Optical measurement of focal offset in tunable lenses

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Abstract: Electrically tunable lenses are becoming a widely used optical tool, and have brought significant innovation to microscopy methods. One current limitation of such systems is the difficulty of directly monitor the focal change in real time. Affordable and reliable feedback for such lenses, compatible with any microscopy setup, represents a much-needed improvement that is still not widely available. We discuss here the implementation and technical performance of an optical device to measure with a high frequency response the displacement of the focal offset of a commercial tunable lens with a precision in the range of the axial Point Spread Function (PSF) of the microscope. The technology presented is cost effective and can be employed on any microscopy setup.

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1. Introduction

Devices to change the focal position of an optical system without any mechanical axial displacement of the optics are gaining growing application in microscopy. Tunable Lenses (TLs), when placed at the back focal plane of the objective of a microscope, allow changing the position of the focal plane by many hundreds of microns without any change in the physical distance between the object and the lens itself [1,2]. In most cases Electrically TLs (ETLs) allow changing the focal plane within a few ms, and acoustically driven devices allow even achieving μs temporal resolution [3].

While extremely fast and relatively cheap, one current disadvantage of ETLs is the absence of a mechanism to detect the actual deformation of the lens and hence the instant focal position. This is of fundamental importance for all applications requiring both long-term, high stability imaging and high repeatability after fast changes of focal plane positions. Deformation of the optical surface in commercial ETLs is affected by thermal effects generated by the electrical current, which makes it difficult to precisely determine actual focal position of the lens based on just the applied current or voltage. To compensate for these
thermal effects some ETL models include temperature sensors and calibration data tables that allow more accurate focal positioning [4], however, it does not provide the real-time actual position. Moreover, these models are bulky and not very suitable for use in commercial microscopes.

Nanopositioning devices such as piezoelectric actuators or stepper motors have the possibility of operating in closed-loop mode by taking advantage of capacitive position sensors or linear encoders. In the case of ETLs, an optical device is needed, and a proprietary built-in optical feedback mechanism allowing for long-term repeatability of <0.02 diopters was recently reported [5]. The obvious advantage of an all-optical device is the high frequency response, in the range of the kHz, as well as the lower cost with respect to a piezo driver, although the only commercial design to date appears expensive, bulky and tailored for laser printing applications rather than microscopy. Custom-made devices based on measuring the back-reflection of a collimated beam impinging on the lens can be implemented with relative ease in custom-made microscopy setups; however, a device fully compatible with any commercial design is still missing.

In this paper, we present a method and a novel design that allows us measuring the position of the focal offset of an ETL with accuracy comparable to the size of the axial Point Spread Function of the microscope with a high frequency response. Focal offsets in μm are used throughout the manuscript, assuming that a 20x, 0.4 NA objective is used. The temporal resolution of the measurement ultimately depends on the signal to noise ratio, which increases proportionally to the amount of light coupled into the system. In the measurements reported here, we achieve 60 Hz. We investigated two possible design variants, meant to be installed close to the back-focal plane of the objective, fitting within the turret space of most modern commercial microscopes.

2. Materials and methods

2.1 Experimental setup

Our fiber-optics based module is built within a custom mechanical ETL holder discussed in a previous publication [1]. We tested the performance of two different designs for the device. In one case, Figs. 1(a), a coupling ring is inserted at the back focal plane of the module, where it threads within a RMS microscope turret. Small holes drilled into the coupling ring allow positioning optical fibers of 500 μm diameter at a controlled angle with respect to the optical axis, close to the window enclosing the ETL surface. The configuration uses one optical fiber to shine light on the surface of the ETL and two, proximal collection fibers, symmetrical with respect to the optical axis of the lens, to collect the reflection. The second configuration is shown in Fig. 1(b) and it uses a retro-reflected configuration where the excitation and collection fibers are placed on the same side of the lens, and a reflective surface on the other side of the lens allows a double passage of the diverging excitation beam, maximizing the change of intensity due to defocus measured at the collection fiber. Sizes and dimensions of the device are illustrated in the diagram of Fig. 1(c). Commercial optical fiber tips were cut and polished to achieve a 45deg angle, allowing them reflecting at 90deg with respect to the fiber axis, as displayed at the bottom of Fig. 1(f).

A Halogen Lamp (TI-PS100W, Nikon, USA) was used as the primary light source for testing purposes. The ETL (#83-921 Edmund Optics, USA), tunable in the range of +50 to +200 mm focal length, was combined with a −75 mm focal length plano-concave offset lens (KPC019AR14 Newport, USA), as described in [1]. The ETL and offset lenses were placed in direct contact to the custom-made holder, which is constructed to minimize the overall thickness of the assembly. The holder has RMS threads and can be directly attached on a revolving microscope turret. Technical characteristics of ETL can be found on the manufacturer website (www.optotune.com) in the provided datasheet.
Fig. 1. Diagram of the configurations tested to implement a feedback module fitting a commercial ETL. 

(a) One fiber is used to provide excitation light on the surface of the ETL. Two stacked fibers are used in detection, to achieve a differential measurement. Orange: plastic ring fitting an ETL custom holder on the side where it threads into any RMS microscope turret. 

(b) Retro-reflected configuration: one excitation and one detection fibers are used, on the same side. The collection fiber measures changes in intensity due to defocus. 

(c) (top) Picture of the electrical lens setup with two optical fibers reaching into the case. (bottom) Plastic ring fitting an ETL custom holder on the side where it threads into a RMS microscope turret with two fibers entering at 45deg. 

(e) (top) Picture of the arrangement illustrated in b. The reflecting surface (aluminum foil here) on the lens side, opposite to the fibers, is clearly visible. (bottom) Picture of a 500 µm fiber cut at 45deg and polished. 

2.2 Materials

Optical fibers (Acrylic, 500 µm core, jacketed) were purchased from Edmunds Optics (#57-097). Detection of the light reflected from the surface of the ETL was done using two Avalanche Photo Diodes (SPCM-AQR-13-FC, Perkin Elmer, USA), as reported in Fig. 2(b). Their output signal was coupled to the data acquisition card (IOTech 3001PCI, MC, USA) of a Personal Computer and the signal was acquired using the SimFCS Software. Detection of the transmitted light was performed using a Hamamatsu PhotoMultiplier Tube (H10721, Hamamatsu, Japan) and either a National Instruments USB6221 data acquisition card to measure the analog voltage output of the device or an IOTech card controlled by the SimFCS software, as reported in Fig. 2(c). The ETL was enclosed in a custom made casing, and coupled to a divergent lens, as previously described [2].

3. Results

3.1 Comparing the performance of two design configurations

We compared the performance of two of the designs discussed in section 2.1 and graphically depicted in Fig. 1, namely the differential detection mode of Fig. 1(a) and the retro-reflected
configuration in Fig. 1(b). In both cases, the current flowing through the lens (i.e. the voltage applied to the driving electronics) was tuned to determine changes in focal position. The relationship between the lens driving current and the focal displacement (according to the objective used) is displayed in Fig. 2(a). The differential measurement allowed a much better baseline stability than the single fiber detection (data not shown). Figure 2(b) shows the signal measured when increments of the lens current of 8mA are applied to the ETL. When the lens is coupled to a 20x, 0.4NA objective this corresponds to a focal offset of approximately 28 μm/step.

**Fig. 2.** Feedback signal measured for two of the configurations discussed in Fig. 1. a) Change in focal distance vs Voltage applied to the ETL for three different objectives. b) (top) Representation of the PSF of the microscope using a 20x 0.4NA objective. The approximate axial waist is 1 μm. (bottom) The graph shows the changes in the measured signal using the differential configuration described in Fig. 2(a). Each point is obtained by averaging the signal over an acquisition of 20s (0.05 Hz). c) Signal measured in reflected transmission geometry while changing the focal distance of the ETL in steps covering over 400 μm (using a 20x, 0.4 NA objective). The linear fit has a slope of −0.5 mV/mA. d) Enlarged view of the linear region of detector output vs lens current. Steps in lens current of 2 mA (corresponding to about 7 μm axial displacement) are clearly resolvable.

Figure 2(c) illustrates the changes of the signal measured by the detection fiber in the retro-reflected configurations upon a focal offset of 400 μm achieved in steps of 2.7 μm, one every 1.5 s. The response is linear up to driving currents of the order of 100 mA (i.e. 400 μm using a 20x, 0.4 NA objective). After this value, due to the changes in position of the reflected spot, correspondence between offset and distance is lost. In the linear regime displayed in Fig. 2(d), steps of the order of, or smaller than, the axial Point Spread Function can be easily resolved.

Using the retro-reflected configuration, with just one detector, the temporal stability of the signal is remarkable Fig. 3(a). Fast modulations of the signal (up to 60 Hz) with an amplitude of a few mA in the driving current, can be clearly resolved Fig. 3(b). This demonstrates the capability of the system to measure minute changes in focal position of the ETL at high
temporal resolution. Figure 3(c) illustrates the modulation of the signal at lower acquisition frequencies (15 Hz).

![Figure 3](image)

**Fig. 3.** a) Stability of lens focal offset signal as a function of time upon modulation (8 Hz) of the lens focal offset with a square wave of 2.7 mA amplitude. b) Enlarged view of a portion of the axial modulation as a function of time acquired at a frequency of 60 Hz, and c) 15 Hz.

### 3.2 Position measurement in a microscope

The retro-reflected configuration was tested on an upright microscope (the DIVER, extensively described elsewhere [7]). Excitation light was coupled into the input fiber after being filtered by a longpass glass filter (600 lp, Edmund Optics, USA), while the retro-reflection was collected by a Photomultiplier tube, as discussed in Fig. 2.

To avoid leakage from the light used to measure the lens position, a glass bandpass filter (UG11, 325 ± 50nm) was used in the emission path of the microscope. Cyan fluorescent beads (1µm diameter, 365nm ex/405nm em) were excited at 750 nm with a multiphoton laser and focused using a µm-sensitive z-stepper motor for the sample in order to find the accurate position of the focal plane while the current driving the ETL was changed. Figure 4(a) displays the measured change in focal position using the PMT signal as a function of the sample axial position. The two solid blue markers indicate the measured signal upon re-focusing at an axial positions of 210 µm and 32 µm respectively, showing perfect overlap.

![Figure 4](image)

**Fig. 4.** Measurement of the focal plane displacement using the add-on in a retro-reflected configuration in a microscope. **a)** Signal measured in reflected transmission geometry while changing the focal distance of the ETL in steps covering over 300 µm (using a 20x, 0.4 NA objective). Error bars are given respectively by the standard deviation of the measured PMT signal (x) and by half the axial PSF width (y). As the current to the lens is changed, the sample is refocused using a stepper motor with µm-step sensitivity, providing the focal offset values in the x-axis. Blue solid markers indicate the measured position signal upon re-focusing at an axial positions of 210 µm and 32 µm respectively, showing perfect overlap. **b)** Microscope objective (20x, 0.4 NA) mounted on the ETL housing containing the position sensitive device as illustrated in Fig. 1(e). The red arrows indicate the input and output optical fibers respectively.
4. Summary

We have demonstrated here the experimental realization of an all-optical device that can be coupled to TLs to measure displacement of the focal offset comparable to the PSF of a microscope at a temporal resolution better than 0.1 s. The retro-reflected configuration appears to be the most effective of the designs we tested in terms of signal to noise ratio. Improvements of the signal to noise ratio, and hence the frequency response of the device, will ultimately depend upon the amount of power that can be coupled to the excitation fiber.

The advantages offered by this device are its compact size, allowing it to fit most commercial microscopes objective turrets, as well as its simple geometry, ease of construction and low cost. In order to be compatible with fluorescence imaging systems, the light used to sense the lens deformation needs to be in a range outside that of the emission spectrum of the sample. A light emitting diode in the IR range, coupled to a photodetector sensitive in the IR (e.g. an InGaAs photodiode) can be used to this purpose. In this case, given the absence of any spectral overlap between visible fluorescence signal and the IR excitation light, the amount of power coupled to the excitation fiber could be increased at will until the desired frequency response is achieved. When coupled to a feedback system this device will allow closed-loop measurements using ETLs, compensating for slow focal plane drifts as well as for fast modulations of the focus employed in most modern microscopes [6,8].

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