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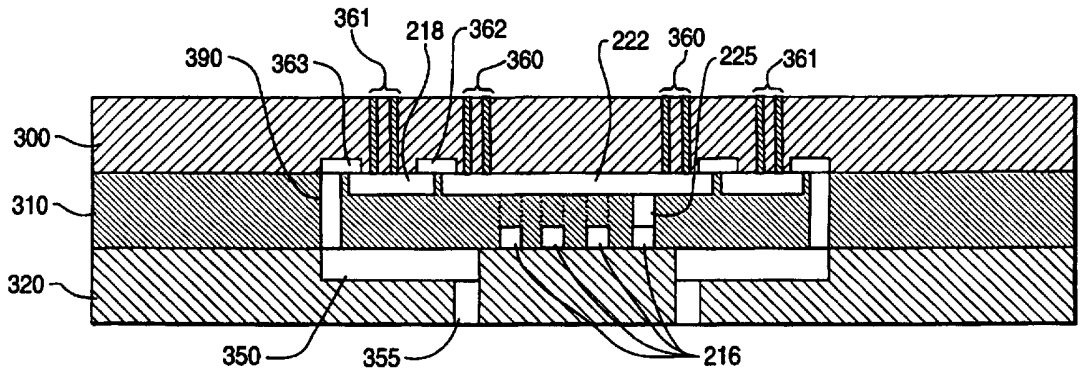

(57) Abstract

The invention provides methods of performing a synthetic process in a liquid distribution system (300, 310 and 320) having reaction cells (in 320), by pumping at least one reagent into a reaction cell using an electrode-based pump (360).

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ELECTROKINETIC PUMPING

This application relates to electrode-based pumps, methods of operating such pumps, and calibration devices for such pumps.

Recently, a number of academic articles have focused on the problems associated with conducting chemical reactions on a micro-scale. This literature has discussed the possibility of managing such reactions on wafer-sized solid supports that have been etched to create microchannels. Reactor systems of this scale could allow multiple diagnostic or drug screening assays to be conducted in a transportable device that uses small amounts of reagents, thus reducing supply and disposal costs.

Combinatorial chemistry seeks to create the large family of compounds by permutation of a relatively limited set of building block chemicals. Preferably, the combinatorial method will create identifiable pools containing one or more synthetic compounds. These pools need not be identifiable by the chemical structure of the component compounds, but should be identifiable by the chemical protocol that created the compounds. These pools are then screened in an assay that is believed to correlate with a pharmacological activity. Those pools that produce promising results are examined further to identify the component compounds and to identify which of the component compounds are responsible for the results.

SUMMARY OF THE INVENTION

The invention provides methods of performing a synthetic process in a liquid distribution system having reaction cells, comprising pumping at least one reagent into a reaction cell. The synthetic processes include chemical reactions including, but are not limited to, nucleophilic or electrophilic substitiutions or a acid-catalyzed or base-catalyized cleavages. Further, the synthetic processes include chemical reactions including, but are not limited to, a catabolic reaction, an anabolic reaction, an oxidation, a reduction, amide formation, Mitsunobu reaction, Suzuki coupling, Stille coupling, alkylation of an amine, acylation of an amine, alkylation of a ketone or nitrile, Schiff base formation, reductive amination, sulfonamide formation, nucleic acid synthesis, protein synthesis, cycloaddition, Mannich reaction, Diels Alder reaction, Wittig reaction, Heck reaction, epoxide elimination, elimination of a leaving group, a condensation reaction, a transesterification, iodolactonization, Grignard reaction, reactions of organometallic compounds with alkyl halides, Michael addition, Ugi reaction, Knoevenagel reaction, oyrazole formation, quinoline formation, thiazolidine formation, pyrazolone formation, isoxazole formation, imine formation, Hantzsch reaction,

Dieckmann reaction, Aldol condensation, Bischler-Hapieralski reaction, acetal/ketal formation, Fischer indole synthesis, Friedel Craft reaction, Carbamate formation or Lactam formation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a voltage pulse pattern used to power an electrode-based pump useful in the liquid distribution system.

Figures 2A and 2B show the field strength and orientation at numerous points about electrode-based pumps.

Figure 3 shows a calibration device.

Figure 4 shows another calibration device.

Figure 5 displays a cut-away view of a liquid distribution system.

Figure 6 displays a distribution plate of the liquid distribution system of Figure 5.

Figure 7 displays an expanded view of a portion of the distribution plate of Figure 6.

Figure 8 shows a capillary barrier between a first distribution channel and a buffer channel.

Figures 9A - 9D show various capillary barrier designs.

Figure 10 shows a device for conducting field assisted bonding of plates.

Figure 11 shows a digital driver for powering the electrode-based pumps.

Figures 12A and 12B show a channel device having electrode-based pumps.

Figure 13 shows a liquid distribution system design pursuant hydrologic liquid distribution system.

Figure 14 shows a reaction cell having a heater and a thermocouple.

Figures 15A and 15B show a valve design.

DEFINITIONS

The following terms shall have the meaning set forth below:

- **addressable** — a reaction cell or channel is "addressable" by a reservoir or another channel if liquid from the reservoir or other channel can be directed to the reaction cell or channel.
- **adjacent** — "adjacent" as used in these situations: (i) a first structure in one of the plates is adjacent to a second structure in the same or another plate if the vertical projection of the first structure onto

	the plate of the second structure superimposes the first structure on the second or places it within about 250 μm of the second; and (ii) groupings of two or more channels are adjacent to one another if each channel is in substantially the same horizontal plane, and all but the outside two channels in the grouping are adjacent (in the sense defined in (i) above) to two neighbor channels in the grouping. Preferably, under item (i), a first structure is adjacent to a second structure if the vertical projection of the first structure onto the plate of the second structure superimposes the first structure on the second or places it within about 150 μm of the second.
• **capillary dimensions**	dimensions that favor capillary flow of a liquid. Typically, channels of capillary dimensions are no wider than about 1.5 mm. Preferably channels are no wider than about 500 μm, yet more preferably no wider than about 250 μm, still more preferably no wider than about 150μm.
• **capillary barrier**	a barrier to fluid flow in a channel comprising an opening of the channel into a larger space designed to favor the formation, by liquid in the channel, of an energy minimizing liquid surface such as a meniscus at the opening. Preferably, capillary barriers include a dam that raises the vertical height of the channel immediately before the opening into the larger space.
• **connected**	the channels, reservoirs and reaction cells of the invention are "connected" if there is a route allowing fluid between them, which route does not involve using a reaction cell as part of the link.
• **directly connected**	reservoirs and horizontal channels are "directly connected" if they are connected and either (1) no other channel is interposed between them or (2) only a single vertical channel is interposed between them.
• **flow preference**	the direction that a liquid pumps under the influence of an electrode-based pump having two symmetrically situated rod-shaped electrodes.
• **hole diameter**	because techniques for fabricating small holes often create holes that are wider at one end than the other (for instance, about 50 microns wider), the hole diameter values recited to herein refer to the narrowest diameter.
• **horizontal, vertical, EW, NS**	indications of the orientation of a part of the distribution system refer to the orientation when the device is in use. The notations "EW axis" and "NS axis" are in reference to Figures 1, 2, 3 and 7, where an EW axis goes from right to left and is perpendicular to the long axis of the page

	and a NS axis is from top to bottom parallel to the long axis of the page.
• **independent**	channels, reservoirs or reaction cells that are not connected.
• **offset**	two sets of channels are "offset" when none of the channels in the first such set is adjacent to any of the channels in the second set.
• **perpendicular**	channels in the distribution plate are perpendicular even if primarily located on separate horizontal planes if their vertical projections onto the same horizontal plane are perpendicular.
• **reservoir**	unless a different meaning is apparent from the context, the terms "reservoir" and "fluid reservoir" include the horizontal extension channels (sometimes simply termed "extensions") directly connected to the reservoir or fluid reservoir.
• **second reservoir extension channels**	these extension channels include the distribution channels that may branch off of these extension channels.
• **substantially the length of one of the horizontal dimensions**	at least about 70% of one of the major horizontal dimensions (e.g. the EW or NS dimensions illustrated in the Figures) of the distribution plate.
• **U-plumbing channel**	a channel designed to connect at least two channels or reservoirs such that the liquid level in one of the connected channels or reservoirs will equalize with the liquid level in the other connected channel or reservoirs due to hydrological forces. U-plumbing channels typically have vertical channels that connect channels or reservoirs located in a higher vertical plane with a substantially horizontal channel segment of the U-plumbing channel located in a lower plane -- these vertical and horizontal segments together comprise the U-plumbing channel. The feeder channels of the invention are typically U-plumbing channels.

DETAILED DESCRIPTION

The invention provides methods of performing a synthetic process, such as a chemical reaction, in a liquid distribution system having reaction cells, comprising pumping at least one reagent into a reaction cell. A "reagent" is defined herein as a substance used in a chemical reaction. Reagents include, but are not limited to, solvents, catalysts and reactants. Any chemical reaction with reaction conditions suitable for the hydrologic

liquid distribution system can be used in the methods of the present invention, including the chemical reactions cited in March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* (John Wiley & Sons, 1992), which is hereby incorporated by reference in its entirety. The chemical reactions include, but are not limited to, a catabolic reaction, an anabolic reaction, an oxidation, a reduction, amide formation, a Mitsunobu reaction, Suzuki coupling, Stille coupling, alkylation of an amine, acylation of an amine, alkylation of a ketone, reductive amination, sulfonamide formation, DNA synthesis, cycloaddition, a Mannich reaction, a Diels Alder reaction, a Wittig reaction, a Heck reaction, elimination of a leaving group and a condensation reaction.

The following publications, all of which are incorporated by reference herein in their entirety, provide a description of chemical reactions for use in methods of the present inventions. For a description of amide formation, see, for example, *Tet. Lett.* 30:1827 (1989), *Tet Lett.* 30:4645 (1989), *Syn. Comm.* 23:349 (1993) and *J. Org. Chem.* 44:5000 (1979). For a description of a Mitsunobu reaction, see, for example, *Tet Lett.* 35:4705 (1994). For a description of Suzuki coupling, see, for example, *Tet Lett.* 49:9177 (1994). For a description of Stille coupling, see, for example, *Tet Lett.* 35:5613 (1994) and *J. Org. Chem.* 60:523 (1995). For a description of alkylation of an amine, see, for example, *J. Org. Chem.* 38:1427 (1995). For a description of acylation of an amine, see, for example, *Ang. Chem. Int. Ed.* 34:907 (1995). For a description of alkylation of a ketone, see, for example, *Ang. Chem. Int. Ed.* 18:221 (1979). For a description of reductive amination, see, for example, *J. Am. Chem. Soc.* 93:2897 (1971), *Synthesis* 135 (1075) and *Tet Lett.* 31:5547 (1990). For a description of sulfonamide formation, see, for example, *J. Org. Chem.* 38:1427 (1995). For a description of cycloaddition reactions, see, for example, *Advanced Organic Chemistry, Part B, "Reactions and Synthesis"* (Plenum Press, NY 1983). For a description of a Mannich reaction, see, for example, *Tetrahedron* 46:1791 (1990) and *Synthesis* 703 (1973). For a description of a Diels Alder reaction, see, for example, *Diels Alder Reactions* (Elsevier, NY 1965). For a description of a Wittig reaction, see, for example, *Org. React.* 25:73 (1977) and *Chem. Rev.* 74:87 (1974). For a description of an oxidation reaction, see, for example, *Oxidations in Organic Chemistry,*

Part C (Academic Press, NY 1978) at page 211. For a description of elimination of a leaving group, see, for example, *The Chemistry of Functional Groups Supplement D, Part 2* (Wiley, NY 1983), at page 1173.

The synthetic processes also include the synthesis of a small molecule, a polymeric organic compound, an oligonucleotide or a peptide. The reagents in these synthetic processes include, but are not limited to, carboxylic acid, carbodiimide, sulfonamide, amine, alcohol, pyridine, azodicarboxylate, carbazole, azobenzene, amino N-oxide, 1,4-benzoquinone or ammonium perruthenate. In certain preferred embodiments, the synthetic process is carried out employing a solid support.

In certain preferred embodiments of the invention, particular reagents are electrokinetically pumped into a reaction cell. For example, when the reaction is an oxidation, the reagents are preferably selected from the group consisting of m-chloroperbenzoic acids, hydrogen peroxide, and $KMnO_4$. When wherein the reaction is a reduction, the reagents are preferably selected from the group consisting of $LiAlH_4$ and lithium borohydride. When the reaction is amide formation, the reagents are preferably selected from the group consisting of o-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate, 2,3-dichloro-5,6-dicyano-1,4-benzoquinonone, 1-hydroxybenzotriazole hydrate, protected amino acids, N-methyl morpholine and diisopropyl carboiimide. Alternatively, in another embodiment, the amide is a urea compound, and two reagents are separately pumped into the reaction cell, with the first reagent being an amine and the second reagent an isocyanate or an isothiocyanate. When the reaction is a Mitsunobu reaction, the reagents are preferably selected from the group consisting of phenol, alcohol, diethylazodicarboxylate, triphenylphosphine and N-methyl morpholine. When the reaction is Suzuki coupling, the reagents are preferably selected from the group consisting of halide, palladium (0) catalyst, aryl boronic acid, triethyl amine and dimethyl formamide. When the reaction is Stille coupling, the reagents are preferably selected from the group consisting of halide, paldium (0) catalyst, aryl stannane, N-methyl pyrrolidinone and triphenyl arsine. When the reaction is alkylation or acylation of an amine, the reagents are preferably selected from the group consisting of tosylate, halide, amine, N-methyl pyrrolidinone and phosgene. When the reaction is alkylation of a ketone, the reagents are preferably selected from the group consisting of amine, ketone, lithium diiosopropyl amine, tetrahydrofuran and alkyl iodide. When the reaction is

reductive amination, the reagents are preferably selected from the group consisting of amine, aldehyde, sodium cyanoborohydride, sodium triacetyoxyborohydride, borohydrate pyridine and methylene chloride. When the reaction is sulfonamide formation, the reagents are preferably selected from the group consisting of amines, sulfonyl chloride and dimethylformamide. When the reaction is DNA synthesis, the reagents are preferably selected from the group consisting of oligonucleotides and phosphates. When the reaction is cycloaddition, the reagents are preferably selected from the group consisting of peracids, substituted alkenes, diazo compounds, azide, nitriles, azomethine ylide, nitrones and carbonyl oxides. When the reaction is a Mannich reaction, the reagents are preferably selected from the group consisting of aldehydes, ketones, amine salts, amides, acids and bases. When the reaction is a Diels Alder reaction, the reagents are preferably selected from the group consisting of substituted alkenes and dienes. When the reaction is a Wittig reaction, the reagents are selected from the group consisting of triphenylphosphines and substituted alkenes. When the reaction is a Heck reaction, the reagents are preferably selected from the group consisting of a palladium (0) catalyst, substituted alkenes, substituted halides and triethylamines. When the reaction is elimination of a leaving group, the reagents are preferably selected from the group consisting of alkyl halides and bases.

For the various reactions which are most preferably employed in the methods of the invention, typically appropriate reagents will be separately pumped or otherwise located in the reaction cell. In some cases, such as where three or more types of reagents are important to the chemistry involved, it can be appropriate, as will be recognized by those of ordinary skill, to pump two or more of the reagents together. Since in one aspect of the invention one reactive moiety will be fixed onto a solid phase located in the reaction cell, in such cases one reagent will not have to be pumped. The reagents pumped are typically pumped in admixture with an appropriate solvent, which solvent can be identified by those of ordinary skill.

The Tabulation below lists non-limiting examples of the types of reagents that are pumped into the reaction cell or fixed to a solid phase in the reaction cell to conduct various reactions according to the method of the invention.

Those of ordinary skill will recognize that some of members of the reagent sets listed below are appropriately pumped concurrently into

the reaction cell, while others are appropriately pumped sequentially.

REACTION	REAGENTS
amide formation	A-1: organic acid A-2: organic amine A-3: dehydration reagents such as O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium-hexafluorophosphate, 2,3-dichloro-5,6-dicyano-1,4-benzoquinonone, 1-hydroxybenzotriazole, protected amino acids, N-methyl morpholine, or diisopropyl carboiimide B-1: reactive ester such an an N-hydroxysuccinimide ester or an ester with 1-hydroxybenzotriazole B-2: organic amine
Mitsunobu reaction	A-1: alcohol A-2: tripheylophosphine A-3: diisopropyl azodicarboxylate A-4: carboxylic acid or substituted phenol
Suzuki coupling	A-1: alkyl or arylboronic acid A-2: alkyl or aryl halide A-3: Pd(0) catalyst A-4: base (triethylamine for example)
Stille coupling	A-1: organotin compound A-2: Pd(0) catalyst A-3: aryl or alkyl halide
alkylation of an amine	A-1: amineA-3: aryl or alkyl halidese A-2: alkyl halide
acylation of an amine	A-1: amine A-2: acyl halide
alkylation of a ketone or nitrile	A-1: ketone or nitrile A-2: strong base such as a metal salt of t-butyl alcohol, or a metal salt of ammonia or a dialkyl amine A-3: alkyl halide
Schiff base formation	A-1: aldehyde or ketone A-2: amine, typically a primary amine
reductive amination	There are many examples A-1: nitro compound reduced to amines with $SnCl_2$/DMF A-2: azides reduced to amines with $SnCl_2$/PhSH/triethylamine/THF A-3: ketones to amines with hydrogen gas and Ni catalyst.
Nucleic acid synthesis	A-1: solid phase with nucleoside base with free 5´ hydroxyl A-2: 3´-phosphoramidite with dimethoxytrityl protecting group on 5´ oxygen A-3: oxidation reagent such as iodine A-4: acid such as dichloroacetic acid
protein	

REACTION	REAGENTS
synthesis	A-1: solid phase with amino acid with free amino terminal A-2: reactive ester formed with t-butoxycarbonyl-amino acid A-3: acid such as trifluoroacetic acid B-1: solid phase with amino acid with free amino terminal B-2: fluorenylmethyloxycarbonyl-amino acid B-3: condensing reagent such as an N-hydroxysuccinimide ester or an ester with 1-hydroxybenzotriazole B-4: base such as triethylamine
cycloaddition	In general, this would be reactions with olefins and Acetylenes with other conjugated systems to form rings
Mannich reaction	A-1: ketone with an active hydrogen A-2: formaldehyde A-3: secondary amine hydrochloride
Diels Alder reaction	A-1: diene such as 1,3-butadiene A-2: dienophile such as maleic anhydride or *p*-benzoquinone
Wittig reaction	A-1: ketone or aldehyde A-2: a ylide such as methylenetriphenylphosphorane
Heck reaction	A-1: olefins or acetylenes A-2: Pd(0) cataylst A-3: aryl or alkyl halides A-4: base (triethylamine for example)
epoxide elimination	A-1: epoxide A-2: strong base such as metal salt of a dialkylamine
dehalogenation reaction	A-1: halo-alkane with an α-hydrogen A-2: base
elimination of an alkyl sulfonate	A-1: alkyl-sulfonate with an α-hydrogen A-2: base
Hofmann elimination	A-1: quaternary ammonium compound A-2: base
transester-ification	A-1: ester A-2: alcohol A-3: acid or base
iodolacton-ization	A-1: organic acid with an unsaturated double bond A-2: iodine, potassium iodate, base
Grignard reaction	A-1: alkyl halide A-2: magnesium A-3: acid, aldehyde, ketone, epoxide, ester or water
reactions of organometallic compounds with alkyl	

REACTION	REAGENTS
halides	A-1: alkyl halide A-2: lithium A-3: copper halide A-4: second alkyl halide
Michael addition	A-1: α,β-unsaturated carbonyl compound A-2: compound that forms a carbanion such as ethyl malonate A-3: base
Ugi reaction	A-1: isocyanides A-2: carboxylic acid A-3: aldehyde or ketone A-4: amine product is a bisamide
Knoevenagel reaction	A-1: aldehyde or ketone A-2: compound that forms a carbanion such as ethyl malonate A-3: base
thiazolidine formation	A-1: amine-thiol compound A-2: aldehyde or ketone
isoxazole formation	A-1: substituted anhydride A-2: NH_2OH
imine formation	A-1: amine A-2: aldehyde or ketone
Dieckmann reaction	A-1: esterified dicarboxylic acid A-2: base such a sodium salt of ethanol
Aldol condensation	A-1: aldehyde or ketone A-2: base
Passerini Reaction	A-1: isocyanide A-2: carboxylic acid A-3: aldehyde or ketone product is a acyloxy amide
acetal/ketal formation	A-1: alcohol A-2: aldehyde or ketone
Fischer indole synthesis	A-1: hydrazines A-2: ketone A-3: ZnCl2 A-4: acetic acid
Friedel Craft reaction	A-1: olefin A-2: acid halide A-3: Lewis acid such as aluminum chloride, boron trifluoride, stannic chloride or zinc chloride B-1: olefin B-2: carboxylic acid B-3: protonic acid such as hydrogen fluoride, sulfuric acid or polyphosphoric acid

REACTION	REAGENTS
Carbamate formation	A-1: p-nitrophenyl carbonate A-2: amine A-3: base (dimethylaminopyridine or diisoproypethylamine)
Pictet-Spengler Reaction	A-1: arylethylamine A-2: aldehyde or ketone product is a hydroisoquinoline
Urea formation	A-1: isocyanates A-2: amines
Pyrazole formation	A-1: substituted anhydride A-2: hydrazine derivative
Horner Emmons/ Wittig Reaction	A-1: Phosphorous ylide A-2: aldehyde or ketone

A. Electrode-based Pumps

At least two types of such electrode-based pumping has been described, typically under the names "electrohydrodynamic pumping" (EHD) and "electroosmosis" (EO). EHD pumping has been described by Bart et al., "Microfabricated Electrohydrodynamic Pumps," *Sensors and Actuators*, A21-A23: 193-197, 1990 and Richter et al., "A Micromachined Electrohydrodynamic Pump," *Sensors and Actuators*, A29:159-168, 1991. EO pumps have been described by Dasgupta et al., "Electroosmosis: A Reliable Fluid Propulsion System for Flow Injection Analysis," *Anal. Chem.*, 66: 1792-1798, 1994. In the present application, pumping effected with electrodes is termed "electrokinetic pumping."

EO pumping is believed to take advantage of the principle that the surfaces of many solids, including quartz, glass and the like, become charged, negatively or positively, in the presence of ionic materials, such as salts, acids or bases. The charged surfaces will attract oppositely charged counter ions in solutions of suitable conductivity. The application of a voltage to such a solution results in a migration of the counter ions to the oppositely charged electrode, and moves the bulk of the fluid as well. The volume flow rate is proportional to the current, and the volume flow generated in the fluid is also proportional to the applied voltage. Typically, in channels of capillary dimensions, the electrodes effecting flow can be spaced further apart than in EHD pumping, since the electrodes are only involved in applying force, and not, as in EHD, in creating charges on which the force will act. EO pumping

is generally perceived as a method appropriate for pumping conductive solutions.

EHD pumps have typically been viewed as suitable for moving fluids of extremely low conductivity, e.g., 10^{-14} to 10^{-9} S/cm. It has now been demonstrated herein that a broad range of solvents and solutions can be pumped using appropriate solutes than facilitate pumping, using appropriate electrode spacings and geometries, or using appropriate pulsed or d.c. voltages to power the electrodes, as described further below.

Useful electrode configurations and operating voltages are described in US Application No. 08/556,423, filed 9-November-1995 (DSRC 11717A), No. 08/645,966, filed 10-May-1996 (DSRC11717B), PCT Application No. US95/14586, filed 9-November-1995 (DSRC11717PCT), and PCT Application No. US95/14590, filed 9-November-1995 (DSRC11402GPCT) which applications are incorporated herein by reference in its entirety.

It is believed that an electrode-based internal pumping system can best be integrated into the liquid distribution system of the invention with flow-rate control at multiple pump sites and with relatively less complex electronics if the pumps are operated by applying pulsed voltages across the electrodes. Figure 1 shows an example of a pulse protocol where the pulse-width of the voltage is τ_1 and the pulse interval is τ_2. Typically, τ_1 is between about 1 μs and about 1 ms, preferably between about 0.1 ms and about 1 ms. Typically, τ_2 is between about 0.1 μs and about 10 ms, preferably between about 1 ms and about 10 ms. A pulsed voltage protocol is believed to confer other advantages including ease of integration into high density electronics (allowing for hundreds of thousands of pumps to be embedded on a wafer-sized device), reductions in the amount of electrolysis that occurs at the electrodes, reductions in thermal convection near the electrodes, and the ability to use simpler drivers. The pulse protocol can also use pulse wave geometries that are more complex than the block pattern illustrated in Figure 1.

Another, procedure that can be applied is to use a number of electrodes, typically evenly spaced, and to use a travelling wave protocol that induces a voltage at each pair of adjacent electrodes in a timed manner that first begins to apply voltage to the first and second electrodes, then to the second and third electrodes, and so on. Such methods are described in

Fuhr et al., *J. Microelectrical Systems*, 1: 141-145, 1992. It is believed that travelling wave protocols can induce temperature gradients and corresponding conductivity gradients that facilitate electric field-induced fluid flow. Such temperature gradients can also be induced by positioning electrical heaters in association with the electrode-based first pumps 360 and second pumps 361.

While not wishing to be restricted to theory, several theoretical concepts are believed to play a role in the mechanics of EHD pumping. The forces acting on a dielectric fluid are believed to be described by:

$$\vec{F} = q\vec{E} + \vec{P} \times \nabla\vec{E} - \frac{1}{2}E^2\nabla\varepsilon + \nabla\left[\frac{1}{2}\rho\frac{\partial e}{\partial p}E^2\right]$$

where **F** is the force density, **q** is the charge density, **E** is the applied field, **P** is the polarization vector, ε is the permittivity and ρ is the mass density. Of the terms in the equation, the first and third are believed to be the most significant in the context of EHD pumping of fluids. The first term (**qE**) relates to the Coulomb interaction with a space-charge region. The third term ($\frac{1}{2}E^2\nabla\varepsilon$) relates to the dielectric force which is proportional to the gradient in permittivity.

In low fields, i.e., the Ohmic region where current is linearly proportional to voltage, the primary source of charges that will be acted upon by the electric field are believed to be primarily due to ions from additives, ions from impurities and ions formed by autodissociation of molecules in the fluid. In intermediate fields, i.e. from beyond the Ohmic region to about 2 V/μm, the charges are believed to be primarily formed by dissociation and electrolylytic processes in the fluid. In higher fields, the charges are believed to be determined by injection processes at the electrodes, which electrodes inject homocharges.

For the purposes of this application, positive (+) flow shall be flow in the direction of the negative electrode, and negative (-) flow shall be flow in the direction of the positive electrode.

In a preferred embodiment of the invention, the controller 10 has a device for storing data and stores the values of voltage and polarity suitable for pumping a number of solvents.

Experimental results indicate that the properties of fluid flow (like direction of flow) correlate well with the solvent's ability to stabilize and solvate the charged species injected or induced from the electrodes. The direction of flow is believed to be determined by the preference of the solvent to solvate either cations or anions. This solvation preference is believed to imply a greater shell of solvent molecules that will be dragged in an electric field, creating fluid movement, when a field is applied to the electrodes of a first pump 360 or a second pump 361. For example, a preferred solvation of cations correlates with a preference for fluid flow from the anode to the cathode (i.e., the positive direction). The degree of such a solvation preference for a solvent is believed to depend on the ability of the molecules within the solvent to accept or donate hydrogen bonds. In one aspect of the invention, for liquids whose pumping behavior has not yet been characterized, the controller will store initial pumping parameters estimated using on the Linear Solvation Energy relationships established by R.W. Taft and co-workers. See, Kamlet et al., *J. Org. Chem.*, 48: 2877-2887, 1983 and Kamlet et al., *Prog. Phys. Org. Chem.*, 13: 485, 1981. These workers have categorized solvents in terms of the following parameters: π, the ability of the solvent to stabilize a stabilize a charge or dipole by virtue of its dielectic properties; α, the hydrogen bond donating ability of the solvent; and β, the hydrogen bond accepting ability of the solvent. These parameters are more fully defined in the above-cited Kamlet et al. publications, from which these definitions are incorporated herein by reference.

Using a one mm capillary of circular cross-section, a pair of 50 micron rod-shaped, platinum electrodes perpendicularly inserted to a depth of 500 microns into the capillary with a 500 micron separation powered by a 400 V field, the direction of flow was determined for several solvents. The direction of flow and the α, β, π, ε and dipole moment values are as follows:

Solvent	direction	α	β	π	ε	dipole moment
ethanol	-	0.83	0.77	.54	24.55	1.69
tetrahydro-furan	+	0	0.55	.58	7.58	1.75
chloroform	-	0.44	0	.58	4.806	1.01
acetone	+	0.08	0.48	.71	20.7	2.69
methanol	-	0.93	0.62	.6	32.7	2.87

2-propanol	+/-	0.76	0.95	.48	19.92	1.66
acetonitrile	+	0.19	0.31	.75	37.5	3.92
N-methyl-pyrrolidone	+	0	0.77	.92	32.0	4.09
diethyl ether	+	0	0.47	0.27	4.335	1.15
1,2 dichloro ethane	-	0	0	0.81	10.36	1.2
DMF	+	0	0.69	.88	36.71	3.86

It is believed that the α and β values reflect the ability of the solvent under an electric field to solvate a negative or positive charged species, with the magnitude of $\alpha - \beta$ correlating with (-) flow, and the magnitude of $\beta - \alpha$ correlating with (+) flow. According to one aspect of the invention, the preferred direction of flow of a liquid can be reversed from that predicted as above if the fluid has a difference in α and β values that is small but not zero and the electrode pair used creates an asymmetric field, such that the acting force on either positive or negative charged species is enhanced. One such electrode pair has an alpha electrode 364 with that points in the direction of intended flow and a beta electrode 365 that lines the walls of the channel in which it is located. Preferably, the alpha electrode 364 sufficiently points in the direction of flow such that its point defines a line that intersects the plane defined by the beta electrode 365. Preferably, the alpha electrode 364 ends in a point or wedge shape. Such an electrode-based pump, fabricated in a 1 mm capillary, has been shown to be effective to pump 2-propanol in the direction pointed to by the alpha electrode 364 either when the voltage applied to the electrodes implied a (-) direction of flow or, with somewhat weaker flow, when the voltage applied to the electrodes implied a (+) direction of flow.

The asymmetric, electrode-based pump effective to pump 2-propanol in the direction pointed to by the alpha electrode 364 is illustrated in Figure 2A. Alpha electrode 364 points from left to right in the figure. Beta electrode 365 is a ring electrode that is flush with the sides of the capillary 501. Using the QuickField program available from Tera Analysis, Granada Hills, CA, the electric field strengths and orientations at various points about the electrodes is indicated by the size and orientation of the arrow at that point. It can be seen from Figure 2A that where alpha electrode 364 acts as a cathode, the area where a solvated negative ion

would be influenced by a strong field pushing it in the (+) direction is greater than the area where a strong field would push a solvated positive ion in the (-) direction. The integration of these forces acting on these solvation spheres explains why asymmetric fields can be used to pump a liquid against its ordinary preferred direction of flow. Thus, depending on the liquids to be pumped, asymmetric fields can be used to assure a given direction of flow.

For comparison purposes, Figure 2B shows the field strength and orientation of a number of points about a more symmetrical pump having alpha electrode 364 and beta electrode 365.

The pumping parameters of a liquid can be calibrated using a plug of the liquid disposed in a capillary that has an electrode-based pump and is angled uphill. If optical devices are associated with the capillary for monitoring the position of the plug, the velocity of pumped flow uphill and the velocity of gravity driven downhill motion can be measured. With these velocities and the angle of the capillary, the pressure applied to the liquid can be calculated. (Fluid resistance, $R = (8 \cdot \mu \cdot l)/\pi r^4$, where μ defines viscosity and l = the length of the fluid plug; Pressure, $P = RA(v_{up} - v_{down})$, where A = cross-sectional area). The efficiency of the pump can also be calculated ($\eta = (q \cdot \rho \cdot Q \cdot N_A)/m \cdot I$, where q = charge of e^-, ρ = density of liquid, Q = flow rate = $v_{up} \cdot A$, m = mass of liquid, and I = current). The velocities can be measured with multiple single point observations of the location of either the front or rear interfaces of the plug using fixed LEDs and optical detectors or in a continuous mode using a light and a silicon photodiode position sensor, such as a SL15 or SC10 position sensor available from UDT Sensors, Inc., Hawthorne, CA. With the latter method, the correlation between the signal produced at the difference amplifier connected to the position sensor must be calibrated prior to experimental use.

Figure 3 shows a calibration device having first optical detector 401, second optical detector 402 and third optical detector 403. These are preferably photodiodes such as the OTS-254 phototransister available from Opto Technnology, Inc., Wheeling, IL. Not shown are corresponding first light source 411, second light source 412 and third light source 413. These are preferably LEDs such as the Super Bright LEDs available from Radio Shack. A capillary 420 is situated in mount 430. Mount 430 can be manipulated to orient capillary 420 at an angle offset from a horizontal orientation. First lead 441 and second lead 442 relay

voltage to the electrode based pump 460 (obscured by mount 430) from the voltage-generating portion 470 of controller 10. Electrical signals correlating with light detection are relayed to the signal processing portion 480 of controller 10 by way of first data lead 481, second data lead 482 and third data lead 483. In operation, the signals from first optical detector 401, second optical detector 402 and third optical detector 403 will show a transition point when the interface of a plug 11 of liquid in capillary 420 goes by the respective detector (401, 402 or 403). The timing of these transitions provides a measure of the velocity of movement of the plug 11.

Figure 4 shows a calibration device having a position sensor 490 which generates electrical signals based on the distribution of light from light source 491 that impacts the surface of the position sensor 490. Light source 491 has a power source 492. Leads 440 that relay voltage to pump 460 are shown schematically. The voltage is controlled by the controller 10 through its pulse generator 471 and voltage driver 472. Electrical output from the position sensor is relayed via leads 484 to the data acquisition module 485 of controller 10. It will be recognized that the signal from the position sensor can be calibrated so that it can be processed to determine the position of the interface of plug 11. This information can be used to calculated the velocity of the movement of plug 11.

The pumping parameters for a number of solvents have been determined in the 1 mm capillary described above, as follows:

Solvent	Flow rate, Q µl/sec	Pressure, P N/m^2	electrical efficiency, η, molecules/e^-
acetone	14.56	16.33	1.9×10^6
methanol	24.46	26.32	9.7×10^4
1-propanol	16.39	74.89	4.2×10^5
diethyl ether	18.44	20.45	5.8×10^8
1,2 dichloroethane	14.24	46.55	2.9×10^7

Another aspect of pumping is the observation that fluids that are resistant to pumping at a reasonable field strength can be made more susceptible to electrode-based pumping by adding a suitable pumping additive. Preferably, the pumping additive is miscible with the resistant fluid and can be pumped at high pressure, P, high flow rate, Q, and good electrical efficiency, η (i.e., molecules pumped per electron of current).

Generally, the pumping additive comprises from about 0.05 % w/w to about 10 % w/w of the resulting mixture, preferably from about 0.1 % w/w to about 5 % w/w, more preferably from about 0.1 % w/w to about 1 % w/w. Carbon tetrachloride and cyclohexane do not pump using the electrode pump situated in a capillary described above at a voltage of 2,000 V. By adding 0.5 % w/w acetone or methanol as a pumping additive, both of these fluids can be pumped at a voltage of 1,000 V. In some cases, it is desirable to reverse the preferred flow direction of a liquid by mixing with it a pumping additive that strongly pumps in the desired direction. In all cases, pumping additives are selected on the basis of their pumping characteristics and their compatibility with the chemistries or other processes sought to be achieved in the liquid distribution system.

The electrode-based pumps of the invention can be operated to as a valve to resist flow in a certain direction by operating the pumps to counter the unwanted flow. To power the electrode-based pumps, one or more digital drivers, consisting of, for example, a shift register, latch, gate and switching device, such as a DMOS transistor, permits simplified electronics so that fluid flow in each of the channels can be controlled independently. Preferably, each digital driver is connected to multiple switching devices that each can be used to control the pumping rate of a separate electrode-based pump.

The invention includes employing an electrode-based pump to move reagent selected from the group consisting solutions of amino acids, protected amino acids, nucleotides, protected nucleotides, carbodiimides, reactive derivatives of N-protected amino acids and phosphoamidite derivatives of nucleotides. The carbodiimides are preferably C2 to C12 aryl carbodiimides. The concentration of these reagents is preferably from about 0.01 M to about 0.2 M.

The invention further provides a method of pumping comprising employing an electrode-based pump to move a reagent selected from the group consisting of organic amines, such as C1 to C10 hydrocarbons substituted with an amino group and carboxylic acids, such as C1 to C10 hydrocarbons substituted with a carboxylic acid group. Preferably, the reagent is dissolved in a solvent. In another preferred embodiment, the solvent, in the absence of the reagent, does not pump using a d.c. powered electrode-based pump at a voltage of 2,000 V/mm, more preferably it does not pump using a d.c. powered electrode-based pump at a voltage of 4,000 V/mm..

Features of other distribution systems described in this application can be applied to this embodiment, irrespective of under which subheading they are described.

B. Hydrologic Liquid Distribution System

One structure in which the invention is usefully employed is a hydrologic liquid distribution system made up of a number of reservoirs and a large number of reaction cells, wherein liquid from any given reservoir can be systematically directed to all or a substantial subset of the reactor cells.

Such a liquid distribution system 100 is illustrated in Figures 5-10. The distribution system is formed of at least three plates, a feedthrough plate 300, a distribution plate 310 and a reaction cell plate 320 (Figure 5). The feedthrough plate 300 is bonded to the distribution plate 310. Most importantly, the feedthrough plate 300 has multiple first electrodes 360 and second electrodes 361 that can be manufactured according to the invention. The reaction cell plate 320 is typically removably fitted to the underside of the distribution plate 310, or the underside of intermediate plate 330 interposed between the distribution plate 310 and the reaction cell plate 320.

Figure 6 shows the layout of a distribution plate 310. Figure 7 shows an expanded view of a portion of a distribution plate 310 that better illustrates some of the features obscured by the scale of Figure 6. Typically, the structures indicated in solid lines will be formed in the top layer of the distribution plate 310, while the structures indicated with dotted lines will be formed in the bottom layer of the distribution plate 310, except that in Figure 6 the reaction cells 350 are indicated by boxes in solid lines even thought these structures are located in a lower plane . Where appropriate, vertical channels connect the structures in the top of the distribution plate 310 with those in the bottom. For convenience, the axis from the top of the illustration to the bottom is designated the NS axis, while the axis from right to left is the EW axis.

At the top of Figure 6 are four first fluid reservoirs 200A, 200B, 200C and 200D, each having a defined fill level. Each of these first fluid reservoirs 200A, 200B, 200C and 200D has two first reservoir extensions 212 extending along substantially all of an EW axis of the distribution plate 310. The ceilings of the first reservoir extensions 212 preferably are at substantially the same elevation as the first fill level. At five staggered locations, A1, B1, C1, D1 and E1, along the EW axis of the first reservoir extensions 212 there are four first vertical channels 214 (not

shown) that connect the first reservoir extensions 212 with four first horizontal feeder channel segments 216 that are formed in the bottom layer of the distribution plate 310. At each staggered location A1, B1, C1, D1 or E1, four adjacent first horizontal feeder channel segments 216, which are connected to separate first reservoir extensions 212, extend along an NS axis to ten positions, A2, B2, C2, D2, E2, F2, G2, H2, I2 and J2. Each position A2, B2, C2, D2, E2, F2, G2, I2 or J2 along the course of each such set of four adjacent horizontal feeder channel segments 216 is adjacent to a pair of reaction cells 350 (not shown). At these positions A2, B2, C2, D2, E2, F2, G2, H2, I2, or J2, the four adjacent first horizontal feeder channel segments 216 are separately connected, via separate second vertical channels 225 (see Figure 8), to each of four perpendicular first distribution channels 222 formed in the top layer of the distribution plate 310. The ceilings of the first distribution channels 222 define a second fill level that is typically substantially the elevation of the first fill level. The fill level of a distribution channel (e.g., the second fill level) is "substantially" the fill level of the connected reservoir (e.g., the first fill level) if they are offset vertically by no more than about 10% of the depth of the channel; even if the fill levels are further offset vertically they are still substantially the same if filling the reservoir to its fill level results in filling the connected distribution channel and the retention of fluid in the connected distribution channel (for instance, retention due to the capillary barriers described further below with reference to Figure 8). The combination of a first vertical channel 214, connected to a horizontal feeder channel segment 216, in turn connected to a second vertical channel 225 makes up a first feeder channel 217 (not identified in the Figures).

If liquids are maintained at a defined first level in a first fluid reservoir 200, then substantially the same level will be maintained in the first distribution channels 222 connected to that first fluid reservoir 200 via first feeder channels 217. This equalization occurs due to the principle that two connected bodies of liquid will tend to seek the same level and, where the size of the channels allows, due to capillary flow. Liquids are maintained at a defined level in the first fluid reservoirs. In the illustrated embodiment, liquid is fed into the fluid reservoir 200 through channels in the feedthrough plate 300 and such liquid that is not needed to fill the fluid reservoirs to the defined level is drained through drains 380. First openings 381 (not shown) are formed in the bottom layer of the feedthrough plate 300 to create a liquid connection or sluice between the first fluid reservoirs 200 and the drains

380. Liquids are constantly feed into the first fluid reservoirs 200 (as well as the second fluid reservoirs 210 and third fluid reservoirs 220) typically by the use of an external pump 15 (not shown), such as the model number 205U multichannel cassette pump available from Watson-Marlow, Inc. Alternatively, a defined level can be maintained by monitoring the level of liquid in the first fluid reservoirs 200 (or second fluid reservoirs 210 or third fluid reservoirs 220) and only activating the pumps feeding liquid to a given fluid reservoir when needed to maintain the defined level.

Each set of four adjacent first distribution channels 222 are adjacent to two buffer channels 218, located to each side of the first distribution channels 222 along the EW axis. Liquid can be pumped from any first distribution channel 222 into the adjacent buffer channel 218 by activating the first pump 360 (indicated in Figure 7 by two filled dots representing the electrodes of one type of pump) of the first distribution channel 222. This pumping creates additional pressure that moves the liquid over capillary barrier 370 (see Figure 8) separating the first distribution channel 222 and the buffer channel 218. Between each first distribution channel 222, second distribution channel 224 or third distribution channel 226 and the adjacent buffer channel 218 and between each buffer channel 218 and its adjacent third vertical channel 390 (described below) there is such a capillary barrier 370 that inhibits liquid flow when the pumps are not activated. Second openings 362 (see Figure 8) are formed in the bottom layer of the feedthrough plate 300 to create a liquid connection or sluice between the first distribution channels 222 and the buffer channels 218. From a buffer channel 218, liquid can be pumped using a second pump 361 (indicated in Figure 8 by two filled dots representing the electrodes of one type of pump) to a third vertical channel 390 that connects with a reaction cell in the reaction cell plate 320. Third openings 363 (see Figure 8) in the bottom layer of the feedthrough plate 300 or the distribution plate 310 serve to create a liquid connection or sluice between the buffer channels 218 and third vertical channels 390.

Figure 8 illustrates a capillary barrier 370, at which a meniscus 371 forms, at the junction between a first distribution channel 222 containing liquid 11 and either a buffer channel 218 or a third vertical channel 390. The meniscus 371 formed at the outlet of first distribution channel 222 into buffer channel 218 will tend to inhibit seepage from the first distribution channel 222, such as the seepage that can result from capillary forces. In some embodiments there are vents (not illustrated) that

extend through the feedthrough plate 300 at the tops of buffer channel 218 or third vertical channel 390.

Note that only a small cut-away of NS oriented horizontal feeder channel segments 216 are shown in Figure 8. Typically, these channels extend inwardly and outwardly from the illustrated cut-away and connect with additional first distribution channels 222 situated to distribute liquid to other reaction cells 350.

Along the right side of the distribution plate 310 are ten second fluid reservoirs 210, each having a second reservoir extension 240 extending along an EW axis. Second distribution channels 224 form "L"-extensions off of second reservoir extensions 240 and are each positioned adjacent to a separate buffer channel 218, such that there are ten second distribution channels 224 extending off of each second reservoir extension 240. Each second distribution channel 224 has a pump 360 that can move liquid from a second distribution channel 224 to the adjacent buffer channel 218. Second openings 362 (not shown) in the bottom of feedthrough plate 300 serve to provide a sluice or route of liquid connection between the second distribution channels 224 and the buffer channels 218. Liquid moves from the buffer channels 218 to the reaction cells as described above. Located adjacent to each second reservoir 210 is a drain 380 (not shown) that operates to maintain a defined third fill level as described above.

As will be described further below in Section D in reference to Figures 9A - 9D, the capillary barriers 370 and sluices created by the second openings 362 or third openings 363 act as a combined valve and pump. The capillary barriers 370 prevent flow to the reaction cell, which flow would be favored by capillary forces, until the first pumps 360 or second pumps 361 provide the extra pressure needed to overcome the capillary barriers 370. Narrowing the sluices can increase the capillary forces favoring flow, thereby reducing the amount of added pressure needed to overcome the capillary barriers 370. The use of the capillary barriers 370 allows flow control to be governed by the first pumps 360 or second pumps 361, which are typically controlled by controller 10.

Located along the bottom edge of the distribution plate illustrated in Figure 6 are ten third liquid fluid reservoirs 220. Horizontal feeder channel segments 230 are connected to the third fluid reservoirs 220 and to third distribution channels 226 via fourth vertical channels 227. The third distribution channels 226 have first pumps 360 which can move liquid into adjacent buffer channels 218 via openings 362 (not shown) in the

feedthrough plate 300. Located adjacent to each third fluid reservoir 220 is a drain 380 (not shown) that operates to maintain a defined fourth fill level as described above. Third fluid reservoirs 220 and connected third distribution channels 226 operate in just the same way as first fluid reservoirs 200 and first distribution channels 222. Those of ordinary skill in the art will readily envision alternative geometries wherein a number of separate third fluid reservoirs 220 can interact with a given buffer channel 218 via a number of third distribution channels 226 positioned adjacent to the buffer channel 218. Located adjacent to each third reservoir 220 is a drain 380 (not shown) that operates to maintain a defined third fill level as described above.

The above discussion describes the distribution system as being formed with a feedthrough plate 300, distribution plate 310 and reaction cell plate 320. However, it will be clear that additional plates can conveniently be incorporated into the distribution system. For instance, a intermediate plate 330 is, in a preferred embodiment, permanently bonded underneath the distribution plate 310 and interposed between the distribution plate 310 and the reaction cell plate 320. The use of the intermediate plate 330 allows for much greater flexibility in the design of the channels the form the distribution system.

C. Controller

The controller 10 will typically be an electronic processor. However, it can also be a simpler device comprised of timers, switches, solenoids and the like. The important feature of controller 10 is that it directs the activity of the first pumps 360 and second pumps 361 and, optionally, the activity of external pumps 171. A circuit of thin film transistors (not shown) can be formed on the liquid distribution system to provide power to the wells via leads and electrodes, and to connect them with the driving means such as the controller 10, so as to move liquids through the array. Pins can also be formed substrate which are addressable by logic circuits that are connected to the controller 10 for example.

D. Capillary barriers

Capillary barriers have been described above with reference to Figure 8. However, more complex design considerations than were discussed above can, in some cases, affect the design of the capillary barrier. In some cases it is desirable to narrow the sluice formed by second opening 362 or third opening 363 to increase the impedance to flow (i.e., the frictional resistance to flow) as appropriate to arrive at an appropriate

flow rate when the associated first pump 360 or second pump 361 is activated. Such a narrowing is illustrated by comparing the sluice of Figure 9A with the narrowed sluice of Figure 9D. The problem that this design alteration can create is that narrower channels can increase capillary forces, thereby limiting the effectiveness of channel breaks.

Thus, in one preferred embodiment, a channel break further includes one or more upwardly oriented sharp edges 369, as illustrated in Figures 9B and 9C. More preferably, a channel break includes two or more upwardly oriented sharp edges 369. In Figure 9B, portion 362A of opening 362 is cut more deeply into first plate 300 to create an open space useful for the operation of upwardly oriented sharp edges 369.

E. Fabrication of Plates, Channels, Reservoirs and Reaction Cells

The liquid distribution systems of the invention can be constructed as described in US Application No. 08/556,423, filed 9-November-1995 (DSRC 11717A), No. 08/645,966, filed 10-May-1996 (DSRC11717B), PCT Application No. US95/14586, filed 9-November-1995 (DSRC11717PCT), and PCT Application No. US95/14590, filed 9-November-1995 (DSRC11402GPCT).

F. Fabrication of Electrode-Based Pumps

In many embodiments, the liquid distribution systems of the invention require the formation of numerous electrodes for pumping fluids through the liquid distribution system as described in described in US Application No. 08/556,423, filed 9-November-1995 (DSRC 11717A), No. 08/645,966, filed 10-May-1996 (DSRC11717B), PCT Application No. US95/14586, filed 9-November-1995 (DSRC11717PCT), and PCT Application No. US95/14590, filed 9-November-1995 (DSRC11402GPCT).

G. Drivers

An analog driver is can be used to vary the voltage applied to the electrode-based pump from a DC power source. A transfer function for each fluid is determined experimentally as that applied voltage that produces the desired flow or fluid pressure to the fluid being moved in the channel. However, an analog driver is required for each pump along the channel and is suitably an operational amplifier. Typically, however, a separate analog driver is required for each electrode-based pump. This is impractical when a large number of channels are to be controlled.

Thus a digital driver having a pulse of suitable voltage amplitude and that can provide gating control to the electrodes is preferred for use herein. Control of fluid flow is accomplished by applying pulses of

different pulse widths and different repetition rates to the electrodes. A typical pulse train is shown in Figure 1 wherein t_1 is the pulse width and t_2 is the distance between pulses.

Figure 11 illustrates one configuration for providing control of fluid flow of a plurality of channels simultaneously and independently. The data generated for the above variables, as obtained experimentally for various fluids and electrodes, is loaded into a controller 10 (not shown), such as a computer. The controller converts the data to instructions for the digital driver to a first pump 360 or second pump 361. The data is transferred to the digital driver and is stored in the shift register 50. Different switching devices 52 attached to each electrode pair can be selected, independently of each other, depending on the state of the latch output. The switching devices are turned on and off by an enabling signal 54 and a latch output signal 55 applied to an AND gate 56. A pulse of a particular width and repetition rate is applied to the enable signal 54 which determines the length of time the switch is on or off. Thus the fluid flow in the channel can be controlled using a signal having constant amplitude but variable pulse width and repetition rate. By preselecting the pulse repetition rate, a predetermined applied voltage is selected for each first pump 360 or second pump 361 in a channel 10.

An array of the above switching devices 52 can be connected to the shift register 50 for controlling the fluid flow of an array of channels, each switching device controlling the fluid flow in a different channel. A single switching device 52', connected to the shift register 50 through a gate 56', an enable signal 54' and a latch signal 55', is shown for simplicity in Fig. 5, but a plurality of switching devices will be used, one for each pump in the array of channels.

I. Miscellaneous Features

In the case where the temperature of a particular well is to be monitored or changed, a means of heating or cooling the well is built into the well, as will be further explained below with reference to Fig. 20. The first well 36 in this example has deposited on its bottom surface a thin film 57 of a suitable metal oxide, such as tin oxide or indium tin oxide. The thin film 57 is connected by means of an electrically conductive metal connection 58 to the end or outer edge of the well 36. The tin oxide coating 57 serves as a heater element for the well 36. The sides of the well 36 have a surface bimetal film 59 and leads 60, suitably made of chromel-alumel alloys, forming a thermocouple to measure the temperature in the well when a

source of current is applied to the tin oxide coating 57 and to the leads 58. A voltage applied to the well 36 via electrodes 56 deposited on the backside as shown regulates the temperature in the well. The amount of current applied can be regulated by the controller 10 in response to the temperature measured through the leads 60.

In some applications of the liquid distribution system a significant vapor pressure may develop in reaction cell 350, causing a back pressure into the distribution plate 310. Thus preformed valves 70 (see Figure 21A) formed of bimetallic materials as described by Jerman et al, "Understanding Microvalve Technology", Sensors, September 1994 pp 26-36 can be situated in third vertical channel 390. These materials have a thermal expansion mismatch. When the temperature in the reaction cell 350 is low, the ball valve 62 is in its normal position permitting free flow of fluids into the well 36 (see Figure 21A). As the temperature in the well 36 increases, the ball valve 62 moves to a cooler position (Figure 21B) blocking the third vertical channel 390 to isolate the reaction cell 350, thereby preventing fluids from passing into and out of the first well 36. Alternatively, a conventional check valve having a bearing, such as a bearing made of quartz or polytetrafluoroethylene polymer can be used to isolate the reaction cell 350. Where it is important to have the capability to have fluid flow counter to the direction established by the check valve, the check valve can have an insulating or magnetic bearing, which can be moved to allow such counter-flow with externally applied electrostatic-or magnetic fields.

Other features of liquid distribution systems are described in an application filed November 9, 1995 entitled, "Liquid Distribution System," U.S. Application No. 08/556,036, which application is a continuation-in-part of U.S. Application No. 08/338,703, titled "A Partitioned Microelectronic and Fluidic Device Array for Clinical Diagnostics and Chemical Synthesis," filed November 10, 1994, a continuation-in-part of U.S. Application No. 08/469,238, titled "Apparatus and Methods for Controlling Fluid Flow in Microchannels," filed June 6, 1995 and a continuation-in-part of U.S. Application No. 08/483,331, titled "Method and System for Inhibiting Cross-Contamination in Fluids of Combinatorial Chemistry Device," filed June 7, 1995. The disclosure of this November 9, 1995 application entitled "Liquid Distribution System" and of all the above-recited priority filings named in the November 9, 1995 application are incorporated herein by reference in its entirety.

EXAMPLES

Example 1 - Liquids pumped with a simple electrode-based pump

Using the 1 mm capillary with a two electrode-pump described above in Section B.ii., a number liquids have been tested, including the following solvents:

Solvent	Flow direction	voltage applied
N-methyl-pyrrolidinone (NMP)	+	1470
Dimethyl formamide (DMF)	+	390
Dichloromethane (DCM)	-	686
Methanol (MeOH)	-	489
Isopropanol (IPA)	+	
Acetone	+	
Acetonitrile	+	

The following solutions in NMP, at 0.1M unless otherwise indicated, have been tested:

Reagent	Flow direction
trans-4-(trifluoromethyl)-cinnamic acid	-
5-benzimidazolecarboxylic acid	-
N,N-dicyclohexylcarbodiimide	+
isobutylamine	+
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	No flow at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)

The following solutions in DMF, all at 0.1M excepting piperidine, which was 20% v/v, have been tested:

Reagent	Flow direction*
p-carboxybenzenesulfonamide	- P
4-fluorophenylacetic acid	- P
4-methoxyphenylacetic acid	- P
m-trifluoromethylbenzoic acid	- P
3-(4-methoxyphenyl)propionic acid	-
4-bromocinnamic acid	- P

Reagent	Flow direction*
terephthalic acid	- P
isophthalic acid	- P
1,3-phenylenediacetic acid	- P
1,4-phenylenediacetic acid	- P
3-(4-carboxyphenyl) propionic acid	- P
1,4-phenylenedipropionic acid	- P
4,4'-oxybis (benzoic acid)	- P
4,4'-dicarboxybenzophenone	- P
piperidine	+
1,3-diisopropylcarbodiimide	+
allylamine	+
butylamine	+
isoamylamine	+
propylamine	+
isobutylamine	+
cyclohexylamine	+
heptylamine	+
benzylamine	+
phenylamine	+ P
3-amino-1-propanol	+ P
2-aminoethanol	+
4-(aminomethyl) pyridine	+ P
4-(2-aminoethyl) morpholine	+ P
1-(3-aminopropyl) imidazole	+
triphenylphosphine	+
4-(aminopropyl) morpholine	+
9-fluorenemethanol	+
p-nitrobenzyl alcohol	+
p-(methylthio) benzyl alcohol	-
o-aminobenzyl alcohol	+
2-methoxybenzyl alcohol	+
2-(triflouromethyl) benzyl alcohol	+
2-amino-3-phenyl-1-propanol	+ P

Reagent	Flow direction*
diethylazodicarboxylate	- P
4-dimethylaminopyridine	+ P
carbazole	+
azobenzene	+
3,4-dihydroxybenzoic acid	- P
4-methylmorpholine N-oxide	+
3-cyanobenzoic acid	No flow
4-nitrophenylacetic acid	No flow, at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	No flow, at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	+ weak
tetrapropylammonium perruthenate	No flow
l-oxo-2,2,6,6-tetramethylpiperdinium chloride	No flow
5-benzimidazolecarboxylic acid	N.D.$^{\delta}$
4-(aminomethyl) benzoic acid	N.D.
4-(aminomethyl) benzoic acid	N.D.
N,N-diisopropylethylamine	N.D.
isobuylamine	N.D.
glutathione (SH)	N.D.

*Those directional indicators (" + " or "-") followed by a "P" indicate that flow was achieved using a pulsed voltage program pursuant to Figure 1, where τ_1 = 0.1 - 1 ms and τ_2 = 3.0 - 10 ms.

$^{\delta}$"N.D.", in this table and the tables below, indicates either that the solute was immiscible with the solvent or that visual inspection suggested that it had decomposed.

The following solutions in DCM, at 0.1M unless otherwise indicated , have been tested:

Reagent	Flow direction*

allylamine	-
butylamine	-
cyclohexylamine	-
1-(3-aminopropyl) imidazole	-
diethylazodiacarboxylate	-
TP Palladium	-
isobutylamine	-
isoamylamine	-
propylamine	-
1-(3-aminopropyl)imidazole	-
p-carboxybenzenesulfonamide	N.D.
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	N.D.

* Those directional indicators ("+" or "-") followed by a "P" indicate that flow was achieved using a pulsed voltage program pursuant to Figure 1, where τ_1 = 0.1 - 1 ms and τ_2 = 3.0 - 10 ms.

The following solutions in methanol, all at 0.1M, have been tested:

Reagent	**Flow direction***
4-fluorophenylacetic acid	-
9-fluorenemethanol	- P
p-(methylthio) benzyl alcohol	-
(R) sec-phenethyl alcohol	-
3-cyanobenzoic acid	No flow
4-nitrophenylacetic acid	- weak
allylamine	No flow
2-aminoethanol	No flow
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	N.D.
isobutylamine	N.D.
isomylamine	N.D.

* Those directional indicators ("+" or "-") followed by a "P" indicate that flow was achieved using a pulsed voltage

program pursuant to Figure 1, where τ_1 = 0.1 - 1 ms and τ_2 = 3.0 - 10 ms.

Example 2 - An electrode-pump based preferential flow system

A channel system was fabricated on two inch by two inch by 20 mil plates of 211 Corning glass (Corning Glass Co., Corning, NY) to confirm that liquids can be switched to a desired flow pathway by controlling the voltages applied to certain electrode-based pumps. As illustrated in Figures 11A and 11B, first channel 804 (2,600 μm long by 150 μm wide by 100 μm deep), second channel 805 (550 μm long by 100 μm wide by 100 μm deep), third channel 806 (800 μm long by 275 μm wide by 100 μm deep), fourth channel 807 (200 μm long by 100 μm wide by 100 μm deep), fifth channel 808 (550 μm long by 100 μm wide by 100 μm deep) and sixth channel 809 (2,600 μm long by 150 μm wide by 100 μm deep) were fabricated on channel plate 810 (not shown). Also fabricated on the channel plate 810 were first well 800A, second well 800B and third well 800C, which were connected by the channels. An electrode plate 820 was overlaid and sealed to the channel plate 810 by field assisted thermal bonding. The electrode plate 820 had openings into first well 800A and second well 800B (not illustrated). Third well 800C included a center drain 855. The electrode plate 820 further had platinum electrodes, fabricated by inserting 25 μm wires. The electrodes included first platinum electrode 801A, second platinum electrode 801B, third platinum electrode 801C, fourth platinum electrode 802A, fifth platinum electrode 802B, third platinum electrode 802C, and the two electrodes comprising gamma electrode-based pump 803. First platinum electrode 801A, second platinum electrode 801B and third platinum electrode 801C make up alpha electrode-based pump 801, while fourth platinum electrode 802A, fifth platinum electrode 802B and sixth electrode 802C make up beta electrode-based pump 802.

Figure 12A shows methanol flowing from first well 800A to second well 800B, while bypassing third well 800C. This is done by applying 160 V to alpha electrode-based pump 801. Figure 12B shows methanol flowing from second well 800B to third well 800C while bypassing first well 800A. This is done by applying 200 V to beta electrode-based pump 802, 100 V to gamma electrode-based pump 803 and 120 V to alpha electrode-based pump 801, where the polarity at beta and gamma electrode-based pumps 802 and 803 favored flow into the third well 800C,

and the polarity at alpha electrode-based pump 801 favored flow away from first well 800A.

Example 3 - Electrode-based pumping past capillary barriers

Figure 13 shows a prototype liquid distribution system fabricated pursuant to the hydrologic liquid distribution system. The distribution system was constructed from three plates of Corning 7740 borosilicate glass, Corning Glass, Inc., Corning, NY which plates became top plate 910, intermediate plate 920 and bottom plate 930. The top of intermediate plate 920 was coated with silicon as described above. In top plate 910 were formed, by laser drilling, first hole 901A, second hole 901B, third hole 902A, fourth hole 902B, fifth hole 903A, sixth hole 903B, seventh hole 904A and eighth hole 904B, which holes each had a diameter of 75 μm . First and second holes 901A and 901B were used to form first model electrode-based pump 961. Third and fourth holes 902A and 902B were used to form second prototype electrode-based pump 962. Fifth and sixth holes 903A and 903B were used to form third prototype electrode-based pump 963. Seventh and eighth holes 904A and 904B were used to form fourth model prototype electrode-based pump 964. The electrodes in each of first through fourth prototype electrode-based pumps, 961 - 964, were separated by 200 μm . By etching, alpha opening 905, beta opening 906 and gamma opening 907 were formed on the underside of top plate 910. By laser drilling, ninth hole 908 and tenth hole 909, each with a diameter of 150 μm, were formed through upper plate 910.

In intermediate plate 920 were formed first prototype channel 911 (made up of segments 911A - 911D) and second prototype channel 912 (made up of segments 912A - 912D). First and second prototype channels 911 and 912 having a depth of 80 μm and a width of 150 μm . The entries into these two prototype channels 911 and 912 are provided by ninth hole 908 and tenth hole 909, respectively. First reaction cell access hole 913 and second reaction cell access hole 914, each with a diameter of 150 μm , were laser drilled through the intermediate plate 920. In the underside of intermediate plate 920, a delta opening 915 was formed, which delta opening 915 connects the reaction cell 950 to first and second prototype drain holes 921 and 922.

In the bottom plate 930, the reaction cell 950 was formed by etching. First prototype drain hole 921 and second prototype drain hole 922 were laser drilled through bottom plate 920. The top plate 910 and

intermediate plate 920 were bonded together by field assisted thermal bonding.

When methanol was introduced into first prototype channel 911, the liquid was stopped from flowing into reaction cell access hole 913 by the capillary barrier formed by the structure at alpha opening 905. Correspondingly, the capillary barrier formed by the structure at beta opening 906 prevented methanol flow into the reaction cell access hole 914. Flow into the reaction cell access holes 913 or 914, by either route, could be initiated by activating the appropriate pumps. For instance, to pump methanol through first prototype channel 911, first prototype electrode-based pump 901 and second prototype electrode-based pump 902 were biased by applying 200 V. Flow through the prototype channel 911 was observed.

IN THE CLAIMS:

1. A method of performing a synthetic process in a liquid distribution system having reaction cells, comprising employing an electrode-based pump for pumping at least one reagent into a reaction cell.

2. The method of claim 1, wherein the process is a nucleophilic or electrophilic substitiution or a acid-catalyzed or base-catalyized cleavage.

3. The method of claim 1, wherein the process is selected from the group consisting of amide formation, Mitsunobu reaction, Suzuki coupling, Stille coupling, alkylation of an amine, acylation of an amine, alkylation of a ketone or nitrile, Schiff base formation, reductive amination, sulfonamide formation, nucleic acid synthesis, protein synthesis, cycloaddition, Mannich reaction, Diels Alder reaction, Wittig reaction, Heck reaction, epoxide elimination, elimination of a leaving group, a condensation reaction, a transesterification, iodolactonization, Grignard reaction, reactions of organometallic compounds with alkyl halides, Michael addition, Ugi reaction, Knoevenagel reaction, oyrazole formation, quinoline formation, thiazolidine formation, pyrazolone formation, isoxazole formation, imine formation, Dieckmann reaction, Aldol condensation, acetal/ketal formation, Fischer indole synthesis, Friedel Craft reaction, Carbamate formation.

4. The method of claim 1, wherein the process is an oxidation and the reagents pumped into the reaction cell are selected from the group consisting of m-chloroperbenzoic acids, hydrogen peroxide, and $KMnO_4$.

5. The method of claim 1, wherein the process is a reduction and the reagents pumped into the reaction cell are selected from the group consisting of $LiAlH_4$ and lithium borohydride.

6. The method of claim 3, wherein the process is amide formation and the reagents pumped into the reaction cell are selected from the group consisting of o-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate, 2,3-dichloro-5,6-dicyano-1,4-benzoquinonone, 1-hydroxybenzotriazole hydrate, protected amino acids, N-methyl morpholine, diisopropyl carboiimide.

7. The method of claim 3, wherein the process is amide formation, wherein the amide formed is a urea compound, and two reagents are separately pumped into the reaction cell, wherein the first reagent comprises an amine and the second reagent comprises an isocyanate or an

isothiocyanate.

8. The method of claim 3, wherein the process is a Mitsunobu reaction and the reagents pumped into the reaction cell are selected from the group consisting of phenol, alcohol, diethylazodicarboxylate, triphenylphosphine and N-methyl morpholine.

9. The method of claim 3, wherein the process is Suzuki coupling and the reagents pumped into the reaction cell are selected from the group consisting of halide, palladium (0) catalyst, aryl boronic acid, triethyl amine and dimethyl formamide.

10. The method of claim 3, wherein the process is Stille coupling and the reagents pumped into the reaction cell are selected from the group consisting of halide, palladium (0) catalyst, aryl stannane, N-methyl pyrrolidinone and triphenyl arsine.

11. The method of claim 3, wherein the process is alkylation or acylation of an amine and the reagents pumped into the reaction cell are selected from the group consisting of tosylate, halide, amine, N-methyl pyrrolidinone and phosgene.

12. The method of claim 3, wherein the process is alkylation of a ketone and the reagents pumped into the reaction cell are selected from the group consisting of amine, ketone, lithium diisopropyl amine, tetrahydrofuran and alkyl iodide.

13. The method of claim 3, wherein the process is reductive amination and the reagents pumped into the reaction cell are selected from the group consisting of amine, aldehyde, sodium cyanoborohydride, sodium triacetyoxyborohydride, borohydrate pyridine and methylene chloride.

14. The method of claim 3, wherein the process is nucleic acid synthesis and the reagents pumped into the reaction cell are selected from the group consisting of oligonucleotides and phosphates.

15. The method of claim 3, wherein the process is cycloaddition and the reagents pumped into the reaction cell are selected from the group consisting of peracids, substituted alkenes, diazo compounds, azide, nitriles, azomethine ylide, nitrones and carbonyl oxides.

16. The method of claim 3, wherein the process is a Mannich reaction and the reagents pumped into the reaction cell are selected from the group consisting of aldehydes, ketones, amine salts, amides, acids and bases.

17. The method of claim 3, wherein the process is a Diels

Alder reaction and the reagents pumped into the reaction cell are selected from the group consisting of substituted alkenes and dienes.

18. The method of claim 3, wherein the process is a Wittig reaction and the reagents pumped into the reaction cell are selected from the group consisting of triphenylphosphines and substituted alkenes.

19. The method of claim 3, wherein the process is a Heck reaction and the reagents pumped into the reaction cell are selected from the group consisting of a palladium (0) catalyst, substituted alkenes, substituted halides and triethylamines.

20. The method of claim 3, wherein the process is elimination of a leaving group and the reagents pumped into the reaction cell are selected from the group consisting of alkyl halides and bases.

21. The method of claim 1, wherein the synthetic process is employed to synthesize a small molecule, polymeric organic compound, an oligonucleotide, or a peptide.

22. The method of claim 1, wherein the reagent is selected from the group consisting of carboxylic acids, carbodiimides, amines, alcohols, pyridines, azodicarboxylates, carbazoles, azobenzenes, amino N-oxides, 1,4-benzoquinone and ammonium perruthenate.

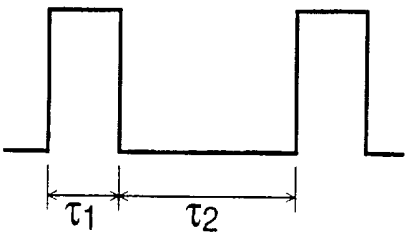


FIG. 1

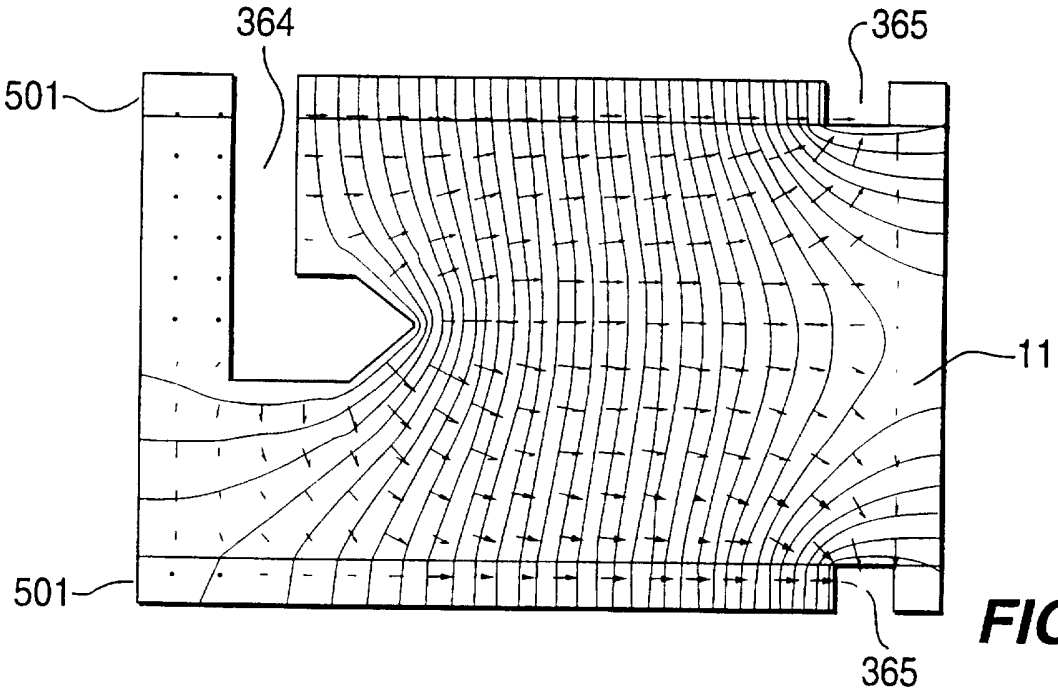


FIG. 2A

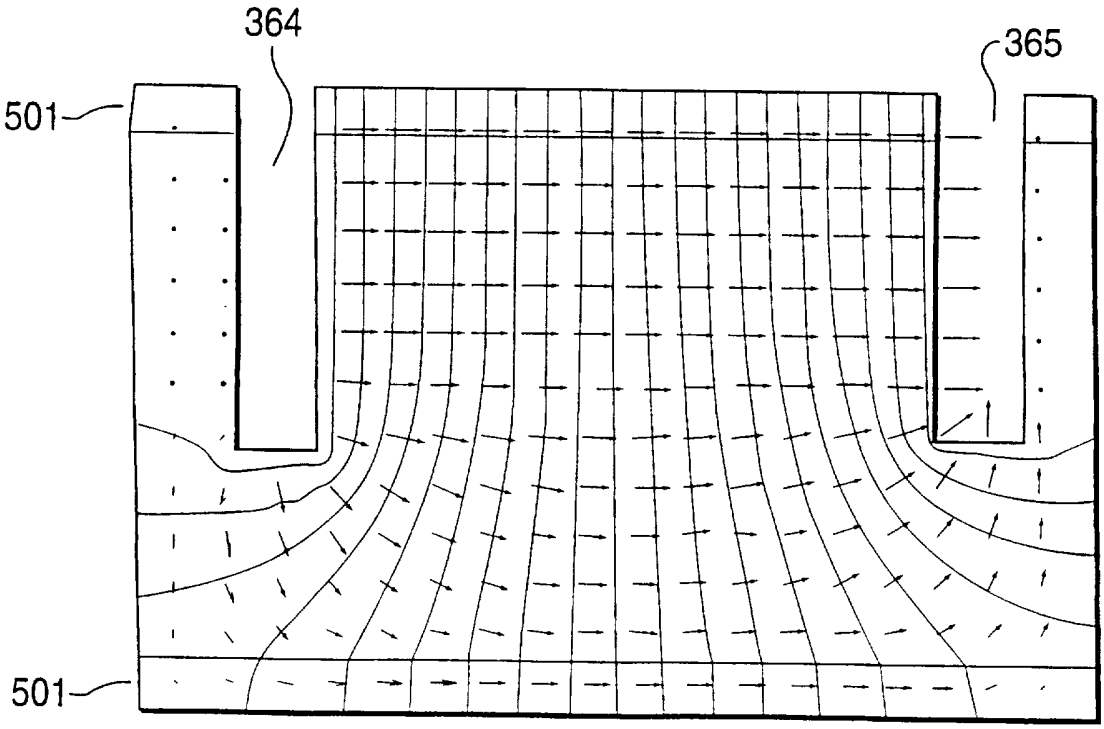


FIG. 2B

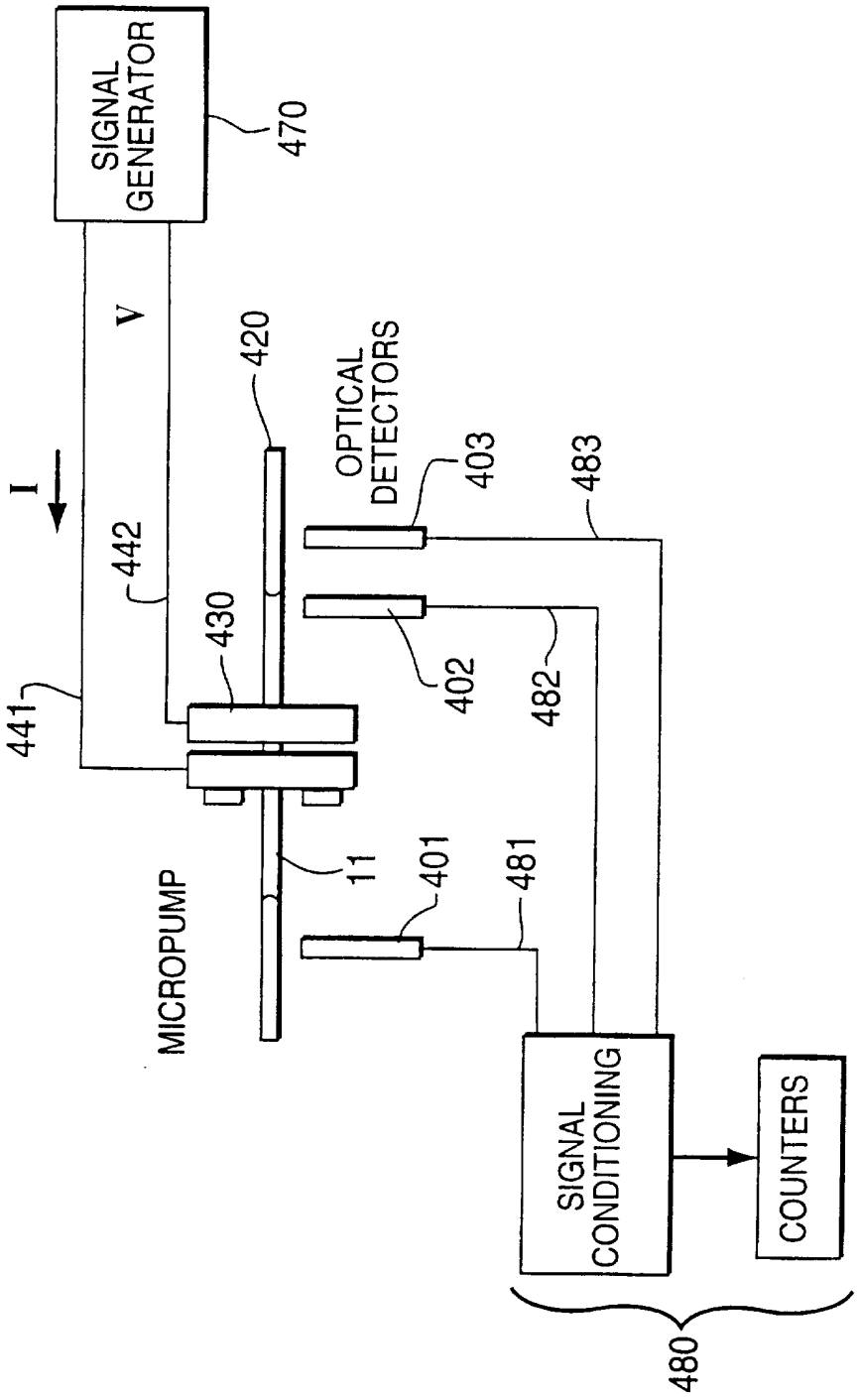
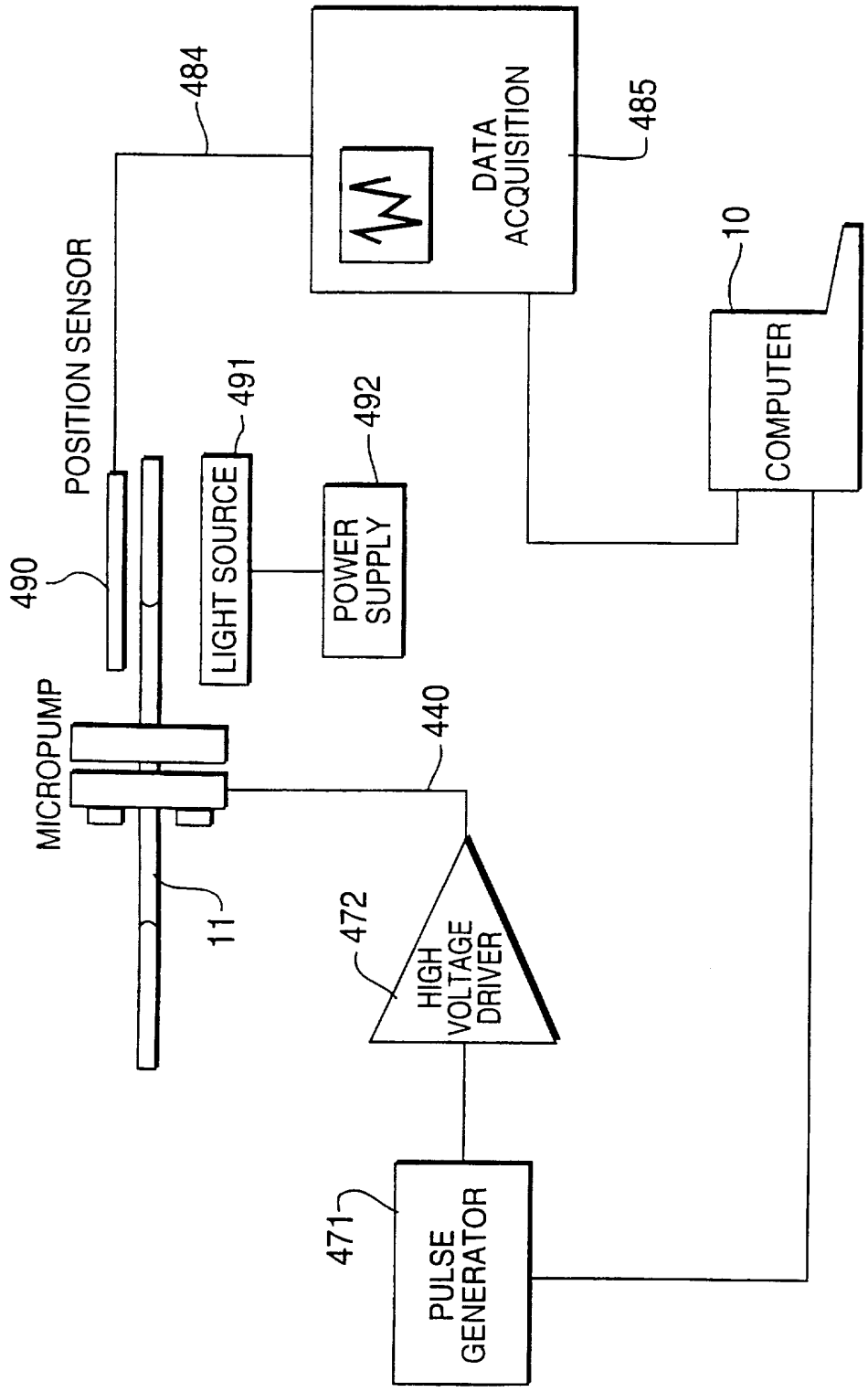

SIGNAL GENERATOR
470
V
I
420
OPTICAL DETECTORS
403
483
442
430
441
402
482
MICROPUMP
11
401
481
SIGNAL CONDITIONING
COUNTERS
480

FIG. 3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DATA ACQUISITION
485
10
COMPUTER
POSITION SENSOR
491
492
490
LIGHT SOURCE
POWER SUPPLY
MICROPUMP
440
11
472
HIGH VOLTAGE DRIVER
471
PULSE GENERATOR

FIG. 4

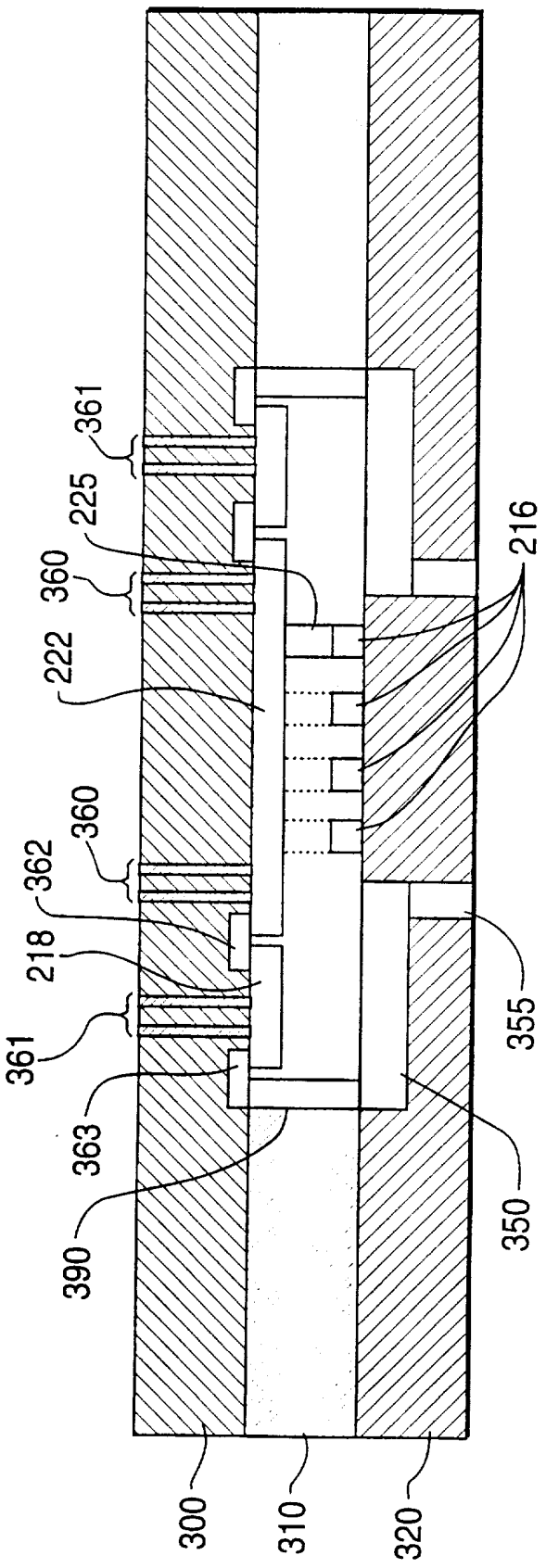
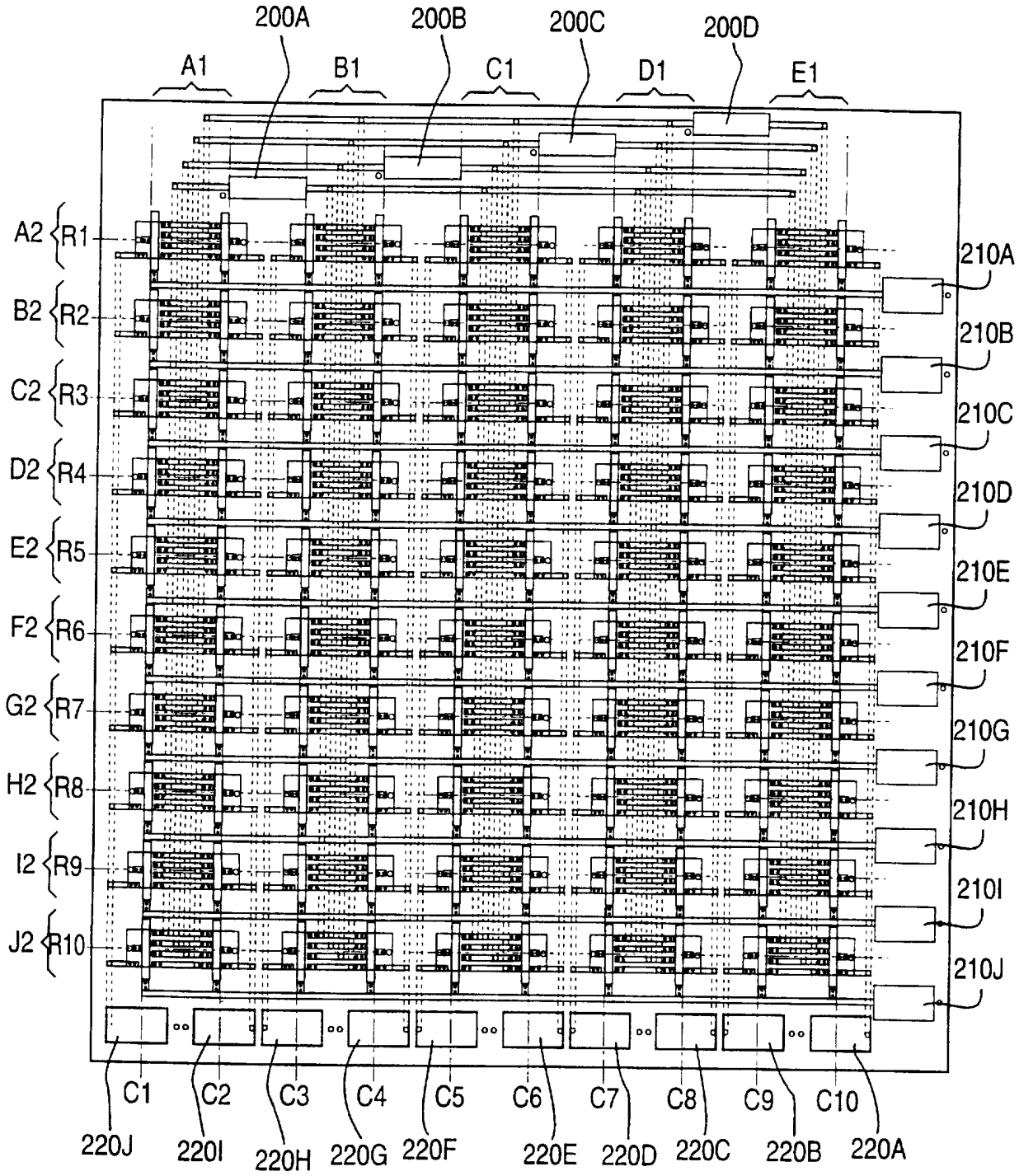


FIG. 5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200C
200D
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B1
C1
D1
E1
A2
R1
B2
R2
C2
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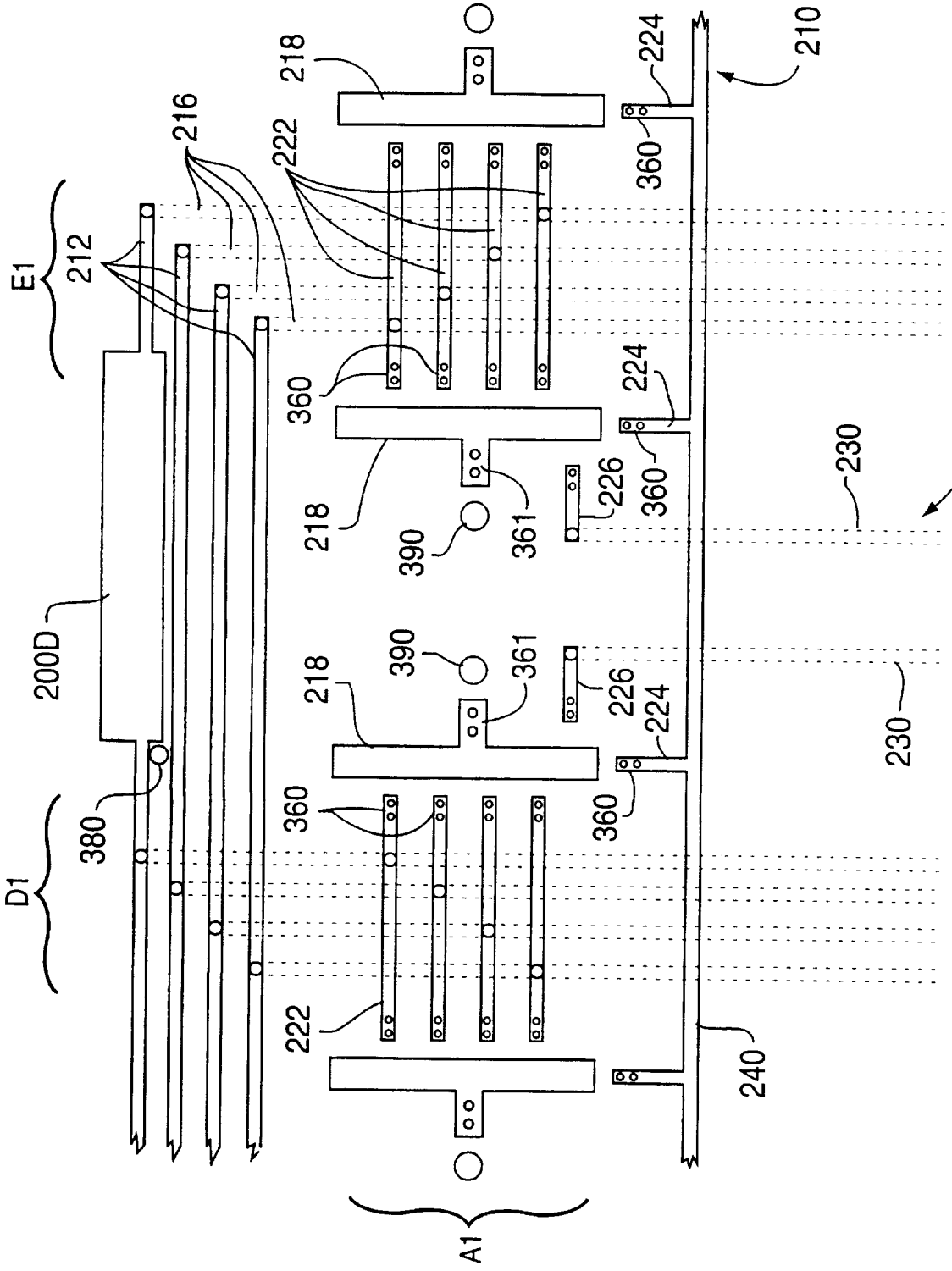
R4
E2
R5
F2
R6
G2
R7
H2
R8
I2
R9
J2
R10
210A
210B
210C
210D
210E
210F
210G
210H
210I
210J
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
220J
220I
220H
220G
220F
220E
220D
220C
220B
220A

FIG. 6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216
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224
210
200D
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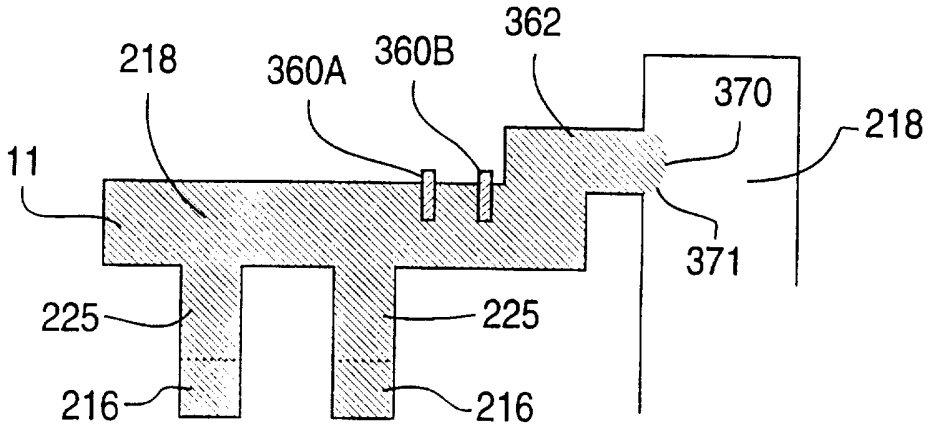
D1
390
361
226
220
230
240
A1

FIG. 7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360A
360B
362
370
218
11
371
225
225
216
216

FIG. 8

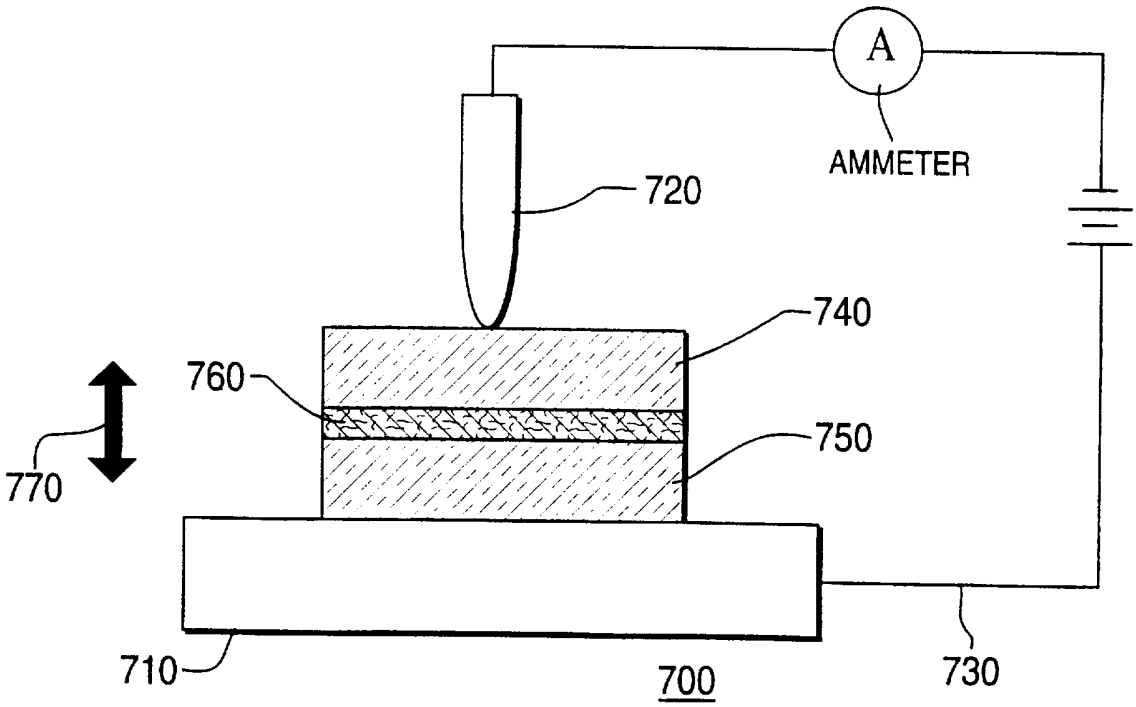
A
AMMETER
720
740
760
750
770
710
700
730

FIG. 10

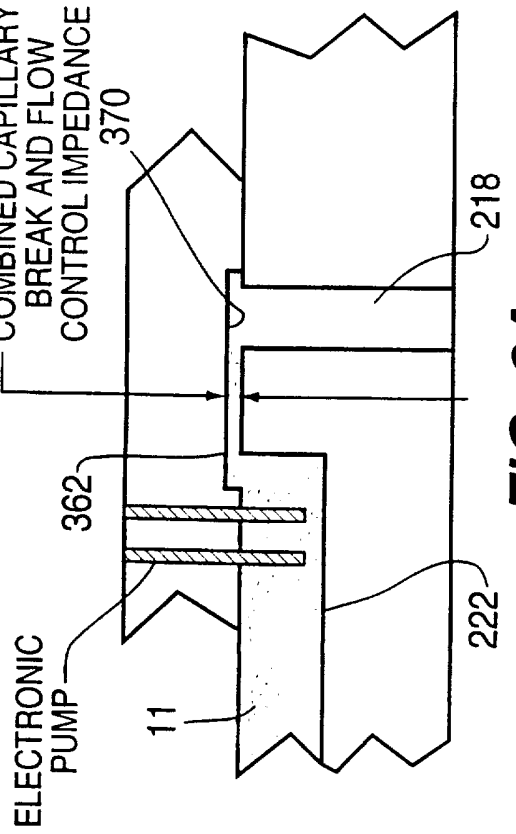

FIG. 9A

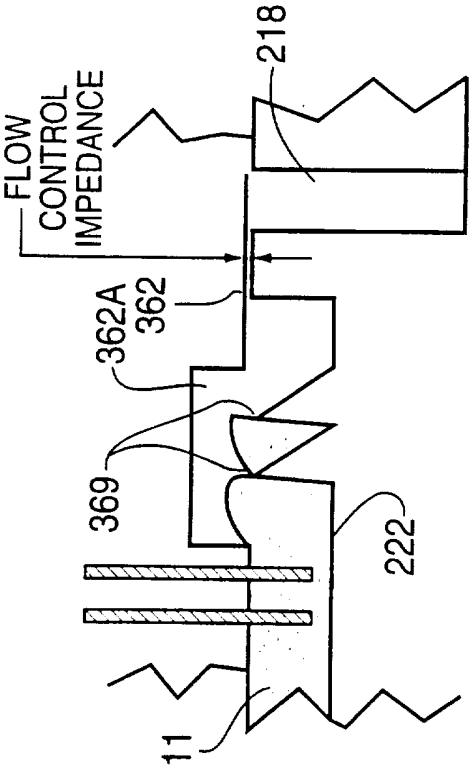

FIG. 9B

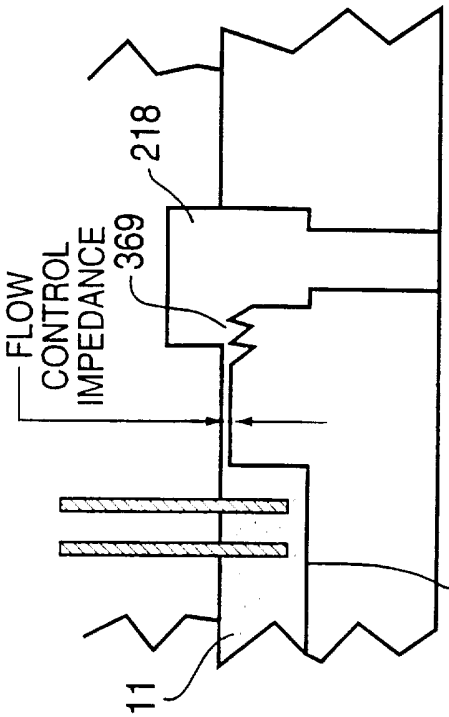

FIG. 9C

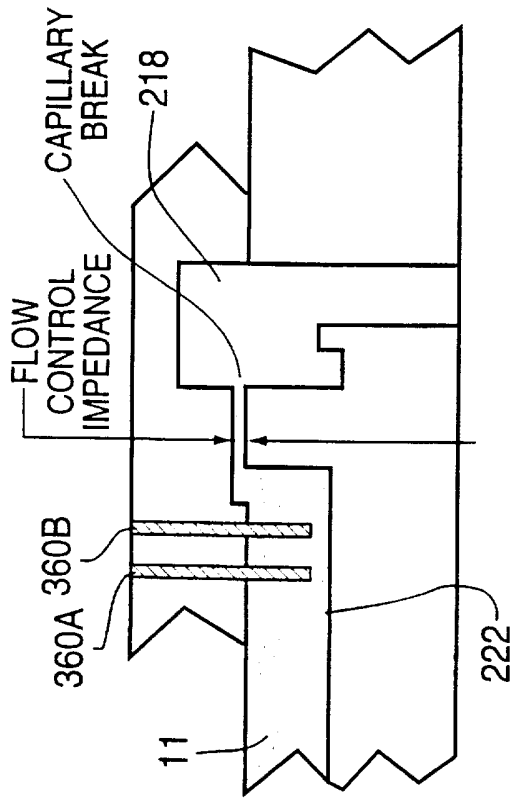

FIG. 9D

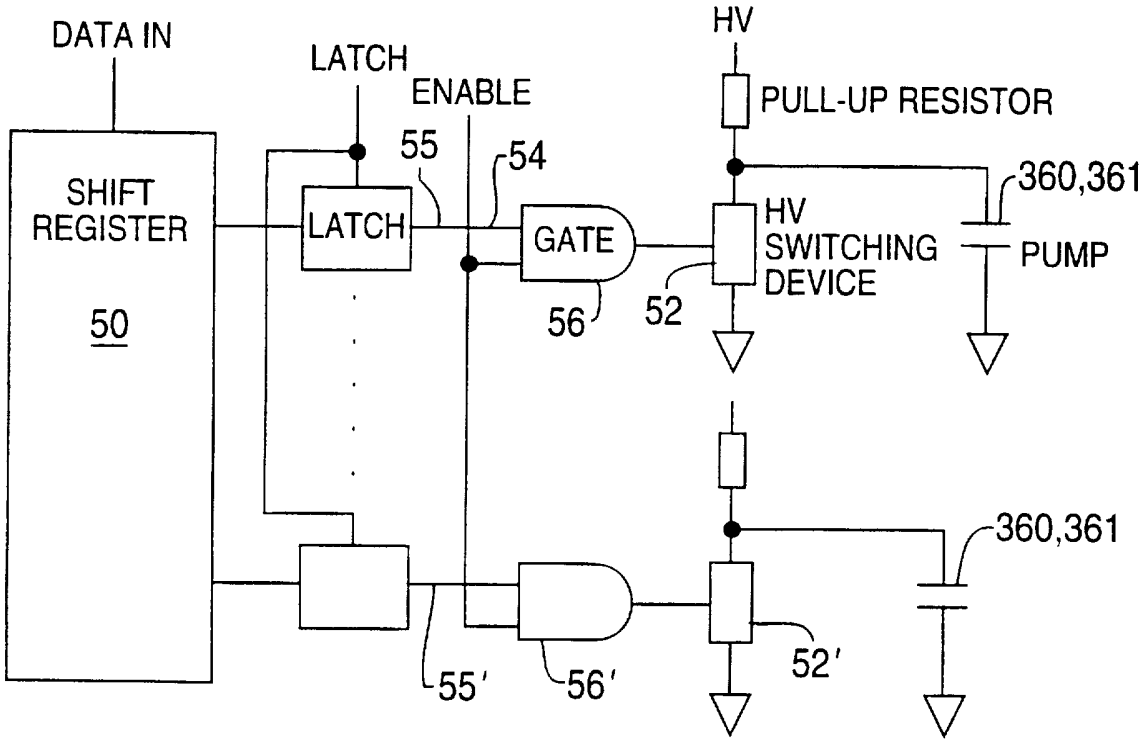

FIG. 11

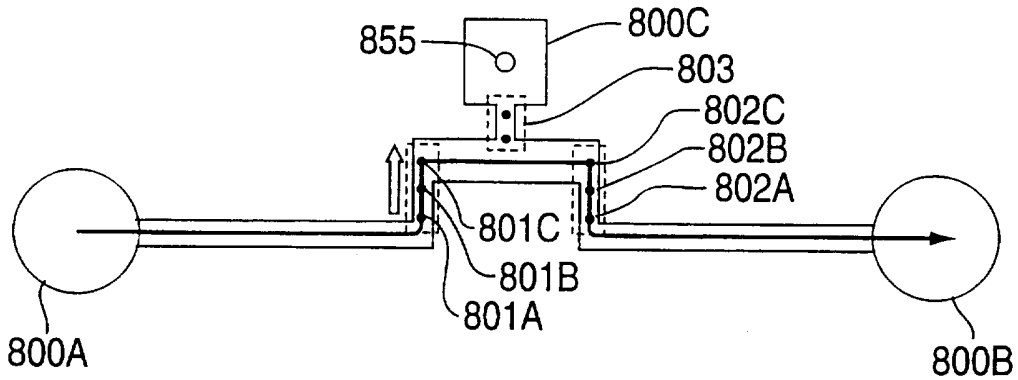

FIG. 12A

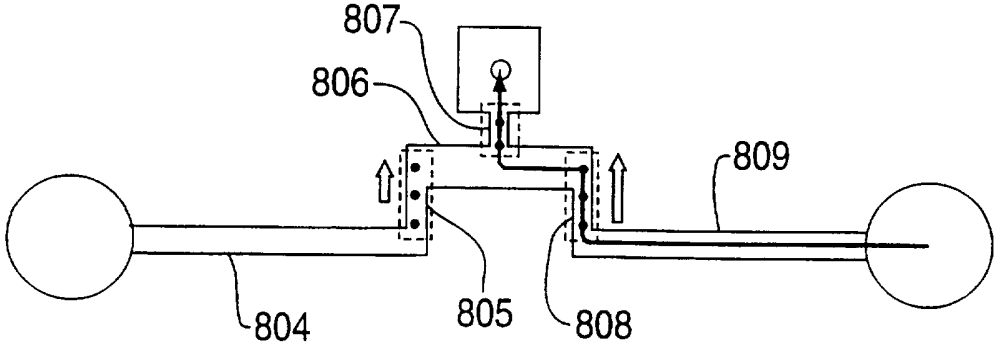

FIG. 12B

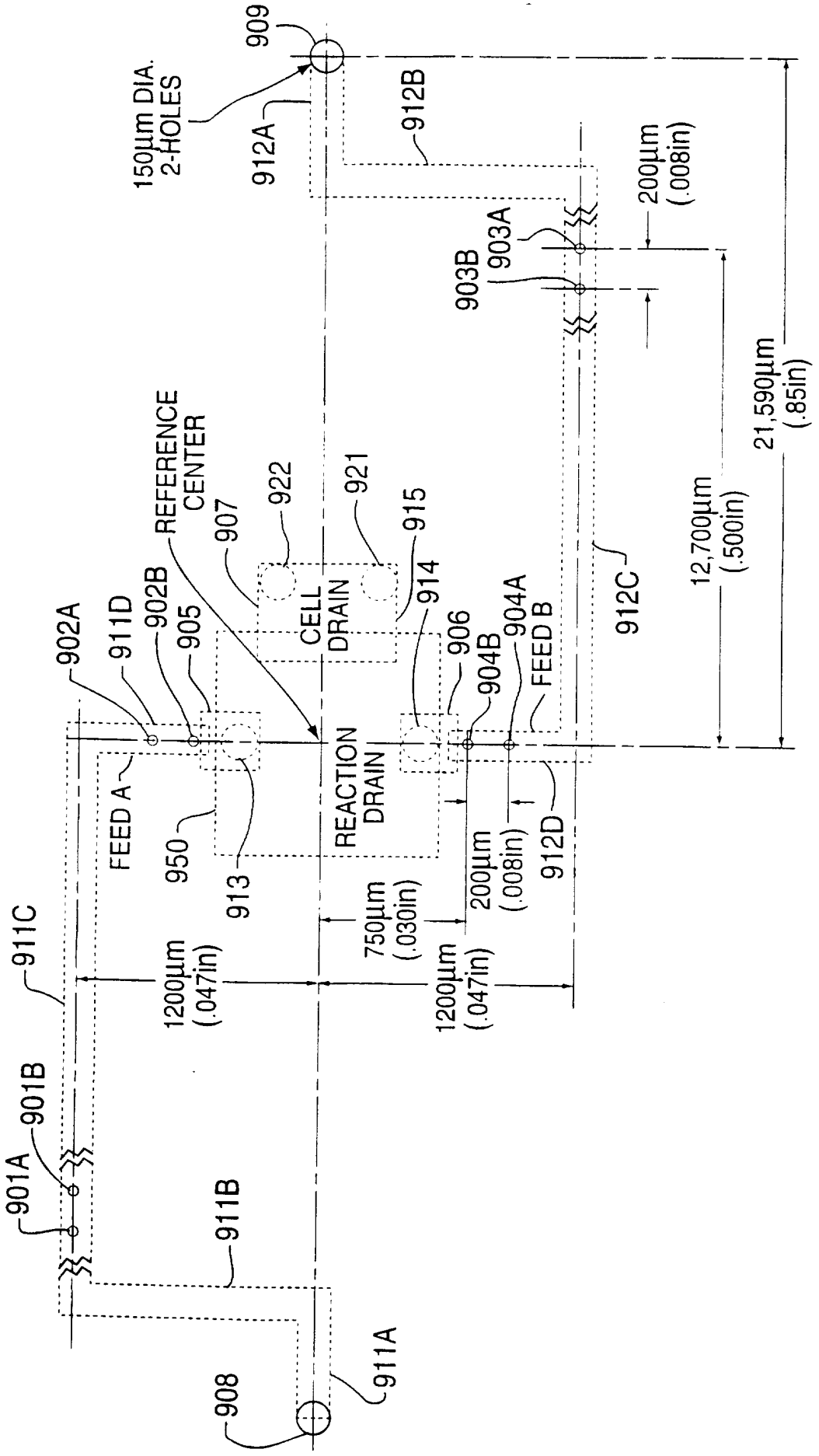
909
150µm DIA.
2-HOLES
912A
912B
200µm
(.008in)
903A
903B
21,590µm
(.85in)
12,700µm
(.500in)
912C
REFERENCE
CENTER
907
922
921
915
914
CELL
DRAIN
902A
911D
902B
905
906
904B
904A
FEED B
FEED A
950
913
REACTION
DRAIN
912D
200µm
(.008in)
750µm
(.030in)
1200µm
(.047in)
1200µm
(.047in)
911C
901B
901A
911B
908
911A

FIG. 13

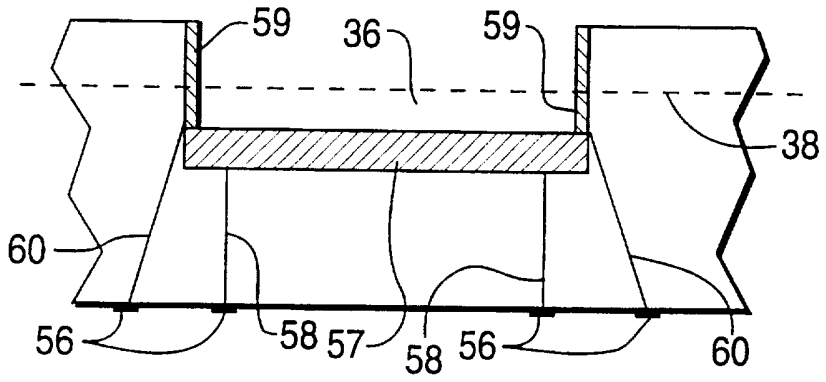

FIG. 14

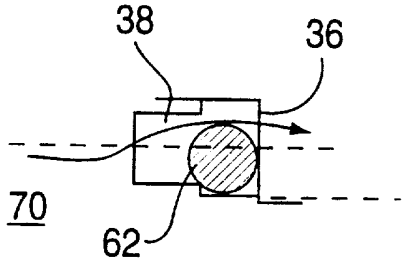

FIG. 15A

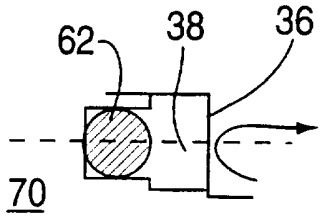

FIG. 15B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07880

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 27/26
US CL : 204/450,600
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)
U.S. : 204/450,600; 422/129, 131, 149,188

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,534,328 A (ASHMEAD ET AL) 09 July 1996 (09/07/96) , column 5, line 5 - column 6, line 7; column 7, line 25; column 7 line 58 - column 8, line 11; column 9, line 10; column 14, line 23-25; column 2, line 31-49; column 3, line 4-23; column 4, line 20-32; column 13, line 22-36; and column 3, line 43-46.	1-22
Y	US 5,480,614 A (KAMAHORI) 02 January 1996 (02/01/96), abstract; and column 1, lines 64-67; column 1,lines 23-3.	1-22.

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 14 AUGUST 1997	Date of mailing of the international search report 05 SEP 1997
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07880

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,384,261 A (WINKLER ET AL) 24 January 1995 (24/01/95), column 1, lines 58-66; column 2, lines 21-27; column 6, lines 6-20; column 8, lines 30-33; column 10, lines 4-11; column 12, lines 1-4; and column 14, lines 9-54; column 9, lines 37-42; column 1, lines 16-31; column 4, lines 28-29, lines 55-62; column 5, lines 25-27; and column 7, lines 7-19.	3,6,11-17, 20-22.
Y	US 5,580,523 A (BARD) 03 December 1996 (03/12/96), column 1,lines 18-38; column 2, lines 32-47; column 2, lines 48-58; column 3, lines 25-30; and column 7, lines 25 - column 9, line 17.	3, 7, 11-16, 17, 20-22.
Y	HUGHES, D.; CAPLUS abstract, The Mitsunobu reaction. Org. React. (N.Y.) 1992, 42, 335-656. See entire document.	3, 8
Y	LUH, Tien-Yau. CAPLUS abstract, Transition metal-catalyzed cross-coupling reactions of unactivated aliphatic C-X bonds. Rev. Heteroat. Chem. 1996, 15, 61-82. See entire document.	3, 9
Y	CIATTINI et al. CAPLUS abstract , An efficient synthesis of 3-substituted indoles by palladium-catalyzed coupling reaction of 3-tributylstannylindoles with organic triflates and halides. Tetrahedron Lett. 1994, 3515), 2405-8. See entire document.	3, 10
Y	AGABAYAN et al., CAPLUS abstract, Amino acids as the amine component in a Mannich reaction. Usp. Khim. 1982, 51(4), 678-95. See entire document.	3
Y	OVERMAN, L.; CAPLUS abstract, Application of intramolecular Heck reactions for forming congested quaternary carbon centers in complex molecule total synthesis. Pure Appl. Chem. (1994), 66(7), 1423-30.	3, 19
Y	MORENO-MANAS et al. The One-pot palladium catalyzed Wittig reaction with allylic alcohols. Scope and limitayions. Synth. Commun. (1986), 16(9), 1003-13.	3, 18

INTERNATIONAL SEARCH REPORT

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PCT/US97/07880

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAPLUS, WPIDS, JAPIO, APS
search terms: microchip, wafer, micromachin?, peptide#, protein#, edman, amino, microscale, micro-scale, nanoscale, mesoscale, mitsunobi, suzuki, names of specified reactions, synthes?, electrokinet? electroosm?, electro-osm?electrohydrodynam?, ion drag, electrode (3a) pump#