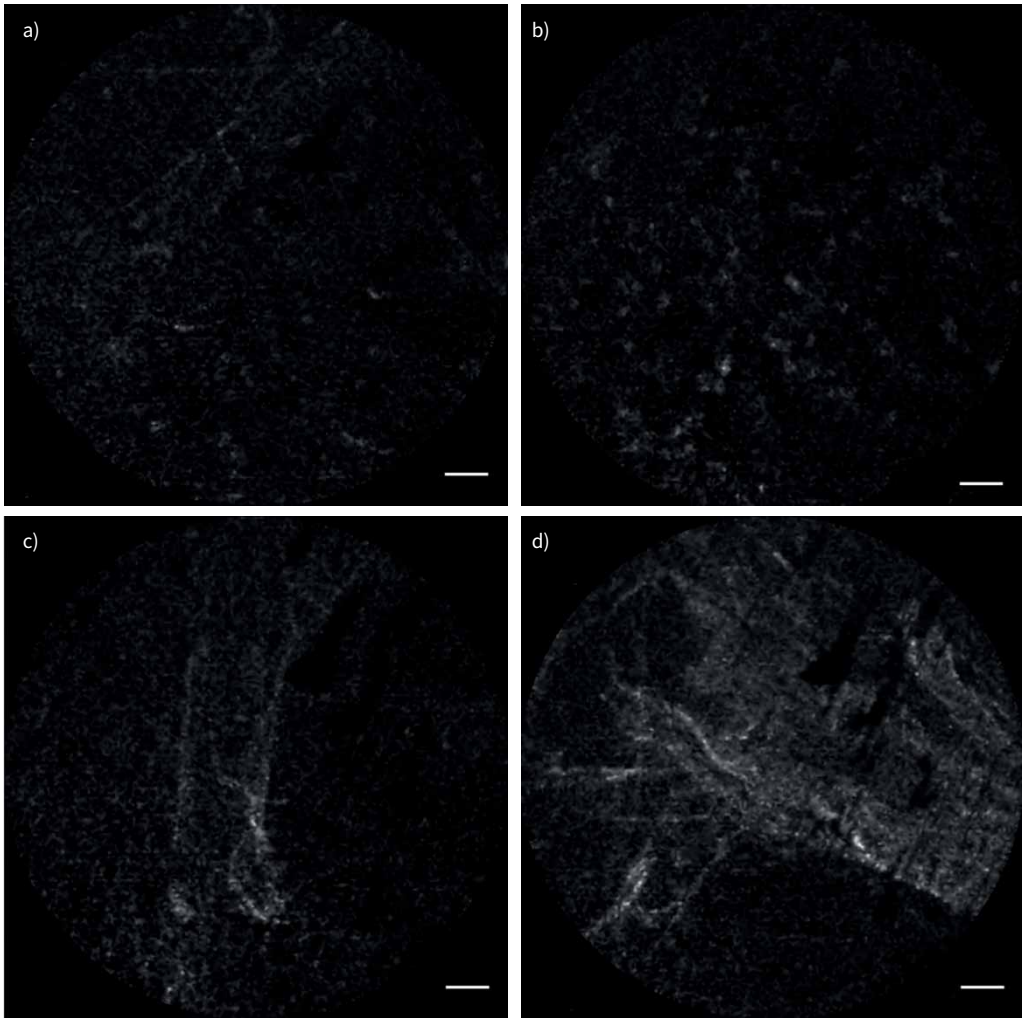

FIGURE 4 Alveolar and perivascular cellular infiltration (PCI). a) Alveoli with minimal to absent alveolar cellularity. b) Abundant alveolar cellularity (AAC). c) Vessels with negative PCI. d) Vessels with positive PCI. Video 3 and video 4 are available as supplementary data to display AAC and vessels with positive PCI separately. Scale bars: 50 μm.

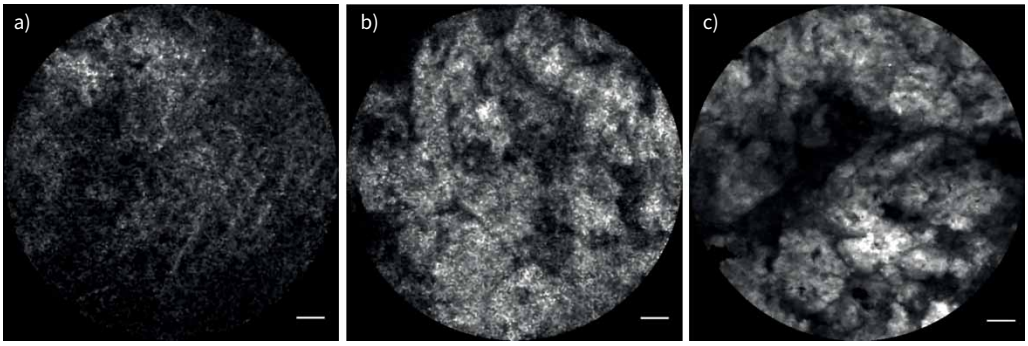

FIGURE 5 Probe-based confocal laser endomicroscopy images showing a) mottled elastin and autofluorescent submucosa in tracheobronchopathia osteochondroplastica, b) unusual dappled with a patchy "cotton wool"-like appearance in bronchial amyloidosis and c) loss of the normal bronchial wall elastin network in endobronchial hamartoma. Scale bars: 50 μm.

COPD

Several studies have indicated that pCLE imaging has the potential to identify the structure–function correlation in COPD. The inverse correlation between forced expiratory volume in one second (FEV_1) predicted and the volume fraction of elastic fibres was firstly described by Black *et al.* [81]. Possibly showing the loss of elastic walls as a result of the destruction of elastin fibres in the extracellular matrix, which has been confirmed in the trail conducted by Newton *et al.* [82]. For the task of quantitative evaluation of the lung microcirculation using pCLE imaging, Salaün *et al* [83] used a rat model with experimental emphysema. It was observed that the elastase group showed higher alveolar facet diameters and intercapillary distances as compared with the controls. The significance of pCLE imaging is further substantiated by Cosío *et al.* [84], who have related specific structural characteristics of pCLE imaging and lung function parameters in patients with COPD. In addition, a recent publication focusing on the usefulness of pCLE imaging in 15 patients with emphysema and 15 healthy controls found an increased cross-sectional area of the alveolar openings and decreased autofluorescence intensity in those with emphysema [85]. However, the value that pCLE imaging adds as a complementary tool of lung function and chest CT to deliver diagnostic information remains to be evaluated.

Other lung diseases

Besides the above findings, pCLE imaging has also been reported in other pulmonary diseases. Yick *et al.* [86] investigated bronchial wall changes using pCLE imaging in a cross-sectional study (eight asthma patients and eight healthy controls) and found a strong relationship between elastic fibre patterns and post-bronchodilator FEV_1 % pred. In addition, real-time and highly detailed pCLE imaging at the bedside appears to be a promising tool for patients with acute respiratory distress syndrome, decreasing the need for open lung biopsy and minimising associated complications [87]. In tracheobronchopathia osteochondroplastica, pCLE imaging showed mottled elastin and autofluorescent submucosa [88] (figure 5a). In bronchial amyloidosis, pCLE examination showed an unusual dappled patchy "cotton wool"-like appearance [89] (figure 5b). Additionally, case reports of pulmonary alveolar microlithiasis [90], endobronchial hamartoma [91] (figure 5c) and metastatic pulmonary calcification [92] were presented in succession. Given that the present findings reviewed above are based on a small sample size, further studies with a larger patient cohort are needed before this technique can be implemented in the clinical field.

Future prospects

At present and although there is increasing interest in translating CLE imaging to enable endomicroscopic exploration in interventional pulmonology, this novel approach has not entered routine clinical settings yet and has found application only in limited expert centres due to various factors such as the lack of trained personnel, limited validation trials and high capital costs. The wider implementation of CLE imaging in the field of pulmonary medicine will require these limitations being addressed.

In the field of lung cancer, efforts are being made to further boost tumour identification. The potential of CLE imaging based on fluorescently labelled tracers has been demonstrated by using polymer dots for real-time high-resolution tracking of macrophage Ana-1 cells in the alveolus of lung and lymph nodes [24]

and fluorescently labelled erlotinib for epidermal growth factor receptor-mutated tumours in xenografted mouse models [23, 93]. Furthermore, a novel hybrid CLE–Raman endomicroscopy system has been developed to enable real-time visualisation of cell/tissue architecture as well as to provide detailed biomolecular compositional information, paving the way for the development of multimodal imaging for diagnostics [94].

In the clinic, manual analysis of CLE images is a very labour-intensive and time-consuming process, and the imaging interpretation has characteristics of subjectivity [71, 95]. There has therefore been great interest in the automated analysis of CLE images, using machine learning tools, to enable accurate predictions with minimal human intervention [12, 18, 59, 71]. Numerous images provided by more related studies with large amounts of patients included are required to build an image library, which will play an essential role in facilitating the future implementation of automated computational analysis of CLE images [96]. Moreover, the advancement of low-cost modular [22] or widefield fibred [70] CLE imaging systems, and single-use disposable packaged CLE fibres [97] may herald the wider application of CLE imaging in the field of pulmonary medicine.

Conclusion

In multiple pulmonary diseases, CLE has been demonstrated to be an effective and minimally invasive technique that enables the real-time visualisation of lesions with a diverse field of view. In the near future, it can be envisaged that a large atlas of CLE images for different pulmonary diseases will be established and can be identified automatically using artificial intelligence; as a result, the role of CLE in facilitating advanced diagnostic capabilities, monitoring and evaluating the treatment response for patients with pulmonary diseases will be further highlighted. Notably, CLE is solely a component of an integrated management in pulmonary medicine; in order to incorporate the benefits and minimise the deficiencies of every single technique, CLE in conjunction with other technologies should be the preferred strategy.

Questions for future research

- How can CLE imaging be standardised (*e.g.* by building an image library or developing automated software) to reduce inter-observer and intra-observer variability relating to diagnostic image interpretation?
- What are the genotoxic effects of acriflavine and methylene in humans? Which contrast agent for CLE imaging is better?
- Can fluorescently labelled tracers broaden the scope of the CLE imaging technique?
- What is the efficacy of the hybrid confocal Raman endomicroscopy system for morpho-chemical tissue analysis in the clinic?
- Can the development of single-use CLE fibres and low-cost modular or widefield fibred imaging systems promote the wider adoption of CLE imaging in the field of interventional pulmonology?
- What is the value of CLE imaging in rare diseases (*e.g.* tracheobronchopathia osteochondroplastica, bronchial amyloidosis and pulmonary alveolar microlithiasis)?
- What is the role of artificial intelligence in CLE imaging?

Provenance: Submitted article, peer reviewed.

Conflicts of interest: All authors have nothing to disclose.

Support statement: This work was supported by 234 Climbing the Discipline Program of first affiliated hospital of Naval Medical University (2020YXK026) and the Interdisciplinary Innovation Centre Program of Medicine and Engineering of University of Shanghai for Science and Technology (1021302410). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Vonder M, Dorrius MD, Vliegenthart R. Latest CT technologies in lung cancer screening: protocols and radiation dose reduction. *Transl Lung Cancer Res* 2021; 10: 1154–1164.
2 Silvestri GA, Bevill BT, Huang J, *et al.* An evaluation of diagnostic yield from bronchoscopy: the impact of clinical/radiographic factors, procedure type, and degree of suspicion for cancer. *Chest* 2020; 157: 1656–1664.
3 Perperidis A, Dhaliwal K, McLaughlin S, *et al.* Image computing for fibre-bundle endomicroscopy: a review. *Med Image Anal* 2020; 62: 101620.
4 Pilonis ND, Januszewicz W, di Pietro M. Confocal laser endomicroscopy in gastro-intestinal endoscopy: technical aspects and clinical applications. *Transl Gastroenterol Hepatol* 2022; 7: 7.

5 Thiberville L, Moreno-Swirc S, Vercauteren T, *et al. In vivo* imaging of the bronchial wall microstructure using fibered confocal fluorescence microscopy. *Am J Respir Crit Care Med* 2007; 175: 22–31.
6 Thiberville L, Salaün M, Lachkar S, *et al.* Human *in vivo* fluorescence microimaging of the alveolar ducts and sacs during bronchoscopy. *Eur Respir J* 2009; 33: 974–985.
7 Sung H, Ferlay J, Siegel RL, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209–249.
8 Glinski L, Shetty D, Iles S, *et al.* Single slide assessment: a highly effective cytological rapid on-site evaluation technique for endobronchial and endoscopic ultrasound-guided fine needle aspiration. *Cytopathology* 2019; 30: 164–172.
9 Filner JJ, Bonura EJ, Lau ST, *et al.* Bronchoscopic fibered confocal fluorescence microscopy image characteristics and pathologic correlations. *J Bronchology Interv Pulmonol* 2011; 18: 23–30.
10 Wellikoff AS, Holladay RC, Downie GH, *et al.* Comparison of *in vivo* probe-based confocal laser endomicroscopy with histopathology in lung cancer: a move toward optical biopsy. *Respirology* 2015; 20: 967–974.
11 Luo W, Zhong C, Chen Y, *et al.* [Preliminary observation of imaging with confocal laser endoscopic in lung cancer]. *Zhonghua Jie He He Hu Xi Za Zhi* 2015; 38: 792–793.
12 Seth S, Akram AR, McCool P, *et al.* Assessing the utility of autofluorescence-based pulmonary optical endomicroscopy to predict the malignant potential of solitary pulmonary nodules in humans. *Sci Rep* 2016; 6: 31372.
13 Kiesslich R, Hoffman A, Goetz M, *et al. In vivo* diagnosis of collagenous colitis by confocal endomicroscopy. *Gut* 2006; 55: 591–592.
14 Liu H, Li YQ, Yu T, *et al.* Confocal endomicroscopy for in vivo detection of microvascular architecture in normal and malignant lesions of upper gastrointestinal tract. *J Gastroenterol Hepatol* 2008; 23: 56–61.
15 Fuchs FS, Zirlik S, Hildner K, *et al.* Fluorescein-aided confocal laser endomicroscopy of the lung. *Respiration* 2011; 81: 32–38.
16 Fuchs FS, Zirlik S, Hildner K, *et al.* Confocal laser endomicroscopy for diagnosing lung cancer *in vivo*. *Eur Respir J* 2013; 41: 1401–1408.
17 Takemura M, Kurimoto N, Hoshikawa M, *et al.* Probe-based confocal laser endomicroscopy for rapid on-site evaluation of transbronchial biopsy specimens. *Thorac Cancer* 2019; 10: 1441–1447.
18 Rakotomamonjy A, Petitjean C, Salaün M, *et al.* Scattering features for lung cancer detection in fibered confocal fluorescence microscopy images. *Artif Intell Med* 2014; 61: 105–118.
19 Sorokina A, Danilevskaya O, Averyanov A, *et al.* Comparative study of ex vivo probe-based confocal laser endomicroscopy and light microscopy in lung cancer diagnostics. *Respirology* 2014; 19: 907–913.
20 Obstoy B, Salaun M, Veresezan L, *et al.* Safety and performance analysis of acriflavine and methylene blue for *in vivo* imaging of precancerous lesions using fibered confocal fluorescence microscopy (FCFM): an experimental study. *BMC Pulm Med* 2015; 15: 30.
21 Megia-Fernandez A, Mills B, Michels C, *et al.* Bimodal fluorogenic sensing of matrix proteolytic signatures in lung cancer. *Org Biomol Chem* 2018; 16: 8056–8063.
22 Krstajić N, Mills B, Murray I, *et al.* Low-cost high sensitivity pulsed endomicroscopy to visualize tricolor optical signatures. *J Biomed Opt* 2018; 23: 1–12.
23 Patout M, Guisier F, Brune X, *et al.* Real-time molecular optical micro-imaging of EGFR mutations using a fluorescent erlotinib based tracer. *BMC Pulm Med* 2019; 19: 3.
24 Tang S, Guo Y, Yang Y, *et al.* High resolution tracking of macrophage cells in deep organs and lymphatics using fluorescent polymer dots. *RSC Adv* 2019; 9: 10966–10975.
25 Park JC, Park Y, Kim HK, *et al.* Probe-based confocal laser endomicroscopy in the margin delineation of early gastric cancer for endoscopic submucosal dissection. *J Gastroenterol Hepatol* 2017; 32: 1046–1054.
26 Terra RM, Trindade JM, Gregório PHP, *et al.* Confocal endomicroscopy and robot-assisted bronchoplasty integration to treat an endobronchial tumour. *Interact Cardiovasc Thorac Surg* 2021; 33: 652–653.
27 Gasparini S, Mei F, Bonifazi M, *et al.* Bronchoscopic diagnosis of peripheral lung lesions. *Curr Opin Pulm Med* 2022; 28: 31–36.
28 Kramer T, Annema JT. Advanced bronchoscopic techniques for the diagnosis and treatment of peripheral lung cancer. *Lung Cancer* 2021; 161: 152–162.
29 Arenberg DA, Gildea T, Wilson D. Proposed classification of probe-based confocal laser endomicroscopy findings for evaluation of inderterminate peripheral lung nodules. *Am J Respir Crit Care Med* 2011; 183: A6097.
30 Shulimzon TR, Lieberman S. Feasibility of confocal laser microscopy in CT-guided needle biopsy of pulmonary and mediastinal tumors: a proof-of-concept pilot study. *J Vasc Interv Radiol* 2016; 27: 275–280.
31 Hassan T, Piton N, Lachkar S, *et al.* A novel method for *in vivo* imaging of solitary lung nodules using navigational bronchoscopy and confocal laser microendoscopy. *Lung* 2015; 193: 773–778.
32 Hassan T, Thiberville L, Hermant C, *et al.* Assessing the feasibility of confocal laser endomicroscopy in solitary pulmonary nodules for different part of the lungs, using either 0.6 or 1.4 mm probes. *PLoS One* 2017; 12: e0189846.

33 Bhutani MS, Koduru P, Joshi V, *et al.* EUS-guided needle-based confocal laser endomicroscopy: a novel technique with emerging applications. *Gastroenterol Hepatol* 2015; 11: 235–240.
34 Wijmans L, de Bruin DM, Meijer SL, *et al.* Real-time optical biopsy of lung cancer. *Am J Respir Crit Care Med* 2016; 194: e10–e11.
35 Su Z, Zhong C, Li S, *et al.* Needle-based confocal laser endomicroscopy in the diagnosis of peripheral pulmonary nodule: a preliminary report. *J Thorac Dis* 2017; 9: 2608–2612.
36 Kramer T, Wijmans L, de Bruin M, *et al.* Bronchoscopic needle-based confocal laser endomicroscopy (nCLE) as a real-time detection tool for peripheral lung cancer. *Thorax* 2022; 77: 370–377.
37 Manley C, Kramer T, Kumar R, *et al.* Robotic navigational bronchoscopy combined with needle-based confocal laser endomicroscopy: case report of a novel approach to diagnose small lung nodules. *Respiration* 2022; 101: 494–499.
38 Fernandes S, Williams G, Williams E, *et al.* Solitary pulmonary nodule imaging approaches and the role of optical fibre-based technologies. *Eur Respir J* 2021; 57: 2002537.
39 Kennedy GT, Azari FS, Bernstein E, *et al.* Targeted detection of cancer cells during biopsy allows real-time diagnosis of pulmonary nodules. *Eur J Nucl Med Mol Imaging* 2022; 49: 4194–4204.
40 Pedersen JH, Saghir Z, Wille MM, *et al.* Ground-glass opacity lung nodules in the era of lung cancer CT screening: radiology, pathology, and clinical management. *Oncology* 2016; 30: 266–274.
41 Giovannini M, Caillol F, Monges G, *et al.* Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy in solid pancreatic masses. *Endoscopy* 2016; 48: 892–898.
42 Benias PC, D'Souza LS, Papafragkakis H, *et al.* Needle-based confocal endomicroscopy for evaluation of malignant lymph nodes – a feasibility study. *Endoscopy* 2016; 48: 923–928.
43 Wijmans L, Yared J, de Bruin DM, *et al.* Needle-based confocal laser endomicroscopy for real-time diagnosing and staging of lung cancer. *Eur Respir J* 2019; 53: 1801520.
44 Bonhomme O, Duysinx B, Heinen V, *et al.* First report of probe based confocal laser endomicroscopy during medical thoracoscopy. *Respir Med* 2019; 147: 72–75.
45 Wijmans L, Baas P, Sieburgh TE, *et al.* Confocal laser endomicroscopy as a guidance tool for pleural biopsies in malignant pleural mesothelioma. *Chest* 2019; 156: 754–763.
46 Zirlik S, Hildner K, Rieker RJ, *et al.* Confocal laser endomicroscopy for diagnosing malignant pleural effusions. *Med Sci Monit* 2018; 24: 5437–5447.
47 Sawada T, Takizawa H, Aoyama M, *et al.* Diagnosis of visceral pleural invasion using confocal laser endomicroscopy during lung cancer surgery. *J Thorac Dis* 2021; 13: 4742–4752.
48 Korevaar DA, Colella S, Fally M, *et al.* European Respiratory Society guidelines on transbronchial lung cryobiopsy in the diagnosis of interstitial lung diseases. *Eur Respir J* 2022; 16: 2200425.
49 Avasarala SK, Wells AU, Colby TV, *et al.* Transbronchial cryobiopsy in interstitial lung diseases: state-of-the-art review for the interventional pulmonologist. *J Bronchology Interv Pulmonol* 2021; 28: 81–92.
50 Salaün M, Roussel F, Hauss PA, *et al. In vivo* imaging of pulmonary alveolar proteinosis using confocal endomicroscopy. *Eur Respir J* 2010; 36: 451–453.
51 Danilevskaya O, Averyanov A, Lesnyak V, *et al.* Confocal laser endomicroscopy for diagnosis and monitoring of pulmonary alveolar proteinosis. *J Bronchology Interv Pulmonol* 2015; 22: 33–40.
52 Mamenko IS, Vasilev IV, Tabanakova IA, *et al.* Probe-based confocal laser endomicroscopy in diagnosis of desquamative interstitial pneumonia in nonsmoker. *Monaldi Arch Chest Dis* 2019; 89: 1052.
53 Salaün M, Roussel F, Bourg-Heckly G, *et al. In vivo* probe-based confocal laser endomicroscopy in amiodarone-related pneumonia. *Eur Respir J* 2013; 42: 1646–1658.
54 Meng P, Tan GL, Low SY, *et al.* Fibred confocal fluorescence microscopy in the diagnosis of interstitial lung diseases. *J Thorac Dis* 2016; 8: 3505–3514.
55 Salaün M, Guisier F, Dominique S, *et al. In vivo* probe-based confocal laser endomicroscopy in chronic interstitial lung diseases: specific descriptors and correlation with chest CT. *Respirology* 2019; 24: 783–791.
56 Wijmans L, Kalverda K, de Bruin D, *et al.* Bronchoscopic journey of *in vivo* real-time microscopic imaging in ILD: a case series. *Respiration* 2022; 101: 1006–1014.
57 Wijmans L, Bonta PI, Rocha-Pinto R, *et al.* Confocal laser endomicroscopy as a guidance tool for transbronchial lung cryobiopsies in interstitial lung disorder. *Respiration* 2019; 97: 259–263.
58 Hetzel J, Maldonado F, Ravaglia C, *et al.* Transbronchial cryobiopsies for the diagnosis of diffuse parenchymal lung diseases: expert statement from the cryobiopsy working group on safety and utility and a call for standardization of the procedure. *Respiration* 2018; 95: 188–200.
59 Bondesson D, Schneider MJ, Silbernagel E, *et al.* Automated evaluation of probe-based confocal laser endomicroscopy in the lung. *PLoS One* 2020; 15: e0232847.
60 Morisse H, Heyman L, Salaün M, *et al. In vivo* and *in situ* imaging of experimental invasive pulmonary aspergillosis using fibered confocal fluorescence microscopy. *Med Mycol* 2012; 50: 386–395.
61 Morisse H, Heyman L, Salaün M, *et al. In vivo* molecular microimaging of pulmonary aspergillosis. *Med Mycol* 2013; 51: 352–360.

62 Akram AR, Avlonitis N, Scholefield E, *et al.* Enhanced avidity from a multivalent fluorescent antimicrobial peptide enables pathogen detection in a human lung model. *Sci Rep* 2019; 9: 8422.
63 Vanherp L, Poelmans J, Hillen A, *et al.* Bronchoscopic fibered confocal fluorescence microscopy for longitudinal *in vivo* assessment of pulmonary fungal infections in free-breathing mice. *Sci Rep* 2018; 8: 3009.
64 Danilevskaya O, Averyanov A, Klimko N, *et al.* The case of diagnostics of invasive pulmonary aspergillosis by in vivo probe-based confocal laser endomicroscopy of central and distal airways. *Med Mycol Case Rep* 2014; 5: 35–39.
65 Shafiek H, Fiorentino F, Cosio BG, *et al.* Usefulness of bronchoscopic probe-based confocal laser endomicroscopy in the diagnosis of *Pneumocystis jirovecii* pneumonia. *Respiration* 2016; 92: 40–47.
66 Mills B, Akram AR, Scholefield E, *et al.* Optical screening of novel bacteria-specific probes on *ex vivo* human lung tissue by confocal laser endomicroscopy. *J Vis Exp* 2017; 129: 56284.
67 Akram AR, Avlonitis N, Lilienkampf A, *et al.* A labelled-ubiquicidin antimicrobial peptide for immediate in situ optical detection of live bacteria in human alveolar lung tissue. *Chem Sci* 2015; 6: 6971–6979.
68 Craven TH, Walton T, Akram AR, *et al.* Activated neutrophil fluorescent imaging technique for human lungs. *Sci Rep* 2021; 11: 976.
69 Mills B, Megia-Fernandez A, Norberg D, *et al.* Molecular detection of Gram-positive bacteria in the human lung through an optical fiber-based endoscope. *Eur J Nucl Med Mol Imaging* 2021; 48: 800–807.
70 Parker HE, Stone JM, Marshall ADL, *et al.* Fibre-based spectral ratio endomicroscopy for contrast enhancement of bacterial imaging and pulmonary autofluorescence. *Biomed Opt Express* 2019; 10: 1856–1869.
71 Eldaly AK, Altmann Y, Akram A, *et al.* Bayesian bacterial detection using irregularly sampled optical endomicroscopy images. *Med Image Anal* 2019; 57: 18–31.
72 Vasilev IV, Mamenko IS, Makarova AV, *et al.* Probe-based confocal laser endomicroscopy in COVID-19. *Adv Respir Med* 2021; 89: 456–459.
73 Greer M, Werlein C, Jonigk D. Surveillance for acute cellular rejection after lung transplantation. *Ann Transl Med* 2020; 8: 410.
74 Arcasoy SM, Berry G, Marboe CC, *et al.* Pathologic interpretation of transbronchial biopsy for acute rejection of lung allograft is highly variable. *Am J Transplant* 2011; 11: 320–328.
75 Keller C, Erasmus E, Alvarez F, *et al.* Preliminary observations in the use of confocal alveolar endomicroscopy in recipients of single lung transplant. *Am J Respir Crit Care Med* 2010; 181: A4316.
76 Yserbyt J, Dooms C, Decramer M, *et al.* Probe-based confocal laser endomicroscopy of the respiratory tract: a data consistency analysis. *Respir Med* 2013; 107: 1234–1240.
77 Yserbyt J, Dooms C, Decramer M, *et al.* Acute lung allograft rejection: diagnostic role of probe-based confocal laser endomicroscopy of the respiratory tract. *J Heart Lung Transplant* 2014; 33: 492–498.
78 Keller CA, Hurst KE, Petrine KD, *et al.* Probe-based confocal laser endo-microscopy (pCLE) can identity perivascular cellular infiltration in patients with acute cellular rejection. *J Heart Lung Transplant* 2014; 33: S69–S70.
79 Keller CA, Khoor A, Arenberg DA, *et al.* Diagnosis of acute cellular rejection using probe-based confocal laser endomicroscopy in lung transplant recipients: a prospective, multicenter trial. *Transplantation* 2019; 103: 428–434.
80 Thiberville L, Salaün M, Bourg-Heckly G. *In vivo* confocal microendoscopy: from the proximal bronchus down to the pulmonary acinus. *Russian J Transpl Artif Organs* 2010; 48: 81–108.
81 Black PN, Ching PS, Beaumont B, *et al.* Changes in elastic fibres in the small airways and alveoli in COPD. *Eur Respir J* 2008; 31: 998–1004.
82 Newton RC, Kemp SV, Yang GZ, *et al.* Imaging parenchymal lung diseases with confocal endomicroscopy. *Respir Med* 2012; 106: 127–137.
83 Salaün M, Modzelewski R, Marie J P, *et al.* In vivo assessment of the pulmonary microcirculation in elastase-induced emphysema using probe-based confocal fluorescence microscopy. *IntraVital* 2012; 1: 122–131.
84 Cosío BG, Shafiek H, Fiorentino F, *et al.* Structure-function relationship in COPD revisited: an *in vivo* microscopy view. *Thorax* 2014; 69: 724–730.
85 Yserbyt J, Dooms C, Janssens W, *et al.* Endoscopic advanced imaging of the respiratory tract: exploring probe-based confocal laser endomicroscopy in emphysema. *Thorax* 2018; 73: 188–190.
86 Yick CY, von der Thüsen JH, Bel EH, *et al. In vivo* imaging of the airway wall in asthma: fibered confocal fluorescence microscopy in relation to histology and lung function. *Respir Res* 2011; 12: 85.
87 Lesur O, Chagnon F, Lebel R, *et al. In vivo* endomicroscopy of lung injury and repair in ARDS: potential added value to current imaging. *J Clin Med* 2019; 8: 1197.
88 Newton R, Kemp S, Zoumot Z, *et al.* An unusual case of haemoptysis. *Thorax* 2010; 65: 309–353.
89 Newton RC, Kemp SV, Yang GZ, *et al.* Tracheobronchial amyloidosis and confocal endomicroscopy. *Respiration* 2011; 82: 209–211.
90 Yserbyt J, Alamé T, Dooms C, *et al.* Pulmonary alveolar microlithiasis and probe-based confocal laser endomicroscopy. *J Bronchology Interv Pulmonol* 2013; 20: 159–163.

91 Shafiek H, Gómez C, Pierola J, *et al.* Probe-based confocal laser endomicroscopy imaging of endobronchial hamartomas. *Respiration* 2014; 88: 484–486.

92 Vasilev I, Mamenko I, Tabanakova I, *et al.* Probe-based confocal laser endomicroscopy in metastatic pulmonary calcification. *J Bronchology Interv Pulmonol* 2018; 25: 60–62.

93 Guisier F, Bohn P, Patout M, *et al. In-* and *ex-vivo* molecular imaging of apoptosis to assess sensitivity of non-small cell lung cancer to EGFR inhibitors using probe-based confocal laser endomicroscopy. *PLoS One* 2017; 12: e0180576.

94 Horgan CC, Jensen M, Chiappini C, *et al.* Hybrid confocal Raman endomicroscopy for morpho-chemical tissue characterization. *Biomed Opt Express* 2022; 13: 2278–2285.

95 Chang TC, Liu JJ, Hsiao ST, *et al.* Interobserver agreement of confocal laser endomicroscopy for bladder cancer. *J Endourol* 2013; 27: 598–603.

96 Perperidis A, Akram A, Altmann Y, *et al.* Automated detection of uninformative frames in pulmonary optical endomicroscopy. *IEEE Trans Biomed Eng* 2017; 64: 87–98.

97 Stone JM, Wood HAC, Harrington K, *et al.* Low index contrast imaging fibers. *Opt Lett* 2017; 42: 1484–1487.