Introduction

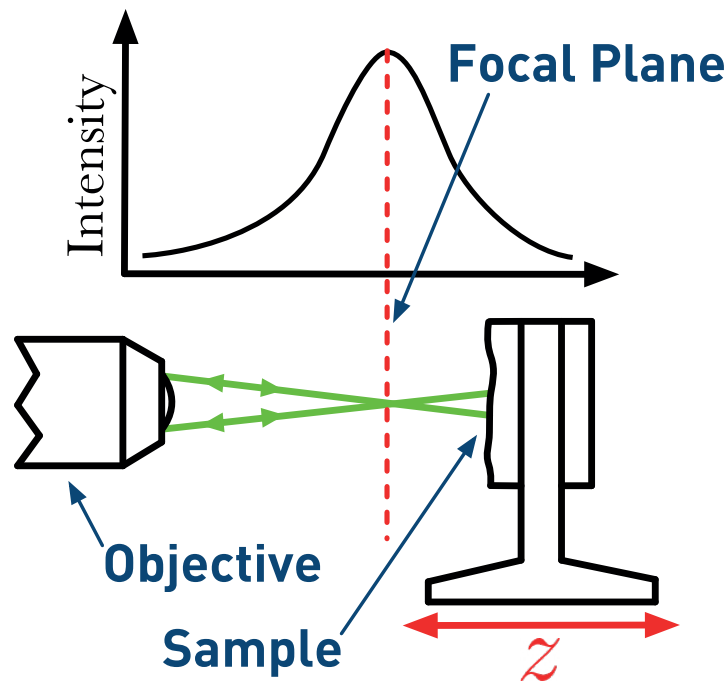
Confocal laser scanning microscopy (CLSM) is an optical imaging technique capable of reconstructing 3D surface maps and full 3D scans made up of several 2D scans at different depths. The initial inspiration for the project came from a YouTube video by “Breaking Taps” [1]. The aim of our project was to construct and investigate the performance of a simple confocal scanning laser microscope using standard laboratory optics and commonly available components.

ZEISS LSM 900
Confocal Micrsocope

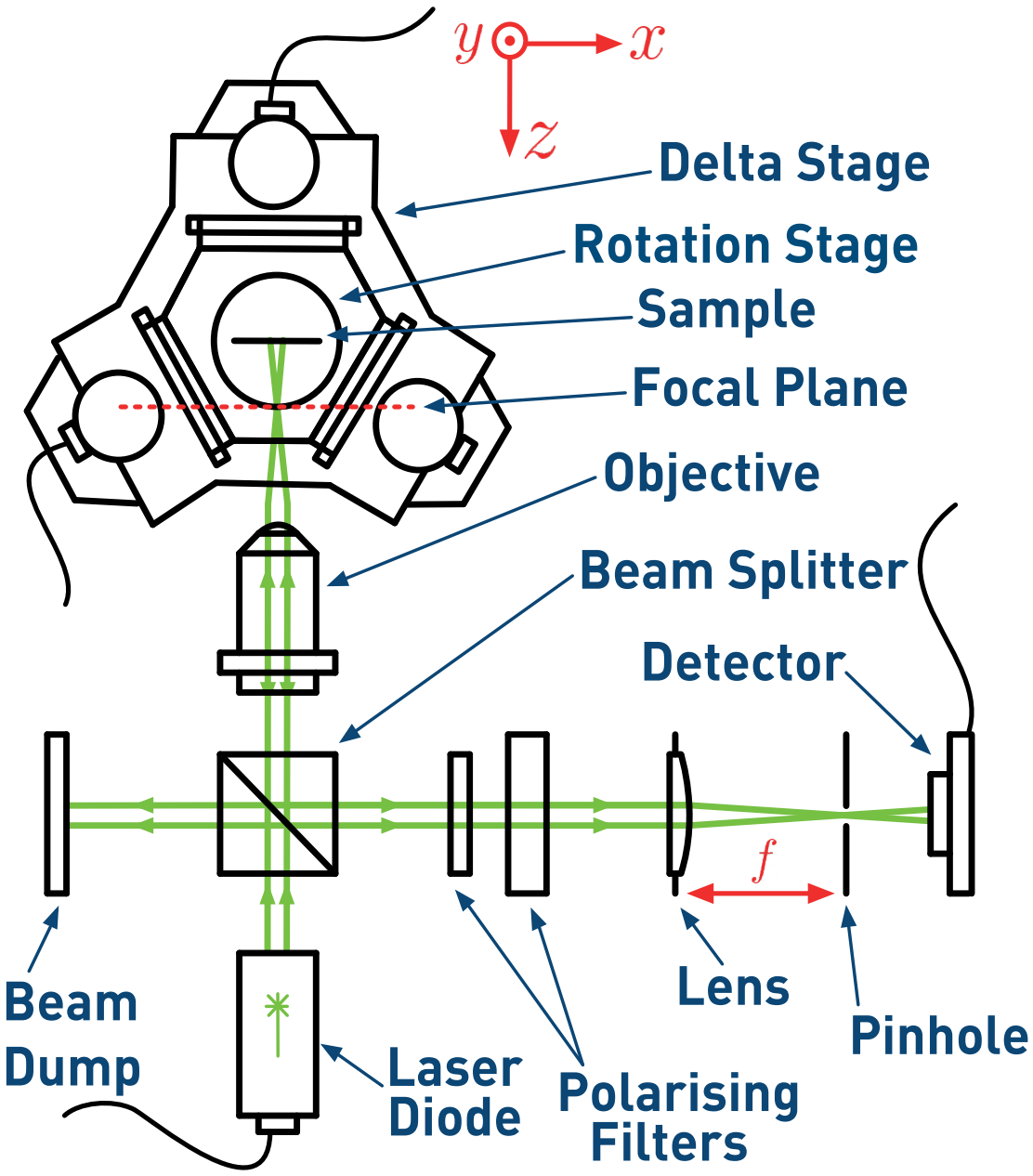
Principles

CLSM works by focusing a laser beam onto a point on the surface being scanned and measuring the intensity of the light reflected back. The reflected light is passed through a spatial pinhole filter such that only the light reflected off of structures in the focal plane is detected. The intensity is then measured while moving the sample in the z-axis, parallel to the laser beam. The relative height of the imaged point can be determined from the position of the peak on a plot of intensity against displacement in the z-direction. This process is then repeated for multiple points with different x, y coordinates to obtain a toplogical map of the scanned surface.

Z-Scans



Optical Setup



A 4.5mW green laser diode is used as a coherent monochromatic beam source. The setup uses a 50:50 beam splitter to simultaneously illuminate the sample and collect the reflected light. The choice of objective has effects on the resolution and range of the setup so multiple objective types have been tested. Best results have been obtained using a low power microscope objective or a simple lense with a short focal length. A pair of linear polarisation filters, one of which is in a rotation mount, are used to control the reflected beam intensity. The spatial filter is created by placing a pinhole at the fourier plane of a plano-convex lens. The reflected beam is then detected using a digital CMOS or CCD camera or a simple photodiode with a transimpedance amplifier.