

A method for characterizing longitudinal chromatic aberration of microscope objectives using a confocal optical system

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Summary

We describe a novel method of characterizing the longitudinal chromatic aberration of microscope objectives by recording a series of axial responses as a function of wavelength as a plane reflector is scanned through the focal region of a confocal microscope. Measurements are presented for a variety of objectives with differing degrees of correction. The use of the chromatic focal shift to measure surface profiles is also discussed.

Introduction

The unique optical properties of the confocal optical system arise from the fact that both the lens which focuses the probe light onto the specimen and that which focuses the reflected or fluorescence light onto the pinhole point detector play an equal role in the image formation (Wilson & Sheppard, 1984; Wilson, 1990). It is this fact which leads to the enhanced lateral resolution and optical sectioning property of confocal microscopes. It is, of course, the latter

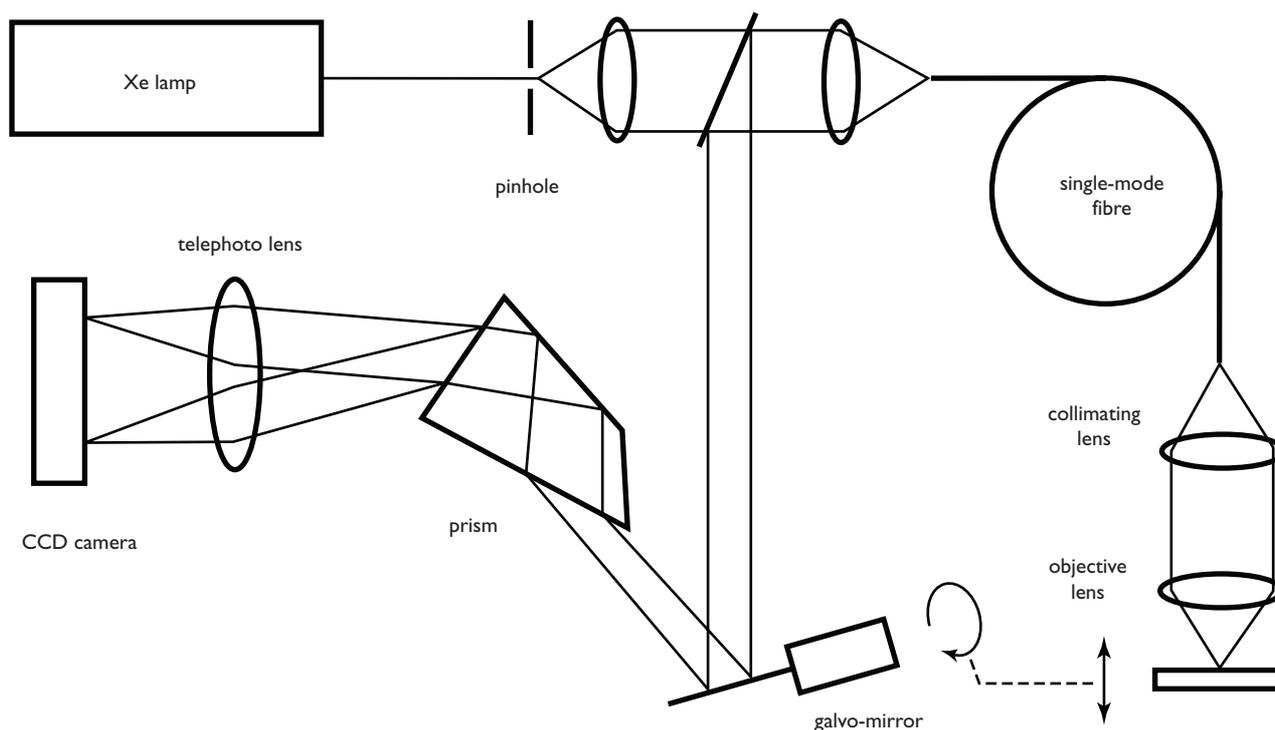


Fig. 1. Schematic of the experimental arrangement.

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effect which permits volume objects to be imaged in three dimensions.

The price one pays for this enhanced imaging performance, however, is that the optical system must be carefully designed. It is particularly important, for example, that the source and detector pinholes are correctly positioned such that their images, in object space, lie exactly on top of each other with no relative spatial displacement. If this is achieved a very sharp effective point spread function with

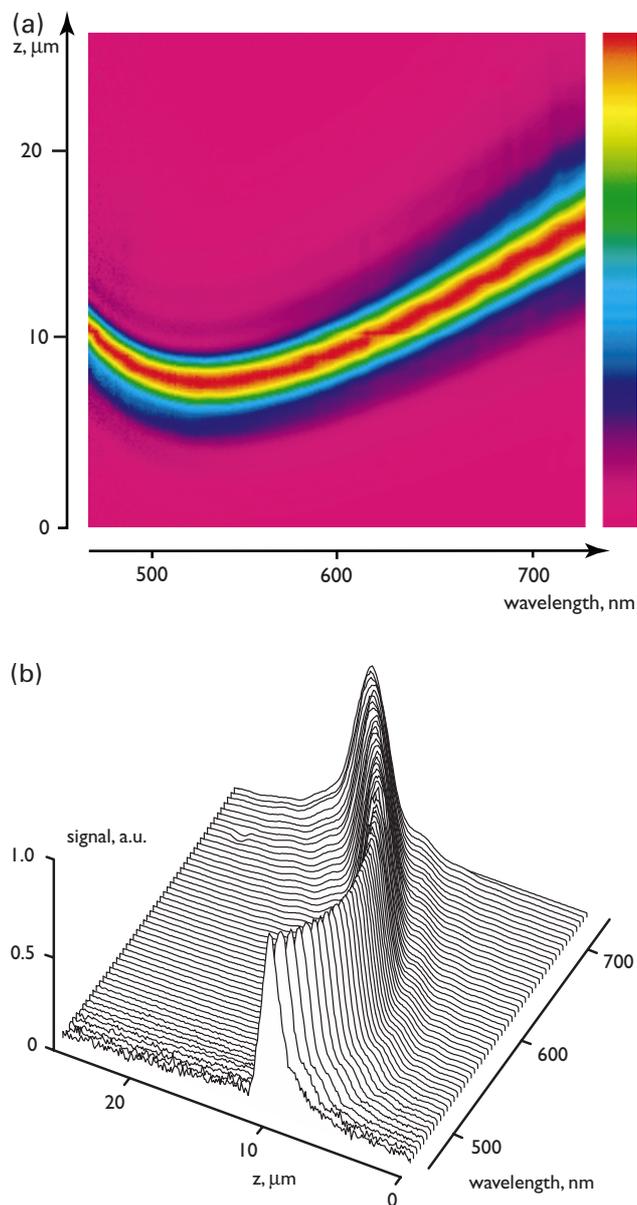


Fig. 2. The axial response as a function of wavelength for a plan-achromat 32× 0.5NA objective (A) in colour-coded form, in (a) and in isometric form in (b). Colour scale in (a) is linear in signal intensity.

very low side lobe levels will result and high quality confocal images will be produced. Since both lenses play an equal part in the imaging the system is particularly sensitive to aberrations, whether specimen induced or inherent in the optical elements used (see, e.g. Pawley, 1995; Fricker & White, 1992). In applications such as single- or multiphoton-confocal fluorescence or second and third harmonic generation imaging where the excitation and fluorescence or harmonic radiation are of different wavelengths it is very important that the objective lenses are corrected as well as possible for chromatic aberration.

In the following we will take advantage of the sensitivity of the confocal optical system to objective lens aberration and present a new method of characterizing longitudinal chromatic aberration in high numerical aperture microscope objectives. The implication of the result in confocal imaging will also be discussed.

The method

A common method to characterize optical sectioning strength of a confocal microscope as well as to check the system alignment is to measure the detected image signal as a plane reflector is scanned axially through the focal region. The maximum signal is detected when the plane reflector lies in the focal plane and correspondingly smaller signals are measured as the reflector is moved further away from the focal plane. This suggests the basis of our method. We measure the axial response as a plane reflector is scanned through focus as a function of wavelength. This will then permit us to determine the change in focal position with wavelength.

In order to do this we use a reciprocal geometry single mode optical fibre-based confocal microscope. In this system, Fig. 1, the same optical fibre (SM450, Fibrecore Ltd, which is single moded at wavelengths greater than 450 nm) is used both to launch light into the microscope system and to detect the confocal signal. The reciprocal geometry is used so as to avoid unnecessary pinhole alignment difficulties. Since we wish to measure the axial response as a function of wavelength we use a Xe arc lamp as a source and, instead of taking measurements at specific wavelengths by the use of appropriate filters, we elect to use a dispersive element so as to capture the entire wavelength spectrum simultaneously. The operation of the system can be seen in Fig. 1. As the plane reflector is scanned axially along the optical axis of the microscope a galvanometer mirror, which is synchronized with the axial z -scan of the plane mirror, causes the reflected confocal light to be deflected up and down in the plane normal to the diagram. A monochromator prism (SF4 glass) then serves to spread the wavelength components in the lateral direction. The chromatic information is finally captured by a cooled 16-bit slow-scan CCD camera (Princeton Instruments with Tektronix TK-512F CCD chip, thermoelectrically

cooled to -30°C). The camera now gives an 'image' in which the horizontal axis represents wavelength, the vertical axis represents the axial z -position of the plane mirror and the image intensity denotes the intensity of the axial response. The z -motion axis was calibrated interferometrically and subsequently linearized, whereas laser lines were used to calibrate and linearize the wavelength axis.

In the following experiments we used five objective lenses from different manufacturers which represented a reasonable cross-section of those available in our laboratory. Since our intention in this paper is to describe only a measurement technique rather than to compare the relative merits of

objectives from different sources we elect to describe the lenses as A, B, C, D and E. All the objective lenses were infinity tube length corrected. Their other details are given in Table 1.

Figure 2 shows the results for an infinity tube length $32\times 0.5\text{NA}$ objective (A), both as a colour coded image, Fig. 2(a), and an isometric plot, Fig. 2(b). In both cases the maximum detected signal has been normalized to unity at each individual wavelength. The presence of longitudinal chromatic aberration is clearly seen in both parts of Fig. 2, which also suggests that this objective is an achromat for which the longitudinal chromatic aberration has been corrected at two wavelengths.

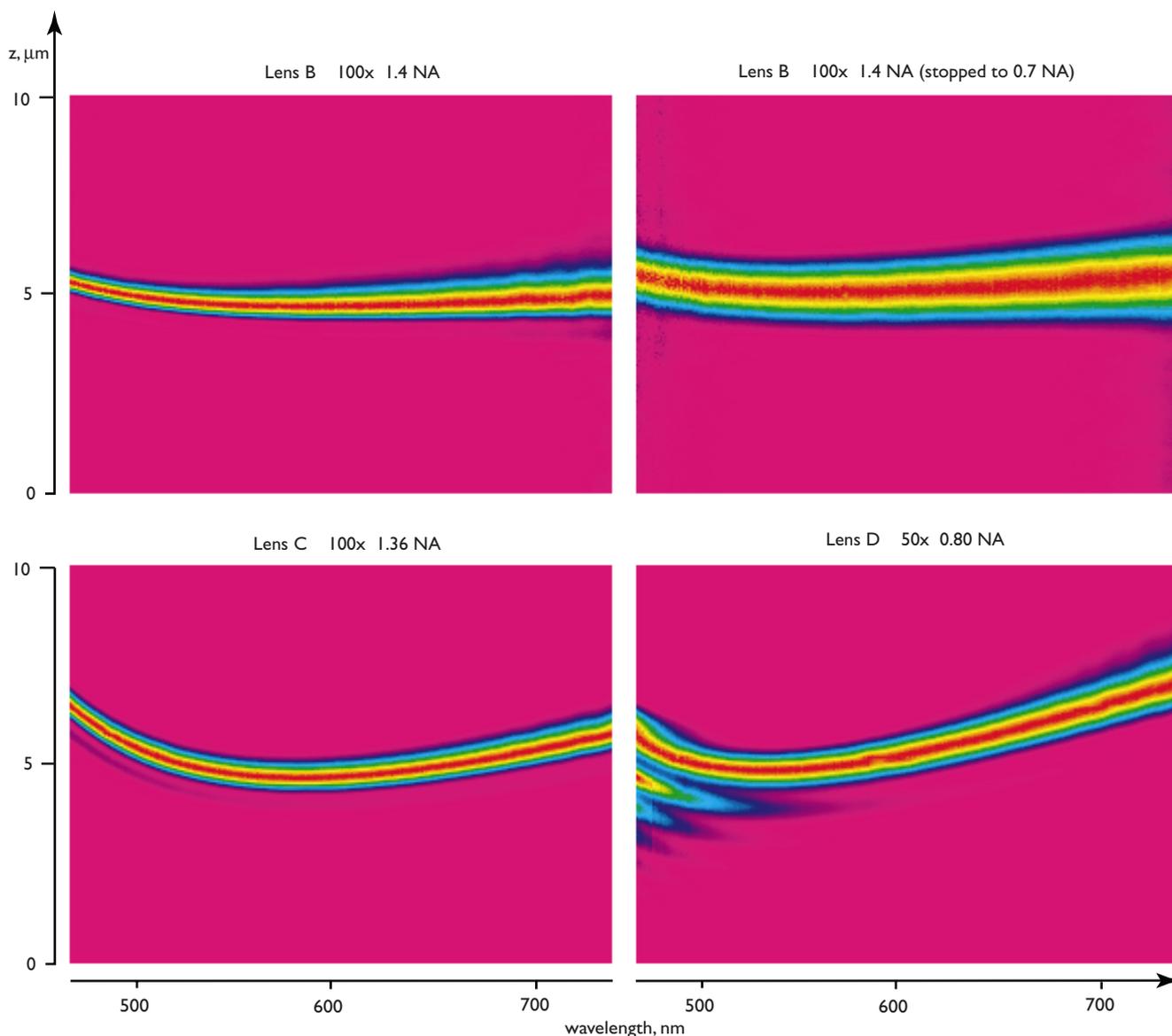


Fig. 3. The axial response as a function of wavelength for a plan-apochromat $100\times$ objective (B) at numerical apertures of 1.4 and 0.7 together with those obtained using a semi-apochromat $100\times 1.36\text{NA}$ objective (C) and a plan-achromat $50\times 0.8\text{NA}$ objective (D). Colour scale is the same as in Fig. 2(a).

Table 1. Details of the objective lenses used in the experiment.

Lens	Type	Magnification	NA
A	plan-achromat	32	0.5
B	plan-apochromat	100	1.4
C	semi-apochromat	100	1.36
D	plan-achromat	50	0.8
E	plan-achromat	20	0.46

Measurements obtained with three other objective lenses are shown in Fig. 3. Two results are shown for the apochromat (B) 100×, one taken at full aperture, nominally 1.4, and a second when stopped down to 0.7NA, where the performance is seen to be slightly improved. It should be noted that this objective is of a much more recent design than the semi-apochromat 100× 1.36NA objective (C), results of which are also presented in Fig. 3. Figure 4(a) shows the variation in focal position as a function of wavelength for a variety of objective lenses, showing the expected improvement in performance of apochromats over achromats and semi-apochromats. It should be noted that the same achromatic collimating lens (Melles Griot 01 LAO 079) was used to obtain the data for all the objective lenses we tested. This lens was chosen because its longitudinal chromatic aberration was well characterized. This permitted us to remove the chromatic effect of the collimating lens from the final results presented in the following figures. It should be noted, however, that for most objective lenses the effect of the collimating lens on the overall longitudinal chromatic aberration was negligible. Indeed, we believe the same would apply to proprietary tube lenses since, to the best of our knowledge, these lenses are intended to correct lateral rather than longitudinal chromatic aberration. The zero in axial shift in the figures has been arbitrarily set to correspond to the 546 nm wavelength correction applied to all the objectives tested. Figure 4(b) presents the same data but in a form more suited to confocal microscopy in the sense that the chromatic focal shift (z) is now expressed in optical units,

$$\frac{8\pi}{\lambda}zn\sin^2\frac{\alpha}{2},$$

where λ is the wavelength and $n\sin\alpha$ the numerical aperture. We choose this set of units since the halfwidth in optical units of the axial response to a plane mirror is given by 2.78 units for all wavelengths and all numerical apertures. This region is also shown in the figure.

We finally note that, although severe longitudinal chromatic aberration is to be avoided in single- and multiphoton confocal fluorescence imaging, it can sometimes be used to advantage. One such case is that of surface

profilometry, where change in focal position with wavelength can be used to obtain depth information (Molesini *et al.*, 1984). Indeed, Boyde and colleagues (Brown *et al.*, 1992; Maly & Boyde 1994) have developed objective lenses with extreme degrees of linear chromatic aberration in order to produce colour-coded surface profiles and real-time stereoscopic display. We plot in Fig. 5(a) the overall longitudinal chromatic aberration of our system when using the 32× 0.5NA objective (A) and note that for wavelengths greater than, say, 575 nm the focal shift varies fairly linearly

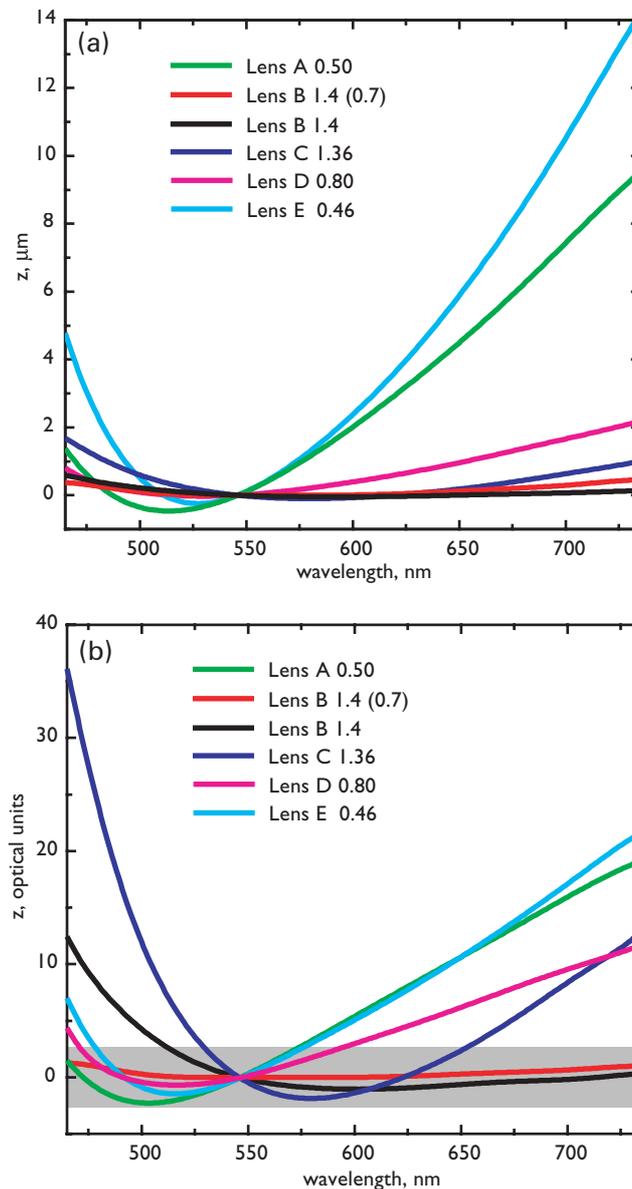


Fig. 4. (a) Focal shift as a function of wavelength for a number of different microscope objectives. (b) Focal shift, measured in optical units, as a function of wavelength for the objectives measured in (a).

with wavelength and hence this standard objective may be used to produce wavelength dependent surface profiles in this wavelength range in a manner similar to that employed by Boyde and co-workers. Moreover, once this overall system calibration curve has been obtained it is a simple matter of using the right software to extract surface profiles using the entire spectral ranges. In order to illustrate the effects of the chromatic focal shift in confocal microscope we show in Fig. 5(b) three confocal images taken at wavelengths of 663, 633 and 607 nm. To observe these images we replaced the CCD camera with a photomultiplier and a pinhole, which could be positioned to select the desired wavelength from the spectrum produced by the specimen. The optical sectioning effect is clear and the refocusing is due entirely to the longitudinal chromatic aberration present in this lens. We note that these images were taken using the Xe arc lamp as source and that the power incident on the specimen was $\approx 0.2 \mu\text{W}$.

Conclusions

We have described a simple technique to characterize the longitudinal chromatic aberration of standard microscope objectives using a confocal microscope. The method permits the chromatic focal shift to be extracted and this, in principle, could be used to obtain depth information and surface profiles from multi-wavelength images.

Finally, we emphasize that the objective lenses we have characterized are representative of those available in our

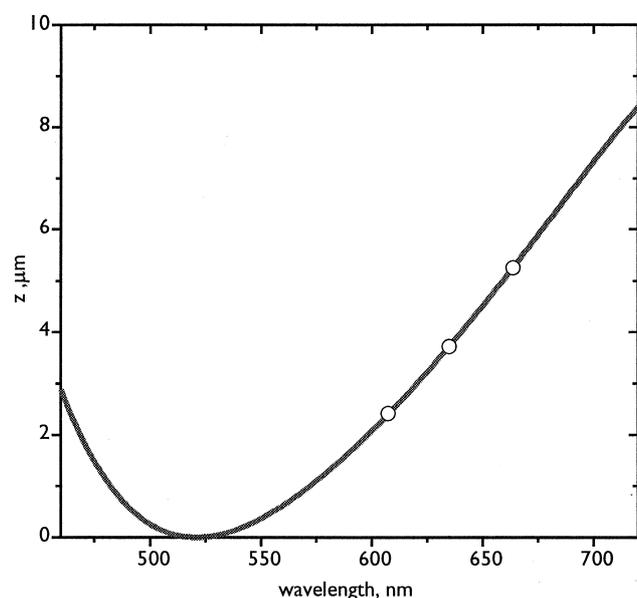
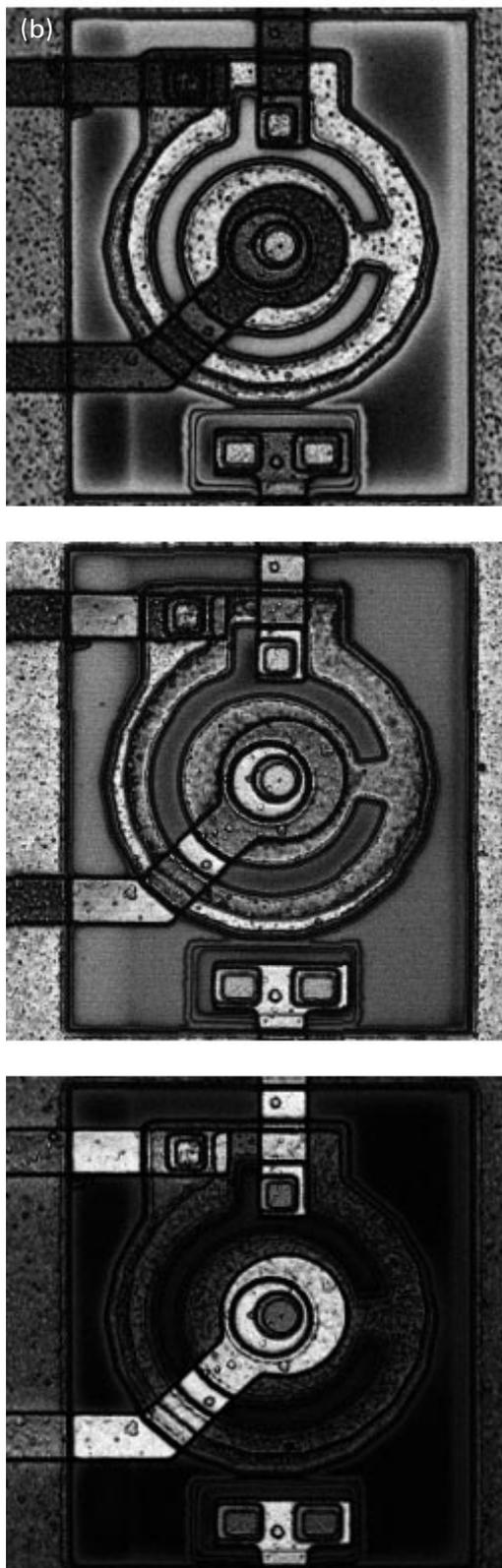


Fig. 5. (a) The focal shift as a function of wavelength for a plan-achromat $32\times 0.5\text{NA}$ objective lens (A). (b) Three confocal images taken with light of wavelength 663, 633 and 607 nm. These wavelengths correspond to chromatical focal shifts of $1.65 \mu\text{m}$ and $2.90 \mu\text{m}$ relative to the 663 nm image.



laboratory and are chosen simply to illustrate the capabilities of the method rather than to compare the relative merits of specific objectives. It is for this reason that we have not specified the manufacturers but rather only the types of objective.

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