9.17
Practical Lab
Feb 11, 2013

Where to Record?

Surface landmarks (occasionally) ← cockroach, fly

*Stereotaxic coordinates (usually for two or three dimensions) ← rat

Physiological indicators (often for third dimension, depth)

Surface landmarks

Sometimes (not usually) the correct location can be identified relative to a specific sulcus (depression or fissure) or gyrus (ridge) or other surface feature.

Diagram of the location and anatomy of the piriform cortex removed due to copyright restrictions. See Figure 1A. Suzuki, Norimitsu, and John M. Bekkers. "Inhibitory Interneurons in the Piriform Cortex." *Proceedings of the Australian Physiological Society* 38 (2007): 9-14.

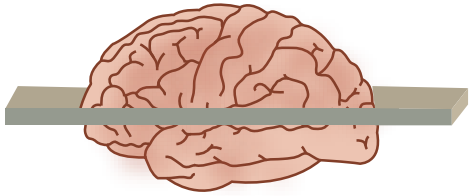
anterior piriform cortex (aPC), between the lateral olfactory tract (LOT) and rhinal fissure (rf).

Diagram removed due to copyright restrictions.

crus IIa, a particular fold of the cerebellar cortex

Planes of the Brain

dorsal (animal)
superior (human)



Horizontal plane
Axial plane
(human)

Image by MIT OpenCourseWare.

ventral (animal)
inferior (human)

Sagittal
plane

lateral (right)

medial

lateral (left)

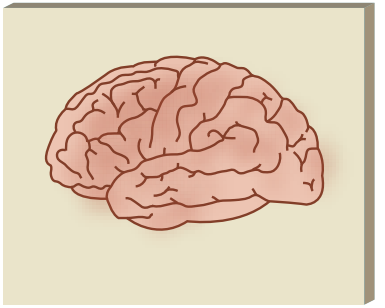
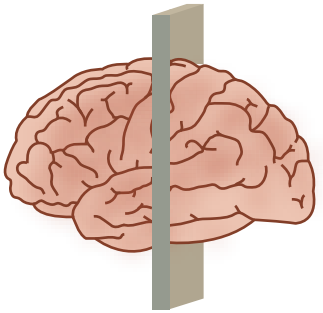


Image by MIT OpenCourseWare.

Coronal plane
Frontal plane



rostral (animal)
anterior (human)

caudal (animal)
posterior (human)

Image by MIT OpenCourseWare.

Vernier Scaling

Named for French mathematician Pierre Vernier.

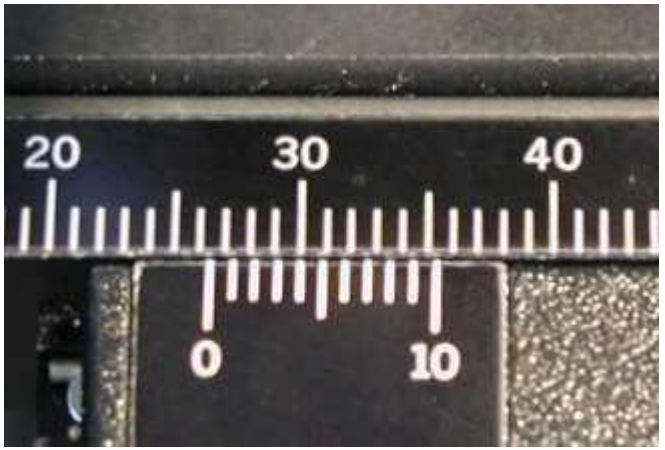
Permits more precise measurements.

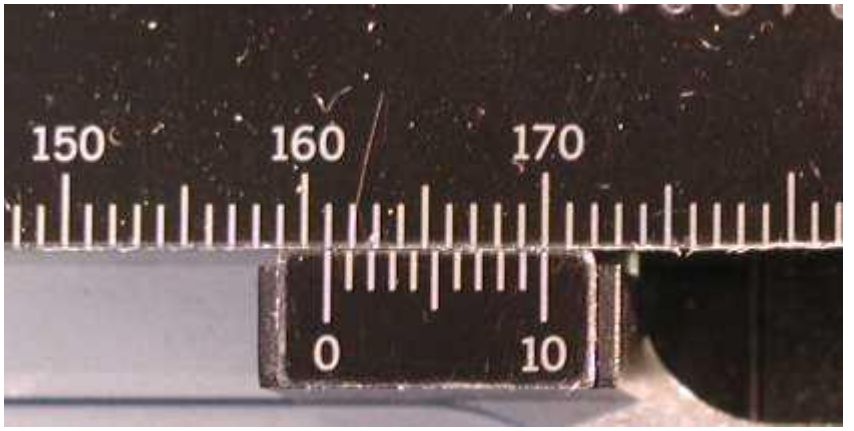
A primary scale corresponding to actual size (e.g. one division equals 1.0 mm), and a sliding scale with 10 divisions per 9 divisions of the fixed scale (e.g. one division equals 0.9 mm).

Read the primary scale number just to the left of (or aligned with) the sliding scale's zero. Then add a decimal corresponding to the sliding scale mark which exactly lines up with a mark of the primary scale.

primary scale

sliding scale





26.4

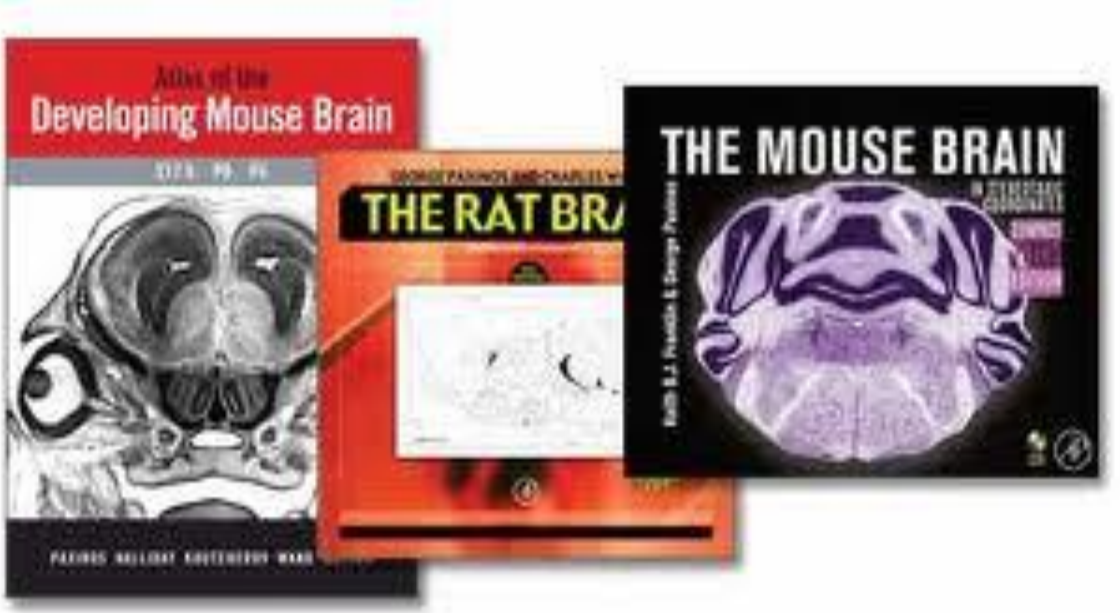
161.0

Choosing coordinates:

Brain atlas

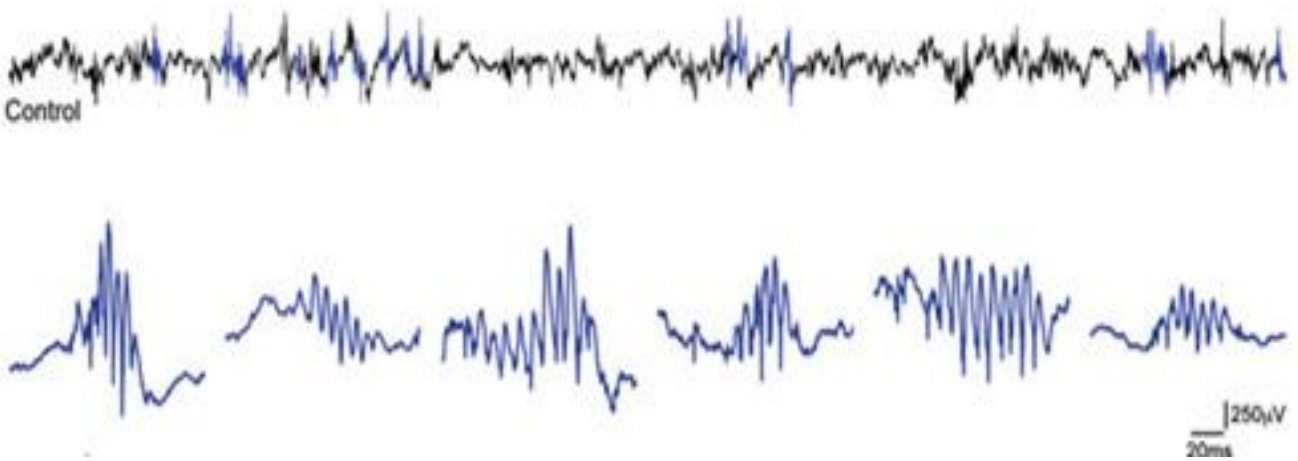
Published reports

Trial and error (your rats may differ...)



Physiological monitoring

Spontaneous local field potentials (LFP)

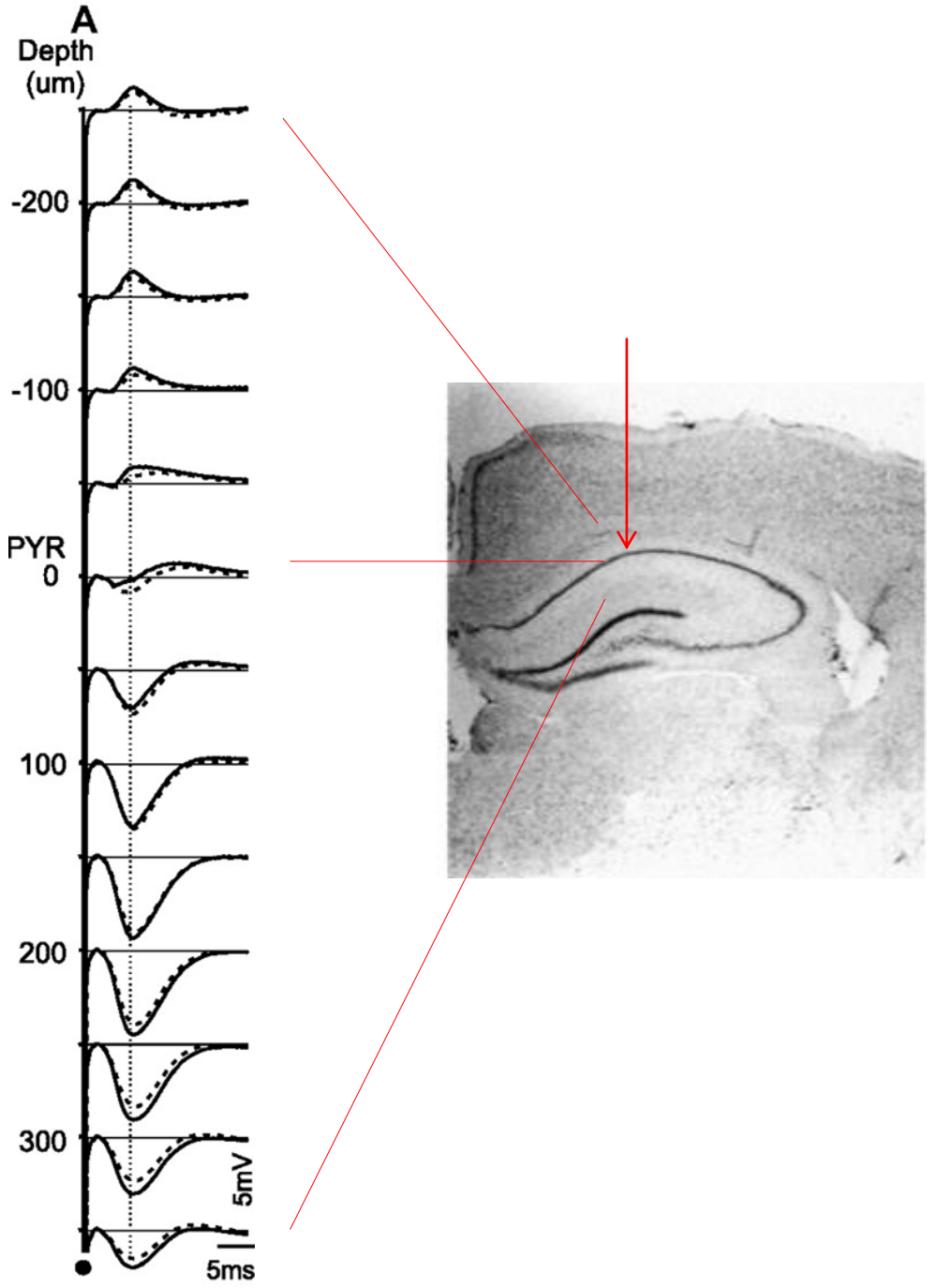


200 Hz “ripples”
localized to pyramidal
cell layer of CA1

Physiological monitoring

Spontaneous local field potentials (LFP)

Evoked field potentials



Field potentials evoked by orthodromic stimulation – note difference in sign above/ below the pyramidal cell layer.

Physiological monitoring

Spontaneous local field potentials (LFP)

Evoked field potentials

Units! Number of cells and their amplitudes will increase as electrode approaches

Physiological monitoring

Spontaneous local field potentials (LFP)

Evoked field potentials

Units! Some cells have distinctive action potentials

Dopaminergic cells in substantia nigra have spikes with unusually long duration, often complex shape.

Where to Record?

Surface landmarks (occasionally)

- Sulci and gyri

Stereotaxy (usually for two or three dimensions)

- Anterior/posterior (a.k.a. rostral/caudal) and medial/lateral from bregma
- Depth from cortical surface
- Vernier scaling allows greater precision

Physiological indicators (often for third dimension, depth)

- Spontaneous LFP
- Evoked potentials
- Units – Number and amplitude
  - Distinctive waveform

How to Record?

Electrode
Filter
Amplifier
Analog/digital converter (A/D)
Display and data storage
Analysis

Remember: Voltages are always defined between two points!

Membrane potential: Intracellular vs. extracellular
(mV or 10s of mV)

Local field potential (LFP) or units: Extracellular vs. distant reference electrode
(10s or 100s of μV)

9.17

Remember: Voltages are always defined between two points!

Membrane potential: Intracellular vs. extracellular
(mV or 10s of mV)

Local field potential (LFP) or units: Extracellular vs. distant reference electrode
(10s or 100s of μV)

Locally generated dipole: Extracellular vs. nearby extracellular
(excludes “volume conducted” signals from other brain areas)

Cortical neurons figure removed due to copyright restrictions. See right half of Figure 5. Aguiar, Paulo, André David, et al. “Eegsolver - Brain Activity and Genetic Algorithms.” *Proceedings of the 2000 ACM Symposium on Applied Computing* 1 (2000): 80-4.

Electrodes – Glass, sharp (*in vitro*, *in vivo* anesthetized; extra- or intracellular)

Also called a “micropipette”.

Pulled from glass tubing to a very fine taper.

Filled with electrolyte solution.

Pros: Fine tip can penetrate small structures
e.g. dendritic branches.
Minimal dialysis of cell contents.

Cons: Cannot effectively voltage clamp.

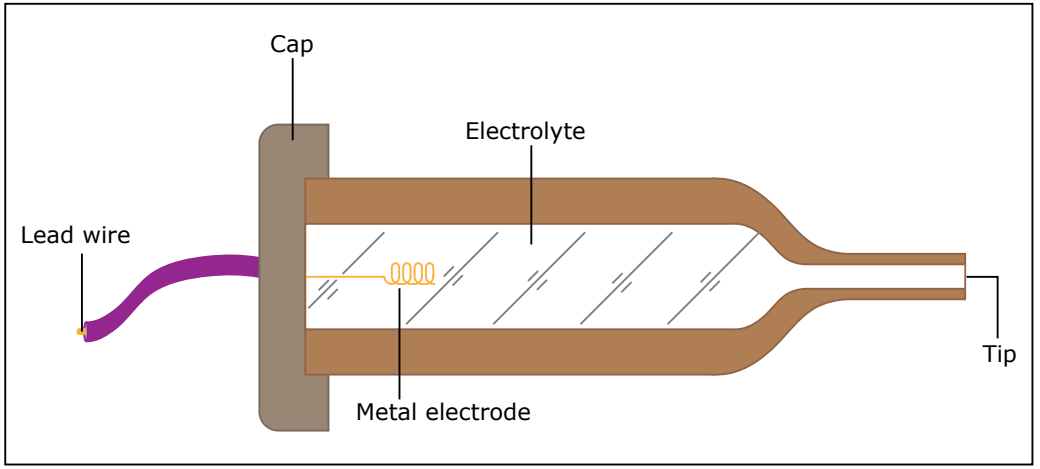


Image by MIT OpenCourseWare.

micropipette puller

PC-10 micropipette puller figure removed due to copyright restrictions.
See: http://products.narishige-group.com/group1/PC-10/injection/english.html.

Electrodes – Glass, patch (*in vitro*, *in vivo* anesthetized (blind); extra- or intracellular)

Pulled from glass tubing to a wider tip, polished.

Brought close to cell membrane, suction applied, forms a very tight seal.

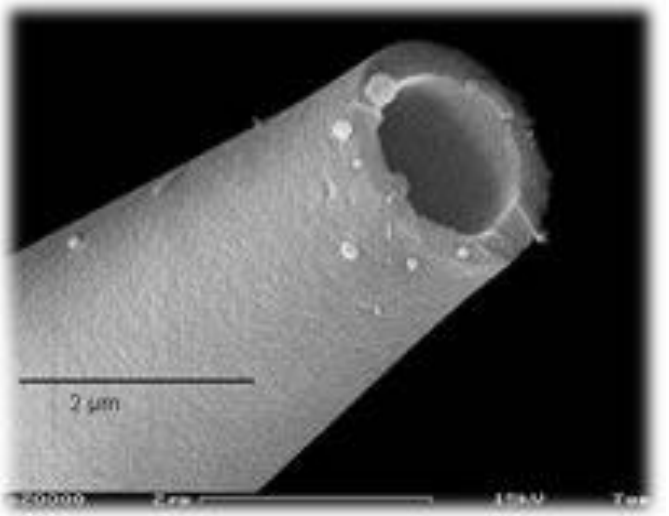
Pros: Can voltage clamp, but only larger cell structures.

Dialysis of cell contents = introduce compounds.

Sub-threshold detection.

Cons: Dialysis of cell contents (can use "perforated patch" to avoid).

Get fewer cells at a time, difficult to do in freely-moving animal.



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scanning electron micrograph (SEM)

infrared differential interference contrast (IR-DIC)

cell filled with Ca^{2+}-sensitive dye

Patch pipette image removed due to copyright restrictions. See figure 2 http://www.leica-microsystems.com/science-lab/the-patch-clamp-technique/.

Figure removed due to copyright restrictions. See: http://stokes.chop.edu/web/coulter/techniques.php.

Electrodes – Metal (*in vitro*, *in vivo* anesthetized or chronic (wires); extracellular)

Tungsten, stainless steel, platinum/iridium. ← rat, fly

Insulated with polymer except at the tip.

Pros: Stiff for recording from deep structures; reusable.

Generally good extracellular unit recordings.

Cons: Extracellular only.

Metal electrode tip guide removed due to copyright restrictions. See: http://www.science-products.com/Products/CatalogG/MPI-Electrodes/MPI.html.

Electrodes – Tetrodes (*in vitro*, *in vivo* anesthetized or chronic; extracellular)

Four thin wires twisted together.

Insulated with polymer, fused.

Pros: Record and isolate multiple single units from one tetrode.

Important where cells are densely packed e.g. CA1

Cons: More recording channels needed.

Electrodes – Tetrodes (*in vitro*, *in vivo* anesthetized or chronic; extracellular)

Spike sorting: Each cell A through D can be detected by each wire 1 through 4, but the amplitude profiles differ. The spikes from a single cell will appear as a "cluster" in the cross-amplitude plots.

Schematic of the relationship between wires of a tetrode and cells in the brain, their waveforms and plots removed due to copyright restrictions. See Figure 6.16.6. Szymusiak, R., and Nitz, D. "Chronic Recording of Extracellular Neuronal Activity in Behaving Animals." *Current Protocols in Neuroscience* 21 (2003): 6.16.1–6.16.24. DOI: 10.1002/0471142301.ns0616s21.

Electrodes – Silicon (*in vitro*, *in vivo* anesthetized or chronic; extracellular)

Etched from silicon substrate; integrated circuit manufacturing methods.

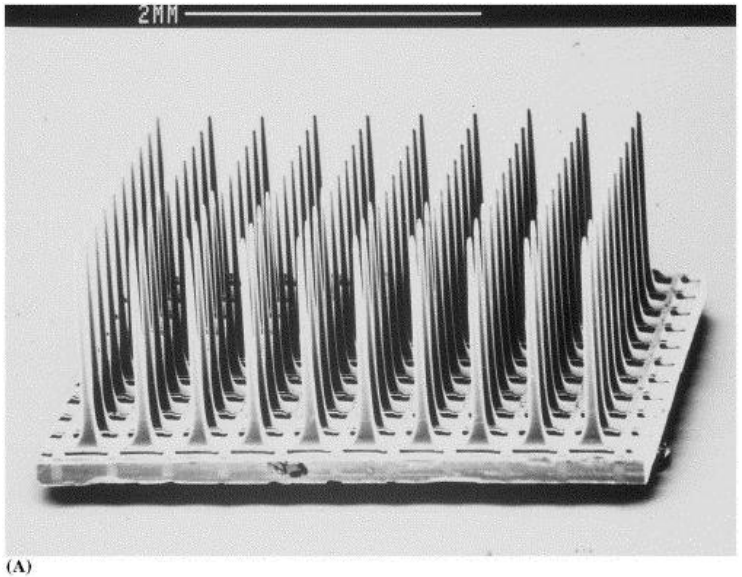
Pros: Multiple recording locations precisely spaced; current source density (CSD) analysis of field potentials.

Potentially, on-board circuitry.

Cons: Poorer unit isolation than tetrodes.

Recording locations not individually adjustable.

"Utah" probe: one recording site at each tip



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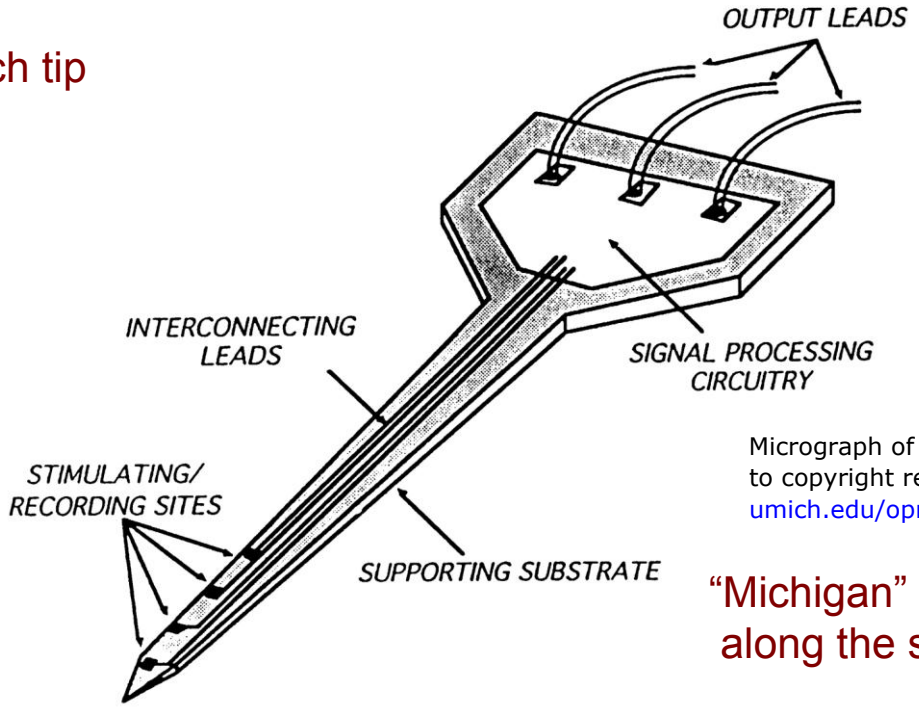


Image: Public Domain. Fig. 1. Micromachined Stimulating Electrodes, Final Report Contract (1999). NIH-NINDS-N01-NS-5-2335.

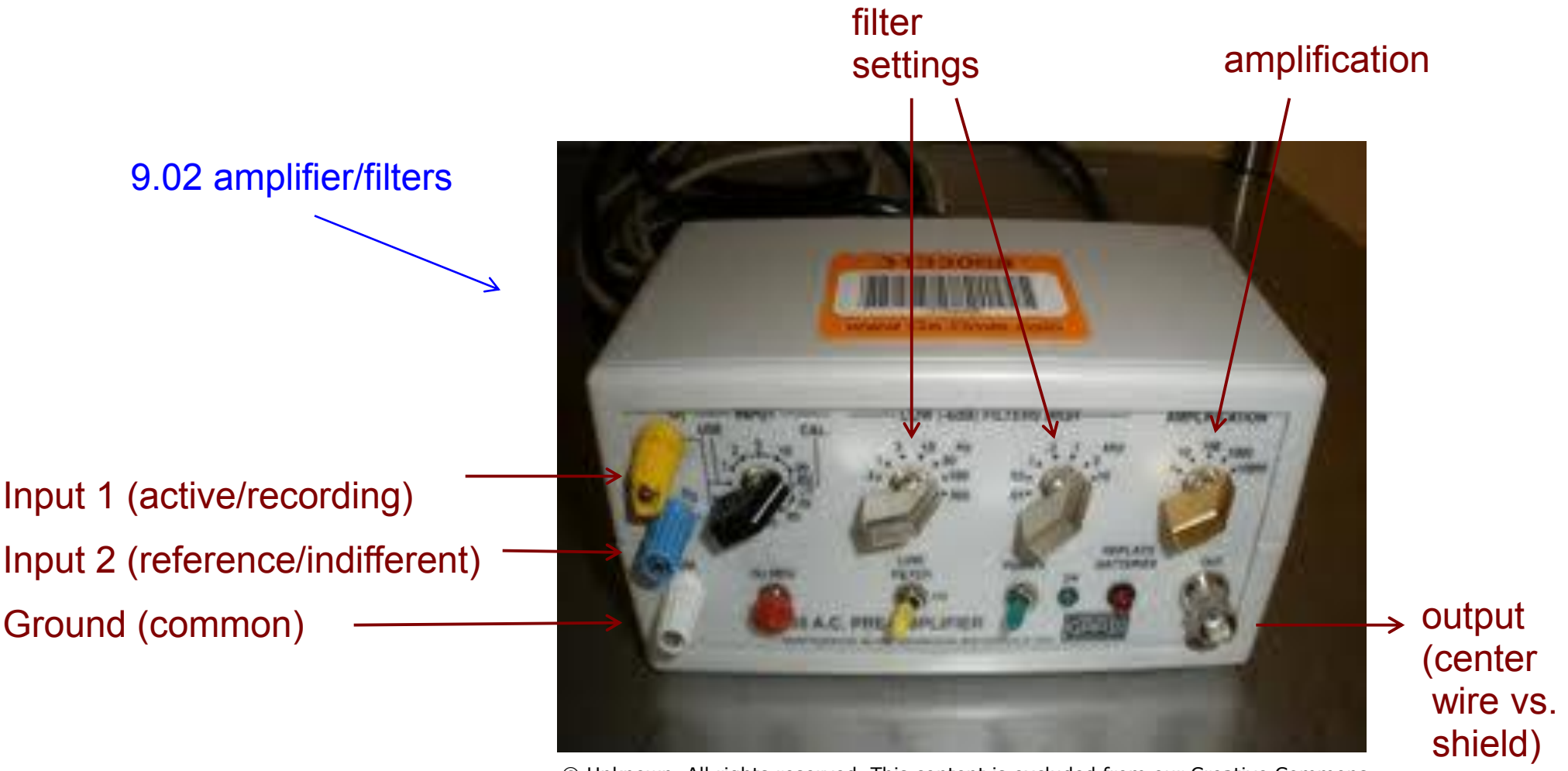
Micrograph of Michigan probe removed due to copyright restrictions. See http://www.med.umich.edu/opm/newspage/2007/hd.htm.

"Michigan" probe: multiple sites along the shaft

Filters and Amplifiers

Filters are often built in to the amplifier

Filtering generally comes first (remove signal components that might cause amplifier to saturate)



Filters and Amplifiers

A unity gain (or “voltage follower”) pre-amplifier is sometimes used right after the electrode. This doesn't change the voltage level of the signal, but reduces impedance to prevent the cable to the amplifier acting as an antenna.

Especially important for behaving animals, which require a long (and moving) cable.

Filtering

What is filtering? What is it good for?

Filtering is a *frequency-domain* operation. It removes part of the signal, corresponding to certain frequencies, and lets other parts of the signal through.

It is useful because we often care about only certain parts of the signal, and consider other parts to be “noise”.

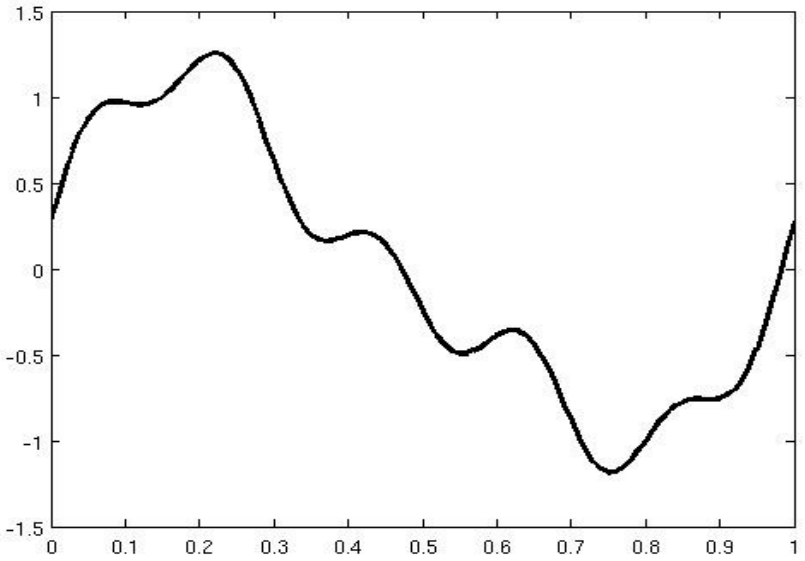
Often, the part of the signal that we care about and the noise occur at different frequencies.

Fourier transforms

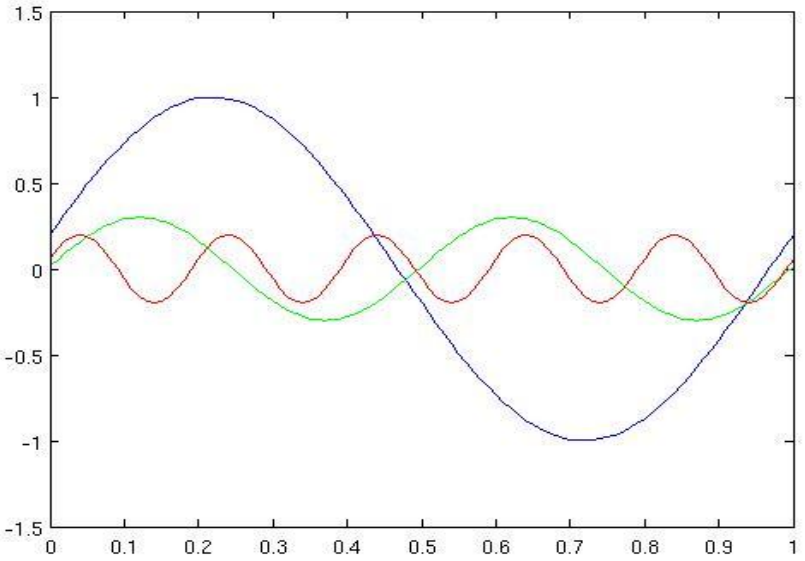
Every signal can be represented as the sum of several sinusoids.

In particular, we can use the fundamental (the sinusoid with period equal to the duration of the signal) and its harmonics (sinusoids with frequencies that are integral multiples of the fundamental frequency).

If we can correctly guess the amplitude and phase for the fundamental and each harmonic, we will fully describe the signal.



time



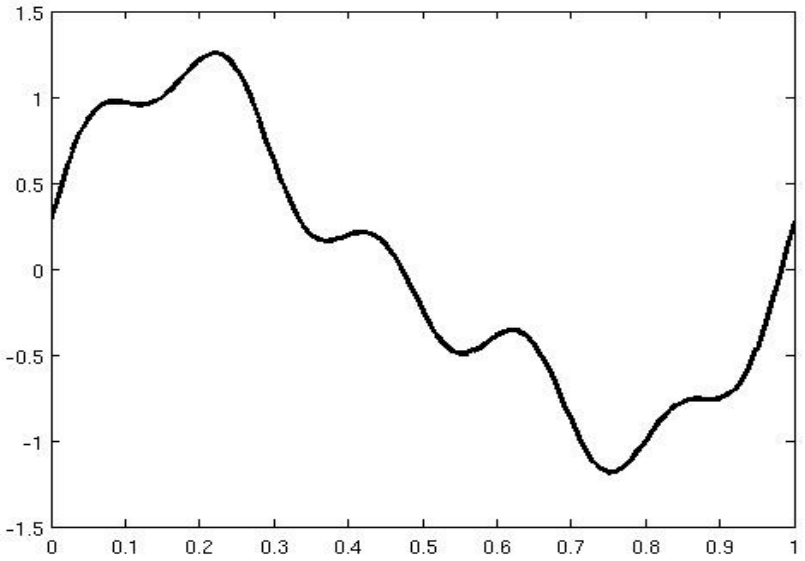
time

1.0 sin(2π t + 0.2)
0.3 sin(4π t + 0.05)
0.2 sin(10π t + 0.1)

Fourier transforms

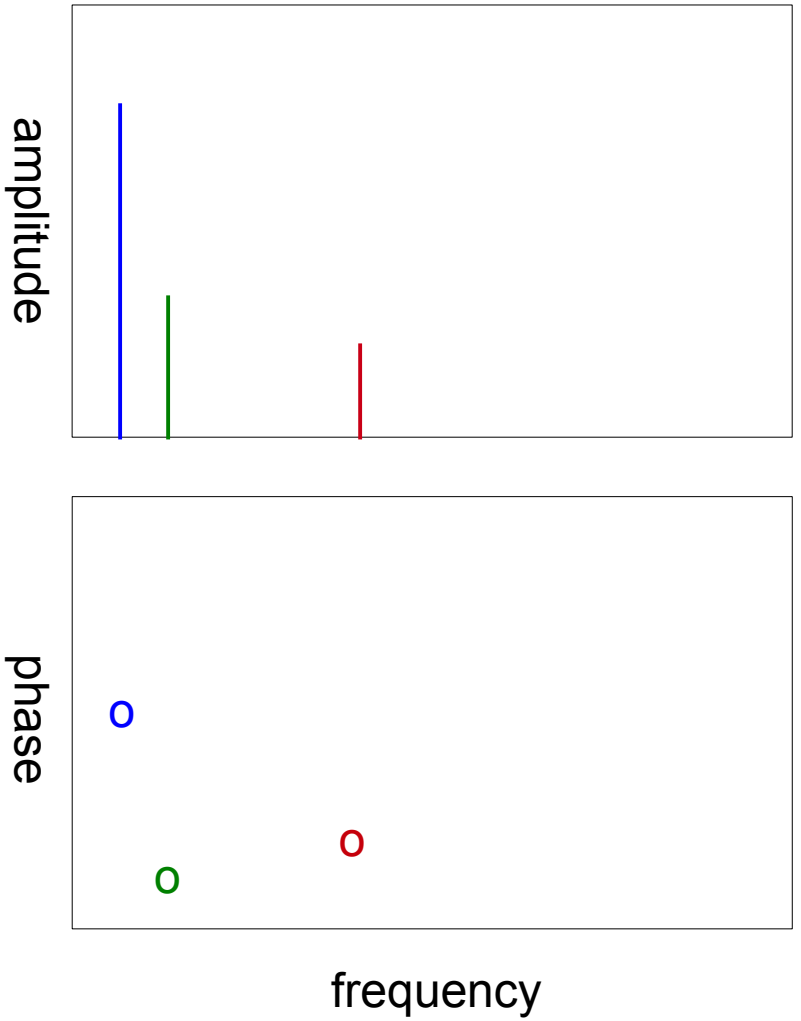
A formula exists that tells us the required amplitudes and phases. This is the **Fourier transform.**

A formula also exists for the inverse operation: the inverse fourier transform.



Fourier transform

inverse Fourier transform



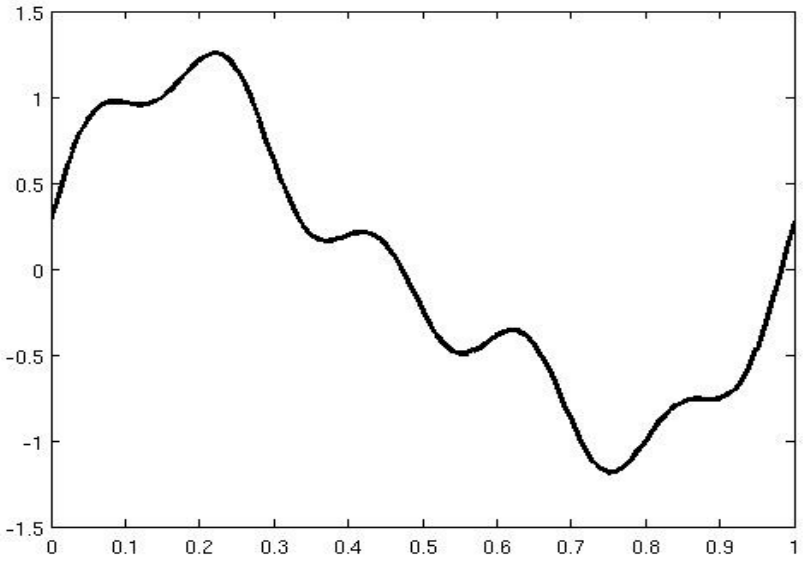
Fourier transforms

We call these two representations “time domain” and “frequency domain”.

They contain exactly the same information!

We refer to frequency domain information as a **spectrum**.

time domain representation

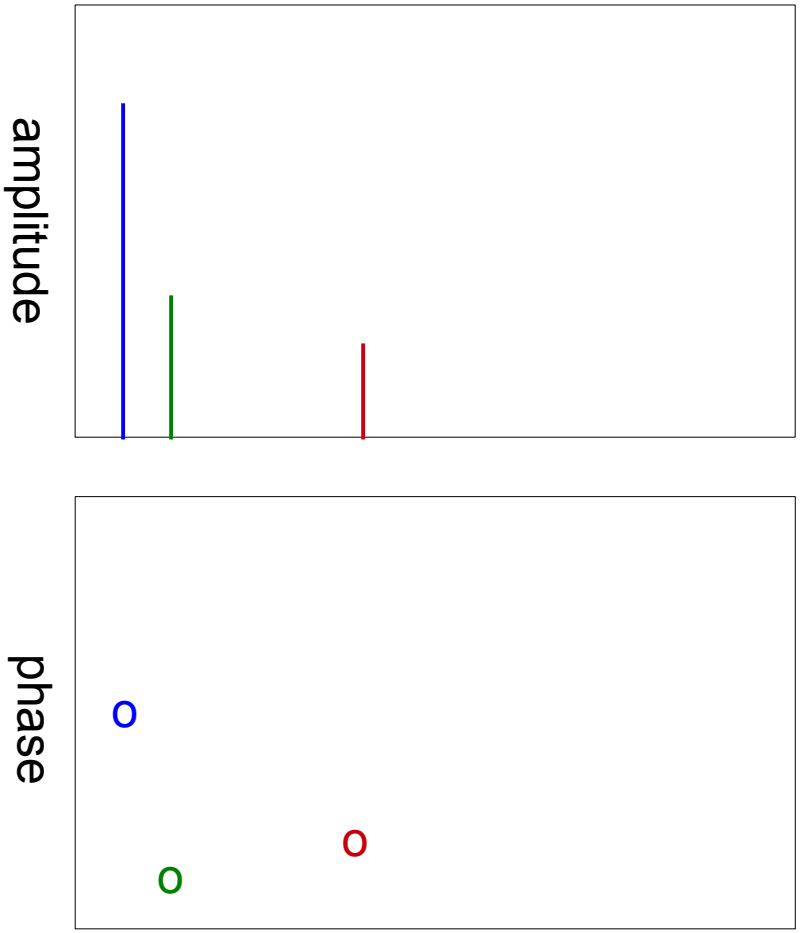


time (s)

Fourier transform

inverse Fourier transform

frequency domain representation



frequency (Hz)

Fourier transforms

$$G(f) = \int_{-\infty}^{\infty} g(t) e^{-i2\pi f t} dt$$

(you do not need to know this formula)

Amplitude, power, phase, coherence --

The Fourier transform returns complex values for each frequency.

The absolute value is the amplitude at that frequency, and collectively they form the amplitude spectrum. More commonly, the square of the amplitude is reported as the power spectrum.

The angle of the complex value describes the phase. For a single signal starting at an arbitrary time, this isn't very useful. What is sometimes described as the “phase spectrum” is actually the cross-phase spectrum, which describes the relative phases between two different signals.

Finally, coherence between two signals resembles a correlation coefficient, in the frequency domain – if the power at e.g. 10 Hz is correlated between the signals (with a consistent phase), their coherence at 10 Hz will be high. This may be taken as an indication of functional coupling between two brain areas, for example.

A few more terms ....

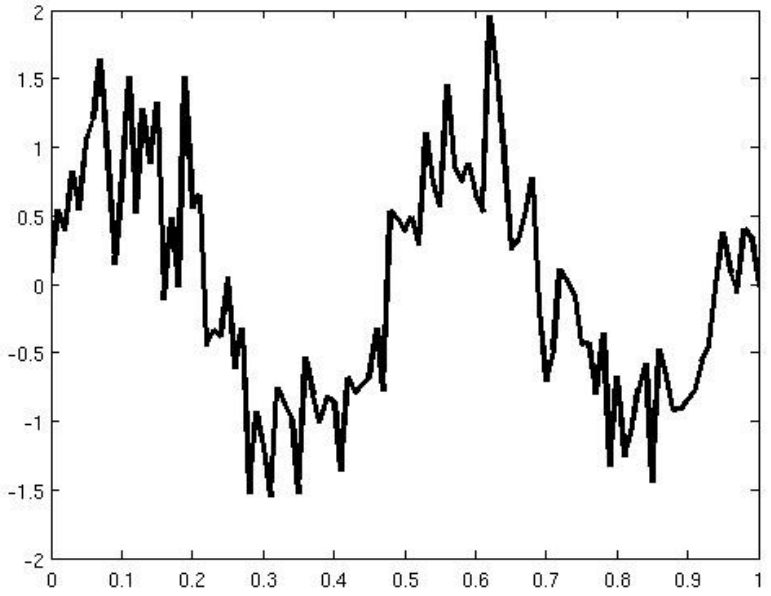
A discreet Fourier transform (DFT) is simply a Fourier transform applied to discreetly sampled data (the voltage is only known at specific timepoints). Used for digitized data.

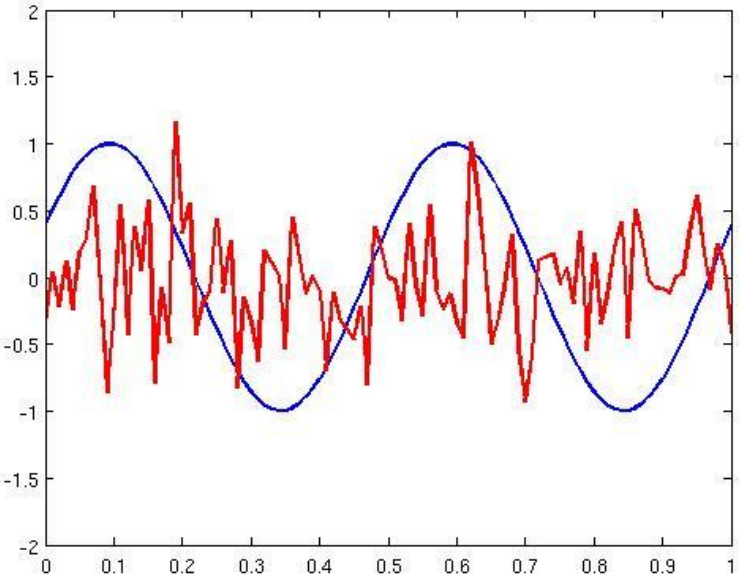
A fast Fourier transform (FFT) is a particular algorithm for implementing the Fourier transform that runs quickly on computers.

Back to Filtering

High-pass filter: Remove low frequency components.

Low-pass filter: Remove high frequency components.





Back to Filtering

High-pass filter: Remove low frequency components.

Low-pass filter: Remove high frequency components.

The frequency-domain view:

The filter will have a cutoff frequency.

Components of the signal at higher frequencies are suppressed (typically exponential fall-off above the cutoff frequency).

Low-pass filter

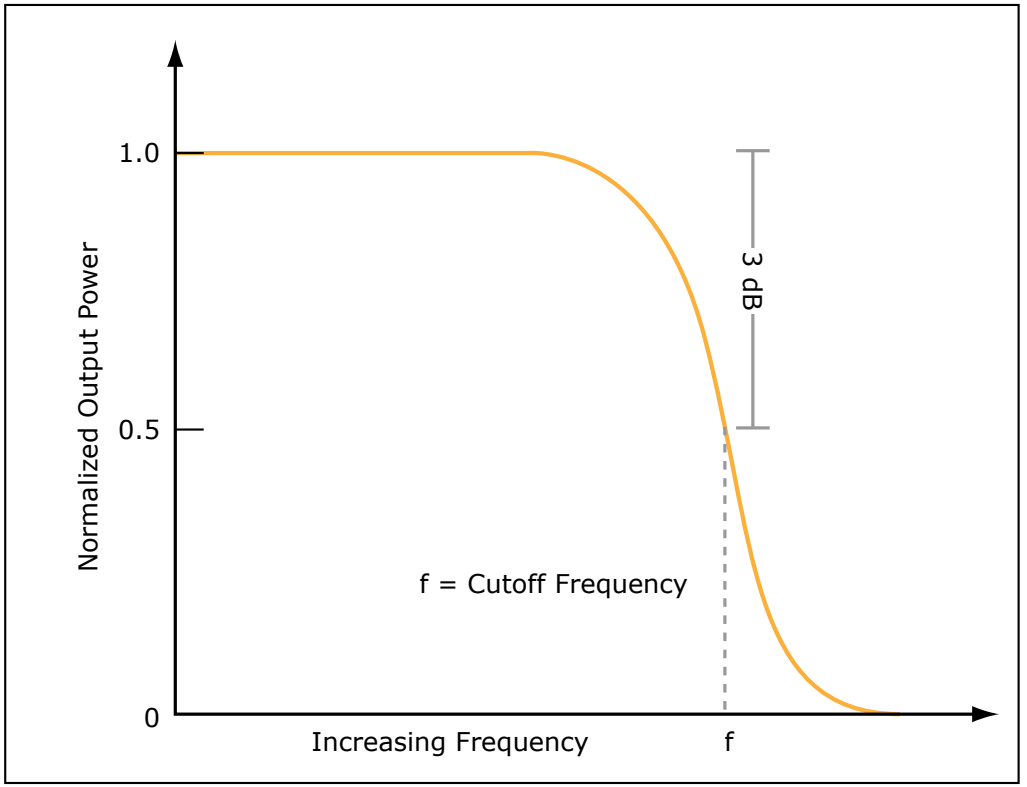


Image by MIT OpenCourseWare.

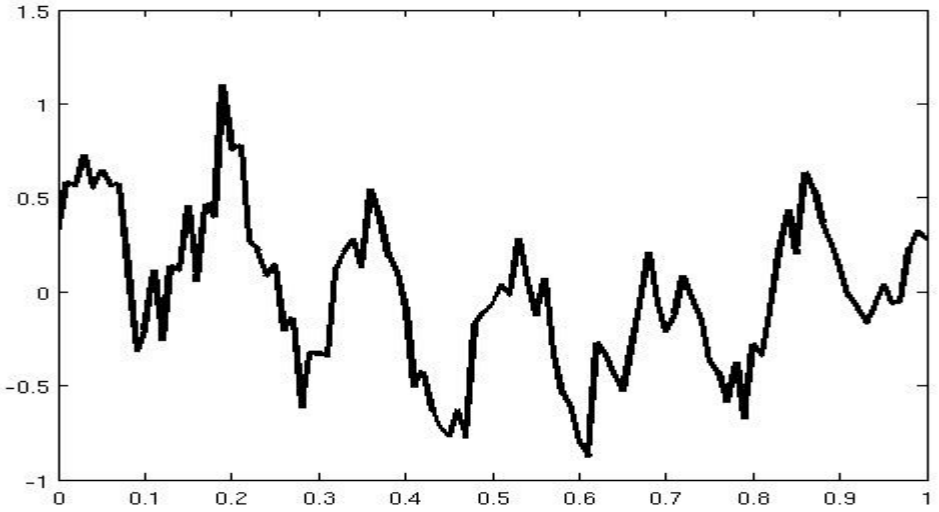
Back to Filtering

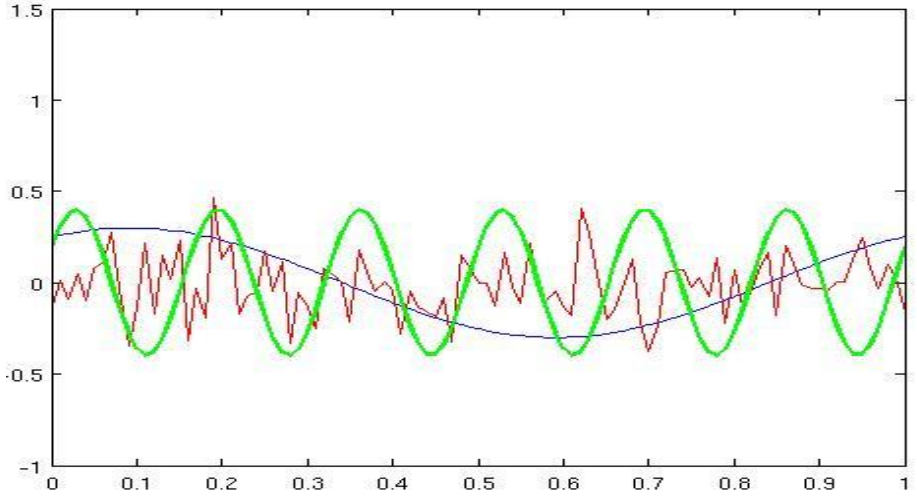
High-pass filter: Remove low frequency components.

Low-pass filter: Remove high frequency components.

Band-pass filter: Remove both low- and high-frequency components, allow frequencies in between.

9.02





Back to Filtering

High-pass filter: Remove low frequency components.

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Band-pass filter: Remove both low- and high-frequency components, allow frequencies in between.

band-pass filter

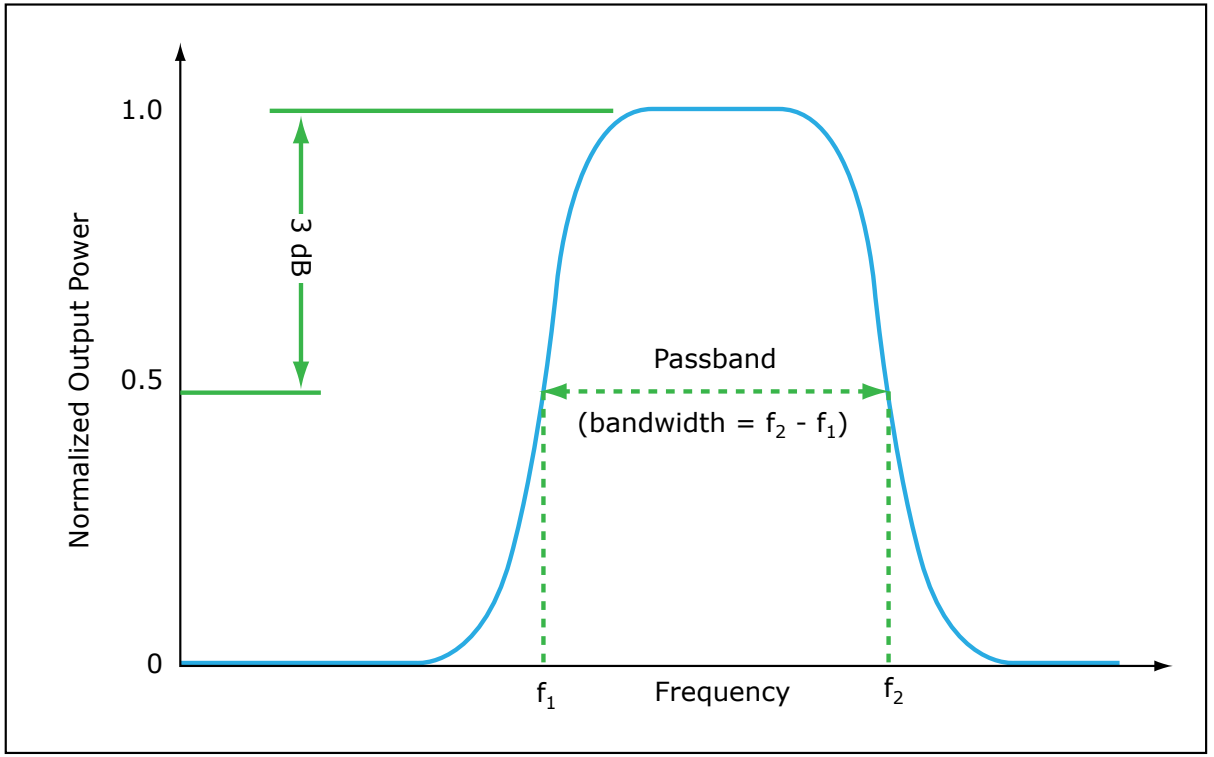

Image by MIT OpenCourseWare.

Back to Filtering

High-pass filter: Remove low frequency components.

Low-pass filter: Remove high frequency components.

Band-pass filter: Remove both low- and high-frequency components, allow frequencies in between.

Band-reject filter or **notch filter**: Remove only a band of frequencies, allow both higher and lower frequency components to pass. Typically used to remove "line noise" at 60 Hz.

our amplifiers have a "line filter"

Typical frequencies of interest

EEG

0.1 – 200 Hz field potentials (synaptic)

There are many “bands” corresponding to natural brain oscillations
e.g. hippocampal theta in rodents is ~7 – 9 Hz.

300 – 3000 Hz units

roach, rat, fly

Analog to digital conversion (A/D)

Continuous signal is sampled at some frequency – now only known at certain timepoints.

Hazards: Sample at too low of a rate, lose information or suffer *aliasing*.
Sample at too high of a rate, requires extra disk space and computing time.

Generally set low-pass filter above your highest expected frequency, then sample at more than twice that.

e.g. when recording units, filter 300 – 3000 Hz and sample at 10 kHz

Analog signal is digitized at some resolution (typically 12 or 16 bits) i.e. rounded.

Hazards: Input exceeds the range of A/D converter – data is lost.
Input is very small -- quantization errors become significant.

Generally set A/D range large, set amplifier accordingly.

e.g. expecting 1 – 2 mV signals, set gain to 1000x and A/D range to +/- 10 V

9.17 Systems Neuroscience Lab

Spring 2013