Spectral Analysis Meets Flow Cytometry

What is Spectral Analysis?

Spectral analysis has been used across many industries over the past half century. It is an analytical technique used to characterize sequenced data by decomposing the data into a different domain that makes it easier to process and analyze the data[1]. In chemistry, spectral analysis is used in spectroscopy, a tool that allows chemists to analyze and measure the different chemical components present in a mixture[2]. To determine the contents of the unknown chemical mixture successfully, the spectral characteristics of pure known samples must first be measured and characterized by the technology. In meteorology, spectral analysis has been used to identify and classify cloud features, and similar applications exist to identify and classify features in MRI, ultrasound, and x-ray images for diagnostic purposes[3]. Other imaging applications include spectral imaging where spectral analysis is used to detect and analyze tumors for tumor classification purposes in living creatures[4]. Geophysicists have leveraged spectral analysis tools to find and characterize magnetic fields and other characteristics in Earth's composition, and astronomers have used spectral analysis to study changes in galaxies and expansion of the universe[5,6].

Early Approaches to Spectral Flow Cytometry

Since the inception of flow cytometry decades ago, advances in the field have focused predominantly on refining and simplifying the use of existing optical schemes. Although helpful to the scientific community, the cytometers created using this approach have not kept pace with the desire to increase data dimensionality from each sample. The cytometers still utilized conventional detection methods with a single dedicated detector and filter pair to detect a single fluorochrome using compensation to eliminate spill-over from neighboring fluorochromes. Additionally, these approaches continued to increase the size and weight of the instrumentation, as well as the cost of the cytometer. To shift the field toward higher dimensionality, several innovative researchers developed different methods to bring spectral analysis to flow cytometry.

A comparison of the hardware components that could be used for conventional cytometry and for spectral cytometry is shown in figure 1. Between 1979 and 2012, spectral flow cytometry methods utilized grating spectrographs or prisms along with detectors such as PMTs or even a vidicon[7] to collect and detect light above and beyond the capability of conventional flow cytometers[8]. The method and design developed by the Robinson group at Purdue University became the first commercially available flow cytometer when Sony licensed their design and launched the SP6800 in May 2013[10].

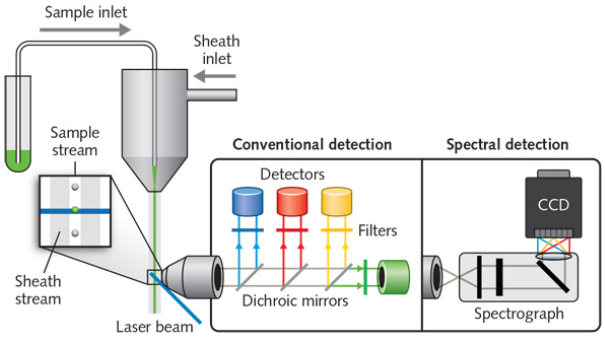

Figure 1: Comparison of conventional flow cytometer detection methods and an example of a spectral flow cytometer detection system. For both types of cytometers, fluorescently-labeled sample (often cells) in suspension passes through one or more lasers. The lasers excite the fluorescent molecules on the sample, causing them to emit light that is captured by the detection system. Figure generated by BioOptics World, based on a figure from John P. Nolan[8,9].

The benefits of the spectral analysis approach compared to the conventional approach to flow cytometry are many. First, spectral analysis provides the researcher more flexibility in their dye choices when designing multicolor panels. Second, the spectral approach captures autofluorescence of any highly fluorescent sample and researchers can remove this autofluorescence from their data, allowing them to better visualize their cells of interest and analyze their results. Third, the

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spectral cytometer has a single optical configuration accommodating a wide array of fluorochromes, eliminating the chance of emission collection mismatch and the time needed to change filter sets to match each user's fluorochrome selections required by conventional flow cytometers. Finally, the spectral cytometer requires fewer lasers to provide higher data dimensionality to the users, and fewer lasers means lower cost to the researchers.

These early spectral approaches have their weaknesses. For starters, all the approaches utilize PMTs which, compared to other detector options, are more expensive, have poorer quantum efficiency in red and near-infrared wavelengths, and have higher electronic noise levels. With PMTs in the picture, the data quality from these early spectral cytometers would not significantly differ from the plethora of PMT-based conventional cytometers on the market. This is because the detection wavelength bandwidths in spectral cytometers are typically narrower than conventional cytometers, hence the sensitivity of each channel tends to be worse than the conventional cytometer when both systems are using PMTs. Second, a significant amount of space is needed to accommodate the prism array or spectrograph, which means a spectral cytometer utilizing those technologies will need a larger footprint to contain all of the parts. Third, some of the designs positioned lasers co-linearly rather than spatially separating them. This reduces the amount of spectral information from a fluorochrome that would otherwise enable differentiation of dyes having similar emission spectra from one laser but different emission from another[11]. Finally, a lot of light is lost as it is dispersed through the gratings to the detectors, losses that are detrimental to the sensitivity and resolution capabilities of the spectral flow cytometer. On the other hand, if the spectral cytometer design uses prisms to disperse the light, then the light is dispersed non-linearly, which will adversely impact the data generated with the system. Further technological advancement was needed to overcome these challenges and generate unmatched high-quality data that flow cytometrists desired in an affordable spectral cytometry platform.

Cytek's Approach to Spectral Flow Cytometry

In 2017, Cytek Biosciences introduced the Cytek® Aurora to the flow cytometry community. The Aurora introduced several fluidic and excitation optics improvements from what existing conventional cytometers were offering: the fluidics system was made modular and accessible for maintenance and repair; the excitation optics used spatially separated beams to reduce excitation spillover with flat-top beam profiles to uniformly energize the fluorochromes on each cell; and the size of the system was kept small

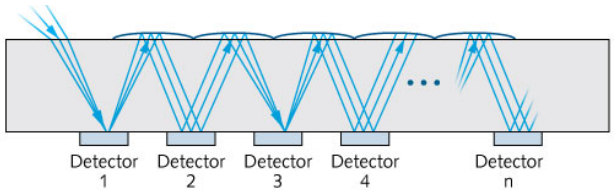

Figure 2: Light emitted from the sample is collected and passes through the optical filter-based coarse wavelength division multiplexing (CWDM) demultiplexer arrays on the Cytek Aurora and Northern Lights cytometers.

to accommodate user's valuable and limited lab spaces.

The powerful uniqueness of the Aurora is in its emission optics design, and how that design is integrated with spectral analytics, enabling users to build larger panels with fewer lasers. Advances in semiconductor detectors, telecom optics, and computation methods integrated together in the Aurora has made full-spectral measurement of 24 colors for high-quality flow data with only 3 lasers possible. Each laser on the Aurora has one patent-pending detector array paired with it[12]. Each array contains an optical filter-

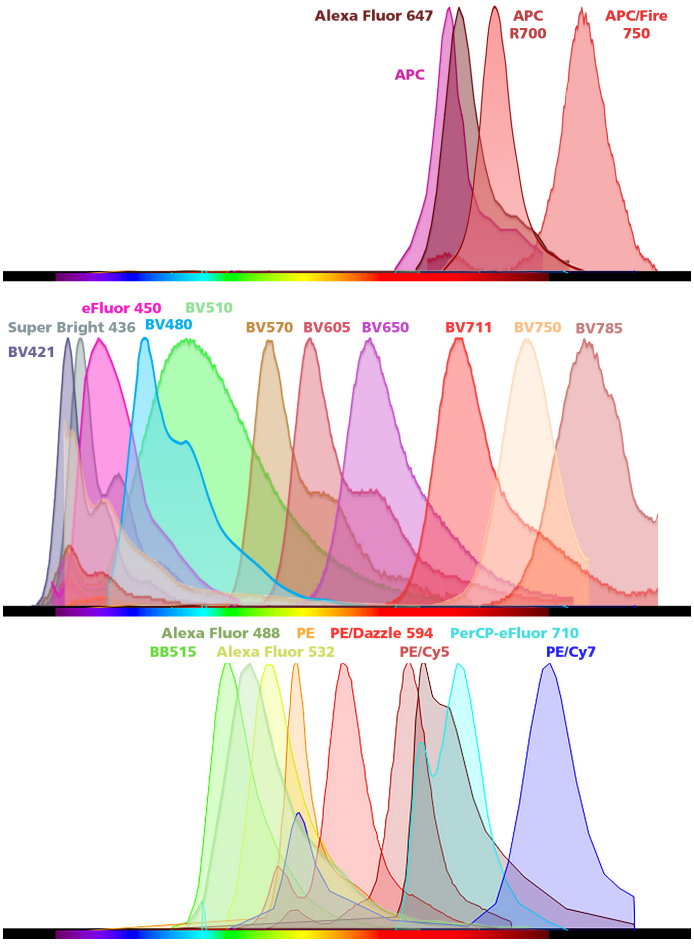

Figure 3: Twenty-four dyes used in combination for a human immunophenotyping panel on a 3-laser Cytek Aurora. For additional information, please visit our website at cytekbio.com.

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based coarse wavelength division multiplexing (CWDM) demultiplexer assembly and avalanche photo diodes (APDs) to collect the light and convert it to electrical signals (figure 2). The benefits of APDs over PMTs are many: 1) they are less noisy than PMTs, which directly correlates to cleaner, better resolved flow data; 2) they are 10 to 100 times more efficient than PMTs at converting photons across a wider range of wavelengths; 3) they are much smaller than PMTs (approximately the size of a pencil eraser), making it easy to fit up to 67 channels onto a 3- to 5-laser Aurora and maintain a small footprint; and 4) they are significantly less expensive than PMTs, allowing flow cytometers utilizing APDs to be manufactured and sold to users at a more affordable price point[13]. The Aurora and Northern Lights design allows for an increased number of detectors per laser without sacrificing the sensitivity of each detector channel with narrow emission bands. As a result, each detector (having 1/3 to 1/2 of the emission band of typical conventional cytometers) has better fluorescence sensitivity than a PMT-based conventional cytometer with wide emission bandwidths. Combining all of these elements together into a spectral cytometer without using any dispersive elements (e.g. gratings or prisms) meant the Aurora could raise the bar in defining what high quality flow data means.

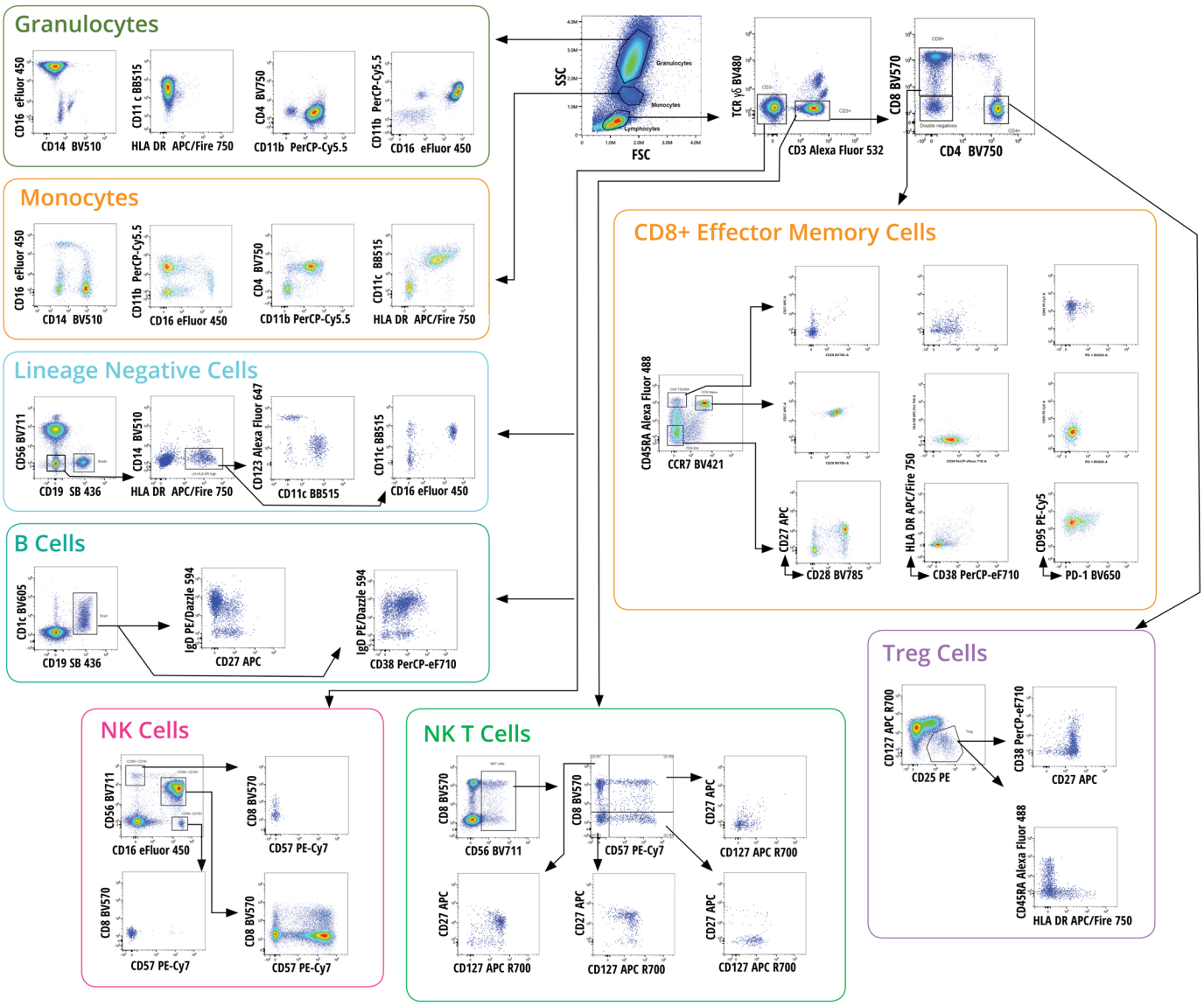

Figure 4: A 24-color immunophenotyping panel demonstrated in a healthy donor using a whole blood lyse wash sample preparation. To view the full gating analysis of all cell subsets in the panel, please visit our website at cytekbio.com and check out the Aurora and Northern Lights product pages.

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Application Advantages with the Cytek Aurora

With a 3-laser Aurora, users can generate a high-quality 24-color human immunophenotyping panel from a single sample tube (figures 3 & 4). High-quality data means that dim or rare populations are easily resolved, and that all cell populations of interest can be visualized and easily gated by the researcher. To achieve this feat on a conventional cytometer, users would need to add more lasers (5 or more) and more detector arrays to their systems to minimize spectral overlap, and design and test filters compatible with the new lasers and the 24 dyes. Finally, in order to reach 24 colors, the addition of a UV laser and expensive UV excitable dyes would likely be required.

Just as adding additional lasers to a conventional cytometer enables users to increase panel complexity, the same story can be true when adding more lasers to the Aurora that allow the use of more dyes (e.g. UV excitable dyes with the UV laser). If 3-lasers can bring 24 color panels to life, imagine what would happen by adding another laser like a UV laser to the Aurora. Examples of these possibilities will soon be available on our website at cytekbio.com.

The Aurora provides more than well resolved high dimensional data. It provides set-up time cost savings (no configuration and filter changes), hardware cost savings (get more with fewer lasers and less expensive detectors), space savings, and sample preparation time and cost savings (24 colors from 1 sample tube versus 2-4 tubes on conventional cytometers, and without using expensive UV dyes). Additionally, Aurora owners have access to ongoing panel design support and troubleshooting assistance from Cytek's growing team of expert scientists and engineers. For more information about the Aurora cytometer, visit www.cytekbio.com or email us at sales@cytekbio.com.

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