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Phase-locked 10 MHz reference signal for frequency domain time-resolved fluorescence measurements

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A complete electronic system that is suitable for use in megahertz frequency domain time-resolved fluorescence instruments based on mode-locked lasers is described. The circuit produces a 10 MHz signal, phase locked to the mode-locked laser pulse frequency, which is required by many commercial frequency synthesizers as the external reference signal. This device is particularly useful in conjunction with ultrafast gated intensified charge coupled device cameras capable of being frequency modulated for time-resolved fluorescence imaging. © *2007 American Institute of Physics.* [DOI: 10.1063/1.2740475]

INTRODUCTION

Time-resolved fluorescence measurements can be recorded in either the time or frequency domain. Both techniques are well established, with frequency domain methods and the time-domain technique of time-correlated single photon counting (TCSPC) being favored particularly by many researchers in the biological and chemical sciences. Under appropriate circumstances, these methods can provide comparable time resolution to one another, and the advantages and disadvantages of these techniques have been well documented elsewhere.[1]

A number of frequency domain instruments with impressive characteristics have been reported in the literature.[2–7] The high frequency components inherent in the stable, high repetition-rate train of picosecond or femtosecond pulses provided by mode-locked lasers and synchrotrons have also been exploited.[2,8] Most mode-locked lasers (and synchrotrons) currently in use operate at pulse repetition rates of many tens of megahertz. Synchronously pumped dye lasers are often cavity dumped, but such a mode is not yet common in commercial titanium:sapphire (Ti:S) laser-based systems, although a number of designs for intracavity dumped Ti:S (Refs. 9–13) (and Cr-forsterite)[14] lasers and optical parametric oscillators[15] have been reported in the literature and are now available commercially.[16,17] Consequently, the majority of these lasers in widespread use operate at the fundamental frequency limited by the oscillator cavity length (usually to within the range of 75–85 MHz). Pulse repetition rates of Ti:S lasers are more commonly reduced through external cavity pulse picking devices based on Bragg[7] or Pockel cell devices.

In frequency domain fluorescence imaging experiments that utilize the high frequency components of mode-locked lasers, a frequency synthesizer must be phase locked to the laser pulse train and used to produce a frequency at which to modulate the gain of the photodetector. This frequency may be such that either homo—or heterodyne (cross correlation) detection may be employed. So *et al.* have published a simple integer divide circuit to reduce the 80 MHz signal from a typical Ti:S laser to 10 MHz.[5] This is an ideal solution if the fundamental laser pulse repetition rate is an integer number of tens of megahertz. However, this is not the case for most commercially manufactured Ti:S lasers that have pulse repetition rates which cannot be easily divided by an integer to produce a reference signal close to 10 MHz. For example, the Spectra-Physics Tsunami operates at 82 MHz, while the Coherent Mira operates at approximately 76 MHz. While these pulse repetition rates can be altered over a limited range (~0.5 MHz) through cavity length adjustment or specified at the time of ordering, the laser pulse repetition rate is also a function of the laser wavelength, so for lasers that are tuned to different operating wavelengths frequently, the pulse repetition rate is rarely fixed to a frequency that is an integer multiple of 10 MHz. In this article, we specifically address this problem and report a system that can provide a 10 MHz reference signal, phase locked to the laser pulse repetition rate, suitable for input into commercial frequency synthesizers, when the laser pulse repetition rate is adjusted to any integer number in megahertz. Pulse repetition rate reducing devices such as cavity dumpers or pulse pickers can still be used. The actual repetition rate of the light source does not matter for the purpose of the approach reported here, as long as the fundamental repetition rate of the laser is an integer number in megahertz (76 MHz in our example). The subsequently selected reduced repetition rate will be synchronized to the fundamental repetition rate and to the phase-locked 10 MHz signal.

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EXPERIMENTAL DETAILS

Laser system

The laser system used in this work is based on a commercial Kerr lens mode-locked Ti:S laser (Coherent Mira 900F). Unlike the system reported by So *et al.*[5] whose laser was operated at 80 MHz, our laser, as delivered, is operated at a pulse repetition rate of approximately 75.5 MHz at 800 nm (corresponding to a cavity length of 1.987 m). In order to increase or decrease this repetition rate to an integer number of tens of megahertz, for example, to achieve a repetition rate of 70 or 80 MHz, the cavity length must be adjusted to 2.143 or 1.875 m, respectively. The available cavity length adjustment provided in this laser is on the order of ±1 MHz (approximately ±25 mm). Significant changes are required to the optical configuration of the cavity in order to alter the cavity length sufficiently to achieve 70 or 80 MHz repetition rates, and thus permit use of the previously published circuit.[5] A retrofit modification kit is available for the Coherent Mira which can achieve this repetition rate, but this is a rather expensive and cumbersome option. Rather than work at 70 or 80 MHz, we chose to run the laser at a predetermined integer number in megahertz. The available cavity length adjustment of the Mira is sufficient to achieve 75 or 76 MHz repetition rates at certain wavelengths. However, the laser repetition rate is wavelength dependent due to the speed of light in the various media within the cavity as a function of the central wavelength. Our laser is often tuned to various operating wavelengths, and an insufficient adjustment was available to achieve even the modest cavity length adjustment required to maintain the 76 MHz repetition rate at all the wavelengths used. We therefore modified the laser in a minor way by installing a 5 cm stainless steel extension tube in which the output coupler was mounted. In this way, the laser pulse repetition rate could be easily adjusted to exactly 76 MHz over the range of wavelengths required.

The repetition rate of this laser can also vary depending on whether the cover is open or closed due partially to a loss in nitrogen purging. The cover should be in place during operation to enable nitrogen purging of the cavity; however, the cavity length adjustment in this laser is only accessible internally. An accurate adjustment of the cavity length with the cover in place is therefore difficult. Furthermore, the laser pulse repetition rate was found to drift slightly over the course of a day's use. In order to facilitate the cavity length adjustments required to maintain the repetition rate at 76 MHz, the fine screw cavity length adjustment standard on this laser was replaced with a motorized actuator system (New Focus Picomotor™; 8303 micrometer-replacement actuator, 8701 single axis driver, 8620 control pad) which enabled an accurate external cavity length adjustment and a computer controlled frequency/cavity length feedback mechanism if necessary. This Ti:S laser can be used directly as the excitation source, or it can be frequency doubled/tripled or used to pump a frequency doubled optical parametric oscillator (APE, Germany). Whichever system is used as the excitation source, a stable 76 MHz source is provided from the Ti:S oscillator with these modifications to the cavity.

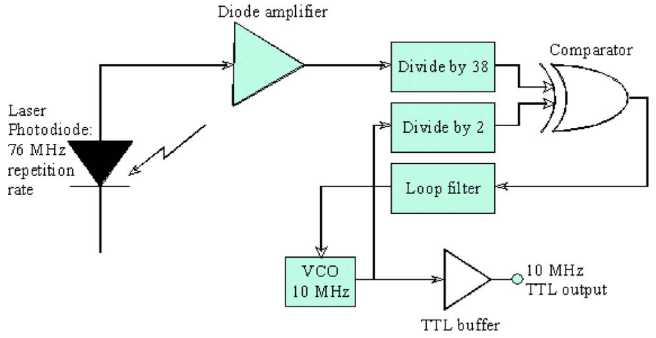

FIG. 1. Schematic circuit of 10 MHz phase-locked frequency source.

Frequency synthesizer and detector

In the frequency domain fluorescence imaging mode, often also referred to as fluorescence lifetime imaging (FLIM), the light source is optically modulated at a frequency appropriate for the time scale of the fluorescence events under investigation, and the gain of an intensified charge coupled device (ICCD) camera is electronically modulated at the same or a slightly different (heterodyne) frequency. A frequency synthesizer is therefore used to provide this modulation to the ICCD, and the synthesizer must be phase locked to the modulation of the light source. The frequency synthesizer used in this work was a Hameg HM8134 programmable rf frequency generator that provides a programmable dc 1.024 GHz output with 1 Hz resolution and power output up to +13 dBm. This cost effective unit, like many other commercial synthesizers such as those used by others, requires an external transistor-transistor-logic (TTL) reference signal of 10 MHz±5 ppm, in phase with the laser source. The output of this synthesizer was sufficient for direct input to the ICCD camera system without further amplification. The ICCD detector used (LaVision, PicoStar HR) was operated in a "rf modulated" mode.

A frequency counter (Hameg HM8122) was used to monitor the laser repetition rate to ~1 Hz accuracy. This frequency was sampled, using the general purpose interface bus (GPIB) interface, by a program (LABVIEW VI) on a personal computer that could also be used to control the cavity length actively through a digital to analog board and the motorized actuator to keep the laser repetition rate fixed at 76 MHz. The counter was also referenced to the frequency generator.

ELECTRONIC DESIGN

Phase-locked 10 MHz reference source

In addition to the simple integer divider circuit reported that allows generation of a 10 MHz signal in phase with the

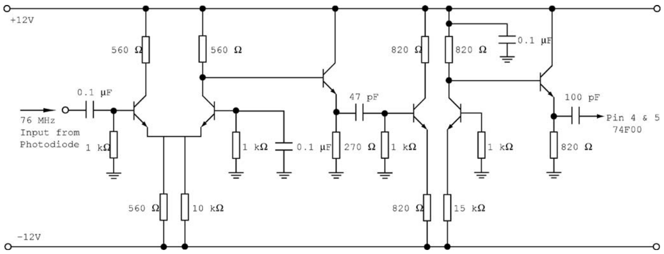

FIG. 2. 76 MHz amplifier.

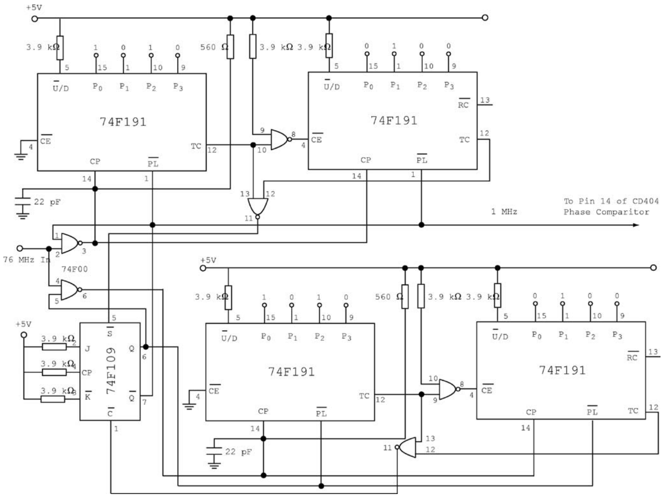

FIG. 3. 76 MHz divider and 1 MHz output.

pulse repetition rate of a 80 MHz Ti:S laser,[5] a device for dividing a recurrent frequency signal by a nonlinear divisor, f, has been patented. However, the design is principally for the case of $f = \mathrm{N} - 1/2$.[18]

The general approach we have adopted is shown schematically in Fig. 1. The use of a phase-locked loop[19–21] (PLL) was inspired by Euclid's algorithm for finding the largest common divisor which, in the present case, is 2. The Coherent Mira 900f Ti:S laser used incorporates a photodiode output that monitors the mode-locked laser pulses internally. The 76 MHz photodiode output was usually sufficient to be fed directly into the input stage of the phase-locked reference frequency circuit, but could be amplified (HP462A) to suitable levels for a TTL logic counter circuit to allow for any diminution of signal (e.g., at the extent of the laser tuning wavelengths). A Colpitts voltage controlled oscillator (VCO) generates a stable 10 MHz signal. Both the laser pulse frequency input and the output of the VCO are sent though digital frequency dividing circuits. The 10 MHz VCO signal is divided by 5 and the output from the laser is divided by 38. These two 2 MHz signals are then further divided by 2. The resulting symmetrical square wave form required for the remainder of the circuitry is inputted to the type-II phase comparator. The output of the phase comparator is fed into a PLL filter. The output of the loop filter goes to the control voltage of the VCO. The output from the buffer amplifier after the VCO minimizes any impact of load upon the VCO. There are two stages for the amplification of the 76 MHz signal input. BFR90 transistors are employed and configured as differential amplifiers with common emitter stage buffering outputs. The stages are signal ac coupled.

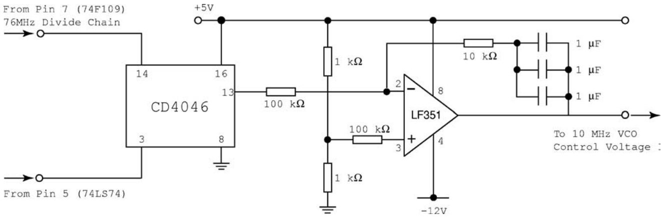

FIG. 4. Type-II phase comparator and loop filter.

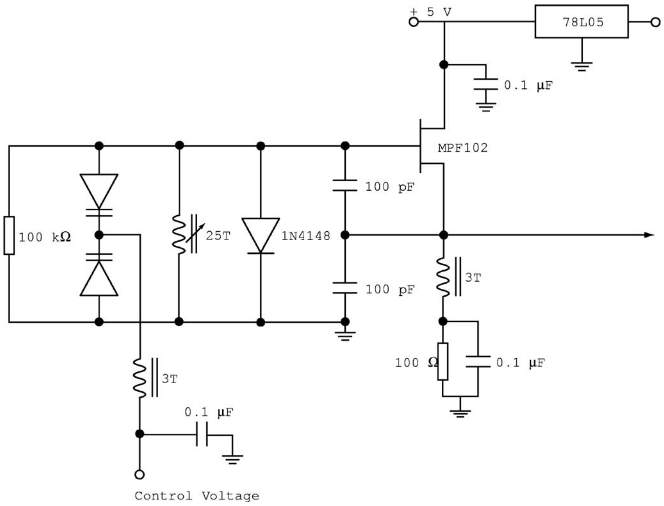

FIG. 5. Colpitts oscillator (VCO) 10 MHz. 3T: Neusid 3500131, 25T: Resonator coil.

The counters were formed using 74F191 (binary up/down counter) series fast TTL devices. They are configured as synchronous divided by "N" counters. The outputs from the two counter chains are further divided by type "D 74LS74" flipflops. This reshapes the digital signals to effect best performance of the type-II phase comparator. The VCO has a narrow lock range. This assists in obtaining a good short-term frequency stability. The VCO feeds a field-effect transistor (FET) buffer stage and then a common emitter (CE) transistor stage to decouple it from the remainder of the circuitry. The output from the CE stage is then fed into a 74LS14 (Hex Schmitt trigger inverter) to further shape, buffer, and split the signal to two signal paths. One path is fed to the VCO divider chain and the other to the line driver output stage as the 10 MHz VCO time base output. Only the phase comparator of the CD4046 PLL integrated circuit (IC) is used. The VCO section of the CD4046 is unable to operate at the needed 10 MHz. The phase comparator is configured as type II since type-II phase comparators are renowned for better phase stability performances than exclusive "OR" type-I devices. The complex logic state diagram for type-II comparators made it more expedient to utilize this from an existing readily available IC. Detailed electronic schematic diagrams of the amplifier, 76 MHz divider, phase comparator/loop filter, Colpitts VCO, 10 MHz divider, oscillator buffer, and amplifier stages, are shown in Figs. 2–7, respectively.

The output is then a stable 10 MHz TTL pulse source, which is fed into the 10 MHz reference input of the fre-

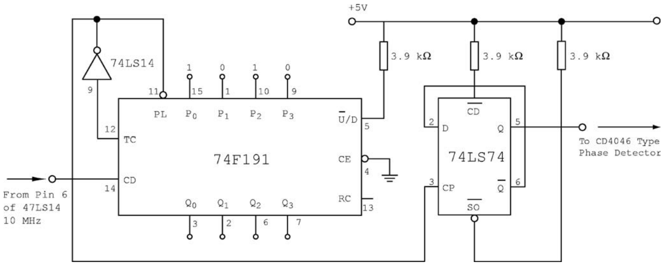

FIG. 6. 10 MHz divided by ten circuits.

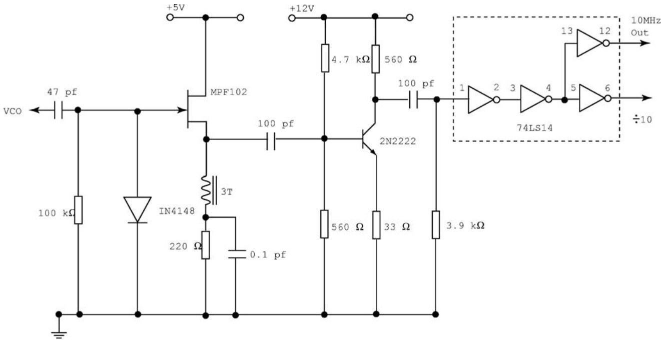

FIG. 7. Oscillator buffer and amplifier stages.

quency synthesizer. Unless the frequency is 10 MHz±5 ppm, the frequency synthesizer will not lock on to the external signal, but the circuitry reported here produces a signal of sufficient stability to maintain the synthesizer's lock.

DISCUSSION

A solution to the problem of interfacing commercial frequency synthesizers with high (megahertz) laser pulse repetition-rate excitation sources for use in high time resolution frequency resolved fluorescence measurements is reported. The system is especially useful when used in conjunction with an ICCD camera operated in a frequency modulation mode for time-resolved fluorescence imaging (or FLIM), which is becoming a widely used technique. The system provides the 10 MHz reference signal required by commercial frequency synthesizers phase locked to the mode-locked laser pulse repetition rate set for an integer number in megahertz. This overcomes the need for expensive modifications to the laser cavity or electronics, or multiple frequency synthesizers. The repetition rate of the laser may be reduced by cavity dumpers and pulse pickers, and the actual repetition rate of the light source does not matter for the purpose of this approach, as long as the fundamental repetition rate of the laser is an integer number in megahertz (76 MHz in our example). The selected repetition rate will be synchronized to the fundamental repetition rate and to the phase-locked 10 MHz signal.

ACKNOWLEDGMENTS

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