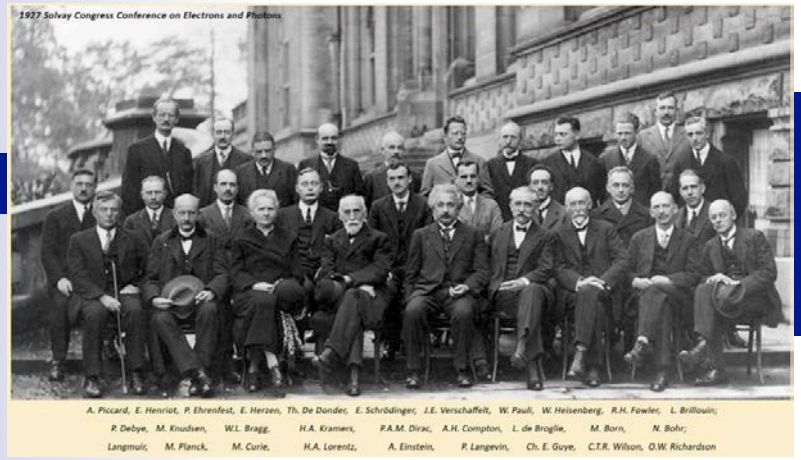
Imaging Basics: with photons/electrons WS 2015

"Small is beautiful...."

Electron Microscopy ETH Zurich (EMEZ), Roger.Wepf@scopem.ethz.ch

www.scopem.ethz.ch



"Small is beautiful...."

Electron Microscopy ETH Zurich (EMEZ), Roger.Wepf@emez.ethz.ch

- Question to audience:
- Did you use any imaging techniques?
- LM..., EM..., MRI..., PET..., X-ray....?
- Who uses Image processing?
- Who is "comfortable" with contrasting/ staining technique?
- Who is familiar with labeling techniques?
- Who can explain the duality of the probes?
- Who is familiar with the resolution range of any mode?
- Do you know the difference between frequency and wavelength...?

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Content:

- why imaging - why 3D...

- Imaging modes...

- Imaging "space"...

- Imaging principle

- Waves or particle - Duality

- Photon - no mass high speed

- Electron - mass and fast

- Imaging modalities e.g in LM and EM....& Applications

- Resolution....

- modern LM/EM imaging techniques.....& sample preparation...

Aim of Imaging is primarily:

Get information on:

- **Structure/ Morphology**
- **Chemical composition/molecular composition**
- **Regional function/ regional dynamic**

As fast as possible, non-invasive and as natural as possible along all length scale to understand structure-function and time domain of life.

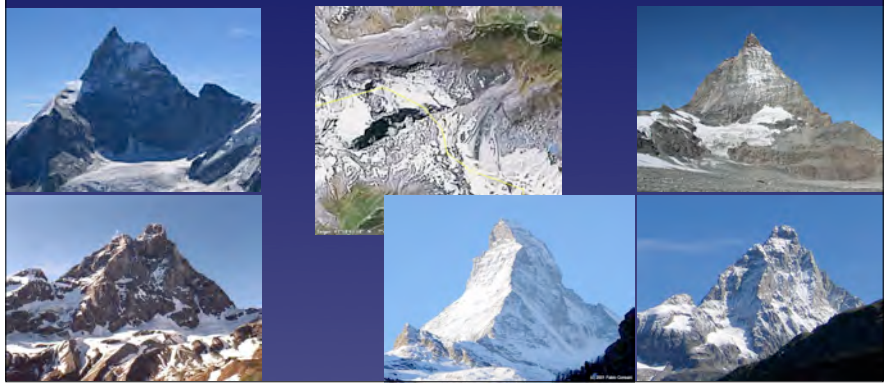
A View - A series of views...:

About 100 years ago, Claude Monet tried to image the "holistic meaning" of a Hay-Stack to be able to capture the wholeness of any object.
He therefor imaged his objects with several images taken from different angle, perspectives, time-points and light conditions etc. He was the first in Art who tried to capture the "wholeness" of an object and was first laugh at....

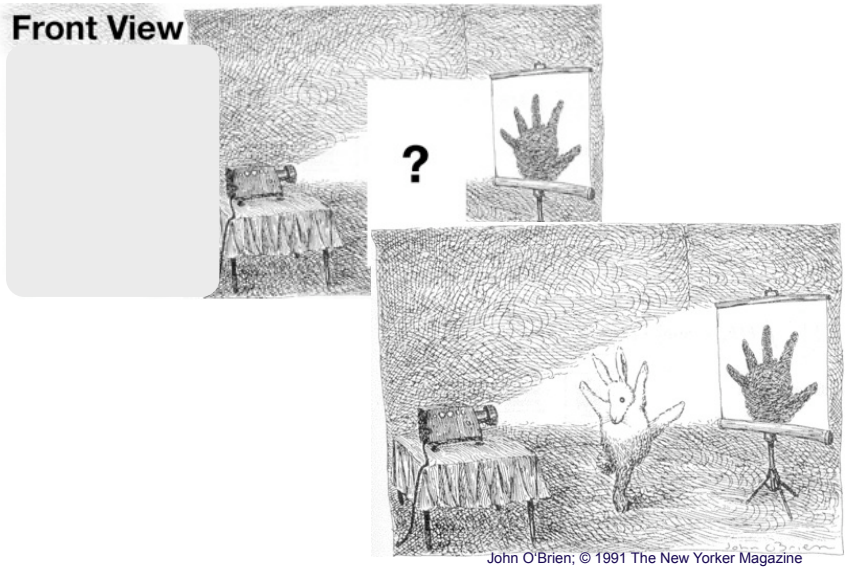
Description of the "whole".....

- A question of perspective and methodic approach...

Perspectives and the truth:
Is there **one** *only Image of a object?*



Why 3D...



John O'Brien; © 1991 The New Yorker Magazine

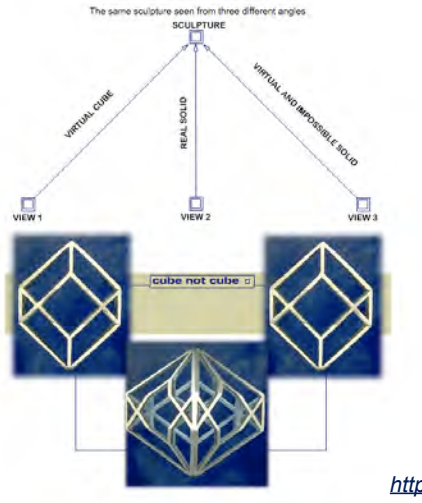
Art: “Imagination - One Image...”

Do you trust your view-point...

-> Guido Morettis Body-Worlds....

http://www.coolopticalillusions.com/artists/guido-moretti/floating-3d-cube.htm

Art: “Imagination - One Image...”



You need more than one view point -
more than one image

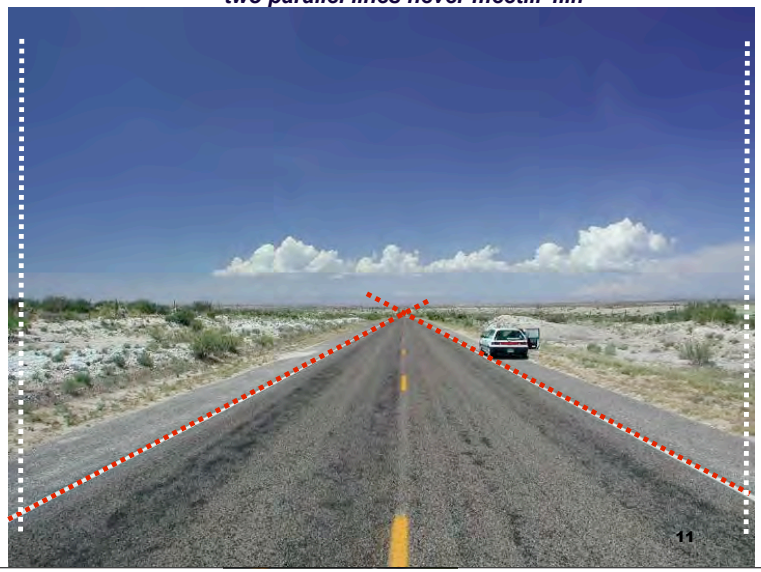
Do you trust your view-point...

-> Guido Morettis Body-Worlds....

http://www.guidomoretti.it/E_illusione.htm

http://www.coolopticalillusions.com/artists/guido-moretti/floating-3d-cube.htm

The real world fools us day by day...The “parallel-paradox”
“two parallel lines never meet...”....:



3D Imaging.....Microscopy:

- Essential to make the “Nano-Word” -
  - “visible, tangible & concrete”
- 3D required because single images may miss-lead you..
  (Viewpoint, Overlap, Irritation, Illusions, miss association....)
- 3D because the “small world” is translated to our “Thinking-Dimension”*....you can turn it and hold it...*
  - Visual perception and brain power.....

A virtual experiment: let's enlarge the world by 1 Million times:

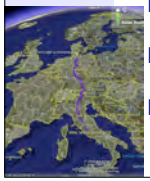
Than the real size of X becomes experienceable in our world

What:	Real Size:	Corresponding Size:	
Atom	0,1-0,3nm	0,1-0,3mm (diameter body hair)	
Protein	2-5nm	2-5mm (Rice corn)	~10^9 to 10^{10}/cell
Zytoskelett Fibers	9-25nm	9-25mm (robe)	
Ribosom	25nm	25mm (2€ piece)	~10^4 to 10^5/cell
Nanoparticle	30-200nm	30-200mm (Walnut - CD)	

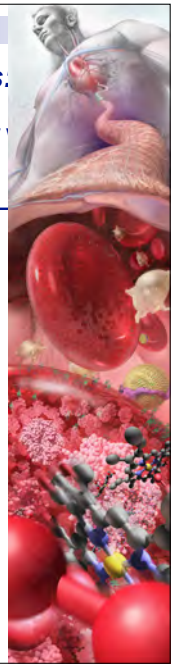
If we enlarge the world by 1 Million times:

Than the real size of X becomes experienceable in our

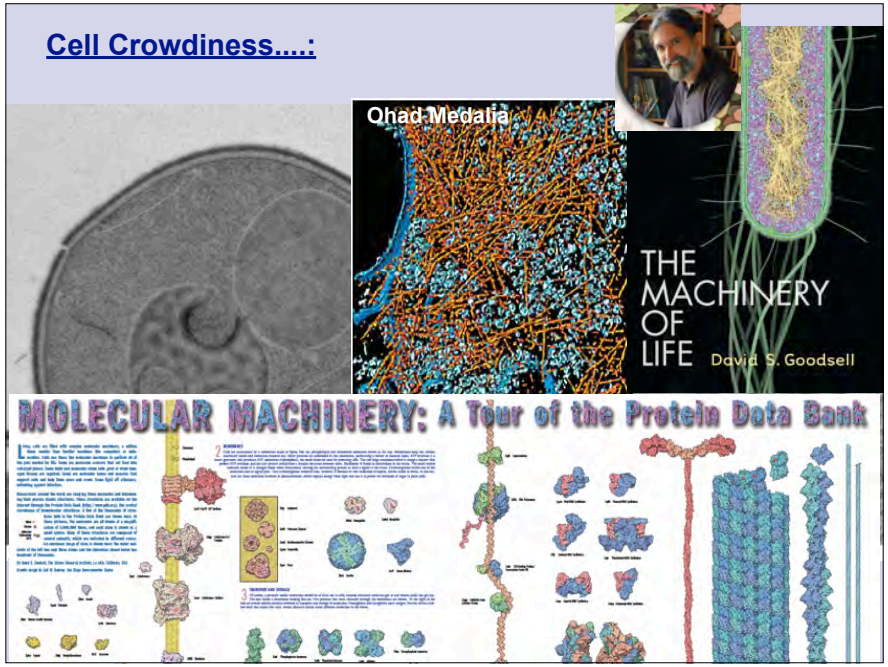
What:	Real Size:	Corresponding Size:
Atom	0,1-0,3nm	0,1-0,3mm (diameter body hair)
Virus	30-400nm	30-400mm (upto a glass bottle)
Bacteria	500-2000nm	0,5-2m
Cell nucleus	5 µm	5m
Human hair	60-100 µm	60-100m (Diameter)
Human eye	4 cm	40km
Human	1,7-1,8 m	1700-1800km



Your feet in Rom - your head in Hamburg if an Atom is just 0,1mm large



Cell Crowdiness....:



Learnings: the chemical & molecular view is not sufficient...

There is a lot of water....

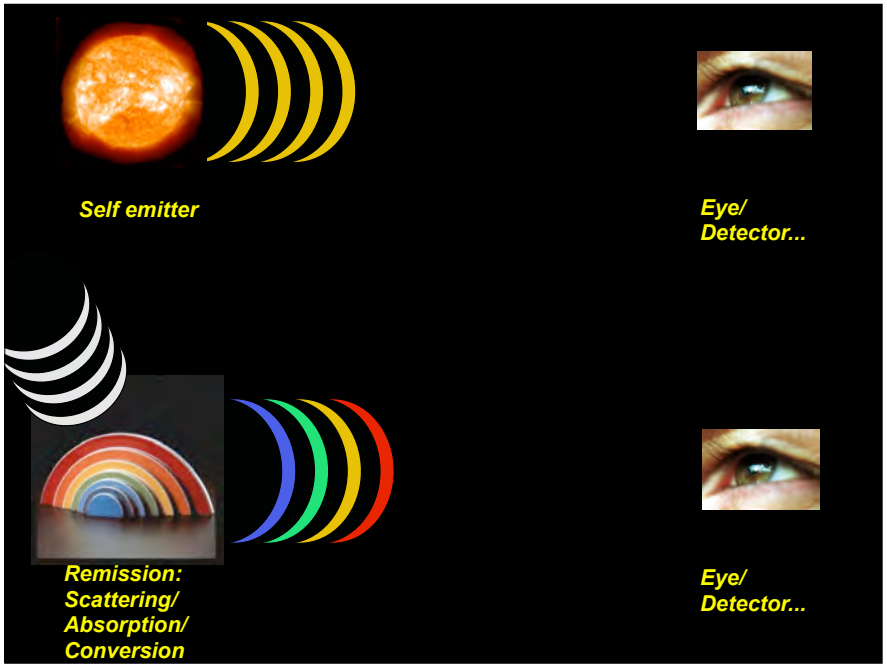
Cell Crowd... ...10^9 in scale....

ca. 80-85% H2O !

We need excellent tools & strategies to cope with all ....

1Mio time:
human body (Rom-HH)
Mitochondria (2m)

How does an image emerge or how is it formed ?

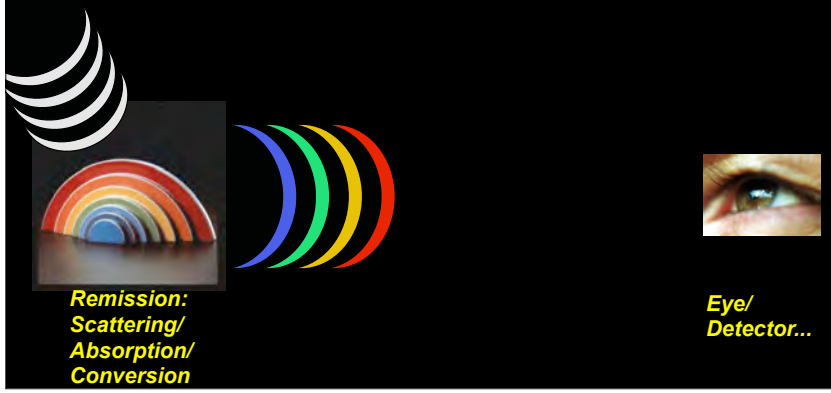


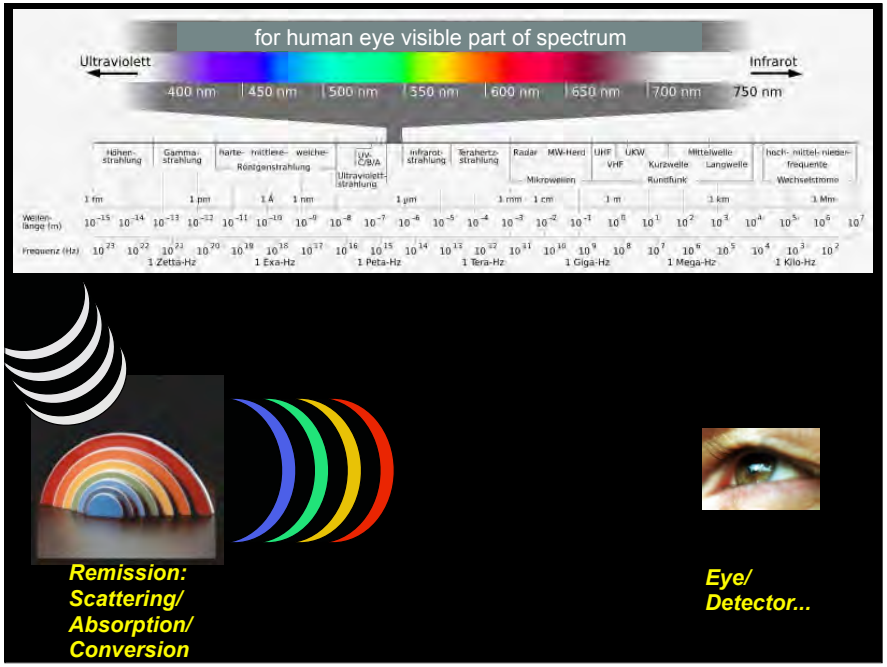
Rays (Waves):

Light (Photons - lampe, laser...)

Particle (Electrons, Ions,...)

Sound (Ultrasound, electromagnetic...)





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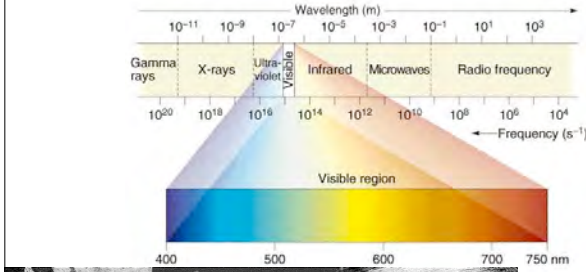
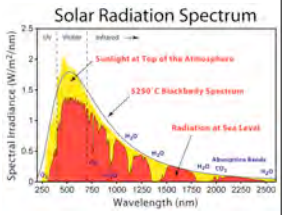
Evolution - human eye & visible spectra...





Imaging Modalities in Life-Science

MRI, MEG, EEG, fMRI

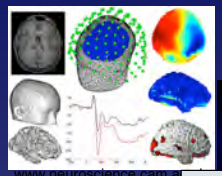
IR, PET

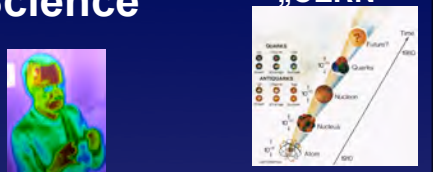
„CERN“

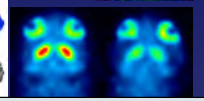
X-ray, NMR

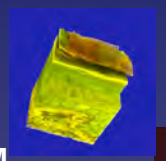
EM, IM SPM

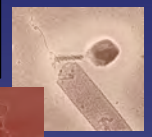
LM, CLSM





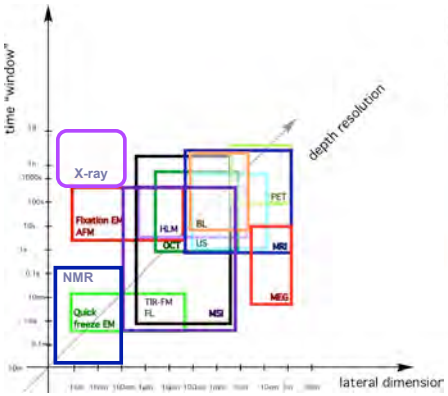


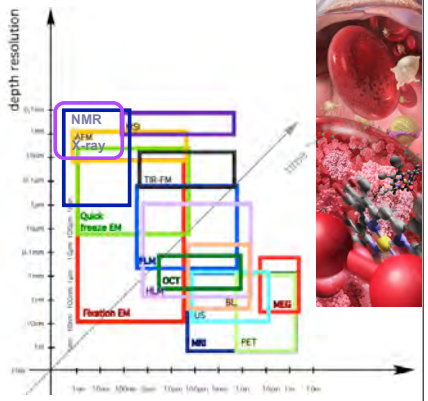




Imaging across the scales - multimodal imaging

“ Imaging Space“ - Time vs. Spacial Resolution





A dream “one tool to zoom from molecular dimension to the whole body” - can only be realized by combining different imaging modes

-> only a correlative approach allows not to get lost in details & keep the large picture „in-focus“

http://emeznas.ethz.ch/datacube2/DataCube.html

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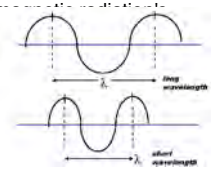
What is Electromagnetic Radiation?

Electromagnetic radiation is energy – we describe it as a wave – visible light is only a small portion

The characteristics which distinguish different types of light are the electro

- wavelength λ
- frequency f
- energy

frequency $f = c / \lambda = \frac{v}{\lambda}$,



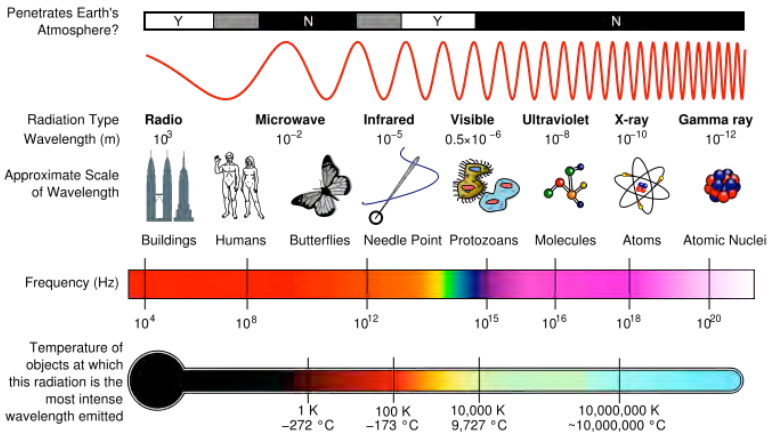
Wavelength: – the distance between two peaks (or two troughs) of the wave.
Frequency: – the number of wavelengths passing a given point in one second.

->The longer the wavelength, the more time it takes for a full wave to pass a given point (or the fewer waves pass the point in a given time).

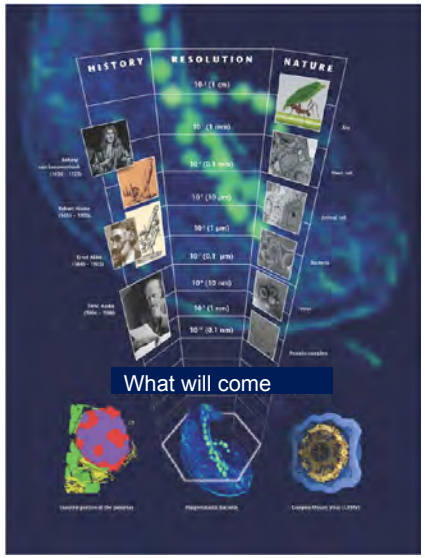
Since frequency is wavelengths per second, as the wavelength becomes longer the frequency decreases, and vice versa.

Energy: - is directly proportional to the frequency-- if the frequency increases, so does the energy of the radiation and vice versa.

The Electromagnetic Spectra.....



Wikipedia....



CLASS	FREQUENCY	WAVELENGTH	ENERGY
γ	300 EHz	1 pm	1.24 MeV
HX	30 EHz	10 pm	124 keV
	3 EHz	100 pm	12.4 keV
SX	300 PHz	1 nm	1.24 keV
	30 PHz	10 nm	124 eV
EUV	3 PHz	100 nm	12.4 eV
NUV	300 THz	1 μm	1.24 eV
NIR	30 THz	10 μm	124 meV
MIR	3 THz	100 μm	12.4 meV
FIR	300 GHz	1 mm	1.24 meV
EHF	30 GHz	1 cm	124 μeV
SHF	3 GHz	1 dm	12.4 μeV
UHF	300 MHz	1 m	1.24 μeV
VHF	30 MHz	1 dam	124 neV
HF	3 MHz	1 hm	12.4 neV
MF	300 kHz	1 km	1.24 neV
LF	30 kHz	10 km	124 peV
VLF	3 kHz	100 km	12.4 peV
VF	300 Hz	1 Mm	1.24 peV
ELF	30 Hz	10 Mm	124 feV

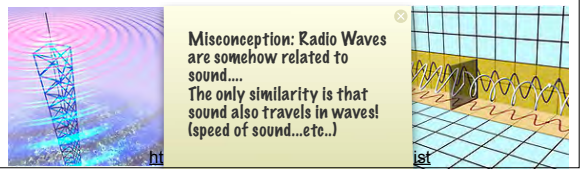
FEI; Wikipedia....

- Duality of “light” - particle or waves ?

The corpuscular properties of a particle are demonstrated when it is shown to have a localized position in space along its trajectory at any given moment.

-> Photon: The smallest (quantum) unit of light/electromagnetic energy.
Photons are generally regarded as particles with zero mass and no electric charge.

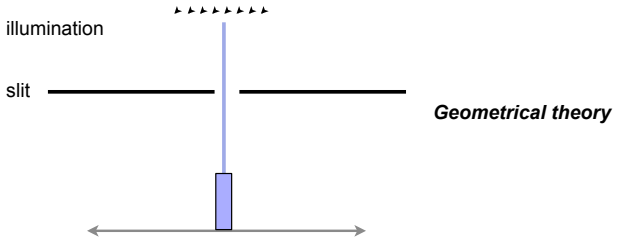
Wave-like nature is observed, for example, when a beam of light is passed through parallel slits and creates interference patterns.



- Duality of “light” - particle or waves ?

The particle concept:
a energy concept with Electronenvolt (eV) - 1eV is the kinetic energy of a charged particle which is accelerated by an electric field of 1 Volt. ....*Newton (1642- 1727)*

The wave concept:
waves with Frequenz (f), Wellenlänge (l) Wavenumber (wz) represent energy-proportional units... *Huygens (1629-1695)&Hooke (1638-1703)*



Geometrical theory

e/Slit http://www

Slit http://www.

Duality of “light” - particle or waves ?

The particle concept:

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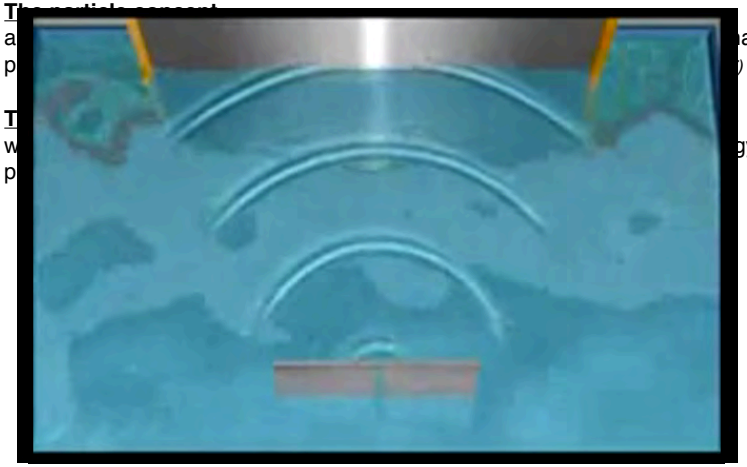

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Duality of “light” - particle or waves ?

The particle concept:

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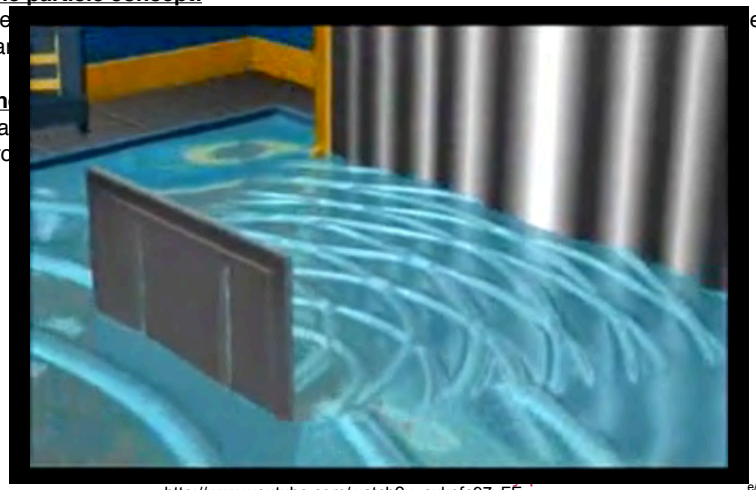


http://www.youtube.com/watch?v=oxknfn97vFE

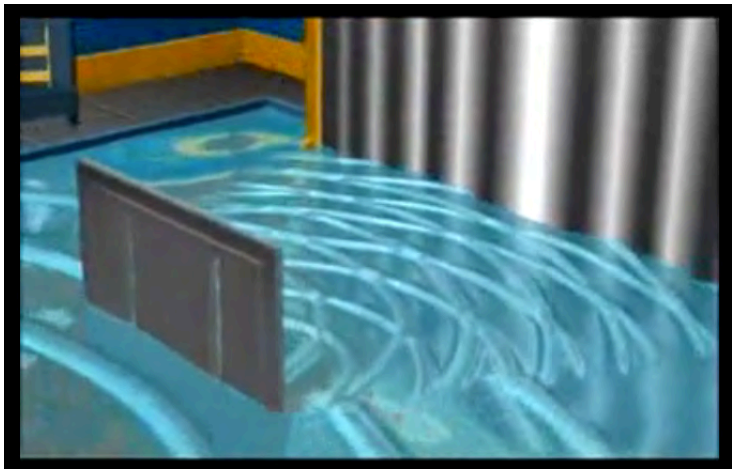
e/Slit http://ww

Slit http://www

-> diffraction of “light” => wave (bending around corners)

Unbelievable.....

....also particles e.g. electrons/ He-ions do it ....



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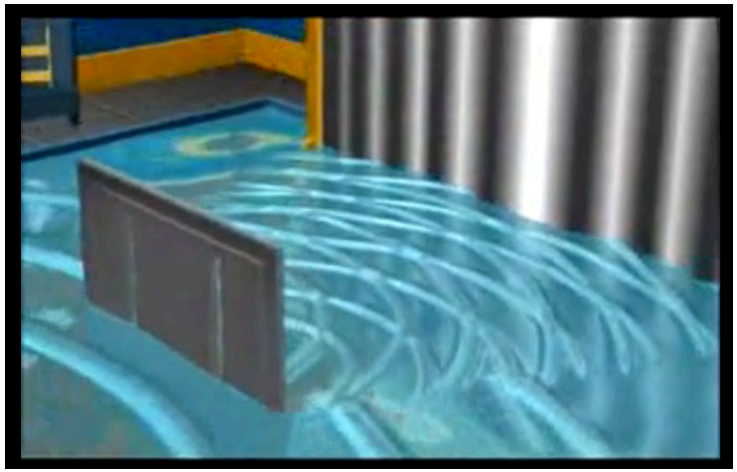

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Duality of “light” - particle or waves.....

The particle concept:
a energy concept with Electronenvolt (eV) - 1eV is the kinetic energy of a charged particle which is accelerated by an electric field of 1 Volt.

-> “classical ray optics”

The wave concept:
waves with frequency (f), wavelength (λ) wave-number (wz) represent energy-proportional units.....

-> “wave optics” (wave front and Schrödinger eq.)

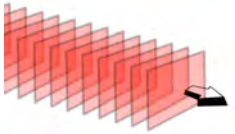
$$E = h \cdot f$$

(h...PLANCK’s constant) directly relates the energy with the frequency of a “ray” Quantum Theory *(Planck & Einstein)*

The relation between the wavelength (λ) of a particle of mass, m, moving at a velocity, v, is given by the ***DeBroglie*** wave equation:

$$\lambda = \frac{h}{p}, \quad \lambda = \frac{h}{mv} \qquad (1)$$

Not one wave but plane waves are used for imaging



In the physics of wave propagation, a **plane wave** is a constant-frequency wave whose wavefronts (surfaces of constant phase) are infinite parallel planes of constant amplitude normal to the phase velocity vector.

Mathematically, a plane wave is a wave of the following form:

$$u(\mathbf{x}, t) = Ae^{i(\mathbf{k}\cdot\mathbf{x}-\omega t)}$$

where *i* is the imaginary unit, **k** is the wave vector, *ω* is the angular frequency, and *A* is the (complex) amplitude.

-> two dimensional sinusoids have a frequency, phase, amplitude and direction!

(This becomes of interest when Fourier Transformations (FFT) are used to analyze images (Amplitude in real part, Phase in imaginary part))

Conversion of energy-> wavelength-> wave-number.....

$$E = h \cdot f$$

The wavelength of light is via the speed of light (c) linked to the frequency (general: $\lambda = \frac{v}{f},$) => λ = c / f

The so called wave-number is the reciprocal of the wavelength (wz, n)
n = 1 /λ (n Wave number usually in cm-1)

-> E = h * c * n

Conversion from one to the other unit:

λ [μm] = 10’000 / n [cm-1] ; λ [nm] = 10’000’000 / n [cm-1]

f [Hz] = 3 * 1010 * n [cm-1]

http://heasarc.gsfc.nasa.gov/cgi-bin/Tools/energyconv/energyConv.pl

E [eV] = 1 / 8065,5 * n [cm-1] http://www.cactus2000.de/de/unit/masswav.shtml

Imaging: Principle

“Information transfer chain”

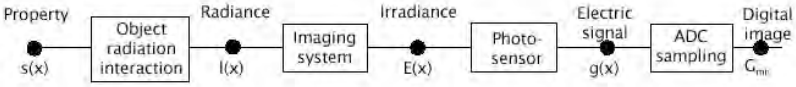


Figure 1.1: *Chain of steps linking an object property to the signal measured by an imaging system.*

To image a certain structure the used wavelength should be in the range of the structure detail to be imaged... if not the wavelength can not interact linearly with the object...

- Conversion in our head.....
  - Visual perception and brain power.....

Watch out what your brain does!

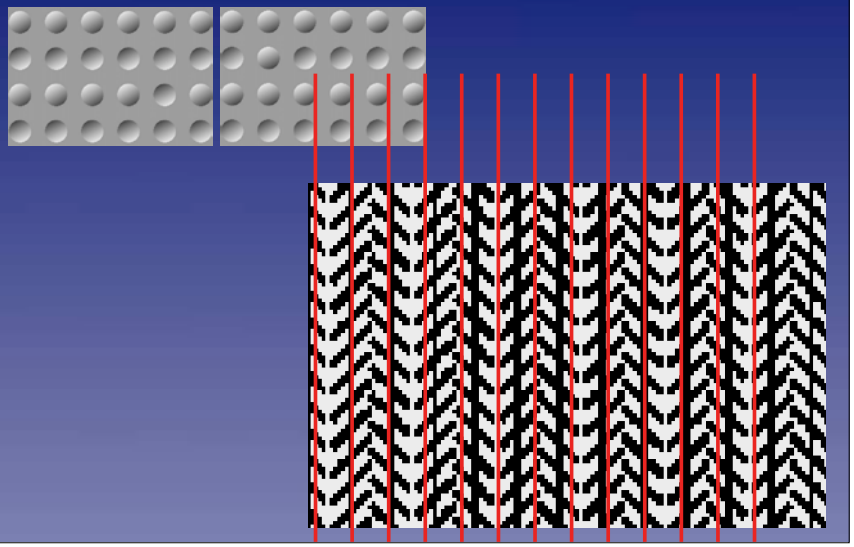
Just start to read (engl. or german).....

Aoccdrnig to a rscheearch at an Elingsh uinervtisy, it deosn't mttaer in waht oredr the ltteers in a wrod are, the olny iprmoetnt tihng is taht frist and lsat ltteer is at the rghit pclae. The rset can be a toatl mses and you can sitll raed it wouthit porbelm. Tihs is bcuseae we do not raed ervey lteter by it slef but the wrod as a wlohe. ceehiro.

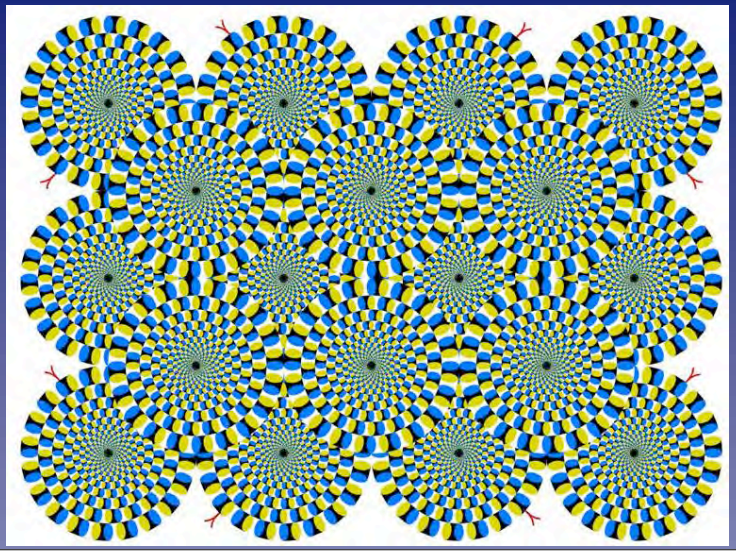
David Harris 2003; David R. Perrott and Caltech senior research fellow Kourosh Saberi, Nature 1999 on speach "Cognitive Restoration of Reversed Speech,"

Afugrnud enier Sduite an enier Elingshcen Unvirestiät ist es eagl, in wlehcer Rienhnelfoge die Bcuhtsbaen in eniem Wrot sethen, das enizg wcihitge dbaei ist, dsas der estre und lzete Bcuhtsbae am rcihgiten Paltz snid. Der Rset knan ttolaer Bölsdinn sien, und du knasnt es torztedm onhe Porbelme lseen. Das ghet dseahlb, wiel wir nchit Bcuhtsbae für Bcuhtsbae enizlen lseen, snodren Wröetr als Gnaezs.

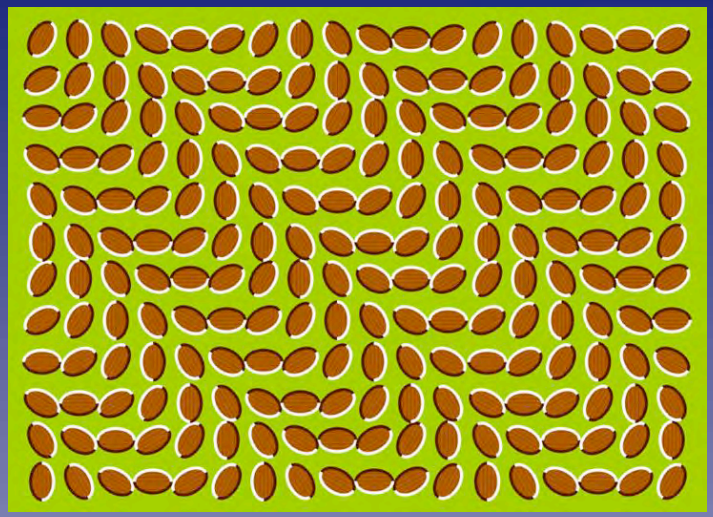
Can you trust what we see…



Nothing is turning - or...?

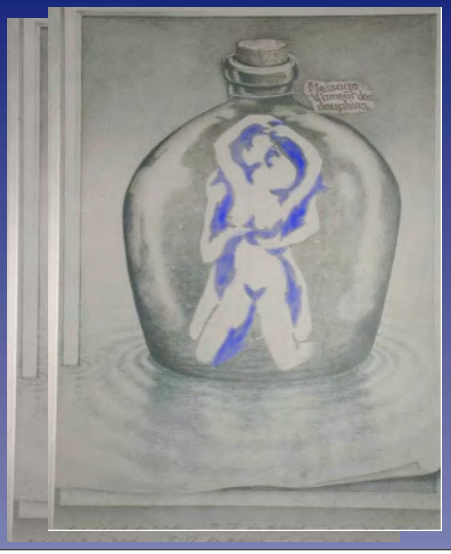


…. an nothing is moving!



Do we trust what we see…Illusionen!

What do you see in this image.....?



Children see only
Dolphins
...what did you see???

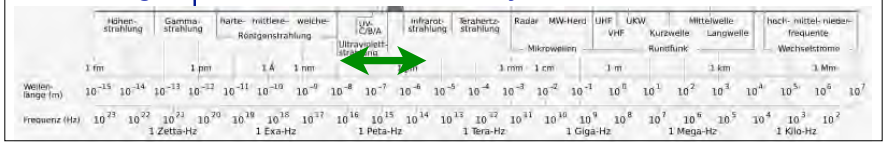
Sandro Del-Prete

"A world were your eyes deceive you"

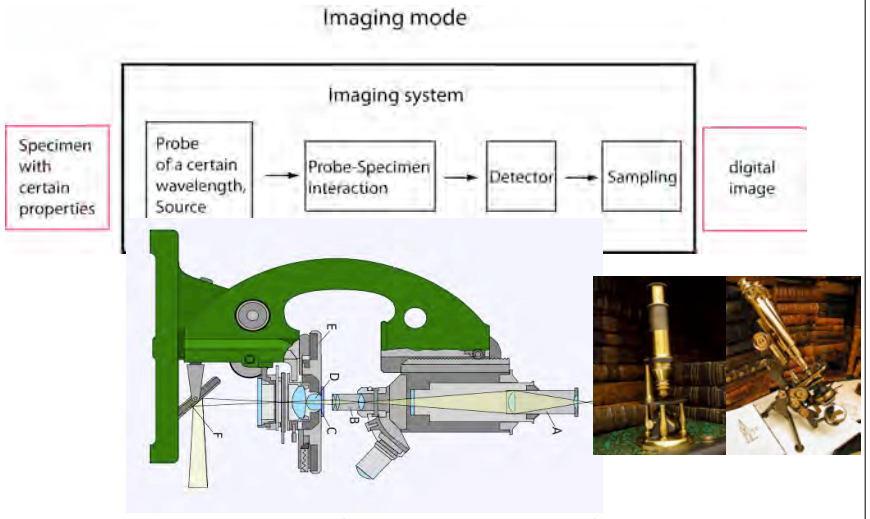
http://www.illusoria.com

Photon..... Properties:

	Photon *(Boson/Eichboson)*	Electron *(Fermion/Lepton)*
Charge:	**0**	
Mass:	**0**	
Resting Energy:	**eV**	
Compton Wavelength:	**-**	
Spin:	**1**	
Radius:	**-**	
Wavelength	**300nm-1600nm in use today...**	

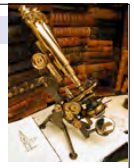


Imaging:

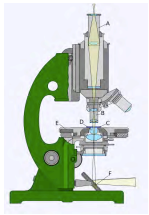


Information transfer.....

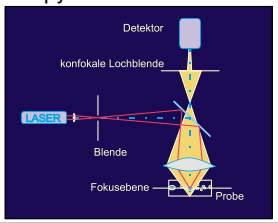
Imaging Mode: Light Microscopy (LM)



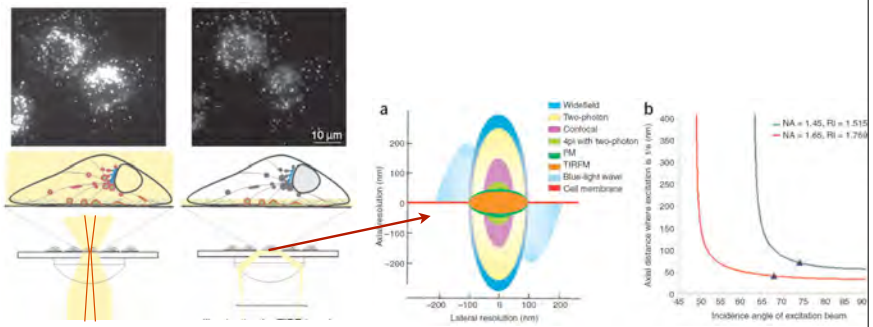
- Full field illumination (Bright field, Phase contrast, Differential Interference Contrast, Fluorescence, Polarisation, Spectroscopy...)
- -> parallel light is exposed to the whole specimen
- -> thickness of specimen is limiting resolution
- -> fast light exposure possible
- -> projection/reflexion images of the exposed area



- Scanning mode (Reflexion, Fluorescence, Spectroscopy - Raman
- -> focused light spot is scanned through the specimen
- -> scanning in x,y and z possible
- -> thickness not so critical
- -> slow for large image area
- -> pixel by pixel images



Light Microscope (LM) Illumination types....



Wide field & confocal illumination

TIRF illumination

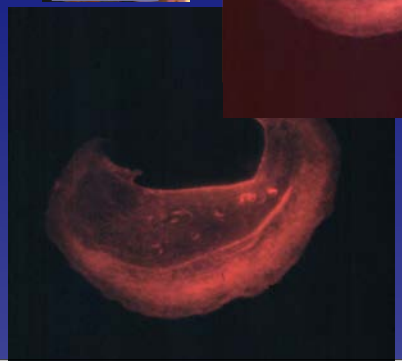
2007; Jyoti K Jaiswa & Sanford M Simon

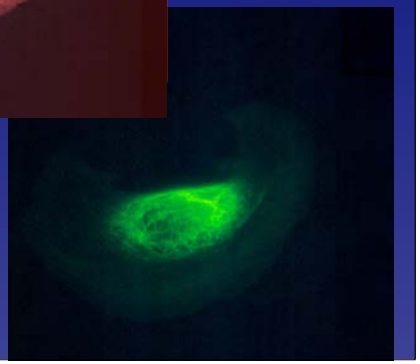
Motile cells (Fish keratocyte) - Fluorescence LM...

(Resolution or Seeing....?)

F-actin (ø 9nm)

Microtubules (ø 25nm)





Imaging Mode: Light Microscopy (LM)

- Probe: bundle of light or focused spot of light
- Wavelength: 300-1000nm

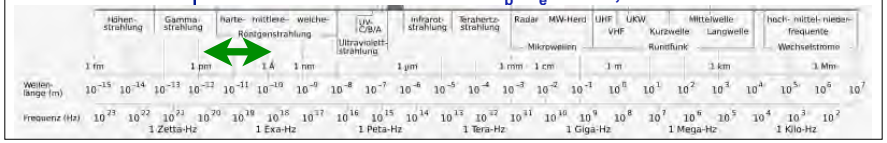
- Probe-Specimen Interaction: Absorption, elastic and inelastic scattering, conversion (fluorescence, Raman), phase shift...

- Modalities:
  - full-field illumination/Interference, Phase contrast, Scattering & Absorption, Emission
  - Scanning probe imaging/ Absorption, Emission, Scattering...

- Resolution: .....
- Application: .....
- Advantage: .....
- Limitation: .....

Photon/Electron Properties:

	Photon *(Boson/Eichboson)*	Electron *(Fermion/Lepton)*
Charge:	**0**	**-1e=-1,602x10^{-19}C**
Mass:	**0**	**5,485x10^{-4}u (1 m$_e$)** **9,109x10^{-31}kg**
Resting Energy:	**eV**	**0,5109 MeV**
Compton Wavelength:	**-**	**2,426x10^{-12}m**
Spin:	**1**	**+/- 1/2**
Radius:	**-**	**2,817x10^{-15}m** **m$_p$/m$_e$= 1836,1**



Electron: Energy & Wavelength

The dualism „wave - particle“ is quantified by the De Broglie equation:

$$\lambda = h/p = h/mv$$

λ : wavelength; h: Planck constant; p: momentum

The energy of accelerated electrons is equal to their kinetic energy:

$$E = eV = \frac{m_0 v^2}{2}$$

V: acceleration voltage
e / m_0 / v: charge / rest mass / velocity of the electron

$$p = m_0 v = (2 m_0 eV)^{1/2}$$

$$\lambda = h / (2 m_0 eV)^{1/2} \; (\approx 1.22 / V^{1/2} \text{ nm})$$

At the acceleration voltages used in TEM, relativistic effects have to be taken into account

$$\lambda = h / [2 m_0 eV (1 + eV / 2 m_0 c^2)]^{1/2}$$

Wavelengths of Electrons

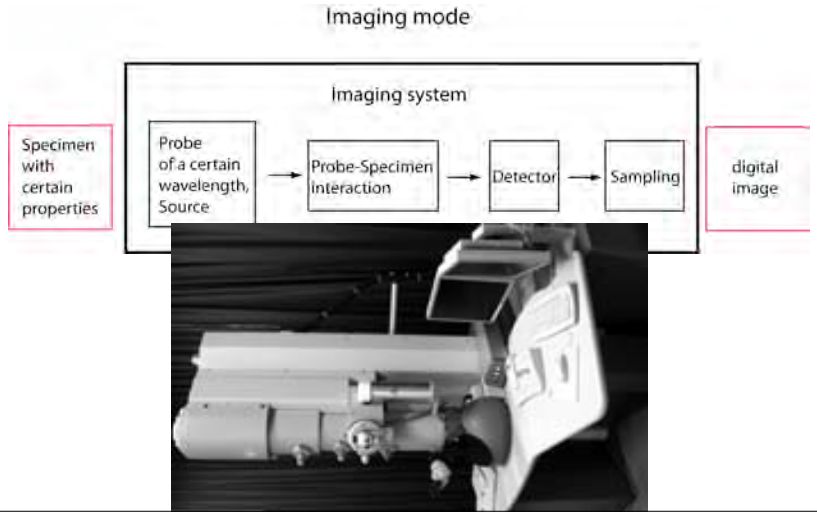
Accelerating voltages: SEM 0.5 – 30 kV
TEM 100 – 1‘000kV

V_{acc} / kV	Nonrelativistic wavelength [nm]	Relativistic wavelength [nm]
1	0.0388	0.0388
40	0.00613	0.0060
100	0.00386	0.00370
300	0.00223	0.00197
1000	0.00124	0.00087

(Atomic distances: ~ 0.1 nm (Å))

Imaging:

"Information transfer chain"

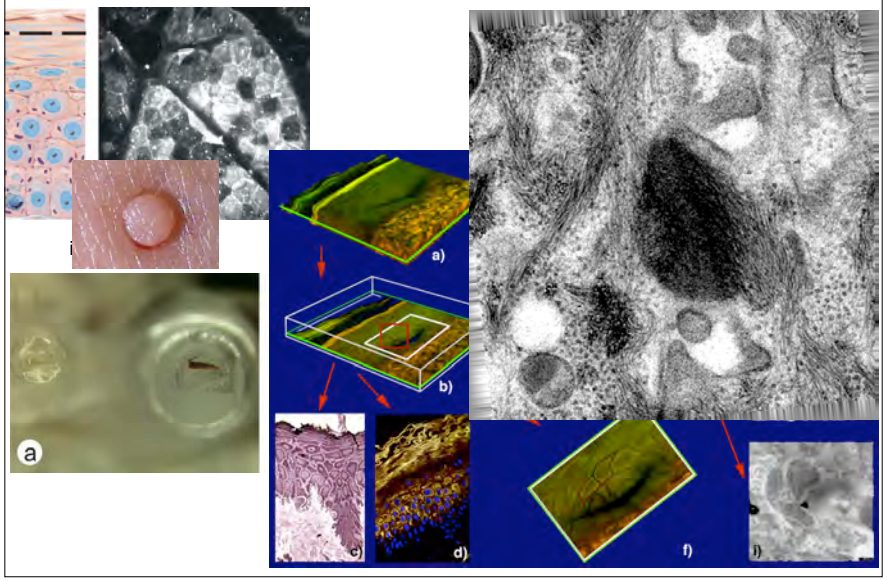


EMEZ

- Imaging Mode: Electron Microscopy (EM)
- Probe: parallel beam or focused spot of electrons
- Wavelength: 10^{-3}-10^{-6} nm
- Probe-Specimen Interaction: Absorption, elastic and inelastic scattering, conversion (fluorescence, luminescence), phase shift, ...
- Modalities:
  - full-field illumination; Interference, Phase contrast, Scattering & Absorption, Emission
  - Scanning probe imaging; Absorption, Emission, Scattering...
- Resolution: .....
- Application: .....
- Advantage: .....
- Limitation: .....

Imaging across scales: Light - & Electron Microscopy

e.g on Human skin.....



Imaging Modalities in Life-Science