HOMOGRAPHICALLY GENERATED LIGHT-SHEETS FOR LARGE SPECIMENS MICROSCOPY

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We compare the performance of linear and nonlinear methods for synchronising the excitation and detection planes throughout large specimen volumes in digitally scanned light sheet microscopy. The simplest nonlinear method involves registering four corner extrema of the imaging volume using a projective transform. We show this improves on the light-collection efficiency of a 3-point affine registration by an average of 42% over a typical specimen volume, whereas increasingly high-order corrections provide more modest returns. The accuracy of illumination/detection registration methods are now very pertinent to biological research in view of current trends towards large sample imaging, at depth, with diffraction limited resolution.

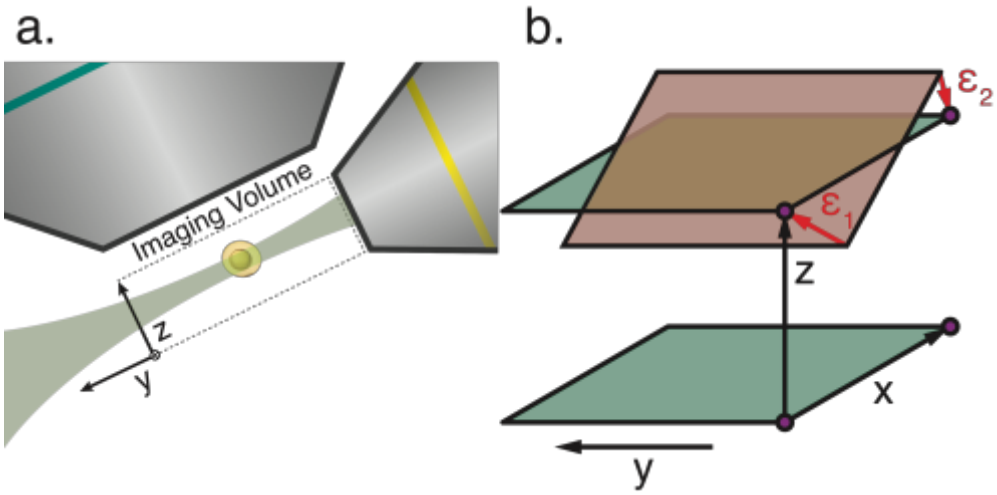

Figure 1 (a) Objective geometry and coordinate system used for light-sheet microscopy, where the light-sheet scans in the x axis. (b) Each green plane represents the desired imaging plane at an arbitrary separation. The red excitation is an exaggerated and skewed plane arising from an imperfect beam scanning system. ε_1, ε_2 are then the correction vectors needed to optimally match imaging and excitation volumes as per the registered purple coordinates taken from the desired imaging volume extrema.