Plant Physiol. (1977) *60*, 30-33

Holographic Interferometric Measurement of Motions in Mature Plants[1]

Received for publication September 7, 1976 and in revised form February 1, 1977

MARTIN D. FOX AND LEROY G. PUFFER
Department of Electrical Engineering and Computer Science, University of Connecticut, Storrs, Connecticut 06268

ABSTRACT

Holographic interferometry has been used to plot graphs of the phototropic and geotropic bending undergone by a mature *Stapelia variegata* Linn over a 5-minute period. The holographic interferometric technique is shown to have the advantage of measuring displacements at least as small as 0.16 micrometers which permits observation of extremely slow plant motions over time periods of a few minutes. In addition, the holographic technique provides a permanent record of displacement information over the entire plant in a single hologram. The short 5-minute period required to produce a holographic interferogram has permitted the monitoring of slow plant motions by recording a series of consecutive holograms at 5-minute intervals over a 75-minute period. The results have been plotted on a graph thereby capturing for the first time such small displacement, velocity, and acceleration of a mature plant as a function of time.

Holographic interferometry is a technique used to measure minute displacements of objects. Since the technique was introduced by Powell and Stetson in 1965 (5), it has been applied almost exclusively to the study of nonbiological systems. In 1967 Williams (10) demonstrated the potential use of holographic interferometry in measuring plant movement by detecting the motion of wheat coleoptiles. Due to low fringe visibility, Williams found it necessary to paint the plants. His primary objective was to determine the feasibility of the biological application and thus no quantitative analysis was made nor were provisions made for detecting the direction of motion.

Coleoptiles, such as used by Williams (10), respond so strongly to phototropic stimuli that their response can be adequately measured using conventional techniques. For this reason coleoptiles have been used extensively to examine the phototropic system. The great sensitivity of the interferometric technique can be better utilized in the quantitative examination of slower moving mature plants to which conventional measuring techniques are relatively insensitive. The use of mature plants also allows the investigation of additional biological systems which are inactive or masked in young, rapidly growing plants.

Recently Fox and Puffer (3) demonstrated that holographic interferometry could be applied to the quantitative analysis of typically slow moving mature plants without applying paint or subjecting the plant to any other perturbing influence. The present research goes beyond previous work by measuring both the magnitude and direction of small plant motions. The results show that by utilizing mirrors to obtain multiple observation points, a single holographic interferogram can be used to measure the precise direction and extent of plant motions in the micron range occurring over time periods of a few minutes. The information extracted from typical holographic interferograms is plotted to illustrate the bending of a *Stapelia variegata* Linn in response to geotropic and phototropic stimuli. In addition, a sequence of holographic interferograms has been used to record plant motion as a function of time.

THEORY

Holography is a well known process which produces three-dimensional images (4, 8). Holographic interferometry requires the production of a double exposure hologram of an object, which in this case is a plant. If a small plant motion occurs between the two exposures, the double exposure hologram will produce two superimposed images of the plant, but because of the small extent of the motion only one image will appear to exist. Due to the coherent nature of the light, interference fringes appear on this image. By examining these fringes it is possible to determine the direction and magnitude of the movement which any point on the plant has undergone during the time interval between the two exposures.

A number of techniques have been developed to interpret interference fringe patterns (1, 6, 7). The multiple observation point approach described by Sollid (6) was selected in this application because a stable reference point could be observed on the object and the small hologram size prohibited the use of any technique requiring moving of the observation point. As shown in Figure 1, the displacement of a point on the object is found by using the vector expression

$$N = \frac{1}{\lambda}(\hat{k}_1 - \hat{k}_2) \cdot \vec{d} \qquad (1)$$

where N is the number of dark fringes counted from a stable reference point at the base of the plant to the moving point, $\hat{k}_1$ is a unit vector in the $\vec{SP}$ direction, $\hat{k}_2$ is a unit vector in the $\vec{PO}$ direction, $\vec{d}$ is the displacement vector, and λ is the wavelength of the illuminating source. Equation 1 can be solved if the number of fringes can be counted and the angle α between $\hat{k}_1$ and $\hat{k}_2$ can be measured. The solution of equation 1 does not determine the location of the moving point, but rather defines a plane surface on which the moving point is located. The precise location of the moving point is found by using a number of observation points each of which is associated with its own equation 1 and corresponding plane surface. The simultaneous solution of this set of equations determines the intersection point of the corresponding plane surfaces and, thereby, defines the location of the moving point.

For small displacements the phototropic and geotropic responses of many plants occur within a plane normal to the axis of

---

[1] This work was supported in part by Grant 35-146 of the University of Connecticut Research Foundation and by the Department of Electrical Engineering and Computer Science of the University of Connecticut.

the plant. In this case only two observation points and two equations are required. If the precise direction of movement is known, as is often the case when dealing with phototropic or geotropic motions, then only one observation point and one equation are required. By arranging the apparatus so that the moving point P, illuminating source S, and observation point O are in a horizontal plane normal to the vertical axis of the plant, and by applying the stimulus so that the displacement vector $\vec{d}$ is toward or away from the laser source S, equation 1 is made to reduce to the scalar expression

$$|d| = N\,\lambda/(\cos\alpha + 1) \tag{2}$$

In this research, even though the direction of motion was known, a second observation point was used to verify that the direction of motion was, indeed, in the direction of the phototropic or geotropic stimulus. The additional observation point was obtained by viewing the plant through a mirror placed next to the plant as shown in Figure 2.

We have found it possible to resolve N and α to within ± 0.1 fringe and ± 10 min, respectively, resulting in errors in d of less than 4%. To monitor the bending along the entire length of the plant, it is necessary to determine the motion of points which are not located on the horizontal plane defined by S, P, and O. If the plant length is less than one-third the distance SP, and if P is initially chosen to lie at the center of the plant axis, equation 2 can be used to determine the displacement of points which lie along the plant axis above and below point P with additional errors in d of less than 1%. A typical variation in α of ± 1 degree due to the examination of points off of the SPO plane introduces an additional error in d of less than 1% if α is taken to be constant. Thus, for many geometries α can be considered to be constant and the displacement becomes a function of a single variable N with a total error in d of less than 6%.

Double exposure holography can determine the direction of the line along which a motion takes place and the extent of motion along that line, but it cannot determine whether the motion was toward or away from the observer. If the direction of the response to the stimulus is not known in advance then the experiment can be repeated with an additional tropic stimulus which is known to elicit a specific response direction. When the initial motion is in the same direction as the known response, the motion will be accentuated so that more fringes will appear, while if the initial motion is away from the known response, that response will retard the plant motion so that fewer fringes will appear.

MATERIALS AND METHODS

The experimental apparatus was as illustrated in Figure 2. In early experiments, a holographic plate holder (10 × 12.5 cm) was used with Agfa-Gevaert 10E75 plates. The plate holder was later replaced by a 35-mm camera and Kodak SO-253 high speed holographic film. The automated camera permitted monitoring plant motions with a series of holographic interferograms. The components were positioned on a steel plate (2.5 × 61 × 100 cm) using magnetic mounts. Vibration isolation was obtained by supporting the apparatus on a number of inflated rubber inner tubes. The room temperature was controlled, and precautions were taken to eliminate air currents and extraneous light.

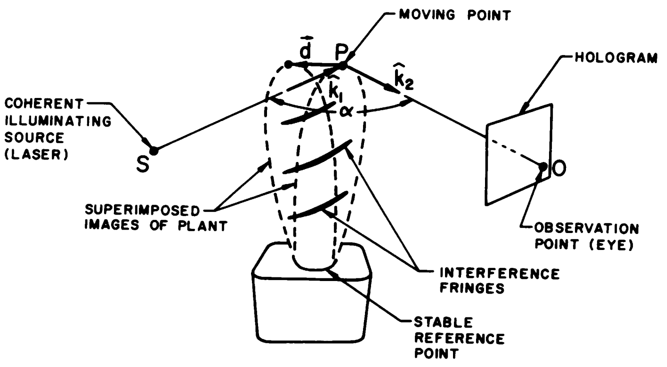

FIG. 1. General geometry for determination of the displacement of a point P on a plant. S, illuminating point source; O, observation point; k_1, unit vector in SP direction; k_2, unit vector in PO direction; d, displacement vector.

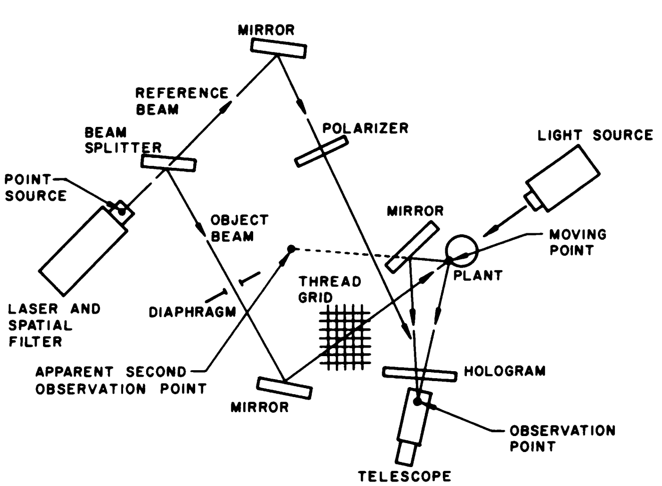

FIG. 2. Experimental apparatus for holographic measurement of plant motion consisted of a He-Ne laser with a spatial filter, a beam splitter for producing the reference and object beams, two mirrors for controlling the beam directions, and polarizer for controlling the reference beam intensity, a diaphragm for eliminating extraneous reflections, a thread grid which cast a shadow grid on the plant thereby producing easily identifiable reference points on the plant, a mirror for obtaining an additional observation point, a telescope mounted on a sextant for measuring angles and counting fringes, and a monochromator for producing the phototropic stimuli.

Following alignment of the apparatus the plant was covered and permitted to reach phototropic, geotropic, and thermal equilibrium over a 6- to 10-hr period. A test interferogram was taken to assure that the plant was undergoing no motion. The stimulus was then applied to the plant. At the appropriate moments the holographic exposures were made. After developing the double exposure hologram, the resulting interference fringes were examined to determine the motion which took place during the time interval between the two exposures.

Stapelia variegata Linn was chosen as the primary subject for investigation because it was easy to maintain in the laboratory, its size and smooth contour resulted in easily discernible fringes which could be observed without the use of magnification, and it typified mature plants in that it was slow moving and contained Chl. Although some holograms of leaves were taken, leaves generally appear to be too unstable to be holographed without taking further precautions to eliminate very small air currents and vibrations.

RESULTS

When measuring minute motions of sensitive plants it is essential that the plant remains uninfluenced by the measuring technique. When a sequence of double exposure holograms was taken of a stapelia over a 0.5-hr period without any tropic stimulus, no plant movement was observed. Thus, the 1,500 erg cm^{-2} 633 nm laser light required for the holographic exposure apparently produced no extraneous thermal motion which could have obscured any tropic response being studied. When using Kodak SO-253 high speed holographic film only 150 erg cm^{-2} is required for the holographic exposure so that thermal responses are even less likely.

Figure 3 is a photograph of a double exposure holographic image and represents the typical results obtained using the holographic technique. Figure 4 shows typical quantitative data obtained. Figure 4A illustrates a phototropic motion of a stapelia over a 5-min period in response to 0.02 mw cm^{-2} of 450 nm light from a monochromator. Figure 4B represents a geotropic movement over a 10-min period following a 10-degree inclination of a stapelia. A small movement in the opposite direction from the over-all tropism was detected near the base of the stem.

Figure 5 represents the phototropic motion of the plant tip as a function of time in response to 0.02 mw cm^{-2} of 450 nm light applied at t = 6 min and removed at t = 51 min. Every 5 min a double exposure hologram was recorded. This was accomplished by taking a 0.5-sec exposure, waiting 4 min while the motion occurred, and then taking a second 0.5-sec exposure. The film was then advanced. One min later, at the beginning of the next 5-min period, the double exposure process was repeated. This procedure resulted in a series of holographic interferograms each similar to that shown in Figure 3. The displacements over a 4-min period were calculated and are given in Table I. These results were further analyzed to obtain the total displacement, velocity, and acceleration as shown in Figure 5. Maximum acceleration is seen to have occurred between 2 and 7 min after initiation of the stimulus. A 15-min period of constant acceleration was followed by a drop in velocity at t = 30 min after which the previous velocity was resumed. Within 2 min following the stimulus termination the plant began a rapid deceleration, followed by a sharp drop in deceleration. The plant then resumed a small constant velocity in darkness. The small constant velocity, which occurred with some plants, might have been associated with a geotropic motion since some stapelia were not totally straight but had a slight curvature. It should also be noted that the humidity was not closely controlled and that a changing rate of evaporation might have played some role in these small motions.

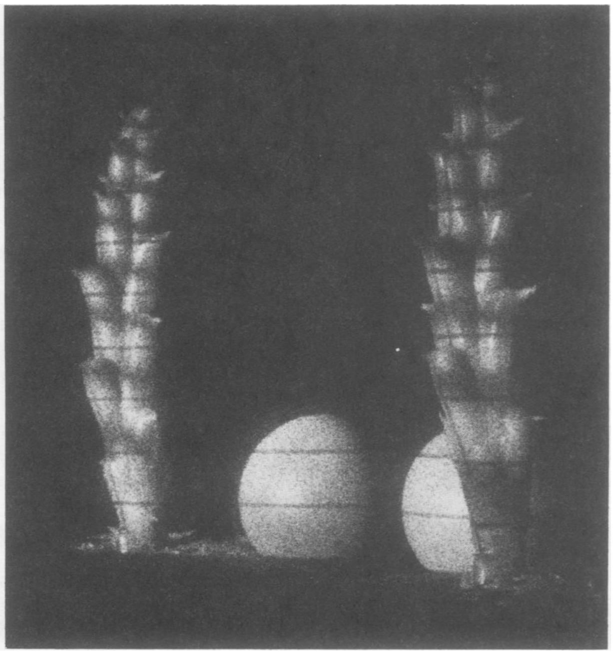

FIG. 3. Typical double exposure hologram of a stapelia. The mirror image is on the left and was used to verify the direction of motion. The sphere behind the plant is a reference object used to detect any unwanted movement of the apparatus. The set of narrow lines, seen on the plant and reference object, is the reference grid. The broad horizontal bands are the interference fringes.

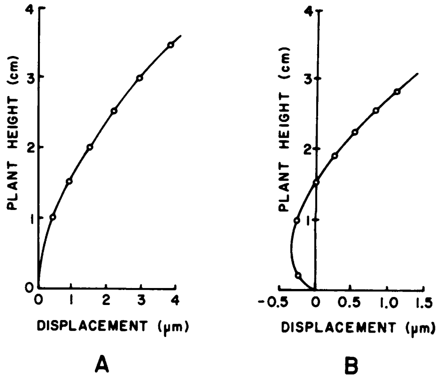

FIG. 4. Quantitative results showing (A) phototropic motion of a stapelia over a 5-min period in response to 0.02 mw cm^{-2} of 450 nm light from a monochromator; and (B) geotropic motion over a 10-min period in response to a 10 degree inclination of stapelia. The vertical axis represents the plant position at the time of the first exposure. The curve represents the plant position at the time of the second exposure.

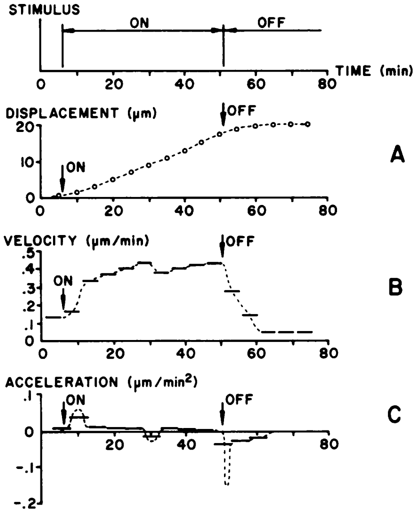

FIG. 5. Phototropic displacement undergone by the stapelia tip in response to 0.02 mw cm^{-2} of 450 nm light from a monochromator. Prior to t = 6 min, the stapelia was undergoing a small constant motion in darkness. The blue light stimulus was applied at t = 6 min and removed at t = 51 min. A: total tip displacement from t = 0 min; B: tip velocity; C: tip acceleration. Horizontal bars in B and C represent average velocity and acceleration over 4- and 5-min periods, respectively. Dotted lines, representing the instantaneous values, were obtained by assuming a smooth response and constructing the lines so that areas above and below the average bars were equal. Extreme values of the acceleration curve were found by measuring the slope of the velocity curve.

Each hologram in the 75-min series can be further analyzed, as was Figure 3, resulting in a series of curves each similar to that shown in Figure 4A. In this manner any irregularities in bending contour or direction can be measured.

DISCUSSION

Red light absorption has previously been related to altered phototropic and geotropic sensitivity in the coleoptile (2, 9). The red light dosage for the holographic exposure is above the threshold dosage for alteration of tropic sensitivity of the coleoptile and is, therefore, a potential problem when studying tropic responses. In any green plant, Chl would absorb red light and would, thereby, reduce the effective dosage so that tropic sensitivity could be much less than in the coleoptile. Any changes in tropic sensitivity may be reduced by masking the plant tip where

Table I. Calculation of stapelia tip displacement, d, in response to 0.02 mW cm^{-2} of 450 nm light applied from t = 6 min to t = 51 min; average velocity, $\bar{V}$, occurring during the 4 min double exposure hologram; and average acceleration, $\bar{A}$, occurring over the 5 min period between successive holograms. N is the number of fringes counted from a stable reference point to the moving point.

Time (min)	N	d over 4 min (μm) ±0.040 μm	d over 5 min (μm) ±0.050 μm	Total d (μm)	$\bar{V}$ over 4 min (μm/min) ±0.010 $\frac{\mu m}{min}$	$\bar{A}$ over 5 min (μm/min^2) ±0.004 $\frac{\mu m}{min^2}$
5	1.3	0.51	0.64	0.64±0.05	0.128	0.0000
10	1.6	0.63	0.79	1.43±0.10	0.158	0.0060
15	3.4	1.34	1.68	3.11±0.15	0.335	0.0354
20	3.8	1.49	1.86	4.97±0.20	0.373	0.0076
25	4.2	1.63	2.04	7.01±0.25	0.408	0.0070
30	4.5	1.77	2.21	9.22±0.30	0.443	0.0070
35	4.0	1.57	1.96	11.18±0.35	0.393	-0.0100
40	4.2	1.65	2.06	13.24±0.40	0.413	0.0040
45	4.4	1.73	2.16	15.40±0.45	0.433	0.0040
50	4.5	1.77	2.21	17.61±0.50	0.443	0.0020
55	2.9	1.14	1.43	19.04±0.55	0.285	-0.0316
60	1.6	0.63	0.79	19.83±0.60	0.158	-0.0254
65	0.6	0.24	0.30	20.13±0.65	0.060	-0.0196
70	0.6	0.24	0.30	20.43±0.70	0.060	0.0000
75	0.6	0.24	0.30	20.73±0.75	0.060	0.0000

red light sensitivity has been found to be maximal in the coleoptile (2). Also, changes in tropic sensitivity in the coleoptile have been found to be far red reversible (2, 9) so that red light effects might be eliminated by far red irradiation following the holographic exposure. This research was carried out using a red light (633 nm) laser primarily because of its availability. It should be noted that the holographic technique can be applied using a green light laser to which phototropic systems would be less sensitive.

Conventional techniques for the measurement of plant motions, such as shadowgraphs and time-lapse photography, have very low resolution and are, therefore, restricted to relatively large movements. Techniques making use of mechanical displacement detectors require physical contact with the plant. Techniques using microscopes can examine only small areas on the plant at a time. All of these conventional techniques lack the accuracy, precision, and dynamic range which are required to produce velocity and acceleration curves, such as shown in Figure 5, B and C, on which the interesting aspects of short term responses can be observed. Perhaps even more importantly, the hologram provides an overview of the entire response pattern of the plant on a single image.

The holographic interferometric technique has the ability to measure displacements at least as small as 0.16 μm with a red laser and 0.13 μm with a green laser within short time periods of a few min. Thus, the rate and extent of plant response to stimuli can be much more finely resolved than is possible with conventional techniques. In addition, a holographic interferogram permanently records information about the entire plant so that the motion of any visible points on the image can be determined by examining the interferogram at a later time. Therefore, a series of holographic interferograms can permanently record the entire plant response from start to finish, including any irregular changes in direction and velocity.

The results obtained thus far not only demonstrate the effectiveness of holographic interferometry in obtaining plant movement data, but they also reveal some interesting facts about short term plant movements. It is apparent from these results that the stapelia, a slowly moving mature plant, can respond to light stimuli within a few min. A relatively constant velocity of 0.4 ± 0.05 μm/min was attained within 6 min after the onset of the stimulus, and was maintained until the stimulus removal 40 min later. This is the first time that such small plant movements have been so precisely measured as a function of time over such a short time interval.

Over-all, these results demonstrate that holographic interferometry can be effectively applied to the quantitative analysis of short term, μm range motions of plants. Due to the sensitivity of holographic interferometry, we are not restricted to the study of young, rapidly moving, dark-grown shoots, but can examine typical slowly moving mature plants in which more than one photosensitive system or movement-producing system is operative. It should be possible, therefore, to examine the interrelationship of different pigment systems on plant movement or detect the presence of other movement-generating systems whose existence has not been detected using less sensitive conventional techniques.

Acknowledgments — The authors would like to thank D. F. Wetherell and J. P. Waters for many helpful suggestions.

LITERATURE CITED

1. Bellani VF, A Sona 1974 Measurement of three-dimensional displacements by scanning a double-exposure hologram. Appl Opt 13: 1337-1341
2. Briggs WR, HP Chon 1966 The physiological *versus* the spectrophotometric status of phytochrome in corn coleoptiles. Plant Physiol 41: 1159-1166
3. Fox MD, LG Puffer 1976 Analysis of transient plant movements by holographic interferometry. Nature 261: 488-490
4. Goodman JW 1968 Introduction to Fourier Optics. McGraw-Hill, New York
5. Powell RL, KA Stetson 1965 Interferometric vibration analysis by wavefront reconstruction. J Opt Soc Am 55: 1593-1598
6. Sollid JE 1969 Holographic interferometry applied to measurement of small static displacements of diffusely reflecting surfaces. Appl Opt 8: 1587-1595
7. Stetson KA 1974 Fringe interpretation for hologram interferometry of rigid-body motions and homogeneous deformations. J Opt Soc Am 64: 1-10
8. Waters JP 1974 *In* RK Erf, ed, Holographic Nondestructive Testing. Academic Press, New York pp 5-59
9. Wilkins MB 1965 Red light and the geotropic response of the *Avena* coleoptile. Plant Physiol 40: 24-34
10. Williams GT 1967 Holographic visualization of plant movement. Nature 215: 1170.