Time Delay Integration: enabling high sensitivity detection for Imaging-in-Flow on the ImageStream® 100 Cell Analysis System

Imaging-in-Flow is a concept with long-standing appeal, coupled with a persistent struggle to achieve adequate fluorescence sensitivity. The ImageStream system solves the sensitivity problem by using a unique charge coupled detector (CCD) camera and a powerful image detection technique called time-delay-integration (TDI). The focus of this report is TDI technology and the way it has been implemented in the ImageStream system to enable Imaging-in-Flow with a sensitivity comparable or superior to conventional flow cytometry.

TDI is a specialized detector readout mode that is used in machine vision applications where there is fast relative movement between the camera and the object being imaged. Examples include semiconductor wafer inspection and assembly line quality assurance. TDI does not read out the entire CCD chip as a single large image after some period of light collection. Instead, the image on the detector is collected and read out continuously, one row of pixels at a time from the bottom of the detector chip. As each row is read out, the signals in the remaining detector pixels are shifted down by one row, causing the latent image to translate down the detector during readout (1). If the readout rate of the detector is matched to the velocity of the object being imaged, the image will not blur.

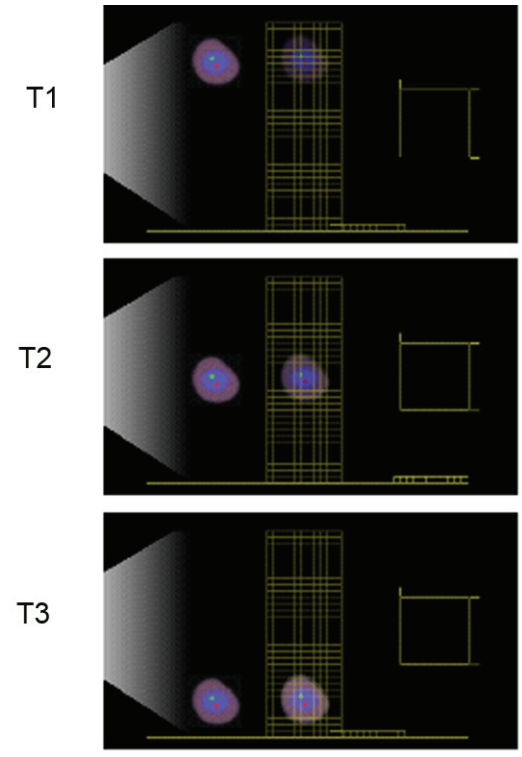

Figure 1A. A cell flows from top to bottom in front of an illumination source and an image is collected on the TDI camera. Each row of pixels on the camera collects light from a corresponding slice of the cell. As the cell progresses downward the signals are passed from each row to the one below it, thereby tracking the cell while continuing to collect more signal. The progressive increase in signal indicated by the latent image's steadily increasing brightness as the cell moves towards the bottom of the detector. Time points T1-T3 are shown in this figure.

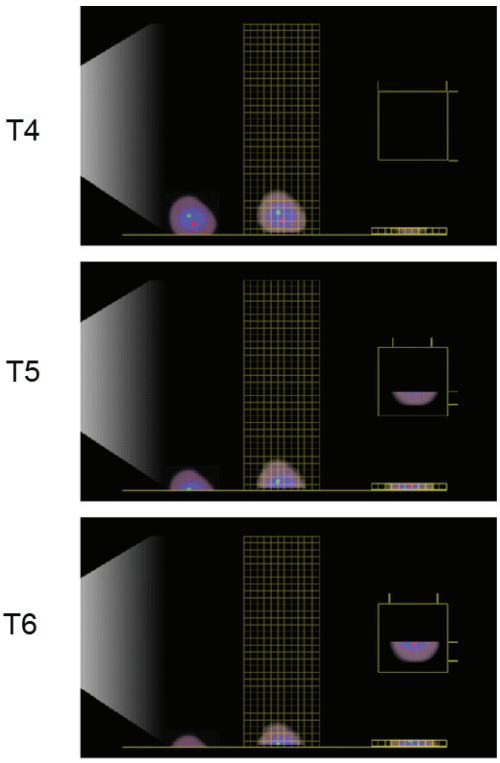

Figure 1B. Time points T4 - T6. As the cell reaches the bottom of the camera, image data are passed to the buffer where an image is built up.

A TDI readout time sequence is illustrated in the series of figures in this report. Please note that Figures 1A-C represent a *single series of sequential time points* (labeled T1-T9) in the capture of a cell image. Each frame in the Figure is divided into four parts, consisting from left to right of an illumination source, a cell in motion from top to

bottom, a detector on which an image of the cell is projected, and an image data buffer. Just before time point T1, a cell enters the field of view, moving towards the bottom of the detector. Light is collected from the cell, generating photoelectrons in the pixels near the top of the chip. A row of image data is read from the bottom of the detector and the photoelectrons are shifted down the detector by one row.

This readout-and-shift process occurs repeatedly, causing the image data to translate continuously down the detector. If the speed of the cell is known, the detector readout rate can be matched to the progress of the cell, as shown in time points T1 - T3, preventing image blur. Eventually, the cell reaches the bottom of the field of view and its image data is read out row by row, as shown in time point T4. Each row of data is analyzed in real time to determine if it contains background or cell imagery. If cell image data is detected, it is placed in the buffer, as shown in time points T5 - T8. After the entire cell image is buffered, T9, a variety of photometric and morphologic parameters are calculated and the cell imagery is stored. All six images of each cell are acquired in parallel using this technique.

The primary advantage of TDI operation is the greatly increased image integration period it affords. In comparison to standard CCD imaging, TDI increases the integrated signal proportional to the number or rows on the detector (512 on the ImageStream 100). (2) The practical limit on the number of rows is determined by the accuracy of the cell velocity measurement, since velocity errors result in cumulative tracking errors. Thus, one of the most important requirements to the success of Imaging-in-Flow is in fact accurate measurement of cell velocity.

The flow velocity detection system employed in the ImageStream platform is accurate to better than one part in one thousand, theoretically permitting the use of TDI detectors with more than 1000 rows. The resulting increased signal levels allow the detection of even faint fluorescent probes within cell images acquired at high speed. Each pixel on the ImageStream's CCD chip is digitized at ten bit resolution, which corresponds to approximately three logs of dynamic range. In practice, higher dynamic range is achieved because each

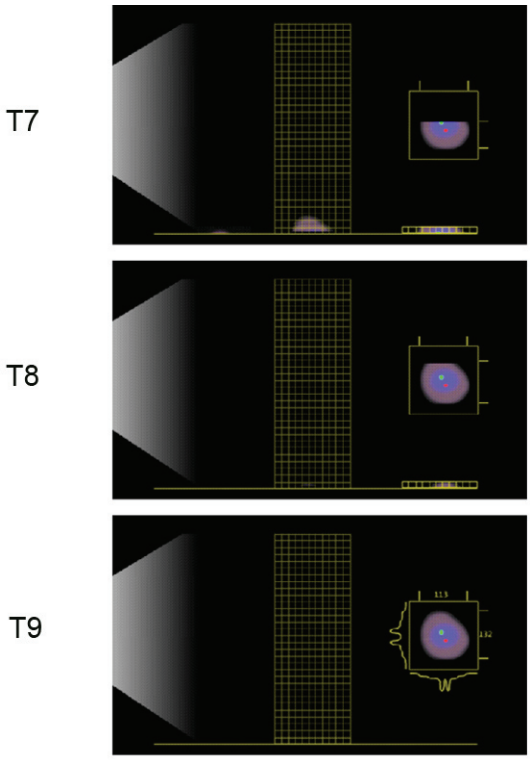

Figure 1C. Time points T7-T9. Once all data have been passed to the buffer photometric and morphometric parameters are calculated.

image generally covers numerous pixels. In the ImageStream 100, a ten micron diameter cell is imaged over approximately 300 pixels, providing a linear dynamic range that generally exceeds four decades. Future technical bulletins will describe Amnis' velocity detection technique and explain in more depth the relationship between sensitivity and image size.

1. Ong S.H., Development of a System for Imaging and Classifying Biological Cells in a Flow Cytometer. Ph.D. Thesis, 1985.
2. Holst, G.C., CCD Arrays, Cameras, and Displays, Second Edition. Winter Park, FL: JCD Publishing and Bellingham, WA: SPIE, 1998