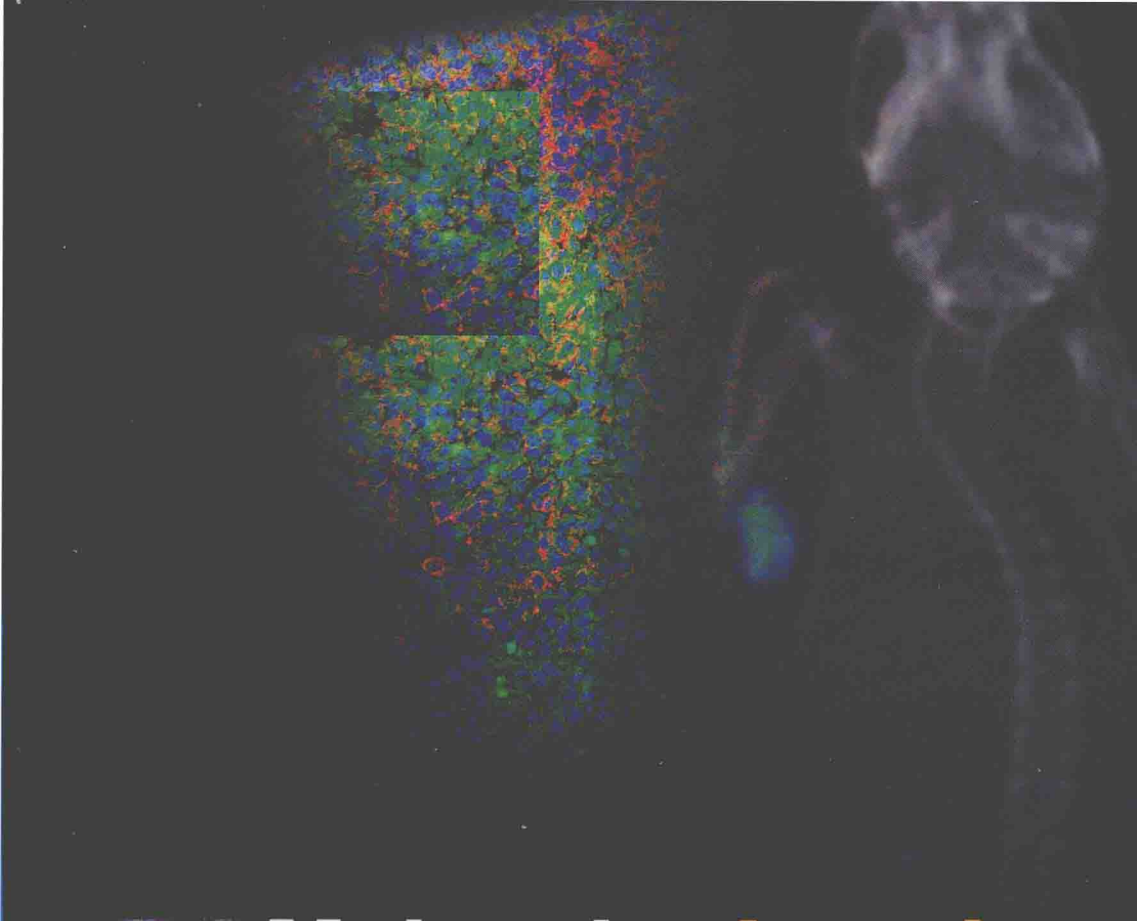

Molecular Imaging

Basic Principles and Applications in Biomedical Research

2nd Edition

>> Markus Rudin

Imperial College Press

2nd Edition

Molecular Imaging

Basic Principles and Applications in Biomedical Research

The area of molecular imaging has matured over the past decade and is still growing rapidly. Many concepts developed for molecular biology and cellular imaging have been successfully translated to *in vivo* imaging of intact organisms. Molecular imaging enables the study of processes at a molecular level in their full biological context. Due to the high specificity of the molecular readouts the approach bears a high potential for diagnostics. It is fair to say that molecular imaging has become an indispensable tool for biomedical research and drug discovery and development today.

This volume familiarizes the reader with the concepts of imaging and molecular imaging in particular. Basic principles of imaging technologies, reporter moieties for the various imaging modalities, and the design of targeted probes are described in the first part. The second part illustrates how these tools can be used to visualize relevant molecular events in the living organism. Topics covered include the studies of the biodistribution of reporter probes and drugs, visualization of the expression of biomolecules such as receptors and enzymes, and how imaging can be used for analyzing consequences of the interaction of a ligand or a drug with its molecular target by visualizing signal transduction, or assessing the metabolic, physiological, or structural response of the organism studied. The final chapter deals with visualization of cell migration, for example in the context of cell therapies.

The second edition covers novel developments over recent years, in particular regarding imaging technologies (hybrid techniques) and novel reporter concepts. Novel biomedical applications have been included, where appropriate. All the chapters have been thoroughly reworked and the artwork updated.

Cover: Imaging hypoxia signaling. SPECT/CT image of mouse with subcutaneously implanted colon tumor visualized using a biotinylated SPECT tracer targeting avidin that is exposed at the surface of cancer cells upon activation by hypoxia signaling (color overlay). Histological image shows tissue hypoxia (green), the level of glucose transporter 1 induced via hypoxia signaling (red), and the cell nuclei (blue). Images courtesy of Dr. Steffi Lehmann, ETH Zürich.

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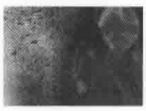

Molecular Imaging

Basic Principles and Applications in Biomedical Research

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Preface to the First Edition

Many scientists state that *molecular imaging is not a new discipline*; nuclear imaging approaches such as positron emission tomography and single-photon emission computer tomography have used molecular imaging concepts for more than a decade to visualize the biodistribution of labeled compounds, including analyses of receptor occupancy. In these studies image contrast is not governed by the anatomical features of the sample but rather by the local concentration of the radiolabeled reporter compound, i.e., by a molecular property.

Other scientists claim that *molecular imaging is a new scientific area merging concepts of molecular biology with noninvasive imaging technologies*. This allows the study of biological processes in a noninvasive manner. These concepts go beyond the labeling of reporter ligands. They involve the development of a battery of reporter assays that are used to probe specific biological questions: Is the expression of a receptor modified under specific pathological conditions? Does the receptor exert its biological activity; does it activate its associated signaling cascade? Can these molecular markers be used as early indicators of a pathological transformation?

In recent years we have experienced rapid growth in the field of molecular imaging. Many of the assay systems originally developed for studying biochemical systems in homogeneous solutions or isolated cells have been successfully translated to the *in vivo* situation, allowing the testing of a biomedical hypothesis in the context of the intact organism. Molecular or target-specific imaging potentially provides a holistic view of a biological system in contrast to the reductionistic approaches of classical molecular biology and biochemistry.

Despite the rapid progress that has been achieved in the last couple of years the field is still in its infancy and major developments are still required. This relates to the imaging technologies, which should provide high sensitivity, high temporal and spatial resolution, and most importantly quantitative data, as well as to the probe design. Highly specific probe constructs should provide the desired information with little interference from non-specific background signals. Multiplexed probes that allow the monitoring of several biological processes simultaneously would be of utmost relevance.

There are considerable expectations associated with molecular imaging techniques. Firstly, the study of molecular events in the intact organism will *enhance our basic molecular biological knowledge*. For instance, specific receptor systems or signal transduction pathways can be studied in their biological context. Secondly, tissue functional or structural aberrations are always preceded by molecular events such as abnormal cellular signaling. It is reasonable to assume that *molecular markers might serve as early indicators of a disease process*, long before a pathomorphological or pathophysiological transformation of tissue occurs. It may furthermore improve the specificity of diagnosis and may help to select the patient population that is most likely to respond to a specific therapy. Thirdly, modern pharmaceutical drugs are designed to interact with a well-characterized molecular target. As clinical drug development is becoming increasingly expensive (today the development of a drug to reach the market will cost approximately US$800 million), early information on clinical efficacy is of crucial importance. Molecular imaging might provide a *direct proof that a therapeutic concept is also valid* in man; e.g., the target enzyme is in fact inhibited by the drug candidate, or inhibition of a specific receptor shuts down the associated molecular signaling cascade. For these reasons, molecular imaging will soon become an indispensable tool in biomedical research, in particular for drug discovery and development.

This book aims to give the reader an introduction into this fascinating and dynamically evolving field. It discusses basic aspects of molecular imaging technology and probe design, which are illustrated with numerous selected examples from the literature. It does not, however, provide a comprehensive review of the current activities in the field. This was never intended, as such a book would probably never

be finished: new exciting applications are being reported almost on a daily basis.

The first part of the book discusses technological aspects of molecular imaging. In Chapter 2, the various imaging modalities such as X-ray computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computer tomography (SPECT), positron emission tomography (PET), fluorescence and bioluminescence imaging, as well as ultrasound imaging, are discussed in some detail. Chapter 3 describes the various reporter systems that are being used for the various imaging approaches, from short-lived radionuclides to microbubbles suited for ultrasound studies. The design of reporter constructs for molecular imaging applications is the topic of Chapter 4. Important aspects to consider are target-specificity, delivery of the probe to the target site, and signal amplification. Most molecular targets are expressed at low concentration (nano- to femtomolar); hence, strategies to enhance the signals produced by the reporter system are essential.

The second part deals with applications of molecular imaging in biomedical research. Knowledge of the drug biodistribution and pharmacokinetics (PK) is highly relevant for drug development as many drug candidates fail due to inadequate PK properties. Chapter 5 deals with drug imaging using PET techniques. In Chapter 6, methods to visualize the expression of the drug targets are discussed. Levels of transcription products can be probed using labeled antisense molecules; receptor imaging relies on the availability of small molecular probes or labeled antibodies. Enzymatic drug targets are attractive for imaging as the enzyme activity can be exploited to activate or trap a reporter substrate, thus yielding high degrees of signal amplification and minimal interference by background signals. A different approach to visualizing gene expression is the use of the reporter genes, which allows unique questions to be addressed. Drug–target interaction will initiate a series of downstream processes. Measurement of these *effector readouts* is the topic of Chapter 7, which discusses methods to visualize protein–protein interactions or apoptotic activity as an example. Further downstream a ligand–receptor interaction will initiate a physiological response that can be visualized using conventional imaging approaches such as the measurement of glucose utilization, energy turnover, tissue perfusion, or second messenger

turnover. Chapter 8 describes techniques for the monitoring of cell trafficking. Myeloid and lymphoid cells are important mediators of inflammation, and visualization of the infiltration of these cells into inflamed tissue is a sensitive indicator of the disease process. Novel therapeutic approaches in degenerative diseases try to exploit the pluripotency of stem cells for tissue repair. Obviously, such therapy concepts will benefit from the ability to monitor the fate of such cells under *in vivo* conditions.

While many of the techniques and applications described will undergo further development in the coming years, most of the basic concepts outlined in this book will remain valid and will also be applicable to these advanced procedures. In this regard, the book is designed as a textbook summarizing basic principles and potential applications of molecular imaging in biomedical research.

Zürich, June 2005

Markus Rudin
University of Zürich

Preface to the Second Edition

The field of molecular imaging has rapidly evolved since the appearance of the first edition of this book: a quick survey of PubMed using the search item '*in vivo* molecular imaging' revealed that the number of articles on the subject has more than tripled between the years 2005 and 2010, from 588 to 2164, following an exponential growth curve. By way of comparison, a PubMed search using the term '*in vivo* molecular imaging 1970' yielded four hits. The amount of recent research in the field illustrates the importance of molecular imaging approaches in biomedical research, as a basic research tool, for enhancing the sensitivity and specificity of diagnostic assays, and for facilitating the development of novel therapeutic interventions. Significant progress has been made with regard to the development of advanced imaging technologies, novel reporter principles, and in particular with regard to applications of the approach in addressing important biological questions. It is beyond the scope of this book to cover the field comprehensively. The second edition includes novel developments if they add new aspects to the overall topic. I have focused on including developments in molecular imaging methodology that are of general conceptual interest and I have included novel applications for illustration purposes.

In terms of imaging technologies, aside from important improvements to well-established tools, there has been a major drive towards the development of hybrid imaging solutions combining two or multiple modalities. This has commonly taken the form of the combination of a structural and a molecular imaging technique. For example, highly sensitive techniques such as positron emission tomography

(PET) or fluorescence tomography (FMT) may be combined with high resolution modalities such as X-ray computed tomography (CT) and magnetic resonance imaging (MRI). PET/CT has become a clinically established tool while PET/MRI is on the verge of introduction into clinics. In contrast with this, optical hybrid approaches will develop as important research tools. In all of these hybrid systems, two instruments are combined into one, allowing for simultaneous measurements without moving the object, thus warranting optimal registration of imaging data sets. As neither of the two measurement principles are affected by the combination, the incorporation of a second modality should be achieved with minimal interference. However, the combination of optical and ultrasound imaging is different. The molecular signal is generated by combining two physical processes: absorption of a light photon leads to a local temperature rise that translates into tissue expansion and thus the generation of a pressure wave that is detected by an ultrasound detector array. In recent years, optoacoustic imaging has evolved as an attractive experimental tool. As a result, Chapter 2 has been adapted to account for these new developments in imaging instrumentation. An additional subsection has been added to discuss various hybrid modalities.

Advances have also been made with regard to reporter systems. An interesting development in MRI probe design is chemical exchange saturation transfer (CEST) probes. Their signal intensity can be modulated by radiofrequency irradiation at the proper frequency, which is essential for detecting the probe-specific signal. A second major development was the introduction of hyperpolarized substrates, the use of which leads to MR signal enhancements by four to five orders of magnitude. Technological solutions have also been introduced that preserve the high degree of polarization attained at very low temperatures during rapid warm-up to body temperature. The spin polarization has been enhanced, typically from a few tens of ppm to the tens of percent range. This tremendous gain in sensitivity will enable attractive new applications in biomedical research. With regard to optical imaging, reporter systems have been optimized. In particular, fluorescent proteins used in reporter gene assay that absorb and emit in the far red-shifted, or even in the near infrared, spectral domain have been developed. In addition, optoacoustic imaging requires probes that are efficient absorbers to produce as much local heat as possible.

Novel developments regarding reporter systems have been included in Chapter 3. In Chapter 4, a subsection on pre-targeting approaches has been included. In particular, click chemistry reactions have been used for this purpose: bioorthogonal ligation reactions are used for introducing an exogenous label (fluorescent dye or radioligand) to a pre-targeted biomolecule.

Chapters 5 to 8, which deal with biomedical applications, have been updated. Some new aspects included relate to drug biodistribution studies using MALDI mass spectrometric imaging, to fluorescent dyes binding to amyloid plaques, ligand designs for intracellular trapping of protease ligands, imaging of hypoxia signaling, or imaging of neurogenesis. Other adaptations have been made throughout the text where appropriate.

Molecular imaging will continue to rapidly evolve in the coming years and is expected to make an impact in multiple fields of biomedical research. Apart from its undoubted role as a research tool, molecular imaging solutions should and will penetrate the clinical arena. This will confront the field with new challenges: methods have to be approved by regulators and economic issues will become increasingly important. Who is taking the risk of developing a project through all the steps required for clinical applications — in particular during a period of ever increasing costs in healthcare? An attractive concept is to 'share' the costs with the development of a therapeutic drug in co-developing a 'companion diagnostics'. Hence, the pharmaceutical industry might play a leading role in the process. Industrial–academic consortia might constitute an approach for reducing both risk and individual costs for such developments.

Though such issues are critical in determining the fate of molecular imaging they are not within the scope of this book, which focuses on general technological and scientific aspects. These should stay valid even in view of the rapid development of the field. The book is meant to be a textbook for professionals and students interested in this dynamic field of biomedical research and noninvasive imaging of molecular processes in the intact organism.

January 2013

Markus Rudin
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Acknowledgments

First Edition

Many colleagues and friends contributed directly and indirectly to this book. Since my early days in imaging I had the privilege to work with partners — NMR/MRI imagers and spectroscopists and biologists — who were eager to discuss not only new ideas, but also to challenge the novel nonvalidated approaches provided by *in vivo* imaging. Together, we have distilled many concepts of imaging, trying to understand what we were looking at on our computer screens.

In particular, I would like to thank all the actual members of the imaging group, with whom I had many discussions concerning molecular imaging and its application to the drug discovery process: Peter Allegrini, Nicolau Beckmann, Hans-Ulrich Gremlich, Rainer Kneuer, Didier Laurent, Martin Rausch, Markus Stöckli, and Jeffrey Tsao. I am also grateful for the support given by the Research Management of the Novartis Institutes for Biomedical Research, and in particular to Rene Amstutz, head of Discovery Technologies. His support and his challenge were essential for the implementation of molecular imaging approaches into our imaging portfolio.

I had the opportunity to discuss many aspects of molecular imaging with the protagonists in the field. These people had made significant contributions to the development of molecular imaging. My special thanks go to Ronald Blasberg (Sloan Kettering Memorial Cancer Center, New York), Sam (Sanjiv) Gambhir (Stanford University, San Francisco), Allan Johnson (Duke University, Durham), Ralph Weissleder (Massachusetts General Hospital, Boston). I am

also grateful to Joachim Seelig (Biocenter University of Basel), who was my tutor and advisor throughout my years in imaging.

Second Edition

Again I have the pleasure and privilege to thank many people who have helped me to rework the manuscript. First and foremost, I am indebted to Dr. York Haemisch, Philips Technologies GmbH, who helped me to rework the sections on Nuclear Imaging (2.3 and 2.4) and who provided me with invaluable information on modern PET systems. Several people have provided me with new material which is acknowledged in the respective passages in the text or figures. I highly appreciate the very stimulating environment within the Animal Imaging Center of the University of Zürich (UZH) and ETH Zürich, the Institute for Biomedical Engineering UZH/ETH, the Institute of Pharmacology and Toxicology UZH, the Center for Imaging Science and Technology of ETH Zürich, the Neuroscience Center UZH/ETH and the Center for Systems Physiology and Metabolic Disease ETH, and the many discussions with my colleagues at these institutions. I am also grateful to the students who attended my lectures on molecular imaging. Their feedback on the clarity of some sections of the book was very useful, hopefully ensuring the book is easily understood by readers.

Last, but by no means least, I would like to thank my wife Verena. Without her invaluable tolerance and her support, it would have not been possible to free the time to write this book.

Zürich, April 2011