Improving Flow Cytometer Reliability Using Sony FlowPoint Technology

Nao Nitta, Ph.D., Greg Veltri, Ph.D., Mark Dessing – Sony Corporation

Abstract: Core flow stability and precise tracking are responsible for the reliable measurement of cells in a flow cytometer. This technical note will explain the engineering behind the flow core stability and optical precision in the Sony SP6800.

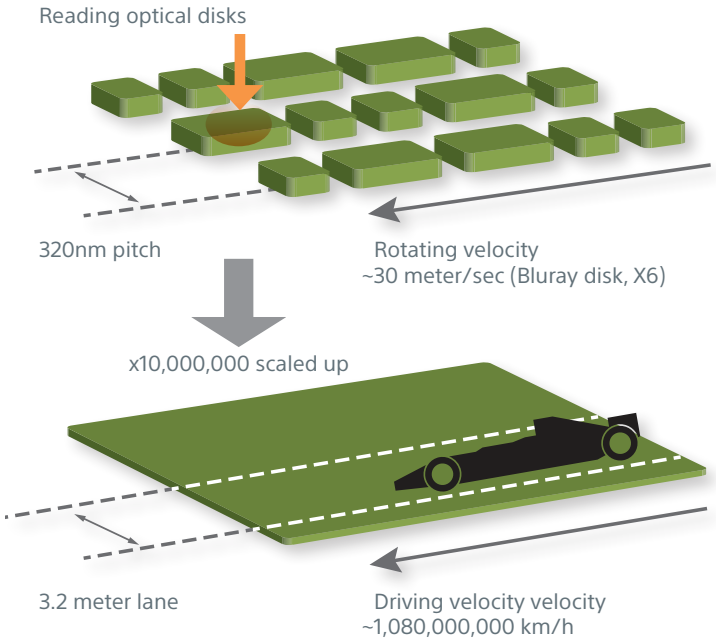

Figure 1. *Auto-tracking on Blu-ray technology.*

Direct monitoring of particle flow status

There are many factors that can affect the reliable measurement of cells in a flow cytometer. One of those factors is core flow instability due to the partial obstruction of the flow cell or the presence of trapped air bubbles. To avoid errors in experiments, direct monitoring of the particle flow status is very valuable. Conventional flow cytometers don't have this capability, and instead depend on skillful operators to identify the problem.

Ensuring reliability of measurements

In optical disk technology, keeping the optics focused and on track is equally important. When watching a Blu-ray movie in a car for example, it's unlikely you're thinking about the precision and speed needed to measure the pits on the Blu-ray disc. Yet to run error free, the disc is running at a track pitch (density) of 320 nano meters and a velocity of 30 meters/per second – comparable to driving a car in a 3.2 meter wide lane traveling at a speed of 1,080,000,000 km/h -- faster than the speed of light (Figure 1). To allow for precise tracking of particles in the flow cell in real-time, the SP6800 spectral analyser uses similar technology to the astigmatic method used in blu-ray technology to improve the reliability of measurements.

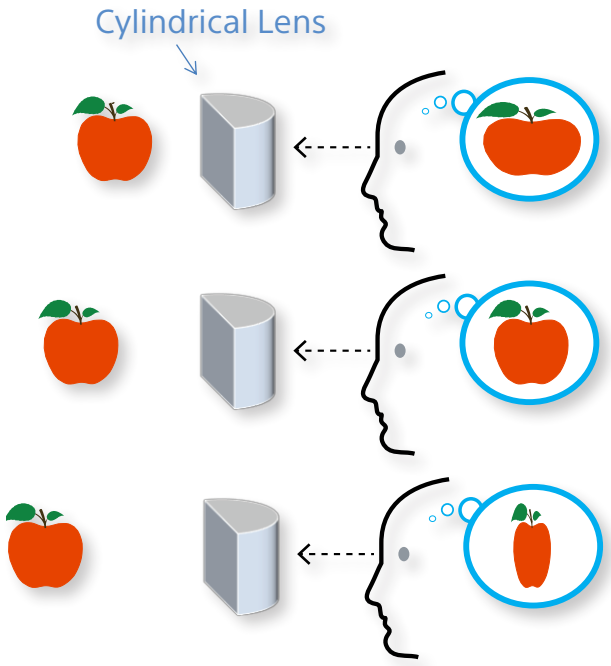

Figure 2. *An illustration of stigmatism.*

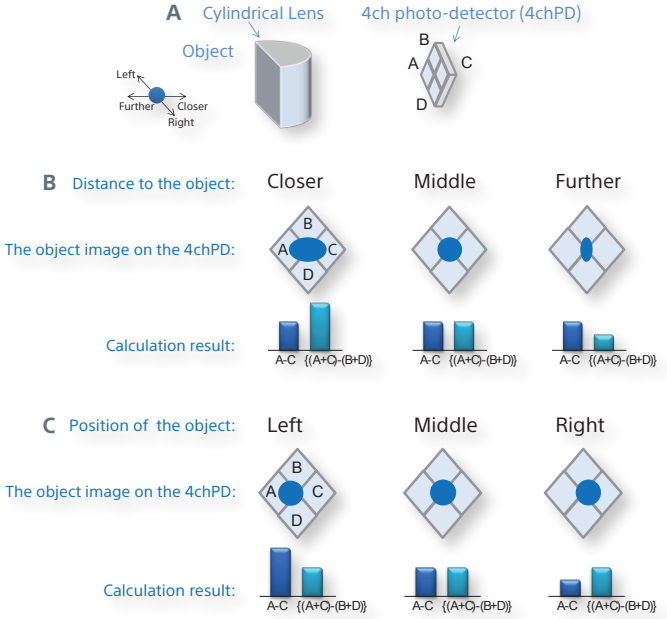

Figure 3. *Astigmatic method to quantify the position of objects.*

A*. Astigmatic method optics shows how the image of the object passes through a cylindrical lens and is projected on a 4-channel photo detector (4chPD), which contains four photo-detection elements (****A****,* ***B****,* ***C*** *and* ***D****).* ***B****. When the object moves closer or further away, the image projected on the 4chPD detector becomes wider or narrower. By monitoring the change of the image shape projected on the 4chPD, the position of the object is measured. A formula {(A+C)-(B+D)} is used to quantify the distance toward the object.* ***C****. To determine the horizontal position detection (left and right), a formula (A-C) is used.*

The "astigmatic method"

Figure 2 shows an individual looking at an apple through a cylindrical lens. The lens changes the image depending on the distance. When the apple is closer to the lens, it appears to be wider. If the apple is moved further away, it looks narrower. This phenomenon is called astigmatism and is a key principal used in the SP6800 flow cytometer.

Figure 3A shows how the astigmatic method quantifies an object. An image of the object passes through a cylindrical lens and is projected on a 4-channel photo detector (4chPD), which contains four photo-detection elements (A, B, C and D). In Blu-ray technology, the object corresponds to the pits on the disc. When the object moves closer or further away, the image projected on the 4chPD detector becomes wider or narrower (Figure 3B), just like the image of the apple observed through the cylindrical lens. By monitoring the change of the image shape projected on the 4chPD, the position of the object is measured. A formula {(A+C)-(B+D)} is used to quantify the distance toward the object. To determine the horizontal position detection (left and right), a formula (A-C) is used (Figure 3C). This simple mechanism provides precise control of optical discs and allows for uninterrupted playback, even in moving conditions.

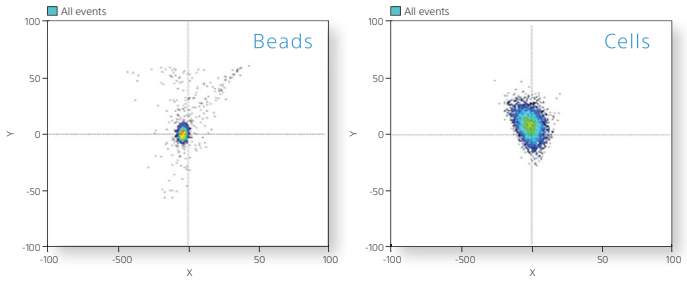

Figure 4. *Typical FlowPoint pattern for beads or living cells.*

Detection system provides a unique insight into the core stream shape and position within the flow cell channel, while providing the cross-sectional position of each passing particle. This information can be plotted and used as part of sample analysis.

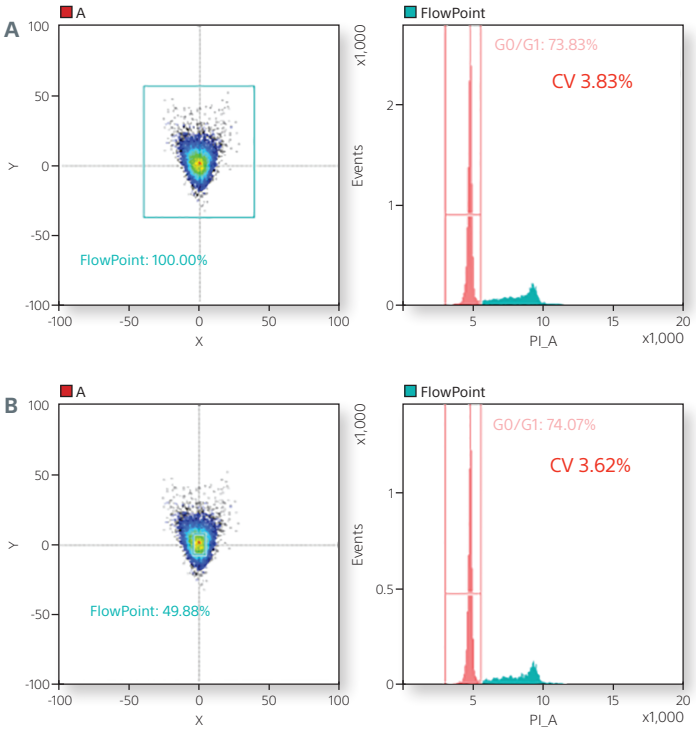

Figure 5. *Gating on FlowPoint improves CV.*

Gating on FlowPoint improves the quality of data. Plots show FlowPoint and PI histograms of the population in the FlowPoint region. ***A****. All events are included in the region.* ***B****. About half of the events are included. By limiting the population the CV in the histogram improved.*

FlowPoint particle position measurement (in flow cytometry)

Based on the astigmatic method, Sony has incorporated a particle position measurement system, called "FlowPoint", used in the SP6800 spectral analyser. It can measure the cross-sectional position of particles flowing through the laminar flow core. Figure 4 shows the typical FlowPoint pattern lows for uninterrupted playback, even in moving conditions.

Cross Sectional Positioning

In figure 4, both beads and cell particles are plotted in the center of a FlowPoint density plot. Plot pattern variation are due to the difference of object size and texture. Regions can also be set regions using FlowPoint parameters.

Figure 5 shows a cell cycle measurement using FlowPoint. When gated on all events on the FlowPoint plot, the CV of G0/G1 population is about 3.83% (A). In this example, when the region is focused only on events passing through the center of the flow cell, the CV is reduced to 3.62%. This is a well-known effect in hydro-dynamical focusing however using FlowPoint this effect can now be monitored, measured and, if necessary, corrected. Thus FlowPoint provides a cleaner result by allowing the user to monitor the status of the fluidics directly. Gating based on time and FlowPoint allows the user to remove erroneous data, caused by air bubbles or cell aggregates, from the measurement.

Conclusion

The astigmatic method has been widely used in optical disc technology and it contributes to high speed and reliable and easy-to-use features of optical disc products. The SP6800 spectral analyser incorporates this technology to increase the accuracy and reliability of flow cytometry based measurements.

North America/International

1730 North First Street
San Jose, CA 95112 U.S.A.
Voice: +1 800-275-5963
FAX : +1 408-352-4130
sales@sonybiotechnology.com
http://www.sonybiotechnology.com

Japan

1-7-1, Konan, Minato-Ku,
Tokyo , 108-0075 Japan
Tel : +0120-677-010
Fax :+0120-388-060
sales_Japan@sonybiotechnology.com
http://www.sony.co.jp/LS

Europe

The Heights, Brooklands.
Weybridge, Surrey, KT13 0XW, UK
Tel: +44 (0) 1932 817448
sales_EU@sonybiotechnology.com

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