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세포 이미징 기능을 겸비한 생체 유세포 분석기

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In vivo Imaging Flow Cytometer

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유(流)세포분석기(flow cytometer)는 일정한 체적 내에 존재하는 세포의 종류 및 개체 수 등을 계측하는 장비로써 생체에서 추출한 유액상태(혈액 또는 림프액)의 세포를 모세관(micro-channel)을 통과시킬 때 발생하는 산란 및 형광 빛을 이용하여 계측한다. 유세포 분석기는 신약의 투여 후 세포수의 증감, 암세포의 전이 및 세포주기의 분석 등을 연구하는 데 사용되며 현재 Becton-Dickinson's 등에서 상용화된 제품을 생산 판매하고 있으며, 계측을 위해서는 생체에서 세포를 추출해야 한다는 단점을 가지고 있다. Harvard 의과대학에서 최근에 개발한 생체 유세포분석기(In vivo Flow Cytometer)는 생체에서 세포를 추출하지 않고 세포의 수를 계측할 수 있다[1]. 레이저가 혈관의 특정한 부위에 조사되고 있고, 이곳을 세포가 통과하면서 발생하는 형광을 계측함으로써 주어진 시간 동안 특정세포군이 얼마나 지나가는 지를 계측할 수 있는 장비이다. 본 특별기사에서는 혈류 가시화 분야의 독자를 위해 최근에 "Optics Express"에 "In vivo imaging flow cytometer"라는 제목으로 최근에 개제된 논문의 내용을 요약하여 소개한다[2].

Abstract. We introduce an *in vivo* imaging flow cytometer, which provides fluorescence images simultaneously with quantitative information on the cell population of interest in a live animal. As fluorescent cells pass through the slit of light focused across a blood vessel, the excited fluorescence is confocally detected. This cell signal triggers a strobe beam and a high sensitivity CCD camera that captures a snapshot image of the cell as it moves down-stream from the slit. We demonstrate that the majority of signal peaks detected in the *in vivo* flow cytometer arise from individual cells. The instrument's capability to image circulating T cells and measure their speed in the blood vessel in real time *in vivo* is demonstrated. The cell signal irradiance variation, clustering percentage, and potential applications in biology and medicine are discussed.

Key Words: Flow cytometer (유세포 분석기), *In vivo* flow cytometer (생체 유세포 분석기),

1. Introduction

An *in vivo* flow cytometer[1)] is a simple confocal microscope that can provide real-time detection and quantitative information of fluorescently labeled cells in circulation in a live animal model. As the labeled cells pass through the beam of a cw laser (termed as "counting laser") that is focused to a slit across a blood vessel, fluorescence is excited and then detected by a photo multiplier tube (PMT) located behind a confocal slit aperture. This confocal detection of cells in circulation makes observing a cell population of interest possible, without the need to extract blood samples.

Despite its unique capability to count the number of circulating cells, the *in vivo* flow cytometer can only identify a cell population based on labeling with a specific marker. In this report, we introduce the *in vivo* imaging flow cytometer that is designed to capture an image of the fluorescently-labeled cell as it moves downstream from the counting laser slit. The velocity of circulating cells was also measured using this new technique.

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2. Method

The *in vivo* imaging flow cytometer is based on the original *in vivo* flow cytometer that is explained in more detail elsewhere[1]. Light from a linearly polarized counting laser is focused to a slit by a cylindrical lens and imaged across the selected blood vessel with a microscope objective lens (Figure 1). Fluorescence is excited as a labeled cell passes through the slit of the counting beam. The emitted fluorescence is collected by the microscope objective and separated from the excitation light by a dichroic beam splitter (BS2). After passing the 50/50 beam splitter, the beam is imaged on a 200 μm×3000 μm mechanical slit, which is confocal with the excitation slit. This confocal arrangement eliminates light from out-of-focus fluorescent and scattering sources. Fluorescence is detected with a PMT placed directly behind the confocal slit. The analog signal from the PMT is digitized and stored on a computer. Converting the existing device into an *in vivo* imaging flow cytometer is made possible by employing a second He:Ne "imaging laser," with a center wavelength at 635 nm that serves as the strobe beam for a wide field microscope. The imaging laser has a power output of 30mW and is linearly polarized. In order to produce strobe pulses of varying duration, the cw imaging laser is gated using an acousto-optic modulator that is controlled by a function generator.

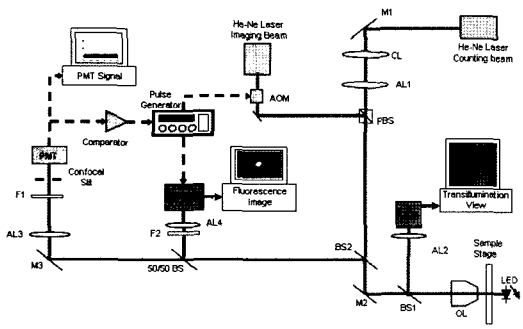

Fig. 1. Schematic diagram of the *in vivo* imaging flow cytometer. M1-M3 : mirrors, AL1-AL4: achromats, BS1-BS2 : dichroic beam splitters , F1-F2: bandpass filters, CL : cylindrical lens, OL: microscope objective lens. Red lines indicate optical path. Dashed black arrows indicate electrical connections.

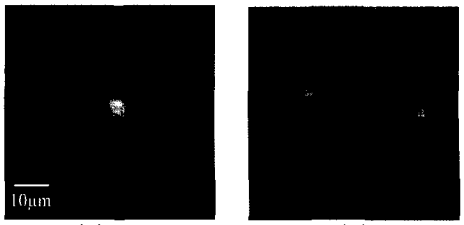

Fig. 2. *In vivo* imaging of a T cell (a) and *In vivo* image of two cells traveling in close proximity to each other (b).

The excited fluorescence is separated by a dichroic beam splitter (BS2) and further split by a 50/50 beam splitter. 50% of the beam are reflected and imaged on the Electron Multiplying CCD through a tube lens with focal length of 400 mm (AL4). A bandpass filter (F2) was placed in front of the tube lens to block unwanted light other than the fluorescence. A PC-based frame grabber captures the fluorescence image and saves it as a digital file.

3. Results

We have constructed an *in vivo* imaging flow cytometer that captures images of fluorescently labeled cells after they have passed a counting slit in a live animal. We have found that the vast majority of cells move as individual cells in the arteries examined, while clusters of cells were observed only rarely. Furthermore, the velocity of the cells was estimated based on the travel distance and imaging pulse delay.

The *in vivo* imaging flow cytometer allowed the identification of DiD-labeled murine T cells circulating in arteries (Figure 2). Most observed cells were circulating as single cells, while clustered T cells were rarely found (Figure 2 (a)). Occasion-

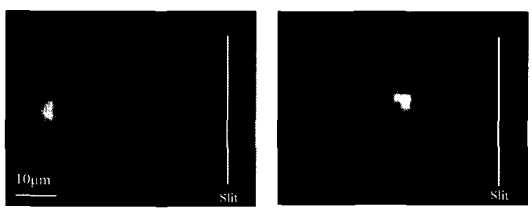

Fig. 3. *In vivo* imaging of cells in circulation with different speed.

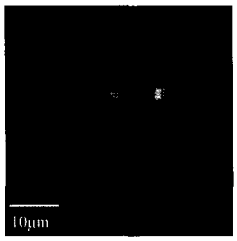

Fig. 4. *In vivo* image of a cell with two consecutive imaging pulses.

ally, two cells traveling in close proximity to each other were imaged with a single exposure of the imaging beam (Figure 2 (b)).

In vivo images of two cells with distinct velocities in Figure 3. The slit of the counting beam is illustrated in order to compare the travel distance of the two cells. The second pulse in both traces is produced by the fluorescence excited by the imaging beam. The imaging beam and EMCCD were triggered 10 ms after the counting beam's PMT signal crosses the threshold (1V). The cell in Figure 3 (a) traveled approximately 34 μm, while the cell in Figure 3 (b) traveled about 19 μm. Travel time was measured to be 9 ms from the counting signal peak to the center of the imaging beam signal in the trace. Assuming cells were traveling along a straight line that is perpendicular to the slit, the speed of cells can be estimated to be 3.8 mm/s and 2.1 mm/s for cells (a) and cell (b), respectively.

An image of a single cell acquired by two consecutive imaging pulses is displayed in Figure 4. The first imaging pulse was triggered 20 ms after the cell signal and the second imaging pulse followed 20 ms later. The trajectory of the cell in the blood vessel can be estimated from the sequence of images. The speed was calculated at 0.67 mm/s, based on the travel distance and time delay between two imaging pulses.

4. Discussion and Conclusion

We have developed an *in vivo* imaging flow cytometer that captures images of fluorescently labeled cells in circulation after they passed a counting laser slit. It became apparent that the vast majority of the signal peaks detected by *in vivo* flow cytometry correspond to single cells, while only a small percentage are potential clusters.

With the *in vivo* imaging flow cytometer, the absolute speed of the cells can be measured precisely based on the travel distance (from the center of the slit to the center of the captured cell image) and the travel time (the delay between the peak of the counting spike and the image pulse), assuming that the cell travels along a straight path that is perpendicular to the counting slit. To determine if the direction deviates from this straight-line assumption, multiple exposures of the imaging beam can be employed (Fig. 4). Multiple illuminations of a single cell within one imaging frame acquired by at least two consecutive pulses can provide additional information on the direction of the flowing cell. The velocity of the cell can be accurately calculated based on the travel distance and the delay between two imaging pulses.

In summary, we have used stroboscopic fluorescence imaging to observe the same cells that are detected by the counting beam. Advantages of the combined imaging flow cytometry include (i) both quantitative and morphologic information can be obtained simultaneously and (ii) in the case of rare events, the trigger signal from the counting beam allows us to capture images of rare cells of interest without having to sort through massive files of high-speed video data, most of which do not contain the rare events of interest. For example, using the combined system, we can begin to investigate whether circulating cancer cells can be distinguished from white blood cells, or activated lymphocytes from their resting counterpart, without the need to inject multiple fluorescent probes that can be problematic in vivo. Future studies will also include imaging and counting cells in an auto-perfused flow chamber and in an artificial microvascular network used for tissue-engineered organs.

References

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