QUICK TECH NOTES
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to quickly demonstrate feasibility

Aerosol Management on a BioSorter Instrument from Union Biometrica

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Objective

To quantify the amount and location of aerosols generated and to explore techniques for containing aerosols on Union Biometrica's BioSorter instrument.

Introduction

In the Biomedical industry it is becoming increasingly popular to take advantage of human induced pluripotent stem cells, for cellular test systems investigating pharmacological and toxicological screenings.(1) This has provided a growing need for automated high throughput screens of thousands of human iPS cells that are too fragile and too large for traditional high pressure cell sorting. Union Biometrica's large particle flow cytometer BioSorter® instrument represents a powerful gentle tool for measuring, selection, sorting and collection of human iPS cell clones in a highly standardized manner and with high throughput at low pressures.(2)

Using human cells puts the user at risk for becoming infected with diseases if they are aerosolized. Aerosols are the likely cause for laboratory-associated infections (7) and infections may occur via aerosol route even if it is not transmitted via aerosol in nature (8, 9). It was known that with jet-in air cell sorters droplets are formed during normal operation, and smaller satellite droplets are also formed from droplet break off. (3) Recently the aerosols that are produced when using high pressure cell sorters were characterized. It was found that increasing the sheath pressure, increases the concentration of aerosols generated from the cell sorter and that concentration decreases as the distance from the source is increased. The concentration of aerosols produced can range from 25,303 particles/cm^3 running at 70 PSI to 6 particles/cm^3 run at 20 PSI. (4) It has therefore become an important process to evaluate risk assessment and to develop standard operating procedures (SOP's) for potential hazards associated with sorting bio hazardous samples and has been recognized by the International Society for Advancement of Cytometry (ISAC) by publishing Biosafety guidelines in 1997 and updated standards in 2007. (5,6) There are no previous studies that characterize the aerosols that the produced from cell sorting instruments that operate at low pressures, like the BioSorter® instrument made by Union Biometrica. That is why it was necessary to evaluate the aerosol generation using a more cost effective and efficient manor using the Cyclex-d cassette technology made by Environmental Monitoring Systems, for risk assessment and to develop SOP's for the BioSorter® instrument.

Materials and Methods

Aerosol concentration and size distributions were done by running the BioSorter instrument using the 250 and 500 FOCA. Acquisition parameters were established using the FlowPilot software to perform a simple 10 minute sorting operation into a bulk container. Due to the many variables that are involved with the BioSorter instrument, it was determined that a simple sorting operation where the time of the sort could be controlled was most effective to evaluate the level of aerosols put into the air by the instrument.

To measure the aerosol levels, an air filtration cassette made by Environmental monitoring systems, called Cyclex-d was used. The Cyclex-d cassette filters air from the environment onto an impact slide at a rate of 20 litters/minute, and collects any particulates suspended in the air onto an impact slide inside the cassette. The impact slide was then imaged and aerosols were counted by hand.

The Cyclex-d cassette was placed at the various distances from the front of the 250 FOCA pointing toward the stream. The sheath in the BioSorter instrument was filled with a 1:500 dilution of a concentrated clear blue dye (CBD), in water that is excited by UV light. Any liquid that is collected onto the Cyclex-d cassette containing the fluorescent dye must have originated from the sheath stream. The sample cup was filled with 15 ml of 4x 42µm control particles, 100µl of one micron orange fluorescent microspheres, and DiH_2O. The 42µm control particles were added so that the instrument had an object to trigger sorting, while the one micron beads were to represent a small biologic, like a cell, that could become aerosolized.

Once the Cyclex-d cassette was placed at the proper distance in front of the 250 FOCA a 10 minute bulk sort was started. The flow rate was set to 50% to achieve 10ml/min for a flow rate and the sample cup pressure was maintained in a range of 4.80-6.10psi to achieve 10events/second passing through the flow cell, for the 250 FOCA. The flow rate was set to 51% for the 500 FOCA to achieve 25ml/min for a flow rate and the sample cup pressure was maintained between 1.90-2.20psi to achieve 10events/second passing through the flow cell. The diverter pressure was set to 2.80psi. The pre-analysis chamber for the 250 FOCA was reading in a range of 4.45-5.01psi during the 10 minute bulk sorts. The pre-analysis chamber for the 500 FOCA was reading in a range of 1.35-to 1.80psi during the 10 minute bulk sorts.

Measurements were taken at 2, 4, 6, and 8cm in front to establish how far the aerosols produced travel and at what level. This simple test was preformed daily over the course of three weeks to build a data set that represents the overall performance of the instrument. This same experiment was performed using the 250 FOCA and with the 500 FOCA to investigate whether increasing the nozzle size and decreasing the pressure results in any change in the level of aerosols produced.

Results

The impact slides from the Cyclex-d cassette for each test performed were analysed using the Zeiss microscope using a 5x objective under 385nm and 555nm excitation light. The images were processed using Zen 2 lite software. The Impact slides were removed from the cassette and then placed into a Petri dish with the sticky surface facing up and then imaged. Each image was then counted by hand for aerosols containing CBD and one micron beads. Due to the fact the impact slide could not be imaged in one frame, multiple frames of the cassette were taken and the count for each frame were averaged to evaluate the total aerosols collected per cassette. The average counts for each cassette at the various locations in front of the BioSorter instrument were then averaged at each position from each day an experiment was performed. The cassettes filtered air for 10 minutes at a rate of 20 litters/minute, filtering a total of 200 litters of air. The data below is presented in the form of number of particles (CBD droplets or one micron beads) per cm^3 of air filtered.

Table 1. Aerosol concentrations produced from the BioSorter instrument during a 10 minute bulk sorting operation using the 250 FOCA.

Distance from 250 FOCA	Particles/cm^3 for CBD droplets	Particles/cm^3 for one micron beads
2cm	$8.31x10^{-4}$ /cm^3 ± $6.80x10^{-4}$	$6.06x10^{-4}$ /cm^3 ± $6.41x10^{-4}$
4cm	$2.66x10^{-4}$ /cm^3 ± $1.69x10^{-4}$	$1.73x10^{-4}$ /cm^3 ± $1.71x10^{-4}$
6cm	$4.68x10^{-5}$ /cm^3 ± $9.70x10^{-5}$	$7.86x10^{-5}$ /cm^3 ± $6.40x10^{-5}$
8cm	$1.40x10^{-5}$ /cm^3 ± $3.91x10^{-5}$	$7.11x10^{-5}$ /cm^3 ± $4.82x10^{-5}$

The table above shows that there is a higher concentration of aerosols with CBD seen closer to the FOCA and decreases exponentially as the cassette was moved further from the stream source. It can be seen that there is a similar level of CBD droplets as compared to the one micron beads at 2 and 4cm, but when the cassette is moved to 6, and 8cm further from the source there are less CBD droplets but more one micron beads. This could be because the CBD droplets are not traveling as far from the source because they have lower velocity and a higher mass and are do not travel as far as a smaller one micron bead. As the sorting operation time becomes longer there is an increased chance to capture more aerosols and so the longer the sort then the higher the concentration of aerosols can be seen on the Cyclex-d cassette.

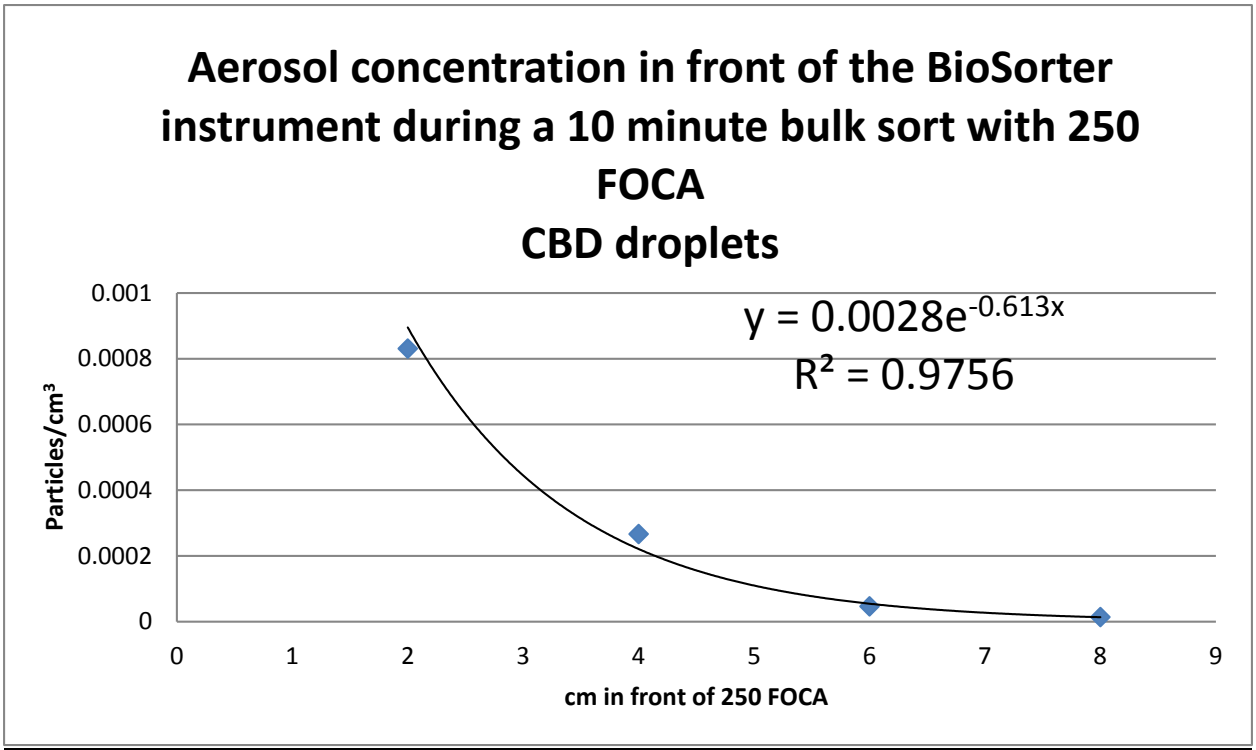

Figure 1. The average aerosol counts seen at various distances in front of the BioSorter instrument. It can be seen that the particles/cm3 follows an exponential decrease as the distance is increased from the front of the 250 FOCA

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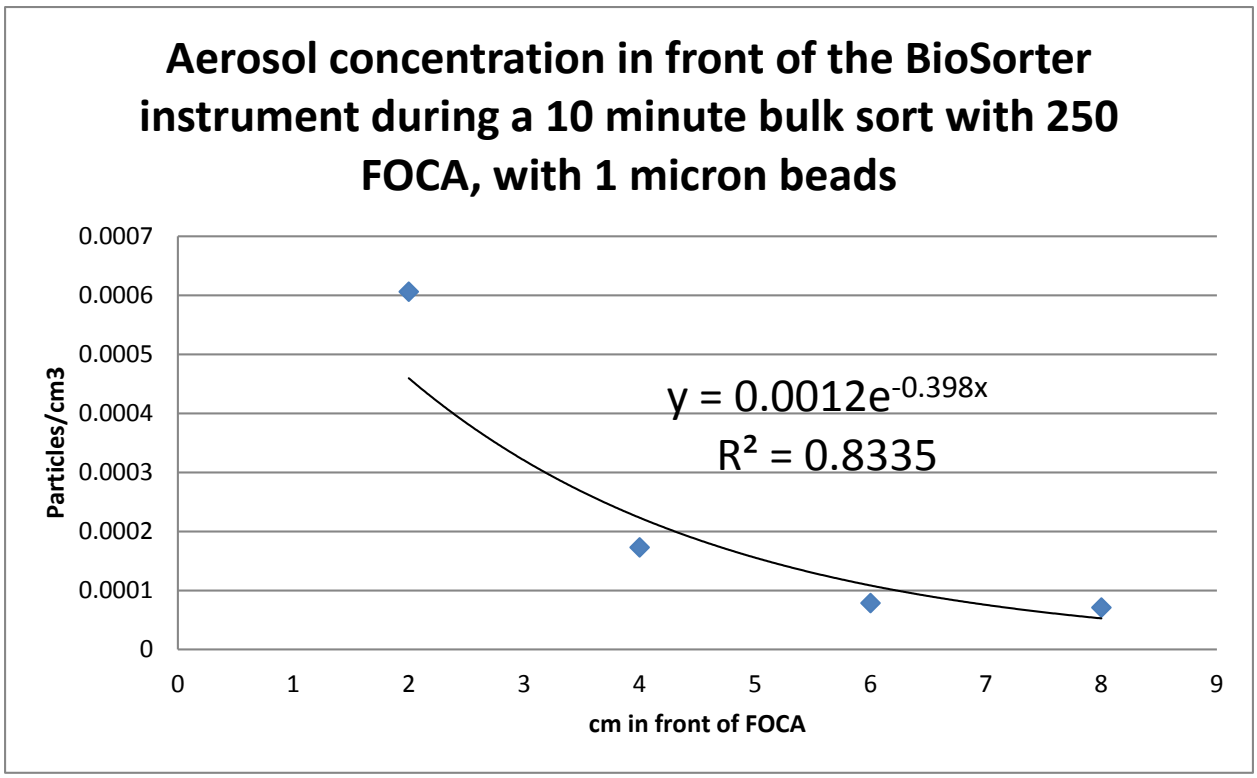

Figure 2. The average aerosol counts seen at various distances in front of the BioSorter instrument. It can be seen that the particles/cm3 follows an exponential decrease as the distance is increased from the front of the 250 FOCA

Table 2. Aerosol concentrations produced from the BioSorter instrument during a 10 minute bulk sorting operation using the 500 FOCA.

Distance from 500 FOCA	Particles/cm^3 for CBD droplets	Particles/cm^3 for one micron beads
2cm	$7.10x10^{-4}$ /cm^3 ± $4.59x10^{-4}$	$1.67x10^{-4}$ /cm^3 ± $1.64x10^{-4}$
4cm	$1.99x10^{-4}$ /cm^3 ± $2.09x10^{-4}$	$1.14x10^{-4}$ /cm^3 ± $1.13x10^{-4}$
6cm	$8.38x10^{-5}$ /cm^3 ± $1.87x10^{-4}$	$2.40x10^{-5}$ /cm^3 ± $1.51x10^{-5}$
8cm	$4.16x10^{-6}$ /cm^3 ± $6.64x10^{-6}$	$1.10x10^{-5}$ /cm^3 ± $8.21x10^{-6}$

The table above shows that there is a higher concentration of aerosols with CBD seen closer to the FOCA and decreases exponentially as the cassette was moved further from the stream source. It can be seen that there is a similar level of CBD droplets as compared to the one micron beads at 2 and 4cm, but when the cassette is moved to 6, and 8cm further from the source there are less CBD droplets but more one micron beads.

The 500 FOCA has more liquid passing through the flow cell than does the 250 FOCA and so it makes sense to see that there are more CBD droplets than 1 micron beads for the 500 FOCA than there are with the 250 FOCA. Also because the drops produced are

larger with the 500 FOCA there are larger aerosol droplets formed and they travel less far compared to the 250 FOCA.

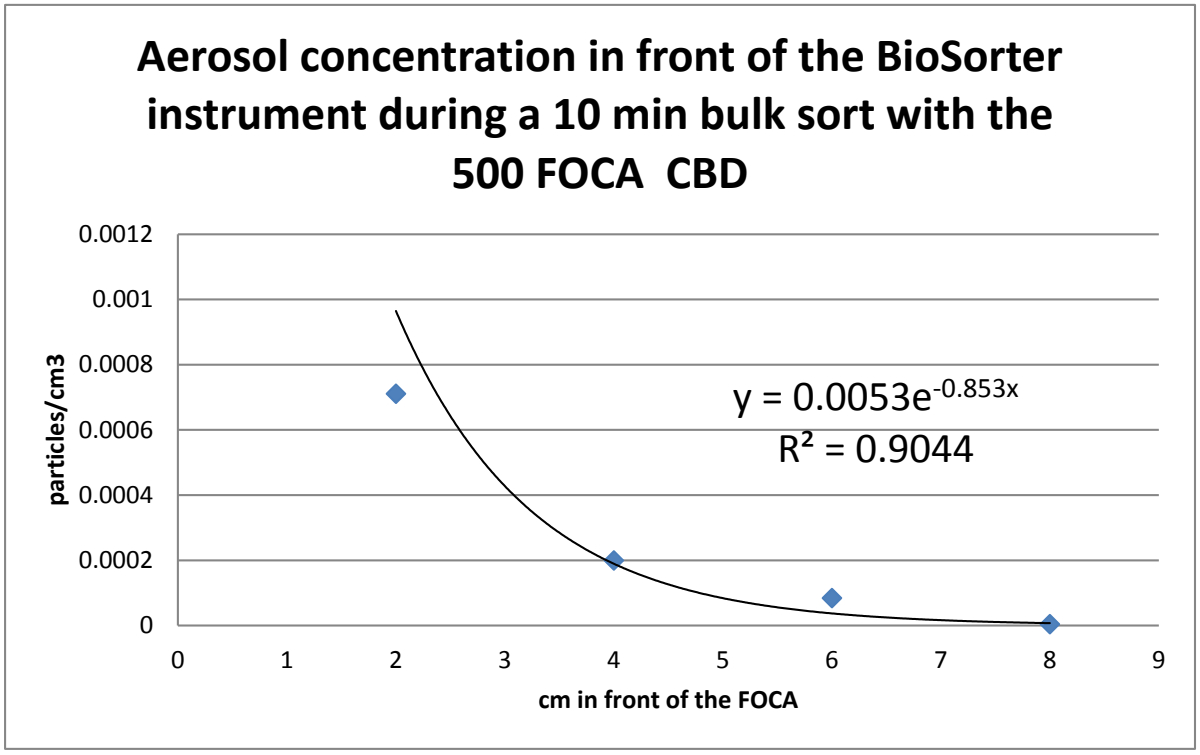

Figure 3. The average aerosol counts seen at various distances in front of the BioSorter instrument. It can be seen that the particles/cm3 follows an exponential decrease as the distance is increased from the front of the 500 FOCA. This could be the result of either the larger droplets not remaining air borne or not being generated.

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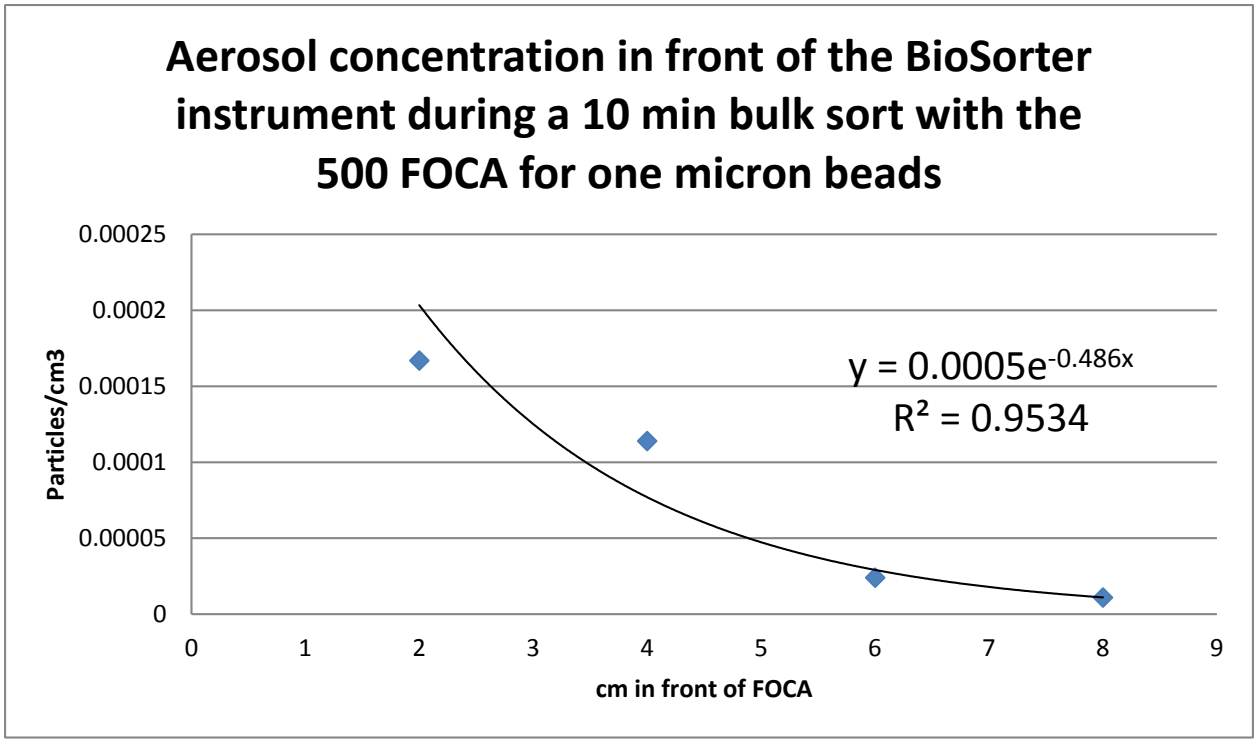

Figure 4. The average aerosol counts seen at various distances in front of the BioSorter instrument. It can be seen that the particles/cm3 follows an exponential decrease as the distance is increased from the front of the 500 FOCA.

2cm 250 FOCA	0-10µm	10-20µm	20-30µm	30-40µm	40-50µm	50-60µm	60µm +	total	
Test 1	9	27	25	9	18	9	17	114	count
	7.894737	23.68421	21.92982	7.894737	15.78947	7.894737	14.91228	100	%total
Test 2	4	25	25	14	9	9	18	104	count
	3.846154	24.03846	24.03846	13.46154	8.653846	8.653846	17.30769	100	% total
Test 3	18	24	32	17	9	7	18	125	count
	14.4	19.2	25.6	13.6	7.2	5.6	14.4	100	%total
Test 4	13	60	67	35	24	16	13	228	count
	5.701754	26.31579	29.38596	15.35088	10.52632	7.017544	5.701754	100	%total
Test 5	11	46	36	13	14	11	9	140	count
	7.857143	32.85714	25.71429	9.285714	10	7.857143	6.428571	100	%total
Test 6	25	126	138	60	25	18	34	426	count
	5.868545	29.57746	32.39437	14.08451	5.868545	4.225352	7.981221	100	%total
Test 7	0	16	15	7	8	2	11	59	count
	0	27.11864	25.42373	11.86441	13.55932	3.389831	18.64407	100	%total
Test 8	14	65	45	35	13	6	27	205	count

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	6.829268	31.70732	21.95122	17.07317	6.341463	2.926829	13.17073	100	%total
SD of %total	4.081572	4.53422	3.574272	3.04199	3.505888	2.225194	4.985351		

4cm	**0-10µm**	**10-20µm**	**20-30µm**	**30-40µm**	**40-50µm**	**50-60µm**	**60µm +**	**total**	
250 FOCA									
Test 9	25	25	21	22	19	13	19	144	count
	17.36111	17.36111	14.58333	15.27778	13.19444	9.027778	13.19444	100	%total
Test 10	6	15	19	8	6	2	6	62	count
	9.677419	24.19355	30.64516	12.90323	9.677419	3.225806	9.677419	100	%total
Test 11	3	13	19	0	5	1	1	42	count
	7.142857	30.95238	45.2381	0	11.90476	2.380952	2.380952	100	%total
Test 12	8	26	20	16	8	5	5	88	count
	9.090909	29.54545	22.72727	18.18182	9.090909	5.681818	5.681818	100	%total
Test 13	11	38	17	11	3	1	1	82	count
	13.41463	46.34146	20.73171	13.41463	3.658537	1.219512	1.219512	100	%total
Test 14	24	73	83	25	18	17	30	270	count
	8.888889	27.03704	30.74074	9.259259	6.666667	6.296296	11.11111	100	%total
Test 15	0	16	15	7	8	2	11	59	count
	0	27.11864	25.42373	11.86441	13.55932	3.389831	18.64407	100	%total
Test 16	8	43	17	16	10	7	11	112	count
	7.142857	38.39286	15.17857	14.28571	8.928571	6.25	9.821429	100	%total
SD of %total	5.038567	8.856183	9.996179	5.456943	3.349367	2.564143	5.751284		

6cm	**0-10µm**	**10-20µm**	**20-30µm**	**30-40µm**	**40-50µm**	**50-60µm**	**60µm +**	**total**	
250 FOCA									
Test 17	19	15	3	4	7	0	2	50	count
	38	30	6	8	14	0	4	100	%total
Test 18	1	5	6	3	1	0	2	18	count
	5.555556	27.77778	33.33333	16.66667	5.555556	0	11.11111	100	%total
Test 19	1	3	1	2	0	0	0	7	count
	14.28571	42.85714	14.28571	28.57143	0	0	0	100	%total
Test 20	9	18	6	0	2	1	0	36	count
	25	50	16.66667	0	5.555556	2.777778	0	100	%total
Test 21	4	2	0	0	0	0	1	7	count
	57.14286	28.57143	0	0	0	0	14.28571	100	%total
SD of % total	20.31096	10.02102	12.65854	12.15777	5.736175	1.24226	6.532682		

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UNION BIOMETRICA
Large Particle Flow Cytometry

2cm	0-10µm	10-20µm	20-30µm	30-40µm	40-50µm	50-60µm	60µm +	total	
500 FOCA									
Test 22	19	78	67	71	28	21	32	316	count
	6.012658	24.68354	21.20253	22.46835	8.860759	6.64557	10.12658	100	%total
Test 23	20	67	40	24	14	8	5	178	count
	11.23596	37.64045	22.47191	13.48315	7.865169	4.494382	2.808989	100	%total
Test 24	13	63	93	83	52	30	74	408	count
	3.186275	15.44118	22.79412	20.34314	12.7451	7.352941	18.13725	100	%total
Test 25	39	103	75	34	12	7	15	285	count
	13.68421	36.14035	26.31579	11.92982	4.210526	2.45614	5.263158	100	%total
Test 26	10	56	78	65	32	14	44	299	count
	3.344482	18.7291	26.08696	21.73913	10.70234	4.682274	14.71572	100	%total
Test 27	5	48	117	70	37	23	39	339	count
	1.474926	14.15929	34.51327	20.64897	10.91445	6.784661	11.50442	100	%total
SD of %total	4.908533	10.3004	4.841154	4.529151	2.985103	1.859261	5.723488		

4cm	0-10µm	10-20µm	20-30µm	30-40µm	40-50µm	50-60µm	60µm +	total	
500 FOCA									
Test 28	1	19	27	24	10	15	7	103	count
	0.970874	18.4466	26.21359	23.30097	9.708738	14.56311	6.796117	100	%total
Test 29	4	18	20	20	8	6	9	85	count
	4.705882	21.17647	23.52941	23.52941	9.411765	7.058824	10.58824	100	%total
Test 30	14	48	53	42	31	10	12	210	count
	6.666667	22.85714	25.2381	20	14.7619	4.761905	5.714286	100	%total
Test 31	4	18	15	12	7	1	4	61	count
	6.557377	29.5082	24.59016	19.67213	11.47541	1.639344	6.557377	100	%total
Test 32	18	37	48	39	13	9	7	171	count
	10.52632	21.63743	28.07018	22.80702	7.602339	5.263158	4.093567	100	%total
SD of %total	3.469289	4.120518	1.72414	1.871265	2.705536	4.831641	2.39273		

6cm	0-10µm	10-20µm	20-30µm	30-40µm	40-50µm	50-60µm	60µm +	total	
250 FOCA									
Test 33	19	15	3	4	7	0	2	50	count
	38	30	6	8	14	0	4	100	%total

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Test 34	1	5	6	3	1	0	2	18	count
	5.555556	27.77778	33.33333	16.66667	5.555556	0	11.11111	100	%total
Test 35	1	3	1	2	0	0	0	7	count
	14.28571	42.85714	14.28571	28.57143	0	0	0	100	%total
Test 36	9	18	6	0	2	1	0	36	count
	25	50	16.66667	0	5.555556	2.777778	0	100	%total
Test 37	4	2	0	0	0	0	1	7	count
	57.14286	28.57143	0	0	0	0	14.28571	100	%total
SD of % total	20.31096	10.02102	12.65854	12.15777	5.736175	1.24226	6.532682		

Aerosol Containment Techniques

There are several approaches to containment that have been explored on the BioSorter instrument. Most involve enclosures that can be constructed to surround either the whole instrument or just an area around the sorting area of the instrument. For the whole instrument, commercially available enclosures can be configured to create either a positive or a negative air-pressure region around the instrument. A positive pressure region around the instrument prevents aerosols and contaminates from entering from the surroundings. A lower pressure region around the instrument prevents aerosols that are generated by the instrument from leaving that area.

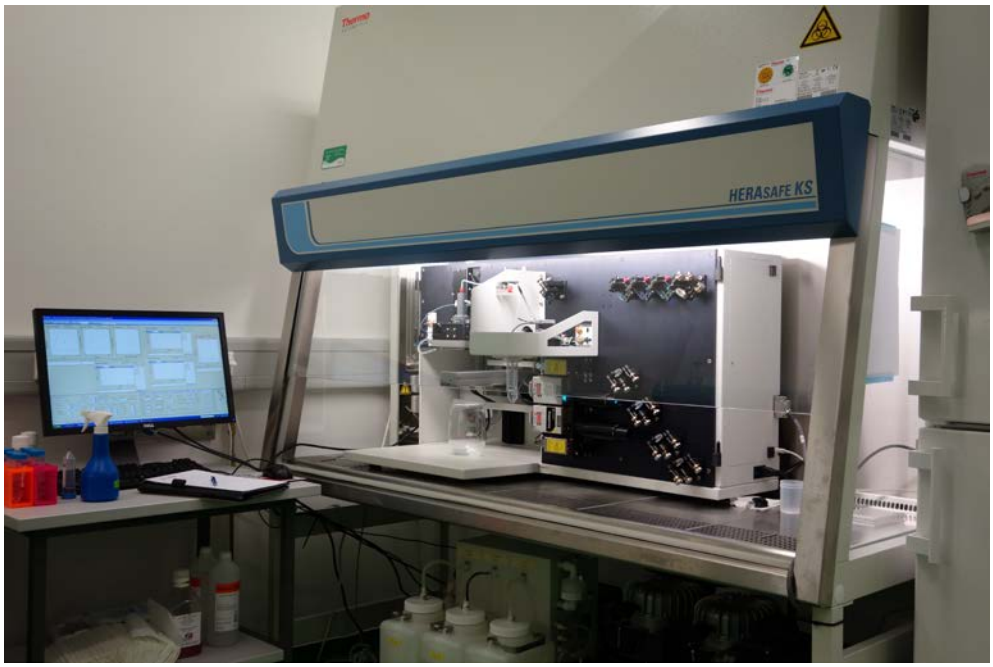

Figure 5: A BioSorter instrument inside a whole system containment enclosure.

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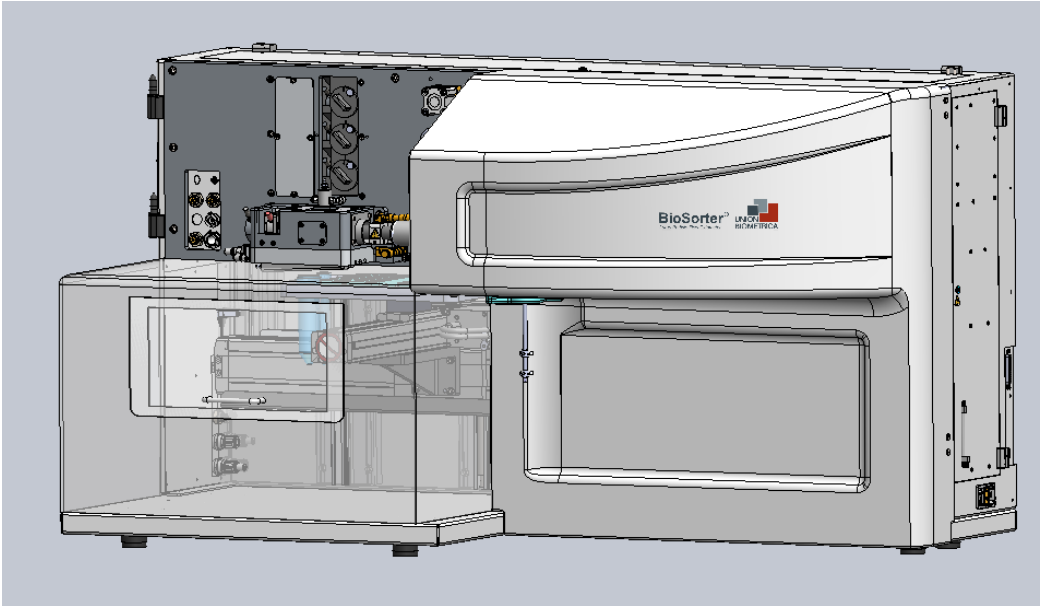

Figure 6: The BioSorter instrument with an enclosure surrounding the stage and sorting area only. A vacuum generator and filter would sit next to the instrument and are not shown.

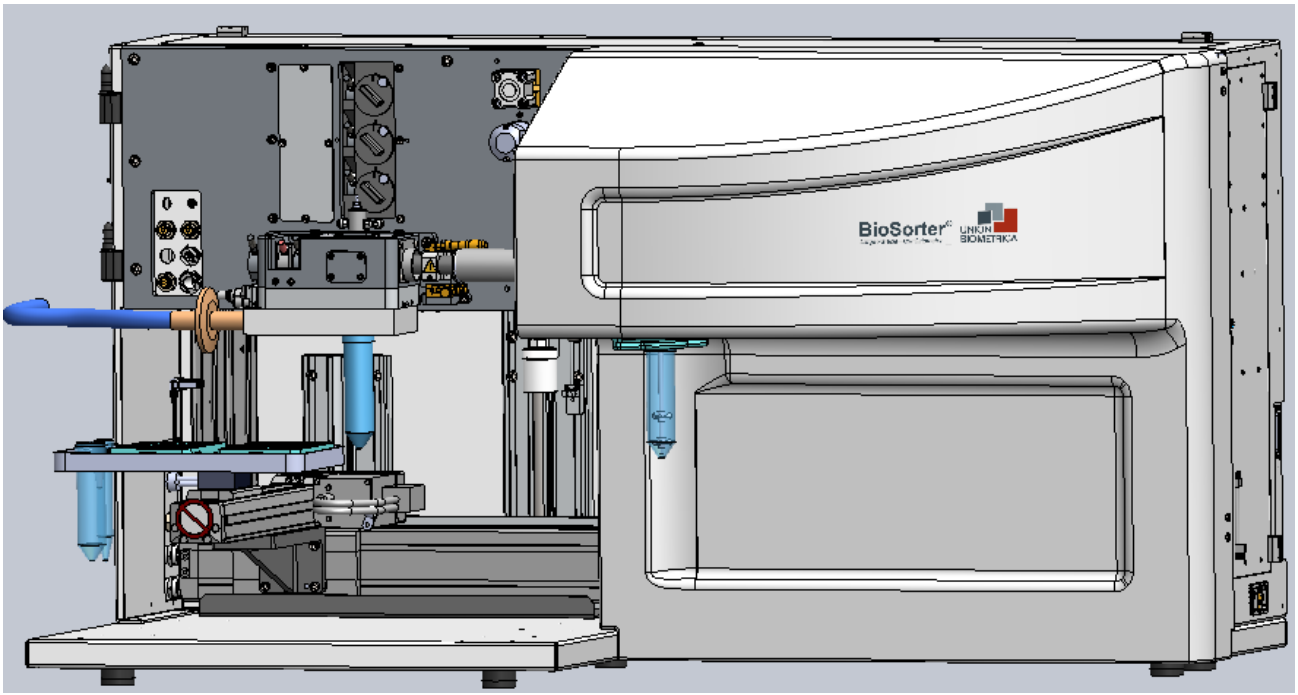

Figure 7: The BioSorter instrument with an enclosure only around the sample sorting area.

All aerosol containment enclosure assemblies consist of three main components: 1) an enclosure that surrounds the area where aerosols are generated; 2) a vacuum generator that takes air out of the enclosure and so creates the negative pressure environment; and 3) a filter between the vacuum generator and the enclosure that collects any

aerosols that leave the enclosure through the vacuum line. For example, an enclosure around the sample sorting area that would enable sorting into collection tubes could be designed as shown in Figure 7 and Figure 8. A larger enclosure like the one shown in Figure 5 and Figure 6 would allow for sorting into multiwell plates in addition to bulk collection into tubes. An idea vacuum generator for a BioSorter instrument would be a low vacuum, high flow device capable of flowing at least 5 CFM (cubic feet per minute) of air. Commercially available vacuum generators and aerosol collection devices can be purchased from companies like Buffalo Filter in Lancaster, NY.

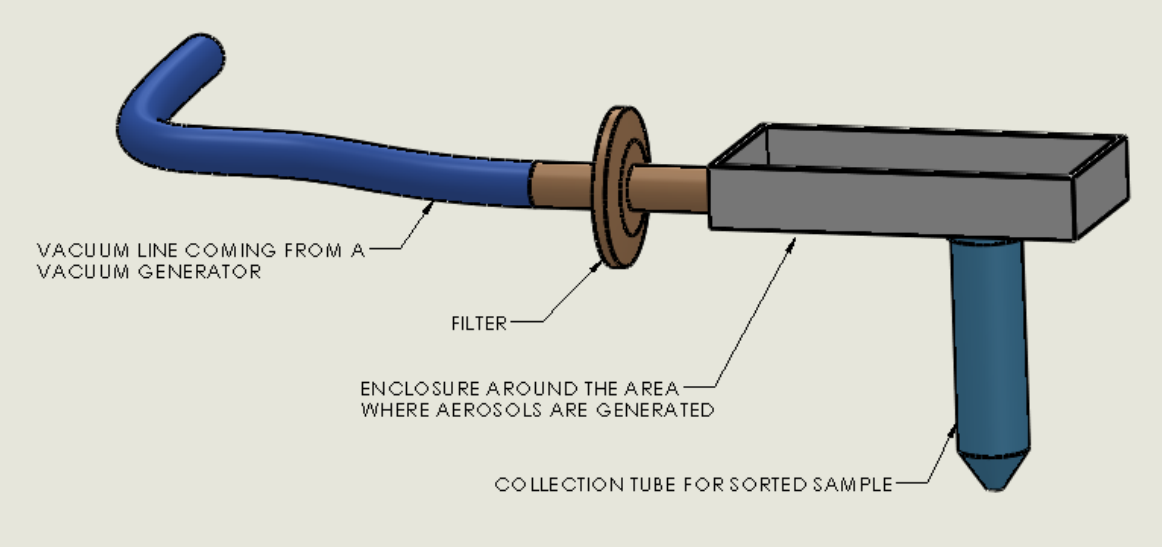

Figure 8: Detail of a typical aerosol containment enclosure

Conclusions

The BioSorter instrument generates aerosols in a limited area around the sorting region at the output of the flow cell. Outside of this 7-8 cm area, our testing has shown that the level of aerosols generated is not detectable above background. Aerosol containment enclosures add an additional layer of protection to the operators of the BioSorter instrument. Three possible designs have been presented here though others designs of physical barriers and vacuum systems could also be implemented. Union Biometrica's aerosol testing is specific to the environment and samples that were run on our instrument in Holliston, MA. Variation in instruments, settings and environments will affect aerosol production. This document is for general guidance only. Union Biometrica recommends that any aerosol containment system be verified in the environment and with the specific instrument and settings used with your sample.

Reference

1. Wanek, Paul, Smet, Francis, Peitz, Michael, Bongaarts, Rico, Pulak, Rock, Zenke, Martin; Automated analysis and sorting of Human Induced Pluripotent Stem cells (HIPS) clusters using large particle Flow Cytometry; Union Biometrica, Geel, Belgium, Institute for Biomedical Engineering, Universitätsklinikum Aachen, RWTH, Aachen, Germany, Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany, Union Biometrica, Holliston, MA, USA; June 14, 2012; ISSCR 2012
2. Wanek, Paul[1], Smet, Francis[2], Haupt, Simone[3], Peitz, Michael[3], Bongaarts, Rico[2], Oliver Brüstle[3], Pulak, Rock[4], Zenke, Martin[1] automated analysis and sorting of human ips cell clusters by large particle flow cytometry;) Institute for Biomedical Engineering, Department of Cell Biology, RWTH Aachen University Medical School, RWTH Aachen University, Aachen, Germany, 2) Union Biometrica, Geel, Belgium, 3) Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany, 4) Union Biometrica, Holliston, MA, USA June 13, 2013 ISSCR 2013
3. Pinkel,D.; Stovel, R. Flowchambers and Sample Handling. In: Van Dilla, MA.; Dean, PN.;Laerum, OD.; Melamed, MR., editors. Flow cytometry: Instrument and data analysis. London: Academic Press. P 77-128.
4. Kevin L. Holmes; Charcterization of aerosols produced by cell sorters and evaluation of containment., flow cytometry section, research technologies branch, national institute of allergy and infectious disease, national institutes of health, Bethesda, MD Cytometry A. 2011 December; 79(12): 1000-1008. Doi; 10.1002/cyto.a21163
5. Schmid I. Biosfatey guidelines for sorting of unfixed cells. Cytometry. 1997; 28:99-117. [pubmed:9181299]
6. Schmid I Lambert C, Ambrozak D, Marti GE, Moss DM, Perfetto Sp. International Society for Analytical Cytology Biosaftey standard for sorting of unfixed cells. Cytometry A. 2007; &1A:414-437. [PubMed: 17385740]
7. US department of health and human services centers for disease control and prevention and national institutes of health. Biosaftey in microbiological and biomedical laboratories. 2009. http://www.cdc.gov/biosaftey/publications/bmbl5
8. Oh M, Kim N, Huh M, et al. Scrub typhus pneumontis acquired through the respitory tract in a labraotry worker. Infection 2001; 29: 54-56. [PubMed:11261762]
9. Pentella, MA.; Kostle, PA.; Desjardin, L.; Gilchrist, MJR. Biosafety for Airborne Pathogens. In: Fleming, DO.; Hunt, Dleditors. Biological Saftey: principles and Practices. 4 Washington, D. C. AMS press; 2006. P 209-220