DEVELOPMENT OF A PROCEDURE FOR DIELECTROPHORETIC (DEP) SEPARATION OF SPERM AND EPITHELIAL CELLS FOR APPLICATION TO SEXUAL ASSAULT CASE EVIDENCE

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ABSTRACT

A critical step in the successful DNA analysis of most sexual assault cases is the effective separation of male sperm and female epithelial cells, which are typically collected on a vaginal swab at the hospital soon after the event. In the differential extraction procedure currently used by most forensic DNA analysts, the swab containing both cell types is re-hydrated, cells are collected and a two-step differential lysis is performed. In the first step, the epithelial cell fraction is removed by a mild chemical lysis (detergent and proteinase), leaving the majority of the sperm heads intact. Cell separation relies on the more robust nature of the sperm head membranes, in particular, on the use of a chemical agent (e.g., DTT) in the second step to reduce disulfide bonds to assist in digesting the sperm membranes. Although the preferential lysis extraction is, by and large, effective, it is labor intensive and not particularly amenable either to automation or to incorporation into the microfluidic devices that are being examined for forensic applications. To address these issues, we began an investigation of the use of dielectrophoresis (DEP) for separating sperm and epithelial cells. DEP is the movement of cells in the presence of a non-uniform electric field. In particular, we investigated the use of a commercially available DEP system, the Silicon Biosystems SlideRunner-DEPSlide™ system, for separating sperm and epithelial cells in a microfluidic, chip-based format. Our results, based on microscopic inspections, demonstrated that DEP can be used to separate sperm and epithelial cells into pure fractions. However, at this stage of development, the standard chemical differential extraction procedure is faster and provides better purity and yield in the sperm cell fraction than the DEP procedures we have examined so far.

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Statement of the Problem:

Sexual assault evidence comprises a large portion of the casework handled in U.S. crime laboratories. A critical step in the successful DNA analysis of most sexual assault cases is the separation of male sperm and female epithelial cells. This separation step, successfully performed, increases the probability for obtaining a clean DNA profile for the male component of the sample, which, in turn, leads to increased success rates for human identification. Current practice in most crime labs is to separate the sperm and epithelial cells using a "differential extraction" method that relies on chemical differences in the proteins that comprise the sperm and epithelial cell membranes. This extraction procedure, which consists of a sequence of labor-intensive digestion, centrifugation, and wash steps, has, by and large, served the forensic adequately for over two decades. However, this manual approach is not readily adaptable to higher-throughput operations (e.g., for automation on a liquid-handling robotic platform), nor is it ideally suited for the kind of reduced-scale operations that will be required for microfluidic-based, "point-of-contact" devices that are currently under development for forensic DNA analysis. Consequently, there is a motivation to examine new approaches for separating sperm and epithelial cells, approaches that could be more readily adapted to high-throughput and/or reduced-scale analysis methods.

Purpose of the Study:

The goal of this study was to examine a chip-based approach, dielectrophoresis (DEP), as an alternative for separating sperm and epithelial cells for sexual assault samples. In the DEP approach, separations rely on differences in the motion of cells when they are placed in a non-uniform electric field. DEP forces on a cell depend upon precisely how electrical charges on a cell re-distribute in the presence of the applied field. Due to differences in cellular size, as well as differences in chemical composition and membrane structure, dissimilar cell populations (e.g., sperm cells vs. epithelial cells) will respond differently to the non-uniform electric field, resulting in differences in DEP mobility that can allow for separation of the populations. To this end, a variety of DEP-based cell separation methods have been developed as research tools for cell biology and medical diagnostic applications. For application to sexual-assault evidence samples, DEP has several potential advantages relative to the standard differential extraction procedure, including: (i) separation in a reduced-scale, microfluidic-compatible format; (ii) adaptability for "hands-off" high-throughput separations; and (iii) increased yield and purity of the sperm-cell fraction. Our purpose was to examine DEP as a method for separating sperm and epithelial cells in comparison to the standard differential extraction procedure.

Design of the Study:

Initially, the study was organized into the following stages: (i) selection and setup of a suitable DEP platform; (ii) establishing DEP conditions appropriate for separating fresh sperm and epithelial cells; (iii) proof-of-principle experiments on mock samples; (iv) optimization and extension of the method to authentic samples; (v) validation. However, our progress did not extend beyond stage (iii), and, consequently, our discussion of the experimental design does not include stages (iv) and (v).

Several criteria factored into our selection of the Silicon Biosystems DEPSlide™ System as the most suitable platform for the study. The system was commercially-available, needing to be supplemented only by a separately purchased microscopic imaging system for documenting the studies. The DEPSlide™ system could handle reduced sample volumes (~5 uL, rather than mL-scale volumes) that would be appropriate for the handling of sexual assault samples containing relatively few sperm cells. The entire separation procedure takes place on a chip that has an active area that is ~1 cm^2 with an active volume of ~15 uL. Moreover, the technical staff at Silicon Biosystems had performed initial experiments demonstrating that the separation of sperm and epithelial cells on the system might be possible, and they were willing to pursue further efforts to aid in modifying separation conditions or components to improve the efficacy of cell separations.

DEP conditions (applied electric field strengths and frequencies) for separating sperm and epithelial cells were based on recommendations by technical staff at Silicon Biosystems, and were based on their experiences developing procedures for other applications.

DEP protocols were tested using mock sexual assault samples consisting of mixtures of previously frozen sperm and female epithelial (buccal) cells suspended in a proprietary isotonic buffer solution. Typically, 5-6 uL of the cell mixture was used per separation, with concentrations of ~400 cells/uL for sperm and ~400 cells/uL for epithelial, as determined by hemacytometry. The protocols were evaluated in several ways. Protocols were evaluated first by using visual methods, i.e., by capturing and inspecting still and time-lapse microscopic images of the cells on the DEP chip as the separation proceeded. These visual inspections were useful for identifying factors (e.g., immobility of cells on the DEP chip) that could reduce the efficiency of the separation. For further evaluations, the separated sperm and epithelial cell fractions were collected, and the DNA from each fraction was extracted and quantified using a custom real-time quantitative PCR (qPCR) assay that was capable of simultaneously measuring total human and male-specific DNA quantities. For some separations, STR profiles were also obtained for the separated fractions. In order to evaluate the effectiveness of the DEP approach, equivalent portions of the same cell mixtures were separated "side-by-side" using the standard differential extraction method so that quantification (or STR) results could be compared directly.

Findings and Conclusions:

Two basic approaches were examined for separating the sperm and epithelial cells using DEP. In the "Left-Right" (LR) approach, a multistep procedure developed by Silicon Biosystems, DEP forces were used to separate the sperm cells onto one side of the microfluidic chip and the epithelial cells onto the other side of the chip. Microscopic examinations indicated that this approach was successful in physically separating the mixture into nearly pure sperm cell and epithelial cell fractions. However, we were unable to successfully remove the pure fractions from the chip. That is, the act of removing the fractions appeared to re-mix the sperm and epithelial cells, in particular reducing the purity of the sperm cell fraction. In addition, the LR approach required ~2.5 hours for each separation, a significant amount of time considering the non-parallel nature of each separation (i.e., one separation is performed at a time). The principal cause for the long separation time was that the DEP forces on the sperm cells are weak, due to the small sizes of these cells. Based on these results, we concluded that though the LR approach had demonstrated the principle of using DEP to separate sperm and epithelial cells, this approach would not be practical to consider for implementation unless the separation time was shortened and the sampling/mixing problem was solved.

In order to address the sampling/mixing issue, Silicon Biosystems designed a new DEP chip to include two additional holes ("arm" holes) on one side of the chip. Using currently available DEP technology, however, it was not feasible to increase the DEP-induced mobility of the sperm cells so as to reduce the separation time of the LR protocol.

Consequently, we began to examine a second separation approach that relied solely on moving the epithelial cells. These cells, due to their larger sizes, experience much stronger DEP forces and give rise to much faster DEP-induced mobilities than sperm cells. In this second approach ("E-Cell Depletion" or (ECD)), the idea was to use DEP to move *only* the epithelial cells to one side of the chip. The concentrated epithelial cells would then be removed from the chip, leaving only sperm cells, which would be washed from the chip in a subsequent step. The main advantage to this "depletion" approach is speed; the epithelial cell fraction could be concentrated in less than an hour. The disadvantage of this approach is that there would inevitably be loss of some portion of the sperm cell fraction to the epithelial cell fraction. Our initial studies indicated that ~30-50% of the sperm cells would be lost to the epithelial cell fraction. These results, coupled with the ~1-hour separation time, suggested that the ECD approach may not be a practical alternative to the standard, chemical differential extraction, which, though tedious and time-consuming, can be performed in parallel and generally provides acceptable purities and yields for sperm-cell fractions.

Implications for Policy and Practice:

This project has demonstrated the *principle* of using DEP to successfully separate sperm and epithelial cells in mock sexual assault samples. However, in our hands at least, DEP has not yet been demonstrated to be a practical alternative for implementation

in a casework laboratory setting. With further developmental efforts to improve the speed of the cell separation, as well as to improve the ability to robustly retrieve pure cell fractions from the DEP chip, it is possible that DEP could become a viable alternative to the standard differential extraction.

Implications for Future Research:

Related directly to the work described in this report, there are several areas that could be investigated to improve separations using the DEPSlide™ system. One area is to improve the procedure for removing the DEP-separated cells from the chip. Some work in this area, using the "arm" chip design, was initiated in our study, but more work could be done to optimize a procedure for removing cells from the chip in a way that is efficient, user-friendly, and that does not reduce the purity of the separated fractions. Another area that deserves further work would be to investigate ways to reduce cellular adhesion in the DEP chips. Reduced adhesion would improve both purity and yields. And a final area would be to work on reducing the time needed for separating the cell fractions.

More generally, though, if DEP is to be implemented in the future for separating sperm and epithelial cells, it seems likely that it would be as one component in a point-of-contact device that would integrate sample collection, cell-separation, DNA extraction, and perhaps even PCR amplification and subsequent detection of suitable genetic markers. Such "lab-on-a-chip" devices are in various stages of development by several research groups, and the issue of separating sperm and epithelial cells is still under investigation. DEP-based methods similar to those described here could play a role in these devices.

I. INTRODUCTION

A critical step in the successful DNA analysis of most sexual assault cases is the effective separation of male sperm and female epithelial cells, typically collected on a vaginal swab at the hospital soon after the event. After collection, the swab sample is dried and later transported to the crime laboratory for analysis. For analysis, the swab containing both cell types is re-hydrated, cells are collected, and a two-step differential lysis is performed (Gill, 1985). This differential lysis of the two cell types is made possible by the presence of protein disulfide bonds in the sperm head membrane, bonds that give the membrane proteins increased resistance to enzymatic hydrolysis by proteases and that make the sperm cells more resistant to enzymatic lysis than the vaginal epithelial cells. This difference is exploited by first lysing epithelial cells with a protease/sodium dodecylsulfate (SDS) solution, pelleting intact sperm cells and removal of the lysed epithelial fraction, followed by lysis of sperm cells in a protease/SDS/dithiothreitol (DTT) solution. The sperm cell lysis is facilitated by DTT, which reduces disulfide bonds in sperm membrane proteins. Prior to lysing the sperm cells, they are subjected to one or more wash steps to maximize the removal of epithelial DNA, thus minimizing female fraction carryover into the sperm fraction. This procedure is, by and large, effective and works fairly well in a large number of cases. However, this manual approach is not readily adaptable to higher-throughput operations (e.g., for automation on a liquid-handling robotic platform), nor is it ideally suited for the kind of reduced-scale operations that will be required for microfluidic-based, "point-of-contact" devices that are currently under development for forensic DNA analysis. Consequently, there is a motivation to examine new approaches for separating sperm and epithelial cells, approaches that could be more readily adapted to high-throughput and/or reduced-scale analysis methods.

There have been a number of attempts to develop an improved separation procedure. For example, some researchers have proposed a sperm antibody capture approach, which would allow sperm cells to be removed from a background of epithelial cells, and which, if effective, would allow for separate DNA extraction of the two fractions thus eliminating the problem of "carryover" from one fraction into the other (Eisenberg, 2002; Herr, 2002). This type of antibody cell capture would be amenable to automation. Unfortunately, research on this approach has not, to date, resulted in an effective method. This lack of success is apparently due, in part, to instability of sperm antigens on the cell surface, especially after drying, which leads to instability of binding and sperm loss during washing steps. Another approach has attempted to exploit the size difference of sperm and epithelial cells by using filtration methods. In one example, the mixed sample is chemically lysed (without DTT), then filtered through a matrix with a pore size that captures sperm cells but that allows for the elution of lysed epithelial cells/DNA (Garvin, 2003). This filtration method appears to suffer from a lack of sensitivity, however, as the starting number of sperm cells needs to be very high to yield enough DNA for a successful typing result. A recent report describes the use of a microfluidic device for separating sperm and epithelial cells (Horsman, 2005). In this work the cellular separation is based on differences in physicochemical properties of the sperm and epithelial cells (e.g., morphological, size, density, and surface adsorption differences).

Due to these differences, the sperm and epithelial cells were shown to have different mobility when traveling through a relatively simple, single-channel microfluidic device, and it was demonstrated that differential separation based on these differences is possible. Another approach is to separate sperm and epithelial cells by laser capture micro-dissection (LCM) (Sanders, 2006). Although current LCM methods are somewhat labor-intensive, it is possible that automated procedures for identifying sperm and epithelial cells will improve this approach. A final alternative is to avoid the separation step altogether and to simply target the Y chromosome for typing, using Y-STRs and/or Y-SNPs. Although this approach will likely prove useful for a limited number of samples, particularly those containing very little sperm, it will not be the preferred choice for the majority of cases because overall discrimination of Y chromosome typing is low compared to standard autosomal STR typing.

We report here an examination of the use of dielectrophoresis (DEP) to separate sperm and epithelial cells for the analysis of sexual assault evidence. In the DEP approach, separations rely on differences in the motion of cells when they are placed in a non-uniform electric field. Importantly, DEP forces on a cell depend upon precisely how electrical charges on a cell re-distribute in the presence of the applied field. Due to differences in cellular size, as well as differences in chemical composition and membrane structure, dissimilar cell populations (e.g., sperm cells vs. epithelial cells) will respond differently to the non-uniform electric field, resulting in differences in DEP mobility that can allow for separation of the populations. To this end, a variety of DEP-based cell separation methods have been developed as research tools for cell biology and medical diagnostic applications (Lapizco-Encinas, 2007). Relative to the standard differential extraction procedure, DEP has several *potential* advantages, including: (i) separation in a reduced-scale, microfluidic-compatible format; (ii) adaptability for "hands-off" high-throughput separations; and (iii) increased yield and purity of the sperm-cell fraction. This report describes the application of a commercial DEP system, the Silicon Biosystems DEPSlide^{TM} system, to separate sperm and epithelial cells for the analysis of sexual assault evidence.

II. MATERIALS AND METHODS

The DEP System: Cell separation experiments used the Silicon Biosystems SlideRunner DEPSlide™ system (see Appendix A) configured with either "Finger W25G5" or "Finger W45G5 Arm" DEPSlide™ chips. DEP separations were based on the "Sperm/Epithelial Cells Separation Protocol with Rights & Lefts," as provided by Silicon Biosystems. This procedure is described in detail in Appendix A.

Microscopic Imaging System: Digital images, still and time-lapse, of the cells on the DEPSlide™ were collected using a Nikon Eclipse 80i microscope equipped with a Qimaging Retiga-SRV black-and-white digital camera system running under Nikon NIS-Elements BR (v.2.30) software. A custom xy-stage (Semprex Corp.) was used to fit the Silicon Biosystems chip adaptor to the microscope. Images were collected in epi-illumination mode using a bright-field filter cube. Typical magnifications used 2x, 4x, or 10x objectives.

Mock Sexual Assault Samples: Mock samples were prepared by mixing male sperm cells (previously frozen either as neat semen or in TE^{-4}) with female epithelial (buccal) cells (fresh or previously frozen in isotonic phosphate-buffered saline (PBS)). Typically, mixtures were prepared so that the final concentrations were ~400 sperm cells/uL and ~400 epithelial cells/uL, as estimated by hemacytometry.

DNA Extraction:

Extraction of DEP-separated, mock sexual-assault samples: The epithelial cell fractions from DEP-separated mock sexual-assault samples were extracted using casework "organic" extraction procedures validated at the California DOJ. These procedures consisted of proteinase digestion in a detergent lysis buffer, extraction into buffered phenol:chloroform:iso-amyl alcohol , with a final concentration and clean-up by centrifugation in Microcon100 filters. Sperm-cell fractions were extracted similarly except that the lysis buffer included dithiothreitol (DTT).

"Standard" differential extraction of mock sexual-assault samples: A casework-validated differential extraction protocol, based on differential lysis due to DTT (Gill, 1985), was used for separating the sperm and non-sperm fractions in mock sexual-assault samples. Separated fractions were extracted using organic and Microcon100 centrifugation procedures, as described above.

Direct extraction of mock sexual-assault samples: For control purposes, a portion of the mixed mock sexual-assault samples was extracted directly using organic extraction procedures that included DTT in the initial lysis step, followed by phenol-chloroform and Microcon100 clean-up and concentration. These "direct" extracts served as controls for estimating the initial sperm and epithelial cell concentrations in the mixtures.

DNA Quantification: DNA extracts were quantified using custom triplex or quadruplex qPCR assays that have been described in detail elsewhere (Swango, 2007; Hudlow,

2008). The quadruplex assay includes a total human target ("nuTH01") and a male-specific target ("nuSRY") that allow for estimations of the ratio of male-to-total-human DNA and the ratio of male-to-female DNA.

STR Analysis: STRs were amplified using the Applied Biosystems Identifiler™ kit according to the manufacturer's instructions. The resulting STR amplicons were resolved and detected on an Applied Biosystems 3130 genetic analyzer according to the manufacturer's instructions.

III. RESULTS AND DISCUSSION

A brief introduction to the theory of DEP is provided in Appendix A. This appendix also includes a description of the Silicon Biosystems DEPSlide™ system, showing how the system can be used to concentrate cells in a chip format by the manipulation of moving DEP "cages."

The "Left-Right" (LR) DEP Separation Protocol

The "Left-Right" (LR) protocol was developed by Silicon Biosystems to separate a mixture of sperm and epithelial cells by using DEP cages to move sperm cells to the right-hand-side of a DEPSlide™ chip and epithelial cells to the left-hand-side of the chip. As described in some detail in Appendix A, the protocol consists of 13 DEP steps and requires ~2.5 hours for each run. Figure 1 shows a photograph of a DEPSlide™ at the beginning of a LR separation protocol. The photograph is of a mixture of cells (5uL total volume) that was injected into the left-hand inlet port of the DEP chip. (NOTE: The microscope image in the figure is reversed view; the left-side of the chip is actually shown on the right-side of the figure.) Although only the epithelial cells are visible at the level of magnification shown in Figure 1, there were an approximately equal number of sperm cells present in the mixed sample. After completing the LR run, Figure 2 shows that the sperm and epithelial cells were concentrated onto the right and left sides, respectively, of the DEP chip. This figure demonstrates that the LR DEP protocol, though somewhat slow, was able to successfully resolve a mixture of sperm and epithelial cells into two fractions that were "pure," at least by visual inspection.

For further analysis of the separated cells, samples were removed from the *right*-hand inlet port (the sperm-cell side) in six sequential aliquots, the first four aliquots of 3.5 uL each, the next two of 10 uL each. Ideally, the first and/or second aliquots should consist of nearly pure sperm cells, while the latter aliquots should include increasing amounts of epithelial cells. Each of the six aliquots was separately extracted (including DTT in the lysis buffer), then quantified using a custom quadruplex qPCR assay, and analyzed using the Identifiler™ STR kit. For comparison, a 5 uL sample of the same initial cell mixture underwent the *standard* chemical differential extraction procedure, and a separate 5 uL sample of the same initial mixture was extracted directly (including DTT in the lysis buffer). These extracts were quantified and analyzed as were the DEP-separated extracts.

Figures 3a-3d show the STR results for the extracted samples, where results for the DEP-separated extracts are shown only for the first two 3.5 uL fractions (i.e., for those fractions expected to consist mainly of sperm cells). The top electropherogram in each figure ("mix") indicates that the initial mixture consisted of a roughly 1:1 ratio of sperm to e-cells. The next two electropherograms in each figure show STR results for the sperm fraction ("diff SF") and non-sperm fraction ("diff NSF") from the standard, chemical differential extraction. Clearly, the standard differential procedure provided nearly pure sperm cell and epithelial cell fractions. The final two electropherograms in each figure show results for the first and second 3.5uL aliquots from the DEP run.

Although the first DEP fraction is enriched in the male profile, relative to the initial mixture, the purity of this fraction is not nearly as good as was obtained for the standard differential extraction. As indicated in Figure 4, based on STR peak intensities, the first DEP fraction was enriched to ~68% male DNA, corresponding to a final sperm:epithelial cell ratio of 4:1, starting from ~34% male DNA in the initial mixture, corresponding to an initial 1:1 cell ratio. Based upon visual inspection of the separated sperm cells on the DEP chip, the final ratio (4:1) was much lower than expected. This result suggested that the act of removing the cells from the DEP chip had re-mixed the sperm and epithelial fractions. Figure 5 shows the DNA yields for each of the extracts, based on qPCR. It is clear that the first DEP aliquot gave a lower yield than the standard differential procedure.

Although the LR DEP procedure provided a visually pure separation of sperm and epithelial cells, it became clear that the procedure needed improvement, particularly to increase the purity and yield of the sperm-cell fraction and to reduce the time of the separation procedure.

In further work (not shown), we noticed that the withdrawal of liquid from an inlet port did not result in the homogeneous sampling of the contents of the DEP chip. That is, as shown in Figure 6, it appeared that liquid was being preferentially removed along a central line connecting the left and right inlet ports. Based on this observation, we began to suspect that this inhomogeneous sampling could be the cause of the reduced purity of the sperm-cell fraction in DEP. That is, epithelial cells located on the left side of the chip and on the axial line could be removed from the right-hand hole in preference to sperm cells located on the right side of the chip but away from the central line. Since it appeared that the standard two-hole DEP chip might not be suitable for removing pure aliquots of separated cells, Silicon Biosystems designed a new DEP chip, shown in Figure 7, to include two additional holes on so-called "arms." As indicated in the figure, the advantage of this arrangement for sampling is that displaced liquid covering the arm holes would "sweep" the desired cells into the outlet hole as liquid is withdrawn. Ideally, the liquid and the cells on the opposite side of the DEP chip would remain largely undisturbed, since there would be no mechanism to displace liquid on this side of the chip.

We have not used the arm chips, yet, in conjunction with the LR DEP separation protocol, although such experiments are planned. Instead, as described in the next section, the new arm DEP chips appeared to provide a means for a faster cell separation approach that would rely on moving only the epithelial cells.

The E-Cell Depletion (ECD) Protocol and the "Arm" Chips

One of the major reasons that the LR separation approach requires 2.5 hours for each run is that the sperm cells experience such weak DEP forces that they move very slowly. The weakness of the DEP forces is largely due to the small volume of the sperm cell (see Appendix A); the much larger epithelial cells experience greater DEP forces and can be moved much more rapidly. The availability of the new "arm" chips allowed us to

consider a separation approach that would rely on using DEP forces to move *only* the epithelial cells, then to remove these e-cells from the chip, leaving only sperm cells. An outline of this so-called e-cell depletion (ECD) approach is shown in Figure 8. The successful application of this approach would require addressing the following issues:

i) the ability to reduce adhesion of the epithelial cells on the DEP chip so that these cells remain DEP-mobile and are successfully concentrated for removal from the chip;

ii) the ability to efficiently aspirate and deplete the epithelial cell fraction while at the same time ensuring that the major portion of the sperm cell fraction remains on the "arm" DEP chip;

iii) the ability to remove the remnant sperm-cell fraction by efficient washing of the cells from the chip in the final step of the separation procedure.

We began to address issues ii) and iii) by performing ersatz "separation" experiments using "mixtures" that contained only sperm-cells (no epithelial cells). In this way, we could assess what portion of the sperm cell fraction could be expected to remain on the DEP chip and what portion could be expected to be efficiently removed from the chip in the ECD procedure. Based on these experiments, qPCR results indicated that with the ECD approach, we could expect to lose 30-50% of the sperm-cell fraction to the epithelial-cell fraction. This level of sperm-cell loss, though possibly acceptable for some authentic mixtures, was not promising for further development of the ECD approach.

IV. CONCLUSIONS

Findings

Our experimental results demonstrated that DEP can be used to separate sperm and epithelial cells. Specifically, evidence from visual inspections (captured microscopic images) indicated that the large majority of cells can be separated into relatively pure fractions on opposite sides of a Silicon Biosystems DEPSlide^{TM} chip. However, when the DEP-separated fractions were collected and subsequently characterized by qPCR and/or STR analysis, the purity of the separated fractions was lower than expected based on visual data. Based on our results to date, the quality of the DEP separation, in terms of purity or yield of the separated fractions, was not as good as that obtained using the standard differential extraction.

Implications for Policy and Practice

This project has demonstrated the *principle* of using DEP to successfully separate sperm and epithelial cells in mock sexual assault samples. However, in our hands at least, DEP has not yet been demonstrated to be a practical alternative for implementation in a casework laboratory setting. With further developmental efforts to improve the

speed of the cell separation, as well as to improve the ability to robustly retrieve pure cell fractions from the DEP chip, it is possible that DEP could become a viable alternative to the standard differential extraction.

Implications for Further Research

Related directly to the work described in this report, there are several areas that could be investigated to improve separations using the DEPSlide™ system. One area is to improve the procedure for removing the DEP-separated cells from the chip. Some work in this area, using the "arm" chip design, was initiated in our study, but more work could be done to optimize a procedure for removing cells from the chip in a way that is efficient, user-friendly, and that does not reduce the purity of the separated fractions. Another area that deserves further work would be to investigate ways to reduce cellular adhesion in the DEP chips. Reduced adhesion would improve both purity and yields. And a final area would be to work on reducing the time needed for separating the cell fractions.

More generally, though, if DEP is to be implemented in the future for separating sperm and epithelial cells, it seems likely that it would be as one component in a point-of-contact device that would integrate sample collection, cell-separation, DNA extraction, and perhaps even PCR amplification and subsequent detection of suitable genetic markers. Such "lab-on-a-chip" devices are in various stages of development by several research groups, and the issue of separating sperm and epithelial cells is still under investigation. DEP-based methods similar to those described here could play a role in these devices.

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VI. DISSEMINATION OF RESEARCH FINDINGS

In addition to presentations at the annual NIJ DNA meetings in 2006-2008, aspects of the work described in this report have been presented in poster format at the 2008 annual AAFS meeting:

Buoncristiani, MR; Timken, MD. Dielectrophoretic (DEP) Separation of Sperm and Epithelial Cells, Abstract of Poster Presentation, AAFS Meeting, Washington, DC, Feb. 20, 2008.

VII. FIGURES FOR MAIN BODY OF REPORT

Figure 1: Composite photomicrograph (2X objective) of DEPSlide™ at start of LR separation protocol, after injection of 5uL mixture into inlet side. In the figure, the typical microscope view is shown, with the left- and right-hand sides reversed so that the "inlet side" is actually the left-hand side of the chip.

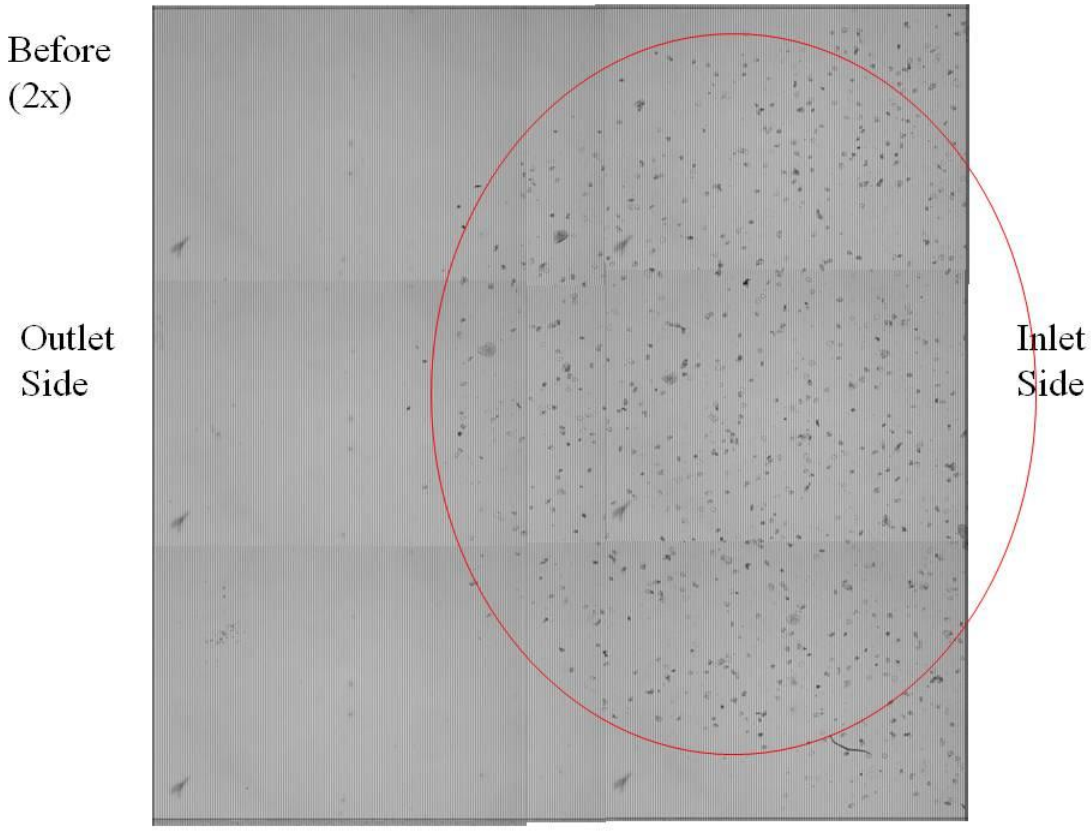


Figure 2: Composite photomicrograph (2X objective) of DEPSlide™ *after* the LR separation protocol. As expected, the epithelial cells were concentrated in a line on the inlet side of the chip (red oval), and the sperm cells in a line on the outlet side of the chip (blue oval).

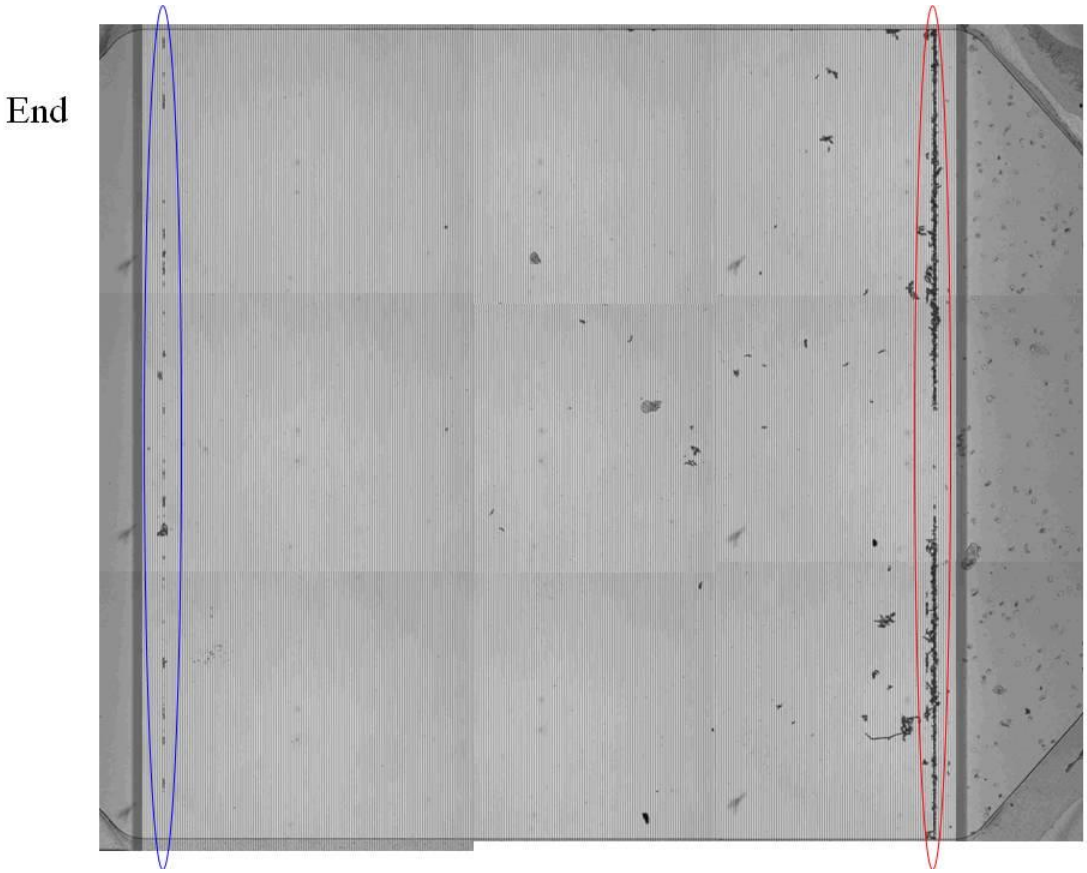


Figure 3a: Identifiler™ STR results ("blue" loci) for LR DEP separation protocol compared to standard differential extraction protocol. The "mix" data is for a direct extraction (using DTT) of the initial mixture of sperm and epithelial cells. The parenthetical "0.8ng" gives the amount used for the 25uL STR amplification. The "diff" samples are the sperm fraction (SF) and non-sperm fraction (NSF) from the standard chemical differential extraction of the mixture. "DEP 1st" represents the first 3.5uL aliquot withdrawn from the chip after the LR DEP separation of the mixture; "DEP 2nd" represents the second 3.5uL aliquot withdrawn.

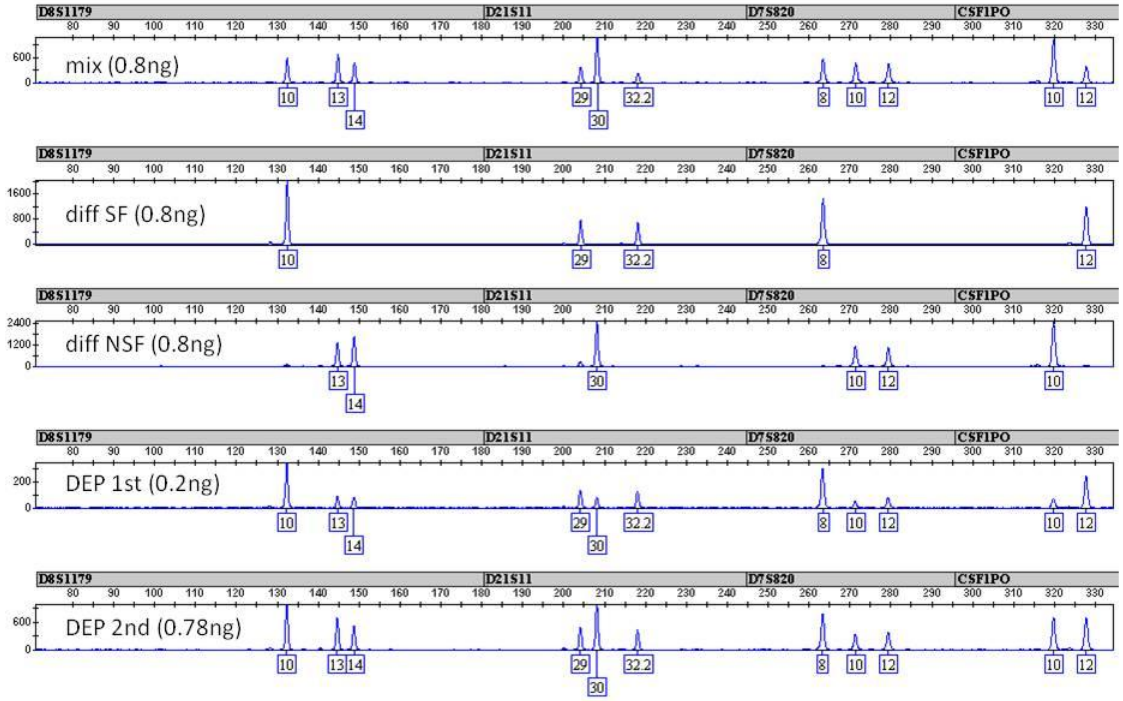


Figure 3b: Identifiler™ STR results ("green" loci) for LR DEP separation protocol compared to standard differential extraction protocol.

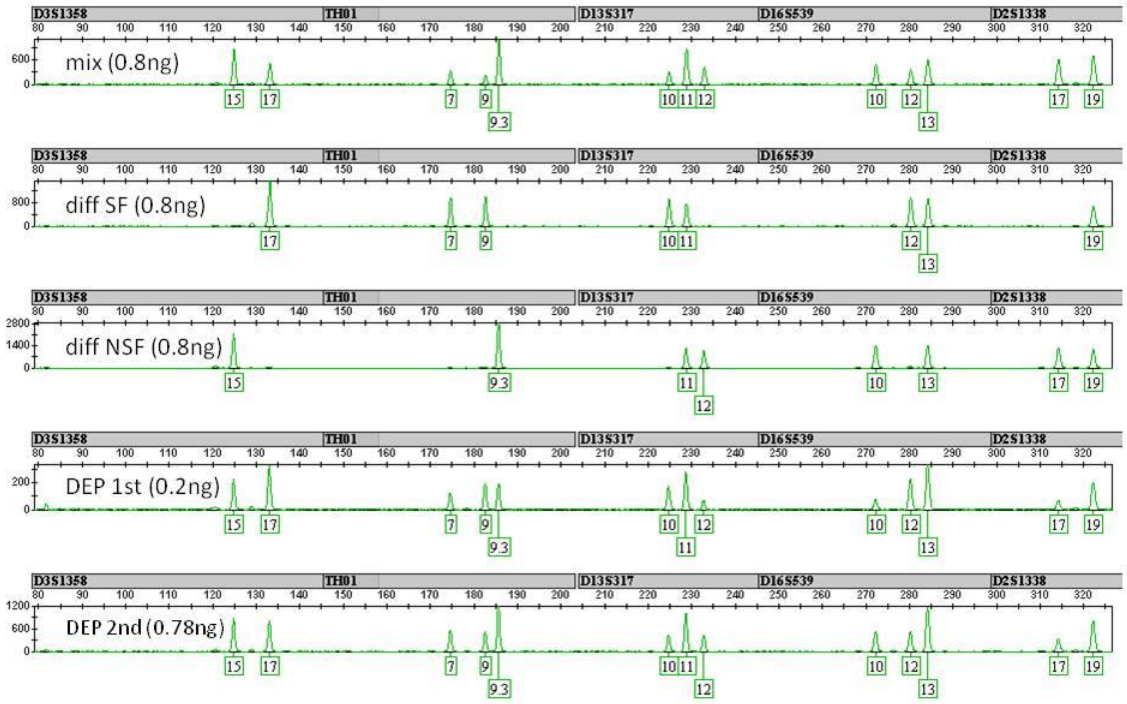


Figure 3c: Identifiler™ STR results ("yellow" loci) for LR DEP separation protocol compared to standard differential extraction protocol.

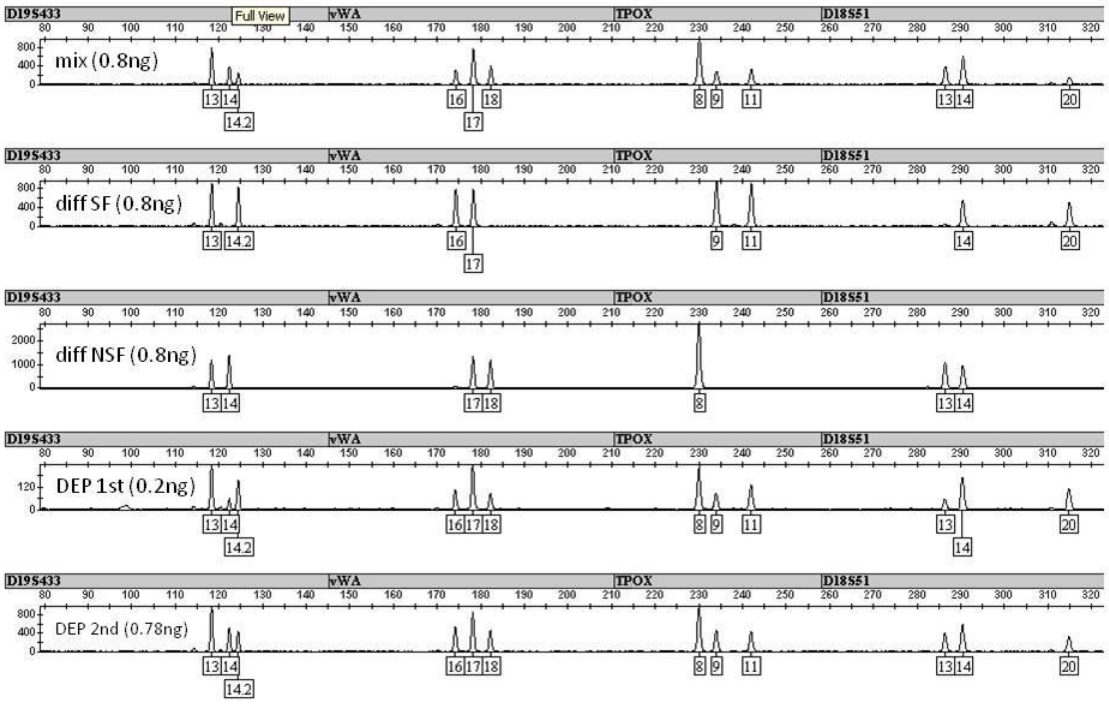


Figure 3d: Identifiler™ STR results ("red" loci) for LR DEP separation protocol compared to standard differential extraction protocol.

Figure 4: Percentages of male DNA in the LR DEP separation experiment, estimated from STR peak heights. The first bar ("MIX") gives the percentage in the initial mixture of sperm and epithelial cells. The next six bars are for the six sequential aliquots from the LR DEP chip. The last two bars (SF and NSF) represent the fractions from the standard differential chemical extraction of the cell mixture.

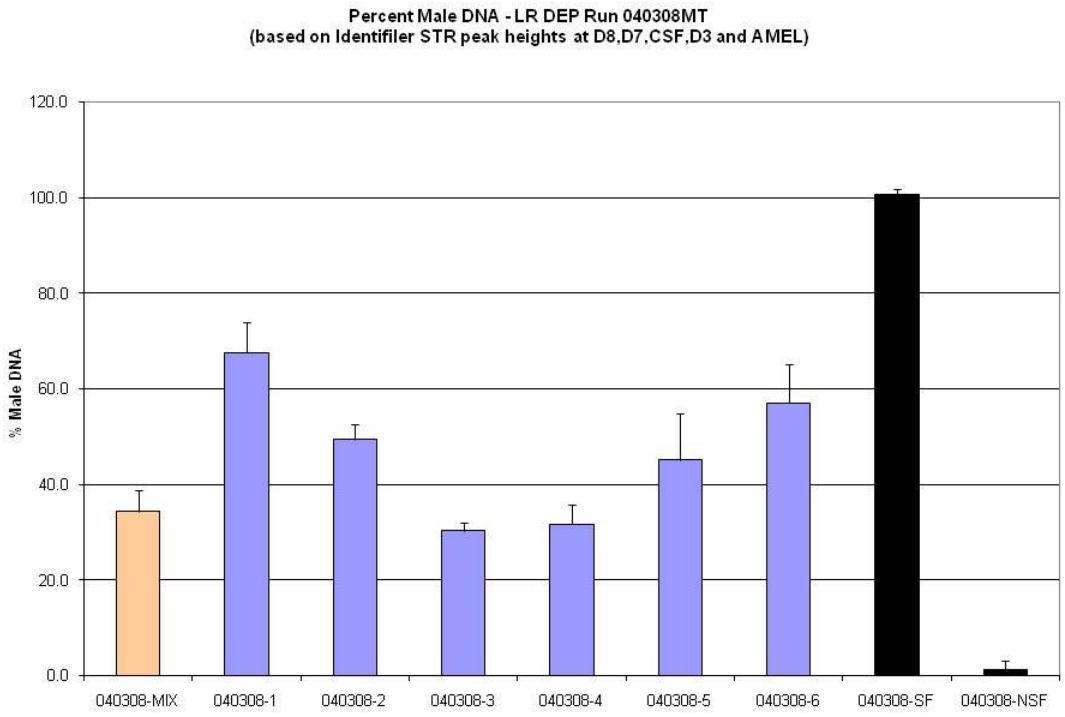


Figure 5: Total human DNA yields, estimated from qPCR data, for the LR DEP experiment. Bars are labeled as described in Figure 4.

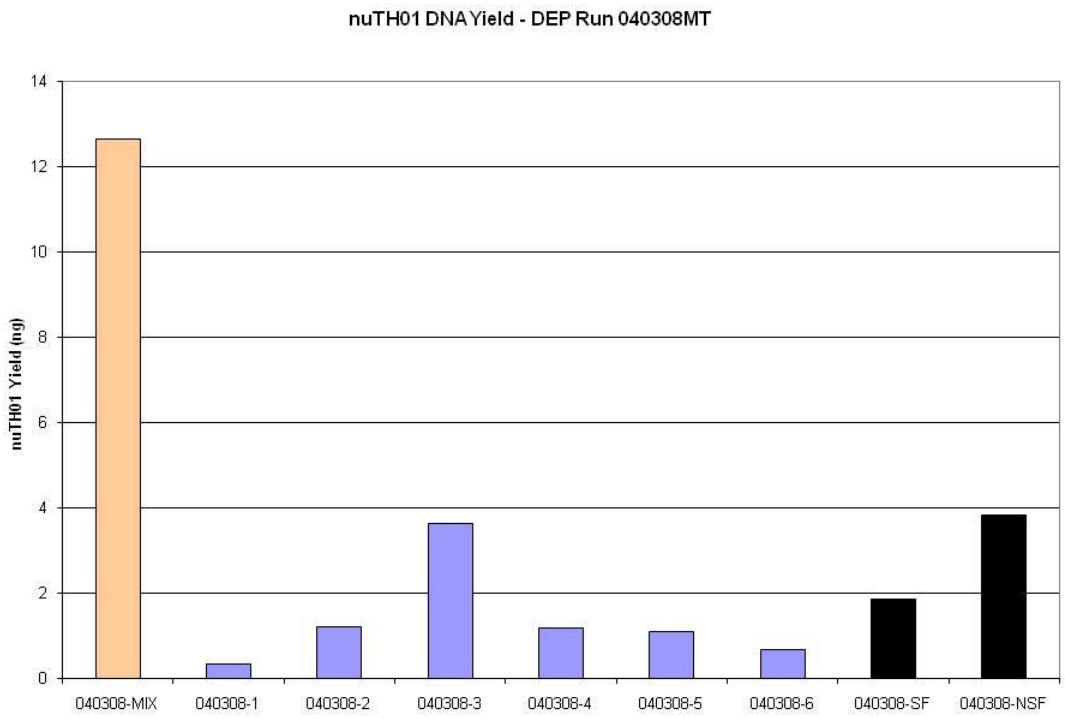


Figure 6: Suspected mechanism for re-mixing of separated sperm (S) and epithelial (E) cells due to a preferential removal of liquid along the inter-hole axis.

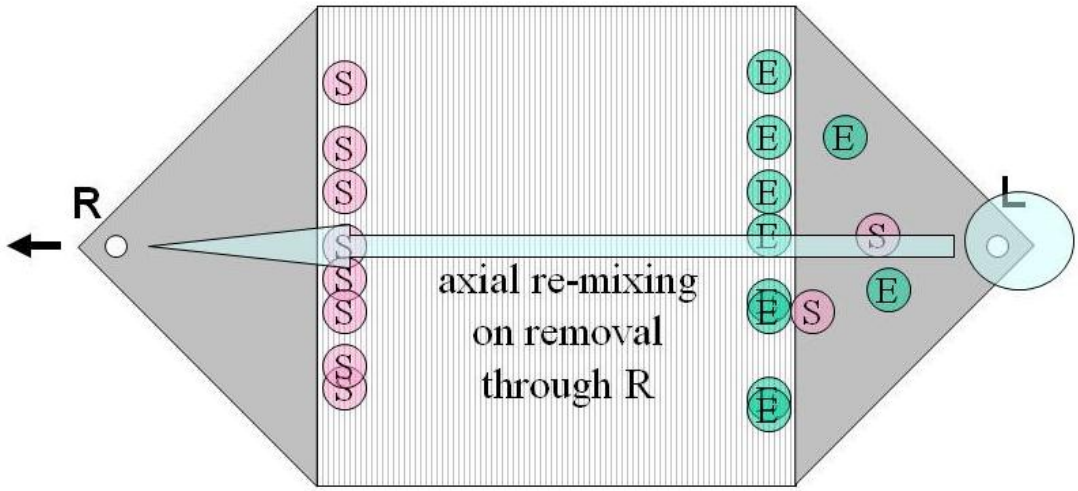


Figure 7: Diagram of DEPSlide™ "arm" chip.

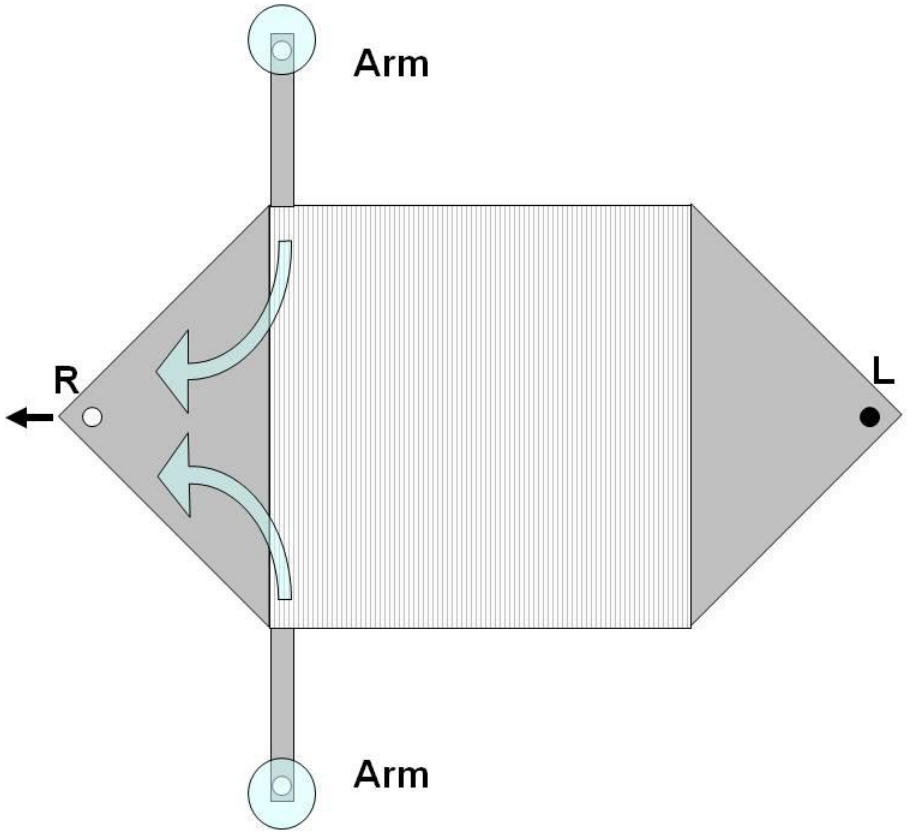
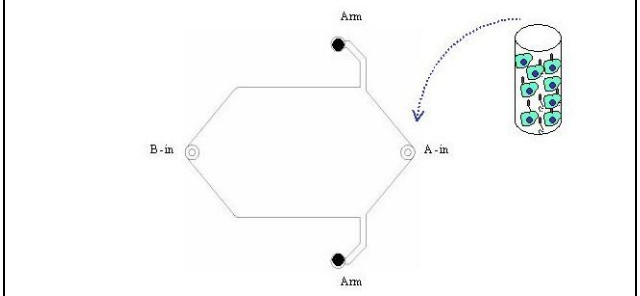
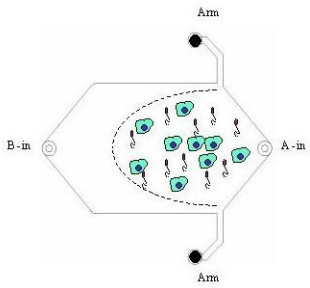
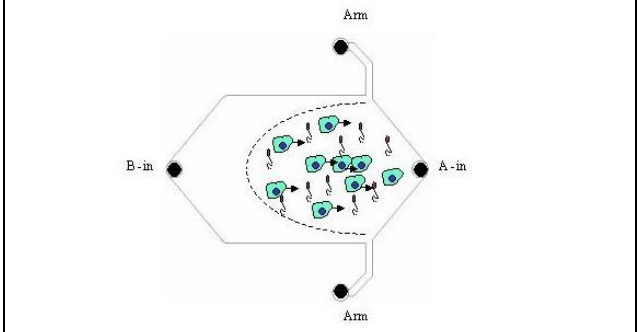
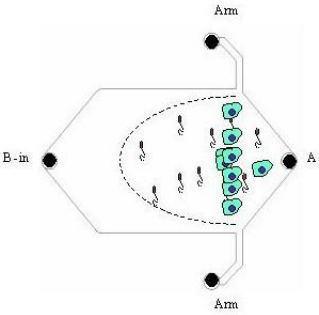
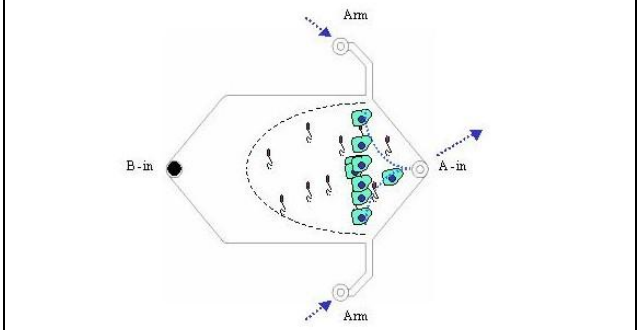
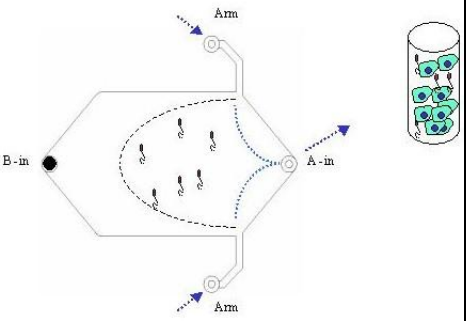
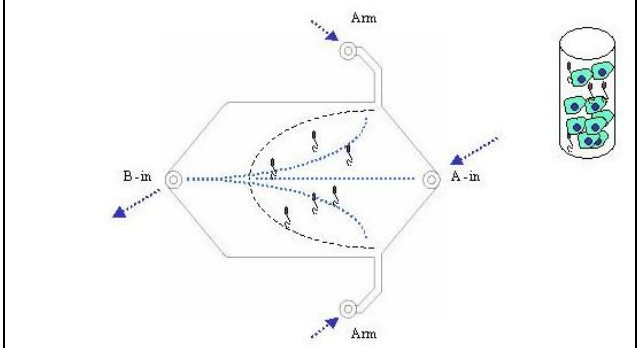
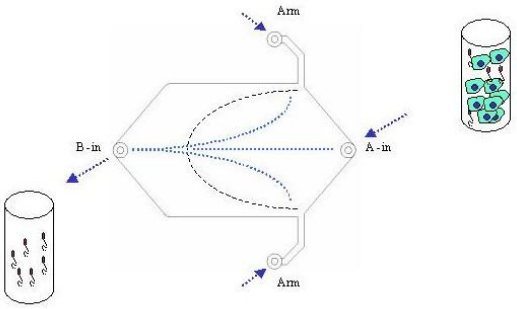


Figure 8: Epithelial Cell Depletion (*ECD*) DEP separation of sperm and epithelial cells (caption/description on next page).

a.		b.	
c.		d.	
e.		f.	
g.		h.	

Caption for Figure 8:

a. Outline of DEP chip (~1cm x 1cm area; ~15uL active volume) showing A-in and B-in sample inlet and outlet holes, as well as the two "Arm" holes on the right side. A ~6 uL volume of mixed epithelial (green) and sperm (red) cells is injected into the A-in hole with both Arm holes closed.

b. After injection, the sample plug of mixed epithelial and sperm cells occupies a parabolic area covering over half the active area of the DEP chip.

c. All holes are closed and the Silicon Biosystems system uses DEP forces (the black arrows) to differentially move the epithelial cells to the right side (towards A-in) of the DEP chip.

d. After DEP, the epithelial cells are concentrated on the right edge of the active area of the DEP chip.

e. The Arm holes are opened and covered with DEP buffer liquid. The A-in hole is opened and ~8uL of DEP buffer is aspirated from the A-in hole. The positions of the opened Arm holes promotes aspiration of those cells primarily at the right edge of the DEP chip (as indicated by the dashed blue arcs shown in the figure).

f. Post-aspiration view of the DEP chip showing e-cell depletion due to enrichment of the aspirated volume in e-cells. Remaining volume of liquid on chip is largely depleted in e-cells.

g. Arm holes and A-in hole are covered with 1%SDS. B-in hole is opened and multiple 10uL volumes are aspirated through B-in.

h. In principle, the multiple aspirations should wash the remaining cells, mainly sperm cells, from the DEP chip. At the end of the procedure, two fractions, one enriched in sperm cells and one in epithelial cells, result for subsequent DNA extraction and analysis.

Appendix A: An Introduction to Dielectrophoresis and the Silicon Biosystems DEPSlide™ System

Basic Principles

Dielectrophoresis (DEP) is the motion of a particle caused by its dielectric polarization in a non-uniform electric field (Pohl, 1978). As shown in Figure A.1, this polarization creates an effective electric dipole on the particle than can interact with a non-uniform electric field to result in a net dielectrophoretic force ($\mathbf{F}_{DEP}$) that causes the particle to move under the influence of the field. Equation A.1 (below) describes the dielectrophoretic force that will develop due to the influence of a non-uniform electric field (**E**) on a spherical particle with radius *r* that is situated in a medium with a dielectric constant (permittivity) ε_m: (Morgan, 1999)

$$\mathbf{F}_{DEP} = 2\pi r^3 \epsilon_m \mathrm{Re}\{K(\omega)\}\nabla|E_{rms}|^2$$

(Equation A.1)

In this equation, Re{$K(\omega)$} is the real component of the Clausius-Mossotti factor ($K(\omega)$) and represents the polarizability of the particle in the medium:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$

(Equation A.2)

In contrast to electrophoresis, the dielectrophoretic force depends upon the polarizability of the particle rather than on its net charge. Consequently, DEP can be used to separate either charged or uncharged particles, whereas electrophoresis can only be used to separate charged particles. Also notice that the DEP force does not depend upon the sign (*i.e.*, the polarity) of the applied electric field **E**. In standard electrophoresis, if the polarity of the electric field is reversed, then the force on the particle will reverse, and the particle will respond by reversing its direction. By contrast, in DEP, when the polarity of the electric field is switched, the particle re-polarizes and the induced dipole re-orients so that the direction of the dielectrophoretic force does not change; the particle will continue moving in its original direction irrespective of the polarity of the field. An important experimental consequence of this difference is that DEP is typically performed using time-dependent alternating current (AC) electric fields (<1kHz to >10MHz), rather than using static direct current (DC) electric fields. The use of AC fields not only removes electrophoretic motion (by effectively time-averaging this motion to zero), but also allows the frequency of the AC field to be used as an independent variable for optimizing DEP separation procedures.

Through the numerator of $K(\omega)$ (see Equation A.2), the sign of $\mathbf{F}_{DEP}$ can be either positive or negative, depending upon the relative magnitudes of the permittivities of the particle (ε^*_p) and of the medium (ε^*_m). If $\varepsilon^*_p > \varepsilon^*_m$, then the force is "positive." This situation is called "positive dielectrophoresis" or pDEP, and corresponds to that previously shown in Figure A.1. Particles experiencing pDEP forces will be attracted to regions of high electric field strength, such as points and edges of electrodes or regions

between electrodes with opposite polarities. On the other hand, if $\varepsilon^*_p < \varepsilon^*_m$, then the DEP force is "negative," and the effect is called "negative dielectrophoresis" or nDEP. Particles experiencing nDEP forces will polarize so that they are attracted to regions of low electric field strength. As shown in Figure A.2, DEP electrodes can be constructed with quadrupolar configurations so that there are inter-electrode regions that have very low or null electric field strengths. Such regions, sometimes called nDEP "cages," can collect or trap particles experiencing nDEP forces. Figure A.3 shows a side-view schematic of the electrode configuration in a Silicon Biosystems DEPSlide™ microfluidic chip. This configuration is similar to the quadrupolar configuration shown in Figure A.2 in that an nDEP cage (the dashed oval) will develop in the region between the lid(+) and any base electrode with a positive voltage. Any particle that experiences sufficient nDEP forces, e.g., the circled "n" in the figure, will be attracted to and "trapped inside" the cages. By manipulating the voltages on the individually adressable base electrodes, the positions of the nDEP cages can be shifted. The result is that the trapped particles will follow the movements of the nDEP cages. Using this principle, the separation of different populations of particles can be achieved if DEP conditions (applied field strengths and/or frequencies) can be found so that one population experiences nDEP forces (and moves with the nDEP cages) while the other population experiences null nDEP forces and so remains stationary. (NOTE: pDEP forces can also be used to manipulate particle movements, although in this case there is a greater tendency for the particles to adhere to the electrodes using pDEP.)

DEP has been used to manipulate and separate such diverse particles as silicon beads, latex spheres, many type of cells, and viruses. (Gascoyne, 2002; Lapizco-Encinas, 2007) As just noted, the separation of two different cell populations relies on finding experimental conditions that lead to different magnitudes or signs of the DEP forces on each population. This is typically possible for different cell populations, because a cell's polarizability depends very sensitively on the cell's size, structure and composition. For example, in Equation A.1, the r^3 term indicates that the magnitude of the DEP force is directly proportional to the cell's volume. (NOTE: The DEP force is also related to the cell's shape, though this difference is not accounted for in the simple spherical model of Equation A.1.) For our DEP-based differential extraction protocol, this term likely plays a significant role in the separation, considering that we are using DEP to separate small sperm cells (sperm head volume <100 μm^3) from much larger epithelial cells (epithelial cell volume >10,000 μm^3). The effective polarizability of a cell, given by Equation A.2, also depends very sensitively upon the structure and composition of the cell. This polarizability represents the frequency-dependent response of the cell to the alternating, non-uniform electric field, *i.e.*, the re-polarization of all of the charges and molecular dipoles on the cell's surface and inside the cell in response to the changing electric field. A variety of cell characteristics influence these re-polarizations, including: the surface morphology (and integrity) of the plasma membrane; the composition of the membrane (lipid composition, presence of electrically charged functional groups); and the internal composition of the cell (cytoplasm composition, nuclear volume and shape). Given the diversity of structures and functions observed for real cells, it is unlikely that any two different cell types will possess identical DEP responses.

The Silicon Biosystems SlideRunner-DEPSlide™ System

Silicon Biosystems has been developing DEP-based separation devices for the past several years. (Manaresi, 2003; Medoro, 2003; Borgatti, 2005) The Silicon Biosystems SlideRunner system is shown in Figure A.4. The heart of the system is the DEPSlide™ chip, which, in the figure, is mounted onto a chip adaptor that itself is mounted onto a microscope stage so that DEP-induced cell movements can be visually monitored. In addition to the DEPSlide™, the SlideRunner system includes a "Power Tower," which is controlled by a programmable DEPSlide™ controller, to provide AC voltages to the ~300 individually addressable electrodes residing on the base of the DEPSlide™ chip, as well as a "Cooling Tower," which is needed to dissipate Joule-effect heat from the chip that is generated during a DEP run.

A close-up photograph of a DEPSlide™ Finger W25G5 chip is shown in Figure A.5, along with a diagram that shows a microscopic view of the electrode arrangement in the chip. The top or "lid" of the chip serves as a single electrode made of glass coated with a thin layer of indium-tin-oxide (ITO), a material that is not only electrically conductive but is also transparent to allow for visual inspection of the chip's contents. The active volume of the chip is accessible, via pipette, by two holes drilled into either end of the lid. These holes are opened and closed simply by using standard office-type transparent tape.

The Silicon Biosystems DEP system can be used to manipulate particle motions based on either pDEP or nDEP forces. For the separation of sperm and epithelial cells, however, our separation procedures were limited to manipulations using nDEP forces. Figure A.6 shows how voltages can be applied to the DEPSlide™ to create nDEP cages at every third base electrode. This figure is directly analogous to the 2-dimensional drawing previously shown in Figure A.3. Figures A.7(a)-(f) show: (a) how a sample is introduced into the chip; (b)-(c) how particles are attracted to the cages due to nDEP forces; (d)-(e) how the particles are conveyed as the nDEP cages shift to the right due to changes in polarities on the base electrodes, and (f) how the particles are concentrated onto the right-hand side of the chip due to nDEP forces. These particles, of course, could just as well have been moved to the left-hand side had the base-electrode polarities been manipulated appropriately.

The "Sperm/Epithelial Cells Separation Protocol with Rights & Lefts"(LR protocol)

Based on these moving nDEP cages, the technical staff at Silicon Biosystems provided a protocol for testing sperm and epithelial cell separations on the W25G5 DEPSlide™ chip. The steps of this protocol, the "Sperm/Epithelial Cells Separation Protocol with Rights & Lefts" (or more simply the "Left-Right" or "LR" protocol), are listed below and are shown in Figure A.8. (In viewing this figure, keep in mind that the chip is shown as it would be seen under a microscope, that is, with the left-hand inlet port ("L") shown on the right side, and the right-hand inlet port ("R") on the left side.)

LR Protocol Steps:

A. Through the left-hand inlet hole ("L"), use a pipettor to fill the active volume of a W25G5 DEPSlide^{TM} chip with DEP buffer (proprietary Buffer 5B).
B. Suspend the sperm/e-cell mixture in Buffer 5B, then add 5 uL of the mixture into the left-hand inlet hole.
C. Close the holes with adhesive tape.
D. Place the chip on the chip adaptor.
E. Turn on the Cooling Tower (set temperature at 10^{o}C). Turn on the Power Tower.
F. Program the DEP Controller to perform the following steps:
    Step 1: "Move Right" by 12 base electrode units
        AC frequency = 200 kHz
        Base Electrode Voltage = 3 V
        Lid Voltage = 2 V
        Wait Time Between Cage Movements = 15 sec
        (used for all "Move Right" steps)
    Step 2: "Move Left" by 140 base electrode units
        AC frequency = 200 kHz
        Base Electrode Voltage = 2 V
        Lid Voltage = 4 V
        Wait Time Between Cage Movements = 3 sec
        (used for all "Move Left" steps)
    Step 3: "Move Right" by 30 units
    Step 4: "Move Left" by 300 units
    Step 5: "Move Right" by 45 units
    Step 6: "Move Left" by 300 units
    Step 7: "Move Right" by 60 units
    Step 8: "Move Left" by 300 units
    Step 9: "Move Right" by 90 units
    Step 10: "Move Left" by 60 units
    Step 11: "Move Right" by 30 units
    Step 12: "Move Left" by 30 units
    Step 13: "Invert Concentration" – concentrate cells to left and right side inlets
        (same settings as for Step 1)
G. Power down the system. Remove the chip from the chip adaptor.
H. Untape the inlet holes. Cover the right-hand inlet hole ("R") with ~10 uL of Buffer 5B.
I. Use a pipette to remove 3.5-10 uL aliquots from the left-hand inlet hole ("L"), adding Buffer 5B to the R hole as this volume is displaced into the chip.

As is evident from Figure A.8, for the "Move *Right*" steps, the DEP voltages and wait times are adjusted so that *both* the sperm and epithelial cell fractions will follow the movement of the nDEP cages. However, for the "Move *Left*" steps, the DEP voltages and wait times are adjusted so that *only* the epithelial cells will follow the movement of the nDEP cages. That is, for the "Move Left" steps, the DEP forces experienced by the sperm cells are insufficient to induce cell motion. By alternating the "Move Left" and

"Move Right" steps, the sperm cells are gradually moved to the right-hand side (R) of the chip, while the epithelial cells are induced to remain on the left-hand side (L) of the chip. In the final step (Step 13), the nDEP cages are manipulated so that any cells on the left-half of the chip will move toward the L inlet port and any on the right-half of the chip will move toward the R inlet port. The times for each step are indicated in Figure A.8. The net time for the separation is ~2.5 hours, largely due to the relatively long wait times (15 sec) needed to move the weakly polarizable sperm cells in the "Move Right" steps.

The idealized cell motion shown in Figure A.8 can be complicated by a number of factors that will generally lead to reduced yields or purities for the cell separation. Cells can become stuck in place, perhaps due to adhesion to the electrodes, and thus unresponsive to DEP forces. Sperm cells can adhere to epithelial cells, resulting in their loss to the epithelial fraction. In addition, if the sperm cells form into clumps, these clumps can respond to the DEP forces as if they were epithelial cells, resulting in their loss to the e-cell fraction.

Appendix A References:

Borgatti, M; Altomare, L; Abonnec, M; Fabbri, E; Manaresi, N; Medoro, G; Romani, A; Tartagni, M; Nastruzzi, C; DiCroce, S; Tosi, A; Mancini, I; Guerrieri, R; Gambari, R. "Dielectrophoresis-based 'Lab-on-a-Chip' Devices for Programmable Binding of Microspheres to Target Cells," *Intl. J. of Oncology*, **27**:1559-1566 (**2005**)

Gascoyne, PRC; Vykoukal, J. "Particle Separation by Dielectrophoresis," *Electrophoresis*, **23**:1973-1983 (**2002**)

Lapizco-Encinas, BH; Rito-Palomares, M. "Dielectrophoresis for the Manipulation of Nanobioparticles," *Electrophoresis*, **28**:4521-4538 (**2007**)

Manaresi, N; Romani, A; Medoro, G; Altomare, L; Leonardi, A; Tartagni, M; Guerrieri, R. "A CMOS Chip for Individual Cell Manipulation and Detection," *Solid-State Circuits, IEEE*, **38**:2297-2305 (**2003**)

Medoro, G; Manaresi, N; Leonardi, A; Altomare, L; Tartagni, M; Guerrieri, R. "A Lab-on-a-Chip for Cell Detection and Manipulation," *Sensors Journal, IEEE*, **3**:317-325 (**2003**)

Morgan, H; Hughes, MP; Green, NG. "Separation of Submicron Bioparticles by Dielectrophoresis," *Biophys J.*, **77**:516-25 (**1999**)

Pohl, HA. *Dielectrophoresis*, Cambridge University Press, Cambridge (**1978**)

Appendix A Figures:

Figure A.1: Simple schematic of DEP.

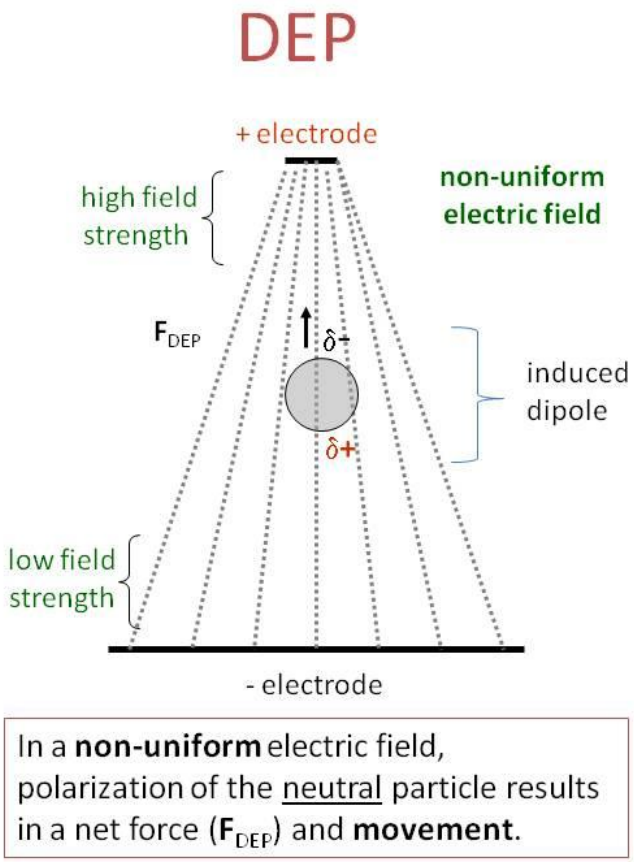


Figure A.2: Quadrupolar electrode arrangement – particles experiencing nDEP forces (encircled "n") will attract to regions of low electric field strength; particles experiencing pDEP forces (encircled "p") will attract to regions of high electric field strength.

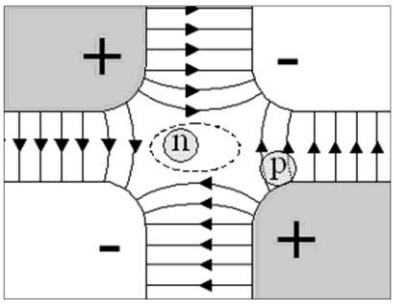


Figure A.3: Simplified side view of the electrode arrangement in a Silicon Biosystems DEPSlide™ chip. nDEP cages are indicated by dashed ovals.

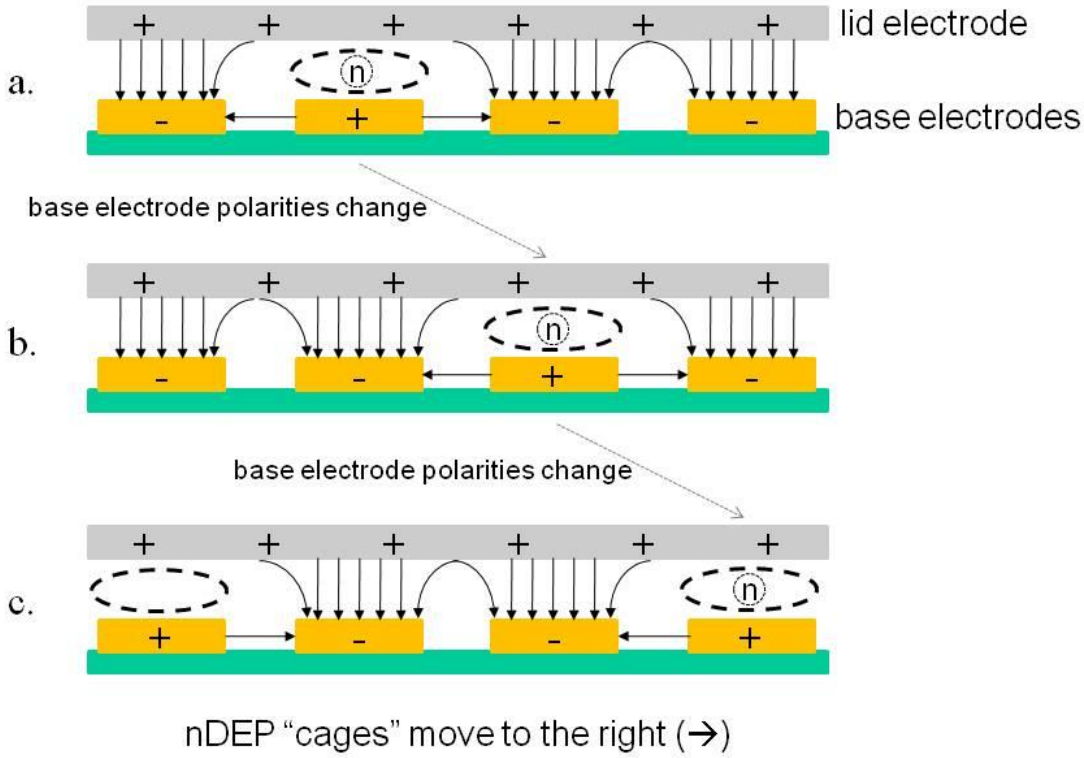
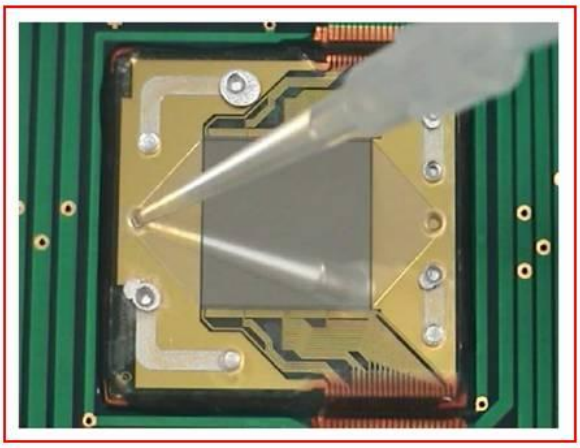


Figure A.4: The Silicon Biosystems SlideRunner-DEPSlide™ system.

Figure A.5: Close-up view of the DEPSlide™ chip.



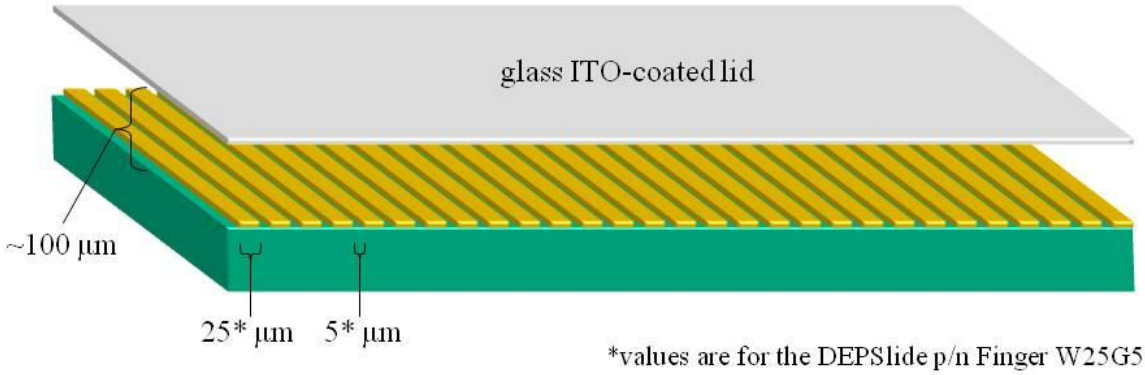
~ 1 cm x 1 cm active chip area

~ 10 µL active volume (~14 µL total vol)

~ 300 programmable electrodes on bottom of chip

- ITO (indium tin oxide) coated glass lid (transparent, conductive)

- inlet/outlet ports on left/right sides of chip



*values are for the DEPSlide p/n Finger W25G5

Figure A.6: Simple view of the development of nDEP cages in the DEPSlide^{TM}.

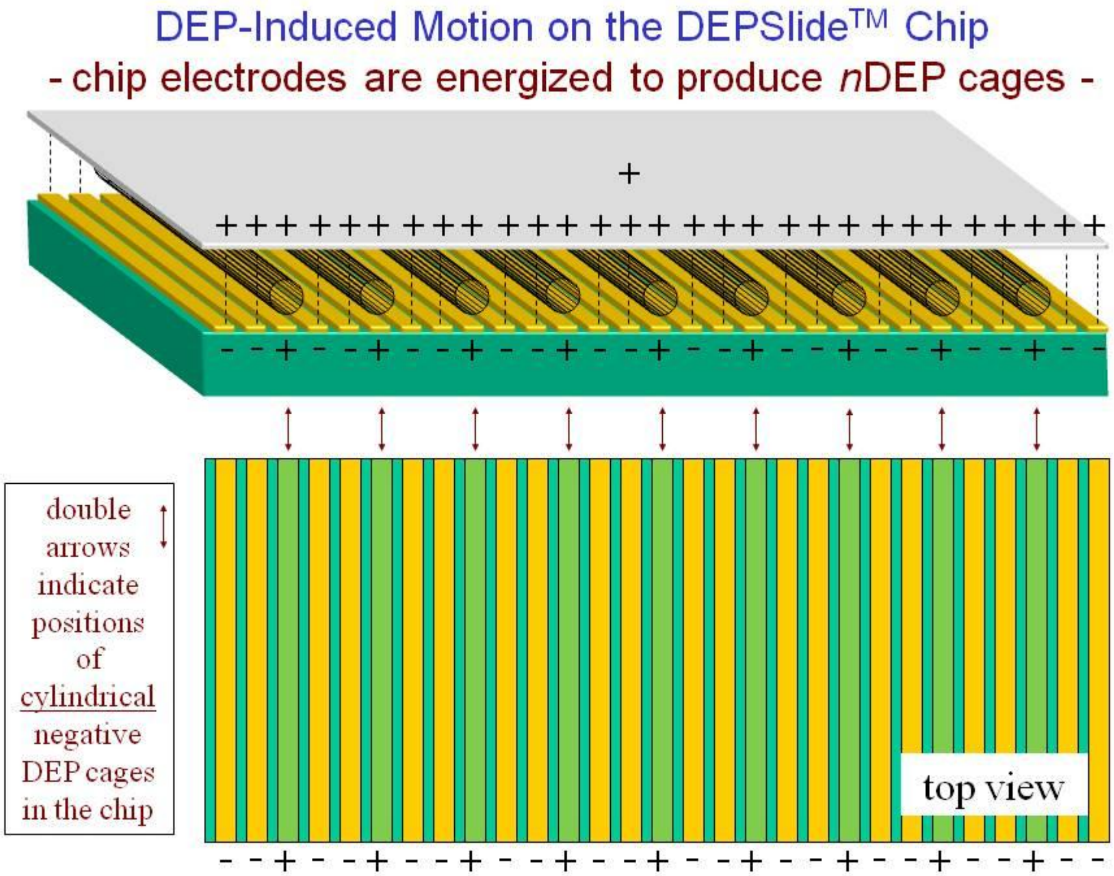


Figure A.7.a: Injection of particles onto the DEPSlide™ chip (no voltages applied).

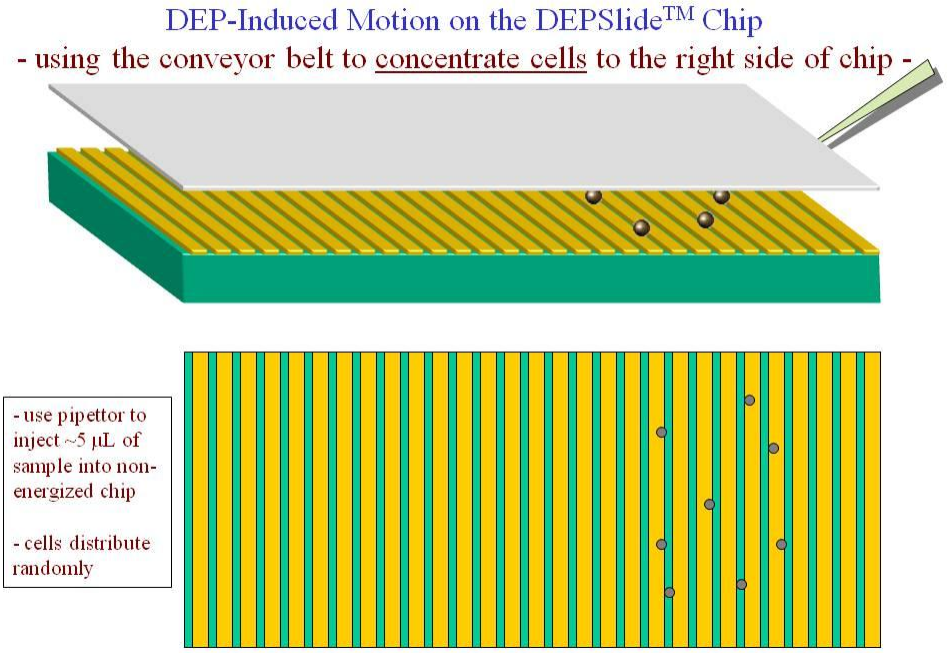


Figure A.7.b: Application of Base and Lid voltages creates nDEP cages.

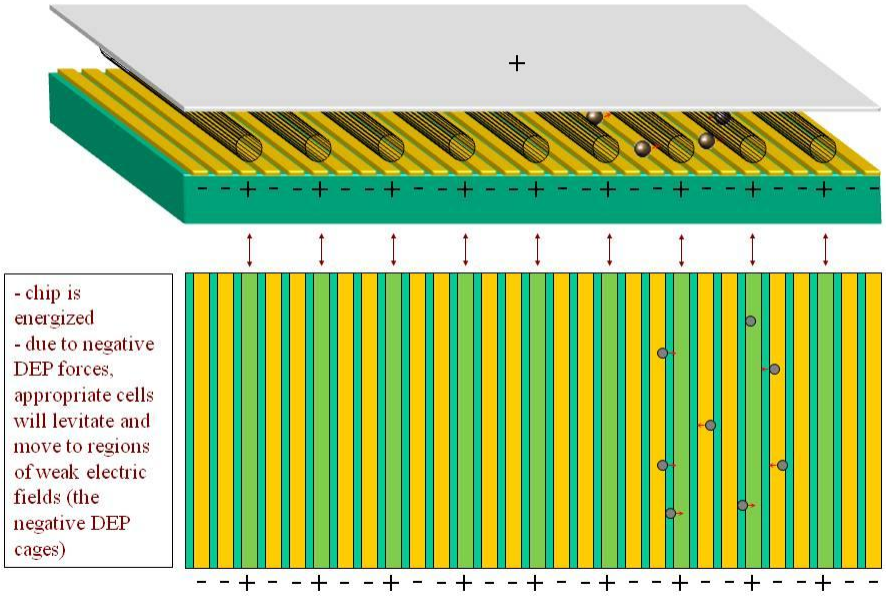


Figure A.7.c: Cells of interest are “trapped” in the nDEP cages.

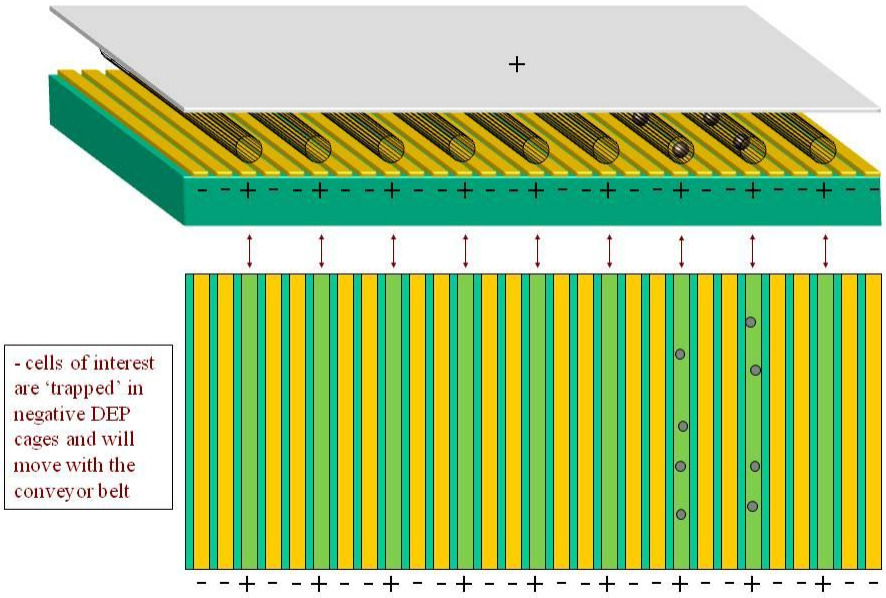


Figure A.7.d: Trapped cells move with the nDEP cages as they shift to the right.

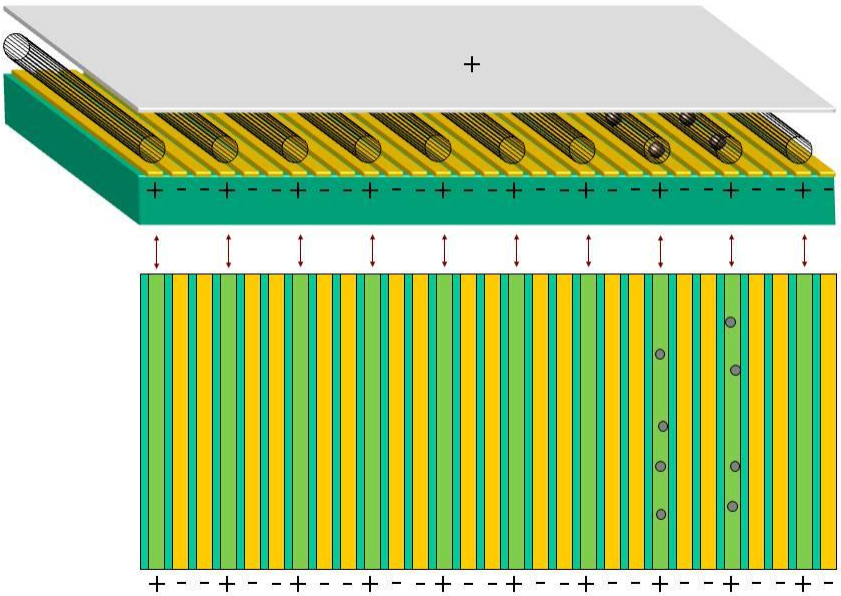


Figure A.7.e: Trapped cells continue to move to the right with the shifting nDEP cages.

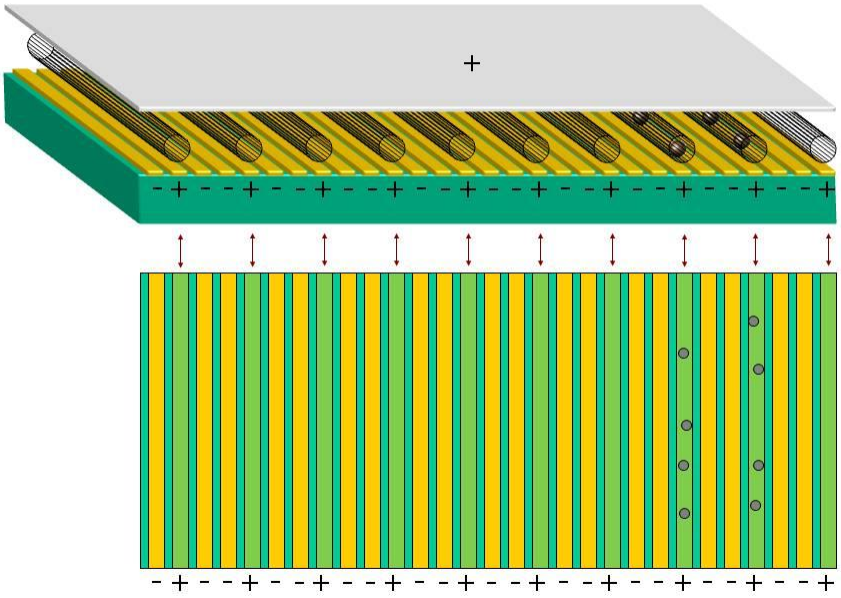


Figure A.7.f: Eventually, the trapped cells are concentrated onto the right side of the chip.

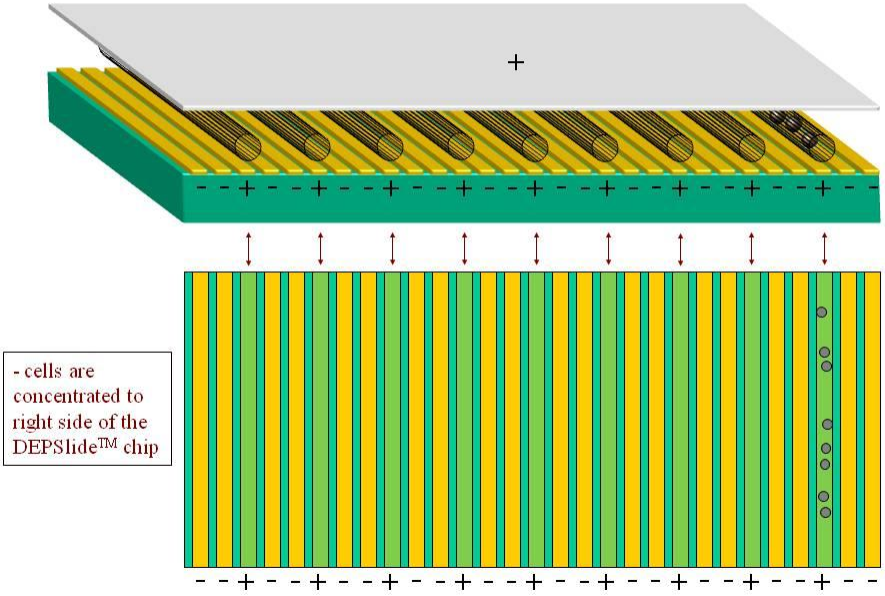
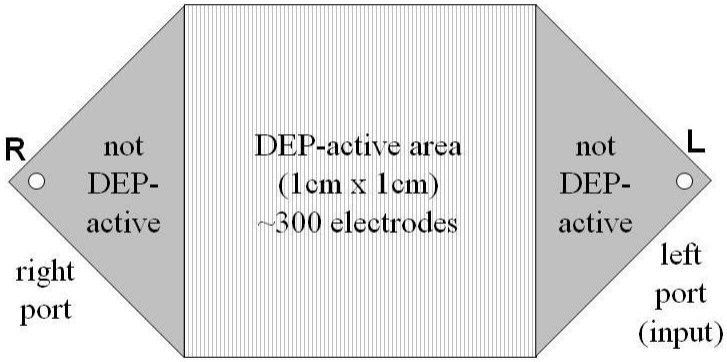
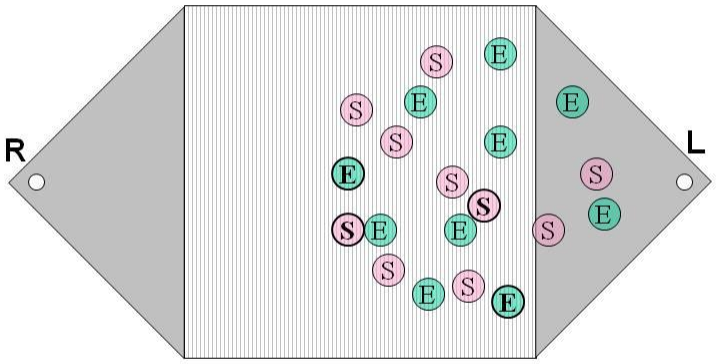


Figure A.8.a: Schematic view of DEPSlide™ (notice that the chip is shown with left and right reversed, as it would be seen under a microscope).



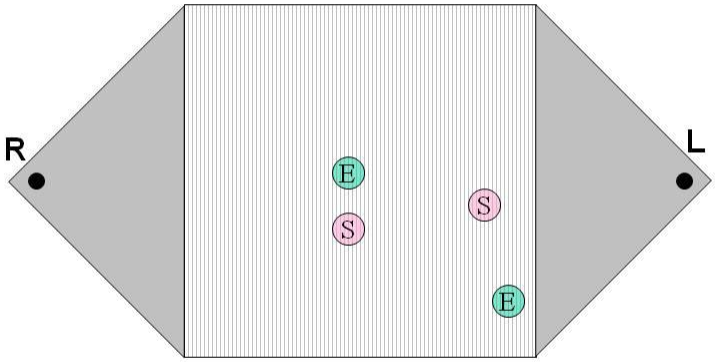
Top View of DEPSlide
(**reverse image, microscope view**)

Figure A.8.b: Distribution of sperm (S) and epithelial (E) cells after injection into L port.



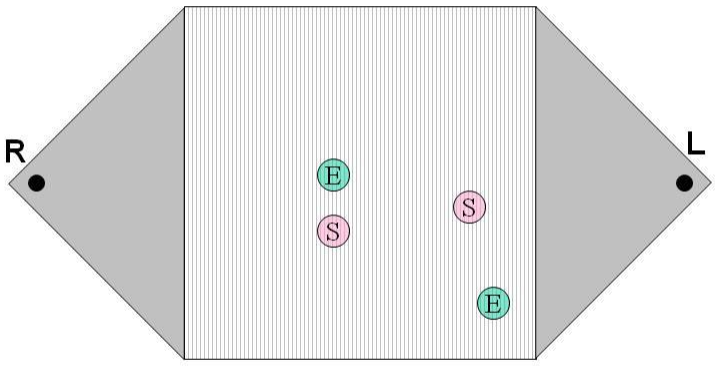
Initial Distribution of Sperm (S) and E-Cells for Separation
(**reverse image, microscope view**)

Figure A.8.c: Same as Figure A.8.b, except focusing on the motions of just **four** of the cells.



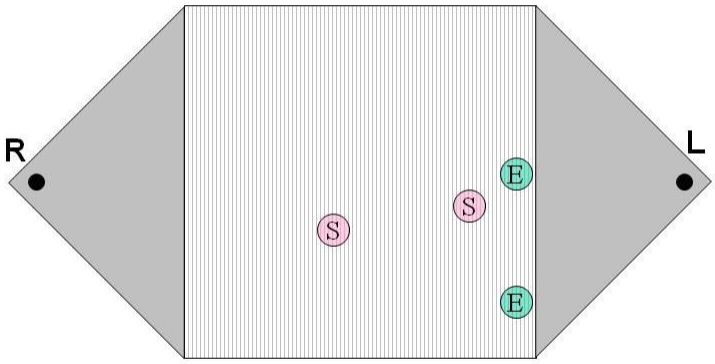
DEP-Induced Movements for 4 Selected Cells

Figure A.8.d: LR Step 1. (This and subsequent figures show idealized motions for LR procedure.)



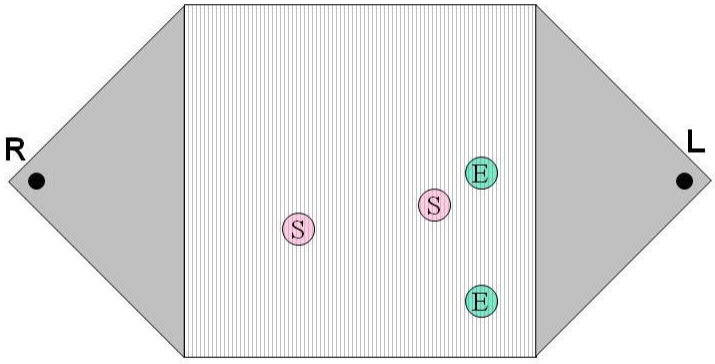
Step 1: All Cells Right (R) by 12 Electrode Steps
(~3 min)

Figure A.8.e: LR Step 2.



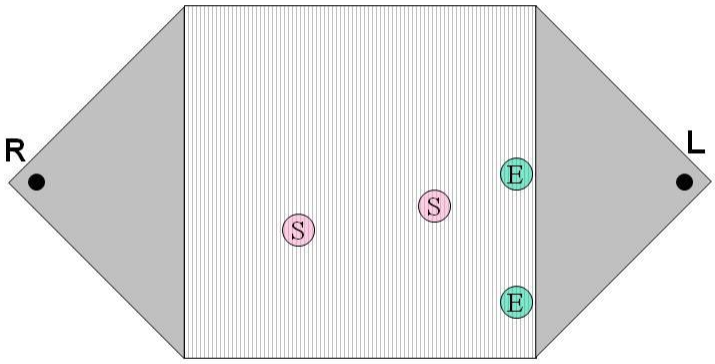
Step 2: E-Cells Only Left (L) by 420 Electrode Steps
(~21 min)

Figure A.8.f: LR Step 3.



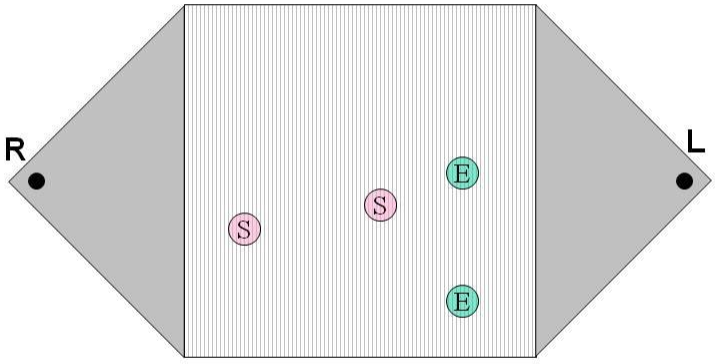
Step 3: All Cells R by 30 Electrode Steps
(~8 min)

Figure A.8.g: LR Step 4.



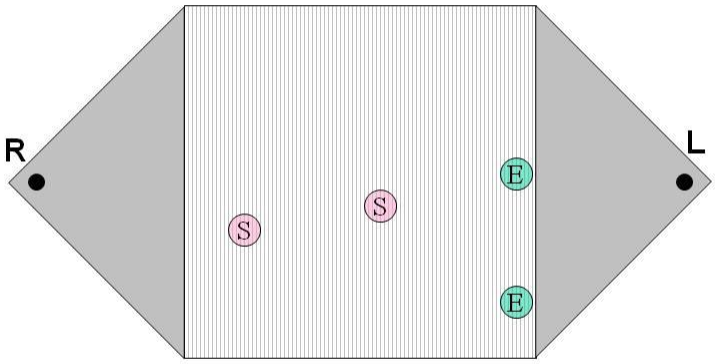
Step 4: E-Cells Only L by 300 Electrode Steps
(~15 min)

Figure A.8.h: LR Step 5.



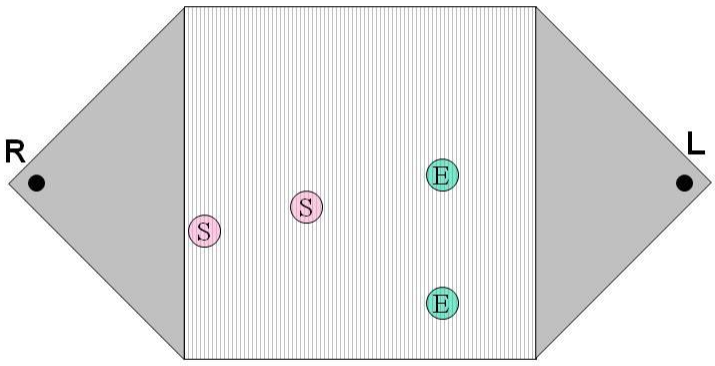
Step 5: All Cells R by 45 Electrode Steps
(~11 min)

Figure A.8.i: LR Step 6.



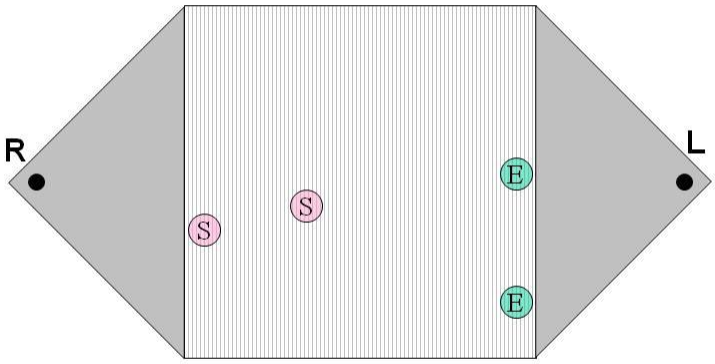
Step 6: E-Cells Only L by 300 Electrode Steps
(~15 min)

Figure A.8.j: LR Step 7.



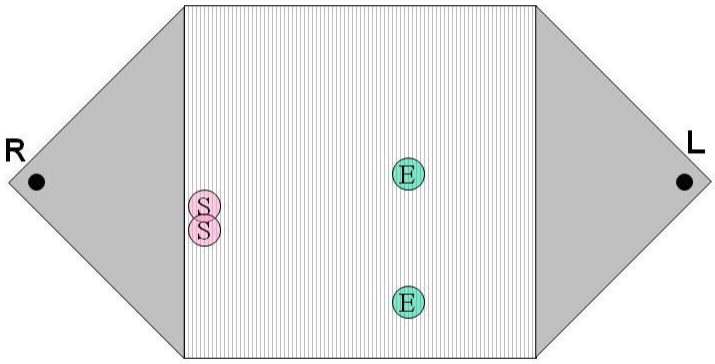
Step 7: All Cells R by 60 Electrode Steps
(~15 min)

Figure A.8.k: LR Step 8.



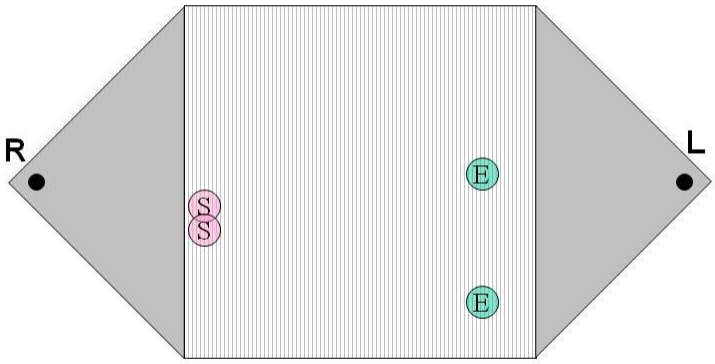
Step 8: E-Cells Only L by 300 Electrode Steps
(~15 min)

Figure A.8.l: LR Step 9.



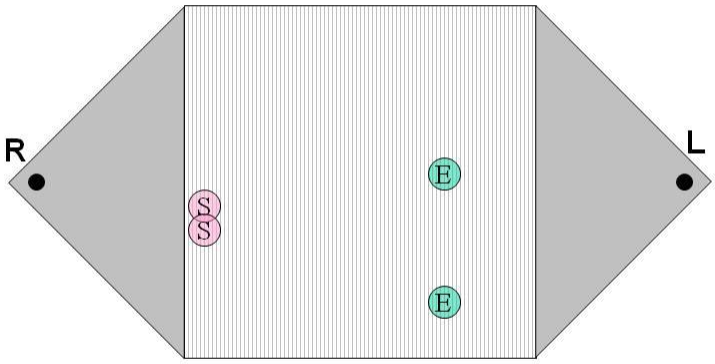
Step 9: All Cells R by 90 Electrode Steps
(~23 min)

Figure A.8.m: LR Step 10.



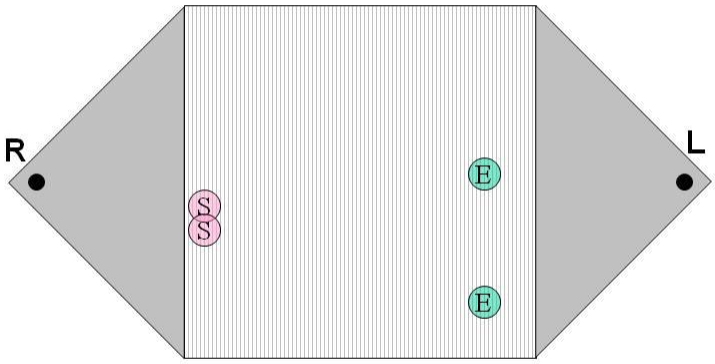
Step 10: E-Cells Only L by 60 Electrode Steps
(~3 min)

Figure A.8.n: LR Step 11.



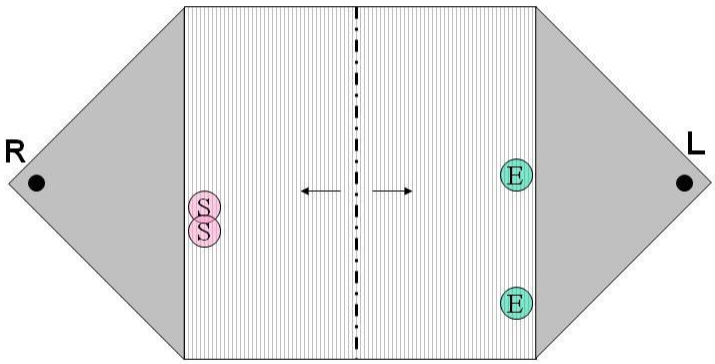
Step 11: All Cells R by 30 Electrode Steps
(~8 min)

Figure A.8.o: LR Step 12.



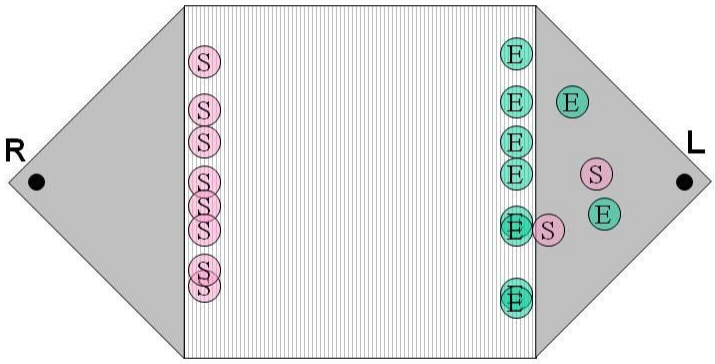
Step 12: E-Cells Only L by 30 Electrode Steps
(~2 min)

Figure A.8.p: LR Step 13.



Step 13: Invert Concentration: Move L-Side Cells L by 55 Steps,
Move R-Side Cells R by 55 Steps (~14 min)

Figure A.8.q: Final distribution of cells after LR DEP separation run. (NOTE: Cells injected into "dead" volume at left-hand-side port did not move.) Sperm cell fraction is removed by withdrawing from the right-hand (R) port.



Final Post-DEP LR Distribution (Ideally) of Cell Mixture
(total DEP time ~ 2.5 hours)