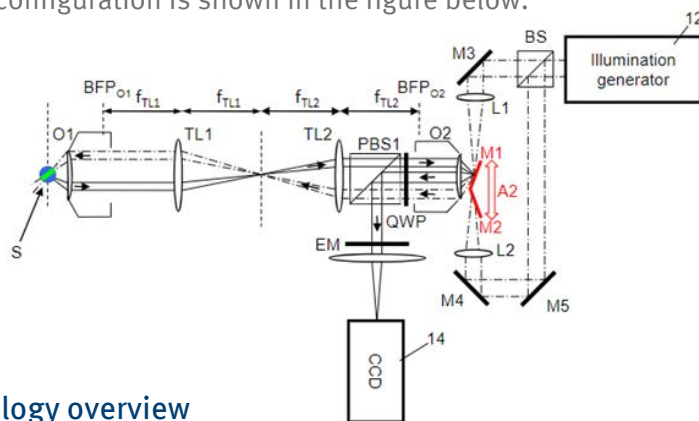


Dual-View Oblique Plane Microscopy

Proposed use

A novel arrangement of oblique plane microscopy is proposed in this invention that folds the illumination and detection arms about a mirror placed at focus of microscope objective 2 (O₂) so as to enable two separate orthogonal views of the specimen to be achieved by translating a pair of tilted mirrors in refocussing space. This approach enables the benefits of dual-view SPIM to be obtained when performing OPM. The dual-view OPM (dOPM) optical configuration is shown in the figure below.



Technology overview

While oblique plane microscopy has all the benefits of light sheet fluorescence microscopy (LSFM), there are a few problems to be addressed:

- Long optical train
- Anisotropic point spread function (PSF) – complicates image analysis
- Shadow/streak artefacts

The above proposed arrangement of oblique plane microscope effectively addresses these problems. This new OPM geometry can acquire two orthogonal views of the sample that can then be fused in post-processing to reduce sample-induced image artefacts. Furthermore, two-view deconvolution can be implemented to obtain a more isotropic PSF and to spatially resolve features not possible with a single view alone. The performance of the system has been demonstrated by imaging 50 spheroids up to 100 μm diameter at 15-minute intervals for 6 days. The fusion and deconvolution of the two views reduces inhomogeneities due to shadow artefacts and provides a more isotropic spatial resolution. Lastly, the use of a folded remote-refocussing setup is compact and only uses two high NA microscope objectives.

Benefits

- It has all the benefits of LSFM (i.e. low light dose to specimen to minimise photobleaching and phototoxicity; no dichroic needed; no out-of-focus background to reject; each optically sectioned image acquired directly so no moving parts and image processing are required).
- It allows two views of the sample to be obtained whilst requiring only two microscope objectives in the remote-refocussing setup, which reduces cost compared to OPM.
- Only one computer-controlled actuator is required to achieve both switching between views and for scanning during acquisition of each view to future reduce cost.
- The folded remote-refocussing geometry allows the numerical aperture of the third microscope objective in the remote-refocussing optics to have the same numerical aperture of the second microscope objective, which increases the spatial resolution achieved.

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