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(54) Title: SPATIAL-TEMPORAL OPTICAL PHASE MODULATION METHOD AND APPARATUS

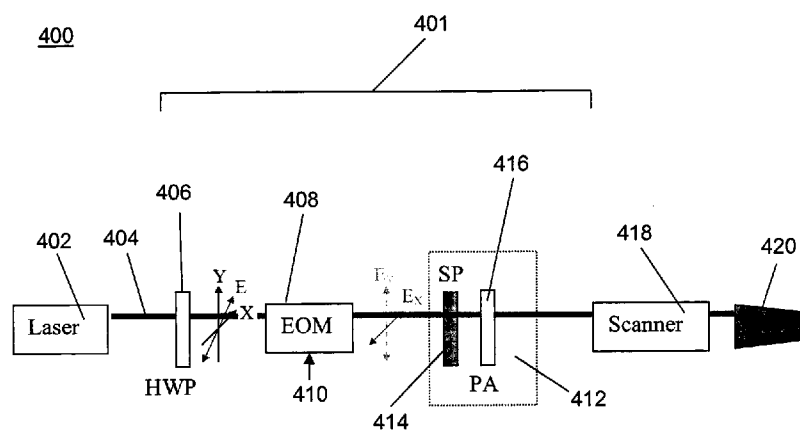


Fig. 4

(57) **Abstract:** A spatial-temporal phase modulator (422) for modulating the phase of a collimated light beam (404) spatially and temporally is disclosed. The output (404) from the laser (402) is linearly polarized and a half wave plate (406) rotates the polarization of the E-field to form a 45 degree angle with the Y-axis. The electro-optical modulator (408) is a polarization dependent device, and provides a variable phase shift on EY and but no phase shift on EX. A RF signal (410) is fed to the EOM (408) to introduce a periodic phase delay between EX and EY. The spatial polarizer (414) selectively blocks EX or EY so that the modulated and non-modulated beams are spatially separated in the output. The polarization analyzer's (416) axis is at 45 degrees with the polarization directions of both modulated and non-modulated beams so that they can interfere with each other.

Spatial-Temporal Optical Phase Modulation Method and Apparatus

Technical Field

This invention is related to laser scanning optical microscopy, in particular focal
5 modulation microscopy.

Background

Confocal microscopy (CM) is the standard tool widely used for the imaging of thick
biological samples. When imaging beyond tens of micron in depth, however, CM
10 suffers from deteriorated resolution and contrast due to background signals from
scattered light. To overcome the limitation of CM, Focal Modulation Microscopy
(FMM) was developed as an imaging method for improved imaging depth. Focal
Modulation Microscopy (FMM) combines focal modulation with confocal detection to
reject background signals from scattering media. FMM has demonstrated a much
15 improved imaging depth than CM: see for example N. Chen, C-H. Wong, C. J. R.
Sheppard, "Focal modulation microscopy," Optics Express 16 (23), 18764-18769
(2008); C. H. Wong, S. P. Chong, C. J. R. Sheppard, and N. Chen, "Simple spatial
phase modulator for focal modulation microscopy," Applied Optics 48 (17), 3238-3243
(2009); and S. P. Chong, C. H. Wong, C. J. R. Sheppard, and N. Chen, "Focal
20 Modulation Microscopy: A Theoretical Study," Optics Letters 35 (11), 1804-1806
(2010).

The main difference between FMM and CM is that a spatial-temporal phase modulator
(SPM) is introduced into the illumination beam path, which results in an intensity
25 modulation in the focal volume where the modulated beam is focused by an objective
lens. The modulated component in the detected signal is related to the ballistic
excitation light only and can be differentiated easily from the background by the use of
lock-in detection.

To ensure proper lock-in detection, the pixel dwell time should be an integer multiple of the modulation period. In modern commercial laser scanning microscopes, the pixel dwell time is usually less than 10 microseconds, which implies that the modulation frequency should be greater than 100 kHz. Two simple spatial-temporal phase modulators have been designed and implemented for a prototype FMM system: Chen *et al.*, and Wong *et al.* However, their modulation frequencies are limited by a few kHz and the corresponding image acquisition time is at least tens of seconds.

A known high-speed FMM system 100, based on acousto-optical modulators (AOM) is shown in Fig. 1. A laser 102 generates a coherent laser beam 104 that is first split by a beam splitter 106 into two beams 108, 110 which then passes through two AOMs 112, 114 having slightly different resonance frequencies (i.e. f_1 and f_2) where they undergo zeroth- and first-order diffractions. The first-order diffracted beams 116, 118 are Doppler shifted by f_1 or f_2 , and are reflected 124, 126 with slight lateral displacement using retroreflectors 120, 122 to return to the same AOMs 112, 114 and then are recombined at the beam splitter 106. The two beams at this moment are Doppler shifted at twice the resonance frequencies of the AOMs 112, 114 respectively as they have passed through the AOMs twice. Part of the combined beam 128 is directed towards a fiber-optic photodetector 130 via a mirror 132 and a lens 134, which generates a reference signal at the optical beating frequency of $2(f_1 - f_2)$. The remaining part of the modulated laser beam 136 is directed to a scanning unit 138 of a conventional CM to excite a biological sample 140 stained with fluorescence probes. The fluorescence emissions are then detected by a PMT 142 behind a confocal pinhole (OBJ) 144. The PMT output is preamplified before feeding to an I/Q demodulator (not shown), where the oscillatory component at the beating frequency is picked up by mixing with the reference signal generated by the photodetector (PD) 1130. The demodulated signal is then used to form the FMM images. The typical frequency of AOMs is tens of MHz. A 10 MHz beating frequency can be readily achieved, which corresponds to a minimum pixel dwell time of 0.1 microseconds.

While the spatial-temporal phase modulator shown in Fig. 1 is fast enough for real-time image acquisition, its aperture, shown in Fig. 2, is far from optimized. Only part of the objective aperture 200 is occupied by the two AOM modulated beams 202, 204. This leads to deteriorated spatial resolution, and more significantly, a small modulation depth.

5 Modulation depth is an important parameter in FMM, which is defined as the ratio of the amplitude of the ac component to the magnitude of the dc component in the detected fluorescence signal:

$$M = I_{ac} / I_{dc}$$

10

In practical situations, the excitation power needs to be maintained at the lowest possible level to reduce photobleaching and phototoxicity. In addition, I_{dc} contributes to shot noise which cannot be completely eliminated by filtering. Consequently, it is desirable to maximize the modulation depth M . The measured modulated depth for the aperture

15 shown in Fig. 2 is only about 0.25.

20

Evaluation of the modulation depth for various other aperture configurations (a) – (h), is shown in Fig. 3. Rays passing the gray zone and the white zone have different phase delays. It has been found that the six-zone annular aperture (f) provides the best performance ($M \sim 0.82$) while the double-D aperture (b) is the worst ($M \sim 0.35$). While multi-zone apertures are preferred for a large M and enhanced FMM signal, such configurations are difficult to implement using the existing approach.

25

As stated, focal modulation microscopy (FMM) is a useful imaging method with improved imaging depth. A spatial-temporal phase modulator is used in the focal modulation microscope to generate an intensity modulation at the focal point, which is important for improving the signal to background ratio. The desired characteristics of the spatial-temporal phase modulator include high-speed, optimal aperture, and compatibility with multiple wavelengths. Such properties, however, are not readily available with

30 commercial products and existing designs.

Summary

This invention seeks to overcome or at least reduce the limitations of existing designs. There is disclosed a spatial-temporal phase modulator having an efficient combination of high-speed temporal modulators with polarization optics. Arbitrary aperture segmentation and configuration can be implemented with time-dependent modulation fast enough for real-time imaging acquisition.

There is disclosed a spatial-temporal optical phase modulator comprising:

- a first optical component receiving a laser beam and generating two resultant beams respectively having orthogonally polarized E-field components;
- an electro-optical modulator, optically coupled to said first optical component, producing a variable phase shift to only one of said E-field component beams; and
- an aperture-forming optical component, optically coupled to said electro-optical modulator, spatially separating said phase shifted and non-phase shifted E-field component beams.

The first optical component can be a half-wave plate.

Preferably, there further comprises a radio frequency modulating signal provided to said electro-optical modulator. The aperture-forming optical component can include a spatial polarizer followed by a polarization analyzer. The aperture-forming optical component can also include a spatial retarder and a polarization analyzer.

There is further disclosed a focal modulation microscopy system including a phase modulator recited above.

There is yet further disclosed a method for generating optical phase modulation both spatially and temporally, comprising:
adjusting the polarization of the input light beam to form an angle of approximately 45 degrees with respect to a reference direction;

generating a temporal phase modulation on a component of the input beam polarized along the reference direction, while the orthogonally polarized component is subject to a constant phase delay;

5 spatially separating the temporally modulated component and the non-modulated component using a spatial polarizer; and
polarizing the output spatial-temporally modulated beam along an optimal direction by using a linear polarizer.

The input light beam preferably is collimated and linearly polarized.

10

There is yet further disclosed a light microscopy system using the above spatial-temporal phase modulation method.

Brief description of drawings

15 Fig. 1 shows a known FMM system having acousto-optical modulators (AOMs).

Fig. 2 shows the effective aperture of two AOM modulated beams of Fig. 1.

Fig. 3 shows configurations of SPM apertures.

20

Fig. 4 shows a FMM system having a single-EOM based spatial-temporal phase modulator embodying the invention.

Fig. 5 shows the configuration of a four-zone spatial polarizer.

25

Detailed description

A high-speed FMM system 400 including a spatial-temporal phase modulator 401 is shown in Fig. 4. Generally speaking, in such a modulator 401 two orthogonally polarized beams are modulated differently with high-speed temporal phase modulators. These two
30 beams are parallel to each other and spatially overlapping before entering the aperture-

forming optics. The aperture-forming optics includes a spatial polarizer that allows only one polarization state to pass through a specific area. The excitation beam after the aperture-forming optics is spatial-temporal modulated with desired properties. A single eletro-optical modulator (EOM) is combined with the polarization optical components.

5

More specifically, the output 404 from a laser 402 is linearly polarized and a half-wave plate (HWP) 406 rotates the polarization of the E-field to form a 45 degree angle with the Y-axis. The two orthogonally polarized components, E_X and E_Y , carry identical power. The EOM 408 is an optical device in which a signal-controlled element displaying electro-optic effect is used to modulate a beam of light. The simplest kind of EOM consists of a nonlinear optical crystal, whose refractive index is a function of the strength of the local electric field. When such a crystal is exposed to an electric field, light will travel more slowly through it. The phase of the light leaving the crystal is directly proportional to the length of time it took that light to pass through it. Consequently, the phase of the laser light exiting an EOM can be controlled by changing the electric field in the crystal. Depending on the type and orientation of the nonlinear crystal, and on the direction of the applied electric field, the phase delay can depend on the polarization direction. The polarization dependent EOM 408 is used to provide a variable phase shift on E_Y and but no phase shift on E_X . A RF signal 410 (~MHz) is fed to the EOM 408 to introduce a periodic phase delay (between 0 to π) between E_X and E_Y . The aperture-forming optics 412 consists of a spatial polarizer (SP) 414 and (linear) polarization analyzer (PA) 416. The SP 414 selectively blocks E_X or E_Y so that the modulated and non-modulated beams are spatially separated in the output. A suitable four-zone annular SP 500 is illustrated in Fig. 5. The gray zones allow vertically polarized light (E_Y) to pass while the white zones pass horizontally polarized light (E_X). The PA's axis is at 45 degrees with the polarization directions of both modulated and non-modulated beams so that they can interfere with each after the PA 416 The output of the PA 416 passes to a scanner 418 and objective lens 420 to excite a biological sample (not shown).

30 The spatial-temporal phase modulator 401, formed by the HWP 406, EOM 408, SP 414 and PA 416, has a number of advantages. First of all, The EOM modulation frequency can easily reach the few MHz range. Secondly, the aperture is defined by the SP 414,

which is easy to configure and fabricate. Thirdly, the modulator 401 can be shared by multiple excitation wavelengths simultaneously. Fourthly, such a design has a high level of flexibility. The aperture-forming optics 412 is compact and can be easily inserted into the scanning head of a standard confocal microscope, while the EOM 408 can be integrated into the laser system 402. The output of the EOM 408 can be linked to the aperture forming optics 412 via a polarization maintaining fiber. Lastly, the aperture forming optics 412 can be miniaturized and integrated into an endoscopic imaging catheter.

10 The spatial-temporal phase modulator 401 is able to generate a modulated excitation beam at a frequency greater than 100 kHz, and the aperture generated leads to a large modulation depth. Embodiments offer one or more of the following advantages:

- 1) High modulation frequency that is adequate for real-time imaging.
- 2) Optimal apertures can be easily implemented.
- 3) Great ease to upgrade existing confocal microscopes.
- 4) Compatible with multiple excitation wavelengths.
- 5) Ready to be miniaturized.

20 In another embodiment, the aperture forming optics 412 can be placed after the scanner 418 and before the objective lens 420.

In yet another embodiment, in the aperture forming optics, the spatial polarizer can be replaced by a spatial retarder. Part of incident beam has its polarization rotated by 90 degrees while the rest of beam remains unchanged. The spatial retarder may be followed by a polarizer.

Preferred aspects of the disclosure are:

1. A spatial-temporal phase modulator (SPM) for modulating the phase of a collimated light beam spatially and temporally. The collimated beam can be focused by a lens and the intensity within the focal volume can be temporally modulated.
2. A method used in the focal modulation microscope (FMM) to generate an intensity modulation at the focal point for improving the signal to background ratio.
3. A method having a high modulation depth value, defined as the ratio of the amplitude of the ac component to the magnitude of the dc component in the detected fluorescence signal.
4. A high-speed design of in excess of several MHz range enabling imaging capture for display in real-time.
5. A method that is compatible with multiple excitation wavelengths.
6. A system including a spatial-temporal phase modulator featuring a combination of high-speed temporal modulators with polarization optics.
7. A system including a spatial-temporal phase modulator featuring arbitrary segmentation of the aperture and fast time-dependent phase modulation within individual segments.
8. A system including a spatial-temporal phase modulator featuring two orthogonally polarized beams which are modulated differently with high-speed temporal phase modulators.

9. These two beams can be parallel to each other and they are spatially overlapping before entering the aperture forming optics.

5 10. The aperture forming optics can include a spatial polarizer that allows only one of the two polarization states to pass through a specific aperture segment, and a polarization analyzer.

10 11. The excitation beam after the aperture forming optics can be spatial-temporal modulated with desired properties.

12. A single eletro-optical modulator (EOM) can be combined with polarization optical components.

15 13. The input light polarization can be arranged to form a 45 degree angle with the fast axis of EOM, which a polarization dependent device.

14. An RF signal (in the range of a few MHz) is fed to the EOM to introduce a periodic phase delay (between 0 to π) between the ordinary and extraordinary waves.

20

15. The aperture-forming optics can consist of a spatial polarizer (SP) and polarization analyzer (PA). The spatial polarizer selectively blocks the ordinary wave or the extraordinary wave so that the modulated and non-modulated beams are spatially separated in the output.

25

16. A polarization analyzer (PA) can have its axis at 45 degrees with the ordinary and extraordinary waves so that they can interfere with each other after the PA.

Claims:

1. A spatial-temporal optical phase modulator comprising:
a first optical component receiving a laser beam and generating two resultant
5 beams respectively having orthogonally polarized E-field components;
an electro-optical modulator, optically coupled to said first optical component,
producing a variable phase shift to only one of said E-field component beams; and
an aperture-forming optical component, optically coupled to said electro-optical
modulator, spatially separating said phase shifted and non-phase shifted E-field
10 component beams.
2. The modulator of claim 1, wherein the first optical component is a half-wave
plate.
- 15 3. The modulator of claim 1, wherein the first optical component is a pair of
acousto-optical modulators.
4. The modulator of any of claims 1 to 3, further comprising a radio frequency
modulating signal provided to said electro-optical modulator.
20
5. The modulator of any of claims 1 to 3, wherein said aperture-forming optical
component includes a spatial polarizer followed by a polarization analyzer.
6. The modulator of any of claims 1 to 3, wherein said aperture-forming optical
25 component includes a spatial retarder and a polarization analyzer.
7. A focal modulation microscopy system including a phase modulator of any one of
claims 1 to 6.
30

8. A method for generating optical phase modulation both spatially and temporally, comprising:

adjusting the polarization of the input light beam to form an angle of
5 approximately 45 degrees with respect to a reference direction;

generating a temporal phase modulation on a component of the input beam polarized along the reference direction, while the orthogonally polarized component is subject to a constant phase delay;

10 spatially separating the temporally modulated component and the non-modulated component using a spatial polarizer; and

polarizing the output spatial-temporally modulated beam along an optimal direction by using a linear polarizer.

9. The method of claim 8, wherein said input light beam is collimated and linearly
15 polarized.

10. A light microscopy system using the spatial-temporal phase modulation method of either of the claims 8 or 9.

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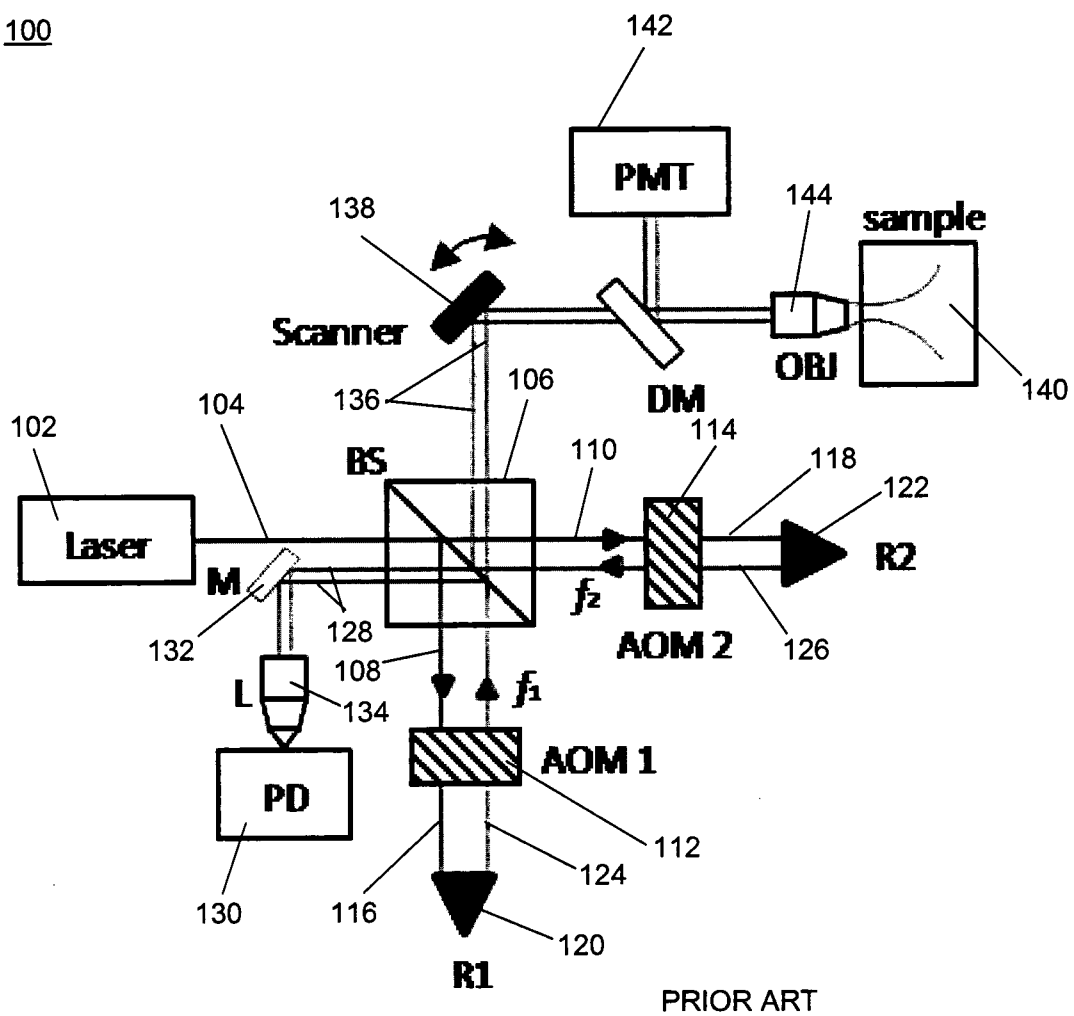
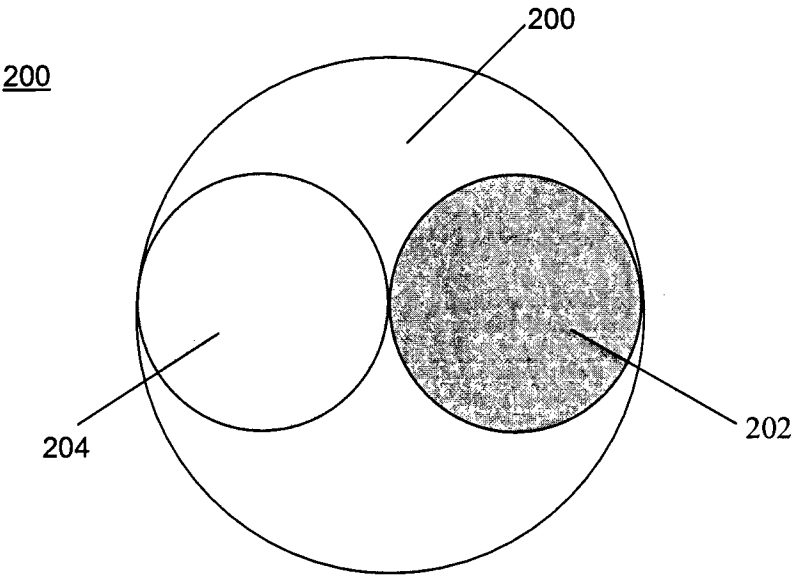


Fig. 1



PRIOR ART

Fig. 2

300

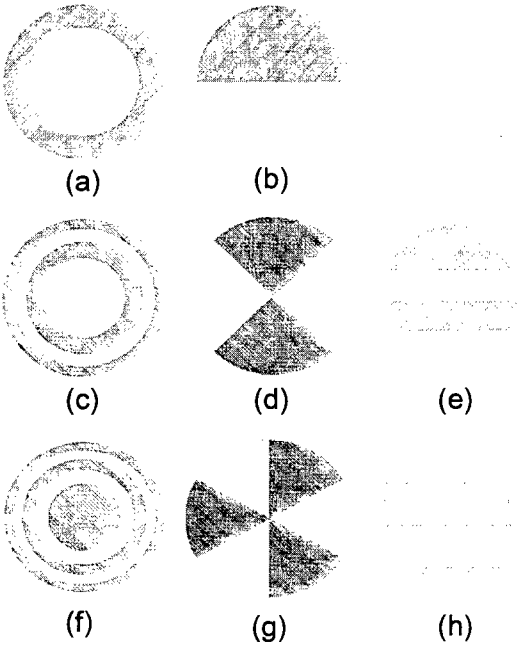


Fig. 3

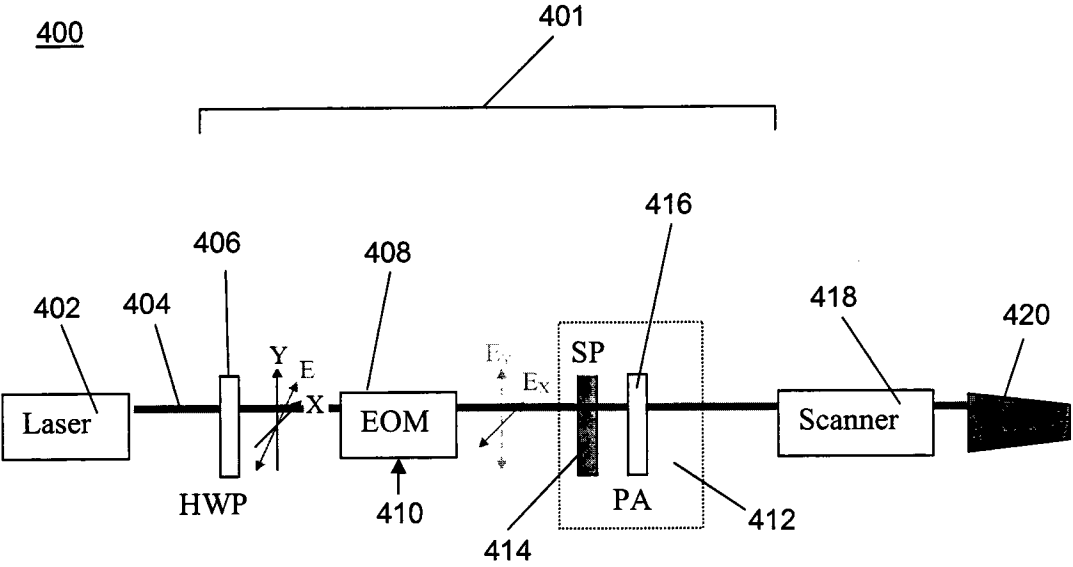


Fig. 4

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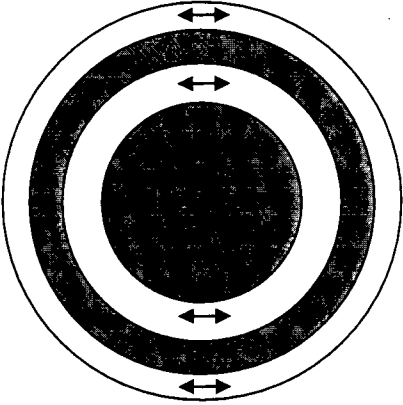


Fig. 5

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI and EPODOC using keywords: phase, modulate, optic, laser, microscope, confocal, fmm, eom, electrooptic, spatial, polarise, retard, delay and similar terms

Google Scholar, Google Patents: spatial, temporal, phase, modulator, microscope, confocal and like terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0214404 A1 (CHEN et al.) 26 August 2010 Abstract; Figures	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2012/000011

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	2010214404	CN	101802675	EP	2171516	JP	2010532878
		KR	20100045964	WO	2009008838		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
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