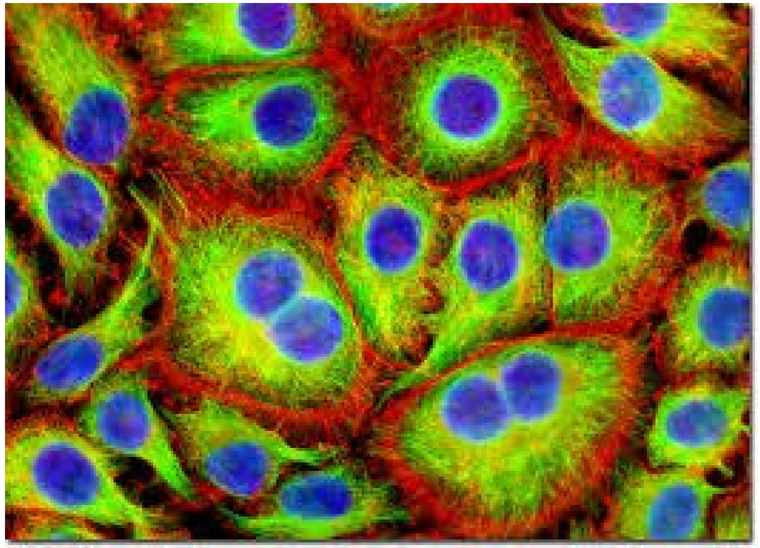
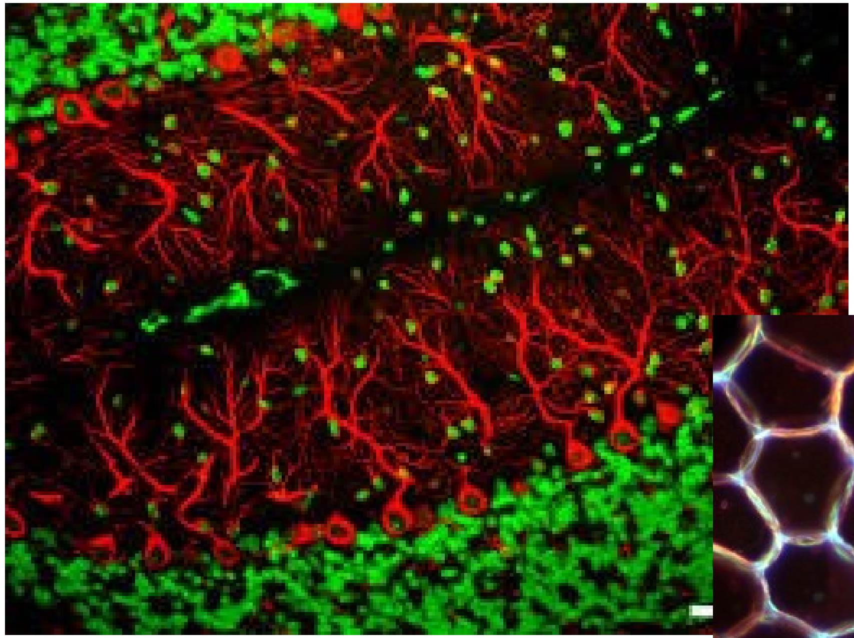
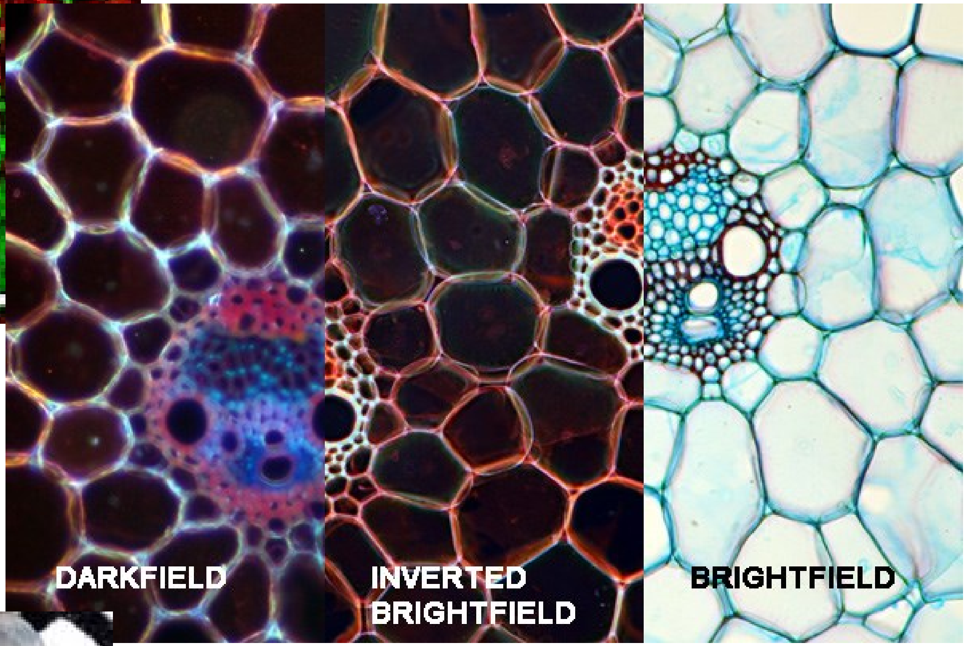
MCDB 4312/5312
Quantitative Optical Imaging

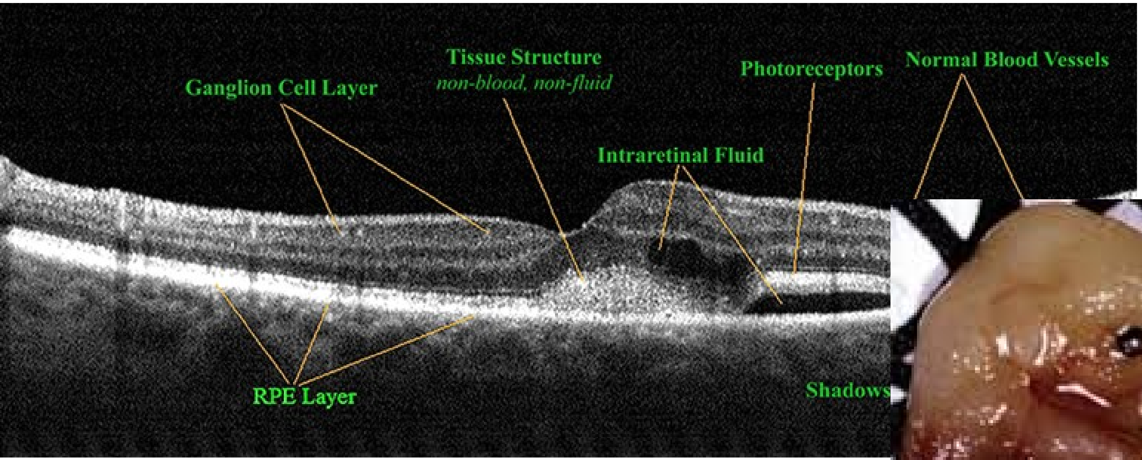
Prof Joel Kralj

Fall 2017






DARKFIELD
INVERTED
BRIGHTFIELD
BRIGHTFIELD


Tissue Structure
non-blood, non-fluid
Ganglion Cell Layer
Photoreceptors
Normal Blood Vessels
Intraretinal Fluid
RPE Layer
Shadows

Course Goals

1. Understand physics of light and optics
2. Explore contrast mechanisms for examine biological systems
3. Know strengths and limitations of fluorescent microscopy
4. Appreciate and understand new imaging techniques

Develop skills in Matlab to perform data analysis

Administrative

- Class held on MWF 11:30 – 12:20 in JSCBB B331
- Grading:
  - 10% Classroom discussion/attendance
  - 40% Homeworks
  - 15% Midterm 1
  - 15% Midterm 2
  - 20% Final exam

Administrative

- Midterms:
  - Sept 27th & Nov 1st
  - Midterms will cover only the immediately preceding section
- Final exam:
  - Scheduled by university registrar
  - Comprehensive across entire course
- Office hours
  - Open space in A-Wing on 3^{rd} floor (Check in A321)
  - Mon – 1-2PM, Thur – 10-11 AM
  - Instructor Email: Quant.Optics@Colorado.edu
    - This is the only email on which I will respond to class questions

Administrative

- Homeworks
  - Quantitative problems MUST HAVE work shown
  - Problem sets will consist of a theory portion and a Matlab portion
  - Homeworks must be turned in on time to receive credit
  - Email Matlab solutions to quant.optics@colorado.edu

- Discussion
  - Definitely ask questions during lectures. It is the best way of learning
  - Your questions will help me refine the course so you can learn more
  - Don’t be upset if timing prohibits me from answering them

Administrative

- Homework gestalt
  - I will ask open ended questions
  - You will have to access additional resources to find the answers
  - You are allowed to work with others in the class
  - Answers turned in must be your own

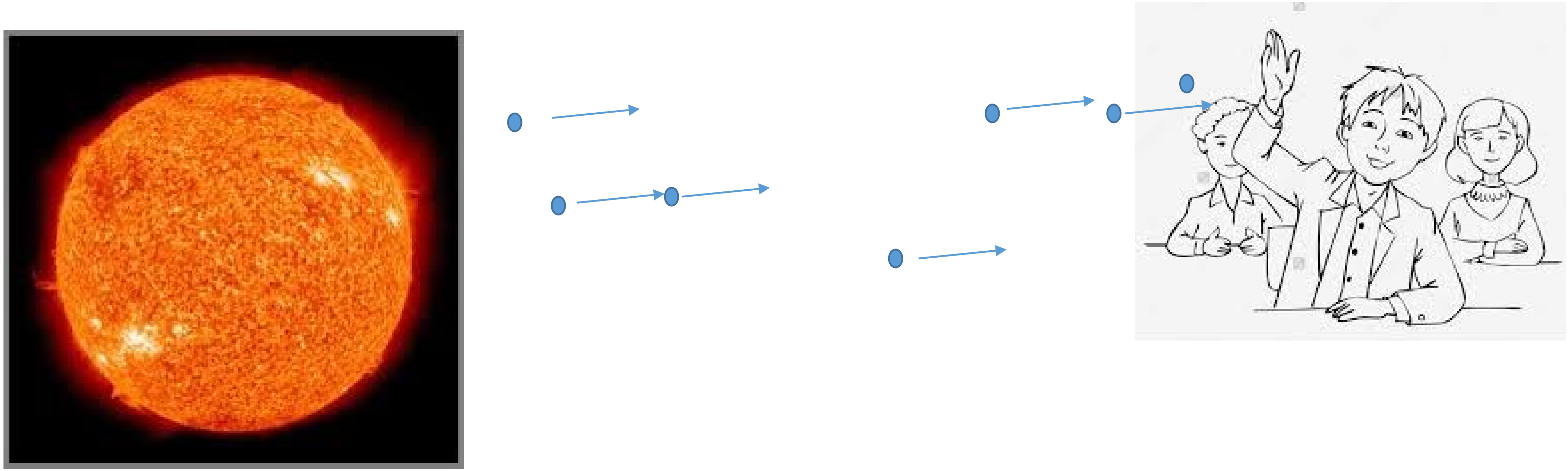
- Class pace
  - I will undoubtedly get excited, and move through material quickly
  - Please stop me and ask questions
  - Really, please stop me and ask if you have questions

Administrative

- Appropriate learning environment
  - Cell phones off
  - Cheating is wrong
- Religious leave
  - Talk to me ASAP

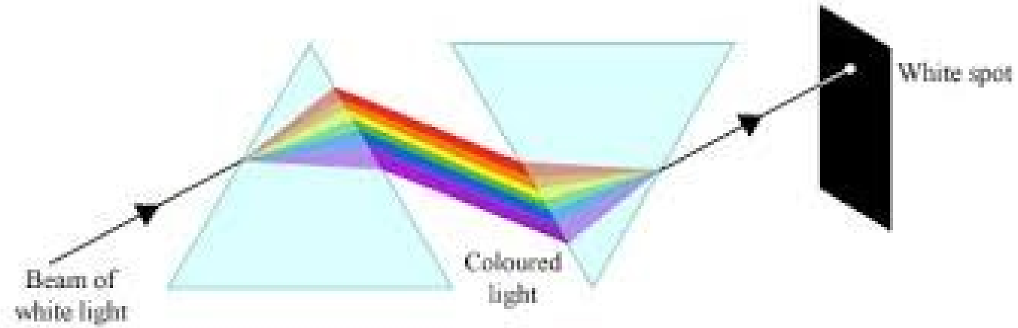
Light, as seen by the ancients

- Transfer of energy across a vacuum
- Little tiny grains of sand, that moves from the sun to us
- Light connects two separated objects



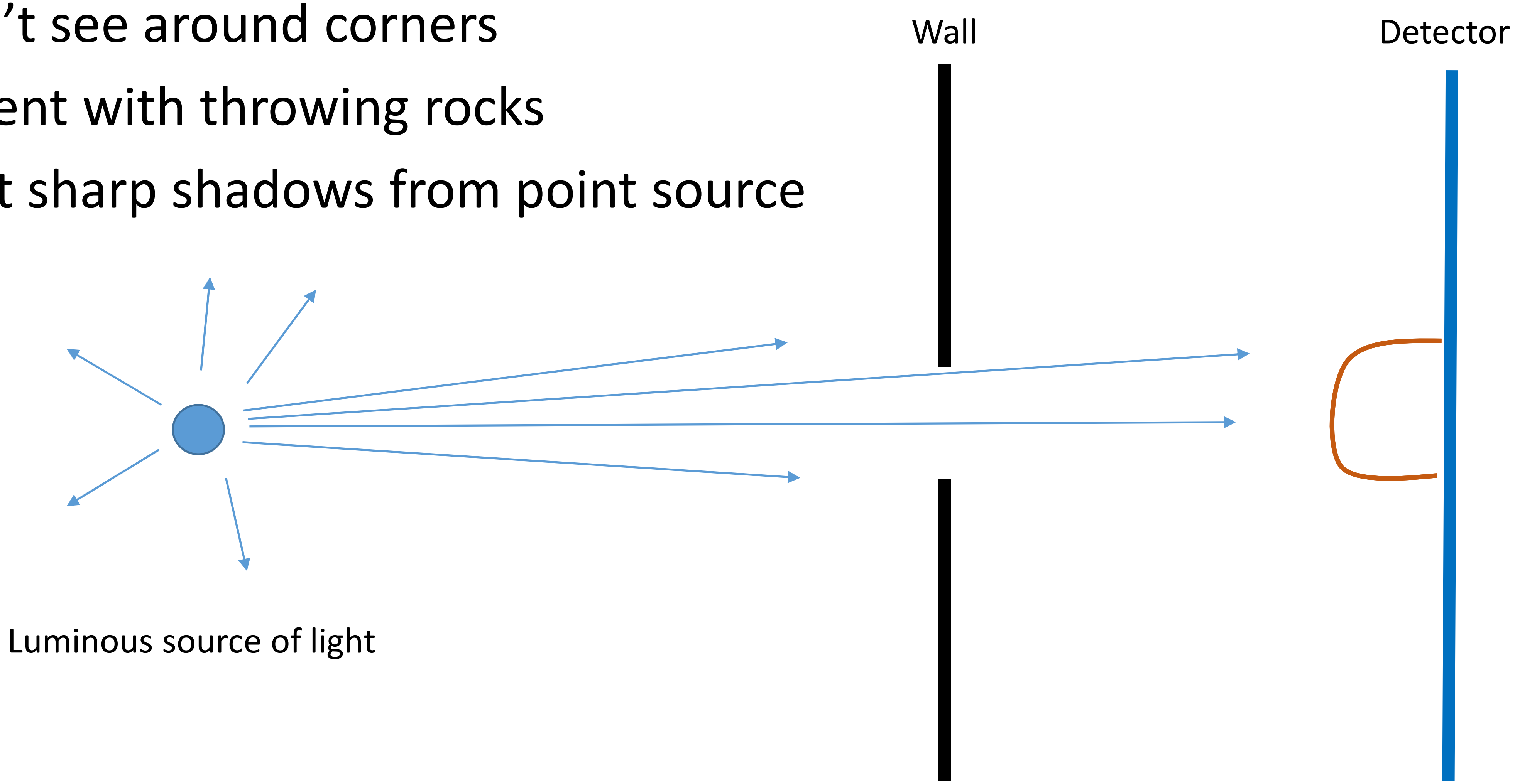
Sunlight is composed of different colors

- Newton showed that white light is composed of unique colors, spread out by a prism
- Individual colors, if isolated, don't spread into other colors
- The rainbow can be re-made back into white light
- **White light is really the individual colors combined**
- **Light interacts with matter in very specific ways**



Light travels in straight paths

- You can’t see around corners
- Consistent with throwing rocks
- You cast sharp shadows from point source

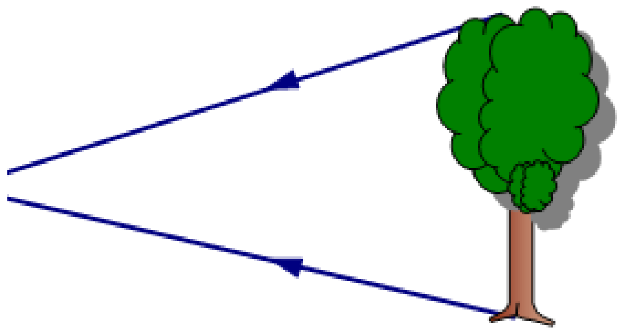


Summing up (~1750):

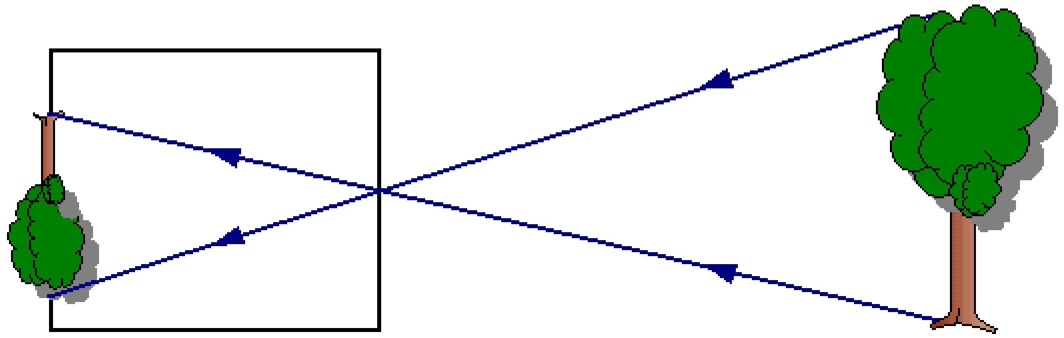
- Light carries energy (across a vacuum)
- Light energy can interact with matter
- White light is composed of different colors
- Individual colors can not be further decomposed
- Light travels in straight lines

Pinhole camera – first attempt at imaging

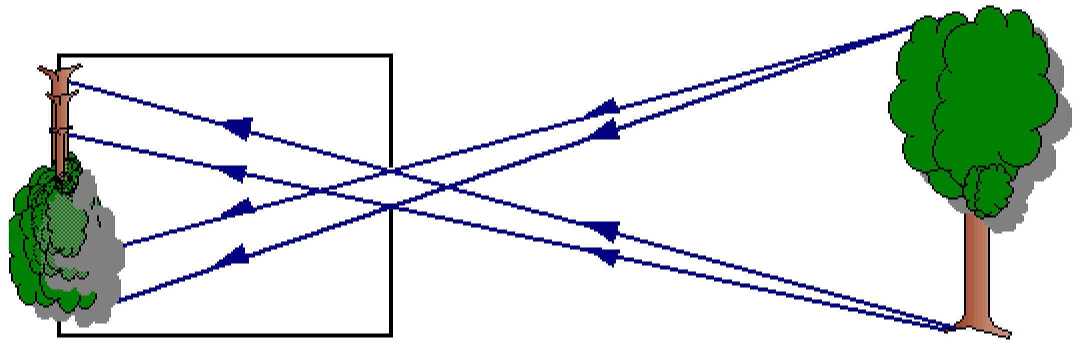
- If there's a small hole, you can project an image onto another surface
- Trade off between light in and sharpness



Clear upside down (inverted) image with a small pinhole



Clear upside down (inverted) image with a small pinhole

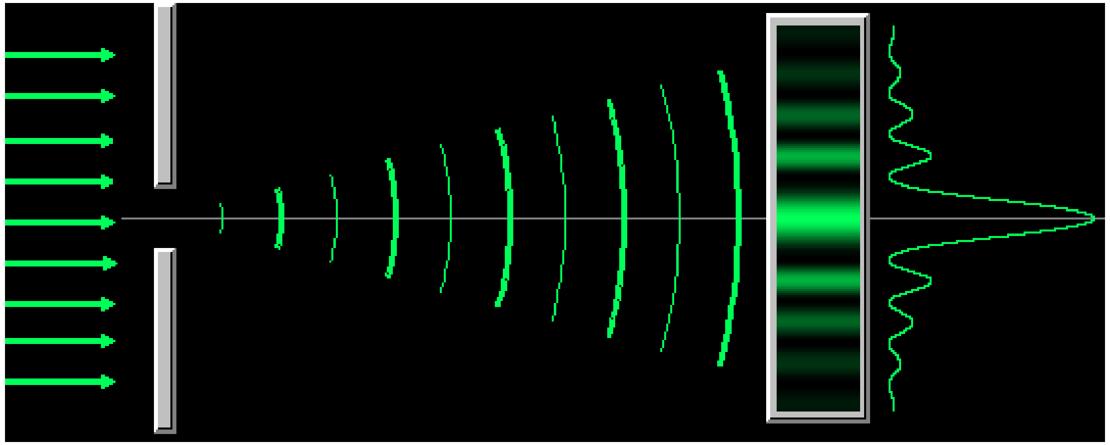


Fuzzy out of focus image with a large pinhole

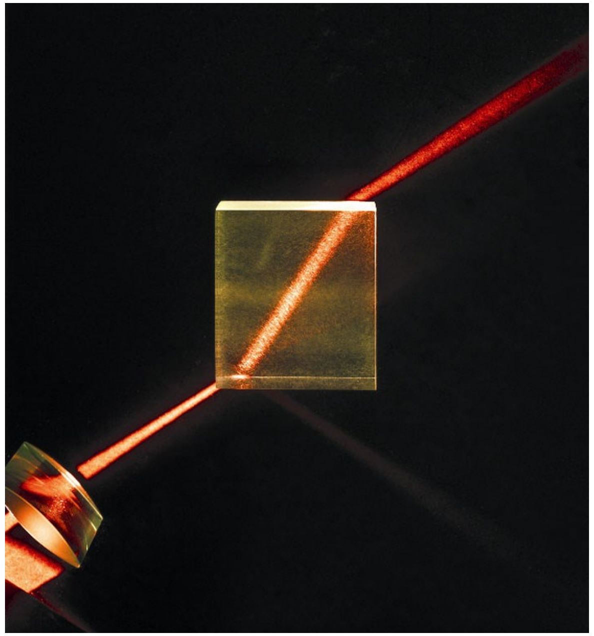
There's a problem

- Light acts like a wave
- Light undergoes diffraction, just like water or sound waves
- Light going through a small opening shows an oscillating pattern
- Light refracts, or bends, when travelling through a medium

Diffraction

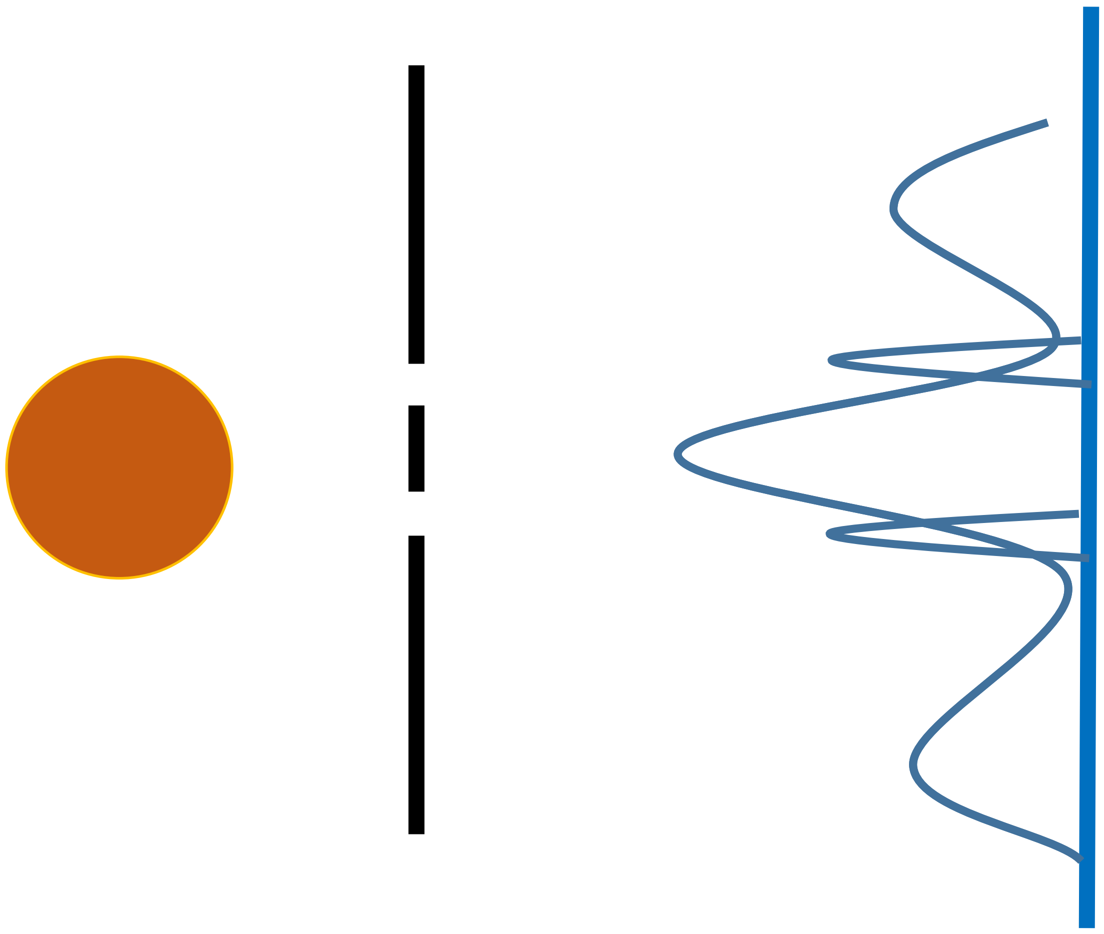


Refraction

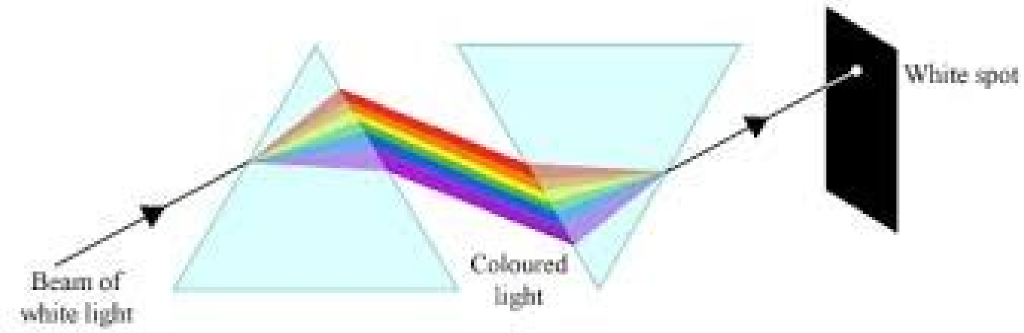


Double slit experiment

- Shows definitively that light is a wave
- Baseballs would behave like there is two independent slits
- Light forms a diffraction pattern if the slits are on the order of the wavelength



Light as a wave



- All waves have an amplitude and frequency, and they travel at a velocity dependent on the medium
- Energy of a wave is carried by amplitude, so brighter light has a bigger amplitude
- Color is carried by the frequency
- Maxwell's equations predict many properties of light, and that is a wave that travels at 3×10^8 m/s
- Light is composed of electric and magnetic fields, and can be created by moving electrons
- **Light displays many properties of waves, triumph of electromagnetic theory**

$$\nabla \cdot \mathbf{E} = \frac{\rho}{\varepsilon_0}$$

$$\nabla \cdot \mathbf{B} = 0$$

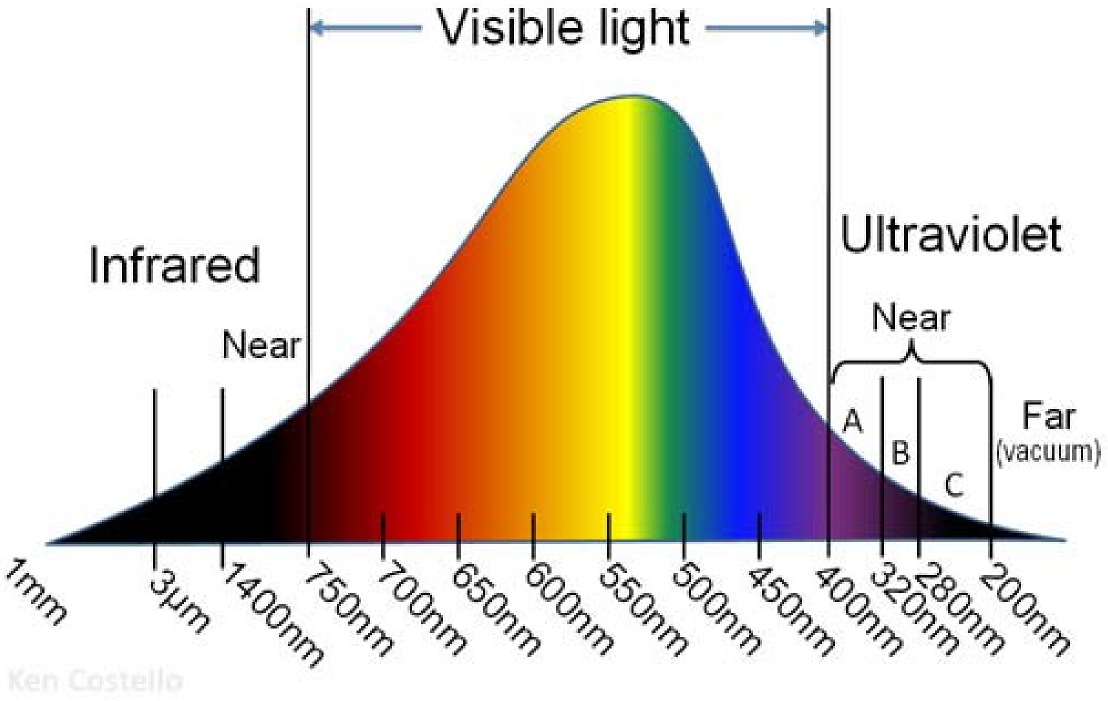
$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

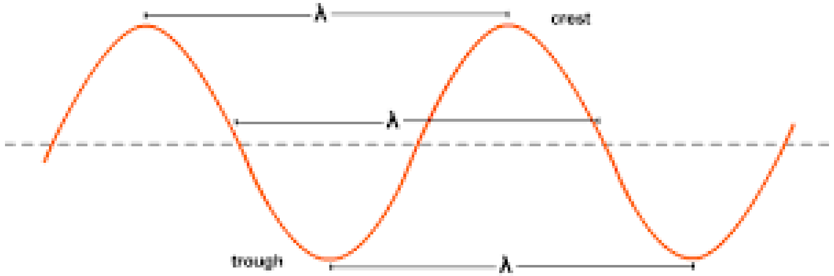
$$\nabla \times \mathbf{B} = \mu_0 \mathbf{J} + \mu_0 \varepsilon_0 \frac{\partial \mathbf{E}}{\partial t}$$

$$c = \frac{1}{\sqrt{\mu_0 \epsilon_0}} = 2.997\ x\ 10^8\ {}^{m}/_{s}$$

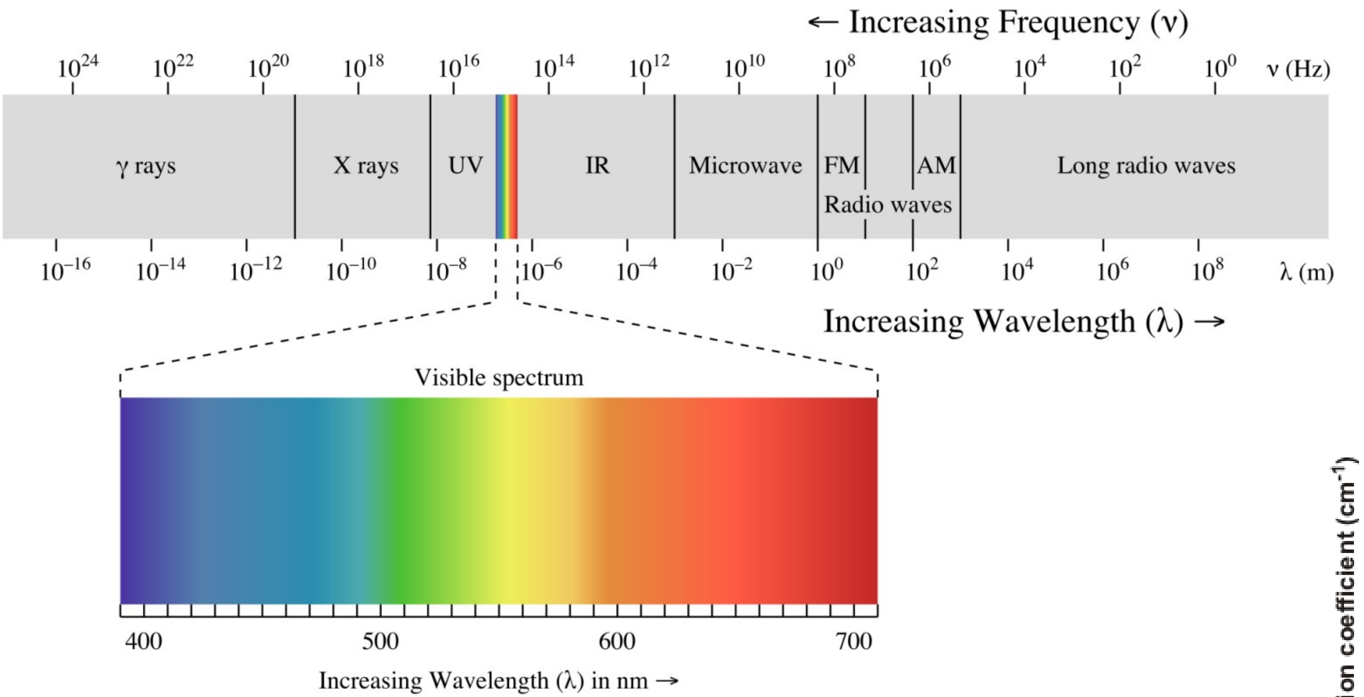
Visible light

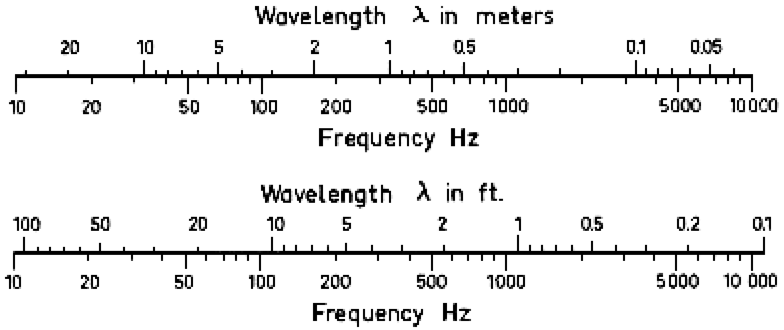
- Light is an oscillating electric and magnetic wave
- Color is defined by wavelength
- ROYGBIV
  - Red Orange Yellow Green Blue Indigo? Violet
- Spans the range from 400 nm – 700 nm

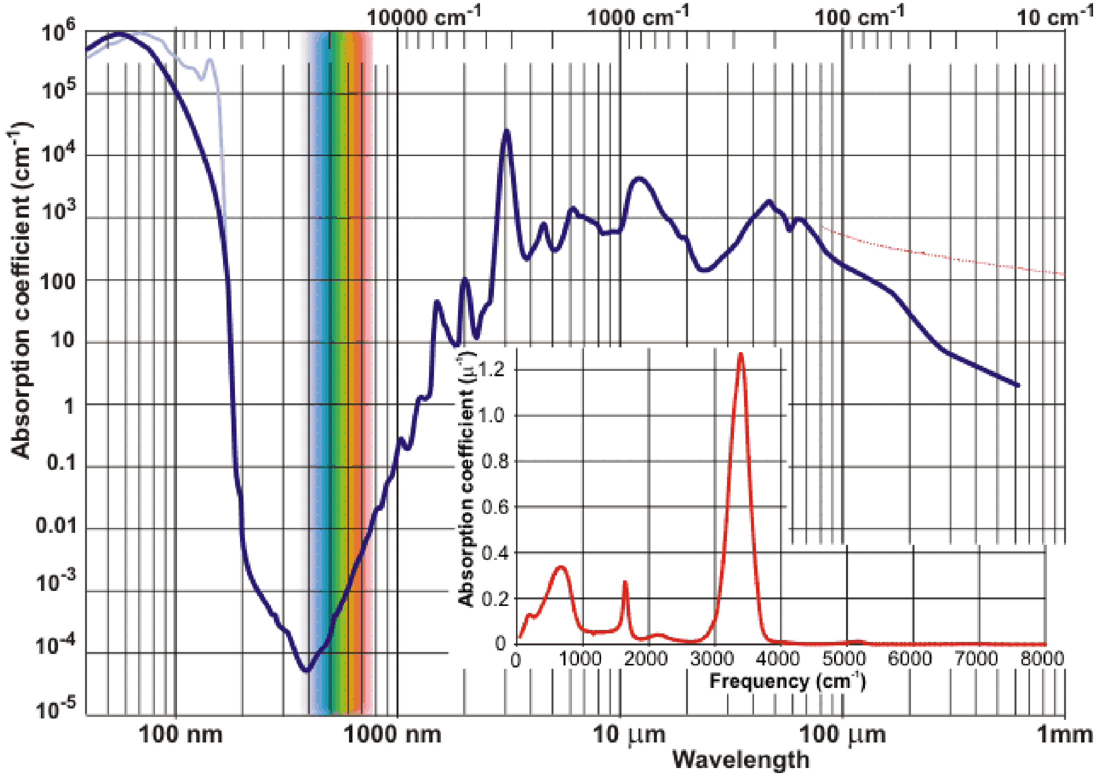




Electromagnetic spectrum







$$\nu = \frac{v}{\lambda} = \frac{c}{\lambda}$$

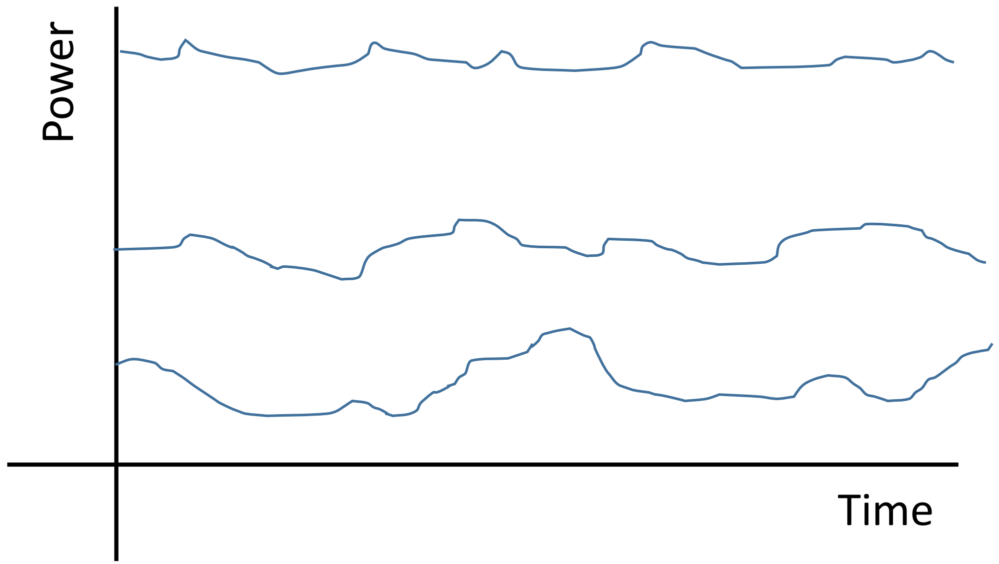
All is well, electricity and magnetism are solved, physics as a discipline starts winding down…

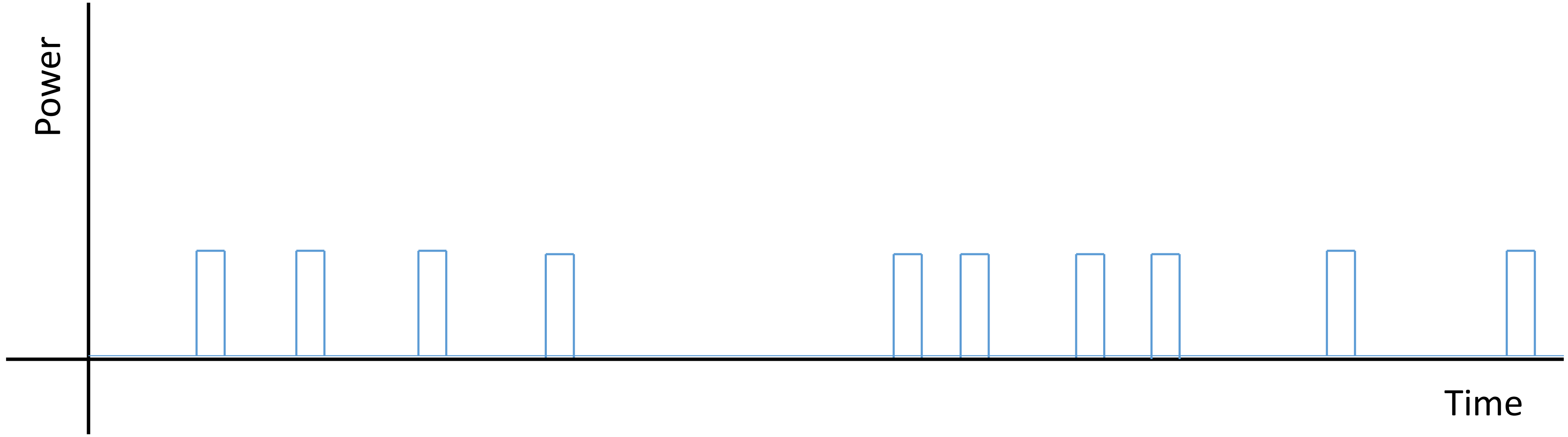
Summing up (1905):

- Light is an electromagnetic wave
- Light carries energy (across a vacuum)
- Light energy can interact with matter
- White light is composed of different colors
- Colors are defined by wavelength (and frequency)
- Light waves travels in straight lines
- Light will diffract and refract

Yet another problem

- Light energy lands on a detector
- Detector will measure for some time, return a power (energy/second)
- Imagine steadily decreasing the light intensity of the source…
- **Light comes in discrete bunches**





Lumpiness is a fundamental property of light

- All sources are lumpy: lasers, sun, candles…
- Lumpiness is not dependent on color or complexity
- Image was scanned with increasing amounts of light
- All images are formed by adding up the number of lumps that land in a particular area

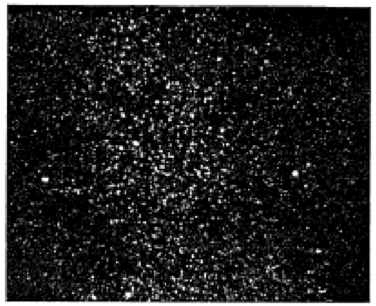


FIG. 1. Number of photons, 3×10^{3}; high-light luminance (foot-lamberts), 10^{-6}.

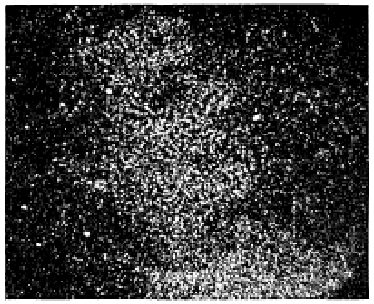


FIG. 2. Number of photons, 1.2×10^{4}; high-light luminance (foot-lamberts), 4×10^{-6}.

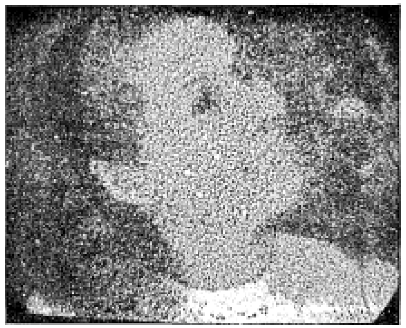


FIG. 3. Number of photons, 9.3×10^{4}; high-light luminance (foot-lamberts), 3×10^{-5}.

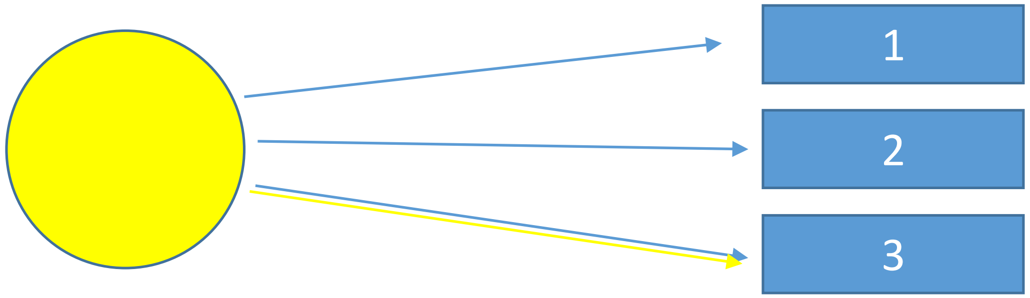
FIG. 4. Number of photons, 7.6×10^{5}; high-light luminance (foot-lamberts), 2.5×10^{-4}.

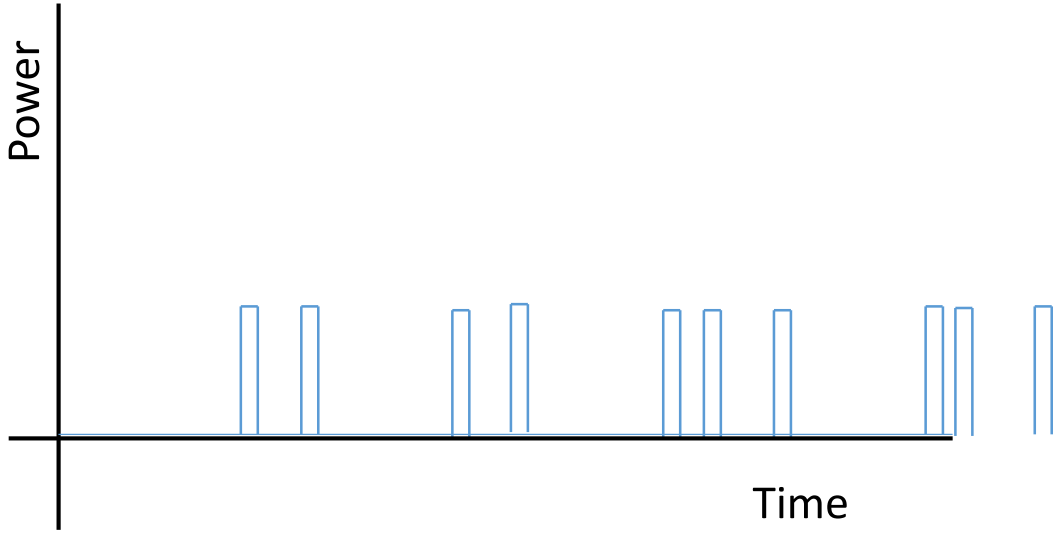
FIG. 5. Number of photons, 3.6×10^{6}; high-light luminance (foot-lamberts), 1.2×10^{-3}.

FIG. 6. Number of photons, 2.8×10^{7}; high-light luminance (foot-lamberts), 9.5×10^{-3}.

Wavelike property is localized

- Imagine a row of detectors in front of a very dim light source
- If this was a water wave, ie buoys in the ocean next to a rock that falls in, all the buoys would bob in synchrony
- Light triggers only individual detectors, with the same energy as if there was only a single detector

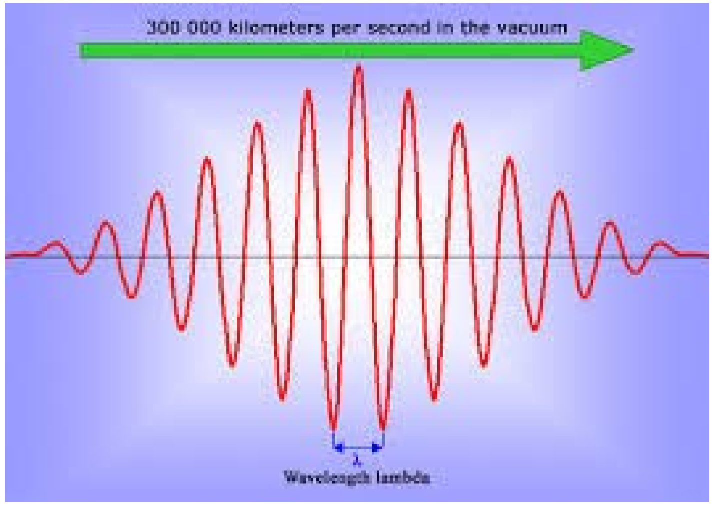




Light has both particle and wavelike features

- The double slit proved light is wavelike, but intensity proves it's lumpy
- Einstein proposed that light comes in discrete packets of energy
- Each packet energy is set by the frequency, and all packets of the same color have the same energy
- Each of these particles are called photons

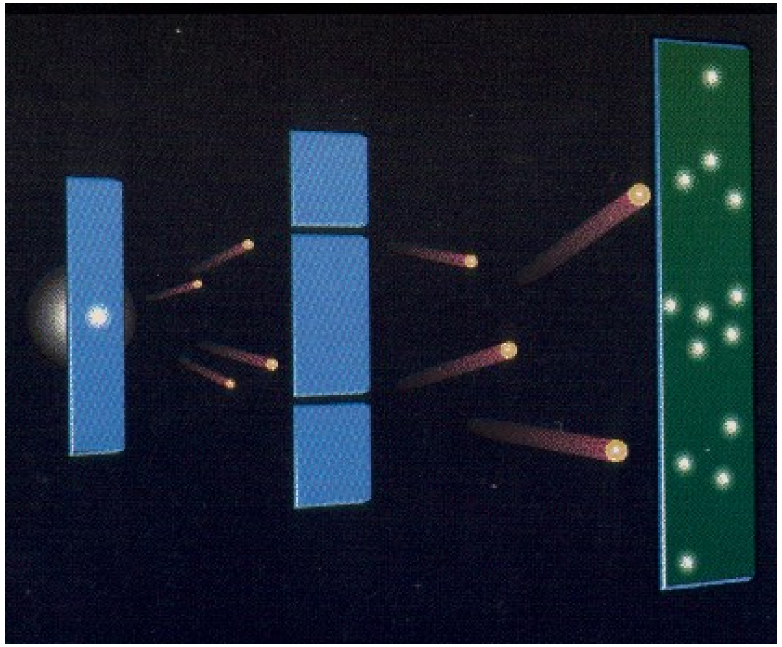
$$E = hf$$
$$= hc/\lambda$$

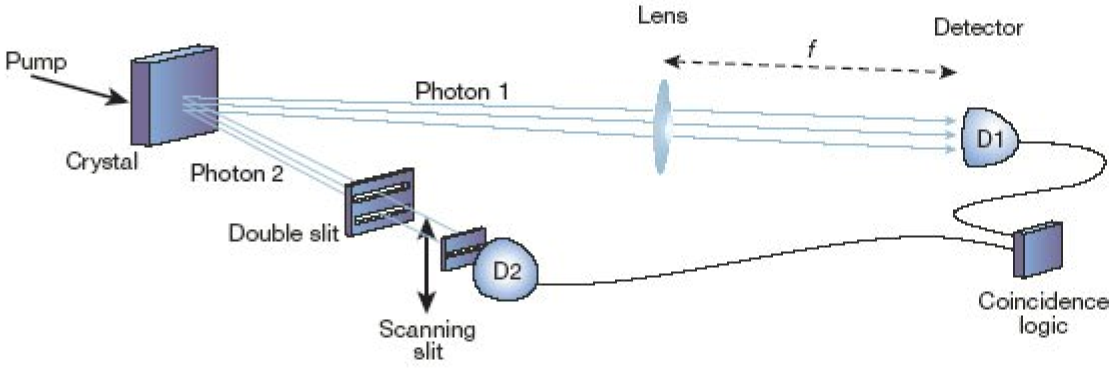


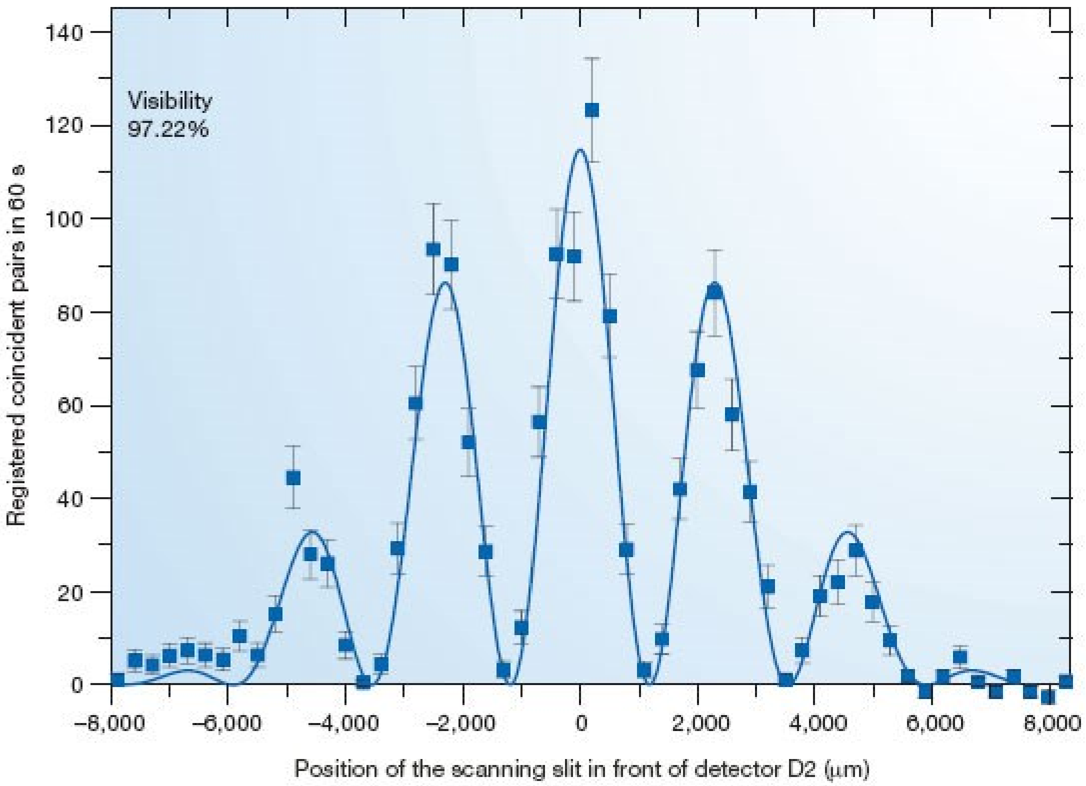
h = Planck's constant
= 6.62×10^{-34} m^2kg/s

Quantum weirdness

- A double slit experiment, even with single photons still produces a diffraction pattern
- Monitoring either slit for the passage of the photon will destroy diffraction pattern
- The act of measurement changes photons from waves to particles







Matlab tutorial

- https://oit.colorado.edu/software-hardware/software-downloads-and-licensing/matlab
- Accessing Matlab from the desktop
- Screen layout