UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NANOCELLECT BIOMEDICAL, INC.,
Petitioner

v.

CYTONOME/ST, LLC,
Patent Owner

Case IPR2020-00550
Patent No. 9,011,797

DECLARATION OF DON W. ARNOLD, PH.D.

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I. INTRODUCTION

1. My name is Dr. Don Wesley Arnold. I have been retained by Cytonome/ST, LLC (the "Patent Owner") as an independent expert consultant in this proceeding before the United States Patent and Trademark Office. I have never testified or otherwise served as an expert witness or consultant in any litigation or any other legal proceeding. Although I am being compensated at my standard consulting rate of $275 per hour for the time I spend on this matter, I have no personal financial interest in any of the entities involved in this proceeding, and my compensation does not depend in any way on my testimony, my conclusions, or the outcome of this proceeding.

2. I have been asked to respond to the opinions expressed by Dr. Bernhard Weigl, the expert witness engaged on behalf of Petitioner, NanoCellect Biomedical, Inc. In that connection, I have been asked to consider and provide my opinions regarding the prior art in relation to the claims of U.S. Patent No. 9,011,797 ("the '797 Patent"), and to compare that prior art to the claims of the '797 Patent. My opinions and the bases for my opinions are outlined below.

3. The testimony I may provide about scientific principles relating to my analysis and opinions, includes subjects relating to, for example, fluids, microfluidics, particle analysis, particle sorting, and device and system design.

4. I reserve the right to revise or supplement my opinions if additional information makes either appropriate or if Petitioner or Dr. Weigl revise or supplement their allegations or opinions.

II. QUALIFICATIONS AND BACKGROUND

5. Attached as Exhibit 2002 is my curriculum vitae, incorporated by reference within this declaration. The following is a brief summary of my qualifications.

6. I received a Bachelor of Arts (B.A.) degree in Chemistry from Cornell University in 1988, where I was a Cornell Tradition Fellow. I then received a Doctorate (Ph.D.) in Physical Chemistry from the University of California, Berkeley in 1994, during which time I received the NSF Pre-doctoral Fellowship. My thesis was titled "Study of Molecules, Clusters, and Transition State Species by Anion Photoelectron Spectroscopy."

7. From 1994 to 1997, I was a postdoctoral research associate at the University of Southern California, where I was the lead researcher in a project involving the study of molecule-surface collision dynamics using jet-cooled molecular beams, surface science techniques, and laser spectroscopy to examine elastic, inelastic, trapping-desorption, and dissociative molecule-surface collisions. Part of my work included updating the apparatuses and components (e.g., electronics, detectors, software, and lasers) used in this study.

8. From 1997 until 2000, I was a Senior Member of the Technical Staff at Sandia National Laboratories ("SNL") in Livermore, CA. There I focused on developing portable microfluidic devices used to detect explosives and chemical or biological agents in liquid samples. My team also developed high-sensitivity detectors and electrokinetic pumps for use in microfluidic devices. While at SNL, I was the named inventor on a number of patents, the author of reviewed publications, and the presenter at international conferences. This work related to microfluidic device fabrication, microscale liquid chromatography, electroosmotic fluid manipulation, and electrokinetic pump-based delivery systems.

9. In May of 2000, I co-founded Eksigent Technologies, LLC ("Eksigent") in Dublin, CA, where I was an employee until 2010. Eksigent focused on microscale liquid chromatography systems for life science applications (proteomics, drug discovery, and development), particle analyzers for diagnostic applications, and electrokinetic pump-based delivery systems for a variety of applications. I contributed in various technical and business roles at Eksigent, including President, VP Advanced Technologies, and VP Business Development. I was also a member of Eksigent's Board of Directors from 2000 to 2010. On the technical side, I was the Manager and Principal Investigator for Eksigent's Advanced Technologies Department, where we developed microfluidic systems with integrated valves, pumps, detection, and connectors for a variety of applications (e.g, HPLC,

LC/MS, biological assays, and diagnostics). In 2002, while at Eksigent, I was an NIST ATP award winner and Principal Investigator for a three-year project entitled "Microfabricated HPLC for High-Throughput Chemical Analysis." During this time, I also managed Eksigent's IP portfolio, licensing efforts, strategic planning, and outside counsel management. While at Eksigent, I was named inventor on several patents, author of reviewed publications, and presenter at international conferences related to microfluidic flow control, particle processing, microfluidic connectors, and microscale detection systems.

10. In February of 2010, Eksigent was acquired by AB SCIEX, LLC (also located in Dublin, CA), and I continued to serve in lead technical and business roles for the Eksigent side of the company until October of 2012. During that time, I was the Vice President and General Manager, and handled all aspects of the Eksigent division of AB SCIEX, including overseeing the manufacturing, R&D, and sales teams, as well as the development and launching of new Eksigent products. From October 2012 until March 2017, I continued to work for SCIEX in their Redwood City, CA location as Vice President and Principal Scientist. In these roles, I continued to lead product development efforts relating to microfluidic devices, including the SCIEX Acoustic Ejection MS product. During this period, I was a named inventor on patents related to microscale fluid manipulation for samples and to particle manipulation using magnetic fields.

11. In March 2017, I founded Veristad, LLC and continue to serve as the CEO. Veristad is a technical and business consulting organization focused on assisting (1) established companies during assessment of early stage technologies and (2) early stage technology companies navigating from start-up through exit via acquisition or IPO. I am also currently a Limited Partner at Tri-Valley Ventures, a venture group focused on establishment and growth of new AI/Data, SaaS, HealthTech, Smart Devices/Hardware, and FinTech companies in the Tri-Valley area of Northern California. I also serve as mentor for the Bay Area Microfluidics Network, where I guide organizers on planning for the quarterly events, connect with experts in the microfluidics field, and serve as moderator for the events.

12. I have published a number of papers in various areas relating to flow cytometry and microfluidic devices, including technical articles in peer-reviewed papers, conference proceedings, and professional publications. I am the inventor or co-inventor on 27 issued United States patents, 6 issued international patents, and several pending applications. A full list of my publications, patents, and patent applications is included in Exhibit 2002.

III. MATERIALS CONSIDERED

13. A list of the documents and materials I have considered in developing my opinions can be found in the Appendix below.

IV. LEGAL PRINCIPLES APPLIED

A. Person of Ordinary Skill in the Art

14. Some of my opinions refer to the perspective of one of ordinary skill in the art ("POSITA" or "POSA") at the time of the invention. Dr. Weigl and Petitioner define a POSA as someone with "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." EX1002 ¶49; Pet. 12. At Cytonome's request, I have adopted and applied that definition herein solely for purposes of this proceeding, with the understanding that neither Cytonome nor I have undertaken an analysis of its accuracy, and that we both reserve the right to revisit its accuracy at a later date if necessary.

B. Claim Construction

15. I understand that patent claim terms are given their ordinary and customary meaning as would be understood by one of ordinary skill in the art. I further understand that the ordinary and customary meaning always applies unless a different meaning is clearly indicated by the patentee (such as by an express definition of a term in the patent specification or clear disclaimer of subject matter during prosecution).

16. Also, I understand that in determining the ordinary and customary meaning, the term should be read in light of "intrinsic" evidence, namely the patent

specification and prosecution history. If intrinsic evidence is not sufficient to inform a person of skill of the ordinary and customary meaning of a term, "extrinsic" evidence may be consulted (e.g., a dictionary).

17. I have applied these claim construction principles to my analysis herein.

C. Obviousness

18. I understand that obviousness is assessed from the view of a person of ordinary skill in the relevant art at the time the invention was made. I understand that the prior art does not need to render obvious every possible embodiment within the scope of the claim. I further understand that in determining whether a patent claim is invalid because of obviousness, one must consider the scope and content of the prior art, the differences between the prior art and the claimed invention, and the level of ordinary skill in the art. In making these assessments, one also considers certain other surrounding circumstances—the so-called "objective indicia" or "secondary considerations."

19. I understand that an invention may be obvious because a skilled artisan merely pursues known options from a finite number of identified, predictable solutions or because the claimed improvement is nothing more than the predictable use of prior art elements according to their established functions. I understand that a claim is obvious if the person of ordinary skill in the art would have had motivation or reason to combine the teachings of the prior art and would have had a reasonable

expectation of success in doing so. And I understand that obviousness does not require absolute predictability of success; all that is required is a reasonable expectation of success.

20. I further understand that, if modifying a base reference or combining it with a secondary reference would result in the base reference changing its "principle of operation" or requiring substantial redesign, then that could serve as a reason or motivation to *not* combine or modify the references.

V. BACKGROUND OF THE TECHNOLOGY

21. The main technologies relevant to the '797 Patent are microfluidics, flow cytometry, and particle sorting. Below, I provide some relevant background about these technologies as they stood at the time of the invention based on my knowledge and experience.

22. "Microfluidics" is the science of manipulating and controlling fluids, usually in the range of microliters (10^{-6}) to picoliters (10^{-12}), in networks of channels with dimensions from tens to hundreds of micrometers (i.e., less than the width of a human hair). This discipline takes its origins in the early 1990's and has grown exponentially. It is viewed as an essential tool for life science research and biotechnologies.

23. "Microfluidic devices" are generally small, flat chips (usually about the size of a credit card) that employ microfluidic technology. Some microfluidic

devices have inlets and outlets connected to small "microchannels." The microfluidic systems at issue in the '797 Patent challenged in the Petition generally flow two types of fluids: (1) a sample fluid that may contain cells or other particles to be analyzed and sorted, and (2) a sheath or "carrier" fluid that surrounds the sample fluid.

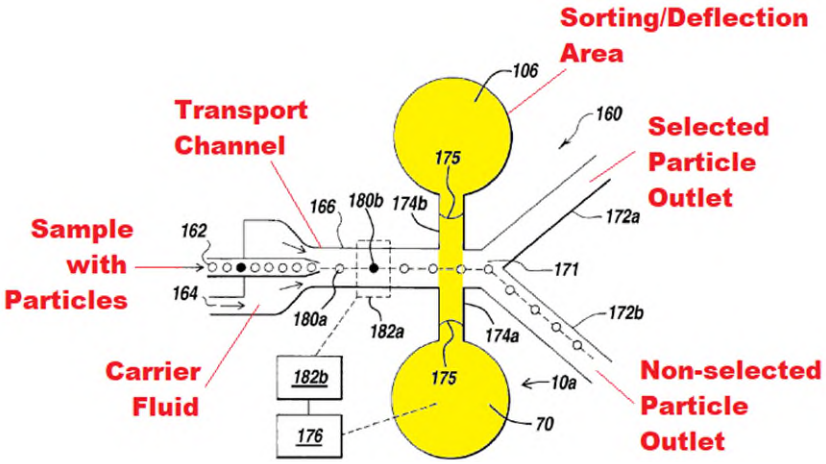
24. Due to the small dimensions of microfluidic devices, the flow of these fluids is almost always "laminar," allowing the sample and carrier fluids to remain in separate layers without mixing (save for some diffusion at the boundary of the layers). This allows the internal sample stream to be narrowed and moved within the cross section of a main transport channel, and it protects the sample (e.g., biological cells) from damage by shear stresses at the transport channel's walls. Because this laminar flow allows samples to be moved within the transport channel, microfluidics can be a useful tool in conjunction with "flow cytometry" and particle sorting, where the narrowed sample stream is often referred to as the "core stream."

25. "Flow cytometry" is a broad term that refers to the analysis of particles, such as cells. Generally, when used in conjunction with a flow cytometer, a microfluidic device narrows the particles into a single-file line in the center of the transport channel, a detector external to the device is arranged to detect a signal for each particle that flows past the detector, and a processor analyzes the signal. A classic example would involve flowing a sample of cells through a microfluidic

device, detecting the size of the cell, and determining whether a particular cell is tumorous based on size or some other indicative characteristic.

26. Microfluidic devices used for flow cytometry are sometimes also used for particle sorting. In the above example regarding tumorous cells, the microfluidic device used for flow cytometry/analysis could include an additional component downstream of the detector that sorts the identified tumorous cells from the non-tumorous cells. In this way, particles of interest can be collected and studied separately.

27. The claims of the '797 Patent that are challenged in the Petition involve microfluidic devices used for particle sorting. Using Figure 16 below to illustrate, the sorting devices generally involve transporting a sample/particles in a carrier fluid through a transport channel where "selected" particles (180b) are eventually deflected so as to be sorted into a keep outlet, while non-selected (not deflected) particles flow into a waste outlet:



EX1001 at Fig. 16 (annotated). The differences between (a) the components and operating principles for achieving the particle sorting in the ’797 Patent and (b) the components and operating principles for achieving particle sorting in the prior art is the main issue discussed herein.

VI. THE ’797 PATENT

A. The Challenged Claims

28. The Petition challenges claims 1–2, 5, 13, 16, and 18–19 of the ’797 Patent. My analysis focuses mainly on independent claims 1 and 5, which are reproduced below.

Claim 1:

> 1. A method for facilitating analysis or processing of a sample, the method comprising:
>
> flowing a sample through a microfluidic flow channel formed in a substrate;
>
> propagating a pressure pulse across the microfluidic flow channel,
>
> wherein the pressure pulse is used to sort one or more particles suspended within the sample.

29. Dependent claim 2 further specifies that the “pressure pulse” is used to “selectively control” the sample.

30. Claim 5:

> 5. A microfluidic device adapted to facilitate analysis or processing of a sample, the microfluidic device comprising:
>
> a microfluidic channel formed in a substrate, the flow channel having a width and a length that exceeds the width; and

a first reservoir in fluid communication with the flow channel adapted for dampening or absorbing a pressure pulse propagated across the width of the flow channel, wherein the first reservoir is otherwise sealed from an exterior environment.

31. The challenged dependent claims that depend from claim 5 further specify various additional features, such as an "actuator" to "generat[e] the pressure pulse" (claim 13) which sorts particles "into a selected branch of the branched portion" of the flow channel (claim 16).

32. The remaining challenged claims disclose a method for "sort[ing] one or more particles" (claim 19) by "propagating a pressure pulse" across the flow channel and "dampening or absorbing" that pressure pulse (claim 18).

33. For all the reasons herein, I disagree with Dr. Weigl's opinion that "each and every element of each of claims 1–2, 5, 13, 16, and 18–19 of U.S. Patent No. 9,011,797 (EX1001) is found in the prior art" and that the challenged claims "recites a method for sample analysis using microfluidics with conventional, well-known features." EX1002 ¶21.

B. The Specification

34. The title of the '797 Patent is "Microfluidic System Including a Bubble Valve for Regulating Fluid Flow Through a Microchannel." EX1001. The patent was filed on June 19, 2012 and issued on April 21, 2015. *Id*. The patent claims priority to Provisional Application No. 60/373,256, which was filed on April 17, 2002. I have used that date as date of the invention throughout this declaration, though my

analysis would not change if the actual date of the invention were found to be slightly later for any particular claim.

35. Although part of the ’797 Patent specification more generally describes controlling and regulating flow, the challenged claims (e.g., claim 1) describe a method for facilitating analysis or processing of a sample. An exemplary particle sorting device to perform this method is depicted in Figure 16 of the patent and reproduced below:

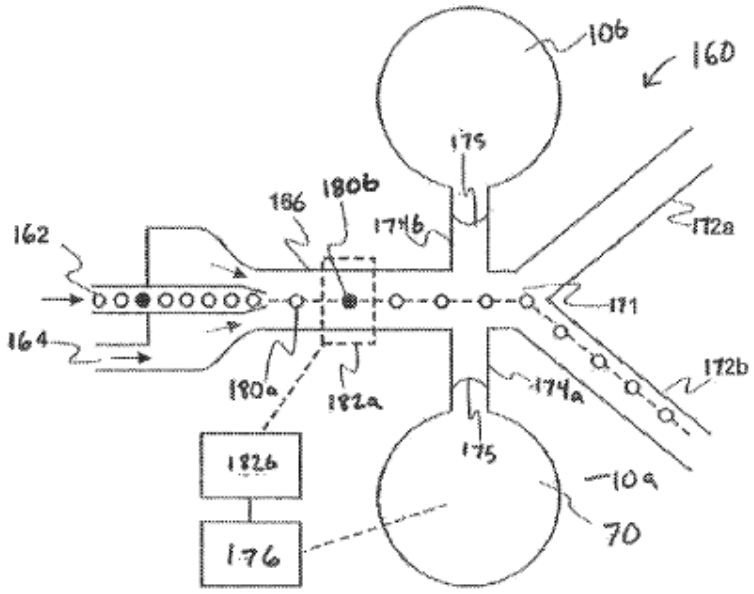
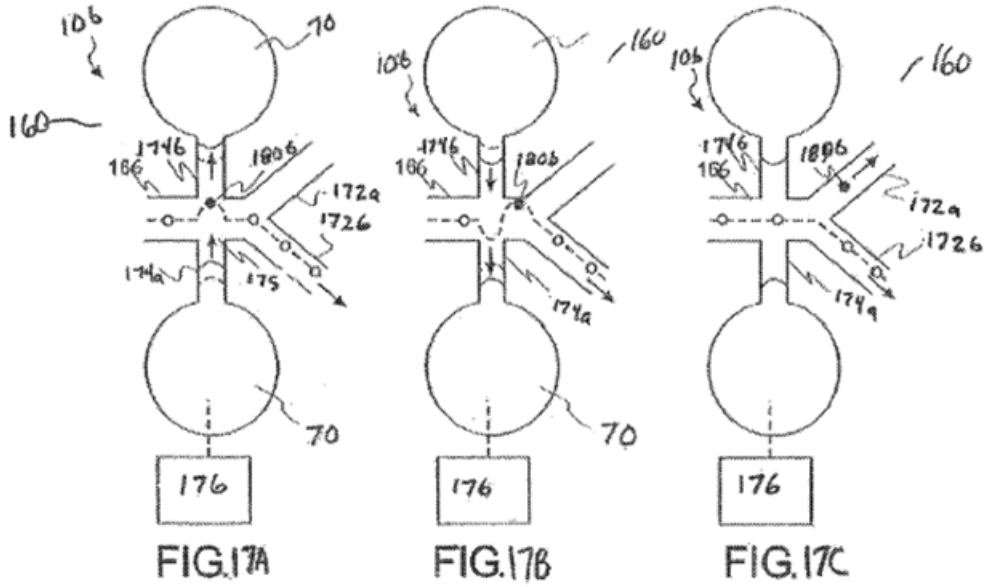


FIG. 16

Id. at Fig. 16.

36. The sorting mechanism and operation is depicted more closely in Figures 17A—17C:



Id. at Figs. 17A–17C.

37. The challenged claims can be understood with reference to these figures (i.e., Figures 16 and 17A–17C) and the accompanying text (*id.* at 11:35–12:65). The Summary of the Invention additionally describes a "particle sorting device" with an "actuator for deflecting the meniscus to create a pressure pulse to selectively deflect a particle having the predetermined characteristic from the stream of particles." *Id.* at 4:31–41.

38. As the patent explains, the actuator "var[ies] the volume of [an associated] reservoir to increase an internal pressure of the reservoir to vary the flow of liquid through the channel." *Id.* at 3:51–59. The positive, increased pressure pulse created by the actuator is a "transient pressure variation" (*id.* at 12:13–15) that "momentarily causes a flow disturbance in the duct to deflect the flow therein . . . ."

(11:58–62). A POSA would understand this to mean that the sorting mechanism described in the patent causes a momentary sideways shift in a spatially-limited portion of the main stream that puts only the selected particle on a divergent path from the remainder of the stream of particles (often referred to in the art as a "core stream").

39. More specifically, the pressure pulse is a positive pressure wave that deflects the particle by causing lateral displacement of the spatially-limited volume of fluid between the actuator and opposed "first reservoir." That lateral displacement is in a direction away from the actuator side of the main transport channel, as illustrated in Figures 17A–17B. This causes the selected particles to flow into a separate outlet from the non-selected particles.

40. The specification describes this pressure-induced transient fluid displacement mechanism and resulting deflection of the selected particle:

> FIGS. **17*a*-17*c*** illustrate the operation of the particle sorting system **160** of FIG. **16**. In FIG. **17*a***, the detector raises a signal to activate the actuator. Upon activation of the actuator, the pressure within the reservoir of the first bubble valve **10*a*** is increased, causing a transient discharge of liquid from the first side passage **174*a*** as indicated by the arrow. The sudden pressure increase caused at this point in the duct causes liquid to flow into the second side passage **174*b*** because of the resilient properties of the reservoir of the second bubble valve **10*b***. This movement of liquid into the second side passage **174*b*** is indicated with an arrow. Resultingly, as can be seen in the figure, the flow through the duct is deflected causing the selected particle of interest **178*b*** located between the first side passage **174*a*** and the second side passage **174*b*** to be shifted perpendicular to its flow direction in the normal state.

Id. at 12:23–37.

41. The patent further explains that the lateral displacement of fluid shown in Figure 17A is reversed during the relaxation cycle (i.e., "relief phase") of the actuator, as shown in Figures 17B–C and described in the '797 Patent at 12:48–65. At the end of the process, there has been no net fluid delivery into the main transport channel (i.e., no fluid has been added to the system for sorting). Although the lateral displacement induced by the pressure pulse effectively shifts the particle into a new flow stream in the transport channel, during the very short time period of the pulse, the fluid continues to flow in the transport channel from the sample source to the outlets under the influence of the pressure delivering the sample stream through the transport channel. By the time the opposed "first reservoir" pushes the fluid back across the channel during the relaxation step, the particle has moved downstream and is not shifted back to its original flow stream. In this way, no fluid is added to the system to deflect a particle, contrary to the "flow-based" sorting operations discussed below. In short, no additional fluid is ever required, other than the fluid provided via the transport channel, to effect the particle sorting process described in the '797 Patent.

42. Moreover, according to the '797 Patent, the actuator used to generate a pressure pulse may be a chamber with a movable membrane and a piezoelectric transducer mounted thereon. *Id.* at 7:17–20, 8:59–62; *see also id.* at 3:4–24. The

opposed "first reservoir" is illustrated as a comparable reservoir (component 70/106 in Figure 16), and it must be sized and configured so as to effectively "dampen[] or absorb[] a pressure pulse propagated across the width of the flow channel" (i.e., across the main transport channel). *Id.* at 14:6–10; *see also id.* at 13:41–51. As the patent explains, "The second side passage **174*b*** and the second bubble valve **10*b*** absorb the transient pressure variations in the measurement duct **166** induced via the actuator **176**." *Id.* at 12:15–18. In other words, according to the specification, the two reservoirs (one an actuating reservoir and one configured to dampen/absorb) act in cooperative fashion with respect to the pressure pulse, with the actuating reservoir first creating the pressure pulse and the opposed reservoir receiving that pressure pulse some short time later, after the pressure pulse has propagated into the channel to deflect the selected particle and then moves on to the opposed reservoir where it is absorbed.

43. The cooperation of these reservoirs—acting in tandem to deflect only selected particles and ensure that no non-selected particles are unintentionally deflected into the wrong outlet by the "pressure pulse"—is critical to the invention. And by dampening or absorbing the pressure pulse, the opposed reservoir further ensures that the pressure pulse does not reverberate throughout the channel and otherwise disrupt the flow.

44. Moreover, unlike systems using electroosmotic flow (described in more detail below), where flow along a particular flow path can be changed without significantly affecting flow rates in connecting channels, a pressure-driven system is intimately connected because pressure is isotropic in nature. If increased pressure is applied to a fluid channel that feeds into an intersection, the increased pressure at the intersection is felt in all directions (i.e., pressure is isotropic). The increased pressure can affect the flow in other intersecting channels to the detriment of the system performance. Such unintended flow disruptions adversely affect system performance for cytometer and particle sorting systems, where a significantly constant flow is important.

45. As taught in the '797 Patent at 12:37–47, to accommodate a cross-channel pulse that does not disrupt the rest of the system, the relative dimensions of the channels in the microfluidic network are carefully selected:

> The flow resistances to the measurement duct **166**, the first branch **172*a*** and the second branch **172*b*** is chosen so that the preferred direction of the flow to and from the first side passage **174*b*** has an appreciable component perpendicular to the normal flow through the measurement duct **166**. This goal can for instance be reached by the first branch **172*a*** and the second branch **172*b*** so that their resistances to flow is large in comparison to the flow resistances of the first side passage **174*a*** and the second side passage **174*b***.

46. This selection of the relative flow resistance for each of the channels in the network provides a means of actuating a pulse of fluid flow from the first side passage to the second side passage and back, timed to deflect only selected particles

in a predictable manner, without introducing a net flow of fluid into the transport channel or disrupting the downstream fluid flow.

47. This can be contrasted to electroosmotic flow, where varying voltage across one channel can increase flow rate in *that* channel without significantly affecting the flow rate in *other* (fluidically-connected) channels. Simply put, unlike pressure-based systems, channels where there is no applied voltage are not impacted by electroosmotic flow.

48. Finally, the addition of fluid from side channels is not a "pressure pulse" as that term is used in the '797 Patent. In several references cited by Dr. Weigl and discussed below, there is a net increase in "hydrodynamic flow" of fluid from the side channel into the transport channel. In the '797 Patent, however, because the system is designed to displace fluid from the actuator reservoir to the opposed reservoir, and then return the same volume of fluid back into the first valve, there is no net flow of fluid (i.e., no fluid is added to the system for sorting, and flow rate is the same in the transport channel before and after a sorting operation). In the '797 Patent's sorting operation, there is just a disruption of the flow streams during the time when the particle is in the selection region of the device. In other words, the '797 Patent teaches how to localize a fluid "flow disturbance" for sorting using an actuator chamber and opposed "first reservoir" that cooperate with respect to

dispensing and absorbing a "pressure pulse" to create that disturbance. This sorting operation is illustrated by the annotated version of Figures 17A–17C below[1]:

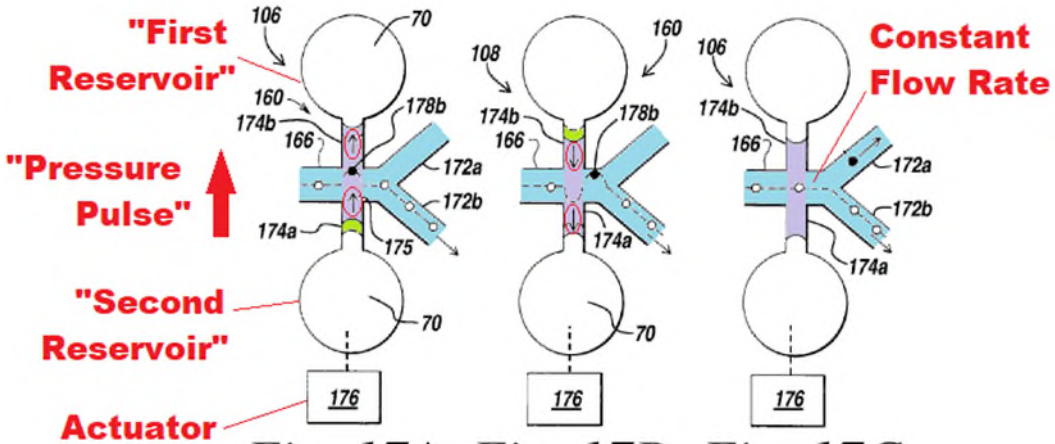


Fig. 17A Fig. 17B Fig. 17C

49. In sum, the principle of operation of the challenged claims of the '797 Patent is momentarily shifting fluid across a transport channel to deflect particles by applying a cross-channel pressure pulse. The "first reservoir" is critical to this operation because it dampens or absorbs the pressure pulse and allows deflection to occur without adding fluid to the system or otherwise disrupting flow. Indeed, as shown below, the components as arranged in the claim to effect this particle sorting operation are novel and innovative, and were not "known in the prior art," as Dr. Weigl suggests. EX1002 ¶28.

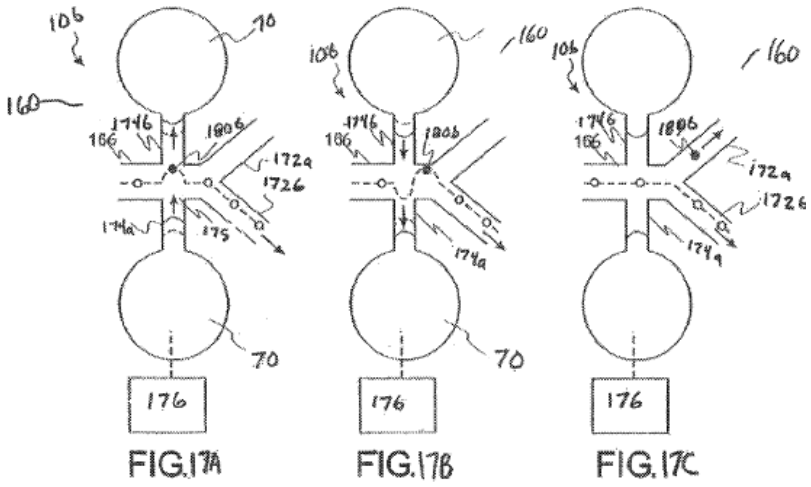
[1] Some annotated figures used herein are from U.S. Patent No. 8,623,295 and are substantively identical. The figures from the '295 Patent were used for clarity.

VII. CLAIM CONSTRUCTION

A. POSA's Understanding of "pressure pulse"

50. I have been asked to opine on the ordinary and customary (aka "plain and ordinary") meaning of the term "pressure pulse" used in the challenged claims, as would have been understood by a POSA at the time of the invention and in light of the specification. After reviewing the specification and claims, my opinion is that the ordinary and customary meaning of the term "pressure pulse" in the '797 Patent is "a transient increase in pressure." A POSA would also understand the claims to require that this pressure pulse travel across the transport channel in order to be dampened or absorbed by a reservoir.

51. A POSA would have understood that the challenged claims are referring to the sorting embodiments in the '797 Patent, which are depicted in Figures 16 and 17A–17C. And those figures depict a "pressure pulse." Specifically, Figure 17A shows an arrow coming from actuator 176 that indicates a positive pressure is applied that pushes a volume of fluid, and thus the selected particle, away from the actuator side:



Id. at Fig. 17A.

52. Figures 17B and C support that the pulse is "transient" because they show the device being relieved of pressure and returning to a ready state immediately after the selected particle passed. A particle is not deflected during this relief state. The description of Figures 17A–17C repeatedly describes the pulse as "transient" and an "increase" in pressure:

> FIGS. 17a-17c illustrate the operation of the particle sorting system 160 of FIG. 16. In FIG. 17a, the detector raises a signal to activate the actuator. Upon activation of the actuator, ***the pressure within the reservoir of the first bubble valve 10a is increased, causing a transient discharge*** of liquid from the first side passage 174a ***as indicated by the arrow***. The ***sudden pressure increase*** caused at this point in the duct causes liquid to flow into the second side passage 174b because of the resilient properties of the reservoir of the second bubble valve 10b. This movement of liquid into the second side passage 174b is indicated with an arrow.

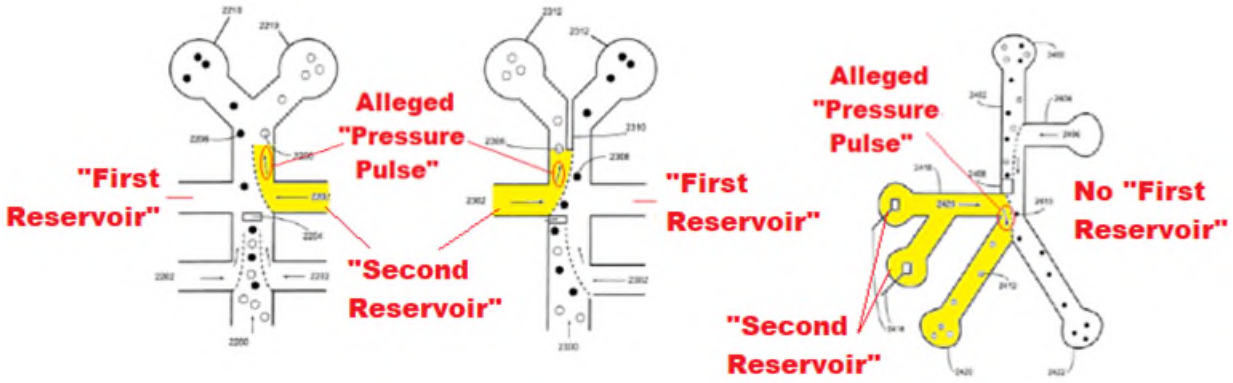
Id. at 12:23–33 (emphases added); *see also* Figs. 16, 17A–17C. The specification further describes that the actuator "causes a transient pressure variation in the first

side passage 174a" that is absorbed, which a POSA would further understand is describing the pressure pulse and its transient nature. *Id*. at 12:9–22.

53. Dr. Weigl incorrectly suggests that a "pressure pulse" is a type of "flow." For example, he attempts to equate Wada's introduction of "hydrodynamic flow" with a "pressure pulse" and explains that, in Wada, an "actuator selectively applies *a pressure pulse represented by arrows 2202 and 2302*"—those arrows (i.e., components 2202 and 2302 in Wada) are defined in Wada as the introduced "hydrodynamic flow." *See* EX1002 ¶133 (emphasis added); EX1006 at 19:23–20:14 (identifying 2202 and 2302 as "hydrodynamic flow"); *see also* EX1002 ¶¶124–125. I disagree with that interpretation because, as used in the patent, a "pressure pulse" is distinct from fluid "flow" taught by Wada. When employing a "pressure pulse" for sorting particles, as per the '797 Patent, there is no net "flow" of fluid into the main fluidic stream of particles as a result of the sorting process. The actuator reservoir and opposed absorption reservoir, when combined with the '797 Patent's microfluidic design constraints, work in tandem to shift the flow streams selectively and transiently in a very localized manner. The volume of fluid displaced by the actuator is accommodated by the opposed absorption reservoir and then the volume is returned to the actuator side once the particle has passed out of the displacement zone. No net fluid is provided from either the actuator or the opposed reservoir because no additional fluid flow leaves the actuator reservoir. In fact, in the devices

claimed in the ’797 Patent, there is no source of fluid (e.g., buffer fluid, gel, or the like) in or near the actuator from which any “flow” could be induced.

54. Moreover, the challenged claims require that the pressure pulse be “propagat[ed] . . . across the microfluidic flow channel” and dampened or absorbed by a “first reservoir” (EX1001 at claim 1). The “hydrodynamic flow” in Wada fails to reach any supposed “first reservoir,” as can be readily seen in Figures 22–24 relied on by Dr. Weigl:



EX1006 at Figs. 22–24 (annotated); EX1002 ¶¶133–48.

55. Thus, Wada’s hydrodynamic flow would not be understood under any reasonable interpretation to be the “pressure pulse” described in the claims.

B. POSA’s Understanding of “dampening or absorbing the pressure pulse using a first reservoir in fluid communication with the microfluidic flow channel”

56. Challenged claim 1 recites “dampening or absorbing the pressure pulse using a first reservoir in fluid communication with the microfluidic flow channel.”

57. The phrase "dampening or absorbing the pressure pulse" provides structure to the "first reservoir." Namely, it tells a POSA that the first reservoir must be configured to "dampen" or "absorb" a pressure pulse, which includes designing the reservoir in terms of materials, fluids, size, and geometry so that it is capable of dampening or absorbing the pressure pulse propagated across the channel by the actuating reservoir.

58. The '797 Patent provides some examples of such design choices that allow for absorption. For example, the patent provides an example in which "the reservoir 70b" of Figure 16 (which a POSA would understand is the "first reservoir") is "a chamber having a resilient wall or contains a compressible fluid such as a gas." EX1001 at 12:18–22. The patent further explains that these "resilient properties allow the flow of liquid from the measurement duct into the second side passage 174 b," which allows the absorbing of the pressure pulse to take place. *Id.* A POSA would further understand that adjusting other features that affect the spring constant of the first reservoir—such as varying the volume of the chamber, the cross-sectional area of the side channel, and/or the stiffness or the thickness of a flexible membrane at the walls of the reservoir/chamber—could also allow for absorption.

59. For these reasons, I disagree with Dr. Weigl's suggestion that mere "align[ment]" of side channels necessarily results in the claimed dampening or

absorbing, which he suggests when he equates Wada's and Marcus's side channel to the "first reservoir." *See* EX1002 ¶¶133–137, 237–41.

VIII. ANALYSIS OF THE PRIOR ART

A. "Electrokinetics" or "Electrodynamics"

60. Some of the prior art references (such as Wada) cited in the Petition and in Dr. Weigl's declaration refer to techniques such as "electrokinetics," "electrodynamics," "electroosmotic flow," or "electrophoresis." To assist with that discussion, I provide some explanations for some terms commonly used related to those techniques. *See, e.g.,* EX1006, EX1018, EX1032; EX1002 ¶¶64–65, 69, 75, 77–78, 80, 93, 116, 123, 130, 141, 191, 239.

61. "Electrokinetics" is also referred to as "electrodynamics." *See, generally,* EX2014 ("Ramsey") for discussion of electrokinetic operations. These terms generally refer to devices that take advantage of the interaction between ions in solution and electric fields. "Electrophoresis" and "electroosmotic flow" are examples of electrokinetics.

62. "Electrophoresis" is the motion of a solvated ion in free bulk solution in the presence of a field. The ion mobility is dependent on the molecule properties, the field and the solvent properties. It is well known from basic electrostatic considerations that a free ion moves in an electric field, and ions of opposite charge move in opposite directions. And if an ion has two charges, it moves twice as fast.

Likewise, the more massive the ion, the slower it moves. If an ion is in solution, the interactions of the charged ions with the uncharged solvent molecules affect the motion. For example, strong ion-solvent interaction (for example, ion-dipole interactions in water) typically slows the ion motion. Because each type of molecule has its own charge, size, shape and interaction with solvents, electrophoresis is used routinely as an analytical tool to separate different types of molecules. Electrophoretic separation devices for separating molecules in microchannels, which are distinct from particle sorters, would have been known to a POSA at the time of the invention. The POSA would also have (correctly) known that, in a bulk solution in an open microchannel, electrophoretic motion of free ions does not generate any pressure in the uniform microchannels. That is because the motive force is based on the charges of the particles and the applied voltage, not any pressure applied to the particle or fluid surrounding it.

63. "Electroosmotic flow" is a fluid transport mechanism widely used in microfluidics, specifically because it allows for pulse-free voltage-controlled fluid delivery with no moving parts, no valves, and no pumps. Electroosmotic flow in microfluidic channels is possible when there is a liquid-solid interface, and the solvated ions form a thin "double-layer" at that interface to balance any charges that may be inherent to the material when wetted. When an electric field is applied along a microfluidic channel with the double-layer, the ions move under the influence of

the field (by electrophoresis) and drag the rest of the fluid along. Since the very thin layer of fluid near the channel walls is providing the motive force, along the entire length of the transport channel, it is as if the walls themselves are moving and dragging the fluid along. The flow is dependent upon the field strength (i.e., applied voltage), the surface, and the ion properties. As the voltage is increased along the microchannel to create a stronger field, the flow rate increases because the ions move faster. Surfaces of microchannels can be negatively or positively charged (e.g, glass is negatively charged; some polymers are positively charged). Microfluidic devices prepared with materials with oppositely-charged surfaces would generate electroosmotic flow that went in opposite directions. Likewise, since the flow rate is dependent upon the ion properties, as described in the electrophoresis section, faster moving ions will generate faster flows because the ions drag the fluid through the channel faster. In all cases, the ions move along the electric field lines, allowing for exquisite fluid motion control by simple variation of applied voltages along the microfluidic channels. No pressure is generated within the channel by the force generating the electroosmotic flow in open channels and no additional applied pressure is required to accomplish fluid motion by electroosmotic flow through an open microfluidic network.

64. Indeed, unlike mechanical pumps, electroosmotic flow requires no moving parts, no valves, no diaphragms, and no actuators. The direct proportionality

of the flow to the applied voltage or current allows for a very smooth, pulseless, easily-controllable fluid flow if a DC voltage is used. As such, unlike mechanical pumps, a system using electroosmotic flow control to deliver the fluid would have no need for any mechanism to absorb oscillations.

B. Flow-Based Particle Sorting

65. In my analysis below, I refer to some of the prior art references as "flow-based" sorting devices that employ a "flow-based" sorting principle. For example, the "electroosmotic flow" described above is one option for inducing the flow (sometimes referred to as "hydrodynamic flow") required to deflect (or "direct" or "nudge") particles in flow-based sorting operations. Here I provide some general background on that principle of operation, using descriptions of the flow-based sorting techniques described in U.S. Patent No. 5,858,187 ("Ramsey," EX2014) that use electrokinetics to induce flow. The same flow-based principles, however, apply to devices that rely on other flow-inducement techniques.

66. In laminar flow systems such as the microfluidic systems at issue here and in Ramsey, the relative width of each "layer" of flow (e.g., sample core stream and outer sheath fluid layer) within the downstream channel is determined by the relative flowrate from each channel that supplies/adds those fluids to the transport channel.

67. As such, the relative width and position of a sample fluid can be adjusted when the sample is first injected into the sheath fluid by adjusting the flow rate of either the sample or the sheath fluid. Generally, a lower sample flow rate relative to the outer sheath fluid flow rate results in a narrower sample core stream. Such "adjustments" can be unintentional. For example, unwanted oscillations emanating from the pumps that supply the fluids can disrupt flow.

68. These adjustments can be made using "side channels" that introduce additional flow. When the flow of additional fluid from a side channel meets the flow of sample (or sheath) fluid already in the transport channel, the fluids do not mix; rather they merge at the intersection, form an interface between the two liquids, and continue downstream together. In a system with two opposed side channels intersecting a transport channel, where equal flow is delivered from the two side channels, the sample will move along the center of the transport channel. This concept is illustrated in Ramsey (EX2014) Figure 2:

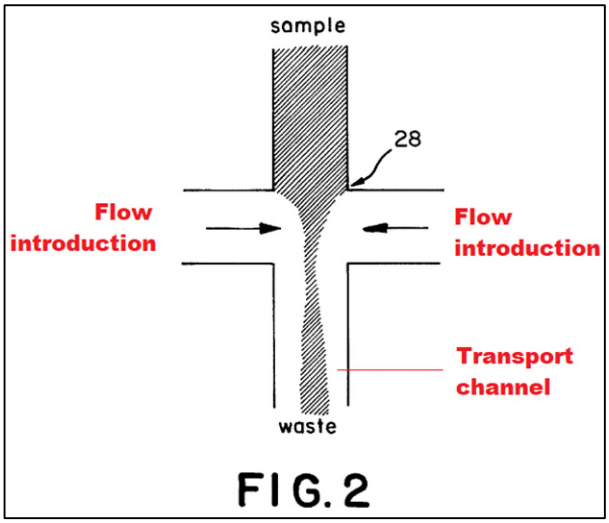


FIG. 2

EX2014, Fig. 2 (annotated; arrows in original to show flow introduction). As Ramsey explains, in Figure 2 "[t]he sample is introduced through the channel on top in a continuous fashion" and can be focused by electrokinetically-induced flow from the side channels, whereby the "[t]he sample stream reaches a minimum transverse dimension and then broadens due to diffusion after leaving the focusing chamber." *Id.* at 4:31–50 (further explaining that the voltages should be adjusted to account for variations in the various channels' dimensions).

69. If both side channel flow rates increase relative to the sample flow rate, the sample remains centered but occupies a narrower section at the center of the channel. This narrowing by adjusting/injecting fluids at small channel dimensions is sometimes referred to as "focusing" or "hydrodynamic focusing." When the sample has been narrowed into a core stream with the particles in a (near) single-file line, individual particles can be detected. Ramsey is again helpful. Figures 4A–4D show various widths of a sample core stream based on increasing fluid flow from two side channels:

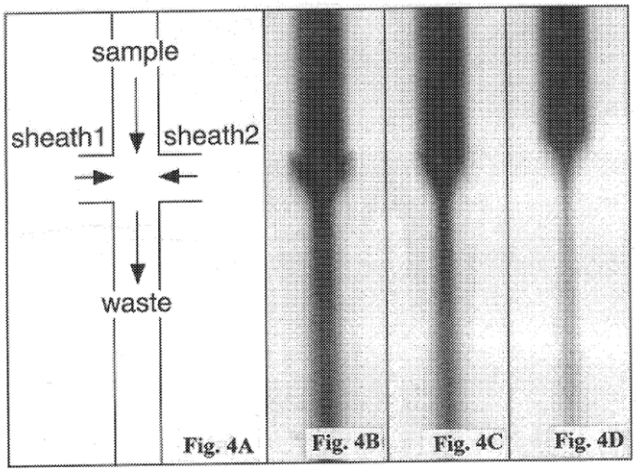


Figure 4B illustrates the lowest ratio of sheath fluid flow (translucent in the photos above) compared to the sample core flow (black in the photos above). In Figure 4D, the highest amount of relative sheath fluid flow was introduced from the side channels. Accordingly, Figure 4D depicts the narrowest sample core stream. EX2014 at 4:51–61.

70. As Ramsey explains, this flow-based narrowing is accomplished by increasing or decreasing the relative voltage applied through the channels. Ramsey further explains that the width of the sample can be adjusted based on adjusting the voltages in the sample channel and sheath fluid introduction side channel because flow rate is directly related to the voltage applied (i.e., "field strength"). *Id*. at 4:62–65, Fig. 5 (graphically illustrating relationship between "sample field strength," "sheath fluid field strength," and sample "stream width"); *see also id*. at 4:66–7:32, Figs. 4A–4D, 6–14 (describing techniques for adjusting sample width in Figs. 4A–4D). In short, an increase in voltage in a channel increases flow rate in that channel, with a relative increase in sheath fluid flow rate compared to sample flow rate effectively narrowing the sample.

71. This same flow-based principle can be used to sort particles by moving selected particles out of alignment with the other channels by increasing flow from *one* of the side channels. In other words, sorting can be accomplished by the net addition of fluid to a laminar-flowing stream in a transport channel so as to change

the transverse location of selected particles within the channel. In this way, particles can be directed into a desired outlet by adding fluid to the transport channel that flows downstream with the particles. In an example involving two side channels, if the relative flow rate from *one* side channel is increased, the flow of sheath fluid on that side increases in width, causing the transverse location of the sample to be shifted *away* from that side. Ramsey is instructive here as well. After describing the use of flow-based techniques to *narrow* the sample core stream, Ramsey describes *sample sorting* using the same electrokinetic-based fluid-introduction operation, as depicted in Figures 16–17:

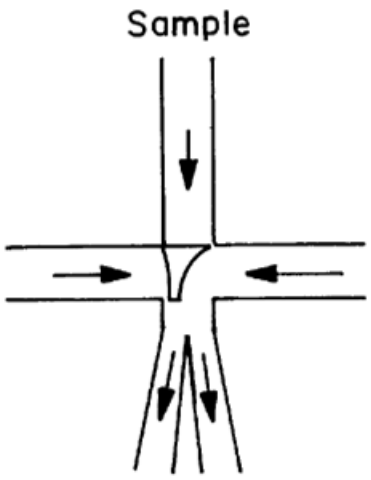


FIG. 16

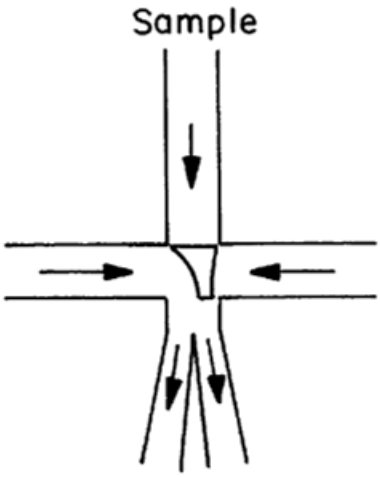


FIG. 17

72. Ramsey describes the "sorting method" depicted in these figures as follows (EX2014 at 7:32–51):

> The sample stream could be further manipulated by controlling the relative strengths of the electric fields in the two focusing channels. An application of such a manipulation procedure is shown in FIGS. 16 and

> 17. The basic four way intersection is again used as a focusing chamber but the lower sample channel is bifurcated into two channels, slightly below the intersection, with either common or separate reservoirs depending on the application.
>
> Electric potentials are applied to achieve sample transport in the direction of the arrows. Two different cases are shown in FIGS. 16 and 17, where the spatial extent of the sample in the focusing chamber is shown by the shading. In FIG. 16, the focusing field strength on the right is larger than on the left as indicated by the relative size of the arrows. The sample profile is pushed to the left and into the left-hand lower channel. Alternatively, the relative magnitude of the focusing field strengths can be reversed to push the sample into the right lower channel. Such manipulations would be useful in cell or molecular sorting experiments. The sample would be interrogated in the focusing chamber or before and depending on the result of that interrogation, the sample would be sent to the right or left lower collection channels. The same result might also be achievable by using separate reservoirs on the lower collection channels and grounding the reservoir in which the sample is to be collected. Using this method of sorting one could either leave the focusing fields fixed or alternate them as described above.

73. In short, both side channels introduce fluid continuously, but their relative flow rates are adjusted so as to sort the sample in one or the other direction. In other words, the flow is modulated. In no event does the sorting fluid retreat back into the side channel or become absorbed by the opposed side channel. The induced flow continues downstream along with the particles. This sorting operation can be further demonstrated using the annotated Figures 16 and 17 below (yellow indicating introduced flow and blue indicating sample):

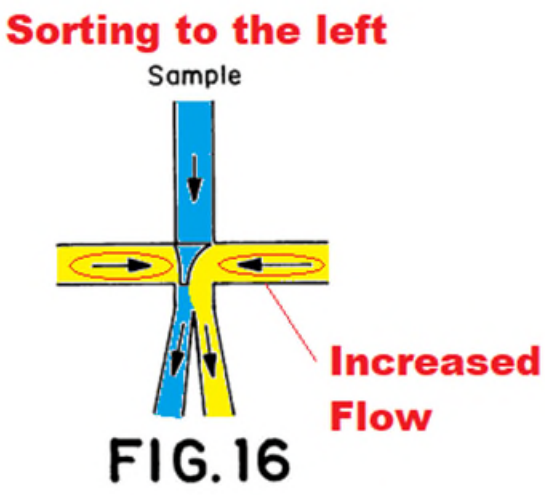


FIG. 16

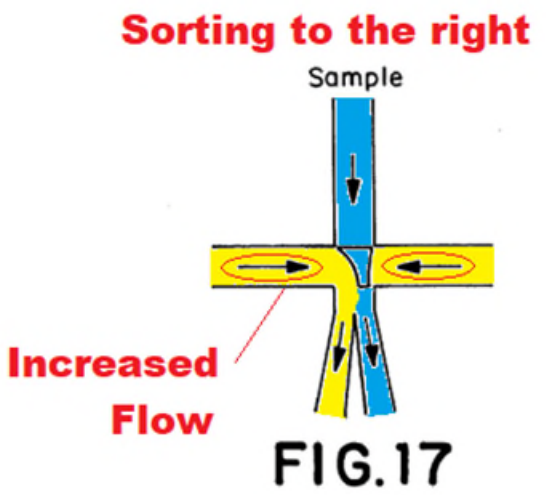


FIG. 17

74. Notably, the motive force for electrokinetic / electroosmotic flow, such as that taught in Ramsey, does not involve pressure when moving liquid. Specifically, there is no pressure gradient applied across the channel and there is no pressure generation or pressure variation in the channels, as long as an external pressure is not applied. The fluid is being transported by ion motion along the entire path of the microchannel given typical situations where the channel has a uniform surface. From a practical standpoint, the motion of the fluid along field lines allows for precise directional flow through microfluidic channel networks. For example, fluid flow can be directed around corners of intersections by application of voltages between a specific inlet / outlet pair of a complex microchannel network.

75. Ramsey chose to employ electroosmotic flow (or, more generally, electrodynamics/electrokinetics) to transport fluid, to increase fluid flow to “focus”

(i.e., narrow) a particle stream (or "core stream"), and to sort the particles in the particle stream. EX2014 at 1:11–2:2.

76. However, electrokinetics is not the only technique for increasing and decreasing flow in a transport channel using side channels. There are various other ways of controlling the net flow through each channel in a microfluidic channel network and taking advantage of the characteristic laminar flow of devices in this size regime to merge fluid flows in ways that precisely control their transverse position and width (such as the Joule heating technique described below for Wada (EX1006)). These approaches can all be used to narrow and manipulate sample streams very precisely in defined portions of a microfluidic channel by simply adjusting the relative net hydrodynamic flow rates of fluids emerging from each of the contributing fluid streams. Once the flow streams are adjusted to establish the sample in a desired section of the microfluidic channel, the location of the core stream can be adjusted by tuning the relative net flow rates of each of the contributing sheath flow channels.

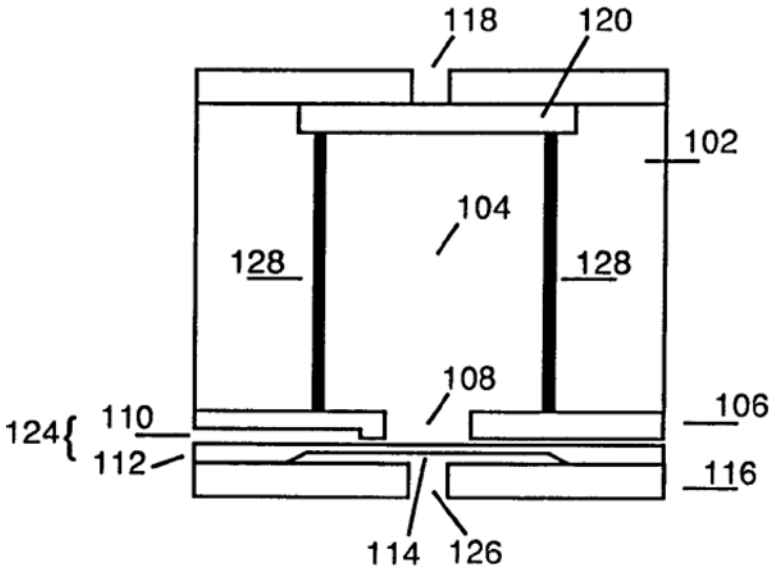
77. One can increase or decrease the flow delivered by the pump connected to a particular sheath flow channel (e.g,. pneumatic source, mechanical pump, electroosmotic pump, etc.); one can adjust the voltage on an electroosmotic sheath flow channel to increase the relative electroosmotic flow introduced from that channel; one can increase the temperature of the fluid in one of the sheath flow

channels to reduce the viscosity and thus increase the flow rate contribution from that sheath flow channel. In all of these cases, the net flow of fluid from a contributing side channel into the transport channel is adjusted, which in turn adjusts flow in the main transport channel. And in all of such cases, the core stream position will be affected in a predictable way such that any particles in the core stream can be directed into a selected outlet channel at a downstream branching intersection, thereby allowing sorting of particles for collection and / or further analysis.

78. As discussed below, this flow-based operation is the principle of operation for several examples of sorting devices cited by Dr. Weigl, and it differs fundamentally from the innovative teachings of ’797 Patent. In short, the flow-based sorting techniques do not rely on applying a “pressure pulse” to the particles to move them into one outlet or another. And the devices claimed in the ’797 Patent do not introduce net flow of fluid into the transport channel and they do not include sources of fluid other than that fluid introduced into, and that flows through, the transport channel. In other words, the sorting devices of the ’797 Patent do not rely on adding fluid flow or changing flow rates for sorting. As such, they are not flow-based sorters. They rely on a cross-channel “pressure pulse” that is absorbed by a “first reservoir” on the opposite side of the transport channel.

C. Anderson (EX1012)

79. Anderson discloses a diagnostic device in which nucleic acid samples are transported between various chambers for "carrying out a variety of preparative and analytical operations" on the samples, including "manipulation, identification and/or sequencing of nucleic acid samples." EX1012 at 3:11–14, 69:30-34, Figs. 5a–5b. The device includes two or more "reaction chambers" and "fluid channels" connecting the various chambers and various microvalves. *Id.* at 32:24–33:4. A representative reaction chamber is shown in Figure 2b:



The reaction chambers "perform a number of varied functions, e.g., as storage chambers, incubation chambers, mixing chambers and the like." *Id.* at 33:4–7.

80. Anderson discloses various fabrication techniques for its "reaction chambers" and various techniques for transporting nucleic acid samples between them, including: diaphragm pump and valve combination (*id.* at 45:3–46:22);

"pressure differentials" set up with vacuum sources, "positive displacement pump[s]," and the like (*id.* at 46:23–52:15); electrohydrodynamic pumps (*id.* at 63:33–64:32); and an assortment of "microvalves" placed between chambers to control flow of the sample (*id.* at 65:33–67:6).

81. Anderson's disclosure is directed mainly to delivering a sample from one chamber to another—i.e., transporting a volume of fluid between chambers. Throughout the disclosure, Anderson states that its device functions to "deliver the sample" between chambers (*id.* at 51:11–13), or "push a sample through the device" (*id.* at 51:31–33), or is used for the "transport" of fluid samples between chambers of the device (46:23–27; 44:15–20). In other words, Anderson's chambers are used exclusively as containers—either source or destination—for the sample of interest.

82. Wada's citation of Anderson in its section titled "Assay Devices" is consistent with this, i.e., that the various devices disclosed in Anderson relate solely to transporting a sample through a device. EX1006 at 42:31–43:14 (citing International Application No. WO 97/02357 (Anderson)). Wada cites Anderson as providing examples of "microfabricated pumps" that may be employed "to provide relatively precise control of the flow rate of the cell suspension" or "for forcing the cell suspension through the analysis channel." *Id*. This part of Wada's disclosure and citation to Anderson are directed to transporting fluids/samples, not particle sorting or providing a "pulse" of flow or pressure.

83. In sum, neither Anderson's chambers, nor its techniques for transporting fluid from one chamber to another, are used to affect an existing "stream of particles in a carrier fluid." More particularly, none of Anderson's microfabricated chambers—or any other of its disclosed structures or devices—are used as pressure pulse generators or reservoirs for receiving a pressure pulse. The chambers serve only as a source and destination for the sample being transported.

84. Finally, I disagree with Dr. Weigl's suggestion that Anderson's discussion of "a separation channel for separating a component of said fluid sample" has any relevance to '797 Patent claims. EX1002 ¶107 (quoting EX1012 (Anderson) at 3:35–4:6)). A POSA would understand that Anderson at 3:35–4:6, which explains 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***" (emphasis added) explicitly refers to electrophoretic separation techniques described by Anderson at 13:11–13:34. As also described above regarding "'Electrokinetics' or 'Electrodynamics,'" these techniques are designed to *separate* a molecular mixture into its components. In any event, as described above, such electrokinetic techniques were understood by POSAs to have no relationship to pressure pulses or particle sorting.

85. In short, Anderson's separation channel is irrelevant to the invention because Anderson is explicitly referring to electrophoretic separation techniques. EX1012 at 13:9–13 ("Electrophoretic methods may also be utilized in the purification of nucleic acids from other cell contaminants and debris."), 13:35–14:10 (referring to "field channels," which are channels that apply a voltage in electrokinetic techniques). As discussed above, such a technique relies on charges inherent to the particles themselves, not flowing fluid. This is an entirely different concept from applying a pressure pulse (or even a hydrodynamic flow) to individually sort particles.

86. Finally, Anderson at 5:32–6:5, which Dr. Weigl describes as "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" has nothing to do with sorting (or particle separation) at all. EX1002 ¶107. A POSA would recognize that Anderson is discussing a means of keeping reagents separated with a bubble (also known in the art as segmented flow) and then removing the bubble in a vented mixing chamber to allow the reagents to mix as part of an assay. Again, this has nothing to do with sorting (or particle separation) at all.

D. Wada (EX1006)

87. As explained below, Wada employs a fundamentally different principle from the claimed invention, and it lacks several requirements from the claims. For

example, Wada fails to employ a "pressure pulse" as claimed. Moreover, Wada does not teach using a "first reservoir" to absorb or dampen a pressure pulse. In fact, such a reservoir is not needed because Wada does not deliver a pressure pulse across a channel to be received by a reservoir in the first place.

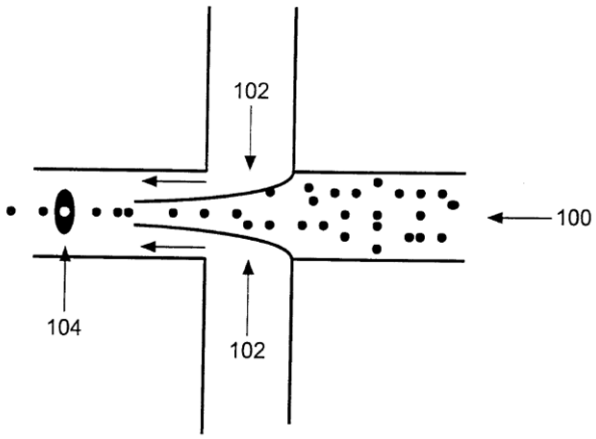
1. Wada's Principle of Operation

88. Wada operates on the same fundamental principal as Ramsey, which is introducing flow by adding fluid into a transport channel from a side channel to move particles away from that side channel. In fact, Ramsey is cited in Wada for its flow based focusing and sorting techniques that rely on electrokinetics. EX1006 at 12:9–14 (citing Ramsey regarding focusing); 18:26–19:9 (citing Ramsey regarding sorting). Wada describes its flow-based sorting operation as introducing a "hydrodynamic flow" of fluid to "nudge" or "direct" the particle stream. EX1006 at 17:10–24:12. Specifically, Wada applies the principles of electrokinetics (for which it primarily relies on Ramsey) or fluid heating (for which Wada gives a more extensive description) to induce this hydrodynamic flow. *Id.* at 12:9–14, 18:26–19:13, 20:15–22.

89. Notably, Wada teaches flow-based sorting techniques to introduce flow into a transport channel via a side channel in ways that increase flow *along* the transport channel so as to deflect particles. Contrary to Dr. Weigl's assertions, Wada does not rely on pressure variations across the channel to effect sorting. *See, e.g.*,

EX1002 ¶¶123–150. The ’797 Patent’s devices, on the other hand, do rely on cross-channel pressure variations. Specifically, pressurizing reservoirs as in the ’797 Patent increases the pressure gradient directly *across* the transport channel to accomplish sorting. And unlike Wada, the ’797 Patent specifically teaches POSAs how to sort particles without adding additional longitudinal flow along the transport channel.

90. Before discussing sorting, Wada first discloses apparatuses and methods for “focusing” (or “pinching”) particles in microchannels using “hydrodynamic flow.” In the embodiment depicted in Figure 1A (reproduced below), a sample of particles is focused near the center of a transport channel by introducing flow from two intersecting channels:



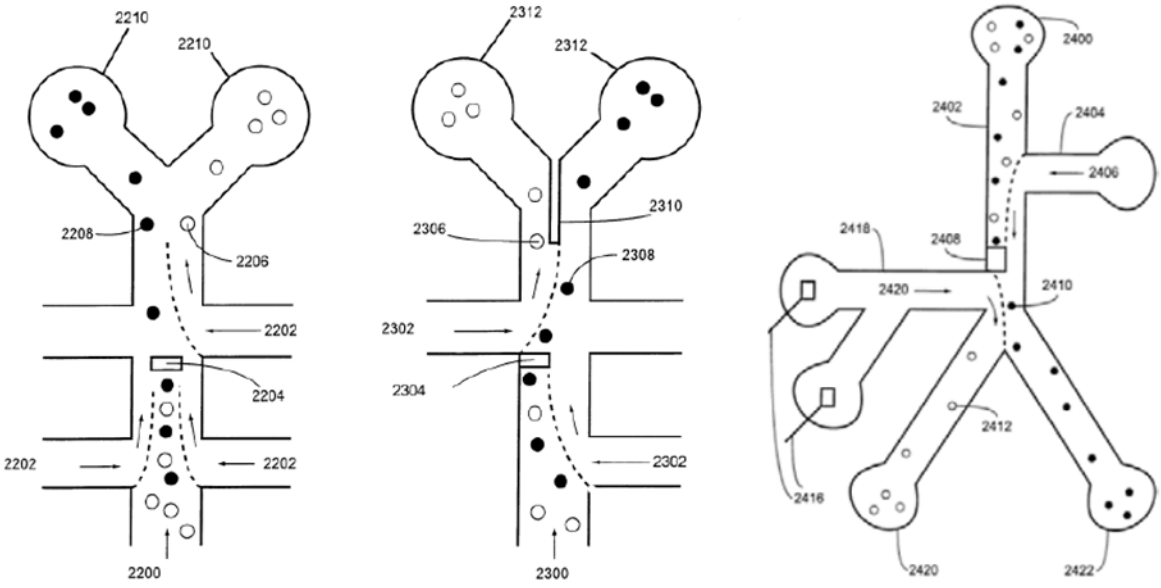
EX1006, Fig. 1A; 9:2–5, 13:11–14:2. Focusing by fluid flow introduction from the side channels into the transport channel is central to Wada’s operation: “the particles are vertically or horizontally focused in the microchannel, e.g., by simultaneously

introducing fluid flow from two opposing microchannels into the first microchannel during flow of the particles in the first channel." *Id.* at 4:11–14. A POSA would recognize that the arrows and lines in Fig. 1A indicate the laminar fluid flow in the intersecting region as the fluid enters from the side channel and makes a turn down the transport channel. And as noted in the description of Figure 1A, this focusing of cells is caused by "simultaneous flow from side channels into a main channel through which the cells are being flowed." *Id. at* 9:3–5. Wada explains that focusing particles in this way allows particles, such as cells, to flow past a detector to be analyzed. *Id. at* 13:28–31.

91. Citing Ramsey, Wada notes that particle stream focusing in microchannels may be achieved by inducing the flow from the side channels through "electrodynamic[] . . . and/or other fluid movement methods," including "Joule heating." *Id. at* 12:7–13. And as explained above, Ramsey describes focusing with fluid injected from side channels, wherein that fluid is caused to flow into and down the transport channel by "electrodynamic" (a.k.a. "electrokinetic") flow induced by a voltage differential applied between the sheath fluid sources and the waste reservoir at the end of the transport channel. EX2014 at 3:61–4:40, Figs. 2, 4A–4D, 15.

92. After discussing "focusing" of particles using hydrodynamic flow (EX1006 at 12:1–15:2), Wada describes using this same "hydrodynamic flow"

principle to sort particles. Specifically, Wada describes inducing flow of fluid from side channels to direct the particle stream, with that added fluid flowing into the waste outlet/container together with the particles. Wada discloses its sorting embodiments solely with reference to Figures 22 through 24:



93. Figures 22 through 24 are discussed in a section titled "Use of Focusing to Sort Particles." *Id.* at 17:10–24:12. In that section, Wada states that its disclosed sorting devices "use hydrodynamic flow to focus and/or sort the particles." *Id.* at 17:31–18:1. Still in that section, Wada describes inducement of sample flow in the transport channel and sample alignment in the center of the channel, noting that the particles can be vertically or horizontally "focused" (i.e., aligned top-to-bottom or side-to-side) in the transport channel "by any method for flowing fluid in a

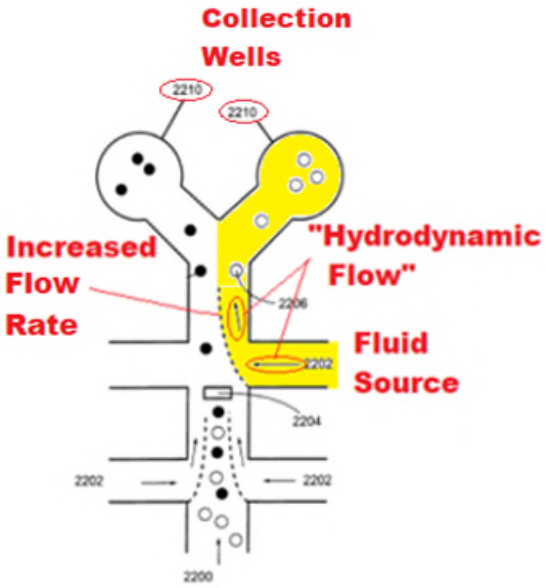
microfluidic device, including electrokinetic flow, pressure-based flow, and the like." *Id.* at 18:18-25.

94. Wada then proceeds to describe the "sorting function," specifically by the use of electrokinetics to "nudge" selected particles across the width of the transport channel. *Id.* at 18:26–19:13. Wada again cites Ramsey for this electrokinetic technique, which teaches the use of electrokinetically-induced flow from the side channels for sorting. Wada explains that Ramsey teaches that such a technique can be used to "nudge" a "sample profile" so as to direct it into one of two downstream collection containers, as explained above for Ramsey. EX2014 at Figs. 16, 17; 7:24–50. As in Ramsey, the electrokinetic "nudging" of flow in Wada does not generate a "pressure pulse" that propagates across the channel and into a "first reservoir." The "hydrodynamic flow" that Dr. Weigl (incorrectly) describes as a "pressure pulse" does not get received or absorbed by either of Wada's side channels. EX1002 ¶¶133–150.

95. With specific reference to Figure 22, Wada describes its sorting technique as the introduction of "hydrodynamic flow" from the side channels:

> A second set of opposing microchannels is typically 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 at 19:29–20:2. Instead of reaching one of the opposed side channels, the hydrodynamic flow enters the transport channel (thereby increasing flow rate in the transport channel), immediately turns downstream, and exits into a collection well. This hydrodynamic flow-based process is depicted below in annotated Figure 22 (introduced hydrodynamic flow shown in yellow):



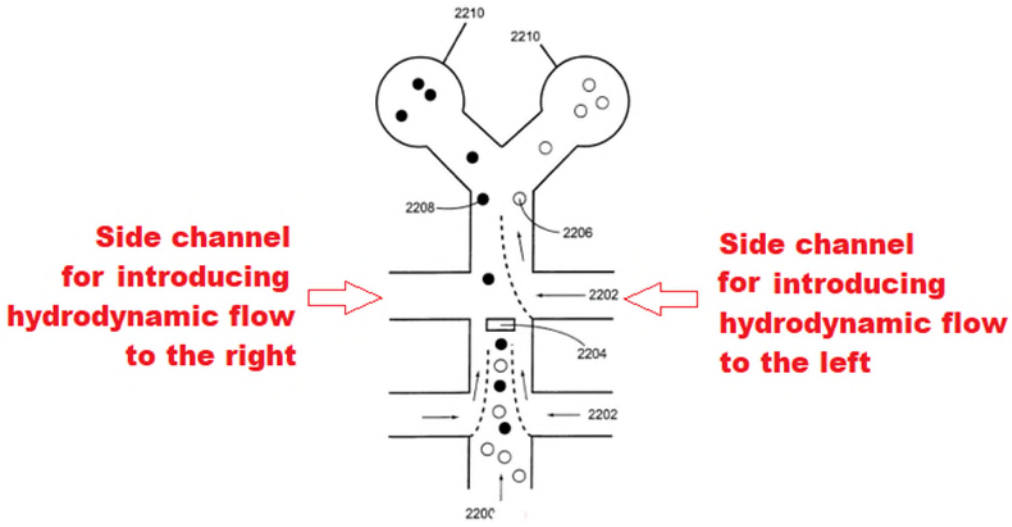
96. The device depicted in Figure 23 is similarly describes as introducing hydrodynamic flow to "direct" particles into a collection well:

> Figure 23 schematically depicts a microchannel configuration that includes one embodiment of separation element 2310. In this example, cells 2300 are typically flowed in a microchannel that intersects with another microchannel located upstream from detector 2304. The upstream intersecting microchannel is optionally used to focus cells 2300 to one side of the microchannel as cells 2300 pass through detector 2304 using hydrodynamic flow 2302. This illustration 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 and into, in this case, one of two microchannels, each terminating in particular collection wells 2312. Separation element 2310 is optionally omitted, with cells 2300 or other particles being directed into destination regions as desired.

EX1006 at 20:3–14. Again, instead of reaching one of the opposed side channels, the hydrodynamic flow enters the transport channel, immediately turns downstream, and exits into a collection well.

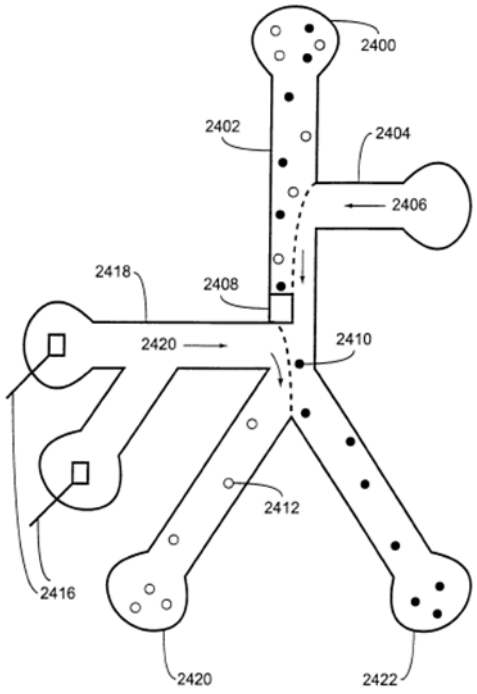
97. Notably, as described above for both Figures 22 and 23, hydrodynamic flow is introduced from *both* side channels for sorting:



To that end, I disagree with Dr. Weigl's assertion that one of the two side channels is a "first reservoir" (for absorbing/dampening) when it is "non-actuated" simply because the channels are aligned geometrically. EX1002¶¶133–150. A POSA would understand that both channels introduce hydrodynamic flow and thus are not passive reservoirs that receive or dampen/absorb fluid. But even if a channel was "non-actuated," the mere presence of liquid in the side channel does not render a side

channel capable of dampening or absorbing a pressure pulse as required by the "first reservoir."

98. After describing Figure 23, Wada provides an extensive discussion of using heating elements (e.g., Joule heaters) to induce the flow from side channels into the transport channel and down into the collection wells with the particles. EX1006 at 20:15–24:12. Wada explains that such heating elements are disposed in the side channels to heat a fluid (e.g., "buffer" liquids or "gels") to induce flow by decreasing the viscosity of that fluid. *Id.; see also id.* at 20:15–22. Wada uses Figure 24 to depict Joule heaters (2416) in the side channels that induce flow (2420):



99. Wada explains the operation of Figure 24 as follows

> The heat produced by current flowing from Joule heating electrodes 2416 raises the temperature of, e.g., a buffer or gel disposed within

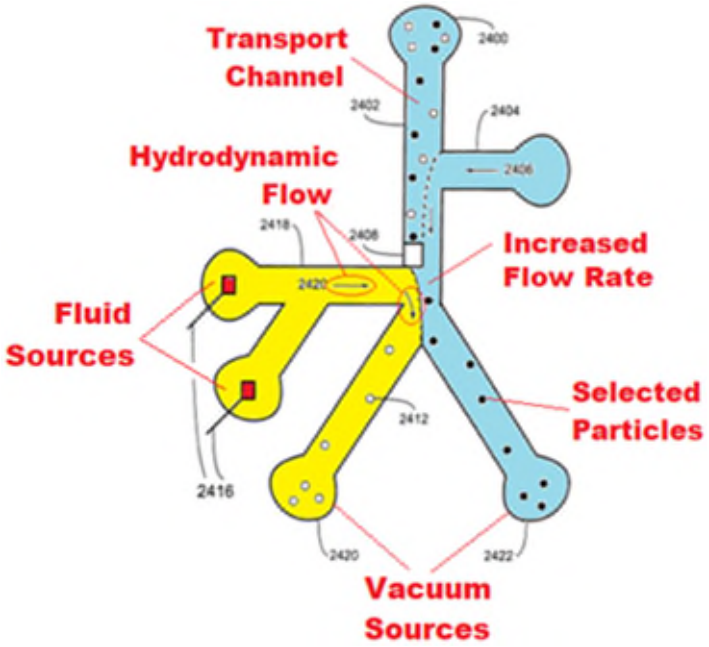
> branched particle sorting microchannel 2418, thus reducing hydrodynamic resistance in the buffer or gel. The reduced viscosity, in conjunction with vacuum source(s) that are typically operably connected to the device at non-selected particle collection well 2420 and/or selected particle collection well 2422, induce or increase flow of the buffer or gel from branched particle sorting microchannel 2418, which intersects main microchannel 2402 downstream from detector 2408. The induced flow of buffer or gel into main microchannel 2402 thus deflects or redirects the flow of selected particle 2410 into selected particle collection well 2422. In this embodiment, non-selected particle 2412 does not trigger Joule heating/redirecting flow and as such, flows unimpeded into non-selected particle collection well 2420.

Id. 23:7-14. In other words, as in Figures 22–23, Figure 24 induces hydrodynamic flow to add fluid to the transport channel to effect sorting, and this fluid flows into one of the collection wells along with the particles. And once again, instead of moving significantly across the channel and displacing fluid into the opposing side channels, the hydrodynamic flow enters the transport channel, immediately turns downstream, and exits into a collection well.

100. Moreover, the "vacuum sources" that are "typically" included at the collection wells are informative to a POSA regarding the operation of Wada Figure 24. The teaching of adding a vacuum (i.e., a suction source) to the collection wells, where the hydrodynamic flow exits, reinforces the notion that Wada employs flow-based sorting. That is, a POSA would recognize that the goal of using a vacuum source is to provide the primary motive force for the outflow of fluid from both the transport channel and the side channel into and down the transport channel (i.e., a longitudinal direction). That is completely different from sending a pressure pulse

across a channel (i.e., a lateral direction). In other words, Figure 24 and its vacuum clarifies the different principle of operations between Wada and the claimed invention. The goal of Wada is to induce a continuous flow from the transport channel and heater wells, the flow rate from the latter being controlled by adjustment of the temperature in order to effect sorting, down the transport channel into the collection wells.

101. The annotated figure below illustrates Figure 24's basic operation described above:



102. Wada also makes one reference to "other alternative techniques for inducing the flow of focusing fluids to sort particles," including "pressure, hydrostatic, wicking, capillary, and other forces," which Dr. Weigl cites. *See* EX1002 ¶¶95, 129–30 (citing EX1006 at 19:9–12). I disagree with Dr. Weigl's suggestion

that this passage has relevance to particle sorting by using a "pressure pulse" as described in the '797 Patent. While using electrokinetic and Joule heating do not rely on pressure at all, using "pressure" to "induc[e] the flow of focusing fluid to sort particles" is referring to the same flow-based principle discussed in Figures 22–24. In such systems, flow from the side channels is modulated by varying the pressure applied at the fluid source, which varies the amount of fluid flowing in streams in the main transport channel parallel to the streams around the particles. In other words, this is fundamentally the same as the principle described in Ramsey and the electrokinetic or Joule heating techniques above. In fact, micropumps and miniature mechanical pumps at the time of the invention were not known to be suitable for sorting operations at all. *See* Zeng (EX1022), Zengerle (1033), and Veenstra (1035) discussed below.

103. Additionally, hydrostatic, wicking, and capillary forces are not appropriate for sorting applications as described by Wada. EX1006 at 19:9–13. These forces would be known to a POSA, as confirmed by Wada EX1006 at 44:3-7, as being only appropriate for continuous flow applications. For example, "hydrostatic" refers to the pressure exerted by a fluid at equilibrium at a given point within the fluid, due to the force of gravity. Hydrostatic pressure increases in proportion to depth measured from the surface because of the increasing weight of

fluid exerting downward force from above. In other words, there is no pressure modulation that can sort particles.

104. In sum, Wada teaches sorting only by introducing fluids into the transport channel from one or more side channels. These introduced fluids can be "buffers, gels, and other materials," (*id.* at 20:15–17), but in all cases they are "induce[d]" to "flow" into the transport channel and the flow rates through the channel networks is adjusted by various means, such as by Joule heating (*id.* at 20:17–22). The fluid from the side channel must be supplied from a "well" or "other cavity within the device" (*id.* at 20:23–25) and proceeds through the transport channel to the outlet. And as taught by Figure 24, a side channel may be outfitted with multiple Joule heaters and fluid sources, so as to increase the available amount of hydrodynamic fluid flow for the sorting function. *Id.* at 23:1–4.

105. Finally, in a later section titled "Assay Devices," Wada describes "[m]icrofluidic devices and assay components which are optionally adapted to the present invention." *Id.* at 37:4–6. Within that section, Wada refers to various components, such as pumps and vacuums, for establishing and controlling the main fluid flow through the device, i.e., for transporting the particles and fluid "across the length of the channel":

> [I]t may be desirable to provide relatively precise control of the flow rate of the cell suspension and/or other particles, e.g., to precisely control incubation times, or the like. As such, in many preferred aspects, flow systems that are more active than hydrostatic pressure

> driven systems are employed. For example, the cell suspension is optionally flowed by applying a pressure differential across the length of a channel. For example, a pressure source (positive or negative) is applied at the cell suspension reservoir at one end of a channel, and the applied pressure forces the suspension through the channel. . . . pressure or vacuum sources are optionally supplied external to the device or system, e.g., external vacuum or pressure pumps sealably fitted to the inlet or outlet of a channel, or they are internal to the device, e.g., microfabricated pumps integrated into the device and operably linked to a channel.

Id. at 42:31–43:13. Here, Wada is discussing the establishing of the main flow through the transport channel for the purpose of accomplishing an assay. As Wada points out, for assays, it is desirable to use a "flow system" that delivers "relatively precise control of the flow rate" for delivery of fluid "through the channel" from, for example, "the suspension reservoir at one end of the channel." *Id.* at 42:31–43:5. A POSA would recognize that all of the techniques or devices are proposed in this section because of their ability deliver fluid at a controlled flow rate, in this case *longitudinally along the main transport channel*. As the claimed pressure pulse actuator does not deliver a net flow of fluid into the channel, a POSA would not look to this section for a pressure pulse actuator. Moreover, Wada already provides extensive discussions on its preferred sorting techniques, namely, using Joule heating and electrokinetics to deliver flow into the transport channel to effect sorting—both of which avoid mechanical pumping as described here.

(a) Heating and Viscosity-Based Flow; Lack of "Pressure"

106. As explained above with respect to "electrokinetics" and Ramsey, electrokinetic-based sorting operations do not rely on pressure modulation at all. The same is true for the Joule heating process described in Wada.

107. Wada refers to the process of heating fluids to selectively divert particles into collection channels. EX1006 at 20:15–24:12, Fig. 24. Dr. Weigl implies that a heating step increases the pressure which, in turn, moves the particles into the selected exit port, and I disagree with that implication. *See* EX1002 ¶127; *see also id.* ¶¶142–46. While heating the fluid entering the transport channel from a side channel does affect the location of the particle, the reason is not the result of a change in pressure. Rather, as Wada repeatedly teaches, heating the fluid takes advantage of "the inverse relationship between temperature and viscosity for various buffers, gels, and other materials . . . to effect particle sorting." EX1006 at 20:15. Just as in electrokinetic and other flow-based operations, Wada's Joule heating embodiment controls the amount of fluid that is introduced from the side channel into the transport channel, which continues downstream.

108. The physical phenomenon responsible for the sorting action, as explicitly discussed by Wada, is based on the change in liquid viscosity with increasing temperature. In layman's terms, viscosity is the "thickness" of a fluid. Syrup, for example, is a "thick liquid" that has a higher viscosity than, say, water. Following with the same example, syrup flows more readily when heated.

109. Given a channel where Area is the cross-sectional area of the channel, Pressure is the pressure drop between two points in the channel separated by the distance defined by the term, Length, it is well known from a simple rearrangement of Poiseuille's equation that the flow rate of a liquid through a pipe is inversely proportional to the viscosity of the fluid as given by

$$\boldsymbol{Flow\ rate} = \frac{Pressure * Area^2}{8 * \boldsymbol{viscosity} * Length}$$

110. Heating reduces the viscosity of most fluids, including those in Wada (as expressly described by Wada). Thus, as is readily apparent from Wada's teachings and the equation, as the fluid temperature increases, the viscosity decreases, and fluid flow rate increases. In the channel configuration given in Wada in Figure 24, the fluid entering from the side channel is heated as it flows from the fluid source through the side channel and into the transport channel. Because the liquid flow is laminar, the fluid from the side channel will not mix with the fluid in the transport channel and will preferentially proceed down the side exit nearest the entrance channel; further, the reduced viscosity will result in an increased flow rate for this fluid even though there is no change whatsoever in the applied pressures on the channels. This increased net flow rate from the side channel into the transport channel adds flow to the transport channel and produces a shift in the position of the sample flow stream and thus diverts the particle. In this case, the addition of flow

into the transport channel is effected by an increase in flow rate from the side channel, the magnitude of the net flow influx being controlled precisely with a small heater that simply lowers the viscosity by increasing the temperature of the transiting fluid, much like the hot syrup flows faster than the cold syrup from a bottle tipped at the same angle.

111. To further address Dr. Weigl's incorrect suggestion that pressure changes in this operation, we can consider the effect of temperature on pressure within the microchannel network. Given that Wada describes heating a localized volume of liquid, the pressure would need to be generated by the expansion of the liquid within the channels. Using the dimensions provided by Wada, the volume of fluid between the heaters located in the channels is on the order of nanoliters (10^{-9} L, as Wada states when discussing the fluid heating process). *See* EX1006 at 20:28–29 (explaining that "nanoliter volumes of fluid have tiny thermal mass"). Given the temperature-change contemplated by Wada (changes up to 100 ºC) and given that the typical Thermal Coefficient of Expansion (TCE) of an aqueous solution is ~10^{-6} / ºC, the miniscule fluid expansion ($10^{-9} * 10^{-6} * 100 = 10^{-13}$ L; or 100 femtoliters) would not generate enough volume change to significantly affect the pressure in the microfluidic channel, certainly not enough to deflect a particle as required for sorting the particles in the devices that are described in Wada. Furthermore, if the Wada sorting process were based on a thermal-expansion-induced pressure change (which

it is not), there would be no reason to include the discussion of the relationship between viscosity, temperature, and flow rates. Rather viscosity changes would have no relevance. Viscosity, and hence the discussion, is only relevant if the particle sorting is dependent upon the change in flow, which ***is*** viscosity dependent.

112. In fact, in a heating-based sorting process, to affect the pressure in the microfluidic channel sufficient to deflect a particle as required for sorting, a person of skill would need to vaporize a liquid (i.e., create an expanding vapor or gas bubble) to get enough volume change to deflect particles. A person of skill would be discouraged from incorporating that into Wada's devices because Wada teaches a person of skill to maintain the fluid used for sorting in a *liquid* state in order to supply a "hydrodynamic flow" to accomplish the particle deflection. In short, generating vapor or gas would impede Wada's sorting operations.

113. A POSA would also appreciate that the use of heat-generated expanding gas bubbles were known to be deleterious to particles such as biological cells. The '797 Patent also notes this deficiency of that type of prior art device. EX1001 at 2:36–53. A POSA would thus have a reason to *avoid* modifying Wada to create such a "pressure pulse" using this technique.

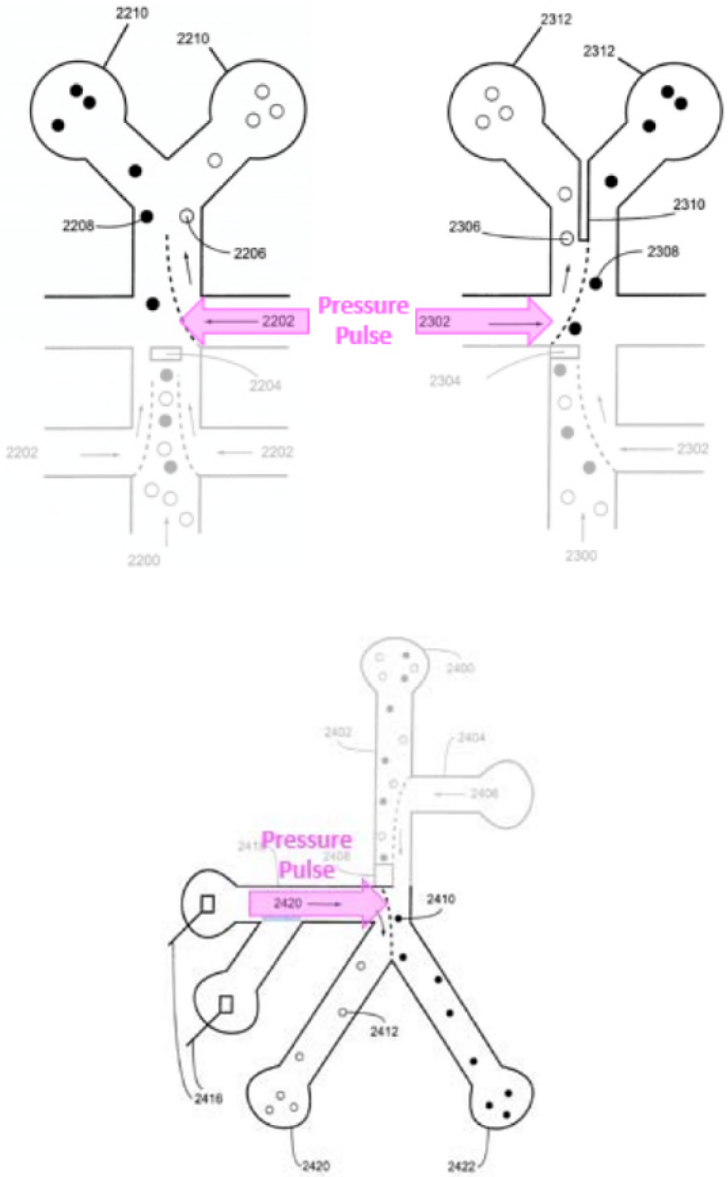
114. As explained above, changing the temperature of a fluid typically changes the viscosity of the fluid. Under constant pressure, the flow rate of a fluid through a microfluidic channel is inversely proportional to the viscosity. Thus, the

flow rate of a fluid through a microfluidic channel can be controlled by heating and / or cooling the fluid. Wada teaches the well-known principle that it is possible to increase the net flow of a liquid through a channel by increasing the temperature of the fluid in that channel (for fluids typically used in cytometry / sorting). As Wada describes, these effects can be very significant and continues to illustrate their utility for microfluidic sorting. Contrary to Dr. Weigl's assertion (i.e., that heating the fluid generates a pressure), and as explicitly described by Wada, for small liquid volumes, reducing the fluid viscosity of the fluid is an effective means of adjusting flow rates through microfluidic channels and provides the basis for sorting particles as described by Wada.

2. Wada Teaches Neither the Claimed "pressure pulse" Nor the "first reservoir" that Dampens or Absorbs the Pressure Pulse

115. I disagree with Dr. Weigl that "Wada teaches a first reservoir in fluid communication with the flow channel adapted for dampening or absorbing a pressure pulse propagated across the width of the flow channel." EX1002 ¶172. Relatedly, I disagree with him that Wada discloses or renders obvious the "actuator or pressurizer" that "generat[es] the pressure pulse." *Id.* ¶192.

116. Dr. Weigl incorrectly contends that Wada's "hydrodynamic flow" is the claimed "pressure pulse" that "propagat[es] across the microfluidic flow channel." *Id.* ¶¶123–132. Indeed, the features labeled 2202, 2302, and 2420 that Dr. Weigl identifies are examples of the "hydrodynamic flow" introduced in Wada:

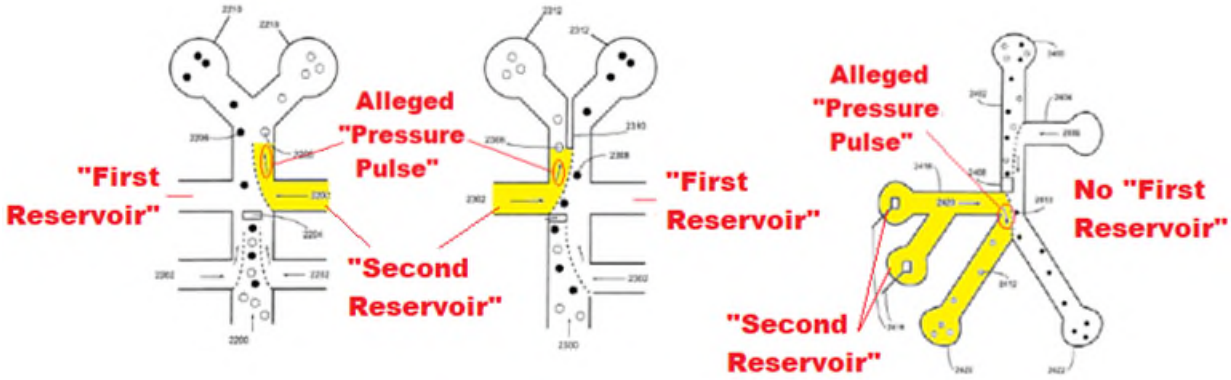


EX1002 ¶124, 126 (Dr. Weigl's annotations); *see also id.* ¶¶127–28 (asserting that Figure 24's introduced flow via Joule heating is also a pressure pulse).

117. Wada explains that these features are simply for introducing flow for a flow-based sorting operation, not a pressure pulse. Regarding Figure 22, the "second set of opposing microchannels is typically 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 at 19:29–20:2 (emphasis added); *see also id.* at 20:8–13 (same regarding Figure 23); *id.* at 23:10–16 ("The reduced viscosity, in conjunction with vacuum source(s) that are typically operably connected to the device at non-selected particle collection well 2420 and/or selected particle collection well 2422, ***induce or increase flow of the buffer or gel*** from branched particle sorting microchannel 2418, which intersects main microchannel 2402 downstream from detector 2408. The induced flow of buffer or gel into main microchannel 2402 thus deflects or redirects the flow of selected particle 2410 into selected particle collection well 2422.") (emphasis added).

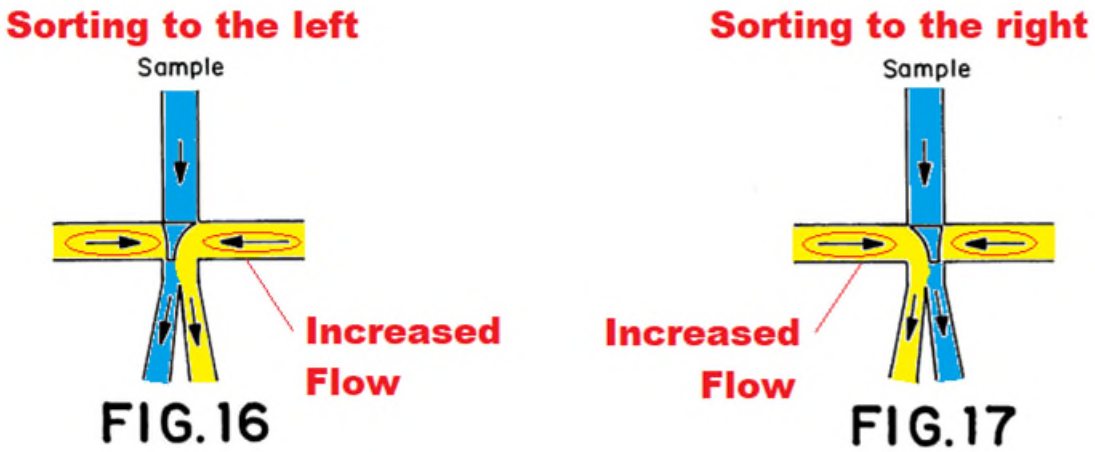
118. I disagree that the features of Wada identified by Dr. Weigl can serve as the claimed "pressure pulse" and thus the "first" or "second" "reservoir" that generates, propagates, dampens, or absorbs it. EX1002 ¶¶133–150. As shown in Wada's Figures 22–24, Wada's "hydrodynamic flow" does not even reach any alleged "reservoir" and thus is not "dampen[ed] or absorb[ed]" by it. A POSA would recognize that the dotted lines used in Wada's figures suggest a laminar flow profile under the conditions where the fluid in the "First Reservoir" is relatively incompressible (e.g., not resilient) and would not receive fluid during the "Alleged Pressure Pulse," much like the case in Fig 24, where there is "No 'First Reservoir'":



119. Instead, as shown (and described in detail above regarding Wada's sorting operation), Wada's hydrodynamic flow enters the transport channel and immediately turns downstream. Moreover, as explained above with respect to Figure 24, "vacuum sources" are "typically" in the collection wells. EX1006 at 23:7–18. This clarifies the different principle of operations between Wada and the claimed invention. That is, the goal of Wada is to induce a continuous flow from the transport channel and heater wells, the flow rate from the latter being controlled by adjustment of the temperature in order to effect sorting, down the transport channel into the collection wells. The electrokinetic approach (as in Ramsey) employs a similar technique and has the same flow-based operating principle. In that approach, the electrical potential between side channel and collection well serves as the analog to the "vacuum source."

120. Unlike the invention, in which the sorting operation relies on no net change in flow (no change in flow rate), Wada's flow-based operation requires fluid sources (sometimes shown as "wells" or "cavities" in Wada) to continuously supply

fluid to the side channels. EX1006 at 17:10–24:13; 20:23–26, 22:30–23:1. Wada's reliance on Ramsey (EX1006 at 19:6–9) to teach the sorting operation employed in Figures 22–23 is instructive, as Ramsey (and thus Wada) teaches POSAs to continuously introduce flow from both side channels to sort particles:



EX2014 at Figs. 16, 17 (annotated), 7:24–50. As shown by the arrows, sorting is achieved by modulating the relative amounts of this continuous flow from each side channel.

121. Even if Wada's side channels were not continuously flowing, "hydrodynamic flow" would still not be a pressure pulse because a "pressure pulse" is a momentary disturbance that deflects a single particle without varying flow rate through the transport channel. Due to being dampened or absorbed by the "first reservoir" in the invention, the pressure pulse does not exist after the moment when the particle is deflected, as depicted below in the annotated version of Figures 2–4 of the '797 Patent:

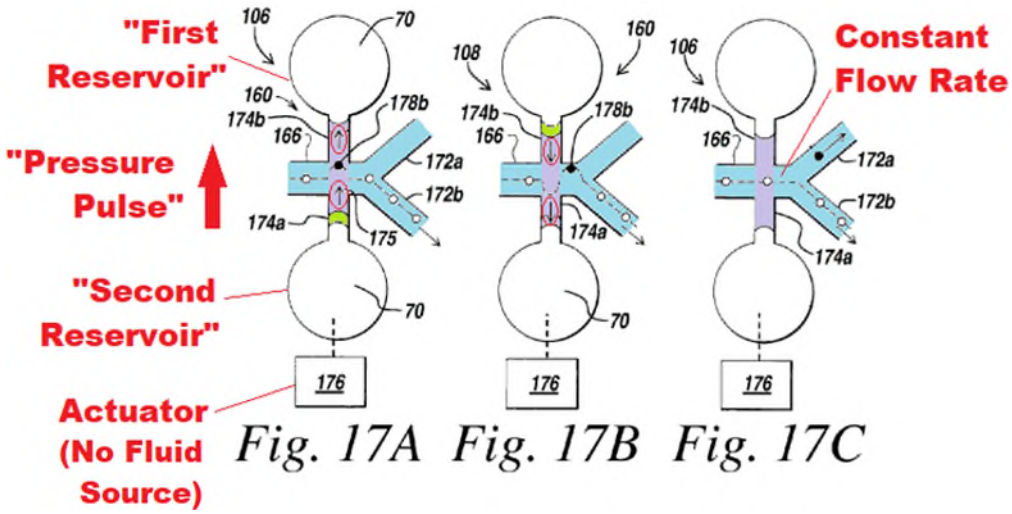
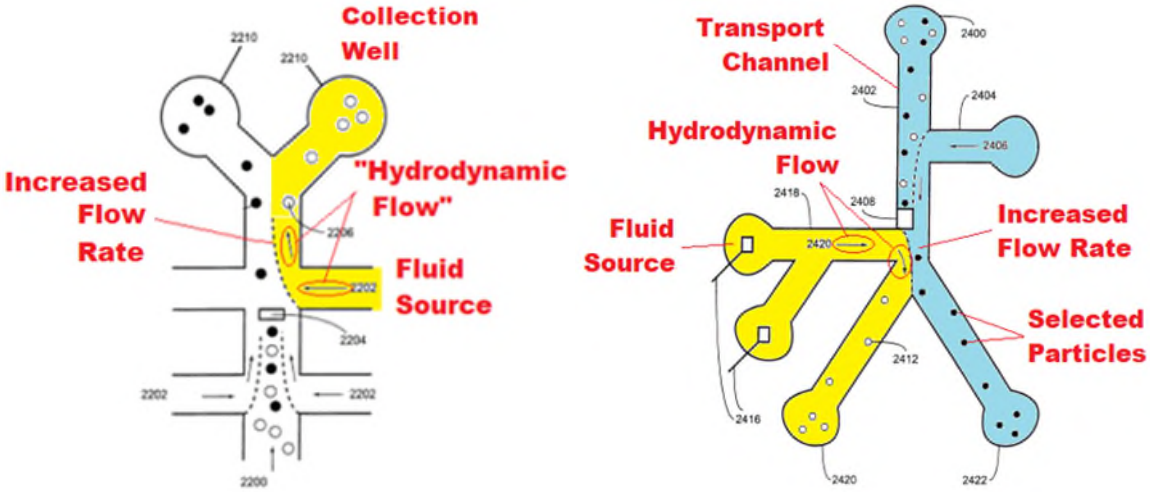


Fig. 17A Fig. 17B Fig. 17C

122. Wada's hydrodynamic flow is not a pressure pulse as described in the invention because it enters the transport channel from a fluid source and turns downstream with the particles before exiting the channel through a collection well, as shown below in the annotated versions of Wada's Figures 22 and 24:



123. Particle deflection (and thus sorting) in Wada is therefore not accomplished by a "pressure pulse" but by the introduction of flow that enters the channel, "nudges" the particles, and then exits the device with the particles instead

of being absorbed or dampened by a "first reservoir." Accordingly, I disagree with Dr. Weigl that any of Wada's components—which are depicted and described solely as delivering "hydrodynamic flow"—can be the claimed "reservoirs" used in delivering a pressure pulse that is to be dampened or absorbed by a reservoir. EX1002 ¶¶123–50.

124. I further disagree with Dr. Weigl's suggestion that Wada's disclosure of other "fluidic direction components" can be an actuator that delivers the claimed "pressure pulse." *See, e.g.*, EX1002 ¶¶93–94. For example, EX1006 at 4:1–5 is outside of Wada's sorting section and is directed at establishing flow through the "first microchannel," i.e., the main transport channel, not a secondary flow from the side channels. In any event, the entire sorting section of Wada concerns sorting by introducing hydrodynamic flow from fluid sources that travels downstream in the transport channel. A POSA would not find Wada's descriptions of "fluidic direction components" and the like as applicable to Wada's hydrodynamic flow-based sorting operation and certainly wouldn't provide a basis to change Wada from employing such an operation.

125. Similarly, I disagree with Dr. Weigl's opinion that Wada's mention of the use of a "pressure source" or "pressure based-flow, electrokinetic flow, or other mechanisms or combinations of mechanisms" can disclose an actuator that delivers a pressure pulse as claimed. *See, e.g.*, EX1002 ¶130. Wada there is explicitly

addressing means for establishing the main flow of cells from the "cell suspension source" and "forcing the cell suspension through the analysis channel." *See* EX1006 at 42:31–43:8. This description is contained within a section on "Assay Devices" and is unrelated to Wada's sorting operation. EX1006 at 37:3–45:2. In any event, these statements relate to the teaching of inducing "flow," as with Wada's description of the use of electrokinetics or Joule heating for its sorting embodiments. *Id.* So to the extent they are relevant, they would simply be employed as part of a flow-based operation, not a pressure pulse-based operation in which the pressure pulse is absorbed by a "first reservoir."

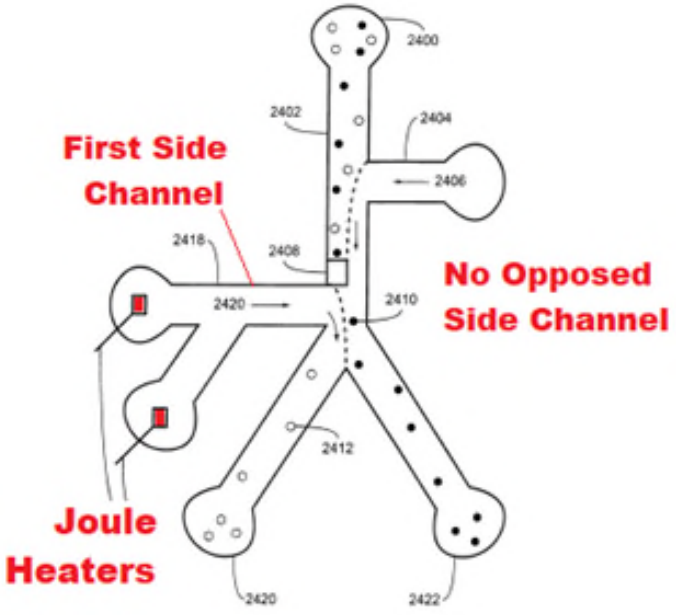
126. In summary, Wada fails to disclose the claimed "second reservoir" because Wada discloses no components configured or capable of "generating" or "propagating" a "pressure pulse" as required in the claims. Wada is also missing the claimed "first reservoir" for dampening or absorbing the pressure pulse for the additional reasons below.

3. Wada's Side Channels are Not "reservoir[s]" for "dampening or absorbing" a Pressure Pulse

127. Dr. Weigl suggests that the side channels in Wada are the claimed "reservoir[s]" that "dampen[] or absorb[]" a pressure pulse as required in claim 1 simply because they are aligned with one another. EX1002 ¶¶133–40. I disagree.

128. Wada's side channels do not act as reservoirs that dampen or absorb in any case. Figure 24 is particularly informative. It has a single side channel that

introduces fluid only to deflect particles, it does not ever draw or suck fluid (or pressure) from the transport channel. EX1006 at 22:23–24:18. In fact, there is *no* structure on the opposite side of the transport channel 2402 from the first side channel (i.e., fluid introduction channel 2418):



EX1006 at Fig. 24 (annotated).

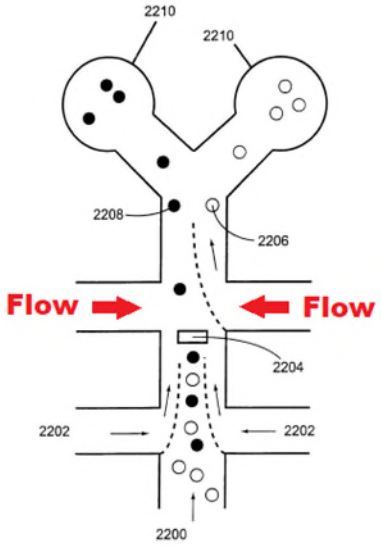
129. In my opinion, Figure 24 and its text would have discouraged a POSA from using a reservoir for dampening or absorbing any pressure or fluid flowing from sorting microchannel 2418 by teaching that the hydrodynamic flow introduced from that channel can be increased by using multiple Joule heaters and associated fluid wells. EX1006 at 23:1–3. By failing to employ an opposed side channel despite including a second Joule heater, Figure 24 suggests that an opposed side channel was not a pressure-pulse absorbing/dampening reservoir essential to the operation of the

Wada sorting device. This is completely consistent with Wada's teaching of the "hydrodynamic flow"-based sorting in the device.

130. Furthermore, as mentioned above, Wada's teaching of adding vacuum sources in the collection wells (EX1006 at 23:10-14) further would have discouraged a POSA from the use of any "reservoir" or other dampening/absorbing structure to take up pressure or fluid. The teaching of vacuums show that Wada has a clear goal of introducing the hydrodynamic fluid into the transport channel and have it flow downstream with the particles. Indeed, this confirms to a POSA that a fundamental part of the overall teaching of Wada is to establish and maintain a flow of liquid from the transport channel and side-channel wells *into the collection wells*. This teaching, by accentuating the flow with vacuum sources, would be understood by a POSA to apply equally to the embodiments of Figs. 22 and 23. Analogously, when using electroosmotic flow for introduction of flow from the side channels into the transport channel, the voltage is applied such that current flows between the side channel and the collection well. In other words, under no reasonable interpretation do the opposed side channels illustrated in Wada's Figures 22–24 absorb a pressure-pulse. Thus, they are not the claimed "first reservoir." The only purpose served by those opposing side channels is as an additional source of sorting fluid. And that is consistent with Wada's disclosure, which only ascribed them the purpose of inducing flow. EX1006 at 19:23–20:14; *see also id.* at 7:20–29, 8:6–24. This is entirely

distinct from the '797 Patent, which does not have any additional source of sorting fluid; only the sample and sheath fluids transit the transport channel.

131. Moreover, contrary to Dr. Weigl's assertions, a POSA would not "recognize that the non-actuated, opposed sorting microchannel structure in each of Figures 22 and 23 . . . includes a reservoir adapted for dampening or absorbing the transient pressure pulse propagated across the microfluidic channel." EX1002 ¶171. Regarding the "opposing microchannels" in those figures, a POSA would not understand Wada's description of them as suggesting they are useful for any purpose other than introduction of "hydrodynamic flow." For Figure 22, Wada says that both cross-channels are suitable for flow introduction, explaining that "[a] second set of opposing microchannels is typically located downstream from detector 2204 for introducing at least one hydrodynamic flow 2202 so as to direct selected cells 2208 . . . [into] collection wells 2210." EX1006 at 19:29–20:2. In other words, when two downstream opposed channels are employed, both are used to introduce flow:

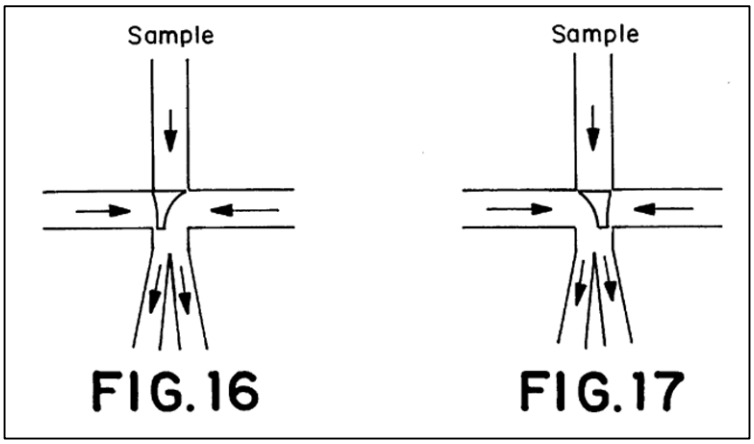


Id. at Fig. 22 (annotated). A POSA, following the teaching of Wada, in light of the reference to Ramsey, would have interpreted the opposing channel as a means of re-positioning the core stream on each side of the centerline by controlling the ratio of flow from each of the side channels. This is reinforced by Wada's teachings with respect to Figure 24 and its vacuum sources, which introduces fluid from only a single side channel that is pulled into the collection outlet. There is not even a structure that could serve as a "first reservoir." Thus, a POSA would not understand any of the side channels to serve such a purpose.

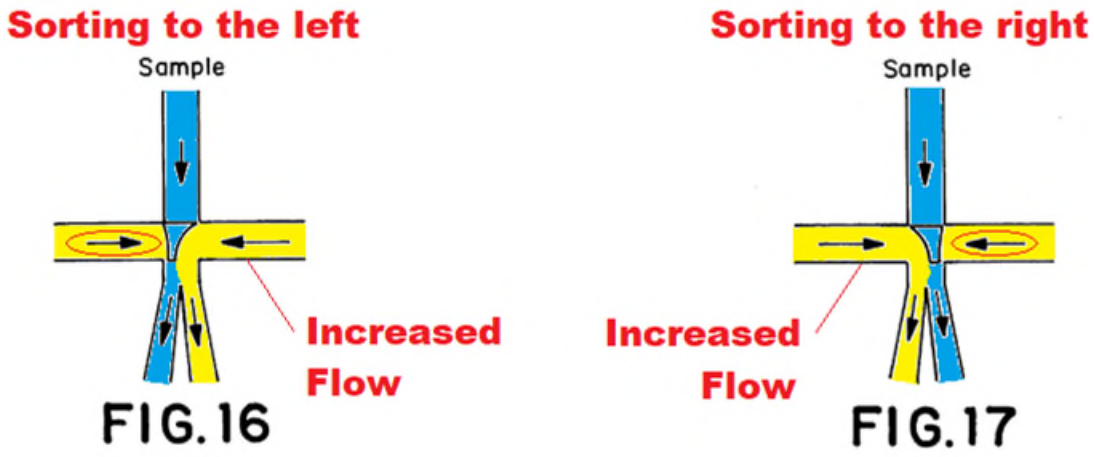
132. Wada teaches the same for Figure 23, describing "a set of opposing microchannels located downstream from the detector for introducing at least one hydrodynamic flow 2302 to direct selected cells 2308 . . . [into] collection wells 2312." *Id.* at 20:8–13.

133. In short, because Wada *introduces* fluid from its side channels, the features Dr. Weigl identifies as "first reservoir[s]" (i.e., the side channels) are not "configured to receive, 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)." EX1002 ¶135.

134. This understanding is consistent with Wada's incorporation of Ramsey to teach electrokinetic-based sorting, whose figures plainly show introduction of flow from each side (albeit in different relative amounts) to effect sorting:



EX2014 at Figs. 16, 17. Ramsey's sorting operation is depicted in the annotated version of Figures 16 and 17 from Ramsey:



135. Wada's cross-channels are only for the purpose of introducing an additional "hydrodynamic flow." EX1006 at 19:29–20:2, 20:8–13. In fact, Dr. Weigl's discussion and illustration of that usage (*see, e.g.*, EX1002 ¶¶133–150) is consistent with this. In short, the sole teaching of Wada is that the opposed microchannel is optional in nature and used only to introduce another "hydrodynamic flow" from that side. Again (as described above with respect to Figure 24 of Wada), by failing to employ an opposed side channel, this suggests that

an opposed side channel was not a pressure-pulse absorbing first reservoir essential to the operation of the device. This is completely consistent with Wada's (and Ramsey's) teaching of a flow-based sorting device.

136. Moreover, I disagree with Dr. Weigl that Wada's "well[s]" or "cavit[ies]" can be the claimed "reservoir." EX1002 ¶¶146. Wada's teachings of "chambers" do not relate to the claimed "reservoir." The chambers described in Wada relate to the "Assay Devices" described in Wada outside of the sorting descriptions. These chambers relate only to manufacturing microfluidic devices in general, for example by explaining that "channels and/or chambers of the microfluidic devices are typically fabricated into the upper surface of the bottom substrate." EX1006 at 39:19–33; *see also id.* at 37:29–32, 38:11–17. A POSA would not understand them to apply to modifications to Figures 22–24 or otherwise suggest using a reservoir that could dampen or absorb a pressure pulse as claimed.

4. A POSA Would Not Have Had Any Reason to Modify Wada to Employ a "pressure pulse" and "reservoir" for Dampening or Absorbing It

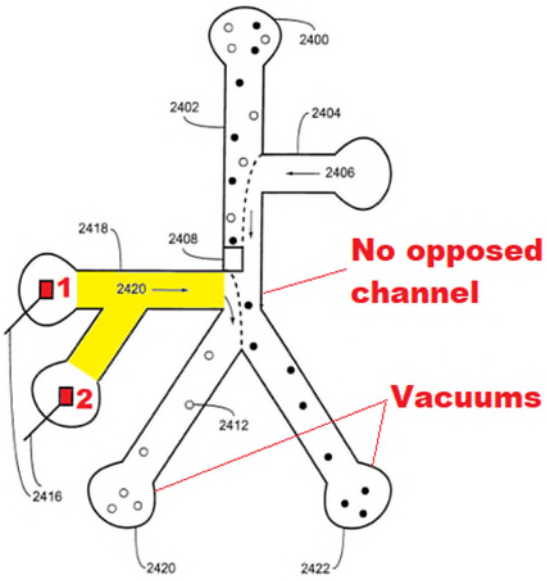
137. I disagree with Dr. Weigl that Wada "provides good reason for one of ordinary skill in the art to reasonably combine those teachings with a reasonable expectation of success of arriving at the claimed microfluidic device." EX1002 ¶108; *see also id.* ¶¶109, 112, 146, 148–50.

138. A POSA would not have had reason to modify Wada to achieve the invention because doing so would require changing Wada's principle of operation from hydrodynamic flow-based sorting (that adds fluid to the transport channel) to pressure pulse-based sorting (that is specifically designed not to add fluid to the channel). As explained above, the only sorting operation described in Wada is via the introduction of flow into the transport channel (from a source) to increase net flow so as to nudge particles against the opposite wall, whereby that flow travels downstream into a collection well. This flow-based operating principle was described extensively and in great detail in the disclosure. EX1006 at 17:10–24:12. This flow-based operation is a completely different sorting principle from shifting a set volume of fluid between an actuator reservoir and a dampening/absorbing "first reservoir"—specifically so as to not increase net flow (i.e., so as to not increase flow rate)—using a pressure pulse.

139. In fact, modifying Wada's sorting mechanisms to achieve the invention would require substantial reconstruction. In Wada's Figures 22–23, one of the side channels would need to be reconfigured to apply a pressure pulse from an actuator instead of flow from a fluid source, which would require a different mechanism (such as a sealed chamber) that is not a fluid source. The opposed side channel would also need to be redesigned from a structure that introduces flow, this time to a "first reservoir" designed so as to absorb that pressure pulse. Simply put, since the '797

Patent claims only add fluid introduced through the main transport channel (and none from the side channels), all of the additional fluid sources included in Wada's devices would need to be removed and replaced with an actuator and opposed absorption/dampening reservoir to provide and receive a pressure pulse across the fluid that is already in the transport channel and in contact with the actuator and opposed "first reservoir." As the patent explains, this requires specific resilient properties or geometry that are not described in Wada or depicted in its figures. *See* EX1001 at 12:18–32, Figs. 16–17C.

140. Additionally, Wada Figure 24, which is solely a Joule heating embodiment, would need to be completely reconstructed to add a "first reservoir" structure where no structure currently exists (i.e., Figure 24 discloses no opposed side channel, as annotated below):



Moreover, Wada's Figure 24's multi-channel heating structure would need be reconfigured to eliminate the vacuum sources and deliver a cross-channel pulse to that new "first reservoir" instead of delivering a heated fluid into and down the transport channel. Joule heating would not be understood to supply such a cross-channel pulse.

141. Finally, even if Wada's "hydrodynamic flow" could be understood to be a "pressure pulse" (which I believe is not the case), a POSA would nonetheless not have any reason to add a "first reservoir" to the opposed side channels. As explained above, the hydrodynamic flow is specifically designed to *not* reach an opposed reservoir because it is intended to flow downstream with the particles so as to adjust the position of the sample stream.

142. Finally, the passages from Wada cited by Dr. Weigl (EX1002 ¶¶121, 129–30) also do not provide any reason to modify Wada to achieve the claimed invention. In fact, several of them do not relate to particle sorting at all but instead main flow through a transport channel; and the ones that do address sorting address Wada's Joule heating and electrokinetic techniques that do not rely on increasing pressure in a chamber. For example, Wada at 3:7–4:5 refers to "methods of providing substantially uniform flow velocity to particles flowing in [the] first microchannel," and this "first microchannel" is the transport channel in Wada. Wada at 42:8 and 43:9–14 similarly relate to fluid flow through the main transport channel and are

completely outside of Wada's sorting disclosure. Furthermore, Wada at 11:8–9 specifically addresses Figure 24, which is a Joule heating embodiment that (as explained above) has nothing to do with "pressure-based flow."

5. A POSA Would Not Have Had Reason to Combine Wada and Anderson to Achieve the Claimed Invention

143. I disagree with Dr. Weigl that Anderson would have provided any reason or motivation to achieve the claimed invention. EX1002 ¶¶206–11. Specifically, I disagree that "[t]he skilled artisan thus would have had good reason to look to Anderson for further discussion regarding actuators for generating pressure pulses" because Anderson has nothing to do with generating pressure pulses, as discussed above in the section titled "Anderson (EX1012)."

144. First of all, Wada does not direct the reader to the Anderson reference for details on how to implement actuators for Wada's particle sorting chips just because Wada cites Anderson. Wada (EX1006) at 43:13–14, which Dr. Weigl cites to support this proposition (EX1002 ¶206), has nothing to do with particle sorting. It is in a different and entirely separate section from Wada's discussion of sorting, specifically as part of a larger/separate discussion of "Assay Devices." It relates only to establishing the main flow through the transport channel. Specifically, Wada at 42:31–43:9 states:

> For example, the cell suspension is optionally flowed by applying a pressure differential across the length of a channel. For example, a pressure source (positive or negative) is applied at the cell suspension

> reservoir at one end of a channel, and the applied pressure forces the suspension through the channel. The pressure source 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, for forcing the cell suspension through the analysis channel. Alternatively, a vacuum source is applied to a reservoir at the opposite end of the channel to draw the suspension through the channel pressure or vacuum sources are optionally supplied external to the device or system, e.g., external vacuum or pressure pumps sealably fitted to the inlet or outlet of a channel, or they are internal to the device, e.g., microfabricated pumps integrated into the device and operably linked to a channel. Examples of microfabricated pumps have been widely described in the art. See, e.g., published International Application No. WO 97/02357 [(Anderson)].

EX1006 at 42:31–43:13 (emphasis added). The phrases "across the length of the channel," "forc[ing] the suspension through the channel," through the analysis channel," and "draw[ing] the suspension through the channel" are clearly referring to flowing a sample of cells through the main transport channel. When Wada cites Anderson, it is providing an example of "microfabricated pumps" that could be used for flowing cells through the transport channel. This has nothing to do with sorting and would not give a POSA a reason to look to Anderson regarding Wada's sorting operations. More specifically, A POSA would not find this applicable to a sorting operation because Anderson's micropumps deal with establishing longitudinal flow through the main transport channel, not lateral pressure pulses. A POSA would in fact be discouraged from using such micropumps as "pulsating pumps" because the goal with these micropumps was to establish a smooth, constant flow. Moreover, Wada already provides extensive discussions on its preferred sorting techniques,

namely, Joule heating and electrokinetics—both of which avoid mechanical pumping as described here. For these reasons, a POSA would not consider this section applicable to Wada's sorting operation.

145. Additionally, a POSA would not have found Wada's description of pumping fluids through the main transport channel in this section (or its reference to Anderson) beneficial to accomplish a sorting operation in part because Wada already extensively teaches sorting techniques, specifically using Joule heating and electrokinetics. A POSA would not have seen any benefit of using Anderson's components for sorting over those disclosed in Wada at the time of the invention.

146. Moreover, Wada's citation of Anderson for pumping fluids through a main transport channel is consistent with Anderson's disclosure. As explained above, Anderson focuses mainly on pumping/transporting fluids and samples from chamber-to-chamber through a transport channel. It has nothing to do with using its micropumps for sorting. For the same reasons, I disagree with Dr. Weigl that, based on Anderson's disclosures, "a person of ordinary skill in the art would have recognized that Anderson further renders obvious . . . 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." EX1002 ¶209. The "micromachined pumps" and "bulging diaphragm" pumps in Anderson have nothing to do with particle sorting—they are meant to establish a smooth,

constant flow through the transport channel (consistent with Wada's citation thereof discussed above). Along those same lines, Anderson's pumps are configured to deliver flow through a transport channel. A POSA would not have thought to reconfigure such a pump to receive (much less dampen or absorb) a pressure pulse.

147. Finally, I disagree with Dr. Weigl's suggestion that Anderson's discussion of "a separation channel for separating a component of said fluid sample" has any relevance to '797 Patent claims. EX1002 ¶107. A POSA would understand that Anderson at 3:35–4:6, which explains 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 volt-age across said separation channel" explicitly refers to electrophoretic separation techniques described by Anderson at 13:11–13:34. As also described above regarding "'Electrokinetics' or 'Electrodynamics,'" these techniques are designed to separate a molecular mixture into it components. In any event, as described above, such electrokinetic techniques were understood by POSAs to have no relationship to pressure pulses generated by a pressure increase in a chamber, or particle sorting at all.

148. Along these lines, Anderson's disclosures of a "separation channel" that "may be used in conjunction with mixing elements described herein, to ensure maximal efficiency of operation" is irrelevant to the invention because Anderson is

again explicitly referring to electrophoretic separation. *See* EX1012 at 13:9–13 ("Electrophoretic methods may also be utilized in the purification of nucleic acids from other cell contaminants and debris."), 13:35–14:10 (referring to "field channels," which are channels that apply a voltage in electrokinetic techniques). In fact, Anderson clarifies here that the "separation" discussed is an "electrophoretic" technique. As discussed above, such a technique relies on charges inherent to the particles themselves, not flowing fluid. This is an entirely different concept from applying a pressure pulse (or even a hydrodynamic flow) to individually sort particles.

149. Finally, Anderson at 5:32–6:5, which Dr. Weigl (EX1002 ¶107) describes as "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" has nothing to do with sorting (or particle separation) at all. A POSA would recognize that Anderson is discussing a means of keeping reagents separated with a bubble (also known in the art as segmented flow) and then removing the bubble in a vented mixing chamber to allow the reagents to mix as part of an assay. This has nothing to do with sorting (or particle separation) at all.

150. Finally, I disagree with Dr. Weigl that "[i]t was thus conventional for a person of ordinary skill in the art to use various actuators, such as a heating element disposed within a pressure chamber formed in a substrate to induce

thermopneumatic pressure," a proposition for which he cites Anderson at 52:1–15 and 65:5–11 (regarding the thermopneumatic pump). The "thermopneumatic pump" described in Anderson is "a resistive heater disposed in a pressure chamber" that contains "a quantity of a controlled vapor pressure fluid." Any pressure increase in a chamber using this technique would result from expansion of that vapor pressure fluid, not the liquid in the chamber. Further, Anderson is describing the use of the thermopneumatic pump as a means of moving fluid from chamber-to-chamber, not as an "actuator" that increases pressure in a chamber to effect a switching operation in a particle-carrying transport channel. Moreover, Wada does not teach anything similar to a specialized vapor pressure fluid such as the one in Anderson that will cause a rapid pressure increase in a chamber when heated together with the buffer/gel fluid.

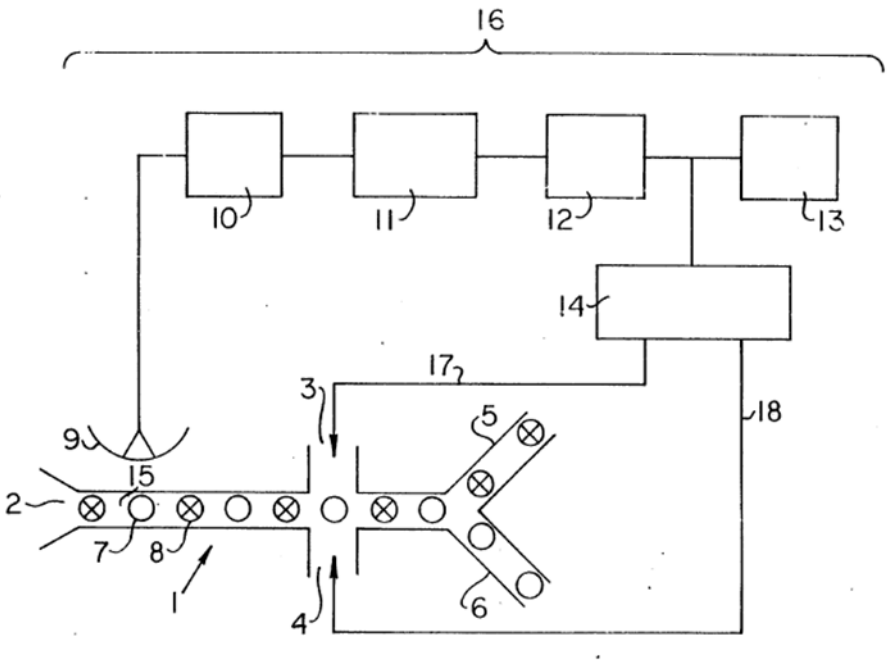
151. A POSA would not have replaced Wada's Joule heating or electrokinetic elements (such as those described in conjunction with Figures 22–24) with Anderson's "thermopneumatic pump" because doing so would require changing Wada's basic principle of operation. Wada's principle of operation is sorting by adding hydrodynamic flow, in the case of Figure 24 specifically by increasing the temperature of fluid in a fluid source. This operation does not result in a pressure increase within a chamber but rather only a reduction in viscosity, and

thus an increase in flow into and down the transport channel. The same is true for electrokinetic-based devices, which also do not rely on modulating pressure.

E. Marcus (EX1005)

152. Marcus's disclosure is very limited in substance, but one thing is made clear to a POSA—Marcus's sorting operation includes two active control "ports," which both receive a sort signal to sort particles. Under no circumstances could these activated ports be, nor are they suggested to be, an actuator and a cooperating passive "first reservoir" for receiving a pressure pulse as claimed. In other words, each control port is roughly analogous to an "actuator," but there is no absorption/dampening "first reservoir." Accordingly, I disagree with Dr. Weigl that Marcus would be configured (in view of Anderson) to disclose the claimed "pressure pulse" and "first reservoir." EX1002 ¶¶214–243.

153. More generally, Marcus discloses a "fluidic sorting device" comprising a "machine vision apparatus" for particle analysis and a "fluidic logic element" to sort particles based on that analysis—depicted respectively as items 16 and 1 in the patent's sole figure:



EX1005, Fig. 1; 3:28–4:3.

154. Most of Marcus's disclosure is aimed at describing the machine vision apparatus, which Marcus describes as follows:

> The machine vision apparatus 16 consists of a machine vision camera lens 9, an image capture device 10, an image interpretation device 11, and a decision control device 12. Said machine vision apparatus may further comprise an optional, conventional counter device 13. Connected to the decision control device 12 is a 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 at 3:38–48.

155. In short, the machine vision apparatus generates a signal according to a visually distinct characteristic detected by a camera and analyzed by an "image interpretation device." That device sends a signal to a "decision control device" and

"fluidic controller device" based on a detected particle characteristic. The fluidic controller device issues a sort signal to generic "control ports" 3 and 4, causing the particles to sort into branched outlets 5 and 6 using non-specific techniques.

156. Marcus provides very little information on its sorting apparatus or its mode of operation. In the Background of the Invention, Marcus describes them both as "conventional":

> The fluidic logic element comprises one input port, two control ports and two output ports. This fluidic logic element is, as stated above, conventional and may be produced using conventional micromachining technology or any other conventional means.

Id. at 2:15–31. Marcus further explains that the invention "makes use of two conventional components," namely "a fluidic sorting device and a machine vision apparatus." *Id.* at 2:7–13. And the sorting apparatus is further described as "commercially available." 3:9–12.

157. The text regarding the operation of the two "control ports" explains only that both ports are actuated to "separate out" particles by type:

> 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. at 3:57–62.

158. Finally, Marcus explains that "[c]onnected to the decision control device 12 is a 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." *Id.* at 3:43–48. In other words, two "leads 17 and 18" from Figure 1 transmit a sort decision signal that "instructs the control ports 3 and 4 as to how the particulate materials 7 and 8 should be sorted."

159. In sum, while generic "control ports" 3 and 4 are depicted as opposed channels intersecting the transport channel through which the particles flow, there is no description of actual side channels, much less any additional components connected to such channels. And the limited disclosure on Marcus's sorting operation indicates that both control ports are actuated to effect sorting. Neither is described as configured or capable of receiving fluid or a pressure pulse.

1. Neither Marcus nor Anderson Teaches Using a "pressure pulse"

160. I disagree with Dr. Weigl that Marcus and Anderson could be combined to disclose the claimed invention because *both* references fail to disclose the claimed "pressure pulse" and "first reservoir" for "dampening or absorbing" the pressure pulse. EX1002 ¶¶227–43.

161. Neither Marcus nor Anderson discloses a "pressure pulse" to sort particles. As explained above, Marcus gives very little detail on its sorting apparatus, explaining essentially that it is comprised of "conventional and commercially

available components" and uses controller 14 to instruct "control ports 4 and 3, respectively" on how to sort particles. EX1005 at 3:9–12, 3:57–62, Fig. 1.

162. Anderson also does not teach or suggest "propagating" a pressure pulse across a channel with a "second reservoir" because (as explained above) Anderson's "actuators and pumps" that Dr. Weigl relies on (at EX1002 ¶¶232, 237–43) are not related to delivering a cross-channel pressure pulse for sorting/deflecting a particle. Specifically, the "actuators and pumps" are "micropumps" that are used in conjunction with an array of separate gating microvalves for "directing, controlling and manipulating fluid" (EX1012; 5:11–13) samples through a sample processing manifold with "storage chambers, incubation chambers, mixing chambers and the like" (*id.* at 33:6–7). Consistent with this, Wada refers to Anderson's pumps as examples of relatively constant pressure pumps that might be suitable for delivery of fluid through the transport channel (EX1006 at 42:31–43:14), not as pumps appropriate for delivering "pressure pulses."

163. Accordingly, the so-called actuators and pumps have nothing to do with sorting, and individual particles are never selected or deflected in Anderson using a "second reservoir" that generates a "pressure pulse." Thus a POSA would not think to use it in Marcus's generic sorting operation.

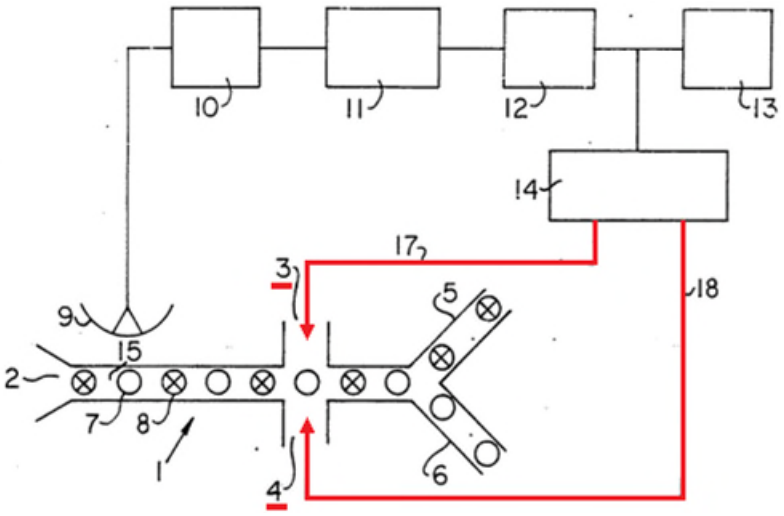
164. In fact, in my opinion, Anderson would discourage a POSA from using its pumps to employ a "pressure pulse" because its goal was to provide a smooth,

constant flow between chambers and because of the close incorporation with additional valves for operation. In other words, a POSA would have been discouraged from using Anderson's pumps to intentionally "pulsate."

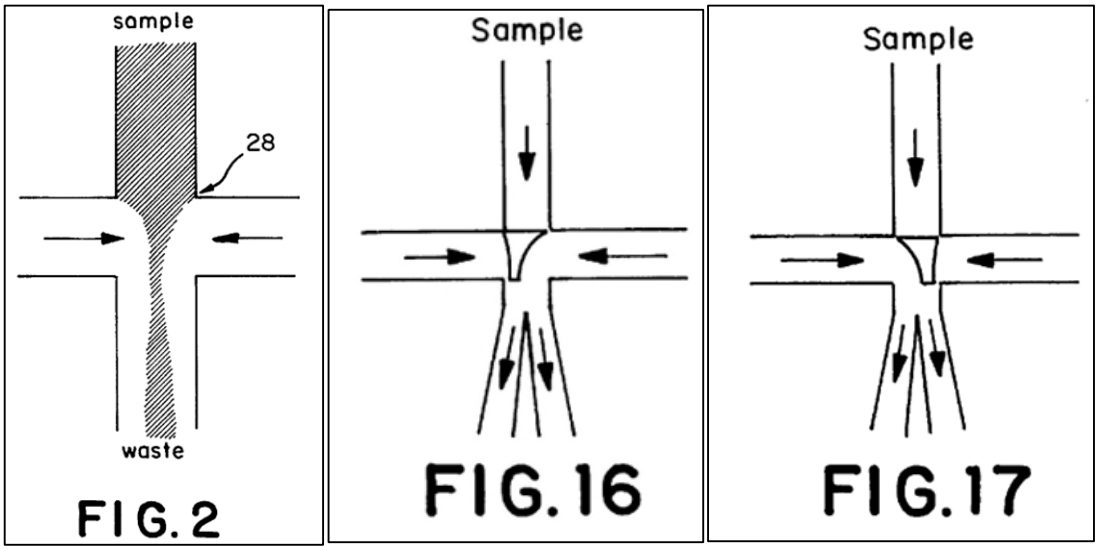
2. Neither Marcus nor Anderson Teaches a "first reservoir" that Dampens or Absorbs a "pressure pulse"

165. Contrary to Dr. Weigl's opinion, neither Marcus's Figure 1, nor any text in Marcus, teaches "dampening or absorbing the pressure pulse using a first reservoir." EX1002 ¶¶237–43. Marcus would in fact discourage a POSA from modifying it to achieve the claimed invention because Marcus instead teaches that both control ports 3 and 4 are actuated by fluidic controller device 14. Thus, neither would be viewed as passive "first reservoirs" for dampening or absorbing a pressure pulse, and it would be understood that modifying it would disrupt the only part of Marcus's operation actually disclosed (namely that two control ports are actuated).

166. As Marcus explains, particle characteristics are "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." EX1005 at 3:57–61. This actuation of both control ports 3 and 4 is illustrated in Figure 1 by the actuating signals represented by arrows (representing two "leads"):



EX1005 at Fig. 1 (annotated). From this, a POSA would understand, for example, that Marcus is employing a sorting apparatus and technique similar to that of Ramsey's electrodynamic focusing technique (depicted below), wherein both cross-channels are supplied with fluid induced from both sides by electrokinetic energy. *See* EX1014 at 6:16–29, 7:24–51, Figs. 2, 15–17.



Such techniques were also employed by Wada and fail to disclose or render obvious the claimed reservoir/pressure pulse mechanism for generating a pressure pulse, propagating it across the transport channel, and then dampening or absorbing it.

167. Dr. Weigl incorrectly suggests that the "opposed microchannel structure" in Marcus would be the claimed "first reservoir" simply because it is "directly across" from "the first microfluidic flow channel." EX1002 ¶¶239–40. As explained above for Wada, the mere fact that two control ports are "aligned" with one another does not mean one of them "dampen[s] or absorb[s]" fluid or pressure. As discussed, this argument is nonsensical because Marcus expressly describes both control ports being actuated. A POSA would understand, as taught in more detailed references such as Ramsey and Wada, that even if "not actuated," the control port would be flowing some amount of fluid into the channel (albeit less than the opposed side so as to effect sorting). As another option, a POSA could valve off the so-called "non-actuated" control port 3 when control port 4 is being actuated (and vice-versa). A valved-off channel stub (to the extent Marcus's generic disclosure is illustrating side channels at all) would not be understood to be the dampening/absorbing "first reservoir" of the '797 Patent. At the very least, the control port (if a side channel at all) would be a side channel filled with fluid, which again (on its own) does not have the characteristics such that it would be understood to be a "first reservoir" as claimed. In short, neither control port would be understood to be capable of or

configured to receive (much less dampen or absorb) fluid or a pressure pulse under any understanding of Marcus's operation.

3. A POSA Had No Reason to Combine Marcus and Anderson or Otherwise Modify Marcus to Achieve the Claimed Invention

168. Contrary to Dr. Weigl's opinion (EX1002 ¶¶214–18, 236), a POSA would have had no reason to incorporate components from the prior art and arrange them as claimed based on a reading of Marcus and Anderson. Marcus's "control ports" 3 and 4 are not described as having any particular structure or any particular function, other than they generically "sort." EX1005 at 3:57–61. Marcus's control ports, for example, are not described as cooperating with one another other in any way to accomplish such sorting or to ensure that a particle ends up in the correct outlet. Specifically with respect to the challenged claims, Marcus's control ports are not described as "generating" or "propagating" and then "dampening or absorbing" a "pressure pulse" in sequential fashion—so as to apply a pressure pulse to a particle to sort it—as the claimed reservoirs are required to do.

169. Because Marcus does not steer a POSA in that direction, a POSA would not have looked to other prior art such as Anderson for the necessary "second reservoir . . . adapted for generating the pressure pulse" and the "first reservoir for dampening absorbing the pressure pulse propagated across the flow channel." In other words, Marcus provides no reason for a POSA to seek a cooperating "second reservoir" and passive "first reservoir" to replace the generic "control ports" 3 and

4. Marcus's dual-actuating apparatus would actually discourage a POSA from doing so.

170. Anderson also fails to provide any reason to reconfigure Marcus because, with respect to the actuators and pumps that Dr. Weigl cites, Anderson is solely geared toward sample transportation, not sorting or using a reservoir to "propagate[] a pressure pulse" across a channel or to dampen or absorb such a pressure pulse.

171. In short, Marcus's referring to "sorting" would not be sufficient to motivate or give reason to a POSA to achieve the claimed invention. In particular, there would have been no reason to incorporate the claimed cooperating reservoirs and pressure pulse. Marcus describes neither generating a pressure pulse to propagate it across a channel nor dampening or absorbing the pressure pulse in a "first reservoir." In fact, upon reviewing Marcus's generic sorting apparatus, a POSA (without knowledge of the '797 Patent and its claims) would have most likely looked to the teachings of Ramsey (and perhaps Wada) for identifying suitable mechanisms for Marcus's "control ports." In fact, "electrokinetic injection" of fluid from cross-channels—as taught in Ramsey and Wada—was a conventional sorting mechanism at the time, which is what Marcus suggests to seek out. *See, e.g.,* EX1018 at 340–41 (Deshpande referring to electrokinetic injection from cross-channels as "classic" and "the simplest switching component"). Moreover, Wada and Ramsey (with their

opposed side channels) are configured very similarly to the schematic of Marcus Figure 1 (with its opposed control ports), and Wada and Ramsey teach sorting by the introduction of hydrodynamic flow from both side channels. As explained above, Wada and Ramsey's techniques entail neither the production of a "pressure pulse," nor a device for "propagating" the pressure pulse across the transport channel, nor a "first reservoir for dampening or absorbing a pressure pulse propagated across the flow channel." Additionally, as explained above, reconfiguring such systems to operate as claimed would require a change in the "principle of operation" and substantial redesign, which negates any reason to combine that may otherwise exists.

IX. OTHER BACKGROUND ART

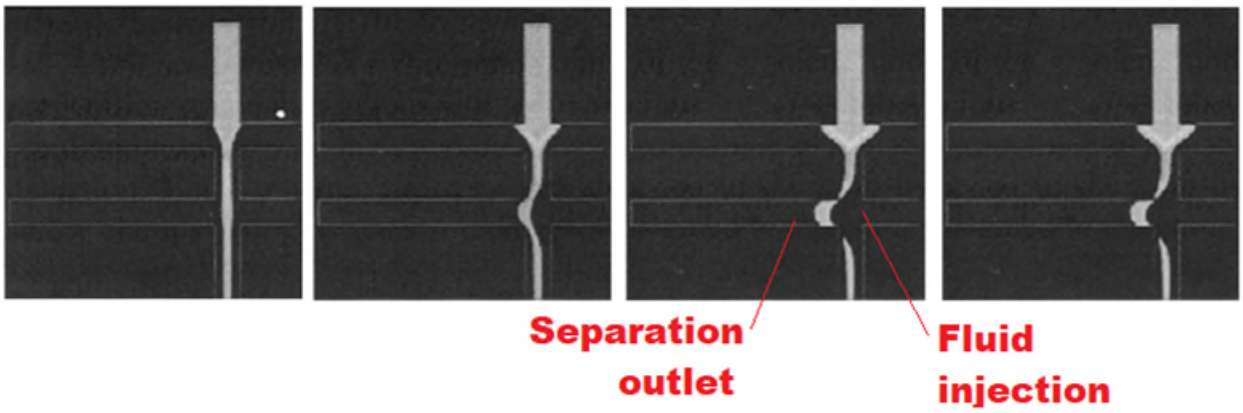
A. Flow-Based Sorting Devices

172. Dr. Weigl cites a number of references to support his assertion that "[a]nother known mechanism for sorting particles involved increasing fluid flow across a flow channel, in effect generating a mechanical pressure pulse" that was a "cross-channel pulse." *See* EX1002 ¶¶75–80. I disagree with Dr. Weigl's assertion because none of the cited references generate a "mechanical pressure pulse" across a channel, much less one that is sent across a channel to deflect a particle and be received and dampened or absorbed by a "first reservoir."

1. Deshpande (EX1018)

173. Deshpande sought to improve electrokinetic-based sorting by using computer-aided design tools that incorporate electrokinetic physics principles.

EX1018 at 339–40 (explaining that the paper is "specifically focusing on one component—the electrokinetic injector"). The device in Deshpande operates by using "electrokinetic injection" from one side channel to "inject" a "plug" of sample into a "separation channel" (an outlet), by "switching" between load and inject states as demonstrated below:



EX1018 at Fig. 5 (annotated), 341–43. As an initial matter, such electrokinetic devices use electrodes at the ends of channels to create a voltage potential to move fluid; thus, they do not have a mechanical "actuator," do not increase pressure in the system, and do not employ a "pressure pulse." In any event, Deshpande discloses nothing resembling the claimed actuation/absorption sequence because, in Deshpande, voltage is *simultaneously* applied across both the injection and separation channels in one direction to push the plug of sample into the outlet. Deshpande also does not disclose anything resembling a "first reservoir" because the opposed separation channel *receives the switched sample volume* (not any alleged pressure pulse).

174. Desphande's flow-based operating principle is the same as described in Wada/Ramsey. Fluid is introduced from a cross-channel into a transport channel to focus the sample and nudge the sample into a desired outlet, and that fluid flows downstream with the non-selected portion of the sample. The main difference is that Desphande uses a second cross-channel as the sample outlet, while Ramsey/Wada use a second cross-channel as an additional sorting channel fluid inlet (with outlets located downstream). This difference only further emphasizes—much like Figure 24 of Wada—that there was no need for cross-channel "reservoirs" in flow-based systems.

175. The motive force for electroosmotic flow as taught in Deshpande (EX1018) does not involve pressure when moving liquid through open microchannel networks. Specifically, there is no pressure gradient applied across the channel and there is no pressure generation or pressure variation in the channels, as long as an external pressure is not applied. The fluid is being transported by ion motion along the entire path of the microchannel given typical situations where the channel has a uniform surface. From a practical standpoint, the motion of the fluid along field lines allows for precise directional flow through microfluidic channel networks. For example, fluid flow can be directed around corners of intersections by application of voltages between a specific inlet / outlet pair of a complex microchannel network.

2. Dubrow (EX1032)

176. Similar to Deshpande, Dubrow discloses an "electrokinetic material transport systems" for "transporting and directing materials within an interconnected channel and/or chamber containing structure, through the application of electrical fields to the materials, thereby causing material movement through and among the channels and/or chambers . . . ." EX1032 at 6:11–18. And as in Deshpande, a "pinched volume" of carrier fluid flowing in the transport channel is "injected into the vertical channel by applying a voltage gradient across the length of the vertical channel," i.e., it is switched into a cross-channel from the main flow channel using a voltage differential applied across both side channels simultaneously. *Id.* at 8:3–6. For the same reasons as Deshpande, there is no "pressure pulse," no "first reservoir operatively associated with the flow channel and adapted for dampening or absorbing a pressure pulse propagated across the flow channel," and no "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."

177. The motive force for electroosmotic flow as taught in Dubrow (EX1032) does not involve pressure when moving liquid through open microchannel networks. Specifically, there is no pressure gradient applied across the channel and there is no pressure generation or pressure variation in the channels, as long as an external pressure is not applied. The fluid is being transported by ion motion along

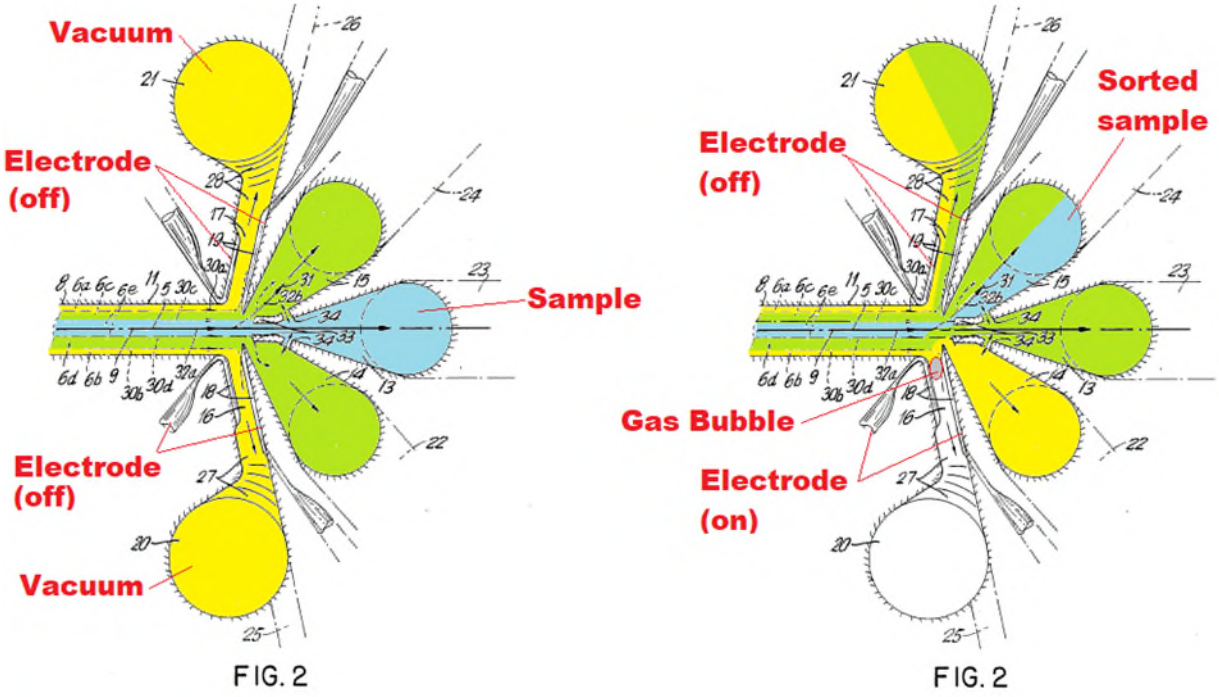
the entire path of the microchannel given typical situations where the channel has a uniform surface. From a practical standpoint, the motion of the fluid along field lines allows for precise directional flow through microfluidic channel networks. For example, fluid flow can be directed around corners of intersections by application of voltages between a specific inlet / outlet pair of a complex microchannel network.

3. Zold (EX1007)

178. Zold (EX1007) is another flow-based device. Zold operates very similarly to Wada, introducing an additional fluid flow through the microfluidic channel network that re-positions the core stream such that is a selected particle is directed to a specific outlet.

179. As illustrated below, Zold sorts a sample using electrodes (18, 19), embedded in the walls of the side channels (16, 17) leading out of the device, that electrolytically convert the fluid from a liquid to gas, thereby creating an expanding, electrolytically-generated gas volume. Zold points out that ideally "the generated gas fills up the volume being left empty by the electrolyte at the same rate the electrolyte flows out" in this case occluding one of two selected exit channels with an *in situ* generated gas flow. EX1007 at 18:67-68. With the fluid flow from the transport channel into the gas-generating channel decreased, or "paralyzed" according to Zold (i.e., replaced by the electrolytically generated gas), the flow

streams in the transport channel re-position and direct the particle stream to the opposing side of the transport channel.



EX1007 at Fig. 2 (annotated), 18:47–54. When sorting (above right), the gas bubble at least partially blocks fluid flowing into the bottom side channel, which normally draws fluid from the transport channel using a vacuum attached to the "buffer cavity" (20 and 21). *Id.* at 19:5–12. The gas bubble therefore increases flow out of one side channel, causing the sample stream to shift in the opposite direction. *Id.* at 19:21–52. After the sorting operation, the gas bubbles are drawn down into the corresponding buffer via the vacuum. *Id.* 17:17–20. There is no physical pressure-actuating structure and thus no "actuator" as claimed.

180. Even if the electrodes could be considered an "actuator," neither the electrodes themselves nor the gas bubble they create applies a pressure pulse to the selected cells. The bubble generation feature is designed to block the flow of carrier fluid into the side channel without having the bubble enter the transport channel. In other words, ideally, the gas bubble causes the pressure differential at the interface of the side channel and the transport channel to be zero—what Zold refers to as "paralysis." That is not a "pressure pulse."

181. Finally, the side channels and associated "buffer cavities" cannot be the claimed "first reservoir for dampening or absorbing a pressure pulse" because 1) they operate independently of one another, 2) they are positioned in an entirely different location relative to the transport channel and the gas-generating electrodes and 3) they serve an entirely different function. Whereas the '797 Patent's claimed "first reservoir" serves to dampen or absorb a "pressure pulse" generated from the opposed side of the transport channel, the "buffering" cavities referred to in Zold are located away from the transport channel, between the gas-generating electrodes and the vacuum and serve to retain and eliminate gas bubbles and processed fluid on their respective sides so as to dampen fluctuations in the applied vacuum, as generated by the sorting operation, and to assure that the processed sample is not sucked into the vacuum pump. EX1007 at 10:20–44.

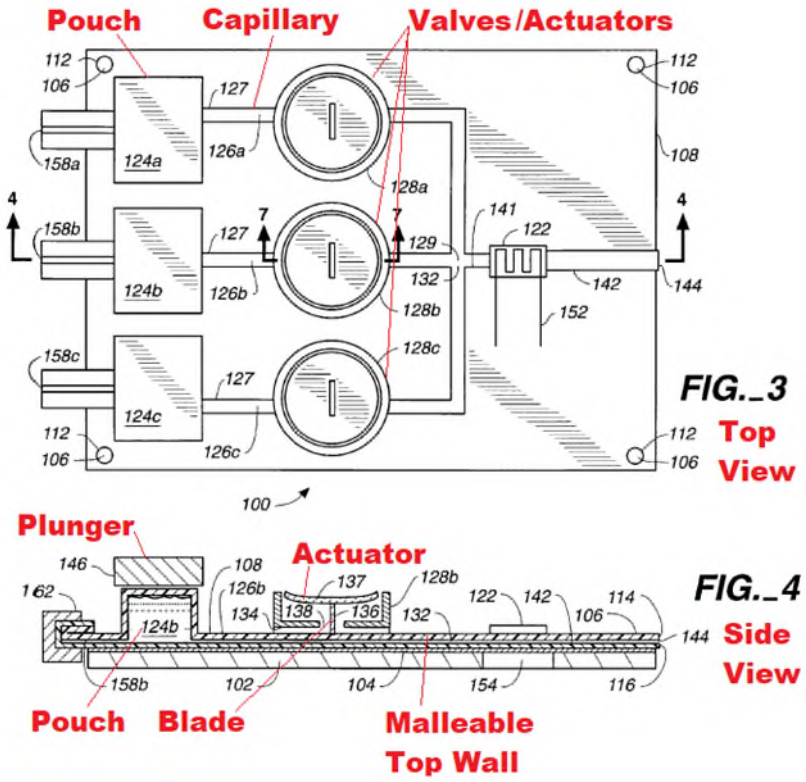
B. Fluid Transportation Devices

182. Dr. Weigl cites several other references to support his opinion that "an actuator is used to propagate a pressure pulse across a microfluidic flow channel was well-known and well reported in the art," suggesting that a POSA would employ such techniques to apply a pressure pulse in a sorting operation. *See* EX1002 ¶¶131–32. I disagree with Dr. Weigl because none of the cited references generate a "pressure pulse" as claimed, particularly not one that is sent across a channel to deflect a particle and be received/absorbed by a "first reservoir." The cited references (instead of delivering a "pressure pulse") are aimed at establishing and / or regulating the main flow through a transport channel, which has nothing to do with sorting or cross-channel pressure pulse generation at all, much less in the way claimed. A POSA would therefore have no reason to employ the teachings of the references in this section to effect particle sorting.

1. Neukermans (EX1020)

183. Neukermans (EX1020) "relates generally to regulating delivery of minute quantities of liquids, and more specifically to microfluidic systems particularly for analytical instruments such as those used for DNA or peptide sequencing and medical or clinical diagnostics." EX1020 at 1:13–17. As an initial matter, Neukermans does not involve particle sorting at all. Instead, Neukermans's devices deliver liquid from a "pouch" into a capillary tube and employ an actuated

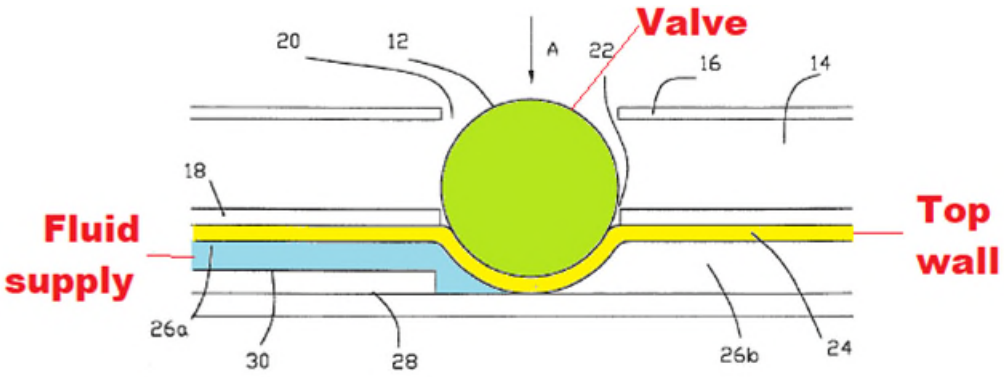
valve above the tube that causes a "blade" to "press firmly" against a malleable wall of the tube to reduce flow therein. EX1020 at 2:60–3:57. As depicted in Figures 3 and 4, these devices can be used in parallel to mix and supply fluids to another instrument:



The excerpts cited by Dr. Weigl merely describe this flow delivery mechanism. *See* EX1002 ¶¶66–67, 131, 235. He fails to explain how or why any it would be incorporated into another device to apply a "pressure pulse" to deflect particles.

2. Williams (EX1023)

184. Like Neukermans, Williams (EX1023) has nothing to do with particle sorting or applying a "pressure pulse" and instead relates only to devices that supply and regulate fluid flow. *See* EX1002 ¶¶72–73, 131, 235. One such device is below:



EX1023 at Fig. 2. The valve in Williams is completely different from the claimed actuator that applies a pressure pulse to deflect a particle. In fact, Williams explains that its valve can be operated "manually using the finger of a human operator," which a POSA would never understand as applicable to particle sorting applications. EX1023 ¶ 0026.

3. Zeng (EX1022)

185. Zeng's (EX1022) "EOF pumps" are "field-induced pumps" that use electroosmotic flow to deliver fluids. Zeng discusses the physics of electroosmotic flow and the relationships between EOF pump characteristics (materials, dimensions, etc) and performance (flow rates, pressures, pumping efficiency, etc.). When operated with DC voltages, EOF pumps do not pulsate. Contrary to Dr. Weigl's suggestion by repeatedly citing to Zeng (EX1002 ¶¶69–71, 131, 235), a POSA would not think to reconfigure Zeng's pumps to pulsate for use in microfluidic particle cytometry and sorting systems because, delivering a smooth/constant flow is desirable for transporting fluids. Moreover, Zeng

discourages POSAs from employing pulsatile mechanical micropumps in microfluidic systems because of their inferiority to electroosmotic (aka "field-induced") pumps: "A clear advantage of field-induced pumps over membrane pumps is that they do not require moving parts, such as check valves, which complicate the fabrication, sealing, and operation of these systems." EX1022 at 107. Indeed, the rest of the article describes and touts strategies for employing electroosmotic flow as smooth, non-pulsing fluid delivery devices. *Id.* at 108–113.

C. Compliance Devices

186. Dr. Weigl further cites a number of references to support his opinion that "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" that was a "cross-channel pulse," to suggest that reservoirs for dampening or absorbing a pressure pulse were well known in the art and would be employed in a particle sorting device as claimed. *See* EX1002 ¶¶81–83, 88–90, 137–38, 241–42. I disagree with Dr. Weigl's assertion because the devices he refers to had to do with controlling problematic vibrations in a main transport channel caused by micropumps that establish variable main flow through the channel. A POSA would not have had reason to employ such components in a sorting operation as claimed.

1. Zengerle (EX1033)

187. Dr. Weigl cites a single page from Zengerle (EX1033 at 198) explaining that in "many applications, the oscillation of pressure and flow in fluid channels has to be suppressed," specifically using "pressure smoothing elements." EX1002 ¶¶81–83, 137–38, 241–42. There, Zengerle is referring to "smoothing" of oscillations associated with pumps providing flow down the length of the transport channel—a common problem with micropumps. EX1033 at 198–99, Fig. 9. Zengerle does not address cross-channel pressure pulses or particle deflection/particle sorting. *Id.*

2. Veenstra (EX1035)

188. Veenstra (EX1035) is no different from Zengerle. *See* EX1002 ¶¶89–90, 138, 242. It addresses only pressure variations in the main flow channel caused by membrane pumps, which produce a "sinusoidal flow" in the channel and reduce the overall throughput. EX1035 at 377. This flow instability would also reduce precision in timing for sorting particles. So-called "compliance chambers," partially filled with air, are fluidically connected at the side of the flow channel to absorb those pressure variations. EX1035 at 377–79, Figs. 2, 3. Veenstra is not directed to cross-channel pulses, particle deflection, or sorting.

3. Chou (EX1034)

189. With respect to pressure smoothing elements and compliance devices, Chou (EX1034) only addresses a "damper" for dampening pressure variations down the main flow channel. EX1034 at 4:5–7. Chou states that its "damper" is a "vent"

or “thin elastic membrane” that “eliminates the fluid oscillation within the fluid channel” caused by pumping the fluid into and through a transport channel. *Id.* at 39:25–33. When used in “sorting applications” (*id.* 43:8–48:19), Chou’s damper is “located . . . before a detector” and other sorting elements (*id.* at 45:15-24; Figure 30A), i.e., it is not involved in particle deflection. Sorting in Chou is accomplished simply by shutting off one of two downstream valves situated between collection and waste receptacles. *Id*.

D. Miscellaneous References

190. Finally, Dr. Weigl cites a couple of other references to suggest that the ’797 Patent simply employs “conventional components” that pre-date the invention. *See, e.g.*, EX1002 ¶59; *see also id.* ¶¶52–59, 74–75, 86, 131, 137–38, 235, 241–42, 264. As explained, below, however, the references he cites have nothing to do with the particle sorting operation of the ’797 Patent, in particular one that employs a “pressure pulse” and a “first reservoir” to absorb it. The references below cited by Weigl employ neither.

1. Chapman (EX1014)

191. Dr. Weigl relies on Chapman (EX1014) to incorrectly contend that a mechanism “wherein an actuator is used to propagate a pressure pulse across a microfluidic flow channel was well-known and well reported in the art.” EX1002 ¶131. The only support Dr. Weigl provides for this contention is that Chapman

mentions "divert[ing] the fluidic stream into the sort collection vessel at the time a particle that fulfilled the sort criteria is detected." *Id.* (quoting Chapman). Chapman is describing two specific commercial sorters—the FACSCalibur and PAS-III—that are unrelated to the claimed invention. EX1014 at 5–6, 9. The FACSCalibur does not employ an actuator to apply a pressure pulse to particles because, as Dr. Weigl acknowledges, the FACSCalibur did not move particles at all—it moved a tube to-and-from the center of the channel to "catch" selected particles. *Id.*, EX1002 ¶74. And the PAS-III was a flow-based sorter that, similar to Zold, closed one outlet with a mechanical valve to create additional flow in the transport channel to shift the sample toward the desired outlet. Such flow-based systems do not employ a "pressure pulse" or a "first reservoir" for absorbing it.

2. Glaettli (EX1016)

192. Glaettli (EX1016) is an ancient sorting device from the 1970s that uses pulleys, a piston, and stepper motor attached to an extraction tube to draw a relatively large volume of fluid containing particles from a channel. A sorting device like Glaettli would not have been considered relevant at the time of the invention. Glaettli itself describes running the extracted sample back through the device "a second time" due to the unintentional extraction of "non-desired cells." EX1016 at 5:39–43. This would have discouraged a POSA from incorporating anything about Glaettli's design in microfluidic sorting devices at the time of the invention.

193. Also, the problem Glaettli was addressing was "inhibiting cavitation." *Id.* at 4:72–5:16. "Cavitation" refers to the generation of bubbles when liquid is accelerated sufficiently quickly to generate low-pressure regions that induce conversion of the liquid to a gas. *Id.* Glaettli is susceptible to cavitation at the junction when the large volume of fluid is rapidly sucked from the transport channel by the piston extraction. *Id*. Cavitation has nothing to do with handling "pressure pulses," and indeed was not relevant to microfluidic particle sorting devices at all by the time of the invention.

X. CONCLUSION

194. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further 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.

Date: 6/3/2020.

By: _____
Don W. Arnold, Ph.D.

APPENDIX—LIST OF MATERIALS

Exhibit No.	Description
1001	U.S. Patent No. 9,011,797 to Gilbert, *et al.*
1002	Declaration of Bernhard H. Weigl
1003	*Curriculum Vitae* of Bernhard H. Weigl, Ph.D.
1004	Prosecution History of U.S. Patent No. 9,011,797 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)
1012	International PCT Patent Publication No. WO 97/02357 to Anderson, *et al.* (published January 23, 1997)
1014	Chapman, *Instrumentation for flow Cytometry*, J. IMMUNOL. METHODS 243 (2000) 3-12
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
1020	U.S. Patent No. 6,068,751 to Neukermans (issued May 30, 2000)
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)
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

1042	Plaintiff's Reply Claim Construction Brief, *Cytonome/ST, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 1:19-00301 (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. 1:19-00301 (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)
1045	Plaintiff's Opening Claim Construction Brief, *Cytonome/ST, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 1:19-00301 (RGA) (D. Del.)
2002	*Curriculum Vitae* of Dr. Arnold
2012	*Markman* Order, *Cytonome/ST, LLC v. NanoCellect Biomedical, Inc.*, C.A. No. 1:19-00301 (RGA) (filed May 26, 2020)
2014	U.S. Patent No. 5,858,187 to Ramsey et al. ("Ramsey") (filed September 26, 1996)
Paper No. 2	NanoCellect Biomedical Inc.'s Petition for *Inter Partes* Review