IN VIVO PRECLINICAL IMAGING

an essential tool in translational research

By Dr John Comley

In vivo imaging of small animals (mainly mice) is increasingly being deployed across the drug development process, particularly in the oncology/cancer therapeutic area. One of the main applications is monitoring the treatment response for early indications of efficacy. The most used imaging modalities are currently optical (bioluminescence and fluorescence), magnetic resonance imaging (MRI) and positron emission tomography (PET). Single modality imaging predominates, with multi-modality currently accessed mainly through co-registration with other imaging modes. The most used imaging combination today is PET+CT (x-ray computed tomography). *In vivo* imaging is expected to have greatest impact in drug development through monitoring disease progression and therapeutic response in longitudinal studies. Bioluminescent markers/reporters (eg luciferins, proluciferins) and PET Tracers (eg Fluorine-18 based) were the most used reagents in imaging studies. Maximising the depth of tissue penetration is perceived as the main limitation associated with optical imaging. From vendor updates it is possible to make some general observations: more compact benchtop imaging systems are being developed to promote accessibility; multi-modality imaging combinations are increasingly being offered: higher spatial resolution imaging is expected to be realised on new imagers: a broader range of imaging and contrasting reagents is under development; imaging systems are heavily reliant on advanced software systems and algorithms for reconstruction of the 3D image and co-registration of multiple imaging modalities; and finally the industry as a whole appears to be focusing on translational research applications. In summary, *in vivo* preclinical imaging is poised to rapidly advance, such that the specification and capabilities of small animal imagers will soon exceed their clinical counterparts.

In vivo imaging is increasingly deployed across the drug development process, with applicability in target identification, compound optimisation and pre-Phase I studies. *In vivo* imaging has been described as bridging the gap between *in vitro* exploratory and *in vivo* clinical research, facilitating the direct and fast transfer of preclinical studies on animal models to clinical investigation in man. There are numerous instances across therapeutic areas where preclinical imaging has proved valuable in drug development, for example: in target localisation, quantification of disease, disease phenotyping, mode of action studies, efficacy studies; *in vivo* pharmacokinetics and dosing and treatment models to name but a few. Non-invasive imaging is a rapidly advancing field, with innovation in dedicated small animal imaging technologies now paralleling their clinical equivalents. Multiple imaging modalities are now available for small animal studies which when combined complement each other to provide information on molecular features, metabolism and function all within the context of anatomical structure and localisation. New imaging systems and developments in animal models, reagent chemistries and biomarkers suitable for cell labelling continue to ensure the rapid growth in the field. With this in mind HTStec initiated in January 2011 a market study on *in vivo* preclinical imaging, as part of its ongoing tracking of emerging life science marketplaces[1]. This survey and the associated vendor contributions serve as the basis for this review.

Key diseases/therapeutic areas targeted

The key diseases/therapeutic areas targeted with *in vivo* preclinical imaging by survey respondents were oncology/cancer (70% targeting). This was followed by inflammatory disease/autoimmune (36% targeting); neurology/CNS/neurodegeneration/pain (32% targeting) and then cardiovascular (25% targeting) (**Figure 1**).

Main applications of *in vivo* preclinical imaging

Monitoring treatment response for early indications of efficacy was rated as the main application of *in vivo* preclinical imaging (68% investigating). This was followed by biodistribution, determining drug/target engagement (59% investigating); cancer cell detection (49% investigating); biomarkers (41% investigating); and then longitudinal studies (39% investigating). Least investigated was epigenetics, monitoring drugs that affect chromatin (4% investigating) (**Figure 2**).

Figure 1: Key diseases/therapeutic areas targeted with preclinical imaging

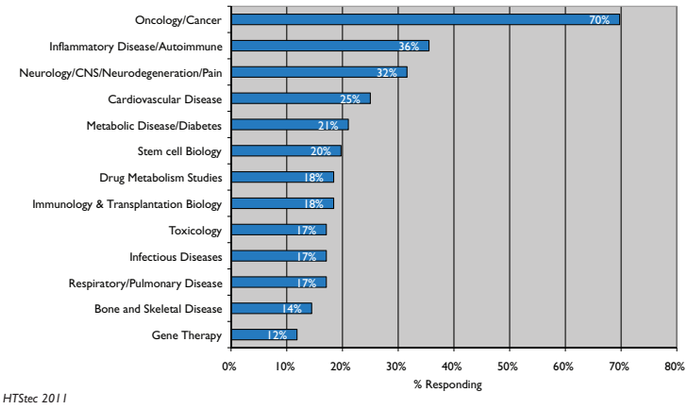


Figure 2: Main applications investigated with *in vivo* preclinical imaging

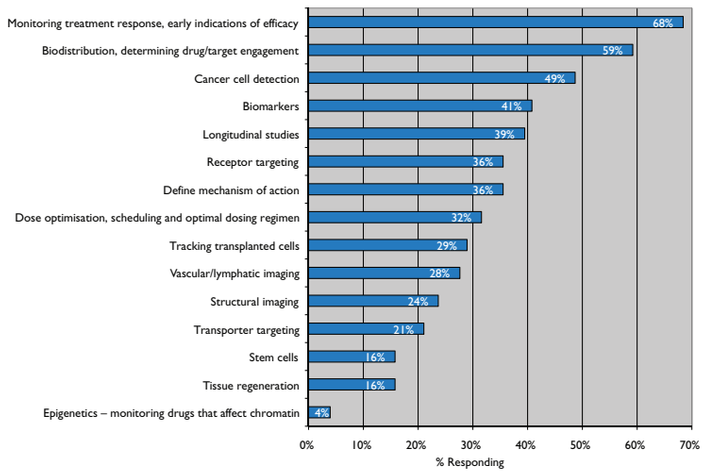


Figure 3: Most used single imaging modality today

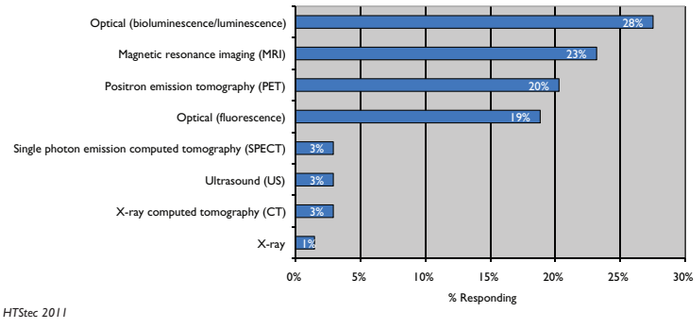


Figure 4: How respondents currently access multi-modality imaging

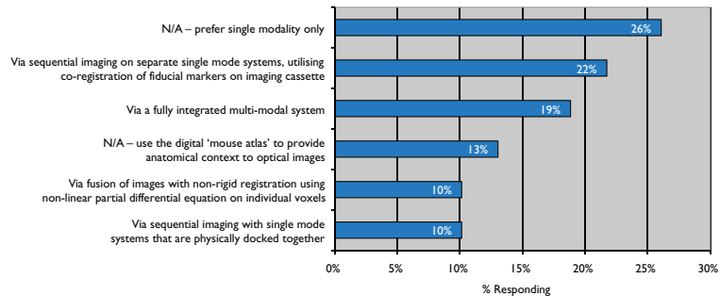


Figure 5: Multi-mode imaging combinations used today

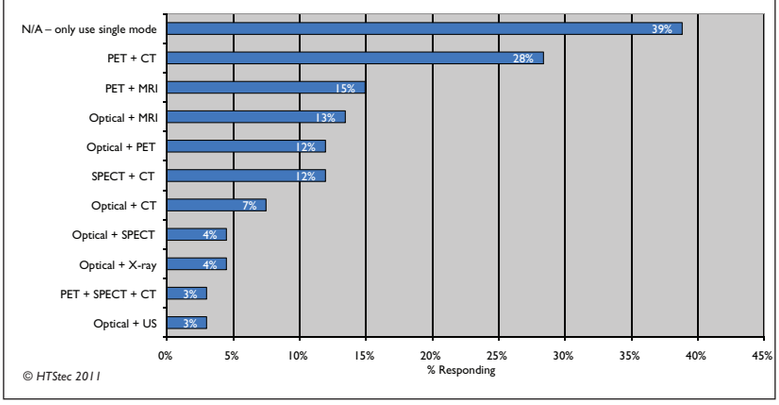
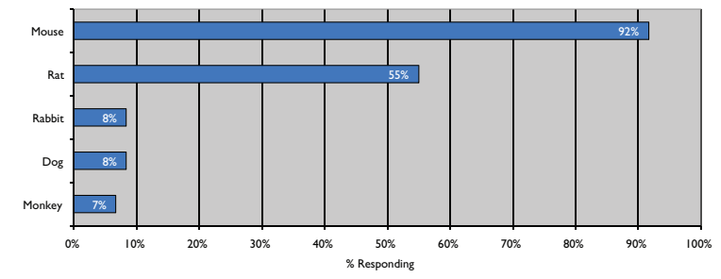


Figure 6: Animal species imaged today and expected for the future



Most used single *in vivo* imaging modality

The single most used *in vivo* imaging modality today (2011) by survey respondents in their preclinical studies was optical (bioluminescence) (28% using). This was followed by magnetic resonance imaging (MRI) (23% using); positron emission tomography (PET) (20% using) and optical (fluorescence) (19%). All other labels had 3% or less use (**Figure 3**).

Multi-modality *in vivo* imaging

26% of survey respondents prefer a single modality instrument for their *in vivo* preclinical studies. Of the remaining 74% preferring multi-mode imaging the greatest preference was shown for sequential imaging on separate single mode systems, utilising co-registration of fiducial markers on imaging cassette as the means to access multi-modality imaging (22% preferring). This was followed by via a fully integrated multi-modal system (19% preferring) and then N/A, ie use the digital 'mouse atlas' to provide anatomical context to optical images (13% preferring) (**Figure 4**). 39% of survey respondents were not using multi-mode imaging combinations today (2011) for their *in vivo* preclinical studies. Of those 61% using multi-mode imaging combinations, the biggest proportion was using PET+CT (28% using). This was followed by PET+MRI (15% using), Optical+MRI (13% using), and then Optical+PET and SPECT+CT (both 12% using). All other combinations were used by less than 10% of respondents (**Figure 5**).

Animal species imaged

The animal species most imaged today (2011) by survey respondents was the mouse (92% using). This followed by the rat (55%) using. All other species (rabbit, dog and monkey) were used by 8% or less than of respondents (**Figure 6**).

How *in vivo* imaging impacts drug development

Monitors disease progression and therapeutic response in longitudinal studies was ranked as how *in vivo* imaging exerts the greatest impact on drug development. This was followed by ensures preclinical *in vivo* data is as predictive as possible of the clinical outcome and then provides additional insight, leading to better decision-making. Least impact was expected on reducing the number of compounds failing in late phases (lower attrition rates) (**Figure 7**).

In vivo preclinical imaging reagents

The reagents most used today by survey respondents in their *in vivo* preclinical imaging studies was bioluminescent markers/reporters, eg luciferins, proluciferins (61% using). This was followed by visible fluorphores/ reporters, eg green fluorescent proteins and PET tracers – Fluorine-18 based (both used by 41%) and then light producing cell lines (39% using). The least used approach was label-free (utilising natural fluorescence of certain molecules like collagen and elastin) (**Figure 8**).

Main limitations of optical *in vivo* imaging

Maximising the depth of tissue penetration was rated the main limitation associated with optical *in vivo* imaging (ie bioluminescent or fluorescent imaging). This was followed by natural scattering of photons by biological tissue; high background signal from surrounding tissue; and then small-animal imaging/resolution does not parallel clinical equivalents. Rated least limiting was data inconsistencies between imagers (**Figure 9**).

Latest developments in preclinical *in vivo* imaging

The following snapshots provide details of how various vendors support work on *in vivo* preclinical imaging through provision of imaging instruments and associated imaging reagents, tracers, contrasting agents and probes.

Aspect Imaging (www.aspectimaging.com) is a world leader in high-performance compact benchtop MRI imaging systems for preclinical research. Aspect's M2™ platform and suite of products enables researchers to harness the power and quantitative insights of MRI for small animal phenotyping and drug development but without the cost, complexity and technical burden of traditional MRI systems. With Aspect's simple-to-use platform, researchers can derive deep insights into their biological questions quickly, easily and cost-effectively. The system that has no fringe magnetic field and because of this the M2 can be placed anywhere in a working lab, including at a scientist's benchtop. The novel underlying technology, (ie the compact high-performance permanent magnets, coils and gradients) addresses the primary obstacles that exist in the current MRI market. These include the high cost of purchasing, operating and maintaining high-field MRI systems, the complexity of operating complex research-based MRI systems, and the physical limitations placed on traditional MRI systems due to their active magnetic

Figure 7: How *in vivo* imaging impacts drug development

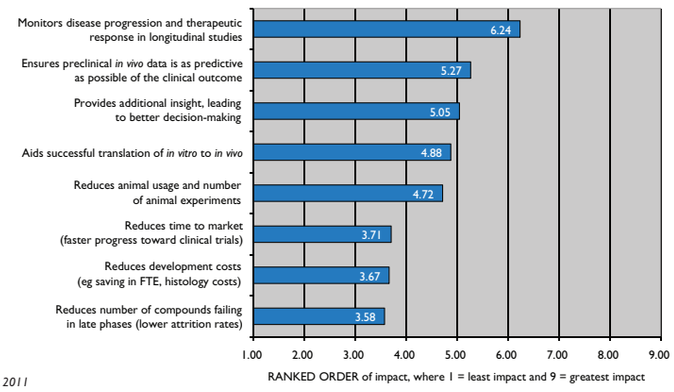


Figure 8: Reagents respondents use today for their *in vivo* imaging studies

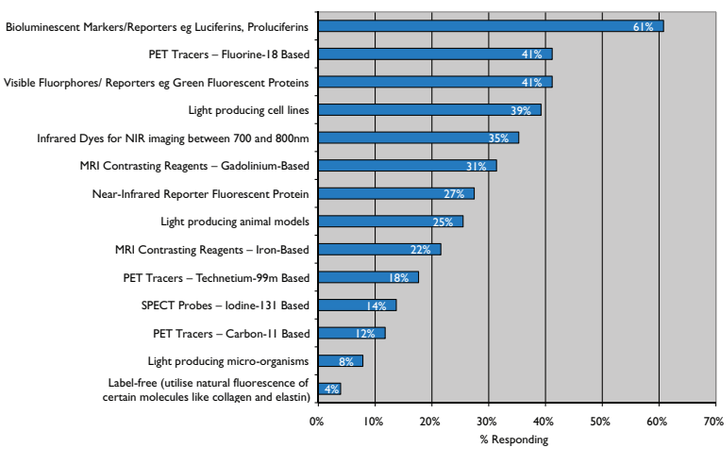
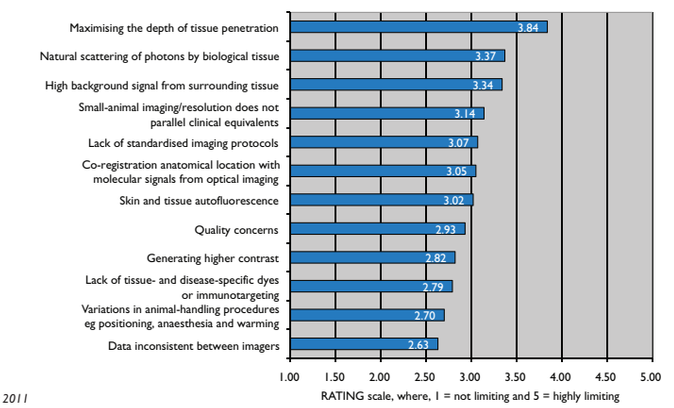


Figure 9: Main limitations associated with *in vivo* optical imaging



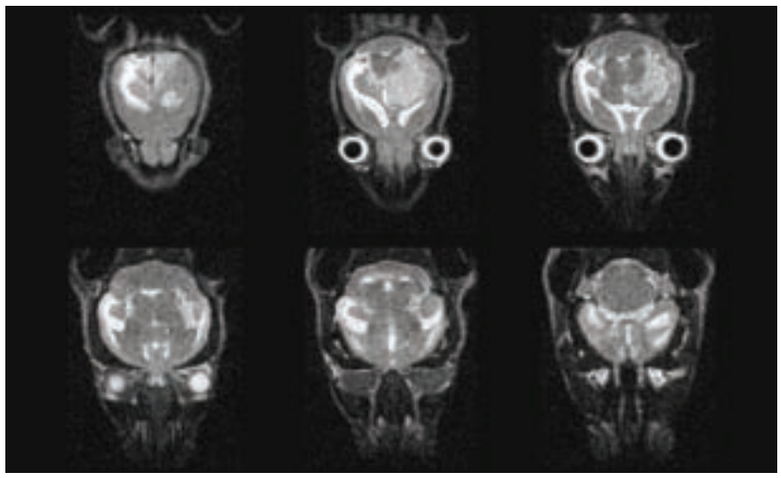


Figure 10: Multi-slice image sequence of Human Glioma in an adult mouse brain using the Aspect Imaging M2 quantitative MRI system. *(Image sequence courtesy of Dr A Johnson, Duke University, NC)*

fields. The Aspect's M2 system solves all these issues as it is affordable to purchase, is virtually maintenance-free, has no active magnetic field so it can be placed anywhere in a research lab and has been designed to be operated by researchers with no prior imaging or MRI experience. Because of this simplicity of operation, the power of MRI as a diagnostic standard is now available to be applied to complex biological questions in many research application areas including cancer research, neurobiology, obesity and diabetes, *in vivo* and *ex vivo* (ie 3D histology) anatomical imaging. The system can leverage a variety of techniques including 2D, 3D and contrast agent acquisition and analysis in addition to participating easily in multi-modality applications and image co-registration (**Figure 10**).

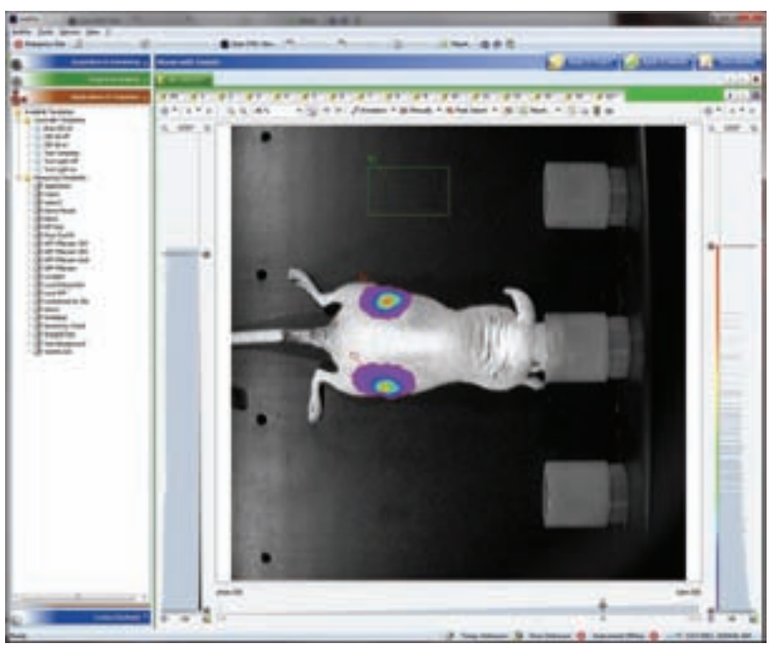


Figure 11: Screenshot of the new indiGO software for *in vivo* imaging now available on Berthold Technologies' NightOWL LB 983

The new easy-to-use indiGO software for *in vivo* imaging available from **Berthold Technologies** (www.berthold.com) for the NightOWL LB 983 has been developed together with users and covers a broad range of laboratory situations. Only three buttons: applications and templates; projects and analysis; and acquisition and scheduling; guide users intuitively through the software. Well organised menus and dialogue boxes help set up experiments easily. All images are saved immediately. The workflow of an application is scheduled by drag and drop. Individual exposures of bioluminescence or fluorescence as well as multi-fluorescence measurements in combination with bioluminescence measurements are possible for different sequences/kinetics. Pre- and post settings for each acquisition are also possible to allow highest flexibility. Multi-fluorescence measurements are shown in different colours according to the used filters. Most effort was spent on the analysis menu. Latest image processing methods such as transparency, combined contrast settings or image overlay have been implemented. The apply function is easy to use and time-saving, as the user selects one image out of the whole measurement series and edits it according to his needs. Afterwards the user applies their chosen settings to all images of the whole measurement series per mouse click. The data export to Excel is done automatically. Different user rights from guest level up to administrator can be implemented, eg to apply new projects, do measurements or evaluations. indiGO software can be installed free of charge on different PCs to allow image analysis at different places. Images from the former WinLight software can be imported for easier data evaluation (**Figure 11**).

Bioscan (www.bioscan.com) is a leading supplier of preclinical nano-tomographic molecular imaging tools that enable significant breakthroughs in the discovery, validation and development of novel therapeutics. The unique imaging acuity enabled by its proprietary technologies make it possible to translate research results from small animal models of human diseases to human clinical trials – driving increased speed to market for drugs. Bioscan's industry-leading nuclear imaging systems generate quantitative, 3D images of biotracers in animals

and are the first nuclear imaging systems to break the sub-mm resolution barrier. Offering sub-mm PET and 10µm CT resolution, only the NanoPET/CT has the sensitivity and image quality to enable truly translational research. The new 'Plus' version of Bioscan's popular NanoSPECT/CT enables users to reconstruct SPECT tomograms in real-time and supports both high-resolution (<350µm) stationary organ imaging and high-throughput whole-body helical scanning. This year, Bioscan will launch NanoFLECT, its groundbreaking fluorescence imaging system. NanoFLECT will be the first optical imaging system featuring true 360° tomography and optional inline X-Ray CT. In addition, Bioscan, together with a consortium of academic and commercial partners in Dijon, France, has been awarded more than €7 million by the French Government to develop a PET/MRI imaging system as well as associated animal models and consumables (**Figure 12**).

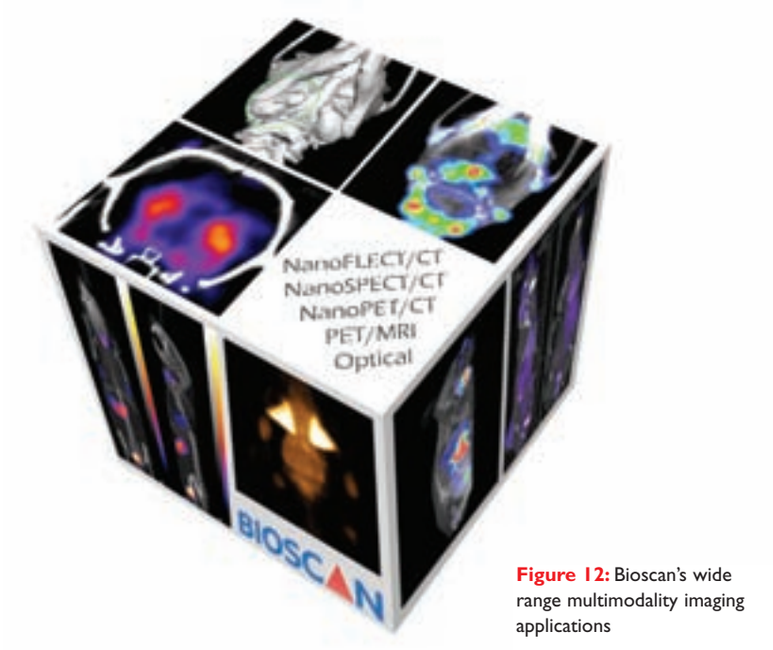


Figure 12: Bioscan's wide range multimodality imaging applications

Caliper Life Sciences (www.caliperls.com) is a leading provider of innovative tools and services for preclinical life science research and drug discovery with leading edge optical technology, integrated x-ray and low dose microCT. It is steadily focused in supporting longitudinal experimental models while maintaining relevant biological settings, translational applications and quantitative credibility. The optical imaging portfolio offers bioluminescence technology in both 2D and 3D with absolute quantitative tools that enable researchers to detect disease down to the number of cells. Caliper's fluorescence technology offers a wide range of tools to detect small amount of either cells or pmol levels of protein by means of spectral unmixing and normalise transmission fluorescence features. Moreover, Dynamic Contrast Imaging (DYCE) offers a translational approach to use either fluorescence or luminescence sources to quantify dynamic distribution of compounds in real time. Caliper's recent introduction of the Quantum FX microCT has set a new standard in low dose

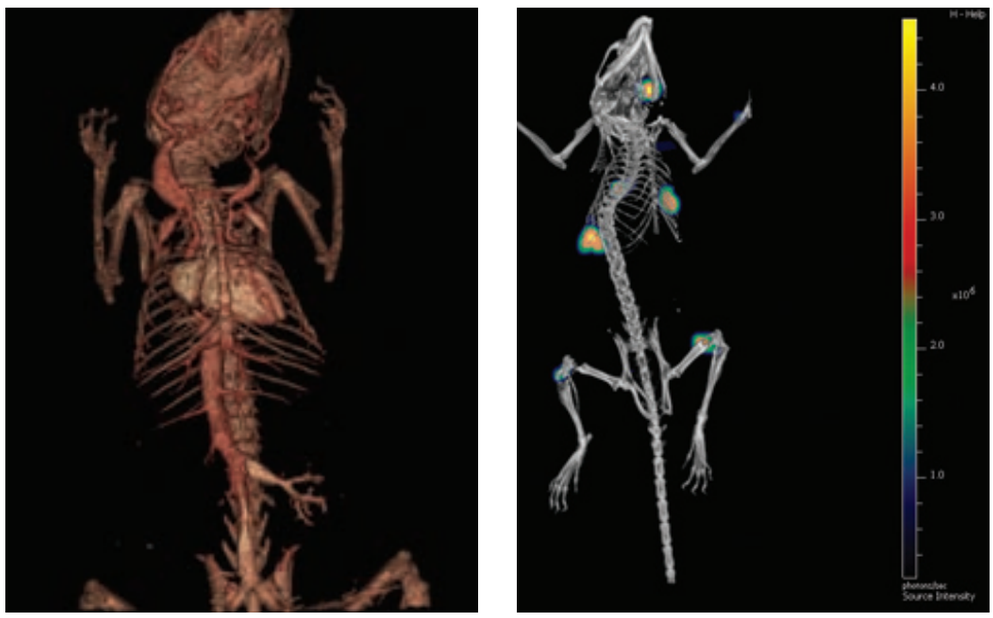


Figure 13
Caliper Life Sciences' Quantum FX uCT scan with a nanoparticle contrast agent to visualise vasculature (left panel), and MB321 metastasis formation identified by 3D bioluminescence and co-registration with uCT (right panel)

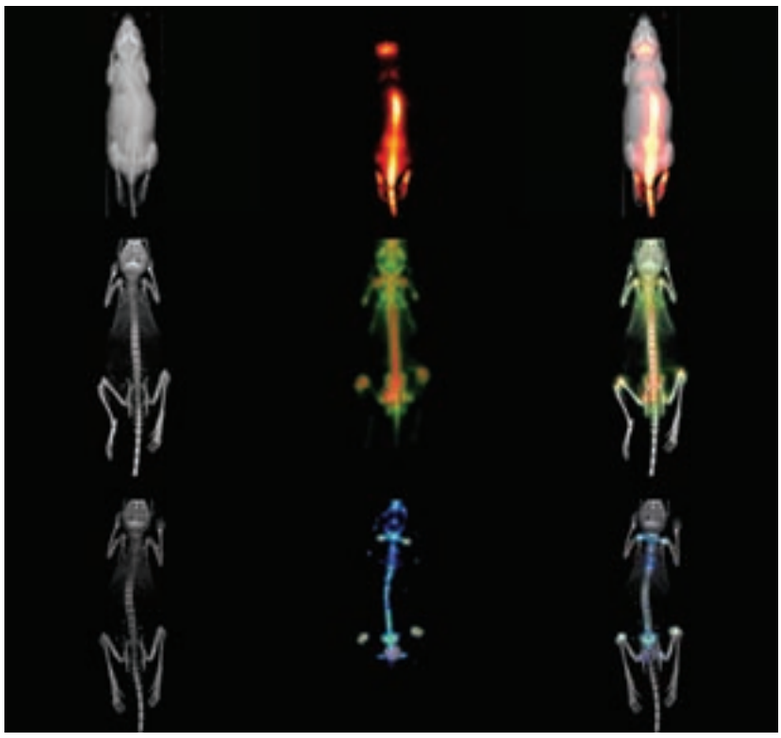


Figure 14: Bone development studied using three probes in the same mouse (top to bottom): NIR fluorescent bisphophonate, 18F-NaF, and 99mTc-MDP. Functional images were automatically co-registered to either x-ray or CT. The mouse was imaged using both the Carestream In Vivo MS FX PRO and the Albira system. *(Images courtesy of Dr W Matthew Leevy, NDIIF, University of Notre Dame)*

microCT for longitudinal studies. With a standard dose level of 13mGy, 17 second scans and one minute reconstruction time, the Quantum FX can image large cohorts of animals at multiple time points throughout an experimental course. The Quantum FX addresses all *in vivo* uCT applications from fat distribution to bone mineral density determinations and elegant vascular imaging procedures. Caliper's multimodality software and imaging shuttles enables co-registration of optical 3D data with CT scans methods to combine absolute functional quantification with precise anatomical localisation *in vivo* (**Figure 13**).

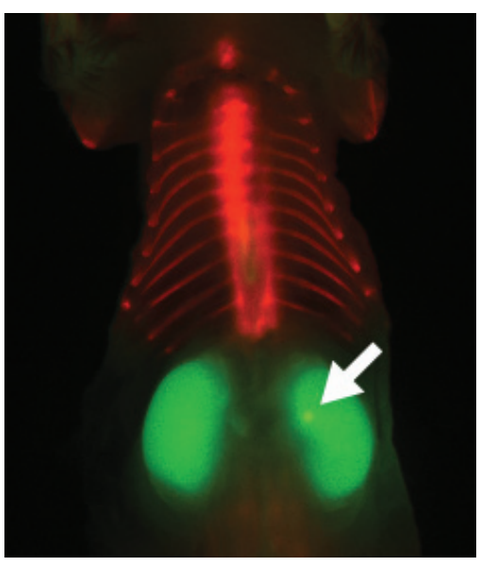


Figure 15
NIR fluorescent imaging of kidney metastasis with LI-COR Pearl® Impulse. Animal received tumour cells intracardially. After 10 weeks, IRDye® 800CW EGF (green) and IRDye 680 BoneTag™ (red) probes were used for multi-target imaging. Animal was surgically examined after sacrifice and imaged. Skeletal structure is visualised in red and metastatic lesion is seen on one kidney (arrow on green object). *(Image courtesy of Dr Melanie A Simpson)*

Carestream Molecular Imaging (http://mi.carestream.com) offers one of the broadest portfolios of preclinical imaging systems in the market. Because no one single imaging modality can fulfill the growing research needs of the drug discovery industry, Carestream offers researchers the ability to use seven distinct modalities – fluorescence, luminescence, radio-isotopic, x-ray, PET, SPECT and CT – available in two compact, cost-effective imaging solutions. With the In Vivo MS FX PRO, an innovative combination of high resolution optical and x-ray imaging, researchers can utilise activatable fluorescent probes, luminescent probes or screen SPECT and PET probes with a high sensitivity radio-isotopic screen. These functional imaging modalities can be automatically co-registered with x-ray images to better localise these signals while simultaneously studying bone and soft tissue phenotypes. The MS FX PRO brings the broadest range of imaging applications to today's researcher – from protein blots to complex, longitudinal studies of disease states *in vivo*. The recently introduced Albira PET/SPECT/CT system is available in six configurations and upgradeable from stand-alone to bi- or tri-modal functionality. This gives researchers the freedom to purchase what they need now, and to upgrade as their needs evolve. Albira's unique detector system uses an exclusive combination of single crystal detectors, PSPMT, and associated advanced electronics to deliver high sensitivity with rapid acquisition of extremely high resolution, quantitative and accurate images. This represents a new, highly innovative alternative to the pixelated crystal technology used in other systems. Ultimately, researchers increasing access to these tools is accelerating preclinical validation and the development and clinical translation of new drugs, medical treatments, imaging probes and contrast agents (**Figure 14**).

LI-COR Biosciences' (www.licor.com/invivo) small animal imaging products make preclinical fluorescence imaging more powerful and affordable than ever. Near-infrared (NIR) fluorescence optical imaging uses highly sensitive fluorescent optical probes to target the molecular changes that underlie disease,

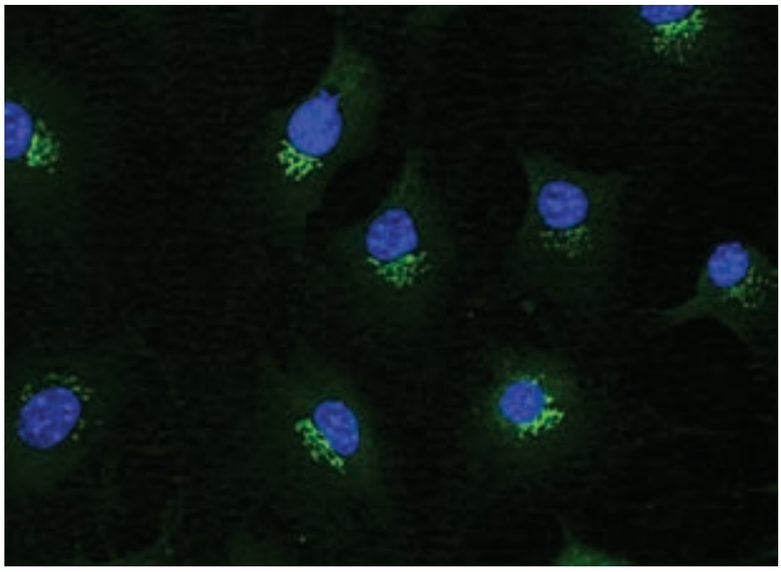


Figure 16: *In vitro* neuronal progenitors cells labelled intracellularly with Viscover FeraTrack (anti-dextran immunofluorescence) reagents from Miltenyi Biotec

and provides strong potential for clinical translation. The Pearl® Impulse Imaging System combines high-performance NIR imaging with easy-to-use image capture and analysis tools that make it easy for new users to get started. Near-infrared laser excitation (685 and 785nm) and FieldBrite™ technology enable an unprecedented 6-log dynamic range and simultaneous multi-target imaging (**Figure 15**). Pearl Impulse is optimised for NIR dyes, including the widely-published IRDye® 800CW dye and IRDye 680RD, a new dye specifically designed for small animal imaging. Ready-to-use BrightSite™ optical agents with IRDye labels target a variety of disease characteristics, so you can start your experiments immediately without developing and characterising new agents. Targets include overexpression of cell surface proteins such as EGFR and β–integrins, glucose metabolism, bone mineralisation, vasculature changes in tumours, and lymphatic tracking. IRDye reactive dyes and labelling kits can be used to develop probes for custom targets. LI-COR Biosciences' growing portfolio of *in vivo* imaging reagents now includes IRDye 680RD optical agents, labelling kits, and reactive dyes suitable for single-target or multi-target imaging.

Figure 17: PerkinElmer's FMT 2500 LX system and the accessories that are typically used with the instrument

Viscover™ from **Miltenyi Biotec** (www.miltenyibiotec.com) represents the first integrated range of *in vivo* contrast agents for preclinical imaging in small animals using the modalities MRI, CT, optical imaging and ultrasound. Its tools include novel molecules and pharmacologies, as well as the application of trusted standards in clinical practice for animal imaging. Viscover developers are committed to animal welfare and the '3 Rs' of animal research: Replace, Reduce, Refine. As replacement is not always feasible, Viscover agents have been optimised to reduce the numbers of animals per study and to refine image technology to yield superior images while reducing time and costs. Its sterile, low viscosity pre-formulation gives you unrivalled convenience and an excellent safety profile in many different species. Contrast enhancement lasts for several hours and because the safety pharmacology of each formulation has been optimised repeat dosing is always an option for the pre-clinical investigator. The new FeraTrack™ superparamagnetic iron oxide contrast agent simplifies intracellular labeling of cells *ex vivo* using a convenient two-component kit. Once labelled with FeraTrack, cells can be tracked in recipient animals using MRI. Intracellular labelling of neural progenitor cells and their injection into the mouse cortex resulted in a group of labelled cells in the left cortex that were clearly identifiable by MRI (**Figure 16**).

PerkinElmer (www.perkinelmer.com) moved into the preclinical imaging market with the acquisition of VisEn Medical in August 2010. This acquisition extended the portfolio of imaging solutions that now extends from confocal microscopy for subcellular image analysis to whole animal imaging *in vivo*. The product solution offered is based on Fluorescence Molecular Tomography (FMT®)

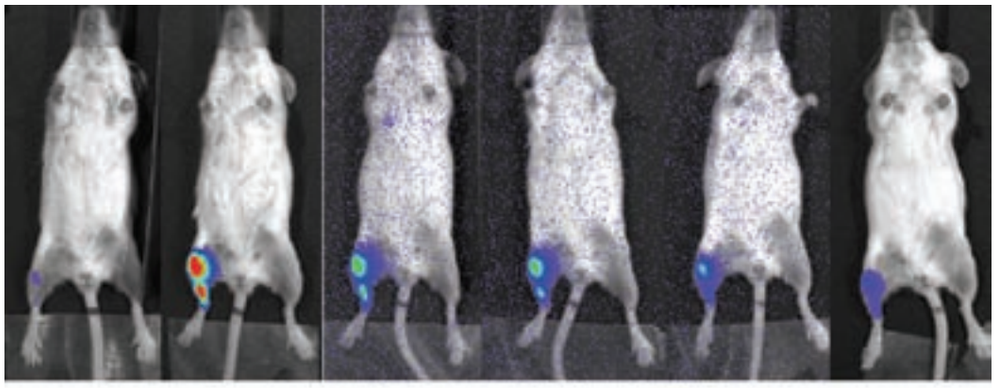


Figure 18
Demonstration of an optimised bioluminescent biosensor for the detection of cell death in living animals. Representative images taken at indicated time points (0 hours through day 5, left to right) of intratibial implanted 1833 reporter cells. TRAIL treatment (8mg/kg) resulted in a 100-200 fold induction of bioluminescence activity that correlated with cell death as demonstrated by increased cleavage of Caspase-3. The high signal to noise and dynamic range of reporter activity provides a sensitive and quantitative surrogate for the evaluation of experimental therapeutics

providing quantitative 3D imaging of biology in context. The 3D image is captured based on transillumination of test animal from laser light excitation, using either two lasers (FMT1500 with excitation at 635 and 745nm) or four lasers (FMT2500LX with excitation at 635, 670, 745 and 790nm). Because photons in the near infrared (NIR) penetrate from 7cm to 14cm *in vivo*, PerkinElmer has designed a broad portfolio of imaging agents labelled in the red and NIR for use on the system. The emission signals are captured by a cooled-CCD and the data is processed using TrueQuant™ software. The algorithm, which processes 10,000 to 100,000 image data points from each scan, takes into account tissue heterogeneity in reconstruction of the 3D image. FMT data can be co-registered with other imaging modes (MR, CT and PET) based on the fiduciary marks on the imaging cassette that holds the test animal. The combined data output extends the utility of the functional data obtained on the FMT. Newly added this year, the Multispecies Imaging Module (MSIM) enables the study of disease models in larger animals (up to 450g). Scans of mice and rats can be done sequentially without effect on the workflow (**Figure 17**).

Promega (www.promega.com), a technical leader in luciferase-based solutions for the life sciences, has continued to develop technologies for assays that can be transferred from the microplate well to the whole animal. Although the firefly luciferase pro-substrate approach has applications for *in vivo* preclinical imaging, a more recent approach involves the use of an engineered form of luciferase designed to act as a cellular biosensor. Commercialised under the trade name 'GloSensor', the biosensor technology has already been used to demonstrate kinetic readouts for proteins (ie GPCRs) that signal through the second messengers, cAMP and cGMP. The technology, in brief, relies on multiple forms of genetically engineered luciferases that are inactive in the absence of analytes of interest (ie cAMP, cGMP, protease etc). In the presence of the specific analyte, the enzyme undergoes a conformational change resulting in an active molecular state and luminescent readout that is proportional to the amount of analyte present. To monitor apoptosis through the activity of caspase-3/7, Promega inserted a protease recognition sequence for caspase-3 into the biosensor such that in the presence of this apoptotic enzyme, the cognate protease recognition sequence is cleaved resulting in an active biosensor. Promega developed proof of concept experiments in cell-based approaches in-house and then collaborated with researchers at the University of Michigan on the detection of apoptosis *in vivo* with the biosensor technology in murine cancer models. At the 2011 AACR Meeting, work was presented that demonstrated the use of the bioluminescent biosensor for imaging Caspase-3 activation in cancer cell lines and mouse models, as well as the evaluation of its usefulness for preclinical treatment efficacy studies[2]. It also demonstrated the biosensor to be extremely sensitive *in vivo* by generating two mouse xenograft models. TRAIL-induced caspase 3 of glioma xenografted animals resulted in bioluminescence activation of 100-fold in just six hours post-treatment. This study highlighted the usefulness of GloSensor for imaging cell death in real time and in kinetic mode in their preclinical mouse models (**Figure 18**).

Siemens Preclinical (www.siemens.com/preclinical) offers its Inveon™ PET, SPECT and CT animal imaging systems in various configurations ranging from standalone PET and CT systems to multi-modal PET/CT, SPECT/CT and PET/SPECT/CT

Figure 19
Siemens Preclinical Inveon CT system that can also be configured as PET/CT, SPECT/CT or PET/SPECT/CT integrated system

systems. The fully-shielded Inveon CT is highly configurable to customer needs and can perform anatomical or high-resolution CT scans, in addition to fast, low-dose CT imaging for longitudinal studies. For added workflow flexibility and throughput, the Inveon platform also offers a unique docking configuration between its standalone PET system and CT or SPECT/CT systems. The Inveon platform includes a complete workflow solution, from acquisition and reconstruction with Inveon Acquisition Workplace™ (IAW) to data analysis with Inveon Research Workplace™ (IRW). IAW includes a total of nine PET reconstruction methods, including the recently introduced OP-MAP algorithm that delivers high quantitative accuracy in low-dose imaging. IRW provides a fully integrated workflow for image visualisation, quantitative analysis and image and data distribution, and is offered with optional upgrades that include pharmacokinetic (PK) modelling and 3D visualisation (3D Vis). The company also offers a broad set of compatible accessories ranging from animal pallets, mouse and rat imaging chambers, physiological monitoring and heating systems and an isoflurane rodent anesthesia system (**Figure 19**).

Figure 20: Sofie Biosciences recently launched GENISYS^4 benchtop preclinical PET system. With the highest sensitivity in the field, researchers can inject less radioactive PET probe into animal models, which will in turn result in less radiation dose to researchers as well

In April 2011 in Orlando, Florida, **Sofie Biosciences** (www.sofiebio.com) launched the industry's first benchtop preclinical PET system, GENISYS^4, at the American Association for Cancer Research Annual Meeting. With its unprecedented compact size (W18"xD19"xH24"), GENISYS^4 can be installed on any laboratory bench with minimal site planning. As PET becomes an essential tool for translational research, Sofie believes it is time for PET technology to be more widely accessible, cost-effective and convenient for integration into a wide variety of labs. To achieve this, it believes PET must reduce its size and complexity while increasing usability and performance. GENISYS^4 was carefully designed to incorporate Sofie's vision to meet all these needs. By utilising a new PET detector architecture in conjunction with advanced software system modelling and reconstruction algorithms, GENISYS^4 demonstrated unmatched sensitivity (>14%) with high spatial resolution (1.4mm), meaning it is the smallest PET imaging system in the industry, yet the most sensitive. The high sensitivity enables researchers to inject less radioactive PET probe into animal models, which will in turn result in less radiation dose to researchers as well. Furthermore, as PET measures physiology of the animal, Sofie believes the animal's condition should be carefully maintained and monitored over the course of the experiment. GENISYS^4 also provides solutions for these needs by employing a novel animal preparation stage called the Docking Station which is integrated with its Imaging Chamber. In addition, the animal respiratory monitoring capability ensures the animal's condition during the study. Sofie hopes GENISYS^4 will enable more investigators to incorporate PET into their research (**Figure 20**).

The **UVP** iBox® Explorer™ Fluorescence Imaging Microscope (http://www.uvp.com/iboxexplorer.

html) is capable of detecting fluorescent proteins and tags in whole organs and individual cells *in vivo*. The high-resolution camera and choice of five magnifications supply maximum flexibility. Fully automated optics permit reproducible and rapid imaging with software presets and macros. Directed dual excitation light paths and specially designed filters allow imaging wavelengths from longwave UV (qDots) and visible to near IR for detection of fluorescent tumours and cells. In addition to tracking the progression of individual cancer cells, the growth of primary and metastatic tumours can also be measured. Quantitation of the dimension *in vivo* measures the relative co-ordinate and length, area and volume of the signal. To accurately acquire these numbers, it is necessary to have contrast between the intensity of the signal and the background level. Researchers define an intensity threshold distinguishing the signal and background based on their spectral characteristic and expected distribution. UVP's VisionWorks®LS Software estimates the tumour region and displays it in graphical and tabular format. Using fluorescent proteins to track small animal tumour growth provides the necessary indicators to visualise, detect, and measure the progression of cancer *in vivo* (**Figure 21**).

Figure 21: The UVP iBox® Explorer™ Fluorescence Imaging Microscope

VisualSonics (www.visualsonics.com) is a world leader in real time, *in vivo*, high-resolution micro imaging systems designed specifically for preclinical research. VisualSonics' product families include the VEVO® LAZR Photoacoustic Imaging platform and VEVO® 2100 and VEVO® 770 high-frequency micro-imaging systems. The VEVO® technology enables *in vivo*, real-time, high resolution (as low as 30 microns) visualisation and assessment of small animal anatomical, functional and structural targets. The VEVO technology features extremely high frame rates, quantification and assessment software tools, advanced imaging features such as: Color Doppler, contrast imaging, strain analysis, multiple imaging and processing modes. These features have found strong utility in advanced preclinical research as related to cardiovascular diseases, drug induced vascular injury, tumour visualisation, imaging and quantification, brain flow imaging among other applications – resulting in more than 700 peer-reviewed publications from researchers across the globe. VEVO LAZR Photoacoustic technology has further expanded *in vivo* imaging techniques with molecular imaging capability. This technology integrates sensitivity of optical imaging with resolution and depth penetration of high-frequency ultrasound, enabling researchers to detect and study cancer in its earliest stages of progression. Researchers are using the Vevo LAZR technology to observe tumour biology, measure hypoxia, evaluate changes in blood flow and quantify data with proprietary software solutions *in vivo* and in real-time. This technology provides researchers with never-before-seen insights into the development of effective therapeutics for treating cancer. In addition to cancer biology, photoacoustic imaging benefits other areas of research such as diabetes and neurosciences, as well as developmental and reproductive biology (**Figure 22**).

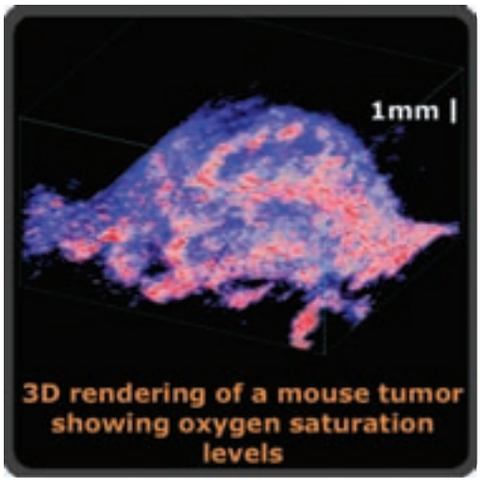


Figure 22
Mouse tumour imaged using VisualSonics Vevo LAZR photoacoustic technology

Table 1: Summary of the in vivo preclinical product offerings of the companies reviewed

Aspect Of In Vivo PreClinical Imaging Supported:	Aspect Imaging	Berthold Technologies	BioScan	Caliper Life Sciences	Carestream Molecular Imaging	LI-COR Biosciences	Miltenyi Biotec	PerkinElmer	Promega	Siemens Preclinical	Sofie Biosciences	UVP	Visual Sonics
Imager Modalities Offered:													
Magnetic resonance imaging (MRI)	X				X					X			
Optical (bioluminescence/luminescence)		X		X	X							X	
Optical (fluorescence)		X	X	X	X	X		X				X	
Positron emission tomography (PET)			X	X[2]	X[3]					X	X		
Single photon emission computed tomography (SPECT)			X		X					X			
X-ray				X	X						X		
X-ray computed tomography (CT)			X	X	X					X			
Ultrasound (US)													X[6]
Multi-Modality Imaging Combinations:													
On the same instrument		X[1]	X	X	X					X	X		
Via co-registration on different imagers	X	X	X	X	X	X		X		X		X	
Imaging Reagents:													
Fluorescent Imaging Reagents (including near IR)			X	X		X	X	X				X	
Bioluminescent Imaging Reagents				X					x			X	
Light producings animals, cell lines or microorganisms				X								X	
PET Radioactive Tracers			X				X[4]			X	X		
MRI Contrasting Reagents	X						X						
X-Ray CT Contrasting Reagents							X						
SPECT Probes			X				X[4]			X			
Ultrasound Contrasting Reagents							X[5]						

Notes: [1] Lumi. + Fluor.; [2] Cherenkov optical of PET tracers; [3] Including radio-isotopic imaging; [4] Coming soon ; [5] Ultrasound microbubbles; [6] Photoacoustic Imaging.

Discussion

In **Table 1** the product offerings of the 12 companies discussed in this article are compared with respect to the imager modalities offered; whether or not they support multi-mode imaging on the same instrument or via co-registration; and the types of imaging reagents, tracers, contrasting agents and probes offered. Some trends were evident from the vendor's contributions:

Compact systems: There seems to be increasing emphasis on building compact benchtop systems that can be placed anywhere in a research lab, which are not constrained by physical limitations eg those placed on traditional MRI systems due to their active magnetic fields. Reducing system complexity such that they can be operated by researchers with no prior imaging experience will further help to promote accessibility, as will the anticipated availability of more cost-effective imaging solutions (Aspect Imaging, Carestream and Sofie).

Multi-Modality: Animal imaging systems that support bi- or even tri-modal combinations (on the same instrument) are on the increase, this is particularly true for the PET, SPECT and CT modalities (Bioscan, Carestream, Siemens and Sofie). In contrast, fixed instruments combinations of optical (fluorescence) imaging are mainly in combination with x-ray CT or micro CT (Bioscan, Caliper & Carestream). Most other optical imaging instruments are single modal and rely on co-registration with other imaging modes (MRI, CT and PET), based on the fiduciary marks on the imaging cassette that holds the test animal, to combine absolute functional quantification with precise anatomical localisation *in vivo* (Aspect, Berthold, Caliper, Carestream, LI-Cor, PerkinElmer and UVP).

Higher Resolution: As new imaging systems incorporate novel detectors, technologies, electronics, advanced software system modelling and reconstruction algorithms, we are seeing higher spatial resolution, unmatched sensitivity with no loss in the speed of acquisition, such that the specifications of some animal imagers will soon exceed their clinical equivalents (Bioscan, Carestream, Siemens, Sofie, VisualSonics).

Better Reagents: Imaging systems that utilise visible fluorescent probes typically have low target-to-background ratios and shallow tissue penetration due to high tissue autofluorescence and light scattering at visible wavelengths. Excitation at near-infrared (NIR) wavelengths achieves high target-to-background ratios with deeper tissue penetration. With this in mind vendors (BioScan, Caliper, LI-Cor, Miltenyi Biotec, PerkinElmer and UVP) have developed a broad range of fluorescent probes labelled in the red and NIR for use in single-target or multi-target optical imaging of cells or organs *in vivo*. Bioluminescent biosensors are also proving very useful in imaging cell death in cancer cell lines and in the evaluation of preclinical efficacy in mouse models (Promega). During *in vivo* imaging it is necessary to have contrast between the intensity of the signal and the background level, this is usually achieved using contrast agents. Several vendors are active in producing *in vivo* contrast agents for preclinical imaging in small animals using the modalities MRI, CT, optical imaging and ultrasound (Aspect, Bioscan, Miltenyi Biotec, Siemens and Sofie).

Advanced Software: All imaging systems are heavily reliant on advanced software systems and algorithms for reconstruction of the 3D image and co-registration of multiple imaging modalities. Some vendors have implemented new software including latest image processing methods such as transparency, combined contrast settings or image overlay (Berthold) and the ability to define an intensity threshold distinguishing the signal and background based on their spectral characteristic and expected distribution (UVP).

Translational Research: Facilitating translational research is emerging as the favoured way of marketing *in vivo* preclinical imaging, with most vendors promoting this concept. In reality, this is nothing new. Bridging the gap between *in vitro* exploratory and *in vivo* clinical research has long been recognised as the niche where *in vivo* imaging plays best. What, however, has changed is the small animal models of human disease or native cells used may now be more conducive to translational research, which when coupled with our ever increasing ability to track cellular or organ changes *in vivo* should allow better translation of research findings to human clinical trials.

In conclusion, the trends highlighted suggest the tools necessary to facilitate significant improvements in small animal imaging are now becoming mainstream and we should expect to see major advances in *in vivo* imaging as applied to translational research over the coming years. DDW

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