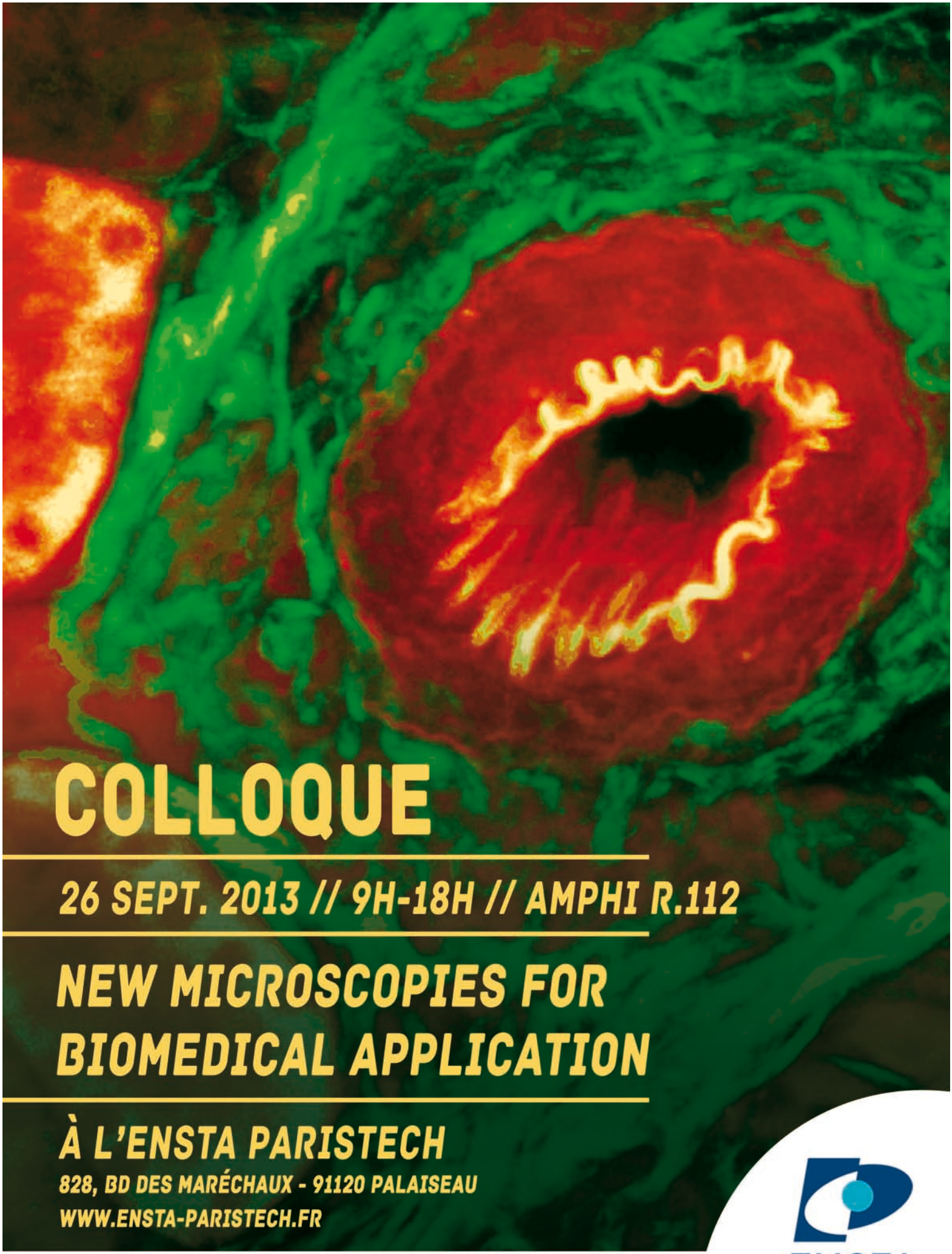

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Fluorescence lifetime imaging across the scales

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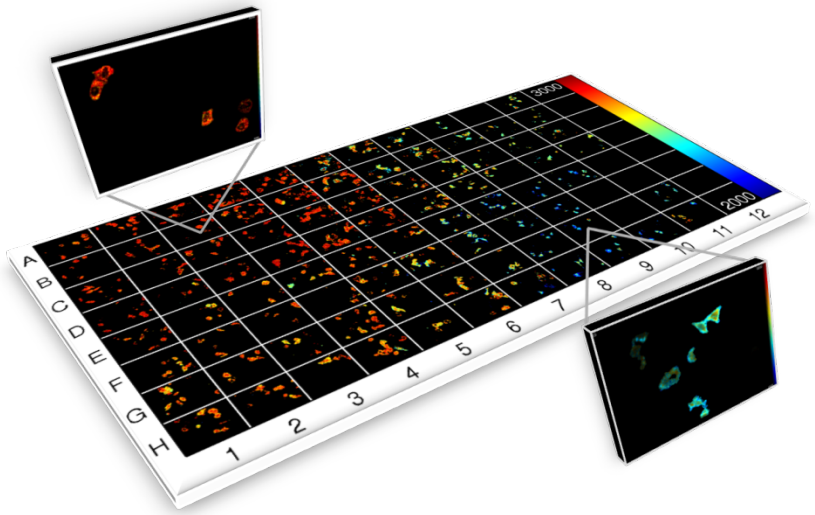
Longer abstract

This talk will review our development and application of fluorescence lifetime imaging (FLIM) and metrology technology applied to microscopy, high content analysis (HCA), endoscopy and tomography, emphasizing the potential to translate molecular readouts across the scales. For cell biology we have developed FLIM microscopes including high-speed optically sectioned FLIM for rapid 3-D imaging, including of FLIM FRET readouts in live cells. For drug discovery we have this to automated optically sectioned FLIM/FRET multiwell plate readers that can "read" a 96 well plate in less than ~15 minutes. With its associated analysis software, this technology makes FLIM a practical tool for HCA including for live cell assays. For drug discovery and for fundamental biomedical research, it is of increasing interest to translate cell-based assays to in vivo studies. Accordingly, we are developing tomographic FLIM instruments including FLIM optical projection tomography, which we have applied to live zebrafish embryos and diffuse FLIM tomography, with which we have demonstrated in vivo FLIM FRET in a mouse model. For imaging larger disease models and patients, we are developing a range of FLIM endoscopes including a FLIM confocal endomicroscope, wide-field FLIM endoscopes and single point fibre-optic multidimensional fluorescence probes to provide more detailed information on complex spectro-temporal autofluorescence signals. These endoscopic instruments are complemented by clinical multiphoton multispectral FLIM tomography, from which we have obtained in vivo data.



Fluorescence lifetime images acquired on an automated multiwell plate reader – from D. Alibhai et al., Automated fluorescence lifetime imaging plate reader and its application to Förster resonant energy transfer readout of Gag protein aggregation, J. Biophotonics 6(5), 398-408 (2013). The DOI is 10.1002/jbio.201200185 (http://onlinelibrary.wiley.com/doi/10.1002/jbio.201200185/abstract)

Unraveling the structural and dynamic properties of macromolecular assemblies in live cells, one molecule at a time

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Single molecule techniques are becoming ubiquitous tools in *in vitro* and *in vivo* assays. In cell biology, they now can be used to count molecules, image with sub-diffraction resolution and track individual molecules as they move in their natural habitat. Thus, single molecule imaging is now a tool of choice to address the dynamics, composition and structural properties of supramolecular assemblies in live cells. In this talk, I will present our effort to develop and apply ultrasensitive fluorescence methods. I will in particular present novel optical methods based on adaptive optics or multifocal imaging to enable the 3D localization and tracking of individual molecules in live cells. All these techniques will be illustrated by experiments made on a variety of biological systems, such as post-synaptic membrane receptors, molecular motors or transcription factors. Finally, I will describe recent experiments in which we used magnetic nanoparticles for locally controlling signaling activity inside cells, demonstrating the interest of using perturbative approach in cell biology.

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Recent publications:

1. Ibrahim Cisse, Ignacio Izeddin, Sebastien Causse, Lydia Boudarene, Adrien Senecal, Leila Muresan, Claire Dugast-Darzacq, Bassam Hajj, Maxime Dahan, Xavier Darzacq, *« Real time dynamics of RNA Polymerase II clustering in live human cells »,* Science (in press)
2. M. El Beheiry and M. Dahan, *"ViSP: tool for visualizing 3D super-resolution data"*, Nature Methods (in press).
3. Christian G. Specht, Ignacio Izeddin, Pamela C. Rodriguez, Mohamed El Beheiry, Philippe Rostaing, Xavier Darzacq, Maxime Dahan and Antoine Triller *"Quantitative nanoscopy at inhibitory synapses: counting gephyrin molecules and receptor binding "*, Neuron (in press).
4. F. Etoc, D. Lisse, Y. Bellaiche, J. Piehler, M. Coppey, M. Dahan, *« Subcellular control of Rac signalling by magnetogenetic manipulation in living cells»,* Nature Nanotechnology (2013) 8, 193–198.
5. S. Abrahamsson, J. Chen, B. Hajj, S. Stallinga, A. Katsov, J. Wisniewski, G. Mizuguchi, P. Soulle, F. Mueller, C. Dugast Darzacq, X. Darzacq, C.Wu, C. I. Bargmann, D. A. Agard, M. Dahan and M.G.L. Gustafsson, *"Fast multi-color 3D imaging using aberration corrected multi-focus microscopy",* Nature Methods (2013) 10(1):60-3.
6. F. Pinaud, S. Clarke, A. Sittner, and M. Dahan, "Probing cellular events, one quantum dot a time", Nature Methods 7, 275-85 (2010).

Superresolution imaging of synapses in brain slices by STED microscopy

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Keywords: Glia and synaptic transmission; brain slices; cellular and developmental neuroscience

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Neuronal synapses are complex structures composed of pre- and postsynaptic membrane specializations ensheated by glia processes, forming elementary functional compartments for rapid and flexible signaling in the central nervous system. Understanding how synapses are built during development and modified by experience is a central theme and challenge for neuroscience.

As synapses and glial processes are typically very small (<< 1 μm), dynamic and reside inside three-dimensional, light-scattering tissue, it is difficult to study them by conventional, diffraction-limited light microscopy.

However, major advances in superresolution imaging and fluorescence labeling are greatly improving our ability to investigate the inner life and dynamics of synapses using life-cell imaging approaches. We have shown that superresolution STED microscopy is a powerful technique for live-cell imaging of synapse morphology using YFP as a genetically encoded volume-label.

We will review our recent progress in developing STED microscopy for live-cell nanoscale imaging of neuronal and glial structures deep inside brain slices and in two colors simultaneously. Specifically, we will demonstrate the powerful potential of these methodological advances for several applications concerning superresolution imaging of synapses: 1) nanoscale imaging up to 100 μm deep below tissue surface in acute brain slices by a novel combination of two-photon and STED microscopy; 2) dual-color nanoscale imaging of synapses interacting with astrocytic and microglial processes; 3) spine structure - function analysis combining nanoscale imaging of spine morphology with two-photon fluorescence recovery after photobleaching (FRAP) measurements.

Digital holographic microscopy: a new coherent imaging modality to quantitatively explore living cell dynamics with nanometer sensitivity: toward resolving local neural network activity

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General Considerations

Digital holographic microscopy (DHM) that we have developed is an interferometric microscopy technique which has the advantage of providing in real-time, the full-field complex wavefront diffracted by the observed specimen. The first idea of reconstructing the wavefront by digitally processing an hologram recorded with a camera dates back to the sixties[1]. The propagation of the wavefront was discovered to be precisely simulated by the numerical computation of the Huyghens Fresnel expression of a diffracted wave [2]. Further on, the basic approach developed for Digital holography has been applied to Microscopy [3, 4 5]. One of the relevant interests of DHM resides in its capacity to provide the quantitative phase measurement of the reconstructed wavefront with a very high accuracy (sub-nanometer). Practically, DHM allows to perform measurements in a very short time interval (acquisition time), as a single hologram is required to achieve the numerical reconstruction. In addition, the acquisition rate can be also very fast (camera limited). Consequently, a large immunity to external perturbations (vibration and ambient light) can be achieved. Otherwise, numerical processing of holograms presents the unique advantage of offering not only the means to reconstruct an exact replica of the wavefront diffracted by the specimen but also the means to reshape it. Such a numerical reshaping allows correcting experimental artifacts including lens defects and aberrations (6, 7, 8, 9,10 ,11).

Quantitative phase imaging of living cells

As far as transparent specimen are considered, the reconstructed quantitative phase images provide accurate measurement of the phase retardation (PR) or optical path difference (OPD) induced by the observed specimen on the transmitted wave front (12). PR which arises from a mismatch Δn between the intracellular integral refractive index (RI) n_c, defined as the mean cellular RI along the optical axis, and the RI of the extracellular medium n_s. PR can be regarded as a powerful endogenous contrast agent, as it contains information about both the thickness and the RI of the transparent sample (13). As far as biological cell are considered the origin of the "phase signal" resides in the refractive index difference generated by the presence of organic molecules: proteins, DNA, organelles, nuclei present in cells. Practically, important biophysical cell parameters can be calculated from the quantitative phase signal, including cell shape and absolute volume (13, 14) dry mass concentration (15), membrane mechanical properties [16], permeability (14), transmembrane water movements (17).

Resolving local neural network activity

Practically, experiments on primary cultures of mouse cortical neurons with a set-up combining electrophysiology and digital holography quantitative phase microscopy (DH-QPM), has allowed to reveal that one of the mechanisms inducing the activity-related modifications of the neuronal intrinsic optical properties are transmembrane water movements, related to transmembrane currents. This result, allowing to perform simultaneous multiple site optical recording of transmembrane currents, opens thus the possibility to non-invasively resolve local neuronal network activity with DH-QPM (18).

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IN SITU VISUALIZATION OF COLLAGEN ARCHITECTURE IN BIOLOGICAL TISSUES USING POLARIZATION-RESOLVED SHG MICROSCOPY

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Multiphoton microscopy has been shown to provide three-dimensional (3D) cell-scale contrasted images of biological tissues. In particular, Second Harmonic Generation (SHG) microscopy has emerged as a unique tool to probe the 3D distribution of collagen fibrils within unstained tissues (see fig. 1) because this second order coherent signal is highly specific for dense non-centrosymmetrical macromolecular structures. Such a 3D imaging technique is of great interest for biological and biomedical studies since collagen is the main component of the extra-cellular matrix in mammals. This structural protein is characterized by triple helical domains, which self-assemble into fibrils that further form fibers, lamellae or other 3D networks essential for the architecture of organs. This hierarchical organization of collagen is responsible for the biophysical and mechanical properties of tissues, for instance the mechanical strength of the tendon, the transparency of the cornea, etc…It is also involved in a variety of pathologies, including genetic diseases such as Elhers-Danlos syndrome, fibrosis, cancer, or any abnormal tissue remodeling, etc…

In the recent years, we focused on the development of polarization-resolved SHG imaging to access the sub-micrometer scale distribution of collagen fibrils [1]. We used a multiscale non-linear optical formalism to show that this technique can probe both the main direction of collagen fibrils and their orientational disorder in the focal volume [2]. We validated this theoretical approach by imaging rat-tail tendons subjected to controlled mechanical stretching, which increasingly straightened and aligned the collagen fibrils. Finally, we used polarization-resolved SHG microscopy to map the 3D distribution of nanometer-sized collagen fibrils in corneal stroma [3]. We successfully retrieved structural information from *ex vivo* human corneas and *in vivo* rat corneas, while raw backward-detected SHG images were spatially homogenous.

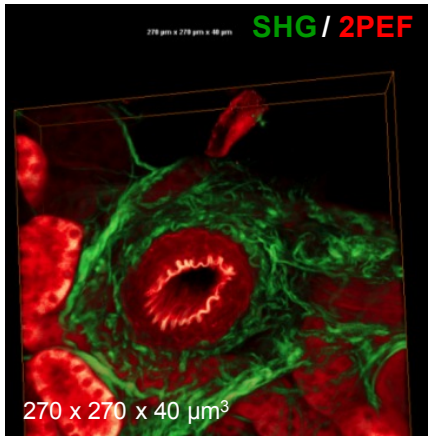

Fig. 1: multiphoton imaging of unstained arcuate artery from fibrotic mice

Our work also aimed to develop *quantitative* SHG imaging of the collagen fibrillar network. As a first step, we measured the nonlinear response of the collagen triple helix using Hyper Rayleigh scattering experiments [4]. Then, we assessed the sensitivity of SHG microscopy by measuring the minimum size of fibrils that can be detected using SHG microscopy. For that purpose, we correlated SHG images to TEM images of the same fibrils, for the first time to the best of our knowledge. It showed that SHG microscopy allows imaging of fibrils with a diameter down to 30 nm in our setup, much below the optical resolution.

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Intraoperative surgery and tissue selection:

What can be done with full field optical coherence tomography?

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Abstract: Optical Coherence Tomography (OCT) is an interferometric technique based on the use of a broad spectrum to virtually "slice" inside scattering materials with a typical resolution in the 10 micrometer range. Full Field Optical Coherence Microscopy provides an endogenous contrast, based on backscattering of ballistic photons, with submicron 3D resolution. We will show how it can be used for ex vivo and in vivo intra operative diagnosis and tissue selection.

A large number of imaging techniques are now available for studying biological tissues at different scales; they provide information about the nature (normal or pathologic), the structure (fat, collagen etc.), composition and evolution in time. Optical techniques could have played a major role in the diagnostic areas, nevertheless light is strongly scattered by tissues that makes the paths of the light rays very tortuous and images difficult to observe. Optical Coherence Tomography (OCT) is an interferometric technique based on the use of a broad spectrum to virtually "slice" inside scattering materials; OCT uses a broadband light source coupled to an interferometric detection. It has been widely used for biological tissues the main domain being the retinal observation. On the opposite the applications of OCT to dense highly scattering tissues such as brain, breast etc. is more reduced.

We have introduced a new approach called Full Field OCT using a Linnik imaging interferometer and a thermal source. The main difference with standard OCT is that we record 2D "en face images" (transverse slices, thickness of about 1 micron) on megapixel cameras without any transverse scanning. This configuration allows sharp focusing in the micrometer range (from 0.5 to 1.5 micron depending on the numerical aperture of the microscope). We will give few examples that of images taken with FF-OCT for intra operative diagnosis and tissue selection.

Figure: *images of virtual slices in the brain of a camel, a mouse and a drosophila*