Processing and Mosaicing of Fibered Confocal Images

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***Abstract*— New imaging technologies allow the acquisition of *in vivo* and *in situ* microscopic images at the cellular resolution in any part of the living body. The automated analysis of these images raises specific issues which require the development of new image processing techniques. In this work, we present some of these issues, and describe some recent advances of microscopic image processing, in particular the automatic construction of very large mosaics from times series of microscopic images to enlarge the field of view. This is also a step to bridge the gap between microscopic and higher resolution images like MRI, PET, SPECT or US. The paper is illustrated with state-of-the-art examples on small animals and humans for biological or clinical applications.**

I. Introduction

Optical technologies are being increasingly applied to perform real time assessment of tissue pathology *in vivo*. One particularly promising new technology is fibered confocal microscopy (FCM) since it allows optical sectioning of the tissue and provides images with micrometric resolution *in vivo* [1]. A striking benefit of this technology can for example be seen in the field of gastrointestinal endoscopy. Millions of biopsies are performed every year on patients during endoscopy in order to be observed afterwards by the pathologist under a microscope. Being able to observe *in vivo* and *in situ* at the microscopic level during endoscopy allows to identify precisely where to perform (or not to perform) the biopsy. This is the reason why academic research teams as well as private companies have dedicated a lot of effort during the past 5 to 10 years to make it happen.

II. Fibered Confocal Microscopy with Real-time Reconstruction

The Cellvizio, developed by Mauna Kea Technologies (MKT) is a complete fibered confocal microscope with a lateral and axial resolution comparable with a standard confocal microscope. It is based on the combination of:

- A flexible optical microprobe consisting in a bundle of tens of thousands of fiber optics, whose overall dimensions are compatible with the accessory channel of a standard endoscope,
- A proximal laser scanning unit, which assembles the functions of light illumination, signal detection, and XY robust and rapid scanning,
- A control and acquisition software providing real-time image processing.

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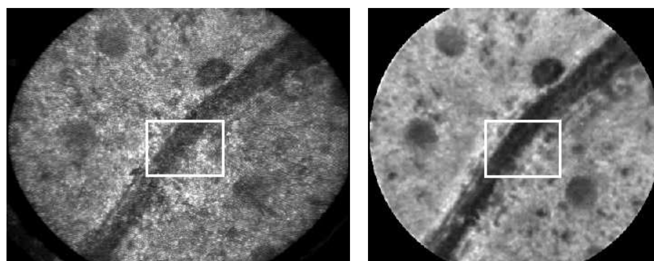
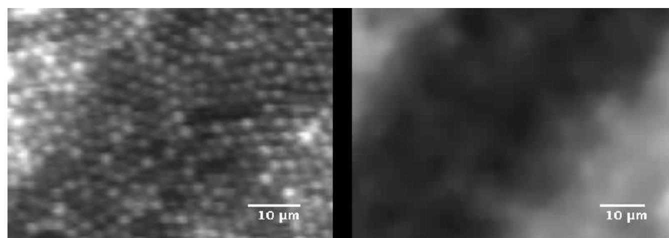
The laser scanning unit, performs a scanning of the proximal surface of the flexible optical microprobe with the laser source by using two mirrors. Horizontal line scanning is performed using a 4 kHz oscillating mirror while a galvanometric mirror performs frame scanning at 12 Hz. A custom synchronization hardware controls the mirrors and digitizes, synchronously with the scanning, the signal coming back from the tissue using a mono-pixel photodetector. When organized according to the scanning, the output of the FCM can be viewed as a raw image of the surface of the flexible image bundle. Scanning amplitude and signal sampling frequency have been adjusted to perform a spatial over-sampling of the image bundle. This is clearly visible on the raw image in Fig. 2 where one can see the individual fibers composing the bundle.



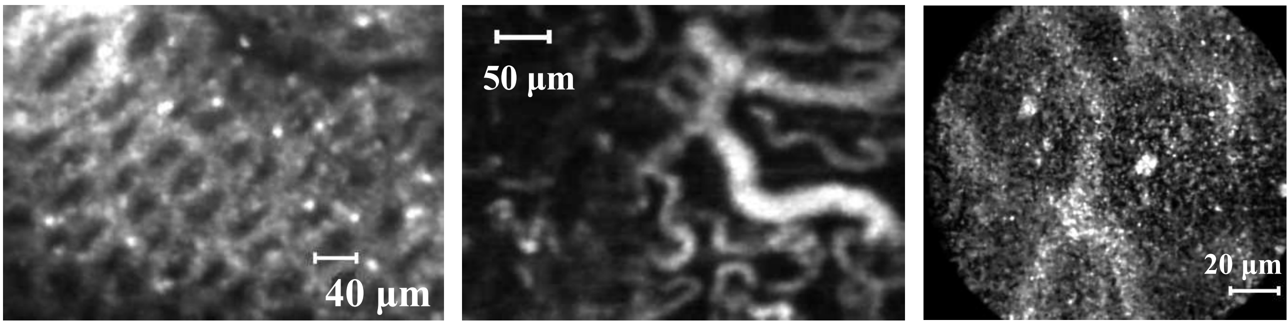
(a) FCM raw data. (b) Reconstructed image.

1: Autofluorescence FCM images of a Ficus Benjamina leaf. Note that the geometric distortions present in the raw data have been corrected in the reconstructed image.

A typical fiber bundle is composed of 30,000 fiber optics, with a fiber inter-core distance of 3.3μm, and a fiber core diameter of 1.9μm. Fiber arrangement is locally quasi hexagonal, but does not show any particular order at larger scales.



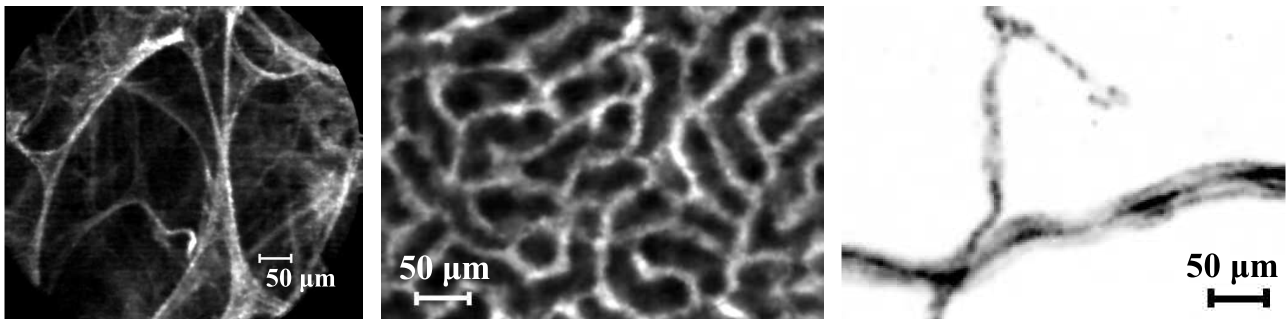
2: Enlarged view of the highlighted region in Fig. 1. Note that the non-uniform honeycomb modulation on the raw data has been corrected in the reconstructed image.



(a) *In vivo* mouse colon after instillation of acriflavine (Courtesy of D. Vignjevic, S. Robine, D. Louvard, Institut Curie, Paris, France).

(b) *In vivo* tumoral angiogenesis in mouse with FITC-Dextran high MW (Courtesy of A. Duconseille and O. Clément, Descartes Image, Université Paris V, Paris, France).

(c) *In vivo* reflectance imaging of human mouth mucosa.



(d) *Ex vivo* Autofluorescence imaging in human lung (Courtesy of Dr P. Validire, Institut Mutualiste Monsouris, Paris, France).

(e) Microcirculation of the peritubular capillaries of a live mouse kidney with FITC-Dextran high MW.

(f) Dendritic receptors in a live Thy1-YFP mouse (Courtesy of I. Charvet, P. Meda, CMU, Geneva, Switzerland and L. Stoppini, Biocell Interface, Geneva, Switzerland).

3: Different types of images acquired with the Cellvizio (Images taken from [3]).

This specific imaging modality raise specific image processing problems. The non-uniform honeycomb pattern and the geometric distortions that appear on the raw data, makes the raw data impracticable for user interpretation or for automated analysis if left untreated. Algorithms that take on the image reconstruction task in real-time have thus been developed in order to provide users with high-quality, smooth-motion video sequences that makes them readily interpretable by the professionals who rely on them for diagnosis and make them readily usable for further automated image processing and analysis [2].

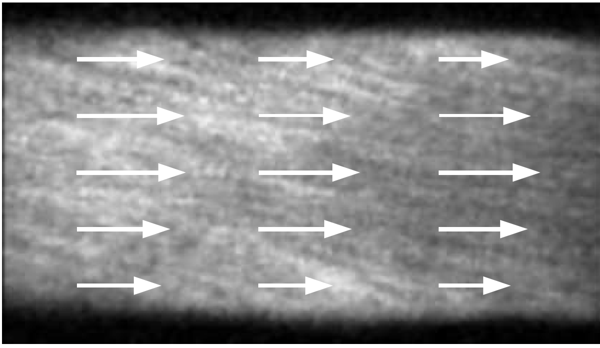
As shown in Fig. 1 and Fig. 2, the task of this on-the-fly image reconstruction module is to restore, at a speed of 12 frames per second, the true physical signal from the raw data by removing the fiber bundle honeycomb modulation. Each fiber of the bundle provides one and only one sampling point on the tissue. Associated with these sampling points comes a signal that depends on the imaged tissue and on the single fiber characteristics. The role of the image processing is first to build a mapping between the FCM raw image and the fibers composing the image bundle. Once the mapping between the raw data and each individual fiber is obtained, characteristics of each fiber are measured and the effective signal coming back from the tissue is estimated. We now have non-uniformly sampled frames where each sampling point corresponds to a center of a fiber in the flexible fiber bundle. An interpolation algorithm is then used to provide the user with smooth images. A collection of typical frames acquired *in vivo* is shown in figure 3.

III. Velocimetry using Motion Artifacts

An interesting point of scanning imaging devices is that the output image is not a representation of a given instant, but a juxtaposition of points acquired at different times [4]. Consequently, if the flexible microprobe moves with respect to the imaged tissue, what we observe is not a frozen picture of the tissue, but a skewed image of it. Each scan line indeed relates to a different instant, and the flexible microprobe can move between each scan line.

This specificity can be used to measure the velocity of blood cells in microvessels from single images. The method exploits the motion artifacts produced by the laser scanning. Although velocity can be estimated from a single image, temporal sequences can then be exploited to increase the robustness and accuracy of the estimation.

Classical methods for velocity measurements of blood cells in microvessels are often based on the processing of 2D temporal image sequences obtained in the field of intravital microscopy. Line shift diagram, spatio-temporal analysis or blood cell tracking are used in such setting to process the sequences generated by CCD-based video microscopes.



4: *In vivo* mouse cremaster microvessel. The arrows are proportional to the estimated velocities computed on a block in the image. Note that, as expected, the velocity does not change along the direction of the microvessel and is maximal at the center.

In contrast, the method presented in [4] uses very specific information about the imaging device. By the combination of its own movement and the laser scanning, a red blood cell should appear almost as a slanting segment, whose length and orientation with respect to the scanning and the microvessel are dictated by its velocity.

In the sequence shown in Fig. 4, the plasma is marked by a fluorescent dye. Distorted red blood cells shapes are then expected to appear as dark trails. But what we observe seems to be bright trails. Our explanation is that there are so many dark trails that it induces a contrast inversion (given the red blood cell concentration, i.e. 4-5 million per microliter of blood, about 1000 red blood cells should be in this image) and what we see are the bright interstices between the dark trails. Segmenting individual traces is thus not possible. We therefore only use the slope of the white ridges that we extract in order to estimate the velocity.

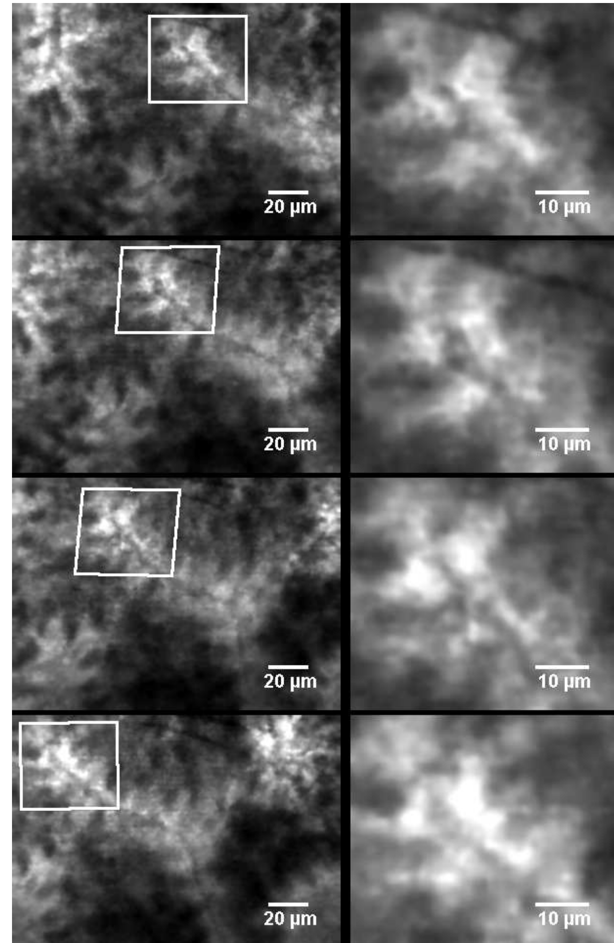
This method has been applied, in Fig. 4, to a real acquisition of mouse cremaster microvessel. The image is divided into a set of blocks. For each block, a robust estimation of the velocity is computed. We can see that our method is able to recover the red blood cell velocity profile within the microvessel. As expected, the velocity does not change along the direction of the microvessel and is maximal at the center.

As this approach is based on a line scan interaction between the laser scanning and the moving cells, higher velocity can be measured than with methods based on analysis of successive temporal frames. When most systems are limited to the measurement of red blood cell velocity inferior to 2 or 5 mm/s, the presented method allows the measurement of velocities up to 30 mm/s.

IV. Region of Interest Tracking for Kinetic Analysis

The high resolution images provided by the Cellvizio are mostly acquired on living organs, therefore specific image processing tools are required to cope with the natural movements of the tissues being imaged. Moreover, if the images of a sequence were stabilized, measurements of various image parameters would become possible or easier and could be carried out for many applications such as gene expression monitoring, drug biodistribution or pharmacokinetics.

Due to the very specific type of images generated by the Cellvizio, classical image stabilization and registration techniques could not be applied here. As a result, we are developing a dedicated region of interest (ROI) tracking tool that takes into account the characteristics of the Cellvizio, and enables an automatic registration, analysis and quantification on sequences of images.



5: Tracking a selected ROI of a mouse colonic mucosa. The tracked ROIs are shown on the left column with the corresponding warped region on the right column.

An additional interesting feature of this tracker is that it also enables the reconstruction of images on the region of interest with an enhanced resolution. This is made possible, when the kinetic of the signal is slow enough, thanks to the noise reduction provided by the processing of several registered noisy images of the same region and to a small remaining aliasing of the input images.

The heart of this tracker is based on the observations that led to the velocimetry measurements and to the mosaicing algorithm : first the fibered nature of the images, but also the motion artifacts induced by the scanning imaging devices. The algorithms that were designed take advantage of these observations to compute efficiently the transformations used to perform a robust registration, while applying motion artifacts

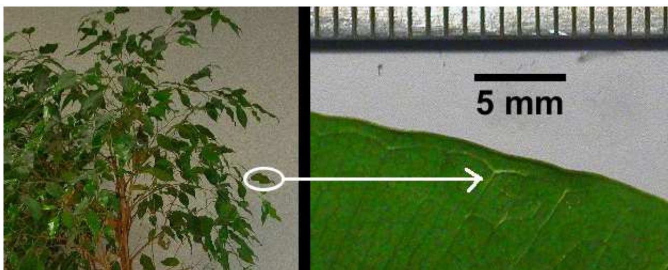
compensation.

In the example showed in Fig. 5, a confocal microprobe was glided along the stained colonic mucosa of a mouse. Once the region of interest is selected (left column), it is tracked and the resulting registered and stabilized ROI is displayed (right column).

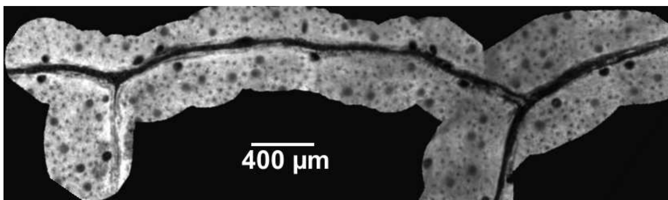
V. Mosaicing: Bridging the Gap between Micro and Macroscopic Scales

We showed that fibered confocal microscopy can unveil in real-time the cellular structure of the observed tissue. However, as interesting as dynamic sequences may be during the time of the medical procedure or biological experiment, there is a need for the expert to get an efficient and complete representation of the entire imaged region. A physician needs, for example, to actually add fixed printed images in the patient's medical record.

Image sequence mosaicing techniques are used to provide this efficient and complete representation and widen the field of view (FOV). Several possible applications are targeted. First of all, the rendering of wide-field micro-architectural information on a single image will help experts to interpret the acquired data. This representation will also make quantitative and statistical analysis possible on a wide field of view. Moreover, mosaicing for microscopic images is a mean of filling the gap between microscopic and macroscopic scales. It allows multi-modality and multi-scale information fusion for the positioning of the optical microprobe.



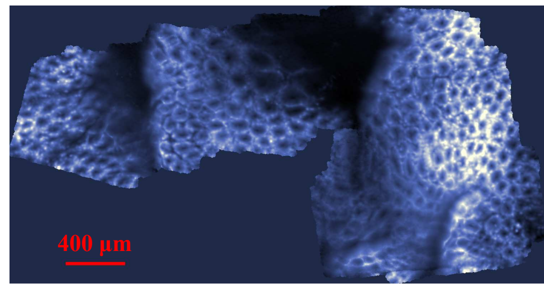
(a) Ficus Benjamina (left). A particular leaf showing a vein triple point (right).



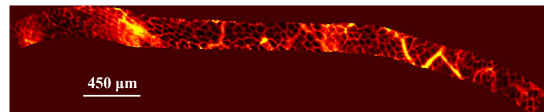
(b) Autofluorescence FCM mosaic.

6: Bridging the gap between macroscopic and microscopic scale. A Ficus Benjamina leaf showing a triple point in a vein was chosen and imaged with fibered confocal microscopy. Note that the triple point can clearly be seen on the reconstructed mosaic.

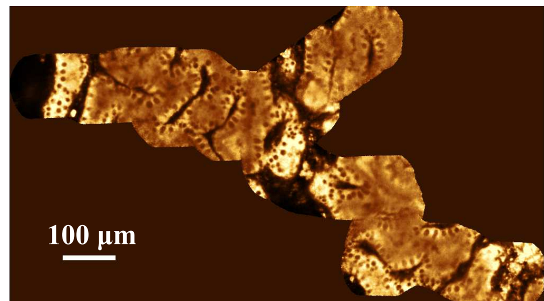
Classical mosaicing algorithms do not take into account the characteristics of fibered confocal microscopy, namely motion distortions, irregularly sampled frames and non-rigid deformations of the imaged tissue. This is the why specific novel algorithms were developed in [5], [3].



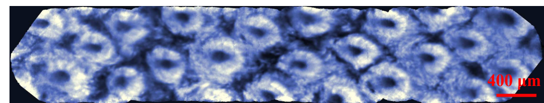
(a) *Ex vivo* mouse colon imaging after instillation of acriflavine (226 input frames). Courtesy of D. Vignjevic, S. Robine and D. Louvard, Institut Curie, Paris, France.



(b) *In vivo* Mouse colon vascularization after injection of FITC-Dextran high MW (300 input frames). Courtesy of M. Booth, MGH, Boston, MA.



(c) *Ex vivo* reflectance imaging of the human colon (1500 input frames).



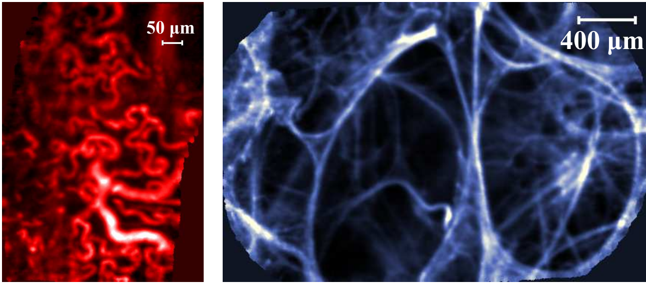
(d) *Ex vivo* imaging of the human colon with methylene blue (51 input frames). Courtesy of P. Validire, Institut Mutualiste Montsouris, Paris, France.

7: Mosaics of the colon (Images taken from [3]).

Our approach in [3] is based on a hierarchical framework that is able to recover a globally consistent alignment of the input frames, to compensate for the motion-induced distortion of the input frames and to capture the non-rigid deformations of the tissue. The global positioning algorithm is tackled as an estimation problem on a Lie group [5]. An efficient optimization scheme is proposed to solve this estimation problem.

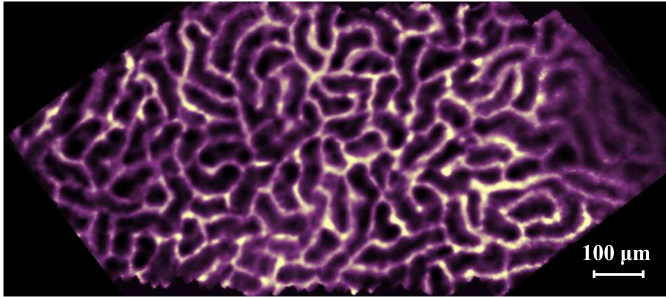
Similarly to the velocimetry problem and the region of interest tracking problems presented above, we use the specificity of FCM to model and use the relationship between the motion distortions and the motion of the optical microprobe. An efficient scattered data fitting method is also used to reconstruct on a regular grid the irregularly sampled images that arise from the inputs and from the mosaic construction process. This reconstruction method is also used to recover the non-rigid deformations with an adapted *demons* algorithm.

As shown in Fig.6, mosaicing for fibered confocal mi-



(a) *In vivo* tumoral angiogenesis in mouse with FITC-Dextran high MW (21 input frames).

(b) *Ex vivo* autofluorescence imaging in human lung (15 input frames).



(c) Microcirculation of the peritubular capillaries of a live mouse kidney with FITC-Dextran high MW (31 input frames).

(d) Dendritic receptors in a live Thy1-YFP mouse (70 input frames).

8: Mosaics using different types of images acquired with the Cellvizio (Courtesy notes appear in figure 3, Images taken from [3]).

croscopy provides a step to bridge the gap between microscopic and macroscopic scales. In this example, a particular leaf of a Ficus Benjamina with a triple point in a vein was chosen and photographed. Autofluorescence fibered confocal microscopy was then used to image the leaf by gliding the probe along the leaf's vein. The chosen triple point can clearly be seen on the reconstructed mosaic.

Our method has been successfully applied to many types of sequences acquired in both mouse and human tissue as shown in Fig. 7 and Fig. 8.

VI. Conclusion

New imaging technologies such as fibered confocal microscopy raise several new image processing and image analysis challenges that cannot readily be addressed using classical approaches. In this paper several dedicated tools were presented. By taking into account the specificity of this imaging modality, the automated algorithms we developed lead to more physiologically relevant data, easier interpretation by the experts and provide an important step to bridge the gap between microscopic and higher resolution images like MRI, PET, SPECT or US.

References

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