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Publication Date

1976

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January 15, 1976

Prepared for the U. S. Energy Research and
Development Administration under Contract W-7405-ENG-48

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Photon Counting System for Subnanosecond
Fluorescence Lifetime Measurements

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Abstract

A new photon counting system has been developed for subnanosecond fluorescence lifetime measurements. The system incorporates a nanosecond light pulser, a dual counter unit, and a constant-fraction discriminator. The operating conditions of the light pulser have been adjusted to minimize the spread of the light pulse waveshape. The discriminator has upper and lower level adjustments and a time walk of no more than ± 35 psec over a 50-mV to 5-V input pulse amplitude variation. The measuring system has the total system time resolution, expressed as the FWHM of the light pulse, of 800 psec and 1480 psec using photomultipliers 8850 and 8852, respectively, with full photocathode illumination and optimized operating conditions. The system will measure both the single and multiple decay components, and it is designed and optimized for experiments involving measurements of decay time constants as short as 90 psec.

Introduction

Among the different techniques available for subnanosecond fluorescence lifetime measurements,¹ the single-photon counting method has gained wide acceptance. A sample is repetitively excited with short light pulses and resulting fluorescent pulses adjusted in intensity so that only one photoelectron is produced at the photocathode of a fast high-gain photomultiplier. It is the most sensitive of techniques for measuring lifetimes, offering excellent signal-to-noise ratio, the measuring system wide dynamic range of several decades of light intensity, and subnanosecond lifetime measurement capabilities. Due to the finite width of the light pulse, the time resolution limitations of the photomultiplier, and the electronic signal processing system the experimental fluorescence lifetime curve is significantly distorted. In order to extract the true value of lifetime parameters from the experimental data, it is necessary to solve the convolution integral.² Generally, the convolution procedure is more accurate if experimental data are measured under identical experimental conditions, particularly concerning the variations of photomultiplier and electronic circuitry parameters, as well as variations of the waveshape of the light pulse. Our measurements have shown that in situations where a subnanosecond lifetime must be accurately measured, the fundamental limitations on the precision of measurements are imposed by the following conditions: the fluctuations in the light pulse waveshape, the single-photoelectron time spread of the photomultiplier, the measuring system dependence on the excitation and emission wavelengths, and the timing error introduced by the discriminator used in the system. Consequently, a high-precision measuring

system operates with the shortest possible light pulses, because the time spread in the light pulse waveshape is in absolute amounts smaller for narrower pulses. Also, the fastest photomultipliers with adequate gain and optimized operating conditions^{3,4} are used, since they generally exhibit a relatively smaller value of the single-photoelectron time spread. To minimize the measuring system wavelength dependence,⁵ the same optical filter for the excitation light pulse is used in recording both the excitation and fluorescence emission profiles, and the difference between excitation and fluorescence wavelength is chosen to be as small as practically possible.

According to our knowledge, fluorescence lifetime measurements using a single-photon counting system for substances with lifetimes smaller than 480 psec have not been reported in the literature. Our system, which uses the above-mentioned components with optimized operating conditions, can measure fluorescence lifetimes as short as 90 psec.

Measuring System Design and Operation

A block diagram of the photon counting system is shown in Fig. 1. Basically, the system electronics can be divided into four main sections: the reference (START) channel, the fluorescence (STOP) channel, the data accumulation channel, and the computer. More specifically, the system consists of a light pulser, a reference constant-fraction discriminator, a fluorescence signal constant-fraction discriminator with upper and lower level adjustments, a photomultiplier with its thermoelectric cooling chamber, delay lines, time-to-pulse amplitude converter, pulse-height analyzer, analyzer-to-computer interface, and a computer. All components used in the system are available commercially with the exception of the

constant-fraction discriminator with upper and lower level adjustments, which increase the accuracy of a measurement. This paper will treat only the discriminator in considerable detail.

An Ortec 9352 nanosecond light pulser, with operating conditions adjusted to minimize the light pulse waveshape spread, is used as the light source. The light pulser operates as a relaxation oscillator and generates light pulses by a spark discharge between tungsten electrodes.

The sample is placed in the cuvette and repetitively excited with nanosecond pulses of light. Colored glass or interference filters A and B are used to isolate the proper wavelength of excitation and fluorescence emission for each individual sample. The fluorescence light is detected at 90° to the exciting light. The lenses A and B, focal lengths 15mm each, focus the exciting light onto the sample and the fluorescence light onto the photomultiplier photocathode, respectively. Both lenses have a diameter of 25 mm. The fluorescence light is attenuated with the variable diaphragm to a low intensity so that the detection system is in the single-photoelectron mode. This mode is obtained when about 10% of the exciting light pulses result in an output pulse from the photomultiplier. The variable diaphragm aperture is typically 20 mm. The photomultipliers used are RCA's 8850 or 8852. The former has a 116 spectral response, and the latter has a 119 ERMA III spectral response which extends into the near-infrared region.

The choice of photomultiplier for a particular experiment depends upon the wavelength of the fluorescent light. In either case the photomultiplier is placed in a thermoelectric cooling chamber to reduce the dark pulse counts. The reference (START) pulse is obtained from an RCA 1P28 photomultiplier, operating at 1.2 kV, which is optically coupled by means of a 12-in.-long American Optical LG 3 light guide to the light pulser.

The sample cuvette, the fluorescence and reference photomultipliers, the thermoelectric cooler, the interference filters, the diaphragm, and the shutter are all mounted in a metal compartment that is light tight and electrical-interference tight. The aperture of the variable diaphragm is adjustable from outside of the compartment. The fluorescence signal photomultiplier output pulses are processed in a specially designed constant-fraction discriminator, with upper and lower level adjustments, which has a time walk less than ± 35 psec over a 50-mV to 5-V input pulse amplitude variation. The constant-fraction discriminator output is applied to the STOP input of a time-to-pulse height converter via an adjustable delay line, used for calibration purposes. The reference channel photomultiplier output pulse is processed in a constant-fraction discriminator to minimize time spread. The first output channel from the discriminator is applied to the START input of the time-to-pulse height converter via a second delay line. The second output channel from the reference channel discriminator is applied to the first input of a two-channel counter with a dual display. Whenever a pulse appears at the STOP input of the time-to-pulse height converter, following a START pulse within a preset interval, an output pulse will be produced at the first channel of the time-to-pulse height converter. This pulse is applied to the second channel of the dual counter, and it is counted as a single photon event. Since the dual counter has a count preset capability from 10^2 to 10^7 counts, a direct percentage readout of the measuring system counting efficiency is readily available. The time-to-pulse height converter is followed by an analog-to-digital converter whose output pulses are applied to a 1024 channel pulse-height analyzer.

After many excitations, the number of counts versus channel number on the multichannel analyzer gives the decay time function of the fluorescence

intensity. The relation between channel number and time is established by calibrated variable delay lines. Since the analysis of fluorescence decay data requires corrections for the shape of the light pulse and evaluation of complex decay functions, the multichannel analyzer memory contents are transferred to the Sigma 2 computer for further numerical analysis. The multichannel analyzer is interfaced with the computer by means of the LBL analyzer-to-computer interface.

Nanosecond Light Pulser Considerations

A critical evaluation of the photon counting system shows that the spread in the light pulse waveshape is one of the fundamental limitations on the precision of the measurements. For an accurate measurement of fluorescence lifetimes and the necessary mathematical corrections, the spread in the light pulse waveshape should be as small as possible in comparison with the value of the sample decay time. This is particularly important in situations where operating conditions of the photomultipliers are optimized to obtain a minimum value of the photomultiplier single-photoelectron time spread.⁶

For the relaxation-type nanosecond light pulser, such as Ortec Model 9352, which uses two tungsten electrodes to form a spark gap, our measurements show that the optimum operating voltage for a minimum light pulse waveshape spread is 5 kV. In this case the full width at half maximum of the light pulse is less than 800 psec. The tungsten electrode spacing, which is adjustable between 0 and about 2 mm by means of a collar near the spark chamber,⁷ is adjusted to a value which gives a light pulse rate of 14×10^3 pulses/sec. Under these operating conditions, the photon yield is approximately 2.8×10^6 photons/pulse. Also, to obtain stable performance of the light pulser with respect to the light pulse and waveshape spread, it is necessary

to gently and continuously flush the spark gap chamber with a flow of dry air during operation. The electrode tips have to be cleaned after every 10 hours of operation and the electrode spacing reset. Also it is necessary to resharpen the pointed electrode into a conical tip after significant erosion has occurred. The maximum photon yield of approximately 1.2×10^7 photons/pulse is obtained by using a nonoptimum operating voltage of 9 kV. Under these conditions, the FWHM of the light pulse is 2.2 nsec, and light pulse waveshape spread is about five times worse than in the case when spark gap is operated with optimum operating voltage. Also, the light pulser performance is significantly degraded with increasing air pressure in the spark chamber.

Optimization of Photomultiplier Operating Conditions for a Minimum Transit Time Spread

The total electron transit time spread of the photomultiplier, used in the subnanosecond photon counting system, represents the second major limitation on the precision of the decay time measurements. The transit time spread of an electrostatically focused photomultiplier consists of the photoelectron transit time spread between the photocathode and the first dynode of the multiplier, the electron transit time spread in the electron multiplier, and that between the electron multiplier and the anode. The major causes of transit time spreads are the distribution of initial emission velocities of photoelectrons and secondary electrons, unequal electron path lengths between different electrodes, and nonuniform electric fields. Generally, the initial stages of a photomultiplier contribute predominantly to the total transit time spread. The transit time spread resulting from the initial velocity distribution is decreased by increasing the voltage between the photocathode and the first dynode. Similar considerations are

valid for the secondary-electron initial velocity in an electron multiplier.

The single-photoelectron time spread and the time response of photomultipliers have improved considerably within the last few years due to better electron-optical design and to the application of new secondary emitters. Secondary emission limitations, which result from the short escape depth of electrons in conventional secondary emitters, are reduced substantially by the use of cesium-activated gallium-phosphide secondary emitters.

Optimization of operating conditions of the RCA 8850 and 8852 photomultipliers for a minimum transit time spread is performed by using the procedure and measuring system described in Ref. 3. For both photomultipliers the single-photoelectron time spread is measured as a function of the voltage ratio between the photocathode-focusing electrode and photocathode-first dynode, $(V_C - V_{FE}) / (V_C - V_{D1})$, to determine the optimum operating conditions. The results of measurements are given in Fig. 2 for full photocathode illumination. For the 8850 photomultiplier the transit time spread has a minimum value FWHM of 0.41 nsec for $(V_C - V_{FE}) / (V_C - V_{D1}) = 0.95$. The 8852 photomultiplier has a minimum time spread of 0.68 nsec for $(V_C - V_{FE}) / (V_C - V_{D1}) = 0.85$. The voltage-divider networks for both photomultipliers are designed according to the above criteria for a minimum time spread. Furthermore, positive voltage supplies are applied to the voltage-divider network so that the photocathode is at the ground potential during operation. This voltage-divider configuration is essential to reduce the number of noise pulses generated by the electroluminescence in the photomultiplier glass envelope and face plate because the thermoelectric cooling chamber components surrounding the photomultiplier are at ground potential.

Constant-Fraction Discriminator Description

The time resolution capabilities of the photon counting system, in addition to the spread in the light pulse waveshape and photomultiplier electron transit time spread, are determined by the time-walk and resolution characteristics of the constant-fraction discriminator. The discriminators used in this system are developed with improved time-walk and resolution characteristics. The photomultiplier signals are properly shaped by an attenuation-subtraction technique producing a pulse with a zero-crossing point and a pedestal added, allowing adjustment of the discriminator to the zero-crossing point. The fast baseline crossover point of a bipolar pulse is relatively independent of the pulse amplitude, and it can be conveniently used to obtain the amplitude-independent timing information. Despite the amplitude-independent crossover point of a bipolar pulse, a leading-edge detector triggered at this point introduces a time walk, in the nanosecond region, when there is a large dynamic range of input pulse amplitude. To overcome this shortcoming, a pedestal is added to shift the bias up to the detector threshold at the right time. By doing this, the detector triggers as soon as the zero-crossing point of the bipolar pulse is reached, producing an almost amplitude-independent timing pulse. To increase the input amplitude dynamic range of the discriminator, the bipolar pulse is additionally amplified and peak limited before being applied to zero-crossing and threshold detectors. All essential functions responsible for time walk in the discriminator, such as threshold discrimination and fast pulse zero-crossing detection, are performed exclusively by means of tunnel diodes with a backward diode as nonlinear load. Other less essential functions, such as limiting, amplification, comparison, and gating, are performed by hot carrier diodes, fast transistors, and emitter-coupled logic, MECL III series.

A schematic diagram of the constant-fraction discriminator is given in Fig. 3. Pertinent waveforms at the specified points in the diagram and the sequence of operation of the discriminator are given in Fig. 4. Referring to both figures simultaneously, the negative unipolar input anode signal, having an amplitude anywhere from 50 mV to 5 V, enters the discriminator at point A where it is split into two parts. The first part of the input signal is delayed for time $t_2 - t_0$, by means of the delay line DL1 at point B. The second part of the input signal is immediately attenuated, by a factor of 5, by an attenuator consisting of resistors R2 and R3 at point C. The value of the resistor R1 is selected experimentally to obtain a discriminator input impedance as close as possible to 50 Ω ; this reduces the amplitude of the reflected signal from the input of the discriminator. Because the photomultiplier, as a signal source, usually is not back-terminated, any reflected signal would be re-reflected back to the input of the discriminator. At high input signal levels the re-reflected signal could be high enough to be processed (by the sensitive fast discriminator and the measuring system) as a single-photoelectron photomultiplier signal, thus yielding spurious data. The attenuated signal is inverted by means of a wideband transformer which has a bandwidth greater than 400 MHz. The delayed and the inverted signals are added, creating the bipolar signal at point D. An amplitude-limiting network, consisting of the hot carrier diodes CR1 and CR2, attenuates high amplitudes of the bipolar signal to a maximum of 400 mV, preventing damage and minimizing the time walk due to the overloading of the following discriminator stages. After limitation, the bipolar signal is amplified by a factor of 3 and inverted by the microwave transistor Q1. (This is indicated by the waveshape at point E.) After amplification, the bipolar

signal is again amplitude limited by means of diodes CR7 and CR8. At this point, the zero-crossover point of the bipolar signal is stable within ± 15 psec through an input signal amplitude dynamic range from 50 mV to 5 V. This input signal amplitude dynamic range is effectively reduced to the 50-400 mV range by the limiter-amplifier-limiter operation. The signals of the 50-400 mV dynamic range are applied to the threshold tunnel-diode zero-crossing discriminator.

The positive portion of the bipolar pulse serves as a trigger pulse for the tunnel diode CR15 threshold detector. The peak current of the diode CR15 is 4.7 mA. The fast crossover slope provides better triggering for the leading-edge threshold discriminator, resulting in a pedestal with less time shift caused by the wide dynamic range of the input signal pulse amplitude. By operating the leading-edge threshold detector in this fashion, the pedestal time shift was found to be approximately 500 psec better than the same detector triggering at the leading edge of the input signal pulse, over a dynamic range of 30:1. The variable resistor R33 is the threshold adjustment of diode CR15. Inductor L14 and the backward diode CR16 serve as the nonlinear load for tunnel diode CR15. Operating a tunnel diode in this mode improves sensitivity and reduces standby power dissipation. The output of this stage in turn triggers the tunnel diode CR18, which is the pedestal generator. This diode has a peak current of 10 mA. A shorting stub is the load for this stage, which generates a 15-nsec-long pedestal (point G). While this is taking place, the bipolar pulse is being delayed for time $t_5 - t_4$, approximately 5 nsec, by the delay cable (point F), before appearing across tunnel diode CR20, the zero-crossing detector. Diode CR20 has a peak current of 10 mA. Variable resistors R47 and R34 are for bias adjustment of the tunnel diode CR20 and the pedestal generator diode CR18,

respectively. The bias pedestal is applied to the zero-crossing detector diode CR20 through inductor L16 and resistor R44. L16 is used to suppress the overshoot at the leading edge of the pedestal. The pedestal raises the bias of CR20 nearly to its threshold level to reduce trigger delay due to different slew rates of the zero-crossing pulses. The zero-crossing detector is a one-shot multivibrator, using an inductor L15 and a backward diode CR19 as a nonlinear load.

The driver stage that follows uses tunnel diode CR23, transformer T2, and the backward diode CR24 as its nonlinear load. Diode CR23 has a peak current of 10 mA. Variable resistor R35 provides bias adjustment for CR23.

The driver stage output signal, at point I, is further delayed by the time $t_7 - t_6$ of 10 nsec. This is done to allow enough time for the gating signal (generated by comparators M2 and M3, operating as leading edge threshold discriminators, and the gate generator, tunnel diode CR13), to be properly applied to the reference input of comparator M4 through the entire signal amplitude range before the driving pulse arrives at M4.

To avoid the degradation of the time-walk characteristics of the discriminator as a whole, the comparator M2 is used as the threshold discriminator that inhibits output pulses below and above certain input signal levels. In this way, the threshold level of the tunnel diode CR15 is adjusted to obtain the best possible time-walk characteristic throughout the entire input pulse amplitude dynamic range, and it stays fixed at that level.

Variable resistors R11 and R8 are the upper and lower threshold adjustments for comparator M2 respectively. When an input pulse exceeds the lower threshold set by R8, the appropriate section of M2 produces an output pulse which is differentiated by C48 and R23 and then delayed by DL2 for 5 nsec before going to pin 5 of comparator M3. Pin 6 of M3 is normally biased at -100 mV because the negative portion of the differentiated output of M2, point L,

is -400mV; M3 would produce an output pulse as long as its reference bias; pin 6 remains at -100 mV. The output of M3 then triggers tunnel diode CR13, which is the enable gate generator for the output driver, M4. The width of the gate generated by CR13 is determined by delay line DL5, which is selected to provide a 25-nsec gate, point P. However, if the input pulse amplitude also exceeds the upper threshold level set by R11, the other half of M2 would produce an output pulse, point M, which is differentiated by C18 and R22, point N. The negative portion of the output pulse at point N in turn triggers tunnel diode CR10 whose output, point O, becomes an inhibit gate for M3. Hence with both the lower and upper sections of M2 operating, M3 will not produce any output and M4 accordingly will not produce an output pulse.

Consequently, by properly adjusting variable resistors R8 and R11, a window can be adjusted to respond only to input pulses within the selected amplitude range. A window of 20 mV is attainable by adjusting R8 and R11.

Figure 5 shows a pulse-height spectrum of the output of 8850. The first peak is the single-photoelectron peak, and the second peak is the two-photoelectron peak; no gating was used. Figure 6 shows the same pulse-height spectrum with the upper and lower threshold settings adjusted to select pulses with pulse height exceeding 50% of the single-photoelectron pulse height.

To evaluate the performance of the constant-fraction discriminator, the system described in Ref. 8 was used to measure the time walk and resolution of the discriminator as a function of input pulse amplitude. Figure 7 shows the result of the measurement. The time walk of the discriminator is ± 35 psec over an input pulse amplitude range from 50 mV to 5 V. The intrinsic time resolution of the discriminator with a constant 2-nsec risetime pulse is 70 psec at 50 mV, decreasing exponentially to 20 psec at 800 mV.

Photon Counting System Wavelength Dependence Considerations

The presence of a significant wavelength dependence in the system response function can seriously limit the measurement accuracy, particularly when very short decay times are measured.⁵ The system wavelength dependence arises in part in the photomultiplier, from the dependence of the kinetic energy of the emitted photoelectrons on the wavelength of the incident photons, and in part in the light pulser emission profile wavelength dependence. The light pulser wavelength dependence can induce a spurious long decay time component in the analysis if the excitation and the fluorescence emission profiles are recorded at widely separated wavelengths. Concerning the light pulser wavelength dependence, we observed that the red emission from the air gap spark discharge is broader in time and has a significantly larger tail component than the ultraviolet emission.⁹ Generally, to minimize the system wavelength dependence, which arises from both photomultiplier and light pulser effect, it is important to use the same light pulser-filter in recording both the excitation and fluorescence profiles and to minimize the difference between the excitation and fluorescence emission wavelength.

Results

Resolution characteristics of photon counting systems are measured with both a non-fluorescent scatterer and with erythrosin in water as the sample. In the first case, the sample is replaced by a piece of white reflecting surface at an angle of 45 degrees to the symmetry axis of both the light pulser and the photomultiplier. The total system time resolution is expressed as the FWHM of the light pulse measured by a particular photomultiplier. With the RCA 8850 operated at the supply voltage of 3000 V and cooled down to 0°C, the system total time resolution is 800 psec FWHM, as shown in Fig. 8. The spectrums are spaced 8 nsec apart by means of the calibrated delay line

and no colored filters are used. The spark gap is cleaned and adjusted before measurements. The resolution decreases to approximately 1 nsec in 30 hours of operating time if a gentle stream of dry air is used for flushing of the spark gap. Without dry air flushing, the resolution decreases to 1 nsec in 15 hours of operating time. The total system resolution is 1.48 nsec when using the 8852 photomultiplier as shown in Fig. 9. The 8852 is operated at the supply voltage of 2800 V, after the photomultiplier is kept in the dark for 24 hours and cooled down to 0°C. During the measurements a Corning range glass filter, No. CS 3-66, is used at the output window of the light pulser. The CS 3-66 is a cutoff filter with a 0.02% transmittance at 540 nm and a 50% transmittance at 575 nm. The system resolution increases to 1.08 nsec when a Corning CS5-58 blue glass filter is used instead of CS3-66. The CS5-58 filter has transmittance of 0.02% at 340 and 480 nm, and a peak transmittance of 40% at 415 nm.

The system resolution and the short fluorescence lifetime measurement capabilities are determined by using erythrosin in water as a sample. By using the 8850 photomultiplier and the light pulser with optimized operating conditions (light pulse waveshape is given in Fig. 10), as well as solving the convolution integral in order to extract lifetime parameters, the fluorescence lifetime of 90 psec is determined for erythrosin in water.⁹ The fluorescence emission is at 550 nm, with excitation wavelength at 470 nm. This result compares favorably with measurement obtained with considerably more expensive and complex mode-locked laser systems.¹⁰

Acknowledgments

This work was performed as a part of the program of the Electronics Research and Development Group of the Lawrence Berkeley Laboratory, and the

Laboratory of Chemical Biodynamics, University of California, Berkeley, and
was supported by the U. S. Energy Research and Development Administration,
Contract No. W-7405-eng-48.

Figure Captions

- Fig. 1. Block diagram of the photon counting system for subnanosecond fluorescence lifetime measurements.
- Fig. 2. Single-electron time spread as a function of the voltage ratio between the photocathode-focusing electrode and photocathode-first dynode for the RCA 8852 and 8850 photomultiplier with full photocathode illumination.
- Fig. 3. Schematic diagram of the constant-fraction discriminator with upper and lower level adjustments.
- Fig. 4. Waveforms at the particular points in the constant-fraction discriminator schematic diagram of Fig. 3.
- Fig. 5. Pulse-height spectrum, showing peaks corresponding to one- and two-electron peaks for the RCA 8850 photomultiplier.
- Fig. 6. Gated pulse-height spectrum of the RCA 8850 photomultiplier.
- Fig. 7. Time-walk characteristics of the constant-fraction discriminator as a function of the amplitude of the input pulse.
- Fig. 8. Measuring system total time resolution by using the RCA 8850 with full photocathode illumination as the photon detector.
- Fig. 9. Measuring system total time resolution by using the RCA 8852 with full photocathode illumination as the photon detector.
- Fig. 10. Light pulse waveshape from the nanosecond light pulser with optimized operating conditions.

Footnotes and References

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FIG. 1

NBL 759-8073

RCA 8850 KO 8845
RCA 8852 K 33393

FIG.2

XBL 759-8074

FIG. 3

XBL 759-8077

FIG. 4

XBL 759-8076

FIG. 5

FIG. 6

XBB 759-6536

FIG. 7

XBL 759-8075

CALIBRATION = 33psec PER CHANNEL
TOTAL SYSTEM TIME RESOLUTION = 800psec
RCA 8850 FULL PHOTOCATHODE OPERATION

FIG. 8

XBB 759-6534

CALIBRATION = 60psec PER CHANNEL
 TOTAL SYSTEM TIME RESOLUTION = 1.48nsec
 RCA 8852 FULL PHOTOCATHODE OPERATION

FIG. 9

XBB 759-6535

FIG. 10

XBL 7512-9877

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