

Chapter 2

Focus on the Distal Assembly of a Nonlinear Endomicroscope: A Gradient Index Lens Coupled with a Piezoelectric Scanner for Fluorescence Lifetime Measurements

Claire Lefort, Hussein Hamzeh, Liu Wei, Frédéric Pain
and Darine Abi Haidar

Abstract Today, prototypes of nonlinear endomicroscopes are appearing in order to transfer techniques of multiphoton characterization of biological samples to humans in vivo in situ. But they require several optical optimizations to be adapted for a clinical use. Among them, the ideal distal lens, coupled with the ideal scanning system is not yet highlighted. In this paper, we are using a gradient index (GRIN) lens coupled with a piezoelectric scanning system (PZT). The main part is dedicated to a spectral analysis in the context of fluorescence lifetime measurements (FLIM), a well-known technic used to complete multiphoton characterization and discriminate healthy and tumors cells. We spectrally highlight the presence of a background fluorescence perturbing the spectral measurement through the GRIN lens in specific conditions of image space working distance. In a second part, we evaluate the resolution available with the GRIN lens and numerically determine the optimal object working distance depending on the position of the fiber imposed by the PZT.

C. Lefort
Laboratoire XLIM, UMR 7252, CNRS, Limoges 87060, France

H. Hamzeh · L. Wei · F. Pain · D. Abi Haidar (✉)
Laboratoire IMNC, UMR 8165, CNRS, Orsay, France
e-mail: abihaidar@imnc.in2p3.fr

D. Abi Haidar
Université Paris 7-Paris DIDEROT, Paris 75012, France

2.1 Introduction

Nowadays, cancer is a disease becoming more and more widespread in the world. Cancer treatments are a ticklish issue, depending on the way of development of the cancerous tumour and its localisation in the patient's body. Currently, the main hope of curing this illness lies in surgery, often completed by chemical treatments or radiotherapy [1]. It is now admitted that if this global process is applied in the early stages of the disease, the chances to heal a patient and extends his hope of life considerably increase. Nevertheless, surgery is a very invasive operation for patients and a risk of relapse associated with a new proliferation of cancerous cells in the treated area still exists. This is often mainly due to a non-complete removal of the cancerous cells in their whole, despite the use of combined modality therapies.

Starting from these alarming observations, mainly true in the case of cancers where it is not possible to remove a large part of the tissues around the cancer area like the brain cancer, there is an need for a tool enabling a fast and accurate discrimination between cancerous and healthy cells.

In this context, a way to achieve this goal has been proposed in recent years. It consists in the development of an endoscope with a microscopic resolution. The first endomicroscopes developed were based on the confocal principle with an excitation source in the visible range [2]. It allows to image endogenous tissue components like elastin, in vivo and in real time. This solution is now clinically used. However, some drawbacks are highlighted, such as an imaging depth limited to at a few tens of micrometres inside the target tissues, mainly due to the strong scattering properties of biological tissues, as well as the small number of mean contrasts available from endogenous fluorescent proteins under linear excitation.

To overcome these restrictions, the current research on endomicroscopy aims at developing multiphoton microscopy through an optical fibre. The main modification compared to confocal endomicroscope stays in the excitation source. Indeed, multiphoton processes are obtained thanks to a near infrared (NIR) excitation with femtosecond pulse (150 fs) having a high repetition rate (80 MHz) [3–5]. The interest of multiphoton imaging techniques compared to the confocal one is not only justified by an increase in imaging depth due to NIR excitation [6] but also by the availability of a new mean of contrast thanks to the second harmonic generation emitted by non-centrosymmetric structures as collagen. This, combined to the absence of confocal pinhole due to the intrinsic localisation of the nonlinear phenomenon in the focal plane of the focusing device, promote a deeper imaging depth within a few hundreds of micrometres.

While nonlinear microscopy is now a very well-known and essential tool for biological tissues characterization, its transposition to endomicroscopy remains problematic. Indeed, endomicroscopy requires several miniaturized elements like scanning system or distal lens of focalization [7]. The laser excitation, coming from a Ti: Sapph oscillator is classically delivered through an optical fibre, flexible and with a small diameter providing access to the internal hollow organs (alveoli, kidney...). The choice of the optimal fibre proves to be complex and must be

realized taking into account all other parameters of the global system. The fibre has to be associated in its upstream by a shaping module for the compensation of linear and nonlinear effects occurring inside this material medium [8].

For the instance, all the parts of the endomicroscope requiring to be miniaturized are problematic and prevent the multiphotonic endomicroscope commercialisation. This is particularly true considering the distal lens of focalization. A recent miniaturized technology, named “GRIN lenses” for gradient index lenses, is based on the gradient of negative refractive index of its material from the centre to its outskirts, glued inside a stainless cylinder steel [9]. Dimensions of classical GRIN lenses range between 0.35 and 2 mm in diameter and between 5 mm and few cm in length, with a numerical aperture (NA) of the object space between 0.2 and 0.8.

In this paper we are studying GRIN lenses for the development of a multiphoton endomicroscope. The first part of this publication is dedicated to the experimental study of a GRIN lens alone. Background fluorescence induced in the GRIN lens is studied under classical UV excitation beam, mimicking the emitted multiphoton fluorescence or second harmonic generation coming from biological samples. Then, the impact of the background fluorescence of the GRIN lens on its imaging ability, spectral detection and fluorescence lifetime measurements is evaluated. The 2D lateral resolution is experimentally measured using 1 μm diameter gold beads. The second part of the paper is dedicated to numerical simulations of the GRIN lens coupled to a specifically designed double clad fibre (DCF) ideal for the assembly of a multiphotonic endomicroscope [4] using Zemax program. Both geometric ray tracing and Gaussian beam approach are considered and compared. Furthermore, the influence of the relative position between the GRIN lens and the DCF tip on the magnification, field of view (FOV), axial and lateral resolutions are evaluated. Finally, choosing a piezoelectric (PZT) stage as scanning device [10], the coupling of the DCF with the PZT and the GRIN lens is characterized through the variation of the image working distance, the coupling efficiency of the fluorescence beam inside the DCF, the size of the FOV and the axial and lateral resolutions.

2.2 Experimental Characterization of the GRIN Lens

2.2.1 Definition of the GRIN Lenses

All the manufactured GRIN lenses have a continuously variable index of refraction between the periphery and the centre of the lens. This optic is glued to a bio-compatible stainless steel ring.

In this study, we have characterized a commercial GRIN lens with a total length of 7.53 mm and a diameter of 1.4 mm (GT-MO-080-018-810, Grintech, Jena, Germany). The definitions of the image and object space and their WD are presented in Fig. 2.1. The object space contains the target biological sample and in the image space lies the DCF delivering the laser and collecting the multiphoton signal emitted by the sample.

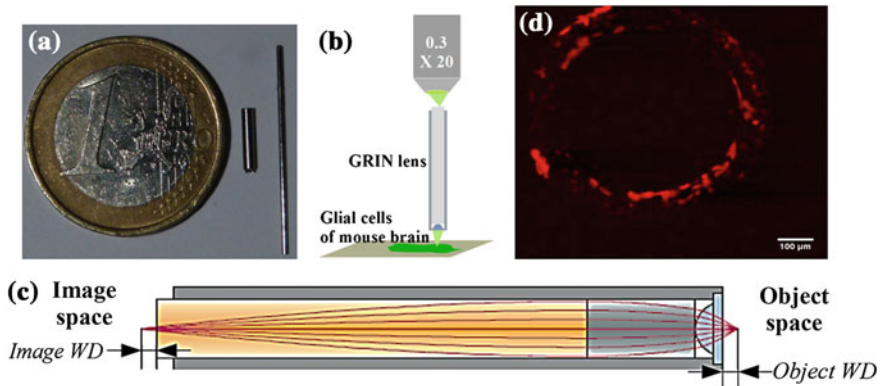


Fig. 2.1 **a** Two GRIN lenses used in this study. **b** Experimental setup for the characterization of GRIN lenses. **c** Definitions of the image and object spaces and the corresponding WD. **d** Fluorescence image of the glue between the GRIN lens and the stainless steel of protection by confocal imaging

2.2.2 Analysis of the Background Fluorescence

2.2.2.1 Background Fluorescence Highlighted

Confocal imaging with a GRIN lens needs an initial step for the characterization of the parasite fluorophores emitting a background. The confocal microscope is focusing a krypton argon laser inside the GRIN lens placed under the objective (Fig. 2.1b). An image of the image face of the GRIN lens is thus shown. Figure 2.1d presents the resulting observation of the image space of the GRIN lens where an inhomogeneous fluorescent signal is visible on Fig. 2.1d and probably results from fluorescent components in the glue fixing the GRIN lens to the steel ring. In a first approach, no impact of the intrinsic fluorescence of the glue on fluorescence images of biological samples is visible.

2.2.2.2 Spectral Analysis of the Background Fluorescence

We have analysed spectrally the background fluorescence of the GRIN lens thanks to the experimental setup presented in Fig. 2.2a.

This experiment involves a pulsed diode laser at 405 ± 10 nm (LDHP-C-405B, Picoquant) injected into one of the two fibres of a bifurcated fibre (Fig. 2.2b). The average power of the laser can be managed, as well as the repetition rate, which can be set between 2.5 and 40 MHz. The excitation source is guided through the fibre to the image space of the GRIN lens. A solution of Rhodamine B (RhB) is placed in

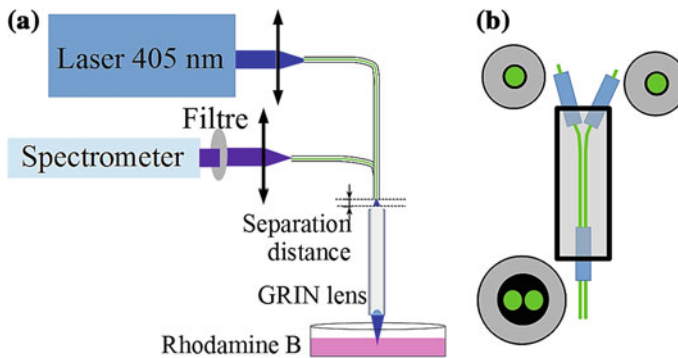


Fig. 2.2 **a** Experimental setup for the spectral characterization of the background fluorescence of the GRIN lens. **b** Structure of the bifurcated fibre

the object space of the GRIN lens. The backward fluorescence, emitted by both the RhB and the GRIN lens, is collected by the second core of the bifurcated fibre. A spectrometer, preceded by a long pass filter to reject the excitation laser beam analyses the result. First of all, the GRIN lens is excited at 405 nm without any sample and without any filter. The idea is to measure the background fluorescence emitted by the GRIN lens alone. The graph of Fig. 2.3a shows the spectrum of the emitted background fluorescence from the GRIN lens. This spectrum covers a wide spectral range of 300 nm in the visible range.

The solution of RhB is then illuminated and its fluorescence emission spectrum collected by the GRIN lens with a separation distance between the lens and the fibre of 2 mm and less than 200 μm (Fig. 2.3b, c, respectively). Figure 2.3d, e resumes the spectra of all the components by comparison with the sum of all the spectrum obtained: glue of the GRIN lens and the RhB solution. The latter was measured using the bifurcated fibre alone so as to eliminate its possible contribution to the fluorescence signal.

The contribution of the fluorescence from the glue around the GRIN lens is now spectrally characterized (Fig. 2.3a, b and d). From comparison between Fig. 2.3b, c, it can be shown that depending on the separation distance between the fibre tip and the GRIN lens, the contribution of the fluorescence of the glue is variable. At a large separation distance between the GRIN lens and the fibre tip (2 mm, Fig. 2.3b), the RhB spectrum is significantly altered by the background fluorescence, while the background fluorescence is hardly noticeable when this separation distance is very short, below 200 μm . This can be explained if one considers the very short image space WD of the GRIN lens (below 100 μm in air at 405 nm). It is also remarkable from Fig. 2.3d that the RhB spectrum in blue recovers successfully the measured spectrum using the GRIN lens corroborated by Fig. 2.3e.

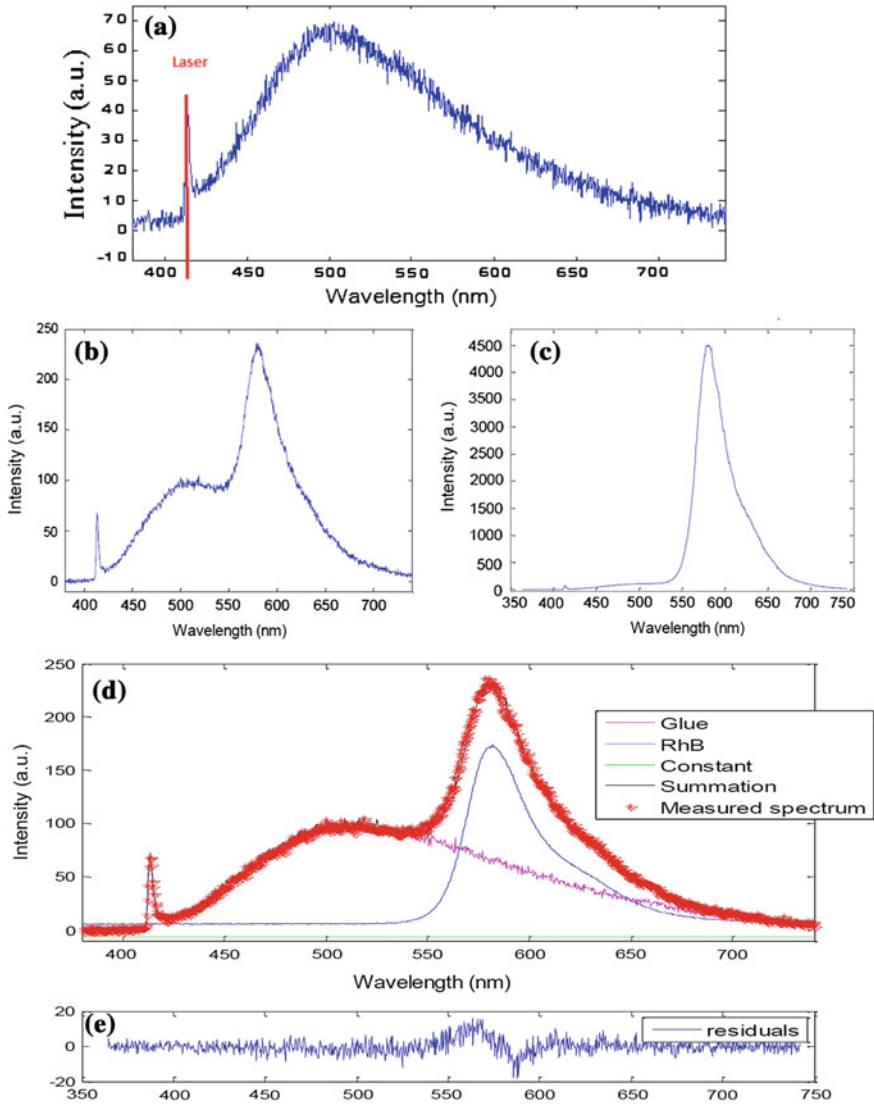


Fig. 2.3 **a** Emission spectrum of the background fluorescence from the GRIN lens alone with at 405 nm. **b** RhB spectrum measured with a separation distance between the fibre and the GRIN lens of 2 mm and **c** of less than 200 μm . **d** Summary of the measured emission spectra and the sum of the RhB and glue emission spectra. **e** Calculation of the difference between the sum of each spectral component and the effective measured spectrum

2.2.2.3 GRIN Lens for Lifetime Measurements

We have shown that background fluorescence is perturbing the spectral measurement through the GRIN lens in specific conditions. In this section, considering ideal conditions of object and image WD, the effect of the GRIN lens position is evaluated on the measurement of fluorescence lifetime of the RhB.

To do so, the spectrometer in Fig. 2.2 is replaced by a photomultiplier tube (PMT) and a Time-Correlated Single Photon Counting (TCSPC) module synchronized with the excitation source. This method detects the exponential decay of fluorescence based on an accurate record of a single photon using a highly sensitive photon detector. The delay time of emission of a photon relative to the laser excitation pulse corresponds to the time spent in the excited state. Then, excitation is repeated several times to maintain the condition that only one photon per molecule is detected at each pulse. A photon counting histogram is generated which represents the fluorescence decay time and the instrument response function (IRF), a Time Harp 200 acquisition card (Pico Quant, Berlin Germany). The timing precision of TCSPC system is characterized by the IRF and should be infinitely narrow for an ideal system. Experimentally, the laser emission is rejected using a 410 nm high pass filter in order to detect fluorescence. The lifetime decays is fitted to an exponential model in order to extract the lifetime information with a reasonable fit quality, assessed by the residuals of the fitting. An exponential model is chosen because it gives a good description of the physics involved in the lifetime decay. Two exponential models have been tested to extract the lifetime information: the multi-exponential tail fit without considering the IRF and the exponential reconvolution considering the IRF. These equations are detailed in (2.1) and (2.2).

$$I(t) = \sum_{i=1}^n A_i e^{-\frac{t}{\tau_i}} \quad (2.1)$$

$$I(t) = \int_{-\infty}^t IRF(t') \sum_{i=1}^n A_i e^{-\frac{t-t'}{\tau_i}} dt' \quad (2.2)$$

where:

$I(t)$ is the measured decay data,

A_i the amplitude of the i th component, in counts,

τ_i the lifetime of the i th component that has to be calculated.

IRF was measured using a mirror instead of the RhB sample and the filter was replaced by an optical density filter in order to let the attenuated laser beam pass to the PMT for IRF detection. The IRFs measurements of the fibre alone, the fibre coupled to a 10× microscope objective and the fibre coupled to the GRIN lens are shown and compared in Fig. 2.4.

The IRFs around 0.65 ns at the full width half maximum (FWHM) are similar for the three conditions. As a result, the GRIN lens does not affect lifetime measurements.

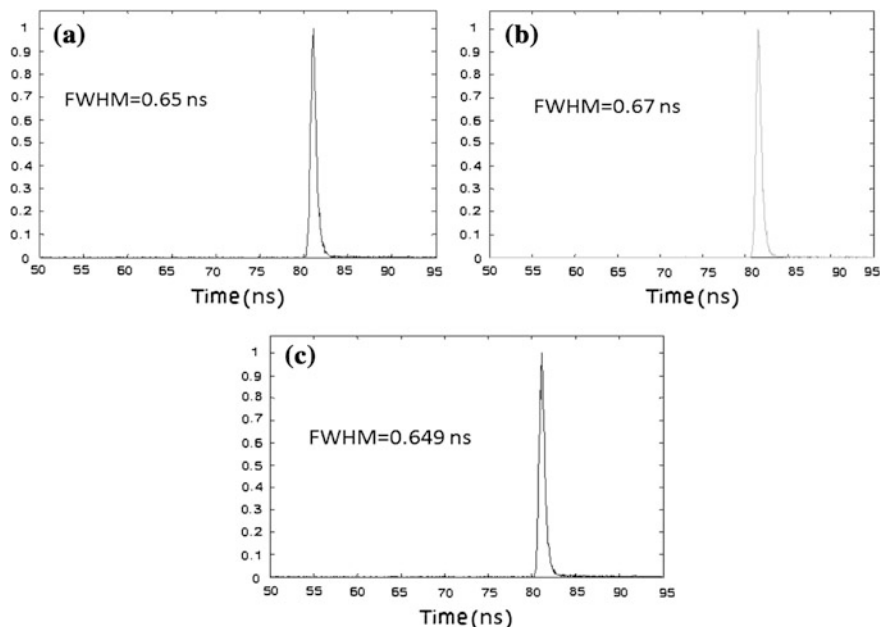


Fig. 2.4 Measurement of the lifetime of the experimental setup. **a** Fibre alone. **b** Fibre and 10 $\times$ objective. **c** Fibre and GRIN lens

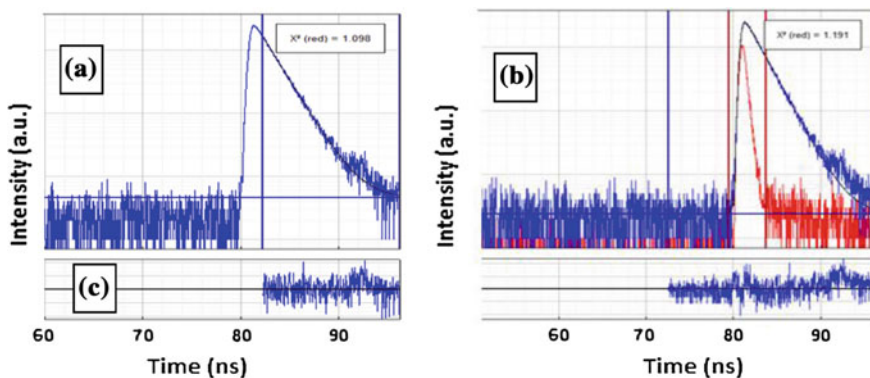


Fig. 2.5 Measured fluorescence decay (blue) coupled with the fitting model (black). **a** Lifetime of RhB without considering the IRF. **b** Lifetime fitting by reconvolution, IRF deconvoluted with the decay data (red). **c** Residuals randomly distributed across the fitting range

Secondly, lifetime measurements using the two fitting methods previously detailed and decay fits of the fluorescence signal of the RhB solution were realized. Figure 2.5a, b displays the fluorescence lifetime of RhB superimposed with the fitting curves.

Table 2.1 Lifetime decay fitting through the setup elements using tail fitting without the IRF and reconvolution fitting with the IRF

Element of the setup	A (counts)	τ (ns)	Fit method
Fibre	1872.4 ± 20.9	1.72 ± 0.02	Tail
	$14,046 \pm 94.7$	1.67 ± 0.01	Reconv
Objective	1638.5 ± 19.3	1.705 ± 0.015	Tail
	3345.3 ± 40.4	1.703 ± 0.014	Reconv
GRIN lens	252.5 ± 9.41	1.628 ± 0.049	Tail
	1835 ± 29.6	1.616 ± 0.019	Reconv

All the lifetime decays of RhB through the different elements of the setup are summarized in Table 2.1.

In the literature [11], the mean lifetime data of the fluorescence lifetime of a solution of RhB in water at 20 °C is $\tau_{\text{RhB, ref}} = 1.72 \pm 0.02$ ns. The fluorescence lifetime is therefore not affected by the presence of the fibre considering the tail fitting method, while the reconvolution method is less relevant. Furthermore, the fluorescence lifetime is not significantly disturbed by the microscope objective if one takes the experimental incertitude into account. Finally, it can be seen that for the GRIN lens, both fitting methods, lead to a decrease of the fluorescence lifetime from 1.7 ns to about 1.62 ns. This may be explained by the detection of fluorescence signal of the glue of the GRIN lens, leading to an average measurement of the lifetime of the RhB and the glue. That point corroborates the previous conclusions in Sects. 2.2.1 and 2.2.2 on the significant perturbation of the signal collected by the GRIN lens due to the background fluorescence of the glue.

2.2.3 GRIN Lens Resolution Measurement

The confocal microscope is used to evaluate the resolution of the imaging system as a whole (10× microscope objective combined with the GRIN lens). Gold beads having a diameter of 1 μm are assumed to be point sources. A Gaussian function depicted in Fig. 2.6a, b and c fits the axial and lateral profiles of their images allowing to calculate the FWHM, equivalent to the optical resolution of the system.

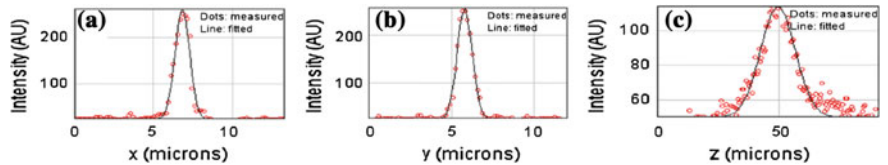


Fig. 2.6 Gaussian fits and beads profile measurements. Resolutions: **a** Lateral in x (μm), **b** Lateral in y (μm), and **c** Axial in z (μm)

The lateral resolution in x and y are both $1\ \mu\text{m}$, in agreement with the best performances described in the literature [3]. This system consequently equals the state of the art of the resolution of a fibred endomicroscope, adding the huge advantage of the miniaturized sizes.

2.3 Numerical Analysis of the Global Endomicroscope

A numerical simulation of the distal end of an optimized nonlinear endomicroscope is proposed, coupling a GRIN lens with a PZT scanner. These simulations are led simultaneously for NIR nonlinear excitation and collection of the multiphotonic signal emitted by the target. The GRIN lens is coupled to the scanning system and the optical fibre. The simulated fibre, a DCF, delivers the nonlinear excitation by the core and collects the multiphotonic emission by the inner cladding. This DCF is simulated with ideal parameters for delivering the multiphotonic excitation through a small core diameter ($5\ \mu\text{m}$) single mode at $800\ \text{nm}$ with a small NA (0.04) and a large inner cladding diameter ($200\ \mu\text{m}$) to collect the maximum of multiphotonic signal from the GRIN lens thanks, to a large NA (0.3).

2.3.1 PZT Scanning System

The scanning system is a tubular PZT. The important advantage of such a scanner stays in its small size not yet equalled by another technology. It consists in a small tubular piezoelectric actuator governed by four electrodes grouped in two pairs, with an optical fibre positioned inside the actuator and electrically glued at its tip. This layout allows driving the fibre tip, exceeding the PZT for several tens of millimetres by application of an arbitrary waveform (triangular or sinusoidal) with a chosen frequency, for each pair of electrodes. This results in a well-defined movement of the fibre tip. The adapted one in the context of endomicroscopy is a circular scanning pattern of the extremity of the fibre. The amplitude is adjustable until a limit depending on the electrical parameters of the PZT, so that the position of the fibre tip can be set on all the surface of a defined circle. The position of the focal point of the excitation beam on different parts of the target relies on the relative position of the fibre with the GRIN lens, as illustrated in Fig. 2.7.



Fig. 2.7 Scheme of the distal end of the endomicroscope containing the DCF, the PZT and the GRIN lens

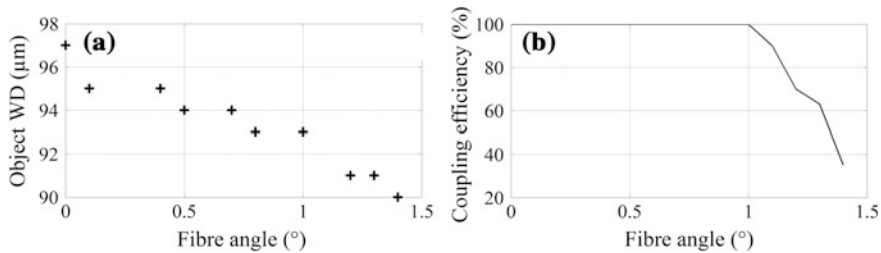


Fig. 2.8 Consequences of the use of a PZT scanner. Evolution of **a** the object WD. **b** the coupling efficiency of the excitation beam with the DCF angle

Several consequences are arising from the use of the PZT scanning system on the fibre tip resulting from the angle between the fibre tip and the GRIN lens: (i) the object WD, (ii) the coupling efficiency between the GRIN lens and the excitation laser, also related to the DCF core diameter and its NA which are necessarily modified by the tilt angle of the fibre. This observation are the subject of this part of numerical simulation realized with Zemax program. Figure 2.8a, b resume the related results.

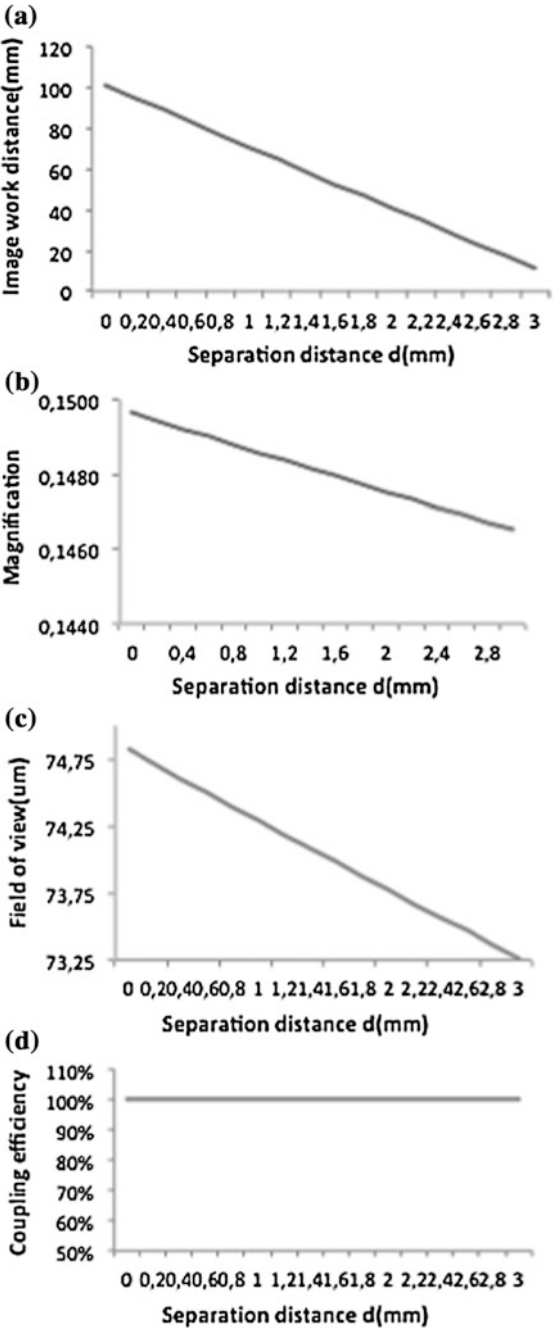
A decrease of the object WD with the fibre angle is shown in Fig. 2.8a, revealing a fibre tip is not really a plan, but describing a semi-sphere. This parameter is not a major drawback but it should be considered for the interpretation of the obtained images or taken in consideration for the numerical reconstruction of the images. An increase of the fibre angle leads to an important decrease of the coupling efficiency between the excitation beam and the GRIN lens (Fig. 2.8b). Indeed, 10 % of the excitation beam is lost for an angle of 1.1° and more than 60 % are not coupled for 1.4°. This results in the fluctuation of the incident average power in the target, involving a proportional fluctuation of the emitted fluorescence. To limit this problem, a reduction of the amplitude of scan is necessary resulting in the reduction of the field of view in the object space.

To conclude, PZT scanning system is interesting by its small diameter ideal for the miniaturization of the distal end, but several alteration of the detected image must be considered to interpret the observed images.

2.3.2 *Simulation of the Optimal Distance Between the DCF and the GRIN Lens*

Several parameters are varying with the distance between the DCF and the GRIN lens (Fig. 2.2a): (i) the focal point position and thus (ii) the object WD, (iii) the magnification properties, (iv) the coupling efficiency and (v) the FOV. The impact of these parameters was numerically simulated and the results are summarized in Fig. 2.9. A distance ranging between 0 and 3 mm between the DCF and the GRIN lens has been tested.

Fig. 2.9 Calculated influence of the separation distance between the GRIN lens and the fibre tip on several parameters. **a** Object WD. **b** Magnification. **c** FOV. **d** Coupling efficiency



The object WD decreases when the separation distance between the fibre and the GRIN lens increases, as expected. And thus, the focal point is under control of this parameter and can be adjusted between 10 and 100 μm , the highest penetration depth reachable by the excitation beam. The magnification and the FOV decrease slowly when the separation distance increases. For example, the FOV, which is very small at the origin (less than 75 μm) declines to about 73.25 μm at 3 mm. In this distance ranges, we can consider these parameters can be considered negligible.

In Fig. 2.9d it is shown that whatever the distance between the fibre and the GRIN lens, after the collection by the GRIN lens, the coupling efficiency of the multiphotonic signal emitted by the sample is not modified. The big inner cladding diameter of 200 μm with the highest possible NA of 0.3 is responsible for this result, justifying the choice of the inner cladding dimension. Consequently, that optimized parameter is crucial for the efficient collection of very weak multiphoton endogenous signals.

In conclusion, if an axial scanning is required, moving the separation distance GRIN lenses are now commonly used in the context of the miniaturization of a. Nevertheless, several modifications must be considered: an important decrease of the object WD as well as a modification of the magnification and the FOV are modifying the resulting images.

2.4 Conclusions

GRIN lenses are now commonly used towards the miniaturization of a distal head of an endomicroscope. In This article, we have consequently characterized a commercial GRIN lens in the context of imaging brain tissues under a confocal microscope. We have identified a potential parasite signal of fluorescent coming from the outlying glue all around the effective area of the lens. Starting from this observation, we have analysed the imaging of glial cells of mouse brain and no influence on the resulting fluorescent image of the sample has been observed. To evaluate the importance of this background fluorescence, spectral analysis and fluorescence lifetime measurements were managed. A non-negligible part of this fluorescence that can be easily identified by its spectrum and by its lifetime were shown for both of them.

In a second step, the GRIN lens lateral and axial resolutions were then established to around respectively 1 and 16 μm , in agreement with the values usually presented in the literature.

Finally, we have numerically simulated the global endomicroscope including this GRIN lens coupled to a homemade DCF fixed with a PZT scanning system. We have highlighted limitations of the use of this scanning device: (i) the focal plan is necessarily not plan but curved and (ii) the coupling efficiency of the excitation beam between the DCF and the GRIN lens is hugely reduced to less than 40 % of coupling when the fibre angle exceeds 1.4° . More, the separation distance between the GRIN lens and the DCF influenced the position of the focal point in the object

plan, presenting a way to perform an axial scanning system, which is still a limiting point for obtaining 3D images. We have nevertheless noticed that a modification of the separation distance is inducing a reduction of the FOV and of the magnification. The best simulated FOV obtained with the GRIN lens is $75 \times 75 \mu\text{m}^2$, which is a very small and far from the ideal situation usually asked by the surgeons of $1 \times 1 \text{ mm}^2$.

For an ideal development of an endomicroscope, all these limitations must be carefully thought for the experimental use of the GRIN lens. Globally, GRIN lens technology remains the only one allowing a distal lens having a diameter smaller than 0.5 mm, being a fundamental importance in nonlinear endomicroscopy in a clinical application.

Acknowledgements This work has been highly supported by INCA Plan Cancer with Physicancer program grants “MEMBO” & “MEVO” and the Institut National de Physique Nucléaire et de Physique des Particules (IN2P3). This work was supported by the L’Oreal Foundation, thanks to the French National Program “For Woman in Science”, distinguishing Claire Lefort for her work on endomicroscopy.

References

1. Miller, A.B., Hoogstraten, B., Staquet, M., Winkler, A.: Reporting results of cancer treatment. *Cancer* **47**(1), 207–214 (1981)
2. Salaün, M., Roussel, F., Hauss, P.-A., Lachkar, S., Thiberville, L.: In vivo imaging of pulmonary alveolar proteinosis using confocal endomicroscopy. *Eur. Respir. J.* **36**(2), 451–453 (2010)
3. Gu, M., Bao, H., Kang, H.: Fibre-optical microendoscopy. *J. Microsc.* **254**(1), 13–18 (2014)
4. Lefort, C., Hamzeh, H., Louradour, F., Pain, F., Abi Haidar, D.: Characterization, comparison and choice of a commercial double-clad fiber for nonlinear endomicroscopy. *J. Biomed. Optics* **19**(7), 076005 (2014)
5. Peyrot, D.A., Lefort, C., Steffenhagen, M., Mansuryan, T., Ducourthial, G., Abi-Haidar, D., Sandeau, N., Vever-Bizet, C., Krulik, S.G., Thiberville, L., Louradour, F., Bourg-Heckly, G.: Development of a nonlinear fiber-optic spectrometer for human lung tissue exploration. *Biomed. Optics Expr.* **3**(5), 840–853 (2012)
6. Cosignani, V., Dvornikov, A., Aguilar, J.-S., Stingari, C., Edwards, R., Mantulin, W.W., Gratton, E.: Deep tissue fluorescence imaging and in vivo biological applications. *J. Biomed. Optics* **17**(11), 116023 (2012)
7. Ducourthial, G., Lefort, C., Peyrot, D.A., Mansuryan, T., Krulik, S.G., Vever-Bizet, C., Thiberville, L., Lacombe, F., Bourg-Heckly, G., Louradour, F.: Label free multiphoton imaging of human pulmonary tissues through two-meter-long microstructured fiber and multicore image-guide. In: *Progress in Biomedical Optics and Imaging—Proceedings of SPIE*, 85750H (2013)
8. Lelek, M., Suran, E., Louradour, F., Barthelemy, A., Viellerobe, B., Lacombe, F.: Coherent femtosecond pulse shaping for the optimization of a non-linear micro-endoscope. *Opt. Expr.* **15**(16), 10154–10162 (2007)
9. Hamzeh, H., Lefort, C., Pain, F., Abi Haidar, D.: Optimization and characterization of nonlinear excitation and collection through a gradient-index lens for high-resolution nonlinear endomicroscopy. *Opt. Lett.* **40**(5), 808–811 (2015)

10. Myaing, M.T.M., MacDonald, D.J., Li, X.: Fiber-optic scanning two-photon fluorescence endoscope. *Opt. Lett.* **31**(8), 1076–1078 (2006)
11. Boens, Noel, Qin, Wenwu, Basaric, Nikola, Hofkens, Johan, Ameloot, Marcel, Pouget, Jacques: Fluorescence lifetime standards for time and frequency domain fluorescence spectroscopy. *Anal. Chem.* **79**, 2137–2149 (2007)

Photoptics 2015

Revised Selected Papers

Ribeiro, P.; Raposo, M. (Eds.)

2016, XVII, 194 p. 119 illus., 30 illus. in color.,

Hardcover

ISBN: 978-3-319-30135-8