Robotic Platform for Real-Time Tracking of a Single Fast Swimming Bacterium

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***Abstract*—In this paper we present a hardware architecture with software implementation able to track free swimming single 2µm in diameter MC-1 bacterium. The computer vision system operates at up to 77 fps at full speed and up to 24 fps when recording full 512×512 pixels frame from a coupled-charge device (CCD) array. Closed-loop control with lock-in tracking is achieved using the Otsu Segmentation Method (OSM) with a cubic spline model-based predictive algorithm. Using the system, speed distribution of MC-1 cells has been recorded showing a mean speed of 200µm/s. Tracking is demonstrated over a range of a few millimeters during 30 sec.**

Keywords-Cell tracking; Otsu Method; real-time tracking; computer vision; cubic spline prediction

I. INTRODUCTION

Cell tracking has become an important tool for understanding the individual behavior of moving cells because this direct measurement method allows intimate observation of the cell interrelation with its surroundings. New approaches in micro-robotic include the use of cells such as magnetotactic bacteria (MTB) to perform task at the micron-scale [1], or to use them for therapeutic applications [2] and for bio-chemical assay [3]. To push research further in this particular field, the MTBs used in previous studies are no longer thought in density or in colony but as a single individual component of a more complex system/environment. This paradigm shift leads to the need to study the microorganism individually and not just with statistical understanding. Our approach is to actively track the cell to obtain visual and behavioral information for relatively long time periods and distances.

We found that there are several human limits to adequately track fast swimming microorganisms under microscope magnification as in [4]. In our previous work, we showed the tracking of relatively slow moving bacterium by optical feedback [5]. Other previous works rely on software implementation to perform post-processing on a pre-recorded movie archive [6], [7], [8], which lacked the capacity of long distance analysis. Some other works achieved real-time image tracking but required the use of extremely expensive equipments [4].

Here, we present the tracking of a single MC-1 MTB that has a mean speed much higher than typical flagellated bacteria at ~200µm/s with a cell's diameter ranging from one to two microns. Our contribution in this field is to demonstrate the architecture needed to make the tracking of those fast swimming microorganisms using optical feedback and to present some preliminary data to validate the approach. We focus on the logical aspect of the problem and will show how a multi-cores computer architecture with appropriate software design can achieve real-time tracking of such fast moving microorganisms.

II. BACKGROUND

There are many challenges to automatically track fast moving MC-1 cells. We can separate them in two categories: the physical ones related to the intrinsic limitations of the system while the logical ones related to the computer technology performance and data handling. Both domains must be optimized to track fast micro-swimmers.

A. Limitations from the Physical Domain

Here we look for the fundamental limits in terms of the minimum size and the maximum speed of the tracked object. For non-fluorescent cells, the light source intensity and the natural diffusion of light by the cell bodies to the sensor give the diffraction limited resolution of the optical system. Increase in sensitivity and resolution can be obtained by the microscopy contrast technique used for optical contour enhancement like bright field, dark field, differential interference contrast (DIC), and phase contrast. In any case, resolution of 300nm ($\lambda/2$) can be considered as a lower limit. For fluorescent cells, resolution can be increased up to the limit of the image sensor sensitivity and noise characteristics. This means that object smaller than the optical diffraction limit can be observed. Beyond this limit, fluorescence would prove to be useful for the tracking of swimming organisms.

Practically, tracking of a single cell will be achieved with other bacteria in the vicinity. As such, the tracking algorithm must be able to operate efficiently within such an environment. Indeed, cell density could impact the tracking algorithm due to the inter-cell interference. In other words, the system needs to be sufficiently robust not to lose the tracked bacterium in the presence of neighboring bacteria.

B. *Limitations from the Logical Domain*

The overall feed-back loop delay for a single tracking, plays a role in the logical limit of the system and must be sufficiently low not to lose the bacterium. Software image manipulation also has to be kept to a minimum because of the high data content coming from the image sensor. A good approach is to first make an image conversion to produce lower data file and then to apply filtering for background noise reduction, contour enhancement and research algorithm.

To be able to perform manual validation and post tracking analysis, the system needs to record the high data content of the original image from the sensor in parallel with the tracking process. While optical system can split the light for two detectors, one for recording and one for tracking, the light intensity will also be split.

III. MATERIAL AND METHOD

A. *System Overview*

The setup is built around two direct-drive Aerotech ALS25030 XY stages and one ALS130-100 Z stage with counter balance system. The stages are driven by Ndrive HLe controller with the A3200 interface software running on a dual core PC operated with WindowsXP RT. These stages have an accuracy of ±1.0 µm with the HALAR option and a maximum acceleration of 30 m/s^2. A custom microscope optical system holds to a bridge on a granite table with four air damping posts. We used a 20× Mitsutoyo M Plan Apo NA 0.42 long distance microscope objective with a working distance of 20 mm and a depth of field of 1.6 microns with a Proximity Series Infinitube interface to connect the CCD. The light source was generated by a homemade array of ultra bright LED placed to produce razing angle beam of light on the MC-1 sample making a dark field contrast image. The main program ran in parallel on two cores of a National Instrument PXI-8108 dual core controller running on WindowsXP in a PXI-1042 eight slots multipurpose acquisition box. One core was used for the tracking algorithm while the other core was dedicated to the recording. The PXI-1042 was equipped with a PXI-1422 multi-camera interface card for the acquisition of the data captured with the CCD (Dalsa 478343). The CCD was an 8-bit grayscale array of 512×512 pixels.

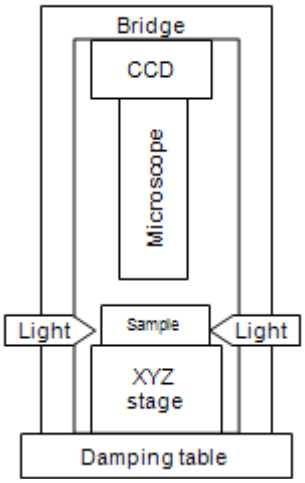

Figure 1. System overview

B. *Overview of the Software Architecture*

We built our software architecture with parallel processing in mind. The parallel processing is of outmost importance to minimize the delays and to process the huge quantity of data generated by the image sensor. We built two main applications called B-Follow and B-Stage. The communication between both applications was done by using two dual core PCs on a local area network. This could also be done on a multi-core system such as a quad-core computer.

NI Labview suite environment was used for the main application design because of its library of basic image manipulation (including brightness and contrast controls), the ease of connecting hardware modules (e.g. CCD) for data acquisition and to produce user interfaces. We also needed to include some C++ and C# subroutines and DLLs for the tracking algorithm. The system was built to easily add new innovative image segmentation methods.

The software information flow in the tracking algorithm is as follow:

- An image is first acquired by the image sensor;
- This image is transferred to the main application;
- The image is converted to a n-bits numerical array of the right size;
- Basic image processing of brightness, contrast, flip, four point rotation and Otsu Segmentation Method are performed;
- The numerical array is then reconverted to an image for display and user adjustment;
- The GUI waits for the user to select one bacterium using the computer's mouse;
- The tracking algorithm processes the image and sends commands to B-Stage in order to maintain the bacterium in the middle of the frame.

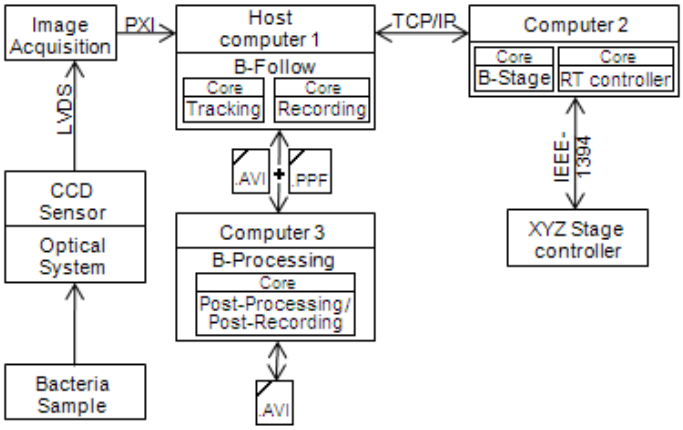

Figure 2. Data flow interconnections showing the multi computers architecture

C. *User Iinterface*

The user interface allows visual observation of the contrast enhanced image by brightness and contrast filters. Pixel to micron ratio can be configured for any optical magnification system with a simple calibration procedure using a non-moving object in the image and by measuring the pixel-micron ratio between two positions of the stage.

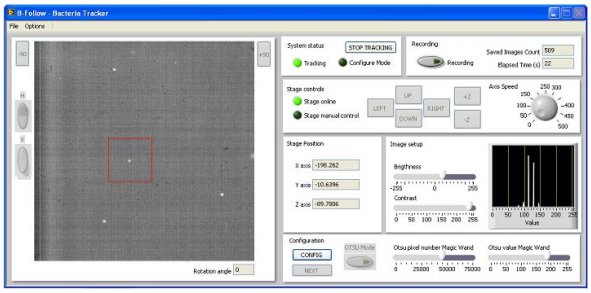

Figure 3. User interface

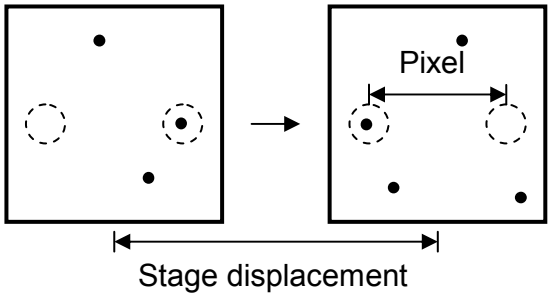

Figure 4. Micomiters to pixels ratio calibration algorithm

D. *Image Manipulation*

Brightness and contrast are the basic image manipulations that are required for the image segmentation to work. While the flip and 90º rotation are convenient to make stage displacement logical to the image, the small angle rotation is very convenient to fine tune the angle between the stages axis and the image sensor array.

We chose the Otsu segmentation algorithm to clean the image from any background noise. This method imported from computer vision techniques works well with images that have a histogram with two distinct regions and perform fine for our white dots on dark background. We developed an interactive user friendly Otsu Magic Wand to configure the sensitivity and the segmentation threshold value to visually adjust the image used by the tracking algorithm.

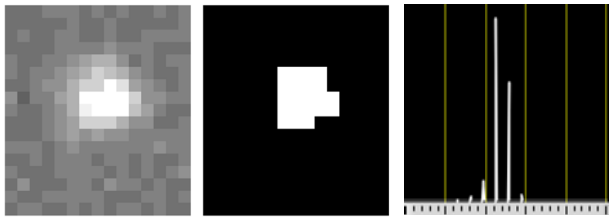

Figure 5. Otsu Segmentation Method applied to a bacterium. Left a bacterium capture with the CCD, center the same bacterium after Otsu segmentation, right the intensity histogram of the original image.

After the image segmentation, the tracking algorithm looks for pixels groups that are now binaries (0 and 1) and uses the position of the four last positions to predict the next position of the bacterium within the next frame using the spline cubic predictor:

$$E = B + AD + 3CD - 3BC, \quad (1)$$

where E is the estimated position and A, B, C, D are the four last measured positions. AD, CD, and BC are the corresponding relative vectors.

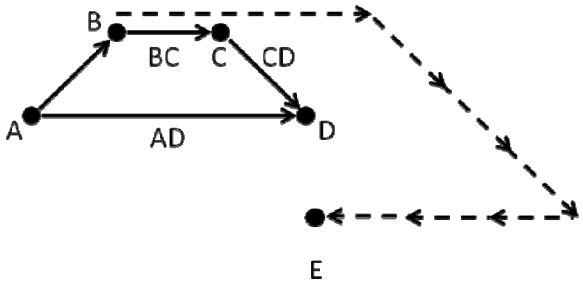

Figure 6. Vectorial representation of the estimator

We used this predictor to be able to follow the instantaneous motion of the swimming MC-1 MTB. Locally, microscopic speed can be very large while the overall long range motion at macroscopic speed will be much lower. This prediction algorithm also helps in the case that two or more bacteria cross or swim in near vicinity. However, collisions and crossing are troublesome for the software so we included a real-time recording function to be able to visually post experimentally confirm that the right bacterium was followed. The rest of the processing like extracting the position value, speed and trajectories are done with another application called B-Processing.

IV. RESULTS

The system was capable of tracking a single bacterium for more than 30 seconds over more than a thousand image frames

along a distance of a few millimeters. The experiments used a low concentration of bacteria sample on a microscope glass slide with cover slip to obtain roughly a ten micron thickness of aqueous medium. Since tracking in 3D is not presently supported, this setup prevented the bacteria to get out of the focusing range of the microscope depth of field.

The software was able to follow any bacterium with a swimming velocity up to 200µm/s even when up to three other bacteria were in its vicinity. The maximum tracking frequency we achieved was 24 frames per second (fps) while recording the CCD raw data of the full frame on the local hard drive. Non-recording experiments can be achieved at speed up to 77 fps. This limitation comes from the maximum frame rate of the CCD and not from the processing power of the computer.

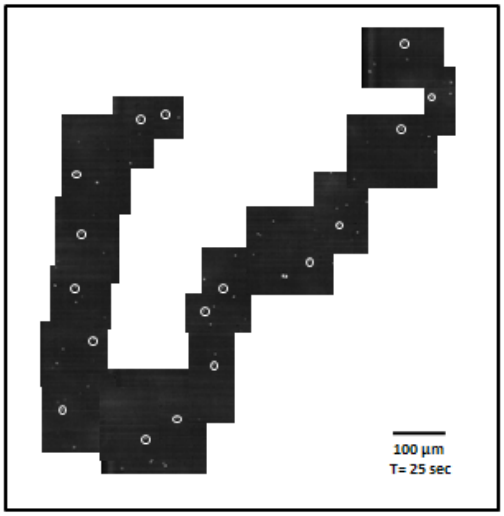

Figure 7. Mosaic image of the tracking of a bacterium on few millimeters distance without magnetic control showing the random motion of the MC-1

When we applied a directional magnetic field from 3 to 50 gauss, the MC-1 MTB changes its motion from random to a general straight line as depicted in Fig. 7. We found that the MC-1 bacteria under magnetic control keep almost the same local instantaneous speed distribution while its overall macroscopic speed is non-zero. Average macroscopic speed was found to be 72µm/s while real average speed was 210µm/s leading to a directional motion control efficiency during this particular experiment of approximately 35%.

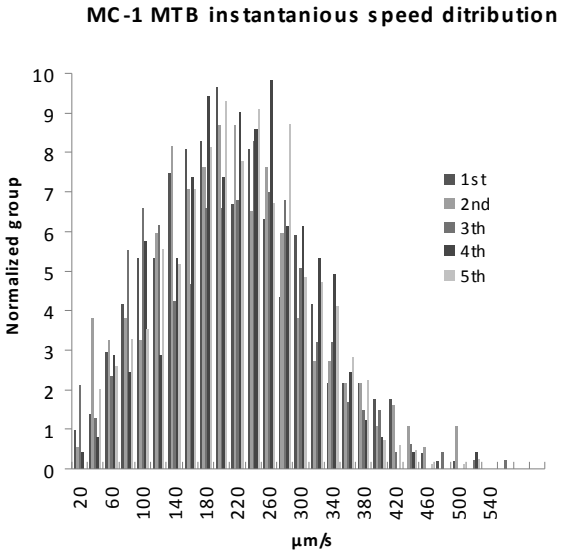

Figure 8. Normalized speed distribution for five different MC-1 under magnetic control.

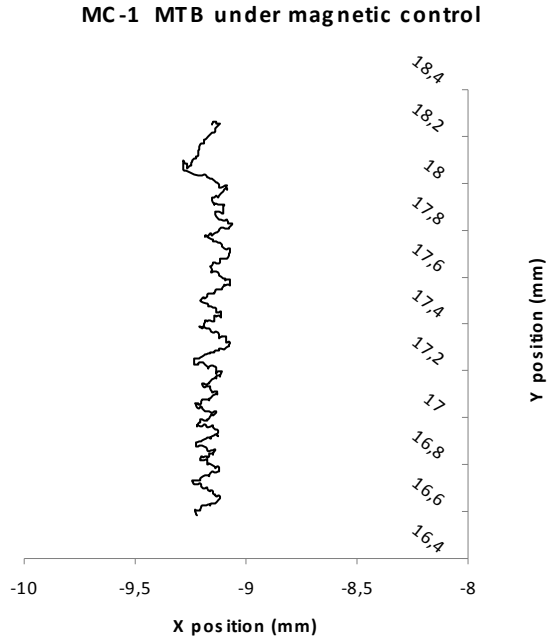

Figure 9. Position data of a tracked MC-1 bacterium under magnetic control showing long range magnetic response.

Easy data extraction can be made through B-Processing or any spreadsheet software. With the absolute stage position, the relative MC-1 MTB position in the image and the time stamp we can obtain individual speed distribution as shown in Fig. 8. The graph roughly shows a Gaussian speed distribution with a mean speed of 210µm/s and a maximum instantaneous speed of more than 500 microns per second.

V. CONCLUSION

We demonstrated the feasibility of fast micro-swimmer automatic tracking for two dimensional samples in noisy environment. Our system is limited on one side by the relative to background cell brightness and on the other side by the frame rate of the imaging sensor and it sensitivity. The data processing is not a limit but this could change for image size that is significantly larger than the ones used here and for significantly higher frame rates. Furthermore, the system would benefit of a Z-axis tracking capability. We found that sometimes, the stage acceleration makes a blur in the image and prevents the tracking algorithm from identifying the bacterium. This situation can be corrected by slowing down the stage acceleration.

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