Chapter 5

Sample Implementation

A functional implementation has been developed as a prototype of the stimulation system by integrating a user interface, the application software running in a processor based board, and a signal conditioning circuitry that delivers to the fluidic device signals, patterns and sequences selected by the user. The user interface allows the selection of signal and operation parameters, the based board system runs the extended version of the application software and shows the functionality of the multi-frequency synthesis methodology, and the conditioning circuitry allows the system to deliver analog voltages in a range that is needed in the majority of AC based electro-kinetics in micro fluidic devices. This prototype implementation include all the configurable parameters for a flexible setting that meets the functional requirements described in the standard extended versions, and is also a portable prototype that can be easily moved to different places or labs.

This chapter details the functionality of the extended version of the application software, defines an experiment to be performed with this prototype, show simulation results for a specific type of particles being manipulated, describe and illustrate the experimental environment and, most important, present the potential of this system in referenced research works about experiments and devices where this stimulation system could be used, as a stand-alone stimulation module or as a block to be integrated at chip level.

5.1 The Running Application Program

The extended version of the application program, developed to run on an arm9 or Cortex-M3 based development board –the LM3S6965-. Extended functionality is added, such as delivering data via an USB port for further analysis of monitored or stored data, mixing different waveforms in a

superimposed signal for more controlled experimental environments and an interactive user interface for configuration and operation.

Data tables for the three signal wave forms (sine, triangle and saw tooth) are displayed at start-up for demonstration purposes of the novel frequency synthesis methodology to show the frequency superposition effect. A requirement for lower frequencies was fulfilled by adding a new routine for frequencies smaller than 400 Hz, where a counter creates wait cycles so the time between samples in the output port is extended. A cyclic delivering of same or different signal patterns are delivered for a specific and individual period of time, is also available in the extended version: multiple tests can be done with sequenced stimulation patterns where each pattern may have different parameters such as frequency, samples per cycle, and exposure time. Additionally, when a particular stimulus pattern is found useful, it can be stored and re-used later so the experiment is repeated without having to set the operation parameters.

Table 5.1 presents a routine list and description of the extended version. The appendix A2 presents a documented version of the program code.

Table 5.1 *Routine List and Description, Extended Version.*

Routine	Description
Store waveform data tables	Stores 256 8-bit values for each of the three waveforms: Sine, Triangle and Saw tooth. See Appendixes A for table content.
Get operation parameters	Get operations parameters via the user interface. Displays parameter list and gets input values.
Validate input values	Check for frequency multiplicity in operation mode 2, check for 2-multiple number of data samples, check for frequency out of range.
Check frequency range	Separate operation into low and frequencies at 400 Hz. Low frequencies use up to 256 data samples per waveform cycle; high frequencies use up to 32.
Calculate Data Separation	Desired output frequency and number of voltage steps determine how many data points will be extracted from the original sine table in order to construct desired output signal. Two data separation parameters are needed for operation modes 2 and 3.

Routine	Description
Calculate base time	According to desired frequency and number of voltage steps, there is a base times that indicates the time between data points are sent to output port.
Create Table-Mode1	Extract data from original sine table needed to construct 1 output signal, 1 single frequency: each 8-bit data from original sine table is stored as the 8 least significant bits of the 32-bit output port.
Create Table-Mode2	Extract data from original sine table needed to construct two output signals, two separated frequencies: if two 8-bit output ports can be stored at the same time with 32-bit data, two data points from original sine tables must be concatenated before stored in buffer memory table.
Create Table-Mode3	Extract data from original sine table needed to construct 1 output signal, two superimposed frequencies: data points for different frequencies should be added to achieve superimposition.
Time match	When in superposition mode, does a time match between data samples for frequencies f_1 and f_2, since period and time between samples are different. See appendix A.
Clock set	Set clock for system and parallel port control from board main oscillator.
Configure port	Enable and configure parallel port A as output, as 8-bit set, output driving current.
Off-line monitor	When in off-line mode, data in final output table is displayed to monitor frequency superposition methodology.
Output Signal Generation, High frequencies.	Continuous, uninterrupted loop, for loading data from buffer memory and storing it on output ports. No memory other than buffer is read, no instructions other than those for signal generation are executed.
Output Signal Generation, Low frequencies.	Loading data from buffer memory and storing it on output ports uses a counter to create wait cycles and extend the time between samples. In this mode of slow output frequencies, the restriction of no computation during synthesis is not necessary.
Generate multiple sequences	When selected on user interface, cyclic delivering of same or different signal patterns is delivered for a specific and individual period of time. Multiple tests can be done with sequenced stimulation patterns.
Start/Stop external interrupt	Start/Stop button is enabled as an external interrupt in two execution moments: at startup to be ready for accepting configuration and operation parameters, and during Output Signal Generation routine to stop signal.

5.2 Experiment Definition

A Carbon-DEP fluidic device is used for these experiments. The fluidic device has 3-dimensional carbon electrodes above a comb-like planar array of electrodes in a chess board arrange. This device was fabricated by pyrolysis of

SU-8 structures defined by a two-step photolithography process following standard C-MEMS techniques.

The electrodes are used to apply an electric potential to the micro-channel in order to produce a non-uniform electric field distribution that will generate DEP traps. The 3-Dimensional carbon structures are 40 μm high with a 12.5 μm radius and a center to center separation of 45 μm and 100 μm in the X and Y axis, respectively.

Deionized water with K_2HPO_4 as buffer solution with a final conductivity of 21 μS/cm was employed. Conductivity was measured with a multi-parameter bench meter, Model HI 255 from Hanna Instruments.

Fluid sample preparation. *Saccharomyces cerevisiae*, 24858 yeast cells from ATCC - a global nonprofit bio-resource center and research organization that provides biological products, technical services and educational programs to industries and labs- were growth in Yeast Malt Broth at 30 °C for 18 hours until late log phase. Cells were then centrifuged and re-suspended with deionized water to remove the excess of culture media within the cells to a final concentration of $6x10^7$ cells/mL. Cells were labeled with Syto® 9 fluorescent (490/520) green stain. For the non-viable yeast cells, a sample of cells from the culture media is centrifuged and washed with deionized water, later to be heated up to 80 °C for 20 minutes. Non-viable cells are then labeled with propidium iodide fluorescent (490/635) color red. Carboxylated fluorescent polystyrene particles with a diameter of 10.14 and Dragon green color (480/520) were employed in this work. Particles were prepared in the buffer solution to a concentration of 2x106 spheres/mL.

Two mixtures, the first containing viable and non-viable yeast cells for experiment 1, and the second containing 10.14 μm polystyrene particles and viable yeast cells for experiment 2, were employed to evaluate the performance of the signal excitation source.

The sample mixtures were introduced into the fluidic micro device using a micropipette. The micro device was mounted under an inverted epifluorescence video microscope for micro fluidics SVM340 from Lab Smith. A personal computer was employed to manipulate the communication and operation of the microscope.

5.3 Simulations

Simulation of crossover frequency spectra for different experimental settings was performed using MATLAB. This allowed for the selection of the best suspending medium conductivity, as well as for the selection of the most adequate AC frequencies to be used on the experiments. Dielectric properties for yeast cells were extracted from and from for polystyrene particles. To compute the equivalent complex permittivity of yeast, the multi-shell model presented in was used.

Finite element method based simulations were carried out using COMSOL Multiphysics in order to obtain predictions of the experimental results. An array of 4x5 electrode posts was considered on a plane located at 30 μm above the channel floor. At this height the effect of the planar electrodes located at the bottom of the channel are negligible. The channel geometry is shown in Figure 5.1. Boundary conditions were set to electric insulation at the channel

walls, and uniform AC electric potential at the electrode posts. The mesh for this geometry consisted of 14,208 elements.

Two different experiments were planned: separation of live and dead yeast cells using an AC signal of $V_{peak\ to\ peak}$ with a frequency of 100 Hz, and separation of live yeast cells and polystyrene beads using an AC signal of 5 $V_{peak\ to\ peak}$ with a frequency of 28 kHz. The geometry section from which this curves were obtained is represented by the red line plotted on Figure 5.1.

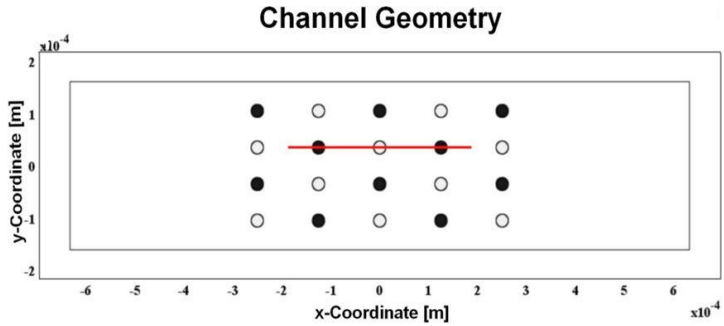

Figure 5.1 *Geometry section of the fluidic device.*

Simulations were performed and estimations of the experimental results are shown in Figure 5.2 where it can be observed that dead cells will experience a positive DEP force, causing the dead cell population to be attracted to the electrode posts. On the other side, live cells will experience a negative repulsive force. However, since the magnitude of this negative DEP force is low, live cells are expected to be found near the posts but not in touch with them. For the second experimental setup, live cells will now experience a strong positive DEP force, while polystyrene beads are expected to be repelled from the posts.

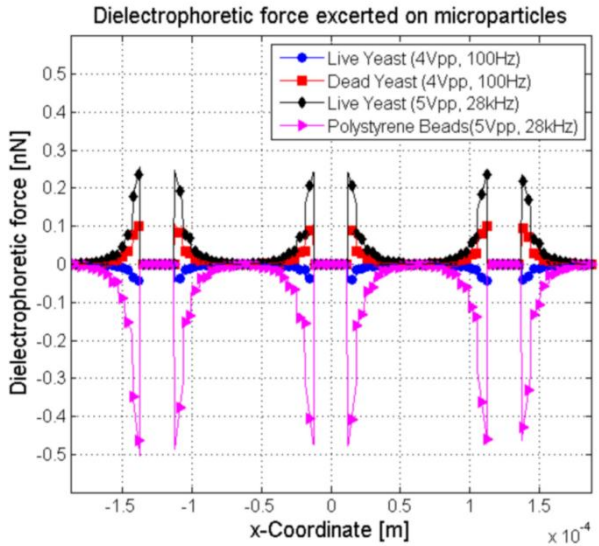

Figure 5.2 *Simulation of dielectrophoretic forces.*

5.4 Experimental Environment

To define reliable experiments the user started by defining test parameters from a previous known base used in manual experiments, like the frequency value known to be effective for a particular manipulation experiment on a specific type of particle. From there, the user can modify parameters such as waveform, frequency, exposure time, or sequence of patterns. These parameters can be changed one at a time or as a set for each test run. Once a set of parameters is found to be effective for a specific manipulation experiment, that test can be precisely repeated with no manual intervention.

This implementation shows a configurable system which delivers single, dual and superimposed $30V_{pp}$ output signals with sinusoidal, saw-tooth and triangle waveforms on frequencies going from 0.01 kHz to 40 kHz. The design is an original application specific architecture which implements a programmable and

configurable dual-frequency and multi-waveform signal generation system. The instrument presented was implemented as a set of components: An application specific user interface, a processor based prototyping board, and a signal conditioning circuit. The C language user interface program was developed to configure the experiment and to control the operation; the processor based development board –the LM3S6965 with an ARM Cortex-M3® processor – runs the application program that generates the electric stimulation signals; the conditioning circuit takes digital data and finally delivers analog signals to a fluidic device.

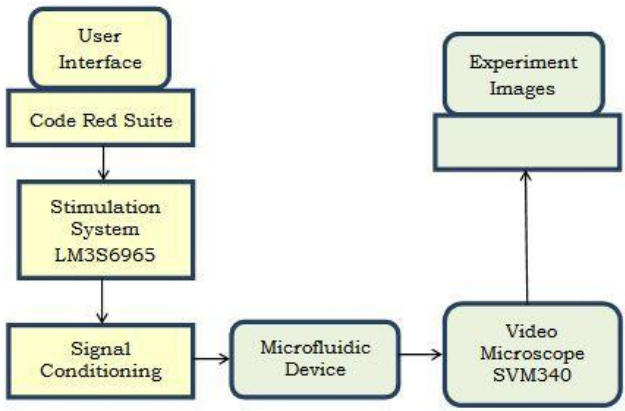

Figure 5.3 *Elements of the board based implementation.*

The core of this instrument is the application software that has been designed specifically for electrical stimulation purposes and has configuration capabilities that allow users to adapt the system to specific tests and applications with no modifications to the hardware or the software. This design can be used as an autonomous stimulation system or can be integrated into Lab-on-Chip designs. Figure 5.3 shows how this stimulation system fits into a particle manipulation setting: the stimulation system running on the LM3S6965 board takes operation

parameters from the User Interface, delivers sine, triangle, and saw tooth wave digital data to the signal conditioning circuitry, which sends analog signals to the micro-fluidic device.

A description of the instrument components is presented:

User interface. Has been developed to define the experiment environment by selecting several operation parameters: select the operation mode between three output options (One single frequency, Two separated frequencies, and Two superimposed frequencies); set the frequencies (base and superimposed) for the experiment; select the number of data samples desired for each frequency; select the exposure time for the test, and start operation when ready for the experiment. Figure 5.4 shows the options for setting operation parameters in the user interface:

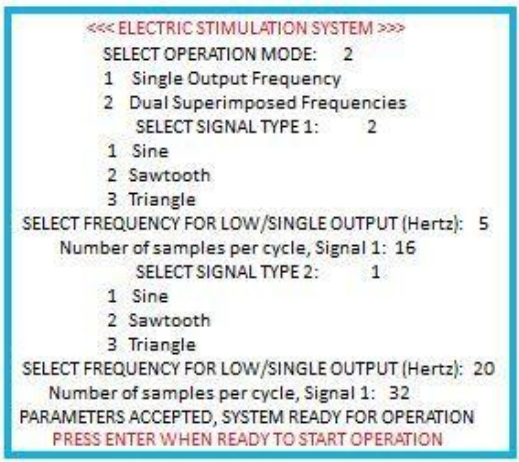

Figure 5.4 *User Interface for the board based implementation.*

Development board. The LM3S6965 - an ARM Cortex-M3® processor based board, shown in figure 5.5 - runs on a 50 MHz clock, has a 256Kb flash memory, 64Kb of SRAM, and up to 42 general purpose output bits grouped in 8-bit output channels. The LM3S6965 stores and runs the application program,

stores processed data needed for the signal generation, and delivers final data to output ports. It has a USB port for the user interface and for re-programming the board. Parallel 8-bit GPIO ports are used to deliver waveform ́s data to the signal conditioning circuitry. The whole system operation is done through the user interface so no manual operation is regularly needed. An emergency start/reset button can be used if an experiment needs to be interrupted before normal operation finishes (Figure 5.5 d).

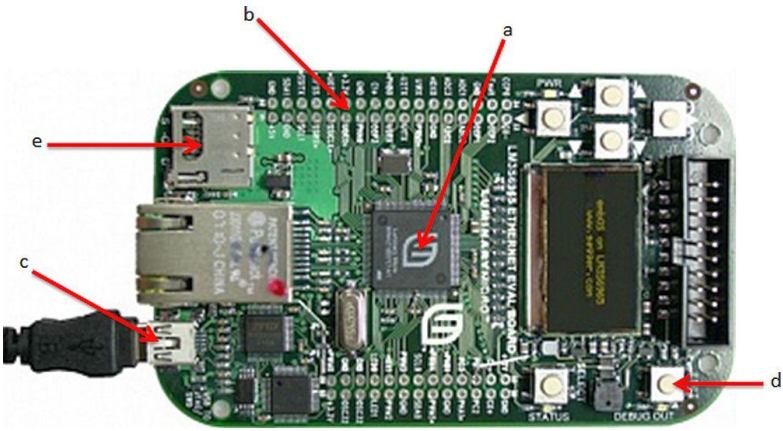

Figure 5.5 *The LM3S6965 prototyping board: a) ARM® Cortex-M3 Processor, b) GPIO port for output digital data, c) USB port for system programming and debugging, and for connecting User interface when in running mode, d) Reset button, e) Memory card slot for extra data and program storage.*

Application Software. Designed for this flexible stimulation environment, it includes the program code and the data tables for the three waveforms. The program contains a frequency synthesis methodology specifically designed for this system, so it can deliver single and dual frequency signals for a more controlled test environment. The program executes operation according to the set of parameters defined by the user and pre-stored data defines the selection of available waveforms. Although, program and data can be modified according to

new users or new needs. Figure 5.6 shows a screenshot of the software development environment, Red code suite ®.

Signal Conditioning. Consists of a digital to analog converter -DAC902- and two AD811 as current-to-voltage converter and voltage amplifiers. Digital data coming from the LM3S6965 board represents single or dual frequency waveforms and are finally converted into a +/-15V analog signal to meet most requirements of current test procedures. The system delivers 2-line analog signals to be applied to the electrodes or stimulation spots in the micro fluidic device.

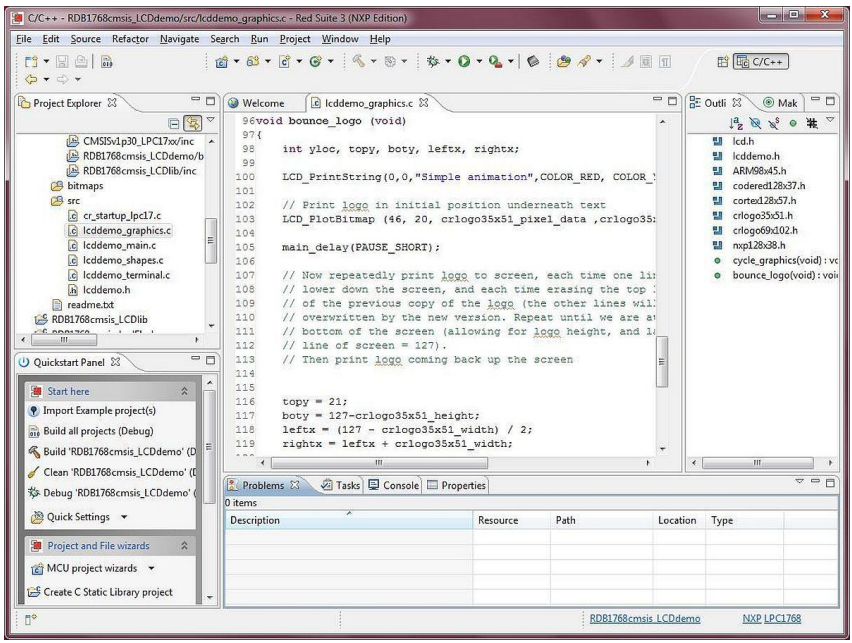

Figure 5.6 *The user interface allows programming, running and debugging the application.*

This system has automated operation: stimulation parameters are selected once, no intervention is needed during execution, and operation can be automatically repeated. This is a programmable implementation since modified

or new applications can be loaded into it. The advantages of this automated, programmable, and intelligent manipulation system are: a) User interface allows to configure and to operate the system for new tests and procedures, b) Previously programmed test parameters for a known test sequence can be stored and accessed later, c) More reliable data results are obtained due to precise reproduction of test parameters, d) Multiple tests can be done and repeated by programming test sequences, e) It can run the current application with the current waveforms or to load and run a different program, and f) It can be integrated to Lab-on-chip implementations or to portable Lab devices.

For experiment continuity, a relevant set of operation parameters can remain loaded in the system for future use: the last set of parameters used for a stimulation experiment is stored in flash memory so the system will perform the last stimulation pattern the next time the system is used, even if it is turned off.

5.5 System Potential

Besides the simulations and experimental results presented, this stimulation system has the potential to be used in a variety of particle manipulation systems.

The flexibility of its operation allows users from different application areas to define a specific stimulation pattern by selecting signal parameters such as frequency, waveform type, superposition, samples per cycle, time of exposure, and sequence.

To show the potential of this system, possible applications have been organized in four types:

- Electric stimulation already in use on particular experiments. Specific experiments from research work that currently use manual or limited electric stimulation show the type of signals used to achieve a particular manipulation effect over a specific type of particles; it is shown here how this system can substitute their stimulation means and improve their research procedures.

- Integrated electric stimulation that can be used in devices currently at proposal or design level. Published particle manipulation systems presented as proposed or demonstration designs that integrate electric stimulation and expose the need of automated stimulation; it is shown here how this system can fulfill those needs by selecting the appropriate set of operation parameters.

- Stimulation presented by theory on ACEK (Alternate Current Electro Kinetics). Existing theory about electric stimulation for particle manipulation presents the possible applications in a variety of fields by using simulations or theoretical demonstrations; it is shown here how this system can be used to comply with almost every application area that needs electric stimulation.

- Applications which use electric stimulation, even if it is not directly related to fluidic systems. Such potential applications go from impedance spectrometry for cell characterization, dielectrophoretic characterization, signal generation for DNA hybridization, or electro-rotation based systems, to completely different research areas such as implantable prosthetics, where new designs of prosthetic devices need to be tested with electric stimulus similar to those received from a live nervous system.

Tables 5.2, 5.3 and 5.4 show referenced research works that could use this stimulation system. For each experiment or device, it can be seen in the tables whether the system can be used as it is or if modifications in the program application are needed to fit in that particular experimental setting.

Experiments. Experiments extracted from the reference list expose a specific manipulation purpose over a particular type of cells or particles, so a specific frequency value or a limited frequency range is used for a particular experiment. Table 5.2 presents the electric stimulation used in actual experiments to show that the stimulation system presented in this work can be used as the stimulation module instead of generic signal generators and manual procedures. Regarding the Voltage amplitude all the experiments require values within the range delivered by this system. Even if higher voltages were needed they could be achieved with additional amplifying stages in the signal conditioning circuitry without modifying the SoC or the platform based design.

Table 5.2 *Particle manipulation experiments.*

Reference	Experiment	Electric stimulation used	Purpose
1992	Experiment: Dual-frequency dielectrophoretic levitation of Canola protoplasts	Sine, f < 1kHz	Compare Single vs. Dual Frequency effect.
2003	Separation of bioparticles using the travelling wave dielectrophoresis with multiple frequencies.	+-6V, 200kHz, 2 superimposed frequencies	Separate red cells from lymphocytes T in a blood sample, simultaneous PDEP and NDEP.
2005	Study of two-frequency dielectrophoresis effects on a linear array.	2 superimposed frequencies: 10Kz+500 Hz, 10- 20 V_{pp}. Large particles NDEP & small particles PDEP. F1<100kHz, f2>300kHz	Main f+low f broaden frequency range for particle separation. T from red blood cells.
2008	Real-time continuous dielectrophoretic separation of malignant cells.	7Vrms, 30-50 kHz	Separate MD231 breast cancer cells in blood.
2008	Micro fluidic Device for DEP Manipulation and Electro-disruption of Respiratory Pathogen Bordetella pertussis.	Sine, 10V, 1 MHz AC and DC sequence patterns.	Manipulation of respiratory pathogens.

Devices. As seen from the State of the Art section in chapter 1, published research works that involve a manipulation device, even if the work is at proposal or design stage, they do not integrate the electric stimulation circuitry in the design, or they talk about a limited integration proposal or a partially configurable demonstration chip. From those device proposals a set of functionality and stimulation parameters were extracted and presented in Table 5.3 to determine if the system presented here can apply to those proposed devices. It was found that the electric stimulation conformed by the signals and patterns delivered by this system are useful in most of the proposed devices. In those cases where V and –V are needed for the device an external high frequency inverter can be used to obtain –V from the original +V.

Table 5.3 *Particle manipulation Devices.*

Reference	Device	Electric Stimulation	Purpose
2000	Micro fabricated multi-frequency particle impedance characterization system	100kHz-10MHz, Sine signal, frequency sweep.	Resistive and reactive impedance measure for characterization of particles and cells
2003	A CMOS Chip for Individual Cell Manipulation and Detection.	2 Sine voltages, phase and counter-phase v1=-v2. Stop and go stimulation for grab & drag. 3.3-9.9V_{pp}, in kHz range.	Detect and manipulate Eukaryotic cells 20-30 μm.
2003	A SoC bio-analysis platform for real-time biological cell analysis-on-a-chip.	Typical DEP stimulation within a frequency range	Multi Bio-analysis.
2003	A programmable dielectrophoretic fluid processor for droplet-based chemistry.	Up to 180V_{pp}, 5-500kHz, varying voltage and frequency	Manipulate contaminants, chemical reagents, virus, and cells.
2005	All CMOS Low Power Platform for Dielectrophoresis Bio-Analysis.	one of four sine signals, 8 different phases, frequency sweep 1kHz-5MHz	Show effect on poly-styrene beads.
2007	A High-Voltage SOI CMOS Exciter Chip for a Programmable Fluidic Processor System Current.	100V_{pp}, up to 200Hz, sine, 0 & 180 °phase. Varying phase, amplitude, and frequency	Use multiple droplets, set a particle route for different particles.

Reference	Device	Electric Stimulation	Purpose
2007	A Programmable Biochip for the Applications of Trapping and Adaptive Multi-sorting.	Sine, $8V_{pp}$, 1MHz, V_1=-V_2, Lab-view controlled	Multi-sorting of proteins and DNA
2009	A robust electrical micro-cytometer with 3-dimensional hydro-focusing.	Sine, 4Vrms, 50kHz	Electrical impedance sensing to detect T cells in blood, for HIV diagnosis.

Theory. From the early research works on particle manipulation to recent publications about more complex stimulation for highly controlled environments, a summary of the AC signals and patterns needed for stimulation are shown in Table 5.4 to illustrate that the mathematical proof and simulations also lead to signals and frequency ranges be covered by this system. Travelling Wave Dielectrophoresis is a specific sequence of Sine signals synchronized to form a travelling electric field that produces a drag effect on the particles within a sample.

Table 5.4 *Particle manipulation Based on Background Theory.*

Reference	Device	Electric Stimulation	Purpose
2003	AC Electro-kinetics—Colloids and Nanoparticles.	Single frequency DEP, TWD, 4 phase Sine	Show mobility effects of AC stimulation
2004	Dielectrophoresis-based programmable fluidic processors.	40V-100V, 1kHz, 2KHZ. 0 and 180 °phase to neighboring electrodes.	Titrating, moving and mixing polar and non-polar, conductive or not, droplets
2004	Sample handling in general-purpose programmable diagnostic instrument.	4 de-phased sine to repel & attract, TWD to concentrate particles in a spiral electrode array.	If f>200kHz all viable cells can be Trapped
2007	Interactions of electrical fields with fluids: laboratory-on-a-chip applications.	2.2Vrms, 100 Hz, 500Hz, 1kHz, $35V_{pp}$ @ 100kHz, $24V_{pp}$ @ 1kHz	Describes ACEK experiments: ACEO, ACDEP, & ACET.
2010	Controlled micro-particle manipulation employing low frequency alternating electric fields.	0.2-1.25Hz, 750V	Show the potential of manipulation using AC fields.

Following Table 5.5 allows visualizing a variety of experimental settings. An experimental setting is defined by selecting the operation parameters for a

specific manipulation purpose. Note that changes in one or several parameters define a whole new experiment setting and purpose.

Parameters that can be changed:

Operation mode: Single or Dual frequency. Waveform type: Sine, Triangle, Saw tooth. Separate or superimposed frequencies. Frequency values for one or two signals. Number of data samples per waveform cycle. Waveform selection for superimposed frequencies.

Table 5.5 *Samples of Experimental Settings.*

Setting	Output channels	Operation mode	Frequencies	Waveform
1	1	Single	50 Hz	Sine
2	1	Dual	Superposition, 10 Hz + 200 Hz	sine over sine
3	2	Dual	Separate, 5 Hz, 400 Hz	Sine and sine
4	1	Single	300 kHz	Triangle
5	1	Dual	Superposition, 2kHz, 10kHz	Saw tooth over saw tooth
6	1	Single	8 kHz	Sine
7	1	Dual	Superposition, 1 kHz + 5 kHz	Triangle over sine
8	2	Dual	Separate, 5 kHz, 10 Hz	Sine, sine
9	1	Single	15 kHz	Saw tooth
10	1	Dual	Superposition, 1kHz, 7kHz	Triangle over saw tooth

Set an experiment through the user interface.

Defining a specific experiment consists of selecting the appropriate set of operation parameters in the user interface. Parameter values can be known from previous experiments or from simulations. At least an idea of the convenient frequency and voltage range is needed. Once the fluid sample has been prepared and put in the fluidic device, the terminals that will carry the electric stimulation

have to be connected to the device. Microscope and camera set have to be ready too.

Here is presented the parameter selection, accessed through the user interface:

Operation Mode. 1 For Single frequency output, 2 for Dual superimposed frequencies, and 3 for Dual separate frequencies.

Waveform for Signal 1. Choose between sine, triangle, and saw-tooth.

Frequency for Signal 1, f_1. Signal 1 is the low frequency signal for the superposition mode.

Number of samples for Signal 1, n_1. The higher the number the less harmonic components are found in the output signal and the lower output frequency can be achieved.

Waveform for Signal 2, requested only if operation mode = 2 or 3.

Frequency for Signal 2, f_2. Is the high frequency to be superimposed on the low frequency Signal 1.

Number of samples for Signal 2, n_2. The amount of memory space needed for the final output table containing sample with both superimposed frequencies could increase significantly if $f_2 >> f_1$. The amount of needed memory space for output buffer table:

$$M_{size} = \left[\left(\frac{f_2}{f_1} \right) n_2 \right] bytes$$

It is recommended that $n_2 < n_1$ to prevent that.

The exposure time for the stimulation, t_{exp}. The exposure time is achieved by defining the number of waveform cycles to be delivered, N. Since f_1 is the base frequency in the case of superimposed frequencies, then

$$N = f_1\left(t_{exp}\right)$$

If a sequence of a different stimulation signal is needed, a similar set of parameters has to be provided.

If the same test or sequence has to be repeated, the same set of parameters is automatically used by the system if re-run.

A set of experiments is described to show the flexibility of this stimulation system.

A specific manipulation experiment.

As shown in tables above, some experiments have already defined the particular set of parameters needed to manipulate a specific type of particles or cells. This set of parameters is introduced once in the user interface and execution are repeated over new fluid samples without changing the parameter set. Another scenario is that the user has an idea about the parameters to be used in an experiment, but not the exact values. In this case user can play with the parameters until the appropriate set of parameters is found.

Once the exact set of parameters is known by achieving the desired manipulation effect, the values can be stored and accessed later to precisely reproduce the experiment.

A particle characterization experiment.

Particle characterization experiments may need a frequency sweep in x10 steps to first determine a smaller range to work. A set of sequences can be defined, and a special case were $f_{new}= 10*f_{old}$ can be defined in program to cover all the frequency range, going through 0.1 Hz, 1 Hz, 10 Hz, 100Hz, 1kHz, 10kHz, …up to the maximum output frequency.

For example, in a sensor measures resistive and reactive impedance of circulating particles. Particle impedance is measured at three or more frequencies simultaneously, enabling the derivation of multiple particle parameters such as blood granulocyte radius, membrane capacitance, and cytoplasmic conductivity.

A frequency sweep experiment.

Some experiments require observing the mobility effect under different frequencies. In those cases the whole frequency range delivered by this system can be swept in user defined steps. An initial f_i frequency is selected, a frequency step f_s is defined, and a time period t_r for each repetition is introduced. This way each following t_r a new frequency $f_{i+1=}f_i+f_s$ is delivered during t_r seconds. In a feasible procedure that uses DEP phenomenon as a method of separation of the abnormal cells from the blood stream is presented.

Negative and positive DEP (NDEP and PDEP) forces generated by a non-uniform electric field are engaged to separate the normal blood cells from the malignant ones. By fine tuning the parameters of the electric field different types of abnormal cells are isolated. It is noticed that at a frequency of 30 kHz all blood cells and the cancer cells experienced a PDEP, and the cells started accumulating in the area of low electric field. Increasing the AC frequency to 50 kHz, the cancer cells experienced PDEP and gathered over the tip of the electrodes array

where the maximum electric filed is present. At the same time the blood cell still with from the electric field. To perform a similar procedure for different cell types within a blood sample, a frequency sweep experiment can be used.

A dual frequency experiment.

If two different particles are present in the same fluid sample, they can be separated by applying two frequencies simultaneously. Particles can be different in type, size, or of the same type but different because one are alive and the others are dead, or because they present a different development stage. In there is an analysis for a mixture of two different types of particles: they choose an angular frequency, w, such that the real parts of the Clausius Mossotti function at U (or Re[G(jw)]) of the two different types of particles have different signs). Then an electric field produces time-averaged dielectric forces in such that the particles with Re[G(jw)]> 0 get attracted to the maximum points of the field, and the particles with Re[G(jw)]< 0 get repelled away from those points.

In a similar analysis they consider an example where the goal is to separate two types of latex balls with a very small difference between both cross-over frequencies, so that the electric field of single frequency is not effective.

A saw-tooth waveform experiment.

It has been shown in previous table that saw-tooth waveforms are useful in a drag-trap effect; during the linear voltage rise the particles are moved to a certain point, and once the voltage exceeds certain level they remain trapped. An interesting effect can be achieved when a saw-tooth over a sine signal is used, because two different types of particles are manipulated, and the more distant the two frequencies, the more different the particles.