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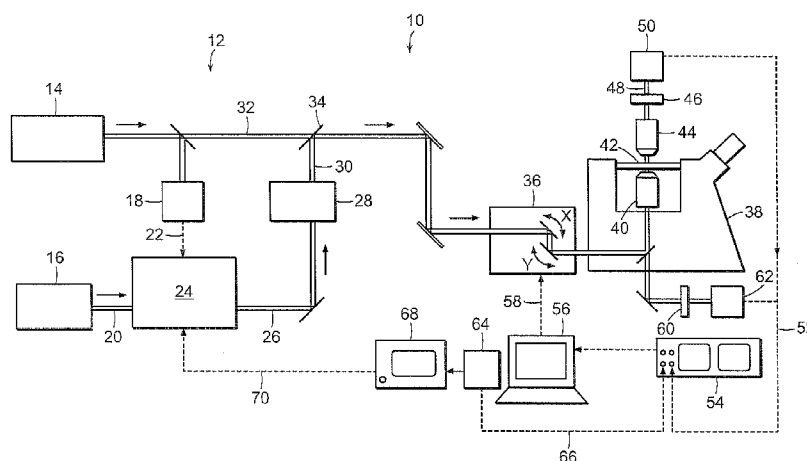


FIG. 1

(57) Abstract: An illumination system is disclosed for use in non-linear modulation transfer microscopy and micro-spectroscopy. The illumination system includes a laser system for providing a first train of pulses at a center optical frequency  $\omega_1$ , and an optical assembly for collinearly combining a first train of pulses with a second train of pulses having a center optical frequency of  $\omega_2$  as synchronized excitation fields for non-linear modulation transfer microscopy and micro-spectroscopy. The laser system includes a continuous wave laser for providing a first continuous wave field, a first chirp modulator system comprising a means to receive the first continuous wave field, a means to receive a trigger signal, a first phase modulator and a first electro-optic modulator; and a first pulse compression unit.

5                   **ON-DEMAND DUAL FREQUENCY LASER SOURCES FOR NON-LINEAR  
                    OPTICAL MICROSCOPY AND MICRO-SPECTROSCOPY IMAGING  
                    SYSTEMS**

**PRIORITY**

                    This application claims priority to U.S. Provisional Patent Application Ser.  
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**BACKGROUND**

                    The invention generally relates to label-free imaging systems, and relates in  
particular to non-linear optical microscopy and micro-spectroscopy imaging systems  
employing efficient dual frequency laser sources.

20

                    Conventional label-free imaging techniques include, for example, infrared  
microscopy, Raman microscopy, coherent anti-Stokes Raman scattering (CARS)  
microscopy and modulation transfer microscopy. Micro-spectroscopy generally  
involves capturing a spectrum from a microscopic volume in a sample, while  
microscopy generally involves capturing an intensity value as well as scanning such  
25   that multiple intensity values are captured to form picture elements (pixels) of a  
microscopy image.

Infrared microscopy involves directly measuring the absorption of vibrationally excited states in a sample, but such infrared microscopy is generally limited by poor spatial resolution due to the long wavelength of infrared light, as well as by a low penetration depth due to a strong infrared light absorption by the water in  
5 biological samples.

Raman microscopy records the spontaneous inelastic Raman scattering upon a single (ultraviolet, visible or near infrared) continuous wave (CW) laser excitation. Raman microscopy has improved optical resolution and penetration depth as compared to infrared microscopy, but the sensitivity of Raman microscopy is rather  
10 poor because of the very low spontaneous Raman scattering efficiency (Raman scattering cross-section is typically on the order of  $10^{-30}$  cm<sup>2</sup>). This results in long averaging times per image, which limits the biomedical application of Raman microscopy.

CARS microscopy, which uses two pulsed laser excitation beams (pump and  
15 Stokes beams), significantly increases the absolute scattering signal due to the coherent excitation. The CARS process, however, also excites a high level of background from the vibrationally non-resonant specimen. Such a non-resonant background not only distorts the CARS spectrum of the resonant signal from a dilute sample but also carries the laser noise, significantly limiting the application of CARS  
20 microscopy to both micro-spectroscopy and micro-sensitivity applications.

One approach to reducing the non-resonant background field in CARS microscopy is to take advantage of the fact that the non-resonant background has different polarization properties than the resonant signal. For example, U.S. Patent No. 6,798,507 discloses a system in which the pump and Stokes beams are properly  
25 polarized and a polarization sensitive detector is employed. Another approach to

reducing the non-resonant background field involves detecting the anti-Stokes field in a reverse direction. U.S. Patent No. 6,809,814 discloses a system in which a CARS signal is received in the reverse direction (epi-direction) from the sample. For transparent samples, however the epi directed signal is significantly smaller than the  
5 forward directed signal.

Modulation transfer microscopy and spectroscopy imaging systems such as stimulated Raman scattering (SRS), spectral excitation of stimulated Raman scattering (SRS Spectral), stimulated emission (SE), ground state depletion (GD), photo-thermal (PT), two-color two-photon absorption (TPA), and stimulated Brillouin scattering  
10 generally involve reliance on the non-linear interaction of two laser beams within a sample, and detection of a characteristic, such as gain or loss, of one of the excitation beams. This is in contrast to detecting a newly generated (new frequency) emission signal as is done, for example, in one-photon and two-photon excited fluorescence, spontaneous Raman scattering, coherent anti-Stokes Raman scattering (CARS),  
15 second harmonic generation, (SHG) and third harmonic generation (THG).

Such modulation transfer microscopy and micro-spectroscopy techniques require a detection scheme that provides for detection of a relatively small signal (e.g., a small gain or loss of an excitation signal) on top of noisy lasers. This is generally achieved in accordance with various embodiments based on modulation transfer - by  
20 modulating a feature of one of the laser excitation beams and measuring the signal of interest with high sensitivity. In particular, the modulation transfers to the other excitation beam due to non-linear interaction within the sample, which facilitates detection of the signal of interest using a modulation sensitive detector. If the modulation frequency is chosen to be faster than the laser noise (e.g., greater than  
25 about 200 kHz), shot-noise limited sensitivity may be achieved. Such modulation

schemes are readily compatible with beam-scanning microscopy and micro-endoscopy, video-rate imaging speeds, and multiplex excitation schemes.

An advantage of these non-linear optical imaging techniques compared to fluorescence microscopy is that they allow for specific image contrast based on  
5 intrinsic spectroscopic properties of the sample rather than extrinsic fluorescent labeling or dye staining. This is particularly important for imaging of small molecules that can be perturbed by labeling and medical diagnostics, for example, if the dyes used introduce toxicities to the sample. In CARS and SRS, chemical contrast is derived from intrinsic molecular vibrations and in TPA, SE and GD microscopy the  
10 information is derived from absorption properties of the molecules constituting the sample.

Common features of these techniques include that each requires (1) pulsed laser beams with a pulse-width shorter than about 10ps, i.e., a spectral bandwidth of at least about 30 GHz, and (2) two synchronized beams that are overlapped in time, i.e.,  
15 the repetition rate of the lasers and the time delay between the pulse-trains must be fixed. Modulation transfer techniques further require that a property (such as intensity, polarization or time delay) of one of the beams is modulated at a rate higher than 100kHz allowing measurement of the modulation transfer from the modulated beam to the second, originally un-modulated beam due to the nonlinear interaction in  
20 the sample

Coherent anti-Stokes Raman scattering (CARS) and modulation transfer microscopy and micro-spectroscopy (MTM) have different laser wavelength requirements. For CARS, Stimulated Raman Scattering (SRS) and SRS Spectral imaging, the difference between the two excitation frequencies ( $|\omega_1 - \omega_2|$ ) is selected to  
25 be resonant with a vibrational frequency of the sample. The specific wavelengths of

the two excitation fields, therefore are not critical as long as the difference frequency is as desired. Such sources are typically chosen to be in the range of about 700 nm to about 1600 nm, for which biological samples are transparent. The tuning of the difference frequency to a vibrational frequency of the sample (about 200  $\text{cm}^{-1}$  to about 5 4,000  $\text{cm}^{-1}$ ) should be to a precision of at least about 1 nm.

Stimulated emission (SE) and ground state depletion (GD) microscopy involve tuning either  $\omega_1$  or  $\omega_2$  to be electronically resonant with the sample. With photo-thermal (PT) microscopy, either  $\omega_1$  or  $\omega_2$  is chosen to match the one or two photon electronic absorption frequency. With two-color two-photon absorption (TPA), the 10 sum of  $\omega_1$  and  $\omega_2$  is chosen to be electronically resonant with the sample.

Many conventional laser systems for CARS and MTM techniques have involved the use of mode-locked lasers in order to achieve pulse widths shorter than 10ps; such pulse-widths cannot be conventionally achieved with an electrically driven laser system. A particular challenge, is that the pulses must be precisely overlapped 15 in time (synchronized), as timing jitter translates into severe noise of the signal if it is larger than the pulse width (i.e., much smaller than the required 10ps).

Certain conventional implementations of CARS microscopy, involve using two Titanium Sapphire (Ti:Sa) lasers whose outputs are electronically locked to one another (e.g., using the Lok-to-Clock system sold by Spectra-Physics, Inc. of 20 Mountain View, California or the Synchrolock system sold by Coherent, Inc. of Santa Clara, California) using feedback regarding the cavity length of one of the lasers. Such systems suffer from severe timing jitter between the pulses, making long-term experiments impossible and limiting day-to-day stability of the system.

Later developed conventional system involved the use of optical parametric 25 oscillators (OPO) for label-free microscopy that are intrinsically locked due to

synchronously pumping the OPO with the same lasers that provides the first beam.

Such OPO laser systems may also be pumped with mode-locked fiber lasers. Long-term stability, complexity and price of OPO laser systems however, remain shortcomings of such systems. Moreover, dual frequency sources employing OPO  
5 laser systems typically include an adjustable translation stage that ensures that the resulting two trains of laser pulses are temporally overlapped. Variations in temperature of the imaging system will also affect the path lengths and therefore synchronization.

There remains a need, therefore, for an efficient dual frequency laser system  
10 with reduced jitter for microscopy and micro-spectroscopy imaging systems.

## SUMMARY

In accordance with an embodiment, the invention provides an illumination system that includes a laser system for providing a first train of pulses at a center  
15 optical frequency  $\omega_1$ . The laser system includes a continuous wave laser for providing a first continuous wave field, a first chirp modulator system, a first pulse compression unit, and an optical assembly. The first chirp modulator system includes a means to receive the first continuous wave field, a means to receive a trigger signal, a first phase modulator for generating the required frequency bandwidth to support  
20 short pulsing, and a first amplitude modulator for carving out the pulses from the phase modulated field. The optical assembly is for collinearly combining the first train of pulses with a second train of pulses having a center optical frequency of  $\omega_2$  as synchronized excitation fields for non-linear modulation transfer microscopy and micro-spectroscopy.

In accordance with another embodiment, the invention provides an illumination system for non-linear modulation transfer microscopy or micro-spectroscopy, including a first laser system, and an optical assembly. The first laser system is for providing a first train of pulses at a center optical frequency  $\omega_1$  responsive to a trigger signal having a non-constant repetition rate, and includes a continuous wave laser for providing a first continuous wave field, a first chirp modulator system, and a first pulse compression unit. The first chirp modulator system is for receiving the first continuous wave field and the trigger signal, and for providing a first train of chirped pulses responsive to the trigger signal. The first chirp modulator system includes a first phase modulator and a first electro-optic modulator, and the chirped pulses have a frequency bandwidth of at least about 30 GHz. The first pulse compression unit is for temporally compressing the first train of chirped pulses to provide the first train of pulses having a temporal pulse width of less than about 10 picoseconds. The optical assembly is for collinearly combining the first train of pulses with a second train of pulses having a center optical frequency of  $\omega_2$  as excitation fields that are synchronized with the trigger signal for the non-linear microscopy or micro-spectroscopy system.

In accordance with a further embodiment, the invention provides an illumination system for non-linear modulation transfer microscopy or micro-spectroscopy, including a first laser system and an optical assembly. The first laser system is for providing a first modulated train of pulses at a first center optical frequency  $\omega_1$  responsive to a trigger signal, and includes a first continuous wave laser for providing a first continuous wave field, a first chirp modulator system, and a first pulse compression unit. The first chirp modulator system is for receiving the first continuous wave field, the trigger signal, and a modulation signal, and provides a first

modulated train of chirped pulses responsive to the trigger signal. The first chirp modulator system includes a first phase modulator and a contrast modulation system that effectively varies a characteristic of some of the chirped pulses responsive to the modulation signal to provide a first modulated train of chirped pulses, wherein the  
5 chirped pulses have a frequency bandwidth of at least about 30 GHz. The first pulse compression unit is for temporally compressing the first modulated train of chirped pulses to provide the first modulated train of pulses having a temporal pulse width of less than about 10 picoseconds. The optical assembly is for collinearly combining the first modulated train of pulses with a second train of pulses having a second center  
10 optical frequency  $\omega_2$  as excitation fields for the non-linear microscopy or micro-spectroscopy system.

In accordance with further embodiments, the invention provides an imaging system for non-linear optical microscopy or micro-spectroscopy, including a first laser system, an optical assembly, and a processor. The first laser system is for  
15 providing a first train of pulses at a first center optical frequency  $\omega_1$  responsive to a trigger signal, and includes a continuous wave laser for providing a first continuous wave field, a first chirp modulator system, and a first chirp compression unit. The first chirp modulator system is for receiving the first continuous wave field and the trigger signal, and for providing a first train of chirped pulses responsive to the trigger  
20 signal. The first chirp modulator system includes a first phase modulator and a first electro-optic modulator, and the chirped pulses having a frequency bandwidth of at least about 30 GHz. The first pulse compression unit is for temporally compressing the first train of chirped pulses to provide the first train of pulses having a temporal pulse width of less than about 10 picoseconds. The optical assembly includes  
25 focusing optics and an optical detector system. The focusing optics is for directing

and focusing the first train of pulses and a second train of pulses having a center optical frequency of  $\omega_2$  through an objective lens toward a common focal volume along an excitation path. The optical detector system includes at least one optical detector for detecting a signal of interest from within the sample that is responsive to  
5 a non-linear optical interaction within the sample, and provides a detector signal. The processor is for providing at least a portion of an image responsive to the detector signal.

In accordance with a further embodiment, the invention provides a method of providing excitation fields for non-linear modulation transfer microscopy or micro-  
10 spectroscopy. The method includes the steps of providing a first continuous wave field, providing a first train of chirped pulses responsive to the first continuous wave field and the trigger signal, wherein the chirped pulses have a frequency bandwidth of at least about 30 GHz; temporally compressing the first train of chirped pulses to provide the first train of pulses having a temporal pulse width of less than about 10  
15 picoseconds, and collinearly combining the first train of pulses having a center optical frequency  $\omega_1$  with a second train of pulses having a center optical frequency of  $\omega_2$  as the excitation fields that are synchronized with the trigger signal for the non-linear microscopy or micro-spectroscopy system.

In accordance with a further embodiment, the invention provides a method of  
20 providing excitation fields for non-linear modulation transfer microscopy or micro-spectroscopy. The method includes the steps of providing a first continuous wave field, providing a first modulated train of chirped pulses responsive to the first continuous wave field, a trigger signal and a modulation signal, such that a characteristic of some of the chirped pulses is varied responsive to the modulation  
25 signal to provide the first modulated train of chirped pulses, wherein the chirped

pulses have a frequency bandwidth of at least about 30 GHz, temporally compressing the first modulated train of chirped pulses to provide the first modulated train of pulses having a temporal pulse width of less than about 10 picoseconds, and collinearly combining the first modulated train of pulses having a first center optical frequency  $\omega_1$  with a second train of pulses having a second center optical frequency  $\omega_2$  as excitation fields for the non-linear microscopy or micro-spectroscopy system.

In accordance with a further embodiment, the invention provides a method of performing non-linear optical microscopy or micro-spectroscopy that includes the steps of providing a first continuous wave field, providing a first train of chirped pulses responsive to the continuous wave field and the trigger signal, the chirped pulses having a frequency bandwidth of at least about 30 GHz, temporally compressing the first train of chirped pulses to provide the first train of pulses having a temporal pulse width of less than about 10 picoseconds, directing and focusing the first train of pulses having a center optical frequency of  $\omega_1$  and the second train of pulses having a center optical frequency of  $\omega_2$  through an objective lens toward a common focal volume along an excitation path, detecting a signal of interest from within the sample that is responsive to a non-linear optical interaction within the sample, and providing a detector signal, and providing at least a portion of an image in a non-linear optical microscopy or micro-spectroscopy system responsive to the detector signal.

The use of the disclosed time-lens illumination systems, which are not based on mode-locking, works surprising well with modulation transfer microscopy and micro-spectroscopy. High-frequency phase-sensitive detection of modulation transfer relies on  $1/f$ -noise, i.e. the noise decreases drastically at high frequencies, and measuring the modulation transfer signal thus has drastically improved sensitivity of

$\Delta I/I < 10^{-5}$  as required for imaging under relevant bio-medical laser excitation conditions. In mode-locked lasers this arises from memory in the cavity due to an out-put coupler with high reflectivity. Time-lens lasers are pulse-on-demand lasers and thus, by definition, do not have a memory in the cavity. Thus it is unexpected to see modulation transfer microscopy work well with the disclosed time-lens illumination systems. The essential element is to provide the modulation of a property of the light field (e.g. amplitude, polarization, time-delay, or frequency) on the time-lens illumination systems at  $\omega_1$  and measure the modulation transfer signal to the second light field at  $\omega_2$  which is provided by a mode-locked laser and thus has the desired noise properties. The trigger signal for the time-lens illumination system is then derived by the pulsing of the second light field to ensured temporal synchronization of the pulses.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The following description may be further understood with reference to the accompanying drawings in which:

Figure 1 shows an illustrative diagrammatic view of an imaging system including illumination system employing a dual-frequency source in accordance with an embodiment of the invention;

Figure 2 shows an illustrative diagrammatic view the chirp modulator system of Figure 1;

Figure 3 shows an illustrative diagrammatic view of the pulse compression unit of Figure 1;

Figure 4 shows an illustrative graphical representation of a continuous wave field that has been modulated to broaden its spectrum;

Figure 5 shows an illustrative graphical representation of a train of chirped pulse in accordance with an embodiment of the invention;

Figure 6 shows an illustrative graphical representation of a train of compressed pulses in accordance with an embodiment of the invention;

5        Figure 7A shows a illustrative graphical representation of a measured spectrum of a train of laser pulses generated by a fiber laser source system, and Figure 7B shows an illustrative graphical representation of a spectrum of the output of the CW laser;

10        Figure 8 shows an illustrative graphical representation of an experimental second order interferometric auto-correlation trace of a pulse, and shows an illustrative graphical representation of a simulated second order interferometric auto-correlation trace of a pulse;

15        Figure 9 shows an illustrative graphical representation of two trains of laser pulses, one of which is amplitude modulated in accordance with an embodiment of the invention;

Figure 10 shows an illustrative graphical representation of two trains of laser pulses, one of which is time-shift modulated in accordance with an embodiment of the invention;

20        Figure 11 shows an illustrative graphical representation of two trains of laser pulses, one of which is frequency modulated in accordance with an embodiment of the invention;

Figure 12 shows an illustrative graphical representation of a Raman intensity response to frequency two pairs of excitation pulses, one of which is resonant with the sample;

Figures 13 and 14 show an illustrative photographic representation of a Stimulated Raman Scattering images acquired with a time lens source synchronized with a Chorent Mira 900 titanium:sapphire laser in accordance with an embodiment of the invention;

5        Figure 15 shows an illustrative diagrammatic view of an imaging system including an illumination system employing an arbitrary trigger signal in a dual-frequency source in accordance with another embodiment of the invention;

Figure 16 shows an illustrative graphical representation of a continuous wave field that has been modulated to broaden its spectrum in the system shown in Figure  
10    15;

Figure 17 shows an illustrative graphical representation of a train of chirped pulse in the system shown in Figure 15;

Figure 18 shows an illustrative graphical representation of a train of compressed pulses in the system shown in Figure 16;

15        Figure 19 shows an illustrative diagrammatic view of an illumination system for microscopy and micro-spectroscopy in accordance with an embodiment of the invention employing single-fiber dual frequency source system;

Figure 20 shows an illustrative diagrammatic view of an illumination system for microscopy and micro-spectroscopy in accordance with a further embodiment of  
20    the invention employing an arbitrary trigger signal in a single-fiber dual frequency source system;

Figure 21 shows an illustrative diagrammatic view of an illumination system for microscopy and micro-spectroscopy in accordance with a further embodiment of the invention employing a dual time-lens source synchronized to a mode-locked laser  
25    in a single-fiber dual frequency source system;

Figure 22 shows illustrative graphical representations of a first train of pulses having a first frequency and a second train of pulses, some of which have a second frequency and some of which have a modified second frequency; and

Figure 23 shows an illustrative diagrammatic view of an illumination system  
5 for microscopy and micro-spectroscopy in accordance with a further embodiment of the invention employing a dual time-lens source using a Mach-Zehnder optical switch in a single-fiber dual frequency source system.

The drawings are shown for illustrative purposes.

## 10 DETAILED DESCRIPTION

The invention provides illumination systems such as an on-demand dual frequency laser source system for use with label-free imaging such as microscopy and micro-spectroscopy. Figure 1, for example, shows an imaging system 10 that employs an on-demand dual frequency laser source system 12 in accordance with an  
15 embodiment of the invention. The laser source system 12 of Figure 1 includes a mode-locked laser 14 and a fiber-based laser source system that includes a fiber Bragg grating (FBG) laser 16. The laser 16 may, therefore, be a single mode semiconductor diode laser providing a continuous wave (CW) output 20. A small portion of the output from the mode-locked laser 14 is diverted (by a beam splitter) to  
20 detector 18, which provides a trigger signal 22 to chirp modulator system 24. The chirp modulator (in accordance with an embodiment) modulates the CW output to introduce periodic frequency variations in the CW output signal responsive to the trigger signal, then selects chirped pulses 26 as described below in more detail with reference to Figures 2 and 4 – 6.

The train of chirped pulses 26 is then passed through a pulse compression unit 28 for temporally compressing the train of chirped pulses to provide a train of pulses 30 having a temporal pulse width of less than for example, about 10 picoseconds as described below in more detail with reference to Figures 3 and 6. The resulting train of compressed pulses 30 is combined with the train of pulses 32 from the mode-locked laser 14 at dichroic mirror 34, and the synchronous collinear trains of pulses 30, 32 are directed to a scan-head 36, and then to a microscope 38. Within the microscope, the trains of pulses 30, 32 are directed via an objective lens 40 toward a sample 42. Collection of the signal of interest may be in a forward direction as shown by collecting illumination at a collection lens 44, then removing illumination at unwanted wavelengths by a filter 46 to provide a filtered signal 48 to a detector 50. The detector output signal 52 (an electrical signal) is then provided to a signal processor 54. The output of the signal processor is provided to a microscopy control computer 56 that also provides user control of the scan-head 36 as shown 58.

15 In certain embodiments, the signal may be detected in a reverse (epi) direction by positioning a filter 60 and detector 62 in the reverse direction with respect to the excitation trains of laser pulses. In such an epi-detection embodiment, either a backward generated signal (e.g., an anti-Stokes signal) may be generated in the reverse direction, and/or in other systems a forward directed signal may be reflected from within a relatively thick sample (e.g., a modulation transfer signal). In accordance with certain epi-detection embodiments, one or more detectors may also be supplied together with the objective lens to capture illumination that surrounds the path of the excitation trains of laser pulses (after being filtered as may be required) as disclosed for example, in Patent Cooperation Treaty Patent Application No.

PCT/US2010/54925, the entire disclosure of which is hereby incorporated by reference in its entirety.

In accordance with some embodiments (e.g., for modulation transfer microscopy and micro-spectroscopy systems), the train of chirped pulses 26 may  
5 further be modulated with a high frequency modulation (e.g., at least about 100 kHz) to provide a contrast mechanism. Generally (and as discussed in more detail below with reference to Figures 7A – 11), a contrast modulation signal generation unit 64 provides a high frequency modulation signal 66 to the signal processor 54 and to a modulation controller 68, which in turn provides a modulation control signal 70 to the  
10 laser source system 12, either by providing the modulation using an existing component of the laser source system 12 (e.g., the switch 76 as shown in Figure 2), or using a separate modulator.

In certain embodiments, the chirp modulator system 24 and the pulse compression unit 28, may be provided within an optical –fiber. The invention  
15 provides therefore, in accordance with certain embodiments, a fiber-based time-lens source that can be synchronized with any free-space or fiber based mode-locked lasers, or other time-lens sources. It can be used for two-color microscopy and micro-spectroscopy, such as coherent anti-Stokes Raman scattering microscopy and modulation transfer microscopy. As for synchronization with a mode-locked laser, an  
20 optical detector detects the optical pulse train from the laser and transforms it into an electrical pulse train that is synchronized with the optical pulse train. This electrical pulse train is used to drive an intensity modulator to modulate a CW laser, hence another optical pulse train can be generated from this time-lens source and automatically synchronized with the original mode-locked laser.

Figure 2 shows an embodiment of a chirp modulator system 24 that includes a phase modulator unit 72 that includes two phase modulators, a pre-amplifier 74, an intensity modulator 76 (e.g., an electro-optic switch), another pre-amplifier 78, a power amplifier 80 that provides the train of chirped pulses 26 to the compression unit 28. The chirp modulator system 24 also includes a power divider 84 that receives the trigger signal 22 (via a radio frequency (RF) delay device 82), and provides a trigger control signal to a narrowband RF filter 86, and to the intensity modulator 76 via another RF delay device 88. The narrowband RF filter 86 is used to derive the Nth harmonic ( $N > 1$ ) of the repetition rate of the mode-locked laser 14 to drive the phase modulator unit 72.

In particular, and with reference to Figures 4 and 5, the phase modulator introduces a periodic frequency variation in the CW output as shown at 75 (which is shown to be the output of the pre-amplifier 74 shown in Figure 2). The period of the frequency variations ( $Nf_r$ ) is based on the Nth harmonic of the repetition rate of the mode-locked laser 14. In other words, the pulse carving is at the repetition rate of the mode-locked laser, and phase modulation is at Nth ( $N > 1$ ) harmonic of the repetition rate, so the period of the carved pulse train is N times the phase modulation period. The intensity modulator 76 provides chirped pulses as shown at 26 in Figure 5. The fiber amplifiers 74, 78 are used compensate for the power loss due to the pulse carving and the insertion loss of the optical components. The third fiber amplifier 80 further boosts the output power.

With reference to Figure 3, the compression unit 28 includes a chirped fiber Bragg grating (CFBG) 92 that provides different path lengths for different frequencies which effectively compresses the chirped pulses (26) having a bandwidth (e.g., 30 GHz) into pulses 30 (e.g., 2 ps) that are narrow in time as shown in Figure 6.

The compression unit 28 is a reflected compression unit in this embodiment, so a directional device 94 ensures and/or significantly reduces any adverse effects from reflected illumination as is well known in the art. By spectral pulse shaping therefore, a parabolic pulse in the time domain may be obtained. By converting such a parabolic pulse into the electrical domain (O/E detector), a nearly perfect time lens can be obtained, improving the pulse quality.

Single-pass lasers compatible with the above synchronization schemes are typically based on Yb- or Er-doped fibers and may thus provide laser beams in the range from 1020nm to 1065nm, and 1530nm to 1580nm, respectively. Er-based designs may be further frequency doubled to cover a range from 765nm to 790nm, which is a commonly used range for certain biomedical imaging. Both single-pass lasers are flexible in the type of oscillator (master clock) to which they are synchronized. As discussed further below, the master clock (or trigger signal) may be provided by a first laser as discussed above, or may be provided by an oscillator or even a source that provides a trigger signal that is non-constant in time as discussed further below.

Two different examples, therefore, may be provided. First, an all-fiber laser system for label-free microscopy can be designed based on synchronizing a Yb-based laser with an Er-based laser. Either may be used as the single-pass laser, with the other used as the oscillator. This combination allows the ability to cover a vibrational frequency range from  $2850\text{cm}^{-1}$  to  $3680\text{cm}^{-1}$ . Second, a hybrid fiber-solidstate system may be designed based on synchronizing a Yb-based single pass laser to a Ti:Sa solid-state laser, which is tunable from 750nm to 1050nm and thus allows us to cover a vibration frequency range from  $0\text{cm}^{-1}$  to  $3800\text{ cm}^{-1}$  (full vibrational range of biological molecules). An additional advantage of this system is that many

microscopes are already equipped with a Ti:Sa laser and may be modified to provide label-free imaging capabilities. The use of time-lens lasers are also compatible with broadband excitation based on picosecond (ps)-lasers and femtosecond (fs)-lasers rather than narrowband excitation based on ps-lasers only. This is required for label-free spectral imaging as disclosed for example in U.S. Published Patent Application Publication No. 2010/0188496, the disclosure of which is hereby incorporated by reference in its entirety.

With regard to synchronization with another time-lens source, the electrical pulse train used to drive the intensity modulator, and the RF tone used to drive the phase modulator can be generated from the same RF source, which automatically guarantees synchronization of the two time-lens sources. Another key feature of this synchronized time-lens source is that tuning of the delay between the two synchronized sources can be readily accomplished through tuning the RF delay line of the time-lens source without movement of any optical components. This improvement eliminates the need to mechanically scan the optical delay line, which could cause misalignment.

To obtain short pulses down to a few picoseconds, a high-order RF tone can be obtained therefore through narrow-band RF filtering of the electrical pulse train, and then used to drive the phase modulators. This leads to a broadening of the spectrum as shown at 75 in Figure 4. After dispersion compensation using fiber based chirped fiber grating or free space based grating pairs, short pulses, e.g., 2 ps, can be obtained as shown at 30 in Figure 6. The order of the phase modulator unit 72, the intensity modulator 76 (e.g., a Mach Zender electro-optical switch) and the Yb-doped fiber amplifiers 74, 78 and 80 may be changed in other embodiments (e.g., the intensity modulator 76 may precede the phased modulator unit 72).

Referring again to Figure 1, the laser 16 may provide a continuous wave output, or in accordance with further embodiments, may provide an output that is discontinuous, yet permits chirped pulses to be created from the output. For example, the laser 16 may provide a CW output for on-periods of, for example, 1 nanosecond  
5 (ns), as long as the selection of the chirped pulses occurs while the laser is on. In order to achieve this, the trigger signal may be provided to a laser controller that controls the laser 16. As used herein, the term “continuous wave” therefore, means that the laser is on at least as long as necessary for chirped pulses to be formed.

Figure 7A shows at 100 the measured spectrum of the train of laser pulses 30  
10 generated by the fiber laser source system 12, and shows at 102 the spectrum of the output 20 of the CW laser 16. The maximum resolution bandwidth of the optical spectrum analyzer used was 0.05 nm. Figure 7B shows at 104 a simulated pulse intensity profile of a pulse in the train of pulses 30. Figure 8 shows an experimental second order interferometric auto-correlation trace (at 110) of the pulse shown at 104,  
15 and shows a simulated second order interferometric auto-correlation trace (at 112) of the pulse shown at 104.

The invention therefore provides a synchronized dual frequency laser source for CARS and MTM based on time-lens lasers that allows for the generation of pulses of for example 2 ps – 10 ps with minimal timing jitter ( $\ll 10$ ps) as required for  
20 CARS and MTM, but is more robust and less expensive to produce. Time lens lasers again rely on electrically locking a second laser to a first laser (clock) but surprisingly have less timing jitter than previous electronic synchronization approaches based on cavity length feedback.

The dual-frequency laser source may be used in an imaging system as shown,  
25 for example, in Figure 1 in accordance with an embodiment of the invention. Micro-

spectroscopy systems generally involve capturing an image for spectroscopic analysis without scanning the sample. Microscopy systems typically generate a picture element (a pixel) from an intensity image and then scan the sample to create an image. Imaging techniques may include coherent anti-Stokes Raman scattering (CARS), or  
5 any of a variety of modulation transfer microscopy techniques including for example, Stimulated Raman Scattering (SRS), SRS Spectral imaging Stimulate Emission (SE), Ground State Depletion (GD), Photo-Thermal (PT), Two-Color Two-Photon Absorption (TPA), and Stimulated Brillouin Scattering.

For a CARS system, the system may provide the first train of laser pulses 30  
10 as a pump beam and the second train of laser pulses 32 as a Stokes beam such that non-linear optical interactions in the sample produce an anti-Stokes field in each of the forward and epi direction when the difference frequency between the pump and the Stokes beams is vibrationally resonant with the sample as disclosed for example in further detail in U.S. Patent No. 6,809,814. With reference to Figure 1, for example,  
15 the train of laser pulses 30 may provide a pump train of pulses and the train of laser pulses 32 may provide Stokes train of pulses. The pump and Stokes trains of pulses are synchronous and collinear, and are directed via a scan-head 36 and an objective 40 toward a sample 42.

Due to the non-linear interaction of the sample with the pump and Stokes  
20 fields, an anti-Stokes field is generated due to the non-linear interaction of the excitation fields with the sample, and the anti-Stokes field is predominantly generated in the forward direction, although a smaller portion (and less background-free portion) is generated in the backward (or epi) direction. When one of the pump and Stokes fields is modulated with a contrast modulation (as disclosed in further detail below),  
25 the detection signal 52 may be processed in connection with the modulation signal to

extract the signal of interest from the overall detection signal. The modulation provides that the difference frequency between the two excitation fields  $\omega_1$  and  $\omega_2$  is resonant with a vibrational resonance of the sample. When one of the excitation fields (e.g.,  $\omega_1$ ) is modulated to be in resonance with and out of resonance with the sample, the detection system may lock into the modulation frequency  $f_m$ , and thereby extract the signal of interest from any background.

A Modulation Transfer Microscopy system also provides the two trains of laser pulses to the sample, except that one of the pulse trains is modulated, and a change in a characteristic of the other of the pulse trains due to the non-linear optical interactions in the sample is detected.

For a Stimulated Raman Scattering (SRS) system for example, the difference frequency between the two excitation pulse trains is selected to be vibrationally resonant with the sample. One of the trains of excitation pulses is modulated, and either a gain or loss of intensity of the other of the trains of excitation fields is detected at the modulation frequency using a lock-in detection system as will be described below in further detail. Further details regarding the operation and components of such an SRS system are disclosed in U.S. Published Patent Application Publication No. 2010/0046039, the disclosure of which is hereby incorporated by reference in its entirety.

With reference again to Figure 1 for example, the dual frequency laser source system may provide collinear pump and Stokes pulse trains 30, 32 as discussed above wherein the difference frequency between the pump and Stokes frequencies ( $\omega_1 - \omega_2$ ) is selected to be vibrationally resonant with the sample. The excitation fields 30, 32 are directed via a scan-head 36 (e.g., that scans in  $x$  and  $y$  directions) and microscope optics (including an objective lens 40) toward a focal volume within a sample 42.

With such an SRS imaging system, one of the trains of laser pulses is modulated at a modulation frequency  $f$  by a modulation system (as discussed in further detail below) that includes, for example, a modulator (e.g., 24), a controller 68 and a modulation source 64. The modulation may be, for example, of every other or every  $n$  pulse. The modulation source 64 provides a common modulation control signal 66 to the controller 54, and the modulation (amplitude, phase or frequency) of the integrated intensity of substantially all of the optical frequency components of the non-modulated train of laser pulses due to the non-linear interaction of the train of laser pulses 30 with the train of laser pulses 32 in the focal volume, is detected by the processor 54 as a contrast mechanism such that the signal gain of the Stokes (or loss of the pump) field may be detected as the signal of interest.

Due to the non-linear interaction of the excitation fields with the sample, the pump beam is depleted and the Stokes beam is increased. Because Raman gain/loss results in an increase/depletion of the excitation beams, the modulation transfer illumination originating from the non-linear optical interaction is directed in the forward direction only. In highly scattering samples such as tissue, the forward traveling illumination is however, back-scattered within the sample. Detection is therefore, possible in the epi-direction for scattering samples, and as such Raman/Gain Loss microscopy therefore has the potential to be implemented for endoscopic imaging.

For SRS Spectral imaging, the difference frequency between the two excitation pulse trains is again selected to be vibrationally resonant with the sample, but for one of the excitation fields, a broadband field is used and is shaped to be selectively resonant with further characteristics of the sample such as multiple difference frequencies. The shaped field may be provided, for example, by a spectral

shaper unit that includes a spectral dispersive element, a lens and a spatial light modulator. The dispersive element spectrally disperses each broadband pulse, and the spatial light modulator then modulates different frequency components of the spectrally disperse broadband pulse to provide a train of shaped pulses. Again one of  
5 the trains of excitation pulses is modulated (e.g., at a frequency of at least about 100 kHz), and either a gain or loss of intensity of the other of the trains of excitation fields is detected at the modulation frequency using a lock-in detection system. Further details regarding the operation and components of such an SRS Spectral imaging system are disclosed in U.S. Published Patent Application Ser. No. 12/690,579 filed  
10 January 20, 2010, the disclosure of which is hereby incorporated by reference in its entirety.

In stimulated emission microscopy, the second beam excites molecules in the sample into an excited state, and when these molecules interact with the first beam, they emit light into that beam with matched polarization and phase, increasing the  
15 brightness of that beam. In ground state depletion microscopy, the second beam removes molecules from the ground state by promoting them to an excited state. In this case, fewer molecules in the sample are in the ground state, and thus the absorption of the first beam is reduced. In order to modify the populations of molecules in the sample, the beams are typically chosen to match the one or two-  
20 photon electronic absorption resonances of the molecule in the ground or excited state. Femtosecond (fs) pulse-width lasers may be used to maximize the signal, and the probe pulse may also be delayed for improving the modulation transfer signal.

For stimulated emission microscopy, the emission of non-fluorescent or weakly fluorescent samples is stimulated to an electronic excited state. If the sample  
25 is fluorescent, a spontaneous fluorescent emission would occur, bringing the energy

level back down to an electronic non-excited state. If the sample is non-fluorescent, a non-radiative decay will occur. Each excitation pulse from the modulated train of excitation pulses causes chromophores in the sample to change energy states from the low (or ground) state to the electronic excited state, and a quickly following  
5 stimulation pulse from the train of stimulation pulses stimulates emission, causing the energy to be released as illumination at the excitation frequency, increasing the total radiative quantum yield by as much as from  $10^{-5}$  to unity. As a result, the originally weakly or non-fluorescent species are turned into highly radiating species. Further details regarding the operation of a stimulated emission system of various  
10 embodiments are disclosed in U.S. Published Patent Application Publication No. 2010/0252750, the disclosure of which is hereby incorporated by reference in its entirety.

In accordance with further embodiments, the modulation transfer technique may employ one and two color photo-thermal (PT) microscopy. Photo-thermal  
15 microscopy relies on modified transmission properties of the sample for a first beam (probe beam) as a result of heating of a sample by the second beam (pump beam), e.g., by thermal lensing or thermal phase shifting. In order to heat the sample volume effectively, the pump beam is typically chosen to match the one or two-photon electronic absorption frequency of the molecule. The probe beam may be either  
20 pulsed or continuous wave (cw). In thermal lens microscopy, an aperture is typically used to convert changes in the beam profile as a result of the non-linear interaction into changes in intensity.

The detection system includes a filter that passes only the probe illumination, a mask, a lens and one or more photo-detectors such as a photodiodes that generally  
25 surround the excitation path of the pump and probe beams. The photo-detector is

coupled to a demodulation unit (e.g., a phase sensitive detection device such as a lock-in amplifier), which is in turn coupled to a computer modulation source. A demodulation unit is also coupled to the modulation source to ensure synchronization.

When the sample exhibits third order non-linear excitation vibrational  
5 resonance with a difference between the pump and Stokes fields, the sample will absorb heat due to the vibrational resonance. When this happens, the index of refraction of the focal area will change, and this will cause the probe beam to diverge. By employing the mask and the demodulation unit, the detection system may detect changes in the probe beam that are due to the changes in the index of refraction.

10 The local heating therefore, induces a local refractive index gradient, because the refractive index of most medium is temperature dependent. Such a local refractive index gradient forms effectively a thermal diverging *lens*, which can be further read out by a probe beam whose intensity is detected behind a mask such as an iris diaphragm. The intensity distribution of this probe beam at the objective focus could  
15 be specially shaped to sensitively sense the non-uniform local refractive index gradient. The objective may, therefore, be designed to control and shape the intensity distribution of the probe illumination so that a change in an index of refraction of a sample may be sensitively probed. Similarly, the mask in front of the far-field detector could also be specially designed to be *conjugate* with the intensity  
20 distribution of the probe beam at the focus.

To optimize the imaging sensitivity, phase sensitive detection may be utilized with modulation of the pump and/or Stokes at a high frequency (>10 kHz). The resulting probe illumination detected behind the mask will carry an intensity modulation at the same modulation frequency, which can be sensitively demodulated  
25 by a lock-in amplifier.

In accordance with further embodiments, a photo-thermal modulation transfer system may provide non-linear optical excitation using a two-photon laser source. The output of the two-photon source is modulated by a modulator at a modulation frequency. The second source provides probe illumination (for example, a continuous wave (CW) laser output or a high repetition rate laser output (e.g., 1 GHz) having a different frequency) that is combined with the modulated trains of laser pulses at a dichroic mirror 58. The combined modulated train of laser pulses and the probe laser output are then all provided to the objective lens that focuses the beams onto a focal area of a sample. The two photon excitation is provided therefore, by having two photons of relatively low energy excite a fluorophore through two-photon absorption of a molecule in the sample, resulting in the emission of a fluorescing photon.

Modulation transfer microscopy and micro-spectroscopy may also be performed in accordance with various embodiments of the invention using two-color two-photon absorption techniques. Two-color two-photon absorption (TPA) techniques rely on the combined absorption of two photons by the sample and involve exciting the molecules into an excited electronic state. Chemical contrast is achieved by tuning the sum energy of the two photons into the energy of the electronic excited state. Femtosecond (fs) pulse-width lasers may be used to maximize the signal, but excitation with picosecond pulses is also possible for certain applications.

Modulation transfer microscopy may further be performed using stimulated Brillouin Scattering microscopy, as well as cross-phase modulation in accordance with further embodiments of the invention.

The contrast modulation system employed for modulation transfer microscopy techniques may include a separate modulation device or may use one of the devices in the dual-frequency laser source system itself for this additional modulation. Again

with reference to the system of Figure 1, the system may include a contrast modulation control system that modifies a characteristic of some of the pulses of the train of laser pulses 30. The modulation control signal 66 is provided to the image processing unit 54, where the signal of interest may be extracted from the detection  
5 signal 52. The modulation may be performed by a separate modulation unit, or may be performed in an existing device in the laser system such as the intensity modulator 70 as shown in Figure 1.

It has further been discovered that the time-lens laser may be intrinsically modulated for MTM by modifying the electric signal derived from the trigger signal  
10 by either modulating the amplitude or temporal delay or frequency of some of the pulses. Because such modulation may be performed before the pulse intensity is amplified, requirements on the laser amplifiers can be reduced.

As shown in Figure 9, in accordance with an embodiment, the modulation system may provide amplitude modulation of one of the excitation pulse trains (e.g.,  
15 the  $\omega_b$  beam) to provide a modulated  $\omega_b$  pulse train as shown at 150 such that only alternating pulses of the  $\omega_b$  pulse train are coincident with the pulses of the  $\omega_a$  pulse train. Such amplitude modulation may be achieved using the intensity modulator 76 shown in Figure 2. In other embodiments, the modulation may be achieved using a Pockel cell and polarization analyzer as the modulator, and a Pockel cell driver as the  
20 controller. Figure 9 shows an illustrative example in which the modulation rate is half the repetition rate of the laser such that every other pulse of the original  $\omega_b$  pulse train is reduced in amplitude to provide that stimulated Raman scattering does not substantially occur in the focal volume with the pulses having the reduced amplitude. If the modulation rate is of the same order of the repetition rate of the laser,  
25 countdown electronics must be utilized to guarantee the synchronization (phase)

between the modulation and the pulse train. A wide variety of different modulation rates are also possible. In further embodiments, the contrast pulses may have an amplitude that is substantially zero by switching off the pulses at the modulation frequency, for example using an electro-optic modulator (such as a MEMs device or a  
5 galvanometric scanner) or an acousto-optic modulator.

Amplitude modulation of the pump or Stokes pulse trains may therefore be achieved, and the increase of the Stokes pulse train or decrease of the pump pulse train may be measured. By modulating the pump train of pulses and then detecting the Stokes train of pulses from the focal volume, Raman gain may be determined by  
10 the processing system. In further embodiments, the Stokes beam may be modulated, the pump beam may be detected from the focal volume, and Raman loss may be determined by the processing system.

The system of the above embodiment of the invention, therefore, provides that stimulated Raman scattering microscopy may be achieved using a modulation of one  
15 of the pump or Stokes beams as a contrast mechanism. Stimulated Raman scattering microscopy bears most of the advantages of the existing methods. In particular, (1) it is an optically stimulated process that significantly enhances the molecular vibrational transition efficiency compared to conventional Raman microscopy that relies on spontaneous scattering; (2) it is a nonlinear process in which the signal is only  
20 generated at the microscopy objective focus, rendering a three-dimensional sectioning ability; (3) it only probes the vibrational resonance, and it is free from interference with the non-resonant background, unlike in the CARS microscopy where non-resonant background is always present; (4) the signal always scales linearly with the solute concentration, allowing ready analytical quantification; (5) the signal can be  
25 free from sample auto-fluorescence; (6) the phase matching condition is always

satisfied for any relative orientations of the beams, unlike in the CARS microscopy; (7) visible and near-IR beams are used resulting in a higher penetration depth and spatial resolution than IR absorption microscopy; and (8) the detection of Raman gain or loss is also unaffected by ambient light, which permits such systems to be used in  
5 open environments.

The process may be viewed as a two photon process for excitation of a vibrational transition. The joint action of one photon annihilated from the pump beam and one photon created to the Stokes beam promotes the creation of the molecular vibrational phonon. The energy of the pump photon is precisely converted to the sum  
10 of the energy of the Stokes photon and the molecular vibrational phonon. As in any two photon optical process, the transition rate is proportional to the product of the pump beam intensity and the Stokes beam intensity. It is obvious that a molecular vibrational level is necessary for this process to happen, as required by the energy conservation. No contribution therefore, from non-resonant background would be  
15 present. This represents a significant advantage over CARS microscopy which is severely limited by non-resonant background which not only distorts the spectrum but also carries unwanted laser noise.

The process may also be treated as a stimulated version of the spontaneous Raman scattering. In spontaneous Raman scattering, the Stokes photon mode is empty  
20 in the initial state and the vacuum field serves as the stimulated Stokes beam, which is why the efficiency is extremely low. The transition rate is only proportional to the pump beam intensity. In stimulated Raman scattering however, the Stokes photon mode has a large number of pre-occupied photons due to the presence of a strong laser beam, and the scattering process becomes stimulated in analogy to the  
25 stimulated emission. As a result, the transition rate is proportional not only to the

pump beam intensity as in spontaneous Raman scattering, but also to the number of pre-occupied photons in Stokes photon mode that is again proportional to the Stokes beam intensity.

The process may also be accounted for as a heterodyne interference between  
5 the pump beam (or the Stokes beam) and a corresponding third-order nonlinear induced radiation at the same optical frequency as the pump beam (or the Stokes beam). These two third-order nonlinear induced polarizations for stimulated Raman gain and loss are different from each other, and are also distinct from the one responsible for CARS generation. If there are no additional electronic resonances  
10 involved, however, their absolute sizes are all the same.

For stimulated Raman loss of the pump beam, this third-order nonlinear induced polarization radiates at the pump beam frequency. The intensity dependence of this nonlinear radiation scales linearly with the pump beam and quadratically with the Stokes beam. Its final phase is 180 degree lag behind that of the input pump beam  
15 at the far field detector. The interference therefore, between this nonlinear radiation and input pump beam results in an attenuation of the pump beam itself, and the intensity dependence of the interference term scales linearly with both the pump beam and the Stokes beam.

For stimulated Raman gain of the Stokes beam, a different third-order  
20 nonlinear induced polarization radiates at the Stokes beam frequency. The intensity dependence of this nonlinear radiation scales quadratically with the pump beam and linearly with the Stokes beam. Its final phase is the same as that of the input Stokes beam at the far field detector. The interference therefore, between this nonlinear radiation and input Stokes beam results in an increase of the Stokes beam itself. The

intensity dependence of the interference term again scales linearly with both the pump beam and Stokes beam.

Two other closely related third-order nonlinear induced polarizations for non-resonant background are also excited by the two input laser beams, and radiate at the pump frequency and the Stokes frequency, respectively. Their relative phases however, are either 90 or 270 degree lag behind those of the pump beam or the Stokes beam at the far field detector. As a consequence of these orthogonal phase relationships, they do not interfere with the input pump beam or the Stokes beam, giving no detectable contribution to the loss or the gain of the signal.

Although the use of amplitude modulation has the highest modulation depth, this approach may introduce a linear background due to a modulation of the temperature or refractive index of the sample due to the intensity modulation on the sample. In accordance with another embodiment, the modulation system may provide time-shifting modulation. Figure 10 shows at 160 the  $\omega_a$  pulse train 20 as well as a modulated  $\omega_b$  pulse train 162 that includes alternating pulses that coincide with a  $\omega_a$  pulse, while the remaining pulses 164 are time shifted (e.g., by  $\Delta t$  as shown) such that they do not coincide with a  $\omega_a$  pulse.

Modulation of one or both of the pump and Stokes beams may also be achieved by frequency modulation as disclosed, for example, in U.S. Patent No. 7,352, 458, the disclosure of which is hereby incorporated by reference in its entirety. As shown in Figure 11, in a frequency modulation system, the frequency of one or both of the pump and Stokes beams is alternately modulated at a modulation frequency such that a difference frequency between the pump and Stokes beams (e.g.,  $\omega_p - \omega_s$ ) is tuned in and out of a vibrational frequency of the sample. The detector then detects the gain/loss that is generated through non-linear interaction of  $\omega_p$  and  $\omega_s$

and the sample responsive to the modulation frequency. An output signal may be passed through a lock-in amplifier such that only changes at the time scale of the modulation period are provided in the final output. In accordance with further embodiments, other modulation schemes may be employed such as time-delay  
5 modulation, spatial beam mode modulation, etc., which will each introduce a modulation of a generated signal.

As shown in Figure 11, in such a frequency modulation system, the  $\omega_b$  pulse train is modulated such that the pulses alternate between frequencies of  $\omega_b$  and  $\omega_b'$  as shown at 170, and the pulses are coincident with the pulses from the  $\omega_a$  pulse train.  
10 As shown in Figure 12, while the difference frequency of  $\omega_b - \omega_a$  yields high Raman intensity at resonance with a sample as shown at 180, the modified difference frequency of  $\omega_b' - \omega_a$  yields only a background signal as shown at 182. Systems for providing frequency modulation of a pulse train may, for example, include an additional laser source system of the invention to provide laser outputs of more than  
15 two frequencies.

In accordance with further embodiments, the laser source system may include two CW lasers, two chirp modulator systems and two pulse compression units, and further, the chirp modulator systems may be governed by a trigger signal that is non-constant. In particular, the trigger signal may be set by a scanning system that moves  
20 either the objective or a sample stage at a non-constant rate (e.g., more slowly when changing directions of scanning). Such a system therefore, provides the use of two such time-lens lasers to produce a laser pulse train with variable repetition rate; this also allows matching of the repetition rate to a pixel clock of a laser scanning microscope or other data acquisition systems. This approach permits the illumination  
25 of each pixel with a fixed number of laser pulses.

Figure 13, for example, shows a Stimulated Raman Scattering image acquired with a time lens source synchronized with a Chorent Mira 900 titanium:sapphire laser with a 2 ps pulse width in both lasers in a system as discussed above with reference to Figure 1. As shown at 190, the image shows the stratum corneum layer near the surface of wild type mouse skin. Hexagonal corneocytes and a few cells of the viable epidermis (as well as hairs) are visible. The image shown at 192 shows two sebaceous glands at a depth of 40 microns below a skin surface. Both images show the density of CH<sub>2</sub> stretching vibrational modes, with predominantly lipid contrast. The images measure approximately 250 microns across, and show that such a system may be used to acquire SRS images. The acquisition time was 30 seconds per frame, and the time lens system was modulated by an internal Mach-Zender electro-optic modulator at 8.8, MHz.

Figure 15 shows an imaging system 200 in accordance with another embodiment of the invention wherein microscopy components 36 – 70 as the same as those discussed above with reference to the system of Figure 1. In Figure 15, the illumination system however, includes two time-lens sources, each of which is responsive to a common trigger signal. In particular, a trigger signal 202 is provided by the scan-head 36. The trigger signal may be constant, or in an embodiment may be non-constant as the scanning of the sample may be executed at a non-constant rate. The trigger signal 202 is provided to a controller 204 that causes the trigger signal to arrive at each of two chirp modulator systems 206 and 208, each of which receives a continuous wave beam from a first CW source 210 (at a first frequency  $\omega_1$ ) and a second CW source 212 (at a second frequency  $\omega_2$ ). As discussed above with reference to Figures 1 and 2, the chirp modulator systems provide first and second trains of chirped pulses that are then each passed through a compression unit 214 and

216 respectively to provide two trains of pulses 224 and 226 at two different frequencies that are combined by a dichroic mirror 220 and provided to the scan-head for imaging as discussed above. In various embodiments, the first and second frequencies ( $\omega_1$  and  $\omega_2$ ) may be chosen to provide CARS imaging, SRS imaging, SRS  
5 Spectral imaging, Stimulated Emission imaging, Ground State Depletion imaging, Photo-Thermal imaging, Two-Color Two-Photon Absorption imaging or Stimulated Brillouin Scattering imaging as discussed above.

Because the dual-frequency laser source system of Figure 15 however, provides the synchronous pulses on-demand, the trigger signal may be non-constant  
10 as mentioned above. The trigger signal, for example, may be provided by the scan-head 36 as the objective or sample are moved during imaging. Such scanning systems may provide non-constant movement, particularly when changing directions. The system provides that irrespective of the time between clock pulses of the trigger signal, the output pulse trains will remain synchronous.

15 Figure 16, for example, shows at 230 a CW output from one of the sources 210, 212 after having been modified by a phased modulation unit as discussed above with reference with Figure 2. Note, however, that the time between adjacent sets of frequency modulation sweeps ( $Nt_1$  and  $Nt_2$  as shown at 230) are not the same; the time  $t_2$  is shorter. The chirp modulator system however, produces a train of chirped  
20 pulses that are also not provided at a constant rate, but rather are provided in synchronization with the trigger signal as shown, wherein adjacent chirped pulses are also separate by times  $t_1$  and  $t_2$  as shown at 240 in Figure 17. Similarly, the compressed pulses 250 are also provided at the same non-constant rate such that adjacent compressed pulses are separate by times  $t_1$  and  $t_2$  as shown in Figure 18.  
25 Both laser systems employ this technique to ensure that each pair of  $\omega_1$  and  $\omega_2$  pulses

are synchronized with each other, yet the pulse trains are provided at a rate that is dynamically provided by the trigger signal 202.

The lasers 210 and 212 may each provide a continuous wave output, or in accordance with further embodiments, may provide an output that is discontinuous, yet permits chirped pulses to be created from each output. For example, each laser 210 and 212 may provide a CW output for on-periods of, for example, 1 nanosecond (ns), as long as the selection of the chirped pulses occurs while the laser is on. In order to achieve this, the trigger signal may be provided to a laser controller that controls the lasers 210, 212.

Dual-frequency sources of the present invention, may further be provided as all-fiber sources. In particular, and with reference to Figure 19, an illumination system 300 may include a CW source 302 and a single-fiber system 304, and control electronics. In certain embodiments, the CW source 302 may be tunable to provide frequency modulation. The single-fiber system 304 may include a mode-locked laser 306, a fiber beam sampler 308, a phase modulation unit 310, a pre-amplifier 312, an intensity modulator 314, another pre-amplifier 316, a power amplifier 318 and a pulse compression unit 320. The pulse compression unit may be a reflective pulse compression unit as described above with reference to Figure 3, or may be a transmission pulse compression unit in various embodiments. The single-fiber system 304 also includes a fiber combiner 322 for combining the fibers carrying the first and second pulse trains to provide the synchronous dual frequency output 324 as discussed above. The control electronics include a detector 326, a power divider 328, RF delay units 330, 332 and a narrow-band RF filter or spectral pulse shaper 334 as discussed above. The illumination system 300 provides dual frequency trains of laser

pulses for microscopy and micro-spectroscopy as described above with reference to Figures 1 and 15, yet is substantially provided within a single optical fiber system.

In accordance with a further embodiment, an illumination system 400 may include a single-fiber system 402, two CW sources 404 and 406 and control  
5 electronics as shown in Figure 20. The single-fiber system includes a first phase modulation unit 408, a pre-amplifier 410, a first intensity modulator 412, another pre-amplifier 414, a power amplifier 416 and a first pulse compression unit 418, which again may be either a reflective or transmissive pulse compression unit in various embodiments. The single-fiber system also includes a second phase modulation unit  
10 420, a pre-amplifier 422, a second intensity modulator 424, another pre-amplifier 426, a power amplifier 428 and a second pulse compression unit 430, which again may be either a reflective or transmissive pulse compression unit.

The single-fiber system 402 also includes a fiber combiner 432 for combining the fibers carrying the first and second pulse trains to provide the synchronous dual  
15 frequency output 434 as discussed above. The control electronics include a trigger or clock signal generator 436 (e.g., a scan-head or other clock signal), a spectral pulse shaper 438 (for controlling the phase modulators 408 and 420), and first and second power dividers 440 and 442 (for controlling the controlling the intensity modulators 412 and 424). The illumination system 400 provides dual frequency trains of laser  
20 pulses for microscopy and micro-spectroscopy as described above with reference to Figures 1 and 15, yet is substantially provided within a single optical fiber system.

Further dual-frequency sources of the present further embodiments of the invention may further be provided as all-fiber sources that include two CW lasers. In particular, and with reference to Figure 21, an illumination system 500 may include  
25 two CW sources 502 and 504 (e.g., 1064 nm and 1030 nm laser diodes) that are

combined by a wavelength division multiplexer 554 and coupled to a current modulator 506, as well as a single-fiber system 508, and control electronics. The single-fiber system 508 may include a mode-locked laser 510 (e.g., Ti:Sa), a fiber beam sampler 512 (e.g. beam splitter), a phase modulation unit 514 for providing  
5 modulation of both laser outputs from the sources 502 and 504 (e.g., two phase modulators), a pre-amplifier 516, an intensity modulator 518 (e.g., a Mach-Zehnder intensity modulator), another pre-amplifier 520, a power amplifier 522 and a pulse compression unit 524. The amplifiers may be, for example, Yb<sup>3+</sup> doped fiber amplifiers. The pulse compression unit 524 may be a reflective pulse compression  
10 unit as described above with reference to Figure 3, or may be a transmission pulse compression unit in various embodiments.

The fiber-based pulse compression unit may include a circulator 548 and two chirped fiber Bragg gratings 544 and 546 (e.g., one for 1064 nm and the other for 1030 nm). The single-fiber system 508 also includes a fiber combiner 526 (e.g.,  
15 dichroic mirror) for combining the fibers carrying the first and second pulse trains to provide the synchronous dual frequency output 528 as discussed above. In accordance with further embodiments, the fiber-based pulse compression unit may be free-space grating pair.

The control electronics include a detector 530, a power divider 538, RF delay  
20 units 532, 534, a narrow-band RF filter or spectral pulse shaper 536 as discussed above, a narrowband RF amplifier 540, and a broadband RF amplifier 542. The illumination system 500 provides dual frequency trains of laser pulses for microscopy and micro-spectroscopy as described above with reference to Figures 1 and 15, yet is substantially provided within a single optical fiber system. The photodetector 530  
25 produces an electronic signal representing the pulse train at the same frequency as the

optical pulse train output by the source 510 (e.g., 76 MHz). The narrow band RF filter 536 provides the narrow band RF amplifier 540 with a sinusoidal output electrical signal (e.g., 9.95 GHz).

All fiber devices are polarization maintained except for the amplifiers, the  
5 outputs of which are passed through polarization controllers 550, 552 as shown in order to align polarization after amplification. The outputs of the two lasers 502, 504 are directly current modulated by the modulator 506 to generate 10 MHz pulse trains. The relative delay of the two pulse trains is half the period of current modulation (e.g., 50 ns for 10 MHz modulation). The RF signals used to drive the two phase  
10 modulators and the intensity modulator are derived from the fast photodetector. In accordance with further embodiments, the two laser sources 502 and 504, the current modulator 506, and the wavelength division multiplexer 544 may be replaced by a fast frequency-tunable laser as disclosed above with reference to Figure 19.

The illumination system 500 provides dual frequency trains of laser pulses for  
15 microscopy and micro-spectroscopy as described above with reference to Figures 1 and 15, yet again, is substantially provided within a single optical fiber system. In a frequency modulation system as discussed above with reference to Figure 11 for example, the  $\omega_b$  pulse train is modulated such that the pulses alternate between frequencies of  $\omega_b$  and  $\omega_b'$  as shown at 170, while remaining coincident with the  
20 pulses from the  $\omega_a$  pulse train. As shown at 172 in Figure 22, in accordance with a further embodiment, the  $\omega_b$  pulse train may be modulated to provide sets of  $\omega_b$  pulses followed by sets of modulated  $\omega_b$  pulses, again while remaining coincident with the pulses from the  $\omega_a$  pulse train.

Frequency-modulating the time-lens laser source and detecting the modulation  
25 transfer to the second field, which can be obtained from a mode-locked laser, is

significant different to quickly modulating the frequency of the second mode-locked laser (e.g. by fast laser tuning), in that it is desirable to measure the modulation transfer to the second field due to concerns with the 1/f-noise discussed above.

Figure 23 shows a dual-frequency source of an illumination system of a  
5 further embodiment of the present invention similar to the system of Figure 21 except that the sources 502, 504, current modulator 506 and wavelength division multiplexer are replaced by sources 602, 604, current modulator 606 and a 2 x 1 Mach Zehnder optical switch 665 that is driven by a 10 MHz pulse train. The remaining elements of Figure 23 are the same as those of Figure 21 and function similarly.

10 Those skilled in the art will appreciate that numerous modifications and variations may be made to the above disclosed embodiments without departing from the spirit and scope of the claims.

What is claimed is:

## CLAIMS

- 1     1.     An illumination system comprising:  
2             a laser system for providing a first train of pulses at a center optical frequency  
3      $\omega_1$ , said laser system including:  
4             a continuous wave laser for providing a first continuous wave field;  
5             a first chirp modulator system comprising a means to receive said first  
6             continuous wave field, a means to receive a trigger signal, a first  
7             phase modulator and a first electro-optic modulator; and  
8             a first pulse compression unit; and  
9             an optical assembly for collinearly combining the first train of pulses with a  
10     second train of pulses having a center optical frequency of  $\omega_2$  as synchronized  
11     excitation fields for non-linear modulation transfer microscopy and micro-  
12     spectroscopy.
- 1     2.     The illumination system as claimed in claim 1, wherein said first train of  
2     pulses is provided responsive to a trigger signal.
- 1     3.     The illumination system as claimed in claim 1, wherein said chirped pulses  
2     have a frequency bandwidth of at least about 30 GHz.
- 1     4.     The illumination system as claimed in claim 1, wherein the first pulse  
2     compression unit temporally compresses the first train of chirped pulses to provide the  
3     first train of pulses having a temporal pulse width of less than about 10 picoseconds.

1     5.     The illumination system as claimed in claim 1, wherein the gain of continuous  
2     wave laser is further modulated electronically in response to the trigger signal.

1     6.     An illumination system for non-linear modulation transfer microscopy or  
2     micro-spectroscopy, said illumination system comprising:

3             a first laser system for providing a first train of pulses at a center optical  
4     frequency  $\omega_1$  responsive to a trigger signal having a non-constant repetition rate, said  
5     first laser system including:

6             a continuous wave laser for providing a first continuous wave field;

7             a first chirp modulator system for receiving the first continuous wave field

8             and the trigger signal, and for providing a first train of chirped pulses

9             responsive to the trigger signal, said first chirp modulator system

10            including a first phase modulator and a first electro-optic modulator,

11            and the chirped pulses having a frequency bandwidth of at least

12            about 30 GHz; and

13            a first pulse compression unit for temporally compressing the first train of

14            chirped pulses to provide the first train of pulses having a temporal

15            pulse width of less than about 10 picoseconds; and

16            an optical assembly for collinearly combining the first train of pulses with a

17     second train of pulses having a center optical frequency of  $\omega_2$  as excitation fields that

18     are synchronized with the trigger signal for the non-linear microscopy or micro-

19     spectroscopy system.

1 7. The illumination system as claimed in claim 6, wherein the first phase  
2 modulator modulates the phase of the first continuous wave field at a modulation rate  
3 that is responsive to the trigger signal to provide first broadband radiation having a  
4 frequency bandwidth of at least about 30 GHz, and the first electro-optical modulator  
5 amplitude modulates the first broadband radiation to produce the first train of chirped  
6 pulses responsive to the trigger signal.

1 8. The illumination system as claimed in claim 6, wherein said chirp modulator  
2 system includes an amplifier.

1 9. The illumination system as claimed in claim 6, wherein the gain of continuous  
2 wave laser is further modulated electronically in response to the trigger signal.

1 10. The illumination system as claimed in claim 6, wherein said system further  
2 includes a second laser system including:  
3 a second continuous wave laser for providing a second continuous wave  
4 field;  
5 a second chirp modulator system for receiving the second continuous wave  
6 field and the trigger signal, and for providing a second train of  
7 chirped pulses responsive to the trigger signal, said second chirp  
8 modulator system including a second phase modulator and a second  
9 electro-optic modulator, and the chirped pulses having a frequency  
10 bandwidth of at least about 30 GHz; and

11                   a second pulse compression unit for temporally compressing the second  
12                   chirped pulses to provide the second train of pulses having a  
13                   temporal pulse width of less than about 10 picoseconds.

1    11.    The illumination system as claimed in claim 6, wherein said trigger signal is  
2    responsive to a pixel clock of a beam-scanning microscope.

1    12.    The illumination system as claimed in claim 6, wherein said collinearly  
2    combined first train of pulses and second train of pulses are provided to one of a  
3    Coherent anti-Stokes Raman Scattering (CARS) microscopy system, or a modulation  
4    transfer microscopy system in which a modulation of one of the first and second trains  
5    of laser pulses is transferred to the other to the first and second trains of laser pulses  
6    due to non-linear optical interactions within a sample.

1    13.    The illumination system as claimed in claim 6, wherein said first laser system  
2    includes two continuous wave lasers and a means to allow fast switching to provide a  
3    frequency modulated pulse train.

1    14.    The illumination system as claimed in claim 13, wherein said first laser system  
2    includes a wavelength division multiplexer and the fast switching is provided by  
3    modulating the gain of the two continuous wave lasers electronically.

1    15.    The illumination system as claimed in claim 13, wherein said first laser system  
2    includes a Mach Zehnder optical switch.

1 16. An illumination system for non-linear modulation transfer microscopy or  
2 micro-spectroscopy, said illumination system comprising:

3 a first laser system for providing a first modulated train of pulses at a first  
4 center optical frequency  $\omega_1$  responsive to a trigger signal, said first laser system  
5 including:

6 a first continuous wave laser for providing a first continuous wave field;

7 a first chirp modulator system for receiving the first continuous wave field,  
8 the trigger signal, and a modulation signal, and for providing a first  
9 modulated train of chirped pulses responsive to the trigger signal,  
10 said first chirp modulator system including a first phase modulator  
11 and a contrast modulation system that effectively varies a  
12 characteristic of some of the chirped pulses responsive to the  
13 modulation signal to provide a first modulated train of chirped  
14 pulses, wherein the chirped pulses have a frequency bandwidth of at  
15 least about 30 GHz; and

16 a first pulse compression unit for temporally compressing the first  
17 modulated train of chirped pulses to provide the first modulated train  
18 of pulses having a temporal pulse width of less than about 10  
19 picoseconds; and

20 an optical assembly for collinearly combining the first modulated train of  
21 pulses with a second train of pulses having a second center optical frequency  $\omega_2$  as  
22 excitation fields for the non-linear microscopy or micro-spectroscopy system.

1 17. The illumination system as claimed in claim 16, wherein the trigger signal is  
2 provided by an electronic clock.

1 18. The illumination system as claimed in claim 16, wherein the second train of  
2 pulses having the center optical frequency of  $\omega_2$  is provided by a second laser, and  
3 wherein said trigger signal is response to the second train of pulses.

1 19. The illumination system as claimed in claim 16, wherein the second train of  
2 pulses having the center optical frequency of  $\omega_2$  is provided by a solid state laser.

1 20. The illumination system as claimed in claim 16, wherein the second train of  
2 pulses having the center optical frequency of  $\omega_2$  is provided by a titanium sapphire  
3 laser.

1 21. The illumination system as claimed in claim 16, wherein the second train of  
2 pulses having the center optical frequency of  $\omega_2$  is provided by a fiber laser oscillator.

1 22. The illumination system as claimed in claim 16, wherein the gain of  
2 continuous wave laser is further modulated electronically in response to the trigger  
3 signal.

1 23. The illumination system as claimed in claim 16, wherein the second train of  
2 pulses having the center optical frequency of  $\omega_2$  is provided by a second laser system  
3 including:

4 a second continuous wave laser for providing a second continuous wave  
5 field;

6 a second chirp modulator system for receiving the second continuous wave  
7 field and the trigger signal, and for providing a second train of

8 second chirped pulses responsive to the trigger signal, said second  
9 chirp modulator system including a second phase modulator and a  
10 second electro-optic modulator, and the second chirped pulses having  
11 a frequency bandwidth of at least about 30 GHz; and  
12 a second pulse compression unit for temporally compressing the second  
13 chirped pulses to provide the second train of pulses having a  
14 temporal pulse width of less than about 10 picoseconds.

1 24. The illumination system as claimed in claim 16, wherein one of the first center  
2 optical frequency  $\omega_1$  and the second center optical frequency  $\omega_2$  is within the gain  
3 spectrum of either an Erbium-doped medium (1500nm – 1600nm) or a Ytterbium-  
4 doped medium (1000nm – 1080nm).

1 25. The imaging system as claimed in claim 16, wherein one of the first center  
2 optical frequency  $\omega_1$  and the second center optical frequency  $\omega_2$  is within the gain  
3 spectrum of either a frequency-doubled Erbium-doped medium (750nm – 800nm) or a  
4 Ytterbium-doped medium (1000nm – 1080nm).

1 26. The illumination system as claimed in claim 16, wherein said trigger signal  
2 has a non-constant repetition rate.

1 27. The illumination system as claimed in claim 16, wherein said first laser system  
2 is provided within an optical fiber.

1 28. The illumination system as claimed in claim 16, wherein said collinearly  
2 combined first train of pulses and second train of pulses are provided to a modulation  
3 transfer microscopy system in which a modulation of one of the first and second trains  
4 of laser pulses is transferred to the other to the first and second trains of laser pulses  
5 due to non-linear optical interactions within a sample.

1 29. The illumination system as claimed in claim 16, wherein the characteristic of  
2 some of the chirped pulses that is varied is intensity.

1 30. The illumination system as claimed in claim 16, wherein the characteristic of  
2 some of the chirped pulses that is varied is time-delay.

1 31. The illumination system as claimed in claim 16, wherein said first laser system  
2 includes two continuous wave lasers and a means to allow fast switching to provide a  
3 frequency modulated pulse train.

1 32. The illumination system as claimed in claim 16, wherein said first laser system  
2 includes a wavelength division multiplexer and the fast switching is provided by  
3 modulating the gain of the two continuous wave lasers electronically.

1 33. The illumination system as claimed in claim 16, wherein said first laser system  
2 includes a Mach Zehnder optical switch.

1 34. An imaging system for non-linear optical microscopy or micro-spectroscopy,  
2 said imaging system comprising:

3           a first laser system for providing a first train of pulses at a first center optical  
4   frequency  $\omega_1$  responsive to a trigger signal, said first laser system including:  
5           a continuous wave laser for providing a first continuous wave field;  
6           a first chirp modulator system for receiving the first continuous wave field  
7           and the trigger signal, and for providing a first train of chirped pulses  
8           responsive to the trigger signal, said first chirp modulator system  
9           including a first phase modulator and a first electro-optic modulator,  
10          and the chirped pulses having a frequency bandwidth of at least  
11          about 30 GHz; and  
12          a first pulse compression unit for temporally compressing the first train of  
13          chirped pulses to provide the first train of pulses having a temporal  
14          pulse width of less than about 10 picoseconds;  
15   an optical assembly including focusing optics and an optical detector system,  
16          said focusing optics for directing and focusing the first train of pulses and  
17          a second train of pulses having a center optical frequency of  $\omega_2$   
18          through an objective lens toward a common focal volume along an  
19          excitation path; and  
20          said optical detector system including at least one optical detector for  
21          detecting a signal of interest from within the sample that is  
22          responsive to a non-linear optical interaction within the sample, and  
23          providing a detector signal and  
24   a processor for providing at least a portion of an image responsive to the  
25   detector signal.

1 35. The imaging system as claimed in claim 34, wherein the second train of pulses  
2 having the center optical frequency of  $\omega_2$  is provided by a second laser system, and  
3 wherein said trigger signal is responsive to the second train of pulses.

1 36. The imaging system as claimed in claim 34, wherein the second train of pulses  
2 having the center optical frequency of  $\omega_2$  is provided by a solid state laser.

1 37. The imaging system as claimed in claim 34, wherein the second train of pulses  
2 having the center optical frequency of  $\omega_2$  is provided by a titanium sapphire laser.

1 38. The imaging system as claimed in claim 34, wherein the second train of pulses  
2 having the center optical frequency of  $\omega_2$  is provided by a fiber laser oscillator.

1 39. The illumination system as claimed in claim 34, wherein the gain of  
2 continuous wave laser is further modulated electronically in response to the trigger  
3 signal.

1 40. The imaging system as claimed in claim 34, wherein the second train of pulses  
2 having the center optical frequency of  $\omega_2$  is provided by a second laser system  
3 including:

4 a second continuous wave laser for providing a second continuous wave  
5 field;

6 a second chirp modulator system for receiving the second continuous wave  
7 field and the trigger signal, and for providing a second train of  
8 second chirped pulses responsive to the trigger signal, said second

9 chirp modulator system including a second phase modulator and a  
10 second electro-optic modulator, and the chirped pulses having a  
11 frequency bandwidth of at least about 30 GHz; and  
12 a second pulse compression unit for temporally compressing the second  
13 chirped pulses to provide the second train of pulses having a  
14 temporal pulse width of less than about 10 picoseconds.

1 41. The imaging system as claimed in claim 34, wherein one of the first center  
2 optical frequency  $\omega_1$  and the second center optical frequency  $\omega_2$  is within the gain  
3 spectrum of either an Erbium-doped medium (1500nm – 1600nm) or a Ytterbium-  
4 doped medium (1000nm – 1080nm).

1 42. The imaging system as claimed in claim 34, wherein one of the first center  
2 optical frequency  $\omega_1$  and the second center optical frequency  $\omega_2$  is within the gain  
3 spectrum of either a frequency-doubled Erbium-doped medium (750nm – 800nm) or a  
4 Ytterbium-doped medium (1000nm – 1080nm).

1 43. The imaging system as claimed in claim 34, wherein said first laser system is  
2 provided within an optical fiber.

1 44. The imaging system as claimed in claim 34, wherein said collinearly  
2 combined first train of pulses and second train of pulses are provided to a Coherent  
3 anti-Stokes Raman Scattering (CARS) microscopy system.

1 45. The imaging system as claimed in claim 34, wherein said system further  
2 includes  
3 a modulator for modulating a property of the first train of pulses at a  
4 modulation frequency  $f$  of at least 100 kHz;  
5 wherein said optical detector system includes at least one optical detector for  
6 detecting a detected intensity of the second pulse train that is back-scattered within a  
7 sample, and for providing an electrical signal representative of the detected intensity  
8 of the second pulse train; and  
9 wherein the processor detects a modulation at the frequency  $f$  of the electrical  
10 signal due to non-linear optical interaction within the common focal volume.

1 46. The imaging system as claimed in claim 34, wherein said processor provides  
2 an output signal that is representative of one of a gain or loss of illumination at the  
3 first optical frequency  $\omega_1$  due to the non-linear optical interaction within the common  
4 focal volume.

1 47. The imaging system as claimed in claim 34, wherein illumination at a  
2 difference frequency between  $\omega_1$  and  $\omega_2$  is resonant with a molecular vibrational  
3 frequency of the sample in the focal volume.

1 48. The imaging system as claimed in claim 46, wherein the second train of pulses  
2 a broadband illumination field.

1 49. The imaging system as claimed in claim 34, wherein one of the first train of  
2 pulses at the first center optical frequency  $\omega_1$  and the second train of pulses at the  
3 second center optical frequency  $\omega_2$  is electronically resonant with the sample.

1 50. The imaging system as claimed in claim 49, wherein the second train of pulses  
2 at the second center optical frequency  $\omega_2$  excites molecules in the sample into an  
3 excited state.

1 51. The imaging system as claimed in claim 49, wherein the second train of pulses  
2 at the second center optical frequency  $\omega_2$  removes molecules from a ground state by  
3 promoting them to an excited state.

1 52. The imaging system as claimed in claim 34, wherein a sum frequency of the  
2 first train of pulses at the first center optical frequency  $\omega_1$  and the second train of  
3 pulses at the second center optical frequency  $\omega_2$  is electronically resonant with the  
4 sample.

1 53. An illumination system for providing a frequency-modulated train of pulses,  
2 comprising:

3 a first continuous wave laser for providing a first continuous wave field at  
4 frequency  $\omega$ ;

5 a second continuous wave laser for providing a first continuous wave field  
6 at frequency  $\omega'$ ;

7 a beam combiner or optical switch for combining the first continuous wave  
8 field and the second continuous wave field and switching between

9                   the two frequencies at high frequency to provide a frequency-  
10                   modulated continuous wave field  
11                a chirp modulator system comprising a means to receive said frequency-  
12                   modulated continuous wave field, a means to receive a trigger signal,  
13                   a first phase modulator and a first electro-optic modulator; and  
14                   a pulse compression unit;

1   54.    A method of providing excitation fields for non-linear modulation transfer  
2   microscopy or micro-spectroscopy, said method comprising the steps of:  
3           providing a first continuous wave field;  
4           providing a first train of chirped pulses responsive to the first continuous wave  
5   field and the trigger signal, wherein the chirped pulses have a frequency bandwidth of  
6   at least about 30 GHz;  
7           temporally compressing the first train of chirped pulses to provide the first  
8   train of pulses having a temporal pulse width of less than about 10 picoseconds; and  
9           collinearly combining the first train of pulses having a center optical  
10   frequency  $\omega_1$  with a second train of pulses having a center optical frequency of  $\omega_2$  as  
11   the excitation fields that are synchronized with the trigger signal for the non-linear  
12   microscopy or micro-spectroscopy system.

1   55.    A method of providing excitation fields for non-linear modulation transfer  
2   microscopy or micro-spectroscopy, said method comprising the steps of:  
3           providing a first continuous wave field;  
4           providing a first modulated train of chirped pulses responsive to the first  
5   continuous wave field, a trigger signal and a modulation signal, such that a

6 characteristic of some of the chirped pulses is varied responsive to the modulation  
7 signal to provide the first modulated train of chirped pulses, wherein the chirped  
8 pulses have a frequency bandwidth of at least about 30 GHz;

9 temporally compressing the first modulated train of chirped pulses to provide  
10 the first modulated train of pulses having a temporal pulse width of less than about 10  
11 picoseconds; and

12 collinearly combining the first modulated train of pulses having a first center  
13 optical frequency  $\omega_1$  with a second train of pulses having a second center optical  
14 frequency  $\omega_2$  as excitation fields for the non-linear microscopy or micro-spectroscopy  
15 system.

1 56. A method of performing non-linear optical microscopy or micro-spectroscopy,  
2 said method comprising the steps of:

3 providing a first continuous wave field;

4 providing a first train of chirped pulses responsive to the continuous wave  
5 field and the trigger signal, the chirped pulses having a frequency bandwidth of at  
6 least about 30 GHz;

7 temporally compressing the first train of chirped pulses to provide the first  
8 train of pulses having a temporal pulse width of less than about 10 picoseconds;

9 directing and focusing the first train of pulses having a center optical  
10 frequency of  $\omega_1$  and the second train of pulses having a center optical frequency of  $\omega_2$   
11 through an objective lens toward a common focal volume along an excitation path;

12 detecting a signal of interest from within the sample that is responsive to a  
13 non-linear optical interaction within the sample, and providing a detector signal; and

- 14 providing at least a portion of an image in a non-linear optical microscopy or  
15 micro-spectroscopy system responsive to the detector signal.

- 1 57. A method for providing a frequency-modulated train of pulses, comprising the  
2 steps of:
- 3 providing a first continuous wave laser for providing a first continuous  
4 wave field at frequency  $\omega$ ;
- 5 providing a second continuous wave laser for providing a first continuous  
6 wave field at frequency  $\omega'$ ;
- 7 combining first continuous wave field and the second continuous wave  
8 field and switching between the two frequencies at high frequency to  
9 provide a frequency-modulated continuous wave field
- 10 providing chirp modulator system comprising a means to receive said  
11 frequency-modulated continuous wave field, a means to receive a  
12 trigger signal, a first phase modulator and a first electro-optic  
13 modulator; and
- 14 compressing the optical pulses.

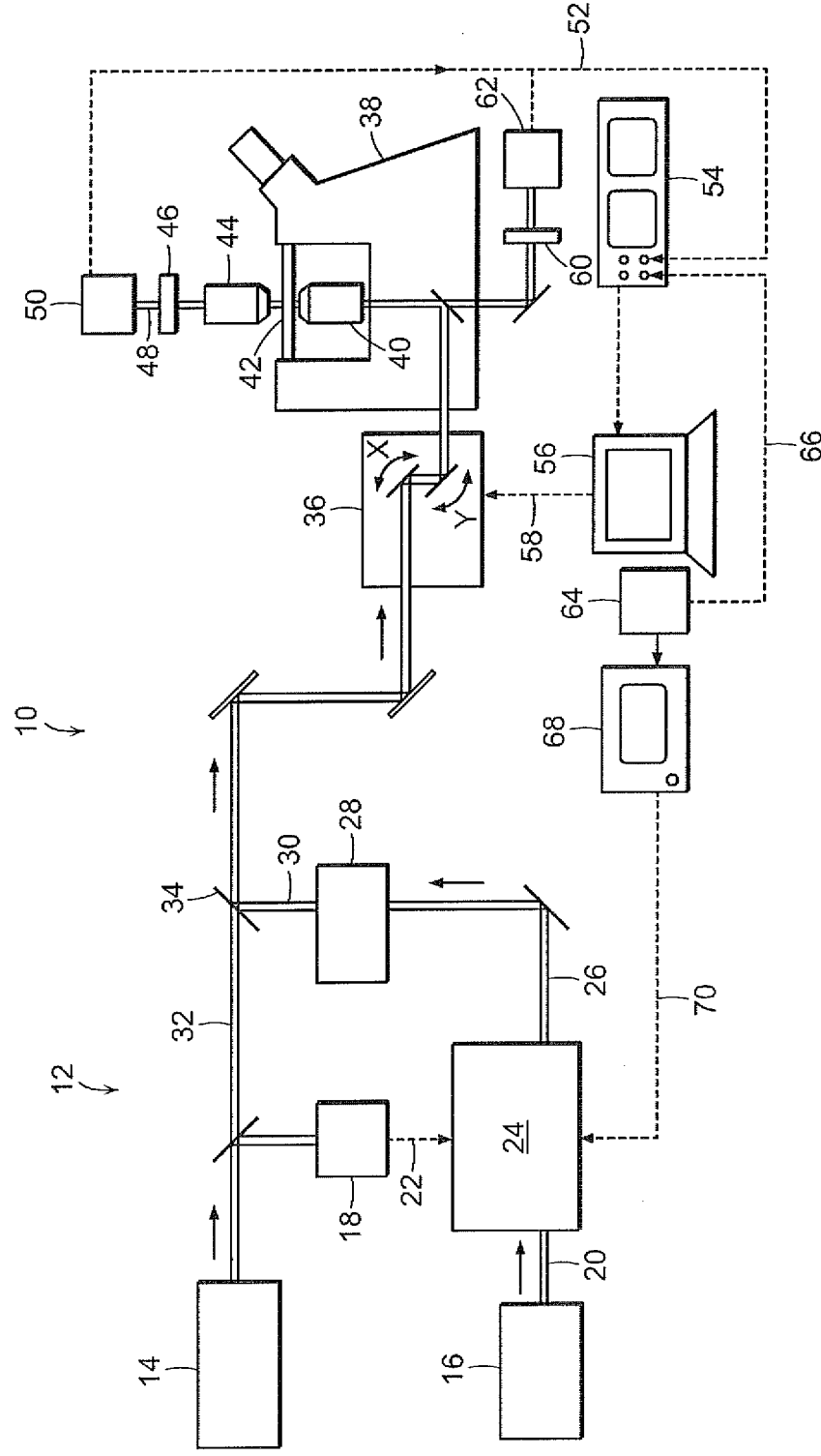


FIG. 1

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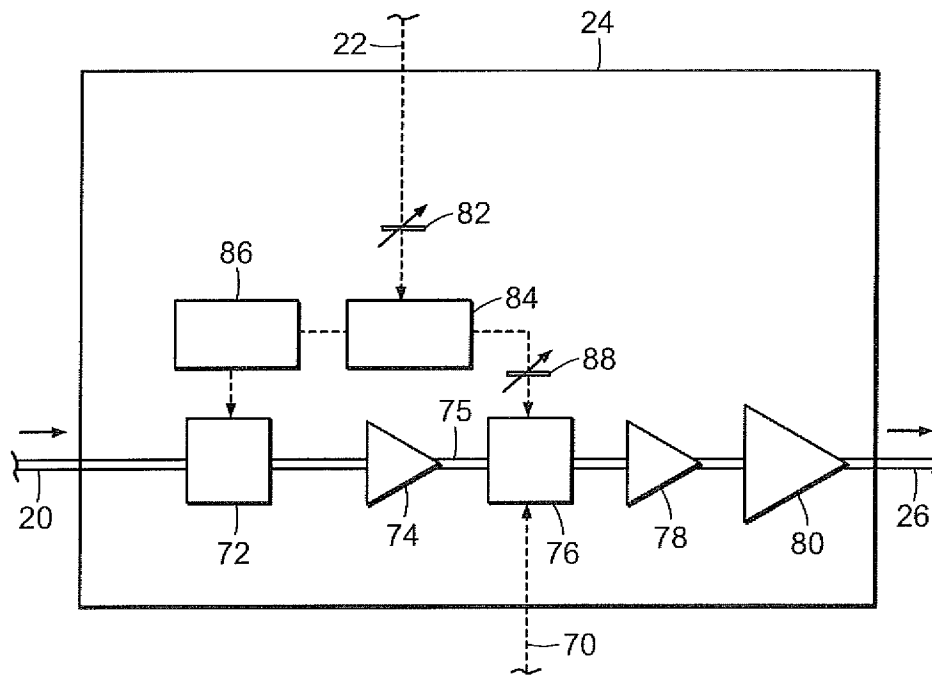


FIG. 2

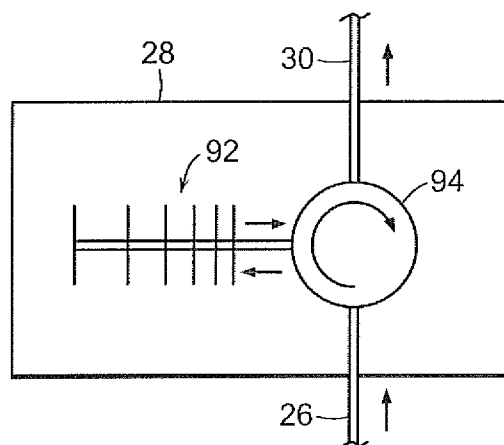


FIG. 3

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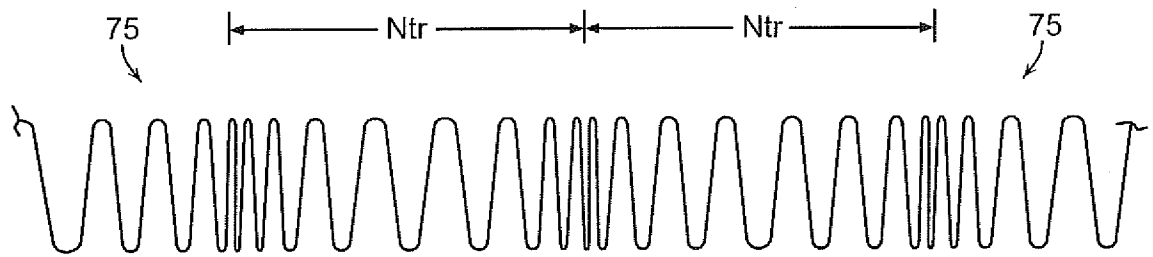


FIG. 4

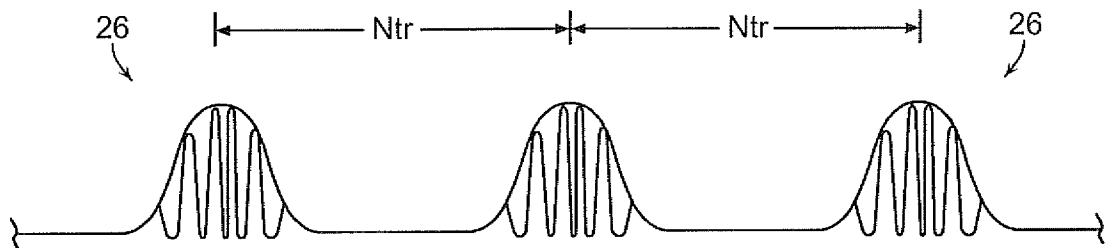


FIG. 5

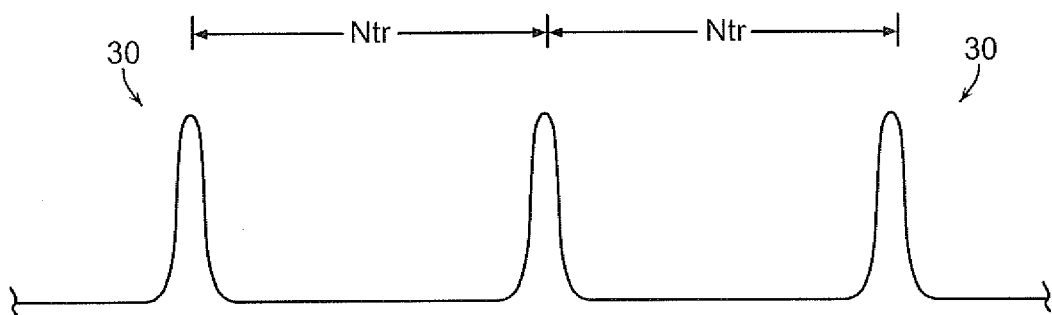


FIG. 6

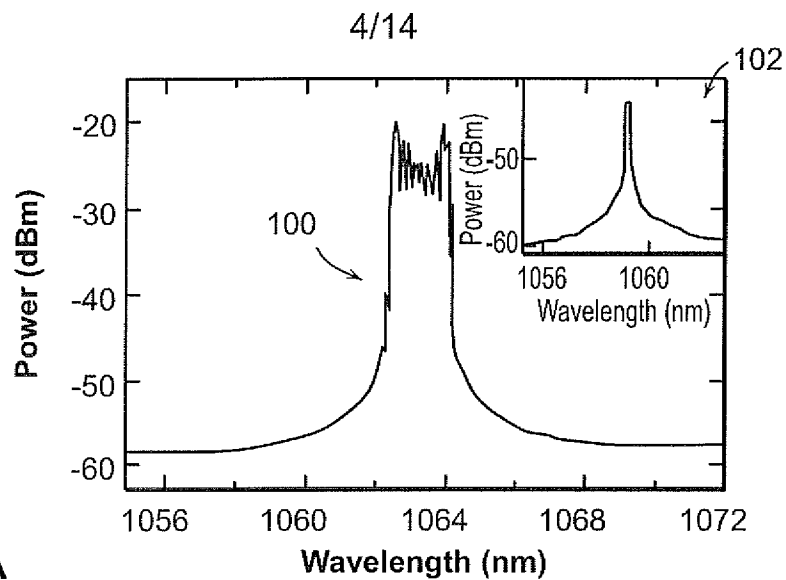


FIG. 7A

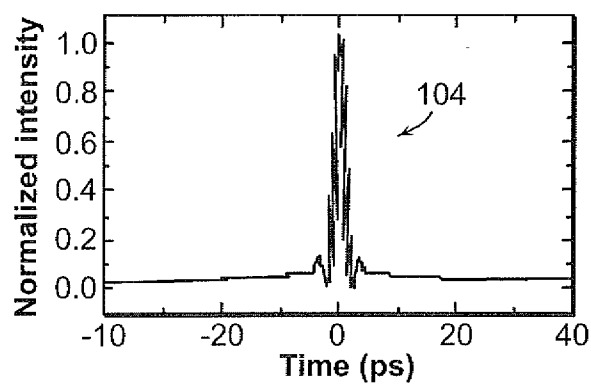


FIG. 7B

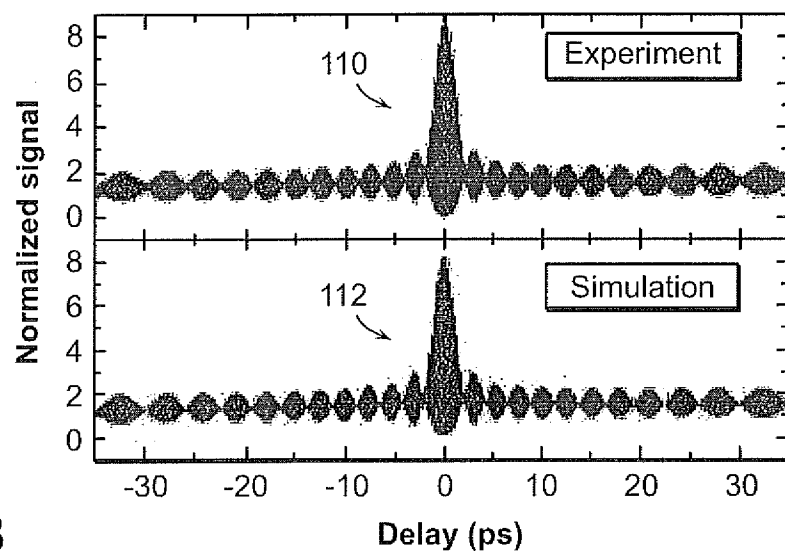


FIG. 8

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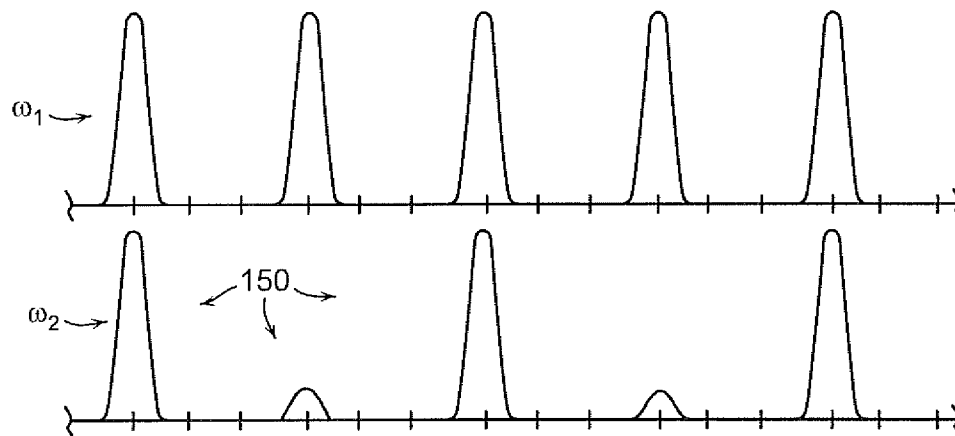


FIG. 9

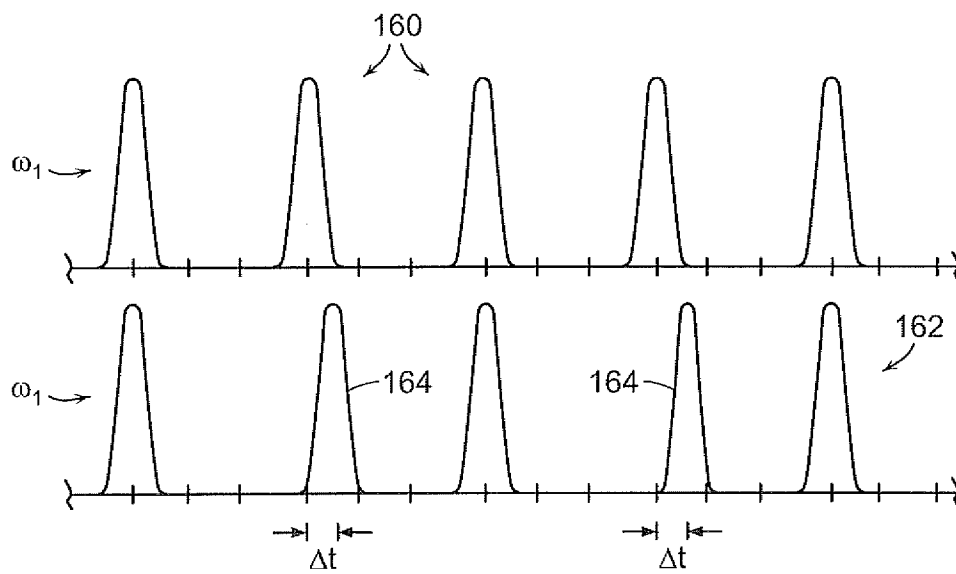


FIG. 10

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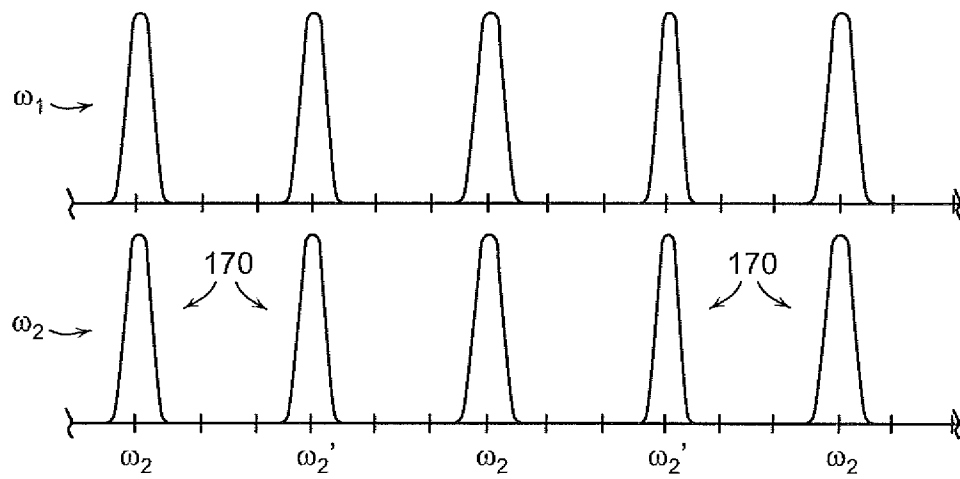


FIG. 11

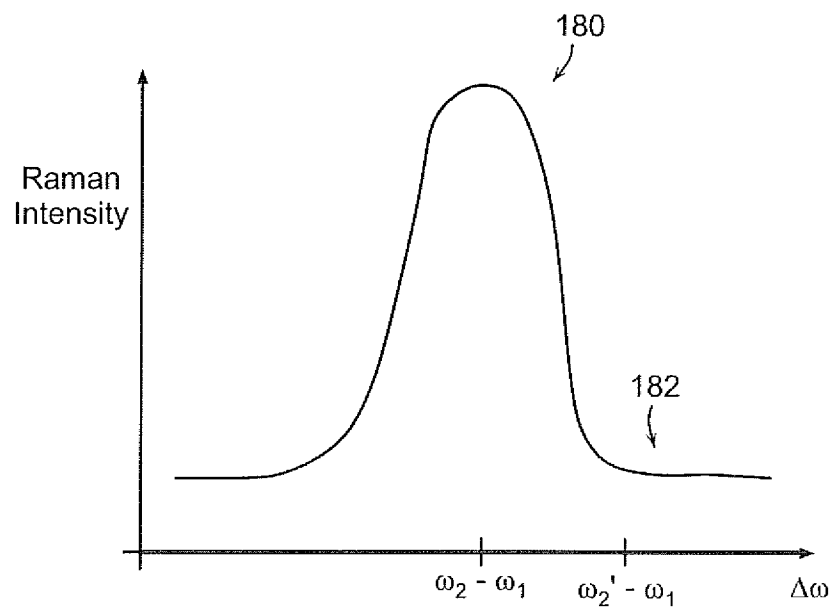
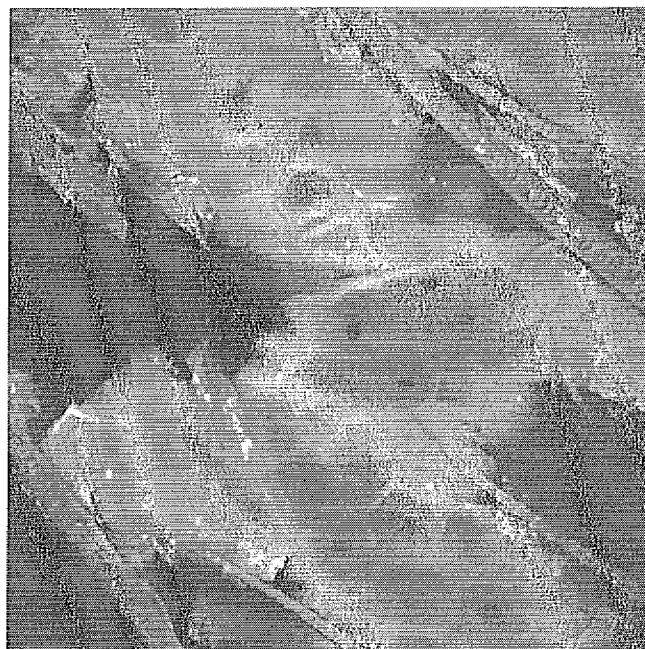


FIG. 12

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FIG. 13



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FIG. 14

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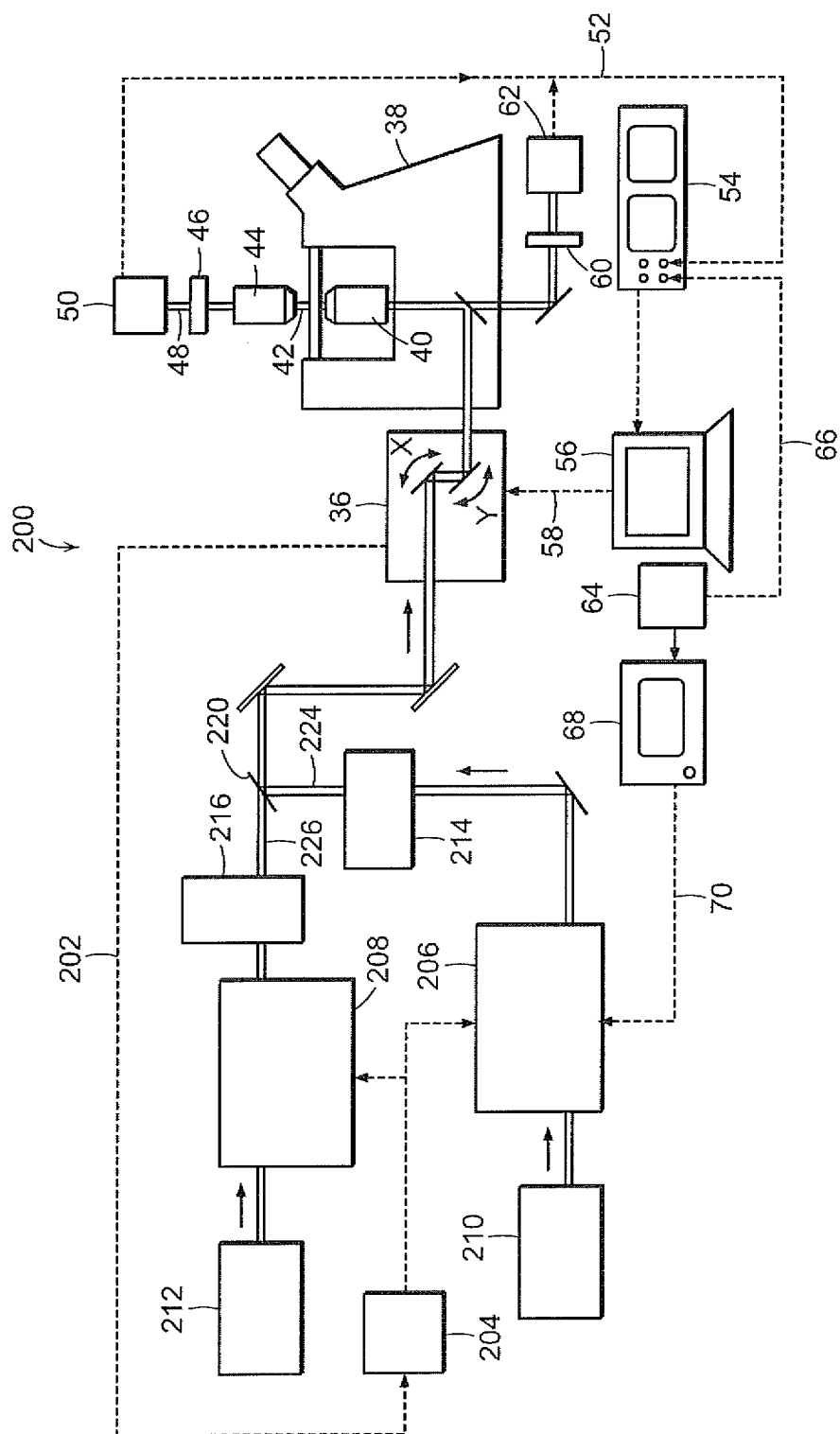


FIG. 15

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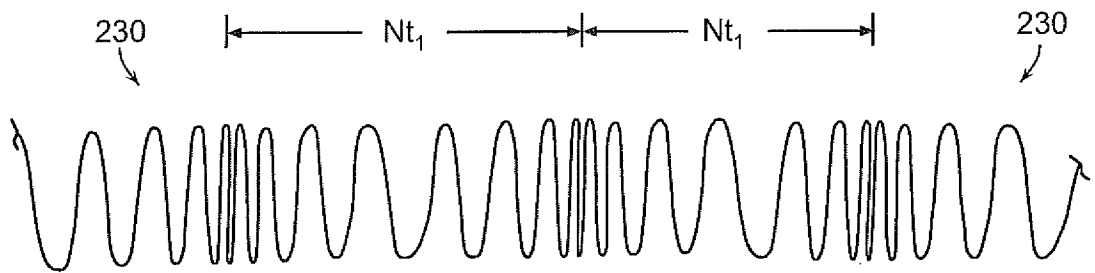


FIG. 16

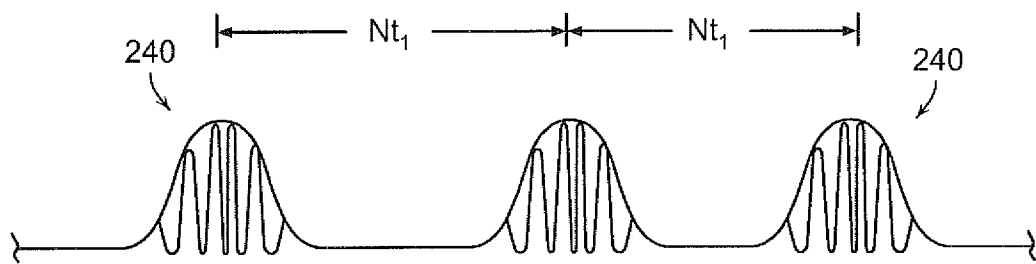


FIG. 17

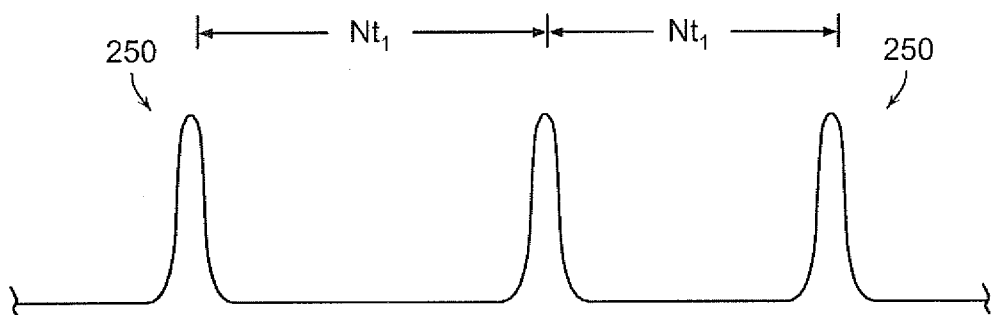


FIG. 18

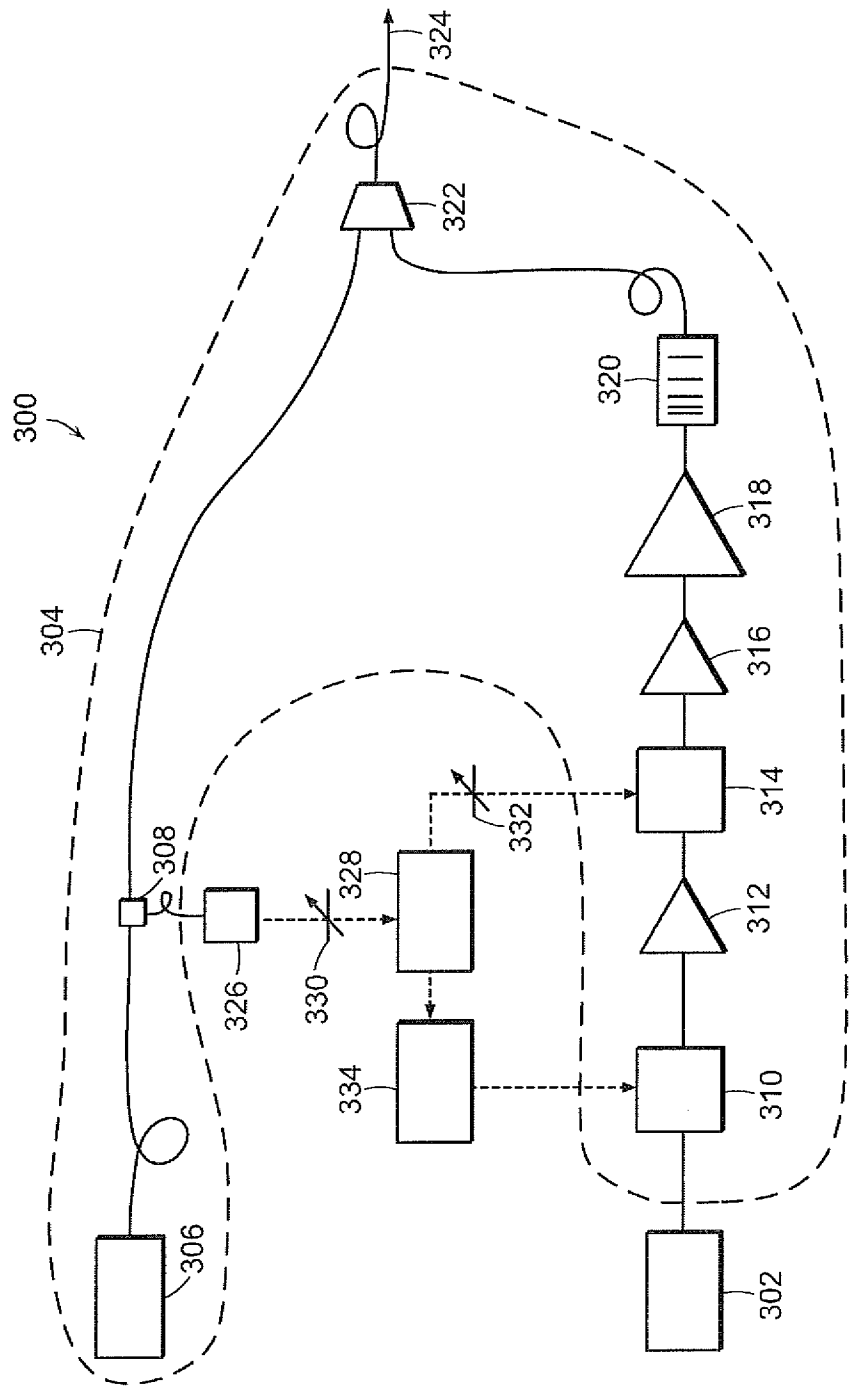


FIG. 19

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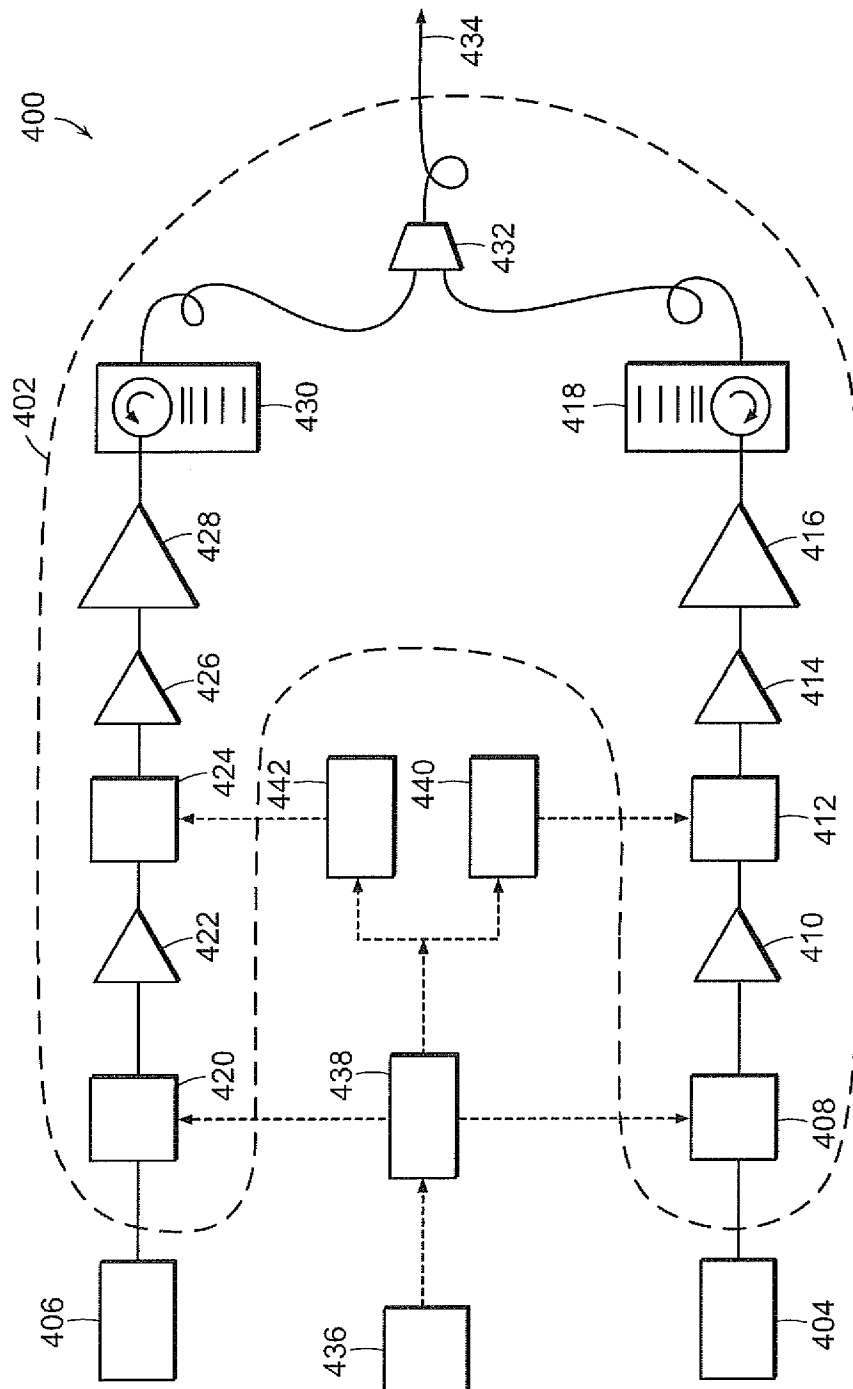


FIG. 20

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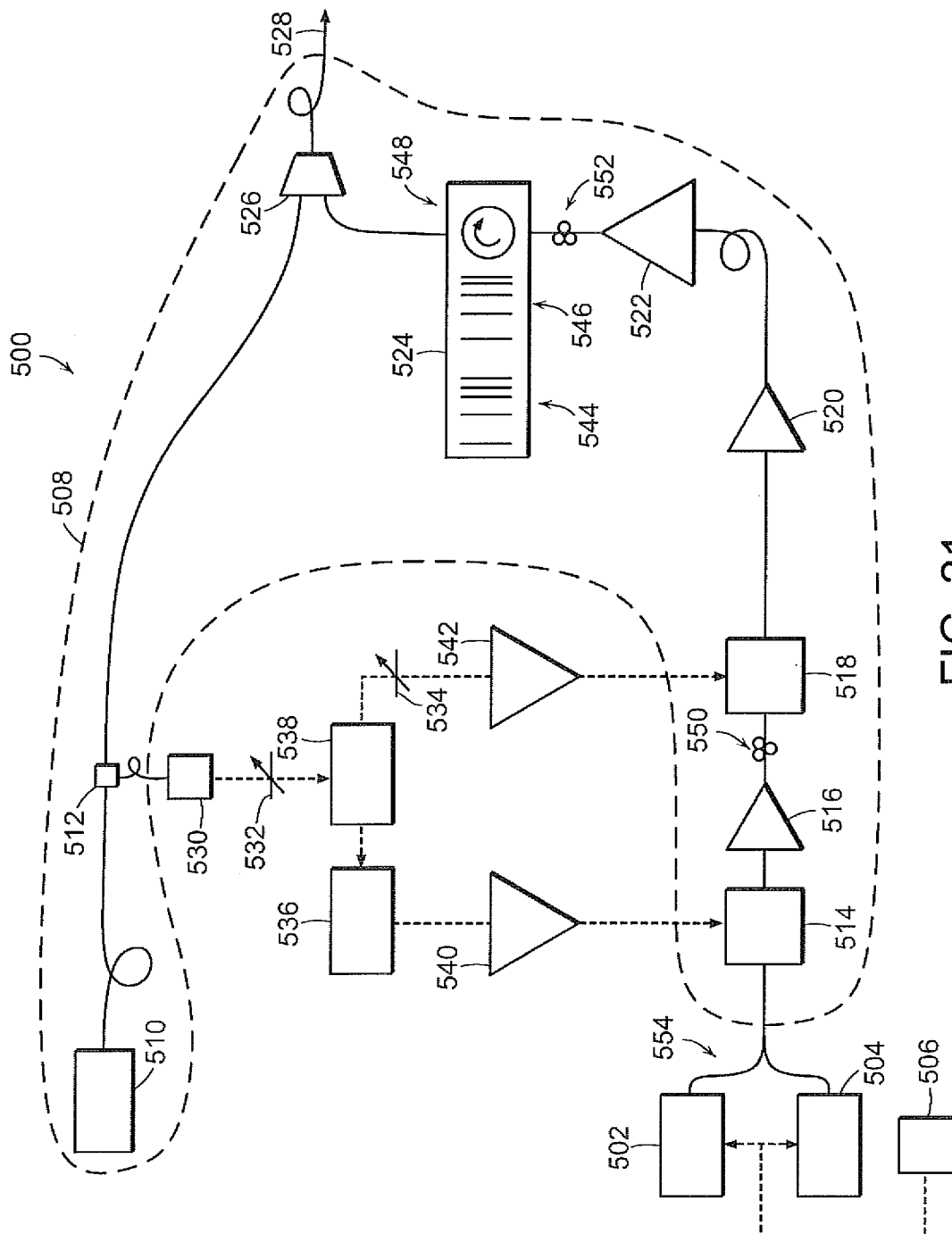


FIG. 21

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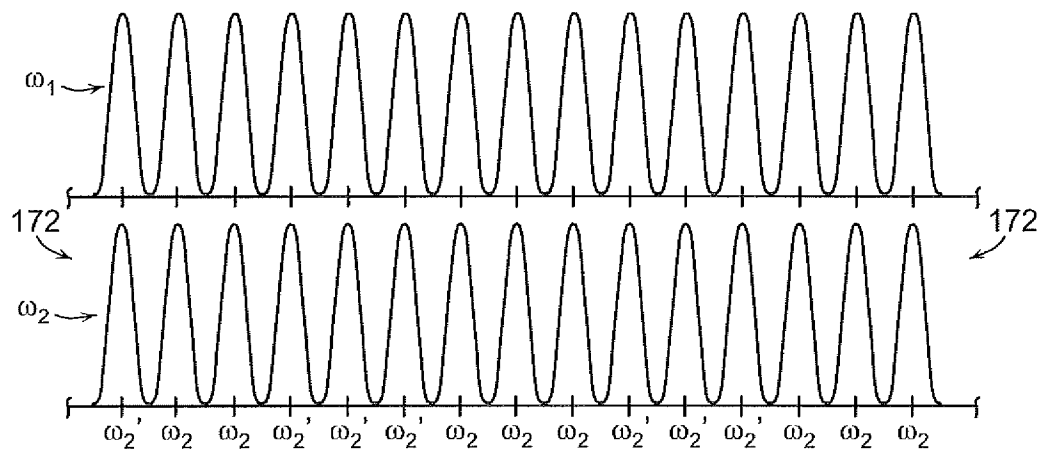


FIG. 22

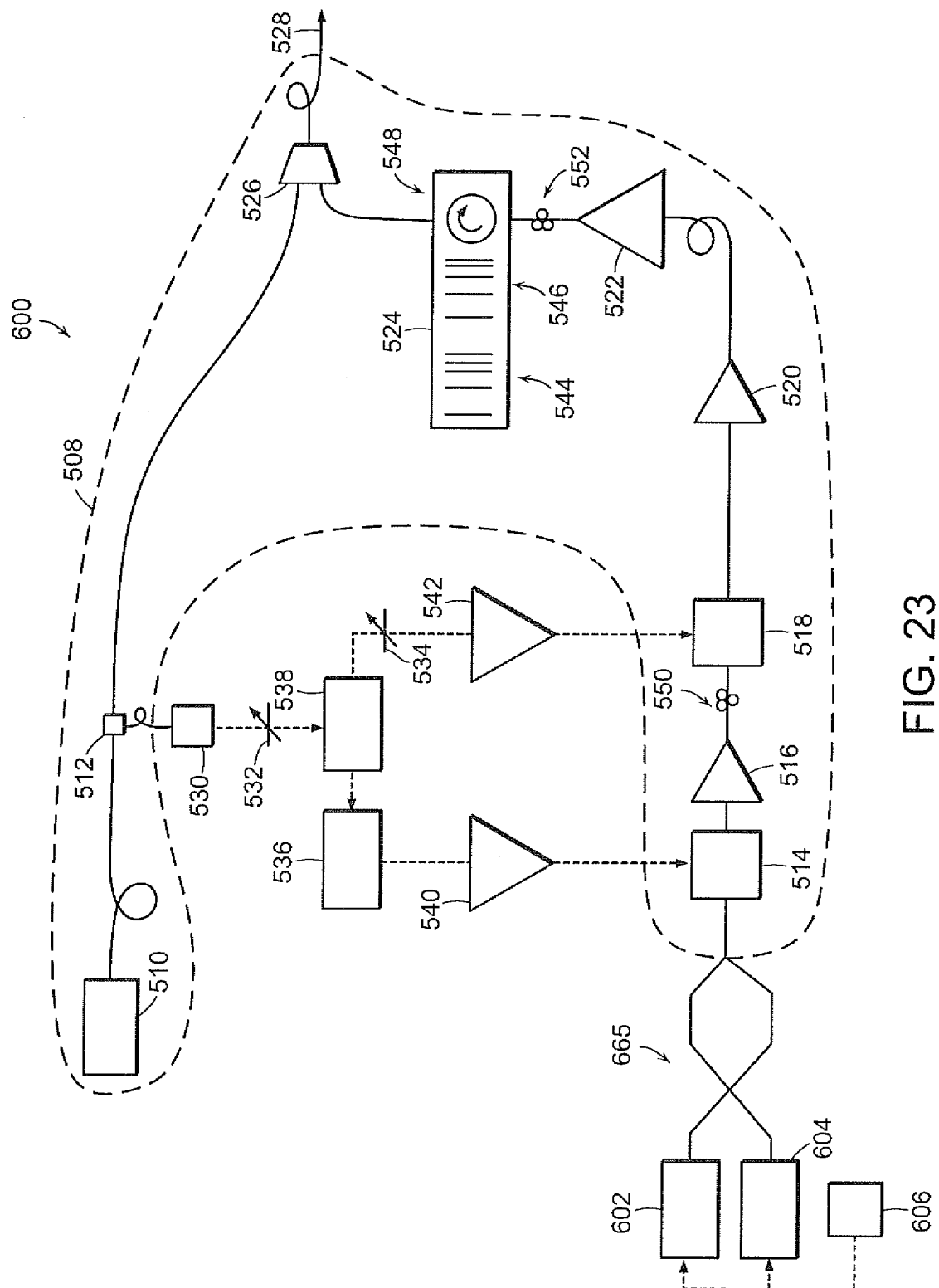


FIG. 23