**Deformable Mirror Light-Sheet Fluorescence Microscope**

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**Project layout**

The repository contains the code for Terry Wright’s PhD at Imperial College. An Alpao DM97-15 was used to refocus a light-sheet fluorescence microscope (LSFM) at video frame rates. The final setup was able to acquire 26.3 volumes/sec with an axial depth of 100 microns.

The scripts which control the deformable mirror (DM), Orca Flash camera, motorised stage and Shack Hartman Wavefront Sensor (SHWS) are in the folders LabVIEW, MATHEMATICA, MATLAB.

The folders ALPAO\_DM, ANDOR\_LUCA, NEWPORT\_ESP\_100, NEWPORT\_SMC\_100, ORCA\_FLASH, SHACK\_HARTMAN contains supporting code for the DMLSFM scripts.

1. Script to measure centre of optical axis on DM – acquires two images, one each side of focus and checks for lateral shift

DMLSFM\_ALIGN\_DM\_WITH\_OPTICAL\_AXIS.m

1. Scripts for flattening the DM – use Shack-Hartmann and DM

DMLSFM\_FLATTENING\_THE\_DM\_WITH\_SHACK\_HARTMAN.m

1. Scripts for flattening the DM – use Shack-Hartmann and DM

DMLSFM\_SCRIPT\_STAGE\_CONTROLLER.m

1. Optimisation of the DM and evaluation of the set of command generated. The DM will play a sequence of poses – at the beginning of the sequence the DM produces a trigger pulse. When the Flash takes an image it produces an output pulse which is high for the length of the exposure (1ms). This is fed into a NIDAQ 6363 which chops down the length of the pulse, which is then attached to the laser. The Matlab processes \_MASTER and \_SLAVE between then control the optimisation and the evaluation with a Boolean variable to decide which mode is used. The 2 Matlab processes communicate via a memory map. The DMLSFM\_SCRIPT\_STAGE\_CONTROLLER.m controls the stage based on a config.xml file. This way other applications can move and change the stage position by adjusting the config.xml file.

DMLSFM\_OPTIMISATION\_AND\_EVALUATION\_MASTER.m

DMLSFM\_OPTIMISATION\_AND\_EVALUATION\_SLAVE.m

DMLSFM\_PULSE\_REDUCER\_FOR\_OPTIMISATION.vi

1. Imaging script – for strobed images at a particular defocus while the DM is oscillating continuously (includes galvo for LSFM). Within each volumetric sweep 1 image is taken with the full FOV of the Orca Flash. The \_MASTER Matlab script controls the DM and the LabView script will provide the correct signals to the Galvo and the Orca Flash. The control of the DM is separate to the camera and the camera could be controlled with the Hamamatsu HCI application or micromanager to acquire the images.

The following Matlab and LabView scripts allow you to change the position of the strobed image at runtime – however the interpose time must be less than 5ms.

DMLSFM\_STROBE\_MASTER.m

DMLSFM\_STROBE.vi

The following Matlab and LabView scripts can be used for interpose time longer than 5 ms, however the position of the strobed image cannot be changed at runtime.

DMLSFM\_STROBE\_MASTER\_SIMPLE.m

DMLSFM\_STROBE\_SIMPLE.vi

1. The following imaging script allows a sequence of images to be taken during a sweep, subject to the bandwidth of the connection between the Orca Flash and the PC.

The following 3 processes must be run in parallel.

DMLSFM\_SCAN\_MASTER.m

DMLSFM\_SCAN\_SLAVE.m

DMLSFM\_SCAN.vi

1. Script to calibrate galvo voltage to actual axial position of illumination sheet

DMLSFM\_CONTROL\_GALVO.vi

**Mathematica Scripts etc**

1. Mathematica scripts for comparing low-NA defocus to high-NA defocus

highNA\_cf\_Prim\_defocus.nb