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Live *C. elegans* Diffraction at a Single Point

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Abstract

Using coherent light, we analyze the temporal diffraction at a single point from real-time living *C. elegans* locomotion in three-dimensional space. We describe the frequency spectrum of single swimming nematodes in an optical cuvette at a single sampling point in the far-field diffraction pattern. An analytical expression of the double slit is used to model the frequency spectra of nematodes as oscillating segments. The frequency spectrum in the diffraction pattern expands discretely and linearly as a multiple of the fundamental frequency with increasing distance from the central maximum. The frequency spectrum of a worm at a single point in the frequency spectrum contains all the frequencies involved in the locomotion and is used to characterize and compare nematodes. The occurrence of resonant frequencies in the dynamic diffraction pattern increases with the distance from the central maximum. The regular spacing of the resonant frequencies is used to identify characteristic swimming frequencies.

Keywords

C. elegans, Nematode, Temporal Diffraction, Live Diffraction, Locomotion

1. Introduction

Tracking the three-dimensional (3D) movement of microscopic species over larger distances is developing with the availability of optical components and increasing computing power [1]. These techniques are highly compact, fast and avoid the issue of the species moving out of the focal plane by using diffraction and holographic methods [2]. Ozcan *et al.*, for example use holographic shadowing to track the path of sperm over a distance of about 10 μm [3]. However, the right and left-handed helical path could not be determined with a two-dimensional (2D) imaging technique where the species travels on a micro-

scope slide.

We have used the transparent nematode, *Caenorhabditis elegans*, to develop methodologies for examining microscopic organismal shape and locomotion in real-time in 3 dimensions without using microscopes [1] [4] [5]. The simple rhythmic motions and small size of these free-living organisms have allowed us to develop techniques and mathematical reconstruction processes to allow us to explore locomotion dynamics.

The analysis of the locomotion of microscopic species typically involves the filming of the species followed by a computationally intense processing of the video [6]. This type of analysis has proven to be useful in characterizing the locomotion of nematodes and comparing to other species by sight. Using these traditional microscopic methods, waveforms, oscillating movement frequencies, etc. can be established with the precision corresponding to the resolution of the microscope and the precision of the specifications associated with the optics. Using diffraction and shadowing methods forces [1] [4], movements on the order of the wavelength are used. The frequency analysis of a live diffraction signal contains information about the cumulative movements of every single point on the microscopic object as the Fraunhofer diffraction pattern is proportional to the magnitude of the Fourier transform (FT) squared of the diffracting nematode shape containing a superposition of the EM radiating from all points on the diffracting object's outline. This point is especially well illustrated by the discrete FT (DFT) [7]:

$$\mathbf{X}_{\vec{k}} = \sum_{\vec{n}=0}^{\vec{N}-1} \mathrm{e}^{-2\pi i \vec{k} \frac{\vec{n}}{\vec{N}}} \boldsymbol{x}_{\vec{n}} \tag{1}$$

where $\mathbf{X}_{\vec{k}}$ is the element in Fourier space denoted by $\vec{k}$, $\vec{n}$ is the element in object space, N represents the total number of elements in a particular dimension and $\boldsymbol{x}_{\vec{n}}$ is the element in Fourier space denoted by $\vec{n}$. The phase sensitivity is on the order of the wavelength of the coherent radiation.

In this paper, we present the single point temporal diffraction model explaining the frequency spectra as related to the locomotion of the nematode; *i.e.*, frequency and amplitude of worm oscillations. The relationship between the worm thickness and thrashing amplitude is related to the number of resonance frequencies as a function of the single point location, where the single point simulates a photodiode (PD) placed in the dynamic diffraction pattern.

2. One Dimensional Single Point Diffraction Model

The *C. elegans* width-to-length ratio is about 1/10 [8] so that the dimensions are similar to a single slit typically discussed in introductory physics texts. The intensity of the Fraunhofer diffraction pattern is proportional to the modulus of the Fourier transform squared [9]:

$$I(p) \propto \left| 2d \operatorname{sinc}(\pi p d) \right|^2 \tag{2}$$

where d is the slit width and p is the position in reciprocal space. Two segments of a *C. elegans* can then be represented by using the shift theorem [9]:

$$F(x+a)+F(x-a) \rightleftharpoons 2\Phi(p)\cos(2\pi pa) \tag{3}$$

where F is the function in object space and Φ its Fourier transform; while x represents position in object space, p the reciprocal variable in Fourier space and a is the distance by which the function F has been displaced. In the case of a dynamic system a is time-dependent a(t).

C. elegans tend to move using a sinusoidal waveform [6] so that there are segments on the worm which move out of phase. To start with a simplified model, the motion of two corresponding segments out of phase can be modeled using a double slit in one dimension (1D), similar to the well-known Young's experiment. Two slits oscillating out of phase produce an oscillating diffraction pattern with an oscillating intensity (**Figure 1**):

$$I(p,t) \propto \left(2d\,\mathrm{sinc}(\pi pd)\cos(2\pi pa_0\sin(2\pi ft))\right)^2 \tag{4}$$

where a_0 is the amplitude of oscillation and f the frequency at which the oscillations of the segments occur.

Placing a photodiode (PD) in the diffraction pattern fixes the position p in the diffraction pattern. The time-dependent signal at $p = 0$ will not vary as the frequency inside the cosine term (Equation (4)) equals zero. Holding the oscillation amplitude steady as the distance of the PD from the central maximum increases the frequency range increases matching the increments of the fundamental frequency; *i.e.*, integer multiples of the frequency at which the slits are oscillating. The amplitude can also increase the frequency of in the diffraction pattern since larger amplitudes will span a larger number of wave-fronts.

Placing a photodiode (PD) in the diffraction pattern fixes the position p in the diffraction pattern. The time dependent signal at the central maximum, $p = 0$, will not vary as the frequency inside the cosine term (Equation (4)) equals zero. Holding the oscillation amplitude steady as the distance of the PD from the central maximum increases the frequency range increases matching the increments of the fundamental frequency; *i.e.*, integer multiples of the frequency at which the slits are oscillating since the sine term inside the cosine term (Equation (4)) in the diffraction pattern dominates. The oscillation amplitude of the worm can also increase the frequency of in the diffraction pattern since a larger amplitude will span a larger number of wave-fronts.

The expected number of higher dominant frequencies is indicated by n in the spectrum can then be determined using the location of the PD at point p is then a factor of the worm thickness, sinusoidal locomotion amplitude and the value of p:

$$n \geq a_0 p/(\pi d) \tag{5}$$

The number of frequencies is indicated by n if n is an integer; otherwise the next larger integer indicates the number of fundamental frequencies included in the signal from the PD. The remaining fraction in n indicates the amplitude of the highest frequency contributing to the cosine while the sine function (Equation (4)) provides the envelope, which can be attributed to the nematode's width.

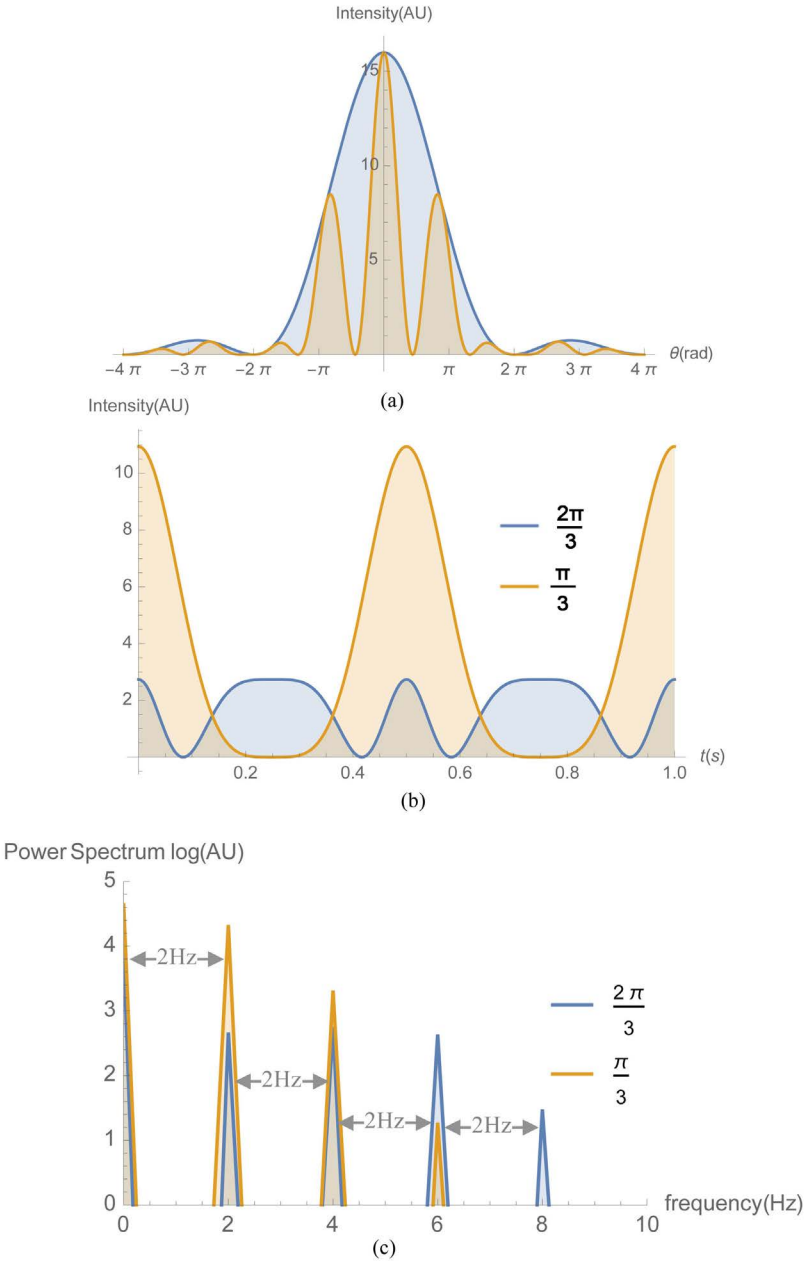

Figure 1. (a) The diffraction pattern due to two single slits oscillating out of phase at a frequency of 1 Hz. The slit width is a third of the slit separation. The outside envelope corresponds to the starting position of overlapping single slits drifting apart at t = 0 s with the spatial frequency of the diffraction pattern increasing as evident in the inner pattern at t = 0.15 s; (b) A PD placed at a phase angle of π/3 produces a 2 Hz signal. The frequency doubles for a PD placed at 2π/3 doubles the frequency. The relative intensities are larger at smaller frequencies for π/3; (c) The frequency spectrum stretches out as the PD is further away from the central maximum.

Overall, the number of frequency components increase with the phase angle as indicated in **Figure 2**. The resonant frequencies are spaced uniformly as dictated by the fundamental frequency. Note that the amplitude corresponds to the maximum worm oscillation (slit separation in our simplified model).

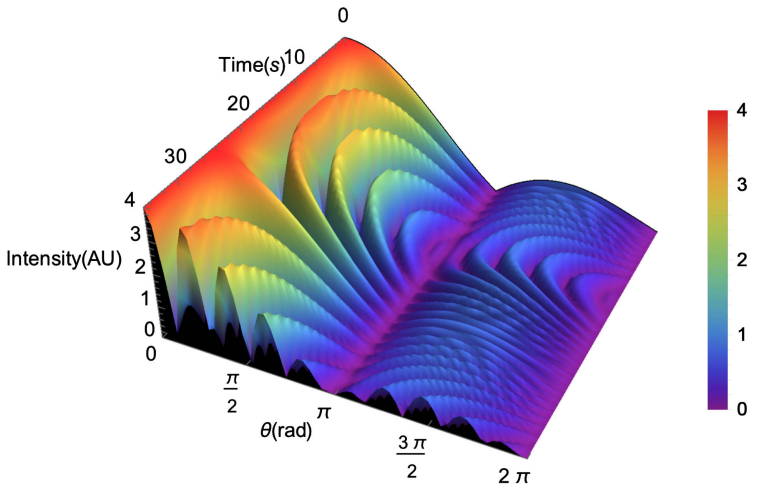

Figure 2. Intensity of PD shown as a function of phase and time. As the sensor moves away from the central maximum, intensity decreases with more frequency components.

3. Experimental Setup and Results

The optical setup was constructed to collect data at one point of the diffraction pattern (**Figure 3**). The Helium-Neon (HeNe) laser at 632.8 nm avoids the blue frequencies, which are known to influence the behavior of the nematodes [10]. The NDF prevents the PD from saturating. An adult (L4) *C. elegans* is placed in a water-filled cuvette. The cuvette is centered between Mirror 1 and Mirror 2 so that the nematode is within the laser beam's path. Mirror 2 and the translational stage control the distance of the PD from the central maximum. Measurements were taken at the 0.18 cm, 0.30 cm, and 0.42 cm away from the central maximum.

The distance from the nematode to Mirror 2 is 3.65 ± 0.05 cm. Mirror 2 is located 18.75 ± 0.05 cm from the photodiode so that the total distance from the diffracting worm to the photodiode is 22.4 ± 0.1 cm. The experimental data sets in **Figure 4** demonstrate that the frequency spectrum spreads out as the distance from the PD from the central maximum increases as indicated by the increasing mean frequency. The mean frequency is estimated using:

$$\langle f \rangle = \frac{\sum f_n \cdot PSD_n}{\sum PSD_n} \tag{6}$$

where f is the frequency, PSD represents the Power Spectral Density and n indicates the matrix element. The experimental spectra are not as distinct in their resonances as the simplified sinusoidal model; nevertheless, the resonances are consistent with known experimental locomotory frequencies of swimming and crawling *C. elegans* [11]. The bottom spectrum in **Figure 4** at 0.42 cm near the third minimum from the central maximum shows a consistent spacing of 0.602 Hz in the major peaks while finding a pattern for the spectrum closer to the central maximum at 0.18 cm near the first minimum is not possible because lower frequencies obscure the fundamental frequency. Placing the PD past the third maximum allows for enough resonances to use the spacing between resonances

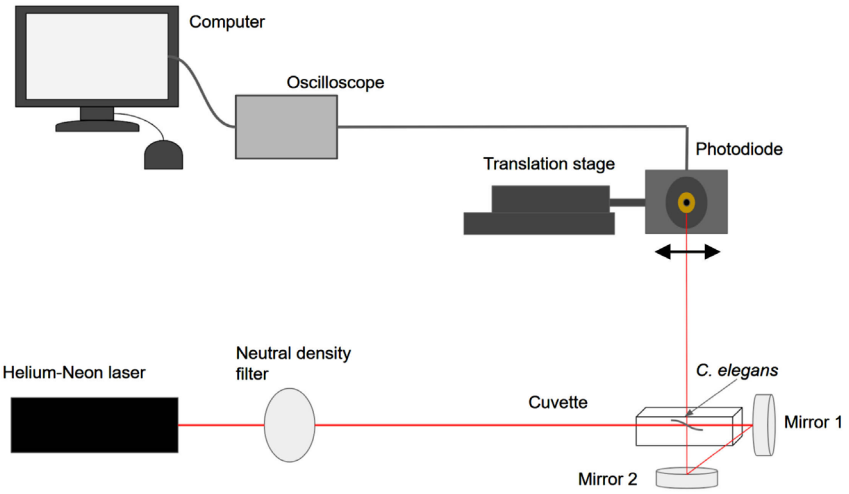

Figure 3. In the optical setup the laser beam travels through a neutral density filter (NDF) to prevent saturating the photodiode. Mirror 1 and Mirror 2 steer the laser beam through the cuvette containing the worm towards the PD. The *C. elegans* diffract the laser beam. Part of the diffraction pattern forms on the PD off center in the diffraction pattern.

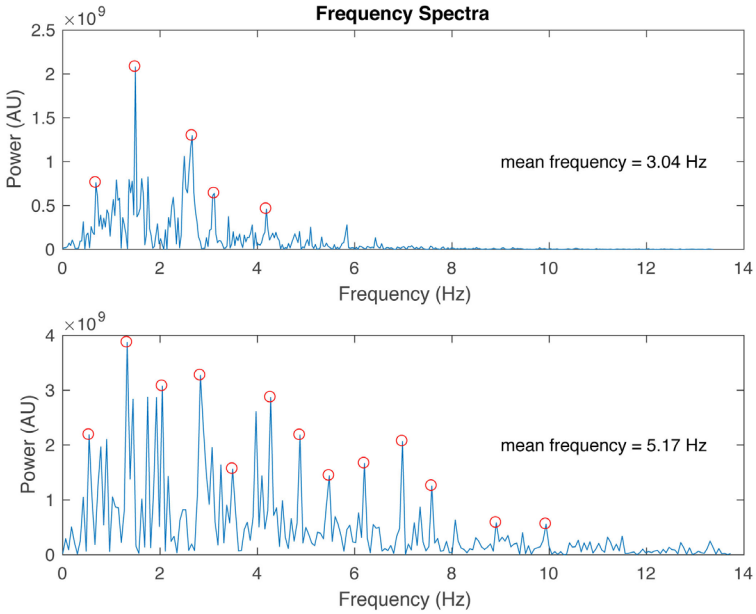

Figure 4. Frequency of intensity at 0.18 cm and 0.42 cm from the central maximum of a single wild type *C. elegans*. The mean frequency was calculated using Equation (6) and increases with distance from the central maximum and the resonance frequencies become more pronounced. The regular spacing of the resonance frequencies becomes apparent, as the PD is placed further away from the central maximum.

to determine the fundamental frequency. Destructive interference can eliminate resonance peaks so that placing the PD strategically allows for a maximum signal. Various worm movements such as head movement and the changing in direction or speed of the nematode can create frequencies. Some of these movements tend to show up in the slower frequencies, diffusing the dominant swimming or crawling frequency.

4. Conclusions

In this case study, we have demonstrated an efficient method for measuring dominant frequencies by strategically placing the PD in a dynamic diffraction pattern. Using this method, the locomotion of various phenotypes and in various environments can be characterized by frequency. A point in the diffraction pattern is used to record the superposition of all locomotory oscillations of the nematode allowing for the analysis of all frequencies embedded in the worm motion through a single time series. The intensities are sensitive to motion on the order of the wavelength of light used in the experiment so that subtle movements that might be missed in a microscopic analysis are detected and measured. Even the newest work in locomotion of *C. elegans* involves traditional microscopy rather than diffraction so that the work published by this group is the only work involving live diffraction of *C. elegans* [12] [13].

Collecting the signal at a distance from the central maximum allows for enough resonant peaks in the FT spectrum to use the spacing to measure the fundamental frequency. The regular spacing of the resonant peaks in the FT helps to identify dominant frequencies in the locomotion precisely. Further studies on separating movements of various worm parts are promising future projects. Dynamic diffraction using power spectral analysis complements traditional microscopic methods. Frequency filters can be employed to isolate various movements and switching mechanisms.

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