

Aberration-free refocusing in high numerical aperture microscopy

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A common requirement in many kinds of microscopy is to obtain a series of high-resolution, through-focus images. An approach often adopted is to move the specimen axially so that different planes are brought into focus. A practical drawback to this approach is that mechanical movement is inevitably slow and, in some applications, may be impractical, for example when the scanning movements affect the specimen. An optical method of refocusing is clearly preferable as this permits imaging to be carried out without disturbing the specimen. Furthermore, such an approach could enable higher axial scan rates than were previously possible. In the simplest case, optical refocusing can be performed by repositioning the detector. However, when used in conjunction with high numerical aperture (NA) objectives, this method produces spherically aberrated images as can be seen in lower regions of Fig.1a.

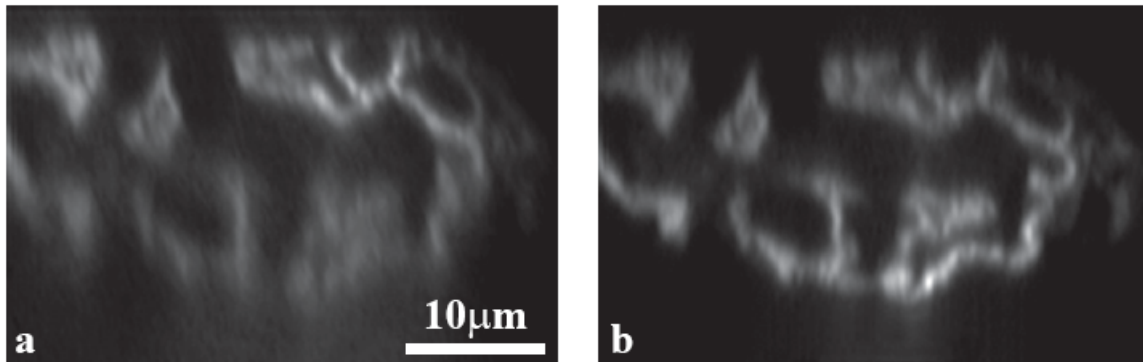


Fig.1 – XZ images of mouse kidney stained with Alexa Fluor 488. a) Refocusing is performed by moving the detector axially. b) Refocusing performed by our new system.

In this paper we present the theory and results of a new system, where the camera remains fixed and refocusing is performed by a different means using a second high NA lens and mirror. We will show that if this is designed correctly then perfect diffraction limited images of all planes in the specimen can be acquired by scanning the reference mirror as can be seen in Fig.1b. In addition to this, we will also present results from a number of different confocal systems that have been built to exploit this principle of operation.

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