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(54) Confocal scanning microscope

(57) A confocal scanning fluorescence light microscope directs light from a laser source (61) along an optical path which includes beam deflecting devices (64, 66) for causing the beam to raster scan the specimen in the object plane of a microscope. The fluorescent beam emitted by the specimen is returned along the same optical path so as to be descanned, and is separated from the incident beam at a dichroic mirror (62) to be incident on the detector (74). Infinity optics in the form of optical telescopes (65, 68) are preferably employed for optical coupling along the optical path of the incident and return beams.

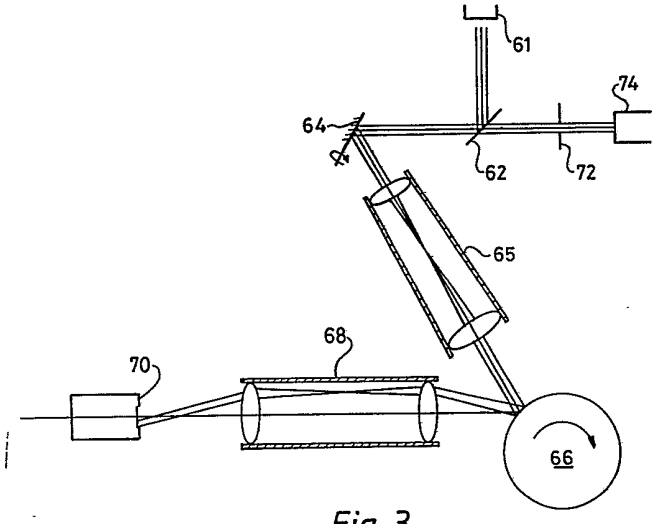

Fig. 3

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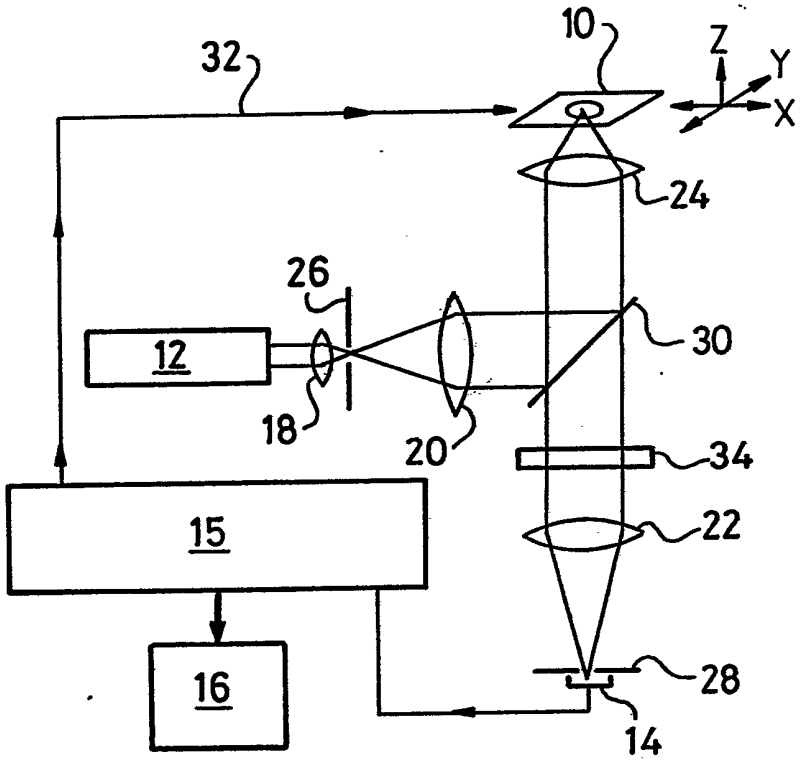

PRIOR ART

Fig.1

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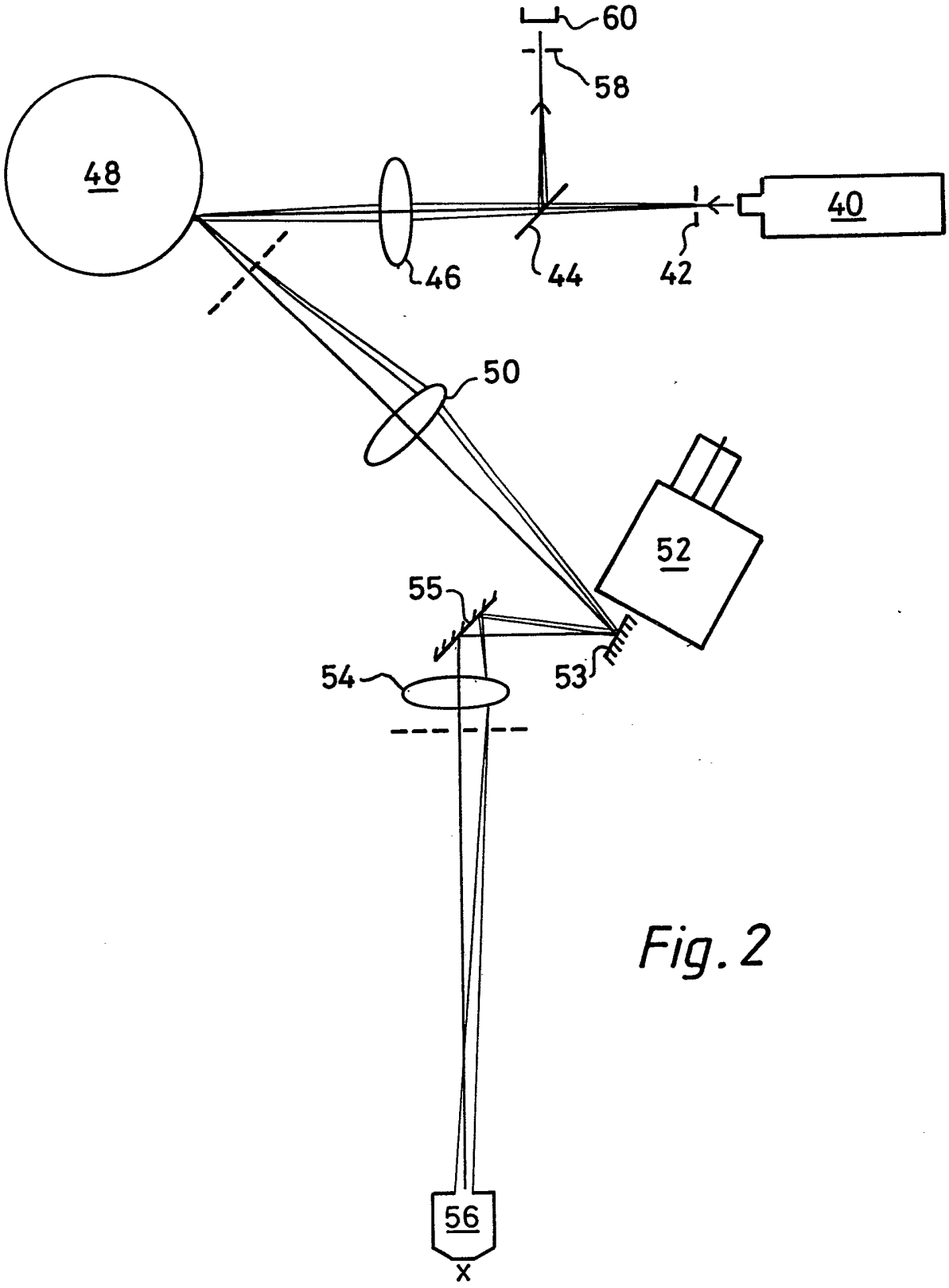

Fig. 2

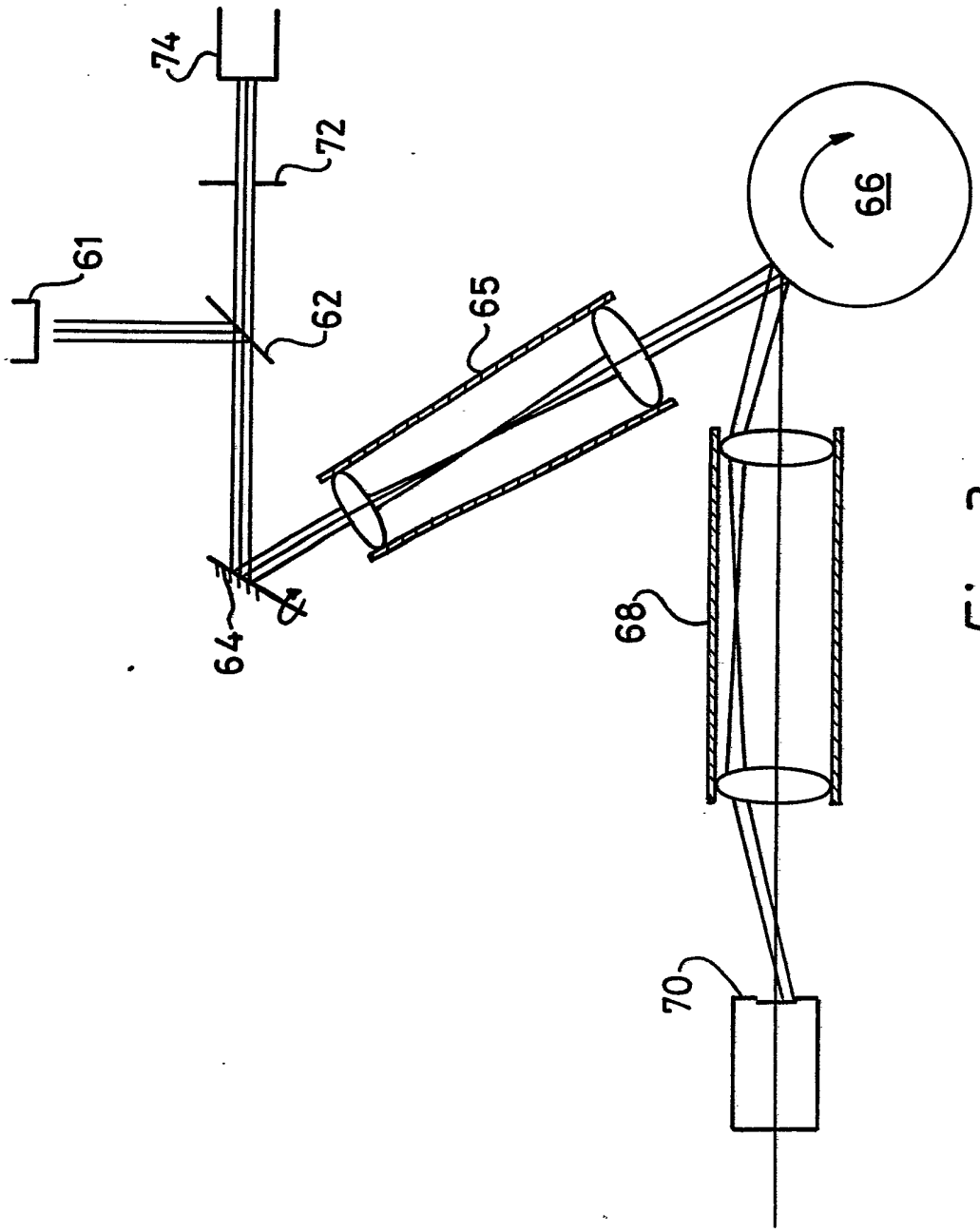

Fig. 3

SPECIFICATION

Confocal Scanning Microscope

Field of the invention

This invention relates to a confocal scanning microscope.

Background to the invention

Fluorescence light microscopy is extensively used in biological research and medical diagnosis. It provides the selectivity necessary to enable specific components of a cell or tissue to be visualised and the spatial organisation of such components to be determined.

A major problem of the technique is that, especially when thick objects are viewed, light emission from out-of-focus regions seriously degrades the signal to noise ratio of the image.

Certain types of scanning system can effect considerable improvements in depth discrimination and in signal to noise ratio and such a system is realisable in the so-called conofocal scanning optical microscope. These advantages are especially significant for fluorescence microscopy.

Previous attempts to remove the out-of-focus signal from fluorescent images have used computational techniques which are intensive and not satisfactory, mainly because underlying assumptions have to be made in relation to optical homogeneity of the specimen which are not generally valid. The potential advantage of the confocal scanning microscope is that the out-of-focus signal is removed at source and imperfect techniques for removing noise from the image do not have to be used.

Making reference to the accompanying drawings, Figure 1 diagrammatically illustrates the optical layout of a known confocal scanning microscope. The specimen at 10 is scanned by a focussed light beam usually obtained from a laser source 12. Scanning is effected by a mechanical drive which displaces the specimen in three coordinate directions. Only the pixel which is being observed is illuminated, thus eliminating interference from adjacent regions. The light emanating from the specimen is focussed at a detector 14, the output signals of which are fed to a computer 15, which processes such signals to provide vision signals for a display device 16. In Figure 1, references 18, 20, 22, 24 denote the lens components of the system, objective 24 being of high numerical aperture, whilst references 26 and 28 denote pinhole apertures and reference 30 denotes a partial (dichroic) reflector. Mechanical scan control 32 is derived from the computer 15. Reference 34 denotes a blocking filter.

Laboratory confocal scanning microscopes constructed hitherto generally in accordance with the arrangement of Figure 1 have shown performances in accordance with theoretical predictions. The disadvantages of these known prototype instruments with fixed beam geometry and scanning movement of the specimen are of a practical nature. In particular, firstly relatively long scanning times are involved and secondly the layout makes it difficult alternately to employ the microscope in a standard, i.e. non-confocal, mode. It is probable that these disadvantages have prevented commercial realisation of the known confocal scanning microscope.

It is an object of this invention to provide a confocal scanning microscope wherein the disadvantages of the known system are substantially avoided or at least minimised.

The invention

According to the invention, there is provided a confocal scanning fluorescence light microscope which comprises a light source, means defining an optical path whereby a diffraction limited spot (pixel) is illuminated on the object, said optical path means including focussing lenses for focussing said light spot at the object and means for deflecting the illuminating beam so that the focussed light spot traces a raster (line scan and frame scan) on the object, a detector which receives light emanating from the object, means for producing vision signals from the output signals obtained from the detector, and a display device receiving the vision signals.

The deflecting means is preferably a rotating polygon mirror for producing the line scan and a galvanometer-driven mirror assembly for producing the frame scan.

Preferably, the light emanating from the object is returned along the same optical path as the incident light so as effectively to be de-scanned, and is separated from the path of the incident light at a semi-reflecting (dichroic) mirror.

The light source is preferably a laser light source, conveniently an argon ion laser, whilst the detector is preferably a photomultiplier detector, preceded by a pinhole spatial filter.

The main advantage arising from the arrangement is that the object, e.g. specimen, may be stationarily located in the object plane of a microscope objective, typically a standard fluorescence microscope, giving a considerably more preacticable instrument overall. According to a further feature of the invention therefore, the confocal imaging system constituted by the above described optical path means is employed as a peripheral to a standard fluorescence microscope. An additional advantage is that, by scanning at high speed and adding synchronising signals to the video signals provided by the detector, the image may be viewed with standard TV equipment.

In a preferred embodiment of the invention, so-called "infinity" optics are employed. Firstly, therefore, an optical telescope is used in the path of the raster-tracing light beam brought to a focus at the object. Secondly, where the deflecting means comprises spaced devices for producing the line scan and the frame scan, the two devices are optically coupled by a second optical telescope.

Use of infinity optics enables the normally required pinhole apertures to be dispensed with, thereby increasing sensitivity as light is not lost due to scatter at the aperture edges. Also adjustments prior to use are minimised.

Brief description of drawings

In the accompanying drawings:

Figure 1 shows the optical system of a prior art confocal scanning microscope;

Figure 2 shows the optical system of a confocal scanning microscope which exemplifies the present

invention; and

Figure 3 shows a preferred embodiment of the invention.

Description of drawings

The known microscope system of Figure 1 has been described heretofore.

Referring now to Figure 2, the microscope system in accordance with the invention comprises an argon ion laser light source 40, the output beam of which passes through a pinhole aperture 42, through a dichroic mirror 44 and a lens 46 (equivalent focal length 40 mm) to a rotating polygon mirror 48 for effecting line scanning. The latter, whilst shown as a circle in the drawing, may for convenience have twenty five facets. The beam from the polygon mirror 48 passes through a lens 50 (equivalent focal length 40 mm) to a galvanometer-driven mirror assembly 52 for effecting frame scanning. The beam from the laser is therefore now performing a raster scanning action. This beam is incident via reflectors 53, 55 and lens 54 (equivalent focal length 40 mm) onto the objective lens of a conventional fluorescence microscope 56 and is brought to a focus at the object plane. At the object, the pinhole aperture 42 is focussed to effect illumination of the diffraction limited spot (pixel). The illuminated pixel moves over the object in accordance with the scanning raster.

Fluorescent light emitted by the object passes along the same path as the incident beam, but in the opposite direction, as far as the dichroic mirror 44, where it is separated out to pass via a pinhole spatial filter 58 to a photomultiplier type detector 60. The return beam is thus effectively de-scanned, i.e. it is incident on a substantially stationary spot on the detector.

Although not shown, the output signals from the detector 60 are processed and, with the addition of synchronising signals, passed to a display device in the form of a conventional TV monitor.

In addition to the advantage of constituting a peripheral to a standard microscope, the invention has the advantage of enabling scanning up to the scanning rate of television frame scan frequencies, and it is this that facilitates display in the aforesaid manner.

Figure 3 shows a preferred embodiment in which infinity optics is employed.

In the preferred embodiment, the beam from an argon ion laser 61 is directed through a dichroic beam splitter 62 on to a galvanometer driven mirror 64. The latter is driven by a sawtooth waveform to cause the laser beam to be deflected in a linear sweep along the y axis. A telescope 65 couples the thus deflected beam on to a motor driven rotating polygon mirror 66 which deflects the beam along the x axis. A second telescope 68 couples the deflected beam to the exit pupil of a conventional microscope eyepiece 70. From this eyepiece the beam passes into a conventional fluorescence microscope (not shown) to be brought to a focus in the objective plane, where the beam traces a raster.

Fluorescent emission from the specimen (object) under examination passes through the optical system in the opposite direction, thereby effectively being de-scanned. The now stationary return beam is separated from the incident beam at the dichroic beam splitter 62 and passes through an iris diaphragm 72 to a photomultiplier detector 74. The signal output is electronically processed and synchronising signals are added to enable the image to be viewed with conventional television equipment.

Various modifications of the illustrated arrangements of Figures 2 and 3 are possible within the scope of the invention as defined by the appended claims.

CLAIMS

1. A confocal scanning fluorescence light microscope which comprises a light source, means defining an optical path whereby a diffraction limited spot (pixel) is illuminated on the object, said optical path means including focussing lenses for focussing said light spot at the object and means for deflecting the illuminating beam so that the focussed light spot traces a raster (line scan and frame scan) on the object, a detector which receives light emanating from the object, means for producing vision signals from the output signals obtained from the detector, and a display device receiving the vision signals.

2. A microscope according to claim 1, wherein the deflecting means comprises separated devices for producing the line scan and the frame scan.

3. A microscope according to claim 2, wherein the said devices are a rotating polygon for producing the line scan and a galvanometer-driven mirror for producing the frame scan.

4. A microscope according to claim 1 or claim 2 or claim 3, wherein the fluorescent light emanating from the object is returned along the same path as the incident beam so as effectively to be de-scanned.

5. A microscope according to claim 4, wherein the return beam is separated from the incident beam, after de-scanning, at a beam splitter.

6. A microscope according to any of claims 1 to 5, wherein the light source is a laser.

7. A microscope according to any of claims 1 to 6, wherein the detector is a photomultiplier.

8. A microscope according to any of claims 1 to 7, wherein means are provided for electronically processing the detector output and adding synchronising signals thereto for enabling the image to be viewed on a TV monitor.

9. A microscope according to any of claims 1 to 8, wherein the optical path means comprises infinity optics.

10. A miscroscope according to claim 9, wherein an optical telescope is employed to couple the raster-scanning incident beam from the deflecting means with the eyepiece of a microscope at which the incident beam is brought to a focus in the object plane.

11. A microscope as claimed in claim 10, when appendant to claim 2 or claim 3, wherein a second optical telescope is employed to couple the two beam deflecting devices.

12. A microscope according to any of claims 1 to 10, comprising a standard fluorescence microscope having said optical path means provided as a peripheral thereto.

13. A confocal scanning fluorescence light microscope substantially as hereinbefore described with reference to Figure 2 or Figure 3 of the accompanying drawings.

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