Spatial lung imaging in clinical and translational settings

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Spatial imaging will provide regional information about a lesion, the disease severity or the disease phenotype in relation to other surrounding cells or tissue structures. Imaging is a translational link between experimental and clinical research. https://bit.ly/463dFgu

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Abstract

For many severe lung diseases, non-invasive biomarkers from imaging could improve early detection of lung injury or disease onset, establish a diagnosis, or help follow-up disease progression and treatment strategies. Imaging of the thorax and lung is challenging due to its size, respiration movement, transferred cardiac pulsation, vast density range and gravitation sensitivity. However, there is extensive ongoing research in this fast-evolving field. Recent improvements in spatial imaging have allowed us to study the three-dimensional structure of the lung, providing both spatial architecture and transcriptomic information at single-cell resolution. This fast progression, however, comes with several challenges, including significant image file storage and network capacity issues, increased costs, data processing and analysis, the role of artificial intelligence and machine learning, and mechanisms to combine several modalities. In this review, we provide an overview of advances and current issues in the field of spatial lung imaging.

Educational aims

- To provide an understanding of current advantages and challenges in spatial lung imaging.
- To give examples of how current imaging modalities can be used in lung imaging together with advanced omics approaches to study the three-dimensional structure of the lung, providing both spatial architecture and transcriptomic/proteomic information at a single-cell resolution.
- To highlight the important translational link between experimental and clinical research, whereby the methodology can be transferred easily between the two settings when employing imaging technologies.

From the first use to today's use of medical imaging

Clinical imaging dates back to the last decade of the 19th century with the introduction of conventional radiography and the first published chest radiography images of the lung in 1896 by F.H. Williams [1]. The very first clinical computed tomography (CT) image was acquired in 1971, in a patient with a brain tumour [2]. The trajectory of magnetic resonance imaging (MRI) followed shortly thereafter, with the initial breakthrough of image formation by P.C. Lauterbur in 1973 [3], leading to one of the first human scans taking place in 1977 [4, 5]. Since then, both techniques have constantly been developed and refined to advance in the imaging field, for example with the introduction of iso-osmolality iodinated contrast, development of multidetectors, using the dual-energy technique, introduction of detectors for photon counting computed tomography (PCCT), gating both cardiac and respiratory signals in both MRI and CT, addition of pulse sequences and rapid pulse sequences in MRI [6], and introduction of imaging-guided surgery [7].

Imaging of the lung is more challenging compared to other organs, primarily due to its constant movement caused by respiration and pulsation. Additionally, the thorax, which comprises approximately one-quarter of the body's volume, contains a wide range of structural densities, spanning air to bone [8, 9], further

complicating the imaging process. The lung is also sensitive to gravity, resulting in density variability depending on the gravitational plane. Imaging with CT takes advantage of the presence of air in the lung, providing a strong contrast to the lung tissue, especially in high-resolution computed tomography (HRCT). Volumetric HRCT with thin sections down to 0.625–1.5 mm in thickness and reconstructed with a bone filter is currently the best imaging method to non-invasively display subtle parenchymal lung changes. HRCT is also the method of choice for imaging interstitial lung disease (ILD), as it allows for visualisation of structures with a magnitude <1 mm [10]. The newly introduced PCCT records every interaction of a photon occurring in a voxel, increasing the granularity of the collected energy, with the potential to reproduce more details with a spatial resolution of 0.2 mm. The new CT systems, therefore, offer the potential to perform examinations with higher spatial resolution, increased contrast-to-noise ratio, multi-energy imaging capabilities and lower radiation doses, making them suitable for the lung [11] or the coronary arteries [12].

In contrast, the presence of air and the lack of protons initially hampered MRI of the lungs. This limitation, however, resulted in the development of dedicated pulse sequences and cardiac and respiratory gating techniques. The spatial resolution in MRI of the lung has also improved, with resolution as fine as 1–3 mm [13], making it a valuable imaging modality, especially in children and young adults due to absence of radiation. However, the strength of MRI lies in its ability to image the chest wall, mediastinum and diaphragm. Recent advancements in MRI include functional MRI [14] and the use of typically three-dimensional (3D) gradient echo sequences with ultrashort echo times. These are considered to be the most robust sequences for acquiring images in various lung diseases [15].

The technical advances in medical and experimental imaging are evolving quickly. There is still a large gap, however, between theoretical imaging knowledge and actual practical live imaging in patients and experimental models. Translation of imaging applications from experimental models to patients could and should occur more in the future to boost advances in patient care. Importantly, though, with improved technology come increased costs. Advanced scan applications of lung CT or lung MRI need advanced technical capacity and support to analyse the image data, develop new software and algorithms and improve workflows to be easily applied in a clinical setting. Recently, several useful tools have been developed for handling potential motion artefacts, for example post-processing and retrospective corrections and breathing-triggered scan protocols can be used to adjust for changes throughout the breathing cycle [16, 17]. With the latest developments and opportunities for artificial intelligence (AI)-based image analysis, the patient diagnostics should be expected to improve, speed up, and become more available in the future [18]. In addition, quantitative imaging biomarkers in CT scans may not be enough for the diagnosis alone. We need to develop models using, for example, AI and machine learning techniques, where the quantitative imaging data can be combined with additional clinical parameters such as lung function tests, serological biomarkers, biomarkers in bronchoalveolar lavage (BAL) samples, and questionnaires [19, 20]. However, the most important reason for the currently limited use of imaging modalities in the clinic is the lack of expert-operator-dependent acquisition and image analysis [21].

Clinical imaging modalities currently available for lung disease imaging

Various imaging modalities are currently available at the clinic, including chest radiography, ultrasound, CT, MRI, single-photon emission computed tomography (SPECT) and positron emission tomography (PET). The chosen modality depends on the clinical request for a suspected diagnosis or follow-up, availability of the modality and sometimes on ability to perform the scan in a particular patient. The most common modalities for clinical imaging of the lungs are chest radiography and CT, which can be used with or without intravenous contrast. These modalities are used for diagnosing the majority of lung diseases, such as infections or malignancies. CT is also an excellent choice for visualising calcifications found in granulomas, cysts, aneurysms and tumours [22], or for monitoring bleeding [23]. HRCT is primarily used in COPD and ILD imaging, due to its high spatial resolution and ability to image detailed morphology [24]. HRCT also enables a distinction between various patterns of interstitial pneumonias, *e.g.* usual interstitial pneumonia or non-specific interstitial pneumonia [10, 24]. Another important consideration when choosing the imaging modality for lungs is to decide between investigating the rapid and dynamic changes of the lung disease or assessing the magnitude or localisation of scar tissue. The broad range of applications among various imaging modalities is indeed undeniable. Regardless, imaging with CT will result in radiation exposure of the patient, in contrast to MRI. The radiation dose from CT is particularly concerning for longitudinal follow-up scans or for young individuals, pregnant women, or patients with compromised immune systems [25]. Through the appropriate application, spatial information can be acquired, allowing for quantification and statistical analysis of disease burden as opposed to disease assessment based on blood or BAL fluid biomarkers alone.

The MRI modality uses strong magnetic fields and radio waves to generate images based on proton density. It is, therefore, suitable for soft tissues and anatomy assessment in general, but should not be underestimated for spectroscopic applications as a complementary technique for anatomical images. Contrast agents can also be used to assess lung disease, especially when generating functional images by sequences adapted for dynamic contrast enhancement (DCE)-MRI. During inflammation and oedema in the lungs, DCE-MRI can be used to visualise pulmonary vascular leak, since the intravenous contrast will reach the lung regions of increased leakage more rapidly compared to the healthy parenchyma [26]. DCE-MRI has also been attempted in fibrotic disease, aiming to identify the so-called "delayed enhancement" in the regions of excessive extracellular matrix deposition and scarring, as the denser lung regions contribute to a delayed contrast flow, thus delaying the signal enhancement [27]. Employing DCE-MRI in lungs can help to distinguish between inflammatory and fibrotic disease by providing spatial information about local regions of inflammation or fibrosis in the lung, with specific implications for the chosen treatment. Another example of functional MRI is the use of inhaled gases as a contrast agent within the lungs. Either pure oxygen inhalation or hyperpolarised gas such as xenon-129 (^{129}Xe) can be used to increase the contrast within the lung [14]. Using oxygen-enhanced MRI or imaging inhaled ^{129}Xe enables the depiction of spatial information within small lung regions without the need for intravenous gadolinium contrast injection. The use of ^{129}Xe in MRI is a relatively novel, yet increasingly employed, technique based on inhalation of ^{129}Xe gas, which is easily transported across the capillaries and taken up by red blood cells. Calculating the red blood cell to tissue ratio allows for unique regions to be identified with compromised perfusion [28].

Other modalities for lung imaging are the nuclear medicine technologies, which can detect injected radioactive tracers to target a specific activity or metabolism in the body of the examined patient. Two techniques are PET and SPECT, which are usually used alongside a standard CT or MRI scan. They cannot be used as stand-alone imaging modalities, since neither the PET nor SPECT data can convey the exact anatomical location of detected signal within the tissue. Therefore, CT or MRI images are overlaid as anatomical background, aiding PET or SPECT in determining the localisation of the tracer uptake. The most commonly employed radiotracer is fluorine coupled to glucose, known as fluorodeoxyglucose (^{18}F-FDG) [29]. Although ^{18}F-FDG is a rather general measure of increased metabolism within the tissue, it is important in identifying disease onset or progression. More specific radiotracers can, however, be employed to assess lung disease by targeting unique events or cells within the lungs, especially for assessment of remodelling and fibrosis pathways, such as tracers targeting fibroblast activation [30, 31] or the tracer targeting αvβ6-integrin [32]. αvβ6-integrin plays a central role in activation of transforming growth factor-β, an important key player within ILD pathology [33] as antifibrotic treatments are improving.

Promising future for imaging techniques: only just the beginning

In recent years, much effort has been put into developing new and advanced imaging techniques, and the number of publications has increased dramatically. There has been significant focus on the development of multimodal imaging approaches to better visualise or track disease progression, enabling the combination of techniques [34, 35]. Multimodality utilises the strengths of two or more modalities such as MRI, CT, PET and SPECT, addressing new research questions with more accuracy. Applying multimodality means that synergistic and additive information can be acquired from the images, leading to improved spatial information and geometrical accuracy when using image post-processing software equipped with intuitive image registration and orientation tools.

Another promising imaging application for lung imaging is optical coherence tomography (OCT). While this technique has traditionally been used in cardiology and oncology, it is increasingly being developed and fine-tuned for respiratory research and diagnostics. This technique has the potential to provide spatial resolution at the cellular level, ranging from 1 to 20 µm [36]. OCT enables microscopic visualisation of the airways and the alveolar structures that could facilitate early detection of pulmonary lesions in lung fibrosis or lung cancer [37, 38].

Nanoparticle molecular imaging is another highly interesting technique [39]. This uses labelled nanoparticles to visualise specific molecular markers or with therapeutically active substances, such as growth factors involved in wound healing processes [40, 41]. The use of nanoparticles is currently mainly employed for visualisation but has the potential to become more frequently used in targeted drug delivery approaches as well.

Another promising lung imaging technique is near-infrared (NIR) imaging technology or functional near-infrared spectroscopy (fNIRS). By determining lung water content, it is possible to measure changes in blood oxygenation within the tissue, thus providing insights into haemodynamics. A recent study in a

lung injury rat model showed the ability to detect increased water content by NIR, representing lung oedema. Lung water content and haemoglobulin oxygenation were, therefore, assessed quantitatively *in vivo* despite only mild alterations identified by histological sections *ex vivo* [42]. Ongoing research aims to enhance the spatial and temporal resolution of fNIRS, which has primarily been explored in brain imaging to date. This application has particular potential for improving surgical procedures and visualisation of small tissue alterations [7, 42].

Ultrasound in lung imaging, which is routinely performed in clinical settings (even more so during the coronavirus disease 2019 pandemic), is useful when fast and accurate lung assessment is needed in the emergency rooms [43]. High-resolution 3D ultrasound, additionally, has potential to improve imaging diagnostics since it offers sufficient spatial resolution and penetration depth to allow for visualisation of small anatomical alterations and disease progression within the lung [44].

The development of imaging applications for lung disease that will become increasingly important for future diagnostics includes PCCT and Xe-MRI, as each currently offer the best spatial accuracy and functional readout, respectively. Furthermore, there is an ongoing development of upright CT (or standing CT) in Japan, providing patient scan acquisition with the lungs imaged in their natural upright position. Being upright clarifies the effects of gravity and could potentially aid objective diagnosis and reduce the access time for examination [45].

Finally, dual-energy CT, which is one of the older technologies but has not been fully incorporated into practice as anticipated, provides opportunities for future improvements and utilisation with radiation reduction strategies and simplified clinical accessibility. Dual-energy CT allows for data acquisition at different X-ray energy levels, which enhances tissue characterisation and facilitates assessment of lung lesions. Dual-energy CT enables quantification of lung ventilation and perfusion, and assessment of the tissue structure [46–48]. Considering targeted imaging applications, such as translational radionuclei probes in PET or SPECT imaging and fluorescence imaging techniques, primarily explored in experimental models, the possibilities for probe design and application are endless. Lung imaging is undeniably advancing, with novel technologies being discovered every decade (figure 1).

The translational link in imaging studies

The significance of imaging is undeniable and it is becoming increasingly applied and available not only in clinical settings but also, importantly, in experimental and translational research. Unique MRI sequences that

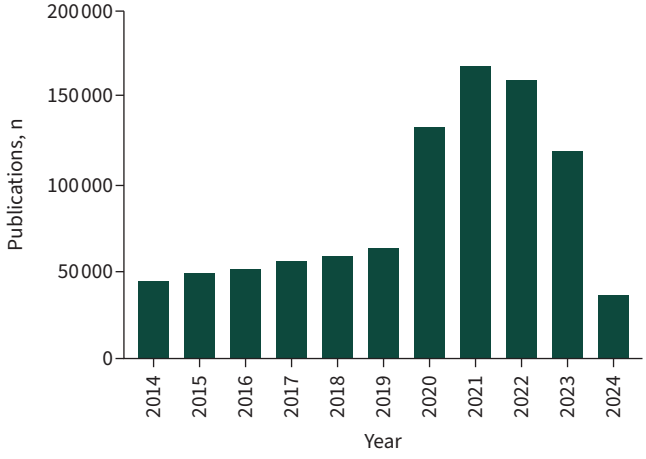

FIGURE 1 Numbers of published articles on imaging techniques in respiratory medicine from 2014 to 2024. Illustration of the increasing frequency of articles employing imaging technology, published over the past decade. The numbers of retrieved articles were generated from the database PubMed using the search terms “imaging technique” [All Fields] AND “chest” [All Fields] OR “lung” [All Fields] OR “pulmonary” [All Fields] OR “respiratory” [All Fields] OR “thorax” [All Fields] OR “thoracic” [All Fields] OR “pneumonia” [All Fields] OR “pneumonitis” [All Fields] OR “bronchiectasis” [All Fields] OR “bronchiolitis” [All Fields] OR “cystic fibrosis” [All Fields] OR “tuberculosis” [All Fields] OR “mycobacteria” [All Fields] OR “asthma” [All Fields] OR “copd” [All Fields] OR “pleural” [All Fields] OR “sarcoidosis” [All Fields] OR “lung fibrosis” [All Fields] OR “ILD” [All Fields] OR “ventilation” [All Fields]. The search was performed on 16 May 2024.

are developed for experimental research are easily implicated in a clinical application with minor modifications. Protocols for CT imaging or specific peptides conjugated to a radionuclide for PET or SPECT readout can often function as applicable tracers for translational imaging, in remodelling and inflammation. Many times, imaging methodology is easily transferred "from mouse to man" [49], or from molecular to patient imaging.

A successful and recent example of a translational approach using PET imaging is the development of a specific tracer targeting collagen type I during an assessment of fibrosis. The research and development were initiated by the group led by Peter Caravan (Harvard University, Cambridge, MA, USA), where they started evaluating variants of small peptides that would target newly synthesised collagen type I [50]. This elegant work eventually resulted in the design of one successful peptide (named CBP8) that was then tested in a mouse model of pulmonary fibrosis [51]. 2 years later, the collagen tracer was validated in a fibrosis rat model, enabling a more accurate spatial mapping of the tracer uptake within the lung because it was a larger animal model [52]. This demonstrated how the PET signal mainly emanated from the borderline of the lesions detected by MRI within the same animal; meanwhile, hotspots of the tracer uptake were evident in regions that did not exhibit apparent fibrosis. Subsequently, *ex vivo* autoradiography followed by histopathology of the rat lungs confirmed the PET imaging results, with the collagen tracer mainly accumulating in the borderline of established fibrotic lesions. Finally, this collagen tracer was employed in a human study with recruitment of idiopathic pulmonary fibrosis (IPF) patients followed by PET-CT imaging. The collagen tracer showed similar results to those obtained in the rat model, whereby the initiated collagen synthesis that was tracked by PET was, conversely, not detected as fibrotic lesions by CT imaging alone [9]. CBP8 is now proposed as one of the major new biomarkers for early detection of ILDs linked to IPF when HRCT or any other imaging modality may have failed to detect early remodelling and fibrogenesis [53]. This collagen tracer for assessment of early fibrotic processes demonstrates the important use of translational imaging applications. By incorporating live imaging, this enabled mapping of spatial information that it would not have been possible to accomplish by invasive methods such as biopsies and use of conventional histology, or by assessment of serological biomarkers or BAL fluid samples. The three PET imaging studies employing the same PET tracer for mapping collagen type I synthesis are shown in figure 2.

In vivo and *ex vivo* imaging applications

Despite the technical advances of imaging applications, including live imaging of organs and full body scans, the need for a gold standard histopathology confirmation remains. Patient biopsies or terminal and invasive animal samples are typically acquired for these verification studies. Conventional histopathological evaluation is still included within the majority of experimental research projects, irrespective of whether imaging is the main readout. Histopathology is sometimes employed as complementary data in patient diagnostics when imaging is not feasible or accessible, or fails to provide sufficient information about the investigated disease [54, 55]. The 3D information gained from imaging will always be superior to the 2D histological assessment from tissue sections, but the ability to diagnose from 3D imaging cannot compete with current image resolutions obtained from complementary biopsies, which can provide magnifications down to the cellular level. When it comes to animal studies that are terminated and where tissues are processed as frozen or paraffin-embedded blocks, or even as whole organ fresh-preserved samples, the imaging of these might provide more spatial information than any 2D-stacked histology readout.

One unique experimental application that is capable of providing detailed spatial information is the light sheet microscopy technique. Light sheet microscopy demands clearing procedures to provide transparent tissue biopsies (*i.e.* from patients) or organs (from animals), in order to facilitate visualisation of the targeted staining within the tissue. This technique is increasingly employed at different research laboratories worldwide and, although various types of clearing protocols have been developed, standardised processing is gradually emerging [56, 57]. The fine tuning of light sheet microscopy methodology may even enable whole organ processing in the future, *e.g.* for assessment of spatial information of rare cell populations within the lung [40]. Moving further down the resolution scale, spatial imaging can be performed within stained precision-cut lung slices or even organoids, with the ability to gain 3D information in the *ex vivo* or *in vitro* applications.

Another novel and increasingly employed technology is spatial omics. Here, advanced technologies such as transcriptomics, genomics or proteomics are combined with advanced imaging methods. This combination of technologies enables our *in situ* understanding by monitoring one or several cells in their natural position in the tissue, while simultaneously identifying and mapping *in vivo* cell interactions. This gives us the opportunity not only to investigate the *in vivo* function, origin and fate of the cells of interest, but also to understand the cell–cell and cell–matrix communication. Nagendran *et al.* [58] utilised a proximity ligation *in situ* hybridisation (PLISH) technology to investigate the expression profiles of cells in

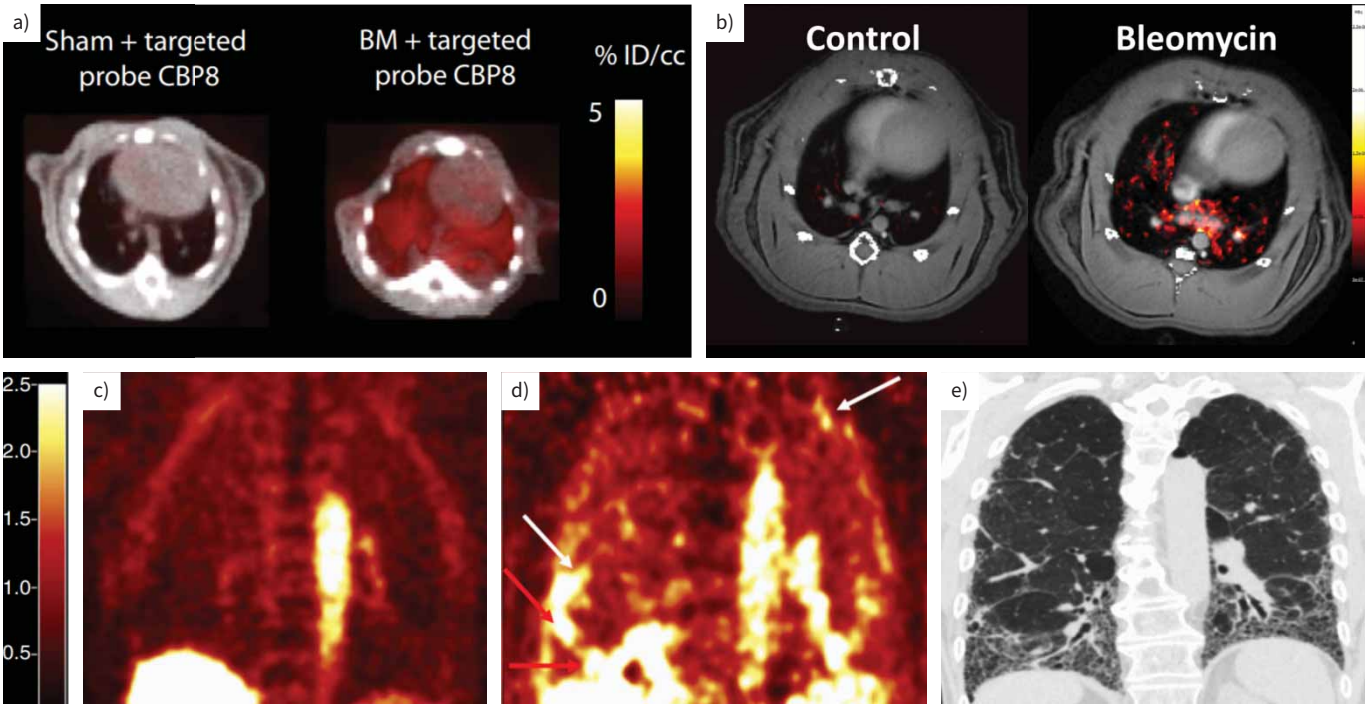

FIGURE 2 Imaging newly synthesised collagen type I. The small peptide, named CBP8, was conjugated to radioactive nuclei for assessment of early fibrogenesis by positron emission tomography (PET) imaging. a) Increased lung uptake of the collagen tracer in a mouse model of fibrosis, comparing a healthy control mouse (sham) to a bleomycin-exposed mouse (BM). Reproduced from [51] with permission. Data are expressed as percent injected dose per cm^3 of tissue (% ID/cc). The tracer uptake for each scan is indicated by the colour bar next to the lung image. b) The same fibrosis model in rats, where the same collagen tracer was employed. Reproduced from [52] with permission. c–e) The first-in-human study where the same collagen tracer was used to assess early fibrogenesis, in terms of collagen type I synthesis. c) A healthy subject was undergoing a PET scan which was compared to d) an idiopathic pulmonary fibrosis (IPF) patient. The increased PET signal at certain hotspots (white arrows) in the IPF patient were not indicated as areas of fibrogenesis within e) the same patient's computed tomography (CT) lung scan. Red arrows show matching disease areas, found by both PET and CT. Reproduced from [9] with permission.

a mouse lung. Using PLISH, they discovered two populations of murine club cells that were molecularly different and anatomically segregated in terminal airways. One advantage of the PLISH technique is that it is compatible with both cryopreserved and formalin-fixed paraffin blocks, making it suitable for most samples routinely obtained in clinical settings. In another study, Fan *et al.* [59] embedded the tissue sample (mouse brain) in a swelling polyelectrolyte matrix on a Visium slide (from 10xGenomics) before capturing the RNA. They employed an expansion spatial transcriptomics protocol, which utilised two different poly-T oligos probes with different melting temperatures. With this new improved protocol, they were able to overcome the resolution limit by using the swollen matrix to better resolve cell types and detect rare transcripts. Although transcriptomic data have the potential to give us important information about cell communication and cell fate, it is well known that the correlation between mRNA and protein expression is not 100% translatable. Much effort, therefore, has been put on developing protocols for assessing the proteome instead at a single-cell resolution. Acknowledging that many improvements and refinements are still needed, several interesting approaches have been published, resulting in substantial advances in the field. Mund *et al.* [60] used a deep visual proteomics (DVP) approach to identify and characterise single cells in a tissue slide with salivary gland acinic cell carcinoma. DVP technology combines laser microdissection (at a single-nucleus level) with ultra-high-sensitivity mass spectrometry. Using a similar approach, Makhmut *et al.* [61] profiled 146 microregions, including lymphocyte niches and cytokines, from formalin-fixed and paraffin-embedded tissue samples in the mouse liver. As mentioned, development and optimisation of method protocols, workflows and data analysis strategies need to be carried out, but the multi-omics technology is here to stay, and it will help us gain insights into the complex cell biology of disease origin and progression.

Harnessing AI and machine learning for interpreting complex lung diseases

Significant development in machine learning-based AI techniques in the respiratory field has recently accelerated, meaning that the adoption of effective and accurate AI approaches in clinical settings is now a

prioritised and essential topic. Several algorithms have been developed for evaluating chest radiographs and CT scans, for example Annarumma *et al.* [62] developed a deep neural network (DNN) system capable of automated real-time triaging of adult chest radiographs. This model detected normal radiographs with a positive predictive value of 73%, a sensitivity of 71% and a specificity of 95%. Importantly, the authors reported that the average reporting delay was reduced by 8.5 days for critical imaging findings (from 11.2 days to 2.7 days) and by 3.5 days for urgent imaging findings (from 7.6 days to 4.1 days) [62]. By introducing a binary classification system into a similar model system, Yates *et al.* [63] achieved an accuracy of 94.6% in the test data. Moreover, several groups have used DNN algorithms to identify specific pathologies on chest radiographs, for example pneumothorax [64], tuberculosis [65], pneumonia [66, 67] and COPD [68]. Tanabe *et al.* [68] established another DNN model in which they converted sharp-kernel images from 30 smokers with and without COPD to soft-kernel-like images. This DNN model provided quantitative measurements of clinical CT indices from the converted images, including the percentage of low-attenuation voxels to those in the entire lungs, percentage of intramuscular adipose tissue, and coronary artery calcium volume. Zhu *et al.* [69] constructed a COPD diagnostic model by integrating deep learning with radiomics features. By combining CT scans with epidemiological questionnaire data obtained from COPD patients, their fusion model outperformed the independent control model, achieving an area under the curve (AUC) of 0.952 for COPD diagnosis compared to the AUC of 0.844 obtained from the control model. Interestingly, Zhou *et al.* [70] also employed a DNN model to retrospectively quantify lung mass density in ILD patients and healthy controls, based on measurements obtained from lung ultrasound surface wave elastography and pulmonary function tests. Despite a limited dataset and small batch size, the authors were able to demonstrate that the magnitude of lung mass density was higher in ILD patients compared to healthy control subjects [70]. While significant advancements have been made in the development of DNNs, current AI models cannot replace radiologists. However, well-trained DNN models may aid the reading, thereby reducing perceptual errors and speeding up the reporting times.

Challenges and opportunities in the field: where do we stand?

The enthusiasm for using advanced imaging technologies, especially at a single-cell resolution, has increased considerably since the very first clinical CT image in 1971 [2] and MRI study in 1977 [4]. Imaging the lung, however, remains a significant challenge due to its complex mechanical nature. With the evolution of AI technology and the ability to train image-processing software, we can minimise and compensate for the motion artefacts using techniques such as breathing triggering and filters. Nevertheless, the rapid progression in data processing and data analysis using AI and machine learning comes with several challenges. These include the fact that the amount of generated data is quickly increasing with each improvement or refinement made. Managing all the data requires safe and effective network systems and storage solutions. Large data files need to undergo further processing and analysis, leading to even larger data files requiring storage. Unfortunately, safe and efficient network systems and storage of huge data files are very expensive. Importantly, this storage also comes with ethical considerations, and we need to ensure that sensitive patient data are securely used and saved, respecting the patient data integrity. Another challenge is the need to educate personnel to handle these types of data, particularly those originating from patients in clinical settings and from preclinical laboratory scans. We need to continue to develop new and improved data processing workflows that are feasible and optimised for clinical settings, where time and costs are limited resources. Nevertheless, it is important to keep in mind both the opportunities and the limitations when working with any lung imaging, from the microscopic level up to the scale of the whole organ.

Despite several remaining challenges with image acquisition and data processing, lung imaging offers numerous advantages that are essential for advances in respiratory research. Importantly, improved and combined technologies have enabled us to observe and investigate the lung in its natural 3D structure at a single-cell resolution. By using the collagen tracer, we now have the possibility to track collagen synthesis, a biomarker currently suggested as an early clinical indicator of increased remodelling and fibrogenesis. Finally, the spatial omics, combining imaging with omics approaches, offers us the opportunity to phenotype patients with lung diseases. This will contribute to driving the progression of precision medicine forward and, hopefully, lead to earlier and less invasive diagnosis of severe lung diseases. Taken together, the opportunities of having more precise imaging technologies and tools in the future will only increase over time. This process can be accelerated further if clinical and experimental scientists work together and collaborate across research disciplines, such as medicine, biology, technical and engineering professions, as well as the environmental research field.

Summary and final remarks

Each advancement in the field of spatial imaging adds another layer to our understanding of the human body in health and disease. We have progressed from imaging molecules and simple 2D structures to

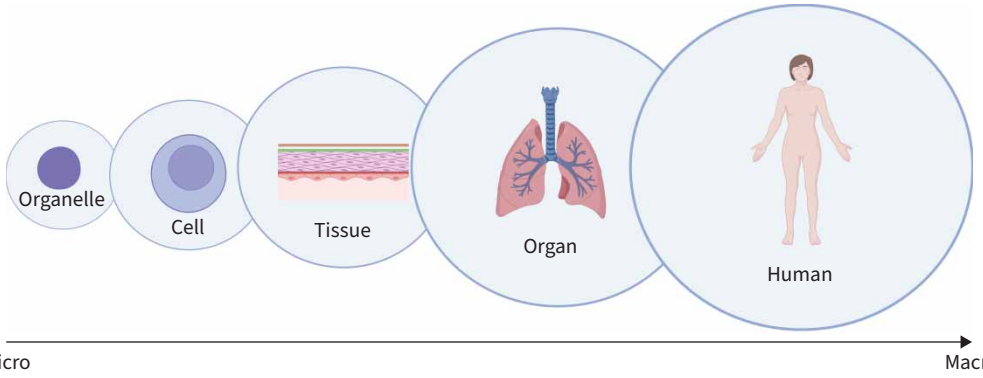

FIGURE 3 Schematic illustration describing how the imaging field has progressed from being able to image simple two-dimensional structures to advanced imaging of whole organs and the human body in three dimensions. Figure created with BioRender.com.

imaging complex organs, such as the lung, and full-body scans in 3D (figure 3). The field has, furthermore, transitioned from imaging structures on a macro scale to achieving very advanced imaging on a micro scale, with high resolution and spatial placement. Combining omics with imaging has significantly pushed the field forward. We can now explore subtypes of cells in their natural location to understand their origin, fate and local communication. Interestingly, spatial omics can also shed light on disease origin and progression, aiding patient phenotyping to enable the development of more personalised treatments. We are convinced that by incorporating advanced imaging, there will be significant achievements and an increased understanding of disease onset and progression in the near future. The development of the collagen tracer, from a basic research discovery to a clinical application through a few animal models, beautifully illustrates the crucial link between basic and translational research and clinical practice. The field of imaging serves as an excellent platform for clinicians and researchers to converge. It is becoming increasingly evident that we require each other's thoughts, expertise and experiences to develop effective diagnostic tools and innovative treatment options for patients of various lung diseases.

After several years of refining technologies, improving clearing protocols, implementing PCCT and AI imaging analysis tools, we are now entering an exciting and rapidly evolving phase of enhanced imaging profiling of lung diseases. This phase holds the promise of significant advances in the field, leading to improved diagnostic tools, precision medicine, and new therapeutic opportunities.

Self-evaluation questions

1. Why is imaging important in the clinical setting?
2. Why is lung imaging needed in experimental science?
3. How is imaging connecting the translational gap between patient care and experimental science?

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Suggested answers

1. Advanced imaging provides spatial information that blood biomarkers cannot reveal, due to systemic and compensatory effects or even diluted signals when measuring systemically for something occurring locally in the lung.
2. Clinical imaging is constantly under improvement and new disease biomarkers need validation; thus, animal models and preclinical imaging are the core in the development of imaging biomarkers for future clinical implications. However, the "3Rs" principle, particularly the "reduce" and "refine" pillars of animal studies, should be considered when repeated imaging is performed in animal disease models instead of terminal sample collection, with the need for larger groups of animals to fulfil the statistical power.
3. Most imaging modalities available clinically are also used in experimental research, and tracers, protocols and image data processing are used similarly for disease assessment and detection, and can even be quantifiable between healthy and non-healthy individuals.