P-65

Development of a multi-beam spectroscopic confocal fluorescence lifetime imaging microscope

［１］Organization
Project Leader：Qiyin Fang
(McMaster University)
Representative at RIE：Keiichiro Kagawa
(Shizuoka University)

［２］Research Progress

In this project, we studied the feasibility of the time-resolved CMOS image sensors developed at Shizuoka University to compact and potentially low-cost multiplexed confocal fluorescence lifetime imaging (FLIM) microscopes. One of the most time-consuming stages of drug discovery is in the identification of drug leads. Modern high-content screening (HCS) microscopes process hundreds of thousands of samples, which have been treated with varied dosages of countless drug leads. Protein-protein interactions, such as a procaspase being cleaved in two, or a multiple proteins forming an oligomer, happen at a scale far below the optical diffraction limit. This makes such interactions invisible in morphological high-content screening. There stands to be a great deal of insight should such interactions be monitored, however, since this would allow the determination of the exact pathway that a candidate drug is modulating. Förster resonance energy transfer (FRET) offers this ability. Fluorescence lifetime imaging microscopy is suitable for measuring FRET (FLIM-FRET), where the presence of FRET can be measured as a reduction in the fluorescence lifetime of the donor fluorophore, making it a more accurate measure of FRET activity – and so protein-protein interaction – within the cell. Confocal (or multiphoton) scanning fluorescence microscopy is still a base requirement for measuring protein-protein interactions by FLIM-FRET. While laser scanning can be performed very quickly and accurately, the time to acquire a confocal scan is slowed down by the collection time: the pixel dwell time must be set such that sufficient emitted light is collected at each position. While the laser power could be increased, in order to reduce the required dwell time, very high laser irradiance could lead to photobleaching and photodamage of the specimen. By employing multiple foci to scan across the sample, each focal spot could be provided with the optimal laser power that would maximize the signal without causing photodamage. The imaging speed of a system like this would then scale with the number of excitation foci.

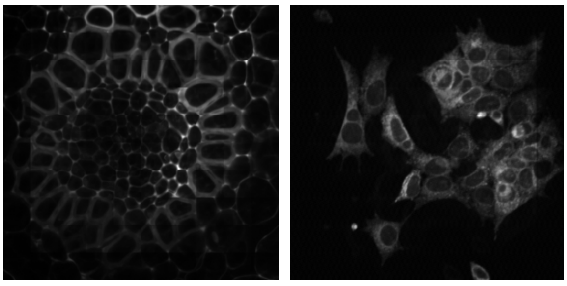

Fig. 1: Sample reconstructed images of multifocal scanning setup. left: Fixed *Convallaria majalis* slide right: MCF-7 breast cancer cell line tagged with mCerulean-3 fluorophore on protein Bcl-XL.

The system has been previously implemented with a scientific CCD imager, where each foci is identified as an ROI on the readout frame. Some of the results of these scans are displayed in Fig. 1. In these cases, a 10x10 foci array was raster scanned with 30x30 steps on the sample, resulting in 300x300 reconstructions. While these sample reconstructions are only intensity images, they show the imaging capabilities that can be made time-resolved with a suitable detector array. Combined, the promise of high-speed FLIM imaging for HCS drug discovery applications appears to be very close to reality.

The streak camera can be an option to implement time-resolving imaging. However, it is

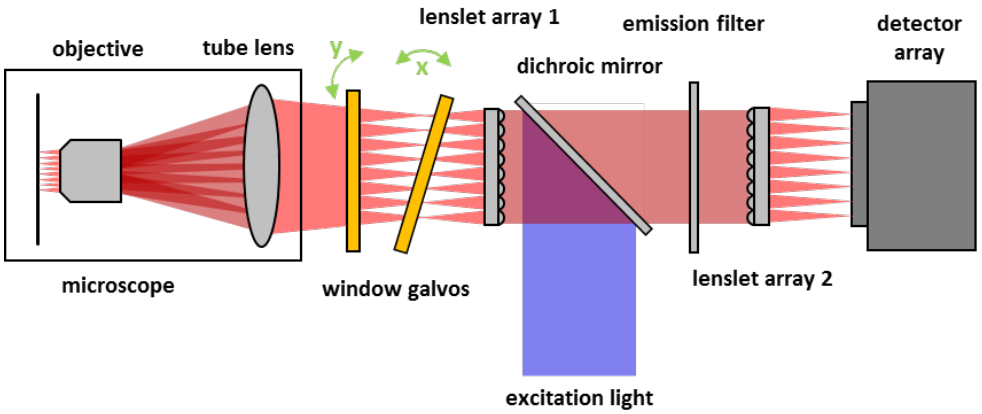

Fig. 2: Experimental setup of multifocal confocal scanning microscope.

bulky, expensive, and difficult to scale up production. In this project, feasibility of Shizuoka University's time-resolving CMOS image senor to multi-beam confocal FLIM has been studied. We had meetings on May 22, 2016 and Nov. 9, 2016 for discussions.

［3］ Results

(3－1) Research results

In order to provide a high-throughput FLIM system, a multifocal scanning system is implemented. Instead of a spinning disk approach, the foci are generated by a lenslet array, and a unique scanner is implemented to uniformly scan the foci array across the sample. The scanner reverses the scan for the returning fluorescent light, such that the fluorescent foci remain stationary, regardless of the current scanning position on the sample. This allows for of a stationary detector array with sparsely arranged pixels to be used for detection. The experimental setup is shown in Fig. 2.

By the advent of multiplexing, each foci is able to independently scan its subregion of the sample, and the final resulting image will stitch each foci's subregion together. The system raster scans the foci array across the sample, and collects one frame for each scan position. If there are MxM foci, and an NxN scan is performed, the resulting reconstructed image will be MNxMN in dimension. A sparse array detector is optimal in this case, due to the small number of foci (maximum 32x32) and the requirement for high-speed readout in order to achieve high-throughput screening.

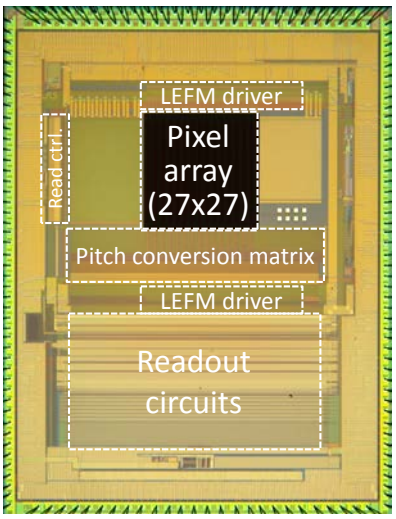

Fig. 3: Microphotograph of a prototype time-resolving CMOS image sensor.

Fig. 3 shows one of the time-resolving CMOS image sensors whose feasibility was tested with the microscope shown in Fig. 2. The lifetimes of several fluorophores such as Coumarine 6 and Flourescein were measured and compared with the reference values.

(3－2) Ripple effects and further developments

The time-resolving CMOS image sensors are suitable for the implementation of multiplexed confocal FLIM, which enhances the throughput of drug discovery. This technology will give a great impact in the field of pharmacy, and happiness of human being.

［4］ Achievements (List of Publications)

No publication this year

Travelling Report（Mention each travel by CRP budget.）

None