Development of a Cost-Effective and High-Precision Microscope Stage Automation System

Degree programme: Master of Science in Engineering
Specialisation: Mechatronics and Automation
Thesis advisor: Prof. Dr. Cédric Bessire

Performing a blood cell counts traditionally requires laboratory setups. A research group at BFH is developing a portable, low-cost device which allows blood counts within minutes. To simplify the required brightfield and fluorescence microscopy a mechatronic system to automate stage and optical focus is developed and evaluated.

Introduction

This thesis focuses on the evaluation and implementation of an accurate, speedy actuation system on three axes of motion, to allow rastering through imaging positions, focusing on cells and capturing multiple images at different focus levels. The rastering system aims for an accumulated positional error per line of less than 10 µm, while Z-axis actuation requires less than 1 µm error to ensure proper focusing. Despite high accuracy demands, the automation hardware including motors should remain as inexpensive as possible. The total image capturing process should take less than ten minutes.

Methods

Following a literature-based component selection, candidates were evaluated with respect to size, cost, motion range, and accuracy. Stepper motors combined with fiducial markers tracked by computer vision enabled precise automated calibration to later compensate for motion errors and ensure reproducible image rastering. A contact sensor was integrated to level the sample on a gimbal stage and prevent damage during Z-motions. Autofocus routines and Z-stacking were optimized to capture sharp images across varying depths. Algorithms were developed in Python for ease of development and implemented in C++ to ensure processing speed.

Results

Following the calibration process, the X- and Y-axes demonstrated a high degree of accuracy, exhibiting an average accumulated error of 4.0 ± 2.3 µm per line. One line of 44 images spans 9.9 mm, resulting in a proportional motion error of just 0.04%. These results of n = 585 lines are displayed in the boxplot of Figure 3. Within ten minutes, more than 1700 images can be captured at different positions. The autofocus algorithm could be optimized to meet the specification of finding the focal plane within 1 µm, as can be seen in Fig. 2. The implementation of all required functionalities was successfully executed within the predetermined stage automation hardware cost below 150.- CHF.

Outlook

With the automated rastering, it was possible to capture more than 80,000 red blood cells in focus within ten minutes. While the autofocusing algorithm currently is limited to red blood cells, it can be easily expanded for white blood cells in fluorescent microscopy, by modifying and optimizing the focus metric. Moreover, the automated system can be used for malaria parasite quantification in infected blood samples. The automated hardware allows rastering for any kind of sample, including biological and non-biological ones.

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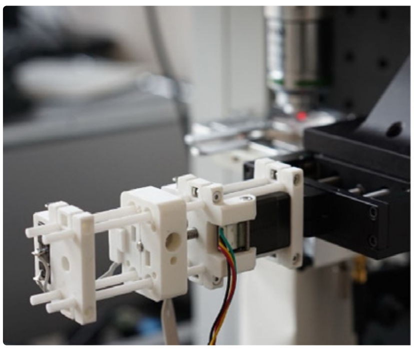

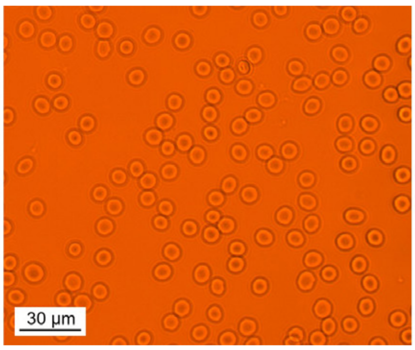

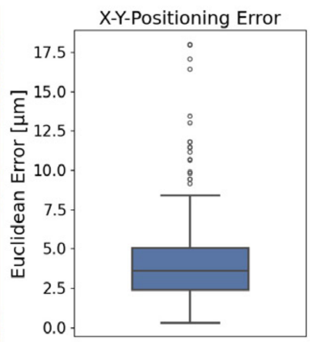

Fig.1: Axis automation setup with a stepper motor. Fig.2: Focused red blood cells captured during rastering. Fig.3: Boxplot of the accumulated motion error along X and Y. The median error is 3.6 µm.