

On line Supplement :

HUMAN IN-VIVO FLUORESCENCE MICROIMAGING OF THE ALVEOLAR DUCTS AND SACS DURING BRONCHOSCOPY.

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SUPPLEMENTARY METHODS

Cellvizio® spectroscopy system

The FCFM system used in this study slightly differs from the commercially available Cellvizio®-Lung device (Mauna Kea Technologies, Paris, France) by the addition of a spectroscopic channel, that allows the simultaneous recording of the spectrum and the microscopic images in the observed field of view. Briefly, the Cellvizio-Spectroscopy system is made of four components : a miniaturized flexible fibered probe, a Laser Scanning Unit, a spectroscopic channel, and a dedicated control and acquisition software that allows the reconstruction of dynamic images in real-time.

The fiberoptic miniprobe (Alveoflex Confocal Miniprobe®, Mauna Kea Technologies, Paris, France) has an overall diameter of 1.4 mm and a length of 3 meters. It is made of a 30,000 fibers bundle and a specific connector that ensures the precise coupling of the fiber bundle with the scanner laser unit. The probe is devoid of distal optics. Its tip is covered with a smooth metallic ferrule that minimizes its aggressiveness on the tissue. The lateral resolution and depth of focus of the probe assessed in the air phase are 3.5 μm and 0-50 μm respectively.

The laser scanner unit is composed of a 488 nm excitation laser-source, scanned by two mirrors on the proximal face of the fiber bundle. This system allows the sequential injection of the laser beam into each fiber core, one after the other. The fluorescent light emitted by the tissue returns back into the same fiber which acts as a point source and a point detector, thereby assuring the confocal properties of the system. The fluorescence signal between 500 nm and 650 nm is collected back for imaging and spectral analysis. The whole system is optimized to ensure that only one fiber is injected at a time, with a resulting frame rate of 9 frames per second for a 896 x 640 pixels image and a circular 600 μm diameter field of view .

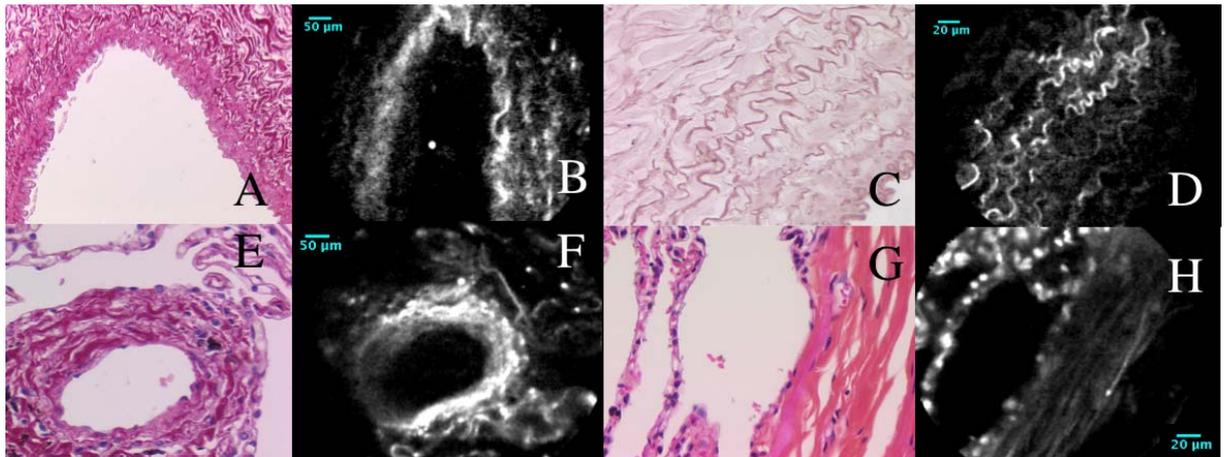
Spectroscopy channel : The detection optical beam is divided by a beamsplitter in two parts, 80% of the fluorescence signal being used for imaging and 20% for spectral analysis. The spectrometer is controlled through the Spectra Suite software (Ocean Optics, Dunedin, FL, USA). The spectral analysis is performed in-vivo in real time, simultaneously to the first 20 frames of the alveolar recording.

Supplementary Results :

Fibred Confocal Microimaging of fresh lung tissue .

Supplementary Fig. 1 shows the FCFM imaging of a human fresh lung tissue section obtained from a lobectomy specimen and the corresponding fixed and stained histology sections. In contrast to the long term fixed tissue sample, FCFM of fresh tissue exclusively images the connective tissue of the distal lung based on its natural autofluorescence. In this figure, the elastin component of extra-alveolar arterial wall is clearly visible (Supplementary fig 1 A B C D). Alveolar walls branching to the connective tissue surrounding the extra alveolar vessel are also clearly identified (Supplementary fig 1 E & F). This experiment confirms that in fresh tissue, fluorescence confocal microscopy does not image the cellular component of the tissue. As a result, the alveolar capillary network is not identifiable, due to the lack of fluorescence of non-fixed erythrocytes.

As a comparison, Supplementary fig 1 (G & H), displays a sub-pleural area where the alveolar cell nuclei have been stained using topical application of acriflavin and imaged using FCFM. By contrast to the unstained tissue section, the use of acriflavin, a potent fluorophore which rapidly penetrates the cell nuclei, revealed an image of the alveolar walls very similar to the conventional histology.



Supplementary Figure 1. Conventional histology and corresponding ex-vivo FCFM images of fresh lung sample (H&E : Haematoxylin-Eosin stain).

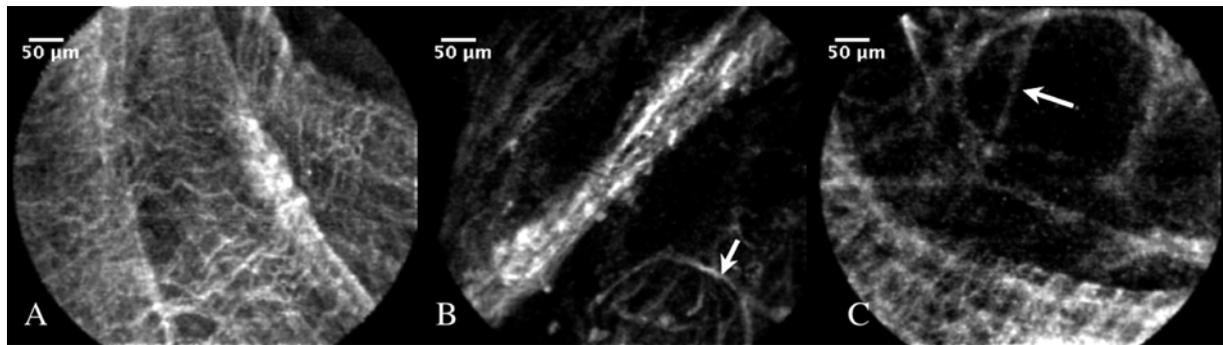
A and B. Arteriole and elastin component of the arteriolar wall (A: H&E, B : FCFM, Alveoflex® miniprobe).

C and D. Same area, details of the arteriolar wall (C: H&E, D : FCFM, High definition miniO® probe)

E and F. Extra alveolar vessel and adjacent alveolar walls (E: H&E, F : FCFM, Alveoflex® miniprobe)

G and H. Subpleural area. In H the nuclei of alveolar cells are stained using acriflavin (G: H&E, H: FCFM, High definition miniO® probe).

In-vivo imaging of distal bronchioles.



Supplementary figure 2. In-vivo imaging of distal bronchioles.

A . FCFM imaging showing the helicoidal imprint of smooth muscles on the inner part of a distal bronchiolar wall (see online video for dynamic sequence)

B and C. In-vivo FCFM imaging of alveolar buds in respiratory bronchioles (white arrowheads). See online video for dynamic sequence.