Optoacoustic spectroscopy and its application to molecular and particle absorption

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1. ABSTRACT

Light absorption in the ocean has been the least studied optical property because of the difficulties in making accurate measurements. With the previously used techniques, large differences have been reported for the specific absorption coefficient of phytoplankton (cultures and natural assemblages). It is difficult to determine if the diversity in these values are methodological or a function of actual variations in absorption. With the renewed interest and activity in optoacoustic spectroscopy (OAS), which accurately measures absorption, some of these discrepancies should be resolved. In this method, as molecules and particles absorb light from a modulated source, they thermally expand and contract, thereby generating acoustic waves, at the modulation frequency, which are detected by a hydrophone. Optoacoustic spectroscopy is ideally suited for measuring dissolved organic material and particle absorptions because of its high sensitivity (10^{-5}m^{-1}) and the negligible effect of scattered light. In this paper the instrumental design for an optoacoustic spectrophotometer (OAS), which specifically measures phytoplankton absorption (420-550nm), is described. The spectral absorption of dissolved organic material and a phytoplankton culture is presented. OAS holds promise in being able to measure absorption without use of either filtration or concentration techniques.

2. INTRODUCTION

The absorption coefficient is a difficult optical property to measure in the ocean. While light absorption by phytoplankton is a major factor controlling global carbon budgets (CO_2) its role is still not clearly understood[1]. The problem in measuring absorption arises from excluding, in conventional transmission and absorption measurements, light attenuated by molecular and particle scattering. Past instrumental designs and methods have tried to correct for these scattering differences[2-7] but usually involve approximations of one type or another.

The total absorption coefficient (a_T), which is an inherent optical property[8], can be partitioned into several components

$$a_T = a_W + a_{DM} + a_{NL} + a_{PH},$$ (1)

where the subscripts W, DM, NL and PH refer to water, dissolved material, non-living (organic and inorganic) particles and viable phytoplankton, respectively. Most published absorption coefficients for phytoplankton have been normalized to chlorophyll a concentrations, and are expressed as specific absorption coefficients [$\text{m}^2(\text{mg chl } a)^{-1}$]. With the specific absorption coefficient, chlorophyll a concentrations can be used to estimate phytoplankton absorption. Specific absorption coefficients have limitations, however, since phytoplankton pigments (porphyrins and carotenoids) other than chlorophyll a absorb light, and the ability of a cell to absorb light is not accurately assessed by extracted pigment concentrations (excludes the sieve, or package, effect)[9].

Comparisons of published absorption and attenuation coefficients, for phytoplankton, indicate large discrepancies (Table 1). It is difficult to determine if the diversity in these values is methodological or a function of actual variations in absorption. Bricaud et al.[6] found, as have other investigators[10-12], that the specific absorption coefficient is species specific.

Table 1. Apparent specific absorption coefficients of cultures and natural assemblages.

Reference	Wavelength (nm)	Apparent Specific Absorption Coefficient $m^2(mg\ chl\ a)^{-1}$
Yentsch[10]	440	0.097
Prieur[11]	440	0.023
Morel & Prieur[12]	440	0.025
Smith & Baker[2]	440	* 0.168, 0.039
Bannister[13]	438	0.031
Dubinsky & Berman[14]	450	0.012
Prieur & Sathyendranath[3]	440	*0.018, 0.070, 0.077

*variable depending on the optical environment or pigment concentration.

Variation in specific absorption coefficients between species is a function of cell size and pigment concentration as well as the composition of the accessory pigments (porphyrins and carotenoids). However, this variability should be reduced in natural assemblages due to the averaging affect induced by the presence of diverse species[6]. Some of the discrepancies in specific absorption coefficient are most likely methodological, as published absorption coefficients for even pure water show differences of a factor of two[15]. Even spectral shapes of published specific absorption coefficients show significant variability (see Fig. 7, Prieur & Sathyendranath[3]). From these data it can be concluded that more information is needed concerning the spectral forms and absolute values of the absorption coefficients of phytoplankton.

3. OPTOACOUSTIC SPECTROSCOPY

Optoacoustic spectroscopy (OAS) measures the acoustic signal generated by light absorption from a modulated light source. As molecules and particles absorb light, heating occurs, thus causing thermal expansion; when the light is off, the molecules and particles cool and contract. This thermal expansion and contraction generates acoustic waves in the medium which can be detected by a hydrophone. These acoustic waves depend upon the absorption coefficient of the sample as well as on geometrical and thermal parameters of the sample, liquid and cell. Because of the enhanced sensitivity and negligible effects by scattered light, OAS is ideal for measuring the absorption of dissolved organic material and particles in oceanic samples.

OAS is insensitive to scattering in the first order[15-17], and is similar to fluorescence in that optoacoustic signals originate against a blank background, making it ideally suited for studying low absorbing substances. Sensitivities as low as 10^{-4} to $10^{-5} m^{-1}$ and with accuracies of $\pm 10\%$ have been reported[15]. With this sensitivity ($10^{-5} m^{-1}$), and a specific absorption coefficient of $0.016 m^2(mg\ chl\ a)^{-1}$ the phytoplankton absorption coefficient of a Sargasso Sea sample would be 80 times higher than reported OAS minimum detection limits [$0.05 mg\ chl\ a\ m^{-3} \times 0.016 m^2(mg\ chl\ a)^{-1} = 80 \times 10^{-5} m^{-1}$]. Assuming an oligotrophic sample contained 10^6 cells l^{-1} (picoplankton included; Law et al.[18]), the phytoplankton cell density within the OAS chamber (1x1x4cm and 2ml volume) is approximately 140 cells per laser beam diameter (3mm). These two calculations indicate that even in oligotrophic conditions OAS will not require concentration techniques (filtering) to measure phytoplankton absorption. By reducing the laser intensity, samples with higher concentrations can be accommodated and the dynamic range of the instrument can span the expected oceanic range of phytoplankton concentrations.

There have been numerous theoretical and analytical analyses on the optoacoustic effect as applied to gases and liquid[19-26]. In general, the acoustic pressure amplitude (p_o) can be characterized by

$$p_o = constant\ [\ (\ \beta\ v_o\ /\ C_p)\ a\ E_o\ ]\ , \tag{2}$$

where β is the coefficient of thermal expansion, v_o is the acoustic velocity, C_p is the specific heat, a is the absorption coefficient and E_o is the laser energy per pulse. Thus, the amplitude of the acoustic signal is directly proportional to the laser pulse

energy and the absorption coefficient. OAS has been used to derive novel information that has not been easily accessible by conventional methods such as; depth profiling of layered chromophores, molecular photodamage, intermolecular energy transfer in vivo and in vitro, intermolecular energy interactions in situ, characterization of components inside a cell, energy-storage processes, photoaction spectra and quantum yields, photothermal dissipation in vivo, adaptation processes, evaluation of photosynthetic oxygen evolution and morphological changes during plant cell differentiation[23]. Some of the advantages of the OAS over conventional methods for measuring absorption are; (1) the ability to accurately measure dissolved organic material and particulate absorptions with (minimal scattering interferences), (2) the enhanced sensitivity, alleviating the need for concentration techniques, which can introduce errors, and (3) the potential for monitoring particle absorption using continuous-flow cells.

3.1. Photochemically stored energy

It has only been in the last decade that the potential for studying photochemical processes (e.g. photosynthesis) by OAS has been addressed[27]. Ortner and Rosencwaig[28] were the first to measure phytoplankton absorption using OAS. Many other investigators[29-34] have successfully used OAS to probe photochemical processes in chloroplast membranes, whole cells and leaves. The usefulness of OAS in studying these processes stems from the fact that as photochemical intermediates store energy, the optoacoustic signal decreases as compared to the sample which has been photochemically inactivated. The easiest way to visualize this is to describe the processes which dissipate absorbed energy in a phytoplankton sample. In a sample of this nature the total energy absorbed (I^t) is equal to

$$I^t = I^{ht} + I^{fl} + I^{pc},\qquad(3)$$

where I^{ht} is the energy dissipated as heat (acoustic signal), I^{fl} is the energy re-radiated as fluorescence and I^{pc} is the photochemically stored energy. Since I^{ht} and I^{fl} can easily be measured using appropriate detectors, the only unknown variable in Eq. 3 is I^{pc}. Very little information is known *a priori* concerning I^{pc} for phytoplankton and therefore, to determine I^t, the energy stored photochemically (I^{pc}) must be blocked.

There are two approaches in blocking or nullifying I^{pc}. One is to use DCMU, a potent inhibitor of Photosystem II, to physically block electron transport and force I^{pc} to zero[29]. This assumes that the inhibitor does not physically damage or change the optical properties of the phytoplankton and requires sample cell changing between poisoning with DCMU. The second approach, which is more appealing, is to illuminate the sample with background light of sufficient intensity to saturate the photochemical processes [31,33]. Any light then absorbed from the modulated laser beam has a reduced probability of being channeled into photochemical energy since the reaction centers are closed. It has also been demonstrated[30] that the optoacoustic response is a linear function of the laser intensity even in the presence of background illumination. This energy in the photochemically inactive sample is then dissipated as heat and fluorescence. The fractional contribution to the dissipation of absorbed energy as I^{ht}, I^{fl} and I^{pc} for phytoplankton is thought to range from 80-90%, 1-3% and 2-18%, respectively[5].

4. INSTRUMENTAL DESIGN AND METHODOLOGY

A block diagram of the instrumental scheme is shown in Fig. 1. The OAS utilizes a pulsed tunable flashlamp pumped dye laser (Phase-R 1200LP; 3mm beam diameter, 10mJ pulse energy, 1μsec pulse width and 10Hz repetition rate). The dye laser is constructed with a valve system enabling up to three different dyes to circulate (15sec changeover period) which allows a scan from 420-535nm to cover the major absorption bands of phytoplankton pigments. The wavelength at which the dye laser operates depends on the angle of the back mirror and the dye that is being used. This rotational angle of the back mirror, and hence the operating wavelength, is controlled by the computer. Light from the laser enters the sample cell, and then falls onto a power meter (Laser Precision 7000), which is also interfaced to the computer. A simplified schematic of the OAS cell design is shown in Fig. 2 and consists of a sample cell equipped with both acoustic and fluorescence detectors. Op-toacoustic signals from the piezoelectric hydrophone (from Tam and Patel[35]) are processed by a pre-amplifier and enter a gated boxcar integrator. Fluorescence signals from the photomultiplier tube follow a similar path into another gated boxcar integrator. Signals from these two boxcars, the power meter and the scan drive go into a computer interface and then to a personal computer for storage and data reduction.

The signal from the piezo-electric transducer is a highly complicated set of damped oscillations as shown in the acoustic envelope in Fig. 3a. Ringing is caused by a combination of acoustic reflections in the sample cell and mechanical ringing of the piezo-electric crystal. The gated boxcar integrator is used to select one of these peaks (Fig 3b) and compute the average
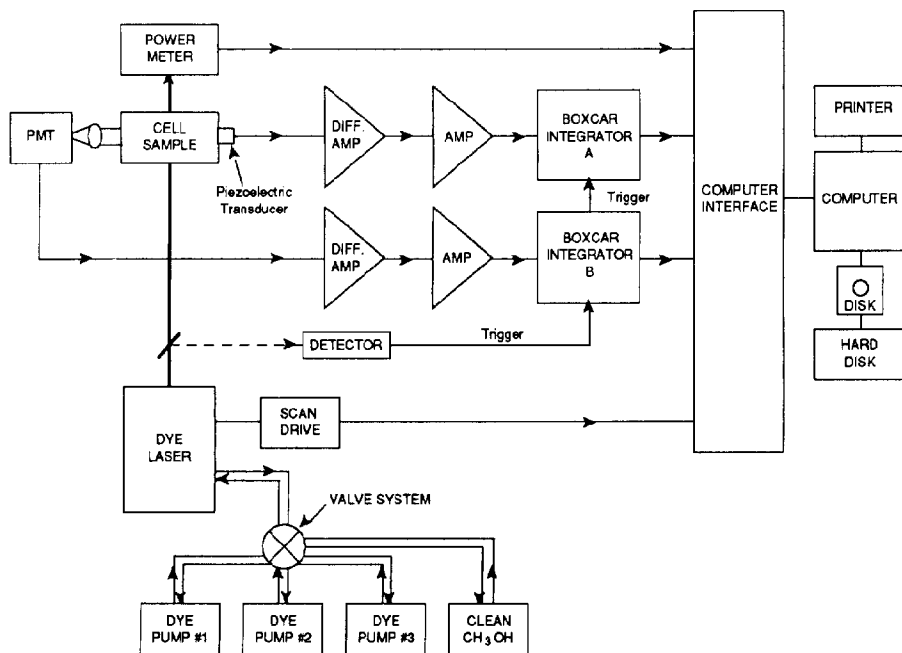
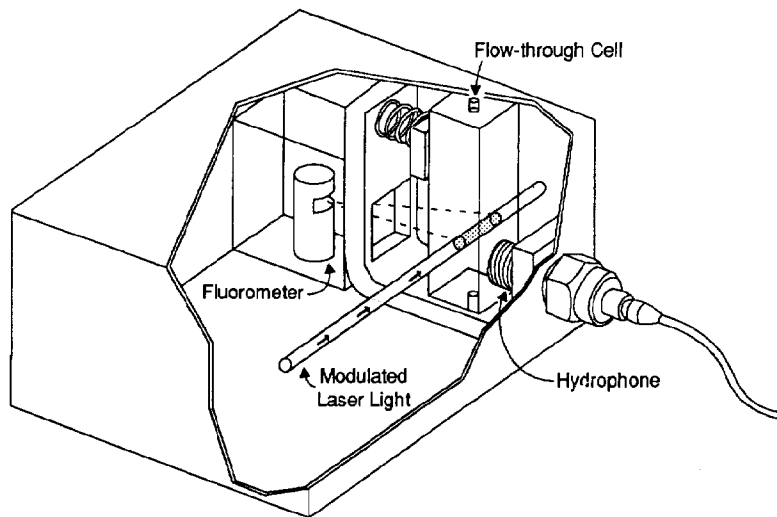Fig. 1. Block diagram of the OAS experimental arrangement.

Fig. 2. Simplified drawing of the OAS sample cell.

signal which is then used as the optoacoustic signal (ρ_o) in the relationship of Eq. 2. A possible source of peak jitter occurs during a spectral scan when movement of the back mirror causes the laser beam to wander in the sample cell. Changing the position of the laser beam causes the peaks to change position relative to the laser pulse due to the acoustic propagation time from the absorption site to the transducer. Special care must be taken in alignment of the laser to prevent this and maintain the alignment of the laser normal to the cell and the rotation axis of the back mirror of the laser. A gated fluorescence signal is shown in Fig 3c.
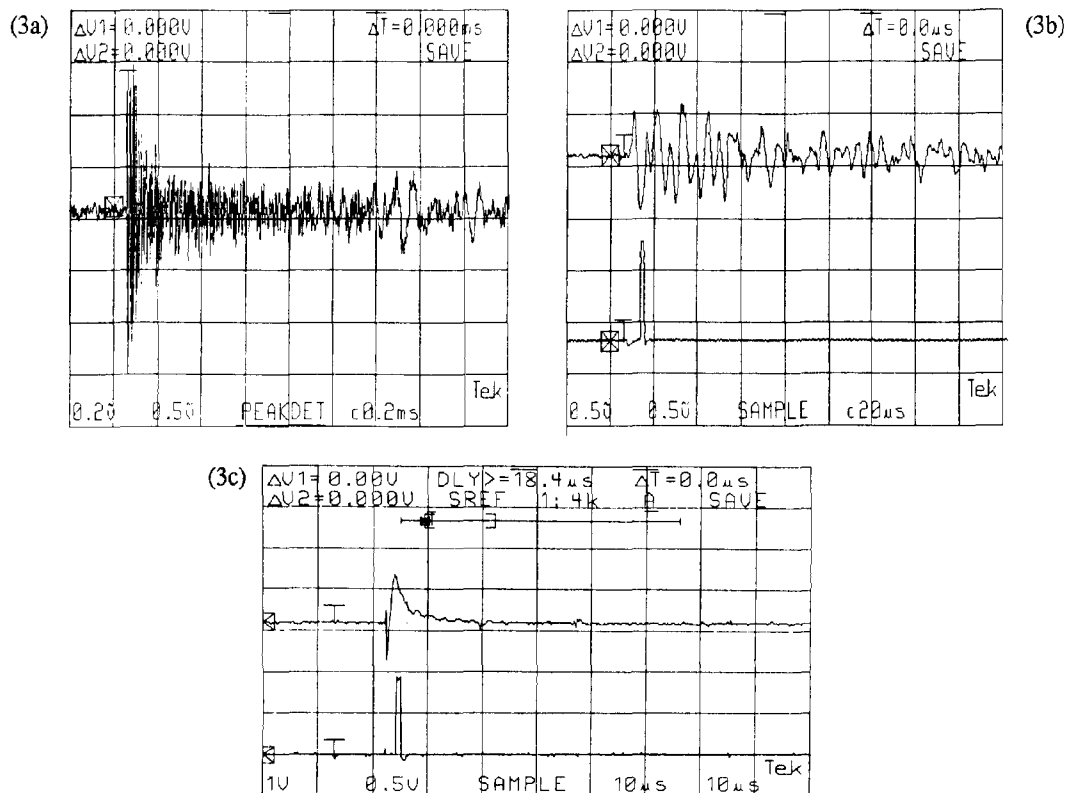
(3a)

(3b)

(3c)

Fig. 3. Example traces of the optoacoustic envelope at 0.2 ms/division (3a), the optoacoustic signal at 20 μs/division with the bottom trace being the gated signal from the boxcar integrator (3b) and the fluorescence signal at 10 μs/division with the gated signal from the boxcar integrator (3c).

The calibration of the DOAS follows that proposed by Tam and Patel[15], where an efficiency factor relating the acoustic spectrum to the absorption spectrum is determined. A dye used must meet the following criteria: highly soluble in the solution and strongly absorbent at the laser wavelengths, non-fluorescent and photochemically inactive. Tam and Patel[15] found $KMnO_4$ a suitable dye when studying absorption coefficients of water. The absorption coefficient of the dye is determined with conventional transmission measurements using long pathlength cells. A sample calibration curve is shown in Fig. 4, illustrating the linearity of the OAS signal. This calibration curve (±2-3%) was used to determine the calibration constant for the data presented below.

4.1. Measurements

High energy pulses (about 1mJ) are required for 10^{-5} m^{-1} sensitivity since the acoustic amplitude is directly proportional to the pulsed energy (Eq. 2). In samples containing phytoplankton these exposures can cause difficulties, especially if the cells are physiologically damaged or optically changed in their ability to absorb light. Unpublished data of J. SooHoo (1985) indicates that, indeed, long term photodamage does occur at high laser energies. Using cultures of Dunaliella tertiolecta grown in f/2 medium and at high light intensities ($300\mu E$ m^{-2}s^{-1}), SooHoo found that cell numbers were reduced at energies of 1J and higher (10ns duration and 1mm diameter with a repetition rate of 20Hz). Short term uptake of ^{14}C was found to be more sensitive in measuring long term exposure effects.

In contrast Ley and Mauzerall[36] found that green algae were not instantaneously photodamaged by high flash laser energies. By simultaneously monitoring both the oxygen flash and relative fluorescence yields as a function of increasing laser energies the shapes of these curves were found to be identical from the minimum up to the maximum yields. This then provides a method for determining the operating energy range of the OAS which will not photodamage the phytoplankton cells during the measurements. An example of a fluorescence trace as a function of laser energy in shown in Fig. 5.
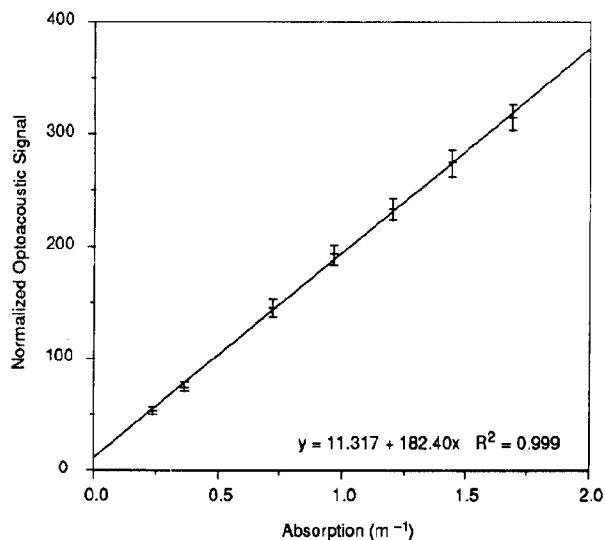
Fig. 4. Calibration of the OA signal
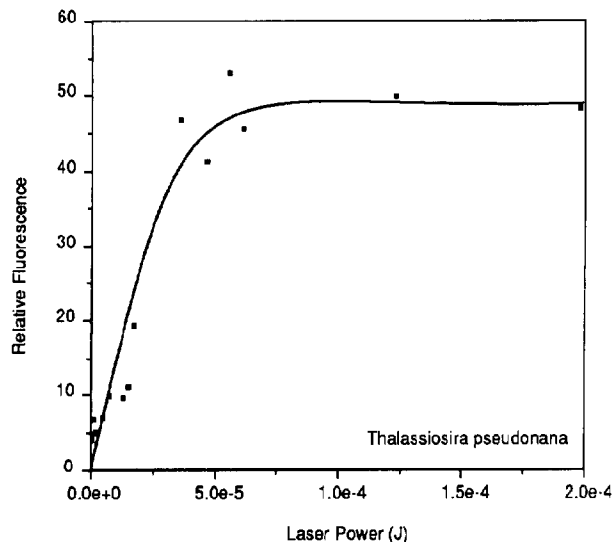(normalized to laser energy) using $KMnO_4$.

Fig. 5. Laser power as a function of relative
fluorescence at 680 nm using the OAS system.

Spectral absorption measurements of dissolved organic material (±2-3%) and phytoplankton cultures (±7-9%) are made in a standard flow-through cell (quartz) to minimize the duration that the solution or phytoplankton cell is in the high intensity laser beam. Spectral scans of a dissolved organic material and a culture are shown in Fig. 6 and 7.

4. CONCLUSIONS

Differential optoacoustic spectroscopy has the potential for accurately measuring light absorption in the ocean. An instrumental design and methodology have been described and data presented of a sample absorption spectra for a phytoplankton culture. The application of OAS to absorption measurements in the ocean would assist in a better understanding of the bio-optical properties and the physiological condition of the phytoplankton.
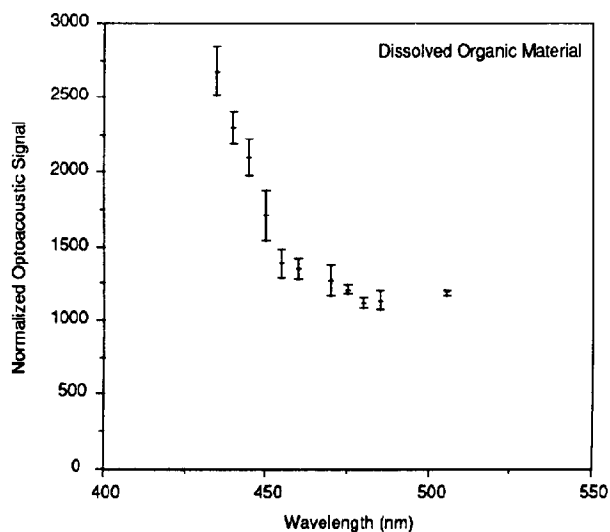

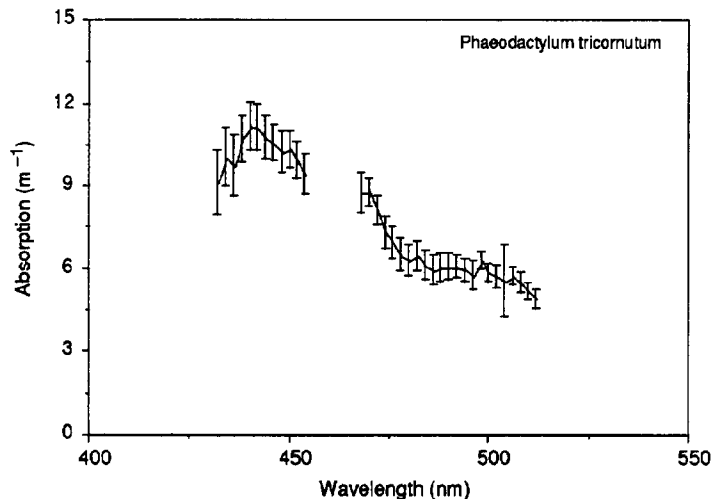Fig. 6. Spectral scan of phytoplankton culture filtrate (DOM).

Fig. 7. Spectral absorption curve of a phytoplankton culture.

5. ACKNOWLEDGMENTS

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