

# (12) Indian Patent Application

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(54) Title: AN IMMUNOFLUORESCENCE DETECTING EQUIPMENT AND A METHOD THEREOF

(57) Abstract: The immunofluorescence equipment disclosed herein comprises a light emitting source 102, a lens tube 104, a microfluidic chip 202, a filter lens 108 and an avalanche photo diode (APD) based detector 106. The light emitting source 102 comprises at least an UV-LED. The UV-LED 102 is placed at a first circular face and a microfluidic chip 202 comprising at least one microfluidic channel 114 containing a biological sample with a biomarker is placed at a second circular face of the lens tube 104. The lens tube 104 comprises at least one biconvex lens 110. Light emitted from the UVLED 102 is converged onto the at least one microfluidic channel 114 by the at least one biconvex lens 110 triggering the biomarker to emit immunofluorescent emissions at one or more wavelengths which are filtered by the filter lens 108 to allow detection of only required immunofluorescent emission.

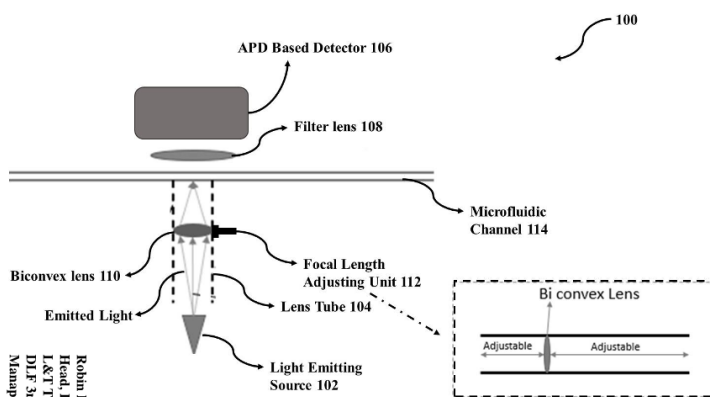


Figure 1

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# **FORM 2**

THE PATENTS ACT 1970  
(39 OF 1970)  
&  
The Patent Rules, 2003  
**Complete Specification**  
(See Section 10 and Rule 13)

## **1. TITLE OF THE INVENTION**

**AN IMMUNOFLUORESCENCE DETECTING EQUIPMENT AND A METHOD THEREOF**

## **2. APPLICANT(S)**

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## **3. PREAMBLE TO THE DESCRIPTION**

**COMPLETE**

The following specification particularly describes the invention and the manner in which it is to be performed.

## DESCRIPTION

### **TECHNICAL FIELD**

[0001] The present invention generally relates to a field of fluorescence spectroscopy and more particularly relates to detection for immunofluorescence from a biomarker in medical applications.

### **BACKGROUND OF INVENTION**

[0002] The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0003] Florescence spectroscopy has been widely used in the field of medical sciences for various applications such as sepsis detection, oncology, bacterial taxonomy, detection of pre-malignant and malignant lesions etc. When used in the field of medical sciences, fluorescence spectroscopy is commonly worded as Immunofluorescence spectroscopy (or microscopy) and is used to detect immunofluorescence from various biomarkers. The most common application of immunofluorescence detection is found in sepsis detection in surgical patients since the mortality rate due to sepsis is very high and it becomes imperative to detect sepsis conditions in surgical patients.

[0004] The very commonly and extensively used setup to detect immunofluorescence for sepsis detection, among other applications, uses laser as a light emitter and a photon counter as a light collector. While the use of laser as a light emitter offers various advantages such as a highly focussed and monochromatic light beam that can easily be used to excite a sample, it also suffers from various disadvantages. Some of the disadvantages include short lifespan of around three years and high cost. In addition, the combination of the laser and photon counter also adds up to the cost of the spectroscopic equipment which ranges between **10000-12000USD**. Further, the size of the equipment is quite large and imposes portability issues. To sum up, the spectroscopic equipments currently used require the laser source to be replaced almost every three years, are costly and also cannot reach to the masses due to portability issues.

[0005] Now, in developed economies, the cost or the size might not be that big a concern as the medical facilities such as hospitals and clinics etc are widespread and the masses have easy access to these facilities. Further, the developed economies make it a point that their citizens have proper health insurances and therefore, the high cost of equipments which indirectly affects the cost of services does not affect the masses in general.

[0006] However, this is not the case with developing economies. Cost-effectiveness and portability of equipments are two major factors that need to be addressed since vast population resides in areas where medical facilities are not highly developed, and majority of population has to pay for treatments out of their pockets. Thus, the existing solutions are not only costly but also technically complex in terms of size, flexibility and portability.

[0007] Therefore, in view of above, it becomes imperative to look for solutions that can provide a reliable, yet cost-effective detection of immunofluorescence from biomarkers for use in various fields of medical sciences.

### **SUMMARY OF INVENTION**

[0008] The present disclosure overcomes one or more shortcomings of the prior art and provides additional advantages discussed throughout the present disclosure. Additional features and advantages are realized through the techniques of the present disclosure. Other embodiments and aspects of the disclosure are described in detail herein and are considered a part of the claimed disclosure.

[0009] In one non-limiting embodiment of the present disclosure, an immunofluorescence detecting equipment is disclosed. The equipment comprises a light emitting source for emitting light in an Ultraviolet (UV) wavelength range. The equipment further comprises a lens tube, having a first circular face and a second circular face, arranged in such a manner that the light emitting source is placed at the first circular face and a microfluidic chip comprising at least one microfluidic channel containing a biological sample with a biomarker is placed at the second circular face. The lens tube comprises at least one biconvex lens configured to converge the light emitted by the light emitting source onto the at least one microfluidic channel in order to trigger the biomarker to emit immunofluorescent emissions at one or more wavelengths. The equipment further comprises an

avalanche photodiode (APD) based detector configured to detect a required immunofluorescent emission.

[0010] In one non-limiting embodiment of the present disclosure, a method of detecting immunofluorescence is disclosed. The method comprises providing a light emitting source for emitting light in an Ultraviolet (UV) wavelength range. The method further comprises providing a lens tube, having a first circular face and a second circular face arranged in such a manner that the light emitting source is placed at the first circular face and a microfluidic chip comprising at least one microfluidic channel containing a biological sample with a biomarker is placed at the second circular face. In one embodiment, providing the lens tube comprises providing at least one biconvex lens configured to converge the light emitted by the light emitting source onto the at least one microfluidic channel in order to trigger the biomarker to emit immunofluorescent emissions at one or more wavelengths. The method further comprises providing an avalanche photodiode (APD) based detector configured to detect a required immunofluorescent emission.

[0011] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

## **BRIEF DESCRIPTION OF DRAWINGS**

[0012] The embodiments of the disclosure itself, as well as a preferred mode of use, further objectives and advantages thereof, will best be understood by reference to the following detailed description of an illustrative embodiment when read in conjunction with the accompanying drawings. One or more embodiments are now described, by way of example only, with reference to the accompanying drawings in which:

[0013] **Figure 1** depicts a block diagram **100** of an immunofluorescence detecting equipment in accordance with an embodiment of the present disclosure;

[0014] **Figure 2** depicts a real-time illustration **200** of the immunofluorescence detecting equipment in accordance with an embodiment of the present disclosure;

[0015] **Figure 3** depicts a circuit diagram **300** of an avalanche photodiode (APD) based detector in accordance with an embodiment of the present disclosure; and

[0016] **Figure 4** depicts a method **400**, by way of a flow diagram, to detect immunofluorescence emission in accordance with an embodiment of the present disclosure.

[0017] The figures depict embodiments of the disclosure for purposes of illustration only. One skilled in the art will readily recognize from the following description that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the disclosure described herein.

### **DETAILED DESCRIPTION**

[0018] The foregoing has broadly outlined the features and technical advantages of the present disclosure in order that the detailed description of the disclosure that follows may be better understood. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present disclosure.

[0019] The novel features which are believed to be characteristic of the disclosure, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying Figures. It is to be expressly understood, however, that each of the Figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

[0020] Disclosed herein is an immunofluorescence detecting equipment and a method thereof. Immunofluorescence detection from biomarkers finds applications in various spheres in the domain of medical sciences including but not limited to sepsis detection, oncology, bacterial taxonomy, detection of pre-malignant and malignant lesions etc. The conventional immunofluorescence detecting equipment uses laser as a light emitter and a photon counter as a light collector. While the use of laser as a light emitter offers various advantages such as a highly focussed and monochromatic

light beam that can easily be used to excite a sample of relatively smaller sizes, it also suffers from various disadvantages such as short lifespan, high cost and portability issues.

[0021] The immunofluorescence detecting equipment disclosed herein overcomes the above-mentioned disadvantages by replacing the laser with a suitable light emitting source capable of emitting light in UV wavelength range and by replacing the photon counter with a single avalanche photo diode (APD) based detector. In addition, the disclosed immunofluorescence detecting equipment does not require any complex circuitry or extensive elements for immunofluorescence detection. The detailed description of said immunofluorescence detecting equipment is described in the subsequent paragraphs.

[0022] **Figure 1** depicts a block diagram **100** of the immunofluorescence detecting equipment (hereafter referred to as “Equipment”) in accordance with an embodiment of the present disclosure. The block diagram **100** of **Figure 1** and the working of various components of the equipment is explained in conjunction with **Figure 2** which depicts a real-time illustration **200** of the equipment.

[0023] The equipment as illustrated in Figures 1 and 2 comprises a light emitting source **102**, a lens tube **104**, a microfluidic chip **202**, a filter lens **108** and an avalanche photo diode (APD) based detector **106**. In one embodiment, the light emitting source **102** comprises at least an ultraviolet-light emitting diode (UV-LED) capable of emitting light in an ultraviolet wavelength range. The lens tube **104** is a cylindrical tube configured to confine the light emitted by the UV-LED **102** within and prevent interference from ambient light. Further, the lens tube **104** comprises a first circular face and a second circular face as is known for any cylindrical structure. In one embodiment, the UV-LED **102** is placed at the first circular face of the lens tube **104** and the microfluidic chip **202** is placed at the second circular face of the lens tube **104**. The microfluidic chip **202** comprises at least one microfluidic channel **114** of a predefined dimension containing a biological sample with a biomarker. In one embodiment, the at least one microfluidic channel **114** has a dimension of around **200** microns. However, it may be known to a skilled person that microfluidic channels of various different dimensions may be fabricated on the microfluidic chip **202**. Further, in accordance with the embodiment, the biological sample comprises of plasma extracted from the blood of a living being.

[0024] In one embodiment, the lens tube **104** further comprises at least one biconvex lens **110** and a focal length adjusting unit **112**. Light emitted from the UV-LED **102** is converged onto the at least

one microfluidic channel **114** by the at least one biconvex lens **110** whose focal length may be adjusted by means of the focal length adjusting unit **112** to obtain a focused beam of light as depicted in the inset of **Figure 1**.

[0025] The light converging onto the at least one microfluidic channel **114** excites the molecules of the biomarker to emit immunofluorescent emissions at one or more wavelengths. Since, the light obtained from the UV-LED **102** is not monochromatic and not as focussed in comparison to that obtained from a laser (used in conventional technique), the immunofluorescent emissions emitted from the biomarker are at different wavelengths. To filter out unwanted immunofluorescent emissions and allow only the required immunofluorescent emission at particular wavelength to pass through the APD based detector **106**, the filter lens **108** is placed between the microfluidic chip **202** and the APD based detector **106**.

[0026] The detection of required immunofluorescence is achieved by the APD based detector **106**. The circuit diagram of the APD based detector **106** is illustrated in **Figure 3**. The APD based detector essentially comprises of three components viz., the APD **302**, a trans-impedance amplifier **304** and a non-inverting amplifier **306**. In accordance with the embodiment, the APD **302** generates a current based on the required immunofluorescent emission detected. The APD **302** operates in a reverse biased region in order to generate current of a reasonable value of the order of a few micron. The current generated by the APD **302** is converted into voltage by a trans-impedance amplifier **304** connected to the APD **302** as shown in **Figure 3**. However, for ease of analysis, the magnitude of the converted voltage is amplified by a non-inverting amplifier **306** connected to the trans-impedance amplifier **304** as shown in **Figure 3**.

[0027] The amplified voltage is in analog form and in one embodiment, is converted into a digital form in order to obtain a voltage reading by means of one or more analog to digital converter (ADC) channels connected to the APD based detector **106**. The resultant voltage reading may be used by a medical professional for detection of either sepsis or lesions or cancerous cells etc depending on the type of application the equipment is used for.

[0028] **Figure 4** depicts a method **400**, by way of a flow diagram, to detect immunofluorescence in accordance with an embodiment of the present disclosure.

[0029] The order in which the method **400** is described is not intended to be construed as a limitation, and any number of the described method blocks may be combined in any order to implement the method. Additionally, individual blocks may be deleted from the methods without departing from the spirit and scope of the subject matter described.

[0030] At block **402**, the method **400** may include providing **402** a light emitting source **102** for emitting light in an Ultraviolet (UV) wavelength range.

[0031] At block **404**, the method **400** may include providing **404** a lens tube **104**, having a first circular face and a second circular face arranged in such a manner that the light emitting source is placed at the first circular face and a microfluidic chip **202** comprising at least one microfluidic channel **114** containing a biological sample with a biomarker is placed at the second circular face. In one embodiment, for providing the lens tube **104**, the method **400** proceeds to blocks **404A** and **404B**.

[0032] At block **404A**, the method **400** may include providing **404A** at least one biconvex lens **110** configured to converge the light emitted by the light emitting source **102** onto the at least one microfluidic channel **114** in order to trigger the biomarker to emit immunofluorescent emissions at one or more wavelengths.

[0033] At block **404B**, the method **400** may include providing **404B** a focal length adjusting unit **112** to facilitate adjustment of a focal length of the at least one biconvex lens **110** in order to obtain a focused beam of light from the light emitting source **102**.

[0034] At block **406**, the method **400** may include providing at least one filter lens **108** between the microfluidic chip **202** and the APD based detector **106**.

[0035] At block **408**, the method **400** may include providing an avalanche photodiode (APD) based detector **106** configured to detect a required immunofluorescent emission.

[0036] A description of an embodiment with several components in communication with each other does not imply that all such components are required. On the contrary, a variety of optional components are described to illustrate the wide variety of possible embodiments of the disclosure.

**[0037]** When a single device or article is described herein, it will be clear that more than one device/article (whether they cooperate) may be used in place of a single device/article. Similarly, where more than one device or article is described herein (whether they cooperate), it will be clear that a single device/article may be used in place of the more than one device or article or a different number of devices/articles may be used instead of the shown number of devices or programs. The functionality and/or the features of a device may be alternatively embodied by one or more other devices which are not explicitly described as having such functionality/features. Thus, other embodiments of the disclosure need not include the device itself.

**[0038]** Finally, the language used in the specification has been principally selected for readability and instructional purposes, and it may not have been selected to delineate or circumscribe the inventive subject matter. It is therefore intended that the scope of the disclosure be limited not by this detailed description, but rather by any claims that issue on an application based here on. Accordingly, the embodiments of the present disclosure are intended to be illustrative, but not limiting, of the scope of the disclosure, which is set forth in the following claims.

**[0039]** While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

## CLAIMS

### We Claim:

1. An immunofluorescence detecting equipment comprising:
  - a light emitting source (102) for emitting light in an Ultraviolet (UV) wavelength range;
  - a lens tube (104), having a first circular face and a second circular face, arranged in such a manner that the light emitting source (102) is placed at the first circular face and a microfluidic chip (202) comprising at least one microfluidic channel (114) containing a biological sample with a biomarker is placed at the second circular face, wherein the lens tube (104) comprises:
    - at least one biconvex lens (110) configured to converge the light emitted by the light emitting source (102) onto the at least one microfluidic channel (114) in order to trigger the biomarker to emit immunofluorescent emissions at one or more wavelengths; and
    - an avalanche photodiode (APD) based detector (106) configured to detect a required immunofluorescent emission.
  
2. The equipment as claimed in claim 1, wherein the light emitting source (102) comprise at least an Ultraviolet-light emitting diode (UV-LED).
  
3. The equipment as claimed in claim 1, wherein the lens tube (104) further comprises a focal length adjusting unit (112) to facilitate adjustment of a focal length of the at least one biconvex lens (110) in order to obtain a focused beam of light from the light emitting source (102).
  
4. The equipment as claimed in claim 1, further comprising:
  - at least one filter lens (108) placed between the microfluidic chip (202) and the APD based detector (106), wherein the at least one filter lens (108) is configured to filter and allow the required immunofluorescent emission to pass through the detector (106), wherein the required immunofluorescent emission comprises an immunofluorescent emission at a particular wavelength of the one or more wavelengths.
  
5. The equipment as claimed in claim 1, wherein the APD based detector (106) comprises:
  - an avalanche photo diode (APD) (302) configured to generate a current based on the required immunofluorescent emission being detected;

a trans-impedance amplifier (304) configured to convert the current generated into voltage; and a non-inverting operational amplifier (306) configured to amplify a magnitude of the voltage obtained by the trans-impedance amplifier (304); and wherein the magnitude of the voltage amplified by the non-inverting amplifier (306) is converted into a digital form by one or more analog to digital converter (ADC) channels connected to the APD based detector (106).

**6.** A method of detecting immunofluorescence, the method comprising:

providing (402) a light emitting source (102) for emitting light in an Ultraviolet (UV) wavelength range;

providing (404) a lens tube (104), having a first circular face and a second circular face arranged in such a manner that the light emitting source is placed at the first circular face and a microfluidic chip (202) comprising at least one microfluidic channel (114) containing a biological sample with a biomarker is placed at the second circular face, wherein providing (404) the lens tube (104) comprises:

providing (404A) at least one biconvex lens (110) configured to converge the light emitted by the light emitting source (102) onto the at least one microfluidic channel (114) in order to trigger the biomarker to emit immunofluorescent emissions at one or more wavelengths; and providing (408) an avalanche photodiode (APD) based detector (106) configured to detect a required immunofluorescent emission.

**7.** The method as claimed in claim 6, wherein the light emitting source (102) comprises at least an Ultraviolet-light emitting diode (UV-LED).

**8.** The method as claimed in claim 6, wherein providing (404) the lens tube further comprises providing (404B) a focal length adjusting unit (112) to facilitate adjustment of a focal length of the at least one biconvex lens (110) in order to obtain a focused beam of light from the light emitting source (102).

**9.** The method as claimed in claim 6, further comprising:

providing (406) at least one filter lens (108) between the microfluidic chip (202) and the APD based detector (106), wherein the at least one filter lens (108) is configured to filter and allow

only the required immunofluorescent emission to pass through the detector (106), wherein the required immunofluorescent emission comprises an immunofluorescent emission at a particular wavelength of the one or more wavelengths.

- 10.** The method as claimed in claim 6, wherein providing the APD based detector (106) comprises:
- providing an avalanche photo diode (APD) (302) configured to generate a current based on the required immunofluorescent emission being detected;
  - providing a trans-impedance amplifier (304) configured to convert the current generated into voltage; and
  - providing a non-inverting operational amplifier (306) configured to amplify a magnitude of the voltage obtained by the trans-impedance amplifier (304);
- and wherein the magnitude of the voltage amplified by the non-inverting amplifier (306) is converted into a digital form by one or more analog to digital converter (ADC) channels connected to the APD based detector (106).

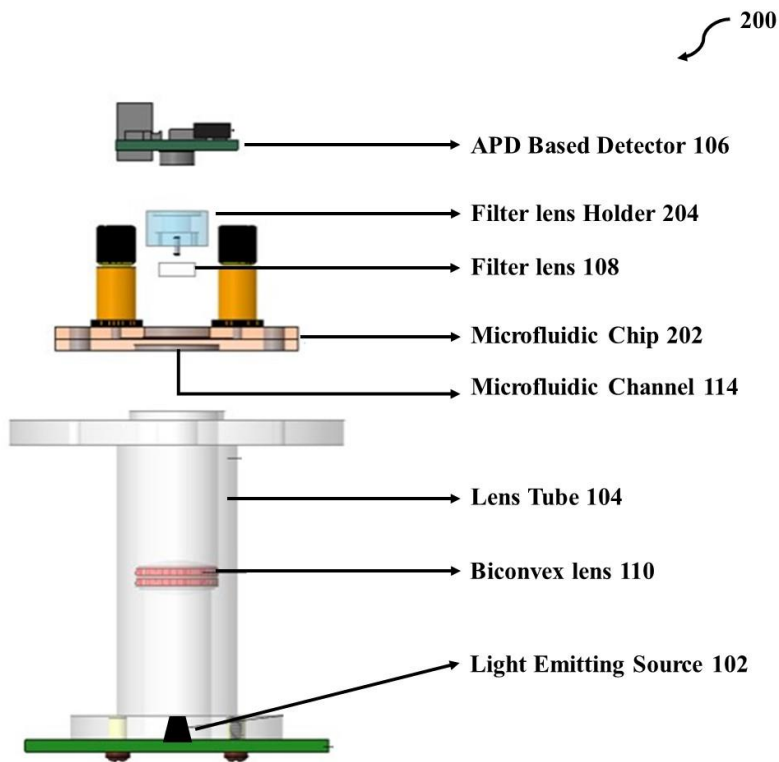
**Dated this 02<sup>nd</sup> Day of December 2021**

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## **ABSTRACT**

### **AN IMMUNOFLUORESCENCE DETECTING EQUIPMENT AND A METHOD THEREOF**

The immunofluorescence equipment disclosed herein comprises a light emitting source **102**, a lens tube **104**, a microfluidic chip **202**, a filter lens **108** and an avalanche photo diode (APD) based detector **106**. The light emitting source **102** comprises at least an UV-LED. The UV-LED **102** is placed at a first circular face and a microfluidic chip **202** comprising at least one microfluidic channel **114** containing a biological sample with a biomarker is placed at a second circular face of the lens tube **104**. The lens tube **104** comprises at least one biconvex lens **110**. Light emitted from the UV-LED **102** is converged onto the at least one microfluidic channel **114** by the at least one biconvex lens **110** triggering the biomarker to emit immunofluorescent emissions at one or more wavelengths which are filtered by the filter lens **108** to allow detection of only required immunofluorescent emission.



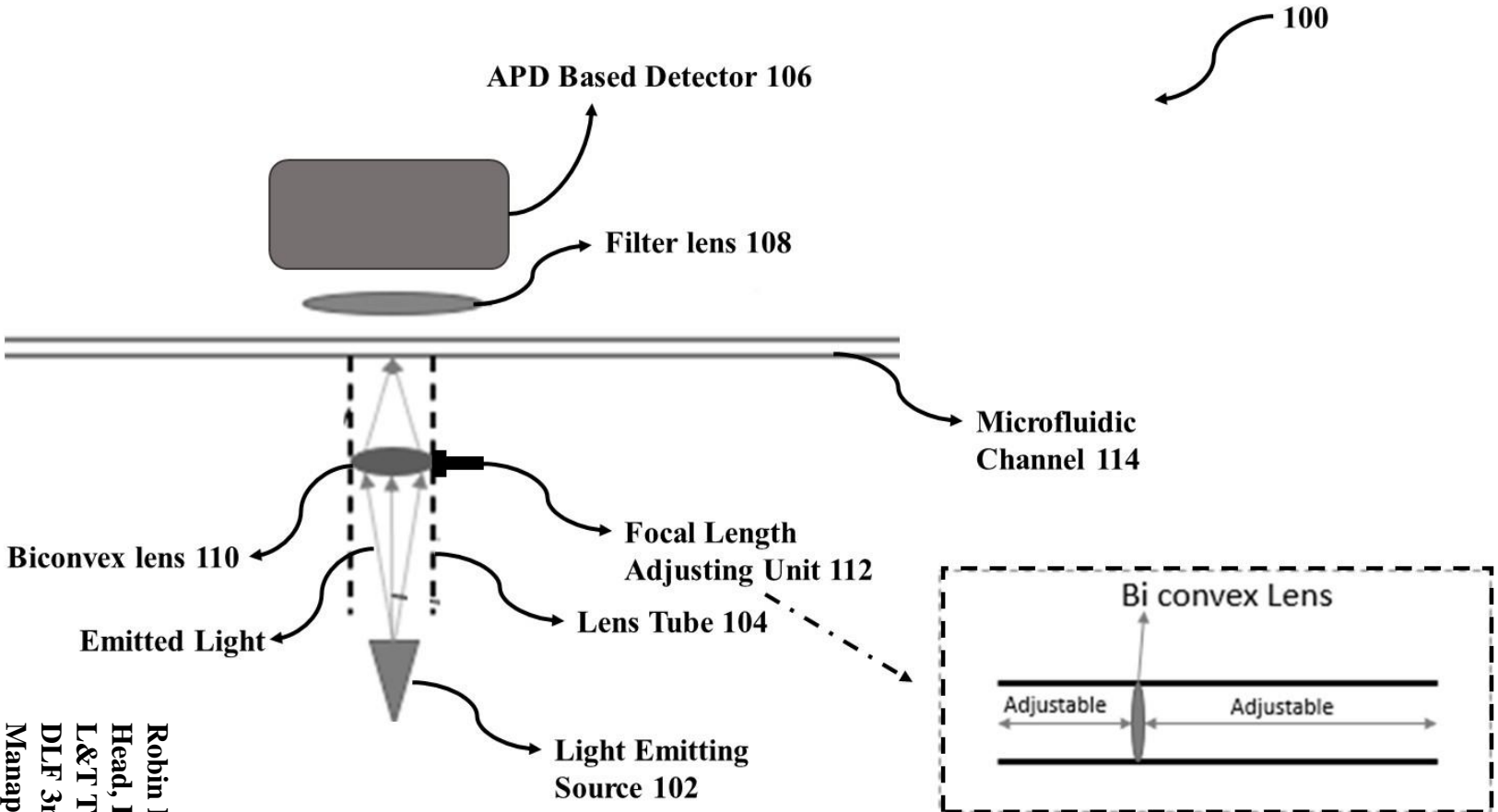


Figure 1

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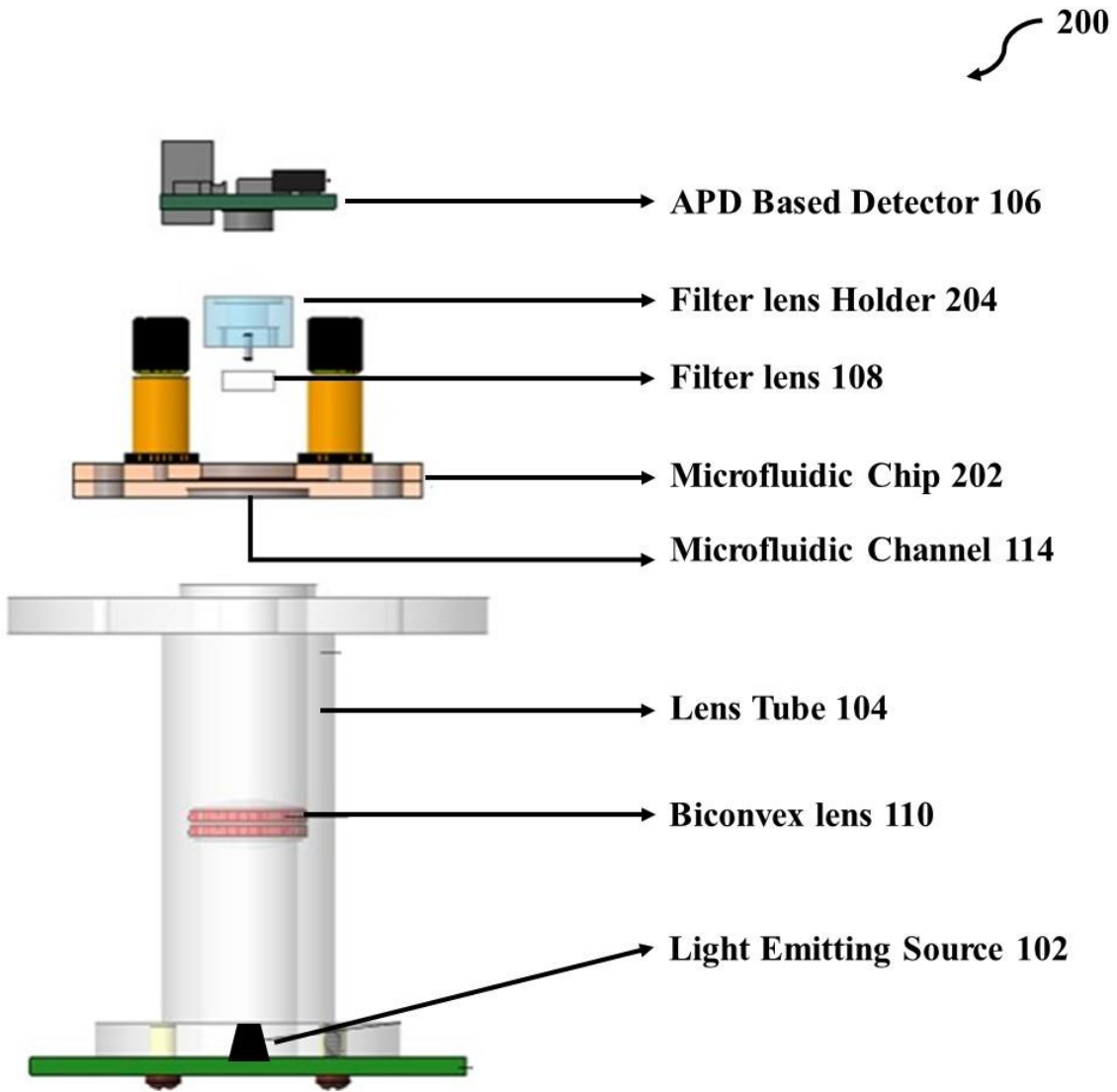


Figure 2

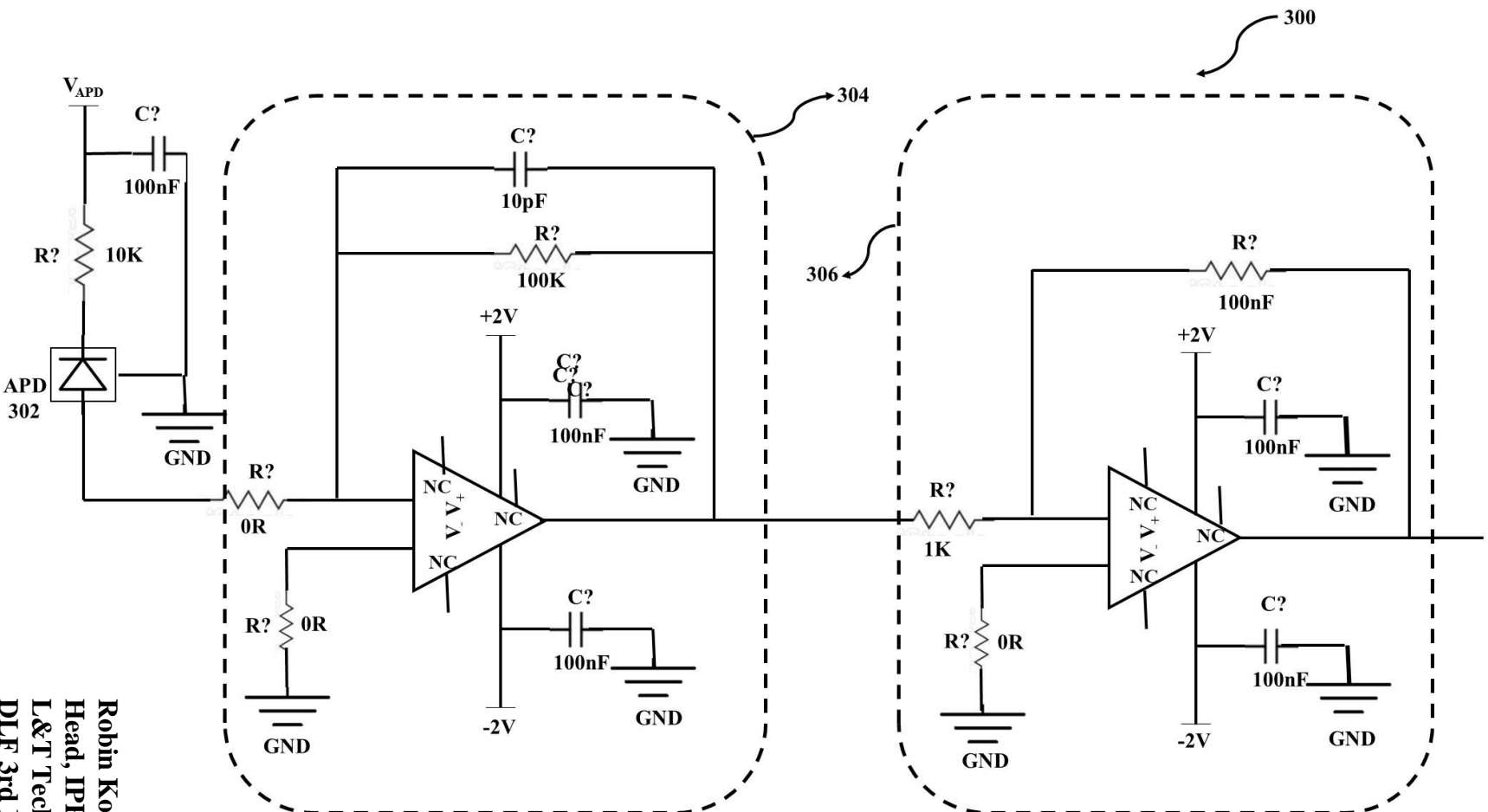


Figure 3

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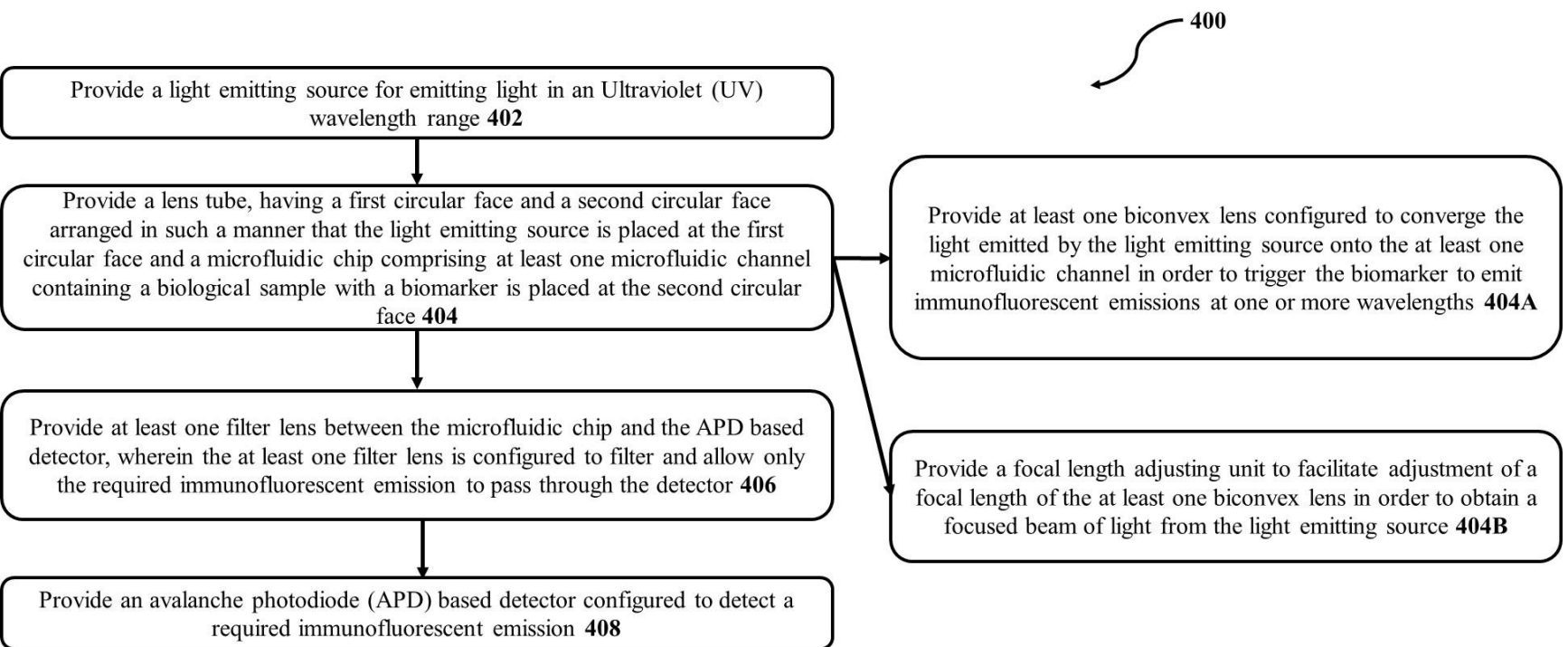


Figure 4

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