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Landscape review of barriers affecting progress in the field of Bioimaging

Final report

Anoushka Davé, Peter Varnai, Rebecca Babb, Bruno Raabe, Marisa Amato

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Executive summary

Introduction

Wellcome has a history of supporting innovation in bioimaging including development of new tools, technologies, infrastructure and data repositories. It aims to build on these investments and the work they have enabled by identifying and then supporting key areas that have the potential to open up new opportunities for advancing the field. To that end, Wellcome commissioned Technopolis to undertake a landscape review of the barriers affecting the field of bioimaging. The review covered bioimaging methodologies, equipment, tools and technology development pertaining to imaging at different scales – from atoms all the way to humans – in the context of discovery research.

The landscape review focused on current global trends with regard to bioimaging technologies, methodologies and tools as well as the challenges and barriers affecting progress in the field of bioimaging from the perspective of high-income and low- and middle-income countries (HICs and LMICs respectively). It also explored solutions to address the key barriers, which has led to recommendations for future Wellcome investment in bioimaging.

Methodology

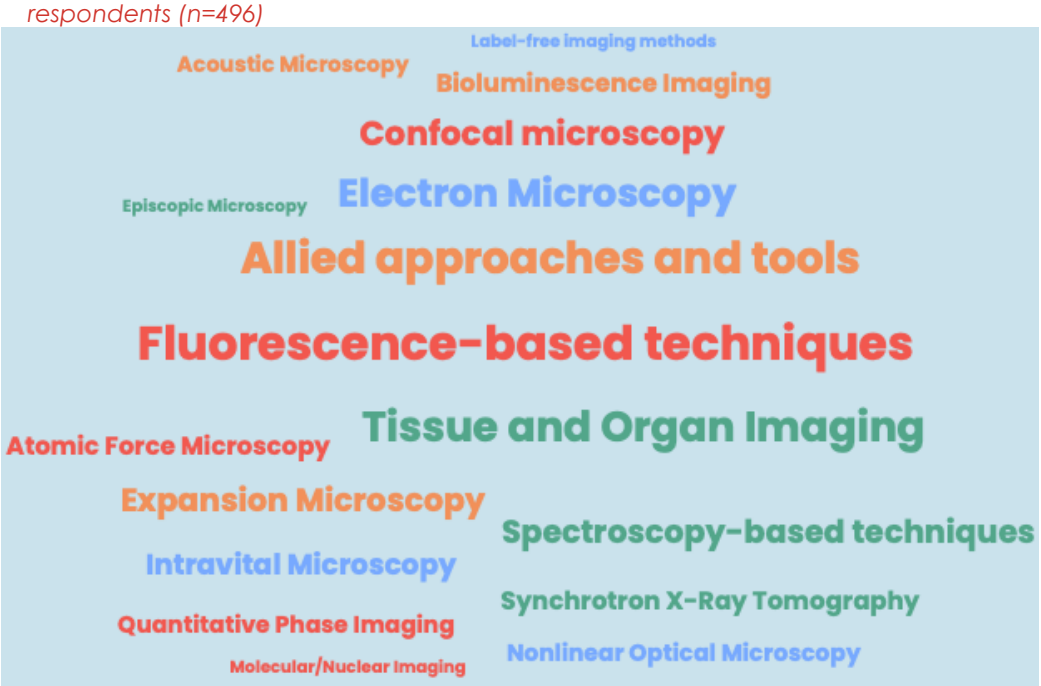
The review followed a mixed methods approach involving desk research (document and literature review), interviews with key experts globally and a survey with the wider bioimaging community.

We received 496 responses (72% HIC vs 28% LMIC; 61% male vs 35% female, 4% others) to our online global survey. In the survey, we asked stakeholders from the bioimaging field about key bioimaging technologies/methodologies that have the potential to transform the field, barriers and challenges to progressing the bioimaging field and interventions that could address these barriers and challenges. We also conducted 51 semi-structured interviews (76% HIC vs 24% LMIC; 55% male vs 45% female) to explore in further depth stakeholders' views on nascent technologies/methodologies and their added benefits, the barriers affecting progress in the field of bioimaging in HICs vs LMICs and potential interventions to mitigate the barriers and/or to support breakthrough and game-changing work in the field. Diversity in terms of geography, gender, discipline/sector and technology was considered in the selection of interviewees.

The key bioimaging technologies/methodologies

The landscape review highlighted three general points with regard to the areas in which the next generation of bioimaging approaches will emerge. First, **integration** is required **across the scales of life** to gain deeper understanding of not only the structures and functions of biological molecules but the wider biological contexts within which they operate. Second, formulation of new hypotheses and breakthroughs will be most effectively enabled by **combining diverse techniques and methodologies** such as in correlative microscopy or multi-modal imaging (or even combination of imaging and other techniques e.g. in spatial transcriptomics/proteomics) rather than a single bioimaging technique. Third, **artificial intelligence (deep learning), big data and image analysis techniques** will play a significant role in supporting image acquisition, data analysis and data integration, and thereby help push bioimaging techniques forward.

Word cloud of "most transformative" bioimaging technologies/methodologies according to survey respondents (n=496)



Note: Only technologies, methodologies or tools chosen by three or more survey respondents are included in the word cloud.

Techniques that are currently transforming bioimaging and the use of which is expected to increase include

- Light sheet microscopy which has high spatiotemporal resolution and allows imaging of tissues and organoids rather than just sections
- Super-resolution microscopy which can provide molecular-level resolution or 3D and fast live-cell imaging
- Correlative microscopy and multi-modal imaging techniques that allow integration across the scales of life such as correlative light and electron microscopy (CLEM); *in vivo* imaging with light sheet microscopy; CLEM and X-ray microtomography; and electron imaging, X-ray imaging and cryogenic electron microscopy (cryo-EM)
- Cryo-EM and volume EM which are powerful structural biology techniques – Cryo-EM is fast becoming a mainstream technique for structural biology, while volume EM allows high resolution imaging of large samples
- Ultra low-field magnetic resonance imaging (MRI) is expected to be rapidly adopted especially in resource-poor settings owing to its lower costs and portability

Challenges, barriers and gaps in the bioimaging field

High costs of equipment/infrastructure, access to infrastructure and imaging software along with lack of availability of appropriate technical expertise and data processing/analysis solutions prevent many researchers from accessing bioimaging techniques. High costs and lack of infrastructure and technical expertise locally or regionally particularly affect access in LMICs, especially to state-of-the-art and powerful techniques like cryo-EM and light sheet microscopy. Lack of appropriate infrastructure e.g. high pressure freezers for cryo-EM or biosafety labs for infectious organisms can also limit choice and use of specific techniques, especially in LMICs.

Common scientific and technological barriers affecting several bioimaging techniques stem from inherent limitations of the imaging technology and complex requirements as regards specimens that can be imaged. These comprise data quality, reproducibility and quantitation; complex sample preparation requirements that may require lengthy optimisation and specialised expertise (e.g. in EM); cellular artefacts observed with standard sample fixation methods at high resolution; and the trade-offs between spatial and temporal resolution and image depth. Such scientific and technological barriers can hinder uptake and use of certain techniques and limit the scope of what can actually be visualised and interpreted with confidence.

The increasing interest in correlative and multimodal imaging has created the need for integrated sample preparation methods that are compatible and comparable across modalities as well as integrated workflows and imaging platforms that can accommodate different sample sizes (e.g. when integrating across nm to cm scales). These approaches also create data integration challenges where datasets from different imaging modalities need to be combined.

High-content and high-throughput imaging techniques e.g. light sheet microscopy and volume EM generate large and complex datasets for which appropriate computing infrastructure and image analysis methods are required. The latter represents a crucial gap in the bioimaging landscape along with lack of access to datasets for reuse. Common agreed metadata standards and implementation of FAIR (findable, accessible, interoperable, and re-usable) principles are keenly required.

There is also a wider sustainability challenge affecting the current bioimaging landscape. Imaging and data scientists do not have appropriate career paths or permanent positions in most countries, making it difficult to retain talent in the field over time and resulting in regular loss of institutional knowledge. There is also a 'brain drain' of skilled individuals to industry. Furthermore, the high cost of maintenance and service contracts means that often expensive imaging equipment becomes unavailable for use after initial service contracts expire.

Overall, the same overarching barriers and challenges exist in HICs and LMICs. The key difference in the LMIC context is that funding for infrastructure, equipment and research is available to a lesser extent and relevant technical expertise (for running and maintaining equipment, training and providing advice to users) is also difficult to find. In this context, availability of resources is the key limiting factor, while access becomes a secondary problem. In HICs, access can be more of a problem than availability, although the extent of the problem is lower compared to that in LMICs. For example, costs to access or use imaging facilities can be prohibitive for LMIC researchers.

Recommendations for Wellcome

1. **Supporting development of bioimaging technologies and methodologies** –

A funding programme to specifically support technology and methodology development in the bioimaging field would be 'low hanging fruit' for Wellcome. The programme's scope should be fairly broad to accommodate innovative ideas from a wide set of stakeholders and not limit development to specific bioimaging modalities or specific types of collaborations (inclusive for monodisciplinary and interdisciplinary/inter-sectoral teams). Preferences could however be indicated for high-priority challenges, e.g. supporting development of image analysis methods and tools or development that leads to low-cost bioimaging solutions which can be easily and widely adopted in resource-poor settings.

Wellcome Discovery Awards provide an opportunity to fund development of bioimaging technologies and methodologies already, but these awards are only available to established

research leaders and groups which will exclude up-and-coming researchers as well as imaging and data scientists. LMIC-based researchers may also struggle to compete for these awards.

2. **Facilitating democratisation of bioimaging technologies and methodologies** –

Funding short-term mobility grants to cover research visits to facilities/labs with the requisite imaging infrastructure and expertise is another idea that Wellcome could explore. We suggest that both the visitors' and hosts' costs are covered (e.g. travel, accommodation, staff/researcher time, instrument time, consumables). Such a scheme would improve access to bioimaging capabilities that are currently out of reach for many, either because of the costs involved or the capabilities not being available locally. Such a scheme would facilitate both knowledge transfer and wider adoption of technologies/methodologies.

Other networks such as Euro-Bioimaging and funders such as the Chan Zuckerberg Initiative provide similar opportunities but these are restricted to a select pool of countries and funding is limited compared to demand.

3. **Creating space for interdisciplinary conversations** –

Wellcome is well-placed to convene and sustain a diverse interdisciplinary and intersectoral community network for the purpose of development and dissemination of novel bioimaging technologies/methodologies owing to the wide spectrum of activities it has supported in the bioimaging and wider biological fields. Mechanisms for kickstarting and supporting such conversations could include activities such as conferences and meetings, webinars, an online networking platform, sandpits and funding programmes.

While some interdisciplinary networks already exist, these are often geographically limited e.g. in specific countries or regions. Existing initiatives that support cross-disciplinary collaboration are mostly focussed on bringing computer scientists and life scientists together towards improving imaging data management and analysis. Wider interdisciplinary activity has usually been intermittent. Thus, there is a gap in the landscape to create and sustain a discipline- or imaging modality-agnostic interdisciplinary community in the bioimaging field. This could be a gap that Wellcome could address considering its global remit and reach.

4. **Promoting reuse and integration of data** –

Wellcome could support open 'hardware' initiatives and data repositories to collate and store imaging data from different modalities in a way that it can be reused, compared and integrated. Common data standards and guidelines for depositing data will be required with need for annotation and curation capabilities. Wellcome is once again well-placed to support establishment of common standards and best practice guidelines by convening international working groups or consensus guideline committees. An alternative would be for Wellcome to support ongoing efforts to develop standards and protocols or to help form consensus in case of duplicate initiatives. Wellcome already supports key imaging data infrastructures and so will be well-placed to help implement the agreed standards and guidelines as well.

A quick win for Wellcome could be adding a requirement for grantees to make their data publicly available or for grant proposals to have data management and sharing plans in line with the recent initiative by the US National Institutes of Health.

1 Introduction

1.1 The review

Wellcome has a history of supporting innovation in bioimaging including development of new tools, technologies, infrastructure and data repositories. Its funding support has extended from individual projects, data repositories (e.g. Electron Microscopy Public Image Archive[1]), research centres (e.g. Wellcome Centre for Integrative Neuroimaging, Oxford) and infrastructure (e.g. Diamond Light Source) to large scale data acquisition endeavours such as the UK Biobank Imaging Studies. It aims to build on these investments and the work they have enabled by identifying and then supporting key areas that have the potential to open up new opportunities for advancing the field. To that end, Wellcome commissioned Technopolis to undertake a landscape review of the barriers affecting the field of bioimaging. The landscape review focussed on the following specific research questions:

1. What are the nascent (emerging) technologies/methodologies in the field of bioimaging that will enable researchers to formulate new hypotheses and address new fundamental questions for life, health, and wellbeing?
2. What are specific barriers (in terms of technology, methodology, hardware, software, and access) that are limiting progress in the field of bioimaging in both High-Income Countries (HICs) and Low/Middle-Income Countries (LMICs)?
3. Who are the key leaders on an international level that are driving development in the field of bioimaging?

The review covered bioimaging methodologies, equipment, tools and technology development pertaining to imaging at different scales – from atoms all the way to humans – in the context of discovery research.

1.2 Methodology

The review followed a mixed methods approach involving desk research (document and literature review), interviews with key experts globally and a survey with the wider bioimaging community.

As part of the desk research, we reviewed peer-reviewed articles, strategic/policy documents, think pieces, commentaries and discussion articles published in journals and on institutional websites in the last 5 years. Relevant literature was identified using a search string of keywords related to bioimaging[2] from databases and search engines including PubMed, Europe PMC, Scopus, Google Scholar and Google.

We received 496 responses (see Figure 1; 72% HIC vs 28% LMIC; 61% male vs 35% female, 4% other) to our online survey (see Appendix A), which was distributed in three ways:

- Via Wellcome and the following bioimaging networks
  - African Bioimaging Consortium
  - BioImaging North America (plus Canada BioImaging and Canadian Network of Scientific Platforms)

1 https://www.ebi.ac.uk/empiar/about/

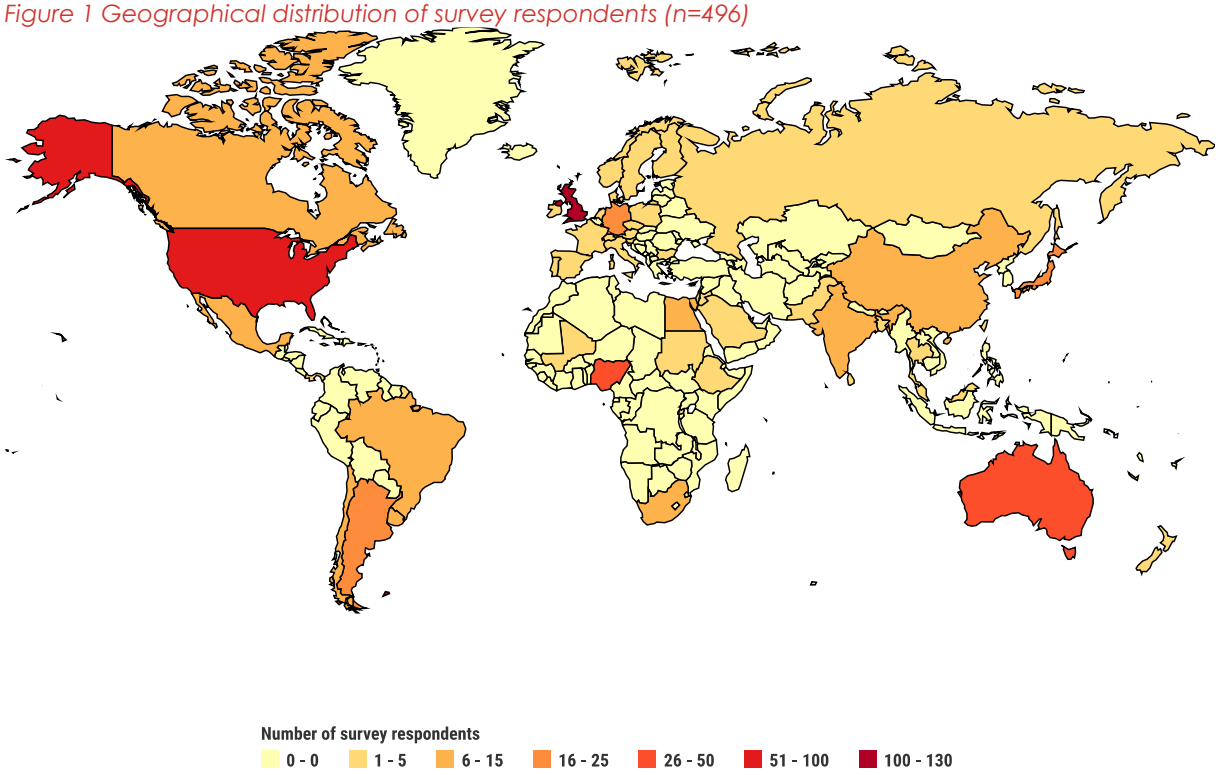
2 List of keywords covering different bioimaging modalities was provided by Wellcome

- Euro-Bioimaging (and Global Bioimaging)
- Latin America Bioimaging
- National Imaging Facility, Australia
- India BioImaging Consortium
- Advanced BioImaging Support, Japan

- By email to corresponding authors of publications in the field of bioimaging
- By snowballing i.e. allowing survey recipients to forward the survey to their personal networks

Further demographics of the survey respondents can be found in Appendix B. Just under a fifth of the respondents (18%, 91) were experienced in whole-body bioimaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT), while the rest most commonly worked with microscopy-based techniques.

Figure 1 Geographical distribution of survey respondents (n=496)



We also conducted a **programme of 51 semi-structured interviews** (Table 1) to explore the views of a range of stakeholders in order to complement gaps in the data gathered by the desk research and survey, and to deepen our understanding of nascent technologies/methodologies and their added benefits, the barriers affecting progress in the field of bioimaging in HICs vs LMICs and potential interventions to mitigate the barriers and/or to support breakthrough and game-changing work in the field.

Table 1 Number of stakeholder interviews conducted by country type and gender

Country type / gender	Scoping interviews	Stakeholder interviews	Total interviews
High-income countries	10	29	39
Males	3	17	20

Females	7	12	19
Low- and middle-income countries	3	9	12
Males	1	7	8
Females	2	2	4
Total	13	38	51

Our interview sample was concentrated on stakeholder types that have high interest as well as influence in progressing the field i.e. technology/methodology developers rather than users. Diversity in terms of geography, gender, discipline/sector and technology was also considered in the sample frame. For country types (HICs vs LMICs) and gender, a 50% target was desired within the sample. While this was almost met for geographical distribution (HIC/LMIC) in the initial longlist, the gender target was not met, owing to the existing gender skew in the field especially in terms of technology/methodology developers in LMICs (see Table 4, Appendix D). Unfortunately, despite a relatively balanced sample frame at the start, our targets could not be fully met owing to lack of availability of proposed interviewees and difficulty in finding like-for-like replacements. Therefore, ultimately our interview sample consisted of 45% women and 24% LMIC respondents (Table 1).

Overall, 12% (n = 6) of the interviewees were experienced in whole-body imaging techniques such as MRI and 16% (n = 8) of the interviewees were associated with computational imaging and data analysis and management. The remaining interviewees (72%, n = 37) were facility managers and researchers predominantly working with different microscopy techniques. There are two reasons for this skew. Firstly, at larger scales of life (e.g. organism level), use of imaging techniques is often biased towards answering clinical research questions such as those related to diagnosis or therapeutics, and discovery research application can be limited. Moreover, there is a greater variety of technologies and methods at the lower scale with applications in many different research areas and recent developments, which also needed to be represented in the sample.

Findings from the different data collection methods were analysed, triangulated and synthesised. Stakeholders with experience in microscopy-based techniques were represented to a greater extent (5 to 6 times more often) in the stakeholder consultations compared to those using whole-body imaging techniques. The landscape findings need to be interpreted with this caveat in mind.

1.3 This report

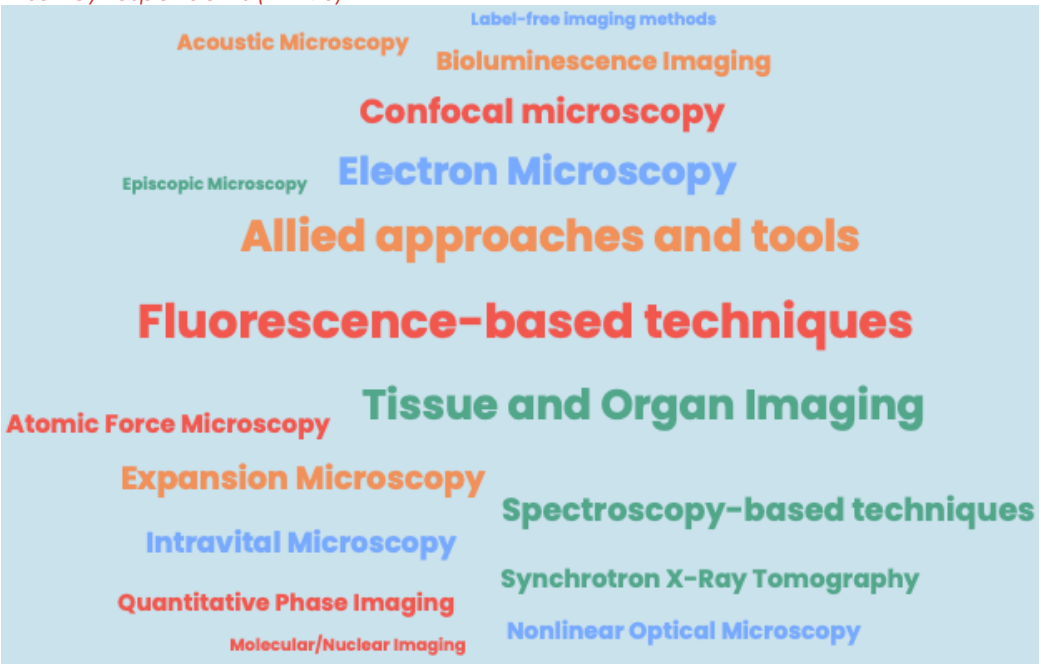
This report describes the findings of the landscape review – the current global trends with regard to bioimaging technologies, methodologies and tools as well as the challenges and barriers affecting progress in the field of bioimaging from the perspective of high-income and low- and middle-income countries (HICs and LMICs respectively). It also presents some suggestions for solutions to overcome the highlighted barriers and specific recommendations for Wellcome.

2 Developments in bioimaging technologies and methodologies

Bioimaging encompasses a range of technologies and methods that can be used to non-invasively visualise biological molecules, processes, cells, tissues and organisms through the use of light, fluorescence, electrons, ultrasound, X-rays, magnetic resonance and positrons among others. As they become increasingly powerful and precise, bioimaging technologies are enabling researchers to visualise and measure biological molecules and processes as never before. Nonetheless, there are opportunities for further development spanning from improved integration across the scales of life and better spatial and temporal resolution to promoting democratisation of novel methods and tools so that researchers all over the world may ask and answer complex and fundamental biological questions that currently remain unanswered.

Before we can identify such opportunities, we need to understand which bioimaging technologies and methodologies have the most potential to transform the field, what their relative advantages and limitations are, and what if anything is holding them back from realising their full potential. To that end, we consulted stakeholders via an online survey and interviews. When asked to choose what in their view were the three "most transformative" bioimaging technologies, methodologies or tools, survey respondents most frequently chose fluorescence-based techniques, allied approaches and tools, tissue and organ imaging, electron microscopy (EM) and confocal microscopy (Figure 2). While the top five areas remained the same for LMIC respondents, HIC survey respondents included expansion microscopy instead of confocal microscopy. It should be noted however that choices may have been influenced by the respondents' bioimaging expertise. For example, almost two-thirds of the respondents have experience with fluorescence microscopy, while just over one-tenth use MRI.

Figure 2 Word cloud of "most transformative" bioimaging technologies/methodologies according to survey respondents (n=496)



Note: Only technologies, methodologies or tools chosen by three or more survey respondents are included in the word cloud.

When we posed a similar question in interviews, most stakeholders made two key points. Firstly, that different bioimaging techniques have their own strengths and limitations, and they enable users to ask and answer different types of research questions. Nevertheless, **new combinations of techniques that allow integration across scales and combine imaging with other methods will be crucial for getting novel insights into biological processes**. Secondly, artificial intelligence (AI) based image analysis and automated workflows are expected to completely transform the field, with benefits expected for all the bioimaging modalities. Additionally, stakeholders highlighted the need to develop image analysis methods and tools in tandem with novel bioimaging technologies to allow the full potential of a new technology or technique to be captured. For example, cryogenic EM (cryo-EM) is now a mainstream technique in structural biology (in HICs) owing to technological advancements in both the hardware and image processing software (See Section 2.2).[3]

Bioimaging techniques and methods that are expected to facilitate key breakthroughs in the understanding of biological phenomena are described in the sections below according to the overarching bioimaging modality or approach.

2.1 Fluorescence-based techniques

Fluorescence microscopy is one of the most commonly used modalities to visualise cellular organelles and track cellular processes as evidenced by the fact that two-thirds (65%) of survey respondents had experience in this modality. Recent developments have sought to address limitations related to photobleaching, low intensity and overlapping spectra. One example is the development of **light sheet microscopy**, which uses a thin sheet of light to excite fluorophores within a focused area. This method allows optical sectioning and restricts photobleaching and phototoxicity while providing high spatiotemporal resolution.[4]

Among fluorescence-based techniques, light sheet microscopy was considered to have the greatest potential to transform bioimaging by both HIC- and LMIC-based survey respondents (Figure 3). This point was confirmed in the interviews. Light sheet microscopy allows imaging of tissues and organoids rather than just sections, thus enabling visualisation at the cellular level. Its use has rapidly expanded and the development of modified versions of the technique (e.g. lattice light sheet) is enabling faster real-time imaging. Commercialisation of lattice light sheet microscopy has helped to make it more widely available and accessible. However, further developments are needed to make commercially available versions compatible with a wider range of samples considering two major drawbacks of the technique – a) expensive and complicated optical setups and b) the trade-off between image quality and image volume.[5]

There have also been developments in other fluorescence microscopy technologies and methodologies. For example, developments in near-infrared (NIR) microscopy now allow bioimaging in the NIR-II channel (1.3–1.7µm wavelength). **NIR-II bioimaging** allows exploration of deep-tissue information in the cm range and µm-level resolution at mm depth, which has

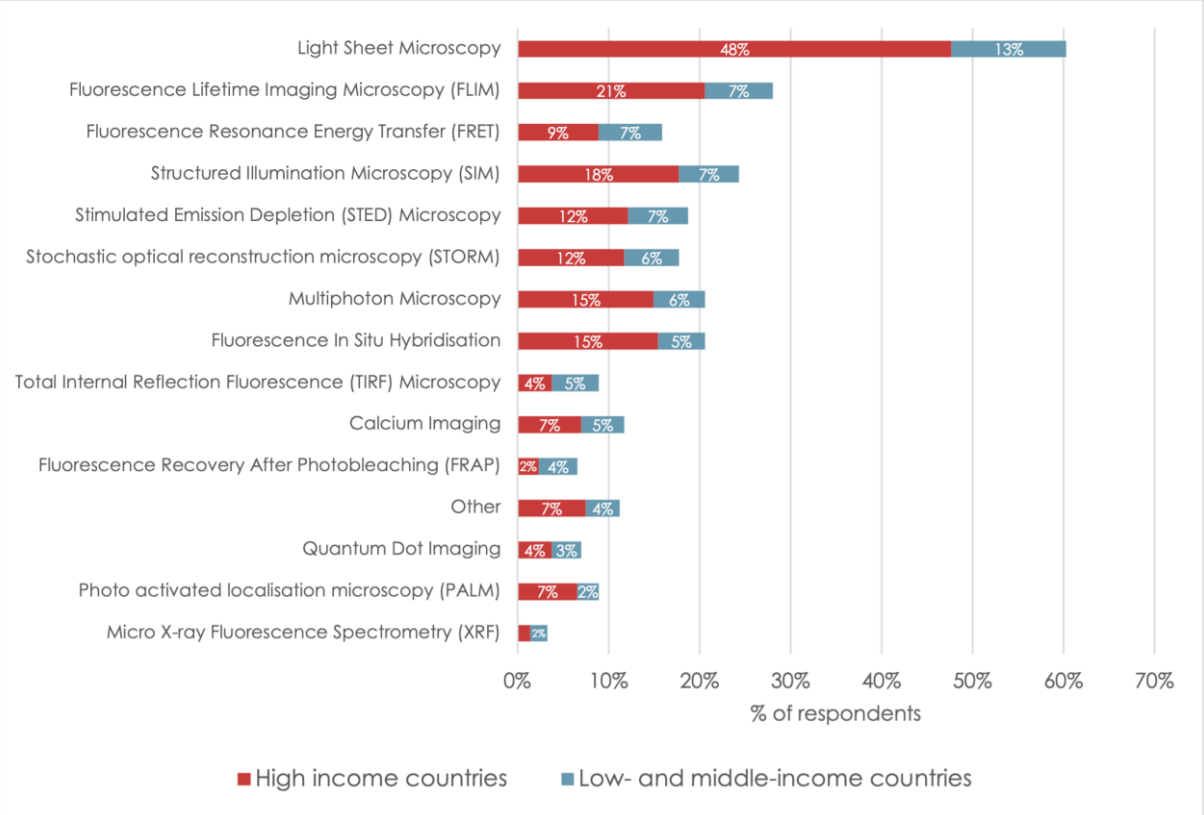
[3] Yip KM, Fischer N, Paknia E, Chari A, Stark H. Breaking the next Cryo-EM resolution barrier – Atomic resolution determination of proteins! bioRxiv; 2020. DOI: 10.1101/2020.05.21.106740.

[4] Stelzer EHK, Strobl F, Chang BJ, et al. Light sheet fluorescence microscopy. Nature Reviews Methods Primers 2021 1:1. 2021;1(1):1-25. doi:10.1038/s43586-021-00069-4

[5] Shi, Fenghua, Wen, Jing and Lei, Dangyuan. "High-efficiency, large-area lattice light-sheet generation by dielectric metasurfaces" Nanophotonics, vol. 9, no. 12, 2020, pp. 4043-4051. https://doi.org/10.1515/nanoph-2020-0227

furthered possibilities to observe samples in 3D using this technique.[6] A key drawback however is the limited progress of NIR-II probes.

Figure 3 Most transformative fluorescence-based techniques by country type (n=214)



Source: Online survey

Many state-of-the-art optical microscopes are based on confocal and multiphoton fluorescence microscopes that focus the light on a specific area of the sample. In contrast, wide-field fluorescence microscopes are able to obtain images from the whole sample. **Optical-sectioning structured illumination microscopy (OS-SIM)** is a type of wide-field microscope that is powerful and relatively low cost compared to confocal and multiphoton microscopes. However, it can only be used for *in vitro* samples owing to the need for thin samples, low signal-to-noise ratio of *in vivo* samples, artefacts and sample-induced distortion of the image-forming light. A new method called adaptive optics when combined with OS-SIM enables live structural and functional imaging at high resolution.[7]

Other techniques mentioned included optogenetic techniques for mouse brain mapping,[8] Swept Confocally Aligned Planar Excitation (SCAPE) microscopy which allows volumetric

[6] Cao J, Zhu B, Zheng K, He S, Meng L, Song J and Yang H (2020) Recent Progress in NIR-II Contrast Agent for Biological Imaging. Front. Bioeng. Biotechnol. 7:487.

[7] Li Z, Zhang Q, Chou SW, et al. Fast widefield imaging of neuronal structure and function with optical sectioning in vivo. *Sci Adv*. 2020;6(19).

[8] Lim, D. H., LeDue, J., Mohajerani, M. H. *et al*. Optogenetic approaches for functional mouse brain mapping. *Front Neurosci* **7**, 54 (2013). doi:10.3389/fnins.2013.00054

imaging of living samples at ultrahigh speeds,[9] and fluorescence fluctuation microscopy which employs a powerful arsenal of analysis tools to investigate molecular heterogeneity in space and time.[10] Structured Illumination Microscopy (SIM) was seen as a very efficient technique for unravelling molecular interactions without the need for special sample preparation.

2.2 Electron Microscopy (EM)

Among techniques involving some form of EM, stakeholders chose **Correlative Light and Electron Microscopy (CLEM)** and cryogenic EM (cryo-EM) as the two techniques with the most transformative potential (Figure 4, interviews).

CLEM combines the benefits of fluorescence microscopy and EM allowing imaging of specific cellular targets along with high resolution ultrastructure context. It could enable analysis of cells and tissue in different pathologies, especially through combination of live cell or tissue imaging techniques such as calcium imaging. CLEM is expected to lead to important and novel morphological insights, provided appropriate data handling and analysis methods are developed to cope with the large amounts of data that will be generated. Survey respondents also highlighted that innovative sample preparation methods have the potential to make CLEM available to more research areas, thus facilitating new breakthroughs and insights.

Cryo-EM is a technique that is increasingly preferred for structural studies of biological macromolecules over techniques such as X-ray crystallography which require crystallisation. It has now become a mainstream technique for determining the structure and function of biomolecules in fixed samples. However, LMIC-based stakeholders noted that cryo-EM has had limited uptake in LMICs since the relevant facilities and expertise are not readily available and establishing new cryo-EM infrastructure is very expensive.

Recent developments in sample preparation methods, hardware and software have further improved structure determination via cryo-EM and promoted its use.[11] For example, advances in computational neural networks and deep learning (DL)-based analysis software have improved cryo-EM analysis, leading to better 3D structures of proteins. Improved protocols for cryo-specimen preparation, data collection and 3D reconstruction for the emerging method of cryo-EM single particle analysis (SPA) can be useful for yielding cryo-EM structures of RNA, which has previously proven difficult to achieve.[12] While recent advances in cryo-EM have led to near-atomic-resolution structures of macromolecular complexes, the associated technique of **cryo-electron tomography (cryo-ET)** can provide insights into these complexes in the context of their natural environment and its use is growing. Unfortunately, cryo-ET requires samples that are thinner than most cells. This limitation can now be circumvented using cryo-focused-ion-beam (cryo-FIB) milling which can be used to carve out thin regions (lamella) in frozen cells. Thus, cryo-FIB milling facilitates structure determination of biomolecules in their native

[9] Bouchard, M., Voleti, V., Mendes, C. *et al.* Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms. *Nature Photon* 9, 113–119 (2015). https://doi.org/10.1038/nphoton.2014.323

[10] Weidemann, T., Mücksch, J., Schwille, P. Fluorescence fluctuation microscopy: a diversified arsenal of methods to investigate molecular dynamics inside cells. *Curr Opin Struct Biol* 28:69-76. DOI: 10.1016/j.sbi.2014.07.008.

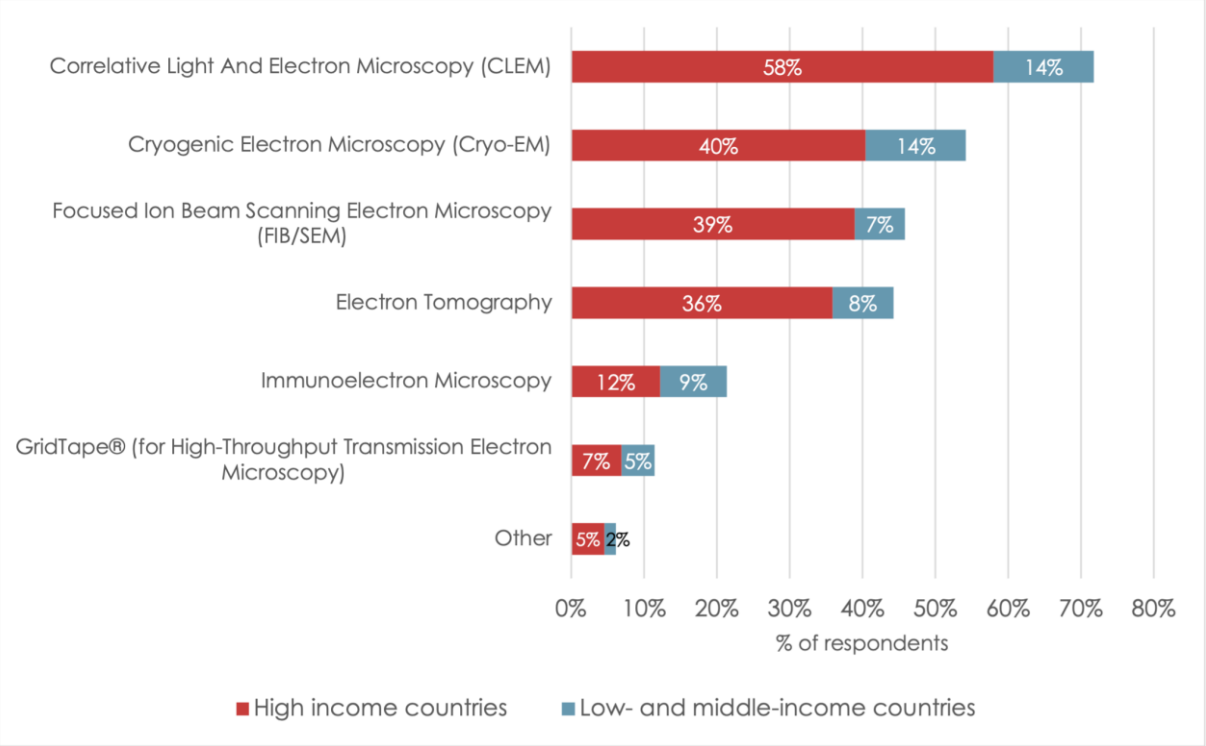
[11] Callaway E. Revolutionary cryo-EM is taking over structural biology. Nature. 2020;578(7794):201. doi:10.1038/D41586-020-00341-9

[12] Li S, Zhang K, Chiu W. Near-Atomic Resolution Cryo-EM Image Reconstruction of RNA. Methods Mol Biol. 2023;2568:179-192. doi:10.1007/978-1-0716-2687-0_12/COVER

environment via cryo-ET. It can also be combined with fluorescence microscopy to identify cells or regions of interest for lamellae creation and cryo-ET imaging.[13]

Another key trend is the wider use of **volume EM** in biomedical research since it can visualise 'large' volumes at high resolution. This is a suite of techniques based on well-established scanning and transmission EM (SEM and TEM) protocols and involving focused ion beam SEM (FIB-SEM), serial block-face SEM and array tomography.[14] Development of systems such as the automatic tape-collecting ultramicrotome, serial block-face SEM and FIB-SEM have allowed automation of serial section techniques which previously the domain of specialists. These improvements have broadened the scope of research areas to which volume EM can be applied (beyond the brain) and enabled better interrogation of cell and tissue ultrastructures, 3D visualisation and nm level resolution using volume EM.[15] Even so, the need for very specialised and costly equipment puts volume EM out of reach for anyone other than large HIC-based facilities with plenty of resources. In our survey, **FIB-SEM** was seen as a key method in HICs, but not in LMICs (Figure 4), which could be due to a combination of reasons ranging from lack of awareness of the technique and unavailability of FIB-SEM equipment and expertise to high costs associated with establishing and performing the technique.

Figure 4 Most transformative Electron Microscopy techniques by country type (n=131)



Source: Online survey

[13] Wagner, F.R., Watanabe, R., Schampers, R. *et al.* Preparing samples from whole cells using focused-ion-beam milling for cryo-electron tomography. *Nat Protoc* 15, 2041–2070 (2020). https://doi.org/10.1038/s41596-020-0320-x

[14] https://www.volumeem.org/what-is-volume-em.html

[15] Kievits AJ, Lane R, Carroll EC, Hoogenboom JP. How innovations in methodology offer new prospects for volume electron microscopy. J Microsc. 2022;287(3):114-137. doi:10.1111/jmi.13134

2.3 Tissue and Organ Imaging techniques

Technological advances over the decades have produced a spectrum of methods for non-invasive imaging of tissues, organs and even whole organisms. These range from X-ray imaging to ultrasound, magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET). Among these, MRI and functional MRI (fMRI) seem to be most commonly used in HICs as evidenced from the survey and interviews. These were also considered the most transformative tissue and organ imaging techniques by HIC-based individuals (Figure 5), although LMIC-based respondents chose single-photon emission computed tomography (SPECT) and PET/CT. PET and SPECT make use of radioactive tracers to provide images of functional processes.[16] Key developments, benefits and limitations of currently used tissue and organ imaging techniques are discussed below.

Currently, advances in the **MRI** field are occurring at opposite ends of the spectrum with both ultra-high field (≥ 7 Tesla) and ultra-low field (< 0.1 Tesla) MRI equipment and methods under development. Advances in MRI scanners that capitalise on the increased signal-to-noise ratio available at ultra-high field are offering better visualisation of organs (e.g. brain) at the functional and structural level.[17] In contrast, development of high-performance low-field MRI systems and deep learning (DL) techniques that remove noise from analysis are making low-field MRI a preferred method, as it is more affordable and portable.[18] Ultra-low field MRI technology is thus expected to democratise MRI, especially in LMICs. Uptake of MRI in LMICs has been low so far owing to the prohibitive costs of scanners which rely on complex designs, expensive infrastructural requirements, high maintenance costs and specialised technicians.[19] Approaches to use nuclei other than hydrogen (non-hydrogen MRI), for example, sodium or deuterium, are being explored as alternatives to conventional MRI. However, it is not possible to get enough signal at standard field strengths with these nuclei just yet and more development is needed.

In contrast to MRI, **PET** offers high sensitivity but only a few centres in UK that offer this technique. Although applicable to a range of research areas, it is predominantly used in neuroscience and oncology research with little uptake in other research areas. One view is that it is held back by lack of investment. Another view is that PET technology is cost-prohibitive – It is expensive and complex compared to other similar modalities, with high costs for scanning and radiosynthesis of tracers.[20] In the CT area, **photon counting** is an emerging CT technique that is transforming cardiovascular research. It can overcome the challenge of 'calcium blooming artefacts' and allow quantification and prediction of vulnerability to heart attacks more readily. Recent engineering and manufacturing advances e.g. in photon counting detectors are expected to scale up use of this technique. Other emerging techniques in this area include dual energy/spectral CT which uses two separate x-ray photon energy spectra and radiomics (extraction of mineable data from medical imaging).

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[16] https://link.springer.com/article/10.1007/s40336-013-0004-4

[17] Dumoulin SO, Fracasso A, van der Zwaag W, Siero JCW, Petridou N. Ultra-high field MRI: Advancing systems neuroscience towards mesoscopic human brain function. Neuroimage. 2018 Mar;168:345-357. doi: 10.1016/j.neuroimage.2017.01.028.

[18] Hori M, Hagiwara A, Goto M, Wada A, Aoki S. Low-Field Magnetic Resonance Imaging: Its History and Renaissance. Invest Radiol. 2021;56(11):669. doi:10.1097/RLI.0000000000000810

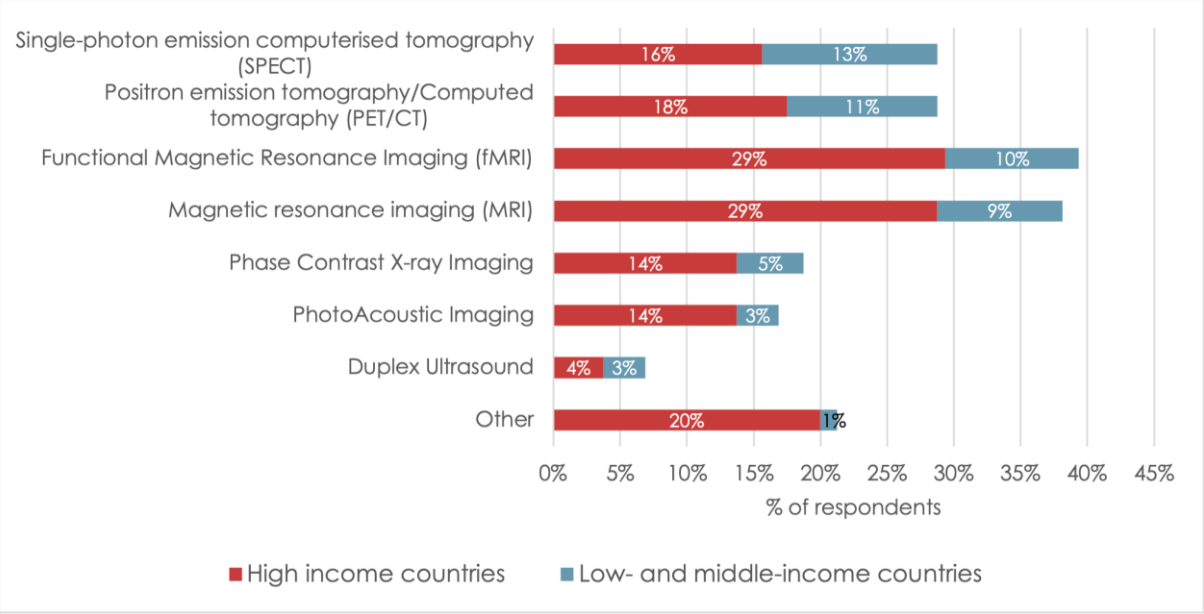
[19] Liu, Y., Leong, A.T.L., Zhao, Y. *et al.* A low-cost and shielding-free ultra-low-field brain MRI scanner. *Nat Commun* **12**, 7238 (2021). https://doi.org/10.1038/s41467-021-27317-1

[20] https://www.ukri.org/wp-content/uploads/2021/12/MRC-011221-ReviewOfPETWithinTheMedicalImagingLandscapeV2.pdf

Photoacoustic imaging combines optical and ultrasound imaging techniques to allow the visualisation of relatively deep biological tissues and provides functional and molecular information when exogenous contrast agents are used. It is thus of interest for both clinical and preclinical applications. Importantly, photoacoustic imaging can be easily integrated into a conventional ultrasound machine, and unlike X-ray CT and MRI, a contrast agent is not necessarily required. One research group has recently developed a new image optimisation platform that aims to provide better user interfaces as well as real-time parameter controls.[21] The impact of these developments on uptake are yet to be seen.

Novel contrast agents for MRI and photoacoustic imaging adapted for different imaging scales could help transform *in-vivo* imaging, promoting use in new research fields. Breakthroughs will also be enabled by correlative multi-modal methods and *in-vivo* measurements (e.g. combining X-ray imaging and X-ray diffraction). 3D imaging analysis will be advanced by combining lab source X-ray CT with spectral detection. Optically Pumped Magnetometers were also noted as having a large potential for paediatric brain imaging, as this technology is more flexible than conventional magnetoencephalography systems. Recent developments in genetically encoded fluorescent sensors and multiplex measurements paired with two-photon microscopy has also advanced 3D *in vivo* imaging.[22]

Figure 5 Most transformative Tissue and Organ Imaging techniques by country type (n=160)



Source: Online survey

The combination of **tissue clearing techniques** with intravital, expansion or light sheet microscopy is helping researchers to look inside whole organs and animals. For example, a novel pipeline, named CUBIC-HistoVIsion, offers opportunities for organ- and organism-scale

[21] Kim J, Park EY, Park B, Choi W, Lee KJ, Kim C. Towards clinical photoacoustic and ultrasound imaging: Probe improvement and real-time graphical user interface. Exp Biol Med. 2020;245:321-329. doi:10.1177/1535370219889968

[22] https://www.laserfocusworld.com/biooptics/article/14286479/twophoton-microscopy-advances-bio-research

histological analysis.[23] The pipeline involves tissue clearing followed by whole-organ/-body 3D tissue staining, imaging with light sheet microscopy and computational image analysis. The method allows imaging of every single cell in an adult mouse brain which has led to a digitalised map of the mouse brain which is awaiting annotation. Whole head imaging is now also feasible, which allows visualisation of the interaction between the brain and the skull.

2.4 Spectroscopy-based techniques

Comparatively, fewer survey respondents chose spectroscopy-based techniques among the three topmost transformative modalities, perhaps owing to very few individuals in the survey sample who are experienced in these techniques. There were differences in how HICs and LMICs ranked spectroscopy-based techniques, with hyperspectral imaging viewed as having more potential in HICs and fluorescence correlation spectroscopy techniques valued more in LMICs (Figure 6).

Hyperspectral imaging is an emerging field which combines optical spectroscopy with 2D optical imaging. Each pixel is captured in the form of different spectral bands (rather than just primary colours), allowing researchers to distinguish between different tissues based on their spectral characteristics. For instance, very small areas of malignant tissue can be detected. The technology however is not fully mature and equipment is expensive, large and difficult to use with low frame rates.[24] One research group has recently developed a hyperspectral multiphoton microscope that can detect up to ten different fluorescent signals in multiple types of *in vivo* preparations.[25]

In contrast, **fluorescence correlation spectroscopy (FCS)** has been widely applied in diverse fields including biomedicine, biophysics and chemistry. FCS allows quantitative evaluation of the concentration, diffusion and interaction of the molecules *in vitro* or *in vivo*. In the last two decades, many variations of FCS have been developed to mitigate problems such as photobleaching of fluorophores and movement of cells. This technique offers high spatial and temporal resolution, short analysis time and high sensitivity and further development of lasers and sensors will make it a more powerful tool.[26]

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) is a unique technique that supports non-invasive exploration of the structure and physiology of the intracellular space *in vivo*. DW-MRS has been shown to be sensitive to changes in cell-specific metabolite diffusion in the brain and in skeletal muscle, and thus can provide cell-specific information from tissues.[27] While the technique is becoming more feasible, measurements are challenging in terms of acquisition as well as analysis and quantification.

Use of **X-ray-free electron lasers (XFELs)** considered to be among the next generation of light sources has the potential to revolutionise structural biology. With this nascent technology, structural changes, ultrafast biological process changes and biological reactions can be

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[23] https Susaki, E.A., Shimizu, C., Kuno, A. *et al.* Versatile whole-organ/body staining and imaging based on electrolyte-gel properties of biological tissues. *Nat Commun* 11, 1982 (2020). https://doi.org/10.1038/s41467-020-15906-5

[24] Schneider, Armin, and Hubertus Feussner. *Biomedical engineering in gastrointestinal surgery*. Academic Press, 2017.

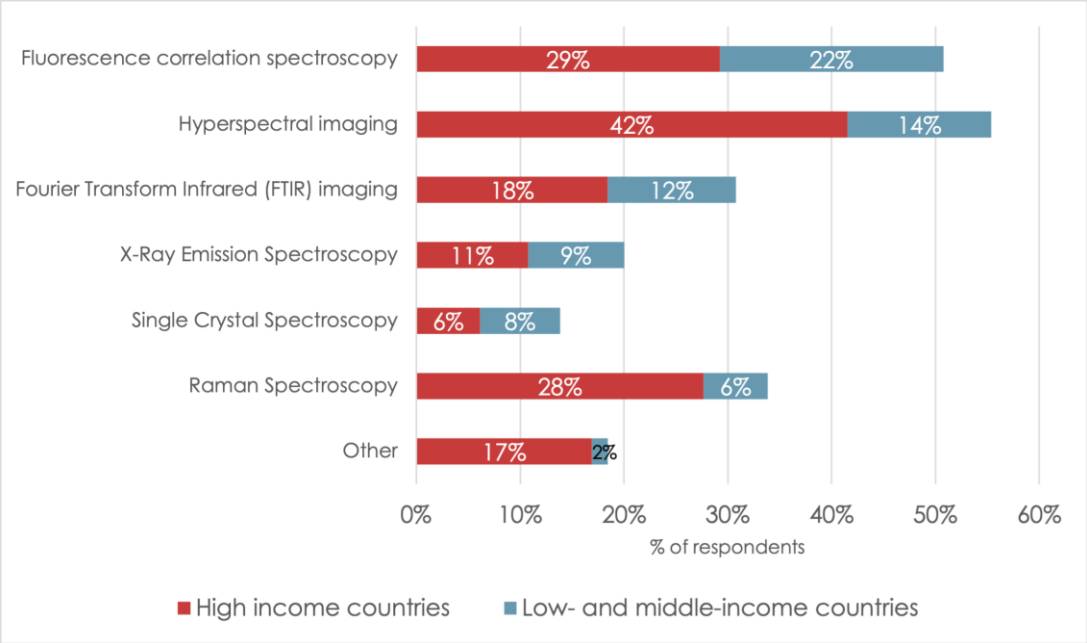
[25] Bares AJ, Mejooli MA, Pender MA, et al. Hyperspectral multiphoton microscopy for in vivo visualization of multiple, spectrally overlapped fluorescent labels. Optica. 2020;7(11):1587-1601. doi:10.1364/optica.389982

[26] Yu, L., Lei, Y., Ma, Y., Liu, M., Zheng, J., Dan, D. and Gao, P., 2021. A comprehensive review of fluorescence correlation spectroscopy. *Frontiers in physics*, 9, p.644450.

[27] de Marco R, Ronen I, Branzoli F, et al. Diffusion-weighted MR spectroscopy (DW-MRS) is sensitive to LPS-induced changes in human glial morphometry: A preliminary study. Brain Behav Immun. 2022;99:256-265. doi:10.1016/J.BBI.2021.10.005

followed, enabling the discovery of the dynamics of biomolecular processes and reactions. The combination of XFELs with ultra-fast spectroscopy could enable the study of molecular dynamics and structural transition simultaneously in real-time.[28] However, this is still a niche technique.

Figure 6 Most transformative Spectroscopy-based techniques by country type (n=65)



Source: Online survey

2.5 Other approaches

2.5.1 Super-resolution microscopy techniques

Super-resolution microscopy is a fast-developing field where new technology has enabled either nm-scale molecular resolution or 3D multi-colour and fast live-cell imaging,[29] allowing the visualisation of subcellular organisation at a level of detail, previously only achievable in fixed cells with EM.[30] It is viewed as a key transformative technique by stakeholders based in both LMICs and HICs (Figure 7) as it allows researchers to visualise proteins in their biological context (their localisation and distribution in cells) and to observe molecular changes in real time. Commercialisation of super-resolution techniques has made them more accessible to the bioimaging community, yet some technologically mature super-resolution techniques e.g. single particle analysis (which produces an averaged model from multiple views of a structure) are yet to be taken up widely according to interviewees. This may be due to the need for

[28] Stelzer EHK, Strobl F, Chang BJ, et al. Light sheet fluorescence microscopy. Nature Reviews Methods Primers 2021 1:1. 2021;1(1):1-25. doi:10.1038/s43586-021-00069-4; Mills G, Bean R, Mancuso AP. First Experiments in Structural Biology at the European X-ray Free-Electron Laser. Applied Sciences 2020, Vol 10, Page 3642. 2020;10(10):3642. doi:10.3390/APP10103642; Huang N, Deng H, Liu B, Wang D, Zhao Z. Features and futures of X-ray free-electron lasers. The Innovation. 2021;2(2). doi:10.1016/J.XINN.2021.100097

[29] Prakash K, Diederich B, Heintzmann R, Schermelleh L. Super-resolution microscopy: a brief history and new avenues. *Philosophical Transactions of the Royal Society A*. 2022;380(2220). doi:10.1098/RSTA.2021.0110

[30] Schermelleh L, Ferrand A, Huser T, et al. Super-resolution microscopy demystified. Nature Cell Biology 2019 21:1. 2019;21(1):72-84. doi:10.1038/s41556-018-0251-8

improvements such as need to achieve high resolution with a wide field of view, to have techniques for high resolution imaging of native unstained biological specimens and to obtain better temporal resolution so that cellular events can be followed in real-time. Notwithstanding these needs, there is also the underlying challenge of identifying the biological questions that actually require information at this scale to uncover insights that otherwise would not be possible. It was also noted that at high levels of resolution, it is important to validate the biological veracity of what is being observed i.e. the image is not just representing an artefact.

Single-molecule localisation methods like MINFLUX (minimal photon fluxes) and RASTMIN (RASTer scanning a MINimum of light)[31] have pushed resolution to molecular dimensions, but have not been taken up widely. MINFLUX can separate the fluorophores in a sample by activating and deactivating them individually, which means researchers can now visualise more than one labelled molecule within live cells in 3D. It has been suggested that MINFLUX can provide 100 times the resolution of a typical confocal microscope.[32] However, the technique is very complex to use and requires dedicated experts, which has limited its use.

2.5.2 Expansion microscopy

Expansion microscopy involves imaging biological specimens that are physically expanded (about 100x in volume) using a chemical process. It allows nanoscale imaging with conventional light microscopes. Sample treatment requirements mean this technique cannot be used for live cell imaging, but it makes specimens transparent and decrowds biomolecules allowing signal amplification.[33] Further development is ongoing, and combining this technique with more powerful microscopes may allow even better resolution and visualisation of DNA, RNA, proteins and lipid complexes. The key potential of the technique lies in the ability to visualise and trace large cellular morphologies and molecular constituents across a whole organism or multiple organisms with nanoscopic resolution.[34]

2.5.3 Fluorescent probes

Development of new fluorescent probes is important to address many challenges such as probes staining cellular structures and selection of appropriate probes for co-localisation of proteins. Novel probes combined with light sheet microscopy and AI-based image analysis could have a significant impact in understanding living organisms, tissues and organoids. Ultimately, this type of combination should also enable non-invasive, low phototoxicity and low laser power imaging that is also sensitive and accurate for imaging low/faint signals over longer periods of time.

Increasing the number of fluorophores/labels that can be used in a single experiment was noted as a key factor that will help advance many fluorescence-based imaging techniques. **Nanoparticles** such as quantum dots are an emerging replacement for fluorescent proteins due to their multiplexing capabilities, unique emission spectrum, high quantum efficiency and chemical stability. However, use of quantum dots in biological applications is limited due to

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[31] Masullo, L.A., Szalai, A.M., Lopez, L.F. *et al.* An alternative to MINFLUX that enables nanometer resolution in a confocal microscope. *Light Sci Appl* **11**, 199 (2022). https://doi.org/10.1038/s41377-022-00896-4

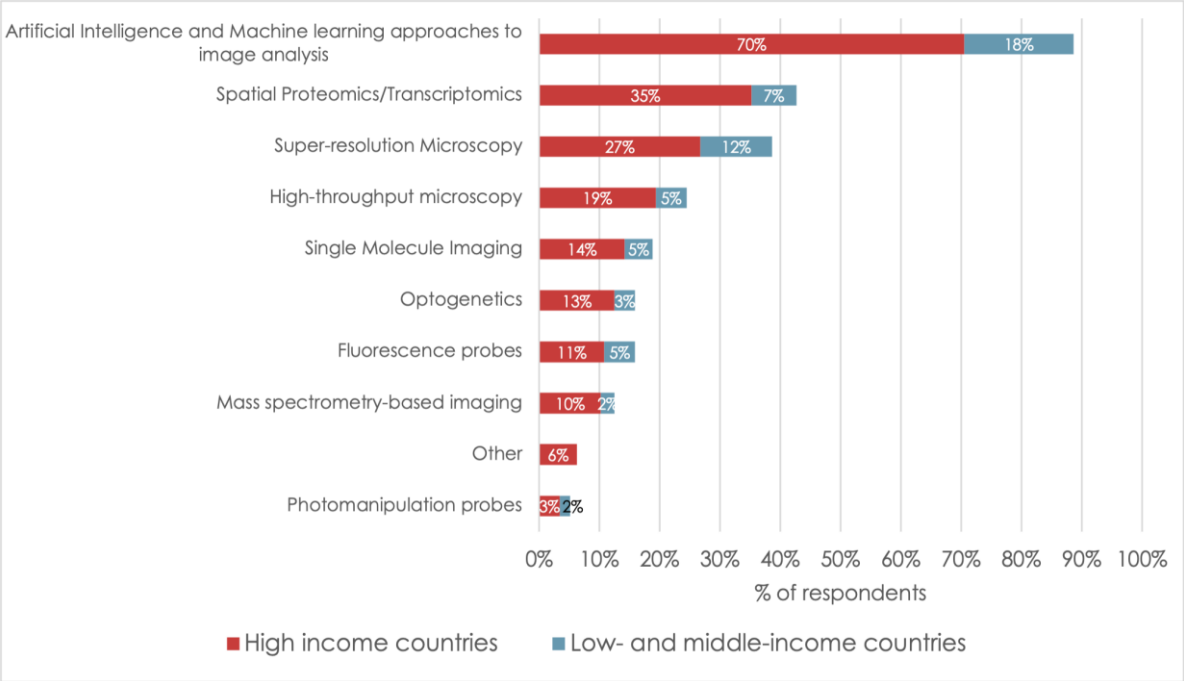
[32] Schmidt R, Weihs T, Wurm CA, et al. MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. Nature Communications 2021 12:1. 2021;12(1):1-12. doi:10.1038/s41467-021-21652-z; New Minflux microscope improves molecule tracking in live cells | Carl R. Woese Institute for Genomic Biology. Accessed November 22, 2022. https://www.igb.illinois.edu/article/new-minflux-microscope-improves-molecule-tracking-live-cells

[33] Wassie, A.T., Zhao, Y. & Boyden, E.S. Expansion microscopy: principles and uses in biological research. *Nat Methods* **16**, 33–41 (2019).

[34] https://focalplane.biologists.com/2020/07/29/expansion-microscopy/

heavy metal toxicity. Carbon quantum dots and graphene quantum dots are emerging as popular and environmentally friendly alternatives, in particular for *in-vivo* and *in-vitro* applications.[35] Other new nanoparticles such as lanthanide-doped nanoparticles (LDNPs) are postulated to improve the longevity of luminescence and resolution.[36]

Figure 7 Most transformative Allied approaches and tools (n=176)



Source: Online survey

2.6 Data-related approaches

2.6.1 *Artificial Intelligence and Machine Learning approaches*

AI and machine learning (ML) approaches to image analysis are widely seen as the key methodologies that will transform the field of bioimaging (Figure 7). These approaches allow for analysis of large imaging datasets, real-time responsive imaging (e.g. AI/ML driven image acquisition), automated workflows, multiplexed automated imaging in live cells, immersive visualization of 3D image datasets (Virtual Reality) and less photo damage according to stakeholders. However, data interoperability needs to improve along with availability of data analysis software (e.g. through open access) for the full potential of AI and ML approaches to be realised.

[35] Molaei MJ. A review on nanostructured carbon quantum dots and their applications in biotechnology, sensors, and chemiluminescence. Talanta. 2019;196:456-478. doi:10.1016/j.talanta.2018.12.042; Jahdaly BA al, Elsadek MF, Ahmed BM, Farahat MF, Taher MM, Khalil AM. Outstanding Graphene Quantum Dots from Carbon Source for Biomedical and Corrosion Inhibition Applications: A Review. Sustainability 2021, Vol 13, Page 2127. 2021;13(4):2127. doi:10.3390/SU13042127

[36] Du P, An R, Liang Y, Lei P, Zhang H. Emerging NIR-II luminescent bioprobes based on lanthanide-doped nanoparticles: From design towards diverse bioapplications. *Coord Chem Rev*. 2022;471.

Application of **deep learning (DL)**, a subset of machine learning, in bioimaging is becoming a powerful analytical tool to transform bioimage analysis.[37,38] Common image analysis tasks that DL can assist with include image restoration whereby images can be enhanced, image segmentation whereby objects of interest (e.g. different cell types) are identified, and image quantification whereby objects are classified, counted and tracked to understand cellular dynamics and underlying biological mechanisms. [39,40,41] With respect to image restoration, DL approaches can 'denoise' bioimages to correct uneven illumination and remove artefacts.[42] In addition, DL approaches are helping to increase the throughput of super-resolution techniques by converting low-resolution images into high-resolution images.[43,44]

2.6.2 *Integrating bioimaging with data sets from other fields*

Genomics, metabolomics, proteomics and other 'omics' approaches have evolved to provide molecular insights at the individual cell level; however, traditional 'omics' methods do not provide spatial information on the location of cells within tissue, the proximity of these cells to other cells and cellular components. Spatial 'omics', an overarching term for approaches combining 'omics' methods and imaging data, is an emerging field that holds the potential to interrogate different biological functions at an unprecedented resolution. Examples include spatial transcriptomics and spatial proteomics.

Spatial transcriptomics affords researchers the ability to locate mRNA in tissues. Recently, image-based methods such as fluorescent in situ sequencing (FISSEQ) and multiplexed error-robust fluorescence in situ hybridisation (MERFISH) have emerged.[45,] FISSEQ enables subcellular interrogation of RNA species, while MERFISH applies probes to tag the RNAs and uses imaging techniques to track them. A kit-based solution requiring less sophisticated processes called Visium Spatial Gene Expression is now available commercially and has potentially lowered the barrier to adoption of the technique.

Mass spectrometry has been a staple tool in studying the molecular composition of proteins, and when combined with imaging technologies, **spatial proteomics** is made possible. Imaging Mass Spectrometry methods, such as matrix-assisted laser desorption ionisation mass spectrometer (MALDI-MS), uses micrometre laser beams to allow the identification of peptides while simultaneously generating tissue images so that the location of the proteins can be

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[37] Meijering E. A bird's-eye view of deep learning in bioimage analysis. Comput Struct Biotechnol J. 2020;18:2312-2325. Published 2020 Aug 7. doi:10.1016/j.csbj.2020.08.003

[38] Hallou A, Yevick HG, Dumitrascu B, Uhlmann V. Deep learning for bioimage analysis in developmental biology. Development. 2021;148(18):dev199616. doi:10.1242/dev.199616

[39] K. de Haan, Y. Rivenson, Y. Wu and A. Ozcan, "Deep-Learning-Based Image Reconstruction and Enhancement in Optical Microscopy," in Proceedings of the IEEE, vol. 108, no. 1, pp. 30-50, Jan. 2020, doi: 10.1109/JPROC.2019.2949575.

[40] Van Valen DA, Kudo T, Lane KM, et al. Deep Learning Automates the Quantitative Analysis of Individual Cells in Live-Cell Imaging Experiments. PLoS Comput Biol. 2016;12(11):e1005177. Published 2016 Nov 4. doi:10.1371/journal.pcbi.1005177

[41] Emami, N., Sedaei, Z. & Ferdousi, R. Computerized cell tracking: Current methods, tools and challenges. Visual Informatic (2020)

[42] Laine RF, Jacquemet G, Krull A. Imaging in focus: An introduction to denoising bioimages in the era of deep learning. Int J Biochem Cell Biol. 2021;140:106077. doi:10.1016/j.biocel.2021.106077

[43] Villoutreix P. What machine learning can do for developmental biology. Development. 2021;148(1):dev188474. Published 2021 Jan 10. doi:10.1242/dev.188474

[44] Weigert M, Schmidt U, Boothe T, et al. Content-aware image restoration: pushing the limits of fluorescence microscopy. Nat Methods. 2018;15(12):1090-1097. doi:10.1038/s41592-018-0216-7

[45] Williams CG, Lee HJ, Asatsuma T, Vento-Tormo R, Haque A. An introduction to spatial transcriptomics for biomedical research. *Genome Med*. 2022;14(1):1-18. doi:10.1186/S13073-022-01075-1/FIGURES/3

identified. However, this technique has many limitations, leading to the development of multiple antibody-based technologies, such as co-detection by indexing (CODEX) that uses mixtures of indexable oligo-tagged antibodies for *in situ* staining.[46] Another emerging technology is called Multi-Omyx, which provides single-cell spatial expression patterns.[47]

Spatial transcriptomics is driving developments in sample preparation, labelling strategies and complex data analysis according to survey respondents. They see potential for implementing such advances in other research areas, with HICs and LMICs ranking spatial proteomics/transcriptomics as their second and third most transformative approach among other/allied bioimaging approaches (Figure 7).

One other example is ***in situ* genome sequencing**, which enables simultaneous sequencing and imaging of genomes in intact samples to understand genome organisation.[48] Previously, scientists combined DNA sequencing with microscopy methods that allow localisation of genomic loci, such as DNA fluorescence in situ hybridisation (FISH). However, these and other similar techniques cannot be applied jointly on the same cell. This method allows DNA sequences to be connected to their native spatial context within and between cells in intact biological samples.

2.7 Multimodal and correlative imaging

No single imaging modality provides all the necessary biological information from molecular structures to events occurring in a biological system. Each bioimaging technology provides different spatial resolution and penetration depth. Multimodal and correlative imaging, the combination of two or more imaging techniques or modalities, has emerged as a strategy to integrate the strengths of individual modalities to build a more complete picture across the scales of life, from biological molecules to whole organisms.

CLEM is the most established multimodal imaging technique and has helped to reveal many new biological insights. However, improvement in sample preparation workflows, labelling approaches and data analysis tools is required so that this technique can reach its full potential.[49] The success of CLEM has prompted developers to combine a wider suite of established bioimaging technologies and methodologies, but this will require further optimisation for integration into a single workflow. For example, correlation of CLEM with non-destructive methods such as X-ray microtomography (which uses X-rays to recreate a 3D model of an object without the need for sectioning) is preferred. Cryo-EM in combination with cross-linking mass spectrometry (XL-MS) has enabled scientists to obtain critical information on specific amino acids and to identify interacting regions of the protein complex, to help deduce the overall protein structure at a much higher resolution and in more detail[50].

Another correlative structural imaging technique that allows researchers to see and understand the effect of the biological process of interest at the cell ultrastructure level is a

[46] Wang N, Li X, Wang R, Ding Z. Spatial transcriptomics and proteomics technologies for deconvoluting the tumor microenvironment. *Biotechnol J*. 2021;16(9). doi:10.1002/biot.202100041

[47] Mund A, Brunner AD, Mann M. Unbiased spatial proteomics with single-cell resolution in tissues. *Mol Cell*. 2022;82(12):2335-2349. doi:10.1016/J.MOLCEL.2022.05.022

[48] Payne AC, Chiang ZD, Reginato PL, et al. In situ genome sequencing resolves DNA sequence and structure in intact biological samples. *Science*. 2021;371(6532). doi:10.1126/SCIENCE.AAY3446

[49] van den Dries K, Fransen J, Cambi A. Fluorescence CLEM in biology: historic developments and current super-resolution applications. FEBS Lett. 2022;596(19):2486-2496. doi:10.1002/1873-3468.14421

[50] Quintana-Gallardo L, Maestro-López M, Martín-Benito J, et al. Combining Electron Microscopy (EM) and Cross-Linking Mass Spectrometry (XL-MS) for Structural Characterization of Protein Complexes. Methods in Molecular Biology. 2022;2420:217-232. doi:10.1007/978-1-0716-1936-0_17/COVER

novel **correlative cryo-3D** imaging technique. This method combines cryo-3D structured illumination microscopy (cryo-3D-SIM) that can pinpoint the location of the molecule of interest and cryo soft X-ray tomography (cryo-SXT) that shows the ultrastructural environment and subcellular localisation. The addition of fluorescence without the need for staining or fixation can have potential benefits in bio-nanomedicine, where locating specific molecules in the cellular environment is desired[51]. This technology is currently available at the Diamond Synchrotron (UK).

Recently, COMULIS (Correlated Multimodal Imaging in Life Sciences), an EU-funded initiative, was launched to support development and adoption of correlated multimodal imaging techniques.[52]

Stakeholders pointed to various combinations of techniques and modalities that have already been tried. For example, combining in-vivo imaging techniques with light sheet microscopy (with spectral deconvolution) is useful to gain insights into pathologies, while PET-CT brings functional and anatomic imaging together. Other imaging modalities that have been combined include electron imaging, X-ray imaging and Cryo-EM; CLEM with volume EM; PET-CT; combinations of Fluorescence Resonance Energy Transfer (FRET) with super-resolution microscopy; and optical and ultrasound imaging (in photoacoustic imaging).

[51] Groen J, Palanca A, Aires A, et al. Correlative 3D cryo X-ray imaging reveals intracellular location and effect of designed antifibrotic protein–nanomaterial hybrids. Chem Sci. 2021;12(45):15090-15103. doi:10.1039/D1SC04183E

[52] https://www.comulis.eu/about-comulis

3 Key barriers and challenges in bioimaging

Many bioimaging modalities require complex sample preparation and/or advanced instrumentation as well as highly trained personnel and high-tech computing infrastructure for data capture, storage and management.[53] Such factors mean that it can be expensive to establish and maintain cutting-edge bioimaging capabilities widely (e.g. in individual research laboratories). Bioimaging is thus often provided as a service within a research institution or in a separate facility which research establishments (academic as well as industry) can have access to. All these aspects require human and financial resources, which along with the inherent scientific and technological limitations of bioimaging techniques may present barriers to the access, use and development of bioimaging modalities and methods, especially in resource-poor contexts. This chapter discusses some of these key barriers and challenges.

3.1 Scientific or technological barriers and challenges

- **Quality, reproducibility and quantitation challenges**

For both HICs and LMICs, quality and reproducibility challenges are limiting progress in the field of bioimaging to the largest extent (Figure 8). The key issues here are the quality, comparability and reproducibility of published data and protocols, of data generated by the same instrument (instrument performance) and of large datasets. A 2020 study, which reviewed 240 research papers across eight journals and found reporting on bioimaging experiments was poor, with less than 20% of papers providing sufficient information to replicate or expand on experiments.[54] Quality and reproducibility issues are also underpinned by the lack of adequate quality control of instruments and lack of quality standards and/or standardised protocols for publishing data analysis, sample preparation, etc.

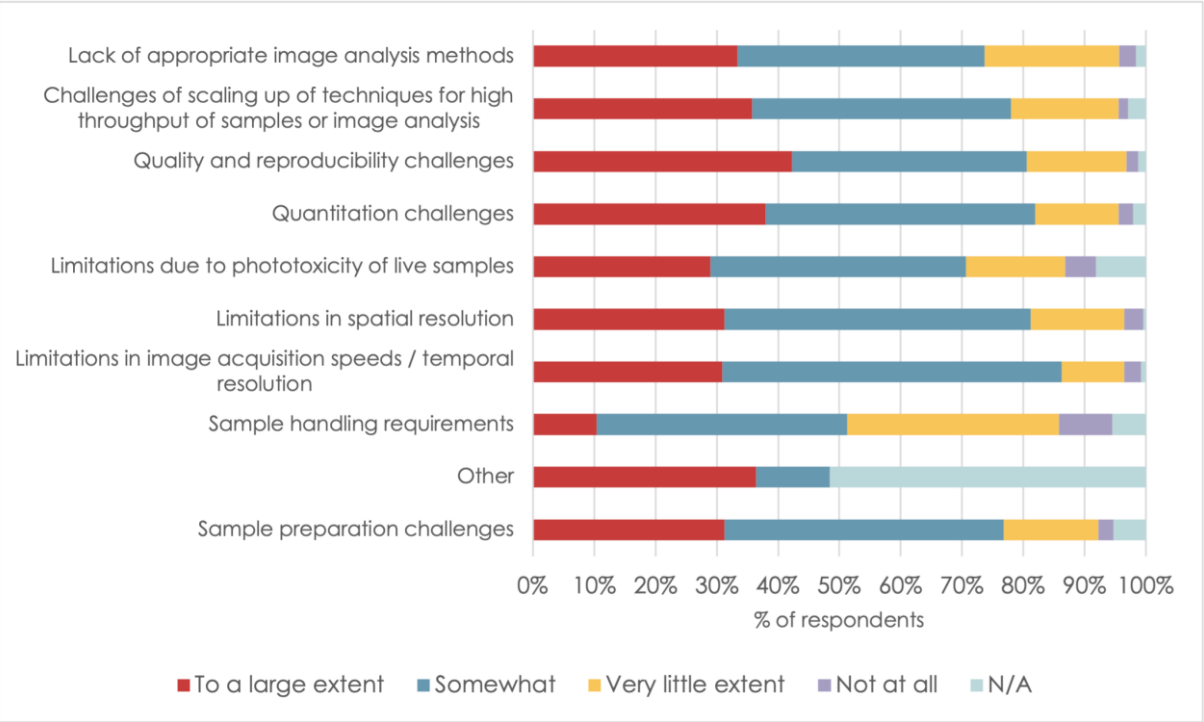
Addressing quality and reproducibility challenges is the top priority (among scientific or technological barriers) for stakeholders across the world. Quantitation challenges are also a priority for HICs, although quantitation is not considered a challenge to the same extent in LMICs. The problem of quantitation goes hand-in-hand with the lack of appropriate image analysis methods to cope with the scale and nature of images captured. Quantitation can present as a problem because of the large and complex data sets as well as technical challenges such as noise, signals being produced in scattering media and need to measure rate of change. Lack of relevant quantitative and analytical skills also adds to the challenge.

[53] https://www.technologynetworks.com/immunology/articles/bioimaging-using-microscopes-challenges-benefits-and-the-future-294744

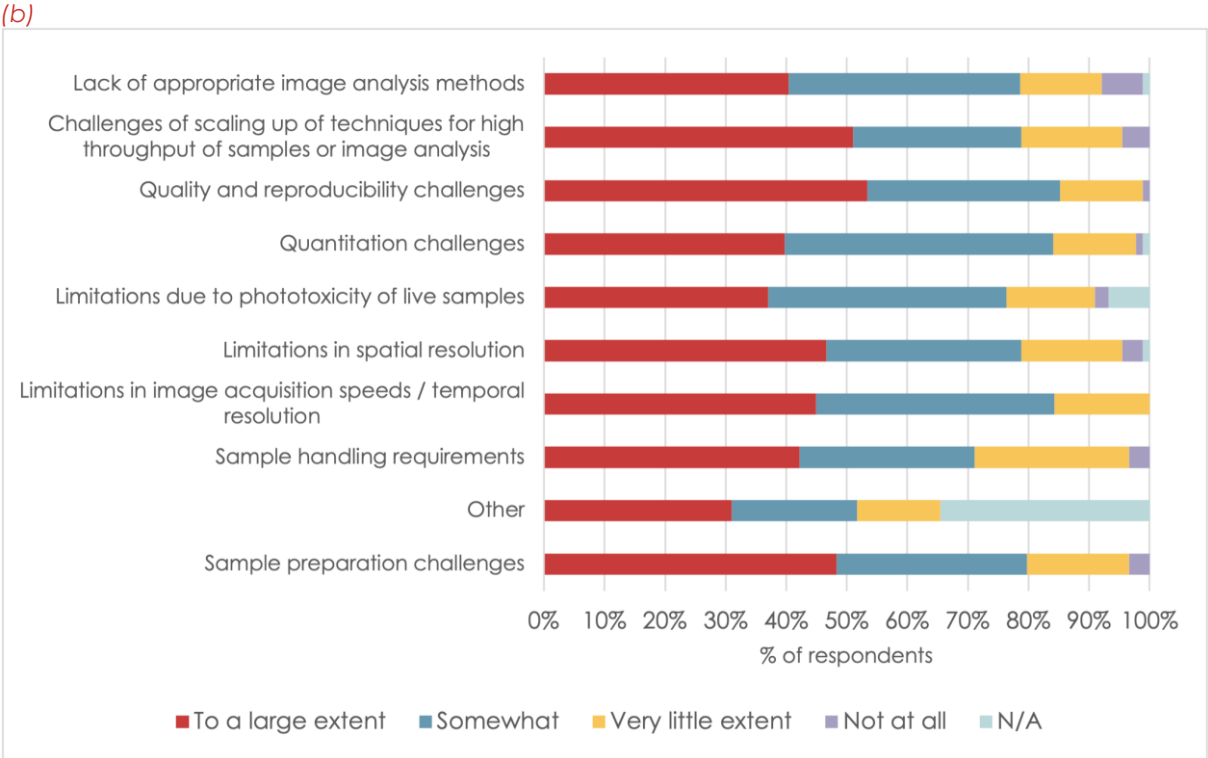
[54] Marqués G, Pengo T, Sanders MA. Imaging methods are vastly underreported in biomedical research. Elife. 2020;9:e55133. Published 2020 Aug 11. doi:10.7554/eLife.55133

Figure 8 The extent to which scientific or technological barriers limit progress in (a) High-income countries (n=261) and (b) Low- and middle-income countries (n=91)

(a)



(b)



Source: Online survey

- **Sample preparation and handling challenges**

Sample preparation remains a major bottleneck for many bioimaging projects. For LMICs, this is one of the top challenges to address in the next 5 to 10 years.

Depending on the availability of standard protocols and the imaging modality, **lengthy optimisation processes** may be required to get samples ready for imaging, hindering widespread adoption of some techniques. For example, challenges related to optimising the freezing conditions of samples on microscopy grids has become a barrier in the cryo-EM pipeline.[55,56] EM sample preparation in general requires a lot of optimisation and specialised expertise, which is often provided by core EM facilities or labs. The visualisation of the 3D structure of cells, tissues and small organisms is also being impeded by sample preparation challenges for techniques such as light sheet fluorescence microscopy and volume EM.[57,58,59] The **lack of standardised protocols** for sample preparation of pathogenic organisms was noted as a key challenge by some interviewees, while one stakeholder reported that the application of single molecule localisation microscopy is limited as the sample preparation protocol is not optimised.

The adoption of super-resolution microscopy techniques capable of nanoscale resolution has revealed that technical advances can bring new sample preparation challenges. Standard sample fixation methods developed for use with conventional microscopes have been shown to create cellular artefacts when viewed at nanoscale resolution (e.g. loss of proteins, clustering of receptors and disruption of cell cytoskeleton).[60] Furthermore, the increasing interest in combining multiple imaging modalities to gain a complete biological picture has created the need for integrated sample preparation methods that are compatible and comparable across modalities.[61,62] The bioimaging community is actively publishing new sample preparation methods that are helping to lower the entry barrier for different imaging modalities, however the diversity of sample preparation methods can make it difficult to determine the most appropriate method.

As regards correlative and multimodal imaging, interviewees highlighted the need for sample preparation protocols that allow transfer and imaging of samples across different types of imaging systems. For example, integrating across nm to cm scales will need different samples e.g. one at a cell level and another at organ or organism level. Workflows and platforms need to be adapted accordingly after considering the logistics of transfer and sample handling requirements. For example, the lack of an optimised protocol to maintain freezing conditions when transferring samples from Cryo-EM to FIB-SEM is limiting use of these techniques. More

[55] Weissenberger G, Henderikx RJM, Peters PJ. Understanding the invisible hands of sample preparation for cryo-EM. Nat Methods. 2021;18(5):463-471. doi:10.1038/s41592-021-01130-6

[56] Xu Y, Dang S. Recent Technical Advances in Sample Preparation for Single-Particle Cryo-EM. Front Mol Biosci. 2022;9:892459. Published 2022 Jun 24. doi:10.3389/fmolb.2022.892459

[57] Weiss KR, Voigt FF, Shepherd DP, Huisken J. Tutorial: practical considerations for tissue clearing and imaging. Nat Protoc. 2021;16(6):2732-2748. doi:10.1038/s41596-021-00502-8

[58] Kievits AJ, Lane R, Carroll EC, Hoogenboom JP. How innovations in methodology offer new prospects for volume electron microscopy. J Microsc. 2022;287(3):114-137. doi:10.1111/jmi.13134

[59] Vieites-Prado A, Renier N. Tissue clearing and 3D imaging in developmental biology. Development. 2021;148(18):dev199369. doi:10.1242/dev.199369

[60] Dankovich TM, Rizzoli SO. Challenges facing quantitative large-scale optical super-resolution, and some simple solutions. iScience. 2021;24(3):102134. Published 2021 Feb 3. doi:10.1016/j.isci.2021.102134

[61] Ando T, Bhamidimarri SP, Brending N, et al. The 2018 correlative microscopy techniques roadmap. J Phys D Appl Phys. 2018;51(44):443001. doi:10.1088/1361-6463/aad055

[62] Heiligenstein X, Lucas MS. One for All, All for One: A Close Look at In-Resin Fluorescence Protocols for CLEM. Front Cell Dev Biol. 2022;10:866472. Published 2022 Jun 30. doi:10.3389/fcell.2022.866472

complex and costly requirements can create barriers to adoption as is the case with cryo-EM in LMICs. Sample handling requirements such as requirements for low temperature storage, biosafety, etc. are a barrier to a much greater extent in LMICs than in HICs (Figure 8).

- **Limitations in spatial and temporal resolution**

Improvement of spatial and temporal resolution is another key challenge, as is increasing the scale and speed at which samples are processed. Over 90% of the stakeholders surveyed agreed that limitations in spatial and temporal resolution were barriers to progress in the bioimaging field at least to some extent.

Several interviewees concurred that improvements in spatial resolution need to be matched with improvements in temporal resolution to allow cellular activity to be captured in real time. Moreover, higher spatial resolution should be combined with larger fields of view to understand morphology and context in the biological system. There is the question of biological interpretability (also organism level interpretability) considering biological context of what is being imaged.

There is a trade-off between high spatial and temporal resolution and image depth. Super-resolution microscopy techniques offer a promising route to combine ultra-high resolution with live specimen imaging.[63] One major drawback of super-resolution microscopy is the difficulty to form a correct image through thick biological samples – a problem that is encountered in many bioimaging techniques. Furthermore, super-resolution microscopy requires high light intensities and long image acquisition times that presents two challenges.[64] [65,66] The first is phototoxicity where light overexposure can cause cells to behave abnormally or die. The second is photobleaching of fluorescent proteins used to label and track cells during biological processes, which limits the timeframe of experiments.

- **Fluorescent labelling**

In addition to the problem of photobleaching, fluorescent proteins used to label targets of interest for fluorescence microscopy can lead to undesired alterations of cellular activity due to their large and hydrophobic nature. Another limiting factor is the ability to multiplex only up to 5 different fluorescent proteins simultaneously to follow multiple cellular targets[60] as it is difficult to find fluorescent proteins that have distinct fluorescent signals under similar imaging conditions. Thus, there is a need for a larger range of probes for multiplexing that are sensitive and do not compromise cellular integrity. For example, in the EM field, there are limited numbers of suitable markers and DNA and RNA probes. Furthermore, alternative labelling techniques such as immunolabelling can compromise cellular morphology and cytochemical staining only allows for detection of one marker.

- **Other scientific and technical challenges**

In the context of live cell studies, survey respondents and interviewees noted challenges with phototoxicity due to light exposure, which are exacerbated when long exposure or image acquisition times are required. In the EM field, there is a dearth of suitable markers and

[63] Schermelleh L, Ferrand A, Huser T, et al. Super-resolution microscopy demystified. Nat Cell Biol. 2019;21(1):72-84. doi:10.1038/s41556-018-0251-8

[64] Wu Y, Shroff H. Multiscale fluorescence imaging of living samples. Histochem Cell Biol. 2022;158(4):301-323. doi:10.1007/s00418-022-02147-4

[65] Bon P, Cognet L. On Some Current Challenges in High-Resolution Optical Bioimaging. ACS Photonics. 2022;9(8):2538-2546. doi:10.1021/acsphotonics.2c00606

[66] Dankovich TM, Rizzoli SO. Challenges facing quantitative large-scale optical super-resolution, and some simple solutions. iScience. 2021;24(3):102134. Published 2021 Feb 3. doi:10.1016/j.isci.2021.102134

DNA/RNA probes – immunolabelling can comprise morphology and cytochemical staining only allows one marker.

3.2 Infrastructural barriers

- **High costs of equipment/infrastructure**

The impact of the high costs of equipment/infrastructure is huge in both HICs and LMICs (Figure 9); the magnitude of this barrier appears to be much greater in LMICs with almost 90% of LMIC stakeholders reporting in the survey that it affected them to a large extent. Therefore, it is unsurprising that stakeholders asked for this barrier to be addresses as a priority.

The costs being discussed include both costs to acquire and maintain expensive instruments/equipment as well as costs to build and maintain bioimaging facilities or infrastructure. The high cost of bioimaging instruments can be a **barrier to availability** of certain imaging techniques, especially in LMICs (e.g. cryo-EM, light sheet microscopy). For LMICs, the lack of availability of appropriate bioimaging equipment/facilities is the main barrier to progress in bioimaging. There is very limited equipment available, in particular advanced microscopes.

A few interviewees explained that techniques such as single molecule microscopy are restricted to a small number of labs as the instrument cost is very high. Adoption of techniques such as MRI, cryo-EM and hyperspectral imaging has been slow in LMICs because of the high costs involved. Manufacturers often charge high prices for technologies where there are few competing suppliers. Government funding often goes to 'big ticket' items rather than basic equipment, maintenance contracts or staff. The size of equipment maintenance contracts and the lack of funding streams to cover service contracts or system upgrades as well as personnel for delivering imaging services and advice puts long-term sustainability of infrastructure in jeopardy. These challenges are experienced in both HICs and LMICs.

One UK-based researcher commented that the discontinuation of Wellcome Trust Infrastructure grants has left a gap in the landscape while a South Africa-based researcher highlighted that because there is **limited regional funding to purchase bioimaging instruments**, often only multipurpose instruments, for example, microscopes that can be modified to have additional functionality are bought. However, additional funds are then required for the customisation, which may then not be forthcoming.

- **Lack of availability of appropriate technical expertise**

The unavailability of appropriate technical expertise has a large impact in both HICs and LMICs, although the effect is felt more keenly in LMICs (Figure 9). Addressing this problem was among the top 3 infrastructural barriers that stakeholders wanted to tackle as a priority.

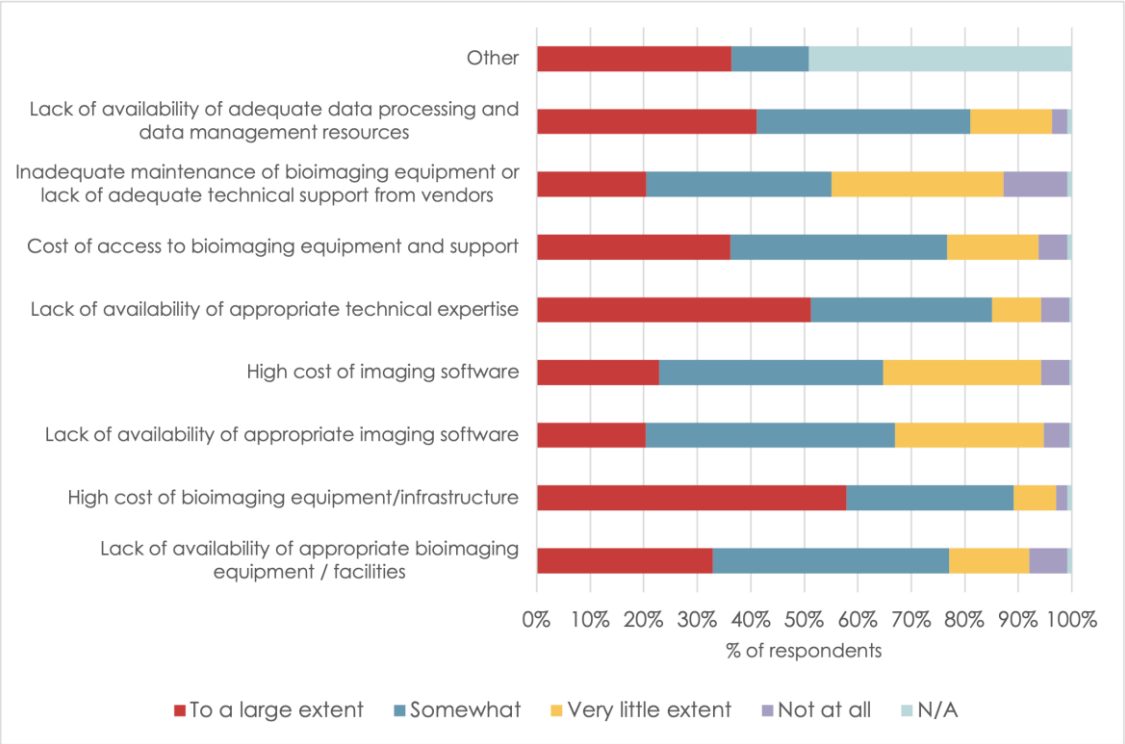
Multiple factors underpin the barriers related to lack of appropriate technical expertise. Firstly, there is a lack of enough trained personnel with the relevant bioimaging expertise to run and maintain infrastructure/equipment, which makes recruitment difficult. Secondly, retaining expertise once individuals have been recruited or trained can be difficult owing to lack of permanent positions/career paths, competitive salaries and recognition. Finally, even if expertise is available, accessing it can be a problem. Lack of appropriate technical expertise can also lead to inefficient utilisation of existing facilities/methods.

- **Access to specialised bioimaging equipment and facilities**

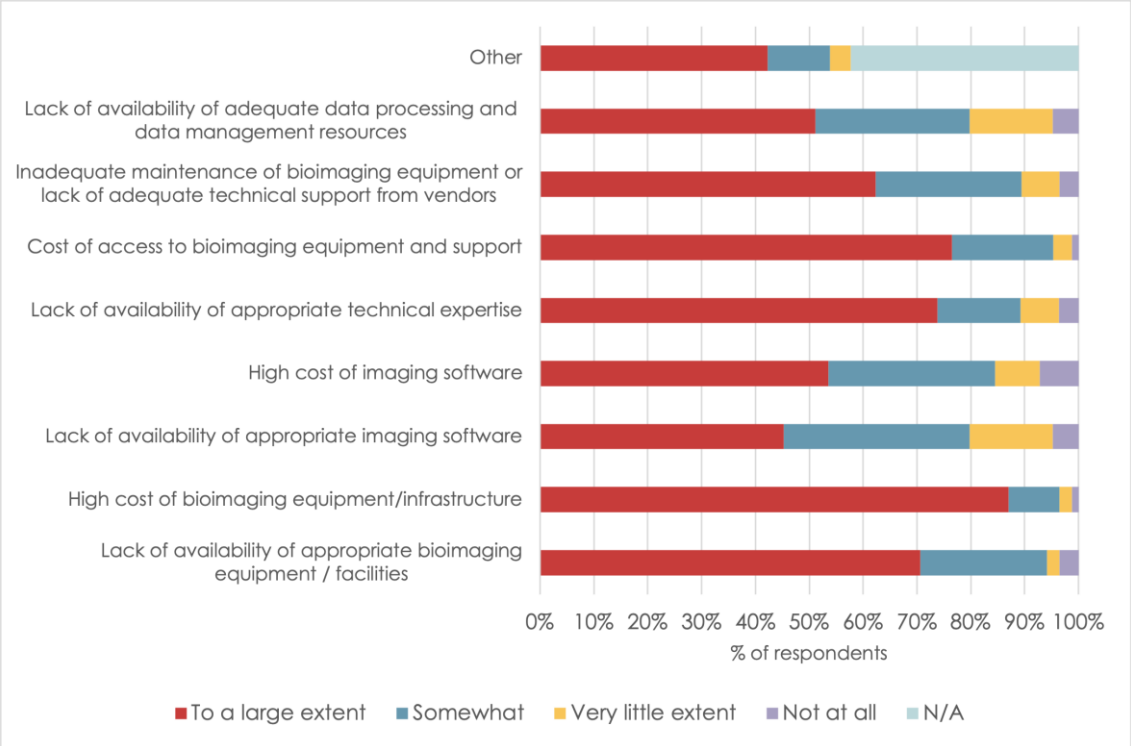
Access is dependent on the availability of facilities/equipment as well as relevant expertise locally or regionally. Availability is the first hurdle in most LMICs and accessing facilities/expertise

Figure 9 The extent to which infrastructural barriers limit progress in (a) High-income countries (n=245) and (b) Low- and middle-income countries (n=86)

(a)



(b)



Source: Online survey

when they are available is the second hurdle. The latter is influenced by location (i.e. distance) as well as costs of access/use.

Lack of availability of trained personnel also affects access to and use of available bioimaging infrastructure. This situation is exacerbated by the fact that there is limited funding for research infrastructure worldwide (including in the UK, Uruguay, South Asian and African regions). Many stakeholders emphasised that smaller research groups (even in HICs) can struggle to get access to state-of-the art imaging technologies through competitive selection processes. Interviewees from HICs and LMICs commented that state-of-the-art bioimaging equipment available in some institutions and labs is not accessible by external users (either because there is lack of awareness or it is only for internal users), which can result in equipment being underutilised although there is potential for other research groups to make use of it.

For imaging facilities that are run on a cost-recovery model, both in HICs and LMICs, certain specialised or complex techniques have high access fees owing to infrequent use or higher instrument times, which creates further barriers to use. LMICs in particular have a 'technology gap' owing to unavailability of the latest imaging technologies more locally, in terms of both equipment and expertise, which results in greater use of traditional microscopy techniques such as confocal microscopy.

Some UK-based interviewees highlighted volume EM and FIB-SEM as imaging modalities that have not quite realised their potential as they are expensive to access and only available in a limited number of facilities. One stakeholder highlighted PET as a technique that offers high sensitivity and allows researchers to follow metabolic processes in real-time but is not widely available. It is further limited by a lack of availability of radiochemistry infrastructure and radioactive tracers.

Researchers working in the infectious diseases area mentioned not being able to fully exploit cutting-edge bioimaging technologies (e.g. cryo-EM and light sheet microscopy) because experiments need to be conducted under containment or with special biosafety procedures. In the UK, only a few cutting-edge bioimaging facilities (e.g. Francis Crick Institute, Pirbright Institute) have adapted set ups to use microscopes safely with infectious organisms.

Access to state-of-the-art imaging facilities is in high demand. However, large scale imaging projects are creating a logistical bottleneck for imaging facilities. These projects present a challenge where nanoscale resolution is needed over an area that is substantially larger than the field of view of the microscope, which can result in experiments requiring days to months of continuous imaging on multiple machines.[67,68] Achieving high throughput for large scale projects is also hindered by lack of automated microscopes to function unsupervised outside of working hours.[67] Therefore, there is a need for automated microscopes that can acquire images of larger areas faster.

Adoption of new or improved imaging instruments may be hindered by a lengthy commercialisation process before they can be deployed in the bioimaging community.[69,73] In some cases, researchers skilled in custom-fitting commercial microscopes can develop new features faster, however even when customisation details are published, the new features remain accessible mostly to the original developers and close collaborators.[67]

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[67] Overcoming the challenges of large-scale electron microscopy (2020). Available here.

[68] Dankovich TM, Rizzoli SO. Challenges facing quantitative large-scale optical super-resolution, and some simple solutions. iScience. 2021;24(3):102134. Published 2021 Feb 3. doi:10.1016/j.isci.2021.102134

[69] Weber, M., & Huisken, J. (2021). Multidisciplinarity Is Critical to Unlock the Full Potential of Modern Light Microscopy. Frontiers in cell and developmental biology, 9, 739015. https://doi.org/10.3389/fcell.2021.739015

- **Lack of availability of adequate data management and processing infrastructure and expertise**

Lack of access to or availability of data processing and management resources is a key barrier for both LMICs and HICs. However, it is a higher priority for HICs than LMICs. There is also a lack of use/development of open-source software that could potentially lower the data processing barrier.

New advancements in bioimaging (e.g. light sheet microscopy, volume EM) have led to an explosion in the volume and complexity of imaging data being produced at the multi-petabyte scale, creating a challenge in terms of the computing infrastructure required to store, annotate and manage large imaging data files as well as the innovative, often custom-made, software tools required for image analysis. [70,71] **Inadequate data infrastructure and computing power** that does not match the size and complexity of the imaging data generated is a key barrier to data processing, not only in LMICs, but also for some HIC institutions. Often LMIC-based scientists who access HIC bioimaging facilities for state-of-the-art facilities and methods cannot process their data in their own country.

Stakeholders highlighted the need for advances in software to analyse large complex bioimaging datasets that are fast, automated, user friendly and generalisable to different research questions to allow novel biological insights to be obtained. Specific data analysis challenges cited included overlaying images, nuclear and cell segmentation and identification of objects of interest. Another problem is that the best image analysis software is often not open source and can be costly, which can put some of the these out of reach for resource-poor labs and countries. Moreover, software maintenance needs to be considered not just software development.

Data integration challenges i.e. combining datasets from different imaging modalities or combining bioimaging data with datasets from other fields e.g. 'omics' was highlighted as a major bottleneck slowing down scientific progress. This is partly due to the lack of agreed metadata standards and also due to lack of robust protocols to combine and analyse large complex bioimaging datasets. There is a pressing need for multimodal deep learning (DL) methods that can integrate data sources from different imaging modalities and other biological data sources (e.g. genomic, proteomic, metabolomic, or other 'omic' data) to enable new biological insights.[61,72] However, integration of bioimaging data is lagging behind due to the lack of common data formats, whereas other fields such as genomics have standardised data formats (e.g. FASTQ).

While DL methods have the potential to improve the speed, cost and accuracy of bioimage data analysis, they also come with limitations. Successful DL approaches rely on the availability of high-quality pre-curated training datasets, which are very time-consuming or expensive to create as they require manual curation of hundreds to thousands of images.[73] This is particularly challenging for 3D imaging datasets or if the biological sample is dynamic and rapidly changing. If the training dataset is inadequate for the desired task, inaccurate results will be obtained that are difficult, if not impossible, to detect without the basis of an original image.

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[70] Ouyang W, Zimmer C: The imaging tsunami: Computational opportunities and challenges. Curr. Opin. Syst. Biol. 2017; 4: 105–113.

[71] https://france-bioimaging.org/node/bioimage-informatics/

[72] Jung YL, Kirli K, Alver BH, Park PJ. Resources and challenges for integrative analysis of nuclear architecture data. Curr Opin Genet Dev. 2021;67:103-110. doi:10.1016/j.gde.2020.12.009

[73] Belthangady C, Royer LA. Applications, promises, and pitfalls of deep learning for fluorescence image reconstruction. Nat Methods. 2019;16(12):1215-1225. doi:10.1038/s41592-019-0458-z

Another challenge is the 'black box' nature of DL methods making it difficult for researchers to understand how decisions are made and therefore interpret the reliability of results.[41,43] Improved transparency and understanding of the limitations and potential pitfalls when analysing and interpreting biological data derived from ML methods is needed before they can be trusted and widely adopted.[40] Furthermore, DL tools are often developed by early career researchers who move on to other projects and therefore tools are not maintained and become redundant.[73] Finally another challenge concerns generality of DL methods so that they can be applied to other studies.

There was also high demand for effective approaches and tools for automation of data processing among stakeholders in both the survey and interviews. An AI model repository for DL, Bioimage.IO, is available but it is not fully used and needs further development.

3.3 Other barriers and challenges

- **Career development and talent retention**

Imaging and data scientists working with bioimaging equipment and data do not have separate career paths or permanent positions in most countries, making it difficult to retain talent in the field of bioimaging over a long time and thus leading to regular loss of institutional knowledge.[74,75] **Lack of career pathways** for technical staff and data scientists working in bioimaging is an important barrier to progress in bioimaging in both LMICs and HICs, with over 50% of survey respondents claiming it affects progress 'to a large extent' (see Figure 10). As such, it was also chosen as a barrier that needs to be addressed as a priority in the coming years.

Many interviewees (from HICs and LMICs) provided examples of '**brain drain**' to industry, especially for PhD graduates and postdoctoral scientists, as universities and institutes cannot compete with the higher salaries offered in the private sector. This also leads to recruitment challenges in terms of employing trained personnel with the requisite skill set (particularly data scientists and imaging technologists). An inability to retain skills and experienced staff also prevents critical mass being reached in some LMICs and limits the use of certain cutting-edge technologies, leaving expensive equipment underutilised.

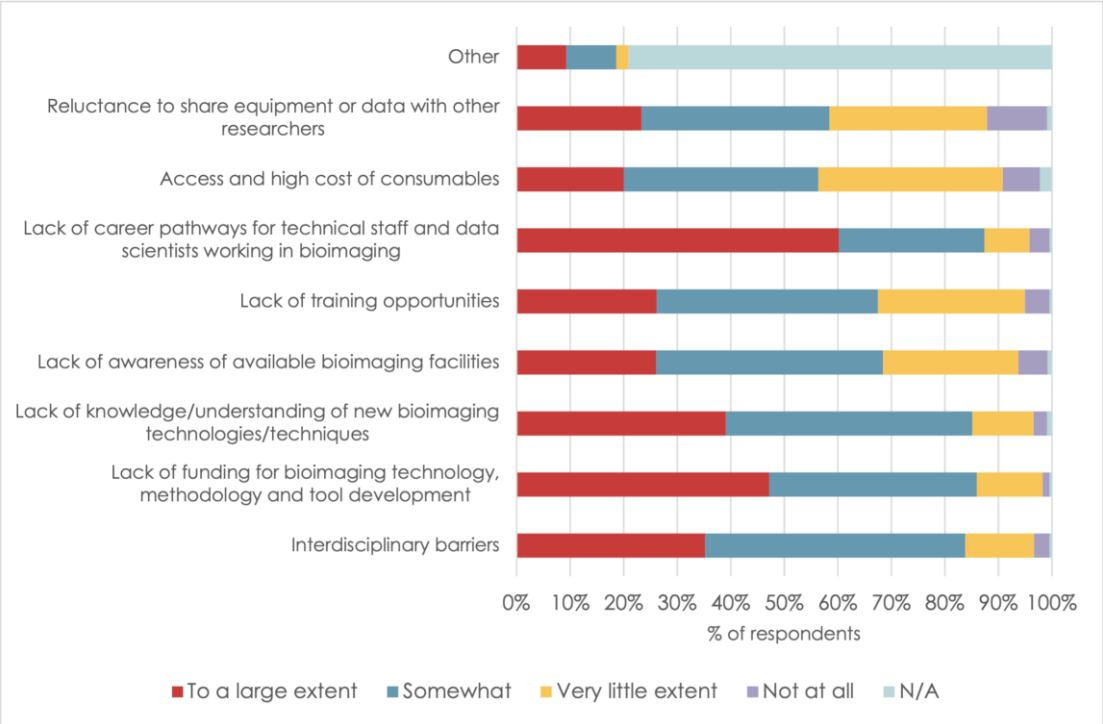
Furthermore, the skills of core facility staff often go unrecognised. For instance, they rarely are first or last authors on academic papers and have to have provided significant scientific input to be included in the first place.

[74] Ravindran S. Core curriculum: learning to manage a shared microscopy facility. Nature. 2020 Dec;588(7837):358-360. DOI: 10.1038/d41586-020-03466-z. PMID: 33293714.

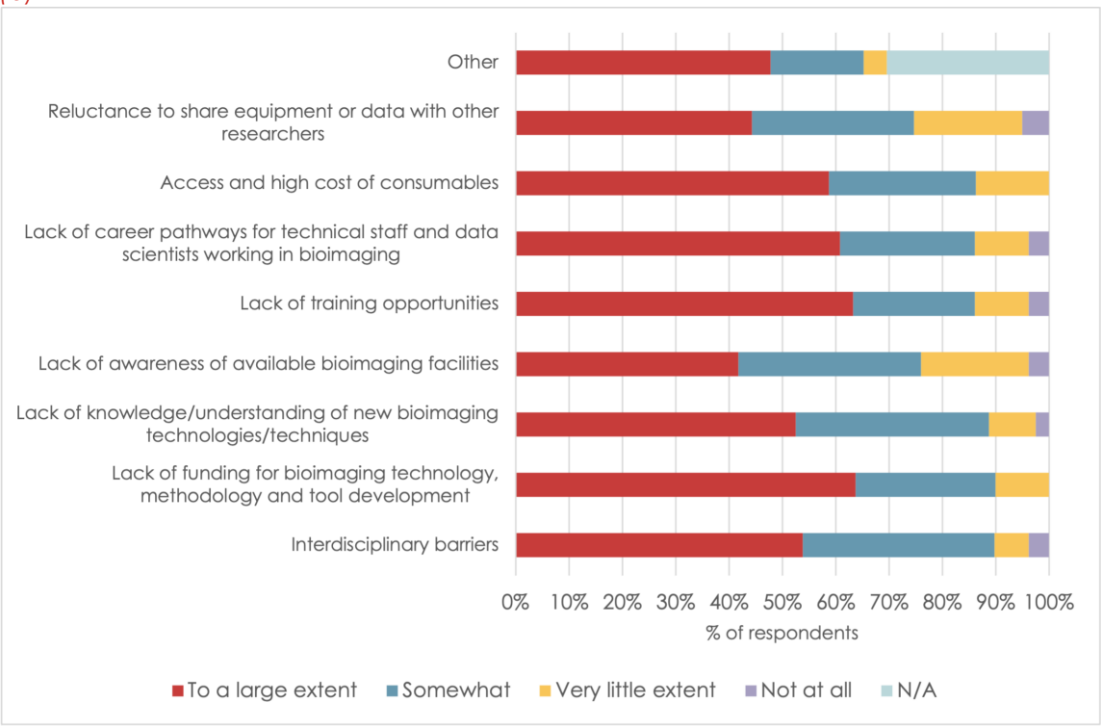
[75] Adami V, Homer N, Utz N, Lippens S, Rappoport JZ, Fernandez-Rodriguez J. An international survey of Training Needs and Career Paths of Core Facility Staff [published online ahead of print, 2020 Nov 20]. J Biomol Tech. 2020;jbt.2021-3201-002. doi:10.7171/jbt.2021-3201-002

Figure 10 The extent to which other barriers limit progress in (a) High-income countries (n=241) and (b) Low- and middle-income countries (n=80)

(a)



(b)



Source: Online survey

- **Interdisciplinary challenges**

Interdisciplinary barriers were reported to have a large impact in LMICs, with both LMIC and HIC stakeholders asking for this barrier to be addressed as a priority.

Modern microscopy requires a diverse skill set that necessitates close collaboration between different disciplines and integration of insights from different scientific fields[69,99,76] For example, an imaging scientist can help a biologist to design a microscopy protocol, while a biochemist may be needed to design specialised fluorophores. Engineers are instrumental in streamlining and developing new microscopes, while computer scientists can design data tools to integrate and analyse data sources from across imaging modalities and fields. However, there is **inadequate communication between different stakeholder groups** (e.g. developers and users, biologists and computer scientists) such that technology development for bioimaging is not always accessible or relevant to user needs – a problem highlighted in our stakeholder consultations. For example, disconnects between bioimaging tool developers and biologists can lead to tools being underutilised as researchers do not understand their importance or cannot adapt them to their research questions.[73] Furthermore, **cultural and technical differences between disciplines** can be a barrier to development and adoption of novel bioimaging technologies and methodologies. Bioimaging pipelines and techniques developed to answer research questions in one research field could in principle benefit other research fields, but such knowledge transfer is not happening. More needs to be done to support cross-fertilisation between disciplines, but it can be challenging to bring expertise other than bioimaging or life science expertise together and funding for such activities is not readily available.

- **Lack of knowledge or understanding of bioimaging technologies/techniques**

The rapid development of imaging techniques and technical breakthroughs makes it difficult for the bioimaging community to stay up-to-date with the latest microscopy advances and assess their suitability for specific imaging experiments.[77] In LMICs, lack of knowledge of new bioimaging techniques was reported to have a large impact (see Figure 10).

Lack of knowledge and awareness of different bioimaging techniques and what they can do can lead to poor utilisation of existing facilities and restricted uptake of novel technologies and methods, with researchers preferring to stick with imaging techniques they know. For example, multiphoton microscopy is a powerful technique for live cell imaging; however according to one interviewee, it has not been adopted widely as researchers do not understand how to use it. There is therefore a demand for well-designed training courses and programmes to meet community needs.[78] Many existing bioimaging courses are oversubscribed e.g. EMBL courses are typically oversubscribed by 50% to 300%.[77] Moreover, the most useful training courses i.e. ones that provide hands-on experience can be very expensive (e.g. subsidised fees for confocal microscopy training courses are around £2000) and difficult to scale. There is also a lack of suitable training materials and reference textbooks for the required bioimaging, computing and engineering skills, which are generally not taught in conventional biology courses.[79] Thus, standardised and equitable access to training needs to be considered.

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[76] Schlaeppi A, Adams W, Haase R, et al. Meeting in the Middle: Towards Successful Multidisciplinary Bioimage Analysis Collaboration. Front Bioinform. 2022;2:889755. doi:10.3389/fbinf.2022.889755

[77] Colón-Ramos DA, La Riviere P, Shroff H, Oldenbourg R. Transforming the development and dissemination of cutting-edge microscopy and computation. Nat Methods. 2019;16(8):667-669. doi:10.1038/s41592-019-0475-y

[78] Biotechnology and Biological Sciences Research Council (2018). Strategic Review of Bioimaging. Available here.

[79] Driscoll MK, Zaritsky A. Data science in cell imaging. J Cell Sci. 2021;134(7):jcs254292. Published 2021 Apr 1. doi:10.1242/jcs.254292

Imaging core facility staff are seen as key knowledge brokers, instrumental in providing experimental scientists with advice, support and training in all aspects of the bioimaging workflow (e.g. choice of imaging modality, experimental design, operating the equipment, through to data analysis and access to data management and analysis resources).[82,80] However, core facility staff positions are often not permanent. Moreover, there is a need for more dedicated training programmes for core facility staff on the latest bioimaging advances so that they can diffuse this knowledge into the wider bioimaging community (train the trainer model).[78]

Other barriers that contribute towards lack of knowledge and understanding of bioimaging techniques are lack of funding for training users and advising on experiment planning and assay development; inadequate technology transfer from HICs to LMICs, and lack of partnerships to exploit technologies.

- **Bioimaging data accessibility and reuse**

As bioimaging enters into an era of 'big data' this has brought new challenges with regards to storage, curation, and distribution of bioimaging data. **Access to open data repositories** that centralise and standardise bioimaging datasets creates opportunities for the wider bioimaging community to reuse and reanalyse data, alone or in combination with other data sets, to answer a plethora of new biological questions, as well as promote openness and reproducibility. Many interviewees highlighted the need for accessible, unified centralised data repositories at national/international level.

A **lack of common/agreed metadata standards to deposit raw bioimaging** data was cited as a barrier limiting the reusability of bioimaging data by several interviewees, although it was noted that some bioimaging modalities (e.g. electron microscopy) already have good metadata standards. The large amount of time required for metadata curation was cited as another barrier to depositing data in repositories. As such, these challenges imply that having data repositories and simple access to raw images is not enough, and metadata curation and annotation are key bottlenecks as these require a large amount of manual effort.

Implementation of FAIR (findable, accessible, interoperable, and re-usable) principles is considered a prerequisite for maximising the reuse of bioimaging data.[81] Findings from a recent survey (2021) that assessed the **adoption of FAIR principles** in the bioimaging community (mainly in Germany) highlighted insufficient metadata guidelines and annotation tools for bioimaging as a key barrier to the adoption of FAIR principles.[82] Lack of guidance on appropriate data repositories and insecurity about the legal aspects of data sharing were cited as other key barriers.

A funding gap was identified in the interviews around the concept of 'making data fair'. Enabling effective management, analysis, sharing and reuse of data is expected to transform bioimaging. However, trust needs to be built before researchers will be willing to share their data for reuse. In addition, agreements on how to cite reused data or on co-authorship of resulting publications will be needed.

- **Research funding gaps**

[80] European Molecular Biology Laboratory (EMBL) (2021) Review of the impacts of EMBL experimental services. Available here.

[81] Wilkinson, M., Dumontier, M., Aalbersberg, I. et al. The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018 (2016). https://doi.org/10.1038/sdata.2016.18

[82] Schmidt C, Hanne J, Moore J et al. Research data management for bioimaging: the 2021 NFDI4BIOIMAGE community survey [version 2; peer review: 2 approved]. F1000Research 2022, 11:638

There is a **lack of dedicated funding** for projects that take nascent bioimaging technologies, methodologies and tools from concept to design, including for commercial projects according to interviewees. Lack of funding for technology and methodology development was also cited as a priority barrier for both LMICs and HICs in the survey (Figure 10).

Current funding schemes are extremely competitive and predominantly focus on achieving impacts on human health. Not enough is done to invest in academics to develop spin-off companies (e.g. in the field of microscopy) or for bioimaging services. There is also a lack of funding for alternative technology development projects such as open hardware initiatives, which requires significant investment to improve the ecosystem of companies that can take a design to a prototype.

4 Solutions to address barriers and challenges in bioimaging

In this chapter, we discuss ongoing efforts and suggested solutions for mitigating barriers and challenges affecting development, adoption and democratisation of bioimaging technologies and techniques. A key point to note is that most solutions are applicable to both HICs and LMICs.

4.1 Addressing scientific and technical barriers

- **Quality and reproducibility challenges**

Suggested solutions for mitigating quality and reproducibility challenges included better development, reporting and sharing of methods and protocols so that experiments can be repeated by others. **Common, agreed standards** need to be established for experimental protocols, sample preparation, publishing data analysis as well as quality control. These standards should be adopted and promoted by publishers and funders.

A global microscopy-user community survey initiated by the European Light Microscopy Initiative (ELMI) in 2019 (across ~200 imaging labs) highlighted inconsistencies when it comes to choosing which microscope Quality Control metrics to record and how frequently they are performed.[83] In 2020, the **QUality Assessment and REProducibility for instruments and images in Light Microscopy (QUAREP-LiMi) initiative** was established, which comprises of imaging scientists from academia and industry who share a common goal to improve the performance and limitations of microscopes and improved Quality Control in light microscopy.[83]

- **Fluorescent probes**

Label-free imaging techniques such as phase-contrast and quantitative phase imaging techniques are being developed to overcome challenges of photobleaching and undesired alterations of cellular activity associated with fluorescent proteins. However, these techniques currently lack resolution and molecular specificity.[64] An emerging application area is the use of **DL in label-free imaging**. DL has been shown to predict labels from other less expensive types of microscopy techniques (e.g. transmitted-light microscopy), however this is currently limited to a small number of labels and not widely used.[43]

The recent development of **fluorescent nanoparticles** is expected to become a powerful alternative to traditional fluorescent proteins used in super-resolution microscopy owing to their small size and high photostability.[84] On the other hand, stimulated Raman scattering is an emerging technique that has enabled multiplexing of vibrational probes to visualise over ten colours in biological samples.[85] The vibrational colour palette has potential to be further expanded if chemical synthesis challenges can be overcome. Thus, this technique has the potential to remove the limitations around the number of fluorescent proteins that can be multiplexed simultaneously. However, further studies are needed to validate the biocompatibility of the technique.

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[83] Nelson G, Boehm U, Bagley S, et al. QUAREP-LiMi: A community-driven initiative to establish guidelines for quality assessment and reproducibility for instruments and images in light microscopy. J Microsc. 2021;284(1):56-73. doi:10.1111/jmi.13041

[84] Li W, Kaminski Schierle GS, Lei B, Liu Y, Kaminski CF. Fluorescent Nanoparticles for Super-Resolution Imaging. Chem Rev. 2022;122(15):12495-12543.

[85] Qian N, Min W. Super-multiplexed vibrational probes: Being colorful makes a difference. Curr Opin Chem Biol. 2022;67:102115. doi:10.1016/j.cbpa.2021.102115

- **Other scientific and technical challenges**

In the survey, automated microscopy protocols were suggested as a way to decrease the phototoxic impact of imaging on a sample. Similarly, better support for sample preparation method development and better sharing of these methods was proposed to tackle sample preparation challenges.

4.2 Addressing infrastructural barriers

- **Improving access to bioimaging technologies and techniques**

Interventions to promote access are the only ones that will particularly require tailoring to national or regional context. In LMICs, availability of infrastructure, equipment and expertise is the main barrier to access, and this aspect will need to be accounted for when designing solutions to improve access. For example, establishing key technologies or infrastructure in LMICs or providing access to state-of-the-art technologies in HICs for LMIC-based researchers may have to be considered.

National imaging facilities or core microscopy services in research institutions can help improve access to and democratise bioimaging techniques. They can host expensive and bulky equipment and provide trained personnel to deliver bioimaging services. Some interviewees noted that centralised purpose-built facilities for bioimaging were perhaps a more efficient, equitable and sustainable way to provide access to a variety of bioimaging modalities to a large number of people than individual labs hosting bioimaging equipment. However, the advantages of having local imaging facilities were also highlighted in terms of lower travel costs, ease of transporting samples and quick iteration of experiments.

To improve access to specialised imaging instruments, expertise, training opportunities and data management services that life scientists might not find at their home institutions or among their collaborators, many countries and regions have developed **bioimaging networks**. For example, Euro-BioImaging,[86] a European network, spans 33 internationally renowned imaging facilities (nodes) that operate in 14 countries and the European Molecular Biology Laboratory (EMBL). It is coordinated by a hub and is the European landmark research infrastructure for biological and biomedical imaging as recognised by the European Strategy Forum on Research Infrastructures (ESFRI). There are similar networks in North America (BioImaging North America), Africa (African Bioimaging Consortium) and Latin America (Latin America Bioimaging), but with different reach and remit. These networks help to connect local research communities to bioimaging facilities and expertise, enabling sharing and more efficient use of existing capabilities.

An international network of imaging infrastructures called Global BioImaging brings all of these networks together, "*recognising that scientific, technical and data challenges are universal rather than restricted by geographical boundaries*".[87] It provides a unique forum for international discussion and cooperation to tackle the practical challenges as well as the strategic questions linked to operating open access infrastructures for cutting-edge imaging technologies in the life sciences. The Chan Zuckerberg Initiative (CZI) is investing in these community and capacity building activities and in 2021 provided dedicated funding to help expand access to bioimaging facilities for LMICs.[88] An HIC-based stakeholder reported how a

[86] https://www.eurobioimaging.eu/about-us/about-eubi

[87] https://globalbioimaging.org/

[88] NewsRoom Chan Zuckerberg Initiative (CZI) (2021) CZI Awards Over $5M to Advance Technologies and Expand Global Access to Bioimaging. Available here.

collaboration with researchers in South Africa to provide remote access to X-ray and electron imaging technologies is helping to build a local user base, which may help build a case for a regional centre in South Africa.

In Finland, there is a national imaging network across five different university cities. They have mapped bioimaging expertise and one unit in the network takes a lead in developing or establishing a specific technology. This leadership is closely coordinated to avoid duplication of effort and ensure most technologies are available across the network through open access. Despite these organisation- and individual-led initiatives, there is still room for further support diverse scientific communities to accelerate development or uptake of novel technologies and methodologies.

One suggested model to improve access to imaging infrastructure and expertise, whether in central or institutional facilities, is to fund **short-term mobility grants** to cover research visits lasting a few weeks to a few months. Individual stakeholders from both HICs and LMICs supported such a scheme which in their view would facilitate greater use of bioimaging technologies to answer novel research questions, more efficient use of resources as well as knowledge transfer and diffusion of new methodologies. Grants or funding pots to cover equipment procurement, core expertise, and maintenance and service contracts were also proposed. This kind of initiative may require multi-funder cooperation. Another suggestion was to have a mechanism for laboratories in HICs to donate older, working instruments to LMICs when they are replaced. The UK Engineering and Physical Sciences Research Council's (EPSRC's) Laser Loan Pool[89] initiative, which ran from 2005 to 2015, loaned equipment to researchers to conduct feasibility experiments prior to grant applications and was cited as an effective mechanism that could be adopted to increase access to imaging technologies.

To overcome barriers related to commercialisation that can delay some newly developed technologies and methodologies from being mainstreamed, many imaging facilities are becoming '**open innovation hubs**' where core facility staff collaborate with end users to develop novel imaging technologies, which can then be made accessible to the wider bioimaging community.[90] However, for imaging facilities to provide access to open innovation opportunities in addition to established bioimaging services, further capacity and resources are required.

4.3 Addressing other barriers

- **Funding**

Stakeholders put forward many ideas for funding programmes to help improve equitable access (democratisation) to bioimaging technologies and methods as well as supporting the development of new bioimaging technologies, methodologies and tools. The most common suggestion was to have **ringfenced funding for development work** as most current grants are focussed on potential for impact on human health rather than developing a new technique or method. The UK Biotechnology and Biological Sciences Research Council's (BBSRC's) 'Better methods, better research'[91] and 'Technology development for the biosciences' grants were given as good examples of grant programmes supporting method and technology development for the wider biosciences research community. The Chan Zuckerberg Initiative

[89] https://www.clf.stfc.ac.uk/Pages/The-Laser-Loan-Pool.aspx

[90] Lippens S, D'Enfert C, Farkas L, et al. One step ahead: Innovation in core facilities. EMBO Rep. 2019;20(4):e48017. doi:10.15252/embr.201948017

[91] https://www.ukri.org/what-we-offer/browse-our-areas-of-investment-and-support/better-methods-better-research/

has had grant schemes to advance technology development in specific areas e.g. visual proteomics, deep tissue imaging and dynamic imaging.

The second most common suggestion was for **infrastructure grants** – for buying new equipment and/or covering maintenance and core staff costs. Stakeholders suggested that such grants/funding could be coordinated at a national or regional level and involve multi-funder cooperation. The idea was that costs would be shared and there would be a few core facilities/labs maintaining and providing open access to specific bioimaging techniques, providing value for money for funders and more sustainable use of equipment.

Innovation grants, grants for commercialisation and scale up of nascent technologies and grants for data repositories, open-source software development and establishing common data standards to improve reuse and integration of data from different sources were also proposed.

Some stakeholders suggested having a 'rolling fund' for small grants to enable rapid access to emerging imaging technologies. These grants would facilitate adoption of these technologies and generation of data to secure future research funding.

- **Improving bioimaging data accessibility, reuse and integration**

National and international funded programmes to unify and harmonise bioimaging data are actively underway, including the creation of bioimaging **data repositories** and recommendations for bioimage **metadata standards**.[92] For example, BioImage Archive and OMERO (Microscopy Environment Remote Objects) are being established as centralised repositories for the bioimaging community to facilitate discoverability and reuse of bioimaging data.[93,94] Most recently, representatives from the light, electron and X-ray microscopy communities developed the Recommended Metadata for Biological Images (REMBI) guidelines.[95] Furthermore, a range of complementary tools for annotating and reporting metadata have been developed, including MetaData Editor for microscopy (MDEmic), Micro-Meta App and MethodsJ2.[96,97,98] However, there is a concern around the sustainability of public funded programmes to maintain bioimaging repositories and complementary tools.[68,99] Furthermore, sharing bioimaging data is often perceived as additional burden that relies on individual researchers.[99]

Open 'hardware' can be a good option for data reuse and accessibility in LMICs and HICs according to some interviewees, as it can reduce costs, facilitate linkage across bioimaging

[92] Ellenberg, J. et al. A call for public archives for biological image data. Nat. Methods 15, 849–854 (2018).

[93] Hartley M, Kleywegt GJ, Patwardhan A, Sarkans U, Swedlow JR, Brazma A. The BioImage Archive - Building a Home for Life-Sciences Microscopy Data. J Mol Biol. 2022;434(11):167505. doi:10.1016/j.jmb.2022.167505

[94] Burel JM, Besson S, Blackburn C, et al. Publishing and sharing multi-dimensional image data with OMERO. Mamm Genome. 2015;26(9-10):441-447. doi:10.1007/s00335-015-9587-6

[95] Sarkans, U., Chiu, W., Collinson, L. et al. REMBI: Recommended Metadata for Biological Images—enabling reuse of microscopy data in biology. Nat Methods 18, 1418–1422 (2021). https://doi.org/10.1038/s41592-021-01166-8

[96] Kunis, S., Hänsch, S., Schmidt, C. et al. MDEmic: a metadata annotation tool to facilitate management of FAIR image data in the bioimaging community. Nat Methods 18, 1416–1417 (2021). https://doi.org/10.1038/s41592-021-01288-z

[97] Rigano, A., Ehmsen, S., Öztürk, S.U. et al. Micro-Meta App: an interactive tool for collecting microscopy metadata based on community specifications. Nat Methods 18, 1489–1495 (2021). https://doi.org/10.1038/s41592-021-01315-z

[98] Ryan, J., Pengo, T., Rigano, A. et al. MethodsJ2: a software tool to capture metadata and generate comprehensive microscopy methods text. Nat Methods 18, 1414–1416 (2021). https://doi.org/10.1038/s41592-021-01290-5

[99] Schlaeppi A, Adams W, Haase R, et al. Meeting in the Middle: Towards Successful Multidisciplinary Bioimage Analysis Collaboration. Front Bioinform. 2022;2:889755. doi:10.3389/fbinf.2022.889755

scales and fill current gaps left by commercial entities. They felt that publishers and funders could set **obligations** to ensure researchers deposit bioimaging data. For example, the US National Institutes of Health requiring all research grants to have a data management and sharing plan was seen as a step in right direction.

There were also suggestions to improve training and share best practice on data reuse, analysis and integration among end-users.

- **Addressing interdisciplinary challenges**

Stakeholders indicated the need for more **cross-disciplinary and cross-sectoral dialogue and collaboration**. In particular, mechanisms for greater cooperation between developers and users towards building new technologies and tools that allow users to ask and answer new fundamental questions about life, health and wellbeing were requested. Stakeholders saw a possible role for Wellcome in facilitating this interdisciplinary communication, either through a new initiative or by bringing together Wellcome grant holders e.g. those working on bioimaging-related projects or those who may have an interest in using or developing bioimaging methods.

Many stakeholders highlighted the need for initiatives to bridge the gap between imaging and computer science fields to advance the development of user-friendly and generalisable image analysis software tools to solve data integration and analysis challenges. Examples of such interdisciplinary initiatives are NEUBIAS (Network of European Bioimage Analysts) and the Electrifying Life Sciences (ELS) project the Rosalind Franklin Institute (funded by Wellcome). NEUBIAS brings together software developers and life scientists to facilitate the use of image analysis in life sciences by designing image analysis workflows with scientific quality and automation.[100] The network also fosters the recognition and career development of Bioimage analysts. The ELS project aims to improve the capability and accessibility of cryo-EM with structural biologists and computer scientists collaborating to create analysis workflows that include correlative imaging and segmentation steps and allow 3D reconstruction of large datasets.[101]

- **Improving understanding and dissemination of bioimaging techniques**

Stakeholders suggested increasing **training opportunities** to promote awareness and adoption of new and existing bioimaging techniques. This could be through funding training platforms or mentoring and training programmes focused on bioimaging. One example of such a scheme is Africa Microscopy Initiative's Partners in Teaching (PiTCH) programme which pairs early career imaging scientists from Africa with an aspiring lecturer or research counterpart from other continents. Upon selection, the pairs are mentored by experienced international imaging scientists to develop and host a week-long microscopy training course at the affiliated African institute. EMBL training courses and Global Bioimaging "Exchange of Experience" meetings were also cited as good examples of training opportunities.

- **Career pathways for imaging and data scientists**

While separate career pathways for imaging and data scientists will be very helpful for retention and career progression of core staff, just acknowledging that their CV looks different than standard researchers and assessing their scientific capabilities and contributions accordingly for funding applications or promotions will go a long way to remedying the situation.

[100] https://f1000research.com/NEUBIAS

[101] https://www.rfi.ac.uk/projects/electrifying-life-sciences/

Permanent positions for core staff and allowing them to apply for R&D grants were other suggestions.

5 Conclusions and recommendations

5.1 Conclusions

5.1.1 The key bioimaging technologies/methodologies

The landscape review highlighted three general points with regard to the areas in which the next generation of bioimaging approaches will emerge in the near future. Firstly, that **integration** is required **across the scales of life** to gain deeper understanding of not only the structures and function of biological molecules but the wider biological contexts within which they operate. Secondly, formulation of new hypotheses and breakthrough in our understanding will be most effectively enabled by **combining diverse techniques and methodologies** such as in correlative microscopy or multi-modal imaging (or even combination of imaging and other techniques e.g. in spatial transcriptomics/proteomics) rather than a single bioimaging technique. Lastly, the significant role of **artificial intelligence (deep learning), big data and image analysis techniques** will play in supporting analysis, linkage of large datasets, as well as pushing bioimaging techniques forward.

Techniques that are currently transforming bioimaging and the use of which is expected to increase include

- Light sheet microscopy which has high spatiotemporal resolution and allows imaging of tissues and organoids rather than just sections
- Super-resolution microscopy which can provide molecular-level resolution or 3D and fast live-cell imaging
- Correlative microscopy and multi-modal imaging techniques that allow integration across the scales of life such as CLEM; in vivo imaging with light sheet microscopy; CLEM and X-ray microtomography; and electron imaging, X-ray imaging and Cryo-EM
- Cryo-EM and Volume EM which are powerful structural biology techniques – Cryo-EM is fast becoming a mainstream technique for structural biology, while volume EM allows high resolution imaging of large samples
- Ultra low-field MRI is expected to be rapidly adopted especially in resource-poor settings owing to its lower costs and portability

5.1.2 Challenges, barriers and gaps in the bioimaging field

Table 2 summarises the key barriers and challenges as well as proposed solutions to mitigate them.

Common scientific and technological barriers affecting several bioimaging techniques stem from inherent limitations of the imaging technology and complex requirements as regards specimens that can be imaged. These comprise data quality, reproducibility and quantitation; complex sample preparation requirements that may require lengthy optimisation and specialised expertise (e.g. in EM); cellular artefacts observed with standard sample fixation methods at high resolution; and the trade-offs between spatial and temporal resolution and image depth. Such barriers can hinder uptake and use of certain techniques and limit the scope of what can actually be visualised and interpreted with confidence.

High costs of equipment/infrastructure, access to infrastructure and imaging software along with lack of availability of appropriate technical expertise and data processing/analysis solutions prevent many researchers from accessing several bioimaging techniques. High cost and lack of infrastructure and technical expertise locally or regionally particularly affect access

in LMICs, especially to state-of-the-art and powerful techniques like cryo-EM and light sheet microscopy. Lack of appropriate infrastructure e.g. high pressure freezers for cryo-EM or biosafety requirements for infectious organisms can limit choice and use of specific techniques.

Furthermore, the increasing interest in correlative and multimodal imaging has created the need for integrated sample preparation methods that are compatible and comparable across modalities as well as integrated workflows and imaging platforms that can accommodate different sample sizes (e.g. when integrating across nm to cm scales). These approaches also create data integration challenges where datasets from different imaging modalities need to be combined.

Table 2 Summary of key challenges or barriers and proposed solutions to address these

Challenge/barrier	**Solutions and/or areas for further studies**
Quality and reproducibility challenges	Support initiatives aimed at improving the performance and quality control of instruments and data Common, agreed standards for experimental protocols, sample preparation, publishing data analysis
Sample preparation and handling challenges	Development and sharing of new methods and protocols Establishment of standardized protocols
High costs of equipment/infrastructure	
Access to bioimaging equipment and facilities	National or core imaging facilities to democratise access Short-term mobility grants for research visits Support for bioimaging networks Grants for equipment procurement, core expertise and maintenance
Funding	Funding for technology or method development Infrastructure and innovation/commercialisation grants
Lack of bioimaging data accessibility, reuse, and integration	Investment into bioimaging data repositories and computing infrastructure Support establishment of common metadata standards Support adoption of FAIR principles Open hardware Funders asking for data management and sharing plan
Interdisciplinary challenges	Support mechanisms for cross-disciplinary and cross-sectoral collaboration e.g. developers and users, imaging and computer science
Lack of availability of appropriate technical expertise / Lack of knowledge of or understanding of bioimaging techniques	Support more training opportunities and courses
Career development and talent retention challenges	Separate career pathway for imaging and data scientists with appropriate performance indicators Recognition through permanent posts, authorship on publications Ability to apply for own funding

Demand for high throughput imaging is creating logistical bottlenecks due to the amount of instrument time needed and automated microscopes and/or workflows are needed to optimise usage of equipment (e.g. data collection outside working hours). High throughput

techniques e.g. light sheet microscopy and volume EM also generate large and complex datasets for which appropriate computing infrastructure and image analysis methods are required. The latter represents a crucial gap in the bioimaging landscape along with lack of access to datasets for reuse. Common agreed metadata standards and implementation of FAIR (findable, accessible, interoperable, and re-usable) principles are keenly required.

There is also a wider sustainability challenge affecting the bioimaging landscape at the moment. Imaging and data scientists do not have appropriate career paths or permanent positions in most countries, making it difficult to retain talent in the field over a long time and resulting in regular loss of institutional knowledge. There is also a 'brain drain' of these skilled individuals to industry owing to higher salaries and not enough replacement personnel being trained. Furthermore, the high costs of maintenance and service contracts means that often expensive imaging equipment becomes unavailable for use after initial service contracts expire.

Overall, challenges in HICs and LMICs do not differ to a great extent. Challenges and barriers that apply to LMICs often apply to smaller research groups/institutions in HICs or relatively less research-intensive countries. The key difference in LMIC contexts is the funding available for infrastructure, equipment and research which is much smaller than that available in HICs and the unavailability of relevant research expertise. In this context, availability of bioimaging equipment and associated technical expertise is the key limiting factor for LMICs and less of a problem in HICs. Access to equipment and expertise is the secondary problem (access is not possible without things being available) and is typically affected by location (distance from user) and cost of access. Since facilities are fewer and far between in LMIC regions, access is a barrier to a larger extent in LMICs than in HICs.

5.2 Recommendations for Wellcome

Based on ongoing efforts and stakeholders' suggestions for interventions to address the key barriers and challenges, we feel Wellcome should consider the following options to facilitate development of new bioimaging technologies/methodologies, democratisation of existing and new bioimaging technologies/methodologies, interdisciplinary communities and bioimaging data reuse and integration.

1. **Supporting development of bioimaging technologies and methodologies** –

A funding programme to specifically support technology and methodology development in the bioimaging field would be 'low hanging fruit' for Wellcome. Such grants can be quite rare because funders' remits or the desire for impact on human health mean bioimaging technology and methodology development is not the key focus.

The programme's scope should be fairly broad to accommodate innovative ideas from a wide set of stakeholders and not limit development to specific bioimaging modalities or specific types of collaborations (inclusive for monodisciplinary and interdisciplinary/inter-sectoral teams). Exceptions could be made however or preferences indicated for supporting development of image analysis methods and tools or development that leads to low-cost bioimaging solutions which can be easily and widely adopted in resource-poor settings. Another consideration for the scope should be whether or not to allow use of grant money for incremental improvements in existing technologies and methodologies. While such work may have limited novelty, it could help improve spatial and temporal resolution, image acquisition, image analysis as well as make the technology/methodology cheaper and/or more accessible.

If a focused, bespoke funding programme is not feasible, development of bioimaging technologies and methodologies could be supported under the Wellcome Discovery Awards which are open to researchers from any discipline who want to pursue cutting-edge research

or development of methodologies, conceptual frameworks, tools or techniques. However, these awards are only available to established research leaders and groups led by them which will exclude up-and-coming researchers and research leaders as well as imaging and data scientists. LMIC-based researchers may also struggle to compete for these awards.

2. **Facilitating democratisation of bioimaging technologies and methodologies** –

Funding short-term mobility grants to cover research visits to facilities/labs with the requisite imaging infrastructure and expertise is another idea that Wellcome could explore. We suggest that both the visitors' and hosts' costs are covered (e.g. travel, accommodation, staff/researcher time, instrument time, consumables). As mentioned, such a scheme would improve access to bioimaging capabilities that are currently out of reach for many, either because of the costs involved or the capabilities not being available locally. Such a scheme would facilitate not only knowledge transfer and wider adoption of technologies/methodologies (where possible) but also could have implications on sustainability of infrastructures and expertise as equipment costs and staff time would be covered providing income for the host facility/lab. Moreover, existing equipment and expertise would be used more optimally perhaps avoiding procurement of expensive equipment where it is not strictly necessary.

Other networks such as Euro-Bioimaging and funders such as the Chan Zuckerberg Initiative provide similar opportunities but these are restricted to a select pool of countries and funding is limited compared to demand.

3. **Creating space for interdisciplinary conversations** –

Wellcome is well-placed to convene and sustain a diverse interdisciplinary and intersectoral community network for the purpose of development and dissemination of novel bioimaging technologies/methodologies. Wellcome's international reach and reputation would encourage engagement from the most innovative minds worldwide. Mechanisms for kickstarting and supporting such conversations could include things like conferences and meetings, webinars, an online networking platform, sandpits and funding programmes.

While some networks already exist, these are often geographically limited e.g. in specific countries or regions – Global Bioimaging is an exception but it is a network of country-/region-based networks and hence does not have complete global coverage. Secondly, existing initiatives that support cross-disciplinary collaboration are mostly focussed on bringing computer scientists and life scientists together towards improving imaging data management and analysis. Wider interdisciplinary activity has usually been in terms of workshops bringing technology developers, imaging scientists and life scientists together but this has been intermittent. Thus, there is a gap in the landscape to create and nurture a discipline- or imaging modality-agnostic interdisciplinary community in the bioimaging field. This could be a gap that Wellcome could try to address considering its global remit and reach as well as interest in supporting technology/methodology development and democratisation of techniques in bioimaging. Wellcome funds and has funded a wide spectrum of activities from bioimaging infrastructure and archives to technology/methodology development and research in a variety of biological fields and even bringing expertise in all these areas (e.g. through current and former grantees and peer reviewers) would be a good foundation to build on.

4. **Promoting reuse and integration of data** –

Wellcome could support open 'hardware' initiatives or data repositories to collate and store imaging data from different modalities in a way that it can be reused, compared and integrated. Common data standards and guidelines for depositing data will be required possibly with need for annotation and curation capabilities. Wellcome is once again well-placed to support establishment of common standards and best practice guidelines by convening international working groups or consensus guideline committees. While there are

several existing initiatives in this area, they are often fragmented and adoption of standards and FAIR data practices is inconsistent. Thus, an alternative would be for Wellcome to support ongoing efforts or to help form consensus in case of duplicate initiatives. Wellcome already supports key imaging data infrastructures (such as the BioImage Archive[102]) and so will be well-placed to help implement the agreed standards and guidelines.

A quick win for Wellcome could be adding a requirement for grantees to make their data publicly available or for grant proposals to have data management and sharing plans in line with the recent initiative by the US NIH. There is a dearth of high-quality pre-curated training datasets for development and validation of AI-based approaches, which is also an area where Wellcome could contribute.

[102] Hartley, M., Kleywegt, G.J., Patwardhan, A., Sarkans, U., Swedlow, J.R. and Brazma, A., 2022. The BioImage Archive–Building a Home for Life-Sciences Microscopy Data. *Journal of Molecular Biology*, p.167505

Appendix A Survey questionnaire

A.1. Introduction

Technopolis is carrying out a global landscape review on behalf of Wellcome to identify new key areas that have the potential to open-up opportunities in the field of bioimaging. The review will cover bioimaging methodologies, tools and technology development across the scales of life, from atoms all the way to humans that are applicable to discovery research.

An important component of the review is a stakeholder survey. Your participation in this survey will help us to understand:

> What are the key emerging bioimaging technologies/methodologies?
>
> What are the main barriers (with a specific focus on novel technology development) to progress and adoption of bioimaging technologies/methodologies?

We welcome contributions to this survey from all researchers working in the field related to bioimaging, from academic research leaders, methodology/technology developers to infrastructure staff. To that end, please feel free to forward the survey link to colleagues, technical staff, postdoctoral researchers and postgraduate/doctoral students.

Wellcome will use the output of this survey to inform its future approaches to support bold, creative and high-quality research activities in the field of bioimaging.

The survey will close on the 4th of November 2022.

All responses and associated personal information will be treated in the strictest confidence, in line with legislation on data protection. Information will only be reported in an anonymised form to Wellcome. Please note that anonymised survey results may be made publicly available in the spirit of Open Science and to allow others to learn from community views.

You have the right to request to amend or delete your response at any time. To do so, please contact the study team at BioimagingLandscapeStudy@technpolis-group.com. For further information on your rights and how to contact us, please refer to Technopolis Group's Privacy Policy.

Thank you for taking the time to complete the survey – your participation is extremely important to inform the review.

Before you begin, please make sure that your browser is maximised. It's easy to navigate through the questionnaire: just click on the answer or answers that apply for each question. You may need to use the scroll bar to see the next question. To continue, click on the next button at the bottom of each page.

The survey contains around 25 questions and should take about 25-30 minutes to complete. You do not have to answer all questions at once – answers will be stored at every page and you can return to the survey at any stage before completing it, provided the same device/browser is used and it is allowed for internet cookies.

Please click 'next' to enter the survey.

A.2. About you

We would like to know a bit more about you and your experience to understand the context of your responses and ensure we include views and perspectives from a diverse set of stakeholders in our landscape review.

1. Which country are you based in most of the time? *[103]

<<Drop down menu with Country List>>

2. What gender do you identify as?*

- Female
- Male
- Nonbinary
- Prefer not to say

3. Which of the following most closely describes your role? *

- Chief Scientific Officer
- Head of Department / Centre Director
- Professor
- Associate Professor / Reader
- Assistant Professor / Lecturer / Research group leader
- Facility manager
- Facility Staff / Imaging Scientist
- Data Scientist / data analyst
- Research and development scientist
- Research Associate
- Technical staff
- Research operations (e.g. programme / project manager)
- Research fellow / Postdoctoral researcher
- Doctoral / postgraduate student
- Other (please specify)

4. Please select your organisation type. *

- Government-funded University / Research Institute
- Private-funded University / Research Institute
- Government agency
- Central Bioimaging Facility
- Hospital / Healthcare Facility
- Independent Research Organisation
- Not for profit organisation
- Industry
- Other (please specify)

5. Please indicate your area of expertise in relation to bioimaging. Please tick all that apply,

- I develop bioimaging technologies.
- I use/apply bioimaging technologies.
- I develop bioimaging methods.
- I use/apply bioimaging methods.

[103] * Indicates a compulsory question. Respondents will not be allowed to continue the survey without answering it.

- I don't use or develop bioimaging technologies and methods.

6. How many years of experience do you have with bioimaging technology and/or methodology? *

- Less than 2 years
- 2 to 5 years
- 6 to 10 years
- 11 to 20 years
- Over 20 years

7. In which research field/s do you predominantly work (please tick all that apply)?*

- Biochemistry
- Bioinformatics and data science
- Biomedical Imaging
- Biophysics
- Biotechnology
- Cancer Research
- Computer Science
- Cell biology
- Developmental Biology
- Drug discovery
- Engineering
- Genetics / Genomics
- Infectious Diseases
- Immunology
- Mental Health / Neurobiology
- Molecular biology
- Veterinary medicine
- Other (please specify)

8. In which bioimaging field/s are you most experienced?

- Light/optical microscopy
- Fluorescence microscopy
- Confocal microscopy
- Computed tomography (CT)
- Electron microscopy
- Infrared imaging
- Magnetic resonance imaging (MRI)
- Positron emission tomography (PET)
- X-ray microscopy
- Image analysis
- Data management / archival
- Other (please specify)

A.3. Emerging technologies and methodologies in the field of bioimaging

We want to identify the emerging methodologies and technologies that will enable researchers to formulate new hypotheses and address new fundamental questions for life, health and well-being.

9. Please choose up to 3 areas of bioimaging that in your view are likely to be most transformative for the field of bioimaging in the future.*

- Acoustic Microscopy
- Atomic Force Microscopy
- Bioluminescence Imaging
- Confocal microscopy
- Episcopic Microscopy
- Expansion Microscopy
- Intravital Microscopy
- Nonlinear Optical Microscopy
- Quantitative Phase Imaging
- Synchrotron X-Ray Tomography
- Electron Microscopy (choice of specific techniques on next page)
- Fluorescence-based techniques (choice of specific techniques on next page)
- Spectroscopy-based techniques (choice of specific techniques on next page)
- Tissue and Organ Imaging (choice of specific techniques on next page)
- Allied approaches and tools (choice of specific techniques on next page)
- Other (please specify)

[Routing: Based on choices above, respondents were given the following additional options to choose from for their chosen modality/modalities.]

10. In your view, which of the following Electron Microscopy techniques are likely to be most transformative for the field of bioimaging in the future? You may choose up to 3 depending on your previous answer.

- Correlative Light And Electron Microscopy (CLEM)
- Cryogenic Electron Microscopy (Cryo-EM)
- Electron Tomography
- Focused Ion Beam Scanning Electron Microscopy (FIB/SEM)
- GridTape® (for High-Throughput Transmission Electron Microscopy [TEM])
- Immunoelectron Microscopy
- Other (please specify)

11. In your view, which of the following Fluorescence-based techniques are likely to be most transformative for the field of bioimaging in the future? You may choose up to 3 depending on your previous answer.

- Calcium Imaging
- Fluorescence In Situ Hybridization
- Fluorescence Recovery After Photobleaching (FRAP)
- Fluorescence Resonance Energy Transfer (FRET)
- Fluorescence Lifetime Imaging Microscopy (FLIM)
- Light Sheet Microscopy / Single Plane Illumination Microscopy (SPIM)
- Micro X-ray Fluorescence Spectrometry (XRF)
- Multiphoton Microscopy

- Photo activated localization microscopy (PALM)
- Quantum Dot Imaging Stimulated Emission Depletion (STED) Microscopy
- Stochastic optical reconstruction microscopy (STORM)
- Structured Illumination Microscopy (SIM)
- Total Internal Reflection Fluorescence (TIRF) Microscopy
- Other (please specify)

12. In your view, which of the following Spectroscopy-based techniques are likely to be most transformative for the field of bioimaging in the future? You may choose up to 3 depending on your previous answer.

- Fourier Transform Infrared (FTIR) imaging
- Fluorescence correlation spectroscopy (FCS/ICS/RICS/N&B)
- Hyperspectral imaging
- Raman Spectroscopy
- Single Crystal Spectroscopy
- X-Ray Emission Spectroscopy
- Other (please specify)

13. In your view, which of the following Tissue and Organ Imaging techniques are likely to be most transformative for the field of bioimaging in the future? You may choose up to 3 depending on your previous answer.

- Duplex Ultrasound
- Functional Magnetic Resonance Imaging (fMRI)
- Magnetic resonance imaging (MRI)
- Phase Contrast X-ray Imaging
- PhotoAcoustic Imaging
- Positron emission tomography/Computed tomography (PET/CT)
- Single-photon emission computerized tomography (SPECT)
- Other (please specify)

14. In your view, which of the following Allied approaches and tools are likely to be most transformative for the field of bioimaging in the future? You may choose up to 3 depending on your previous answer

- Artificial Intelligence and Machine learning approaches to image analysis
- Fluorescence probes
- High-throughput microscopy
- Imaging-based Spatial Proteomics/Transcriptomics
- Mass spectrometry-based imaging (MSI)
- Photomanipulation probes
- Optogenetics
- Single Molecule Imaging
- Super-resolution Microscopy
- Other (please specify)

15. Please elaborate on your choices to explain why and how the relevant methodologies/technologies will be transformative and/or what research fields/questions they will contribute to. Please feel free to provide evidence in the form of weblinks, DOIs, etc.

<<Free text box>>

A.4. Barriers and challenges limiting progress in the field of bioimaging

In this section, we wish to identify the barriers and challenges affecting uptake and use of bioimaging technologies/methodologies.

16. Based on your own experience to what extent do you think the following **scientific or technological barriers** limit progress in the bioimaging field generally?

	To a large extent	Somewhat	Very little extent	Not at all	Don't know	N/A
Sample preparation challenges						
Sample handling requirements (e.g. low temperature, storage, biosafety level)						
Limitations in image acquisition speeds / temporal resolution						
Limitations in spatial resolution						
Limitations due to phototoxicity of live samples						
Quantitation challenges						
Challenges of scaling up of techniques for high throughput of samples or image analysis						
Lack of appropriate image analysis methods						
Other (please specify)						

17. In your view which **scientific or technological barrier** needs to be addressed as a priority in the next 5 to 10 years to advance the field of bioimaging?

<<Drop-down menu of the barriers mentioned above>>

18. Please explain why you think this barrier or challenge should be prioritised. Please provide details of any bioimaging methodologies/technologies this barrier specifically applies to and how it is affecting progress.

<<Free text box>>

19. In your view, what are some of the possible solutions to address this barrier?

<<Free text box>>

20. Based on your own experience to what extent do you think the following **infrastructural barriers** limit progress in the bioimaging field generally?

	To a large extent	Somewhat	Very little extent	Not at all	Don't know	N/A
Lack of availability of appropriate bioimaging equipment / facilities						
High cost of bioimaging equipment/infrastructure						
Lack of availability of appropriate imaging software (image capture, analysis)						

High cost of imaging software (image capture, analysis)						
Lack of availability of appropriate technical expertise (e.g. experienced imaging scientists)						
Cost of access to bioimaging equipment and support						
Inadequate maintenance of bioimaging equipment or lack of adequate technical support from vendors						
Lack of availability of adequate data processing and management resources (e.g. computing capacity, repository/archives)						
Other (please specify)						

21. In your view which **infrastructural barrier** needs to be addressed as a priority in the next 5 to 10 years to advance the field of bioimaging?

<<Drop-down menu of the barriers mentioned above>>

22. Please explain why you think this barrier or challenge should be prioritised. Please provide details of any bioimaging methodologies/technologies this barrier specifically applies to and how it is affecting progress.

<<Free text box>>

23. In your view, what are some of the possible solutions to address this barrier?

<<Free text box>>

24. Based on your own experience to what extent do you think the following **other barriers** limit progress in the field of bioimaging generally?

	To a large extent	**Somewhat**	**Very little extent**	**Not at all**	**Don't know**	**N/A**
Interdisciplinary barriers (e.g. between technology developers and users; data scientists and biologists) affecting development and application of new bioimaging technologies, methodologies and tools						
Lack of funding for bioimaging technology, methodology and tool development						
Lack of knowledge/understanding of new bioimaging technologies/techniques (resulting in reliance on known methods)						
Lack of awareness of available bioimaging facilities (e.g. location, type of equipment/methods available)						
Lack of training opportunities						
Lack of career pathways for technical staff and data scientists working in bioimaging						
Access and hiigh cost of consumables						
Reluctance to share equipment or data (to enable Open Science) with other researchers						
Other (please specify)						

25. In your view which barrier (from the list above) needs to be addressed as a priority in the next 5 to 10 years to advance the field of bioimaging?

<<Drop-down menu of the barriers mentioned above>>

26. Please explain why you think this barrier or challenge should be prioritised. Please provide details of any bioimaging methodologies/technologies this barrier specifically applies to and how it is affecting progress.

<<Free text box>>

27. In your view, what are some of the possible solutions to address this barrier?

<<Free text box>>

28. Please describe any additional barriers/challenges affecting the field of bioimaging in the space below, if they have not been covered already.

<<Free text box>>

29. [For Facility Managers, Staff / Imaging Scientists / Technical Staff] What would be the potential **impact on your work** if the barriers identified above were addressed?

	Significant impact	**Moderate impact**	**Very little impact**	**No impact at all**	**Don't know**
I would receive more requests for bioimaging services					
It would bring more revenue to my facility, allowing investment in new equipment, maintenance of existing equipment and training					
It would increase the quality and reproducibility of our imaging services					
It would enable me to help researchers answer new hypotheses and address new fundamental questions about biological processes and mechanisms					
It would encourage/enable me to work on bioimaging technology, methodology and tool development					
My work would directly contribute to improved health and wellbeing					
My work would directly contribute to enhanced economic impact					
My work would directly contribute to enhanced societal impact					
My work would directly contribute to environmental sustainability					
Other (please specify)					

30. [For Users of bioimaging technology & methods] What would be the potential **impact on your work** if the barriers identified above were addressed?

	Significant impact	**Moderate impact**	**Very little impact**	**No impact at all**	**Don't know**

I would use bioimaging in my research work more often					
It would increase my interest in bioimaging technology, methodology and tool development					
It would increase the quality and reproducibility of my research outputs					
It would enable me to formulate new hypotheses and address new fundamental questions about biological processes and mechanisms					
My research would directly contribute to improved health and wellbeing					
My research would directly contribute to enhanced economic impact					
My research would directly contribute to enhanced societal impact					
My research would directly contribute to environmental sustainability					
Other (please specify)					

31. [For Developers of bioimaging technology, methods or both] What would be the potential **impact on your work** if the barriers identified above were addressed?

	Significant impact	**Moderate impact**	**Very little impact**	**No impact at all**	**Don't know**
It would increase the quality and reproducibility of my research outputs					
It would enable me to develop novel bioimaging technologies more readily					
Lowering of interdisciplinary barriers will enable me to better focus my development work on user needs					
It would enable me to formulate new hypotheses and address new fundamental questions about biological processes and mechanisms					
I would use bioimaging in my research work more often					
My work would directly contribute to improved health and wellbeing					
My work would directly contribute to enhanced economic impact					
My work would directly contribute to enhanced societal impact					
My work would directly contribute to enhanced environmental impact					
Other (please specify)					

32. What would be the single most important impact of overcoming the barriers and challenges discussed above?

<<Free text>>

A.5. Final remarks

Thank you for your response. We appreciate your input so far. We would like to conduct short follow-up interviews (by telephone or videoconference) with a subset of survey respondents to explore the responses in more depth and develop short case studies.

33. If you are willing to be contacted for further information by the study team, please provide your contact details below.

Name	
Email address	
Comments	

Please be assured that your contact details will not be shared outside the study team, and will be deleted on completion of the study. Full details on how the study team will handle your data are available at http://www.technopolis-group.com/privacy-policy/.

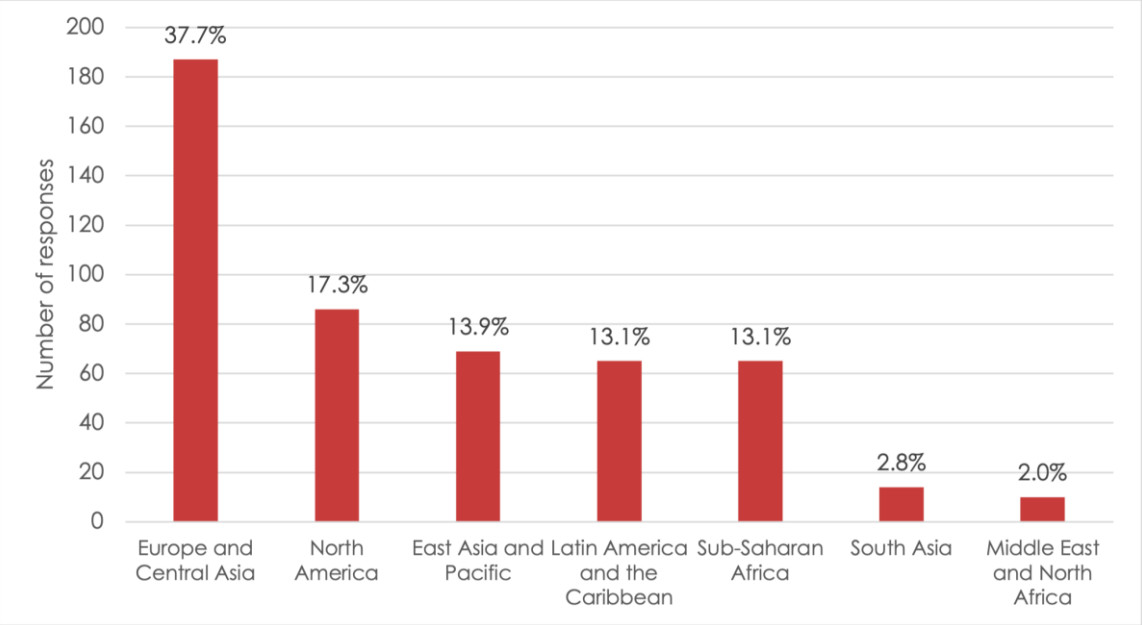
Appendix B Supplementary survey data

B.1. Demographics of survey respondents

- **Location of respondents (by region and country)**

As per Figure 11, just under 40% of respondents were based in Europe (38%, n = 187), followed by North America (17%, n = 86), East Asia and Pacific (14%, n = 69), Latin America and the Caribbean (13%, n = 65), Sub-Saharan Africa (13%, n = 65), South Asia (3%, n = 14) and the Middle East and North Africa (2%, n = 10).

Figure 11 Survey responses by region (n=496)



The total number of responses per country and region is outlined in Table 3. High income countries (HICs) represented 72% of respondents (n = 358) while low- and middle-income countries (LMICs) represented 28% (n = 138).[104] Respondents based in the United Kingdom (n = 129), United States (n = 73), Nigeria (n = 49) and Australia (n = 37) collectively represented over half of the survey respondents (58%).

Table 3 Number of responses by region and countries (n=496)

Region and countries	Total responses	% Of total
Europe and Central Asia	**187**	**37.7%**
United Kingdom	129	26.0%
Germany	19	3.8%
France	5	1.0%

[104] Classification of High income countries and low- and middle-income countries were based on the Organisation for Economic Co-operation and Development (OECD) as per Wellcome's guidance available here: https://wellcome.org/grant-funding/guidance/low-and-middle-income-countries

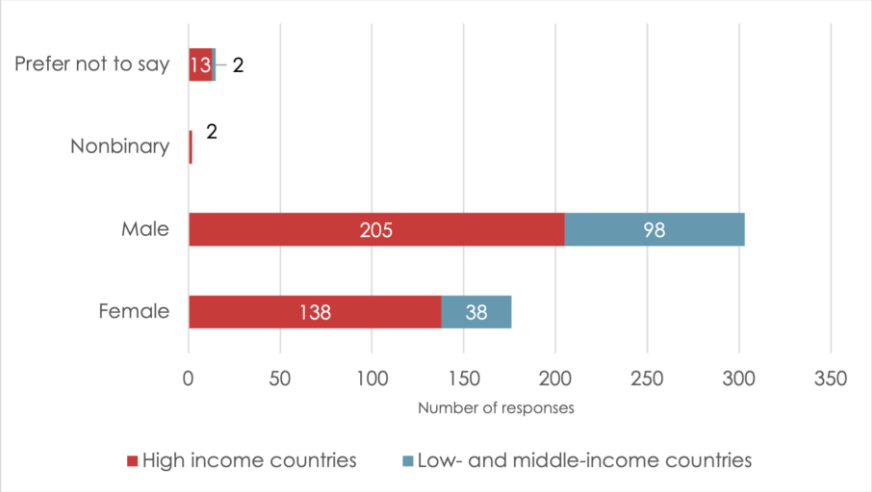
Austria	4	0.8%
Netherlands	4	0.8%
Switzerland	4	0.8%
Ireland	3	0.6%
Italy	3	0.6%
Portugal	3	0.6%
Czech Republic	2	0.4%
Finland	2	0.4%
Spain	2	0.4%
Bulgaria	1	0.2%
Denmark	1	0.2%
Norway	1	0.2%
Poland	1	0.2%
Russia	1	0.2%
Slovenia	1	0.2%
Sweden	1	0.2%
North America	**86**	**17.3%**
United States of America	73	14.7%
Canada	13	2.6%
East Asia and Pacific	**69**	**13.9%**
Australia	37	7.5%
Japan	19	3.8%
China	6	1.2%
Taiwan	2	0.4%
Hong Kong	1	0.2%
Malaysia	1	0.2%
New Zealand	1	0.2%
Singapore	1	0.2%
Thailand	1	0.2%
Latin America and the Caribbean	**65**	**13.1%**
Argentina	22	4.4%

Uruguay	15	3.0%
Mexico	11	2.2%
Brazil	9	1.8%
Chile	7	1.4%
Panama	1	0.2%
Sub-Saharan Africa	**65**	**13.1%**
Nigeria	49	9.9%
South Africa	12	2.4%
Ethiopia	1	0.2%
Mali	1	0.2%
Rwanda	1	0.2%
Sudan	1	0.2%
South Asia	**14**	**2.8%**
India	11	2.2%
Bangladesh	1	0.2%
Pakistan	1	0.2%
Sri Lanka	1	0.2%
Middle East and North Africa	**10**	**2.0%**
Egypt	7	1.4%
Israel	1	0.2%
Jordan	1	0.2%
Saudi Arabia	1	0.2%

- **Gender of respondents by country type**

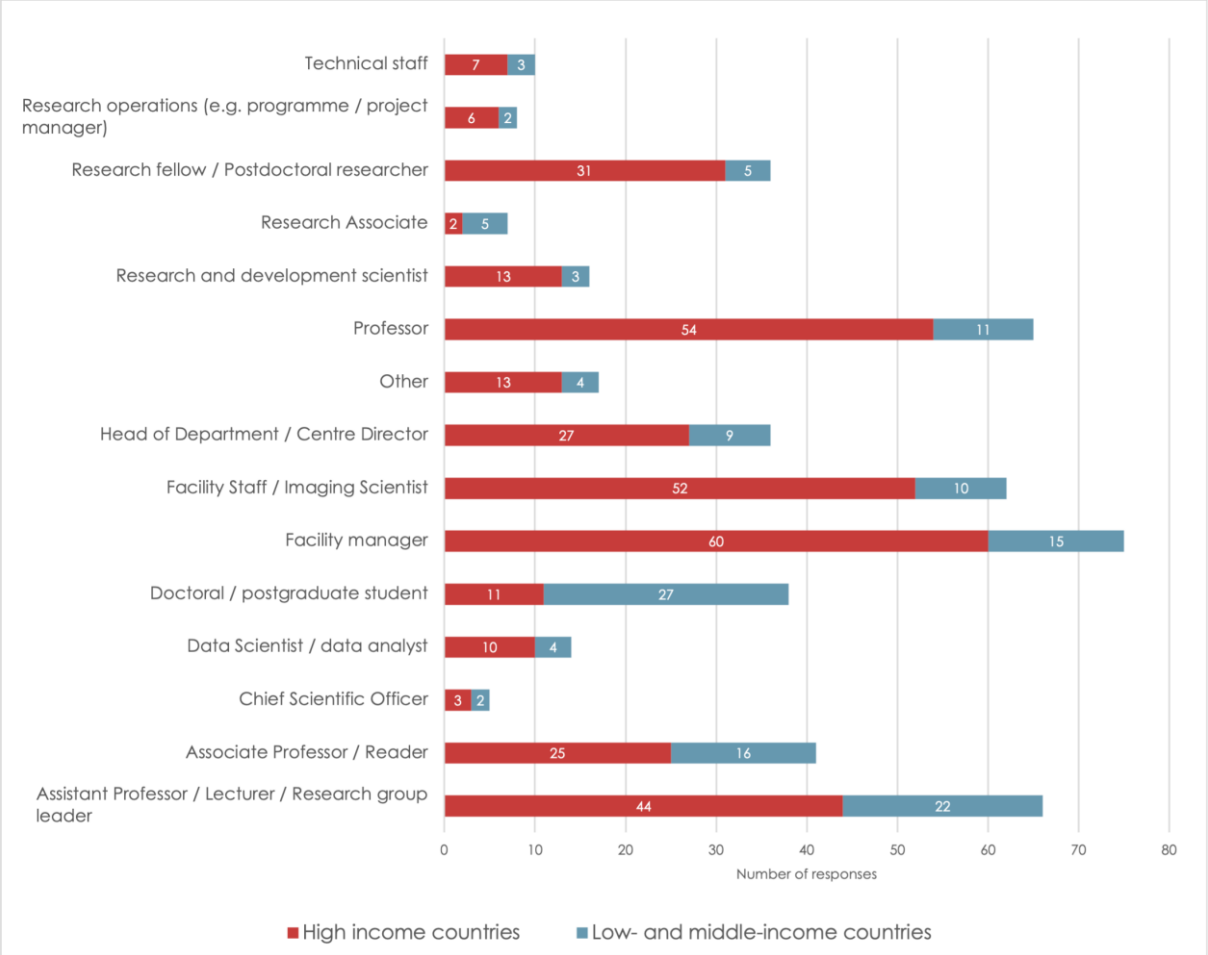
Figure 12 shows the gender of respondents by country type. The majority of responses (61%, n = 303) came from people that identify themselves as 'Male'. 39% (n = 138) of HIC respondents and 28% of (n = 38) LMIC respondents self-identified as female.

Figure 12 Gender of respondents by country type (n=496)



- **Role of respondents by country type**

Figure 13 Role of respondents by country type (n=496)



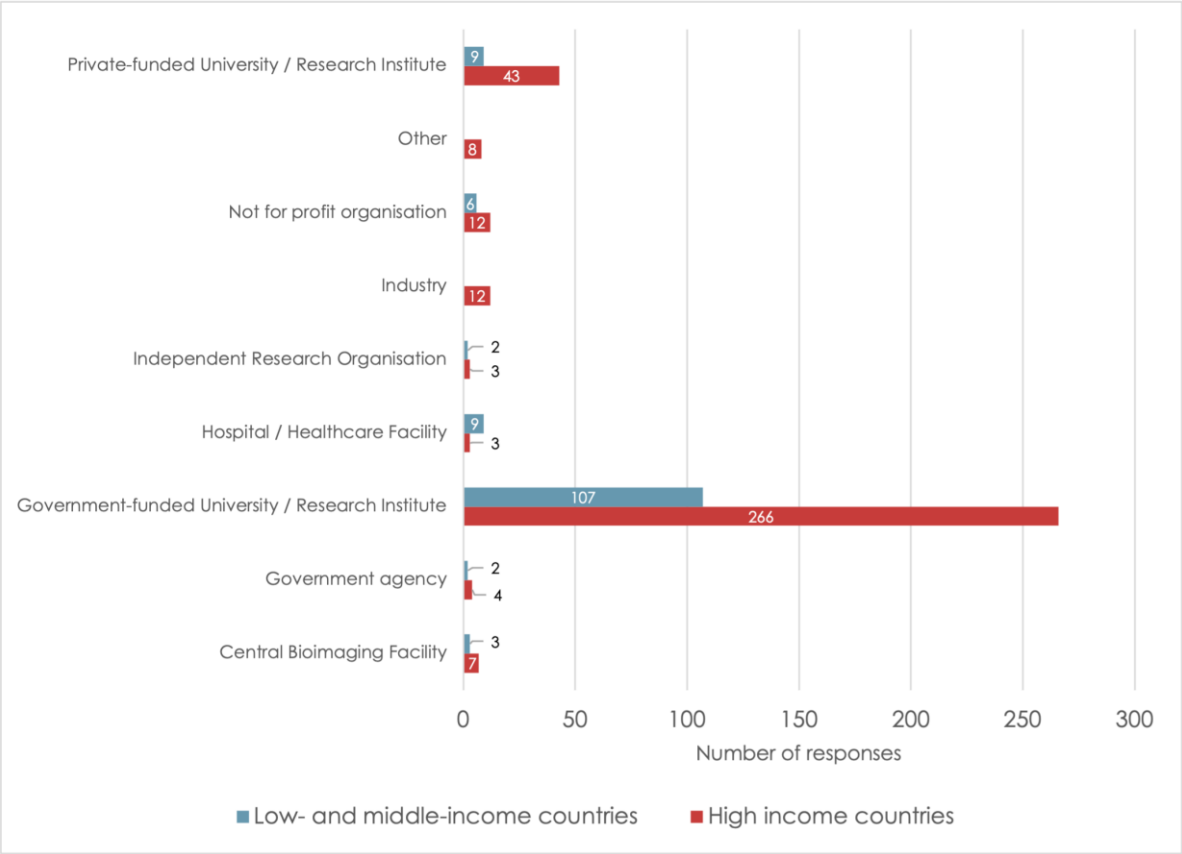
Facility managers (n = 75), assistant professors / lecturers / research group leaders (n = 66), professors (n = 65) and facility staff / imaging scientists (n = 62) together accounted for the majority (54%) of survey respondents. As shown in Figure 13, a greater number of doctoral /

postgraduate students and Research Associates from LMICs (n = 27[105] and 5 i.e. 20% and 4% of LMIC respondents respectively) responded to the survey than those from HICs (n = 11 and 2 i.e. 3% and 1% of HIC respondents respectively). The 'other' category represented under 4% of total answers and included undergraduate & masters students, a research software engineer, a director of research infrastructure, consultants and a data steward among other roles.

- **Affiliation of respondents by country type**

Over three-fourths of respondents were based in a government-funded university / research institute, of which HICs accounted for 54% (n = 266, 75% of HIC respondents) and LMICs 22% (n = 107, 78% of LMIC respondents).

Figure 14 Affiliation of respondents by country type (n=496)



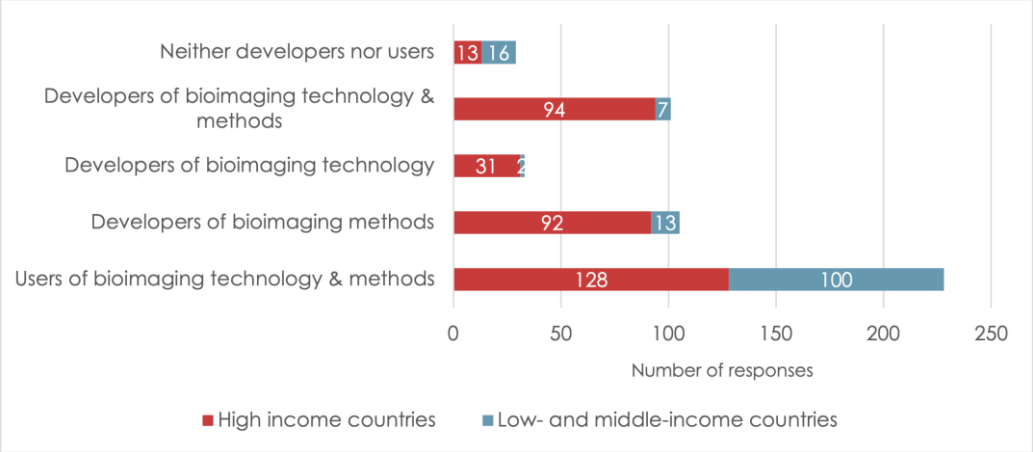
- **Expertise of respondents by country type**

When asked whether they use and/or develop bioimaging methods and/or technologies, 6% of respondents (n = 29)[106] stated they were neither developers nor users. Users of bioimaging technologies and/or methods represented 46% (n = 228) of total responses (Figure 15). Notably, the vast majority of LMIC respondents (72%, 100 of 138) were users rather than developers. Thus, technology/methodology development expertise in the survey was heavily dominated by respondents from HICs (n = 217 i.e. 43% of total respondents, 60% of HIC respondents and 91% of developers).

[105] 20 of the 27 doctoral / postgraduate students were from Nigeria

[106] 'Neither developers nor users' are respondents who selected the option: 'I don't use or develop bioimaging technologies and methods'.

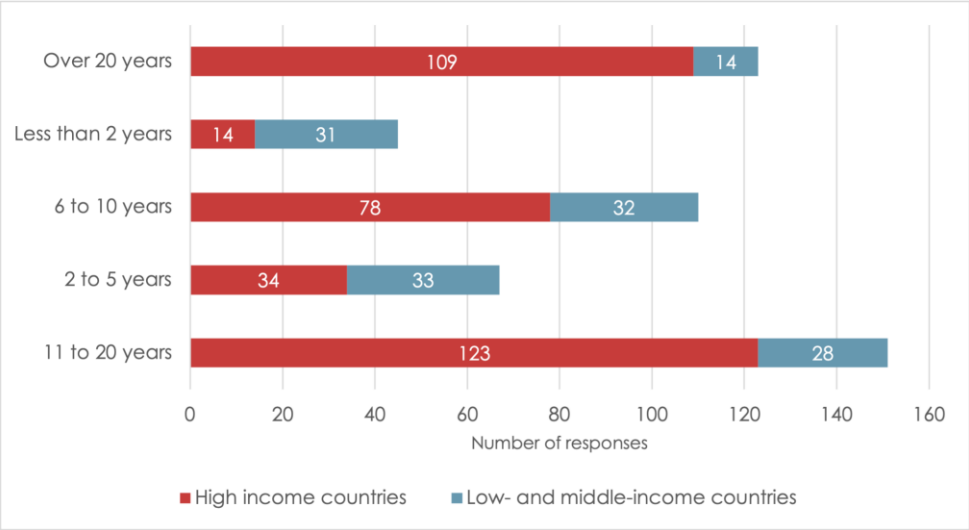
Figure 15 Expertise of respondents by country type (n=496)



- **Years of experience of respondents by country type**

Over half of the respondents had at least 11 years of experience (55%, n = 274). LMIC respondents tended to be less experienced (65 of 138 i.e. 47% having 2 to 10 years' experience) compared to HIC respondents (232 of 358 i.e. 65% having 11 or more years' experience).

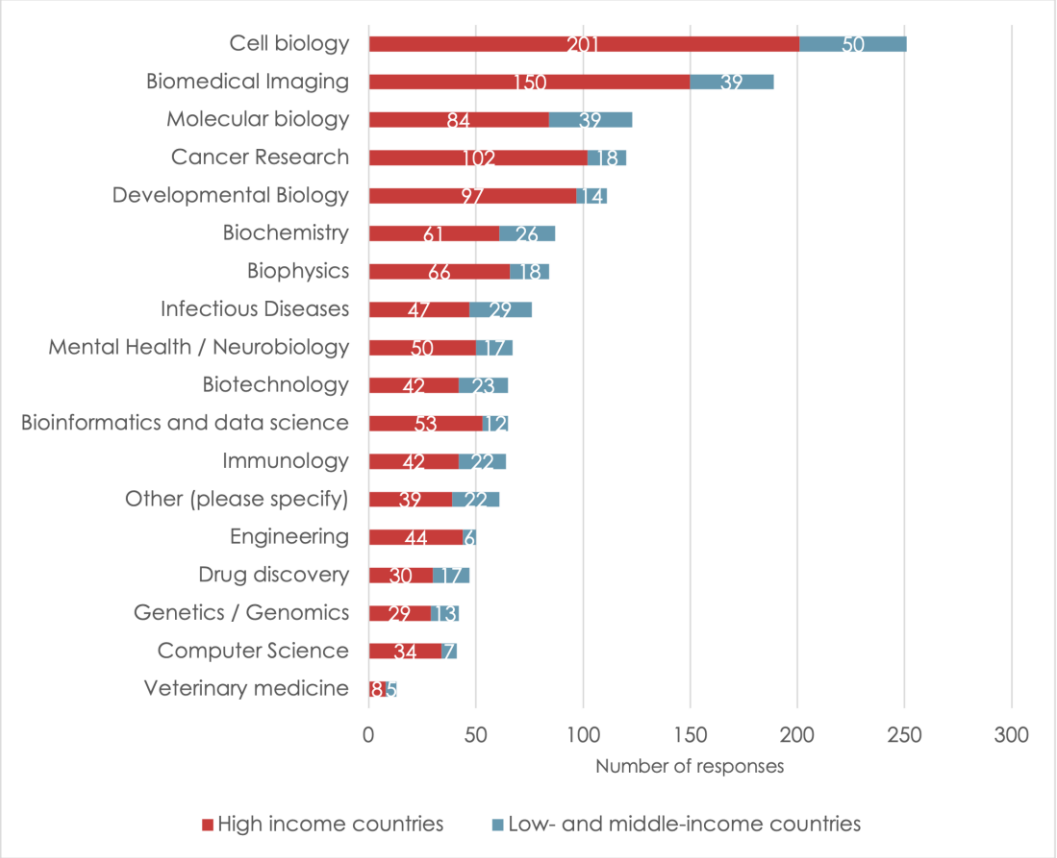
Figure 16 Years of experience of respondents by country type (n=496)



- **Research field of respondents by country type**

Respondents were asked to select at least one research field they were active in. The five most commonly selected fields overall were: cell biology (n = 251, 51%), biomedical imaging (n = 189, 38%), molecular biology (n = 123, 25%), cancer research (n = 120, 24%) and developmental biology (n = 111, 22%). While the top three fields were identical for HIC and LMIC respondents, infectious diseases and biochemistry comprised the fourth and fifth most common research fields for LMIC respondents. The 'other' option represented under 4% of total answers and included areas such as indigenous health, medical geography, materials science, social studies, environmental engineering, nanotechnology, reproductive biology, mathematics and zoology.

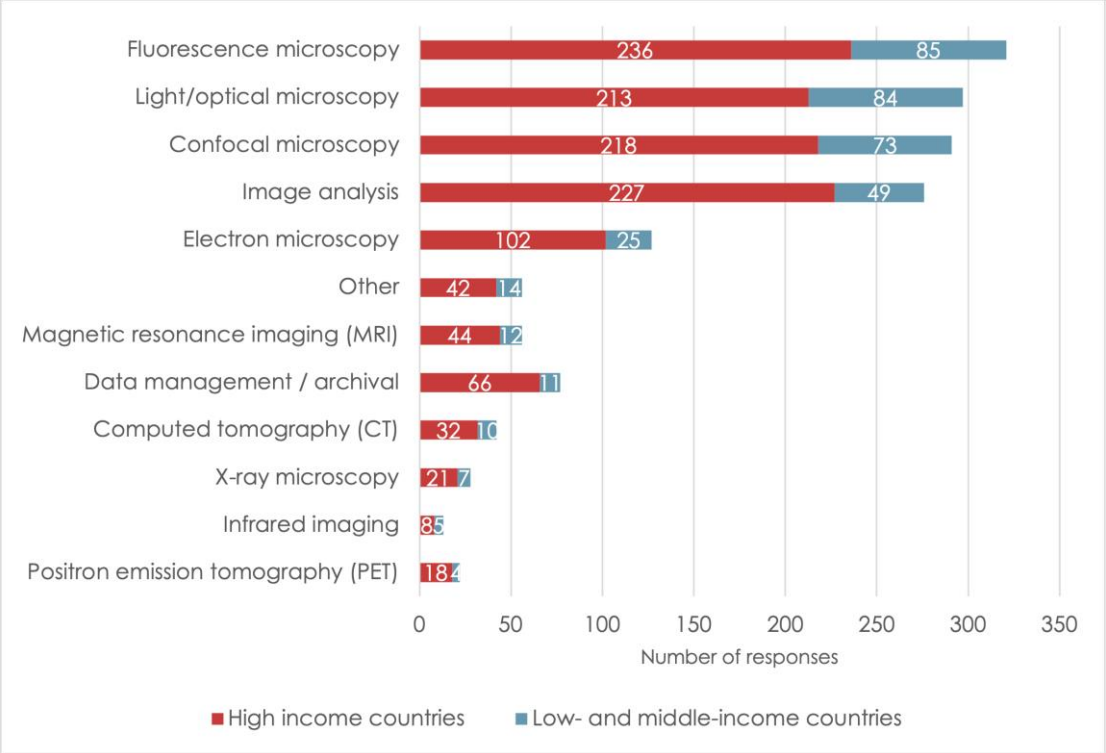
Figure 17 Research field of respondents by country type (n=496)



- **Bioimaging expertise of respondents by country type**

Respondents were asked to select at least one field of bioimaging that they are experienced in and the top four fields, each selected by at least 56% of the respondents, comprised: fluorescence microscopy (n = 321, 65%), light/optical microscopy (n = 297, 60%), confocal microscopy (n = 291, 59%) and image analysis (n = 276, 56%). The top four fields for both LMIC and HIC respondents were the same. Overall, 82% (n = 406) of the respondents were experienced in microscopy-based methods, while 18% (n = 91) of respondents were experience in organ and body imaging methods such as magnetic resonance imaging (MRI) and computed tomography (CT).

Figure 18 Bioimaging expertise of respondents by country type (n=496)



Appendix C Interview guide

C.1. Introduction

Wellcome has a history of supporting innovation in bioimaging, with a portfolio of investments that ranges from new tools and technologies to infrastructure and data repositories. It aims to build on these investments by identifying and then supporting new key areas with the potential to be transformative for the field. To aid identification of these key areas, Wellcome's Discovery Research team has commissioned us i.e. Technopolis Ltd, an independent policy research and consulting organisation, to conduct a global landscape review covering bioimaging methodologies, tools and technology development across the scales of life from atoms all the way to humans. The study needs to equally cover both High-Income Countries (HICs) and Low/Middle-Income Countries (LMICs) to understand the current situation, needs and challenges in both settings. It is also more focussed on discovery / basic research applications of bioimaging than clinical applications.

Within the review, we will be conducting a stakeholder survey and interviews to answer two key questions:

(1) What are the key emerging bioimaging technologies/methodologies?

(2) What are the main barriers to progress and adoption of bioimaging technologies/methodologies in High-Income versus Low/Middle-Income Countries?

Do you have any questions for me before we start?

We will report this information, for example, opinions and views expressed, in aggregate to Wellcome. Where your contribution may be identifiable, we will ask for your permission to include this information in the report. Do you agree to this?

C.2. About the interviewee

1. To start, could you please provide some information on your area of research and experience related to bioimaging?
   i) What research questions are you answering? What bioimaging techniques do you use? What has been the result?

C.3. Novel/emerging areas

2. What are the new emerging technologies, methodologies and tools in the field of bioimaging that will enable researchers to formulate new hypotheses and address new fundamental questions for life sciences and health?
   - What do you think is at the leading edge of the field?

     We are interested in emerging areas or even gaps which when filled will be transformative for biological research or will help democratise bioimaging technologies. This includes technologies, methodologies or tools that could help to increase spatial and temporal resolution limits or improve analysis, storage and access of imaging datasets.

   i) Are there any emerging interdisciplinary technologies, techniques or tools, for example those that will help integration across the scales of life?

   ii) How mature are these technologies?
3. How and why are these novel/emerging technologies, methods or tools better than the current state-of-the-art methodologies? In what way will they be transformative for the field?

- What type of information is currently achievable with current technology/methods and what needs to be improved and why?
- Low hanging fruit vs long term transformative outcomes?
- Hot topics right now and topics that could become more relevant in the future?

4. How should these novel/emerging technologies, methods or tools be supported to enable their full potential to be achieved? What is needed to further develop them or to allow equitable access?
   i) What nascent areas are funders not funding yet?
5. What specific scientific/research questions are researchers unable to answer because the necessary technology, methodology or tool has not been developed yet?

C.4. Barriers

6. In your view, what is limiting progress in the field of bioimaging?

For example,

- Technological barriers e.g. image acquisition speed, spatial and temporal resolution
  - Are there specific questions that cannot be answered because there is a limitation in the technology?
- Scientific and methodological barriers e.g. quantitation, sample preparation
- Infrastructural barriers e.g. hardware, software, etc.
- Barriers to access to specialised equipment / imaging datasets
- Barriers related to relevant skills, knowledge and research culture
- Barriers in HICs and LMICs
- Sustainability issues i.e. any issues with sustaining imaging technology for the future (so that it's of use to the field)

7. What barriers limit equitable access and use to novel methodologies, for example, those that work across scales of life?
   - Are there any untapped communities (other research fields)/resources (computing power etc) that would be of benefit to the development of better imaging tools/technologies?
   - Is the wider research landscape/field adequately engaged with the technologies and methodologies?
   - Do those utilising or engaging with the technologies and methodologies have the skills that they need to advance the field?
8. Can you give an example of a state-of-the-art methodology/technology that is not being fully used? Why is it not achieving its full potential?
9. What solutions could be implemented to address the barriers we just discussed?
   i) How can research funders better support new interdisciplinary and diverse scientific communities to accelerate development/uptake of novel technologies/methodologies?
   ii) What activities could help to scale up the use of well-established methodologies and/or lower their cost allowing to address unanswered biological questions?
   iii) How have funders been able to support you in unlocking your imaging technology? Was there a specific type of support which worked well? And which didn't work so well?

iv) What could Wellcome do to remove barriers to progress? What could Wellcome do to facilitate the development of new technology/access to cutting edge technology?

C.5. Key leaders

10. Who are the key leaders (individuals or organisations) on an international level that are driving development in the field of bioimaging?
    - Any up-and-coming research leaders/post docs making gains in the landscape?
    i) Would you like to recommend any interviewees for this study?

C.6. Close

11. Do you have any other comments with regard to the study or any suggestions? Are there any aspects you would like to discuss that we may have missed?

Thank you very much for your time and insights; this is extremely helpful to inform the study.

Appendix D Interviewees

D.1. Stakeholder interview invitations

Table 4 Number of invitations sent and stakeholder interviews conducted by country type and gender

Country type / gender	Initial invitation list	**Interviews conducted**	Additional invitations, including from survey & recommendations	**Interviews conducted**	Total stakeholder interviews invitations sent	**Total stakeholder interviews conducted**
High-income countries	22	7	67	22	89	29
Male	12	4	37	13	49	17
Female	10	3	30	9	40	12
Low- and middle-income countries	18	4	9	5	27	9
Male	11	4	6	3	17	7
Female	7	0	3	2	10	2
Total	**40**	**11**	**76**	**27**	**116**	**38**

D.2. Interviewee list

Below is the list of 51 interviewees who were consulted for the study.

Interviewee	**Country**	**Gender**	**Job title/ role**	**Research area and/or bioimaging expertise**
Alex Sossick	UK	M	Facility manager	Developmental biology, cancer research; confocal, light sheet, spinning disk and high-throughput microscopy
Alexandra Kerbl	UK	F	Postdoctoral Research Fellow	Morphology with focus on nervous systems; Light, confocal, electron microscopy
Anna Kreshuk	Germany	F	Group leader	Machine learning applied to imaging
Antje Keppler	Germany	F	Director of facility	Imaging Infrastructure Strategy Development
Arne Seitz	Switzerland	M	Facility manager	Light microscopy

Beth Cimini	US	F	Senior Group Leader	Computational biology, Imaging Platforms, Image analysis workflows
Caron Jacobs	South Africa	F	Researcher	Cell biology, Infectious Diseases; Confocal microscopy, Light and super-resolution microscopy
Chris Wood	Mexico	M	Facility manager	Confocal microscopy, multiphoton imaging, whole animal imaging, AI algorithm development
Christopher Rowlands	UK	M	Researcher	Cell biology; new types of optical systems for use in biology
Claire Brown	Canada	F	Director of facility	Quantitative Bioimaging, Fundamental and advanced light microscopy
Dave Stuart	UK	M	Director of Life Sciences	Structural Molecular Biology, viral crystallography
David Newby	UK	M	Professor	Cardiology; CT, PET/CT, Angiography
Ejia Jokitalo	Finland	F	Adjunct Professor	Electron Microscopy, Advanced imaging
Federico Lecumberry	Uruguay	M	Associate Professor	Signal Processing and Machine Learning, Cryo-EM
Florian Jug	Italy	M	Group Leader	Computer vision and ML for bio-image analysis
Gustavo Menezes	Brazil	M	Professor	Cell Biology, Gastrointestinal Biology; Intravital microscopy
H Krishnamurthy	India	M	Director of facility	Reproductive biology; Flow cytometry
Hiroki Ueda	Japan	M	Team leader	Circadian biology; Whole-brain imaging with single-cell resolution
Ian Dobbie	US	M	Professor / Director of facility	Super-resolution imaging, correlative cryo-fluorescence imaging
Jemima Burden	UK	F	Technical staff	Electron microscopy
Jens Rittscher	UK	M	Professor	Engineering science; Microscopy image analysis
Julia Schnabel	Germany	F	Professor	Computational imaging in medicine, AI / computational techniques

Laurence Lejeune	Canada	M	Researcher	Cytometry, Core Facility Management, Infrastructure Support
Leandro Lemgruber Soares	UK	M	Imaging technologist	Infectious diseases, Immunology; super-resolution and cryo-microscopy, correlative microscopy
Leonel Malacrida	Uruguay	M	Professor	Cell biology; FLIM, Hyperspectral imaging, Phasor plots
Liangyi Chen	China	M	Group leader	Neuroscience; Super-resolution microscopy, two-photon microscopy, light sheet microscopy
Lize Engelbrecht	South Africa	F	Facility manager	Fluorescence microscopy
Lucy Collinson	UK	F	Facility manager	Volume electron microscopy, correlative imaging techniques, cryo-microscopy, X-ray microscopy, image analysis, and microscope design and prototyping
Madeline Parsons	UK	F	Professor	Cell Biology; Super-resolution microscopy
Mahmoud Bukar Maina	Nigeria	M	Research Fellow	Neuroscience; Confocal microscopy, Electron microscopy
Mara Cercignani	UK	F	Professor	Neurobiology; Quantitative MRI
Maria Harkiolaki	UK	F	Beamline Scientist	Correlative cryo-imaging
Mark Lythgoe	UK	M	Head of Department / Centre Director	Neurobiology, Biophysics, Engineering, Drug discovery, Cancer Research, Computer Science; Light, fluorescence microscopy; CT, MRI, PET, ultrasound, photoacoustic imaging, SPECT
Markus Barth	Australia	M	Professor	Information Technology and Electrical Engineering; MRI
Matthew Hartley	UK	M	BioImage Archive Team Leader	Structural Molecular Biology; Data repositories for molecular imaging
Menattallah Elserafy	Egypt	F	Assistant Professor	Yeast Genetics; Confocal microscopy
Meriem el Karoui	UK	F	Professor	Single molecule imaging

Michael Reiche	South Africa	M	Director of facility	Infectious Diseases; Confocal and super-resolution microscopy
Neil Ranson	UK	M	Head of Department / Centre Director	Infectious Diseases, Structural molecular biology; Cryo-EM
Peijun Zhang	UK	F	Professor	Structural biology of human pathogens; combination of high-resolution cryo-EM and cryo-electron tomography with computational and biophysical/biochemical methods
Peter O'Toole	UK	M	Director of facility	Advanced Microscopy and Flow Cytometry
Petra Schwille	Germany	F	Professor	Cellular and molecular biophysics; Fluorescence Correlation Spectroscopy, Atomic Force Microscopy, Single Molecule, Synthetic Biology
R. M. G. Rajapakse	Sri Lanka	M	Professor	Chemistry and nanotechnology; creating new probes and sensors for ultrasound and MRI
Richard Bowman	UK	M	Researcher	Optical microscopy, designing new instruments including 3D printing for LMIC instrument design
Steve Lee	Australia	M	Group Leader	Biological Physics, Optical physics, Cell Physiology; AI and image processing; development of adaptive, low phototoxicity imaging tools; biomedical instrumentation
Susan Cox	UK	F	Researcher	Development of super-resolution localisation microscopy techniques
Theresa Ward	UK	F	Associate Professor	Infectious diseases, Immunology; Confocal microscopy
Valeria Piazza	Mexico	F	Professor	Cell biology, Neurobiology; Confocal microscopy, development of new optical microscopy techniques
Wojtek Goscinski	Australia	M	Professor	Imaging informatics, Neuroinformatics, Infrastructure and standards
Yara Reis	Germany	F	Manager	Imaging Infrastructure
Yaw Aniweh	Ghana	M	Senior Research Fellow at WACCBIP	Cell Biology of infectious pathogens; Confocal microscopy, Cryo-EM

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