1 Wellcome Bioimaging Landscape Review Survey analysis

1.1 Overview

The survey was distributed in three ways:

1. Via Wellcome and the following 7 bioimaging networks
   - African Bioimaging Consortium
   - BioImaging North America (plus Canada BioImaging and Canadian Network of Scientific Platforms)
   - Euro-Bioimaging (and Global Bioimaging)
   - Latin America Bioimaging
   - National Imaging Facility, Australia
   - India BioImaging Consortium
   - Advanced BioImaging Support, Japan
2. By email to corresponding authors of publications in the field of bioimaging – Using keywords search queries in Europe PMC, a total of 20,150 corresponding author emails were extracted from academic publications, covering researchers from all continents. Invitations were sent to 7,381 of these email addresses. Geographical regions not covered by the bioimaging networks or those with low numbers of responses were preferentially contacted through this route.
3. By snowballing, allowing survey recipients to forward the survey to their personal networks

The survey launched on 2nd October 2022 and closed on 4th November 2022. Following the initial invitation, two reminders were sent to encourage responses. A total of 496 responses were received.

1.2 Demographics of survey respondents

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

Just under 40% of respondents (Figure 1) 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).

The total number of responses per country and region is outlined in Table 1. High income countries (HICs) represented 72% of respondents (n = 358) while low- and middle-income countries (LMICs) represented 28% (n = 138)[1]. 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%).

[1] 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

Figure 1 Survey responses by region (n=496)

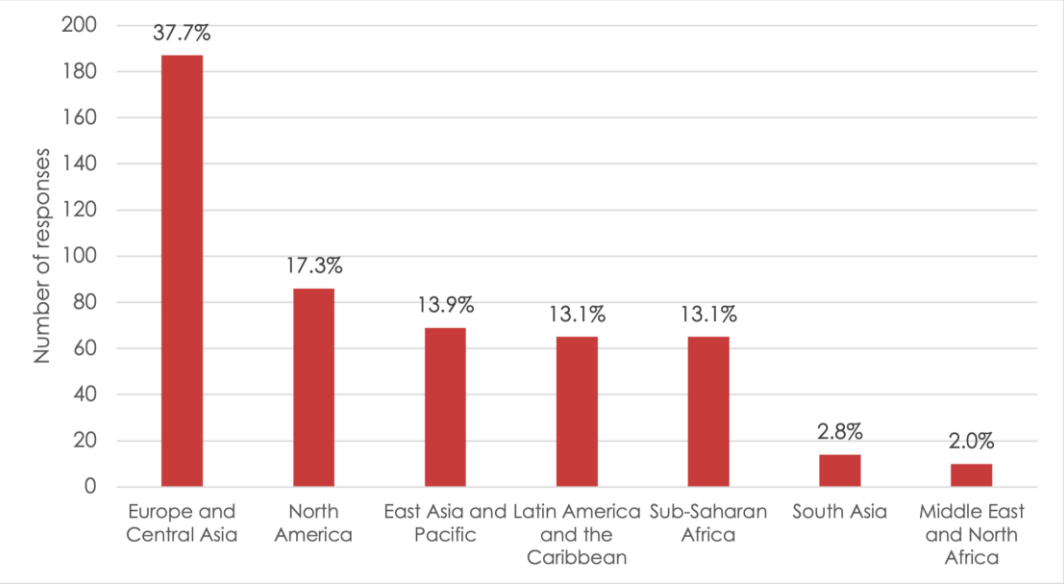


Table 1 Number of responses by region and countries (n=496)

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%
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%

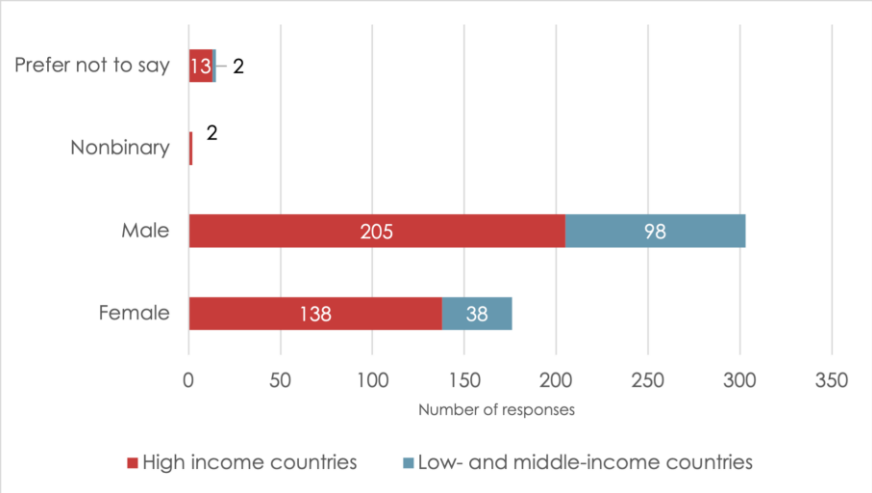
Slovenia	1	0.2%
Sweden	1	0.2%
North America	**86**	**17.3%**
United States of America	73	14.7%
Canada	13	2.6%
East Asia and Pacific	**69**	**13.9%**
Australia	37	7.5%
Japan	19	3.8%
China	6	1.2%
Taiwan	2	0.4%
Hong Kong	1	0.2%
Malaysia	1	0.2%
New Zealand	1	0.2%
Singapore	1	0.2%
Thailand	1	0.2%
Latin America 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 2 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 2 Gender of respondents by country type (n=496)

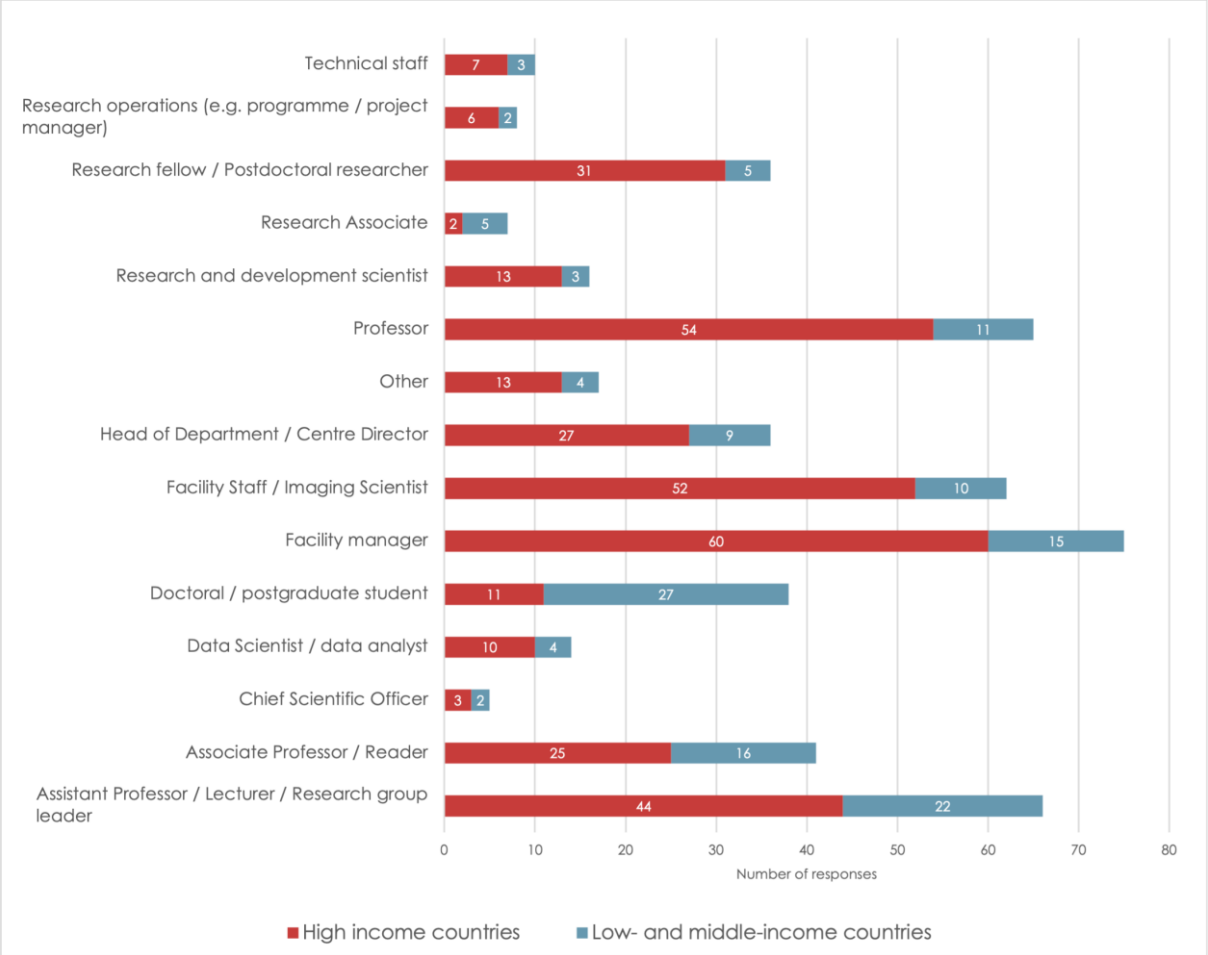


- **Role of respondents by country type**

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 3, a greater number of doctoral / postgraduate students and Research Associates from LMICs (n = 27[2] 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.

[2] 20 of the 27 doctoral / postgraduate students were from Nigeria

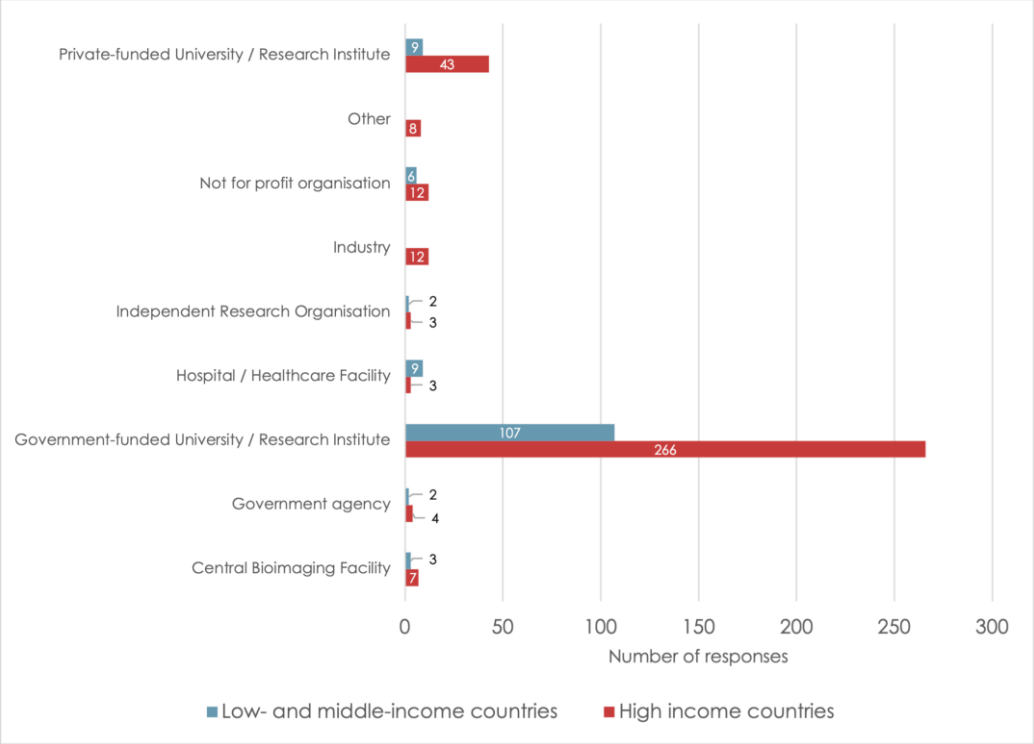
Figure 3 Role of respondents by country type (n=496)



- **Affiliation of respondents by country type**

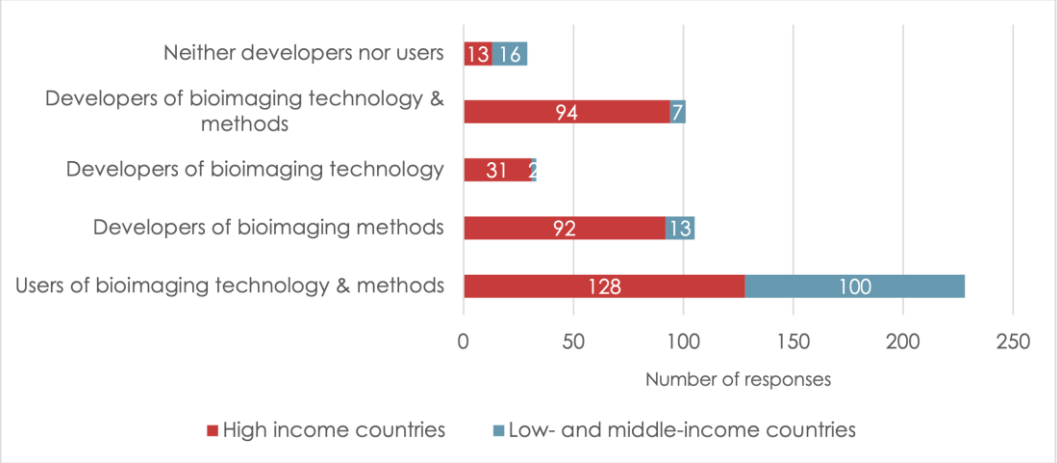
Over three-fourths of respondents were based in a government-funded university / research institute, of which HICs accounted for 54% (n = 266, 75% of HIC respondents) and LMICs 22% (n = 107, 78% of LMIC respondents).

Figure 4 Affiliation of respondents by country type (n=496)



- **Expertise of respondents by country type**

Figure 5 Expertise of respondents by country type (n=496)



When asked whether they use and/or develop bioimaging methods and/or technologies, 6% of respondents (n = 29)[3] stated they were neither developers nor users. Users of bioimaging technologies and/or methods represented 46% (n = 228) of total responses (Figure 5); 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

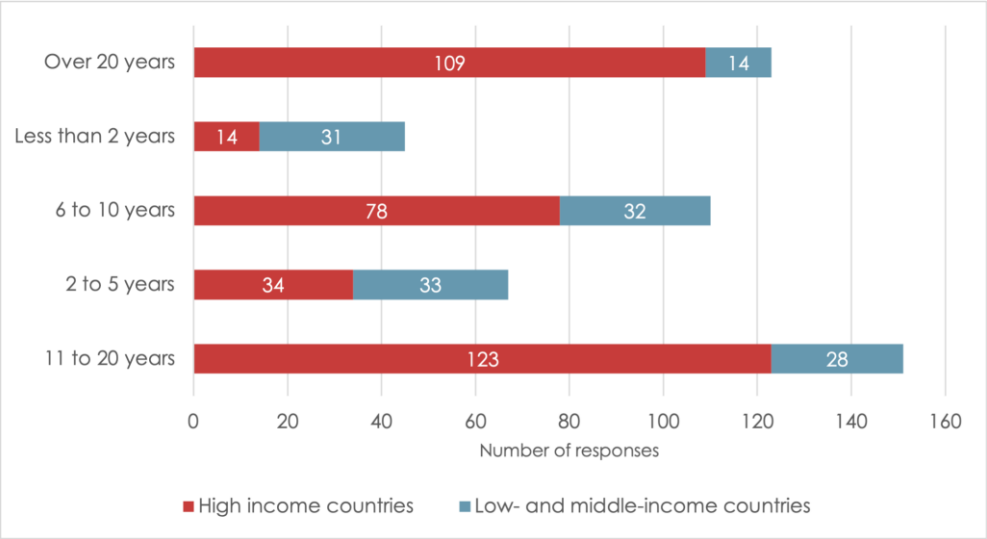
[3] 'Neither developers nor users' are respondents who selected the option: 'I don't use or develop bioimaging technologies and methods'.

respondents from HICs (n = 217 i.e. 43% of total respondents, 60% of HIC respondents and 91% of developers).

- **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).

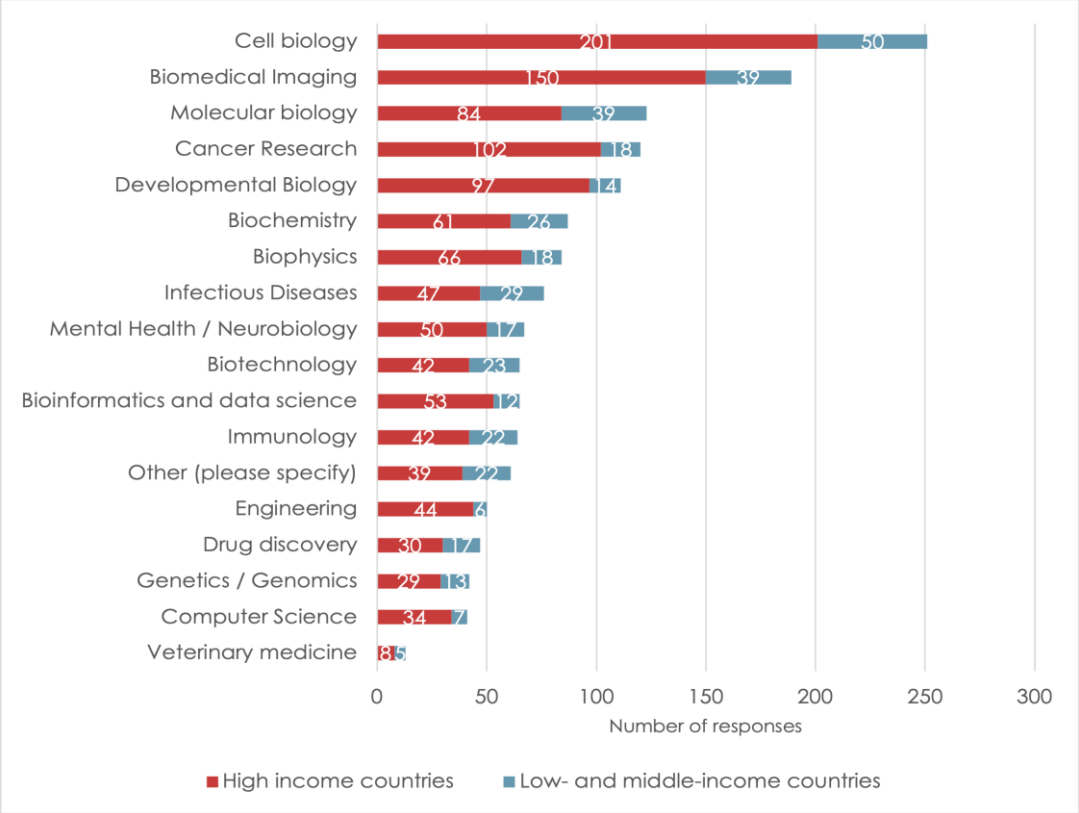
Figure 6 Years of experience of respondents by country type (n=496)



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

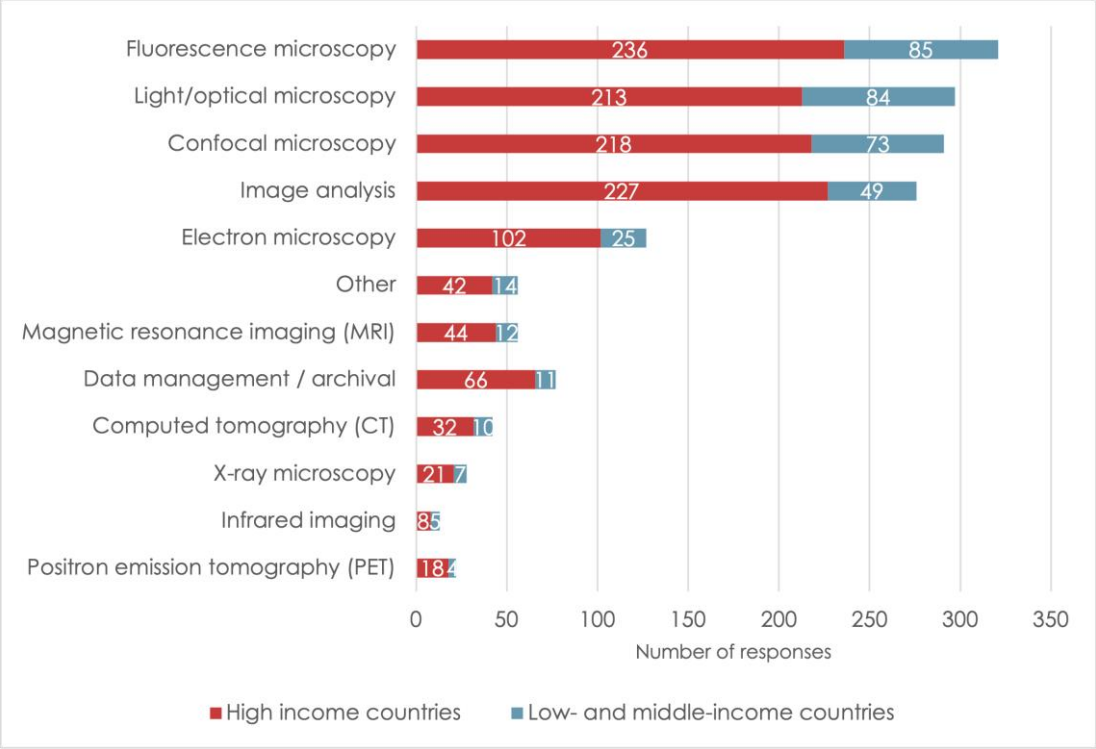
Respondents were asked to select at least one research field they were active in. The five most commonly selected fields overall were: cell biology (n = 251, 51%), biomedical imaging (n = 189, 38%), molecular biology (n = 123, 25%), cancer research (n = 120, 24%) and developmental biology (n = 111, 22%). 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 7 Research field of respondents by country type (n=496)



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

Figure 8 Bioimaging expertise of respondents by country type (n=496)



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.

1.3 Novel and emerging areas of bioimaging

- **The most transformative bioimaging technologies and methodologies**

Respondents were asked to select up to 3 bioimaging modalities that in their view are most likely to transform the field of bioimaging. Overall, the top five areas selected were fluorescence-based techniques (n = 222, 45%), allied approaches and tools (e.g. AI, probes, super-resolution) (n = 188, 38%), tissue and organ imaging (n = 164, 33%), electron microscopy (n = 135, 27%) and confocal microscopy (n = 92, 19%). The top five areas selected did not change across stakeholder types, with the exception of *developers of bioimaging methods* and *developers of both bioimaging technology and methods* whose top five included spectroscopy-based techniques instead of confocal microscopy. Similarly, the only difference in the top five areas for LMICs and HICs was that HIC respondents included expansion microscopy instead of confocal microscopy. Table 2 outlines the bioimaging areas selected by stakeholder group, including additional areas mentioned under the 'other' category.

Table 2 Most transformative areas of bioimaging by stakeholder group

Areas of bioimaging	Developers of technology & methods (n=101)	Developers of methods (n=105)	Developers of technology (n=33)	Neither developers nor users (n=29)	Users of technology & methods (n=228)	Total (n=496)
Fluorescence-based techniques	45 (44.6%)	47 (44.8%)	16 (48.5%)	28 (96.6%)	86 (37.7%)	222 (44.8%)
Allied approaches and tools (e.g. AI, probes, super-resolution)	38 (37.6%)	40 (38.1%)	15 (45.5%)	20 (69.0%)	75 (32.9%)	188 (37.9%)
Tissue and Organ Imaging	39 (38.6%)	38 (36.2%)	10 (30.3%)	28 (96.6%)	49 (21.5%)	164 (33.1%)
Electron Microscopy	20 (19.8%)	32 (30.5%)	5 (15.2%)	24 (82.8%)	54 (23.7%)	135 (27.2%)
Confocal microscopy	10 (9.9%)	13 (12.4%)	6 (18.2%)	25 (86.2%)	38 (16.7%)	92 (18.5%)
Expansion Microscopy	17 (16.8%)	16 (15.2%)	4 (12.1%)	7 (24.1%)	29 (12.7%)	73 (14.7%)
Spectroscopy-based techniques	20 (19.8%)	18 (17.1%)	4 (12.1%)	13 (44.8%)	17 (7.5%)	72 (14.5%)
Intravital Microscopy	13 (12.9%)	12 (11.4%)	4 (12.1%)	4 (13.8%)	16 (7.0%)	49 (9.9%)
Atomic Force Microscopy	3 (3.0%)	1 (1.0%)	3 (9.1%)	8 (27.6%)	17 (7.5%)	32 (6.5%)
Bioluminescence Imaging	6 (5.9%)	5 (4.8%)	4 (12.1%)	3 (10.3%)	12 (5.3%)	30 (6.0%)
Synchrotron X-Ray Tomography	6 (5.9%)	9 (8.6%)	1 (3.0%)	4 (13.8%)	10 (4.4%)	30 (6.0%)
Nonlinear Optical Microscopy	8 (7.9%)	6 (5.7%)	4 (12.1%)	2 (6.9%)	7 (3.1%)	27 (5.4%)
Quantitative Phase Imaging	8 (7.9%)	4 (3.8%)	2 (6.1%)	6 (20.7%)	7 (3.1%)	27 (5.4%)
Acoustic Microscopy	8 (7.9%)	3 (2.9%)	1 (3.0%)	3 (10.3%)	6 (2.6%)	21 (4.2%)
Episcopic Microscopy	2 (2.0%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	1 (0.4%)	4 (0.8%)

Label free imaging methods e.g. SRS	2 (2.0%)	1 (1.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	4 (0.8%)
Molecular/Nuclear Imaging	0 (0.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)	2 (0.9%)	3 (0.6%)
Optically Pumped Magnetometers applied to MEG	1 (1.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.4%)
AC Impedance Analysis	0 (0.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Functional neuroimaging	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Imaging flow cytometry (esp with sorting)	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Imaging of live specimen over time	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Magnetic Particle Imaging	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Metalens design and it applications in biological sciences	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Multimodal/multi length scale	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Near Infrared Spectroscopy	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Optical trap/tweezers	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Optically pumped magnetoencephalography	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
Paediatric neuroimaging	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
persistent luminescent imaging	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Simultaneous multi focal plane capture	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Spectral CT/ Photon counting CT	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Theranostics	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Total Body PET	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Ultra-high field (e.g.7T)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)
X-ray Diffraction tomography	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)

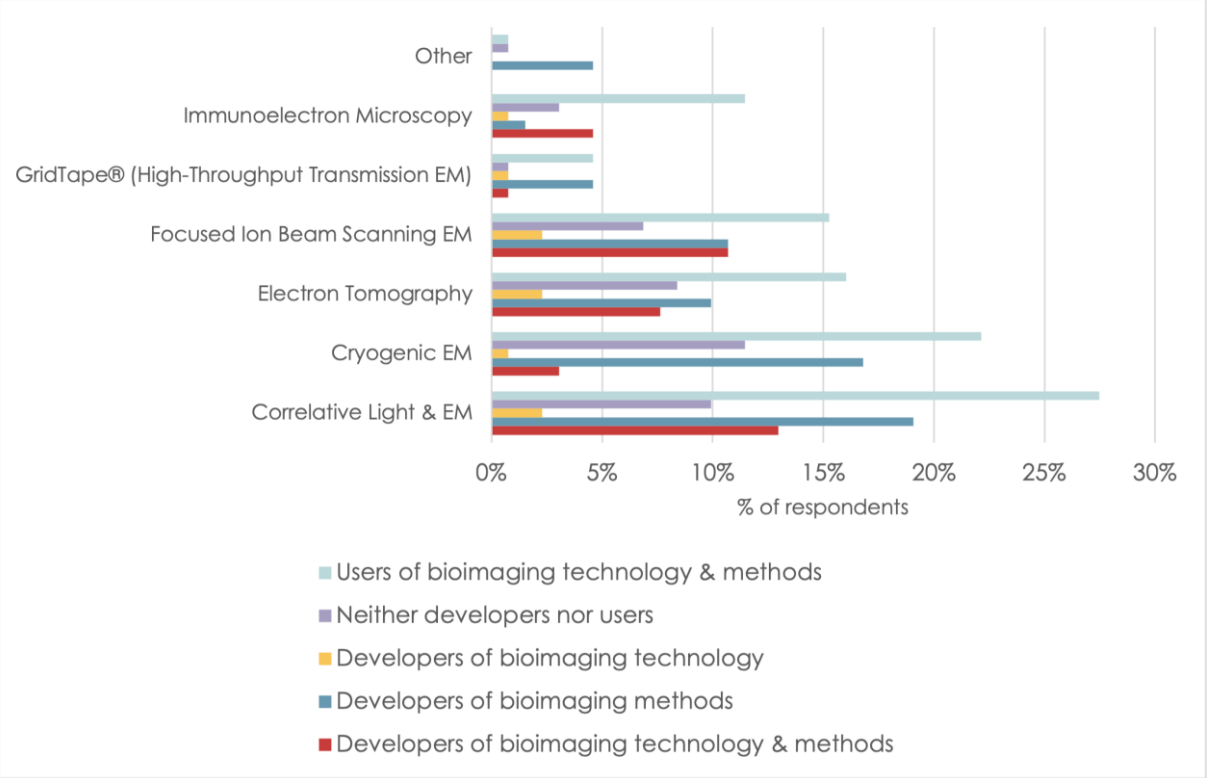
Respondents that selected electron microscopy, fluorescence-based techniques, spectroscopy-based techniques, tissue and organ imaging techniques or allied approaches and tools as the most transformative area(s) for bioimaging, were asked to further narrow down the specific method or technology within their selected area(s). In addition, respondents had the opportunity to provide context to their answers. One key point noted by respondents was that linking different methods will be a critical factor for advancing the bioimaging field, for example, linking protein/gene expression analysis with functional/structural analysis, deploying analytical chemical measurements (from the field of spectroscopy) as imaging techniques that can handle spatiotemporal information and extending spatial transcriptomics to super-resolution or live-imaging techniques.

- **The most transformative Electron Microscopy (EM) techniques**

Developers and users chose Correlative Light and Electron Microscopy (CLEM) as the most transformative EM technique. Technology developers ranked CLEM, Focused Ion Beam

Scanning Electron Microscopy (FIB/SEM) and electron tomography equally at the top. Users and method developers chose Cryogenic EM (Cryo-EM) as the second most transformative technique, while developers of both bioimaging technology and methods chose FIB/SEM. Additional techniques mentioned by respondents included volume EM (e.g. array tomography), 4D scanning transmission electron microscopy (4D STEM) and serial section array.

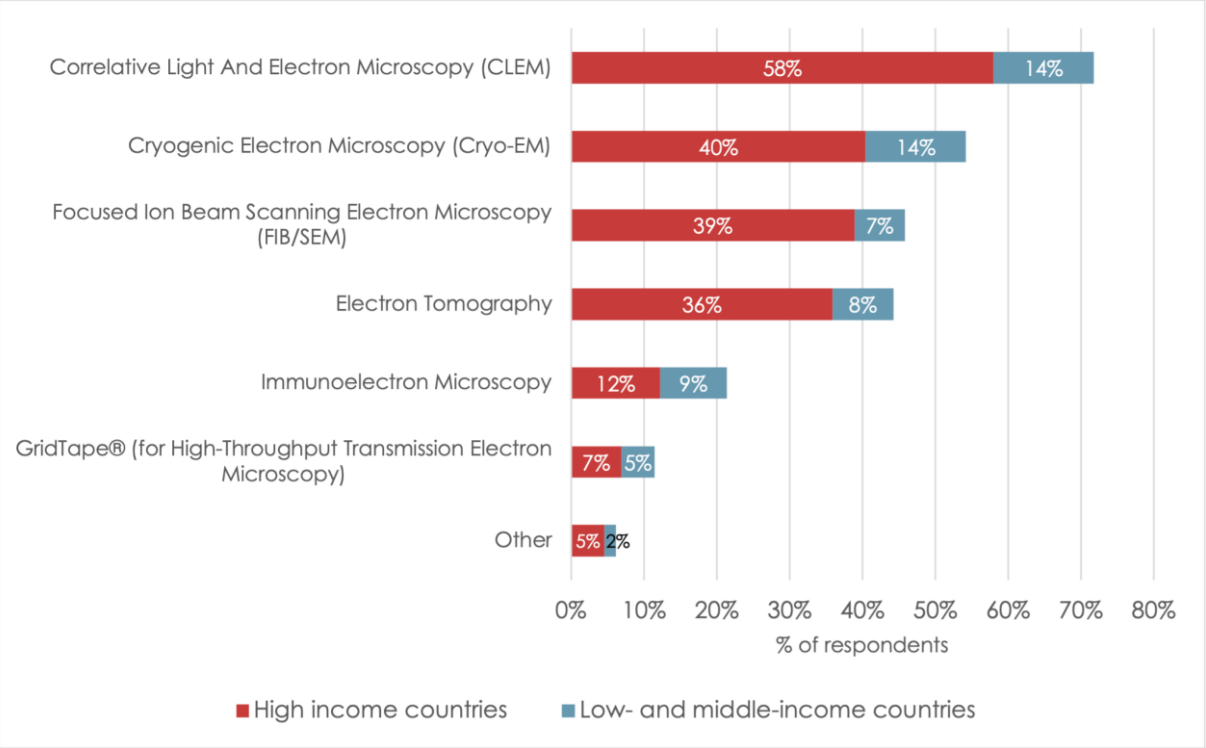
Figure 9 Most transformative Electron Microscopy techniques by stakeholder group (n=131)



Both HIC and LMIC respondents agreed that CLEM and Cryo-EM will be the two most transformative techniques in EM (Figure 10).

Respondents noted that CLEM will enable analysis of disease-relevant cells and tissue e.g. retinal pathologies as well as understanding of pathophysiological mechanisms (for example, through combination of live-cell or -tissue imaging techniques such as calcium imaging). CLEM is expected to lead to important and unexpected morphological insights, provided there are improvements in data handling and analysis methods to cope with the large amounts of data that will be generated. Respondents also highlighted that innovative sample preparation methods have the potential to make high-resolution imaging available to more research areas and noted that sample preparation aspects need more attention from researchers and funders. The potential of cryo-EM to help improve understanding of protein structure and accelerate drug discovery was mentioned several times by researchers from LMICs, but they also highlighted that access to such high-end technologies is still extremely scarce.

Figure 10 Most transformative Electron Microscopy techniques by country type (n=131)



- **The most transformative Fluorescence-based techniques**

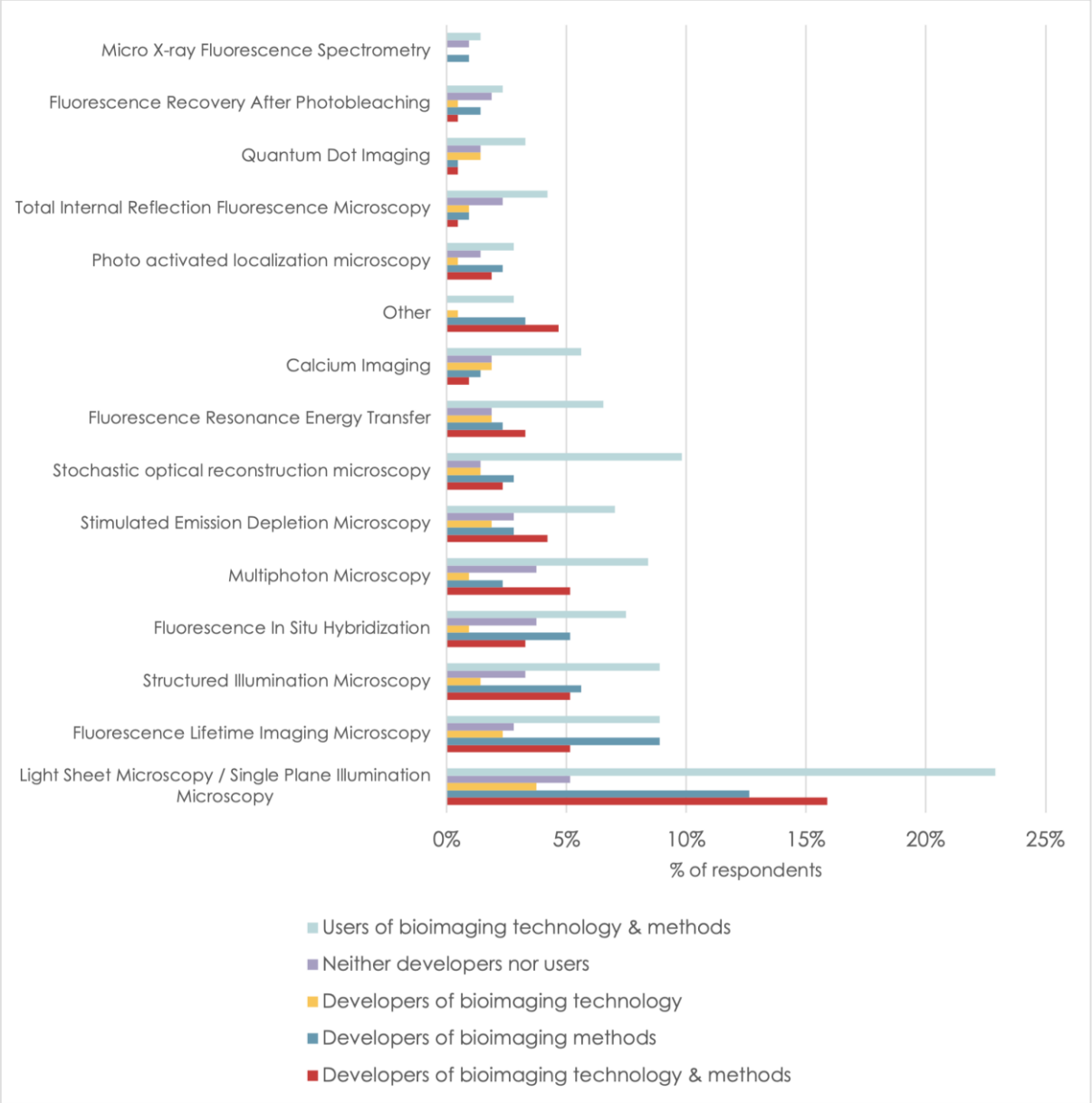
There was overall agreement across stakeholder groups that Light Sheet Microscopy / Single Plane Illumination Microscopy (SPIM) is going to be the most transformative fluorescence-based technique going forward. Among the remaining techniques, developers chose Fluorescence Lifetime Imaging Microscopy (FLIM) as the second most transformative technique, whereas users chose Stochastic Optical Reconstruction Microscopy (STORM) (Figure 11). Developers of both technology and methods also picked multiphoton microscopy and Structured Illumination Microscopy (SIM) as joint second with FLIM. 'Other' techniques mentioned included single-molecule localisation methods like MINFLUX (minimal photon fluxes) and RASTMIN (RASTer scanning a MINimum of light)[4], optogenetic techniques for brain mapping[5], Swept Confocally Aligned Planar Excitation (SCAPE) microscopy which allows volumetric imaging of living samples at ultrahigh speeds[6], high-resolution (submicron) multifunctional imaging of systems (whole organs or organ-system interactions) using functional fluorescence markers with positional labelling methods, label-free microscopy, fluorescent in situ sequencing (FISSEQ), spatial 'omics', spectral deconvolution, combinations of Fluorescence Resonance Energy Transfer (FRET) with super-resolution fluorescence microscopy, metabolic imaging, computational nanoscopy, holographic imaging and fluorescence fluctuation microscopy.

[4] 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

[5] 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

[6] 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

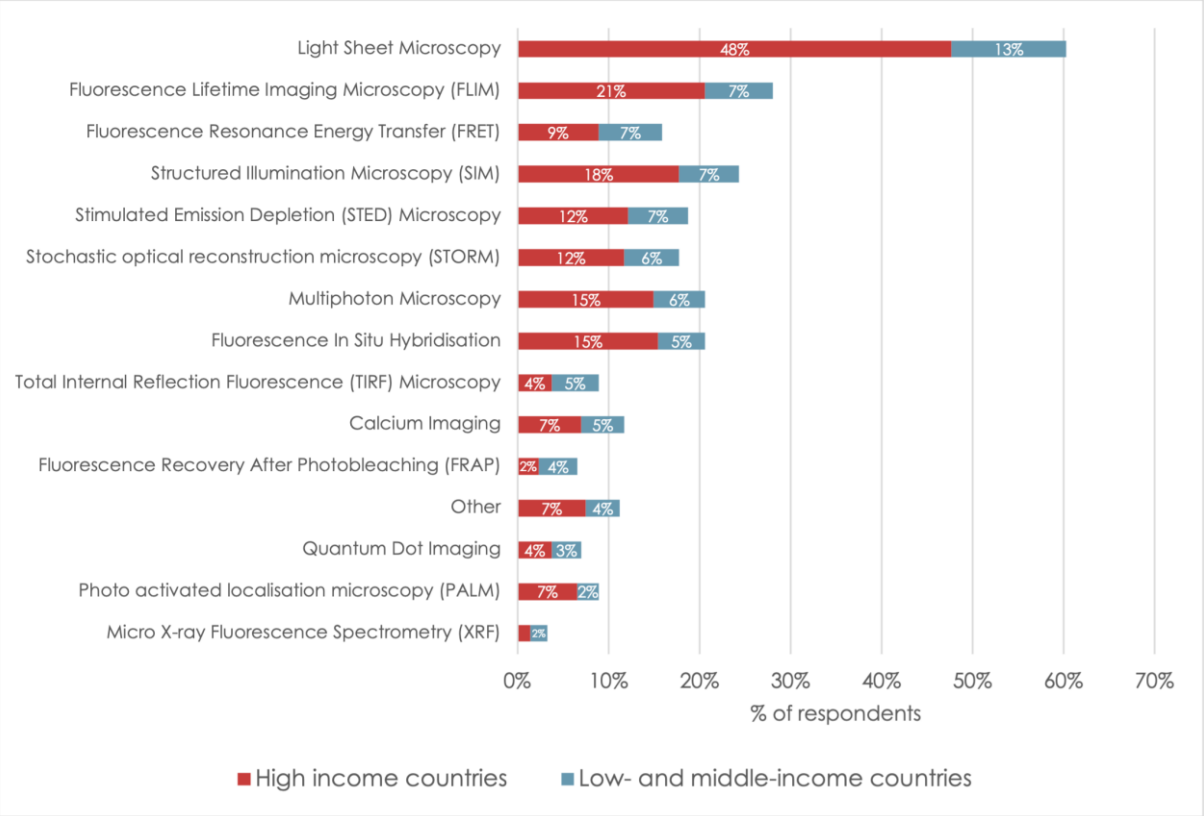
Figure 11 Most transformative Fluorescence-based techniques by stakeholder groups (n=214)



Respondents from HICs and LMICs also chose light sheet microscopy as the most transformative fluorescence-based technique, followed by FLIM and SIM for HICs and FLIM, SIM, Stimulated Emission Depletion (STED) microscopy and FRET for LMICs (Figure 12).

Respondents further discussed the use and transformative potential of their chosen techniques. In situ hybridisation was seen as important to understand changes in mRNA, increasing the demand for labs to set up such techniques. For light sheet microscopy, respondents highlighted the need and challenge of imaging tissues and organoids rather than just sections to enable visualisation at the cellular level. Increasing the number of fluorophores/labels that can be used in a single experiment was noted as a key factor that will help advance FLIM as a technique. The association of spectroscopy techniques with both FLIM and light sheet microscopy will potentially lead to major leaps in the understanding of molecular dynamics. Similarly, the combination of in-vivo imaging using light sheet microscopy with spectral deconvolution was noted as important for gaining insights into pathologies. SIM was seen as a very efficient technique for unravelling molecular interactions without the need for special sample preparation.

Figure 12 Most transformative Fluorescence-based techniques by country type (n=214)



- **The most transformative Spectroscopy-based techniques**

Hyperspectral imaging was seen as having the most potential overall among spectroscopy-based techniques (56%, 36 of 65 respondents) followed by fluorescence correlation spectroscopy (51%, 33 of 65 respondents). The first choice however differed across stakeholder groups. Hyperspectral imaging was the first choice for those who develop either methods or technologies (exclusively), while fluorescence correlation spectroscopy was seen as most useful by users and those who develop both bioimaging technology and methods (Figure 13). 'Other' techniques mentioned by respondents included mass spectroscopy, magnetic resonance spectroscopic techniques (in-vivo), Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), atom probe tomography and human metabolic spectroscopy techniques.

Respondents from HICs and LMICs also assessed the potential of specific spectroscopy-based techniques differently, as shown in Figure 14. Hyperspectral imaging, fluorescence correlation spectroscopy and Raman spectroscopy received the most endorsements from HICs, while fluorescence correlation spectroscopy, hyperspectral imaging and Fourier-Transform Infrared Spectroscopy (FTIR) were the top choices for LMICs.

Figure 13 Most transformative spectroscopy-based techniques by stakeholder groups (n=65)

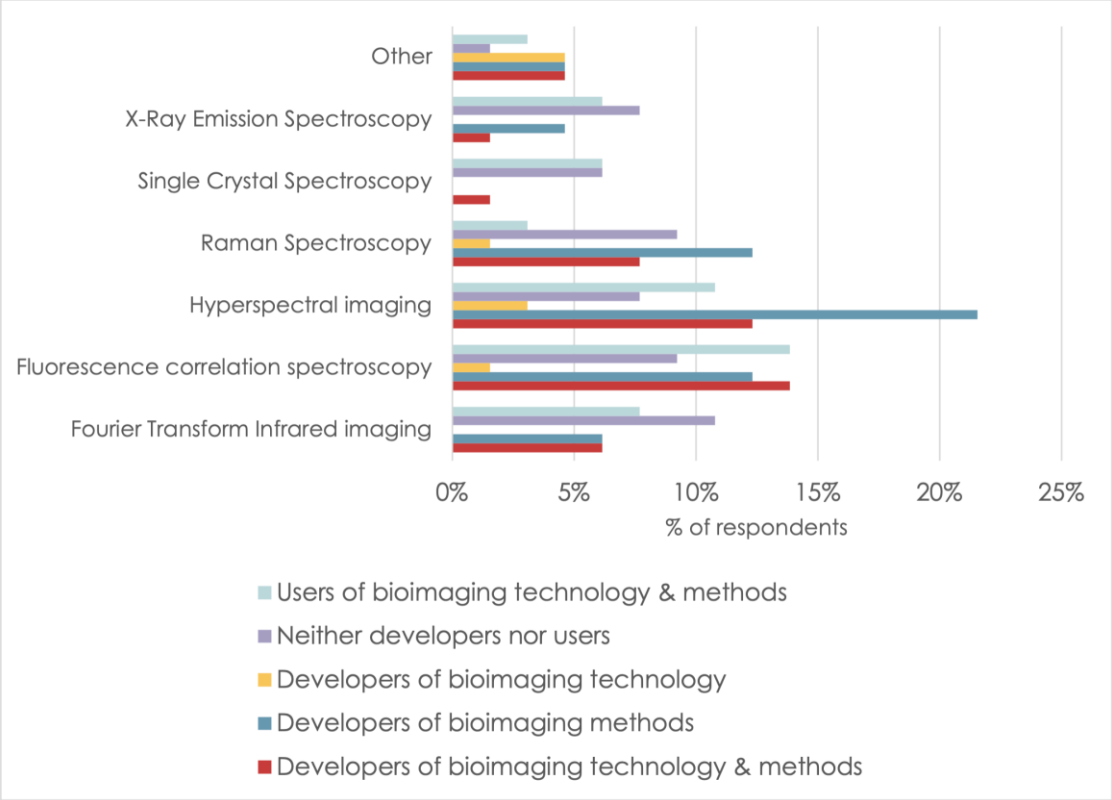
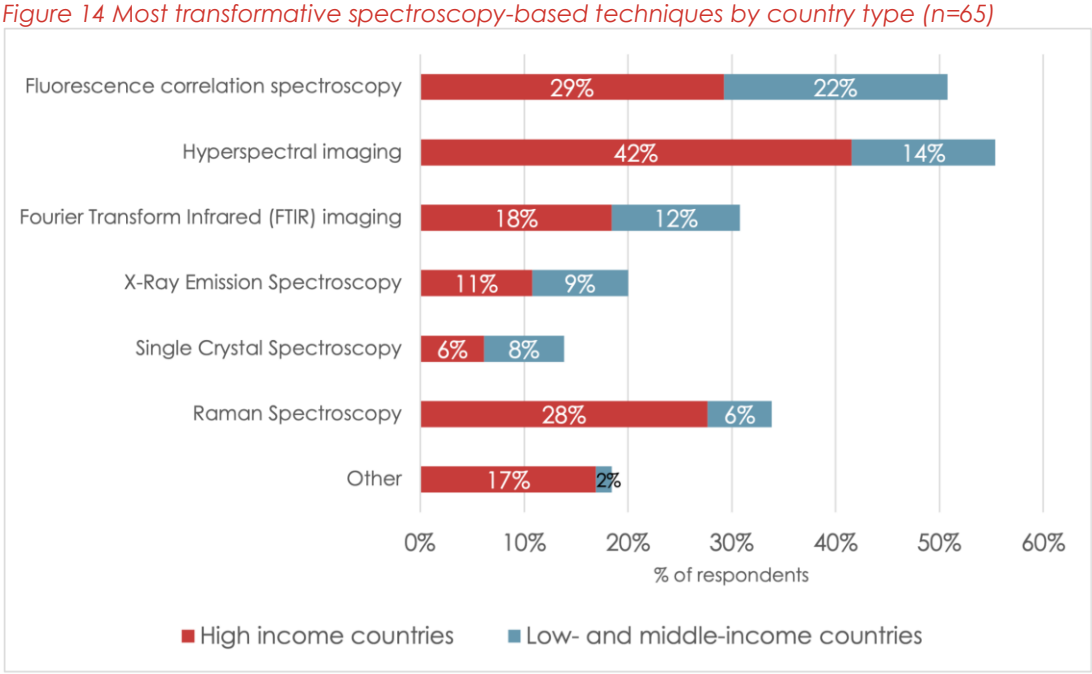


Figure 14 Most transformative spectroscopy-based techniques by country type (n=65)



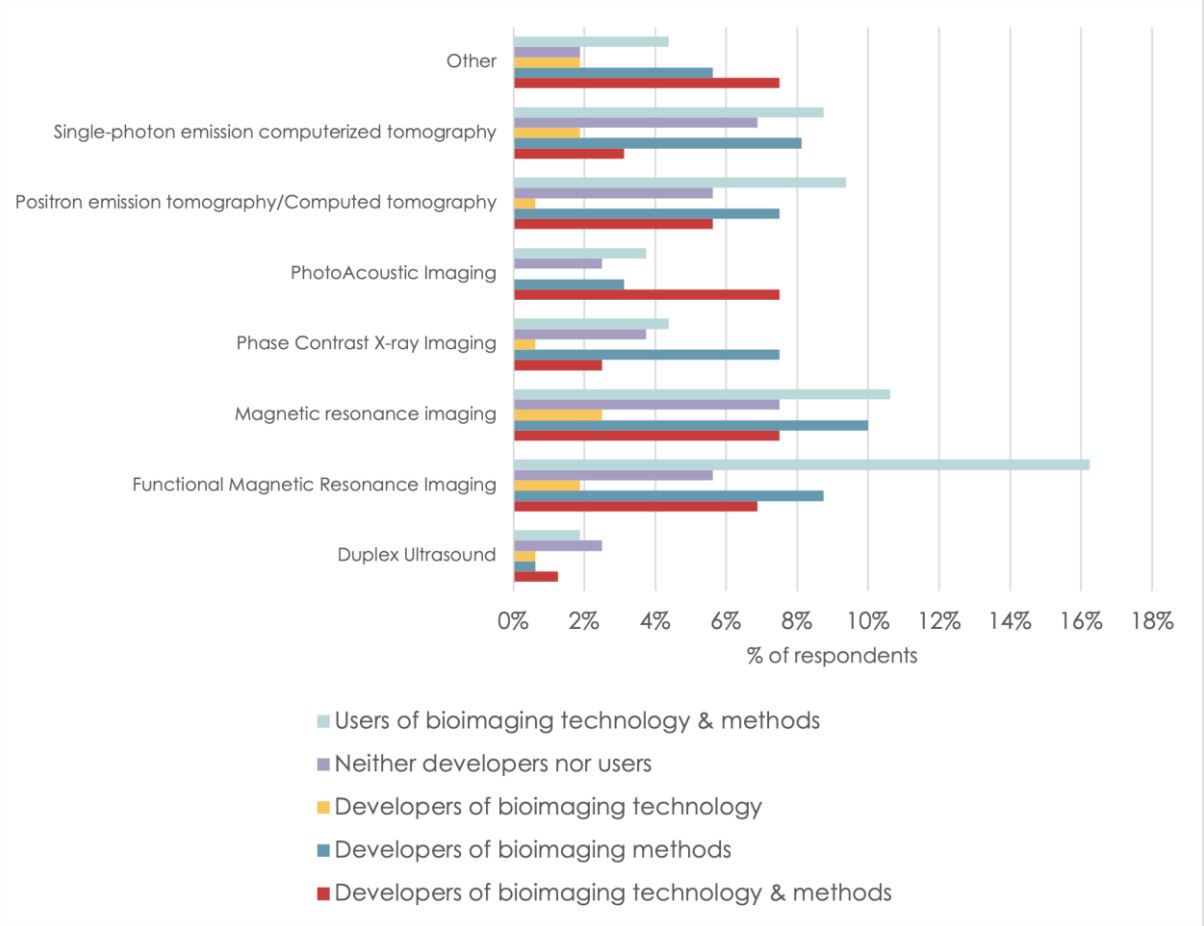
- **The most transformative tissue and organ imaging techniques**

Overall, functional Magnetic Resonance Imaging (fMRI) and Magnetic Resonance Imaging (MRI) were considered the most transformative tissue and organ imaging techniques. However, there were differences across stakeholder groups as shown in Figure 15 below. While the

transformative potential of fMRI and MRI was noted by most stakeholder groups, photoacoustic imaging was also picked as a key technique by developers of both technologies and methods.

Additional techniques mentioned included dual energy CT, echocardiography, next generation ultrasound, in-vivo multiphoton microscopy and connectomics. The combination of tissue and organ clearing with expansion microscopy was also mentioned as a potentially transformative approach.

Figure 15 Most transformative Tissue and Organ Imaging techniques by stakeholder group (n=160)

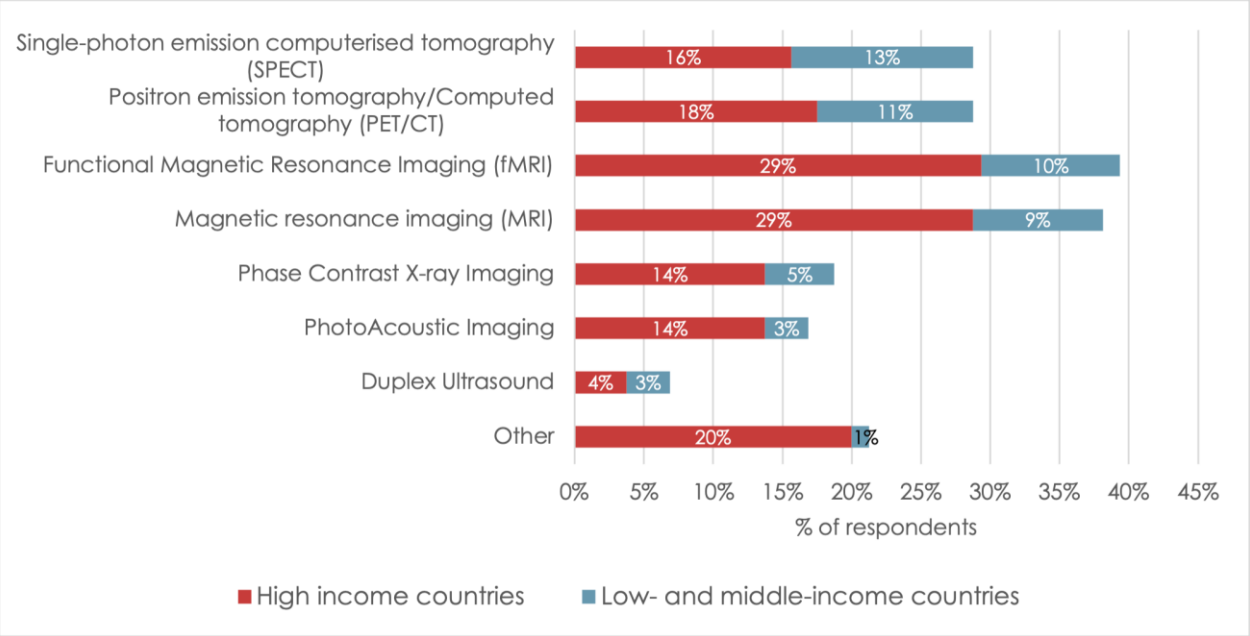


There was no agreement between HICs and LMICs respondents about the most transformative tissue and organ imaging techniques, with LMICs ranking single-photon emission computerised tomography (SPECT) as their first option, while HICs preferred fMRI (Figure 16).

Respondents noted that imaging of in-vivo processes with high signal-to-noise methods will be the key to transforming tissue and organ imaging. Developing techniques for visualisation at intracellular level, both in-vivo or ex-vivo, will enable better understanding of cell activity and contribute to neuroscience, cancer research and many other fields. Novel contrasts for MRI and photoacoustic imaging that can be adapted for different imaging scales was highlighted as a key need to further the impact of these techniques. Breakthroughs are also expected through the use of correlative, multi-modal methods and in-vivo measurements (e.g. combining X-ray imaging and X-ray diffraction or X-ray CT and spectral detection). Improvements in quality checks for phototoxicity, technical expertise and data analysis tools will collective help improve live cell, organ and tissue imaging. Optically pumped magnetometers were also noted as having great potential to change paediatric brain

imaging, as this technology is more flexible than conventional magnetoencephalography systems.

Figure 16 Most transformative Tissue and Organ Imaging techniques by country type (n=160)



- **The most transformative allied approaches and tools**

Artificial Intelligence (AI) and Machine Learning (ML) approaches to image analysis was considered the most transformative allied bioimaging approach / tool across all stakeholder groups and country types. Imaging-based spatial proteomics/transcriptomics, super-resolution microscopy and high-throughput microscopy were next in line of importance, although there were some minor differences in ranking across stakeholder groups and country types. Approaches and tools under the 'other' category included: correlative microscopy, single-pixel imaging, automation (e.g. AI/ML driven image acquisition), adaptive optics, integration across modalities into searchable atlases, theranostics, novel diagnostic radiopharmaceuticals and immersive visualisation of 3D image datasets (virtual reality).

The benefits of some of these allied approaches and tools were discussed at length by respondents. AI and ML can help improve performance of microscopes and real-time imaging, allowing multiplexed automated imaging in live cells. ML could also help to identify specific relevant events and thereby minimise photodamage as well as to analyse large imaging datasets e.g. from high-throughput imaging. 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.

Development of novel fluorescent probes and combining light sheet microscopy and AI techniques is expected to have significant impact in the understanding of living organisms, tissues and organoids through enabling 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. Spatial transcriptomics is driving developments in sample preparation, labelling strategies and complex data analysis, and there is potential for implementing such advances in other research areas. Another area highlighted was Quantitative Phase Imaging (label-free and quantitative methods to map refractive index of live cells and tissues), a non-destructive approach with applications in biopharmaceuticals and cancer therapies.

Figure 17 Most transformative Allied approaches and tools by stakeholders (n=176)

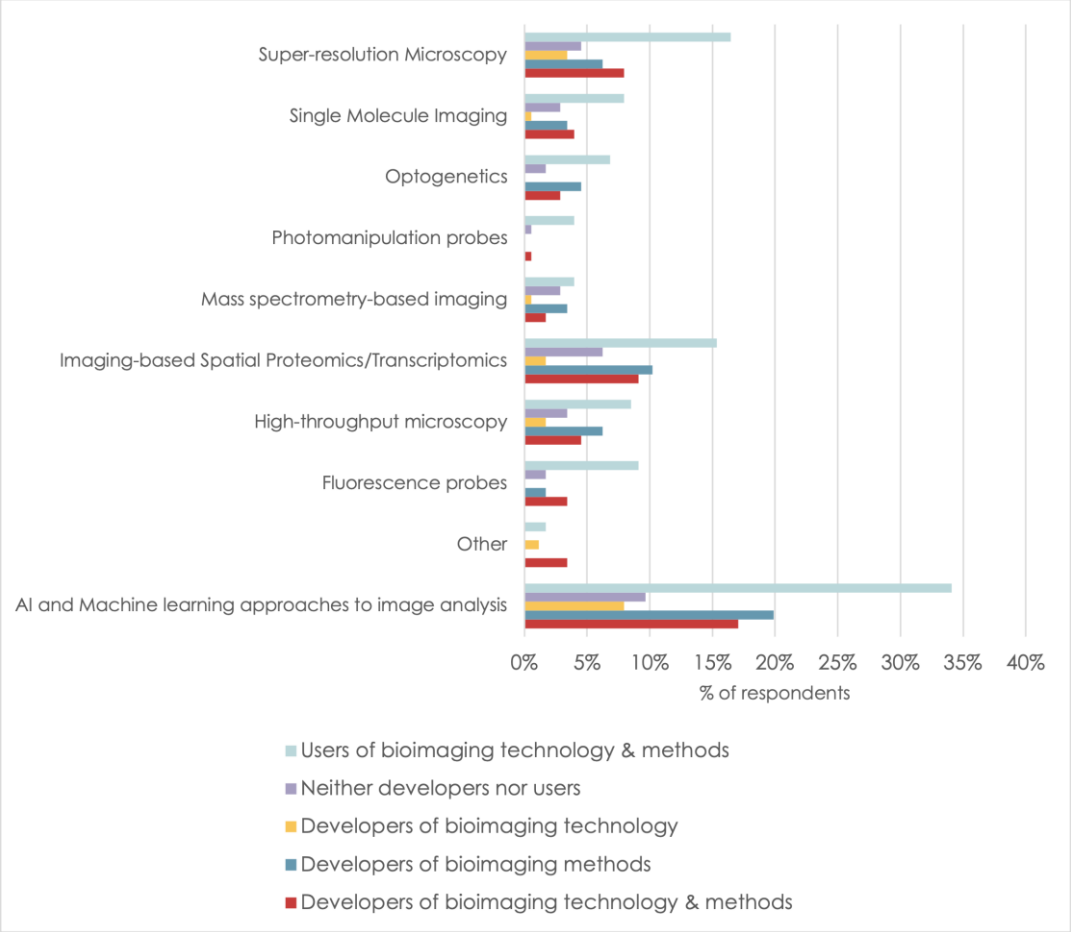
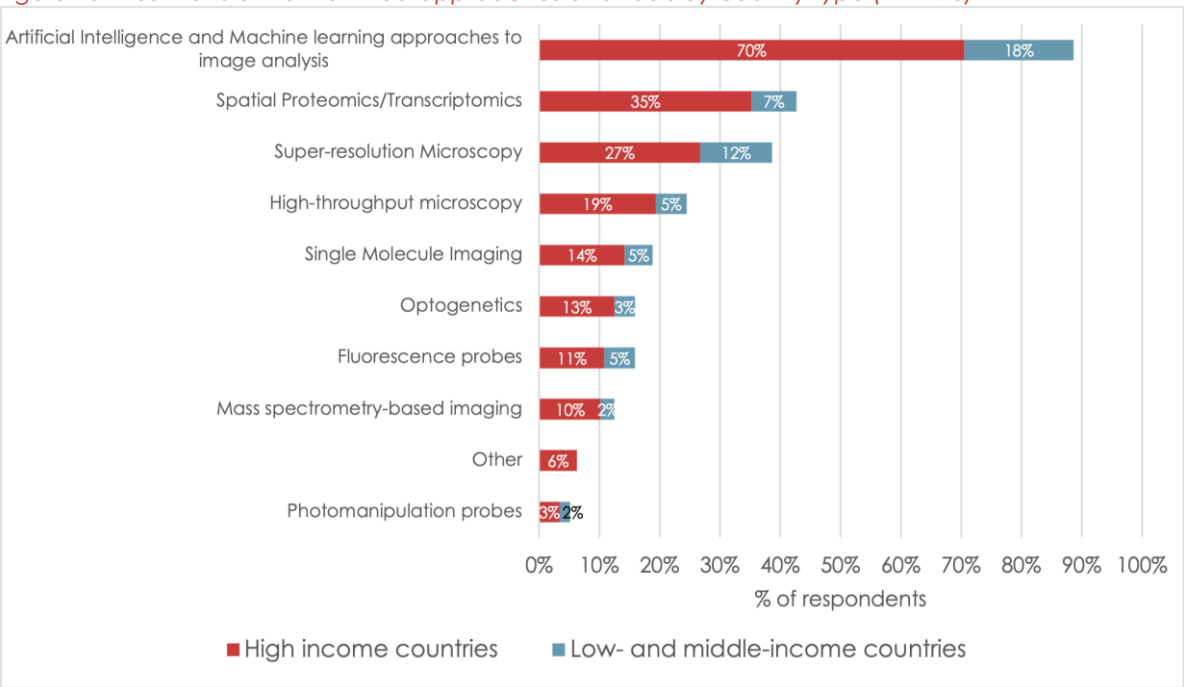


Figure 18 Most transformative Allied approaches and tools by country type (n = 176)



Super-resolution microscopy is expected to enable better understanding of sub-resolution structural changes in biological systems. Improving super-resolution methods through new labelling strategies and combination with FRET imaging could lead to more insights on sub-10 nanometre scales.

1.4 Barriers limiting progress in the field of bioimaging

- **Scientific or technological barriers**

For both respondents from HICs and LMICs, 'quality and reproducibility challenges' are limiting progress in the field of bioimaging to the largest extent (Figure 19 and Figure 20). Challenges in scaling up of techniques for high throughput of samples or image analysis were the second most important barrier for LMICs, whereas for HICs the second most limiting barrier was quantitation challenges.

Figure 19 The extent to which scientific or technological barriers limit progress in High-income countries (n=261)

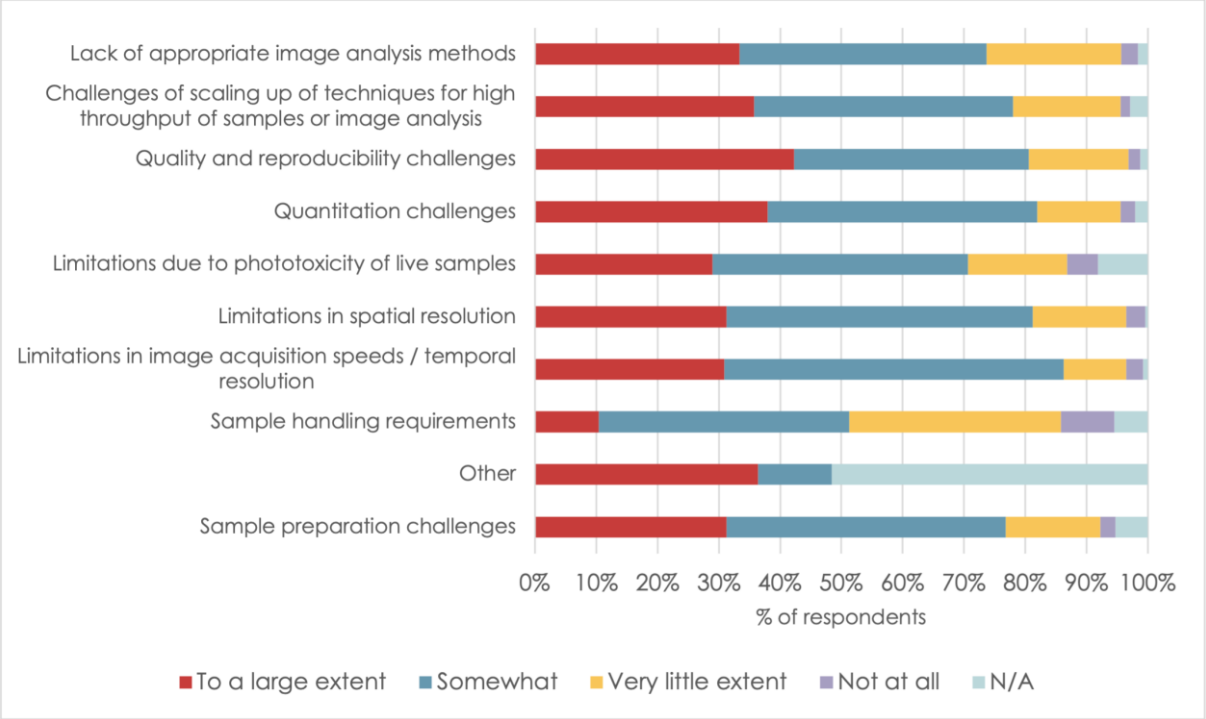
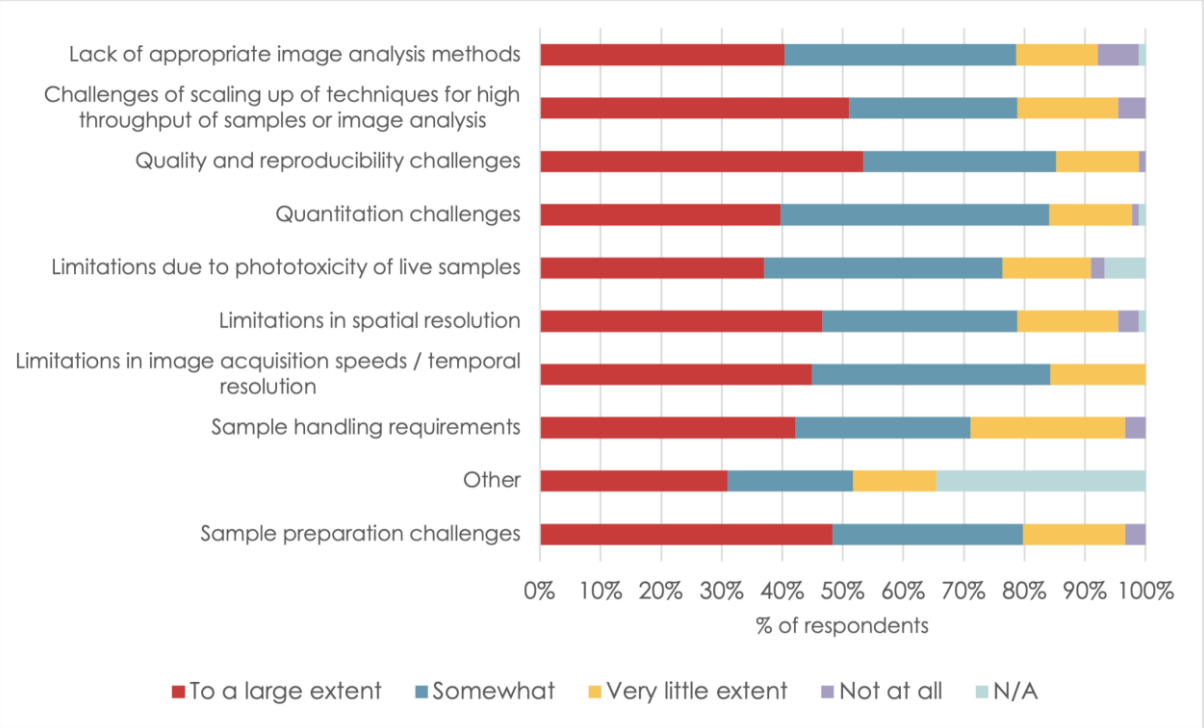


Figure 20 The extent to which scientific or technological barriers limit progress in Low- and middle-income countries (n=91)



Respondents were also asked to select the scientific or technological barrier that needs to be addressed as a priority in the next 5 to 10 years (n = 340). For HICs (n = 253), the top three barriers selected were

1. Quality and reproducibility challenges (n = 54, 21%)
2. Lack of appropriate image analysis methods (n = 43, 17%)
3. Quantitation challenges (n = 28, 11%)

Table 3 below provides a summary of the open-text responses from HICs that delineate the issues underlying the priority scientific or technological barriers and potential solutions to address them.

Table 3 Top three scientific or technological barriers limiting the field of bioimaging in high-income countries

Barrier	Key issues	Solutions
Quality and reproducibility challenges	Lack of comparability and reproducibility of published data / lack of reproducibility of protocols (n = 21)	Collaboration across stakeholders (scientists, developers, etc) (n = 6)
		Better development, reporting and sharing of methods and protocols (n = 4)
		Share primary data and imaging workflows (n = 2)
		Funding for repeat studies and technical experts to do peer reviews, appropriate communication/recording of metadata (n = 4)
	Lack of quality control and reproducibility for instruments, Lack of quality standards /	Better monitoring of microscopy performance (e.g see QUAREP initiative) (n = 4)
		Stringent quality control requirements from publishers and funders (n = 4)

	standardised protocols (n = 9)	Standards for publishing data analysis, sample preparation and development of interoperable tools (n = 14)
Lack of appropriate image analysis methods	Appropriate image analysis methods to cope with scale and nature of images captured or reduce burden of image acquisition (n = 8)	Further implementation of AI and deep learning in image analysis/acquisition (n = 9)
		Sharing of data and computer codes (n = 2)
	Large data sets and complex data analysis required (n = 8)	Training & best practise guidelines (n = 7)
		Funding for experts in image analysis, bioinformatics and research software engineers to developing more functionalities for users of bioimaging technologies (n = 3)
	Lack of adequate data management and software (n = 4)	Dedicated resources for data management and image analysis projects (n = 4)
		New (open) software, data hubs and imaging modalities (n = 3)
Quantitation challenges	Lack of training on quantitative and image analysis methods, Lack of maths and computing skills among bioscientists (n = 3)	Imaging analysis training courses (n = 3)
	Technical challenges e.g. noise, quantifying signals in scattering media, quantifying rate of change, large datasets (n = 6)	Standards for data and imaging analysis across different providers of equipment, international standards (n = 3)
		Better sample size and preparation controls (n = 1)
		More collaboration across disciplines (physics, maths, etc) (n = 1)

For LMICs (n = 87), the main barriers are as follows:

1. Quality and reproducibility challenges (n = 13, 15%), sample preparation challenges (n = 13, 15%)
2. Challenges of scaling up of techniques for high throughput of samples or image analysis (n = 9, 10%), limitations in spatial resolution (n = 9, 10%), limitations due to phototoxicity of live samples (n = 9, 10%)
3. Quantitation challenges (n = 8, 9%), limitations in image acquisition speeds / temporal resolution (n = 8, 9%)

Table 4 below provides a summary of the key issues in LMICs that contribute to the main scientific or technological barriers, including potential solutions suggested by respondents.

Table 4 Top scientific or technological barriers limiting the field of bioimaging in low- and middle-income countries

Barrier	Key issues	Solutions
Quality and reproducibility challenges	Lack of comparability and reproducibility of published data / lack of reproducibility of protocols (n = 5)	Formalisation of appropriate validation tests for techniques (n = 1)
		More publication of standards, detailed sample preparation methods and imaging processing analysis (n = 1)

		Open storage and better procedures to record metadata (n = 1)
Sample preparation challenges	Lack of consistent and reliable sample preparation (n = 3)	Sample preparation method development (incl. support for such development), better reporting/sharing of methods (n = 3)
		State of the art equipment and facilities to allow better sample preparation (n = 2)
		More training (n = 3)
Challenges of scaling up of techniques for high throughput of samples or image analysis	Lack of training (n = 1)	Training in data acquisition and analysis methods (n = 3)
	Need for high throughput or automated methodologies for microscopy and image processing (n = 3)	Hardware and software development (n = 3)
Limitations in spatial resolution	Limitations of imaging equipment (n = 1)	Funding for state-of-the-art bioimaging equipment, upgrading equipment and better training (n = 3)
Quantitation challenges	Technical challenges e.g. noise, large datasets (n = 2)	Funding for experts and training in image analysis, bioinformatics and research software (n = 3)

- **Infrastructural barriers**

For both respondents from HICs and LMICs, the high cost of bioimaging equipment/infrastructure was the barrier limiting progress to the largest extent (Figure 21 and Figure 22). While lack of availability of appropriate technical expertise (e.g. experienced imaging scientists) was also an important barrier for both LMICs and HICs, the availability and cost of bioimaging equipment and expertise limits progress in the bioimaging field to a larger extent in LMICs. Most of the infrastructural barriers impact to a large extent in LMICs (over 50% of respondents) compared to HICs where the impact is more varied (to a large extent, somewhat, to a very little extent). Other infrastructural challenges included access challenges (specialist imaging/gatekeeping), high cost of service contracts and sustainability of facilities, lack of training on new techniques and dissemination of knowledge, lack of support/expertise in data management and analysis and not enough use/development of Open-Source software.

Figure 21 The extent to which infrastructural barriers limit progress in High-income countries (n=245)

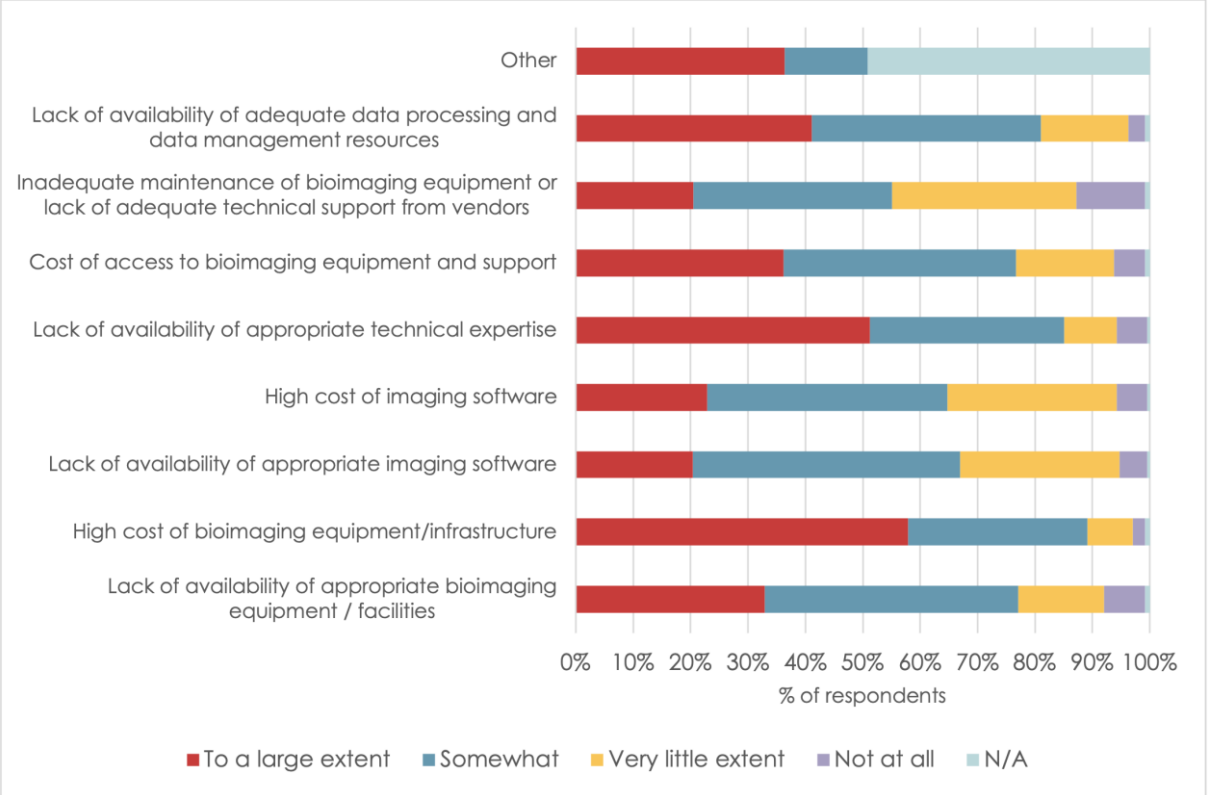
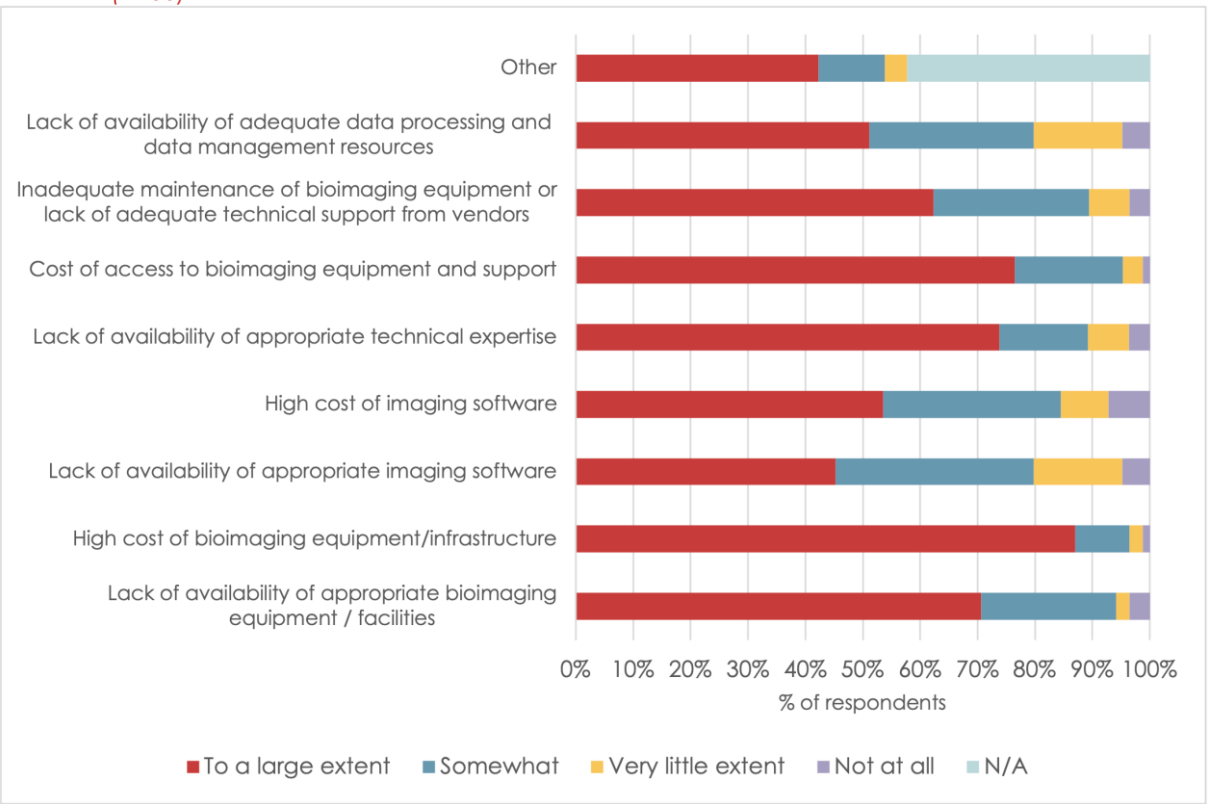


Figure 22 The extent to which infrastructural barriers limit progress in Low- and middle-income countries (n=86)



For HICs (n = 243), the top three barriers are

1. Lack of availability of appropriate technical expertise (e.g. experienced imaging scientists) (n = 71, 29%)
2. High cost of bioimaging equipment/infrastructure (n = 47, 19%)
3. Lack of availability of adequate data processing and data management resources (e.g. computing capacity, repository/archives) (n = 43, 18%)

Table 5 below provides a summary of the open-text responses from HICs that provide context to the top three infrastructural barriers that should be addressed as a priority in the next 5 to 10 years and potential solutions to address these.

Table 5 Top three infrastructural barriers limiting the field of bioimaging in high-income countries

Barrier	Key issues	Solutions
Lack of availability of appropriate technical expertise (e.g. experienced imaging scientists)	Lack of expertise to run and maintain infrastructure/equipment; Poor (inefficient) utilisation of existing facilities/methods due to lack of knowledge/awareness (n = 13)	More funding for postdocs and bioimaging scientist/analyst roles (incl. training grants, studentships, postdoc funding) (n = 11)
		Mentoring and training programmes for bioimaging (also image processing and analysis); funding for platforms to educate experts in bioimaging (n = 11)
		More BSc and MSc degree programmes for imaging (n = 3)
	Lack of permanent positions and career paths/funding for staff/recognition (n = 14)	Dedicated career path for imaging facility staff (n = 12)
		Recognition and Promotion for Imaging Scientists (n = 8)
		Competitive salaries (n = 3)
	Lack of available experts (in imaging analysis) / lack of retention of expertise (n = 10)	Grants for centres of excellence (n = 4)
	Interdisciplinary challenges (n = 2)	Collaboration and training including across disciplines (incl. imaging processing) or sectors (n = 5)
High cost of bioimaging equipment/infrastructure	Lack of financially sustainable model for imaging facilities; lack of funding for equipment/infrastructure (n = 4)	Funding for core facilities to include long-term institutional commitment for equipment and salaries for staff (n = 6)
		New leasing and maintenance models, include replacement costs in financial planning of facilities (n = 2)
	High costs of state-of-art equipment and maintenance (n = 18)	Pool funding & sharing of resources, funding for researchers to access existing infrastructure (n = 6)
		More centralised imaging facilities with appropriate support (e.g. AI imaging analysis) (n = 3)
		Funding for small institutions (n = 2)
Lack of availability of adequate data processing and data management resources (e.g. computing capacity, repository/archives)	Data management and metadata challenges (incl. lack of data storage infrastructure, lack of standardised approaches) (n = 10)	Community-led data and metadata formats and tools to promote adoption (n = 1)
		Standardised analysis pipeline (n = 1)

	Large and complex datasets (n = 13)	Require data management plans for grants; encourage FAIR data for publications (n = 1)
		More funding for digital infrastructure, data integration and analysis hubs; long-term funding for imaging repositories (n = 7)
		Incentivise data sharing and build infrastructure to enable global data sharing (n = 2)
		Funding for central computing facilities for image processing (n = 1)
		Integration of data from microscopy vendors with laboratories and cloud service providers (n = 1)
	Lack of data analysis expertise particularly among end-users (n = 3)	Funding for Training & best practices, esp. for biologists (n = 5)
	Lack of standardised or automated data processing (n = 2)	Make use of AI and deep learning approaches (n = 1)

For LMICs (n = 85), the top three barriers are

1. Lack of availability of appropriate bioimaging equipment / facilities (n = 34, 40%)
2. High cost of bioimaging equipment/infrastructure (n = 14, 17%)
3. Lack of availability of appropriate technical expertise (e.g. experienced imaging scientists) (n = 12, 14%)

Table 6 below summarises the key issues underpinning the key barriers in LMICs and potential solutions provided by respondents.

Table 6 Top three infrastructural barriers limiting the field of bioimaging in low- and middle-income countries

Barrier	Key issues	Solutions
Lack of availability of appropriate bioimaging equipment / facilities	Lack of bioimaging infrastructure (e.g. very limited equipment available, in particular advanced microscopes) (n = 10)	Funding for core/regional facilities (n = 1)
		Funding for infrastructure and (expensive) equipment (n = 11)
		Imaging consortiums, sharing platforms (n = 1)
		Open hardware & 3D printing (n= 1)
		Donation of imaging equipment (n =1)
High cost of bioimaging equipment/infrastructure	High costs of state-of-art equipment and maintenance (n = 7)	Funding for upgrading equipment/infrastructure (e.g. PET, SPECT, MRI) (n = 1)
	Lack of access to training (n = 1)	Funding for capacity development (n = 3)
	Lack of opportunities for researchers (n = 1)	Funding for researchers to access infrastructure (n = 2)
	Lack of awareness and institutional support to	Funding for buying and maintaining entry-level imaging equipment (n = 1)

	advance bioimaging (n = 2)	Funding for core facilities to include long-term institutional commitment for equipment and salaries for staff, more centralised facilities (n = 2)
Lack of availability of appropriate technical expertise (e.g. experienced imaging scientists)	Poor utilisation of existing facilities due to lack of knowledge/awareness (n = 3)	Funding for platforms to educate experts in bioimaging (n = 6)
	Lack of expertise to run and maintain infrastructure/equipment (n = 2)	Mentoring and training programmes for bioimaging (also image processing and analysis) (n = 4)

- **Other barriers**

Lack of career pathways for technical staff and data scientists working in the bioimaging is an important barrier in both LMICs and HICs, with 60% of HIC respondents confirming that it impacts 'to a large extent' (Figure 23). For LMICs, several barriers were noted to have a large effect, including access and high costs of consumables, lack of training opportunities and lack of funding for bioimaging technology, methodology and tool development (Figure 24). Other challenges and barriers mentioned by individual respondents included

- lack of funding for consistent access to infrastructure, for hiring dedicated specialist staff to run imaging equipment, for training users and advice on experiment planning and assay development as well as for maintenance of equipment
- lack of understanding of certain techniques leading to wrong conclusions
- not enough transfer of technology from HICs to LMICs
- limited access to local expertise and equipment (in particular in LMICs)
- lack of financial sustainability for universities to upgrade systems
- lack of partnerships to exploit technologies and accelerate the development of new solutions

Figure 23 The extent to which other barriers limit progress in High-income countries (n=241)

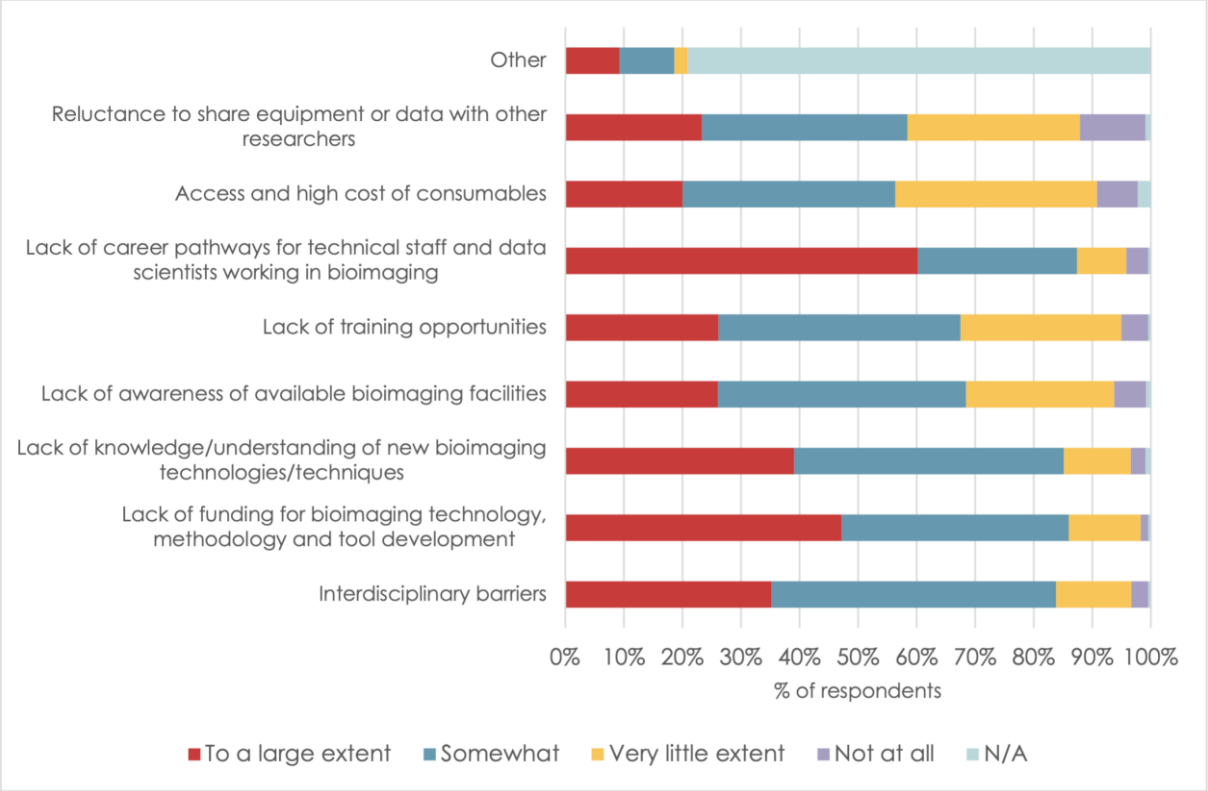
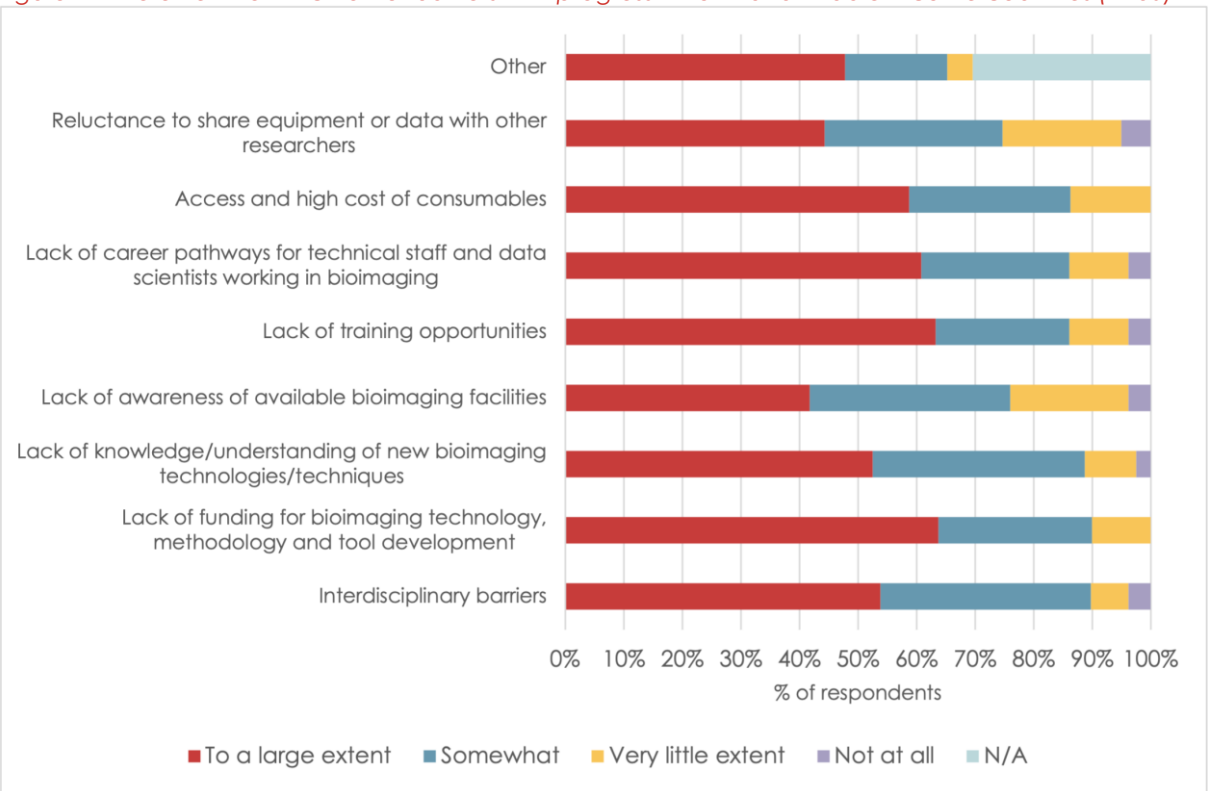


Figure 24 The extent to which other barriers limit progress in Low- and middle-income countries (n=80)



Respondents had a chance to select another barrier that needs to be addressed as a priority in the next 5 to 10 years to advance the field of bioimaging (n = 314). Overall, the most selected

barrier was Lack of funding for bioimaging technology, methodology and tool development, however, across the Developers stakeholders, Lack of career pathways for technical staff and data scientists working in bioimaging was the most important barrier to be addressed. In terms of country type,

For HICs (n = 234), the top three barriers that need to be prioritised in the next 5 to 10 years are

1. Lack of career pathways for technical staff and data scientists working in bioimaging (n = 72, 31%)
2. Lack of funding for bioimaging technology, methodology and tool development (n = 50, 21%)
3. Interdisciplinary barriers (e.g. between technology developers and users; data scientists and biologists) affecting development and application of new bioimaging technologies, methodologies and tools (n = 46, 20%)

Table 7 below provides a summary of the key issues and potential solutions to address these barriers.

Table 7 Top three 'other' barriers limiting the field of bioimaging in high-income countries

Barrier	Key issues	Solutions
Lack of career pathways for technical staff and data scientists working in bioimaging	High interdisciplinary barriers (n = 4)	Funding for career paths that bridge different fields (n = 4)
		Partnerships with industry to foster learning (e.g. engineering) (n = 1)
	Lack of opportunities (n = 15)	Commitment from funders and/or universities for more stable careers for facility staff; Appropriate titles/positions (e.g. Professor) and tailored key performance indicators (n = 19)
		Funding opportunities for own-career development (e.g. small projects, training courses, etc) (n = 2)
		Communicate the importance of bioimaging technologies to students and wider audiences, graduate programmes for career in bioimaging (n = 3)
		Support for workshops and conferences to improve professional qualifications (n = 3)
	Lack of recognition (n = 24)	Authorship and/or acknowledgements in academic publications (n = 4)
		Appropriate salaries for retaining people in the long-term (e.g. cryoTEM should have around 8 years of technical staff funding) (n = 6)
		Demand from funders that imaging specialists are included in grants, steering groups and other leadership positions (n = 1)
Lack of funding for bioimaging technology, methodology and tool development	Lack of funding for maintenance & repair including for maintaining software and tools (n = 9)	Funding for training staff and developing appropriate support to researchers; Training of new scientists (n = 2)
		Sharing facilities and equipment across institutions (n = 2)
		Funding for maintaining software and tools (n = 1)
		Creation of permanent positions staff to maintain key software infrastructure (n = 3)

		Funding regional bioimaging facilities (n = 2)
	Lack of opportunities/funding for research and development of new technologies and methods; Underfunding of certain areas e.g. radiochemistry, whole body imaging, in vivo preclinical imaging (n = 8)	Grant opportunities for neglected research areas (n = 3)
		Long-term funding for technology development, funding for response mode grants (n = 2)
	Lack of funding for small businesses (n = 2)	Grants for technology development for SMEs (n =2)
Interdisciplinary barriers (e.g. between technology developers and users; data scientists and biologists) affecting development and application of new bioimaging technologies, methodologies and tools	Challenges for collaboration across disciplines and lack of collaboration across imaging facilities (n = 8)	Improve networks / bring people together through workshops/meetings (e.g. users and developers), more funding for collaboration (n = 13)
		Creation of interdisciplinary research centres to foster education and technical development (n = 1)
	Challenges related to domain expertise (n = 3)	Funding for bioimaging analysists and research software engineers (n = 1)
		Open-source coding (n = 1)
		Incentives for knowledge exchange (biologists to learn about imaging technology and vice-versa, tool development awards) (n = 1)
		Funding for joint degrees and courses in interdisciplinary research / training at undergraduate level (n = 3)
	Lack of user-friendly methods and equipment; Lack of user-friendly imaging technology (n = 1)	User friendly solutions to facilitate knowledge exchange and adoption (n = 1)

For LMICs (n = 80), the top three barriers that should be addressed as a priority are

1. Lack of funding for bioimaging technology, methodology and tool development (n = 34, 43%)
2. Interdisciplinary barriers (e.g. between technology developers and users; data scientists and biologists) affecting development and application of new bioimaging technologies, methodologies and tools (n = 11, 14%)
3. Lack of training opportunities (n = 10, 13%)

Table 8 below outlines some of the key issues underpinning these barriers and some solutions to address them.

Table 8 Top three 'other' barriers limiting the field of bioimaging for Low- and middle-income countries

Barrier	Key issues	Solutions
Lack of funding for bioimaging technology, methodology and tool development	Lack of opportunities for research and lack of funding for development of new methods and tools (n = 6)	Funding regional bioimaging facilities (n = 1)
		Funding for training staff and developing appropriate support to researchers; Training of new scientists (n = 3)

		Grants specific for LMICs to nurture bioimaging community or access imaging facilities (n = 2)
		Collaborative research grants for novel technologies (n = 1)
	Lack of funding for maintenance & repair, lack of sustainable funding for maintaining software and tools (n = 7)	Sharing facilities and equipment across institutions (n = 3)
		Donations of equipment and support to open-source initiatives (n = 1)
Interdisciplinary barriers (e.g. between technology developers and users; data scientists and biologists) affecting development and application of new bioimaging technologies, methodologies and tools	Challenges for collaboration across disciplines and lack of collaboration across imaging facilities (n = 3)	Improve networks / bring people together (e.g. in Latin America, users and developers) – more funding for collaboration (n = 2)
	Challenges related to domain expertise (n = 1)	Manufacturers to improve user friendliness of equipment/software (n = 1)
Lack of training opportunities	Lack of funding for training facility staff (n = 2) Lack of funding for training early career researchers (n =1)	Specialised training courses and workshops (including online) for leader facilities to spread expertise regionally (n = 2)
		Support grants / investment into training (lower barriers to training) (n = 1)

Respondents from LMICs highlighted that without focused and sustainable support for developing bioimaging infrastructure in under-resourced regions, the research questions that are critical to these regions will remain unanswered. Importantly, a case needs to be built about the importance of the availability of / access to bioimaging infrastructure for supporting research in LMICs. Thus, long-term support for the development of local bioimaging capacity that will run and support bioimaging facilities is key as is support for national and regional policies and strategies that will enable the establishment and use of bioimaging infrastructure.

1.5 Scale of potential impact if barriers are addressed

Respondents were probed on the potential impact of addressing the barriers discussed. The questions were tailored according to the role of the respondent and/or their bioimaging expertise.

- **Facility managers, facility staff, imaging scientists and other technical staff**

Whether based in LMICs or HICs, bioimaging facility staff, imaging scientists and other technical staff stated that addressing the key barriers in the bioimaging landscape would enable them to help researchers answer new hypotheses and address new fundamental questions about biological processes and mechanisms, increase the quality and reproducibility of their imaging services and help increase revenues for their facility (Figure 25 and Figure 26).

Other potential impacts noted by a few HIC-based respondents included better application of research in healthcare, better return on investments in science and more trained staff available to maintain existing research infrastructure. Several LMIC-based respondents noted that overcoming the barriers discussed would allow researchers to address local research questions, such as in the area of neglected tropical diseases. However, this will require democratisation of access and an increase in research productivity. A few LMIC respondents mentioned that overcoming barriers would lead to less reliance on international collaboration and more focus on building research infrastructure that is financially sustainable and that can support advances in diverse fields of work, such as food security and health.

Figure 25 Scale of potential impact on facility managers, facility staff, imaging scientists and other technical staff in high-income countries if barriers are addressed (n = 88)

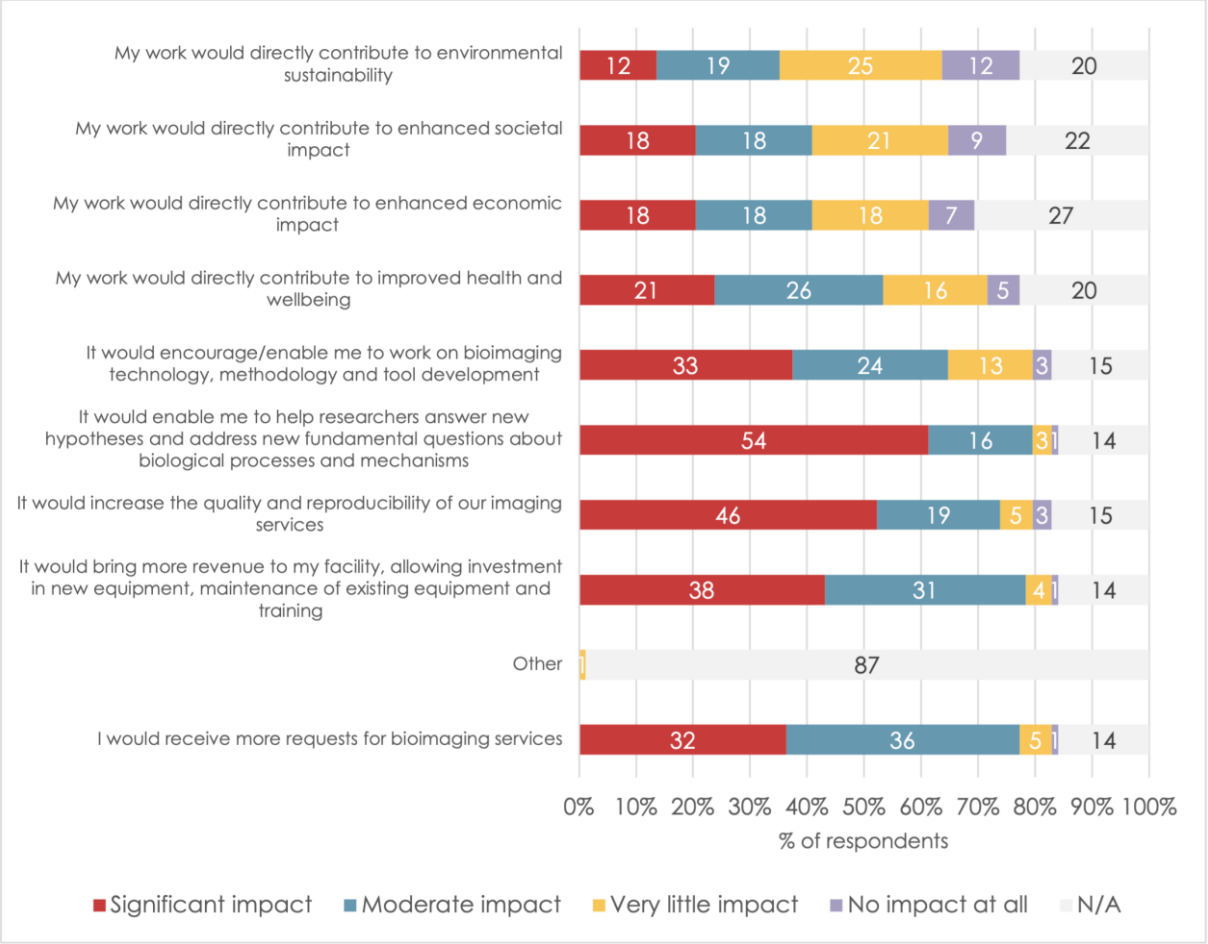
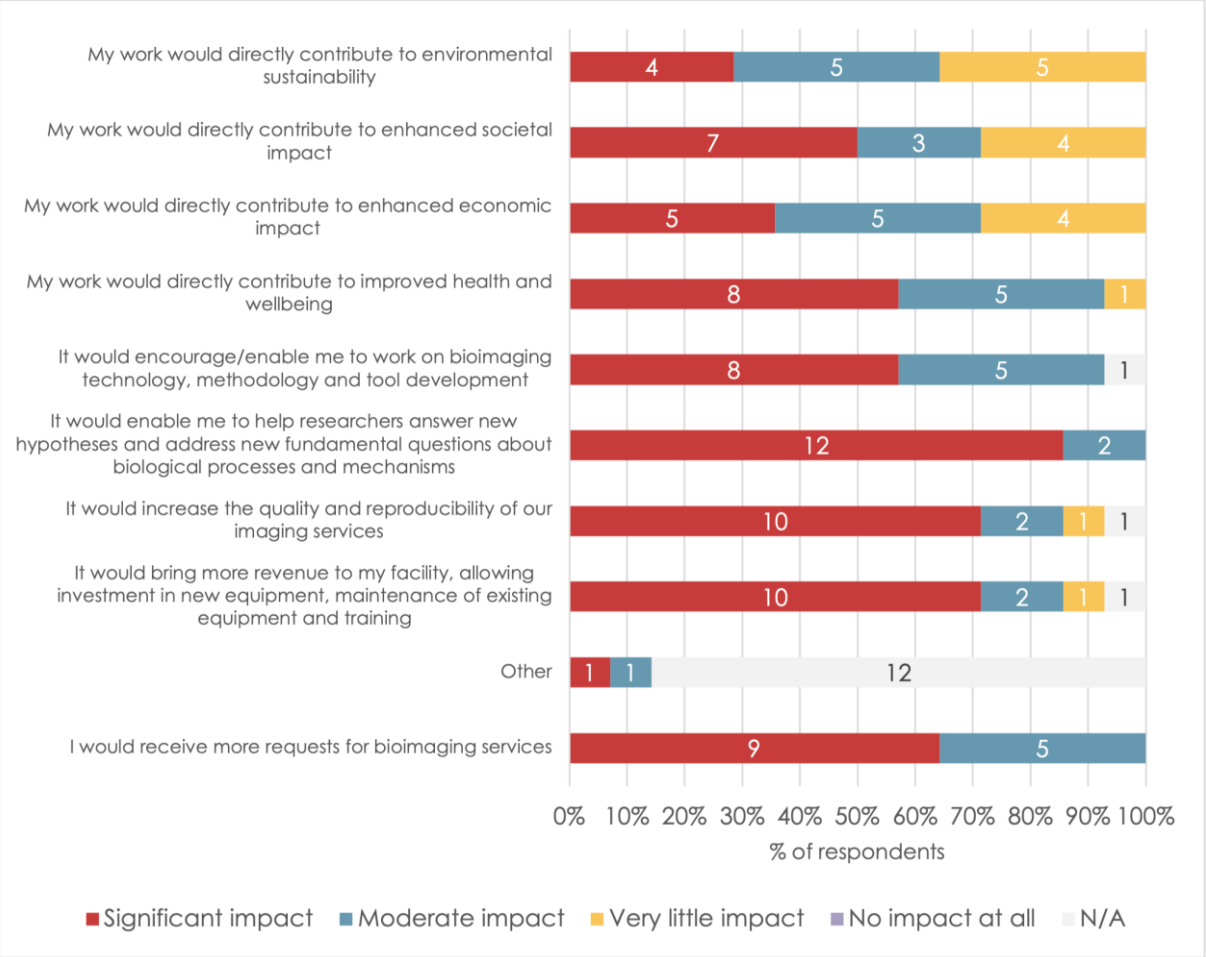


Figure 26 Scale of potential impact on facility managers, facility staff, imaging scientists and other technical staff in low- and middle-income countries if barriers are addressed (n=14)



- **For users of bioimaging technology and methods**

Users from HICs too feel that addressing the key barriers will enable them to formulate new hypotheses and address new fundamental questions in biology as well as increase the quality and reproducibility of their research (Figure 27). They also hope to use bioimaging techniques more readily in their research. The latter will the key impact for users from LMICs who state that they would use bioimaging in their research work more often (Figure 28). LMIC-based respondents also expect to be able to test new hypotheses and explore new research questions and improve the quality and reproducibility of their research. However, they also see a direct impact in terms of contributing to improved health and wellbeing in their region.

A few HIC respondents highlighted that open data and use of shared image resources will improve research, make it more equitable and support research in currently neglected areas. They also noted that new hypothesis and discoveries would be made possible through the incorporation of new techniques in areas such as molecular mechanistic biology, neuroscience, medical imaging and viral infections and diseases. Other effects included better reputation of science and technology projects in society and improved quality of training available. A few LMIC respondents noted that democratising access to bioimaging technologies will encourage more interest in bioimaging in LMICs and thus allow new ideas to be tested in different fields of expertise. Others also noted that more opportunities for scientific training of early career researchers and technical staff may become available.

Figure 27 Scale of potential impact on users of bioimaging technology and methods in high-income countries if barriers are addressed (n = 48)

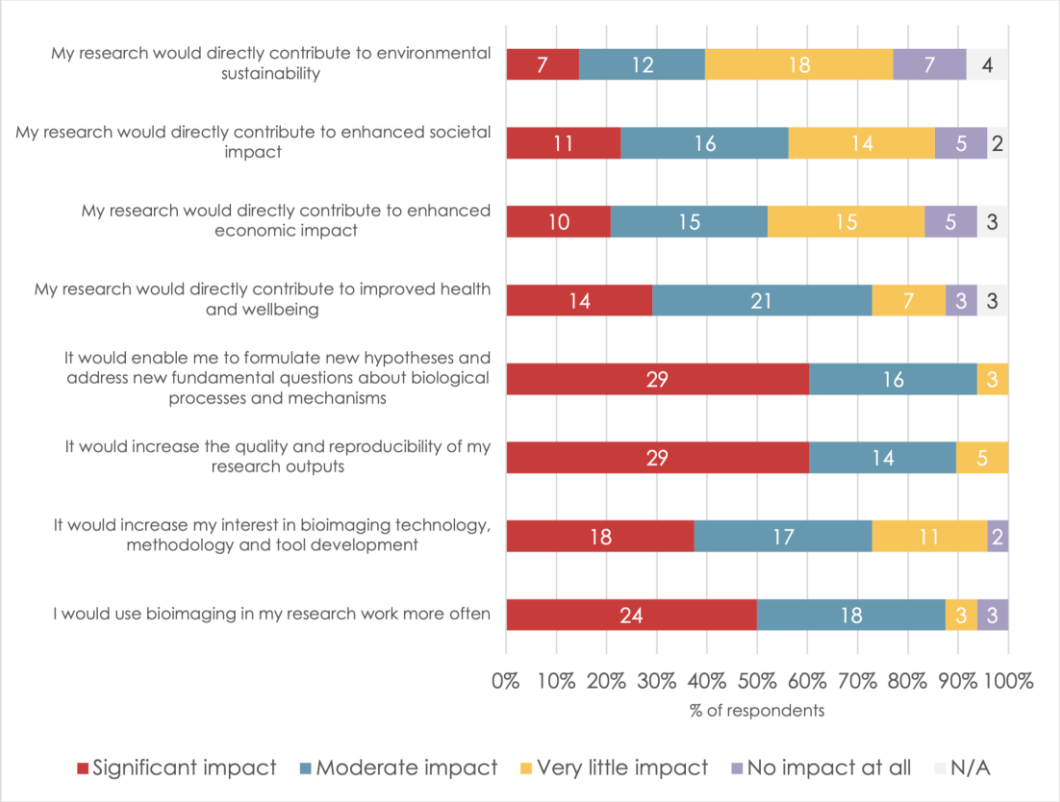
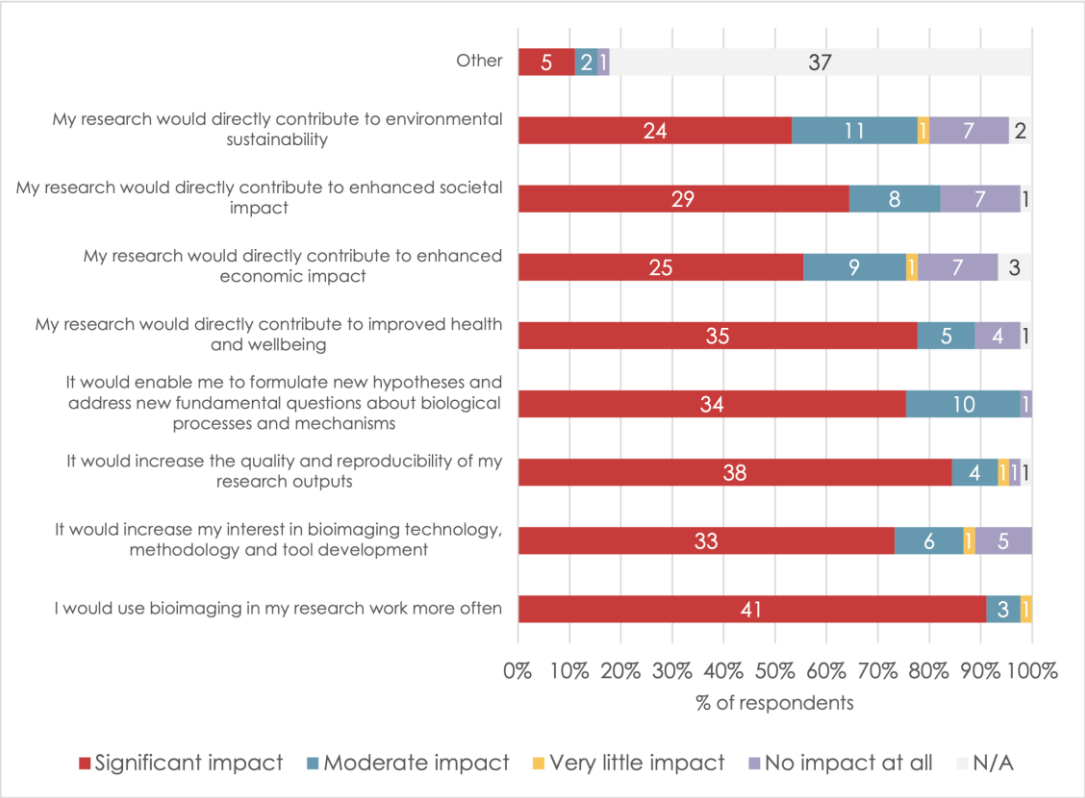


Figure 28 Scale of potential impact on users of bioimaging technology and methods in low- and middle-income countries if barriers are addressed (n = 45)



- **For developers of bioimaging technology and/or methods**

For methodology/technology developers from both LMICs and HICs, addressing the barriers discussed would have a significant impact on their ability to formulate (or help other researchers formulate) new hypotheses and address new fundamental questions about biological processes and mechanisms, and enable them to develop novel bioimaging technologies/methodologies more readily (Figure 29 and Figure 30). Developers from HICs also impact significant impact on the quality and reproducibility of their research outputs, while those from LMICs indicate that they will see significant impact from lowering of interdisciplinary barriers which will allow them to better focus their development work on user needs and from contributions to improving health and wellbeing.

Several respondents from HICs noted that the most important impact of overcoming barriers to progress in the bioimaging field would be to facilitate new discoveries, in particular by enabling the use of bioimaging technology in novel ways and ensuring the availability of appropriate tools to answer complex scientific questions in areas such as neuroscience, infectious and cardiovascular diseases, drug development, 3D imaging and in-vivo preclinical imaging. The potential positive impact of retaining researchers and specifically imaging scientists in the bioimaging field was also mentioned by a few respondents. Democratisation of science was also a likely impact according to both HIC and LMIC respondents. For LMICs respondents, overcoming barriers in the bioimaging field is expected to lead to better research outputs with more societal impact.

Figure 29 Scale of potential impact on developers of bioimaging technology and/or methods in high-income countries if barriers are addressed (n = 102)

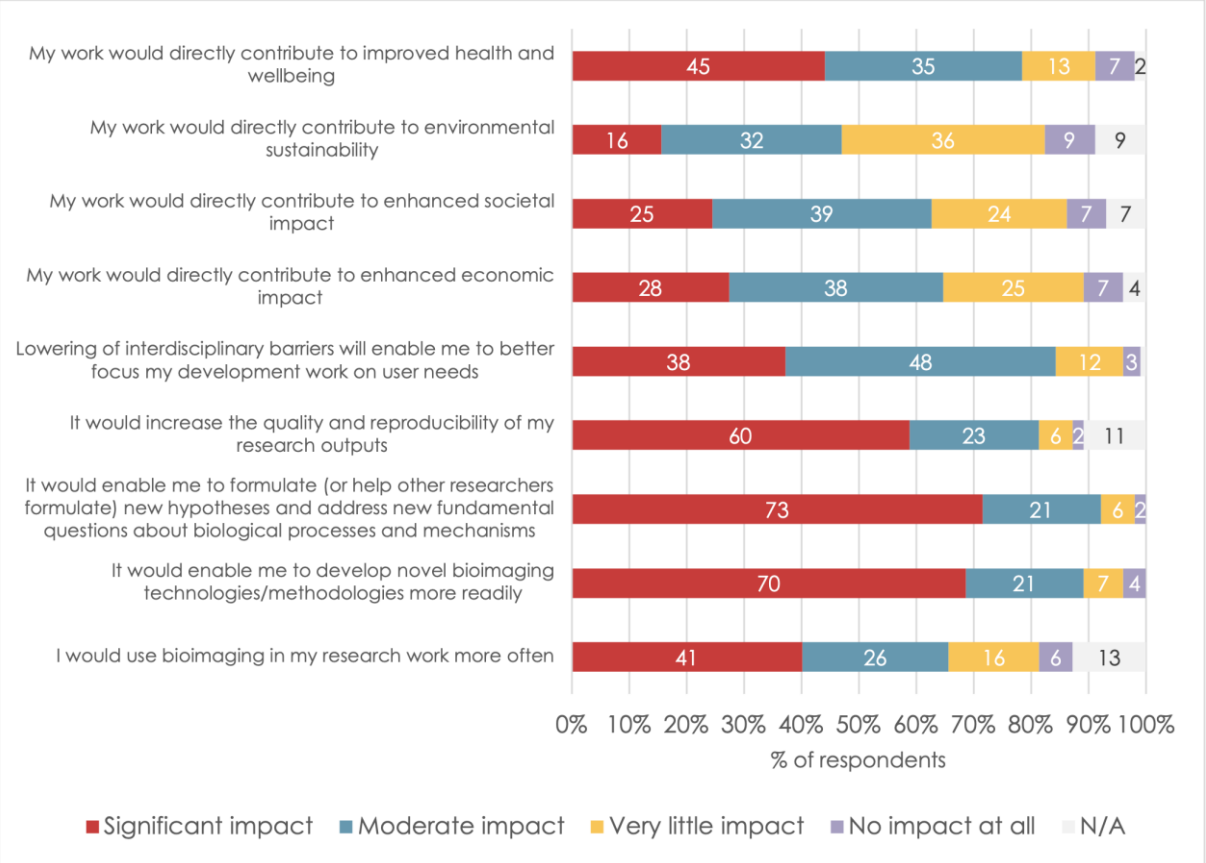


Figure 30 Scale of potential impact on developers of bioimaging technology and/or methods in low-middle-income countries if barriers are addressed (n=10)