

High resolution microscopy with fast focus control

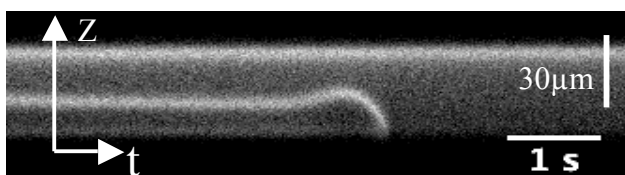
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A common requirement in high-resolution optical microscopy is to obtain a three-dimensional representation of the object under investigation. This is typically achieved by using an optical sectioning technique to obtain a clean in-focus image of a single plane within the sample and then repeating this process a number of times at different focal settings to produce a full three dimensional image stack of the object. In dynamic studies of biological processes, a series of such image stacks are often acquired in quick succession and a computer reconstruction used to observe biological movements in three dimensions. Clearly, the speed of the fastest biological process that can be observed in this way is limited by how quickly each constituent stack in the time series can be acquired.

Developments in scanning techniques now permit practical microscope systems to acquire a single in-focus image of the specimen very quickly and the process of refocusing usually provides the real bottleneck when trying to acquire three dimensional image stacks quickly. For fundamental optical reasons, the only satisfactory method for refocusing high NA microscope systems, up until now, has been to change the distance between the objective and specimen mechanically. This is generally a slow process as it involves moving either the specimen stage or objective lens, which are both relatively heavy. This method also suffers from additional disadvantages, such as specimen agitation, which make the imaging of delicate samples, such as live cell cultures, very difficult.

Previously, we have suggested an alternative focusing method that does not involve mechanical movements near the specimen^{1,2}. This enables refocusing to be carried out remotely without the introduction of systematic aberrations. In this paper, we will present a number of practical applications of this method to structured illumination microscopy as well as a two photon microscopy. This work is expected to have impact in fields as wide ranging as liquid crystal research to neuroscience. The image opposite displays z-t data in an application where high speed remote axial scanning (focusing) is necessary so as not to disturb the specimen environment.



1. E. Botcherby, R. Juškaitis, M. Booth and T. Wilson, "Aberration-free optical refocusing in high numerical aperture microscopy", *Opt. Lett.* **32**(14), pp. 2007-2009, (2007)
2. E. Botcherby, R. Juškaitis, M. Booth and T. Wilson, "An optical technique for remote focusing in microscopy" *Opt. Comm.* **281**, (2008)
3. E. Botcherby, M. Booth, R. Juškaitis and T. Wilson, "Real-time extended depth of field microscopy", *Opt. Express* **16**, 21843-21848, (2008)