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Synthesizers' phase noise in frequency-domain fluorometry

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The influence on the final phase and amplitude determination of the phase noise of frequency synthesizers used in frequency-domain fluorometry is analyzed. It is shown that the phase noise exactly cancels out due to the differential method used for the phase measurement. The only consequence of the phase noise is the detuning of the cross-correlation frequency with respect to the center of the filter used to analyze the cross-correlation signal. This detuning can have severe adverse effects on the phase and modulation values. By using higher values of the cross-correlation frequency, these effects can be minimized. The performance of different synthesizers is tested and compared. The results support the general prediction that the noise of the synthesizer has little influence on the phase noise of actual measurements.

INTRODUCTION

Frequency-domain fluorometry has become a valid alternative to time-domain fluorometry because of its high sensitivity, rapid data acquisition, and excellent time resolution.[1] Investigations to further improve the performance of frequency-domain instruments are ongoing. Recently, the frequency range of these instruments has been increased to several Gigahertz, providing time resolution that is comparable to streak-camera kinds of devices.[2,3] The cross-correlation method,[4] introduced by Weber and Spencer, is used in all state-of-the-art frequency-domain fluorometers. The original apparatus described by Spencer and Weber operated at only two frequencies. The development of high-resolution frequency-domain fluorometers was accomplished by the introduction of a method to obtain the cross-correlation over a wide band of frequencies.

The first practical continuous multifrequency cross-correlation phase and modulation fluorometer (MPF) was described in 1983.[5] Although successful improvements[2,3,6,7] increased the frequency range of the original instrument, the basic layout has not changed. The crucial element in this design was the introduction of coupled frequency synthesizers, which allow selection of the modulation frequency and cross-correlation frequency over a wide band of frequencies. Using synthesizers, the generation of frequencies coherent to the phase of a common crystal can be obtained with great accuracy from a few Hertz to several Gigahertz. All frequency-domain fluorometers use this basic configuration. In multifrequency phase fluorometry, the phase noise of the frequency generators has been the major concern. There are essentially two classes of synthesizers: direct-synthesis and phase-locked loop (PLL) synthesizers. In the first class, a given frequency is obtained by proper filtering of a series of harmonics directly derived from a common quartz crystal. The simplest PLL synthesizer uses a voltage-controlled oscillator (VCO), a divider chain and a phase detector which compares and matches the output frequency at a VCO divided by an integer N, to the frequency of a quartz crystal divided by M (Fig. 1). By changing N and M, different frequen-

cies can be obtained. More sophisticated division schemes can be used to obtain very high frequency resolution. The quality of the VCO and the electronic scheme used for the phase detector are important for the stability of the output frequency. It has long been suggested that direct-synthesis frequency synthesizers are essential for a good performance of the MPF instrument since they can provide optimum phase-noise performance.[5] A thorough analysis of how the phase-noise is processed by the electronics and optics of the MPF instrument shows that low-cost, phase-locked loop frequency synthesizers with relatively high-phase noise are equally efficient.

I. OPERATION OF AN MPF

The block diagram of an MPF is shown in Fig. 2. A light beam, amplitude-modulated by a light modulator (Pockels cell, acousto-optic modulator, etc.), or carrying a harmonic content (mode-locked laser, synchrotron radiation, pulsed sources etc.), irradiates the sample. The phase shift and the demodulation of the fluorescence with respect to the incoming light beam are the physical observables used to record the fluorescence decay and to determine the lifetime of the excited electronic level of the fluorophore.[1] The accurate measurement of these observables is obtained as follows. A small percentage of the incoming amplitude modulated light
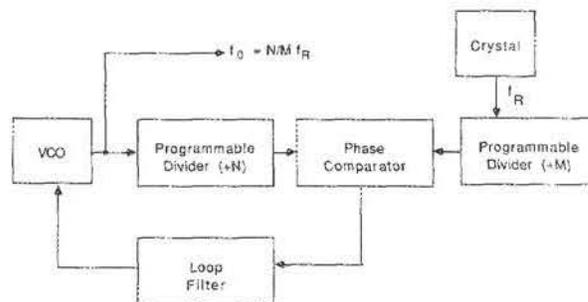
FIG. 1. Block diagram of a typical phase-locked loop synthesizer.
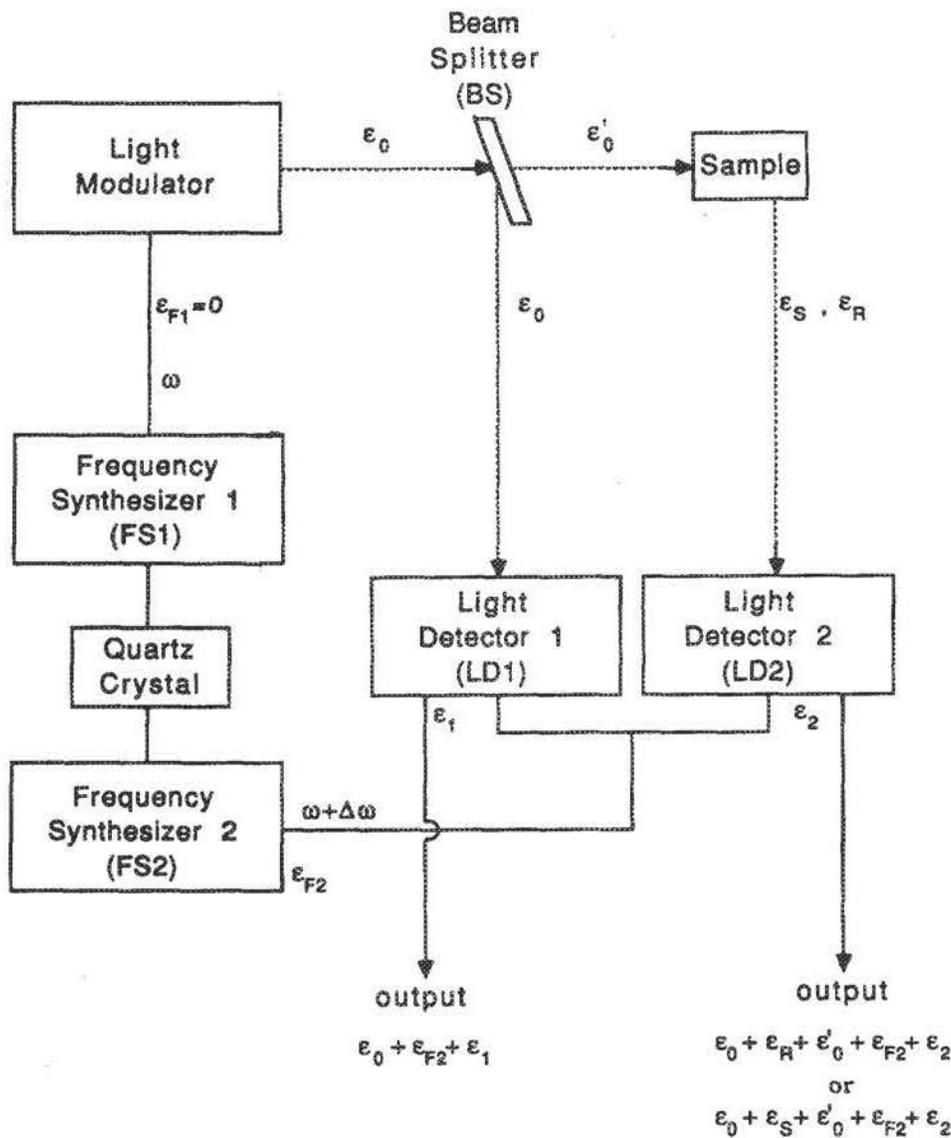
FIG. 2. Block diagram of a multifrequency phase fluorometer.

beam is diverted by a beam splitter (BS) into a light detector (photomultiplier tube, photovoltaic detector, microchannel plate, etc.) (LD1). A second light detector (LD2) collects the sample's fluorescence. The phase delay, due to the persistence of the excited state, is measured by the difference between the phase of the sample's fluorescence and that of the signal from a scattering solution (glycogen in water, etc.) or from the fluorescence of a fluorophore whose lifetime (and consequently phase delay) is well known under experimental conditions. Both the phase from the sample's fluorescence and that from the reference solution are measured with respect to the phase of the signal detected by LD1. The frequency synthesizer (FS1) in Fig. 1, in conjunction with a light modulator, amplitude modulates the incoming light beam at a frequency ω and the frequency synthesizer (FS2) modulates the gain of the light detector at a frequency $(\omega + \Delta\omega)$. While ω spans from 100 Hz up to several GHz, $\Delta\omega$ has a smaller, fixed value, usually between 25 and 40 Hz, depending on the particular implementation. This method is called the "cross-correlation technique".[4]

The main purpose of the cross-correlation technique is to shift the frequency of light modulation, generally in the MHz range, to a very low frequency, 25–40 Hz, where measurements of phase and modulation are easy to perform using accurate digital techniques. A key point is that the two frequency synthesizers must be phase-locked in order to provide phase-coherent signals to both the light modulator and the light detectors. The beating between the signal at frequency ω and the signal at frequency $(\omega + \Delta\omega)$ in the light detectors, provides signals at the sum and difference frequencies, respectively. The low-frequency component of the signal, $\Delta\omega$, is filtered out and processed by the instrument electronics to measure the phase shift and the demodulation of the fluorescence signal. The analysis of the phase and modulation data to obtain the parameters associated with the fluorescence decay has been extensively discussed in the literature.[8,9]

The stability of the cross-correlation frequency at 40 Hz is essential to the performance of the instrument. In fact, if the two generators are not phase locked, at 100 MHz a center

frequency stability of 10^{-8} will cause the cross-correlation frequency to shift by about 1 Hz. This shift will have two major effects: (1) The filter used to isolate the cross-correlation frequency generally has a bandwidth of about 0.5 Hz. Consequently the cross-correlation frequency will shift out of the filter center causing severe alteration of the value of the phase and amplitude of the signal. (2) A shift of the cross-correlation frequency of 1 Hz over 40 Hz will cause a shift of about 9° in the phase value due to the change in the period length.

Generally, phase angles are measured with an accuracy of $\pm 0.2°$ and modulation amplitudes with an accuracy of ± 0.004. This high accuracy provides picosecond time resolution at modulation frequencies on the order of 100 MHz. It was commonly assumed that in order to get such results, the phase noise of the synthesizers had to be below 0.2° and the amplitude noise below 0.004. Over a wide frequency range, only direct synthesis frequency synthesizers or very expensive phase-locked loop synthesizers can provide phase noise levels below this limit. When the frequency range of MPF instruments was extended well above 1 GHz, the search for adequate synthesizers became more difficult. Above 1 GHz only commercially available synthesizers costing well over $20 000 provide a phase noise level below 0.2°. In this article, we show that the noise of the synthesizer plays a minor role in determining the accuracy of a phase and modulation fluorometer.

II. ANALYSIS OF PHASE MEASUREMENT IN AN MPF

Let us assume we want to determine the lifetime of a fluorophore, using a glycogen solution as a reference. The phases (ϵ_i) of the optical and electronic signals at different points in the instrument are reported in Fig. 2 and in Table I. Phases are measured with respect to the common quartz crystal. In a typical measurement, we first measure the phase of the sample at time t_1:

1st measurement: (sample)

$$(\epsilon_0 + \epsilon_0' + \epsilon_S + \epsilon_{F2} + \epsilon_2) - (\epsilon_0 + \epsilon_{F2} + \epsilon_1)$$
$$= \epsilon_S - \epsilon_1 + \epsilon_2 + \epsilon_0'. \tag{1}$$

After the sample, we measure the phase of the reference solution with respect to the phase of the signal detected by LD1, at the time t_2:

TABLE I. The phase of the signals at different points of an MPF. ϵ_1, ϵ_2 = constant→dependent on the length of cables connecting the light detectors to the frequency synthesizer FS2. ϵ_0' = constant→dependent on the light path between the beam splitter and the sample cuvette. $\epsilon_{F1} = 0$ by asumption. The fluorescence phase shift is $\phi = \epsilon_S - \epsilon_R$.

	Phase	Δ phase with respect to LD1
output LD1	$\epsilon_0 + \epsilon_{F2} + \epsilon_1$	0
output LD2 ref	$\epsilon_R + \epsilon_0' + \epsilon_0 + \epsilon_{F2} + \epsilon_2$	$\epsilon_R + \epsilon_0' + \epsilon_2 - \epsilon_1$
output LD2 samp	$\epsilon_S + \epsilon_0' + \epsilon_0 + \epsilon_{F2} + \epsilon_2$	$\epsilon_S + \epsilon_0' + \epsilon_2 - \epsilon_1$

2nd measurement: (scattering reference)

$$(\epsilon_0 + \epsilon_0' + \epsilon_R + \epsilon_{F2} + \epsilon_2) - (\epsilon_0 + \epsilon_{F2} + \epsilon_1)$$
$$= \epsilon_R - \epsilon_1 + \epsilon_2 + \epsilon_0'. \tag{2}$$

The phase shift due to the fluorescence is obtained as the difference between the sample and the reference phase measurements:

$$\phi = \epsilon_S - \epsilon_R. \tag{3}$$

Since ϵ_R is known, relationship (3) allows the measurement of ϵ_S and, hence, the lifetime of the excited electronic level using well-known expressions.[1]

It is a property of the optical and electronic layout of the instrument that during each measurement represented by Eqs. (1) and (2), the phase ϵ_{F2} of the frequency synthesizer FS 2, and the phase ϵ_0 of the signal after the light modulator, cancel out exactly at every instant of time. The phases ϵ_1 and ϵ_2 are given by the difference in cable length at the two detectors and are rigorously constant. The phase ϵ_0' is also constant since it is due to the different light path between the beam splitter and the sample. As a consequence, the phase noise associated with each of the two synthesizers' signal, also cancels out. Following the above derivations, the noise of the synthesizer should not play a role in the determination of the phase (and modulation) of the sample. However, as we shall see in the next section, the noise of the frequency synthesizers can have other important consequences. We have not shown the effect of the amplitude noise of the synthesizers on the modulation measurement; generally it is negligible compared to the effect we discuss next. It must be noted from the relations giving the phase of the signal at different points of the instrument (Table I), that the phase noise of the frequency synthesizer used for the cross-correlation product at the light detectors is not important, since the phase jitter is alway equal and opposite on the two channels and only the difference between the two channels is measured. The phase noise is important only for the frequency synthesizer sending the signal to the light modulator. Ultimately, this imposes a constraint on the allowed phase noise of the frequency synthesizers.

III. EFFECT OF THE CROSS-CORRELATION PHASE NOISE DUE TO THE BANDWIDTH OF THE ANALOG FILTER

In this section, we analyze how the bandwidth of the filter, which is used to isolate the cross-correlation frequency, determines the maximum allowed phase noise of the synthesizers. In the absence of sources of noise other than the frequency synthesizer's phase noise, the instantaneous value of the cross-correlation frequency is given by

$$\omega_c = \Delta\omega + \frac{d\epsilon_0}{dt}. \tag{4}$$

Equation (4) imposes constraints on the characteristics of the synthesizers in relation to the response function of the electronic filter used to isolate the cross-correlation frequency. Usually narrow-bandwidth filters have been used, since

low-frequency harmonics must be rejected.[5] For example, the commercial MPF GREG 200 by I.S.S. Inc. uses filters centered at 40 Hz with $Q \simeq 80$ (the bandwidth is 0.5 Hz). Specifically, these are 6-pole active band-pass filters. The response of such a filter is reported in Fig. 3. This figure shows that, if the cross-correlation frequency moves from the center value of the filter, both phase and amplitude values are strongly affected due to the filter response. In order to find the maximum allowed phase noise of the synthesizer, we observe that a change of 0.2 Hz modifies the phase by 10° and the amplitude by 0.80, as seen in Fig. 3. At a cross-correlation frequency of 40 Hz, a change of 0.2 Hz corresponds to a phase shift of 1.8°. Consequently, the shift of the cross-correlation frequency, due to the term $d\epsilon_0/dt$, must be smaller than the bandwidth of the analog filter. Therefore, it is important to examine the magnitude of the term $d\epsilon_0/dt$ for different synthesizers.

IV. PHASE NOISE OF SOME COMMERCIAL SYNTHESIZERS

We have measured the phase noise of some synthesizers using the method shown in Fig. 4. If the two frequencies at the R and L input of the mixer are exactly the same, then the output of the mixer should be a dc voltage proportional to the phase difference between the two signals. Any difference in phase as a function of time [the term $d\epsilon_0/dt$ in Eq. (4)] should appear as a voltage variation at the mixer output. This variation, which is proportional to $\sin d\epsilon_0/dt$, is analyzed using a low-frequency signal analyzer. It is a property of frequency synthesizers due to the phase correction of the phase-locked loop that the average frequency is always within 1/4 of a period with respect to a corresponding harmonic of the quartz crystal that drives the phase-locked loop. This implies that there is no average frequency shift, but only phase noise due to the electronic nature of the phase-locked loop.

Experimentally, one finds that direct-synthesis frequency synthesizers are less noisy than phase-locked loop frequency synthesizers. Figure 5 reports the time analysis and

FIG. 4. Experimental arrangement for the measurement of the phase noise of different frequency synthesizers.

frequency analysis of the phase noise of different frequency synthesizers measured at 10 MHz using the method of Fig. 4. A Hewlett Packard, model 8590A, spectrum analyzer was used for the plots reported in Fig. 5(B). The full scale
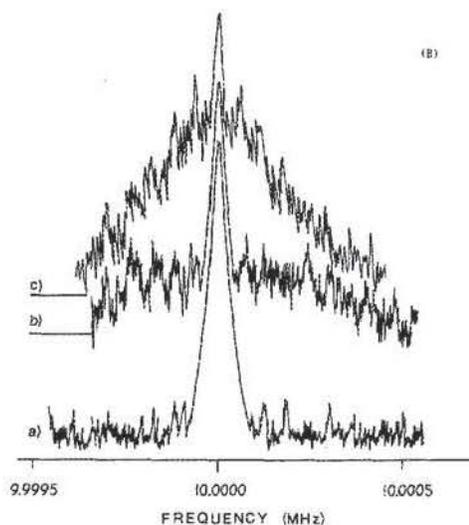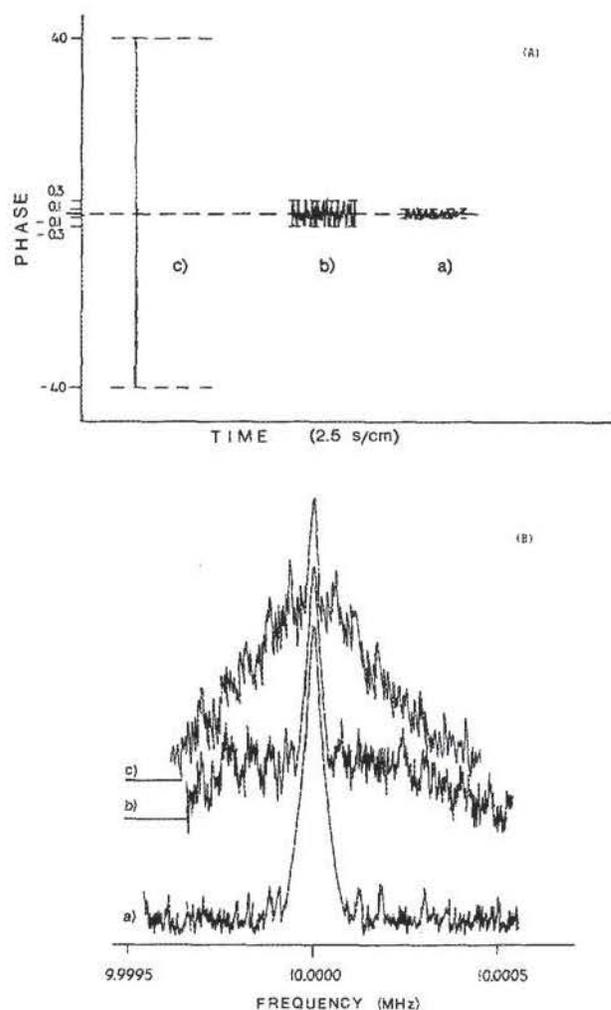
FIG. 5(A). Time variation of the phase of different frequency synthesizers. (c) Model SI-160 (Synthest Corporation); (b) Model 2022C (Marconi Instruments, Ltd.); (a) PTS 500 (Programmed Test Sources, Inc.). Note the scale variation for (c) with respect to (b) and (a). (B). Power spectrum of the phase noise of different frequency synthesizer at 10 MHz.

FIG. 3. Response of the electronic filter used on the I.S.S. Inc. GREG 200 to isolate the cross-correlation frequency.

spanned was 1 kHz around the 10-MHz frequency carrier, with a 10-Hz frequency window. The direct-synthesis PTS-500 (Programmed Test Sources, Inc.) synthesizer has the lowest phase noise. The other frequency synthesizers examined were the Marconi, model 2022 (Marconi Instruments, Ltd.) and model SI-160 by Synthest Corporation. All instruments are phase-locked loop frequency synthesizers.

The phase noise level of the Marconi 2022 and the Synthest SI-160 synthesizers, as shown in Fig. 5, is well above the 0.2° limit that was assumed to be the maximum allowed for the good performance of a MPF.

V. EFFECT OF CROSS-CORRELATION FREQUENCY ON PHASE ERROR MEASUREMENT

In Table II, we report measurements performed with the cross-correlation multifrequency phase and modulation fluorometer described by Gratton and Limkeman.[5] The experiment uses 5-s integration and compares a scattering glycogen sample versus another scattering glycogen sample. The absolute phase difference is close to zero since the two samples are identical and the standard deviation of 10 phase and modulation determinations is well below the required limit of $\pm 0.2°$ and ± 0.004 for the phase and modulation, respectively, for all the synthesizers used. These phase measurements have a much lower phase noise with respect to the phase noise of the synthesizers, in accordance with the derivation of the equations of the previous section. A closer inspection of the standard deviation of the phase and modulation measurements reveals that, under the specific conditions of the measurement, the quality of the synthesizers used is important, although the results are satisfactory in all cases.

The phase noise of the synthesizers is largely independent of the cross-correlation frequency. Consequently, the ratio between the phase noise and the cross-correlation frequency decreases as the cross-correlation frequency is increased. This results in a lesser effect of the phase noise of the frequency synthesizer with respect to the bandwidth of the filter used to isolate the cross-correlation frequency in the measurements performed by a MPF. In order to minimize the effect due to filter detuning, the ratio of the cross-correlation frequency to the filter bandwidth should be increased. In Fig. 6, we report the standard deviation of a series of ten measurements of phase and modulation as a function of the cross-correlation frequency for the same sample used in the previous measurement. For this experiment, the filter used
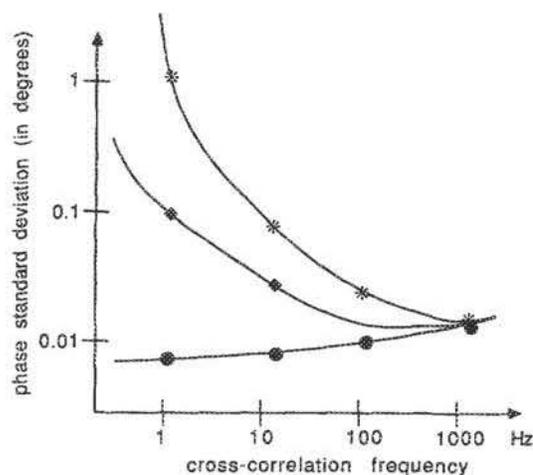
FIG. 6. Standard deviation as a function of the cross-correlation frequency for three different synthesizers ◆ = Marconi 2022, ● = PTS 500, and * = Synthest SI-160.

to isolate the cross-correlation frequency is a digital filter with a constant $Q = (f/\Delta f) \simeq 200.$[10] From this study, it appears that the phase noise of the synthesizers becomes less important at higher cross-correlation frequencies. This is due to the fact that the filter used has a constant Q; therefore, by increasing f, Δf also increases and the effect of filter detuning is less important.

VI. DISCUSSION

The conclusion of this study is that low-cost phase-locked loop synthesizers can be used in frequency-domain fluorometry without degrading the performance of the instrument. However, the value of the cross-correlation frequency must be high enough for the ratio of the phase noise to the cross-correlation frequency to be smaller than the bandwidth of the filter used for isolating the cross-correlation frequency. Table III reports minimum acceptable values for the cross-correlation frequency for different synthesizers. For a system that uses a photomultiplier tube for light detection, a high-frequency limit for the cross-correlation frequency is imposed by the current-to-voltage converters used to analyze the PMT output signal. Generally, it is inconvenient to use cross-correlation frequencies higher than 1 kHz, due to digital analysis of the phase and modulation signal. For systems that use a microchannel plate detector, it is possible to use higher cross-correlation frequencies without degrading the system's performance.

TABLE II. Measurements performed using a glycogen solution at 10-MHz modulation frequency using a cross-correlation frequency of 40-Hz. The synthesizer driving the pockel cell modulator was an HP3525B.

Synthesizer	Phase (in degrees)	Modulation
PTS 500	0.010 ± 0.012	1.000 ± 0.001
Marconi 2022	-0.009 ± 0.009	1.000 ± 0.001
Synthest SI-160	$+0.012 \pm 0.055$	0.999 ± 0.002

TABLE III. Limits imposed by the digital filter used in the experiment.

Synthesizer	Minimum cross-correlation frequency
PTS 500	~1 Hz
Marconi 2022	⩾10 Hz
Synthest SI-160	⩾100 Hz

ACKNOWLEDGMENTS

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