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(54) **OPTICAL DETECTOR FOR ENZYME ACTIVATION**

(75) Inventors: **Orhan Soykan**, Shoreview, MN (US); **Maura G. Donovan**, St. Paul, MN (US); **Amy E. Thompson**, Coon Rapids, MN (US)

(73) Assignee: **Medtronic, Inc.**, Minneapolis, MN (US)

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See application file for complete search history.

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Primary Examiner—Jon P Weber
Assistant Examiner—Bin Shen
(74) *Attorney, Agent, or Firm*—Michael C. Soldner; Carol F. Barry; Steve Bauer

(57) **ABSTRACT**

Activation of an enzyme in a bodily fluid is detected based on the amount of cleavage of a substrate for the enzyme. The substrate is tagged with two fluorescent dyes—a donor and an acceptor. The tagged substrate is presented to the bodily fluid. A device emits energy at a first wavelength into the bodily fluid, and detects energy at second and third wavelengths emitted by the dyes in response to the energy at the first wavelength. Prior to enzymatic cleavage of the substrate, the acceptor emits energy at the third wavelength in response to energy at the second wavelength received through fluorescent resonant energy transfer (FRET) from the donor. After enzymatic cleavage of the substrate, the donor emits energy at the second wavelength. The device can determine the concentration of activated enzyme within the bodily fluid based on the relative intensities of energy at the second and third wavelengths.

20 Claims, 9 Drawing Sheets

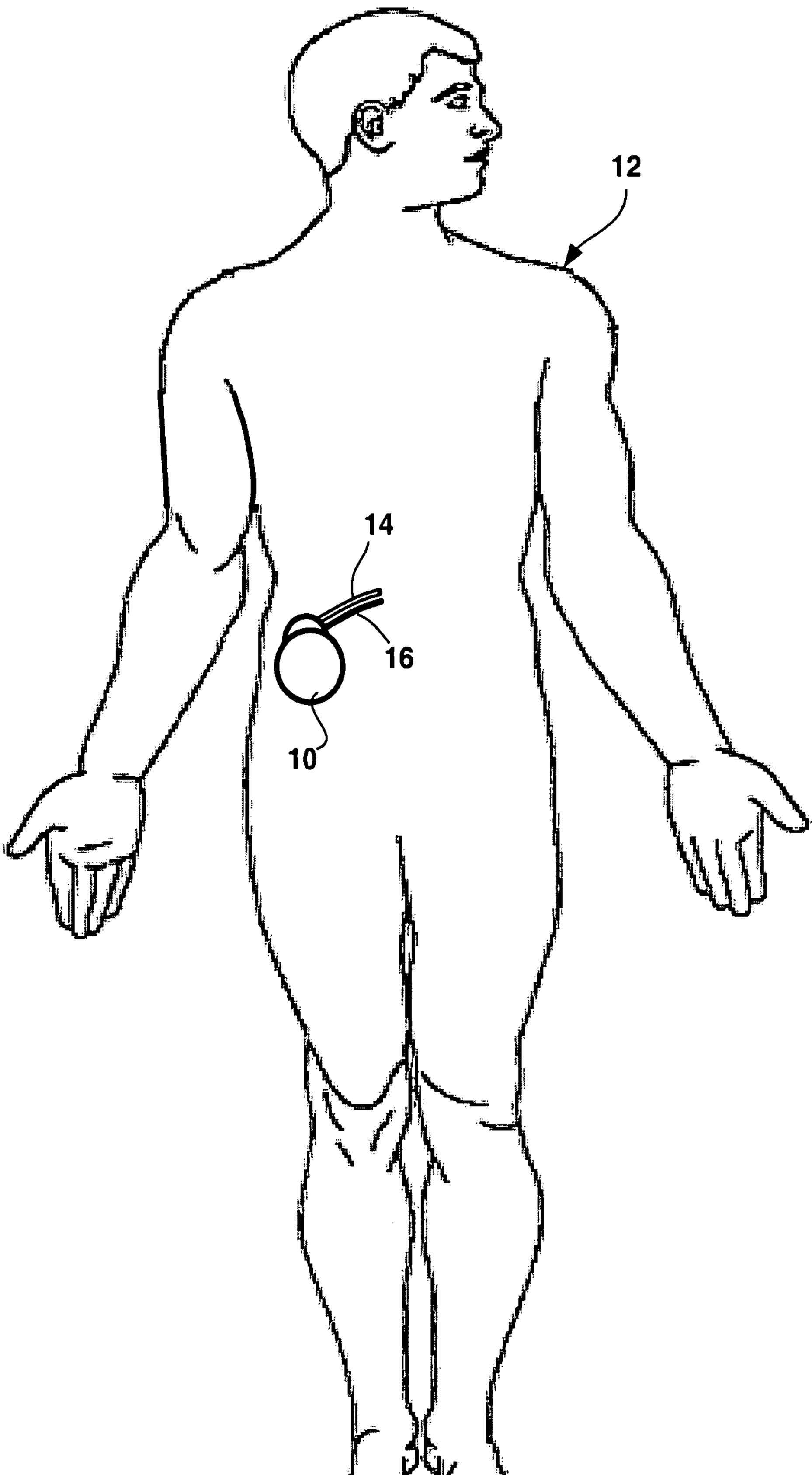
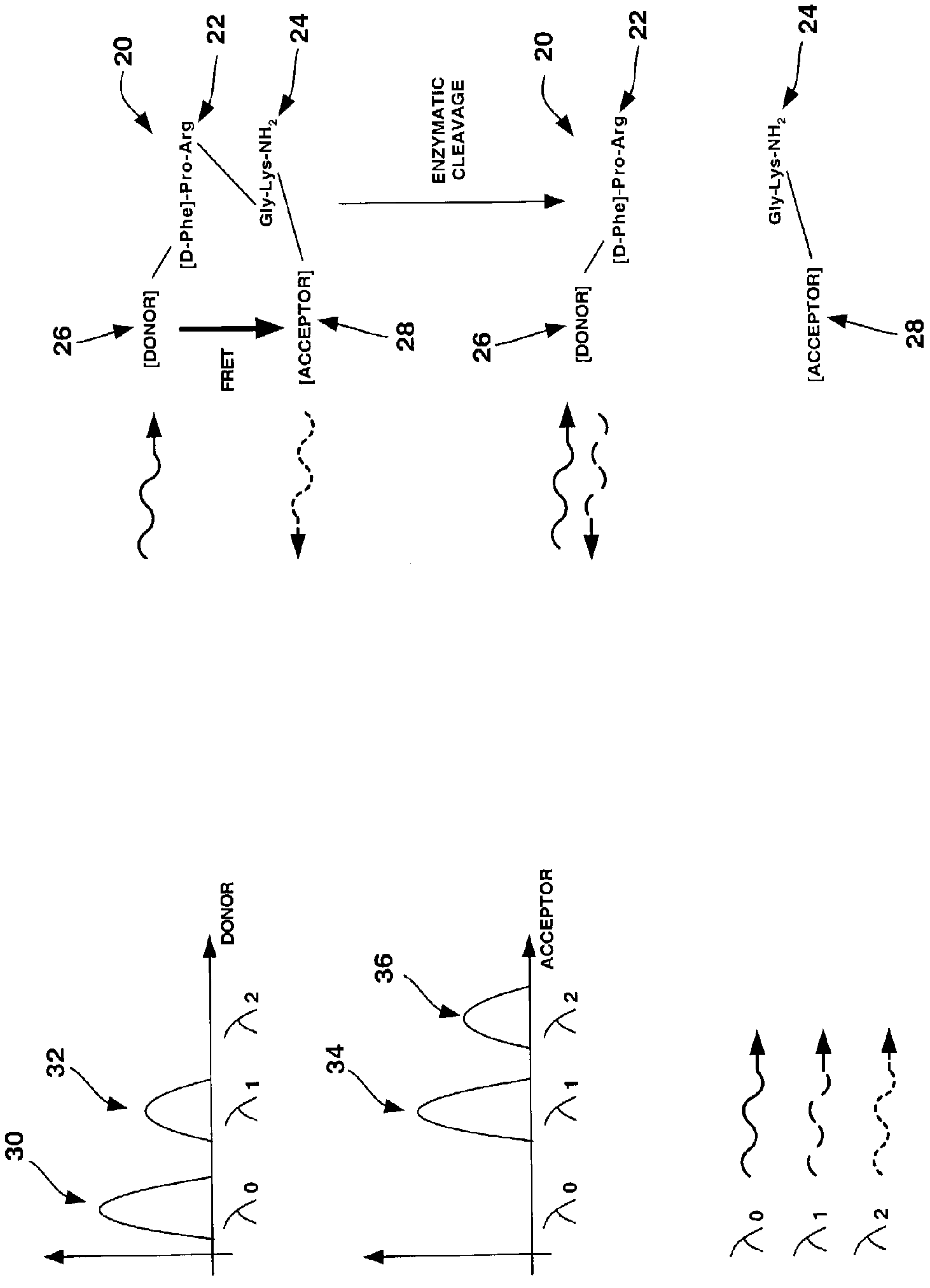


FIG. 1

FIG. 2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22
24
[D-Phe]-Pro-Arg
Gly-Lys-NH2
26
[DONOR]
FRET
[ACCEPTOR]
28
ENZYMATIC
CLEAVAGE
30
32
34
36
DONOR
ACCEPTOR
λ0
λ1
λ2

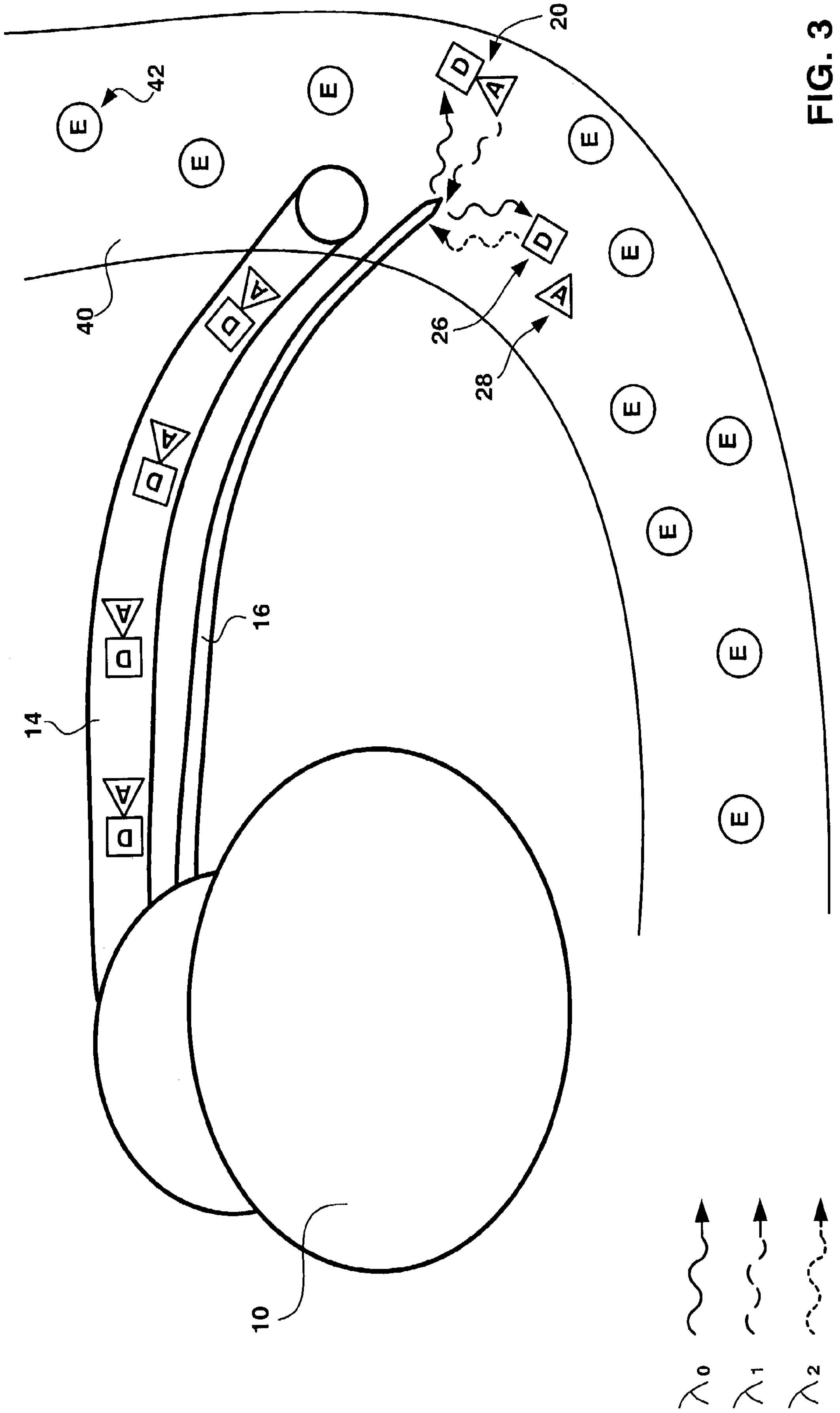
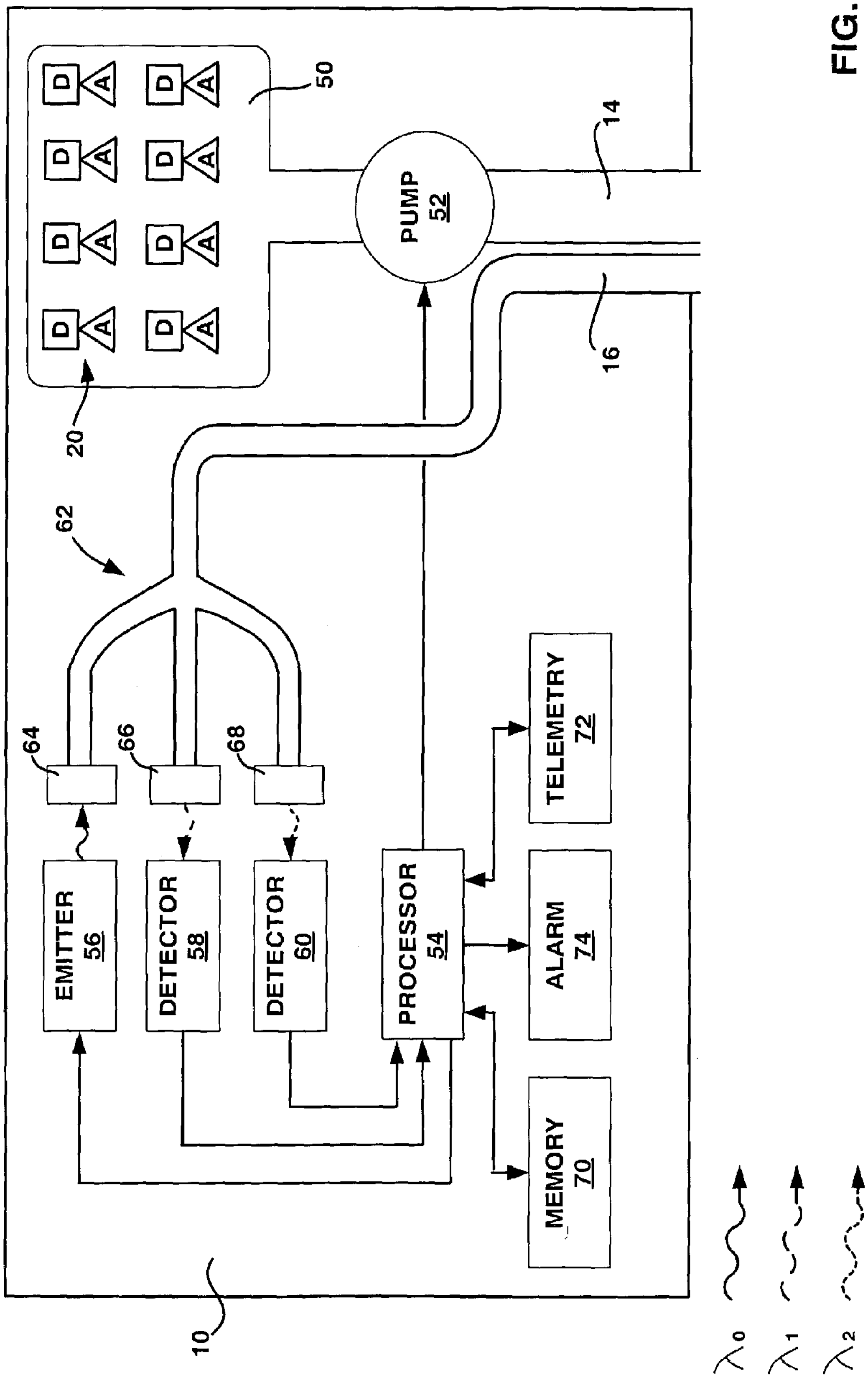


FIG. 3



D
A
50
20
PUMP
52
14
16
62
64
66
68
EMITTER
56
DETECTOR
58
DETECTOR
60
PROCESSOR
54
TELEMETRY
72
ALARM
74
MEMORY
70
10
λ_0
λ_1
λ_2

FIG. 4

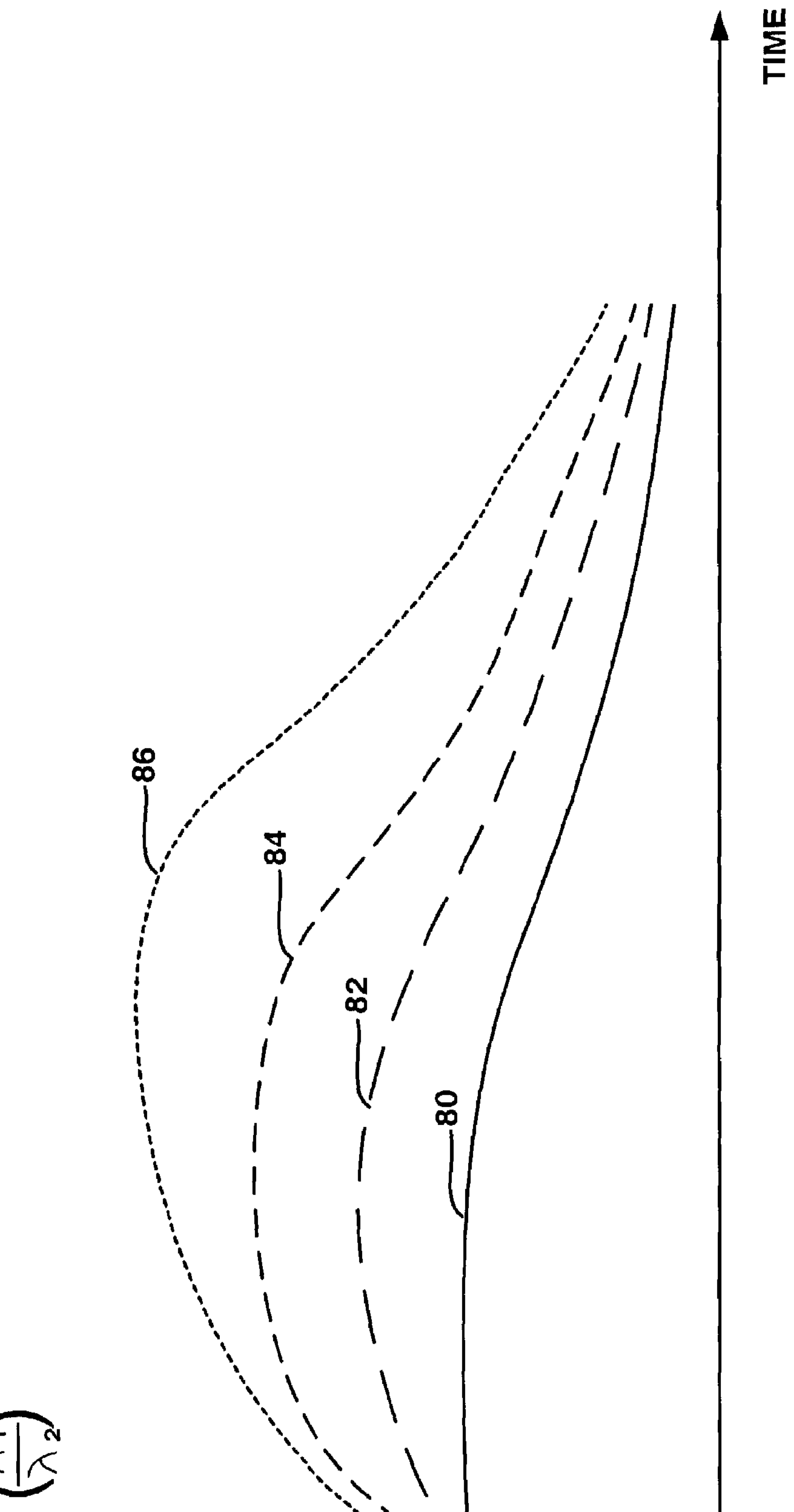


FIG. 5

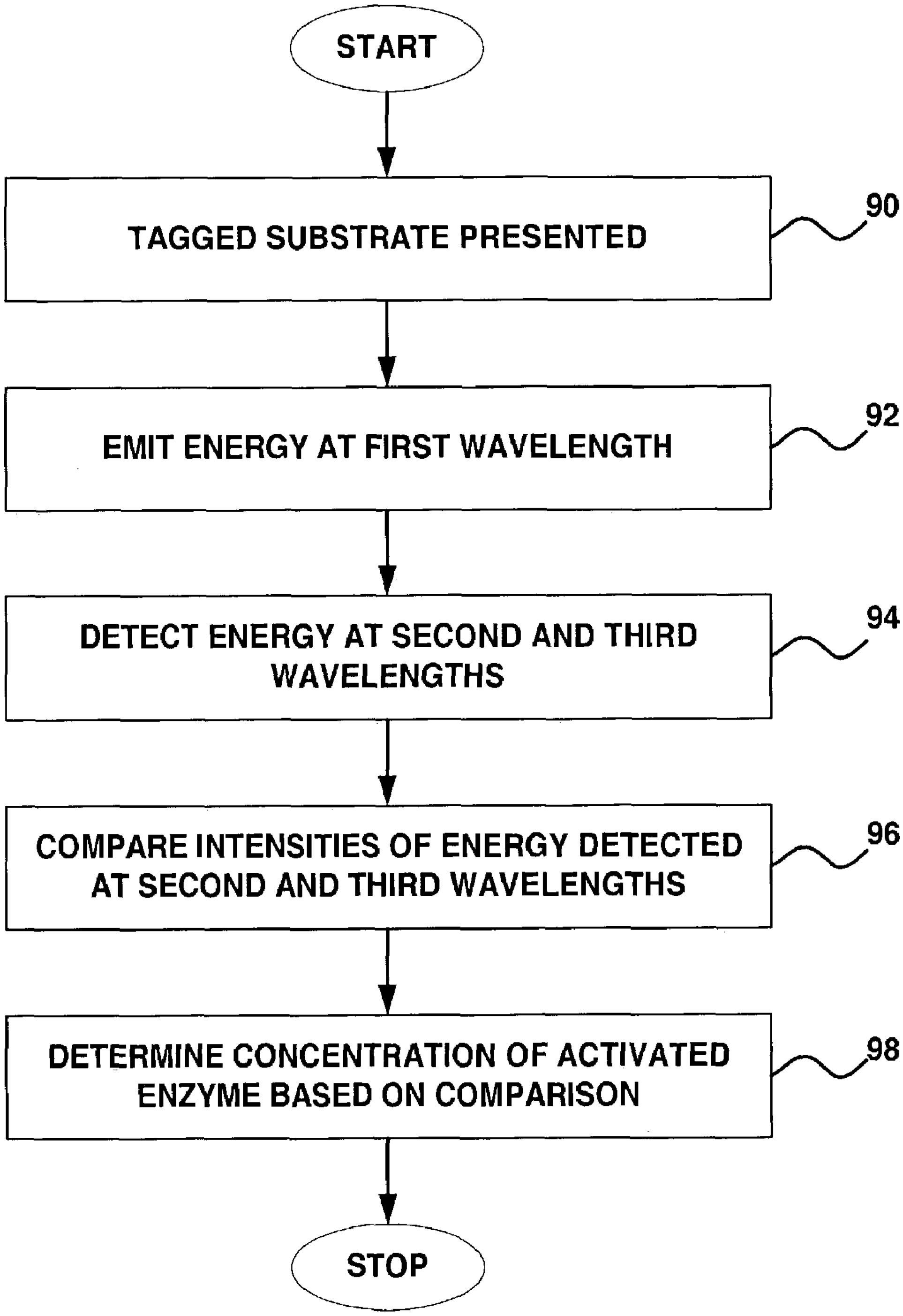

START
TAGGED SUBSTRATE PRESENTED
90
EMIT ENERGY AT FIRST WAVELENGTH
92
DETECT ENERGY AT SECOND AND THIRD WAVELENGTHS
94
COMPARE INTENSITIES OF ENERGY DETECTED AT SECOND AND THIRD WAVELENGTHS
96
DETERMINE CONCENTRATION OF ACTIVATED ENZYME BASED ON COMPARISON
98
STOP

FIG. 6

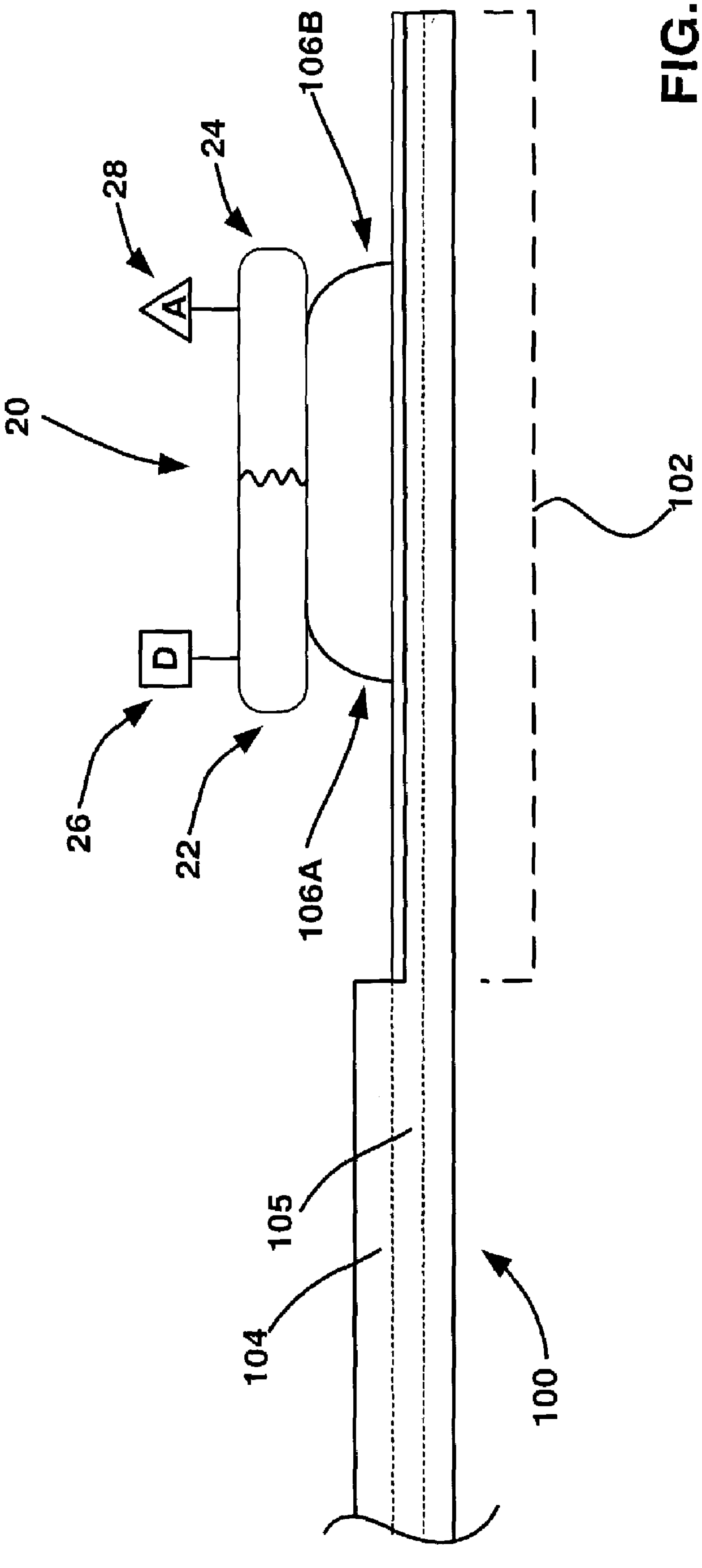
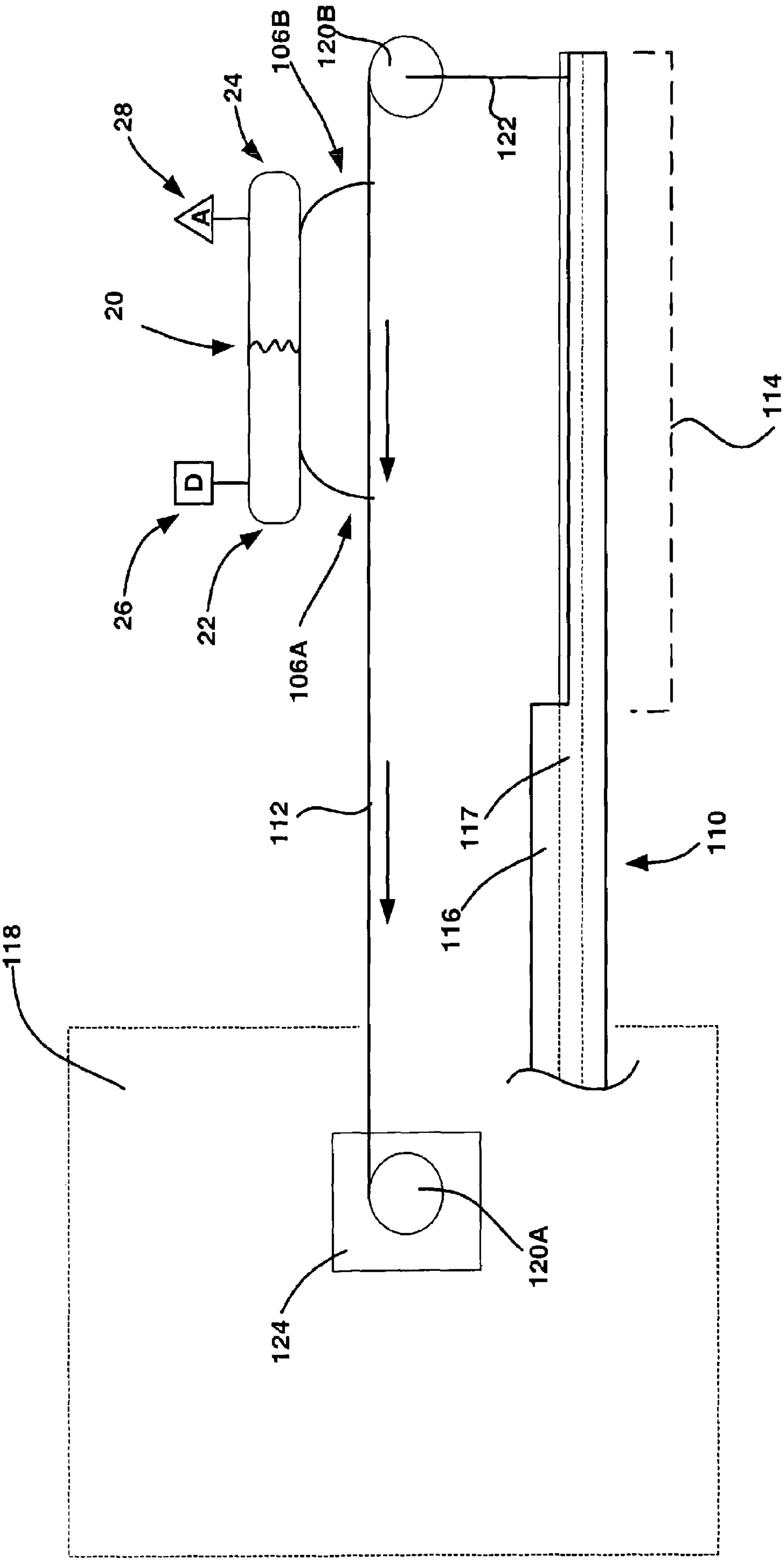


FIG. 7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106A
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110
116
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120A
124

FIG. 8

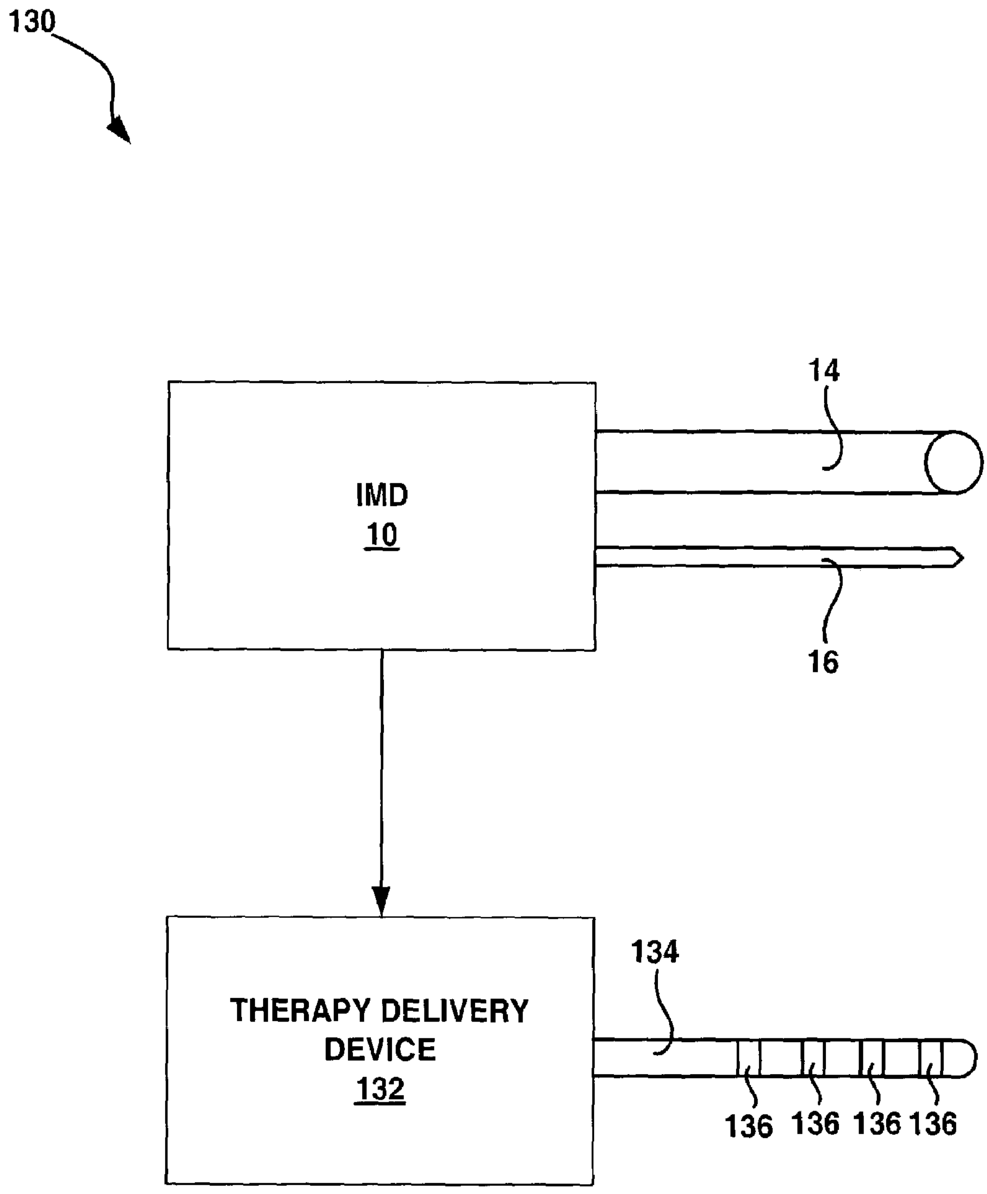
130
IMD
10
14
16
THERAPY DELIVERY
DEVICE
132
134
136
136
136
136

FIG. 9

OPTICAL DETECTOR FOR ENZYME ACTIVATION

TECHNICAL FIELD

The invention relates to medical devices, and more particularly, to medical devices that detect enzymes within bodily fluid.

BACKGROUND

In general, the activities of various enzymes within bodily fluids, such as the blood or lymph, are of clinical interest. Some enzymes exist in bodily fluids as an inactive precursor until an event or condition triggers their activation. For example, the enzymes kallikrein and thrombin are present in blood as their respective inactive precursors, prekallikrein and prothrombin, until activated. The activation of enzymes, such as kallikrein and thrombin, can indicate clinically significant underlying events or conditions.

Kallikrein is involved in the processes of signaling painful events or stimuli to the nervous system via the extrinsic pain pathway. When an agent, such as heat, force, or radiation, causes a cell to rupture, that cell releases its internal components into the surrounding environment. Among these cell components are proteins that activate kallikrein, i.e., convert inactive prekallikrein to kallikrein. Kallikrein in turn activates a protein called bradykinin, which acts on free nerve endings to signal pain in the area. Bradykinin can also induce pain by causing tissue to swell, e.g. by causing edema.

Thrombin is involved in the process of blood coagulation. Specifically, thrombin converts fibrinogen into fibrin, which in turn forms thrombi. Prothrombin is converted to thrombin as part of an intricate cascade of enzymatic activities. Generally speaking, patients with conditions that lead to pooling of the blood within vessels, such as atrial fibrillation, or patients with implanted foreign matter exposed to blood flow, such as artificial heart valves, are at increased risk of developing potentially life-threatening thrombi. Such patients often receive anticoagulants to reduce the likelihood of thrombus formation.

SUMMARY

In general, the invention is directed to techniques for optically detecting activation of an enzyme within a bodily fluid. The bodily fluid can be blood, and the detected enzyme can be, for example, kallikrein or thrombin. Activation of the enzyme can indicate a medically significant event or condition. In some embodiments, detecting activation of the enzyme within the bodily fluid according to the invention enables clinical diagnosis, chronic monitoring, and/or closed-loop therapy delivery.

Activation of the enzyme in a bodily fluid is detected based on the amount of cleavage of a substrate for the enzyme. The substrate is tagged with two fluorescent dyes, such that each of two products that will result from cleavage of the substrate by the enzyme is tagged with one of the dyes. The absorption spectrum of one of the dyes, the acceptor, overlaps the emission spectrum of the other dye, the donor.

The tagged substrate is presented to the bodily fluid. A device emits energy at a first wavelength into the bodily fluid, and detects energy, emitted by the dyes in response to the energy emitted by the device at the first wavelength, at a second and a third wavelength. The donor absorbs energy emitted by the device at the first wavelength. Prior to enzymatic cleavage of the substrate, the acceptor receives energy at the second wavelength from the donor through fluorescent resonant energy transfer (FRET), and emits energy at the third wavelength. After enzymatic cleavage, FRET does not occur and the donor emits energy at the second wavelength in response to absorbing energy at the first wavelength.

The same device that emits energy at a first wavelength can also present the tagged substrate to the bodily fluid from a reservoir using a pump and a catheter. The device can emit and detect energy via an optical fiber with a distal end located in the bodily fluid. In some embodiments, the substrate is presented to the bodily fluid on a substrate tape, or is linked to the optical fiber.

The rate of cleavage of the substrate is determined based on the relative intensities of energy at the second and third wavelengths over time. In some embodiments, the device determines a ratio between the intensities of energy at the second and third wavelengths, and determines the amount of activated enzyme within the bodily fluid based on the value of the determined ratio over time. Information describing relationships between amount of activated enzyme and ratios can be stored within a memory as one or more look-up tables, equations, curves, or the like. The amount of activated enzyme can, for example, be expressed as a concentration of activated enzyme, e.g., units per milliliter of bodily fluid. Information describing relationships between concentration of activated enzyme and ratios over time can be determined experimentally.

In some embodiments, the device stores determined amounts of activated enzyme in the memory for later retrieval by a clinician. In some embodiments, the device stores one or more thresholds in the memory and activates an alarm if the amount of activated enzyme or a rate of change of the amount of activated enzyme exceeds or falls below the threshold. Therapy, such as anticoagulant or pain relieving drug therapy, or neurostimulation, may be delivered based on determined amounts of activated enzyme.

In one embodiment, the invention is directed to a method in which energy is emitted at a first wavelength into a bodily fluid. A tagged substrate within the bodily fluid emits energy at a second wavelength and a third wavelength in response to the energy at the first wavelength, and the energy emitted by the tagged substrate is detected. Activation of an enzyme that cleaves the substrate within the bodily fluid is detected based on the detected energy. A ratio between a first detected intensity of energy at the second wavelength and a second detected intensity of energy at the third wavelength may be determined, and the concentration of activated enzyme within the bodily fluid may be determined based on the ratio.

In another embodiment, the invention is directed to a device that includes an emitting element to emit energy at a first wavelength into a bodily fluid, and a detector to detect energy emitted at a second wavelength and a third wavelength by products of a tagged substrate in the bodily fluid in response to the energy at the first wavelength. The device further includes a processor to control the emitting element to emit energy at the first wavelength, receive indications of intensities of energy detected by the detector, and detect activation of an enzyme that cleaves the substrate within the bodily fluid based on the indicated energy intensities. The device may also include an optical fiber optically coupled to the emitting element and the detector. A distal end of the optical fiber may be located in the bodily fluid. The emitting element may emit energy into the bodily fluid via the optical fiber, and the detector may detect energy emitted by products of the tagged substrate in the fluid via the optical fiber.

In another embodiment, the invention is directed to a system that includes a first medical device to optically detect

activation level of an enzyme within a bodily fluid of a patient, and a second medical device to deliver a therapy to the patient based on the detection of enzyme activation. The second medical device may be, for example, a drug pump that delivers drugs to the patient based on the concentration of activated enzyme within the bodily fluid, or a neurostimulation device that delivers neurostimulation therapy to the patient based on the concentration of activated enzyme within the bodily fluid.

In another embodiment, the invention is directed to an optical fiber that includes a core and a cladding. The cladding is partially removed from a section of the fiber to expose a section of the core, and a substrate for an enzyme within a bodily fluid is linked to the exposed section of the core.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** is a schematic diagram illustrating an example implantable medical device for optically detecting enzyme activation.

FIG. **2** is a conceptual diagram illustrating use of fluorescent resonant energy transfer to detect enzyme activation according to the invention.

FIG. **3** is a schematic diagram illustrating an example operation of the implantable medical device of FIG. **1** to detect enzyme activation.

FIG. **4** is a block diagram illustrating an example configuration of an implantable medical device for optically detecting enzyme activation.

FIG. **5** is a timing diagram illustrating example ratio curves used to determine an amount of activated enzyme.

FIG. **6** is a flowchart illustrating an example method for detecting enzyme activation.

FIG. **7** is a perspective diagram illustrating an example optical fiber that includes a substrate for use in optically detecting enzyme activation.

FIG. **8** is a perspective diagram illustrating an example optical fiber and a substrate tape for use in optically detecting enzyme activation.

FIG. **9** is a block diagram illustrating an example system for optically determining an amount of activated enzyme and delivering therapy to a patient based on the determined amount of activated enzyme.

DETAILED DESCRIPTION

FIG. **1** is a perspective diagram illustrating an example implantable medical device (IMD) **10** implanted within a patient **12**. IMD **10** optically detects enzyme activation within a bodily fluid of patient **12**. The bodily fluid can be blood within a blood vessel (not shown) of patient **12**. Further, IMD **10** can, in some embodiments, detect activation of protease enzymes, such as kallikrein or thrombin, within blood.

IMD **10** is coupled to a catheter **14** and an optical fiber **16**. The distal ends of catheter **16** and optical fiber **18** extend into the bodily fluid to enable IMD **12** to detect activation of the enzyme within the bodily fluid. For example, the distal ends of catheter **14** and optical fiber **16** can extend into a blood vessel so that IMD **10** can detect activation of kallikrein or thrombin within the blood of patient **12**.

In general, when the target enzyme is activated within the bodily fluid, it acts to cleave a specific protein within the bodily fluid. For example, kallikrein cleaves kininogen to release bradykinin, and thrombin cleaves fibrinogen to release fibrin. According to the Michaelis-Menten model, the amount of cleaved protein depends on the concentration of activated enzyme, the turnover rate of the enzyme, and the amount of accessible protein.

IMD **10** uses a substrate to detect activation of the target enzyme. In addition to cleaving the protein, the target enzyme cleaves the substrate. IMD **10** determines the level of enzyme activation based on the extent of substrate cleavage. The substrate is presented to the bodily fluid via catheter **14**.

The enzyme cleaves the protein and the substrate by breaking selected bonds within the molecular structures of the protein and the substrate. Specifically, both kallikrein and thrombin are serine-proteases and selectively target the peptide bonds between arginine and lysine or arginine and glysine. Thus, a suitable substrate for kallikrein or thrombin includes a peptide bond that can be broken by the target enzyme to cleave the substrate.

Examples of suitable substrates for kallikrein and thrombin are listed in Tables 1 and 2, respectively. However, the invention is not limited to detection of enzyme activation using these exemplary substrates. Nor is the invention limited to detection of the activation of kallikrein or thrombin. Suitable substrates for detection of the activation of any particular enzyme can be identified based on the bond selectivity of that particular enzyme.

TABLE 1

Kallikrein Substrates
H-D Pro-HHT-Arg-pNA
H-D-Pro-Phe-Arg-pNA
H-D-But-CHA-Arg-pNA.2AcOH
Pro-Phe-Arg-MCA
H-D-Val-Leu-Arg-AFC, 2HCL

TABLE 2

Thrombin Substrates
Protein C
Secretin
Lysozyme
Growth Hormone
Actin
d-FPRX (where x = paranitroanilide)
d-FPKX (where x = paranitroanilide)
d-FGRX (where x = paranitroanilide)
d-VGKX (where x = paranitroanilide)

IMD **10** detects the amount of substrate cleavage, and thus enzyme activation, optically. IMD **10** emits energy into the bodily fluid via optical fiber **16** when the substrate is presented to the bodily fluid. The substrate is tagged with fluorescent dyes. IMD **10** detects energy that is emitted by the fluorescent dyes in response to absorbing the energy emitted by IMD **10** via optical fiber **16**. As will be described in greater detail below, IMD **10** detects the amount of substrate cleavage based on the level of fluorescent resonant energy transfer (FRET) between the dyes. The energy emitted and detected by IMD **10** may be visible light.

The distal ends of catheter **14** and optical fiber **16** can, as shown in FIG. **1**, be located substantially proximate to each other within the bodily fluid. In some embodiments, catheter **14** and optical fiber **16** are contained within a common sheath (not shown) that facilitates and maintains their placement proximate to each other. However, catheter **14** and optical fiber **16** need not be collocated. For example, IMD **10** can present the tagged substrate to a blood flow via catheter **14** at a point that is "upstream" from the location of optical fiber **16**. Moreover, in some embodiments IMD **10** is not coupled to a catheter **14**. In such embodiments, the tagged substrate can be presented to the bodily fluid via manual injection using a hypodermic syringe and needle, via an external drug pump delivering an intravenous bolus, via a substrate tape, or linked to optical fiber **16**.

FIG. **2** is a conceptual diagram illustrating use of FRET to detect enzyme activation according to the invention. An exemplary substrate molecule **20** includes a first product **22** and a second product **24**. The first product **22** of substrate molecule **20** is tagged with a first fluorescent dye, which is a donor dye **26**. The second product **24** of substrate molecule **20** is tagged with a second fluorescent dye, which is an acceptor dye **28**.

An absorption spectrum **30** and emission spectrum **32** for donor dye **26** are illustrated in FIG. **2**. An absorption spectrum **34** and emission spectrum **36** for acceptor dye **28** are also illustrated. As illustrated, donor dye **26** emits energy at a wavelength λ_1 in response to the absorption of energy at a wavelength λ_0. Acceptor dye **28** emits energy at a wavelength λ_2 in response to the absorption of energy at a wavelength λ_1. Thus, the absorption spectrum of acceptor dye **28** overlaps the emission spectrum of donor dye **26**.

When donor dye **26** and acceptor dye **28** are sufficiently proximate, e.g., between approximately 10 and 100 Angstroms, and their dipole orientations are approximately parallel, acceptor **28** will resonantly receive energy at wavelength λ_1 from donor **26** via FRET. Acceptor **28** will emit energy at wavelength λ_2 in response to absorbing the energy at wavelength λ_1. As illustrated in FIG. **2**, donor **26** and acceptor **28** are sufficiently proximate and properly oriented prior to enzymatic cleavage of substrate molecule **20**, and acceptor **28** emits energy at wavelength λ_2 in response to donor absorbing energy at wavelength λ_0. When substrate molecule **20** is enzymatically cleaved, donor **26** and acceptor **28** are no longer sufficiently proximate or properly oriented, and donor **26** emits energy at wavelength λ_1 in response to absorbing energy at wavelength λ_0.

FIG. **3** is a perspective diagram illustrating an example operation of IMD **10** to detect enzyme activation. The distal ends of catheter **14** and optical fiber **16** are deployed within a blood vessel **40**. The blood within blood vessel **40** includes enzyme **42**. Enzyme **42** includes activated enzyme molecules and inactive precursor molecules for the enzyme. For example, enzyme **42** can include prekallikrein and kallikrein molecules, or prothrombin and thrombin molecules. For ease of illustration, only a single enzyme molecule **42** is labeled.

IMD **10** presents substrate **20**, which includes a product tagged with donor dye **26** and a product tagged with acceptor dye **28**, to the blood flow within vessel **40** via catheter **14**. As shown in FIG. **3**, activated enzyme molecules **42** cleave substrate **20** such that the products tagged with dyes **26** and **28** are separated. For ease of illustration, only a single substrate molecule **20** and a single instance of products tagged with donor and acceptor dye **26** and **28** are labeled.

IMD **10** emits energy at wavelength λ_0 into the blood stream of vessel **40** via optical fiber **16**. Donor dye **26** absorbs energy at wavelength λ_0. When substrate molecules **20** are intact in the blood stream, acceptor dye **28** will, through FRET, emit energy at wavelength λ_2 in response to the donor **26** absorbing energy at wavelength λ_0, as described above. When activated enzyme molecules **42** cleave substrate molecules **20**, dyes **26** and **28** are physically separated preventing FRET from occurring. When substrate molecules **20** are cleaved, donor **26** emits energy at wavelength λ_1 in response to absorbing the energy emitted by IMD **10** at wavelength λ_0.

IMD **10** detects energy emitted by donor **26** at wavelength λ_1 and energy emitted by acceptor **28** at wavelength λ_2 via optical fiber **16**. The intensities of energy detected at wavelengths λ_1 and λ_2 are related to the amount of cleaved and uncleaved substrate **20** within vessel **40**. The amount of cleaved and uncleaved substrate **20** within vessel **40** depends on the amount of activated enzyme **42** within the blood.

IMD **10** detects activation of enzyme **42** based on the ratio between the intensities of energy detected at wavelengths λ_1 and λ_2. By detecting activation of enzyme **42** based on a ratio, IMD **10** is less susceptible to errors caused by signal degradation, e.g., fibrous tissue growth on optical fiber **16**. In some embodiments, IMD **10** can monitor the overall intensity of the detected energy and compensate for reduced signal intensity by increasing the intensity of the emitted energy.

Suitable donor **26**/acceptor **28** pairs for use with the exemplary substrates listed in Tables 1 and 2 include AMCA/FITC and FITC/TRITC. The invention is not, however, limited to these dye pairs. Suitable dye pairs include any dye pair that has an acceptor whose absorption spectrum overlaps the emission spectrum of the donor. Suitability of a dye pair may also depend on its ability to bond to the products of a selected substrate.

FIG. **4** is a block diagram illustrating an example configuration of IMD **10**. In the example configuration of IMD **10** illustrated in FIG. **4**, IMD **10** includes a reservoir **50** and a pump **52** in fluid communication with reservoir **50** and catheter **14**. Reservoir **50** contains tagged substrate **20**. A processor **54** controls pump **52** to present substrate **20** to bodily fluid via catheter **14**. With respect to the presentation substrate **20**, IMD **10** can correspond substantially to an implantable drug pump, such as Synchromed™ drug pumps sold by Medtronic Corporation. Reservoir **50** can be refillable, e.g., via a syringe, to enable chronic implantation and enzyme activation detection.

IMD **10** also includes an emitter **56** and detectors **58** and **60** that are optically coupled to optical fiber **16**. Processor **54** controls emitter **56** to emit energy at wavelength λ_0 in conjunction with the presentation of substrate **20** by pump **52**. Detector **58** detects energy emitted by substrate **20** in the bodily fluid at wavelength λ_1, and detector **60** detects energy emitted by substrate **20** in the bodily fluid at wavelength λ_2. Processor **54** receives an indication of the intensities of the energy at wavelengths λ_1 and λ_2 from detectors **58** and **60**.

Detectors **58** and **60** can take the form of electro-optical transducers. In some embodiments, a single detector detects energy at wavelengths λ_1 and λ_2. In such embodiments, the detector can include analog or digital signal processing components in addition to an electro-optical transducer to determine the intensities at wavelengths λ_1 and λ_2 and indicate the intensities to processor **54**.

An optical coupler **62** couples emitter **56** and detectors **58** and **60** the fiber **16**. A filter **64** filters energy emitted by emitter **56** to assure that energy at wavelength λ_0 is delivered to the bodily fluid. Filters **66** and **68** filter energy received from substrate **20** in the bodily fluid to assure that detectors **58** and **60** receive energy at wavelengths λ_1 and λ_2, respectively. In some embodiments, filters **64**-**68** take the form of dichroic band-pass filters.

Energy delivered to detectors **58** and **60** can be amplified. One or more amplifiers (not shown), such chopper stabilized amplifiers, can be used to amplify the energy to improve the signal to noise ratio for the energy delivered to detectors **58** and **60**.

Processor **54** detects activation of an enzyme, and can, in some embodiments, determines the amount of activated enzyme within a bodily fluid based on the indicated intensities at wavelengths λ_1 and λ_2. Specifically, processor **54** can calculate a ratio between the intensities at wavelengths λ_1 and λ_2, and determine the amount of activated enzyme based on the ratio. The amount of activated enzyme can be expressed as a concentration of the activated form of the enzyme within the bodily fluid, e.g., units per milliliter of bodily fluid.

Processor **54** can store the determined ratio in a memory **70**. Memory **70** also stores information used by processor **54** to determine an amount of activated enzyme based on the ratio. For example, in some embodiments, memory **70** includes a look-up table or equation that describes a relationship between the ratio and enzyme concentration.

The determined amount of activated enzyme, e.g., concentration of activated enzyme, can be stored in memory **70** for later retrieval by a clinician. In exemplary embodiments, the clinician retrieves one or more concentrations from memory **70** using a programmer via a telemetry circuit **72**, as is known in the art. Where the target enzyme is kallikrein, the clinician can use the concentrations to more accurately identify the intensity and frequency of pain, for example, and, depending on the position of optical fiber **16**, the location of pain. This information may allow the clinician to more accurately diagnose the underlying cause of the pain, and prescribe more effective pain therapies for patient **12**. Where the target enzyme is thrombin, the clinician can use the enzyme concentrations collected over time to, for example, better determine a dosage for anticoagulant therapy for patient **12**. Telemetry can also be used to communicate information relating to the status or performance of IMD **10**, such as battery and reservoir status, to clinician.

Memory **70** can store threshold values, and processor **54** can activate an alarm **74** when the ratio, concentration, rate of change of the ratio over time, or rate of change of concentration over time, exceeds or falls below a threshold value. Alarm **74** is detectable by patient **12**, e.g., audibly or through vibration. Alarm **74** can be used to cause patient **12** to seek immediate medical attention when enzyme activation concentration of activated enzyme indicates an emergency medical condition, such as a thrombin concentration that indicates a dangerously high probability of thrombi formation. Increased thrombin activation can in some cases indicate a coagulation cascade preceding a heart attack.

Memory **70** can also store program instructions that control processor **54** to perform the functions ascribed to it herein. Memory **70** may include a variety of magnetic, optical, or electronic media, such as random access memory (RAM), read-only memory (ROM), electronic erasable programmable read-only memory (EEPROM), flash memory, or the like. Processor **54** may include one or more microprocessors, digital signal processors (DSPs), application specific integrated circuits (ASICs), field-programmable gate arrays (FPGAs), or the like.

In some embodiments, processor **54** controls delivery of therapy to patient **12** based on determined amounts of activated enzyme. In some embodiments, IMD **10** includes an additional reservoir, pump and catheter for the delivery of one or more drugs to patient **12**. Processor **54** can control the additional pump to initiate delivery of a drug or change the dosage for a drug based on the determined amount of activated enzyme. For example, delivery of a pain relieving drug may be initiated upon determination of increased kallikrein activation, or the dosage of an anticoagulant, such as heparin, may be altered based on a determination of increased thrombin activation. In other embodiments, IMD **10** is part of system that includes a therapy delivery device that is either controlled by processor **54** or acts in response to activated enzyme amounts received from processor **54**, as will be described in greater detail below. In this manner, IMD **10** can enable closed-loop delivery of therapy to patient **12**.

FIG. **5** is a timing diagram illustrating example ratio curves **80-86** used to determine activated enzyme amount. Each of ratio curves **80-86** is associated with an amount of activated enzyme, e.g., a concentration of activated enzyme. Ratio curves **80-86** are generated by measuring λ_1/λ_2 ratios over a period of time, e.g., one to ten minutes, after introduction of a known concentration of activated enzyme with a tagged substrate. In some embodiments, memory **70** stores information representing curves **80-86**, and processor **54** uses the information to determine the current activation level based on one or more λ_1/λ_2 ratios calculated at known times subsequent to presentation of substrate **20** to the bodily fluid. The information describing curves **80-86** may be stored as one or more look-up tables or equations.

FIG. **6** is a flowchart illustrating an example method that may be employed by IMD **10** to detect enzyme activation. Tagged substrate **20** is presented to the bodily fluid (**90**). In some embodiments, processor **54** controls pump **52** to present substrate **20** via catheter **14**, as described above. IMD **10** then emits energy at wavelength λ_0 by, for example, processor **54** controlling emitter **56** to emit energy at wavelength λ_0 (**92**).

IMD **10** detects energy at wavelengths λ_1 and λ_2, which is emitted by donor and acceptor dyes **26** and **28** tagged to substrate **20** in response to absorption of the energy emitted by IMD **10** at wavelength λ_0 (**94**). For example, in some embodiments, processor **54** receives indications of the intensities of energy at wavelengths λ_1 and λ_2 from detectors **58** and **60**, as described above. Processor **54** compares the intensities of energy at wavelengths λ_1 and λ_2 (**96**), e.g., by calculating a λ_1/λ_2 ratio, and determines an amount of activated enzyme, e.g. concentration of activated enzyme, as a function of the comparison (**98**).

The frequency with which the method is performed can depend on the application. For example, the method can be performed one or more times during a clinic visit as a diagnostic test under the control of a clinician using a programmer. In some embodiments, the method may be performed periodically as indicated by the condition or enzyme being monitored. For example, the method may be performed one or more times per day or week, or may be performed hourly. The invention is not limited to any particular frequency of enzyme activation detection.

FIG. **7** is a perspective diagram illustrating an example optical fiber **100** that can be coupled to IMD **10** according to some embodiments of the invention. As shown in FIG. **7**, optical fiber **100** includes substrate **20** linked thereto. Optical fiber **100** is used to present substrate **20** to a bodily fluid. Optical fiber **100** can, for example, be used with embodiments of IMD **10** that do not include a pump **52** and reservoir **50** for presenting substrate **20** to the bodily fluid.

Substrate **20** is linked to a region **102** of optical fiber **100** where a cladding **104** of fiber **100** is partially removed to expose a core **105** of optical fiber **100**. In general, cladding **104** reflects and refracts energy to direct the energy to travel within core **105** to the distal end of fiber **100**. At region **102** where cladding **104** is partially removed, energy may exit and enter core **105** of fiber **100**. In other words, IMD **10** may emit energy at wavelength λ_0 into a bodily fluid via region **102** such that the donor **26** presented to the bodily fluid may absorb the energy. When substrate **20** is uncleaved, as illustrated in FIG. **7**, the acceptor **28** emits energy at wavelength λ_2 through FRET, as described above. The energy at wavelength λ_2 emitted by the acceptor **28** enters fiber **100** via region **102** for detection by IMD **10**.

Donor tagged product **22** and acceptor tagged product **24** of substrate **20** are linked to core **105** at region **102** by cross-linkers **106**A and **106**B, respectively (collectively "cross-linkers 106"). Cross-linkers **106** can take the form of hetero-bifunctional cross-linkers, such as SPDP (N-Succinimidyl-3-(2-pyridyldithio)propionate). Region **102** of fiber **100** is treated with aminosilanes, such as 3-aminopropyltriethoxysilane, or proteins with modified amine groups that yield sulfhydryl-reactive intermediates to promote binding of cross-linkers **106** to region **102**.

Substrate **20** is exposed to the bodily fluid, which contains an enzyme. When the enzyme cleaves substrate **20**, donor tagged product **22** and acceptor tagged product **24** are separated such that FRET no longer occurs between dyes **26** and **28**. The separation between donor tagged product **22** and acceptor tagged product **24** can be due in part to a springing action of cross-linkers **106**. When substrate **20** is cleaved, the donor **26** will emit energy at wavelength λ_1 in response to energy emitted by IMD **10** at wavelength λ_0 via region **102**, as described above.

In some embodiments, fiber **100** can be used a single time and thereafter discarded. Thus fiber **100** is particularly suitable for use with external devices capable of practicing the invention as described herein. Fiber **100** may be used percutaneously or in an in vitro sample of bodily fluid. Although it is understood that multiple substrate molecules **20** are linked to the exposed region **102**, a single substrate molecule **20** is shown for ease of illustration. Further, region **102** need not be located at the distal end of fiber **100** as illustrated in FIG. **7**.

FIG. **8** is a perspective diagram illustrating an example optical fiber **110** and a substrate tape **112** for use in optically detecting enzyme activation according to some embodiments of the invention. As shown in FIG. **8**, fiber **110** may include a region **114** of optical fiber **110** where a cladding **116** of fiber **100** is partially removed to expose a core **117** of fiber **110**. As described above with reference to FIG. **7**, an implantable medical device **118** may emit energy and detect energy emitted by dyes **26** and **28** via region **114**.

Substrate **20** is linked to tape **112** via cross-linkers **106**. Tape **112** travels between reels **120**A and **120**B. Tape **112** can travel on a path that is substantially parallel and proximate to optical fiber **110**, such the substrate **20** and dyes **26** and **28** are proximate to region **114**. Reel **120**B can be attached to fiber **110** by a support member **122** that allow reel **120**B rotational freedom in a single direction. Reel **120**A is coupled to a reel rotation mechanism **124** that rotates reel **120** to move tape **112** along the indicated path between reels **120**A and **120**B.

A processor (not shown) of IMD **118** can control reel rotation mechanism **124** to move tape **112** in order to present new substrate **20** for each new determination of enzyme activation. Tape **112** is spooled on reel **120**B, and substrate molecules **20** are insulated from the bodily fluid while spooled on reel **120**B. Substrate molecules **20** become exposed to the bodily fluid as the tape is drawn from reel **120**B by the action of reel rotation mechanism **124**.

IMD **118** can include components, such as a processor, emitter and detectors similar to IMD **10**, as described above with reference to FIG. **4**. However, for ease of illustration, only reel rotation mechanism **124** and reel **120**A are shown in FIG. **8**. Although it is understood that multiple substrate molecules **20** are linked to tape, a single substrate molecule **20** is shown for ease of illustration. Further, region **114** and reel **120**B need not be located at the distal end of fiber **110** as illustrated in FIG. **8**.

FIG. **9** is a block diagram illustrating an example system **130** for optically detecting enzyme activation and delivering therapy to patient **12** based on the determined level of enzyme activation. System **130** includes IMD **10** coupled to catheter **14** and optical fiber **16**. IMD **10** optically determines an amount of activated enzyme as described above.

System **130** also includes a therapy delivery device **132** for delivering therapy to patient **12** based on amounts of activated enzyme as determined by IMD **10**. Therapy delivery device **132** can be an implantable or external device. Therapy delivery device **132** can be, for example, an implantable drug pump that delivers a drug as a function of determined enzyme activation levels, such as a Synchromed™ drug pump. In other embodiments, therapy delivery device **132** takes the form of an implantable neurostimulation device, such as an implanted pulse generator. In such embodiments, therapy delivery device **132** delivers neurostimulation to patient **12** via one or more electrodes **136** included on a lead **134**.

Drug or neurostimulation therapy can be provided to treat pain. When operated in concert with IMD **10**, therapy delivery device **132** can treat pain in a closed-loop fashion, with adjustments to pain therapy based on an objective assessment of pain. In other embodiments, anticoagulation therapy is provided via a drug pump embodiment of therapy delivery device **132**. In general, anticoagulant dosage is adjusted only after the coagulation status of the patient is measured, which occurs during infrequent clinic visits. Infrequently made measurements may miss changes to the condition of the patient that would require a change in the anticoagulant dosage. Too much anticoagulant can cause problematic bleeding, while too little can leave patient subject to formation of thrombi. System **130** may enable a more accurate, closed-loop determination of proper coagulation dose.

Processor **54** of IMD **10** can control therapy delivery device **132** to deliver therapy based on determined enzyme activation levels, or in other embodiments can simply communicate determined activated enzyme amounts to therapy delivery device **132** which in turn adjusts the therapy based on the communicated amounts. Processor **54** can control or communicate with device **132** via wired or RF telemetry communications. In some embodiments, IMD **10** and device **132** are a single device sharing one or more processors, or a single device with processors dedicated to either the enzyme activation detection functions or therapy delivery functions described herein.

Various embodiments of the invention have been described. For example, implantable medical devices for optically detecting enzyme activation have been described. However, one skilled in the art will recognize that the invention is not limited to the described embodiments, and that various modifications can be made to the described embodiments without departing from the scope of the invention. For example, although the invention has been primarily described herein as detecting the activation of the enzymes kallikrein and thrombin, the invention may be used to detect the activation of any enzyme within bodily fluid.

Further, although the invention has been described with reference to an implantable medical device, non-implanted embodiments are within the scope of the invention. For example, devices according to the invention can optically detect enzyme activation via percutaneous leads, or within bodily fluid samples in vitro. Some external device embodiments within the scope of the invention can utilize an optical fiber **100** linked to substrate **20** as described with reference to FIG. **7**. Such embodiments may take the form of diagnostic devices within clinics for use with multiple patients or fluid samples. A new optical fiber **100** can be used for each patient or fluid sample. These and other embodiments are within the scope of the following claims.

The invention claimed is:

1. An implantable medical device comprising:

an emitting element to emit energy at a first wavelength into a bodily fluid that contains an enzyme and a substrate tagged with at least two fluorescent dyes;

a detector to detect energy emitted by a first one of the dyes at a second wavelength based on fluorescent resonant energy transfer from a second one of the dyes in response to the emitted energy at the first wavelength; and

a processor to control the emitting element to emit energy at the first wavelength, receive an indication of an intensity of energy detected by the detector, and detect enzyme activation based on the indicated energy intensity.

2. The device of claim 1, wherein the detector detects energy emitted by the second one of the dyes at a third wavelength in response to the emitted energy at the first wavelength, and

wherein the processor receives an indication of an intensity of energy detected by the detector at the second and third wavelengths, determines a ratio between a first detected intensity at the second wavelength and a second detected intensity at the third wavelength, and determines a concentration of activated enzyme within the bodily fluid based on the ratio.

3. The device of claim 2, wherein the substrate includes a first product and a second product, the first product tagged with the first one of the dyes, and the second product tagged with the second one of the dyes, and

wherein the enzyme cleaves the substrate, and the first one of the dyes absorbs energy received from the second one of the dyes at the third wavelength via fluorescent resonant energy transfer and emits energy at the second wavelength in response to receiving the energy at the third wavelength prior to cleavage of the substrate by the enzyme.

4. The device of claim 2, wherein the detector comprises a first detector to detect energy emitted at the second wavelength and a second detector to detect energy at the third wavelength, the device further comprising:

a first filter to pass energy at the second wavelength to the first detector; and

a second filter to pass energy at the third wavelength to the second detector.

5. The device of claim 1, further comprising an optical fiber optically coupled to the emitting element and the detector, wherein a distal end of the optical fiber is located in the bodily fluid, the emitting element emits energy into the bodily fluid via the optical fiber, and the detector detects energy emitted by the first one of the dyes via the optical fiber.

6. The device of claim 5, wherein the fluid is blood and the distal end of the optical fiber is located within a blood vessel of a patient.

7. The device of claim 5, wherein the optical fiber includes a cladding, and wherein the optical fiber includes a region at which the cladding is removed from the optical fiber, and wherein the substrate is linked to the region of the optical fiber where the cladding is removed.

8. The device of claim 7, wherein the region is a distal region of the fiber.

9. The device of claim 5, further comprising:

a first reel;

a second reel; and

a reel rotation mechanism to move a tape between the first reel and the second reel along a path that is substantially parallel and proximate to the optical fiber,

wherein the substrate is linked to the tape, the optical fiber includes a cladding, the cladding is removed from a portion of the optical fiber, and the processor controls the reel rotation mechanism to move the tape such that uncleaved substrate is proximate to the unclad portion of the optical fiber.

10. The device of claim 9, wherein at least one of the first and second reels is coupled to the optical fiber.

11. The device of claim 1, further comprising:

a reservoir that contains the substrate;

a pump in fluid communication with the reservoir; and

a catheter that includes a proximal end in fluid communication with the pump and a distal end located in the bodily fluid,

wherein the processor controls the pump to present the substrate to the bodily fluid via the catheter.

12. The device of claim 11, wherein the bodily fluid is blood and the distal end of the catheter is located in a blood vessel of a patient.

13. The device of claim 1, wherein the energy is visible light.

14. The device of claim 1, wherein the enzyme is a protease enzyme.

15. The device of claim 14, wherein the enzyme is one of thrombin and kallikrein.

16. The device of claim 1, further comprising:

a memory to store information relating to the detection of enzyme activation; and

a telemetry circuit,

wherein the processor delivers the information to a user via the telemetry circuit.

17. The device of claim 1, further comprising an alarm, wherein the processor activates the alarm based on the detection of enzyme activation.

18. The device of claim 1, further comprising:

a reservoir containing a drug;

a pump in fluid communication with the reservoir; and

a catheter with a proximal end in fluid communication with the pump and a distal end located within a patient,

wherein the processor controls the pump to deliver the drug to the patient via the catheter based on the detection of enzyme activation.

19. The device of claim 18, wherein the drug comprises one of an anticoagulant and a pain relieving drug.

20. The device of claim 1, wherein the device is implanted in a patient.

* * * * *

专利名称(译)	用于酶活化的光学检测器		
公开(公告)号	US7473548	公开(公告)日	2009-01-06
申请号	US10/423112	申请日	2003-04-25
[标]申请(专利权)人(译)	美敦力公司		
申请(专利权)人(译)	美敦力公司，INC.		
当前申请(专利权)人(译)	METRONIC INC.		
[标]发明人	SOYKAN ORHAN DONOVAN MAURA G THOMPSON AMY E		
发明人	SOYKAN, ORHAN DONOVAN, MAURA G. THOMPSON, AMY E.		
IPC分类号	C12M1/34 A61B5/00 C12Q1/37 C12Q1/56 G01N21/64 G01N21/85 G01N33/86		
CPC分类号	A61B5/0071 A61B5/7203 A61B5/0084 A61B5/14546 A61B5/4839 C12Q1/37 C12Q1/56 G01N21/6428 G01N21/8507 G01N33/86 A61B5/0075 G01N2021/6415 G01N2021/6441 G01N2021/6484 G01N2021/8528		
助理审查员(译)	沉，BIN		
其他公开文献	US20040215134A1		
外部链接	Espacenet USPTO		

摘要(译)

基于酶的底物的切割量来检测体液中酶的活化。用两种荧光染料 - 供体和受体标记基质。标记的基质呈现给体液。装置以第一波长将能量发射到体液中，并且响应于第一波长的能量检测由染料发射的第二和第三波长的能量。在酶促切割底物之前，受体通过来自供体的荧光共振能量转移（FRET）接收的第二波长的能量发射第三波长的能量。在酶促切割底物后，供体以第二波长发射能量。该装置可以基于第二和第三波长处的能量的相对强度来确定体液内活化酶的浓度。

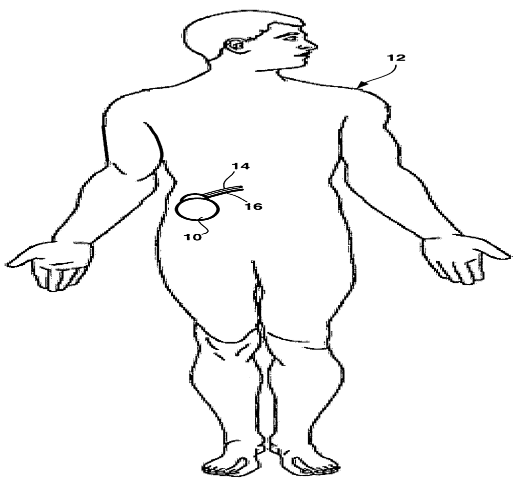

FIG. 1