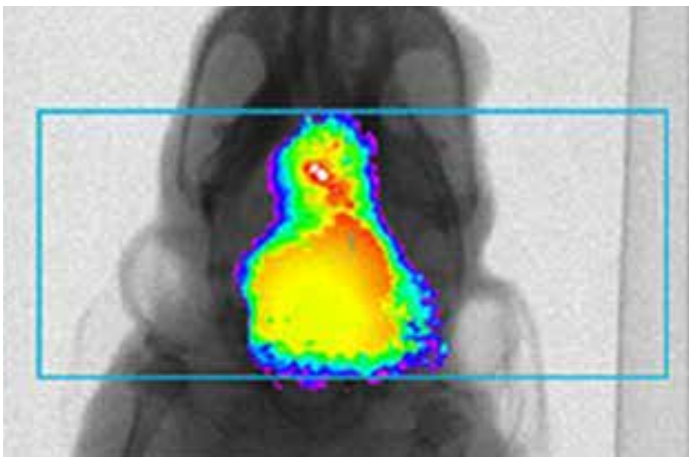

In Vivo Optical Neuroimaging

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Abbreviations

BBB	Blood Brain Barrier
CCI	Controlled Cortical Impact
DiR	1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide
FLI	Fluorescent Imaging
IV	Intravascular
MMAB	Multimodal Animal Bed
MARS	Multimodal Animal Rotation System
NIR	Near Infrared
PARACEST	Paramagnetic Chemical Saturation Transfer
PS	Phosphatidylserine
ROI	Region of Interest
TBI	Traumatic Brain Injury
Zn-DPA	Zinc (II)-dipicolylamine

Preclinical optical neuroimaging in small animals has been used in a wide range of research applications including: general pathology studies, development of clinically relevant disease models, testing of novel optical probes, and pharmacokinetic evaluation of therapeutics, including those designed to cross the BBB[1,2]. Optical imaging has several technical advantages over other imaging modalities. It is easy to use, allows high animal throughput, has low infrastructure cost, and can make use of numerous, commercially available, target-specific probes including: antibody conjugate probes that recognize specific antigenic targets, and various small molecule and/or activatable probes that are designed to detect markers of specific physiological phenomenon (e.g. matrix metalloproteinase enzymes as markers of inflammatory sites). Absorption and scattering of light signals in tissue does limit *in vivo* optical imaging to a depth of 2cm or less. However, the speed and sensitivity of optical imaging make it an optimal research tool for the development of disease-specific molecular probes and for cell tracking. Research findings from preclinical optical imaging may ultimately be translatable to clinical modalities/applications. In this article, we highlight some studies of optical neuroimaging using the Bruker In-Vivo optical imaging platforms.

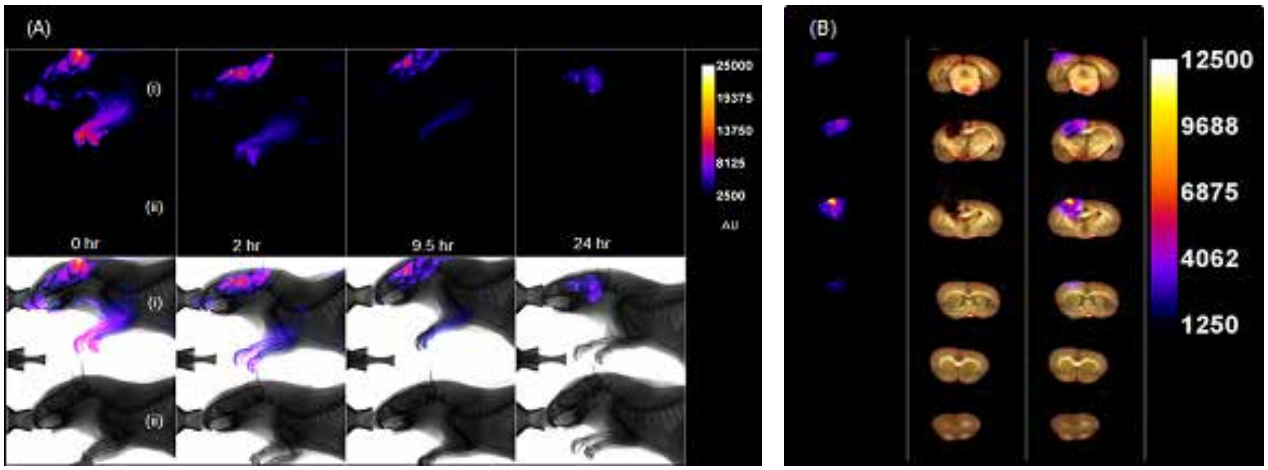
Several groups are actively evaluating candidate probes for Alzheimer's disease. Ran et al.[1] developed a class of Amyloid–β specific optical probes and validated these probes in a mouse model. In this study the author reported on the synthesis, design and testing of the NIR fluorescent curcumin analogue probe CRANAD-2 (named for the first author (C.RAN) and Alzheimer's disease (AD). Mice were shaved and injected with CRANAD-2 (5.0 mg/kg in DMSO and polypropylene glycol solution). Fluorescent images were recorded prior to injection and at time intervals of 30, 60, 120 and 240 minutes post-injection. Probe uptake (determined by *in vivo* FLI) correlated well with histological analysis performed *ex vivo*. These results have paved the way for future developments of CRANAD-2 for research, diagnostic and/or therapeutic applications.

The BBB restricts the transfer of molecules to the brain[3]. Several techniques to facilitate the transfer of target compounds across the BBB have been proposed. Many methods for BBB transfer are physiologically invasive and include injection or permeabilization of tight junctions, osmotic disruption with agents (e.g. mannitol) and biochemical openings (e.g. via RMP-7 Alkermes, histamine). More specific and targeted approaches for delivery of agents have been evaluated. Phamet al. (2005) proposed the use of a docking and transfer mechanisms using myristoylated polyarinine peptide (MPAP)[2]. To evaluate this approach, mice were injected with MPAP conjugated to Cy5.5. MPAP-Cy5.5 probe signal peaked (determined by *in vivo* FLI) in the brain at 24 hours post-injection. Probe kinetics were also assessed via confocal microscopy. Transcytosis of the molecule was observed within the first hour. MPAP-Cy5.5 passed the endothelium and accumulated primarily in neuronal cells in almost all regions of the brain including the cortex, hippocampus and thalamus. In another study (Meng et al., 2010), a myristic acid (MC) polyethylenimine (PEI) NIR probe was evaluated for brain imaging[4]. The probe (MC-PEI-IR820) was first characterized using NMR spectroscopy at 400 MHz and later injected in experimental and control mice. *In vivo* FLI was performed at 0.5, 6, 12, 24 and 48 hours post injection. Accumulation of the MC-PEI-IR820 in the brain increased from 0.5 to 24 hours post injection. Recently, Hao et al. (2013) demonstrated that liposomes modified with P-aminophenyl-α-D-mannopyranoside could be used as a fluorescent dye carrier. DiR labeling and *in vivo* FLI was used to track the carrier system across the BBB[5]. The complex was tracked from cortex to cerebellum and finally to the brainstem, allowing the researchers to plot a distinct spatio-temporal pattern for the distribution of this carrier in the brain.

Clinically, FLI has probably the greatest potential for use in endoscopy and tumor margin detection for diagnostic and surgical applications. Recently, an orthotopic hypervascular glioma was detected and monitored *in vivo* using a dual PARACEST and fluorescent contrast agent[6]. This study showed a potential for this dual modality probe for non-surgical glioma detection via MRI and clinical surgical excision interventions via fluorescence detection. In many studies, optical neuroimaging information is enhanced by anatomical co-registration with X-ray or reflectance images. In addition, multimodal imaging can be extended with cross-platform MR imaging as above. Using the Bruker MMAB for cross-platform transfer/imaging, optical studies can now be directly combined with PET, SPECT, MRI and CT to investigate multimodal research applications. The MMAB is compatible with the Bruker In-Vivo Xtreme II Optical/X-ray system, Bruker BioSpec MRI systems, ICON MRI, Bruker Albira Si PET/SPECT/CT and Bruker microCT 1176 and 1278.

The prevalence of TBI in civilian life, professional sports and the military arena globally has prompted the development of preclinical models to study the nature, extent and possible treatments for TBI. Non-invasive optical imaging of TBI in rats has been successfully conducted by Dr. Chu Chun et. al. (Temple University, personal communication). Here, the extent of cerebral tissue damage was assessed using a NIR probe, PSVue 794, which contains a polycationic Zn-DPA moiety that binds to exposed anionic PS residuespresent in high concentrations in the membranes of apoptotic, dead or dying cells. At 24 hrs post CCI trauma, either PSVue 794 (3 mg/kg), or PBS (at an equal volume) was administered IV. Animals were imaged at 0, 2, 9.5 and 24 hours following administration of probe (Figure 1A). *In vivo* analysis showed that signal persisted at the site of CCI. Sectional ex vivo FLI showed that the locations of signal were well-correlated with visible TBI-derived hemorrhagic lesions (Figure 1B).

Figure 1



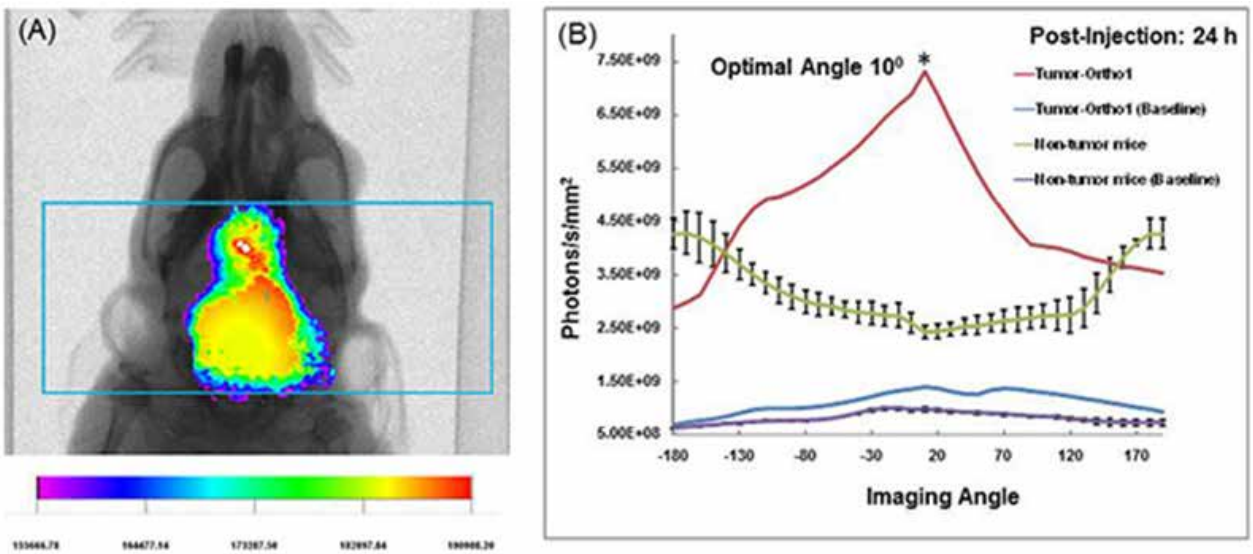
A) At 24 hrs post CCI,animals received: (i) PSVue 794 NIRF probe (3 mg/kg) or (ii) PBS (at equal volume). FLI (fire) and X-ray (gray) imaging was performed at 0, 2, 9.5 and 24 hrs after probe administration. Non-specific probe clearance, and cerebral retention was optimal at 24 hrs.
B) FLI (fire) and Reflectance (RGB) imaging of ex vivobrain tissue slices was subsequently performed. Fluorescent signal localized to brain lesions.

Rotational imaging can provide optimized detection for neurological applications. Using the Bruker MARS imaging platform, test mice can be incrementally and reproducibly rotated to acquire multimodal images at designated positions. In a study reported by Chu et al. (2013), MARS rotational imaging permitted longitudinal monitoring ofU87-ΔEGFR-Luc brain tumors in mice[7]. The tumors were detected using a Sapocin C– dioleoylphosphatidylserine nanovesicles CellVue Maroon (SapC-DOPS-CVM) fluorophore probe and FLI (Figure 2).

Conclusion

Preclinical optical imaging has been used in studies of neuro-specific probe development, Alzheimer's disease, TBI, and neurooncology. The Bruker In-Vivo Xtreme II provides optical/X-ray imaging for flexible detections that are supported by rotation detections and cross-platform imaging.

Figure 2



A) Peak fluorescent signal (rainbow) at an optimal imaging angle of a brain tumor.
B) A corresponding graph shows the effect of imaging angle on ROI analysis results.Non-tumor mice and tumor mice are shown.
Measurements are made before SapC-DOPS-CVM injection (baseline data) and 24 hr after injection. Imaging angle at 10° showed the greatest ROI intensity.

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