Label-free Optical Techniques for Biomedical Diagnostics & Imaging: Challenges and Opportunities for Clinical Translation

Lead Authors:

Paul French, *Imperial College, United Kingdom;* **Laura Marcu**, *University of California - Davis, United States;* **Robert J. Nordstrom**, *National Institute of Health, United States;* **Juergen Popp,** *Leibniz Institute of Photonic Technology, Jena, Germany;* **Brian Wilson**, *University Health Network, Canada*

This white paper is based on the many contributions to an OSA Incubator held on 16-18 September 2015 at OSA Headquarters, 2010 Massachusetts Ave. NW, Washington, DC, USA. The Incubator (osa.org/label_free_incubator) was organized by the lead authors, the following participants contributed to discussions at the Incubator and to this white paper.

Anant	Agrawal	US FDA	USA	Ian	McDowall	Intuitive Surgical	USA
Robert	Alfano	City College of New York	USA	Mary-Ann	Mycek	University of Michigan	USA
Stefan	Andersson-Engels	Lund University	Sweden	Robert	Nordstrom	National Institute of Health	USA
Sanford	Asher	University of Pittsburgh	USA	Rakesh	Patalay	Imperial College of London	UK
Vadim	Backman	Northwestern University	USA	Francesco	Pavone	European Lab for Non-Linear Spectroscopy	Italy
Mikhail	Berezin	Washington University School of Medicine	USA	Brian	Pogue	Dartmouth College	USA
Rohit	Bhargava	University of Illinois at Urbana–Champaign	USA	Gabriel	Popescu	University of Illinois at Urbana–Champaign	USA
Irving	Bigio	Boston University	USA	Juergen	Popp	Leibniz Institute of Photonic Technology, Jena	Germany
Ji-Xin	Cheng	Purdue University	USA	Francisco	Robles	Duke University	USA
Richard	Conroy	NIH	USA	Kathleen	Schmeler	MD Anderson Cancer Center	USA
Max	Diem	Northeastern University	USA	Behrouz	Shabestari	National Institute of Health	USA
Wolfgang	Drexler	Medizinische Universität Wien	Austria	Lingyan	Shi	City College of New York	USA
Gregory	Faris	SRI International	USA	Jonathan	Sorger	Intuitive Surgical	USA
Michele	Follen	Brookdale University Hospital	USA	Paola	Taroni	Politecnico di Milano	Italy
Gerald	Fraser	NIST	USA	Taranjit	Tatla	Imperial College of London	UK
Paul	French	Imperial College of London	UK	Gary	Tearney	Wellman Center for Photomedicine	USA
Irene	Georgakoudi	Tufts University	USA	Bruce	Tromberg	University of California, Irvine	USA
Ewa	Goldys	Macquarie University	Australia	Alexandre	Tumlinson	Carl Zeiss Meditec	USA
Richard	Levenson	University of California, Davis	USA	Alex	Vitkin	Ontario Cancer Institute	Canada
Xingde	Li	Johns Hopkins University	USA	Lihong	Wang	Washington University in St Louis	USA
Kristen	Maitland	Texas A&M University	USA	Warren	Warren	Duke University	USA
Laura	Marcu	University of California	USA	Adam	Wax	Duke University	USA
Thomas	Mayerhofer	Leibniz Institute of Photonic Technology, Jena	Germany	Min	Xu	Fairfield University	USA

OSA Incubator Meeting
Collaborate • Innovate • Discover

This Incubator was supported in party by:

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH · UNITED STATES AIR FORCE

INTUITIVE SURGICAL®

NIST National Institute of Standards and Technology U.S. Department of Commerce

1. TABLE OF CONTENTS

2. Introduction……3

3. Overview of the Key Label-free Optical Techniques……5

A Transmitted/reflected/scattered light based techniques 5

A1. Mid-infrared spectral imaging for histopathology 5

A2 White light imaging 6

A3 Elastic-scattering spectroscopy and diffuse reflectance spectroscopy (at short distances) 7

A4 OCT 8

A5 Quantitative Phase imaging..... 10

A6 Imaging and spectroscopy with scattered light 11

A7 Photoacoustic imaging and tomography 12

B. Fluorescence-based techniques..... 14

B1 Steady-state autofluorescence readouts..... 14

B2 Time resolved autofluorescence readouts 16

C Nonlinear microscopy and Raman techniques..... 16

C1 Multiphoton fluorescence microscopy 16

C2 Harmonic generation..... 17

C3 Raman techniques 18

C4. Cerenkov imaging 20

D. Opportunities for multimodal application 20

4. Barriers/hurdles to translation……21

Technical challenges 22

Regulatory challenges 23

Clinical uptake challenges..... 24

5. Comercial perspectives……25

6. Next steps & Reccomendations……26

2. INTRODUCTION

While optical imaging and sensing is often viewed as a field where microscopy is the major contribution, clinical macroscopic or mesoscopic imaging of extended tissue areas is already an enormous biomedical market. Optical imaging is routinely used in endoscopy, colposcopy, colonoscopy, otolaryngology, dermatology, laparoscopy as well as primary care to visualise tissue *in situ*. Companies manufacturing light sources, fiber optics, specialized cameras and displays contribute to a multi-billion-dollar market and advances in each of these areas continue to improve the state-of-the-art. However, the demand for access to healthcare - and the cost of providing it - continue to increase beyond what current resources can accommodate, and there is considerable interest in new paradigms for detecting and treating disease at earlier stages and for increasing the effectiveness of therapies through personalised medicine. Furthermore, optical techniques are increasingly enhanced by digital computational solutions related to disease pattern recognition, and this can facilitate a degree of automation to allow expansion of diagnostic utility to "novice" operators and environments presently not contemplated: i.e. community allied health professionals.

Optical technologies can provide structural and molecular contrast in biological tissues beyond what is immediately visible, and there is a widespread aspiration to advance biomedical optics beyond visualisation of tissue towards "optical biopsy" to provide earlier, more accurate screening and diagnosis of disease. Moreover, such quantitative diagnostic tools lead to objective determinations of disease, rather than subjective interpretation by practitioners with variable skills. There is also considerable scope to guide intervention, e.g. by defining disease margins to avoid or minimize damage to critical tissues, so that normal function can be preserved as far as possible. This could be applicable to all treatment modalities: e.g. surgical, laser, PDT, radiotherapy. Convenient optical techniques could also help enhance the safety and quality of care delivery (e.g. optical sensing to ensure optimal positioning and placement of catheters, tubes, implants etc.). The potential to develop compact optical instruments is particularly important for minimally invasive procedures, including robotic procedures, while the potential for relatively low cost instrumentation is important for point-of-care applications.

While many optical techniques employ contrast agents – as is particularly exemplified in histopathology - label-free optical techniques that exploit endogenous contrast are of particular interest, since they can potentially be more directly translated to the clinic than is possible with techniques that require biological or chemical agents to be introduced into patients. This potential has stimulated many scientists and engineers to investigate and develop label-free optical techniques for clinical imaging and diagnostic spectroscopy, and the ability to acquire rich multidimensional molecular information has been demonstrated using a wealth of modalities, as summarised in Section 2. The same technologies may also be applied in conjunction with contrast agents, and thereby benefit from prior knowledge of the optical properties of the molecular probes, which is usually much more specific than prior knowledge of intrinsic tissue optical properties; but considerations of potential hazards can present major challenges when using exogenous contrast. Table 1 below summaries the relative advantages and limitations of optical techniques based on endogenous and exogenous contrast.

	Endogenous	Exogenous
Advantages	Absence of administered "dye" ⇒ lower cost ⇒ no potential toxicity ⇒ reduced regulatory barriers ⇒ no dependence on pharmacokinetics/biodistribution properties Access to intrinsic structural & functional biomarkers and analytes, both normal and diseased Direct correlation with histopathology for validation	Strong signal ⇒ reduced imaging time &/or ⇒ less sensitive /expensive detectors &/or ⇒ reduced excitation intensity Known optical spectrum ⇒ simplifies measurement ⇒ easier quantification Multiplexed detection Target known biomarkers Monitor therapeutic agents that have optical signatures Use of a "consumable" may enhance commercial value of technique
Limitations	Spectral 'signature' not known a priori ⇒ characterization studies required May be signal-to-noise or background-limited May require sophisticated analytic algorithms	Need to optimize dose and timing ⇒ uncontrolled variation with disease site/stage and between patients Need for FDA-approved safety studies Cost/toxicity/regulatory barriers Restricted biomarkers accessible

Table 1. Comparison of endogenous (label-free) and exogenous contrast for biomedical optical imaging and spectroscopy

Besides direct application to patients, label-free optical techniques also have the potential to add value to histopathology, where optical microscopy using exogenous contrast underpins the gold standard for clinical diagnosis, by enabling a faster turnaround. Label-free techniques can also be important for preclinical studies and assays, particularly where such techniques enable real-time observation and study of disease progression in cell-based or animal models. In addition to providing significant scientific and commercial value for clinical research and for drug discovery, these capabilities would also be of great utility for veterinary applications.

However, any clinical (optical) technique should be correlated to the current gold standard for validation, which is histopathology for most indications, and mapped to all the patient pathways in which it might be used. The costs and impact can then be discussed in the appropriate context, which will require investment for multidisciplinary clinical input. Considerations of currently incurred pathway costs, delays and "wastage," will help define appropriate requirements for new optical imaging tools that may achieve clinical and commercial impact. Unfortunately, to date, few label-free optical techniques have achieved significant clinical or commercial success. This white paper aims to review the label-free approaches to biomedical optical imaging and spectroscopy, and to outline the barriers and hurdles to clinical translation, with a view to suggesting how to accelerate such translation.

A key issue for optical measurements and imaging in biological tissue is the strong scattering of visible and near infrared (NIR) radiation, which severely compromises measurement and imaging of thick tissue samples – and therefore in vivo application. There have been many approaches devised to address this challenge, particularly exploiting various properties of laser radiation including its high degree of directionality, spectral brightness, polarization, coherence and potential for to provide high intensity with low average power from ultrashort pulse trains – the latter also enabling accurate time-resolved measurements. Many of these approaches are outlined in the following section. Scattering of optical radiation in biological tissue tends to decrease with wavelength and the advent of convenient laser technology, particularly ultrafast Ti:Sapphire lasers, stimulated research on techniques exploiting the transmission NIR window of biological tissue (~650-1000 nm). Today there is increasing interest to develop techniques to exploit "windows" between water absorption bands in the short wavelength infrared radiation (SWIR, ~1000-2500 nm) region, particularly the "golden window" (~1620 – 1880 nm).

3. OVERVIEW OF THE KEY LABEL-FREE OPTICAL TECHNIQUES

A Transmitted/reflected/scattered light based techniques

A1. Mid-infrared spectral imaging for histopathology

MIR spectroscopy has a long tradition in being employed to investigate the conformation and other spatial and compositional aspects of biomolecules. For application to biological tissue, broadband IR radiation with wavelengths between about 2 and 20 μm is employed to provide molecular contrast derived from vibrational energy level absorption signatures that provide quantitative information on the (bio-)chemical constituents of a sample. Mid-infrared spectral histopathology (SHP) thus offers comparable sensitivity and specificity to other vibrational spectroscopic modalities such as Raman or pump probe spectroscopy. Mid-IR spectra typically comprise superpositions of ~30-50 independent spectral features (with $S/N>10^3$) that mainly represent differences in the proteome and metabolome. These complex fingerprints present challenges in terms of decomposition into individual spectral signatures but can leverage advances in computing power to provide powerful chemometric analysis. Laboratories in Europe and the US have collected and analyzed data sets with thousands of patients and comprising over 109 tissue spectra. Predictive accuracies of about 92 – 95 % have been achieved, as compared to classical histopathology augmented with immuno-histochemistry. The very high absorption of water in the MIR spectral range means that MIR techniques are not well suited for in vivo applications due to the high water content of cells and tissue but the technique is appropriate for analysis of thin histological sections. Owing to the relatively long wavelengths used, the spatial resolution is not sufficient for sub-cellular imaging. Nevertheless, even without imformation on cell morphology, MIR spectroscopy can distinguish dozens of tissue types and disease states and is therefore particularly well suited for histopathology, achieving results that are comparable with the existing gold standards. The lack of requirements for staining and potential to apply to fresh ex vivo tissue sections make MIR-SHP appropariate for inter-operative applications such as Moh's procedure.

Advantages/opportunities:

- Highly sensitive/specific vibrational molecular contrast
- Ability to direcetly replace established (H&E) hisopathology with superior speed/performance

Disadvantages/challenges:

- Light sources and detectors are expensive
- Water absorption limits depth in tissue
- Vibrational signatures are complex, requiring significant computation

Clinical applications:

- SHP is applicable to a wide variety of organs and statistically significant results have been reported for lung, breast and prostate cancer, along with pilot studies on many other organs (including brain, cervix, esophagus, colon, lymph nodes).

There are no commercial products as yet.

References

Molecular Pathology via Infrared and Raman Spectral Imaging by M. Diem et al. in *Ex-vivo and In-vivo Optical Molecular Pathology*, J. Popp (Editor), ISBN: 978-3-527-33513-8. Wiley-Blackwell (2014).

Infrared spectroscopic imaging protocols for high-throughput histopathology by R. Bhargava, I. W. Levin, *Vibrational Spectroscopy for Medical Diagnosis*, M. Diem, P. R. Griffiths, J. Chalmers, eds., John Wiley and Sons, Chichester, UK (2008)

A2 White light imaging

Current endoscopic-based clinical techniques are based on white light imaging. They enable visualization of various parts of the body including gastrointestinal tract (GI tract), the respiratory tract, the urinary tract and the female reproductive tract. Endoscopic assessment of these tracts with tissue sampling (biopsy) serves as primary diagnostic for diseases in these tracts including cancer. Moreover, endoscopy plays an important role in guiding various interventions and surgical procedures in orthopaedics, endodontics, spinal, abdominal cavity, etc. Over the past two decades, numerous methods were evaluated and implemented for image enhancement by modifying the conventional endoscopes. For example, narrowband imaging (NBI) enhances the visualization of micro-vessels morphology in superficial neoplastic lesions or small irregularities associated with non-neoplastic inflammatory changes. High definition or high resolution endoscopy allows for visualization of subtle mucosal details. Miniaturization of fiber arrays and cameras has permitted the ability to image body channels which were previously only accessed invasively or through small fiber ports. The fill or “capsule” camera is an example of such innovation. Recent include hyperspectral imaging and spatial frequency modulation. There are many instances of hybrid systems that integrate traditional white light imaging with optical modalities based on fluorescence, scattering, and absorbion and non-optical modalities providing more specific molecular contrast.

Advantages/opportunities:

- White light endoscopy is considered safe
- No advanced training required for standard-definition or high-definition endoscopy

Disadvantages/challenges:

- Standard endoscopy does not provide much molecular specificity and diagnosis usually requires confirmation by biopsy with the risk or missing regions of diseased tissue
- More advanced techniques such as chromoendoscopy and endomicroscopy require additional procedure, additional procedure time, dedicated equipment, and advanced training.

Clinical applications:

- Standard endoscopy including high-definition is now part of standard of care
- Cancer management (screening, diagnosis, monitoring response to therapy) in multiple tracts (GI, respiratory, urinary, etc)
- Surgical guidance including laparoscopy procedures, orthopaedic surgery, etc

Broad commercialization

References

Multicenter, prospective trial of white-light imaging alone versus white-light imaging followed by magnifying endoscopy with narrow-band imaging for the real-time imaging and diagnosis of invasion depth in superficial esophageal squamous cell carcinoma, Gastrointestinal Endoscopy, Masahide Ebi, Takaya Shimura, Tomonori Yamada, Takashi Mizushima, Keisuke Itoh, Hironobu Tsukamoto, Kenji Tsuchida, Yoshikazu Hirata, Kenji Murakami, Hiroshi Kanie, Satoshi Nomura, Hiroyasu Iwasaki, Mika Kitagawa, Satoru Takahashi, Takashi Joh, 81(6): 1355 – 1361, 1015

Sensitivity of Narrow Band Imaging Compared With White Light Imaging for the Detection of Endometriosis, Journal of Minimally Invasive Gynecology, Fermin F. Barrueto, Kevin M. Audlin, Lisa Gallicchio, Charles Miller, Ryan MacDonald, Edgar Alonsozana, Mary Johnston, Kathy J. Helzlsouer, Volume 22(5): 846-852, 2015

A3 Elastic-scattering spectroscopy and diffuse reflectance spectroscopy (at short distances)

In elastic scattering spectroscopy (ESS) tissue is illuminated by light typically in the spectral range between 300 – 750 nm, with the light usually being delivered to the sample and collected using optical fibres that are typically separated by less than 0.1 cm. The scattering and absoprption of the light depends on the properties of the sample and the wavelength, and ESS can provide information about the absorbance and scattering properties of the sample and, therefore, about the tissue microstructure and biochemistry, making ESS potentially useful for histopathology and optical biopsy. The offset between delivery and collection optical fibres (both in optical contact with the tissue surface) ensures that the light that is collected has undergone a few elastic scattering events (i.e. without a change in wavelength or energy) but has not been specularly reflected from the tissue surface. For this reason elastic scattering spectroscopy is often known also as diffuse reflectance

spectroscopy, although the light collected does not meet the usualy definitions of “diffuse,” given the small source-detedctor separation. The degree of scattering and its angular dependence are influenced by the size and the shape of the scatterers and will directly impact the detected spectrum if the collecting fiber is sufficiently close to the illuminating fiber. (For separations ≥ 0.5 cm, the diffusion approximation is usually valid and sensitivity to scatterer size and shape is lost.) While ESS is generally aimed at extracting micro-architectural properties of cells and tissues, it is also sensitive to strong absorbing species, including chromophores that are not fluorescent (such haemoglobin), which can yield additional diagnostic information. One emerging technique which transforms elastic-scattering spectroscopy and diffuse reflectance spectroscopy at short distances into an imaging modality is high spatial frequency modulated imaging (HSFDI) discussed in A6.

Advantages/opportunities:

- Low cost
- Compact probes
- Rapid readout
- Configurable with neglible phototoxicity

Disadvantages/challenges:

- Localised interrogation region
- May not be sensitive to subtle biochemical or metabolic changes preceding structural changes

Clinical applications:

- Main clinical application is *in situ* histopathology, where it can be used to detect the cellular changes that accompany cancer (and other pathologies) and which can be used to discriminate nondysplastic and dysplastic tissue and cancer.
- Diffuse reflectance spectroscopy at short source-detector separations has also been applied to monitor the local pharmacokinetics of chromophoric drugs in animal models, and has been used in the pharmaceutical industry, e.g. to identify and quantify active pharmaceutical ingredients in tablets.

Limited commercialization

References

Spectroscopic Sensing of Cancer and Cancer Chemotherapy, current status of translational research Irving J Bigio and Stephen G. Bown, *Cancer Biology and Therapy* **3**, no.3, 259-267 (2004).
Elastic-Scattering Spectroscopy and Diffuse Reflectance, J. R. Mourant and I. J. Bigio, Biomedical Photonics Handbook, T. Vo-Dinh, ed., CRC Press (Jan. 2003).
Quantitative Biomedical Optics: Theory, Methods, and Applications by Irving J. Bigio and Sergio Fantini, Cambridge University Press (2016).

A4 OCT

Optical coherence tomography (OCT) – the non-invasive optical analogue to ultrasound –is based on low coherence interferometry and is non-invasive, simple to implement with

integrated fibre-optics components and enables high sensitivity (> 90 dB), high dynamic range and high speed, decoupling lateral and depth resolution to permit 4D imaging with sub-µm axial and lateral resolution in real time. This is especially advantageous in ophthalmology (retinal imaging) due to the low numerical aperture of the human eye. Since the necessary key technological components (light source, detector, etc.) are relatively compact and originate from telecommunication industry, they are relatively low-cost with proven technology. In addition, the well established complementary ecosphere involving academics and industry engaging in research and development significantly drives OCT innovation and its rapid translation. The already considerable clinical success of OCT could be enhanced by extending the imaging depth, speed and functional contrast while reducing cost and size. Imaging depth can be increased by moving to longer wavelength radiation but this is limited by available light sources. There is likely to be a trend from spectral domain OCT @ 800 nm versus swept source OCT @ 1060 nm and future developments will see systems operating at wavelngths longer than 1500 nm, taking advantage of lower attenuation (absorption and scattering) in biological tissue. For more widespread clinical application, this will need fast (>100kHz), cheap (<$5k) swept sources over broad bandwidth (>100 nm) with sufficient power (> 10 mW) that ideally will be akinetic for higher reliability. Higher scanning/imaging rates will require increased parallelization (multiple beams, line field, full field). However, this will increase the challenges associated with data analysis and visualisation, which need to be real-time. More cost-effective, compact or even hand held OCT systems will find increasing areas of application including in needles and in multimodal systems. Increased functional contrast, e.g. using nanoparticles or microbubbles will further extend applications.

Advantages/opportunities:

- Broadly applicable depth resolved imaging to ~ 2 mm depth in tissue with high axial resolution
- High speed
- Compact and integrated with fibre-optic using proven technologies from optical telecommunications
- Configurable with neglible phototoxicity
- Enormous development and application communities
- Already used in humans thus standards and methodologies for clinical applications have been established
- Several devices have been commercialized thus a history of regulatory approval exits

Disadvantages/challenges:

- Mainly sensitive to morphology but not to chemical composition.
- Limited penetration depth since it needs to detect ballistic light signals

Clinical applications:

- Ophthalmology, cardiology, dermatology, gastrointestinal, dentistry, oncology, digital pathology
- Further application fields could include intraoperative, (intra)surgical OCT – therapy monitoring, surgical guidance ...

With several million A-scans per second and up to 1-2 mm penetration depth. OCT (especially in ophthalmology) is considered as one of the most innovative and successfully translated imaging techniques with substantial economic impact (more than 50 companies involved; > 10 patents granted; > 12k publications, $ 1.5 bn in 2020 with about 10% CAGR and more than 70% market share among the optical imaging techniques) and clinical acceptance in many fields.

References

Biomedical Imaging using optical coherence tomography, J. G. Fujimoto et al. in *Biomedical optical imaging* by J. G. Fujimoto and D. L. Farkas (Editors), Oxford University Press 2009.
W. Drexler and J.G. Fujimoto, Editors, Optical Coherence Tomography: Technology and Applications , 2nd edition, ISBN 978-3319064185, Springer Series in Biological and Medical Physics, Biomedical Engineering, Springer Verlag, Berlin, Heidelberg (2015).

A5 Quantitative Phase imaging

QPI uses interferometry to measure optical path differences to nanometer precision and can be implemented in scanning or wide-field imaging configurations to provide real time phase images, e.g. of cellular dynamics, that can provide readouts of physiological function, such as dynamics in tumour models, cell growth, intracellular traffic, cytoskeleton dynamics and cell differentiation. It can also be applied to study and quantify nanoarchitecture of tissue slices, which can be used for diagnosis and prognosis. Other applications include blood testing in unstained smears. It can be implemented on existing microscopes to provide complementary information, e.g. to fluorescence imaging. Like OCT, it is a non-resonant optical technique and so photobleaching and phototoxicity are not major issues. Similarly, QPI lacks the molecular specificity of fluorescence or vibrational spectroscopic techniques. Recent advances include tomographic phase microscopy, diffracted light tomography and the use of phase retrieval techniques.

Advantages/opportunities:

- Enables extended live cell imaging without photoxicity and photobleaching
- Quantitative measurement of cell growth and tissue architecture
- Nanoscale sensitivity, e.g. to measure membrane fluctuations, nanovesicle transport and nanoarchitecture in tissues
- 3D tomographic imaging of unlabeled cells
- Integration with existing (fluorescence) microscopes

Disadvantages/challenges:

- Lack of molecular specificity
- Phase stability issues due to mechanical vibrations, air fluctuations, etc., although common path methods can address this
- Resolution can be limited by speckle noise when using lasers but white light approaches can address this
- Limited to thin specimens and tissue sections

Clinical applications:

- Blood testing: QPI can produce a complete blood count from a smear without staining; diagnosis of malaria and sickle cell disease has been demonstrated
- Cancer diagnosis: the sensitivity to tissue nanoarchitecture provides diagnostic value in unlabelled biopsies and there are potential applications in cytometry. Recent studies show that QPI can distinguish between aggressive and indolent tumours.

Limited commercialisation to date but this is chaging rapidly with at least ten companies actively developing products.

References

Cell refractive index tomography by digital holographic microscopy, F. Charriere, A. Marian, F. Montfort, J. Kuehn, T. Colomb, E. Cuche, P. Marquet and C. Depeursinge, Opt. Lett., 31, 178-180 (2006)
Quantitative phase imaging of cells and tissues, G. Popescu (McGraw-Hill, New York, 2011)
White-light diffraction tomography of unlabeled live cells, T. Kim, R. J. Zhou, M. Mir, S. D. Babacan, P. S. Carney, L. L. Goddard and G. Popescu, Nature Photonics, 8, 256-263 (2014)

A6 Imaging and spectroscopy with scattered light

Deep tissue (up to several centimetres) spectroscopy and imaging is a significant field of development where physiology can be monitored in real time, and the signals can be combined with different eletrophysiological or mechanical information streams to provide predictive or diagnostic information. Diffuse optical spectroscopy (DOS) can be applied to the local measurement of tissue optical properties and diffuse optical imaging (DOI) or diffuse optical tomography (DOT) can be used to evaluate the spatial distribution of these properties. Numerous studies have demonstrated that deep (~1-10 cm) tissue measurement of signals originating from hemoglobin, oxygen saturation, water, lipids, collagen, betacarotine, bilirubin are possible, along with scattering signatures that originate from sub-microscopic cellular/structural components. Such measurements frequently utilise the NIR "golden windows" of low tissue attenuation. The scattering spectrum, angular and coherence emission patterns in tissue can provide measurements of tissue ultrastructure with innovation continuing to improve cellular/matrix level sensitivity. Current advances in coherence, angular and spatial frequency modulation will all contribute to new methods for disease diagnosis based upon morphological differences. Temporal characteristics of the optical signals can be associated with time-varying and dynamic physiological processes that in turn yields potential diagnostic information. Imaging with spatial frequency domain imaging (SFDI) is an emerging methodology for wide field quantification of tissue scattering properties and molecular absorption features *in situ* and *in vivo*. This imaging approach is particularly useful to quantify pathological features which have a cellular or tissue matrix transformation to the surface detectable by sub-diffuse scattering changes. HSFDI can be regarded as the imaging counterpart for elastic-scattering spectroscopy and diffuse reflectance spectroscopy at short distances. The phase function of the tissue sensitive to its sub-cellular and cellular structure can also be potentially quantified.

Advantages/opportunities:

- High penetration depth ranging from a few centimetres to over 10 cm depending on tissue type

- Relative simple and inexpensive experimental apparatus
- Configurable with neglible phototoxicity
- Already used in humans thus standards and methodologies for clinical applications have been established
- Several devices have been commercialized thus a history of regulatory approval exits
- Can be compact and so suitable point-of-care and/or portable devices, continuous monitoring
- HSFDI can provide high resolution real time images of tissue cellular or tissue matrix transformation near the surface

Disadvantages/challenges:
- Deep tissue imagin is computationally complex – often with long data analaysis times whereas imaging limited to near surface is much simpler and can be done real time.
- Some measurement approaches require complex calibration models
- Limited functional contrast

Clinical applications:
- Pulse oximetry – now broadly used in clinical settings (success story).
- Tissue oximetry with applications to: 1) skeletal muscle studies (oxygen consumption and muscle metabolism), 2) brain pathological hemodynamics and development in infants.
- Brain functional imaging
- Breast cancer detection, diagnosis, imaging, monitoring of the efficacy of therapy.
- Skin cancer screening
- Prostate cancer detection
- Histological evaluations and cancer margin detection using HSFDI

Limited commercialization but this is chaging rapidly with at least one companies actively developing products relating to SFDI.

References

Quantitative Biomedical Optics by I. J. Bigio and S. Fantini, Cambridge University Press 2016.
Diffuse Optical Spectroscopy: Frequency-Domain Techniques, A. E. Cerussi and B. J. Tromberg in Biomedical Photonics Handbook Volume II by Tuan Vo-Dinh, CRC PressTaylor & Francis Group 2015.
Diffuse optical imaging using spatially and temporally modulated light, T. D. OSullivan, A. E. Cerussi, D. J. Cuccia, and B. J. Tromberg. J. Biomed. Opt., 17(7), 071311 (2012).
Diagnosis of the phase function of random media from light reflectance, Min Xu. Sci. Reports, 6, 22535 (2016)
Biomedical Photonic, Spectroscopy ,and Microscopy, David Andrews,Wiley 2015, Chapter 9 - light scattering in turbid media

A7 Photoacoustic imaging and tomography

Photoacoustic imaging (PAI) and photoacoustic tomography (PAT) use an ultrashort pulsed laser (with power restricted below the safety limit) to produce ultrasound molecular vibrations via a transient thermal absorption in soft tissue that is converted in mechanical

energy or ultrasound. Thus inner structure is visualized by reconstructing the initial pressure distribution in the object and, because scattering of ultrasound in biological tissue is 100-1000 times less than that of photons, this approach can provide optical absorption contrast with higher-resolution and/or deeper penetration depth compared with all-optical imaging modalities. PAI and PAT can be used to image numerous endogenous biological molecules including oxyhemoglobin, deoxyhemoglobin, melanin and lipids. At depths beyond the optical diffusion limit, the spatial resolution is primarily determined by the center frequency and the bandwidth of the ultrasonic detection system, but the worse the ultrasonic penetration depth decreases as the frequency increases. Multiscale imaging from organelles to organ level is possible but spatial resolution decreases with imaging depth.

Advantages/opportunities (both microscopy and macroscopy):

- Avoids constraints of optical scattering on imaging depth – reaching 7 cm depth or 14 cm in transmission
- Multiscale imaging achieved by scaling depth and resolution
- High resolution (>90 nm) at shallow depths – able to resolve capillaries, cells and organelles
- Can detect fluorescent or non-fluorescent chromophores
- Spectral discrimination of multiple chromophores
- Functional imaging derived from endogenous chromophores including metabolic rate of oxygen and glucose uptake
- Molecular imaging of targeted contrast agents
- Real-time imaging

Disadvantages/challenges:

- Requires direct contact between ultrasonic transducers and the biological tissue
- Need to overcome the skull aberration for adult human brain imaging (ultrasound suffers significant attenuation and phase distortion in thick bones)
- Still to demonstrate clinical efficacy for targeted applications

Clinical applications:

- Breast cancer detection and imaging (now in clinical trials)
- Melanoma cancer screening
- Intravascular catheter imaging
- Neonatal brain imaging
- Prostate cancer detection
- Guided sentinel lymph node needle/core biopsy for breast cancer staging

Commercialization of PA technique is focussed primarily on products for preclinical and basic research at different locations world, particularly providing scanning PAT imaging products based on a high-frequency, high-resolution B-mode ultrasound imaging system.

References

Photoacoustic Imaging and Spectroscopy, Lihong V. Wang (Editor), CRC Press 2009.

B. Fluorescence-based techniques

Fluorescence provides a powerful means of achieving molecular contrast in a wide range of biological and medical applications. Label-free approaches can utilise the label free autofluorescence from naturally occurring (endogenous) fluorophores in biological tissues that can provide contrast between different states of diseased tissue. This could be useful for diseased tissue in-situ diagnosis and histopathology – and also for inter-operative surgical applications such as the determination of tumour margins. Targeted probes (exogenous) can also be applied that localize in specific cellular locations, e.g. binding to specific receptors on the cell membrane. There are many fluorescence imaging and metrology modalities single-photon or multi-photon excitation with detection techniques including steady-state or time-resolved intensity measurements with spectroscopic analysis based on multispectral or hyperspectral detection, polariation and fluorescence lifetime.

Tissue autofluorescence originates primarily from specific endogenous fluorophores with absorption spectrum in UV-VIS wavelength range. This includes amino acid trypotophan, structural proteins (elastin and collagen and cross-links), co-enzyme involved in cellular metabolism (reduced NADH, oxidized flavins (FAD and flavin mononucleotide), lipofuscin, keratin, lipids, lipoproteins and porphyrins. Autofluorescence measurements using single-photon excitation allows for interrogation of tissue composition within 300-400 micrometers depth. As discussed in section C1, the use of multiphoton excitation enable increased depth of interrogation up to 1-1.5 mm. While autofluorescence can be measured or imaged using conventional intensity measurements, it is challenging to make sufficiently quantitative measurements for *in vivo* diagnostic applications since the autofluorescence intensity signal may be affected by fluorophore concentration, variations in temporal and spatial properties of the excitation flux, the angle of the excitation light, the detection efficiency, attenuation by light absorption and scattering within the tissue, and spatial variations in the tissue microenvironment altering local quenching of fluorescence. For ex vivo histopathology of thin biopsy tissue sections, however, these limiting factors may not be significant. Clinical applications would be enhanced by databases correlating the fluorescence signatures with distinct pathologic features. However, the development of such database requires recruitment of 100's of patients, and require several years with studies conducted in parallel at multiple medical centres.

B1 Steady-state autofluorescence readouts

Because the origins of tissue autofluorescence are so complex, simple intensity mesurements are not able to provide useful quantitative information. Spectrally resolved imaging of autofluorescence has therefore received significant attention and the use of two or more spectral emission windows can be used to improve quantitation through ratiometric means, although the heterogeneity in the distribution of tissue fluorophores and their broad, heavily overlapping, emission spectra can limit the discrimination achievable. Algorithms are being developed to spectrally unmix different components, which could recover the absolute fluorophore concentration maps from autofluorescence images of thin tissue sections. For cancer the use of ultraviolet excitation radiation (e.g. 290 nm, 300 nm, 340 nm and 380 nm) is showing promise for the diagnosis of cancer.

Advantages/opportunities

- Steady-state instrumentation is relatively and low-cost and already tested and used in clinical settings.
- Simple instruments can be miniaturized, including for development of portable or point-of-care devices.
- Can provide fast characterization of tissue composition to aid diagnosis
- Light can be delivered-collected through fiber-optic probes and imaging bundles for straightforward integration with endoscopes, biopsy needles and other surgical instruments

Disadvantages/challenges:

- Steady-state techniques have relatively low specificity and are typically are used in conjunction with reflectance spectroscopy,
- Require complex calibration methods and computational models to account for factors that affect the non-uniform illumination, changes to fluorescence excitation-collection geometry, presence of endogenous absorbers etc.
- Shallow light penetration for high resolution imaging (with cellular resolution) although diffuse optical imaging methods can also be adapted for fluorescence. Diffraction-limited imaging depth can be extended with multiphoton excitation but this requires relatively expensive and complex instrumentation.
- Lack of standards for calibration
- It is challenging to obtain the absolute fluorophore concentrations for *in vivo* measurements.
- Poor understanding of and limited guidance for permissible light (exposure/radiation) dose for internal organs.

Clinical applications:

- Mainly oncology: Steady-state techniques currently applied to cancer diagnostics (tumor detection, staging and grading, border delineation) for GI tract (oral cavity, oesophagus, colon), respiratory tract (lung/bronchi, larynx) cervical cancer, brain, cervix, breast, skin). Several commercial devices and clinical trials.

Some commercial instruments including endoscopes and dermoscopes utilise steady-state autofluorescence readouts

References

Anita Mahadevan-Jansen and Steven C. Gebhart *Steady State Fluorescence Spectroscopy for Medical Diagnosis* (Chaper 20, pp: 761-798) in Optical-Thermal Response of Laser-Irradiated by Ashley J. Welch and Martin J.C. van Gemert (Editors), 2nd Edition Springer 2011.
Laser induced fluorescence spectroscopy from native cancers and normal tissues , Ralfano et al. IEEEJ Quantum Electron, 20,1507(1984);
Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. Ramanujam, N.. Neoplasia (New York, N.Y.), 2(1-2), 89117. (2000)

B2 Time resolved autofluorescence readouts

There is increasing interest in exploiting fluorescence lifetime contrast to analyze tissue autofluorescence since fluorescence lifetime measurements are inherently ratiometric and therefore largely unaffected by many factors that can compromise autofluorescence intensity measurements. Time-resolved measurements can be used to enhance diffuse light modalities and fluorescence lifetime imaging (FLIM) can provide quantitative molecular contrast

Advantages/opportunities

- Lifetime techniques have demonstrated ability to improve the specificity of measurement. Currently tested in clinical settings by a few research groups.
- FLIM is easily implemented in systems already ustilising multiphoton excitation
- Fluorescence lifetime can provide information on metabolic changes (e.g. for cancer diagnosis) and changes in tissue matrix components

Disadvantages/challenges:

- Time-resolved /lifetime systems require relatively expensive instrumentation (pulsed lasers, cameras, detectors) and there are limited options for the required pulsed (sub-ns) light sources).
- Complex computational models and powerful computers may be required to analyse tissue autofluorescence decay profiles
- Multiphoton FLIM cannot be easily adapted to fiber-optic probes or endoscopes and is currently limited to skin diseases.
- Lack of standards for calibration and poor understanding of permissible light (exposure/radiation) dose for ultrashort pulsed readiation, particularly for internal organs.

Clinical applications:

- Mainly oncology with clinical trials for various cancers (brain, head and neck, skin, colon). Studies are underway exploring applications to heart disease and osteoarthritis.

Limited commercialisation with commercial device for multiphoton tomography of skin.

References

Fluorescence Lifetime Spectroscopy and Imaging: Principles and Applications in Biomendical Diagnostics, by Laura Marcu, Paul M. W. French, Daniel S. Elson (Editors), Taylor & Francis Group CRC Press 2014

C Nonlinear microscopy and Raman techniques

C1 Multiphoton fluorescence microscopy

Multiphoton microscopy can be envisaged as an extension of laser scanning confocal microscopy where the use of multiple, longer wavelength photons to nonlinearly excite tissue fluorophores provides advantages in terms of reduced attenuation of the light by biological tissue. Combined with the opportunity to realise optical sectioning with no confocal detection pinhole, which is important when imaging in scattering media, these

advantages make multiphoton fluorescence microscopy a powerful technique for *in vivo* optica biopsy. Tradiationally mutlitphoton microsopy has utilised ultrashort pulsed radation in the 750-1150 nm window, typically employing femtosecond Ti:Sapphire lasers. To increase the imaging depth in tissue, there is much recent interest to utilise the optical windows further into the NIR where tissue attenuation is lower, including the "golden window" at 1600 to 1870 nm, with two photon or three photon excitation.

Advantages/opportunities

- Ability to use optimal wavelengths to maximise tissue penetration
- Multiphoton fluorescence microscopes can be combined with other modalities such as harmonic microscopy and CARS

Disadvantages/challenges:

- Requirement for ultrafast lasers that increases cost of instruments
- High intensities require laser scanning modality that is typically slower than real-time

Clinical applications:

- Mainly oncology with clinical trials for various cancers. Studies are underway exploring applications to ophthalmology.

Limited commercialisation with commercial device for multiphoton tomography of skin.

References

Nonlinear magic: multiphoton microscopy in the biosciences. Zipfel, W.R., Williams, R.M., and Webb, W.W., Nat. Biotechnol., 21, 1369–1377 (2003).

Transmission in near infrared optical windows for deep brain imaging , Lingyan Shi , Laura Sordillo, A Rodriques –Contreras, R Alfano, J Biophotonics 9,38-43(2016).

C2 Harmonic generation

Second harmonic generation (SHG) and third harmonic generation (THG) of radiation is routinely achievable in multiphoton microscopes using ultrashort pulses in the femtosecond range that provide electric fields high enough to stimulate non-linear polarization in biological tissue. The quadratic term of the nonlinear polarization results in SHG and the cubic term in THG.

A key advantage of harmonic generation microscopy is the possibility to use light with a convenient wavelength, e.g. with a large penetration depth in tissue, and the generated frequency-doubled or -tripled light can easily be separated from the exciting light or light arising from other effects such as fluorescence. In general, image acquisition is performed employing a laser scanning microscope and similar lateral and axial resolutions are achieved as for multiphoton microscopy.

The generated frequency-doubled or tripled light is coherent and in isotropic materials it is suppressed by destructive interference between the incident and generated light. When the irradiated tissue is anisotropic and lacks a centre of inversion, however, the destructive interference does not occur and the generated SHG or THG signals can provide information concerning the anisotropy. SHG is generated in materials where the anisotropy is intrinsic

on a wavelength scale, e.g. in tissues that contain periodic collagen structures, while THG is generated in materials presenting anistropy on the scale of the focused beam waist, such as boundaries that present a difference in the dielectric constant. Accordingly it is possible to use SHG and THG to investigate a multitude of collagen-rich tissues, such as the cornea, tendons, arteries, and human skin – where information can be obtained concerning the orientation of the fibrils, keloidal alterations, fibrosis, alterations in thermally treated skin, and changes in collagen in the microenvironment of tumors. The collagen organization in this microenvironment can be related to the tumor invasiveness and can be used to assess and to predict the development of a tumor from an in situ to an invasive stage. Harmonic generation techniques may therefore be useful for in-vivo diagnosis or staging of cancer.

Advantages/opportunities

- Ability to use optimal wavelengths to maximise tissue penetration
- Easily implemented on multiphoton fluorescence microscopes and can be combined with other modalities such as CARS

Disadvantages/challenges:

- Lack of chemical information
- Requirement for ultrafast lasers that increases cost of instruments
- High intensities require laser scanning modality that is typically slower than real-time

Clinical applications:

- Mainly oncology with clinical trials for various cancers. Studies are underway exploring applications to ophthalmology.

Limited commercialisation with commercial device for multiphoton tomography of skin.

References

Multiphoton Microscopy and SHG by R. Cicchi et al. in *Advanced Biophotonics Tissue Optical Sectioning*, edited by R. K. Wang and V. V. Tuchin, Taylor & Francis Group (2014).
Biomedical Photonic, Spectroscopy ,and Microscopy, David Andrews,Wiley 2015, chapter 9

C3 Raman techniques

Raman spectroscopy in its various forms reads out molecular vibrational energy level signatures and can provide information on the chemical composition of tissue using instrumentation that can be fast, contactless, precise, label-free, non-invasive and non-destructive. All molecular markers can be probed and Raman-based imaging techniques can provide both morphological and chemical contrast. Raman signatures can be correlated with individual molecular markers and signals from tissue samples can be decomposed into specific markers with multiplexing providing enhanced specificity. A single Raman spectrum can be acquired in seconds, which is sufficient to characterize homogenous specimens and small (below spatial resolution limit) species such as bacteria, which makes Raman spectroscopy a particular attractive technique for pathogen detection. It is also a well-established technique in pharmaco-analytics. Spontaneous Raman scattering can also be applied to single point (i.e. spatially integrated) measurements of tissue samples. It is, however, an intrinsically weak process and therefore sensitivity to low concentrated marker

molecules is relatively low such that it is usually too slow for many in vivo imaging applications, such as tumor margin delineation staging and grading of tumors. Raman spectra can be acquired by scanning over ~cm^2 areas with diffraction limited resolution within minutes, which is suitable for histopathology applications. For some applications the sensitivity can be enhanced by using surface enhanced Raman spectroscopy although this approach suffers from poor reproducibility, which is a problem for quantitative applications. For faster imaging there exist a variety of stimulated Raman scattering modalities, such as Coherent Anti-Stokes Raman Scattering (CARS) or stimulated Raman Scattering (SRS) that can provide imaging speeds up to real-time, although with reduced sensitivity.

Advantages/opportunities

- For spontaneous Ramans scattering, the components (light source, detector, etc.) are relatively low-cost
- CARS and SRS can be implemented in systems already ustilising multiphoton excitation and combined with other nonlinear microscopy techniques such as SHG, THG.

Disadvantages/challenges:

- Stimulated Raman scattering systems require relatively expensive instrumentation (pulsed lasers, cameras, detectors) and there are limited options for the required pulsed (sub-ns) light sources).
- Differences in Raman spectral signatures are relatively small and so sophisticated chemometric methods are needed in order to interpret the Raman data and extract relevant clinical information. Consequently, data processing is relatively demanding.
- Background signals such as tissue autofluorescence and room light can be a problem for clinical applications
- Lack of standards for calibration and poor understanding of permissible light (exposure/radiation) dose for ultrashort pulsed radiation, particularly for internal organs.

Clinical applications:

- Potential clinical applications include pathogen detection (infectious diseases), cancer diagnostics (tumor detection, staging and grading, border delineation) and plaque composition detection (cardiovascular diseases). Spontaneous Raman spectroscopy has been applied in diagnosis of cancers including skin , breast, cervical, gastrointenstinal and oral cancer.

Limited commercialisation with commercial device for multiphoton CARS of skin.

References

Human brain cancer studied by resonace Raman spectroscopy, Yan Zhou ,CH Liu, et al J, Biomedical Optics , 17,116021(2012)

Vibrational Spectroscopic Imaging of Soft Tissue, C. Krafft and J. Popp in *Infrared and Raman Spectroscopic Imaging*, R. Salzer and H. W. Siesler, eds.,Wiley-VCH Verlag Weinheim, Germany (2014).

Coherent Raman for Medical Diagnosis, Jeffrey L. Suhalim and Eric Potma in *Ex-vivo and In-vivo Optical Molecular Pathology*, J. Popp (Editor), ISBN: 978-3-527-33513-8. Wiley-Blackwell (2014).

C4. Cerenkov imaging

The recent discovery that low level light detection methods with high dynamic range rejection of background signals can be used to recover images of Cherenkov emission from isotopic and x-ray therapy has stimulated the exploration of a number of medical applications including in vivo dosimetry for radioisotope and radiotherapy research. Cerenkov imaging provides an opportunity to bridge the optical and nuclear gap using approved tracers and therapeutic agents. Both endoscopy and surgery could benefit from the translation of optical imaging techniques to visualize either tumors labelled by conventional radiotracers intraoperatively and thus provide real-time information to guide surgical resection. Currently there are limited clinically approved targeted probes for use with targeted fluorescence imaging.

Advantages:

- No need for an excitation light source. Also, using a radiotracer as an internal light source can reduces non-specific background signal for the object being imaged.
- Direct visualization of radiation dose delivery – more effective radiation therapy

Disadvantages/challenges:

- Cerenkov radiation provides weak intensity of optical signal (nW/cm^2 to PW/cm^2), thus it requires advanced and sensitive cameras for detection – and makes it difficult to conduct studies at room light.
- Cerenkov radiation intensity decreases exponentially with wavelength (high in UV, low in red), thus low penetration depth of the UV photons in tissue limits the range of utility.

Clinical applications:

- Dosimetry – breast cancer radiation therapy. There will also be preclinicalapplications for small animal imaging

References

G. S.. Mitchell. R. K.. Gill. D. L.. Boucher. S. R.. Cherry. *Physical Transactions of the Royal Society A*. 369. 4605. 2011. In vivo Cerenkov luminescence imaging: a new tool for molecular imaging. 1955. 10.1098/rsta.2011.0271. 2011RSPTA.369.4605M.

Kaveh Tanha, Ali Mahmoud Pashazadeh, Brian W, Review of biomedical Čerenkov luminescence imaging applications

Pogue, *Biomed Opt Express*. 2015 August 1; 6(8): 3053–3065. Published online 2015 July 28. doi: 10.1364/BOE.6.003053

D. Opportunities for multimodal application

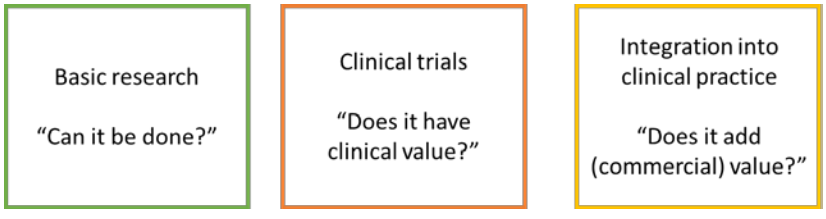
Although several label-free optical modalities have had significant success in distinguishing diseased tissues, a tool that matches the performance of histopathology (approaching 100% sensitivity, 100% specificity) is likely to be multi-modal, usually combining morphological information with spectroscopic readouts, i.e. chemical information. It is often practically useful to also combine larger (~cm) field of view imaging with more targeted (higher resolution and/or spectroscopically richer) techniques and to minimise noise – particularly intra-patient variability of parameters. Multimodal instruments are not restricted to the combination of optical techniques - non-optical modalities can be combined with optical

techniques to overcome the disadvantages of individual techniques. An example is ultrasound combined with Raman spectroscopy used e.g. to localize and quantify cholesterol and calcium salts in atherosclerotic coronary arteries.

For both clinical and health service procurement there is decreasing tolerance for multiple commercial clinical devices that differ only superficially yet are marketed for separate clinical specialty applications. More modular designs where common platforms could be adapted/modified to allow application across different organ systems and specialties could be commercially and clinically attractive. Different modules could include, e.g. microscopy, endoscopy and single point (needle) optical biopsy, potentially with robotic-adjunct delivery, including to deeper solid-organ masses. Such a sysematic approach would reduce the need to design devices from scratch for every application and potentially allow collaboration and commercialisation opportunities for specific new modular solutions that could “plug in” to established platforms. This would help accelerate the evolution and dissemination of solutions to common hurdles for clinical applications such as managing motion artefact, image registration, tissue targeting and tracking, large data handling. It could also allow the development of combination light-based diagnostic and therapeutic solutions in a single product (e.g. multi-modal optical diagnostics coupled with therapeutic lasers).
A well-known example for a multimodal clinical approach is the combination of high-resolution white-light imaging, autofluorescence imaging and narrow-band imaging in endoscopy, which could potentially help to better detect early neoplasia in case of Barrett's esophagus. High-resolution white-light imaging allows to improve the detection of lesions and autofluorescence imaging is likely to improve the sensitivity. The lesions detected could then be investigated by narrow band imaging which allows to distinguish early neoplasia from nondysplastic Barrett's esophagus.

Further examples also consist of techniques that provide mainly morphological information like OCT, SHG and THG with techniques that provide chemical information such as MIR, fluorescence or Raman contrast. Of particular interest are combinations that rely on the same light source such as the combination of CARS with SHG and multiphoton fluorescence as this allows more easily to minimize space requirements and helps to keep the costs down, as expensiveness is one of the major trade-offs of multimodal solutions.

4. BARRIERS/HURDLES TO TRANSLATION



A simple model of translational research can be considered as three distinct activities. In the first stage, the basic research functions are undertaken to design, construct, and bench test a device. The motivating question being addressed is “Can it be done?” Many researchers confine their work to this domain without considering a transition to the next stage - the

clinical trials and research activities that attempt to answer the question, “Does it have clinical value?” Here, the goal is to test the device, drug or therapy treatment on patients in order to determine safety and efficacy. This requires regulatory approval (e.g. from the FDA) so that all concerns must be adequately answered by the investigators before the clinical studies can begin. Having proven clinical value, the next stage is to show that the device or technique can be integrated into clinical practice and provide a net benefit. This includes **cost/benefit** considerations and physician acceptance of the procedure. For commercial success it needs to become a “standard of care” such that the costs will be reimbursed by insurance companies or state agencies (in the USA this entails obtaining a CMS payment code and AHRQ product indorsement).

These regulatory and clinical hurdles are beyond the interest and/or resources of many academic investigators who are more interested in solving the scientific and engineering questions than in moving through the translational maze. As a result, many novel biomedical device ideas are given only a cursory pass through a clinical study before a few papers are published and new projects get started.

Technical challenges

As can be seen from Section 2, there are some generic technical challenges for label-free techniques that will present hurdles for translation to the clinic.

In general, the contrast achievable is generally lower than is available using specifically designed or selected exogenous probes. This may be mitigated by multiparameter/multimodal approaches where several lower contrast techniques can be combined to increase sensitivity/specificity.

Some modalities may require the use of challenging spectral regions. For example, label-free readouts based on autofluorescence often require UV/blue excitation (e.g. for NADH) and at these wavelengths it is common to observe unwanted background fluorescence signals, e.g. from glass components such as lenses or fibres (endoscopes). There is also almost no data or guidance concerning safe exposure levels for UV/blue irradiation of internal tissues. This can be mitigated through the use of specialised glasses or designs incorporating free space propagation and reflective optics. In the mid infrared (MIR) contrast is available through absorption but the signatures are complex and require sophisticated computer algorithms to disentangle.

A key challenge for all optical imaging is scattering and addressing this has been the subject of much research over the last few decades. The deleterious effects of scattering can be reduced by selecting ballistic light, e.g. using spatial filtering, coherent detection or time gating or polarisation gating but this approach is limited to typically mm penetration depths and requires endoscopy or surgical (minimally invasive robotic) intervention to reach internal tissues. There is some prospect to penetrate deeper into tissue using NIR windows; in particular , 1600 nm to 1870 nm that present reduced optical scattering and have not been widely exploited to date. Alternatively, statistical models of photon transport can be employed to obtain information from greater depths at the expense of reduced spatial resolution.

Many of the more sophisticated approaches such as time-gating for nonlinear optical techniques to improve molecular contrast or resolution are expensive and/or cumbersome. They can also entail relatively high light exposure for patients.

Photoacoustic tomography offers a means to improve the spatial resolution/depth trade-off but at the expense of reduced molecular contrast and increased complexity and expense compared to fluorescence imaging.

As optical techniques get more sophisticated and data gets richer and larger, there are increasing technical challenges associated with the acquisition, analysis and management of data. With the development of sCMOS cameras and other very high data rate detectors, the data volumes associated with a single measurement can exceed Gb and routinely generating Tb/day is in sight if not already here. The transmission, storage and curation of such large data volumes presents significant challenges that have not yet been addressed and the use of data compression raises important ethical and regulatory issues. However, the ability to access remote resources for data analysis and sharing offers many opportunities for improved diagnostic capabilities.

Most of the above challenges can be and are being addressed through the ongoing development of new technology, techniques and computational tools. Miniaturization of optical and electronic technology, increased computational power and new algorithms combined with opportunities to leverage consumer electronics and the internet are creating new opportunities and enhancing the potential of label-free optical technologies. Automation and robotics combined with label-free optical techniques offer the potential to reduce costs through reducing the need for expert practitioners. Nevertheless, they often have to compete with exogenous probe-based techniques that may offer simpler and lower cost “readers” compared to label-free techniques.

Regulatory challenges

Clarification and Revision of Safety Standards for Optical Radiation

Optical radiation can be hazardous in terms of photo-induced biochemistry and/or thermal damage. Its use is regulated by guidelines largely derived from considerations of hazard thresholds for dermal or ocular exposure. These are not necessarily appropriate for many clinical contexts such as internal organs that are not routinely exposed to light.
Clearly further systematic phototoxicity measurements are required but the parameter space that needs to be addressed is large (e.g. in terms of wavelength, power, pulse duration, peak intensity, illumination time, etc.) and the sample size should be large to address interpatient variation. It is difficult to get funding to support such measurements in an academic context and there is a shortage of scientists with the expertise and resources required to undertake them.

Certification

Medical instruments need to be certified for application in the clinic, e.g. by the FDA, which requires developers to implement quality management protocols that include standard operating procedures (SOP), good laboratory practices, document control, and validation testing. Academic researchers interested in the science and engineering of new ideas for biomedical applications often have little or no experience and/or expertise in this area and

it can be difficult to fund such work through standard research sponsors for whom successful proposals require innovation and adventure – with translational research often being condemned as “incremental”.

In order to facilitate certification, it is important to develop Standards and Traceability in Label Free Imaging. Again it is difficult to fund such research through conventional academic channels and so this important work probably needs to be addressed by government agencies.

Clinical uptake challenges

As well as the regulatory challenges discussed above, the lack of published early data from clinical pilot studies, together with a paucity of defined and relevant clinical outcome measures, makes it challenging to engage clinicians and to obtain investment for clinical translation. This is compounded by a tendency of developers to publish “methods” papers in technical journals rather than clinically useful results in clinical journals, i.e. developers talk to each other much more than their potential users. Developers of new instruments and techniques should publish confidence intervals in their sensitivity/specificity data. To obtain reasonable results requires significant sample sizes beyond those typically encountered in most work to date. Many preliminary clinical studies have been conducted and published for the purpose of grant proposal submission.

The relatively high cost of much label-free optical technology and the scarcity of translational funding makes it difficult to deploy on a large scale or with non-research orientated clinicians. Consequently, there are relatively few clinical pilot studies and fewer large scale clinical trials, which makes it difficult to get clinical acceptance.
Translational research is usually labour-intensive, repetitive, protracted, costly, and can be judged by proposal reviewers to be incremental and lack innovation. It can take months-years to obtain regulatory approval for a clinical trial – particularly if the (academic) investigator is not familiar with the process. Devices have to be demonstrated to be safe (e.g. in terms of optical radiation hazard, sterilization, mechanical robustness, etc.). For technology developers, it is necessary to work with clinical champions who are prepared to invest significant time and potentially reputation in a translational project. Clinical partners need to have access to suitable patient cohorts to test/validate new instruments and techniques. This can require large or specialist (e.g. tertiary referral) hospitals to ensure sufficient patient numbers during the duration of a study.

Validating a new technique or instrument may entail many years gathering data from 100’s – 1000’s cases and at the end publish a single paper, which may not be in a high-profile journal. It is not uncommon for statistically robust trials to present negative outcomes, even though small pilot studies have been successful. Such considerations can deter potential investors and translation partners. It can also be challenging to enlist graduate students and postdocs who plan to pursue academic careers to translational projects. Furthermore, it can be challenging for academic investigators to take the time required for clinical translation from their academic duties and research activities.

However, at the National Institutes of Health (NIH) there is increased interest in improving rigor and reproducibility in research, encouraging researchers to demonstrate robust results

using methods that avoid bias and can be reproduced under well-controlled conditions. This is a move toward many of the activities found in the regulatory quality management schemes endorsed by the FDA.

5. COMMERCIAL PERSPECTIVES

Within the technology developer community there is dismay at apparent reluctance of investors and large companies to invest in optical diagnostics - whether label-free or not – in spite of the large and growing body of clinical research data amassed by the biomedical optics community. This may be due in part to the reluctance of academic investigators to progress from the first stage of translation and thereby "de-risk" the investment required from industry. Academics are often reluctant to leave university positions to start or drive start-up companies. Translation is therefore often undertaken with large companies that have the internal expertise and resources to execute the technology translation using the academic inventors/developers as consultants. Commercial companies typically require IP to be licensed from the universities – usually with exclusive licences. This can prevent others from translating the licensed IP and delay/prevent new technology/techniques from reaching the market and clinic.

The **Carl Zeiss** experience with marketing label free imaging was presented by Alexandre Tumlinson, with particular respect to the commercialisation of optical coherence tomography (OCT), which is widely recognised as one of the key examples of successful clinical translation of optical label-free medical technology: Carl Zeiss has been developing and marketing optical innovation for 150 years including the (now) blockbuster success of OCT, systems still in potentially interesting states of development and many ideas that were never successfully brought to market.

Alexandre offered the following observations from Carl Zeiss perspective:

- Academics need to honestly evaluate the readiness of their technology
    - Technology readiness level (TRL) can act as a useful checklist and communication tool
    - Clearly identify what is known and what is a leaps of faith
        - Technical risks – will further development yield quality data?
        - Clinical risk – will quality data yield clinical benefit?
        - Regulatory risk – are risks of procedure or potential misdiagnosis identified, and outweighed by benefits?
        - Market risk – will clinical benefit translate into sales, justifying investment?
    - Developers need to design experiments to address remaining unknowns

- Note that even a blockbuster medical technology like OCT can have a long adoption cycle:
    - 5yrs OCT patent → lab tool
    - 5yrs lab tool → clinical work horse (and profitability)
    - 5yrs profitability → mature industry with significant competition
    - A strong patent can encourage a company to leap in – a confusing patent 'thicket' can discourage investment

- Medical technologies that have proved very successful may still have had periods of uncertainty:
    - First OCT images had unconvincing clinical benefit, and project was nearly cancelled
    - Interaction between company and zealous early adopters developed the product
    - Ability to quantify data → clear clinical benefit → billing codes →market sustainability
    - OCT benefitted from being able to inform care decisions for several medical conditions in one setting. This is still a primary driver of OCT growth over 'single use' technologies

The discussions about the path to success of OCT in ophthalmology indicated that there was some degree of "faith" by early translation partners in keeping the product development alive, and in rapidly adopting improved versions. Such risk-taking seems to be much less common since 2008. There is also a lot of current uncertainty about the direction of the health-care insurance industry in the US, and the methods that will be imposed for cost-containment. This probably also has an impact on available investment for new medical technologies.

The experience of Intuitive Surgical was presented by Jonathan Sorger, who also reported that the path to its current commercial success had included a period where the amalgamation of two competing start-ups had been necessary to save the translation of this technology to the market and clinical practice.

In the USA, the Preservation and Incorporation of Valuable Innovations (PIVI) standards of the FDA for the adoption of novel technologies could be a powerful driver towards clinical translation. While medical targets are always moving, the kind of consensus guidance from clinical specialties that is developing for clinical endoscopy could help reduce the risk for industry when considering new technology in this field. OSA and other learned societies could facilitate communications with counterpart clinical professional organizations to encourage development of similar standards across other medical specialties.

6. NEXT STEPS & RECCOMENDATIONS

It seems desirable to move from the current situation where much of the development of new instruments and techniques for label-free optical imaging and spectroscopy is "technology-push", i.e. communities of scientists and engineers mainly talking to each other and following an essentially opportunistic approach to solving technical challenges. Rather this field should be addressed by more broadly multidisciplinary teams within networks and communities that are more driven by **unmet clinical need**. This should entail collaboration between academia, clinicians, industry and entrepreneurs from the outset in order to prioritise the development of new tools that will be clinically fit for purpose and commercially viable. It is particularly important to address the mind-sets of clinicians and other stakeholders who are not experts in optical technology and identify it predominantly with microscopy and not with macroscopic imaging, which in practice is already more widespread outside histopathology and potentially of greater impact.

These teams should be driven by "*what is clinically useful*" rather than "*what can be done with optical technology*" and define the specific clinical challenges to be addressed (including who will use the technology, what segment(s) of the global healthcare market are being targeted, what are the patient expectations and to what extent can the instrument or technique address an unmet need or replace current practice – and what is the likelihood). In reality, most label-free optical modalities are only suitable for a small set of clinical applications. For each application (unmet need) teams should identify or define clinically useful outcome measures – with input from clinicians, industrial partners and regulators at the earliest opportunity. This process should help define the necessary parameters of new (label-free optical) medical devices in terms of, e.g. the required sensitivity/specificity, physical constraints, data acquisition speed, complexity of the user interface/parameter output to end-users and safety requirements.

The following specific recommendations and suggestions emerged from the discussions at the OSA incubator meeting:

- Biomedical optics conferences should always strive to include clinicians to provide necessary context for new technical innovations, the label-free optical diagnostic community should attend clinical meetings and new hybrid focused meetings could be established to specifically created the required multidisciplinary networks and teams.

- Although there are significant other hurdles to translation, technical challenges remain significant. In particular, progress continues to be limited by our lack of understanding of light-tissue interaction, which results in inappropriate physical models and inconsistent results – that discourage potential end-users and investors. The community should identify and acknowledge the technical areas that need more understanding and make it clear to funding agencies that further basic science is needed to underpin translational research.

- To realise the potential of new (label-free optical) medical technologies, research funders need to support basic underpinning studies of, e.g. tissue optical parameters and phototoxicity, with in vivo measurements undertaken on sufficiently large scales to be statistically robust. Such studies will necessarily be incremental but will facilitate subsequent innovation and clinical impact.

- It is difficult to publish such data where it does not underlie new innovations and/or discoveries. Consequently, such studies are often undertaken in parallel with little communication. The label-free optical community should work together to share such data – perhaps in a central global repository and the academic community and funders should recognise the value of this type of research. This would increase the statistical power and reduce the cost of campaigns to establish safety limits and standards. It was suggested that the FDA could publish such data when it is submitted to support requests for approval of clinical trials.

- Cross-cultural meetings and networks including scientists and engineers, clinicians, industry and entrepreneurs are required to create and reinforce a research community driven by unmet clinical need rather than technology-push publications.

- Clinically significant trials of new technology need to be undertaken on a scale sufficient to produce statistically significant results and to meet regulatory safety and standardization requirements. To achieve the required reproducibility in device performance, SOP, documentation, etc. requires different expertise and personnel than the research undertaken by PhD students and post-docs. Gary Tearney presented his translation activity at MGH where a “product engineering” team is embedded in the research programme to produce devices for clinical trials. Such programmes are challenging to fund funded through regular research channels but are critical to clinical impact. Research funders should devise funding mechanisms to support such translational work.

- Academic researchers should engage industrial partners and other potential funders at the earliest stage. However, they should not rush to exclusive licensing of the IP. Commercialisation partners should recognise that the market and clinical acceptance is often enhanced when there are multiple players. A more open approach can help de-risk a new technique or technology that can be prematurely killed if it is only being exploited by a single company. Incubators with multiple investors may provide a suitable environment for early stage development, clinical trials definition of the product, thereby de-risking the investment proposition.