Could you start by giving a synopsis of your project on multispectral fluorescence molecular tomography? What are the principle aims and objectives?

The goal of our lab is to develop new quantitative thick-tissue optical imaging platforms for preclinical and clinical applications. We focus on three main areas: designing new optical tomographic imaging instruments; developing new reconstruction algorithms for quantitative volumetric imaging; and investigating optimal experimental and theoretical parameters for functional, molecular and dynamical optical imaging.

We are pioneering wide-field optical tomography based on time-resolved patterned illumination and detection for biomedical applications. We demonstrated that quantitative high-resolution fluorescence molecular tomography (FMT) can be performed by exploiting a combination of illumination patterns and a time-gating detection technique associated with reconstruction algorithms based on the Monte Carlo method.

The first implementation of this new imaging paradigm focuses on preclinical molecular imaging. Such an innovative approach will allow us to perform three-dimensional visualisation of multiple biomarkers with high sensitivity at unprecedented acquisition speeds (<5 min from neck to tail).

One method of imaging you have looked at is known as fluorescence lifetime imaging (FLI). How is it used? And how do you hope to develop these techniques?

Imaging spectra and lifetime can be used to investigate many biomedical applications. Fluorescence imaging allows indirect monitoring of cell or tissue biomarkers, or direct monitoring of endogenous fluorophores within the sample. Recording the spatial distribution, concentration and lifetime of these markers can reveal information regarding their metabolic activity, binding state or molecular interactions.

Whilst these techniques have been well established in microscopy, their translation to in vivo application is still somewhat nascent. Optical imaging is limited in its depth penetration to a few millimetres in the visible range, which is the most common spectral range used in microscopy. Light is absorbed by endogenous chromophores found in living tissue (e.g. haemoglobin, melanin and lipids) with the lower absorption exhibited by tissues in the near infrared (NIR) region (780-900 nm). Hence, there is a need to red-shift optical imaging for non-invasively imaging thick tissues such as in preclinical and clinical applications.

Our goal is to use FLI in vivo to provide multiplexing and therefore the ability to follow multiple physiologically important targets during different normal and altered processes, such as tumor growth. Another possibility provided by FLI is to detect protein-protein interactions using Forster Resonance Energy transfer based assays.

What are some of the challenges associated with developing cutting-edge technology such as yours? Following this, how have you overcome these?

Developing such cutting-edge technology is considered to be high-risk and high-impact. Hence, there are some challenges that need to be overcome. Foremost, it is challenging to garner resources to directly support the development of these new technologies at the onset. When we first proposed the new idea of using wide-field illumination and detection, we were faced with scepticism. For instance, in the early steps of attempting to secure funding for this project, a summary statement from one of the federal agencies stated that the main concern they had about our proposal was that the technique was too novel, and hence that it would not be embraced by the community, and so was seen as too high-risk.

Hence, it is crucial to tie the development of this new technology with an outstanding biological/clinical problem very early on. In this regard, we have been very lucky to interact with Dr Margarida Barroso at Albany Medical College, who is an expert in fluorescence resonance energy transfer (FRET) imaging and supports the translation of FRET assays to small animal imaging as a very exciting new application.

Finally, you propose the use of in vitro and in vivo techniques for validation. Could you please describe what this will entail?

There are two facets to this: Firstly, the drug development pipeline requires in vitro and preclinical work before translating to the clinic. The vast majority of the work at the in vitro stage is performed using fluorescence imaging techniques. However, to continue to the preclinical stage, another technology is employed (such as MRI, ultrasound or PET). The development of NIR assays will allow the same markers/metrics to be used from in vitro to in vivo.

Secondly, as with every new technique that aims at being quantitative, we need to benchmark its performance. In this regard, our in vivo results were consistent with the in vitro data generated by confocal microscopy. Thus, our novel approach offers the possibility to employ well-characterised quantitative metrics used in FRET microscopy, but with the additional benefit of a seamless technological platform to perform quantitative in vitro and in vivo assays. We expect that technology continuity will accelerate screening and validation in drug discovery.
The golden ticket

The ability to visualise biological processes as they happen in a live animal could greatly enhance the knowledge of disease processes and lead to the development, as well as optimisation, of novel drugs.

Ever since Zaccharias Janssen and his son Hans accidentally created the first light microscope in Holland over 500 years ago, the golden chalice of optical imaging has been to observe biological processes as they happen in vivo. Whilst the field of microscopy has evolved at a stupefying rate over the last half century, scientists of today are still craving the possibility to image centimetre-thick specimens.

With that said, Dr Xavier Intes from Rensselaer Polytechnic Institute’s Center for Biotechnology and Interdisciplinary Studies in New York believes his new approach to time-resolved diffuse optical tomography (DOT) might be a critical milestone towards imaging nanometre-scale protein-protein interactions in live subjects.

Diffuse optical tomography

Fundamentally, DOT is a method that retrieves the volumetric bio-distribution of an optical contrast (such as fluorescence, absorption or scattering) which can be visualised in sections. This is achieved by reconstructing images using light (usually in the 700-1,000 nm region) transmitted through the centimetrewell-thick sample and picked up by sensitive photodetectors. Employing mathematical modelling, the volumetric distribution of the biomarker can be recovered in three dimensions. DOT is a very sensitive, cost-effective technique that can be easily fused with traditional medical imaging modalities. These features have led to it becoming one of the most promising forms of biomedical imaging for preclinical and clinical studies.

DOT has been an intense area of research since the mid-1990s, with primary applications in optical mammography and functional brain imaging. In the last decade, DOT has also been developed for preclinical applications; especially, to help develop and optimise new drugs. Among the different DOT methods devised, time domain (TD) DOT has been established to provide the best imaging performances (higher quantification accuracy, improved spatial resolution, multiplexing of biomarkers). However, in the past these advantages have come at too high a price in terms of instrument complexity and acquisition time which have hindered its dissemination.

In a 2010 paper that was published in Optics Letters, Intes and his team proposed a new instrumental paradigm based on wide-field patterned illumination and detection strategies that made TD DOT a fast and robust imaging technique. This study was a breakthrough, as it paved the way for time-resolved tomographic studies of thick tissues that Intes hoped would be successful for high-resolution volumetric functional and molecular imaging applications. Importantly, the method allows the acquisition of dense spatial, temporal and spectral datasets at unmatched speeds (a few minutes compared to hours).

Fluorescence molecular tomography

An important evolution in the field came in 2000 when researchers began to investigate fluorescence molecular tomography (FMT) of tissues. This new avenue of research was motivated by the development of fluorescent imaging biomarkers that can be used to assess disease progression and drug response at the molecular, cellular and target levels. FMT paired with appropriate near infrared (NIR) imaging agents can be used to assess disease progression, drug response at the molecular, cellular and target levels, or can be used as a discovery screening tool in live subjects.

The advantages of FMT over traditional imaging techniques such as ultrasound, magnetic resonance imaging or positron emission tomography are high sensitivity (picomolar range), low cost, with the possibility to image numerous molecular targets with multiple distinct agents similar to fluorescence microscopy and cytometry in tissue in vivo.

Utilising fluorescence

FMT can be applied for fluorescence lifetime imaging (FLI). In its most basic sense, FLI is a technique that produces an image based on differences in the exponential decay rate of the fluorescence from a fluorescent sample (ie. one that contains fluorophores). This, as explained by Intes, makes it an ideal candidate for pre-clinical and clinical applications: “FLI is particularly powerful because the use of fluorescent labels can yield a high specificity and can provide information about the environment of the fluorophore molecule, as well as their location.”

Whilst this technique has been around for some time now, recent advances in both label and detection technology have greatly enhanced its impact in the field of biomedicine. One such area has come in biomedical imaging, where it has been found that FLI can provide greater specificity than intensity-based fluorescence imaging through additional contrast mechanisms, which Intes is now hoping to exploit to observe discrete and sometimes subliminal biological processes in vivo.

Wide-field time-resolved optical tomography of murine model. (A) Transverse slice (y = 21.5 mm), (B) coronal slice (x = 6.5 mm) of 3D fluorescence reconstruction overlaid with microCT imaging, (C) 3D visualisation of FMT and microCT results.
MULTISPECTRAL FLUORESCENCE MOLECULAR TOMOGRAPHY WITH STRUCTURED LIGHT

OBJECTIVES

The application of optical techniques for biomedical imaging in preclinical and clinical settings, concentrated on functional imaging of the breast and brain, fusion with other modalities and fluorescence molecular imaging.

The goal is to develop quantitative thick-tissue optical imaging platforms by focusing on three main areas: design of new optical tomographic imaging instrumentation; developing new reconstruction algorithms for quantitative volumetric imaging; and investigating optimal experimental and theoretical parameters for functional, molecular and dynamical optical imaging.

PARTNERS

Dr Margarida Barroso
Assistant Professor, Center for Cardiovascular Sciences
Albany Medical College
47 New Scotland Avenue
Albany, New York

CONTACT

Dr Xavier Intes
Associate Professor, Biomedical Engineering
Co-Director, Biomaging Center
Biomedical Engineering Department
Rensselaer Polytechnic Institute
110 8th Street Troy,
NY 12180, USA
T +1 518 276 6964
E intexx@rpi.edu
http://intes-lab.bme.rpi.edu/index.shtml

As this imaging method can elucidate the presence of fluorophores (either endogenous or labelled) within certain tissues, it is often used to show co-localisation and, thus, allows protein interactions to be monitored. With that said, there are limitations, as explained by Intes: “Although super-resolved imaging microscopy techniques can break the diffraction limit, imaging of protein interaction is not directly achievable, but can be sensed via fluorescence resonance energy transfer (FRET). FRET is the radiationless transfer of energy from an excited donor fluorophore to an appropriate acceptor in close proximity. “Energy transfer only occurs between fluorophores separated by less than ~10 nm, allowing for the visualisation of protein interactions on the nanoscale.”

FRUITFUL COLLABORATION

With this in mind, Intes teamed up with Dr Margarida Barroso from Albany Medical College to try to demonstrate how FRET FMT, in combination with near infrared (NIR) FRET pairs, may be able to non-invasively image protein-protein interactions in murine models. By comparing fluorescent lifetimes (FL) of FRET donors (in close proximity to acceptor) and non-FRET donors, Intes’ team harnessed the fact that the FRET donor will exhibit a shorter FL when compared to its non-FRET counterpart to be able to distinguish between extracellular (non-FRET) and receptor-bound ligand-labelled molecules (FRET fraction – FD).

In a breakthrough 2012 paper that appeared in Biomedical Optics Express, Intes was able to show the quantitative accuracy of FRET FMT using a tissue-mimicking hydrogel phantom. Once this had been achieved, the team turned their attention to proving the accuracy of the method in vivo. Using a murine model with implanted fluorescent markers, the researchers were able to validate that the quantitative accuracy was retained from in vitro to in vivo settings (~5 per cent accuracy).

Since then, the technique has been successfully benchmarked against traditional microscopy and validated in live animal bearing breast cancer xenograft. These studies, to appear in PloS One, were the first to successfully demonstrate quantitative assay imaging from in vitro to in vivo to monitor receptor dimerisation of transferrin receptor bound to labeled transferrin molecules. This is something Intes now hopes to build on with his group and in close corroboration with Barroso to improve anti-cancer drug efficacy by providing a new quantitative imaging tool to distinguish in vivo between drug internalisation and non-specific accumulation due to the enhanced permeability retention effect (EPR).

FROM BENCH TO BEDSIDE

As with many forms of medical imaging research, the overall goal is to optimise the diagnostic and therapeutic applications. The ability to visualise biological processes as they happen in vivo is a realistic end-goal for Intes’ FMT approach, as he explains: “It could provide a critical theranostics tool by discerning protein-protein interactions in the native in vivo environment, conferring a tremendous increase in accurately understanding true physiological mechanisms underlying diseases such as cancer”. He continues: “We also believe it may be useful in other applications, such as monitoring stem cells survival and proliferation post-transplantation or in vivo assessments of tissue-engineered grafts for instance”.

Top: intermolecular fluorescence lifetime NIR FRET in vitro. The FRET cell-based assay was analysed by microscopy and timeresolved FMT. Bottom: ex vivo imaging of five organs (liver, kidney, spleen, brain and heart). Tomographic estimate of intermolecular fluorescence lifetime NIR FRET in a mouse model. Both in vitro, ex vivo and in vivo results are quantitatively consistent.