the extra technical support available in a full-service core. The ever changing needs of the flow cytometry community makes it difficult, if not impossible for one standard type of core to meet those needs.

After thorough examination of each of the 3 core types described here, it can be seen that they can all be successful and serve unique needs. All 3 types of cores can coexist and work together to meet the needs of the growing cytometric community. All would benefit from having some shared resources (for example a shared data storage service), but each can equally benefit by having enough autonomy to serve their unique niches appropriately. An in-depth discussion of this topic can help to resolve issues that have arisen between different types of cores and ensure that the community moves forward.

I propose to open up this topic for discussion in the core management workshop with the data collected from this study, and would welcome input from others as to the best way to do this.

# Flow Cytometry and Sorting (B118 – B135)

**224/B118**

## A Modified Baker Bioprotect III Jr. Biosafety Cabinet Provides Rigorous BSL-2 Containment for Sorting with a JSAN Cell Sorter

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**Introduction:** Conventional cell sorting has long been identified as a source of aerosols that can potentially contaminate cellular products as well as contaminate the environment. For example, infectious agents that can be spread by aerosol (e.g., *Toxoplasma*, various bacteria, etc.) require a minimum of BSL-2 containment. This requires placing the instrument in a biosafety cabinet that can meet BSL-2 conditions. We wanted to demonstrate that we can maintain BSL-2 containment with the JSAN operating under normal conditions as well as conditions where a large amount of aerosol would be produced. The primary focus is to provide personnel and product protection against contaminants or hazardous agents under normal laboratory applications.

**Methods:** Instrumentation: JSAN Flow Cytometer (Bay bioscience, Co., Ltd., Kobe, Japan)

BioProtect III Jr. (The Baker Company, Sanford, Maine)

Procedure: All tests were performed to the NSF/ANSI Biosafety standard 49, 2010. The modified performance testing (15 minute exposure vs. 5 minute exposure) is a more stringent test and exceeds the NSF standards by increasing the test challenge duration. The tests incorporated an air deflector on top of the cytometer and the instrument was operating throughout all test durations. The challenge cylinder simulating a person's arm or obstruction was in place for all tests.

**Results:** Instrument placement for optimal airflow: The JSAN cytometer was inserted into the cabinet on top of the movable cart. The cart was centered inside the cabinet for proper ergonomic alignment of the cytometer on the cart, focus was on how close to the front of the cabinet the cytometer needed to be for accessing the stream chamber without compromising safety.

Testing under normal sorting conditions

Personnel Protection Test
The cabinet was challenged with *Bacillus subtilis* spores at 5.0 x 10^8 spores/ml for each test run. Each run was 30 minutes with a nebulizer time challenge of 15 minutes. The filter count of "CFUs" represents number of *B. subtilis* colony forming units that are obtained after filtering (6) glass impingers (AGIs) and incubating on a soy agar plate. Pass/Fail criteria </= 10 cfu on a filter after any given test. There are also two slit samplers with 150mm soy agar sampling plates, which provide evidence of spores in the environment. After incubation no more than 5 cfu can be found on the two plates.

Product Protection Test
The cabinet was challenged with *B. subtilis* spores at 5.0 x 10^6 spores/ml. Each run was 30 minutes with a 15 min. nebulizer challenge. Pass/Fail criteria is </= 5 cfu per test on the soy agar sampling plates.

Testing inside the stream chamber (door open)

Another condition of tests was performed inside the stream chamber. Agar sampling plates were placed inside chamber for product protection and the nebulizer placed inside the chamber for personnel protection. Both test conditions provided good passing results with the cytometer operating.

**Conclusion:** The above test results demonstrate that the BioProtect III Jr. with the JSAN cytometer operating meets the biotest passing range of plus/minus 15 fpm from nominal set point of 55 fpm down flow and 105 fpm intake. The cabinet provided safe operating conditions under static and under standard operational conditions of the cytometer. The opened door to the stream chamber and challenge cylinder in place showed that safety wasn't compromised during daily user functions.

**225/B119**

## Optical Super-Resolution with Polarized Light Scattering for Precise Characterization of Nonspherical Particles by Flow Cytometry

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Analysis of individual microscopic particles requires one to solve identification and characterization problems. The latter requires substantial scientific research to establish a mathematical relation between experimental signals and morphological characteristics of a particle. A scanning flow cytometer was improved to provide large amount of data necessary for solution of such characterization problems. The current device measures angle-resolved intensity of light scattered by individual particles (light-scattering profiles, LSPs) in regular and polarized states. It was fabricated by CytoNova Ltd., Novosibirsk, Russian Federation. (http://cyto.kinetics.nsc.ru).

First, we measured regular LSPs of individual spherical beads to verify proper alignment of the laser beam and the flow. The solution of the inverse light-scattering problem was applied for these LSPs to retrieve bead sizes and refractive indices. The bead sizes were determined with uncertainty of about 10 nm – an exeptionally high precision for optical methods. Second, we developed a method to characterize polymer bead dimers, as an example of non-spherical particles, based on regular and polarized LSPs. Characteristics of a dimer, such as sizes and refractive indices of constituent monomers, were successfully retrieved from the solution of the inverse light-scattering problem. Orientation of each dimer in a flow relatively to the direction of the incident laser beam were also determined. Both ordinary and polarized measured LSPs are in good agreement with T-matrix simulations, which leads to average uncertaintly of 50 nm for determined bead sizes in a dimer.

Measurement of the polarized LSP opens the way for optical characterization of particles with complex shape and internal structure. For example, this approach looks promising for detailed characterization of red blood cells (RBCs). Implementation of this approach into a hematological analyzer should lead to substantial decrease of systematic errors in RBC indices. The polarized LSP can also be used for assessing the homogeneity of cell nucleus and for analysis of blood platelets microaggregates.