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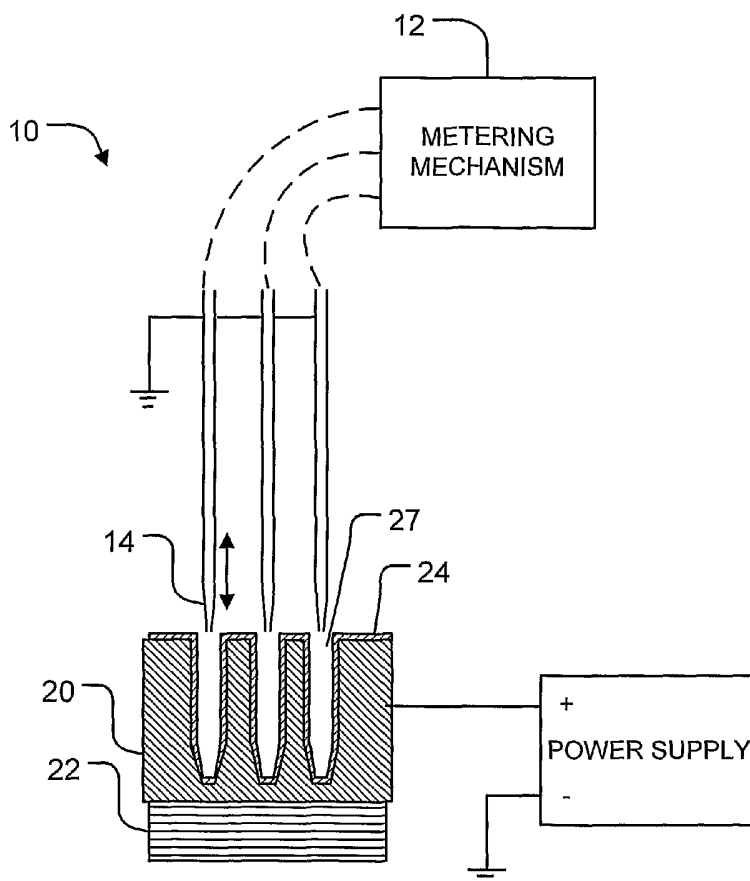
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[Continued on next page]

(54) Title: SUB-MICROLITRE ELECTROSTATIC DISPENSER



(57) Abstract: Sub-microlitre droplets are dispensed by a metering mechanism. After being dispensed, the droplets are pulled from the dispensing tip by an electric field. The electric field may be applied by grounding the dispensing tip and applying a high potential to a container or a structure located behind a container into which the droplet will be dispensed.



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SUB-MICROLITRE ELECTROSTATIC DISPENSER

Technical Field

[0001] This invention relates to dispensing sub-microlitre droplets of fluid. The invention has application, for example, in dispensing samples comprising nucleotides, reagents, or the like, in biological research, drug development, testing for the presence of substances in samples, or the like.

Background

[0002] In biological research it is often desired to dispense small droplets of sample, reagents or the like. Such droplets may be expelled from a pipette. In many cases, biological experiments are performed simultaneously on arrays of samples. In such cases it may be necessary to dispense a large number of droplets of the same material. For example, biological experiments are often done in a plate which has an array of wells in it. Some typical plates have 96 or 384 wells in them. Measured volumes of fluid materials can be dispensed into the wells using a pipette. Some pipettes permit materials to be dispensed simultaneously into a number of wells. A device called a hydra may be used. A hydra has a large number of dispensing pipette tips. Typically a hydra will have the same number of dispensing pipette tips as there are wells in a plate in which a process is to be performed. A hydra can therefore be used to simultaneously dispense droplets of material into all of the wells of a plate.

[0003] It is desirable to conduct certain biological processes on a small scale due to the great expense of certain reagents and/or biological materials that may be desired to be used in such biological processes. Conventional pipettes or hydra devices suffer from the disadvantage that they cannot reliably dispense tiny droplets. A reason for this is that sub-microlitre droplets have very small masses. Therefore, the gravitational force which acts on such tiny droplets is not sufficient to overcome the adhesive force which acts between the tiny droplets and the tip of the pipette (typically the inner pipette wall) from which the droplets are being dispensed.

[0004] There is a need for practical apparatus which can be used to dispense sub-microlitre quantities of material, especially for practicing biological processes. Similar needs may exist also in other areas.

Brief Description of the Drawings

[0005] In drawings which illustrate non-limiting embodiments of the invention:

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Figure 1 is a schematic view showing apparatus according to an embodiment of the invention.

Figure 2 is a schematic diagram of a 15kV power supply as used in a prototype embodiment of the invention.

5 Figure 3 is a schematic diagram of a power supply circuit used in an experimental embodiment of the invention.

Description

10 [0006] Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

15 [0007] This invention provides a method and apparatus for dispensing sub-microlitre samples. The invention uses a standard pipette, hydra, or the like to define the volume of sample to be dispensed. An electric field is created at the dispensing tip. The electric field pulls the sub-microlitre droplet of sample off of
20 the tip of the pipette or other dispensing mechanism. In example embodiments of the invention the electric field is created between the dispensing tip and a container into which the sample is to be deposited.

25 [0008] In one embodiment of the invention, an electric field is created between the dispensing tips of a multi-tipped hydra or other dispensing device and wells in a plate into which tiny droplets of sample are to be dispensed from the dispensing device. In a prototype embodiment of the invention the wells are wells in a multi-well plate of plastic or other dielectric material. The multi-well plate is supported on an electrically conducting base.

30 [0009] In the prototype embodiment, the base comprised an aluminum plate having an array of holes drilled in it. The wells of the multi-well plate were each received in a hole in the electrically conducting base. A power supply was then connected between the dispensing tips of a hydra and the electrically conducting base. In the
35 prototype embodiment a potential difference of approximately 15kV was established between the conducting base and the dispensing tips. The electrically conducting

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base was mounted on a block of electrical insulator in order to prevent arcing from the plate. The dispensing tips of the hydra were maintained at ground potential.

5 [0010] Figure 1 is a schematic view showing apparatus according to an embodiment of the invention. Shown in Figure 1 are a multi-tipped dispensing device 10 having a metering mechanism 12 which causes each of a plurality of dispensing tips 14 to dispense a droplet of a sample. Each of the droplets have a volume defined by metering mechanism 12. Apparatus 10 further includes an electrically conducting base 20 supported on a block of electrical insulator 22. A multi-well plate 24 is
10 disposed on the electrically conducting base 22. Multi-well plate 24 has a plurality of wells 27. Each of wells 27 corresponds with one dispensing tip 14. A power supply 30 is connected to raise base 22 to a high electrical potential relative to dispensing tips 14.

15 [0011] Figure 2 is a schematic diagram of a 15kV power supply as used in the prototype embodiment of the invention. Power supply 40 had a 15V power supply 42 connected to a 10k Ω potentiometer 44 which acted as a voltage divider. The output of the voltage divider was connected to a voltage follower circuit 46. The output of voltage follower circuit 46 was supplied to a voltage controlled voltage
20 source 48 (the prototype of the invention used a SC-150VCVS device available from American High Voltage). Voltage controlled voltage source 48 was connected through a 1G Ω resistor 52 to base plate 20.

[0012] The negative output of voltage controlled voltage source 48 was grounded
25 (the dispensing tips were also grounded) and the positive output of voltage source 48 was connected to base 20 through resistor 52. The 1G Ω resistor 52 served as a current limiting resistor in case of a short circuit at the output. Current limiting resistor 52 was potted in a block of acrylic filled with silicone elastomer in order to prevent arcing between its input and output leads.

30 [0013] A prototype apparatus according to one embodiment of the invention comprises a hydra unit having a 384 channel base plate mounted in it below dispensing tips 14. Power supply 40 is located in a housing near to the hydra dispensing unit. The dispensing tips of the hydra are aligned so that they can be
35 inserted into corresponding wells of a microtiter plate being supported by base plate 20.

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[0014] Various modes of operation are possible. In one mode of operation, an electric field is applied by charging the base plate to a high potential relative to the dispensing tips. For example, the potential may be greater than 10kV, or in some cases, 15kV or more. With the electrical field applied, the dispensing function of the hydra is actuated. Actuating the dispensing function causes the base plate to be lifted to a preset height in which the dispensing tips are near the bottoms of the corresponding wells of the microtiter plate. The hydra then expels droplets from each of the dispensing tips. The electric field arising due to the potential difference between base plate 20 and the dispensing tips causes the tiny droplets of sample to be pulled from the dispensing tips into the corresponding wells. After a sample expulsion has been completed the hydra plate is returned to its resting position.

[0015] In another embodiment of the invention, the dispensing cycle of a hydra or other similar dispensing device may be activated and the electric field may be applied once the droplets of sample are already on the ends of the dispensing tips.

[0016] It has been found possible to transfer droplets of samples into the corresponding wells having volumes as small as 100nL with reasonable success rates. For example, in experiments with the prototype device, it was found that there was a 95 % to 100 % success rate in dispensing 100nL samples; a 99.5 % to 100 % success rate in dispensing 200nL samples; and a 100 % success rate in dispensing 500nL samples. Without the applied electric field, the same device was not capable of reliably dispensing sample droplets having volumes smaller than approximately 3.5 μ l. For these prototype experiments, the dispensing tips were Teflon-coated steel needles of a 384 channel hydra. The samples were dispensed into the wells of a 384-well clear optical plate from Applied Biosystems. 10 μ l of water was placed in each well prior to dispensing the samples.

[0017] In the prototype experiments it was noted that there was no significant reverse transfer of fluid from the well to the dispensing tips under the applied voltage.

[0018] The dispensing tips may be made very sharp to provide enhanced electric field strength near the dispensing tips so as to apply increased force to the sample droplets. In cases where it is desired to use a plate made of a thicker material or a material with a higher dielectric strength it may be desirable to increase the voltage applied to base 20 to values greater than 15kV.

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[0019] Especially in cases where the material being dispensed tends to wet the dispensing tips it may be desirable to turn on the electric field and to operate the metering mechanism to dispense droplets of sample at about the same time with the dispensing tips already positioned in the wells of a multi well plate.

[0020] In the above description, the electric field applied to the tips of the dispensing tips is a DC electric field. In some embodiments of the invention, an AC field may be used to cause the tiny droplets of sample to leave the dispensing tips. In some embodiments of the invention the electric potential applied to base 20 is varied with time so that the resulting electric field near the dispensing tips is time-variable with a frequency that is a reasonably close match to a mode of vibration of the droplets after they have been dispensed before the droplets have left the dispensing tips.

[0021] In some embodiments of the invention the dispensing mechanism is activated and, after activating the metering mechanism, the electric field is modulated by an AC signal having a frequency component matching a frequency of vibration of the suspended tiny droplets.

[0022] Table I summarizes results of a number of experimental trials of the methods of the invention.

Table I

Sample Volume: 100nL				
	Water Volume in Wells	Voltage	Gap	Success Rate
Trial #1	10 μ l	15 - 16kV	1.5mm	100% (384/384)
Trial #2	10 μ l	15.5 - 16.5kV	1.5mm	95.1% (365/384)
Trial #3	10 μ l	16 - 17kV	1.5mm	95.3% (366/384)

Sample Volume: 200nL				
	Water Volume in Wells	Voltage	Gap	Success Rate
Trial #1	10 μ l	16 - 17kV	1.5mm	100% (384/384)
Trial #2	10 μ l	15.5 - 16.7kV	1.5mm	95.5% (382/384)
Trial #3	15 μ l	15.8 - 16.8kV	1.4mm	99.5% (382/384)

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Sample Volume: 500nL				
	Water Volume in Wells	Voltage	Gap	Success Rate
Trial #1	10 μ l	15.5 - 16.8kV	1.5mm	100% (384/384)

- 5 [0023] The following passages describe some experiments performed using an apparatus according to an experimental embodiment of the invention:

10 Multichannel automated syringe pipettors are ubiquitous in high-throughput molecular biology laboratories and are capable of aspirating and dispensing sample volumes as low as 100 nL (www.artrobbinsinstruments.com), but encounter difficulties transferring such volumes to a dry microtiter plate or to a fluid sample without contact. At submicroliter scales surface tension dominates over gravity, and drops remain attached to the pipettor needles. Touch-off methods (contact of the needles with the target well or fluid after the dispense from the pipettor is complete, or direct contact between the needles and the plate during dispense) are typically used to transfer small volumes to dry plates, but such contact techniques are highly sensitive to tip cleanliness, alignment, length, and flexibility, and often result in high variability of the transferred volume. In cases where the target well is filled with fluid, contact methods raise concerns of cross-contamination between runs. Piezo electric, ink-jet, or solenoid-actuated devices capable of ejecting small volumes exist (www.deerac.com; www.cartesiantech.com; www.cybio-ag.com; www.gilson.com; www.biodot.com; www.auroradiscovery.com), but are expensive, often less flexible, and are typically limited to relatively few channels since each channel needs an individual actuator, while syringe pipettors are available up to 384 channels (www.artrobbinsinstruments.com).

30 We present a low cost and highly adaptable method of sample transfer for generic pipettors based on electrostatic forces. Metering of the sample is performed by the pipettor; however, an electric potential is applied between the pipettor needles and the target plate providing an electrostatic force that pulls the drops into the target well. We present results with common reagents dispensed using a Hydra® (Art Robbins Instruments, Sunnyvale, CA, USA) that show this to be effective for volumes as low as 100 nL.

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5 The electrostatic device according to an experimental embodiment of the invention comprises an adjustable high voltage power supply and a microtiter plate charger. The charger consists of an aluminum plate (the anode) partly encased in an acrylic shell. The target plate is placed onto the exposed face of the aluminum plate. As a safety precaution, the aluminum plate can be covered with a thin insulating coating to prevent user contact with the anode, without compromising the performance of the device.

10 The Hydra-96 Microdispenser (Art Robbins Instruments), which was used for all experiments, is composed of 96 actuated pipettors arrayed on a standard microtiter plate spacing and, for these experiments, is fitted with two types of pipettor needles: (i) 48 Teflon® - coated stainless steel tips and (ii) 48 Teflon-coated Nitinol® tips (a nickel-titanium alloy). It should be noted that the methods and device we present can be used with any pipettor, consisting of any
15 number of channels, provided that the tips are electrically conductive or that there is a ground path to the fluid inside the tip. For pipettors with disposable tips, use of carbon-filled tips is sufficient to meet this criterion.

20 Fluorescent dye was used to assess dispensing performance. Test solutions included: distilled water, 95% ethanol, 25% polyethylene glycol (PEG), and BigDye® Mix (Applied Biosystems, Foster City, CA, USA) (2:1:7 of BigDye Ready Reaction Premix, BigDye sequencing buffer, and distilled water respectively). Each solution was mixed with fluorescein at a concentration of 10 mg/mL. Multiple experiments were conducted with each solution at 100, 300,
25 and 600 nL nominal volumes. Skirted 96-well, V-bottom, polypropylene microtiter plates (MJ Research Microseal® 96; Bio-Rad Laboratories, Hercules, CA, USA) were used for all experiments. After the sample was dispensed into the microtiter plate, the plate was centrifuged for 1 min at 2755× g prior to being imaged on an AlphaImager® 3400 (Alpha Innotech Corporation, San Leandro,
30 CA, USA). Experiments were performed confirming that the pH of the fluorescein solution (which impacts its quantum yield) remained stable, within 0.5 pH units, when exposed to air for the duration of the experiment.

35 Both wet-well and dry-well dispensing tests were performed. In wet-well tests, the target wells were prefilled with 10 µL of the same solution as was being dispensed (without fluorescein). In the case of BigDye Mix, the 10-µL target solution was distilled water. This volume was chosen because it is representative

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of volumes used in high-throughput molecular biology. However, greater or smaller volumes would not affect the performance of the electrostatic device, as the applied potential can be adjusted to compensate for a varying air gap between the fluid and the pipet tip. It should be noted that the target fluid volume is a low-impedance part of the electric circuit, and only the size of the air gap above the volume, rather than the height of the fluid column, affects the electric potential required for drop transfer.

Three types of tests were performed for the wet-well dispensing experiments. A control test in which contact was made between the pipettor needles and the solution during dispense, a no contact test in which the needles did not contact the solution during dispense, and an electrostatic test in which no contact was made with the solution, but an electrostatic potential was applied while the sample was being dispensed. In tests that involved contact between the needles and the solution or plate, the plate was also manually touched-off to the tips after the dispense. A typical wet-well electrostatic dispense protocol is as follows: aspirate 2 μ L air, aspirate 100–600 nL sample, apply stated electric potential between tips and the plate charger, raise the target plate such that the tips are within 2 mm of the solution in the wells, dispense sample and air volumes, pulse voltage to 20 kV for 250 ms, and lower the plate away from tips.

Dry-well dispensing involved similar tests: dispense with contact between the needles and plate, dispense without any contact between the needles and plate, and dispense without any contact, but with an applied electrostatic potential. In all cases after dispensing into a dry well, each well was filled with 10 μ L solution for direct comparison to the wet-well control. The dry-well electrostatic dispense protocol is similar to the wet-well protocol, except that during dispense, the tips are brought to within 3 mm of the dry-well bottom. For distilled water, each test was performed three times at each nominal sample volume.

In all experiments, control samples containing fluorescein were exposed to similar conditions as the test samples to ensure that evaporation, nonlinearity in fluorescence versus dye concentration, or decay of the dye over time did not skew experimental results. Tests with dispensing of fluorescein in ethanol were performed repeatedly over a period of 6 h to ensure that evaporation during the experiment time (typically 1 h) did not significantly alter the calibration of the solutions. The temporal fluctuation in the fluorescence appeared random, as

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opposed to monotonic, and is attributed to stochastic measurement error, which is approximately 10%. This is comparable to our well-to-well measurement variation.

- 5 Fluorescence readings were calibrated against reference volumes metered with a Hamilton syringe attached to a 149 μm inner diameter glass capillary. Volumes were determined by accurate measurement of the length of the fluid slug inside the capillary.
- 10 Application of an electric potential between the dispenser needles and the target plate during and immediately following the dispense results in highly successful transfer of submicroliter drops from the pipettor needles to the target wells. For stainless steel tips in dry wells with a volume of 100 nL distilled water, electrostatic transfer results in 85% of drops transferring to their targets,
- 15 compared with only 50% with contact dispensing. As the sample volume is increased to 600 nL, the electrostatic transfer success rate reached 100%, while contact dispensing only resulted in a 91% transfer rate. Similar results are observed with the Nitinol tips. BigDye Mix and 25% PEG performed similarly to distilled water. Results for 95% ethanol, however, were poor at the 100 nL
- 20 sample volume scale. For stainless steel tips in dry wells at a 100 nL, electrostatic transfer results in 25% of drops transferring compared with 48% with contact dispensing. The difficulty in dispensing ethanol stems from its ability to wet hydrophobic surfaces. The ethanol sample tended to wet the sides of the pipettor needle (as opposed to forming a droplet at the tip) upon
- 25 dispensing. By adhering to the needle walls, the sample moved out of the region of strong electric field, resulting in less successful electrostatic transfer. At ethanol volumes of 300 and 600 nL, however, significant drops form at the needle ends, and the electrostatic transfer rate improves to 83% and 98% success, respectively (compared with 69% and 77% with contact dispensing). In
- 30 summary, electrostatic-assisted transfer performs better than contact methods, is likely to be less sensitive to tip alignment and position, and allows transfer without contamination of the needle tips.
- 35 Transfer results for distilled water (dH_2O), 95% Ethanol, 25% polyethylene glycol (PEG), and BigDye Mix from 48 stainless steel and 48 Nitinol Tips of a Hydra-96 under various conditions for both wet and dry contact wells are shown in Tables II, III, IV and V, respectively. Successful transfers represent

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transferred volumes within 30% of the mean of all transferred volumes above 50% of the nominal volume. % CV is the coefficient of variation of dispense over the entire 48 tips. Plate mean is the mean over all wells. Nominal volume is the intended dispense volume. SD (% successful transfers) is the standard deviation in the percentage of successful transfers over three sets.

Table II

dH₂O

		Stainless Steel Tips	Nominal Volume	100 nL	300 nL	600 nL
10	Control	Plate mean (nL)		116.9 ± 5.6	343.6 ± 15.1	645.5 ± 22.6
		Wet-well	% CV	11.4	9.0	7.6
		contact	% Successful	99.3	100.0	100.0
			SD (% successful transfers)	1.2	0.0	0.0
	Wet-well no contact	Plate mean (nL)	26.6 ± 23.4	42.9 ± 22.0	157.6 ± 109.2	
		% CV	126.7	231.4	149.4	
		% Successful	26.6	12.5	22.4	
		SD (% successful transfers)	23.8	5.5	14.0	
15	Wet-well electrostatic	Plate mean (nL)	109.8 ± 9.8	337.8 ± 30.1	633.6 ± 45.1	
		% CV	16.9	11.6	11.0	
		% Successful	96.5	98.6	99.3	
		SD (% successful transfers)	2.4	1.2	1.2	
		DC voltage (kV)	5.8	5.8	5.8	
	Dry-well no contact	Plate mean (nL)	53.7 ± 11.0	182.0 ± 34.8	480.8 ± 106.2	
		% CV	74.3	85.2	56.8	
		% Successful	59.0	50.7	70.1	
SD (% successful transfers)		9.6	7.9	16.2		
20	Dry-well contact	Plate mean (nL)	42.0 ± 16.1	215.0 ± 65.3	653.6 ± 6.6	
		% CV	81.3	52.7	26.6	
		% Successful	50.0	68.8	91.0	
		SD (% successful transfers)	14.6	21.1	6.0	
	Dry-well electrostatic	Plate mean (nL)	78.6 ± 10.2	363.2 ± 7.0	668.8 ± 93.3	
		% CV	29.2	13.9	8.6	
		% Successful	84.7	96.5	100.0	
		SD (% successful transfers)	9.4	2.4	0.0	
	DC voltage (kV)	5.8	5.8	5.8		

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Nitinol Tips	Nominal Volume	100 nL	300 nL	600 nL
Control	Plate mean (nL)	105.7 ± 5.9	325.9 ±	614.4 ± 22.1
Wet-well	% CV	13.4	10.7	9.7
contact	% Successful	98.6	100.0	100.0
	SD (% successful transfers)	1.2	0.0	0.0
Wet-well no	Plate mean (nL)	3.2 ± 3.3	1.6 ± 1.4	20.2 ± 21.3
contact	% CV	296.7	58.0	343.7
	% Successful	2.6	0.0	2.1
	SD (% successful transfers)	3.1	0.0	2.4
Wet-well	Plate mean (nL)	91.4 ± 9.2	313.3 ±	625.1 ± 43.5
electrostatic	% CV	22.5	12.2	11.1
	% Successful	91.0	100.0	100.0
	SD (% successful transfers)	5.2	0.0	0.0
	DC voltage (kV)	5.8	5.8	5.8
Dry-well no	Plate mean (nL).	4.6 ± 2.0	13.6 ± 2.2	22.0 ± 10.9
contact	% CV	341.8	394.1	280.8
	% Successful	4.2	2.8	1.4
	SD (% successful transfers)	2.1	1.2	1.2
Dry-well	Plate mean (nL)	16.5 ± 4.5	211.2 ±	545.8 ± 175.2
contact	% CV	133.5	54.6	41.3
	% Successful	11.8	73.6	76.4
	SD (% successful transfers)	2.4	7.3	23.2
Dry-well	Plate mean (nL)	65.1 ± 7.6	353.3 ± 9.5	634.1 ± 80.8
electrostatic	% CV	35.4	11.8	12.9
	% Successful	72.9	100.0	99.5
	SD (% successful transfers)	13.7	0.0	1.0
	DC voltage (kV)	5.8	5.8	5.8

Table III

95% Ethanol

Stainless Steel Tips		Nominal Volume	100 nL	300 nL	600 nL
5	Control	Plate mean (nL)	87.74	241.22	526.39
	Wet-well	% CV	15.96	15.12	6.87
	contact	% Successful	96.88	96.88	100.00
10	Wet-well no contact	Plate mean (nL)	1.88	1.93	22.31
		% CV	555.85	425.39	424.20
		% Successful	4.17	0.00	4.17
15	Wet-well electrostatic	Plate mean (nL)	10.97	158.39	473.04
		% CV	125.80	25.00	8.51
		% Successful	8.33	89.58	100.00
		DC voltage (kV)	4.00	4.00	7.80
20	Dry-well no contact	Plate mean (nL)	7.31	100.43	288.49
		% CV	207.39	99.36	75.74
		% Successful	0.00	45.83	54.17
25	Dry-well contact	Plate mean (nL)	31.83	135.59	402.69
		% CV	48.76	46.32	47.11
		% Successful	47.92	68.75	77.08
30	Dry-well electrostatic	Plate mean (nL)	32.26	166.71	429.28
		% CV	60.26	22.50	14.77
		% Successful	25.00	83.33	97.92
		DC voltage (kV)	4.00	4.00	7.80

Nitinol Tips		Nominal Volume	100 nL	300 nL	600 nL
20	Control	Plate mean (nL)	83.95	232.42	504.15
	Wet-well	% CV	13.07	10.45	8.02
	contact	% Successful	98.96	100.00	100.00
25	Wet-well no contact	Plate mean (nL)	0.00	0.07	11.48
		% CV	N.A.	1700.00	610.71
		% Successful	0.00	0.00	2.08
30	Wet-well electrostatic	Plate mean (nL)	19.82	176.40	460.69
		% CV	52.42	13.89	10.79
		% Successful	0.00	100.00	100.00
		DC voltage (kV)	4.00	4.00	7.80
30	Dry-well no contact	Plate mean (nL)	1.24	25.84	303.14
		% CV	395.16	246.87	50.55
		% Successful	0.00	10.42	58.33
30	Dry-well contact	Plate mean (nL)	27.25	173.93	427.36
		% CV	69.94	29.57	35.17
		% Successful	50.00	89.58	83.33
30	Dry-well electrostatic	Plate mean (nL)	34.18	178.58	434.69
		% CV	55.97	16.13	12.56
		% Successful	31.25	95.83	97.92
		DC voltage (kV)	4.00	4.00	7.80

Table IV

25% PEG

	Stainless Steel Tips	Nominal Volume	100 nL	300 nL	600 nL
5	Control	Plate mean (nL)	104.47	296.41	562.83
	Wet-well	% CV	12.01	13.15	9.21
	contact	% Successful	97.92	100.00	98.61
	Wet-well no contact	Plate mean (nL)	48.10	148.66	266.69
		% CV	66.09	63.03	101.38
		% Successful	66.67	64.58	39.58
10	Wet-well electrostatic	Plate mean (nL)	71.77	212.40	635.99
		% CV	29.52	16.37	13.21
		% Successful	79.17	95.83	97.92
		DC voltage (kV)	4.00	4.00	4.00
	Dry-well no contact	Plate mean (nL)	66.14	182.64	445.74
		% CV	75.34	35.92	37.44
		% Successful	58.33	85.42	85.42
15	Dry-well contact	Plate mean (nL)	62.05	202.69	559.70
		% CV	20.90	14.58	8.09
		% Successful	91.67	95.83	100.00
	Dry-well electrostatic	Plate mean (nL)	79.25	202.83	587.02
		% CV	31.05	12.72	7.82
		% Successful	87.50	97.92	100.00
		DC voltage (kV)	4.00	4.00	4.00
	Nitinol Tips	Nominal Volume	100 nL	300 nL	600 nL
20	Control	Plate mean (nL)	96.44	286.78	533.08
	Wet-well	% CV	16.03	15.30	10.85
	contact	% Successful	97.92	97.92	99.31
	Wet-well no contact	Plate mean (nL)	6.08	32.61	46.82
		% CV	300.16	205.15	288.94
		% Successful	8.33	8.33	6.25
25	Wet-well electrostatic	Plate mean (nL)	62.46	199.99	647.81
		% CV	33.09	16.88	11.90
		% Successful	75.00	100.00	97.92
		DC voltage (kV)	4.00	4.00	9.80
	Dry-well no contact	Plate mean (nL)	18.57	83.60	191.92
		% CV	175.71	110.28	115.27
		% Successful	16.67	39.58	35.42
30	Dry-well contact	Plate mean (nL)	44.01	152.91	507.81
		% CV	50.90	54.17	20.20
		% Successful	64.58	72.92	95.83
	Dry-well electrostatic	Plate mean (nL)	38.06	180.46	553.12
		% CV	74.22	19.38	10.60
		% Successful	35.42	91.67	100.00
		DC voltage (kV)	4.00	9.80	4.00

Table V

BigDye Mix

	Stainless Steel Tips	Nominal Volume	100 nL	300 nL	600 nL
5	Control	Plate mean (nL)	83.12	214.68	471.50
	Wet-well	% CV	13.85	7.83	7.38
	contact	% Successful	93.75	100.00	100.00
10	Wet-well no contact	Plate mean (nL)	44.71	112.07	266.03
		% CV	75.64	68.68	71.98
		% Successful	58.33	64.58	58.33
15	Wet-well electrostatic	Plate mean (nL)	72.90	168.01	427.54
		% CV	18.16	9.38	8.25
		% Successful	95.83	100.00	97.92
20		DC voltage (kV)	4.00	4.00	4.00
	Dry-well no contact	Plate mean (nL)	47.27	175.84	350.31
		% CV	54.60	41.79	52.42
25		% Successful	72.92	81.25	72.92
	Dry-well contact	Plate mean (nL)	57.00	178.96	435.70
		% CV	21.60	31.91	23.81
30		% Successful	93.75	85.42	91.67
	Dry-well electrostatic	Plate mean (nL)	54.38	198.72	471.83
		% CV	22.16	11.33	9.07
35		% Successful	91.67	97.92	97.92
		DC voltage (kV)	4.00	4.00	4.00
40	Nitinol Tips	Nominal Volume	100 nL	300 nL	600 nL
	Control	Plate mean (nL)	69.19	201.76	457.33
	Wet-well	% CV	17.47	8.97	8.25
45	contact	% Successful	85.42	100.00	100.00
	Wet-well no contact	Plate mean (nL)	31.56	16.54	212.36
		% CV	95.88	286.03	97.31
50		% Successful	45.83	8.33	45.83
	Wet-well electrostatic	Plate mean (nL)	61.63	161.34	420.54
		% CV	25.38	9.69	8.35
55		% Successful	83.33	100.00	100.00
		DC voltage (kV)	4.00	4.00	4.00
60	Dry-well no contact	Plate mean (nL)	19.18	90.47	217.66
		% CV	140.35	105.56	98.49
		% Successful	25.00	41.67	45.83
65	Dry-well contact	Plate mean (nL)	50.78	170.86	401.36
		% CV	19.20	45.45	36.19
		% Successful	93.75	81.25	87.50
70	Dry-well electrostatic	Plate mean (nL)	40.40	198.17	464.34
		% CV	30.67	9.77	8.22
		% Successful	62.50	100.00	100.00
75		DC voltage (kV)	4.00	4.00	4.00

Description of Circuit Diagram

Figure 3 is a circuit diagram of a power supply in the experimental embodiment of the invention. The electrostatic charger can be operated in automatic or manual mode. In automatic mode, the high voltage is controlled by means of an analog input ranging from 0–5 V, corresponding to 0–20 kV at the high voltage side of the device. In our case, a laptop running NI Labview 7.1® and a DAQCard 6062E® (National Instruments, Austin, TX, USA) was used to provide the analog control signal. The 0–5 V control signal is fed into a noninverting amplifier with a gain of 2.6, whose output is directly supplied to the SC-150® high voltage power supply (American High Voltage, Elko, NV, USA), and DPM 3AS-B® digital LCD display module (Martel Electronics, Londonderry, NH, USA). In manual mode, analog control of the high voltage is performed through a potentiometer. The potentiometer output is sent through a voltage follower to the SC-150 high voltage power supply and digital LCD display module.

The SC-150 high voltage power supply is a voltage-controlled voltage source (VCVS) that provides an output voltage proportional to the input voltage (www.ahv.com). The maximum output is 20 kV, which is supplied to the microtiter plate charger via a 25kV high voltage cable (Belden Electronics Division, Richmond, IN, USA).

An FSK15 Series® AC/DC converter (V-Infinity, Beaverton, OR, USA) provided the 15 V DC required for both automatic and manual operation.

The high voltage side contains the output pins of the SC-150 and the high voltage current limiting resistor (1 GΩ), which is inside a groove in an acrylic block. The groove is filled with a silicone rubber of high dielectric strength to prevent arcing. The high voltage side is supported on a 1/8" acrylic plate with the output pins of the SC-150 and the acrylic block epoxied to the acrylic plate. The separation of the high voltage side from the low voltage side in the illustrated manner ensures that the high voltage components are completely prevented from arcing to any other circuitry. The metal housing that encompasses the circuit is grounded.

Description of Microtiter Plate Charger

5 The microtiter plate charger comprises an aluminum plate (the anode) partly encased in an acrylic shell, which insulates the charged aluminum for safety and to prevent arcing. The high voltage cable connects to the aluminum plate via an aluminum connector and is securely fastened by a cord grip. The microtiter plate is placed onto the face of the aluminum, such that the wells are in contact with the aluminum. Two spring plungers and four stoppers are present to ensure that the microtiter plate is securely positioned. 10 The holding force of the spring plungers may be adjusted to accommodate for different styles of microtiter plates. This particular microtiter plate charger was designed to fit the Hydra®-384 (Art Robbins Instruments, Sunnyvale, CA, USA). To enable compatibility with the Hydra-96, a 0.6" thick plastic spacer of similar dimensions to the Hydra-96 tray table was 15 secured to the bottom of the microtiter plate charger.

The overall dimensions of the aluminum plate are 4.415" × 2.915" × 0.420" (L × W × H). The dimensions of the base of the acrylic shell are 5.025" × 3.375" × 0.75" (L × W × H). The overall dimensions of the lip of the 20 acrylic shell are 5.555" × 3.905" × 0.125" (L × W × H). The overall height of the microtiter plate charger is 0.875".

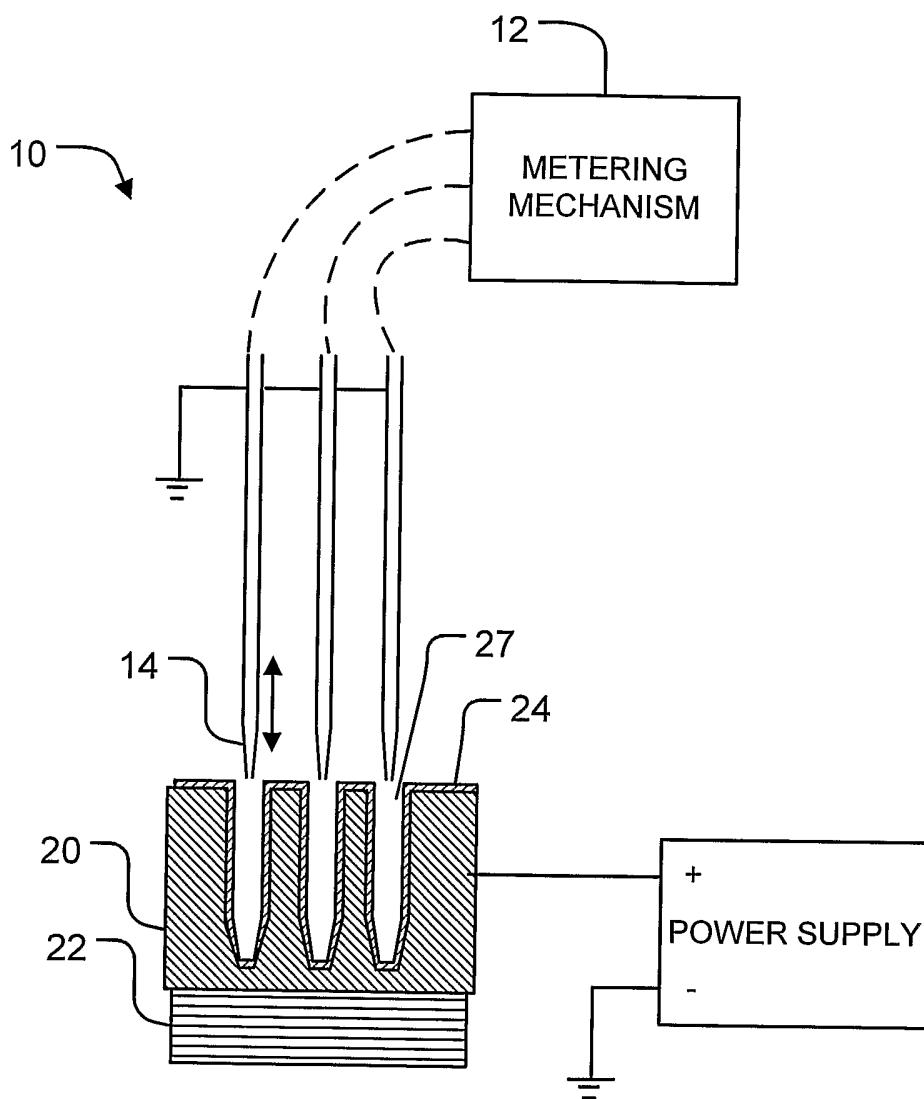
[0024] While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, 25 additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

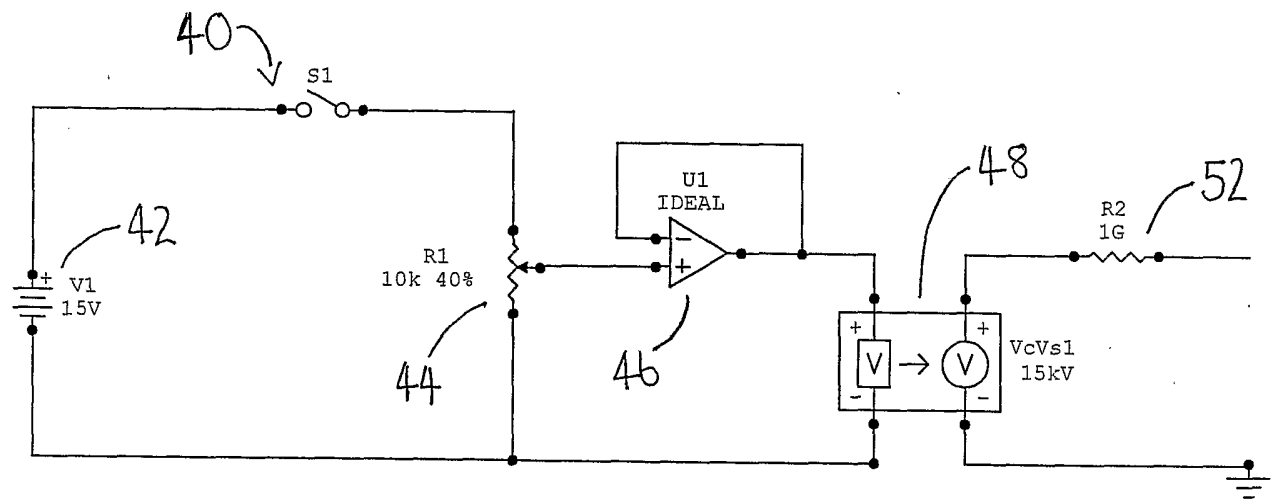
CLAIMS:

1. A method for dispensing a sub-microlitre droplet of fluid, the method comprising:
5 metering a sub-microlitre droplet of fluid at a dispensing tip; and
 applying an electric field to the dispensing tip to pull the droplet off of the dispensing tip.
2. A method according to claim 1 wherein metering the sub-microlitre droplet
10 comprises mechanically metering the droplet.
3. A method according to claim 1 or 2 wherein the electric field is pulsed after the sub-microlitre droplet has been metered.
- 15 4. A method according to claim 3 wherein the electric field is pulsed for approximately 250 milliseconds.
5. A method according to any of claims 1 to 4 wherein the electric field is at least 10 kV.
20
6. A method according to any of claims 1 to 4 wherein the electric field is at least 15kV.
7. A method according to any of claims 1 to 6 wherein the sub-microlitre
25 droplet of fluid has a volume of at most 600 nL.
8. A method according to any of claims 1 to 6 wherein the sub-microlitre droplet of fluid has a volume of 100 nL.
- 30 9. A method according to any of claims 1 to 8 wherein applying the electric field comprises applying a DC electric field.
10. A method according to any of claims 1 to 8 wherein applying the electric
35 field comprises applying an AC electric field.

11. A method according to claim 10 comprising varying the AC electric field with a frequency that is a reasonably close match to a mode of vibration of the sub-microlitre droplet before the sub-microlitre droplet has left the dispensing tip.
- 5
12. A method according to any of claims 1 to 11 wherein applying the electric field comprises applying an electrostatic potential between the dispensing tip and a base plate comprising a well.
- 10
13. A method according to claim 12 comprising positioning the base plate such that the dispensing tip within 3 millimeters of a bottom of the well before applying the electric field.
- 15
14. A method according to claim 12 wherein the well contains a solution, the method comprising positioning the base plate such that the dispensing tip within 2 millimeters of the solution in the well before applying the electric field.
- 20
15. An apparatus comprising a dispensing tip, a metering mechanism connected to deliver metered quantities of fluid to the dispensing tip; and a power supply connected to cause an electric field at the dispensing tip, the electric field having a gradient and strength at the dispensing tip sufficient to pull droplets dispensed by the metering mechanism off of the dispensing tip.
- 25
16. An apparatus for dispensing sub-microlitre droplets of fluid, the apparatus comprising:
- a plurality of dispensing tips;
 - a metering mechanism connected to deliver sub-microlitre droplets of fluid to the dispensing tip;
 - 30 a base plate located below the dispensing tips for supporting a microtiter plate comprising a plurality of wells; and,
 - a power supply connected to apply an electrostatic potential between the dispensing tips and the base plate.
- 35
17. An apparatus according to claim 16 wherein the power supply comprises a voltage-controlled voltage source.

18. An apparatus according to claim 17 wherein the voltage-controlled voltage source comprises an input ranging from 0 to 5 V and an output ranging from 0 to 20 kV.
- 5 19. An apparatus according to any of claim 16 to 18 wherein the power supply comprises a current limiting resistor.
20. An apparatus according to claim 19 wherein the current limiting resistor has a resistance of 1 G Ω .
- 10 21. An apparatus according to any of claims 16 to 20 comprising means for varying a distance between the base plate and the dispensing tips.

**FIGURE 1**

**FIGURE 2**

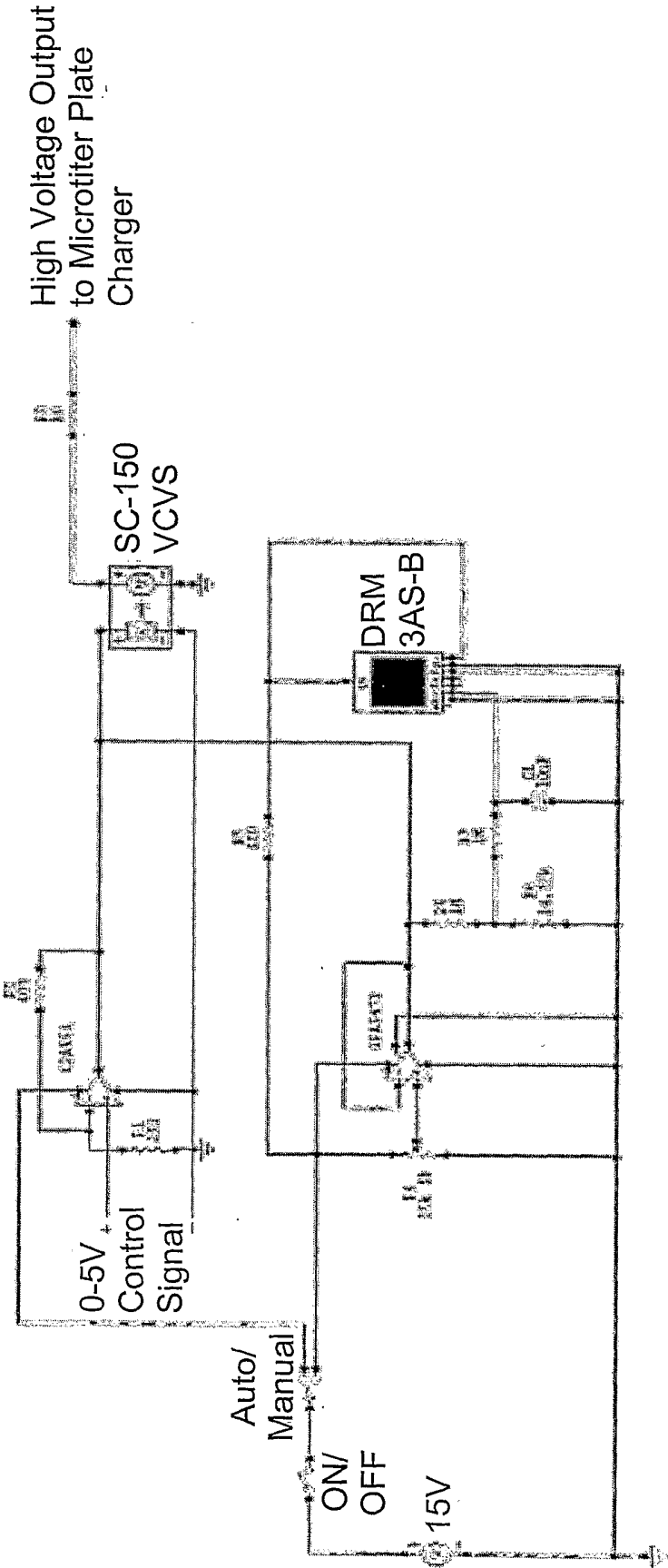


FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/000201

A. CLASSIFICATION OF SUBJECT MATTER IPC: <i>G01N 35/10</i> (2006.01) , <i>G01N 1/28</i> (2006.01) , <i>C40B 60/00</i> (2006.01) , <i>B01L 3/02</i> (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC: <i>G01N 35/10</i> (2006.01); <i>G01N 35/00</i> (2006.01); <i>G01N 35/02</i> (2006.01); <i>G01N 35/04</i> (2006.01); <i>G01N 35/08</i> (2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Canadian Patent Database, Delphion, WEST, Google Scholar Keywords: droplet, fluid, electric, field, tip, dispensing, pipette, electrowetting, electrohydrodynamic		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2 434 305 Brock et al. 15 August 2002 (15-08-2002)	1 to 4, 7 to 9, 12, 15 to 17 and 21
Y	*abstract, pages, 4, 7, 9 and 11 *	13, 14 and 18 to 20
X	EP 1 157 737 A2 Tanaami 28 November 2001 (28-11-2001)	1 to 3, 7, 9, 12, 15 to 17 and 21
Y	* abstract, col. 2 and 3 *	13, 14 and 18 to 20
X, P	US 2005/0214799 A1 Cho et al. 29 September 2005 (29-09-2005) * abstract, pages 2 and 4 *	1 to 3, 5, 6, 9, 10, 12, 15 to 17 and 21
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 12 April 2006 (12-04-2006)		Date of mailing of the international search report 18 May 2006 (18-05-2006)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476		Authorized officer Wendy Stewart (819) 934-2674

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
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