Curriculum on Optical Imaging I

Optical Imaging

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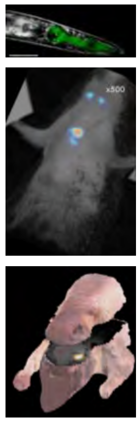
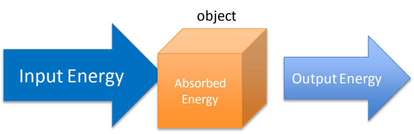
Universidad Carlos III, Madrid, Spain



During this first part we will try to explain:

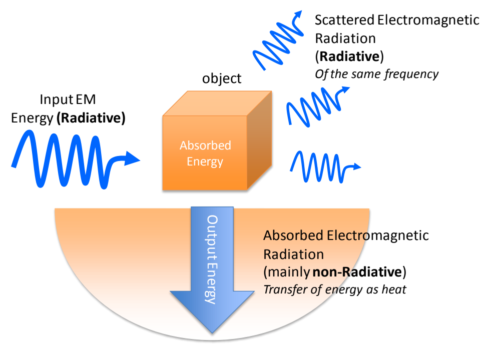
The Basics: Energy Conservation

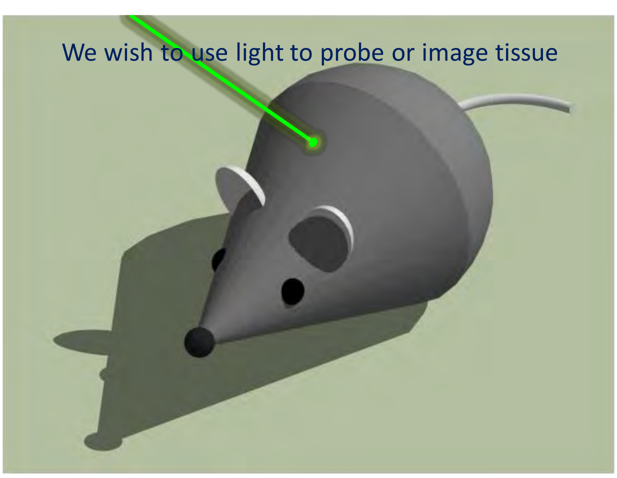


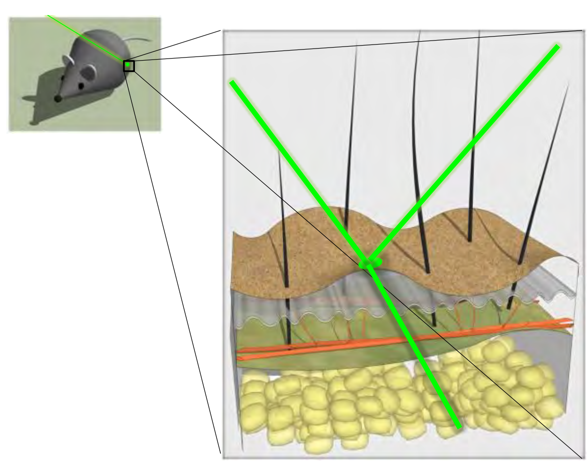
This interaction can have many forms, as many as different forms of energy exist (thermal, electrical, chemical, electromagnetic, kinetic, magnetic, mechanical, nuclear or any combination) . What always must hold true is:

Total Input Energy = Total Energy Absorbed by Object + Total Output Energy

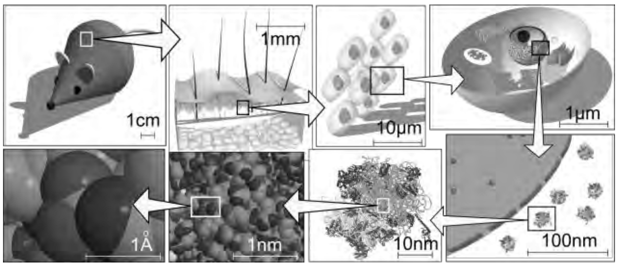
Light Interaction







Characteristic Sizes



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Optical Properties depend on the collective properties:

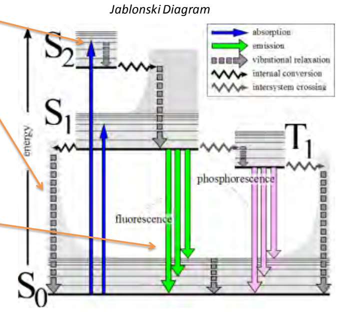
Depending on the levels (orbitals) accessible, this extra energy can be given back to the system either as **non-radiative** or **radiative** emission. It is the radiative emission we're interested in right now.

We managed to excite the electron to this higher energy level (in this case, a singlet state)

It can then return to the ground state (state of minimum energy) either non-radiatively (heating up its environment, for example)

Or radiatively, i.e. emitting EM radiation, as in Fluorescence or Phosphorescence.

Jablonski Diagram



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Light Scattering

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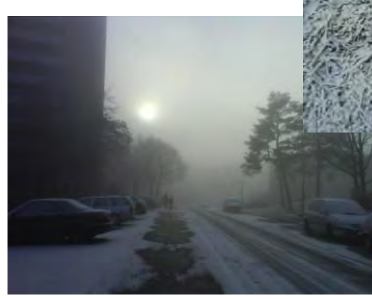
Light Scattering



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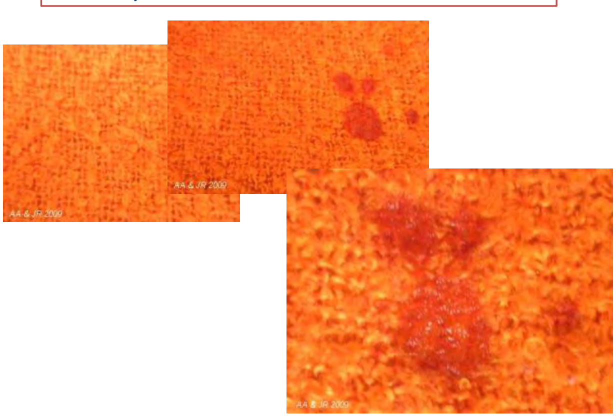
Q:Why does fabric look darker when wet?



Interaction with a single particle

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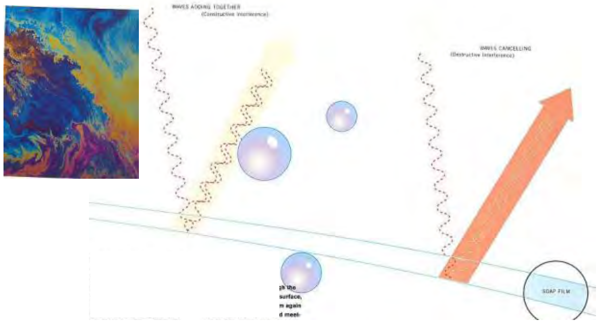
Bubbles



From "the Colors of Nature"

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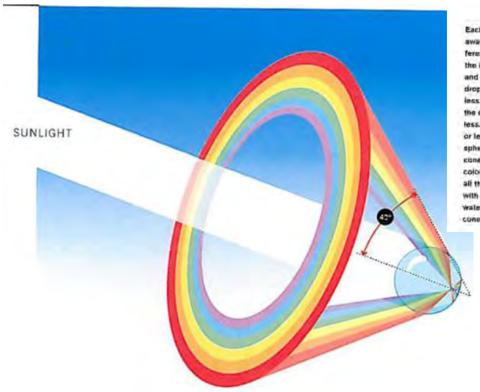
The Rainbow



Each of the rainbow colors bounces away from a raindrop at a slightly different angle relative to the path of the incoming light. Red light bends and reflects so that it leaves a water drop at an angle of 42 degrees or less. Green light bounces away from the drop at an angle of 41 degrees or less. Blue light reflects at 40 degrees or less. Because the water drop is spherical, the reflecting light forms a cone, with the drop at the tip. Each color makes its own cone of light, and all these cones fit inside each other, with the red cone on the outside. The water drop is at the tip of all these cones of colored light.

Color and Light in Nature by David K. Lynch and William Livingston

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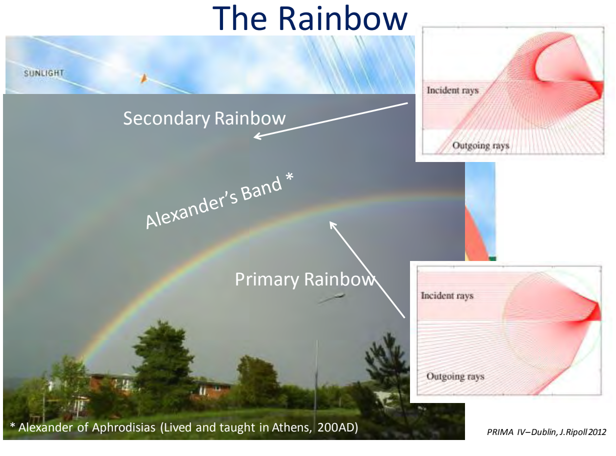
The Rainbow



* Alexander of Aphrodisias (Lived and taught in Athens, 200AD)

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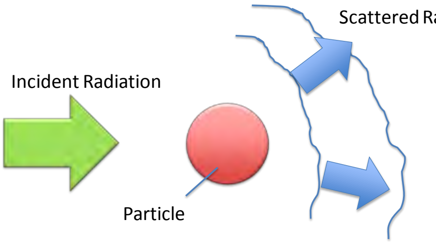
Quantifying scattering



How much energy is scattered by the particle?

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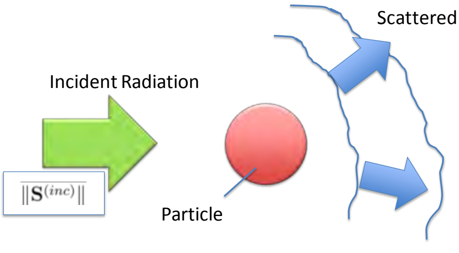
And the total scattered power then is:



Total Scattered Power:

$$\bar{P}_{sc} = \sigma_{sc} \|\mathbf{S}^{(inc)}\| \quad (Watts)$$

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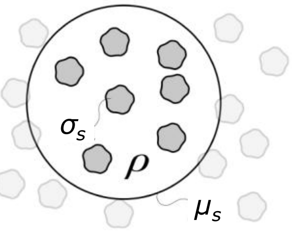
Statistical Description of Optical Properties



In the case where we have a collection of these particles at a certain density, the amount of scattering will depend on the density of particles.

Scattering Coefficient

$$\mu_s = \rho\sigma_{sc}, \qquad \left(cm^{-1}\right)$$

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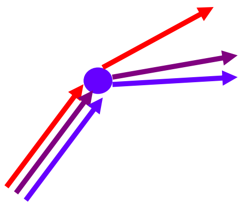
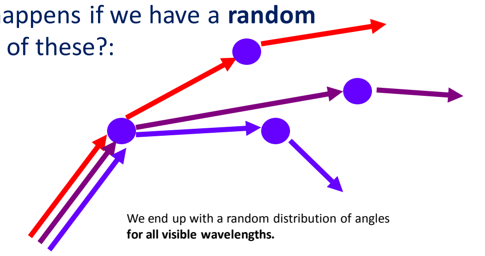
Scattering

- We have seen that each wavelength is scattered at a different angle:



Scattering

- So what happens if we have a **random** collection of these?:



We end up with a random distribution of angles **for all visible wavelengths.**

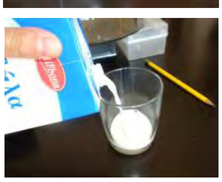
So, even though each particle may be **transparent** on its own, an ensemble of these will randomize light's angular distribution mixing all colors in all directions: *diffuse white light*

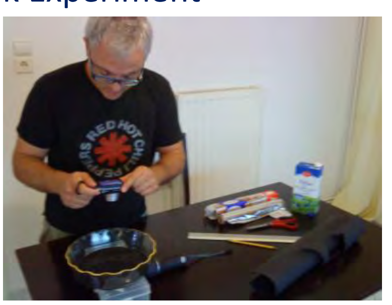
Milk Experiment

How does scattering affect light propagation?

Material

Milk Experiment

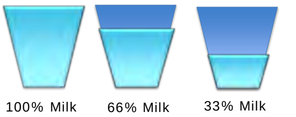




Milk Experiment

Using different proportions of milk for the same volume (1cm high):

3 cups milk | 2 cups milk 1 cup water | 1 cup milk 2 cup water



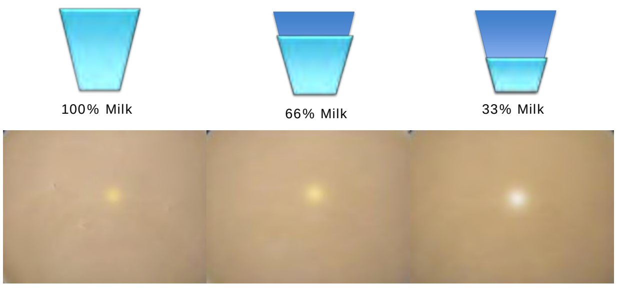
100% Milk | 66% Milk | 33% Milk

$$\mu_s = \rho\sigma_{sc}$$

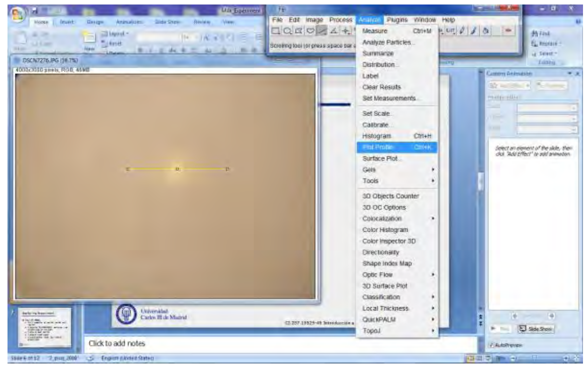
We're effectively changing the density of scatterers

Milk Experiment

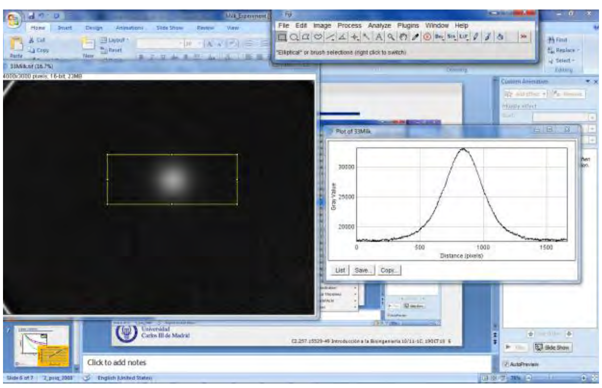
For 1cm depth:



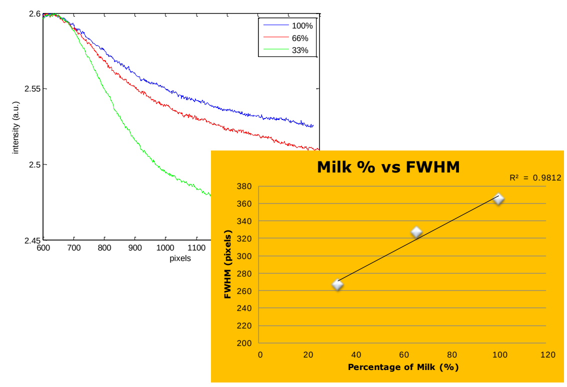
Analysis



Analysis

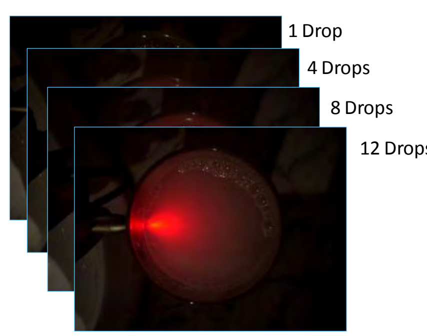


Analysis: 100% Milk

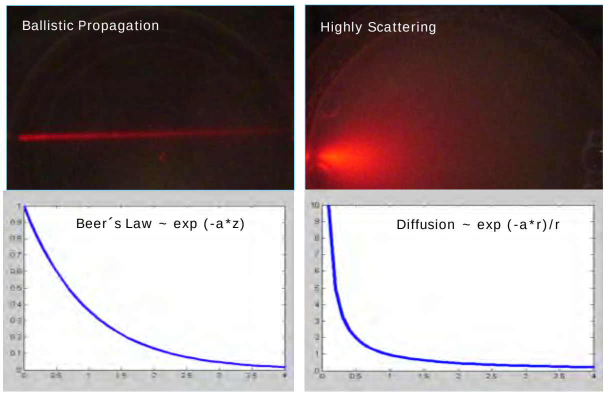


A second example

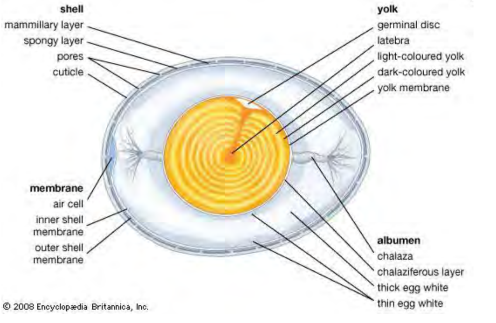
Scattering



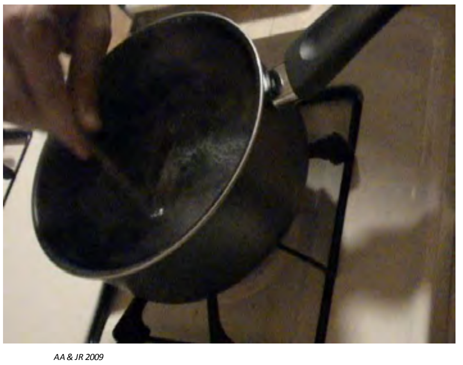
Scattering



The Egg… from ballistic to multiple scattering

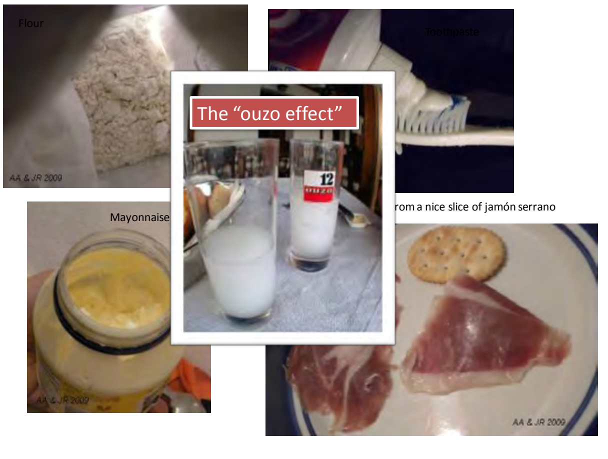


Boiling an egg

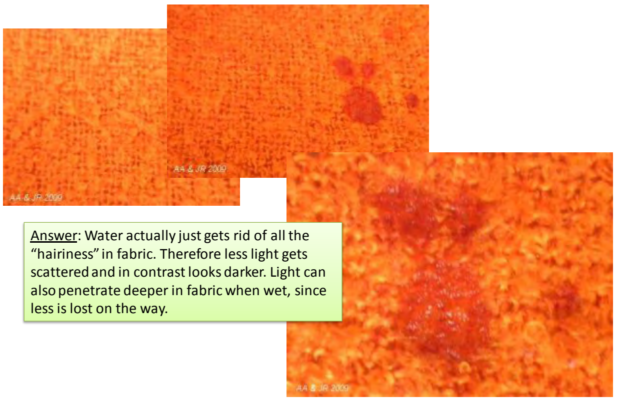


AA & JR 2009

Other stuff that multiply scatters light



Q:Why does fabric look darker when wet?



Answer: Water actually just gets rid of all the “hairiness” in fabric. Therefore less light gets scattered and in contrast looks darker. Light can also penetrate deeper in fabric when wet, since less is lost on the way.

Light Absorption

Flamingos are pink because their feathers contain carotenoids, pigments that are responsible for many of the reds, oranges, golds, and yellows of plants and animals. Though carotenoid pigments are among the most widespread of animal pigments, animals can't synthesize these compounds but must obtain them from their diet. The yellow color of butter, which comes from a carotenoid, depends on what the cow has been eating; the yellowness of an egg yolk depends on the hen's diet. The pink of the flamingo's feathers comes from pigments in the crustaceans it eats. The crustaceans, in turn, obtain their pigment from algae. If captive flamingos don't get sufficient pigment in their diet, they'll lose their pink color and fade to white. *From "The Colors of Nature".*

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Light Absorption

Additive Primaries
(adding light)

Substractive Primaries
(adding absorption)

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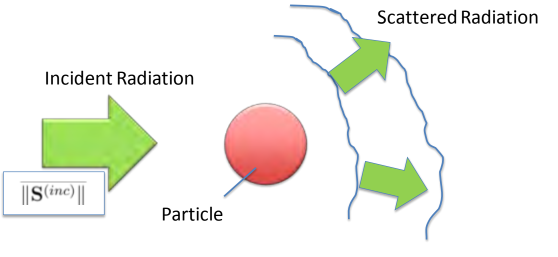
Quantifying Absorption:



Total Absorbed Power:

$$\bar{P}_{abs} = \sigma_a \|\mathbf{S}^{(inc)}\| \quad (Watts)$$

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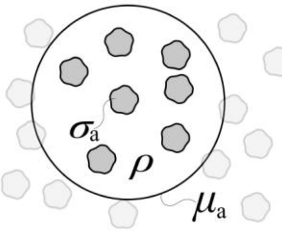
Statistical Description of Optical Properties



In the case where we have a collection of these particles at a certain density, the amount absorption will depend on the density of particles.

Absorption Coefficient

$$\mu_a = \rho\sigma_a. \qquad (cm^{-1})$$

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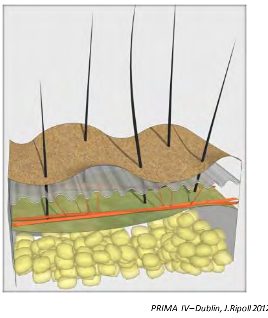
Tissue Absorption

- Main Absorbers in Tissue:
  - Blood
  - Water
  - Skin (melanin)



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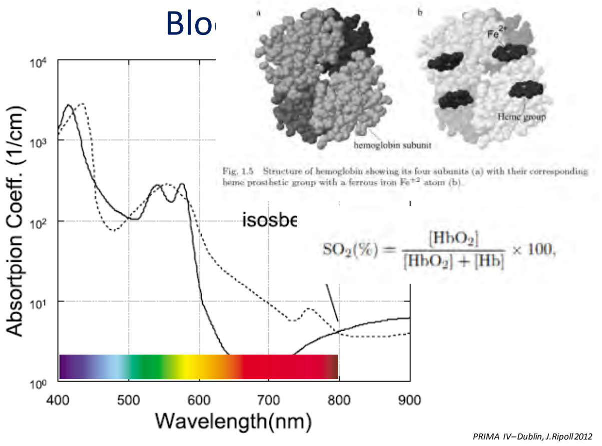
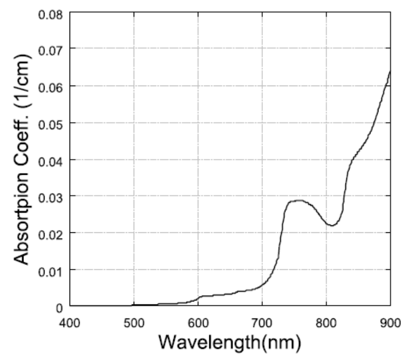
Blo

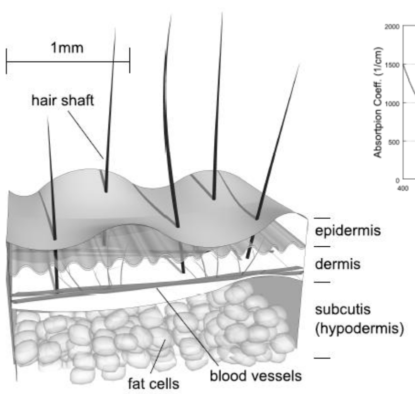


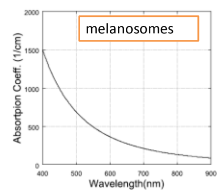
$$SO_2(\%) = \frac{[HbO_2]}{[HbO_2]+[Hb]} \times 100,$$

Absorption of Water

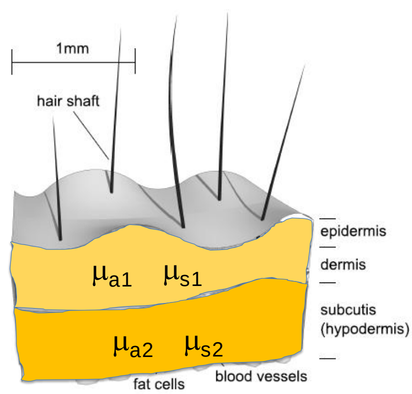


Effect of Skin

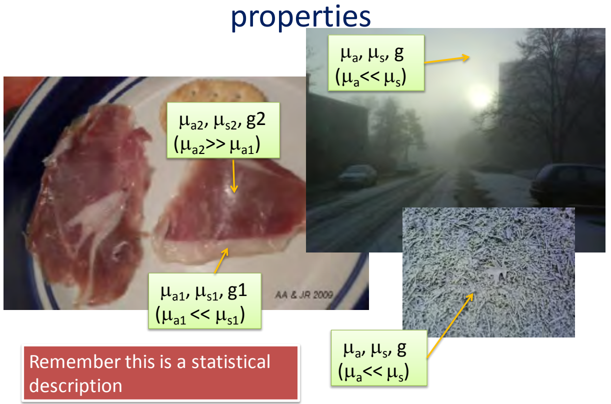




Statistical Description of optical properties



We can now characterize the optical properties



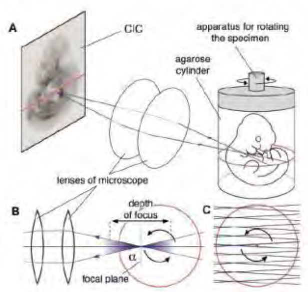
Remember this is a statistical description

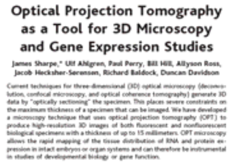
Universidad Carlos III de Madrid · BiiG · SaludMadrid · Hospital General Universitario Gregorio Marañón

Optical Projection Tomography (OPT)

Slightly Scattering Tissues

Principles of OPT

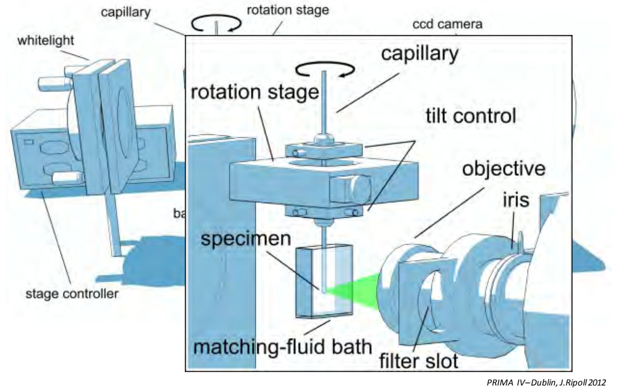



Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies

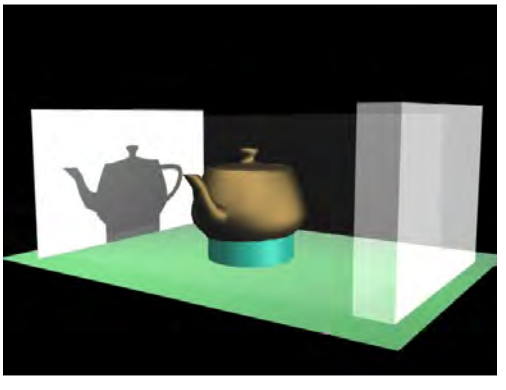
James Sharpe,* Ulf Ahlgren, Paul Perry, Bill Hill, Allyson Ross, Jacob Hecksher-Sørensen, Richard Baldock, Duncan Davidson

J. Sharpe et al, Science **296** 2002

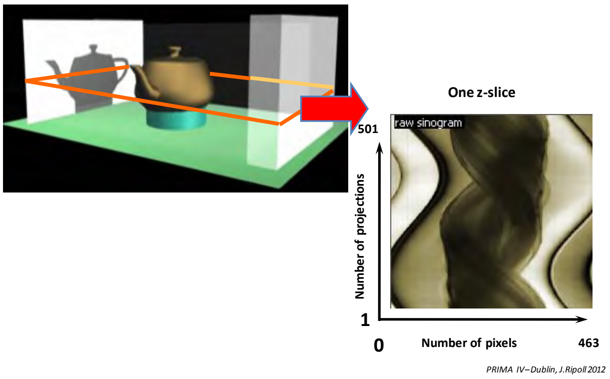
OPT Setup



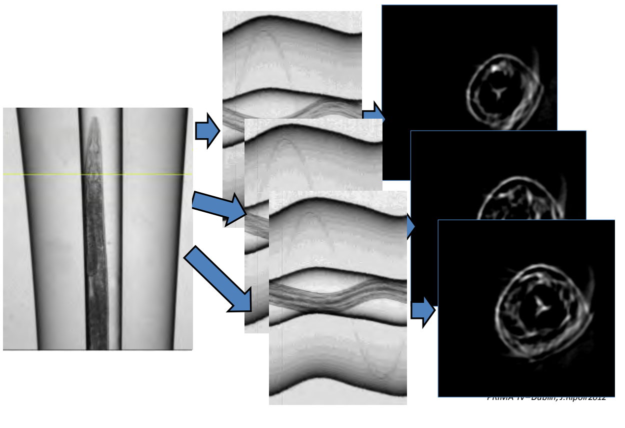
Radon Transform



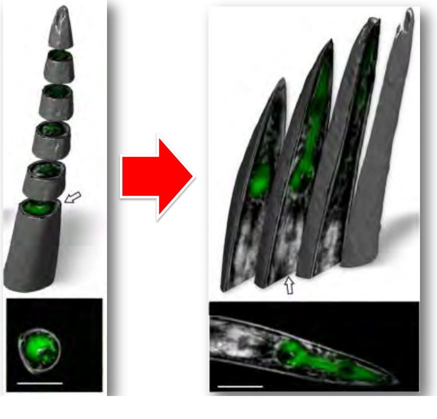
Radon Transform



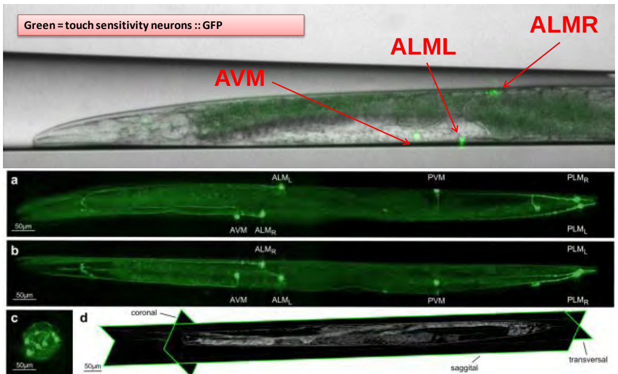
OPT Reconstruction



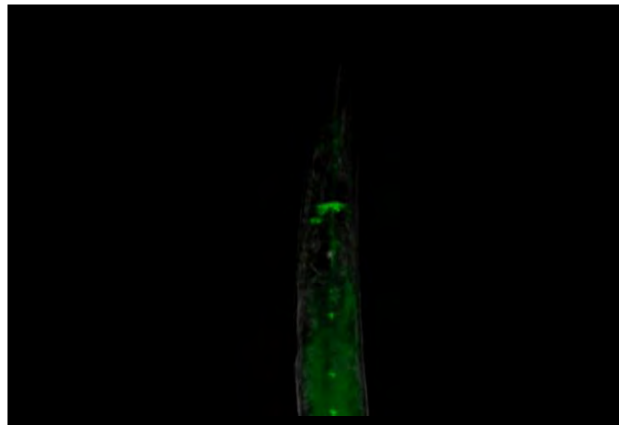
OPT



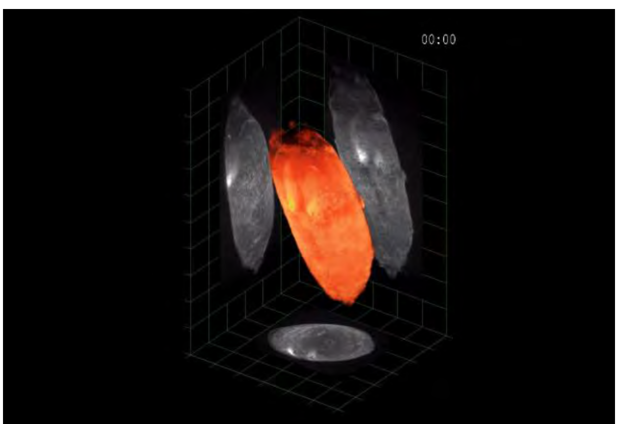
In-vivo *C. elegans*



M. Rieckher *et al*, PlosOne (2011)



Dopamine neurons expressing GFP
M. Rieckher *et al*, PlosOne (2011)



3D Time-lapse Imaging of *D. melanogaster* development = ~500 OPT datasets
CUDA Implementation: Dong Di and Shouping Zhu. Specimen: S. Oehler and B. Savakis (Fleming)

Funding
E.U. Integrated Project "Molecular Imaging"
E.U. EST – MOLEC IMAG
Bill & Melinda Gates Foundation
E.U. Collaborative Project „FMT-XCT"

FORTH Institute of Electronic Structure and Laser

Oii group

IMBB

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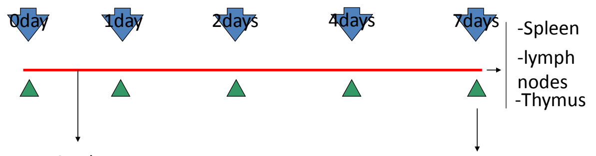
Assessment of stroke-induced immune depression

C. Mamalaki A. Planas, A. Martin

Dimitris Kioussis

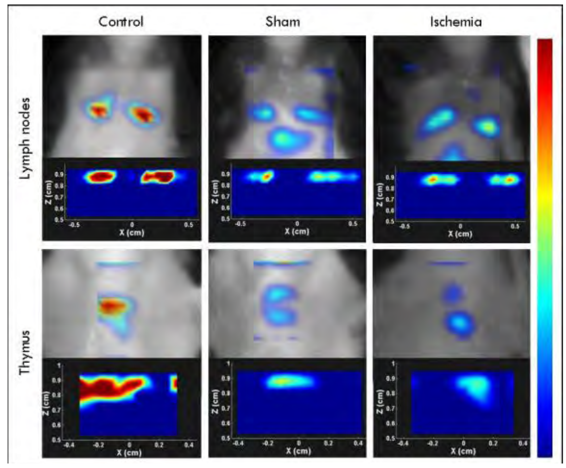
Protocol

FMT "Fluorescence Molecular Tomography-Optical Imaging

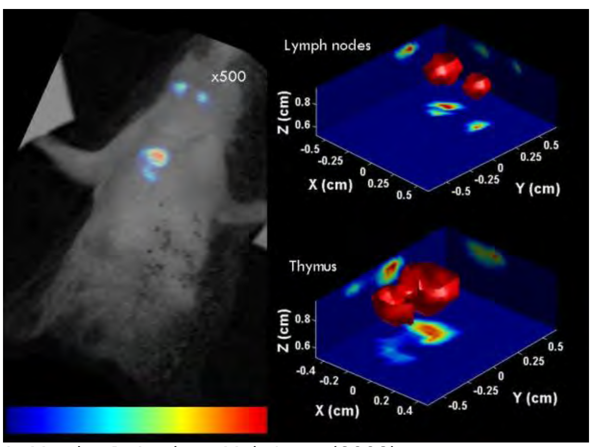


Blood samples

FACS "Fluorescence Analizer Cell Sorter".

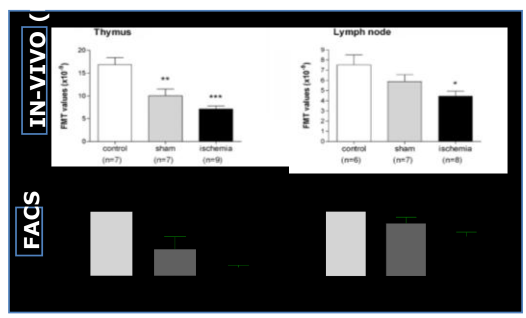


A. Martin, J. Aguirre, et al, Mol. Img (2



A. Martin, J. Aguirre, Mol. Img. (2008)

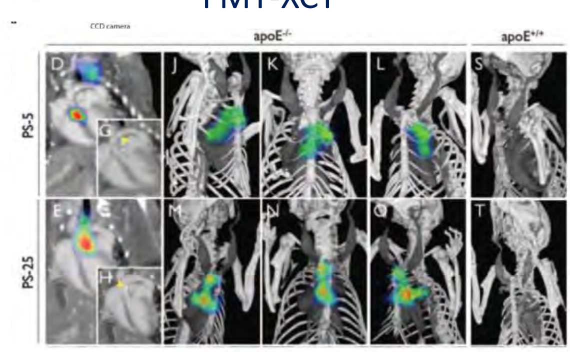
Stroke-induced immune depression



A. Martin, J. Aguirre, et al, Mol. Imag (2008)

Hybrid Systems

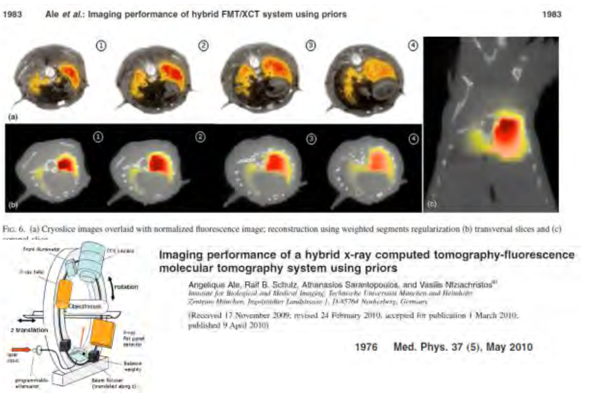
FMT-XCT



Imaging of artherosclerotic plaques in mice

Nahrendorf, et al, *Artherosclerosis,*

FMT-XCT



FMT-MRI

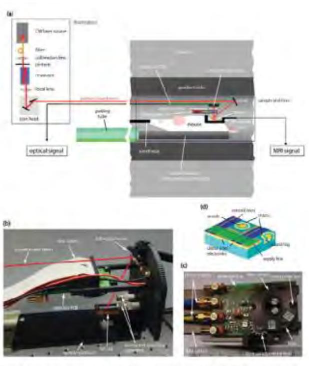
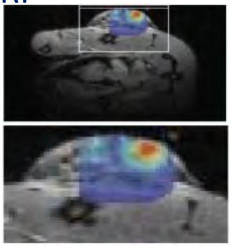


Fig. 1. Experimental setup. (a) Schematic of the FMT/MRI instrumentation. (b) Side view of the sample platform. (c) Bottom view of the detector PCB. (d) Sketch of the SPAD architecture.



Stuker et al, IEEE Trans. *In press*(2011)

High scattering samples

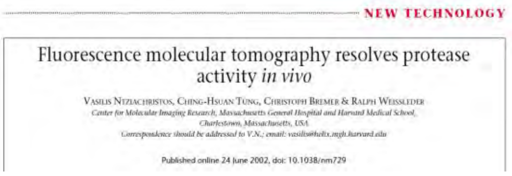
EXAMPLES

Fluorescence Molecular Tomography (FMT)

High Scatte

History of FMT

- Evolved from Diffuse Optical Tomography (DOT), in fluorescence mode also termed f-DOT developed by *A. Yodh, B. Chance, B. Pogue, S. Arridge, J. Schotland*, amongst others during the 90's.
- Developed by *V. Ntziachristos* in the context of Molecular Imaging as FMT in 2002.



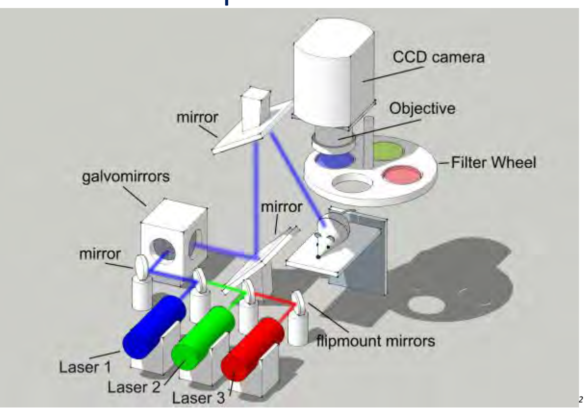
NEW TECHNOLOGY

Fluorescence molecular tomography resolves protease activity *in vivo*

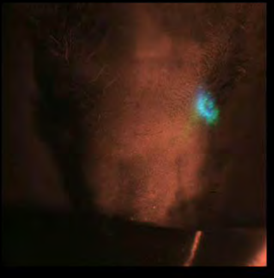
Vasilis Ntziachristos, Ching-Hsuan Tung, Christoph Bremer & Ralph Weissleder

Published online 24 June 2002, doi: 10.1038/nm729

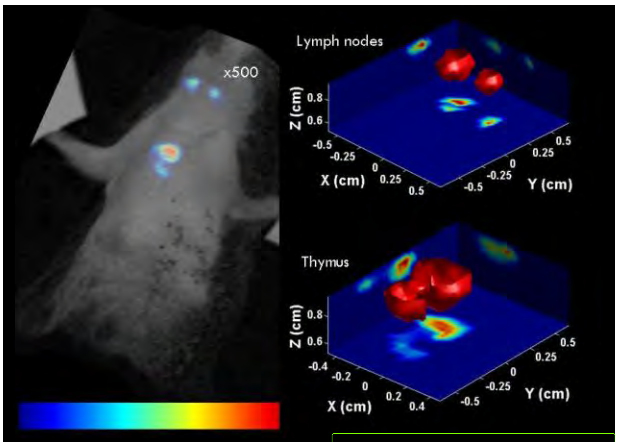
FMT Setup at FORTH & ETH



Raw Data – GFP exc/emm



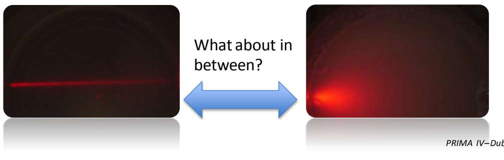
FMT Data: J. Aguirre, A. Martin (FORTH)
GFP Mouse: C. Mamalaki (FORTH) and D. Kioussis (MRC)



A. Martin, J. Aguirre, Mol. Img. (2008)

In Summary

- Account for Scattering using appropriate model
- In low scattering conditions: ***ballistic propagation*** (OPT, SPIM) and traditional microscopy
- In high scattering media: ***diffusive propagation*** (FMT)



Funding
E.U. Integrated Project "Molecular Imaging"
E.U. EST – MOLEC IMAG
Bill & Melinda Gates Foundation
E.U. Collaborative Project „FMT-XCT"

FORTH
Institute of Electronic Structure and Laser

Oii group

IMBB

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Former members
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Udo Birk (Post-doc)
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Anna Planas and Abraham Martin*
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Dimitris Kioussis

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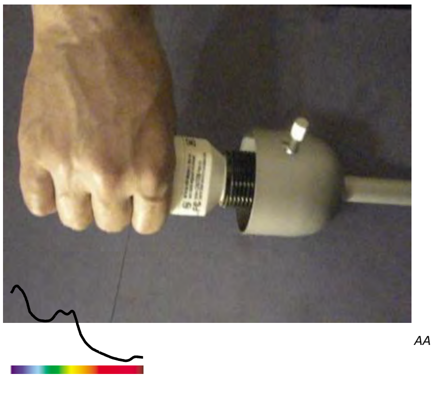
Nektarios Tavernarakis
Matthias Rieckher (PhD student)

FLEMING
Stephan Oehler and Babis Savakis

C.A.S. & Xidian University
Dong Di, Shouping Zhou and Jie Tian

ETH Zurich (AIC)
Markus Rudin, Florian Stuker

Blood Absorption

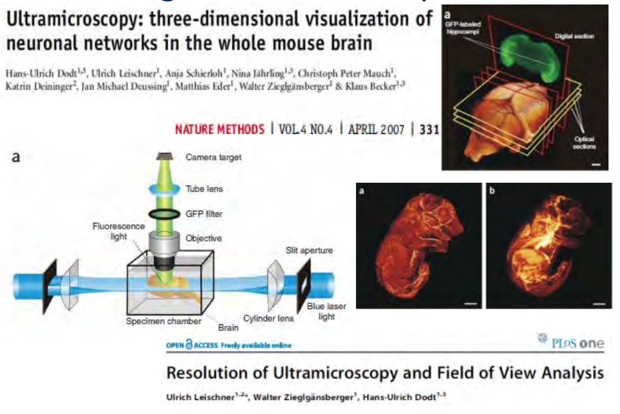


AA & JR 2009

Note: About the Quantitative Values of Absorption

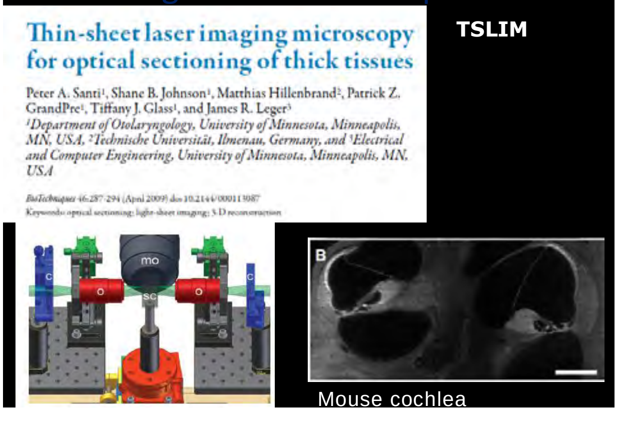
Throughout this part you might have noticed that all values presented deal with the main absorbers present in tissue, but the average absorption properties of tissue itself have not been presented. As a matter of fact, even though we do know the absorption spectra of most components present in tissue, each tissue/organ has a completely dierent combination of these absorbers not only from subject to subject, but within the same subject if measured at dierent times. Placing this into context, the total absorption due to blood will depend on the total blood volume present (which we do not know), and on its oxygenation state (which we do not know either). We might `assume' some parameters as an indication (i.e. we do know what organs contain more blood), but the truth of the matter is that we do not know optical properties of whole tissue *a priori, even if we might know their anatomical* distribution. Fitting for the actual *in-vivo values is still a matter of research, and a* quick search through the literature reveals huge discrepancies between the assigned values.

Light Sheet Techniques

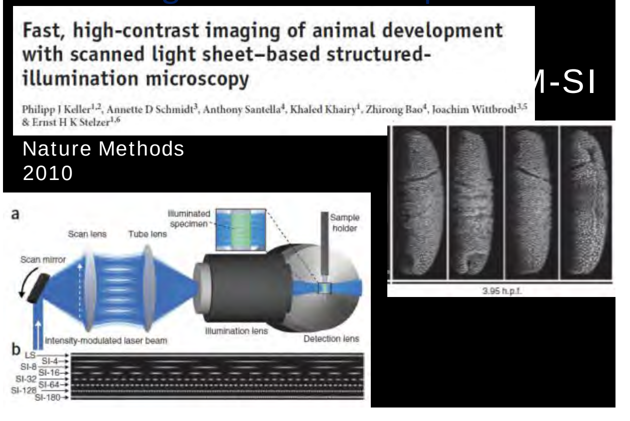


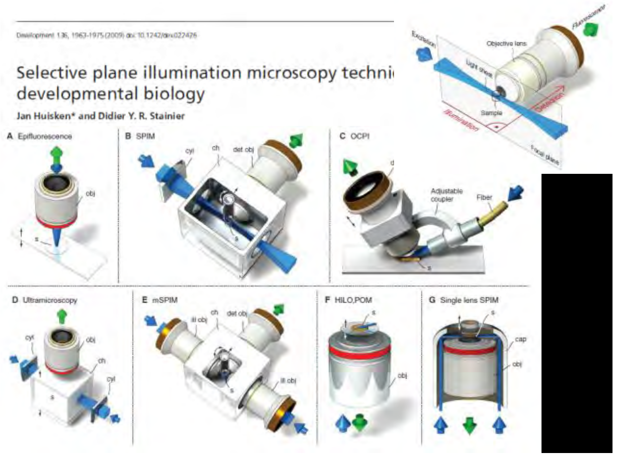
Light Sheet Techniques

Light Sheet Techniques



Light Sheet Techniques





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Some extra interesting stuff

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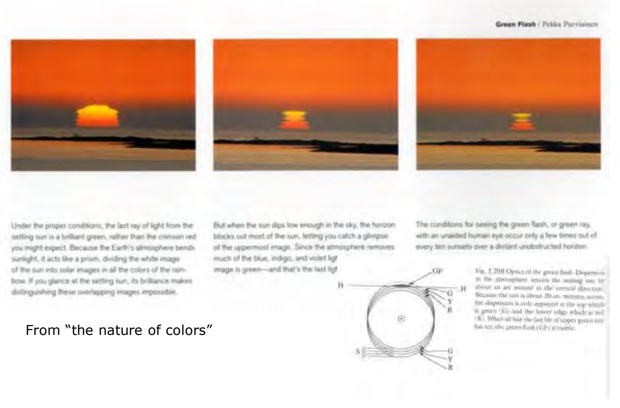
The Green Flash



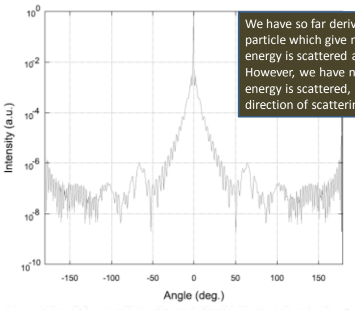
From “the nature of colors”

Huge Moons

Doesn’t the moon look larger when close to the skyline (buildings, for example) than when its up in the sky? Its an optical illusion!

Fig. 7.10A Time exposure of the rising full moon. Departure from the horizon is noticeable in the loss of reddening. Counter to the moon illusion, we find no size change in this photograph.

Scattering Anisotropy



We have so far derived two main properties of the particle which give magnitudes related to how much energy is scattered and how much is absorbed. However, we have no information on *how* this energy is scattered, i.e. if there is a preferential direction of scattering

Fig. 1.13 Approximation of what the angular intensity distribution would look like for a fat cell, assuming it is a sphere of radius 50 times the wavelength of the incident light (i.e. 80μm diameter for a 800nm incident wavelength) with index of refraction 1.4 surrounded by water.

Scattering Anisotropy

This information on the average *angular* distribution of energy is provided by the *Anisotropy Scattering Factor*, *g*:

$$g =< \cos\theta >= \frac{\int_S |\langle \mathbf{S}^{(sc)} \rangle| \hat{\mathbf{s}} \cdot \hat{\mathbf{s}}_0 \mathrm{d}S}{\int_S |\langle \mathbf{S}^{(sc)} \rangle| \mathrm{d}S}$$

Which is simply the averaged cosine of the scattered angle.

Scattering Anisotropy

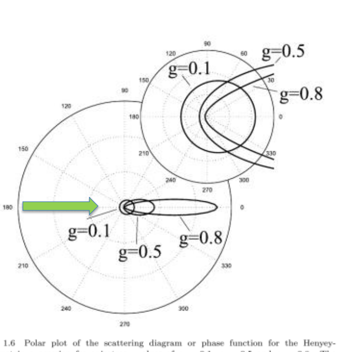
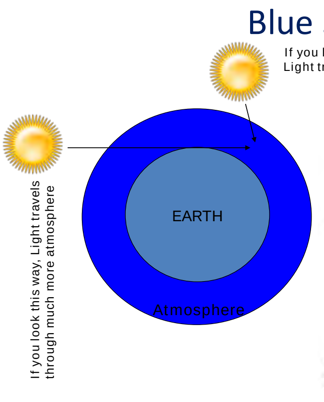


Fig. 1.6 Polar plot of the scattering diagram or phase function for the Henyey-Greenstein expression for anisotropy values of g = 0.1, g = 0.5 and g = 0.8. The inset shows in greater detail the difference for backscattering angles (note that light is incident from the left).

Blue Sky



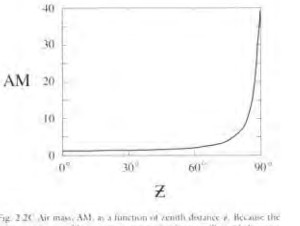
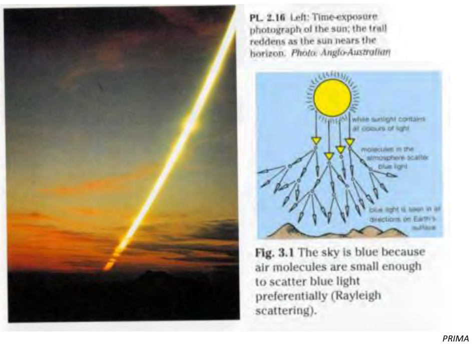


Fig. 2.2C Air mass, AM, as a function of zenith distance z. Because the air mass varies roughly as secant z, it is near unity at small zenith distances and slowly changes until zenith distances of greater than about 45°

And what happens with light at the atmosphere?

Rayleigh scattering primarily occurs through light's interaction with air molecules
Some of the scattering can also be from aerosols of sulfate particles



PL. 2.16 Left: Time-exposure photograph of the sun; the trail reddens as the sun nears the horizon. *Photo: Anglo-Australian*

Fig. 3.1 The sky is blue because air molecules are small enough to scatter blue light preferentially (Rayleigh scattering).

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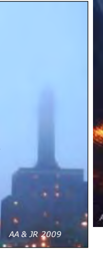
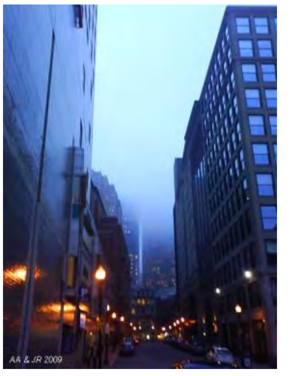
Scattering

Fog





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Sand Storm

So, multiple scattering (and absorption and high reflectivity for certain wavelengths) explain therefore the color of a sand storm:

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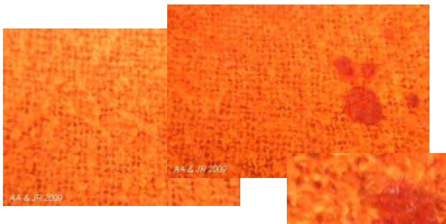
Why does fabric (like your jeans for example) look darker when you spill water on them?



Water actually just gets rid of all the "hairiness" in fabric. Therefore less light gets scattered and in contrast looks darker. Light can also penetrate deeper in fabric when wet, since less is lost on the way.

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Light Emission

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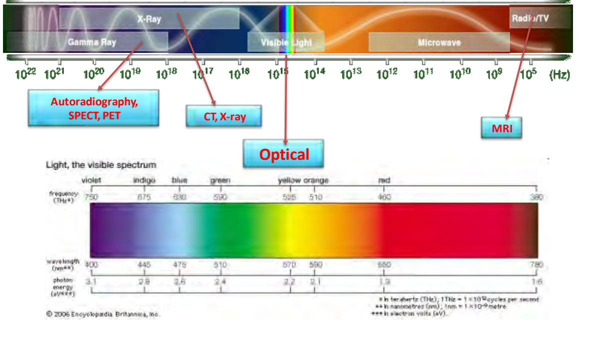
Radiative Energy Spectrum



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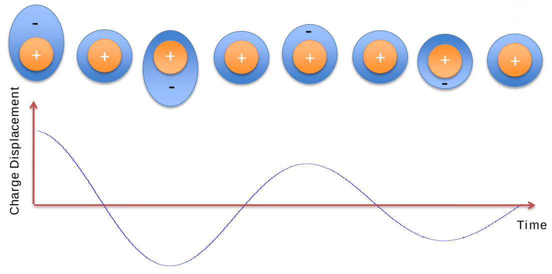
Light Emission

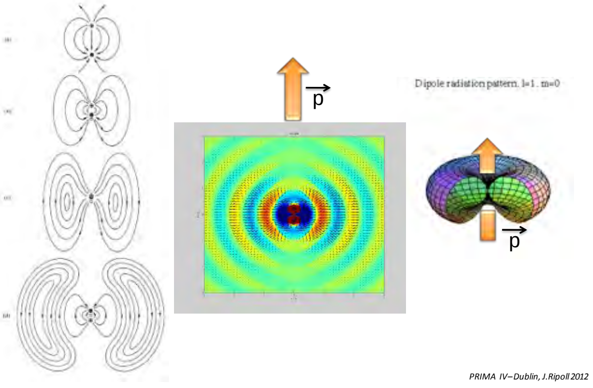
Imagine the evolution of the excited state to the ground state as a damped spring:



The frequency of this oscillation only depends on the difference between energy levels, and as shown before it is given by Plank's relation:

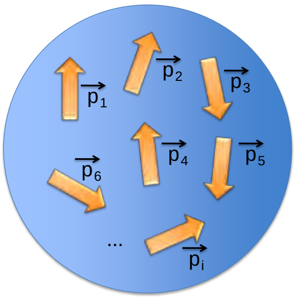
$$\upsilon = E_{emission}/h$$

Emission from a Dipole



Light Emission

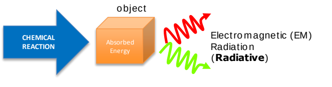
So far we approximated how the atom/molecule will de-excite emitting radiative energy and how this de-excitation can be approximated to an oscillator with a specific dipole moment. Consider now a collection of these atoms/molecules…



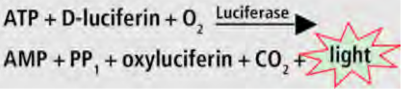
Light Emitted from this collection of randomly-oriented dipoles will be **incoherent**

Chemoluminescence

If the source of energy is *chemical*, we have *Chemoluminescence:*



In the specific case when this is produced by a *living organism* we have *Bioluminescence:*



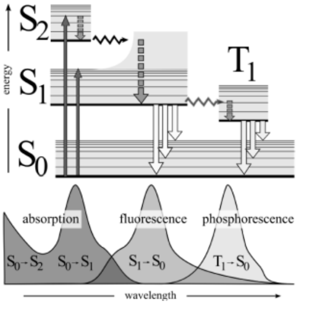
Light Emission

Bioluminescence therefore explains:

Photinus pyralis

Fluorescence

In the case of *Fluorescence*, if the emission is from a *triplet state*, we have *Phosphorescence*:

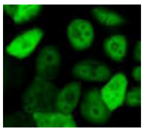


Phosphorescence is persistent fluorescence since the T_1 –S_0 is (in principle) prohibited and is possible due to spin-orbit coupling. Due to this reason, Fluorescence life-times are in the order of nanoseconds, whereas Phosphorescence life-times can be in the order of seconds or even more.

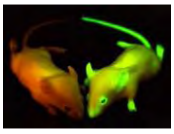
Fluorescence

Important note: In order to see Fluorescence, we need a filter to "remove" the excitation light.

Fluorescence, is capable of explaining*:*



Green Fluorescent Protein (GFP) - expressing cells



GFP-expressing mice

And here is the culprit:

Aequorea victoria

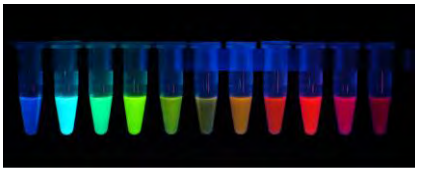
The Nobel Prize in Chemistry 2008
Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

Fluorescence

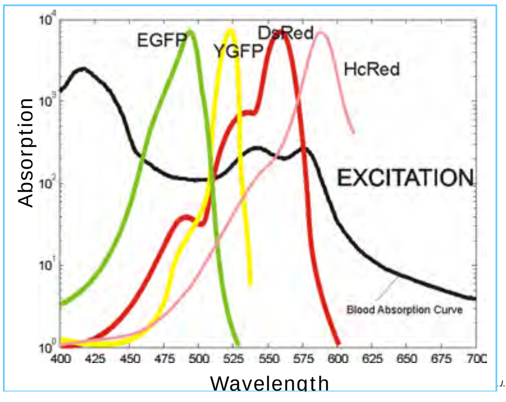
Thanks to the cloning of the GFP we have now a great number of fluorescent proteins to choose from:

Development of nerve cells. Each nerve cell expresses a combination of fluorescent proteins. Each one, needs to be visualized with its own filter.

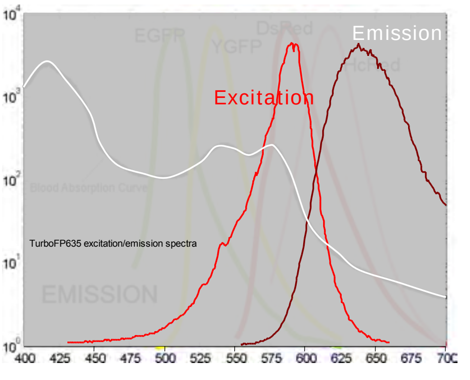
Fluorescent Proteins from Tsien's lab:



Blood Absorption

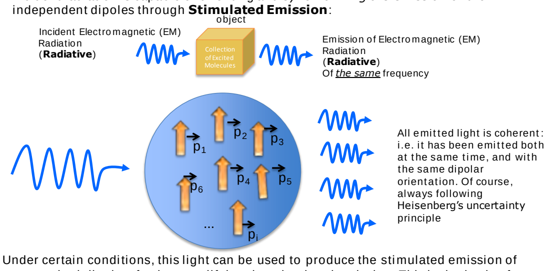


Blood Absorption



Coherent Light Emission: The laser

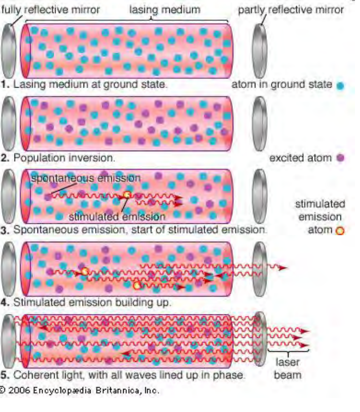
But what happens is somehow the source of energy – *once the system is in its excited state* - is capable of somehow acting on the independent dipoles in a *coherent way*? This occurs under very special conditions, but specifically, when the incident radiation is capable of orienting and *synchronizing the emission* of the independent dipoles through **Stimulated Emission**:



Under certain conditions, this light can be used to produce the stimulated emission of more excited dipoles, further amplifying the stimulated emission. This is the basis of ***the laser*** (Light Amplification through Stimulated Emission Radiation).

Light Emission

But don't forget! The Laser needs enough atoms/molecules in their excited state in order for it to work. And how are atoms excited (population inversion its termed – see Fig. 2)? Exactly as we have seen in the previous slides: either through thermal energy, electromagnetic energy or chemical energy, mainly.



Further Reading

- van de Hulst, H.C., "Light Scattering by Small Particles," Chapters 9 and 10, Wiley, New York, 1957.
- Born and Wolf, "Principles of Optics"
- P. Murphy, "The Color of Nature: An Exploratorium Book"
- Color in Nature: A Visual and Scientific Exploration by Penelope A. Farrant
- Color and Light in Nature by David K. Lynch and William Livingston
- The Physics and Chemistry of Color, by Kurt Nassau
- Living Lights, by E. N. Harvey