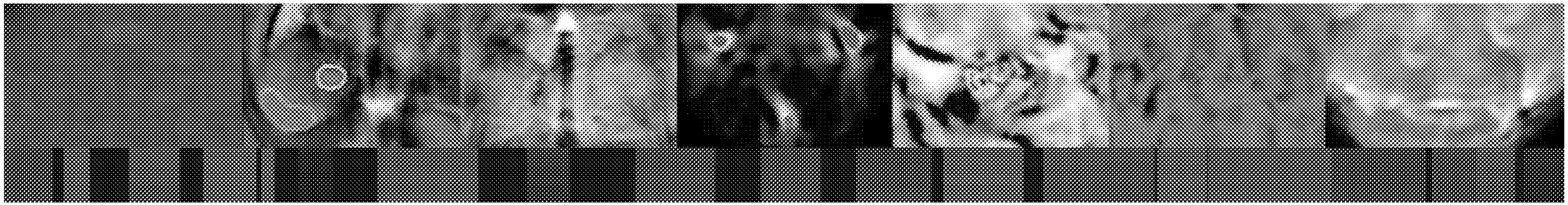
Imaging by Listening to Molecules: From translational research to drug development

Presented by Rui Wang, PhD
Cold Spring Biotechnology
24.09.2015



Agenda

- Company overview
- MSOT technology
- Selection of MSOT applications
- Handheld system and its clinical outlook

Co-founder: Vasilis Ntziachristos

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Major professional appointments

2007- **Professor & Chair for Biological Imaging**
Technische Universität München, Germany
School of Medicine and School of Electrical Engineering
Director, Institute for Biological and Medical Imaging (IBMI)
Helmholtz Zentrum München, Munich, Germany

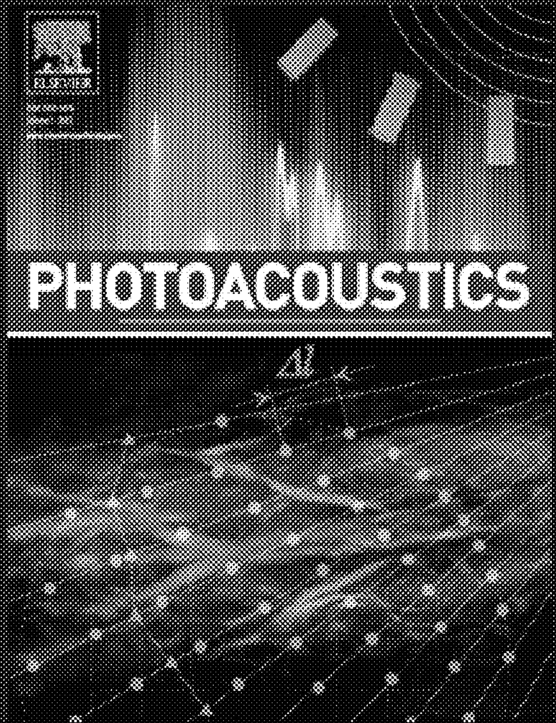
2002-2007 **Assistant Professor**
Director, Laboratory for Bio-optics and Molecular Imaging (LBMI)
Harvard University, School of Medicine & Massachusetts General Hospital, Boston MA

Selected professional activities

2013- **Established Photoacoustic editorial**
2010- Advisory Board, **Journal of Contrast Media & Molecular Imaging**
2008- Council Member, **Society for Molecular Imaging**
2006- Topical Editor for **Optics Letters**, Optical Society of America
2005- Associate Editor, **IEEE Transactions on Medical Imaging**
2005- Associate Editor, **International Journal of Biomedical Imaging**

Medical

NEW JOURNAL – to promote a Photoacoustics community
http://ees.elsevier.com/pacs/



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http://elsevier.com/locate/pacs

iThera Medical – overview

iThera Medical
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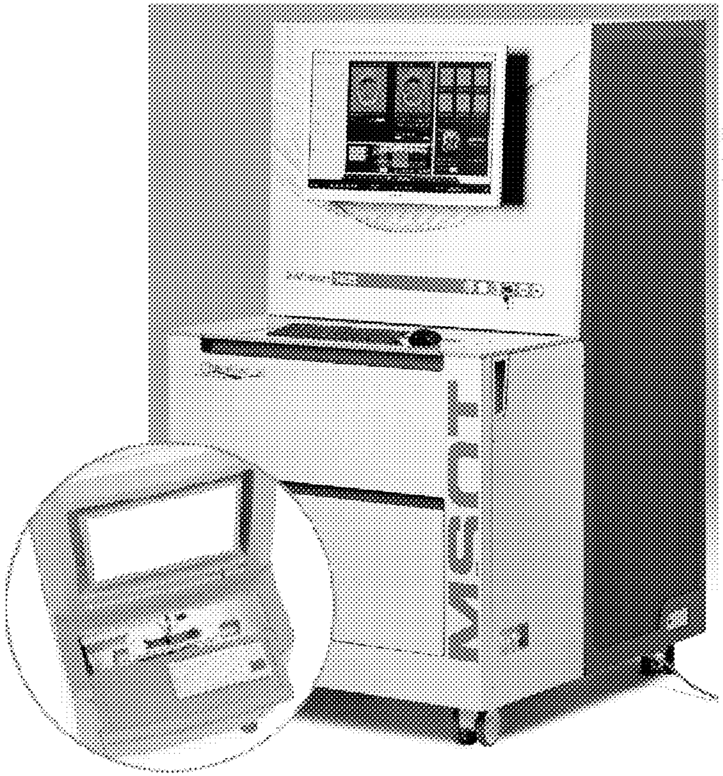
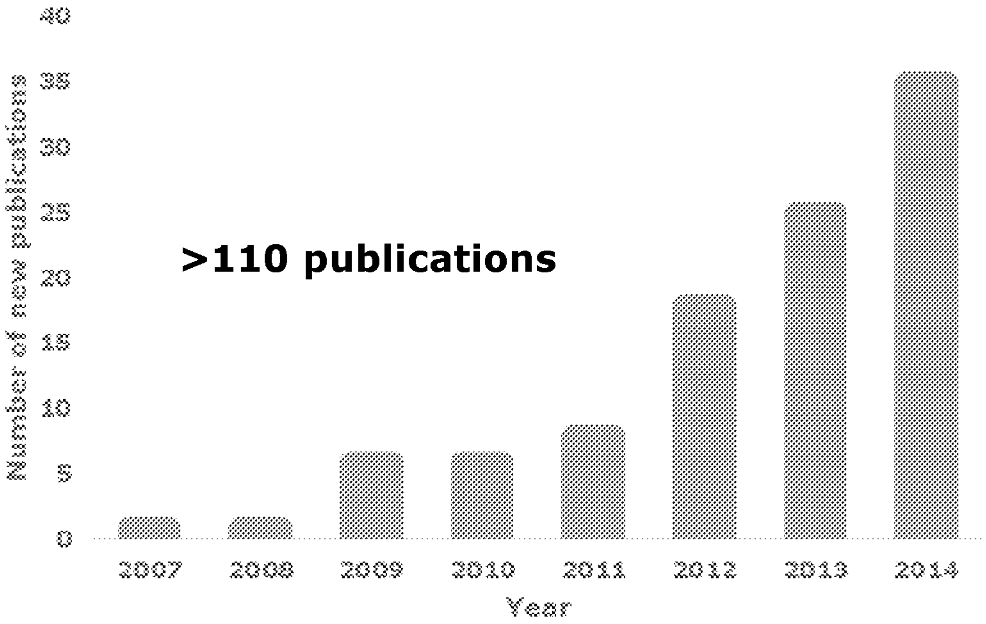
- Founded in 2010 as a spin-off from Helmholtz Centre Munich
- Developing and marketing novel molecular imaging technology
- Launched first optoacoustic preclinical scanner in 2010
- Supported by BMBF, awarded multiple times as innovative young company

Bundesministerium für Bildung und Forschung GO-Bio | Science 4 Life | bob best of biotech | BioVaria Munich 2010



MSOT-based publication each year



MSOT: Next-gen biomedical imaging

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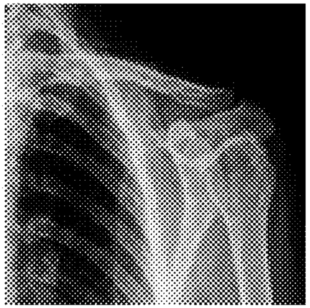
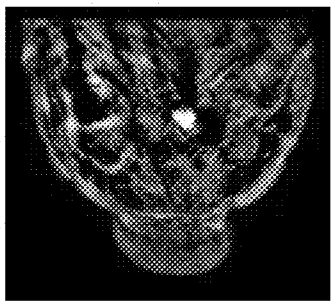
1895	1940s	1970s		1980s	1990s	2010
X-Ray	Ultra-sound	CT	MRT	PET/ SPECT	Optical	Opto-acoustic

Anatomical information → **Functional information** → **Molecular information**

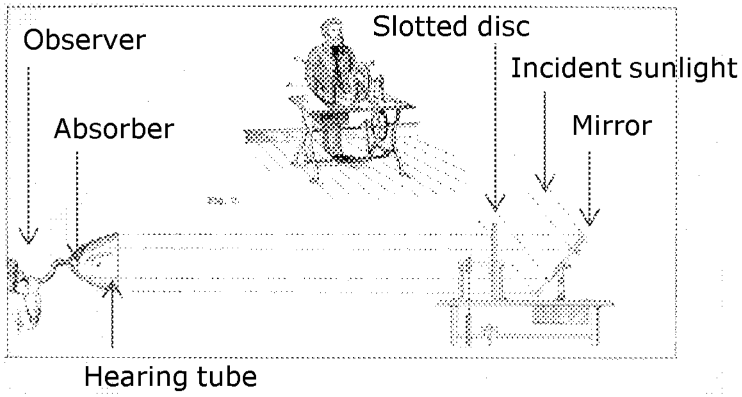




Historical background of PA imaging

Alexander Graham Bell first published on the photoacoustic effect in 1881

(A. G. Bell, "The Production of Sound by Radiant Energy," *Science,* vol. 2, pp. 242-53, May 28 1881)



- Incident sunlight was reflected to and rapidly interrupted by a rotating slotted disc
- A thin absorbing solid in the path of the filtered light was connected to a hearing tube
- Bell demonstrated that the strength of the acoustic signal depended on the intensity of the incident light

Availability of adequate lasers, ultrasound detectors, acquisition electro-nics, algorithms and computing performance now enable practical use

Agenda

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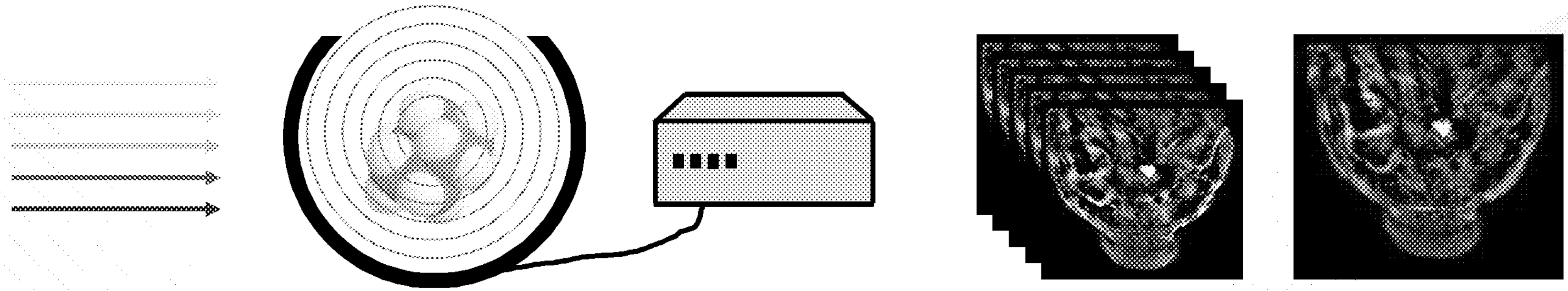
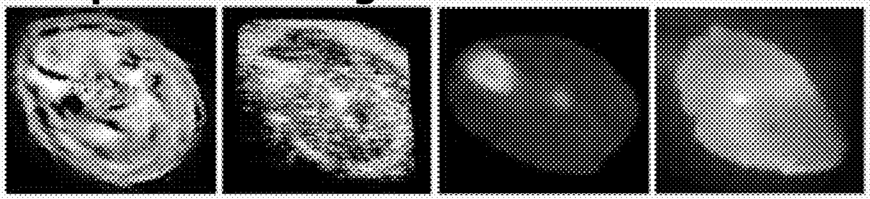
- Handheld system and its clinical outlook

Technology and benefits of “MSOT”

Laser excitation (NIR region) → Thermoelastic expansion → Ultrasound signal detection → Image reconstruction → Spectral decomposition



Comparative images – MSOT & others



MSOT* **Ultrasound** **CT** **Epi-fluoresc.**

User benefits

- 10 x **resolution** vs. PET & Optical
- **Molecular specificity** and **real time**
- **Safe** and **cost-efficient**

* MSOT = Multispectral Optoacoustic Tomography (cross-section of mouse leg)

Advancing biology AND medicine!

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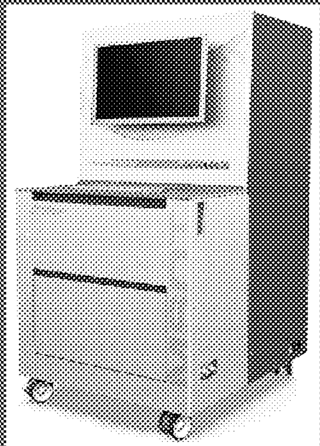
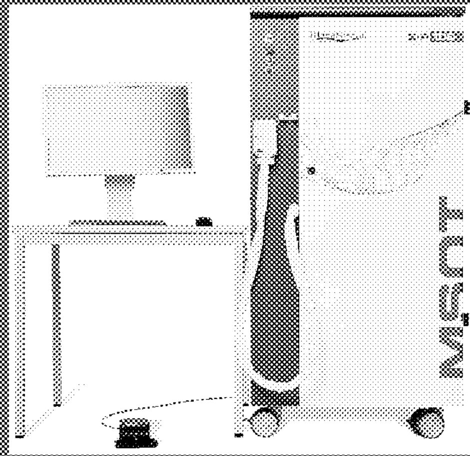
Preclinical



- Small animal scanner "inVision"
- Proven technology
- Wide range of applications

Clinical



- Handheld system "EIP"
- CE and FDA mark in 2015
- Clinical studies ongoing

Preclinical imaging with "MSOT inVision"

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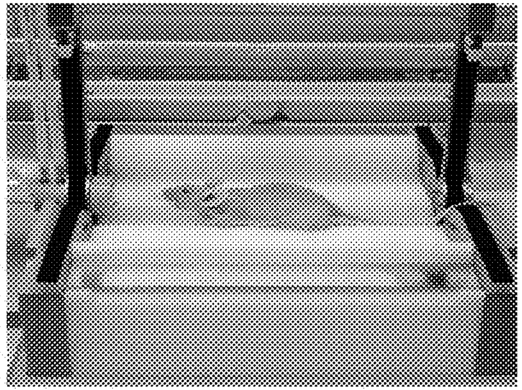
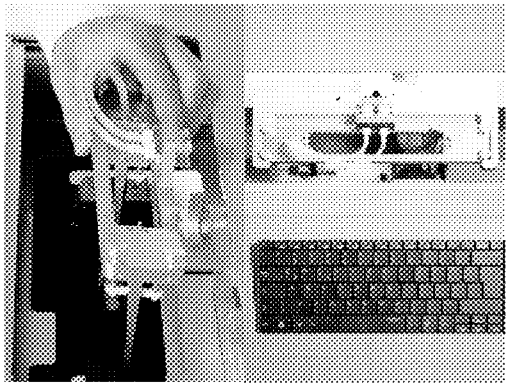
Animal preparation



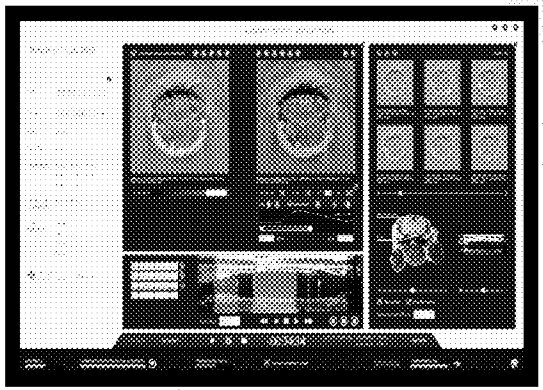
- Defined / repeatable positioning
- Nose cone with anesthesia supply
- Foil membrane for signal coupling

Image acquisition



- Chamber filled with water
- Stages for x-y-z positioning
- Ultra-fast image acquisition

Data processing

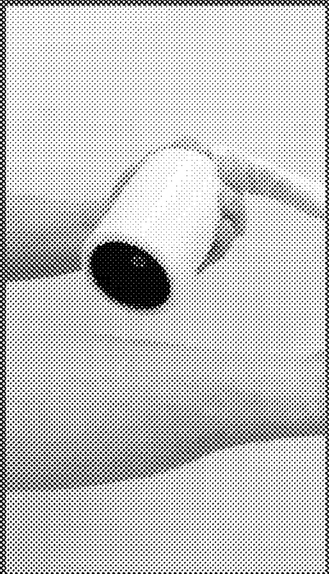


- Quantitative image reconstruction
- Multispectral / kinetic data analysis
- Export of images, videos, graphs

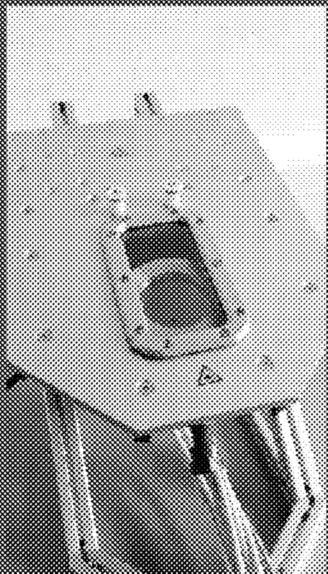
Clinical imaging with “MSOT EIP”

Two modes of EIP operation

Handheld

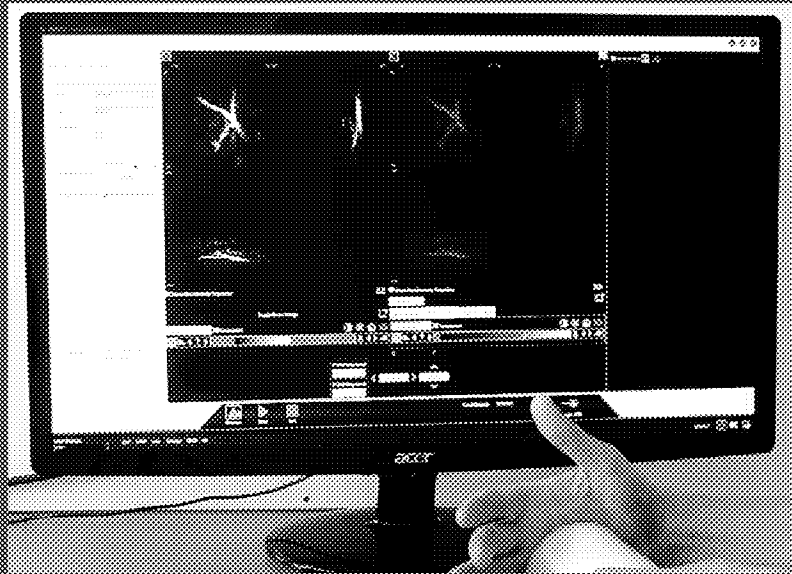


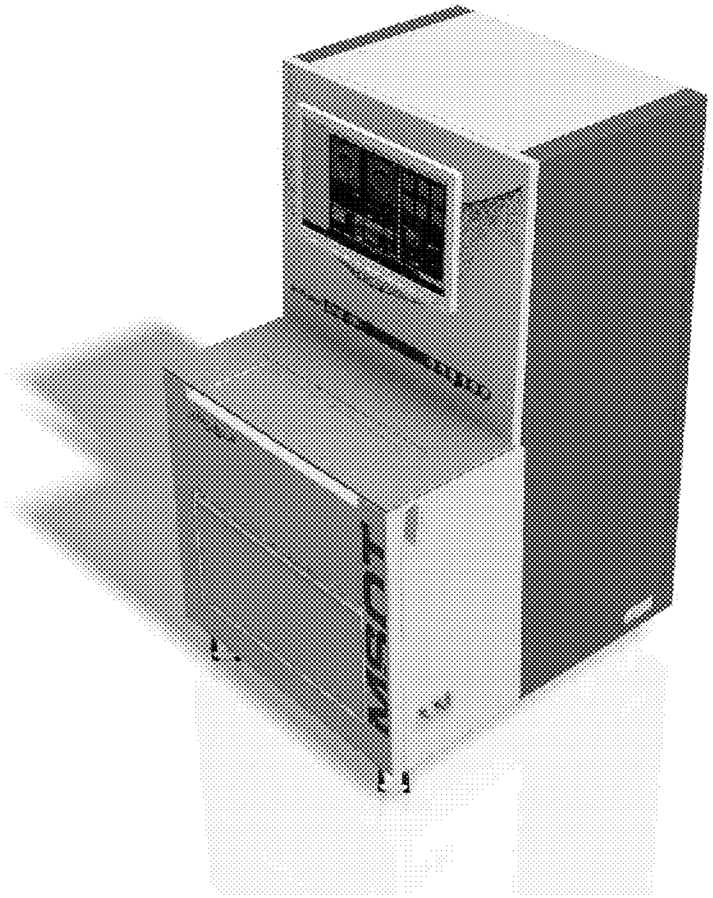
Stage-controlled



Live 3D visualization

Screen display during acquisition





Information acquired with MSOT

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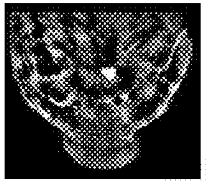
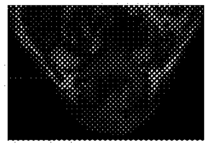
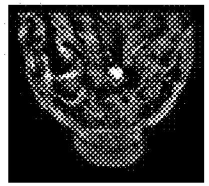
Anatomical information — Optical absorption of tissue



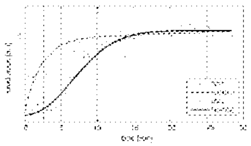
Functional information — Perfusion, oxygenation



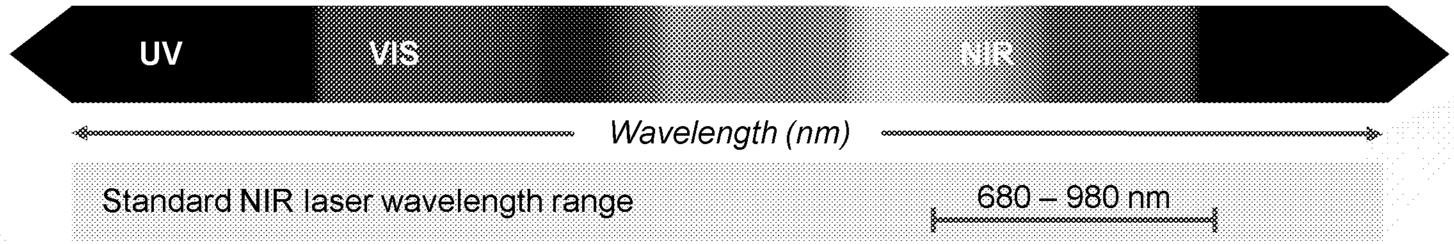
Molecular information — (Targeted) probes / FPs



Kinetic information — PK/biodistribution data



Detection of probes with MSOT



Implications of wavelength range for NIR optoacoustic imaging

1. Organic dyes: Cy5.5, IRDye, ICG, AF750 and similar dyes absorb in the NIR
2. Bioinorganic nanoparticles can be synthesized to absorb in the NIR (e.g. AuNR)
3. Fluorescent proteins: iRFP* can be detected using a NIR laser; blue-shifted fluorescent proteins (e.g. GFP, YFP, CFP, mCherry) require illumination at wavelengths in the visible range

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Imaging anatomy with MSOT

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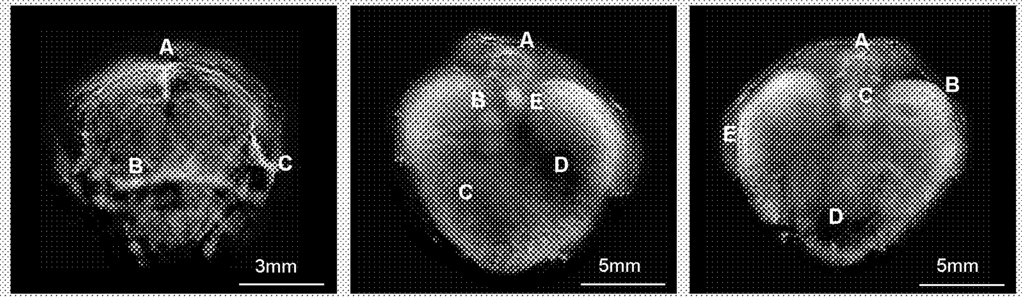
Experiment	Top row shows single-wavelength MSOT images at different characteristic cross-sections. Bottom row shows histology for reference.
Application	MSOT can visualize optical contrast at high resolution throughout the entire animal cross-section. The main absorber in tissue is blood. This yields an excellent contrast especially for highly perfused regions such as kidney, liver, and spleen.

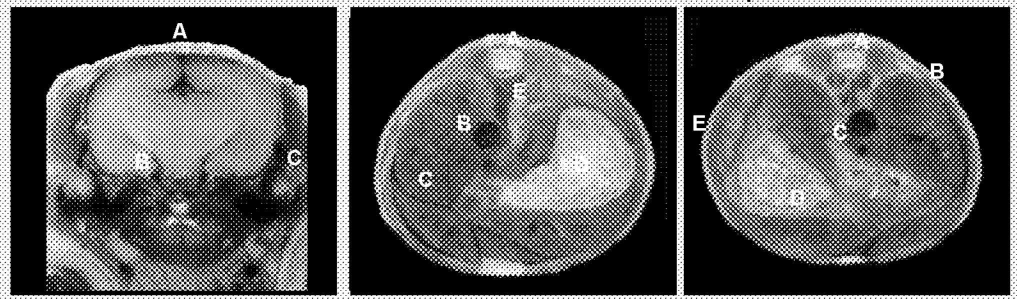
Brain | **Liver** | **Kidney/Spleen**



A: superior sagittal sinus
B: posterior cerebral art.
C: temporal artery

A: spinal cord
B: vena cava
C: liver
D: stomach
E: aorta

A: spinal cord
B: right kidney
C: vena cava
D: intestines
E: spleen



三维小鼠全身结构扫描

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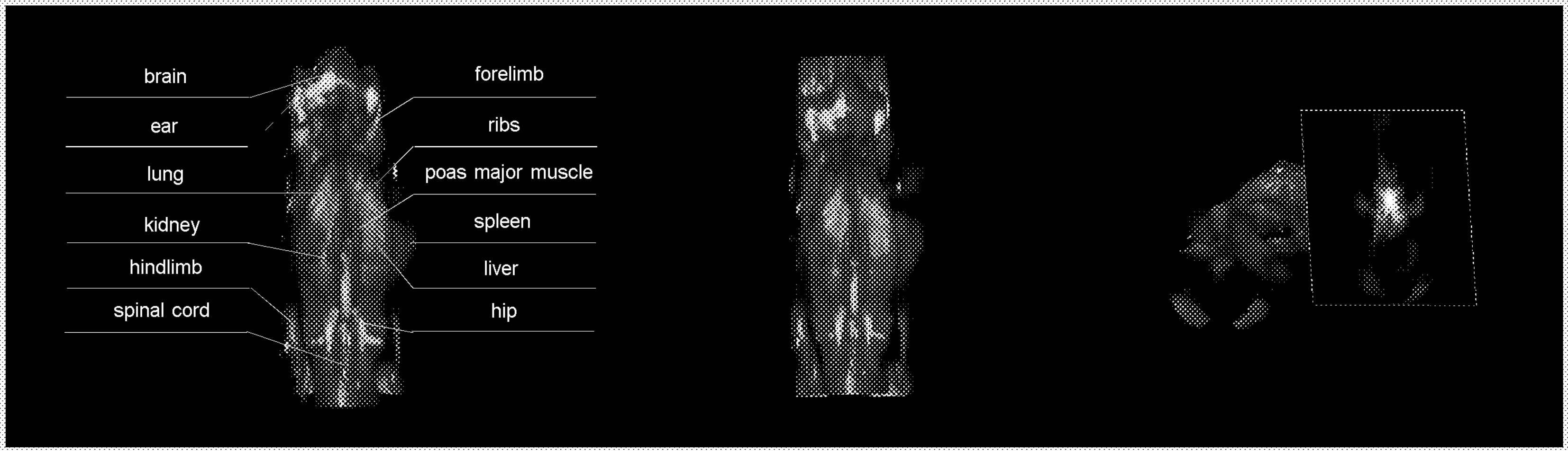
Anatomy | **Rotation** | **Fly-through**



Anatomy Cancer Cardio Brain Kinetics Inflam

Injection of microspheres into liver

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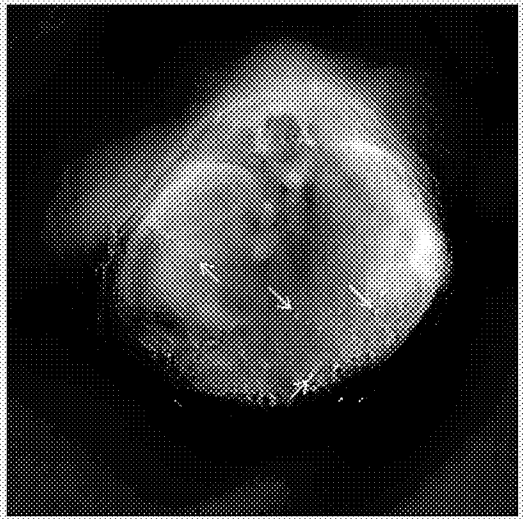
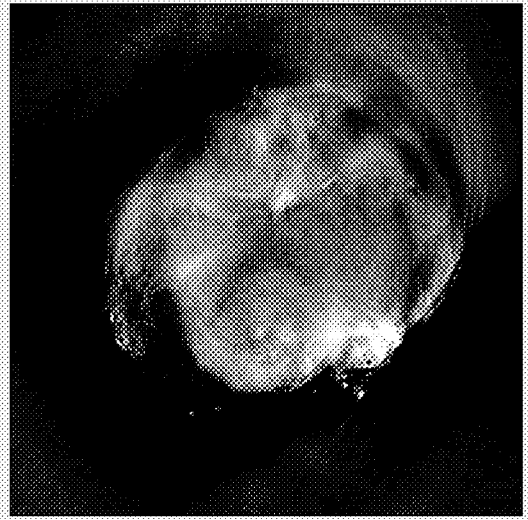
Experiment	100µm microspheres were injected into the ileo colic vein to introduce small spheres with high absorbance into the liver. The mouse was then scanned *in vivo* via MSOT, visualizing the distribution of these spheres
Application	The visualization of microspheres of 100µm diameter demonstrates the capacity of MSOT to detect structures the size of micro-metastases throughout the mouse, especially in hemoglobin-rich organs such as the liver which provide high background absorption.

Single slice

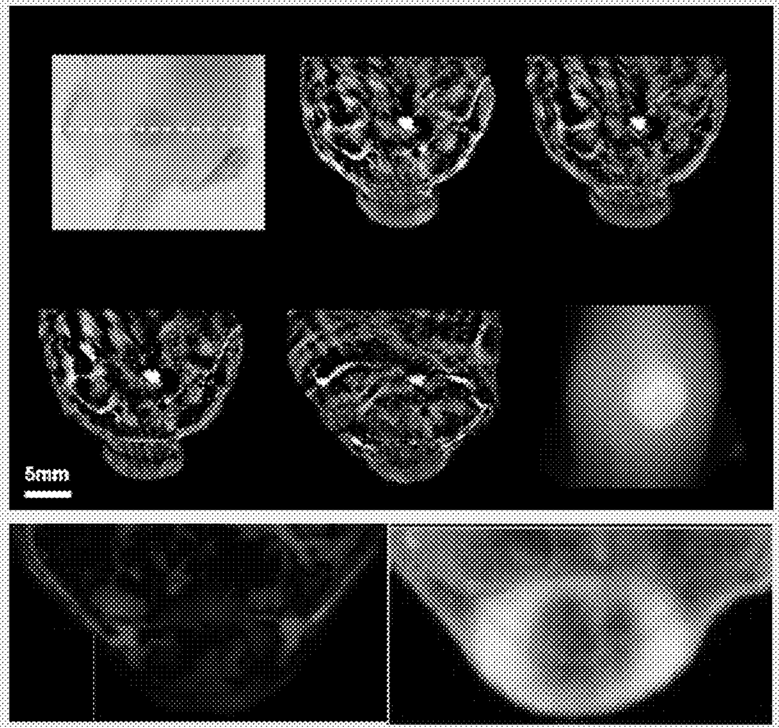


Fly-through video



In collaboration with Lacey R. McNally PhD, University of Louisville, USA

Assessing probe delivery to tumor area



Courtesy of Helmholtz Zentrum Muenchen (Hertzog et.al. 2011, submitted for publication)

Quantifying perfusion heterogeneity

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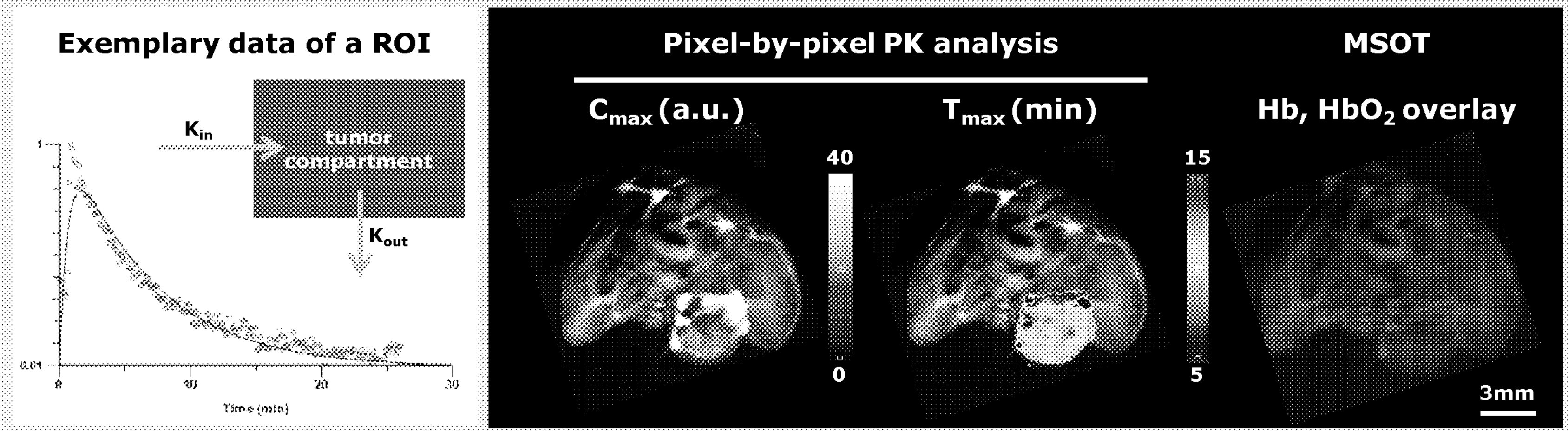

Experiment	Mice with orthotopic 4T1 tumors were injected with 40 nmoles of liposomal ICG and images were acquired for 30 mins. ROI analysis was performed on a per-pixel basis and MSOT-signal vs. time data (red circles) was fitted (blue curve) to a PK model (cartoon). Derived PK parameters (e.g. C_{max} and T_{max}) can now be visualized in parametric images and areas of reduced perfusion can be compared to areas of relative hypoxia (blue areas, right panel).
Application	Fast dynamic processes, such as perfusion heterogeneity throughout tumors, can be visualized by MSOT using dynamic contrast enhancement (DCE). This is important for the analysis of tumor perfusion, to compare the extent of EPR effect during tumor growth, or to predict compound delivery to tumor tissue.

Analysis of αvβ3-integrin targeting

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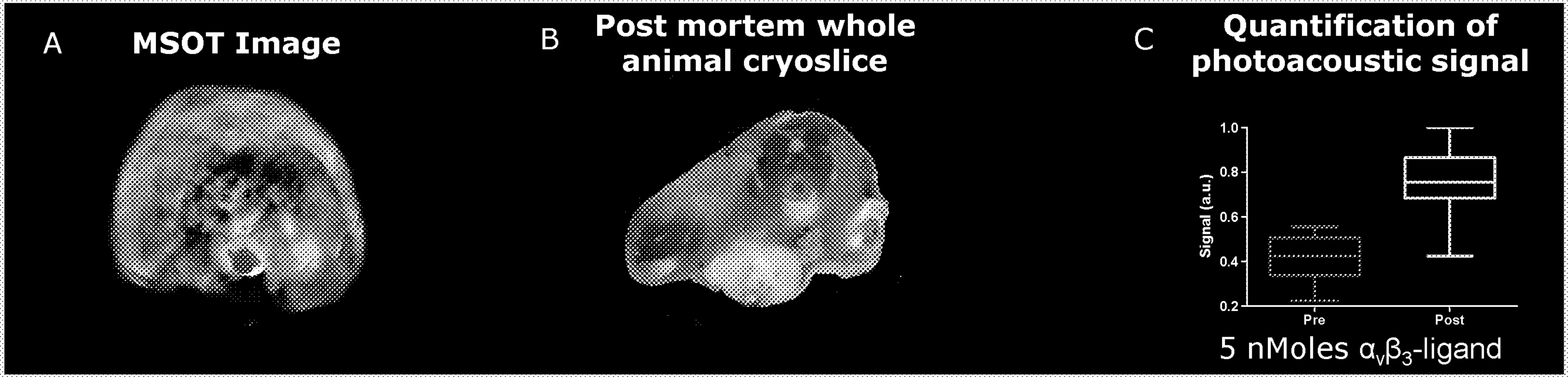
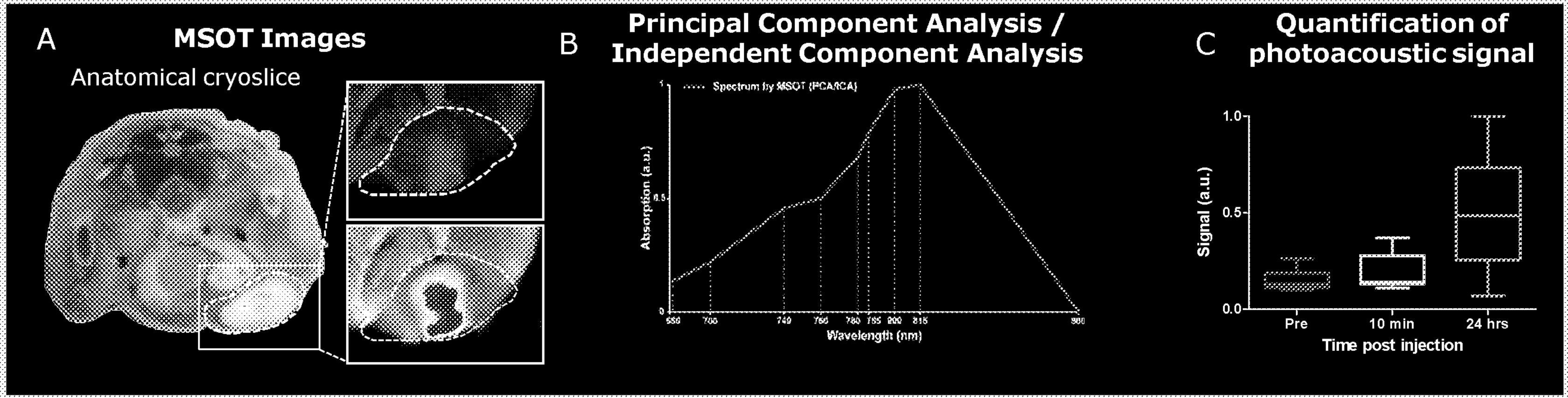


Study performed in collaboration with Florian Rechenmacher, Stefanie Neubauer, Prof. Dr. Horst Keller (Technische Universität München)

Experiment	Female BALB/c nude mice were injected with 0.5x10^6 4T1 mouse breast tumor cells in the abdominal mammary fat pad. Ten days post implantation, 5 nMoles of Cy5.5 labeled $\alpha_v\beta_3$-ligand was administered by tail vein injection and allowed to circulate for 1 hour. Tumor accumulation of probe was visualized (A) and quantified (C) by applying linear regression component analysis using ViewMSOT™ software. Localization of MSOT signal was validated by post mortem whole animal cryoslicing (B). The fluorescent signals (B) were in good accordance with MSOT images (A).
Application	Using MSOT technology receptor expression can be assessed *in vivo* using short imaging regimens. The targeting to expressed receptors can accurately be visualized and quantified.

Cell tracking：巨噬细胞靶向



Experiment	Bone marrow cells were isolated from the femur of a BALB/c nude mouse and differentiated into macrophages by tumor cell conditioned media. Cells were then labeled using the CellVue® NIR 815 cell labeling kit and injected systemically ($0.5x10^6$ cells) in a BALB/c nude mouse bearing an orthotopic 4T1 breast tumor. MSOT imaging was performed before and after injection (10 min. and 24 hrs). Macrophage accumulation in the tumor was visualized after 24 hrs (A). Determination of the key components within the MSOT data by PCA/ICA analysis confirmed that the main signal consisted of labeled cells (B). Quantification of macrophage signal by linear regression showed a significant accumulation of cells after 24 hrs (C).
Application	Using MSOT technology cells can be tracked *in vivo* over prolonged periods of time. The biodistribution of labeled cells can be visualized and quantified using spectral unmixing.

Imaging apoptosis markers

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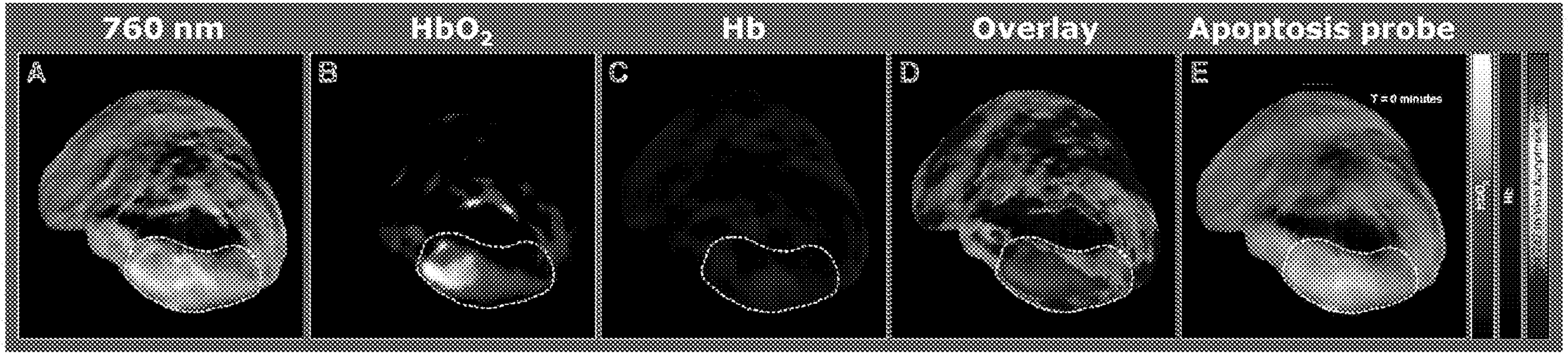
Analysis of apoptotic process heterogeneity *in vivo*



- **Caspase-targeting apoptosis detection reagent injected i.v.**
- **Hypoxic regions within the tumor identified by spectral Hb/HbO_2 unmixing**
- **Strong apoptosis signals detected in more hypoxic regions in the tumor**

Imaging of apoptosis in tumor research

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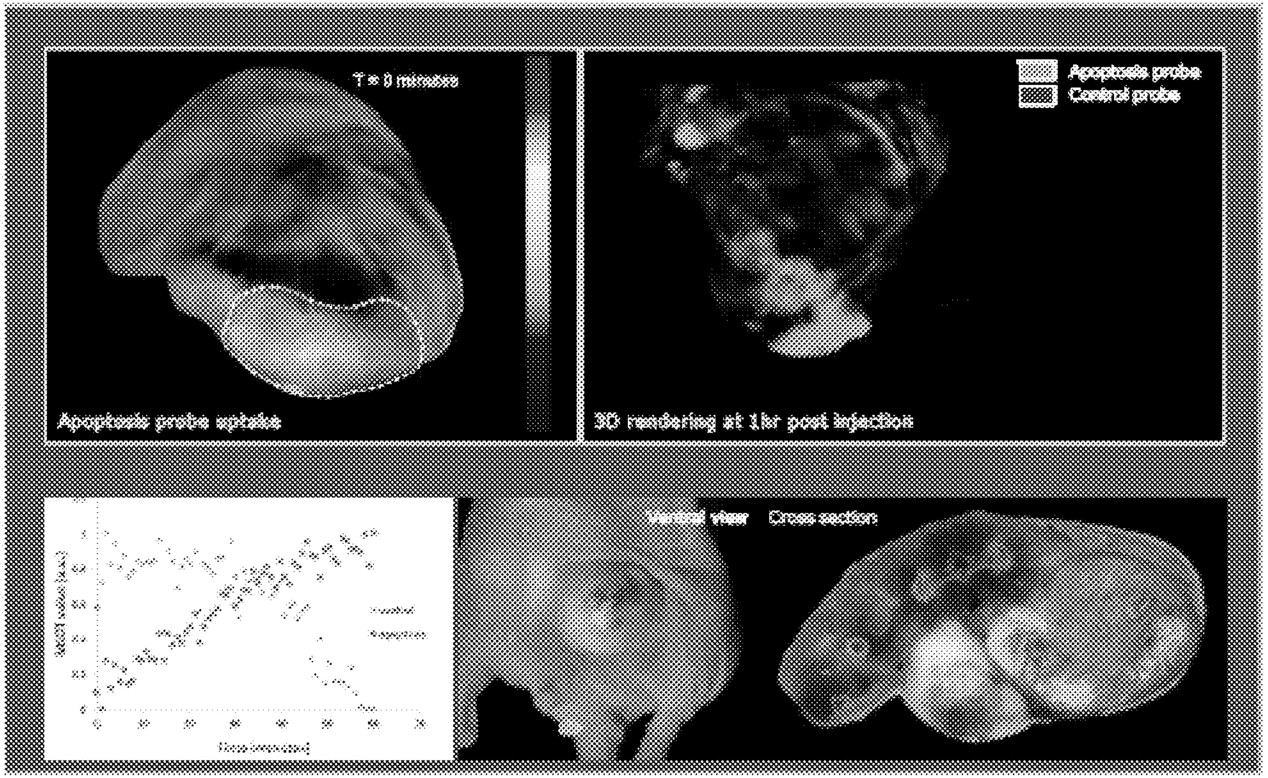

- Simultaneous injection of caspase-targeting apoptosis probe and control probe
- Spectral unmixing of biodistribution and tumor targeting of both probes
- Accumulation of targeted probe vs. wash-out of control probe

EGFR-targeting in pancreatic tumor

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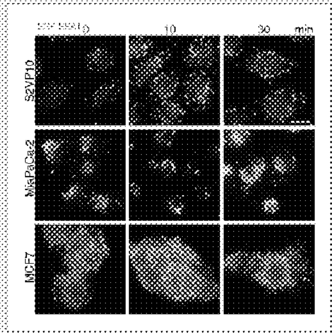
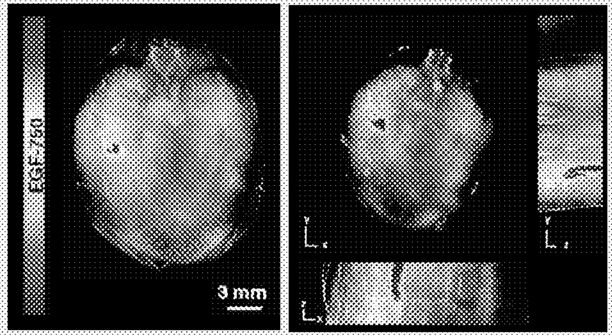
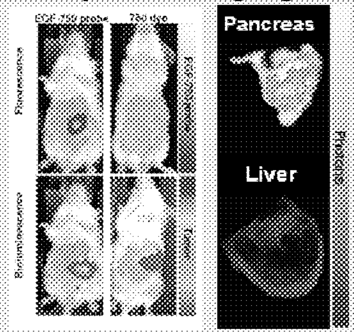
Microscopy



Optoacoustic imaging



Optical imaging



*Hudson SV et al., **Targeted non-invasive imaging of EGFR-expressing orthotopic pancreatic cancer using Multispectral Optoacoustic Tomography (MSOT)**, Cancer Res November 1, 2014 74:6271-6279. DOI: 10.1158/0008-5472.CAN-14-1656.*

Experiment	Mice were orthotopically implanted with S2VP10 (derived from human pancreas carcinoma) luciferase cells. A peptide-based probe targeting the EGF receptor was injected i.v., and the mice were analyzed by optical imaging and optoacoustic tomography
Application	Multiple modalities can track optically labeled tumors. However, MSOT offers high resolution spatial imaging at depth in orthotopic models of pancreas cancer. Tumor volumes can be calculated by 3D rendering of sequential axial data sets.

Imaging probe targeting to tumor

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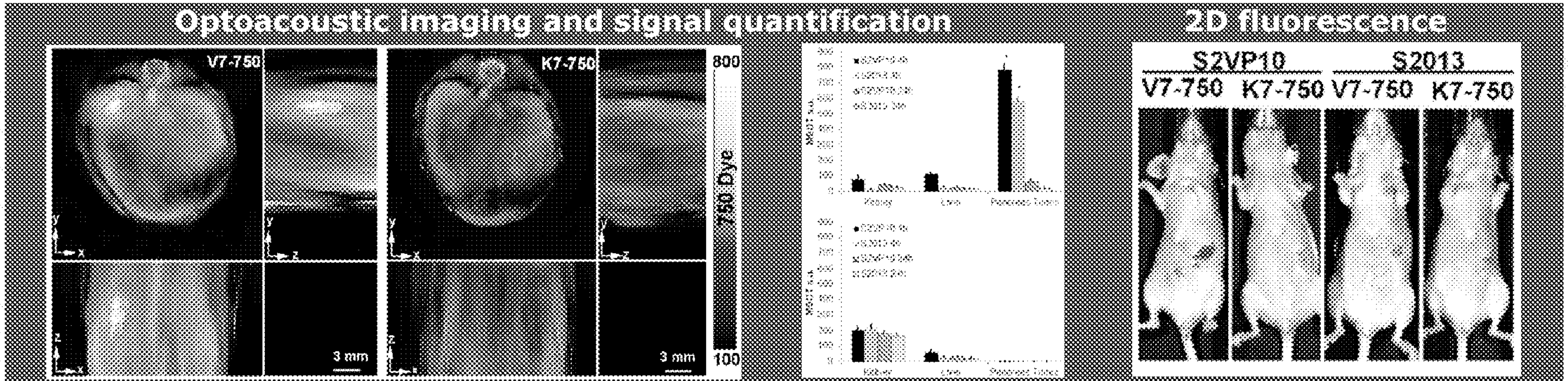
pH-sensitive insertion peptide for tumor targeting

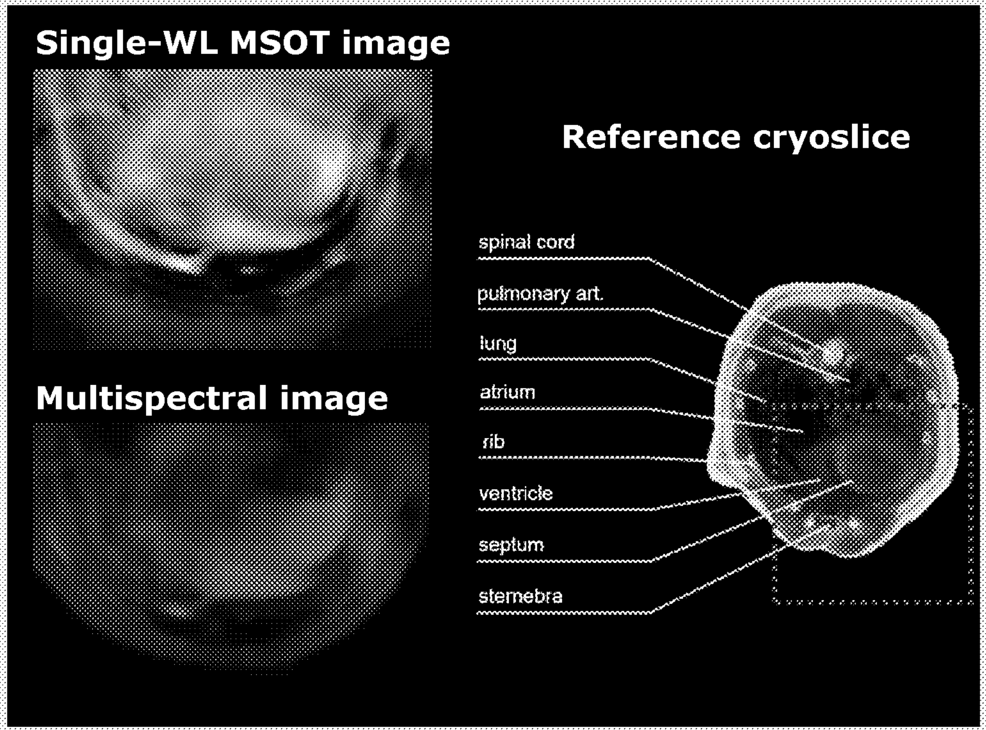


Kimbrough CK, Khanal A, Zeiderman M, Khanal BR, Burton NC, McMasters KM, Grizzle WE and McNally LR. ***Targeting Acidity in Pancreatic Adenocarcinoma: Multispectral Optoacoustic Tomography Detects pH-low Insertion Peptide Probes in vivo.*** *Submitted and under review process*

- **Mice were implanted with human pancreatic cancer cells and a pH-low insertion peptide V7-750 or control K7-750 were injected i.v.**
- **Multiple optical imaging methods were used to track V7 and K7 bio-distribution.**
- **MSOT accurately shows the location of the tumor in deep tissue**

心脏区域血红蛋白分析

Experiment	Left, single-wavelength MSOT image of the heart and multispectrally processed image showing HbO_2 and Hb. Right, reference anatomy
Application	Spectral decomposition of HbO_2 and Hb shows regions of highly oxygenated Hb in the heart, allowing a functional characterization of cardiac activity *in vivo*. Myocardial infarction can thereby be visualized in real time.



10 vs. 50 Hz analysis of the heart beat

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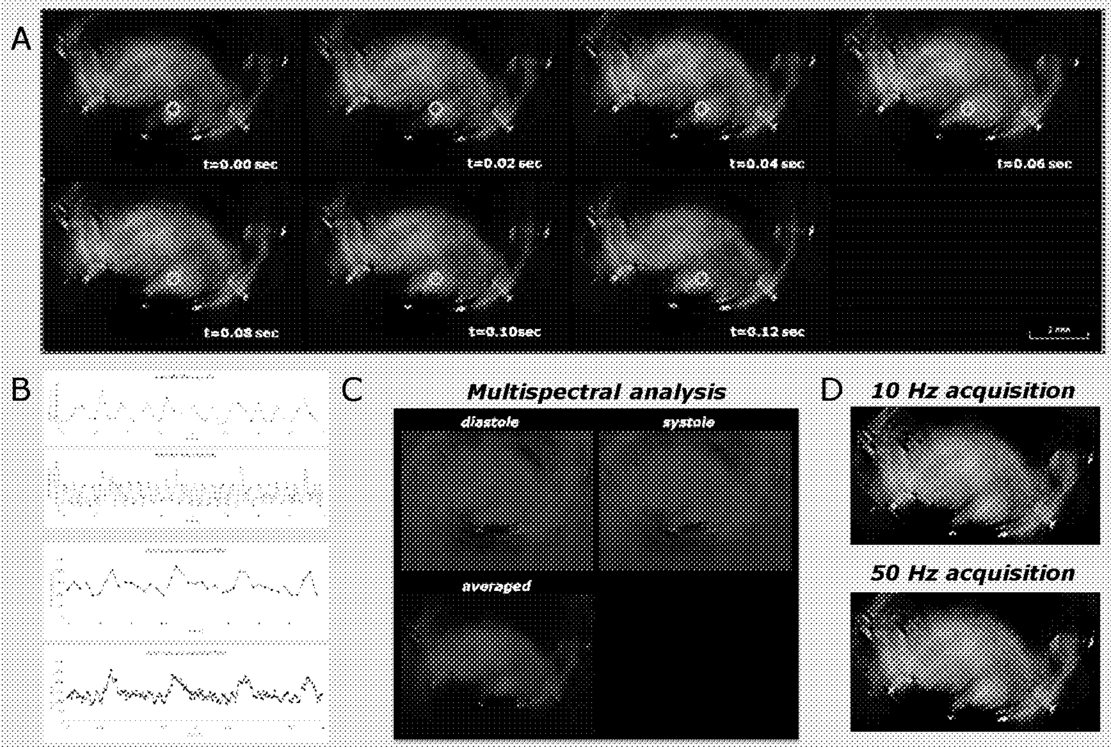
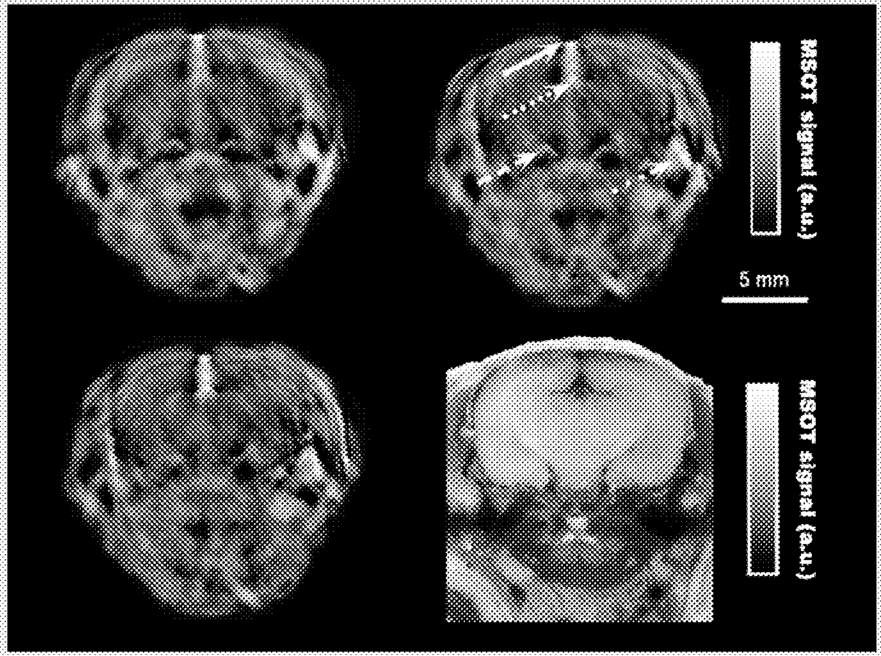
Experiment	The heart of a 12 day old mouse was scanned by MSOT at 10 and 50Hz. Panel A shows the heart imaged at 50Hz, with a blue ROI drawn within the left ventricle. Panel B shows ROI analysis from A, with the blue ROI data emphasizing signal change from the heart beat, and the red ROI emphasizing breathing and heart beat. Panel C shows multispectral analysis of Hb and HbO_2 in the heart at 50Hz (top) or by averaging multiple sequential slices (bottom), which blurs the image. Panel D shows a single-wavelength video of the heart beat at 10 and 50Hz.
Application	50Hz acquisition allows the observation of the full cardiac cycle via MSOT, enabling the calculation of the heart and breathing rate. Faster acquisition also obviates the need for averaging, allowing maximal spatial resolution of multispectral absorbers such as Hb and HbO_2.



Imaging vascular anatomy in the brain

Experiment	Non-invasive coronal brain showing superficial vasculature and blood vessels within the brain, at multiple levels of the mouse brain Note: all images acquired through intact skin and skull Solid arrow: Superior sagittal sinus; dotted arrow: Temporal artery; long dash: inferior cerebral vessel; short dash: 3rd ventricle
Application	MSOT can be used to accurately reveal the vascular anatomy of the brain through intact skin and skull. MSOT enables the visualization of anatomical and structural changes associated with the presence of lesions, tumors and hydrocephalus.



Burton NC et al., ***Multispectral Optoacoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma***, *Neuroimage, 2012 Sep 28; pii: S1053-8119(12)00963-9*

3D vasculature of head / brain

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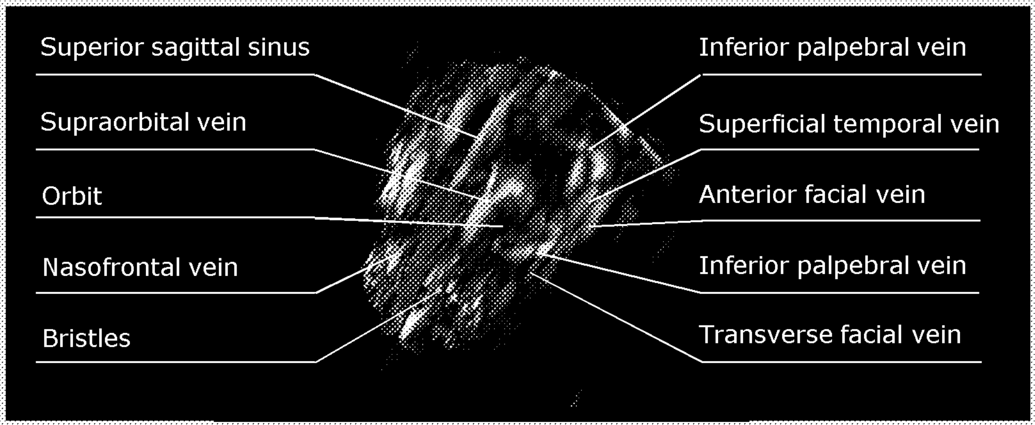

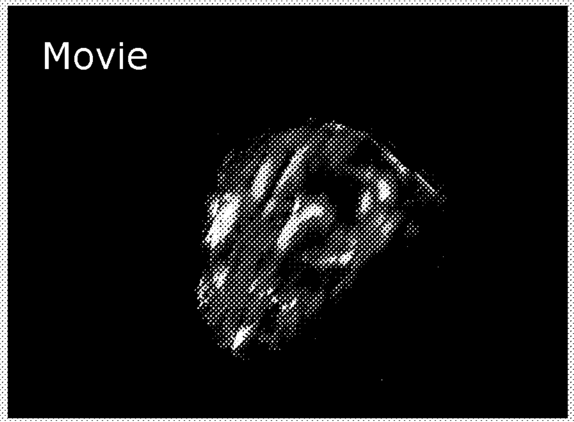

Detecting probes deep in the brain

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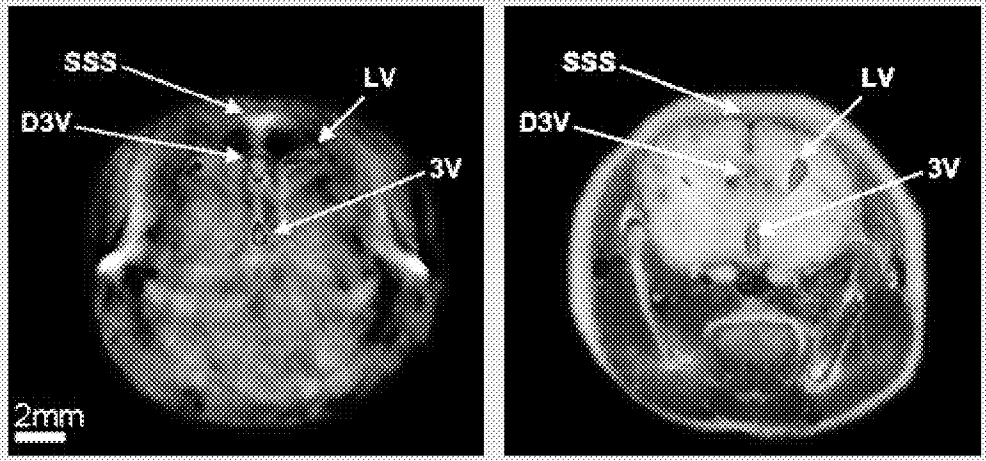
Experiment	Left: Grayscale background is an optoacoustic image taken at 900nm. Green overlay represents multispectrally resolved probe. Right: Cryosection with green overlay showing fluorescence from NIR dye797 SSS: superior sagittal sinus D3V: dorsal 3rd ventricle LV: lateral ventricle 3V: 3rd ventricle
Application	MSOT can be used to accurately determine the spatial biodistribution of probes in the brain through an intact skin and skull. In combination with specific probes, this provides the capacity to study molecular features of neurological disease *in vivo*.

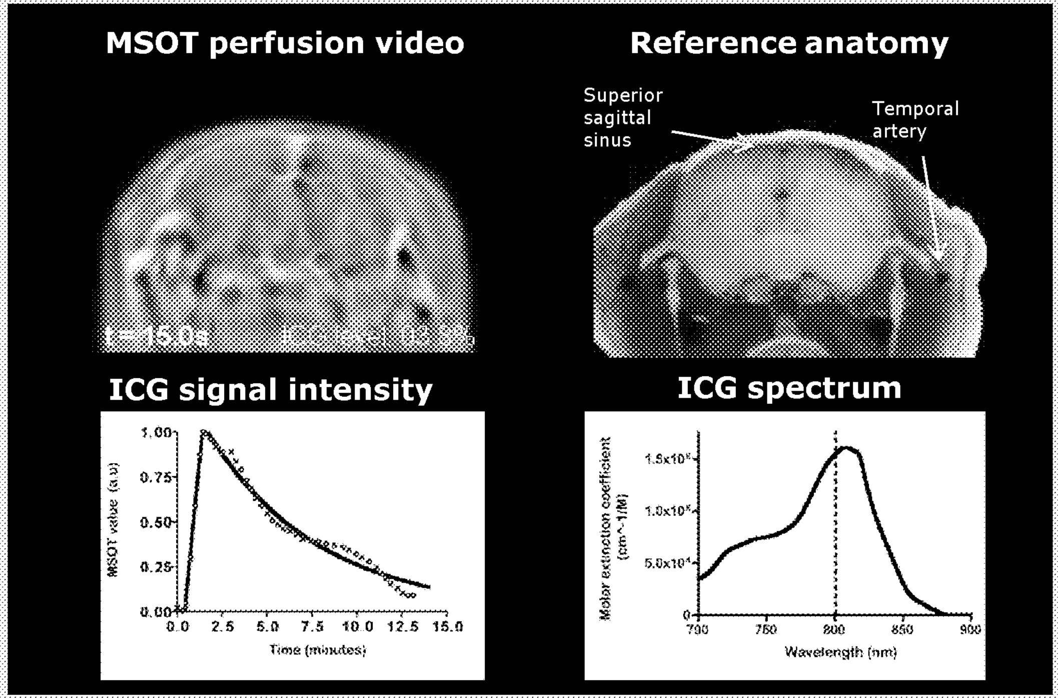
Multispectral MSOT image | **Reference cryoslice**



Lozano N et al., ***Liposome-gold Nanorod Hybrids for High-resolution Visualization Deep in Tissues****, J Am Chem Soc, 2012 Aug 15;134(32):13256-8.*

Monitoring perfusion in the brain

Experiment	ICG was injected via the tail vein and the accumulation in the blood vessels of the head and brain was monitored in real time.
Application	The accumulation and clearance of probes in the brain can be monitored in real time, allowing the direct visualization and calculation of the pharmacokinetics of molecular probes in the brain. MSOT can be used to determine the half-life of drugs and the localization of disease markers in the brain.



Burton NC et al., ***Multispectral Optoacoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma****, Neuroimage, 2012 Sep 28; pii: S1053-8119(12)00963-9*

2D monitoring oxygenation in the brain

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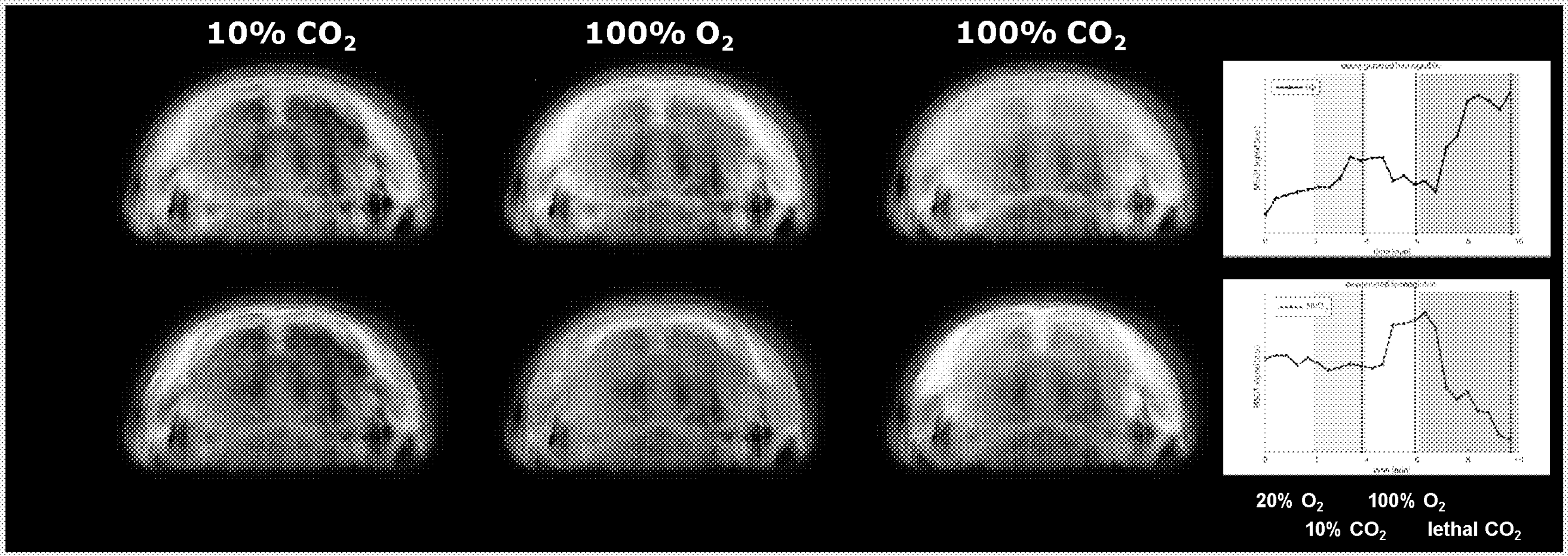
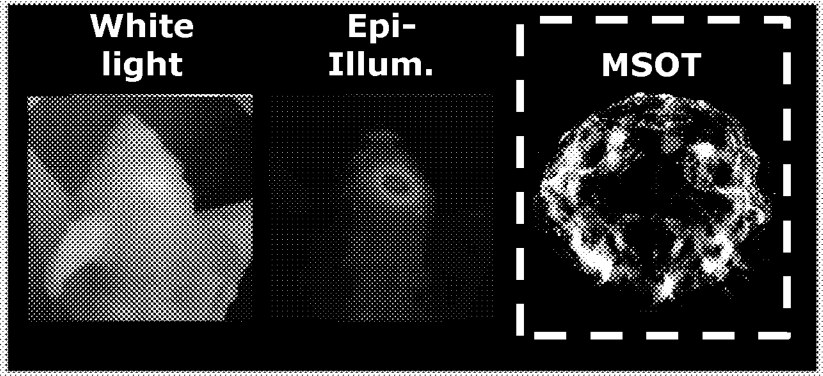


*Burton NC, Patel M, Morscher S, Driessen W, Claussen J, Beziere N, Jetzfellner T, Taruttis A, Razansky D, Bednar B, Ntziachristos V, **Multispectral Optoacoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma**, Neuroimage, 2012 Sep 28; pii: S1053-8119(12)00963-9*

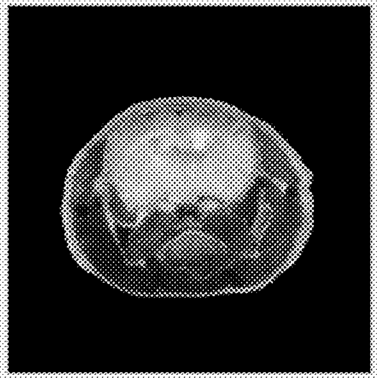
Experiment	A mouse was anesthetized and challenged with carbon dioxide during MSOT imaging
Application	Changes in blood oxygenation, such as those that occur during ischemic injury or tumor growth, can be measured in real time by MSOT.

Imaging iRFP-transfected brain tumor

***In vivo* imaging**



***Ex vivo* validation**



Adapted from: Deliolanis et al. 2014, Deep-Tissue Reporter-Gene Imaging with Fluorescence and Optoacoustic Tomography: A Performance Overview, Mol Imaging Biol. 2014 Mar 8.

Experiment	Mice were implanted with U87 glioblastoma cells transfected with a near-infrared-absorbing protein and imaged *in vivo* by multiple modalities, including epi-illumination / trans-illumination fluorescence and MSOT, and compared to *ex vivo* cryo-sectioning with fluorescence detection.
Application	Compared to established optical imaging modalities, MSOT more accurately determines the distribution of fluorescent protein in the brain *in vivo* and with high resolution, thus providing 3D information about the tumor volume, without losing resolution with increasing imaging depth.

MSOT anatomy of metabolic organs

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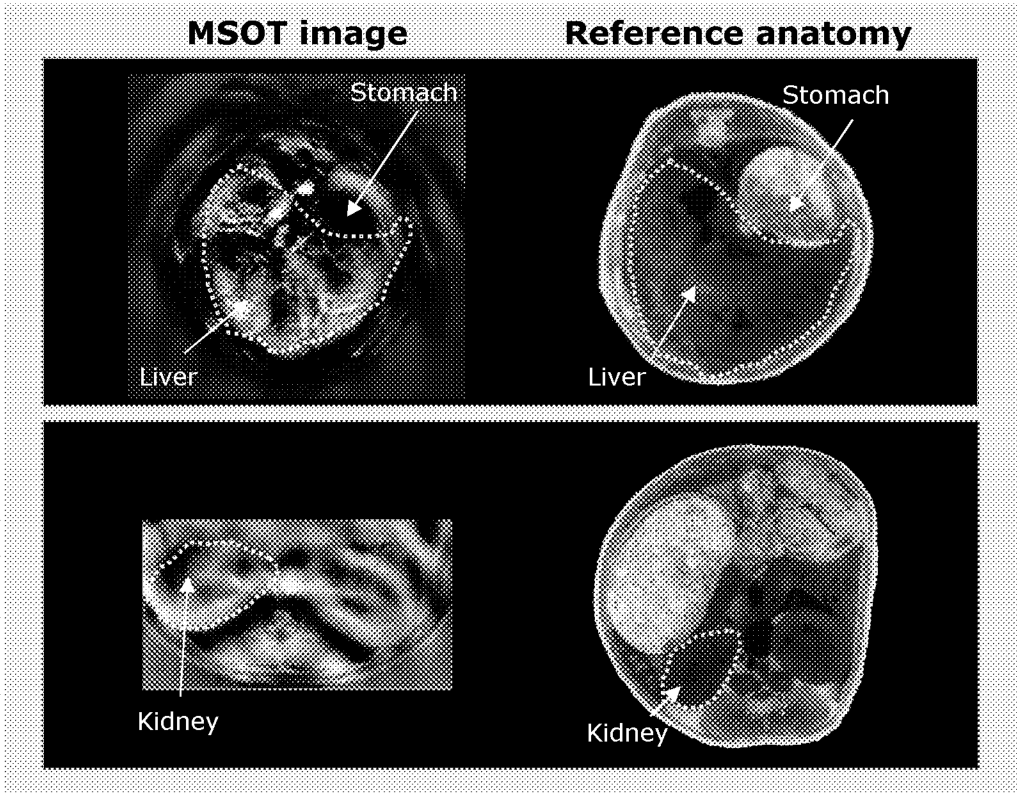

Preclinical imaging in drug discovery

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MSOT imaging in the drug discovery stage

Molecular imaging:

- **Target validation (localization and expression levels)**
- **Imaging drug localization and efficacy (characterization of pathology and response to treatment)**

Pharmacokinetics and biodistribution imaging:

- **Identification of PK issues in Hit-to-lead stage and optimization of animal PK profiles**
- **PK/PD relationships in preclinical studies**
- **Absorption, Distribution, Metabolism, Excretion (ADME) analysis**
- **Toxicity screening and TK**

Measuring probe PK properties with MSOT

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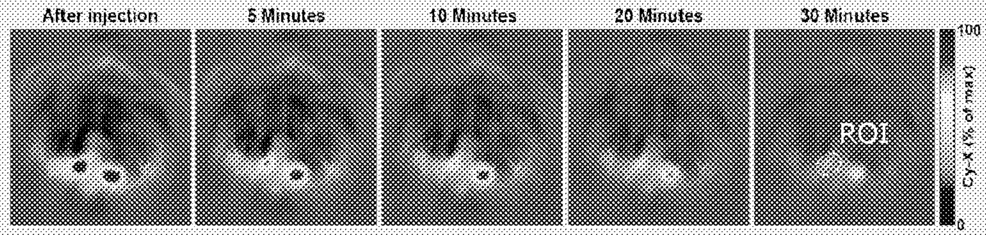
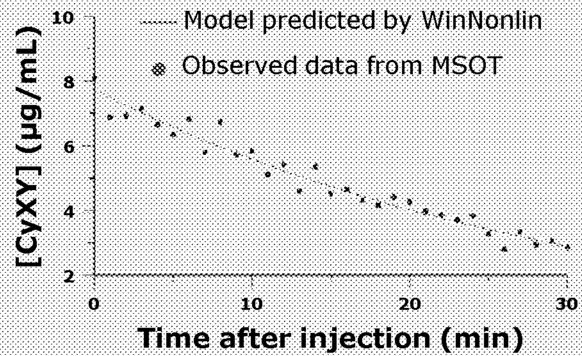
Experiment	After i.v. injection of probe (Cy-X; 25nMoles) the jugular veins of mice were monitored over 30 minutes by MSOT. After ROI analysis concentration-time curves were determined and non-compartmental analysis was performed. This allows for the calculation of important pharmacokinetic parameters such as half-life, clearance and AUC, all without having to bleed or sacrifice the animals.
Application	By MSOT, PK parameters of NIR-absorbing agents can be determined non-invasively, thus reducing the number of animals and saving time. PK analysis can aid in the optimization of dosing schedules and imaging time points.





Parameter	Value	Unit
R^2	0.9591	-
$T_{1/2}$	21.16	min
AUC_{inf}	236.7	min*µg/mL
Cl	0.06845	mL/min
MRT	30.55	min

*Study performed in collaboration Dr. Young-tae Chang, A*STAR, SBIC, Singapore*

Determining probe pharmacokinetics

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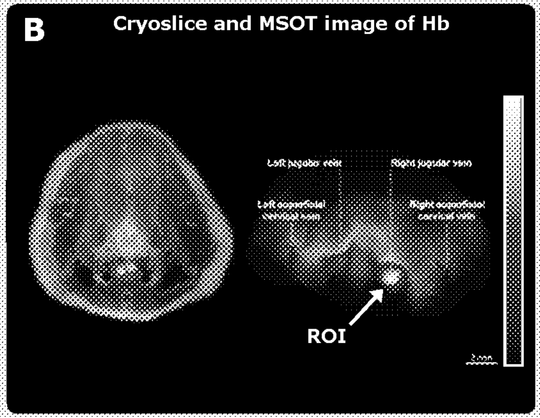
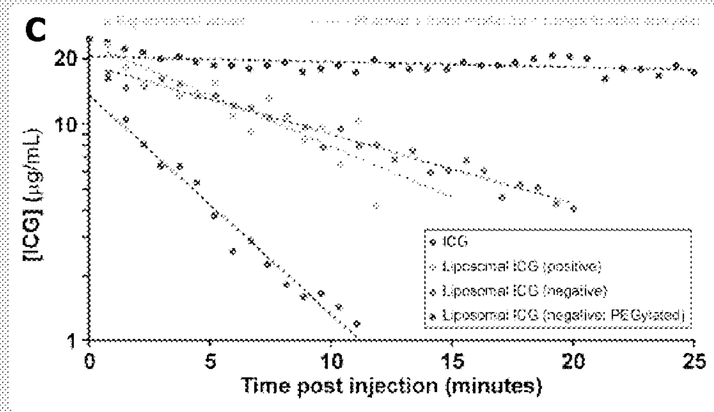
A

Formulation	Size (nm)	ζ-potential (mV)
Negative PEGylated	92.8	-39.63
Negative	77.0	-43.53
Positive	72.0	+61.78





Experiment	The neck region of a BALB/c nude mouse was imaged and the jugular vein was located (B). Free ICG and three different formulations (Table A) of liposomal ICG (50 nmoles) was then injected systemically and the neck region was continuously imaged for 30 minutes. ICG signal was unmixed by linear regression and a region of interest was placed over a large vessel in the MSOT image (B) to determine the strength of optoacoustic signal. Values were converted to ICG concentrations by determining ICG plasma levels at T=30 minutes by fluorescence spectroscopy. The experimental data was modeled by non-compartmental analysis (C) and half-life was calculated from the fitted curves. $T_{1/2}$ = ICG (3 minutes), positive liposomes (6 minutes), negative liposomes (9 minutes) and negative PEGylated liposomes (≥14 minutes)
Application	Using MSOT technology pharmacokinetic behavior can be studied non-invasively. PEGylated liposomal ICG is an excellent intravascular contrast agent.

Quantitative analysis of kidney function

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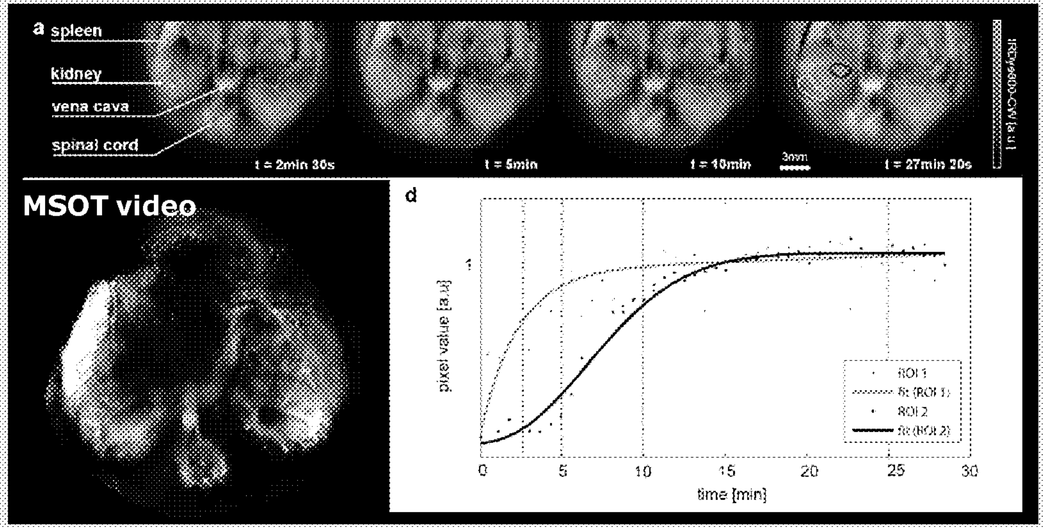
Experiment	CD1 nude mice were intravenously injected with 20nmol IRDye800-CW (LI-COR), a near infrared dye that is excreted by the kidneys. The kidney region was observed by MSOT over a period of 30 minutes (a) and PK processes were analyzed for the renal cortex and pelvis (d). The ROI in the renal cortex shows fast signal pickup through filtration whereas the renal pelvis features delayed uptake. The animals were afterwards sacrificed for cryoslice/ fluorescence validation for different time points (b/c).



Taruttis A, Morscher S, Burton NC, Razansky D, Ntziachristos V, ***Fast Multispectral Optoacoustic Tomography (MSOT) for Dynamic Imaging of Pharmacokinetics and Biodistribution in Multiple Organs****, PLoS ONE 2012, 7(1):e30491.*

Application	MSOT imaging offers the capability to monitor pharmacokinetic and pharmacodynamic processes in real time and high spatial resolution of 150µm. With that, MSOT reveals a unique performance in tracing the fate of optical agents for drug discovery and assessing chronic renal diseases *in vivo*.

Whole body biodistribution imaging

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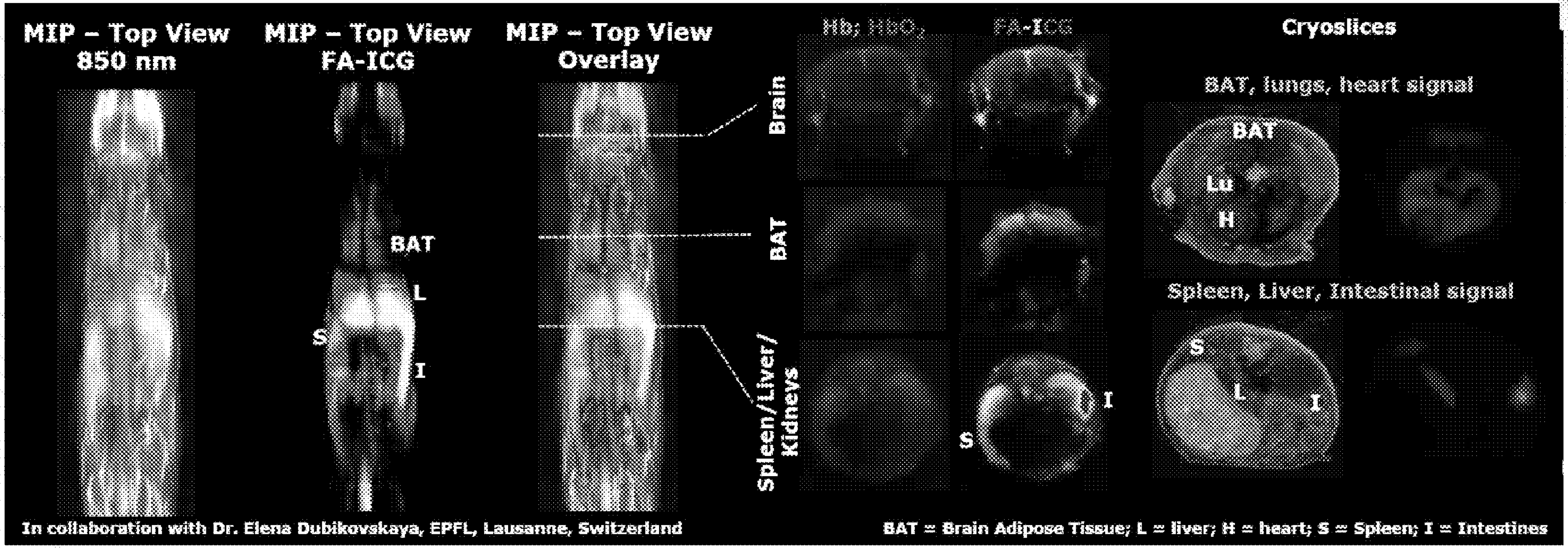
ICG-labeled fatty acid distribution throughout the mouse



- Mouse scanned from head to base of tail (10 minute scans)

Assessment of renal damage

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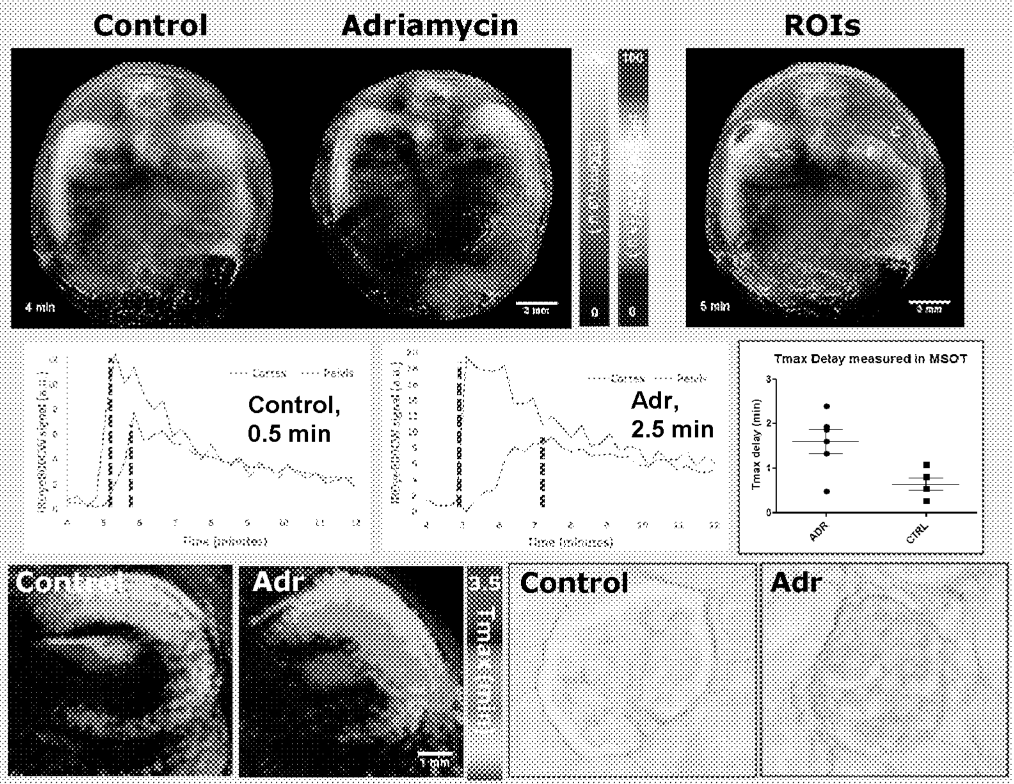
Experiment	Renal clearance of IRDye800CW was assessed by MSOT in saline and adriamcyin-treated (6.5mg/kg) mice.
Application	ROI analysis of dye clearance can differentiate kinetics in the renal cortex and pelvis, allowing the observation of a T_{max} delay that provides a surrogate marker for glomerular function. This correlates with histological markers (Picrosirius Red) of renal damage.

Scarfe L et al., ***Novel techniques for non-invasive measurements of glomerular kidney damage.*** *Submitted for publication.*



Analysis of urinary excretion

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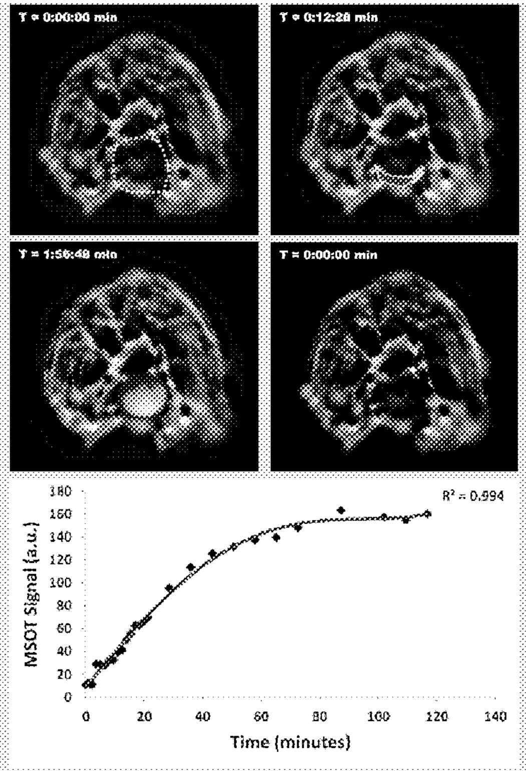
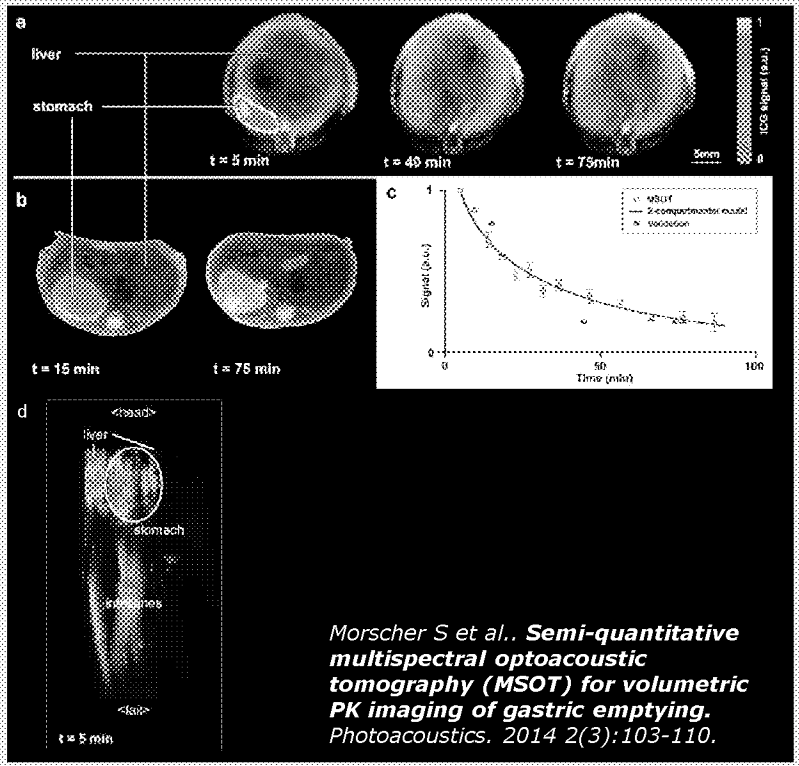
Experiment	A catheter was inserted into the tail vein of a nude mouse, and the mouse was imaged by MSOT. During image acquisition, an organic NIR-absorbing dye (AlexaFluor 750) conjugate was injected i.v., and the accumulation of this dye in the bladder was monitored over a two hour period. Shown are individual time points, with a single wavelength MSOT image (800nm) in greyscale and the spectrally unmixed AlexaFluor750 overlaid in jet. Also shown is a time-lapse video showing the accumulation over a two hour period. The graph shows experimental values (black dots) as well as the modeled data (red line) in the ROI indicated in the first panel.
Application	The high temporal resolution of MSOT combined with high spatial resolution imaging through the entire cross-section of the mouse allows an investigator access to organs involved in excretion as well as the ability to quantify these physiological processes.



In vivo assessment of gastric emptying

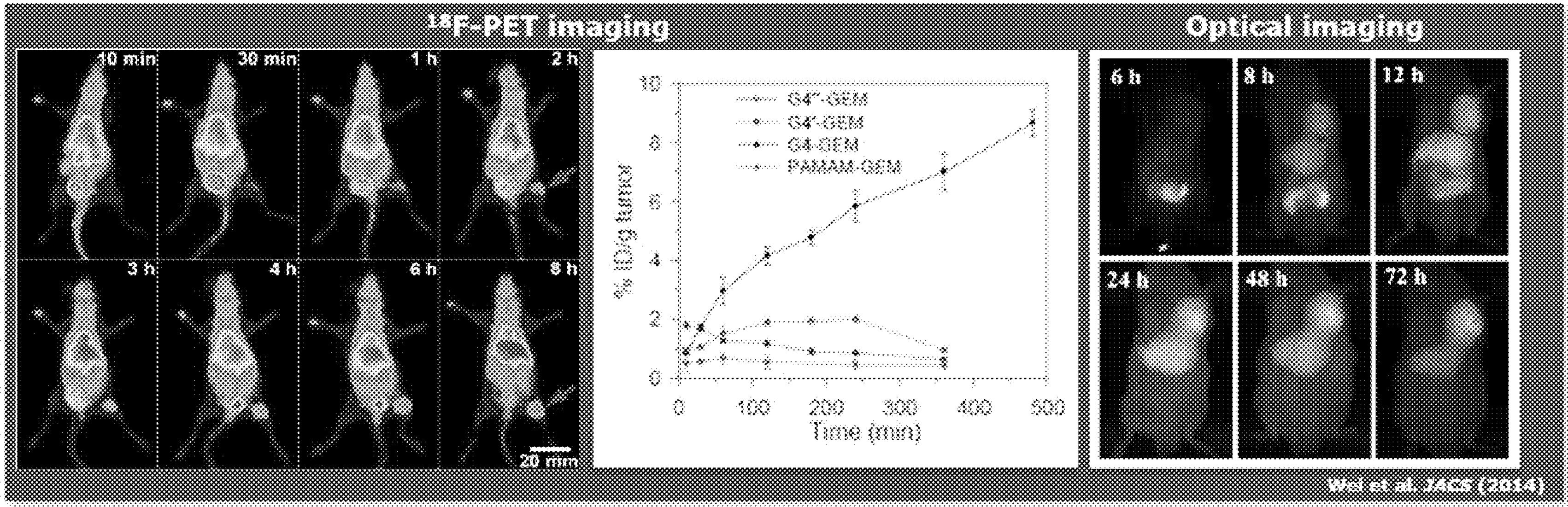
Experiment	10 nMoles of ICG was administered by oral gavage to BALB/c nude mice. MSOT images were acquired every 8-10 minutes post administration for 120 minutes. ICG signals are superimposed onto single-wavelength optoacoustic images (850nm; grayscale) and quantified (panel a). Clearance kinetics are determined by fitting the MSOT data to a 2-compartmental pharmacokinetic model that reveals a clearance half-life from the stomach of ~22 minutes (panel b). Post-mortem validation by fluorescence imaging is in good correspondence with MSOT (panel c). Panel (d) shows real-time distribution and clearance of ICG signals in the stomach and liver.
Application	MSOT technology offers the potential to monitor physiological regulation and pharmacological modulation of gastric emptying.



*Morscher S et al.. **Semi-quantitative multispectral optoacoustic tomography (MSOT) for volumetric PK imaging of gastric emptying.** Photoacoustics. 2014 2(3):103-110.*

Nanoparticle distribution

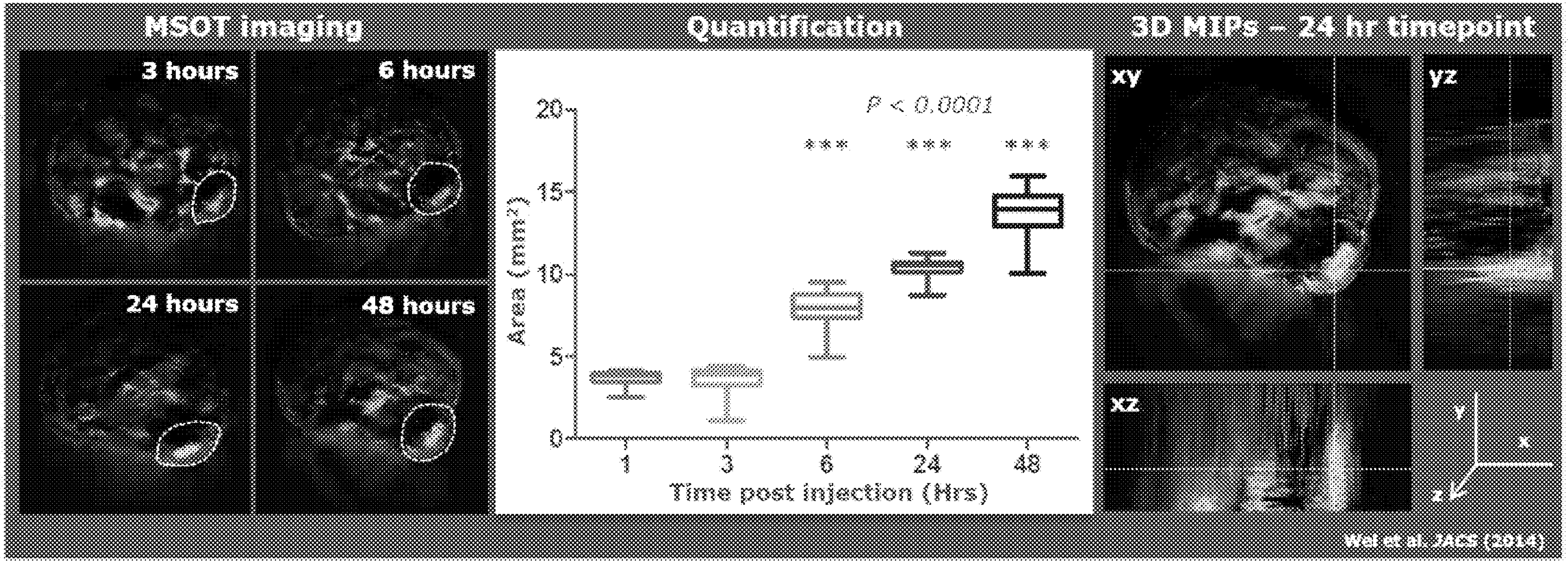
Accumulation of dendrimer particles in tumor-tissue



- ^{18}F PET imaging offers sensitive quantification, but poor resolution and limited ability for longitudinal studies
- Optical imaging offers the ability for longitudinal studies, but with poor resolution

Nanoparticle distribution

Accumulation of dendrimer particles in tumor-tissue



- **MSOT offers high-resolution longitudinal imaging in 3D**

Nanoparticle distribution

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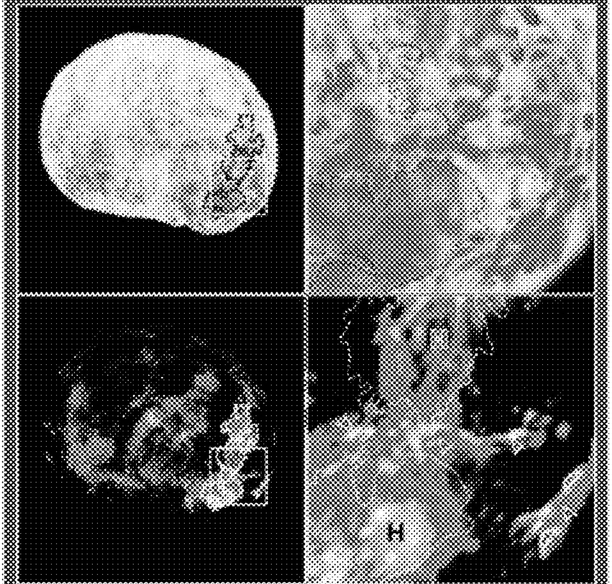
Heterogenous accumulation of dendrimer particles in tumor-tissue

Colocalization with hypoxic regions



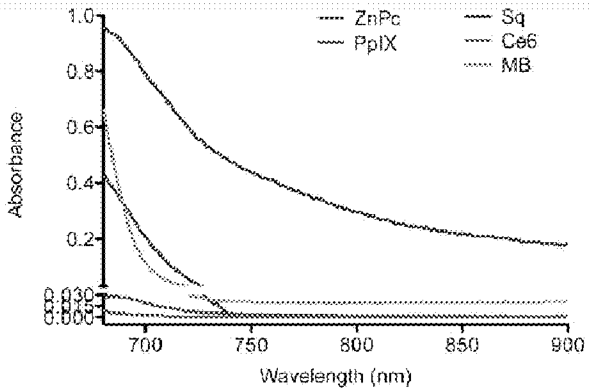
Wei et al. JACS (2014)

- **By MSOT distribution imaging it is possible to monitor nanoparticle accumulation in tumors longitudinally**
- **In contrast to ^{18}F PET imaging, the accumulation can be tracked for multiple days, in this case the more crucial time points, as the dendrimer continues to accumulate post 6hrs**
- **In contrast to optical imaging, MSOT imaging offers high resolution imaging in 3D**
- **Uniquely, MSOT offers the capability to combine the imaging of injected probes with functional imaging (Hb and HbO_2)**

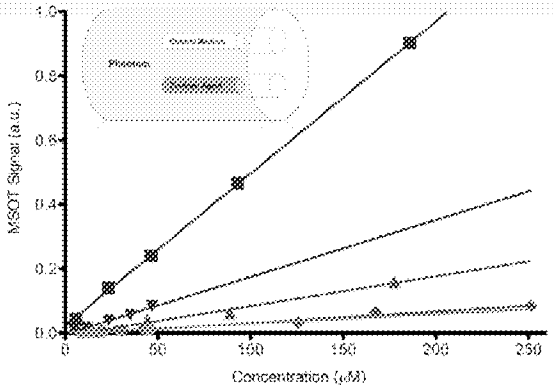
Photosensitizer-based MSOT contrast

in vitro analysis allows for quick prioritization

Absorbance spectra



MSOT signal linearity



Relative MSOT intensity

PDT	φF	φΔ	φP
ZnPc	0.06	0.62	0.47
PpIX	0.16	0.56	0.093
Sq	0.037	0.61–0.74	0.18
Ce6	0.19	0.65	0.036
MB	0.02	0.55	0.029

φF=fluorescence QY; φΔ=singlet oxygen QY; φP=MSOT QY

Ho & Balasundaram et al. *Scientific Reports* (2014)

- **Five PDT agents of different classes were evaluated for MSOT signal generation:**
  - Zinc phthalocyanine (ZnPc), protoporphyrin IX (PpIX), 2,4-bis[4-(N,N-dibenzylamino)-2,6-dihydroxyphenyl] squaraine (Sq), chlorin e6 (Ce6) and methylene blue (MB)

In vivo biodistribution analysis

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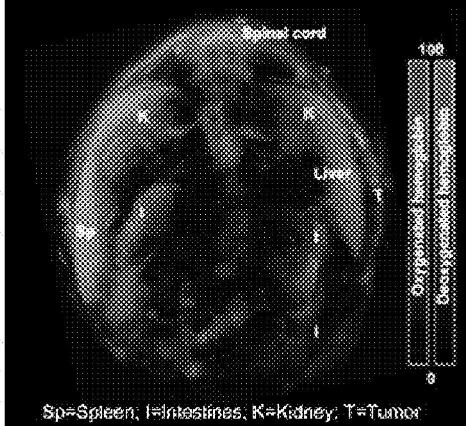
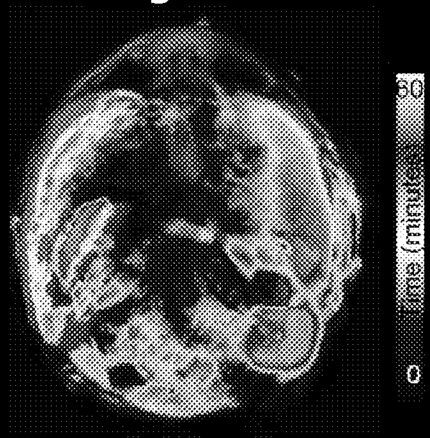
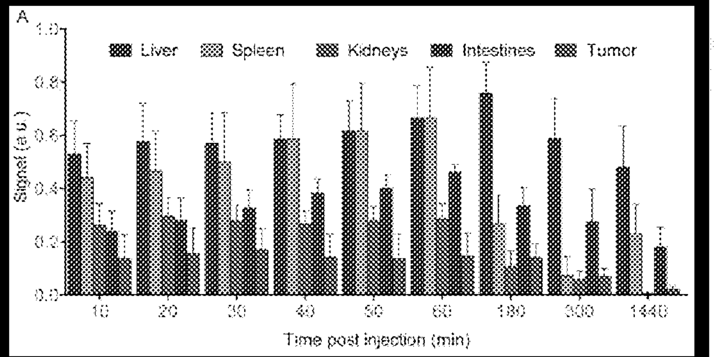
Hb/HbO_2 image



ZnPC signal over time



Biodistribution analysis



Ho & Balasundaram et al. *Scientific Reports* (2014)

- **MSOT imaging allows visualization and quantification of distribution of injected agents**
  - ZnPC reaches a maximum Tumor-Background ratio at T=60mins
- **Quick optimization of theranostic approaches**

Upconversion Nanoparticles as a Contrast Agent for Photoacoustic Imaging in Live Mice

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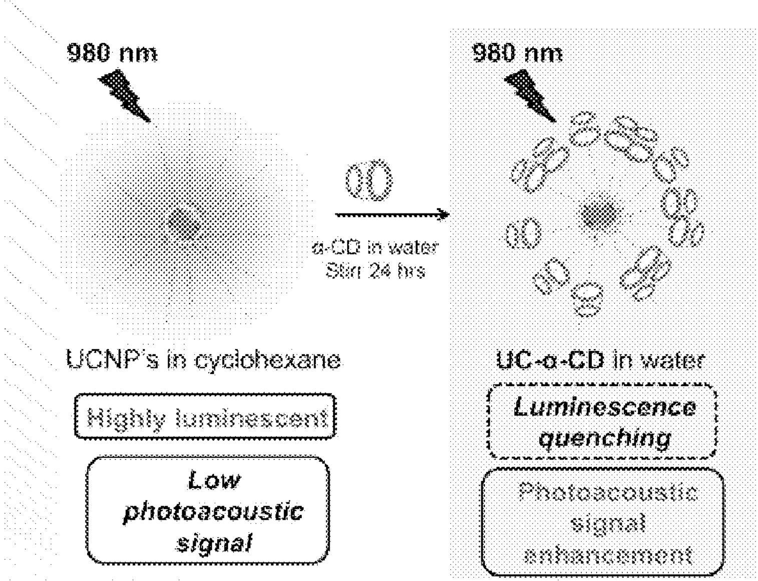
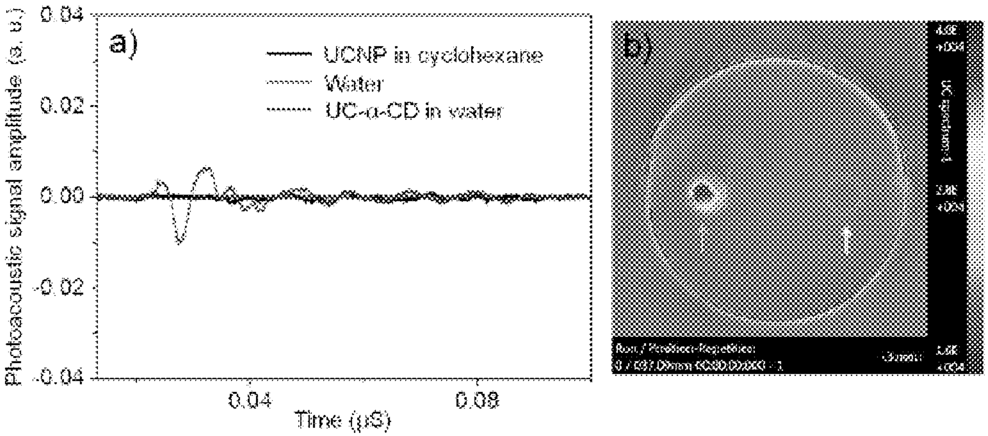
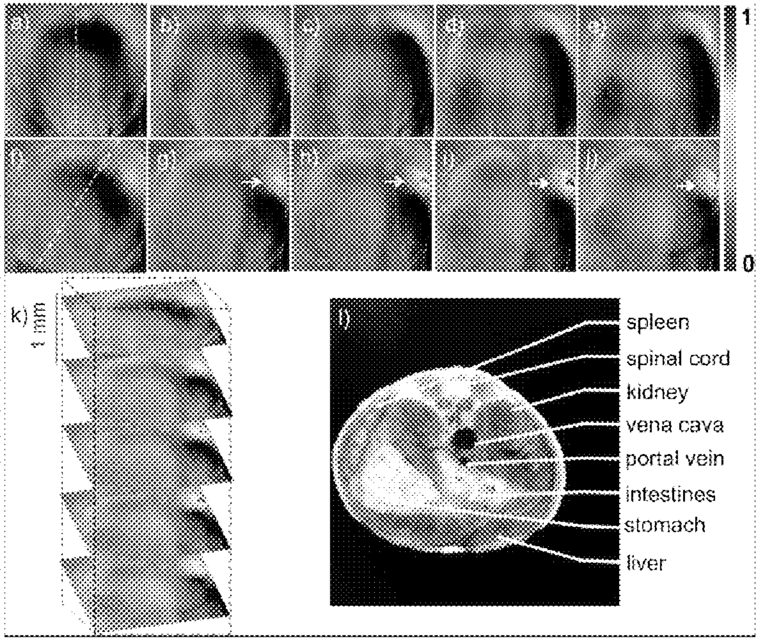
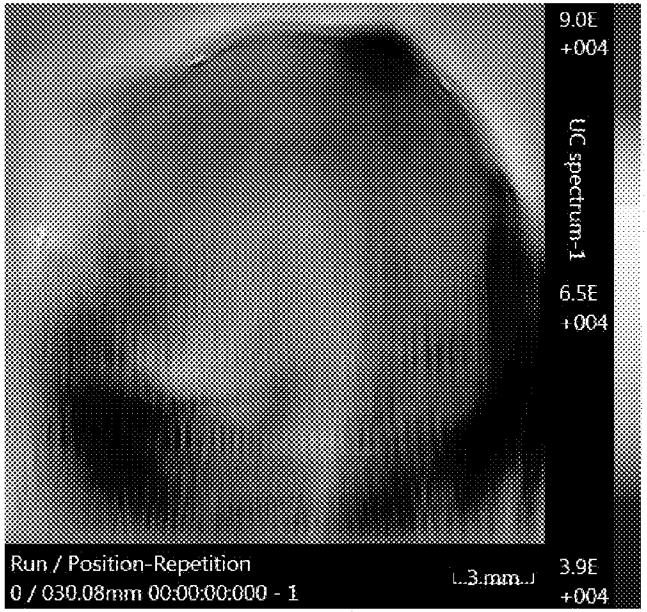




PAI of UCNP in live mouse





Simultaneously resolving two agents

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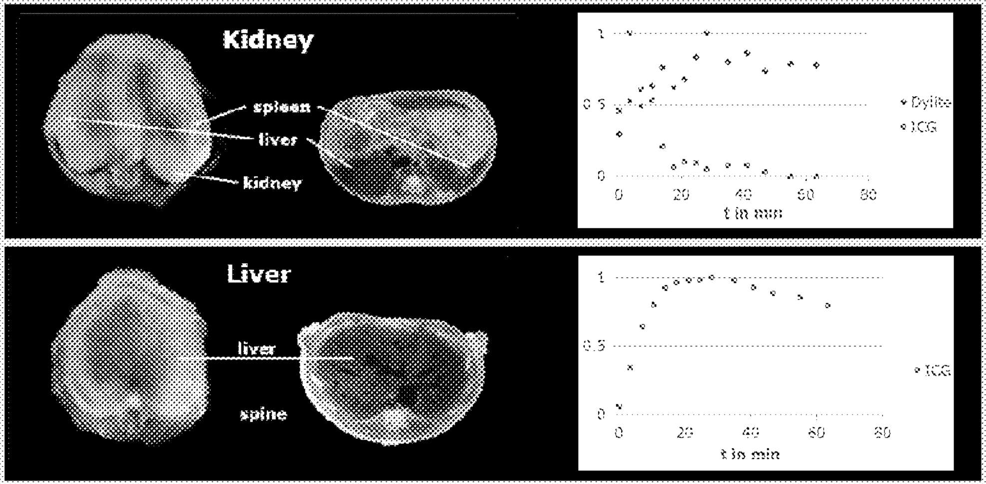
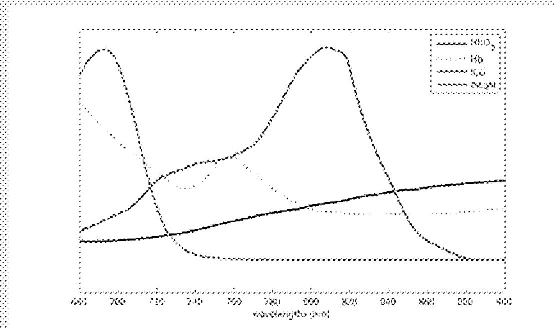


Absorption spectra



Experiment	20nmoles of ICG and 20nmoles of DyLite-690 were injected intravenously into a mouse as a cocktail. The mouse was imaged continuously by MSOT before, during and after injection, allowing quantification of kidney and liver concentration of each dye.
Application	Multispectral unmixing in MSOT enables simultaneous quantification of multiple contrast agents, allowing an investigator to monitor multiple excretory pathways in a single imaging session.

Imaging of inflammation in the knee

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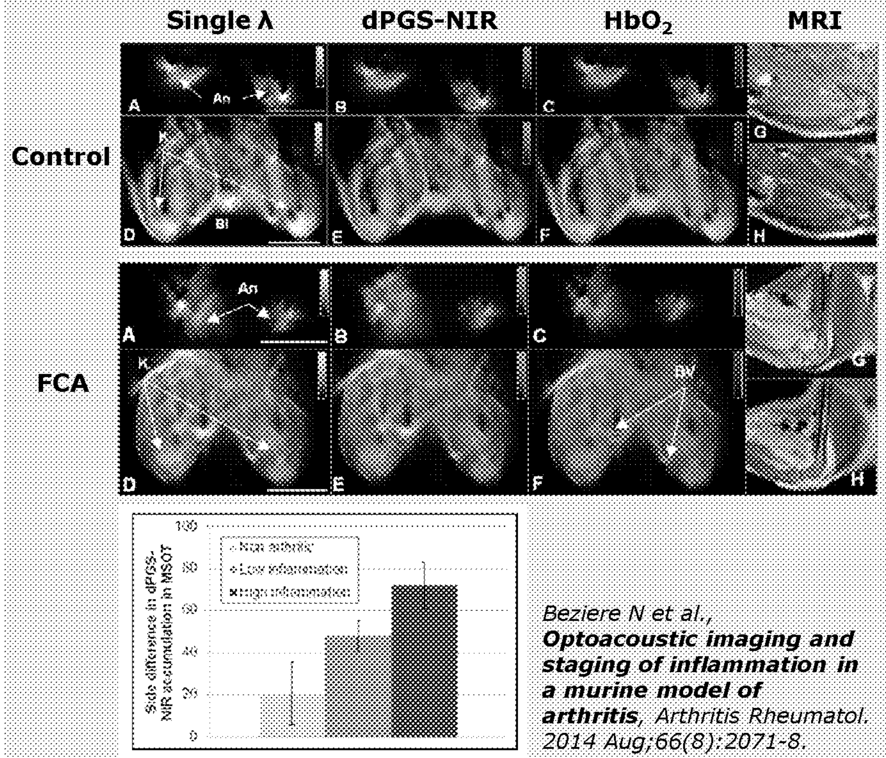
Experiment	12 mice were injected with Freund's Complete Adjuvant (FCA) into the left knee. Three weeks later, mice were imaged by MRI (Gd-enhanced, T1-weighted). Mice showing induction of FCA-induced inflammation were then further injected with dPGS-NIR, which targets P and L selectins indicative of inflammation, and imaged by MSOT
Application	MSOT offers structural and functional imaging of joints involved in arthritis. Further, molecular imaging via MSOT enables visualization and quantification of P and L selectins, allowing MSOT-guided staging of inflammation in a mouse model of arthritis.



Beziere N et al.,
Optoacoustic imaging and staging of inflammation in a murine model of arthritis*, Arthritis Rheumatol. 2014 Aug;66(8):2071-8.*

Agenda

- Company overview
- MSOT technology
- Selection of MSOT applications
- Handheld system and its clinical outlook

MSOT Experimental Imaging Platform (EIP)

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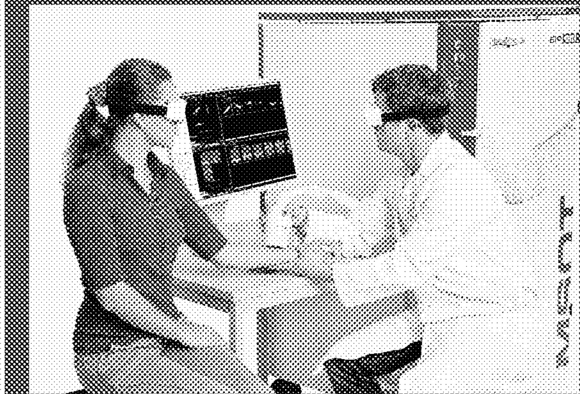
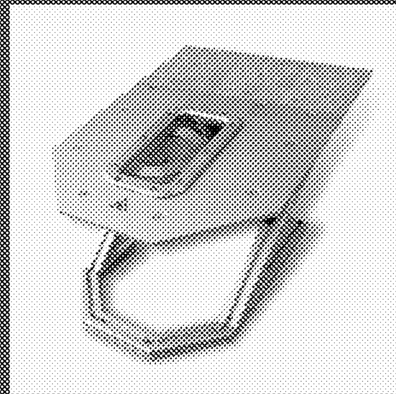
Two modes of EIP operation

Handheld



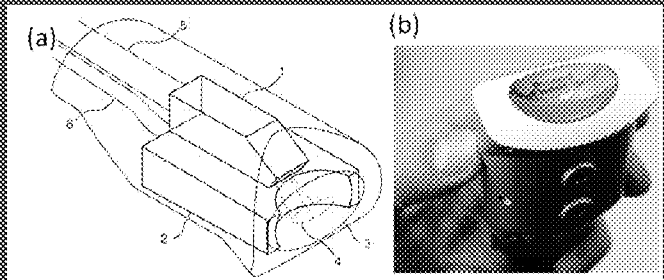
Stage-controlled



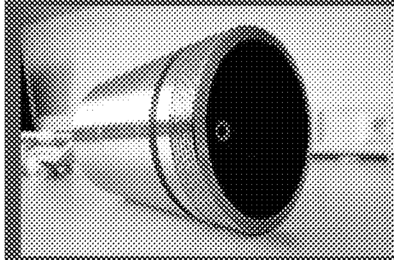
Current EIP detector options

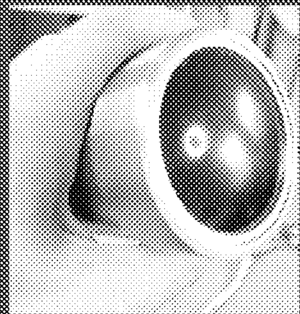
# Elements	Geometry	Central frequency	Resolution	Field of view (xyz)
128	2D	8 MHz	115 µm	5 x 10 mm
256	2D	4 MHz	200 µm	20 x 30 mm
384	3D	2.5 MHz	300 µm	15 x 15 x 25 mm
384	3D	4 MHz	200 µm	10 x 10 x 15 mm
512	3D	10 MHz	80 µm	5 x 5 x 7 mm

2D MSOT



3D MSOT





Clinical applications for MSOT EIP

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Shortlist of currently assessed applications

Clinical study ongoing
Clinical study upcoming

Clinical application	Incidence	MSOT value
Malignant melanoma	230K	Non-invasive, improved detection
Sentinel node detection	>2.000K	Non-radioactive procedure
Peripheral vascular disease	5.000K	Assess tissue hypoxia as primary burden
Rheumatoid arthritis	1.000K	Point-of-care inflammation monitoring
Breast cancer	1.700K	Non-invasive lesion assessment
Head and neck cancer	640K	Plan radio-/chemotherapy, monitor ablation
Diabetic wound healing	15.000K	Assess progress of chronic ulcer healing
Alopecia	>100.000K	Assess follicle composition for hair growth
Colon anastomosis	>1.000K	Predict anastomotic leakage
Neonatal brain injury	>1.000K	Accurate hemorrhage / ischemia detection

Dynamic 5D mouse brain imaging

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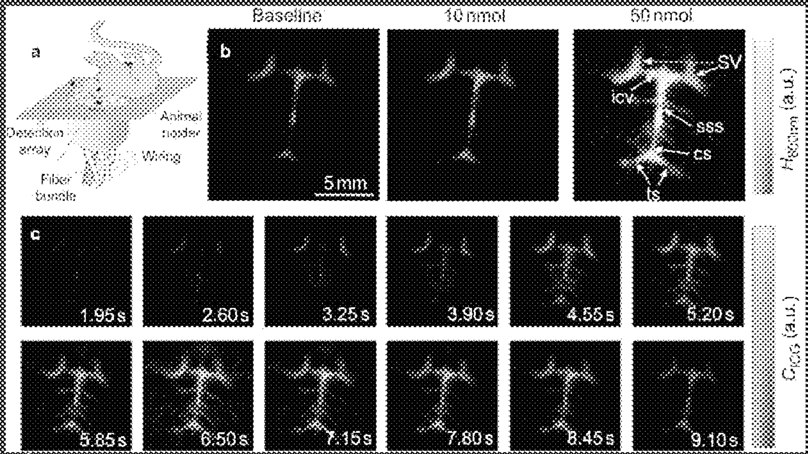
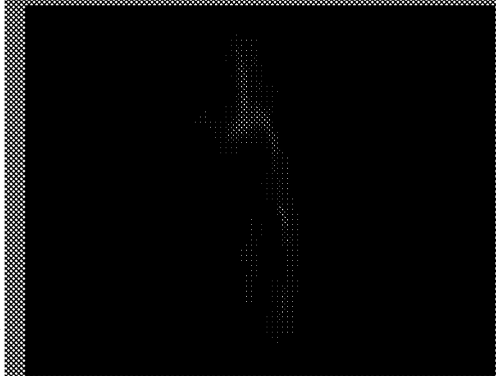
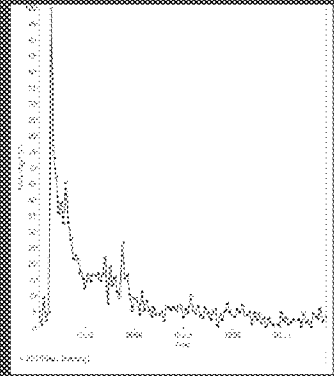
Dynamic ICG contrast (brain)



5D rendering



ICG quantification



Dean-Ben, Razansky, ***Adding fifth dimension to optoacoustic imaging: volumetric time-resolved spectrally enriched tomography***, Light: Science&Applications, 2014

- **Dynamic contrast enhancement via ICG injection enables quantification of fast kinetics in the brain**
- **5D imaging allows spatial and kinetic imaging with specificity**

Tumor imaging with the EIP100

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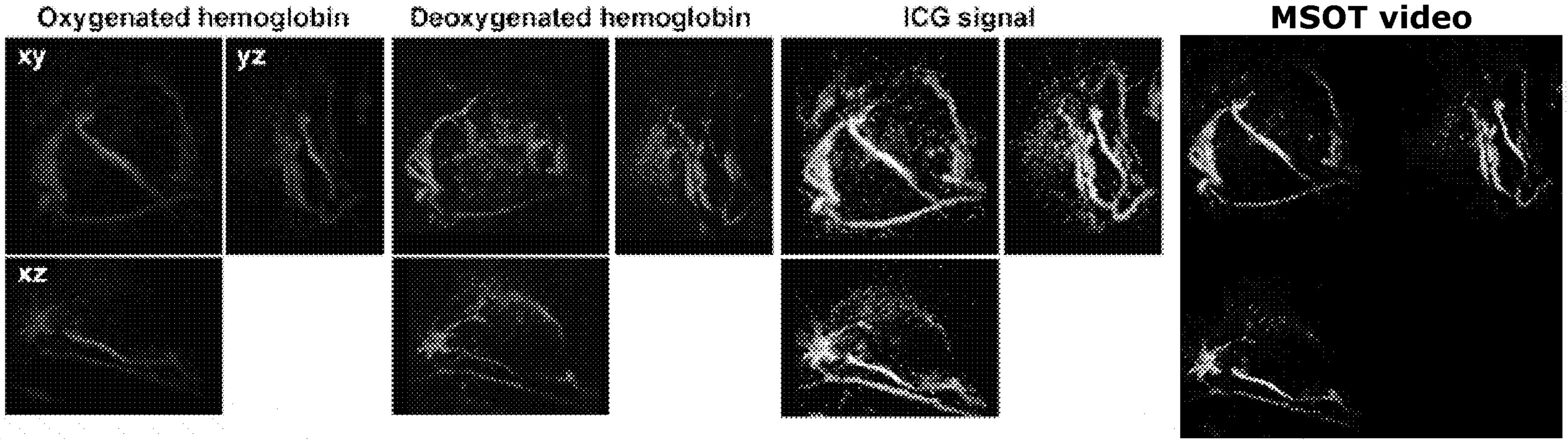
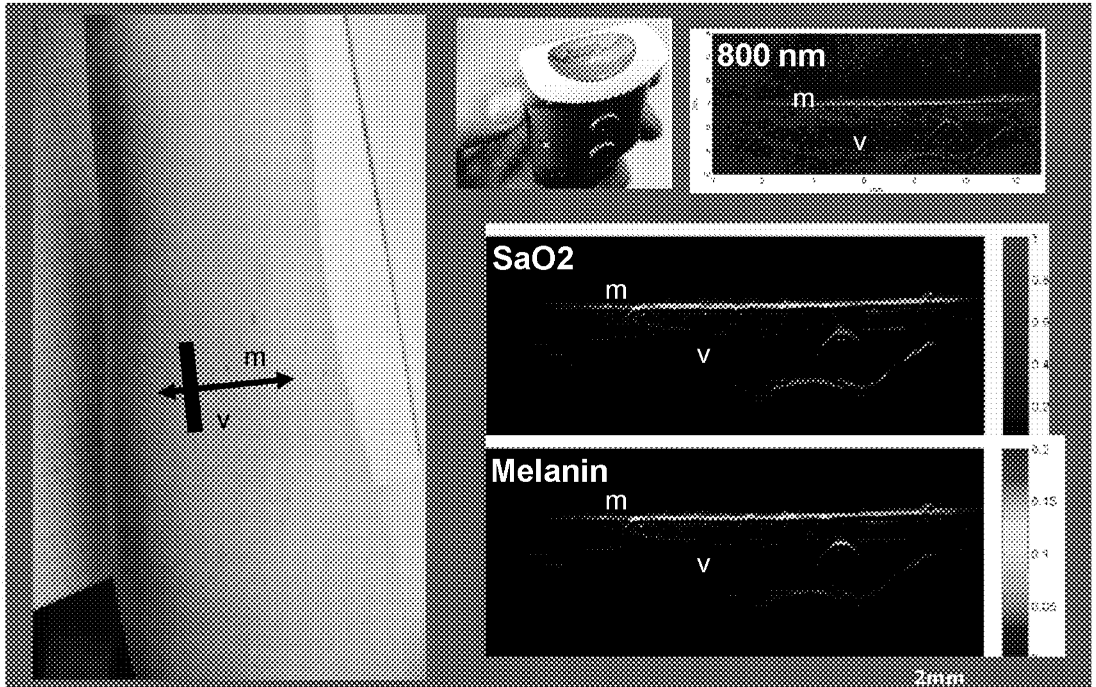
4T1 tumors imaged with the 512-element cup (10MHz)



- **Mice bearing orthotopic 4T1 tumors were imaged using the EIP 100 system with a cup-shaped transducer with 512 US elements (10MHz central frequency)**
- **The laser was operated at a frequency of 25Hz, allowing for fast multispectral data collection (5Hz, for 5 wavelengths)**

Live spectral analysis in 2D

Spectral unmixing of blood oxygenation and melanin



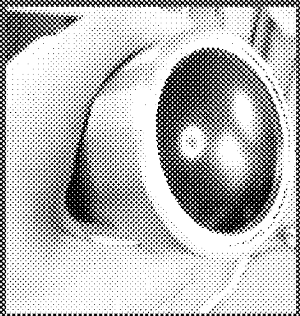
*Buehler A et al., **Real-time handheld multispectral optoacoustic imaging.** Opt Lett. 2013 May 1;38(9):1404-6. DOI: 10.1364/OL.38.001404.*

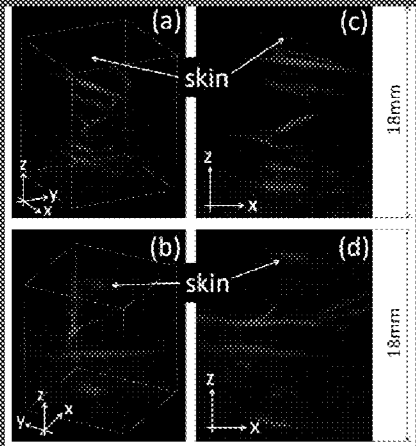
- **Besides real-time reconstruction, tissue chromophore distribution can be spectrally unmixed in real time**
- **Oxy- and deoxy-Hb as well as melanin are among the most inter-esting intrinsic markers for MSOT imaging**
- **Chromophore concen-tration is visualized as overlay on background tissue absorption**

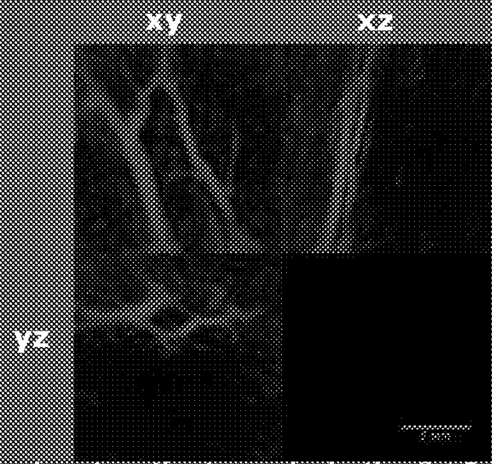
3D high-resolution vascular imaging

Imaging depth ~2 cm

80µm resolution







Deán-Ben et al., ***Portable, Spherical array probe for volumetric real-time optoacoustic imaging at centimeter-scale depths***, Opt Express. 2013 Nov 18

- **Structures can currently be visualized up to approx. 2 cm depth**
- **10 MHz detector 80µm resolution and visualization of microvasculature**

Functional angiography with MSOT

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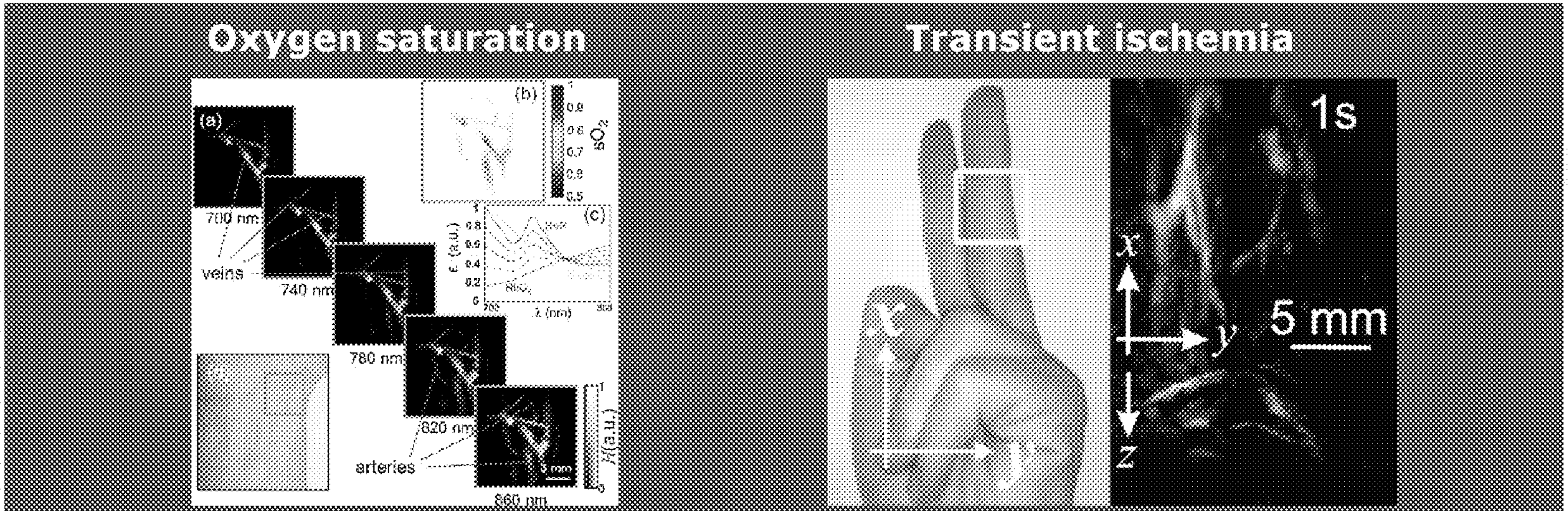
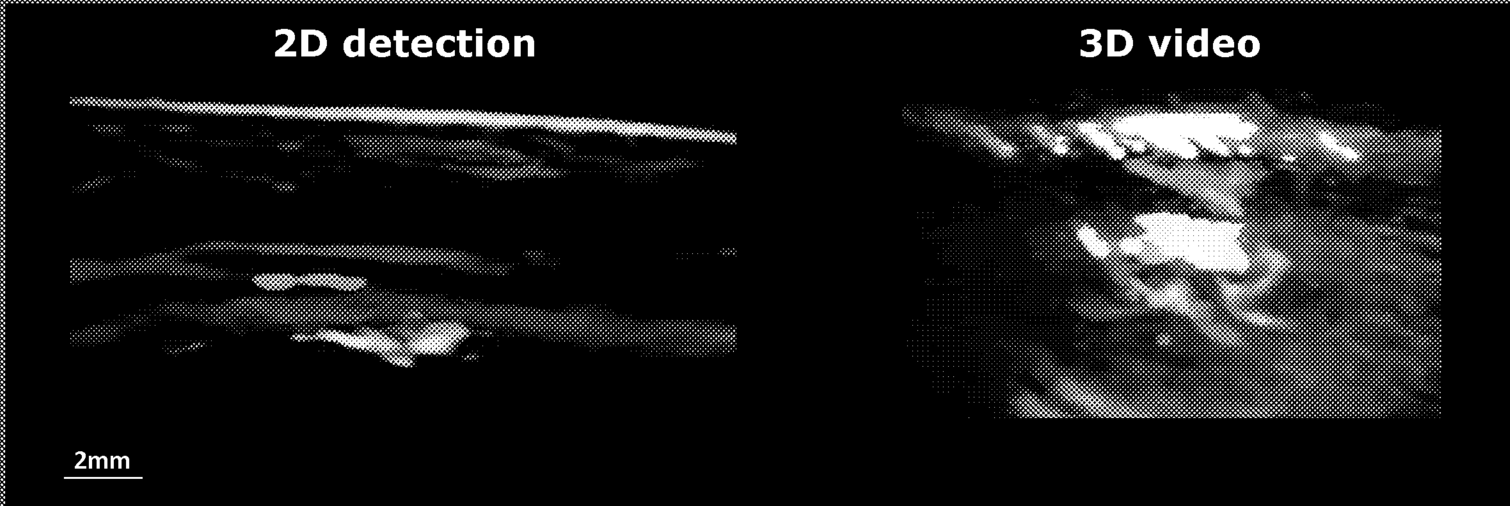
Listening to Molecules



Deán-Ben et al., ***Functional optoacoustic human angiography with handheld video rate three dimensional scanner.*** *Photoacoustics 2013; 1(3-4):68-73.*

- **Oxygen saturation is quantified via spectral unmixing of Hb and HbO_2**
- **Changes in perfusion and oxygenation can be visualized in real time**

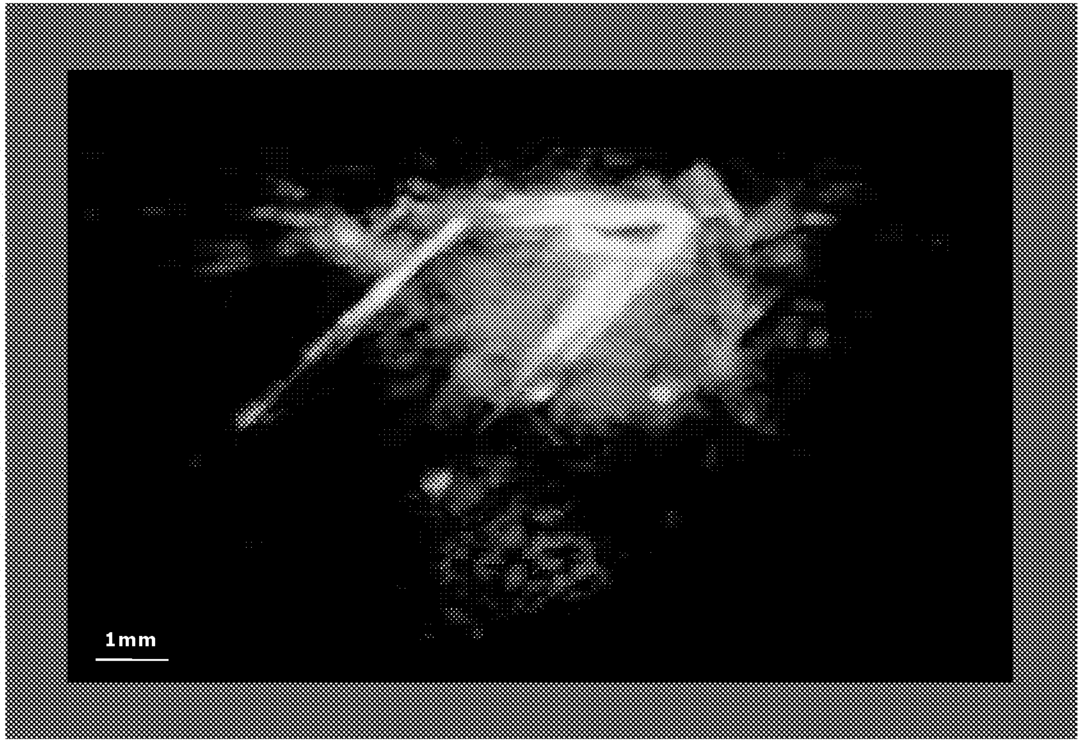
SLN imaging in melanoma patients



Klode J. et al. ***Clinical application of noninvasive and nonradioactive determination of microscopic lymph node tumor status by multispectral optoacoustic imaging.*** *Submitted for publication to the Lancet Oncology*

- **A melanoma patient was scanned with MSOT before undergoing surgical sentinel lymph node (SLN) biopsy**
- **MSOT enables the detection of SLNs via subcutaneous ICG injection and the assessment of melanin presence, potentially indicative of LN metastasis**

Multispectral imaging of hair follicles

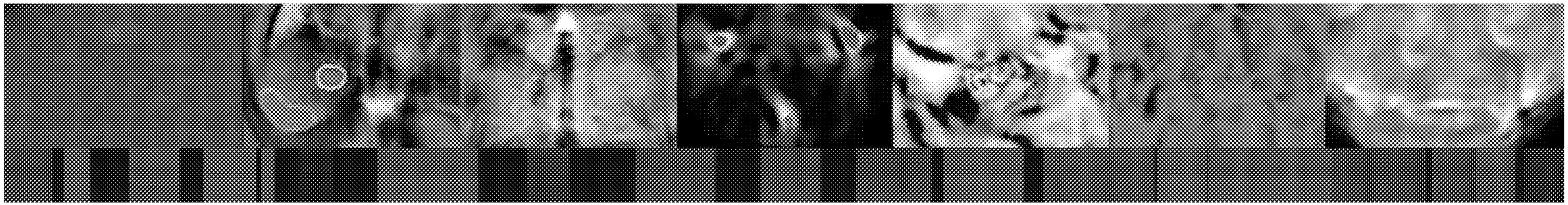


- **The morphology of hair follicles as well as their micro-environment are determinants for continued hair growth**
- **Endogenous chromophoric substances including hemoglobin, melanin and lipids are critical for hair growth**
- **MSOT can assess morphology and endogenous chromophore distribution related to hair growth and alopecia**

iTheraMedical
Listening to Molecules

MSOT inVision 512-echo

Optoacoustic UltraSound (OPUS)



Hybrid Optoacoustic/Ultrasound (OPUS)

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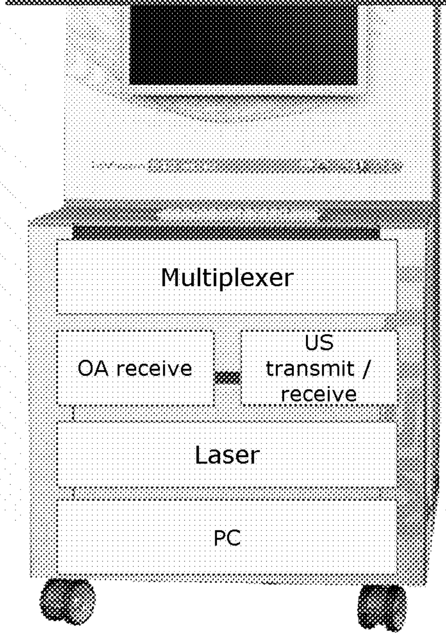
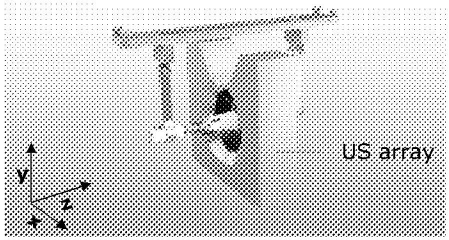
MSOT inVision 512-echo

OPUS imaging system



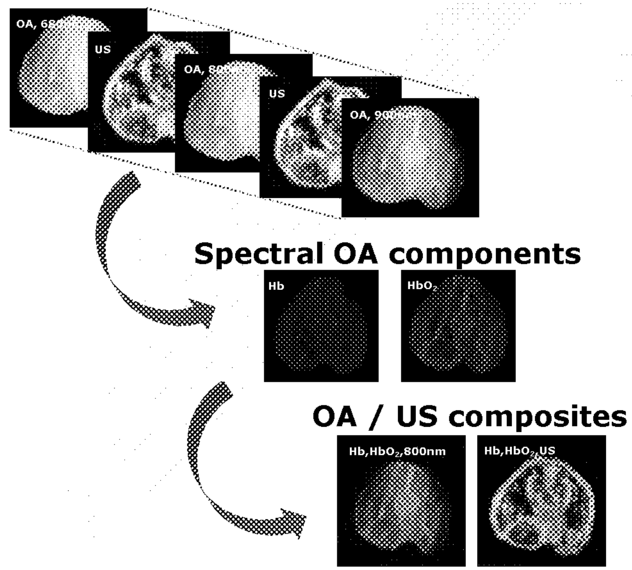
Imaging chamber



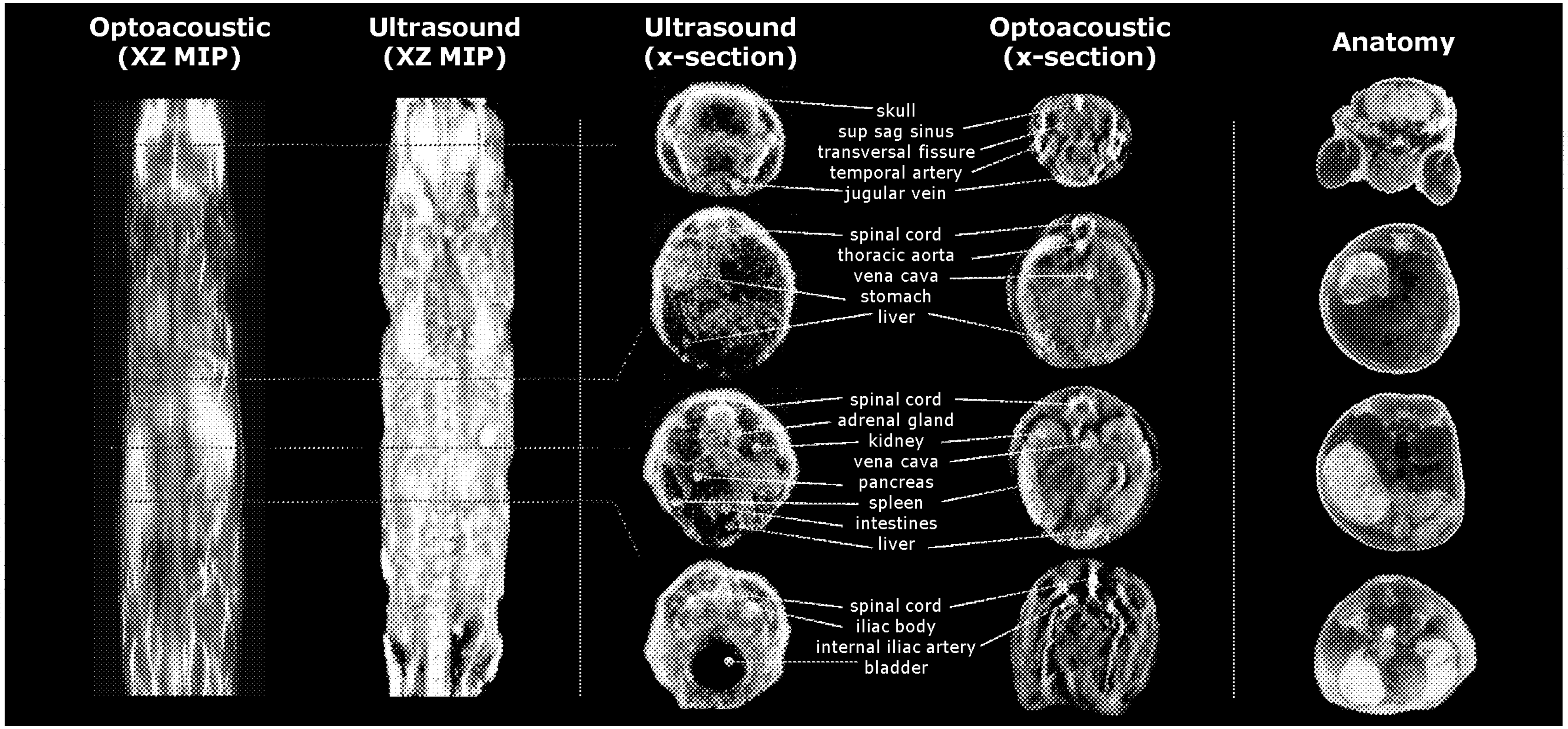
Technical parameter	Specifications
Center frequency	5 MHz
Number of elements	512
Angular coverage	270°
Radius / focus	40 / 37 mm
Height / pitch	15 / 0.37 mm
Resolution OA / US	150 / 300 µm

Interleaved image acquisition

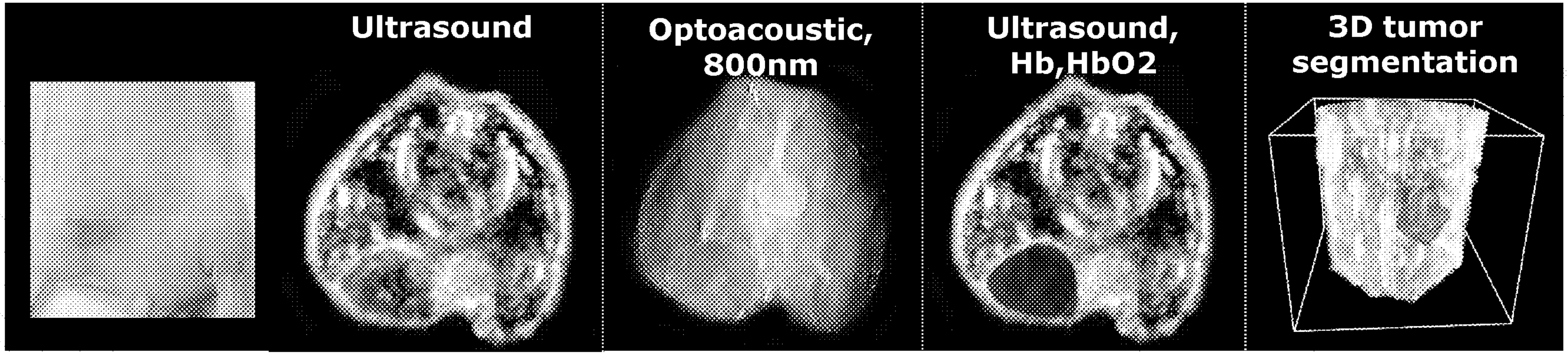
Single-wavelength OA and US images



Imaging mouse anatomy with OPUS

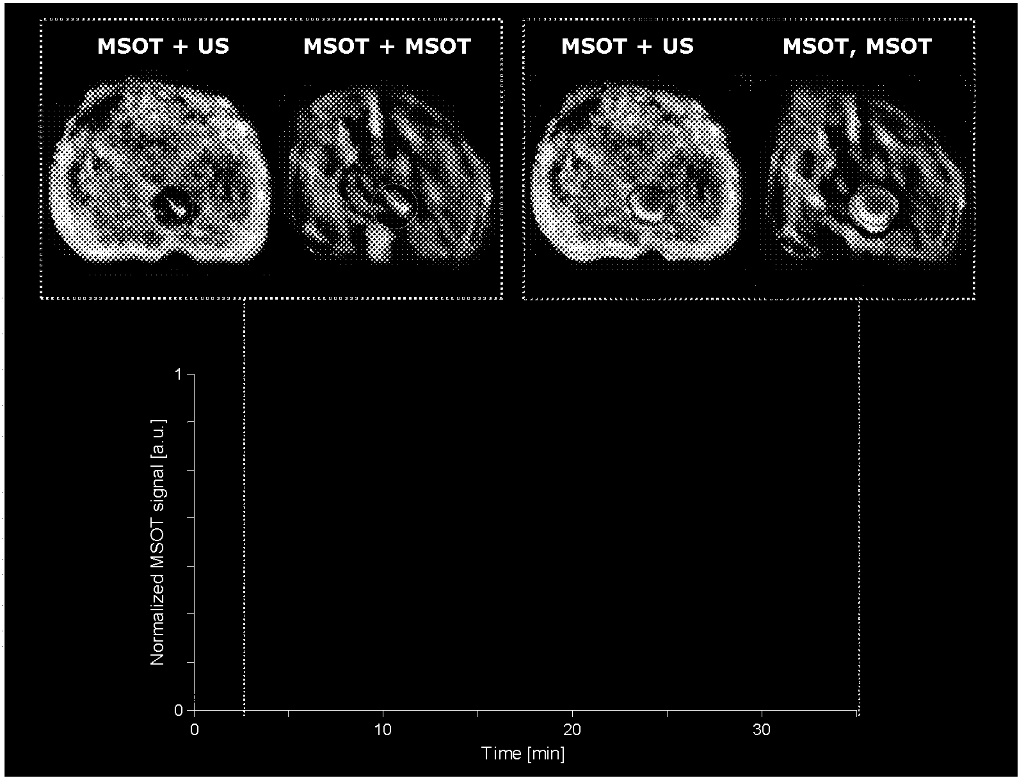


Tumor imaging with OPUS



- 4T1 mammary tumors were implanted in the mammary fat pad
- Ultrasound imaging shows clear tumor boundaries, allowing segmentation
- MSOT imaging allows functional oxygenation analysis within the tumor

Imaging probe accumulation in bladder



- **The bladder was imaged during i.v. injection of IRDye-800CW**
- **Ultrasound imaging allows identification of the bladder, invisible in MSOT in naïve mice, prior to injection**
- **Bladder uptake of dye reflects glomerular filtration and excretion, enabling functional analysis of the kidney**

Selection of MSOT publications

- Shanice V Hudson et al., **Targeted Non-invasive Imaging of EGFR-expressing Orthotopic Pancreatic Cancer using MSOT**, Cancer Res. 2014 Sep 12. DOI: 10.1158/0008-5472.CAN-14-1656.
- N. Beziere et al., **Optoacoustic Imaging and Staging of Inflammation in a Murine Model of Arthritis**, Arthritis Rheumatol. 2014 Aug;66(8):2071-8. DOI: 10.1002/art.38642
- X. Luís Deán-Ben et al., **Adding fifth dimension to optoacoustic imaging: volumetric time-resolved spectrally enriched tomography**, Light: Science & Applications (2014) 3, e137; doi:10.1038/lsa.2014.18.
- Stritzker J et al., **Vaccinia Virus-mediated Melanin Production Allows MR and Optoacoustic Deep Tissue Imaging and Laser-induced Thermotherapy of Cancer**, PNAS February 26, 2013 vol. 110 no. 9 3316-3320.
- Buehler A et al., **Real-time handheld multispectral optoacoustic imaging,** Opt Lett. 2013 May 1;38(9):1404-6. doi: 10.1364/OL.38.001404.
- Burton NC et al., **Multispectral Opto-acoustic Tomography (MSOT) Brain Imaging and Characterization of Glioblastoma**, Neuroimage, 2012 Sep 28; pii: S1053-8119(12)00963-9 .
- Herzog E at al., **Optical Imaging of Cancer Heterogeneity with MSOT**, Radiology. 2012 May;263(2):461-8.
- Taruttis A et al., **Fast Multispectral Optoacoustic Tomography (MSOT) for Dynamic Imaging of Pharmacokinetics and Biodistribution in Multiple Organs**, PLoS ONE 2012, 7(1):e30491.
- Razansky D et al., **Volumetric Real-time Multispectral Optoacoustic Tomography (MSOT) of Biomarkers**, Nature Protocols 6, 1121-1129 (2011).

Current Users

1. Institute for Biological and Medical Imaging (IBMI),Helmholtz Zentrum München, Munich, Germany
2. Institute for Radiology,Klinikum rechts der Isar, Technische Universität München, Munich, Germany
3. ZMB/Faculty of Medicine, University Hospital Esssen, Germany
4. Department of Surgery,University Medical Center Groningen, The Netherlands
5. Bioorganic Chemistry and Molecular Imaging (LCBIM),École Polytechnique Fédérale de Lausanne, Switzerland
6. Centre for Drug Delivery Research,UCL School of Pharmacy, University College London
7. Department of Imaging,Merck Research Laboratories, Merck Inc., Philadelphia, USA
8. Roche Diagnostics, Penzberg,

1. National Center for Nanosciences and Technology
2. Chang Chun Institute of Applied Chemistry Chinese Academy of Sciences
3. Institute of Automation, CAS
4. Beijing University of Chemistry technology
5. Institute of Materia Medica
6. Henan University of Traditional Chinese Medicine
7. Soochow Univeristy
8. Beijing University of Technology
9. IMPLAD
10. SCUT
11. The Hong Kong Polytechnic University
12. West China Hospital

Conclusions, outlook

- **Multispectral Optoacoustic Tomography(MSOT) provides:**
  - …anatomical, functional and molecular information
  - …in real time
  - …at high spatial resolution, in deep tissue
- **The range of preclinical/research applications in pharmacokinetic research spans wide…**
  - Plasma-concentration time curves of absorbers
  - Whole body biodistribution at high spatial and temporal resolution
  - Longitudinal studies of heterogeneous accumulation allow for the visualization and quantification of ADME processes
  - Toxicity assessment
  - Dynamic Contrast Enhanced(DCE) MSOT
- **Clinical translation is within reach!**

Conclusions, outlook

- **OPUS is the world's first hybrid tomographic optoacoustic / ultrasound imaging technology, featuring unparalleled high-resolution image quality, user-independent and whole-body**
- **Incorporation of ultrasound into MSOT was achieved through innovation of the detector and by developing novel acquisition and image reconstruction algorithms for reflection-mode ultrasound computed tomography (R-UCT)**
- **The addition of anatomical ultrasound contrast expands the utility of MSOT in a wide range of applications, including cancer and pharmacology**

PARTICIPATE IN AN IMAGING REVOLUTION.

Introducing MSOT, the next generation in molecular imaging.