

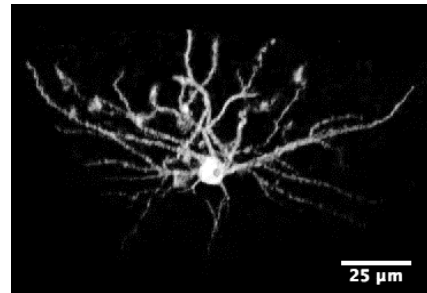
## Two-photon microscope with near-isotropic scan rate for functional imaging of neurons.

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There are a number of situations in two-photon microscopy where it is not necessary to acquire entire three-dimensional image stacks to study the functional dynamics of a biological process. For instance, in neuroscience, when imaging calcium signals as they propagate along the dendritic processes of a neuron cell, it is only necessary to scan the focal spot along the dendrites themselves as scanning the surrounding areas does not yield any useful information about the overall function of the cell. This reduces the number of points through which the focal spot must pass during each scan which should, in principle, lead to a vast improvement in scan rate and ultimately an improvement in the temporal resolution of any data acquired. In



practice, however, there is one problem with this approach. For fundamental optical reasons, commercial systems are only able to scan laterally in the focal plane at high speed (e.g. up to 4 kHz). In contrast, scanning the spot along the axis is much slower as this is usually done by physically moving the objective lens, a process that limits the scan speed in this direction to about 10Hz because of the weight of the lens. As can be seen from the image reconstruction of a neuron above, the dendritic processes do not generally lie in the focal plane, so to scan along any of these trajectories necessarily requires the system to be refocused axially, which in turn restricts us to a temporal resolution of 10Hz - a speed far too slow for measuring meaningful data about calcium propagation.

Previously, we have suggested an alternative focusing method that does not involve mechanical movements of the objective lens<sup>1,2</sup>. Instead, focusing is carried out remotely using a small mirror. We have built a two-photon microscope to demonstrate this approach using a mirror weighing 0.1g and measured axial frequency responses up to 3.5kHz, an improvement by a factor of 350. The rate at which the focal spot can be scanned around the specimen is therefore nearly isotropic, i.e. it is almost independent of the direction in which it is travelling. In this paper, a number of practical applications of this system will be demonstrated.

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)