Measuring cell and tissue mechanics

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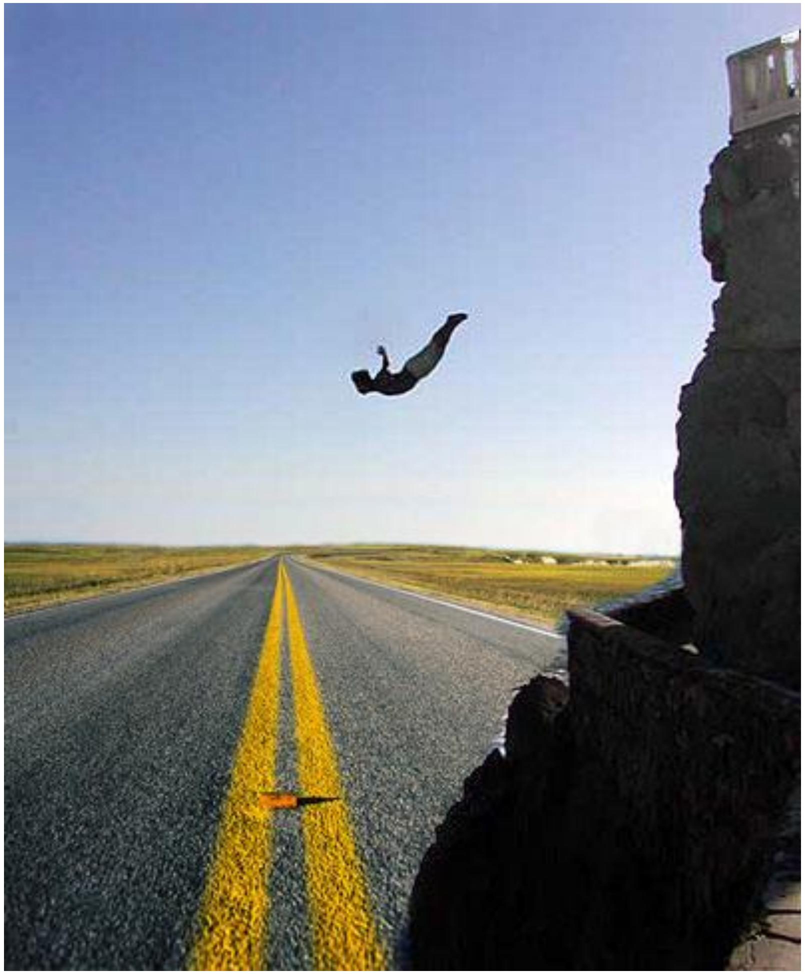
Forces vs. mechanical properties



Definitions

Force F: vector [N]

Tension: pulling force [N] (measured parallel to the string on which it applies)

Stress σ: a measure of the internal forces acting within a deformable body: $\sigma = F/A$ [Pa]

Strain γ: proportion of deformation to original size: $\gamma = \Delta l / l$

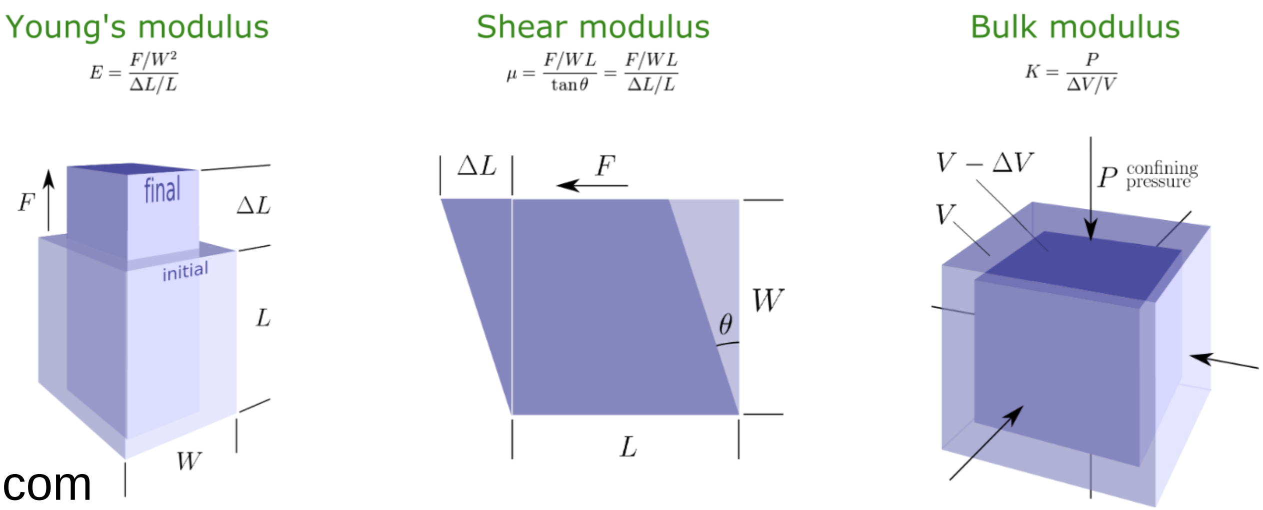
Elastic modulus: a measure of resistance to deformation [Pa], depends on load direction (= σ / γ)

Mechanical testing

Direct measurements	Indirect measurements
• e.g. AFM, rheology • Advantage: direct mechanical testing (force applied, deformation measured or vice versa) • Disadvantage: contact required	• e.g. MRE, Brillouin • Advantage: contact free • Disadvantage: assumptions, interpretability

Direct mechanical testing

- **strain can be measured, stress needs to be calculated**
- usually linear, homogeneous, isotropic materials considered
- in praxis, uniaxial deformations applied:
  - perpendicular to the surface: **normal force (tension or compression)** $E = \sigma/\gamma$ **E = Young's modulus**
  - parallel to the surface: **shear force; G = Shear modulus**
  - Application of uniform compression yields the **bulk modulus *K***



Adapted from agilescientific.com

Conversion

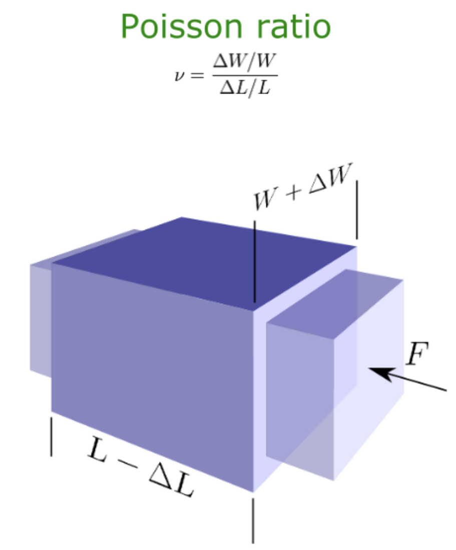
for linear, homogeneous, isotropic materials:

$$E = 2G(1+\nu) = 3K(1-2\nu)$$

ν = **Poisson‘s ratio**: ratio of transverse strain and axial strain

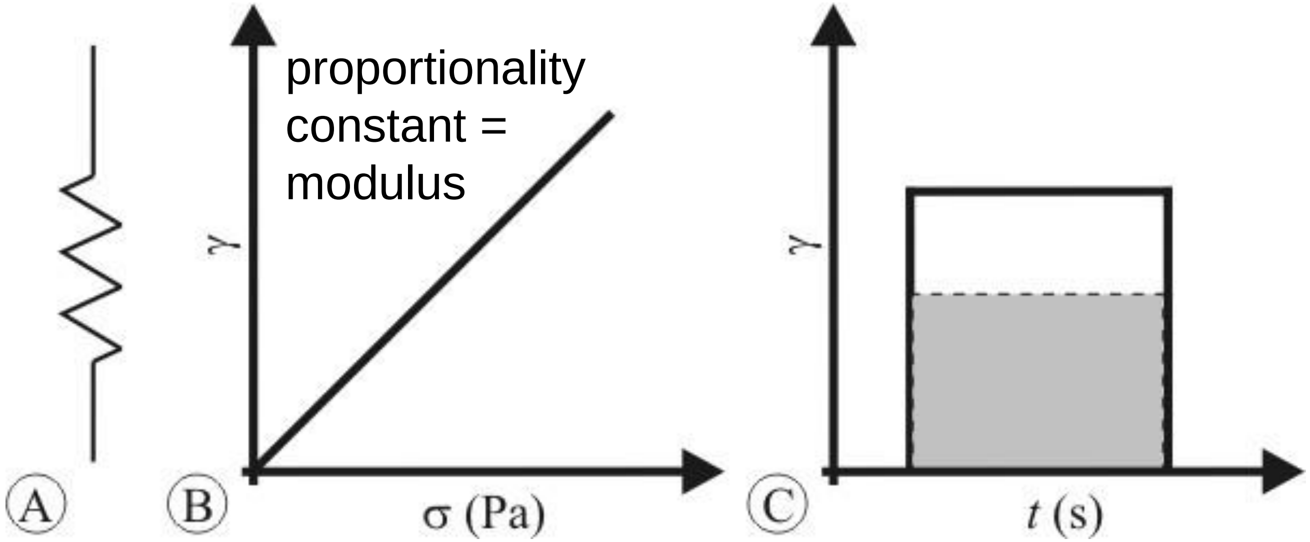
(-1 ≤ ν ≤ 0.5; most materials: 0 ≤ ν ≤ 0.5)

$$\nu = -\frac{\Delta W / W}{\Delta L / L}$$



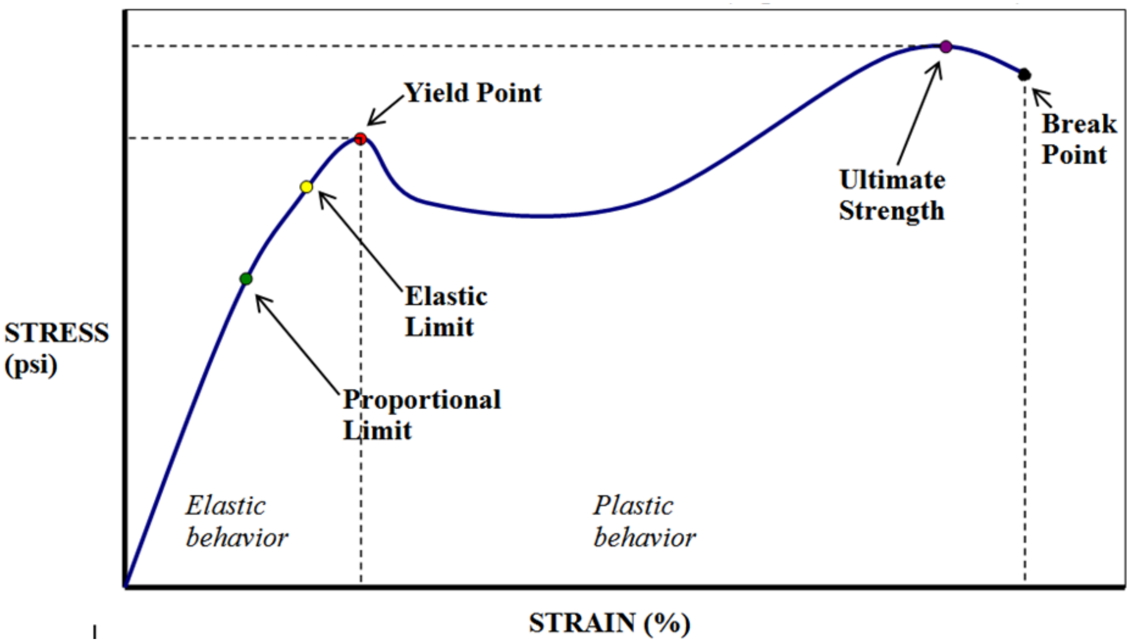
1. Elastic response

- applied energy is stored in the material
- in elastic solids, stress is exclusively a function of strain
  - rigid (Euclidian) solids (no deformation)
  - **linear elastic (Hookian) solids** ($\sigma \propto \gamma$) (see below)
  - nonlinear elastic solids



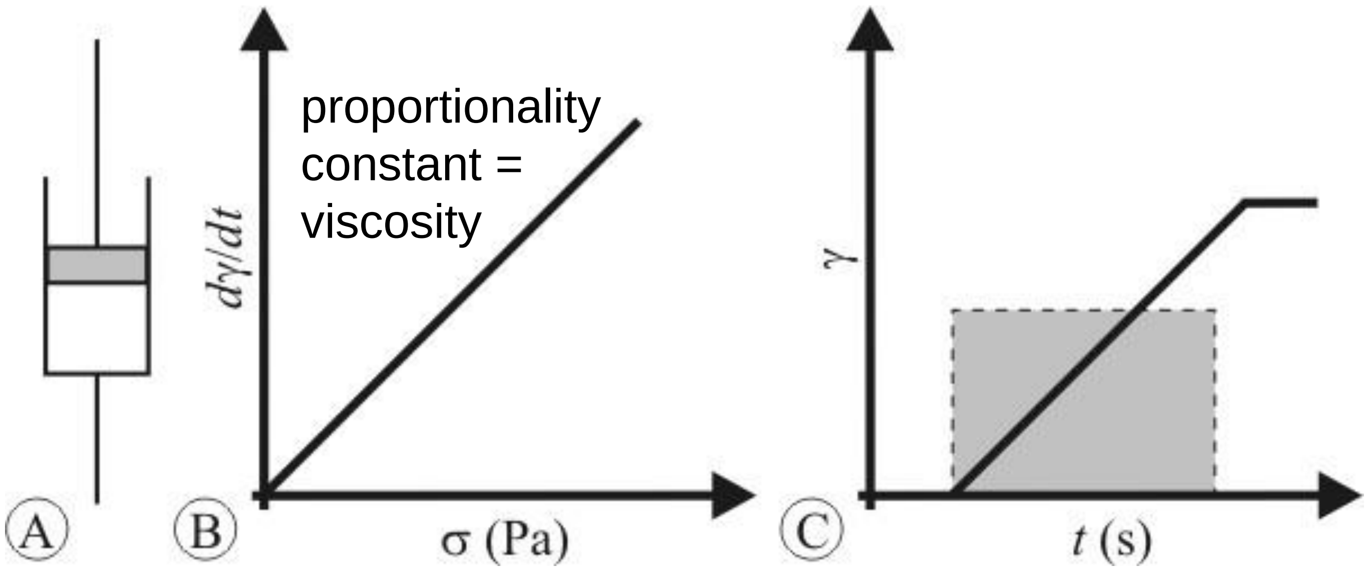
2. Plastic response

- deformation of a material undergoing non-reversible changes of shape in response to applied forces
- **yield strength** (or **yield point**): stress at which a material begins to deform plastically



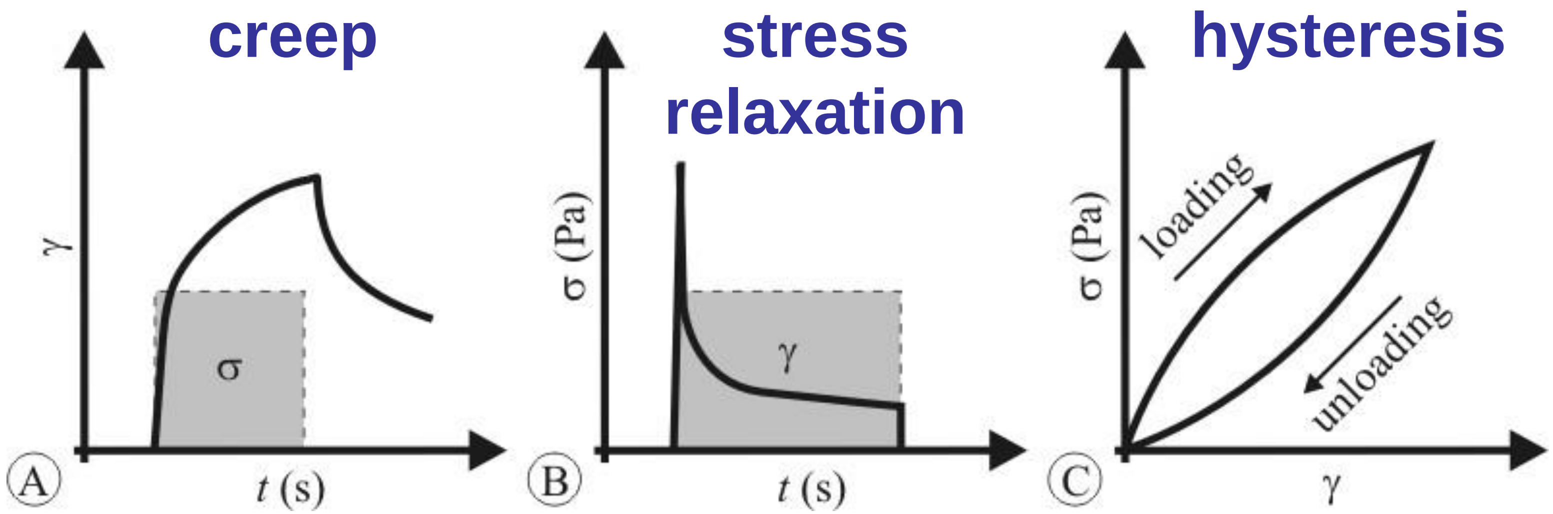
3. Viscous response

- applied energy is dissipated in the material
- in viscous fluids, stress is a function of strain rate
    - inviscid (Pascalian) fluids (no stress)
    - **linear viscous (Newtonian) fluids** ($\sigma \propto d\gamma/dt$)
    - nonlinear viscous (non-Newtonian) fluids

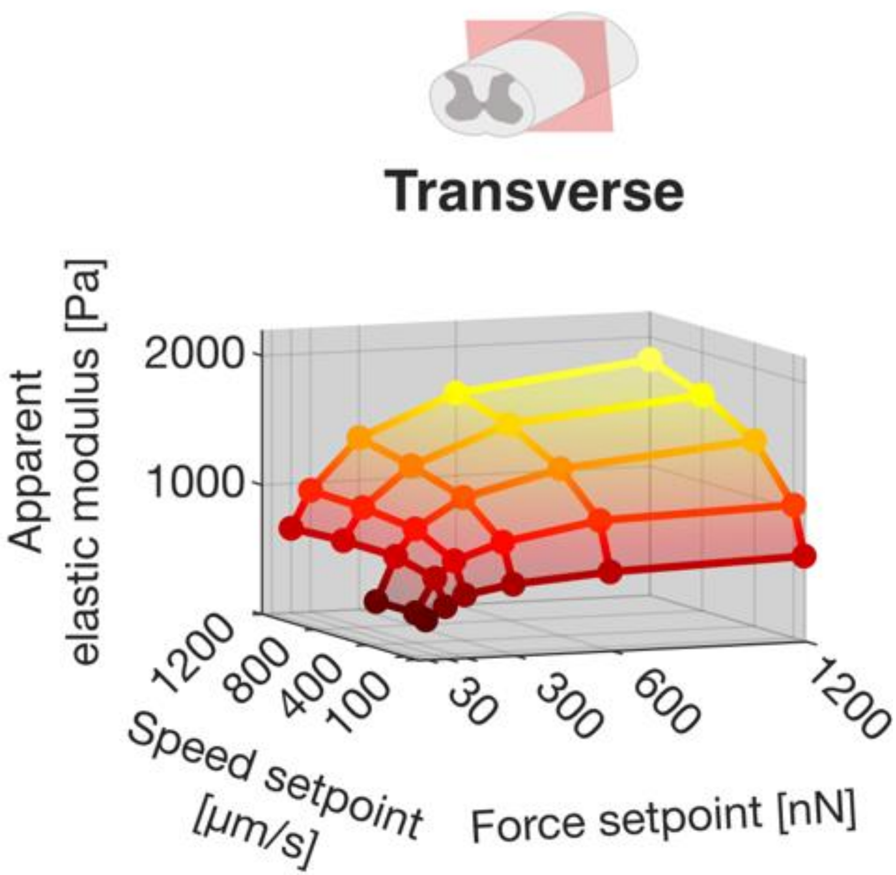


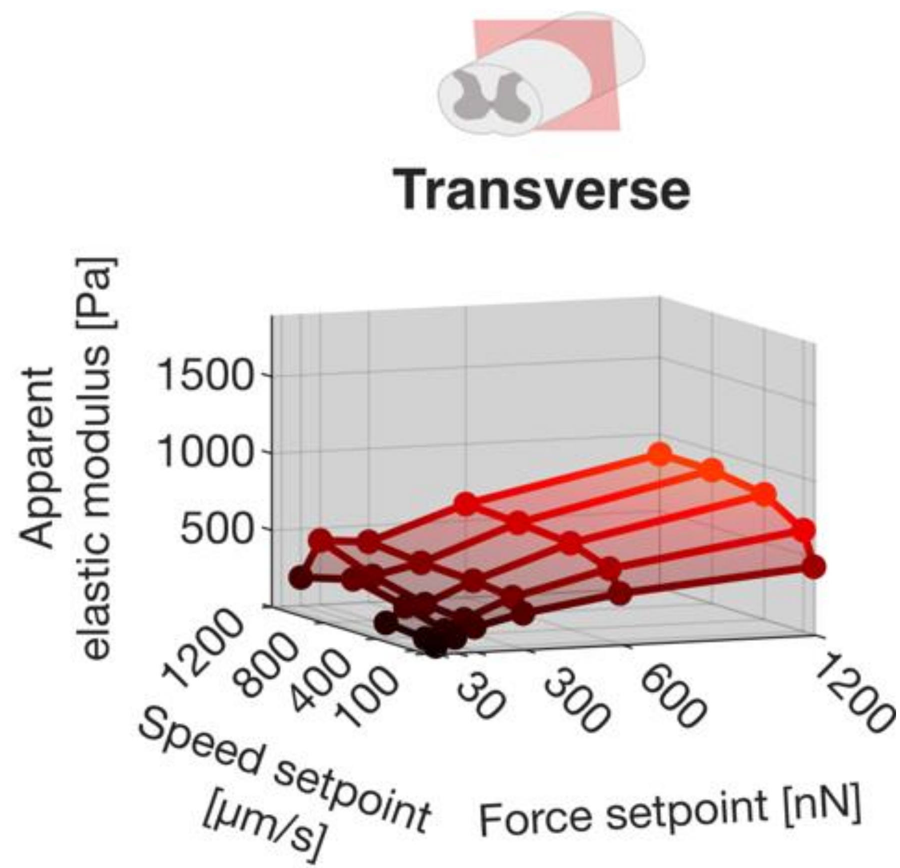
4. Viscoelastic response

- combination of solid- and fluid-like behaviour
- relationship between stress and strain **changes with time**
- stress-response depends on both the strain applied and the strain rate at which it is applied
- viscoelastic material is characterized by three typical responses to external loads:



The complex mechanical response of CNS tissue as measured by AFM





Grey matter Courtesy of Julia Becker White matter

Applying forces to measure mechanical properties of cells

Experimental approach

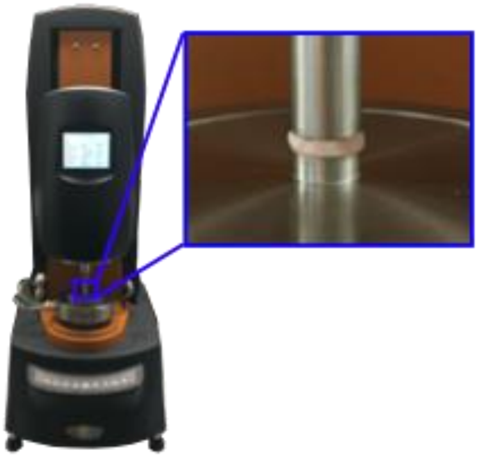
- *static methods*:
  - imposition of a step change in stress (*creep experiments*) or strain (*relaxation experiments*)
  - observation of the subsequent development of the strain or stress as a function of time
- *dynamic methods*:
  - application of harmonically varying stresses or strains
  - for linear viscoelastic materials:
    - amplitudes of stress and strain are proportional
    - stress alternates sinusoidally at the same frequency, but it is out of phase with the strain
    - lag between the applied strain and the resultant stress defines the phase shift δ (depends on viscosity)

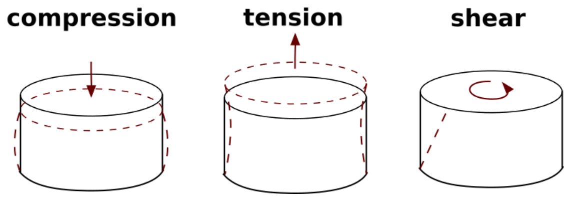
Challenges

1. **Time scale dependence**
   - Chose the right time scale
   - Infinity? (relaxation modulus)
2. Sample **heterogeneity**
   - Chose the right method
   - Chose the right probe size
3. **Anisotropy**
   - Know the underlying structure of your sample
   - Measure all three dimensions?
4. **Non-linearity**
   - Apply small strains

Rheometer

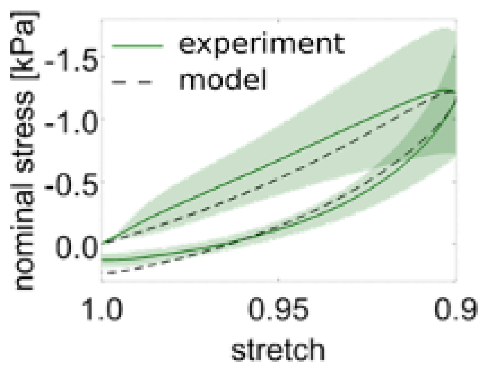
- **multi-modal** analysis: compression, tension, and shear experiments on the tissue scale
- **quasi-static and dynamic** experiments
- **small strain regime**: frequency sweep → (frequency-dependent) modulus values that are easy to compare
- **large strain regime**: cyclic and stress relaxation tests → information on nonlinear, time-dependent (hysteretic) behavior for material modeling





Advantages

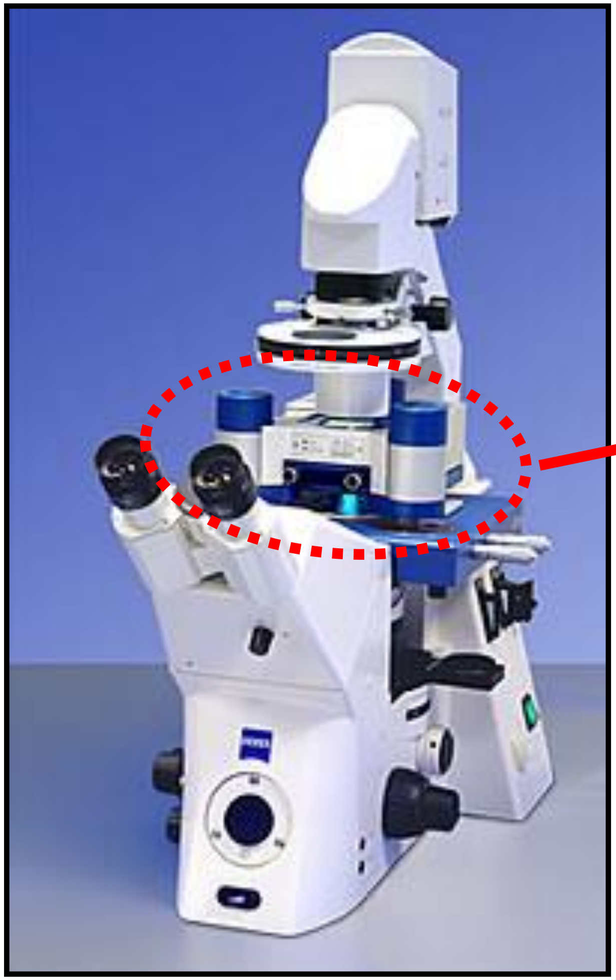
- controllable deformation → data set for calibrating (hyper-/visco-/poro-elastic) material models
- different loading modes, small and large strains, quasi-static and dynamic loading conditions
- controllable temperature

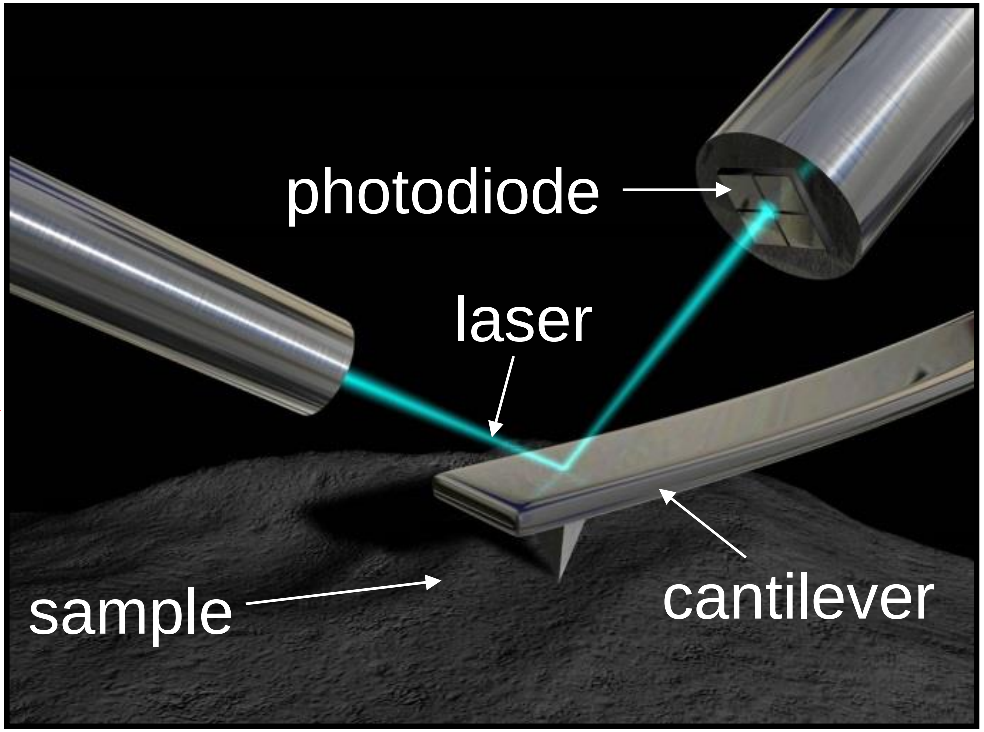


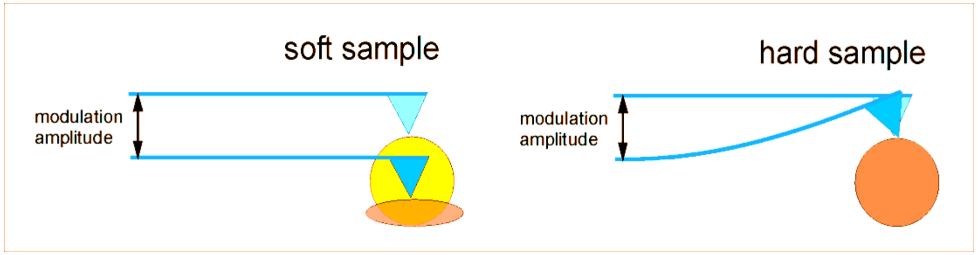
Disadvantages

- *ex vivo* measurements
- homogenized response → no information on cell scale
- large specimens required (mm range)

Atomic force microscopy

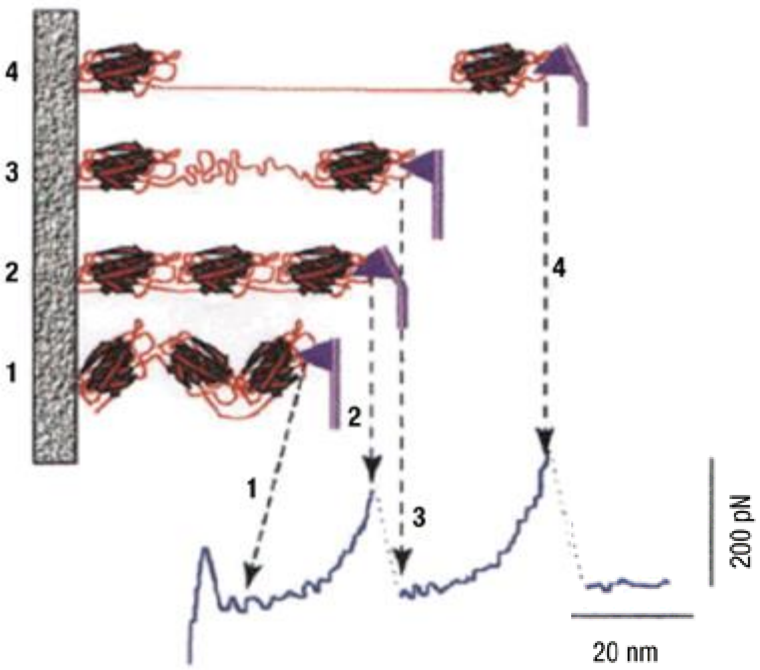




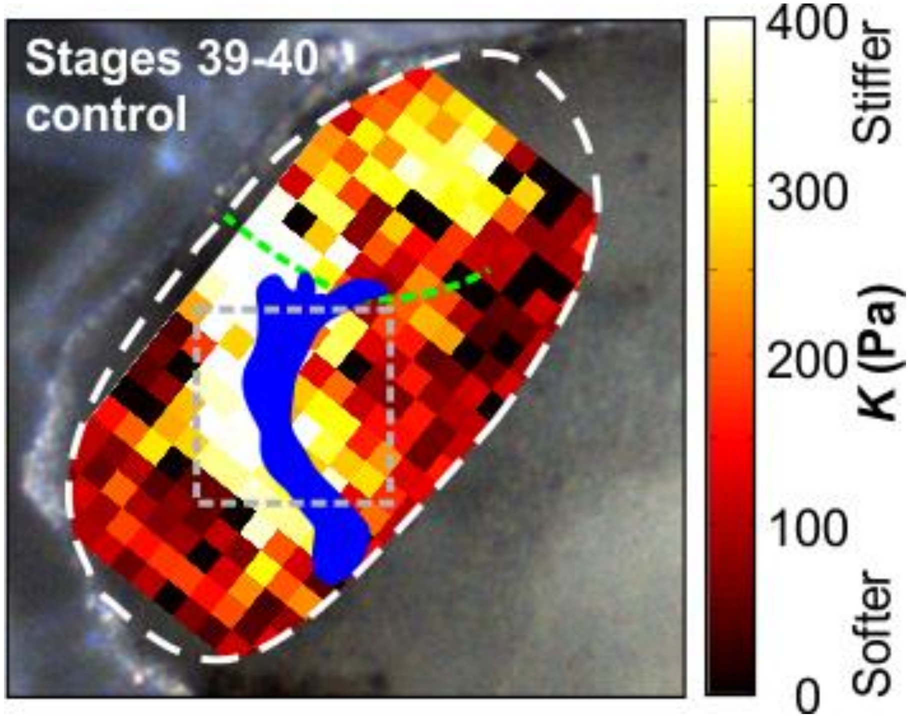


Franze, Curr Opin Gen Dev, 2011

Atomic force microscopy

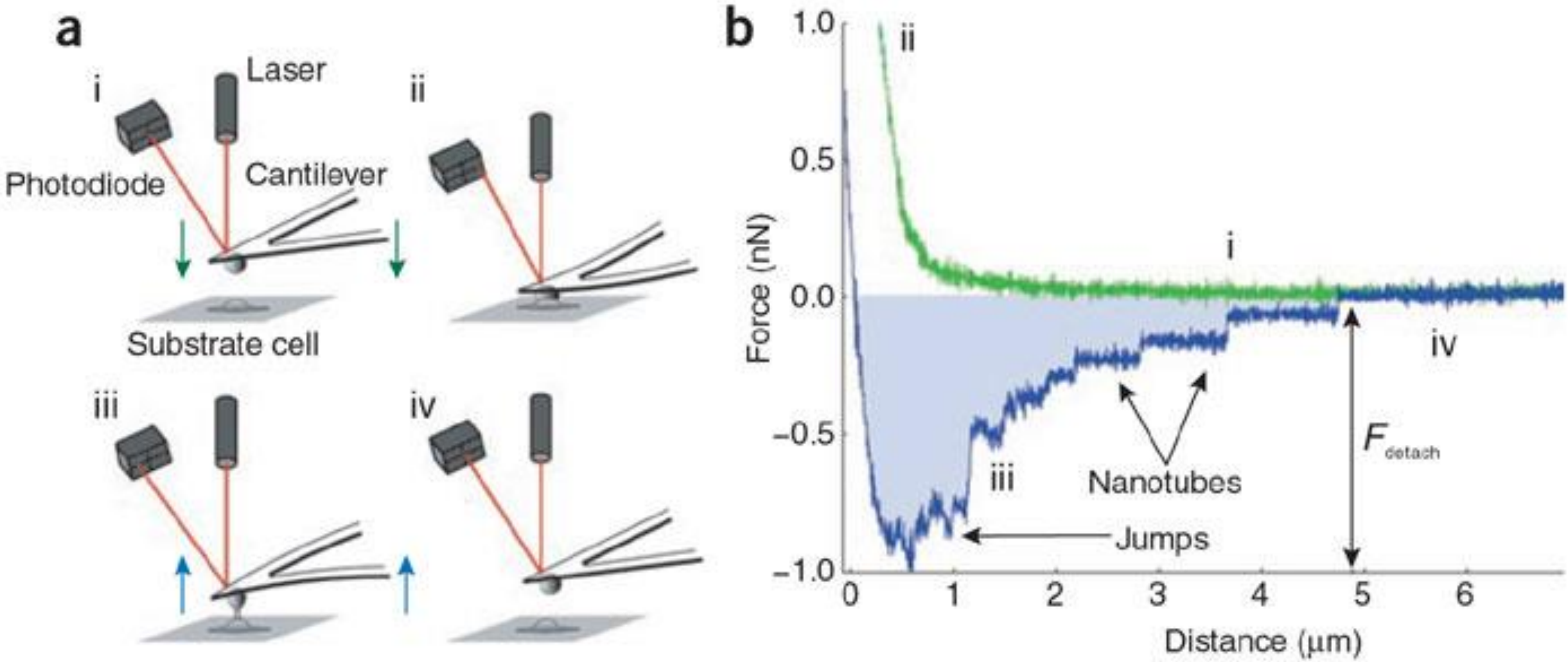


Unfolding of Tenascin-C



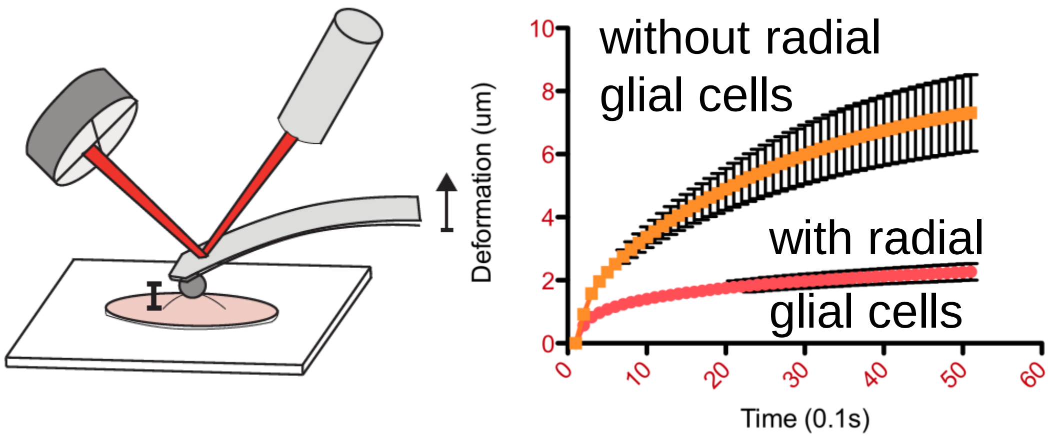
In vivo time laps stiffness mapping in the developing frog brain

AFM-based cell adhesion measurements

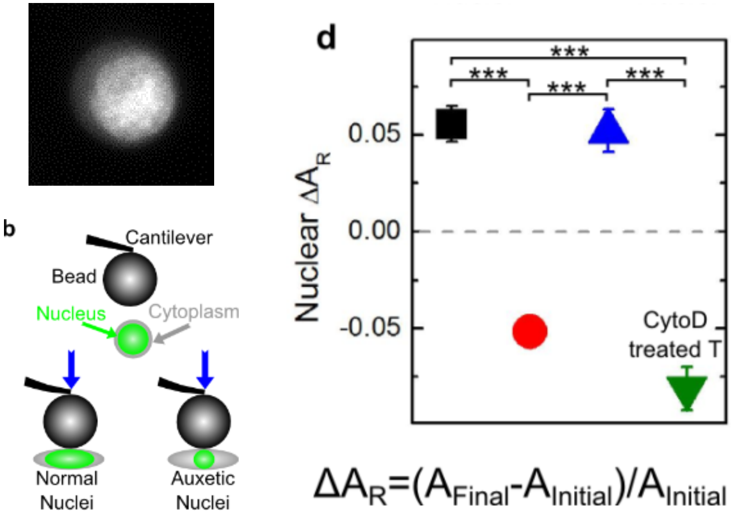


Müller et al., Nature Chemical Biology 5, 383 - 390 (2009)

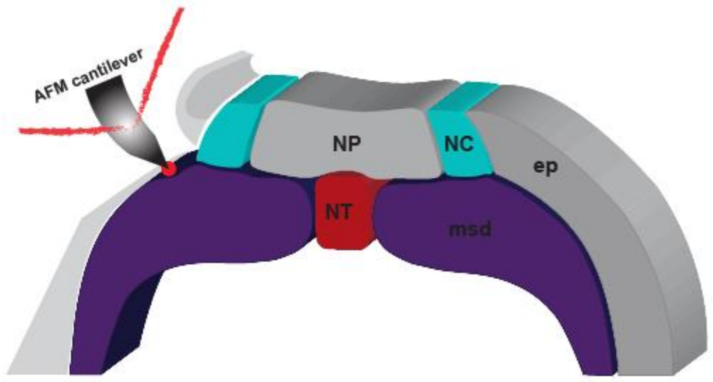
Other applications of AFM



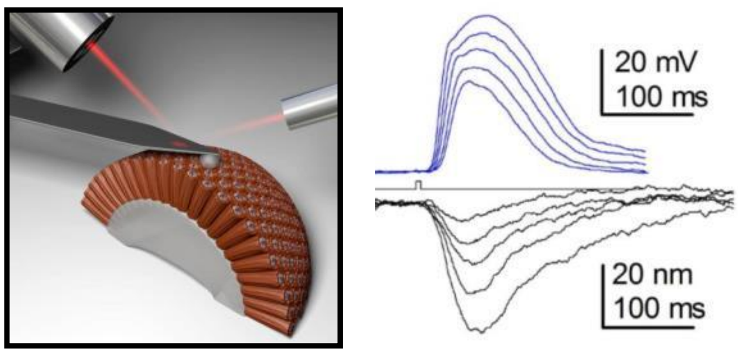
MacDonald et al., J Cell Biol, 2015



Pagliara et al., Nat Mater, 2014



Barriga et al., Nature, 2018



Hardie & Franze, Science, 2012

AFM

AFM (tissue, cells): quasi-static / creep or 0.1-100Hz,
measurement parameters: apparent elastic (Young's) modulus, fluidity / viscosity,
spatial resolution: ~um for tissue, sub um for cells,
hypotheses: Hertz contact mechanics
(with adaptations for different measurement regimes)

Pros:

- direct mechanical measurement (you can tell from raw data!)
- Magnitude and time scale of applied forces similar to forces applied by cells in the tissue

→ Measurement of what cells feel at that time scale

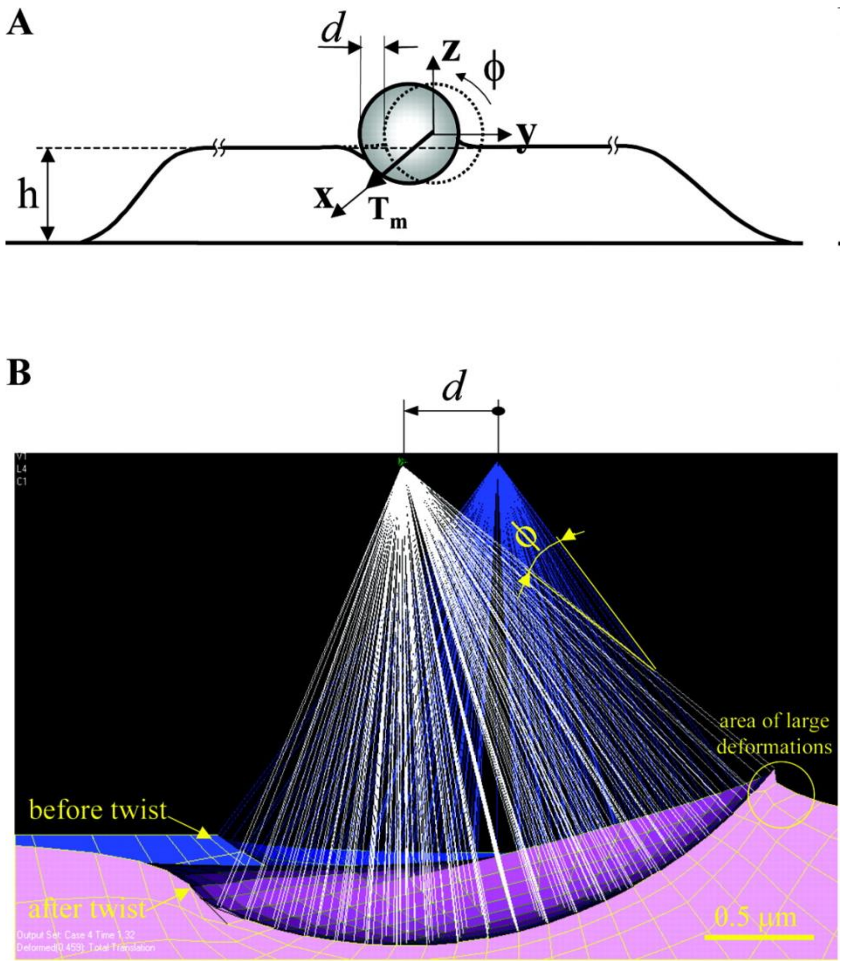
Cons:

- Measurements restrained to surfaces

→ Cannot look into tissues or embryos, tissues often have to be sliced

- Rather low throughput

Mechanics measurements: Magnetic bead twisting



T_m = applied magnetic torque
h = cell height
φ = angular rotation of the beads
d = bead lateral translation

Comparison cortical and deep cytoskeletal structures:

cortical CSK:
E = 63-109 Pa
η = 7- 18 Pa s

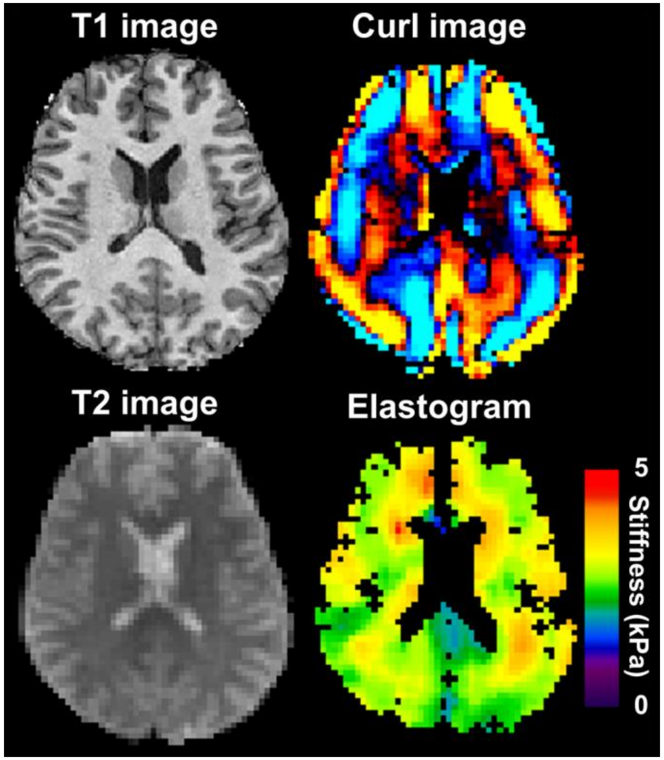
deep CSK:
E = 95-204 Pa
η = 760-1967 Pa s

Mijailovich et al., 2002

Laurent et al., 2003

MRE

1. a mechanical vibrator is used on the surface of the patient's body to generate shear waves that travel into the patient's deeper tissues
2. an MRI acquisition sequence measures the propagation and velocity of the waves
3. iformation is processed by an inversion algorithm to quantitatively infer and map tissue stiffness in 3-D



MRE

MRE (in vivo humans): 10 to 60 Hz,
measurement parameters: shear modulus, viscosity (loss angle),
spatial resolution: 2x2x2 mm³ (3T) 1x1x1 mm³ (7T),
hypotheses: fluid/solid interactions (CSF, blood), viscoelastic network interactions (neurons, oligodentrocytes, astrocytes), visocelastic damping (ECM)

MRE (in vivo mice): 800 to 1600 Hz,
measurement parameters: shear wave speed, viscosity (loss angle),
spatial resolution: 0.06 mm x 0.06 mm x 0.8 mm³,
hypotheses: fluid/solid interactions (CSF, blood), viscoelastic network interactions (neurons, oligodentrocytes, astrocytes), visocelastic damping (ECM)

MRE (ex vivo brain tissue): 800 to 6000 Hz,
measurement parameters: shear wave speed, viscosity (loss angle),
spatial resolution: 0.04 mm x 0.04 mm x 0.8 mm³,
hypotheses: viscoelastic network interactions (neurons, oligodentrocytes, astrocytes), visocelastic damping (ECM)

BM

(BM = Brillouin microscopy)

- empirical spectroscopy technique
- uses inelastic scattering of light when it encounters acoustic phonons in a crystal, a process known as Brillouin scattering, to determine phonon energies and therefore interatomic potentials of a material
- scattering occurs when an electromagnetic wave interacts with a density wave, photon-phonon scattering
- Brillouin scattering depends on density, refractive index, and longitudinal modulus
- Longitudinal modulus is related to bulk modulus

BM (zebrafish in vivo, any tissue ex vivo): GHz,

measurement parameters: direct measurement of local sound velocity (acoustic phonons) and dissipation, with knowledge of refractive index and mass density (can be measured simultaneously with ODT) the longitudinal elastic modulus and viscosity can be derived,

spatial resolution: 1 µm (spatial extent of an acoustic phonon), 3D distribution inside materials,

hypotheses: sensitive to nm-scale intermolecular interactions, water mobility,

scales with shear modulus as measured with AFM in some sample but not in others

Pros: in live animals, in deep tissues

Cons: quantitative interpretation difficult

Responsible structures

- **cell wall**
- **cytoskeleton**
  - Microtubules (rigid rods, persistence length L_p ~ mm)
  - actin („microfilaments“) (semiflexible, L_p ~ 10 µm)
  - intermediate filaments (cell type specific, e.g., keratin, desmin, vimentin, GFAP, neurofilaments, lamins) (flexible, L_p < 1 µm)
- **extracellular matrix**
  - tissue-dependent
  - combinations of different proteins, glycoproteins and polysaccharides such as collagens, laminins, proteoglycans, fibronectin and glycosaminoglycans
- **cell organelles, membranes**
- **hydrostatic pressure**
- **active forces**