Design of an endomicroscope including a resonant fiber-based microprobe dedicated to endoscopic polarimetric imaging for medical diagnosis

COLMAN BUCKLEY,1 MARC FABERT,1 DAMIEN KINET,2 VYTAUTAS KUCIKAS,1,3 AND DOMINIQUE PAGNOUX1,*

1University of Limoges, CNRS, Xlim, UMR 7252, F-87000 Limoges, France
2University of Mons, Faculté Polytechnique, Bd Dolez 31, B-7000 Mons, Belgium
3RWTH Aachen University, Institute for Molecular Cardiovascular Research (IMCAR), Aachen, Germany
*dominique.pagnoux@xlim.fr

Abstract: We report on a novel endomicroscope, to the best of our knowledge, designed for achieving full 4×4 Mueller polarimetric images of biological tissues through a fiber endoscope for medical diagnosis. The polarimetric technique is based on a previously published two-wavelength differential method (TWDM). A key component of the endomicroscope is a resonant fiber-based microprobe including a highly-selective fiber Bragg grating (FBG), free of detrimental polarimetric effects, photo written in the core of the fiber, near the output face. By means of the TWDM, and using the specially designed microprobe (diameter 2.9 mm, length 30 mm), full Mueller images of 250×250 pixels were produced at the rate of 1 image/2 s through a 2 m single mode fiber, paving the way to in vivo applications in polarimetric endomicroscopy.

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1. Introduction

Among the 100,000 known proteins, collagen proteins are by far the most abundant in the animal kingdom. For example, they represent more than a quarter of the protein mass of a mammal. Collagens are structural proteins mainly located in the extracellular matrix of multicellular animal. Their most common form is type I collagen, which represents about 90% of the total collagen of a vertebrate, largely present in common connective tissue of tendons, ligaments, cornea, skin and also in all major internal organs such as heart, liver, lung or kidney [1].

The fibrillar structure of type I collagen can be affected at the submicronic scale, due to the development of various pathologies in the tissue, such as fibrosis or certain cancers [2]. A large number of recent works have shown that, due to these structural changes, the optical polarimetric characteristics of a tissue measured in a pathological region can be significantly different from that of a healthy region [3–5]. Thus, optical polarimetric imaging can constitute a particularly attractive diagnostic tool, in particular at the early stages of the disease, when it develops in the depth of the tissue and when it is not yet discernable with conventional imaging techniques [4].

Several polarimetric characteristics can be simultaneously sensitive to the structural modification of type I collagen, in particular the linear phase retardance induced by the linear birefringence of the tissue and the spatial depolarization due to the more or less advanced destructurization of the fibrillar arrangement [6]. Among the existing optical polarimetry techniques, Mueller polarimetry is of special interest as it is the only one capable of measuring all the polarimetric effects experienced by the probe beam when interacting with the tissue: linear and circular retardance, linear and circular diattenuation and depolarization [7]. For this reason, it is often proposed for applications in medical diagnosis [8]. The method is based on the following: four predefined polarization states are generated by a polarization state generator (PSG) in successive manner and directed on the area of interest of the tissue (also called “sample” in the following).
Each of the four transmitted states is analyzed via four successive configurations of a polarization state analyzer (PSA). Suitable linear combinations of the 16 measurements make it possible to build the $4 \times 4$ Mueller matrix, representing the polarimetric response of the sample [7]. Finally, the decomposition of this matrix into a product of elementary matrices of pure polarimetric effects allows to identify and to quantify all the polarimetric effects generated in the observed sample [9]. It should be noted that such decomposition is based on the assumption that the polarimetric effects occur successively in the tissue. In practice, they are actually entangled in a complicated manner, therefore the suitable order of the elementary matrices in the product is still a subject of intense discussions in the literature [9,10]. Nevertheless, the most commonly accepted decomposition is the Lu and Chipman decomposition, stating that the probe beam experiences diattenuation first while entering the tissue, then retardance due to the possible birefringence of this tissue and finally depolarization due to volume diffusion [11]. Thus, the measured Mueller matrix $M$ is then decomposed as follows:

$$M = M_\Delta M_R M_D$$

(1)

where $M_\Delta$, $M_R$, and $M_D$ are Mueller matrices of a depolarizer, a retarder, and a diattenuator respectively. Let us also note that the measured polarimetric characteristics are dependent on the penetration depth of light in the tissue which is itself a function of the wavelength of the probe beam. Most of the time, the working wavelength is chosen in the visible domain (penetration depth of few hundred microns in the green to few millimeters in the red, typically) [4].

In the most common implementation of Mueller polarimetry, the tissue of interest is directly enlightened by the probe beam, and the direct reflection of light is analyzed by the PSA. In these conditions, the technique only works for analyzing biopsies, external tissues such as skin, and cervix if implemented on a special colposcope [12]. However, for clinical trials early diagnosis of diseases on deep organs is of paramount importance. Therefore, a remote measurement must be performed by endoscopic means. In this configuration, the main challenge is to separate the polarimetric effects induced by the optical waveguide which can dramatically affect the measurement. Over the last decade, a few techniques overcoming this problem have been reported in the literature. One approach is to employ a rigid endoscope, which drastically limits the reachable regions in the body [13]. Others, based on the use of flexible optical fibers, provide only part of the polarimetric parameters of interest [14–16]. There are two recent studies reporting remote Mueller polarimetry through a flexible fiber endoscope by putting both PSG and PSA at the distal end of the fiber, i.e. between the output end of the fiber and the tissue [17–18]. One system consists of 6 fibers assembly, each one having a linear polarizer positioned at its output [17]. By considering successively 9 pairs of fibers with their attached polarizers (one fiber of the pair acting as a PSG and the other one acting as a PSA) one can build a $3 \times 3$ Mueller matrix. However, this device can only achieve a one pixel measurement and the measurement duration of several minutes is far too long for practical application. The second system is based on an optical head set at the output end of the fiber, including a sophisticated arrangement of two rotating polarizers (one acting as a PSG and the second acting as a PSA), each driven by a servo-motor [18]. A CMOS HD sensor, integrated in this optical head after the PSA, provides intensity images required for building a $3 \times 3$ Mueller matrix. Despite the miniaturization of the components of the optical head, the overall diameter of the device remains larger than 13 mm, far too large for most of the applications in usual endoscopy. In particular, it cannot be inserted in the working channel of classical endoscopes, which is generally smaller than 3 mm.

In previous works, we reported a promising method allowing to measure the full $4 \times 4$ Muller matrix of the tissue, through a single mode fiber, the PSG and PSA being set at the proximal end of the fiber. In this method, so-called “two-wavelength differential method (TWDM)”, we simultaneously launch two probe beams at two very close wavelengths $\lambda_1$ and $\lambda_2$ in the fiber (Fig. 1) [19]. The beam at $\lambda_1$ is totally reflected by a dichroic mirror set at the output end of
the fiber, making it possible to measure the Mueller matrix of this fiber only, on a round trip of the light. The beam at \( \lambda_2 \) is totally transmitted through the dichroic mirror and focused on the sample, part of the light being reflected into the fiber towards the PSA and detection system. Thus, at the wavelength \( \lambda_2 \), we measure the Mueller matrix of the assembly “fiber (forward path) + sample + fiber (backward path)”. We take advantage of the fact that a single mode optical fiber can be considered as a concatenation of a large number of thin linear birefringent plates with some arbitrary orientations [20], without depolarization and diattenuation. In these conditions, as shown in [21], Mueller matrix \( M_1 \) of the fiber in double pass measured at \( \lambda_1 \) is given by:

\[
M_1 = R(\theta_{a1}).D(2\delta_1).R(-\theta_{a1})
\]

where \( R(\theta) \) and \( D(\delta) \) are respectively the rotation matrix of angle \( \theta \) and the Mueller matrix of a linear retarder of retardance \( \delta \), \( \theta_{a1} \) and \( \delta_1 \) being the orientation angle of one eigenaxis of the fiber at the proximal side, and the linear retardance of this fiber, respectively, at \( \lambda_1 \). From the decomposition of matrix \( M_1 \), taking into account the general form of matrices \( R \) and \( D \), \( \theta_{a1} \) and \( \delta_1 \) can be calculated.

For its part, Mueller matrix \( M_2 \) of the assembly fiber + sample, measured at \( \lambda_2 \), is:

\[
M_2 = R(\theta_{a2}).D(\delta_2).R(-\theta_{b2}).M_{S2}.R(\theta_{b2}).D(\delta_2).R(-\theta_{a2})
\]

where \( \theta_{a2}, \theta_{b2} \) and \( \delta_2 \) are the orientation angles of one eigenaxis of the fiber at the proximal side and at the distal side, and the linear retardance of this fiber respectively, at \( \lambda_2 \), and \( M_{S2} \) is the Mueller matrix of the sample in the lab frame.

If the two wavelengths are close enough (difference lower than 1%), we can consider that \( \theta_{a2} \approx \theta_{a1} \) and \( \delta_2 \approx \delta_1 \) [19]. In this case, we can calculate the matrix \( M_E \) given by:

\[
M_E = R(-\theta_{b2}).M_{S2}.R(\theta_{b2}) = D^{-1}(\delta_1).R(-\theta_{a1}).M_2R(\theta_{a1}).D^{-1}(\delta_1)
\]

Matrix \( M_E \) is the Mueller matrix of the sample in a frame defined by the unknown orientation of the fiber axis at the distal side. However, this does not affect values of the polarimetric parameters of the sample. Thus, they can be extracted from \( M_E \) except for an absolute orientation of the sample eigenaxis.

In a previous work, for the proof of principle of this TWDM, we used two laser diodes, one centered at \( \lambda_1 = 634 \text{ nm} \) for characterizing the fiber only, and the second centered at \( \lambda_2 = 640 \text{ nm} \) for characterizing the assembly “fiber + sample” [19]. These diodes will be referred respectively as to reference diode and to probing diode in the following. At the distal side of the fiber, we used a bulk dichroic filter for separating the two wavelengths (Semrock LP02-633RE-25) and a
lens for focusing the light on the sample. In this configuration, the device was only able to make a single pixel characterization. For achieving images of samples, the fiber was kept motionless in front of the lens and the sample was translated pixel per pixel in the focal plane of the lens by means of a two-axis motorized translation stage. For each pixel, the intensities exiting the PSA at $\lambda_1$ and $\lambda_2$ were measured for the 16 successive combinations of the PSG/PSA, resulting in a measurement time of 32 ms/pixel. Taking into account the additional time required for the translation between two pixels, even an image of few pixels required a long acquisition time. For example, the acquisition time for an image of only $40 \times 40$ pixels exceeded 1 min. Therefore, in its present state, this imaging process was not suitable in view of minimally-invasive in vivo imaging, for three major reasons: (i) the scanning process was obviously inappropriate, (ii) the components and devices at the distal end of the fiber (dichroic filter, lens and two-axis translation stage) were far too bulky, and (iii) the measurement time was too long.

In this paper, we report on an endomicroscope including a novel microprobe specially designed for addressing all these issues while remaining compatible with the implementation of the TWDM, i.e. involving two close wavelengths, one being reflected in the fiber and the other being transmitted towards the sample. The microprobe is founded on the use of a piezoelectric-based resonant fiber for scanning operation (so-called microscanner in the following), including a photo written fiber Bragg grating (FBG) for achieving the wavelengths separation. In the following, the general design of the microprobe is first described. Then, the measurements of polarimetric effects in different FBG and their detrimental effects are discussed. Subsequently, a microprobe including an optimized FBG with negligible polarimetric effects is fabricated and the performance of the whole device (TWDM-based polarimeter and dedicated microprobe) is reported, in the perspective of applications in operational endoscopy. Finally, some ex vivo images of calibrated and biological samples are presented and discussed.

2. Overall design of a microprobe for endoscopic polarimetric imaging by the two-wavelength differential method

In order to drastically reduce the acquisition time of a Mueller polarimetric imaging, the process for the intensities acquisition must be revised. Previously, 16 intensities of different PSG and/or PSA states were successively measured for each pixel in the image. While the transition of the PSG or PSA state is relatively time consuming, the method proposed in this paper consists in performing 16 successive scans of the sample, the PSG and/or the PSA being switched only between each scan. In this case, one polarimetric image is obtained with only 16 switches of the PSG/PSA, independently of the number of pixels in the image. Therefore further reduction of frame time is primarily dependent on the performance of the probe scanner. This probe must fulfill several mandatory conditions:

- (i) The frame rate of the scanner must be as high as possible in order to allow achieving polarimetric images at high rate. Our targeted goal in this work is to get a complete Mueller image in 2 s, which requires scanner working at 8 fps (frames per second);

- (ii) the scanner must ensure a thoroughly reproducible path of the beam on the sample for each of the 16 successive scans, in order to guarantee that all the 16 measured intensities used for calculating the Mueller matrix of a given pixel correspond to light reflected by exactly the same and single pixel;

- (iii) as already mentioned, the scanner principle must be compatible with the implementation of the TWDM;

- (iv) if placed at the distal end of the optical guide, after the dichroic filter, the probe must cause minimal polarimetric effects on the transmitted beams, both back and forth. Indeed, in the TWDM principle, any element positioned after the dichroic filter is considered as a part
of the sample. In other words, any polarimetric effects of the probe would be combined with those of the sample, resulting in a harmful uncertainty on the measured polarimetric characteristics of this sample:

- (v) at last, if situated at the distal end of the optical guide, the probe must be miniaturized enough for being compatible with operational endoscopy. More precisely, depending on the targeted organs, the outer dimensions of this so-called microprobe should be smaller than 5 to 13 mm in diameter typically, and shorter than 50 mm in length for being used at the end of a dedicated fiber endoscope. For being inserted in the working channel of an existing conventional endoscope, the requirements on the dimensions are even more stringent as the probing head should be smaller than 3 mm in diameter and 40 mm in length.

There are three different approaches to implement scanning in the optical endoscopic systems, based on fiber bundle (proximal scanning), microelectromechanical systems (MEMS) (mirror scanning) and piezo electric actuation (fiber cantilever scanning). Fiber bundle approach is based on the use of a multi core imaging fiber as an endoscopic optical guide coupled with a bulk scanner positioned at the proximal end. Additionally, for polarimetric imaging with the TWDM, a miniaturized dichroic mirror fixed at the output for reflecting the wavelength $\lambda_1$ into the incoming core and for transmitting the wavelength $\lambda_2$ must be implemented. This so-called bundle of fibers consists of a large number of non-coupled light guiding elements (from 1500 to 100,000 typically) which preserve the spatial relationship between the entrance and the output. Thus, the scanning procedure can be shifted from the distal end to the proximal end of the bundle, each core corresponding to one pixel of the image. Such device has already been implemented in various endomicroscopes designed for achieving linear [22] or non-linear [23] imaging modalities. However, with this technique, the image resolution is severely limited due to inter-core coupling if the gap between individual fiber cores is too small, or due to low core density if the gap is increased to avoid inter-core coupling. In order to achieve low inter-core coupling and high core density simultaneously, the index contrast between the core and the cladding should be increased and, above all, structural variations in the size and shape of the neighboring cores should be accepted [24]. It should be noted that even slight structural non-uniformity results in various polarimetric effects, especially affecting retardance magnitude and orientation in each core. Furthermore, the principle of the TWDM states that the polarimetric effects in the waveguide must be the same back and forth, both at $\lambda_2$ and $\lambda_1$. In other words, because of the structural non uniformity in the bundle, the backward path at $\lambda_2$ must be achieved in the same fiber as the forward path, this fiber being also the one in which the reference beam at $\lambda_1$ makes its back and forth path. But in fact, it will not be the case since the light at $\lambda_2$ which is guided towards the sample by a given core will be diffused by this sample and will be likely collected and guided back towards the detection system by another or different other core(s). Therefore, this leads to the conclusion that the TWDM is not compatible with an imaging technique based on the use of a bundle of fibers.

Since achieving scanning operation from the proximal side is not suitable for our application, one must consider the design of a microscanner operating at the distal side. One approach can be the use of MEMS scanning mirrors placed inside a distant probe, after a miniaturized dichroic mirror [25]. However, scanners based on MEMS remain relatively bulky due to the size of the actuator and the associated electronics [26]. Another drawback specific for the TWDM is the fact that the reflection phenomenon on the tilted mirror of the MEMS is likely to induce polarimetric effects on both the incident beam and the beam reflected by the sample, especially significant phase retardance. This retardance depends on both the material constituting the mirror and the incidence angle [27,28]. As stated above, the possible polarimetric effects of the mirror would be combined with those of the sample, impeding a proper characterization of the sample.
In order to fulfill all the required conditions, we designed a novel microprobe including a microscanner based on the mechanical resonance of a fiber cantilever induced by means of a piezoelectric tube (PZT) [29]. By properly modulating the PZT driving voltage, one produces an outer spiral path of the free fiber end. After being focused by a miniaturized optical lens system, the output beam at $\lambda_2$ sweeps the sample with a spiral trajectory. Part of the back diffused light is collected in the fiber core and guided towards the PSA and detection system. To replace the bulky dichroic filter, a FBG is photowritten in the core of the fiber, near the output end. The schematic of this microprobe is shown in Fig. 2.

![Fig. 2. Layout of the microprobe designed for implementing the TWDM for polarimetric endomicroscopy.](image)

Let us note that microscanners based on this mechanical resonance principle have already been reported in the literature for various imaging modalities such as optical coherent tomography [29], two photon fluorescence and/or second harmonic generation [30,31] and coherent anti-Stokes Raman scattering [32]. However, specific features are additionally required for the application to polarimetric imaging by means of the TWDM:

- the optical fiber must guide light in the single mode regime at $\lambda_1$ and $\lambda_2$, i.e. in the red in our experiment. Accordingly, its numerical aperture $NA$ and core radius $a$ must be small enough in order to fulfill this condition. This results in a reduced coupling efficiency of the light backscattered by the sample into the fiber, i.e. a low signal level back guided to the PSA. Thus, particular attention must be paid to minimize any reflection at $\lambda_2$ on the different components and interfaces in the experimental setup since it would be added to the useful signal and would distort the measurement;

- as said above, the fiber cantilever must include a FBG for separating the wavelengths $\lambda_1$ and $\lambda_2$. This FBG must be very effective since it must be able to selectively reflect the wavelength $\lambda_1$ while reflection at the close wavelength $\lambda_2$ must remain negligible compared to the useful low level signal reflected by the sample and collected by the fiber;

- strict repeatability of the probing path on the sample must be guaranteed over the 16 successive scans required for each Mueller image.

In the next section, we discuss the design, fabrication and characterization of the FBG which is a key component of the microprobe.
3. Design, fabrication and characterization of the FBG to be written at the output of the endoscopic fiber

3.1. Required specifications

At every moment, the PSA analyses a reference signal at $\lambda_1$ and a probe signal at $\lambda_2$. Ideally, the reference signal comes exclusively from the reflection on the FBG, and the probe signal comes exclusively from the sample. Indeed, any signal at $\lambda_1$ coming from the sample and any signal at $\lambda_2$ reflected by the FBG constitute unwanted parasitic signals which add to the useful ones and can cause erroneous measurements. In other words, ideally, the transmission coefficient of the FBG at $\lambda_1$ must be zero (reflection at $\lambda_1 = 100\%$) and its reflection coefficient at $\lambda_2$ must be zero (transmission at $\lambda_2 = 100\%$). However, in practical conditions, one can tolerate some parasitic signals provided that their level is low enough for not perturbing the measurements. This results in less demanding spectral characteristics of the FBG. The acceptable level of parasitic signals and the ensuing required spectral characteristics of the FBG are determined below.

Let us consider an incident power $P_0$ on the FBG at a given wavelength $\lambda$, as depicted in Fig. 3. $R$ being the reflection coefficient of the FBG, the reflected power is $P_{FBG} = R.P_0$ whereas the power transmitted towards the sample is $P_1 = (1 - R).P_0$. After the reflection on the sample, the fraction of $P_1$ which is coupled back in the fiber is $P_2$ given by $P_2 = \eta P_1$, where the coefficient $\eta$ will be designated as the “recoupling efficiency” in the following. Finally, the power from the sample transmitted through the FBG towards the analysis system is $P_{SAM} = (1 - R).P_2$.

![Fig. 3. Reflection of the probing beam on the FBG and on the sample.](image)

Let us call $P_u$ and $P_p$ the useful and the parasitic signals respectively and let us call $X = P_u / P_p$ the minimum ratio required for ensuring an undistorted measurement. At $\lambda_1$, $P_u = P_{FBG}$ and $P_p = P_{SAM}$ so that the required condition to be respected is:

$$\frac{R(\lambda_1)}{[1 - R(\lambda_1)]^2.\eta} > X_1$$  \hspace{1cm}  (5)

where $X_1 = X(\lambda_1) = P_{FBG}(\lambda_1)/P_{SAM}(\lambda_1)$

The resolution of Eq. (5) results in the following condition on the reflection coefficient of the FBG at $\lambda_1$:

$$R(\lambda_1) > R_1 = \frac{A_1 - \sqrt{A_1}}{2}$$  \hspace{1cm}  (6)
where $A_1 = 2 + \frac{1}{\eta X_1}$ and $\Delta_1 = A_1^2 - 4$. At $\lambda_2$, $P_u = P_{SAM}$ and $P_p = P_{FBG}$ so that the required condition at this wavelength is:

$$\frac{[1 - R(\lambda_2)^2] \eta}{R(A_2)} > X_2$$

(7)

where $X_2 = X(\lambda_2) = P_{SAM}(\lambda_2)/P_{FBG}(\lambda_2)$

The resolution of Eq. (7) results in the following condition on the reflection coefficient of the FBG at $\lambda_2$:

$$R(\lambda_2) < R_2 = \frac{A_2 - \sqrt{\Delta_2}}{2}$$

(8)

where $A_2 = 2 + \frac{1}{\eta X_2}$ and $\Delta_2 = A_2^2 - 4$.

With the aim of calculating the practical values of $R_1$ and $R_2$, we conducted some specific measurements for determining the parameters $X$ and $\eta$. First, in order to determine the parameter $X$, we used the setup shown in Fig. 1 in which we put a 2 m piece of single mode fiber (with no FBG in its core) in place of the endoscopic fiber, we removed the dichroic filter and we put a mirror in place of the sample in order to reflect light into the core of the fiber, towards the PSA. The laser diode emitting at $\lambda_2$ was turned off. In these conditions, we measured the Mueller matrix of the fiber over a round trip at $\lambda_1$. The analyzed signal was composed of a useful part reflected by the mirror (power $P_u$) and a spurious part due to unwanted reflections on different interfaces like the input face of the fiber and the beam splitter faces (power $P_p$). When the mirror was well-aligned in order to get the highest $P_u$, the Mueller matrix of the fiber was that of a pure retarder with rotated main axes, as expected. Then, we progressively decreased $P_u$ by changing the alignment of the mirror, whereas the power $P_p$ was unchanged. The retardance of the fiber and the orientation of the main axes were found unchanged until the ratio $P_u/P_p$ reached 8 dB. As this ratio was gradually decreased below 8 dB, the measured values of these quantities began to change and the drift became larger and larger, showing that the minimum acceptable ratio was reached. This experiment was repeated with other pieces of fibers, giving similar results. Thus, by arbitrary adding a 2 dB security margin, we considered that a parasitic signal up to 10 dB below the useful signal is acceptable.

At this point, it is important to note that, in the operational measurement device with the endoscopic fiber including the FBG, this parasitic signal due to reflections on the different interfaces will be carefully suppressed by means of classical precautions, namely 8° angle-cleaving of the fiber faces, slightly tilting the beam splitter and achieving a strict spatial filtering of the parasitic signals just before the PSA. Thus, when implementing the TWDM in this endoscopic fiber, the only remaining parasitic signals will be the signal at $\lambda_1$ coming from the sample and the signal at $\lambda_2$ reflected by the FBG. These two parasitic signals cannot be spatially filtered as they are perfectly superimposed on the useful ones. From the above measurements, the value of $X = X_1 = X_2$ is set at 10.

In a second step, with the same setup as previously, we measured the highest reachable recoupling efficiency $\eta_{\text{max}}$ when the mirror was carefully aligned in order to optimize the power coupled back into the fiber. We found $\eta_{\text{max}} \approx 0.3$ (i.e. -5 dB). Then, we replaced the mirror by different samples of biological tissues and we measured the recoupling efficiency $\eta$ at different points of each sample. We found that the minimum $\eta$ was $\eta_{\text{min}} \approx 10^{-3}$ (i.e. -30 dB).

According to Eq. (6), $R(\lambda_1)$ must exceed the highest $R_1$ in order to be suitable in any experimental conditions. This highest $R_1$ is found when $\eta = \eta_{\text{max}}$. With the above experimental values, we calculated that this corresponds to the condition $R(\lambda_1) > 0.57$. Similarly, according to Eq. (8), $R(\lambda_2)$ must be lower than the lowest $R_2$ which is found when $\eta = \eta_{\text{min}}$. This results in the condition $R(\lambda_2) < 10^{-4}$. In the end, the final specification concerning the reflection coefficient of the FBG will be the following: $R(\lambda_1) > 0.6$ and $R(\lambda_2) < 10^{-4}$. 
The polarimetric effects in the FBG is another point to be carefully considered. Indeed, the FBG is a distributed reflector which downstream part is crossed by the probe signal at \( \lambda_2 \) but not by the reference signal at \( \lambda_1 \) which was reflected by the upstream part. Thus, according to the TWDM principle, this downstream part of the FBG will be seen as part of the sample. Polarimetric effects may occur in this FBG, namely possible retardance due to linear birefringence induced by photo-writing operation [33]. If we call \( \delta_{\text{FBG}} \) the retardance which can be measured over one round trip path in the downstream part of the FBG, this terminal part behaves like a pseudo-waveplate of retardance \( \delta_{\text{FBG}}/2 \). However, as already stated with relation (4), determining the orientation of the eigenaxes of this pseudo-waveplate is not possible. Consequently, assuming that the retardance of the sample is \( \delta_s \), the overall measured retardance will be comprised between \( |\delta_s + \delta_{\text{FBG}}| \) (in case the fast axis of the FBG is parallel to that of the sample), and \( |\delta_s - \delta_{\text{FBG}}| \) (in case the fast axes are perpendicular). Therefore, the retardance \( \delta_{\text{FBG}} \) corresponds to the uncertainty introduced on the measure of the retardance of the sample. To avoid ambiguities when interpreting measures in the view of the diagnosis of pathologies, this uncertainty must be lower than the gap between the retardance measured in healthy regions and that measured in pathological ones. The careful analysis of data published in the literature shows that this gap depends on both the considered organ and pathology. On the basis of papers previously published on breast carcinoma [34], liver fibrosis [35], colon cancer [4] or polyps on the cervix [13], the acceptable uncertainty will be set at \( \pm 5^\circ \). In other words, the retardance induced by the FBG over a round trip of the light must be lower than \( 5^\circ \).

3.2. Fabrication and characterization of the FBG

The two light sources involved in the experimental setup shown in Fig. 1 were two 100 mW CW laser diodes fabricated by Oxxius, and centered respectively at \( \lambda_1 \sim 633.7 \) nm (reference diode LBX-633-100-ISO-PP) and \( \lambda_2 \sim 640 \) nm (probe diode LBX-638-100-CSB-PP). Typical spectra of these sources are displayed in Fig. 4. Let us note that, as these diodes are longitudinally multimode, their spectrum can slightly change when adjusting the emitted power or the internal temperature.

![Fig. 4. Spectra of the laser diodes at 633.7 nm and at 640 nm involved in the experimental device depicted in Fig. 1.](image)

The optical fiber used in this setup was a Ge-doped-core single mode fiber (Thorlabs 630HP, cutoff wavelength = 580 nm). Several 2.5 m long pieces of this fiber were prepared. Prior the
photowriting step, they were hydrogen loaded in order to increase their photosensitivity. This stage was done by placing the optical fibers in a vessel under high hydrogen gas pressure (200 bar) and high temperature (68 °C) for 26 hours until an equilibrium of concentrations inside and outside the fiber was reached. In each piece, a FBG was photo-written in the core, 2 m from the entrance. We tested two techniques: (i) either the Lloyd mirror technique [36], implemented with a continuous UV laser emitting at 244 nm (quadrupled 976 nm fiber laser from Azurlight System) (Fig. 5), (ii) or the phase mask technique [37] implemented with an excimer laser emitting at 193 nm (Noria system from NorthLab Photonics). The photo-writing conditions of four selected FBG (so-called FBG #i (i = 1,4)) are specified in Table 1. After desorption of hydrogen by annealing at 100 °C for 24 hours, the transmitted and reflected spectra were measured, for each FBG. The transmission spectra are displayed in Fig. 6 and the main spectral characteristics are reported in Table 1.

![Fig. 5.](image1) Lloyd mirror system for writing fiber Bragg gratings (FBG): (a) overall view of the device and (b) zoom in on the interference area on the fiber.

![Fig. 6.](image2) Transmission spectra of 4 FBG photowritten in order to efficiently reflect $\lambda_1 = 633.7$ nm and totally transmit $\lambda_2 = 640$ nm.

As expected, the Bragg wavelength properly coincided with that of the main peak of the reference laser diode at $\lambda_1 = 633.7$ nm, except for FBG #1 which Bragg wavelength is slightly
Table 1. Photo-writing conditions and spectral characteristics of 4 selected FBGs

| Bragg grating | Technique used | Insolation duration (s) | Length of the grating (mm) | Bragg wavelength $\lambda_B$ (nm) | Transmission coefficient at $\lambda_B$ (dB) | FWHM (nm) |
|---------------|----------------|-------------------------|---------------------------|----------------------------------|----------------------------------|-----------|
| FBG #1        | Lloyd mirror   | 600                     | 5                         | 633.4                            | -21                              | 0.9       |
| FBG #2        | Lloyd mirror   | 200                     | 3                         | 633.7                            | -20                              | 0.7       |
| FBG #3        | Lloyd mirror   | 200                     | 4                         | 633.7                            | -21                              | 0.8       |
| FBG #4        | Phase mask     | 105                     | 3                         | 633.7                            | -21                              | 0.7       |

lower (633.4 nm). The transmission coefficient at $\lambda_1$ was -20 dB and the FWHM was $\sim$ 0.8 nm. The part of the light from the reference diode which was reflected by the FBG, over the entire spectrum, was measured to be about 90%, i.e. much more than the required 60%. The transmission coefficient at $\lambda_2$ was very close to 100% and the reflection coefficient was lower than the required $10^{-4}$ value. Thus, with these FBGs, the targeted specifications were achieved.

Then, to measure the polarimetric characteristics of one given FBG, the fiber was first cleaved a few mm after the grating with an angle of 8° in order to avoid back guiding of the Fresnel reflections from the output face. Then, it was installed in the setup shown in Fig. 1, with a mirror placed instead of the sample. The other possible reflections on the various interfaces in the setup were also carefully suppressed, as explained previously. By means of the TWDM, we measured the Mueller matrix of the components situated downstream the reflection at $\lambda_1$. As the Mueller matrix of the mirror is an identity matrix, the measured Mueller matrix was that of the grating. Respective Mueller matrix was decomposed according to the Lu and Chipman method [12] for each of the characterized FBG. Only linear retardance was noticed in any of the FBGs, with no diattenuation, no depolarization and no circular birefringence. The retardance measured over a round trip of the light for each FBG is reported in Table 2.

Table 2. Measured retardance and calculated tilt of the 4 characterized FBG

| Bragg grating | Measured linear retardance (°) | Computed tilt of the grating planes (°) |
|---------------|--------------------------------|---------------------------------------|
| FBG #1        | 64                             | 1.5                                   |
| FBG #2        | 7                              | ~0                                    |
| FBG #3        | 3                              | ~0                                    |
| FBG #4        | 15                             | 1                                     |

Significant retardance can be noticed, especially in FBG #1 and FBG #4. The reason for this retardance can be found by carefully observing the transmission spectra of these FBG (Fig. 6). Indeed, in transmission spectra, a deep secondary dip was measured at a shorter wavelength, between 632 nm and 633 nm. Such dip is generally a signature of a slight tilt of the grating planes [38,39], which is likely to introduce phase retardance between the polarization oriented in the planes parallel and perpendicular to the grating planes [40]. It is well-known that the larger the tilt is, the larger the retardance it induces. For verifying this tendency in our FBGs, we conducted simulations by means of Optigrating software from Optiwave Photonic software [41] with the aim of finding the optogeometrical parameters of the FBGs providing spectra in the closest agreement with the measured one. The comparison of measured and calculated spectra for one of the gratings (FBG #1) is shown in Fig. 7. The best fitting spectrum was computed with the index contrast $\Delta n \sim 7.2 \times 10^{-4}$, the length of FBG 5 mm as evaluated experimentally, and the tilt of the grating planes of 1.5°. We conducted similar calculations for all other FBG. The computed tilts of the grating planes are reported in the Table 2. It can be seen, as expected, that larger linear retardance is induced by FBG exhibiting higher tilt of the grating planes. High sensitivity of retardance to the tilting of FBG planes in both simulated and measured domains highlights the
importance of high precision in FBG photo-writing. Therefore, in further experiments, particular attention will be paid for reducing this tilt. Let us note that residual retardance can also be attributed to the fabrication conditions which can introduce some inner birefringence in the material [33].

Fig. 7. Transmission spectrum of the grating FBG #1: measured (black) and calculated with suitable parameters for obtaining the best possible fit (magenta).

Considering gratings FBG #3 and FBG #2, which fabrication conditions were very similar, they initially exhibited the same retardance of 7° over one round trip. However, by slightly changing the temperature of the laser diode at $\lambda_1$, we noticed that the retardance of FBG #3 was diminished down to 3°. This improvement was induced by a slight change in the spectrum of the laser beam. To our conviction, it results in a longer mean penetration distance of the light at $\lambda_1$ in the grating, and thus in a shorter length of non-traveled birefringent terminal part. Finally, this grating FBG #3 fulfills the specification requirement of the retardance lower than 5° expressed in section 3.1.

4. Fabrication and characterization of the microprobe

Due to its satisfactory spectral and polarimetric characteristics, the fiber with the FBG #3 was selected for manufacturing the microprobe. In this fiber, the 8° cleaved end face was at a distance shorter than 5 mm from the end of the grating. The fiber was inserted and glued in a X-Y piezo actuator and the length of the cantilever was set to 9.2 mm, corresponding to both a high resonance frequency of the fundamental mode (1064 Hz) and a large amplitude of the vibration (> 500 $\mu$m) for high speed and extended field of view (FOV). This assembly was encapsulated in a maintaining biocompatible metallic tube (2.9 mm outer diameter). At the output, we set a micro optic imaging system consisting of a series of two achromatic doublets by Edmund Optics (diameter = 2 mm, effective focal length = 3 mm) with anti-reflective coating. In addition to reduced spherical aberration and large numerical aperture, these optical components exhibit no polarimetric effects, which is of great importance for our application. With these optics, the core of the fiber was imaged on the sample with a magnification of 1, so that the resolution was similar to the mode field diameter of the fundamental mode of the fiber ($\sim 4 \mu$m). By means of a calibrated magnification system and of a beam profiler (Thorlabs BP109-VIS), the resolution was
measured to be 4.1±0.2 µm. The overall length of the manufactured microprobe was 30 mm (Fig. 8).

When applying two sinusoidal voltage ramps $V_x$ and $V_y$ at the 1064 Hz resonance frequency with the phase shift of $\pi/2$ on the actuators, the fiber cantilever was mechanically actuated so that the output end follows an outgoing spiral path (maximum amplitude = 40 V for an attainable FOV of ~ 1 mm in diameter). The duration of this path until reaching the maximum amplitude was 85 ms. Then, by inducing a phase shift for both voltages, a braking phase of 40 ms was applied to the fiber until it comes back to its resting position. An entire cycle taking 125 ms, 8 sweeps by the fiber were achieved each second. In the principle of the experimental setup, one outgoing spiral sweep is used to acquire one intensity image associated with one configuration of the PSG/PSA. Then, the configuration of the PSG/PSA is switched to the next one during the braking phase. Thus, the 16 intensity images necessary for building one Mueller image, corresponding to the 16 successive configurations of the PSG/PSA, can be acquired within 2 s. In other words, the frame rate of this polarimetric endoscopic imaging system is 1 image/2 s.

For the following, the maximum amplitude of the driving voltages of the actuator was set to 25 V, so that the diameter of the FOV reaches 400 µm. To characterize the path of the probing beam at $\lambda_2$ in the focal plane of the microprobe, this focal plane was imaged on a position sensing detector (PSD Hamamatsu C10443-04) which accurately provides two quantities proportional to the $(x,y)$ coordinates of the barycenter of the pattern versus time (22,000 points over one outgoing spiral path). As already mentioned in section 2, one mandatory working condition of

![Fig. 8. Microprobe fabricated for in vivo implementation of the TWDM (length = 30 mm, diameter = 2.9 mm).](image)

![Fig. 9. (a) paths on a PSD of the probing beam delivered by the microprobe shown in Fig. 8, for 3 successive sweeps; (b) zoom in on the center of the 3 paths shown in (a); (c) intensity image of a reference grid reconstructed from the registered path of the probing beam.](image)
the microscanner is that the reproducibility of the probing beam path must be excellent for each of the 16 successive scans. This was verified by recording and comparing successive paths on the PSD, as shown in Figs. 9(a) and 9(b). It can be seen in these figures that even the small irregularities of the path at the very beginning of the sweep (center of the spiral) are very well reproduced from one path to the other, as required.

Simple processing of the recorded trajectory of the probing beam allows for the x-y reconstruction of any intensity image of a sample. Figure 9(c) is an example of such reconstruction of an image of a reference grid (Thorlabs Grid Array R1L3S3P, lines spacing 50 µm). We carefully compared successive intensity images of this grid and, as expected, no measurable changes over time were detected. However, we can see on this figure that the mean intensity of light is higher at the bottom than at the top. The reason is that the fiber in the microprobe is angle cleaved by 8°, resulting in the fact that the output beam exits a little off the microprobe axis. In the case of Fig. 9(c), it is rather directed downwards. However, this asymmetry has no undesirable effect on the polarimetric measurements because the Mueller matrix of each pixel is normalized by a quantity proportional to the incident power on this pixel, so that the coefficient m_{11} of any Mueller matrix is m_{11} = 1.

Another important point to be considered for achieving images of uniform quality is the balance of the density of the points over the entire FOV. When a sinusoidal voltage ramp with an increasing linear envelope was applied to the actuator (Fig. 10(a)), the dynamics of the fiber

Fig. 10. Voltage applied to the actuator in the X direction, (a) with an increasing linear envelope, (b) with an abrupt step at t=0; path of the probing beam delivered by the microprobe: (c) when applying the voltage (a) which results in a higher density of measured points in the center), (d) when applying the voltage (b) which results in a more uniform density of measured points; (e) zooms in on the path obtained with the abrupt step of the envelope at the periphery (top) and in the center (bottom)
output tip along the spiral resulted in a significantly higher density of points in the center of the image than in the periphery, as shown in Fig. 10(c). Improvements in the uniformity of the density of points were obtained with steeper envelopes at the origin, the best uniformity ((Figs. 10(d) and 10(e)) being obtained when applying an abrupt voltage step (Figs. 10(b)). Let us remark that the tests on the reproducibility of the paths shown in Fig. 9 were achieved with this abrupt voltage step.

Considering the \( \sim 4 \mu m \) resolution of our imaging system, and the 400 \( \mu m \) circular FOV, we fixed the square image size to 250 \( \times \) 250 pixels (i.e. 1 pixel = square with 1.6 \( \mu m \) side). The 22,000 intensities measured during one 85 ms outgoing sweep were then distributed among the \( \sim 40,000 \) pixels of the circular central region corresponding to the FOV. In these conditions, assuming that a single intensity measurement is assigned to one pixel, 45\% of the pixels in the FOV should be empty. However, several intensities may fall in the same pixel in particular in the center of the image, further increasing the overall number of empty pixels. That is the reason why the fraction of empty pixels was found significantly higher (61\%). In the case of several intensities falling in the same pixel, the final intensity assigned to this pixel was the mean value of the intensities. On the other hand, inverse distance weighted interpolation was used in order to assign an intensity value to empty pixels \([42]\). The ratio of non-empty pixels being only 39\% could be considered as somewhat low. However, thanks to the fact that the polarimetric effects do not drastically change from one pixel to the other in biological tissues, this fraction of non-zero pixels is quite sufficient for making proper polarimetric images, as shown in the next section.

5. Polarimetric images realized with the microprobe by means of the TWDM

We first implemented the TWDM through the 2 m endoscope fiber ending with the microprobe described in section 4, for making the remote polarimetric image of a simple mirror. Same Mueller matrices were obtained for all the pixels of the image, a typical one being given in relation (9):

\[
M_m = \begin{pmatrix}
1.0000 & -0.0196 & 0.0054 & -0.0004 \\
-0.0425 & 1.0237 & 0.0360 & 0.0292 \\
-0.0110 & 0.0054 & 1.0025 & 0.0101 \\
-0.0069 & -0.0194 & 0.0124 & 1.0096
\end{pmatrix}
\]

As expected, this Mueller matrix is very close to the identity matrix. The decomposition by the Lu and Chipman method shows that it corresponds to a linear retardance of \( \sim 1.4^\circ \), null circular retardance, and negligible diattenuation and depolarization. The mean retardance, linear diattenuation and depolarization over the entire image of the mirror are respectively \( \sim 1.4^\circ \), \( \sim 0.027 \) (standard deviation \( \sim 0.0073 \)) and \(-0.009 \) (standard deviation \( \sim 0.077 \)). The slightly negative depolarization comes from some diagonal coefficients in the Mueller matrices which exceed 1, due to uncertainty in the measurements of intensities. However, as the uncertainty on the measured depolarization is evaluated to \( \sim 10^{-2} \), we can conclude that the value of the depolarization is actually zero.

Then, we put a \( \lambda/8 \) waveplate between the output face of the microprobe and the mirror and we characterized this waveplate over a round trip of the light. In these conditions, the only expected polarimetric effect is a 90\° linear retardance. The obtained image of the retardance is shown in Fig. 11(a). For a better assessment, the color toolbar range is limited to the range [80\° 100\°]. The mean measured retardance was actually 89\°, within the \( \pm 3^\circ \) uncertainty due to the retardance of the FBG. However, a careful observation of the image shows the existence of slight fringes in this image, modulating the retardance between 88\° and 90\°. These fringes were also seen when characterizing the mirror only (modulation of \( \pm 0.3^\circ \) of the measured retardance). They have the appearance of equal inclination fringes with very low visibility, and they move...
when the mirror is slightly tilted. Therefore, they simply originate from a highly unbalanced Fabry-Perot interferometer between the reflecting front face and the air-silica rear face of the mirror. Furthermore, slightly lower values of the retardance, down to $87^\circ$, were measured at the top of the image. This distortion was attributed to the fact that the intensities collected from this region are very low, as can be seen in Fig. 9(c). In this case, the ratio $X_2$ defined in Eq. (7) may become too low for allowing an accurate measurement. Fortunately, this situation occurs only with a non-diffusive reflecting sample such as a mirror. With scattering biological samples, light from any point of the FOV will be partially collected, even if the mean level over the entire sample is reduced.

![Fig. 11. Polarimetric images of a $\lambda/8$ waveplate over one round trip of the probing beam (250 x 250 pixels), achieved with the microprobe shown in Fig. 8: (a) linear retardance, (b) diattenuation, (c) depolarization, (d) orientation of the eigenaxis ((d1) arbitrary orientation measured with a first measurement; (d2) and (d3) new orientations measured after a rotation of the waveplate of $+20^\circ$ and of $-20^\circ$ respectively, with respect to its original orientation).](image-url)

From the retardance matrix, we also extracted the orientation $\theta$, of the eigenaxis of the sample, which is a quantity with no absolute physical meaning since it depends on the conditioning of the fiber as stated in section 1. However, provided that the conditioning of the fiber is not modified, it allows relative comparisons of the measured orientations. This orientation measured for the first orientation of the waveplate was $\sim 44^\circ$ and it was uniform over the entire FOV, as expected (Fig. 11(d1)). Then, we rotated the waveplate in the plane of its faces respectively $+20^\circ$ and then $-20^\circ$ with respect to its original orientation, and we measured both the retardance and the new orientations of the eigenaxis for the two new positions, without changing the conditioning of the fiber between each measurement. Thanks to the TWDM, the measured retardance was found unchanged for the 3 measurements. The new measured orientations where respectively $\sim 64^\circ$ and $\sim 24^\circ$, in very good accordance with the expected values (Figs. 11(d2) and 11(d3)).

The images of the diattenuation and of the depolarization are respectively displayed in Figs. 11(b) and 11(c). We found that these quantities were negligible over the entire FOV (mean values respectively equal to 0.016 and 0.02). However, at this point, a special comment is required on this result concerning the measured depolarization. Since the probing beam is the fundamental mode exiting the fiber, and thus uniformly polarized and spatially coherent, one can expect to always measure a Mueller-Jones matrix that represents a non-depolarizing medium,
as demonstrated in [43]. In other words, any spatial depolarization cannot be revealed by the direct decomposition of a Mueller matrix measured through a single mode fiber. To overcome this serious limitation, a modified measurement process is currently being investigated in our laboratory.

Finally, we imaged a biological sample consisting of a slice of rat tendon which is mainly composed of birefringent Type I fibrillar collagen (thickness $\sim 30\,\mu m$). The intensity image of a $1.8\,mm \times 2\,mm$ region of this tendon is displayed in Fig. 12(a). We first characterized this sample with the TWDM implemented with the setup depicted in Fig. 1, involving the former bulk assembly of the dichroic filter and the point by point imaging system already reported and validated in our previous study [19]. With this system, we achieved polarimetric images (180 $\times$ 200 pixels) of the entire $1.8\,mm \times 2\,mm$ region with a 10 $\mu m$ resolution and

Fig. 12. Images of a Type I collagen sample: (a) intensity image with 3 identified regions; (b) retrieved retardance and (c) orientation of the eigenaxis of linear retardance from a 2 mm $\times$ 1.8 mm Mueller image obtained with the point by point imaging device (180 $\times$ 200 pixels); (d) retrieved retardance and (e) orientation of the eigenaxis of linear retardance from a 400 $\mu m$ diameter Mueller image (region 2 of image a)), obtained with the microprobe shown in Fig. 8 (250 $\times$ 250 pixels).
the measurement time exceeded 1 hour. The images of the retardance and of the orientation of the eigenaxis are respectively displayed in Figs. 12(b) and 12(c). Similarly to the sample already described in Ref. [21], we noted that, depending on the considered region, significant retardance (∼110° in Region 1 and ∼70° in Region 2) or low retardance (<30° in Region 3) were measured (Fig. 12(b)). In Regions 1 and 2, the high retardance can be attributed to the well oriented structure of collagen fibers, as confirmed by the constant orientations measured in the horizontal sub-patterns in these areas (Fig. 12(c)). On the contrary, no preferential orientation can be identified in Region 3 of Fig. 12(c). This is surely due to the fact that the medium in this region is not fibrillar collagen but rather non-birefringent ground substance of connective tissue. This assumption is well supported by the low retardance measured in this area (Fig. 12(b)). The distinction between the different tissues cannot be done in the intensity image (Fig. 12(a)).

Then we selected the region of the sample with intermediate retardance (Region 2, diameter 400 µm) and we performed the Mueller image of this region (250 × 250 pixels) by means of the TWDM implemented with the microprobe shown in Fig. 8. As for previous images made with this device, the measurement duration was limited to 2 s. The measured diattenuation and the depolarization were negligible. The retardance displayed in Fig. 12(d) is in good accordance with that measured using the former scanning device (Fig. 12(b)). The orientations of eigenaxis cannot be directly compared, as the endoscopic fiber has been changed between the two measurements. To make a valid comparison, we calculated the angular offset and applied it to the orientation of the central pixel of Region 2, so that it matches the orientation measured for the corresponding pixel in Fig. 12(c). Then, this offset was applied to all the pixels of Region 2, resulting in the image shown in Fig. 12(e). This orientation image is in very good accordance with that displayed for Region 2 in Fig. 12(c).

6. Conclusion

In this paper, we reported on a novel endomicroscope designed for implementing the two wavelength differential method (TWDM) which has been previously demonstrated to be able to achieve full 4 × 4 Mueller images through a fiber endoscope that could potentially be used for in vivo polarimetric characterization of inner tissues. This endomicroscope includes a small size cylindrical microprobe (30 mm in length and 2.9 mm in diameter) containing a piezoelectric-based resonant fiber. Thanks to its small size, this microprobe could be used in a specially dedicated fiber endoscope or as an additional tool inserted in the working channel of a classical endoscope. The circular FOV in our experiments was 400 µm in diameter but we showed that the reachable FOV could be extended to 1 mm × 1 mm. With this device, we obtained Mueller images of 250 × 250 pixels, with a resolution of ∼4 µm. A key component in this microprobe was a photowritten fiber Bragg grating (FBG) enabling efficient separation of two very close probe wavelengths at the output end of the endoscopic fiber. Besides its spectral selectivity, one of the mandatory features of this FBG was that inner polarimetric effects must be negligible. Therefore, we carried out a complementary study in order to fulfill this condition. A FBG with a retardance of at most 3° over one round trip of the light and negligible diattenuation and depolarization was manufactured and selected for being used at the output of the fiber endoscope. Measurements on calibrated samples such as a λ/8 waveplate were conducted and proved the ability of the device to provide accurate polarimetric characterizations. Then, other measurements on biological samples confirmed this statement.

Full Mueller polarimetry requires 16 intensity measurements for each pixel of the image, each measurement being achieved with one given configuration of a polarization state generator (PSG) and a polarization state analyzer (PSA). In our work, we proposed another procedure for accelerating the data acquisition, consisting in successive measurements of 16 intensity images, each corresponding to one configuration of the PSG/PSA. Therefore, very good reproducibility of successive probing beam paths was necessary. This feature was verified thanks to a careful
analysis of these successive paths by means of a position sensing detector (PSD). With this procedure, the frame rate was increased from at most few images per hour to 1 image / 2 s.

The development of this novel setup constitutes a significant step towards the actual implementation of the technique for in vivo polarimetric imaging of inner tissues, in the view of minimally invasive early diagnosis of diseases affecting these tissues. Thanks to the small size of the microprobe, the endomicroscope can apply in any application where a flexible endoscope of 3 mm in diameter or more can be used. In particular, this includes applications in gastrointestinal tract imaging (oesophagus, stomach, intestine, colon), respiratory tract imaging (larynx, bronchi), or female reproductive system imaging ( cervix, uterus, fallopian tubes).

Nevertheless, we name several improvements that would be beneficial to the current system. First of all, since we noted that the polarimetric features of the FBG were sensitive to the slight changes in the spectrum of the longitudinally multimode reference diode used for probing the fiber, a single mode laser diode would be preferred for emitting a much more stable spectrum. Secondly, even if the frame rate has been significantly increased, a 2 s duration for one image may be too long for measurements free of motion artefacts, caused by uncontrollable means (patient breath, heartbeat, . . .). For stabilizing the sample during the measurement, the extremity of the maintaining tube could be simply pressed against the targeted region. Of course, in this case, the focalization optics should be set back into this tube, at a distance equal to the 3 mm effective focal length. Finally, a last improvement concerning the penetration depth of the light into the sample should be considered. Specifically, for comparing results obtained for different penetration depths, other pairs of wavelengths, namely in the green and in the blue, should be added to the device, at the expense of some sophistication in the setup. Indeed, in addition to the coupling of these new wavelengths in the setup, two other FBG should be written near the existing one in order to reflect the green and the blue reference wavelengths. These improvements are under consideration in our laboratory.

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The authors declare that there are no conflicts of interest related to this article.

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