Bioinformatics II
Winter Term 2016/17

Rheinische
Friedrich-Wilhelms-
Universität Bonn

Chapter 7: Biological Imaging

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December 20, 2016

Job Opportunity

- We are looking for an experienced **web designer** who is willing to set up a relatively simple page for the VMV 2017 symposium

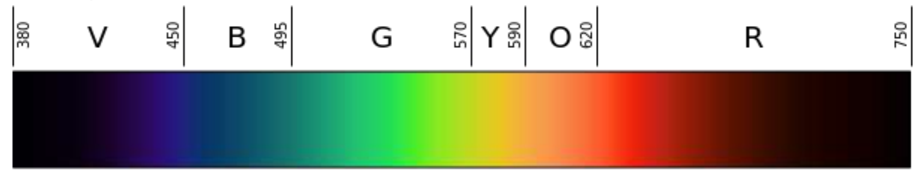
Forming Groups for Second Project

- Second project will be due on January 31
- Again, you can work in **groups of two**
  - Groups of three are acceptable **if it helps avoid forcing people to work alone**
  - Please tell Shekoufeh **by January 9** if you have not found a group, but would like to be part of one
  - We will only allow you to form groups of three if nobody is left alone; we will announce this via the mailing list and in the lecture

7.1 Light Microscopy

Optical Microscopy

- Developed in 17^{th} century
- Based on visible light

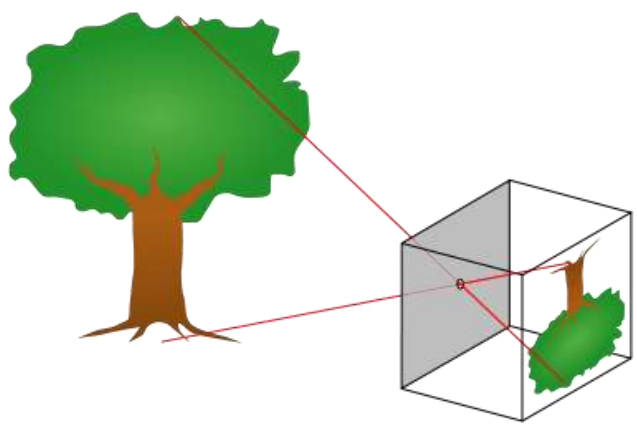


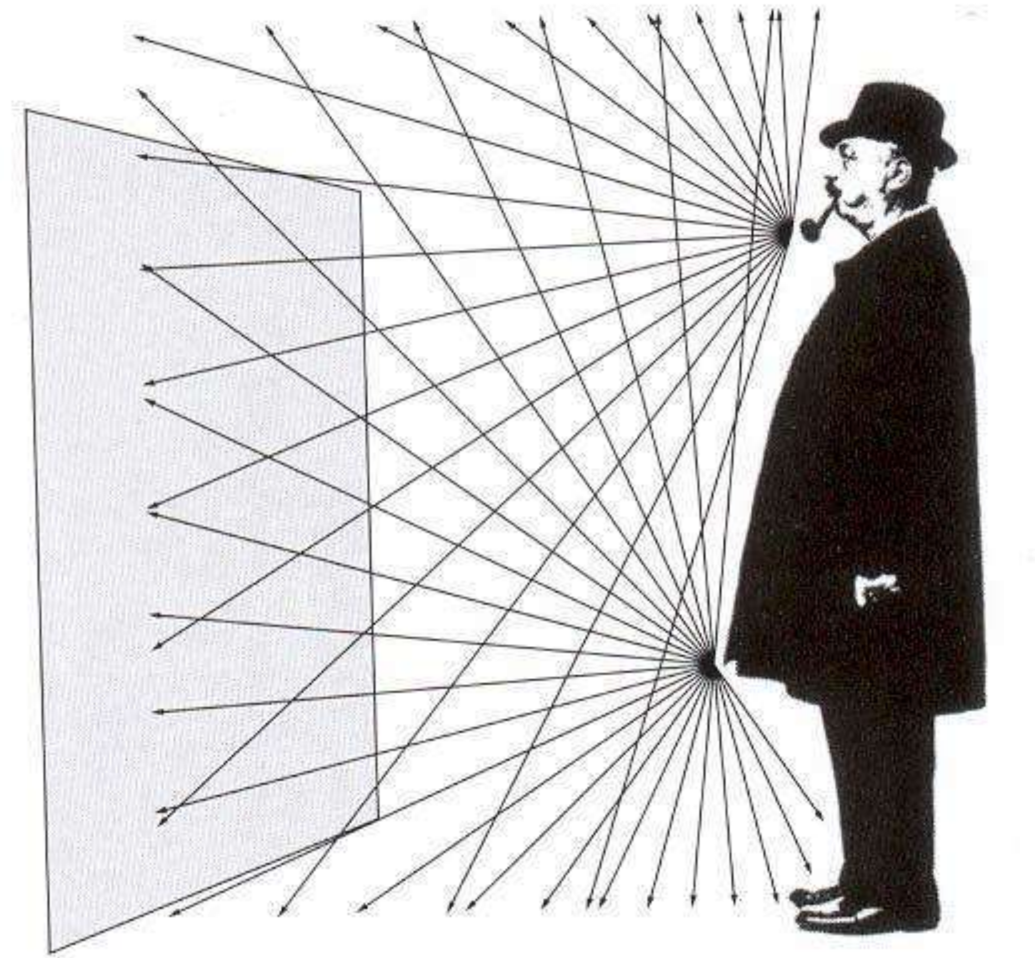
  - Objects illuminated from above (reflected) or below (transmitted)
  - Often combined with CCD camera
- Magnification through lenses

Imaging Without Optics?

Images from [London/Upton 1998] / wikimedia

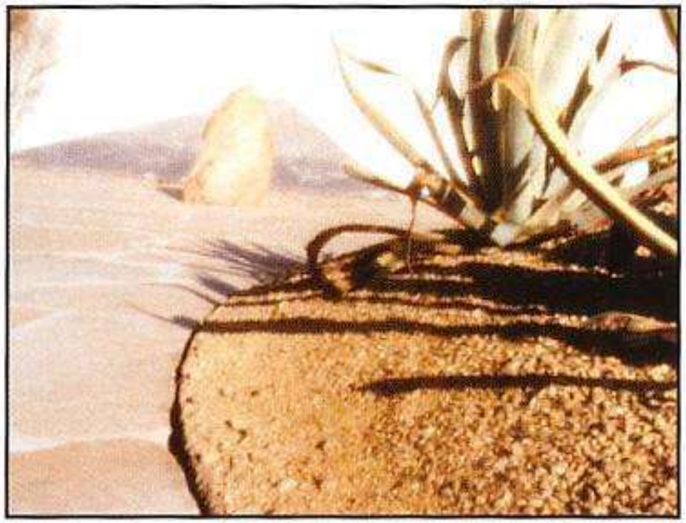
- No sharp image without optics
- Simplest remedy: Pinhole camera

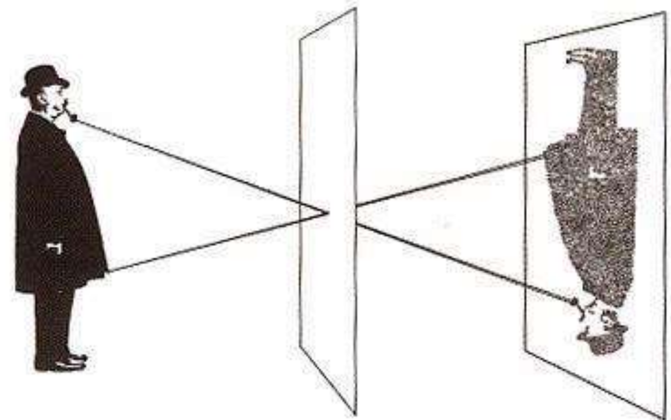




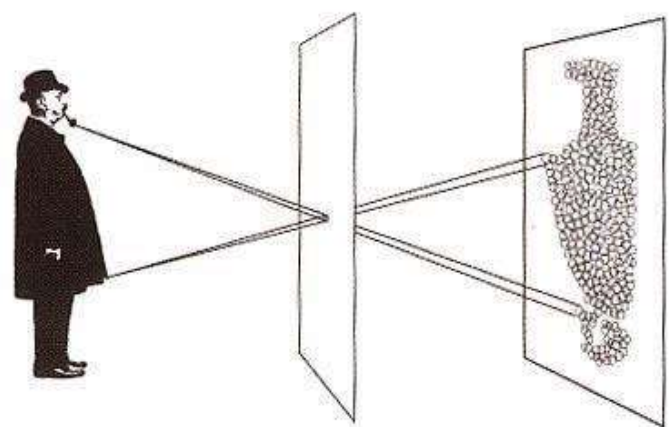
Imaging with Pinhole Camera

Photograph made with small pinhole





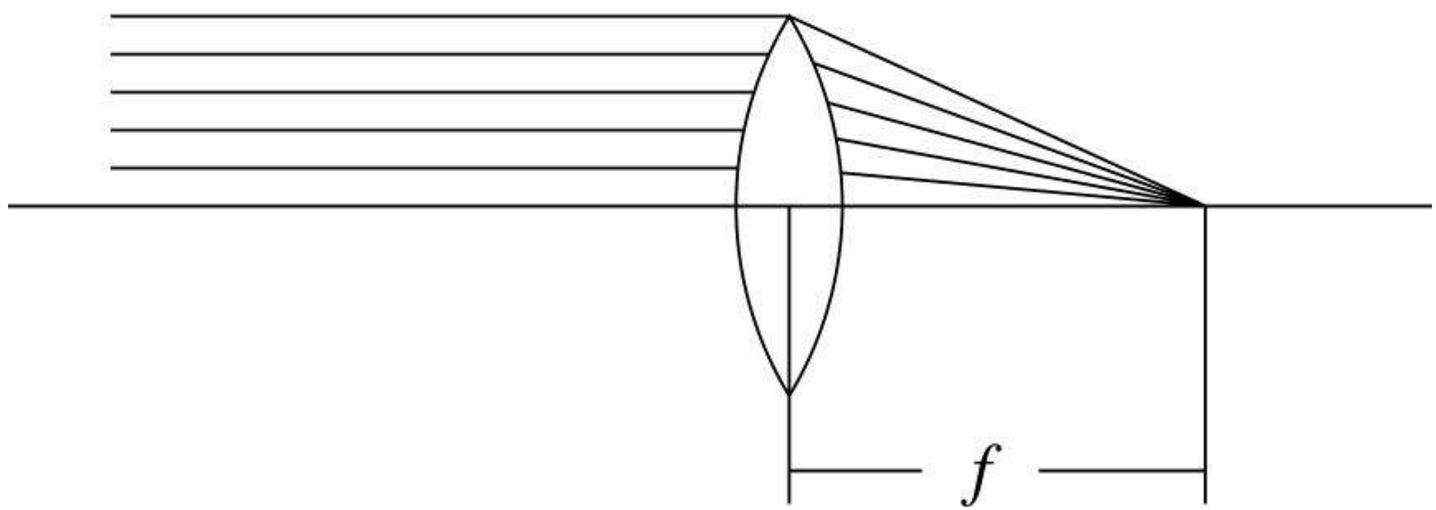
Photograph made with larger pinhole



Images from [London/Upton 1998]

Importance of Lenses

- **Convex lenses** allow you to create a bright *and* sharp image
- Rays parallel to the optical axis get focused to a point on the axis, with focal length *f*



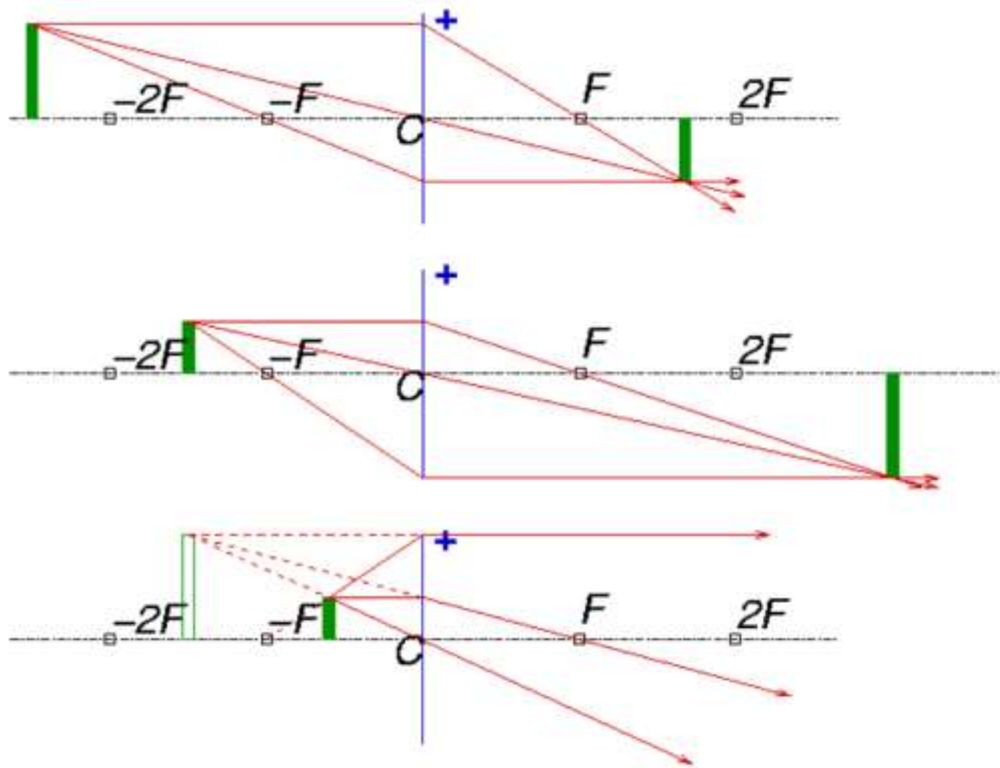
Images from Matthias Hullin

Lens Optics

- **Thin Lens Equation:** Point at distance *s* from the lens and *h* from the axis (upward) is transferred to image point at distance *s'* from lens and *h'* from axis (downward):

$$\frac{1}{f} = \frac{1}{s} + \frac{1}{s'}$$

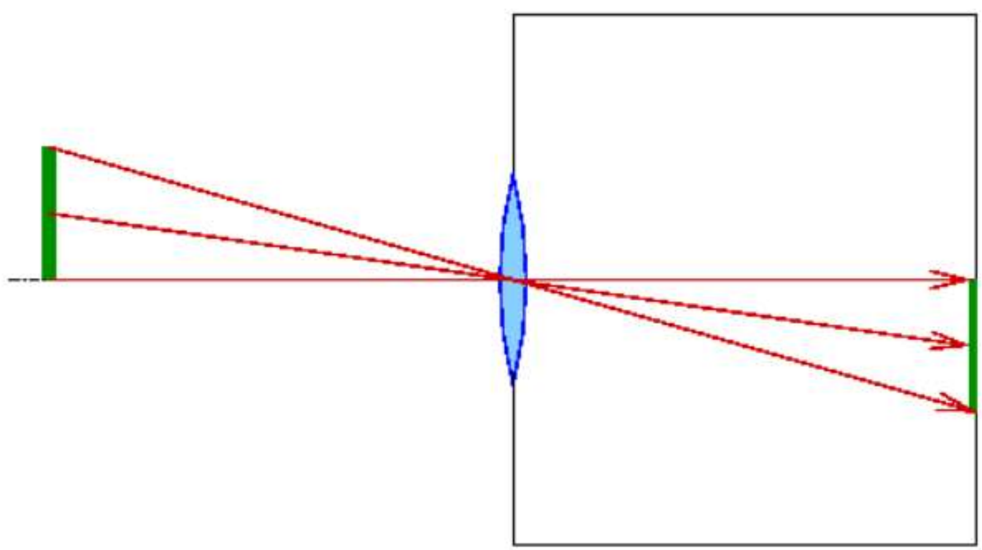
$$\frac{h}{s} = \frac{h'}{s'}$$

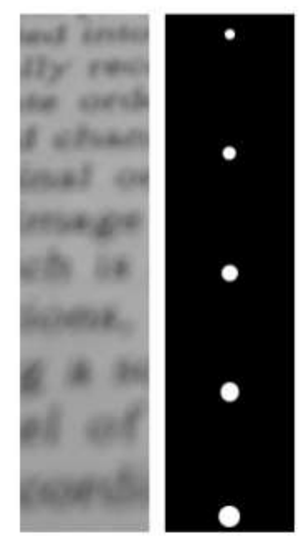


Images from Martin Welk

Imaging with Lenses

- Objects within the focus plane are sharp, all others get increasingly blurred
- Change focal plane by varying distance between lens and sensor
- Most optical systems work with multiple lenses, partly to reduce aberrations

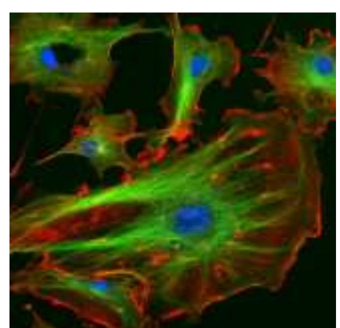


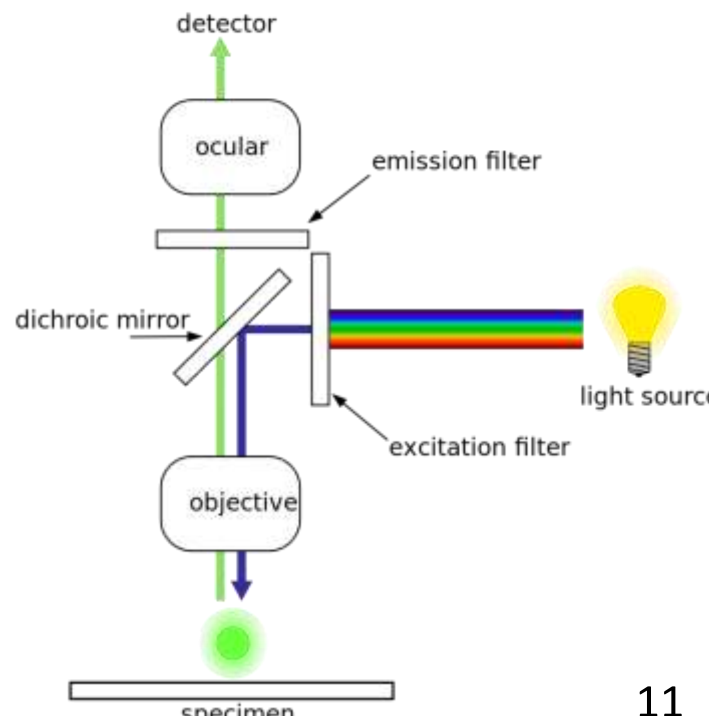


Images from Martin Welk

Fluorescence Microscopy

- **Fluorescence:** Property of some substances to absorb light at a certain wavelength, and emit light at a longer wavelength
  – Fluorescence microscopy uses only those wavelengths
  – Large selection of often very selective fluorescent stains in biology
  – Can use multiple stains with different wavelengths

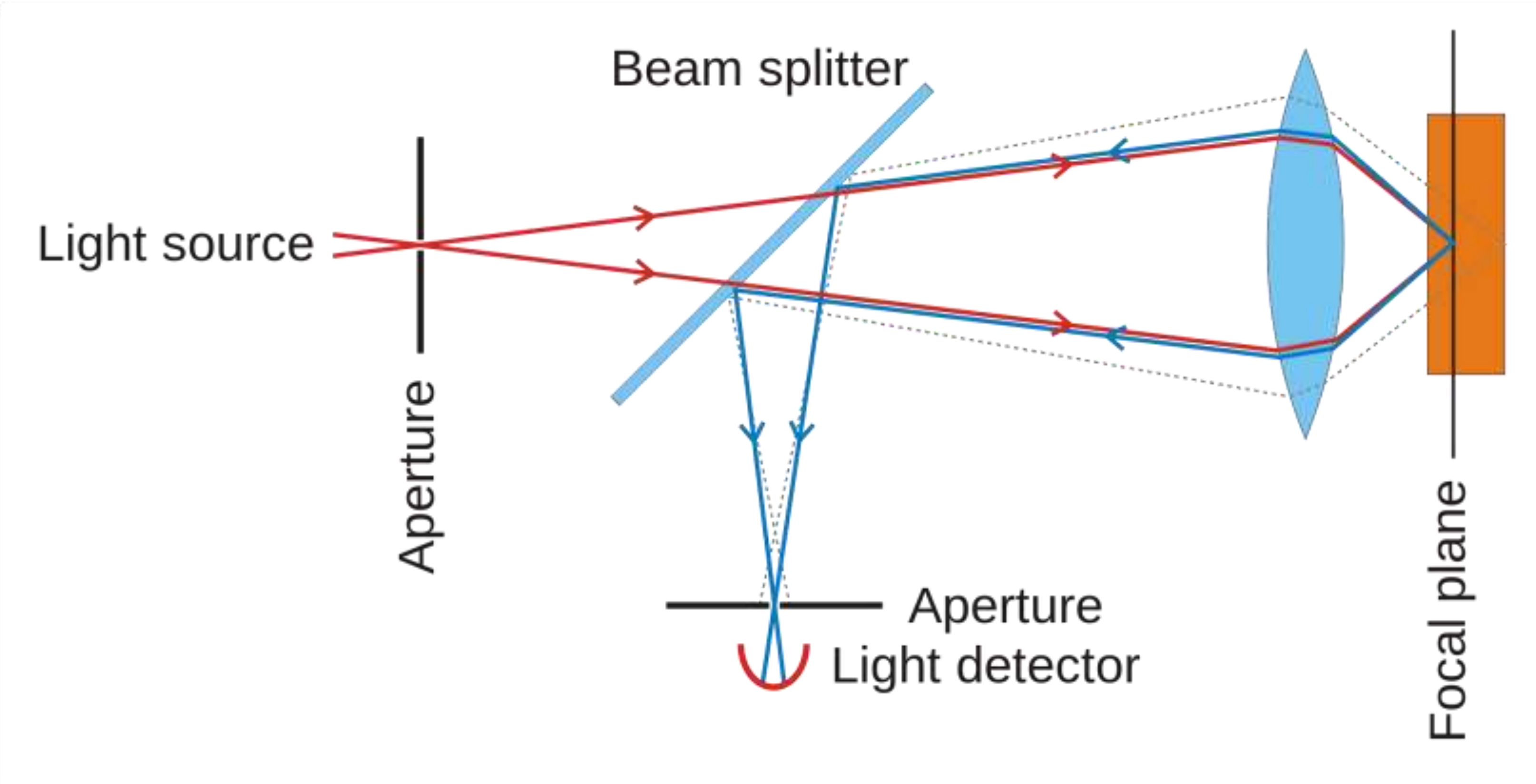




Images from Henry Mühlpfordt / wikimedia

Confocal Microscopy

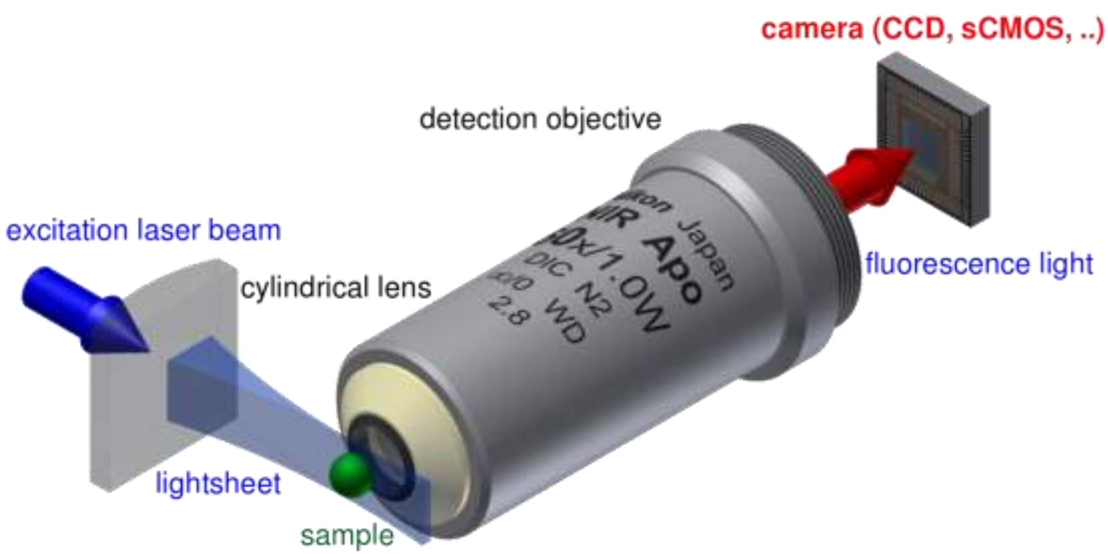
- 3D fluorescence microscopy
  - Scan with a point light source (e.g., laser)
  - Block light from outside focal plane

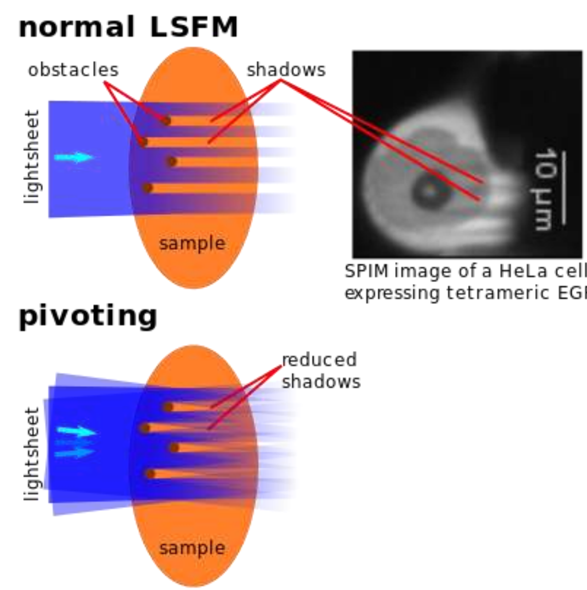


Images from danh (wikimedia)

Light Sheet Fluorescence Microscopy

- Fluorescence microscopy with illumination from the side
- Faster than confocal, resolution often worse
- Reduce stripe artifacts by pivoting

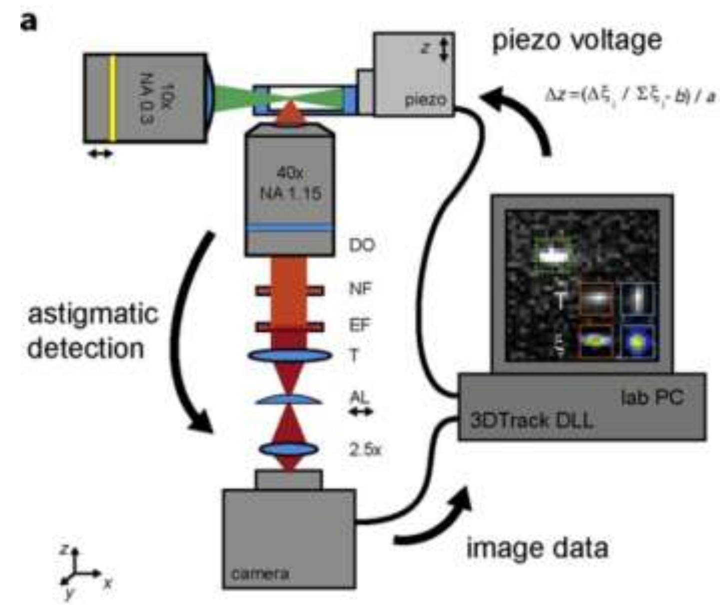


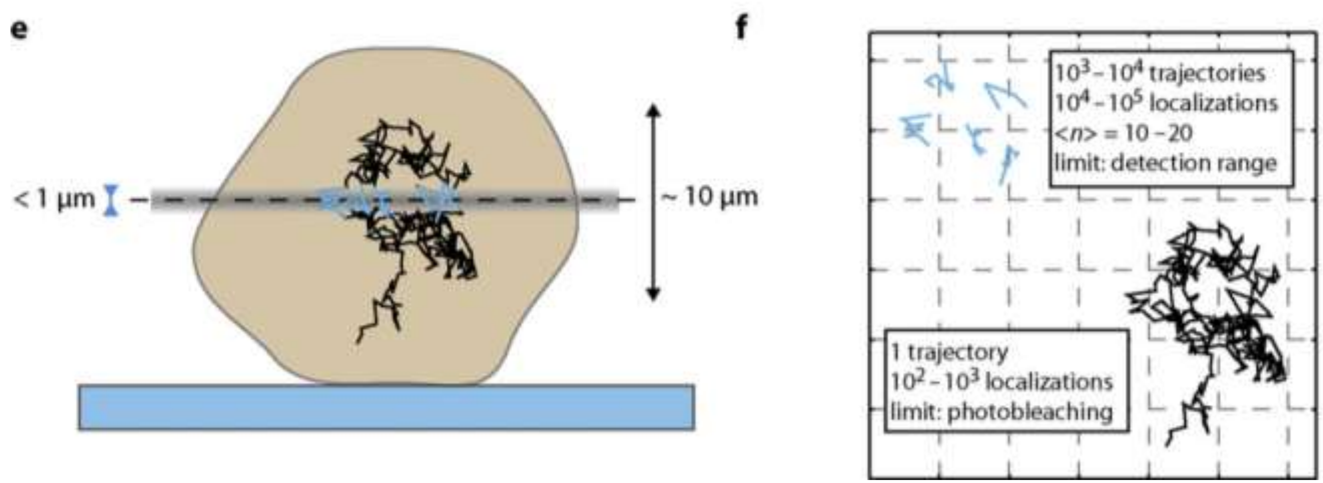


Images from Jan Krüger (wikimedia)

LSFM with 3D Feedback Tracking

- **Astigmatic lens** leads to anisotropic out-of-focus blur, can detect axial position!
  - Real-time feedback allows for tracking

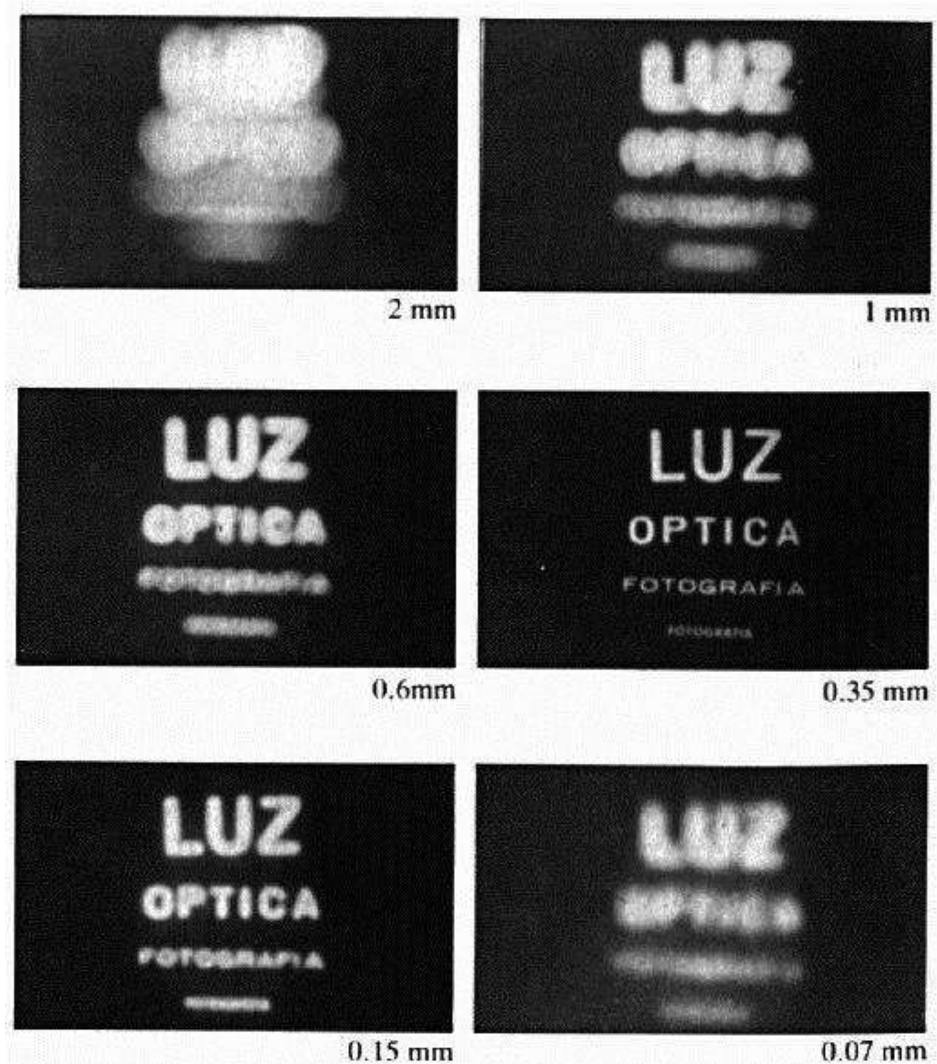




Jan-Hendrik Spille et al. Nucl. Acids Res. 2015;43:e14

Diffraction Limit in the Pinhole Camera

- **Observation:** Decreasing the pinhole size beyond an optimal value again introduces blur

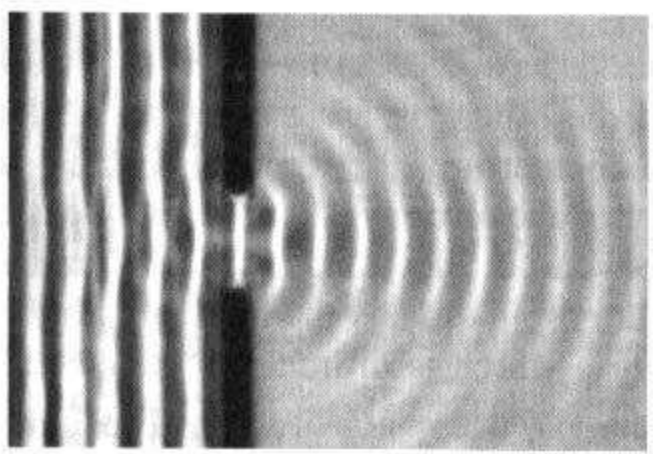


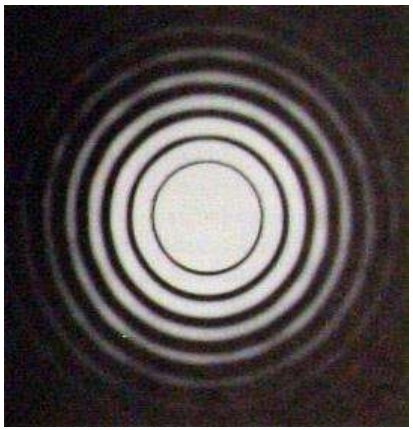
Images from [Hecht 1987]

Diffraction

- Light has **wave characteristics**
  - *Huygens Principle:* Every point on a wavefront can be considered as a source of spherical wavelets
- Imposes **resolution limit** for light microscopy (≈200nm, magnification below 2000x)
  - Abbe's formula (wavelength λ, refractive index n, aperture angle α):

$$d = \frac{\lambda}{2n \sin \alpha}$$





Images from [Hecht 1987]

7.2 Electron Microscopy

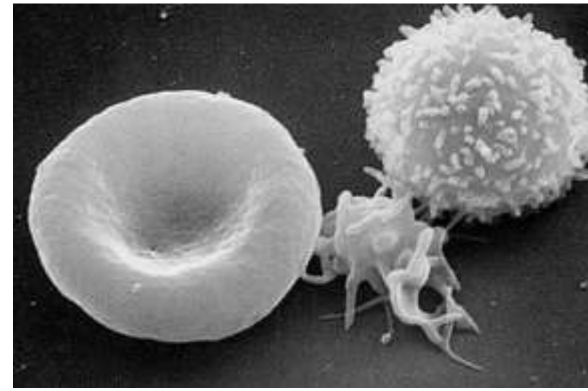
Electron Microscopy

- Developed in 1930s
- Uses beams of focused, highly accelerated electrons instead of light
  - Have wave characteristics with wavelengths on the order of picometers (10^{-12} m)
  - According to Abbe's formula, this greatly increases the achievable resolution
- Transmission Electron Microscopy (TEM)
  - Similar principle as optic microscope, with electromagnetic coils replacing lenses
  - Requires ultra-thin samples (down to 50nm)
  - Samples have to tolerate vacuum
    - Dehydration, plastic substrates, rapid freezing
  - Imaging is often destructive

Image from J. Brew

Scanning Electron Microscopy (SEM)

- First commercialized in 1960s
- Focused electron beam scans the object
  - Emitted electrons are recorded and information across the scan is combined into an image
    - Mostly: Secondary electrons, emitted by atoms that were excited by the electron beam
  - Resolution worse than with TEM, around 1nm
  - No ultra-thin sectioning, but chemical fixation of biological tissue and coating with electrically conductive material



Red / White Blood Cells

Tongue of a butterfly

Imaging the Connectome at Cellular Level

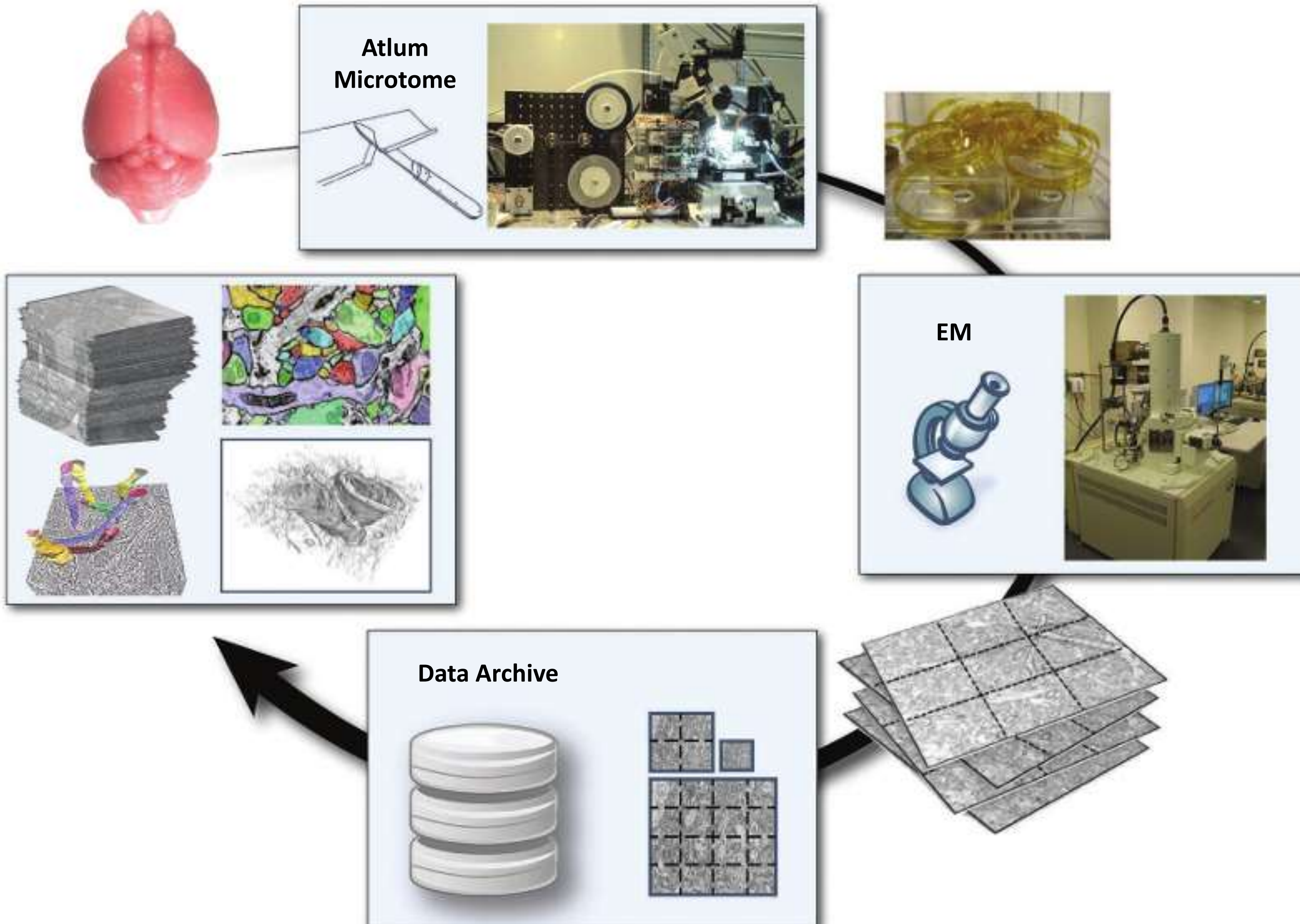


Image from [Beyer et al. 2013]

Cellular-Level Connectomics

- Ultra-Thin Serial Section Electron Microscopy
- 1mm^3 of brain tissue amounts to 20,000 slices à 40 gigapixel, total of 800 TB of image data

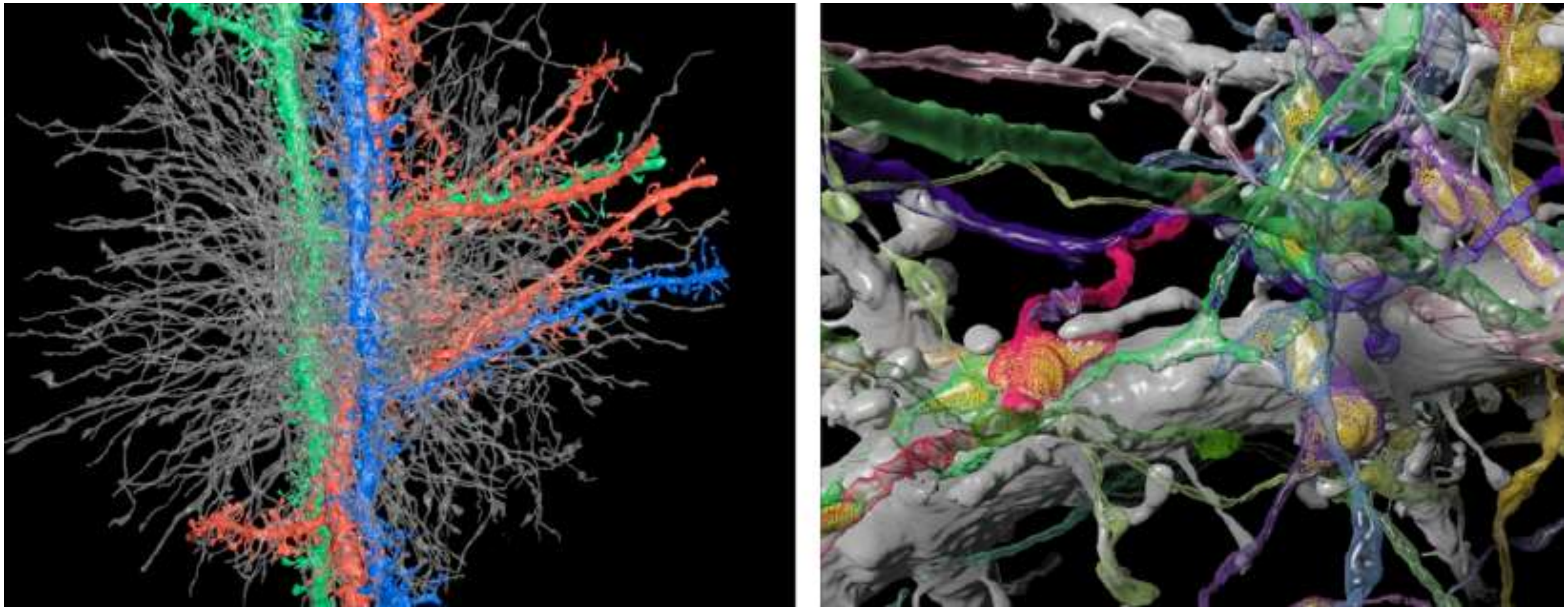
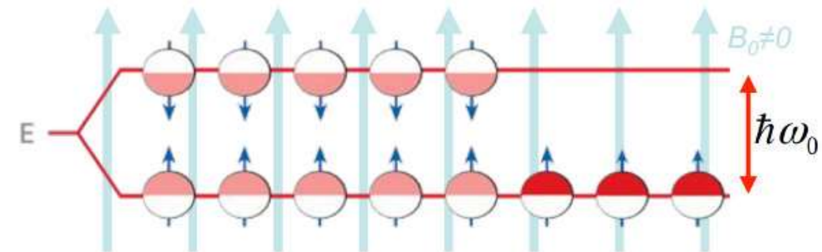


Image Source: Pfister et al., Visualization in Connectomics, arXiv 2012

7.3 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI)

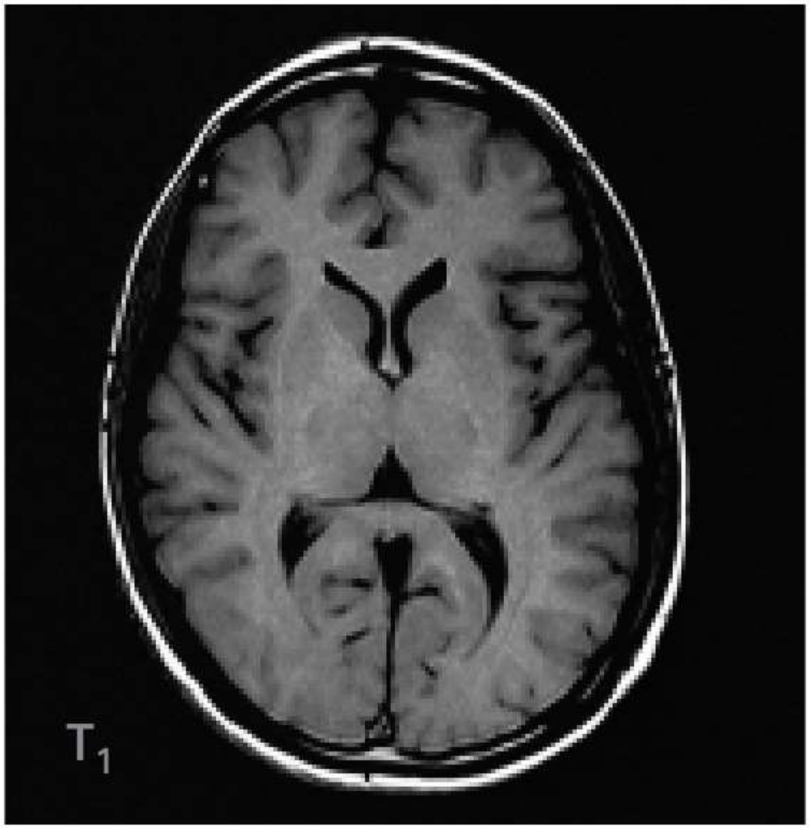
- Based on the magnetic resonance of atomic nuclei (mostly hydrogen)

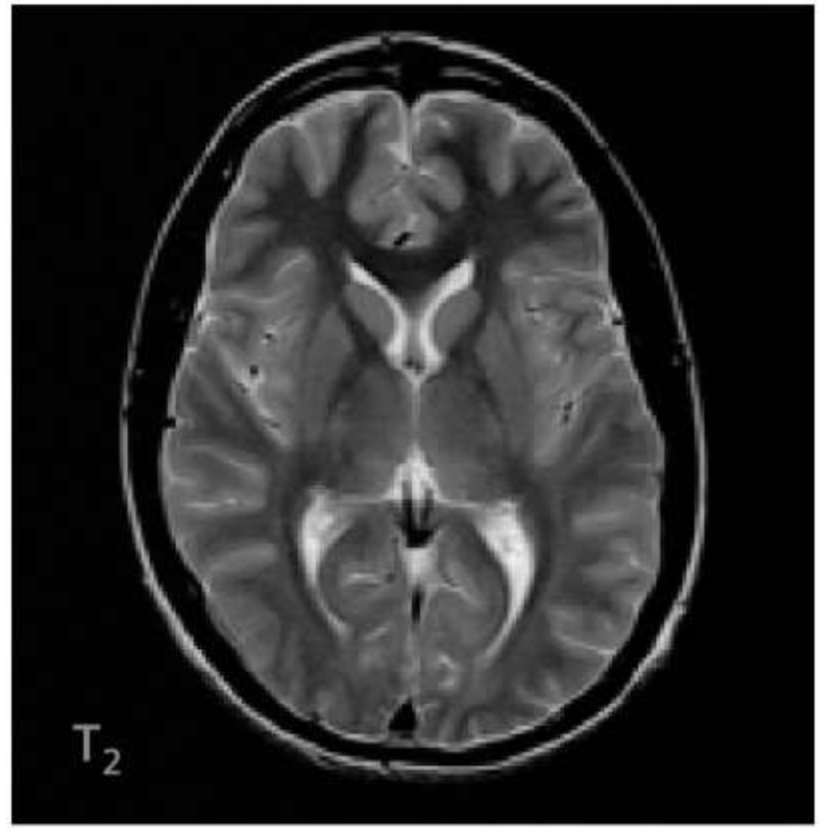


- Requires a strong static magnetic field, and high-frequency electromagnetic pulses
- Nondestructive, safe on volunteers, flexible contrasts, 3D imaging

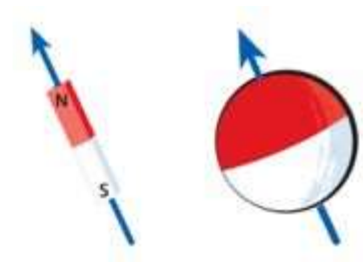
- Typical image resolution: Several mm^3

Examples: Multi-Contrast (T1/T2-weighting)

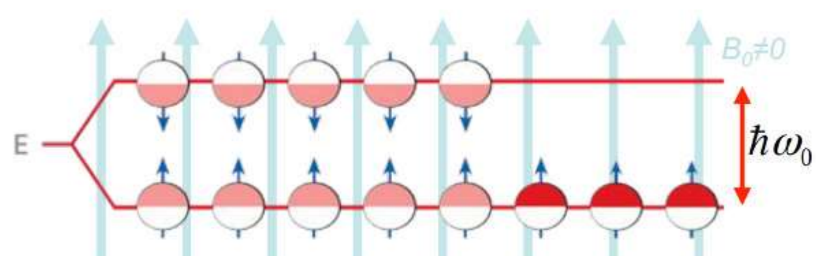




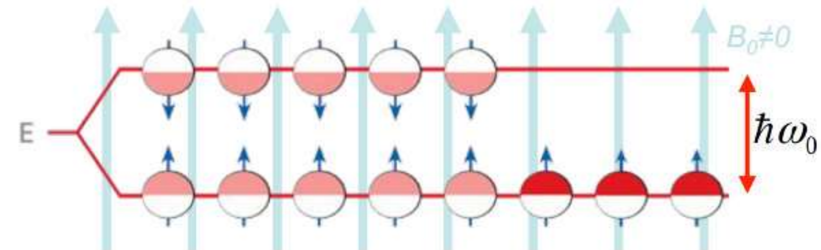
Nuclear Magnetic Moment



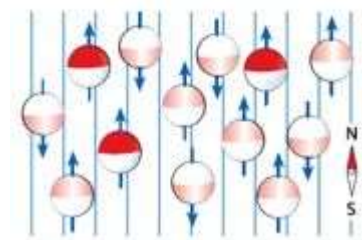
- Human body consists of ≈70% $H_2 0$
- Hydrogen atoms possess a **nuclear magnetic moment** $\boldsymbol{\mu}$
- When exposed to a static external magnetic field $\mathbf{B}_0$, the z-components of the nuclear magnetic moments align with $\mathbf{B}_0$.
- Two possible orientations of the magnetic moments: parallel and anti-parallel



Nuclear Magnetic Resonance



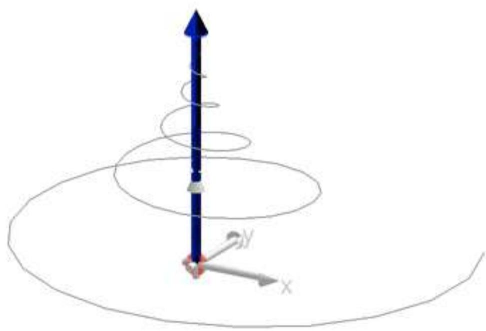
- At equilibrium, there is a tiny excess of spins in the lower energy state



- The ensemble average of all nuclear magnetic moments is a measurable **macroscopic magnetization** $M_0 \sim B_0$
- **Nuclear Magnetic Resonance:**
  - Electromagnetic wave perpendicular to $\boldsymbol{B_0}$ at ω_0 lifts spins into the higher energy state
  - Magnetization starts to precess with

$$\omega_0 \sim B_0$$

Relaxation



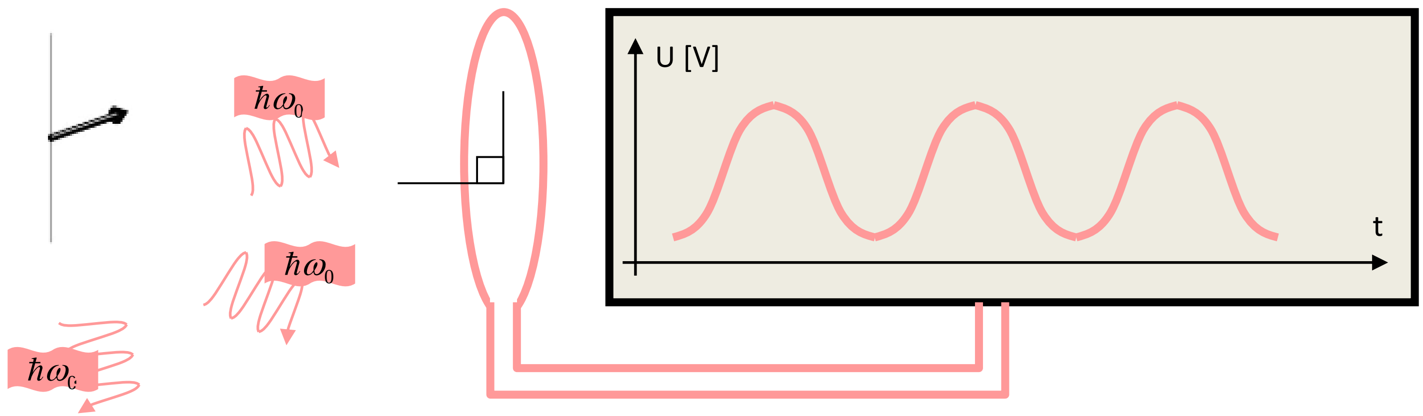
- Following an excitation, the magnetization returns to its equilibrium state
- Longitudinal magnetization regains its equilibrium value M_0
- Transverse magnetisation decays to zero
- Both happen exponentially, transverse decay is faster

MR Signal Reception

- Faraday's law: "A changing magnetic flux induces a voltage in a conductive loop."

(cf. bicycle dynamo)

- Maximize the area of the loop by orienting it perpendicular to the transverse plane

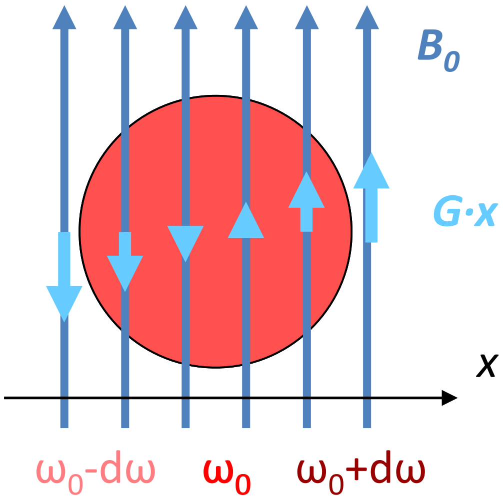


Spatial Encoding

- The basic idea of MRI:

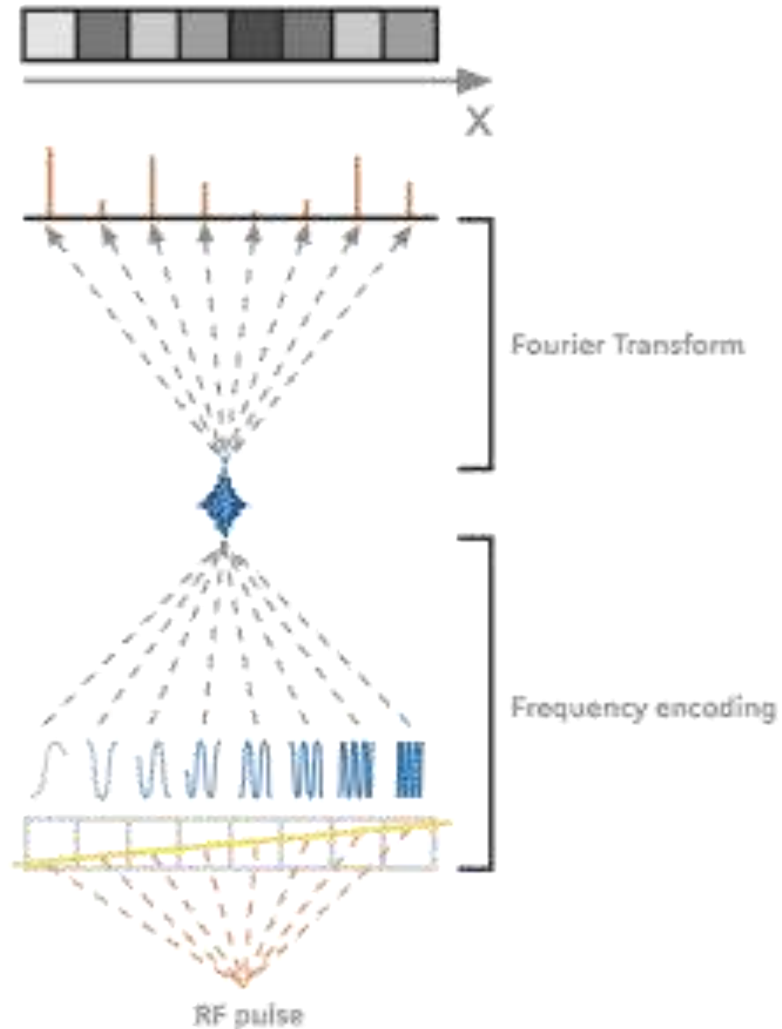
Make the precessional frequency a function of space!

- The "spectrum" then reflects spatial distribution.
- Linear field gradients of the B-field in z-direction, e.g. $G_x = dB_z/dx$



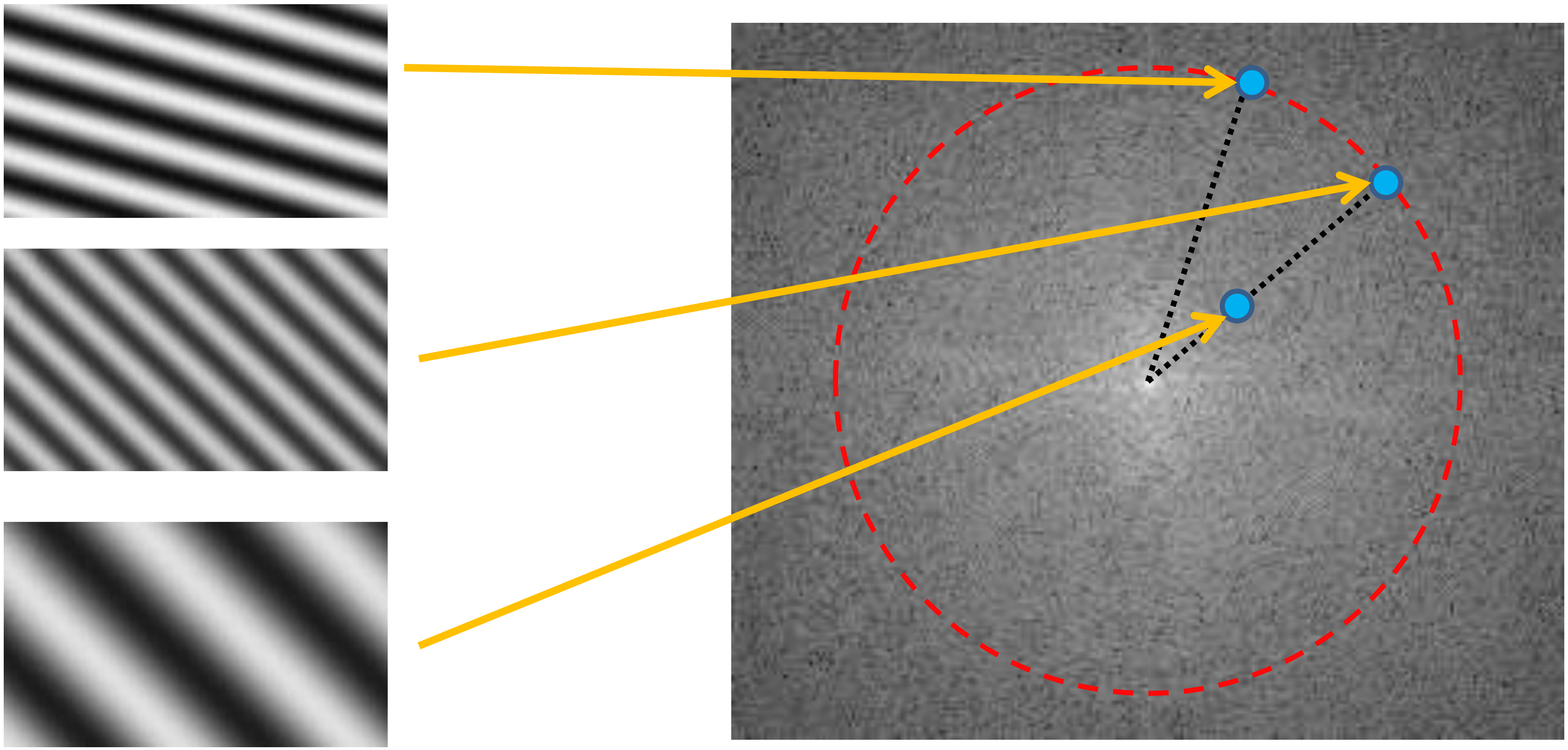
Frequency Encoding

- The Fourier Transformation allows us to transform that frequency information back to spatial location

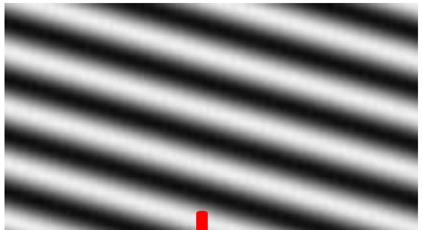


Source: Siemens, Magnete, Spins und Resonanzen

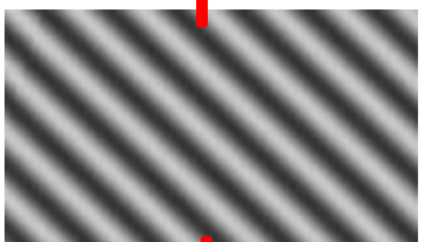
Spatial Frequency



Images in Frequency Space

$\alpha_1 \cdot$ 

+

$\alpha_2 \cdot$ 

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$\alpha_3 \cdot$ 

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...

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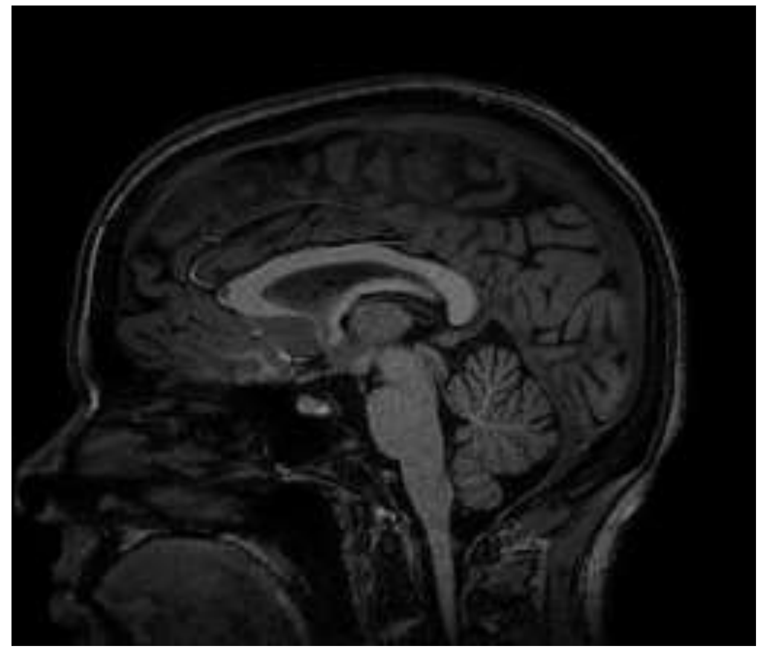
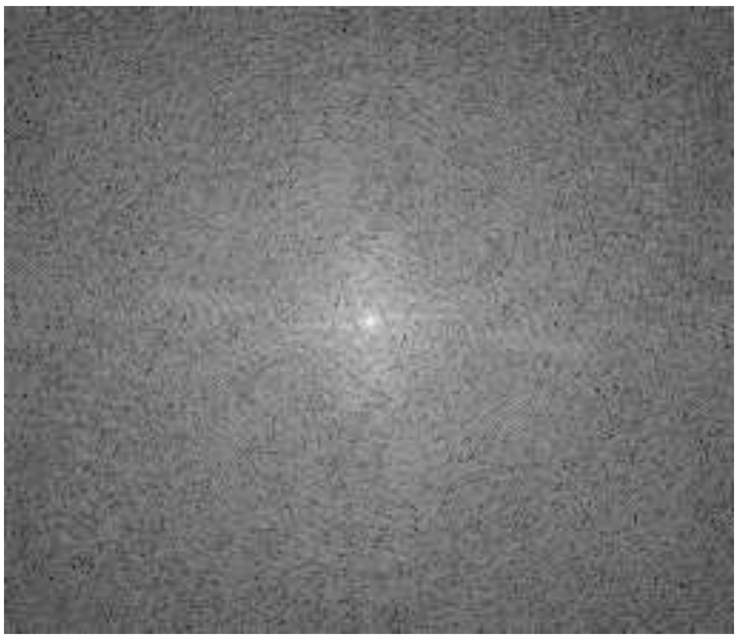


Image Reconstruction

Application of inverse 2D Fourier Transform:



k-space

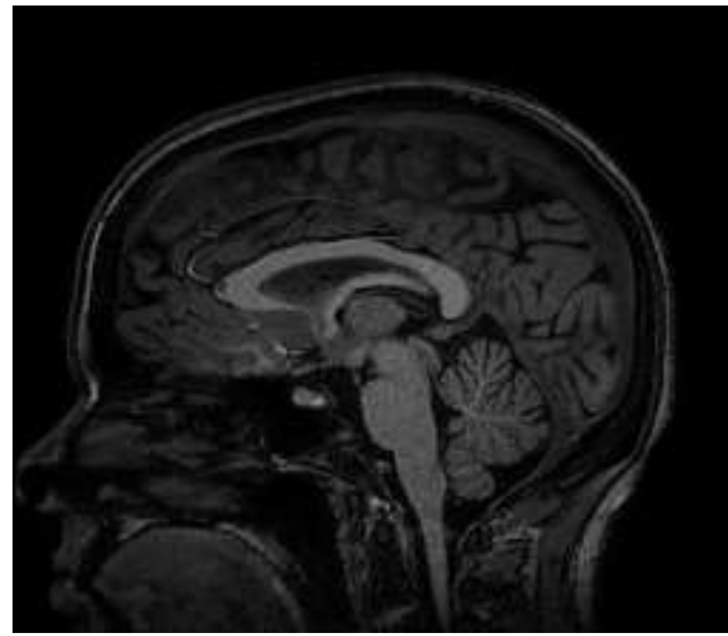
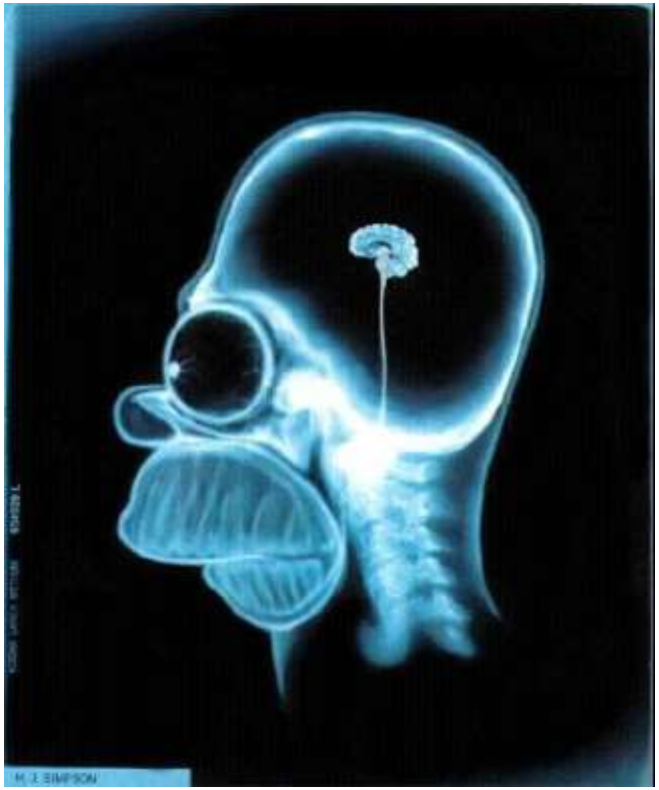
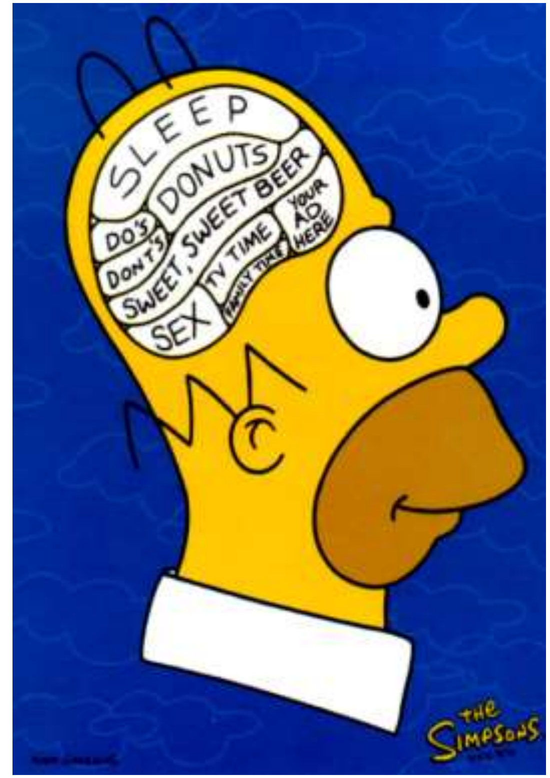


Image space

Structural vs. Functional MRI





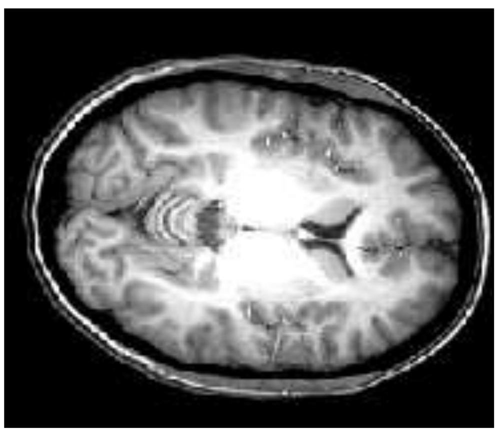
Structural MRI studies brain anatomy

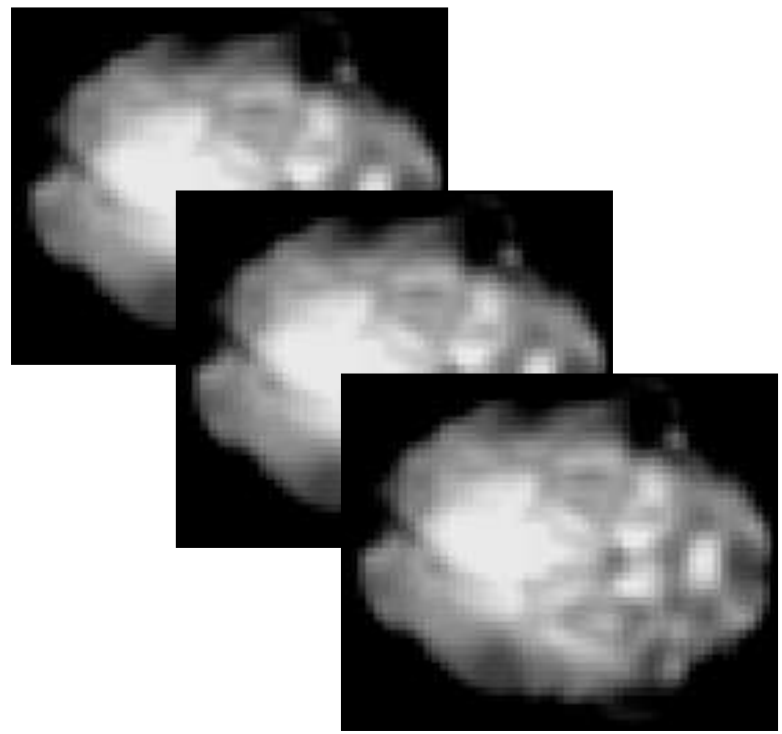
Functional MRI studies brain function

Slide from Jody Culham

fMRI: The Raw Data

- In **structural MRI,** we take one high-resolution image (e.g., 1x1x1 mm^3)
- In **functional MRI,** we repeatedly (e.g., every 2 sec) take low-resolution images (e.g., 3x3x5 mm^3)

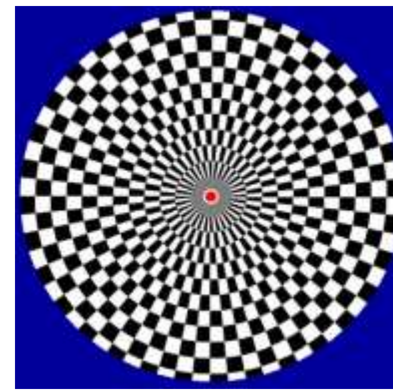


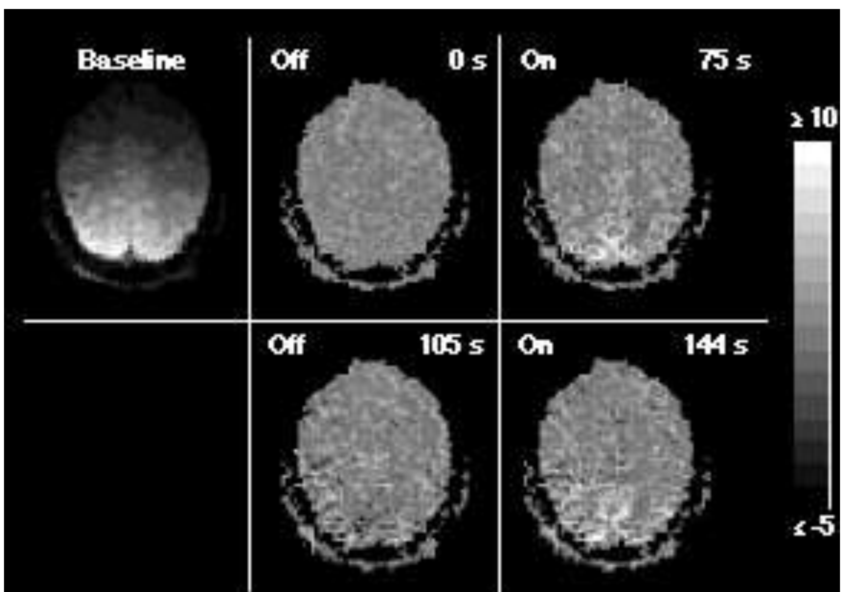


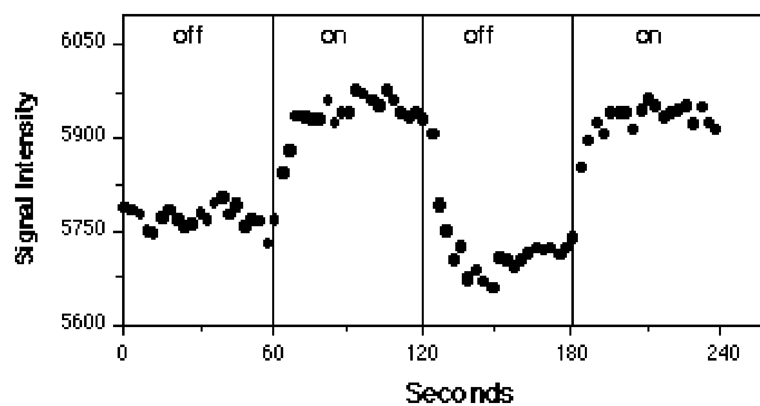
Images from Jody Cullham

fMRI: Activations

- In fMRI, an **activation** is measured by taking the difference between the signal for different tasks
- **Example stimulus:** Checkerboard
  OFF (60 sec) – ON (60 sec) – OFF …
- **Example MR response:**



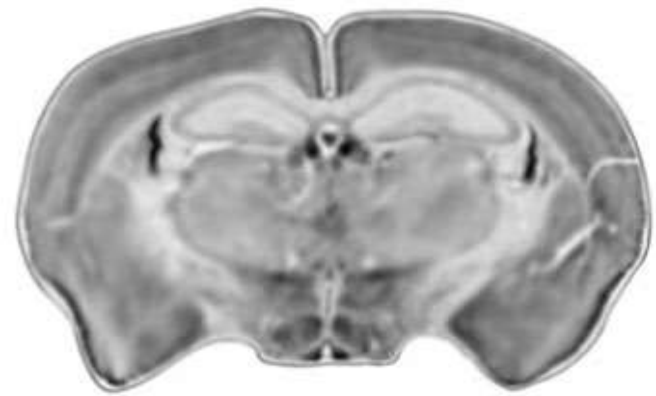




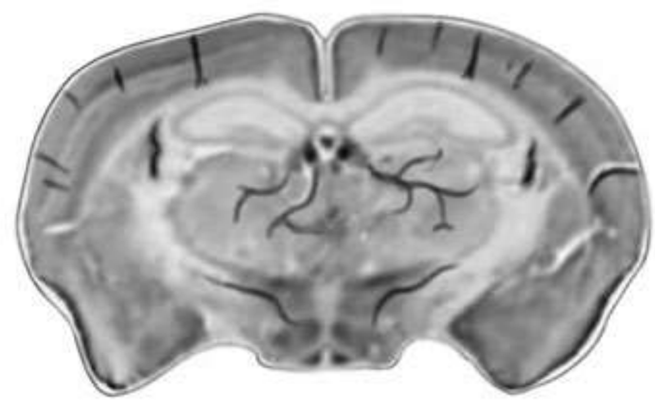
Images from Kwong et al., 1992

Where Does the Signal Come From?

- **BOLD effect:** MR intensity is **B**lood **O**xygenation **L**evel **D**ependent
- **Oxygenated Blood**
  - **Diamagnetic:** (weakly) counteracts the local magnetic field
  - Effect similar to water
  - (Almost) no change in signal
- **Deoxygenated Blood**
  - **Paramagnetic:** (slightly) enhances the field
  - Decreases T_2^*
  - Attenuates the MR signal



rat breathing pure oxygen



rat breathing normal air

Images from Ogawa et al., 1990

Temporal Complexity of BOLD Response

MR response to flashing visual stimulus

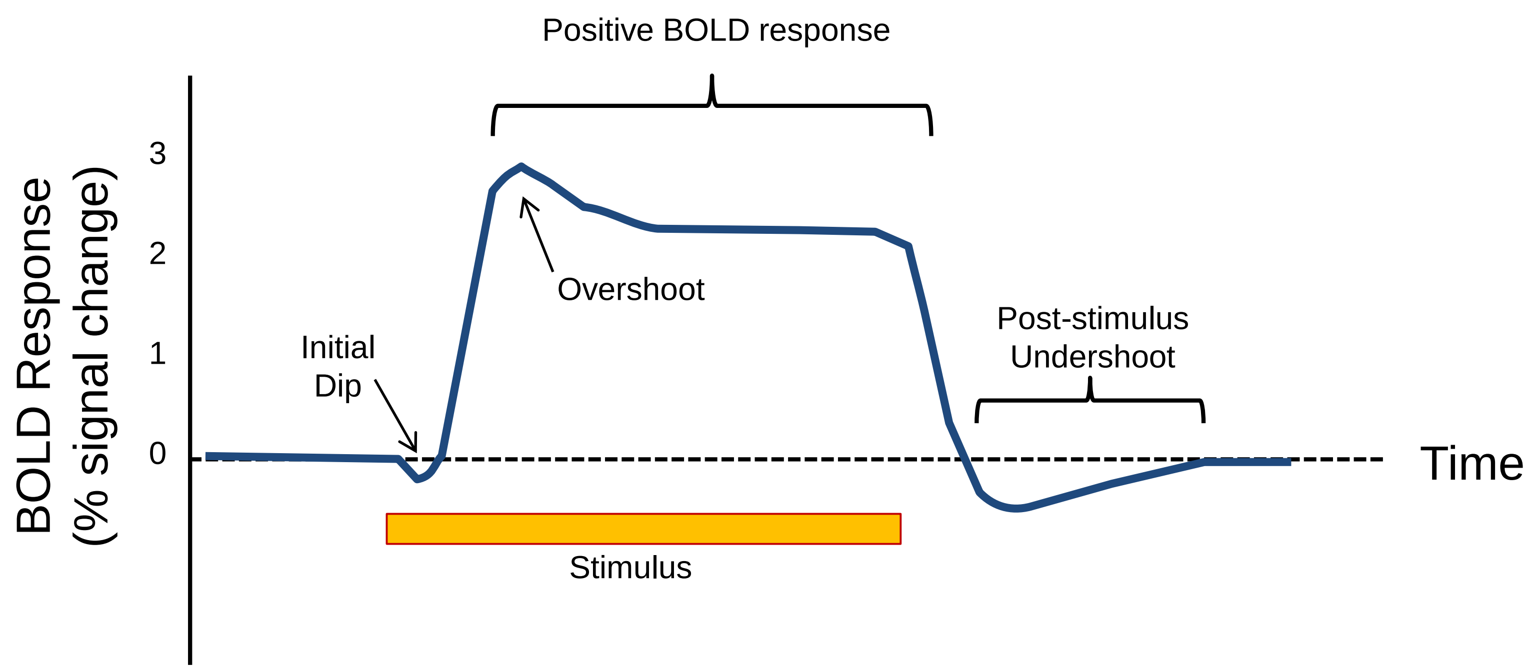
Red: Increased MR Signal

Blue: Decreased MR Signal

Images from Hu et al., 1997

How Does BOLD Relate to Neuronal Activity?

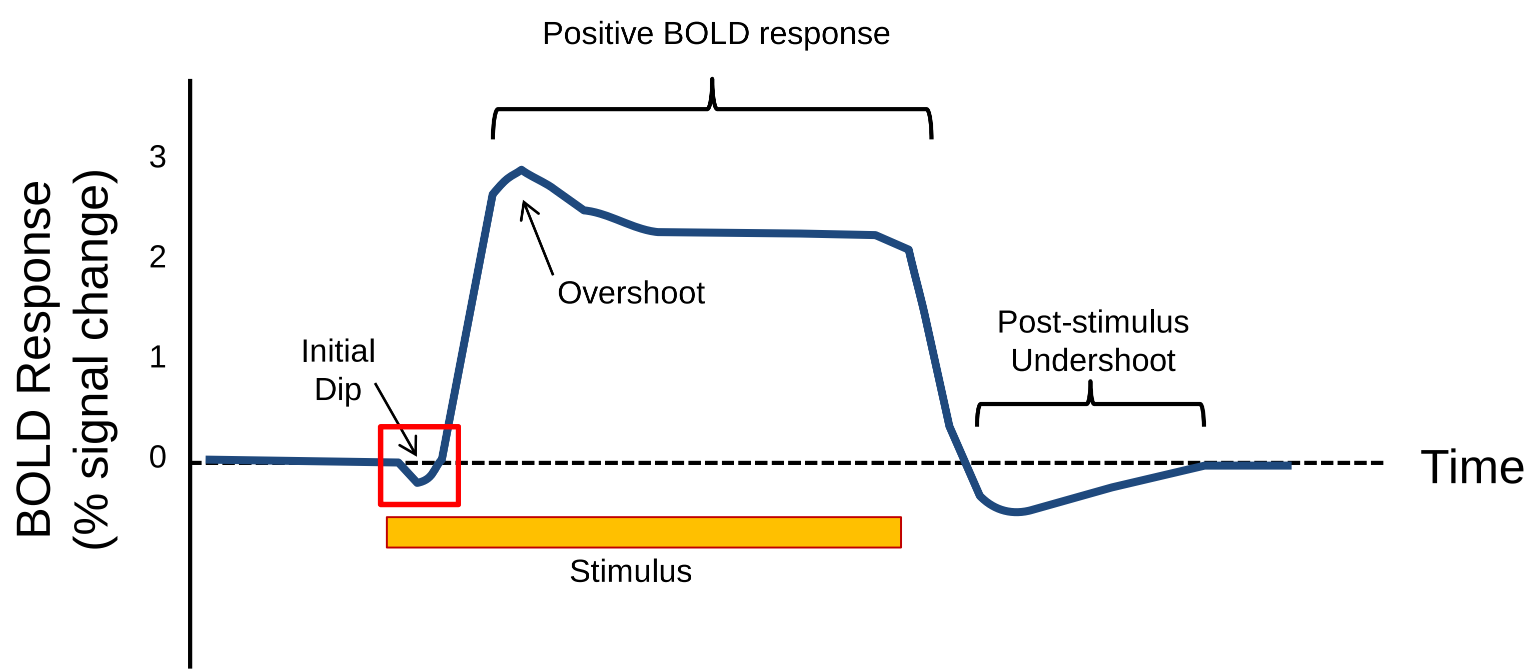
- Typical example of the time course of the MR signal change in response to an extended stimulus:



Plot from Jody Culham

Mechanism: Initial Dip

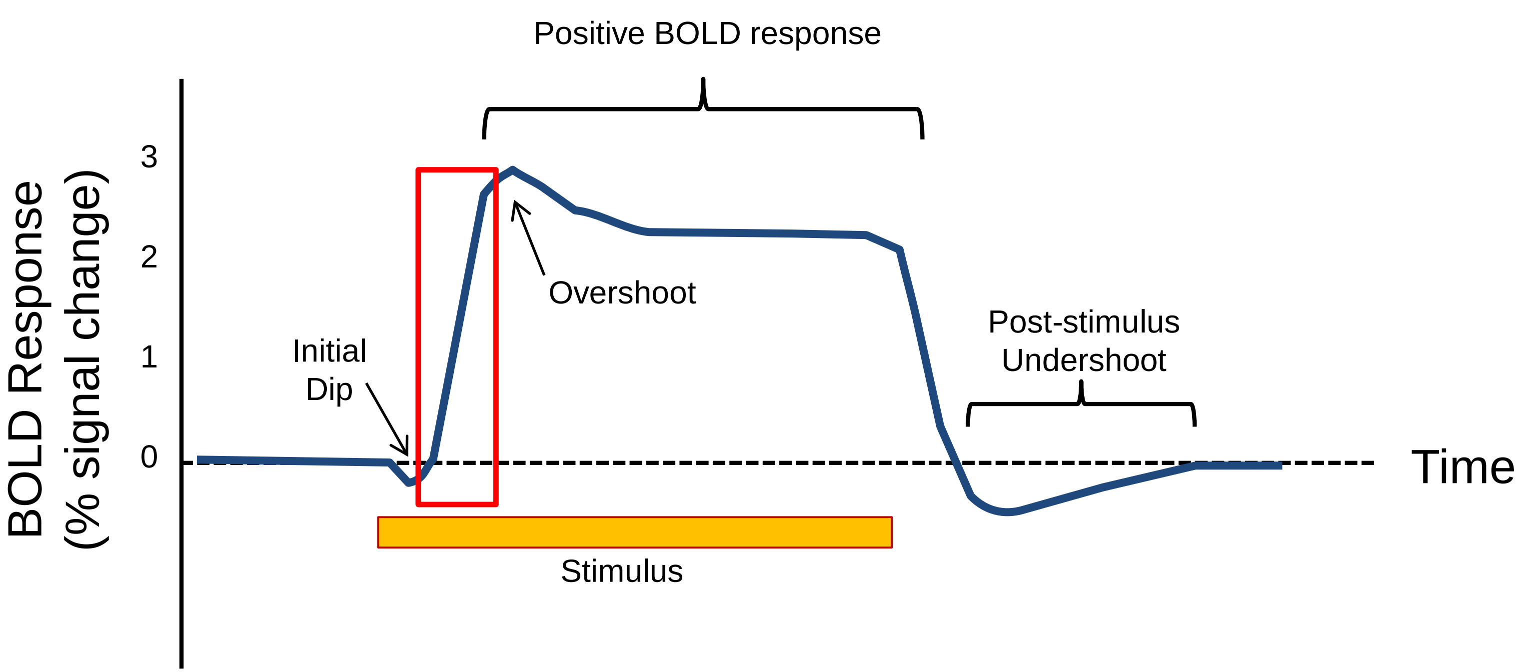
- Stimulus **increases neuronal activity**, which requires more oxygen and increases the amount of deoxygenated blood
  - Leads to a decrease in MR signal intensity
- **“Initial dip”** rather weak and not observed in all studies
  - If observed, often spatially more restricted than main peak



Plot from Jody Culham

Mechanism: Rise

- **Local blood flow increases,** providing more oxygenated blood and **overcompensating** the additional need from neural activity
  - Change in signal strength between 5% (primary sensory stimulation) and 0.1-0.5% (cognitive tasks)
  - Basis of most fMRI studies

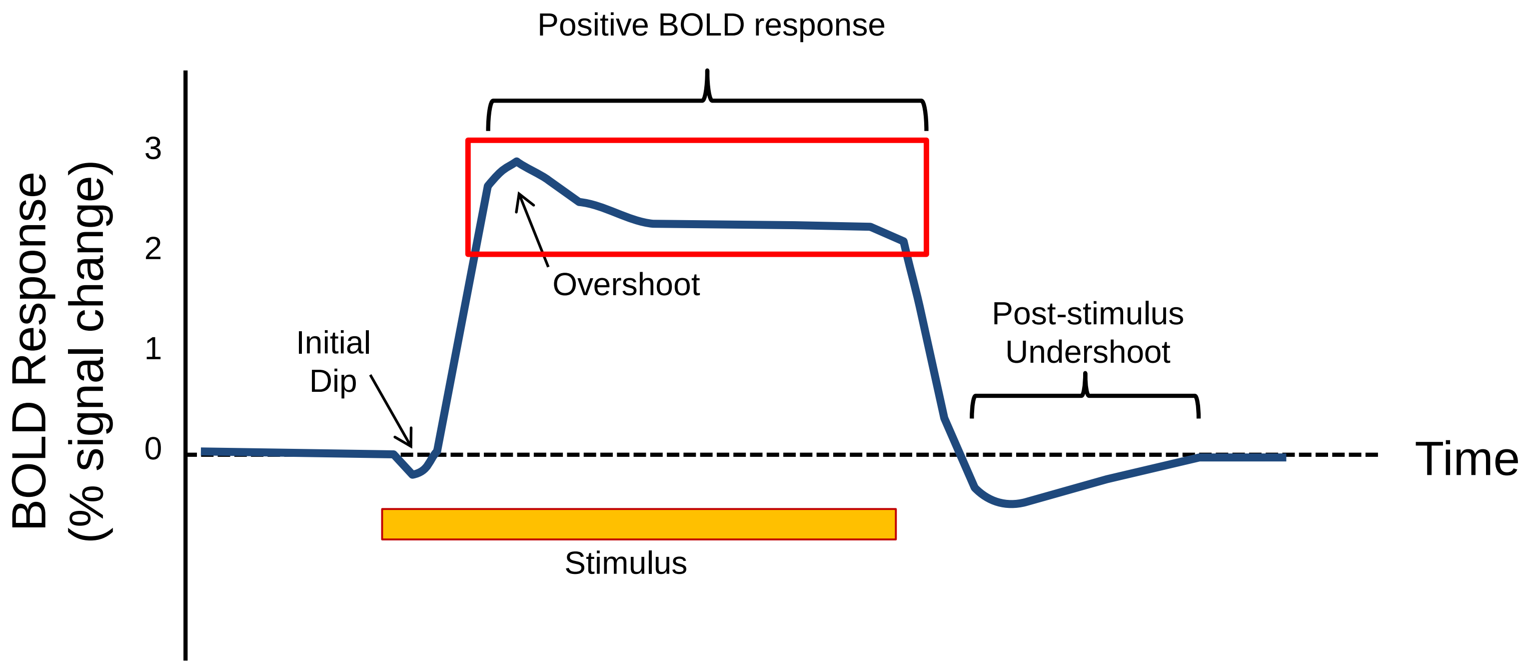


Plot from Jody Culham

Mechanism: Overshoot / Plateau

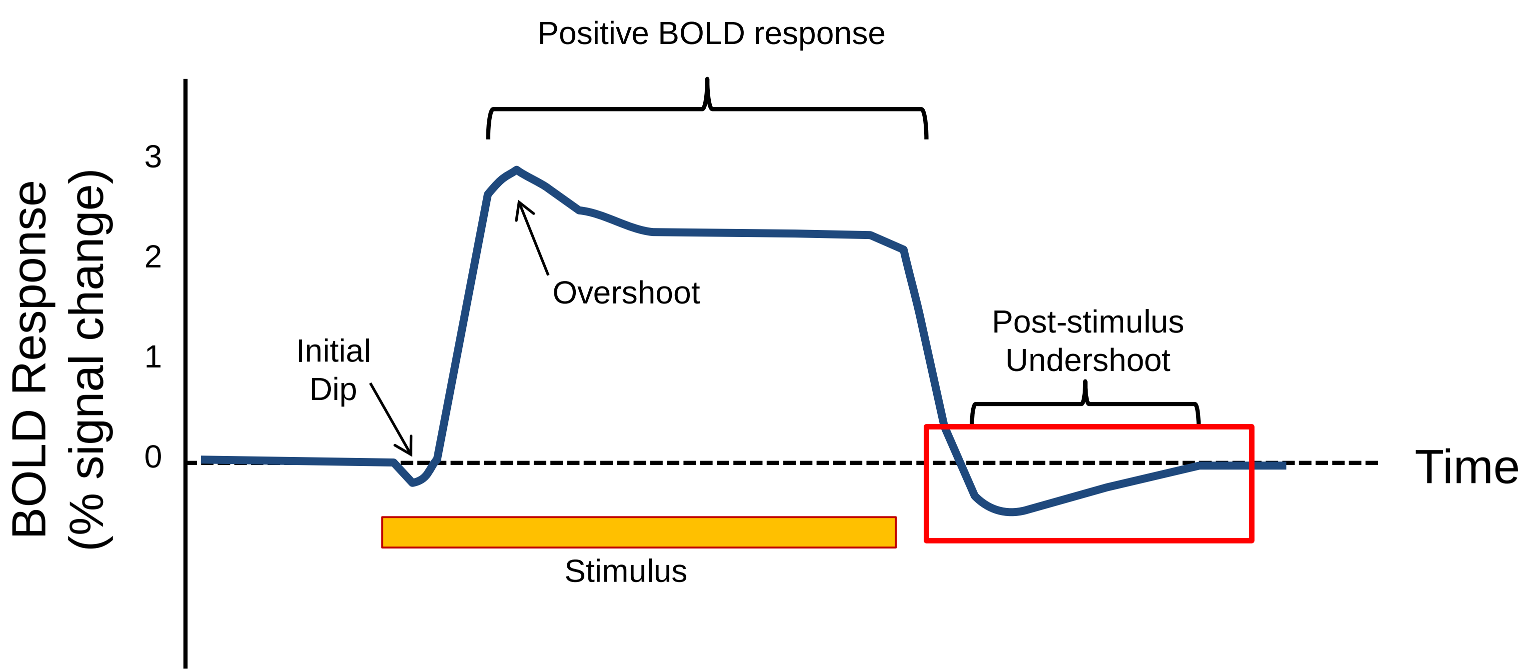
- While neuronal activity persists, increased oxygen uptake continues to be overcompensated
  - In “blocked” fMRI design with extended phases of stimulation, a plateau follows an initial overshoot

Plot from Jody Culham



Mechanism: Post-Stimulus Undershoot

- Many studies observe a **post-stimulus undershoot** in MR intensity that often persists for tens of seconds
  - Mechanism still not fully agreed upon [van Zijl et al., 2012]
  - May indicate uncoupling of metabolic and blood flow response:
    - Blood flow returns to baseline earlier than need for oxygen
    - Much oxygen used while "cleaning up" after activity



Plot from Jody Culham

Mechanism: Overview

- fMRI provides a rather **indirect measure** of neuronal activity

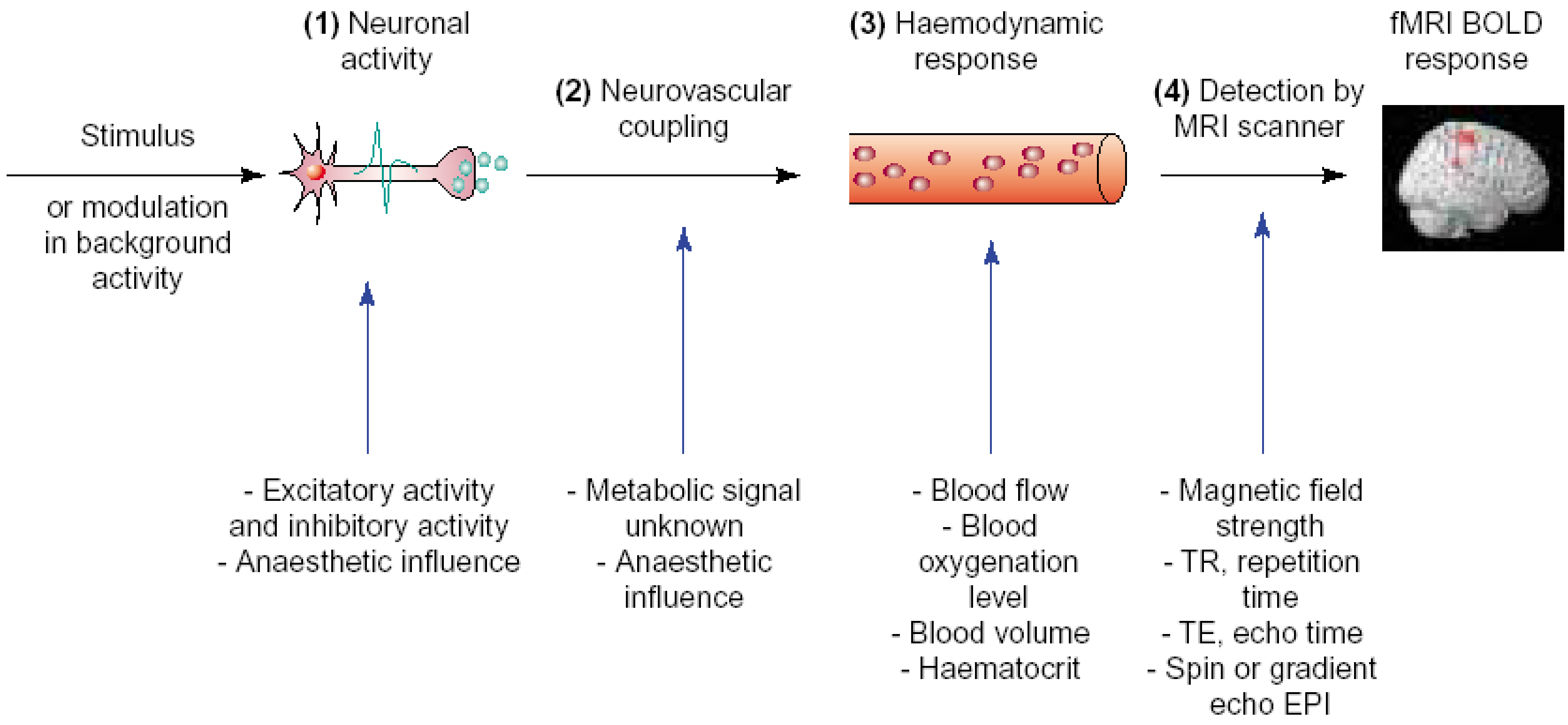
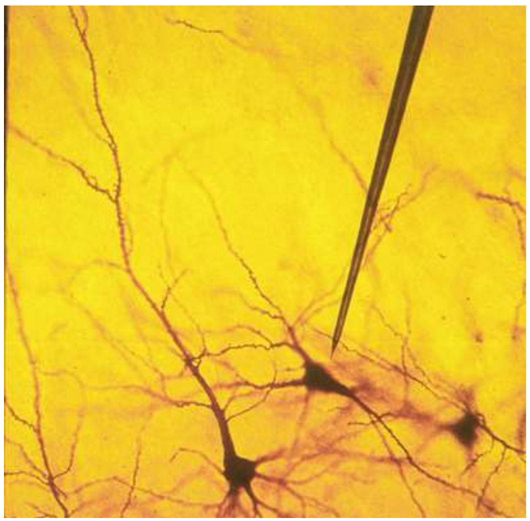


Image from Arthurs / Boniface 2002

Electrophysiology

- Taking **electrical measurements** in the cortex allows us to monitor the neuronal activity:



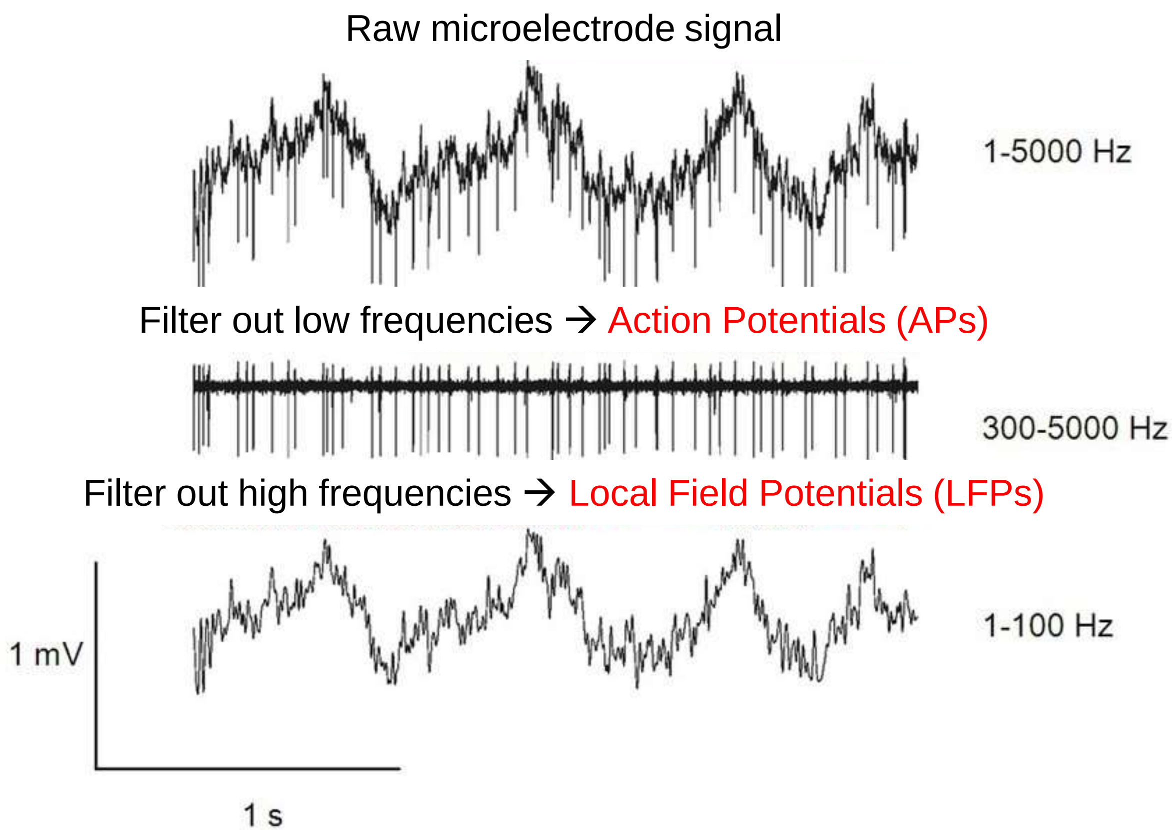
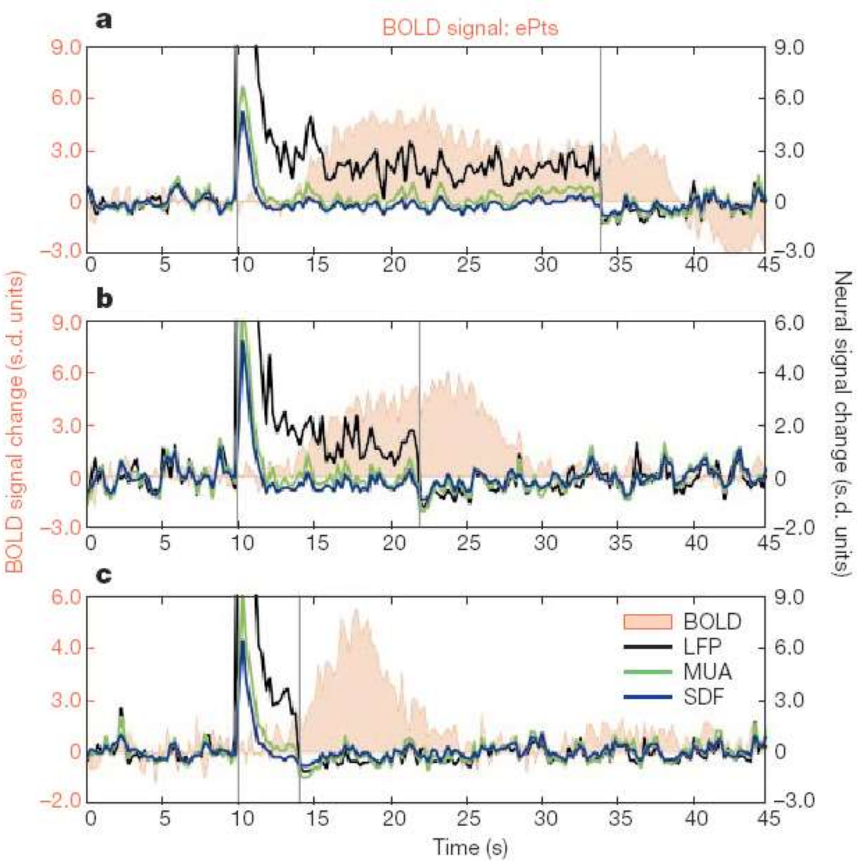


Image from CIN, University of Tübingen

What Does BOLD Measure?

- **Logothetis et al. 2001** performed simultaneous electrophysiology and fMRI in monkeys



Conclusion:

- BOLD correlates more clearly with
  - **local field potentials** (post-synaptic potentials; input) than with
  - **multi-unit activity** (action potentials, output)

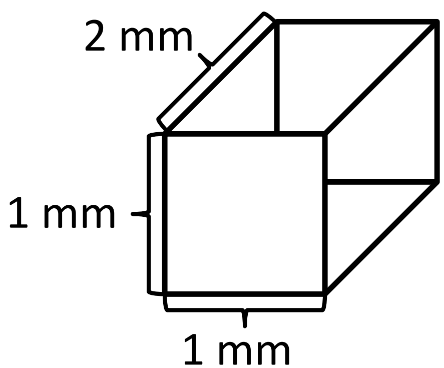
Summary: Functional MRI

- **BOLD effect:** MR intensity reduced by presence of deoxygenated blood
- Amount of deoxygenated blood changes during **neuronal activity**
  - After initial dip, additional need for oxygen is overcompensated, leading to a stronger signal
  - Mechanism still not known in detail, but found to correlate mostly with local field potentials
- **Observing signal change over time** allows us to draw conclusions about brain activity
  - Tradeoff between temporal and spatial resolution

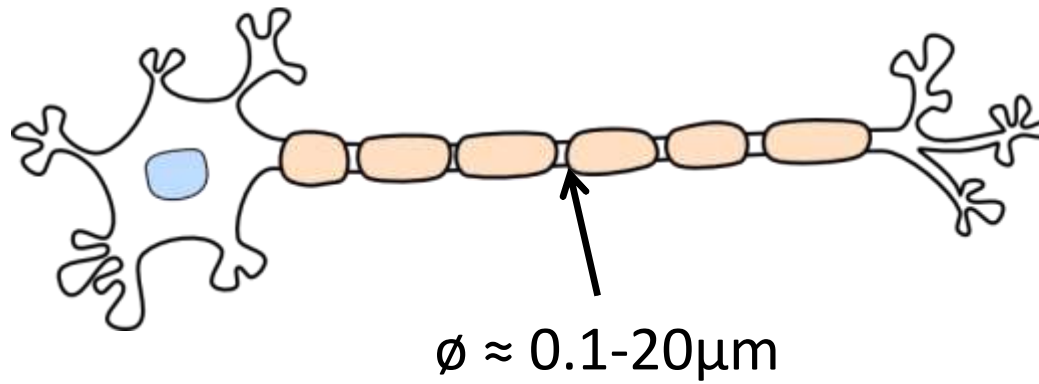
Introduction to Diffusion MRI

Goal: Investigate the microstructure of biological tissue using Magnetic Resonance Imaging (MRI)

Challenge: Voxel size is far too large to resolve the structures of interest



Voxel size



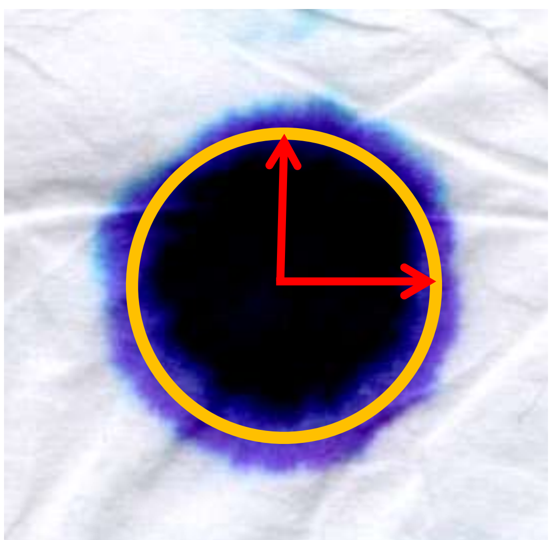
Axon (nerve fiber) size

Introduction to Diffusion MRI

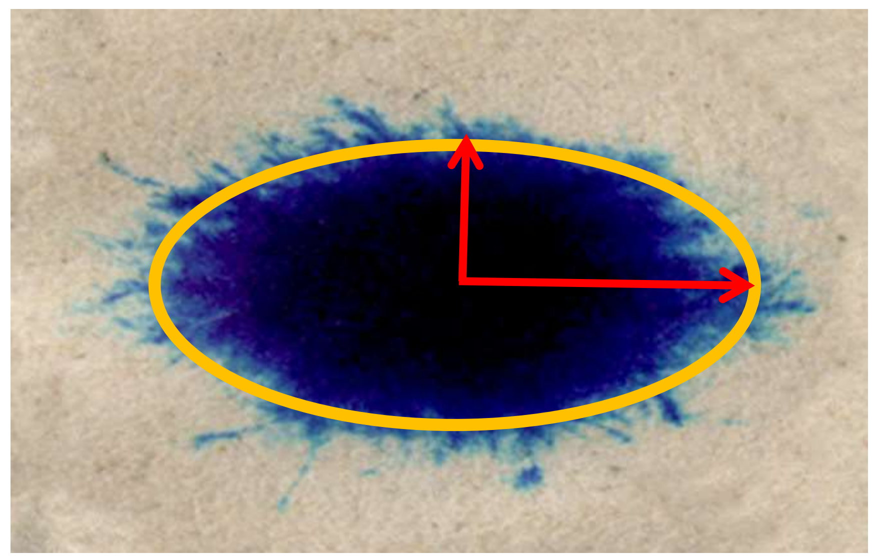
Approach: Use water molecules as a contrast agent

- Exploits their spontaneous heat motion at the desired spatial scale

Analogy: Observe diffusion of ink on paper



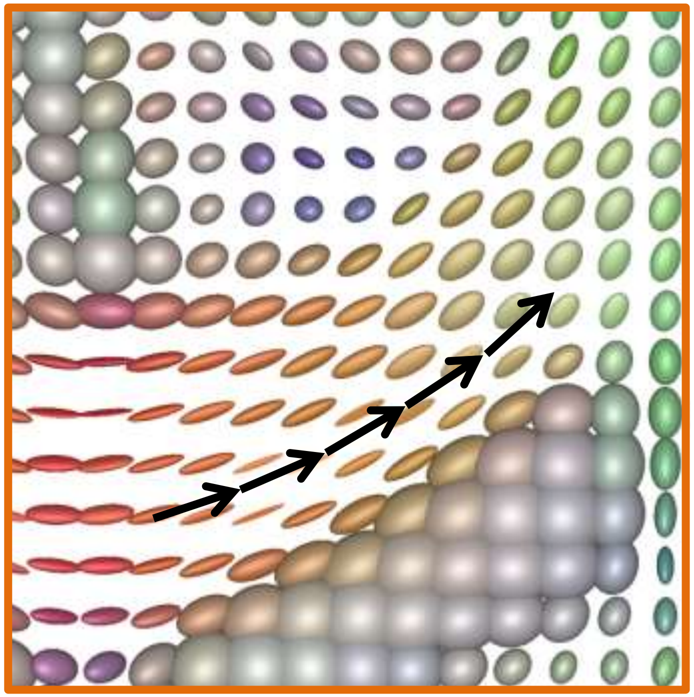
Kleenex

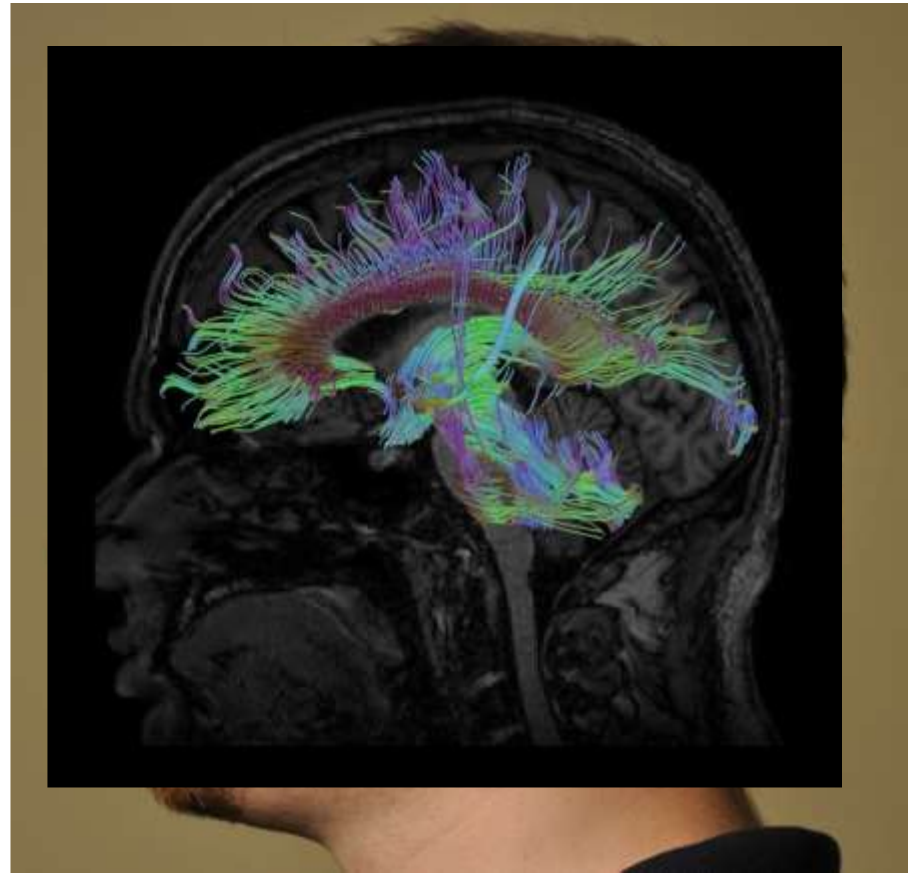


Newspaper

Images by Gordon Kindlmann

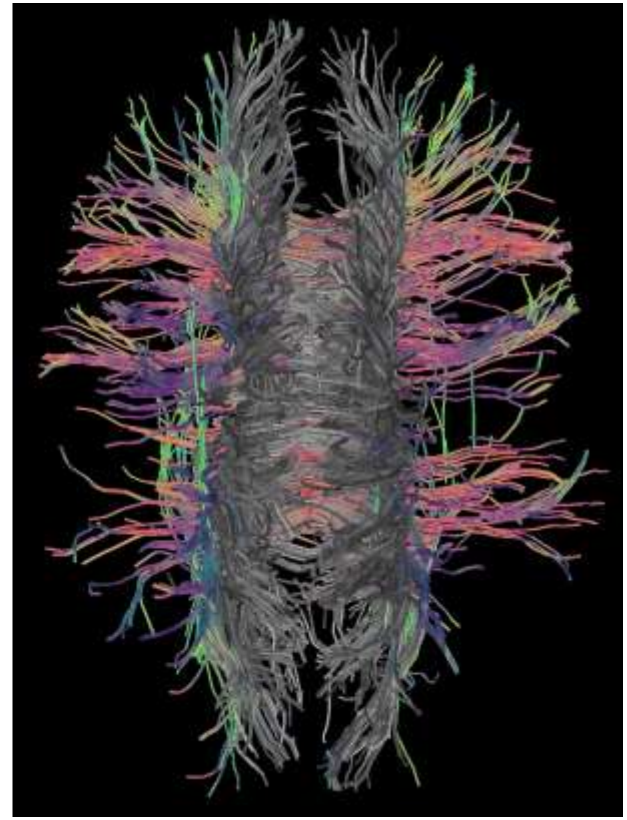
DTI: Glyphs and Tractography





Studying the Human Connectome

- Connectome = complete map of the connections in the human brain
- Even larger-scale than Genome (3×10^9 base pairs vs. $\approx 10^{14}$ synapses)
- NIH Human Connectome Project (1,500 subjects)



[Schultz/Seidel 2008]

- Rhineland Study (Bonn): Prospective study with 30,000 participants, piloting now

Summary

- **Advantages of MRI** as an imaging modality:
  - **No ionizing radiation**
  - **No contrast agents** (except for specific use cases)
  - **Well-suited for soft tissue** such as brain
    - Signal from protons, abundant in H_20
  - Fast enough for **functional imaging**
  - Flexible sequences offer **variety of contrasts**
    - different physical properties (T_1, T_2, diffusion, etc.)
  - Can be made **quantitative** (with some effort)
  - **Flexible orientation** of slices

7.4 Other Imaging Modalities

Computed Tomography (CT/CAT)

- Introduced in 1968, Nobel Prize in 1979
- Based on measuring X-ray absorption from many different directions
  - Algorithmic reconstruction of a slice image
  - Multiple slices combined into a 3D volume
    - Often acquired simultaneously
- Can achieve sub-mm voxel size
  - e.g., inspect fine fractures

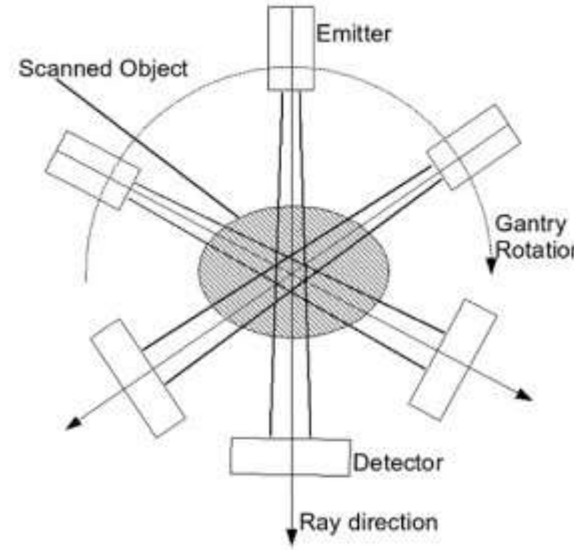


Image from Siemens Medical

MRI vs. CT: Applications

- CT ideally suited for **skeletal structures**
- MRI provides a larger variety of contrasts for **soft tissue** (e.g., brain)
- Unlike MRI, CT is **quantitative**
  - Disclaimer: Some variants of MRI can also be made quantitative
- CT **cheaper** and **more widely available**

CT scan

MRI scan

MRI vs. CT: Safety

- X-ray radiation in CT is **ionizing** and potentially harmful to biological tissue
  – Rules on allowable dose
- MRI has **no known harmful effects,** safe to use on healthy volunteers
  – Footnote: Beware of implants

...and office chairs

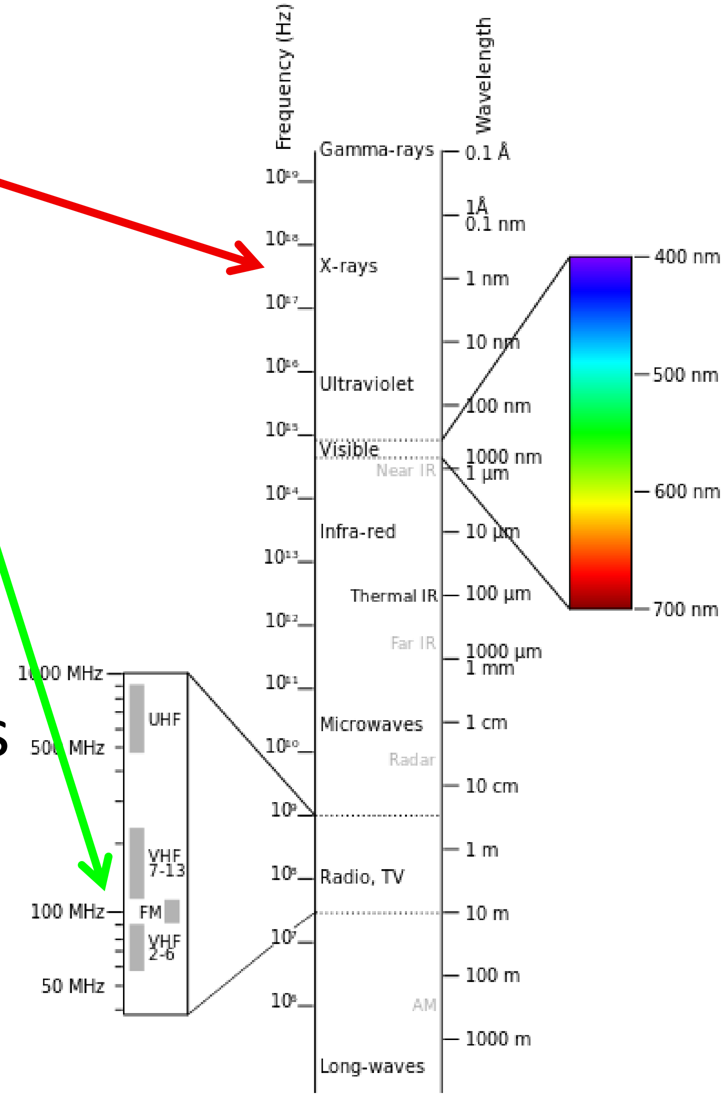
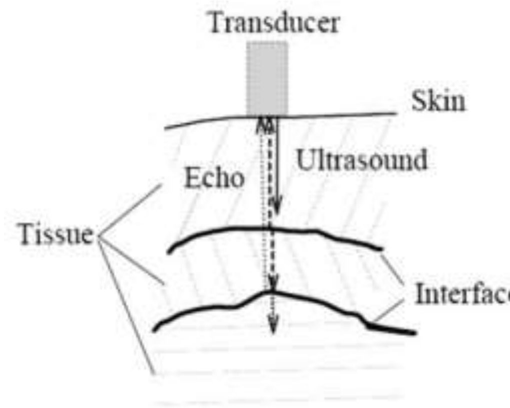
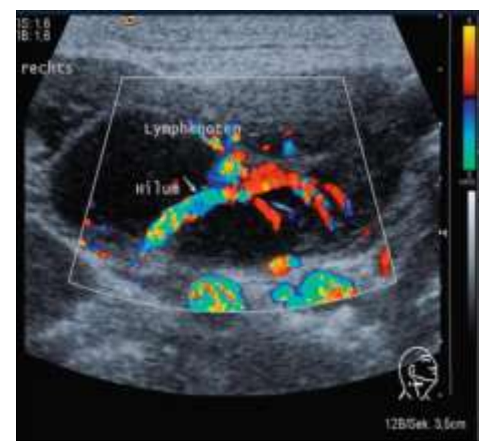


Image Sources: Victor Blacus / www.impactednurse.com

Ultrasound

- Based on transmitting **high-frequency** (MHz) **acoustic waves** and measuring their echo
- Simple, cheap, safe, but relatively poor image quality (noise level, sharpness, shadows…)
- **Real-time results**
- Combining measurements from a tracked ultrasound probe allows for **3D reconstructions**





Images from Preim / Botha

Summary

- The wide range of **imaging techniques used in biology** includes
  - Light microscopy (few hundred nm resolution)
    - Fluorescence microscopy (high specificity, 3D)
  - Electron microscopy (down to pm resolution)
  - Magnetic Resonance Imaging (cm resolution, but noninvasive, 3D, functional / diffusion available)
  - CT
  - Ultrasound

References

- B. Preim, C. Botha: *Visual Computing for Medicine: Theory, Algorithms, and Applications,* Morgan Kaufmann, 2014
  - **E-book available within UBonn network!**
- M. A. Flower (Ed): *Webb‘s Physics of Medical Imaging,* 2nd edition, CRC Press 2012
- *Magnets, Spins and Resonances,* Siemens Healthcare (available online)