EVANESCENT WAVE MICROSCOPY

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3 Introduction

In neuroscience, evanescent wave microscopy is a fairly new technique that is gaining extensive popularity because of its spectacular signal-to-noise ratio in comparison with confocal microscopy. A measure of the novelty of evanescent wave microscopy is that it was *not* included in the microscopy short-course offered by the Society for Neuroscience Meeting in 2002. The design of a typical evanescent waver microscopic experiment will be presented after a brief introduction to the physics of evanescent waves.

The physical nature of an evanescent wave–an electromagnetic wave traveling parallel to the interface of two material with different refractive indices, but decaying exponentially in amplitude with respect to distance into the medium of lower index of refraction–can be derived from Maxwell's equations in a fairly straightforward way for the case of infinite plane waves incident on the interface. For bounded waves that occur in an experimental situation the derivation is not so straightforward and results in the somewhat surprising and prediction of the obscure by very real Goos-Hanchen effect.

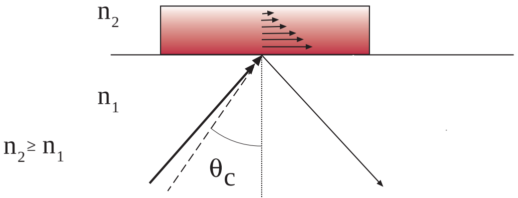

FIG. 1. Evanescent waves are cool.

Evanescent waves have applicability in many areas of physics. As proof of this point searching for *evanescent* on any ol' database I found 1000 hits.

Let us examine the basic experimental design of an evanescent wave microscopy experiment:

- GOAL: to determine the spatial relationship between proteins in exo- and endo cytosis. (Due to the previously defined nature of evanescent waves having only sensible intensities slightly beyond the boundary of total internal reflection, evanescent wave microscopy can only be used for protein-protein interactions near the surface of a cell.)

Endo- and exocytosis are interesting questions because they are the processes which govern chemical transmission of brain signals–action potentials. First it is fine to recall that in the dawn of anatomy and physiology it was perfectly clear that the electrical signals of the body had no chemical component–especially in the case of neuron-to-neuron connections as opposed to neuro-muscular junctions–until about 1937 when Cajal made his discoveries [[Synapses p.8]].

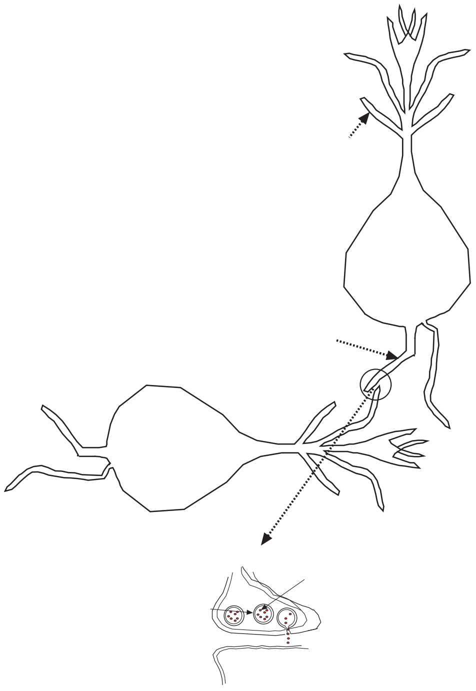

FIG. 2. Two pyramidal neurons interacting. The axon of the vertical neuron is represented as making synaptic contact with the dendrite of the horizontal neuron.

Our experimental design is lacking just one component. To colocalize the proteins involved in synaptic transmission we are going to use the approach of seeing them in the microscopic by forcing them to emit light that we will collect with the microscope. We will use the method of fluorescence.

Fluorescence is an emission of photons of at a well-defined band of wavelengths as a process of de-exciting a molecule–generically known as a *fluorophore*. In the case of fluorescence the fluorophore is brought to the excited state by a band of wavelengths of photons of higher energy than the band of emitted wavelengths–due to the conservation of energy. There are other way to de-excite a molecule. The core of the experimental design is to use evanescent waves to excite the fluorophores. Still unanswered is the question as to how the fluorophores become associated with the proteins.

Fusion protein–two proteins forced together by techniques in microbiology– technology probably has a fascinating history that we must forego for the moment, but a brief review of transgenic cells lines is in order for the complete comprehension of the experimental design. A fusion protein that includes a fluorophore as one of the components is designed by taking the DNA of the protein whose position we wish to know and ligating that sequence with the sequence of a fluorophore. For example: ligate DsRed with the clathrin light chain. Transfect that total DNA sequence into the cell line of interest,say, fibroblasts and allow the new cells to grow and express DsRed-clathrin as determined in an appropriately illuminated microscope. So, now we should be able to see the clathrin-coated pits at the cell surface of fibroblasts.

The final set up for an evanescent wave microscopy experiment is shown in Figure 3.

4 Review of Maxwell's Equations

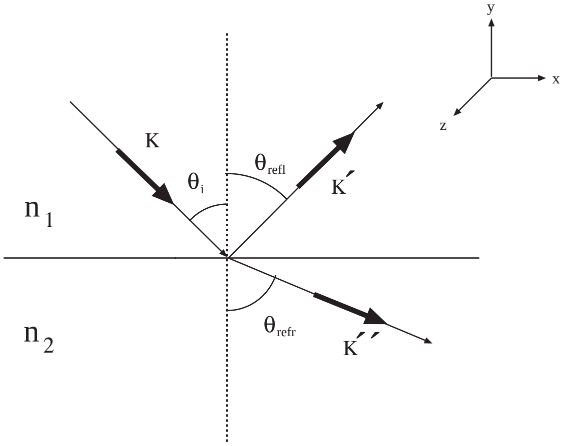

FIG. 4. Basic definitions for a derivation of the $\vec{E}_{trans}$ for an evanescent wave.

$$\text{Static electricity} \qquad \vec{\nabla} \cdot \vec{E} = \frac{\rho}{\epsilon_0} \tag{1}$$

$$\text{Bicycle light of the generating kind} \qquad \vec{\nabla} \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \tag{2}$$

$$\text{No magnetic dipoles} \qquad \vec{\nabla} \cdot \vec{B} = 0 \tag{3}$$

$$\text{Oersted's great experiment with a compass} \qquad \vec{\nabla} \times \vec{B} = \mu_0 \vec{j} + \epsilon_0 \mu_0 \frac{\partial \vec{E}}{\partial t} \tag{4}$$

Let's review how derive the wave equation for, say $\vec{E}$.

1. Take the curl $\nabla\times$ of Faraday's Law, Eq. 2.

2. Apply the BAC CAB rule from vector algebra.

3. Set $\vec{\nabla} \cdot \vec{E} = 0$ since the RHS of Eq.1 is zero in free space by definition.

4. Similarly set $\vec{j}$ equal to zero and substitute the thusly revised version of Eq. 4 in the RHS of Faraday's Law.

5. And you have $\vec{\nabla}^2(\quad) = \frac{1}{c^2}\frac{\partial^2(\quad)}{\partial t^2}$.

5 Two types of evanescent waves

So ... what is the difference between ideal and nonideal evanescent waves. The difference is only theoretical; ideal evanescent waves are produced by light that is modeled by infinite planes waves. Where as real evanescent waves are produced by bounded plane waves. For now we consider the spatial and temporal dependence of light to be the following,

$$\text{incident wave} \quad e^{i\left(\vec{k}\cdot\vec{r}-\omega t\right)}, \tag{5}$$

$$\text{reflected wave} \quad e^{i\left(\vec{k'}\cdot\vec{r}-\omega t\right)}, \tag{6}$$

$$\text{and a transmitted wave} \quad e^{i\left(\vec{k''}\cdot\vec{r}-\omega t\right)}. \tag{7}$$

For all points of the boundary for all times the arguments of the exponentials must be equal at the boundary between n_1 and n_2. This means–for the time dependent term–that the $e^{i\omega t}$s all cancel, since the frequency of all these waves was determined once and for this entire problem at the source of the radiation. For the space-dependent term one only needs to solve Problem 8.15 in David Griffiths's *Introduction to Electrodynamics* which asks the alert reader to show that

$$a = b = c$$

for

$$Ae^{iay} + Be^{iby} = Ce^{icy},$$

$$\vec{k}\cdot\vec{r} = \vec{k'}\cdot\vec{r} = \vec{k''}\cdot\vec{r}. \tag{8}$$

In agreement with experience the implication of the above set of equations is that all the ks are coplanar. Furthermore their projections on the boundary plane are equal since $\vec{r} = x\hat{i} + y\hat{j} + z\hat{k}$ and $y = 0$ for the case at hand.

$$ksin\theta_i = k'\sin\theta_{refl} = k''\sin\theta_{refr}. \tag{9}$$

In medium with index of refraction n_1 (since $\theta_i = \theta_{refr}$

$$k = k'. \tag{10}$$

In light of (10) the first expression in (9) yields the law of reflection;

$$\sin\theta_i = \sin\theta_{refl}. \quad \textbf{Law of reflection}$$

(This law may derived from the principle of least action in a very elegant manner, the interested reader is referred to Lancoz text on analytical mechanics.) Eq. 9 also produces Snell's raw of reflection by considering the expressions in k and k'',

$$k\sin\theta_i = k''\sin\theta_{refr}.\textbf{Law of refraction} \tag{11}$$

Equation 11 combined with the generic dispersion relation for light,

$$\frac{\omega}{k} = \frac{2\pi\nu}{2\pi/\lambda} = \nu\lambda = \frac{c}{n}, \tag{12}$$

produces the famous form of Snell's law. Eq. 12 in (11) with attention to the proper subscripts on the indices of refraction and some algebra produces.

$$n_2\sin\phi = n_1\sin\theta. \quad \textbf{Snell's Law}$$

Let's use the following form of Snell's law;

$$\frac{\sin\theta}{\sin\phi} = \frac{n_2}{n_1} \equiv n. \tag{13}$$

Where $\phi \equiv \theta_{refr}$.

Let us now discuss the amplitudes of the of the electric and magnetic fields so that we can satisfy the boundary conditions for Maxwell's equations. $\vec{E}$, $\vec{E}'$, and $\vec{E}'$ represent electric fields that correspond to the appropriate wavevectors k. The tangential components of the total electric field must be continuous, and the same is true of the magnetic field H. Let us consider only the case in which that electric field vectors are perpendicular to the x-y plane–this case is referred to as the transverse electric (TE) polarization.

The continuity of the tangential components of E are expressed as follows,

$$E + E' = E''. \tag{14}$$

It is crucial for the derivation of the form of the evanescent wave to also consider the continuity of the tangential components of $\vec{H}$. Recall that the relationship between magnetic fields in vacuum and matter can be expressed by removing the zero subscript on the permeability of free space in the following constitutive relation $\vec{H} = \frac{1}{\mu}\vec{B}$. The components of H obey the following continuity requirement–remembering the law of reflection and examining the accompanying figure that indicates why the cosines, instead of sines appear–we have,

$$-H\cos\theta_i + H'\cos\theta_i = H''\cos\theta_{trans}. \tag{15}$$

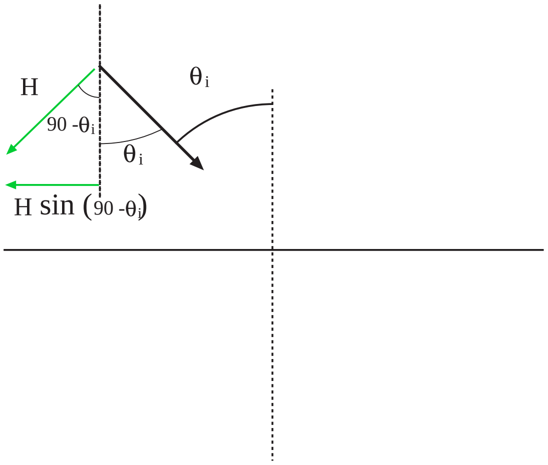

FIG. 5. The argument for cosines in the boundary condition for H

In that case of Maxwell's equations in a medium with permeability μ Faraday's law looks like

$$\vec{\nabla} \times \vec{E} = -\mu\frac{\partial \vec{H}}{\partial t}.$$

Substituting into the above equation the functional forms of the $\vec{E}$ and $\vec{H}$ produces the following form,

$$\vec{k} \times \vec{E} = \mu\omega\vec{H}$$

because $\nabla \to i\vec{k}$ and $\frac{\partial}{\partial t} \to -i\omega$. The $i$canceling on both side of Faraday's law in a medium.

As part of derivation we show why evanescent waves are always associated with total internal reflection. (have you ever heard of *external reflection*?) Under what conditions might one be convinced that the modifier *total* is deserved? Certainly if we could show that the reflection coefficient–the ratio of reflected intensity to incident intensity–is one ... you would be convinced. In order to make progress toward that goal we need to calculate

$$r = \left[\frac{E'}{E}\right].$$

The square of modulus of r is the reflection coefficient. From Eqs. 14 and 15 and Faraday's law—$\vec{k} \times \vec{E} = \mu\omega\vec{H}$—we can eliminate E'' towards the end of obtaining the ratio which is r. From (14) and yet another application of the dispersion relationship for light in a medium of index of refraction of n; $\frac{k}{\omega} = \frac{n}{c}$ we find,

$$\frac{E'}{E} r \equiv \frac{\cos\theta - n\cos\phi}{\cos\theta + n\cos\phi}, \tag{16}$$

$$\frac{n_2}{n_1} \equiv n = \frac{\sin\theta}{\sin\phi}. \tag{17}$$

Using simply substituting n from (17) into (16) and multiplying numerator and denominator by $\sin\phi$ in combination with the high-school-esque multiple angle formulas of trigonometry produces another form of r that we will call r;

$$\mathsf{r} = -\frac{\sin(\theta - \phi)}{\sin(\theta + \phi)}. \tag{18}$$

One more form of reflection amplitude puts at the final destination that's really worth the effort. To arrive at that equation eliminate functions of ϕ in (16) in favor of functions of θ by manipulation of (17) and basic trigonometric identities. The procedure goes as;

- express $\cos\phi$ as a function of $\sin\phi$ using $\cos\phi^2 + \sin\phi^2 = 1$
- now Snell's law allows us to eliminate $\sin\phi$ in favor of $\sin\theta$ in a straightforward manner. Leading to the following result

$$\mathrm{r} = \frac{\cos\theta - \sqrt{n^2 - \sin^2\theta}}{\cos\theta + \sqrt{n^2 - \sin^2\theta}}. \tag{19}$$

Examining Equation 19, for $n > 1$

$$r = \frac{x - \epsilon}{x + \epsilon} < 1,$$

which means no total internal reflection.

Now we are perfectly positioned to understand the difference between *external reflection* and *internal reflection.* External reflection is mathematically defined as the case when $n > 1$ for our figure that means that external reflection is the situation when the light source is in the more rarified medium. Eq. 19 tells us that *no* angle of incidence in external reflection will ever produce any argument of the square root function that isn't the standard positive number. Internal reflection, however, is another–more interesting story–because of (19). Eq. 19 tells us that when $n < 1$ –the definition of internal reflection–the argument of the square root of (19) is negative for certain values of θ the condition of which is easy to determine. Set the argument of the square root function equal to zero;

$$n^2 - \sin^2\theta = 0. \tag{20}$$

Since $\sin\theta$ increases as θ increases the LHS of (20) will become negative–causing fun with the square root function–for angles greater than that defined by (20). Since this angle is special it is referred to as the *critical angle.* One can easily calculate the critical angle for the interesting case–for a swimmer–of air and water,

$$\theta_{critical} = \sin^{-1}\frac{n_{air}}{n_{water}}$$

$$1.3 \approx n_{water}$$

$$1 \approx n_{air}$$

What do we know about sine of angles? Well, the sine of 45° is $\frac{1}{\sqrt{2}}$ and since 1.3 is just about equal to $\sqrt{2}$ (1.4) and the $\theta_{critical}$ in the swimming pool would be a tad more that 45°.

In the case that $\theta_i > \theta_{critical}$ (19) rewritten below for convenience becomes complex,

$$\mathrm{r} = \frac{\cos\theta_i - \sqrt{n^2 - \sin^2(\theta_i)}}{\cos\theta_i + \sqrt{n^2 - \sin^2(\theta_i)}}.$$

Rewriting (19) will permit–finally–us to understand what is *total* about total internal reflection.

$$\frac{E'}{E} \equiv \mathrm{r} = \frac{\cos\theta_i - i\sqrt{\sin^2(\theta_i) - n^2}}{\cos\theta_i + i\sqrt{\sin^2(\theta_i) - n^2}}. \tag{21}$$

Taking the square of the modulus of (21) will give the intensity of reflected light. The RHS of (21) is of the form;

$$\frac{a+ib}{a-ib},$$

Which when the complex conjugate is taken and multiplied gives

$$\frac{a+ib}{a-ib}\frac{a-ib}{a+ib},$$

which in turn gives the cool result of,

$$\frac{a^2+b^2}{a^2+b^2}$$

when applied to (19);

$$\mathrm{R}=1, \qquad \textbf{Total internal reflection}$$

and one is about as *total* as it gets in this business.

6 The ideal evanescent wave

The solution to Maxwell's equations in the medium of index of refraction n_2 is,

$$E_{transmitted} = E'' e^{i\left[\vec{k''}\cdot\vec{r}-\omega t\right]}. \tag{22}$$

So we need to figure out the meaning of $\vec{k''}\cdot\vec{r}$.

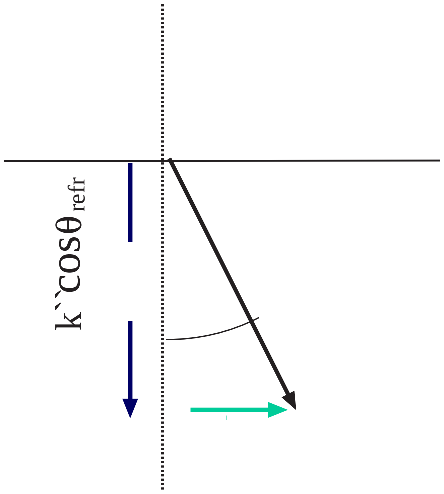

FIG. 6. Geometry of the spatial argument of the exponential for E_{trans}.

Doing the inner product:

$$\vec{r} = x\hat{i} + \hat{j},$$

and

$$\vec{k''}\cdot\vec{r}$$

$$\vec{k''}\cdot\vec{r} = k''\sin\phi x - k''\cos\phi y, \tag{23}$$

where $\phi = \theta_{refr}$.

Repeating the bulleted procedure above for arriving at (20) expressing cosϕ in terms of sinθ (23) becomes.

$$\vec{k''} \cdot \vec{r} = k''\mathrm{sin}\phi x - k''i\left[\frac{\mathrm{sin}^2\theta}{n^2} - 1\right]^{\frac{1}{2}} y. \tag{24}$$

The alert reader will realize that all the trump are out now, but let's just play out the hand. Substituting (24) into (22) yields,

$$E_{trans} = E''e^{i\left[k''\mathrm{sin}\phi x - k''i\left[\frac{\mathrm{sin}^2\theta}{n^2} - 1\right]^{\frac{1}{2}} y - \omega t\right]}. \tag{25}$$

Defining a term makes the final expression much more appealing,

$$k''i\sqrt{\frac{\mathrm{sin}^2\theta}{n^2} - 1} \equiv \alpha. \tag{26}$$

(26) into (25) and considering the multiplication of the complex numbers and the fact that y is negative in the region that we are considering gives,

$$E_{trans} = E\,''e^{-\alpha\,|y|}e^{i\,[k''\,\sin{(\phi)}\,x - \omega t]}. \tag{27}$$

So at long last Eq. 27 is the mathematical representation of the evanescent wave at the very beginning of this section. Note how the argument of the exponential that has the k'' indicates an electromagnetic wave moving in the x direction, *i. e.* parallel to the boundary, but whose amplitude decreases exponentially with position into the medium with lower index of refraction.

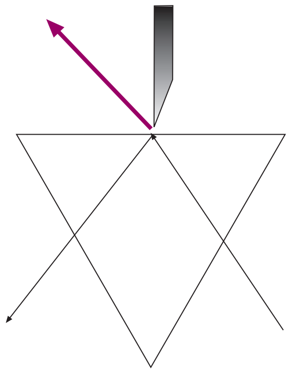

FIG. 7. Raman's demonstration of the existence of evanescent waves.

7 Non-ideal evanescent waves

The next level of complexity of theory in evanescent waves involves treating the light that is incident as non-infinite–bounded. When such waves are considered for the case of total internal reflection the *Goos-Hanchen effect* is predicted. In this effect the reflected light in the case of total internal reflection does *not* emanate from the point where the incident wave strikes, but rather emanates some non-negligible distance from the that point. In the case of microwave radiation shown in the figure below.

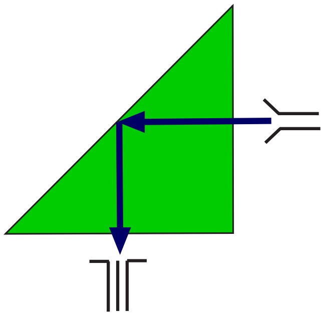

FIG. 8. The Goos-Hanchen Effect is best observed in the microwave region.