

Understanding Light Propagation Characteristics of a Single GFP Transfected Neuron with Simulations

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Abstract

This work uses tapered optical fibers to probe neuronal projections, called axons, and test if they are able to guide light. The nuclei of the neurons are transfected with green fluorescent proteins (GFP) to generate light to propagate down the axon. A tapered optical fiber is brought beside the axon to measure its evanescent field and determine the light guiding properties of the axon. A decreasing oscillatory behavior was expected to be measured when bringing the fiber probe in close contact with the axon and moving it along the axon, away from the nucleus. However, an increase in signal was determined as the probe moved further from the GFP-transfected nucleus. We are utilizing COMSOL Multiphysics to gain insights into how the cell surroundings impacted the measurements and get a better understanding of how light propagates and scatters in a cellular environment.

Keywords: neuron, axon, evanescent coupling

Introduction

Cells have been reported to emit ultraweak photons, commonly referred to as biophotons [1]. Biophotons are typically a byproduct of metabolic processes. However, it has been noted that there is a significant increase in biophoton production in the brain as opposed to other regions of the body [2]. Neurons are generating a disproportionate number of photons, leading to speculation that biophotons may be used in intercellular signaling. It has been reported that biophoton production increases significantly in damaged or stressed neurons [3]. While photons are capable of carrying unique bits of information in an efficient manner, they must be able to reach their desired target in order to effectively work as a signal. Axons possess a refractive index contrast relative to their surroundings and a geometry similar to that of optical fibers [4]. As a result, they may be able to function as a waveguide and carry both chemical and photonic signals to surrounding neurons.

Experimental Setup

To confirm if an axon is guiding light as opposed to scattering light, an evanescent field must be present. Evanescent coupling is achieved between two waveguides of similar size and refractive index. As

a result, the probe developed to measure the axon's evanescent field was a tapered optical fiber with a diameter of $1.59\mu\text{m}$, close to an axon's $2\mu\text{m}$ diameter. The refractive index of the silica fiber is assumed to be close to the axon's refractive index of 1.44. Since the probe's traits are similar to those of an axon, effective evanescent coupling is expected. If light were being scattered, there would be an exponentially decreasing function of signal with respect to distance as the probe is pulled further from the light source. Evanescent fields, on the other hand, would produce oscillatory behavior as a function of signal with respect to distance. The oscillations indicate that light is coupling back and forth between the axon and the probe, whereas an exponential decrease would indicate light leaving the system via scattering.

Surrogate Measurements

On-Chip waveguides were used as surrogate axons for initial measurements. The waveguides are single mode, made from a 300nm SiN layer buried in $4\mu\text{m}$ of SiOx cladding. The SiN waveguides have a similar refractive index and geometry to an axon, making them a constructive indicator of how an axon may interact with a probe. Light was butt-coupled from an optical fiber to the on-chip waveguide and measured with a tapered optical fiber probe on top of the cladding. These experiments, shown in Figure 1,

yielded an oscillatory and decreasing signal as the probe was run along the waveguide, further from the source point. This is expected because oscillations are indicative of evanescent coupling and a decrease is a result of propagation losses.

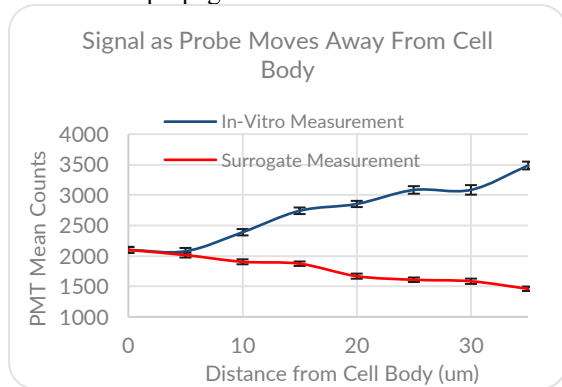


Figure 1. Photon counts measured at different points along the axon and surrogate waveguide.

In-Vitro Measurements

Light-emitting proteins were introduced to the neurons by transfecting their nuclei with GFP. This allows the cell to act as a light source for the axon waveguide. A cell with a long, clear axon and a nucleus containing GFP was located and excited using two-photon microscopy.

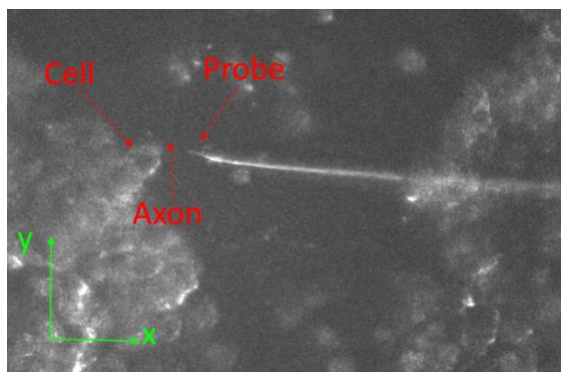


Figure 2. Bright field image of experimental set up.

The tapered optical fiber was placed alongside the axon of the cell of interest. The probe was run along the axon, measuring the signal as the probe was swept away from the excited cell of interest. Contrary to the surrogate measurements, an increasing signal was detected (Figure 1). However, both the surrogate and in-vitro measurements display signs of slight oscillations above the noise level. These oscillations could be indicative of the axon waveguiding. However, the increasing signal indicates that additional light is being detected, raising the question of its origin. The increase in signal may be due to: (1) the surrounding cells scatter light from the cell of interest into the probe, or (2) the two-photon excitation laser light scatters to

neighboring cells which act as secondary emitters. In order to better understand the measurement, the experiment was recreated in COMSOL Multiphysics.

Simulations

The cells were created using Image Processing in COMSOL from the bright field image shown in Figure 2. The cells were outlined in a bright white outline and uploaded to COMSOL. The outline helped isolate the cells from the background, creating one large domain consisting of the cell bodies and a separate domain for the background. The cell groups were split into separate domains so they could be treated individually.

In the experiment, the cell of interest was located on the edge of a larger cell cluster. A circular domain was modeled in its place to act as the nucleus transfected with GFP. The cell domains were set to have a refractive index of 1.44, whereas the surrounding media has a refractive index of 1.35 similar to water. The probe was assumed to have the same index as the cells.

Typically, a single cell nucleus contains between 5,000- 10,000 GFP molecules. The high density of individual emitters can be modeled as a single domain emitter using the External Current Density Condition with an arbitrary current in z-direction. From the two-photon microscopy image (Figure 3), potential secondary emitters were located and modeled accordingly.

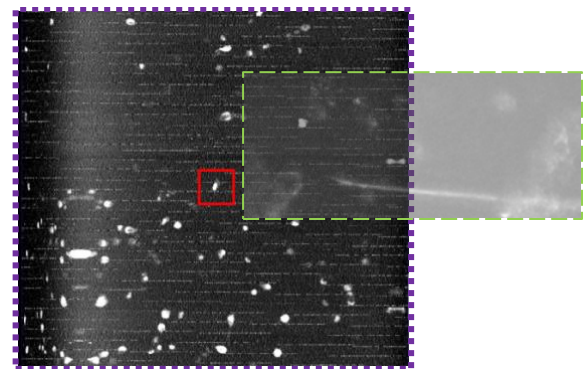


Figure 3. Brightfield image of computation domain (dashed green outline) overlaid with two-photon image (dotted purple outline) of the experiment with the cell of interest highlighted in red.

For each case, the average power in x-direction (P_{oavx}) was monitored at the probe output and normalized to the power at the axon entrance. This highlights light entering the probe through pathways other than direct coupling from the nucleus. Perfectly matched layers were used to avoid back reflections at the simulation boundaries. Three cases were simulated: (1) a single emitter without surrounding cells, (2) a single emitter with

surrounding cells, and (3) additional secondary emitters in neighboring cells.

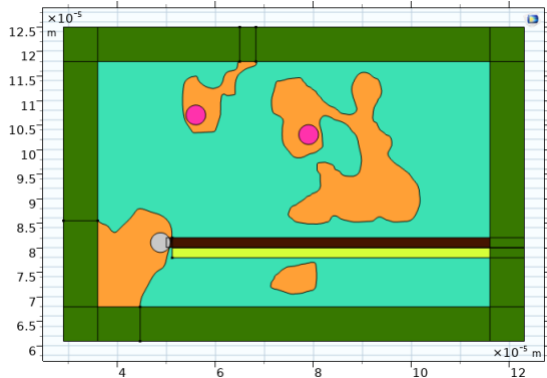


Figure 4. COMSOL Multiphysics geometry recreating the experiment. Cell bodies depicted in orange, emitters shown in pink, cell of interest in grey, axon in brown, probe in yellow, water in turquoise, and PML in green.

Case 1: Removing Surrounding Cells

The refractive index of the cell domains (orange) were matched to that of water (turquoise domains) eliminating their optical effect. This depicts the ideal scenario of light-coupling from axon to the probe without any interference from the surroundings.

Case 2: Single Emitter with Surrounding Cells

The surrounding cells (orange domains) were introduced into the simulation by changing their index to match that of the cell of interest. As a result, stray light will scatter when hitting the cells. This replicates the experiment under the assumption that the two-photon microscope only excites the cell of interest (grey domain).

Case 3: Secondary Emitters

The simulated light emission of the cell of interest and the subsequent scattering to the surrounding cells suggests that the incident two-photon excitation light may be scattered. This scattered light has a chance to excite nuclei with GFP in the surrounding cells, making them to secondary emitters. The light emitted from the surrounding nuclei could be detected by the probe adding to the increase in the signal seen as the probe moves towards these other cells and away from the cell of interest.

Simulation Results

The two single emitter cases exhibit extremely similar behavior indicating that the presence of cells doesn't scatter the light significantly. Both single emitter cases never exceed a coupling of 1, certifying that no external light is entering the system. The case of multiple emitters exceeds a coupling of 1,

demonstrating how light from the surrounding cells is entering the probe.

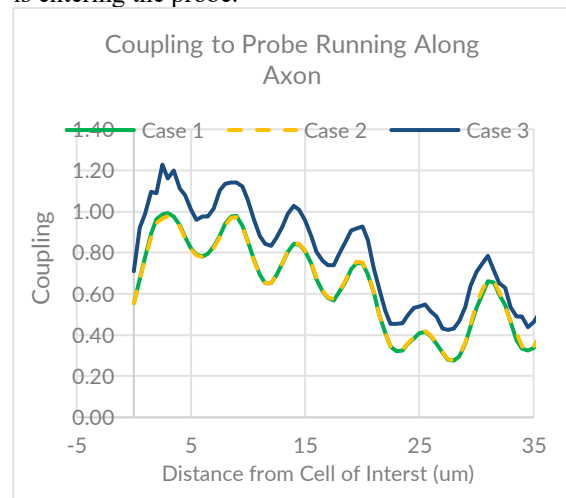


Figure 5. Coupling from the axon to the probe for the three cases.

The oscillations present in all three cases show that despite the scattering occurring, evanescent coupling is present with a coupling period of $5\mu\text{m}$. Therefore, the simulated axon is successfully acting as a waveguide. From the field plots (Figure A1), it becomes apparent the axon is reminiscent of a multimode waveguide, as has been previously recorded [5].

The general decrease of intensity in all three cases is due to the probe moving away from the main emitter. Even though the probe is moving closer to other surrounding emitters in Case 3, the axon stands between these emitters and the probe. As a result, the axon may be acting as a block to the probe making its effects appear less prominent.

To get a greater understanding of how the addition of cells and emitters impacts the coupling, cases 2 and 3 were normalized to the ideal case of a single cell emitter without surrounding cells (Figure 6). The addition of cells is shown to positively affect the coupling. As the probe moves further from the main emitter, there is an increase in the coupling. This is likely due to the probe approaching cells, which are acting as scattering elements and redirecting light into the probe. The case of multiple sources and cells

has an even greater impact on the coupling due to the increase in surrounding light entering the system.

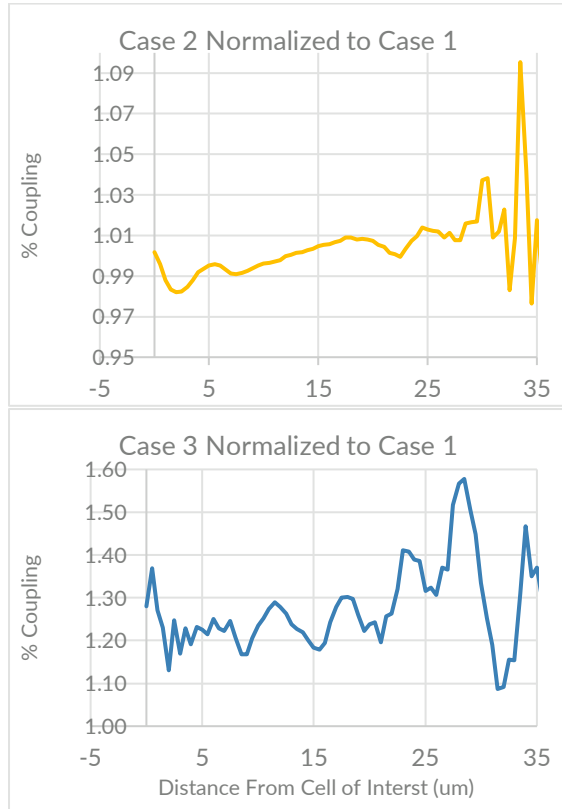


Figure 6. Cases 2 & 3 normalized to the ideal case of single emitter with no cells present (Case 1).

Decreasing the strength of the secondary emitters, reduces the impact on the power measured at the probe. Specifically, the intensity of the high frequency oscillations is reduced, as can be seen in Figure A2, leading to a smoother signal. Therefore, strong secondary emitters could be the origin of the slight oscillations measured in the experiments.

Conclusions

The simulated scenarios highlight how the presence of surrounding cells impacts the coupling. This effect is exemplified by the presence of neighboring emitters and cells that may be lying out of the simulated 2D-plane. Our model doesn't account for the several layers of cells above and below our working plane, which may be impacting the probe's detection through scattering and extra GFP emission. The oscillations in the simulations show that the axon is capable of waveguiding, however, these are very idealized models.

The performed simulations inform the design of subsequent experiments, emphasizing the need for lower cell densities and well-isolated axons to

provide better measuring conditions. Furthermore, the experimental measurements were likely dominated by scattering and are not indicative of evanescent fields. The presence of slight oscillations in the in-vitro measurements could be a result of evanescent coupling or interference from multiple secondary emitters. Upcoming experiments will be conducted with finer sampling to resolve the coupling period.

References

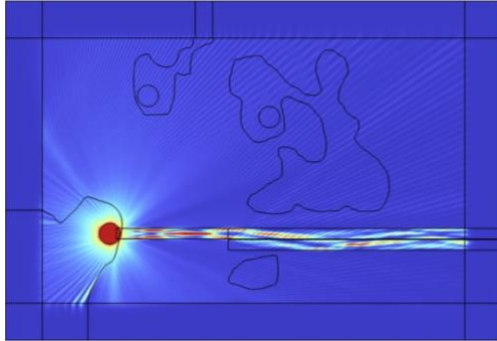
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Acknowledgements

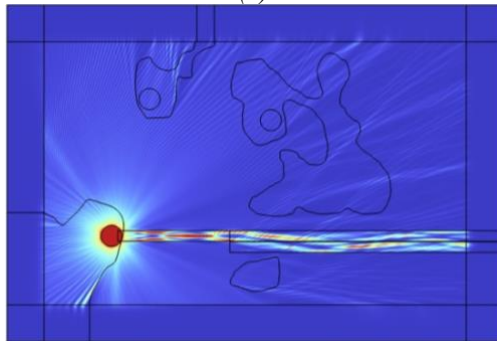
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Appendix

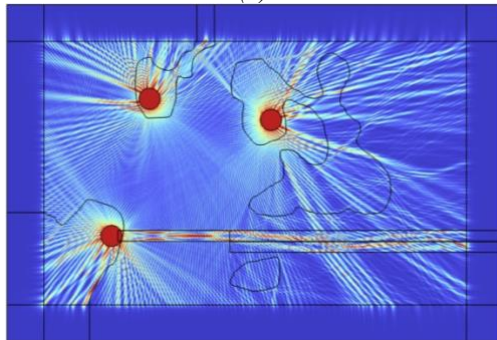
The power in both the x and y components for the three cases (single emitter with no cells, a single emitter with cells, and multiple emitters with cell) were displayed. The probe is 20 μ m from the cell of interest and all emitters have a current density of 1A.



(a)



(b)



(c)

Figure A1. (a) Single emitter with no surrounding cells (b) Single emitter with surrounding cells present (c) Multiple emitters with surrounding cells present.

When using two-photon microscopy, the beam is highly concentrated to a small spot size to excite a single cell. If multiple cells were being excited, it may be due to scattering of incident light on the main emitter or from the spot size being larger than anticipated. If the spot size encompasses multiple cells they would emit with a relatively equal strength. However, if surrounding cells are being excited by stray laser light, the surrounding emitters would be significantly weaker. These two cases are

modeled to illustrate the effects of different strengths for the secondary emitters.

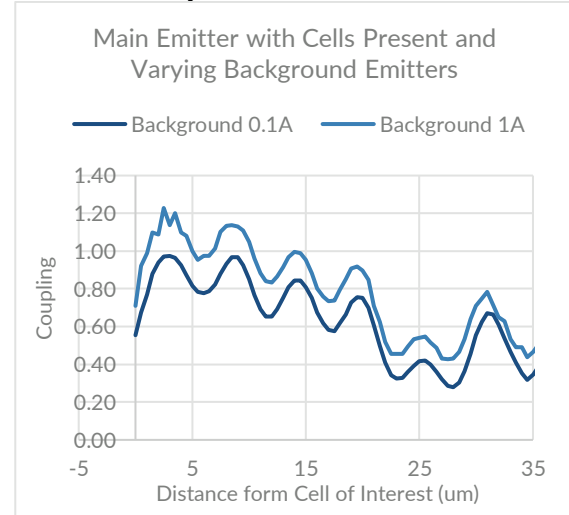


Figure A2. Probe coupling with a main emitter at 1A and secondary emitters at 0.1A strength and 1A strength.