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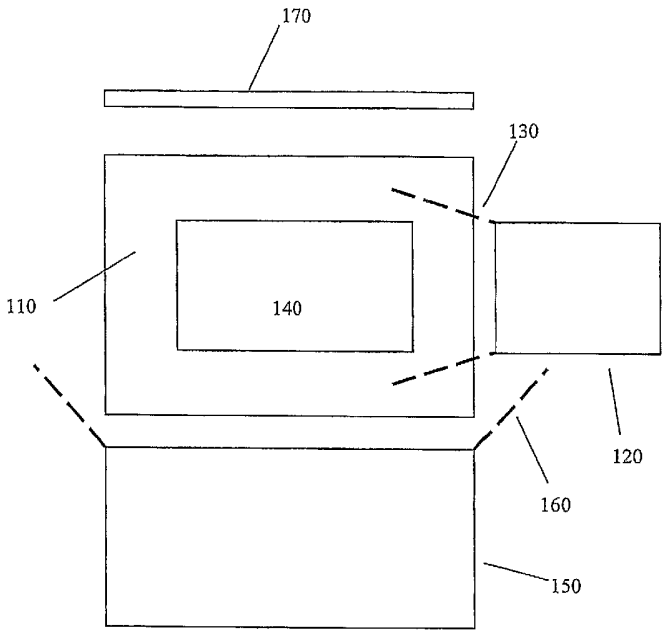
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(54) **Title:** MINIATURE LASER-INDUCED FLUORESCENCE DETECTOR



(57) **Abstract:** A microfluidic detection cell for stimulated radiation measurements, such as fluorescence or phosphorescence can have a detection volume of 1 μL or less and a sample can be excited using two excitation wavelengths. The detection cell can include a 5 mm to 10 cm long capillary tube and an excitation fiber proximate to the capillary tube. A detection fiber is also proximate to the capillary tube, and the detection fiber has a diameter the same size or larger than the internal diameter of the capillary tube. A plurality of either or both of excitation and detection fibers can be used.

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MINIATURE LASER-INDUCED FLUORESCENCE DETECTOR

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The invention relates to a miniature laser-induced fluorescence detector having an in-line microfluidic detection cell. The detection cell finds application in High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Mass Spectroscopy (MS) applications, among others.

Description of the Related Art

[0002] Advanced analytical methodologies are constantly requiring ever reduced sample sizes in such fields as neurochemistry, pharmacology, biochemistry, molecular biology, immunology, trace analysis of drugs in biological samples, environmental analysis, toxicology, oncology, cosmetology, food, and forensics. The analytical methodologies include high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry (MS). A detection method associated with these methodologies is fluorescence.

[0003] Fluorescence is the emission of a photon by a molecule following its excitation by an electromagnetic

radiation of a precise wavelength. This excitation induces an energy jump from a fundamental electronic state S0 to an upper electronic energy level S1. If the molecule comes back to its fundamental energy level via a radiative process, a photon is emitted. The photon energy corresponds to the energy difference between the two levels S0 and S1. Some of the excitation energy is dissipated by rotational and vibrational quanta, and so the excitation energy is thus always greater than the emission energy. Consequently the emitted fluorescence is always at a longer wavelength than the exciting light.

[0004] Phosphorescence occurs when the excitation is to a state that decays by a "prohibited" electronic transition. In this instance the lifetime of the excited state can be quite long, and there can be substantial delay between the excitation event and the emission event.

[0005] Conventional cells for measuring fluorescence are typically unsuited for in-line analysis of small samples. These cells are typically based on the filling of a cuvette, followed by the excitation and analysis of a static sample.

[0006] In order to more efficiently measure fluorescence and reduce sample size, cells have been developed using capillary tube technology. Figure 1 shows a related art column capillary detector. Figure 1 shows a laser 10 that

generates a laser beam carried by an optical fiber 20 to an optical bench, where a dichroic mirror 30 reflects the laser beam to be focused by an objective lens 40 to a cell 50, which is fed by column capillaries 60. The fluorescence signal is collected by the same lens 40 and passes through the dichroic mirror 30. After a series of filters 70, the signal reaches the photomultiplier tube (PMT) 80, which transforms the fluorescence signal into an electrical signal.

[0007] A capillary detection cell has been proposed by Chervet et al. (U.S. Patent 5,423,513). This method uses a bent capillary detection cell in which an external UV/visible light beam is directed into an elongated section of the detection cell from a bend thereof. However, this type of bent capillary detection cell is difficult to manufacture and therefore expensive. Therefore, this type of cell is not well suited for use as a disposable cell. Also, this is a relatively high volume detection cell (up to 20 nanoliters) with a path length of less than 10 mm.

[0008] However, the related art detection cells are hampered by the large required measurement volume. The related art capillary cells typically have a sample volumes in the range of 1 μL. However, this large volume is unsuitable for such goals as the measurement of sub-cellular components such as mitochondrial proteins. For measurement

on these types of samples, a sample volume of 10 nL or less is desirable.

[0009] Another disadvantage of the related art detection cells arise from the need for frequent cleaning. An expensive cell must be cleaned and reused. However, an inexpensive cell can be considered a consumable that can be thrown away after use.

[0010] An additional disadvantage of the related art technology arises from the reduction in sensitivity as the sample size decreases. Modern research requires detection of signals from samples at a concentration of 0.5 picogram/mL or even lower.

[0011] Another disadvantage of the related art technology is that there are large optical components, such as a microscope objective, dichroics, filters, or photomultiplier tubes, which must be positioned adjacent to the detection cell. These components are required for the coupling of light into and/or out of the detection cell in the existing architectures. It is then much more difficult to incorporate the detection cell into an existing instrument, such as a mass spectrometer, without substantially increasing the overall capillary length.

[0012] Accordingly, modern technology requires new detection cells that are easy to construct, inexpensive and

provide high sensitivity and utilize very small sample volumes. For optimal flexibility, these cells should also have optical fiber coupling from both the excitation and the emitted light.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The accompanying drawings, which are included to provide a further understanding of the invention and are incorporated in and constitute a part of this application, illustrate embodiments of the invention and together with the description serve to explain the principle of the invention.

[0014] In the drawings:

[0015] Fig. 1 shows a diagram of a related art fluorescence measurement apparatus; and

[0016] Fig. 2 shows a cross sectional view of a miniature detection cell in accordance with the invention.

[0017] Figure 3 shows a plan view of a bottom piece of a detection cell in accordance with the invention.

[0018] Figure 4 is a plan view of a top cover (top piece) of the inventive detection cell.

[0019] Figure 5 shows a diagram of a fluorescence spectrometer incorporating a microfluidic detection cell in accordance with the invention.

[0020] Figure 6 is a photograph of the inventive microfluidic detection cell mounted in a frame.

[0021] Figure 7 is photograph showing a different view of the mounted microfluidic detection cell.

[0022] Figure 8 shows the laser and photomultiplier tubes of the inventive fluorescence spectrometer.

[0023] Figure 9 shows a fluorescence chromatogram obtained using the inventive detection cell.

[0024] Figures 10A and 10B show a plan view and edge view, respectively, of a bottom piece of a second embodiment of a detection cell in accordance with the invention.

[0025] Figures 11A and 11B show a plan view and edge view, respectively, of a top piece of a second embodiment of a detection cell in accordance with the invention.

[0026] Figure 12 shows a photograph of a second embodiment of a detection cell in accordance with the invention in assembled form.

DETAILED DESCRIPTION OF THE INVENTION

[0027] Features and advantages of the invention will be set forth in the description which follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the invention will be realized and attained by

the structure particularly pointed out in the written description and claims hereof as well as the appended drawings.

[0028] It is to be understood that both the foregoing general description and the following detailed description of the invention are exemplary and explanatory of the invention as claimed. The scope of the invention is defined by the claims.

[0029] Accordingly, the invention is directed to producing a miniature laser-induced fluorescence detector that substantially obviates one or more problems due to limitations and disadvantages of the related art. Any particular embodiment of the invention might not solve every problem of the related art described above.

[0030] In one aspect, the invention provides a miniature detection cell comprising a flow cell portion formed from a capillary tube. The capillary tube portion of the detection cell can be about 5 mm to 10 cm in length and will typically have fluidic fittings at each end capable of easy and repeated insertion into or removal from an existing flow path. The detection cell can constitute part of a micro-flow chromatography system, a nano-flow chromatography system or a capillary electrophoresis system, and the detection cell is usable as a removable and reinsertable element in systems

such as mass spectrometers, electrochemical detectors, refractive index detectors and ultra-violet detectors.

[0031] The invention, in part, pertains to a detection cell comprising a capillary tube and an "excitation fiber" having an end proximate to the capillary tube and also a "detection" or "emission fiber" having an end proximate to the capillary tube. The excitation fiber(s) couple light from a source into the capillary tube for exciting a fluorescent molecule or other light emitting moiety to be detected. The detection fiber(s) couple emitted light from the capillary to a photodetector.

[0032] The excitation fiber can have a diameter the same size or larger than the inner diameter of the capillary tube. A detection fiber also has an end proximate to the capillary tube, and the detection fiber has a diameter the same size or larger than an inner diameter of the capillary tube. Such a relative diameter provides for maximal collection of emitted light. By proximate, a distance of 1 mm or less (including actually touching) is meant. In a preferred embodiment of the invention, the excitation fiber, the emission fiber and the capillary are all mutually perpendicular. The excitation and emission fibers are preferably located at the same cross sectional plane along the capillary, but together can be arranged at any appropriate location along the length of the

detection cell, provided that an optical window, i.e. a clear portion of the capillary that can transmit light, is present at such place on the capillary. (The entire capillary may be clear, in which case the entire length may serve as an optical window.) The detection volume is determined by the volume of the capillary excited with light from the excitation fiber. The total volume of the flow cell is determined by the length of the capillary needed for fluidic connections to a larger system. This length can be from 5 mm to 10 cm, preferably from 1 cm to 5 cm, and may be as short as 1 cm to 2 cm. In a preferred embodiment of the invention, excitation and detection fibers are located at corresponding locations of the detection cell so that the optimum amount of emitted fluorescent radiation can be collected by the detection fiber. That is, the detection fiber is preferentially located along the capillary in the same cross sectional vicinity as the excitation fiber, but arranged so that the numerical aperture of the detection fiber does not overlap that of the excitation fiber, as is illustrated in Fig. 2. In a preferred embodiment of the invention, the excitation fiber and the detection fiber are juxtaposed at about the same cross sectional plane of the capillary tube and oriented so that their numerical apertures do not overlap.

In this fashion, there is no excitation light directly incident on face of the detection fiber.

[0033] In the invention, the excitation fiber can have a numerical aperture the same size or smaller than the numerical aperture of the detection fiber. The diameter of the excitation fiber can be the same size or smaller than the diameter of the detection fiber. The capillary tube can have a circular or polygonal cross section, preferably, a square or rectangular cross section. The capillary tube can be formed from fused silica and may have an inner layer of fused silica and an outer layer of a polymer such as polyimide. In the instance of a polyimide-coated capillary, optical windows are formed at areas where light enters or leaves the capillary in operation of the excitation fiber and the detection fiber.

[0034] The detection volume of the flow cell is defined by the volume of the flow capillary that is illuminated by the excitation light. In the invention, the detection cell can have a detection volume of about 10 nL or less. The detection volume can be as small as one nL or even smaller, e.g. down to 0.25 nL in the instance of a capillary having a 50 μm diameter and a 100 μm diameter excitation fiber.

[0035] The detection cell can be used so that fluorescence signals at two wavelengths can be simultaneously excited and

measured. The excitation fiber can be attached to the detection cell at about a 90° angle from the detection fiber. The detection cell can have about a 100 μm by 100 μm inner dimension, that is, a cross section of about 10,000 μm^2.

[0036] In the invention, optical coupling between the capillary tube, the excitation fiber and the detection fiber can be optimized by placing an index matching fluid between the capillary window and the excitation fiber and the detection fiber. The index matching fluid will reduce or eliminate changes in refractive index as light traverses the capillary wall into the detection fiber and/or from the excitation fiber.

[0037] The invention, in part, pertains to a multi-wavelength detection cell that includes a capillary tube and at least one excitation fiber having an end proximate to the capillary tube, where the excitation fiber has a diameter a same size or larger than an inner diameter of the capillary tube. One or more detection fibers have an end proximate to the capillary tube, each with a diameter a same size or larger than an inner diameter of the capillary tube.

[0038] The detection cell of the invention can be used with two or more excitation wavelengths. In such applications, light of each excitation wavelength may be carried along a separate excitation fiber. Alternatively

light at two or more excitation wavelengths can be coupled into and carried along a single excitation fiber. Also, if light at two or more emission wavelengths is to be detected, there can be two or more detection fibers, with each detection fiber used to detect light at one of the emission wavelengths. Alternatively, one detection fiber can be used to detect light at two or more emission wavelengths, by use of beam splitters and thin-film filters at the far end of the fiber.

[0039] The invention, in part, also pertains to a method for manufacturing a detection cell. A method for manufacturing the detection cell of the invention includes providing a capillary tube having a glass or fused silica interior layer and a polymer outer layer; removing the polymer outer layer to form a window to accommodate the excitation fiber and the detection fiber; bringing an excitation fiber end proximate to the excitation window; and bringing a detection fiber end proximate to the detection window. The excitation fiber typically has a diameter the same size or larger than the inner diameter of the capillary tube, and the detection fiber also typically has a diameter a same size or larger than an inner diameter of the capillary tube.

[0040] Any polymer coating the outside of the capillary can be removed by heating methods known to the art, to form a window around the whole or part of the circumference of the capillary. In a multiple fiber detection system, each excitation/detection fiber pair would need a separate window. In some embodiments of the invention multiple detection fibers may be used to detect a signal excited by one excitation fiber. Multiple groups of fibers can be used to provide excitation at multiple wavelengths and detection at multiple wavelengths.

[0041]

[0042] Reference will now be made in detail to the preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings. The figures and preferred embodiments should not be considered as limiting of the invention; the scope of the invention is defined only by the claims following.

Figure 2 shows a cross sectional view of a microfluidic detection cell in accordance with an embodiment of the invention. The detection cell is formed from a capillary 110. The capillary 110 is formed from any material that can transmit visible, infrared and/or ultraviolet (UV) light. Preferably the capillary 110 is formed from glass or fused silica and may be coated with a polymer support, such as

polyimide. The capillary 110 can have any appropriate shape, but a circular, rectangular or square cross section is preferred. The preferable length of the capillary may be from 5 mm to 10 cm. By microfluidic, one means a cell capable of handling volumes in the μL range or less. The invention has the capability to evaluate samples in the range of 1 nL or less.

[0043] An excitation fiber 120 is brought proximate to the capillary 110. By proximate, from 5 mm to touching is meant. Preferably the excitation fiber 120 is from 1 mm to touching the capillary 110. The excitation fiber has a numerical aperture (indicated by dashed lines 130) sufficient to encompass the inner diameter 140 of the capillary 110. That is, the numerical aperture 130 need only be so large as to irradiate the interior of the capillary cell through which the sample flows. When the sample is irradiated, the sample becomes excited and fluoresces or phosphoresces. The emitted radiation is captured by the detection capillary 150. Since the fluorescence or phosphorescence is emitted in all directions, the detection fiber 150 should have a numerical aperture 160 as large as possible so that the maximum amount of radiation can be detected. In addition, the diameter of the excitation fiber 120 should be the same size or larger than the inner diameter of the capillary tube 110, and the

diameter of the detection fiber can be the same size or larger than the inner diameter of the capillary tube 110.

[0044] In order to enhance the efficiency of collection of the emitted light, mirrors such as 170 can be placed along surfaces of the capillary tube 110 that are not covered by excitation or collection fibers. The mirror 170 can be placed in a holder or made part of the bottom cover 200 and/or the top cover 260. Also, the interiors of the bottom cover 200 and/or the top cover 260 can be coated with a mirroring material such as silver. Also, the exterior of the capillary tube 110 can be coated with a mirroring material. In another embodiment, mirroring material can be placed between the fused silica interior layer and the polymer exterior layer of the capillary tube 110. For optimum coupling of the emitted light into the fiber 150, curved mirrors may be used.

[0045] In one preferred embodiment of the invention, the capillary 110 can have an outer dimension of about 363 ± 15 μm with an inner dimension of about 100 μm by 100 μm (± 5 μm). The excitation fiber 120 can preferably have a diameter of about 200 μm and a numerical aperture of about 0.22. The detection fiber can preferably have a diameter of about 600 μm and a numerical aperture of about 0.4. The capillary 110 can have different dimensions, typically 50 μm x 50 μm, 75 μm

x 75 μm and 100 μm x 100 μm ((± 5 μm). Regarding the excitation fiber, a multimode fiber no larger than 200 μm is preferable, and a numerical aperture of 0.22 is standard. The emission fiber can be from 100 μm to 1 mm in diameter, preferably from 350 μm to 1 mm in diameter, with a numerical aperture of from 0.37 to 0.66 being preferred. However, the invention is not restricted to the aforesaid dimensions, and any suitable dimensions can be used.

[0046] The capillary 110 can be a square capillary such as those available from Polymicro Technologies. The capillary can have an OD (outer diameter) of 300 μm, and when coated with polyimide, have a total OD of 363 ± 15 μm. The ID (inner diameter) of the capillary can be (measured from flat-to-flat) 50 ± 5 μm, 75 ± 5 μm or 100 ± 5 μm. In a preferred embodiment of the invention, the 100 μm ID is used. This dimension can result in a flow-through cell having a 100 μm by 100 μm (10,000 μm^2) inner cross sectional area. However, the capillary can also have a circular or even oval cross section.

[0047] The capillary tube 110 can be formed from glass (e.g., borosilicate glass) if visible wavelengths are of interest. The capillary tube 110 can also be fused silica to extend the range of wavelengths into the ultraviolet. A polymer such as polyimide can cover the capillary tube 110.

The capillary tube is therefore translucent or opaque. Therefore, a window must be formed by removing the polymer at the area where the fibers are to be placed. For multiple excitation/detection fiber pairs, corresponding multiple windows are to be formed. The polymer can be burnt off. In a preferred embodiment of the invention, the polymer can be burnt off using a heated wire. A nichrome wire heated by an electric current can be used. Also, a laser can be used to remove the polymer. The window goes, wholly or partly, around the circumference of the capillary.

[0048] The excitation fiber 120 and the detection fiber 150 can be joined proximate to the glass or fused silica face of the capillary window. By proximate, a distance from about 1 mm to touching is meant. However, the light transmission can be enhanced if a drop of water, glycerin or other index matching fluid is placed between the fiber and the capillary. Ideally, an immersion fluid that has a refractive index equal to the glass or fused silica in the fiber and/or capillary should be used. The refractive index of fused silica varies from 1.40 to 1.55 through the transmission range. While air has a refractive index of 1.00, room temperature water has a refractive index of 1.33. Glycerine, with a refractive index of 1.473, is also a good index matching fluid. In a preferred embodiment of the invention, enhanced light

collection was observed when a drop of liquid (water, glycerin, index matching fluid or immersion oil) was placed between the capillary 110, the excitation fiber 120, and the detection fiber 150. Also, wax or optical cement can be used to attach the excitation fiber 120 and the detection fiber 150 to the capillary 110. If the surfaces to be joined are very flat, then optical contacting techniques can be used, where the capillary and fiber or thoroughly cleaned, physically joined and then sealed around the edges with a shellac or cement. However, cementing may negatively impact the disposability of the cell.

[0049] Also, when water is used as the immersion fluid, the inventors have observed that a regime of optimized optical signal is observed as the water evaporates, probably due to meniscus formation. In this way, a roughly 10-fold increase in signal was observed.

[0050] In a preferred embodiment of the invention, the excitation fiber 120 and the detection fiber 150 are joined to a square or rectangular capillary at different faces at an angle of about 90° to one another. This normal angle arises naturally from the square geometry of the capillary 110. However, the invention is not restricted to right angle (90°) joinder of the excitation fiber 120 and detection fiber 150 to the capillary 110, since the measurement is of light

radiation, typically fluorescence, that is emitted in all directions. Any appropriate angle can therefore be used to join the excitation fiber 120 and detection fiber 150 to the capillary 110. Preferably, laser light from the excitation fiber will not be directly coupled into the detection fiber. This constraint, coupled with the cell geometry, thus translates to a range of joinder angle of from about 10° to about 120°.

[0051] The arrangement of the fibers and the capillary tube can be very versatile. For example, the fibers can be arranged about a single cross sectional area of the capillary tube. That is, the excitation fiber and the detection fiber can be arranged about the capillary tube so that they are aligned at the same cross sectional plane of the capillary tube. Also, more than one excitation (or detection) fiber can be placed at the same cross section. On the other hand, the fibers can be arranged along the linear length of the capillary tube. However, for any given excitation/emission fiber pair, it is preferred that both elements in the pair be aligned to the same cross-section of the capillary, as is done in the exemplary holder/device.

[0052] Figure 3 shows a plan view of a bottom piece 200 of a detection cell in accordance with the invention. The bottom cover 200 has two grooves 210, 220 cut at right (90°)

angles. The two grooves 210, 220 are to hold the excitation fiber 120 (not shown in the figure 3) and the capillary 110 precisely positioned and at right angles to each other. A recess 230 defines the area in which the fiber 150 is to be joined to the capillary 110 and the fiber 120. The recess is on the opposite side from the grooves, and is present to provide access for tightening and loosening the sub-miniature type-A (SMA) coupling of the detection fiber. A circular opening 240 defines a window into which the detection fiber 150 is to be attached to the bottom cover 200 at an orientation normal to the surface of plane surface of the bottom cover 200. The bottom cover 200 also has screw or bolt holes 250 for fastening the bottom cover 200 to the top cover 260 so as to provide a holder for the detection cell.

[0053] Although not restricted to these dimensions, the bottom piece 200 can have a length of 1.4 inches. Groove 220, for holding the capillary 110, can have a dimension of 0.015 inches wide and 0.012 inches deep. Groove 210, for holding the excitation fiber 120, can be 0.025 inches wide by 0.016 inches deep. This groove should be circular or wedge shaped so as to hold the excitation fiber precisely in the center of the groove. The recess 230 can have a diameter of 0.5 inches and a depth of 0.125 inches. The circular opening 240 can

have a radius of 7/32 (0.22) inches. The screw or bolt holes 250 were tapped at 2.5 mm.

[0054] Figure 4 is a plan view of a top cover (top piece) 260 of the inventive detection cell. Groove 270 accommodates the excitation fiber 120, and allows the center of the excitation fiber 120 to coincide precisely with the center of the capillary. An oblong or oval window 280 is formed at the center of the top cover 260 to avoid compression of the capillary at the optical window in the coating polymer. Screw or bolt holes 290 correspond to the screw or bolt holes 250 of the bottom cell. The top cover can have a dimension of 1.4 inches to a side. The groove 270 can be 1/8 (0.125) inches wide and .004 inches deep. The screw or bolt holes 290 can be 2.5 mm holes. It is important that the groove 270 be somewhat wider than the excitation fiber 120 so as not to interfere with the horizontal position already determined by the position of the groove 210 in the bottom piece 200. The depth of the grooves are chosen such that the center of the excitation fiber 120 coincides precisely with the center of the capillary 110. The fibers will in some embodiments be mechanically protected, e.g. by a Tefzel® (DuPont) buffer, and for practical reasons, the excitation fiber and/or detection fiber may be larger in outer diameter than the outer diameter

of the capillary, or vice versa. The depths of the grooves are then adjusted accordingly.

[0055] The size of the screw and bolt holes is not important. The overall 1.4 inch dimension is not critical, but was chosen as a compromise between ease of adjustment (bigger is easier) and flow cell length (smaller is better).

[0056] The bottom cover 200 and the top cover 260 of the holder of the inventive detection cell can be milled from aluminum. Anodizing the aluminum is preferred. However, a less expensive and thus disposable detection cell and holder can be constructed from a suitable plastic such as polypropylene, nylon, polyethylene terephthalate, polytetrafluoroethylene, etc. Also, the bottom cover 200 and the top cover 260 can be made by injection molding.

[0057] In practice, the detection cell can be assembled in a highly efficient fashion. The bottom cover 200 and the top cover 260 can be first bolted together loosely. Then the capillary tube 110, and the fibers 120, 150 can be inserted into the channels formed by the matching grooves in the bottom cover 200 and the top cover 260, and the bolts tightened to clamp everything in place. To match the refractive index of the capillary tube with the fibers, the ends of the fibers, 120, 150 can be wetted with an index matching fluid (such as water, glycerine or immersion oil).

The application of the index matching fluid is preferably performed after fiber insertion, through an opening such as the window 280. Alternately, the capillary tube 110 and the fibers 120, 150 can be aligned in the corresponding grooves of the bottom cover 200 before the top cover 260 is bolted on.

[0058] Of course, if a plurality of excitation and/or detection fibers are used, then the arrangement of grooves for holding the fibers is modified accordingly.

[0059] Figure 5 shows a diagram of a fluorescence spectrometer incorporating a microfluidic detection cell in accordance with the invention. An excitation laser 300 emits laser light for exciting the fluorophore(s). Any appropriate wavelength laser can be utilized, such as a 409 nm laser. Other laser wavelengths can include, but are not restricted to 266 nm, 308 nm, 330 nm, 375 nm, 405 nm, 470 nm, 532 nm, 633 nm and 660 nm. Also, a tunable laser can be used. The laser radiation passes through a condensing lens or objective 310 to be coupled into the excitation fiber 320. The excitation fiber enters the microfluidic detection cell 330 to irradiate the rectangular or square capillary 340.

[0060] The detection fiber 345 carries light from the cell 330 to a collimating lens 350 and through thin film filters 360, which filter out the excitation wavelengths. The wavelength of the emitted light is dependent upon the

particular signaling moiety used and the selection of appropriate filters and detection fibers would be apparent to one of ordinary skill in the art.

[0061] The filtered fluorescence signal is then incident to a photomultiplier tube (PMT) 370 powered by a power supply 380. The electronic signal generated by the PMT is then processed to produce fluorescence data. Although not shown, the cell 330 is typically connected in-line with an analytical device such as a HPLC or CE apparatus.

[0062] The capillary 110 of the microfluidic cell can be connected to a liquid line using any appropriate fitting or piping arrangement. Preferably, the connection is made using a compression-type fitting that minimizes or eliminates any dead space. The connection can be accomplished using easily disconnectable microfluidic connectors known to the art, such as NANOTIGHT or MICROTIGHT connectors from Upchurch Scientific, Oak Harbor, Washington. However, any suitable compression fitting or connector can be used.

[0063] Figure 6 is a photograph of the inventive microfluidic detection cell mounted in a holder or frame. The capillary can be observed to be entering the left and right sides of the cell. The excitation fiber can be seen to be entering the top of the cell. The detection fiber leaves

the back of the cell and is not seen. A United States quarter is seen at the bottom to indicate the scale.

[0064] Figure 7 is photograph showing a different view of the mounted microfluidic detection cell. In this view, the detection fiber and be seen connected to the rear, i.e., bottom piece, of the detection cell. The excitation fiber enters the detection cell from the top. The light colored fittings indicate the position of the capillary.

[0065] Figure 8 shows the laser and photomultiplier tubes of the inventive fluorescence spectrometer. At the bottom of Figure 8, a 409 nm excitation laser is positioned to emit light through a lens to the excitation fiber. At the top of Figure 8 is the photomultiplier assembly, which has a detection fiber, collimating lens, filter and PMT.

[0066] To assemble a detection cell of the invention, a bulkhead sub-miniature type-A (SMA) connector is positioned (screwed into) tapped hole 240 so that the SMA-terminated detection fiber will be proximate to the capillary 110 (roughly 20-100 μm distant). Detection fiber 150 was inserted for positioning, but is then removed, as further assembly is easier if the holder (bottom piece) 200 can lie flat on that face. The capillary 110 is cut to length, and an optical window is made in the capillary (using a heated coil). The bottom cover 200 of the aluminum holder (shown in Fig. 3)

is already bolted into a black plastic box (shown in Figs 6-7). The capillary 110 is threaded through the Microtight fittings (including compression sleeves used with Microtight fittings) already in place in the holes in the black box, and lightly pressed into groove 210 with the optical window centered over opening 240. The excitation fiber 120 is cleaved and placed in groove 220. Exact positioning is not important here, though the end should be close to capillary 110. The top cover 260 (Fig. 4) is placed over the bottom cover 200 and loosely attached to the bottom cover 200 (Fig. 3) using 4 screws through holes 290 and tapped holes 250, taking care to tighten the four screws evenly to keep plates parallel. An excitation fiber 120 is slid along groove 210/270 until the end is proximate (20-100 μm distant) to the capillary, as can be seen through the openings 280 and 240. One then checks that the optical window of the capillary is still in the correct position. Then, the screws are tightened (evenly) until the fibers can no longer slide. Microtight fittings (which until now have not been tightened, and so can slide freely over the capillary) are now tightened on the capillary per manufacturer's instructions. Set screws at holes in the black plastic box are tightened to hold the Microtight fittings in place (mostly to provide strain relief). Then, the SMA-terminated detection fiber 150 is

screwed into the bulkhead fitting. At this point, the Microtight fittings (which can be chosen to be unions for HPLC PEEK tubing) can be attached to the flow path in order to insert the detection cell into whatever large system is being used.

[0067] Figures 10A and 10B show the bottom plate 400 and Figures 11A and 11B show the top plate 460 of one embodiment of a detector having two excitation fibers and two emission fibers. Dimensions of the various grooves, screw holes and openings for fittings are similar to those for the single emission fiber embodiment described above, and the detector is assembled in a similar fashion.

[0068] In Figures 10A and 10B, the groove 410 holds the capillary 110. The two excitation fibers are laid in grooves 421 and 422. The excitation fibers are brought into proximity to the capillary; a portion of the exciation fiber in groove 421 may be unsupported by the groove. The opening 441 is tapped to allow coupling of a SMA fitting or other fitting holding an emission fiber. The opening 431 is left open to provide tool access for installing the fitting holding the emission fiber. The center of the opening 441 is aligned with the point defined by the crossing of the centerlines of the grooves 410 and 421. Holes 450 are provided for inserting a means for connecting the top piece

400 and bottom piece 460 of the detection cell. Holes 451 are optionally provided to allow mounting of the detection cell onto another item.

[0069] In Figures 11A and 11B, the holes 490 may tapped so as to allow tightening of the top and bottom plates together after the detector is assembled, as described above. Alternatively, the tightening mechanism may be provided by the fitting piece itself, e.g. as when a bolt or sliding collar is used. The opening 442 is made to engage a fitting holding a second emission fiber in the manner similar to the opening 441. The center of the opening 442 is aligned with the point defined by the crossing of the centerlines of the grooves 410 and 422. Similarly to the hole 431, the opening 432 is made to provide tool access to the fitting inserted into the opening 442.

[0070] Figure 12 is a photograph of a detection cell of the two excitation fiber, two detection fiber embodiment described above. The capillary is shown horizontally, and is clearly seen on the left side; the exit of the capillary is in shadow. The two excitation fibers can be seen entering the cell from the top. One of the detection fibers is shown perpendicular to the plane of the detector. The other detection fiber is hidden behind the detector.

[0071] The optical fibers used in the invention can be plastic clad silica (PCS) that have a silica core, a silicone resin optical cladding and a fluoropolymer, such as ethyltetrafuoroethyene (ETFE), coating. The core diameter of the fibers can vary from 200 to 1000 μm (e.g., 200 μm, 300 μm, 600 μm or 100 μm). Commercial optical fiber technology does make available additional optical fiber choices. For example, a 400 μm diameter Teflon®-clad fiber is available, which is useful as a detection fiber. Silica-clad silica fibers are available in 200 μm and 100 μm diameters that are useful as excitation fibers.

[0072] The numerical aperture of the optical fibers can be as high as 0.4 or higher. For example a Teflon®-clad fiber may have a numerical aperture as high as 0.66.

[0073] The fiber connectors can be for single mode or multimode fibers. SMA connectors are one of the industry standard connectors for coupling fibers to instruments or to each other. Other connectors can be used, such as fiber channel (FC) or straight tip (ST) connectors. The optical fibers can be cut and the ends polished using methods known in the art.

[0074] The invention is not restricted to utilizing excitation by a single laser wavelength. The excitation fiber can carry two or more excitation wavelengths to thus

afford a more thorough fluorescence analysis. The downstream optics can also then include a beam splitter to separately analyze the fluorescence emissions. Also, more than one excitation fiber can be used to inject excitation radiation into the detection cell. Additionally, several of the inventive detection cells can be placed in line in parallel or series configurations to permit multiple analyses.

[0075] When more than one wavelength is used, the cell can take on several configurations. If two excitation wavelengths are used, both wavelengths can be transmitted using a single excitation fiber, where instead of a single laser 300, two lasers are used and their beams made collinear using mirrors and a dichroic. Alternately, a separate excitation fiber and laser can be used for each excitation wavelength. Also, multiple emission wavelengths (which may arise from multiple fluorophores or phosphors excited at the same or at different wavelengths) can be measured using the same detection fiber. Alternately, a different detection fiber can be used for each emission wavelength. Also, a mirror or second detection fiber coupled to a single PMT detector can be placed at the window 280 to improve sensitivity.

[0076] In an additional embodiment of the invention, multiple, independent detection cells can be used for each

wavelength. The detection cells can be placed in-line in either parallel or series configurations. The low cost and compact size of the inventive cell makes this option attractive when analysis of multiple wavelengths is desired.

EXAMPLE

[0077] The inlet adaptor (Upchurch F720 Microtight union) of an assembled flow cell was attached to a Micro-Tech Protein 5-C18W-100 micro-bore column via 5-cm of 100-μm internal diameter PEEK tubing. The outlet adaptor (Upchurch F720 union) of the flow cell was attached to a 20-cm length of identical tubing, which ran to waste. The analytical column was attached to a Dionex LC Packing Ultimate dual gradient Micro-flow HPLC and the separation run at 8 μL per minute. Twenty femtomoles in 1 μL of a commercially available (Sigma/Aldrich Chemical Company) tryptic digest of bovine serum albumin was injected and the elution monitored on-line via the detector. The output of the photomultiplier was fed into a World Precision Instrument Duo-18 data acquisition interface and recorded on a PC as a virtual recorder. The chromatogram was screen captured and printed using Corel Draw 7 Software Suite. The resulting chromatogram is shown in Fig. 9.

[0078] The detection cell of the present invention provides for linear detection of analytes, which allows for

quantitation of the analyte, at least through the 20 fmole to 500 fmole range in a detection volume of 4 nl.

[0079] Therefore, embodiments of the invention offer many clear advantages over the conventional art detection cells, which have high cost, large sampling volumes and insufficient sensitivity. In contrast, the invention utilizes a low volume microcapillary and an excitation fiber that has a diameter and numerical aperture sufficiently small to illuminate only the interior of the microcapillary, and a detection fiber having a diameter and numerical aperture larger than that of the excitation fiber. The resulting inventive detection cell thus is very advantageous for the analysis of proteins and sub-cellular materials. The invention has a detection volume as low as 1 nL or less in comparison to the 2000 nL sample volume typical of the conventional art. For example, a 1 cm x 50 μm x 50 μm capillary has a detection volume of 250 pL. If a smaller capillary is used, a smaller diameter optical fiber for excitation would be needed. The invention therefore offers a disposable detection cell having one or more advantages of compactness, sensitivity, ease of manufacture and low cost.

[0080] Also, the inventive detection cell is very versatile. It can be part of a micro-flow chromatography system, a nano-flow chromatography system or a capillary

electrophoresis system. The inventive detection cell is usable in combination with detection systems that can be, for example a mass spectrometer, an electrochemical detector or an ultraviolet detectors. The detection flow cell would be connected in-line with the flow path of the other/larger instrument via the Microtight fittings seen in, e.g., Figs. 6-7. The associated optics and electronics then run independently, and can be placed several meters away (the distance limited only by the length of the optical fibers).

[0081] It will be apparent to those skilled in the art that various modifications and variations can be made in the invention as described hereinabove without departing from the spirit or scope of the invention. It is intended that the invention covers the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

What Is Claimed Is:

1. A detection cell, comprising:

a capillary tube having a length of 5 mm to 10 cm;

at least one window in the capillary tube;

a fluidic fitting mounted at each end of the capillary tube;

at least one excitation optical fiber having an end proximate to the window of the capillary tube; and

at least one detection optical fiber having an end proximate to the window of capillary tube.

2. The detection cell of claim 1, wherein the at least one excitation fiber has a numerical aperture the same size or smaller than a numerical aperture of the detection fiber.

3. The detection cell of claim 1, wherein the diameter of the at least one excitation fiber is the same size or smaller than the diameter of the detection fiber.

4. The detection cell of claim 1, wherein the capillary tube has a circular, square or rectangular internal cross section.

5. The detection cell of claim 1, wherein the capillary tube has a circular, square or rectangular external cross section.

6. The detection cell of claim 1, wherein the capillary tube is formed from fused silica or glass.

7. The detection cell of claim 1, wherein the capillary tube is formed from an inner layer of fused silica and an outer layer of polymer, and optical windows are formed at areas facing the excitation fiber and the detection fiber.

8. The detection cell of claim 7, wherein the polymer is polyimide.

9. The detection cell of claim 1, wherein the capillary has a detection volume of 1 μL or less.

10. The detection cell of claim 1, wherein the capillary has a detection volume of 10 nL or less.

11. The detection cell of claim 1, wherein the at least one excitation fiber is placed proximate to the detection cell at a same cross section of the capillary as at least one paired detection fiber at about a 90° angle from the paired detection fiber.

12. The detection cell of claim 1, wherein the capillary tube has a cross section of about 10,000 μm^2.

13. The detection cell of claim 1, wherein optical coupling between the capillary tube, the excitation fiber and the detection fiber is optimized by placing a refractive index matching fluid between the capillary and aperture of the excitation fiber and between the capillary and the aperture of the detection fiber, wherein a refractive index refractive index matching fluid approximates the refractive index of the capillary tube, the refractive index of the excitation fiber and the refractive index of the detection fiber.

14. The detection cell of claim 1, wherein the fluidic fittings are capable of easy and repeated insertion into or removal from an existing flow path.

15. The detection cell of claim 1, wherein the detection cell comprises part of a micro-flow chromatography system, a nano-flow chromatography system or a capillary electrophoresis system, and the detection cell is usable in combination with detection systems selected from the group consisting of mass spectrometer, electrochemical detectors, refractive index detectors and ultra-violet detectors.

16. The detection cell of claim 1, wherein there are a plurality of excitation fibers each having an end proximate to the capillary tube, each excitation fiber having a diameter a same size or larger than an inner diameter of the capillary tube.

17. The detection cell of claim 16, wherein there are a plurality of detection fibers each having an end proximate to the capillary tube, each detection fiber having a diameter a same size or larger than an internal diameter of the capillary tube.

18. A detection method, comprising:

providing the detection cell of claim 1;

introducing a liquid sample into the capillary tube;

transmitting light of at least one excitation wavelength into the detection cell via the at least one excitation fiber; and

measuring emitted light transmitted through the at least one detection fiber.

19. The detection method of claim 18, wherein two or more excitation wavelengths are transmitted along one excitation fiber.

20. The detection method of claim 18, wherein there are a plurality of excitation fibers, and a different wavelength is transmitted along each excitation fiber.

21. The detection method of claim 18, wherein there are two excitation fibers.

22. The detection method of claim 18, wherein there are a plurality of detection fibers.

23. The detection method of claim 19, wherein there are a plurality of detection fibers, each coupled to a detector for detecting emitted light of a different wavelength.

24. The detection method of claim 20, wherein there are a plurality of detection fibers, each paired to a different excitation fiber and each coupled to a detector for detecting emitted light of a different wavelength.

25. The detection method of any one of claims 18-24, in which the emitted light is fluorescent light.

26. A method for manufacturing a detection cell, comprising:

providing a 5 mm to 10 cm long capillary tube having a glass or fused silica interior layer and a polymer outer layer;

removing the polymer outer layer to form an excitation window and a detection window;

bringing an excitation fiber end proximate to the excitation window; and

bringing a detection fiber end proximate to the detection window,

wherein the detection fiber has a diameter a same size or larger than an internal diameter of the capillary tube.

27. A method for manufacturing a detection cell, comprising:

providing a top cover having a groove for a capillary tube having a length of 5 mm to 10 cm, a groove for an excitation fiber and a port for a detection fiber;

attaching a bottom cover to the top cover, to form a cavity for the capillary tube and a cavity for the excitation fiber;

removing a polymer outer layer from the capillary tube to form a window;

inserting the capillary tube into the cavity for the capillary tube;

inserting the excitation fiber into the cavity for the excitation fiber so that the excitation fiber has an end proximate to the excitation window; and

inserting the detection fiber into the port to bring a detection fiber end proximate to the detection window,

wherein the detection fiber has a diameter a same size or larger than an external diameter of the capillary tube.

28. The method according to claim 26, wherein the polymer is removed by heating the polymer.

29. The method according to claim 27, wherein the polymer is removed by heating the polymer.

Fig. 1

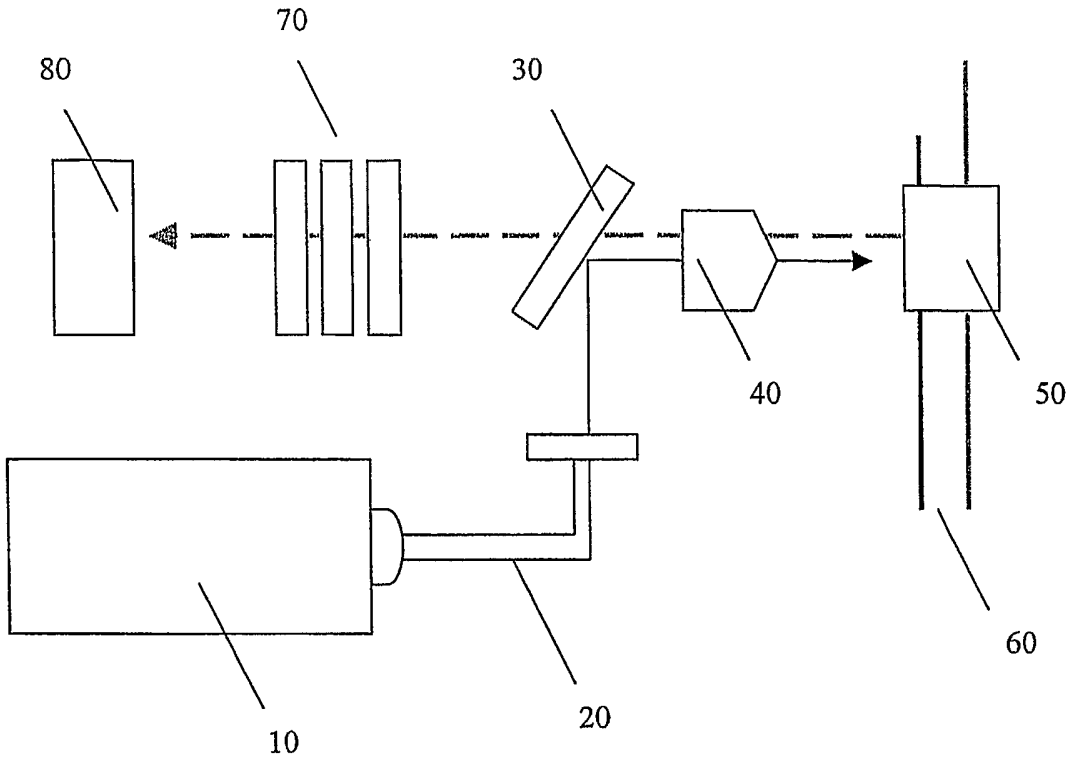


Fig. 2

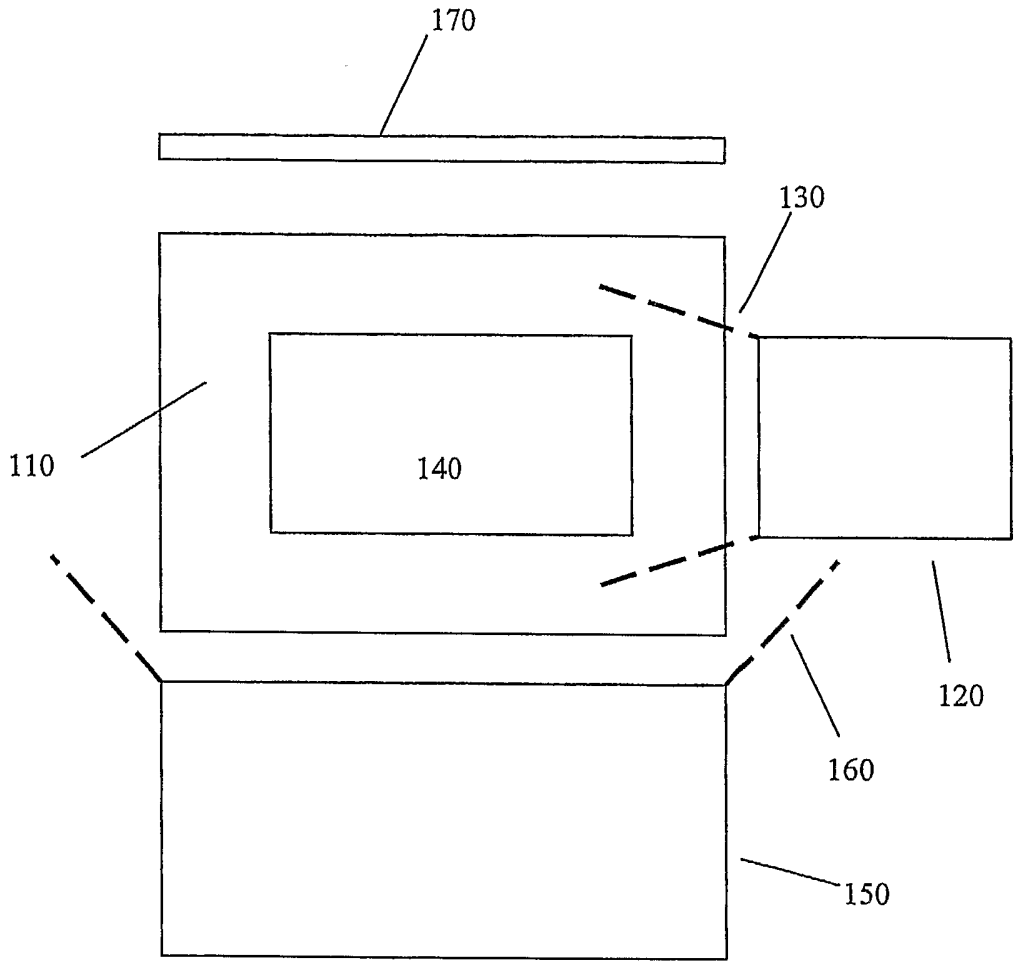


Fig. 3

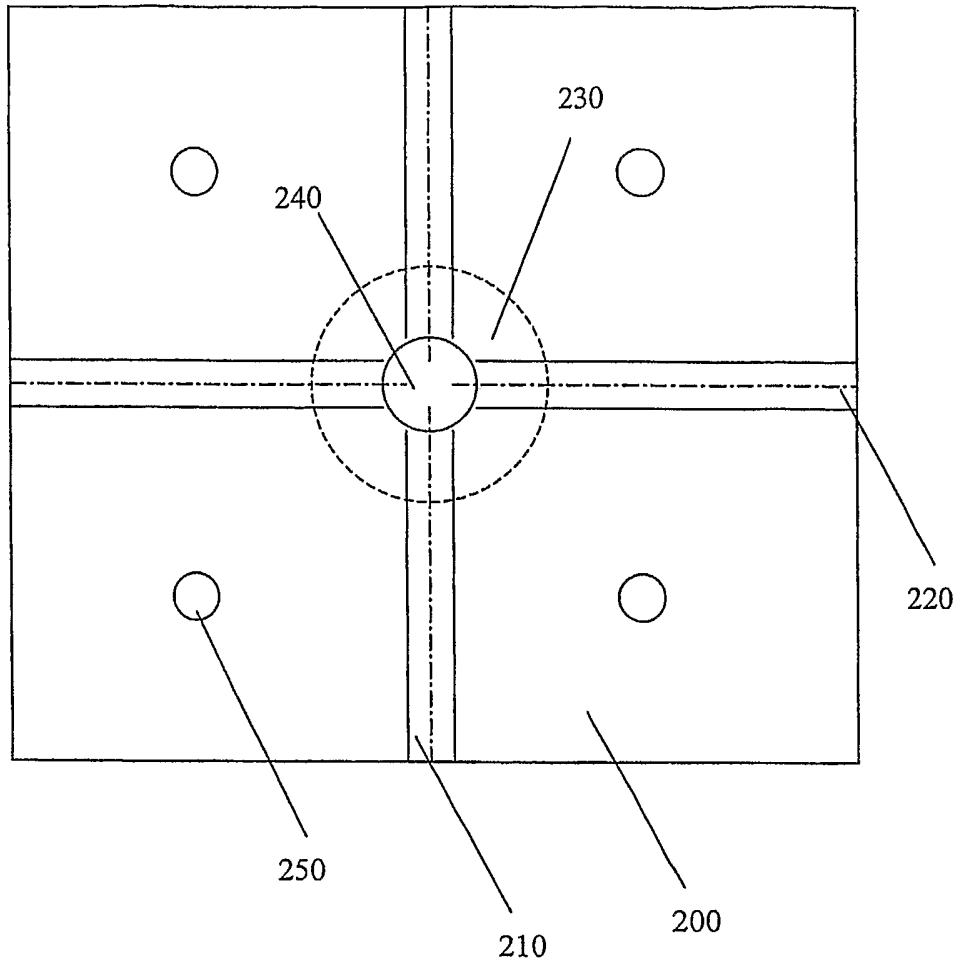


Fig. 4

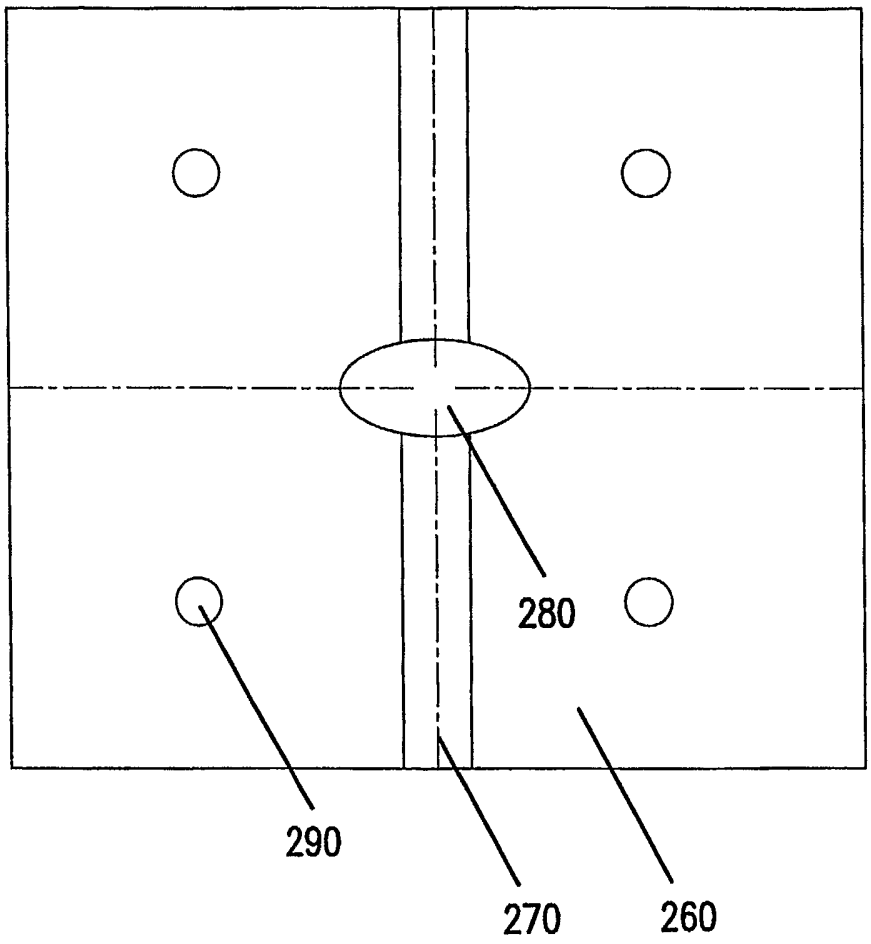


Fig. 5

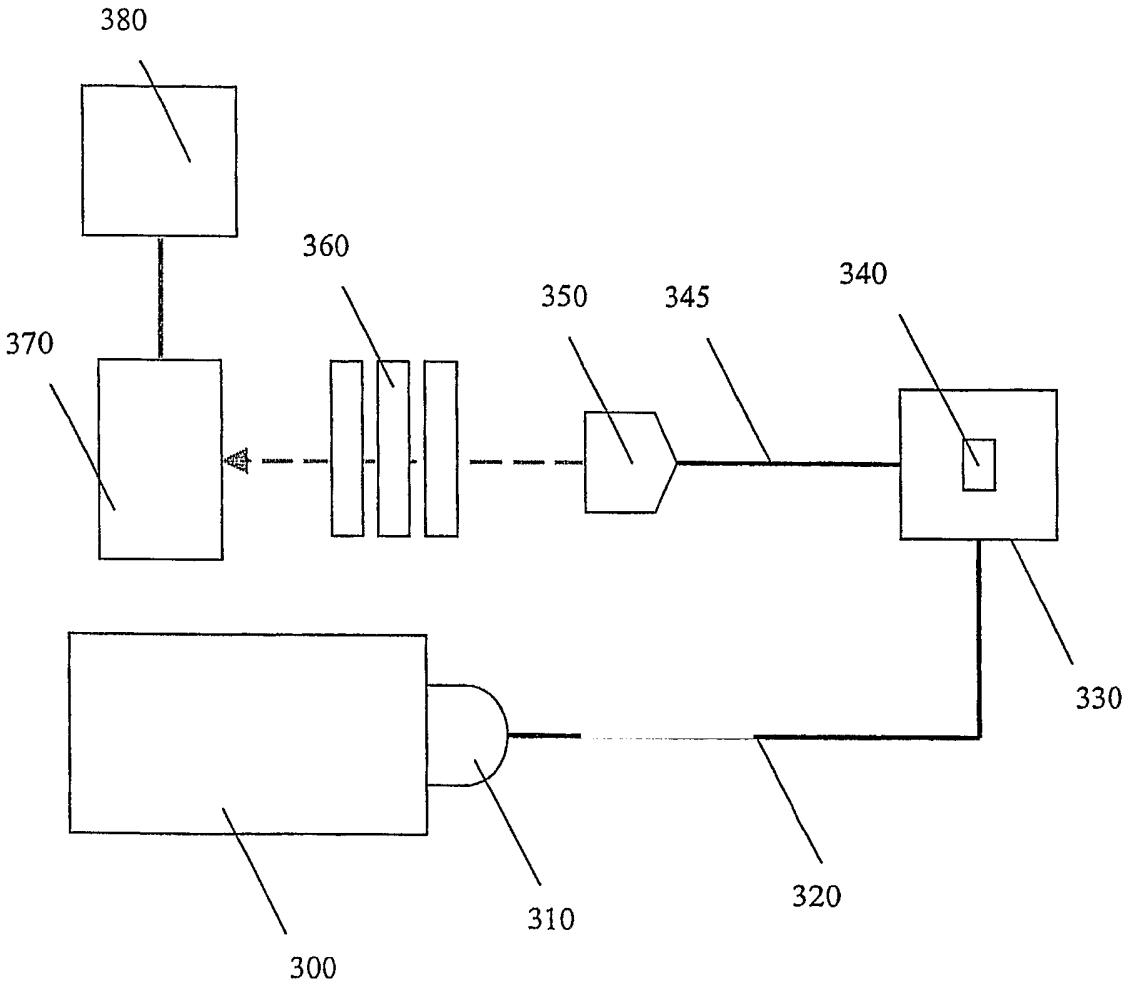


Fig. 6

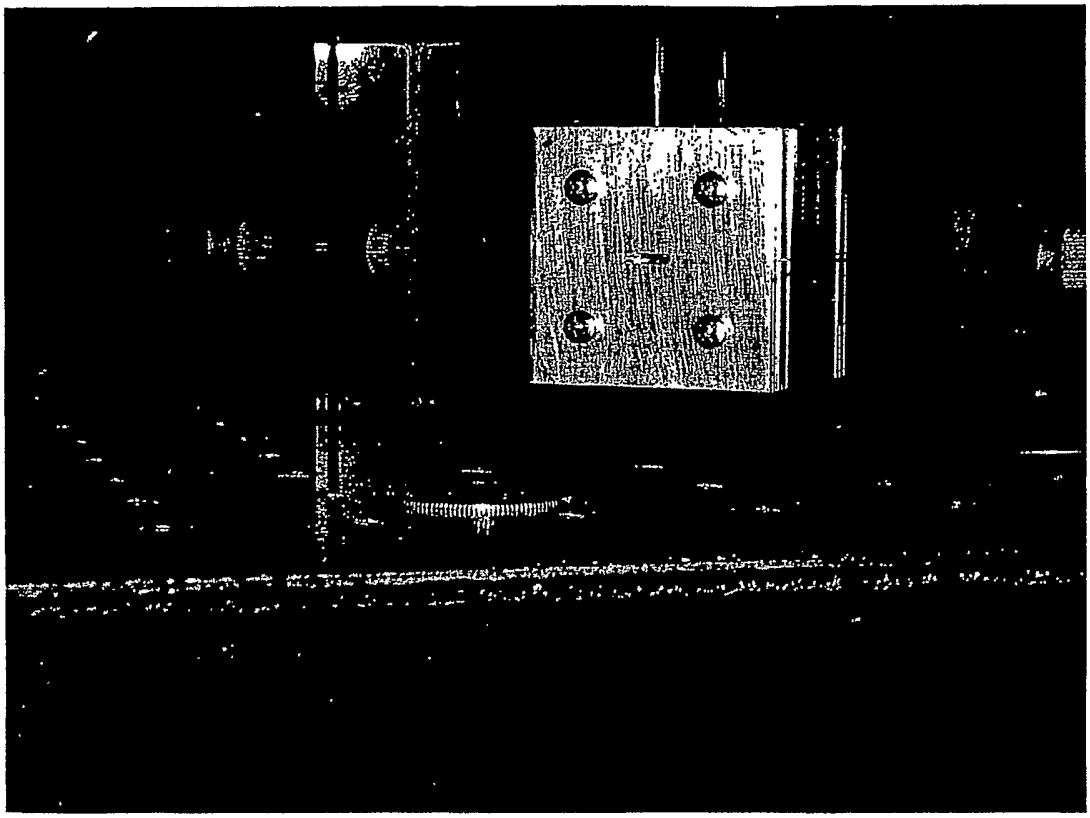


Fig. 7

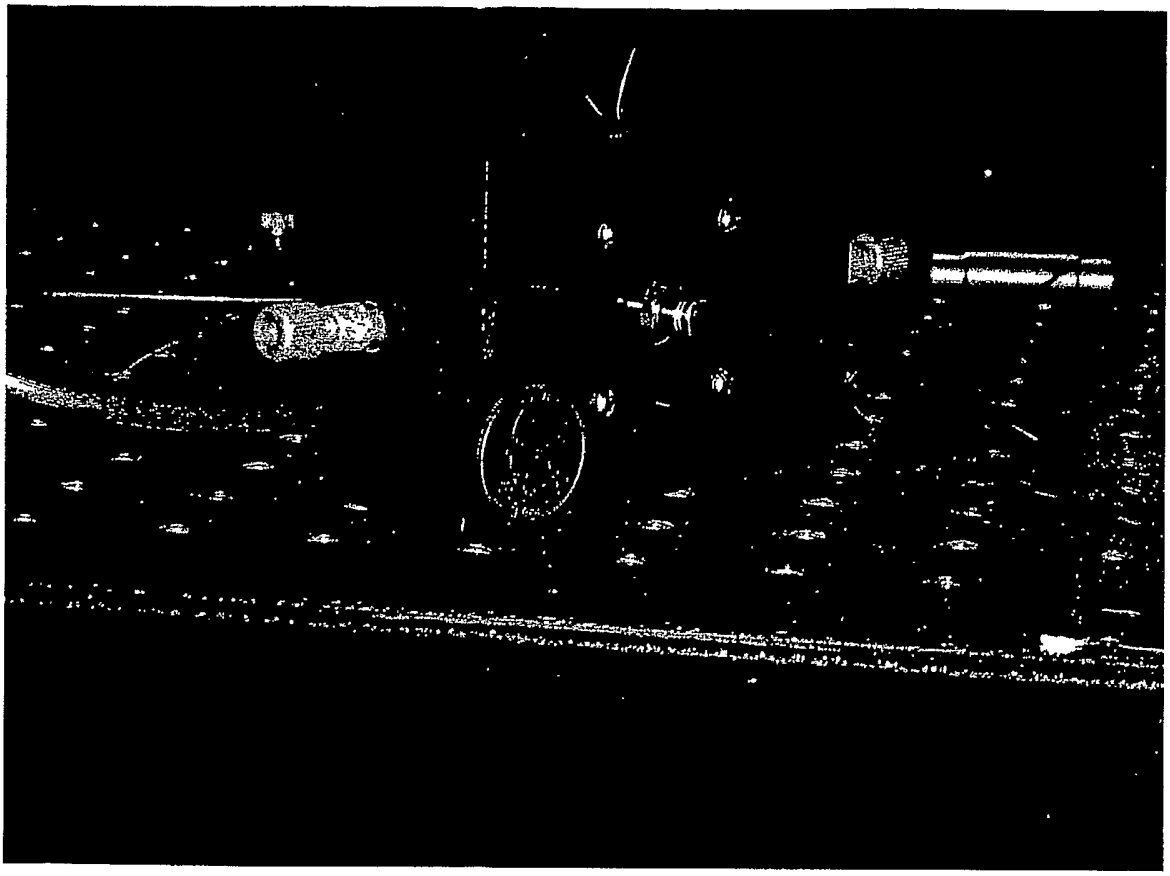


Fig. 8

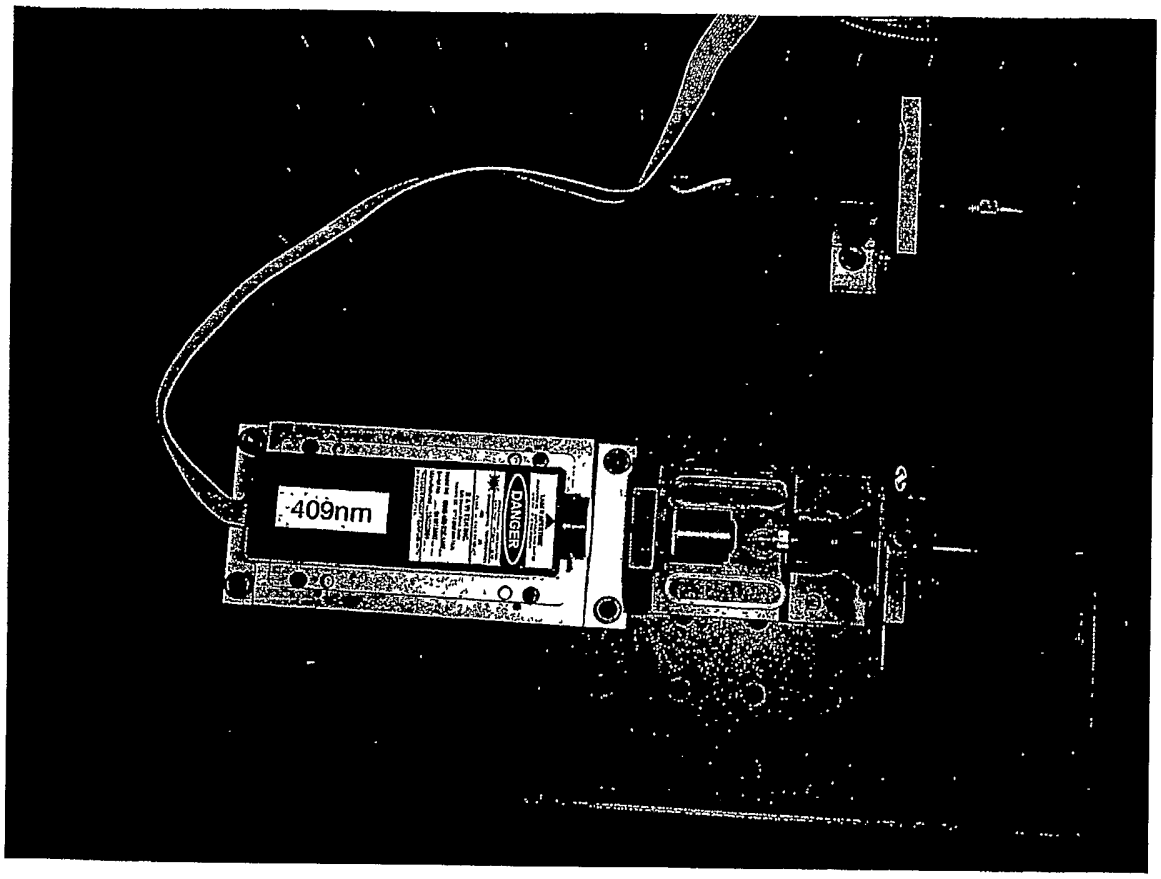
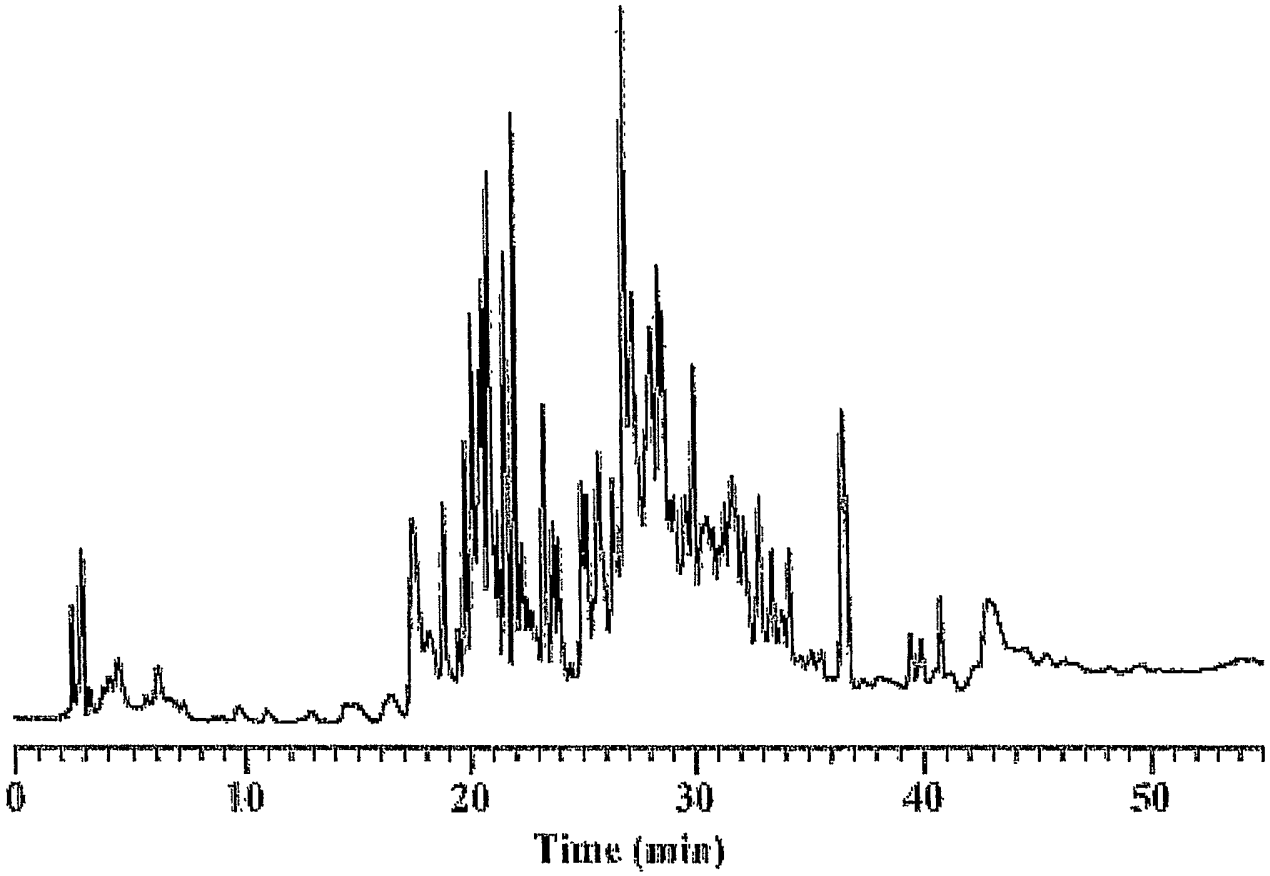


Fig. 9



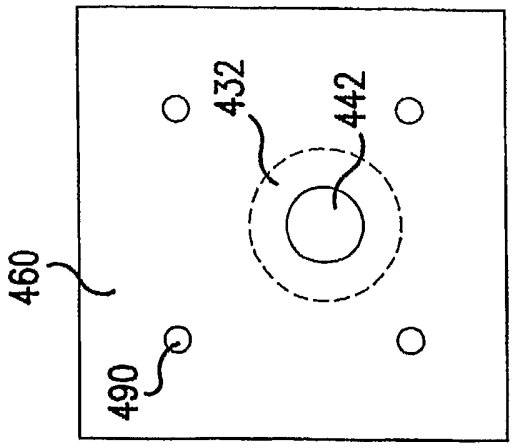


Fig. 11A

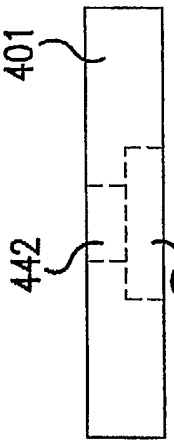


Fig. 11B

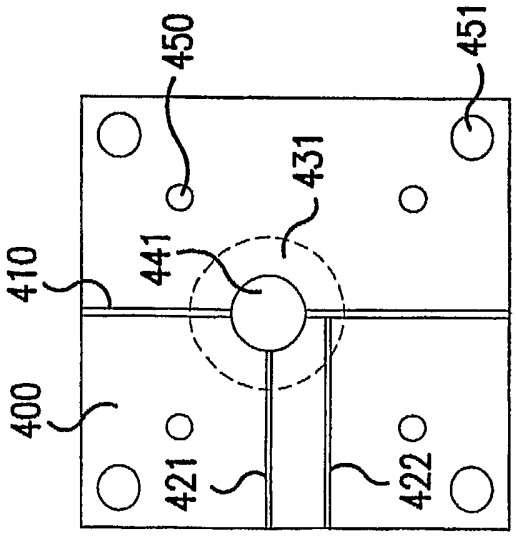


Fig. 10A

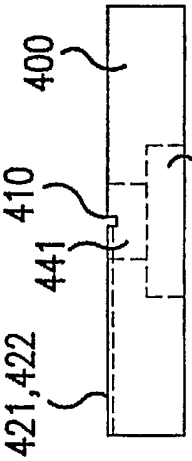


Fig. 10B

Fig. 12

