Multi Spectral BARDOT

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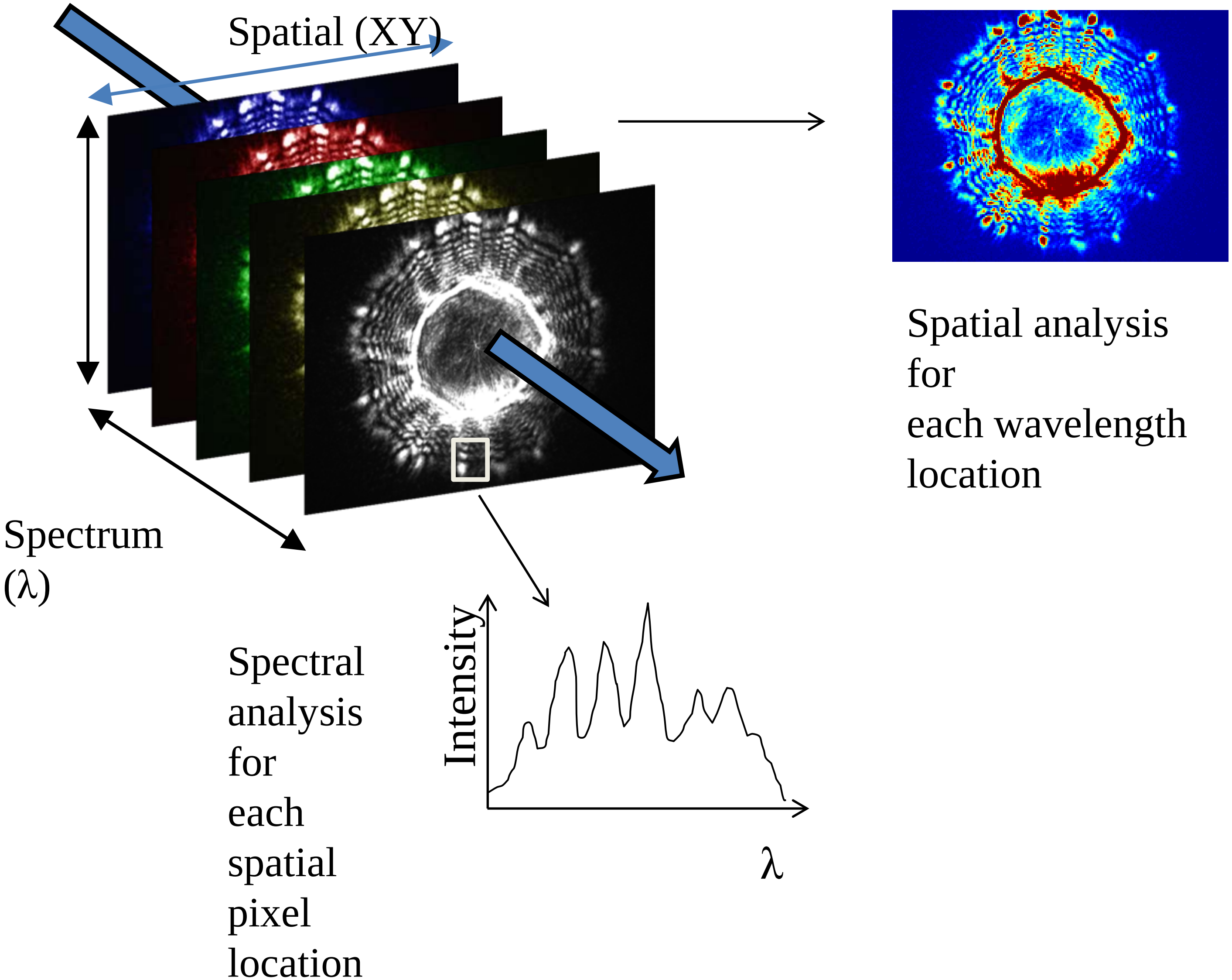
- Rapid identification and classification of food-borne pathogens are critical to ensure the sound operation for food industries and public safety. Conventionally, there were several methods that have been used to complete this task: the serological method, proteomics, and genomics to name a few. However, these methods were time consuming, costly, and required artificial labels such as biochemicals or dye to provide required specificity. Recently, many research groups are investigating rapid, cost-effective, and label-free techniques to discriminate the bacterial species. Forward scattering pattern analysis is one of the promising techniques based on elastic light scattering theory. The effectiveness has been reported to work on several types of bacterial genera and species. However, previous results were relying solely on single wavelength laser which render us to explore the possibility of using multi-spectral forward scattering since many physical parameters depended on the incident wavelength. From our research, it was elucidated that each bacterial genera had unique light absorption patterns along the wave length. Also, many different optical characteristics, such as number of diffraction rings, both width and gap of each diffraction rings, and maximum diffraction angle were affected by incident wave length. Multi-spectral BARDOT used these physical phenomena to offer additional information for identifying and classifying each bacterial genus.

- In our current research, predictions of forward scatting patterns of a bacterial colony for multiple wavelengths are presented to demonstrate the possibility of identifying bacterial species based on their optical characteristics, induced notable phase shift, and different numbers of diffraction.

INTORDUCTION

- Rapid identification and classification of food-borne pathogens are critical
- Forward scattering pattern analysis is based on elastic light scattering theory
- Incident wavelength determines several parameters in forward scattering pattern
- Cost effective, Time saving

OVERVIEW

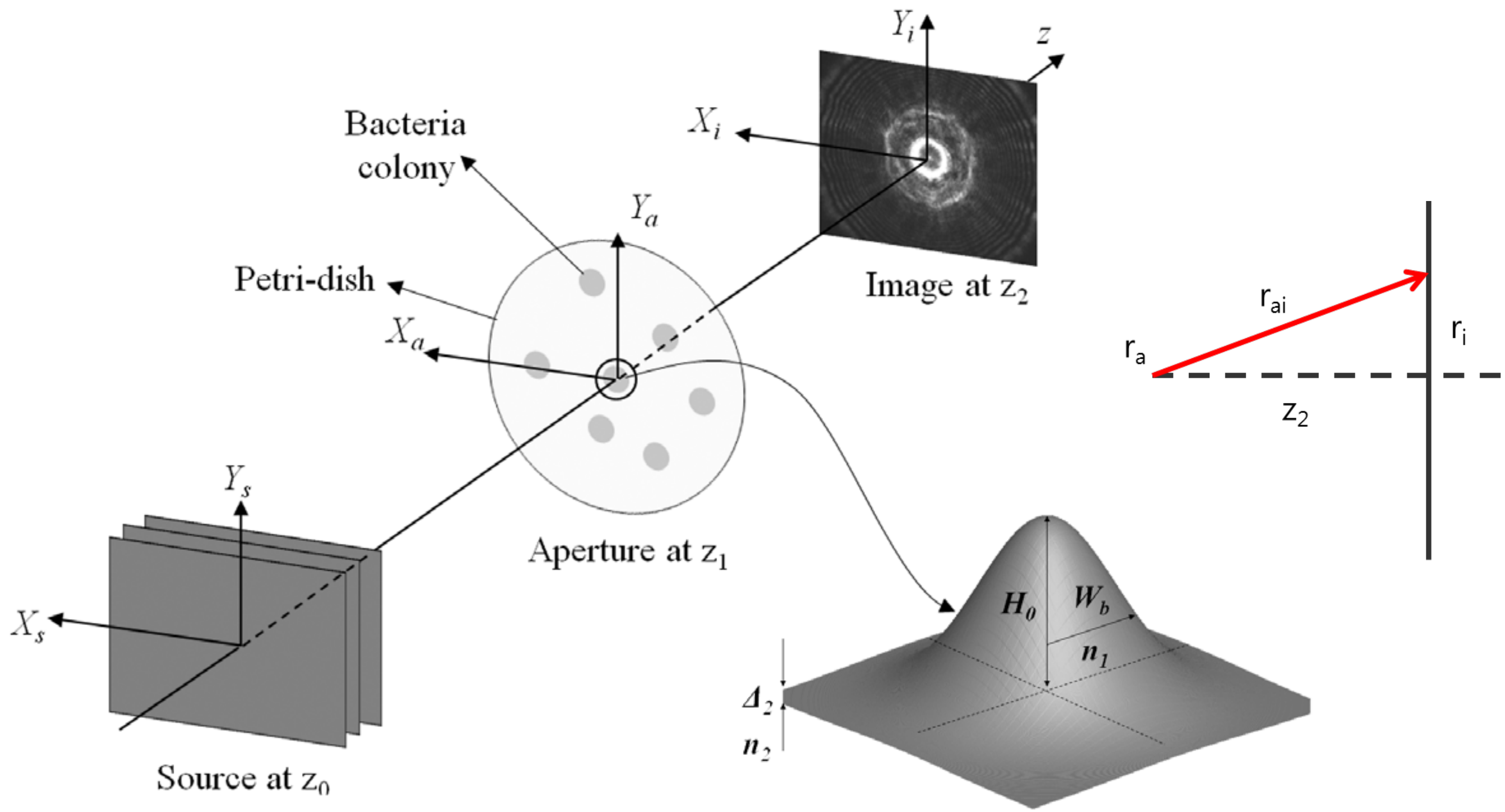


Spatial (XY)
Spectrum (λ)
Spatial analysis for each wavelength location
Spectral analysis for each spatial pixel location
Intensity
λ

MODELING AND THEORY

Overall modeling

We define the coordinate systems for the source, the colony, and the imaging coordinate as x_s , y_s; x_a , y_a; and x_i , y_i, respectively

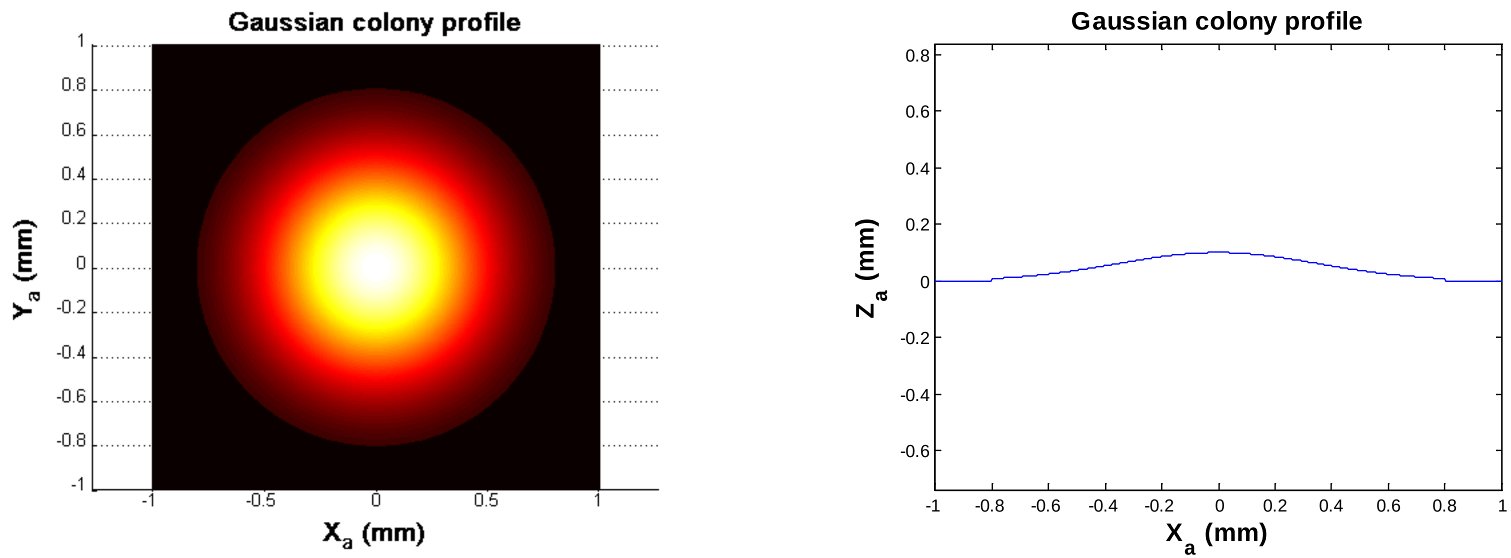


<Fig. 1> Colony modeling

Bacterial colony modeling

Various researchers mathematically modeled the bacterial colony growth phenomena using different assumptions and conditions to model the uniqueness of the spatial aggregation pattern. From our previous analysis on the morphology of the bacterial colonies, a Gaussian profile with 1:10 aspect ratio (height and diameter ratio) was suitable to describe the actual bacterial colony shape. Therefore, we adopted a Gaussian profile as a colony shape to predict the multi-wave length forward scattering pattern of the bacterial colony. Two critical parameters for the colony profile were diameter, and central thickness H_0.

$$\text{Colony}(x_a, y_a) = H_0 \exp\left[-\frac{(x_a^2 + y_a^2)}{r_c^2(z_1)}\right]$$



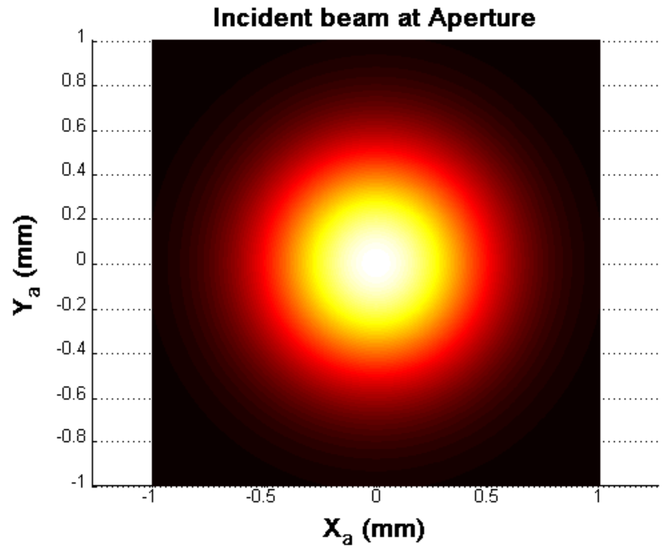
<Fig. 2> Colony modeling

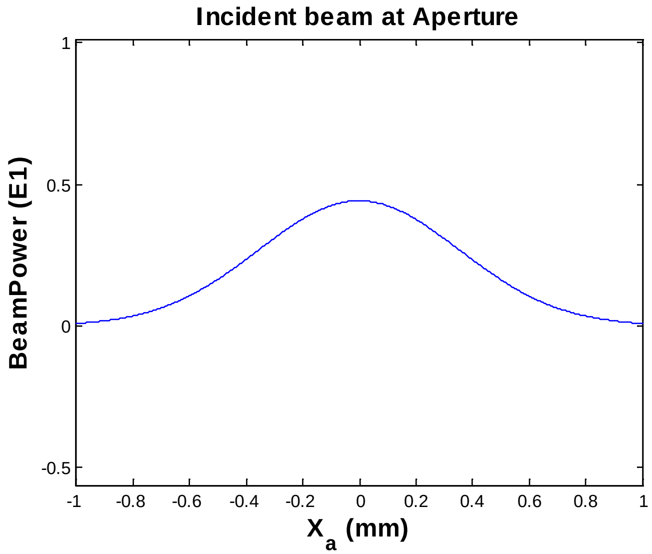
Modeling at aperture plane

Assuming a TEM00 mode of laser beam centered on the z axis, electric field on the aperture plane E_1 can be expressed as

$$E_1(x_a, y_a, z_1) = E_0 \exp\left[-\frac{(x_a^2 + y_a^2)}{\omega^2(z_1)}\right]\exp(ikz_1)\exp\left[ik\frac{(x_a^2 + y_a^2)}{2R(z_1)}\right]$$

where E_0 is the on-axis field strength; three terms account for variations of amplitude of field, longitudinal phase, and radial phase, respectively; x_a and y_a are the coordinates in the aperture; and $w(z_1)$ and $R(z_1)$ are the beam waist and radius of the wavefront in the colony plane ($z = z_1$), which is defined by





<Fig. 3> Modeling at aperture plane

Modeling at image plane

From aperture plane modeling, the electric field E_2 at the imaging plane can be expressed as

$$E_2(x_i, y_i) = \frac{1}{i\lambda}\iint t(x_a, y_a) E_1(x_a, y_a) \exp\left[ik\Phi(x_a, y_a)\right] \frac{\exp\left[ikr_{ai}\right]}{r_{ai}} \cos\theta \mathrm{d}x_a \mathrm{d}y_a$$

t is 2-D transmission coefficient, is 2-D modulation factor, and rai is distance between aperture and image plane. We can assume that cosθ=r01/z2. We can approximate

$$r_{a1} = \left[z_2^2 + (x_a - x_i)^2 + (y_a - y_i)^2\right]^{\frac{1}{2}} \quad \text{to} \quad r_{01} \simeq z_2\left[1 + \frac{1}{2}\left(\frac{x_a - x_i}{z_2}\right)^2 + \frac{1}{2}\left(\frac{y_a - y_i}{z_2}\right)^2\right]$$

Plug these approximations to the electric field equation, then

$$\iint \frac{\exp\left[ikr_{ai}\right]}{r_{ai}} \frac{r_{ai}}{z_2} \mathrm{d}x_a \mathrm{d}y_a = \iint \frac{1}{z_2} \exp\left[ikz_2\left[1 + \frac{1}{2}\left(\frac{x_a - x_i}{z_2}\right)^2 + \frac{1}{2}\left(\frac{y_a - y_i}{z_2}\right)^2\right]\right] \mathrm{d}x_a \mathrm{d}y_a$$

$$= \frac{e^{ikz_2}}{z_2} \exp\left[\frac{ik}{2z_2}\left(x_i^2 + y_i^2\right)\right] \iint \exp\left[\frac{ik}{2z_2}\left[x_a^2 + y_a^2 - 2\left(x_a x_i + y_a y_i\right)\right]\right] \mathrm{d}x_a \mathrm{d}y_a$$

$$= \frac{e^{ikz_2}}{z_2} \exp\left[\frac{ik}{2z_2} \left(x_i^2 + y_i^2 \right) \right] \iint \exp\left[\frac{ik}{2z_2} \left(x_a^2 + y_a^2 \right) \right] \exp\left[-2\pi i \left(f_x x_a + f_y y_a \right) \right] \mathrm{d}x_a \mathrm{d}y_a$$

where $f_x = \dfrac{x_i}{\lambda z_2}$, $f_y = \dfrac{y_i}{\lambda z_2}$

To sum up the whole $E_2(x_i, y_i)$ then,

$$E_2(x_i, y_i) = C_1 \iint t(x_a, y_a) \exp\left[-\frac{(x_a^2 + y_a^2)}{\omega^2(z_1)} \right] \exp\left[ik \frac{(x_a^2 + y_a^2)}{2R(z_1)} \right] \exp\left[i\Phi(x_a, y_a) \right]$$

$$\exp\left[ik \frac{(x_a^2 + y_a^2)}{2z_2} \right] \exp\left[-i2\pi(f_x x_a + f_y y_a) \right] \mathrm{d}x_a \mathrm{d}y_a$$

where $C_1 = \dfrac{E_0 \exp(ikn_2\Delta_2) \exp\left[ik(z_1 + z_2) \right] \exp\left[\dfrac{ik}{2z_2} (x_i^2 + y_i^2) \right]}{i\lambda z_2}$

Amplitude and Phase component

The two important components in the electric filed equation at image plane are amplitude component and phase component.

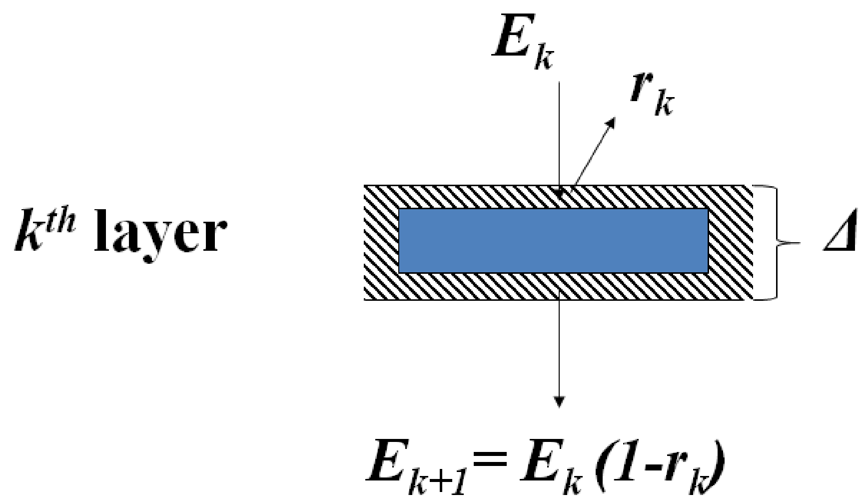
Amplitude component is $t(x_a, y_a) = \dfrac{E_{out}}{E_0} = (1 - r_1)(1 - r_k)^l (1 - r_2)$

r_1, r_2, r_k is reflection between air-bacterium, reflection between bacterium-agar, and inter-bacterium reflection respectively. l is defined like $l = \dfrac{\text{ColonyProfile}(x_a,\ y_a)}{\Delta}$

where Δ means thickness (Bacterium +extracellular material)

$$r_1 = \left|\frac{n_0 - n_1}{n_0 + n_1}\right| \quad r_k = \left|\frac{n_1 - n_{ec}}{n_1 + n_{ec}}\right| \quad r_2 = \left|\frac{n_1 - n_2}{n_1 + n_2}\right|$$

n_0, n_1, n_2, n_{ec} is reflective index of air, bacteria agar, and, extracellular materials respectively.



<Fig. 4> Multiple layer model

The phase component of the diffraction integral is governed by OPD term

$$\Phi(x_a, y_a) = n_1 H\left(x_a, y_a\right) + \left[H_0 - \text{ColonyProfile}(x_a, y_a)\right]$$

$$= (n_1 - 1) H_0 \exp\left[-\frac{(x_a^2 + y_a^2)}{r_c^2(z_1)}\right] + H_0$$

From E_2, $\exp\left[ik\Phi(x_a, y_a)\right]\exp\left[ik\frac{(x_a^2 + y_a^2)}{2z_2}\right]\exp\left[ik\frac{(x_a^2 + y_a^2)}{2R(z_1)}\right]$

$$= \exp\left[ik\left((n_1 - 1)H_0 \exp\left[-\frac{(x_a^2 + y_a^2)}{r_c^2(z_1)}\right] + H_0 + \frac{(x_a^2 + y_a^2)}{2z_2} + \frac{(x_a^2 + y_a^2)}{2R(z_1)}\right)\right]$$

$$= \exp(ikH_0)\exp\left[ik\Phi'(x_a, y_a)\right]$$

where $\Phi'(x_a, y_a) = k\left((n_1 - 1)H_0 \exp\left[-\frac{(x_a^2 + y_a^2)}{r_c^2(z_1)}\right] + \left(\frac{1}{2z_2} + \frac{1}{2R(z_1)}\right)\left(x_a^2 + y_a^2\right)\right)$

$$E_2(x_i, y_i) = C_2 \iint t(x_a, y_a)\exp\left[-\frac{(x_a^2 + y_a^2)}{\omega^2(z_1)}\right]\exp\left[i\Phi'(x_a, y_a)\right]\exp\left[-i2\pi(f_x x_a + f_y y_a)\right]\mathrm{d}x_a\mathrm{d}y_a$$

where $C_2 = C_1 \exp(ikH_0)$.

Intensity of the electric field (Power) is $I = \frac{1}{2}c\varepsilon\left|E_2\right|^2$

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