SAPIENZA Università di Roma
Laurea magistrale in Ingegneria delle Nanotecnologie
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Biophotonics Laboratory Course

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Applications of optics and photonics

Microscopic Techniques

- Conventional Wide-Field Fluorescence
- TIRF
- FLIM
- FRET, FRAP
- Confocal
- Two-Photon
- Second Harmonic
- Super-resolution (SNOM, STED, PALM, STORM)

Non-Microscopic Label-free

- Surface plasmon Polaritons (SPP)
- Photonic crystals (PC)
- Raman , CARS
- Quantum dots

Non-Microscopic Techniques

- Citofluorimetry
- ELISA
- DNA-Chip
- Cycle-sequencing
- SOLID

Other non Microscopic Techniques

- Southern
- Western
- Northern

All of them make use of the emission of luminescent markers (labels)

LECTURE 1
Basics of Linear Optics

Basics of Linear Optics

Main results from Classical Electro-Magnetism (Macroscopic Theory)

Maxwell's Equations

$$\text{div}\,\vec{D} = \rho$$

$$\text{div}\,\vec{B} = 0$$

$$\text{curl}\,\vec{E} = -\frac{\partial \vec{B}}{\partial t}$$

$$\text{curl}\,\vec{H} = \vec{J} + \frac{\partial \vec{D}}{\partial t}$$

Constitutive Equations

$$\vec{D} = \varepsilon_0 \cdot \vec{E} + \vec{P}$$

$$\vec{B} = \mu_0 \cdot (\vec{H} + \vec{M})$$

$$\vec{P} = \vec{f}(\vec{E})$$

$$\vec{M} = \vec{g}(\vec{H}) = \chi^{(1)}_{magn} \vec{H}$$

For homogeneous media the Maxwell's equations give rise to the wave equation (non magnetic media, J=0, ρ=0):

$$\nabla^2 \vec{E} - \mu_0 \varepsilon_0 \frac{\partial^2 \vec{E}}{\partial t^2} = \mu_0 \frac{\partial^2 \vec{P}}{\partial t^2}$$

Basics of Linear Optics

Vacuum

$$\vec{P} \equiv 0 \quad \Rightarrow \quad \nabla^2 \vec{E} - \mu_0 \varepsilon_0 \frac{\partial^2 \vec{E}}{\partial t^2} = 0$$

Predicts that an electric field perturbation propagates with the speed:

$$c = 1 / \sqrt{\varepsilon_0 \mu_0} \approx 3 \cdot 10^8 \, m/s$$

Isotropic medium under linearity conditions

If the electric field intensity is sufficiently small, one can approximate the dependency of the polarization on the electric field with a linear function:

$$\vec{P} = \varepsilon_0 \cdot \chi_{el}^{(1)} \cdot \vec{E} \quad \Rightarrow \quad \nabla^2 \vec{E} - \mu_0 \varepsilon_0 \frac{\partial^2 \vec{E}}{\partial t^2} = \mu_0 \varepsilon_0 \chi_{el}^{(1)} \frac{\partial^2 \vec{E}}{\partial t^2}$$

$$\nabla^2 \vec{E} - \mu_0 \varepsilon_0 (1 + \chi_{el}^{(1)}) \frac{\partial^2 \vec{E}}{\partial t^2} = 0$$

ε_r

The propagation speed is modified:

$$v = 1 / \sqrt{\varepsilon_0 \varepsilon_r \mu_0} = 1 / \sqrt{\varepsilon_0 \mu_0} \cdot 1 / \sqrt{\varepsilon_r} = \frac{c}{\sqrt{\varepsilon_r}} = \frac{c}{n}$$

$n = \sqrt{\varepsilon_r}$ Refractive Index

Basics of Linear Optics

Linear Case

1) The medium could be anisotropic therefore:

$$P_i = \varepsilon_0 \cdot \chi^{(1)}_{el,ij} \cdot E_j \qquad i, j = x, y, z$$

And the electric susceptivity $\overline{\overline{\chi}}^{(1)}_{diel}$ will be a rank 2 tensor (‘’array 3x3’’).

2) The $\overline{\overline{\chi}}^{(1)}_{diel}$ components could depend on the angular frequency ω of the field:

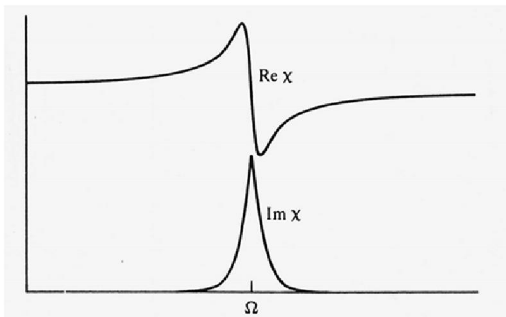
$$\overline{\overline{\varepsilon}}(\omega) = \overline{\overline{1}} + \overline{\overline{\chi}}^{(1)}_{el}(\omega) \quad \Rightarrow \quad n = n(\omega)$$

Giving rise to the dispersion.

3) $\overline{\overline{\chi}}^{(1)}_{el} \in C$ could be complex:

$$\tilde{n} = \tilde{n}(\omega) = n + i\kappa$$

n:= refractive index (refraction)
κ:= extinction coefficient (absorption)



Basics of Linear Optics

Linear Case

More precisely, the polarisation of a medium should be described in the time and space domain:

$$\vec{P}(\vec{r},t) = \varepsilon_o \int_V \int_{-\infty}^{t} \mathbf{R}^{(1)}(t-t',\vec{r}-\vec{r}') \bullet \vec{E}(\vec{r}',t')dt'dx'dy'dz'$$

The response of a medium at a time and in one spatial position can depend on the values taken by the electric field at preceding times and in different positions. Assuming locality of the response (absence of spatial dipendency, i.e. no spatial disperson):

$$\vec{P}(t) = \varepsilon_o \int_{-\infty}^{t} \mathbf{R}^{(1)}(t') \bullet \vec{E}(t-t')dt' = \varepsilon_o \int_{-\infty}^{+\infty} \mathbf{R}^{(1)}(t') \bullet \vec{E}(t-t')dt'$$

In terms of cartesian components we have:

$$\begin{cases} P_x(t) = \varepsilon_o \int_{-\infty}^{+\infty} \left[R_{xx}^{(1)}(t')E_x(t-t') + R_{xy}^{(1)}(t')E_y(t-t') + R_{xz}^{(1)}(t')E_z(t-t')\right]dt' \\ P_z(t) = \varepsilon_o \int_{-\infty}^{+\infty} \left[R_{yx}^{(1)}(t')E_x(t-t') + R_{yy}^{(1)}(t')E_y(t-t') + R_{yz}^{(1)}(t')E_z(t-t')\right]dt' \\ P_z(t) = \varepsilon_o \int_{-\infty}^{+\infty} \left[R_{zx}^{(1)}(t')E_x(t-t') + R_{zy}^{(1)}(t')E_y(t-t') + R_{zz}^{(1)}(t')E_z(t-t')\right]dt' \end{cases}$$

Basics of Linear Optics

Linear Case

We can use the Fourier transform to transfer the problem to the angular frequency domain:

$$\vec{E}(t)=\int_{-\infty}^{+\infty}\vec{E}(\omega)e^{-i\omega t}d\omega \qquad \vec{E}(\omega)=\frac{1}{2\pi}\int_{-\infty}^{+\infty}\vec{E}(t)e^{i\omega t}dt$$

Then we have:

$$\vec{P}(t)=\varepsilon_o\int_{-\infty}^{t}\mathbf{R}^{(1)}(t')\bullet\vec{E}(t-t')dt'=\varepsilon_o\int_{-\infty}^{+\infty}\mathbf{R}^{(1)}(t')\bullet\left[\int_{-\infty}^{+\infty}\vec{E}(\omega)e^{-i\omega(t-t')}d\omega\right]dt'=$$

$$=\varepsilon_o\int_{-\infty}^{+\infty}\left[\int_{-\infty}^{+\infty}\mathbf{R}^{(1)}(t')e^{i\omega t'}dt'\right]\bullet\vec{E}(\omega)e^{-i\omega t}d\omega=\varepsilon_o\int_{-\infty}^{+\infty}\chi^{(1)}(-\omega_\sigma;\omega)\bullet\vec{E}(\omega)e^{-i\omega_\sigma t}d\omega$$

where the linear electric suscetivity tensor is 2π the Fourier transform of the response function:

$$\chi^{(1)}(-\omega_\sigma,\omega)=\int_{-\infty}^{+\infty}\mathbf{R}^{(1)}(t')e^{i\omega t'}dt'$$

In these equations $\omega_\sigma=\omega$. However we introduce ω_σ now because when we shall discuss nonlinear optics it will be useful for the comprehension.

Basics of Linear Optics

Linear Case

Comparing the expression obtained for the polarization and its Fourier expansion:

$$\vec{P}(t) = \varepsilon_o \int_{-\infty}^{+\infty} \boldsymbol{\chi}^{(1)}(-\omega_\sigma;\omega) \bullet \vec{E}(\omega) e^{-i\omega_\sigma t} d\omega$$

$$\vec{P}(t) = \int_{-\infty}^{+\infty} \vec{P}(\omega) e^{-i\omega t} d\omega$$

we retrieve the basic physics textbook expression given above (for every position in the space):

$$\vec{P}(\vec{r},\omega) = \varepsilon_o \boldsymbol{\chi}^{(1)}(\vec{r},\omega_\sigma;\omega) \bullet \vec{E}(\vec{r},\omega)$$

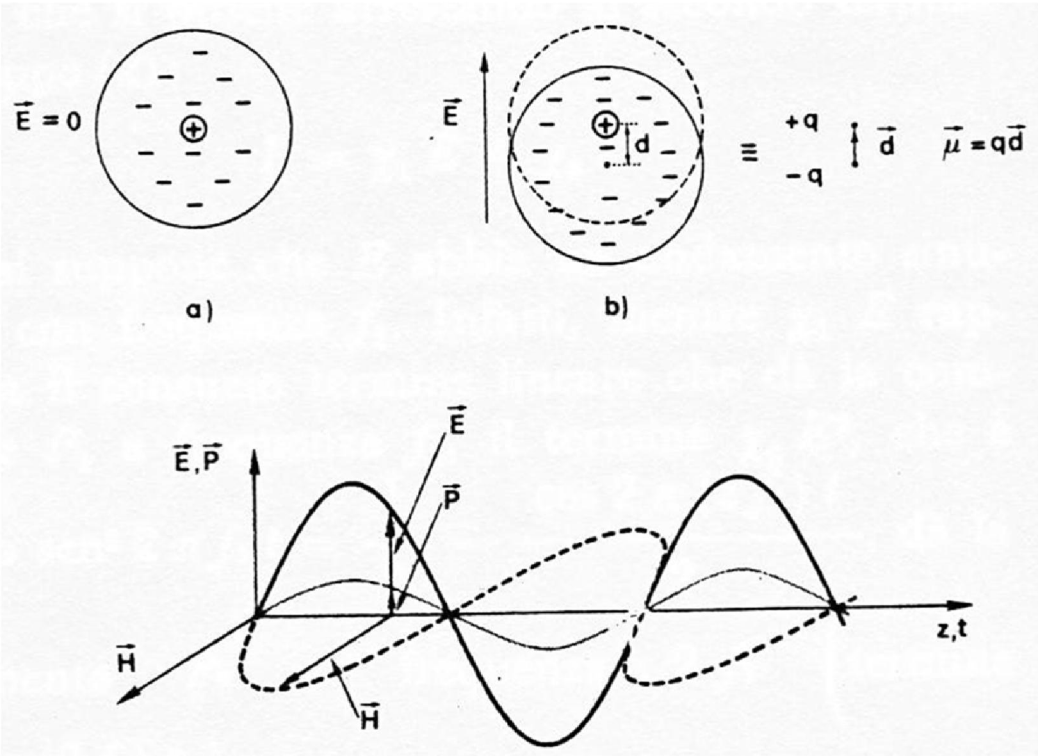
Where now it is clear that the Fourier component at ω of the polarization is proportional to the component of the electric field at the same ω and to the value of the linear elecric susceptivity at that ω.

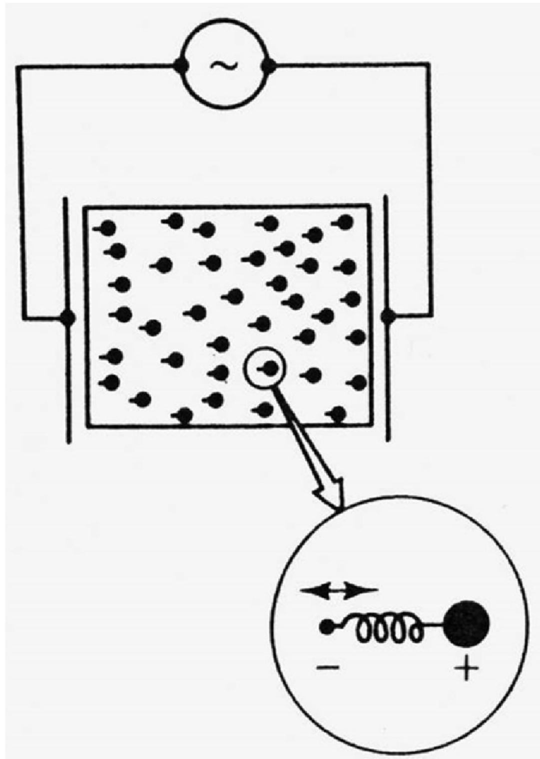
The expressions we gave at the beginning (without ω dependency) are valid in case of a monochromatic or quasi-monochromatic electric field or in the case of a medium with a dispersionless electric susceptivity $\chi \neq \chi(\omega)$

Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

We can describe the linear response of a dielectric medium by means of a simplified model based on the **harmonic oscillator** in classical physics (**Lorentz**). Let's suppose that the medium is composed of identical harmonic oscillators, which are 1D for the sake of simplicity.





Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

Let's suppose that the oscillators are reached by a local electric field oscillating at ω, and express the field in complex notation:

$$E(t) = E_o \cos(\omega t) = \frac{1}{2} E_o \left[e^{-i\omega t} + e^{+i\omega t} \right] = \frac{1}{2} E_o \exp^{-i\omega t} + \text{c.c.}$$

The displacement x(t) with respect to the equilibrium position is a solution of the damped harmonic oscillator equation:

$$\vec{F}_{el} + \vec{F}_{visc} + \vec{F}_{ext} = m\vec{a} \quad \xrightarrow{\text{1D}} \quad -kx(t) - bv(t) - eE(t) = ma(t)$$

$$-kx(t) - b\frac{dx(t)}{dt} - eE(t) = m\frac{d^2x(t)}{dt^2} \qquad \Omega = \sqrt{\frac{k}{m}} \qquad \Gamma = \frac{b}{2m}$$

$$m\left[\frac{d^2x(t)}{dt^2} + 2\Gamma\frac{dx(t)}{dt} + \Omega^2 x(t) \right] = -eE(t)$$

The solution is a harmonic oscillation at ω given by:

$$x(t) = x_0 \cos(\omega t + \varphi) = \frac{1}{2} x_0 e^{-i(\omega t + \varphi)} + \text{c.c.} = \frac{1}{2} \tilde{x}_0 e^{-i\omega t} + \text{c.c.}$$

Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

Calculating the derivatives and substituting x(t), x'(t) and x''(t) in the differential equation we can find the expression of x(t).

$$x'(t) = \frac{1}{2}\tilde{x}_0(-i\omega)e^{-i\omega t} + c.c.$$
$$x''(t) = \frac{1}{2}\tilde{x}_0(-i\omega)^2 e^{-i\omega t} + c.c.$$

$$\Rightarrow \quad m\left[\frac{d^2x(t)}{dt^2} + 2\Gamma\frac{dx(t)}{dt} + \Omega^2 x(t)\right] = -eE(t)$$

$$m\left[\frac{1}{2}\tilde{x}_0(-i\omega)^2 e^{-i\omega t} + 2\Gamma\frac{1}{2}\tilde{x}_0(-i\omega)e^{-i\omega t} + \Omega^2\frac{1}{2}\tilde{x}_0 e^{-i\omega t}\right] = -e\frac{1}{2}E_0 e^{-i\omega t}$$

$$\tilde{x}_0\left[-\omega^2 - 2i\Gamma\omega + \Omega^2\right] = \frac{-eE_0}{m} \quad \Rightarrow \quad \tilde{x}_0 = \frac{-e/mE_0}{\Omega^2 - 2i\Gamma\omega - \omega^2}$$

$$x(t) = \frac{1}{2}\tilde{x}_0 e^{-i\omega t} = \frac{-eE_o}{2m}\frac{e^{-i\omega t}}{\Omega^2 - 2i\Gamma\omega - \omega^2} + c.c.$$

Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

The induced microscopic dipole moment is:

$$p(t) = -e \cdot x(t)$$

and:

$$p(t) = \frac{e^2}{2m} \frac{E_o \exp(-i\omega t)}{\Omega^2 - 2i\Gamma\omega - \omega^2} + c.c.$$

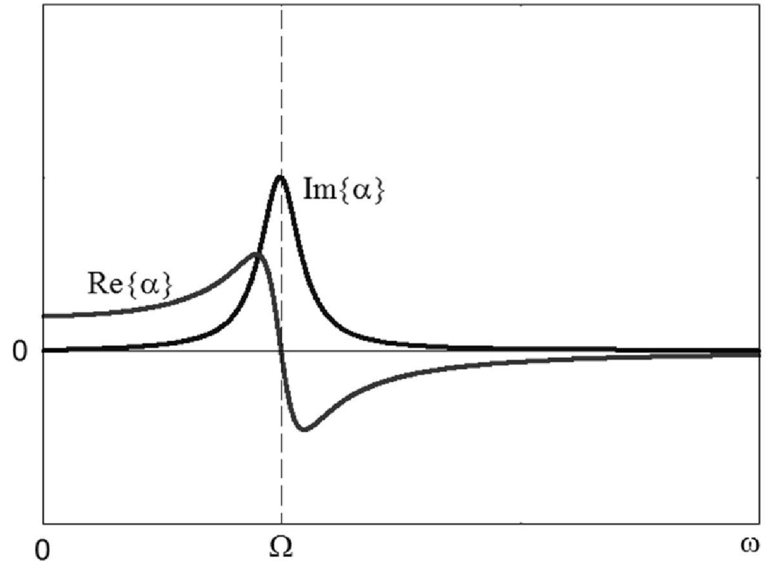
that can be compared to the classical expression that makes use of the polarizability α:

$$p(t) = \tfrac{1}{2}\tilde{\alpha}(\omega) E_o \exp(-i\omega t) + c.c.$$

Providing the expression:

$$\tilde{\alpha}(\omega) = \frac{e^2}{m} \cdot \frac{1}{\Omega^2 - 2i\Gamma\omega - \omega^2}$$

Where Ω is the characteristic resonance angual frequency of th oscillator and Γ is the so-called damping coefficient taking into account losses.



For $\omega \rightarrow 0$:

$$\tilde{\alpha}(\omega) = \alpha = \frac{e^2}{m\Omega^2} = \frac{e^2}{mk/m} = \frac{e^2}{k}$$

$$p = \alpha E_o = \frac{e^2 E_0}{k} = \frac{e x_{stat}}{k x_{stat}} F_{electr} = e x_{stat} \frac{F_{electr}}{F_{elast}} = e x_{stat}$$

Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

The induced dipole moment is:

$$p(t) = -e \cdot x(t)$$

Assuming that there are N dipoles per unit volume:

$$P(t) = Np(t) = -Nex(t)$$

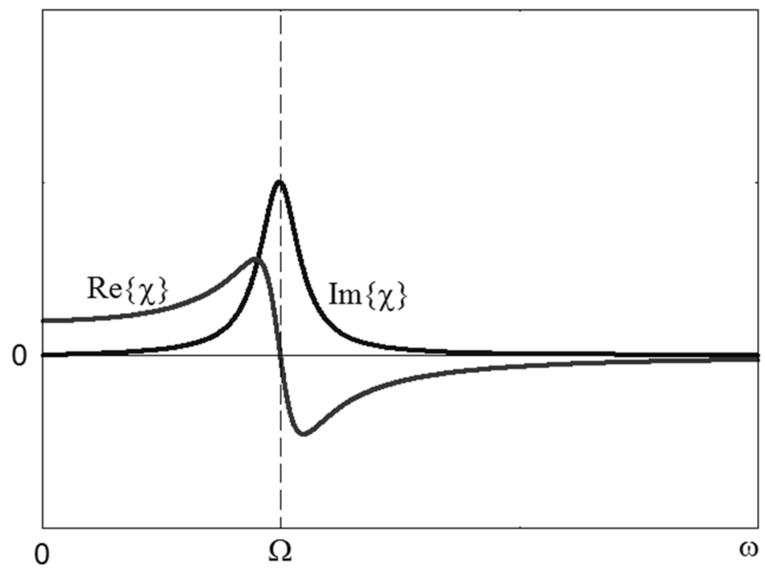
$$P(t) = \frac{Ne^2}{2m} \frac{E_o \exp(-i\omega t)}{\Omega^2 - 2i\Gamma\omega - \omega^2} + c.c.$$

That can be compared to the monochromatic expression of the polarization we derived previously:

$$P(t) = \tfrac{1}{2}\varepsilon_o \tilde{\chi}(\omega) E_o \exp(-i\omega t) + c.c.$$

Giving as a result the expression of the linear susceptibility :

$$\tilde{\chi}(\omega) = \frac{Ne^2}{\varepsilon_o m} \cdot \frac{1}{\Omega^2 - 2i\Gamma\omega - \omega^2}$$



Complex linear susceptivity

Basics of Linear Optics

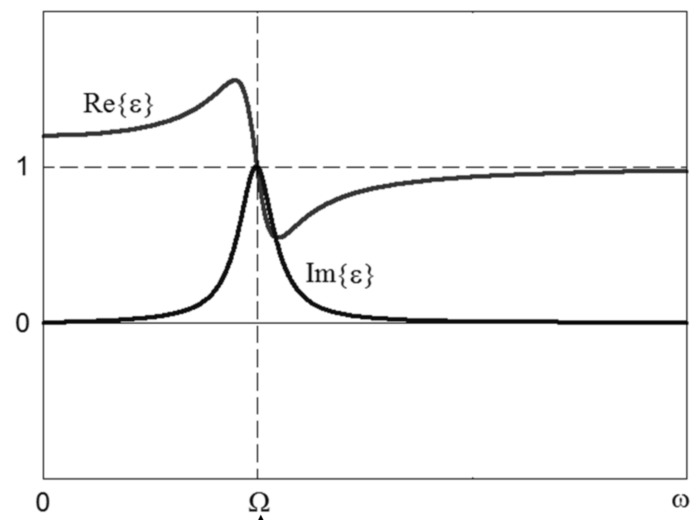
Origin of the dielectric response – Microscopic model – Linear Case

We can calculate the relative dielectric constant:

$$\tilde{\varepsilon}(\omega) = 1 + \tilde{\chi}(\omega) = 1 + \frac{Ne^2}{\varepsilon_o m} \cdot \frac{1}{\Omega^2 - 2i\Gamma\omega - \omega^2}$$

That is a complex quantity characterized by real and an imaginary parts:

$$\begin{cases} \varepsilon_R(\omega) = 1 + \mathrm{Re}\{\chi\} = 1 + \dfrac{Ne^2}{\varepsilon_o m} \cdot \dfrac{\Omega^2 - \omega^2}{(\Omega^2 - \omega^2)^2 + 4\Gamma^2\omega^2} \\ \varepsilon_I(\omega) = \mathrm{Im}\{\chi\} = \dfrac{Ne^2}{\varepsilon_o m} \cdot \dfrac{2\Gamma\omega}{(\Omega^2 - \omega^2)^2 + 4\Gamma^2\omega^2} \end{cases}$$



In general we have that :

$$\tilde{\varepsilon}(\omega) = \varepsilon_R(\omega) + i\varepsilon_I(\omega)$$

Complex dielectric constant

Giving as a result the expression of the linear susceptibility :

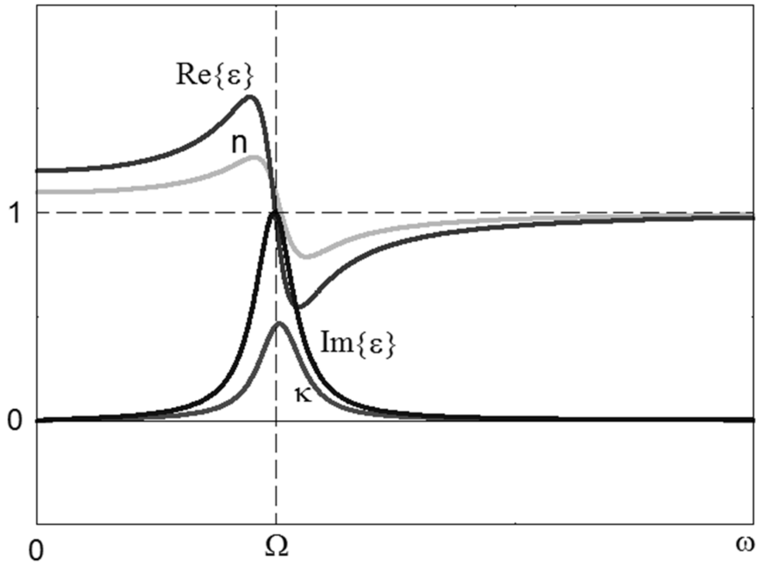
Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

As a result the refractive index is a complex quantity and is frequency dependent:

$$\tilde{n}(\omega) = n(\omega) + i\kappa(\omega)$$

$$\tilde{n}^2 = \tilde{\varepsilon} \qquad\qquad (n(\omega) + i\kappa(\omega))^2 = \varepsilon_R + i\varepsilon_I$$



From the definiton of n we have:

$$n^2(\omega) - \kappa^2(\omega) + 2i\kappa(\omega)n(\omega) = \varepsilon_R + i\varepsilon_I$$

n < 1 ?

v > c ????

and:

$$\begin{cases} \varepsilon_R(\omega) = n^2(\omega) - \kappa^2(\omega) \\ \varepsilon_I(\omega) = 2\kappa(\omega)n(\omega) \end{cases}$$

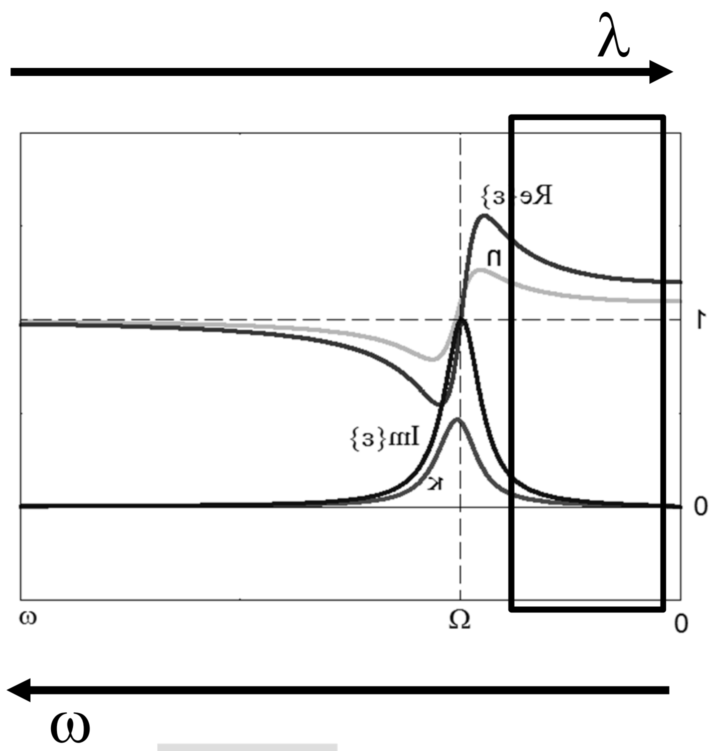
We can work out the inverse formulas

$$\begin{cases} n(\omega) = \sqrt{\dfrac{\varepsilon_R + \sqrt{\varepsilon_R^2 + \varepsilon_I^2}}{2}} \\ \kappa(\omega) = \sqrt{\dfrac{-\varepsilon_R + \sqrt{\varepsilon_R^2 + \varepsilon_I^2}}{2}} \end{cases}$$

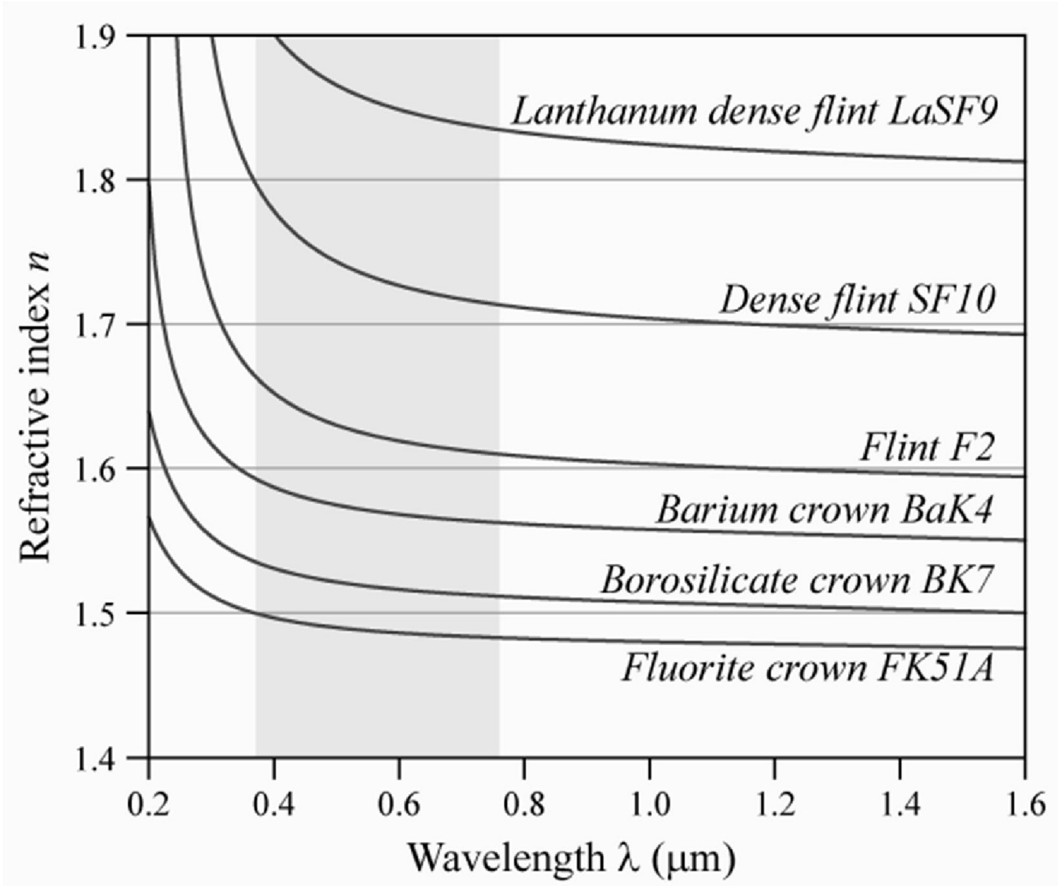
Basics of Linear Optics

Origin of the dielectric response – Microscopic model – Linear Case

EXAMPLE: Glasses are characterized by absorption peaks at Ω in the UV. In the visible range for $\omega < \Omega$ and $\lambda > \lambda_{UV}$, the real part of the refractive index decreases with λ

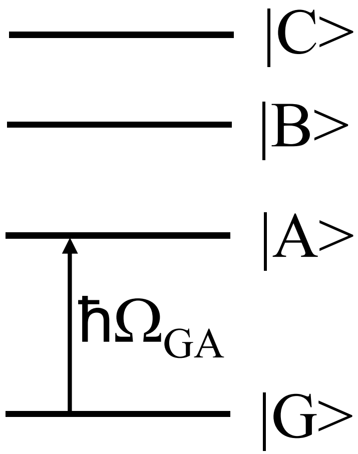


$$\lambda = \frac{2\pi c}{\omega}$$ in vacuum



Basics of Linear Optics

Quantum description



We can describe a molecule by means of its energy levels system, whose energies can be calculated by solving the Schroedinger equation:

$$\hat{H}_0|A\rangle = \hbar\Omega_A|A\rangle$$

The Hamiltonian that is describing the interaction of a molecule with monochromatic fileds is given by

$$\hat{H}_1 = \sum_j \frac{e_j}{2m_j c}\left[\vec{A}(\vec{r}_j,t)\cdot\vec{p}_j + \vec{p}_j\cdot\vec{A}(\vec{r}_j,t)\right] + \frac{e_j^2}{m_j c^2}A^2(\vec{r}_j,t)$$

vector potential   momentum of the particle j

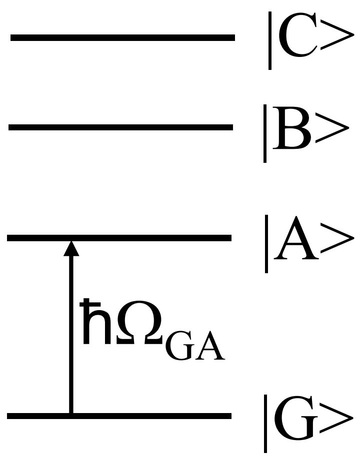
The interactino Hamiltonian can be written as a multipolar expansion:

$$\hat{H}_1 = -(\vec{\mu}\cdot\vec{F} + \vec{m}\cdot\vec{H} + \vec{Q}\cdot\nabla\vec{F} + ...)$$

Electric dipole   Magnetic dipole   Electric quadrupole..

Basics of Linear Optics

Quantum description



If one introduces the perturbation of the field can calculate the expression of the molecular polarizability, which takes into account all possible transitions between couples of energy levels:

$$\tilde{\alpha}(\omega)=\sum_{A}\left[\frac{\langle G|\vec{\mu}|A\rangle\langle A|\vec{\mu}|G\rangle}{\hbar\left(\omega-\Omega_{AG}+j\Gamma_{AG}\right)}+\frac{\langle G|\vec{\mu}|A\rangle\langle A|\vec{\mu}|G\rangle}{\hbar\left(\omega+\Omega_{AG}-j\Gamma_{AG}\right)}\right]$$

that is obtained considering only the electric dipole contribution to the interaction Hamiltonian (Fermi, Golden rule)

Basics of Linear Optics

Kramers–Kronig relations

The complex response function of a physical system obeys to some particular relations between its real and imaginary parts given by Kramers and. Kronig.

In mathematical analysis it is found that the real and imaginary parts of any complex function that is analytic in the upper half-plane obey such relations.

Given that for physical systems the causality principle implies the analyticity condition, all response function of real system obey to K-K.

Given:

$$\tilde{\varepsilon}(\omega) = \varepsilon_R(\omega) + i\varepsilon_I(\omega)$$

Then:

$$\varepsilon_R(\omega) = \frac{1}{\pi} P \int_{-\infty}^{+\infty} \frac{\varepsilon_I(\omega')}{\omega' - \omega} d\omega' \qquad \varepsilon_I(\omega) = -\frac{1}{\pi} P \int_{-\infty}^{+\infty} \frac{\varepsilon_R(\omega')}{\omega' - \omega} d\omega'$$

where P is principal value of the integral (Cauchy). Therefore, the real and imaginary parts are not independent, and one can retrieve one of them if he know the other one for every ω.

This is true also for α, χ, n (but also for the transfer function of an electronic amplifier!)