Abstract:

Devices and methods for immobilizing nucleic acids on a substrate include a voltage source, and a substrate coupled to the voltage source, in which hydrophobicity of the substrate changes in response to an applied electric field, and a surface of the substrate is coated with a substance that retains nucleic acids.
DEVICES AND METHODS FOR IMMOBILIZING NUCLEIC ACIDS

Related Application
The present application claims the benefit of and priority to U.S. nonprovisional application serial number 13/190,947, filed July 26, 2011, the content of which is incorporated by reference herein in its entirety.

Field of the Invention
The present invention generally relates to devices and methods for immobilizing nucleic acids on a substrate.

Background
Physical genomic mapping using restriction endonucleases can provide accurate information about the nucleic acid sequences of various organisms. Optical mapping can be used to produce ordered restriction maps that are visualized using fluorescence microscopy. In optical mapping, nucleic acids are digested by restriction enzymes on a glass surface. The nucleic acids are fixed and elongated on the surface to provide access to restriction sites for the enzymes. Generally, a microchannel is temporarily sealed to a charged glass substrate by mechanical placement, a small volume of nucleic acid solution is flowed into the resulting microchannels, excess nucleic acid solution is removed from the surface/channel interface, followed by the removal of the microchannel to continue processing for optical mapping protocols.

A problem with deposition techniques that use microchannels is that the manual intervention required significantly hinders development of high throughput optical mapping protocols. Further, standard microchannel protocols are not optimal with respect to scalability and automation for high-throughput optical mapping.

Previous attempts to automate the optical mapping process have relied on microfluidic chips that include vents, valves, and pumps. Those chips have many moving parts and have proved to be unreliable and inefficient for fluid movement during the optical mapping process.

There is a need for methods that provide a mechanism of delivering stretched individual nucleic acid molecules to a substrate for high throughput optical mapping.
Summary

The present invention generally provides devices and methods that use electrowetting to control fluid flow for deposition and elongation of nucleic acids on a charged substrate.

Electrowetting involves modifying the surface tension of liquids on a solid surface using a voltage. By applying a voltage, the wetting properties of a hydrophobic surface can be modified and the surface becomes increasingly hydrophilic (wettable). Devices and methods of the invention are driven by voltage, thus eliminating moving parts (e.g., vents, valves, and pumps), and also eliminating the need for bulk flow based nucleic acid deposition techniques and the need for microchannels. Methods of the invention allow for the automated immobilization and characterization of nucleic acids that have been fixed and elongated on a surface.

In certain aspects, devices of the invention include a voltage source, and a substrate coupled to the voltage source, in which hydrophobicity of the substrate changes in response to an applied electric field and a surface of the substrate is coated with a substance that retains nucleic acids. Exemplary substrates include a flow cell or a channel. An exemplary surface includes glass, preferably glass that is coated with silanes. The surface also includes a conductive material, such as metal. Devices of the invention may also include reservoirs that are fluidically coupled to the substrate.

Other aspects of the invention provide methods for elongating nucleic acids on a substrate. Methods of the invention include temporarily applying an electric field to temporarily decrease hydrophobicity of a charged substrate, thereby causing a sample fluid comprising a nucleic acid to temporally flow onto the charged substrate, and maintaining the electric field for a time sufficient to allow the nucleic acid to interact with the charged substrate and become elongated and fixed on the substrate. The nucleic acid may be DNA or RNA and may have any origin, e.g., human or microorganism.

Other aspects of the invention provide methods for characterizing nucleic acids. Such methods include temporarily applying an electric field to temporarily decrease hydrophobicity of a charged substrate, thereby causing a sample fluid comprising a nucleic acid to temporally flow onto the charged substrate, maintaining the electric field for a time sufficient to allow the nucleic acid to interact with the charged substrate and become elongated and fixed on the substrate so that the nucleic acid remains accessible for enzymatic reactions, intermittently re-applying the
electric field to cause reagents to flow to and from the substrate to wash, enzymatically digest, and stain the nucleic acid to obtain one or more restriction digests of the nucleic acid, and imaging the restriction digests, thereby characterizing the nucleic acid. Methods of the invention may further include constructing an optical map from the restriction digests.

The nucleic acid may DNA or RNA and may have any origin, e.g., human or microorganism. In particular embodiments, the nucleic acid is from a microorganism, such as a bacterium. The bacterium may be any bacterium. In certain embodiments, the bacterium is a species of *E. coli* or *S. aureus*. The strain of *S. aureus* may be a community-acquired methicillin-resistant strain of *S. aureus* or a hospital-acquired methicillin-resistant strain of *S. aureus*. The nucleic acid may include substantially all genomic DNA of the bacterium. In certain embodiments, the nucleic acid includes a transcriptome of the bacterium.

**Detailed Description**

In certain aspects, the invention provides devices for depositing and elongating nucleic acids on a charged substrate. Devices of the invention may include a voltage source and a substrate coupled to the voltage source, in which hydrophobicity of the substrate changes in response to an applied electric field and a surface of the substrate is coated with a substance that retains nucleic acids.

Devices of the invention utilize electrowetting to control movement of fluid.

Electrowetting-on-dielectric microfluidics is based on the actuation of droplet volumes up to several microliters using the principle of modulating the interfacial tension between a liquid and an electrode coated with a dielectric layer. An electric field established in the dielectric layer creates an imbalance of interfacial tension if the electric field is applied to only one portion of the droplet on an array, which forces the droplet to move.

Devices of the invention are based on charge control manipulation at the solution/insulator interface of discrete droplets by applying voltage to a control electrode. Devices of the invention exhibits bilateral transport, are electrically isolated, use a gate electrode for charge-controlled transport, have a threshold voltage, and are a square-law device in the relation between droplet velocity and gate actuation voltage.

Further description of devices that utilize electrowetting to control fluid movement and methods of using electrowetting for fluid manipulation are shown for example in Fair
(Microfluidics and Nanofluidics, 3:245-281, 2007); Fair et al. (IEEE Design & Test of
Computers, 24:10-24, 2007); Song (Microfluidics and Nanofluidics, 7:75-89, 2009); Chakrabarty
(IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems, 29:1001-
1017, 2010); Pollack et al. (Applied Physics Letters, 77(11), 2000); Pollack et al. (Lab on a Chip,
2:96-101, 2002); Su et al. (Proc. IEEE International Test Conference, 1192-1200, 2003); Fair et
al. ("Electrowetting-based On-Chip Sample Processing for Integrated Microfluidics", IEEE Inter.
Electron Devices Meeting (IEDM), 2003); Ren et al. (Sensors and Actuators B (Chemical),
B98:3 19-327, 2004); Fei et al. (IEEE Transactions on Computer-Aided Design of Integrated
Circuits and Systems, 25:211-223, 2006); Pamula et al. (U.S. patent numbers 6,911,132;
7,329,545; 7,439,014; and 7,569,129); Kolar et al. (U.S. patent number 6,989,234); and Pollack
et al. (U.S. patent number 7,759,132). The content of each of the above is incorporated by
reference herein in its entirety.

In certain embodiments, devices of the invention include a metal coated insulator that is
about 1 to about 20 micrometers thick. The insulator is coated with a hydrophobic material at a
thickness of about 0.1 to about 1 micrometer. Exemplary materials include Paralyene C and
Teflon. These components are combined with a charged surface and held together by glue to
build the device. The charged surface may be glass that is derivatized with silanes. The device
further includes at least one solution port for connection to at least one fluid reservoir. The metal
compartment of the device and the solution port are connected to a controllable voltage source.

The voltage source is coupled to a controller. The controller is used to set the voltage and
the timing, allowing for devices of the invention to flow several solutions onto the charged
surface at defined sequences and times. Electrowetting drives the movement of fluid onto the
charged substrate.

Devices of the invention may be used to generate optical maps. Optical mapping is a
single-molecule technique for production of ordered restriction maps from a single DNA
molecule (Samad et al., Genome Res. 5:1-4, 1995). During some applications, individual
fluorescently labeled DNA molecules are elongated and fixed on the surface using methods of
the invention. The added endonuclease cuts the DNA at specific points, and the fragments are
imaged. Id. Exemplary endonucleases include BgIII, Ncol, Xbal, and BamHI. Exemplary
combinations of restriction enzymes include:

AfIII  ApaLI  BgIII
Afln  BglII  Ncol
ApaLI  BglII  Ndel
AATI  BglII  Mlul
AATI  BglII  PacI

5  AflII  Mlul  Ndel
BglII  Ncol  Ndel
AflII  ApaLI  Mlul
ApaLI  BglII  Ncol
AflII  ApaLI  BamHI

10  BglII  EcoRI  Ncol
BglII  Ndel  PacI
BglII  Bsu36I  Ncol
ApaLI  BglII  Xbal
ApaLI  Mlul  Ndel

15  ApaLI  BamHI  Ndel
BglII  Ncol  Xbal
BglII  Mlul  Ncol
BglII  Ncol  Pad
Mlul  Ncol  Ndel

20  BamHI  Ncol  Ndel
BglII  Pad  Xbal
Mlul  Ndel  Pad
Bsu36I  Mlul  Ncol
ApaLI  BglII  Nhel

25  BamHI  Ndel  Pad
BamHI  Bsu36I  Ncol
BglII  Ncol  PvuII
BglII  Ncol  Nhel
BglII  Nhel  Pad
Restriction maps can be constructed based on the number of fragments resulting from the digest. \textit{Id.} Generally, the final map is an average of fragment sizes derived from similar molecules. \textit{Id.}


Optical Maps are constructed as described in Reslewic et al., Appl Environ Microbiol. 2005 Sep; 71 (9):551-22, incorporated by reference herein. Briefly, individual chromosomal fragments from test organisms are immobilized on derivatized glass by virtue of electrostatic interactions between the negatively-charged DNA and the positively-charged surface, digested with one or more restriction endonuclease, stained with an intercalating dye such as YOYO-1 (Invitrogen) and positioned onto an automated fluorescent microscope for image analysis. Since the chromosomal fragments are immobilized, the restriction fragments produced by digestion with the restriction endonuclease remain attached to the glass and can be visualized by fluorescence microscopy, after staining with the intercalating dye. The size of each restriction fragment in a chromosomal DNA molecule is measured using image analysis software and identical restriction fragment patterns in different molecules are used to assemble ordered restriction maps covering the entire chromosome.

An exemplary protocol for using devices of the invention to generate an optical map is described herein. A sample containing nucleic acids is obtained. The sample may be a human tissue or body fluid. A tissue is a mass of connected cells and/or extracellular matrix material, e.g. skin tissue, nasal passage tissue, CNS tissue, neural tissue, eye tissue, liver tissue, kidney tissue, placental tissue, mammary gland tissue, placental tissue, gastrointestinal tissue, musculoskeletal tissue, genitourinary tissue, bone marrow, and the like, derived from, for example, a human or other mammal and includes the connecting material and the liquid material in association with the cells and/or tissues.

A body fluid is a liquid material derived from, for example, a human or other mammal. Such body fluids include, but are not limited to, mucous, blood, plasma, serum, serum derivatives, bile, blood, maternal blood, phlegm, saliva, sweat, amniotic fluid, mammary fluid,
urine, and cerebrospinal fluid (CSF), such as lumbar or ventricular CSF. A sample may also be a fine needle aspirate or biopsied tissue. A sample also may be media containing cells or biological material.

The sample may also be an environmental sample such as water, air, dirt, rock, etc. In other embodiments, the sample is a food sample.

Nucleic acid is then extracted from the sample. Methods of extracting nucleic acids and methods of purifying biological samples are known in the art. See for example, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y. (1982).

The process further involves capture and elongation of nucleic acid on a charged substrate. A solution containing the extracted nucleic acids is loaded into a first fluid reservoir and the reservoir is coupled to a solution port of the device. Due to the hydrophobicity of the surface of the device, the solution remains in the reservoir and does not flow onto the substrate.

The voltage source of the device is turned on, resulting in the hydrophobic surface becoming more hydrophilic, which causes the solution containing the nucleic acid to flowed from the first reservoir onto the charged substrate. The charged substrate can be composed of any material that is suitable for optical mapping and is compatible with nucleic acids. Exemplary materials include polymers, ceramics, glass, or metals. In a preferred embodiment, the surface is glass, such as a microscope slide. Because a net positive charge is require to capture/retain nucleic acids, the surface includes silanes to impart a net positive charge to the surface. Interaction between the nucleic acid in the solution and the charged substrate results in capture and elongation of the nucleic acid on the substrate.

After a sufficient time is allowed for the nucleic acid solution to interact with the charged substrate, the voltage is turned off and the solution returns to the reservoir because the surface changes from hydrophilic to hydrophobic. This process may optionally be repeated several time to ensure that a sufficient amount of nucleic acid has been fixed and elongated on the charged substrate.

After fixation and elongation of the nucleic acids on the charged substrate, the first fluid reservoir is disconnected from the device and a second fluid reservoir is connected to the device. The second fluid reservoir includes a buffer, such as TE buffer. The voltage is turned on, causing the TE buffer to flow from the second reservoir onto the charged substrate. The voltage is maintained for a time sufficient to rinse the substrate and remove nucleic acids that have not
become fixed to the substrate. An exemplary amount of time is one minute. The voltage is then turned off, and the TE buffer returns to the second reservoir.

The second fluid reservoir is disconnected from the device and a third fluid reservoir is connected to the device. The third fluid reservoir includes a solution of restriction enzymes in a suitable buffer. Enzymes for use with optical mapping are discussed above. The voltage is turned on, causing the digestion solution to flow from the third reservoir onto the charged substrate. The voltage is maintained for a time sufficient to allow the restriction enzymes to interact with the nucleic acids and digest the nucleic acids on the substrate. Since nucleic acids have charge, the digestion of the nucleic acids may be manipulated by the applied voltage. After a sufficient time has elapsed, the voltage is turned off and the digestion solution returns to the third reservoir.

After digestion, the third fluid reservoir is disconnected from the device and a fourth fluid reservoir is connected to the device. The fourth fluid reservoir includes a nucleic acid staining solution, such as YOYO-1. The voltage is turned on, causing the staining solution to flow from the fourth reservoir onto the charged substrate. The voltage is maintained for a time sufficient to allow the staining solution to interact with the digested nucleic acids. After a sufficient time has elapsed, the voltage is turned off and the staining solution returns to the fourth reservoir. The substrate is now ready for image analysis.

Restriction mapping, e.g., optical mapping, can be used in a variety of applications. For example, the methods featured herein can be used to determine a property, e.g., physical and/or chemical property, e.g., size, length, restriction map, weight, mass, sequence, conformational or structural change, pKa change, distribution, viscosity, rates of relaxation of a labeled and/or non-labeled molecule, e.g., an amplicon (e.g., PCR product), of a portion of a genome (e.g., a chromosome), or of an entire genome.

Optical mapping can also be used to identify various organisms, e.g., viruses and prions, and various microorganisms, e.g., bacteria, protists, and fungi, whose genetic information is stored as DNA or RNA by correlating the restriction map of a nucleic acid of an organism with a restriction map database. Such identification methods can be used in diagnosing a disease or disorder. Methods of identifying organisms by restriction mapping are described, e.g., in a U.S. Patent Application Serial Number 12/120,586, filed on May 14, 2008, incorporated herein by reference. The methods featured herein can also be used in other diagnostic applications, for
example, imaging specific loci or genetic regions for individuals or populations to help identify specific diseases or disorders. Other uses of the methods will be apparent to those skilled in the art.

The methods described herein can be used in a variety of settings, e.g., to identify an organism in a human or a non-human subject, in food, in environmental sources (e.g., food, water, air), and in industrial settings. The featured methods also include methods of diagnosing a disease or disorder in a subject, e.g., a human or a non-human subject, and treating the subject based on the diagnosis. The method includes: obtaining a sample comprising an organism from the subject; imaging a nucleic acid from the organism; obtaining a restriction map of said nucleic acid; identifying the organism by correlating the restriction map of said nucleic acid with a restriction map database; and correlating the identity of the organism with the disease or disorder.

As discussed above, various organisms can be identified by the methods discussed herein and therefore various diseases and disorders can be diagnosed by the present methods. The organism can be, e.g., a cause, a contributor, and/or a symptom of the disease or disorder. In one embodiment, more than one organism can be identified by the methods described herein, and a combination of the organisms present can lead to diagnosis. Skilled practitioners would be able to correlate the identity of an organism with a disease or disorder. For example, the following is a non-exhaustive list of some diseases and bacteria known to cause them: tetanus - *Clostridium tetani*; tuberculosis - *Mycobacterium tuberculosis*; meningitis - *Neisseria meningitidis*; botulism - *Clostridium botulinum*; bacterial dysentry - *Shigella dysenteriae*; lyme disease - *Borrelia burgdorferi*; gastroenteritis - *E. coli* and/or *Campylobacter spp.*; food poisoning - *Clostridium perfringens*, *Bacillus cereus*, *Salmonella enteriditis*, and/or *Staphylococcus aureus*. These and other diseases and disorders can be diagnosed by the methods described herein.

Once a disease or disorder is diagnosed, a decision about treating the subject can be made, e.g., by a medical provider or a veterinarian. Treating the subject can involve administering a drug or a combination of drugs to ameliorate the disease or disorder to which the identified organism is contributing or of which the identified organism is a cause. Amelioration of the disease or disorder can include reduction in the symptoms of the disease or disorder. The drug administered to the subject can include any chemical substance that affects the processes of the mind or body, e.g., an antibody and/or a small molecule. The drug can be administered in
the form of a composition, e.g., a composition comprising the drug and a pharmaceutically acceptable carrier. The composition can be in a form suitable for, e.g., intravenous, oral, topical, intramuscular, intradermal, subcutaneous, and anal administration. Suitable pharmaceutical carriers include, e.g., sterile saline, physiological buffer solutions and the like. The pharmaceutical compositions may be additionally formulated to control the release of the active ingredients or prolong their presence in the patient's system. Numerous suitable drug delivery systems are known for this purpose and include, e.g., hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Treating the subject can also include chemotherapy and radiation therapy.

**Incorporation by Reference**

References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

**Equivalents**

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.
What is claimed is:

1. A device for elongating a nucleic acid, the device comprising:
   a voltage source; and
   a substrate coupled to the voltage source, wherein hydrophobicity of the substrate changes in response to an applied electric field and a surface of the substrate is coated with a substance that retains nucleic acids.

2. The device according to claim 1, wherein the substrate is a channel.

3. The device according to claim 1, wherein the substrate comprises glass.

4. The device according to claim 3, wherein the glass is coated with silanes.

5. The device according to claim 4, wherein the substrate further comprises metal.

6. The device according to claim 1, further comprising at least one reservoir fluidically coupled to the substrate.

7. A method for elongating a nucleic acid on a substrate, the method comprising:
   temporarily applying an electric field to temporarily decrease hydrophobicity of a charged substrate, thereby causing a sample fluid comprising a nucleic acid to temporarily flow onto the charged substrate; and
   maintaining the electric field for a time sufficient to allow the nucleic acid to interact with the charged substrate and become elongated and fixed on the substrate.

8. The method according to claim 7, wherein the nucleic acid is DNA.

9. The method according to claim 7, wherein the nucleic acid is from a microorganism.
10. The method according to claim 9, wherein the microorganism is a bacterium.

11. A method for characterizing a nucleic acid, the method comprising:
   temporarily applying an electric field to temporarily decrease hydrophobicity of a
   charged substrate, thereby causing a sample fluid comprising a nucleic acid to temporarily flow
   onto the charged substrate;
   maintaining the electric field for a time sufficient to allow the nucleic acid to interact
   with the charged substrate and become elongated and fixed on the substrate so that the nucleic
   acid remains accessible for enzymatic reactions;
   intermittently re-applying the electric field to cause reagents to flow to and from the
   substrate to wash, enzymatically digest, and stain the nucleic acid to obtain one or more
   restriction digests of the nucleic acid; and
   imaging the restriction digests, thereby characterizing the nucleic acid.

12. The method according to claim 11, further comprising constructing an optical map from the
    restriction digests.

13. The method according to claim 11, wherein the nucleic acid is DNA.

14. The method according to claim 11, wherein the nucleic acid is from a microorganism.

15. The method according to claim 14, wherein the microorganism is a bacterium.

16. The method according to claim 15, wherein the bacterium is at least one species selected
    from the group consisting of *E. coli* and *S. aureus*.

17. The method according to claim 16, wherein the *S. aureus* is a community-acquired
    methicillin-resistant strain of *S. aureus*.

18. The method according to claim 16, wherein the *S. aureus* is a hospital-acquired methicillin-
    resistant strain of *S. aureus*.
19. The method according to claim 15, wherein the nucleic acid comprises substantially all genomic DNA of the bacterium.

20. The method according to claim 15, wherein the nucleic acid comprises a transcriptome of the bacterium.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48, 21/00 (2012.01)
USPC - 436/94, 435/6.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): G01N 33/48, 21/00 (2012.01)
USPC: 436/94, 435/6.1

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

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

PubWEST (DB=PQRP,USPT,USOC,EAPB,JAPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents

Search Terms Used: hydrophobicity, change, reverse, glass, siloxane, silane, voltage, DNA, nucleic acid, elongation, pore, aperture, opening, channel, linear, stretch, PDMS, microfluidic, optical, mapping, NOA63, Jiang OPGEN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>Kim et al. &quot;Simple fabrication of hydrophilic nanochannels using the chemical bonding between activated ultrathin PDMS layer and cover glass by oxygen&quot; Lab Chip; 201 1; Vol. 11; pg 348-353. esp: abstract, pg 352 section entitled &quot;DNA stretching in the nanochannels?&quot;; pg 349 last line of second column; pg 348 second column first complete paragraph; pg 351 first column, first line; pg 7351 bottom of second column; Fig. 1, Fig. 4, Fig. 5.</td>
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<td>Y</td>
<td>Dimalanta et al. &quot;A Microfluidic System for Large DNA Molecule Arrays&quot; Anal. Chem.; 2004; Vol. 76; pg. 5293-5301. esp: abstract, pg 5296 section entitled &quot;DNA Elongation and Deposition&quot;; pg 5295 section entitled &quot;MicroChannel Fabrication&quot;; pg 5301 section entitled &quot;Applications to Optical Mapping&quot;; pg 5297 section entitled &quot;Deposition of DNA Molecules onto Derivatized Surfaces&quot;; pg 5297 section entitled &quot;Results and Discussion&quot; Fig. 5.</td>
<td>11-20</td>
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X Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
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Date of the actual completion of the international search
21 September 2012 (21.09.2012)

Date of mailing of the international search report
26 OCT 2012

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