ePetri PLATFORM FOR CONTINUOUS ON-CHIP MONITORING OF MICROORGANISMS

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ABSTRACT

We present ePetri, a self-imaging culture platform for continuous and long-term monitoring of microorganisms. The ePetri achieves on-chip microscopy by using complimentary metal-oxide semiconductor (CMOS) image sensors as a culture substrate. For motile microorganisms, we can perform pixel super-resolution image reconstruction by using the inherent motion of the cells, allowing high-resolution imaging without any optical element. We also demonstrate longitudinal imaging of *Euglena gracilis* cultured in ePetri with various image-based analysis for culture monitoring. As a miniaturized and automated culture monitoring platform, this ePetri technology can greatly improve studies and experiments.

KEYWORDS

ePetri dish, chip-scale microscopy, longitudinal imaging, pixel super-resolution reconstruction

INTRODUCTION

Imaging based long term monitoring of a biological culture provides useful information of the culture by visualizing the morphological and behavioral changes of each individual cell. Miniaturization and automation of an imaging system can greatly benefit many scientific researches and clinical applications by reducing the cost and streamlining the culture experiments. Recently, we have reported a self-imaging Petri dish - the ePetri [1] - for continuous monitoring of live specimen in a large field of view over a long period of time. CMOS image sensors were used as the substrate for cell culture, which allows for taking direct shadow images of the cells as they grow. In order to boost the optical resolution beyond the pixel size of the sensor, we raster scanned a light source to create sub-pixel-shifted low-resolution shadow images, which are later combined into a single high-resolution image with the pixel super-resolution image reconstruction.

In continuation of this work, we demonstrate an imaging method on ePetri for motile microorganisms, such as flagellated protozoa. The inherent motion of these microorganisms can be used as a means to obtain the sub-pixel shifts that are required for pixel super-resolution reconstruction without scanning of the light source. We name this technique, sub-pixel motion microscopy (SPMM) [2]. Using SPMM, We first demonstrate high resolution imaging by taking advantage of the swimming behavior of motile protozoa Euglena gracilis. Then, we conduct longitudinal study of Euglena gracilis, demonstrating microorganism counting, tracking and statistical analysis capabilities using the described ePetri system. Through the proof-of-concept experiments, we show that the ePetri platform using SPPM method allows for inexpensive, miniaturized and minimum-labour culture monitors, that can benefit clinical and scientific researches.

RESULTS AND DISCUSSION

The SPMM ePetri dish platform takes a very simple geometry; a bare CMOS image sensor and a PDMS well mounted on top (Fig 1). Each ePetri chip containing the sample is loaded into a camera board and the shadow images of the cells are continuously captured through the sensor. We used CMOS image sensors with the pixel size of 2.2 μm, which has the total imaging area of 6.3 mm × 4.8 mm. The optical resolution of the ePetri is the highest at the floor of the image sensor. In order to keep the microorganisms as close to the sensor surface as possible, we dispensed a small amount (< 5 μL) of culture medium containing the specimen into an ePetri chip, and a drop of oil was put on top of the culture medium on order to prevent drying during the experiment.

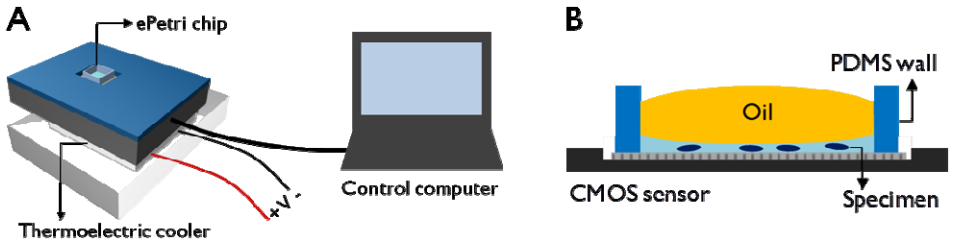

Figure 1. (a) the ePetri dish imaging platform is composed of an ePetri chip, camera module and a control computer. A Peltier module and a heat sink are used to maintain the temperature. (b) Schematic diagram of a single ePetri dish chip. Sample is dispensed into a chip and a drop of oil is used to prevent evaporation of the medium.

SPMM ePetri system acquires low-resolution shadow images as the microorganisms swim across the pixel array of the CMOS image sensor and combine these sub-pixel shifted images into a single high resolution image. The resolution of the raw images is fundamentally limited to twice the pixel size by the Nyquist criterion (the pixel size is 2.2 μm in our experiment.). We can boost the optical resolution beyond this limit by using pixel super resolution reconstruction, where low resolution images of the same object with subpixel shifts are combined to a higher

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resolution image. The raw images captured through the sensor can be considered as undersampled images of the original object with a known subpixel shift between each frame. Upon reconstruction, the low resolution images are enhanced by n times, meaning that the physical size that a single pixel represents decreases by 1/n times in the reconstructed high resolution image. This subpixel shift ((d_{kx},d_{ky}), for kth image in the sequence) determines the vector in which n^2 different low resolution shadows are redistributed within the denser pixel grid of a single high resolution image. To obtain the precise sub-pixel shift for each microorganism, we trace the motion of all microorganisms throughout the entire sequence and perform image reconstruction separately for each moving cell.

We used Euglena gracilis for demonstration of SPMM imaging technique. Euglena gracilis is an ellipsoidal protozoan with the dimensions of approximately 60 × 10 × 10 µm and moves in a rototranslatory motion with the speed of about 100-400 µm/s. The motion vector for each Euglena gracilis cells were calculated using a simple image processing technique. With this method, we have imaged motile Euglena cells with 950 nm optical resolution, which is comparable with the resolution of a 0.30 NA 10x objective lens (0.92 µm resolution), and the field-of-view of over 6 mm × 4 mm area (Fig. 2).

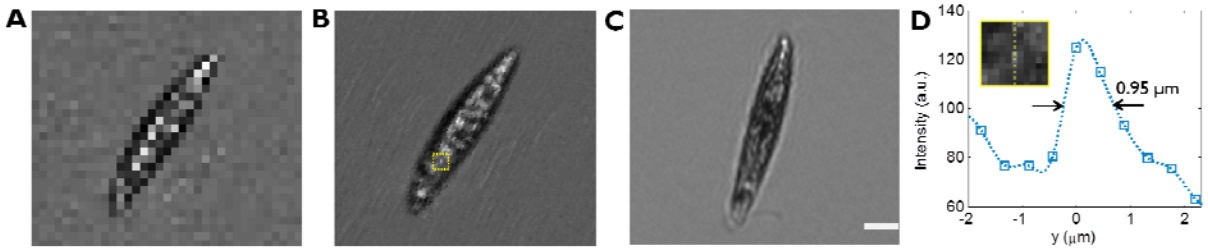

Figure 2. (a) Raw and (b) reconstructed images of *Euglena gracilis* using SPMM. (c) A conventional 10x microscope image. (Scale bar 10 µm) (d) Line trace of a small feature in the area highlighted in (b) shows FWHM of 0.95 µm

Motion artifacts such as rotational blur can be computationally removed by rotation compensated pixel super-resolution reconstruction. Each low resolution frame is re-rotated by the angle $-\Theta_k$ during the reconstruction, where the rotation angle is measured by the orientation or the trajectory of each cell (Fig. 3). The rotation compensation process can be described as below;

$$i_{kr}(l_x, l_y) = i_k(l_x \cos\theta_k - l_y \sin\theta_k + nd_{kx},\ l_x \sin\theta_k + l_y \cos\theta_k + nd_{ky})$$

where i_k represents k[th] low resolution image up-sampled by the factor of n and i_{kr} represents k[th] low resolution image up-sampled by n, shifted and rotated for reconstruction. Figure 3-a3 and 3-b3 shows the reconstructed high resolution image using the rotation compensation with the same set of low resolution sequence for fig.3-a2 and 3-b2. Note that the stripe-patterned artifacts are removed.

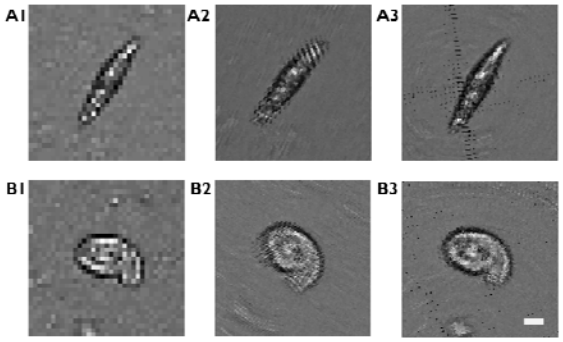

Figure 3. Cells with large rotation showing artifacts in the image (A2, B2). Reconstructed images with rotation compensation (A3, B3). (Scale bar 10 µm)

To demonstrate longitudinal imaging and motion analysis capabilities, we cultured *Euglena gracilis* protist in our ePetri system. Long term culture monitor and image based motion analysis with ePetri system can benefit many biological assays and scientific studies using various microorganisms. For the imaging and anlaysis, we used two image acquisition modes; we monitored the whole field-of-view at low frame rate for statistical information of the culture, such as cell counting, motion and size parameters (Fig. 4A) . Cell counting results show exponential increase in the cell population, indicating that the ePetri provides adequate culture environment for *Euglena gracilis*. analysis for cell counting was performed by first identifying each cell via thresholding the raw gray scale images (euglena cells or the boundary of euglena cells appear darker than the background), converting into binary image and then counting the number of areas within a predefined size range to filter out debris and noises. From the counting results, the growth of the cells showed clear increase with the doubling time of approximately 5 hours. To assure the accuracy of our cell counting software, our results were compared with manual counting results of 2 observers. The results showed the average percent difference of 3.0 % between the manual and the machine count, where percent difference between the observers is 3.1 %.

Next we performed differential experiment with two sets of euglena cultures. We used spring water as medium for culture A and the euglena culture medium for culture B. Two cultures were kept in ePetri for > 5 hrs under same temperature and illumination conditions. We used our analysis software to obtain the distribution of the motility and

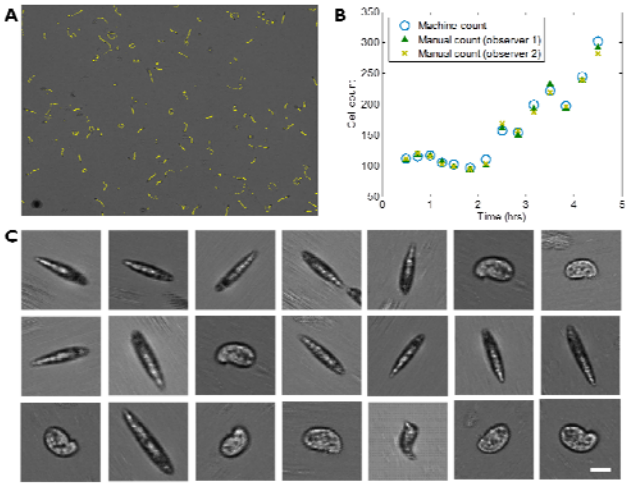

Figure 4. Longitudinal imaging of Euglena gracilis on ePetri dish. (a) Large FOV images of Euglena gracilis. (b) Growth in cell population counted with our cell counting software. (c) High resolution images of the cells (Scale bar 20 μm)

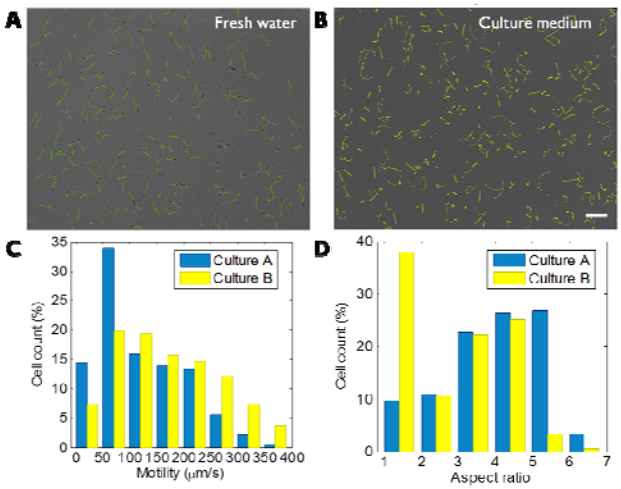

Figure 5. Motion tracking and shape analysis using ePetri. Euglenas cultured in spring water (a) and in Euglena medium(b) (Scale bar 200 μm) showing different motility(c) and shape(d) distribution.

the cell shapes. The result shows that the cells cultured in fresh water have more population of elongated cell types with high aspect ratio and lower motility, whereas larger population of Euglena gracilis cultured in the medium showed faster motion and rounded shapes.

CONCLUSION

We demonstrated the imaging of motile microorganisms in an ePetri platform by using the inherent motion of the cells for the pixel superresolution reconstruction. This work shows the versatility of our SPMM ePetri system in the choice of the sample and the type of information collectible through our system. The key advantage of our SPMM ePetri is that it lowers the labor and the cost of culture experiments by miniaturizing and automating the monitoring system. It can also improve the quality of experiments a by improving the temporal resolution of the monitoring and allowing for streamlined and parallel process.

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