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(54) **Title**: FLUORESCENCE FILTERING SYSTEM AND METHOD FOR MOLECULAR IMAGING

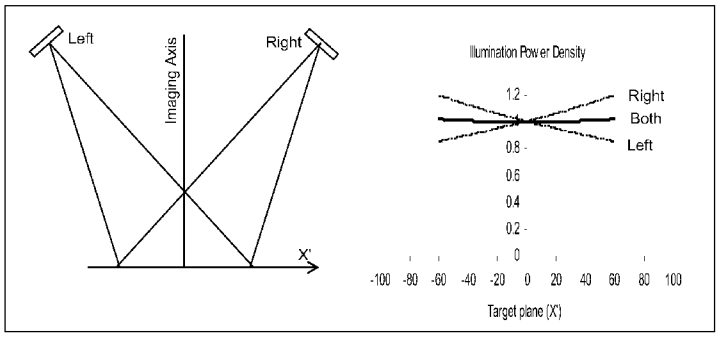


Figure 18a

(57) **Abstract**: An optical system is disclosed that can be used for fluorescence filtering for molecular imaging. In one preferred embodiment, a source subsystem is disclosed comprising a light source and a first set of filters designed to pass wavelengths of light in an absorption band of a fluorescent material. A detector subsystem is also disclosed comprising a light detector, imaging optics, a second set of filters designed to pass wavelengths of light in an emission band of the fluorescent material, and an aperture located at a front focal plane of the imaging optics. A telecentric space is created between the light detector and the imaging optics, such that axial rays from a plurality of field points emerge from the imaging optics parallel to each other and perpendicular to the second set of filters

WO 2009/142976 A2

FLUORESCENCE FILTERING SYSTEM AND METHOD FOR MOLECULAR IMAGING

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 61/055,885, filed on May 23, 2008 and U.S. Patent Application No. 12/191,976 filed August 14, 2008. The disclosures of both applications are hereby incorporated by reference in their entireties for all purposes.

BACKGROUND

[0002] The present invention relates generally to optical imaging, and more particularly to systems and methods for providing uniform illumination for optical imaging applications such as fluorescence imaging .

[0003] A fluorescence optical system illuminates a fluorophore-labeled target with light whose wavelength content falls within the absorption band and collects light whose wavelength content is in the emission band. An emission filter placed in front of a detector filters light that is not in the emission band. One challenge with emission filters is that unwanted photon rejection depends on the angle at which light traverses the filter. Specifically, as the angle of incidence increases, the transmission/reflection of the filter shifts to lower wavelengths. Accordingly, even if the field of view is a single point that provides an axial ray at a 0 degree angle, other rays of the same light beam will pass through the filter at non-0 degree angles and, accordingly, may experience different amounts of filtering.

[0004] This situation is addressed in Hwang et al., "The influence of improved interference filter performance for molecular imaging using frequency domain photon migration measurements," Optical Tomography and Spectroscopy of Tissue VI, SPIE vol. 5693, pp. 503-512. Hwang et al. describes an optical system in which a collimator is placed between imaging optics and an emission filter. The collimator ensures that all rays in a light beam originating from a certain point in the image field will pass through the filter at a 0 degree angle and, thus, will receive the same type of filtering. However, if a relatively large field of view is used, light beams emanating from the edge of the field, while still collimated, will

pass through the filter at an angle. This results in different amounts of excitation leakage across the field.

[0005] There is a need, therefore, for a fluorescence filtering method and system that will overcome this problem.

[0006] Imaging systems targeting quantitative measurement applications such as fluorescence imaging also typically require uniform illumination of the targeted area. The various types of illumination methods in use today share a common set of limitations: they generally suffer from low efficiency and/or produce 'hot' areas, typically in the center of the field of view. Some methods use light sources that emit into a large angular extent in order to improve the uniformity within the usable smaller field of view. These methods tend to suffer from low overall efficiency, not so great uniformity, and produce a significant amount of stray light that can present other challenges to obtaining good quality imaging. Other methods take the approach of using more condensed illumination techniques, such as using light-guides, that can be more efficient but do not generally produce uniform illumination.

[0007] One other factor that plays an important role in how well an illumination method will work is the type of light source being used. Broadband sources, such as lamps and LEDs, which tend to have a broad angular extent, lend themselves more towards uniformity than efficiency. Laser sources, on the other hand, have limited angular extent and therefore can be managed more efficiently but present more challenges to producing uniform illumination. For fluorescence imaging applications, laser illumination offers a number of advantages over broadband sources and is typically the preferred type of source to use.

[0008] Accordingly, there is also a need for efficient ways of producing uniform laser illumination, especially for fluorescence imaging applications.

BRIEF SUMMARY

[0009] Various embodiments described herein relate to an optical system that can be used for fluorescence filtering for molecular imaging. In one embodiment, a source subsystem is disclosed comprising a light source and a first set of filters designed to pass wavelengths of light in an absorption band of a fluorescent material. A detector subsystem is also disclosed comprising a light detector, imaging optics, a second set of filters designed to pass wavelengths of light in an emission band of the fluorescent material, and an aperture located at a front focal plane of the imaging optics. A telecentric space is created between the light

detector and the imaging optics, such that axial rays from a plurality of field points emerge from the imaging optics parallel to each other and perpendicular to the second set of filters. Other embodiments are provided, and each of the embodiments described herein can be used alone or in combination with one another.

[0010] The present invention also provides systems and methods for providing uniform illumination for optical imaging applications. Embodiments are particularly useful for fluorescence imaging applications.

[0011] Various embodiments provide highly efficient and uniform methods of illumination. The methods are particularly suitable for wide-field fluorescence imaging with laser excitation. When coupled with the field-uniform filtering methods described herein, these methods produce field independent quantitative fluorescence measurement.

[0012] According to one aspect, an illumination system is provided that typically includes a sample region defining an optical detection axis, a first illumination module configured to generate a first illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the first module is positioned such that the first illumination pattern impinges on the sample region at an angle relative to the optical detection axis. The system also typically includes a second illumination module configured to generate a second illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the second module is positioned such that the second illumination pattern impinges on the sample region at said angle relative to the optical detection axis, and wherein the component of the second module is symmetrically positioned around said optical detection axis relative to the component of the first module. In a typical operation, a power density of the cumulative illumination of the first and second illumination patterns on at least a portion of the sample region is substantially uniform across that portion of the sample region.

[0013] According to another aspect, an illumination system is provided that typically includes a sample region defining an optical detection axis, and an illumination module configured to generate an illumination pattern and having one or more components configured and positioned such that the illumination pattern impinges on at least a portion of the sample region at an angle relative to the optical detection axis and with a power density that is substantially uniform across the illuminated portion of the sample region.

[0014] According to yet another aspect, an illumination system is provided that typically includes a sample region defining an optical detection axis, a dichroic mirror element positioned along the optical detection axis, and an illumination module configured to generate an illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the first module is positioned relative to the dichroic mirror element such that the illumination pattern is redirected by the dichroic mirror element along the optical detection axis and such that the illumination pattern impinges on at least a portion of the sample region with a power density that is substantially uniform across the illuminated portion of the sample region.

[0015] According to yet another aspect, an illumination system is provided that typically includes a sample region defining an optical detection axis, and a plurality, N, of illumination modules, each configured to generate an illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of each module is positioned such that the illumination pattern impinges on the sample region at an angle relative to the optical detection axis, and wherein the components of the modules are spaced around said optical detection axis such that a power density of the cumulative illumination of the illumination patterns on at least a portion of the sample region is substantially uniform across that portion of the sample region.

[0016] Reference to the remaining portions of the specification, including the drawings and claims, will realize other features and advantages of the various embodiments. Further features and advantages of the various embodiments, as well as the structure and operation of various embodiments, are described in detail below with respect to the accompanying drawings. In the drawings, like reference numbers indicate identical or functionally similar elements.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figures 1A and 1B are graphs showing wavelength shifting of a band-pass filter due to incident angular variation.

[0018] Figure 2 is an illustration of an optical arrangement in which an emission filter is placed in front of imaging optics.

[0019] Figure 3 is an illustration of an optical arrangement in which an emission filter is placed between the imaging optics and a detector.

[0020] Figure 4 is an illustration of an optical arrangement using a collimator.

[0021] Figure 5 is an illustration of a detector system of a preferred embodiment.

[0022] Figure 6 is an illustration of a detector system with a filter wheel of a preferred embodiment.

[0023] Figure 7 is an illustration of a fluorescence filtering system of a preferred embodiment.

[0024] Figure 8 is an illustration of a fluorescence filtering system of another preferred embodiment.

[0025] Figure 9 is a graph of transmission curves for excitation and emission filters of a preferred embodiment.

[0026] Figure 10 is a graph showing the same data as in Figure 9 but in log scale.

[0027] Figure 11 is a graph showing reduction in residual leakage using the filtering architecture shown in Figure 7.

[0028] Figure 12 is a graph showing a horizontal cross-section from a fluorescence image obtained with a prototype system of a preferred embodiment with one band-pass filter placed in front of the lens at T = 5 s.

[0029] Figure 13 is a graph showing a horizontal cross-section from a fluorescence image obtained with a prototype system of a preferred embodiment with one band-pass filter placed behind the lens at T = 5 s.

[0030] Figure 14 is a graph showing a horizontal cross-section from a fluorescence image obtained with a prototype system of a preferred embodiment at T = 5 s.

[0031] Figure 15 is a graph showing a horizontal cross-section from a fluorescence image obtained with a prototype system of a preferred embodiment at T = 120 s.

[0032] Figure 16 illustrates illumination of a target plane with a non-normal impinging illumination beam as well as the illumination power density gradient for the target plane.

[0033] Figure 17 illustrates power density angular components useful for computing power density along an angular plane.

[0034] Figures 18a and b illustrate one embodiment of an illumination system having two symmetrically located (about an optical detection or imaging axis) illumination modules as well as the illumination power density gradients for both sources on the target plane and the cumulative power density on the target area.

[0035] Figures 19a-c illustrate the power density for illumination from different sides of an optical imaging axis and cumulative power density on the target area.

[0036] Figure 20 illustrates another embodiment using a single illumination source with components configured to compensate for the angular incidence of the illumination beam on the target plane.

[0037] Figure 21 illustrates another embodiment using a single illumination source configured to match the aspect ratio of the field of view when using a dichroic mirror element to direct the illumination along the imaging axis.

DETAILED DESCRIPTION

[0038] Fluorescence detection is a tool for molecular imaging. It enables researchers to detect particular components of complex bio-molecular assemblies, such as in live cells. Fluorescence is a photo-physical process that involves the interaction of light with certain molecules called fluorophores or fluorescent dyes. It consists of the absorption of light energy at the appropriate wavelength by such molecules and the subsequent emission of other light photons at longer wavelengths. The wavelength ranges that a fluorophore molecule can absorb and emit at are called absorption and emission bands, respectively.

[0039] A fluorescence optical system illuminates a fluorophore-labeled target with light whose wavelength content falls within the absorption band and collects light whose wavelength content is in the emission band. The source(s) and optics that generate the illumination part of the system are called the "excitation optics," and the optics used to collect the fluorescence emission are called the "emission optics." Since it is rarely possible to find a light source that has a spectral content (i.e., wavelength range) that exactly matches every fluorophore absorption band, special optical filters (usually band-pass filters) are used along with the light sources to limit the range of illuminating wavelengths to that of the absorption band and not the emission band. At the same time, other filters are used in the emission path to allow light with wavelengths in the emission band only to reach the detector.

[0040] The task of a fluorescence optical system design is to make sure that photons with wavelengths in the absorption band only reach the target, and photons with wavelengths in the emission band only reach the detector. If not, photons from the light source will wrongly be considered as fluorescence, and, therefore, a wrong measure of the amount of fluorophore dye results. This can be a tough task if the amount of emitted fluorescence is much less than the amount of excitation light scattered by the target surface (i.e., not absorbed). This is usually the case for in-vivo imaging, such as in small animal imaging, since there are a number of challenges to achieving good signal-to-noise performance when imaging fluorescence targets deep inside small animals.

[0041] One challenge is that the amount of excitation light that reaches the inside of an animal is usually quite low because of the significant absorption and scattering caused by the various body parts (skin, muscle, fat, bone, etc.). For example, the transmission through "shaved skin + fat layer + whole rib cage + abdominal wall" is in the order of 10^{-6} and varies with the thickness and composition of each of those parts. The emitted fluorescence will have to traverse a comparable tissue path back up towards the detection system. Thus, the level of fluorescence is $<<10^{-12}$ times that of the excitation signal. So, for example, if a flux density of $1mW/cm^2$ impinges upon the outside of a mouse or other small animal, only a sub-nano Watt optical signal actually reaches dye-labeled cells inside the abdomen, and, in turn, only sub-femto Watt of fluorescence signal reaches the detector. The low amount of emitted fluorescence is further reduced by absorption and scattering as it makes its way out towards the detector. This means that the scattering from the excitation light that occurs at the outer parts of the animal can cause much higher levels than the fluorescence signal itself. At the same time, existing optical filter technology (e.g., thin-film emission filters, such as multi-cavity designs) can, at best, provide rejection of unwanted photons only in the order of OD6 (10^{-6}). So, standard fluorescence methods would allow through high non-fluorescent background levels and, in turn, result in low Signal-to-Background (SBR) and Signal-to-Noise (SNR) ratios.

[0042] Another challenge is that unwanted photon rejection also depends on the angle at which light traverses the filter, as the spectral properties of optical thin film filters vary with the angle of incidence of light. Specifically, as the angle of incidence increases, the transmission/reflection of the filter shifts to lower wavelengths ("blue shift"). This shift can be described by

$$\lambda(\theta) = \lambda_o \sqrt{1 - \left(\sin\theta / \overline{n}\right)^2}$$

where θ is the angle deviation from the normal to the filter, and $\overline{n}$ is the effective index of refraction of the thin-film. The value of $\overline{n}$ is typically in the range of 1.5 to 2.5 and varies with polarization.

[0043] Figures 1A and 1B are graphs (transmission and transmission (dB), respectively) showing wavelength shifting of a band-pass filter due to a varying angle of incidence (0, 10, and 20 degrees). As shown in these graphs, as the angle of collected light increases relative to the normal to the filter, the effective transmission band shifts to lower wavelengths, and the amounts of transmitted fluorescence and background signals change accordingly. Light from the target spans a significant range of field angles when a relatively large field of view is imaged, such as in the case of small animal imaging. Therefore, in small animal imaging where a relatively large field of view is imaged, the resulting emission filtering (i.e., transmitted SBR) is non-constant across the image. Accordingly, it is desired to use special spectral filtering solutions in order to improve the rejection of non-fluorescence light across the whole field of view (i.e., where light is collected at different angles).

[0044] Many current area fluorescence imaging techniques use the same excitation and emission filters designed for microscopy and scanning systems and use arrangements where the emission filter 5 is placed in front of the imaging optics 10 (as in Figure 2) or behind it (as in Figure 3, where the emission filter 5 is between the imaging optics 10 and the detector 15 (here, a CCD)). (The horizontal lines from which the emission is originating in these and other figures herein represent a target, such as mouse or other small animal.) These filters are typically multi-cavity interference filters optimized for maximum rejection in the excitation band and maximum transmission in the emission band. As discussed earlier, the spectral properties of such filters vary with the angle of incidence of light. Because, in Figure 2, the axial ray 20 (i.e., the "chief" or center ray of a light beam) of light beam 25 is at a 0 degree angle to the filter 5, while the axial ray 30 of light beam 35 exceeds a 10 degree angle of incidence onto filter 5, the filter 5 will provide different photon rejection characteristics of the axial rays 20, 30. This is also true in the arrangement in Figure 3. In Figure 3, the filter 5 is behind the imaging optics 10. Because the pupil plane (i.e., the plane at which axial rays of all light beams cross) is in the center of the imaging optics 10, the axial ray passes through the imaging optics 10 without changing direction. Accordingly, the filter 5 in Figure 3, like

the filter 5 in Figure 2, will provide different photon rejection characteristics of the axial rays 20, 30. Accordingly, in both arrangements, the angular spectral dependence of the filter 5 results in a significant amount of excitation leakage that both limits the achievable SBR and is non-constant across the image.

[0045] It should be noted that, even in the instance where the axial ray 20 passes through the filter 5 at a 0 degree angle, other rays of the light beam 25 pass through the filter 5 at a non-0 degree angle. Accordingly, even if the field of view is a single point that provides an axial ray at a 0 degree angle, other rays of the same light beam will pass through the filter 5 at non-0 degree angles and, accordingly, may experience different amounts of filtering by the filter 5 due to the angular spectral dependence problem.

[0046] This situation is addressed in Hwang et al., "The influence of improved interference filter performance for molecular imaging using frequency domain photon migration measurements," Optical Tomography and Spectroscopy of Tissue VI, SPIE vol. 5693, pp. 503-512. Figure 4 is an illustration of the arrangement disclosed in Hwang et al. As shown in Figure 4, a collimator 40 is placed between imaging optics 45 and band-pass and holographic filters 50, 55. (Hwang suggests the use of a holographic notch filter 55 to enhance the rejection capability of the band-pass filter 50.) A lens 60 focuses the light beams passing through the filters 50, 55 onto a CCD detector 65. The collimator 40 causes the rays of each of the light beams to exit the collimator 40 parallel to each other. As a result, unlike the situation noted above, if the field of view is a single point that provides an axial ray 70 through the filters 50, 55 at a 0 degree angle, other rays of the same light beam 75 will also pass through the filters 50, 55 at a 0 degree angle because of the effect of the collimator 40. However, as shown in Figure 4, if a relatively large field of view is used, a light beam 80 emanating from the edge of the field, while still collimated, traverses the filters 50, 55 at an angle. This is because the pupil plane is in the center of the imaging optics 45, and the axial ray 85 of light beam 80 passes through the imaging optics 45 without changing direction. Accordingly, light from different field points enter the filters 50, 55 at different angles and, therefore, results in different amounts of excitation leakage across the field.

[0047] Figure 5 is an illustration of a detector system 100 of a preferred embodiment that minimizes field dependence and maximizes the Signal to Background Ratio (SBR) performance of spectral filtering. The detector system 100 comprises a light detector 105 (such as a CCD), imaging optics 110 with an equivalent focal length F, a set of filters 115

positioned between the light detector 105 and the imaging optics 110, and an aperture 120 located at a front focal plane of the imaging optics 110. As used herein, the term "imaging optics" refers to one or more optical elements whose function collectively is to project a scene onto a detector (e.g., a sensor array) such as a CCD camera. Imaging optics can comprise a single lens if its placement allows it to project the picture of a given scene onto the detector. Imaging optics can also comprise two or more lenses together in such a way that they all work together to produce the same function (i.e., project the image of a scene onto a detector). The term "imaging optics" can be used interchangeably with the terms "imaging lens" and "imaging lens assembly." Further, imaging optics can include components other than lenses (e.g., mirrors). As also used herein, a "set" can include one or more than one member. Accordingly, a set of filters, for example, can contain a single filter or a plurality of filters. In this way, one can stack one or more filters to achieve the desired background rejection.

[0048] By locating the aperture 120 in front of the imaging optics 110, the pupil plane (i.e., the plane at which axial rays of all light beams cross) is not in the center of the imaging optics 110, and axial rays that hit the imaging optics 110 at non-0 degree angles will change direction when exiting the imaging optics 110. Further, because the pupil aperture 120 located at a front focal plane of the imaging optics 110, the pupil plane is in the front focal plane of the imaging optics 110, and a telecentric space is created between the imaging optics 110 and the light detector 105. This will cause the axial rays from a plurality of field points (i.e., locations in the imaged target) to emerge from the imaging optics 110 parallel to each other and perpendicular (i.e., at a 0-degree angle) to the set of filters 115. (A telecentric approach also eliminates otherwise unavoidable ghost images when the set of filters 115 comprises more than one filter.) As a result, each of the axial rays will receive the same filtering from the set of filters 115. While the non-axial rays of each light beam will hit the set of filters 115 at non-0 degree angles and, hence, be subject to varying filtering effects due to the angular dependence problem, such rays from each light beam will see the same effect. In other words, in the telecentric space, all the field points (light emanating from different parts of the image) traverse the set of filters 115 in the same manner, centered around the zero-degree angle. This minimizes the angular variation across the field and, thus, the resulting spectral filtering variation. Accordingly, unlike with the optical arrangement in Figure 4, light from different field points entering the set of filters 115 at different angles will result in substantially the same amount of excitation leakage across the field.

[0049] Figure 6 is an illustration of a detector system 200 of another preferred embodiment. This system 200 is similar to the system 100 in Figure 5, and common components are labeled the same. However, the system 200 in Figure 6 has an additional set of filters 210 in front of the imaging optics 110. Preferably, the set of filters 210 comprises one or more dichroic filters. This system 200 takes advantage of the fact that rays that traverse a filter placed in front of imaging optics at large angles will traverse a filter placed behind the imaging optics at smaller angles and vise versa. This has the effect of balancing out any residual leakage and, thus, flattening the field. Therefore, by placing the additional set of filters 210 in front of the imaging optics 110, the angular effect from the first set of filters 115 is balanced out more evenly across the field. Although not necessary, the additional set of filters 210 in this embodiment is located on a filter wheel 230 comprising at least one additional set of filters (not shown). Similarly, the set of filters 115 can be placed in a filter wheel 240 comprising at least one additional set of filters (not shown). This allows different "colors" of filters to image different labels.

[0050] Turning again to the drawings, Figure 7 is an illustration of a fluorescence filtering system 300 of another preferred embodiment. This system 300 comprises a source subsystem 310 comprising two light sources 320, 330, each with a set of filters 340, 350 designed to pass wavelengths of light in an absorption band of a fluorescent material. (As discussed above, a filter may leak wavelengths of light in other bands.) The system 300 also comprises a detector subsystem 360, identical to the detector system 200 in Figure 6 (components are labeled the same). Preferably, rejection performance of the set of excitation filters 340, 350 in the excitation paths matches the rejection performance of the set of emission filters 115. Since the detector 105 responds to all the photons that pass through the excitation as well the emission bands, the rejection by both the set of excitation and emission filters 115, 340, 350 is preferably matched so that leakage from the set of excitation filters 340, 350 in the emission band will have the same effect as a comparable leakage from the set of emission filters 115 in the excitation band. It should be noted that, while Figure 7 shows two light sources 320, 330, three or more light sources can be used. Also, the number of light sources does not have to match the number of sets of filters. For example, one can use one light source with one filter set and then split the output to act like separate sources. Alternatively, one can split the output to more than one port and put filter sets in front of each port.

[0051] Figure 8 is an illustration of an alternate system 400, in which a single source 410 is used with a dichroic splitter 420. The dichroic splitter 420 is positioned such that light from

the light source 410 illuminates a target and light emitted from the target reaches the detector 430. The dichroic splitter 420 also has filtering properties like the set of filters 210 in Figures 6 and 7. However, the advantage of using the set of filters 210 in Figures 6 and 7 is that they prevent any possible specular reflections from getting into the collection optics.

[0052] In one presently preferred embodiment of the system 300 shown in Figure 7, the detector is a Hamamatsu ORCA_AG detector, the imaging optics 110 is a Canon 50mm/ F2.0 lens, the set of emission filters 115 are Omega 822DF20 filters, and the second set of filters 210 is a Semrock 800LP filter, operating at a nominal zero-degree angle of incidence. The excitation sources preferably consist of two fiber-coupled, symmetrically-positioned identical laser diode sources (782nm) as the light sources 320, 300 and a set of two excitation filters 340, 350 in front of each laser 320, 330. Both excitation and emission filters have about OD6 rejection each.

[0053] Turning again to the drawings, Figure 9 is a graph showing transmission curves for the excitation and emission filters. Figure 10 shows the same data in log scale so that the rejection level can be better evaluated. Tests were conducted to confirm that rejection with a configuration of (2, 2) excitation and emission filter sets is better than (1, 1), (1, 2), and (2, 1) configurations. Of course, if further rejection is needed, one can use (3, 3), (4, 4), etc. Figure 11 is a graph showing reduction in residual leakage from the filtering architecture shown in Figure 7. A comparison between Figures 10 and 11 show the theoretical level of reduction in background leakage that can be achieved by doubling the rejection capability of both the excitation and emission filters.

[0054] Figures 12-15 show horizontal cross-sections from images obtained with the prototype system described above. The target is a nitro-cellulose membrane with 5 IRDye®800 labeled fluorescent spots. The membrane produces a significant amount of scattering from the excitation laser and is thus used to obtain a measure of the rejection capability of the filters and the flatness of the residual background. The cross-section is arbitrarily chosen to pass through a fluorescent spot located near the center of the image. Such fluorescent spot is used to measure the fluorescence transmission efficiency. This way, a measure of Signal-to-Background (SBR) can easily be obtained. In each figure, the graph is displayed in log-scale in order to enhance the levels of the background.

[0055] In Figures 12 and 13, only one emission filter was placed in front and in the back of the lens, respectively. This is similar to what is done in most prior small animal imaging

solutions. It also shows how the non-flatness of the background in both cases complements each other, and, therefore, by placing filters on both sides of the lens, a more balanced rejection is obtained. Figures 14 and 15 show the image with filters configured according to the preferred embodiment of Figure 7. In Figure 15, the exposure time is increased to 120s in order to enhance the detection of any residual background leakage. As is clear from the image, even though the fluorescent signal is much higher than saturation, the leakage is still flat and non-significant. The SBR improvement in this case is estimated to be about 30x.

Uniform Illumination

[0056] According to one embodiment, two illumination modules are arranged so that their outputs are symmetrical around a detection optical axis. In this embodiment, the cumulative illumination of the two modules provides a substantially uniform power density distribution across at least a portion of an illuminated sample or sample region. In certain aspects, the illumination modules include laser sources. Although it is understood that other illumination sources may be used, the remainder of this document will discuss various embodiments using laser modules. In one aspect, the output of the laser modules are substantially identical (and complementary as will be described further below). Each of the laser modules produces a uniform square pattern illumination as shown in Figure 16 (inset). A uniform square illumination pattern can be obtained, in one aspect, by using a diffractive diffuser such as an "Engineered Diffuser" provided by Thorlabs. Inset of Figure 16 shows typical uniformity produced by such diffusers. Alternative refractive means such as a combination of Powel lenses can also be used to redistribute the laser intensity profile to produce a uniform square pattern. When the illumination path of a substantially square pattern light source is intercepted by a target plane normal to the imaging optical axis, a power density with a gradient results as shown in Figure 16. The power density gradient is higher at the side closer to the illumination module and lower towards the other end.

[0057] It will be appreciated that the various embodiments produce illumination having a substantially rectangular pattern. One example is a substantially square-shaped pattern. In general, the optical elements producing the illumination pattern may create some rounding, or other edge effects, that will produce a pattern that is not fully rectangular. Also, effects from light impinging at an angle may also modify the pattern, upon intersection with a target plane, from being fully rectangular in shape.

[0058] With reference to Figure 17, the power density along the angular plane X' intersecting a uniform square illumination pattern can be described by the following relationship

$$P(X') = \frac{P_o}{[2tan\theta.Z_o]^2}\left[\frac{1}{1+\frac{sin\alpha}{Z_o}X'}\right]^2 \qquad -Z_o\frac{sin\theta}{cos(\theta+\alpha)} \le X' \le Z_o\frac{sin\theta}{cos(\theta+\alpha)},$$

where P_o is the optical power at the output of the diffuser, θ is half-divergence angle of the diffractive diffuser, Z_o is distance to the target plane, and α is the angle between illumination direction and target plane.

[0059] When two similar laser modules are placed at equal distances from the center of the targeted area and with equal but symmetrical angles from the imaging optical axis, each side produces an illumination with a gradient as shown in Figures 18a-b and Figures 19a-b. When both sides are used together, the cumulative power density that results is uniform as shown in Figures 18b and 19c. This embodiment has the added advantage of using angular illumination as a way to direct specular reflections away from the detection path. Although two symmetrically located light sources are shown, it should be appreciated that more than two light sources may be used. For example, multiple light sources symmetrically spaced around the imaging optical axis may be used (e.g., 2π/Nspacing - 3 sources with 120° spacing, 4 sources at 90° spacing, etc.). As another example, where pairs of sources are used, each source in a pair can be located 180° relative to each other around the imaging axis, but between pairs, the spacing around the imaging axis can be arbitrary.

[0060] It should be understood that the laser modules may each include many optical elements. In certain aspects, the last optical element or component, e.g., mirror, lens element, etc., of each laser module is symmetrically spaced around the imaging optical axis. Hence, other components of the laser module may be positioned as desired, with the final component arranged and positioned such that the illumination provided to the target plane impinges at an appropriate angle.

[0061] According to another embodiment, a single laser module may be placed off of the imaging optical axis and aimed at the target plane as shown in Figure 20. In this case, the diffractive diffuser is designed to produce a square pattern but with a brightness uniform when intercepted by a plane at the same geometry of the interception of the target plane. This embodiment also has the benefits of angular illumination and can have the further added advantage of cost and size savings. In certain aspects, therefore, it is desired to make the

pattern generated by each illumination module square in shape so that when they intercept the sample plane the resulting illuminated area is rectangular and matches the area being imaged.

[0062] According to yet another embodiment, a laser module is used that generates a square or rectangular uniform pattern that matches the aspect ratio of the field of view and a dichroic to combine the illumination path with the detection optical axis as shown in Figure 21. This has the advantage of normal illumination incidence onto the target plane. The uniform pattern can be generated by a diffractive diffuser as described above but with the appropriate aspect ratio.

[0063] Advantageously, the various embodiments provide an illumination uniformity of less than or equal to about +/-10% using either a diffractive diffuser or Powel lenses, and an efficiency of greater than about 85% using either a diffractive diffuser or Powel lenses. The efficiency can also be improved further by coating diffuser (lenses) with an anti-reflective optical coating.

[0064] There are several alternatives that can be used with these embodiments. For example, while embodiments have been illustrated above with respect to an application for fluorescence filtering for molecular imaging, these embodiments can be used in an suitable application. Accordingly, the filters do not have to be designed to pass wavelengths of light in absorption and emission bands of fluorescent materials. Also, while these embodiments were illustrated in terms of imaging a small animal, such as a mouse, they can be used to image other targets. Additionally, any suitable light source, detector, filter, imaging optics, and aperture can be used. Further, any of the embodiments disclosed herein can be used by itself or in combination with any of the other embodiments disclosed herein. Finally, each of the excitation filter sets can pass wavelengths in more than one excitation band, and emission filter sets can pass wavelengths in more that one emission band. Also, any of the sets of filters disclosed herein can be placed on a filter wheel.

[0065] While the invention has been described by way of example and in terms of the various embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. To the contrary, it is intended to cover various modifications and similar arrangements as would be apparent to those skilled in the art. Therefore, the scope of the appended claims should be accorded the broadest interpretation so as to encompass all such modifications and similar arrangements.

WHAT IS CLAIMED IS:

1. An illumination system, comprising:

a sample region defining an optical detection axis;

a first illumination module configured to generate a first illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the first module is positioned such that the first illumination pattern impinges on the sample region at an angle relative to the optical detection axis; and

a second illumination module configured to generate a second illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the second module is positioned such that the second illumination pattern impinges on the sample region at said angle relative to the optical detection axis, and wherein the component of the second module is symmetrically positioned around said optical detection axis relative to the component of the first module,

wherein a power density of the cumulative illumination of the first and second illumination patterns on at least a portion of the sample region is substantially uniform across that portion of the sample region.

2. The system of claim 1, wherein each of the first and second illumination modules include laser illumination sources.

3. The system of claim 1, wherein the first illumination module includes a pair of Powel lenses oriented so as to produce the substantially uniform rectangular-shaped illumination pattern.

4. The system of claim 1, wherein the first illumination module includes an engineered diffuser configured to produce the substantially uniform rectangular-shaped illumination pattern.

5. The system of claim 1,wherein each of the components of the first and second modules includes one of a mirror element, a lens element or a diffuser element.

6. The system of claim 1, wherein each of the first and second illumination patterns are substantially square-shaped.

7. The system of claim 1, wherein the first illumination module includes a diffractive diffuser configured to produce the substantially uniform rectangular-shaped illumination pattern.

8. An illumination system, comprising:

a sample region defining an optical detection axis; and

an illumination module configured to generate an illumination pattern and having one or more components configured and positioned such that the illumination pattern impinges on at least a portion of the sample region at an angle relative to the optical detection axis and with a power density that is substantially uniform across the illuminated portion of the sample region.

9. The system of claim 8, wherein the portion of the sample region, where the power density of the illumination is substantially uniform, substantially matches the field of view of an imaging system.

10. The system of claim 8, wherein the illumination module includes an engineered or diffractive diffuser configured to produce the substantially uniform illumination pattern at the field of view.

11. An illumination system, comprising:

a sample region defining an optical detection axis;

a dichroic mirror element positioned along the optical detection axis; and

an illumination module configured to generate an illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of the first module is positioned relative to the dichroic mirror element such that the illumination pattern is redirected by the dichroic mirror element along the optical detection axis and such that the illumination pattern impinges on at least a portion of the sample region with a power density that is substantially uniform across the illuminated portion of the sample region.

12. The system of claim 11, wherein the portion of the sample region, where the power density of the illumination is substantially uniform, substantially matches the field of view of an imaging system.

13. The system of claim 11, wherein the illumination module includes an engineered or diffractive diffuser configured to produce the substantially uniform illumination pattern at the field of view.

14. The system of claim 11, wherein the illumination module includes a set of Powel lenses configured to produce the substantially uniform illumination pattern at the field of view.

15. The system of claim 11, wherein the illumination pattern is substantially square-shaped.

16. The system of claim 1, wherein the first illumination pattern is substantially square-shaped, and wherein the second illumination pattern is substantially square-shaped.

17. The system of claim 8, wherein the illumination pattern is substantially square-shaped.

18. The system of claim 1, wherein the portion of the sample region, where the cumulative illumination is substantially uniform, substantially matches the field of view of an imaging system.

19. The system of claim 1, wherein the component of the second module is positioned about 180° around said optical detection axis relative to the component of the first module.

20. An illumination system, comprising:

a sample region defining an optical detection axis;

a plurality, N, of illumination modules, each configured to generate an illumination pattern that is substantially uniform and rectangular-shaped, wherein a component of each module is positioned such that the illumination pattern impinges on the sample region at an angle relative to the optical detection axis; and wherein the components of the modules are spaced around said optical detection axis such that a power density of the cumulative illumination of the illumination patterns on at least a portion of the sample region is substantially uniform across that portion of the sample region.

21. The system of claim 20, wherein the modules are equally spaced at 360/N° around the optical detection axis.

22. The system of claim 20, wherein the portion of the sample region, where the cumulative illumination is substantially uniform, substantially matches a field of view of an imaging system.

23. The system of claim 20, wherein the illumination pattern of each module is substantially square-shaped.

24. A fluorescence filtering system, comprising:

a source subsystem, comprising:

a light source; and

a first set of filters designed to pass wavelengths of light in an absorption band of a fluorescent material; and

a detector subsystem, comprising:

a light detector;

imaging optics;

a second set of filters positioned between the light detector and the imaging optics, the second set of filters designed to pass wavelengths of light in an emission band of the fluorescent material; and

an aperture located at a front focal plane of the imaging optics, wherein a telecentric space is created between the light detector and the imaging optics, such that axial rays from a plurality of field points emerge from the imaging optics parallel to each other and perpendicular to the second set of filters.

25. A detector system comprising:

a light detector;

imaging optics;

a set of filters positioned between the light detector and the imaging optics; and

an aperture located at a front focal plane of the imaging optics, wherein a telecentric space is created between the light detector and the imaging optics, such that axial

rays from a plurality of field points emerge from the imaging optics parallel to each other and perpendicular to the set of filters.

26. A method for fluorescence filtering, the method comprising:

(a) illuminating a target comprising a fluorescent material with light in an absorption band of the fluorescent material, wherein, in response to absorbing the light in the absorption band, the fluorescent material emits light in an emission band of the fluorescent material;

(b) causing axial rays of light beams from a plurality of field points in the target to emerge from imaging optics parallel to each other and perpendicular to a set of filters designed to pass wavelengths of light in the emission band; and

(c) detecting light passed through the set of filters.

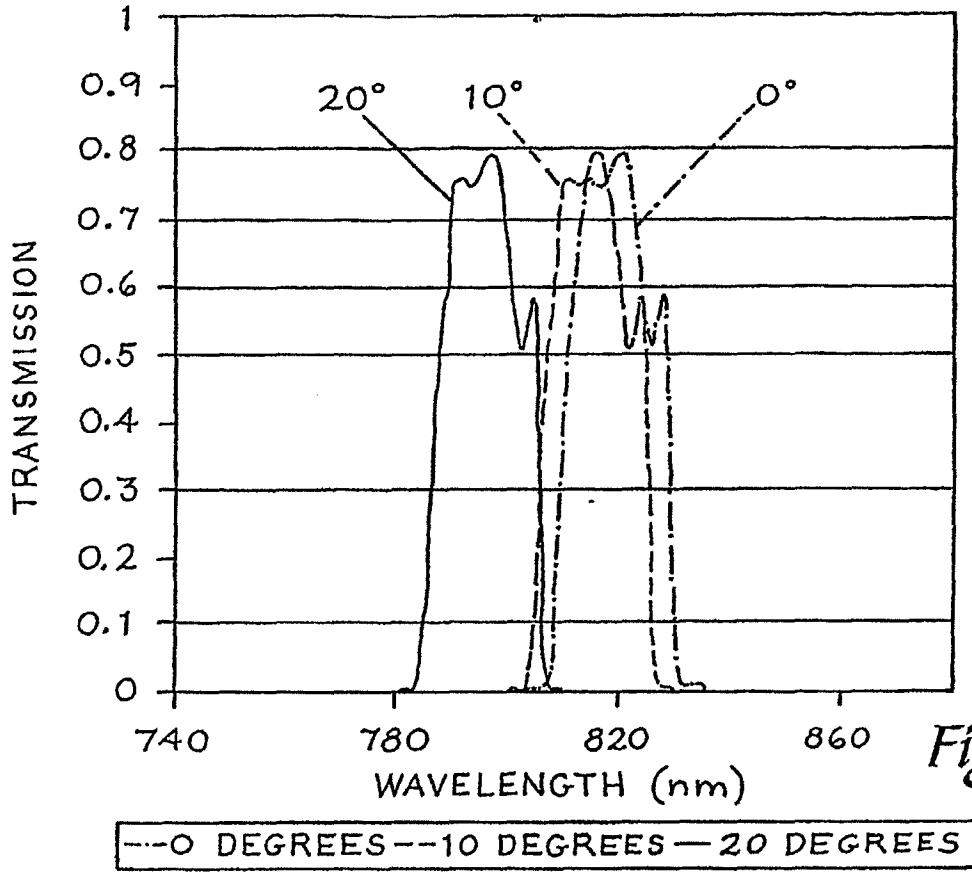

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
TRANSMISSION
20°
10°
0°
740
780
820
860
WAVELENGTH (nm)
---0 DEGREES--10 DEGREES—20 DEGREES

Fig. 1A

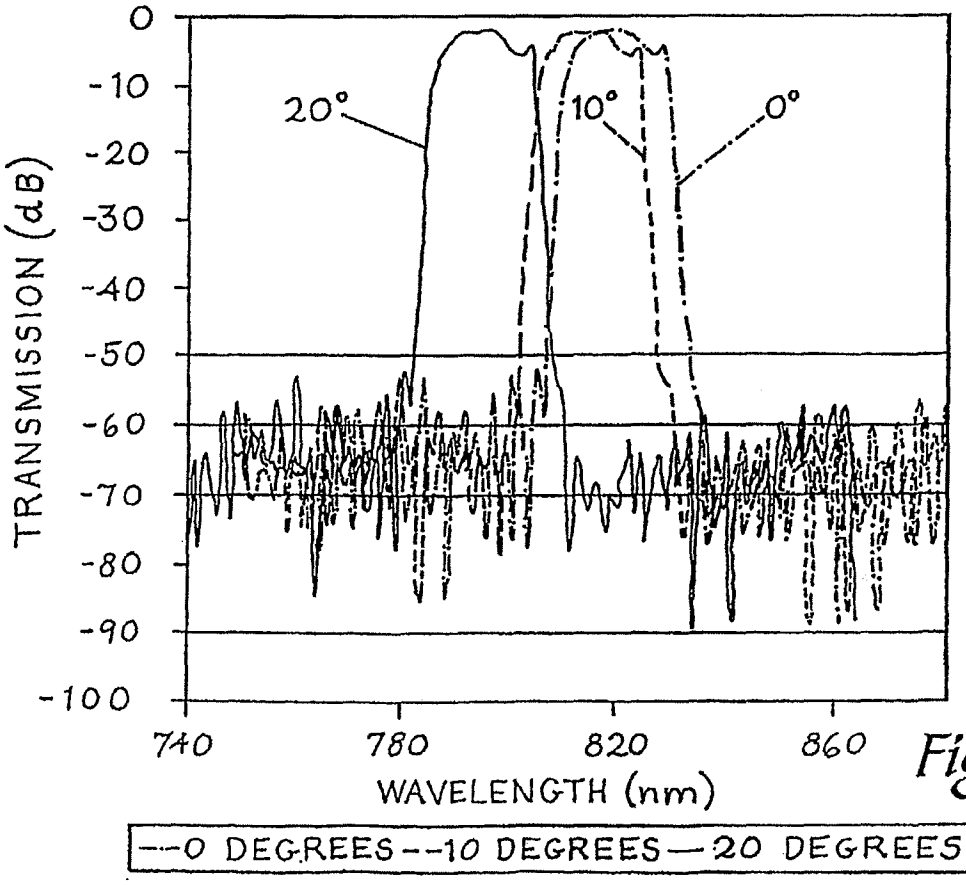

0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
TRANSMISSION (dB)
20°
10°
0°
740
780
820
860
WAVELENGTH (nm)
---0 DEGREES--10 DEGREES—20 DEGREES

Fig. 1B

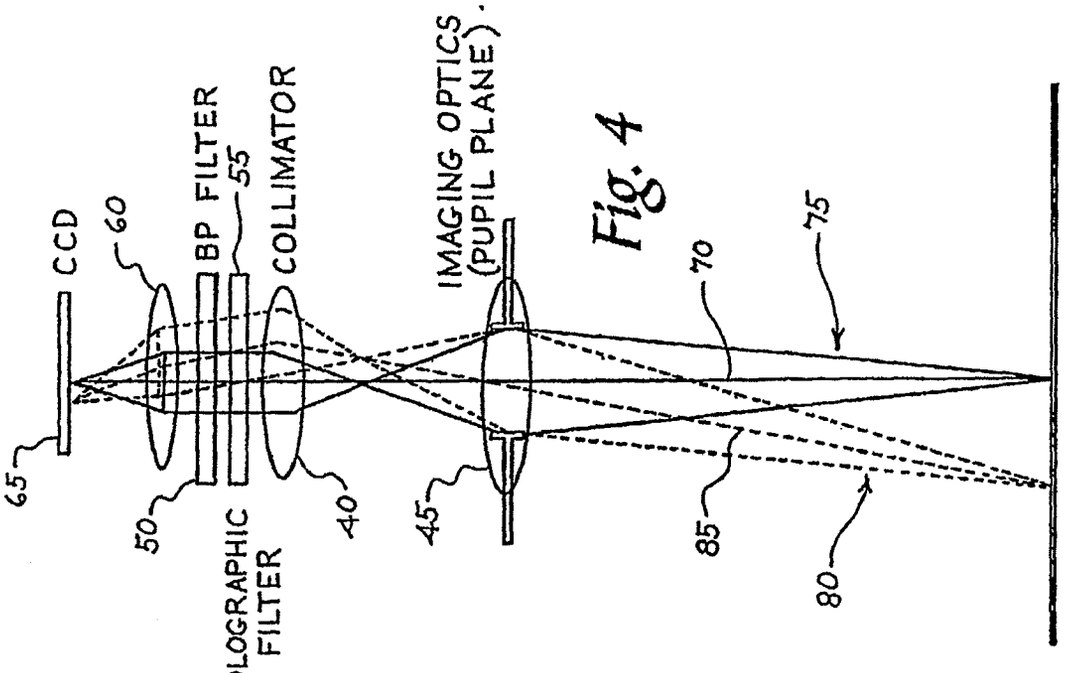


Fig. 4

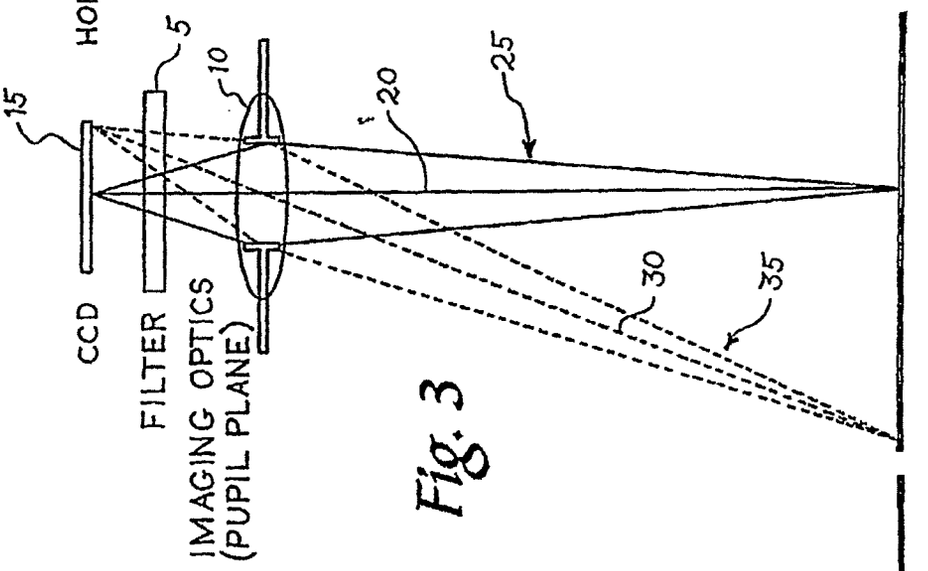


Fig. 3

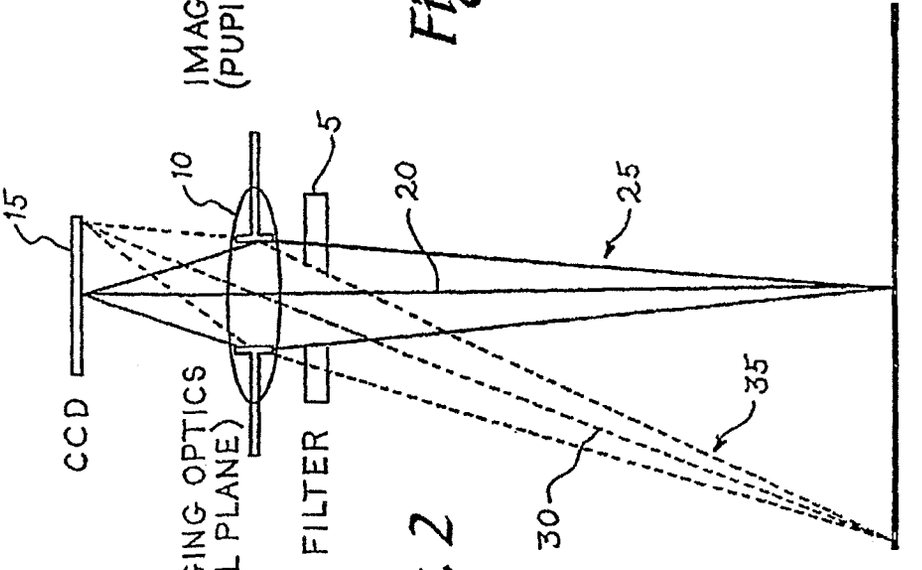


Fig. 2

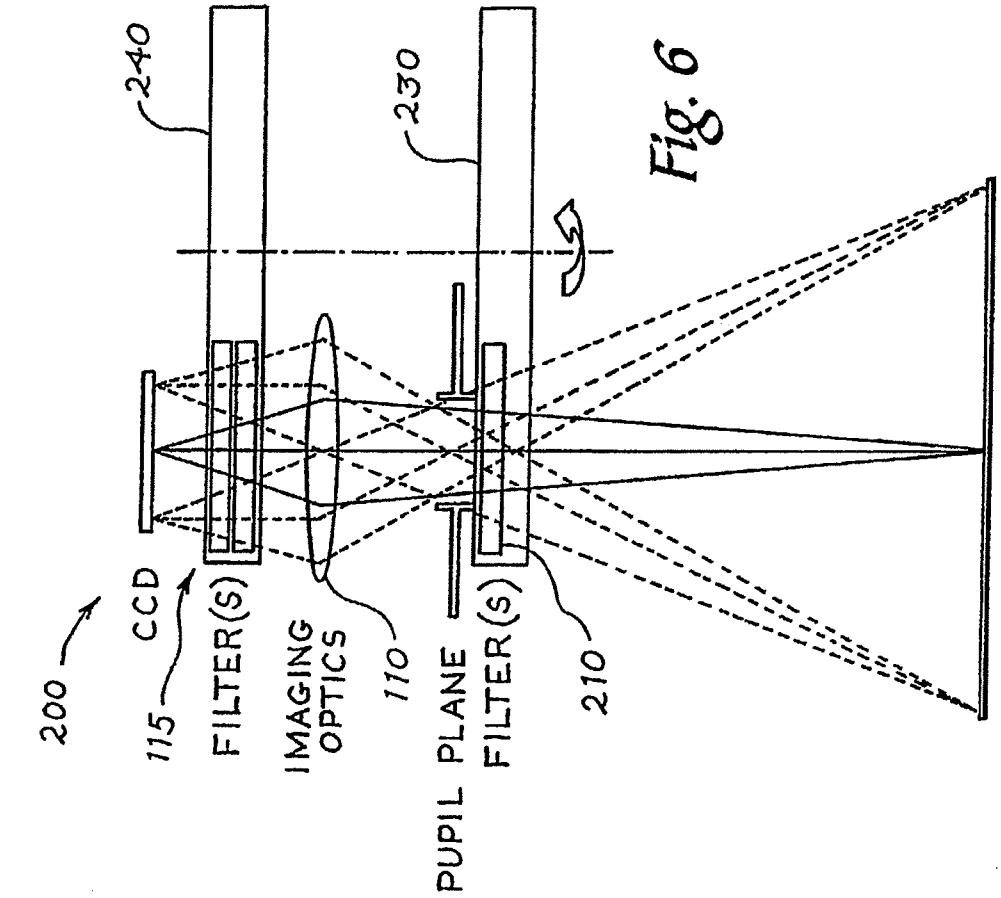

240
230
200
CCD
115
FILTER(S)
IMAGING OPTICS
110
PUPIL PLANE
FILTER(S)
210

Fig. 6

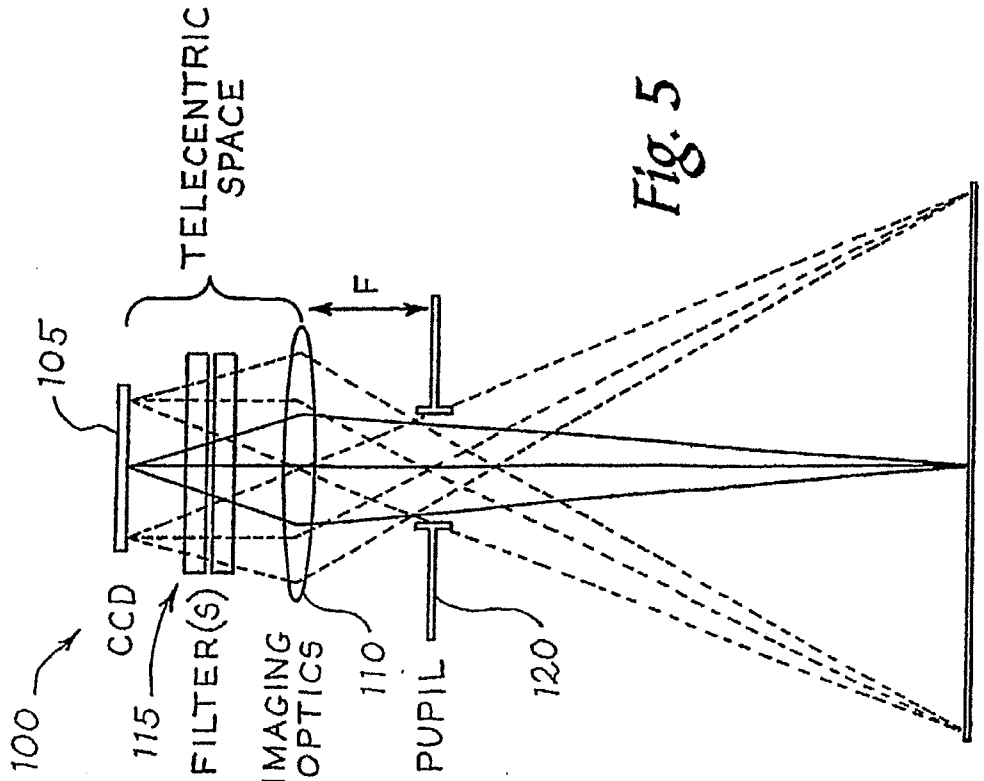

TELECENTRIC SPACE
F
105
100
CCD
115
FILTER(S)
IMAGING OPTICS
110
PUPIL
120

Fig. 5

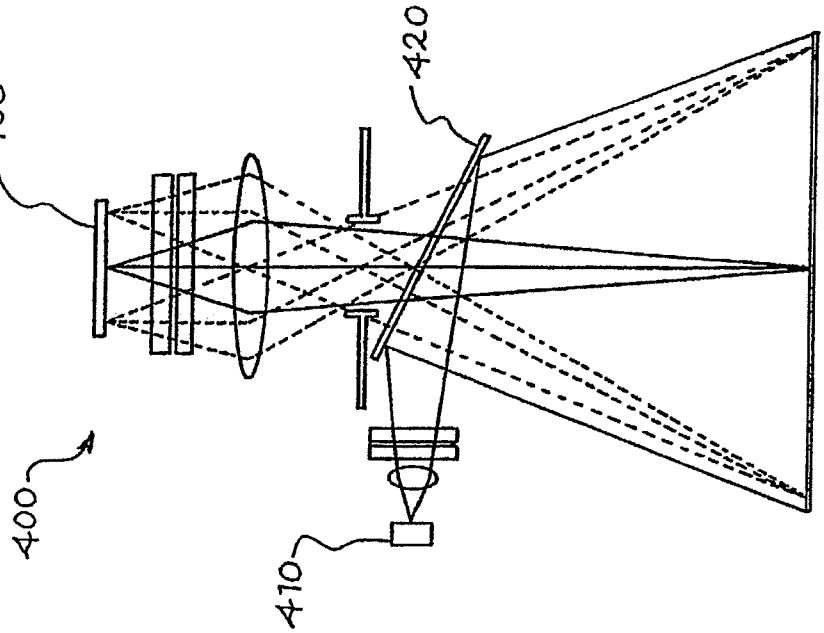


Fig. 8

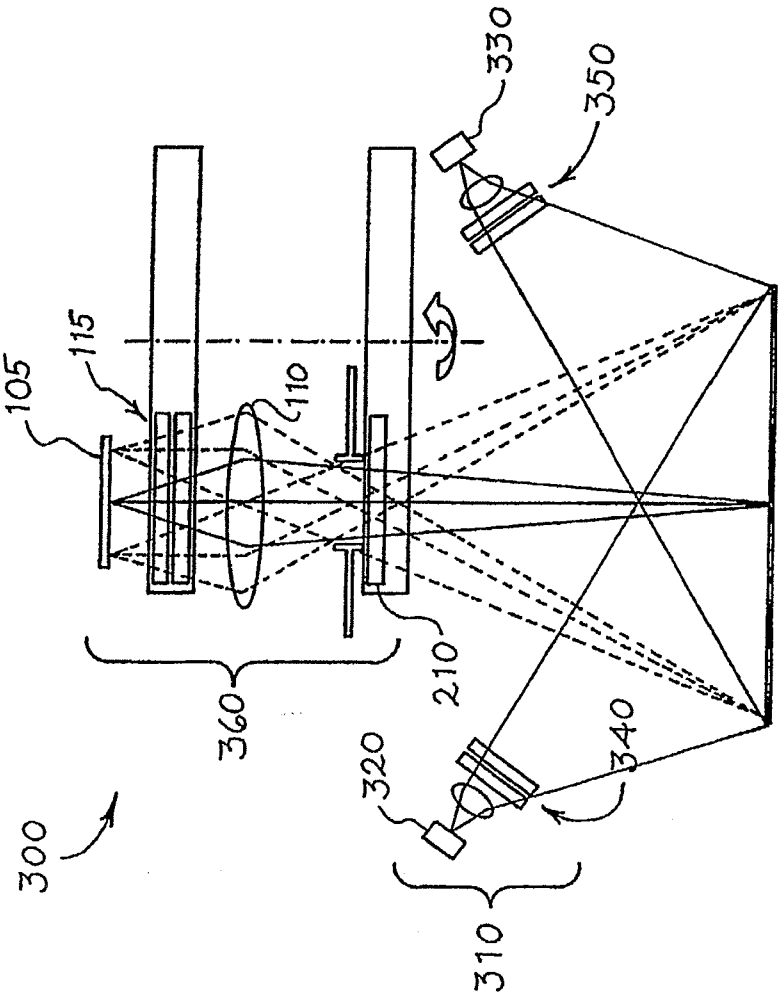


Fig. 7

Fig. 9

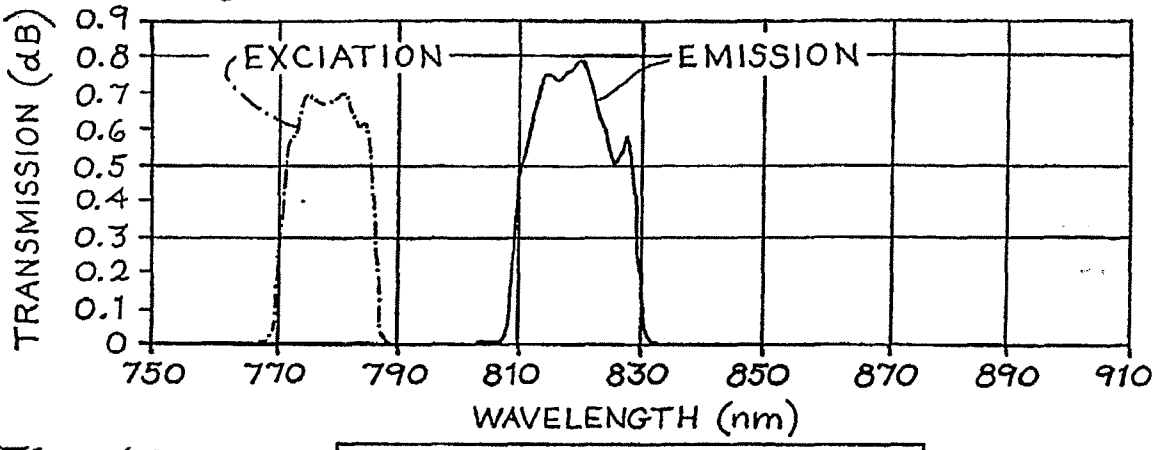


Fig. 10

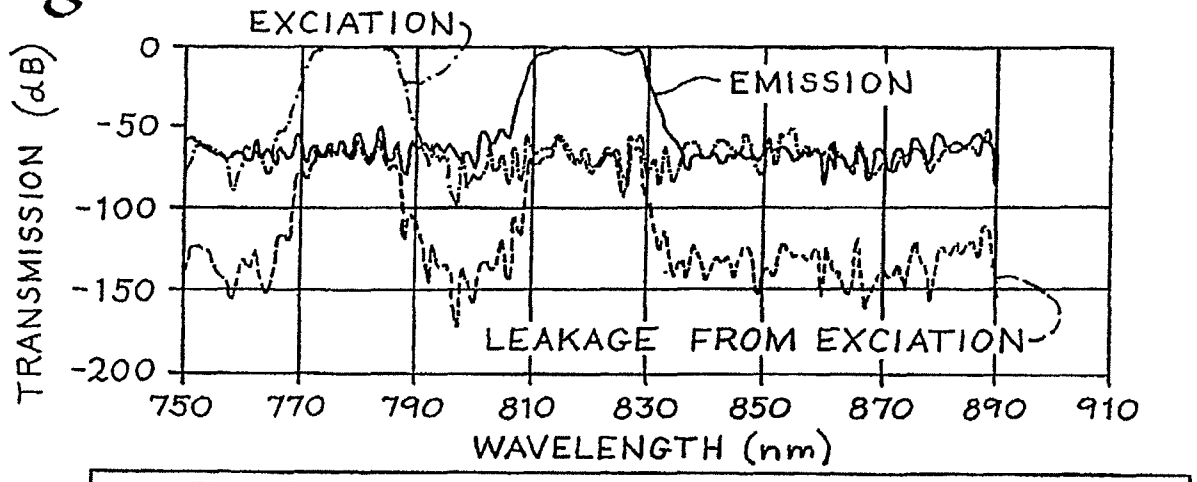


Fig. 11

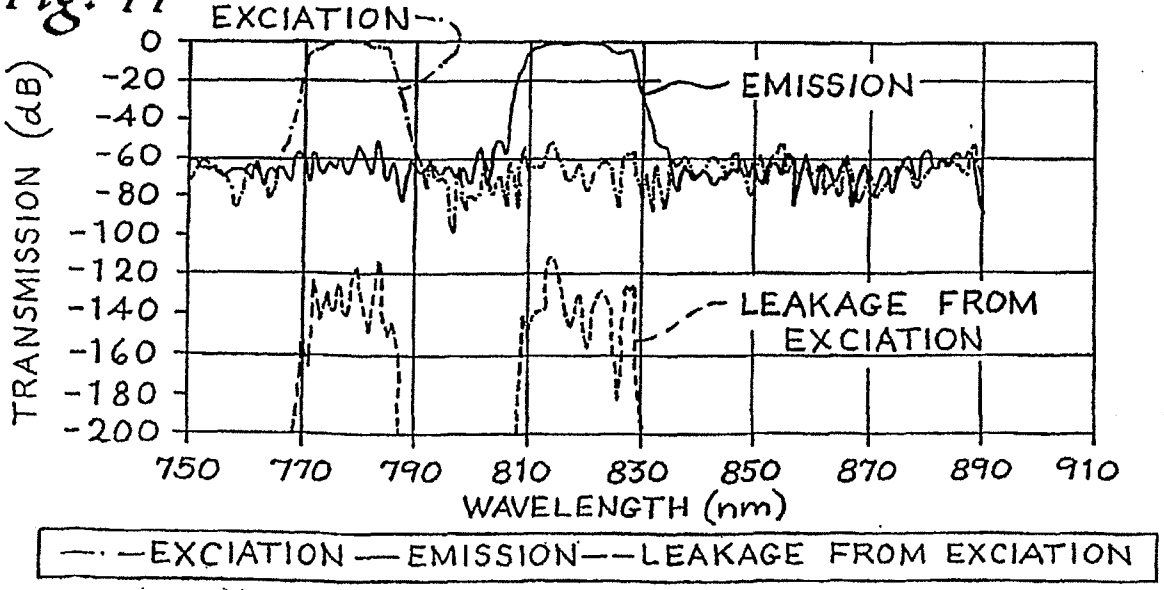


Fig. 12

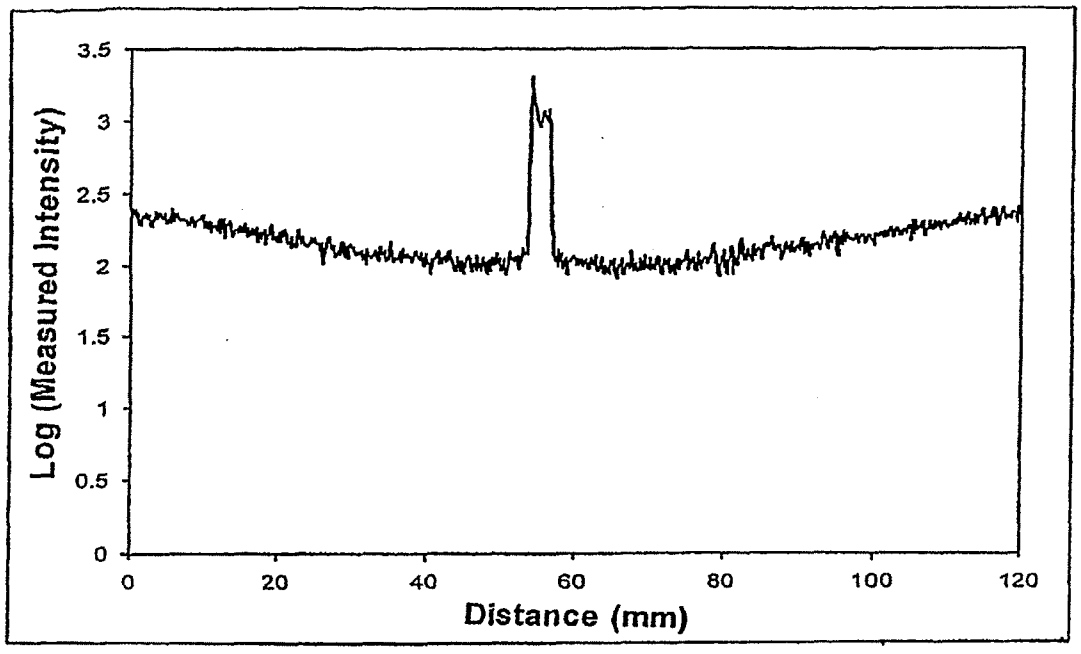

Log (Measured Intensity)
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Distance (mm)

Fig. 13

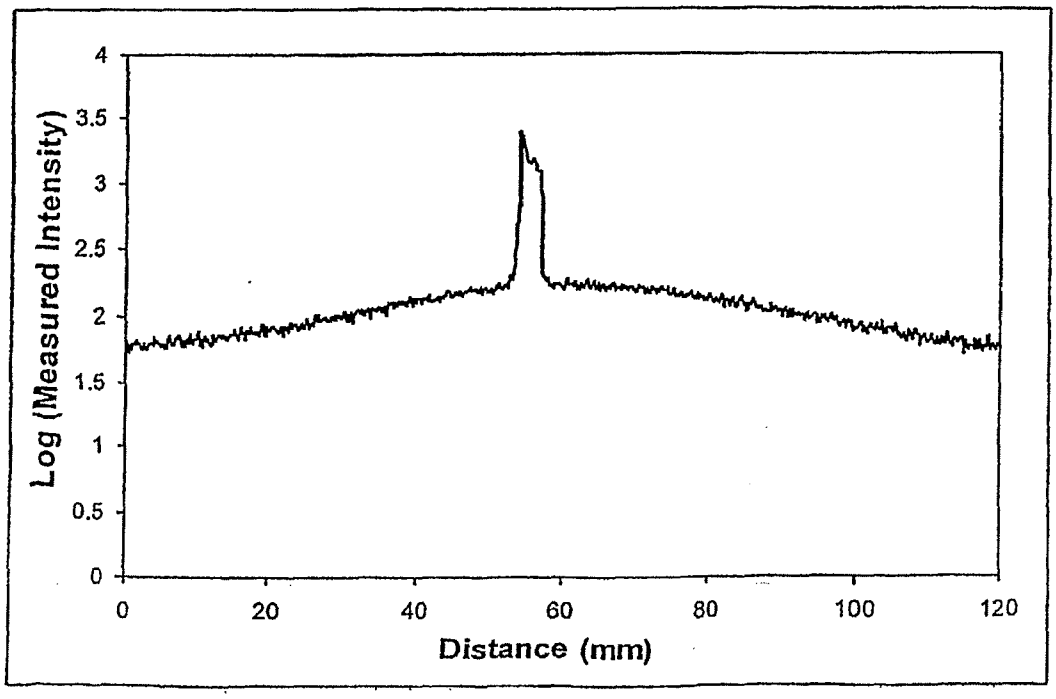

Log (Measured Intensity)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Distance (mm)

Fig. 14

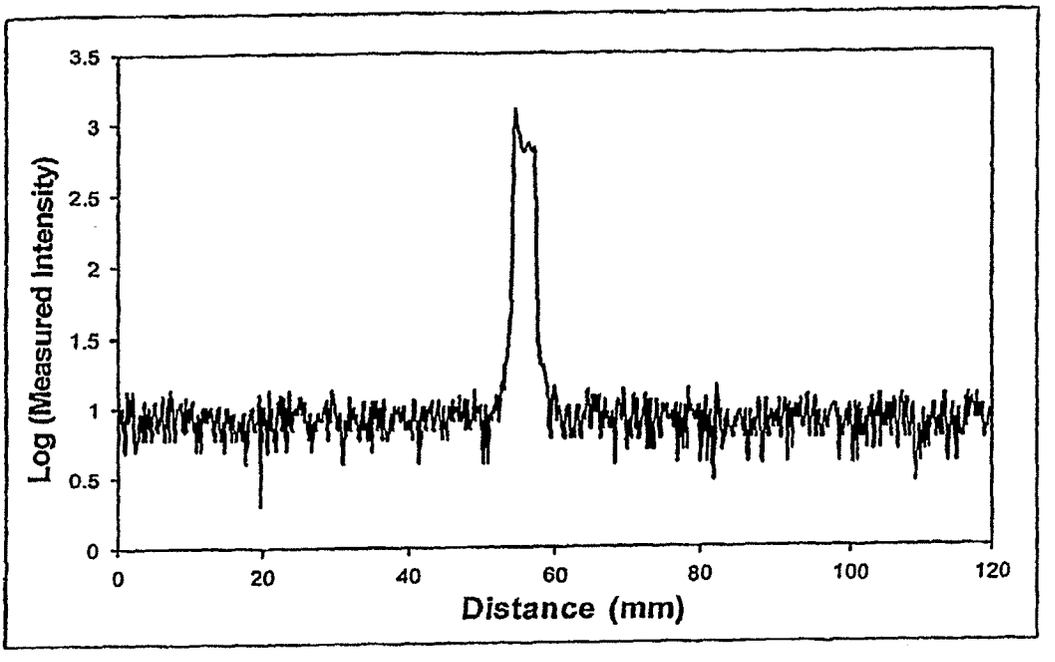
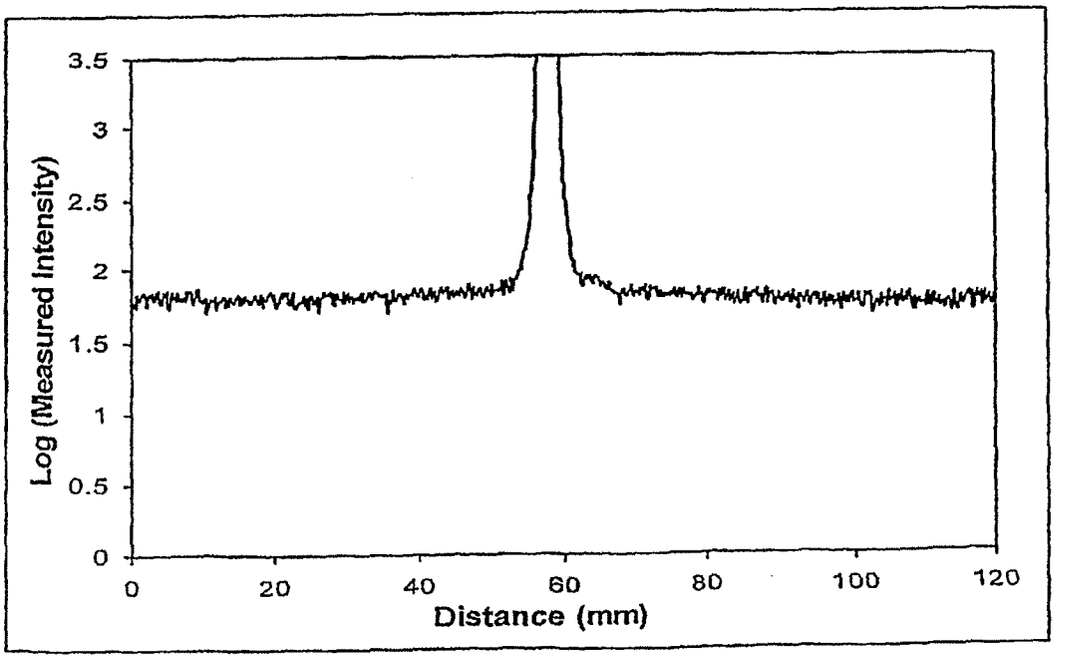

Log (Measured Intensity)
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Distance (mm)

Fig. 15


Log (Measured Intensity)
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Distance (mm)

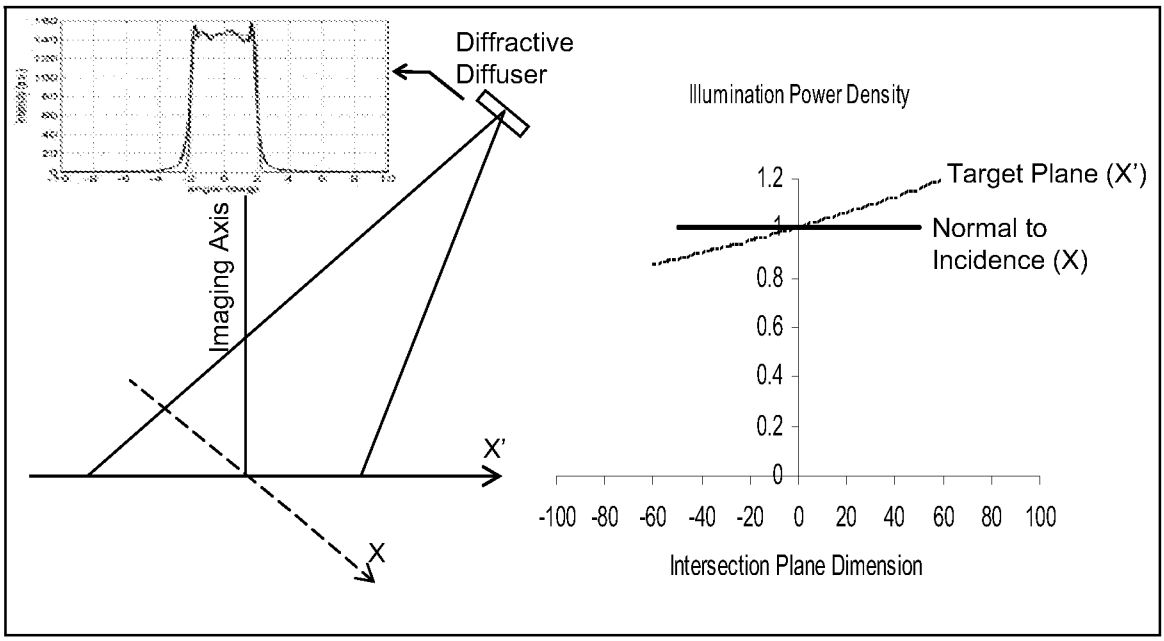


Figure 16

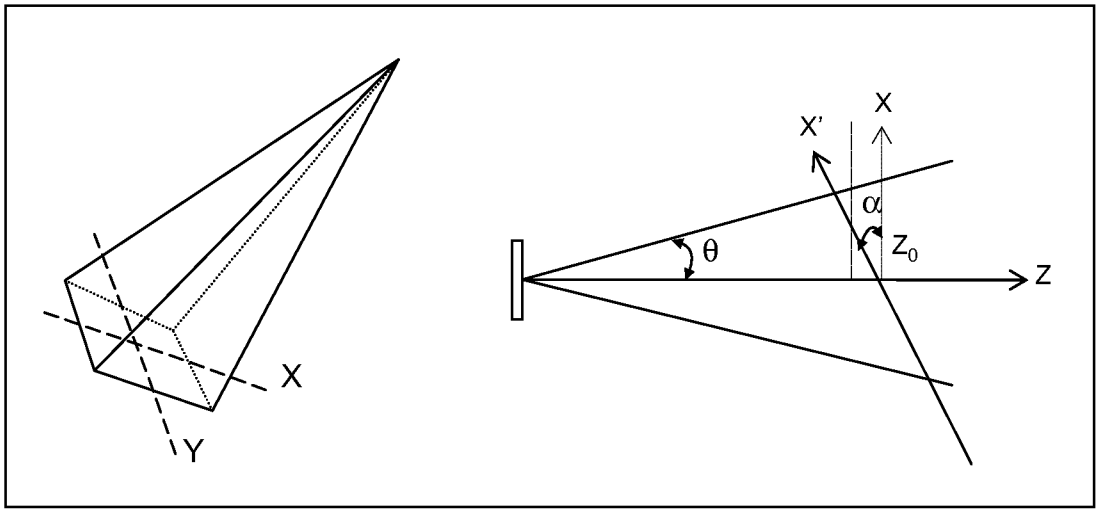


Figure 17

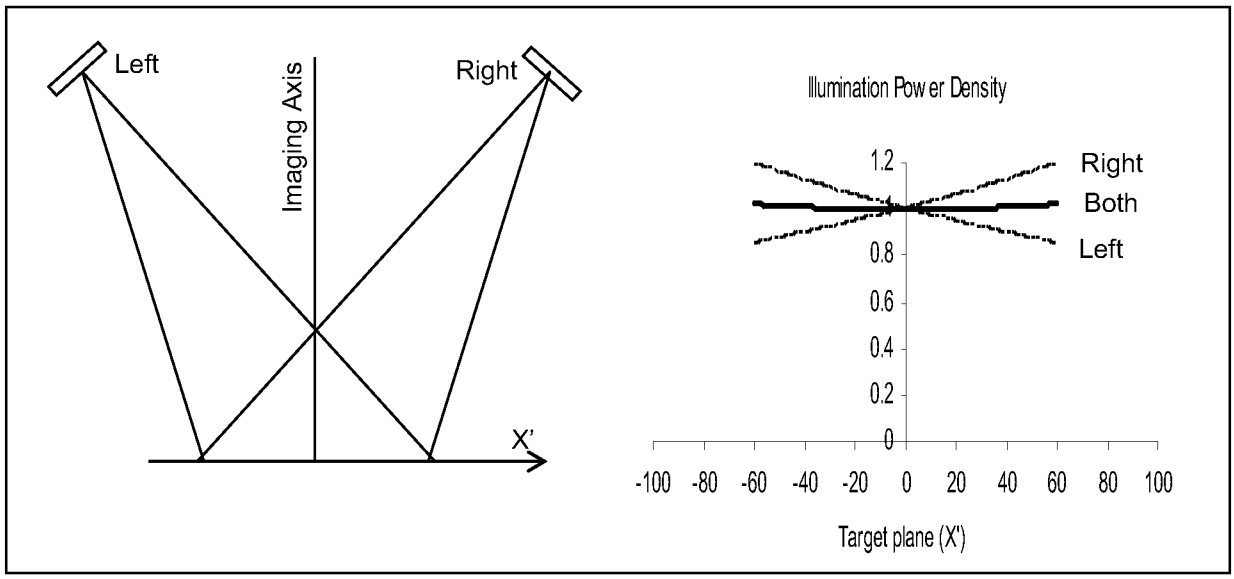


Figure 18a

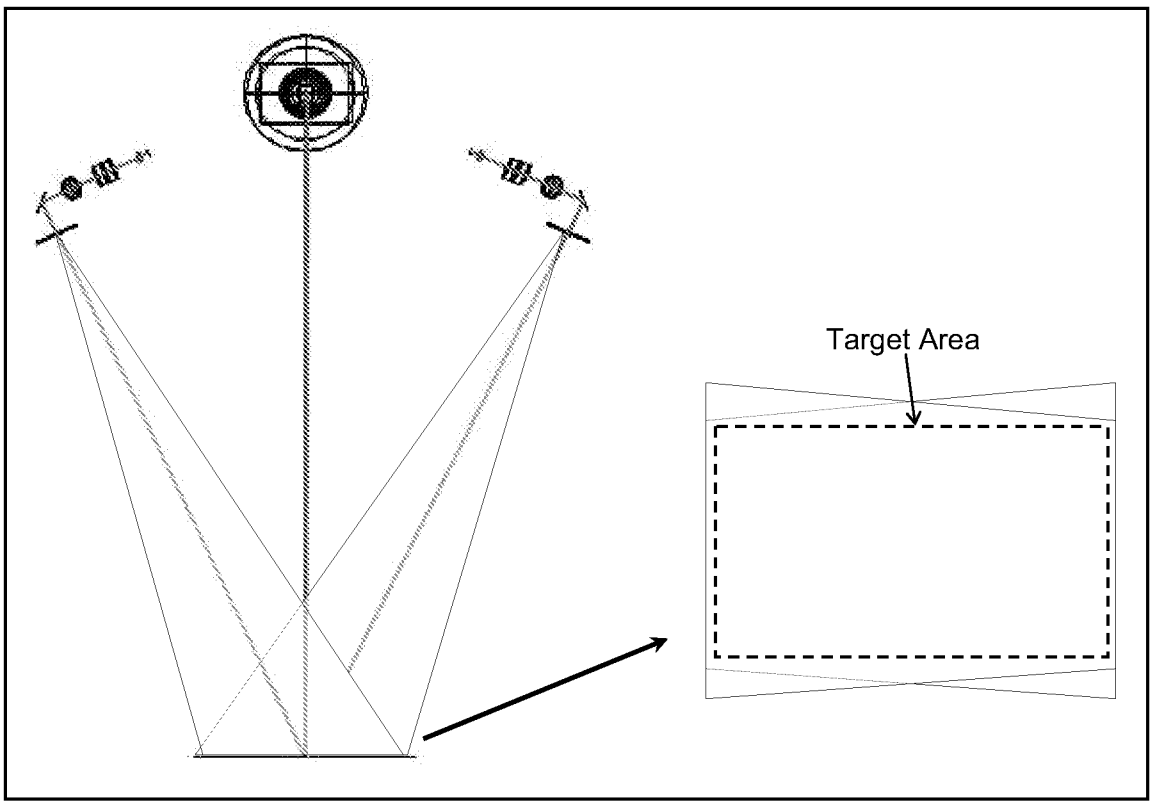


Figure 18b

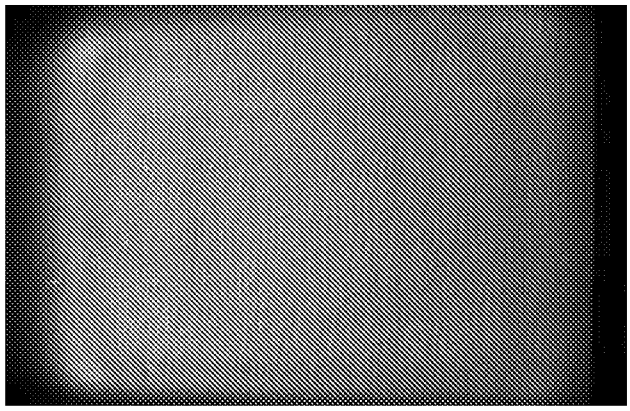


Figure 19a: Illumination from left

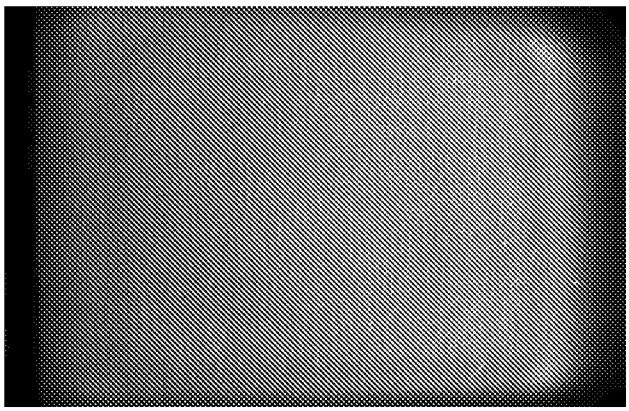


Figure 19b: Illumination from right

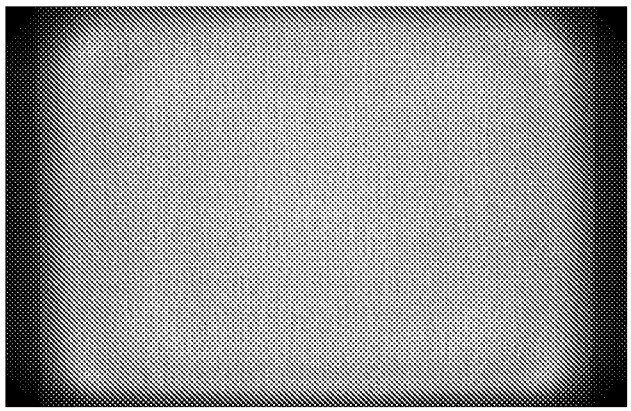


Figure 19c: Illumination from both: left + right

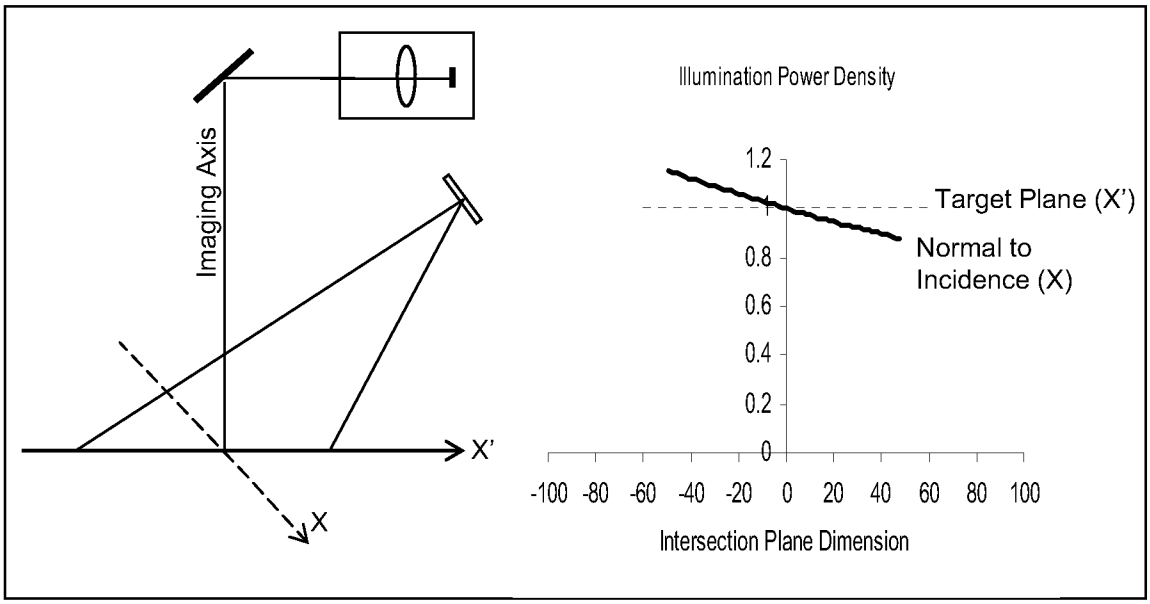


Figure 20

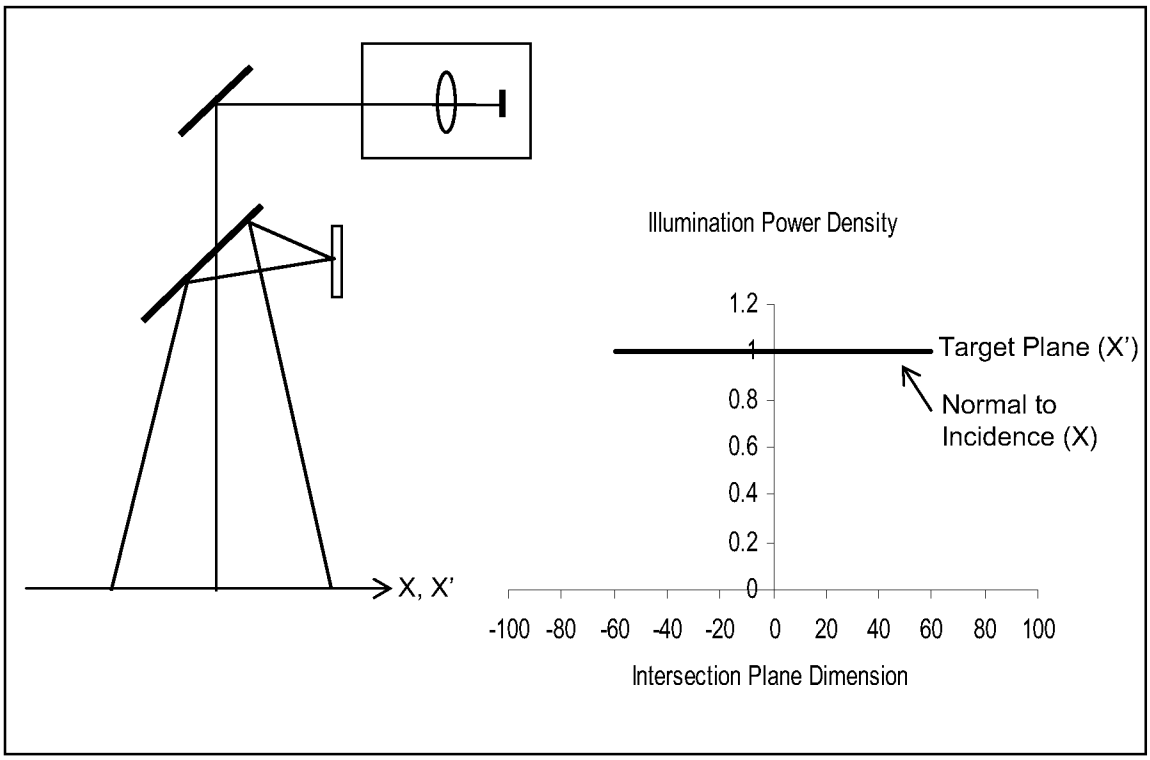


Figure 21