The Educational Programme 2012

ROOM	Wicklow 1		Liffey 2	
SESSION	Chemistry of contrast media		What life scientists should know about imaging modalities	
07:45 - 08:00	**Basics - probes and suitable imaging modalities** Fabian Kiessling & Twan Lammers - Aachen, Germany	Basics in pharmacokinetics Twan Lammers, Aachen	**Optical Imaging, Ultrasound, Photoacoustics** Jorge Ripoll - Madrid, Spain & Georg Schmitz - Bochum, Germany	Physics of Ultrasound Imaging - Georg Schmitz, Bochum
08:00 - 08:15				
08:15 - 08:30		Physical limits: Sensitivity, specificity and quantitation - Bernd Pichler, Tuebingen & Fabian Kiessling, Aachen		Advanced microscopy technologies - Udo Birk, Heidelberg
08:30 - 08:45				
08:45 - 09:00	**Biologicals** Tony Lahoutte - Brussels, Belgium	Monoclonal Antibodies, Antibody Fragments and Peptides - Nick Devoogdt, Brussels		Optical tomography - Jorge Ripoll, Madrid
09:00 - 09:15				
09:15 - 09:30		Protein and Oligonucleic Acid Scaffolds - Imaging using Affibody molecules Vladimir Tolmachev, Uppsala		
09:30 - 09:45				
09:45 - 10:00		Reporter gene imaging - Veerle Baekelandt, Leuven		Photoacoustic imaging - Stanislav Emelianov, Austin
10:00 - 10:15				
10:15 - 10:30	**coffee break**			
10:30 - 10:45				
10:45 - 11:00	**Small molecules** Dean Sherry - Dallas, US	Hyperpolarised probes - Damian Tyler, Oxford	**MR Fundamentals for Life Scientists** Michal Neeman - Rehovot, Israel & Joseph Ackerman - St. Louis US	Introduction to MR Physics - Andrew Fagan, Dublin
11:00 - 11:15				
11:15 - 11:30		Chelate complexes for imaging - Dean Sherry, Dallas		Introduction to MR Hardware - Dominik von Elverfeldt, Freiburg
11:30 - 11:45				
11:45 - 12:00		Small molecules for nuclear medicine - Danielle Vugts, Amsterdam		Contemporary MR: Pushing the Limits - Joel Garbow, St. Louis
12:00 - 12:15				
12:15 - 12:30	**lunch break**			
12:30 - 12:45				
12:45 - 13:00				
13:00 - 13:15				
13:15 - 13:30				
13:30 - 13:45				
13:45 - 14:00	**Particles and polymers** Klaas Nicolay - Eindhoven, Netherlands	Basic considerations on the use of particles and polymers - Hisataka Kobayashi, Bethesda	**Nuclear Imaging** Steven Meikle - Sydney, Australia	Principle of PET and SPECT - Steven Meikle, Sydney
14:00 - 14:15				
14:15 - 14:30		Established particles and polymers - Willem Mulder, New York		Detector technologies - Dennis Schaart, Delft
14:30 - 14:45				
14:45 - 15:00		Advances in particles and polymers - Sanjiv Sam Gambhir, Stanford		Hybrid imaging systems Roger Fulton, Sydney
15:00 - 15:15				
15:15 - 15:30	**Meet the Experts Chemistry of contrast media**		**Meet the Experts What life scientists should know about imaging modalities**	
15:30 - 15:45				
15:45 - 16:00				
16:00	**Opening Ceremony**			

ROOM	Wicklow 2 Wicklow 2		Auditorium	
SESSION	Postprocessing and Cross Validation		Biology and Pathology	
07:45 - 08:00	**Modeling and quantification** Adriaan Lammertsma - Amsterdam, Netherlands	Basic principles of quantification using PET Mark Lubberink, Uppsala	**Cardiovascular** René Botnar - London, UK	Introduction to Biology and Treatments - Atherosclerosis Michael McConnell, Stanford
08:00 - 08:15				
08:15 - 08:30		Basic principles of quantification using MR - Markus Rudin, Zuerich		Introduction to MR and PET imaging - Atherosclerosis Renè Botnar, London
08:30 - 08:45				
08:45 - 09:00		Basic principles of quantification using optical techniques - Adrian Taruttis, Munich		Introduction to Molecular Contrast Agents and New Devices - Klaas Nicolay, Eindhoven
09:00 - 09:15				
09:15 - 09:30		Basic principles of tracer kinetic modelling Adriaan Lammertsma, Amsterdam	**Systems biology and its link to MI** Hermann-Georg Holzhütter - Berlin	Systems Biology of single cells - Hermann-Georg Holzhütter, Berlin
09:30 - 09:45				
09:45 - 10:00				
10:00 - 10:15				
10:15 - 10:30	**coffee break**			
10:30 - 10:45				
10:45 - 11:00	**Basics of image processing** Wiro Niessen - Rotterdam, The Netherlands	Image segmantation: methodology and validation Wiro Niessen - Rotterdam	**Cancer** Markus Rudin - Zuerich, Switzerland & Robert Gillies - Tampa, US	Image Analysis and Informatics - Robert Gillies, Tampa
11:00 - 11:15				
11:15 - 11:30				Biomarkers - Oncology and Inflammation - Markus Rudin, Zuerich
11:30 - 11:45				
11:45 - 12:00				Oncology and Inflammation - Zaver Bhujwalla, Baltimore
12:00 - 12:15				
12:15 - 12:30	**lunch break**			
12:30 - 12:45				
12:45 - 13:00				
13:00 - 13:15				
13:15 - 13:30				
13:30 - 13:45				
13:45 - 14:00	Focus Session on **new imaging tools: Cerenkov luminescence imaging** Jan Grimm - New York, US	Faster than the speed of light - applications for Cerenkov imaging Jan Grimm, New York	**CNS** Doris Doudet - Vancouver, Canada	Pathophysiology and imaging of neurodegenerative diseases - Karl Herholz, Manchester
14:00 - 14:15				
14:15 - 14:30		Cerenkov Luminescence Endoscopy: Feasibility and Challenges - Zhen Cheng, Stanford		Neuroplasticity - Annemie van der Linden, Antwerp
14:30 - 14:45				
14:45 - 15:00		Cerenkov specific contrast agents - Edward J. Delikatny, Philadelphia		Pathophysiology and imaging of stroke - Mathias Hoehn, Cologne
15:00 - 15:15				
15:15 - 15:30	**Meet the Experts Postprocessing and Cross Validation**		**Meet the Experts Biology and Pathology**	
15:30 - 15:45				
15:45 - 16:00				
16:00	**Opening Ceremony**			

The Educational Concept – A New Format

THE EDUCATIONAL CONCEPT 2012-2014

"Traditionally" the World Molecular Imaging Congress – WMIC starts with the educational sessions.

In 2012 we are excited to emphasise the importance of the educational part by developing a new concept based on a **3-year-curriculum-format** covering the main topics:

- Chemistry of contrast media,
- What life scientists should know about imaging modalities,
- Biology and Pathology, and
- Postprocessing and Cross Validation.

All in all eleven sessions are scheduled. The eleven curricula – developed by outstanding scientists – are describing the **talk titles** and the **expected contents** of the lectures in order to avoid overlaps and to present a consistent eduactional programme. Nevertheless the concept should be also seen as **basis for improvements and developments** according to the requirements of the MI research community. **Seventeen** internationally recognised experts intensely worked on the composition of their curriculum. Their dedication to this mission is gratefully acknowledged – **thank you:**

CHEMISTRY OF CONTRAST MEDIA

Fabian Kiessling - Aachen, Germany
Tony Lahoutte - Brussels, Belgium
Twan Lammers - Aachen, Germany
Klaas Nicolay - Eindhoven, Netherlands
Dean Sherry - Dallas, US

WHAT LIFE SCIENTISTS SHOULD KNOW ABOUT IMAGING MODALITIES

Joseph Ackerman - St. Louis, US
Steve Meikle - Sydney, Australia
Michal Neeman - Rehovot, Israel
Jorge Ripoll - Madrid, Spain
Georg Schmitz - Bochum, Germany

POSTPROCESSING AND CROSS VALIDATION

Adriaan Lammertsma - Amsterdam, Netherlands
Wiro Niessen - Rotterdam, The Netherlands

BIOLOGY AND PATHOLOGY

René Botnar - London, UK
Robert Gillies - Tampa, US
Hermann-Georg Holzhütter - Berlin, Germany
Markus Rudin - Zuerich, Switzerland
Vesna Sossi - Vancouver, Canada

We are confident that you enjoy our educational programme, find it comprehensive and inspiring. We are looking forward to the **feedback** from the speakers, the people who developed the three-year-outline, and from **you** - the attendees of **YEAR 1**. We are eager to continously improve our concept in order to provide not only early-stage resaerchers with the most recent knowledge but also to ensure a flow of expertise between the diverse disciplines in MI.

Fabian Kiessling – Chair of the Educational Sessions 2012

Content

1 – Chemistry of Contrast Media

1A – BASIC CONSIDERATIONS ABOUT SUITABLE MODALITIES & PROBES

chaired by Fabian Kiessling & Twan Lammers – Aachen, Germany

TALK 1: BASICS IN PHARMACOKINETICS

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LEARNING OBJECTIVES:

- Understanding basic pharmacokinetic principles and processes
- Recognizing the most important pharmacokinetic parameters
- Discriminating optimal pharmacokinetic properties of drugs vs. contrast agents

Pharmacokinetics (PK) is a sub-discipline within pharmacology which focuses on the use mathematical models to describe and predict the time-course of drugs and imaging agents in the body. The four most important PK processes are absorption, distribution, metabolism and elimination (ADME). Absorption deals with the uptake of a molecule from the gastro-intestinal system into the blood. Distribution encompasses the delivery of diagnostic or therapeutic molecules to pathological and healthy sites all over the body via systemic circulation. Metabolism refers to the degradation of the drug or imaging agent, and primarily takes place in the liver. Elimination finally results in the removal of the agents from the body, either via renal excretion, or via hepatobiliary clearance. In case of therapeutic molecules, all four PK processes are highly important for determining the efficacy and the toxicity of the drug. In case of diagnostics, PK parameters predefine the potential and the specific use of the imaging agent. In the present lecture, I will briefly introduce the basic principles, processes and parameters involved in pharmacokinetics, I will outline and compare optimal pharmacokinetics properties of drugs vs. imaging agents, and I will provide several examples showing that tailoring the pharmacokinetic properties of drugs and imaging agents can be used to enhance therapeutic efficacy and enable novel imaging applications.

TALK 2: PHYSICAL LIMITS: SENSITIVITY, SPECIFICITY, AND QUANTITATION

Kiessling, Fabian & Pichler, Bernd
Aachen, Germany & Tübingen, Germany

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LEARNING OBJECTIVES:

- Quantification limitations in µCT and nuclear imaging
- Limiting factors impacting the spatial resolution in small animal imaging
- Factors how the imaging probe specificity can impact quantification

Imaging modalities significantly differ in their ability to display anatomy and to quantify the accumulation of probes. MRI and CT, for example provide an excellent tissue contrast but have low sensitivity for contrast agents. Furthermore, quantification is more complex with MRI compared with CT. Nuclear medicine techniques and optical imaging are highly sensitive to probes but do not provide much anatomical information. In addition, while PET and SPECT data can considerably easy be quantified, quantification of optical data is challenging. Ultrasound displays a good tissue contrast and is sensitive to probes, however, visualization of deep lesions can be difficult and demand for experienced observers. Furthermore, the reproducibility is lower as compared with other tomographic technologies. Besides a general overview on the strengths and limitations of imaging technologies, this educational session will focus on data quantification with µCT and nuclear medicine technologies. PET, SPECT: Nuclear imaging methods rely on the detection of radioactive decay emitted by radiolabeled biological probes. This approach provides data yielding reproducibility in the range of 95% and a detection sensitivity in the pico-molar range. However, as the spatial resolution of PET and SPECT is limited to around 1 mm, the

reconstructed image data suffer from partial volume effects (PVE) and restrictions to resolve tiny lesions. The PVE results in an underestimation of the activity concentration in the target volume of interest. This educational talk will review the different effects of PET and SPECT limiting the accurate tracer quantification and image resolution. It will further provide an overview about the importance of specific radiolabeled probes with high specific activity levels. µCT: The voxel intensity of µCT data sets is proportional to the absorption of the voxel with respect to the x-ray wavelength, which in turn depends linearly on the elemental composition of the tissue. However, when dealing with µCT-systems one should know that many µCT systems are not calibrated and display CT numbers not Houncefield units (HU). Furthermore, background noise levels are much higher in µCT than clinical systems and can reach values of 30 HU and more. This has to be considered when performing dynamic contrast enhanced CT-scans. Injecting contrast agent (CA) doses that are equivalent to those used in clinics may thus not be sufficient to generate a sufficient change in contrast. Blood pool CA allow longer scan times and CA injection can be made outside the CT-system. Taking the change in contrast intensity before and after CA injection in tumor tissue and dividing it by the contrast change in a feeding vessel relative blood volume in tumors can be quantified accurately. If the applied x-ray dose is a critical factor the pre-scan can be discarded when using dual energy scans. Single source CT-scanners allow quantitative decomposition of tissue into two components, e.g. into soft tissue and calcium. When a third component is present (e.g. CA), iodine and calcium based enhancements may be hard to distinguish. Dual energy µCT systems acquire two data sets at two different wavelengths, potentially allowing quantitative decomposition of three components. This can, for example, be used to distinguish calcified atherosclerotic plaques from iodine enhanced blood vessels.

1B – BIOLOGICALS

chaired by Tony Lahoutte - Brussels, Belgium

TALK 1: MONOCLONAL ANTIBODIES, ANTIBODY FRAGMENTS AND PEPTIDES

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LEARNING OBJECTIVES

- discover the power of antibodies and antibody fragments for biotechnological applications, biomedical research and clinical programs.
- understand the relationship between antibody/fragment structural, biochemical and pharmacokinetic properties.
- learn about the art to generate imaging tracers derived from antibodies and its engineered fragments

Antibodies are professional antigen-binding molecules that are present in all mammals as part of their natural host immune system. As such they can be used to target membrane receptors and soluble proteins for imaging applications in preclinical models and in patients. Through biotechnological methods, antibodies can be further reformated into smaller molecules such as scFv's, diabodies, minibodies and nanobodies. Antibodies and derivatized fragments have their particular properties in regard to protein structure, size, stability, affinity, specificity and pharmacologic behavior. In this session we will further evaluate their labeling methods and technologies to isolate binders of interest. Finally, we will discuss important aspects to progress an antibody-derived imaging tracer into clinical translation.

TALK 2: PROTEIN AND OLIGONUCLEIC ACID SCAFFOLDS: IMAGING USING AFFIBODY MOLECULES

Tolmachev, Vladimir
Unit of Biomedical Radiation Sciences, Uppsala University, Sweden

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LEARNING OBJECTIVES:

- Scaffold structure of Affibody molecules permits obtaining of robust binding proteins with subnanomolar affinities;
- Robust structure of Affibody molecules permits efficient labeling in harsh conditions (pH range of 3.6-11.5; temperature up to 100°C, presence of lipophilic solvents) without losing binding capacity;
- Small size (6-7 kDa) of Affibody molecules enables rapid extravasation and tumor penetration, and rapid clearance of unbound tracer, permitting high contrast imaging shortly after injection;
- Rich labeling chemistry permits optimizing of biodistribution of Affibody molecules and increasing of imaging contrast;
- Clinical studies demonstrated that Affibody molecules can image HER2-expressing breast cancer metastases with high sensitivity.

Affibody molecules are a new class of small (6-7 kDa) affinity proteins based on a three-helical scaffold. Randomization of 13 amino acids on the surfaces of helices 1 and 2 provides large (>10^{10} members) libraries enabling molecular-display selection of binders. The robust Affibody scaffold enables high affinity of selected proteins. Affibody molecules with picomolar affinity have been developed for binding to such therapeutic cancer-associated targets as HER2, EGFR, HER3, IGF-1R and PDGFRβ. Di- and multimeric forms of Affibody molecules and fusion proteins can be easily produced by recombinant expression in E. coli. Since the Affibody scaffold does not contain cysteines, a unique cysteine residue can be introduced in a desirable position allowing site-specific coupling of prosthetic groups or chelators using thiol-directed chemistry. Monomeric forms of Affibody molecules can be made by peptide synthesis allowing site-specific incorporation of chelators or prosthetic groups as well as unnatural amino acids. One of the key feature of Affibody molecules is very rapid (3 µs) re-folding at physiological pH and molarity, permitting application of harsh conditions during labeling and purification (pH range of 3.6-11.5, temperature up to 95°C and the use of lipophilic solvents). During optimization of targeting properties, Affibody molecules were labeled with 16 different nuclides suitable for imaging (e.g. ^{18}F, ^{68}Ga, ^{99m}Tc, ^{111}In, ^{124}I) and therapy (e.g. ^{90}Y, ^{177}Lu, ^{131}I, ^{186}Re, ^{211}At), using over 50 different nuclide-chelator/linker combinations. The binding capacity was preserved in all cases.

In vivo, monomeric Affibody molecules clear rapidly via kidneys. In the case of abundant targets, such as e.g. HER2, a tumor uptake of 20-25% ID/g and tumor-to-blood ratio up to 200 can be obtained in mouse xenograft models at 4h after injection. However, good contrast is possible also in the case of a low target expression (< 30000 molecules/cells). Targeting is highly specific (as shown by the use of pre-saturation, by the use of non-expressing xenografts or non-specific Affibody molecules). An unspecific tumor accumulation due to EPR effect is negligible, at the level of less that 1% of specific uptake.

Genetic fusion of Affibody molecules with the albumin-binding domain (ABD, Albumod) enables appreciable (up to 80-fold) extension of their residence time in circulation and reduces renal uptake, which broaden their use for therapeutic applications. The use of near-infrared fluorescent dyes or fusion with fluorescent proteins facilitates optical in vivo imaging using Affibody molecules. Furthermore, affibody molecules can also be used for specific targeting of different nanocarriers, such as liposomes or SPIO.

Preclinical studies have demonstrated a high potential of radiolabeled affibody molecules for in vivo imaging of expression of therapeutic targets and for monitoring of response to targeted therapy. Clinical studies have demonstrated that the ^{111}In- and ^{68}Ga-labeled anti-HER2 Affibody molecules can image HER2-expressing metastases in breast cancer patients. So far, anti-Affibody molecule antibodies were not detected in clinics.

TALK 3: REPORTER GENE IMAGING

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LEARNING OBJECTIVES:

- To get acquainted with different reporter gene systems for different imaging modalities
- To learn about preclinical applications of reporter gene imaging in vivo
- To get insight into the clinical translation of reporter gene imaging

A variety of imaging techniques have been developed that allow non-invasive detection of gene expression or cells within intact mammals, ranging from mouse to man. The basic concepts of these imaging

techniques will be discussed. Different imaging reporter genes have been developed that can be detected and quantified by these imaging techniques which allow to unravel the temporospatial dynamics of gene expression or cells within the intact living animal. We will explain methods to introduce reporter genes in cells and in vivo. Applications of reporter gene imaging can be found in different research fields. We will give some examples of the use of imaging reporter genes in preclinical neuroscience. We will highlight the potential impact of reporter gene imaging in the preclinical evaluation and clinical implementation of gene and cell therapy.

1C – SMALL MOLECULES

chaired by Dean Sherry – Dallas, USA

TALK 1: HYPERPOLARISED PROBES

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LEARNING OBJECTIVES

- To understand the general principles of hyperpolarization
- To overview the main techniques used to generate a hyperpolarized state
- To detail how hyperpolarization can be utilized in magnetic resonance imaging experiments
- To review the main areas in which hyperpolarized techniques have been applied

Metabolic imaging, where imaging technology is coupled with metabolic probes to detect disease–specific biomarkers, is transforming our approach to disease detection and treatment. In recent years the development of magnetic resonance imaging (MRI) has provided a valuable new approach for the assessment of tissue structure and function. However, despite the enormous technical developments that have taken place, MRI remains an inherently low sensitivity technique and the low signal levels obtained limit its application for the assessment of metabolism. More recently, the development of a range of techniques, which can be gathered under the umbrella term of 'hyperpolarization', has offered potential solutions to this low sensitivity. Such hyperpolarization methods, including dynamic nuclear polarization (DNP), parahydrogen induced polarization (PHIP) and optical pumping of the noble gases, have been demonstrated to increase the sensitivity of MRI to detect metabolic tracers by more than 10,000–fold, thereby allowing in vivo substrate uptake and metabolism to be measured in real–time and at repeated time–points during disease progression. Various studies have been performed in different pre–clinical models of disease demonstrating unprecedented visualization of the biochemical mechanisms of normal and abnormal metabolism. With the emerging clinical application of hyperpolarized helium and xenon imaging of the human lungs and the recent landmark demonstration that DNP enhanced MRI can be safely applied in humans in the study of prostate cancer, the potential for clinical application of hyperpolarized MRI in the assessment of many different diseases has become a reality.

This talk will provide a general introduction to the principles of hyperpolarization with a detailed overview of the different hyperpolarization techniques and their potential areas of application. The talk will also provide a review of the existing literature, summarizing the key experimental findings and the potential application areas for clinical translation.

TALK 2: CHELATE COMPLEXES FOR IMAGING

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LEARNING OBJECTIVES:

- How to choose the proper ligand for any given metal ion reporter (largely MR versus nuclear imaging applications)
- Compare and contrast thermodynamic and kinetic principles for metal chelate complexes
- "Hard" versus "soft" metal ions; why does the type of ligand donor atom matter

Metal ions and their complexes play an important role in medical imaging. About 85% of all diagnostic nuclear imaging procedures use a complex of the gamma emitting metastable isotope ^{99m}Tc and approximately half of all MRI exams are performed using a Gd^{3+}-based T_1 contrast agent. Due to their favorable nuclear properties, complexes of $^{68}Ga^{3+}$ and $^{64}Cu^{2+}$ are emerging as promising new radiopharmaceuticals for PET imaging. To avoid potential toxicity, these ions are always administered in chelated form. In addition to lowering toxicity, the ligand can influence the biodistribution, alter the chemical properties such as relaxivity (r_1) or water exchange rates (PARACEST), or have a reactive functional group for covalent attachment of the complex to a targeting vector (e. g. antibody or peptide). Thus, the choice of the ligand is critical in the design of new metal ion-based imaging agents. Gd^{3+} and Eu^{3+} belong to the lanthanides ($4f^7$ and $4f^6$) and have an ionic radius of around 0.95Å. They are hard Lewis acids and prefer F, O and N donor atoms. Their preferred coordination number is 8 or 9 and the coordination geometry is typically determined by the ligand. They form highly stable chelate complexes with polyamino polycarboxylate ligands with a matching number of donor atoms. It is therefore not surprising that the macrocyclic ligand DOTA and its derivatives are exceptionally well suited as chelates for these ions. As a result of the rigid, preorganized nature of the ligand, Gd(DOTA) has high thermodynamic stability and kinetic inertness. The complex has one inner sphere water molecule in fast exchange with bulk water molecules, an important consideration for MRI applications. Substitution of glycine amide groups for the carboxylates of DOTA gives rise to a closely related ligand, $DOTA(gly)_4$. The weaker amide oxygen donor atoms in $EuDOTA(gly)_4^-$ lead to a complex with lower stability but, interestingly, higher kinetic inertness than $GdDOTA^-$.

The aqueous chemistry of Ga^{3+} ($3d^{10}$) is governed by its hard Lewis acid character. The Ga^{3+} ion has a smaller ionic radius than the Ln^{3+} ions and tends to form stable octahedral complexes. For this simple reason, octadentate chelators such as DOTA are not ideal for this ion despite the fact that DOTA derivatives are widely used to chelate Ga^{3+} because it is more widely available. Ga^{3+} complexes formed with the smaller, hexadentate macrocyclic ligand, NOTA, has exceptionally high kinetic inertness and thermodynamic stability compared to $GaDOTA^-$.

The chemical properties of Cu^{2+} are quite different from the Ln^{3+} and Ga^{3+} ions. It possesses borderline softness, and therefore, prefers soft donor atoms (N and S) over harder donors such as O. Being a transition metal, there is significant metal-ligand orbital overlap in Cu^{2+} complexes. The most common coordination geometry in Cu^{2+} complexes is square planar or distorted octahedral. Cu^{2+} forms very stable complexes with the rigid cross-bridged cyclam derivatives. These complexes have extremely high kinetic inertness and are well suited for nuclear medicine applications.

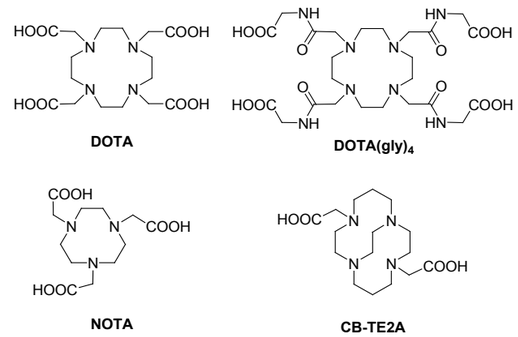

Fig. 1. Some common ligands used in metal ion-based imaging.

TALK 3: LABELING OF (BIO)MOLECULES WITH RADIOMETALS

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LEARNING OBJECTIVES:

- Have knowledge about the most important SPECT and PET metal ion radionuclides and their characteristics
- Be able to select the right radiometal for a (bio)molecule
- Be able to select the appropriate chelate for a particular radiometal
- Have knowledge about labeling conditions for the different radiometals in combination with chelates and (bio) molecules

Throughout history, metals and metal compounds have been used for treatment of various diseases, such as arthritis and cancer. However, the use of radiometals in radiopharmaceuticals for medical imaging is a relatively new area. Radiometals are of particular interest for the development of radiopharmaceuticals due to their broad range of nuclear properties (type of radiation, gamma ray or beta particle energy and half-life), and their rich coordination chemistry.

In this teaching session the different radiometals that are used in practice will be discussed with respect to their physical characteristics. Both SPECT and PET tracers will be discussed and an overview will be given about the different radionuclides that are used mostly in clinical practice. Next, the different chelates that can be used to complex these radiometals will be discussed as well as how these chelates can be coupled to (bio)molecules. In addition, labeling conditions for the differentradiometal-chelate combinations will be discussed.

Finally, examples of clinical trials using these radiometals will be discussed.

1D – PARTICLES AND POLYMERS

chaired by Klaas Nicolay – Eindhoven, The Netherlands

TALK 1: BASIC CONSIDERATIONS ON THE USE OF PARTICLES AND POLYMERS IN MOLECULAR IMAGING

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LEARNING OBJECTIVES:

- To review the *in vivo* behavior of macromolecular imaging agents
- To understand how to optimize the pharmacokinetics of nano-sized imaging agents?
- To evaluate the unique toxicities anticipated for of macromolecular imaging agents

From the biological and chemical perspective, macro-molecules, polymers, and particles that are used in imaging agents, can be categorized as non-biodegradable or biodegradable. Non-biodegradable materials generally have covalently-bound, single macromolecular structures that do not have bio-cleavable bonds such as ester- disulfide- or amide-bonds, that may be degraded by specific enzymes in the body. In contrast, biodegradable materials are single molecules with bio-cleavable bonds including bio-degradable polymers or self-assembled particles which compose of charge or biphasic (hydrophobic-hydrophilic) molecules such as liposomes and viral capsids or metal crystals including iron oxide nano-particles. Imaging agents made by non-biodegradable materials can be designed in a straightforward fashion because injected agents are not metabolized and are excreted unchanged from the body. In contrast, imaging agents based on biodegradable materials will form metabolites which may have different rates of excretion compared with the parent compound. These factors add complexity to the design of biodegradable imaging agents. On the other hand, metabolites may enable more rapid overall excretion than non-biodegradable agents.

To effectively target cells in the body, macromolecular imaging agents must be able to evade the reticuloendothelial system (RES). If not, such agents will be rapidly trapped by the liver and the spleen and will not be available for the target. Moreover, rapid sequestration in the liver and spleen increases the likelihood of immunogenicity. In order to evade the RES, agents should be hydrophilic and close to neutral charge. Therefore, to ensure this behavior the surface of “stealth” agents are generally coated with hydroxyl group-rich moieties such as polyethylene glycol (PEG). In general, excretion rates and routes are determined by the size of agents or their breakdown products. If agents or their breakdown products are small enough (<6nm in diameter), those agents will be filtered through the glomerulus and rapidly excreted into the urine (unless there is binding to the proximal tubules). In some cases, agents composed of softer, more flexible molecules, can be larger than 6nm but still be reliably excreted by the kidneys. . In contrast, larger agents will circulate longer in the blood and will be trapped and excreted through the liver to the bile resulting in slow clearance. Agents with rapid clearance are generally preferable due to their higher safety profile than those with slow clearance. However, toxicity is also dependent on the nature of the degradation products. Additional complexity is added when the agents or their degradation products interact with plasma proteins such as albumin and macroglobulins where there are added concerns regarding clearance and immunogenicity. Ultimately, the value of a macromolecular imaging agent will be determined by its ability to leak into a tumor, be retained that called enhanced permeability and retention (EPR) effect, yet be cleared by the background organs as quickly as possible. This represents the supreme challenge of designing and testing larger molecular weight imaging agents.

TALK 2: ESTABLISHED PARTICLES AND POLYMERS

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LEARNING OBJECTIVES:

- Self-assembly of amphiphilic molecules and aggregate morphology
- Synthetic nanoparticles, natural nanoparticles, hybrid nanoparticles, complex structures
- One step synthesis methods
- Incorporation of imaging labels and/or nanocrystals
- Surface modification

The nanoparticle platforms that have been investigated most extensively for biomedical purposes are self-assembled nanoparticulate lipid aggregates. Liposomal nanoparticles have thus far been most successful, which has resulted in the FDA approval and clinical application of several liposomal formulations of cytostatic agents. Micelles and microemulsions are thoroughly investigated for the delivery of hydrophobic compounds, while lipids and other amphiphilic molecules can also function as coating for solid nanoparticles. The relative ease of preparation and functionalization, the possibility to create a variety of lipid aggregate morphologies, and importantly, the ability to combine multiple amphiphilic molecules with different functionalities are the most important reasons for the popularity of lipidic nanoparticles.

In the field of (molecular) imaging lipid-based nanoparticle platforms allow the inclusion of a variety of imaging agents ranging from fluorescent molecules to chelated metals for MRI and nuclear imaging, and nanocrystals, including QDs, FeO and gold. There are several methods to incorporate the aforementioned molecules and materials in lipidic nanoparticles. The most straightforward strategy is to use an amphiphilic version of the molecule of interest. This may be accomplished by conjugating acyl chains to e.g. a fluorescent dye or a metal chelator. In addition, small molecules that are inherently amphiphlic or lipophilic may spontaneously incorporate in lipidic nanoparticles. Amphiphiles tend to nestle and mix with the lipids, whereas lipophilic (or hydrophobic) compounds can be included in the core of micelles and microemulsions. The inclusion of hydrophobic compounds is not limited to small molecules only. Nanocrystals like QDs, capped with hydrophobic ligands, have also been demonstrated to efficiently incorporate in the core of micelles and microemulsions.

In the past decade a variety of multifunctional lipid-based nanoparticles for molecular imaging and targeted therapy have been developed and tested preclinically. These nanoparticles differ in size, morphology, multimodal imaging ability, and specificity for biological markers. In this educational the synthesis and characterization of different platforms as well as their application for multimodality (molecular) imaging and targeted therapy will be discussed. Functionalization methods, surface modification and the effect on pharmacokinetics are highlighted. In addition, recent developments in the area of endogenous nanoparticles, like lipoproteins and exosomes, modified to carry different diagnostically active materials for multimodality imaging will be discussed. Lastly, some key applications in experimental cancer and cardiovascular disease as well as a pathway for clinical translation will be shown.

TALK 3: ADVANCES IN PARTICLES AND POLYMERS

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LEARNING OBJECTIVES:

- Relative advantages of fluorescence, Raman optical imaging, and photoacoustic imaging
- Novel nanoparticles for optical and photoacoustic imaging
- Learn about applications of fluorescence, Raman, and photoacoustic imaging

The development of optical fluorescence, optical Raman, and photoacoustic imaging are allowing for rapid advances in the field of molecular imaging. Driving these changes are improvements in instrumentation, novel molecular imaging agents, and new small animal as well as clinical applications. This talk will review all of these strategies including the

use of novel nanomaterials for enhancing the signal from low copy number of target biomarker molecules. Understanding the physical principles of each of these imaging modalities to guide molecular imaging agent development will be stressed. The effect of imaging agent geometry on the desired signal will be detailed. The use of multiplexing to derive information from multiple biomarkers will be highlighted especially with the Raman based technique which may allow up to 10-20 simultaneous signals. Combining several of these modalities in order to take advantage of their relative strengths (e.g., depth penetration, multiplexing) will also be stressed. Several new clinical applications under exploration including for imaging of the gastro-intestinal tract and for intraoperative imaging will be discussed. Finally, future challenges for the field and areas in need of active exploration will also be highlighted.

My notes & questions

for the "MEET THE EXPERT SESSION" subsequent to this educational session on "CHEMISTRY OF CONTRAST MEDIA"

2 – Biology and Pathology

2A – CANCER BIOLOGY

chaired by Robert Gillies – Tampa, USA & Markus Rudin – Zuerich, Switzerland

TALK 1: IMAGE ANALYSIS AND INFORMATICS

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LEARNING OBJECTIVES:

- Describe the motivation underlying analyses of tumor heterogeneity
- Describe the role of Image 'omics in Oncology
- List the different levels of biomarker qualification

Image "Omics" involves the high throughput extraction of quantitative imaging features with the intent of creating mineable databases from images (1). Such profound analyses and mining of image feature data will reveal quantitative predictive or prognostic associations between images and medical outcomes. In cancer, current quantitative measurements are limited to dimensional measurements of tumor size via one (RECIST) or two (WHO) dimensional long axis measures (2). These measures do not reflect the complexity of tumor morphology or behavior, nor, in many cases, are changes in these measures predictive of therapeutic benefit (3). When additional quantitative measures are performed, they generally average values over an entire region of interest (ROI).

In focused studies, texture features have been shown to provide significantly higher prognostic power than ROI-based methods (4-7). This is reflective of the fact that tumors are highly heterogeneous systems, and that such heterogeneity has high prognostic power (8). Profound analyses of such image features can improve prediction of clinical CT (9), MR (10) or PET (11) images. Although paradigm-shifting, these analyses have been performed manually and the studies were underpowered. In order to qualify as a clinically useful biomarker, such studies have to be performed with larger cohorts in prospective, multi-institutional trials. In the current iteration of radiomics, image features have to be extracted automatically and with high throughput, putting a high premium on novel machine learning algorithm developments.

The goal of radiomics is to convert images to mineable data, with high fidelity and high throughput. The radiomics enterprise can be divided into five processes with definable inputs and outputs, each with its own challenges that need to be overcome: (i) image acquisition and reconstruction; (ii) image segmentation and rendering; (iii) feature extraction and feature qualification (iv) databases and data sharing; and (v) ad hoc informatics analyses (12). Each of these steps must be developed de novo and, as such, poses discrete challenges that have to be met. For example, optimum protocols for image acquisition and reconstruction have to be identified and harmonized. Segmentations have to be robust and involve minimal operator input. Features have to be generated that robustly reflect the complexity of the individual volumes, but cannot be overly complex or redundant. Informatics data bases that allow incorporation of image features and image annotations, along with medical and genetic data have to be generated. Finally, the statistical approaches to analyze these data have to be optimized, as radiomics is not a mature field of study. Variation in results may come from variations in any of these individual processes. Thus, after optimization, another level of challenge is to harmonize and standardize the entire process, while still allowing for improvement and process evolution.

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TALK 2: BIOMARKERS - ONCOLOGY AND INFLAMMATION

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LEARNING OBJECTIVES :

- Understanding the concept of biomarkers
- Hallmarks of Cancer

In a seminal paper, Hanahan and Weinberg [1] defined characteristics of cancerous tissue. Those included insensitivity to antigrowth signal, self sufficiency in growth signals, limitless replication potential, evasion of apoptosis, angiogenesis and infiltration and metastasis formation. This list was later extended to include deregulation of cellular energetics, avoidance of immune destruction, genomic instability and tumor promoting inflammation [2]. Many of these phenotypic hallmarks can be assessed using imaging, which is attractive both with regard to improved diagnosis as well as for therapy management complementing the established structural readouts based on computer tomography (CT) and magnetic resonance imaging (MRI). While clinical diagnosis is commonly based on qualitative interpretation of imaging data, assessment of therapy response relies on quantitative readouts. In oncology response criteria for solid tumors (RECIST) have been established and recently revised [3]. Yet, structural readouts are in general late indicators of response. In contrast, it has been shown that physiological and metabolic tissue parameters are highly susceptible indicators of the tissue state and can be used as biomarkers of prognostic quality. Such biomarkers are of course of high interest for therapy evaluation and management. Three potential readouts which are currently being used in this context are positron emission tomography in combination with 2-[^{18}F]-fluoro-2-deoxyglucose (FDG-PET) to assess glucose utilization, dynamic contrast enhanced MRI (DCE-MRI) to assess vascular leakage as measure of angiogenesis, or PET using [18F]-fluoro-thymidine to assess proliferation.

. Commonly, such biomarkers do not substitute for a clinical endpoint, which might be tumor shrinkage or patient survival, but rather provide early information that the patient is responding to treatment. They are typically used in proof-of-concept studies, clinical studies with a small group of patients demonstrating the validity of the pharmacological strategy/mechanism. For example, it has been shown with FDG-PET studies that treatment of patients suffering from gastro-intestinal stromal tumors with a tyrosine kinase inhibitor [4] or of melanoma with B-raf inhibitor [5] led to significant reduction of tumor glucose utilization within days after treatment onset. Similarly, it has been shown that anti-angiogenic drugs reduce tumor vascular permeability with a few days of treatment [6].

Critical aspects in biomarker studies are aspects such as robustness, accuracy, and reproducibility, implying a careful validation of the imaging approach. Given the heterogeneity of the patient population the typical study design consists of pre- and post-treatment measurements in the same individual. Recording two baseline data sets is strongly recommended. Also, proof-of-concept studies may be conducted at different sites which demands for stringent standardization of data acquisition and analysis procedures as well as rigorous quality assurance/quality control in order to deliver comparable results. Guidelines worked out by consortia of stakeholders should warrant this (for DCE-MRI see [6]). Of course these guidelines are under constant revision as novel opportunities emerge, e.g. the implementation of analysis methods that explicitly account for tumor heterogeneity.

The availability of validated biomarkers providing early information on potential outcome will be of tremendous value for patient management and will become an indispensible tool for the treating physician and for the therapy developer.

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TALK 3: ONCOLOGY AND INFLAMMATION

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LEARNING OBJECTIVES

- Understanding the role of inflammation in the cancer phenotype
- Imaging phenotypic changes induced by inflammatory pathways in cancer
- Image-guided targeting of inflammatory pathways in cancer

Inflammation is a characteristic response of living vascularized tissue to injury and induces the formation of eicosanoids. Three phospholipases, phospholipase A_2 (PLA_2), phospholipase C (PLC) and PLD, participate in the formation of free arachidonate from membrane phospholipids in response to mechanical, chemical and physical stimuli. The arachidonic acid (AA) formed is converted to various eicosanoids by the action of lipoxygenases (LOX) and cyclooxygenases (COX) that impact on cell motility, invasion, vascular characteristics and metastatic dissemination. Most solid tumors, including breast cancers, exhibit inflammatory properties characterized by increased levels of prostaglandins and other proinflammatory molecules that are secreted by tumor cells, stromal cells, and specialized immune cells during inflammation. Such an upregulation of inflammatory characteristics is not surprising in view of the similarities between physiological conditions in injured tissue, such as hypoxia and low extracellular pH (pHe), and the physiological environment of solid tumors. COX-1 and COX-2 are cytoplasmic enzymes that convert PLA_2-mobilized AA into the lipid signal transduction molecules prostaglandins and thromboxanes. One major product of the COX-2–catalyzed reaction is prostaglandin E_2 (PGE_2), an inflammatory mediator participating in several biological processes, including development, pain, immunity and angiogenesis, and cancer. COX-2 function has been the target of pharmaceutical intervention in a multitude of widespread degenerating conditions, including autoimmune diseases, gastric inflammation, and several different cancers, such as gastric, lung, breast, and colon cancer. Its expression is induced by proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and its promoter contains a cyclic AMP response element, a nuclear factor-κB binding site, and two nuclear factors for IL-6 target sequences. The activation of several genes that form the adaptive response of cells to hypoxia is mediated through the binding of HIF-1 to HRE that regulate the transcription of these genes. Under oxygenated conditions HIF-1 is rapidly degraded but under hypoxic conditions HIF-1 is stabilized. In addition to the oncogenic effects of PGE_2 in COX-2 expressing tumors, PGE_2 may also act through the HIF-1 axis with the multitude of effects such as drug resistance, increased invasion, increase of Chk, and the emergence of an aggressive phenotype even under well oxygenated conditions. Molecular and functional imaging provide a range of abilities to investigate the role of inflammation in cancer with a span that covers intact cells in culture to preclinical models to clinical translation. These opportunities will be discussed within the context of quantitative imaging data and data analysis. Examples will be provided of insights into oncology and inflammation obtained with multi-modal molecular and functional imaging and their potential for image-guided treatment strategies.

2B – CARDIOVASCULAR DISEASES

chaired by René Botnar – London, UK

TALK 1: INTRODUCTION TO BIOLOGY AND TREATMENTS - ATHEROSCLEROSIS

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LEARNING OBJECTIVES:

- Understand the key biological processes involved in atherosclerosis
- Learn about promising approaches for targeted molecular imaging of atherosclerosis
- Understand the main treatments for atherosclerosis – from statins to stents

Atherosclerosis is the primary underlying cause of cardiovascular disease (heart attacks, strokes, and peripheral vascular disease) and involves multiple cell types and biological processes. It typically begins in early adulthood and progresses silently over decades, but can present suddenly with high morbidity and mortality. A multitude of risk factors (high cholesterol, cigarette smoking, hypertension, diabetes) contribute to injury and inflammation in the blood vessel wall, resulting in endothelial dysfunction, lipid deposition, macrophage accumulation, and smooth muscle cell proliferation. This atherosclerotic plaque formation can progress, initially with preservation of the vessel lumen due to outward vascular remodeling. More advanced plaques can develop necrotic cores, neovascularization, intraplaque hemorrhage, and calcification. More biologically active (so-called “vulnerable”) plaques are prone to disruption of the fibrous cap separating the lumen from the necrotic core, resulting in thrombosis/occlusion of the vessel lumen, inducing downstream ischemia and organ cell death (infarction).

Molecular imaging approaches have aimed at detecting these biological processes, including early endothelial activation, macrophage infiltration, proteolytic activity, angiogenesis, and thrombosis. The primary treatment is prevention through regular physical activity and a heart-healthy diet. In patients at increased risk and with elevated cholesterol, statin therapy has been shown to regress atherosclerosis (modestly) and prevent heart attacks and strokes (substantially). Aspirin and other platelet inhibitors are used in high-risk patients to reduce thrombosis and thus reduce heart attacks and strokes. In patients with advanced plaques that narrow the lumen enough to cause symptoms, stents can be placed to restore normal blood flow or surgery can be performed to bypass the narrowings (coronaries) or remove plaque (carotids).

The ongoing challenge in atherosclerosis has been to identify more precisely the patients (and their plaques) that are at high risk for near-term clinical events in order to optimize preventive therapy. Additionally, molecular imaging could provide crucial feedback that the therapeutic interventions chosen for a specific patient have converted their atherosclerotic plaques to a more “benign” phenotype.

TALK 2: INTRODUCTION TO MR AND PET IMAGING - ATHEROSCLEROSIS

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LEARNING OBJECTIVES:

- Understand basic MR sequences for atherosclerosis imaging
- Understand basic contrast mechanisms in MRI (T1, T2, proton density, magnetization transfer contrast)
- Understanding basic principle of contrast enhanced MRI (inversion recovery, Look Locker, T1 and T2/T2* mapping, positive contrast)
- Understand basic motion compensation approaches
- Understand basic PET quantification and partial volume correction

Despite advances in diagnosis and treatment complications due to atherosclerosis remain the number one killer in the western world and developing countries. Current diagnostic tests focus on the detection of lumen stenosis either using angiography or by measuring myocardial or cerebral blood flow but have failed to predict future cardiovascular events. This is because most myocardial infarctions and strokes are caused by rupture of an unstable atherosclerotic plaque and often occur suddenly in patients without previous symptoms of cardiovascular disease. Autopsy studies have shown that features characteristic for disrupted plaques include a thin heavily inflamed fibrous cap, a large lipid core, large plaque burden, expansive positive remodeling, neovascularization and intraplaque hemorrhage. Other features include dysfunctional endothelial function, endothelial activation, inflammation, extracellular matrix remodeling, hypoxia, activation of proteases and endothelial erosion. To detect these vessel wall changes several new imaging techniques have been developed with MRI and PET being one of the most promising non-invasive approaches. In this lecture the basic principles of MR and PET imaging of atherosclerosis will be introduced. This will include measurement of tissue properties by use of MR prepulses, visualization and quantification of MR contrast agents, discussion of the basic principles of MR contrast agents and concluding with discussion of motion correction techniques. PET imaging of atherosclerosis will be only touched on briefly, discussing signal quantification, partial volume correction and the basic differences between PET tracers and MR contrast agents.

TALK 3: INTRODUCTION TO MOLECULAR CONTRAST AGENTS AND NEW DEVICES – ATHEROSCLEROSIS

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LEARNING OBJECTIVES:

- Describe the role of molecular imaging in diagnosis and therapy of atherosclerosis
- Describe target-specific contrast agents for key processes in plaque formation, destabilization and rupture

Many mechanisms are known to play a role in the development of atherosclerosis and the associated thrombotic clinical events. These insights have led to significant improvements in disease outcome, as exemplified by the development of statin therapy. Nevertheless, cardiovascular disorders remain a prime cause of death worldwide. Conventional anatomic imaging techniques (such as X-ray contrast angiography for assessing arterial stenosis and MRI for visualizing plaque dimensions) have limited utility in atherosclerosis diagnostics, as lesions that do not cause a flow-limiting stenosis are the main cause of fatal myocardial infarctions. Thus, there is a pressing need for imaging methodologies that go beyond stenosis visualization and are capable of informing specifically on cellular and molecular processes that govern atherosclerotic lesion development. In recent years, molecular imaging has emerged as a powerful tool to assess biological aspects of atherosclerotic plaques that are not captured by anatomical imaging (for excellent recent reviews see Refs. 1 and 2). This educational lecture highlights the basics of molecular and cellular imaging as applied in the setting of atherosclerosis research, the key requirements in terms of contrast agents, as well as the relative strengths of the major imaging methods that are used in this field.

Molecular and cellular imaging of atherosclerosis exploit all major imaging modalities [2], *i.e.* X-ray CT, PET, SPECT, MRI, optical imaging and contrast-enhanced ultrasound, each presenting inherent strengths and weaknesses. The nature, location and abundance of the molecular and cellular targets to a large extent determine the types of contrast agent as well as the imaging modalities that are most effective. As an example, the activation of endothelial cells lining arteries is associated with an increased expression of adhesion molecules, such as VCAM-1 and ICAM-1, which are involved in the initiation of local inflammation and therefore are considered important targets of atherosclerosis molecular imaging. Ligands for targeting these factors include peptides and antibodies. All major imaging modalities have been used to probe adhesion molecule up-regulation in atherosclerosis. Nanoparticle contrast agents have proven very effective [3], as they are largely restricted to the blood pool such that there is little background signal from contrast material that has permeated (non-target) tissue beyond the vasculature. Another benefit of nanoparticles is they carry a high payload of contrast material, thus enabling the visualization of low levels of target expression. A third virtue of nanoparticles is that they are also well suited as drug carrier devices for theranostic approaches [1]. Nanoparticle contrast agents that have been successfully used include iron oxides for MRI, lipid-based materials such as liposomes and micelles for MRI and nuclear imaging, as well as micro-bubbles for contrast-enhanced ultrasound. Several examples of other contrast agent-imaging modality combinations will be given to illustrate their use for the detection of key features of atherosclerotic lesions, including the presence of inflammatory cells, metabolic activity, cell death, oxidative stress, extracellular matrix components and thrombus formation.

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2C – CENTRAL NERVOUS SYSTEM

chaired by Doris Doudet – Vancouver, Canada

TALK 1: PATHOPHYSIOLOGY AND IMAGING OF NEURODEGENERATIVE DISEASES

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LEARNING OBJECTIVES:

- Overview on current imaging techniques related to the pathophysiology of human neurodegenerative disease
- Insight into the perspectives and challenges for imaging in translational research

Most neurodegenerative diseases are characterized and probably caused by pathological protein depositions. The most frequent is amyloid deposition in Alzheimer's disease (AD), which can now be imaged clinically with specific PET tracers, including 11C-PIB, 18F-PIB (flutemetamol), and 18F-florbetapir. Other pathological proteins are phosphorylated tau in neurofibrillary tangles (AD) and Pick bodies (Frontotemporal Dementia), and alpha-synuclein in Parkinson's disease (PD) and Dementia with Lewy bodies (DLB). Tracers for these are under development. PET and SPECT imaging techniques have also been developed for impaired neurotransmitter systems in PD and DLB (dopamine synthesis, transport and vesicular storage; vesicular monoamine transporter) and AD and DLB (acetylcholine esterase, nicotinic receptors). Changes in functional activity and connectivity are being imaged by FDG PET, techniques for blood flow measurement (PET, SPECT, ASL), fMRI (activation and resting state studies), MR tractography, EEG and MEG. Most techniques are applicable to experimental animals and humans, thus providing essential tools for translational research and "reverse translation" by stimulating experimental research on issues detected primarily in human disease.

TALK 2: NEUROPLASTICITY

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LEARNING OBJECTIVES:

- Convince the audience how in vivo imaging tools could increase our understanding of neuroplasticity.
- Learn the audience which imaging tools visualizes which phenomena/aspects of neuroplasticity.

The brain's ability to act and react in ever-changing ways is known as neuroplasticity. This special characteristic allows the brain to constantly modify the pathways for neural communication throughout life, thereby aiding the processes of learning, memory, adaptation through experience and adjusting to a new environment. While plasticity enables the brain to recover from injuries (such as stroke or brain trauma) or overcome cognitive disabilities it can also disable the normal functioning of the brain as it can lead to abnormal wiring of certain brain circuits leading to disorders such as epilepsy, schizophrenia and autism spectrum disorders.

The term neuroplasticity clearly captures a wide diversity of phenomena at the neuronal level ranging from activity-dependent changes in synaptic physiology (e.g., Long Term Potentiation and Long Term Depression) to morphological changes which include pine formation, dendrite branching , axonal outgrowth and even generation of new neurons, finally resulting in neuronal circuitry modulations. Recent evidence indicates that genetic but also epigenetic mechanisms may serve as a contributing mechanism in memory formation and storage as well as neuronal plasticity. Understanding these mechanisms may be relevant for healthy brain plasticity but also more generally in the control of a variety of clinical conditions such as Alzheimer's disease, depression, anxiety, schizophrenia, just to mention a few.

Some of the most dramatic examples of plasticity in brain structure and function have been identified in the neural structures that control vocal production in songbirds. The brain of songbirds (oscines) displays an unusual plasticity both during ontogeny and, for most species, across seasons. This plasticity directly relates to the acquisition and expression of songs and thus provides a novel and useful model for understanding the neural plasticity and relationships between perception, cognition, behavior, and the underlying cellular and molecular processes in the nervous system. For all these reasons song birds have become THE best model for studying neuroplasticity.

In this lecture we will demonstrate how different aspects and diverse phenomena of neuroplasticity such as dendrite branching, axonal outgrowth, generation of new neurons and the resulting modified neuronal circuitry can be assessed or monitored in time using different in vivo imaging techniques. The capabilities of these imaging tools (mainly MRI and BLI) will be demonstrated in songbird studies as an unusually auspicious model of neuroplasticity and cognition/learning/memory but also in mice and rat models for different neuropathologies. The MRI focus will be on manganese enhanced (ME) MRI, Diffusion sensitive MRI (DTI and DKI), Resting state functional (rsf)MRI and functional (f)MRI but also on BLI using LV transfection of neurogenic niches in the adult brain. This should convince the audience how in vivo imaging tools could increase our understanding of neuroplasticity in the first place but also how it could contribute in monitoring the loss but also the recovery of connections because of trauma or disease.

TALK 3: PATHOPHYSIOLOGY AND IMAGING OF STROKE

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LEARNING OBJECTIVES:

- The audience will learn about the pathophysiological cascade of events following a stroke
- The audience will learn to discriminate the different relevant phases of pathophysiological developments in the acute, subacute and chronic time window following the ischemic incident
- The audience will learn the prerequistes to study stroke and to assess therapeutic strategies in the experimental field, with focus on translational relevance. This includes animal models, experimental monitoring of animal status, tool to investigate various aspects, and assessment of therapeutic effectiveness of approaches

The different forms of stroke – ischemic and hemorrhagic – will be discussed and their clinical relevance. This will have substantial influence on the optimal choice of animal model for the investigation on ideal treatment. Directly following the primary event of an occluded (ischemic case) or ruptured (hemorrhagic case) vessel, a series of pathophysiological events take place being the cause for the final tissue loss and the clinically relevant functional deficit. These cascades of events are divided into various phases, beginning with the acute phase during which the metabolic disturbance will lead to anaerobic glycolysis, tissue acidosis, and finally electric silence of the neuronal cellular network. This will result in tissue necrosis (and apoptosis to minor extent) within several hours if reperfusion and nutrient supply are not rapidly restored. The second phase includes secondary toxic events caused by toxic molecules as result of the anaerobic metabolic disturbance and intracellular imbalance. The next phase lasts for 1-2 weeks and is best characterized by inflammation as response to the tissue loss. Macrophages from the vascular system will invade the lesioned territory, followed by activated microglia to take care of the dead cell debris and to seal the lesion against the healthy tissue by a gliosis scar reaction. In a later phase, a second round of inflammatory reaction is discussed, considered to be predominantly constructive and supportive in regenerative processes. This leads to the final phase, the potential regeneration phase. Here we will discuss mechanisms of action, both of endogenous processes as well as therapeutic approaches. Of course, also a delayed destructive process is still active during this time window: contributions from delayed retrograde degeneration which will affect remote tissue areas originally untouched by the primary ischemic event.

We will present animal models best suited for the investigation of appropriate therapeutic interventions to these above mentioned phases of pathophysiological events. In this context, the tools to describe, monitor and assess the various steps of pathophysiological events will be presented, with focus on using invasive and noninvasive imaging modalities, doing justice to the fact that stroke represents a regionally heterogeneous process which consequently requires a region-resolved characterization of the relevant processes.

Finally, assessment of functional deficit and functional improvement will be discussed in the quest for outcome improvement during therapeutic interventions. Furthermore, the pitfalls in the assessment of functional improvement in the animal model will be discussed when using behavioural test batteries. Here, often times, care is needed to distinguish between compensation for lost function and true functional improvement. Here again, the role of noninvasive imaging, particularly fMRI and functional connectivity fMRI will be presented.

2D – SYSTEMS BIOLOGY AND ITS LINK TO MI

chaired by Hermann-Georg Holzhütter – Berlin, Germany

SYSTEMS BIOLOGY OF SINGLE CELLS

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LEARNING OBJECTIVES:

- understanding the background and motifs for the establishment of systems biology as a new research field in life sciences
- getting familiar with specific methods and approaches used in cellular systems biology
- conceiving the enormous potential of cellular SB for biotechnological purposes and the prevention, diagnosis and treatment of diseases

This talk introduces systems biology (SB) as a new fascinating research field in life sciences. SB aims at integrating observations and measurements gathered at various levels of cellular and organismic organization into holistic paradigms. The final goal of SB of humans is to decipher the complete causal chain of molecular events by which genomic information and environmental conditions of an individual translate into features on the phenotype level such as, for example, aging, susceptibility to certain diseases or the efficacy and side effects of a special medications. In this first lecture of a planned series of lectures on SB, I will focus on SB at cellular level thereby outlining novel techniques and approaches that have evolved during the past 15 years to monitor cellular processes and structures simultaneously at all hierarchical levels of organization and to consistently lump together this wealth of information by the use of complex mathematical models. I will demonstrate how the enormous progress in the development and improvements in the spatial resolution and acquisition frequency of imaging techniques like confocal microscopy have paved the way for studying the dynamics an d structural endowment of single cells.

My notes & questions

for the "MEET THE EXPERT SESSION" subsequent to this educational session on "BIOLOGY & PATHOLOGY"

3 – What life scientists should know about imaging modalities

3A – OPTICAL IMAGING, ULTRASOUND, AND PHOTOACOUSTICS

chaired by: Jorge Ripoll – Madrid, Spain & Georg Schmitz – Bochum, Germany

TALK 1: PHYSICS OF ULTRASOUND IMAGING

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LEARNING OBJECTIVES:

- Gain basic understanding of ultrasound B-mode imaging
- Ability to select correct settings for imaging
- Understand resolution, penetration depth and artifacts
- Realize advantages and drawbacks in comparison to other modalities
- Get an understanding of new development trends in ultrasonic imaging

Owing to the very sensitive detection of microbubble contrast media, ultrasonic imaging has become a modality for molecular imaging. Additionally, the new imaging modality of photoacoustic imaging is also based on ultrasound detection and allows to image dye or nanoparticle contrast media as well as metabolic changes like oxygen saturation. With the availability of high-frequency small animal ultrasound scanners, B-mode and contrast imaging have become important research tools.

The current talk gives an introduction to the underlying principles of ultrasound imaging used in clinical scanners and laboratory small animal scanners. The physics of mechanical waves in the ultrasound range are discussed first avoiding too much mathematical detail. While the pulse/echo-principle in ultrasound is easy to understand, the scanner properties influencing the resolution and contrast of the images are often less well understood. The talk focusses on presenting the basic relationships in image formation that explain image quality differences e.g. between different systems. Typical artifacts – for example the speckle pattern disturbing ultrasound images – are explained and demonstrated.

A major component of the image formation is ultrasound array beamforming: traditionally, the ultrasound system addresses a one-dimensional array of ultrasound transducers in the scan-head with different amplitude and delay to focus the sound beam along one scan-line, which is then moved electronically. This principle will be discussed together with new acquisition schemes needed for real-time three-dimensional imaging with two-dimensional arrays, e.g. in current cardiovascular ultrasound systems.

Application examples for anatomical and molecular imaging with microbubble contrast media will be given and discussed. Additionally, an overview of novel developments coming to the clinical and preclinical ultrasound systems will be given and used to apply what has been learned in the first part of the talk.

TALK 2: ADVANCED MICROSCOPY TECHNOLOGIES

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LEARNING OBJECTIVES:

- derive what limits the resolution in microscopy
- list several high- and super-resolution techniques
- discuss pros and cons of advanced fluorescence microscopy techniques

More than 100 years ago, Ernst Abbe has theoretically shown that diffraction of the detected light in any optical microscope fundamental limits the resolution to about half the wavelength used for imaging. Using visible light (with a wavelength between 400 and 700nm), fine object structures below 200nm could not be resolved in these instruments. In recent years however, various high- and super-resolution microscopy devices have been developed in order to measure and visualize structural details of fluorescent objects with unprecedented quality down to the nanometer range. Starting with considerations of light as a wave and of its propagation, we will take a close look at some of the optical elements that make up a microscope in order to get an idea of the image formation process. We will then point out which parts are limiting the resolution of the final images, and what physical effects can be used to overcome this limitation. Several examples of high- and super-resolution microscopes will be given with an explanation as to how each of these particular microscopic techniques circumvents the classical resolution limit. Example applications of these microscopes to biological specimens will be shown, illustrating the enormous progress that far field optical microscopy has recently made.

TALK 3: OPTICAL TOMOGRAPHY

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LEARNING OBJECTIVES:

- Basis of Scattering and absorption
- Light propagation and the role of scattering
- Approaches to 3D optical imaging

During this talk we shall first discuss the basis of absorption and scattering, with emphasis on the optical properties of tissue. Once this has been covered, we will analyze the basis of light propagation and study it in the context of scattering where two extremes can be identified: ballistic propagation, the approach used in microscopy and the basis of direct imaging; and diffuse propagation, the approach used in diffuse optical tomography in which case we need to resort to indirect imaging. Once these two extremes have been identified, we shall cover different approaches currently being used for 3D optical imaging in-vivo based on the different contribution of scattering.

TALK 4: PHOTOACOUSTIC IMAGING

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LEARNING OBJECTIVES:

- Understand the fundamental principles of photoacoustic imaging
- Knowing major components of photoacoustic imaging system
- Knowing how photoacoustic images are from and how to interpret photoacoustic images
- Understand how imaging contrast agents assist contrast and penetration depth in photoacoustic imaging
- Understand the ability of photoacoustic imaging system to visualize anatomical, functional and molecular properties of imaged tissue
- Identify the role of photoacoustic imaging in basic science and clinical applications

Photoacoustic (also known as optoacoustic and, more generally, thermoacoustic) imaging or tomography – a non-ionizing, non-invasive, real-time imaging technique capable of visualizing optical absorption properties of tissue at reasonable depth and high spatial resolution, is a rapidly emerging biomedical and clinical imaging modality. Photoacoustic imaging is regarded for its ability to provide in-vivo morphological and functional information about the tissue. With the recent advent of targeted contrast agents, photoacoustics is capable of in-vivo molecular imaging, thus facilitating further molecular and cellular characterization of tissue.

This presentation is designed to provide both a broad overview and a comprehensive understanding of photoacoustic imaging, sensing, and spectroscopy. With a brief historical introduction, we will examine the foundations of photoacoustics, including discussion of governing equations. We will also review relevant optical/acoustic properties of the tissues and the related topics of laser-tissue interaction. The experimental aspects of photoacoustic imaging will then be discussed with emphasis on instrumentation, i.e., system hardware and signal/image processing algorithms. Specifically, penetration depth and spatial/temporal resolution of photoacoustic imaging will be anlyzed in relationship to a laser source, an ultrasound transducer and other components of the photoacoustic imaging system. Integration of photoacoustic and ultrasound imaging systems will be discussed. Techniques to increase contrast and to differentiate various tissues in photoacoustic imaging will be presented including image reconstruction algorithms. Furthermore, design, synthesis and optimization of imaging contrast nanoagents to enable molecular/cellular photoacoustic imaging will be presented. Special emphasis will be placed on contrast agents capable of multiplexed imaging, multi-modal imaging and image-guided therapy including drug delivery and release. The presentation will continue with an overview of several commercially available and experimental systems capable of photoacoustic imaging. Regulatory aspects of photoacoustic imaging systems and imaging contrast agents will be presented. Finally, current and potential biomedical and clinical applications of photoacoustics will be discussed.

3B – MR FUNDAMENTALS FOR LIFE SCIENTISTS

chaired by Joseph Ackerman – St. Louis, USA & Michal Neeman – Rehovot, Israel

TALK 1: INTRODUCTION TO MR PHYSICS

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LEARNING OBJECTIVES:

- Understanding of the underlying physics of MRI
- Familiarity with the main components of an MRI scanner
- Introduction to the basics of image acquisition

The MRI technique depends on the phenomenon of Nuclear Magnetic Resonance (NMR) which, as the name suggests, is based on a fundamental magnetic property of certain atomic nuclei and their resonant interaction with externally-applied electromagnetic photons. The use of the word “resonant” is critical in this context, and refers to the fact that the energy of the photons must be very exact for the interaction to occur. The specific energy required is governed by the properties of the nuclei in question (usually hydrogen) and the magnetic field in which the sample containing these nuclei are situated (usually several Tesla in magnitude). Thus we may “excite” a sample of, say, water (contains many 1H nuclei) by irradiating the sample with photons with energies in the radiofrequency range; the trick is then to observe how the signal we measure decays over time, and also to observe how the sample relaxes back to its unexcited, equilibrium state. These decay and relaxation times are exquisitely dependent on the molecular environment within each tissue type, and hence we can design experiments wherein we use these inherent tissue properties to determine the magnitude of our signal and ultimately to produce images with a wide variety of contrasts (for example, to enhance white matter signal over grey matter, etc.).

Although the NMR phenomena has been known to physicists and chemists since the 1950’s, it was not until the 1970’s that the idea to adapt it to imaging was conceived. The core concept enabling us to spatially-localise the measured signal involves the application of additional magnetic fields whose magnitude varies with position – the so-called “magnetic field gradients” – which add to the existing large static magnetic field, effectively rendering the resonant condition dependent on position in 3D space. This allows us to selectively measure a signal from every position within the sample, a process which requires the repetitive application of gradients of varying strength and direction, typically resulting in image acquisition times of many tens of seconds (although sub-second scans are also possible, albeit with lower image quality).

The inherent dependence of the measured signal on the molecular properties of the constituent tissues in the sample enable us to design experiments, so-called imaging pulse sequences, to acquire images with contrast influenced or weighted by factors such as tissue density, the ability of water molecules to diffuse through tissue, the flow of blood through large vessels or its perfusion through tissue, the blood oxygenation level, the local temperature and/or pH levels, and many more physical mechanisms.

This talk with provide an introduction to the world of MRI, explaining some of the underlying physics of the NMR phenomenon, giving a brief overview of the main components in a modern scanner, and describing the basics of image acquisition.

TALK 2: INTRODUCTION TO MR HARDWARE

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LEARNING OBJECTIVES:

- the basic principles of MRI RF-coil function
- the main facts in SNR optimization
- different application adapted coil designs
- and the issues when reducing coil sizes towards animals and microscop

This lectures aim is to give an overview on RF coils in MRI. It will cover the basic physics of LC circuits in strong magnetic fields, the principles of sample excitation and signal reception as well as issues determining and optimizing the achievable SNR. The most common coil geometries including phased arrays with their design trade-offs, advantages and limitations for specialized applications will be introduced. Furthermore the miniaturization of MRI RF-coils from man to mouse and further to MR microscopy will be discussed in terms of design and SNR optimization

TALK3: CONTEMPORARY MR: PUSHING THE LIMITS

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Learning objectives:

- Understand the importance of contrast in MR imaging.
- Describe T1, T2,and T2* relaxation phenomena and how they are measured.
- Understand relaxation as a source of image contrast.
- Understand the role of magnetization exchange in relaxation and as a source of contrast (MT).
- Describe more technically advanced, endogenous-contrast experiments (diffusion, perfusion, flow, BOLD, CEST).
- Describe the role of exogenous contrast agents.
- Describe more technically advanced, exogenous-contrast experiments (DCE, DSC, ParaCEST).

The vast majority of clinical magnetic resonance (MR) imaging experiments involve 1H imaging of the water, which is ubiquitous in biological tissue. Water has a wide variety of biophysical magnetic signatures that are characteristic of specific tissues and organs. The exquisite sensitivity of water's MR properties to its local environment can be used to enhance image contrast and provide detailed structural information. MR image contrast distinguishes organs and soft tissues and helps to identify normal vs. abnormal, healthy vs. damaged, and viable vs. pathologic tissue. The key to tissue MR contrast is effective encoding of the physical properties of tissue into the MR image.

The question of which physical property to encode often reduces to questions of "What are we trying to measure?" and "What MR property generates the best contrast?" Herein, we will discuss different MR contrast mechanisms, including longitudinal relaxation (T1), transverse relaxation (T2 and T2*), diffusion, velocity (perfusion and flow), and blood oxygen level dependence (BOLD) MRI, the mechanism that serves as the underpinning for functional MRI. We will explore the role of exogenous agents in generating contrast and describe their use in dynamic MR experiments that can provide measures of perfusion and vascular permeability.

We begin with a phenomenological discussion of T1 and T2 relaxation and a description of how the corresponding relaxation rate constants, R1 (=1/T1) and R2 (=1/T2), are measured. The manner in which tissue differences in R1 or R2 can provide a source of image contrast will be illustrated. Exchange processes are central to MR and MR relaxation. Contrast arising from magnetization transfer (MT), in which 1H magnetization is exchanged between macromolecules not visible by MR and mobile water molecules, will be discussed. We will then describe the basic role of MR contrast agents. Built around paramagnetic centers, MR contrast agents are fundamentally different than optical tracers or PET probes, in that the agents themselves are never directly observed. Instead, MR contrast agents function by changing the 1H relaxation properties in nearby water molecules. The consequences of such indirect observation of these agents in terms of contrast and quantitative detection and modeling will be explored. Examples of both T1 agents (e.g., Gd-based), which brighten images, and T2/T2* agents (e.g., iron oxides), which darken images, will be provided. The use of contrast agents in a static mode – inject an agent and wait for it to be distributed within the body – provides a powerful and versatile source of contrast. However, these same agents can also be used in a dynamic mode to provide information about vascular structure and function. Two such experiments, dynamic contrast enhanced (DCE) and dynamic susceptibility contrast (DSC) MRI, will be described. Both DCE and DSC experiments begin with injection of a bolus of contrast agent – the DSC experiment focuses on measuring tissue perfusion, while DCE highlights both perfusion and vascular permeability (vessel "leakiness"). The use of iron-oxide contrast agents to label and track cells will also be reviewed.

3C – NUCLEAR IMAGING: PHYSICAL PRINCIPLES AND INSTRUMENTATION

chaired by Steven Meikle – Sydney, Australia

TALK 1: PRINCIPLES OF PET AND SPECT

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LEARNING OBJECTIVES:

- Be able to explain the tracer principle and its importance in PET and SPECT molecular imaging.
- Be able to explain to your peers how radiation emitted from the body is detected externally using SPECT and PET instrumentation.
- Be able to explain the key principles in forming a reconstructed image of the tracer distribution in the body.

This unit of study explores the principles and methods that underpin two key molecular imaging techniques based on the radioactive tracer principle: single photon emission computed tomography (SPECT) and positron emission tomography (PET). Topics covered include the radioactive tracer principle, radioisotope production and decay, radiation transport in tissue, radiation detection, PET and SPECT instrumentation and tomographic image reconstruction. On completion of this lecture, students will have a basic understanding of the imaging chain as it relates to PET and SPECT, starting with the emission of radiation in the body, leading to its external detection and, finally, a reconstructed image of the radioactive tracer distribution in the body. The factors affecting the accuracy and noise properties of molecular images will be briefly explored. Students will also have an appreciation of how to use these imaging technologies to exploit the properties of the radioactive tracer principle and make estimates of important physiological parameters.

TALK 2: DETECTOR TECHNOLOGIES

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LEARNING OBJECTIVES:

- To obtain a basic understanding of the physical principles of radiation detection
- To learn the essential principles of inorganic scintillators and their most important properties
- To acquire basic knowledge of photosensors and readout electronics relevant to radiation detection

This lecture provides a basic introduction into the physical principles of the detection of gamma radiation in nuclear medicine imaging (in particular PET and SPECT). The emphasis lies on scintillation detectors, which are the most commonly applied type of detector. The interaction of the gamma quanta with the scintillation material forms the basis for the signal formation. Therefore, a basic understanding of the different types of interaction that may occur is required to understand the operation and performance of a scintillation detector. Furthermore, the physics underlying the conversion of the energy of a gamma quantum into scintillation photons will be briefly discussed. The next step in the detection chain is the conversion of the relatively weak light signal emitted by the scintillator into an electronic signal by means of a photosensor. An important aspect of detector design is the optimization of the crystal-photosensor geometry in order to achieve a good balance between multiple and often conflicting requirements on the detector performance at reasonable costs. The classical and still most widely used photosensor is the photomultiplier tube (PMT). For a long time, the relatively large internal gain and low noise of these devices made them the first, if not only, choice for the detection of very small amounts of light, down to the single photon level. However, advances in semiconductor technology have recently given birth to several new types of low-level light sensor, some of which have distinct advantages compared to PMTs for certain applications. Examples include avalanche photodiodes (APDs) and silicon photomultipliers (SiPMs). These solid state devices enable new crystal-sensor geometries, as well as new combinations of imaging modalities such as PET and MRI in highly integrated multimodality systems. For optimum detector performance, the use of dedicated front-end electronics, adapted to the specific properties of the scintillator-photosensor combination, is paramount. The readout electronics and data acquisition (DAQ) architecture furthermore need to be tailored to the imaging modality and application, as the corresponding requirements may vary greatly. The overall detector performance, expressed in terms of parameters such as spatial resolution, energy resolution, timing resolution, and detection efficiency, is the result of the design choices made with respect to each of the above components making up the detection chain (scintillation material, photosensor, detector geometry, electronics). Since the performance of the detectors impose ultimate limits on the image quality achievable with any scanner, it is not surprising that many research groups work on new and better detectors. Some of these recent developments will be briefly highlighted in this lecture.

TALK 3: HYBRID IMAGING SYSTEMS

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LEARNING OBJECTIVES:

- To become acquainted with a range of hybrid imaging methods, their rationale and principles.
- To develop an appreciation of the applications of clinical and preclinical hybrid imaging.
- To gain an understanding of technical challenges facing designers of hybrid imaging systems, and recent technological innovations.
- To be familiar with potential sources of artifacts in hybrid imaging and how they may be counteracted.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are nuclear imaging modalities that provide unique spatial and temporal information about biochemical, functional, and molecular processes *in vivo*. Both are used extensively to evaluate diseases in humans, and as research tools in studies with experimental animals. Nuclear imaging techniques are however poor at providing anatomical information. Before the advent of hybrid imaging techniques, anatomical information to aid the interpretation of PET and SPECT images was provided by viewing them side-by-side with CT or MRI images obtained in another department. Spatial correlation was limited by differences in scanner geometry and displacement of mobile structures between scans, as well as the ability of the viewer to perceive 3D relationships.

Hybrid imaging systems largely overcome the problem of spatial correlation by combining two different imaging technologies, e.g. PET/CT, PET/MRI, SPECT/CT, SPECT/MRI, and PET/Optical, in a single imaging system. The imaging procedure on a hybrid system yields images with complementary information that are spatially matched. Combining these images provides unique and readily interpreted information not available from either image alone.

The best known example of a hybrid imaging system is PET/CT. Fusion of the functional and anatomical images provided by these modalities provides both accurate anatomical localization of abnormalities seen on the PET image, and low noise transmission data that can be used with minimal preprocessing to correct measured PET emission data for attenuation before reconstruction. Although both images are acquired while the patient remains on the scanning couch, they are not acquired simultaneously and there is a possibility the patient may move between scans. This and other potential sources of artifacts will be discussed, along with recent innovations in PET/CT technology. The unprecedented success of PET/CT led quickly to the introduction of the first SPECT/CT systems. Here the combination with CT has proven to be as valuable as it was for PET.

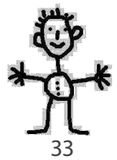
PET/MR, like PET/CT, provides spatially matched functional/anatomical images. However there are important differences. Replacing CT with MR means that there is no CT-related radiation dose. MRI also offers much better soft tissue contrast than CT. As will be shown, it is feasible to design scanners capable of simultaneous PET/MR imaging, to completely eliminate the possibility of spatial misregistration. In addition to anatomical information, the MR component can provide useful functional information, and details of the chemical composition of selected regions, via fMRI and MR spectroscopy techniques, respectively. On the down side however, the MR image cannot provide information about photon attenuation, preventing its use for attenuation correction directly. Thus accurate attenuation correction in PET/MR remains a topic of current research interest.

Optical imaging uses light to interrogate cellular and molecular function in living tissue.

Contrast is derived through the use of exogenous agents, endogenous molecules with optical signatures, and reporter genes. Its use in combination with PET or SPECT enables imaging of two molecular targets simultaneously. Examples of the use of this and other hybrid imaging techniques in translational research will be presented.

My notes & questions

for the "MEET THE EXPERT SESSION" subsequent to this educational session
on "WHAT LIFE SCIENTISTS SHOULD KNOW ABOUT IMAGING MODALITIES"



4 – Postprocessing and Cross Validation

4A – MODELING AND QUANTIFICATION

chaired by Adriaan Lammertsma – Amsterdam, The Netherlands

TALK 1: BASIC (PHYSICS) PRINCIPLES OF QUANTIFICATION USING PET

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LEARNING OBJECTIVES:

- To know the principles of detection
- To know which technical parameters determine the quantitative accuracy of PET measurements
- To know which corrections are necessary in order to obtain quantitative data

The objective of this presentation will be to introduce the audience to the basics of quantitative imaging with positron emission tomography (PET). PET is inherently a quantitative method, not only imaging the distribution of positron-emitting isotopes but also measuring their absolute concentration in Bq per ml. However, physical aspects and intrinsic factors in the design of PET scanners affect and limit its quantitative capabilities, and a number of corrections have to be applied to measured data to obtain quantitative images.

Principles of detection: PET is based on the principle of coincidence detection of annihilation photons. The principles of photon detection and scanner design will be discussed.

Limiting factors: A number of physical limit the accuracy of PET images. Spatial resolution, that is, the minimum distance required between two points to distinghuish them as separate points in the image, is determined mainly be the energy of the emitted positrons, the size of the detector elements, and the non-collinearity of the annihilation photons. Sensitivity of the scanner is defined as the number of measured true coincidences per actual decay, and is mainly affected by the geometry of the scanner and the detector materials. Since a higher sensitivity results in better counts statistics and a higher signal to noise ratio, sensitivity is also of importance for quantitative accuracy.

Correction factors: During image reconstruction, PET data has to be corrected for a number of effects. Firstly, the sensitivity of each individual detector pair, or line of response, is different, and this has to be corrected by a normalisation procedure. Correction methods for random coincidences will be discussed. Then, a correction has to be applied for coincidences in which one or both of the annihilation photons have scatter on their path between decay and detectors. Although the choice of a detector material with a high energy resolution can limit the scatter coincidence fraction using energy discrimination, the scatter coincidence fraction is still in the order of several tens of percent. Other correction factors include those for detector dead time.

Quantification and quality control: Once all appropriate corrections are applied and the image reconstruced, the PET images are in units of counts per ml per second. A cross-calibration measurement, generally using a uniform cylindrical phantom containing a solution with a known radioactivity concentration, is used to measure a calibration factor, converting counts per ml per second into Bq/ml. Although an absolute calibration can in principle be obtained, it is mainly important that the PET scanner is properly cross-calibrated with the dose calibrator used to measure syringes prior to patient administration, as well as for example detectors used for measurement of blood radioactivity concentration. In addition to routine daily quality control, the scanner's calibration has to be validated on a regular, e.g. monthly, basis.

TALK 2: BASIC (PHYSICS) PRINCIPLES OF QUANTIFICATION USING MR

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LEARNING OBJECTIVES:

- Basic principles of MRI
- Factors determining intensity and contrast in MRI
- Contrast agents in MRI

Nuclei with an odd number of protons or neutrons possess and non-zero magnetic moment, the most important representative being the hydrogen nucleus. These nuclear properties are exploited in nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI). MR images represent the weighted distribution of (hydrogen) nuclei within biological tissue, with weighting factors given by the relaxation properties of the nuclear magnet (describing how rapidly excess energy is dissipated and how strongly it interacts with neighboring nuclei), by its microscopic motion in an inhomogeneous magnetic field (diffusion, perfusion), and potentially by its involvement in chemical reactions. The resonance frequency depends on the local magnetic field at the site of the nucleus; hence spatial information is obtained by rendering the magnetic field strength location dependent through the transient application of magnetic field gradients.

MRI is inherently insensitive due to the low quantum energy involved. For typical field strength used in preclinical and clinical imaging only 0.001 to 0.005% of the nuclear magnets will contribute to the signals detected. This limitation governs important imaging parameters such as spatial and temporal resolution. For high resolution structural imaging, typical voxel dimensions are 50mm^3 to 100 mm^3 for small animal and 1mm^3 for clinical imaging. Temporal resolution may range from less than 1s to hours depending on the type of data acquired. It is important to realize that sensitivity, spatial, and temporal resolution constitute canonical quantities: improving one of them would inevitably compromise the other ones.

The MR signal intensity is governed by parameters such as relaxation times, diffusion properties, and microcirculation, which all depend on the local tissue characteristics. This is the source of the high soft tissue contrast observed in MRI. By tailoring the data MRI acquisition, individual parameters may be emphasized such that a given structure can be represented with high contrast. With regard to quantitative characterization of tissue the measurement of these parameters becomes attractive: T1, T2 or diffusion maps represent tissue features and changes in these parameters indicate alterations in tissue structural or physiological properties.

The MRI signal intensity and hence contrast can be modulated by administration of contrast agents (CA), compounds that affect the relaxation properties of endogenous tissue (hydrogen) nuclei. Effective CAs must exert a strong local magnetic field. This is achieved by using compounds that contain at least one unpaired electron, the electron magnetic moment being 650 times that of the hydrogen nucleus. Typical CAs used in MRI are gadolinium (III) complexes (with 7 unpaired electrons) or Fe_xO_y nanoparticles (with up to 5 unpaired electrons per Fe). By coupling CAs to targeting moieties specific probes can be designed, which opens the door to molecular imaging applications. The effect of CAs on MRI relaxation rates is directly proportional to the total amount of CA in a voxel; hence generation of maps of the absolute relaxation times allows the quantitative determination of local concentrations of the CA provided the proportionality factor (the molar relaxivity) and the CA's distribution volume are known. The option to derive quantitative information is an important feature that is attractive for molecular imaging studies using MRI.

TALK 3: BASIC (PHYSICS) PRINCIPLES OF QUANTIFICATION USING OPTICAL TECHNIQUES

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LEARNING OBJECTIVES:

- To understand the limitations and pitfalls related to quantification in optical imaging.
- To gain an overview of optical techniques suitable for robust quantification in different imaging scenarios.

Optical imaging has come into widespread use in research laboratories. In particular, approaches based on near infrared fluorescence have enabled versatile and convenient whole-body imaging of small animals. However, the simple photographic techniques most commonly applied today ignore the effects of light attenuation in tissue and can produce misleading results if not properly understood. This talk will cover the principles of light propagation and detection of optical signals to provide the audience with a fundamental understanding of the physics involved. Techniques for optical imaging will be analyzed to provide an understanding of their performance with respect to quantification accuracy. Advanced approaches to correction and quantitative volumetric imaging will be introduced.

TALK 4: BASIC PRINCIPLES OF TRACER KINETIC MODELLING

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LEARNING OBJECTIVES:

- Understanding of first principles of tracer kinetic modelling
- Basic understanding of the use of compartment models
- Understanding the need for dynamic scanning to extract physiological parameters from images
- Understanding how to measure perfusion, volume of distribution and binding potential

A typical tracer (imaging) experiment involves injection of that tracer followed by measurement of its concentration in the organ or tissue of interest. The tracer itself is selected because of its properties, i.e. its association with a physiological, biochemical or pharmacokinetic process, such as blood flow, metabolism, receptor density, etc.

From a clinical point of view it is interesting to inject the tracer in a quiet (dedicated) room, wait until there is sufficient uptake of the tracer, and then position the patient in the scanner and perform a single (static) scan to measure the distribution of the tracer. However, as the tracer is injected in the bloodstream, its uptake in tissue will be determined by a number of different physiological processes, i.e. delivery to the tissue, (molecular) interactions within the tissue and clearance from the tissue. Delivery to the tissue, in turn, depends on the time course of the arterial tracer concentration, tissue perfusion and extraction fraction. It will be clear that, in general, the relationship between total signal (uptake) and specific signal (i.e. the signal related to the actual process under study) in an image will not be linear and that this relationship will vary over time.

Qualitative imaging may be sufficient for diagnostic purposes (e.g. areas of increased or decreased uptake, left/right differences, etc). Quantification, however, is needed for pathophysiological studies that are carried out to obtain a better understanding of disease (mechanisms), and it is essential for studies aimed at following progression of disease and its subsequent modification by an intervention (e.g. medication). For true quantification of a physiological or molecular process a tracer kinetic model is essential, together with dynamic scanning in which uptake and clearance (i.e. kinetics) of the tracer are followed over time. Only in this way it is possible to extract the tissue specific signal from the total measured signal that is affected by the various processes mentioned above.

A tracer kinetic model is a mathematical description of the fate of a tracer in the body, with emphasis on the organ of interest. Using such a model, observed tissue uptake and clearance can be related to various model parameters, provided the input function is also known. As most tracers are injected intravenously, they reach tissues through the bloodstream. Consequently, in most cases, measurement of the arterial plasma concentration over time is also needed. Although other types of models have been proposed, in practice compartmental models are used. In these models, the distribution of activity is allocated to a number of (not necessarily physical) discrete compartments. The resulting operational equation primarily contains (unknown) rate constants, describing the rate of exchange of tracer between the various compartments, which are estimated by fitting the measured data to a suitable model using non-linear regression.

In this contribution the basic principles of tracer kinetic modelling will be presented, together with the most commonly used compartment models. In addition, it will be shown how these models can be used to measure perfusion, volume of distribution and binding potential.

4B – BASICS OF IMAGE PROCESSING

chaired by Wiro Niessen – Rotterdam, The Netherlands

IMAGE SEGMENTATION: METHODOLOGY AND VALIDATION

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LEARNING OBJECTIVES:

- To get a comprehensive overview of different image segmentation techniques
- To understand how prior knowledge is integrated in various model-based image segmentation techniques
- To learn about standardization and validation of image segmentation techniques

In this talk, a comprehensive overview of image segmentation techniques will be provided. The talk will first introduce low-level, unsupervised techniques, which utilize image information, such as intensity, gradients, and textures, for voxel classification. Subsequently, segmentation techniques that utilize prior information will be discussed. Prior information can relate to object smoothness, the physics of image formation, anatomy of physiology, or on image appearance. The following segmentation techniques will be discussed in some detail: (i) Pixel/voxel based classification, (ii) deformable model based segmentation, (iii) active shape and appearance based models, (iv) atlas-based segmentation, and (v) graph cuts. Different examples in the field of biomedical and biological image analysis will be shown. Finally, the importance for validation and standardization is discussed, and different frameworks for standardized validation, and consolidation of image segmentation techniques will be discussed.

My notes & questions

for the "MEET THE EXPERT SESSION" subsequent to this educational session on "POSTPROCESSING AND CROSS VALIDATION"

Focus Session on new imaging Tools – Cerenkov Luminescence Imaging

NEW IMAGING TOOLS: CERENKOV LUMINESCENCE IMAGING

chaired by Jan Grimm – New York, USA

FASTER THAN THE SPEED OF LIGHT - APPLICATIONS FOR CERENKOV IMAGING

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Cerenkov radiation is the blue-light produced by particles traveling faster than the speed of a light through a diaelectric medium. While this phenomenon has been described originally in the early 20th century and rewarded with the Nobel Price for Physics in 1958 it was only recently that this phenomenon was recognized as utilizable tool for optical in vivo imaging of nuclides. In this context Cerenkov Luminescence Imaging (CLI) is a new, emerging modality that utilizes the light produced by radionuclides for in vivo imaging using optical equipment. CLI requires highly sensitive optical cameras to detect the low amount of photons emitted compared to other optical imaging modalities. However, it offers several compelling advantages. The imaging equipment remains still cheaper than a PET scanner; CLI allows imaging of nuclides that cannot be imaged otherwise such as [90)Y or 225[Ac] and it offers some compelling advantages that might suggest CLI for clinical applications once technical challenges are overcome.

CERENKOV LUMINESCENCE ENDOSCOPY: FEASIBILITY AND CHALLENGES

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Recently Cerenkov Luminescence Imaging (CLI) has risen as a novel, attractive molecular imaging technique. It bridges nuclear and optical imaging and has demonstrated many applications in biomedical research, thus CLI has attracted much attention in the molecular imaging community. More interestingly, the combination of CLI with endoscopy techniques could result in Cerenkov Luminescence Endoscopy (CLE) techniques which may be very useful in clinic. In this presentation, our efforts on developing CLE systems will be introduced. We will present the feasibility of using CLE for tumor detection and surgery monitoring in small animal models. The challenges that this new imaging modality faces will be discussed as well.

CERENKOV SPECIFIC CONTRAST AGENTS

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LEARNING OBJECTIVES:

- Contrast agents can be synthesized to exploit the unique properties of Cerenkov imaging
- These radiolabeled contrast agents selectively absorb a band Cerenkov radiation dependent upon a specific biological function
- Cerenkov specific contrast agents provide complementary molecular information to anatomical and functional data provided by PET

A number of recent publications have demonstrated the feasibility of detecting Cerenkov radiation using optical imaging techniques. Cerenkov radiation occurs when a travelling charged particle, such as an electron or positron, exceeds the speed of light in the medium, emitting the excess energy in the form of a photon. While the sensitivity of Cerenkov imaging is limited by a number of factors including absorption and scattering of visible photons by tissue, limited depth penetration and low signal to noise associated with the low photon release rate of most radioisotopes, there are also a number of distinct advantages to this method. First, Cerenkov radiation is emitted by both positrons and electrons, enabling the imaging of a number of nuclei that have not previously been accessible. Secondly, optical scanners can typically image 3-5 mice simultaneously, thus allowing the imaging of radioisotopes with higher throughput in animal models. Third, because Cerenkov imaging measures photon release, optical imaging techniques such as photon quenching and resonant transfer to longer wavelength fluorophores can be utilized. Finally, Cerenkov radiation is multispectral, emitting continuously across the bandwidth of 300-800 nm with intensity proportional to $1/\lambda^2$.

In this presentation we will discuss contrast agents that we have designed specifically for Cerenkov imaging. These contrast agents exploit the optical and multispectral properties of Cerenkov light to add functional imaging capabilities, thus enhancing the information available from radiolabeled tracers. The functional contrast is based on selective bandwidth quenching of the Cerenkov emission spectrum. Both intermolecular (tracer on a different molecule) and intramolecular (tracer on the same molecule) selective bandwidth quenching are possible. As proof of principle, we present the synthesis and characterization of ^{18}F labeled pH indicators. A change from acidic to basic environment causes a color change in the indicator, resulting in increased photon absorption and a bandwidth selective reduction in the Cerenkov emission. The PET signal remains invariant. Using ratiometric imaging, or a scaling of the emitted intensity from different bandwidths, we can directly estimate the pH *in vitro* and *in vivo*.