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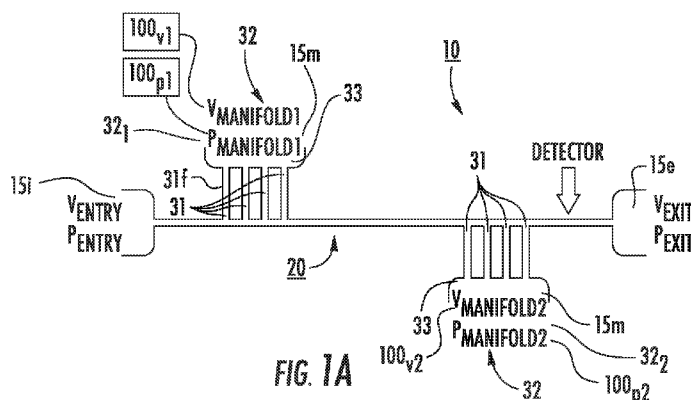


FIG. 1A

(57) Abstract: Fluidic devices with a primary transport nanochannel(s) intersected by at least one nanoscale manifold for active control of capture, manipulation and transport of analyte molecules. The at least one manifold can be an array or network of nanochannels, nanoslits or nanoelectrodes joined to a common voltage or pressure source. A respective nanoscale manifold can be configured to allow for precise and active control of driving forces applied to the primary transport nanochannel(s) to drive molecular transport through the various regions along the transport nanochannel(s). The at least one manifold can generate monotonic force gradients with a limited or reduced number of independent input potentials and/or pressures applied to the device.

FLUIDIC DEVICES WITH NANOSCALE MANIFOLDS FOR MOLECULAR TRANSPORT, RELATED SYSTEMS AND METHODS OF ANALYSIS

Related Applications

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application Serial Number 62/159,503, filed May 11, 2015, the contents of which are hereby incorporated by reference as if recited in full herein.

Statement of Federal Support

[0002] This invention was made with government support under Grant No. 1R01HG007407-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

Field of the Invention

[0003] This invention relates to "lab on a chip" fluidic devices and may be particularly suitable for molecular transport along a fluidic nanochannel for analyzing various target analytes in samples.

Background of the Invention

[0004] There has been considerable recent interest in the incorporation of nanoscale components in lab-on-a-chip fluidic devices. This interest owes its origin to several advantages (and differences that may be advantageously leveraged) in moving from the micron scale to the nanoscale. These differences include, for example, double-layer overlap (DLO) and its effect on electro-osmosis and charge permselectivity, localized enhancement of electric fields, higher surface to volume ratios, confinement effects on large synthetic and bio-polymers, and the emerging importance of entropic effects. *See, e.g.,* Yuan et al., *Electrophoresis* 2007, 28, 595-610; Schoch et al., *Rev. Mod. Phys.* 2008, 80, 839-883; and Kovarik et al., *Anal. Chem.* 2009, 81, 7133-7140. Historic examples of nanoscale devices include the use of porous media and gels in chromatographic separations and filtration membranes with nanoscale pores. *See, e.g.,* Lerman et al., *Biopolymers* 1982, 21, 995-997; and Tong et al., *M. Nano Lett.* 2004, 4, 283-287. Recent efforts, however, have been focused on engineering geometrically well-defined conduits for fluid and analyte transport and

seamlessly integrating them into devices. *See, e.g.,* Volkmuth et al., *Nature* 1992, 358, 600-602; and Striemer et al., *Nature* 2007, 445, 749-753. The advantage of such regular structures is the relative simplicity of pressure and field gradients, fluid flow, and molecular motion contained within, in contrast to these properties in more tortuous networks. The capability to define, characterize, and easily model these systems can allow a better understanding of separation mechanisms and single molecule physics, for example. *See, e.g.,* Volkmuth et al., *Nature* 1992, 358, 600-602; Reisner et al., *Phys. Rev. Lett.* 2005, 94, 196101; and Salieb-Beugelaar et al., *Lab Chip* 2009, 9, 2508-2523.

[0005] Recently FIB milling techniques have been described to form nanofluidic devices. *See, Menard et al., Fabrication of Sub-5 nm Nanochannels in Insulating Substrates Using Focused Ion Beam Milling, Nano Lett.* 2011, 11, 512-517 (published Dec. 20, 2010); and U.S. Provisional Patent Application Serial No. 61/384,738, filed September 21, 2010 (and related PCT Application PCT/US2011/052127), entitled, Methods, Systems And Devices For Forming Nanochannels, the contents of which are hereby incorporated by reference as if recited in full herein. In addition to FIB milling, a variety of other methods suitable for nanochannel fabrication can be used, including, for example, electron beam lithography, nanoimprint lithography, photolithography, templating or molding strategies, and other methods understood by one of ordinary skill in the art.

[0006] A number of nanofluidic devices have been proposed, including those with integrated miniature electrodes (nano- or micro- scale) for single-molecule sensing and/or nucleic acid sequencing. However there remains a need for alternative designs.

Summary of Embodiments of the Invention

[0007] Embodiments of the invention provide fluidic devices with one or more nanoscale manifolds for incorporation within fluidic lab-on-a-chip devices that are designed for the active control of the capture, manipulation, and transport of analyte molecules. The nanoscale manifolds, e.g., arrays or networks of nanochannels, nanoslits, or nanoelectrodes, are joined to a common voltage and/or pressure source, and are interfaced to at least one primary transport nanochannel. Each bank of nanoscale manifolds can be configured to allow precise and active control over the forces driving molecular transport in the various regions of the primary nanochannel(s). This allows for control over the transport of molecules along discrete regions of the primary nanochannel(s). The manifold structures can permit the establishment of designed monotonic force gradients along the length of the primary fluidic nanochannel(s) while limiting the required number of independent input

potentials or pressures applied to the device. These tunable force gradients can be used for the manipulation of small molecules and macromolecules spanning many orders of magnitude in size while minimizing the occurrence of force gradient induced molecular fragmentation. Through the decoupling of the force magnitudes required to capture and then transport molecules through a nanochannel, precise spatio-temporal control over the molecules can be obtained.

[0008] Some embodiments are directed to fluidic devices that include at least one fluidic transport nanochannel; and at least one nanoscale manifold having a plurality of nanoscale elements. At least a plurality of the nanoscale elements directly interface with the at least one fluidic transport nanochannel. At least a plurality of the nanoscale elements of the nanoscale manifold are configured to be concurrently controlled by a common externally applied pressure and/or voltage to generate a force gradient within the at least one transport nanochannel

[0009] The fluidic device can be a chip.

[0010] The device can further include a plurality of reservoirs including at least one reservoir in fluid communication with at least one microfluidic inlet to at least one transport nanochannel and at least one reservoir in fluid communication with a plurality of the nanoscale elements of the at least one nanoscale manifold.

[0011] The at least one nanoscale manifold can include at least first and second nanoscale manifolds interfaced to a first one of the at least one transport nanochannel.

[0012] The first nanoscale manifold can be upstream of the second nanoscale manifold.

[0013] The first nanoscale manifold can face the second nanoscale manifold across the first fluidic transport nanochannel.

[0014] The plurality of nanoscale manifold elements can be between 2-10,000 adjacent elements that directly interface with at least one transport nanochannel. At least some of the adjacent elements can be spaced apart by 10 nm to 1 cm.

[0015] The plurality of nanoscale manifold elements for a respective transport nanochannel can be one of: (a) between 2-10, (b) between 10-100, (c) between 100-1000, or (d) between 1000-10,000.

[0016] The nanoscale manifold elements can include nanoslits.

[0017] The nanoscale manifold elements can include nanoelectrode elements.

[0018] The nanoscale manifold elements can include nanochannels.

[0019] The nanoscale manifold can include at least one cross-channel that connects at least two parallel nanoscale manifold elements that directly interface with at least one of the at least one transport nanochannels.

[0020] The nanoscale manifold elements can include at least one diagonal element.

[0021] The at least one diagonal manifold element can extend outward from at least one straight manifold element.

[0022] The at least one diagonal element can extend from a voltage and/or pressure input junction or channel segment associated therewith to the at least one transport nanochannel.

[0023] The at least one fluidic transport nanochannel can be a plurality of discrete, spaced apart fluidic transport nanochannels. The at least one nanoscale manifold can interface with at least two of the plurality of transport nanochannels.

[0024] The at least one nanoscale manifold can interface with first and second spaced apart segments of at least one of the at least one fluidic transport nanochannel.

[0025] The at least one transport nanochannel may have a serpentine shape with first and second legs that may be parallel. At least one of the at least one nanoscale manifold can interface with both the first and second legs.

[0026] The device can be in combination with power and/or pressurized gas supplies and at least one controller in communication with the power and/or pressurized gas supplies.

[0027] Each nanoscale manifold can be controlled by a separate voltage and/or pressure input and are addressed individually.

[0028] A single pressure and/or voltage input to a respective nanoscale manifold can feed only its respective manifold elements.

[0029] Each primary transport nanochannel can have voltage and/or pressure inputs that are separate from a respective nanoscale manifold inputs.

[0030] Other embodiments are directed to analysis systems. The systems include: (a) a fluidic analysis device comprising: at least one fluidic transport nanochannel; at least one nanoscale manifold having an array of nanoscale elements, at least some of the nanoscale elements interface directly with the at least one fluidic transport nanochannel; (b) a first power and/or first pressurized gas supply in communication with a single one of the at least one nanoscale manifolds to feed voltage and/or pressure to its respective nanoscale elements so that the nanoscale manifold applies a force gradient on an analyte in the transport nanochannel; and (c) at least one additional pressurized gas supply and/or power supply in communication with the transport nanochannel proximate an entrance and exit end portion

thereof. Voltages and/or pressures at the entrance and exit of the transport nanochannel and at each respective nanoscale manifold can be independently controlled.

[0031] The at least one nanoscale manifold can optionally include first and second spaced apart nanoscale manifolds that both interface with at least one of the at least one fluidic transport nanochannels.

[0032] Each nanoscale manifold can have an independent pressurized gas supply and/or voltage input whereby each nanoscale manifold is independently addressable.

[0033] Still other embodiments are directed to methods of controlling the transport of molecules within a fluid for the purposes of analysis. The methods can include providing a fluidic analysis device, the analysis device having at least one primary transport nanochannel and at least one nanoscale manifold with a plurality of nanoscale elements that intersect or interface with at least one primary transport channel; and automatically concurrently applying a single pressure and/or single voltage input to multiple nanoscale elements of a respective nanoscale manifold to generate a force profile on the at least one transport nanochannel to carry out at least one of: accelerate, decelerate, compress, stretch, collect/separate or trap/concentrate an analyte as the analyte flows through the at least one primary transport nanochannel proximate the nanoscale manifold.

[0034] The at least one nanoscale manifold can be a plurality of nanoscale manifolds, each interfacing with at least one of the at least one transport nanochannel. Each nanoscale manifold can be independently controlled to apply a force gradient to a respective transport nanochannel.

[0035] The nanoscale manifold elements can include fluidic nanoscale elements.

[0036] The nanoscale elements can include nanoelectrodes.

[0037] The method can further include controlling molecular transport in the at least one transport nanochannel by selectively dynamically changing force gradients applied by the nanoscale magnitude.

[0038] The controlling can be carried out by changing the force gradient in one or more of magnitude, shape, and polarity using a single external voltage input and/or a single pressure input to each of the plurality of nanoscale manifolds.

[0039] The controlling can include varying a single external voltage input and/or a single pressure input to each of a plurality of nanoscale manifolds in a time-dependent manner such as stepping to different voltages or pressures, transiently pulsing to different voltages or pressures, continuously ramping at various linear or non-linear rates, or varying according to a sinusoidal or other wave function.

[0040] It is noted that aspects of the invention described with respect to one embodiment, may be incorporated in a different embodiment although not specifically described relative thereto. That is, all embodiments and/or features of any embodiment can be combined in any way and/or combination. Applicant reserves the right to change any originally filed claim and/or file any new claim accordingly, including the right to be able to amend any originally filed claim to depend from and/or incorporate any feature of any other claim or claims although not originally claimed in that manner. These and other objects and/or aspects of the present invention are explained in detail in the specification set forth below. Further features, advantages and details of the present invention will be appreciated by those of ordinary skill in the art from a reading of the figures and the detailed description of the preferred embodiments that follow, such description being merely illustrative of the present invention.

Brief Description of the Drawings

[0041] **Figures 1A and 1B** are schematic illustrations of a fluidic analysis device according to embodiments of the present invention.

[0042] **Figure 1C** is an equivalent circuit diagram of the fluidic devices shown in **Figures 1A and 1B** according to embodiments of the present invention.

[0043] **Figure 2A** is an enlarged view of a portion of a fluid device with dual nanoscale manifolds with associated greatly enlarged insets with a grayscale of intensity representing field strength (V/cm) or velocity ($\mu\text{m/s}$) of an analyte with exemplary voltage controlled operation according to embodiments of the present invention.

[0044] **Figure 2B** is an enlarged view of a portion of a fluid device with dual nanoscale manifolds with associated greatly enlarged insets with a grayscale of intensity representing linear flow rate ($\mu\text{m/s}$) with exemplary pressure controlled operation according to embodiments of the present invention.

[0045] **Figures 3A-3F** are schematic illustrations of a fluidic analysis device with exemplary different actions using a nanoscale manifold to generate a defined force profile for a respective action according to embodiments of the present invention.

[0046] **Figures 4A-4V** are enlarged exemplary nanoscale manifold configurations with nanochannels and/or nanoslits according to embodiments of the present invention.

[0047] **Figure 4W** is an enlarged illustration of an exemplary nanoscale manifold with nanochannels and/or nanoslits interfaced to an array of discrete, separate primary transport nanochannels according to embodiments of the present invention.

[0048] **Figures 4X and 4Y** illustrate exemplary nanoscale manifolds with nanochannels and/or nanoslits interfaced to multiple different segments of a common primary transport nanochannel according to embodiments of the present invention.

[0049] **Figures 5A-5V** are enlarged exemplary nanoscale manifold configurations with nanoelectrodes according to embodiments of the present invention.

[0050] **Figure 5W** is an enlarged illustration of an exemplary nanoscale manifold with nanoelectrodes interfaced to an array of discrete, separate primary transport nanochannels according to embodiments of the present invention.

[0051] **Figures 5X and 5Y** illustrate exemplary nanoscale manifolds with nanoelectrodes interfaced to multiple different segments of a common primary transport nanochannel according to embodiments of the present invention.

[0052] **Figures 6A-6G** illustrate exemplary geometries for nanoscale manifold elements according to embodiments of the present invention.

[0053] **Figure 7A** is schematic illustration of a fluidic analysis device with a nanoscale manifold configured to decrease the velocity of molecular transport in a primary transport nanochannel (the arrow indicates the direction of molecular transport; in this example, the analyte is a molecule of DNA) according to embodiments of the present invention.

[0054] **Figure 7B** shows adjacent images/panels (b), (c) and (d) of the transport nanochannel shown in **Figure 7A** with a 10kbp long DNA molecule stained with intercalating fluorescent dye according to embodiments of the present invention. Image (b) is a bright-field micrograph showing the entry region, the primary nanochannel and the manifold nanoslits. Image (c) is a representative fluorescence image of a DNA molecule in the primary nanochannel. Image (d) is a kymogram (images recorded at 330 frames/second) showing the change in the DNA molecule's position over time as it is driven through the primary transport nanochannel. The position of the nanoslits in **Figure 7B** are indicated by the vertical broken lines.

[0055] **Figure 8A** is schematic illustration of a fluidic analysis device with a nanoscale manifold configured to increase the velocity of molecular transport in a primary transport nanochannel (indicating the direction of transport of a DNA molecule) according to embodiments of the present invention.

[0056] **Figure 8B** shows adjacent images/panels (b), (c) and (d) of the transport nanochannel shown in **Figure 8A** with a 10kbp long DNA molecule stained with intercalating fluorescent dye according to embodiments of the present invention. Image (b) is

a bright-field micrograph showing the entry region, the primary nanochannel and the manifold nanoslits. Image (c) is a representative fluorescence image of a DNA molecule in the primary nanochannel. Image (d) is a kymogram (images recorded at 330 frames/second) showing the change in the DNA molecule's position over time as it is driven through the primary transport nanochannel. The position of the nanoslits in **Figure 8B** are indicated by the vertical broken lines.

[0057] **Figure 9** is a plot of velocity ($\mu\text{m/s}$) and calculated electric field (V/cm) versus distance from entrance of the primary nanochannel (μm) for six different experimental conditions showing the tunability of analyte transport velocity using one or more nanoscale manifolds interfaced to the primary transport nanochannel according to embodiments of the present invention.

[0058] **Figure 10** is a graph of measured velocity ($\mu\text{m/s}$) versus calculated electric field (V/cm) showing characteristics of voltage-controlled transport of analyte molecules through a primary nanochannel for six different experimental conditions according to embodiments of the present invention.

[0059] **Figure 11** shows adjacent images/panels (a), (b), (c), (d) and (e) of a device with a nanoscale manifold operated in an accelerating capacity to transport fluorescently stained T4-phage DNA molecules (165 kbp, with a fully extended length of 73 μm) in a primary transport nanochannel. Image (a) is a bright-field micrograph of a portion of the device. Image (b) is a representative fluorescence image of two T4-phage DNA molecules in the primary nanochannel (the leftmost molecule is approaching the nanoscale manifold and the rightmost molecule spans the manifold). Image (c) is a representative fluorescence image of the leftmost molecule in (b) as it is stretched in the region of the nanoscale manifold. Image (d) is a representative fluorescence image of the molecule in (c) after it has exited the region of the manifold. Image (e) is a kymogram (images collected at 252 frames/second) showing the change in the DNA molecule's position over time as it is driven through the primary transport nanochannel. The position of the nanoslits of the manifold are indicated by the vertical broken lines.

[0060] **Figure 12** is a flow chart of methods of operating a fluidic analysis device according to embodiments of the present invention.

[0061] **Figure 13** is an example of a fluidic analysis system according to embodiments of the present invention.

Description of Embodiments of the Invention

[0062] The present invention will now be described more fully hereinafter with reference to the accompanying figures, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Like numbers refer to like elements throughout. In the figures, certain layers, components or features may be exaggerated for clarity, and broken lines illustrate optional features or operations unless specified otherwise. In addition, the sequence of operations (or steps) is not limited to the order presented in the figures and/or claims unless specifically indicated otherwise. In the drawings, the thickness of lines, layers, features, components and/or regions may be exaggerated for clarity.

[0063] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms, "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises," "comprising," "includes," and/or "including" when used in this specification, specify the presence of stated features, regions, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, regions, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items. As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y." As used herein, phrases such as "from about X to Y" mean "from about X to about Y."

[0064] It will be understood that when a feature, such as a layer, region or substrate, is referred to as being "on" another feature or element, it can be directly on the other feature or element or intervening features and/or elements may also be present. In contrast, when an element is referred to as being "directly on" another feature or element, there are no intervening elements present. It will also be understood that, when a feature or element is referred to as being "connected", "attached" or "coupled" to another feature or element, it can be directly connected, attached or coupled to the other element or intervening elements may be present. In contrast, when a feature or element is referred to as being "directly connected", "directly attached" or "directly coupled" to another element, there are no intervening elements present. Although described or shown with respect to one embodiment, the features so described or shown can apply to other embodiments.

[0065] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.

[0066] Spatially relative terms, such as "under", "below", "lower", "over", "upper" and the like, may be used herein for ease of description to describe one element or feature's relationship to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if the device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus, the exemplary term "under" can encompass both an orientation of over and under. The device may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly", "downwardly", "vertical", "horizontal" and the like are used herein for the purpose of explanation only unless specifically indicated otherwise.

[0067] It will be understood that, although the terms first, second, etc. may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. These terms are only used to distinguish one element, component, region, layer or section from another region, layer or section. Thus, a first element, component, region, layer or section discussed below could be termed a second element, component, region, layer or section without departing from the teachings of the present invention.

[0068] The term "nanochannel" refers to a channel or trench having a critical dimension that is on the order of nanometers. The nanochannel has sidewalls and a floor. The nanochannel can be formed into a solid substrate to have an open top surface and a closed bottom surface with the sidewalls extending therebetween. A cover may be used to seal or otherwise close the upper surface of the nanochannel(s). The term "primary dimension" (also known as the "critical dimension") refers to a width and/or depth dimension. The primary dimension of a transport nanochannel can be between about 1 nm to about 900

nm, and is more typically between about 10 nm to about 500 nm. Different nanochannels on any particular substrate can have different primary dimensions and can change in magnitude over its length.

[0069] The term "about" refers to parameters that can vary between +/- 20% or less, such as +/- 10%.

[0070] The terms "transport nanochannel" and "primary transport nanochannel" and "fluidic transport nanochannel" are used interchangeably herein and refer to a nanochannel through which an analyte flows for manipulation and/or analysis.

[0071] The analyte can be any analyte of interest including, for example, single analyte molecules including synthetic and biological macromolecules, nanoparticles, small molecules, DNA, nucleic acids/polynucleic acids, peptides, proteins and the like. The transport through the nanochannel can be carried out using electrokinetics, concentration polarization and/or hydraulic pressure (forced pressure or pressure gradients) with at least one nanoscale manifold.

[0072] The term "nanoscale manifold" refers to an array or network of nanoscale elements that include one or more of nanochannels, nanoslits or nanoelectrodes. The nanoscale manifolds can be comprised of a single type of nanoscale element or combinations of two or more of these three types of nanoscale elements. At least some, and in some embodiments, all, the nanoscale elements of a respective nanoscale manifold are joined to a common voltage and/or pressure source and interfaced to one or more primary transport nanochannels, e.g., a single primary transport nanochannel, a plurality of primary transport nanochannels, or an array of primary transport nanochannels. The nanoscale manifold can have nanoscale elements that directly interface with a respective transport nanochannel or nanoscale elements that indirectly interface with a respective transport nanochannel by way of nanoscale elements that directly interface with the transport nanochannel as will be discussed below. The nanoscale manifold can have a plurality of nodes. The term "node" refers to a point at which nanoscale elements, e.g., nanoelectrodes, nanoslits or nanochannels, intersect or branch between a voltage and/or pressure source and a respective primary transport nanochannel.

[0073] The term "nanoslit" refers to channels that have nanometer scale depths but micrometer or larger widths. Nanoslits can have a depth between about 1 nm to about 900 nm, and more typically between about 10 nm to about 500 nm and the depth and/or width can be constant or vary over a length of the nanoslit. Nanoslits have typical depth:width aspect ratios between about 1×10^{-8} to about 0.2, more typically between about 1×10^{-5} to about 0.01.

[0074] The term "nanoelectrode" refers to a structure having a critical dimension that is on the order of nanometers consisting of an electrically conductive solid and/or non-flowable material including, for example, metals, metal carbides, carbon, semiconductors, conductive polymers, and gels. The critical dimension (or primary dimension) refers to a width and/or depth dimension. The primary dimension of a nanoelectrode can be between about 1 nm to about 900 nm, and is more typically between about 10 nm to about 500 nm. Different nanoelectrodes on any particular substrate can have different primary dimensions and can change in magnitude over its length.

[0075] The term "upstream" indicates a relative position that is closer to the ingress end of the primary transport nanochannel. The term "downstream" indicates a relative position that is closer to the egress end of the primary transport nanochannel.

[0076] The term "circuit," when referring to a control system for the fluidic chip, refers to an entirely hardware embodiment or an embodiment combining software and hardware using one or more controller.

[0077] The term "size", when referring to a sample, means that fragments are pulled into a segment of the transport channel (which can be identified as a detection nanochannel at that location), creating separation from neighbors for the determination of the size of fragments by detecting electrical or optical signal duration or amplitude.

[0078] The term "chromosomal DNA" means an entire chromosome's complement of DNA or a fragment of same.

[0079] Embodiments of the invention are directed to genomic mapping of DNA in a nanofluidic device but other uses are contemplated.

[0080] Analytes of interest that can be evaluated using a fluidic device with at least one nanoscale manifold include small molecules, nucleic acids, proteins, peptides, polysaccharides, viruses, ribosomes, micelles, and nanoparticles, for example.

[0081] The fluidic devices contemplated by embodiments of the invention can be configured to be able to characterize and/or controllably transport nucleic acids to various reservoirs associated with the fluidic devices with at least one nanoscale manifold, which may be useful for pooling genomic elements for assembly into synthetic genomes.

[0082] Systems using one or more of the fluidic devices contemplated by embodiments of the invention can include one or more detectors for detecting signals of onboard analytes. The detected signal may be analyzed to determine, for example, the identity of an analyte, structural conformation, size, positions of targeted or functional sites,

or sequence of component units (e.g., nucleotides in polynucleic acids, amino acids in proteins, prosthetic groups, and blocks in block co-polymers).

[0083] **Figures 1A and 1B** illustrate exemplary fluidic analysis devices **10**. **Figures 1A and 1B** illustrate the fluidic analysis devices **10** can have at least one nanoscale manifold **32** that interfaces with (e.g., are in electrical and/or fluid communication with) at least one primary transport nanochannel **20**.

[0084] The primary transport nanochannel **20** can be linear or curvilinear and may have a width and/or depth that changes at the entrance and/or exit or at other segments along lengths thereof. For example, the transport nanochannel **20** can have a reaction channel segment that is deeper and/or wider than a detection channel segment.

[0085] As shown in **Figures 1A and 1B**, there are first and second spaced apart manifolds **32₁**, **32₂**, each with a plurality of nanoscale elements **31** which can form pathways with nodes. As shown in these examples, the nanoscale elements **31** can each directly interface with the primary transport channel **20**. At least a plurality of nanoscale elements **31** of each respective manifold **32** extend from a common (single) voltage or pressure source junction **33**, typically a microchannel **15m**, to interface with at least one primary transport channel **20**. Thus, a nanoscale manifold **32** comprises a common voltage and/or pressure junction **33** for a plurality of nanoscale elements **31**. The voltage and/or pressure junction **33** can be attached to a manifold reservoir **50₂** (**Figure 13**) or other input connector for external input to supply pressure **P** (**100p₂**, **Figure 13**) and/or voltage **V** (**100v₂**, **Figure 13**) concurrently to a plurality of nanoscale elements **31** of a respective nanoscale manifold **32**. In the embodiment shown in **Figures 1A and 1B**, the manifold **32** has a pressure/voltage junction **33** that concurrently supplies voltage and/or pressure to all nodes **31n** of a respective nanoscale manifold **32**.

[0086] Although each manifold **32** in **Figures 1A and 1B** is shown with four nanoscale elements **31** with four nodes **31n**, more or fewer directly interfacing nanoscale elements **31** with more or fewer nodes **31n** may be used. Where more than one manifold **32** is used, each can have the same or different numbers of nanoscale elements **31**, but at least two nanoscale elements **31** that directly interface with the primary nanochannel **20** at a corresponding number of spaced apart locations.

[0087] **Figure 1A** illustrates the nanoscale elements **31** can be fluidic nanoscale elements **31f** such as nanochannels or nanoslits with voltage **V** and/or pressure **P** at the microchannel **15m** for supplying the input to the nodes **31**. In some embodiments, the fluidic nanoscale elements **31** can have a shallow channel depth, which, in some embodiments, can

prevent DNA molecules from entering the respective manifold nanochannel due to an energy barrier because of the additional confinement. This allows a relatively high field in the manifold element, e.g., nanochannel, which is desirable for significantly slowing down (or speeding up) the molecular transport. However, the fluidic nanoscale element **31f** may also have a deep but narrow channel for similar function.

[0088] **Figure 1B** illustrates a device **10** similar to **Figure 1A** but with nanoelectrodes **31e** as the nanoscale elements **31** that can include a non-flowable conductive medium or material **35** (shown by the darker background in the elements **31**). Nanoelectrodes **31e** as the manifold elements **31** can be constructed from a variety of non-flowable materials including, for example, metals, metal carbides, carbon, semiconductors, conductive polymers, and gels. Such electrodes **31e** can be patterned using lift-off lithography, electron beam induced deposition, ion beam induced deposition, lithography and subtractive etching, dielectrophoretic or direct manipulation of nanoelectrodes, templated growth of electrodes within nanochannels or nanoslits, injection of melted or suspended conductive or semiconductive media into nanochannels or nanoslits, *in situ* polymerization or gelation within nanochannels or nanoslits, and other methods known to those of skill in the art.

[0089] Each manifold **32** can be individually addressable by a common or an independent voltage and/or pressure source **100v₁**, **100v₂** and/or **100p₁**, **100p₂**, as shown schematically in **Figure 1A**. Thus, the nanoscale manifold **32** can allow multiple control elements **31**, e.g., nodes **31n**, to be addressed from the same voltage and/or pressure input. The multiple interfaces/intersections of the nanoscale elements **31** to the primary nanochannel(s) **20** can result in a series of nodes **31n** with separate characteristic voltages or pressures, as shown schematically in an equivalent circuit diagram in **Figure 1C**.

[0090] The voltage and/or pressure differences between nanoscale elements **31** can drive the electrokinetic or pressure driven flow, respectively, of analyte molecules in the nanochannel **20**. **Figures 1A** and **1B** show an exemplary fluidic device **10** with two nanoscale manifolds **32₁**, **32₂** interfaced to a single transport nanochannel **20**. It should be understood that any number of nanoscale manifolds **32** can be interfaced to a single and/or a plurality of primary transport nanochannels **20** as will be discussed below.

[0091] A respective manifold **15m** can have any suitable number of manifold elements **31**, such as between 2-10, between 10-100, between 100-1000, between 1000-10,000 (or even more depending on the application).

[0092] **Figure 1A** is an example of a fluidic device **10** where the forces driving transport are controlled by the application of voltages (V) and/or pressures (P) at the

microchannels **15m** accessing the primary nanochannel entrance **15i**, its exit **15e**, and the nanoscale manifold elements **31**. In **Figure 1A**, the nanoscale manifold elements **31** are nanoslits or nanochannels. In **Figure 1B**, the device **10** has nanoscale manifold elements **31** that have a non-flowable, electrically conductive medium (indicated in shading/darker background). Here, transport control can be carried out by applying voltages **V** to at least the manifold inputs **15m**. Voltages and/or pressures can be applied at the primary nanochannel entrance and exit **15i**, **15e**, respectively, to affect molecular transport.

[0093] **Figure 1C** is an equivalent circuit showing how the applied voltages and/or pressures **V**, **P** can be divided across the various manifold nodes **31n** and/or nanoscale elements **31** and different segments of the primary transport nanochannel **20**. Resistance to the electrical current is equal to the resistivity (ρ) of the medium in the segment multiplied by the segment's length (L) and divided by its cross sectional area (A); $R_{\text{electrical}} = \rho L / A$. Resistance to hydrodynamic flow depends on the geometry of the fluidic nanoscale element. An example is for channels with circular cross-sections with radius a : $R_{\text{hydrodynamic, circular-channel cross-section}} = L / a^4$. Another example is for channels with rectangular cross-sections having width w and depth h : $R_{\text{hydrodynamic, rectangular channel cross-section}} = L / (wh^3 (1 - 0.63h/w))$.

[0094] Generally stated, each nanoscale manifold **32** can be controlled by a separate voltage and/or separate pressure source. Additionally, the primary transport nanochannel(s) **20** at an entrance portion **15i** and exit portion **15e** can each be addressed by its own pressure and/or voltage source(s). Thus, each manifold **32** can be modular. That is, each manifold **32** can be addressed individually, a single pressure or single voltage control (or a combination single voltage and pressure control) affects many elements **31** in a given manifold **32**. Device **10** performance can be engineered by fabricating/assembling multiple manifolds **32** that are interfaced to the primary transport nanochannel **20** or a plurality of primary transport nanochannels **20**. For example 2, 3, 4, 5, 6, 7, 8, 9, 10, 10s, 100s, 1000s, or more nanoscale manifolds **32** can be interfaced to the primary transport nanochannel **20** or a plurality of primary transport nanochannels **20**.

[0095] The spacing of the nanoscale manifold elements **31**, their dimensions, their connectivity, and their conductivity can be designed to control their resistances (electrical or hydraulic) and shape the force profiles within the primary transport nanochannel(s) **20** and within nanoscale elements **31**.

[0096] The spacing between adjacent nanoscale elements **31** and/or nodes **31n** of a respective manifold **32** can vary or be the same. The spacing between adjacent nanoscale

elements **31** can be nm size spacing, (100s of) μm size spacing and, even mm size spacing. Typically, the spacing between adjacent nanoscale elements **31** and/or nodes **31n** is between about 10 nm to about 20 μm , more typically between 0.5 μm and 10 μm such as 0.5 μm , 0.6 μm , 0.7 μm , 0.8 μm , 0.9 μm , 1 μm , 2 μm , 3 μm , 4 μm , 5 μm , 6 μm , 7 μm , 8 μm , 9 μm and 10 μm . In other embodiments, the spacing between adjacent nodes **31** for a respective manifold **32** can be between 100 nm and 6000 nm, such as 100 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, 150 nm, 155 nm, 160 nm, 165 nm, 170 nm, 175 nm, 180 nm, 190 nm, 195 nm, 200 nm, 210 nm, 220 nm, 230 nm, 240 nm, 250 nm, 260 nm, 270 nm, 280 nm, 290 nm, 300 nm, 310 nm, 320 nm, 330 nm, 340 nm, 350 nm, 360 nm, 370 nm, 380 nm, 390 nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, 1000 nm, 1100 nm, 1200 nm, 1300 nm, 1400 nm, 1500 nm, 2000 nm, 2250 nm, 2500 nm, 3000 nm, about 3500 nm, about 4000 nm, about 4500 nm, about 5000 nm, about 5500 nm, and about 6000 nm and any increment therebetween, whether explicitly listed or not.

[0097] The device **10** can have a body that is a planar chip with a pattern of microchannels **15i**, **15e** and **15m** (the manifold microchannel **15m** can merge into nanoscale elements **31**) that communicate with the at least one nanochannel **20** as shown in **Figures 1A** and **1B**. The device **10** can include a cover that can be attached (typically bonded) to a substrate with the pattern of channels. In some embodiments, some features, *e.g.*, one or more of the micro and/or nanochannels **15i**, **15e**, **20** and/or one or more of the manifolds **15m** and/or nanoscale manifold elements **31** can be patterned on the substrate while others can be patterned on the cover plate. Reservoirs **50₁**, **50₂**, **50₃** may be mounted on the device **10** at various microchannel interfaces, **15i**, **15m**, **15e** (**Figure 13**), for example.

[0098] The transport nanochannel **20** can be deeper and more narrow (in width) than the depth and/or width of all or some of the nanoscale elements **31** of a respective nanoscale manifold **32**.

[0099] In some embodiments, the nanoscale elements **31** can be shallow. The term "shallow" refers to manifold nanochannel elements **31** with depths that are less than a depth of an associated transport nanochannel **20** and can be smaller than analyte macromolecules' hydrodynamic sizes. With respect to the depth, a shallow manifold nanochannel or nanoslit **31** can have a depth that is typically less by at least a factor of two (2), such as by between 2-100X less than that of an associated transport channel **20**.

[00100] The at least one manifold **32** can be configured to produce a defined monotonic gradient in the electric field (**Figure 2A**) or pressure (**Figure 2B**), which may be particularly important for some uses/analysis. The field strength "**E**" V/cm or velocity "**U**"

$\mu\text{m/s}$ of an analyte with a mobility $1 \times 10^{-4} \text{ cm}^2/(\text{V}\cdot\text{s})$ is illustrated by the vertical graduated scale to the right of **Figure 2A**. The linear flow rate "F" $\mu\text{m/s}$ for the pressure-driven flow is shown in **Figure 2B** with the appended scale to the right of the figure.

[00101] **Figures 2A and 2B** provide examples of operation of a fluidic device **10** where the manifold elements **31** are nanoslits or nanochannels. The drawings are not to scale. In this example, the primary nanochannel **20** is 320 nm wide x 440 nm deep with a total length of 275 μm . In this example, each of the nanoscale manifold elements **31** is 550 nm wide x 65 nm deep x 18 μm long. The spacing between (node) nanochannel elements **31** within a manifold **32** is 5 μm for this example. **Figure 2A** illustrates exemplary voltages for the manifolds **32₁**, **32₂** for a voltage controlled operation. The electric field strengths (and analyte velocity) in the vicinity of the manifolds **32₁**, **32₂** are indicated in the enlarged insets by the grayscale intensity. These were calculated using the voltages provided in the schematic. **Figure 2B** illustrates pressure-controlled operation of the manifold **32**. The linear flow velocities in the vicinity of the manifolds are indicated by the grayscale intensity. These were calculated using the pressures provided in the schematic. All calculations were performed using finite element analysis implemented in COMSOL Multiphysics® modeling software, a general-purpose software platform, based on advanced numerical methods, for modeling and simulating physics-based problems from Comsol Inc., Burlington, MA and Los Angeles and Palo Alto, CA.

[00102] Several examples of use of fluidic devices **10** with exemplary force profiles ("force profiles" is used interchangeably with "force gradients") P_F that can be applied by a nanoscale manifold **32** to the transport nanochannel **20** are shown in **Figures 3A-3F** for analyte molecules **A**, represented by solid circles, arcuate lines and a longer curvilinear "flex" line (the latter for flexible macromolecule **M**). For example, by appropriately increasing or decreasing the electric field strength within a primary transport nanochannel **20**, an analyte **A** such as a flexible macromolecule can be accelerated (**Figure 3B**) or decelerated (**Figure 3A**) without inducing shearing, i.e., covalent bond breakage. These features allow gentle handling of fragile molecules, reducing shear forces across a wide range of transport velocities within a primary nanochannel. **Figure 3C** illustrates compression, **Figure 3D** illustrates stretching, **Figure 3E** illustrates collection/separation and **Figure 3F** illustrates trapping/concentration. The number and architecture of the nanoscale manifold elements can be tailored to the application. The simplicity of device design and fabrication may be the primary consideration when the anticipated analyte is mechanically robust. More complex

manifolds **32** might be more suitable for the most fragile analytes, where a premium is placed on very gradually changing the transport driving forces.

[00103] Through the application of electric potentials and/or pressures to the entrance and exit of the primary nanochannel **15i**, **15e** and/or nanoscale manifold elements **31** and/or nodes **31n** of the manifold **32**, each nanoscale manifold **32** can establish an independent decelerating or accelerating force gradient within the primary transport nanochannel(s) **20** for a desired force profile P_F . By way of example, in operation, a manifold **32** placed close to the entry location **15i** of an analyte into the transport channel **20** (e.g., left manifold **32₁** in **Figure 1A**) can decelerate analyte macromolecules **A** after they are initially forced into a transport nanochannel **20**. This "close" spacing can be between 100 nm and 20 μm , for example. Given the entropic barrier to entry into a transport nanochannel **20** a relatively strong force may be required to thread macromolecules into the primary nanochannel **20**. This force can inject molecules into the primary nanochannel **20** at high velocity, resulting in their elongation and exposure to significant shear forces. Use of the manifold **32** can significantly and gradually decrease the magnitude of the transport driving force within the primary nanochannel **20**, minimizing the shear force on the macromolecules and decreasing their velocity within the transport nanochannel **20**. Downstream of the first manifold **32₁**, such as at an exit end portion of the transport nanochannel **15e**, a second manifold **32₂** (**Figures 1A, 1B**) can be employed to accelerate the molecules out of the primary nanochannel **20**, for example past an optical detector "**Di**" and/or electrical detector "**De**" (**Figure 1A, 13**) for analysis. The applied forces can be held constant throughout a respective experiment or can be switched or otherwise varied during the experiment to alter the dynamics of molecule transport.

[00104] Generally stated, input voltages and/or pressures at the entrance and exit of the transport nanochannel **15i**, **15e** and at the nanoscale manifold(s) **15m** can be independent of each other and controlled and output adjusted independently.

[00105] In some embodiments, the primary transport nanochannel **20** can have an aspect ratio ("AR")(depth:width) of ~ 1 ; typically the primary transport nanochannel **20** has an AR that is greater than 0.5 and less than 2. In some embodiments, the nanoscale elements **31** (nanoslits or nanochannels) can have aspect ratios less than unity and less than the transport channel **20**. However, manifold nanochannels and nanoslits **31f** can have a variety of aspect ratios.

[00106] When nanochannels or nanoslits are used as the nanoscale manifold elements **31**, however, care should be taken to avoid unwanted molecular transport out of the primary

transport nanochannel(s) **20** and into the manifold fluidic conduits **31f**. This can be achieved by selecting the driving forces such that the forces that drive continued transport of a target analyte molecule through the primary nanochannel(s) **20** are greater than those driving transport down the manifold nanoscale fluidic elements **31f**.

[00107] When the analytes of interest are large macromolecules, the use of manifold nanochannels or nanoslits **31f** that have either widths or depths that are significantly (e.g., shallow depths) less than those of the primary transport nanochannel(s) **20** can also assist in ensuring that analytes remain in the primary nanochannel **20**. This results from an entropic free energy barrier that inhibits macromolecule entry into a more constraining conduit. Correspondingly, manifold nanoscale fluidic elements **31f** could also have the reciprocal AR to utilize entropic free energy barriers to keep macromolecules in the primary transport nanochannel(s) **20** but typically it is easier to fabricate low AR features than high AR features. Such considerations are not typically a concern when nanoscale electrodes are the manifold elements **31** used to generate transport-driving electric fields.

[00108] A variety of geometries for the nanoscale manifold elements **31** are possible, allowing the device **10** to be customized or tailored for specific sample and operational needs. **Figures 4A-4Y** show examples of manifold array geometries with nanofluidic elements **31f** coupled to one pressure and/or voltage interface **33** or coupled to more than one spaced apart interface, with each interface communicating directly with at least two nanoscale manifold elements **31**. **Figures 5A-5Y** illustrate corresponding manifold array geometries with nanoelectrodes **31e**. **Figures 4M, 5M, 4N, 5N, 4O, 5O, and 4R, 5R** illustrate cooperating pairs of nanoscale manifolds **32₁, 32₂**, positioned to face each other across the transport channel **20**.

[00109] **Figures 4E, 5E, 4H, 5H, 4L, 5L, 4N, 5N and 4O, 5O** illustrate diagonal elements **31d**, some that directly interface with the transport nanochannel **20** at a discrete node intersection (**Figures 4H, 5H, 4N, 5N, 4O, 5O**) and others that merge into another manifold element **31** to form a common, shared node (**4E, 5E, 4L, 5L**) at the intersection with the transport nanochannel **20**.

[00110] **Figures 4F, 5F, 4I, 5I, 4J, 5J, 4K, 5K, 4L, 5L, 4Q, 5Q, 4S, 5S, 4T, 5T, 4U, 5U and 4V, 5V**, for example, illustrate at least one cross-channel **31c** connecting different elements or forming a series of nodes (shown as perpendicular to the nanoscale elements **31**). Some of the cross-channels **31c** can indirectly interface with the transport nanochannel **20** via other nanoscale elements **31**.

[00111] **Figures 4L and 5L**, for example, illustrate the manifold **32** can have both diagonal and cross elements **31d**, **31c**.

[00112] **Figures 4W and 5W** illustrates that the manifold **32** can be interfaced to a plurality of different transport nanochannels **20₁**, **20₂**, **20₃** (e.g., an array of primary transport nanochannels).

[00113] **Figures 4X, 5X and 4Y, 5Y** illustrate the nanoscale manifold **32** can interface with a single transport nanochannel **20** at a plurality of spaced apart segments, typically where the transport channel **20** has a curvilinear or serpentine shape as shown so that the nanoscale elements **31** can interface with parallel legs **22** of the transport channel **20**.

[00114] **Figures 6A-6G** show examples of nanoscale manifold elements **31** with various geometries that may be configured to provide additional control over manifold design. Such geometries can help shape the voltage or pressure gradients present in the primary transport nanochannel **20**. In addition to width variations, the individual elements **31** can vary in their depth (for nanoslit or nanochannel manifold elements **31f**) or thickness (for nanoelectrode manifold elements **31e**). For example, the depth (thickness) of each element **31** can increase from the voltage or pressure source to the primary nanochannel **20**, decrease from the voltage or pressure source to the primary nanochannel **20**, decrease and then increase one or multiple times, or increase and then decrease one or multiple times. These variations in nanoscale element depth **31f** or thickness **31e** can occur in a step-wise fashion (e.g., a 100-nm change in feature depth over a length of 1 nm) or more gradually (e.g., a 100-nm change in feature depth over a length of 1 μ m). A manifold **32** can consist of nanoscale elements **31** of identical geometries and dimensions or of nanoscale elements **31** having a variety of geometries and dimensions. Thus, **Figures 6A-6G** are greatly enlarged schematic top views of some of the many geometries that can be used for individual nanoscale manifold elements **31**. In addition to variations in width shown, the depth of fluidic nanoscale elements **31f** (for nanoslit and nanochannel manifold elements) or the thickness of nanoelectrode elements **31e** can be varied along their lengths.

[00115] **Figures 7A and 7B** illustrate a fluidic device **10** with a nanoscale manifold **32** used to decrease the velocity of molecular transport within a primary nanochannel. In this example the analyte is a 10-kbp long DNA molecule stained with an intercalating fluorescent dye. **Figure 7A** is a schematic of the device **10** used to collect the data shown in the images in **Figure 7B**. Image (b) in **Figure 7B** is a bright-field micrograph showing the entry region, primary nanochannel **20**, and manifold nanoslits **31f**. The field of view is restricted to the first ~ 80 μ m of the primary nanochannel **20**. Image (c) is a representative fluorescence image

of a DNA molecule in the primary nanochannel **20**. Image (d) is a kymogram (images recorded at 330 frames per second) showing the change in the DNA molecule's position over time as it is driven through the primary transport nanochannel. The positions of the manifold nanoslits are indicated by the dashed lines. The molecule is pulled into the primary nanochannel by a 186 V/cm magnitude electric field. The field strength in the primary channel is gradually lowered by the action of the nanoscale manifold to a magnitude of 32.7 V/cm to the right of the fourth manifold nanoslit. The total relative decrease in velocity is 5.68-fold. The 10-kbp DNA molecule (1 kbp = 1,000 base pairs) underwent rapid injection into a primary nanochannel **20**, followed by a ~ 6-fold reduction in velocity upon passage through the region interfaced to the nanoscale manifold **32**. **Figure 7B** illustrates adjacent different DNA images (b), (c), (d). The bright-field microscope image of the device (b) shows the location of the primary nanochannel entrance and the junctions with the four nanoslits comprising the nanoscale manifold nearest to the entrance. The fluorescence image of a DNA molecule stained with an intercalating dye at image (c) shows the molecular conformation from a single video frame. The time series or kymogram (d) shows the position of the DNA molecule at multiple time points as it traverses the primary nanochannel. This time series reveals the rapid transport of the DNA molecule in the initial region of the primary nanochannel. In each of the nanochannel segments of the transport channel **20** located between a pair of manifold nanoslits **31**, the molecule's velocity is decreased. This stepwise reduction results in the apparent curvature in the region demarcated by dashed lines in (image (d)) of **Figure 7B**. A lower, nearly constant velocity characterizes the subsequent transport of the DNA molecule.

[00116] **Figures 8A and 8B** illustrate the use of a nanoscale manifold **32** to increase the velocity of molecular transport within a primary nanochannel **20**. In this example the analyte is a 10-kbp long DNA molecule stained with an intercalating fluorescent dye. **Figure 8A** is a schematic of the device used to collect the data shown in **Figure 8B**. In **Figure 8B**, image (b) is a bright-field micrograph showing the primary nanochannel and a second set of manifold nanoslits. Image (c) is representative fluorescence image of a DNA molecule in the primary nanochannel. Image (d) is a kymogram (images recorded at 330 frames per second) showing the change in the DNA molecule's position over time as it is driven through the primary transport nanochannel. The positions of the manifold nanoslits are indicated by the dashed lines. The molecule is initially driven through the primary nanochannel (segment 1 in panel (d)) by a 32.7 V/cm magnitude electric field. The field strength in the primary channel is gradually increased by the action of the nanoscale manifold to the following values in each

of the segments numbered in panel (d): 2, 127.2 V/cm; 3, 230.8 V/cm; 4, 350.7 V/cm; 5, 495.5 V/cm. The total relative increase in velocity is 15.13-fold.

[00117] **Figure 8A** shows the same device **10** as in **Figure 7A** but operated differently as noted above. **Figure 8B** illustrates adjacent different images (b), (c), (d). Image (b) shows the location of the primary nanochannel exit end and the junctions with the four nanoslits comprising the nanoscale manifold. The fluorescence image of a DNA molecule stained with an intercalating dye per image (c) shows the molecular conformation from a single video frame, while the time series or kymogram (image (d)) shows the position of the DNA molecule at multiple time points as it traverses the primary nanochannel. This time series reveals the relatively slow transport of the DNA molecule in the initial region of the primary nanochannel shown in the figure. In each of the transport nanochannel segments located between a pair of manifold nanoslits **31**, the molecule's velocity is increased. This stepwise increase results in the apparent curvature in the region demarcated by dashed lines in image (d) of **Figure 8B**. Transport of the molecule in the rightmost segment of the primary nanochannel, after (to the right of) the nanoscale manifold, is characterized by the molecule's relatively high, constant velocity. Overall the DNA velocity increased ~15-fold as it moved from left to right through the second manifold region.

[00118] The behavior of the device **10** that is shown in **Figures 7A** and **8A** (representative of a class of devices shown schematically in **Figure 1A**) was characterized for a wide range of molecule deceleration and acceleration conditions that were controlled by the input voltages driving electrokinetic DNA transport. For each condition, the electric field strength in each segment of the primary transport nanochannel was estimated from the equivalent circuit shown in **Figure 1C**, where the resistances were the resistances to the ionic current in the electrolyte solution calculated from the dimensions (width, depth, length) of each segment of the primary nanochannel and each nanoslit in the nanoscale manifolds. DNA velocity in each segment of the primary nanochannel was determined from fluorescence images in time series similar to those shown in **Figures 7B** and **8B** (image (d)).

[00119] **Figure 9** shows how the DNA velocity was affected at various positions along the primary nanochannel **20** in each of the six experiments. These experiments varied the four input voltages such that DNA molecules experienced comparable deceleration after entry into the primary nanochannel in each of the experiments. In each experiment, however, DNA molecules experienced significantly different degrees of acceleration near the exit (right) end of the primary nanochannel. Thus, the plot/graph in **Figure 9** shows the tunability of analyte transport velocity. The velocities of 10-kbp DNA molecules were measured from

kymograms such as those shown in **Figures 7B** and **8B**. The average velocity of several molecules was determined within each segment of the primary transport nanochannel. In this demonstration, the entry electric field strength and the initial deceleration of the molecules were fixed for six different experimental conditions (different voltages applied to the primary nanochannel ends and to the two nanoscale manifolds). The change in velocity of the molecules at the exit end of the primary nanochannel was different for each of the experiments. The relative change in a DNA molecule's velocity was $15.1\times$ for condition 1, $10.6\times$ for condition 2, $6.2\times$ for condition 3, $3.2\times$ for condition 4, $1\times$ for condition 5, and $0.5\times$ for condition 6.

[00120] **Figure 10** shows these same measured velocities as a function of the electric field strength estimated for each segment of the primary nanochannel using an equivalent circuit diagram. The linear dependence of velocity on estimated electric field strength indicated that interfacial effects or local perturbations to molecular conformations did not strongly affect DNA mobility under the conditions investigated. The plot/graph in **Figure 10** shows the characteristics of voltage-controlled transport of analyte molecules through a primary transport nanochannel. Transport velocities of 10-kbp DNA molecules were measured under six different experimental conditions in the various segments of the primary transport nanochannel. The linear dependence of transport velocity on electric field strength indicates that a similar electrophoretic mobility is measured in each experiment [$1.2(\pm 0.2) \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$]. Deviation from linearity (most evident for data collected under experimental condition 1, black squares) is believed to be the result of pressure gradients established by high electro-osmotic flow rates under these experimental conditions. 3

[00121] Representative devices were also used to control the transport of long ($>50 \text{ }\mu\text{m}$ or $>120 \text{ kbp}$) macromolecules. T4-phage DNA molecules (165-kbp long) have a fully extended length ($73 \text{ }\mu\text{m}$) exceeding the total widths of the nanoscale manifolds ($15 \text{ }\mu\text{m}$) on this device. Because the analyte macromolecule spanned multiple sections of the primary transport nanochannel having different electric field strengths, the nanoscale manifolds could be used to not only alter the overall velocity of the molecule's transport but also affect molecular compression and stretching (**Figure 11**). The device used to collect these data employed nanoscale manifold elements with a staggered spacing (indicated by the vertical dashed lines in **Figure 11**), in contrast to the evenly spaced manifold elements of the device shown in **Figures 7** and **8**. **Figure 11** illustrates device performance for large DNA molecules. Fluorescently stained T4-phage DNA molecules (165 kbp, with a fully extended length of $73 \text{ }\mu\text{m}$) are transported across a nanoscale manifold operated in an accelerating

capacity. These molecules are long enough to span the entire width (15 μm) of the manifold during transit. Image (a) is a bright-field micrograph of the device. Image (b) is a fluorescence image of two T4-phage DNA molecules. The leftmost molecule has not yet reached the nanoscale manifold while the rightmost molecule spans the manifold. Image (c) is a fluorescence image of the leftmost T4-phage DNA molecule as it is stretched in the region of the nanoscale manifold. Image (d) is a fluorescence image of the leftmost T4-phage DNA molecule after it has exited the region of the nanoscale manifold. As it continues transport down the primary nanochannel, the tension imparted on the molecule by its passage through the accelerating region relaxes and the molecule's end-to-end length decreases. Image (e) is a kymogram (images collected at 252 frames per second) showing the entire transport process for the two molecules. The positions of the manifold elements (here nanoslits) are indicated by the dashed lines.

[00122] **Figure 12** is a flow chart of exemplary actions that can be used to operate a fluidic analysis device. A fluidic analysis device is provided, the device having a primary transport nanochannel and at least one nanoscale manifold with a plurality of nanoscale manifold elements that intersect or interface with the primary transport channel (block **150**). A single pressure and/or voltage input is concurrently applied to at least some of the different nanoscale elements of a respective nanoscale manifold to generate a force profile (e.g., force gradient) to carry out at least one of: accelerate, decelerate, compress, stretch, collect/separate or trap/concentrate an analyte as the analyte flows through the primary transport nanochannel (block **160**).

[00123] The at least one nanoscale manifold can be at least two nanoscale manifolds (block **152**). The nanoscale manifold elements can comprise nanoslits (block **153**). The nanoscale manifold elements can comprise nanoelectrode elements (block **154**). The nanoscale manifold elements comprise nanochannels (block **155**).

[00124] The nanoscale manifold elements can have between 2-10,000 adjacent elements, at least some of which are spaced apart by 10 nm to 1 cm (block **156**). The manifolds can have between 2-10, between 10-100, between 100-1000, between 1000-10,000 (or even more depending on the application).

[00125] The nanoscale manifolds **32** can allow the generation of a wide variety of precisely controlled force gradients using only a few input forces. The manifolds **32** can generate distributed force gradients (e.g., a force profile P_F) as opposed to large discrete changes in force magnitudes using a cross-channel, for example, and the ability to generate these gradients without relying on independent voltage or pressure control for each control

element that intersects the primary transport nanochannel or nanochannel array. Gradients can be achieved over an arbitrarily large number of nodes (intersections between the primary transport nanochannel(s) and the nanoslits, nanochannels, or nanoelectrodes of a nanoscale manifold). This is believed to provide an unprecedented ability to establish user-defined force gradients, thereby affording control in each segment of the primary nanochannel(s) over the transport of analytes ranging from small molecules to centimeters-long stretched macromolecules.

[00126] Nanoscale manifolds have additional benefits for the manipulation of analyte molecules. In devices in which a single control nanoslit or nanochannel intersects the primary transport nanochannel(s), the degree to which the velocity of the molecule can be altered may be limited. Examples of fluidic devices with primary transport nanochannels are described in US Patent Application Serial Number 14/190,520, the contents of which are hereby incorporated by reference as if recited in full herein. Overaggressive attempts to alter the velocity with a single control nanoslit or nanochannel can result in the molecule's entry into the control structure, as opposed to the continued transport down a primary nanochannel. In a device containing a nanoscale manifold, in contrast, the gradual variation of the forces driving molecular transport can minimize the likelihood of transport down one of the nanoscale manifold elements. As illustrated in **Figures 3A-F, 4, 5 and 6**, there are a large number of design parameters that can be varied to engineer the desired device performance. Design choices can affect the shape of the force gradients, their sensitivity to the control voltages or pressures, and the range of control settings over which the device can be stably operated. Each of the aforementioned characteristics contributes to the tunability of the device performance. Nanoscale manifolds can be considered as modular elements, enabling several to be linked together to create complex, sometimes repeating force profiles to drive molecular transport. The control forces applied to the device may be held at constant values throughout operation or they may be varied in time, (e.g., stepped to different voltages or pressures, transiently pulsed to different voltages or pressures, continuously ramped at various linear or non-linear rates, varied according to a sinusoidal or other wave function). This possibility provides greater opportunities for complex transport control. Furthermore, nanoscale manifolds enable a small number of time-varying control forces to impact a large number of nodes in the primary transport nanochannel or nanochannel array with precisely controlled cooperative timing at each node.

[00127] Nanoscale manifolds have extremely broad applicability and can be integrated into a variety of nanofluidic devices in which a high degree of control over the transport of

analyte molecules is desired. Devices can incorporate such manifolds to facilitate the analysis of polynucleic acids such as RNA and DNA. Possible applications include optically or electrically characterizing nucleic acids, assessing nucleic acid samples for polydispersity, mapping the positions of specific sequences, assessing environmentally caused damage, quantifying epigenetic modifications, quantifying protein binding, determining the secondary structure of single-stranded nucleic acids or single-stranded regions of nominally double-stranded nucleic acids, directly reading genomic sequence at single-nucleotide resolution, and identifying pathogens based on nucleic acid characterization. In other implementations the ability to hydrodynamically or electrostatically stretch macromolecules can be used to characterize proteins at the single-molecule level, mapping protein structure or locating the positions of enzyme active sites. More generally, such devices can be used to conduct force-response characterizations of any macromolecule, biological or synthetic. The electrophoretic mobility of denatured or native proteins within primary nanochannels can be measured to characterize a mixture of proteins. The ability to apply large forces at the primary nanochannel entrance facilitates high throughput operation while the ability to decrease analyte velocity within the nanochannel allows more sensitive detection of sample components.

[00128] Devices with nanoscale manifolds may facilitate the analysis of sample mixtures, either using single molecule sorting or sample preconcentration followed by ensemble separation techniques. As noted above, analytes of interest in such applications include small molecules, nucleic acids, proteins, peptides, polysaccharides, viruses, ribosomes, micelles, and nanoparticles, for example. The ability to characterize and controllably transport nucleic acids to various reservoirs using nanochannel devices could also be useful for pooling genomic elements for assembly into synthetic genomes.

[00129] Where multiple nanoscale manifolds **32** are used for a respective primary transport nanochannel **20**, each bank or series of nanoscale elements **31** and/or nodes **31n** can allow precise and active control over the forces driving molecular transport in the various regions of the primary nanochannel(s). This allows for control over the transport of molecules along discrete regions of the primary nanochannel(s) **20**. The manifold structures, such as those described, permit the establishment of designed monotonic force gradients along the length of the primary fluidic nanochannel(s) while limiting the required number of independent input potentials or pressures applied to the device. These tunable force gradients make the structures ideal for the manipulation of small molecules and macromolecules spanning many orders of magnitude in size while minimizing the occurrence of force gradient

induced molecular fragmentation. Through the decoupling of the force magnitudes required to capture and then transport molecules through a nanochannel, precise spatio-temporal control over the molecules is obtained.

[00130] Fluidic devices **10** can be fabricated in a variety of substrates including silicon, glass (silica), quartz, plastics, thermoplastics, and elastomers or a combination thereof. Various nanopatterning methods known to those of skill in the art can be used independently or in combination to form the nanoscale features, such as high resolution photolithography, electron beam lithography, focused ion beam (FIB) milling, nanoimprint lithography, templating or molding strategies, wet or dry etching, molding, embossing, or machining. See, e.g., Guo L. J.; Cheng, X.; Chou C.-F. Fabrication of size-controllable nanofluidic channels by nanoimprinting and its application for DNA stretching. *Nano Lett.* **2004**, *4*, 69; WO2013/119765 and US2014/0360877; and WO2013/195723, the contents of which are hereby incorporated by reference as if recited in full herein. See also, Mijatovic, D.; Eijkel, J. C. T.; van den Berg, A. Technologies for nanofluidic systems: top-down vs. bottom-up - a review. *Lab Chip* **2005**, *5*, 492-500; Perry, J. L.; Kandlikar, S. G. Review of fabrication of nanochannels for single phase liquid flow. *Microfluid. Nanofluid.* **2006**, *2*, 185-193; Chantiwas, R. et al. Flexible fabrication and applications of polymer nanochannels and nanoslits. *Chem. Soc. Rev.* **2011**, *40*, 3677-3702; and Utko, P.; and Persson, F.; Kristensen, A.; Larson, N. B. Injection molded nanofluidic chips: Fabrication method and functional tests using single-molecule DNA experiments. *Lab Chip* **2011**, *11*, 303-308. The contents of which are hereby incorporated by reference as if recited in full herein. The ability to use wafer-scale processing can provide for a high impact, low cost technology.

[00131] Once the nanoscale and microscale elements are fabricated in the top surface of the substrate, a cover plate can be attached, typically bonded to the substrate to form the enclosed fluidic network using, for example, fusion bonding, anodic bonding, or bonding with an adhesive film between the bottom substrate and cover plate. The microchannels can be accessed through vias that pass through the bottom substrate and/or top cover plate.

[00132] Referring to **Figure 13**, the system **200** can include a voltage input **100v** (shown as three independent voltage sources/power supplied) **100v₁**, **100v₂**, **100v₃** and/or a pressure input **100p** (shown as three independent pressurized gas supplies **100p₁**, **100p₂**, **100p₃** and respective supply lines **101**), one each for a respective manifold **15m**, an entrance end portion **15i** of a primary transport nanochannel **20** and an exit end portion of the primary transport nanochannel **20**. One voltage and/or pressurized gas source **100v₂**, **100p₂** can provide the input for the manifold reservoir **50₂** of a manifold junction **33** for the associated

nanoelectrodes **31e** and/or fluidic elements **31f** (**Figures 1A, 1B**) for controllably applying voltage **V** and/or pressure **P** to achieve a force profile **P_F** along the primary transport nanochannel **20** proximate the nanoscale manifold **32**.

[00133] Reservoirs **50** (shown as **50₁, 50₂, 50₃**) can be affixed to the device **10** to facilitate liquid handling. Input electrodes **50e** from a power source **100v** can be inserted into all or selected reservoirs **50**. The reservoirs **50** have vias **50v**. The input electrodes **50e** apply voltages across the various fluidic elements, e.g., transport channel ingress **15i**, transport channel egress **15e**, and manifold **32**. Pressurized air or vacuum lines **101** can be coupled to the reservoirs **50** or vias to apply positive pressure or vacuum from the pressurized gas source **100p** to the fluidic elements and drive pressure-driven fluid flow. One or more reservoirs **50** can have both a pressurized gas/vacuum line **101** and an electrode input **50e**.

[00134] The system **200** can have a circuit **100c** with at least one controller **110** that can apply the pressure and/or electrical input to a respective manifold **32** for the force profile **P_F**. The controller **110** can comprise at least one processor programmed to provide a menu of different operational timing diagrams and voltage/pressures for the force profile. The controller **110** can direct a desired voltage and/or pressure input timing program or algorithm that can also communicate with or includes a detection circuit with an optical and/or electrical detector **Di, De** associated with an imaging system **250I** and/or electrical detection system **250E**. The imaging system **250I** can include the detector **Di** and an excitation source that can take a series of images of an analyte molecule in the detection channel. The imaging system **250** can be any suitable imaging system. The system **250** can include an excitation light source (typically for generating light that excites fluorescently labeled molecules) (which can optionally include a mirror, beam splitter, polarizer, lens, and/or other optical elements) and image generating device or detector **D** such as one or more of a camera, photomultiplier tube or photodiode. An objective/lens, where used, can reside under or over a primary surface of the transport channel of the device. The electric inputs/outputs and flow operation can reside on an opposing side of the device **10**. The device **10** may also be flipped to operate on its side (with the flat primary surfaces being upright or angled) rather than substantially horizontal.

[00135] The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included

within the scope of this invention as defined in the claims. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED:

1. A fluidic device, comprising:
at least one fluidic transport nanochannel; and
at least one nanoscale manifold having a plurality of nanoscale elements, wherein at least a plurality of the nanoscale elements directly interface with the at least one fluidic transport nanochannel, and wherein at least a plurality of the nanoscale elements of the nanoscale manifold are configured to be concurrently controlled by a common externally applied pressure and/or voltage to generate a force gradient within the at least one transport nanochannel.
2. The device of Claim 1, wherein the fluidic device is a chip, the device further comprising a plurality of reservoirs held by the chip, including at least one reservoir in fluid communication with at least one microfluidic inlet to at least one transport nanochannel and at least one reservoir in fluid communication with a plurality of the nanoscale elements of the at least one nanoscale manifold.
3. The device of Claim 1, wherein the at least one nanoscale manifold includes at least first and second nanoscale manifolds interfaced to a first one of the at least one transport nanochannel.
4. The device of Claim 3, wherein the first nanoscale manifold is upstream of the second nanoscale manifold.
5. The device of Claim 3, wherein the first nanoscale manifold faces the second nanoscale manifold across the first fluidic transport nanochannel.
6. The device of Claim 1, wherein the plurality of nanoscale manifold elements is between 2-10,000 adjacent elements that directly interface with at least one transport nanochannel, wherein at least some of the adjacent elements are spaced apart by 10 nm to 1 cm.

7. The device of Claim 1, wherein the plurality of nanoscale manifold elements for a respective transport nanochannel is: (a) between 2-10, (b) between 10-100, (c) between 100-1000, or (d) between 1000-10,000.

8. The device of Claim 1, wherein the nanoscale manifold elements comprise nanoslits.

9. The device of Claim 1, wherein the nanoscale manifold elements comprise nanoelectrode elements.

10. The device of Claim 1, wherein the nanoscale manifold elements comprise nanochannels.

11. The device of Claim 1, wherein the nanoscale manifold comprises at least one cross-channel that connects at least two parallel nanoscale manifold elements that directly interface with at least one of the at least one transport nanochannels.

12. The device of Claim 1, wherein the nanoscale manifold elements include at least one diagonal element.

13. The device of Claim 12, wherein the at least one diagonal manifold element extends outward from at least one straight manifold element.

14. The device of Claim 12, wherein the at least one diagonal element extends from a voltage and/or pressure input junction or channel segment associated therewith to the at least one transport nanochannel.

15. The device of Claim 1, wherein the at least one fluidic transport nanochannel is a plurality of discrete, spaced apart fluidic transport nanochannels, and wherein the at least one nanoscale manifold interfaces with at least two of the plurality of transport nanochannels.

16. The device of Claim 1, wherein the at least one nanoscale manifold interfaces with first and second spaced apart segments of at least one of the at least one fluidic transport nanochannel.

17. The device of Claim 1, wherein the at least one transport nanochannel has a serpentine shape with first and second legs that are parallel, and wherein at least one of the at least one nanoscale manifold interfaces with both the first and second legs.

18. The device of any of Claims 1-17, in combination with power and/or pressurized gas supplies and at least one controller in communication with the power and/or pressurized gas supplies, wherein each nanoscale manifold is controlled by a separate voltage and/or pressure input and are addressed individually, wherein a single pressure and/or voltage input to a respective nanoscale manifold feeds only its respective manifold elements, and wherein each primary transport nanochannel has voltage and/or pressure inputs that are separate from a respective nanoscale manifold inputs.

19. An analysis system, comprising:

(a) a fluidic analysis device comprising:

at least one fluidic transport nanochannel;

at least one nanoscale manifold having an array of nanoscale elements, wherein at least some of the nanoscale elements interface directly with the at least one fluidic transport nanochannel;

(b) a first power and/or first pressurized gas supply in communication with a single one of the at least one nanoscale manifolds to feed voltage and/or pressure to its respective nanoscale elements so that the nanoscale manifold applies a force gradient on an analyte in the transport nanochannel; and

(c) at least one additional pressurized gas supply and/or power supply in communication with the transport nanochannel proximate an entrance and exit end portion thereof,

wherein voltages and/or pressures at the entrance and exit of the transport nanochannel and at each respective nanoscale manifold are independently controlled.

20. The system of Claim 19, further comprising a controller in communication with the power and/or pressurized gas supply to independently control the voltages and/or pressures, wherein the at least one nanoscale manifold includes first and second spaced apart nanoscale manifolds that both interface with at least one of the at least one fluidic transport

nanochannels, and wherein each nanoscale manifold has an independent pressurized gas supply and/or voltage input whereby each nanoscale manifold is independently addressable.

21. A method of controlling the transport of molecules within a fluid for the purposes of analysis, comprising:

providing a fluidic analysis device, the analysis device having at least one primary transport nanochannel and at least one nanoscale manifold with a plurality of nanoscale elements that intersect or interface with at least one primary transport channel; and

automatically concurrently applying a single pressure and/or single voltage input to multiple nanoscale elements of a respective nanoscale manifold to generate a force profile on the at least one transport nanochannel to carry out at least one of: accelerate, decelerate, compress, stretch, collect/separate or trap/concentrate an analyte as the analyte flows through the at least one primary transport nanochannel proximate the nanoscale manifold.

22. The method of Claim 21, wherein the at least one nanoscale manifold is a plurality of nanoscale manifolds, each interfacing with at least one of the at least one transport nanochannel, and wherein each nanoscale manifold is independently controlled to apply a force gradient to a respective transport nanochannel.

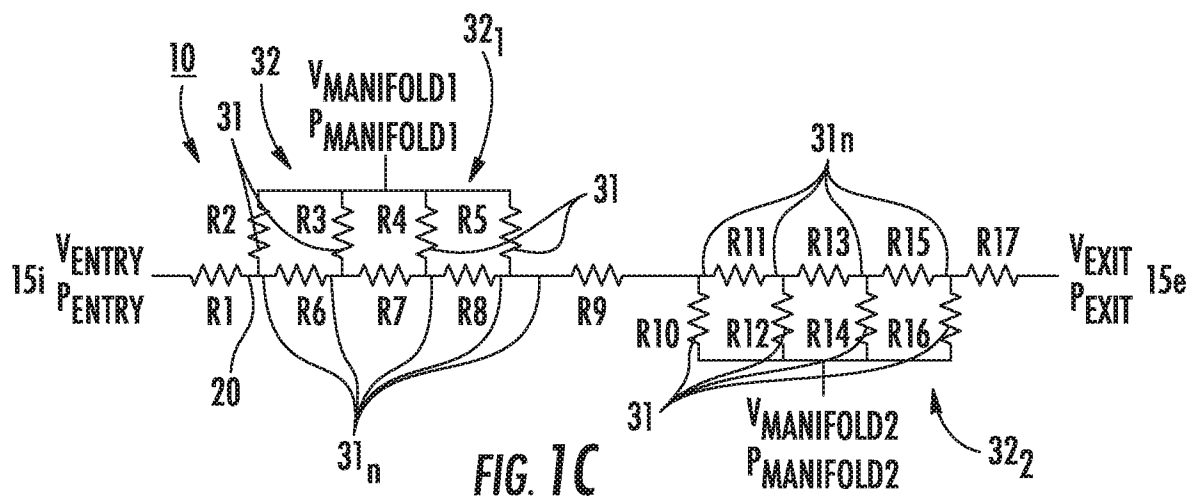
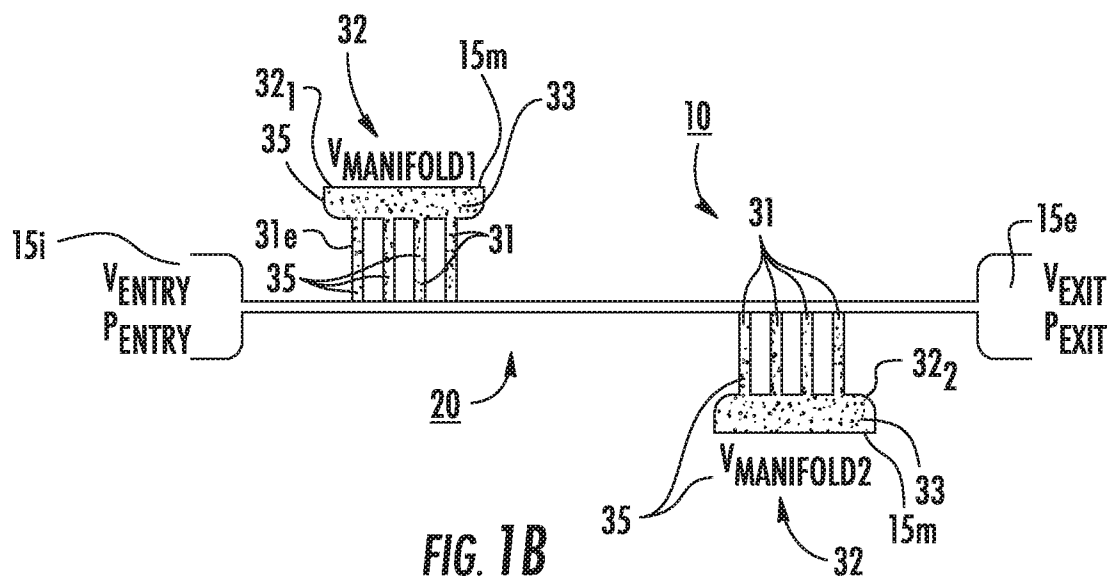
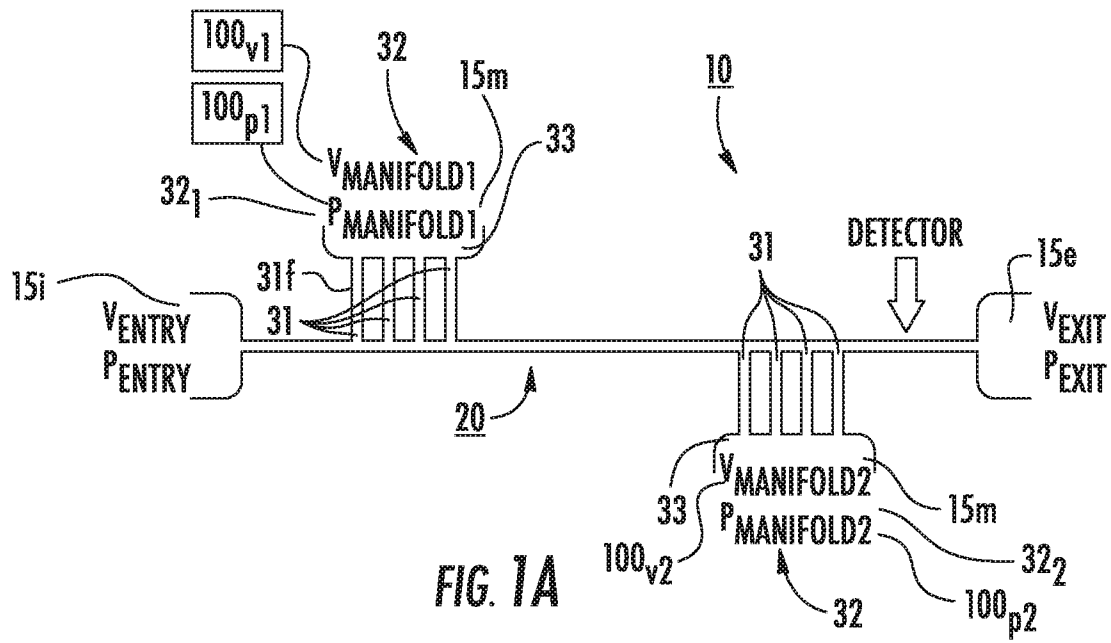
23. The method of Claim 21, wherein the nanoscale manifold elements are fluidic nanoscale elements.

24. The method of Claim 21, wherein the nanoscale elements comprise nanoelectrodes.

25. The method of Claim 21, further comprising controlling molecular transport in the at least one transport nanochannel by selectively dynamically changing force gradients applied by the nanoscale magnitude.

26. The method of Claim 25, wherein the controlling comprises changing the force gradient in one or more of magnitude, shape, and polarity using a single external voltage input and/or a single pressure input to each of the plurality of nanoscale manifolds.

27. The method of Claim 25, wherein the controlling comprises varying a single external voltage input and/or a single pressure input to each of a plurality of nanoscale manifolds in a time-dependent manner such as stepping to different voltages or pressures, transiently pulsing to different voltages or pressures, continuously ramping at various linear or non-linear rates, or varying according to a sinusoidal or other wave function.



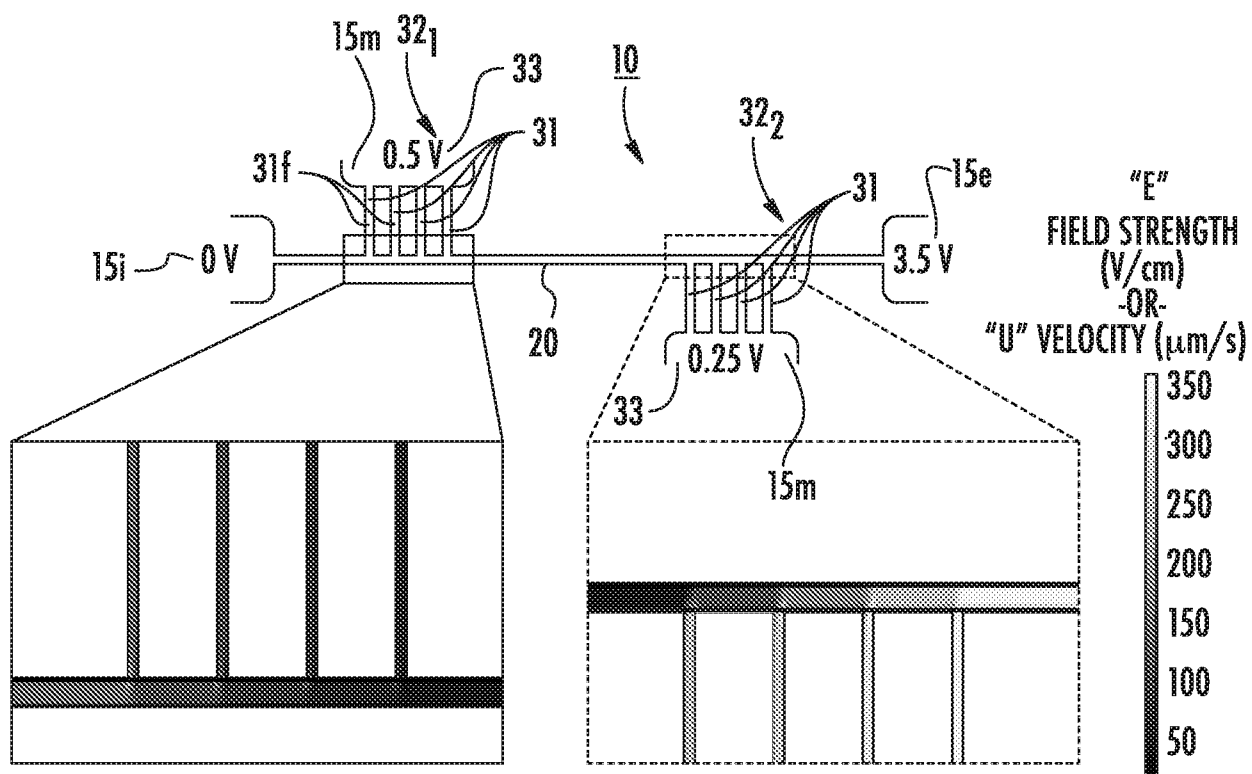


FIG. 2A

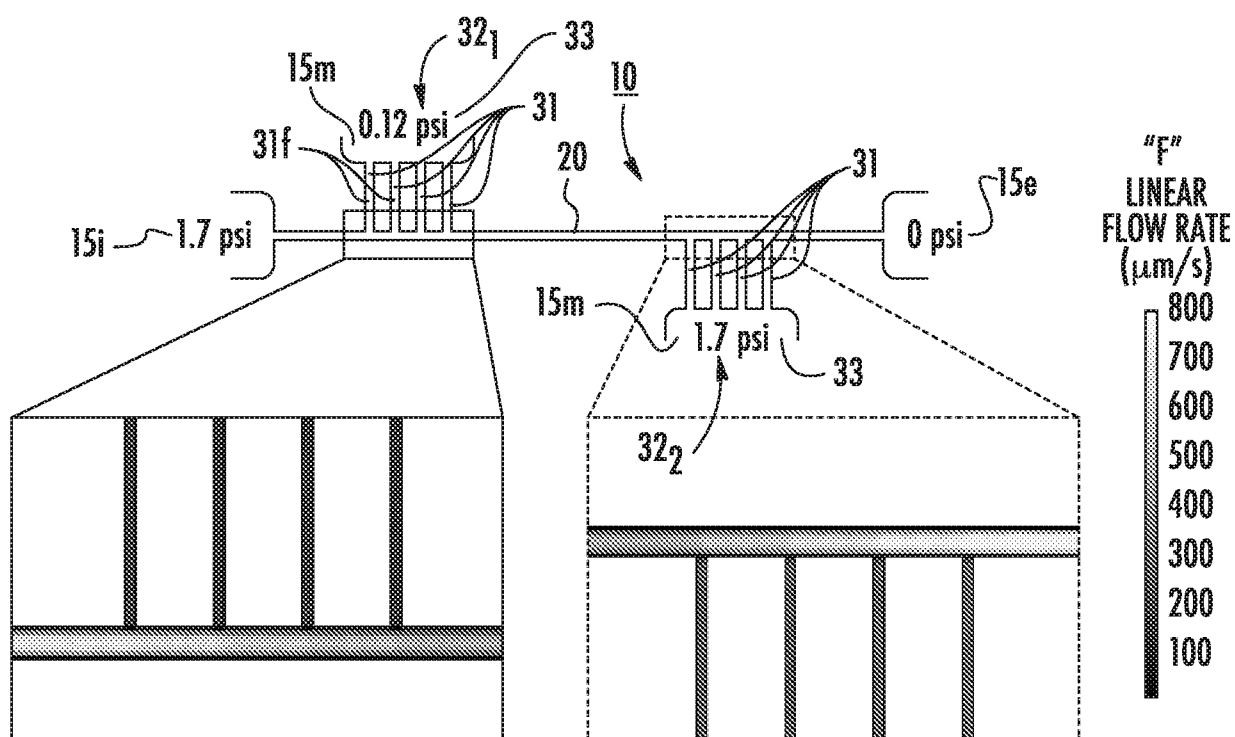
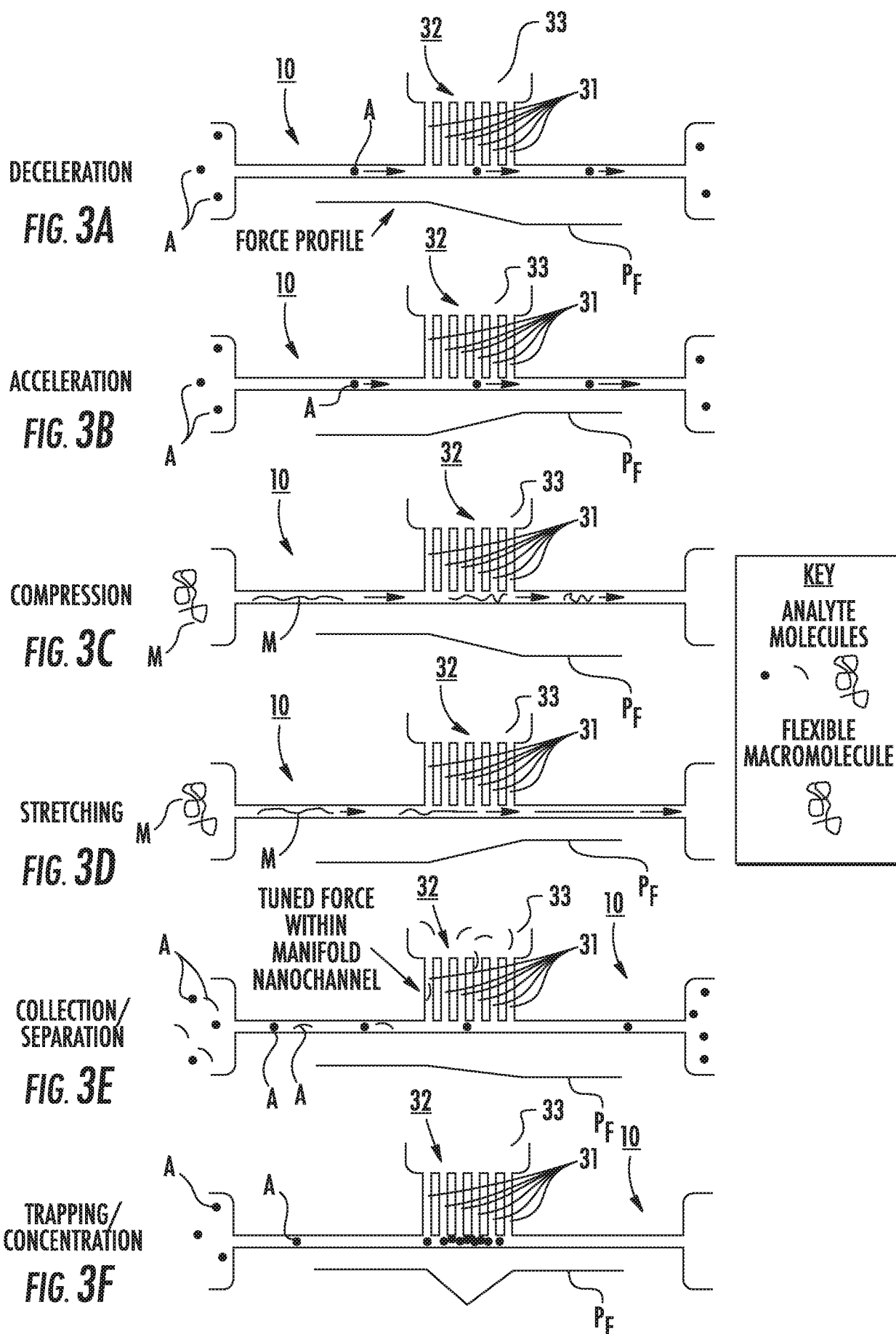
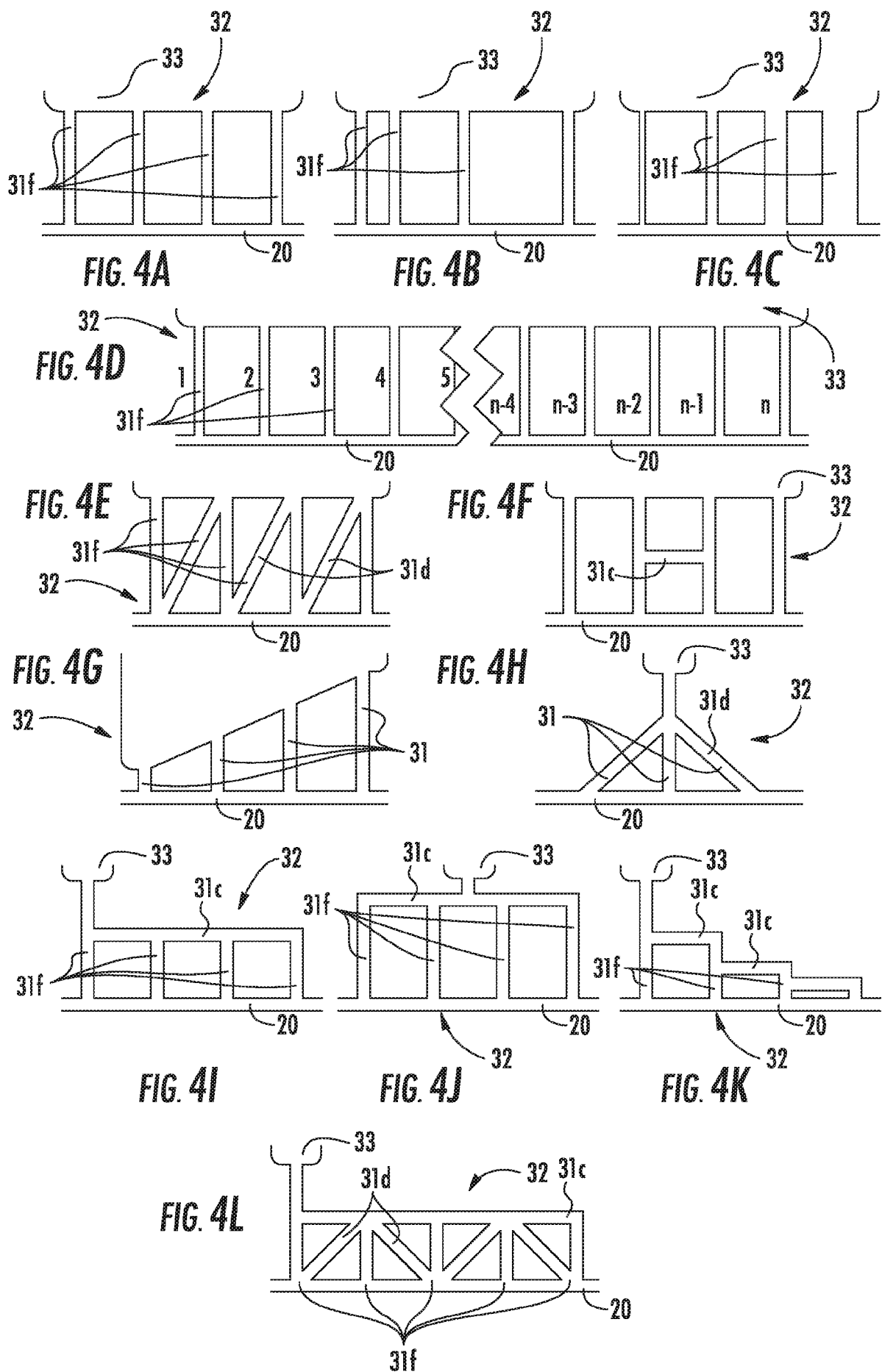
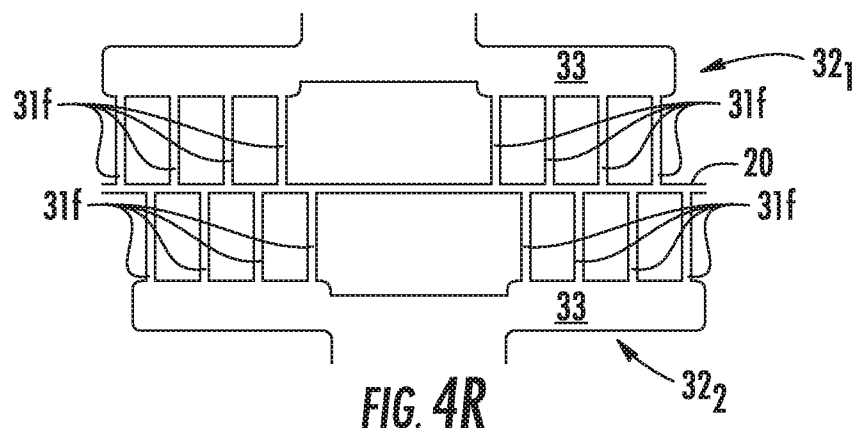
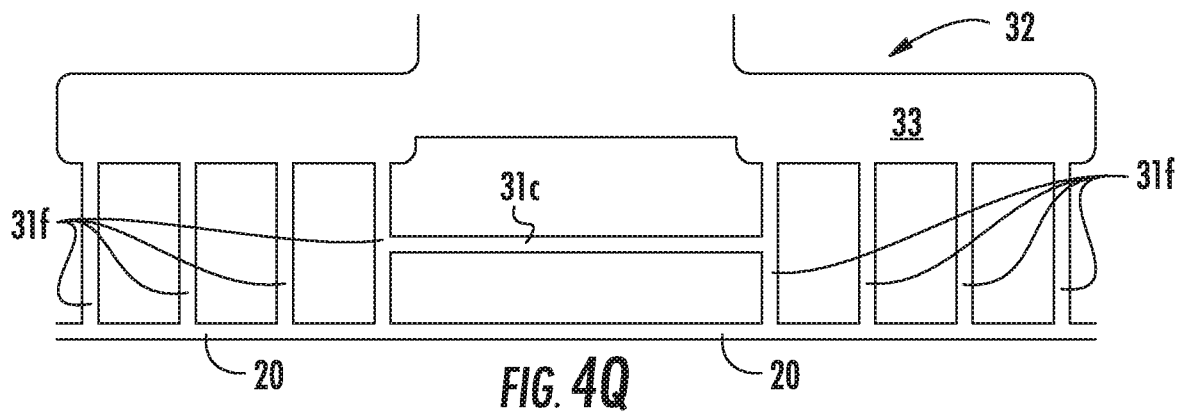
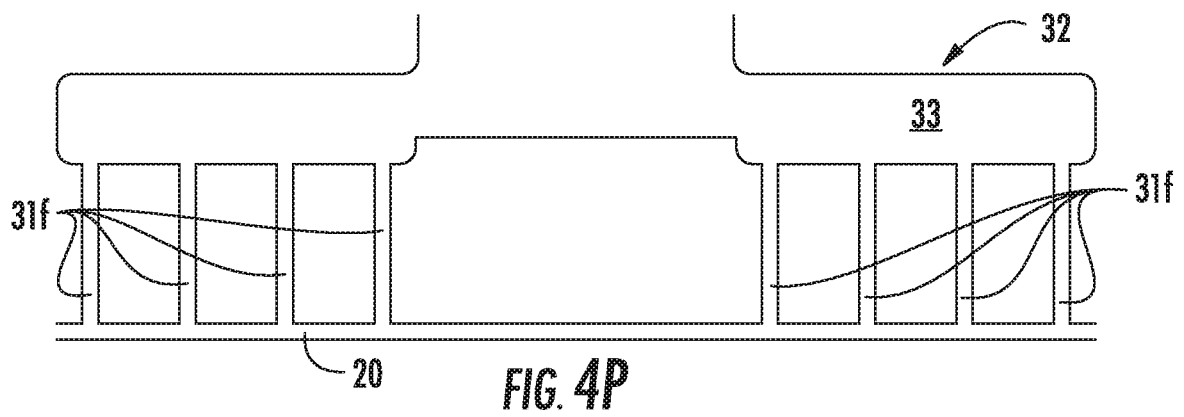
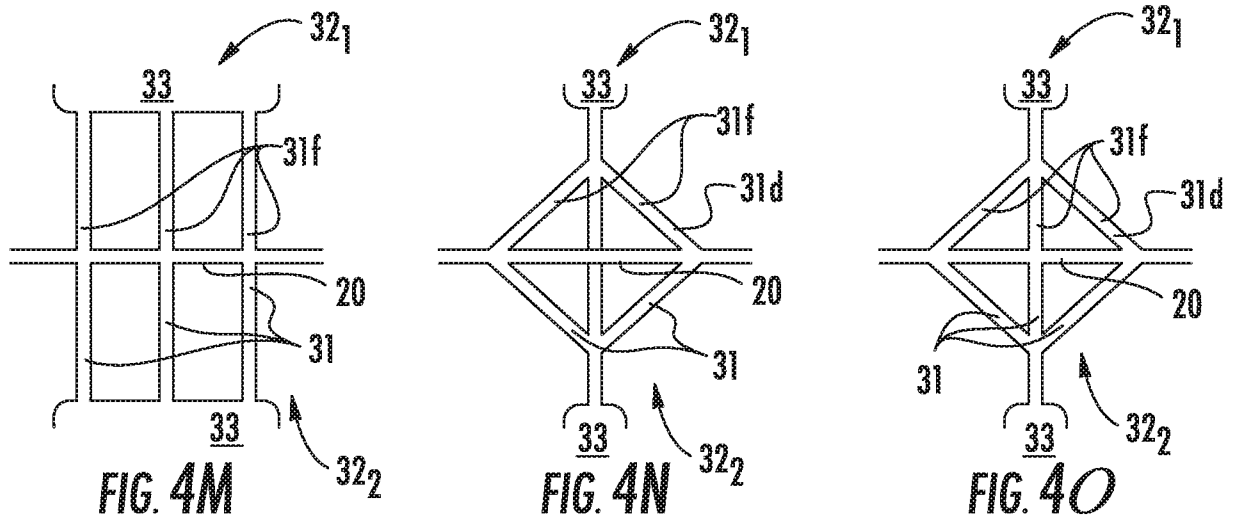
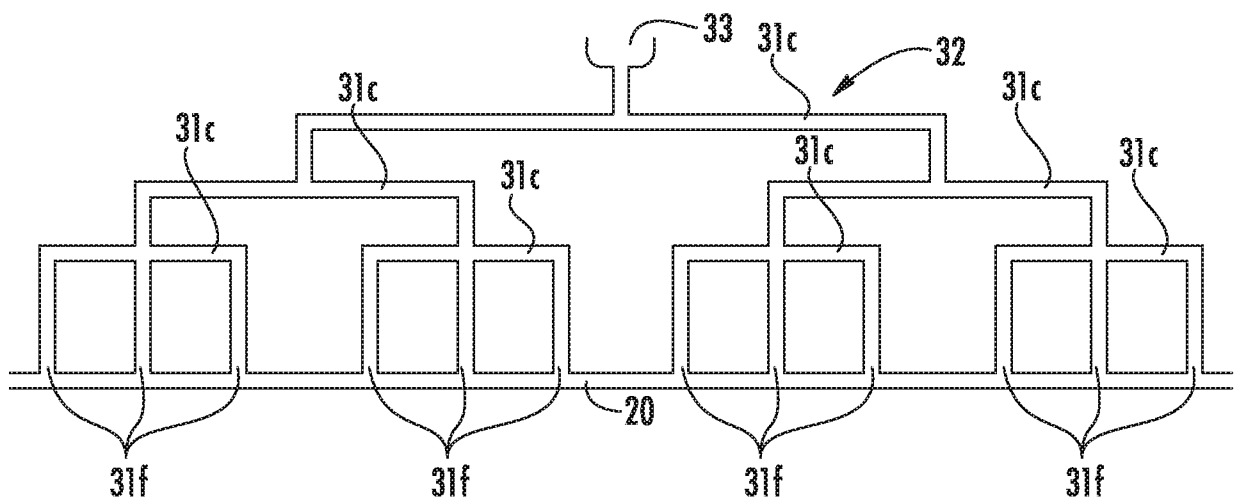
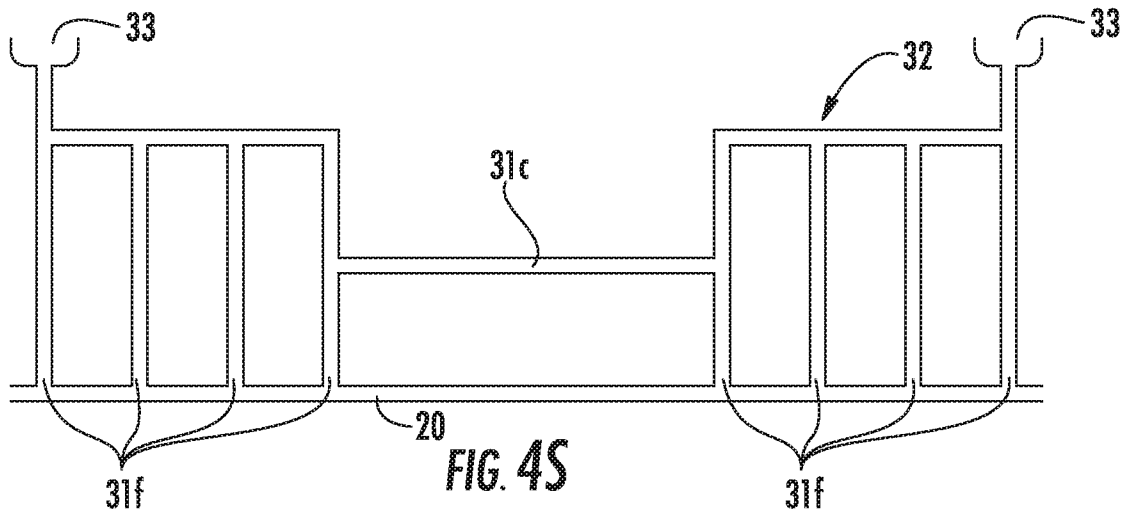


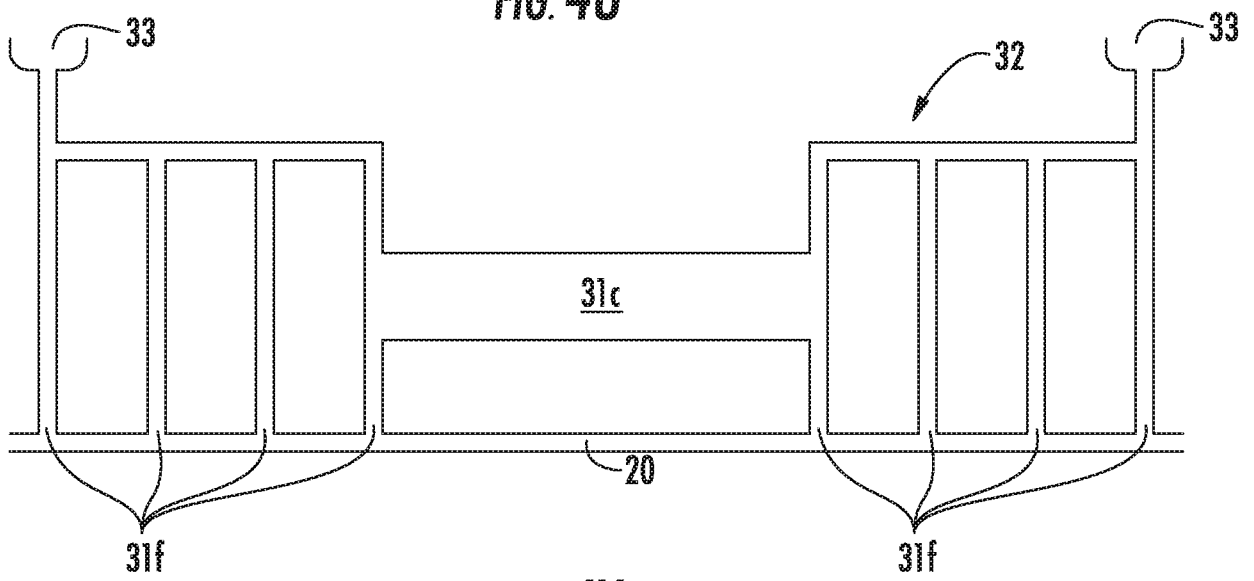
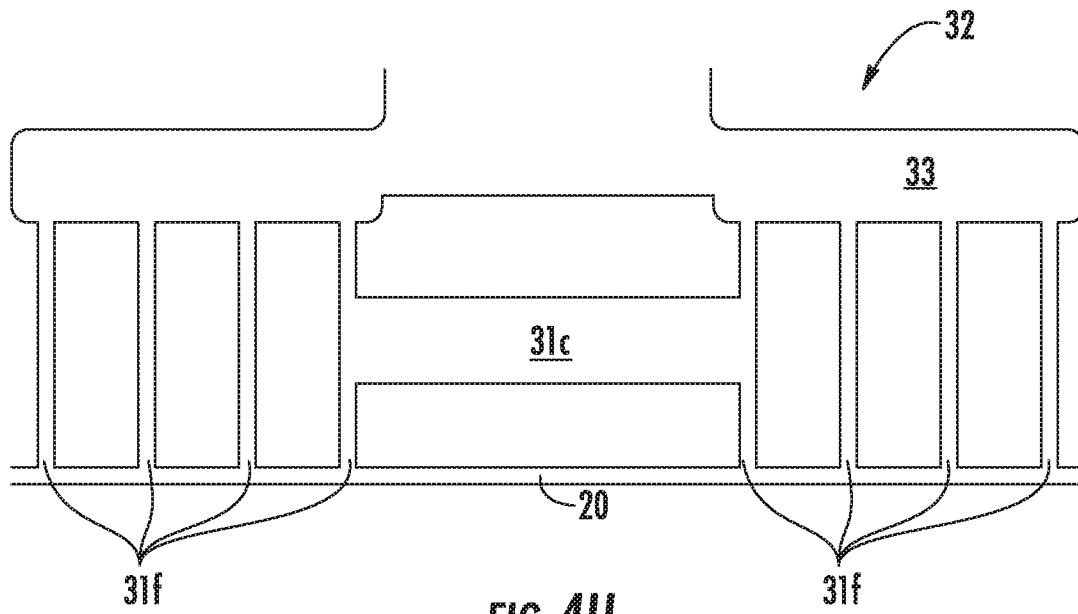
FIG. 2B











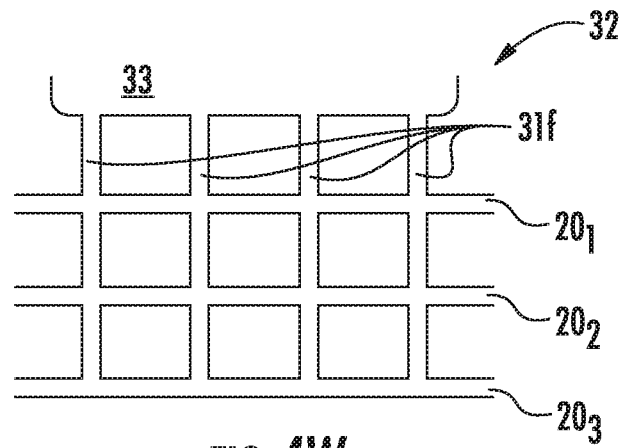


FIG. 4W

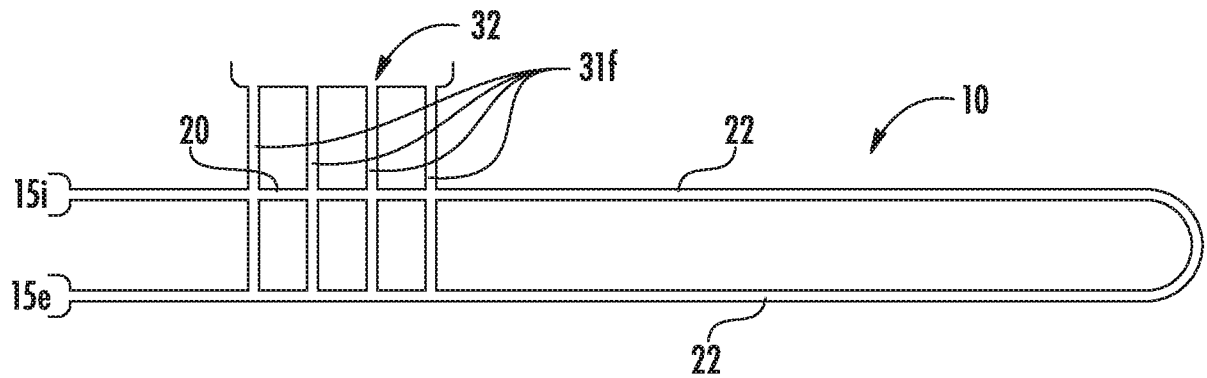


FIG. 4X

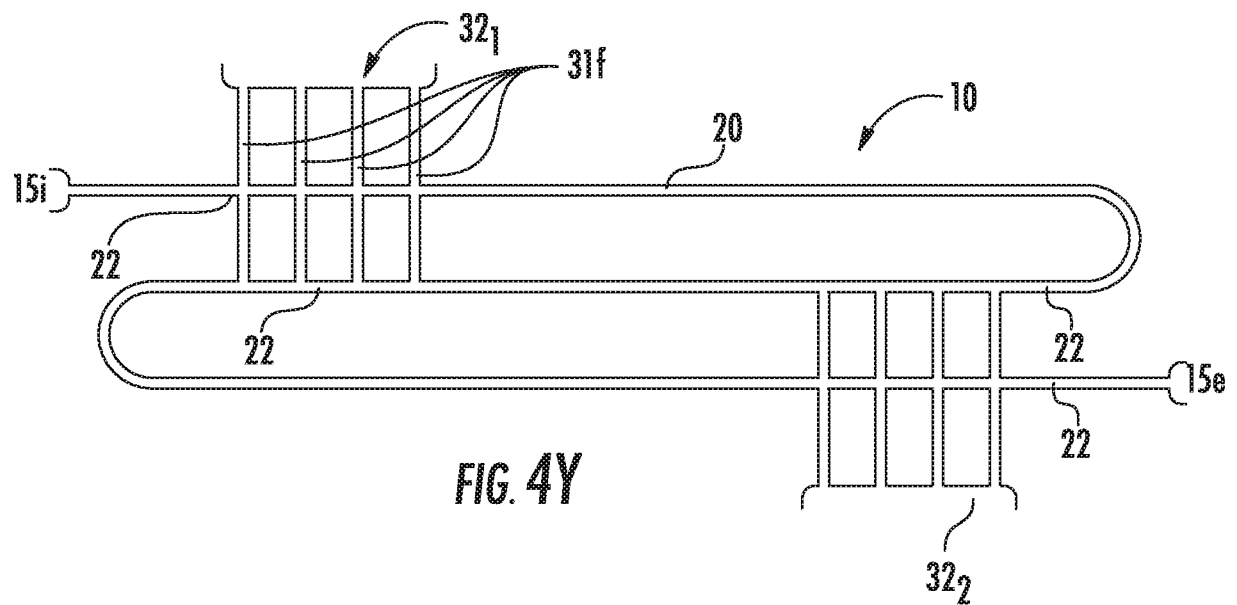
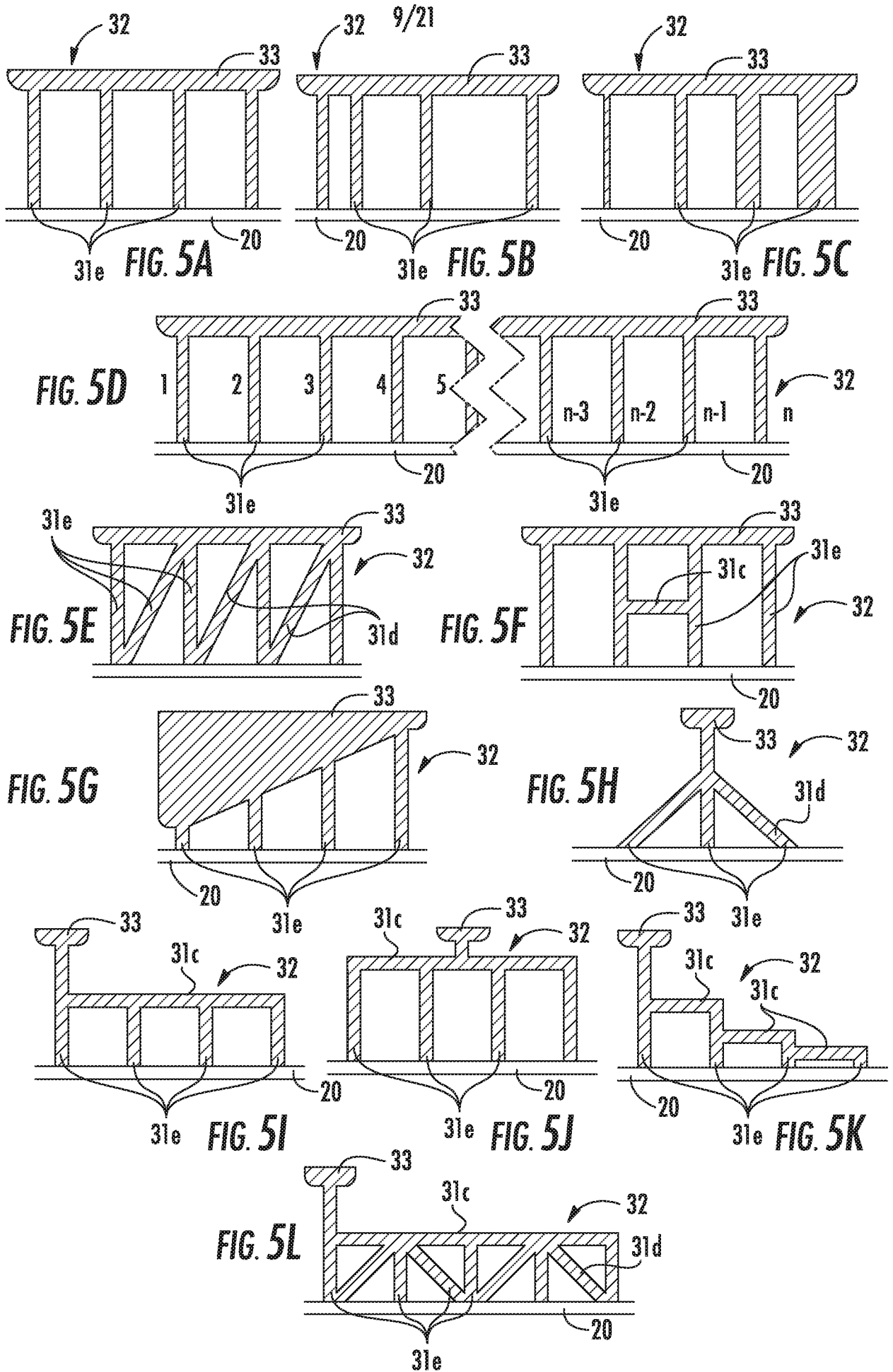


FIG. 4Y



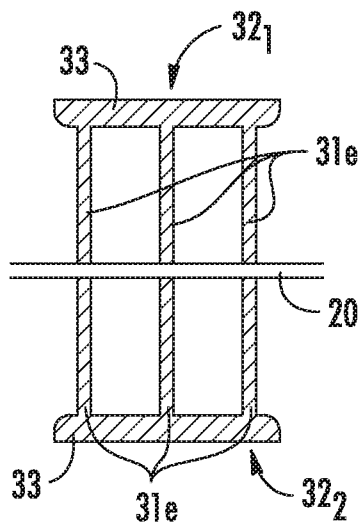


FIG. 5M

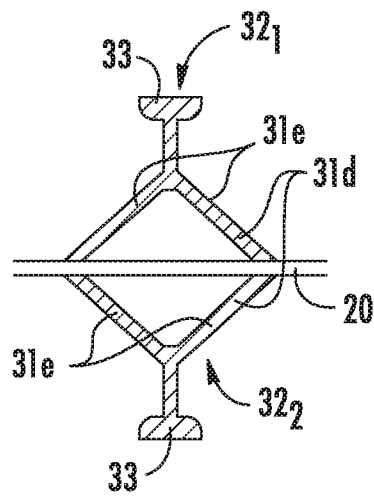


FIG. 5N

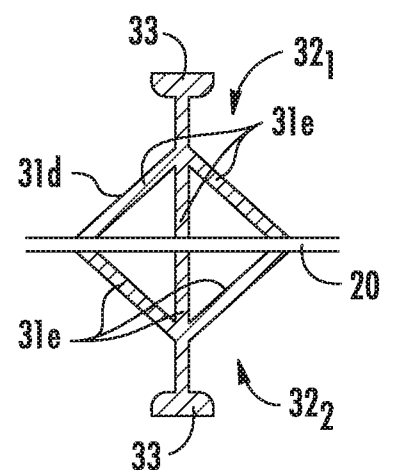


FIG. 5O

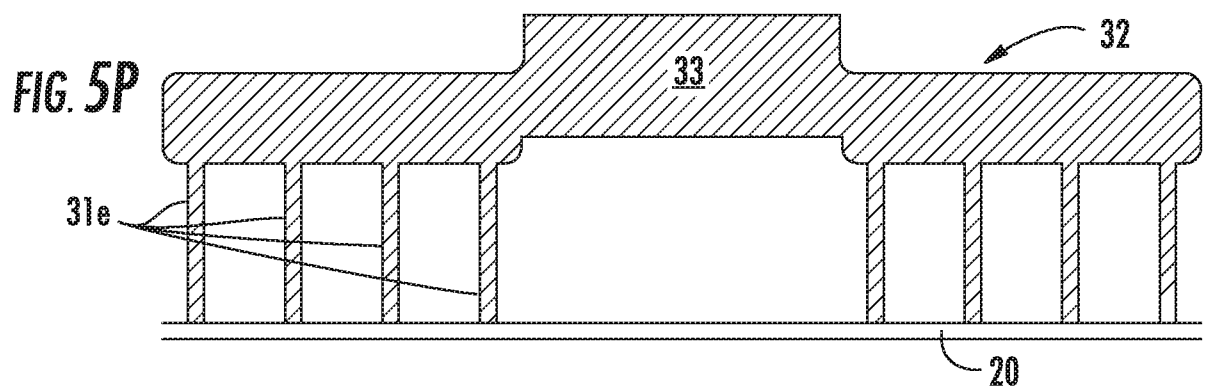


FIG. 5Q

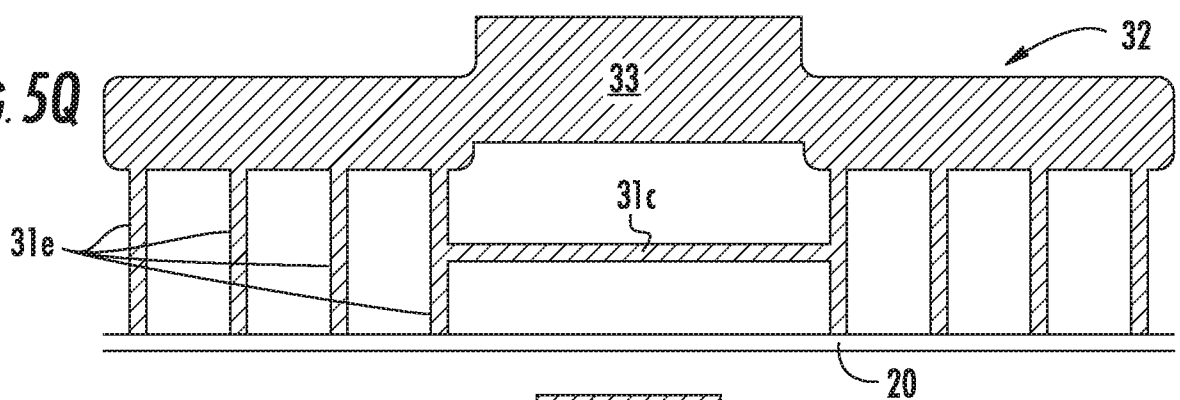
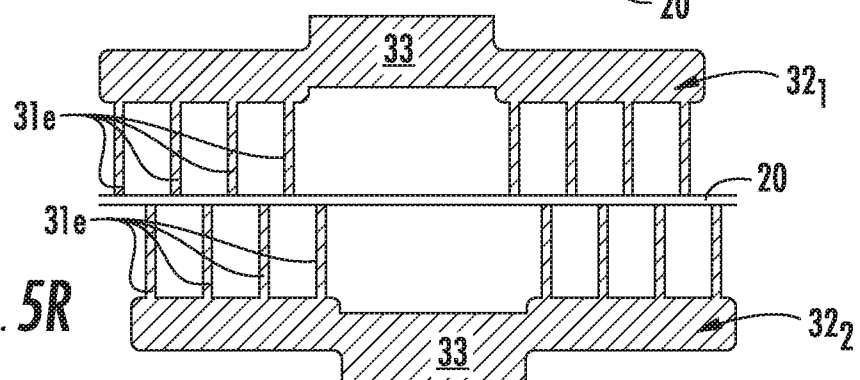
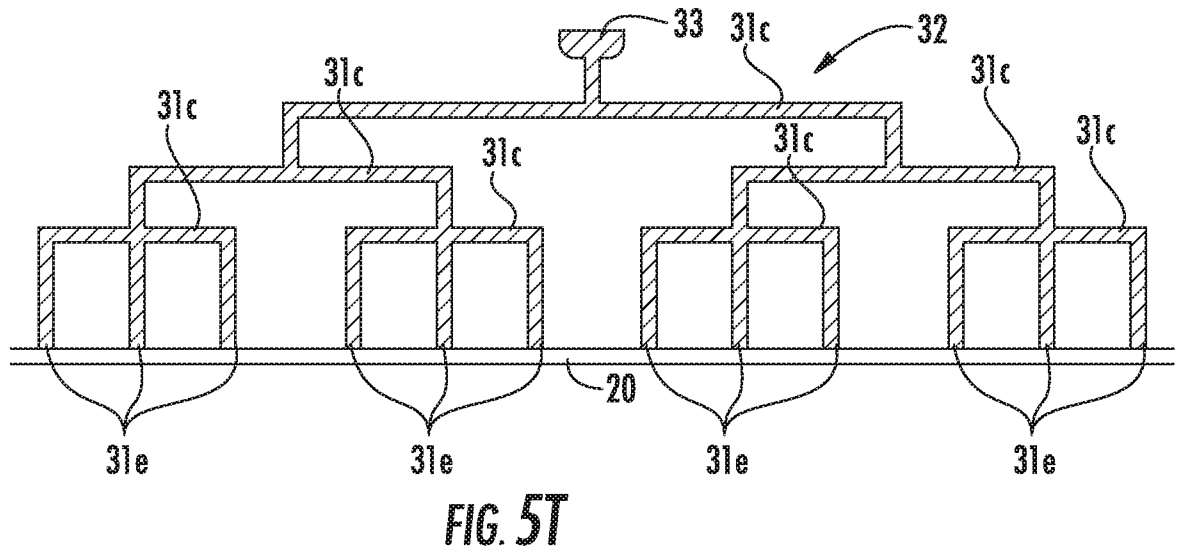
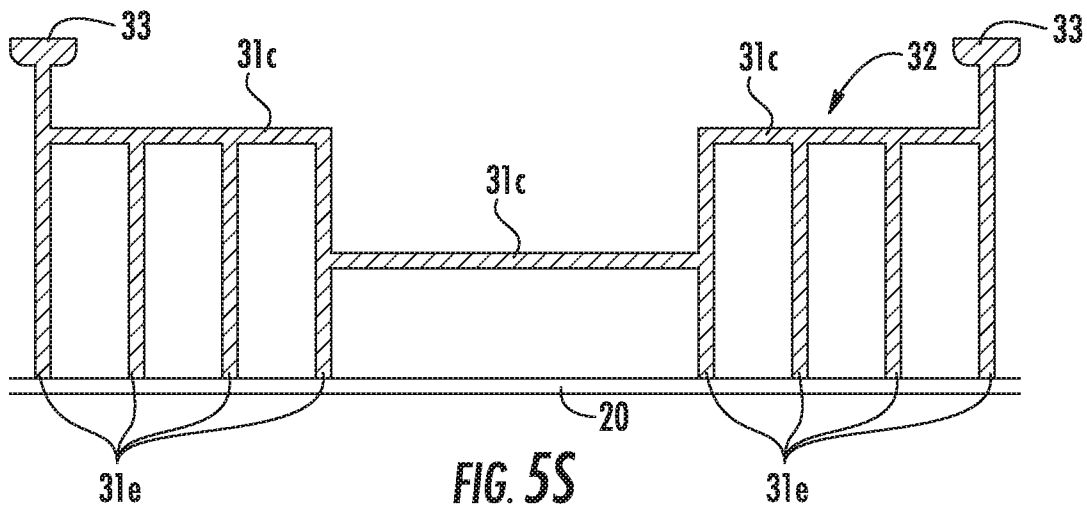
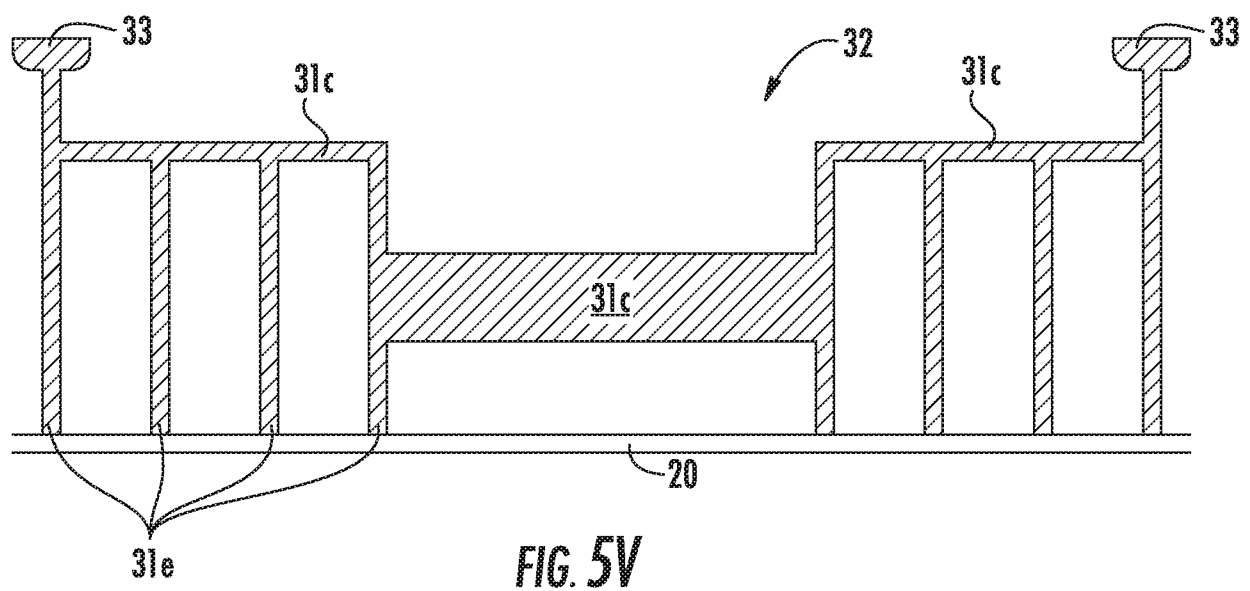
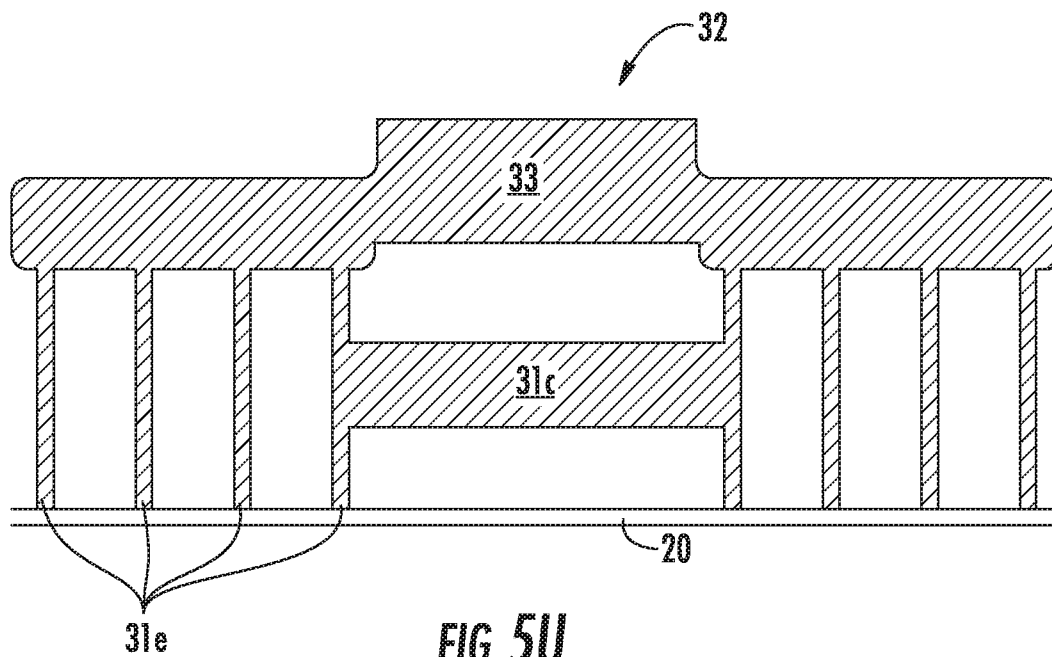
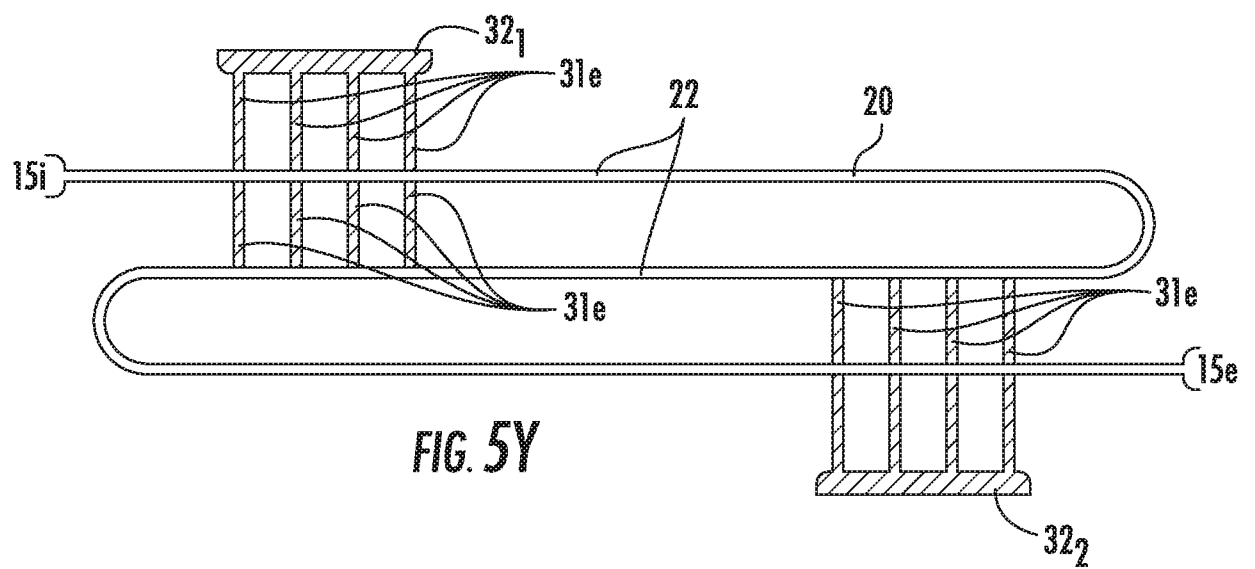
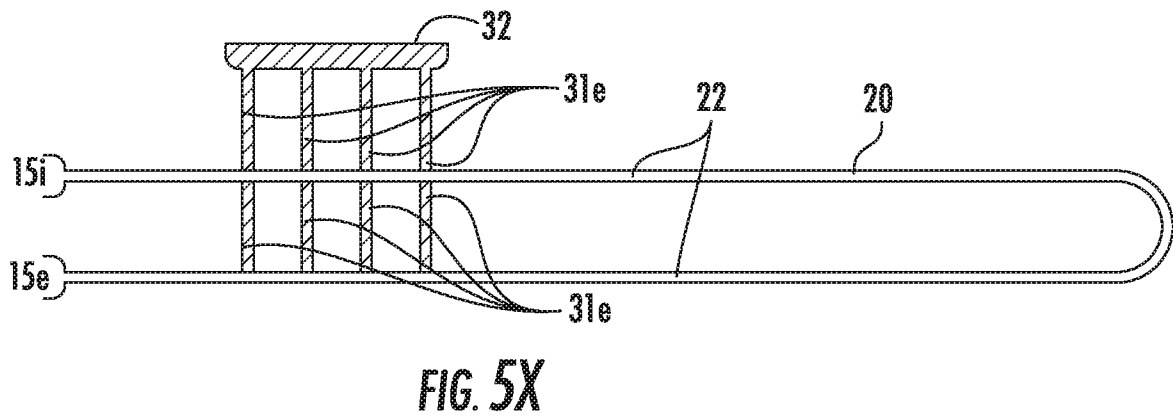
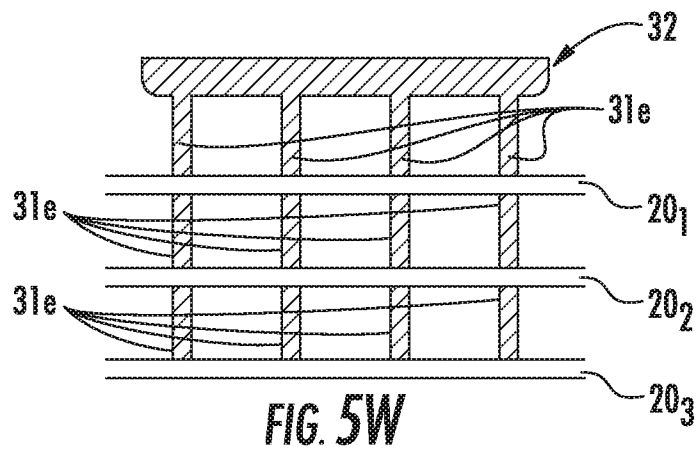


FIG. 5R









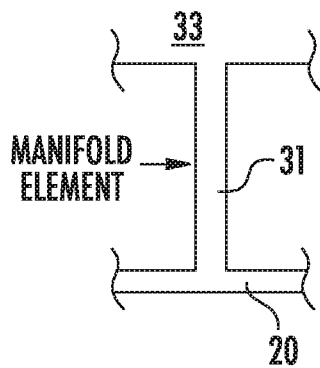


FIG. 6A

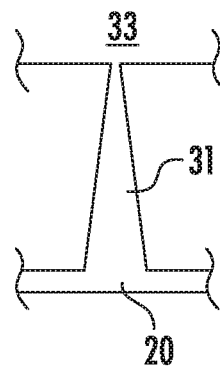


FIG. 6B

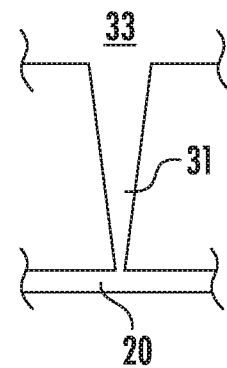


FIG. 6C

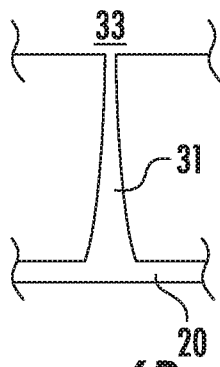


FIG. 6D

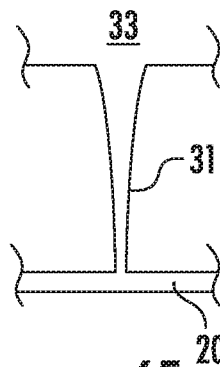


FIG. 6E

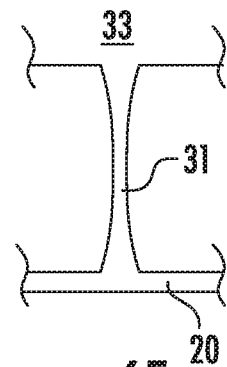


FIG. 6F

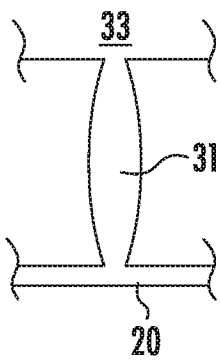
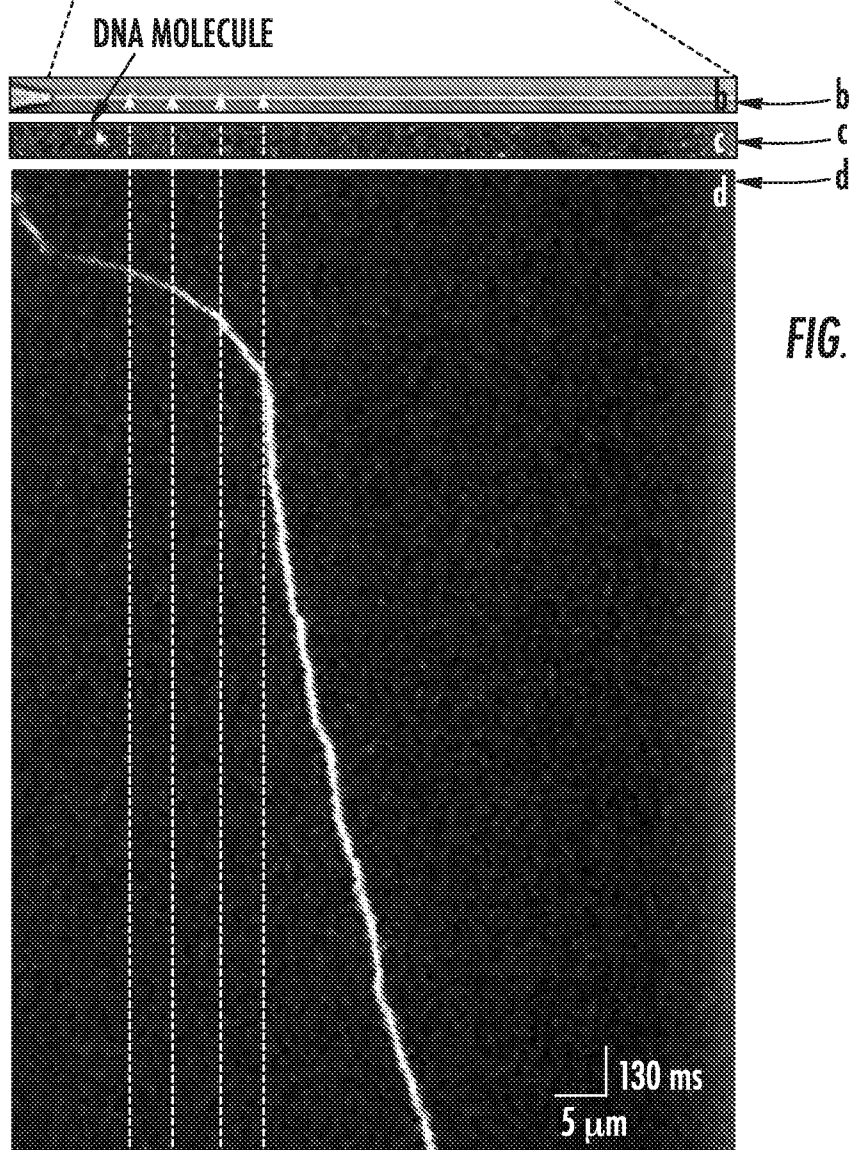
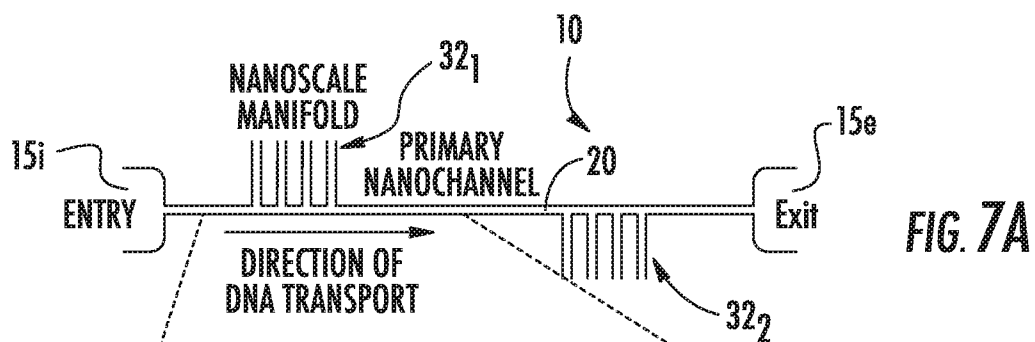


FIG. 6G



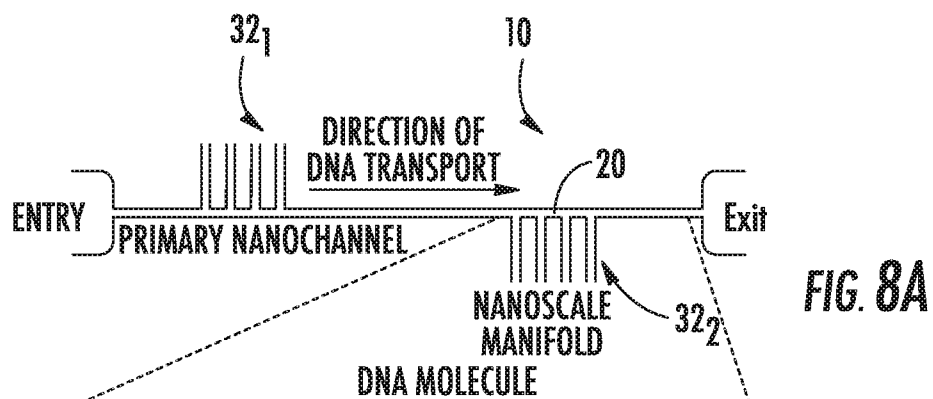


FIG. 8A

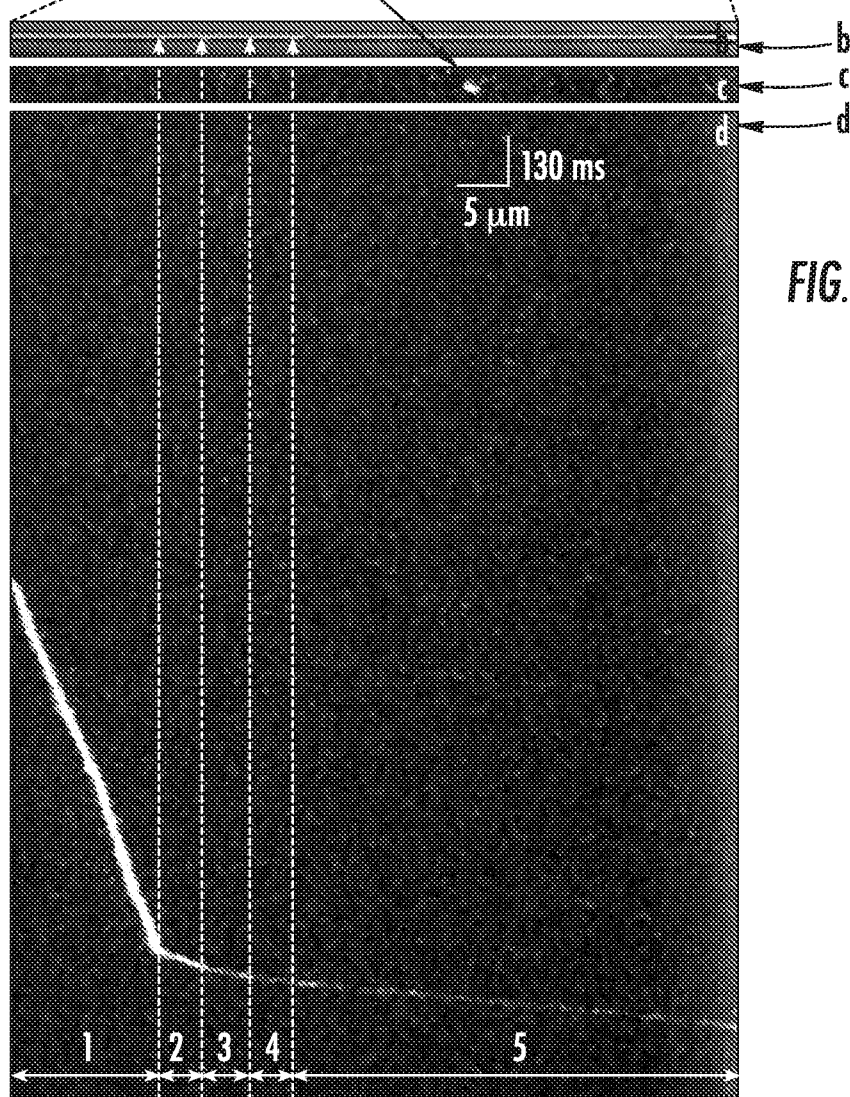


FIG. 8B

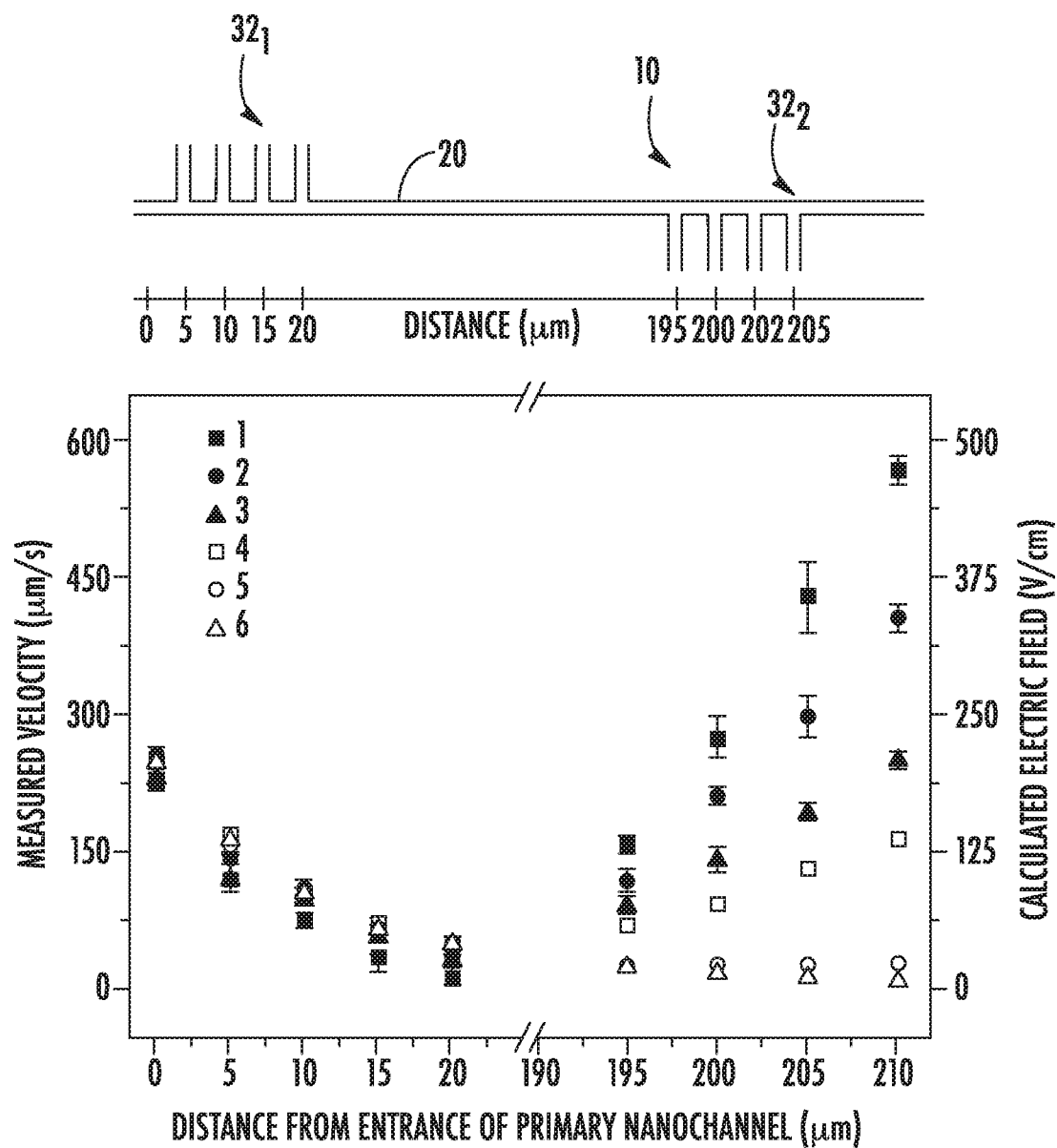


FIG. 9

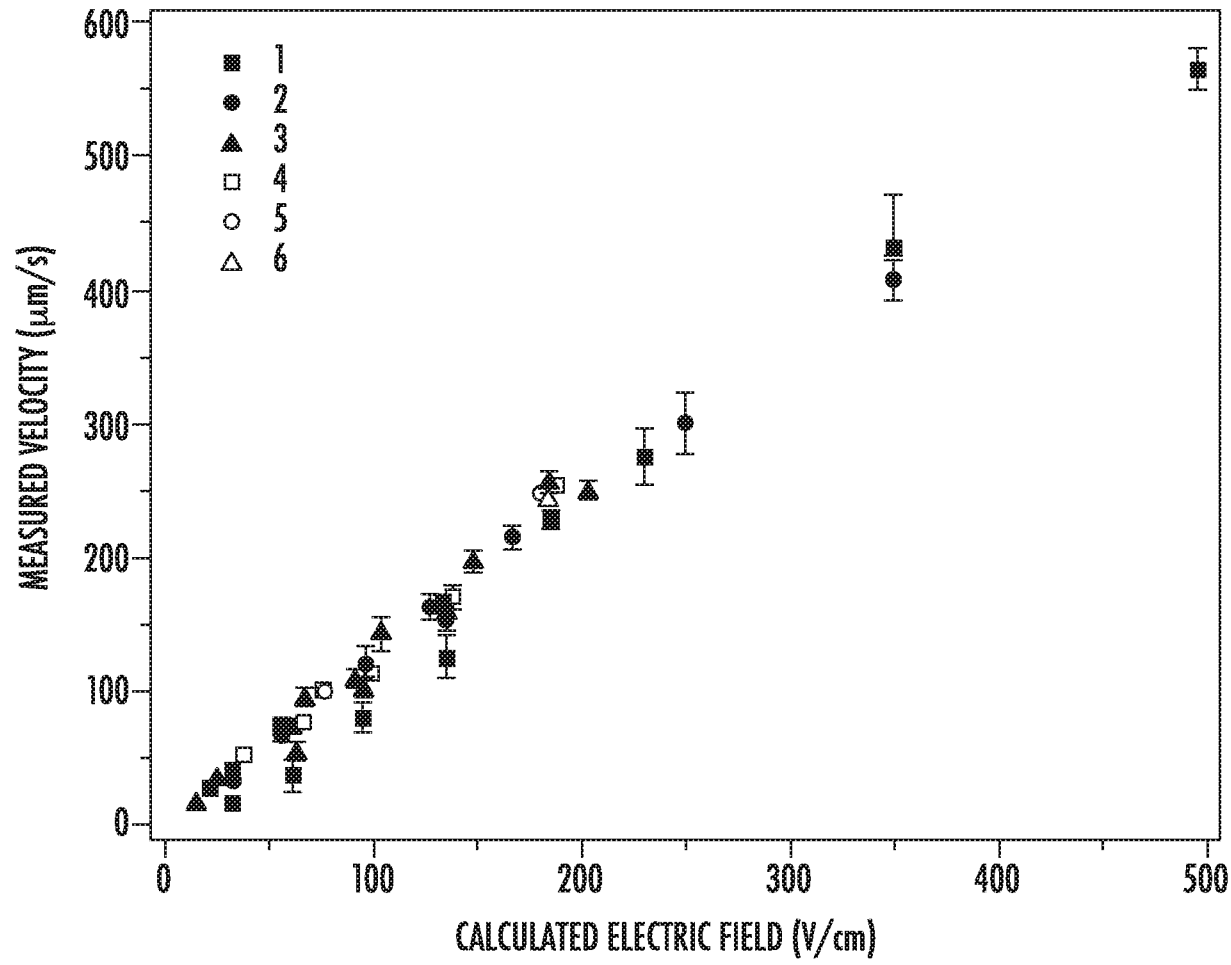


FIG. 10

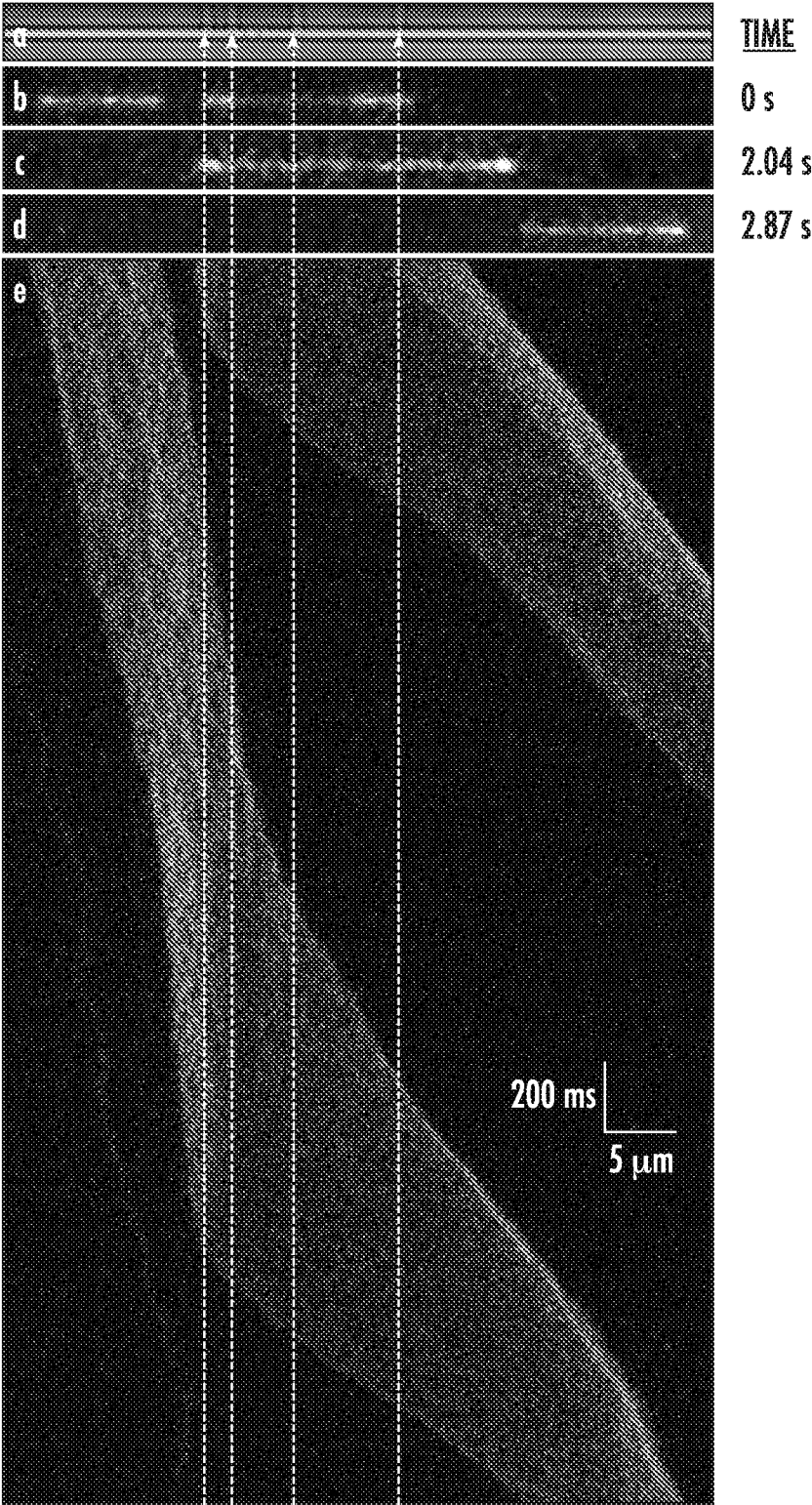


FIG. 11

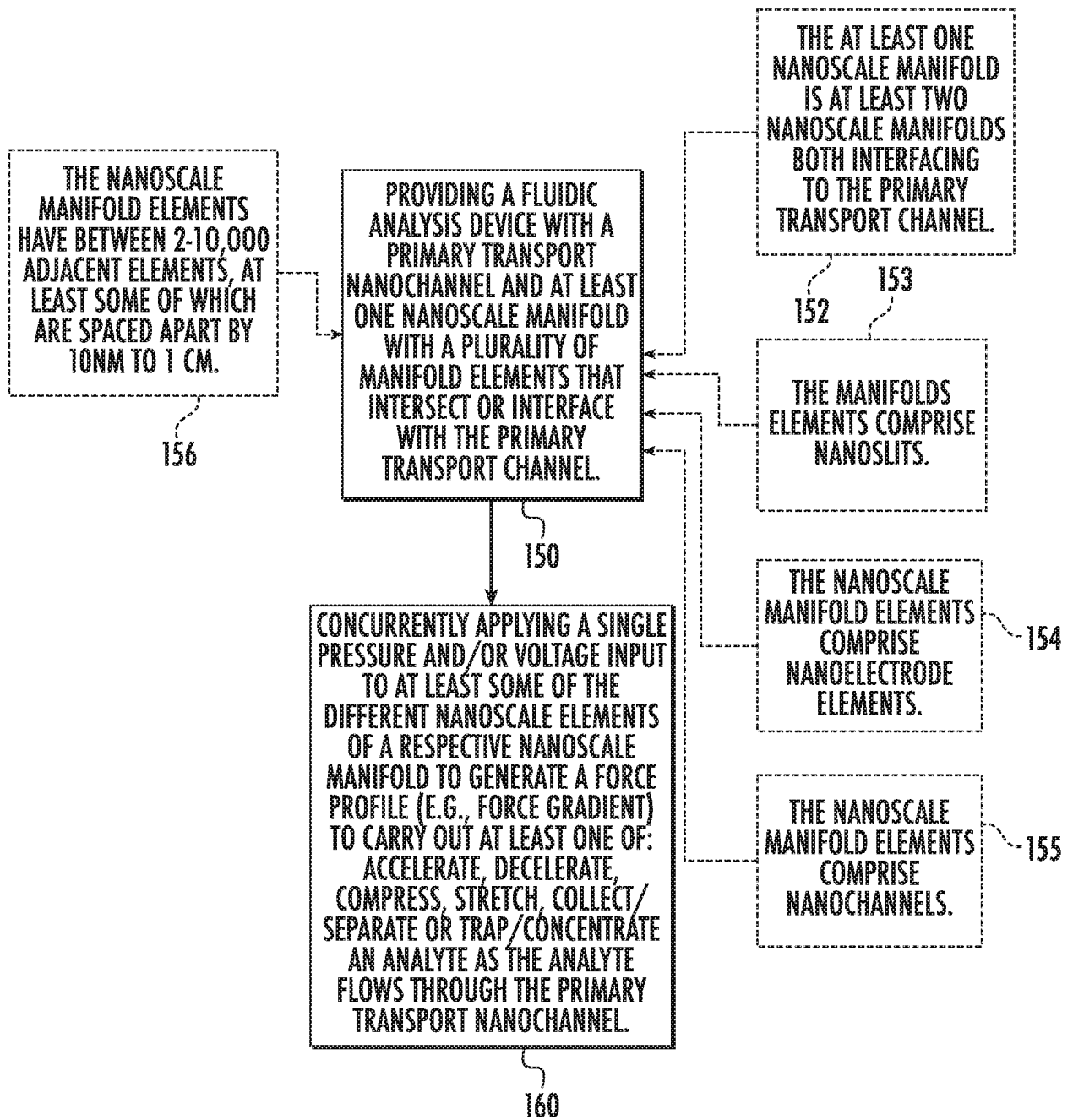
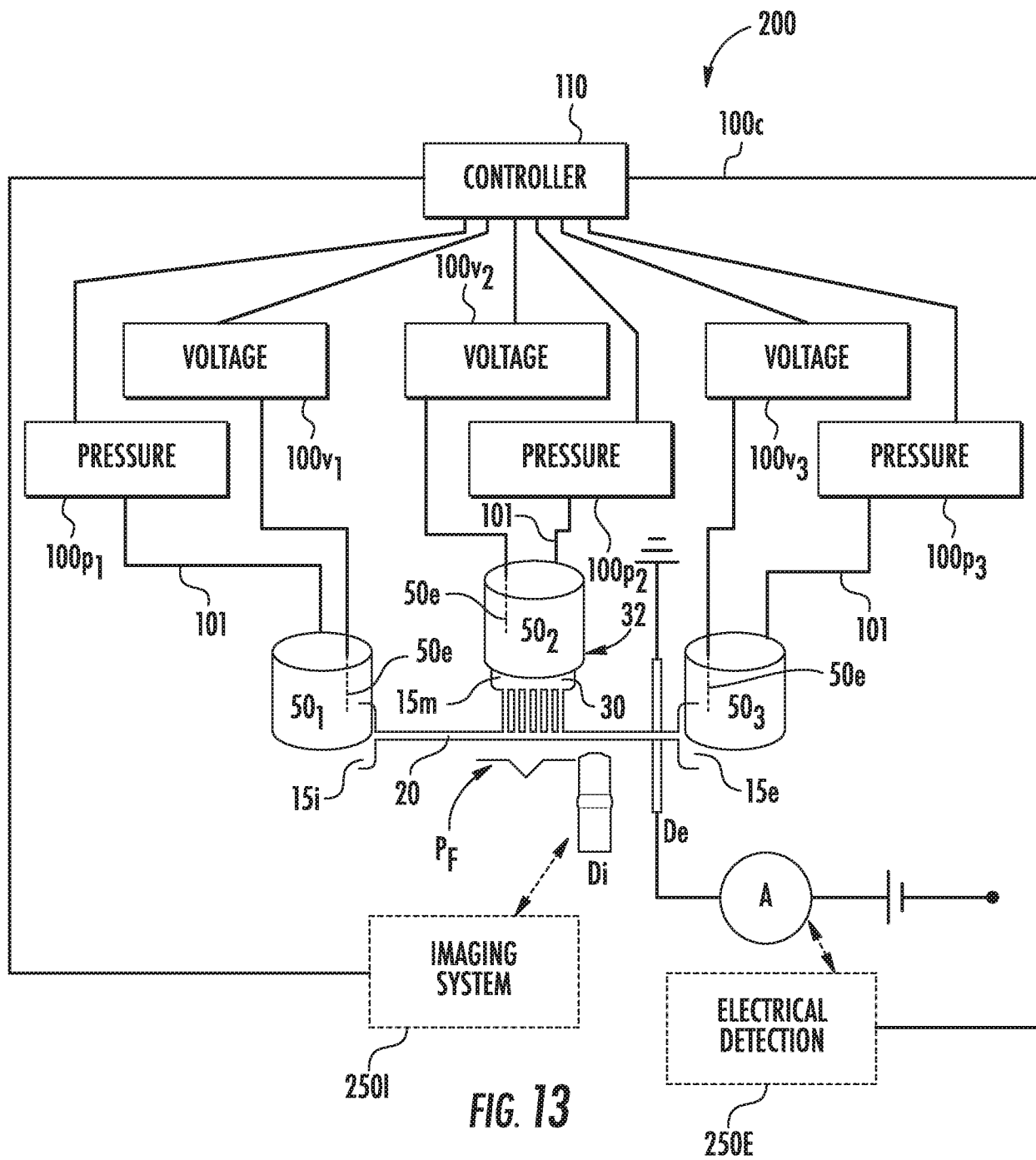


FIG. 12



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/030702**A. CLASSIFICATION OF SUBJECT MATTER****G01N 1/18(2006.01)i, G01N 11/04(2006.01)i, G01N 30/38(2006.01)i**

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)

G01N 1/18; G01N 27/447; G01N 33/58; C25B 15/00; A61M 1/16; C12Q 1/68; G01N 11/04; G01N 30/38

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: nanochannel, manifold, pressure, voltage, force profile and gradient

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014-0238856 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 28 August 2014 See abstract, paragraphs [0060]-[0079], [0105]-[0113], claims 1-12 and figures 1-2B, 10A-12.	1-10, 12-16, 18-27
Y		11, 17
Y	US 2014-0249039 A1 (BIONANO GENOMICS, INC.) 04 September 2014 See abstract and figure 11.	11, 17
A	US 2014-0197105 A1 (THE CHARLES STARK DRAPER LABORATORY, INC.) 17 July 2014 See abstract and figure 1A.	1-27
A	US 2015-0008124 A1 (NABSYS, INC.) 08 January 2015 See abstract and figure 1.	1-27
A	US 2013-0068618 A1 (HARRER et al.) 21 March 2013 See abstract and figure 1.	1-27

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

16 August 2016 (16.08.2016)

Date of mailing of the international search report

17 August 2016 (17.08.2016)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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Information on patent family members

International application No.

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		WO 2013-037240 A1	21/03/2013