Accessible low-cost, long range, optical autofocus module for open-source multiwell plate and slide scanning microscopy

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Background incl. aims
We have previously presented novel optical autofocus modules that simultaneously provide extended range of operation (>100 µm) and high precision (<600 nm) using machine learning [1] in a 2-step approach or providing closed loop "single-shot" operation over up to ±37 µm with <50 nm accuracy [2]. These levels of performance are realised by focusing an infrared laser beam onto the microscope coverslip with the back reflection being imaged on a dedicated autofocus camera. We derive a metric that quantifies defocus from the size of the light distribution at the autofocus camera that can be independent of laser power and insensitive to drift in the optical alignment. The operating range and precision depend on the confocal parameter of the autofocus laser beam after being focused by the objective lens. This can be adjusted by changing the diameter of the autofocus laser beam incident at the objective lens. By contriving a different beam diameter in two orthogonal planes using either a rectangular aperture [1] in the collimated autofocus laser beam, or using different orthogonal cylindrical lenses [2] to collimate the autofocus laser beam emerging from the single mode fibre that delivers it to the autofocus module, we can maximise precision (with maximum beam diameter) and extend operating range (reducing orthogonal beam diameter), making both measurements simultaneously by resolving the autofocus camera image along orthogonal directions.

While these two approaches can provide months of stable operation, they each have their drawbacks. The machine learning approach with the rectangular apertured beam [1] requires a convolutional neural network to be trained to determine magnitude and sign of defocus from the autofocus camera image and we found it necessary to train it over ~10 days to make it independent of any system variations impacting the autofocus camera image. For the second approach [2], we slightly offset the collimation of the cylindrical lenses such that the measured defocus is different for the two planes defined by the orthogonal cylindrical lenses, and this enables the magnitude and sign of the defocus to be calculated from a single autofocus camera image following calibration of the system. However, while the system

reported in [1] utilised a low-cost single-mode fibre (SMF)-coupled laser diode, we used a superluminescent diode (SLD) in the system reported in [2] since its performance was impacted by interference between the autofocus laser beam reflected from the coverslip and unwanted beam(s) reflected from other surfaces in the optical system. Using the SLD removed this interference. Unfortunately, SLDs are significantly more expensive than laser diodes, and availability can be intermittent. Accordingly, we are redesigning the optical system and analysis method to enable the closed-loop approach of [2] to be used with a simple fibre-coupled diode laser for implementation in slide scanning and automated multiwell plate microscopy.

Methods

We determined that the primary source of unwanted back reflections of the autofocus laser beam were from the microscope objective lens, and we modified the optical system such that the curvatures of the unwanted back-reflected wavefronts are different from the desired autofocus beam reflected from the coverslip. Utilizing a modified background subtraction and signal processing algorithm we were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens that was controlled using MicroManager [3]. To independently measure the performance of the autofocus system, we configured the microscope for brightfield transillumination imaging of a USAF test chart and imaged the edge of a bar to derive a metric of defocus from the steepness of the gradient of this edge. We are also working on a fluorescent bead image-based approach utilising machine learning to determine defocus from a single bead image for real-time monitoring.

Results

We were able to achieve stable operation of this autofocus using a simple SMF-coupled diode laser implemented on an openFrame-based microscope [2] with a 100x oil immersion objective lens. When imaging a test chart in transillumination, focus was maintained in closed loop within 200 nm over 5000 seconds – and within < 50nm over 500 seconds. The autofocus system can recover focus with single-shot operation within a range of ~60 µm and up to ~80 µm in a multi-step mode [2]. We are working to improve the autofocus precision and range and are cross-validating the measurement of defocus between the autofocus readout, the transillumination edge measurement and the machine learning approach applied to bead images. We will also explore using higher power multimode laser diodes that exhibit shorter coherence lengths [4] although these present additional laser safety considerations.

Conclusions

We have demonstrated that we can implement an optical autofocus using a low-cost diode laser that can provide closed loop “single-shot” operation and is suitable for multiwell plate imaging and slide scanning. This is important for

the development of cost-effective instrumentation, including modular openFrame-based instruments for pathology and high content analysis. We will present the latest design together with methods to independently validate the correction of defocus.

Graphic:

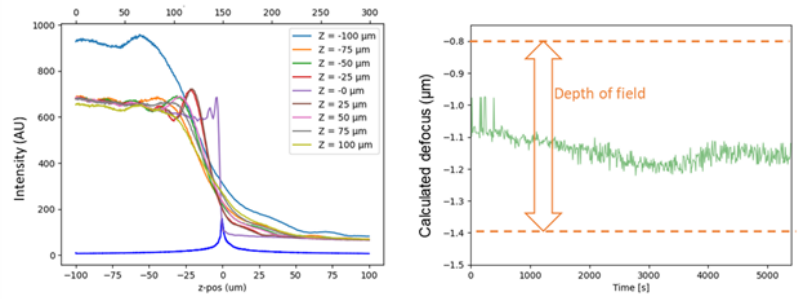

Figure caption: (a) line profiles though edge of a test chart bar in z-stack imaged in transillumination (b) defocus calculated from gradient of test chart bar edge measured as a function of time.

Keywords:

Optical microscopy, autofocus, slide-scanning, characterisation

Reference:

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