

Optical design for a fiber-coupled, single-molecule detection instrument**BACKGROUND OF THE INVENTION**

[0001] The disclosed invention relates to a system for a fiber-coupled, single molecule detection instrument for measurement of rare protein concentrations in a prepared assay solution via a 2-PLEX optical design.

5 [0002] The invention belongs to the technical fields of Protein Detection.

[0003] Instrumentation for detection of proteins can take a variety of forms and utilize a variety of techniques. In general, proteins are isolated from a sample and labeled for detection. Several principal techniques include:

10 [0004] 1. Western blot, a protein identification methodology and a semi-quantitative approach which is used for screening.

[0005] 2. ELISA, a workhorse standard in industry with a typical sensitivity 10 pg/mL.

[0006] 3. Bead-based sandwich ELISA (e.g. Luminex) which offers high multiplexing potential (up to 80 markers) for low sample volumes; used for screening with a typical sensitivity of ~ 1-10 pg/mL.

15 [0007] 4. Immunoassay qPCR (e.g. ProQuantum), which is typically a single plex approach, with a quantitative, sensitivity as low as 0.01 pg/mL

[0008] With the exception of chemiluminescent ELISAs, most of these techniques employ fluorescently labeled targets and measurement using fluorescence detection instrumentation.

20 [0009] Broadly, fluorescence detection instrumentation utilizes a light source of a specific wavelength, typically a laser, to excite and promote fluorescence of the label, and a detector, such as a photodetector, avalanche photodiode, or photomultiplier tube. Optical filters are used, particularly in the detection path, to sample a small band of the label's fluorescence spectrum.

[0010] The highest sensitivity fluorescence detection instruments target single molecule accuracy, hence the single molecule detection (SMD) category. One approach for SMD is to limit the interrogated sample volume, often called the confocal volume, typically by use of a spatial pinhole filter. By reduction of the detection volume, out of focus light is blocked. This provides enhanced signal-to-noise and improved ability to identify single fluorescently labeled target molecules.

[0011] One example is a confocal volume of $1 \mu\text{m}^3$. This is a small volume compared to many detection systems, and yet such a volume will still contain over 3.3×10^{10} H₂O molecules, assuming a primarily aqueous buffer. At low target concentrations, weak 'background' emission (Raman, autofluorescence from impurities, etc.) from the solution matrix can be significant due to the high ratio of background-to-target molecules. Target molecules will be greatly outnumbered, and as such even weak contributions from the background can become significant, compete with the fluorescence signal, and obscure the target. By shrinking the confocal volume, a single molecule's fluorescence signature and intensity can better compete with the matrix and enable more sensitive detection [1].

[0012] High-sensitivity ELISA assays can quantify targets as low as 10 pg/mL concentrations. However, single-molecule detection instruments can typically achieve orders of magnitude lower sensitivity.

[0013] The SMCxPRO® and SMC® assay product line is the current state of the art platform for detection of ultra-rare protein targets using the single molecule detection technique. The SMCxPRO® utilizes assays created using proprietary preparation techniques, and along with its confocal detection architecture can resolve concentrations as low as 0.01 pg/mL.

[0014] This known state of the art still possess some disadvantages and limitations, like non-optimized signal-to-noise ratio, complex setup, and non-optimized detection efficiency.

[0015] To comply with and enhance the state of the art it would therefore be desirable to find a new and advanced SMC® instrument optical design.

BRIEF SUMMARY OF THE INVENTION

- [0016] This task can be solved by a detection system for detecting protein concentrations in a prepared assay solution consisting of several components, arranged after each, the components being a fiber coupled laser for emitting laser light, a laser tube lens to focus the laser light, a laser cleanup filter for blocking non-laser wavelengths, a first dichroic filter for passing the laser light and reflecting fluorescence light, an objective lens to focus the laser light to a small excitation volume, the objective also collecting fluorescence light emitted from a sample, a pre-confocal and a post-confocal lens with a pinhole positioned between them to focus and re-focus the fluorescent light, a second dichroic filter, a focus photodiode for auto-focusing on the sample, at least one bandpass filter for spectrum filtering of the fluorescent light, at least one fiber focus lense for coupling the fluorescent light to an optical fiber, at least one fiber patch cables to couple fluorescently focused light to at least one computer-based detector for signal detection, wherein the sample is located after the objective lens, the second dichroic filter, the focus photodiode and the at least one bandpass filter are forming a De-Multiplexer for the incoming fluorescence light and the at least one bandpass filter, at least one fiber focus lense, at least one fiber patch cables and at least one computer-based detector always consist of same the number and one of each of them are located along a respective fluorescence light. This system provides has several advantages like a sensitive detection of single molecules in solution, meaning that the confocal design is optimized for detection of rare, single molecule labels versus rejection of background from the buffer and other sources. The next generation optical filter designs were optimized for highest signal-to-noise for each detection band. Another advantage is a 2-PLEX detection capability, which means a 2-channel laser and 2-channel detection sorter enables measurement of two independent fluorescent labels and thus biomarkers in solution. A third advantage is the mentioned fiber coupled excitation and detection system which means that the excitation and detection paths are fiber-coupled, enabling streamlined servicing and replacement of components in the field, without resorting to time-consuming optical alignments.
- [0017] Advantageous and therefore preferred further developments of this invention emerge from the associated sub claims and from the description and the associated drawings.

[0018] One of those preferred further developments of the disclosed system comprise that the De-Multiplexer is a detection multiplex sorter for fluorescence light which is configured to take light from two fluorescent regions in the light stream and splits the light according to its color / wavelength.. This has the advantage of efficiently separating and
5 sorting multiple fluorescence light streams, allowing simultaneous detection of different biomarkers in the solution.

[0019] Another one of those preferred further developments of the disclosed system comprise that two Bandpass filters are used to separate the spectra of the incoming fluorescence light with the first Bandpass filter being positioned in front of a first Fiber
10 focus lense and the second Bandpass filter in front of a second Fiber focus lense. This enables precise spectral filtering for each fluorescence light stream and ensures accurate detection and analysis of specific fluorescence signals.

[0020] Another one of those preferred further developments of the disclosed system comprise that the first bandpass filter is a 780/30 bandpass and the second bandpass
15 filter is a 687/34 bandpass. That offers the advantage of tailored spectral filtering, allowing specific wavelengths of interest to pass through each filter while reducing unwanted background noise. It therefore enhances the accuracy and sensitivity of the protein concentration detection.

[0021] Another one of those preferred further developments of the disclosed system
20 comprise that the dichroic filters are positioned in a 45-degree angle of incidence and reflect the fluorescence light stream while the laser light streams are transmitted, wherein the transmission bands are for wavelengths of around 642 nm and 730 nm, while the Reflection bands for fluorescence are between 660 nm and 710 nm and between 760 nm and 830 nm. That has the advantage of efficiently separating the
25 fluorescence and laser light paths. The transmission and reflection bands of the dichroic filters are optimized to allow specific wavelengths of laser light to pass through and reflect the fluorescence light, enhancing the signal-to-noise ratio for fluorescence detection.

[0022] Another one of those preferred further developments of the disclosed system
30 comprise that the Fiber coupled laser emits laser lines of 642 nm and 730 nm wavelength. It provides the advantage of specific excitation wavelengths tailored for specific fluorescent labels and optimal fluorescence excitaiton and detection of the labels. This ensures efficient excitation of the fluorescent labels in the solution.

[0023] Another one of those preferred further developments of the disclosed system comprise that the at least one computer-based detector comprises of at least one sensor receiving the fluorescence light stream which is connected to a computer which computes the protein concentrations by analyzing the signal from the at least one
5 sensor. This offers the advantage of flexibility and efficiency in protein concentration computation. In this case, if the sensor is connected to a computer, the advantage lies in the ability to process the signal from the sensor separately, enabling complex analysis algorithms to be applied for accurate protein concentration determination.

[0024] Another one of those preferred further developments of the disclosed system
10 comprise that the laser tube lens is a color corrected lens with a focal length (f) of 40 mm and a numerical aperture (NA) of 0.10 and/or the objective lens is a color corrected lens with a focal length (f) of 5 mm and a numerical aperture (NA) of 0.58 and/or the pre-confocal lens is a color corrected lens with a focal length (f) of 40 mm and a numerical aperture (NA) of 0.10 and/or the post-confocal lens is a color corrected lens with a focal
15 length (f) of 12 mm and a numerical aperture (NA) of 0.10 and/or the at least one fiber focus lens is a color corrected lens with a focal length (f) of 10 mm and a numerical aperture (NA) of 0.10. This offers the following advantages: The color corrected laser tube lens ensures accurate focusing and alignment of confocal volumes of multiplex excitation lines, enhancing the overall optical performance of the system. Also the color
20 corrected objective lens, with a smaller focal ratio and higher numerical aperture, allows for high-resolution imaging of the sample. It captures and maximizes the fluorescence signals emitted from the excited sample, resulting in improved sensitivity and detection capabilities. Furthermore the color corrected pre- and post-confocal lenses provide precise focusing and re-focusing of the fluorescence light, enabling enhanced confocal
25 detection and reducing background noise, resulting in improved signal quality. The color corrected fiber focus lens now ensures efficient coupling of the fluorescent light into the optical fibers. It optimizes the light transmission, minimizing losses and maintains signal integrity throughout the fiber-based detection system. Overall, these lens characteristics contribute to improved image quality, sensitivity, and accuracy in protein concentration
30 detection and analysis within the system.

[0025] Another one of those preferred further developments of the disclosed system comprise that the pinhole is a spatial filter to create confocal detection volume with a diameter of 80 μm . It ensures the advantage of selective and precise detection. By

restricting the detection volume, it eliminates out-of-focus fluorescence, enhancing the signal-to-noise ratio and overall detection sensitivity.

[0026] Another one of those preferred further developments of the disclosed system comprise that between the fiber coupled laser and the laser tube lens a turning mirror is positioned for adjusting the laser light into a specific direction. The inclusion of a turning mirror between the fiber-coupled laser and the laser tube lens enables the advantage of fine adjustment of the direction of laser light. This allows for flexibility in the system setup and alignment, facilitating efficient utilization of the laser lines within the system.

[0027] A further component of the claimed invention is a method for detecting protein concentrations in a prepared assay solution comprising the following steps of Emitting laser lines with a fiber coupled laser; a laser tube lens to focus the laser light; Blocking non-laser wavelengths with a laser cleanup filter, Passing the laser light through a first dichroic filter; Collecting fluorescence light emitted from a sample with the protein concentrations in the prepared assay solution by using an objective, wherein the sample is excited by the laser light and an objective lens is used to focus the laser light to a small excitation volume; Passing the collected fluorescence light through the first dichroic filter and reflecting it; Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal detection volume in the fluorescent light positioned between them; Separating the fluorescence light from other components using a second dichroic filter; Performing peak finding in the laser light and auto-focusing with a focus photodiode; Filtering the fluorescent light spectrum using at least one bandpass filter; Coupling the fluorescent light to an optical fiber patch cable using at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector; Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable.

[0028] Another one of those preferred further developments of the disclosed method comprise that the laser light is turned into a rectangular direction away from the fiber coupled laser by using a turning mirror which is positioned between the fiber coupled laser and the laser tube lens.

[0029] Another component of the disclosed invention is a Computer program comprising instructions which cause the involved computers to control the respective configured hardware components to carry out the following method steps of Turning on

laser excitation from the fiber coupled laser to emit laser light; Focusing the laser lines into a laser stream using a laser tube lens; Blocking non-laser wavelengths with a laser cleanup filter; Passing the laser stream through a first dichroic filter and focusing to the sample using an objective lens; Collecting fluorescence light emitted from a sample with the protein concentrations in the prepared assay solution by using an objective, wherein the sample is excited by the laser stream and an objective lens is used to focus the laser stream to a small excitation volume; Passing the collected fluorescence light through the objective and to the first dichroic filter and reflecting it; Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal detection volume in the fluorescent light positioned between them; Separating the fluorescence light from other components using a second dichroic filter; Performing peak finding in the laser stream and auto-focusing with a focus photodiode positioned behind the second dichroic filter; Filtering the fluorescent light spectrum using at least one bandpass filter; Coupling the fluorescent light to an optical fiber patch cable using at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector; and Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable. . The program parts responsible for the single method steps are running on the respective computer parts. How the program itself is partitioned depends on the computer hardware being involved. It is possible to use a main software running on one of the mentioned computers or a separate computer which controls local client programs. Other options include equal instances of the software who communicate with each other and so on.

[0030] Only requirement for this computer program to perform the whole method as described is, that the used program and its respective hardware components together are able and configured to perform the method completely and automatically. Such a program can then be stored on a Computer-readable storage medium and/or data carrier signal which cause the involved computers to carry out the method steps of Emitting laser lines with a fiber coupled laser; a laser tube lens to focus the laser light; Blocking non-laser wavelengths with a laser cleanup filter, Passing the laser stream through a first dichroic filter; Collecting fluorescence light emitted from a sample with the protein concentrations in the prepared assay solution by using an objective, wherein the sample is excited by the laser light and an objective lens is used to focus the laser light to a small excitation volume; Passing the fluorescence light through the first dichroic filter and

reflecting it; Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal detection volume in the fluorescent light positioned between them; Separating the fluorescence light from other components using a second dichroic filter; Performing peak finding in the laser light and auto-focusing with a focus photodiode; Filtering the fluorescent light spectrum using at least one bandpass filter; Coupling the fluorescent light to an optical fiber patch cable using at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector; Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable. The storage medium can be stored on any suitable digital memory like an usb drive, a harddisk, a flashdrive and so on. From that memory it can also be provided via remota communication means using respective data carrier signals, like ethernet, wired or wireless, or any other suitable network transmission means, for transmitting the software to its target hardware.

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BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

[0031] Figure 1: a schematic of the optical layout, up to the detection multiplexed sorter

[0032] Figure 2: a schematic of the interior of the De-Multiplexer detection multiplex sorter

[0033] Figure 3: a ray diagram showing the excitation path of the laser lines

[0034] Figure 4: a ray diagram showing the fluorescence emission detection path with detection multiplexed sorter omitted at engineering break

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DETAILED DESCRIPTION OF THE INVENTION

[0035] The invention will be explained in more detail by presenting one preferred exemplary embodiment of the next generation SMC instrument optical design including the new laser, optical lenses, and optical filter designs.

[0036] The design is based on an epi-fluorescence confocal layout as shown schematically in Figure 1. A further breakout of the fluorescence sorter mechanism, in the following called DeMux, is shown in Figure 2.

[0037] The Excitation Path of the laser lines is highlighted in Figure 1 using cartoon light rays. A breakdown and description of major elements in this path is listed as follows – in the order from laser to objective:

[0038] First comes a fiber coupled laser with 642 nm and 730 nm laser lines.

- 5 [0039] Next is a turning mirror to turn the emitted laser lines into the desired direction. This is not critical to the system, and could be eliminated with a re-orientation of the laser source in an alternative working example.

[0040] The next part is the laser tube lens which is used to collimate and focus the laser lines to infinity.

- 10 [0041] Afterwards a color corrected lens is positioned with a focal length $f=40$ mm, and a numerical aperture N.A.=0.10.

- [0042] Then comes a laser cleanup filter which is an optical filter for blocking any non-laser wavelengths. Its transmission bands are around 642 nm and 730 nm and are adapted to the laser source. The blocking bands on the other hand are outside of the
15 laser wavelengths and specifically at detection bands to limit any laser crosstalk.

[0043] Next part is a dichroic filter, which is a further optical filter which lets the laser lines pass and reflects any fluorescence light. It is located at a nominal 45-degree angle of incidence and has transmission bands around 642 nm and 730 nm. Its reflection bands for fluorescence are minimally 660-710 nm, 760-830 nm.

- 20 [0044] The following objective lens is used to focus the laser lines to a small excitation volume. It is a color corrected, high-numerical aperture objective with a focal length $f=5$ mm and a numerical aperture N.A.=0.58

[0045] After the objective the sample is excited with the focused laser and emits fluorescence light which is re-collected with the objective.

- 25 [0046] The Detection Path is now highlighted in Figure 1 using cartoon rays to show the fluorescence signal. The further breakout of detection of each fluorescent signature using the De-Multiplexer is shown in Figure 2.

[0047] A breakdown and description of the following major elements up to the De-Multiplexer sorter follows now:

[0048] The Objective is used to collect the emitted fluorescence light from the sample. The itself sample is held in a container and scanned via exposure to the focused laser lines. Excitation and collection are done through the clear substrate of a well plate.

5 [0049] Afterwards the emitted fluorescence light from the sample is collected by the objective, passes to the dichroic filter again which, with it's Nominal 45-degree angle of incidence, reflects the fluorescence light into a different direction.

[0050] The reflected fluorescence light then passes a pre-confocal lens which is used to focus fluorescent light to pass through a pinhole. The pre-confocal lens is a color corrected lens with a focal length $f=40$ mm and a numerical aperture $N.A.=0.10$. The
10 Pinhole is a spatial filter to create confocal detection volume with a $80\text{ }\mu\text{m}$ diameter.

[0051] After the pinhole a post-confocal lens is positioned. It is used to re-focus fluorescent light to infinity. It is color corrected lens as well with a focal length $f=12$ mm and a numerical aperture $N.A.=0.10$.

[0052] The Detection Path after the De-Multiplexer, meaning the fluorescence multiplex
15 sorter, is shown graphically in Figure 2. Figure 1 highlights the separation of the focus rays, the remnants from excitation laser line, and Figure 2 highlights the separation of the two fluorescence light sources in the De-Multiplexer. A breakdown and description of major elements is as follows:

[0053] A second Dichroic optical filter separates entering light into the De-Multiplexer.
20 Remnants of laser lines are passed through while fluorescence light is reflected at nominally 45 degrees, caused by the nominal 45-degree angle of incidence. The transmission bands are again around 642 nm and 730 nm and the reflection bands for fluorescence light are minimally 660-710 nm, 760-830 nm.

[0054] The subsequent focus photodiode positioned behind the second dichroic optical
25 filter works as follows: Excitation light from the laser passed through the dichroic and is aligned on photodiode plane. Its source is the reflection of laser lines from the objective focus, such as at bottom of well plate plane. Significant portions of light will reach the photodetector and may be used for peak finding and auto-focusing to the bottom of the well plate.

30 [0055] Next in the line of the fluorescence light are two Bandpass filters. The fluorescence light is reflected from the second dichroic filter and will contain mixed spectra. The two bandpass filters are utilized to further separate the spectra. Therefore a

687/34 bandpass and a 780/30 bandpass are used. Upon incidence to the first bandpass, as shown left in Figure 2, the fluorescence of light from one band is passed, and the other band is reflected. The reflected band continues until it reaches the second bandpass, as shown right in Figure 2, and is passed. The Bandpass positions are
5 interchangeable but preferably the 780/30 bandpass is in position 1, and the 687/34 bandpass is in position 2.

[0056] After each bandpass is a fiber focus lens installed for coupling the fluorescence light into an optical fiber. It is a color corrected lens, with a focal length $f=10$ mm and a numerical aperture $N.A.=0.10$.

10 [0057] The optical fibers are realized in form of fiber patch cables. After each fiber focus lens is a fiber located by typically a ferrule (eg. FC/PC type), to individually couple the fluorescently focused light to the detector.

[0058] The Detector are used for the signal detection and are preferably a photodetector, avalanche photodiode, or photomultiplier tube. Their detection results will
15 afterwards be analyzed by a computer which also provides the information to any user and/or stores it in a respective database.

[0059] To further provide understanding of the light path for excitation and detection, meaning the path of the excitation laser lines and detection fluorescence light, two further optical ray diagrams are provided in Figures 3 and 4.

20 [0060] A ray diagram describing the excitation path is shown in Figure 3. The dichroic is not shown here as it does not substantially affect excitation path rays.

[0061] Another ray diagram describing the detection path is shown in Figure 4. The De-Multiplexer components including dichroic and bandpass filters are not shown for simplicity. A single detection path is followed to the fiber lens and focus to fiber at the
25 leftmost portion of the figure.

[0062] The invented detection system has several advantages over the known prior art:

[0063] First: Sensitive detection of single molecules in solution. The confocal design is optimized for detection of rare, single molecule labels by way of rejection of background from the buffer and other sources. These next generation optical filter designs were
30 optimized for high signal-to-noise for each detection band.

[0064] Second: 2-PLEX detection capability. A 2-channel laser and 2-channel detection sorter enable measurement of 2 independent fluorescent labels, and thus biomarkers, in solution.

[0065] Third: Fiber coupled excitation and detection systems. The excitation and
5 detection paths are fiber-coupled, enabling streamlined servicing and replacement of components in the field, without resorting to time-consuming optical alignments.

CLAIMS

1. A detection system for detecting protein concentrations in a prepared assay solution consisting of several components, arranged after each other, the system comprising a fiber coupled laser to thereby emit laser light, a laser tube lens to thereby focus the laser light, a laser cleanup filter to thereby block non-laser wavelengths, a first dichroic filter to thereby pass the laser stream and reflecting fluorescence light, an objective lens to thereby focus the laser light to a small excitation volume to thereby collect fluorescence light emitted from a sample, a pre-confocal and a post-confocal lens with a pinhole positioned between them to focus and re-focus the fluorescent light, a second dichroic filter, a focus photodiode to thereby auto-focus on the well plate sample holder, at least one bandpass filter to thereby filter the spectrum of the fluorescent light, at least one fiber focus lense to thereby couple the fluorescent light to an optical fiber, at least one fiber patch cables to thereby couple fluorescently focused light to at least one computer-based detector to thereby detect signals in the fluorescent light, wherein the sample is located after the objective lens, the second dichroic filter, the focus photodiode and the at least one bandpass filter are forming a De-Multiplexer for the incoming fluorescence light streams and the at least one bandpass filter, at least one fiber focus lense, at least one fiber patch cables and at least one computer-based detector always consist of same the number and one of each of them are located along a respective fluorescence light stream.
2. The System according to claim 1, wherein the De-Multiplexer is a detection multiplex sorter for fluorescence light streams, which is configured to take light from two fluorescent

regions in the light stream and splits the light according to its color / wavelength.

- 5 3. The System according to claim 1, wherein
two Bandpass filters are used to separate the spectra of the
incoming fluorescence light with the first Bandpass filter being
positioned in front of a first Fiber focus lense and the second
Bandpass filter in front of a second Fiber focus lense.
- 10 4. The System according to claim 3, wherein
the first bandpass filter is a 780/30 bandpass and the second
bandpass filter is a 687/34 bandpass.
- 15 5. The System according to claim 1, wherein
the dichroic filters are positioned in a 45-degree angle of incidence
and reflect the fluorescence light stream while the laser light
streams are transmitted, wherein the transmission bands are for
wavelengths of around 642 nm and 730 nm, while the Reflection
bands for fluorescence are between 660 nm and 710 nm and
20 between 760 nm and 830 nm.
- 25 6. The System according to claim 1, wherein
the Fiber coupled laser emits laser lines of 642 nm and 730 nm
wavelength.
- 30 7. The System according to claim 1, wherein
the at least one computer-based detector either comprises of at
least one sensor receiving the fluorescence light stream which is
connected to a computer which computes the protein
concentrations by analyzing the signal from the at least one sensor.

8. The System according to claim 1, wherein
the laser tube lens is a color corrected lens with a focal length (f) of
40 mm and a numerical aperture (NA) of 0.10 and/or the objective
lens is a color corrected lens with a focal length (f) of 5 mm and a
numerical aperture (NA) of 0.58 and/or the pre-confocal lens is a
color corrected lens with a focal length (f) of 40 mm and a numerical
aperture (NA) of 0.10 and/or the post-confocal lens is a color
corrected lens with a focal length (f) of 12 mm and a numerical
aperture (NA) of 0.10 and/or the at least one fiber focus lense is a
color corrected lens with a focal length (f) of 10 mm and a numerical
aperture (NA) of 0.10.
9. The System according to claim 1, wherein
the pinhole is a spatial filter to create confocal detection volume with
a diameter of 80 μm .
10. The System according to claim 1, wherein
between the fiber coupled laser and the laser tube lens a turning
mirror is positioned for turning the laser lines into a specific
direction.
11. A method for detecting protein concentrations in a prepared assay
solution comprising the following steps:
- Emitting laser line excitation with a fiber coupled laser.
 - Focusing the laser lines into a laser stream using a laser tube lens.
 - Blocking non-laser wavelengths with a laser cleanup filter.
 - Passing the laser stream through a first dichroic filter and focusing to
the sample using an objective lens.
 - Collecting fluorescence light emitted from a sample with the protein
concentrations in the prepared assay solution by using an objective,

wherein the sample is excited by the laser stream and an objective lens is used to focus the laser stream to a small excitation volume.

- Passing the collected fluorescence light through the objective and to the first dichroic filter and reflecting it.
 - 5 • Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal detection volume in the fluorescent light positioned between them.
 - Separating the fluorescence light from other components using a second dichroic filter.
 - 10 • Performing peak finding in the laser stream and auto-focusing with a focus photodiode positioned behind the second dichroic filter.
 - Filtering the fluorescent light spectrum using at least one bandpass filter.
 - Coupling the fluorescent light to an optical fiber patch cable using at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector.
 - 15 • Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable.
 - 20 •
12. The method of claim 11, wherein
- the laser lines are turned into a rectangular direction away from the fiber coupled laser by using a turning mirror which is positioned
- 25 between the fiber coupled laser and the laser tube lens.
13. A Computer program comprising instructions which cause the involved computers to control the respective configured hardware components to carry out the following method steps:
- 30 • Turn on laser excitation from the fiber coupled laser to emit laser light.

- Focusing the laser lines into a laser stream using a laser tube lens.
- Blocking non-laser wavelengths with a laser cleanup filter.
- Passing the laser stream through a first dichroic filter and focusing to the sample using an objective lens.
- Collecting fluorescence light emitted from a sample with the protein concentrations in the prepared assay solution by using an objective, wherein the sample is excited by the laser stream and an objective lens is used to focus the laser stream to a small excitation volume.
- Passing the collected fluorescence light through the objective and to the first dichroic filter and reflecting it.
- Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal detection volume in the fluorescent light positioned between them.
- Separating the fluorescence light from other components using a second dichroic filter.
- Performing peak finding in the laser stream and auto-focusing with a focus photodiode positioned behind the second dichroic filter.
- Filtering the fluorescent light spectrum using at least one bandpass filter.
- Coupling the fluorescent light to an optical fiber patch cable using at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector.
- Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable.

14. A Computer-readable storage medium and/or data carrier signal having stored thereon the computer program of claim 13 which cause the involved computers to control the respective configured hardware components to carry out the following method steps:
- 5 • Turn on laser excitation from the fiber coupled laser to emit laser light. Focusing the laser lines into a laser stream using a laser tube lens.
 - Blocking non-laser wavelengths with a laser cleanup filter.
 - Passing the laser stream through a first dichroic filter and focusing
10 to the sample using an objective lens.
 - Collecting fluorescence light emitted from a sample with the protein concentrations in the prepared assay solution by using an objective, wherein the sample is excited by the laser stream and an objective lens is used to focus the laser stream to a small
15 excitation volume.
 - Passing the fluorescence light through the objective and to the first dichroic filter and reflecting it.
 - Focusing and re-focusing the reflected fluorescent light using pre- and post-confocal lenses with a pinhole for creating a confocal
20 detection volume in the fluorescent light positioned between them.
 - Separating the fluorescence light from other components using a second dichroic filter.
 - Performing peak finding in the laser stream and auto-focusing with a focus photodiode positioned behind the second dichroic
25 filter.
 - Filtering the fluorescent light spectrum using at least one bandpass filter.
 - Coupling the fluorescent light to an optical fiber patch cable using
30 at least one fiber focus lens and transmitting the fluorescently focused light via the at least one fiber patch cable to at least one computer-based detector.

- Computing the protein concentrations signal using at least one computer-based detector coupled to the optical fiber via a fiber patch cable.