Cancer imaging by optical coherence tomography: preclinical progress and clinical potential

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Abstract

The last decade has seen dramatic technological advances in the field of optical coherence tomography (OCT) imaging. These advances have driven commercialization and clinical adoption in ophthalmology, cardiology, and gastrointestinal cancer screening. Recently an array of OCT-based imaging tools developed for preclinical intravital cancer imaging applications have yielded exciting new capabilities to probe and monitor cancer progression and response *in vivo*. Here, we review these results, forecast the future of OCT for preclinical cancer imaging and discuss its exciting potential to translate to the clinic as a tool for monitoring cancer therapy.

Introduction

Optical coherence tomography (OCT) provides non-invasive and label-free imaging of living tissues and organisms[1–4]. While it is a relatively new technology, OCT is based on principles of optical interferometry that have been developed for several hundred years (Box 1). The way in which the image is generated in OCT (through the use of interferometric sectioning (Box 1)) is unique compared with other *in vivo* imaging modalities, and gives OCT distinctive capabilities. First, contrast in OCT is derived from the light scattering properties of cells, stroma, and other tissue structures. As such, OCT is a label-free optical imaging modality. Second, OCT imaging utilizes longer wavelength in the near infrared region. Because scatter decreases with increasing wavelength, OCT can image at high resolutions deeper into tissues than possible using shorter wavelength optical microscopy. Third, the mechanical components that carry out OCT can be miniaturized. Although fluorescence microscopes have also been miniaturized, OCT can be operated with low numerical aperture lenses, which facilitate the integration of OCT optics into small probes, catheters, and endoscopes for imaging at internal sites.

Box 1

Principles of OCT

In its simplest form, an optical interferometer is an instrument capable of measuring the differential distance travelled by two paths of light (See the figure, part a). It operates by splitting a light source into two beams, each of which is made to travel one of the two

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paths that are to be compared. When these beams are recombined, their resulting interference signal depends on the distance (at the sub-micron scale) travelled by each beam. In OCT, this concept is extended using one of the two beams of the interferometer to illuminate living tissue (See the figure, part b). As the beam propagates into the tissue, scattering by cells and other structures redirects some light backward. This scattered light is collected and made to interfere with the second (mirror-reflected) beam. Using the resulting interference signal, the depth of all the tissue-scattered light can be measured to the limit of the resolution of the interferometer, which is typically 5–10 μm in OCT systems. Transverse resolution is limited by the focused beam size and varies from 5–30 μm depending on the system design. Repeating these measurements across three-dimensions allows an OCT instrument to map optical scattering across the tissue volume with near histological resolution. By analyzing these optical scattering measurements, properties including anatomy, blood vasculature (see the figure, part b), lymphatic vasculature, and tissue viability can be measured.
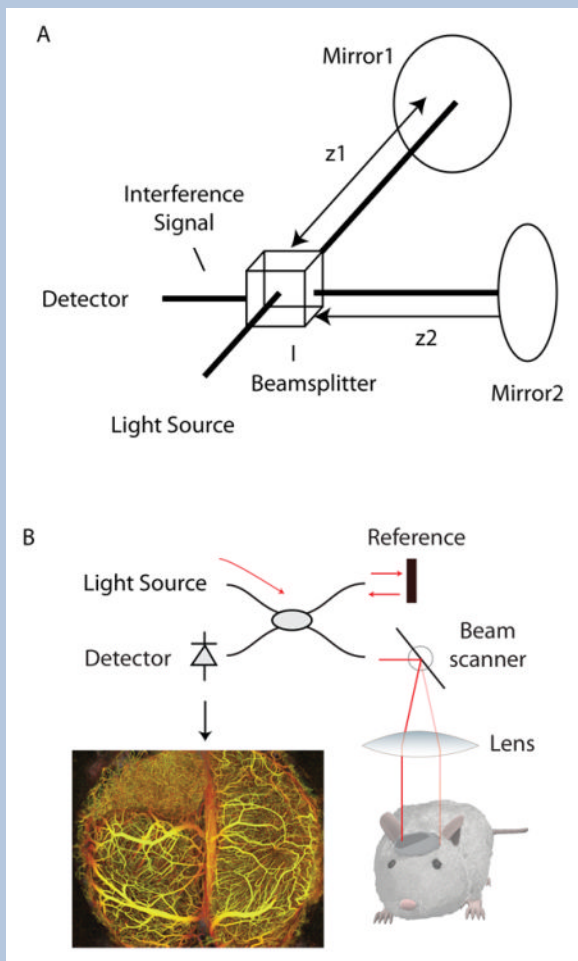
These factors have combined to motivate adoption of OCT into several diagnostic applications. Opthalmic OCT is now standard-of-care in the U.S. and Europe[5], intravascular OCT has been commercialized world wide and is rapidly becoming a standard-of-care in interventional cardiology[6], and endoscopic OCT is being commercialized as an adjunct to conventional white-light endoscopic examination[2]. OCT is also applied to preclinical imaging. Within the past several years, exciting achievements in intravital cancer imaging,

i.e., imaging of cancers in living mouse models, underscore a growing role for OCT in preclinical imaging. In this Innovation article, we highlight how recent advances in OCT have provided new imaging capabilities in cancer biology, and how these capabilities contrast to those provided by existing technologies. We highlight successful preclinical applications of OCT from multiple laboratories for imaging different aspects of the biology of tumours and their microenvironments, and how OCT can be integrated with complementary imaging modalities to enhance the information obtained from imaging studies. Finally, we discuss the potential of using OCT to monitor the clinical treatment of cancer.

Optical Coherence Tomography Instrumentation

OCT is one of many techniques for imaging tissues and cells in a living organism (Table 1). An OCT system typically comprises a light source, an interferometer, and a microscope or imaging catheter that delivers light to and collects reflections from the tissue to be imaged (Box 1). Because of optical scattering in tissue, OCT signals cannot penetrate beyond 2 mm in most tissues. This necessitates selection of appropriate animal models. Often, as with fluorescence microscopies that have limited imaging depths, benchtop (non-endoscopic) imaging is performed using window models at the skin, mammary fat pad, or brain sites[7–9]. OCT can also be used for subcutaneous models by transdermal imaging, or can be applied to surgically exposed internal sites[10]. Benchtop imaging employs a microscope similar in design to those used in fluorescence microscopy wherein anesthetized animals are placed under the imaging objective and galvanometric beam scanners translate the imaging beam. As an alternative to benchtop OCT, endoscopic OCT allows imaging at internal sites within the animal[11, 12]; here the probe is placed in contact with or very near to the tissue to be imaged. This miniaturization typically does not induce significant degradation in imaging sensitivity or resolution. Beyond providing access to internal sites in small animals, these endoscopic embodiments are also the likely pathway for potential future clinical adoption of OCT tools with which to monitor cancer therapy.

Image Contrast Applications of OCT

OCT fundamentally measures how light propagates in tissue and scatters from tissue structures. Over the past decade, our ability to translate these measurements to physiological and anatomical parameters has expanded substantially. The methods relating OCT signals to anatomical measures relevant to cancer are summarized here and contrasted with similar measurements performed by alternative intravital approaches.

Microstructural imaging

Structural (anatomical) imaging is used to measure tumour volume, locate tumours anatomically, or define the tumour microenvironment. Intravital ultrasound[13] and micro computed tomography (μCT)[14] are commonly used for this purpose. Both have the advantage of large imaging fields and deep penetration but each is also hindered by relatively poor soft-tissue contrast that limits the interpretation of the anatomy.

Microstructural images can be generated from OCT measurements by using the log-scale magnitude of optical scattering. Because optical scattering is more varied across soft tissues than either acoustic scattering or x-ray absorption, microstructural OCT images generally provide greater contrast than ultrasound and contrast-free CT. This increases the ability to detect tumour margins, and more broadly elucidates the microenvironment at the tumour site. Because OCT penetration is limited to 2 mm, measuring the volume of larger tumours is more suited to ultrasound and CT methodologies. Microstructural OCT imaging can be

performed rapidly: an 8 mm × 8 mm × 2 mm field at 10 μm resolution requires less than 5 seconds to acquire using current instrumentation[14].

Viability imaging

Imaging viability within a tumour model helps to elucidate the spatially heterogeneous response to therapy. Methods for intravital imaging of viability however are limited. Micro positron emission tomography (μPET) scanners using 18-fluorodeoxyglucose (FDG) labelling can map viability in whole-body imaging studies but their limited resolution (typically greater than 1 mm) is not well matched to the size scale of tumours in small animal models[15]. Fluorescent deoxyglucose analogs[16] have been shown to allow optical monitoring of glucose uptake activity in culture[17] and *in vivo*[18, 19], and may provide alternatives to PET-based methods. GFP expressing tumours can be used to monitor viability as the fluorescent protein carrier has an approximately 36 hour half-life[20–22], but these techniques are restricted by imaging field and depth. Luciferase reporter bioluminescence (e.g., firefly, gaussia, renilla luciferase) can also be used to image cell viability at lower spatial resolutions[23].

OCT can discriminate between viable and non-viable compartments of the tumour through correlated changes in optical scattering[10]. While the underlying changes in tissue structure that modulate optical scattering have not been identified, a correlation between high scattering and loss of viability has been confirmed by registered images and histology. However, caution must be taken when associating scattering changes to viability status as other processes could manifest similar scattering changes. OCT-based viability imaging has the advantage of being label-free, and can be easily performed concurrently with other OCT imaging modalities, e.g., microstructural imaging; both contrast methods operate on the same acquired dataset and are differentiated only by post-processing. The paucity of robust methods for imaging viability in preclinical settings makes this a compelling application for OCT.

Lymphangiography

Lymphatic vessels play a central role in solid tumour growth and metastasis[24]. The disruption of normal lymphatic function by solid tumours contributes to the high interstitial fluid pressure within the solid tumour and hinders convective drug transport[25]. On the other hand, peri-tumour lymphatic vessels provide a route for metastasis. There is therefore a need to image lymphatic vessels in solid tumour microenvironments and in response to cancer therapies. However, lymphatic imaging remains one of the most significant challenges in intravital microscopy because, unlike the blood vasculature, the lymphatic system cannot be easily labelled systemically. The most common technique currently available requires tracer injection into the site (e.g., tumour or surrounding host tissue)[26]. As lymphatic vessels collect and are filled with the injected tracers, they can be imaged by either wide-field cameras or fluorescent microscopy. This approach, however, only reveals a partial network and perturbs the lymphatic vessel physiology.

OCT can be used for label-free lymphangiography[10, 27]. Contrast for lymphatics is derived from the difference in optical scattering between lymph fluid, which is nearly transparent, and tissue, which is highly scattering. The lymphatic network appears in three-dimensional datasets as hypoechoic (low-scattering) regions; three-dimensional connectivity and characteristic valve and lymphangion morphology make identification of lymphatics relatively straightforward. Because some fraction of the lymphatic vasculature is collapsed at any given time in normal physiology, comprehensive mapping is not always possible. Higher resolution embodiments of OCT that are able to detect lymphatic vessels with smaller open luminal areas are technically feasible and would mitigate this limitation.

Angiography

Understanding tumour angiogenesis and the response of tumours to vascular targeting therapies has been a major theme in cancer research over the past decade[28–30]. Current approaches for intravital angiography include Doppler ultrasound, micro magnetic resonance imaging (μMRI), μCT, photoacoustic tomography[31–34], and fluorescent microscopy. Of these, ultrasound, μMRI and μCT-based methods cannot resolve single vessels due to limited resolution. To date, fluorescence angiography has been adopted most broadly to study angiogenesis in tumour models at the resolution of individual vessels[35]. Fluorescent methods however require systemic labelling of the vasculature through intravenous injection which carries known limitations in longitudinal studies[8].

Using microstructural OCT, some large vessels exceeding 100 μm in diameter can be visualized. To extend contrast to smaller vessels, alternative methods based on blood flow are commonly used in OCT. Unlike microstructural, viability, and lymphatic methods of contrast, angiographic imaging relies on the measurement of scattering dynamics. Flowing blood modulates optical scattering, and this modulation can be detected and used to differentiate between intravascular and extravascular compartments of the tumour. Unlike fluorescent approaches, OCT-based angiography is label-free. OCT and fluorescence angiography are further differentiated by their fields, imaging depths, and resolutions (see Table 1), with fluorescence microscopy providing smaller fields and higher resolution than OCT.

Unlike architectural, viability, and lymphatic OCT imaging, angiographic OCT monitors scattering changes over time and hence requires strict animal immobilization. In a typical setup, the site to be imaged must be mechanically immobilized through use of a window chamber models or other mechanical restraint. When immobilizing the site, however, care must be taken to prevent occlusion of the vasculature and loss of vascular contrast in response to mechanical pressure. Angiographic OCT methods also require longer imaging times to capture the scattering dynamics at each location. Depending on the algorithm used and blood flow sensitivity required, imaging of an 8 mm × 8mm × 2mm field at 10 μm resolution takes 1–20 minutes, compared to ~5 seconds for non-dynamic contrast modes of OCT[10]. Longer imaging times allow for more extensive sampling of the signal dynamics which lead to improved image contrast. Refinements in algorithms are likely to reduce this time in the near future.

Additional contrast modes in OCT

Several additional imaging contrast modes are available in OCT but are at earlier stages of development or have not yet been shown to have relevance in cancer studies, and are therefore not discussed in detail in this article. These include techniques for detecting exogenous labels which could enable OCT-based measurements of drug distribution within tumours[36]. Also possible is the use of OCT to identify specific tissue components, such as collagen and muscle fibres by measuring their influence on the polarization of the incident light field[37]. This polarization-sensitive OCT approach is relatively mature, and may find utility in probing tumour-stroma interactions. Finally, careful measurements of scattering dynamics may yield contrast for cellular processes such as apoptosis[38] or allow for comprehensive quantification of blood flow velocity in a tumour vascular network.

OCT in Preclinical Cancer Research

Investigators have adopted these OCT methods to study cancer biology and evaluate drug response in preclinical settings. A survey of recent accomplishments is presented here with an emphasis on applications where OCT provided measurements that would be difficult or impossible to obtain using alternative modalities.

The utility of microstructural OCT has been demonstrated in several studies. In a first study, OCT was used to measure the volume of tumours implanted in dorsal skinfold chambers[10]. This study used a manual segmentation approach to delineate the tumour margins in three-dimensions (Figure 1a). From these margins, the tumour volume was calculated and used both to define consistent therapeutic start points across animals in an anti-angiogenic drug study, and to demonstrate growth delay in response to vascular endothelial growth factor receptor 2 (VEGFR2) blockade. By acquiring architectural and vascular OCT images simultaneously, the authors were further able to use these tumour margins to segment intratumoural and peritumoural vessels, allowing for differential analysis of vascular response at these sites. The expanded field, imaging depth and multimodal imaging provided by OCT make these analyses possible.

Microstructural OCT was also deployed endoscopically to image disease progression in induced models of colorectal cancer[11]. These authors were able to comprehensively map and longitudinally track disease progression in the mouse colon (Figure 1b). In this work, they also demonstrated that microstructural OCT-based contrast could be used to image nanoshells that were targeted to epidermal growth factor receptor (EGFR). This endoscopic OCT system was also combined with fluorescence imaging to correlate early disease (identified by microstructural OCT), with increased VEGFR2 expression (identified by fluorescence labelling). In these endoscopic applications, the resolution and soft tissue contrast afforded by OCT allowed detection of lesions earlier than they would be possible in μCT and μMRI.

The ability of OCT to monitor cell-targeted therapy was demonstrated by viability imaging in xenograft models given a diphtheria toxin, which served as a proxy for a tumour cell-targeted therapy. The toxicity of diphtheria toxin is highly species-specific, hence it is acutely toxic to the human tumour cells but not to the mice[10]. Changes in scattering within the tumour volume were quantified across time to track changes in tumour viability (Figure 1c). Again, simultaneous architectural and vascular imaging provided correlated measures of tumour volume and vascular response.

OCT-based lymphangiography has been used to map lymphatic vessels in the peri-tumour space in both dorsal skinfold chamber models and in ear models[10]. Quantification of peri-tumour lymphatic hyperplasia was shown to be correlated with the distance to the tumour margin in human sarcoma models, and longitudinal tracking of single peri-tumour lymphatic vessels was demonstrated[10].

Some of the most compelling applications of OCT in preclinical cancer research have been in the area of angiography. The ability of OCT to repeatedly image over broad fields makes it ideal for studies of tumour angiogenesis and vascular response across a variety of sites[10]. In a recent study, a novel therapeutic strategy using a bioengineered von Hippel-Lindau (VHL) protein was used to induce degradation of hypoxia-inducible factor 1α (HIF1α) and HIF2α. OCT was employed to characterize angiogenesis in xenograft models treated by this VHL protein[39]. In combination with fluorescence microscopy, which was used to confirm adenovirus delivery of the VHL protein, the study demonstrated robust inhibition of angiogenesis and increased tumour necrosis following treatment. In a further example of hybrid studies combining OCT with other modalities, an instrument capable of dynamically imaging oxygen supply and demand in tumours was demonstrated[40]. Here, OCT was used to measure blood flow while spectroscopic techniques provided measures of blood oxygenation (using hemoglobin spectroscopy) and metabolic demand (using "redox ratio" fluorescence measurements).

The wide-field imaging of OCT reveals the morphological nature of tumour vascular networks with a unique clarity. This capability was used to highlight the significant role of the microenvironment on tumour vasculature (Figure 2)[10]. Frequent repeated angiography — which is not practical using fluorescence microscopy due to imaging time and accumulation of extravasated label — is possible using OCT. OCT techniques were used to perform time-lapse angiography and microstructural imaging of tumours in response to VEGFR2 blockade[10] (Supplementary information S1 (movie)). OCT imaging was performed every two hours for a period of 48 hours to capture the rapid vascular changes induced by VEGFR2 blockade. Microstructural imaging confirmed that, despite these vascular changes, the vascular-targeted therapy did not affect the tumour growth during this time.

OCT in clinical treatment response monitoring

Many preclinical imaging approaches, while very effective, are not translatable because they rely on genetic modifications or would require regulatory approval of a new label. OCT has moved rapidly into diagnostic applications largely because it is label-free and thereby circumvents these translational challenges. With the growth in OCT-based imaging of cancer demonstrated in preclinical settings, there is a strong argument for efforts to translate OCT to clinical monitoring of cancer therapies. Early work has demonstrated in animal models that OCT-based angiography can provide sensitive feedback on the effect of photodynamic therapy in models of prostate cancer and oesophageal carcinoma[41–44]. Efforts to translate OCT techniques to clinical response monitoring are now underway.

Conclusions and Perspectives

OCT is an established diagnostic method and is being increasingly adopted in preclinical cancer research. OCT provides imaging capabilities that fill specific gaps left by existing intravital approaches, and may therefore become a commonplace technology in the biological laboratory. Of course, the anatomical contrast provided by OCT would be complemented by the molecular insight provided by labelled approaches. Multi-modality imaging should therefore be used in preclinical settings. The growth of both the research and commercial industry backing OCT will continue to yield advances in performance and broaden access to the instrumentation, further driving adoption.

A need for imaging biomarkers in cancer offers an exciting role for OCT in clinical therapy monitoring. Currently, vascular imaging biomarkers derived from clinical whole-body imaging modalities such as MRI are used to assess therapies[45–49]. With the development of appropriate probes, it may be possible to use OCT techniques to provide a higher resolution visualization of the vasculature within tumours. Early studies that confirm a correlation between OCT-derived images and therapeutic response are needed. Accessible cancers that require minimal probe development, such as skin and oral cancers, may present near-term opportunities for these studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Biographies

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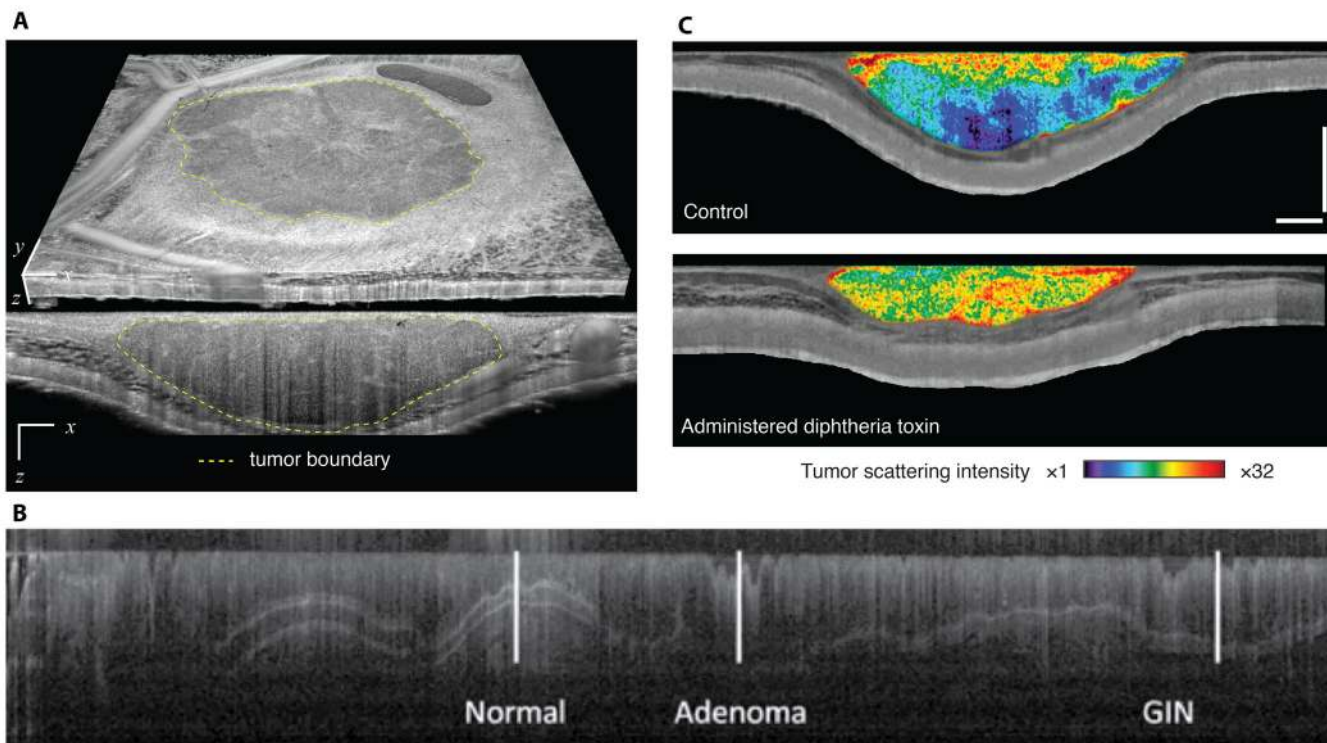
Figure 1.
Microstructural OCT imaging of tumours. (a) Using microstructural contrast, tumour tissue (an allotransplanted MCaIV mammary adenocarcinoma) can be differentiated from surrounding host subcutaneous and muscle tissues in a dorsal skinfold chamber model[10]. By defining the tumour margins, the three-dimensional tumour volume can be calculated. (b) Endoscopic microstructural OCT of azoxymethane-induced colorectal cancer in the mouse colon. High soft-tissue contrast and near histology resolution allows imaging of colonic epithelial microstructure[12]. The image shows the contrast at different tissue depths (shown in the vertical direction) versus distance along the colon (shown in the horizontal direction). Disease progression — from normal tissue to gastrointestinal intraepithelial neoplasia (GIN) to adenoma — can be monitored through hallmark modifications, such as the loss of tissue stratifications. (c) By quantifying scattering in OCT microstructural datasets, tumour viability can be monitored during cytotoxic interventions. Here, representative viability images of a LS174T human colorectal adenocarcinoma xenograft in a dorsal skinfold chamber model are presented two days following administration of diphtheria toxin (lower panel) or untreated (upper panel)[10]. Increases in scattering indicating significant loss of viability are evident in the diphtheria toxin administered animal. Transverse extent in a: 5 mm (x), 4.4 mm (y). Scale bars in c: 500 μm. Figure 1a and 1c are adapted from ref [10]. Figure 1b is adapted from reference [12] with permission.
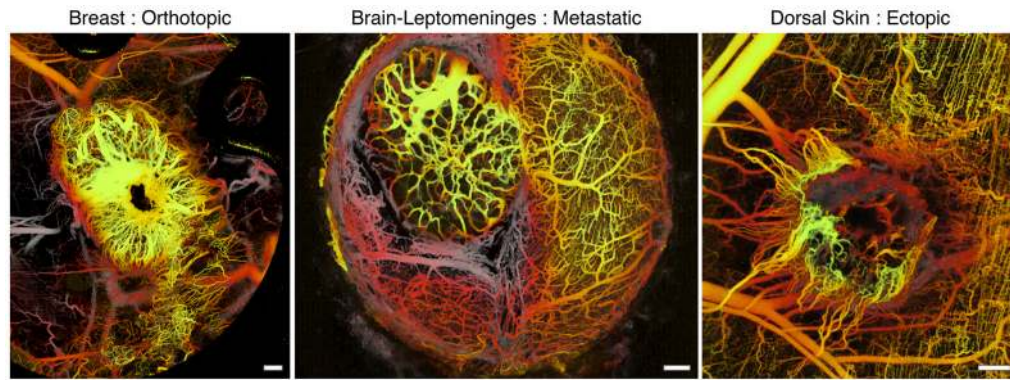
Figure 2.
Imaging of tumour angiogenesis using OCT. OCT-based angiography reveals strikingly different vascular networks in an MCaIV murine mammary carcinoma grown in different anatomical sites. Scale bars, 500 µm. In these images, three-dimensional vascular signals are projected into a single image and the colormap is used to encode the vessel depth. Reproduced from [10].

Table 1

Overview of intravital imaging approaches in preclinical cancer research

	Type of Wave	Tissue Penetration Depth	Resolution	Supports label-free	Supported labels
Optical Coherence Tomography	near infrared light	1–2 mm	1–20 µm	Yes	nanoparticles magnetic particles near infrared dyes
Fluorescence Microscopy	visible/near infrared light	~300–800 µm	1–5 µm	Limited	visible and near infrared fluorophores
Ultrasound	sound	cm	50 µm	Yes	Microbubbles
Computed Tomography	x-ray	no limit	50 µm	Yes	Iodine
Positron Emission Tomography	gamma rays	no limit	1–2 mm	No	Radionuclides (e.g., F-18)
Magnetic Resonance Imaging	RF	no limit	10–100 µm	Yes	magnetic/paramagnetic
Bioluminescence	visible light	cm	60 µm - cms	No	luciferins
Photoacoustic Imaging	visible/near infrared light and sound	cm	5 µm – 1 mm	Yes	near infrared dyes nanoparticles