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Progress in Physics (43)

Extended-focus optical-coherence microscopy – seeing the brain at work

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The 2-dimensional (2D) scanning of brain structures with an optical Bessel beam allows for coherent 3D microscopy of living mouse brains up to 500 μm depth, while providing high lateral and axial resolution of $< 2 \mu\text{m}$. This is a minimally invasive imaging technique allowing to map brain structures, vascularisation and to perform a full assessment of quantitative blood flow at unprecedented acquisition rates.

During the last centuries, microscopy enriched biology and medicine with an ever-increasing image quality, providing high resolution with an increased contrast for a visualization of tissue and cell structures. Our biology textbooks testify with many images this undeniable impact of optical imaging. Besides this, imaging of tissue and cell processes has received increasing attention during the last decades, as these processes control and regulate tissue and cell function. But imaging of dynamic processes generates new challenges for optical image acquisition. As an important example, imaging blood flow and disease-induced alterations requires rapid three-dimensional imaging, where classical microscopy concepts meet their technical and physical limitations.

By the end of the 1980s, A. Fercher [1] and his co-workers at the University of Vienna (Austria) invented optical-coherence tomography (OCT), a low coherence interferometric imaging modality. In close analogy to ultrasound, OCT generates tomograms, so-called "depth profiles", which are sequentially acquired for synthetic tomograms. This interferometric imaging technique leads to a fast acquisition rate with an increased penetration depth. Consecutive tomograms are assembled to render a full 3D imaging stack. Due to the interferometric nature of OCT, the OCT contrast mechanism is given by the small variations of the index of refraction of tissues or cells causing a small sample reflection. Moreover, through the intrinsic optical amplification of these sample reflections, a strong signal can be detected. This results in a label-free imaging modality with an access to phase information. These features pave the route towards optical-coherence microscopy with an ever-growing field of novel applications.

In this article, we describe the current state of the art in optical-coherence microscopy (OCM) with a particular emphasis on brain and Alzheimer imaging. The promising outlook on future OCM developments [2-4] is a clear indication of the dynamism and breadth of potential contributions that OCM may make in the next coming future to biology and medicine.

The method – optical-coherence imaging

The set-up of an OCM instrument is based on a Mach-Zehnder interferometer (figure 1). A beam splitter divides the beam of a broadband light source into a reference (red in figure 1) and an illumination beam (blue in figure 1). The sample is laterally scanned in the x-y direction and the back-scattered sample beam interferes with the reference beam. The resulting interference signal is detected by the spec-

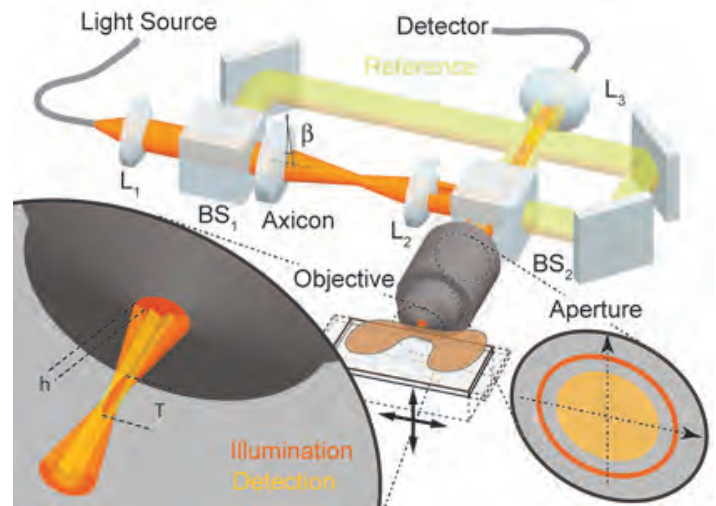


Figure 1: General set-up of an xFOCM system with Mach-Zehnder interferometer and spectral detection (spectral interferometer)

trometer. The crucial element in this interferometric set-up is the broadband source: its small temporal coherence leads to a "coherence gating" enabling a high axial resolution (coherence length $l_c \propto \lambda_c^2 / \Delta\lambda$). The interference resulting from the different tissue structures and layers manifests itself as a modulation on the spectrum due to constructive and destructive interference (spectral interferogram) [5, 6]. Applying a Fourier transform to these spectral interferograms (the so called k -spectrum) results in a depth profile. The entire depth profile is thus acquired in a single spectrum. This multiplexing advantage (no depth scanning needed), corresponding to the so-called Fellgett advantage, generates a synthetic 3D image of the sample by fast lateral scanning. Thus, fast 3D imaging is made possible by this coherent imaging and opens the door towards minimally invasive structural and functional *in-vivo* imaging of small animals.

Spatial resolution

For most optical microscopy methods, spatial resolution is of utmost importance. In OCM, one illuminates the sample with a weakly focused Gaussian beam so that the probed sample receives a nearly uniform illumination over the whole depth. As in classical microscopy, the lateral resolution is given by the Abbe criterion and is a function of the numerical aperture (NA), whereas the temporal coherence length determines the decoupled axial resolution. Maintaining a uniform resolution over the whole field depth is more complicated than at first glance. The increased NA leads effectively to an increased lateral resolution, but the uniform sample illumination is squeezed by a strongly reduced

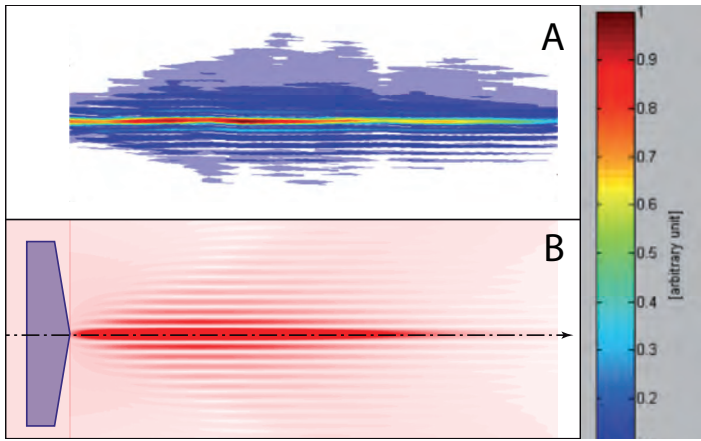
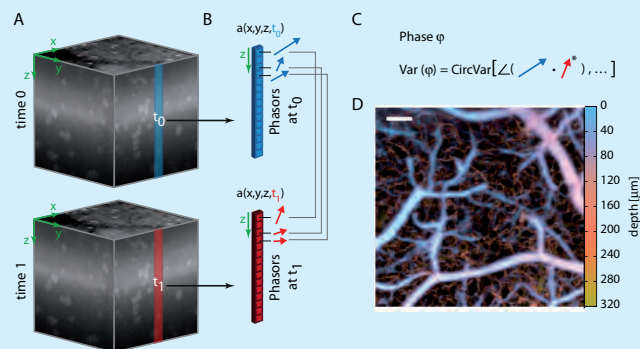


Figure 2: Bessel beam
 (A) measured illumination (Ti:Sa laser 130 nm bandwidth / 50 nm depth of focus)
 (B) simulated Besselbeam with an elongated illumination and side lobes

Rayleigh range. What is needed is a so-called "optical needle" with an almost constant illumination profile over an extended depth range. A so-called Bessel beam (figure 2) [7], generated by an axicon, fulfils these requirements and ensures a uniform narrow illumination profile over an extended depth. The combination of tube lens and objective "demagnifies" this Bessel beam to a lateral extent of about $1.5 \mu\text{m}$ over a depth range of approximately $500 \mu\text{m}$ ideally suited for the multiplexed OCM imaging approach.

Phase-variance Angiography

In OCT, each voxel of the image can be represented by a phasor describing the scattering strength (length) and the position of the scattering particles within the resolution volume (phase). In theory, if these particles are static, the corresponding phase will be constant over time. In the case of erythrocytes flowing through a capillary and due to the movement of these scatterers, the phase of their corresponding voxels will change with time. Therefore by analysing the temporal evolution of the phase, the dynamic and the static components can be separated to generate angiographic maps. In practice this is done by acquiring a series of B-scans (typically 4) at the same lateral x-position and calculating the spatial variance of their phase difference (see figure below).



Overview phase variance method: (A,B) Two time samples are taken at the same lateral location. To isolate the dynamic component, the circular variance over the phasors is calculated (C). As depicted, the presented method allows rendering angiographic maps of a rodent's cerebral cortex (D).

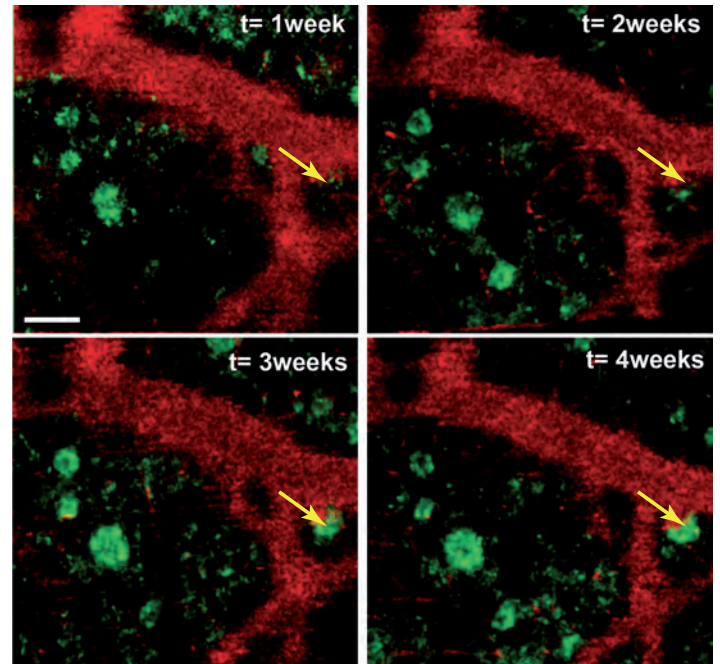


Figure 3: Longitudinal study of Alzheimer's disease in an APPS1-mouse

OCM and Alzheimer's disease research

In Alzheimer's disease, extracellular amyloid plaques, i.e. water-insoluble aggregates made of amyloid-beta (Abeta) proteins, are considered a major neuropathological hallmark of Alzheimer's disease. The exact toxic effects of these highly scattering proteins aggregates are however not yet fully understood. OCM allows a longitudinal label-free monitoring of amyloid plaque formation in the living mouse brain,

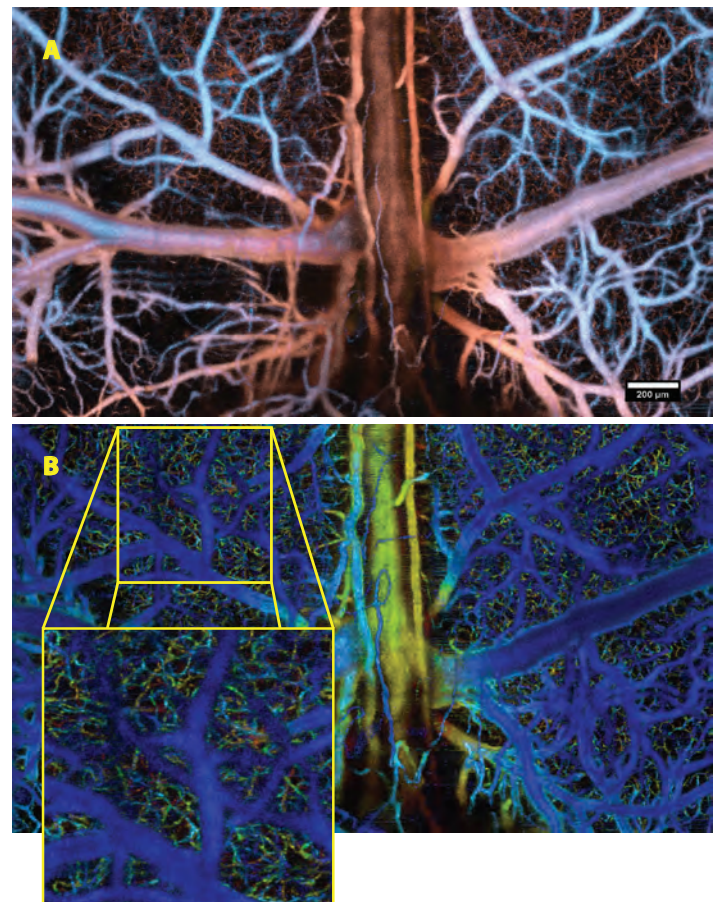
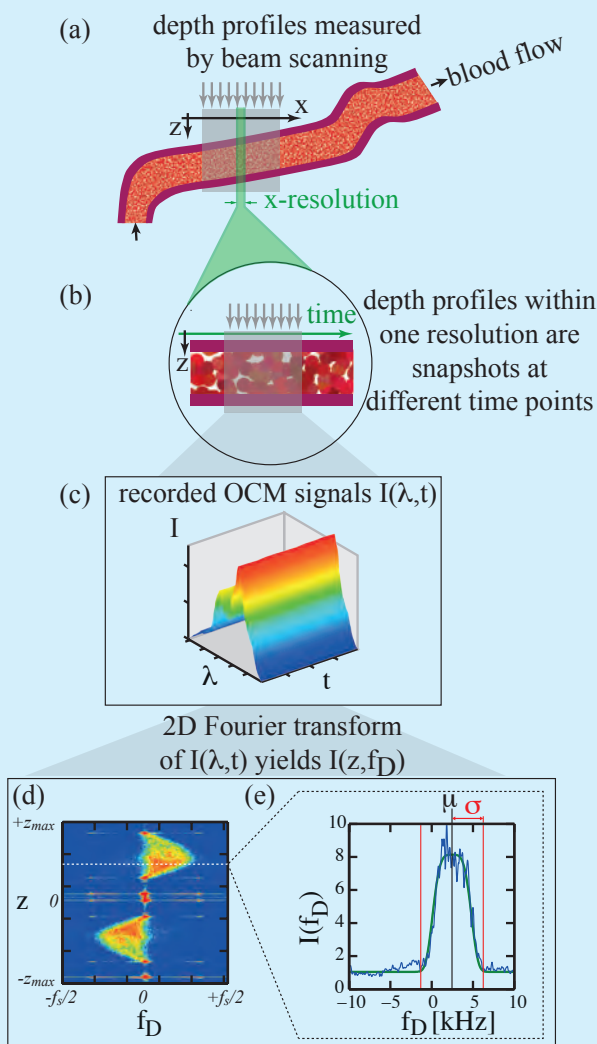


Figure 4: OCM phase-variance image of a mouse brain showing the vascularisation with micro-vessel structures measuring up to $8 \mu\text{m}$ across (corresponding to the diameter of a red blood cell)

Quantitative blood flow measurement

Complementary to angiography (see Infobox 1), velocimetric techniques perform a quantitative estimation of the blood flow speed by measuring the Doppler frequency shift induced by moving scatterers. Most velocimetric OCT methods only measure the velocity component along the optical axis. However, in most biological applications, many vessels and capillaries are directed almost perpendicularly to the optical axis. Therefore, we developed a technique for measuring the parallel and perpendicular flow components by quantifying the whole Doppler frequency spectrum (as opposed to only a single Doppler frequency), which is created when a high-resolution OCM system images moving scatterers. This Doppler spectrum can be measured by acquiring depth profiles in a densely sampled x-scan (oversampling), such that many (typically, 32) such profiles are acquired within one resolution element (a). Hence, the depth profiles recorded within this resolution element represent the same spatial position in the sample, but were recorded at different time-points (b). Therefore, for each spatial position, the OCM signal $I(\lambda, t)$, is obtained as a function of wavelength λ and time t (c). By applying a 2D Fourier transform on these data, a Doppler spectrum is obtained for each depth position (d). Fitting this Doppler Spectrum and extracting its mean and standard deviation (e) allows calculating the parallel and perpendicular velocity components.



without prior administration of an amyloid-binding dye or a radioactive tracer [8]. As shown in figure 3, these scattering structures (diameter about 20–200 μm) increase in size and numbers during an observation period of four weeks.

The underlying interferometric principle of OCM allows an access to the optical phase. (Box 1) A simple reprogramming of the scan-protocol allows extracting the vasculature with a high spatial 3D-resolution. The vascularisation shown in figure 4 is a 3x4 mm section of a mouse brain. The fine deeply lying micron-sized vessels can be identified with a diameter of about 8 μm , corresponding to the size of individual erythrocytes. The re-location of these structural changes in affected areas of the brain can easily be retrieved with high accuracy while using the vascular network as a reference road map.

Further exploiting this phase information allows assessing the blood flow quantitatively (Box 2). The blood flow is modulated by the natural heartbeat cycle, but contains additional flow components, which are induced by neuronal brain activity. The localized blood flow increase in response to external stimulation is an indirect measurement of stimuli responses. In close analogy to functional MRI, OCM can be applied to functional brain imaging with an increased spatial resolution (typically 2 order of magnitude). For brain research, this results in the possibility to assess the functional responses induced by external stimuli or distortions, with high spatial and temporal resolution (see figure 5).

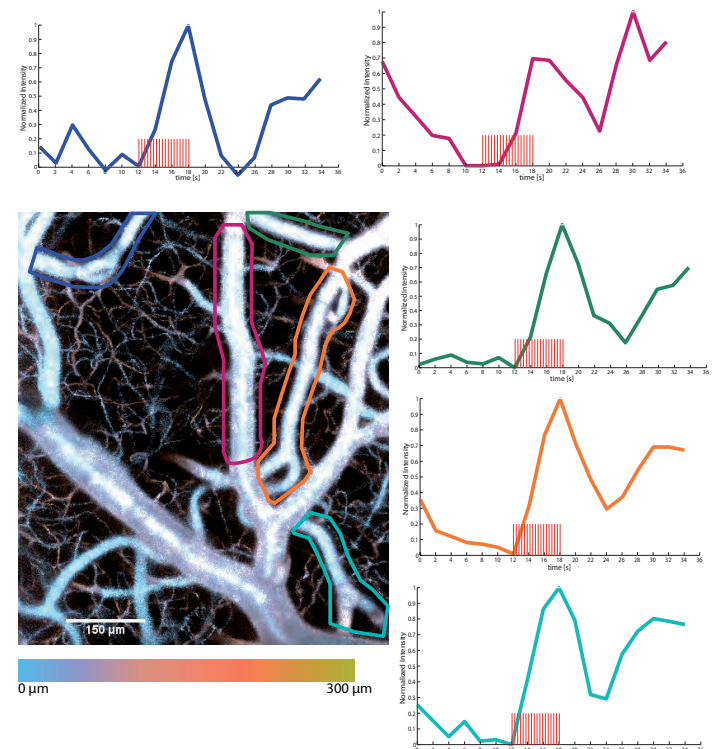


Figure 5 Mouse brain, somatosensory cortex showing vascular evoked responses to electrical stimulation of the contralateral hind-paw. Stimulation period is indicated by the red train of electrical pulses.

Outlook

The potential contributions and application areas of OCM are steadily growing. Due to this high sensitivity, gold nanoparticles can be detected down to a size of 2 nm. These

findings have been recently exploited for photo-thermal optical lock-in OCM (poliOCM) [2]. This contrast enhancement adds high specificity to selected proteins and sub-cellular structures in a similar fashion to confocal fluorescence microscopy. Very recently, OCM has been extended to the visible wavelength range, which opens up completely new perspectives for high resolution cell imaging.

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