**Serial No.:**   09/886,051

**Filing Date**:   23 June 2000

**Inventor**:   Martin C. Peckerar

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> WASHINGTON DC 20375

# PATENT APPLICATION/TECHNICAL DIGEST PUBLICATION RELEASE REQUEST

**FROM:** Associate Counsel (Patents) (1008.2)
**TO:** Associate Counsel (Patents) (1008.2)

**Via:** (1) <u>Martin C.Peckerar</u>  (Code<u> 6860 </u>)
     (2) Division Superintendent (Code<u> 6800 </u>)
     (3) Head, Classification Management & Control (Code 1221)

**SUBJ:** Patent Application/Technical Digest entitled:
**"MICROELECTRONIC DEVICE AND METHOD FOR LABEL-FREE DETECTION AND QUANTIFICATION OF BIOLOGICAL AND CHEMICAL MOLECULES"**
request for release for publication.

**REF:** (a) NRL Instruction 5510.40C
     (b) Chapter 6, ONRINST 5870.1C

**ENCL:** (1) Copy of patent Application/Technical Digest

1. In accordance with the provision of references (a) and (b), it is hereby requested that the subject Patent Application/Technical Digest be released for publication.

2. It is intended to offer this Patent Application/Technical Digest to the National Technical Information Service, for publication.

3. This request is in connection with Navy Case No. **82,541**

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(date)

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JOHN J. KARASEK
Associate Counsel (Patents)

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**FIRST ENDORSEMENT**            Date:

**FROM:** Martin C. Peckerar (Code 6860)
**TO:** Division Superintendent (Code 6800)

1. It is the opinion of the Inventor(s) that the subject Patent Application/Technical Digest (is) (is not) classified and there is no objection to public release.

_____
Inventor's Signature

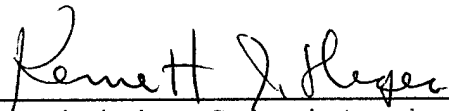**SECOND ENDORSEMENT**                              Date: 7/9/01

**FROM:** Division Superintendent (Code 6800)
**TO:**   Classification Management & Control (Code 1221)

1. Release of Patent Application/Technical Digest (is) (is not) approved.

2. To the best knowledge of this Division, the subject matter of this Patent Application/Technical Digest (has) (has not) been classified.

3. This recommendation takes into account military security, sponsor requirements and other administration considerations and there in no objection to public release.

_____   for G.M. Borsuk
          Division Superintendent

**THIRD ENDORSEMENT**                              Date:

**FROM:** Head, Classification & Control (Code 1221)
**TO:**   Associate Counsel (Patents) (1008.2)

1. This Patent Application/Technical Digest is authorized for public release.

_____
Head, Classification, Management & Control

1        MICROELECTRONIC DEVICE AND METHOD FOR LABEL-FREE DETECTION

2        AND QUANTIFICATION OF BIOLOGICAL AND CHEMICAL MOLECULES

3

4        [0001]    This application claims the benefit of provisional application U.S. Serial No.

5        60/213,471, filed June 23, 2000, the disclosure of which is incorporated herein by reference.

6

7        **Background of the Invention**

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9        **1. Field of the Invention**

10       [0002]    The present invention is a microelectronics-based sensor, specifically, a

11       microelectronics-based transducer for molecular recognition-based sensors for detection or

12       quantification of charged and uncharged target molecules.

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14       **2. Description of the Background Art**

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16       [0003]   Molecular recognition-based sensors are important research tools because of the

17       wide range of target molecules for which receptors can be harvested or synthesized. They

18       have proven indispensable in genomic and pharmaceutical research and development

19       configured in high-density arrays of thousands of individual sensor elements, each element

20       being responsive to a specific target molecule by immobilization of a corresponding

21       molecular receptor. Such arrays enable simultaneous detection of thousands of target

22       molecules from mixtures. Currently, the detection of chemical and biochemical species

1

1  utilizing molecular recognition elements is dependent upon the use of labels or reagents

2  including, but not limited to, fluorophores, radioisotopes, and enzymes that generate a

3  measurable signal to report binding of target molecule by the receptor molecule. The use of

4  such labeling reagents is labor intensive, equipment intensive and is prone to human and

5  equipment error. Further, the difficulty in the use of labeling reagents has prohibited

6  widespread application of molecular receptor recognition-based sensing outside research and

7  development environments. Further, the use of labeling reagents precludes real time

8  diagnostics in the field.

9  [0004]  Further, the current methods for the detection of chemical and biochemical species

10  utilizing microelectronics-based transducers (i.e., field effect transistors (FETs)) depend upon

11  a change in FET gate charge as the sensing approach. Many target molecules of interest are

12  uncharged, therefore they are not detectable using the current microelectronics-based

13  transducers.

14  [0005]  Therefore, there is a strong need in the field to provide devices and methods capable

15  of sensitive and accurate detection of target molecules without the use of labeling reagents.

16  Further there is a strong need for devices and methods for label-free assays capable of real-

17  time analysis *in vitro* and *in vivo*. Further, there is a strong need for arrays for simultaneous

18  detection of multiple target molecules without the use of labeling reagents.

19  [0006]  Further, there is a strong need in the art for devices and methods to detect both

20  charged and uncharged target molecules.

21

22  **Objects of the Invention**

1    [0007]  Accordingly, it is an object of the present invention to provide molecular

2    recognition-based electronic devices and methods capable of sensitive and accurate detection

3    of target molecules without the use of labeling reagents.

4    [0008]  It is another object of the present invention to provide molecular recognition-based

5    electronic devices and methods of label-free assays capable of real-time analysis *in vitro* and

6    *in vivo*.

7    [0009]  It is another object of the present invention to provide for arrays for simultaneous

8    detection of multiple target molecules without the use of labeling reagents.

9    [0010]  It is another object of the present invention to provide devices and methods to detect

10   both charged and uncharged target molecules.

11   [0011]  Additional objects, advantages and novel features of the invention will be set forth

12   in part in the description which follows, and in part will become apparent to those skilled in

13   the art upon examination of the following or may be learned by practice of the invention.  The

14   objects and advantages of the invention may be realized and attained by means of the

15   instrumentalities and combinations particularly pointed out in the appended claims.

16

17   **Summary of the Invention**

18

19   [0012]  According to the present application, the foregoing and other objects and advantages

20   are attained by providing a sensor comprising a gateless, depletion-mode, field effect

21   transistor (FET) having a source implant and a drain implant that are spatially arranged within

22   a semiconductor structure and separated by an active channel.  A dielectric layer covers the

1    active channel between the source and drain, and the dielectric layer surface is modified with

2    immobilized molecular receptors. The receptor-modified dielectric layer surface contacts a

3    sample solution. The immobilized molecular receptors are available to bind target molecules

4    present in the sample solution. The FET-based sensor is imbedded in a substrate with its

5    receptor-modified dielectric layer exposed, and electrical connections are available for

6    applying bias between the source and drain and between a reference electrode in the sample

7    solution and the FET semiconductor substrate. The sensor detects the presence of target

8    molecules in the sample solution by measuring the change in current between the source and

9    drain that occurs due to either the change in capacitance of the receptor-modified dielectric

10   film/sample solution interface when target molecules bind to the molecular receptors, or

11   when charged molecules bind to the receptor-modified dielectric film/sample solution

12   interface.

13   [0013]    Another aspect of the invention provides for a sensor array, with at least two sensors

14   as described above. Each sensor is fabricated into a common substrate with each individual

15   sensor's respective receptor-modified dielectric layer exposed. Each sensor is individually

16   modified with molecular receptors. The sensors operate in parallel and are individually

17   electrically addressable.

18   [0014]    Another aspect of the invention provides for a method for detecting a target

19   molecule species by contacting the sensor of the present application with a sample solution,

20   which creates an interface between the sample solution and the receptor-modified dielectric

21   layer. The binding of target molecules to the receptor molecules immobilized on the

22   dielectric layer changes the interfacial capacitance that exists between the sample solution and

1    the receptor modified dielectric layer. This change of interfacial capacitance changes the

2    conductivity of the active channel. The conductivity of the active channel modulates an

3    externally supplied current flowing through the active channel when a terminal bias is applied

4    between the source and the drain. The modulation of the externally supplied current flowing

5    through the active channel can be measured to detect binding of the target molecules to the

6    molecular receptors.   The methods and devices of the invention remove the dependency of

7    molecular-recognition-based sensors on labeling reagents, which enables a wider scope of

8    practical and worthwhile utilization of recognition-based sensors. The removal of

9    dependency of molecular recognition-based sensors on labeling reagents also makes possible

10   *in vivo* application of miniaturized sensor arrays for medical research and real time treatment

11   assessment.

12

13   **Brief Description of the Drawings**

14   [0015]   A more complete appreciation of the invention will be readily obtained by reference

15   to the following Description of the Preferred Embodiments and the accompanying drawings

16   in which like numerals in different figures represent the same structures or elements, wherein:

17          Fig 1. is a schematic, not to scale, cross sectional representation of a single sensor.

18          Fig.2 shows the typical response of a 100 $\mu$m-dimensional electronic sensor in

19   aqueous solutions.

20

21   **Description of the Preferred Embodiments**

22   [0016]   The device of the present application is a depletion mode field effect transistor

1    consisting of source and drain n+ implants, an n- depletion-mode channel implant, and an

2    oxide/nitride insulating bilayer. In conventional embodiments of FET technology, a

3    conductive layer, i.e. a gate, is installed above the active channel. No such gate is present in

4    the present invention, therefore, this device is referred to as a "gateless" FET. This

5    oxide/nitride layer can be modified with immobilized molecular receptors including, but not

6    limited to, proteins, antibodies, antigens, peptides or oligonucleotides. The molecular

7    receptors can be immobilized on the dielectric layer by any of several processes, including,

8    but not limited to, providing a thiol reactive layer by either AuPd plasma sputter co-

9    deposition or Cr/Au evaporation.

10    [0017]    The observed channel conductivity responds to changes in gate-channel capacitance

11    as well as solution potential, and such response is immediate and substantial. In practice, a

12    sample solution containing no, one, or more target molecule species is allowed to contact the

13    gate region. A reference electrode is inserted in the solution. The consequent solution

14    potential (with respect to the substrate) represents a gate bias that couples capacitively to the

15    active channel, itself biased by the source and drain applied potentials. Binding of target

16    molecules (if present) by the immobilized receptor molecules reduces the capacitive coupling

17    between the channel and the solution, and thus channel conductivity. A device such as this

18    can be miniaturized and fabricated by standard microelectronic techniques in high-density

19    arrays for simultaneous detection of multiple target molecules, with sensitivity increasing

20    with miniaturization. Examples of potential uses include, but are not limited to, a genetic

21    assay based in a point of care environment requiring limited instrumentation and performed

22    by non-technically trained personnel to provide important genetic information rapidly and

1   cost-effectively.

2   [0018]   A schematic, not to scale, cross-section of the device is shown in Fig. 1.

3   Semiconducting regions of n+ carrier type, including, interchangeably, a source 12 and a

4   drain 14, are fabricated into a semiconducting substrate material 30. An active

5   semiconducting channel 20 is between the source 12 and drain 14. A $SiO_2$ "gate oxide" layer

6   35 covers and is in contact with the active channel 20. A body contact 40 is also fabricated

7   into the substrate 30. Electrodes 50 connect to the source 12, drain 14, and body contact 40.

8   A second $SiO_2$ layer 60 isolates the electrodes 50 from the substrate 30. A $Si_3N_4$ layer 70

9   covers the first $SiO_2$ layer 35 and the second $SiO_2$ layer 60. A third $SiO_2$ layer 80 isolates the

10  electrodes 50 from the test solution 90. Molecular receptors 100 cover the active sensing

11  region 110. A reference electrode 120 is used to bias test solution 90.

12  [0019]   The sensor described here is based upon a depletion-mode (normally on) field-effect

13  transistor. The use of this type of FET is important in that the base-line bias over the active

14  channel of the FET is supplied by the sample solution. The sample solution itself effectively

15  provides the gate bias, and it is not required that a threshold voltage be overcome in order to

16  supply source-to-drain current. The target molecule may be therefore be either charged or

17  uncharged.

18  [0020]   In a conventional n-channel depletion mode MOSFET in the ohmic state, the

19  source-drain current $i_{SD}$ is given by

20  $$i_{SD} = \frac{k}{2}\left[2(v_{GS} - V_{th})v_{SD} - v_{SD}^2\right],$$

21  where the transconductance parameter $k$ is given by

1    $$k = \frac{W}{L}\mu_n C_{ox},$$

2

3    $\mu_n$ is the electron mobility (1900 cm$^2$V$^{-1}$ s$^{-1}$ in Si), $C_{ox}$ is the gate oxide capacitance per unit

4    area, $v_{GS}$ and $v_{SD}$ are the gate-source and drain-source biases, respectively, and $V_{th}$ is the

5    threshold bias of the device. Here, where the gate "oxide" is ultimately a bilayer, the net

6    capacitance is given as a series capacitor network by

7    $$C = \left[ \sum_{i=1}^{n} \frac{d_i}{\varepsilon_0 \varepsilon_i} \right]^{-1}$$

8    Substituting values for layer thickness given above, using values for the dielectric constant $\varepsilon_i$

9    of 3.0 and 12.7 for SiO$_2$ and Si$_3$N$_4$, respectively, and for $\varepsilon_0$ the value 8.85 x 10$^{-15}$ F cm$^{-2}$, the

10   layer capacitance is 4.8 nF cm$^{-2}$. Thus we find an expected intrinsic (untreated, dry) value

11   for $k$ in our devices of 39 $\mu$A V$^{-1}$. Collecting terms, we find that

12   $$i_{SD} = C_{ox} \frac{W}{L} \frac{\mu_n}{2} \left[ 2(v_{GS} - V_{th}) - v_{SD} \right] v_{SD}.$$

13   This indicates several features of the utility of a depletion mode FET as the basis for this

14   device, essentially functioning as a first stage signal transducer. An accumulation of even

15   low levels of charge on the gate affects the current equivalent to a change in gate bias. A

16   change in capacitance due to an accumulation of molecular species equivalent to a change in

17   gate oxide thickness has a linear effect on current. In comparison to an enhancement mode

18   FET, a substantial current flows even a zero gate bias, decreasing the requisite power

19   requirements and increasing the operational flexibility.

20   [0021]    In the configuration in which these devices are used, the channel may be thought of

1    as one electrode in an electrochemical cell.  As such, the gate capacitance is a combination of

2    the gate oxide, any molecular layer on the surface, and the double layer capacitance of the

3    solution used during testing at both the device surface as well as the reference electrode.

4    Effects, including, but not limited to, changes in temperature, ionic strength, and pH,

5    attributable solely to changes in the test solution (or equivalently at the reference electrode-

6    solution interface) are observable dynamically in many ways including, but not limited to, the

7    use of control devices modified with receptors whose targets are not present in solution.

8    [0022]   The immobilized biological and chemical molecular receptors of the FET include,

9    but are not limited to, single strand DNA, single strand PNA, binding proteins, antibodies,

10    DNA aptamers, PNA aptamers, RNA aptamers, antigens, enzymes, peptides, chelating

11    molecules, molecular assemblies with chelating functional groups, and reagents for covalent

12    attachment of target molecules.

13    [0023]   The charged or uncharged biological and chemical target molecules of the FET

14    include, but are not limited to, single strand DNA, proteins, antigens, bacteria, viruses,

15    biological molecules with functional groups that covalently bound to reactive species,

16    chemical species for which biological receptors exist, chemical species for which PNA

17    receptors exist, chemical species for which RNA receptors exist, chemical species for which

18    DNA receptors exist, chemical species which are bound by chemical chelators, and metal

19    ions.

20    [0024]   The advantages of the invention include, but are not limited to, the ability to

21    integrate the sensor with a pre-amplifier, to make a sensor as small as 2 $\mu$m by 2 $\mu$m ($10^{-8}$

22    $cm^2$) which is capable of detecting one or more target molecules, to configure arrays of

1　　sensors containing thousands of individually addressable and discrete sensor elements

2　　occupying a total active area as small as 1 cm$^2$, and to provide for label-free continuous multi-

3　　analyte detection of charged and uncharged target molecules that has high sensitivity, high

4　　resolution, and is cost-effective.  The FET-based sensor of the present application provides

5　　electrical gain.  Therefore, a small change in the extent of occupancy of the immobilized

6　　receptors by target molecules modulates a large (microamp to milliamp) externally supplied

7　　current.  The FET-based sensor integrates the pre-amplifier with the sensor element therefore

8　　significantly reducing electrical noise. Multiple sensing elements allow for signal averaging

9　　and utilization of cross-reactive responses.

10

11　　**EXAMPLES**

12　　**[0025]**　The following examples illustrate certain embodiments of the present invention.

13　　However, they are not to be construed to limit the scope of the present invention in any way.

14
15
16　　Example 1

17　　**[0026]**　A source and drain were formed by a P implant in a semiconductor structure at 80

18　　keV to an areal density of 1 X 10$^{15}$ cm$^{-2}$. The channel region is 32 μm x 140 μm and P

19　　implanted at 60 keV to an areal density of 6 x 10$^{11}$ cm$^{-2}$. Body contacts were formed by B

20　　implantation at 80 keV to 1 x 10$^{15}$ cm$^{-2}$. A 63 nm thermal oxide layer was followed by a 30

21　　nm LPCVD Si$_3$N$_4$ layer.  Following a Cr/Au contact metallization, a 600 nm APCVD oxide

22　　layer was formed over all. The present example used 36 devices per wafer arranged in 9

23　　groups of 4 on a 6.5 mm pitch. Each device had a separate and independent source and drain

1    contact. Additionally, 14 gated and ungated test structures were included on the wafer for use

2    as reference standards and process and instrumentation diagnostic tools.

3    [0027]    Immobilization procedures have relied on the facile attachment of thiol (-S) ligands

4    to Au evaporated onto the dielectric layer by means of molecular self-assembly. To

5    accomplish this, a patterned photoresist layer defined the gate region for a thin (<10 nm)

6    PdAu layer sputter deposited on the wafer and subsequently lifted off in acetone. In one

7    example, a dodecane thiol layer was formed by immersion into a dilute solution of the

8    precursor in ethanol. In another example, a 15-mer sequence of a DNA single strand,

9    modified with a thiol ligand, was self-assembled from a binding buffer solution onto the gate

10   area.

11   [0028]    Before use, a Plexiglas fluid cell was fastened to the wafer to confine a phosphate

12   buffer solution (PBS) to each cell, allowing independent testing and dosing. An Ag reference

13   electrode was used to establish the solution potential. Testing used a Keithley 617

14   electrometer to establish the source drain bias ($v_{SD}$) and measure the source drain current ($i_{SD}$),

15   and a second Keithley 617 electrometer was used to establish the gate (reference electrode)

16   bias ($v_{GS}$). The devices were simultaneously contacted by a custom epoxy ring probe card and

17   demultiplexed into the electrometer in a Keithley 7002 matrix switcher and 7012 4 x 10

18   matrix cards.

19   [0029]    Dodecane thiol ($C_{12}H_{25}HS$) creates an inert, non-reactive surface that can be used to

20   observe non-specific adsorption of proteins. Here, streptavidin was used as the target

21   molecule, dissolved at 1 μg/ml in PBS and allowed to absorb overnight from solution to the

22   surface. Protein species will absorb to the alkane surface nonspecifically. The addition of

11

1   streptavidin to the cell was observed to lower $i_{SD}$ by 35 – 45 %. The absorption of protein to

2   the dodecane surface displaces solution ionic charge from the surface, lowering the interfacial

3   capacitance, and thus $i_{SD}$.

4   Example 2

5   [0030]   In contrast to Example 1, the gold modified dielectric layer was in turn modified

6   with thiol terminated single strand DNA. The sensors were exposed to either a solution with

7   1fM of single stranded DNA that was complementary to the DNA on the surface, a solution

8   with 1fM of single stranded DNA that contained a one base pair mismatch to the DNA on the

9   surface, or a blank buffer solution. At this concentration, response to the target DNA was a

10  drop in current of 1.2 +/- 0.5%, while the current increased 1.2 +/- 0.5% for the devices

11  exposed to the control buffer solution and 3.0 +/- 0.3% for those exposed to the mismatch

12  DNA. Fig. 2 shows the typical response of the sensors to the three different solutions plotted

13  as the fractional change in current. The fractional change in source to drain current is $(i_{SD}^{before}$

14  $- i_{SD}^{after})/i_{SD}^{before}$.

15  [0031]   It is understood that the foregoing detailed description is given merely by way of

16  illustration and that many variations may be made therein without departing from the spirit of

17  this invention.

18

1

## ABSTRACT

3

Molecular recognition-based electronic sensor, which is gateless, depletion mode field effect transistor consisting of source and drain diffusions, a depletion-mode implant, and insulating layer chemically modified by immobilized molecular receptors that enables miniaturized label-free molecular detection amenable to high-density array formats. The conductivity of the active channel modulates current flow through the active channel when a voltage is applied between the source and drain diffusions. The conductivity of the active channel is determined by the potential of the sample solution in which the device is immersed and the device-solution interfacial capacitance. The conductivity of the active channel modulates current flow through the active channel when a voltage is applied between the source and drain diffusions. The interfacial capacitance is determined by the extent of occupancy of the immobilized receptor molecules by target molecules. Target molecules can be either charged or uncharged. Change in interfacial capacitance upon target molecule binding results in modulation of an externally supplied current through the channel.
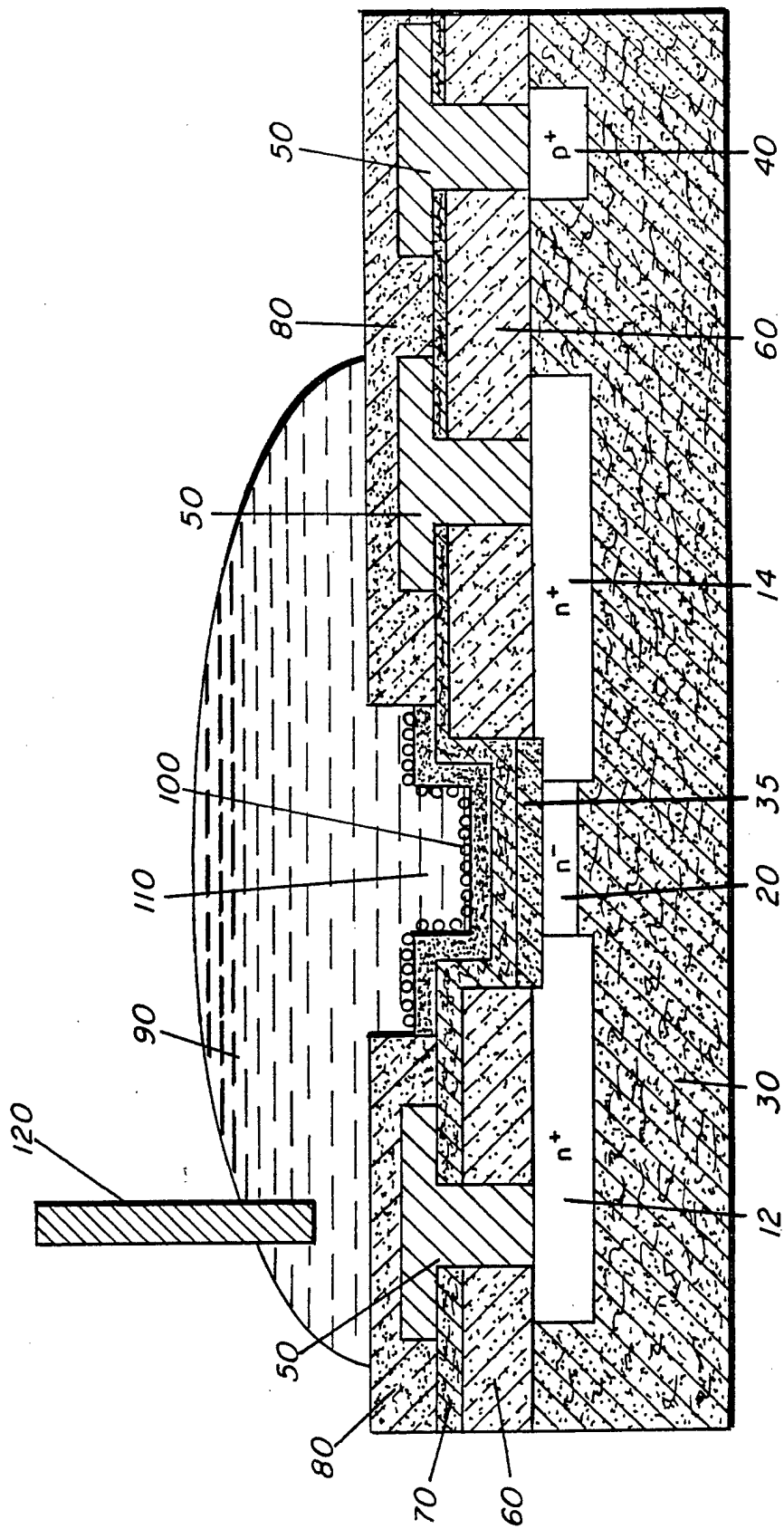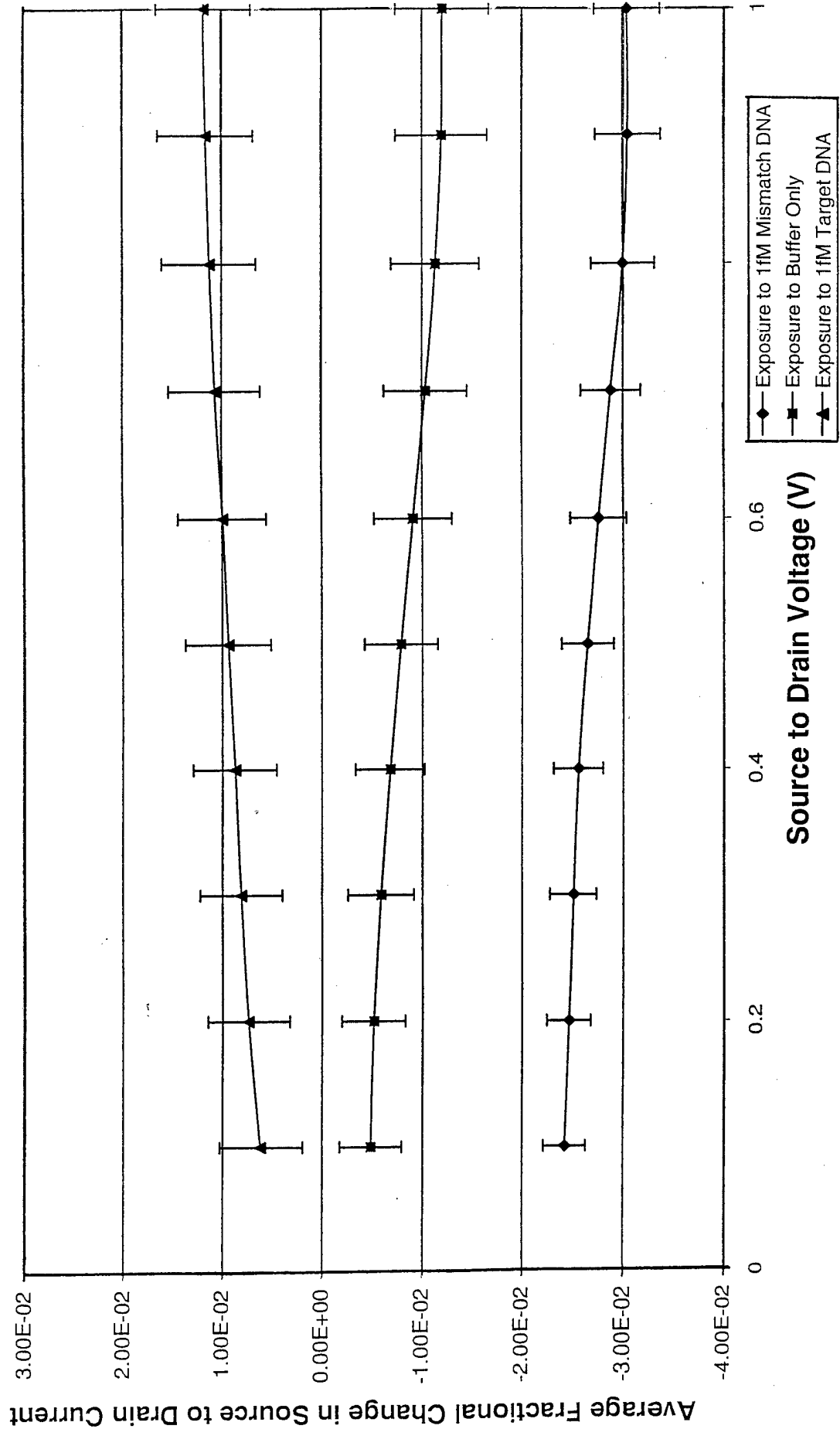
FIG. 1

FIG. 2