Construction of a two-photon microscope with near-isotropic scan rates for imaging biological tissues.

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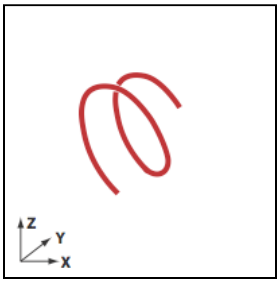
There are a number of situations in two-photon microscopy where it is not necessary to acquire a series of entire three-dimensional image stacks to study the functional dynamics of a biological process. Indeed, it might only be necessary to scan the focal spot over relatively few points in the specimen to gather all the information that is required. In principle, one might think that by only imaging the functional parts of a specimen this should lead to a considerable reduction in scan time and therefore a vast improvement in temporal resolution. However, in practice, such improvements are rarely realised. This is because commercial microscope systems usually possess an extremely non-isotropic behaviour when scanning the focal spot along different directions in the specimen. For instance, while scanning the focal spot laterally in the specimen frequency responses greater than 4kHz can be achieved, however, for any other scan involving axial movements, the frequency response becomes severely limited to about 10Hz because scanning along this direction requires the objective lens to be moved physically. This a process that is generally slow due to the excessive weight of the lens. Clearly, this situation is not ideal for all but a small number of situations where the dynamic processes are confined to a single plane and even in these cases, it is not always simple or even possible to orient the sample correctly so as to image this plane of interest. For all other situations, the non-isotropic scan behaviour leads to a considerable restriction on the temporal resoltion of any data recorded in time lapse studies.



Previously, we have suggested an alternative method for scanning the focal spot along the axis that does not involve mechanical movements of the objectitve lens[1,2]. Instead, scanning is carried out remotely using a small mirror. We have now built a two-photon microscope based on this approach using a mirror weighing 0.1g and measured axial frequency responses of around 3.5kHz, an improvement by a factor of 350. The near-isotropic scan response of the scanning in this system can be exploited in a number of ways, for instance, the focal spot can now be driven along non-linear trajectories in three dimensions such as a spiral shown in the figure. In this poster, we will demonstrate the practical application of this system, with its near-isotropic scan behaviour, and show some examples of its use.

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)