UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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NANOCELLECT BIOMEDICAL, INC.,
Petitioner,

v.

CYTONOME/ST, LLC,
Patent Owner.

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Case No. IPR2020-00548
Patent No. 8,623,295

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# DECLARATION OF BERNHARD H. WEIGL

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I, Bernhard H. Weigl, declare as follows:

## I. ENGAGEMENT

1. I have been retained by counsel for NanoCellect Biomedical, Inc. as an expert witness in the above-captioned proceeding. I have been asked to provide my opinion about the state of the art of the technology described in U.S. Patent No. 8,623,295 to Gilbert, *et al.* ("the '295 patent," EX1001) and on the patentability of claims 1-3, 9, and 17-18 of this patent. The following is my written testimony on these topics.

2. I am being compensated at a rate of $250 per hour for my study and other work in this matter. I am also being reimbursed for reasonable and customary expenses associated with my work in this investigation. My compensation is not contingent upon the outcome of this matter or the specifics of my testimony.

## II. QUALIFICATIONS

3. I am currently employed as the Director of the Center for In-Vitro Diagnostics and a Principal Investigator at Intellectual Ventures/Global Good (IV/GG). The Global Good unit in which I serve is directly funded by Bill Gates. I also serve as an Affiliate Professor in the Department of Bioengineering at the University of Washington.

4. I joined Intellectual Ventures in 2015. Before that, starting in 2004, I worked at PATH in Seattle, Washington, serving as the Portfolio Leader in Diagnostics for Non-Communicable Diseases, as well as Group Leader for

Diagnostics, and Director of the Center for Point of Care Diagnostics for Global Health. Between 1995 and 1997 I was a Post-Doctoral Fellow in Professor Paul Yager's group in the Department of Bioengineering at the University of Washington, and in 1997 a co-founder and until 2003 Director of Microfluidic Applications for the microfluidics-based point-of-care diagnostics company Micronics, Inc. of Redmond, Washington (now part of Sony America). I also worked at the microfluidic engineering firm MicroPlumbers MicroSciences of St. Paul, Minnesota during this time.

5. My primary research interests include traditional and paper-based microfluidics as well as any assay platform that allows simplification and integration of previously complex assays. As chronic diseases, and especially diabetes, are emerging as a major health threat in developing countries, I am now also focusing on their diagnosis, screening, and treatment. Other areas of interest include diagnostics-related health systems topics such as more rational diagnostic algorithms and the evaluation and selection of the most appropriate diagnostic tool for a particular application using metrics beyond just sensitivity and specificity.

6. I received my bachelor's degree in Chemistry, my M.Sc. degree in Analytical Chemistry (1991), and my Ph.D. on the topic of Optical Chemical Assays (1993) from the University of Graz, Austria. Thereafter, I completed my post-doctoral studies at the University of Southampton, UK and the University of

Washington, Seattle, Washington. I also received a Certificate in Technology Management from the University of Washington in 1998.

7. I am listed as an inventor or co-inventor on at least 35 issued U.S. patents and am the co-author of over 120 scientific publications. My work has been published in numerous journals, including *Nature*, *Science*, *Nature Biotechnology*, *Biomedical Microdevices*, *Sensors and Actuators B*, *Lab on a Chip*, *Analytical Chemistry*, *Microchimica Acta*, and *Microfluidics, BioMEMS, and Medical Microsystems*. I am a member of SPIE, ASTMH, AACC, and AAAS, and have served on the editorial board of proceedings and journals published by SPIE and IEEE. I have also provided manuscript reviews for publications for other journals and have authored or co-authored numerous book chapters.

8. The fields of endeavor at issue in this case are microfluidic and particle sorting devices. I have spent over 30 years studying, researching, and developing microfluidic systems, including microfluidic particle sorting devices. I have presented and published over 100 research papers on microfluidic devices, many of which were designed to sort particles. This research has included performing three-dimensional focusing in microfluidic disposable-based flow cytometry. I have also received many research grants and have been the principal investigator on many research ventures involving microfluidics and microfluidic systems.

9. For a more detailed listing of my credentials and publications, please see my *curriculum vitae*, attached as EX1003.

## III. LEGAL PRINCIPLES

10. I have been advised that a claimed invention is not patentable as anticipated under 35 U.S.C. §102 if each and every element of the claim is disclosed, either expressly or inherently, in the claimed configuration in a single prior art reference. I have also been advised that a claimed invention is not patentable under 35 U.S.C. §103 if the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the claimed invention was made. I understand that the obviousness analysis does not require that all elements of the challenged claim be disclosed in a single prior art reference.

11. I further have been advised that the ultimate conclusion of whether a claim is obvious should be based upon several factual determinations. That is, a determination of obviousness requires inquiries into: (1) the level of ordinary skill in the field; (2) the scope and content of the prior art; (3) what difference, if any, existed between the claimed invention and the prior art; and (4) any secondary evidence bearing on obviousness.

12. I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in

the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

13. I have also been advised that, in determining the scope and content of the prior art, a reference may be relevant if it is in the same field as the claimed invention or is from another field to which a person of ordinary skill in the field would have looked to solve a known problem.

14. I have been advised that, in considering the obviousness of a patent claim composed of several elements, I should consider whether there is a reason that would have prompted a person of ordinary skill in the field to combine the elements or concepts from the prior art in the same way as in the claimed invention.

15. I have been further advised that obviousness may be demonstrated by showing the claim was merely the result of applying common sense and ordinary skill to solve a known problem. I have been advised that market forces or other design incentives may be what produced a change, rather than true inventiveness. I understand that I may consider whether the claimed subject matter was merely the predictable result of using prior art elements according to their known functions and whether the claimed innovation applies a known technique that had been used to improve a similar device or method in a similar way. I may also consider whether there is some teaching or suggestion in the prior art to make the

modification or combination of elements claimed in the patent. I may also consider whether the claimed invention would have been obvious to try, meaning that the claimed innovation was one of a relatively small number of possible approaches to the problem with a reasonable expectation of success by those skilled in the art.

16. I have also been advised, however, that I must be careful not to determine obviousness using the benefit of hindsight; many true inventions could seem obvious after the fact, in view of what we know today. Thus, in my analysis below, I put myself in the position of a person of ordinary skill in the field at the time the claimed invention was made and did not consider what is known today or what is learned from the teaching of the patent in reaching my conclusions.

17. Finally, I have been advised that any obviousness rationale for modifying or combining prior art should include a showing that a person of ordinary skill would have had a reasonable expectation of success.

18. With regard to secondary considerations of nonobviousness, I have been advised that objective evidence may be considered for an indication that the claimed invention would not have been obvious at the time the claimed invention was made. I understand that the purpose of secondary considerations is to prevent a hindsight analysis of the obviousness of the claims.

19. I have been advised that there are several factors that may be contemplated as a secondary consideration. These factors include the commercial

success of the invention, industry praise for the invention, skepticism of those in the field regarding the invention, licensing of the invention, copying of the invention, a long-felt need that the invention solved, failure of others to achieve the invention, and unexpected results of the invention.

20. I have been further advised that in order for evidence of secondary considerations to be significant, there must be a sufficient nexus between the claimed invention and the evidence of secondary considerations. I understand that this nexus must provide a link between the merits of the claimed invention and the evidence of secondary considerations provided.

## IV. SUMMARY OF OPINIONS

21. In my opinion, each and every element of each of claims 1-3, 9, and 17-18 of U.S. Patent No. 8,263,295 (EX1001) is found in the prior art. As I set forth in more detail below, each of these claims recites a microfluidic device with conventional, well-known features. Examples of such devices are described in, *e.g.*, (1) International PCT Publication No. WO 00/70080 to Wada, *et al.*, entitled "Focusing of Microparticles in Microfluidic Systems," published on November 23, 2000 ("Wada," EX1006); (2) U.S. Patent No. 5,101,978 to Marcus (issued April 7, 1992) ("Marcus," EX1005); and (3) International PCT Patent Publication No. WO 97/02357 to Anderson, *et al.*, entitled "Integrated Nucleic Acid Diagnostic Device," published on January 23, 1997 ("Anderson," EX1012).

22. Below are exemplary grounds upon which I believe claims 1-3, 9, and 17-18 of the ’295 patent to be unpatentable.

| Ground | Claims | References |
|---|---|---|
| **1** | 1-3, 9, 18 | Obvious over Wada |
| **2** | 1-3, 9, 17-18 | Obvious over Wada in view of Anderson |
| **3** | 1-3, 9, 17-18 | Obvious over Marcus in view of Anderson |

## V. SUMMARY OF THE ’295 PATENT

23. The ’295 patent is entitled “Microfluidic System Including a Bubble Valve for Regulating Fluid Flow through a Microchannel” and is assigned to Cytonome/ST, LLC (referred to herein as “Cytonome,” “Applicant,” or “Patent Owner”).

24. The ’295 patent describes a microfluidic system that uses a bubble valve and an actuator to “separate[e] particles having a predetermined characteristic from particles” that do not. EX1001 (the ’295 patent), Abstract, 4:31-34. The ’295 patent readily notes that microfluidic systems that use bubble valves and actuators were known in the prior art. For example, the ’295 patent describes a microfluidic device comprising a chip, a liquid flow channel, and a bubble valve that controls the flow of liquid in the channel:

> U.S. Pat. No. 6,062,681 describes a bubble valve for a liquid flow channel in which the flow of a liquid is controlled by the generation of a gas bubble in the channel using a heater placed in the liquid. As the

heater is activated, a bubble is formed which can be enlarged or reduced in size by increasing or decreasing, respectively, the temperature of the heater.

EX1001 (the ’295 patent), 2:27-55 (noting “[o]ther valves in the prior art use electrochemical means to produce a bubble in a liquid”). The specification of the ’295 patent sets forth the use of an externally operated mechanical or pneumatic actuator bubble valve for regulating fluid flow through a channel without heating the fluid as the point of novelty. *Id.*, 2:36-3:3.

25. The ’295 patent has 18 claims. Of the six claims upon which I have been asked to focus (claims 1-3, 9, and 17-18), only claim 1 is an independent claim. Specifically, claim 1 of the ’295 patent recites:

1. A microfluidic system comprising:

a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;

a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel; and

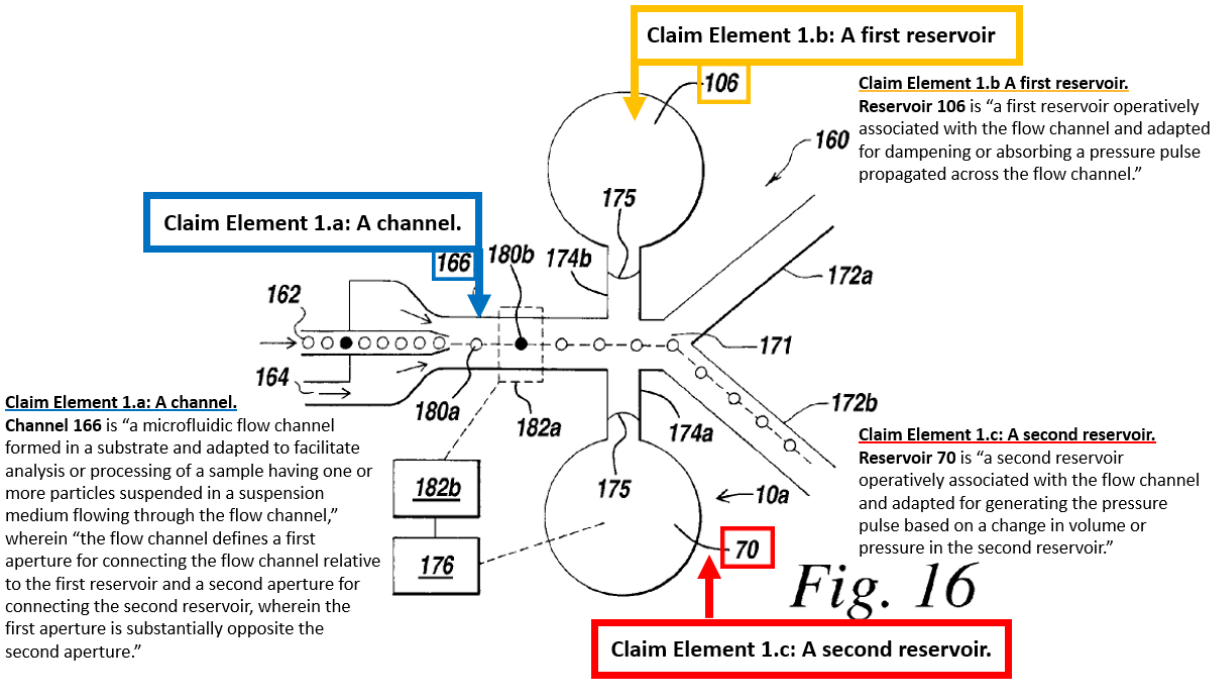
a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir;

wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for

connecting the flow relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.

EX1001 (the ’295 patent), 13:42-14:3.

26. Thus, claim 1 recites a microfluidic system that comprises three main elements: (a) a microfluidic flow channel; (b) a first reservoir; and (c) a second reservoir. These three elements are shown on the annotated Figure 16 from the ’295 patent, below, for convenience. *See* EX1001 (the ’295 patent), FIG. 16.




27. The use of the claimed microchannel geometry with a channel, chamber, or reservoir opposing the source of the pressure pulse to absorb the pressure pulse was well known and well documented in the art. *See*, *e.g.*, U.S. Patent No. 5,101,978 to Marcus (issued April 7, 1992) (“Marcus,” EX1005), FIG.

1; EX1006 (Wada), FIGS. 22-24; and U.S. Patent No. 4,936,465 to Zöld (issued June 26, 1990) ("Zöld," EX1007), FIG. 2.

28. Claims 2-3, 9, and 17-18 each depend from independent claim 1. In my opinion, these dependent claims fail to recite anything that was not already known in the existing art. For instance, claim 2 recites the known use of an actuator or pressurizer for generating a pressure pulse by varying the volume or pressure in the second reservoir. EX1001 (the '295 patent), 14:4-14:7. Claim 3 recites known arrangements wherein the flow channel includes a branched portion, and wherein the pressure pulse is used to sort the particles into a selected branch of the branched portion. *Id.*, 14:8-14:11. Claim 9 further adds that the first reservoir includes a resilient wall for dampening or absorbing of the pressure pulse, which was also known in the art. EX1001 (the '295 patent), 14:25-14:27. Claim 17 adds a deflectable flexible membrane in the second reservoir. EX1001 (the '295 patent), 14:46-14:49. Lastly, claim 18 merely recites that the change in volume or pressure of the second reservoir is a transient change. *Id.*, 14:50-14:52.

**A. File History of the '295 Patent**

29. The '295 patent states on the front page that it was filed on September 26, 2011 as U.S. Patent Application No. 13/245,331 to Gilbert *et al.* EX1001, [12], [21], [22]. I understand that the earliest claimed priority date for the '295 patent is

April 17, 2002, the filing date of U.S. Provisional Patent Application No. 60/373,256 to Gilbert, *et al.*

30. During examination, the underlying application that issued as the ’295 patent was rejected in view of some prior art, including European Patent No. 1,065,378 to Unger, *et al.* EX1004 (the ’295 file history), 0045-46.

31. I understand that no assertions of secondary considerations, such as commercial success of the invention, industry praise for the invention, skepticism of those in the field regarding the invention, licensing of the invention, copying of the invention, a long-felt need that the invention solved, failure of others to achieve the invention, or unexpected results of the invention were made during prosecution.

32. In order to distinguish over Unger and gain allowance, Applicant amended the independent claims to recite “a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir” and “wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.” EX1004 (the ’295 file history), 0027-29. However, as discussed in more detail below, these features were all well-known and well documented in the art.

33. A Notice of Allowance for the underlying application issued on August 28, 2013. *Id.*, 0009.

**B. Claim Terms**

34. I have been advised that the claims terms of the ’295 patent are to be given their plain and ordinary meaning, *i.e.*, the meaning that the terms would have to a person of ordinary skill in the art at the time of the invention.

35. I understand that this analysis focuses on intrinsic evidence, including how the patentee used the claim term in the claims, specification, and prosecution history. I also understand that dictionaries or other extrinsic sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is unambiguous from the intrinsic evidence. I have followed these principles in my analysis throughout this declaration.

36. Several claims of the ’295 patent recite a reservoir. For example, independent claim 1 recites a microfluidic system comprising a first reservoir “for dampening or absorbing a pressure pulse” and a second reservoir “adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.” EX1001 (the ’295 patent), 13:42-53. The first and second reservoir are respectively connected to the flow channel by first and second apertures that are substantially opposite one another. *Id.*, 13:54-14:3.

37. Claims 4 and 10 respectively recites that the first and second reservoirs include a gas pocket, the compression of which gas facilitates dampening or absorbing of the pressure pulse. Claims 6 and 11 further recite a fluid filled region interfacing with the flow channel and the gas pocket includes a portion of the first and second reservoirs, respectively. Claims 7 and 12 respectively depend from claims 6 and 11 and recite the fluid filled region includes a side channel connecting the first and second reservoirs, respectively, to the flow channel. Claim 15 depends from claim 11 and further recites the fluid filled region forms a meniscus with the gas pocket. Claim 16 depends from claim 15 and recites the change in volume or pressure in the second reservoir deflects the meniscus into the flow channel. Claim 9 depends from claim 1 and recites the first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse. Claim 17 depends from claim 1 and recites the second reservoir includes a deflectable flexible membrane, the deflection of which increases the pressure in the second reservoir.

38. The differences in language between the independent and dependent claims suggests that the term reservoir in claim 1 is broader than the specific reservoirs recited in the dependent claims. In other words, the reservoir of claim 1 need not necessarily comprise a gas pocket, a fluid-filled region interfacing with the flow channel and the gas pocket, a side channel connecting the reservoirs to the

flow channel, a meniscus, a resilient wall, or a deflectable flexible membrane. Although the dependent claims suggest that a reservoir may comprises these features, they also suggest that these features are not definitive for a structure to constitute a "reservoir" as that term is used in claim 1.

39. In one embodiment, the '295 patent states: "Basically, the reservoir 70*b* of the second bubble valve 10*b* is a chamber having a resilient wall or contains a compressible fluid such as a gas." EX1001 (the '295 patent), 12:18-20. Consistent with the dependent claims demonstrating that a reservoir need not comprise a resilient wall or contain gas, the '295 patent points identifies a "chamber" as a structure that constitutes a "reservoir."

40. I understand that, according to the Patent Owner, the term "reservoir" in the '295 patent means "a physical structure that contains fluid." EX1045, 9. I understand that the Patent Owner also concedes that a resilient wall and a deflectable flexible membrane are structural features "that can be included in the reservoir" but are not prerequisites for a structure to constitute a reservoir. *Id.*, 10.

41. I also understand that the Patent Owner engaged a consultant named Dr. Giacomo Vacca to address what a person of ordinary skill in the art would have understood with respect to the meaning of the terms reservoir, buffer, chamber, absorbing, and dampen as used in the '295 and the other patents upon which NanoCellect has asked me to opine. *See* EX1043 (Vacca declaration), ¶1.

42. I understand that Dr. Vacca has opined that the term "reservoir" in claim 1 of the '295 patent refers to "a physical structure that contains fluid." EX1043 (Vacca declaration), ¶25. I understand that Dr. Vacca has opined that the term reservoir had a commonly understood meaning in the field of microfluidics in 2002, consistent with the reservoir structures described in the '295 patent. *Id.*, ¶26. I understand that Dr. Vacca points to "well" 1602 in Figure 15 of U.S. Patent No. 6,592,821 to Wada e*t al.* as a reservoir within the meaning of the '295 patent. *Id.*, ¶27 ("This is a typical example of a fluid reservoir formed as (often) a shallow cylindrical well in one or more layers of a substrate. Reservoirs are typically formed in this way in microfluidic devices due to the ease of fabrication, but the exact geometry may vary."). I understand that Dr. Vacca has opined that a person of ordinarily skill in the art would have understood the term reservoir as used in claims 1 and 18 of U.S. Patent No. 9,011,797 and claims 1, 5, and 15-16 of U.S. Patent No. 10,029,263 patent to have the same meaning as in the '295 patent. *Id.*, ¶¶31, 33-34. I further understand that Dr. Vacca has opined that the term "chamber" as used in, *e.g.*, claims 1 and 5-6 of U.S. Patent No. 10,029,283 and in claims 10-11 of U.S. Patent No. 9,339,850 has the same meaning as the term "reservoir," as in the claims of the '295 patent, namely a physical structure that contains fluid. *Id.*, ¶¶44-48.

43. I understand that the Patent Owner defines the fluid in the reservoir to include any liquid or gas, such that even when a reservoir, buffer, or chamber is "empty" it still contains a gas (*i.e.*, air) and thus contains a fluid. EX1045, 20.

44. I understand that Dr. Vacca has opined that the term "absorbing" as used in claims 18 and 22 of U.S. Patent No. 6,877,528, in claims 1 and 9 of the '295 patent, in claims 1 and 18 of the '797 patent, in claim 1 of U.S. Patent No. 9,339,850, and in claims 1 and 15 of the '263 patent, means to receive, take up, or take in the pressure pulse. EX1043 (Vacca declaration), ¶¶50-52, *see also* EX1042, 1, 7-8, 18-19.

45. I understand that Dr. Vacca has opined that the term "dampen" or "dampening" as used in claims 1 and 9 of the '295, in claims 1 and 18 of the '797 patent, in claim 1 of the '850 patent, and in claims 1 and 15 of the '263 patent, means to meaningfully dissipate so as to prevent a pressure wave from affecting the flow of the remaining particles in the stream of particles. EX1043 (Vacca declaration), ¶¶56-57; EX1042, 13-14.

## VI. PERSON OF ORDINARY SKILL IN THE ART

46. I have been asked to consider the level of ordinary skill in the art as of April 17, 2002, which I understand is the earliest priority date claimed by the '295 patent.

47. As I noted earlier in Section III, I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

48. In my opinion, a person of ordinary skill in the art at the relative time would have had a bachelor's or master's degree in the field of bioengineering, mechanical engineering, chemical engineering, or analytical chemistry; or a bachelor's or master's degree in a related field and at least three years of experience in designing or developing microfluidic systems. Those of ordinary skill in the art would have been familiar with the state of the art as described below in Sections VII and VIII.

49. As set forth below, the claims of the '295 patent do not add anything new or non-obvious over the prior art and thus, a person of ordinary skill would not have needed particularly significant experience in the field, any additional specialty training, or an extensive post-graduate education.

## VII. BRIEF OVERVIEW OF FLOW CYTOMETRY, PARTICLE SORTING, AND MICROFLUIDIC DEVICES IN THE PRIOR ART

50. The discussion below summarizes the relevant state of the art as of April 17, 2002, the earliest claimed priority date of the '295 patent. This overview identifies exemplary disclosures of some of the different types, components, and

operating mechanisms of flow cytometers, particle sorters, and microfluidic devices known in the art prior to the '295 patent. Based on my experience, those of ordinary skill in the art were familiar with the state of the art as outlined below and were capable of implementing the features discussed below as part of their routine, day-to-day responsibilities.

**A. A Brief History of Flow Cytometry and Cell Sorting**

51. Flow cytometers are instruments that measure one or more properties of particles in a flowing stream. *See* Chapman, *Instrumentation for flow Cytometry*, J. IMMUNOL. METHODS 243 (2000) 3-12 ("Chapman," EX1014), 3.

52. Flow cytometers before the earliest claimed priority date were used for cell sorting "to extremely high purities (<99%)." *See* EX1014 (Chapman), 3. Particle sorters are often categorized as either droplet or microfluidic sorters. *Id.*, 5. Droplet sorters use a vibrating nozzle to break the laminar sheath stream of the flow cytometer into droplets. In comparison, fluidic sorters use a mechanism, such as a mechanical force or pulse oriented perpendicularly to the main flow channel, to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected." *Id.* Early on, those of ordinary skill in the art recognized that "[f]luidic sorters have a distinct advantage over droplet sorters," especially for biohazardous samples, which should often remain sealed within the chambers and channels of the particle sorting device. *Id.*

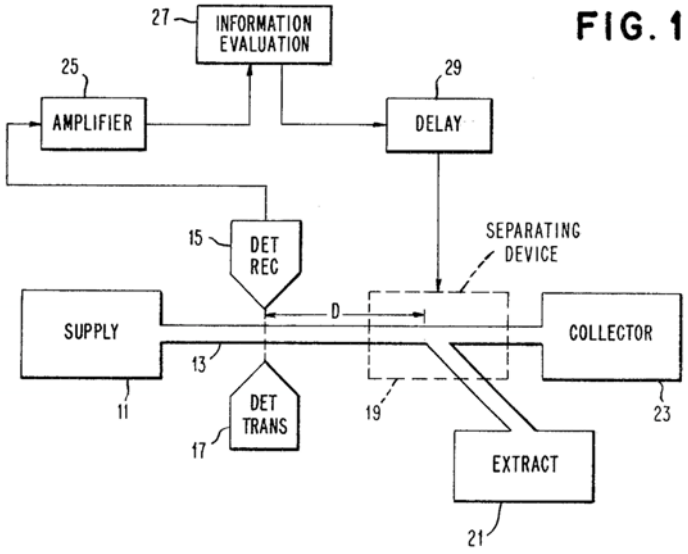
53. Glaettli, for example, describes a cell sorter "for the detection of cells of special interest if they exhibit automatically measurable properties distinguishing them from other cells in which they are mixed." U.S. Patent No. 3,508,654 to Glaettli (issued April 28, 1970) ("Glaettli," EX1016), 1:51-2:19. Glaettli's particle sorting system comprises channels with inner diameters of just 100 μm, making it an early microfluidic device, and "provides for fast and reliable separation of particles being selected according to certain criteria from other particles not exhibiting these criteria." *Id.*, 2:62-67, 4:47-49.

54. More specifically, Glaettli describes:

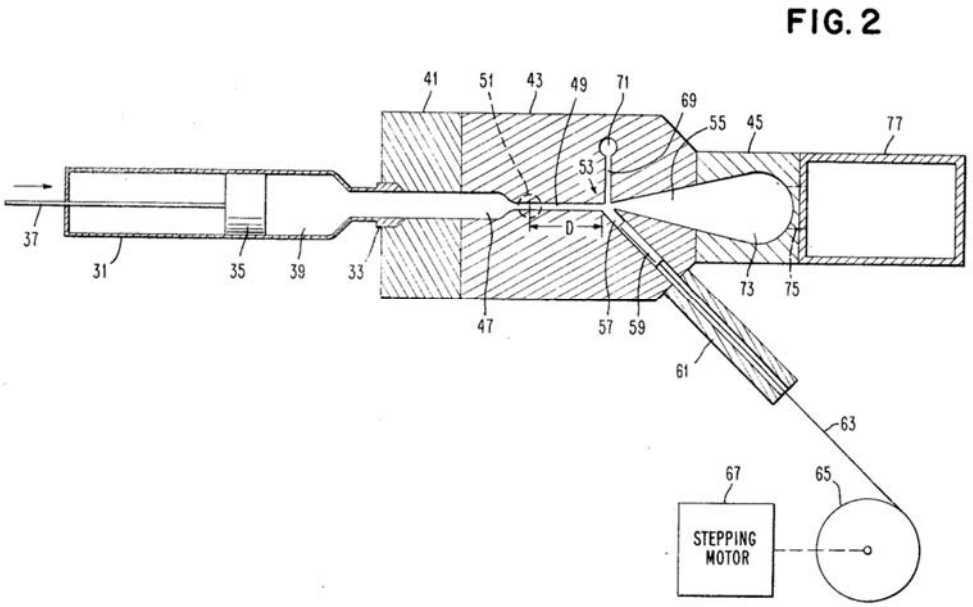
> A particle separating apparatus for separating suspended biological bodies having specified indication criteria which has a main channel upstream of a junction from which three branches emanate. A closed channel containing an air bubble at its closed end extends substantially perpendicularly from the main channel, while a diffuser channel of gradually widening size extends from the junction along the same axis as the main channel. An extraction channel extends from the junction preferably at an included angle of 135º from the axis of the main channel and contains a piston within the channel which, when actuated at a desired time, removes a biological body appearing at the junction from the junction and causes it to enter the extractor channel. The separated particles are subsequently removed from the extraction channel for examination.

EX1016 (Glaettli), 1:14-29.

55. Figure 1, which provides a block diagram of the Glaettli particle sorter, is reproduced below. *Id.*, FIG. 1.



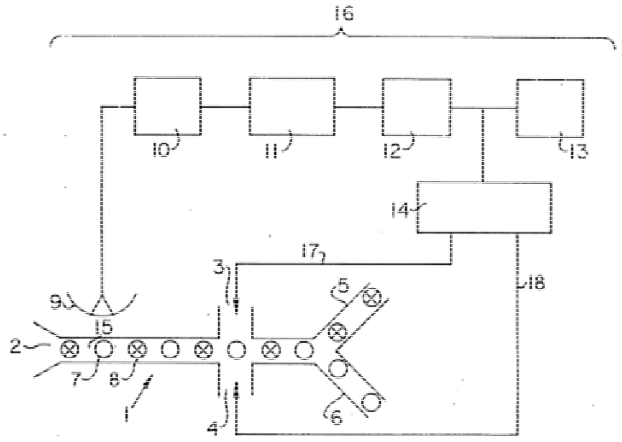

56. Figure 2, reproduced below, provides a more detailed view of the separating device that sorts particles in the microchannel into the "extract" and "collector" outputs of Figure 1. EX1016 (Glaettli), FIG. 2.



57. As can be seen above, the automated particle sorter taught by Glaettli includes an actuator, in this case, a stepping (*i.e.*, stepper) motor connected to a piston, to introduce a pulse within the main flow channel, "separating selected individual particles from a larger number of similar particles." *Id.*, 2:22-25.

58. Even today, microfluidic separations operate using the same basic principles and conventional components, all of which pre-date the '295 patent by decades. And by the 1990s, particle sorting flow cytometers were not just known in the art, but also widely commercially available. The Partec PAS-III and Dako Galaxy PRO, for example, were popular fluidic particle sorters that "use fluid switching to sort single populations from a sample." EX1014 (Chapman), 9. Both of these fluidic sorters used piezoelectric valves that, when actuated, "control[led] the fluid stream to either waste or the sort collection tube." *Id.*

59. Marcus similarly describes a fluidic sorting device for separating particles, such as different cells in blood. Marcus describes the combined "use of two conventional components…a fluidic sorting device and a machine vision apparatus," noting that "[n]o novelty lies in either one" of these components. EX1005 (Marcus), 2:7-13. Figure 1, reproduced below, illustrates an exemplary microfluidic particle sorter taught by Marcus.




*Id.*, FIG. 1.

60. Marcus teaches that the fluidic logic element (labeled generally as "1," above) comprises an inlet (2), two control ports (3 and 4) for sorting particles (7 and 8) within a fluid carrier (15), and two outlet branches (5 and 6). EX1005 (Marcus), 2:14-18, 3:30-62. The fluidic logic unit is described as "conventional" and able to "be produced using conventional micromachining technology or any other conventional means." *Id.*

61. Marcus teaches that a machine vision apparatus (16) that includes a machine vision camera lens (9), an image capture device (10), an image interpretation device (11), and a decision control device (12), identifies particles with visually distinct features and subsequently conveys a "message to control ports in the fluidic logic element" to sort particles 7 and 8. EX1005 (Marcus), 2:49-66, 4:30-39. More specifically, a signal from the decision control device (12) is sent to a fluidic controller device (14) that transmits the signal through leads (17

and 18) in order to actuate the fluidic element control ports (3 and 4). *Id.* Marcus teaches that a conventional counter device (13) may also be included. *Id.*, 3:41-43. A second fluidic logic element may also be added to allow for sorting based on multiple, distinct characteristics. *Id.*, 4:58-64.

**B. Particle Sorting Chips**

62. By the early 1990s, microfabrication techniques allowing for the manufacture of more complex microfluidic components became widely available and the "lab-on-a-chip" revolution blossomed. *See* Figeys, *et al.*, *Lab-on-a-Chip: A Revolution in Biological and Medicinal Sciences*, ANAL. CHEM. (2000) 330-35 ("Figeys," EX1017), 330.

63. Early microfluidic chips were designed with relatively simple flow patterns that could be etched into the surface of a substrate like glass or silicon, using a technique called photolithography. EX1017 (Figeys), 330-31. Despite their simplicity, these chips could be used for complex analytical techniques and could be run in a "massively parallel" fashion, "attract[ing] considerable attention" from those in the field. *Id.*, 331; *see also* Deshpande, *et al.*, *Novel Designs for Electrokinetic Injection in μTAS*, MICRO TOTAL ANAL. SYS. (2000) 339-42 ("Deshpande," EX1018), 339 (noting "a wide interest in micron-scale integrated chemical/biochemical analysis or synthesis systems, also referred to as lab-on-a-chip or μTAS").

64. The advent of computer-aided design (CAD) in the mid-to-late 1990s further enabled better and more efficient micro-total analysis system (μTAS) designs. EX1018 (Deshpande), 330. As explained by Deshpande, those of ordinary skill in the art recognized that:

> The real advantage of CAD tools, however, lies in their ability to design – that is, to create new designs or extend and optimize existing designs to make the components better.…Insertion of CAD into the design cycle can therefore reduce both the number of expensive experimental iterations and the time required for the design.

*Id.* (describing the design of microfluidic switch components used in particle sorting as "well understood and predictable using CAD tools"); *see also id.*, 341 (noting the performance of a CAD-optimized microfluidic switch component was confirmed via experiments that "verified the behavior observed in the simulations and have demonstrated significantly higher separation efficiency compared to the classic injector"). Thus, the design of microfluidic devices soon became appreciably and routinely easier.

65. As microfluidic devices became more popular, disposable chips approximately the size of credit cards were manufactured en-mass. The chips of these plug-and-play cartridge systems could be run serially or in parallel for rapid analytical work on a microscale. For example, Neukermans teaches portable, fully integrated, and completely automated microfluidic systems. U.S. Patent No.

6,068,751 to Neukermans (issued May 30, 2000) ("Neukermans," EX1020), 1:42-64. As explained by Neukermans, such a system allows for detection means and other reusable instrumentation to be housed in a main body, while the "microfluidic circuit[s], where all liquid passages, valve seats, reaction and mixing chambers are simply integrated into an inexpensive container." *Id.*, 2:31-34, 2:51-54; *see also id.*, 2:41-44 (noting that the "actuator portion of valves are reusable without cleaning"). More specifically, Neukermans teaches microfluidic cartridges that use valves comprising a layer of malleable material and "an electrically-powered actuator secured within the valve housing which, upon application of an electrical signal to the electrically-powered actuator, extends towards or retracts from the layer of malleable material." *Id.*, 2:60-3:13.

66. Neukermans teaches that this type of valve may be provided upon a disposable microfluidic cartridge and is "particularly adapted for use with a pouch that includes a layer of malleable material." EX1020 (Neukermans), 3:14-17. A "blade," which functions as an extendable arm of the microfluidic valve, "is coupled to the electrically powered actuator and shaped so that extension of the electrically-powered actuator toward the layer of malleable material presses the blade against the layer of malleable material." *Id.*, 2:60-3:13. As explained by Neukermans, the pouch serves as a "reservoir that is adapted for holding a quantity of liquid," and thus "pressure applied to the reservoir urges the liquid to flow out

of the pouch" and into a microfluidic channel. *Id.*, 3:14-36; *see also id.*, 16:6-13 (noting that the reservoirs may be "used directly with any fluid, i.e. both liquids and gasses"). Neukermans notes different actuators could be used in these devices, explaining that "[p]ressure may be applied to the reservoirs" through "mechanical springs," "external pneumatic means," or using a "piezo-electric actuator" that is "secured within the valve housing." *Id.*, 5:55-65, 8:29-37, 10:48-65, 12:16-29, 13:46-48.

### C. Flow-control in Microfluidic Systems

67. Prior to the filing of the '295 patent, those in the art recognized that a major driving factor behind the Lab-on-a-Chip "revolution" was the ability to control the movement of fluids on the sub-microliter level. EX1017 (Figeys), 331-32. This ability to precisely control the movement of liquid within microfluidic channels is due, in part, to inherently laminar flow patterns. As explained by Figeys:

> [L]aminar flow…is the smooth flow of liquids without the turbulence that generally occurs at low velocities. As the velocity of the fluid increases, its inertia overcomes the frictional force of the fluid, and turbulence occurs.
>
> Laminar flow occurs at Reynolds numbers (Re) below ~2000.…Contrary to macroscale devices, ***laminar flow is the norm in microfabricated devices*** because of their narrow channel diameters and

the low velocity required to move fluids across their short channels in a reasonable time.

*Id.*, 331-32; *see also* Zengerle, *et al.*, *Simulation of Microfluid Systems*, J. MICROMECH. MICROENG. 4 (1994) 192-204 ("Zengerle," EX1033), 192 (noting that "it is a common practi[c]e to calculate the Reynolds number Re and compare the result with a translational number of 1000-2500," in "order to decide, whether the fluid flow through a miniaturized channel is laminar or turbulent," and teaching that the "Reynolds numbers are usually considerably smaller" in "microminiaturized fluid systems. Therefore the microscopic flow through the channels can be assumed as laminar and the pressure dependence of the flow characteristic is linear"). Microfabricated microfluidic channels thus conventionally have Re numbers below 10 and the fluid flow therein is laminar.

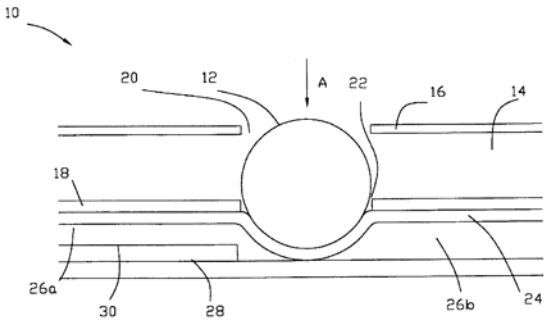
68. Before 2002, a variety of different mechanisms by which flow could be manipulated within micromachined fluid channels were well understood and described in the prior art. Many of these mechanisms involved the use of a micropump. Micropumps can be classified as either membrane-displacement pumps or field-induced flow pumps. Zeng, *Fabrication and characterization of electroosmotic micropumps*, SENS. ACTUAT. B, 79 (2001) 107-14 ("Zeng," EX1022).

69. Membrane pumps may be classified based on how the membranes are actuated. Actuators commonly used with membrane-displacement pumps include

piezoelectric, electrostatic, thermopneumatic, electromagnetic, and photothermical-type actuators. EX1022 (Zeng), 107; *see also* EX1012 (Anderson), 38:1-39:4 (noting "[d]eflection of the diaphragm valve may be carried out by a variety of methods including, e.g., application of a vacuum, electromagnetic and/or piezoelectric actuators coupled to the diaphragm valve, and the like").

70. As membrane-displacement pumps provide a pulsing action through the deflection of a microfabricated membrane, such as a flexible silicone diaphragm or the interface of a fluid/gas meniscus, they may be further classified in view of the "valving schemes often used to rectify the flow through the pump, such as dynamic passive valves." EX1022 (Zeng), 107; *see also* EX1012 (Anderson), 38:27-30 (teaching that, to "allow for a deflectable diaphragm," one wall of an actuatable chamber "will typically be fabricated, at least in part, from a flexible material"), 62:18-63:13 (describing pumping devices).

71. Williams, for example, teaches a valve that "uses a spherical member, such as a ball bearing, to depress an elastomeric member, to selectively open and close a microfluidic channel." U.S. Patent Publication No. 2001/0054702 to Williams (published December 27, 2001) ("Williams," EX1023), Abstract. Such a valve is shown in Figure 2, reproduced below.




*Id.*, FIG. 2.

72. As shown above, a membrane of flexible material (24) rests below the sphere (12) to which pressure is applied. Thus, following actuation, a force imparts stress to the elastomeric material, which deflects towards the flow channel (26), allowing for control of "the flow of a fluid traveling within channel 26." EX1023 (Williams), ¶0026. As noted by Williams, this "force may be applied manually using the finger of a human operator, or by any suitable mechanical means as known in the art." *Id.*; *see also id.*, ¶0032 (noting that actuation may "be accomplished using common electrical, magnetic, or pneumatic means, as is well known in the art," as well as by "external control means," involving the use of a computer, cartridges, etc.). Then, when the force is removed, the system "is shifted back to its unactuated position as shown in FIG. 1 by virtue of the elastomeric property of the membrane." *Id.*, ¶0026.

73. By 2002, multiple mechanisms for manipulating the flow of a stream of particles to sort particles within a microfluidic chip were known. For example, the "Partec PAS-III and Dako Galaxy PRO" used "fluidic switching to sort single

populations from a sample" via actuation of "a piezoelectric valve" to "control the fluid stream to either waste or the sort collection tube." EX1014 (Chapman), 9. The "Beckton Dickinson FACSCalibur™," when "equipped with the fluidic switching sort option," sorted particles using a "sort collection tube which is normally placed eccentrically," but "can be rapidly moved into the centre of the stream to 'catch' the particle to be sorted." *Id.*

74. Another known mechanism for sorting particles involved increasing fluid flow across a flow channel, in effect generating a mechanical pressure pulse. This cross-channel pulse was known to those in the art as the "simplest switching component," which those in the art regarded as "surprisingly powerful tools that enable the definition of sample plugs at the picoliter level." EX1018 (Deshpande), 340. Generation of this mechanical pulse within the flow channel to promote sorting or otherwise manipulate fluid flow within a microchannel is accomplished by a switching device comprising an actuator. As noted above, Glaettli taught the use of a mechanical actuator (a stepper motor) within a switching device for particle sorting back in 1970. EX1016 (Glaettli), 4:66-5:38.

75. Increased flow in a cross-channel to apply a pulse in a microfluidic flow channel was also performed via the generation of a gas bubble or in the deflection of the meniscus at a gas-fluid interface. In 1990, for example, Zöld described a microfluidic device that relies upon a "gas bubble" to generate a

"momentary pressure increase" to deflect a particle of interest into a desired outlet. EX1007 (Zöld), Abstract.

76. As explained by Zöld, using a mechanical pressure pulse to sort particles provided advantages over relying upon electroosmotic flow patterns. Notably, by sorting with mechanical pulses in a closed microfluidic system, the particle sorter of Zöld avoids the "instability" and "unreliability" of open flow systems that instead relied upon electrostatic deflection to sort particles. EX1007 (Zöld), 2:16-42.

### D. Improving Sorting Precision and Avoiding Flow Disruption

77. Persons of ordinary skill in the art recognized that, while cross-channel pulses were effective at deflecting particles or redirecting microfluidic flow, the deflected portion left room for optimization, particularly because of the size and asymmetry of the deflected portion. EX1018 (Deshpande), 341. The figure below illustrates the asymmetry of the portion deflected with a pulse of increased fluid flow from the right-side cross-channel during a switching operation. *Id.*, FIG. 2.

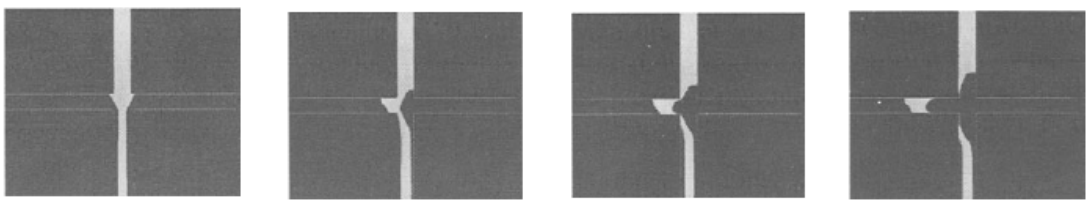


**Figure 2: Simulations of electrokinetically Switched injection (the classic injector). The switching sequence has two phases (load and switch).**

78. Deshpande teaches, however, that toggling pulses across the channel (*i.e.*, issuing a pulse moving from left to right, followed immediately by a stronger pulse moving right to left) could improve separation. As can be seen in the figure below, by implementing a tri-phase switching operation involving "load, pull-back and switch phases," in effect allowing the pulse to be toggled across the microchannel, more precise flow manipulations can be achieved. *Id.*, 342 (comparing the smaller size and heightened symmetry of the deflected flow fraction in Figure 4 with the larger, asymmetrical fraction deflected using the method depicted in Figure 2).

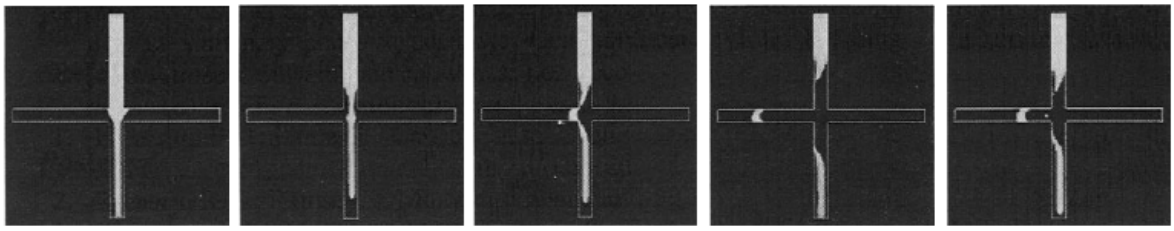


**Figure 4: Reverse Injection Process showing Pullback prior to switching. The cycle is accomplished by a three-phase switch in the field – the load, pull-back and switch phases.**

79. Dubrow similarly teaches the utility of adding a "pull back" phase to a switching operation in a microfluidic device, stating that for "any bleeding over of material" during the injection of fluid into the cross channel, "a low level of fluid is directed back into the side channels resulting in a 'pull back' of the material from the intersection." International PCT Publication No. WO 99/43432 to Dubrow, *et al.* (published September 2, 1999) ("Dubrow," EX1032), 8:4-8.

80. Relatedly, in order to promote even faster and more precise movement of fluid in microfluidic devices, those in the art also looked towards various compliance devices and chip designs that avoided significant flow disruption. *See* EX1033 (Zengerle), 198 (noting that, for "many applications, the oscillation of pressure and flow in fluid channels has to be suppressed"). Thus, as noted earlier, most devices relying upon pressure pulses comprised some sort of vent, chamber, passageway, or other compliance devices as "pressure smoothing elements" to isolate the effects of output loading from control flow characteristics. *See id.* (describing "pressure smoothing" in microfluidic systems as "[a]nalogous to the procedure with macroscopic pumps," wherein "the oscillations can be avoided by the use of elastic elements" that are "in contact with the oscillating fluid").

81. A person of ordinary skill in the art thus would have understood that these vents or compliance elements (also often called fluidic capacitors) were useful to avoid both the oscillation (causing lateral disturbance in the particle-containing stream that can affect the ability to sort) as well as the translation of a lateral pressure pulse into a change in flow speed of the particles (that can affect the ability to detect and subsequently deflect the particle of interest).

82. Those of ordinary skill in the art also readily recognized that, "[a]nalogous to the procedure with macroscopic pumps, the oscillations can be avoided by the use of elastic elements." EX1033 (Zengerle), 198. Thus, rather than

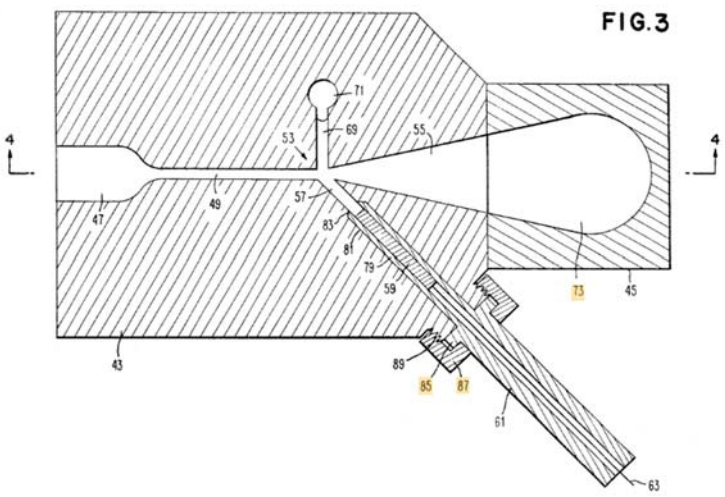
having a shockwave or pressure fluctuation reverberate throughout a microchannel, it would instead be absorbed or at least dampened by an elastic element provided for in the microchip design. As explained by Zengerle, these elastic "elements have to be in contact with the oscillating fluid" to allow for "a smoothing of pressure and flow signals. *Id.* (teaching that "an enclosed gas volume is used as an elastic element very often," as are "deflectable diaphragm[s] with a large fluid capacitance").

83. Zöld teaches that these undesirable flow fluctuations can be mitigated with oppositely-facing buffer cavities. EX1007 (Zöld), 21:68-22:7 (describing pressure-smoothing cavities to be "of paramount importance"). In Zöld's microfluidic particle sorter, gas bubbles generated by electrodes were used to generate a cross-channel pulse to deflect a particle of interest into a desired outlet. *Id.*, Abstract.

84. By including in the side channels cavities that housed oppositely-facing bubble generators for particle sorting, Zöld teaches that pressure fluctuations caused by the "pressure increase for a short time interval, at the entrances of preselected ducts…caused at the ducts either by injection of another fluid or gas through the injection duct…or by generation of a gas bubble or vapor between electrodes situated at the entrance of these ducts" can be mitigated

through the stabilization of fluid flow and "the decoupling of the very fast fluctuating suspension." EX1007 (Zöld), Abstract, 21:44-22:7.

85. Glaettli addressed similar concerns in 1970 by designing a particle sorter wherein pulses could be dampened using a gas bubble located inside a side channel to the flow channel located across from the pressure source. EX1016 (Glaettli), 5:44-54. This gas bubble is labelled in Figure 3, reproduced below, with the number 71, in side channel 69, which is opposite the source of the pressure that generates the pulse for sorting. *Id.*, FIG. 3.




86. The presence of the gas bubble at the cross junction "considerably reduces the length of the column of fluid material that must be accelerated during the extraction process. As a result, cavitation effects are inhibited which tend to increase the response time and could possibly destroy the cells to be extracted." *Id.*; *see also id.*, 7:21-24, claim 7 ("In a system according to claim 1 wherein said means for inhibiting cavitation effects includes a closed channel containing a gas

bubble at the closed end thereof the open end of which is disposed opposite said extraction channel.").

87. Chou teaches microfluidic devices comprising flexible membranes that use similar principles for particle sorting. International Patent Publication No. WO 02/29106 to Chou, *et al.* (filed October 2, 2001, published April 11, 2002) ("Chou," EX1034), Abstract. For example, Chou describes a microfluidic device for sorting particles that comprise "thin membranes of [an] elastomer material deflectable into the flow channel to provide pump or valve functionality." *Id.*, 2:23-34; *see also id.*, 26:9-31 (teaching one electrode "positioned on (or in)" the flexible membrane and a second electrode "on (or in) planar substrate," such that, once actuated, "attractive force between the two electrodes" causes the flexible membrane "to deflect downwardly, thereby closing the 'valve'"). A "damper" or an "energy absorber" can then be "configured to experience a physical change in response to an oscillation within the flow channel" as a result of sorting particles. *Id.*, 39:25-34, 40:20-25. The damper may comprise "a flexible membrane, a pocket filled with a compressible fluid, and/or flexible walls of the flow channel itself." *Id*. As explained by Chou, "[p]referably, the damper is an encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas." *Id.*

88. Veenstra similarly describes ways to dampen pressure variations that it describes as "compliance structures for the use in liquid-based microfluidic systems." Veenstra, *et al.*, *The Design of an In-Plane Compliance Structure for Microfluidical Systems*, SENS. ACTUATORS B, 81 (2002) 377-83 ("Veenstra," EX1035), 377. As explained by Veenstra, these compliance structures are fluidically coupled to the flow channel itself for dampening pressure variations:

> The basic principle is that these structures contain air bubbles that dampen the flow and pressure variations that may arise from a micropump. The compliance structures were specifically designed to work with the no moving parts valve (NMPV) Pump. The structures were modeled and simulated. From the results of these simulations and the model, design rules for the compliance are formulated….Working with two sets of pumps showed that pumps without the compliance structure needed an external compliance in order to get them to work, whereas the pumps with the on-chip compliance pumped right away.

*Id.*

89. Veenstra notes that increased "pressure in the system results in the compliance taking in fluid. The amount of fluid that is absorbed by the compliance matches theory perfectly." EX1035 (Veenstra), 382-83. Moreover, these gas-based compliance structures allowed "the pump to deliver almost four times as much fluid per unit time," which would not only allow for increased sorting and flow precision, but for these operations to be performed faster as well. *Id*.

## VIII. BRIEF OVERVIEW OF PRIOR ART

### A. Wada (EX1006)

90. International PCT Publication No. WO 00/70080 to Wada, *et al.*, entitled "Focusing of Microparticles in Microfluidic Systems," published on November 23, 2000. Accordingly, I understand that Wada is prior art.

91. Wada teaches systems for focusing particles in microchannels for particle sorting applications. EX1006 (Wada), 3:13-15, 7:5-8:8. These microchannels are taught to be "integrated into the body structure of a microfluidic device" that "comprises an aggregation of two or more separate layers which when appropriately mated or joined together, form the microfluidic device of the invention." *Id.*, 37:11-29. As explained by Wada, the microfluidic devices typically comprise top and bottom portions and "an interior portion, wherein the interior portion substantially defines the channels and chambers of the device." *Id.*, 38:11-17; *see also id.*, 3:13-15, 7:5-29.

92. A flow control regulator is taught to allow for the focusing of the particles within the microchannel, prior to sorting. EX1006 (Wada), 4:1-5 (listing types of fluid direction components known in the art, including "a fluid pressure force modulator, an electrokinetic force modulator, a capillary force modulator, a fluid wicking element, or the like").

93. Once focused, a "signal detector typically detects a detectable signal produced by a selected member of the particle population." EX1006 (Wada), 7:13-24. For example, as depicted in Figures 22-24, Wada teaches methods of sorting cells wherein the cells "flow past a detection point to obtain accurate fluorescent readings.…Thereafter, cells (e.g., viable and non-viable cells) are optionally sorted similarly using hydrodynamic flow." *Id.*, 26:26-27:2; *see also id.*, 19:23-24:12, FIGS. 22-24. Once detected by the signal detector, a computer that is "operably linked to the first or other fluid direction component(s), the flow control regulator, and the signal detector," directs the introduction of a fluid from a microchannel perpendicular to the main flow channel in which the particles were focused. *Id.*, 7:18-29. In so doing, the computer triggers the flow control regulator to manipulate the detected particle into a designated output microchannel. *Id.*, 26:26-27:2.

94. Wada also teaches "other alternative techniques for inducing the flow of focusing fluids to sort particles," including "pressure, hydrostatic, wicking, capillary, and other forces." EX1006 (Wada), 19:9-12. This includes heating methods that may involve directing "light from a laser source through one or more fiber optic cables" or "[h]eat from other external sources." *Id.*, 24:9-12.

95. Figures 22-24, reproduced below, illustrate exemplary microfluidic particle sorters taught by Wada. *Id.*, FIGS. 22-24.

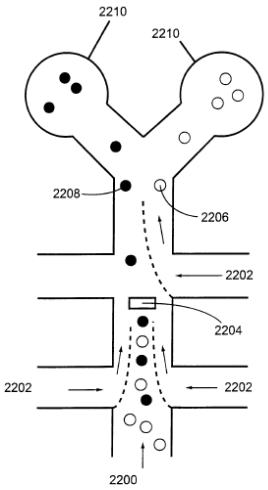



Fig. 22

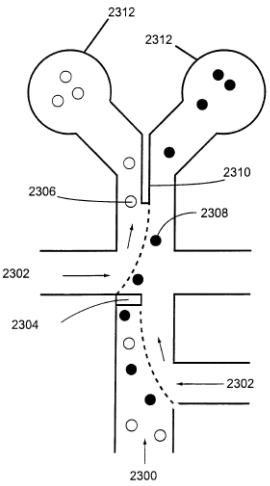



Fig. 23

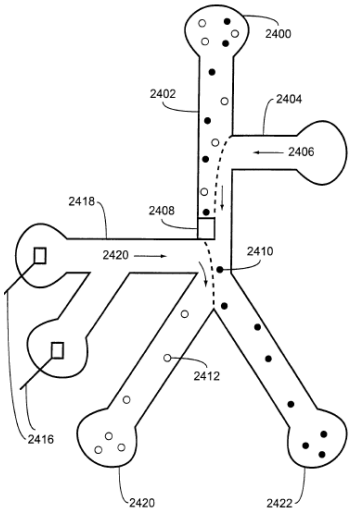
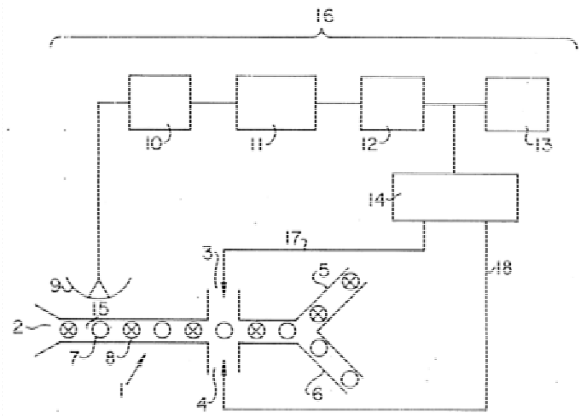



Fig. 24

**B. Marcus (EX1005)**

96. U.S. Patent No. 5,101,978 to Marcus, entitled "Fluidic Sorting Device for Two or More Materials Suspended in a Fluid," issued on April 7, 1992. Accordingly, I understand that Marcus is prior art.

97. Marcus describes a fluidic sorting device for separating particles, such as different cells in blood. Marcus describes the combined "use of two conventional components…a fluidic sorting device and a machine vision apparatus," noting that "[n]o novelty lies in either one" of these components. EX1005 (Marcus), 2:7-13. Figure 1, reproduced below, illustrates an exemplary microfluidic particle sorter taught by Marcus.




*Id.*, FIG. 1.

98. Marcus teaches that the fluidic logic element (labeled generally as "1," above) comprises an inlet (2), two control ports (3 and 4) for sorting particles (7 and 8) within a fluid carrier (15), and two outlets (5 and 6). EX (Marcus), 2:14-18, 3:30-62. The fluidic logic unit is described as "conventional" and able to "be produced using conventional micromachining technology or any other conventional means." *Id.*

99. Marcus teaches that a machine vision apparatus (16), which includes a machine vision camera lens (9), an image capture device (10), an image interpretation device (11), and a decision control device (12), identifies particles with visually distinct features and subsequently conveys a "message to control ports in the fluidic logic element" to sort particles 7 and 8. EX1005 (Marcus), 2:49-66, 4:30-39. More specifically, a signal from the decision control device (12) is sent to a fluidic controller device (14), which transmits the signal through leads (17 and 18) in order to actuate the fluidic element control ports (3 and 4). *Id.*

100. Marcus also teaches that a conventional counter device (13) may also be included within the particle sorting system. *Id.*, 3:41-43. A second fluidic logic element may also be added to allow for sorting based on multiple, distinct characteristics. *Id.*, 4:58-64.

**C. Anderson (EX1012)**

101. International PCT Patent Publication No. WO 97/02357 to Anderson, *et al.*, entitled "Integrated Nucleic Acid Diagnostic Device," published on January 23, 1997. Accordingly, I understand that Anderson is prior art.

102. Anderson teaches microfluidic devices, noting that "advances in the semiconductor manufacturing arts have been translated to the fabrication of micromechanical structures, e.g., micropumps, microvalves and the like, and microfluidic devices including miniature chambers and flow passages." EX1012 (Anderson), 2:29-34; *see also id.*, 28:1-29 (noting that "[m]icrofabrication of microfluidic devices…has been discussed in detail in" the prior art). This fabrication is taught to typically involve photolithography, which "etch[es] micron scale channels on a silica, silicon or other rigid substrate or chip." *Id.*, 28:3-29; *see also id.*, 34:5-21 (noting that "[p]hotolithographic methods of etching substrates are particularly well suited for the microfabrication of these substrates and are well known in the art" and include shining an electromagnetic radiation source "through a photolithographic mask to expose the photoresist in a pattern which reflects the

pattern of the chambers and/or channels on the surface of the sheet," and subsequent etching), 37:13-28 (similar). A first etched plate may then be sealed with a "second thin polymeric member having ports corresponding to the termini of the capillary channels disposed therethrough." *Id.*, 28:3-29.

103. Anderson teaches that fluid may be moved from a first channel or chamber in a microfabricated device into another by applying positive pressure to the first chamber (*i.e.*, a first reservoir, *see* ¶¶39, 42, above). EX1012 (Anderson), 5:14-31. As explained by Anderson, this "positive pressure forces said fluid sample from the [at] least first chamber into the common chamber or channel." *Id.*; *see also id.*, 46:23-29 (teaching "transportation of fluid within the device" that may "be affected by the application of pressure differentials provided by either external or internal sources. Alternatively, internal pump elements which are incorporated into the device may be used to transport fluid samples through the device"), 46:30-48:16 (providing examples of moving fluid using pulses via the application of positive or negative pressure), 62:18-63:13.

104. Anderson teaches that chambers may comprise inlet and outlet ports, such that the chamber may be sealed "to retain a fluid sample therein." EX1012 (Anderson), 38:1-39:4; *see also id.*, 53:20-23 (noting that closure of such ports allows the fluidic operation to proceed "without influencing or influence from elements outside of the chamber"). Chambers etched into a substrate may be sealed

by placing a second layer comprising a diaphragm valve upon the substrate layer defining the chamber. *Id.* This diaphragm valve may then "overlap with the fluid channel 110 such that deflection of the diaphragm results in a gap between the first and second planar members, thereby creating a fluid connection between the reaction chamber 104 and the fluid channel 110, via the inlet 108." *Id.*

105. Anderson teaches that chambers may function as "pumping chamber[s]." EX1012 (Anderson), 44:28-46:44. A pumping chamber may have "a diaphragm pump as one surface of the chamber, and in preferred aspects, will have a zero displacement when the diaphragm is not deflected." *Id.* (noting that the "diaphragm pump will generally be fabricated from any one of a variety of flexible materials, e.g., silicon, latex, teflon, mylar, and the like"). Anderson notes that "[d]eflection of [a] diaphragm valve may be carried out by a variety of methods including, e.g., application of a vacuum, electromagnetic and/or piezoelectric actuators coupled to the diaphragm valve, and the like." *Id.*, 38:23-27; *see also id.*, 63:1-13 (teaching "pumps which hav[e] a bulging diaphragm, powered by a piezoelectric stack"), 65:18-23 (teaching that, upon activation of a chamber with a flexible membrane, "fluid is then expelled from the pump chamber").

106. Anderson also teaches microfluidic particle sorters that comprise "a separation channel for separating a component of said fluid sample." EX1012 (Anderson), 3:35-4:6 (explaining that the "separation channel is fluidly connected

to at least one of the chambers and includes at least first and second electrodes in electrical contact with opposite ends of the separation channel for applying a voltage across said separation channel"); *see also id.*, 13:9-14:10 (noting the separation channel "may be used in conjunction with mixing elements described herein, to ensure maximal efficiency of operation"), 5:32-6:5 (teaching methods of mixing in which "fluid components are then introduced into the channel separated by a gas bubble," and mixed upon removal of the bubble from the channel)

## IX. GROUND 1: CLAIMS 1-3, 9, AND 18 ARE OBVIOUS IN VIEW OF WADA.

107. As discussed in detail below, Wada discloses each and every element of each of claims 1-3, 9, and 18. In my opinion, at least some of the embodiments of Wada would reasonably be viewed as anticipating the claimed subject matter. Though it is my opinion that Wada provides embodiments that provide all of the claimed elements in the claimed configuration, I also address how the full scope of the Wada disclosure teaches each and every element of each claim, as well as provides good reason for one of ordinary skill in the art to reasonably combine those teachings with a reasonable expectation of success of arriving at the claimed microfluidic device.

108. Indeed, as set forth in more detail below, Wada provides a complete description of all of the limitations of claims 1-3, 9, and 18, and, in teaching that "all the techniques and apparatus described above may be used in various

combinations," Wada provided one of ordinary skill in the art with good reason to combine the embodiments of its disclosures. EX1006 (Wada), 49:13-20; *see also*, *e.g.*, *id.*, 23:20-28 (teaching combining features of Figure 24 with features of Figure 22). For example and as discussed in further detail below, the embodiment represented by Wada Figure 22 satisfies all of the limitations of independent claim 1.

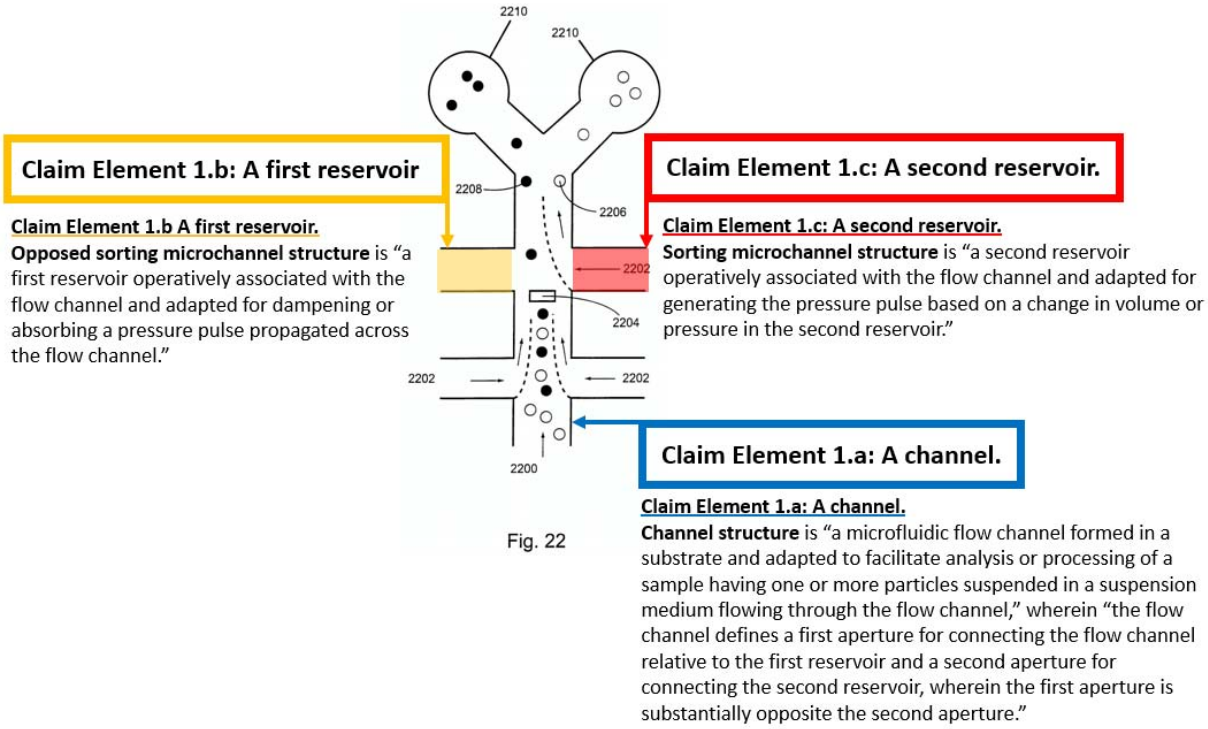


Fig. 22

*See* EX1006 (Wada), FIG. 22.

109. More specifically, Wada's "invention relates to particle focusing to improve assay throughput," including vertical and horizontal focusing "within the microscale systems of the invention." EX1006 (Wada), 11:15-16, 25-27. Wada teaches that "[m]icrofluidic hydrodynamic focusing is a highly effective technique

when used, e.g., in flow cytometry applications" in which fluid flow streams "are 'pinched' or 'focused' into a narrow region of a microchannel to facilitate single particle or narrow streamline detection." *Id.*, 12:1-9.

110. Wada discloses that the present invention is used to focus particles in microchannels into a stream near one side of the microchannel or in the center of the microchannel. EX1006 (Wada), 3:13-18. Wada states that the "present invention provides microfluidic devices and methods for sorting particles (e.g., fluorescently-labeled particles) that use hydrodynamic flow to focus and/or sort the particles." *Id.*, 17:30-18:1. The invention typically sorts members of a particle (*e.g.*, cell) population by "flowing members of the particle population in a first microchannel," and focusing the members of the particle population horizontally and/or vertically in the first microchannel "such that selected individual members are directed into at least a second microchannel that intersects with the first microchannel." *Id.*, 18:1-8.

111. I address below each of claims 1-3, 9, and 18 on an element-by-element basis as exemplary support demonstrating that Wada discloses each element of each claim and renders obvious each claim as a whole. Though it is my opinion that Wada discloses all of the claimed elements, it is also my opinion that Wada provides good reason for one of ordinary skill in the art to combine its teachings with a reasonable expectation of success in a manner that satisfies each

of claims 1-3, 9, and 18 as a whole. Indeed, it is my opinion that those in the art at the time typically would read Wada as a whole for all that it discloses and would not mechanically limit its teachings to individual embodiments only as specifically illustrated. This is particularly true for Wada, given that it expressly teaches that its embodiments are cooperative and integral.

**A. Claim 1**

**1.[preamble] A microfluidic system comprising:**

112. Wada's disclosure is directed to microfluidic systems. Entitled "Focusing of Microparticles in Microfluidic Systems," Wada teaches "systems for particle focusing to increase assay throughput in microscale systems." EX1006 (Wada), Title, Abstract. Wada's systems "provid[e] substantially uniform flow velocity to flowing particles in microfluidic devices." *Id*., Abstract. Further, the microfluidic systems allow for sorting of "particle populations, such as cells and various subcellular components." *Id*.; *see also id*., 3:13-33, 12:3-14.

113. Example schematics of Wada's microfluidic systems can be seen throughout its disclosure, including as represented in Figures 1A, 22, 23, and 24.

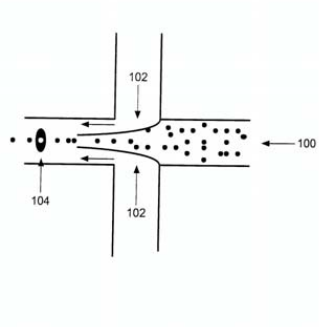



Fig. 1A

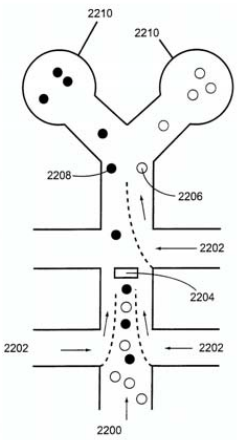



Fig. 22

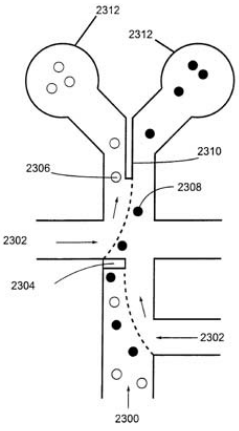



Fig. 23

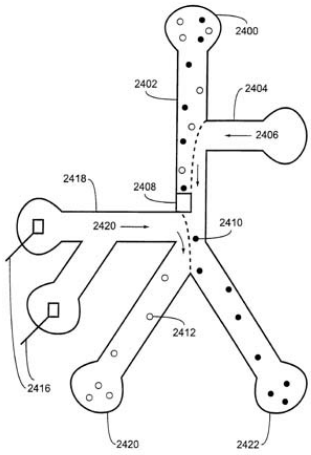



Fig. 24

114. Thus, a person of ordinary skill in the art would have understood Wada's disclosure as teaching a microfluid device, as recited in the preamble of claim 18.

**[1.a] a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;**

115. Wada discloses a microfluidic flow channel formed in a substrate and adapted to facilitate analysis and processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel. For example, Wada teaches microfluidic flow channels that are "integrated into the body structure of a microfluidic device." EX1006 (Wada), 37:23-32; *see also id.*, 38:1-31 (microscale channels formed in body structure, especially in the substrate using photolithography and other techniques), 39:19-33 (channels fabricated into

upper surface of the bottom substrate). These microscale channels can be formed "on almost any type of substrate material," including glass, plastic, silicon, composite, multi-layered materials, and combinations thereof. *Id.*, 21:3-5.

116. It is my opinion that a person of ordinary skill in the art would have understood the microchannel configurations disclosed in the figures of Wada to provide for a microfluidic flow channel formed in a substrate. For instance, Wada expressly teaches that the "***channels*** and/or chambers of the microfluidic devices are typically ***fabricated in the upper surface of the bottom substrate*** or portion of the device" and sealed by mating a top layer "to the planar surface of the bottom substrate, covering and sealing the grooves and/or indentations in the surface of the bottom substrate, to form the channels and/or chambers (i.e., the interior portion) of the device at the interface of these two components." EX1006 (Wada), 21:3-5; *see also id.*, 37:23-32 (microchannels are "integrated into the body structure of a microfluidic device"), 38:1-31 (microscale channels formed in body structure, especially in the substrate using photolithography and other techniques), 39:19-33 (channels fabricated into upper surface of the bottom substrate). And as set forth in Section VII, fabrication of microfluidic channels formed in a microfluidic chips was routine, and well within the abilities of those of ordinary skill in the art. *See id.*; *see also*, *e.g.*, EX1017 (Figeys), 330-31.

117. Wada also discloses that its microfluidic flow channel is adapted to facilitate analysis and processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel. As Wada explains, its devices and methods for sorting particle populations "are carried out ***within fluidic channels***, ***along which the cell suspensions and/or other particles are flowed***." EX1006 (Wada), 37:24-28. Wada teaches achieving "uniform flow velocity to particles flowing in a first microchannel." *Id.*, 3:22-23. Wada also teaches "horizontally and vertically focusing a ***particle stream*** to position the ***stream within a desired region of a microchannel***." *Id.*, 12:30-32; *see also id.*, 12:1-14 (teaching that this focusing aligns cells "into a narrow region of a microchannel to facilitate single particle or narrow streamline detection"). Flow of the cells along of the channels of the device may be carried out by a number of mechanisms, including pressure-based flow, electrokinetic flow, or other mechanisms or combinations of mechanisms. *Id.*, 42:8-24.

118. In my opinion, a person of ordinary skill the art would have appreciated that suspensions of cells or other particles were routinely conveyed in the microfluidic device in a carrier fluid. Indeed, Wada clearly contemplates such embodiments. For instance, in screening various test compounds, Wada teaches that "the test compounds may be provided, e.g., injected, free in solution, or may be attached to a carrier, or a solid support, e.g., beads." EX1006 (Wada), 34:14-16.

As another example, Wada explains that a particle being sorted "typically includes, e.g., a soluble or non-soluble component of a fluid mixture, such as a solubilized or suspended molecule." *Id.*, 11:19-20; *see also id.*, 45:14-23 (preparing assays to be sorted by suspending particles).

119. Various examples of the microfluidic flow channel as recited in element **1.a** are depicted in the figures of Wada. For example, Figure 1A of Wada depicts a microfluidic system in which "cells 100 (or other particles) are typically flowed from one microchannels into a cross-junction and focused by introducing hydrodynamic flows 102 from two orthogonal microchannels." EX1006 (Wada), 13:11-23. Figure 1A depicts these cells as being "constrained to the center of a detection microchannel downstream from the two orthogonal microchannels by hydrodynamic flows 102 introduced from both sides as cells 100 pass through detector 104." *Id.*, 13:28-31. The left-hand side of Wada Figure 1A depicts the stream of particles (*e.g.*, cells 100) conveyed through a microfluidic flow channel (*e.g.*, horizontal microchannel) suspended in a carrier fluid in the center of the channel after passing by the cross-junction in the direction of the detector 104.

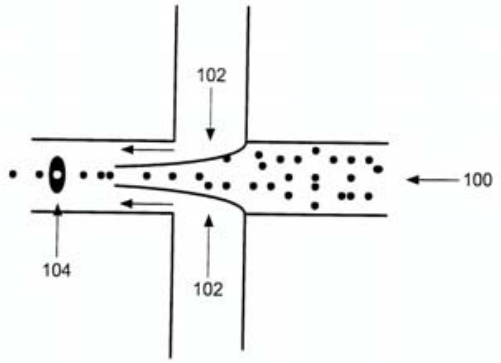



Fig. 1A

EX1006 (Wada), FIG. 1A.

120. As another example, Figure 21 of Wada a microfluidic system for focusing particles in which cell suspensions are loaded into wells (2101) and focused using a cross-configuration as in Figure 1A or a T-junction as in Figure 16 before reaching a detection zone. EX1006 (Wada), 14:3-14. That is, Figure 21 provides for a microfluidic flow channel formed in a substrate wherein particles suspended in a suspension medium flow through said channel.

121. Similarly, Figure 22 of Wada depicts a microfluidic system comprising a microfluidic flow channel for conveying a stream of particles in a suspension. In Figure 22, the schematic diagram illustrates a particle sorting configuration using sets of opposing microchannels to focus and direct the flow of members of a cell population to achieve cell sorting. EX1006 (Wada), 11:3-5, 19:23-20:2. The microfluidic flow channel comprises a vertical main

microchannel, with the main flow direction moving from the bottom of the figure to the top of the figure. As can be seen below, Figure 22 depicts particles (*e.g.*, cells 2200) in a stream in the center of the channel (*e.g.*, vertical main microchannel) after passing by the cross-junction of opposing side channels with hydrodynamic flow (2202), and proceeding toward a detector (2204).

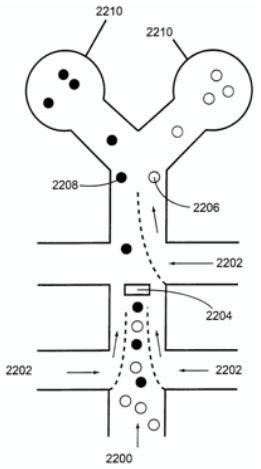



Fig. 22

EX1006 (Wada), FIG. 22.

122. Figure 23 of Wada provides another example of a microfluidic flow channel for analyzing a sample of particles suspended in a suspension medium. In Figure 23, the schematic diagram illustrates a particle sorting configuration using a microchannel orthogonal to the channel to focus the flow of members of a cell population to achieve cell sorting. EX1006 (Wada), 11:6-7, 20:3-14. The microfluidic flow channel comprises a vertical main microchannel, again, with the main flow direction moving from the bottom of the figure to the top of the figure.

As can be seen below, the particles (*e.g.*, cells 2300) in a stream on the left side of the channel, are flowed through the microfluidic flow channel (*e.g.*, vertical main microchannel), after passing by the T-junction of the side channel with hydrodynamic flow (2302), and proceeding toward a detector (2304).

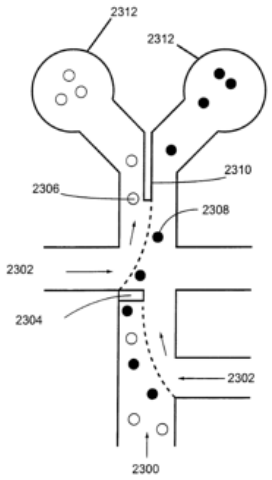



Fig. 23

EX1006 (Wada), FIG. 23.

123. Figure 24 of Wada also depicts a microfluidic system having a microfluidic flow channel for facilitating the analysis or processing of particles suspended in a suspension medium flowing through the flow channel. Specifically, a microchannel orthogonal to the channel focuses the flow of members of a cell population to achieve cell sorting. EX1006 (Wada), 8:6-23, 11:8-9, 18:26-19:21, 22:23-23:18. The channel comprises a vertical main microchannel 2402, with the main flow direction moving from the top of the figure to the bottom of the figure. As can be seen below, Figure 24 depicts the particles (*e.g.*, 2410 and 2412, as from

particle well 2400) in a stream on the left side of the channel, as they are flow through a portion (*e.g.*, vertical main microchannel 2402) of the channel.

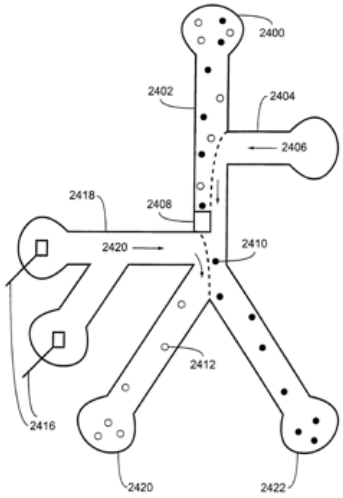



Fig. 24

EX1006 (Wada), FIG. 24.

124. For the reasons discussed above, Wada teaches claim element **1.a**. Accordingly, it is my opinion that a person of ordinary skill in the art reasonably would have utilized a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel.

**[1.b] a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel; and**

125. Wada teaches a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel. For example, Wada Figures 22 and 23 (reproduced below)

depict a reservoir in the form of an opposing microchannel structure (labeled in annotations below) disposed opposite of the actuated sorting microchannel structure (through which the actuator selectively applies a pressure pulse represented by arrows 2202 and 2302) disposed downstream of the detectors 2204 and 2304. EX1006 (Wada), 19:23-20:14; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same); *see also*, ¶¶44-45, above (discussing the terms "absorbing" and "dampening"); EX1042, 13-14, 18-20 (construing "absorbing" and "dampening"); EX1043, ¶¶37-38, 40-43, 50-52, 56-57 (construing "buffer," "absorbing," and "dampening"). Figures 22 and 23 (reproduced below with annotation) disclose to a person of ordinary skill in the art that the opposing microchannel structure is, in each case, a physical structure that contains fluid, receives a pressure pulse, and is operatively associated with the flow channel via its fluid communication with the flow channel.

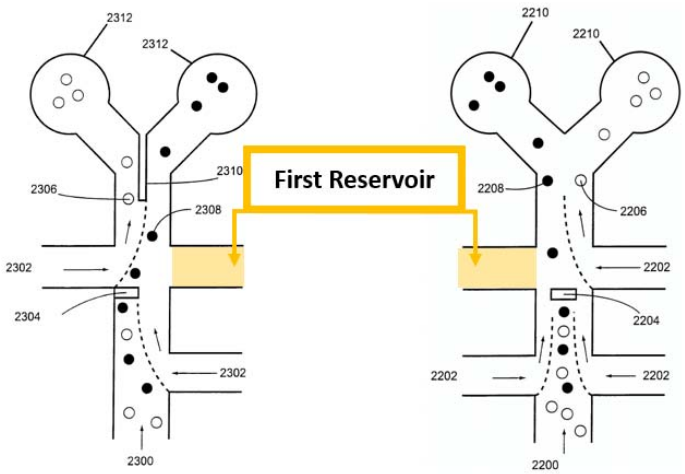



Fig. 23 Fig. 22

EX1006 (Wada), FIGS. 22-23.

126. Each of Figures 22-23 discloses a pressure pulse propagated across the flow channel (flow 2202 or 2302) to deflect a selected particle from one side of the microchannel into a selected branch on the other side of the flow channel. *See* EX1006 (Wada), FIGS. 22-23; *see also id.*, 18:26-19:13 (describing that the fluid direction components "'nudge' materials across the width of a first channel" in order to "direct sample flow towards or away from" a first flow channel, and "into, e.g., an additional intersecting channel or into a channel region"). One of ordinary skill in the art would have understood the opposed microchannel structure in each of Figures 22 and 23 is configured to receive the pressure pulse and to dampen and absorb the pressure pulse because the structure opening is aligned with the direction of the pressure pulse (substantially perpendicular to the main microchannel). The opposed microchannel structure is thus not only a reservoir,

but it is also a structure that is adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

127. As discussed above in Section VII, the use of a microchannel structure geometry with a channel, chamber, or reservoir structure across from the source of the transient pressure pulse for dampening or absorbing the transient pressure pulse was well-known and well documented in the art. *See*, *e.g.*, EX1005 (Marcus), FIG. 1; EX1007 (Zöld), FIG. 2; EX1016 (Glaettli), 1:49-2:19, FIG. 1; EX1033 (Zengerle), 198 (noting that, "[f]or many applications, the oscillation of pressure and flow in fluid channels has to be suppressed").

128. And, as also discussed in Section VII, the use of compliance devices, including fluid-filled regions for dampening or absorbing pressure disturbances in microfluidic flow channels was also well-known and well reported in the prior art. *See*, *e.g.*, EX1033 (Zengerle), 198 (teaching that pressure-induced flow "oscillations can be avoided by the use of elastic elements" that are "in contact with the oscillating fluid," describing that the use of "an enclosed gas volume is used as an elastic element very often" in the "macroscopic world," and noting "pressure smoothing" in microfluidic systems as "[a]nalogous to the procedure with macroscopic pumps"); EX1034 (Chou), 2:23-34, 39:25-34, 40:20-25 (describing a "damper" or an "energy absorber" that is "configured to experience a physical change in response to an oscillation within the flow channel" as a result of

sorting particles, wherein the damper comprises "a pocket filled with a compressible fluid, and/or flexible walls"); EX1035 (Veenstra), 377, 382-83 (describing the use of compliance structures that "contain air bubbles that dampen the flow and pressure variations that may arise from a micropump"); EX1016 (Glaettli), 5:44-54, FIG. 1 (using a gas bubble located inside a side channel to the flow channel located across from the pressure source). In my opinion, a person of ordinary skill in the art would have recognized that Wada's first reservoir is configured for absorbing or dampening the pressure pulse, as recited in claim element **1.b.**

129. Wada specifically depicts the opposed microchannel structure in Figures 22 and 23. In my opinion, Wada's teaching of the opposed microchannel structure is not limited to Figures 22 and 23 but applies equally to Figure 24. Wada expressly teaches that "all the techniques and apparatus described above may be used in various combinations." EX1006 (Wada), 49:13-20. A person of ordinary skill in the art would have understood the use of a second reservoir disclosed in Wada was equally applicable to the embodiment represented in Figure 24, as I have indicated below:

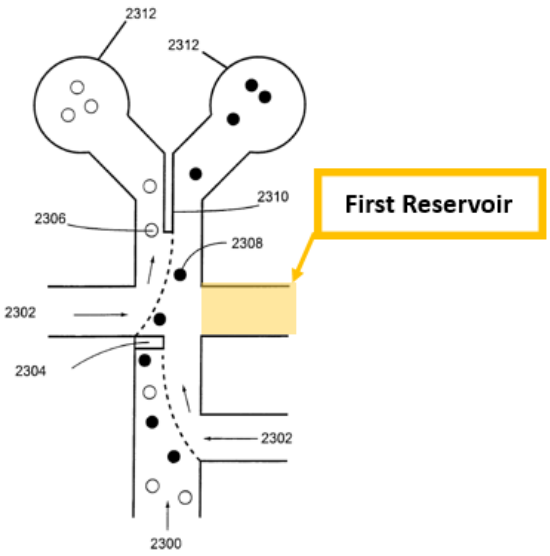



Fig. 23

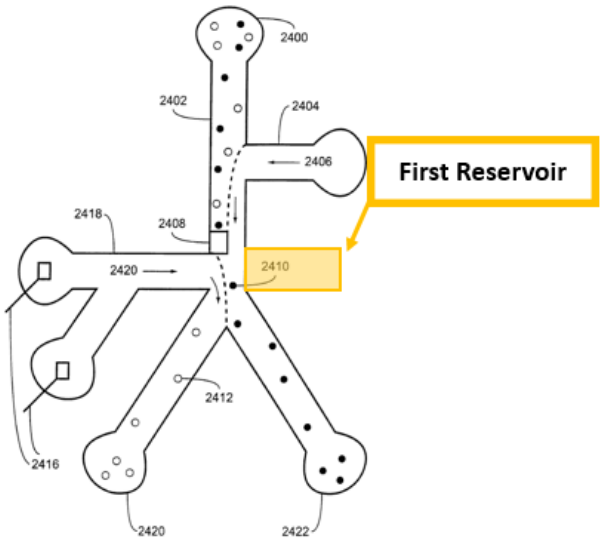



Fig. 24

EX1006 (Wada), FIGS. 23-24.

130. Indeed, this symmetrical geometry, wherein opposing ends of a cross channel are adapted to individually actuate and individually dampen or absorb the resulting transient pressure pulse, was well-reported and well known in the art as allowing for more precise flow manipulations during particle sorting. *See*, *e.g.*, EX1018 (Deshpande), 341-42; EX1032 (Dubrow), 8:4-8.

131. For the reasons discussed above, in my opinion, a person of ordinary skill in the art would have understood that Wada's opposing channel structure in Figures 22 and 23 and as applied to Figure 24 is a reservoir adapted for absorbing or dampening the pressure pulse propagated across the flow channel.

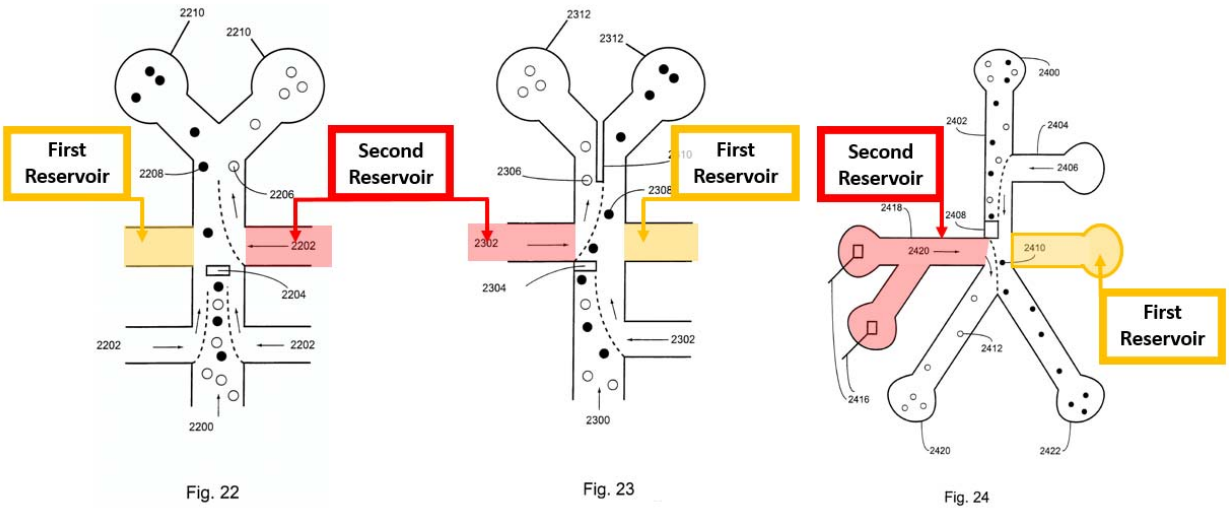
132. It is also my opinion that Wada discloses the use of a chamber or reservoir located at the distal end of the opposed microchannel that is operatively

associated with the flow channel and adapted for dampening and absorbing a pressure pulse propagated across the flow channel. Wada discloses an actuator (including a Joule heating electrode) may be disposed in a well on the distal end of each of the opposing sorting channels and being operatively associated with the flow channel. *See*, *e.g.*, EX1006 (Wada), 7:25-29. This disclosure applies to the microfluidic devices represented in Wada Figures 22 and 23, as well as the device represented in Wada Figure 24 in which an actuator is illustrated in a well at the distal end of a sorting microchannel. *See*, *e.g.*, *id.*, 8:12-23 (actuators, including Joule heating electrodes, may be disposed in each of the third and sixth microchannels or within the wells that fluidly communicate with them to sort the particles), 22:30-23:3 (Joule heating electrode 2416 is disposed in a well), 23:19-24 ("although not shown in Figure 22, one or both microchannels of the second set of opposing microchannels located downstream from detector 2204 optionally include one or more Joule heating electrode"). As Wada teaches actuators associated with each of the opposed sorting microchannels downstream of the detector, a person of ordinary skill in the art would have understood Wada as disclosing an actuator in a fluid-containing well at the end of each of the fluid-containing opposed sorting microchannels.

133. In this opposed microchannel structure disclosed by Wada, the well at the distal end of the opposed sorting microchannel is a first reservoir operatively

associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel. A well placed at the distal end of the opposed sorting microchannel as described in Wada is a physical structure that contains fluid and receives the pressure pulse. A person of ordinary skill in the art would have recognized that this first reservoir, because of its fluid connection with the opposed microchannel, is adapted for dampening and absorbing a pressure pulse propagated across the flow channel.

134. A person of ordinary skill in the art would have understood that, when the actuator in only one of the first and second reservoirs (*i.e.*, one of the two opposed sorting microchannel structures) is activated at a given time, as illustrated in each of Figures 22-24, the reservoir at the distal end of the opposite (non-actuated) sorting microchannel is adapted for dampening or absorbing the pressure pulse propagated across the flow channel. I have added annotations to Figures 22-24 to illustrate this disclosed embodiment:




EX1006 (Wada), FIGS. 22-24.

135. Wada discloses that the actuator associated with the opposed microchannel structure may be a disposed in a well, channel, or cavity. As explained by Wada, "Joule heating is typically produced by flowing current through an electrode or other conductive component positioned within a well, microscale channel, or other cavity within the device." EX1006 (Wada), 20:23-25. The term "other cavity within the device" suggests a chamber (*i.e.*, a first reservoir, *see* ¶¶39, 42, above; EX1043 (Vacca declaration), ¶¶44-48), as Wada specifically teaches creating chambers in the substrate. EX1006 (Wada), 37:29-32, 38:11-17, 39:19-33. In view of Wada, a person of ordinary skill in the art thus would have had good reason to place the Joule heating electrode on the substrate within a reservoir at the distal end of each opposed sorting microchannel to generate a pressure pulse that will propagate across the flow channel downstream of the detector a deflect a selected particle into a selected branch of the flow channel.

136. A person of ordinary skill in the art would have recognized that each reservoir at the distal end of an opposed sorting microchannel, because of its fluid connection with the flow channel via an opposed microchannel, is operatively associated with the flow channel. When the actuator in only one of the two reservoirs is activated at a given time, as illustrated in each of Figures 22-24, a person of ordinary skill in the art would have understood that the reservoir at the distal end of the opposite sorting microchannel is adapted for dampening or absorbing the pressure pulse propagated across the flow channel.

137. For the reasons discussed above, in my opinion, a person of ordinary skill in the art would have had good reason to employ a well or chamber structure that contains fluid and receives the pressure pulse at the distal end of each of Wada's opposing sorting microchannels and that this well or chamber is a reservoir operatively associated with the flow channel and adapted for absorbing or dampening the pressure pulse propagated across the flow channel.

138. In addition to Joule heating electrodes, Wada suggests using other pressure sources as an actuator including "a pressurized gas, or alternatively…a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir" that would pump the contents of the reservoir into the adjacent channel when actuated. EX1006 (Wada), 43:5-9. Wada further teaches that actuator pressure sources may be "***internal to the device***, e.g., microfabricated pumps

integrated into the device and operably linked to a channel." *Id.*, 43:9-14. A person of ordinary skill in the art thus would have had good reason in view of Wada to employ an actuator comprising a reservoir and a pressurized gas (*i.e.*, a compressible fluid) at the distal end of each of the opposed sorting microchannels. In my opinion, a person of ordinary skill in the art would have recognized that this reservoir comprising pressurized gas at the distal end of the non-actuated, opposed sorting microchannel, as suggested by Wada, is a reservoir operatively associated with the flow channel and adapted for dampening and absorbing the pressure pulse propagated across the flow channel, as recited in claim element **1.b.** For the reasons discussed above, Wada teaches claim element **1.b** and it would have been obvious to provide, in a microfluidic system, a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

**[1.c] a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir;**

139. As discussed above, Wada teaches reservoirs as part of opposing sorting microchannel structures. When actuated, the opposed sorting microchannel structure, including its well or chamber from which the pressure pulse enters the flow channel, is a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or

pressure in the second reservoir. For example, both Figures 22 and 23 of Wada depict second reservoirs as part of opposing microchannel structures (labeled in annotations below). EX1006 (Wada), 19:23-20:14; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). A person of ordinary skill in the art would have recognized that these second reservoirs are operatively associated with the microfluidic flow channel because they are in fluid communication with it and generate a transient fluid flow (*e.g.*, 2202, 2302) into the flow channel.

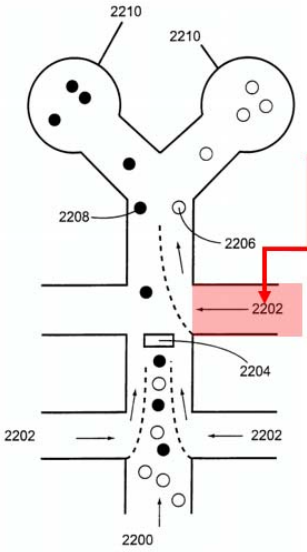



Fig. 22

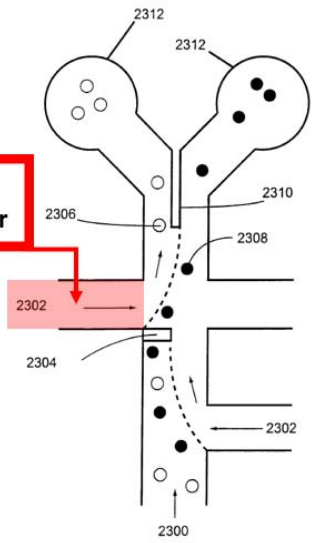



Fig. 23

EX1006 (Wada), FIGS. 22-23.

140. Further, Wada specifically teaches that these reservoir can be actuated using an actuator to generate a pressure pulse based on a change in volume or

pressure in the second reservoir. For example, Wada's fluid direction components "'nudge' materials across the width of a first channel" in order to "direct sample flow towards or away from" a first flow channel, and "into, e.g., an additional intersecting channel or into a channel region." EX1006, 18:26-19:13. "[A]lthough not shown in Figure 22, one or both microchannels of the second set of opposing microchannels located downstream from detector 2204 optionally include one or more Joule heating electrodes (e.g., 1, 2, 3, 4, 5, or more electrodes)." *Id.*, 23:18-24. Wada explains that using such an actuator allows for deflecting or redirecting of the flow of target particles. Specifically, the introduction of various fluids or particles into the microchannels of the microfluidic device can be controlled by "activating a heating element," such as "a Joule heating electrode" located in one or both of the opposed sorting microchannels or in wells or chamber/cavity at the distal ends of the opposed sorting microchannels. *Id.*, 7:25-29; *see also id.*, 8:12-23, 20:23-25, 22:30-23:3, 37:29-32, 38:11-17, 39:19-33, FIG. 24. In use, detection of a selected particle triggers the actuator to redirect the selected particle from one side of the flow channel into a selected outlet branch on the other side of the flow channel. In contrast, a non-selected particle would not trigger the actuator and would flow unimpeded into a collection well for the non-selected particles. *Id.*, 23:10-18.

141. A person of ordinary skill in the art would have recognized that the heating of the fluid in the sorting microchannel by Joule heating electrode selectively generates and applies a pressure pulse based on a change in pressure in the second reservoir and propagates it across the flow channel to deflect a particle into a selected branch of the flow channel. Wada describes this pressure pulse in terms of a decrease in the viscosity of the heated fluid resulting in flow of the fluid, but this is, in fact, a pressure pulse. *See*, *e.g.*, EX1006, 20:30-31 (describing temperature oscillation period as short as 100 milliseconds), 21:27-32 (describing applying the power supply to generate the heat as a "pulse"), 21:32-22:2 (describing the change in temperature that generates the sorting fluid flow as "extremely rapid and easily controlled"). This pressure pulse is selectively generated and applied when the detector indicates the particle is selected for deflection from the stream of particles.

142. A person of ordinary skill in the art would have appreciated that Wada's disclosures are not limited solely to embodiments where the actuator (*e.g.*, 2416 of Figure 24) is in the form of a heating element. Indeed, Wada broadly teaches "techniques for inducing the flow of focusing fluids to sort particles" using fluid direction components and flow control regulators. EX1006 (Wada), 19:9-13. Wada teaches that particle sorting may be achieved via focusing, and that particles may be focused "using one or more ***fluid direction components*** (e.g., a ***fluid***

***pressure force modulator***, an electrokinetic force modulator, a capillary force modulator, a fluid wicking element, or the like)." *Id.*, 4:1-5; *see also id.*, 7:18-29 (describing its particle sorting embodiments as comprising "***fluid direction components***" and a "***flow control regulator***," noting that the "flow control regulator…further regulates flow" in the microchannels associated with sorting), 42:8-30 (teaching that the "flowing of the suspension of cells or other particles along one or more channels of the devices described herein is optionally carried out by a number of mechanisms, including ***pressure based-flow***, electrokinetic flow, or other mechanism or combination of mechanisms"). Wada teaches that these pressure sources may be "***internal to the device***, e.g., microfabricated pumps integrated into the device and operably linked to a channel." *Id.*, 43:9-14.

143. A person of ordinary skill in the art would have recognized that these other actuators also would be useful to selectively generate and apply a pressure pulse across the flow channel to deflect a particle into a selected branch of the microchannel. For example, Wada teaches that the pressure source provided by the actuator of the particle sorting chip "is optionally pneumatic, e.g., a pressurized gas, or alternatively is a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir" that would pump the contents of the reservoir into the adjacent channel when actuated. EX1006 (Wada), 43:5-9.

144. A person of ordinary skill in the art thus would have had good reason in view of Wada to employ an actuator comprising a reservoir and a pressurized gas (*i.e.*, a compressible fluid) at the distal end of each of the opposed sorting microchannels.

145. Indeed, this sorting mechanism, wherein an actuator is used to actuate a reservoir in a microfluidic system was well-known and well reported in the art. *See*, *e.g.*, EX1020 (Neukermans), 1:42-64, 2:31-3:17 (describing "an electrically-powered actuator" that "upon application of an electrical signal to the electrically powered actuator, extends towards or retracts from the layer of malleable material" associated with a reservoir on a disposable microfluidic cartridge, wherein the "pressure applied to the reservoir urges the liquid to flow out"); EX1023 (Williams), ¶¶0026, 0032 (describing a similar mechanism of deflecting or blocking "the flow of a fluid traveling with [a] channel" via actuation of a sphere, such as a ball-bearing, wherein the "force may be applied manually using the finger of a human operator, or by any suitable mechanical means as known in the art," including actuation "using common electrical, magnetic, or pneumatic means, as is well known in the art," as well as by "external control means," involving the use of a computer, cartridges, etc.); *see also* EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is

detected"); EX1016 (Glaettli), FIG. 2 (depicting particle sorting using a stepper motor to vary volume and pressure to sort particles); EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps).

146. In my opinion, a person of ordinary skill in the art would have recognized that this reservoir comprising pressurized gas at the distal end of the non-actuated, opposed second side microchannel, as suggested by Wada, when actuated, is a second reservoir operatively associated with the flow channels and adapted for generating a pressure pulse based on a change in volume in the second reservoir and propagating it across the flow channel, as recited in claim element **1.c** of the '295 patent.

147. Based on these disclosures, it is my opinion that Wada teaches element **1.c**. That is, it would have been obvious to provide, in a microfluidic system, a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

**[1.d] wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.**

148. Wada teaches the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow relative to the second reservoir and the first aperture is substantially opposite the second aperture, as recited in element **1.d**. As discussed above, the first reservoir and second reservoir are operatively associated with the flow channel. Indeed, in describing Figure 22, Wada explains that the "opposing microchannels [are] located downstream from detector 2204 for introducing at least one hydrodynamic flow 2202 so as to direct selected cells 2208 (e.g., fluorescently-labeled cells) and non-selected cells 2206 into, in this case, one of two microchannels, each terminating in particular collection wells 2210." EX1006 (Wada), 19:30-20:2; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). Similarly, the microfluidic system of Figure 23 "also includes a set of opposing microchannels located downstream from the detector for introducing at least one hydrodynamic flow 2302 to direct selected cells 2308 (e.g., fluorescently-labeled cells) and non-selected cells 2306 to either side of separation element 2310." EX1006 (Wada), 20:8-11. As discussed above in ¶129, employing a set of opposing sorting microchannels located downstream of the detector in the microfluidic system represented by Figure 24 would have been readily apparent to an ordinarily skilled artisan based on the teachings of Wada.

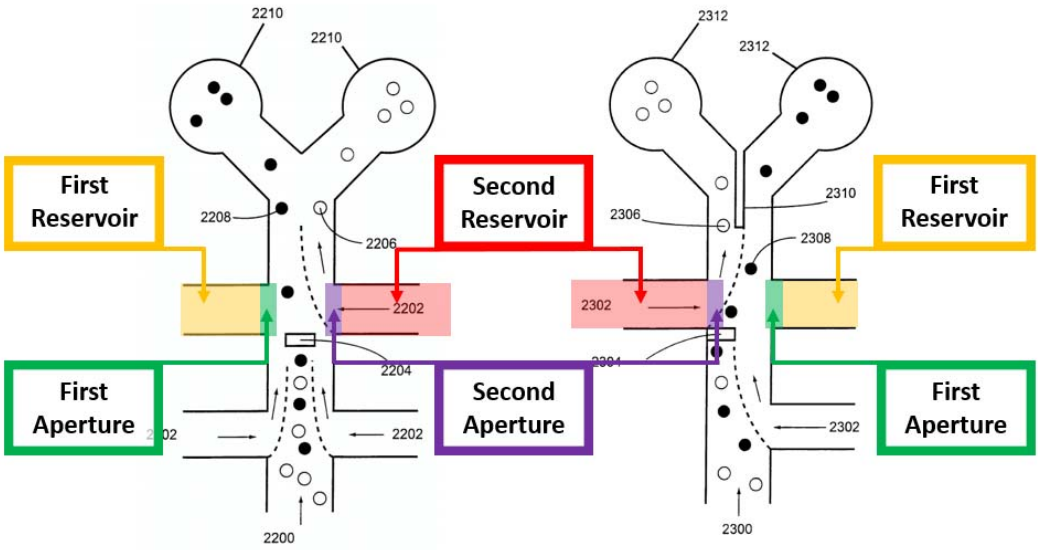



Fig. 22 Fig. 23

EX1006 (Wada), FIGS. 22-23.

149. Based on Wada's disclosure, a person of ordinary skill in the art would have recognized that the first reservoir opens into the microfluidic flow channel and, similarly, the second reservoir opens into the microfluidic flow channel. That is, the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir. The first and second aperture are depicted in the annotated versions of Figures 22 and 23. As seen in these figures, the first aperture is substantially opposite to the second aperture. As the embodiment of Figure 24 comprising a first reservoir also provides the first reservoir opening into the microfluidic flow channel, for the same reasons as those discussed above with respect to Figures 22-23, it too would comprise a flow channel that defines a first

aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow channel relative to the second reservoir, wherein the first aperture is substantially opposite to the second aperture. Wada thus discloses element **1.d.**

150. As set forth above, Wada teaches each and every element of claim 1. To the extent those elements are not found in a single embodiment of Wada, it would have well within the level of skill of the ordinary artisan to use all the elements in a single embodiment. That is expressly recognized by Wada, which teaches "all the techniques and apparatus described above may be used in various combinations." EX1006 (Wada), 49:13-20; *see also*, ¶108, above. Accordingly, it is my opinion that Wada renders obvious the subject matter of claim 1.

### B. Dependent Claims 2-3, 9, and 18

151. As I set forth below, it is my opinion that Wada, in addition to teaching each and every element of claim 1, as discussed above, also teach each and every element of claims 2-3, 9, and 17-18, which depend directly or indirectly from claim 1. My discussion above with respect to claim 1 set forth how, in my opinion, Wada provided a person of ordinary skill in the art with good reason to use the claimed elements in the claimed combination as well as a reasonable expectation of success in so doing. Those same teachings and rationales apply to my discussion of claims 2-3, 9, and 17-18.

**2. The system of claim 1 further comprising an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir.**

152. Wada teaches the microfluidic system of claim 1 further comprising an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir. As discussed above for element **1.c**, Wada teaches the use of an actuator in association with the second reservoir. Specifically, Wada uses an actuator to generate a pressure pulse and propagate the pressure pulse across the flow channel based on a change in pressure in the second reservoir to deflect a particle and direct the selected particle into a selected outlet branch. *See*, *e.g.*, EX1006 (Wada), 7:18-8:23. For the same reasons discussed above, the actuator is operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the pressure in the second reservoir.

153. As one example, Wada uses of an actuator in the form of Joule heating electrode. EX1006 (Wada), 7:25-27; *see also id.*, 8:19-22, FIG. 24. A person of ordinary skill in the art would have recognized that the heating of the fluid in sorting microchannel 2418 by Joule heating electrode 2416 selectively applies a pressure pulse to the stream by varying the pressure in the second reservoir.

154. Wada's disclosures are not limited to embodiments where the actuator (*e.g.*, 2416 of Figure 24) is in the form of a heating element. For instance, particle sorting may be achieved via focusing, and particles may be focused "using one or more ***fluid direction components*** (e.g., a ***fluid pressure force modulator***, an electrokinetic force modulator, a capillary force modulator, a fluid wicking element, or the like)." EX1006 (Wada), 4:1-5; *see also id.*, 7:18-29 (describing its particle sorting embodiments as comprising "***fluid direction components***" and a "***flow control regulator***," noting that the "flow control regulator…further regulates flow" in the microchannels associated with sorting), 42:8-30 (teaching that the "flowing of the suspension of cells or other particles along one or more channels of the devices described herein is optionally carried out by a number of mechanisms, including ***pressure based-flow***"). Notably, Wada teaches that these actuators can be included in "one or both microchannels of the second set of opposing microchannels located downstream from detector." *Id.*, 23:21-22.

155. In addition to Joule heating electrodes, Wada suggests using other pressure sources as an actuator including "a pressurized gas, or alternatively…a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir" that would pump the contents of the reservoir into the adjacent channel when actuated. EX1006 (Wada), 43:5-9. Wada further teaches that actuator pressure sources may be "***internal to the device***, e.g., microfabricated pumps

integrated into the device and operably linked to a channel." *Id.*, 43:9-14. A person of ordinary skill in the art thus would have had good reason in view of Wada to employ an actuator comprising a reservoir and a pressurized gas (*i.e.*, a compressible fluid) at the distal end of each of the opposed sorting microchannels. In my opinion, a person of ordinary skill in the art would have recognized that an actuator comprising a reservoir and pressurized gas in the opposed microchannel structure, as suggested by Wada, is an actuator that is a pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir, as recited in claim 2.

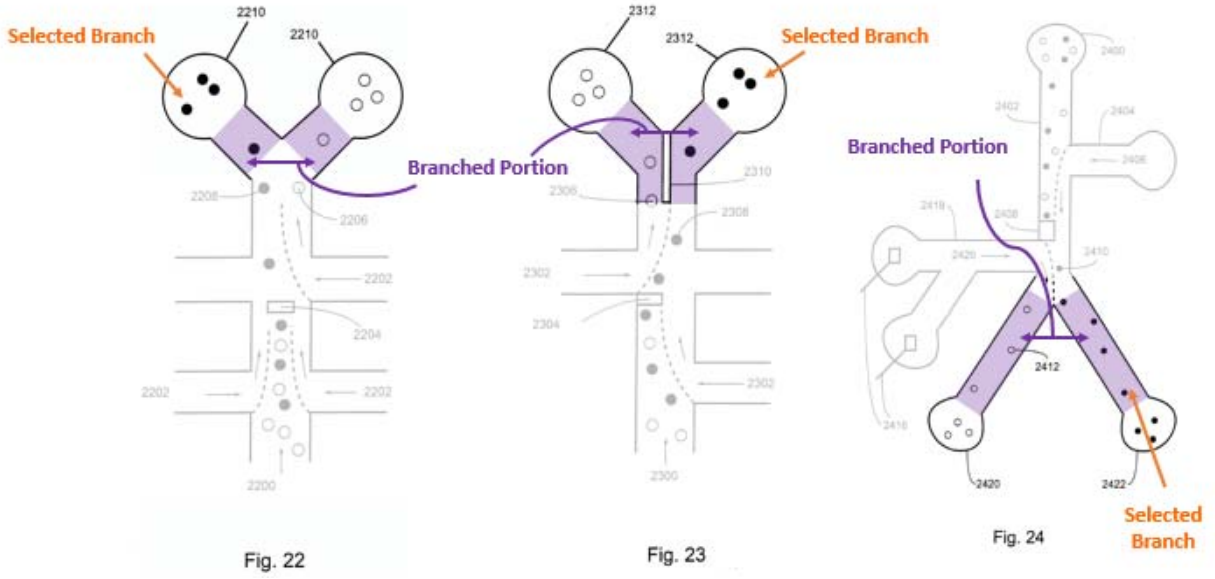
156. This type of actuation mechanism was well-known and well-reported in the art. *See*, *e.g.*, EX1016 (Glaettli), FIG. 2 (depicting particle sorting using a stepper motor to vary volume and pressure to sort particles); EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps); EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected"); EX1020 (Neukermans), 10:48-65, 12:16-19, 13:46-48 (teaching actuation means wherein "[p]ressure may be applied to the reservoirs" using a "piezo-electric actuator").

157. Accordingly, it is my opinion that Wada teaches the microfluidic system of claim further comprising an actuator or pressurizer operatively

associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir. Wada thus renders obvious claim 2 as a whole.

**3. The system of claim 1, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.**

158. Wada teaches that the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion. For example, as discussed for claim 1, Wada discloses sorting particles into one of two selected branches within a branched portion downstream of the first reservoir, each terminating in a collection well (*e.g.*, 2210, 2312, 2420, 2422). *See*, *e.g.*, EX1006 (Wada), 19:23-23:26 & FIGS. 22-24. A person of ordinary skill in the art would have recognized that the two outlet branches that each terminate into a particular collection well as described by Wada together constitute a branched portion of the flow channel, wherein the pressure pulse is used to sort a particle into a selected branch of the branched portion. Indeed, such a branched portion is depicted in each of Figures 22, 23, and 24 of Wada.




EX1006 (Wada), FIGS. 22-24.

159. Further, Wada makes clear that the pressure pulse is used to sort a particle into a selected branch of the branched portion across the flow channel. For instance, in describing Figure 22, Wada explains that the fluid flow is induced by heating, which creates a pressure pulse (*see d*iscussion re element **1.c** and claim 2). As a result of the pressure pulse, "selected cells 220 (e.g., fluorescently-labeled cells) and non-selected cells 2206" are directed into "one of two microchannels, each terminating in particular collection wells 2210." EX1006 (Wada), 19:29-20:2. That is, the pressure pulse applied across the flow channel in Figure 22 is used to sort one or more particles into a selected branch of the branched portion.

160. Similarly, in describing Figure 23, Wada explains that the pressure pulse is used to "direct selected cells 2308 (e.g., fluorescently-labeled cells) and non-selected cells 2306 to either side of separation element 2310 and into, in this

case, one of two microchannels, each terminating in particular collection wells 2312." EX1006 (Wada), 20:10-14.

161. Similarly, in describing Figure 24, Wada explains that the pressure pulse "deflects or redirects the flow of selected particle 2410 into selected particle collection well 2422" whereas non-selected particle 2412 "flows unimpeded into non-selected particle collection well 2420." EX1006 (Wada), 22:23-23:18.

162. Accordingly, Wada teaches the microfluidic system of claim 1, wherein the flow channel includes a branched portion, and wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.

**9. The system of claim 1, wherein first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.**

163. As discussed above, Wada discloses a first reservoir formed in the substrate that is adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

164. As also discussed above, in addition to Joule heating electrodes, Wada suggests using other pressure sources as an actuator including "a pressurized gas, or alternatively…a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir" that would pump the contents of the reservoir into the adjacent channel when actuated. EX1006 (Wada), 43:5-9; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and

"chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same); *see also*, ¶¶44-45, above (discussing the terms "absorbing" and "dampening"); EX1042, 13-14, 18-20 (construing "absorbing" and "dampening"); EX1043, ¶¶37-38, 40-43, 50-52, 56-57 (construing "buffer," "absorbing," and "dampening").

165. Wada further teaches that actuator pressure sources may be "***internal to the device***, e.g., microfabricated pumps integrated into the device and operably linked to a channel." EX1006 (Wada), 43:9-14. A person of ordinary skill in the art thus would have had good reason in view of Wada to employ an actuator comprising a reservoir and a pressurized gas (*i.e.*, a compressible fluid) at the distal end of each of the opposed sorting microchannels.

166. As discussed above with respect to claim element **1.b**, a person of ordinary skill in the art would have recognized that this reservoir comprising pressurized gas at the distal end of the non-actuated, opposed second side microchannel, as suggested by Wada, is a reservoir operatively associated with the flow channels and adapted for dampening and absorbing the pressure pulse propagated across the flow channel.

167. Wada explains the device materials are "generally selected for their compatibility with the full range of conditions to which the microfluidic devices are typically exposed, including extremes of pH, temperature, salt concentration, and application of electric fields." EX1006 (Wada), 38:24-27.

168. As discussed above in Section VII.D, reservoirs comprising a resilient wall for dampening and absorbing a pressure pulse were commonly used in the art. *See*, *e.g.*, EX1033 (Zengerle), 198 (teaching that "an enclosed gas volume is used as an elastic element very often," as are "deflectable diaphragm[s] with a large fluid capacitance"); EX1034 (Chou), 39:25-34, 40:20-25 (describing a "damper" or an "energy absorber" that then can be "configured to experience a physical change in response to an oscillation within the flow channel" as a result of sorting particles, the damper comprising "a flexible membrane, a pocket filled with a compressible fluid, and/or flexible walls of the flow channel itself."); *id*. ("the damper is an encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas").

169. Accordingly, it is my opinion that Wada teaches or suggests the microfluidic device of claim 1 further comprising a first reservoir having a resilient wall for facilitating dampening or absorbing of the pressure pulse.

**18. The system of claim 1, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.**

170. Wada discloses that the change in pressure in the second reservoir is a transient change in pressure. For instance, when using Joule heating in the second reservoir to facilitate sorting, Wada notes that "transitions between temperatures

are typically extremely short." EX1006 (Wada), 20:28-31; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). A person of ordinary skill in the art would have recognized that, because the duration of the temperature change is extremely short, the corresponding change in pressure is also short-lived, meaning transient. *See*, *e.g.*, EX1006, 20:30-31 (describing temperature oscillation period as short as 100 milliseconds), 21:27-32 (describing applying the power supply to generate the heat as a "pulse"), 21:32-22:2 (describing the change in temperature that generates the sorting fluid flow as "extremely rapid and easily controlled").

171. Thus, it is my opinion that claim 18 is obvious in view of Wada.

## X. GROUND 2: CLAIMS 1-3, 9, AND 17-18 ARE OBVIOUS IN VIEW OF WADA AND ANDERSON.

172. As discussed in Ground 1, Wada renders obvious the microfluidic system of claims 1-3, 9, and 18. The disclosures of Anderson further demonstrate the obviousness of these claims as well as claim 17. The analysis above of Ground 1 is included here for each of the claims analyzed.

### A. Claim 1 and Dependent Claims 2-3

173. Wada teaches that many "techniques for inducing the flow of focusing fluids to sort particles according to the methods of the present invention" may be used. EX1006 (Wada), 19:9-13. Sorting forces disclosed by Wada include

"pressure, hydrostatic, wicking, capillary, and other forces." *Id.* As discussed above, based on Wada's disclosure, a person of ordinary skill in the art would have recognized that Wada provides for a first reservoir used for dampening or absorbing a pressure pulse and a second reservoir used for generating the pressure pulse. For more specifics regarding actuators, Wada points to and incorporates by reference International PCT Patent Publication No. WO 97/02357 (Anderson, EX1012). EX1006 (Wada), 43:1-14. The skilled artisan thus would have had good reason to look to Anderson for further discussion regarding actuators for generating pressure pulses.

174. For example, Anderson teaches "[p]umping devices that are particularly useful in a variety of ***micromachined pumps***," including those with "***a bulging diaphragm.***" EX1012 (Anderson), 63:1-13. One example provided by Anderson, for example, includes a chamber that "incorporate[s] a ***diaphragm pump as one surface of the chamber***." *Id.*, 45:3-12 (noting that this "diaphragm pump will generally be fabricated from any one of a variety of flexible materials, e.g., silicone, latex, teflon, mylar and the like"); *see also id.*, 38:17-27, 45:3-12 (noting that the flexible diaphragm pump is "similar to the valve structure" comprising a flexible diaphragm, noting that "[d]eflection of [a] diaphragm valve" may also be achieved using "piezoelectric actuators coupled to the diaphragm valve, and the like"). Based on these disclosures, a person of ordinary skill in the

art would have recognized that Anderson further renders obvious elements **1.b** and **1.c**, directed to the dampening or absorbing a pressure pulse propagated across the flow channel, and the generating of a pressure pulse based on a change in volume or pressure in the second reservoir.

175. Since Wada expressly points to Anderson for teaching applicable pumps for incorporation into its microfluidic systems, a person of ordinary skill would have had good reason to employ, in the microfluidic systems taught by Wada, a micropump comprising a flexible membrane, as taught by Anderson, in each of the opposing sorting microchannel structures (*i.e.*, first and second reservoirs), including at the distal end of the structures. When one of the opposed sorting microchannels is actuated and the other is not, as disclosed at least in Wada Figures 22 and 23, the actuated opposed sorting microchannel structure will generate a pressure pulse to sort a particle in the microfluidic flow channel and be a second reservoir. In contrast, the non-actuated opposed sorting microchannel structure will dampen or absorb the pressure pulse and be a first reservoir. It would have been obvious for each of the reservoirs to include a resilient wall, including a deflectable membrane, for facilitating dampening or absorbing of the pressure pulse (in the non-actuated reservoir) and for increasing pressure (in the actuated reservoir).

176. In view of the teachings of Wada and Anderson, a person of ordinary skill in the art thus would have had good reason and a reasonable expectation of success to use flexible membranes, as taught by Anderson, in the microfluidic system of claims 1-3.

177. Based on the above disclosures, it is my opinion that the combination of Wada and Anderson further demonstrates the obviousness of claims 1-3.

### B. Dependent Claims 9 and 17-18

**9. The system of claim 1, wherein the first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.**

178. Anderson discloses using a diaphragm pump "as one surface of the chamber" in microfluidic device. EX1012 (Anderson), 45:3-12; *see also*, ¶¶36-43, above (discussing the term "reservoir"; EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same); *see also*, ¶¶44-45, above (discussing the terms "absorbing" and "dampening"); EX1042, 13-14, 18-20 (construing "absorbing" and "dampening"); EX1043, ¶¶37-38, 40-43, 50-52, 56-57 (construing "buffer," "absorbing," and "dampening"). In my opinion, a person of ordinary skill in the art would have understood that a diaphragm pump has a resilient wall (*e.g.*, the diaphragm) that can dampen or absorb a pressure pulse.

179. As discussed above for claim 1 as to Ground 1, the first reservoir of the microfluidic system is provided for absorbing a pressure pulse propagated across the flow channel from the second reservoir. A person of ordinary skill in the art would have appreciated that the diaphragm pump of Anderson has a resilient wall (*e.g.*, the diaphragm) that absorbs pressure. In view of Wada's express invitation to turn to the teachings of Anderson, as well as Anderson's teachings regarding resilient surfaces, a person of ordinary skill in the art would have had good reason to use a diaphragm pump in the first reservoir of the microfluidic system for dampening or absorbing a pressure pulse propagated across the flow channel.

180. Using a resilient wall as taught by Anderson was conventional in the art before the earliest claimed priority date. As discussed above in Section VII.D, reservoirs comprising a resilient wall were commonly used in the art. *See*, *e.g.*, EX1033 (Zengerle), 198 (teaching that "an enclosed gas volume is used as an elastic element very often," as are "deflectable diaphragm[s] with a large fluid capacitance"); EX1034 (Chou), 39:25-34, 40:20-25 (describing a "damper" or an "energy absorber" that then can be "configured to experience a physical change in response to an oscillation within the flow channel" as a result of sorting particles, the damper comprising "a flexible membrane, a pocket filled with a compressible fluid, and/or flexible walls of the flow channel itself."); *id*. ("the damper is an

encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas").

181. Based on at least these teachings in Wada and Anderson, it is my opinion that claim 9 as a whole would have been obvious.

**17. The system of claim 1, wherein the second reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the second reservoir.**

182. As discussed above, Wada points to Anderson for teachings about actuators for its microfluidic devices. Anderson teaches "[p]umping devices that are particularly useful in a variety of ***micromachined pumps***," including those with "***a bulging diaphragm.***" EX1012 (Anderson), 63:1-13. One example provided by Anderson, for example, includes a chamber that "incorporate[s] a ***diaphragm pump as one surface of the chamber***." *Id.*, 38:17-27, 45:3-12 (noting also that this "diaphragm pump will generally be fabricated from any one of a variety of flexible materials, e.g., silicone, latex, Teflon, mylar and the like" and that the flexible diaphragm pump is "similar to the valve structure" comprising a flexible diaphragm, noting that "[d]eflection of [a] diaphragm valve" may also be achieved using "piezoelectric actuators coupled to the diaphragm valve, and the like"). Based on these disclosures, a person of ordinary skill in the art would have recognized that Anderson teaches the use of diaphragm pump for increasing the pressure in the microfluidic system. Furthermore, a person of ordinary skill in the

art would also appreciate that a diaphragm pump comprises a deflectable flexible membrane (*e.g.*, the diaphragm) wherein deflection of the membrane increases the pressure in the second reservoir.

183. Since Wada expressly points to Anderson for teachings regarding micropumps for incorporation into its microfluidic system, a person of ordinary skill would have had good reason to employ, and a reasonable expectation of success of using, a diaphragm pump, as taught by Anderson, in the microfluidic system taught by Wada. *See*, *e.g.*, EX1006 (Wada), 43:1-14; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). Doing so would provide the second reservoir with a deflectable flexible membrane (*e.g.*, the diaphragm) wherein deflection of the membrane increase the pressure in the second reservoir when the flexible membrane flexes into the chamber volume.

184. This type of pressure-based actuation mechanism was well-known and well-reported in the art. *See*, *e.g.*, EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps); EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse, which results in a pressure pulse that "divert[s] the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected"); EX1020 (Neukermans), 10:48-65, 12:16-19, 13:46-48 (teaching

actuation means wherein "[p]ressure may be applied to the reservoirs" using a "piezo-electric actuator").

185. Based on at least these teachings in Wada and Anderson, it is my opinion that claim 17 as a whole would have been obvious.

**18. The system of claim 1, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.**

186. As discussed above, a person of ordinary skill in the art would have had reason to, along with a reasonable expectation of success in, incorporating the diaphragm pump of Anderson in the second reservoir of the microfluidic system of Wada. Further, a person of ordinary skill in the art would have appreciated that deflection of a diaphragm pump provides a corresponding change in volume or pressure. Such change is not permanent, however, and therefore the change in volume or pressure is a transient change in volume and pressure. Accordingly, the combination of Wada and Anderson renders obvious the microfluidic system of claim 1 wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure, as recited in claim 18.

## XI. GROUND 3: CLAIMS 1-3, 9, AND 17-18 ARE OBVIOUS OVER MARCUS IN VIEW OF ANDERSON.

187. As discussed in detail below, it is my opinion that Marcus, together with Anderson, teaches each and every element of each of claims 1-3, 9, and 17-18 of the '295 patent. It is also my opinion that a person of ordinary skill in the art

would have had good reason to use the claimed elements in the claimed combination, in view of the combined teachings of Marcus and Anderson. I also explain why a person of ordinary skill in the art would have done so with a reasonable expectation of success.

188. Marcus describes a fluidic sorting device for separating particles, such as different cells in blood. EX1005 (Marcus), 2:7-13. As explained by Marcus, the fluidic logic element of the fluidic sorting device (labeled generally as "1," in Figure 1, above), comprises an inlet (2), two control ports (3 and 4) for sorting particles (7 and 8) within a fluid carrier (15), and two outlets (5 and 6). EX1005 (Marcus), 2:14-18, 3:30-62.

189. Marcus teaches that machine vision apparatus (16), which includes decision control device (12), identifies particles conveyed in a stream of particles within the main channel of the device with visually distinct features and subsequently conveys a "message to control ports in the fluidic logic element" to actuate the control ports using electronic leads 17 and 18 in order to deflect a particle in the stream of particles from the stream of particles and into selected outlet branch (5) or (6). EX1005 (Marcus), 2:49-66, 4:30-39; *see also id.*, 4:57-64 (teaching that a second fluidic logic element may also be added to allow for sorting based on multiple, distinct characteristics).

190. Marcus explains that its system operates by combining a conventional "fluidic sorting device," that is "produced using conventional micromachining technology" (*i.e.*, a microfluidic sorting device) and a conventional "machine vision apparatus." EX1005 (Marcus), 2:1-20 (noting that "[n]o novelty lies in either one of the components per se"); *see also id.*, 3:8-12. Thus, it would have been readily apparent to a person of ordinary skill in the art to look to other available teachings, including those of Anderson regarding thermopneumatic and piezoelectric micropumps, to sort individual particles in a stream of particles flowing through a microfluidic flow channel of a microfluidic device, as disclosed in Marcus. Given the conventional nature of the components taught by Marcus and Anderson, one of ordinary skill in the art would have found it routine and well within their abilities to implement or practice the device of Marcus using conventional microfluidic actuator components, such as those disclosed in Anderson.

191. As set forth in more detail below, it is my opinion that the teachings of Marcus and Anderson provide a complete description of all of the limitations of claims 1-3, 9, and 17-18. I address below each of claims 1-3, 9, and 17-18 on an element-by-element basis as exemplary support demonstrating that Marcus and Anderson disclose each element of each claim. Further, as explained in greater detail below, it is my opinion that one of ordinary skill in the art would have found

it apparent to and would have had a reasonable expectation of success in combining the teachings of Marcus and Anderson. Using known and conventional components as disclosed by Anderson and suggested by Marcus would result in the subject matter recited in claims 1-3, 9, and 17-18. *See*, *e.g.*, EX1005, 2:9-20, 3:63-4:3, 5:1-11.

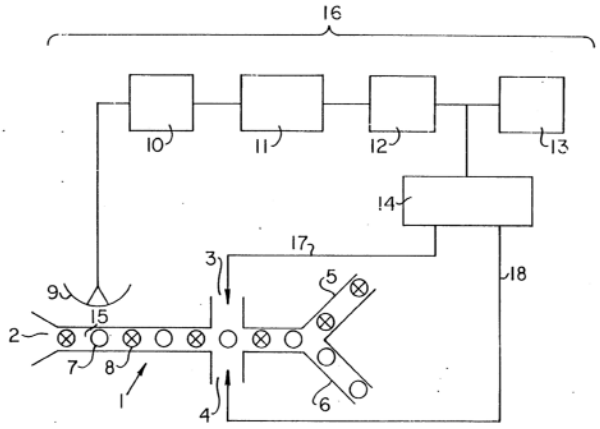
**A. Claim 1**

**1.[preamble] A microfluidic system comprising:**

192. Marcus's disclosure is directed to microfluidic systems. Entitled "Fluidic Sorting Device for Two or More Materials Suspended in a Fluid," Marcus teaches "a fluidic sorting device for the separation of two or more visually different materials." EX1005 (Marcus), Abstract. Further, the microfluidic device allows for sorting of "a particle or material" via "value judgements" made by a machine vision apparatus that "decides as to whether the particle is to be separated out." *Id.* Marcus explains that the machine vision apparatus "conveys this message" of which particle to sort to "a fluidic controller" within a "fluidic logic element." *Id.*, 2:49-66. The "fluidic logic element" is "conventional and may be produced using conventional micromachining technology." *Id.*, 2:15-20; *see also id.*, 1:62-68 (noting the device sorts "blood cells or chromosomes" as well as many other "different types and sizes of materials or particles"), 3:65-4:3. In my opinion, the implementation of conventional micromachining technology to make a fluidic

sorting device provides a microfluidic system, and a person of ordinary skill in the art would have understood as much in view of the teachings of Marcus.

193. An example schematic of Marcus's microfluidic system can be seen throughout its disclosure, including as represented in Figure 1, reproduced below.




194. Thus, a person of ordinary skill in the art would have understood Marcus's disclosure as teaching a microfluid device, as recited in the preamble of claim 18.

**[1.a] a microfluidic flow channel formed in a substrate and adapted to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel;**

195. Marcus discloses a microfluidic flow channel formed in a substrate and adapted to facilitate analysis and processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel. For example, Marcus teaches microfluidic flow channels that are within the fluidic

logic element that is "***produced using conventional micromachining technology*** or any other conventional means…so as to accommodate the sizes of the particles to be sorted." EX1005 (Marcus), 2:14-30. As Marcus explains, its systems for sorting particle populations involve the use of a fluid logic element that has a main microfluidic flow channel (*e.g.*, horizontal microfluidic flow channel) that "comprises one input port, two control ports and two output ports." *Id.* I have provided an annotated Figure 1 below, highlighting these components of the fluidic logic element of Marcus Figure 1.

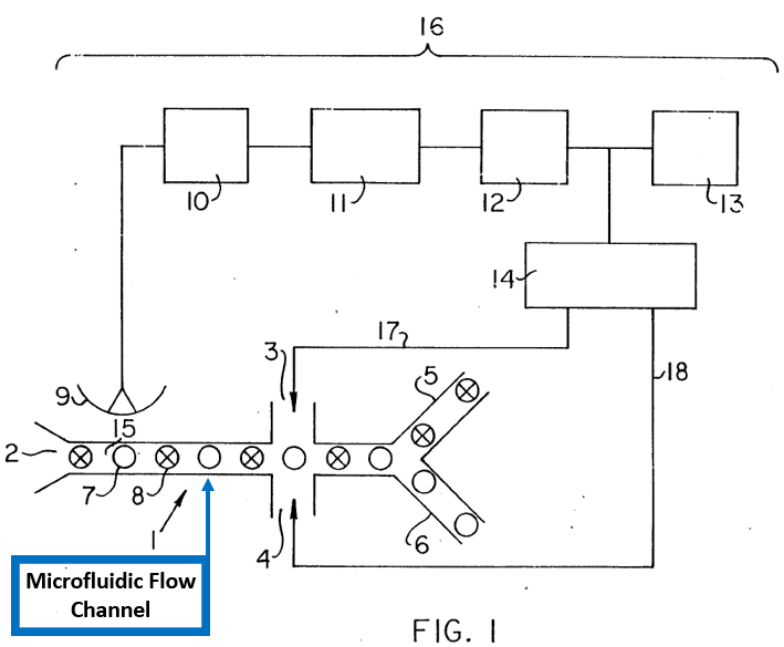


FIG. 1

*See* EX1005 (Marcus), FIG. 1.

196. Marcus further explains that the substrate of the fluidic logic element "may be composed of semi-conductor materials, such as silicon and gallium arsenide." *Id.* (explaining also that, in "order for the machine vision apparatus to

capture an image of the particles flowing therethrough, the top surface cover over the fluidic logic device is composed of a transparent material"). Thus, Marcus teaches the formation of a microfluidic flow channel using micromachining technology in a substrate such as, *e.g.*, silicon, wherein the microfluidic flow channel is covered with a transparent material. As set forth in Section VII, fabrication of microfluidic flow channels formed in a substrate was routine, and well within the abilities of those of ordinary skill in the art. *See id.*; *see also*, *e.g.*, EX1017 (Figeys), 330-31.

197. Marcus also discloses that its microfluidic flow channel is adapted to facilitate analysis and processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel. As Marcus explains, its devices and methods for sorting particle populations "utilize[] a continuous flow of suspended materials or particles in a liquid means." EX1005 (Marcus), 1:37-53.

198. In my opinion, a person of ordinary skill the art would have appreciated that suspensions of cells or other particles were routinely conveyed in the microfluidic device in a carrier fluid. Indeed, Marcus clearly contemplates such embodiments. For instance, Marcus teaches that the microfluidic flow channel "***utilizes a continuous flow of suspended materials or particles in a liquid means***." EX1005 (Marcus), 1:37-53. Marcus also teaches achieving particle

sorting using a machine vision apparatus, which is "programmed to identify and interpret different images ***suspended in a fluid medium***." *Id.*, 2:49-66.

199. An example of the microfluidic flow channel as recited in element **1.a** is depicted in Marcus Figure 1, reproduced above. Marcus Figure 1 shows an example microfluidic system wherein "element 1 broadly depicts the fluid logic element. Said fluidic logic element 1 comprises input flow port 2 through which a suspension containing particulate matter is forced. Fluidic logic element 1 further comprises control port A and control port B, 4 and 3, respectively; and output port A and output port B, 5 and 6, respectively." EX1005 (Marcus), 3:30-37.

200. Figure 1 depicts a "suspension containing at least two visually different particular matter [that] is forced through input flow port 2. The suspension contains particle type A, 8, particle type B, 7, and a fluid carrier 15." *Id.*, 3:49-62. As can be seen above, Marcus Figure 1 depicts the stream of particles (particles 7 and 8) conveyed through a microfluidic flow channel (*e.g.*, horizontal microchannel) suspended in a fluid carrier (15) in the center of the channel, passing by the detector (*e.g.*, machine vision camera lens 9) and flowing towards the sorting control ports (3 and 4).

201. For the reasons discussed above, Marcus teaches claim element **1.a**. Accordingly, it is my opinion that a person of ordinary skill in the art reasonably would have utilized a microfluidic flow channel formed in a substrate and adapted

to facilitate analysis or processing of a sample having one or more particles suspended in a suspension medium flowing through the flow channel.

**[1.b] a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel; and**

202. Marcus in view of Anderson discloses teaches a first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

203. As to be discussed below in claim element **1.c**, Marcus in view of Anderson teaches propagating a pressure pulse across the flow channel. Marcus teaches "***fluidic controller device 14*** which transmits using leads 17 and 18 the input signal from the control decision device 12 to the fluidic element control ports 3 and 4. The input signal ***instructs the control ports 3 and 4 as to how the particulate materials 7 and 8 should be sorted***." EX1005 (Marcus), 3:37-48; *see also id.*, 3:49-62. More specifically, Marcus discloses the fluidic controller device 14 receives an "input decision from the control decision device 12" of the machine vision apparatus (16), which detects a particle of interest at a detector (machine vision camera lens 9)." *See*, *e.g.*, *id.*, 3:43-48. *Id.* As explained by Marcus:

**Machine vision camera lens 9 reads the appearance of said particles 7 and 8. It transmits what is read to an image capture device 10 which forwards what it sees to an image interpretation device 11**

**which is able to interpret and register a distinction between particles 7 and 8. This distinction is then transmitted to a control decision device 12 which signals a** *fluidic controller device 14 to instruct control ports A and B, 4 and 3 respectively, to separate out said particles 7 and 8. These particles 7 and 8 are then sorted through output port A and output port B, 5 and 6***.**

*Id.*

204. I have added annotations to Figure 1 (reproduced below) to label the fluidic controller device 14 and the control ports (3 and 4):

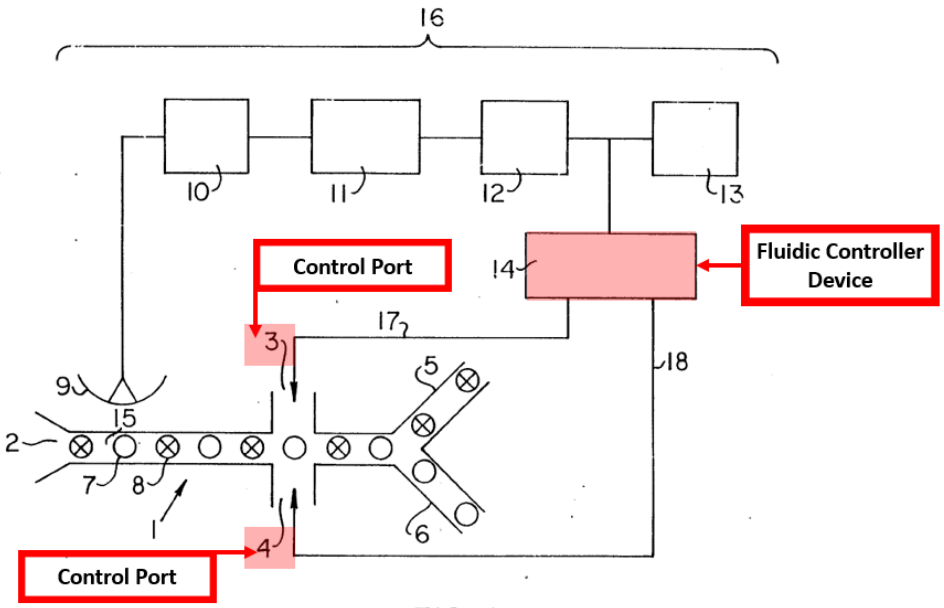



FIG. 1

EX1005 (Marcus), FIG. 1.

205. Marcus thus discloses an actuator that facilitates analysis or processing of a particle sample.

206. To the extent that Marcus does not expressly state that the mechanism being used is a pressure pulse, such implementation would have been conventional in the art. Marcus teaches its microfluidic device is comprised of conventional components. *E.g.* EX1005 (Marcus), 3:8-12. Thus, it is my opinion that it would have been routine for one of ordinary skill in the art to look to other references for specific teachings regarding such components. As discussed above, Anderson provides disclosures regarding conventional actuators and pumps that were adopted by microfluidic devices during the relevant time. EX1012, 2:29-34; *see also id.*, 28:1-29 (noting that "[m]icrofabrication of microfluidic devices…has been discussed in detail in" the prior art), 34:5-21, 37:13-28; *see also* EX1006 (Wada), 43:14 (directing persons of ordinary skill in the art to reference Anderson's teachings regarding pumps for use in microfluidic devices).

207. Anderson describes select advances in "the fabrication of micromechanical structures, e.g., micropumps, microvalves and the like, and microfluidic devices including miniature chambers and flow passages." EX1012, 2:29-34. As one example, Anderson teaches a chamber that serves as a pumping chamber with "a diaphragm pump as one surface of the chamber" with "zero displacement when the diaphragm is not deflected." EX1012 (Anderson), 44:28-46:44. The diaphragm pump may be activated via heat (*e.g.*, using a "thermopneumatic pump" comprising a "small scale resistive heater disposed in

[the] pressure chamber") or via physical displacement (*e.g.*, piezoelectric stack). EX1012 (Anderson), 20:9-17, 38:17-27, 45:3-12, 52:1-15, 63:1-13, 65:6-11. A person of ordinary skill in the art would have recognized that these conventional actuators were suitable for implementing Marcus's microsorter. Accordingly, it is my opinion that it would have been apparent to incorporate the pump of Anderson as the sorting mechanism in Marcus.

208. In view of the teachings of Anderson and Marcus, a person of ordinary skill in the art would have recognized that fluidic controller device 14 direct fluid control ports 3 and 4 to propagate a pressure pulse across the flow channel to facilitate analysis or processing of a particle sample.

209. Indeed, this mechanism, wherein an actuator is used to apply a pressure pulse in a microfluidic device was well-known and well reported in the art. *See*, *e.g.*, EX1020 (Neukermans), 1:42-64, 2:31-3:17 (describing "an electrically-powered actuator" that "upon application of an electrical signal to the electrically powered actuator, extends towards or retracts from the layer of malleable material" associated with a reservoir on a disposable microfluidic cartridge, wherein the "pressure applied to the reservoir urges the liquid to flow out"); EX1023 (Williams), ¶¶0026, 0032 (describing a similar mechanism of deflecting or blocking "the flow of a fluid traveling with [a] channel" via actuation of a sphere, such as a ball-bearing, wherein the "force may be applied manually

using the finger of a human operator, or by any suitable mechanical means as known in the art," including actuation "using common electrical, magnetic, or pneumatic means, as is well known in the art"); s*ee also* EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected"); EX1016 (Glaettli), FIG. 2 (depicting particle sorting using a stepper motor to vary volume and pressure to sort particles); EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps).

210. Marcus in view of Anderson also teach use of a first reservoir operatively associated with the flow channel to dampening or absorbing the pressure pulse that is propagated across the flow channel.

211. For example, Marcus Figure 1 (reproduced below) depicts a reservoir in the form of an opposing microchannel structure (labeled with yellow annotation below) disposed opposite the actuated sorting microchannel structure (through which the actuator selectively applies a pressure pulse represented by the arrow associated with fluidic control port 4) disposed downstream of the detector, machine camera lens 9. EX1005 (Marcus), 3:28-62; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same); *see also*, ¶¶44-45, above

(discussing the terms "absorbing" and "dampening"); EX1042, 13-14, 18-20 (construing "absorbing" and "dampening"); EX1043, ¶¶37-38, 40-43, 50-52, 56-57 (construing "buffer," "absorbing," and "dampening"). Figure 1 discloses to a person of ordinary skill in the art that the opposing microchannel structure is, in each case, a physical structure that contains fluid, receives a pressure pulse, and is operatively associated with the flow channel via its fluid communication with the flow channel.

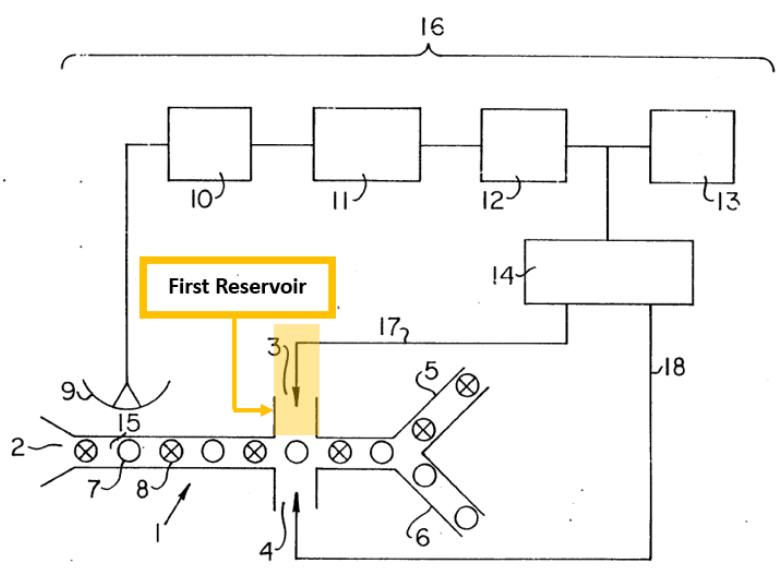



FIG. 1

EX1005 (Marcus), FIG. 1.

212. Based on the teachings of Marcus and Anderson, one of ordinary skill in the art would have understood the opposed microchannel structure (3) in Figure 1 is configured to receive the pressure pulse (flow from fluidic control port 4) and to dampen and absorb the pressure pulse because the structure opening is aligned

with the direction of the pressure pulse (substantially perpendicular to the main microchannel). The opposed microchannel structure is thus not only a reservoir, but it is also a structure associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

213. When the actuator ("fluidic controller device 14") actuates only one of the two opposed sorting microchannel structures at a given time (*e.g.*, control port A, 4), as illustrated in each of Figure 1, a person of ordinary skill in the art would have understood that the opposite (non-actuated) sorting microchannel (3) is adapted for dampening or absorbing the pressure pulse propagated across the flow channel. I have added annotations to Figure 1 (reproduced below) to illustrate this disclosed embodiment:

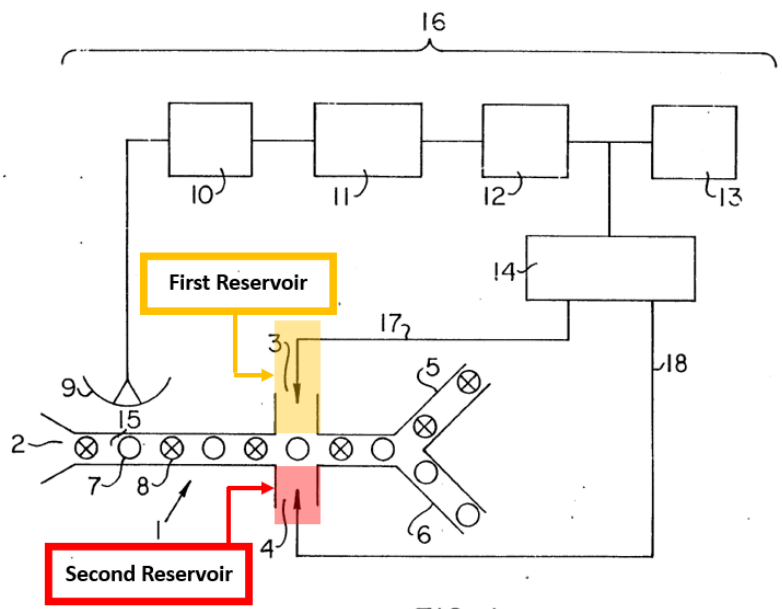



FIG. 1

*See* EX1005 (Marcus), FIG. 1.

214. Indeed, this symmetrical geometry, wherein opposing ends of a cross channel are adapted to individually actuate and individually dampen or absorb the resulting transient pressure pulse, was well-reported and well known in the art as allowing for more precise flow manipulations during particle sorting. *See*, *e.g.*, EX1018 (Deshpande), 341-42; EX1032 (Dubrow), 8:4-8.

215. As discussed above in Section VII, the use of a microchannel structure geometry with a channel, chamber, or reservoir structure across from the source of the transient pressure pulse for dampening or absorbing the transient pressure pulse was well-known and well documented in the art. *See*, *e.g.*, EX1016 (Glaettli), 1:49-2:19, FIG. 1; EX1033 (Zengerle), 198 (noting that, "[f]or many applications, the oscillation of pressure and flow in fluid channels has to be suppressed").

216. And, as also discussed in Section VII, the use of compliance devices, including fluid-filled regions for dampening or absorbing pressure disturbances in microfluidic flow channels was also well-known and well reported in the prior art. *See*, *e.g.*, EX1033 (Zengerle), 198 (teaching that pressure-induced flow "oscillations can be avoided by the use of elastic elements" that are "in contact with the oscillating fluid," describing that the use of "an enclosed gas volume is used as an elastic element very often" in the "macroscopic world," and noting "pressure smoothing" in microfluidic systems as "[a]nalogous to the procedure with macroscopic pumps"); EX1034 (Chou), 2:23-34, 39:25-34, 40:20-25

(describing a "damper" or an "energy absorber" that is "configured to experience a physical change in response to an oscillation within the flow channel" as a result of sorting particles, wherein the damper comprises "a pocket filled with a compressible fluid, and/or flexible walls"); EX1035 (Veenstra), 377, 382-83 (describing the use of compliance structures that "contain air bubbles that dampen the flow and pressure variations that may arise from a micropump"); EX1016 (Glaettli), 5:44-54, FIG. 1 (using a gas bubble located inside a side channel to the flow channel located across from the pressure source). In my opinion, a person of ordinary skill in the art would have recognized that Marcus's first reservoir is configured for absorbing or dampening the pressure pulse, as recited in claim element **1.b.**

217. For the reasons discussed above, in my opinion, a person of ordinary skill in the art would have had good reason to employ Marcus's opposing sorting microchannels, wherein one opposing sorting microchannel (*e.g.*, 3) is a reservoir operatively associated with the flow channel and adapted for absorbing or dampening the pressure pulse propagated across the flow channel. Accordingly, Marcus in view of Anderson teaches claim element **1.b**.

**[1.c] a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir;**

218. As discussed above relative to claim element **1.b**, Marcus in view of Anderson teaches reservoirs as part of opposing sorting microchannel structures. When actuated, the opposed sorting microchannel structure, including its well or chamber from which the pressure pulse enters the flow channel, is a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

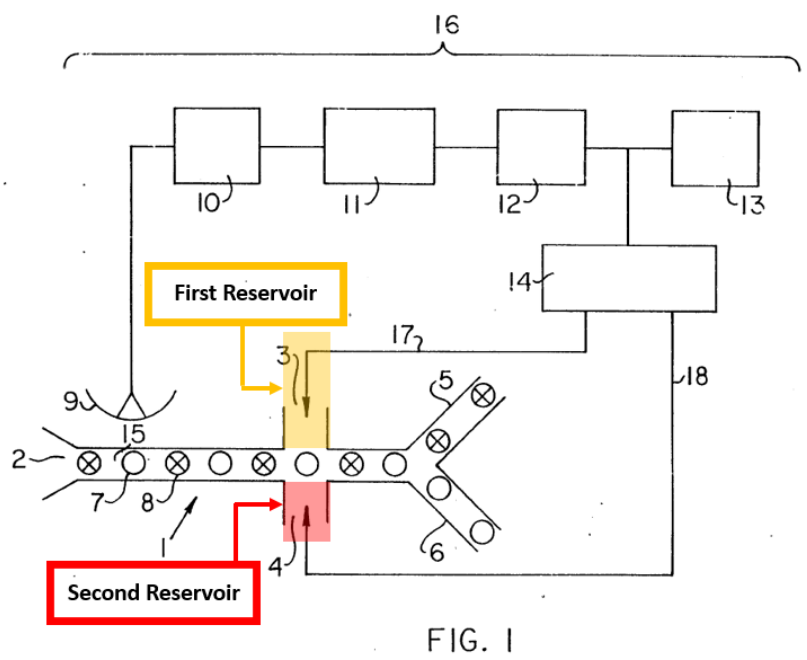


FIG. 1

*See* EX1005 (Marcus), FIG. 1.

219. For example, Figure 1 of Marcus depicts a second reservoir as part of opposing microchannel structures (labeled in annotations). EX1005 (Marcus), FIG. 1; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). A person of ordinary skill in the art would have recognized that these

second reservoirs are operatively associated with the microfluidic flow channel because they are in fluid communication with it and generate a transient fluid flow (*e.g.*, flow from fluidic controller port 4) into the flow channel.

220. Further, as discussed above, Marcus teaches the second reservoir (*e.g.*, 4) can be actuated using an actuator (fluidic controller device 14) to generate a pressure pulse based on a change in volume or pressure in the second reservoir. As Marcus explains,

> Machine vision camera lens 9 reads the appearance of said particles 7 and 8. It transmits what is read to an image capture device 10 which forwards what it sees to an image interpretation device 11 which is able to interpret and register a distinction between particles 7 and 8. This distinction is then transmitted to a control decision device 12 which signals a ***fluidic controller device 14 to instruct control ports A and B, 4 and 3 respectively, to separate out said particles 7 and 8. These particles 7 and 8 are then sorted through output port A and output port B, 5 and 6***.

EX1005 (Marcus), 3:43-48.

221. Marcus explains that using such an actuator facilitates analysis or processing of a particle sample. Specifically, the introduction of various fluids or particles into the microchannels of the microfluidic device can be controlled by the "control ports in the fluidic logic element." *Id*., 2:49-66. In use, detection of a selected particle (*e.g.*, particle A, labelled number 8 in Figure 1) triggers the

actuator to redirect the selected particle from the stream of particles into a selected outlet branch (5). In contrast, a non-selected particle (*e.g.*, particle B, labelled number 7) flows into the outlet branch for the non-selected particles (6). *Id.*, Abstract, 2:49-66, 3:49-62; *see also id.*, 5:13-6:2 (claim 1).

222. A person of ordinary skill in the art would have recognized that the fluidic controller device (14) associated with the opposed sorting microchannels (fluidic control ports 3 and 4) generates and applies a pressure pulse based on a change in pressure in the second reservoir and propagates it across the flow channel to deflect a particle into a selected branch of the flow channel. Marcus describes this pressure pulse in terms of "fluidic control" actuated through "leads 17 and 18" "instruct[ing] the control ports 3 and 4 as to how the particulate materials 7 and 8 should be sorted," but a person of ordinary skill in the art would have recognized this as a pressure pulse. *See* EX1005 (Marcus), 3:37-48. This pressure pulse is selectively applied when the detector indicates the particle is selected for deflection from the stream of particles. *Id.*, 3:28-62.

223. Indeed, this sorting mechanism, wherein an actuator is used to actuate a reservoir in a microfluidic system was well-known and well reported in the art. *See*, *e.g.*, EX1020 (Neukermans), 1:42-64, 2:31-3:17 (describing "an electrically-powered actuator" that "upon application of an electrical signal to the electrically powered actuator, extends towards or retracts from the layer of malleable material"

associated with a reservoir on a disposable microfluidic cartridge, wherein the "pressure applied to the reservoir urges the liquid to flow out"); EX1023 (Williams), ¶¶0026, 0032 (describing a similar mechanism of deflecting or blocking "the flow of a fluid traveling with [a] channel" via actuation of a sphere, such as a ball-bearing, wherein the "force may be applied manually using the finger of a human operator, or by any suitable mechanical means as known in the art," including actuation "using common electrical, magnetic, or pneumatic means, as is well known in the art," as well as by "external control means," involving the use of a computer, cartridges, etc.); *see also* EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected"); EX1016 (Glaettli), FIG. 2 (depicting particle sorting using a stepper motor to vary volume and pressure to sort particles); EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps).

224. In my opinion, a person of ordinary skill in the art would have recognized that control port 4 in the opposed second side microchannel, as suggested by Marcus, when actuated, is a second reservoir operatively associated with the flow channels and adapted for generating a pressure pulse based on a

change in volume in the second reservoir and propagating it across the flow channel, as recited in claim element **1.c** of the ’295 patent.

225. Based on these disclosures, it is my opinion that Marcus and Anderson teach element **1.c**. That is, it would have been obvious to provide, in a microfluidic system, a second reservoir operatively associated with the flow channel and adapted for generating the pressure pulse based on a change in volume or pressure in the second reservoir.

**[1.d] wherein the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow relative to the second reservoir, wherein the first aperture is substantially opposite the second aperture.**

226. Marcus teaches the flow channel defines a first aperture for connecting the flow channel relative to the first reservoir and a second aperture for connecting the flow relative to the second reservoir and the first aperture is substantially opposite the second aperture, as recited in element **1.d**. As discussed above, the first reservoir and second reservoir are operatively associated with the flow channel.

227. Indeed, in describing Figure 1, Marcus explains that the machine vision apparatus “signals a fluidic controller device 14 to instruct control ports A and B, 4 and 3 respectively, to separate out said particles 7 and 8. These particles 7

and 8 are then sorted through output port A and output port B, 5 and 6." EX1005 (Marcus), 3:43-48; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). As discussed above, employing a set of opposing sorting microchannels located downstream of the detector in the microfluidic system represented by Figure 1 would have been readily apparent to an ordinarily skilled artisan based on the teachings of Marcus.

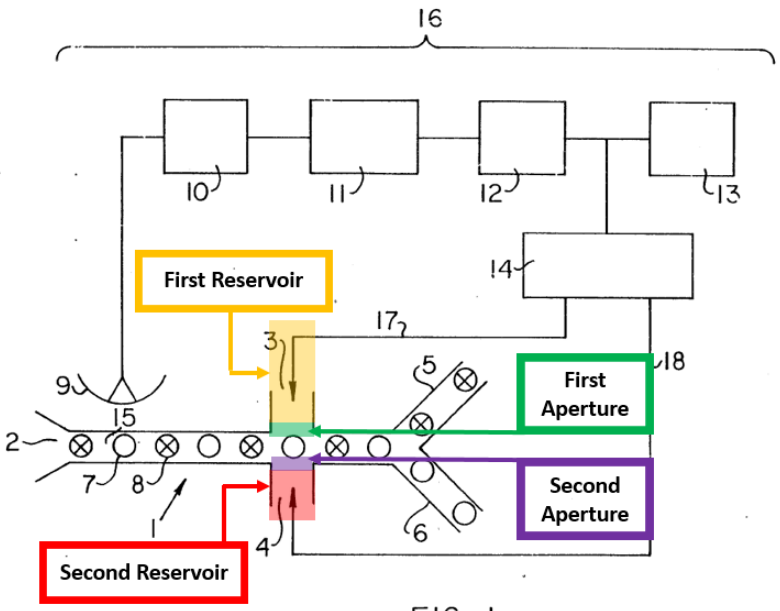


FIG. 1

*See* EX1005 (Marcus), FIG. 1.

228. Based on Marcus's disclosure, a person of ordinary skill in the art would have recognized that the first reservoir opens into the microfluidic flow channel and, similarly, the second reservoir opens into the microfluidic flow channel. That is, the flow channel defines a first aperture for connecting the flow

channel relative to the first reservoir and a second aperture for connecting the flow relative to the second reservoir. The first and second aperture are depicted in the annotated versions of Figure 1. As seen in these figures, the first aperture is substantially opposite to the second aperture. Marcus thus discloses element **1.d.**

229. As set forth above, Marcus teaches each and every element of claim 1. *See also* ¶¶191, 207-11, above (discussing Marcus's teachings of a microfluidic device that comprises conventional components and Anderson's teachings of said conventional components). Based on at least these teachings in Marcus and Anderson, it is my opinion that claim 1 as a whole would have been obvious.

### B. Dependent Claims 2-3, 9, and 17-18

230. As I set forth below, it is my opinion that Marcus and Anderson, in addition to teaching each and every element of claim 1, as discussed above, also teach each and every element of claims 2-3, 9, and 17-18, which depend directly or indirectly from claim 1. My discussion above with respect to claim 1 set forth how, in my opinion, Marcus and Anderson provided a person of ordinary skill in the art with good reason to use the claimed elements in the claimed combination as well as a reasonable expectation of success in so doing. Those same teachings and rationales apply to my discussion of claims 2-3, 9, and 17-18.

**2. The system of claim 1 further comprising an actuator or pressurizer operatively associated with the second reservoir and**

**adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir.**

231. Marcus in view of Anderson teaches the microfluidic system of claim 1 further comprising an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir. As discussed above for elements **1.b** and **1.c**, Marcus and Anderson teach the use of an actuator or pressurizer in association with the second reservoir. In my opinion, a person of ordinary skill in the art would understand Marcus in view of Anderson as teaching an actuator or pressurizer for generating a pressure pulse and propagating the pressure pulse across the flow channel based on a change in pressure in the second reservoir. *See*, *e.g.*, EX1005 (Marcus), 3:28-62; EX1012 (Anderson), 20:9-17, 38:17-27, 45:3-12, 52:1-15, 63:1-13, 65:6-11. For the same reasons discussed above, the actuator is operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the pressure in the second reservoir.

232. As one example, Marcus uses of an actuator in the form of "fluidic controller device 14 which transmits using leads 17 and 18 the input signal from the control decision device 12 to the fluidic element control ports 3 and 4. The input signal instructs the control ports 3 and 4 as to how the particulate materials 7 and 8 should be sorted." EX1005 (Marcus), 3:28-62, FIG. 1.

233. As discussed above, a person of ordinary skill in the art would have found it apparent to look to the teachings of Anderson for specific implementations of the fluidic controller device or actuator using conventional components. As one example, Anderson teaches an actuator or pressurizer in the form of a pumping chamber with "a diaphragm pump as one surface of the chamber" with "zero displacement when the diaphragm is not deflected." EX1012 (Anderson), 44:28-46:44. The diaphragm pump may be activated via heat (*e.g.*, using a "thermopneumatic pump" comprising a "small scale resistive heater disposed in [the] pressure chamber") or via physical displacement (*e.g.*, piezoelectric stack). EX1012 (Anderson), 20:9-17, 38:17-27, 45:3-12, 52:1-15, 63:1-13, 65:6-11. A person of ordinary skill in the art would recognize that these actuators or pressurizers propagate a pressure pulse by varying the volume or pressure in the second reservoir, as recited in claim 2.

234. Indeed, the use of such actuators or pressurizers was well-known and well-reported in the art. *See*, *e.g.*, EX1016 (Glaettli), FIG. 2 (depicting particle sorting using a stepper motor to vary volume and pressure to sort particles); EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps); EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse to "divert the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is

detected"); EX1020 (Neukermans), 10:48-65, 12:16-19, 13:46-48 (teaching actuation means wherein "[p]ressure may be applied to the reservoirs" using a "piezo-electric actuator").

235. Accordingly, it is my opinion that Marcus in view of Anderson teaches the microfluidic system of claim further comprising an actuator or pressurizer operatively associated with the second reservoir and adapted for generating the pressure pulse by varying the volume or pressure in the second reservoir. Claim 2 is thus obvious in view of Marcus and Anderson.

**3. The system of claim 1, wherein the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.**

236. Marcus teaches that the flow channel includes a branched portion, wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion. As discussed for claim 1, Marcus discloses sorting particles into one of two microchannels output port A (5) and output port B (6). *See*, *e.g.*, EX1005 (Marcus), 3:49-62, FIG. 1. A person of ordinary skill in the art would have recognized that the two microchannels defining output ports A and B (5 and 6) as described by Marcus together constitute a branched portion of the flow channel, wherein a particle is sorted into a selected branch of the branched portion. Indeed, such a branched portion is depicted in Figure 1 of Marcus.

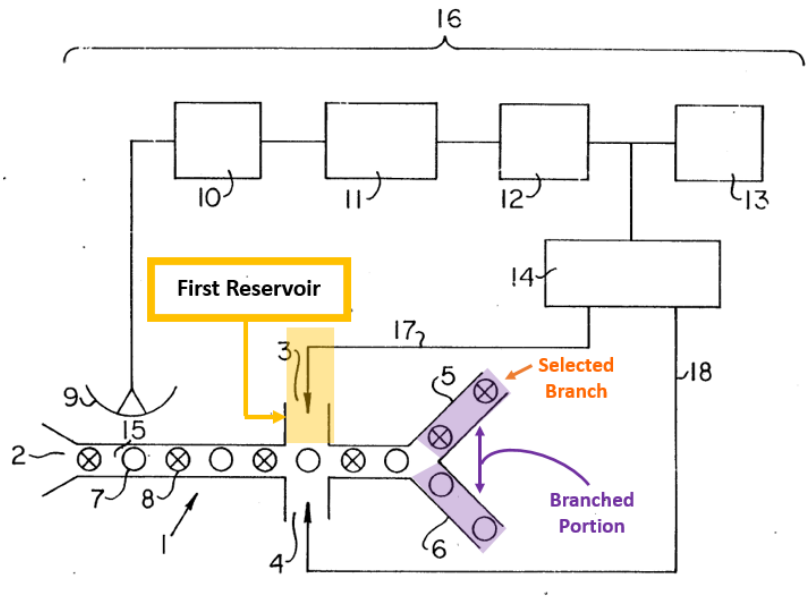



FIG. 1

*See* EX1005 (Marcus), FIG. 1.

237. As discussed above, a person of ordinary skill in the art would have found it apparent to use conventional components to implement such particle sorting, including the actuators and pressure pumps described in Anderson. *See* analysis of claim elements **1.b** and **1.c** above. Based on the teachings of Marcus in view of Anderson, a person of ordinary skill in the art would understand that the pressure pulse is used to sort a particle into a selected branch of the branched portion across the flow channel. For instance, in describing Figure 1, Marcus explains that the fluid flow is induced by fluidic controller device 14, which creates a pressure pulse at controller port 4 (*see* my discussion of element **1.c** and claim 2, above). As a result of the pressure pulse, "particles 7 and 8 are then sorted through output port A and output port B, 5 and 6." EX1005 (Marcus), 3:43-48.

That is, the pressure pulse applied across the flow channel in Figure 1 is used to sort one or more particles into a selected branch of the branched portion.

238. Accordingly, Marcus and Anderson teach the microfluidic system of claim 1, wherein the flow channel includes a branched portion, and wherein the pressure pulse is used to sort one or more particles into a selected branch of the branched portion.

**9. The system of claim 1, wherein first reservoir includes a resilient wall for facilitating dampening or absorbing of the pressure pulse.**

239. As discussed above, Marcus and Anderson teach a first reservoir formed in the substrate that is adapted for dampening or absorbing a pressure pulse propagated across the flow channel.

240. As also discussed above, Marcus Figure 1 depicts an embodiment in which an actuator (fluidic controller device 14) is used for selectively applying a pressure pulse to the stream to deflect a particle in the stream of particles from the stream of particles. In use, detection of a selected particle (*e.g.*, particle A, labelled number 8 in Figure 1) triggers the actuator to redirect the selected particle from the stream of particles into a selected outlet branch (5). In contrast, a non-selected particle (*e.g.*, particle B, labelled number 7) flows into the outlet branch for the non-selected particles (6). *Id.*, Abstract, 2:49-66, 3:49-62; *see also id.*, 5:13-6:2 (claim 1).; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34,

44, 48 (same); *see also*, ¶¶44-45, above (discussing the terms "absorbing" and "dampening"); EX1042, 13-14, 18-20 (construing "absorbing" and "dampening"); EX1043, ¶¶37-38, 40-43, 50-52, 56-57 (construing "buffer," "absorbing," and "dampening").

241. As discussed above with respect to claim element **1.b**, a person of ordinary skill in the art would have recognized that fluidic controller device 14 selectively applies a pressure pulse to the stream to deflect a particle in the stream of particles from the stream of particles. *See* EX1005 (Marcus), 3:28-62. As also discussed above as to claim 1, a person of ordinary skill in the art would have recognized that this non-actuated, opposed sorting microchannel structure is a reservoir operatively associated with the flow channels and adapted for dampening and absorbing the pressure pulse propagated across the flow channel because it is aligned with the direction of the pressure pulse (substantially perpendicular to the main microchannel).

242. A person of ordinary skill in the art would have had good reason to employ a resilient wall for facilitating dampening or absorbing of the pressure pulse by the first reservoir. For example, an ordinarily skilled artisan would have recognized that a resilient wall in the reservoir would further facilitate dampening and absorbing of the pressure pulse.

243. Indeed, Marcus itself suggests including a resilient wall in the reservoirs. For example, Marcus explains that the substrate for the microfluidic system, in which the first reservoir is formed, may be made from a resilient material. Specifically, the substrate materials are "produced using conventional micromachining technology or any other conventional means" and may be "composed of semi-conductor materials, such as silicon and gallium arsenide." EX1005 (Marcus), 2:20-30 (noting that the substrate may also be comprised of "metallic laminates," and additionally noting that a "top surface cover" is provided "over the fluidic logic device" that is "composed of a transparent material," the composition of which "is not critical"). Marcus thus teaches making the substrate out of flexible and resilient materials.

244. For example, silicon is used in elastomers and other flexible materials, medical devices, and microfluidic chips. *See*, *e.g.*, EX1022 (Zeng), 107 (describing "membrane displacement pumps," wherein "the deflection of microfabricated membranes (*e.g.*, silicon diaphragms) provides the pressure work for the pumping of liquids"); EX1023 (Williams), ¶0007 (describing the use of a "silicon micromechanical non-reverse valve" that is "integrally formed within the silicon wafer such that the beam can be shifted to control flow within channels of the microfluidic structure"); EX1033 (Zengerle), 193 (describing "passive check valves, made by micromachining of silicon"), 199 (describing "flow channels with

elastic walls (for example silicon tubes)"), 201 (describing "the deflection of a 800 μm wide and 0.4 μm thick silicon carbide diaphragm"); EX1034 (Chou), 8:18-25, 13:17-27 (describing "elastomeric layers [that] are composed of different elastomeric materials," including "RTV 615 silicone," which "is a two-part addition-cure silicone rubber. Part A contains vinyl groups and catalyst; part B contains silicon hydride (Si-H) groups"). Thus, it would have been conventional for at least one wall of the reservoir to be formed by a substrate comprised of silicon, as taught by Marcus, which is resilient and flexible. Marcus thus provides good reason for the first reservoir to include a resilient wall for facilitating dampening or absorbing of the pressure pulse.

245. Using a resilient wall for dampening and absorbing a pressure pulse was conventional in the art. As discussed above in Section VII, reservoirs comprising a resilient wall for dampening and absorbing a pressure pulse were commonly used in the art. *See*, *e.g.*, EX1033 (Zengerle), 198 (teaching that "an enclosed gas volume is used as an elastic element very often," as are "deflectable diaphragm[s] with a large fluid capacitance"); EX1034 (Chou), 39:25-34, 40:20-25 (describing a "damper" or an "energy absorber" that then can be "configured to experience a physical change in response to an oscillation within the flow channel" as a result of sorting particles, the damper comprising "a flexible membrane, a pocket filled with a compressible fluid, and/or flexible walls of the flow channel

itself."); *id*. ("the damper is an encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas.").

246. Based on Marcus and Anderson's disclosures, it is my opinion that Marcus and Anderson teach the microfluidic device of claim 1, further comprising a first reservoir having a resilient wall for facilitating dampening or absorbing of the pressure pulse. Accordingly, claim 9 is obvious in view of Marcus and Anderson.

**17. The system of claim 1, wherein the second reservoir includes a deflectable flexible membrane wherein deflection of the membrane increases the pressure in the second reservoir.**

247. As discussed above, Anderson teaches "[p]umping devices that are particularly useful in a variety of ***micromachined pumps***," including those with "***a bulging diaphragm.***" EX1012 (Anderson), 63:1-13. One example provided by Anderson, for example, includes a chamber that "incorporate[s] a ***diaphragm pump as one surface of the chamber***." *Id.*, 38:17-27, 45:3-12 (noting that this "diaphragm pump will generally be fabricated from any one of a variety of flexible materials, e.g., silicone, latex, teflon, mylar and the like" and noting that the flexible diaphragm pump is "similar to the valve structure" comprising a flexible diaphragm, noting that "[d]eflection of [a] diaphragm valve" may also be achieved using "piezoelectric actuators coupled to the diaphragm valve, and the like").

248. In my opinion, one of ordinary skill in the art would have recognized from these disclosures that Anderson teaches the use of diaphragm pump for increasing the pressure in the microfluidic system. Furthermore, one of ordinary skill in the art would also appreciate that a diaphragm pump provides for a deflectable flexible membrane (*e.g.*, the diaphragm) wherein deflection of the membrane increases the pressure in the second reservoir.

249. The components that Anderson describes were conventional and well-known in the art. One of ordinary skill in the art would have been familiar with the usage and incorporation of such components, including the diaphragm pump and the flexible membrane. *See*, *e.g.*, EX1022 (Zeng), 107 (categorizing micropumps as either membrane-displacement pumps or field-induced flow pumps); EX1014 (Chapman), 4, (teaching that fluidic particle sorters use a mechanical force or pulse, which results in a pressure pulse that "divert[s] the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected"); EX1020 (Neukermans), 10:48-65, 12:16-19, 13:46-48 (teaching actuation means wherein "[p]ressure may be applied to the reservoirs" using a "piezo-electric actuator").

250. Accordingly, it is my opinion that one of ordinary skill would have had good reason to employ, and a reasonable expectation of success of using, a diaphragm pump, as taught by Anderson, in the microfluidic system taught by

Marcus. *See*, *e.g.*, EX1006 (Wada), 43:1-14; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same). Doing so would provide the second reservoir with a deflectable flexible membrane (*e.g.*, the diaphragm) wherein deflection of the membrane increase the pressure in the second reservoir when the flexible membrane flexes into the chamber volume.

251. Based on at least these teachings in Wada and Anderson, it is my opinion that claim 17 as a whole would have been obvious.

**18. The system of claim 1, wherein the change in volume or pressure in the second reservoir is a transient change in volume or pressure.**

252. Marcus and Anderson teach that the change in pressure in the second reservoir is a transient change in pressure. For instance, when actuating the second reservoir to facilitate sorting, Marcus notes that the "timing and flow rate control of the suspensions should be such as to allow the suspended materials to be properly diverted to the desired port." EX1005 (Marcus), 4:4-7; *see also*, ¶¶36-43, above (discussing the term "reservoir"); EX1042, 9-10, 18-20 (construing "reservoir" and "chamber"); EX1043, ¶¶25-27, 31, 33-34, 44, 48 (same).

253. A person of ordinary skill in the art would have recognized that, because Marcus's fluidic sorting device identifies "a particle," "decides as to whether the particle is to be separated out," and then provides a pressure pulse to

sort said selected particle (*e.g.*, particle A, 8) into a selected output (*e.g.*, output port A), while non-selected particle (*e.g.*, particle B, 7) flows into a second output (*e.g.*, output port B), the pressure pulse otherwise described above as to claim 1 is short-lived, *i.e.*, transient. EX1005 (Marcus), Abstract; *see also id.*, FIG. 1 (depicting every other particle in the suspension medium flowing through the flow channel before the branched portion of the fluidic logic element as a selected particle and showing these selected particles sorted into selected particle output A (5) from the non-selected particles, which instead flow into non-selected particle output B (6)). Thus, it is my opinion that claim 18 is obvious in view of Marcus and Anderson.

## XII. CONCLUDING STATEMENTS

In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: February 12, 2020 By: /Bernhard H. Weigl/
Bernhard H. Weigl, Ph.D.

## XIII. APPENDIX – LIST OF EXHIBITS

| Exhibit No | Description |
|---|---|
| 1001 | U.S. Patent No. 8,623,295 to Gilbert, *et al.* |
| 1003 | *Curriculum Vitae* of Bernhard H. Weigl, Ph.D. |
| 1004 | Prosecution History of U.S. Patent No. 8,623,295 to Gilbert, *et al.* |
| 1005 | U.S. Patent No. 5,101,978 to Marcus (issued April 7, 1992) |
| 1006 | International PCT Publication No. WO 00/70080 to Wada, *et al.* (published November 23, 2000) |
| 1007 | U.S. Patent No. 4,936,465 to Zöld (issued June 26, 1990) |
| 1008-11 | *Intentionally left blank* |
| 1012 | International PCT Patent Publication No. WO 97/02357 to Anderson, *et al.* (published January 23, 1997) |
| 1013 | *Intentionally left blank* |
| 1014 | Chapman, *Instrumentation for flow Cytometry*, J. IMMUNOL. METHODS 243 (2000) 3-12 |
| 1015 | *Intentionally left blank* |
| 1016 | U.S. Patent No. 3,508,654 to Glaettli (issued April 28, 1970) |
| 1017 | Figeys, *et al.*, *Lab-on-a-Chip: A Revolution in Biological and Medicinal Sciences*, ANAL. CHEM. (2000) 330-35 |
| 1018 | Deshpande, *et al.*, *Novel Designs for Electrokinetic Injection in μTAS*, MICRO TOTAL ANAL. SYS. (2000) 339-42 |
| 1019 | *Intentionally left blank* |

| Exhibit No | Description |
|---|---|
| 1020 | U.S. Patent No. 6,068,751 to Neukermans (issued May 30, 2000) |
| 1021 | *Intentionally left blank* |
| 1022 | Zeng, *Fabrication and characterization of electroosmotic micropumps*, SENS ACTUAT. B, 79 (2001) 107-14 |
| 1023 | U.S. Patent Publication No. 2001/0054702 to Williams (published December 27, 2001) |
| 1024-31 | *Intentionally left blank* |
| 1032 | International PCT Publication No. WO 99/43432 to Dubrow, *et al.* (published September 2, 1999) |
| 1033 | Zengerle, *et al.*, *Simulation of Microfluid Systems*, J. MICROMECH. MICROENG. 4 (1994) 192-204 |
| 1034 | International Patent Publication No. WO 02/29106 to Chou, *et al.* (filed October 2, 2001, published April 11, 2002) |
| 1035 | Veenstra, *et al.*, *The Design of an In-Plane Compliance Structure for Microfluidical Systems*, SENS. ACTUATORS B, 81 (2002) 377-83 |
| 1036-41 | *Intentionally left blank* |
| 1042 | Plaintiff's Reply Claim Construction Brief, *Cytonome/ST, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 19-301 (RGA) (D. Del. filed Jan. 31, 2020) |
| 1043 | Exhibit A: Declaration of Giacomo Vacca, Plaintiff's Reply Claim Construction Brief, Cytonome/ST, LLC v. NanoCellect Biomedical, Inc., C.A. No. 19-301 (RGA) (D. Del. filed Jan. 31, 2020) |
| 1044 | Complaint, *Cytonome/St, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 1:19-00301 (UNA) (D. Del., filed Feb. 12, 2019) |

| Exhibit No | Description |
|---|---|
| 1045 | Plaintiff's Opening Claim Construction Brief, *Cytonome/St, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 1:19-00301 (RGA) (D.Del.) |