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Progress in Multimodal *En Face* Imaging: feature introduction

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Abstract: This feature issue contains papers that report on the most recent advances in the field of *en face* optical coherence tomography (OCT) and of combinations of modalities facilitated by the *en face* view. Hardware configurations for delivery of *en face* OCT images are described as well as specific signal and image processing techniques tailored to deliver relevant clinical diagnoses. The value of the *en face* perspective for enabling multimodality is illustrated by several combination modalities.

Multimodal optical imaging vastly enriches the amount of information that can be retrieved from biological tissue. Towards this purpose, many different approaches have been reported to date, which include combining optical coherence tomography (OCT), microscopy, scanning laser ophthalmoscopy (SLO), fluorescence imaging, multi-photon imaging, Raman spectroscopy, microperimetry, electrophysiology, oximetry, photo-acoustics, and others.

Depending on the underlying imaging technology, images with different preferential orientations have been combined with varying degrees of success. In OCT, for example, one-dimensional depth profiles (A-scans) are typically acquired and are then combined into a cross sectional image (B-scan). This presents a specific set of challenges for a combination of this technique with e.g. confocal microscopy (CM) or scanning laser ophthalmoscopy (SLO), as the image acquisition of both latter methods lies in the *en face* plane, that is orthogonal to that of the more conventional cross sectioning OCT imaging.

The year 2018, when the initiative of creating this special issue was taken, marked 20 years since the publication of a first report [1] on a combination of the original SLO [2], dating back from 1980 and OCT [3], a newer imaging technique, introduced in 1991. What initially appeared challenging to achieve, fusion of the two different modalities to simultaneously display a contiguous SLO image and a thin fragmented *en face* OCT image, was demonstrated successfully in 1998. The *en face* OCT [4,5] display of a thin section of the eye [6] together with its SLO counterpart [1] opened new diagnostic avenues. Apart from providing complementary anatomic data, the combination of these two methods additionally affords the opportunity for motion correction since different imaging planes are usually recorded with different speeds. Of particular advantage is the fact that many structures of interest are best viewed in the *en face* plane. Cone and rod photoreceptor mosaics of the retina recorded with adaptive optics (AO) assisted imaging methods, are a good example. The regularity of the mosaic is best revealed in the *en face* imaging plane. Vascular imaging is another example where only the *en face* display reveals tissue specific patterns and disease related loss of perfusion. Most strikingly, OCT angiography (OCTA), a product of OCT acquisition speed progress, which has attracted widespread interest for its non-invasive,

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Biomedical Optics EXPRESS

quantitative imaging of retinal vasculature, could only have come into being as a product of the *en face* perspective.

These are some of the reasons why *en face* display is so intimately linked to multimodality, hence the theme of this feature issue. The multimodality seed launched by the OCT/SLO combination is reflected in the more than 5 million web search hits generated by the acronym.

This feature issue contains reports on the most recent advances in the field of *en face* OCT and of combination of modalities facilitated by its perspective. The special issue celebrates the past 20 years of engineering creativity in the field of multimodal and *en face* imaging and the spark of clinical necessity that inspired the creation of combinations of diverse imaging modalities.

A comprehensive review by Rainer Leitgeb [7] from Medical University Vienna, presents the hardware evolution of technology, as well as the adoption of terminology over the years. As OCT addressed a problem in imaging the eye and more than 50% of reports on OCT are on the eye, this review focuses on ophthalmology. The review concludes by outlining exciting technological prospects of en face OCT based both on time domain as well as on spectral (Fourier) domain OCT.

Wes M. Allen et al. [8] describe an automated segmentation algorithm to fuse *en face* micro-elastograms with OCT images to provide dual contrast images. The procedure enables rapid visualization of *en face* images required for intraoperative assessment. In order to validate the contrast in micro-elastograms, a method is presented that enables co-registration of *en face* images with histology of wide-local excision specimens, sectioned in the orthogonal plane, without any modification to the standard clinical workflow. The results presented demonstrate the potential of optical coherence micro-elastography for imaging tumor margins.

Acner Camino, et al. [9] present an algorithm to identify shadowed areas in OCTA due to obstacles to the propagation of light such as vitreous floaters or the pupil boundary. This algorithm is applied to healthy subjects as well as patients with diabetic retinopathy, uveitis and age-related macular degeneration. The aim is to exclude the shadowed areas from analysis so that the overall OCTA parameters are minimally affected by such artefacts.

Michaal Chlebiej et al. [10] present a method of OCT angiography (OCTA) data filtering for noise suppression and improved visualization of the retinal vascular networks in *en face* projection images. The core of the method is based on application of directional filtering to the C-scans, i.e. one-pixel thick sections of the 3-D data set, perpendicular to the direction of the scanning OCT beam. The method uses a concept of structuring, where directional kernels of shapes match the geometry of the image features. Rotating ellipses are employed to find the most likely local orientation of the vessels and use the best matching ellipses for median filtering of the C-scans.

Arash Dadkhah et al. [11] present a multimodal microscopic optical imaging system with the capability of providing comprehensive structural, functional and molecular information of living tissues. This imaging system integrated photoacoustic microscopy, OCT, optical Doppler tomography and confocal fluorescence microscopy in one platform. Focusing adjustment is achieved using an OCT-guided surface contour scanning methodology.

Mayank Goswami et al. [12] present a novel mouse ocular xenograft model of glioblastoma, where the resultant xenografts are imaged *in vivo* with combined fluorescence SLO and volumetric OCT for a period up to several months. The ocular model shows potential for examining the relationships between neoplastic growth, neovascularization and other features of the immune microenvironment, and for evaluating treatment response longitudinally *in vivo*. The paper demonstrates quantitative tracking of tumor development, of the delivery of theranostic nanoparticles, and of tumor and tissue response to optically stimulated nanotherapeutic treatment.

Van Phuc Nguyen et al. [13] present a multimodal system to evaluate optical properties of retinal vein occlusion and retinal neovascularization (RNV) in living rabbits, using multi-wavelength *en face* photoacoustic microscopy (PAM) and a spectral domain OCT. They monitored changes in the retina morphology and vessels after vein occlusion. The OCT provides useful information to quantify RNV and classify different layers of RNV, retina pigment epithelium, choroid, and sclera that supplements the information provided by PAM. Both *en face* PAM and OCT can visualize the dynamic changes in the retinal thickness. In addition, spectroscopic PAM image helps to select the optimal wavelength for monitoring and quantification of the RNV structure.

Tristan T. Hormel et al. [14] quantitatively compared the different projection schemas used in OCTA, such as maximum and mean value projections. They demonstrate that maximum value projection achieves a consistently higher signal-to-noise ratio and higher image contrast across multiple vascular layers, in both healthy eyes and for each disease examined.

Giselle Lynch et al. [15] demonstrate that *en face* OCT reflectance images provide useful anatomic baselines of structural foveal avascular zone (FAZ) morphology prior to the onset of diabetic retinopathy. In this study, within-subject FAZ area enlargement was obtained by the comparison of structural FAZ area to the functional FAZ area using simultaneously-acquired, corresponding *en face* OCT reflectance and OCT angiography images.

Justin V. Migacz et al. [16] demonstrate 23% average improvement in the contrast of OCTA of the human choriocapillaris and choroid images when using a 1.64 MHz swept source OCT system in comparison with a slower rate system operating at 100 kHz. They speculate that the improvement is due to the better suppression of the noise contribution from eye motion when scanning fast.

Mircea Mujat et al. [17] present a multimodal platform that combines OCT for measurement of retina/choroid structure and ocular blood flow with line-scanning Doppler flowmetry for a wide-field semi-quantitative global flow visualization. They show that such combination enables comprehensive assessment of blood flow in the retina and choroid in animals and human subjects for diagnostic purposes. Ultra-widefield vasculature visualization is demonstrated based only on the motion of particles within the vasculature.

Elena Goas-Salas et al. [18] present an adaptive optics flood illumination ophthalmoscope using near infrared (NIR) light. This is configured to work both in bright-field and dark-field modalities to generate flood-illumination NIR video sequences of erythrocytes, or red blood cells (RBC). They developed a new computational method relying on a spatio-temporal filtering of the sequences to isolate blood flow from noise in low-contrast sequences. By temporally filtering the image sequence, several perfusion maps are generated, each one showing capillaries with blood flowing at a different speed range.

Sam Osseiran et al. [19] present a combination of two complementary methods, corneometry, a bulk assessment tool, and coherent Raman scattering (CRS) microscopy, a modality with subcellular resolution. The last one is implemented in an *en-face* tissue imaging setup. These techniques are used to measure uptake and efficacy of topical compounds in order to better understand their mode of action and improve therapeutic applications. Using such a system, *ex vivo* human skin explants undergoing dehydration and humectant-induced rehydration were measured. They noticed that the imaging data and corneometer readings show differences under the experimental rehydration conditions.

Shaohua Pi et al. [20] have integrated wide-field visible-OCT retinal angiography with oximetry to study the organization of the arterial and venous retinal circulation in rats. Arterioles were found predominant in the superficial vascular plexus whereas veins tended to

Biomedical Optics EXPRESS

drain capillaries from the deep capillary plexus. The oxygen metabolism supported by the retinal microcirculation was determined by combining retinal vessel oxygen saturation and flow flux measurements.

Adrian Podoleanu et al. [21] introduces down-conversion of the photodetected signal based on the Master Slave concept using a mixer and a physical interferometer to produce the master signal. This strategy allows production of an *en face* OCT image in real time with no need to compensate for the chirp in the signal. The bandwidth of the signal to be processed is the same irrespective of the depth in the sample, commensurate to the sweeping rate, i.e. at a downconverted scale in comparison with the bandwidth of the photodetected signal.

Jules Scholler et al. [22] describe recent technological progress in multimodal *en face* full-field optical coherence tomography that has allowed detection of slow and fast dynamic processes in the eye. High-resolution imaging of the retina and anterior eye with temporal resolution from milliseconds to several hours are obtained by combining static, dynamic and fluorescence contrasts. This has allowed probing biological activity at subcellular scales inside 3D bulk tissue. Its contactless and non-destructive nature is shown to be effective for both following *in vitro* sample evolution over long periods of time and for imaging of the human eye in vivo.

Jia-Pu Syu et al. [23] proposed a multimodal system consisting in ultrahigh-resolution multi-contrast OCT integrated with fundus photography for *in vivo* retinal imaging of rodents. High resolution was achieved by using a supercontinuum light source and birefringence characterization using a polarization sensitive architecture with two spectrometers. Using the proposed system, three types of tissue contrast are simultaneously obtained based on: backscattered intensity, phase retardation and microvasculature at a capillary level. The retardation contrast provides the information of the changes in choroid and sclera, and depolarization properties from melanin. *In vivo* imaging results were validated with histology.

Daniel J. Wahl et al. [24] present a multi-modal Sensorless Adaptive Optics (SAO) *en-face* retina imaging system that includes OCT, OCT-Angiography, confocal SLO, and fluorescence detection. A compact lens-based imaging system design is reported that allows for a 50-degree maximum field of view, which can be reduced to the region of interest to perform SAO AO with the modality of choice. The system was demonstrated on mice and transgenic mice, including volumetric cellular imaging of microglia throughout the inner retina.

Sarah Walters et al. [25] present a combination of a confocal SLO, OCT and high-resolution retinal imaging for observation of retina of non-human primates with induced photoreceptor degeneration. Photoreceptors were imaged at single-cell scale using three modalities: traditional confocal reflectance, a non-confocal offset aperture technique and two-photon excited fluorescence. Assessment of photoreceptor structure and function using these imaging modalities revealed a reduction in retinoid production in cone photoreceptor outer segments while inner segments appeared to remain present. The authors propose this combination of imaging modalities to deliver both the structural integrity and function of photoreceptors as well as for evaluation of efficacy of future cell and gene-based therapies for vision restoration.

Julia Walther et al. [26] present depth-resolved (i.e. local) birefringence of healthy human oral mucosa *in vivo* as a potential prognostic indicator in a variety of cancers and other diseases accompanied by fibrosis. Using a polarization-sensitive optical coherence tomography (PS-OCT) set-up with local phase retardation, local collagen organization is revealed. Compared to *en face* views of intensity or conventional cumulative phase retardation, they show that this novel approach offers improved visualization of the mucosal connective tissue layer in general, and reveals the collagen fiber architecture in particular.

Jin Wang et al. [27] assessed the full retinal thickness, the vessel density and the flow area in several retinal layers of healthy and coronary heart disease (CHD) patients using OCT and OCTA. They concluded that OCTA is sensitive to detect a decrease in the vessel density in all retinal/choroidal layers and choroidal flow area before any clinical fundus sign showed in CHD patients, except for the superficial and deep fovea.

Jie Wang et a [28]. addresses the problem of aberrations in the eye affecting stitching together several OCTA images. They propose to make use of an automated registration of contiguous OCTA images based on invariant features. The invariant features were used to register the overlapping areas between adjacently located scans by estimating the affine transformation matrix needed to accurately stitch them. The proposed method could montage the angiograms seamlessly and provided a wide-field of view of retinal vasculature.

Yiyang Wang et al. [29], present a machine learning classification approach to explore whether hyper-spectral images offer an improved outcome compared to standard red-green-blue images. They demonstrate that the classifier performs better on hyper-spectral images with an improved accuracy and sensitivity for drusen classification compared to standard imaging. The paper provides further evidence that hyper-spectral retinal image data are uniquely suited for computer-aided diagnosis and detection techniques.

Hao Zhou et al. [30] present a swept source optical coherence tomography (SS-OCT) equipped with attenuation correction to facilitate accurate automatic segmentation of the choroid. This allows visualization of the choroidal vasculature without performing OCT angiography. An algorithm that segmented the choroid from attenuation compensated B-scans, achieved significantly higher accuracy when compared with an automated segmentation performed on regular OCT scans. *En face* images of choroidal vessels are obtained with less artefacts from retinal vessels and measurements of mean choroidal thickness and vessel density showed high repeatability.

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