UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TRANS OVA GENETICS, LC
Petitioner
v.
XY, LLC
Patent Owner

U.S. Patent No. 7,723,116
Issued: May 25, 2010
Filed: May 25, 2006
Inventors: Kenneth Evans *et al.*

Title: Apparatus, methods and processes for sorting particles and for providing sex-sorted animal sperm

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Case IPR2018-00247

PETITION FOR *INTER PARTES* REVIEW

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1002	Declaration of Jonathan H. Hartnett
1003	Declaration of J. Paul Robinson, Ph.D
1004	*Curriculum vitae* of J. Paul Robinson, Ph.D
1005	WO 01/85913, published November 15, 2001, to Evans et al. ("Evans")
1006	Tardif et al., "Use of Hoechst 33342 Stain to Evaluate Live Fresh and Frozen Bull Sperm by Computer-Assisted Analysis," *J. Androl.*, 19:201-206 (1998) ("Tardif")
1007	U.S. Patent No. 5,135,759, issued August 4, 1992, to Johnson ("Johnson")
1008	U.S. Patent No. 5,504,337, issued April 2, 1996, to Lakowicz ("Lakowicz")
1009	WO 92/08120, published May 14, 1992, to Piper et al. ("Piper")
1010	U.S. Patent No. 5,173,740, issued December 22, 1992, to Fukuda ("Fukuda")
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1012	U.S. Patent No. 5,660,997, issued August 26, 1997, to Spaulding ("Spaulding")
1013	U.S. Patent No. 5,985,216, issued November 16, 1999, to Rens ("Rens")
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1022	December 2, 2009 Amendment & Response to Office Action
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I. INTRODUCTION

Trans Ova Genetics, LC ("Petitioner") requests IPR and cancellation of claims 1-26, 33-34, and 38-41 of U.S. Patent No. 7,723,116 ("the '116 patent").

The challenged claims are drawn to a method of flow-sorting sperm cells comprising staining sperm cells with a dye, flowing them past a radiation source that causes the dye to excite and emit fluorescence, measuring the resulting signal, and using the difference in signal to discriminate between the X- and Y-bearing sperm cells (because X-bearing cells contain more DNA, they give off more signal).

This general method had been known for many years and was detailed in Evans more than a year before the '116 patent's earliest priority date. Unlike Evans' method, however, which uses a continuous wave ("CW") radiation source to irradiate the stained sperm, the '116 patent discloses and claims the use of a pulsed radiation source. But the use of pulsed radiation sources in flow cytometry, while not expressly disclosed in Evans, also had been known for years.

The patent claims issued nonetheless, in part because the Patent Owner never had to address the most pertinent prior art, like Evans. This was almost certainly due to the fact that Patent Owner submitted over 1,500 references during prosecution, thereby burying the most relevant art (including that discussed in this Petition) under a mountain of mostly irrelevant references. Notably, none of the

references relied on in this Petition were raised by the Office during prosecution. Instead, the principal prior art reference discussed during prosecution was a patent publication that the Patent Owner ultimately overcame by antedating. Had the Office been aware of the presently-cited art, it never would have allowed the patent claims.

II. MANDATORY NOTICES

Pursuant to 37 C.F.R. § 42.8(b), Petitioner states as follows:

A. Real Parties In Interest. Trans Ova Genetics, LC and Intrexon Corporation are real parties in interest. No other parties exercised, or could have exercised, control over this Petition; no other parties funded or directed this Petition. *See* Office Patent Trial Practice Guide, 77 Fed. Reg. 48,759-60.

B. Related Matters. A separate petition for IPR of claims 42-59, 65-67, and 69 of the '116 patent is being filed concurrently (IPR2018-00248). Petitioner requests that both petitions be reviewed by the same panel.

The '116 patent is the subject of pending litigation in the United States District Court for the District of Colorado (*XY, LLC et al. v. Trans Ova Genetics, LC,* No. 1:17-cv-00944) ("Colorado litigation"), which was transferred from an earlier filed case in the United States District Court for the Western District of Texas between the same parties (No. 6:16-cv-00447). Petitioner will soon also be filing petitions for IPR on two other patents owned by the Patent Owner: U.S.

Patent No. 6,372,422 (IPR2018-00249) and U.S. Patent No. 8,652,769 (IPR2018-00250).

C. Lead and Backup Counsel. Petitioner identifies the following:

Lead Counsel	Backup Counsel
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D. Service Information. Please address all correspondence to lead counsel shown above. Petitioner consents to electronic service by email to dkelly@hunton.com and gyao@hunton.com.

III. REQUIREMENTS FOR IPR REVIEW

Pursuant to 37 C.F.R. § 42.104, Petitioner states as follows:

A. Grounds for Standing. Petitioner certifies that the '116 patent is available for IPR and that Petitioner is not barred or estopped from requesting review on the grounds identified herein. The Director is authorized to charge the fee specified by 37 C.F.R. § 42.15(a), and any other fees necessary, to Deposit Account No. 50-0206.

B. Identification of Challenge. Pursuant to 37 C.F.R. § 42.104(b) and § 42.22(a)(1), Petitioner requests review and cancellation of claims 1-26, 33-34, and

38-41 of the '116 patent pursuant to the following statement of the precise relief requested:

Ground	Claims	Basis	References
I	1	§ 102	Evans (Ex. 1005) and Piper (Ex. 1009)
II	1-26, 33-34, 39-41	§ 103	Evans (Ex. 1005), Piper (Ex. 1009), and Tardif (Ex. 1006)
III	12	§ 103	Evans (Ex. 1005), Piper (Ex. 1009), Tardif (Ex. 1006), and Lakowicz (Ex. 1008)
IV	38	§ 103	Evans (Ex. 1005), Piper (Ex. 1009), Tardif (Ex. 1006), and Fukuda (Ex. 1010)

Pursuant to 37 C.F.R. § 42.22(a)(2), Petitioner sets forth a full statement of the reasons for the relief requested below in Section VIII. These grounds are not redundant with those filed in the co-filed sister petition, which seeks IPR and cancelation of claims 42-59, 65-67, and 69. Petitioner's arguments are supported by a Declaration from Dr. J. Paul Robinson (Ex. 1003), SVM Professor of Cytomics and Professor of Bioengineering at Purdue University, who has over 40 years of relevant experience, detailed in his C.V. (Ex. 1003.)

IV. LEVEL OF ORDINARY SKILL IN THE ART

The '116 patent claims priority (through a continuation application) to U.S. Provisional Patent App. No. 60/471,509, filed on May 15, 2003. Without conceding that this priority claim is valid, Petitioner and its expert declarant, Dr. Robinson, use May 15, 2003, as the relevant date for analysis of the level of skill and knowledge of a person of ordinary skill in the relevant art ("POSA").

The '116 patent relates to methods of flow cytometry to separate (*i.e.*, "sort") sperm cells based on whether they carry an X or Y chromosome. A POSA for purposes of the '116 patent is someone with at least a Bachelor's of Science degree in the biological sciences or closely related discipline and at least 5 years' experience in one or more of the following areas: flow cytometry, sperm sorting, and/or biomedical engineering. Ex. 1003 ¶ 25. Dr. Robinson is qualified to opine from the perspective of a POSA. *Id*.

V. STATE OF THE RELEVANT ART

A. Methods Of Using Flow Cytometry To Sort Sperm Were Well-Known.

Evans details the use of flow cytometry to sort sperm based on differences in DNA content. Ex. 1005, 16:6-18:12. In Evans' method, sperm are stained with a fluorochrome dye, such as Hoechst 33342, deposited within a nozzle of a flow cytometer, and funneled through the nozzle (via fluid hydrodynamics) using a sheath fluid. *Id.,* 16:6-15, Figs. 1 & 2. As the stream of sperm cells flow out of the

nozzle, an oscillator acts upon it, breaking the stream into droplets, each containing a single sperm cell. *Id.,* 16:15-23, Fig. 2.

The sperm-containing droplets then pass through a radiation source, such as a split laser, whose beams excite the dye and cause the sperm cells to emit a fluorescent light. *Id.,* 16:24-17:9, 31:5-19, Figs. 1, 2 & 24. Because the X chromosome contains more DNA than the Y chromosome, X-bearing sperm emit more fluorescent light than Y-bearing sperm. *Id.,* 17:9-14. The emitted fluorescent light is sensed by a detector, such as a photomultiplier tube (PMT), located near the stream, converted into an electronic signal and multiplied, and then analyzed and displayed as single or multiple parameter histograms. *Id.,* 17:15-19, 18:13-15, 20:6-22, Figures 1-3.

The difference in signal then permits discrimination and separation of X- and Y-bearing sperm. *Id.,* 17:12-21. Specifically, the sensor detects how much light each sperm-containing droplet emits and transmits that information to an analyzer coupled to a droplet charger. *Id.,* 17:15-19. The droplet charger then charges each droplet depending on the chromosomal makeup: X-bearing sperm get one charge, and Y-bearing sperm get a different charge (positive or negative). *Id.,* 17:19-18:3 The charged droplets are then passed through a pair of opposing electrostatic deflection plates, which deflect the differentially-charged X and Y-bearing droplets into respective collection containers. *Id*.

In this way, the sperm cells are sorted. Evans teaches that the sperm may be sorted at a rate of 2,500 intact sperm per second with a collection rate of 4,000-10,000 sperm per second. *Id.,* 21:10-12 and 27:5-6. The sexual purity of the sperm is 90% or greater. *Id*. 27:6-8. In addition, Evans teaches that the sorted sperm may be used to produce sex-selected offspring in procedures such as artificial insemination ("AI") and *in vitro* fertilization ("IVF"). *Id.,* 7:5-10.

B. Methods Of Using Less Than The "Maximum" Amount Of Stain Were Well-Known.

Evans discloses staining the sperm with the fluorochrome dye Hoechst 33342. Ex. 1005, 4:5-7, 18:25-27. Evans cautions, however, not to use too much dye. For one, the dye could be toxic to the sperm. *Id.,* 5:15-18 (noting that UV light "may affect the viability of the spermatozoa," and thus "it may be preferable to use a method that requires less or no stain"). Second, Evans warns that too much dye can exacerbate the background noise. *Id.,* 18:25-27 ("The amount of background signal can be further exacerbated when fluorochrome such as Hoechst 33342 can be used to label the nuclear DNA of sperm cells.").

Evans also expressly incorporates other references that similarly caution against using too much dye. For example, Johnson specifically warned that, while the dye concentration should be "sufficient to stain sperm uniformly and to detect the small differences in the DNA of X and Y sperm with minimal variation," it must be "minimal to avoid toxicity." Ex. 1007, 4:31-35. To that end, Johnson

observed that a "suitable concentration was found to be 5 μg/ml, but this may be varied from 4 to 5 μg/ml." *Id.,* 4:35-36. Thus, it was known that too much dye could harm the sperm and, thus, it was preferable to use less than the "maximum" amount of dye when staining sperm.

C. Methods Of Using Pulsed Radiation Sources In Flow Cytometry And Other Applications Involving Irradiating Hoechst 33342 Dye-Stained Cells Were Well-Known.

Evans does not expressly disclose a pulsed excitation source. The use of such, however, was widely reported in the prior art. Piper, for example, taught the advantages of pulsed radiation sources over CW radiation sources for flow cytometry, including that they are cheaper to purchase and operate, have high instantaneous illumination intensities, relaxed beam focusing requirements, good beam uniformity, and wavelength versatility. Ex. 1009, 1:38-2:4, 3:19-23, 5:33-35. Similarly, Tardif taught that a pulsed radiation source was preferable over a continuous radiation source in applications involving Hoechst 33342-stained sperm, because a pulsed radiation source reduced the time the sperm cells were exposed to harmful UV illumination. Ex. 1006, 205. And Lakowicz taught the use of pulsed lasers to irradiate Hoechst 33342-stained sperm, and disclosed that, for such applications, the laser should be operated at "325-355 nm (UV)," which is the dye excitation/absorption wavelength for Hoechst 33342.

VI. SUMMARY OF THE CLAIMED INVENTION

A. Brief Description Of The Challenged Claims

Claims 1-26, 33-34, and 38-41 are being challenged. Claim 1, the lone independent claim, recites:

> 1. A method of flow cytometry sperm processing comprising the steps of:
>
> establishing a sheath fluid;
>
> flowing said sheath fluid into at least one nozzle;
>
> providing a percentage of a fluorescent dye stain as compared to a standard control, said percentage of stain selected from a group consisting of about 90%, about 80%, about 70%, and about 60% of maximum stain;
>
> staining sperm cells with said percentage of said fluorescent dye stain to provide irradiatable stained sperm cells;
>
> injecting said irradiatable stained sperm cells into said sheath fluid;
>
> utilizing one radiation source;
>
> splitting a radiation into at least two beams;
>
> directing each of said beams to said at least one nozzle and said sperm cells;
>
> multiply subjecting said irradiatable sperm cells to said radiation for a first amount of time;
>
> multiply terminating said radiation of said irradiatable sperm cells for a second amount of time;
>
> multiply exciting said irradiatable sperm cells with said radiation;

wherein said step of multiply subjecting said irradiatable sperm cells to said radiation for said first amount of time and said step of multiply terminating said radiation of said irradiatable sperm cells for said second amount of time and said step of multiply exciting said irradiatable sperm cells with said radiation are performed in a sequential manner then repeated in order at a repetition rate;

emitting fluorescence from said excited sperm cells;

detecting an amount of said emitted fluorescence from each of said sperm cells;

evaluating said amount of emitted fluorescence from each of said sperm cells;

selecting an electrical condition based on the amount of emitted and detected fluorescence from each of said sperm cells to be associated with each of said sperm cells in said sheath fluid flow;

charging a stream of said irradiatable sperms cell and sheath fluid based on the amount of emitted and detected fluorescence from each of said sperm cells in said sheath fluid flow;

forming a charged drop having one of said sperm cells located therein;

isolating said charged drop from said sheath fluid flow;

deflecting said charged drops;

sorting said sperm cells; and

collecting said sorted sperm cells.

Claims 2-26, 33-34, and 38-41 all depend from claim 1. Each recites one or more steps that are conventional, trivially-limiting, and/or inherent in the method of claim 1. These steps include: irradiating and exciting the sperm (claims 11-12,

15); using lower power radiation (claims 16-19); using a pulsed laser (claims 40-41); measuring the fluorescence (claim 2) to differentiate between X and Y-chromosomes (claims 3 and 20); sorting or collecting sperm at specific rates, purities, and resolution (claims 4-5, 21-26); using elements to split the beam (claims 6 and 7), detect the signal (claims 8 and 9), or oscillate the sheath fluid to create a drop (claim 33); utilizing at least two nozzles (claim 38); staining for a specific amount of time (claims 13 and 14); utilizing mammalian sperm (claim 34); and using the sperm to inseminate a female mammal (claim 39).

B. Summary Of The '116 Patent Specification

The '116 patent describes the basics of flow cytometry and, more specifically, conventional methods for flow-sorting dye-stained sperm, which included the following known steps: establishing a sheath fluid and flowing it into a nozzle (*id.,* 11:47-56); staining sperm cells with less than the "maximum" amount of Hoechst 33342 dye (*id.,* 16:24-37); injecting the stained sperm cells into the sheath fluid (*id.,* 11:44-56); irradiating the stained sperm with a split beam laser (*id.,* 8:64-9:5); exciting the stained sperm cells, thereby causing them to emit fluorescent light (*id.,* 12:11-17); detecting and evaluating that light (*id.,* 12:45-53); charging the X and Y-bearing sperm with either a positive or negative charge (*id.,* 12:53-65); forming charged drops entraining an X or Y-bearing sperm (*id.,* 12:66-13:1); and using deflection plates to deflect the X and Y drops into different

collectors (*id.,* 13:1-7), thereby separating (*i.e*., sorting) the sperm cells based on sex (*id.,* 13:7-14).

Notably, every feature of flow-sorting dye-stained sperm disclosed in the '116 patent had been known and described in one or more of the ***1,500+ references*** cited during prosecution. *Id.,* pp. 1-21. One feature, however, that the '116 patent touts as distinguishing its methods over the prior art is the use of a ***pulsed*** excitation source (e.g., laser) in place of the more "traditional" CW laser. *Id.,* 2:41-45 ("The traditional type of laser used for the analysis of particles in flow cytometry is a continuous wave (CW) laser. Often this provides a beam of constant intensity. However, in some instances, CW lasers can have particular disadvantages for applications as discussed here."); *id.,* 1:15-18 ("More specifically, the invention relates to the use of a pulsed laser on a flow system for particle analysis which results in more accurate quantification of measurable properties of individual particles.").

However, as detailed below, pulsed excitation sources had been known for years, including for use in flow cytometry applications and other applications involving dye-stained sperm. *See* Section VIII below; *see also* Ex. 1001, 2:53-3:18 (describing use of pulsed lasers in flow cytometry). Even the specific pulsed laser disclosed in the '116 patent—the "Vanguard Laser"—had been used for years before the '116 patent was filed. *Id.,* 17:37-40 (explaining that the Vanguard Laser

was "manufactured by Spectra-Physics" and was described in "Laser Forefront, Spectra-Physics, No. 30 (2001)"); *see also id.,* 21:35-43, Fig. 17, Tables 1 & 2.

C. Summary Of The Relevant Prosecution History

The '116 patent issued from U.S. App. No. 11/442,735, filed on May 25, 2006, and claims the benefit (through a continuation application) to U.S. Provisional App. No. 60/471,509, filed on May 15, 2003.

The '735 application was filed with 143 claims. Ex. 1015, 59-126. After an initial Office Action rejecting all the claims as anticipated by U.S. Publication No. 2005/0112541 to Durack et al. ("Durack"), the applicants responded by cancelling all then-pending claims and replacing them with a new set of claims drawn to: (A) a "method of flow cytometry sperm processing" (claims 144-199); and (B) a "flow cytometry system for sperm" (claims 200-243). Ex. 1016, 3-7; Ex. 1017, 2-18.

In April 2008, the Office rejected all the claims as not only indefinite for various reasons, but also as anticipated by Durack, which the Office asserted taught a flow cytometry method using a pulsed laser and beam splitter to sort and classify particles according to one or more characteristics. Ex. 1018, 2-8.

In October 2008, the applicants responded by amending the claims, including adding the following limitation after the three "multiply" steps:

> wherein said step of multiply subjecting said irradiatable sperm cells to said radiation for said first amount of time and said step of multiply terminating said radiation of said irradiatable sperm cells for said

> second amount of time and said step of multiply exciting said irradiatable sperm cells with said radiation are performed in a sequential manner then repeated in order at a repetition rate;

Ex. 1019, 3. Although the applicants did not offer an explanation for the amendment, it appears to have been in response to the Office's indefiniteness rejections. *See id.,* 19. The applicants also added the following limitations to overcome the prior art rejection:

> staining sperm cells with a fluorescent dye;
>
> providing a percentage of stain as compared to a standard control, said percentage of stain selected from a group consisting of about 90%, about 80%, about 70%, and about 60% of maximum stain.

Id., 3. They argued that Durack "does not disclose the use of less stain, e.g., about 90%, about 80%, about 70%, and about 60% of maximum stain as presented in claims 144-200." *Id.,* 20.

In November 2008, the Office maintained the indefiniteness rejections over most of the pending claims and modified the anticipation rejection to obviousness based on the amendment. Ex. 1020, 2-6. The Office contended that, while Durack does not explicitly teach the use of the recited amounts of the dye, it would have been obvious for a POSA to use a lower amount of dye since Durack teaches that it is advantageous to use less dye to minimize any harmful impact thereof. *Id*.

In December 2009, the applicants submitted a Rule 131 Declaration from inventors Evans and Gilligan, who averred conception before Durack's March 28, 2003, priority date and diligent reduction to practice. Ex. 1021. The applicants also argued that the invention was non-obvious over Durack, which they alleged failed to teach using less stain with a pulsed laser. Ex. 1022, 19-20.

The application was subsequently allowed in view of the inventors' Rule 131 Declaration and, according to the Examiner, because the prior art did not teach or suggest staining sperm cells with less than 100% of a maximum amount of fluorescent dye used to stain a standard control material. Ex. 1023, 2.

VII. CLAIM CONSTRUCTION

In an IPR, claim terms are interpreted according to their broadest reasonable interpretation ("BRI") in view of the specification in which they appear. 37 C.F.R. § 42.100(b).[1]

[1] The BRI of claim terms here may be different from the construction that those same terms may receive following claim construction proceedings in district court. *See, e.g., In re Trans Texas Holdings Corp.*, 498 F.3d 1290, 1297 (Fed. Cir. 2007). Thus, the claim constructions presented in this Petition, including where Petitioner does not propose an express construction, do not necessarily reflect the claim constructions that Petitioner believes should be adopted by a district court under *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005).

A. "providing a percentage of a fluorescent dye stain as compared to a standard control, said percentage of stain selected from a group consisting of about 90%, about 80%, about 70% or about 60% of maximum stain;" (claims 1-26, 33-34, 38-41)

This limitation is not self-explanatory and thus resort to the specification is necessary to shed light on its meaning. The support for the limitation appears to derive principally from the Examples. Specifically, in Examples 1-10, samples of bull sperm are "stained in standard conditions with DNA binding stain Hoechst 33342." Ex. 1001, 26:19-31:7. Examples 5 and 8 further specify that the sperm are stained "with the standard concentration of Hoechst 33342 ***being defined as*** 100% level of stain (control)." *Id.,* 28:4-9 (Ex. 5) (emphasis added), 29:20-25 (Ex. 8). In these two examples, some samples of bull sperm are also "stained with 80% or 60% of the amount of Hoechst 33342 stain, respectively." *Id.,* 28:9-11 (Ex. 5), 29:25-28 (Ex. 8); *see also id.*, Tables 6 and 7. Similarly, in Example 9, bull sperm are stained either with 100% or 80% stain. *Id.,* 30:18-39.

Thus, the specification seems to equate "the standard concentration" of a dye with being the maximum amount of that stain, *i.e.*, "100% level of stain (control)." Ex. 1003 ¶ 60. Put another way, the terms "maximum stain" and "standard concentration of the dye" appear to mean the same thing. *Id*. The patent, however, fails to specify a ***particular*** concentration of dye as being the standard concentration (maximum stain). This is for good reason, as the standard concentration of any dye used in flow cytometry will necessarily turn on a variety

of factors, including the particular dye used, the specific conditions in which the dye is used (*e.g.*, temperature, incubation time, media used, etc.), the species of sperm (*e.g.*, human, bovine, equine, etc.), the nature of the sperm being stained (*e.g.*, fresh, frozen-thawed, etc.), and the precise number of sperm cells in the sample. *Id*. ¶ 61. As such, there is no universal standard control; rather, there is a wide range of dye concentrations that a POSA might select for any given experiment, depending on the foregoing factors.[2] *Id*.

The fact that even the same dye can have varying "standard concentrations" is evidenced by the very documents the '116 patent incorporates by reference, such as Spaulding (Ex. 1012), Rens (Ex. 1013), and Seidel (Ex. 1014). *See* Ex. 1001, column 52. Each of these incorporated patents discloses a process for sorting sperm into X- and Y-bearing populations, just like the '116 patent, yet each discloses a different "standard concentration" (maximum stain) of Hoechst dye, as well as different staining conditions. Ex. 1003 ¶ 62. Spaulding treats mammalian sperm with 10 µg/ml (*i.e.*, ***22 µM***) Hoechst dye for two hours. Ex. 1012, 8:60-9:8.

[2] Petitioner is mindful that indefiniteness is not an appropriate ground of invalidity in an IPR and, to that end, has done its best to ascribe a definite construction to this claim limitation. Petitioner, however, reserves the right to challenge the definiteness of this limitation in the co-pending Colorado litigation, which can address issues beyond those available in an IPR, including indefiniteness.

Rens treats mouse and human sperm with ***7.1 μM*** Hoechst dye for 40 minutes at 32°C, and uses a similar dye preparation diluted with propidium iodide for bull sperm. Ex. 1013, 5:2-10. And Seidel treats bull sperm with ***38 μM*** Hoechst dye for 1 hour at 34°C. Ex. 1014, 15:2-5.

Accordingly, the maximum stain for any given dye appears to be the highest amount of effective stain used in any particular experiment (or set of experiments). Ex. 1003 ¶ 63. And a "percentage of [maximum] stain" is simply some amount less than that. *Id*. Thus, the phrase "providing a percentage of a fluorescent dye stain as compared to a standard control" means "providing some amount of dye less than the standard concentration." *Id*. That amount of dye is further specified by the claim as "about 60%, about 70%, about 80%, or about 90%" of the maximum stain. *Id*.

In sum, a POSA reading the claims in view of the specification would understand the limitation "providing a percentage of a fluorescent dye stain as compared to a standard control, said percentage of stain selected from a group consisting of about 90%, about 80%, about 70% or about 60% of maximum stain" to mean, simply, "providing about 60%, 70%, 80%, or 90% of the highest concentration of dye shown to be effective at staining the sperm in any particular experiment (or set of experiments)." Ex. 1003 ¶ 64.

B. "radiation source" (claims 1-26, 33-34, 38-41)

The term "radiation source" is not defined in the '116 patent, but is used interchangeably with the terms "irradiation source," "light source," "source light," and "illumination source." Ex. 1001, 1:50-51, 2:17-19, 3:30-31, 11:16-17, 13:43-44, 17:62-63, 18:49-50, 18:53-56, 19:40-41. Thus, a POSA would understand this term to mean "a source that emits or transmits energy, including electromagnetic radiation, such as light." Ex. 1003 ¶ 65.

C. "about [X]%" (claims 1-26, 33-34, 38-41)

The term "about" in the phrase "about 90%, about 80%, about 70%, and about 60%" is not defined in the '116 patent. However, the use of the term "about" is generally understood to "avoid[] a strict numerical boundary" and must, rather, "be interpreted in its technologic and stylistic context." *Pall Corp. v. Micron Separations, Inc*., 66 F.3d 1211, 1217 (Fed. Cir. 1995). Here, a POSA reading the '116 patent would understand the term "about" in the phrase "about [X]%" to embrace a variance of at least 10% of the recited value. Ex. 1003 ¶ 66. This is because, for a fluorescent dye to be excited within a cell, the molecules must be "saturated" by the radiation, and saturation would require a moderately broad concentration related to the intensity of the radiation. *Id*.

Thus, a POSA would understand that the phrases "*about* 60%" would range from, *e.g.*, 54% to 66%; "*about* 70%" would range from, *e.g.*, 63% to 77%; "*about*

80%" would range from, *e.g.*, 72% to 88%; and "*about* 90%" would range from, *e.g.*, 81% to 99%. That is, each recited value would have a ±10% variance. *Id*. ¶ 67.

D. "multiply" (claims 1-26, 33-34, 38-41)

The claims recite several steps that are performed "multiply," *i.e.*, "***multiply*** subjecting said irradiatable sperm cells to said radiation for a first amount of time; ***multiply*** terminating said radiation of said irradiatable sperm cells for a second amount of time; [and] ***multiply*** exciting said irradiatable sperm cells with said radiation." A POSA would understand this term to have its plain and ordinary meaning, *i.e.*, "more than once." Ex. 1003, ¶ 68. This meaning of "multiply" is also consistent with the term's usage in the specification. Ex. 1001, 10:23-27, 12:14-17.

E. "wherein said step of multiply subjecting said irradiatable sperm cells to said radiation for said first amount of time and said step of multiply terminating said radiation of said irradiatable sperm cells for said second amount of time and said step of multiply exciting said irradiatable sperm cells with said radiation are performed in a sequential manner then repeated in order at a repetition rate" (claims 1-26, 33-34, 38-41)

The above phrase, when read in conjunction with the three antecedent "multiply" steps, requires that each of the three "multiply" steps be "performed in a sequential manner then repeated in order." As such, this limitation on its face requires first subjecting sperm to radiation multiple times; followed by terminating that radiation multiple times; followed, finally, by exciting the sperm multiple

times; then repeating those three steps in the same order. Taken literally, this would render the claimed method inoperable as it is nonsensical to subject sperm cells to irradiation multiple times ***before*** terminating the radiation multiple times and, further, to ***complete both of those*** steps before ever exciting the sperm. Ex. 1003, ¶ 69.

In any event, for purposes of this IPR, Petitioner has endeavored to ascribe an operable construction to the claims.[3] To that end Petitioner assumes that the claims drafter intended this limitation to be read in conjunction with the three preceding "multiply" limitations to cover a process in which the sperm cells are irradiated in a pulsed fashion at a given repetition rate, thus causing the sperm cells to excite at regular intervals. This construction, while seemingly at odds with the claim's literal meaning, is at least in line with the patent's description of using an "intermittingly punctuated radiation" emitter, such as a "pulsed laser." Ex. 1001, Abstract, 8:26-31, 9:49-51, 10:19-27, 10:44-59, 11:60-62, 12:11-17, 17:65-18:1, Example 1-13.

Accordingly, Petitioner proposes that the Board adopt the following construction of this limitation: "wherein the sperm cells are irradiated in a pulsed

[3] Petitioner reserves the right to offer a more literal construction of the claim language in the co-pending Colorado litigation, which can address issues beyond those available in an IPR, including non-infringement and indefiniteness.

fashion at a given repetition rate, thus causing the sperm cells to excite at regular intervals."

VIII. DETAILED DISCUSSION OF HOW EACH GROUND RAISES A REASONABLE LIKELIHOOD OF UNPATENTABILITY

Pursuant to Rule 42.104(b), this section demonstrates that the challenged claims are unpatentable. (Ex. 1003, ¶ 78-157.)

A. Each of the Relied-Upon References is Authentic, Admissible Prior Art to the '116 Patent

Petitioner relies on the following references:

1. Evans (Ex. 1005) - Evans is a PCT application that published in November 2001, and is thus prior art to the '116 patent claims under pre-AIA 35 U.S.C. § 102(b). Exhibit 1005 is an authentic, admissible copy of the Evans reference under the Federal Rules of Evidence. Ex. 1002.

2. Piper (Ex. 1009) - Piper is a PCT application that published in May 1992, and is thus is also prior art to the '116 patent claims under pre-AIA 35 U.S.C. § 102(b). Exhibit 1009 is an authentic, admissible copy of the Piper reference under the Federal Rules of Evidence. Ex. 1002.

3. Tardif (Ex. 1006) - Tardif published in the March/April 1998 volume of the *Journal of Andrology*, and is thus prior art to the '116 patent claims under pre-AIA 35 U.S.C. § 102(b). As detailed in the accompanying declaration of Jonathan H. Hartnett, a librarian with the law firm of Hunton & Williams (Ex.

1002), Exhibit 1006 is an authentic, admissible copy of the Tardif reference under the Federal Rules of Evidence.

4. Lakowicz (Ex. 1008) - Lakowicz is a United States patent issued in April 1996. As such it is prior art to the '116 patent claims under pre-AIA 35 U.S.C. § 102(b). Exhibit 1008 is an authentic, admissible copy of the Lakowicz reference under the Federal Rules of Evidence. Ex. 1002.

5. Fukuda (Ex. 1010) - Fukuda is a United States patent that issued in December 1992. As such, it is prior art to the '116 patent claims under pre-AIA 35 U.S.C. § 102(b). Exhibit 1010 is an authentic, admissible copy of the Fukuda reference under the Federal Rules of Evidence. Ex. 1002.

Each one of Exhibits 1008 (Lakowicz), 1009 (Piper), and 1010 (Fukuda) is over 20 years old, and each was prepared before January 1, 1998, and thus each qualifies as an ancient document under Fed. R. Evid. 803(16), both before and after the pending Dec. 1 2017 amendment to the Rule. Moreover, each of these Exhibits, as well as Exhibits 1005 (Evans) and 1006 (Tardif), also meets the residual exception to hearsay under Fed. R. Evid. 807 as each: (i) has equivalent circumstantial guarantees of trustworthiness, (ii) is offered as evidence of a material fact, (iii) is more probative on the point for which it is offered than any other evidence that Petitioner can obtain through reasonable efforts, and as (iv) admitting the Exhibit will best serve the purposes of the Federal Rules of Evidence

and the interests of justice. Additionally, Exhibits 1005 (Evans) and 1009 (Piper) are Publications of the World Intellectual Property Organization and Exhibits 1008 (Lakowicz) and 1010 (Fukuda) are issued United States Patents. As such, these documents meet the public records exception to hearsay under Fed. R. Evid. 803(8).

B. Summary Of The Grounds Of Rejection

The principal reference in the proposed grounds of rejection, Evans, shares a co-inventor with the '116 patent and discloses strikingly similar methods of flow-sorting sperm. Embodiments of Evans' method are recited in claims 11 and 14-15, appearing at the end of the document. As can be seen, these claims, which are reproduced below, contain ***nearly every element*** of the challenged '116 patent method claims:

> 11. A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 2, further comprising the steps of:
>
> a. introducing said sperm cells into a fluid stream;
>
> b. analyzing said sperm cells entrained in said fluid stream;
>
> c. forming droplets a plurality having one of said sperm cells entrained;
>
> d. charging each of said droplets differentially based upon said sex differentiation characteristic of said sperm cells entrained in said droplets;

e. deflecting each of said droplets; and

f. differentially collecting each of said droplets based upon said sex differentiation characteristic of said sperm cells entrained in said droplets.

14. A method of isolating X-chromosome beaming sperm cells and Y-chromosome bearing sperm cells as described in claim 13, further comprising the step of irradiating stained DNA within said nucleus of said sperm cell.

15. A method of isolating X-chromosome bearing sperm cells and Y-chromosome bearing sperm cells as described in claim 14, further comprising the step of detecting fluorescent light emitted from irradiated stained DNA within the nucleus of said sperm cell.

Ex. 1005, 54:8-55:4 (claims 11-15).

The foregoing methods, as well as various alternatives and preferred embodiments, are detailed in Evans, which specifically describes performing sperm-sorting on a "Cytomation SX MoFlo® sorting flow cytometer." *Id.,* 20:6-23:16; *see also id.,* Figs. 3-12. Notably, this is the very same machine that Petitioner uses to sort sperm, and which forms the basis of Patent Owner's assertions of infringement in the co-pending Colorado litigation. Ex. 1011. In other words, the Patent Owner is accusing Petitioner of infringing the '116 patent claims by performing ***the same methods*** on ***the same machine*** that Evans disclosed more than a year before the '116 patent's earliest priority date.

In any event, as detailed below, Evans expressly discloses or suggests every element in the challenged '116 patent claims. Any claimed feature not expressly recited in Evans was conventional and/or expressly taught by other, closely-related art.

For example, Piper and Tardif both taught the use of a pulsed radiation source, as well as its advantages over conventional, continuous wave (CW) radiation sources. Piper specifically taught the use of a pulsed laser in flow cytometry applications, and Tardif taught the use of a pulsed radiation source in applications involving evaluating Hoechst 33342 dye-stained sperm cells. Tardif also taught using the pulsed radiation source at a wavelength of 327 to 395 nm. Moreover, Lakowicz, which also disclosed a pulsed laser, taught that cells stained with Hoechst 33342 should be irradiated at 325-355 nm. And Fukuda disclosed a flow cytometer comprising two nozzles, which it taught increased the efficiency of the cytometer.

C. Ground 1: Claim 1 Is Obvious Over Evans In View Of Piper.

Claim 1 (preamble): A method of flow cytometry sperm processing comprising the steps of:

Evans discloses a method of flow-sorting stained sperm cells. Ex. 1005, 16:6-18:12 (detailing method for flow-sorting sperm); 8:22-9:2; *see also id.,* 54:8-55:4 (claims 11-15). Figure 1 depicts Evans' system for flow-sorting sperm:

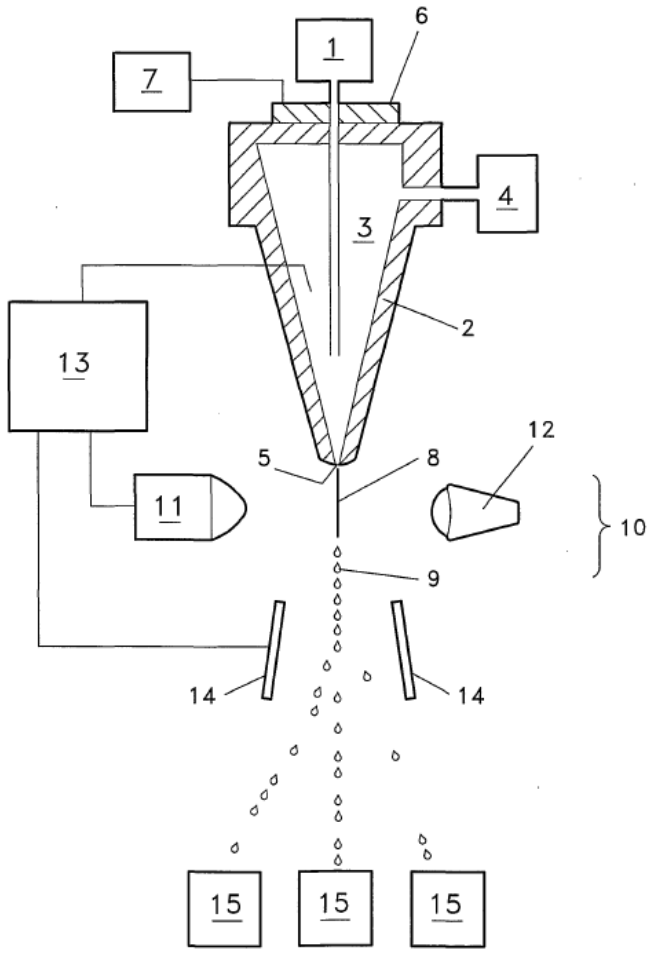

Fig. 1

Key:

(1) = Cell source
(2) = Nozzle
(3) = Fluid stream / Sheath fluid
(4) = Sheath fluid source
(5) = Nozzle orifice
(6) = Oscillator
(7) = Oscillator control
(8) = Exiting stream
(9) = Drops
(10) = Cell sensing system
(11) = Detector / sensor
(12) = Irradiation source (laser)
(13) = Analyzer
(14) = Deflection plates
(15) = Collection containers

Id., Fig. 1; *see also id.,* 16:6-17:23 (describing the parts of Fig. 1).

Claim 1(a): establishing a sheath fluid;

Evans expressly discloses this step. Ex. 1003 ¶ 82. Referring to Figures 1 and 2, Evans states:

> The particles or cells are deposited within a nozzle (2) in a manner such that the particles or cells are ***introduced into a fluid stream or sheath fluid*** (3). . . . In this manner it can be easily understood how

> ***the sheath fluid (3) forms a sheath fluid environment for the particles or cells.***

Id., 16:6-15 (emphasis added); *see also id.,* 54:8-17 (claim 11).

> ***Claim 1(b): flowing said sheath fluid into at least one nozzle;***
>
> *See* Claim 1(a) above.
>
> ***Claim 1(c): providing a percentage of a fluorescent dye stain as compared to a standard control, said percentage of stain selected from a group consisting of about 90%, about 80%, about 70%, and about 60% of maximum stain;***

As discussed in Section VII above, this limitation means "providing about 60%, 70%, 80%, or 90% of the highest concentration of dye shown to be effective at staining the sperm in any particular experiment (or set of experiments)." Although Evans does not expressly disclose the concentration of dye used in its experiments, Evans expressly (and repeatedly) incorporates Johnson (Ex. 1007)—widely considered to be one of the pioneering patents in the field of flow sorting.[4] Ex. 1005, 2:15-25; *see also* Ex. 1003 ¶ 84.

[4] The Johnson patent is so foundational to the field of flow-sorting sperm that Evans incorporates it by reference not once, but ***three different times.*** *See* Ex. 1005, 1:11-12, 2:24-25, and Table on pg. 38, row 3.

As Johnson is expressly incorporated into Evans' disclosure, it is deemed a part of that disclosure.[5] *See Liebel-Flarsheim Co. v. Medrad, Inc.*, 481 F.3d 1371, 1382, n.3 (Fed. Cir. 2007) ("We have further explained that material incorporated by reference 'is effectively part of the host document as if it were explicitly contained therein.'") (internal citation omitted); *see also Callaway Golf Co. v. Acushnet Co*., 576 F.3d 1331, 1346-47 (Fed. Cir. 2009) (holding that material properly incorporated by reference into a prior art document is generally treated as being within the "four corners" of the document itself).

Like Evans, Johnson describes methods of flow-sorting Hoechst 33342 dye-stained sperm. Ex. 1003 ¶ 85. Additionally, Johnson specifically teaches using lower amounts of Hoechst 33342 to avoid harming the sperm. Ex. 1007, 4:27-36. Johnson explains:

> Concentration of the fluorochrome ***must be minimal to avoid toxicity***, and yet be sufficient to stain sperm uniformly and to detect the small differences in the DNA of X and Y sperm with minimal variation. A suitable concentration was found to be 5 µg/ml, but this may be varied ***from 4 to 5 µg/ml.***

[5] Should the Board determine that Johnson should not be treated as a part of Evans' disclosure, Petitioner respectfully requests that the Board, in that case, treat Johnson as a secondary reference to be combined with Evans.

Id., 4:27-36 (emphasis added). Thus, Johnson provides both the motivation to use lower amounts of stain than the maximum, as well a ***specific*** range of concentrations, namely, "from 4 to 5 μg/ml." Significantly, this range encompasses from 80% to 100% of the maximum amount of dye disclosed by Johnson as "suitable" for staining X and Y sperm. Ex. 1003 ¶ 85. Johnson, therefore, both recommends using less than the maximum amount ***and*** discloses an amount of dye (4 μg/ml) that reads directly onto the claimed "about 80%" maximum stain. *Id*.

Accordingly, a POSA practicing Evans' method of flow-sorting sperm would have been prompted by Johnson, which is expressly incorporated into Evans' disclosure, to use less than the maximum amount of dye necessary or suitable to stain sperm. Ex. 1003 ¶ 86. Further, the POSA would have been instructed by Johnson to choose, for example, 80% of maximum stain, as taught therein. *Id*. Guided by Johnson's instruction and informed by its own teaching, the POSA would have reasonably expected this concentration to be both effective in staining the X and Y chromosomes, yet generally safer for sperm than using the maximum amount. *Id.*

Claim 1(d): ***staining sperm cells with said percentage of said fluorescent dye stain to provide irradiatable stained sperm cells;***

Evans discloses this step. Ex. 1003 ¶ 87. Specifically, Evans teaches staining the sperm cells with the fluorochrome dye, Hoechst 33342. Ex. 1005, 22:23-28

("[T]he isolated [sperm] heads were washed, fixed with 2% formalin and then stained with Hoechst 33342."); 24:15-19; *see also id.,* 54:8-55:4 (claims 11-15).

***Claim 1(e):* injecting said irradiatable stained sperm cells into said sheath fluid;**

Evans discloses this step. Ex. 1003 ¶ 88. Specifically, Evans discloses injecting the stained sperm cells into a sheath fluid. Ex. 1005, 27:2-5 ("Spermatozoa introduced into the laminar flow of sheath fluid maintained by an embodiment of the injector tube invention having the paddle shaped beveled blade allows for a 20%, 30%, 40%, 50% or even greater increase in spermatozoa sorting rates over conventional injection tube technology."); *see also id.,* 54:8-55:4 (claims 11-15).

***Claim 1(f):* utilizing one radiation source;**

Evans discloses this step. Ex. 1003 ¶ 89. Specifically, Evans discloses:

> ***An electromagnetic radiation source*** (43) generates electromagnetic radiation or a beam of electromagnetic radiation (44) . . . . The electromagnetic radiation which can be laser light, but could also be numerous types of electromagnetic radiation including, but not limited to, microwave radiation, ultraviolet radiation, or the like."

Ex. 1005, 31:5-11 (emphasis added); *see also id.,* 32:8-9.

Claim 1(g): ***splitting a radiation into at least two beams;***

Evans discloses this step. Ex. 1003 ¶ 90. Specifically, Evans discloses "a beam splitter (50) to ***split up the beam into two***, or more, components . . ." Ex. 1005, 32:17-19 (emphasis added); *see also id.,* 33:3-5.

Claim 1(h): ***directing each of said beams to said at least one nozzle and said sperm cells;***

This step was conventional. Ex. 1003 ¶ 91; *see also* Ex. 1001, 4:5-7 ("In conventional flow cytometry, Hoechst 33342-stained spermatozoa traverse a laser beam resulting in a fluorescent light emission."). Moreover, Evans specifically discloses this step. Ex. 1003 ¶ 91. More specifically, Evans discloses entraining particles of stained sperm in a fluid droplet as they exit the nozzle, whereupon they pass through a radiation source, such as a laser beam, and are excited. Ex. 1005, 16:24-17:7. Evans also depicts several embodiments in which the sperm pass through a radiation source upon exiting the nozzle. *See, e.g., id.,* 31:26-32:21 & Fig. 24 (describing and showing an electromagnetic source (43) directing a laser beam (12) at sperm (28) contained in a fluid stream (8) as the fluid stream (8) exits the nozzle (2)).

Claim 1(i), 1(j), 1(k), and 1(l): ***multiply subjecting said irradiatable sperm cells to said radiation for a first amount of time; multiply terminating said radiation of said irradiatable sperm cells for a second amount of time; multiply exciting said irradiatable sperm cells with said radiation; wherein said step of multiply subjecting said irradiatable sperm cells to said radiation for said first amount of time and said step of multiply terminating said radiation of said irradiatable sperm cells for said second***

amount of time and said step of multiply exciting said irradiatable sperm cells with said radiation are performed in a sequential manner then repeated in order at a repetition rate;

As discussed in Section VII above, a POSA would most likely understand these steps as requiring alternately irradiating the stained sperm cells and terminating that radiation, thus exciting the stained sperm at regular intervals. Ex. 1003 ¶ 92. This could be done with an "intermittingly punctuated radiation" emitter, such as a "pulsed laser." Ex. 1005, Abstract, 8:26-31, 9:49-51, 10:19-27, 10:44-59, 11:60-62, 12:11-17, 17:65-18:1, Examples 1-13. Evans specifically discloses irradiating and exciting stained sperm. *Id.,* 2:18-19 ("As stained particles or cells pass through the excitation or irradiation source, the fluorochrome emits fluorescent light."). Yet Evans does not expressly indicate whether the radiation source can be pulsed.

Pulsed radiation sources, however, were widely reported in the art of flow-sorting cells. For example, Piper disclosed a pulsed radiation source in 1992. Ex. 1009. More specifically, Piper disclosed a method of flow-sorting cells using a high-repetition pulsed laser. *Id.,* 1:3-4 ("The invention pertains to flow cytometry and more particularly to a pulsed laser light source in a flow cytometer."); *id.,* 3:37-32. Significantly, Piper explained the disadvantages of continuous lasers in flow cytometry, including that they are more expensive to purchase, install, and operate (*id.,* 1:33-2:3), have a relatively short laser tube lifetime (*id.,* 2:3-10), and

are difficult to align (*id.,* 2:11-20). "[H]igh repetition rate pulse lasers," by contrast, "have particular advantages in application as flow cytometry sources[,] including high instantaneous illumination intensities (giving high signal levels), relaxed beam focussing [*sic*] requirements, good beam uniformity[,] and especially wavelength versatility[,] which can be effectively utilised by temporal multiplexing techniques." *Id.,* 3:27-32.

Piper noted that other advantages of certain pulsed lasers, such as neodymium:YAG lasers, is that their "output may be frequency-doubled to generate high repetition rate pulsed output in the visible [spectrum]." *Id.,* 3:17-23. Such devices, Piper explained, "are expected to increasingly dominate in laser applications requiring long-term reliability in the low-power regime; their compact size and projected low cost are especially appropriate to applications requiring multiple sources as in flow cytometry."[6] *Id.,* 3:24-27. Piper also disclosed splitting the laser beam. *Id.,* 10:7-8 (claim 10: "the laser source comprises a multi-wavelength laser whose beam is split using a beam splitter into separate beams of individual wavelengths").

[6] Piper notes that a perceived difficulty with the use of pulsed lasers had been the difficulty in ensuring that each cell is illuminated as it passes through the interaction region; however, Piper explains, it had overcome that difficulty by synchronizing the pulse rate with the droplet flow rate and by adjusting the focal spot size of the beam. *Id.,* 3:33-4:11.

In view of the above, it would have been obvious for a POSA to modify Evans' flow cytometry method by using Piper's pulsed laser. Ex. 1003 ¶ 97. The POSA would have been motivated to make this substitution given the many advantages of pulsed lasers taught by Piper, including lower costs and increased performance, higher instantaneous illumination intensities, relaxed beam focusing requirements, good beam uniformity, and wavelength versatility. *Id.* Moreover, the POSA would have reasonably expected Piper's pulsed laser to work for flow cytometry applications because Piper specifically taught that its pulsed laser was particularly useful and effective in flow cytometry methods like that taught by Evans. *Id*.

Claim 1(m): emitting fluorescence from said excited sperm cells;

When fluorescently-stained sperm cells are irradiated, they naturally emit fluorescence. Ex. 1003 ¶ 98. Moreover, Evans specifically discloses this natural phenomenon. Ex. 1005, 2:18-19 ("As stained particles or cells pass through the excitation or irradiation source, the fluorochrome emits fluorescent light."); *see also id.,* 54:8-55:4 (claims 11-15).

Claim 1(n): detecting an amount of said emitted fluorescence from each of said sperm cells;

Evans discloses this step. Ex. 1003 ¶ 99. Specifically, Evans discloses detecting an amount of fluorescence emitted from each of the sperm cells. Ex. 1005, 25:25-29 ("***the light emitted*** (31) by laser excitation of fluorochrome(s)

bound to the DNA contained ***within spermatozoa*** can be ***detected*** by photomultiplier tubes (32) situated at 0 and 90 degrees relative to the flat surface of the sperm head (28) as it flows through the excitation laser beam pattern." (emphasis added); *see also id.*, 16:26-28 (describing a "detector or sensor (11) which responds to the particles or cells contained within fluid stream (8)."); 54:8-55:4 (claims 11-15).

Claim 1(o): evaluating said amount of emitted fluorescence from each of said sperm cells;

Evans discloses this step. Ex. 1003 ¶ 100. Specifically, Evans discloses that, "by ***measuring*** the fluorescence emitted by the bound fluorochrome upon excitation, it is possible to differentiate between X-bearing spermatozoa and Y-bearing spermatozoa." Ex. 1005, 17:12-14 (emphasis added); *see also id.,* 54:8-55:4 (claims 11-15).

Claim 1(p): selecting an electrical condition based on the amount of emitted and detected fluorescence from each of said sperm cells to be associated with each of said sperm cells in said sheath fluid flow;

Evans discloses this step. Ex. 1003 ¶ 101. Specifically, Evans discloses:

> [T]he flow cytometer acts to separate the particle or cells (16) by causing them to be directed to one or more ***collection containers*** (15). For example, when the analyzer differentiates sperm cells based upon a sperm cell characteristic, the droplets entraining X-chromosome bearing spermatozoa can be ***charged positively*** and thus ***deflect in one direction***, while the droplets entraining Y-chromosome bearing

> spermatozoa can be ***charged negatively*** and thus ***deflect the other way***, and the wasted stream (that is droplets that do not entrain a particle or cell or entrain undesired or unsortable cells) can be left uncharged and thus is collected in ***an undeflected stream*** into a suction tube or the like . . . .

Id., 17:15-29 (emphasis added); *see also id.,* 54:8-55:4 (claims 11-15).

Claim 1(q): ***charging a stream of said irradiatable sperms cell and sheath fluid based on the amount of emitted and detected fluorescence from each of said sperm cells in said sheath fluid flow;***

See Claim 1(p) above.

Claim 1(r): ***forming a charged drop having one of said sperm cells located therein;***

See Claim 1(p) above.

Claim 1(s): ***isolating said charged drop from said sheath fluid flow;***

See Claim 1(p) above; *see also* Ex. 1005, 14:5-6 ("The invention involves isolated high purity X-chromosome bearing and Y chromosome bearing populations of spermatozoa or sperm cells.").

Claim 1(t): ***deflecting said charged drops;***

See Claim 1(p) above; *see also* Ex. 1005, 17:19-21. ("In this manner the separation discrimination system or analyzer (13) acts to permit the ***electrostatic deflection*** plates (14) to deflect drops (9) based on whether or not they contain the appropriate particle or cell.") (emphasis added); *id.,* 54:8-55:4 (claims 11-15).

Claim 1(u): sorting said sperm cells; and

See Claim 1(p) above. Additionally, it should be noted that "sorting" sperm cells is the whole object of flow-sorting methods, such as those detailed in Evans. Ex. 1003 ¶ 106; *see also* Ex. 1005 at 6:22-24 ("Another significant object of particular embodiments of the invention can be ***to sort spermatozoa*** into X-chromosome bearing and Y-chromosome bearing populations having high purity even at high separation rates.") (emphasis added); *id.,* 54:8-55:4 (claims 11-15).

Claim 1(v): collecting said sorted sperm cells.

See Claim 1(p) above; *see also* Ex. 1005 at 54:8-17 (claim 11: "differentially collecting each of said droplets based upon said sex differentiation characteristic of said sperm cells entrained in said droplets.").

D. Ground 2: Claims 1-26, 33-34, And 39-41 Are Obvious Over Evans In View Of Piper And In Further View of Tardif.

1. Tardif provides additional motivation to use the claimed staining conditions and pulsed radiation source recited in claim 1.

Claim 1(c):

If any additional motivation was needed to use less than the "maximum" amount of stain—*see* Claim 1(c) above—Tardif provides it. Ex. 1003 ¶ 109. Tardif studied how to improve procedures for evaluating Hoechst 33342 dye-stained bull sperm. Ex. 1006, Abstract. To that end, Tardif analyzed how factors such as dye concentration, dye exposure time, media content, and pulse illumination affected

the sperm. *Id.*, Abstract, 201-202. In Experiments 2 and 3, Tardif reported that frozen-thawed samples of bull sperm stained with 40, 50, and 60 μg/ml of Hoechst 33342 dye and pulse-illuminated for 20 minutes all showed similar sperm motility. *Id*., 202-205; Figs. 1 & 2. Tardif thus concluded that 40 to 60 μg/ml of Hoechst 33342 was the "optimal" range to when staining frozen-thawed bull sperm. *Id*., Abstract.

Thus, Tardif discloses both (i) a ***range*** of "optimal" Hoechst 33342 dye concentrations (*i.e.*, "40 to 60 μg/ml"), as well as (ii) several ***specific*** concentrations falling within that range, namely, 40, 50, and 60 μg/ml. Ex. 1003 ¶ 110. As 60 μg/ml is the highest effective concentration of dye disclosed in Tardif, it is the "maximum stain." *Id*. As such, the disclosed range of "40 to 60 μg/ml" encompasses about 67% to 100% of the maximum stain, which nearly completely subsumes the claimed ranges of "about 60%, about 70%, about 80% or about 90%" maximum stain. *Id*. This alone is sufficient to render the claimed ranges *prima facie* obvious. *See, e.g., In re Wertheim*, 541 F.2d 257, 267 (CCPA 1976) (claimed ranges *prima facie* obvious where they "overlap or lie inside ranges disclosed by the prior art"); *see also In re Geisler*, 116 F.3d 1465, 1469-71 (Fed. Cir. 1997) (prior art disclosure of "not less than about [100 Angstroms]" rendered obvious "50 to 100 Angstroms").

In addition, Tardif's explicit disclosure of staining sperm with 40 and 50 µg/ml of Hoechst 33342 constitutes a ***specific*** disclosure of two of the claimed ranges. Specifically, 40 µg/ml is 67% of the maximum stain disclosed in Tardif, or "***about*** 70%"; and 50 µg/ml is 83% of maximum stain, or "***about*** 80%." Ex. 1003 ¶ 111 (explaining that a POSA would understand 40 and 50 µg/ml to constitute "about 70%" and "about 80%", respectively, of the maximum stain disclosed in Tardif); *see also, e.g., In re Woodruff*, 919 F.2d 1575 (Fed. Cir. 1990) (prior art disclosure of "about 1-5%" deemed to overlap with the claimed range of "more than 5%.").

Moreover, even if 67% and 83% are deemed just outside the claimed concentrations of "about" 70% and 80%, they are sufficiently close to render those concentrations *prima facie* obvious, as there is no indication that the claimed concentrations produce a result different in kind to the disclosed concentrations. *See, e.g.*, *In re Huang*, 100 F.3d 135, 139 (Fed. Cir. 1996) (claimed thickness of 0.180 or more obvious over prior art disclosure of 0.111 to 0.142 where the modification did not "produce a new and unexpected result which is different in kind and not merely in degree from the results of the prior art"); *In re Aller*, 220 F.2d 454, 455 (CCPA 1955) (claimed process performed at 40° to 80°C and an acid concentration of 25% to 70% was obvious over prior art method performed at 100°C and an acid concentration of 10%).

Here, very little experimentation would have been required to decrease the dye concentration from, say, 50 µg/ml (*i.e.*, 83% of maximum stain), as taught by Tardif, to 48 µg/ml (*i.e.*, 80% of maximum stain), as claimed in the '116 patent. Ex. 1003 ¶ 112; *see, e.g.*, *In re Kulling*, 897 F.2d 1147, 1149 (Fed. Cir. 1990) (claimed range obvious where increasing and decreasing concentration was "a matter of routine optimization"). Moreover, there was ample motivation in the art to do so. Ex. 1003 ¶ 112. For example, Johnson—incorporated by reference into Evans—specifically recommended using "minimal" concentrations of dye "to avoid toxicity." Ex. 1007, 4:27-36. Thus, there was incentive for a POSA to experiment with decreasing the dye concentration. Ex. 1003 ¶ 112. Moreover, given the modest differences in dye concentrations between those disclosed by Tardif and those claimed, the POSA would have reasonably expected the claimed amounts to work just as well in distinguishing X and Y chromosomes. *Id*.

Claim 1(i), 1(j), 1(k), and 1(l):

If any additional motivation was needed to use a pulsed radiation source (like that expressly taught in Piper) on fluorescently-stained sperm—*see* Claim 1(i), (j), (k), and (l) above—Tardif also would have provided this. Ex. 1003 ¶ 113. Tardif discloses using a strobed (*i.e.*, pulsed) radiation source to evaluate Hoechst 33342 dye-stained bull sperm. Ex. 1006, Abstract; *see also id.,* 201 (explaining that the instrument is "equipped with strobe light UV illumination to be used with the

DNA-specific Hoechst 33342 stain for CASA [Computer-Aided Sperm Analysis]."). Tardif explained that the advantage of using a pulsed radiation source on stained sperm instead of a conventional continuous radiation source was that it minimized the time sperm are subjected to potentially harmful UV exposure:

> Although continuous exposure of fluorescently stained cells to UV illumination is ***often harmful***, the illumination in the Hamilton Thorne IVOS unit is not continuous. The exposure to the strobe light is about 10 to 15 microseconds, with a frequency of 60 cycles/second, so sperm are exposed ***very briefly***.

Id., 205 (emphasis added). Thus, Tardif not only disclosed the use of a pulsed radiation source to evaluate Hoechst 33342 dye-stained sperm (as is done in flow-sorting), but also provided explicit motivation for substituting the pulsed radiation source for the more conventional continuous radiation source. Ex. 1003 ¶ 113.

In view of the above, in addition to the advantages of a pulsed radiation source taught by Piper, a POSA would have been further prompted by Tardif to use a pulsed radiation source when evaluating fluorescently-stained sperm, such as in Evans' flow-sorting method. *Id*. ¶ 114. More specifically, a POSA would have sought to minimize the harm to the sperm known to be caused by the UV light of a radiation source. *Id*. To that end, the POSA would have been motivated by Tardif to replace Evans' continuous radiation source with a pulsed one. *Id*. Moreover, the POSA would have reasonably expected such a modification to succeed in a flow-

sorting method given Tardif's teaching that it worked well for irradiating and evaluating Hoechst 33342-stained sperm. *Id*.

2. Claims 2-26, 33-34, and 39-41 are similarly obvious over Evans in view of Piper and Tardif.

As detailed below, claims 2-26, 33-34, and 39-41 recite trivial modifications to the method of claim 1. As such, each is also rendered obvious by Evans in view of Piper and (if needed) Tardif.

> ***Claim 2:*** ***A method of flow cytometry sperm processing according to claim 1 wherein said step of detecting an amount of said emitted fluorescence from each of said sperm cells comprises the step of quantitatively detecting an amount of said emitted fluorescence from each of said sperm cells.***

See Ground 1, Claim 1(n) above. Additionally, Evans specifically discloses that, "by measuring the fluorescence emitted by the bound fluorochrome upon excitation, it is possible to ***differentiate*** between X-bearing spermatozoa and Y-bearing spermatozoa." Ex. 1005, 17:9-14 (emphasis added); *see also id.,* 54:8-55:4 (claims 11-15).

> ***Claim 3:*** ***A method of flow cytometry sperm processing according to claim 2 wherein said step of quantitatively detecting an amount of said emitted fluorescence from each of said sperm cells comprises the step of distinguishing between a X-chromosome bearing sperm and a Y-chromosome bearing sperm wherein said X-chromosome bearing sperm emits a different fluorescence from said Y-chromosome bearing sperm.***

See Ground 2, Claim 2 above.

Claim 4: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of sorting said sperm cells comprises the step of rapidly sorting said sperm cells at a rate greater than 500 cells per second.***

See Ground 1, Claim 1(u) above. Additionally, Evans specifically discloses:

> The mean sort rates of live spermatozoa after the above-mentioned conversion was about 40.3 x 10^6/ 4.5 hour sort (i.e. about ***2,500 sorts per second per stream***) at about 90.8% purity with a range of 89% to about 92%. The events per second were 13,000, 15,000, and 19,500 respectively for the three sorts."

Ex. 1005, 21:10-12:13 (emphasis added).

Claim 5: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of sorting said sperm cells comprises the step of rapidly sorting said sperm cells at a rate selected from a group consisting of: greater than 1000 cells per second; greater than 1500 cells per second; greater than 2000 cells per second; and greater than 3000 cells per second.***

See Ground 2, Claim 4 above.

Claim 6: ***A method of flow cytometry sperm processing according to claim 1 and further comprising the step of utilizing a beam manipulator.***

See Ground 1, Claim 1(g) above. As discussed, Evans specifically discloses "a beam splitter (50) to split up the beam into two, or more, components . . ." Ex. 1005, 32:17-19. Evans also discloses a "mirror (57) [that] breaks up the light (44)" (*id.,* 34:2-3), an "objective lens (45)" that can focus the light "onto a detector" (*id.,* 31:11-14), and "additional filters (48), such as color filters" (*id.,* 31:24-25). Each of these elements is a "beam manipulator" as set forth in the '116 patent. Ex. 1001, 8:39-44 ("Beam manipulators may include mirrors, optically reflective or even

refractive mirrors, partially mirrored surfaces, deflectors, beam splitters, refractive objects, lenses, filters, prisms, lenses, or the like.").

Additionally, Piper also expressly discloses using a beam splitter, optical lenses and a prism/diode array—all "beam manipulators" under the patent. Ex. 1009 at 4:27-29, 6:15-16, 10:7-8.

> ***Claim 7:*** ***A method of flow cytometry sperm processing according to claim 6 wherein said step of utilizing a beam manipulator selected from a group consisting of mirrors, deflectors, beam splitters, prisms, refractive objects, lenses and filters.***
>
> *See* Ground 2, Claim 6 above.
>
> ***Claim 8:*** ***A method of flow cytometry sperm processing according to claim 1 wherein said step of detecting an amount of said emitted fluorescence from each of said sperm cells with a detection system.***

See Ground 1, Claim 1(n) above. Additionally, Evans specifically discloses a "particle or cell sensing system involv[ing] at least some type of ***detector*** or sensor (11) which responds to the particles or cells contained within fluid stream (8)." Ex. 1005, at 16-26-28 (emphasis added). Evans also discloses that "the light emitted (31) by laser excitation of ***fluorochrome***(s) bound to the DNA contained within spermatozoa can be ***detected by photomultiplier tubes*** (32) situated at 0 and 90 degrees relative to the flat surface of the sperm head (28) as it flows through the excitation laser beam pattern." *Id.,* 25:25-29 (emphasis added); *see also id.,* 54:8-55:4 (claims 11-15).

Claim 9: ***A method of flow cytometry sperm processing according to claim 8 wherein said step of detecting an amount of said emitted fluorescence from each of said sperm cells with a detection system comprise the step of utilizing a photomultiplier tube.***

See Ground 2, Claim 8 above. Moreover, if any additional motivation were needed to use a photomultiplier tube with a pulsed radiation source, Piper discloses this. Ex. 1009 at 4:36-5:1 ("The collection optics 15 images the collected light onto a detection device 16 such as a ***photomultiplier tube*** where an electrical signal is generated for each incident pulse.") (emphasis added).

Claim 10: ***A method of flow cytometry sperm processing according to claim 1 wherein step of staining said sperm with said fluorescent dye comprises the step of staining said sperm with fluorochrome.***

See Ground 1, Claim 1(d) above.

Claim 11: ***A method of flow cytometry sperm processing according to claim 1 wherein step of multiply subjecting said irradiatable sperm cells to radiation for a first amount of time comprises the step of multiply subjecting said irradiatable sperm cells to radiation having a wavelength appropriate to activate fluorescence in said irradiatable sperm.***

This limitation is inherent in any method that uses a pulsed radiation source to irradiate sperm, thus causing the sperm to fluoresce. Ex. 1003 ¶ 126. Thus, this step is obvious for the same reasons Claim 1 is obvious. *See, e.g.*, analysis of Ground 2, Claim 1(i), (j), (k), and (l); *see also* Ground 1, Claim 1(m).

> ***<u>Claim 12:</u> A method of flow cytometry sperm processing according to claim 11 wherein said step of multiply subjecting said irradiatable sperm cells to radiation having a wavelength appropriate to activate fluorescence in said irradiatable sperm comprises the step of providing a wavelength of said radiation of 355 nm.***

See Ground 2, Claim 11 above. Additionally, while Evans does not expressly disclose irradiating the Hoechst 33343 dye-stained sperm at a wavelength of 355 nm, such would have been obvious. Tardif, for example, discloses that when irradiating sperm cells stained with Hoechst 33342 dye with a pulsed radiation source, a wavelength of 327 to 395 nm should be used:

> For all experiments, the IVOS (version 10 model, Hamilton Thorne Research) equipped with UV illumination was used. This unit has a strobed xenon light source with a double band-pass filter. For fluorescence, the 50% excitation band-pass is ***327 to 395 nm.***

Ex. 1006, 202 (emphasis added). Tardif's disclosure that the excitation range for Hoechst 33342 dye is "327 to 395" nm renders obvious the claimed range of 355 nm, which falls directly in the middle of the disclosed range. *See*, *e.g., Wertheim*, 541 F.2d 257 (claimed range lying inside disclosed range is *prima facie* obvious).

> ***<u>Claim 13:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of staining said sperm with said fluorescent dye comprises the step of said step of staining said sperm with said fluorescent dye comprises the step of staining said sperm for a reduced staining time of less than about 40 minutes.***

See Ground 2, Claim 1(c) above. As discussed, Tardif discloses the use of a pulsed radiation source to irradiate and excite sperm cells stained with Hoechst

33342 dye. Ex. 1003 ¶ 128. More specifically, Tardif describes several staining sperm (for 10, 20, or 30 minutes), and observes that staining for 20 minutes was "optimal". Ex. 1006, Abstract ("In Experiments 2a and 2b … it was determined that 40 to 60 µg/ml of dye in EYGT or WMG, with UV illumination for 20 minutes, was optimal."); *id.,* 202-203 and Figs. 1 & 2 (in Experiments 2a and 2b, sperm were stained with 10, 20, 40, and 60 µg/ml of dye and "incubated at 37°C for 0, 10, 20, 30, and 45 minutes."); *id.,* 203 and Table 2 (in Experiment 3, sperm were stained with 40, 50, and 60 µg/ml of Hoechst 33342 dye and "incubated at 37°C for 20 minutes."); *id.,* 204 ("A dye concentration of 20 µg/ml or greater with an exposure time of 20 minutes was required to provide uniform staining of the sperm heads. No increase in intensity of the stain was detected visually after 20 or 30 minutes.").

Accordingly, a POSA would have been prompted by Tardif to stain the sperm for the "optimal" period of 20 minutes, particularly as staining for longer periods of time did not lead to an increase in the intensity of the stain. Ex. 1003 ¶ 129. The POSA also would have had a reasonable expectation of success in using sperm stained in this manner for flow cytometry applications, which require uniform staining of sperm, given Tardif's teaching that staining sperm in this manner allowed for uniform staining. *Id*.

<u>Claim 14:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of staining said sperm with said fluorescent dye

comprises the step of staining said sperm for a reduced staining time selected from a group consisting of: less than about 35 minutes; less than about 30 minutes; less than about 25 minutes; less than about 20 minutes; less than about 15 minutes; less than about 10 minutes; and less than about 5 minutes.

See Ground 2, Claim 13 above.

Claim 15: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of multiply exciting said irradiatable sperm cells with said radiation comprises the step of sufficiently hitting said sperm with said radiation to cause said irradiatable sperm to emit fluorescence.***

As with claim 11, this claim limitation is inherent in any method that uses a pulsed radiation source to irradiate sperm, thus causing the sperm to fluoresce. Ex. 1003 ¶ 131. Thus, this step is obvious for the same reasons Claim 1 is obvious. *See, e.g.*, analysis of Ground 2, Claim 1(i), (j), (k), and (l); *see also* Ground 1, Claim 1(m).

Claim 16: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of multiply subjecting said irradiatable sperm cells to radiation for a first amount of time comprises the step of multiply subjecting said irradiatable sperm cells to low power radiation of less than 300 mW.***

See Ground 2, Claim 11 above. Additionally, Evans specifically discloses use of a SX MoFlo® flow cytometer with a laser operating at 100 mW of power. Ex. 1005, 21:23-26 ("Upon using the invention, the SX MoFlo® flow cytometer was operated at about 262 volts at the photocathode, with the laser adjusted to about ***100 mW***, a gain of 4X, without the neutral density filter, at about 10,000 separable events per second.") (emphasis added).

Claim 17: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of multiply subjecting said irradiatable sperm cells to radiation for a first amount of time comprises the step of multiply subjecting said irradiatable sperm cells to low power radiation selected from the group consisting of: less than 350 milliwatt; less than 200 milliwatt; less than 175 milliwatt; less than 100 milliwatt; less than 88 milliwatt; less than 50 milliwatt; and less than 25 milliwatt.***

See Ground 2, Claim 16 above.

Claim 18: ***A method of flow cytometry sperm processing according to claim 1 wherein said step of splitting said radiation into at least two light beams comprises the step of subjecting said sperm cells with a reduced power of radiation than which was originally emitted from a laser source.***

See Ground 2, Claim 6 above. Moreover, this claim limitation is inherent in any method that uses a beam splitter to split the laser into two beams. Ex. 1003 ¶ 134. Each beam would necessarily have about half the power as the original single beam. *Id*.

Claim 19: ***A method of flow cytometry sperm processing according to claim 18 wherein said step of subjecting said sperm cells with a reduced power of radiation than which was originally emitted from a laser source comprises the step selecting said reduced power from a group consisting of a half, a fourth and an eighth said originally emitted power.***

See Ground 2, Claim 6 above. Moreover, this limitation is inherent in any method that uses a beam splitter to split the laser into two beams. Ex. 1003 ¶ 135. Each beam would necessarily have about half the power as the original single beam. *Id*. If those beams are in turn split, then each of the four resulting beams would necessarily have about one-fourth the power as the original single beam, and so on. *Id*.

<u>Claim 20:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of detecting an amount of said emitted fluorescence from each of said sperm cells comprises the step of distinguishing between a X chromosome bearing sperm and a Y chromosome bearing sperm wherein said X chromosome bearing sperm emits a different fluorescence from said Y chromosome bearing sperm.

See Ground 1, Claim 1(n) above. Additionally, Evans specifically discloses:

> Because X-chromosome bearing spermatozoa contain more DNA than Y-chromosome bearing spermatozoa, the X-chromosome bearing spermatozoa can bind a greater amount of fluorochrome than Y-chromosome bearing spermatozoa. Thus, by measuring the fluorescence emitted by the bound fluorochrome upon excitation, it is possible to ***differentiate between X-bearing spermatozoa and Y-bearing spermatozoa.***

Ex. 1005, 17:9-14 (emphasis added); *see also id.,* 54:8-55:4 (claims 11-15).

<u>Claim 21:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of collecting said sorted sperm cells comprises the step of collecting a sorted population of X chromosome bearing sperm and collecting a sorted population of Y chromosome bearing sperm.

See Ground 1, Claim 1(v) above. Additionally, Evans specifically discloses that "the flow cytometer acts to separate the particle or cells (16) by causing them to be directed to one or more ***collection containers*** (15)." Ex. 1005, 17:15-29 (emphasis added); *id.,* 54:8-17 (claim 11: "differentially ***collecting*** each of said droplets based upon said sex differentiation characteristic of said sperm cells entrained in said droplets.") (emphasis added).

> ***<u>Claim 22:</u> A method of flow cytometry sperm processing according to claim 21 wherein said step of collecting a sorted population of X chromosome bearing sperm and collecting a sorted population of Y chromosome bearing sperm comprises the step of collecting said populations at a high purity, wherein said high purity is selected from a group consisting of: greater than 85% purity; greater than 90% purity; greater than 95% purity; greater than 96% purity; and greater than 98% purity.***
>
> *See* Ground 2, Claim 21 above. Additionally, Evans specifically discloses:
>
> > The mean sort rates of live spermatozoa after the above-mentioned conversion was about 40.3 x 10^6/ 4.5 hour sort (i.e. about ***2,500 sorts per second*** per stream) at ***about 90.8% purity with a range of 89% to about 92%.*** The events per second were 13,000, 15,000, and 19,500 respectively for the three sorts.

Ex. 1005, 21:10-12:13 (emphasis added); *see also id.,* 27:6-8 ("High purity (90% or greater) of the X-chromosome bearing and Y-chromosome bearing populations can be established at even these high sort rates.").

> ***<u>Claim 23:</u> A method of flow cytometry sperm processing according to claim 21 and further comprising the step of providing high resolution of said sorted sperm, said high resolution selected from a group consisting of: greater than 7.0; greater than 7.5; greater than 8.0; greater than 8.5; greater than 9.0; and greater than 9.2.***
>
> *See* Ground 2, Claim 21 above. Additionally, while Evans does not disclose particular resolution numbers, a "significant object" of Evans' invention was to "increase the apparent resolution of chromatograms or histograms resulting from sorting fluorochrome stained sperm . . . having small differences in emitted light flux upon excitation of the bound fluorochrome(s)." Ex. 1005, 8:15-18. Moreover,

in comparing the resolution of a pulsed laser against that of a continuous wave ("CW") laser like the one used by Evans, the '116 patent discloses that the CW laser provides a resolution of 7.6 (as compared to a resolution of between 8.0 to 9.8 for the pulsed laser). Ex. 1001, 29:40-58. Thus, the '116 patent establishes that the CW laser by Evans inherently "provid[es] high resolution of said sorted sperm . . . of greater than 7.0." Moreover, based on the '116 patent's teaching, substituting a pulsed laser in place of Evans' CW laser would only have led to higher resolutions, not lower. *See id.; see also* Ex. 1003 ¶ 140.

> ***Claim 24:*** ***A method of flow cytometry sperm processing according to claim 21 wherein said step of collecting a sorted population of X chromosome bearing sperm and collecting a sorted population of Y chromosome bearing sperm comprises the step of collecting said populations at a high collection rate selected from a group consisting of: greater than 2400 sperm per second; greater than 2600 sperm per second; greater than 2900 sperm per second; greater than 3000 sperm per second; and greater than 3100 sperm per second.***

See Ground 2, Claim 21 above. Additionally, Evans discloses that "[h]igh speed sorting of spermatozoa at rates of about 4,000 to about 10,000 sorts of each sex per second can be accomplished." Ex. 1005, 27:5-6; *see also id.,* 21:10-13 (disclosing "about 2,500 sorts per second per stream"); 22:15-16 (disclosing "separation rates or sort rates exceed[ing] 4,800 sorts per second average at 12,000 events per second") *id.,* 80:11-81:2 (claim 147, reciting sperm separation rates ranging from 500 to 11,000 per second).

<u>Claim 25:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of sorting said sperm cells comprising the step of sorting said sperm cells at a low coincidence rate selected from the group consisting of: less than 4400; less than 4000; less than 3700; and less than 3600.

See Ground 1, Claim 1(u) above. Additionally, while Evans does not disclose particular coincidence rates, another "significant object" of Evans' invention was "to provide beam shaping optics which minimizes coincidence of objects within the excitation/detection path." Ex. 1005, 9:11-13. Moreover, in comparing the coincidence rate of a pulsed laser against that of a CW laser like the one used by Evans, the '116 patent discloses that the CW laser provides a coincidence rate of 4380 (as compared to a coincidence rate of between 3500 and 3600 for the pulsed laser). Ex. 1001, 29:40-58. Thus, the '116 patent establishes that the CW laser by Evans inherently "sort[s] said sperm cells at a low coincidence rate . . . of less than 4400." Moreover, based on the '116 patent's teaching, substituting a pulsed laser in place of Evans' CW laser would only have led to lower coincidence rates, not higher. *See id.; see also* Ex. 1003 ¶ 143.

<u>Claim 26:</u> A method of flow cytometry sperm processing according to claim 1 wherein said step of detecting an amount of said emitted fluorescence from each of said sperm cells comprises the step of detecting said sperm cells at an event rate of between about 10,000 to about 60,000 sperm cells per second.

See Ground 1, Claim 1(n) above. Additionally, Evans discloses that "separable event rates" can be "as high as about 35,000 per second." Ex. 1005,

30:5-7; *see also id.,* 19:21-20:5. In one example, "[t]he separable event rate was 22,000, 23,000, and 20,000 respectively for the three sorts." *Id.,* 21:8-9; *see also id.,* 30:24-29 ("[H]igh purity X-chromosome bearing and Y-chromosome bearing populations of bovine spermatozoa can be isolated at high purity of 92% to 93% by achieving sortable event rates of about 15,000-20,000 sortable events per second or higher as described above").

> ***Claim 33:*** ***A method of flow cytometry sperm processing according to claim 1 wherein step of forming a charged drop comprises the step of oscillating said sheath fluid to form said charged drop.***

See Ground 1, Claim 1(p) above. Additionally, Evans specifically discloses oscillating the sheath fluid to form the charged drop:

> By providing some type of ***oscillator*** (6) which may be very precisely controlled through an oscillator control (7), pressure waves may be established within the nozzle (2) and transmitted to the fluids exiting the nozzle (2) at nozzle orifice (5). ***Since the oscillator (6) acts upon the sheath fluid (3)***, the stream (8) exiting the nozzle orifice (5) eventually and ***regularly forms drops (9).***"

Ex. 1005, 16:16-20 (emphasis added); *see also id.,* 76:8-15 (claim 121).

> ***Claim 34:*** ***A method of flow cytometry sperm processing according to claim 1 wherein said step of injecting irradiatable sperm cells into said sheath fluid comprises injected sperm cells selected from a group consisting of mammals, bovine sperm cells, equine sperm cells, porcine sperm cells, ovine sperm cells, camelid sperm cells, ruminant sperm cells, and canine sperm cells.***

See Ground 1, Claim 1(e) above. Additionally, Evans discloses that "spermatozoa from any male of a species of mammal" can be utilized in the invention, "including, but not limited to, spermatozoa from humans and spermatozoa from commonly known animals such as bovids, equids, ovids, canids, felids, goats, or swine, as well as less commonly known animals such as elephants, zebra, camels, or kudu." Ex. 1005, 14:13-21; *see also id.,* 1:8-10. More specifically, most of Evans' own work was done on bovine and equine sperm. *See, e.g., id.,* 11:14-16 ("Figure 5 shows univariate and bivariate histograms illustrating improved resolution between X-chromosome bearing and Y-chromosome bearing populations of ***bovine spermatozoa*** using a particular embodiment of the amplification invention.") (emphasis added); *id.,* 12:46 ("Figure 9 shows univariate and bivariate histograms illustrating the improved resolution between X-chromosome bearing and Y-chromosome bearing populations of ***equine spermatozoa*** using a particular embodiment of the amplification invention.") (emphasis added).

> ***Claim 39:*** ***A method of flow cytometry sperm processing according to claim 1 and further comprising the step of utilizing said collected sorted sperm for insemination of female mammals.***

Evans discloses this step. Ex. 1003 ¶ 147. Specifically, Evans discloses that a "significant object" of this invention was "to preselect the sex of offspring of females inseminated with high purity artificial insemination samples." Ex. 1005,

7:11-14; *see also id.,* 7:5-10; *id.,* 14:22-24 ("High purity separated spermatozoa from the various species of mammals can be incorporated into products that can be used with artificial insemination protocols").

> ***Claim 40:*** ***A method of flow cytometry sperm processing according to claim 1 wherein said steps of multiply subjecting said irradiatable sperm cells to radiation for a first amount of time and multiply terminating said radiation of said irradiatable sperm cells for a second amount of time comprises the step of utilizing a pulsed laser.***

See Grounds 1 & 2, Claim 1(i), 1(j), 1(k), and 1(l), which details how the art, including, *e.g.*, Piper, would have prompted a POSA to utilize a pulsed laser in place of the CW laser used by Evans, as well as a reasonable expectation that the pulsed laser would work at least equally as well as a continuous wave laser. Ex. 1003 ¶ 148.

> ***Claim 41:*** ***A method of flow cytometry sperm processing according to claim 40 wherein said step of utilizing a pulsed laser comprises the step of a utilizing said pulsed laser selected from a group consisting of Nd:YAG and Nd:YVO4.***

See Ground 2, Claim 40 above. Additionally, as discussed above, Piper expressly discloses using a pulsed laser, such as "neodymium:YAG" laser, whose "output may be frequency-doubled to generate high repetition rate pulsed output in the visible [spectrum]." Ex. 1009, 3:17-23. Such devices, Piper predicts, will likely "increasingly dominate in laser applications requiring long-term reliability in the low-power regime; their compact size and projected low cost are especially appropriate to applications requiring multiple sources as in flow cytometry." *Id.,*

3:24-27. Thus, it would have been obvious to use an Nd:YAG pulsed laser, like the one taught by Piper, in Evans' flow-sorting method. Ex. 1003 ¶ 149.

E. Ground 3: Claim 12 Is Obvious Over Evans In View Of Piper And Tardif And In Further View Of Lakowicz.

Claim 12 recites irradiating the sperm at a wavelength of 355 nm. As discussed in Ground 2 above, this claim is rendered obvious by Evans in view of Piper and Tardif. Tardif, for example, teaches using a pulsed radiation source on Hoechst 33342 dye-stained sperm at a wavelength of 327 to 395 nm. Ex. 1006, 202. However, should any additional motivation be necessary to irradiate Hoechst dye-stained DNA with a pulsed radiation source at 355 nm, Lakowicz expressly discloses doing so. Ex. 1008, Table 1.

More specifically, Lakowicz discloses using a flow cytometer to differentiate fluorescently-dyed cells. *Id*., Abstract; 1:10-12. Lakowicz stains the cells with Hoechst 33342 and irradiates them from a periodically pulsed laser. *Id.,* 2:11-30; 8:62-64, Figs. 1 and 2; Table 1. The laser "can produce pulses at a repetition frequency in the Mhz-range which is sufficient to illuminate each cell with a pulse of light at least one time as it passes through the observation point of flow chamber 3." *Id.,* 9:1-5; Fig. 2. Importantly, Lakowicz teaches that, for cells stained with Hoechst 33342 dye, the laser should be operated at "***325-355 nm (UV)***," which is the "dye excitation/absorption wavelength" for Hoechst 33342. *Id.*, Table 1.

Thus, a POSA practicing Evans' flow-sorting method utilizing a pulsed radiation source on Hoechst 33342 dye-stained DNA would have known, from Tardif's general disclosure or Lakowicz's more specific disclosure, to use a beam of light providing a 355 nm wavelength of radiation. Ex. 1003 ¶ 152. The POSA would also have had a reasonable expectation of success in exciting Hoechst 33342 dye-stained sperm using light at this wavelength given that it falls right in the middle of the range provided by Tardif and is specifically taught by Lakowicz to be appropriate for exciting Hoechst 33342 dye-stained DNA. *Id*.

F. Ground 4: Claim 38 Is Obvious Over Evans In View Of Piper And Tardif, And In Further View Of Fukuda.

Claim 38 depends from claim 1 and further recites "the step of subjecting said radiation to at least two nozzles of identical flow cytometers." As detailed in Grounds 1 and 2 above, Evans, as modified by Piper and (if needed) Tardif, teaches flow-sorting dye-stained sperm using a pulsed radiation source. Neither of these disclosures, however, expressly mentions irradiating a fluid stream flowing from "at least two nozzles." This feature, however, is expressly taught by Fukuda. Ex. 1010.

Fukuda describes an improved flow cytometer containing two oppositely-opposed nozzles from which a sample fluid may flow into a flow cell. *Id.,* 2:9-46; Figures 1-9. Fukuda teaches that a benefit to using two nozzles is increased efficiency. *Id*., Abstract (explaining that with "two liquid specimen nozzles []

disposed opposite to each other . . . the analysis processing time can be notably shortened."); *see also id.,* 5:21-27 (same). As depicted in Figure 1 of Fukuda, the fluid sheath streams from both nozzles (labeled 18) are irradiated by the "light emitting element such as a laser source" (labeled 29) inside a flow cell (11). *Id*., Fig. 1; *see also id.,* 3:42-4:22 (Embodiment 1).

Accordingly, a POSA seeking to increase the efficiency of Evans' flow-sorting method, would have been motivated by Fukuda to modify the method by increasing the number of nozzles used to disperse the sheath fluid and sample. Ex. 1003 ¶ 155. The POSA would also have had a reasonable expectation of success in using the modified two-nozzle flow cytometer given Fukuda's disclosure of a working example. *Id*. Of course, the POSA would also have known how to make any necessary modifications to Fukuda's two-nozzle approach should such be necessary to maximize the use of two nozzles for flow-sorting sperm. *Id*.; *see also KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 420-21 (2007) ("[I]n many cases a person of ordinary skill will be able to fit the teachings of multiple patents together like pieces of a puzzle. . . . A person of ordinary skill in the art is also a person of ordinary creativity, not an automaton.").

IX. CONCLUSION

For the foregoing reasons, Petitioner respectfully requests that trial be instituted and that claims 1-26, 33-34, and 38-41 of the '116 patent be cancelled.

Dated: November 30, 2017

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CERTIFICATION OF WORD COUNT UNDER 37 CFR § 42.24(d)

Pursuant to 37 C.F.R. § 42.24, I certify that the foregoing **PETITION FOR *INTER PARTES* REVIEW** contains 13,934 words (as calculated by the word processing system used to prepare the Petition), excluding the parts of the Petition exempted by 37 C.F.R. § 42.24(a)(1).

Dated: November 30, 2017

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CERTIFICATE OF SERVICE

I hereby certify that on this 30th day of November, 2017, a copy of this **PETITION FOR *INTER PARTES* REVIEW** has been served by Federal Express at the following address for the Patent Owner:

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