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Reimagining Flow Cytometric Cell Sorting

Suwan N. Jayasinghe

In this review, a brief history of this unrivaled technology, flow cytometry, is provided, highlighting its past and present advances, with particular focus on "flow cell" technologies. Flow cytometry has truly revolutionized high-throughput single cell analysis, which has tremendous implications, from laboratory to the clinic. This technology embodies what is truly referred to as cross fertile research, merging the physical with the life sciences. This review introduces the recent notable advancements in flow cell technology. This advancement sees the complete removal of liquid sheath flow, which has advanced the technology with the possibility of both the reduction in its foot print, while also simplifying the flow cells explored in cytometry. Interestingly, the novel sheathless flow cell technology demonstrated herein has the flexibility for handling both heterogeneous cell populations and whole organisms, thus demonstrating a versatile flow cell technology for both flow cytometry and fluorescent-activated cell sorting.

1. Introduction

Flow cytometry is a fluorescent-activated cell sorting (FACS) technology. This technology has seen advancements largely in laser sciences and fluid dynamics coupled with molecular and cellular biology, unearthing a powerful diagnostic platform, having transformative implications to healthcare. Literature demonstrates this technology was first described in 1934[1] and was then referred to as a photoelectric technique for counting microscopic cells. Since then the technology has undergone a rapid developmental journey, driven by the possible value to medicine. This has seen each element of this approach undergoing focused development.

The technology has many elements that contribute to its operation, namely, ranging from the fluidics, electrics, lasers to the basic molecular and cellular biology to finally automation through computer control, via both software and hardware. Each aspect plays its crucial role for successfully and accurately assessing the dynamics of single cells within a heterogeneous cell population. The reader should note that the author has chosen to describe flow cytometry first and later FACS. The author's reason for explaining the techniques this way round is primarily as this review is designed for lay readers, thus understanding the simplified equipment arrangement, prior to considering the more complex operation that takes place in the cell purification process.

Briefly, the cells and/or particles start their journey in flow cytometry as suspensions. Essentially, the fluidics (flow cell) component sees the technology take a near homogenously suspended cellular population, aspirating it in laminar flow conditions, for forming a liquid jet having a near single cell diameter. This is created by the suspension fed into a flow cell accommodating a needle, centrally placed in a cylindrical chamber, having a converging base, and exit, which is coupled with a short jet stabilizing nozzle. This chamber has another input, namely, the sheath flow. With the sheath and sample flowing, the exiting sample is squeezed by the sheath flow, for it to eventually pass through both the converging architecture at the base of the chamber and subsequently flowing past the jet stabilizing exit nozzle.[2] The sheath squeezing effect, both focuses and narrows the ensuing composite jet to nearly the diameter of the cells in suspension. This process is referred to as hydrodynamic focusing.[3] This exiting fine composite jet breaking into droplets subsequently passes the point referred to as the interrogation point, where it is exposed to the laser. There are two angles in which the laser assesses these cells while they are in the jet, namely, through forward and side scatter. The forward scatter assessment process identifies the cells by way of their size. Simply put, if one was to take two objects of different shapes and sizes and place in front of a source of light, their shapes and sizes would scatter different amounts of light. This would be evident by the reflective image of the shapes created of themselves seen on some collector, placed opposite to the light source. Forward scatter works on this principle and has a detector placed on the opposite side of the laser. Similar in some respects, side scatter works with a fluorophore which is tagged using some antibody on one side while the opposite end of the antibody is attached to the molecule of interest. Note that many years of research into molecular and cell biology has given rise to the development of advanced antibodies and fluorophores which enable many cellular components to be assessed in real

Prof. S. N. Jayasinghe
BioPhysics Group
Centre for Stem Cells and Regenerative Medicine
Institute of Healthcare Engineering and Department of Mechanical Engineering
University College London
Torrington Place, London WC1E 7JE, UK
E-mail: s.jayasinghe@ucl.ac.uk

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time. The enormous contributions from molecular and cellular biologists have seen the technology possess the ability to assess more than ten cellular components simultaneously. This has been with the developmentals, including those which have embraced new chemistries exploited for precise and specific molecular tagging for their dynamic assessment over a given course of time. In addition, side scatter assessment is via the scattered light undergoing identification by way of detectors. The analysis of forward and side scatter is then fed into a computer which not only automates the system but also controls the fluidic velocities and allows one to focus on either a single cell type within a heterogeneous population or the integrity of a selected molecule(s). The software deciphers the large datasets generated and makes sense of these data, by plotting a wide range of graphs for understanding the cellular wellbeing or the integrity of a molecule(s), etc., at a given time point. It is important to note that the lasers and detectors have many options, which allow the identification of a wide range of wavelengths using a selection of optical filters. This fine jet is subsequently broken down by way of vibrations into droplets. The vibrating force allows the jet to transition from a composite liquid meniscus to droplets, modulated depending on the cellular/particulate suspension properties. These postassessed cells are then collected into a waste curvet. At present flow cytometry has an analysis rate ranging from ≈1000 to 70 000 events s^{-1},[4] the variation exists depending on the machine costs and/or footprint, to other aspects such as number of lasers etc.

For FACS,[5] the flow cytometry equipment arrangement and operational process is modified where the assessed cells within the jet are broken into single-cell-bearing droplets using vibratory motion controlled via the software and hardware. These single cell droplets are then passed through a charging ring which is computer controlled in combination with a set of deflector plates. Here, gated cellular populations identified via the software within a heterogeneous cellular population could be charged differently at the charging ring via a high and/or low positive or negative charge. These differently charged droplets containing different cell types on entering the electric field generated through these deflector plates change their flight trajectories and are thus collected in separate curvets, hence purifying cell types. FACS has the ability to generate from four to six populations of purified cell types. At present sorting ranges from about ≈10 000–200 000 cells s^{-1},[6] which as in the case of flow cytometry varies with instrument capabilities etc. Although cell sorters and flow cytometers have the capacity to analyze cells, in true high-throughput fashion many aspects need to be carefully considered for harnessing accurate counts/analysis.[7] Both flow cytometry and FACS were developed for the analysis of blood,[8] and this however with the many advancements has seen these technologies widely being used for assessing both a wide range of cells and fertilized whole embryos.[9]

In this review, the author wishes to particularly focus on the flow cell technologies explored in both FACS and flow cytometry, and thus will discuss the many manifestations of flow cells. These manifestations of flow cells will be introduced, discussed in detail, and will end with the introduction of a recent development, which demonstrates a new flow cell technology, shown to operate without a liquid sheath. In the author's opinion, this has many advantages, such as the reduction in costs and foot print, to others which enable the flow cell to be interchangeable with ease, such that the analysis could include not only heterogeneous cell populations but could also assess whole fertilized embryos.

Suwan N. Jayasinghe leads the efforts of the BioPhysics Group at University College London. His group has made several discoveries in the direct handling of living cells and whole organisms for a wide range of applications, spanning the laboratory and the clinic.

The author wishes to group the flow cell technologies discussed herein into two categories they are, namely, channel/capillary-based and nonchannel/capillary-based flow cell technologies. For the purpose of clarity to the reader, the author refers to channel/capillary-based flow cells as those that use manifestations of lab on chip type flow channel or capillary systems.

2. Flow Cell Technology

Fluidics play a critical role in both the single cell analysis (flow cytometry) and their purification (FACS) as briefly described. In particular these cells to be either analyzed or sorted are presented in a single fold of cells which are near equally spaced. Essentially these cells when presented in this way for interrogation, i.e., exposure to the laser for analysis they cannot be resident anywhere within the jet. Meaning the laser is focused generally to the center of the ensuing composite jet. Therefore, if the cells are on the extremities or placed noncentrally within the jet, the analysis of these cells is affected. This cell positioning within the jet has a term associated to it, namely, the coefficient of variation.[10] Thus, if the coefficient of variation is narrow then the results have greater accuracy. Additionally, precise control of the jet and its break-up is crucial for cell purification in the case of FACS. Therefore, the involved fluidics are significant for both the accurate analysis of the dynamics of the cells and for their purification.

2.1. Channel/Capillary-Based Flow Cells

In this review, the author stresses this category or class of flow cells as those that have the architecture generally framed and referred to as a manifestation of lab on a chip, which sometimes are referred to as microfluidic systems.[11] Thus, containing fine channel/capillary(s) for controlling precisely, volumes of liquids/suspensions through architectures for forming singular cells within droplets or a stream of cells, centrally placed within a flowing stream of media. Note that in these systems (namely channel/capillary-based flow cells) the laser and analysis of the

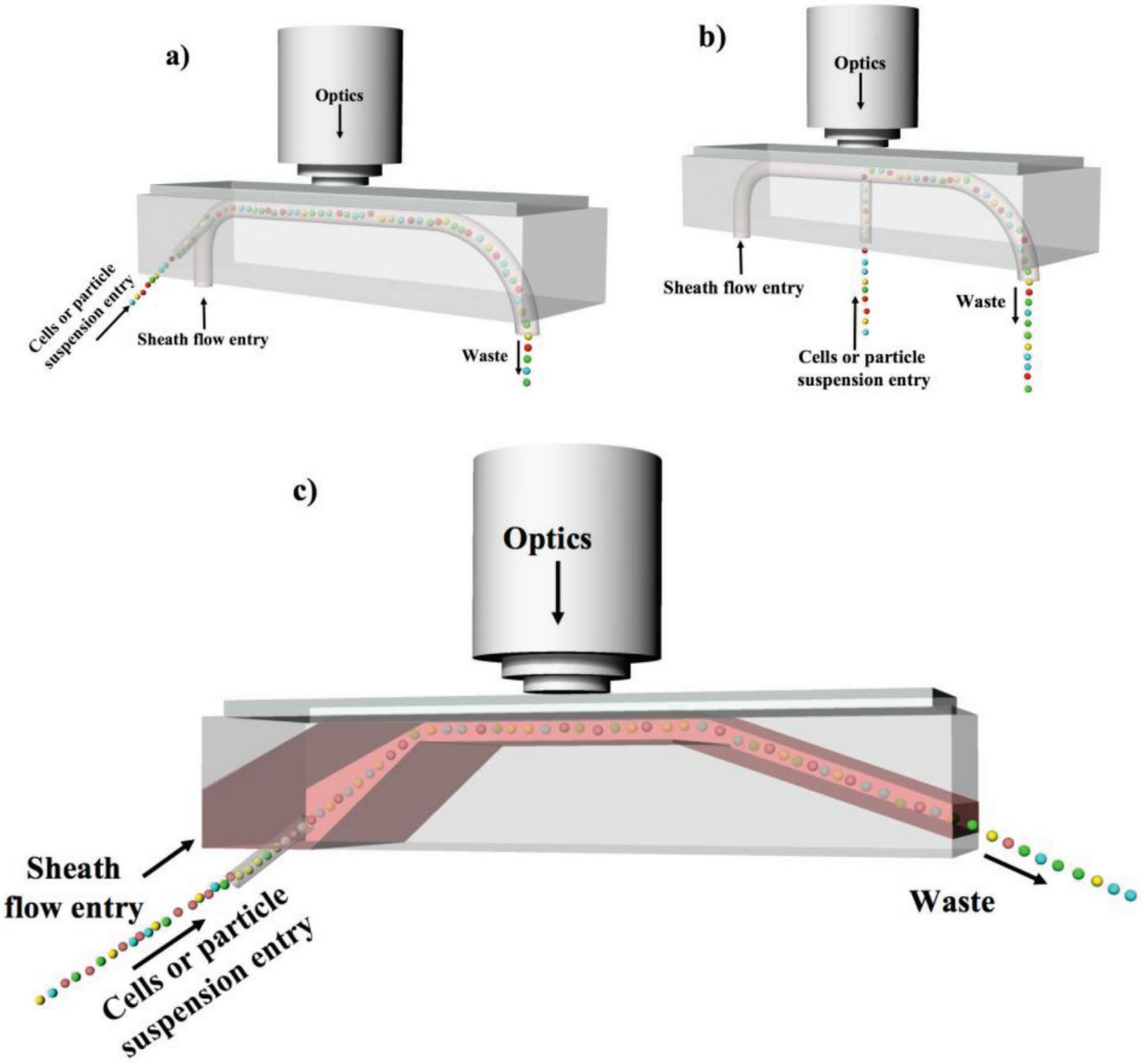

Figure 1. a–c) Characteristic depictions of three manifestations of lab-on-chip-based flow cytometry systems. Note that these three systems demonstrate the variation, of mixing the sheath and sample flows, early on a,c) or later b), giving rise to the ability to form a single fold of cells having a near equal spacing between cells. This single cell stream is later interrogated.

cells/particulates are carried out whist the samples are flowing, within the realms of a channel or capillary. **Figure 1** depicts many such channel-based flow systems[12] which have previously been explored and in some cases are still being explored. There are various manifestations of these architectures which differ in the supply of both the sample and the sheath. However, the overall flow and their coflow are somewhat similar.

Architectures depicted in Figure 1 and their manifestations[13] coupled with the sheath and sample velocities have given rise to some unique systems, allowing the user to visualize and analyze single cells and to further image the cell dynamics,[14] at that given time course. Such architectures have been multiplexed for batch processing of cells, which have shown their capacity and their ability to process increased cell numbers.[15] Similar in design there have been approaches where two needles facing and centrally in-line with each other have been fitted with a given spacing in between them. The spacing between the two needles has the supply and an exit for some biocompatible or an inert fluid continuously flowing through this space. The needles facing each other happen to have different internal bore diameters. The larger bore diameter accommodates the flow of the sample, while the fluid flowing in the spacing acts as the sheath flow. The finer needle aspirates the sample exiting the larger bore needle through the sheath flow within the spacing, thus focusing the sample through the fine needle, in a singular fold through for analysis.[16] A recent advancement

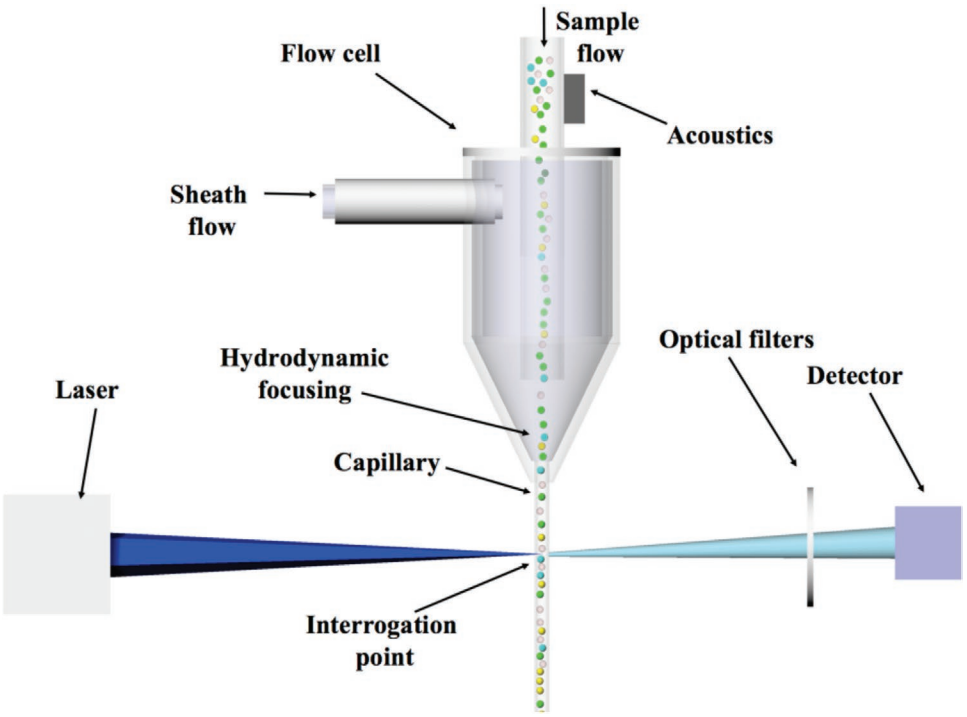

Figure 2. A representation of a sheath-flow-assisted acoustic flow cytometer. Here, the acoustically preorganized cells or particles flowing are accurately further aligned for interrogation by the laser.

in this category is where acoustics have been coupled with cells and particulates flowing within a capillary. Flow of such a suspension either containing cells or particulates generally would flow in a random fashion with either the cells or particles clustered and/or unevenly distributed within the capillary. However, when the acoustics are switched on, they generate waves (>2 MHz), which focus the cells or particulates within the carrier liquid, and subsequently these prefocused cells/particles are combined with a sheath liquid to further focus the cells/particles to the interrogation point (**Figure 2**). This technology is known as acoustic-focusing cytometry.[17]

Recently, the wealth of the knowledge generated through the above and many other systems in this class of flow cells have given rise to the technology referred to as fluorescence-activated droplet sorting (FADS).[18] Here an interestingly simple architecture commonly referred to as a T-junction has been employed for encapsulating the cells requiring purification. Once those cells have been compartmentalized or encapsulated with a given liquid and molecule, the sample is reinjected into another channel system coupled with AC electric fields to sort droplets into two channels. Note that the process here exploits dielectrophoresis for sorting. Although this technology is interesting, the additional steps is not ideal in a laboratory nor in a clinical setting. There are many different architectures explored in this endeavor.[19] That being said[20] demonstrated microchannel driven focusing as an interesting possibility for these architectures to be explored for single cell analysis. These advances have provided some inspiration for such systems to possibly be used for low viscosity or low density based samples to be analyzed. As present such approaches have not grabbed the community as they are limited to low viscosity samples, and therefore is seldom in their exploration in the clinic. However, such systems could be useful for analyzing rare heterogeneous cellular populations, which are generally found in low concentrations. The reader should also note well that this category of flow cells is primarily restricted to the analysis of the cells or molecules and not explored for purification or the sorting of cells.

2.2. Nonchannel/Capillary Based Flow Cells

There have been many difference designs in this category explored for utility as flow cells in both cytometry and sorting. These have ranged from ink-jet-driven approaches[21] to those which are commonly explored to date and referred to as hydrodynamic focusing.[22] Ink-jets have been investigated for its utility in this endeavor, but was seldom in its adoption as the processed biological molecules and cells have been found to get damaged through this processing approach and is limited in terms of the cell densities processable.[23] Therefore, the hydrodynamic focusing technology has been the sole principal which is used in a majority of flow cells.[24]

In this category of flow cells, the sample is generally made to flow in some centrally placed needle, within a converging chamber, which has the supply of sheath flow. The sheath fluid essentially flows around the needle accommodating the sample. The sheath flow influences the exiting sample through its needle, to draw into a liquid cone. As the sheath continues to flow it further encourages the sample cone to ensue a jet from its apex, which is later passed through the converging chamber exit at its base having a stabilizing nozzle. Following this the composite jet enters the atmosphere. The explored

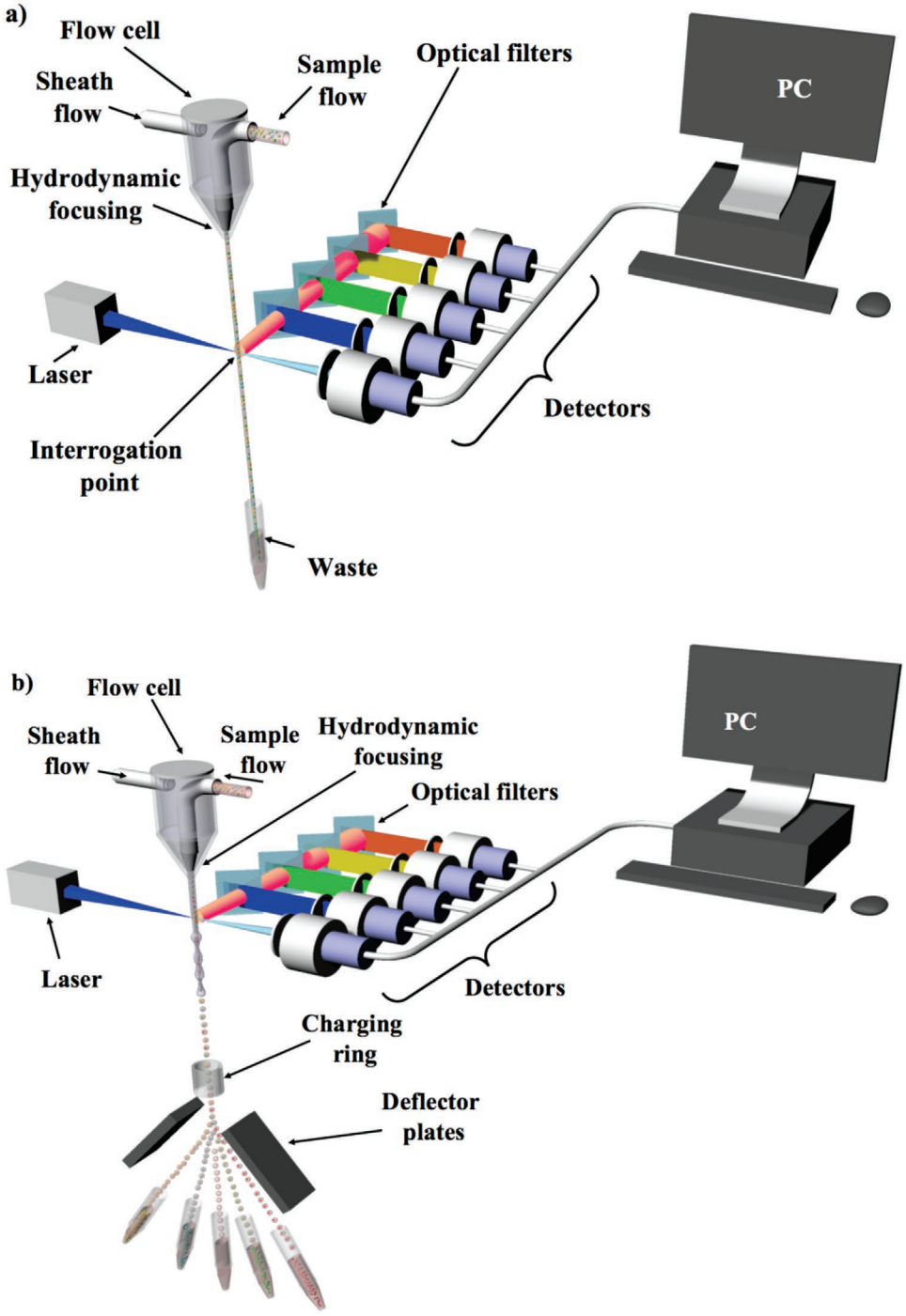

Figure 3. Characteristic drawings showing a) flow cytometry and b) fluorescent-activated cell sorting systems. The equipment arrangements and their individual elements (namely the flow chamber design, etc.) in both may vary from cytometer/sorter manufacturer to manufacturer but the working principle is largely similar. The reader should note that in FACS, the cells are analyzed first within the composite jet, consequently broken down into single-cell-bearing droplets for charging (at the ring), deflection (by the deflector plates) to finally purification. It should be noted that both these systems can also batch process, even larger volumes of samples. For this purpose, they have additional pieces of equipment, such as advanced liquid handling systems, allowing the feed of multiple samples for analysis.

sheath flow in these flow cells has several uses, from breaking cellular clusters within the sample to the narrowing of the sample into a meniscus roughly the diameter of the cell within the suspension. Thus, hydrodynamically focusing also assists the cells within the sample to be in a single fold and nearly equispaced. This stable composite jet (sheath and sample) now on entering the atmosphere is passed through the interrogation point and assessed for many cellular parameters (**Figure 3**a).

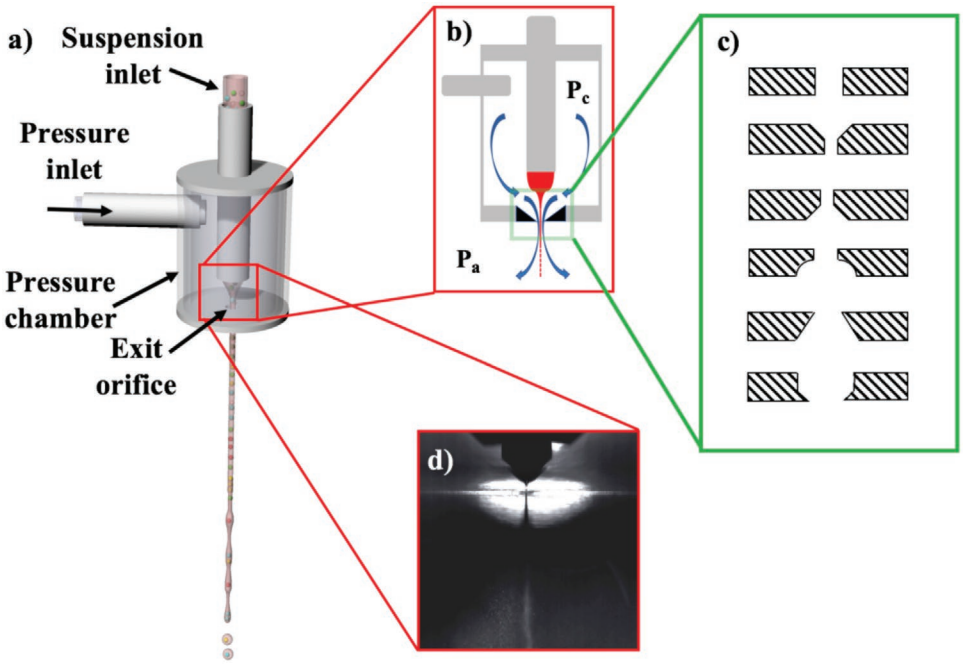

Figure 4. a) 3D drawing portraying the aerodynamically assisted biojetting process and b) a cross-sectional view of the chamber showing the pressure drop at the exit orifice as $P_c \gg P_a$, where P_c is pressure within the chamber and P_a is atmospheric pressure. Panel (c) depicts the variation in exit orifice geometries and panel (d) illustrates a captured high-speed digital image of the AABJ process in action, in particular the cone and jet formation within the chamber with the jet leaving the exit orifice.

The reader should note that this is carried out in combination with antibodies and fluorophores. The postassessed cells are then passed into a waste curvet. These systems are the most robust and allow the analysis of a wide range of high viscosity samples containing heterogeneous cell populations. A modification of this equipment arrangement enables this flow cytometry system to identify cells and purify them, into a given respective cell population. This fluorescent active cell sorting technology identifies cells through their size and markers with the aid of tagged fluorophores. Note that once the cells have been identified the user is allowed to gate the cell population of choice/interest through the software, thus enabling allocation of those selected cells to a given aliquot. Later those cells identified will be charged accordingly and deflected with the aid of the charging ring and deflector plates into that respective aliquot. FACS systems today allow the gating from four to six populations of cells to be purified from a heterogeneous cellular sample (Figure 3b). This form of sheath-driven hydrodynamics coupled with advanced optics and fast data acquisition has seen this system dominating both the flow cytometry and fluorescent-activated cell sorting industry.[25]

Hydrodynamically focused nonchannel/capillary based fluidic systems are well known and established for handling the largest populations of cells, implying they possess the capacity to handle highly viscous cell containing samples for both analysis and sorting. Hence, this together with advancements in optics, rapid data acquisition, and hardware enables these systems to be the go-to systems for high-throughput single cell analysis and sorting. Therefore, these systems have overcome all the obstacles faced by their rival systems (channel/capillary-based).

The flow cytometry and FACS arena is teaming, with many different flow chambers inspired by the hydrodynamic focusing concept. The reader should note well that we are referring to this focusing concept as applied to the nonchannel/capillary-based flow systems. Therefore, in this review, the author as mentioned previously takes this opportunity to introduce a manifestation of this principle, with the significant replacement of the liquid sheath with a pressurized sheath of air. The technology introduced herein is referred to as aerodynamically assisted biojets (AABJ). This technology results from the wealth of knowledge generated during the design and development, of jetting systems studied by the flow cytometry and FACS communities.[26]

The AABJ system works on the principle of a pressure variation, causing flowing liquid to be drawn out into a jet subsequently transitioning into droplets (**Figure 4**). The jetting process takes place as follows: the vertical needle as seen in Figure 4a accommodates the flow of a suspension either containing living cells or particles. Generally, a syringe pump feeds this to the suspension inlet. Simultaneously, pressurized air is fed into the pressure inlet, causing a rise in pressure within the chamber, with reference to the surrounding atmospheric pressure (Figure 4b). Pressure travels from high to low, and in this case from within the pressurized chamber through to the atmosphere across the exit orifice. This pressure drop over the exit orifice draws out the suspension flowing within the needle. This drawing effect results in a cone forming at the suspension needle exit, which further yields a jet from its apex (Figure 4a,b). The resulting jet is further squeezed by the pressure within the chamber to exit the chamber through the exit orifice (Figure 4a–c). The exit orifice geometries in this

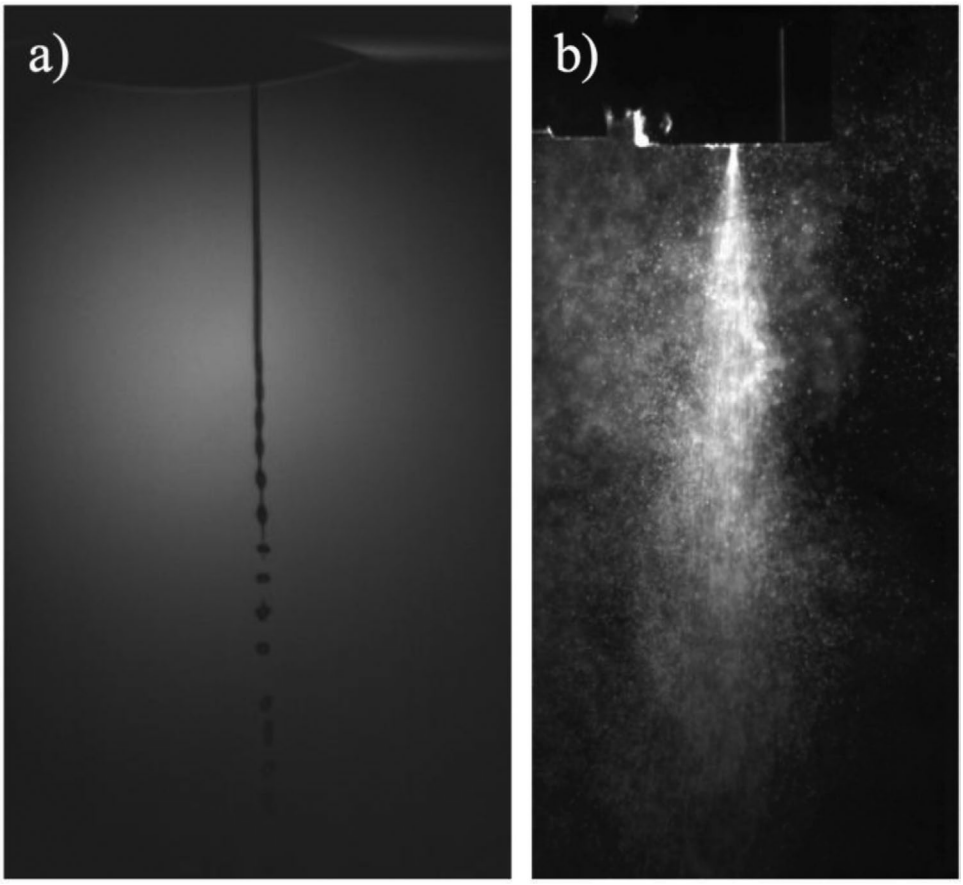

Figure 5. Typical high-speed digital images captured at 10 000 frames per second of a) the AABJ process in stable jetting conditions while panel (b) shows the formation of a spray plume at the exit orifice which is at elevated high applied pressures to the chamber. Similar jetting behaviors are exhibited in AAJ.

technology could be varied widely as shown in Figure 4c. The jet emanating through the exit orifice (Figure 4d) undergoes a variety of forces including air drag that assists the jet to transition into droplets through the jet whipping, thus forming a 3D conical spray plume. Note in this process the applied pressure to the chamber, the flow rate of the suspension to the needle, and its rheological and other properties together with chamber dimensions and exit orifice geometry allow the process to be versatile and fine-tuned. Hence, for given applied pressures to flow rates for a given device design and suspension properties, the emanating jet could be tweaked to form a conical spray plume or a laminar flowing jet which undergoes controlled droplet formation (**Figure 5**).

Prior to the discovery of AABJ in 2007,[27] this technology was referred to as AAJ (aerodynamically assisted jetting/jets) and was explored for the direct handling of a wide range of nonbiological materials.[28,29] These varied from nanotubes to nanoparticulates, for the purpose of controlled deposition for forming architectures, for both surface treatment to the profiling of surfaces for a multitude of applications, ranging from surface repellents, antimicrobial surfaces, drug containing patches to many others. In fact, AAJ has also been explored by Jayasinghe et al. as a novel materials processing technology for printing 3D architectures to forming compartmentalized multimaterial architectures.[30] These have been made possible as this technology is extremely versatile in allowing the material inlets (as suspensions), to be varied from single, coaxial to tri-needle systems[31] with the variation in chamber dimensions to the mixing and matching of exit orifice geometries. Interestingly, AAJ/AABJ has also been developed for the handling of viscosities ranging from high (>5000 mPa s) to ultraviscous liquids (>1000 000 mPa s), where the latter was for forming scaffolds from those continuous fibers generated, through the sister technology referred to as aerodynamically assisted threading and biothreading (AAT/AABT).[32] These studies allowed both the clear understanding of this jetting and threading process, to fully appreciate the device dimensions and the selection of options in its geometries best suited for adopting as a flow cell. Figure 5 illustrates the variation in operational parameters, allowing the control of the formation, of either a fine meniscus of liquid undergoing droplet break up and formation, to the complete removal of the existence of a jet thus forming a conical 3D mist of droplets. The reader should note that the chamber design parameters, such as the distance between the suspension needle exit to the exit orifice to the exit orifice geometry have significant effects on the formation and stability of these ensuing jets and their subsequent mechanisms of break up into droplets.

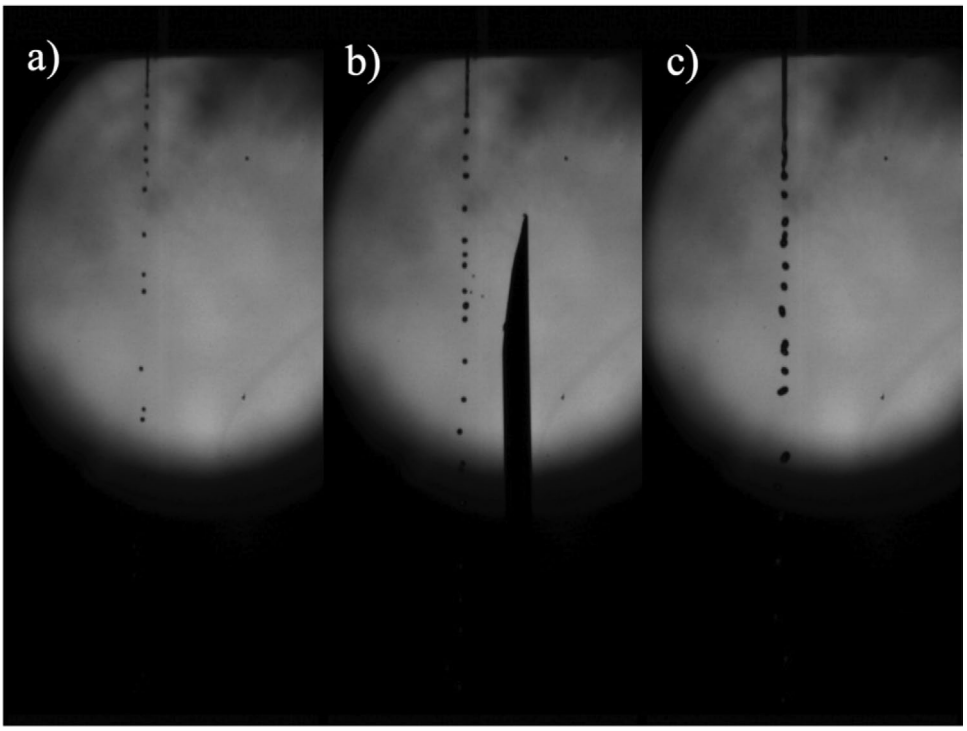

Figure 6. Representative digital high-speed images captured at 10 000 fps. Panels (a)–(c) depict the gradual reduction in applied pressure to the chamber for a given suspension flow rate to the needle. The reduction in applied pressure is shown to increase jet diameter and length, and increase the droplets sizes, together with reducing the droplet generation frequency. The hypodermic needle used for scaling in panel (b) is applicable to both panels (a) and (c) and is 34 gauge.

The effects of these operational parameters could also be used for controlling, jet diameter and length, the resulting droplet sizes to their production rates. **Figure 6** illustrates a parametric study which shows the ability to maintain a continuous suspension flow to the chamber, while decreasing the applied chamber pressure. The decrease in chamber pressure for a constant flow rate is seen to increase the jet diameter and its length, with the resulting generation of larger droplets at a lesser frequency (Figure 6). In Figure 6, the hyperdermic needle used in panel (b) is applicable for panels (a) and (c). All three digital images were captured at the same frame rate and magnification using a Phantom III high-speed digital camera system. Similarly, if the applied pressure was kept constant to the chamber, and the flow rate of suspension to the needle was increased, the jet would increase in both diameter and length and generate larger droplets at lower frequency. That being said a point would be reached where the pressure is too small for the increased flow rate that the process would reach instability and form unstable jets, which would later result in pulsating, thus forming a polydispersity distribution of droplets. This scenario is reversed and found in different operating conditions where the flow rate of the liquid was kept constant and the applied pressure to the chamber was reduced and increased respectively. Hence, these studies showed that for a given loading within a suspension the operational parameters allowed a working operational window to exist, which would permit the process to be fine-tuned for achieving the finest loaded droplets to be generated. Moving to either increasing or decreasing beyond these operational window space would see the jetting process reaching an unstable condition, thus generating polydispersed fine droplets to the forming of a spray without a jet as shown in Figure 5b.

From the demonstration of using this technology for both the processing of a wide range of advanced materials, spanning the structural and functional classes of materials, subsequent investigations established the ability for aerodynamically assisted jets to directly handle a wide range of cells and whole fertilized embryos.[33,34] The processed cells through this technology have undergone rigorous studies for assessing their viability and functionality from a molecular level upward, thus characterized via well-established biological protocols such as gene microarrays to those karyotypic studies.[34,35] Continued investigations have shown these post processed cells have been transplanted into mouse models, which have engrafted without rejection and thus have been comparable to those controls.[36] These studies together with our other investigations demonstrated AABJ's capability to directly handle highly concentrated cellular suspensions.

Having demonstrated through the many detailed studies we have carried out with AABJ, for directly handling a wide range of living cells, ranging from immortalized, primary (including stem cells and human spermatozoa) to whole fertilized embryos. This technology has been shown as possessing the ability to handle such sensitive materials without altering them from a molecular level upward, thus unlike its rival technology, namely, ink-jet printing. Therefore, this technology has the capability for its utility as a flow cell. Bearing this in mind, our preliminary studies have shown the ability to charge the jets

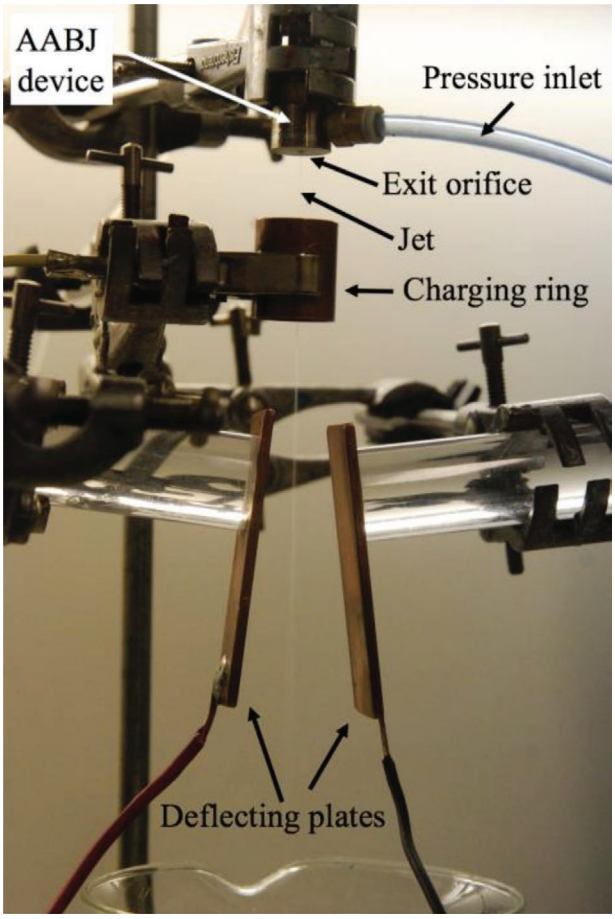

Figure 7. Digital image of the AABJ system incorporated into a simple arrangement coupling a charging ring with deflector plates.

with different voltages through the charging ring (**Figure 7**), subsequently deflecting them by way of charged defector plates.

The equipment arrangement explored in Figure 7 was set up, and a wide range of applied pressures to flow rates to charges applied to the ring together with the field applied to the deflector plates were studied. As mentioned previously many different operational spaces were seen to exists with the variation in the operational parameters (applied pressure, sample properties and flow rate, distance between sample needle exit and exit orifice, exit orifice geometry and diameter to the chamber volume to many others). **Figure 8** demonstrates the variation in droplet trajectories with increasing applied voltage at the deflector plates for a given applied charge at the charging ring. Closer examination of the jetting process was seen to not only generate primary cell bearing droplets but was also periodically seen to generate blank (noncell bearing) satellite droplets, which generally evolves at the droplet pinching and breaking off stage. The generation of these satellite droplets takes form when the jet undergoes the stable varicose behavior as shown in Figure 5a; here, the droplet break-up process sometime gets disturbed due to some droplets having multiple cells as oppose to a single cell. Hence, an increase in either cells/particulates results in a greater volume of liquid than those containing a single cell/particle. The excess

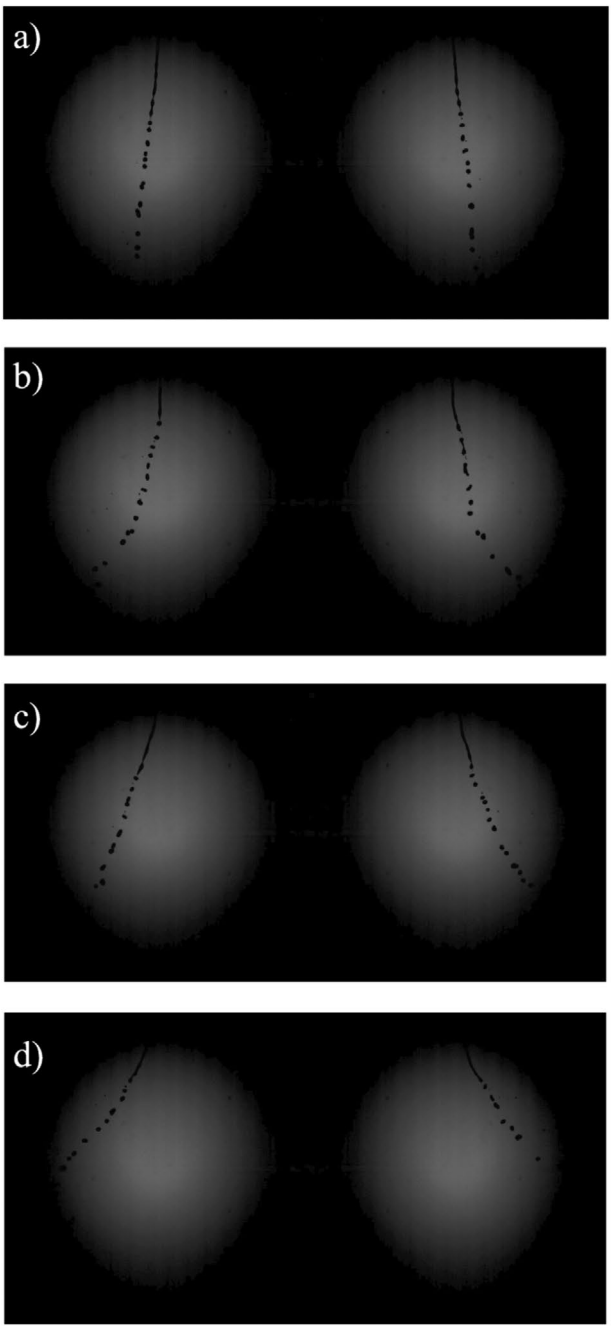

Figure 8. Characteristic digital images captured using a high-speed camera at the top end of the deflector plates. Panels (a)–(d) show droplet deflections for an applied voltage of ±4, ±5, ±6, and ±7 kV to the deflector plates, respectively, for an applied voltage to the ring at ≈±2 kV.

volume of liquid when narrowing down (sometimes referred to as pinching) just before break-up is drawn in two directions resulting in a small volume of liquid separating from the varicose jet and the detaching primary cells/particulates containing droplet. This small volume of liquid contracts and recoils rapidly during its formation as a result of its generation from the droplet being drawn from both sides, namely, the varicose jetting side and the droplet detaching side, but later forms a small

spherical droplet much smaller in size and volume in comparison to the primary droplet. The frequency at which such satellite droplets were generated was low. Another event observed during the close examination of the droplet production process was that some satellite droplets once again not very many were seen to coalesce with the primary droplets while in flight. We are currently investigating these effects on the droplet production process and hope to minimize this further, if not to completely eliminate the production or the occurrence of such events. Interestingly, such events are present even in those jets formed with a liquid sheath.[37]

The ring electrode we explored was also varied in both thickness and height and it was understood that this also has effect on the droplet charging process. Jetting behaviors were studied at the point where the jet was breaking up in the ring, this would cause disruption of the break up process and the charging was not accurate as the individual droplet were not getting charged accurately and individually thus effecting their flight trajectories. In this scenario, we also observed at elevated applied voltages to the charging ring, and at high flow rates, the detaching droplets would sometimes exhibit coulomb fission.[38] A process where the charge on the droplet surface exceeds the surface tension of the liquid, resulting in the droplet undergoing an exploding effect, forming a multiple number of smaller droplets of polydispersity, to sometimes jets from droplets which generate daughter droplets. Such events are frequently observed in electrosprays.[39] These investigative studies demonstrated that these effects can all be avoided with the equipment arrangement modified. An additional observation we noted during our studies was where we deemed the inlet of the applied pressure to the chamber to be directly above and not from the side as shown in Figures 4 and 7. In fact, since the AABJ devices could be modified with ease, we were able to alter the applied air pressure to the chamber vertically, while introducing the sample from the side with the sample accommodating needle still remaining centrally placed within the chamber and in-line with the exit orifice. This was seen to assist the stable jetting operational space to expand as the swirl angle of the applied air pressure, within the chamber had a lesser effect on the sample jetting process. Many other designs are currently being tested for investigating whether there is a wider operational space thus enabling this technology to afford a larger handling capability than those operating with liquids sheaths.

These investigative studies on AABJ provide an interesting alternative to those liquid sheath flow systems. Thus, allowing the unveiling of a multipurpose flow cytometric cell sorting system previously hither to unknown.

As a testament to the growing interests and constant advancement to flow cytometry and FACS, recently a technology referred to as mass cytometry was unveiled. Bendell et al.[40] in 2011 pioneered and coined mass cytometry, which is a technology manifested from mass spectrometry working with induvial coupled plasma and time of flight mass spectrometry for analyzing cells. Here unlike in classical flow cytometry and FACS antibodies are conjugated with isotopically pure elements, where these antibodies are explored for tagging cellular proteins. These labeled cells are subsequently introduced via a spray into an argon plasma, which burns the cells and ironizes the conjugated antibodies. The signals from the pure elements are then analyzed through a time-of-flight mass spectrometer. Mass cytometry has the promise of overcoming spectral overlapping faced by the use of fluorophores in flow cytometry with the ability to analyze >50 cellular parameters in a single experiment. That being said this platform is extremely expensive, is limited to the analysis of low cell counts, and does not allow the cells to be harvested postanalysis, which could be a disadvantage. Nevertheless, in the author's opinion this technology has its place in the cell analysis endeavor, where mass cytometry alongside conventional flow cytometry would be able to contribute for identifying many aspects of cells and embryos thus permitting one to see the face of the enemy and its manifestations over a time course.

3. Applications and Future

This novel liquid sheathless flow cell technology allows the operation to be more economical as it does not require the sheath liquid, and it therefore reduces the concern of contamination of samples where analyzed samples would be required for transplantation. AABJ also allows the user to have a wider space in which one could modify the device settings for handling a widespread range of heterogeneous populations of cells to those whole fertilized embryos. The author envisages the embryo purification capability having most applications in the agriculture (sexing) and aquaculture industries at present, but has the potential in the foreseeable future for assessing the quality of human embryos, which could possibly have a larger percentage of guaranteeing successful fertility in the case of IVF to those many other fertility issues faced. Additionally, in a laboratory setting such a device would enable a larger user base to explore one machine as opposed to having those that could only handle cells or embryos.

4. Conclusions

In this review, the author has provided the reader with a brief history of both flow cytometry and FACS. The approaches have been described with the author separating the many manifestations of flow cells explored in either cytometry and sorting into two categories, based on there working principles. This has also included expressing in the author's opinion, advantages and disadvantages of both categories. Moreover, the review goes on to unveil a novel liquid sheathless flow cell technology, referred to as aerodynamically assisted biojets, which operates on the principle of a pressure gradient over an exit orifice. The technology evolution was described with its working principles, while demonstrating its applicability as a flow cell technology for handing heterogeneous cell population and fertilized whole embryos. Our studies demonstrate the promise this liquid sheathless technology has as a flow cell platform, for cytometry and cell sorting. Ongoing efforts are looking at equipment development investigations in those aspects elucidated herein and hope to see this technology soon incorporated into both flow cytometry and FACS. The sheathless nature of this flow

cell technology not only reduces its footprint but also permits the user to vary the equipment setups, thus enabling the analysis of a wide range of heterogeneous cellular populations and fertilized whole embryos. The flexible nature of this technology is unrivaled and has much relevance in both the laboratory and the clinic.

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Conflict of Interest

The authors declare no conflict of interest.

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