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研究課題名（和文）Simultaneous high-frame-rate recognition of cells fast-flowing in microchannels toward ultra-fast cell sorting

研究課題名（英文）Simultaneous high-frame-rate recognition of cells fast-flowing in microchannels toward ultra-fast cell sorting

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研究成果の概要（和文）：本研究でフレームストラドリング高速ビジョンプラットフォーム上へ複数対象の特徴量抽出アルゴリズムを実装し超高速セルソーティングに向けたマイクロ流路内を高速異動する細胞の高フレームレート計測を実現した．画像処理ベースの細胞特徴量はハードウェアロジックを用いて実時間抽出が可能である．細胞の認識と分類も実時間で行われる．マイクロ流路内を高速移動する細胞の高分解能なROI領域は1000fpsで保存、解析が可能である．また超高速な解析，検査，ソーティングがバイオアッセイ効率を上げた．Lab-on-a-chip従事者は赤血球やiPS細胞等の様にマイクロ流内で高速移動する細胞のソーティングの応用が容易である．

研究成果の概要（英文）：In this research project, we have achieved high-frame-rate recognition of cells fast-flowing in microchannels toward ultra-fast cell sorting by implementing a parallel multi-object feature extraction algorithm on a frame-straddling high-speed vision platform. Image-based cell features can be extracted in real-time by using hardware circuits. Recognition and classification of cells can be performed in real-time. High resolution ROI regions of fast-flowing cells in microchannels could be stored and analyzed at 1000 fps or higher. And ultra-fast analysis, inspection, and cell sorting could be achieved to increase production efficiency in bio-assembling. Lab-on-a-chip practitioners can easily apply it to real-time cell sorting of cells fast-flowing in microchannels, such as red blood or iPS cells.

研究分野：Engineering

キーワード：High-speed vision　Cell sorting　Hardware implementation

１．研究開始当初の背景

Lab-on-a-chip (LOC) is a high-throughput device that integrates laboratory functions on a single chip of a few square centimeters or less in size. Several non-vision cell analysis systems have been developed to extract the shape and motion of cells in LOC; however, the detectable geometrical properties of cells have been limited by several technical factors, such as poor spatial resolution and difficulty conducting quantitative measurements at such small scales. Vision-based cell analysis systems have been proposed for shape and motion analysis of cells in microchannel flow such as red blood cells (RBC) and cancer cells, and offline high-speed cameras have recently been used for vision-based cell analysis in microchannel flows. However, offline processing cannot satisfy the rapid growth of demand on ultra-fast cell sorting.

２．研究の目的

To overcome this restriction, we want develop the next generation real-time recognition system for cells fast-flowing in microchannels by utilizing our experience on real-time high-frame-rate vision-based sensing technology. If the shapes of cells fast-flowing in microchannels could be simultaneously detected for long-term observation on high-speed vision platform, the performance of vision-based cell analysis could be remarkably improved for LOC-based automated mass sorting of cells in proportion to the speed of microchannel flow.

３．研究の方法

In vision-based cell analysis, the speeds of cells flowing in microchannels can be measured by calculating their displacements between frames in corresponding processes for the cell regions extracted in images. High-speed vision systems can reduce the image displacement between frames, however, there still remains too large an image displacement to calculate the speeds of cells fast-flowing in microchannels because the fast-flowing cells can be observed only in a single frame. Moreover, the image displacement between frames increases as the microscopic view is magnified using a higher power object lens or a smaller pixel pitch image sensor. There is a trade-off between computational cost and the measurable range of microchannel flow speed when a vision system operates at a higher frame rate, and it is extremely difficult to improve the measurable range of speed in high-frame-rate video analysis by only increasing the frame rate of the vision system. To overcome this restriction, in this research, we introduce a frame-straddling function for high speed vision system, which can synchronize two camera inputs with the same view with only a tiny time delay on the sub-microsecond timescale, as shown in figure on the right. By setting the frame-straddling time in a certain range to avoid large image displacements between the two camera inputs, our frame-straddling high-speed vision platform can perform simultaneous shape and motion analysis of cells in fast microchannel flows at several meters per second. High-frame-rate real-time video processing can be performed in hardware logic by extracting the moment and shape features of multiple cells for the two camera inputs. Dynamic behavior of the cells in fast-flowing in microchannels can be observed with high spatial resolution and quantified based on cell features extracted by hardware circuits, such as size, transparency, and inner texture of cells. Fig. 1 shows the flow concept of frame-straddling multi-object feature extraction. And, Fig. 2 shows the system configuration of our system.

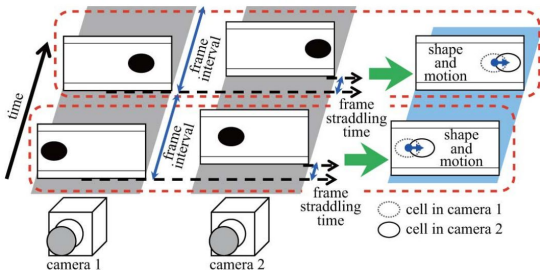

Figure 1. Concept of frame-straddling camera

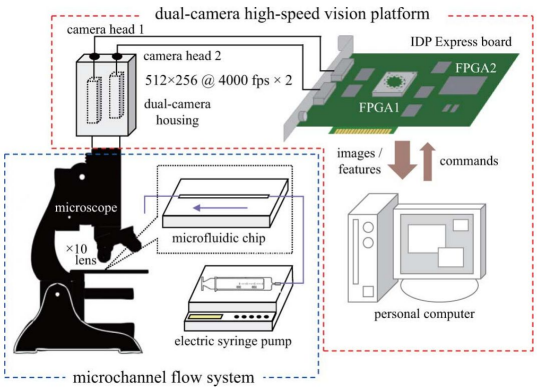

Figure 2. System configuration

The final target of this research is to accelerate the speed of vision-based cell sorting to 1000 fps level by utilizing real-time high-speed vision based sensing technique.

4．研究成果

The biggest contribution of this research is to provide us an ultra-fast recognition platform for cells fast-flowing in microchannels, which will accelerate the development speed of bio assembling. We realized vision-based simultaneous high-frame-rate recognition system for cells fast-flowing in microchannels toward ultra-fast cell sorting. We designed multi-object extraction hardware circuits for two camera heads to accelerate the feature extraction speed of cells. The schematic data flow and timing chart of our implemented circuits are shown in Fig. 3 and 4, respectively.

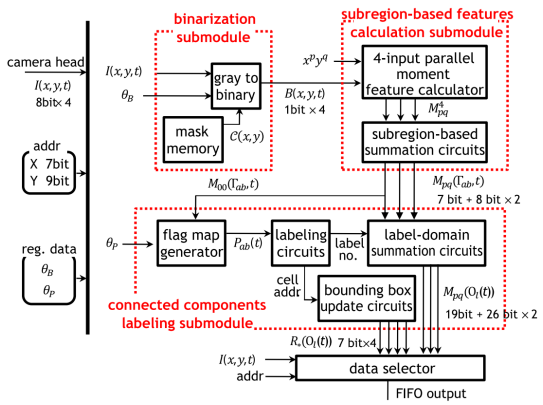

Figure 3. Schematic data flow.

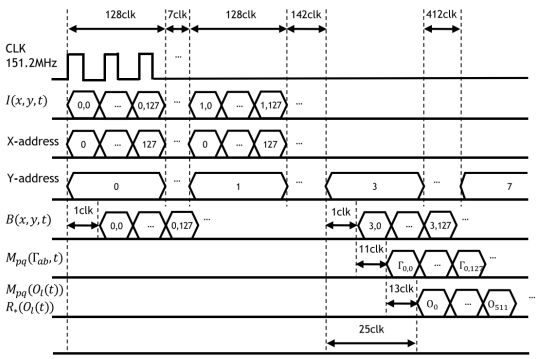

Figure 4. Timing chart.

We also used frame-straddling concept to improve the measurable range of flow speed to 1 m/s or more. We used sea urchin eggs to evaluate the performance of our developed system, as shown in Fig. 5. In our developed system, image based features of cells can be extracted in real-time by hardware circuits; recognition and classification of cells can be performed in real-time; high resolution ROI region of cells fast-flowing in microchannels can be stored and analyzed at 1000 fps or more; ultra-fast analysis, inspection, and cell sorting can be realized to increase production efficiency of bio assembling.

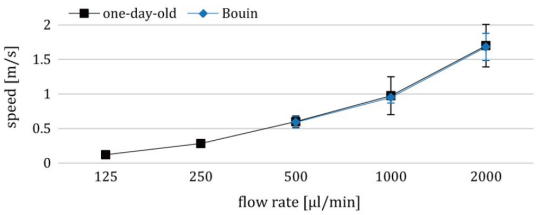

Figure 5. Speeds of sea urchin egg cells when observing them at different flow rates.

Fig. 6 shows snapshots of sea urchin embryos at different times after fertilization. All of the snapshots were captured in a 500 μl/min microchannel flow from left to right. Different stages of sea urchin embryo development are shown: signal cell, first division, second division, …, blastula, gastrula, pluteus.

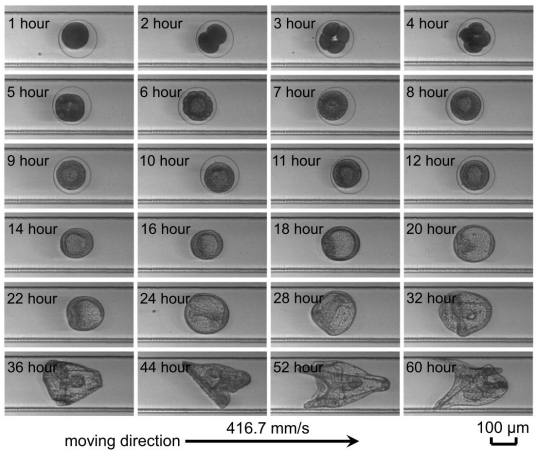

Figure 6. Snapshots of sea urchin embryos

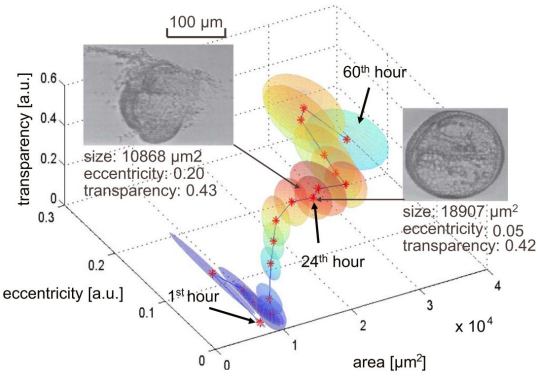

Figure 7. 3D development path of sea urchin embryo from 1 to 60 h after fertilization.

Fig. 7 shows the 3D development path of sea urchin embryos at 1–60 (start from the blue region) h after fertilization with regard to size, eccentricity, and transparency feature space, in which spheres indicate standard deviations in the 3D feature space. Two cell examples are shown in Fig. 7 to illustrate the experimental results in the 24th hour after fertilization of the sea urchin embryo. The upper left panel shows a damaged cell in the microchannel, and the lower right panel shows a normal cell in the 24th hour after fertilization. By using this type of multi-parametric view, biologists can easily explore the dynamic properties of cells.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　2　件）

① Qingyi Gu, Tadayoshi Aoyama, Takeshi Takaki, Idaku Ishii, Simultaneous vision-based shape and motion analysis of cells fast-flowing in a microchannel, **査読有**, IEEE Transactions on Automation Science and Engineering, 12(1):204-215.

② Qingyi Gu, Tomohiro Kawahara, Tadayoshi Aoyama, Takeshi Takaki, Idaku Ishii, Ayumi Takemoto, Naoaki Sakamoto, LOC-Based high-throughput cell morphology analysis system, **査読有**, IEEE Transactions on Automation Science and Engineering, 2015, 12(4):1346-1356.

〔学会発表〕（計　2　件）

① Qingyi Gu, Tadayoshi Aoyama, Takeshi Takaki, Idaku Ishii, Rapid vision-based shape and motion analysis system for fast-flowing cells in a microchannel, (ICRA 2014) IEEE International Conference on Robotics and Automation, 5848-5853, June 4, 2014, Hong Kong China.

② Qingyi Gu, Tadayoshi Aoyama, Takeshi Takaki, Idaku Ishii, Ayumi Takemoto, Naoaki Sakamoto, Real-time LOC-based morphological cell analysis system using high-speed vision, (IROS 2014) IEEE/RSJ International Conference on Intelligent Robots and Systems, 822-827, September 15, 2014, Chicago U.S.A.

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