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SHORT COMMUNICATION

Real-time three-dimensional imaging of macroscopic structures

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Summary

We describe an extremely simple method of obtaining optically sectioned images with conventional low-power imaging systems in real time. A single spatial frequency grid pattern is projected onto an object. Images taken at three spatial positions of the grid projection are processed to provide 3D images of macroscopic structures.

The confocal optical system is very simple and merely involves focusing light from a point source onto an object and refocusing the reflected or fluorescent light onto a point detector. The point detector, or pinhole, acts as a physical obstruction which prevents light from out-of-focus planes contributing efficiently to the image. This is the origin of the optical sectioning or depth discrimination property. The system we have just described merely probes one point of the object – in order to form an image it is necessary to introduce scanning. It is relatively simple to build a scanning system suitable for microscopy and the most common application of the confocal principle has probably been in the form of the confocal microscope (Wilson & Sheppard, 1984; Wilson, 1990; Pawley, 1995). However, the principle applies equally well to the imaging of larger objects and confocal macroscopes may also be built (Dixon *et al.*, 1995a,b). The optics in these systems need careful design and usually a telecentric $f^*\theta$ laser scan lens is used to permit large specimens to be scanned quickly. An alternative method of producing optically sectioned images in real time using simple white light sources has recently been proposed for microscopy (Neil *et al.*, 1997) and we will show in this short communication that this approach also permits a simple, cheap, optically sectioning macroscope to be built with the minimum modification of existing low-power imaging systems.

The basis of the approach is to realize that in a conventional microscope it is only the zero spatial frequency in the transfer function which does not attenuate with defocus – all the other spatial frequencies do attenuate with defocus and hence we can see that a conventional microscope may be thought to exhibit optical sectioning for all spatial frequencies imaged apart from zero. Our method therefore is to modify the illumination system so as to project a one-dimensional single spatial frequency fringe pattern onto the object. The microscope will then image the fringe pattern efficiently only on those portions of the object that are in focus. We can therefore obtain an optically sectioned image by extracting only those parts of the image where the fringe pattern is visible. Let us assume that the single spatial frequency fringe pattern which illuminates the object may be written as

$$s(x, y) = 1 + m\cos(2\pi\nu x + \phi_0) \tag{1}$$

where m denotes the modulation depth, ν the spatial frequency and ϕ_0 an arbitrary spatial phase. A full theory is presented elsewhere (Neil *et al.*, 1997) but it will suffice for our purposes to claim that this illumination leads to an image of the form

$$I = I_0 + I_s\cos(2\pi\nu x + \phi_0) \tag{2}$$

where I_0 denotes a conventional image due to the constant term in Eq. (1) and I_s is the desired sectioned image modulated by the fringe pattern.

A simple way to extract I_s and I_0 is to take three images I_1, I_2 and I_3 which correspond to three relative spatial phases $\phi_0 = 0°$, 120° and 240°. We then find that

$$I_s = [\sqrt{(2)}/3][I_1 + I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2]^{1/2} \tag{3}$$

and

$$I_0 = (I_1 + I_2 + I_3)/3 \tag{4}$$

which permits us to extract both the sectioned image and the conventional image from the same three raw images. We emphasize that Eq. (2) is approximate for the purposes

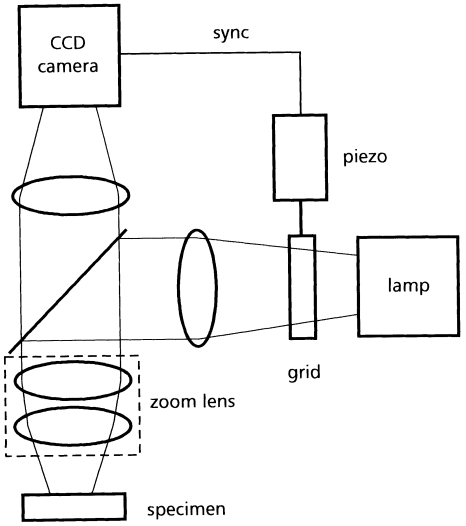
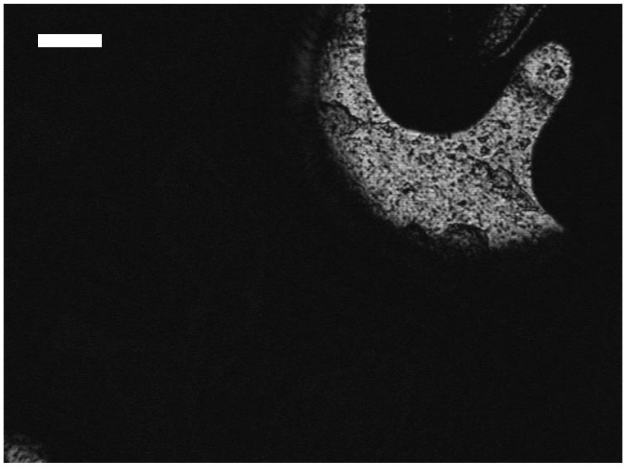


Fig. 1. Schematic diagram of the macroscope system.



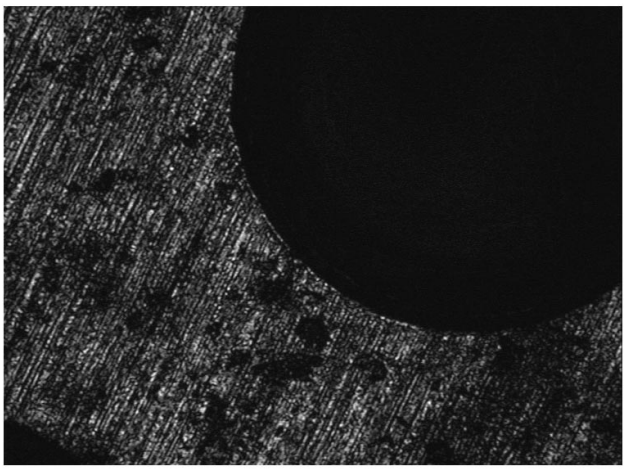


Fig. 2. Optically sectioned images, taken at focal settings 1·25 mm apart, of a section of a wrist watch mechanism: the scale bar depicts a length of 100 μm.

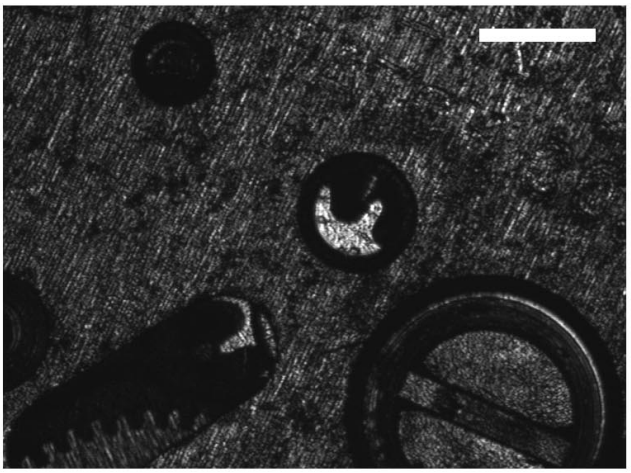


Fig. 3. Three autofocus images of the wrist watch mechanism at relative zoom settings of 1·25, 3 and 7. The scale bar depicts a length of 1 mm in the 1·25 zoom image.

of explanation but that Eqs. (3) and (4) also follow from a more detailed theory (Neil *et al.*, 1997).

Equations (3) and (4) are the basis of our approach. We introduce a one-dimensional grid pattern into the illumination path of our low-power imaging system so that a grid (fringe) pattern is imaged onto the object. We then obtain three images at three spatial positions of the grid and hence compute the sectioned and conventional images according to Eqs. (3) and (4).

Figure 1 shows a schematic of the system which was build around an Optem Zoom 70 magnifier which could be set to magnifications between 0·75 and 5·25. We merely introduced a 40-line mm^{-1} one-dimensional grid into the illumination path. A standard 15-W tungsten halogen lamp was used as the light source, together with a green filter (100 nm bandwidth). Images were recorded with a CCD camera and transferred to a Matrox Meteor frame grabber. The grid was moved in a simple sawtooth fashion synchronized to the CCD camera frame rate such that three successive camera images correspond to a spatial shift of one-third of a period of the projected image of the grid. Optically sectioned images were obtained from Eq. (3), together with a look-up table which mapped all possible combinations of I_1, I_2 and I_3 from our 8-bit camera to I_s.

In order to demonstrate the optical sectioning capacity of our macroscope we show in Fig. 2 two images taken at focal settings 1·25 mm apart of a portion of a wrist watch mechanism. The optical sectioning is clearly demonstrated. Finally on Fig.. 3 we show three autofocus images, obtained by displaying the maximum image intensity at each pixel throughout the entire image volume, at relative zoom settings of 1·25, 3 and 7.

In conclusion, we have described a very simple modification to a low-power imaging system which results in an optical sectioning capability which permits three-dimensional imaging of macroscopic structures to be performed in real time.

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