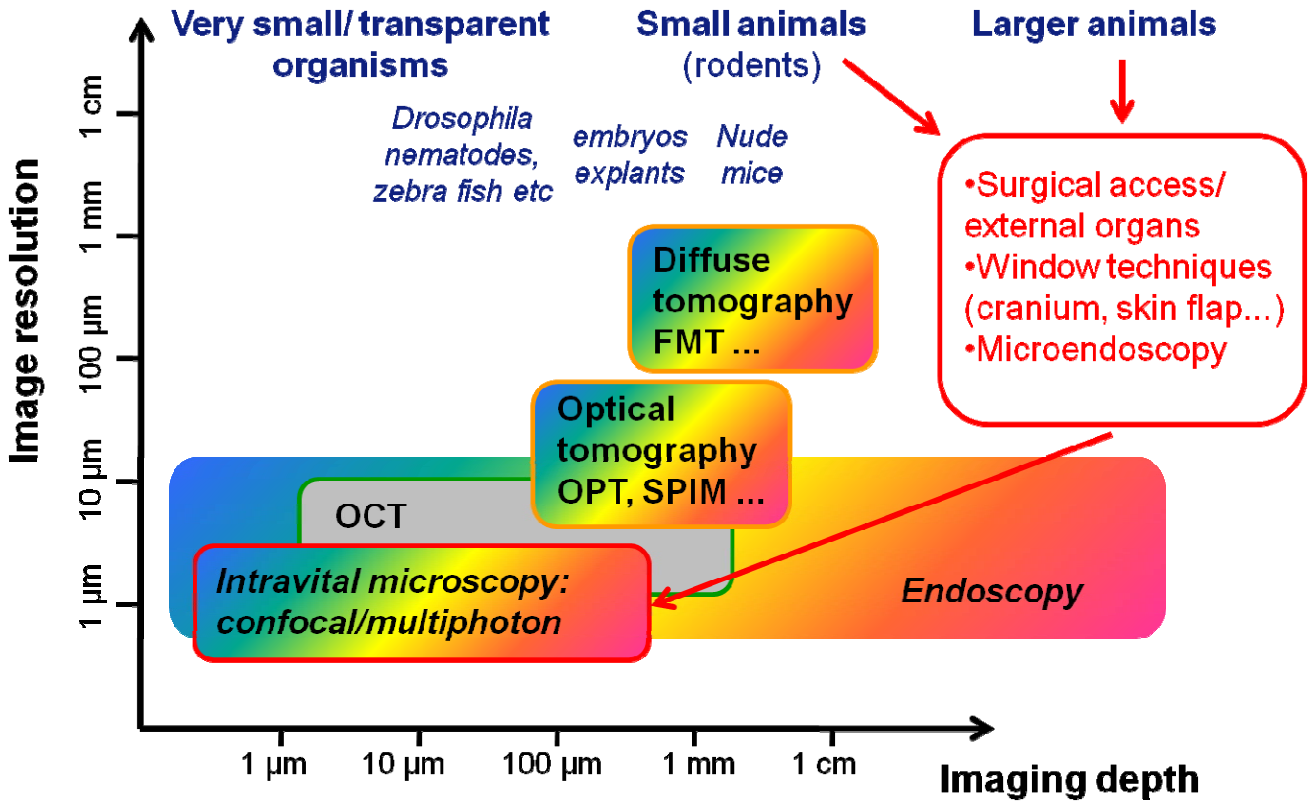
EPSRC Mid Range Facility for *in vivo* optical imaging (FIVOI)

1. Please state the type of facility and give a *brief* description of its function.

In vivo optical imaging is one of the most exciting frontiers of modern science – where advances in optical technology are being harnessed to advance our understanding of the molecular mechanisms and manifestations of disease. This emerging multidisciplinary field draws on optical science, bioengineering, chemistry, molecular biology and physiology, requiring complex research teams and advanced technological instrumentation that is rapidly evolving. New optical imaging techniques are translating molecular cell microscopy experiments from *in vitro* cultures to live organisms and animals to increase their value and to enhance the efficiency of drug discovery, toxicology and the development of biomarkers – as well as to reduce the numbers of animals used. Fluorescence and bioluminescence can elucidate protein localisation with single molecule sensitivity and sophisticated spectroscopic approaches can provide real-time optical molecular contrast for studying protein interactions in signalling networks, as well as physiological changes in metabolism and tissue structure. Fluorescence, in particular, is an immensely powerful means of obtaining molecular contrast with the exquisite specificity of labelling techniques including genetically expressed fluorophores to label live organisms and animals, complemented by designer dyes, quantum dots and nanoparticles. Förster resonance energy transfer (FRET) can be used to readout out protein interactions and protein conformational changes to report on signalling networks and calcium sensors can report on metabolic activity. Increasingly, autofluorescence is providing label-free readouts of metabolites like NADH and flavoproteins and is also being used to contrast different types and states of tissue matrix. Optical techniques such as optical coherence tomography (OCT) and polarisation-resolved imaging can provide further structural information and this can be complemented by second and third harmonic generation (SHG/THG) microscopy to probe macroscopic and microscopic structure. Such optical readouts are non-invasive or minimally invasive and so can be used for longitudinal, extended time course studies, potentially reducing the numbers of animals required where otherwise a series would be sacrificed at different endpoints.

Unfortunately the full potential of these new optical imaging modalities are beyond the reach of most life scientists in the UK, being technologically complex and requiring a level of investment and expertise beyond individual institutions. The situation is more challenging because in vivo optical imaging technology is evolving rapidly – particularly to address the formidable challenges associated with obtaining optical information from beneath the surface of an animal. In (highly scattering) biological tissue multiphoton microscopy can provide diffraction limited resolution at depths approaching ~1 mm, with confocal and wide-field microscopy imaging to significantly less depth, and internal organs must be accessed using surgical preparations, window techniques or endoscopy. Whole animal imaging techniques like IVIS using wide-field bioluminescence or fluorescence imaging can report on localisation of specific proteins but the image resolution is low and it is difficult to probe much below the surface. Optical tomography techniques such as optical projection tomography (OPT) or selective plane illumination microscopy (SPIM) can provide high resolution 3-D images of ~transparent samples, such as embryos and diffuse optical tomography (DOT) or fluorescence molecular tomography (FMT) can provide images, albeit with significantly reduced resolution, in scattering biological samples such as live mice. The information from these optical imaging techniques can be correlated with other modalities such as electrophysiology, PET, MRI, ultrasound and x-ray CT. Often modalities like X-ray or MRI can establish a baseline anatomical image and optical techniques can be used to detect or monitor functional dynamics, sometimes taking advantage of the anatomical data to improve reconstruction of optical images. It is also useful to relate optical imaging, which can provide molecular contrast on cellular and subcellular scales, to larger scale anatomical and functional images to provide aligned complementary information. Established *in vivo* imaging modalities can provide anatomical orientation, allowing navigation back to the same point in the tissue for precisely targeted optical studies.

In vivo optical imaging thus provides vital opportunities to extend molecular biological imaging to physiologically relevant samples, e.g. to live mouse models, and is likely to be of great importance for drug discovery, toxicology and fundamental studies of disease mechanisms. For example in oncology alone, in vivo optical imaging could dramatically impact on the detection of cancer, post therapy monitoring of residual cancer at the cellular level, and monitor cancer metastases. Current methodologies and associated technology require a high level of skill in instrumentation, data analysis and curation, sample labelling and preparation and animal handling that is not accessible to most potential life science users. It therefore is a highly fitting theme for a multidisciplinary EPSRC Mid-range facility. Such a facility should provide access to a wide user base and include core staff with the required multidisciplinary skill sets. Given the rapid advances in optical imaging technology, the facility should include or be linked to a strong technology development group able to implement the latest advances and customise the advanced optical imaging instrumentation for specific user requirements. It should also have strong links with experts in data analysis and image reconstruction. In addition it will be essential to have strong links with animal handling facilities, with established animal imaging technologies such as MRI and x-ray CT, and with teams developing probes and labelling techniques.



The essence of this proposed mid-range facility is that it should offer a range of complementary techniques for in vivo optical imaging, ranging from sub cellular resolution for protein dynamics – ideally with single molecule sensitivity or super resolution – through 3D imaging of embryos, tissue explants and small organisms to longer term longitudinal studies of mice and larger animal models. The range of spatial scales should be complemented by a strategically customised multimodal approach to imaging, combining a range of instrumentation to provide structural and functional information' such that life scientists can address different aspects of their biological questions and develop a more "holistic" perspective. Life scientist users would typically interact with the facility over extended timescales (months to years) to address particular biological questions.

2. Is this an existing UK facility or is it a new facility? If it is a new facility, please explain why this facility is now needed or will be needed in the future.

Looking forward, it is clear that *in vivo* validation will increasingly become a desirable, if not essential, part of any biological discovery process, in academic research as well as in the pharmaceutical and other industrial and medical sectors. Furthermore, developing a holistic approach to address biological questions by imaging on a range of scales is vital for systems biology, which is a major priority in the UK and elsewhere. There is currently no such in vivo optical imaging facility in the UK. There are UK groups, e.g. at Imperial College London, Newcastle and UCL, who are developing a subset of optical imaging techniques and applying them to biological questions and other UK groups who are using a limited range of commercially available optical instruments alongside other established animal imaging modalities like x-ray CT and MRI but there is no UK institution where life scientists have an open choice of optical imaging tools with the opportunity to adapt and customise them such that they can design an imaging strategy to best fit their biological questions. Multimodal imaging centres combining optical imaging with other modalities are being established outside the UK, e.g. in the USA (e.g. UC Davis, UCLA, MGH ...) and Europe (e.g. Heidelberg, Leipzig, Paris). This proposal therefore concerns a new UK mid-range facility that would be of immediately use and would develop an increasing strategically importance – particularly as such capabilities become available to our international competition.

3. What facilities of this type already exist (a) at the university level, (b) at the national or regional level and (c) at the international level. How accessible are these existing facilities to UK academics?

There are no such facilities in UK universities or at the national or regional level. UK universities often have strength in one or two optical imaging modalities, such as multiphoton microscopy or OCT, usually being developed by a group for a specific class of biological questions and not usually available to outside users or for customisation or adaptation. There are not yet any such facilities in Europe although there is increasing demand for *in vivo* optical imaging and growing strengths in Germany (e.g. Heidelberg, Leipzig) and France (e.g. Paris). Outside the UK, the main groups are in the USA where NIH does fund mid-range facilities in California, Boston etc. Such facilities are mainly available to UK academics through bilateral collaborations.

4. Please describe who will benefit from the existence of this facility, including the number and type of researchers in the UK who are likely to want to use it and the research disciplines that it will benefit. Please indicate what level of usage such a facility would get in a year.

Life scientists across the UK would benefit from such a facility. Clearly access would be more straightforward for local users but as long as the facility were to be sited close to a large, open access animal house, projects could be initiated and monitored remotely with life scientists coming to the facility for specific experiments. It is clear that collocation with other imaging modalities would also be important as traditional users of e.g., microPET or microCT could then add optical techniques to their imaging armoury and life scientists could make correlative studies. A mid-range centre for *in vivo* optical imaging would enable instrumentation developers to trial their prototypes and also stimulate the development of new probes and labelling techniques by chemists and other interdisciplinary scientists.

5. Please explain why this facility is a "mid range facility" and what the benefits are of EPSRC supporting this facility. That is, why the facility needs to be supported at a national or regional level, rather than at a University or international level.

Given the complexity and cost of the proposed facility, the complex issues associated with animal handling and the extended timescales over which users would access the facility, it should be clear that the required investment would be too great for most individual universities or institutions in the UK – in terms of the cost and of the range of skills required to make it effective. Nevertheless, this consultation has revealed a wide enthusiasm for access to such a facility that is likely to significantly increase as in vivo optical imaging technology develops along with the imperative for life scientists to use it. It will not be feasible to meet this demand with a single European facility, or even with a single national UK facility and we believe that a number of regional mid-range facilities will be required. In the first instance, however, a pilot mid-range facility, located alongside a significant animal imaging centre with the resource and infrastructure to support extended collaborations, would be of great interest and utility for a wide spectrum of users.
It is appropriate, if not essential, that EPSRC support this facility because an *in vivo* optical imaging centre needs to be much more than an assembly of commercially available instrumentation. The field is changing very rapidly and facility would become quickly outdated without significant ongoing technological development – in terms of imaging hardware, software tools, molecular probes and imaging protocols. This significant technological development should dovetail into the design of imaging experiments and the customisation of capabilities to address specific biological questions. Thus partnership between the imaging technologists, animal handling specialists, data analysts and life science users is a vital requirement for success. The capabilities of imaging technology available today will not meet the in vivo imaging needs of 5 years time and it is important that the UK has a facility that can develop to meet this challenge.

6. How long should the facility be supported for?

The facility should be supported for 5 years in the first instance. It could be set up within ~ 18 months, initially providing commercially available equipment with a high degree of customisation alongside a development stream of new technology, probes and data analysis and curation tools. We believe there will be sustained and increasing demand for this facility but consider that the capabilities would need to be revised in light of the rapidly changing landscapes of technology and funding,. This facility should be managed by an external advisory board drawn from UK life science users, technology developers, industry and foreign experts.

7. Please indicate what the facility should provide to be of maximum benefit to the research community and estimate the likely cost of the facility. For example, indicate what size should it be, what technologies should it have available, how many staff would it need. You should prioritise these requirements in terms of "must have" and "desirable". In addition, please highlight any features that would be detrimental.

The facility should provide a spectrum of in vivo imaging instrumentation ranging from microscopy to tomography. Initially the instrumentation could include at least two confocal/multiphoton microscope systems, one with electrophysiology capabilities and one for imaging deep in mammals, e.g. using specialised "stick" lenses. It should also include OPT and SPIM set ups for tomographic imaging of near transparent samples, such as developing embryos, and IVIS and FMT systems for imaging mice and other

animals. There should also be at least two endoscopy systems, including microconfocal endoscopy. Several groups have also expressed the desire for in vivo super-resolved imaging using e.g. Stimulated Emission Depletion (STED) microscopy to reach the nanometre scale. These high-end instruments should be complemented by wide-field CCD-based microscopes and single channel probe-based instruments for in vivo point measurements. Other optical instrumentation would include OCT for correlating structure with spectroscopic information and tools for monitoring physiological and metabolic parameters such as Doppler angiography for blood flow measurement.

The instrumentation for *in vivo* optical imaging to set up this mid-range facility, together with the associated data processing and archiving resources, is likely to cost £5M. It would also require at least two full time staff to install, commission, maintain and operate the instrumentation and at least two further staff for data analysis and curation and for animal handling and preparation. As discussed above, this mid-range facility must be linked to strong optical technology research groups with joint appointments and an integrated strategy for developing and customising instrumentation.

This optical imaging instrumentation must be collocated with appropriate animal facilities including capabilities for sample preparation such as animal surgical facilities and isolated organ bath preparations for *in vivo* and *ex vivo* studies. It would be useful to be able to provide real-time behavioural monitoring of animals, e.g. prior to or after *in vivo* imaging, as an adjunct to include as part of phenotypic analysis. It should also be collocated with an imaging centre providing the more established imaging modalities including MRI, PET, SPECT, ultrasound and x-ray CT. These techniques can be used non-invasively in humans in support of target validation and evaluation of potential new drugs. Thus there should be facilities to optimise co-registration of multi-modality imaging and development of multi-platform probes/tracers/contrast agents. For work with dangerous human pathogens it would be highly desirable to establish some instrumentation under Cat 3 containment.

It would be detrimental not to have the necessary computational power (data processing and analysis) to deal with the enormous amount of images generated. Also surgery facilities, animal holding rooms and permission to shuttle animals to/from the imaging facility must be in place. It would be very detrimental for the facility not to be linked to ongoing technology and software development programmes to drive advances for *in vivo* optical imaging. Another critical issue would be the ongoing model to fund access to this facility, which needs to be core funded for UK academics to enable a good uptake and real impact on our research capabilities. One approach would be to establish bursaries managed by the Scientific Board to enable very fast access whilst at the same time ensuring that the facility is run in a proactive manner and not sit back for 5 years. The “pay-as-you-go” model would be very detrimental in terms of enabling timely and wide access.

8. If EPSRC was unable to support this facility, what would the research community do? (for example, in terms of looking for other sources of financial support or seeking access to non-UK facilities)

Other possible sponsors could include the Euro-BioImaging initiative, the Wellcome Trust and perhaps the BBSRC and/or MRC although EPSRC is in many ways the most appropriate UK research council because of the strong technological element and the need for ongoing development and customisation of instrumentation and analysis.

9. Please make any other comments that you think are relevant to the statement of need for the facility.

The development and customisation of optical probes and contrast agents would be important for the success of this facility and it should establish a network of probe developers as well as commercial vendors.

10. Who was involved in preparing this statement of need? Please list name, institution and research interests.

Paul French	*Imperial College London*	Microscopy endoscopy and tomography
Jo Hajnal	*Imperial College London*	Tomography
Danny Altmann	*Imperial College London*	Multiparameter bioluminescence imaging to look in vivo at immune cell fate decisions; in vivo imaging of CNS neuroinflammation in Alzheimer's disease
Brian Robertson	*Imperial College London*	*In vivo* imaging for tuberculosis in mice using both fluorescent and luminescent reporters (IVIS/FMT)
Guy Rutter	*Imperial College London*	Multiphoton microscopy, OPT, STED
Michael Schneider	*Imperial College London*	Imaging mice with either adverse (heart failure) phenotypes or beneficial ones (resistance to injury, improved regeneration).
Simon Schultz	*Imperial College London*	*In vivo* brain imaging in mice
Tony Magee	*Imperial College London*	*In vivo* light microscopy and animal welfare
Martin Spitaler	*Imperial College London*	*In vivo* light microscopy
Matthew Fuchter	*Imperial College London*	Imaging cancer and cardiovascular disease
Alexander Lyon	*Imperial College London*	Cardiac Optical Mapping and Microscopy
Maggie Dallman	*Imperial College London*	Whole organism immunology, inflammation and infection studies using tomographic imaging of genetically engineered zebra fish'
Christina LoCelso	*Imperial College London*	Study of stem cell niches in the whole organism using optical/multiphoton imaging
Julia Buckingham	*Imperial College London*	Understanding the functions of genes and their products in health and disease using a multimodal imaging approach including microPET, fMRI, intravital microscopy, spectroscopy/bioluminescence

		and fluorescence (endogenous proteins, e.g. GFP, and exogenous probes) and the capacity to exploit emerging technologies; Director of Centre for Integrative Mammalian Physiology and Pharmacology (CIMPP)
Philip “Eddie” Edwards	*Imperial College London*	Guidance of therapy, relating optical imaging to larger scale anatomical imaging
Nick Long	*Imperial College London*	Probe design and synthetic chemistry for in vivo imaging
Ramon Vilar Compte	*Imperial College London*	Development of novel probes for optical imaging
Vincenzo De Paola	*Imperial College London*	*In vivo* multiphoton imaging of the diseased nervous system
Rainer Heintzmann	*King’s College London*	High Resolution Microscopy and Image Processing
Gregory J Bancroft	*London School of Hygiene and Tropical Medicine*	IVIS, FMT, multiphoton microscopy, for work on dangerous human pathogens
Michael Taggart	*Newcastle University*	*In vivo* tissue and organ remodelling in pregnancy, microscopy, biomolecular trafficking and blood flow.
Paul Flecknell	*Newcastle University*	Behavioural and other indicators of pain and distress in animals, real-time assessment of animal behaviour, development of anaesthetic techniques for in-vivo imaging
Maya Sieber-Blum	*Newcastle University*	*In vivo* monitoring of survival and migration of transplanted neural crest stem cells in animal models of human disease (IVIS).
Ross Maxwell	*Newcastle University*	Positron Emission Tomography, X-Ray Computed Tomography, Magnetic Resonance Spectroscopy and Imaging; non-invasive imaging methods for evaluation of new cancer treatments.
Derek Mann	*Newcastle University*	*In vivo* analysis of inflammation and NF-kB signaling in the context of models of acute and chronic liver disease (IVIS).
Olaf Heidenreich,	*Newcastle University*	*In vivo* monitoring of cancer stem cells, siRNA delivery (incl. in vivo pharmacokinetics), cancer stem cell-directed therapeutic approaches; bioluminescence and –fluorescence, CT;.

Michael Taggart	*Newcastle University*	*In vivo* tissue and organ remodelling in pregnancy, microscopy, biomolecular trafficking and blood flow.
Trevor Jackson	*Newcastle University*	Bioluminescence and fluorescence microscopy approaches to investigate kinase signalling pathways as targets of intervention in models of cancer. Informatics tools for bioimaging
Simon Walker	*The Babraham Institute, Cambridge*	*In vivo* cellular fluorescence imaging
Simon Waddington	*UCL*	Bioluminescence imaging
Simon Arridge	*University College London*	Diffuse Optical, Photo Acoustic and Optical Projection Tomography
Angus Lamond	*University of Dundee*	Multiphoton microscopy, FLIM, FRET analysis, live cell multi-wavelength fluorescence time-lapse imaging, photobleaching and photoactivation analysis
Jason Swedlow	*University of Dundee*	Imaging chromosome proteomics and mitosis; data analysis and curation
Peter O'Toole	*University of York*	Probes and technologies for correlative high resolution imaging, flow cytometric developments and imaging facilities