

US Patent & Trademark Office

Patent Public Search | Text View

United States Patent	12390810
Kind Code	B2
Date of Patent	August 19, 2025
Inventor(s)	Heller; Michael J. et al.

Electrokinetic microelectrode devices and methods for biomarker analysis

Abstract

Disclosed are miniaturized electronic systems, devices and methods for biomarker analysis, which can be incorporated into blood collection tubes and other containers that enable the immediate isolation, concentration, analysis and storage of disease related biomarkers upon blood draw. In some aspects, a miniaturized electronic system includes a high-surface area folded or sandwiched electrokinetic microelectrode array chip device that allows both AC dielectrophoretic (DEP) and DC electrophoretic based separation and isolation and other processes to be used for the concentration and biomarkers.

Inventors:	Heller; Michael J. (Portland, OR), Heineck; Daniel (Tigard, OR), Ibsen; Stuart D. (Portland, OR), Esener; Sadik (Portland, OR), Lewis; Jean M. (San Diego, CA)
Applicant:	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA (Oakland, CA)
Family ID:	1000008764442
Appl. No.:	17/753176
Filed (or PCT Filed):	August 21, 2020
PCT No.:	PCT/US2020/047519
PCT Pub. No.:	WO2021/041259
PCT Pub. Date:	March 04, 2021

Prior Publication Data

Document Identifier	Publication Date
US 20220274111 A1	Sep. 01, 2022

Related U.S. Application Data

us-provisional-application US 62891204 20190823

Publication Classification

Int. Cl.: **B01L3/00** (20060101); **A61B5/157** (20060101); **B03C5/00** (20060101); **B03C5/02** (20060101); **C12Q1/686** (20180101); **G01N27/447** (20060101)

U.S. Cl.:

CPC **B01L3/502761** (20130101); **A61B5/157** (20130101); **B01L3/502715** (20130101); **B03C5/005** (20130101); **B03C5/02** (20130101); **G01N27/44721** (20130101); **G01N27/44791** (20130101); B01L2200/0647 (20130101); B01L2300/0636 (20130101); B01L2300/0645 (20130101); B01L2400/0415 (20130101)

Field of Classification Search

CPC: B01L (2300/0636); B01L (2400/0415); G01N (27/44721); C12Q (1/686)

References Cited

U.S. PATENT DOCUMENTS

Patent No.	Issued Date	Patentee Name	U.S. Cl.	CPC
5849486	12/1997	Heller et al.	N/A	N/A
5929208	12/1998	Heller et al.	N/A	N/A
6017696	12/1999	Heller	N/A	N/A
6048690	12/1999	Heller et al.	N/A	N/A
6051380	12/1999	Sosnowski et al.	N/A	N/A
6129828	12/1999	Sheldon et al.	N/A	N/A
6238624	12/2000	Heller et al.	N/A	N/A
6245508	12/2000	Heller et al.	N/A	N/A
6306348	12/2000	Havens et al.	N/A	N/A
6379897	12/2001	Weidenhammer et al.	N/A	N/A
6403367	12/2001	Cheng et al.	N/A	N/A
6488832	12/2001	Heller	N/A	N/A
6492122	12/2001	Weidenhammer et al.	N/A	N/A
6518022	12/2002	Sosnowski et al.	N/A	N/A
6569382	12/2002	Edman et al.	N/A	N/A
6582660	12/2002	Heller et al.	N/A	N/A
6652808	12/2002	Heller et al.	N/A	N/A
6682936	12/2003	Kovacs	N/A	N/A
6706473	12/2003	Edman et al.	N/A	N/A
6780584	12/2003	Edman et al.	N/A	N/A
6824740	12/2003	Sheldon et al.	N/A	N/A
7060224	12/2005	Edman et al.	N/A	N/A
7101661	12/2005	Heller et al.	N/A	N/A
7172864	12/2006	Heller et al.	N/A	N/A

7172896	12/2006	Cheng et al.	N/A	N/A
7300757	12/2006	Edman et al.	N/A	N/A
7314708	12/2007	Heller et al.	N/A	N/A
7704726	12/2009	Heller et al.	N/A	N/A
7857957	12/2009	Cheng et al.	N/A	N/A
7947486	12/2010	Heller et al.	N/A	N/A
8114589	12/2011	Sosnowski et al.	N/A	N/A
8202689	12/2011	Heller et al.	N/A	N/A
8313940	12/2011	Heller et al.	N/A	N/A
8389212	12/2012	Heller et al.	N/A	N/A
8603791	12/2012	Krishnan et al.	N/A	N/A
8815554	12/2013	Krishnan et al.	N/A	N/A
8815555	12/2013	Krishnan et al.	N/A	N/A
8871481	12/2013	Krishnan et al.	N/A	N/A
8877470	12/2013	Krishnan et al.	N/A	N/A
8932447	12/2014	Heller et al.	N/A	N/A
8932815	12/2014	Krishnan et al.	N/A	N/A
8969059	12/2014	Krishnan et al.	N/A	N/A
9005941	12/2014	Krishnan et al.	N/A	N/A
9034578	12/2014	Krishnan et al.	N/A	N/A
9034579	12/2014	Krishnan et al.	N/A	N/A
9206416	12/2014	Krishnan et al.	N/A	N/A
9387489	12/2015	Charlot et al.	N/A	N/A
9499812	12/2015	Krishnan et al.	N/A	N/A
9682385	12/2016	Charlot et al.	N/A	N/A
9827565	12/2016	Krishnan et al.	N/A	N/A
10006083	12/2017	Krishnan et al.	N/A	N/A
10232369	12/2018	Turner et al.	N/A	N/A
2008/0047832	12/2007	Cheng et al.	N/A	N/A
2019/0090791	12/2018	Siu et al.	N/A	N/A

FOREIGN PATENT DOCUMENTS

Patent No.	Application Date	Country	CPC
2008138006	12/2007	WO	N/A

OTHER PUBLICATIONS

Barrese, J.C. et al. "Scanning electron microscopy of chronically implanted intracortical microelectrode arrays in non-human primates", J. Neural Eng., vol. 13, No. 2, p. 026 003, Jan. 2016. cited by applicant

Barrett, A.N. et al. "Implementing prenatal diagnosis based on cell-free fetal DNA: Accurate identification of factors affecting fetal DNA yield", PLoS One, vol. 6, No. 10, e25202, 2011. cited by applicant

Benke, G. et al. "The electrochemical dissolution of platinum", Hydrometallurgy, vol. 64, No. 3, pp. 205-218, Jun. 2002. cited by applicant

Bentley, R. et al. "The alternating current electrolysis of concentrated acids", Journal of Applied Chemistry, vol. 7, No. 11, pp. 619-626, May 1957. cited by applicant

Ben-Yaakov, D. et al. "Dielectric decrement as a source of ion-specific effects", The Journal of Chemical Physics, vol. 134, No. 7, p. 074 705, 2011. cited by applicant

Bisceglia, E. et al. "A generic and label free method based on dielectrophoresis for the continuous separation of microorganism from whole blood samples", Sensors and Actuators B: Chemical, vol.

212, pp. 335-343, Jun. 2015. cited by applicant

Black, R.C. et al. "Dissolution of smooth platinum electrodes in biological fluids.", eng, Appl Neurophysiol, vol. 42, No. 6, pp. 366-374, 1980. cited by applicant

Briner, E. et al. "Recherches sur l'electrolyse avec courant ondule. III. observations sur l'attaque des electrodes de platine et la formation de l'ozone sous l'e et du courant alternatif et du courant ondule, a differentes frequences et a differentes concentrations des so", Helvetica Chimica Acta, vol. 26, No. 6, pp. 1829-1835, Oct. 1943. cited by applicant

Brown, M.A. et al. "Effect of electrolyte concentration on the stern layer thickness at a charged interface", Angewandte Chemie International Edition, vol. 55, No. 11, pp. 3790-3794, Feb. 2016. cited by applicant

Brown, S.A. et al. "Electrochemical corrosion in saline and serum" Journal of Biomedical Materials Research, vol. 14, No. 2, pp. 173-175, Mar. 1980. cited by applicant

Castellanos, A. et al. "Electro-hydrodynamics and dielectrophoresis in microsystems: Scaling laws", Journal of Physics D: Applied Physics, vol. 36, No. 20, p. 2584, 2003. cited by applicant

Castro, E.R. et al. "Present state of microchip electrophoresis: State of the art and routine applications", Journal of Chromatography A, vol. 1382, pp. 66-85, Feb. 2015. cited by applicant

Chen, D.F. et al. "Simulation studies on electrothermal fluid flow induced in a dielectrophoretic microelectrode system", Journal of Micromechanics and Microengineering, vol. 16, No. 11, p. 2411, 2006. cited by applicant

Cheng J, et al. "Preparation and Hybridization Analysis of DNA/RNA from *E. coli* on Microfabricated Bioelectronic Chips", Nature Biotechnology, vol. 16, pp. 541-546, 1998. cited by applicant

Cheng, J. et al. "Isolation of Cultured Cervical Carcinoma Cells Mixed with Peripheral Blood Cells on a Bioelectronic Chip", Analytical Chemistry, vol. 70, #11, pp. 2321-2326, 1998. cited by applicant

Chin, C.D. et al. "Commercialization of micro fluidic point-of-care diagnostic devices", Lab on a Chip, vol. 12, No. 12, p. 2118, 2012. cited by applicant

Chuang, C.H. et al. "Lab on a chip for multiplexed immunoassays to detect bladder cancer using multifunctional dielectrophoretic manipulations", Lab Chip, vol. 15, No. 14, pp. 3056-3064, 2015. cited by applicant

Craggs, M.D. et al. "Performance of platinum stimulating electrodes, mapped on the limitvoltage plane", Medical & Biological Engineering & Computing, vol. 24, No. 4, pp. 424-430, Jul. 1986. cited by applicant

Czarnetzki, L.R. et al. "Formation of hypochlorite, chlorate and oxygen during NaCl electrolysis from alkaline solutions at an RuO₂/TiO₂ anode", J Appl Electrochem, vol. 22, No. 4, pp. 315-324, Apr. 1992. cited by applicant

Daniel M. et al. "Selective DNA attachment of particles to substrates" Journal of Materials Research, vol. 17, No. 2, Feb. 2002, pp. 473-478. cited by applicant

Dehlinger D. et al. "Next Generation Microelectronic Array Devices", in Handbook of Nanotechnology, Bhushan, B (Ed). Part B, Chapter 14, Springer, pp. 401-413, 2006. cited by applicant

Dehlinger D. et al. "Reconfigurable CMOS Electronic Microarray System for the Assisted Self-Assembly of Higher-Order Nanostructures", in Nanomanufacturing Handbook, Busnaina A (Ed), Chapter 5, CRC Press, pp. 107-126, 2006. cited by applicant

Dehlinger DA et al. "Electric Field Assisted Assembly of Functionalized Quantum Dots into Multi Layer Thin Films", NISTI-Nanotech 2006, vol. 1, Chap. 4, pp. 389-392, 2006. cited by applicant

Delgado, V.A. et al. "Measurement and interpretation of electrokinetic phenomena (IUPAC technical report)", Pure and Applied Chemistry, vol. 77, No. 10, Jan. 2005. cited by applicant

Edman, C. et al. "Electric Field Directed Nucleic Acid Hybridization on Microchips" Nucleic Acids Research, vol. 25, #24, pp. 4907-4914, 1997. cited by applicant

Edman, C.F. et al. "Electric Field Directed Assembly of an InGaAs LED onto Silicon Circuitry", IEEE—Photonics Technology Letters, v12, #9, 1198-2000, 2000. cited by applicant

Ellison, W. et al. "New permittivity measurements of seawater", Radio Science, vol. 33, No. 3, pp. 639-648, May 1998. cited by applicant

Esener, S. et al. "DNA Assisted Micro-Assembly: A Heterogeneous Integration Technology For Optoelectronics," Proc. SPIE Critical Reviews of Optical Science and Technology, Heterogeneous Integration, Ed. A. Hussain, CR70, Chapter 7, Jan. 1998. cited by applicant

Fang, X. et al. "Electrophysiological and histological studies of chronically implanted intrapapillary microelectrodes in rabbit eyes", Graefes Arch Clin Exp Ophtholmo, vol. 244, No. 3, pp. 364-375, Aug. 2005. cited by applicant

Fernandez, R.E. et al. "Aptamer-functionalized graphene-gold nanocomposites for label-free detection of dielectrophoretic-enriched neuropeptide y", Electrochemistry Communications, vol. 72, pp. 144-147, Nov. 2016. cited by applicant

Franks, W. et al. "Impedance characterization and modeling of electrodes for biomedical applications", IEEE Transactions on Biomedical Engineering, vol. 52, No. 7, pp. 1295-1302, Jul. 2005. cited by applicant

Gahan, P.B. "Circulating nucleic acids in early diagnosis, prognosis and treatment monitoring" Advances in Predictive, Preventive and Personalised Medicine. Springer, 2015, vol. 5. cited by applicant

Gonzalez, A. et al. "Electrothermal flows generated by alternating and rotating electric elds in microsystems", J. Fluid Mech., vol. 564, p. 415, Sep. 2006. cited by applicant

Gu, W.Y. et al. "Diffusivity of ions in agarose gels and intervertebral disc: Effect of porosity", Annals of Biomedical Engineering, vol. 32, No. 12, pp. 1710-1717, Dec. 2004. cited by applicant

Gurtner C, et al. "Microelectronic Array Devices and Techniques for Electric Field Enhanced DNA Hybridization In Low-Conductance Buffers" Electrophoresis 2002, 23, 1543-1550. cited by applicant

Gurtner, C. et al. "Photoelectrophoretic Transport and Hybridization of DNA Oligonucleotides on Unpatterned Silicon Substrates", J. Am. Chem. Soc., v122, #36, pp. 8589-8594, 2000. cited by applicant

Hartmann et al. "Stable 16-year storage of DNA purified with the QIAamp ® DNA Blood Mini Kit" Oct. 2016. 4 pages. cited by applicant

Haus, H.A. et al. "Electromagnetic fields and energy" Prentice-Hall: Englewood Clis, NJ, 1989. cited by applicant

Hayes, M. et al. "Aggregation effects on the electrocatalytic activity of platinum", Journal of Catalysis, vol. 53, No. 1, pp. 88-95, 1978, issn: 0021-9517. cited by applicant

Heineck D.P. et al. "Electrokinetic device design and constraints for use in high conductance solutions", Electrophoresis, 2017. cited by applicant

Heller M, et al. "Electric Field Devices for Assisted Assembly of DNA Nanocomponents and Other Nanofabrication Applications", in BioMEMS and Biomedical Nanotechnology VI, Heller MJ, Ozkan M, and Ferrari M (Eds), Springer, vol. 2, Chapter 6, pp. 139-159, 2006. cited by applicant

Heller M. "DNA Microarray Technology: Devices, Systems and Applications", In Annual Review of Biomedical Engineering, 2002, 4:129-53. cited by applicant

Heller M. "Integrated Microfabricated Biodevices: Advanced Technologies for Genomics Drug Discovery, Bioanalysis, and Clinical Diagnostics" 2001. 462 pages. cited by applicant

Heller M. et al. "Detection of Cancer Related DNA Nanoparticulate Biomarkers and Nanoparticles in Whole Blood" TechConnect World 2010 Proceedings, Nanotechnology 2010, vol. 3, Chap. 6, Cancer Nanotechnology pp. 372-375. cited by applicant

Heller M. et al. "Parallel Assisted Assembly of Multilayer DNA and Protein Nanoparticle Structures Using a CMOS Electronic Array" DNA-Based Nanoscale Integration Symposium, W. Fritzsche (Ed), American Institute of Physics, NY, pp. 73-81, 2006. cited by applicant

Heller, M. "An Active Microelectronic Device For Multiplex DNA Analysis", IEEE Engineering In Medicine And Biology, pp. 100-104, Mar./Apr. 1996. cited by applicant

Heller, M. et al. "Active Microelectronic Arrays for DNA Hybridization Analysis" in DNA Microarrays: A Practical Approach, Edited by M. Schena, Oxford University Press, pp. 167-185, 1999. cited by applicant

Heller, M. et al. Active Microelectronic Chip Devices Which Utilize Controlled Electrophoretic Fields for Multiplex DNA Hybridization and Other Genomic Applications, Electrophoresis 2000, vol. 21, pp. 157-164, Jan. 2000. cited by applicant

Heller, M. et al. "An Integrated Microelectronic Hybridization System for Genomic Research and Diagnostic Applications", in Micro Total Analysis Systems 98, Edited by D. J. Harrison and A. van den Berg, Kluwer Academic Publishers, pp. 221-224, 1998. cited by applicant

Heller, M. et al. "Self-Assembling DNA Photonic Nanostructures", in Artificial Self-Assembling Systems for Gene Delivery, American Chemical Society, pp. 72-83, 1996. cited by applicant

Heller, M.J. et al. "Electric Field Process for the Fabrication of Higher Order Structures from Biomolecule Derivatized Nanoparticles" NISTI-Nanotech 2007, vol. 1, Chap.4, 269-271, 2007. cited by applicant

Hirschorn, B. et al. "Determination of effective capacitance and film thickness from constant-phase-element parameters", Electrochimica Acta, vol. 55, No. 21, pp. 6218-6227, Aug. 2010. cited by applicant

Hodko D. et al. "CMOS Microarrays for Diagnostic and Nanotechnology Applications", in CMOS Biotechnology, Lee H, Ham D and Westervelt (Ed.), Chapter 7, pp. 179-206, 2007. cited by applicant

Hoerter, J.A. et al. "Sirna-like double-stranded rnas are specifically protected against degradation in human cell extract", PloS one, vol. 6, No. 5, e20359, 2011. cited by applicant

Horbett, T.A. et al. "Cell adhesion to a series of hydrophilic-hydrophobic copolymers studies with a spinning disc apparatus", Journal of Biomedical Materials Research, vol. 22, No. 5, pp. 383-404, May 1988. cited by applicant

Huang Y. et al. "Electric Manipulation of Bioparticles and Macromolecules on Microfabricated Electrodes", Analytical Chemistry 2001, (73):1549-59. cited by applicant

Huang, Y. et al. "Dielectrophoretic Cell Separation and Gene Expression Profiling on Microelectronic Chip Arrays", Anal. Chem. 2002, 74, 3362-71. cited by applicant

Hudak, E.M. et al. "Platinum for neural stimulation: Voltammetry considerations", J. Neural Eng., vol. 7, No. 2, p. 026 005, Mar. 2010. doi: 10.1088/1741-2560/7/2/026005. cited by applicant

Ibsen S. et al. "Rapid Isolation and Detection of Exosomes and Associated Biomarkers from Plasma", ACS Nano 2017, DOI: 10.1021/acsnano.7b00549. cited by applicant

Ibsen, S. et al. "Recovery of drug delivery nanoparticles from human plasma using an electrokinetic platform technology", Small, vol. 11, No. 38, pp. 5088-5096, Aug. 2015. doi: 10.1002/sml.201500892. cited by applicant

Inci, F. et al. "Nanoplasmonic quantitative detection of intact viruses from unprocessed whole blood" ACS Nano, vol. 7, No. 6, pp. 4733-4745, Jun. 2013. doi: 10.1021/nn3036232. cited by applicant

Irimajiri, A. et al. "A dielectric theory of multi-stratified shell model with its application to a lymphoma cell", Journal of Theoretical Biology, vol. 78, No. 2, pp. 251-269, May 1979. cited by applicant

ISA, International Search Report and Written Opinion for PCT Application No. PCT/US2020/047519. Mail Date: Nov. 24, 2020. 10 pages. cited by applicant

Jones, T. "Basic theory of dielectrophoresis and electrorotation", IEEE Eng. Med. Biol. Mag., vol. 22, No. 6, pp. 33-42, Nov. 2003. doi: 10.1109/memb.2003.1304999. cited by applicant

Juchniewicz, R. "The influence of alternating current on the anodic behaviour of platinum", Platinum Metals Review, vol. 6, No. 3, pp. 100-105, 1962. [Online]. Available:

<http://www.ingentaconnect.com/content/matthey/pmr/1962/00000006/00000003/art00009>. cited by applicant

Juchniewicz, R. "The influence of increasing superimposed 50 c/s a.c. on the anodic dissolution of platinum in 3% sodium chloride", *Corrosion Science*, vol. 6, No. 2, pp. 69-77, Jan. 1966. doi: 10.1016/s0010-938x(66)80026-4. cited by applicant

Kaatze, U. "Complex permittivity of water as a function of frequency and temperature", *Journal of Chemical & Engineering Data*, vol. 34, No. 4, pp. 371-374, Oct. 1989. cited by applicant

Kassengne SK, et al. Numerical Modeling of Transport and Accumulation of DNA on Electronically Active Biochips, *Sensors and Actuators* 2003, B 94, 81-98. cited by applicant

Kong, F.Y. et al. "A paper disk equipped with graphene/polyaniline/au nanoparticles/glucose oxidase biocomposite modified screen-printed electrode: Toward whole blood glucose determination", *Biosensors and Bioelectronics*, vol. 56, pp. 77-82, Jun. 2014. cited by applicant

Krishnan R. et al. "Alternating current electrokinetic separation and detection of DNA nanoparticles in high-conductance solutions" *Electrophoresis*, v. 29, #9, pp. 1765-1774, May 2008. cited by applicant

Krishnan R. et al. "An AC electrokinetic method for the enhanced detection of DNA nanoparticles", *J. Biophotonics*, V2, #4, pp. 253-261, 2009. cited by applicant

Krishnan R. et al. Rapid Isolation and Detection of Cell Free Circulating DNA and Other Disease Biomarkers Directly from Whole Blood, in *Circulating Nucleic Acids in Plasma and Serum*, 2011, Chap. 34, pp. 247-257. cited by applicant

Krishnan, R. et al. "Interaction of nanoparticles at the {dep} microelectrode interface under high conductance conditions", *Electrochemistry Communications*, vol. 11, No. 8, pp. 1661-1666, 2009. cited by applicant

Kuhn, A. et al. "The behaviour of platinum, iridium and ruthenium electrodes in strong chloride solutions", *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, vol. 41, No. 3, pp. 329-349, Feb. 1973. cited by applicant

Lee, M.G. et al. "Label-free cancer cell separation from human whole blood using inertial micro fluidics at low shear stress", *Analytical Chemistry*, vol. 85, No. 13, pp. 6213-6218, Jul. 2013. cited by applicant

Lewis J.M. et al. "Integrated Analysis of Exosomal Protein Biomarkers on AC Electrokinetic Chips Enables Rapid Detection of Pancreatic Cancer in Patient Blood", *ACS Nano* (2018). cited by applicant

Lewis, J.M. et al. "Detecting cancer biomarkers in blood: Challenges for new molecular diagnostic and point-of-care tests using cell-free nucleic acids", *Expert review of molecular diagnostics*, vol. 15, No. 9, pp. 1187-1200, 2015. cited by applicant

Llopis, J. "Corrosion of platinum metals and chemisorption", *Catalysis Re-views*, vol. 2, No. 1, pp. 161-220, Jan. 1969. cited by applicant

Llopis, J. et al. "Electrochemical corrosion of platinum in hydrochloric acid solutions", *Journal of The Electrochemical Society*, vol. 108, No. 8, p. 720, 1961. cited by applicant

Llopis, J. et al. "Study of the impedance of a platinum electrode in the system $\text{Cl}_2/\text{Cl}-(\text{HClO}_4\text{aq})$ ", *Electrochimica Acta*, vol. 8, No. 3, pp. 163-173, Mar. 1963. cited by applicant

Malmberg, C. et al. "Dielectric constant of water from 0 to 100 C", *J Res Nat Bureau Stand*, vol. 56, pp. 1-8, 1956. cited by applicant

Manouchehri, S. et al. "Dielectrophoretic recovery of DNA from plasma for the identification of chronic lymphocytic leukemia point mutations", *Int. J. Hematol. Oncol.*, vol. 5, No. 1, pp. 27-35, May 2016. cited by applicant

Mathew, B. et al. "Dielectrophoresis based cell switching in continuous flow micro fluidic devices", *Journal of Electrostatics*, vol. 84, pp. 63-72, Dec. 2016. cited by applicant

McCanna, J.P. et al. "Low level epifluorescent detection of nanoparticles and DNA on dielectrophoretic microarrays" *J. BioPhotonics*, 1-11 (2013). cited by applicant

Odde, C. et al. "Stability study of 81 analytes in human whole blood, in serum and in plasma", *Clinical Biochemistry*, vol. 45, No. 6, pp. 464-469, Apr. 2012. cited by applicant

Park, K. et al. "Dielectrophoretic lab-on-CMOS platform for trapping and manipulation of cells", *Biomedical Microdevices*, vol. 18, No. 1, Jan. 2016. cited by applicant

Patrick, E. et al. "Corrosion of tungsten microelectrodes used in neural recording applications", *Journal of Neuroscience Methods*, vol. 198, No. 2, pp. 158-171, Jun. 2011. cited by applicant

Pethig, R. "Dielectrophoresis: Status of the theory, technology, and applications", *Biomicrofluidics*, vol. 4, No. 2, p. 022 811, 2010. cited by applicant

Pourbaix, M. et al. "Electrochemical properties of the platinum metals", *Platinum metals review*, vol. 3, No. 2, pp. 47-53, 1959. cited by applicant

Prasad, A. et al. "Comprehensive characterization and failure modes of tungsten microwire arrays in chronic neural implants", *J. Neural Eng.*, vol. 9, No. 5, p. 056 015, Sep. 2012. cited by applicant

Pyell, U. "Characterization of nanoparticles by capillary electromigration separation techniques", *Electrophoresis*, vol. 31, No. 5, pp. 814-831, Mar. 2010. cited by applicant

Ramo, S. et al. *Fields and waves in communication electronics*. John Wiley & Sons Inc, Feb. 11, 1994, 864 Seiten. cited by applicant

Ramos, A. "Electrokinetics and electrohydrodynamics in microsystems" Springer Science Business Media, 2011. cited by applicant

Ramos, A. et al. "AC electric-field-induced fluid flow in microelectrodes", *Journal of colloid and interface science*, vol. 217, No. 2, pp. 420-422, 1999. cited by applicant

Rand, D. et al. "A study of the dissolution of platinum, palladium, rhodium and gold electrodes in 1 m sulphuric acid by cyclic voltammetry", *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, vol. 35, No. 1, pp. 209-218, Mar. 1972. cited by applicant

Sanghavi, B.J. et al. "Ultrafast immunoassays by coupling dielectrophoretic biomarker enrichment in nanoslit channel with electrochemical detection on graphene", *Lab Chip*, vol. 15, No. 24, pp. 4563-4570, 2015. cited by applicant

Saucedo-Espinosa, M.A. et al. "Polarization behavior of polystyrene particles under direct current and low-frequency (1 kHz) electric elds in dielectrophoretic systems", *Electrophoresis*, vol. 37, No. 4, pp. 635-644, Dec. 2015. cited by applicant

Saville, D.A. "Electrohydrodynamics:the Taylor-melcher leaky dielectric model", *Annu. Rev. Fluid Mech.*, vol. 29, No. 1, pp. 27-64, Jan. 1997. cited by applicant

Scott, M. et al. "Theoretical model of electrode polarization and AC electroosmotic fluid flow in planar electrode arrays", *Journal of Colloid and Interface Science*, vol. 238, No. 2, pp. 449-451, Jun. 2001. cited by applicant

Sharma, A. et al. "Rapid electrical immunoassay of the cardiac biomarker troponin i through dielectrophoretic concentration using imbedded electrodes", *Biosensors and Bioelectronics*, vol. 82, pp. 78-84, Aug. 2016. cited by applicant

Shepherd, B. et al. "Scanning electron microscopy of platinum scala tympani electrodes following chronic stimulation in patients", *Biomaterials*, vol. 12, No. 4, pp. 417-423, May 1991. cited by applicant

Song, H. et al. "Continuous-flow sorting of stem cells and differentiation products based on dielectrophoresis", *Lab on a Chip*, vol. 15, No. 5, pp. 1320-1328, 2015. cited by applicant

Song, Y. et al. "Device for dielectrophoretic separation and collection of nanoparticles and DNA under high conductance conditions", *Electrophoresis*, vol. 36, No. 9-10, pp. 1107-1114, Apr. 2015. cited by applicant

Sonnenberg A. et al. "Dielectrophoretic Isolation and Detection of Cancer Related Circulating Cell Free DNA Biomarkers from Blood and Plasma", *Electrophoresis*, V35, 12-13, pp. 1828-1836, Jul. 2014. cited by applicant

Sonnenberg A. et al. "Rapid Electrokinetic Isolation of Cancer-Related Circulating Cell Free DNA Directly from Blood", *Clinical Chemistry*, 60:3, pp. 500-509, 2014. cited by applicant

Sonnenberg, A. et al. "Dielectrophoretic Isolation and Detection of cfc-DNA Nanoparticulate Biomarkers and Virus form Blood", Electrophoresis, V34, pp. 1076-1084, 2013. cited by applicant

Sonnenberg, A. et al. "Dielectrophoretic Isolation of DNA and Nanoparticles from Blood", Electrophoresis V33, 2482-90, 2012. cited by applicant

Sosnowski, R. et al. "Active Microelectronic Array System for DNA Hybridization, Genotyping and Pharmacogenomic Applications", Psychiatric Genetics 2002, 12:181-192. cited by applicant

Sosnowski, R. et al. "Rapid Determination of Single Base Mismatch in DNA Hybrids by Direct electric Field Control", Proc. Nat Acad. Sci. USA, vol. 94, pp. 1119-1123, 1997. cited by applicant

Sukhorukov, V.L. et al. "A single-shell model for biological cells extended to account for the dielectric anisotropy of the plasma membrane", Journal of Electrostatics, vol. 50, No. 3, pp. 191-204, Feb. 2001. cited by applicant

Sun, M. et al. "Continuous on-chip cell separation based on conductivity-induced dielectrophoresis with 3D self-assembled ionic liquid electrodes", Analytical Chemistry, vol. 88, No. 16, pp. 8264-8271, Aug. 2016. cited by applicant

Tsikritsis, D. et al. "Label-free biomarkers of human embryonic stem cell differentiation to hepatocytes", Cytometry, vol. 89, No. 6, pp. 575-584, May 2016. cited by applicant

Vitagliano, V. et al. "Diffusion coefficients for aqueous solutions of sodium chloride and barium chloride", Journal of the American Chemical Society, vol. 78, No. 8, pp. 1549-1552, 1956. cited by applicant

Wei, C. et al. "From bench to bedside: successful translational nanomedicine: Highlights of the Third Annual Meeting of the American Academy of Nanomedicine", Nanomedicine: Nanotechnology, Biology and Medicine, V3, Issue 4, pp. 322-331, Dec. 2007. cited by applicant

Wei, X.F. et al. "Impedance characteristics of deep brain stimulation electrodes in vitro and in vivo", J. Neural Eng., vol. 6, No. 4, p. 046 008, Jul. 2009. cited by applicant

Weng, P.Y. et al. "Size-dependent dielectrophoretic crossover frequency of spherical particles", Biomicrofluidics, vol. 10, No. 1, p. 011 909, Jan. 2016. cited by applicant

Yang, X. et al. "Integrated separation of blood plasma from whole blood for micro fluidic paper-based analytical devices", Lab Chip, vol. 12, No. 2, pp. 274-280, 2012. cited by applicant

Zhou, D. et al. "Electrochemistry in neural stimulation by biomedical implants", Electrochemistry, vol. 17, No. 3, pp. 249-262, 2011. cited by applicant

Primary Examiner: Kim; Young J

Attorney, Agent or Firm: Perkins Coie LLP

Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This patent document is a U.S.C. § 371 National Stage application of International Application No. PCT/US2020/047519 entitled "ELECTROKINETIC MICROELECTRODE DEVICES AND METHODS FOR BIOMARKER ANALYSIS" filed on Aug. 21, 2020 which further claims priority to and benefits of U.S. Provisional Patent Application No. 62/891,204 entitled "ELECTROKINETIC MICROELECTRODE DEVICES AND METHODS FOR BIOMARKER ANALYSIS" filed on Aug. 23, 2019. The entire contents of the aforementioned patent applications are incorporated by reference as part of the disclosure of this patent document.

TECHNICAL FIELD

(1) This patent document relates to analyte sensors.

BACKGROUND

(2) Dielectrophoresis (DEP) is an electrokinetic phenomenon in which a force is exerted on a dielectric particle (e.g., polarizable particle, including molecules and nanoscale particles) in a suspending medium when the particle is subjected to a non-uniform electric field.

Dielectrophoresis can be used to attract and separate various particles in aqueous media, depending on the dielectric response of the particle in the presence of the non-uniform electric field. Although particles in general can exhibit dielectrophoretic activity in the presence of an electric field, the magnitude of the dielectrophoretic force depends on the type of medium, certain properties of specific particles, e.g., electrical properties and shape and size, and the frequency of the electric field exerted on the particles.

SUMMARY

(3) Disclosed are miniaturized electronic systems, devices and methods for biomarker analysis, which can be incorporated into blood collection tubes and other containers that enable the immediate isolation, concentration, analysis and storage of disease related biomarkers upon blood draw. The miniaturized electronic systems can become activated and immediately carry out in-situ sample preparation, processing and storage of biomarkers for later down-stream analysis in the clinical laboratory or other settings. Such systems are able to carry out in-situ detection, identification and analysis of specific biomarkers and other entities that require more immediate results.

(4) In some embodiments, the miniaturized electronic systems include a specially designed high surface area folded or sandwiched electrokinetic microelectrode arrays (also called microarrays, chips, or devices) that allows both AC dielectrophoretic (DEP) and DC electrophoretic based separation and isolation and other processes to be used for the concentration and storage of the biomarkers.

(5) In some embodiments, an electronic device for biomarker analysis in a biofluid includes an electrokinetic microelectrode array chip operable to separate and isolate a biomarker in a biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces, the electrokinetic microelectrode array chip including: a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array chip includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel, and wherein the electrokinetic microelectrode array chip device is able to be incorporated within a container to hold the biofluid.

(6) In some embodiments, a method of analyzing one or more biomarkers in a biofluid includes obtaining a biofluid from a subject, wherein the biofluid is placed in a collection tube including an electrokinetic microelectrode array including a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel; contacting the biofluid with the electrokinetic microelectrode array to separate and isolate the one or more biomarkers in the electrokinetic microelectrode array, wherein the electrokinetic microelectrode array separates and isolates the one or more biomarker(s) from the biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces; and analyzing the one or more biomarkers in the biofluid.

(7) The subject matter described in this patent document can be implemented in specific ways that provide one or more of the following features.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

- (1) FIG. 1 shows a diagram illustrating a conventional technique or present pathway for blood biomarkers going to the clinical laboratory for molecular diagnostic and clinical chemistry analyses.
- (2) FIG. 2 shows a diagram illustrating an example embodiment of a miniaturized electronic system in accordance with the present technology.
- (3) FIG. 3 shows a diagram illustrating the example miniaturized electronic system (shown in FIG. 2) being incorporated into a blood draw tube.
- (4) FIG. 4 shows a diagram illustrating example blood draw and biomarker isolation steps in some implementations of the example miniaturized electronic system.
- (5) FIG. 5 shows a diagram illustrating an example of an overall method where the miniaturized electronic systems are able to carry out: (a) immediate blood processing and biomarker isolation; (b) if so desired, the rapid in-situ/on-chip detection, identification and analysis of certain specific biomarkers collected on the electrokinetic microarray surface, as well analytes in the blood is carried out; (c) the reporting via RF or other transmitting device of the on-chip analysis results; (d) the protection and storage of the concentrated biomarkers for transport; and (e) the final release of the biomarkers into the clinical laboratory system or other diagnostic systems for more complete final analysis.
- (6) FIGS. 6A and 6B show a diagram illustrating an enlarged view of the example embodiment of the sandwiched electrokinetic microarray chip & detector-sensor chip.
- (7) FIG. 6C shows a diagram illustrating detail of features and components of the example electrokinetic microarray chip and detector-sensor chip.
- (8) FIG. 7 shows a diagram illustrating an example implementation of the example electrokinetic microarray device.
- (9) FIG. 8 shows a block diagram illustrating an example embodiment of a battery-powered device control system.
- (10) FIG. 9 shows an illustrative diagram of example embodiments of an insulator-based electrokinetic DEP microelectrode devices integrated in a blood draw tube.

DETAILED DESCRIPTION

- (11) The increasing interest in molecular diagnostics for research or clinical applications, e.g., forms of personalized or precision medicine, is concomitant with the rapid improvements in sequencing technologies and amplification techniques. This progress puts greater burden on sample preparation, throughput, yield, and maintaining sample integrity. Yet little has changed with fundamental techniques and best practices in a several decades. Dielectrophoretic separations offer tremendous benefits over more traditional techniques, and hold promise to positively affect these critical metrics.
- (12) Disclosed are miniaturized electronic systems, devices and methods for biomarker analysis, which can be incorporated into blood collection tubes and other containers that enable the immediate isolation, concentration, analysis and storage of disease related biomarkers upon blood draw. The miniaturized electronic systems can become activated and immediately carry out in-situ sample preparation, processing and storage of biomarkers for later down-stream analysis in the clinical laboratory or other settings. Such systems are able to carry out in-situ detection, identification and analysis of specific biomarkers and other entities that require more immediate results. Furthermore, the disclosed systems, devices and methods immediately overcome many of

the problems related blood draw logistics, including blood degradation and biomarker degradation due to delay in processing, storage and many other adverse effects that occur before the blood is actually analyzed.

(13) In some embodiments, the miniaturized electronic systems include a specially designed high surface area folded or sandwiched electrokinetic microelectrode array (e.g., microarray) chip device that allows both AC dielectrophoretic (DEP) and DC electrophoretic based separation and isolation and other processes to be used for the concentration and storage of the biomarkers.

(14) In some embodiments, the miniaturized electronic systems can be incorporated into a detection-sensor chip that is flip-chip bonded to the electrokinetic chip. This chip incorporates uv/vis light emitters, detectors and other sensors that allow more immediate analysis of certain specific biomarkers and other blood constituents. Other components of the miniaturized system can include a control unit with frequency generator and RF transmitter; a battery power unit; micro-pumps and control valves; and/or buffer and reagents reservoirs.

(15) In some implementations, the disclosed systems and devices are designed to isolate for storage and/or analysis a wide range of disease related biomarkers that can include, but are not limited to cell free (cf) DNA, RNA, nucleosomes, exosomes, extracellular vesicle (EVs), drug delivery nanoparticles, cell organelles (mitochondria, etc.) proteins/enzymes, protein complexes, virus, bacteria, cancer cells and other important analytes and entities.

(16) In addition to blood draw tubes, blood collection bags and other blood storage containers, the example devices can be incorporated or simply added (dropped into) urine collection tubes, and other collection tubes and containers used for clinical, biological and research applications, for example. In addition to undiluted blood, the example systems and devices can also be operated in other high conductance solutions ~0.5 to 1.5 Siemens/m (S/m) including but not limited to: buffy coat blood, plasma, serum, urine, saliva, cerebrospinal fluid (CSF), and other biological fluids, buffers and environmental samples.

(17) Conventional blood draw logistics and techniques for collecting, storage and transport of blood can create problems for later down-stream biomarker analysis in the clinical lab setting. This is true for many molecular diagnostic biomarkers such as cf-DNA, RNA, exosomes, cancer cells, and other entities that are labile and present in only very low amounts. In these cases, blood processing and extended delay or storage of the blood can lead to biomarker loss and the release of blood cell components that later interfere with analysis of the desired biomarkers. Even, temporary cooling of the blood may have adverse effects. Also, choices of blood draw tubes must be made regarding whether the tubes contain additives such as heparin, EDTA, etc., used for the downstream preparation of plasma or serum. These additives are often incompatible with many of the different biomarkers that are to be later analyzed.

(18) The disclosed systems, devices and methods address these problems and provide unique solutions. For example, in some implementations of the example miniaturized electronics system including the electrokinetic microelectrode array chip device, the system immediately upon blood draw collects, concentrates, and stores all the desired biomarkers “in-situ,” on the electrokinetic microarray device surface. As a result, the biomarkers are now both protected and prevented from being further degraded, mixed and diluted with the numerous interfering substances that are released by red and white blood cell lysis and other ongoing cellular and biochemical/enzymatic processes—challenges often associated with conventional blood draw and analysis techniques.

(19) In comparison, for example, while some existing techniques, such as adding RNase inhibitors to the blood, can help prevent RNA degradation, they do nothing to prevent release of relatively large amounts of other RNA from white blood cells which begin to lyse soon after blood collection. Release of these competing biomolecules makes subsequent analysis of specific disease related RNA's much more difficult. The immediate isolation of the molecular biological biomarkers also assures that whatever additive is being used in the blood tube will have only negligible effects on the biomarkers being collected and stored inside the system.

(20) The disclosed systems, devices and methods are also capable of carrying out the more immediate analysis of certain specific biomarkers. By way of just one example, the immediate measurement and reporting of the cf-DNA level in the blood is valuable as this often indicates that serious pathology or other problems exists. The disclosed systems, devices and methods can be implemented in un-diluted blood and other high conductance sample solutions, which is another major advantage over conventional techniques.

(21) The disclosed systems, devices, and method are also capable of carrying out direct separation and analysis of targeted biomarkers with a blood sample without the need to go through centrifugation, chemical isolation, and precipitation steps associated with conventional techniques. For example, in some implementations, the miniaturized electronics system including the electrokinetic microelectrode array chip device can be added directly to a tube containing a blood sample. The disclosed systems, devices, and method are then able to carry out a rapid analysis of the biomarkers in the blood sample without degradation of the blood.

(22) The disclosed systems, devices, and method are also capable of carrying out direct separation and analysis of targeted biomarkers from a single blood sample. Conventional techniques often require obtaining one blood sample for each test to be performed on the sample. This requires multiple blood draws from the patient, resulting in a more arduous and time extensive process. The disclosed systems, devices, and method enable performing multiple tests on a single blood sample drawn from the patient.

(23) Example embodiments and implementations of the miniaturized electronic systems and electrokinetic microelectrode array chip device are disclosed below.

(24) It is well understood that the blood draw process for collecting, storage and transport of blood can frequently cause problems for later down-stream biomarker analysis in the clinical lab setting. This is true for many molecular diagnostic biomarkers (e.g., cf-DNA, RNA, exosomes, cancer cells, etc.) that are often labile and present in only very low amounts. In these cases, blood processing and extended delay can lead to biomarker loss and the release of blood cell components that later interfere with analysis of the desired biomarkers. Also, choices of blood draw tubes must be made regarding whether the tubes contain, heparin, EDTA, or other additives which are often not compatible with one or more of the many different biomarkers that are later to be detected and analyzed.

(25) FIG. 1 shows a diagram illustrating a process **100** of a conventional technique used for obtaining blood biomarkers to be sent to the clinical laboratory for molecular diagnostic and clinical chemistry analyses. The process **100** includes an initial Step **101** of collecting and transporting the blood sample. After collection and transport, the sample is centrifuged in Step **102**, separating the sample into three layers: plasma (top layer), white blood cells (WBCs) and circulating tumor cells (CTCs) (middle layer), and red blood cells (bottom layer). Each of the three layers are then prepared for separation and analysis of the individual constituents comprised in each layer (e.g., clinical chemistry analytes, cf-DNA, exosomes, and CTC isolation). In Step **103**, the prepared samples can then be analyzed using an array of different techniques to include digital polymerase chain reaction (PCR), next generation sequencing (NGS), methylation, imaging, and immunotyping. Lastly, the process **100** includes a Step **105**, where the samples can be used for a variety of applications to include prenatal testing, pharmacogenomics, genotyping, cancer diagnostics, and allograft acceptance. However, in using the process **100**, from the time of the blood draw, samples can experience degradation and the loss of important biomarkers during temporary storage and the time required for shipment and later preparation of plasma, serum and for specific cell isolation.

(26) The example miniaturized electronic systems is envisioned to overcome many of the blood draw problems and logistics that occur in the collecting and storage of blood before later down-stream biomarker analysis in the clinical lab. The example miniaturized electronic allows for direct separation of targeted biomarkers without the need to go through centrifugation, chemical isolation,

and precipitation steps often required using conventional techniques for analyzing biomarkers.

(27) FIG. 2 shows a diagram illustrating an example embodiment of a miniaturized electronic system **200** in accordance with the present technology. Example features and components of the example miniaturized electronic system **200** includes a specially designed high surface area folded or sandwiched electrokinetic microelectrode array device **260** that allows both AC dielectrophoretic (DEP) and DC electrophoretic based separation and isolation and other processes to be used for the concentration and storage of the biomarkers.

(28) In some embodiments, the electrokinetic microelectrode array device **260** includes a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure. In some implementations, the electrokinetic microelectrode array device **260** includes a folded or a sandwiched configuration of the array of microelectrodes, which are labeled in FIG. 2 as **261**, **262**, **263**, **264**, and **265** on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel.

(29) In some example embodiments of the electrokinetic microelectrode array device **260**, like that shown in FIG. 2, the device **260** can be configured as a flip-chip bonded to a second key component, which is a detection-sensor chip device that allows in-situ detection and analysis of the biomarkers that are collected and stored on the surface of the electrokinetic chip, as well as other analytes still in the blood itself.

(30) The electrokinetic microelectrode array device **260** can be configured with other components of the miniaturized electronic system **200**. As shown in FIG. 2, the blood sample including the target biomarkers is drawn into the inlet port **250** by the micropump & control valve unit **230**. Once drawn into the inlet port **250** controlled by the inlet control valve **280a**, a fluidic sensor **240** is activated, signaling the introduction of the blood into the miniaturized electronic system **200**. The blood sample including the targeted biomarkers then travels through the inlet port **250** and flows over the electrokinetic electrode array chip device **260**. The blood passes over the electrokinetic electrode array chip device **260**, while the biomarkers remain, isolated on the surface of the electrokinetic electrode array chip device **260**. After passing over the electrokinetic electrode array chip device **260**, the blood exits the miniaturized electronic system **200** through the outlet port **290** controlled by outlet control valve **280b**. The miniaturized electronic system **200** can now flow from the buffer and reagent reservoirs **220**, a stabilizing buffer onto the electrokinetic electrode array chip device **260** containing the isolated biomarkers. The miniaturized electronic system **200** further includes a unit **201** including a battery power unit and AC/DC converter, a unit **210** including a control unit, frequency generator, and RF transmitter that are in operative communication with the electrokinetic electrode array chip device **260**.

(31) This microarray chip device **260** allows both AC dielectrophoretic (DEP) and DC electrophoretic based separation and other processes to be used for the rapid isolation, concentration, and in-situ or on-chip storage of the biomarkers. The electrokinetic microelectrode array chip **260** is operable to isolate directly from undiluted whole blood a wide range of disease related biomarkers that can include, but are not limited to cell free (cf) DNA, RNA, nucleosomes, exosomes, extracellular vesicle (EVs), drug delivery nanoparticles, cell organelles (mitochondria, etc.) protein complexes, virus, bacteria, cancer and other cells and other important analytes and entities.

(32) The example electrokinetic microelectrode array chip device **260** is flip-chip bonded to a detection-sensor chip device that allows in-situ detection and analysis of the biomarkers that are collected and stored on the surface of the electrokinetic chip, as well as other analytes still in the blood itself. In some embodiments, the detector-sensor chip incorporates uv/vis light emitters, mini/micro size fluorescent detectors and/or other chemical sensors that allow more immediate

analysis of certain specific biomarkers and other blood constituents or conditions. This may include but is not limited to some biomarkers being isolated on the electrokinetic chip (cf-DNA, exosomes, rare cancer cells, etc.), which can be detected and/or analyzed by using of fluorescent dyes (DNA or RNA dyes, etc.) or other fluorescent based reagents (fluorescent antibodies, etc.). Other analytes in the blood that are not being isolated, but whose immediate detection and/or analyses may be desirable can include but are not limited to: glucose, pH, electrolytes, enzymes, hormones, lipids (fats), other metabolic substances, and proteins. A variety of mini/micro sensor devices, well known in the art, have been designed for these purposes. Such sensor devices can be incorporated into the detector-sensor chip device. Other components of the miniaturized systems include: a control unit with frequency generator and RF transmitter; a battery power unit; micro-pumps and control valves; and buffer and reagents reservoirs.

(33) In some implementations, the example miniaturized electronic systems can be incorporated into blood collection tubes and other containers that enable the immediate isolation, concentration, analysis and storage of disease related biomarkers upon blood draw.

(34) FIG. 3 shows a diagram illustrating the example biomarker analysis system **300** that incorporates the miniaturized electronic system **200** into a blood draw tube **310**. The overall size of the systems that would be used in 1 mL to 5 mL blood draw tubes **310** would be from about 1.5 cm to 2.5 cm in height by 0.5 cm to 1.5 cm in width by 0.5 cm to 1.5 cm in depth. However, present fabrication technology would allow both smaller micro-systems to be built, as well as larger systems could easily be fabricated.

(35) In the example shown, the miniaturized electronic system **200** is mounted/bonded inside near the bottom of the blood collection tube **310**. The miniaturized electronic system **200** includes an example embodiment of the electrokinetic microelectrode array device **260** that is configured as a high-surface area folded AC/DC microarray with a flip-chip bonded detector and sensor chip, labeled as **260'** in FIG. 3. The whole miniaturized electronic system **200** is encapsulated/packaged in a protective covering **320** that prevents liquid (e.g., blood) from entering the device at any other location but the input port, which have been designed for bringing the blood into the system. The miniaturized electronic system **200** is programmed via a special blood sensor to become activated and immediately carry out in-situ sample preparation, processing, analysis and storage of biomarkers for more complete down-stream analysis in the clinical laboratory or other settings.

(36) FIG. 4 shows a diagram illustrating example blood draw and biomarker isolation process **400** using any of the example embodiments of the electrokinetic microelectrode array device **260** incorporated into an embodiment of the miniaturized electronic system **200**, such as the example miniaturized electronic system **403** shown in FIG. 4. In the example implementation, the process **400** includes a Step **401** of drawing the blood from the user into a tube **405**, where the blood contains the biomarkers **402** to be isolated (e.g., cf-DNA, RNA, exosomes, EVs, nucleosomes, CTC's, etc.). Step **410** includes the system activation when the blood sensor of the miniaturized electronic system **403** detects that the blood has been drawn. Step **420** is the collection of cf-DNA, exosomes, EVs, nucleosomes, CTC's and other biomarkers.

(37) In Step **420** the blood is drawn into the system by the micro-pump at the inlet port as indicated by the arrows **404**; the blood flows through the sandwiched AC/DC electrokinetic microelectrode array devices and exits at the outlet port. The blood can transit (e.g., flow through) the device at a rate that could range from about 1 $\mu\text{l/sec}$ (60 $\mu\text{l/min}$) to 20 $\mu\text{l/sec}$ (1200 $\mu\text{l/min}$). At a rate of 10 $\mu\text{l/sec}$ (600 $\mu\text{l/min}$), a 1 ml blood sample could be processed in about less than two minutes.

(38) During this transit process, the biomarkers are isolated and concentrated on the microarray surface as shown in Step **430**. Generally, in the AC mode for DEP based isolation, an AC frequency in the range of from 1 kHz to 20 kHz and AC voltages in the range of about 1 V to 20 V AC are used to isolate, concentrate and firmly hold biomarkers in the range of 20 nm to 800 nm in the DEP high-field regions areas on or near the edges of the microelectrodes. AC voltages in the range of 1 volt to 50 volts could be applied, with AC voltages in the range of from 3 volts to 15 volts being

more suitable. In the AC mode, the microelectrodes would be biased in checkerboard or other fashion as to create areas or regions of high field DEP and low field DEP which are suitable for collecting the various biomarkers in the sample. In the DC mode for electrophoretic isolation, a voltage of from 1 volt to 50 volts could be applied, with DC voltages in the range of from 2 volts to 20 volts being more suitable. In the DC or electrophoretic mode any of the negatively charged entities would be attracted to and collected on the positively biased electrodes. The system now pumps from the buffer reservoir, a stabilizing buffer into the chamber containing the microarrays with biomarkers concentrated on their surface.

(39) Step **430** is the completion of the process showing that each of the biomarkers are isolated and concentrated on the microarray surface; and at Step **440** the tube **405** is now ready for storage, and/or transport to the clinical lab. If on-chip analyses of certain biomarkers is desired (e.g., pre-analyses), then the detector-sensor chip component can be activated to carry out rapid detection of the specific biomarkers.

(40) FIG. 5 shows a diagram illustrating an example of the overall process **500** where the miniaturized electronic systems are able to carry out: (a) immediate blood processing and biomarker isolation (Step **501**); (b) if so desired, the rapid in-situ/on-chip detection, identification and analysis of certain specific biomarkers collected on the electrokinetic microarray surface, as well analytes in the blood is carried out (Step **510**); (c) the reporting via RF or other transmitting device of the on-chip analysis results (Step **520**); (d) the protection and storage of the concentrated biomarkers for transport (Step **530**); and (e) the final release of the biomarkers into the clinical laboratory system or other diagnostic systems for more complete final analysis (Steps **540-560**). Such final molecular diagnostic analyses can include but are not limited to immunofluorescent detection of exosome and other protein biomarkers, various PCR assays, RT PCR analysis of RNA, DNA sequencing and epigenetic analyses, DNA genotyping and many other types of biomarker and specific cell analyses.

(41) In the example implementation, process **500** includes a Step **501** in which a tube containing the blood is obtained and ready for immediate blood processing and biomarker isolation. A miniaturized electronic system is then added directly to the blood tube. If desired, the process **500** further includes a Step **510** where a rapid in-situ/on-chip detection, identification and analysis of certain specific biomarkers collected on the electrokinetic microarray surface, as well analytes in the blood is carried out. In Step **520**, results from the on-chip analysis in Step **510** can be quickly reported and/or received via an RF signal (e.g., to a cellular device) to a user, providing initial blood chemistry and biomarker information. The process **500** includes a Step **530** where the concentrated biomarkers are safely stored and prepared for transport to the clinical lab for further analysis. After transportation to the clinical lab, the process includes a Step **540** in which the concentrated biomarkers are removed from the tube via a sample extraction device. In Step **550**, the concentrated biomarkers are then transferred to a clinical laboratory system or other diagnostic systems for more complete final analysis in Step **560**.

(42) In addition to blood draw tubes, blood collection bags and other blood storage containers, the example devices of the disclosed technology can be incorporated or simply added (e.g., dropped into) into urine collection tubes, and other collection tubes and containers used for clinical, biological and research applications. Also, in addition to undiluted blood, the systems and devices in accordance with embodiments of the disclosed technology can be operated in other high conductance solution ~ 0.5 to 1.5 Siemens/m (S/m) as well as lower conductance solutions < 0.5 S/m. Other types of sample solutions include but not limited to: buffy coat blood, plasma, serum, urine, saliva, CSF, and other biological fluids, buffers and environmental samples.

(43) The example embodiments of methods, systems and device are envisioned to enable isolation and concentration, in-situ or on-chip sample preparation and processing, detection, identification, analysis and storage of important biomarkers. To enable these advantages and properties, the AC/DC electrokinetic microelectrode array chip component can include folded or sandwich chip

structures with provide an overall larger surface area to improve the performance of the system and enable isolation of relatively larger amounts of the biomarkers that would be present in the more common one to five milliliter blood draw tubes. The folded/sandwiched structures can be designed with from five to ten or more 1 cm by 1.5 cm electrokinetic microarrays/detector-sensor chips sandwiched together within the system.

(44) FIGS. **6A-6C** show diagrams illustrating an enlarged view of an example embodiment of a sandwiched electrokinetic microarray chip & detector-sensor chip **610** in accordance with example embodiments of the electrokinetic microelectrode array device **206**. As shown in FIG. **6A**, the example sandwiched electrokinetic microarray chip & detector-sensor chip **610** is included in a miniaturized electronic system **600** in accordance with example embodiments of the miniaturized electronic system **200**. The example AC/DC electrokinetic microelectrode array chip & detector-sensor chip **610** includes both an electrokinetic microarray chip **620** and a detector-sensor chip **630**. The sandwiched electrokinetic microarray chip & detector-sensor chip **610** can include features with folded or sandwich electrokinetic microarray chips **620** and a detector-sensor chips **630**, where the folded or sandwiched structure provides an overall larger surface area to improve the performance of the miniaturized electronic system **600** and enables isolation of relatively larger amounts of the biomarkers that would be present in the more common one to five milliliter blood draw tubes. The blood sample travels through the channels formed by the folded or sandwich electrokinetic microarray chips **620** and a detector-sensor chips **630**. In some implementations, the channels **615** are about 0.2 mm to 0.3 mm wide.

(45) The folded or sandwiched structures can be designed with from five to ten or more 1 cm by 1.5 cm electrokinetic microarrays chips **620** and detector-sensor chips **630** sandwiched together within the miniaturized electronic system **600**. The electrokinetic microarrays chips **620** and detector-sensor chips **630** are fabricated on a substrate **611** (e.g., a silicon substrate). The detector-sensor chip **630** incorporates one or more of uv/vis light emitter(s), mini/micro size fluorescent detector(s) and other chemical sensor(s) labeled as **614** that allow more immediate analysis of certain specific biomarkers and other blood constituents or conditions. The electrokinetic microarrays chips **620** incorporates one or more electrode(s) **612**. In some implementations the one or more electrode(s) **612** can include a noble metal such as platinum (Pt), gold (Au), and/or palladium (Pd). In some implementations, the one or more electrode(s) **612** is a carbon (C) electrode. FIG. **6A** shows that the one or more electrode(s) **612** is encapsulated by a permeation or porous layer **613**. The permeation or porous layer **613** ameliorates the adverse effects of electrolysis products formed during the biomarker collection and storage process. To prevent the targeted biomarkers from directly contacting the one or more electrode(s) **612** (and formed electrolysis products), the permeation or porous layer **613** creates a barrier that prevents the biomarkers from reaching the surface of the one or more electrode(s) **612**. While barrier prevents the biomarkers from diffusing to the surface of one or more electrode(s) **612**, the porous nature of the porous or permeation layer **613** enables the movement of electrolytes to and from the surface of the one or more electrode(s) **612**.

(46) Enlarged front views of the AC/DC electrokinetic chip **620** and the detector-sensor chip **630** are also shown in FIG. **6B**. As shown in FIG. **6B**, the electrokinetic microarray chip **620** surface includes a plurality of electrodes **621** fabricated on a substrate **622** (Shown in FIG. **6A**, labeled as one or more electrode(s) **612** and substrate **611**). The detector-sensor chip **630** includes detector sensors **631**, UV-VIS emitters **632**, and fluorescent detector and other sensors **633** also fabricated on a substrate **634**, which allows for more immediate analysis of certain specific biomarkers and other blood constituents or conditions.

(47) If desired, both smaller and larger microarrays can be fabricated for other applications. For example, in some embodiments, each electrokinetic microarray can contain anywhere from 100 to 1000 microelectrodes. The microelectrodes could range in size from 10 μm (microns) to 100 μm (microns), with center to center spacing of from 30 μm (microns) to 500 μm (microns). In some

implementations, for example, the microelectrodes are circular in shape, but other shapes (e.g., oval, square, rectangular, triangular or other) may be used for certain applications. However, both electrode sizes and spacing could be smaller $<10\text{ }\mu\text{m}$ and larger $>100\text{ }\mu\text{m}$ if desired for other applications. The spacing between the sandwiched electrokinetic/detector-sensor microarrays could range from about 0.1 mm to 1 mm, with about 0.2 mm to 0.3 mm spacing being more optimal for blood flow from one section into the next section. By way example, the size of each of the sandwiched electrokinetic/detector-sensor microarrays could be approximately 1.5 cm high by 0.5 cm wide by 0.5 mm in thickness.

(48) In some implementations, the microelectrode array and the sensor array can be fabricated by photolithographic methods on a silicon base or other support material (e.g., glass, plastic, ceramic, etc.), resulting in an electrokinetic/detector-sensor microarray that is about $100\text{ }\mu\text{m}$ (microns) to about 1.0 mm (millimeters) in thickness, preferably about 0.2 mm to about 0.5 mm. In some implementations, the permeation layer is applied later in a separate process (e.g., after the fabrication of the microelectrode array and sensor array). In some implementations, the binding/adhesions layers, insulating and dielectric (e.g., silicon dioxide, silicon nitride) material between microelectrodes, the Pt, Au, or Pd microelectrodes and final permeation layer have a thickness between about $50\text{ }\mu\text{m}$ (microns) to about $250\text{ }\mu\text{m}$ (microns). In some implementations, the binding, dielectric layers and sensor device structures on the reverse side of the silicon base would also be about $50\text{ }\mu\text{m}$ (microns) to $100\text{ }\mu\text{m}$ (microns) or less in thickness. By way of one example, about five electrokinetic/detector-sensor microarrays (each approximately 1.5 cm high by 0.5 cm wide by 0.5 mm in thickness) sandwiched together with a spacing of 0.2 mm would be suitable for a 2 mL blood draw tube.

(49) The overall size and total surface area from the example embodiment of the five microarrays (e.g., ~ 3.75 square centimeters) can provide enough capability to collect and concentrate all the desired biomarkers (e.g., cf-DNA, exosomes, etc.) from a 2 mL blood sample. In some implementations, the five sandwiched electrokinetic/detector-sensor microarrays are incorporated into the overall device with associated buffer reservoirs, battery, pumps etc. For a larger 5 mL blood draw tube, about ten to twelve electrokinetic/detector-sensor microarrays (e.g., each approximately 1.5 cm high by 0.5 cm wide by 0.5 mm in thickness) sandwiched together with a spacing of 0.2 mm is suitable. However, devices of many other sizes and shapes can be fabricated and designed to properly fit into the particular blood draw tube and effectively collect the biomarkers from the given amount of blood in the tube.

(50) The example devices, such as that shown in FIGS. 6A-6C, show one arrangement of the sandwiched electrokinetic/detector devices to allow blood flow from the inlet port through each microarray sections and then exit via the outlet port. Different sets of the electrodes and microelectrodes on the device can be arranged in unique ways to carry out many different functions. Microelectrodes can be wired individually or in groups to form various asymmetric patterns for AC DEP type isolation of biomarkers. Microelectrode wiring designed for DC applications allows certain electrodes to be biased positive while other are biased negative. Also, more sophisticated CMOS electrokinetic/detector-sensor arrays could be designed with on-chip control for each electrode being either on or off, being biased $+/-$ and to control the AC and DC voltage and current levels. CMOS and other types of semiconductor on-chip control would also allow any microelectrode geometry or pattern to be produced on the chip surface.

(51) FIG. 6C shows a diagram illustrating detail of features and components of the example electrokinetic microarray chip **620** and detector-sensor chip **630**. The basic electrokinetic microelectrode chip **620** can be fabricated on a silicon base **624**, with electrode structures **621** (e.g., Pt, Pd, Au, C, or graphite electrodes) about 20 nm to $100\text{ }\mu\text{m}$ (microns) in thickness, or more ideally, e.g., 50 nm to 500 nm. The electrodes structures **621** are insulated from one another by an insulator **622** (e.g., silicon dioxide or silicon nitride). The overall microelectrode array surface is over-coated with a porous coating **622** or permeation layer **622**. Generally, this can be an agarose or

polyacrylamide gel coating of from about 1 μm (microns) to 500 μm (microns) in thickness, or preferably ideally from 10 μm (microns) to 250 μm (microns) in thickness. However, other types of porous coating are acceptable providing they allow electrolyte contact with the surface of electrode structures **621**, are robust and bond to the surface of the electrode structures **621** reasonably well, and can be impregnated with binding agents such as streptavidin, biotin, or other binding entities. In some implementations, the permeation layer **622** or porous layer **622** can include “lawns” of surface chemicals (i.e., layers, such as self-assembled monolayers), e.g., of polyethylene glycol and other hydrophilic polymers linked to the surface. Such lawns could also contain other polymers with amine, sulfhydryl, biotin and other groups that would allow attachment of affinity binding groups such as DNA probes and antibodies.

(52) In some implementations the permeation layer **622** or porous layer **622** are porous, sintered or fritted glass permeation layers which would add structural stability and overall robustness not possible with conventional permeation layers. For example, ultra-thin porous glass structures from about 10 μm to 500 μm in thickness can be positioned and mounted over the microelectrodes and serves as the permeation layer **622** or porous layer **622**. In some implementations, pore sizes can range from 50 nm to 50 μm , preferably ranging from 100 nm to 1 μm . Such pore sizes effectively limit or significantly impede the further transport of biomarkers to the electrochemically active microelectrode surface. In some implementations, the pore space or pore volume accounts for about 10% to 40% of the overall structure, assuring contact of the electrolyte with at least the same proportion of microelectrodes. The microporous or fritted glass is available commercially or can be created from a thin solid glass coating by treatment with hydrogen fluoride (HF) gas or liquid HF. In some implementations, the thin solid glass covering over the microelectrode array can be masked by applying a HF resistant coating (e.g., photolithographically), and then HF gas or liquid applied to etch out the pores, and followed by removal HF resistant coating.

(53) In some implementations, permeation layer **622** or porous layer **622** are sintered glass permeation layers prepared from micron size glass or silica particles. Such sintered glass can be thermally (e.g., heat) fused to the surface of the microelectrode array and then HF treatment used to control pore size. Methods for modifying glass surfaces with other covalent binding or affinity binding entities that include but are not limited to DNA probes, antibodies, etc.

(54) In some implementations the permeation layer **622** or porous layer **622** are porous permeation layers made from sintered quartz. Unlike glass, quartz is conductive and imparts novel semi-conductive properties up into the permeation layer itself, with the added benefit of reduced electrochemical effects.

(55) In some examples, the electrode structures **621** are insulated from one another by an insulator **624** such as silicon dioxide or silicon nitride. The electrode structures **621** are generally deposited by sputtering process or other deposition process. However, in some implementations, robust electrodes that are resistant to degradation from electrolysis products (e.g., H^+ , OH^- , O_2 , H_2 , free radicals, etc.) are used. Also, such electrodes can be operated at higher AC and DC voltages, for longer periods of time and in higher conductivity solutions ($>0.5 \text{ S/m}$).

(56) In addition to sputtering other deposition methods for Pt, Au, and Pd electrodes include but are not limited to: (1) Ebeam Evaporation; (2) Thermal Evaporation; (3) Electrodeposition (requires a strike layer, usually Titanium base layer); (4) Electroless deposition (various substrates, but generally need an adhesion promoter, e.g. Titanium, but maybe Tungsten/Molybdenum); (4) ALD, but may be excessive and too slow (useful for monolayers), same for MBE.

(57) To ensure robust films that can withstand the corrosive electrochemical environment at the electrode interface, a continuous, smooth, defect-free surface is needed. Pt, Au, and Pd layers may be added via many different deposition methods broadly separable into physical and electrochemical. Physical vapor deposition (PVD) techniques include RF and DC sputtering; electron-beam evaporation, and thermal evaporation. Electrochemical deposition methods such as electroplating and electroless deposition on various substrates are generally lower cost, more

common for larger parts, and require specific surface preparation for smaller features.

Electrochemical deposition of Pt, Au, and Pd forms large grains, thus a rough surface, and may result in undesirable pinholes, albeit highly unlikely in thick films. However, thick films (e.g., $>1\text{ }\mu\text{m}$) are easily and economically achieved and the thick film has more material to corrode away before failure.

(58) For micron-sized patterned electrodes, a lift-off technique is commonly used, where photoresist is applied and patterned before a PVD layer is applied. The photoresist is subsequently dissolved, and the metal remains only in the photoresist windows. For thicker films (e.g., $>50\text{ nm}$), chemical vapor deposition, molecular beam epitaxy and atomic layer deposition techniques are unfavorable due to their slow growth rate and expensive precursors. Local surface annealing can be carried out by (1) Laser annealing; or (2) Spot contact annealing (via some sort of inductive heater). Annealing is shown to increase surface roughness (as the atom mobility increases) with temperature and one increases crystallinity (so will reform into larger metallic crystals) as opposed to get smoother. However, a Pt/Ti layer above Si will form into a low impedance contact. In which case a simple rapid thermal annealing would work well. Sputtered films/evaporated films are $<0.5\text{ nm}$ RMS roughness, which are suitable when the film's thickness is 100 nm or greater. Sputtering under elevated temperatures versus room temperature (RT) does not appear to make a major difference in roughness, and higher temperatures lead to slightly rougher surfaces. Laser annealing using laser annealers can affect the surface properties, or to rapidly anneal (e.g., lattice align) implanted dopants in semiconductors without giving them a chance to diffuse into the bulk. That said, these have heat zones on the order of 100 nm to $10\text{ }\mu\text{m}$, depending on the purpose. In some examples, a very low dose can ensure that only the surface is melted. Yet in some cases, ablation may be a concern. Notably, a fabrication method can involve using electrochemical deposition to achieve a thick film followed by a pulsed laser surface melt to achieve greater robustness and flatness.

(59) The overall microelectrode array surface can be over-coated with a porous coating or permeation layer **623**. In some embodiments, the porous coating or permeation layer includes agarose or polyacrylamide gel coating of from about $1\text{ }\mu\text{m}$ to 1 mm . In some embodiments, the porous coating or permeation layer has a thickness about $10\text{ }\mu\text{m}$ to $50\text{ }\mu\text{m}$. However, other types of porous coating are acceptable providing they allow electrolyte contact with the surface of the electrode structures **621**, are robust and bond to the electrode surface reasonably well, and can be impregnated with binding agents such as streptavidin, biotin, etc., that allow attachment of other entities such as oligonucleotide probes, etc.

(60) Example methods and constructs are used with electrokinetic devices that combine AC dielectrophoretic (DEP) and DC electrophoresis to first rapidly isolate and collect biomarkers from the blood onto specific electrodes, microelectrode or defined areas of a microarray chip device. Generally, depending for AC frequencies used entities in the range of from about 20 nm to $20\text{ }\mu\text{m}$ can be isolated when in the alternating current (AC) or DEP mode.

(61) FIG. 7 shows a diagram illustrating an example implementation of the example electrokinetic microarray device **700**. In the example, the electrokinetic microarray is shown being operated at: (a) low AC frequency conditions (labeled **710**); (b) high AC frequency conditions (labeled **720**); and (c) under DC electrophoretic conditions (labeled **730**). Under lower AC frequency conditions, biomarkers that can be isolated include cf-DNA, RNA, exosomes, EVs, and nucleosomes (labeled **710**, FIG. 7). For example, in the lower AC frequency range of from about 1 kHz to 20 kHz , using voltages in the range of about 1 V to 20 V AC, biomarkers in the range of 20 nm to 800 nm can be moved, concentrated and firmly held in the DEP high-field regions areas on or near the edges of the microelectrodes. These would include but are not limited to high molecular weight (hmw) cell free (cf) DNA, RNA, protein aggregates, nanoparticles, exosomes, EVs, and other entities. Under these conditions, larger entities greater than 800 nm (e.g., some cells, etc.) can be attracted to the DEP low-field regions, however, modest fluid flow will remove the cells from the low-field areas

and also wash away the much smaller molecules and biomolecules (panel **710**, FIG. 7). Generally, entities less than about 20 nm or 10,000-20,000 MW less effected by the DEP fields. Under high AC frequency conditions, specific cells, such as rare cancer cells can be isolated from red and white blood cells in the blood sample. For example, operation at higher AC frequencies that can range from about 20 kHz to 1000 kHz and AC voltages in the range of about 1V-30V allows larger entities of about 1 μm to 20 μm in size to now be isolated onto the DEP high-field regions. Selection of specific frequencies will allow isolation of specific cells, such as cancer cells to be carried out (**720**, FIG. 8). Because the electrokinetic device is a folded or sandwiched structure many different separation patterns can be achieved by changing the AC frequencies and voltages/currents the individual microarrays or sections of the microarray device. By way of example, one section of the device can be run at the AC frequencies of from 1 kHz to 20 kHz that allows isolation of the biomarkers in the 20 nm to 800 nm range, while another section is operated at AC frequencies in the 20 kHz to 1000 kHz range that now allow isolation of different cell types (e.g., cancer cells) to be isolated. In addition to AC operation, the electrokinetic chip device can also be operated in the direct current (DC) mode for electrophoretic isolation of biomarkers or other entities. In this case, the biomarkers and entities are attracted by charge; negatively charged entities attracted to the positively biased microelectrodes and the positively charged entities attracted to the negatively biased electrodes; and liked charged entities are repelled from the respectively biased electrodes (**730**, FIG. 7). Generally, the electrophoretic process is achieved by applying a DC voltage in the range of from about 1V to 30V.

(62) FIG. 8 shows a block diagram illustrating an example embodiment of a battery-powered device control system **800** with a microarray chip device **870**, which includes an example embodiment of the electrokinetic microelectrode array (such as the microarray chip device **260**, shown in FIG. 2). The microarray chip device **870** includes an AC/DC electrokinetic chip **870a** and a sensor-detector chip **870b**. The battery-powered device control system **800** is powered by a miniature lithium ion battery **810** (e.g., a 3.7V lithium ion battery) that is in operative communication with a battery/power controller and converter **820** to regulate the rate at which electric current is added to or drawn from lithium ion battery **810**. The battery/power controller & converter **820** is then in operative communication with a microcontroller unit **830**, which directs a signal to a direct digital synthesizer **840** to produce controlled wave forms of various frequencies. The direct digital synthesizer **840** is in operative communication with a variable gain amplifier **850** that passes the signal to a fixed gain output amplifier **860**. The fixed gain output amplifier is then in operative communication with the microarray chip device **870**, where the biomarkers are in the blood samples pass through. In some embodiments, the example battery-powered device control system **800** includes microfluidics **880**, which can comprise the micropump & control valve unit **230**, the fluidic sensor **240**, and/or the buffer and reagent reservoirs **220**.

(63) In some implementations, for example, some embodiments of the electrokinetic microelectrode array device **260** can use porous membranes as mechanical filters and to create DEP high/low field regions. These can be made from conductive materials to serve as interleaved electrode layers (e.g., foil/wire mesh) and/or from insulating materials doing i-DEP. For example, applying a dielectrophoretic force through a porous media can serve as an effective filter to selecting positive-DEP particulate, even when the identically-sized particle are unable to diffuse through the media passively. This can add an additional layer of size selection and obviates concerns about capturing desired particulate within a tip.

(64) In some implementations, for example, some embodiments of the electrokinetic microelectrode array device **260** can utilize insulation-based DEP topologies as a good match to high conductivity solutions. One can design the electrodes to be large, thus minimize local current density and its attendant corrosive effects. Electric fields may instead be constricted into large gradients in regions far away from the electrodes. Additionally, nonreactive insulating materials may be selected for the constrictive regions instead of chemically-active metals, further reducing

device degradation. Constrictions in insulation-based DEP may be sufficiently distributed to ensure that any local Joule heating may be diffused over a much greater area, lowering peak temperatures and minimizing thermal gradients. Most efforts into insulator-based DEP has been focused into small devices where that require extensive lithographic patterning, which greatly limits overall active surface area and is expensive. Bulk structures, where the material itself can reliably formed into small geometries without need for patterning, allow for scaling device size over several orders of magnitudes.

(65) FIG. 9 shows an illustrative diagram of example embodiments of an insulator-based electrokinetic DEP microelectrode devices **900A** and **900B** integrated in a blood draw tube **902**, e.g., sealable with a cap **901** and/or a septum **905**. Architecturally, both example devices **900A** and **900B** operate on the same principle, where a porous, open-cell, membrane **950** made of a dielectric medium is placed as to separate the blood draw tube **902** into a blood side and a buffer side. The devices **900A** and **900B** include electrodes **960** (e.g., such as microelectrodes) that are placed in each of the blood medium **903** and the buffer medium **904**, respectively, such that the electric field applied between the electrodes **960** is locally constrained through the porous membrane **950**, thus drawing positive-DEP particles to the region. Diffusion of even small particles across the barrier is extremely slow, essentially inhibiting any non-targeted transport. By applying an electric field across the membrane, local high field gradients are realized throughout the porous structures, drawing particles, e.g. cell-free DNA, RNA or exosomes, into the membrane via positive-DEP.

EXAMPLES

(66) Some example embodiments of systems, devices and methods in accordance with the disclosed technology include the following example features, whether alone or in any combination with another or other features.

(67) In some example embodiments, a system, device and/or method includes a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip.

(68) In some example embodiments, a system, device and/or method includes a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip.

(69) In some example embodiments, a system, device and/or method includes a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip, that is incorporated and packaged into an electronic system for isolation of biomarkers from undiluted blood.

(70) In some example embodiments, a system, device and/or method includes a packaged electronic system containing a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip and other components that is incorporated into a blood draw collection tube.

(71) In some example embodiments, a system, device and/or method includes a packaged electronic system containing a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip and other components that is incorporated into a blood draw collection tube, that isolates, concentrates and protects biomarkers for later analysis in the clinical laboratory.

(72) In some example embodiments, a system, device and/or method includes a packaged electronic system containing a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip and other components that is incorporated into a blood draw collection tube, that isolates, concentrates and protects biomarkers, where the detector-sensor chip component can carry out more immediate detection of biomarkers.

(73) In some example embodiments, a system, device and/or method includes a packaged electronic system containing a high surface area folded or sandwiched AC/DC electrokinetic microelectrode array chip flip-chip bonded to a detection-sensor chip and other components that can be added or dropped into other containers with other sample solutions.

(74) In some example embodiments, a system, device and/or method includes a method involving the use of electrochemical deposition to achieve a thick film followed by a pulsed laser surface melt to achieve greater flatness, robustness and resistance to degradation by electrolysis products.

(75) The disclosed systems, devices and methods can be applied in a variety of applications. Some examples include blood draw, storage and transport to the clinical laboratory. For example, the disclosed devices are capable of carrying out the more immediate analysis of certain specific biomarkers. By way of just one example, the immediate measurement and reporting of the cf-DNA level in the blood is valuable as this often indicates that serious pathology or other problems exists. For example, the example systems and devices can be operated in un-diluted blood and other high conductance sample solutions provides major advantages over conventional systems. Other example attributes include that, in addition to blood draw tubes, blood collection bags and other blood storage containers, the disclosed devices can be incorporated or simply added (dropped into) urine collection tubes, and other collection tubes and containers used for many other clinical, biological and/or research applications.

(76) In some embodiments in accordance with the present technology (example 1), an electronic device for biomarker analysis in a biofluid includes an electrokinetic microelectrode array chip operable to separate and isolate a biomarker in a biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces, the electrokinetic microelectrode array chip including: a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array chip includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel, and wherein the electrokinetic microelectrode array chip device is able to be incorporated within a container to hold the biofluid.

(77) Example 2 includes the device of any of examples 1-20, wherein the one or more detectors, sensors, and/or emitters includes at least one of a uv/vis light emitter, a fluorescent detector, or a chemical sensor.

(78) Example 3 includes the device of any of examples 1-20, wherein the electronic device further includes a housing that surrounds the electrokinetic microelectrode array chip, a power unit, and an electronic control unit.

(79) Example 4 includes the device of any of examples 1-20, wherein the electronic control unit includes a frequency generator and an RF transmitter.

(80) Example 5 includes the device of any of examples 1-20, wherein the electronic control unit includes a processing unit comprising a processor and a memory.

(81) Example 6 includes the device of any of examples 1-20, wherein the electronic device further includes a fluidic sensor operable to detect a fluidic parameter of the biofluid.

(82) Example 7 includes the device of any of examples 1-20, wherein the electronic device further includes a microfluidic pump operable to draw the biofluid into and/or out of the electrokinetic microelectrode array chip.

(83) Example 8 includes the device of any of examples 1-20, wherein the electrokinetic microelectrode array chip provides a high surface area based on the folded or sandwiched configuration.

(84) Example 9 includes the device of any of examples 1-20, wherein the electrokinetic microelectrode array chip is flip-chip bonded to a detection-sensor chip.

(85) Example 10 includes the device of any of examples 1-20, wherein the electrokinetic microelectrode array chip is incorporated and packaged in a fluid collection tube.

(86) Example 11 includes the device of any of examples 1-20, wherein the fluid collection tube includes a blood draw collection tube, and the device is operable to isolate, concentrate and protect

the biomarker for analysis, including a later analysis in a clinical laboratory.

(87) Example 12 includes the device of any of examples 1-20, wherein the fluid collection tube includes a blood draw collection tube, and the detector-sensor chip component is operable to carry out an immediate detection of the biomarker.

(88) Example 13 includes the device of any of examples 1-20, wherein the permeation layer includes one or more of porous glass, sintered glass, or silica.

(89) Example 14 includes the device of any of examples 1-20, wherein the permeation layer includes porous and sintered quartz and has conductive properties.

(90) Example 15 includes the device of any of examples 1-20, wherein the biofluid is undiluted blood.

(91) Example 16 includes the device of any of examples 1-20, wherein the biomarker is one or more of cell free (cf) DNA, RNA, nucleosomes, exosomes, extracellular vesicle (EVs), drug delivery nanoparticles, cell organelles, proteins, enzymes, protein complexes, virus, bacteria, and cancer cells.

(92) Example 17 includes the device of any of examples 1-20, comprising from five to ten electrokinetic microelectrode array chips in the folded or sandwiched configuration.

(93) Example 18 includes the device of any of examples 1-20, wherein the electrokinetic microelectrode array chips are 1 cm by 1.5 cm.

(94) Example 19 includes the device of any of examples 1-20, wherein the microfluidic channel between the five to ten electrokinetic microelectrode array chips is between about 0.1 mm to about 1 mm.

(95) Example 20 includes the device of any of examples 1-19, wherein the microfluidic channel between the five to ten electrokinetic microelectrode array chips is between about 0.2 mm.

(96) In some embodiments in accordance with the present technology (example 21), a method of analyzing one or more biomarkers in a biofluid includes obtaining a biofluid from a subject, wherein the biofluid is placed in a collection tube including an electrokinetic microelectrode array including a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel; contacting the biofluid with the electrokinetic microelectrode array to separate and isolate the one or more biomarkers in the electrokinetic microelectrode array, wherein the electrokinetic microelectrode array separates and isolates the one or more biomarker(s) from the biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces; and analyzing the one or more biomarkers in the biofluid.

(97) Example 22 includes the method of any of examples 21-31, comprising introducing a stabilizing buffer onto the electrokinetic electrode array containing the one or more biomarkers to stabilize the one or more biomarkers for storage, transport, or both.

(98) Example 23 includes the method of any of examples 21-31, wherein electrokinetic microelectrode array is operable to carry out an immediate detection of the one or more biomarkers.

(99) Example 24 includes the method of any of examples 21-31, wherein the immediate detection of the one or more biomarkers is reported via an RF signal to a cellular device.

(100) Example 25 includes the method of any of examples 21-31, comprising transporting the one or more biomarkers in the electrokinetic electrode array to a clinical setting, removing the one or more biomarkers from the electrokinetic electrode array, and analyzing the one or more biomarkers.

(101) Example 26 includes the method of any of examples 21-31, wherein the analysis includes one

or more of immunofluorescent detection, PCR, RT PCR analysis of RNA, DNA sequencing and epigenetic analyses, or DNA genotyping.

(102) Example 27 includes the method of any of examples 21-31, wherein the one or more biomarkers is one or more of cell free (cf) DNA, RNA, nucleosomes, exosomes, extracellular vesicle (EVs), drug delivery nanoparticles, cell organelles, proteins, enzymes, protein complexes, virus, bacteria, or cancer cells.

(103) Example 28 includes the method of any of examples 21-31, wherein the biofluid is blood, buffy coat blood, plasma, serum, urine, saliva, and cerebrospinal fluid (CSF).

(104) Example 29 includes the method of any of examples 21-31, wherein about 1 mL to about 5 mL of the biofluid is obtained from the subject.

(105) Example 30 includes the method of any of examples 21-31, wherein the biofluid is unprocessed blood.

(106) Example 31 includes the method of any of examples 21-30, wherein about 1 mL to about 5 mL of unprocessed blood is obtained from the subject.

(107) In some embodiments in accordance with the present technology (example P1), an electronic device for biomarker analysis in a biofluid includes an electrokinetic microelectrode array chip operable to separate and isolate a biomarker in a biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces for concentration and/or storage of the biomarker, wherein the electrokinetic microelectrode array chip device is incorporated within a container to hold the biofluid.

(108) Example P2 includes the device of any of examples P1-P10, wherein the electrokinetic microelectrode array chip includes: a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array chip includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, and/or emitters on the second side of the support structure that forms a microfluidic channel.

(109) Example P3 includes the device of any of examples P1-P10, wherein the one or more detectors, sensors, and/or emitters includes at least one of a uv/vis light emitter, a fluorescent detector, or a chemical sensor.

(110) Example P4 includes the device of any of examples P1-P10, wherein electrokinetic microelectrode array chip includes one or more of: a device substrate, a power unit, an electronic control unit including a frequency generator and an RF transmitter, one or more fluid reservoirs to store a buffer fluid and/or a reagent fluid, a fluidic sensor, and/or a microfluidic pump.

(111) Example P5 includes the device of any of examples P1-P10, wherein the electrokinetic microelectrode array chip provides a high surface area based on the folded or sandwiched configuration.

(112) Example P6 includes the device of any of examples P1-P10, wherein the electrokinetic microelectrode array chip is flip-chip bonded to a detection-sensor chip.

(113) Example P7 includes the device of any of examples P1-P10, wherein the electrokinetic microelectrode array chip is incorporated and packaged in a fluid collection tube.

(114) Example P8 includes the device of any of examples P1-P10, wherein the fluid collection tube includes a blood draw collection tube, and the device is operable to isolate, concentrate and protect the biomarker for analysis, including a later analysis in a clinical laboratory.

(115) Example P9 includes the device of any of examples P1-P10, wherein the fluid collection tube includes a blood draw collection tube, and the detector-sensor chip component is operable to carry out an immediate detection of the biomarker.

(116) Example P10 includes the device of any of examples P1-P9, wherein the biofluid is undiluted blood.

(117) Implementations of the subject matter and the functional operations described in this patent document can be implemented in various systems, digital electronic circuitry, or in computer software, firmware, or hardware, including the structures disclosed in this specification and their structural equivalents, or in combinations of one or more of them. Implementations of the subject matter described in this specification can be implemented as one or more computer program products, i.e., one or more modules of computer program instructions encoded on a tangible and non-transitory computer readable medium for execution by, or to control the operation of, data processing apparatus. The computer readable medium can be a machine-readable storage device, a machine-readable storage substrate, a memory device, a composition of matter effecting a machine-readable propagated signal, or a combination of one or more of them. The term “data processing unit” or “data processing apparatus” encompasses all apparatus, devices, and machines for processing data, including by way of example a programmable processor, a computer, or multiple processors or computers. The apparatus can include, in addition to hardware, code that creates an execution environment for the computer program in question, e.g., code that constitutes processor firmware, a protocol stack, a database management system, an operating system, or a combination of one or more of them.

(118) A computer program (also known as a program, software, software application, script, or code) can be written in any form of programming language, including compiled or interpreted languages, and it can be deployed in any form, including as a stand-alone program or as a module, component, subroutine, or other unit suitable for use in a computing environment. A computer program does not necessarily correspond to a file in a file system. A program can be stored in a portion of a file that holds other programs or data (e.g., one or more scripts stored in a markup language document), in a single file dedicated to the program in question, or in multiple coordinated files (e.g., files that store one or more modules, sub programs, or portions of code). A computer program can be deployed to be executed on one computer or on multiple computers that are located at one site or distributed across multiple sites and interconnected by a communication network.

(119) The processes and logic flows described in this specification can be performed by one or more programmable processors executing one or more computer programs to perform functions by operating on input data and generating output. The processes and logic flows can also be performed by, and apparatus can also be implemented as, special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application specific integrated circuit).

(120) Processors suitable for the execution of a computer program include, by way of example, both general and special purpose microprocessors, and any one or more processors of any kind of digital computer. Generally, a processor will receive instructions and data from a read only memory or a random access memory or both. The essential elements of a computer are a processor for performing instructions and one or more memory devices for storing instructions and data. Generally, a computer will also include, or be operatively coupled to receive data from or transfer data to, or both, one or more mass storage devices for storing data, e.g., magnetic, magneto optical disks, or optical disks. However, a computer need not have such devices. Computer readable media suitable for storing computer program instructions and data include all forms of nonvolatile memory, media and memory devices, including by way of example semiconductor memory devices, e.g., EPROM, EEPROM, and flash memory devices. The processor and the memory can be supplemented by, or incorporated in, special purpose logic circuitry.

(121) It is intended that the specification, together with the drawings, be considered exemplary only, where exemplary means an example. 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. Additionally, the use of “or” is intended to include “and/or”, unless the context clearly indicates otherwise.

(122) While this patent document contains many specifics, these should not be construed as

limitations on the scope of any invention or of what may be claimed, but rather as descriptions of features that may be specific to particular embodiments of particular inventions. Certain features that are described in this patent document in the context of separate embodiments can also be implemented in combination in a single embodiment. Conversely, various features that are described in the context of a single embodiment can also be implemented in multiple embodiments separately or in any suitable subcombination. Moreover, although features may be described above as acting in certain combinations and even initially claimed as such, one or more features from a claimed combination can in some cases be excised from the combination, and the claimed combination may be directed to a subcombination or variation of a subcombination.

(123) Similarly, while operations are depicted in the drawings in a particular order, this should not be understood as requiring that such operations be performed in the particular order shown or in sequential order, or that all illustrated operations be performed, to achieve desirable results.

Moreover, the separation of various system components in the embodiments described in this patent document should not be understood as requiring such separation in all embodiments.

(124) Only a few implementations and examples are described and other implementations, enhancements and variations can be made based on what is described and illustrated in this patent document.

Claims

1. A method of analyzing one or more biomarkers in a biofluid, comprising: obtaining a biofluid from a subject, wherein the biofluid is placed in a collection tube comprising therein an electrokinetic microelectrode array including: a support structure, an array of microelectrodes coupled to a first side of the support structure, a permeation layer at least partially formed on the array of microelectrodes over the support structure, and one or more detectors, sensors, and/or emitters coupled to a second side of the support structure, wherein the electrokinetic microelectrode array includes a folded or a sandwiched configuration of the array of microelectrodes on the first side of the support structure and the one or more detectors, sensors, emitters, or combination thereof on the second side of the support structure that forms a microfluidic channel; contacting the biofluid with the electrokinetic microelectrode array to separate and isolate the one or more biomarkers in the electrokinetic microelectrode array, wherein the electrokinetic microelectrode array separates and isolates the one or more biomarker(s) from the biofluid using one or both of AC dielectrophoretic (DEP) and DC electrophoretic based forces; and analyzing the one or more biomarkers in the biofluid.
2. The method of claim 1, comprising introducing a stabilizing buffer onto the electrokinetic electrode array containing the one or more biomarkers to stabilize the one or more biomarkers for storage, transport, or both.
3. The method of claim 1, comprising: transporting the one or more biomarkers in the electrokinetic electrode array to a clinical setting, removing the one or more biomarkers from the electrokinetic electrode array, and analyzing the one or more biomarkers.
4. The method of claim 1, wherein the analysis includes one or more of immunofluorescent detection, PCR, RT PCR analysis of RNA, DNA sequencing and epigenetic analyses, or DNA genotyping.
5. The method of claim 1, wherein the one or more biomarkers is one or more of cell free (cf) DNA, RNA, nucleosomes, exosomes, extracellular vesicle (EVs), drug delivery nanoparticles, cell organelles, proteins, enzymes, protein complexes, virus, bacteria, or cancer cells, and wherein the biofluid is blood, buffy coat blood, plasma, serum, urine, saliva, or cerebrospinal fluid (CSF).
6. The method of claim 1, wherein about 1 mL to about 5 mL of the biofluid is obtained from the

subject.

7. The method of claim 1, wherein the biofluid is unprocessed blood.
