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:

\[
\text{Total Input Energy} = \text{Total Energy Absorbed by Object} + \text{Total Output Energy}
\]

**Light Interaction**

- **Absorbed Electromagnetic Radiation (mainly non-Radiative)**
  - Transfer of energy as heat
- **Scattered Electromagnetic Radiation (Radiative)**
  - Of the same frequency

We wish to use light to probe or image tissue.
Characteristic Sizes

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 radiation 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.

Light Scattering

Jablonski Diagram

Q: Why does fabric look darker when wet?

Interaction with a single particle
Bubbles

The Rainbow

From "the Colors of Nature"

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

The Rainbow

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

Primary Rainbow

Secondary Rainbow

Alexander’s Band

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

Quantifying scattering

And the total scattered power then is:

Total Scattered Power:

\[ P_{sc} = \sigma_{sc} || \mathbf{S}(inc) || \ (Watts) \]

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

\[ \mu_s = \rho \sigma_{sc} \left( cm^{-1} \right) \]
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?

Milk Experiment

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

- 3 cups milk
- 2 cups milk
- 1 cup water

We're effectively changing the density of scatterers:

\[ \mu_s = \rho\sigma_{sc} \]
Milk Experiment
For 1cm depth:

- 100% Milk
- 66% Milk
- 33% Milk

Analysis

Analysis: 100% Milk

R² = 0.9812

A second example

Scattering
Scattering

Beer’s Law $\sim \exp (-a*z)$

Diffusion $\sim \exp (-a*r)/r$

The Egg... from ballistic to multiple scattering

Boiling an egg

Other stuff that multiply scatters light

Sugar

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.
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 lose their pink color and fade to white. From “The Colors of Nature.”

**Light Absorption**

Additive Primaries (adding light)

Subtractive Primaries (adding absorption)

**Quantifying Absorption:**

\[
\hat{P}_{\text{abs}} = \sigma_a \| S^{(inc)} \| \left( \text{Watt} \cdot \text{s} \right)
\]

**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

\[
\mu_a = \rho \sigma_a, \quad \left( \text{cm}^{-1} \right)
\]

**Tissue Absorption**

- **Main Absorbers in Tissue:**
  - Blood
  - Water
  - Skin (melanin)
We can now characterize the optical properties

\[ \mu_a, \mu_s, g \quad (\mu_a << \mu_s) \]

Remember this is a statistical description

Optical Projection Tomography (OPT)
Slightly Scattering Tissues
Principles of OPT

J. Sharpe et al., Science 296, 2002

Radon Transform

OPT Setup

OPT Reconstruction
Merging the raw data of fluorescent and white light images combines the visualization of anatomical features and fluorescent expression patterns (A).

Green = touch sensitivity neurons :: GFP

Dopamine neurons expressing GFP

In vivo D. melanogaster

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)

Assessment of stroke-induced immune depression

C. Mamalaki, A. Planas, A. Martin

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NIMR - MRC

Dimitris Koussis
**Protocol**

**FMT** "Fluorescence Molecular Tomography-Optical Imaging"

0day 1day 2days 4days 7days

- Spleen
- Lymph nodes
- Thymus

**Blood samples**

**FACS** "Fluorescence Analyzer Cell Sorter"

**Stroke-induced immune depression**


**Hybrid Systems**

**FMT-XCT**

Imaging of atherosclerotic plaques in mice

Nahrendorf, et al, Artherosclerosis
**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. Atzchiachristos in the context of Molecular Imaging as FMT in 2002.
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)

### Blood 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 different combination of these absorbers not only from subject to subject, but within the same subject if measured at different 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**

Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain

Hans-Chris Pedd, Chad Hunkeler, Vita McIlvain, Wei-Min Chen, Jinho Nam, Kay Celma, Karien Joanne, Ivan Michael Daouass, Nathan Ziel, Fabian Dollar, and Jaffar Shaikh

**Resolution of Ultramicroscopy and Field of View Analysis**

Ute Lechene, Will Dibnah, and Ulrich Duhl

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**Light Sheet Techniques**

Orthogonal Plane Fluorescence Optical Sectioning: a technique for 2-D imaging of biomedical specimens

J. Beruart, E. Decamps, B. Blauw, and J. Bersts

Laboratory of Biological Physics, University of Leuven, Leuven, Belgium

**OPFOS**


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**Light Sheet Techniques**

Thin-sheet laser imaging microscopy for optical sectioning of thick tissues

Peter G. 14, Sue R. 14, John R. 15, Jennifer H. 15, and Jennifer R. 15

Department of Ophthalmology, University of Minnesota, Minneapolis, MN, USA; University of Heidelberg, Germany, and Helmholtz and Computer Engineering, University of Minnesota, Minneapolis, MN, USA

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**Light Sheet Techniques**

Fast, high-contrast imaging of animal development with scanned light sheet-based structured-illumination microscopy

Philipp J. Klocke, E. Dechmick, Anthony Thompson, Michael Martin, François Bank, and Michael Wobbrock

Nature Methods 2019

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Some extra interesting stuff
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 it's up in the sky? It's an optical illusion!

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.

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

$$
g = \frac{\int_0^\infty \frac{S(\theta)}{|S(\theta)|} |\mathbf{S} \cdot \mathbf{S}_0| dS}{\int_0^\infty \frac{|S(\theta)|}{|S(\theta)|} dS}
$$

Which is simply the averaged cosine of the scattered angle.

Blue Sky

If you look this way, light travels through less atmosphere.
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.

**Sand Storm**

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

*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.

**Light Emission**

Radiative Energy Spectrum
Light Emission

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

\[ \omega = \frac{E_{\text{emission}}}{h} \]

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

\[ \omega = \frac{E_{\text{emission}}}{h} \]

Charge Displacement

Time

Emission from a Dipole

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:

Bioluminescence therefore explains:

Photinus pyralis

Fluorescence

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

Fluorescence life-times are in the order of nanoseconds, whereas Phosphorescence life-times can be in the order of seconds or even more.

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.

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:

Chemoluminescence therefore explains:

Photinus pyralis

Fluorescence

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

- Green Fluorescent Protein (GFP) expressing cells
- GFP-expressing mice

And here is the culprit:

Aequorea victoria

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

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

**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**:

- Collection of Excited Molecules
- Emission of Electromagnetic (EM) Radiation (Radiative)

Of the same frequency

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 is 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

- 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