



European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *with international search report*

Apparatus and Method for Monitoring Cell Motility

Technical Field

Disclosed is an apparatus and method for monitoring
5 cell motility in response to a chemical agent.

Background

Cell motility in response to a chemical agent is
fundamentally important in many biological processes such
10 as cancer metastasis, embryogenesis and wound healing. The
study of cell motility in response to specific chemical
agents can lead to the identification of agents or
receptors for the agents which are potential therapeutic
targets for use in the treatment of wounds, cancer and
15 inflammation.

For a cell to become motile there are discrete steps
of detection of a chemical agent, protrusion, adhesion of
the protrusion to a substrate, the release of adhesion at
the posterior of the cell and contraction that pulls the
20 cell forward into the protrusion.

There are many types of cells capable of motility
including bacteria, protozoa, amoeba, cellular slime
moulds (*Dictyostelium discoideum*), fungi, sperm,
fibroblasts, phagocytes, cancer cells, neurons, epithelial
25 cells, endothelial cells, and cells of the immune system
including mast cells, neutrophils, lymphocytes, monocytes,
macrophages, leukocytes, T-cells, B-cells, eosinophils and
basophils.

The different cell types demonstrate variations in
30 motility. For example, neutrophils and *Dictyostelium* are
typically fast moving and are able to maintain a direction

- 2 -

of movement once determined even if concentrations of a chemical agent fluctuate. In contrast, cancer cells typically move 10-15 times slower than cells, such as neutrophils and Dictyostelium, and lack the ability to
5 maintain a direction of movement and will often make numerous changes in direction of movement under conditions of fluctuating concentrations of the chemical agent.

The most informative way to study cell motility is through monitoring the motility cycle of a cell in
10 response to a chemical agent of interest.

However, the currently available methods and apparatus for monitoring cell motility are only suitable for certain cell types such as fast moving neutrophils or leukocytes and are not adaptable for other cell types such
15 as slow moving cancer cells.

Summary

In one aspect, there is provided an apparatus for monitoring cell motility in response to a chemical agent
20 comprising

a chamber for containing a medium;
a cell viewing surface arranged in the chamber so as to be submersed in the medium when in use;
a fluid delivery arrangement in communication with
25 the chamber for delivering the chemical agent to a portion of the cell viewing surface;

wherein the fluid delivery arrangement is arranged relative to the cell viewing surface for directing the chemical agent such that, when monitoring cell motility,
30 the chemical agent assumes a concentration gradient in relation to the cell viewing surface portion.

- 3 -

The apparatus may be used for monitoring any type of cell motility in response to a chemical agent. Cell motility includes, but is not limited to chemotaxis, haptotaxis, chemokinesis, motility by contact priming
5 (with substrate patterning) and invasion.

The term "chemical agent" as used herein refers to any type of agent to which a cell may respond. The chemical agent may be synthetic, for example a drug or drug compound. The chemical agent may be a biological
10 factor, for example a sugar, amino acid, peptide or protein. The chemical agent may be a chemoattractant and chemorepellent or a chemotactic agent.

The chamber of the apparatus may be made from any material suitable for monitoring cell motility in response
15 to a chemical agent. In one form the chamber is made from an optically transparent material suitable for use with video microscopy. For example, the chamber may be made from glass or plastic.

In use, the chamber can contain a medium suitable to
20 monitoring cell motility. The medium may be a liquid medium. The medium may be a matrix. In one form the matrix is a gel. The gel may comprise collagen, fibronectin, other connective tissue matrix components, other naturally-derived and tumour-derived matrix. The
25 medium may be a buffer such as MEM, DMEM, L15, RPMI, HEPES-buffered medium.

In use, the cells to be monitored are placed onto the cell viewing surface, with the cell viewing surface being arranged so as to be submersed in the medium. The
30 apparatus is suitable for monitoring any cell type. Examples of cell types that may be monitored using the apparatus include but are not limited to cancer cells,

- 4 -

spermatozoa, bacteria, fungi, epithelial cells, neurons, endothelial cells, fibroblasts, mast cells, neutrophils, dictyostelium cells, lymphocytes, monocytes, macrophages, leukocytes, T-cells, B-cells, eosinophils, basophils and
5 combinations thereof.

The fluid delivery arrangement of the apparatus can be arranged in any configuration relative to the cell viewing surface provided that the configuration is suitable for directing the chemical agent such that, when
10 monitoring cell motility, the chemical agent assumes a concentration gradient in relation to the cell viewing surface portion.

In one form, the fluid delivery arrangement comprises one or more apertures arranged to release the flow of the
15 chemical agent to the medium in the vicinity of the cell viewing surface portion. In this form, the one or each aperture can have a configuration that influences a direction to the flow of chemical agent through the aperture. For example, the side wall of the one or each
20 aperture may influence a direction to the flow of chemical agent through the aperture.

The apertures may be located at any position relative to the cell viewing surface portion provided they are capable of releasing the flow of the chemical agent to the
25 medium in the vicinity of the cell viewing surface portion. For example, the apertures may be located in the cell viewing surface.

In another form, the fluid delivery arrangement may further comprise a barrier construction arranged relative
30 to the cell viewing surface portion such that, when a flow of chemical agent is delivered by the fluid delivery arrangement via the one or more apertures to the cell

- 5 -

viewing surface portion, the flow interacts with the barrier construction to impart the concentration gradient.

In one form, the barrier construction diverts the flow of chemical agent to impart the concentration
5 gradient. Typically, diverting the flow of chemical agent modulates any one or more of the following: the rate at which the gradient is formed; the steady-state shape of the gradient; the stability of the gradient; the steepness of the gradient.

10 The barrier can be defined by a physical means such as a dam. The barrier can be defined by a photonic means such as through optical trapping. Methods for optical trapping and other photonic means are described in, for example, Dholakia and Reece (2006) Optical
15 Micromanipulation Takes Hold, Nanotoday Vol 1(1): 18-27; Mach, P. et al. (2002) Tunable microfluidic optical fiber, Applied Physics Letters, vol. 80(23): 4294-4296. The barrier can be defined by solid-phase chemistry as described in Lim et al. (2004) Retinal Pigment Epithelial
20 Cell Behaviour is Modulated by Alterations in Focal Cell-Substrate Contacts, Investigative Ophthalmology & Visual Science, vol. 45(11): 4210-4216).

In one embodiment, the cell viewing surface is a platform that defines a wall in the chamber, with the wall
25 in turn defining the dam. The wall may extend across the chamber from one chamber side to the other.

The fluid delivery arrangement of the apparatus may further comprise one or more conduits in fluid communication with a source for the chemical agent, one or
30 each conduit extending to one or each respective aperture.

In one embodiment, the conduits are one or more channels in a substrate that each extend to a respective

- 6 -

aperture positioned in, or in relation to, the substrate. The channels can be any dimension suitable for delivery of the chemical agent to the cell viewing surface portion. For example, the channels can be as deep as the depth of
5 view of the lowest magnification objective lenses and as wide as the width of the chamber. In one embodiment, the channels are 50 μ m in depth and 50 μ m in width.

In another embodiment, the one or each conduit is defined by a respective one or more micropipettes, with
10 each aperture being defined by an outlet of the respective micropipette.

The apertures of the apparatus may be any size suitable for directing the chemical agent such that, when monitoring cell motility, the chemical agent assumes a
15 concentration gradient in relation to the cell viewing surface portion. The apertures can be as wide as the width of the chamber and as narrow as 0.02 μ m. In one embodiment, the apertures are 1 μ m in diameter.

The chemical agent may be delivered to the cell
20 viewing surface portion under pressure. The chemical agent may be delivered at a constant and continuous pressure. Alternatively, the chemical agent may be delivered under pulsed pressure. The chemical agent may be delivered at any pressure that is suitable for delivering the chemical
25 agent. For example, when the chemical agent is delivered at 0 hPa constant pressure, the chemical may diffuse freely into the medium in the chamber. When the chemical agent is delivered at 0 hPa pulsed pressure, backpressure or pressure less than 0 hPa may be applied to stop the
30 flow of chemical agent at intervals. Typically, the chemical agent is delivered at a pressure of 0 hPa or more. In one embodiment, the chemical agent is delivered

- 7 -

at a pressure of 20hPa.

In a second aspect, there is provided apparatus for monitoring cell motility in response to a chemical agent comprising

- 5 a chamber for containing a medium;
- a cell viewing surface arranged in the chamber so as to be submersed in the medium when in use;
- a fluid delivery arrangement in communication with the chamber for delivering the chemical agent to a
- 10 portion of the cell viewing surface, the fluid delivery arrangement comprising a barrier construction that is arranged, relative to the cell viewing surface portion, such that when a flow of chemical agent is delivered by the fluid delivery
- 15 arrangement to the cell viewing surface portion, the flow interacts with the barrier construction to impart a concentration gradient of the chemical agent in the medium.

The barrier construction of the second aspect may be

20 defined by the aperture wall(s) or dam of the first aspect. The apparatus of the second aspect may otherwise be as defined as per the first aspect.

In a third aspect, there is provided a method for monitoring cell motility in response to a chemical agent

25 in a medium, comprising the step of delivering the chemical agent to one or more cells in a manner such that, when monitoring the motility of the one or more cells, the chemical agent has assumed a concentration gradient in the medium.

30 The concentration gradient of the chemical agent can be shaped by physically directing the flow of chemical agent to the medium in the vicinity of the one or more

- 8 -

cells.

In one form, the flow of the chemical agent is directed through one or more apertures arranged in the medium. A side wall of one or each aperture may physically
5 direct the flow of the chemical agent through the aperture.

In another form, the flow of chemical agent interacts with a barrier arranged in the fluid medium to physically direct the flow of the chemical agent. The barrier can be
10 defined by a dam.

The method for monitoring cell motility may be performed using the apparatus as described in the first and second aspects.

15 **Brief Description of Drawings**

Notwithstanding any other forms that may fall within the scope of the method and apparatus as defined in the Summary, specific embodiments of the method and apparatus will now be described, by way of example only, with
20 reference to the accompanying drawings in which:

Figure 1A shows a perspective view of one form of the apparatus for monitoring cell motility employing a single micropipette;

Figure 1B shows a side sectional view of the
25 apparatus shown in Figure 1A;

Figure 2A shows a perspective view of the apparatus shown in Figure 1 employing multiple micropipettes 2a;

Figure 2B shows a perspective view of the
30 apparatus shown in Figure 1 employing a micropipette 2a having a broad tip;

- 9 -

Figure 3 shows a perspective view of another form of the apparatus for monitoring cell motility in which:

Figure 3A shows a perspective view of an upper substrate 1 containing apertures 1a;

5 Figure 3B shows a perspective view of a lower substrate 2 containing channels 2a;

Figure 3C shows a perspective view of the assembled product of surfaces 1 and 2, with superimposition of the substrates and the upper
10 apertures 1a and lower channels 2a;

Figure 3D shows an enlarged sectional view of the apparatus shown in Figure 3C showing the relative positions of a single aperture 1a in the upper substrate 1, and the opening 2b of the channel 2a in the lower
15 substrate 2;

Figure 3E shows an enlarged view of a single aperture of the upper substrate shown in Figure 3A, where the side walls of the aperture direct the flow of chemical agent (the concentration gradient 7 forms at
20 the rim of the aperture and its direction is generally perpendicular to the vertical flow along the walls of aperture 1b);

Figure 3F shows various alternative embodiments for the configuration of aperture 1a;

25 Figure 3G shows a perspective view of the apparatus shown in Figure 3C incorporating a single elongate aperture;

Figure 3H shows a perspective view of the apparatus shown in Figure 3C in which the alignment of
30 the aperture(s) is intermediate the channel(s) and not at the channel termini, and where the termini of the

- 10 -

channel(s) are sealed; and with additional aperture set(s) being provided along the length of the channel;

Figure 4 shows various embodiments of the apparatus shown in Figure 3 in which:

5 Figure 4A depicts a single channel;

Figure 4B depicts more than one channel;

Figure 4C depicts more than one channel 2a, each housed in a sub-chamber 3;

10 Figure 4D depicts groups of more than one channel in a single sub-chamber 3; and

Figure 4E depicts groups of channels where each group is housed in its own sub-chamber 3;

Figure 5 shows a perspective view of another form of the apparatus for monitoring cell motility;

15 Figure 6 shows a plan view of yet another form of the apparatus for monitoring cell motility;

Figure 7 shows a steady-state concentration profile of a chemical agent for the apparatus shown in Figures 1 and 2;

20 Figure 8 shows a steady-state concentration profile of a chemical agent for the apparatus shown in Figure 3; and

Figure 9 shows the concentration gradient of a chemical agent at various pressures.

25

Detailed Description of Specific Embodiments

Referring firstly to Figures 1, 2 and 7, one embodiment of an apparatus for monitoring cell motility is shown. This embodiment is typically employed for
30 monitoring slow moving cell types, such as cancer cells.

- 11 -

The apparatus shown comprises a chamber 3 housing two optically-transparent substrates, an upper cell viewing substrate 1 and lower substrate 2 forming a floor of the chamber 3. The substrates are approximately 100 microns in thickness (t). The substrates 1, 2 are bonded together either permanently or non-permanently to form a sealed unit. The wall 1a of the cell viewing surface 1 defines a dam extending across the chamber from one side to the other. One or more micropipettes 2a extend to an aperture in the form of a distal outlet of the micropipette, which in use is positioned to abut the dam 1a. The outlet diameter of the micropipette is typically 0.5µm.

The aperture of each micropipette can also be held in place against dam wall 1a via a suitable adjustable jig.

In use, the cells to be monitored are seeded onto an upper surface of the cell viewing substrate 1, and the chamber filled with a suitable buffer. The micropipette(s) 2a is filled with a chemical agent and then positioned at a 40° angle (θ) to the dam 1a. The 40° angle has been found to be optimal for concentration gradient generation in the apparatus depicted, but of course can and may vary with chamber variation. The micropipette(s) is lowered into the chamber 3, manoeuvred onto the upper surface of the cell viewing substrate 1, drawn over the edge, lowered to a distance (H) of 17µm from the top edge of the cell viewing substrate 1 and pushed gently against the side of the wall 1a. Again, the 17µm distance has been found to be optimal, but can vary.

The micropipette(s) are then connected to a compressor (not shown), for example a Femtojet micromanipulator 5171 (Eppendorf) compressor. The compressor produces an adjustable, continuous pressure (P)

- 12 -

of approximately 20 hPa. This pressure induces the flow of the chemical agent through and out of the micropipette 2a. After leaving the micropipette the flow of agent in the medium reaches the top edge of the cell viewing substrate 1 and, as it progresses over the edge, it has imparted thereto via the wall 1a and the wall edge a concentration gradient of chemical agent, which advances into the portion of the upper surface of the cell viewing substrate 1 where the cells are located.

10 The position of the micropipette 2a and the pressure at which the chemical agent is delivered as described above result in a flow of chemical agent, and a moving gradient of concentration of the agent, which is generally perpendicular to the path of the moving cells on the surface of the cell viewing substrate. The concentration gradient that forms has two phases (Figure 7). The first phase spans the 17 μ m distance from the tip of the micropipette 2a to the upper surface of the cell viewing substrate 1. This phase typically has a high diffusion rate. The second phase spans from the edge of cell viewing substrate 1 across the upper surface of the cell viewing substrate 1. This phase typically has a slower diffusion rate. Thus the dam wall 1a provides for the rapid establishment of a stable and steep gradient, typically within 20 seconds of micropipette agent release, that results in a slow rate of diffusion of growth factors across the upper surface of the cell viewing substrate.

The cell motility is typically then monitored by time-lapse video microscopy for approximately 1 hour.

30 The apparatus shown in Figures 1 and 2 may be used with a single micropipette as shown in Figures 1A and 1B, with multiple micropipettes as shown in Figure 2A, or with a broad tipped pipette as shown in Figure 2B. One

- 13 -

advantage of using multiple micropipettes or a broad
tipped pipette is the resultant increase in the area of
the concentration gradient that the cells are exposed to.
This increases the number of cells that can be monitored,
5 and therefore, the throughput of an experiment.

Referring now to Figures 3, 4 and 8, where like
reference numerals are used to denote similar or like
parts, another embodiment of the apparatus for monitoring
cell motility is shown. This form is typically suitable
10 for monitoring slow moving cell types, such as cancer
cells.

The apparatus shown comprises a chamber 3 with two
optically-transparent substrates, an upper cell viewing
substrate 1, as shown in Figure 3A and a lower substrate 2
15 as shown in Figure 3B. The substrates are microfabricated
to contain either apertures 1a or channels 2a.

The substrates are then bonded together either
permanently or non-permanently to form a sealed unit as
shown Figure 3C. Once sealed, the cell viewing substrate 1
20 serves as a cover and to enclose the channels 2a. The
cell viewing substrate 1 contains apertures 1a that
overlay the termini of the channels 2a in the lower
substrate.

The channels 2a are connected to an inlet 6, which
25 connects to a compressor (not shown) and allows the
delivery of a chemical agent to the cell viewing substrate
1 (Figures 3A and 3C).

Figure 3D shows a detailed view of the relationship
between an aperture 1a in the cell viewing substrate and
30 the opening 2b of a channel 2a in the lower substrate that
combine to direct the chemical agent to the upper surface
of the cell viewing substrate 1. The side wall of an

- 14 -

aperture causes the flow of the chemical agent through the aperture to form a concentration gradient 7 which then fans over the top edge of the aperture as shown in Figure 3E. Figure 8 shows the concentration profile of a chemical agent obtained using the apparatus as shown in Figure 3.

The apertures 1a and the termini of the channels 2a may be provided with various shapes, several examples of which are shown in Figure 3F. Figure 3G shows an embodiment in which the aperture 1a is provided in the form of an elongate groove within the cell viewing substrate 1. The aperture(s) 1a can be positioned at more than one location along and in the cell viewing substrate relative to the channels 2a as shown in Figure 3H.

The apparatus shown in Figure 3 can be modified to accommodate high-throughput experiments. Figure 4 depicts alternative configurations for the apparatus, including Figure 4A: a single channel 2a within a single chamber 3; Figure 4B: multiple channels 2a within a single chamber 3; Figure 4C: multiple channels 2a within individual sub-chambers 3; Figure 4D: groups of two or more channels 2a within a single sub-chamber 3; and Figure 4E: groups of two or more channels 2a within individual sub-chambers 3.

Figure 5 shows another embodiment of the apparatus for monitoring cell motility. Figure 5C shows the assembled apparatus.

Figure 5A shows an upper substrate 1 supporting a chamber 3 thereover, and with apertures 1a formed in the substrate. Figure 5B shows a lower substrate 2 with channels 2a defined therein. The termini 2b of the channels 2a in this embodiment are triangular in shape (but could be any shape), and are 50µm wide and 50µm deep. The apertures 1a are 1µm in diameter. The channels can

- 15 -

vary from $1\mu\text{m} \times 1\mu\text{m}$ up to $50\mu\text{m} \times 50\mu\text{m}$ in size without effecting the concentration profile. However, to avoid interaction between flow from each aperture 1a, the apertures are spaced on the cell viewing substrate 1 at
5 least $40\mu\text{m}$ apart, for embodiments where the channels 2a are $50\mu\text{m} \times 50\mu\text{m}$ in size.

Figure 6 shows another embodiment of the apparatus in which the chamber 3 is circular in shape and the inlet 6 from the chemical agent is located in the centre. The
10 apertures 1a are located around the periphery of the chamber 3 with radially extending channels 2a extending to each respective aperture 1a from inlet 6.

Figure 7 shows the steady state concentration profile of a chemical agent formed by diverting the flow of
15 chemical agent using a barrier as described in above and in Figures 1 and 2. As can be seen from Figure 7, dam 1a diverts the flow of chemical agent from micropipette 2a to impart a concentration gradient. By diverting the flow of chemical agent, the rate of formation of the gradient,
20 the shape and steepness of the gradient, and/or the stability of the gradient can be modulated.

Figure 9 shows the concentration profile of a chemical agent as it varies with pressure. The Figure indicates that at pressures ranging from 10,000 to 400,000
25 Nm^{-2} the concentration gradient of the chemical agent remains steep, dropping from a concentration of 0.000001M to 0M over a $40\mu\text{m}$ distance.

It will be apparent to a person skilled in the art that while the method and apparatus have been described
30 herein in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments described herein may be made without

- 16 -

departing from the scope of concept disclosed in this specification.

5 In the claims which follow and in the preceding description, except where the context requires otherwise due to express language or necessary implication, "comprise" and variations such as "comprises" or "comprising" are used in an inclusive sense (i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features).

10 It is understood that a reference herein to a prior art document does not constitute an admission that the document forms part of the common general knowledge in the art in Australia or any other country.

- 17 -

CLAIMS:

1. Apparatus for monitoring cell motility in response to a chemical agent comprising

- 5 a chamber for containing a medium;
 a cell viewing surface arranged in the chamber so as to be submersed in the medium when in use;
 a fluid delivery arrangement in communication with the chamber for delivering the chemical agent to a
10 portion of the cell viewing surface;

wherein the fluid delivery arrangement is arranged relative to the cell viewing surface for directing the chemical agent such that, when monitoring cell motility, the chemical agent assumes a concentration gradient in
15 relation to the cell viewing surface portion.

2. Apparatus according to claim 1, wherein the fluid delivery arrangement comprises one or more apertures arranged to release the flow of the chemical agent to the
20 medium in the vicinity of the cell viewing surface portion.

3. Apparatus according to claim 2, wherein the one or each aperture has a configuration that influences a direction
25 to the flow of chemical agent through the aperture.

4. Apparatus according to claim 3, wherein the side wall of the one or each aperture influences a direction to the flow of chemical agent through the aperture.

- 18 -

5. Apparatus according to claim 4, wherein the apertures are located in the cell viewing surface.

6. Apparatus according to claim 2, wherein the fluid
5 delivery arrangement further comprises a barrier construction arranged relative to the cell viewing surface portion such that, when a flow of chemical agent is delivered by the fluid delivery arrangement via the one or more apertures to the cell viewing surface portion, the
10 flow interacts with the barrier construction to impart the concentration gradient.

7. Apparatus according to claim 6, wherein the barrier is defined by a physical means.

15

8. Apparatus according to claim 7, wherein the physical means is a dam.

9. Apparatus according to claim 6, wherein the barrier is
20 defined by a photonic means.

10. Apparatus according to claims 6, wherein the barrier is defined by solid-state chemistry.

25 11. Apparatus according to claim 8, wherein the cell viewing surface is a platform that defines a wall in the chamber, with the wall in turn defining the dam.

12. Apparatus according to claim 11, wherein the wall
30 extends across the chamber, from one chamber side to the

- 19 -

other.

13. Apparatus according to any one of the preceding
claims, wherein the fluid delivery arrangement further
5 comprises one or more conduits in fluid communication with
a source for the chemical agent, one or each conduit
extending to one or each respective aperture.

14. Apparatus according to claim 13, wherein the conduits
10 are one or more channels in a substrate that each extend
to a respective aperture positioned in, or in relation to,
the substrate.

15. Apparatus according to claim 14, wherein the channels
15 are as deep as the depth of view of the lowest
magnification objective lenses and as wide as the width of
the chamber.

16. Apparatus according to claim 15, wherein the channels
20 are 50 μ in depth and 50 μ in width.

17. Apparatus according to claim 16, wherein the
apertures in the cell viewing surface are spaced at least
40 μ m apart.

25

18. Apparatus according to 13, wherein one or each
conduit is defined by a respective one or more
micropipettes, with each aperture being defined by an
outlet of the respective micropipette.

30

- 20 -

19. Apparatus according to any preceding claim wherein the one or more apertures is as wide as the width of the chamber and as narrow as 0.02 μ m.
- 5 20. Apparatus according to any preceding claim wherein the one or more aperture is 1 μ m in diameter.
21. Apparatus according to any preceding claim wherein the chemical agent is delivered to the cell viewing
10 surface under pressure.
22. Apparatus according to claim 20 wherein the chemical agent is delivered at a pressure of 0hPa or more.
- 15 23. Apparatus according to claim 20 wherein the chemical agent is delivered at a pressure of 20hPa.
24. Apparatus for monitoring cell motility in response to a chemical agent comprising
20 a chamber for containing a medium;
a cell viewing surface arranged in the chamber so as to be submersed in the medium when in use;
a fluid delivery arrangement in communication with the chamber for delivering the chemical agent to a
25 portion of the cell viewing surface, the fluid delivery arrangement comprising a barrier construction that is arranged, relative to the cell viewing surface portion, such that when a flow of chemical agent is delivered by the fluid delivery
30 arrangement to the cell viewing surface portion, the

- 21 -

flow interacts with the barrier construction to impart a concentration gradient of the chemical agent in the medium.

5 25. Apparatus according to claim 21, wherein the fluid delivery arrangement comprises one or more apertures arranged to release the flow of the chemical agent to the medium in the vicinity of the cell viewing surface portion.

10

26. Apparatus according to claim 25, wherein the one or each aperture has a configuration that influences a direction to the flow of chemical agent through the aperture.

15

27. Apparatus according to claim 25 or 26, wherein the barrier construction is defined by a side wall of the one or each aperture, with each side wall influencing a direction to the flow of chemical agent through the
20 aperture.

28. Apparatus according to claim 24, wherein the barrier construction is defined by a dam in the chamber.

25 29. Apparatus according to claim 28, wherein the dam is defined by a wall that subtends from the cell viewing surface in the chamber.

30 30. Apparatus according to any one of claims 24 to 29, wherein the fluid delivery arrangement is otherwise as

- 22 -

defined in any one of claims 2 to 23.

31. A method for monitoring cell motility in response to a chemical agent in a medium, comprising the step of
5 delivering the chemical agent to one or more cells in a manner such that, when monitoring the motility of the one or more cells, the chemical agent has assumed a concentration gradient in the medium.

10 32. A method according to claim 31, wherein the concentration gradient of the chemical agent is assumed by physically directing the flow of chemical agent to the medium in the vicinity of the one or more cells.

15 33. A method according to claim 31, wherein the flow of the chemical agent is physically directed through one or more apertures arranged in the medium.

20 34. A method according to claim 33, wherein a side wall of one or each aperture physically directs the flow of the chemical agent through the aperture.

25 35. A method according to claim 31, wherein the flow of chemical agent interacts with a barrier arranged in the fluid medium to physically direct the flow of the chemical agent.

36. A method according to claim 35, wherein the barrier is defined by a dam.

- 23 -

37. A method according to any one of claims 31 to 36 that employs the apparatus as defined in any one of claims 1 to 27.

1/13

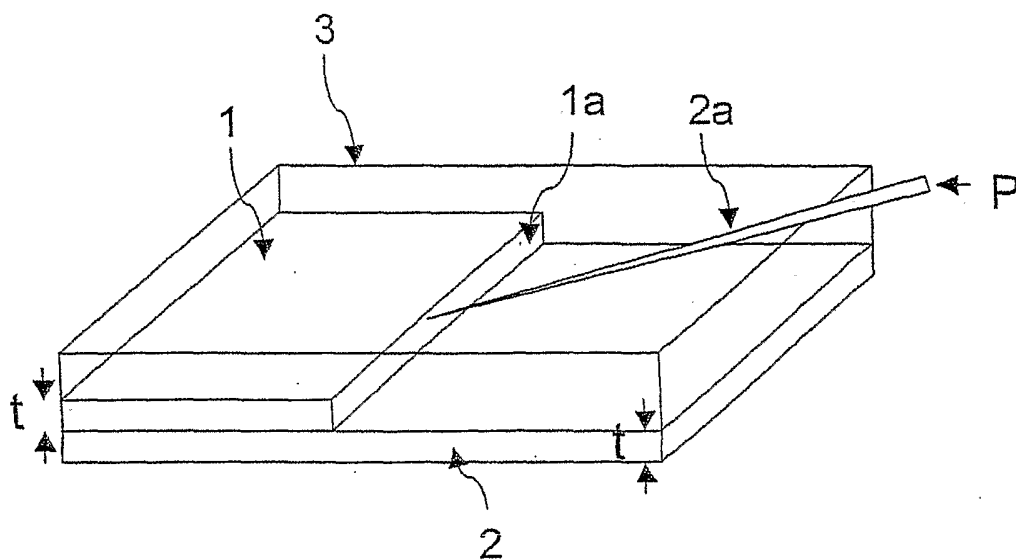


FIG. 1A

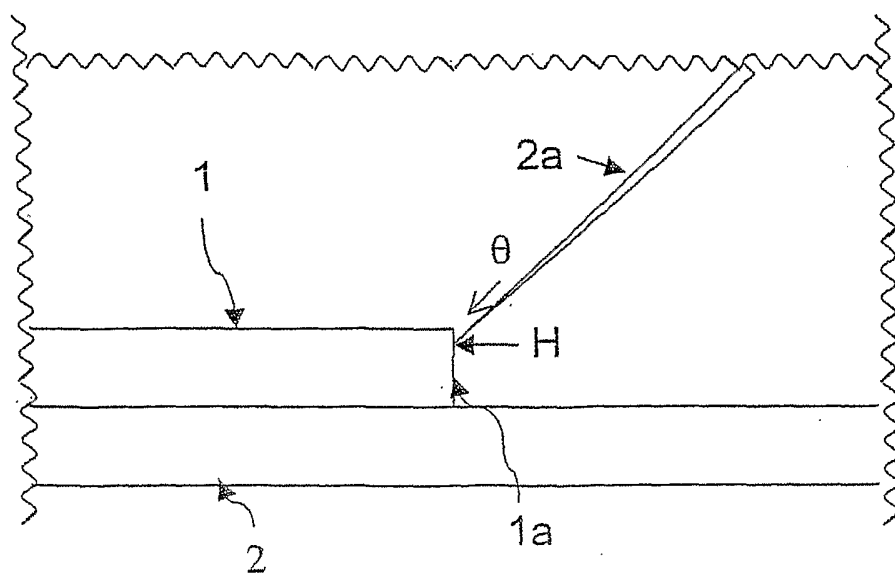


FIG. 1B

2/13

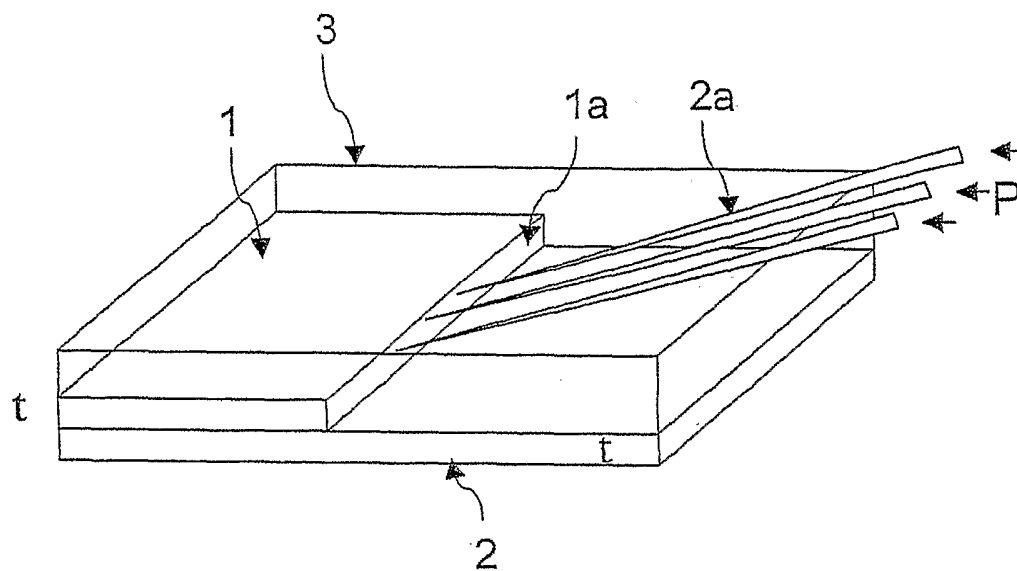


FIG. 2A

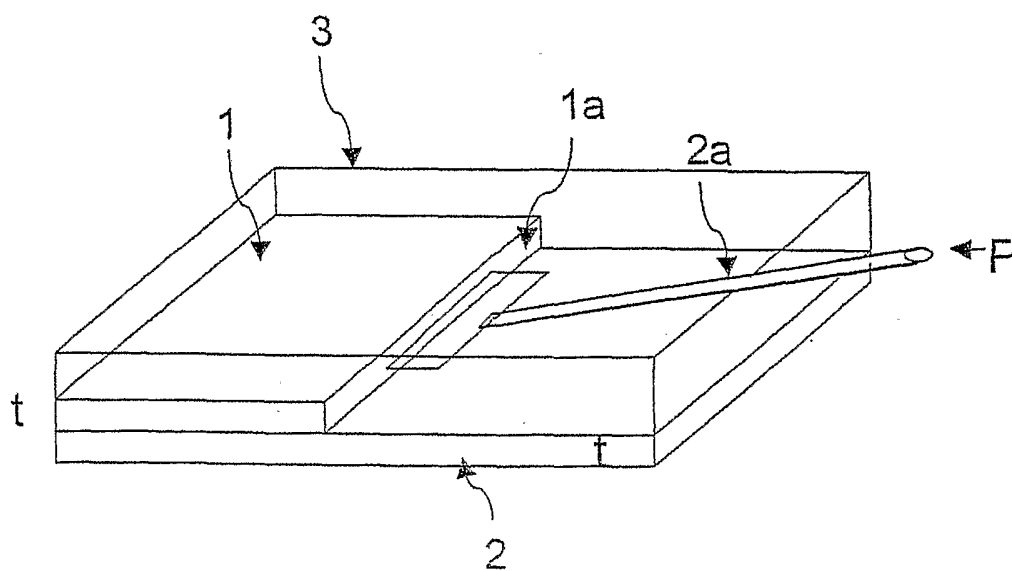


FIG. 2B

3/13

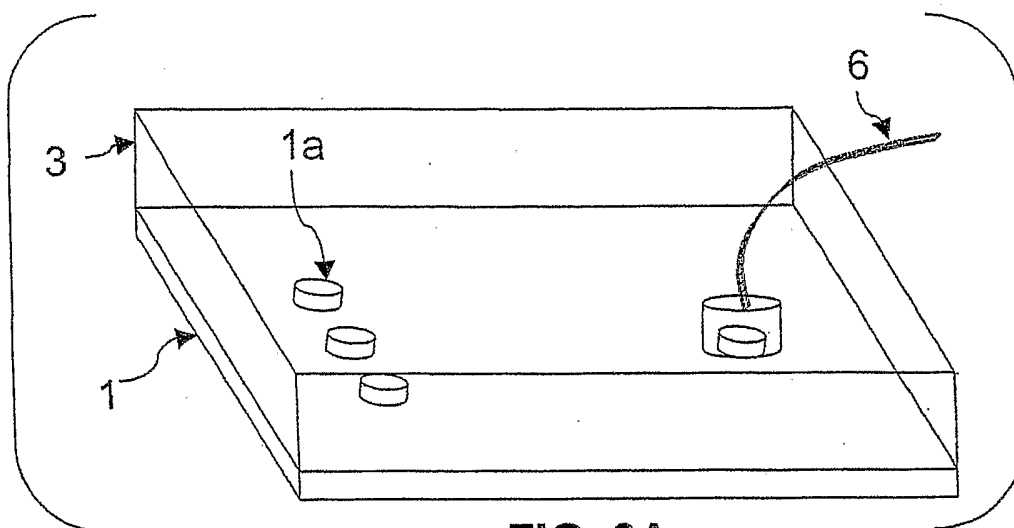


FIG. 3A

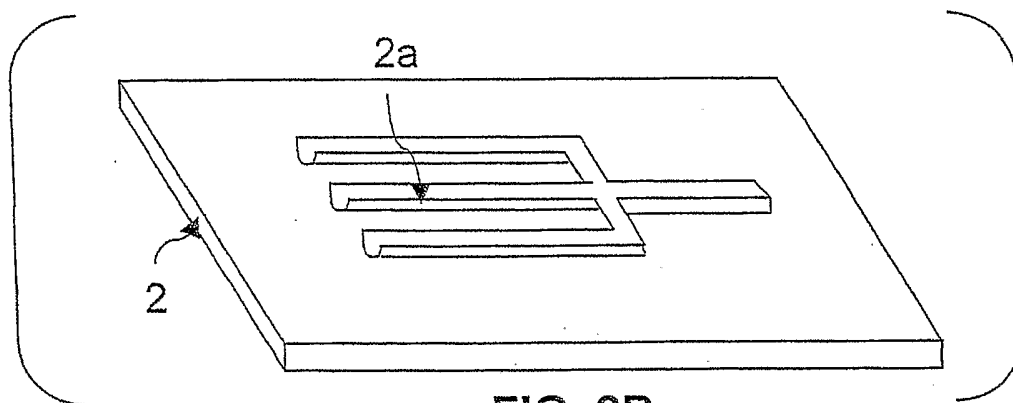


FIG. 3B

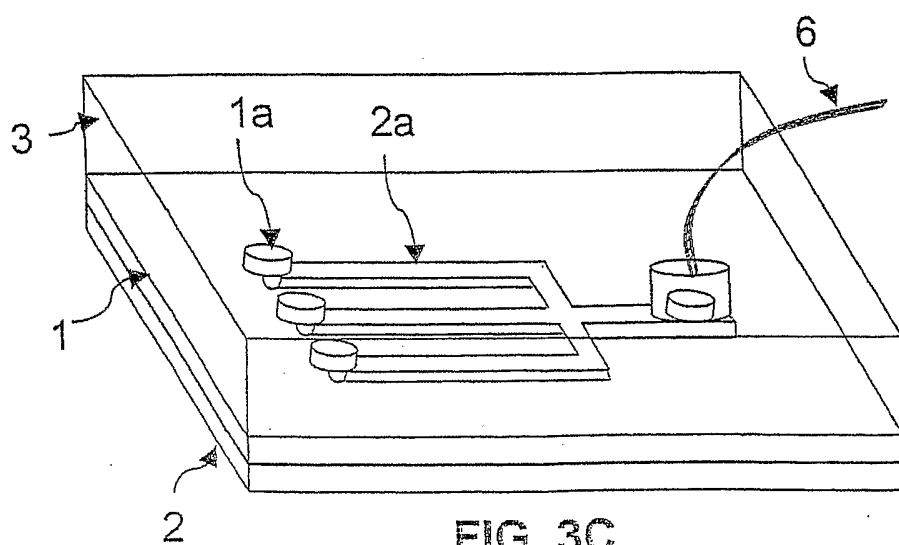


FIG. 3C

4/13

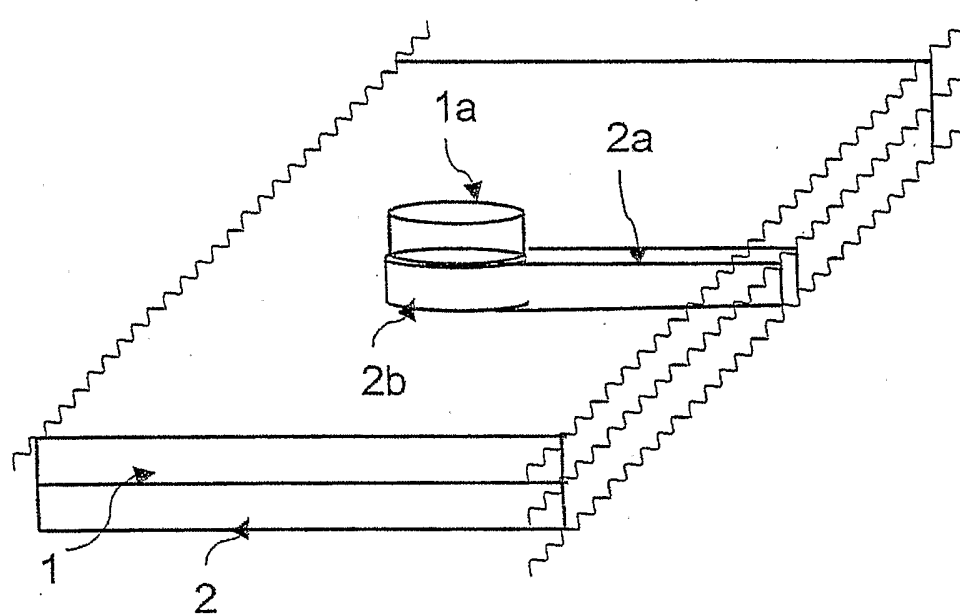


FIG. 3D

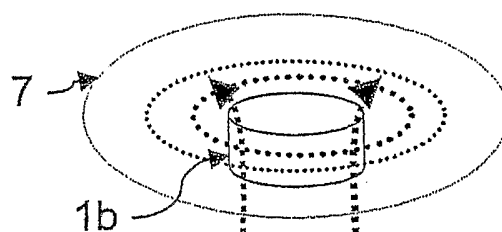


FIG. 3E

5/13

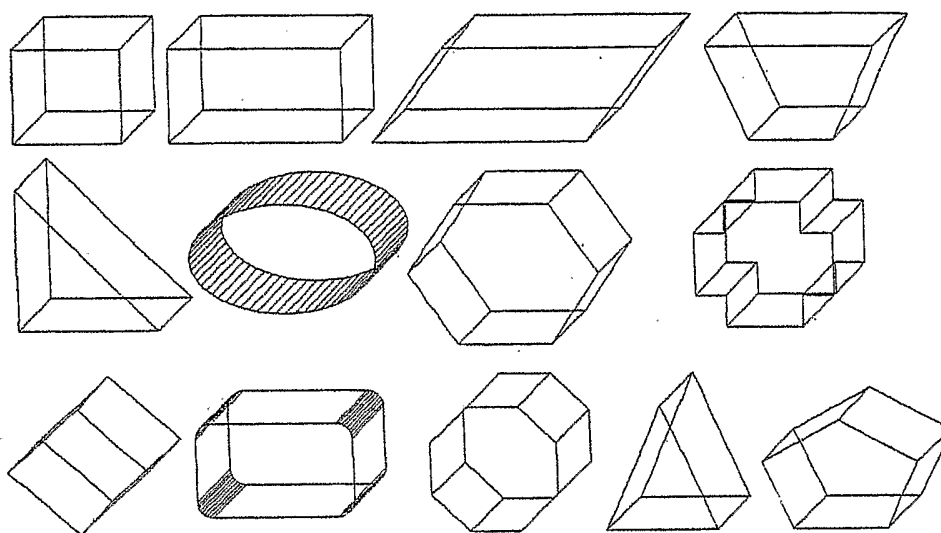


FIG. 3F

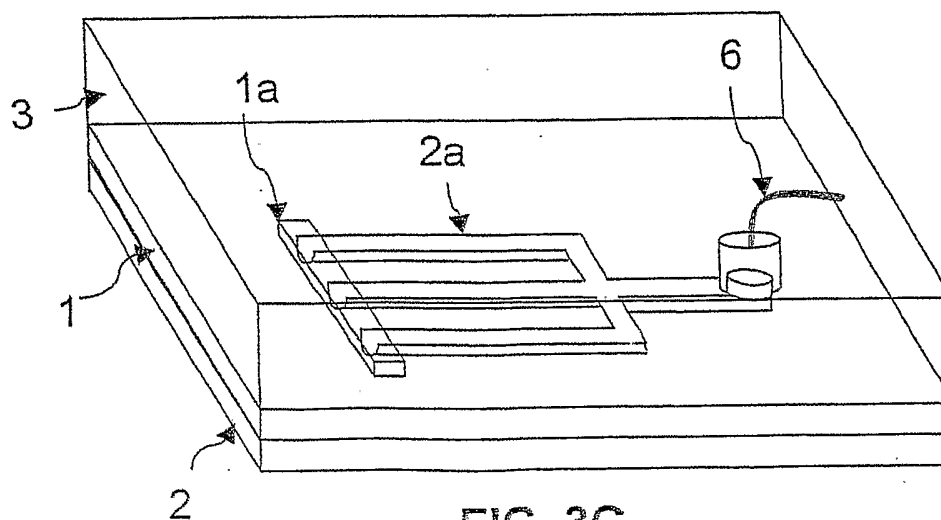


FIG. 3G

6/13

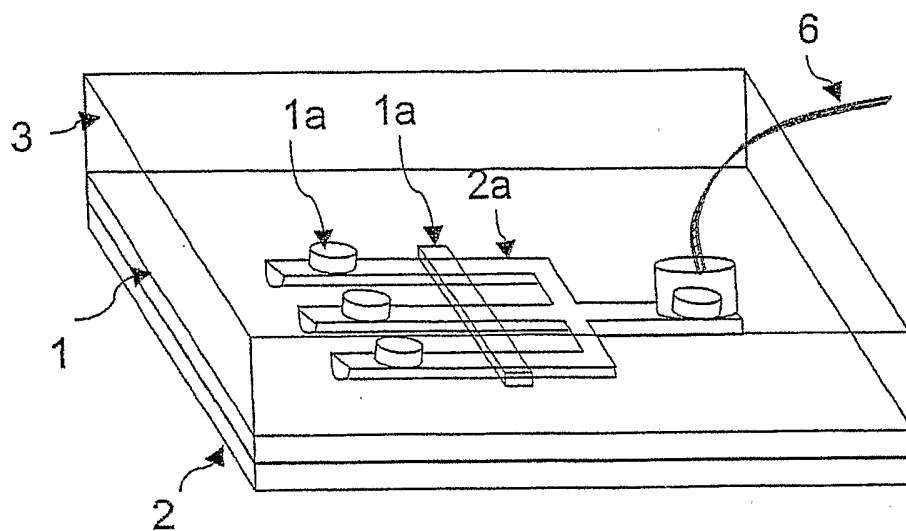


FIG. 3H

7/13

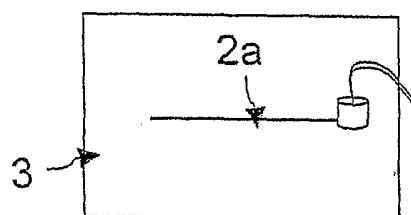


FIG. 4A

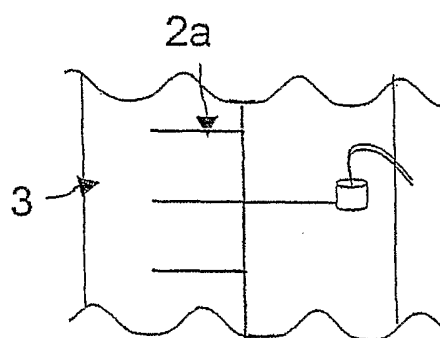


FIG. 4B

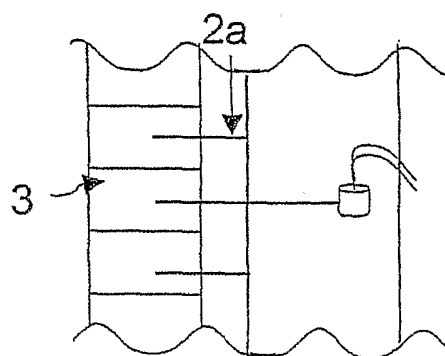


FIG. 4C

8/13

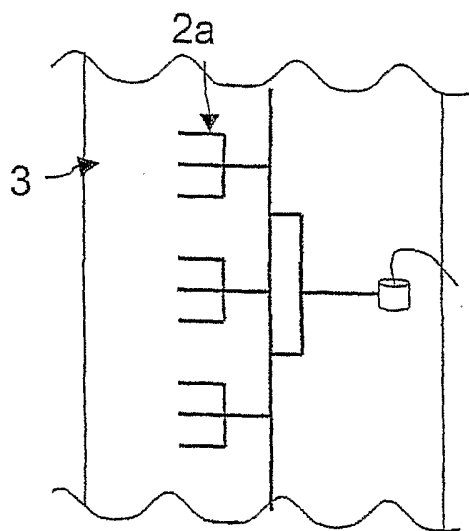


FIG. 4D

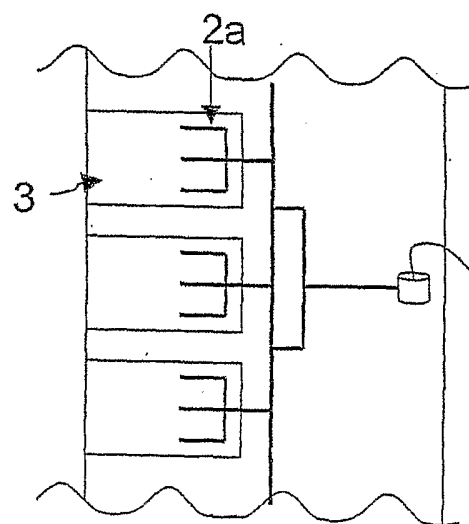
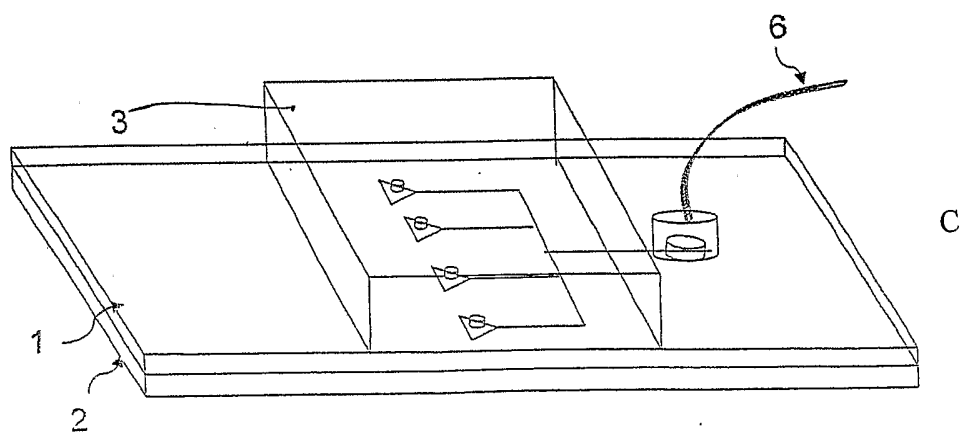
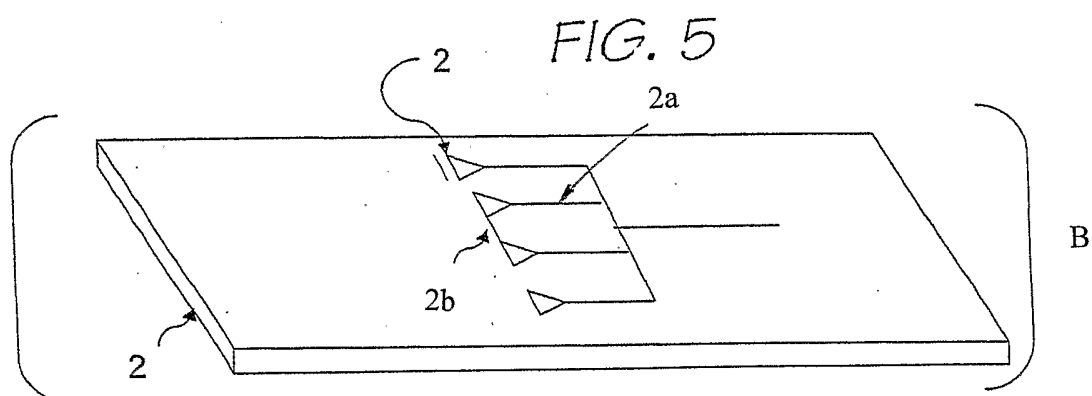
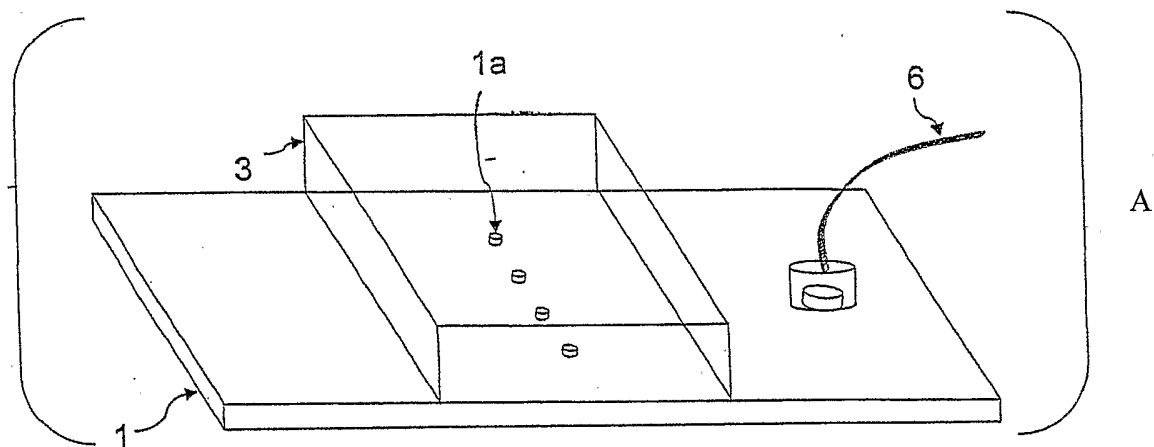


FIG. 4E

9/13



10/13

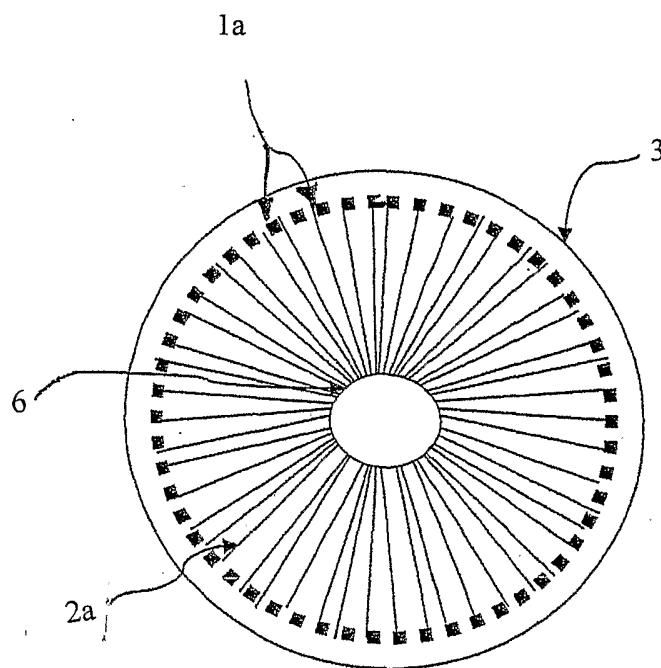


FIG. 6

11/13

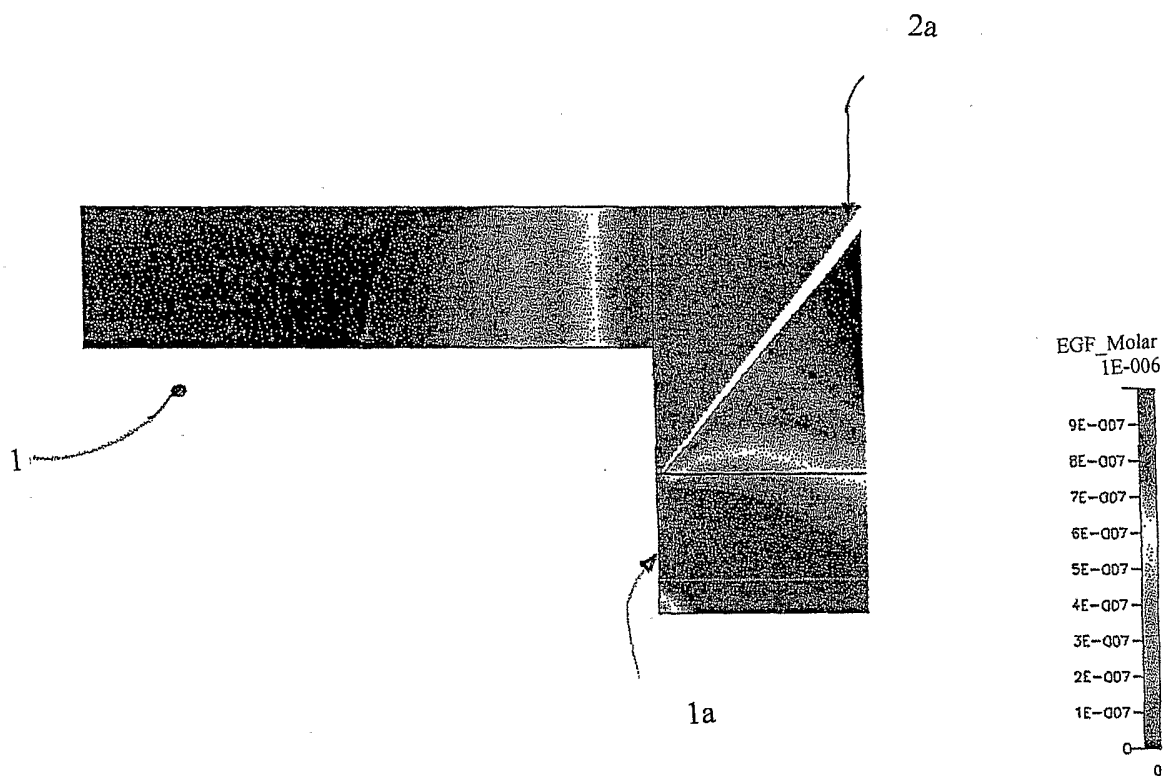


FIG. 7

12/13

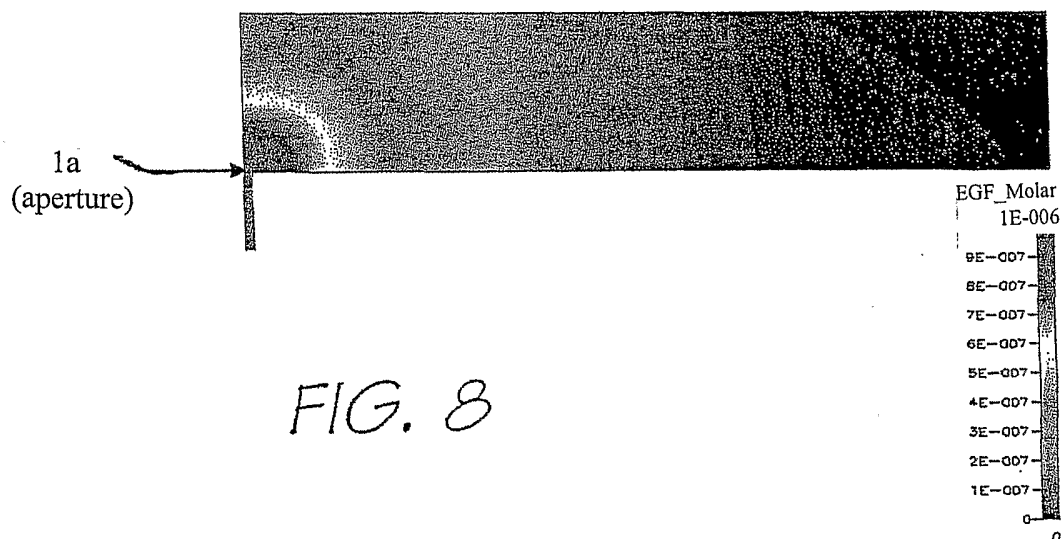


FIG. 8

13/13

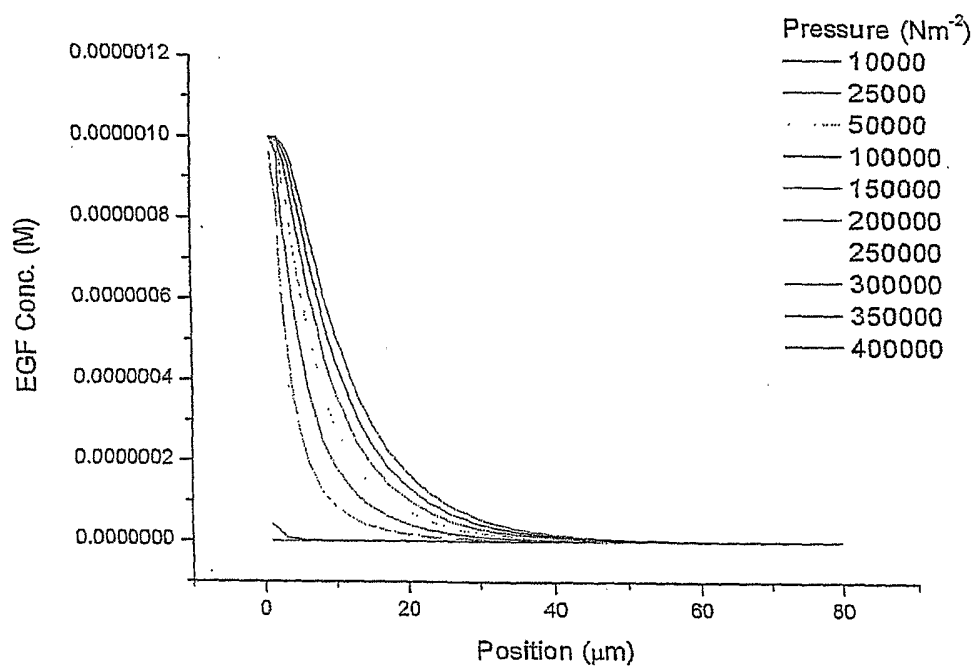


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2006/000116

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12M 1/34 (2006.01) G01N 33/48 (2006.01)

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)

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)

DWPI: C12M, B01L, G01N and (motil+ or chemotax+) and concentrat+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0022269 A (KIRK et al) 30 January 2003 See especially column 7 paragraph 58	1-5, 13-23, 31-35, 37
X	US 5302515 A (GOODWIN et al) 12 April 1994 See especially column 5 lines 12-20	1-5, 13-23, 31-35, 37
X	WO 2000/007007 A (BIOMETRIC IMAGING INC. et al) 10 February 2000 See whole document	31-35
A	WO 2004/053465 A (ADVANCED FLUIDIX LABORATORIES LLC) 24 June 2004 See whole document	1-37

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

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
15 February 2006Date of mailing of the international search report
20 FEB 2006Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustalia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

ROGER HOWE

Telephone No : (02) 6283 2159

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2006/000116

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5916801 A (LoPINTO et al) 29 June 1999 See whole document	1-37

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2006/000116

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	2003022269	AU	43397/02	AU	2003213834	AU	2003223722
		AU	2003282950	AU	2003287170	CA	2429056
		CA	2484058	CA	2503186	CA	2503203
		EP	1339838	EP	1502108	EP	1558076
		EP	1578194	US	6699665	US	6803205
		US	6811968	US	6818403	US	6844184
		US	6864065	US	6893851	US	6921660
		US	6967074	US	6982171	US	2002168757
		US	2003017582	US	2003021457	US	2003022153
		US	2003022197	US	2003022362	US	2003030184
		US	2003032046	US	2003032048	US	2003032076
		US	2003036188	US	2003040031	US	2003040033
		US	2003040087	US	2003068637	US	2003073228
		US	2004002131	US	2004142408	US	2004142411
		US	2005100974	US	2005250097	WO	0248676
		WO	03012392	WO	03012726	WO	03062920
		WO	03078565	WO	03091730	WO	2004038367
		WO	2004038368				
		US	5302515				
		WO	0007007	EP	1101106	US	6238874
WO	2004053465	AU	2003297757	CA	2507031	EP	1581798
		US	6929945	US	2004146850	US	2005083522
		US	2005130122	WO	2005037085		
US	5916801						
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							