the physical and biochemical limits, we have confidence that such instrumentation will become available in the coming years. Moreover, such instrumentation may also be useful to detect other small particles, such as bacteria, and viruses.

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Evaluation of Cell Sorting Aerosols and Containment by an Optical Airborne Particle Counter

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Understanding aerosols produced by cell sorting is critical to biosafety risk assessment and validation of containment efficiency. Characterization of the aerosols produced by cell sorters has been performed using an Aerodynamic Particle Sizer, but the device is prohibitively expensive for the widespread, routine evaluation and monitoring of aerosol containment. In this study an Optical Airborne Particle Counter was used to analyze aerosols produced by the BD FACSAria and to assess the effectiveness of its aerosol containment. The suitability of using this device to validate containment was directly compared to the Glo-Germ method put forth by the International Society for Advancement of Cytometry (ISAC) as a standard for testing.

It was found that high concentrations of aerosols ranging from 0.3 µm to 10 µm can be generated in failure mode, with most less than 5 µm. Although the aerosols are effectively contained in the Biosafety Cabinet (BSC), the Aerosol Management System (AMS) operated at the recommended settings fails to contain small aerosols (<0.5 µm) from escaping to the BSC. In most cases, while numerous aerosols smaller than 5 µm were detected by the Optical Particle Counter, no Glo-Germ particles were detected. The results indicate that small aerosols are able to escape AMS containment and are likely underevaluated by the Glo-Germ method.

Our data in this study has clearly illustrated that

1. BD FACSAria Cell sorters are able to generate aerosols in the range from 0.3 µm to 10 µm and the data strongly suggest that containment of small (<5 µm) aerosol particles needs to be validated for infectious material sorting. This has so far not been clearly addressed in the ISAC guidelines. Our study has advocated that the cell sorting aerosol containment validation protocol developed in the Ragon BL3 core facility using a Fluke 985 airborne particle sizer could practically fulfill this task well.

2. Both the BD AMS at 20% and the integrated AMS of the Bioprotect IV in 'high or low' mode were not able to completely contain aerosols generated in failure mode, especially if the tube holder was not installed. Small aerosols were still detected. This does not present an immediate risk to the operators if the cell sorter is housed inside of a BSC, but it does stress the importance of using a BSC and a need of proper enforcement of additional PPE for arms/hands (such as a sleeve and additional layer of gloves) when reaching into the inside of the BSC if biohazardous cell sorts are conducted.

3. The importance of the tube holder or splash guard in containing aerosols is strongly illustrated in our testing.

4. The Fluke 985 airborne particle counter can be used routinely as an aerosol containment testing tool for cell sorting. It is portable, gives a rapid reading of aerosol sizes and concentrations, and is more sensitive than the Glo-Germ method. It can also be used to periodically check the sort chamber area to test aerosol containment without interrupting the ongoing operation. We believe this device is an invaluable tool for flow cytometry and strongly suggest others to implement its use.

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Measurement of Light Scattering in Backward Hemisphere: A New Way for the Study of Platelet Aggregation with Scanning Flow Cytometry

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Background: Blood platelets play a central role in hemostasis and are involved in many diseases, including thrombosis, hemorrhage, inflammation, and cancer. Many methods for the evaluation of platelet function are based on platelets aggregation testing. These 'gold standard' among those methods is light-transmission aggregometry. However, the alterations are not noticeable on the very first stage of aggregation, the dimerization of platelets, while this process precedes clot formation and is critically important for the hemostasis. Single-particle technique, such as flow cytometry, may help to overcome this limitation. However, the identification of platelet monomers and dimers from flow-cytometric data is impossible due to high variability of cells volume and shape.

Methods: We use the scanning flow cytometry, which is based on the measurement of angle-resolved light-scattering profiles (LSPs) of individual particles. The range of scattering angles where the LSP is measured was 10-70°. We measure LSPs with the Scanning Flow Cytometer fabricated by CytoNova Ltd. (Novosibirsk, Russia, http://cyto.kinetics.nsc.ru) during platelet aggregation. The aggregation was initiated by the addition of adenosine diphosphate (ADP).

For the simulation of LSPs of single platelets and their aggregates we used discrete dipole approximation software ADDA v 1.1. Each platelet were modeled as an oblate spheroid, and platelet aggregates were modeled as spheroids in contact.

Results: LSPs of platelets and their aggregates were measured with the SFC in several time points, i.e. 0, 1, 5, 10 and 15 min after the addition of ADP. The overall intensity of LSPs slightly increased with the increasing aggregation time, while the structure was approximately the same. This fact is non-trivial, because the LSP structure strongly depends on particle shape. We explained this effect by the numerical simulation, which showed that the scattering by platelets in aggregate can be considering single. Also we showed that the interference structure along the azimuthal scattering angle φ, which is present only for aggregates, is washed out by the integration over φ. This leads to the additivity of platelets LSPs, which results in the identical structure of monomer and dimer LSPs.
Numerical simulations showed several ways to overcome this problem. The simpler way is probably the measurement of LSPs in backward hemisphere. In this range of scattering angles (110-170°) LSPs of monomer and dimers differ from each other, which is promising for the identification of aggregates. The novel version of SFC with extended angular range is now in the process of manufacturing and testing.

Conclusions: The features of light scattering by platelets aggregates make it impossible to separate monomers from dimers with existing instruments, including modern flow cytometers. The device capable of measuring light scattering in backward hemisphere is needed for this purpose. The modernized version of the SFC may shed a light on the process of platelet aggregation.

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Tunable Green Fiber Lasers with Expanded Wavelength Ranges for Flow Cytometry

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