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Reply to "Comment on 'Digital parallel acquisition in frequency domain fluorometry' " [Rev. Sci. Instrum. 60, 2929 (1989)]

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The duty cycle problem referred to by Alcala has been discussed in our original manuscript, and we calculated the reduction of the signal quite explicitly. His comments on the harmonic content are in error, and the correct values were given in the original paper.

The comment of Ricardo Alcala touches on two ideas presented in our article, "Digital parallel acquisition in frequency domain fluoremetry" [Rev. Sci. Instrum. **60**, 2929 (1989)]. The first is that the advantages due to the parallel acquisition scheme are decreased by the low duty cycle of the instrument and the second is that the harmonic content we have obtained is greater than would be expected based on his theoretical arguments. We will discuss both of these issues with respect to our original manuscript and Dr. Alcala's letter, as well as clarify several details that apparently have been misunderstood by Dr. Alcala.

With respect to the low duty cycle, we have always been aware of this problem and it is discussed at length in our paper. On p. 2934, there is a detailed description of the duty cycle problem and our solution to it based on a trade-off between duty cycle and the number of harmonics that can be collected. Dr. Alcala was a student in our laboratory when we began to develop this technique, and was present at several meetings where this problem was discussed. Therefore, it is quite surprising that he was unable to recognize the discussion of the duty cycle problem while reading this section of the paper. We believe that we have stressed the importance of the problem and thoroughly discussed it in our article. Thus, we consider Dr. Alcala's note of clarification superfluous.

Regarding the second point, the comment of Dr. Alcala is not redundant, but wrong. Figure 8 in our paper shows the experimental data of the output of a cross-correlated PMT signal arising from our cavity-dumped laser. Examination of this plot shows that the amplitude of the ninth harmonic is about $\frac{1}{9}$ that of the fundamental, not the $\frac{1}{5}$ found by Dr. Alcala. (In fact, the only factor of five reduction that we discuss, on p. 2934, second column, 5th line from the bottom, refers to the reduction of duty cycle from $\frac{1}{2}$ to $\frac{1}{10}$ and not a reduction in signal strength.) Dr. Alcala states that his Eq. (2) is in agreement with Fig. 8 of the paper, and the signal $F(t)C(t)$ "is what is used to temporally resolved the luminescence." However, he fails to realize that since $F(t)$ is linear with the excitation in our experiments and that the harmonic content of the excitation is flat over at least the first ten harmonics (see our Fig. 7), the function $F(t)$ will also be flat, and therefore the signal of interest will be proportional to $C(t)$. This is indeed in excellent agreement with the experimental data reported in our paper. Furthermore, Dr. Alcala's Eq. (3) is not relevant to the problem, as even he states $F(t)C(t)$ is the signal output of the detection system. For the cross-correlation signal, it is the voltage signal and not the electrical power that counts, so his main reasoning used in the second part of his comment is erroneous.

There are several other mistakes in Dr. Alcala's comment. The evaluation of the 25 W of average power needed to provide 50 V of peak-to-peak output is certainly wrong! Dr. Alacala is confusing peak-to-peak voltages with RMS voltages. The correct calculation shows that only about 6.5 W are needed to generate the 50 V signal. Another example of the kind of careless analysis that permeates Dr. Alcala's letter is his statement that the "...low frequency signals are filtered, digitized and Fourier transformed... ." While it is true that in a standard phase fluorometer the filtering takes place directly after the PMT, in our application the signal is digitized as soon as possible, and all of the signal processing is done by software. Indeed, one of the major points of our paper is that the software filtering is superior to existing analog filters and that by doing only the current-to-voltage with analog electronics conversion we bypass many potential noise sources.

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