Clinical translation of optical and optoacoustic imaging

BY VASILIS NTZIACHRISTOS*

Chair for Biological Imaging and Institute for Biological and Medical Imaging (IBMI), Technische Universität München and Helmholtz Zentrum München, Munich, Germany

Macrosopic optical imaging has rather humble technical origins; it has been mostly implemented by photographic means using appropriate filters, a light source and a camera yielding images of tissues. This approach relates to human vision and perception, and is simple to implement and use. Therefore, it has found wide acceptance, especially in recording fluorescence and bioluminescence signals. Yet, the difficulty in resolving depth and the dependence of the light intensity recorded on tissue optical properties may compromise the accuracy of the approach. Recently, optical technology has seen significant advances that bring a new performance level in optical investigations. Quantitative real-time multi-spectral optical and optoacoustic (photoacoustic) methods enable high-resolution quantitative imaging of tissue and disease biomarkers and can significantly enhance medical vision in diagnostic or interventional procedures such as dermatology, endoscopy, surgery, and various vascular and intravascular imaging applications. This performance is showcased herein and examples are given to illustrate how it is possible to shift the paradigm of optical clinical translation.

Keywords: optoacoustic; imaging; clinical; intravascular; multi-spectral; near-infrared

1. Introduction

Optical methods, such as microscopy or fluorescence assays, have been widely employed in biology to reveal information on otherwise invisible cellular and sub-cellular components. In parallel, the engineering of photo-absorbing nanoparticles, fluorescent proteins and probes for in vivo use has allowed the study of dynamic processes in their unperturbed environments [1,2]. The use of externally administered optical agents can impart high contrast, improve detection sensitivity and enable the visualization of several biological targets or biomarkers simultaneously. In addition, the use of non-radio-decaying agents allows measurements over extended periods of time and enables longitudinal studies and safe operation.

With the availability of disease-specific agents developed for in vivo use, such as fluorescence-based probes, engineered dyes or photo-absorbing nanoparticles, optical imaging has also become an attractive modality for pre-clinical and

*v.ntziachristos@tum.de

One contribution of 20 to a Theo Murphy Meeting Issue ‘Illuminating the future of biomedical optics’.
Clinical optical and optoacoustic imaging

Clinical optical imaging however comes with two major complications. First, in contrast to clear solutions or ultra-thin (5–10 μm) tissue slices, intact tissue scatters light significantly and leads to loss of resolution and challenging image formation [4]. Second, optical attenuation owing to absorption and scattering modifies the signal intensity coming from the optical agent in a nonlinear manner [5,6]. Therefore, the variation of optical properties in tissue modulates the recorded signals potentially leading to inaccurate readings [7]. Solutions that are appropriate for clinical imaging and can account for these limitations can be grouped into three general categories. The first includes epi-illumination or transillumination and tomographic imaging methods that take measures to correct for the effect of variation of tissue optical properties on fluorescence signals and offer accurate imaging over photographic approaches. The second strategy uses optoacoustic approaches that are effectively sensitive only to absorption changes and they can, through multi-spectral unmixing, decompose the presence of fluorochromes and other photo-absorbing agents over the background tissue absorption [8]. Finally, a third strategy uses tissuesectioning microscopy, for example confocal or two-photon microscopy, which disentangles the effects of scattering and absorption [2,9]. In this case, diffraction-limited resolution can be achieved at the expense of reduced fields of view and depth. Optical coherence tomography (OCT) [10] also qualifies under microscopy but instead uses scattering as the prime contrast mechanism. Scattering in this case nevertheless reduces the signal available to the method past a few millimetres deep; but otherwise gives rise to the contrast observed on OCT images.

Following on from the opportunity of the truly remarkable 2010 Royal Society Scientific Discussion Meeting on Biomedical Optics, where I had the honour of participating, I give herein a perspective on the first two and perhaps less-explored imaging strategies, i.e. normalized fluorescence imaging and optoacoustic imaging. I present examples on state-of-the-art optical imaging performance achieved with these methods, which explain the potential and high promise for clinical use. Performance is mentioned in the context of imaging ability, i.e. accuracy, depth and resolution, which, owing to the progress with multi-spectral and optoacoustic methods and corresponding data-processing schemes, yields truly unparalleled performance over macroscopic optical imaging of the past. Accordingly, clinical optical imaging herein refers more specifically to methods that operate under high light-scattering conditions and use in vivo ‘staining’ methods to improve upon the diagnostic or sensing sensitivity and specificity over intrinsic tissue contrast.

2. Imaging in the near-infrared

Light in the visible wavelength range is routinely used in diagnostic and interventional imaging. Surgery, various endoscopies and laparoscopies, dermatological inspections and even the first ‘look’ at a patient presenting with disease symptoms are all performed with a white-light source and the human eye as the detector. Human vision however is limited in terms of penetration depth (only the first few tens of micrometres are visible under white-light epi-illumination conditions) and importantly contrast. The human eye is a rather poor spectral detector, especially with regard to quantitatively identifying
possible spectral differences between healthy and diseased tissues, for example in benign versus malignant tumours, even though basic features such as largely de-oxygenated tissues, melanomas or increased levels of bilirubin can be detected with the naked eye.

Correspondingly, various optical techniques attempt to improve upon the performance of human vision. For probing deep under the surface, a well-known strategy is operation in the near-infrared (NIR) spectral region, where light propagating in tissues undergoes less attenuation compared with the visible. In this case, it is possible to achieve depths of several centimetres depending on the tissue type. Typically, greater than 3 cm can be achieved in muscle and brain and greater than 10 cm in less-attenuating organs such as the human breast [11]. Correspondingly, several molecules with the ability to reveal biological processes associated with disease and treatment are labelled with NIR fluorescent dyes. Besides fluorochromes, other dyes and nanoparticles with absorption in the NIR have also been considered as optical probes, in particular as regards optoacoustic imaging. Collectively, the use of externally administered optical agents is a second strategy to improve upon the limitations of human vision, by improving on the detection sensitivity and specificity. Depending on the detection method employed, the sensitivity can also be very high, typically less than 100 fmol of an organic dye can be detected. Imaging in the NIR region has the added advantage of minimizing the tissue auto-fluorescence present, which can improve target to background ratios in fluorescence imaging.

3. Normalized epi-illumination imaging

(a) Limitations of photographic approaches

Epi-illumination ‘photographic’ imaging probably qualifies as the simpler form of optical imaging. The method uses light with a defined spectral bandwidth, such as a filtered white light, a laser source or a laser diode; an exact bandwidth selection being important especially to excite fluorochromes when the method is applied for fluorescence imaging. The source light is expanded and emitted towards tissues in order to generate an illumination field. Light incident on the surface of interest can propagate for several millimetres in tissues, especially in the far-red and NIR (630–900 nm). The corresponding photon field established in the tissue has a depth-dependent strength and can excite tissue fluorochromes in its path. Fluorescence signals generated by this process can similarly propagate to the tissue surface, where they are recorded with a highly sensitive charge-coupled device camera through appropriate filters. Applied in epi-illumination or reflectance mode, i.e. a geometrical arrangement where illumination and detection are at the same side of the tissue imaged, the method is appropriate for open surgery (figure 2) or for endoscopic procedures, for example colonoscopy or laparoscopy.

Epi-illumination optical imaging achieved in the NIR comes with complications; in particular, as it relates to the effects of light diffusion on the images collected. In fluorescence imaging, a major challenge is that images collected with simple photographic approaches do not reflect fluorescence biodistribution [7,12,13]. Instead, they are the coupled interaction of fluorescence, absorption and scattering; a nonlinear mixing process. Besides the loss of
resolution, scattering and absorption modify the intensity and spatial appearance of fluorescence signals resulting in image distortion and quantification errors [14]. For example, areas with higher vascularization will offer higher attenuation of fluorescence light compared to areas with less blood content. It is therefore possible that a vascular, and therefore absorbing, diseased area with significant fluorochrome accumulation may appear darker than an adjacent benign area that is only scattering, for example an area containing adipose tissue, even if small amounts of fluorochrome are present in the non-absorbing area. These effects have been generally noted [7,15] and can lead to false negatives and false positives; such an example is shown in figure 1.

‘Photographic methods’ have been regardless extensively employed for fluorescence and bioluminescence imaging of small animals as they offer very practical features, including the simplicity of implementation and operation and the safe, non-ionizing energies employed. Under controlled conditions, for example when imaging the same animal model over time, the results can reflect relative changes in a biological process and lead to a non-invasive evaluation of the underlying activity. In more complex schemes, however, for example in clinical measurements where the size, depth and optical properties of the area visualized are less accurately known, the accuracy of the method can deteriorate. Therefore, for applications in mainstream medical imaging and importantly for obtaining appropriate approvals for medical use, it is important to use systems that are accurate, i.e. they decompose the effects of tissue optical properties on the fluorescence images and in this way eliminate the appearance of false positives and false negatives that is possible on raw (uncorrected) fluorescence images.

An emerging strategy for improving the accuracy of photographic imaging is the development of normalization techniques that can compensate for the effects of optical properties on the resulting images. Normalization refers to cancelling the effects of tissue on the collected images; for example, the effects of tissue optical properties on corresponding fluorescence images collected. For being relevant to clinical use, such a method needs to be executed without compromising the scan times or simplicity of operation of conventional photographic imaging [7,13]. In this case, it becomes important to independently detect the fluorescence contributions and the contributions of optical properties, typically by employing multi-spectral imaging methods.

Multi-spectral imaging generally refers to imaging methods that capture multiple images at different spectral regions or methods that can resolve multiple spectra. The spectral regions can be selected adjacent and consecutive over a wider spectral window or have a different pattern for detecting particular fluorochromes of chromophores. In epi-illumination imaging, the multi-spectral decomposition can be applied either to the back-reflected (excitation) light or the fluorescence light. The normalization methods considered so far use at least on aspect of this multi-spectral analysis to provide data for correction. One normalization method divides images obtained at a fluorescence wavelength with images obtained at the excitation wavelength [16]. This approach has recently shown significant accuracy improvement over uncorrected images in the case of varying tissue absorption [7], while corrections for scattering variations or depth are also possible. Figure 1c depicts the effects of correction on the raw fluorescence image shown in figure 1b. Of importance for clinical use in this case is the simultaneous collection of images at different spectral bands of interest.
Figure 1. Example of normalized versus non-normalized fluorescence imaging. (a) Postmortem colour image of the surgically exposed abdominal area of a mouse in epi-illumination mode. The two white arrows show the lumbar lymph nodes injected with a mixture of Cy5.5 fluorescence dye and India ink, around the inferior vena cava (double line arrow). The orange arrow indicates an area of adipose tissue. (b) Raw fluorescence image showing low signal intensity from the lymph nodes compared with bright background signals because of internal light attenuation owing to the India ink, but increased fluorescence intensity from the adipose tissue owing to scattering of fluorescent light escaping laterally from the nodes. (c) Corrected image showing markedly improved fluorescence quantification, correctly resolving the underlying fluorescence activity in the nodes. Reproduced from Themelis et al. [7]. (Online version in colour.)

recorded over an identical field of view. For this reason, a multiple camera system visualizing through a common optical lens was developed and used, as shown in figure 2. Typically, all endoscopic or surgical imaging operates at video rate, often with the physician changing continuously the position of the endoscope or camera.
Figure 2. Example of epi-illumination imaging. (a) Real-time multi-spectral epi-illumination fluorescence imaging system developed for clinical imaging. The system uses three charge-coupled device (CCD) cameras that can simultaneously capture the same field of view. In this manner, different co-registered spectral bands can be collected in real time. (b) Colour image obtained with this system. (c) Corresponding normalized fluorescence image after the injection of the fluorescent agent IntegriSense (Visen Medical, Woburn, MA, USA; now PerkinElmer). Images courtesy of George Themelis, IBMI. Reproduced from Themelis et al. [7]. (Online version in colour.)

Therefore, the ability to collect multi-spectral images and process them at video-rate speeds to offer normalized performance needs to occur within a few tens of milliseconds to achieve video-rate operation. The system in figure 2a can collect colour images and two more channels that can be selected in different spectral bands, for example one collecting a fluorescence image and the other collecting an image at the excitation wavelength. Figure 2b shows a colour image of a mouse tumour and figure 2c shows correspondingly a normalized fluorescence image, rendered in pseudocolour and superimposed on the colour image. Similarly, other normalization methods have been proposed to improve the accuracy of normalized imaging over raw photographic fluorescence images [13,17].

Normalized epi-illumination imaging is an ideal tool for interventional imaging and it has been already applied in clinical studies using targeted and non-targeted fluorescence agents. The placement of our system in the operating room is shown in figure 3, which also summarizes measurements from imaging lymph nodes in a cervical carcinoma patient, as described in the study of Crane et al. [18]. The approach can operate in real-time, video-rate mode and attains large field of...
view rendering it appropriate for screening and overall for inspecting large tissue areas, which is necessary in surgery, colonoscopy, etc. Similarly, the method generally offers simplicity of operation, safety and high sensitivity for optical contrast of cancers close to the surface. Typical acquisition times range from a few milliseconds to a few seconds. Normalized epi-illumination systems can be made portable and to have small space requirements to be ideal for the laboratory bench or the operating room. They also naturally relate to the physician’s vision and field of view so that they can naturally integrate in several clinical settings.

4. Multi-spectral optoacoustic tomography

Another potent approach for detecting fluorochromes and other chromophoric agents is the use of multi-spectral optoacoustic methods. This technology has recently resolved, with high resolution, fluorescent and other photo-absorbing agents through several millimetres to centimetres of tissue [3,8]. The method
Clinical optical and optoacoustic imaging

Clinical optical and optoacoustic imaging is based on optoacoustic (photoacoustic) principles \cite{19–21} and can be applied to overcome major limitations of conventional optical imaging while retaining many of the advantages of photonic methods. This is achieved by combining the highly versatile optical contrast resolved with multi-spectral methods with ultrasonic resolution.

Multi-spectral optoacoustic tomography (MSOT) operates on the identification of the spectral signatures of reporter molecules and their decomposition from the spectra of intrinsic molecules such as haemoglobin, melanin and other natural tissue absorbers. A characteristic of the method is that it does not have strong sensitivity to scattering, which is an advantage as it can achieve resolution typical of ultrasound imaging and not of optical imaging. The detection process itself relies on optoacoustics, i.e. the detection of ultrasonic signals induced by the absorption of pulsed light emitted at multiple wavelengths. The amplitude of the generated broadband ultrasound waves reflects local optical absorption properties, multiplied by the local photon fluence. Correspondingly, MSOT images also retain a dependence on tissue optical properties, as the photon fluence generated in a tissue volume is directly related to its optical properties. This dependence is more evident with depth and, similar to epi-illumination imaging approaches, normalization or correction becomes important for achieving accurate performance. In this case, the availability of multiple wavelengths also offers possibilities for normalization; an area of current research developments.

Optoacoustic imaging has high potential for clinical translation, as it enables the detection of optical contrast with characteristics analogous to normalized epi-illumination imaging. A recent literature review from our group outlines many scanners developed for clinical use \cite{13}. It can offer real-time sensing, portability and the use of non-ionizing radiation. In addition, however, it offers high-resolution three-dimensional performance which is not available to normalized epi-illumination imaging. This aspect is illustrated in figure 4, showing results from real-time imaging of a female, eight-week-old CD1 mouse, which was anaesthetized with a mixture of ketamine and xylazine and injected intravenously with 0.3 \textmu mol of indocyanine green (ICG) solution. The results show the anatomical imaging ability of the method, resolving in this case optical differences demarcating the kidneys. Importantly, they also show the ability to resolve the ICG biodistribution in the kidneys as a function of time. The acquisition sequence in this case consisted of single pulse per slice and wavelength images; i.e. each single frame is collected with a single laser pulse, a process that lasts approximately 50 \mu s. Correspondingly, this approach can result in true ‘real-time’ imaging applications, as the sampling time is significantly shorter than most time scales common to biological changes.

In addition to the single-wavelength measurements shown in figure 4a, multi-spectral scanning over nine wavelengths (i.e. 750, 770, 790, 810, 830, 850, 870, 890 and 910 nm) was also performed. These data can be processed either by taking the difference of images acquired over time versus a baseline measurement at time $t = 0$ or by multi-spectral analysis in order to identify the ICG absorption spectral signature. The ability for spectral unmixing is important as, in this case, the detection can be achieved without baseline measurements, which are not generally available in clinical imaging. Conversely, multi-spectral scanning slows down the acquisition times. With current laser wavelength scanning times, the collection rate is approximately 1 s per frame which does not qualify as real-time.
scanning and it may be prone to movement errors. Correspondingly, the need for faster scanning technologies is important for realizing the full clinical potential of the technology.

MSOT operates optimally by selecting fluorochromes, other chromophores and photo-absorbing nanoparticles with distinct absorption spectra, in particular, spectra with steep changes over narrow wavelength bands. When using fluorescent

Figure 4. (a) Cross-sectional optoacoustic images at different time points of the kidneys of a female CD1 mouse illuminated at 800 nm after having injected 0.33 μmol of ICG. (b) Superposition of the difference image and single-wavelength image before injection. From Buehler et al. [22]. (Online version in colour.)
dyes, the emphasis is on low-quantum-yield fluorochromes, which convert a large part of the absorbed energy to thermal energy. The absorption spectrum of ICG has notable changes in the spectral window at 750–850 nm, compared with the absorption variation of the spectra of intrinsic tissue chromophores in this spectral region. Therefore, tissue contrast can be readily decomposed from the spectrum of ICG or another externally administered photo-absorbing agent. The use of dyes or photo-absorbing nanoparticles with large absorption cross sections is particularly helpful for optoacoustic signal generation as they can increase the sensitivity per injected particle; however, the ultimate determination of sensitivity depends not only on the ability of the agent to efficiently absorb light but also its ability to localize in appropriate amounts in the intended lesion. The latter is better achieved by agents of small size, such as dyes and organic molecules versus large particles. Therefore, the optimal MSOT agent should balance absorption cross section and size for achieving optimal sensitivity.

Optoacoustic imaging typically uses transducers in physical contact with tissue, similar to ultrasound imaging. Therefore, the method is not well suited for inspecting large fields of view, in analogy to lens-based detection using a camera, but it can generally offer much larger fields of view compared with optical microscopy. By detecting physiological parameters such as blood content and hypoxia, resolving anatomical contrast and imaging and quantifying the biodistribution of extrinsically administered agents, MSOT is therefore expected to find clinical application that resembles operation ultrasound applications. Detection of vessels and vascular diseases, characterization of the oxygenation status of various tissues and detection of disease biomarkers, especially using externally administered agents, for example in cancer or atherosclerosis, are areas where MSOT can bring important visualization solutions. Importantly, the method may be combined with normalized epi-illumination imaging for three-dimensionally resolving tissue structures at areas where epi-illumination imaging has identified suspicious lesions based on the normalized fluorescence signals.

5. Fibre-based catheter imaging

Fibre-based fluorescence imaging is an emerging imaging method that is suited for interventional cardiology procedures, in particular, intravascular imaging. The development of catheter-based fluorescence imaging has been showcased recently [23,24]. Fluorescence imaging can illuminate the underlying biology of atherosclerosis using a combination of molecular- and cellular-targeted imaging probes, together with sensitive, clinically applicable hardware detection modalities. Intravascular imaging is common, most notably through intravascular ultrasound and OCT. While these modalities offer primarily anatomical images, there is a high synergistic effect in the combination with fluorescence detection in order to offer physiological and molecular imaging of atheromas and stents. Important questions in this case are the performance in characterizing plaque risk and the overall condition of carotids, coronaries and other similar-sized vessel parts in vivo.

Recently, the use of NIR fluorescent molecular probes with sensitivity to inflammation has shown promise for sensitive and specific detection of inflammatory proteolytic activity in atherosclerosis [25]. While original
observations were based on one-dimensional manual pullback systems, rotational fibre systems were more recently developed and have shown in vivo imaging ability [24]. For clinical measurements, this basic approach should be expanded to normalization (correction) and the MSOT method in order to improve the accuracy of the observation and offer a robust assessment of biomarkers independently of the presence of blood and the vessel size.

6. Discussion

While human vision has been a standard of care in everyday healthcare applications, notably in endoscopies, surgeries and other interventions, a number of novel optical methods are emerging that have the potential to significantly enhance human vision. Besides microscopic methods such as OCT or confocal microscopy that are suitable for surface and sub-surface interrogations, deeper tissue imaging also becomes possible through optical and optoacoustic methods that correct for the nonlinear dependencies of light propagating in tissues in order to offer medical-grade performance ensuring accurate (quantitative) methods that eliminate the appearance of positive and negative artefacts.

Herein, through my own examples, I have attempted to illuminate optical systems with the potential ability to improve healthcare procedures. Even though OCT methods have been considered for deep tissue imaging for more than a decade, large field of view normalized fluorescence imaging methods, operating at video rates, appear to have more immediate clinical propagation potential in surgical and endoscopic procedures. Similarly, fibre-based fluorescence imaging may become an add-on method to existing intravascular imaging approaches. High-resolution MSOT also has high potential for key applications in surgery, dermatology and endoscopy by assessing the three-dimensional appearance of vessels, oxygenation status and disease biomarkers in suspicious lesions.

The combination of adept fluorescent and other photo-absorbing agents with the above imaging methods can shift the paradigm of many current human vision-based clinical procedures. Already one such study performed by the Technische Universität München and Helmholtz Zentrum München, and the University Medical Center Groningen has achieved the clinical translation of a folate-targeting fluorescent agent for identifying ovarian cancer during surgery; a study that will be published soon. Important to the practical propagation of fluorescence interventional imaging is the availability of additional fluorescent agents with molecular specificity for clinical use. There are several important agents that would be appropriate for clinical use safety-wise and can improve the identification of currently invisible lesions and processes. Many labelled antibodies and fragments have been widely used in biological research and have shown no toxicity in experimental studies. While such observations need to be documented accurately with appropriate studies, on a per labelled agent basis, there is a mounting possibility to explore such agents in clinical studies—in analogy to investigations ongoing in nuclear imaging applications. Overall, the approval for such agents for clinical use can be a lengthy process but the clinical use of carefully selected and validated agents can radically change the accuracy of interventional procedures, when compared with the current standard of clinical decision-making in this case, i.e. primarily human vision.
References


