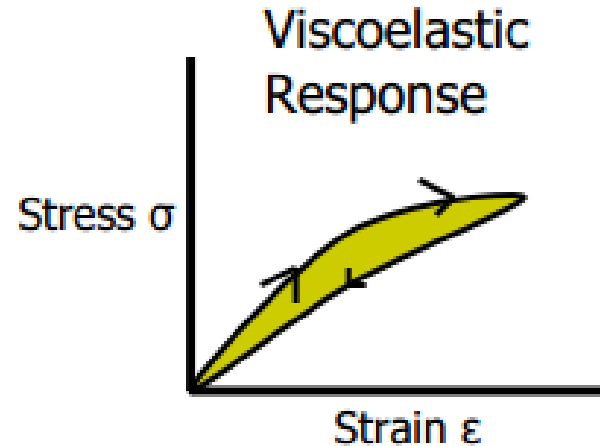
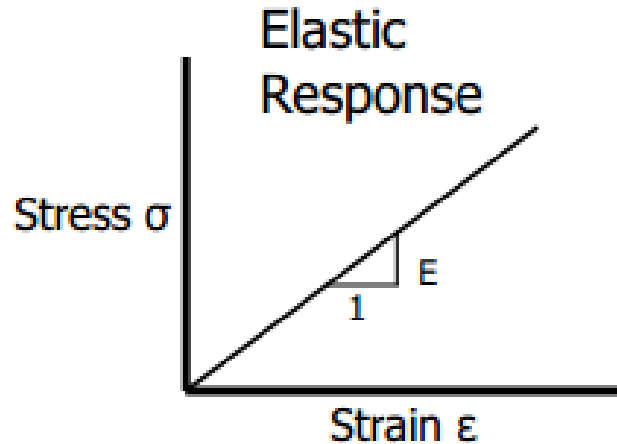


Nano-mechanics for Intelligent Materials (2/2)

Giorgio MATTEI

- **Viscoelastic materials** exhibit the characteristics of both elastic and viscous materials
 - Viscosity → resistance to flow (damping)
 - Elasticity → ability to revert back to the original shape
- **Elastic vs. viscoelastic** stress-strain response



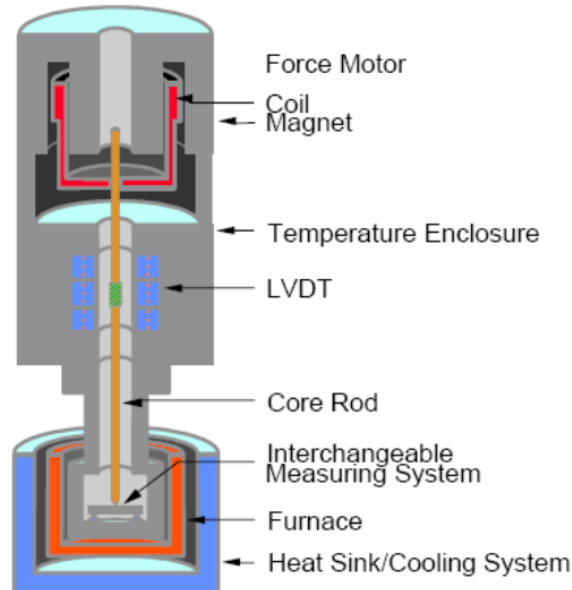


Methods to characterise viscoelasticity

- **Time** domain
 - Creep response
 - Stress relaxation
- **Frequency** domain
 - Dynamic mechanical analysis (DMA)
 - Dynamic mechanical thermal analysis (DMTA)
- **Strain-rate** domain
 - Epsilon dot Method
- **Stress-rate** domain
 - Sigma dot Method

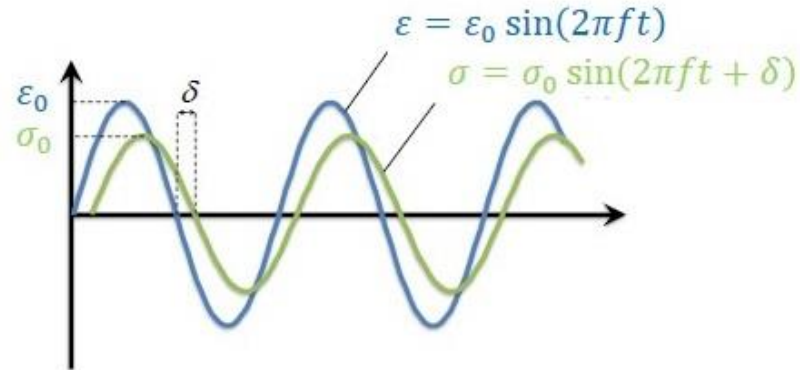
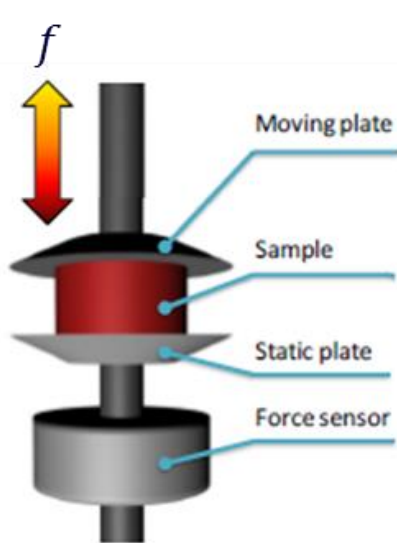
Dynamic mechanical analysis (DMA)

- Dynamic mechanical analysis (DMA) is a standard **force-triggered method** to **determine viscoelastic properties** of materials by **applying a small amplitude cyclic strain** on a sample and **measuring the resultant cyclic stress response**.



Dynamic mechanical analysis (DMA)

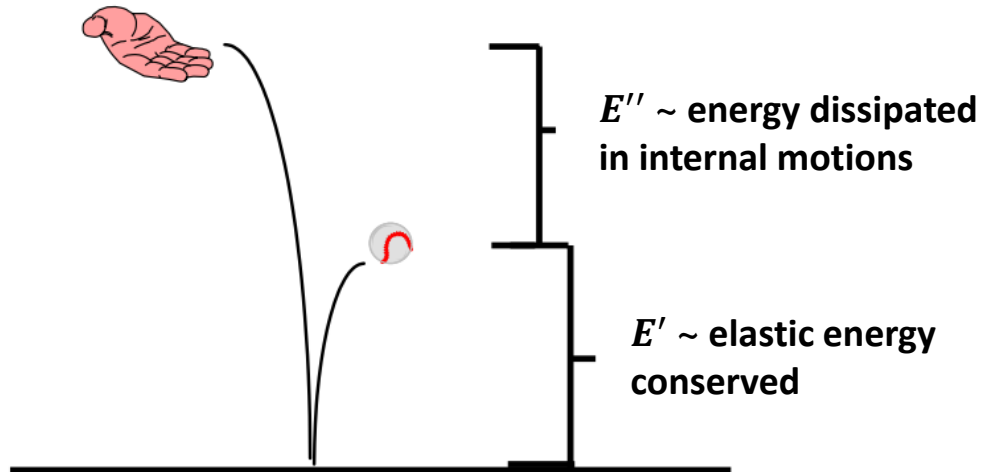
- For a given **sinusoidal strain input** the resulting **stress will be sinusoidal** if the applied strain is **small enough** so that the tissue can be approximated as **linearly viscoelastic**.



Viscoelastic material response is characterised by a **phase lag (δ)** between the strain input and the stress response, which is comprised **between 0° (purely elastic) and 90° (purely viscous)**. This phase lag is **due to the excess time necessary for molecular motions and relaxations** to occur.

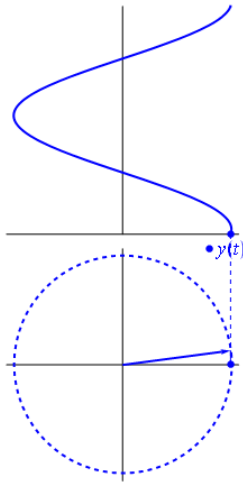
Complex, storage and loss moduli

- The dynamic mechanical properties are quantified with the **complex modulus** (E^*), which can be thought as an **overall resistance** to deformation under dynamic loading. The complex modulus is composed of the **storage** (E' , elastic component) and the **loss** (E'' , viscous component) moduli, that are **additive under the linear theory of viscoelasticity** ($E^* = E' + iE''$).

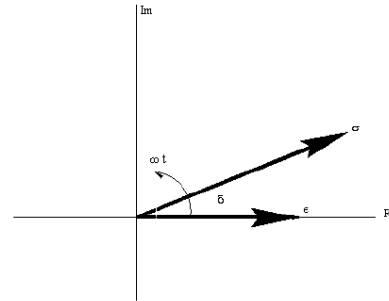


- It is convenient to represent the sinusoidal stress and strain functions as complex quantities (called rotating vectors, or **phasors**) with a **phase shift** of δ .

$$\varepsilon = \varepsilon_0 e^{i\omega t} \quad \sigma = \sigma_0 e^{i(\omega t + \delta)}$$



Rotating vector representation of harmonic stress and strain



Observable σ and ε can be viewed as the projection on the real axis of vectors rotating in the complex plane at the same frequency ω

$$\begin{aligned} E^* &= \frac{\sigma}{\varepsilon} = \frac{\sigma_0}{\varepsilon_0} e^{i\delta} = \\ &= \frac{\sigma_0}{\varepsilon_0} (\cos \delta + i \sin \delta) = \\ &= \mathbf{E}' + i\mathbf{E}'' \end{aligned}$$

↙
↘

Storage modulus Loss modulus
 $E' = E^* \cos(\delta)$ $E'' = E^* \sin(\delta)$

$\tan(\delta) = E''/E'$ Damping factor

$\eta' = E''/\omega$ Dynamic viscosity

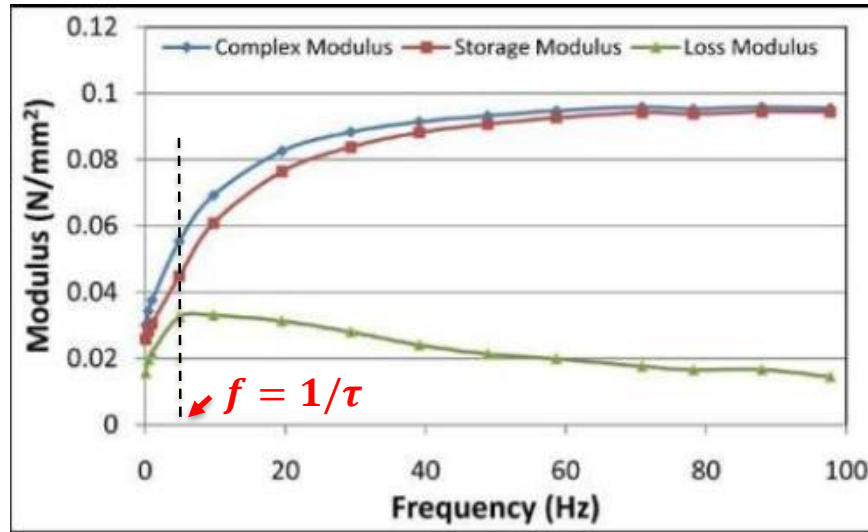
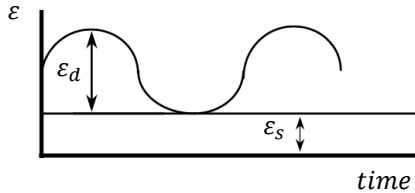


- **Temperature sweep:** Modulus and damping are recorded as the sample is heated
- **Frequency sweep:** Modulus and damping are recorded as the sample is loaded at increasing (or decreasing) frequencies
- **Stress amplitude sweep:** Modulus and damping are recorded as the sample stress is increased
- **Strain amplitude sweep:** Modulus and damping are recorded as the sample strain is increased
- **Combined sweep:** Combinations of above methods

- A sample is held to a **fixed temperature** and tested at **varying frequency**.

Test parameters:

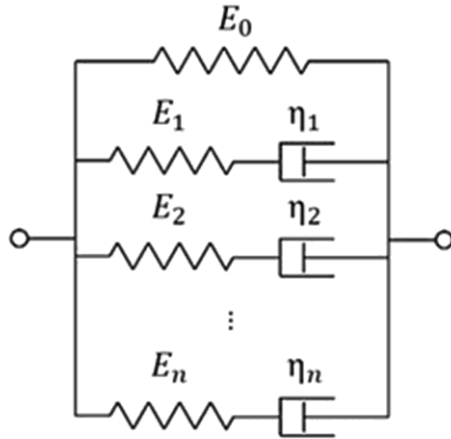
- Temperature (T)
- Frequency range (f)
- Static strain (ε_s)
- Dynamic strain (ε_d)



- **Peaks** in $\tan(\delta)$ or E'' with respect to frequency identify the **characteristic relaxation frequencies** of the viscoelastic sample under testing, defined as $f = 1/\tau$ (where τ is the **characteristic relaxation time**)

Lumped models to describe linear viscoelastic response

- The most general form of linear viscoelastic model is called the **Generalised Maxwell (GM)** model and consists of a **pure spring (E_0)** with **n Maxwell arms** (i.e. spring E_i in series with a dashpot η_i) assembled **in parallel**, thus defining a set of **n different characteristic relaxation times** (i.e. $\tau_i = \eta_i/E_i$)



$$H_{GM}(s) = \frac{\bar{\sigma}}{\bar{\epsilon}} = E_0 + \sum_{i=1}^n \frac{E_i \eta_i s}{E_i + \eta_i s}$$

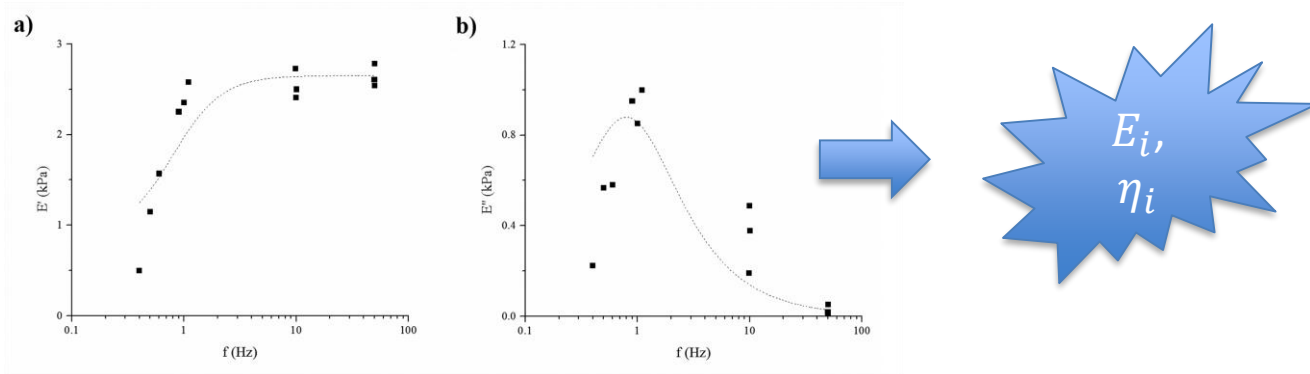
GM model transfer function in the Laplace domain

Lumped parameters derivation from frequency sweep

- Calculate the **complex conjugate of the GM modulus** (E_{GM}^*) by substituting $s = i \omega = i 2\pi f$ in $H_{GM}(s)$, then **split the expression into its real (Re)** and **imaginary (Im)** parts to obtain the **frequency-dependent relations** for the **storage** and **loss** moduli, respectively

$$E_{GM}^*(f) = \underbrace{\left(E_0 + \sum_{i=1}^n \frac{4 E_i \eta_i^2 f^2 \pi^2}{E_i^2 + 4 \eta_i^2 f^2 \pi^2} \right)}_{E'(f)} + i \underbrace{\left(\sum_{i=1}^n \frac{2 E_i^2 \eta_i f \pi}{E_i^2 + 4 \eta_i^2 f^2 \pi^2} \right)}_{E''(f)}$$

- Global fitting with shared parameters** (χ^2 minimisation)



- Experimental data obtained for a given frequency can be used to compute the frequency-dependent storage (E') and loss (E'') moduli as:

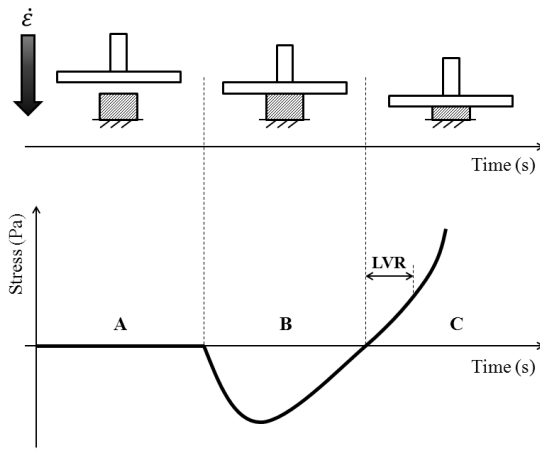
$$\frac{E'(f)}{(1 - \nu^2)} = \frac{P_0}{h_0} \cos(\phi) \frac{1}{\sqrt{hR}}$$

$$\frac{E''(f)}{(1 - \nu^2)} = \frac{P_0}{h_0} \sin(\phi) \frac{1}{\sqrt{hR}}$$

Herbert EG et al, *J. Phys. D. Appl. Phys.* 41 (2008)

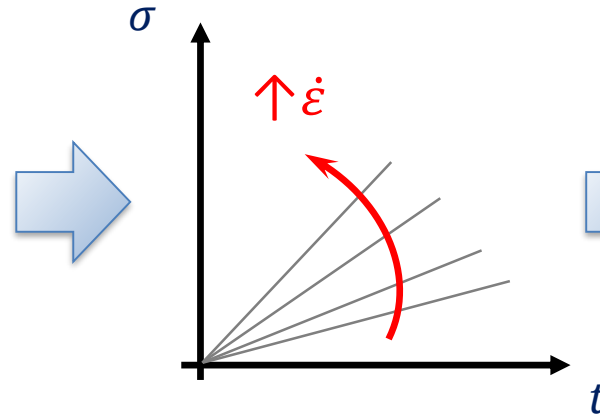
- Frequency spectra of storage and loss moduli can then be fitted to lumped parameter rheological models to derive material viscoelastic constants as previously described

$\dot{\epsilon}M$ paradigm: characterise the material **viscoelastic behaviour** testing samples at **different constant strain rates** ($\dot{\epsilon}$), then **analyse $\sigma(t)$ curves** within the **LVR**

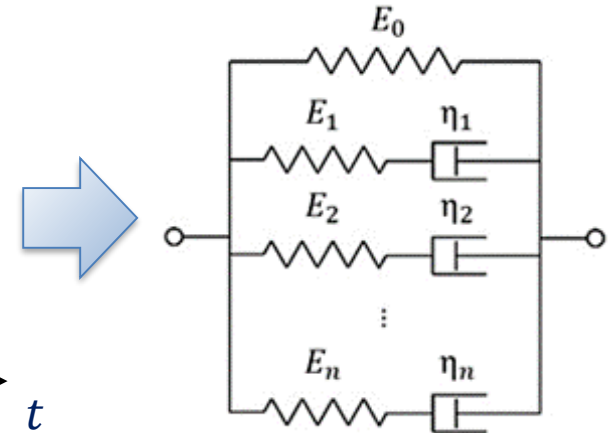


1. Bulk test at constant $\dot{\epsilon}$

- ✓ **Force-displacement time recording** starts **prior to sample contact** → **no pre-load**
- ✓ **Short test duration** → **no sample deterioration**
- ✓ **LVR determined through measured σ - ϵ curves**



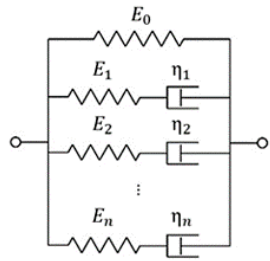
2. **Global fitting** of LVR stress-time (σ - t) series measured **at different $\dot{\epsilon}$**



3. **Lumped parameter estimation**

$\sigma(t)$ response to a constant $\dot{\epsilon}$ for the $\dot{\epsilon}M$ global fitting

1. Calculate the **transfer function** of a **lumped parameter model** in the Laplace domain



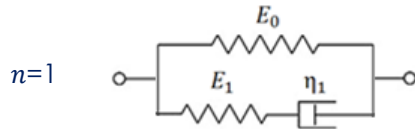
$$\tau_i = \eta_i/E_i \quad i^{\text{th}} \text{ relaxation time}$$

$$H_{GM}(s) = \frac{\bar{\sigma}}{\bar{\epsilon}} = E_0 + \sum_{i=1}^n \frac{E_i \eta_i s}{E_i + \eta_i s}$$

2. Derive the **model response** to a **constant $\dot{\epsilon}$ input with amplitude $|\dot{\epsilon}|$** in the Laplace domain

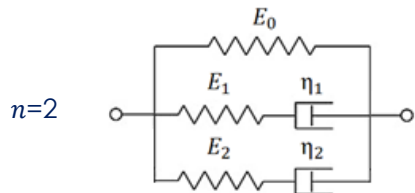
$$\bar{\sigma} = H_{GM}(s) \cdot \frac{|\dot{\epsilon}|}{s^2}$$

3. Get the **$\sigma(t)$ response** through **Inverse Laplace transformation**



Maxwell Standard Linear Solid (**SLS**)

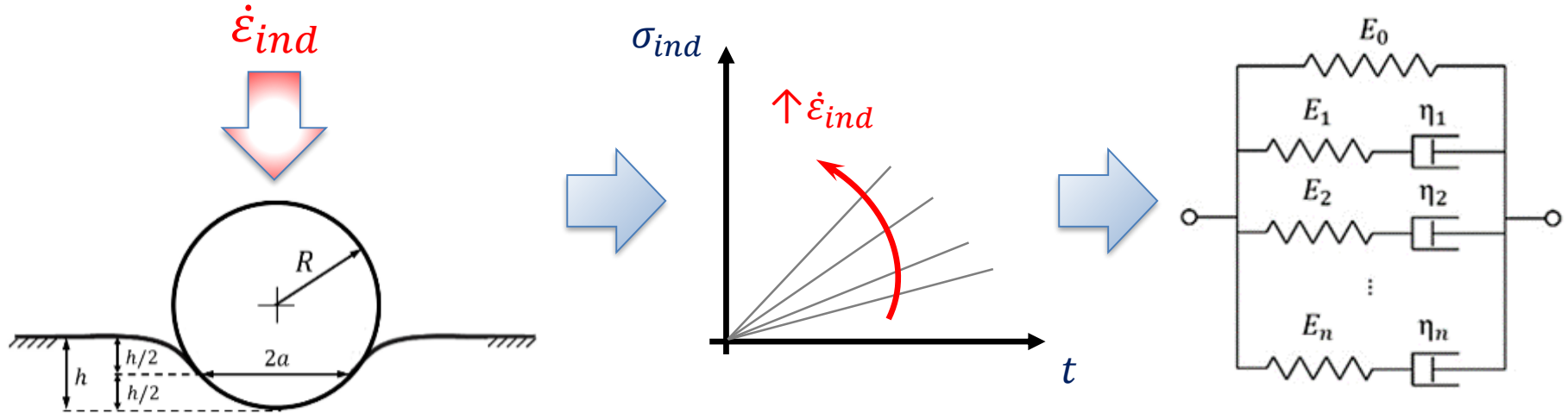
$$\sigma_{ind}(t) = \dot{\epsilon}_{ind} \left[E_0 t + \eta_1 \left(1 - e^{-\frac{E_1}{\eta_1} t} \right) \right]$$



2-arm generalised Maxwell (**GM2**)

$$\sigma_{ind}(t) = \dot{\epsilon}_{ind} \left[E_0 t + \eta_1 \left(1 - e^{-\frac{E_1}{\eta_1} t} \right) + \eta_2 \left(1 - e^{-\frac{E_2}{\eta_2} t} \right) \right]$$

- Estimate material viscoelastic constant through **nano-indentation** at **different constant strain rates** ($\dot{\epsilon}_{ind}$), then **analyse** $\sigma_{ind}(t)$ **curves** within the **LVR**



1. Nano-indentation at constant $\dot{\epsilon}_{ind}$

- ✓ Force-displacement time recording starts prior to sample contact → **no pre-load**
- ✓ Short test duration → **no sample deterioration**
- ✓ LVR determined through **measured** σ_{ind} - $\dot{\epsilon}_{ind}$ curves

2. Global fitting of LVR stress-time (σ_{ind} - t) series measured at different $\dot{\epsilon}_{ind}$

3. Lumped parameter estimation

How to get σ - ϵ curves from P - h measurements?

- **1st issue:** identifying the **initial contact point**

Commercial load-controlled nano-indenters

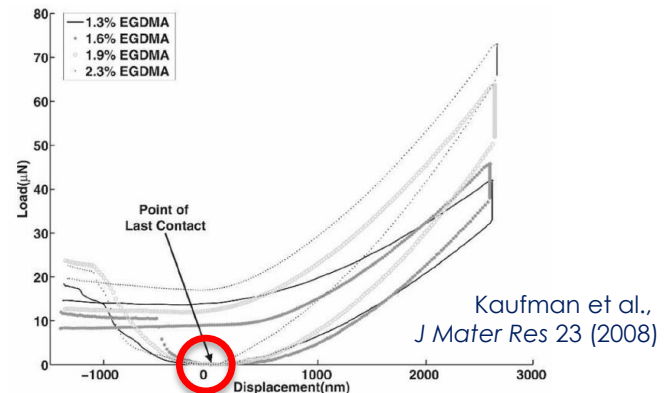
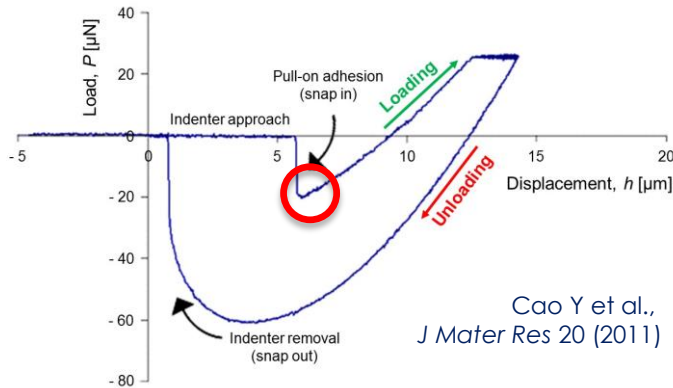


Load-based contact determination



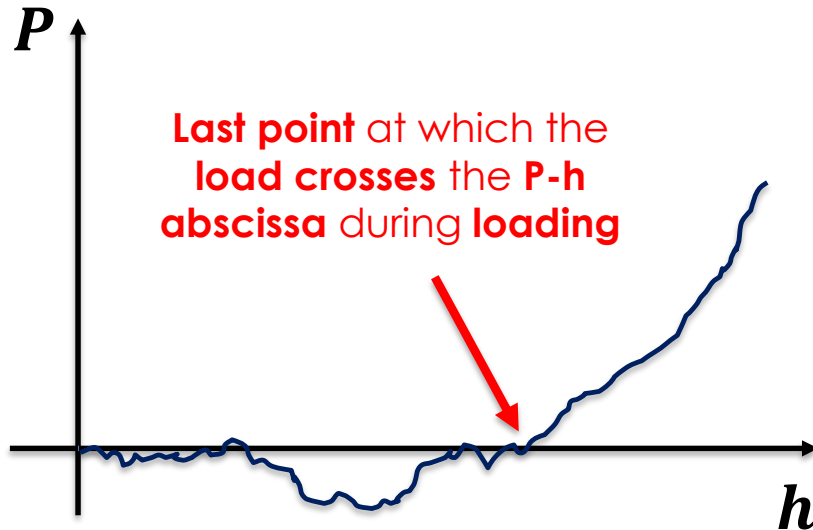
Even **small trigger load** can cause **significant pre-stress** on **soft samples**

Ideal tests should **start out of contact with the sample** \Rightarrow need of **displacement-controlled experiments** and **post-measurement identification** of the **initial contact point**



How to get σ - ε curves from P - h measurements?

- **1st issue:** our solution



Unique identification of the **contact point** both when

- ✓ **Snap into contact** is **poorly evident**
- ✓ **Noise around zero load** is present



How to get σ - ε curves from P - h measurements?

- **2nd issue:** nano- $\dot{\varepsilon}M$ needs $\sigma_{ind}(t)$ response to constant $\dot{\varepsilon}_{ind}$

Most of the studies define $\dot{\varepsilon}_{ind} = \dot{h}/h$ ^{1,2} and $\varepsilon_{ind} = \sqrt{h/R}$ ³, **but...**

- $\dot{\varepsilon}_{ind} \neq \frac{\partial \varepsilon_{ind}}{\partial t}$
- both $\dot{\varepsilon}_{ind} = \dot{h}/h$ and $\frac{\partial \varepsilon_{ind}}{\partial t} = \dot{h}/2\sqrt{Rh}$ are **depth-dependent** functions

NOT suited for the nano- $\dot{\varepsilon}M$

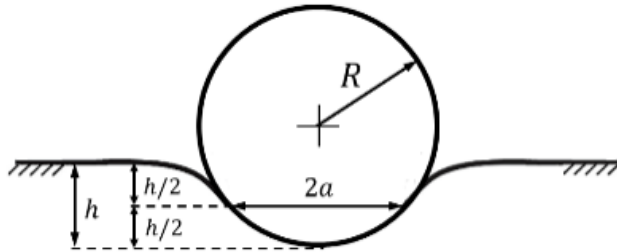
¹ Haghshenas M et al., *Mater Sci Eng* 572 (2013)

² Maier V et al., *J Mater Res* 26 (2011)

³ Basu S et al., *J Mater Res* 21 (2006)

How to get σ - ε curves from P - h measurements?

- **2nd issue:** our **solution**



Hertz model $P = \frac{4}{3} E_{eff} R^{1/2} h^{3/2}$

Sneddon relation $h = \frac{a^2}{R}$

$$\left. \begin{array}{l} \text{Hertz model} \\ \text{Sneddon relation} \end{array} \right\} \frac{h}{a} \cdot \frac{P}{\pi a^2} = \frac{4}{3\pi} E_{eff} \left(\frac{a}{R} \right) \cdot \frac{h}{a}$$

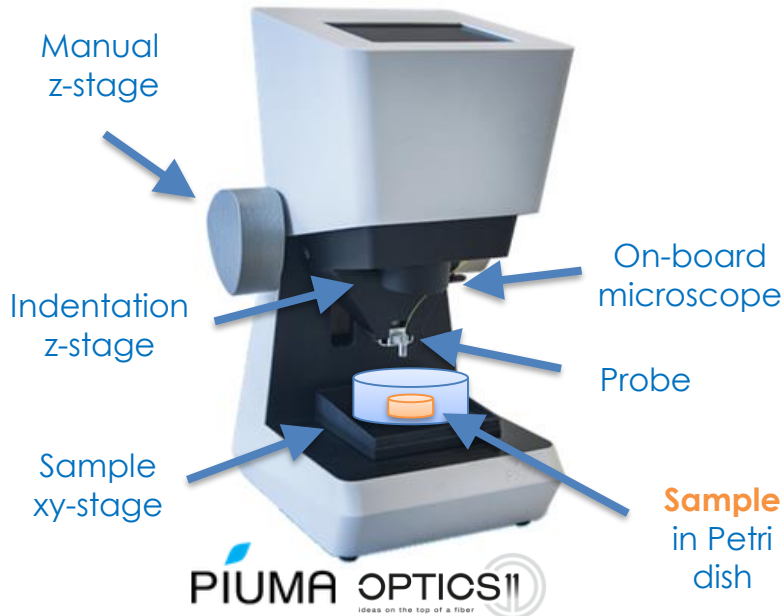
$$\left(\frac{1}{E_{eff}} = \frac{1-\nu^2}{E} + \frac{1-\nu'^2}{E'} \approx \frac{1-\nu^2}{E} \right)$$

$$\left. \begin{array}{l} \sigma_{ind} = \frac{P}{R\sqrt{hR}} \\ \varepsilon_{ind} = \frac{4}{3(1-\nu^2)} \left(\frac{h}{R} \right) \end{array} \right\}$$

- ✓ $\sigma_{ind}/\varepsilon_{ind} = E$ (in case of soft materials where $E' \gg E$)
- ✓ $\dot{\varepsilon}_{ind} = \frac{\partial \varepsilon_{ind}}{\partial t} = \frac{4}{3(1-\nu^2)} \left(\frac{\dot{h}}{R} \right)$ A **constant indentation rate** (\dot{h}) yields a **constant strain rate** ($\dot{\varepsilon}_{ind}$)

An example: PDMS and gelatin characterisation

- **2 different samples: PDMS** (Sylgard 184, 10:1 base to catalyst), **5% w/v gelatin** (type A)
- **Constant $\dot{\epsilon}_{ind}$ tests in dH_2O at RT** using the **PIUMA Nanoindenter** (Optics11)
- **Measurements started above the sample surface to avoid pre-stress** (different tests on different surface points spaced by 200 μm)

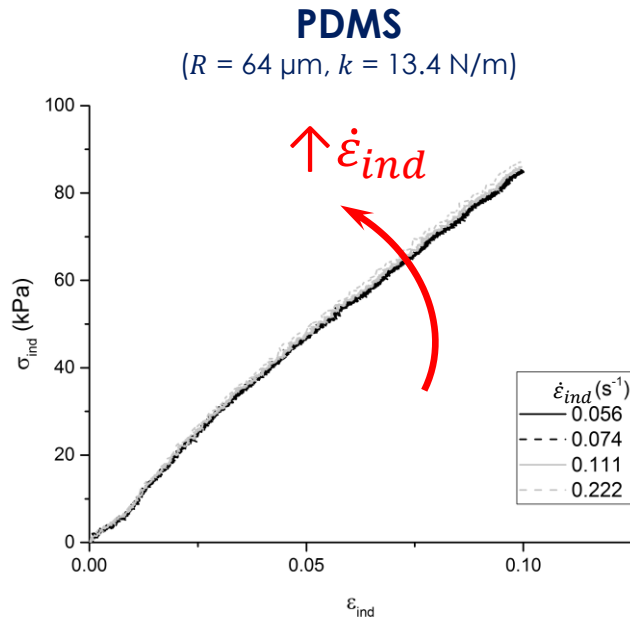


Indentation rate \dot{h} to obtain a given indentation strain rate $\dot{\epsilon}_{ind}$

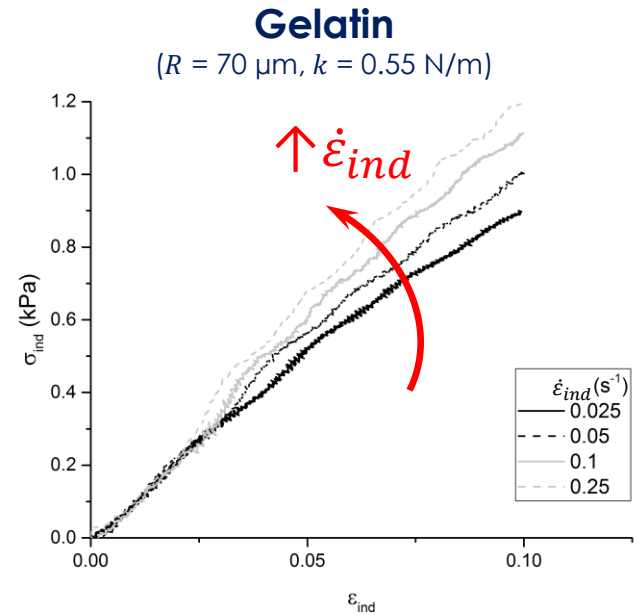
$$\dot{h} = \frac{4}{3(1 - \nu^2)} \left(\frac{\dot{\epsilon}_{ind}}{R} \right)$$

Indentation stress-strain curves at different $\dot{\epsilon}_{ind}$

- Tests at **4 different indentation strain rates** ($n = 10$ per each $\dot{\epsilon}_{ind}$)



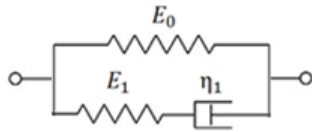
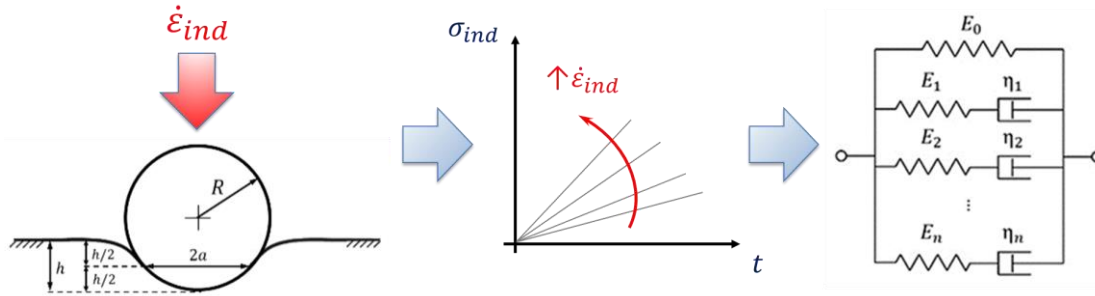
- ✓ **LVR** up to **10% ϵ_{ind}**
- ✓ Fairly **rate-independent** behaviour



- ✓ **LVR** up to **10% ϵ_{ind}**
- ✓ Pronounced **rate-dependent** behaviour

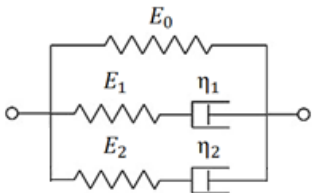
Viscoelastic lumped parameter estimation

- Indentation stress-time data within LVR obtained at different $\dot{\epsilon}_{ind}$ were analysed with $\dot{\epsilon}M$ global fitting procedure sharing the viscoelastic parameters to estimate



Maxwell Standard Linear Solid (**SLS**)

$$\sigma_{ind}(t) = \dot{\epsilon}_{ind} \left[E_0 t + \eta_1 \left(1 - e^{-\frac{E_1}{\eta_1} t} \right) \right]$$



2-arm generalised Maxwell (**GM2**)

$$\sigma_{ind}(t) = \dot{\epsilon}_{ind} \left[E_0 t + \eta_1 \left(1 - e^{-\frac{E_1}{\eta_1} t} \right) + \eta_2 \left(1 - e^{-\frac{E_2}{\eta_2} t} \right) \right]$$

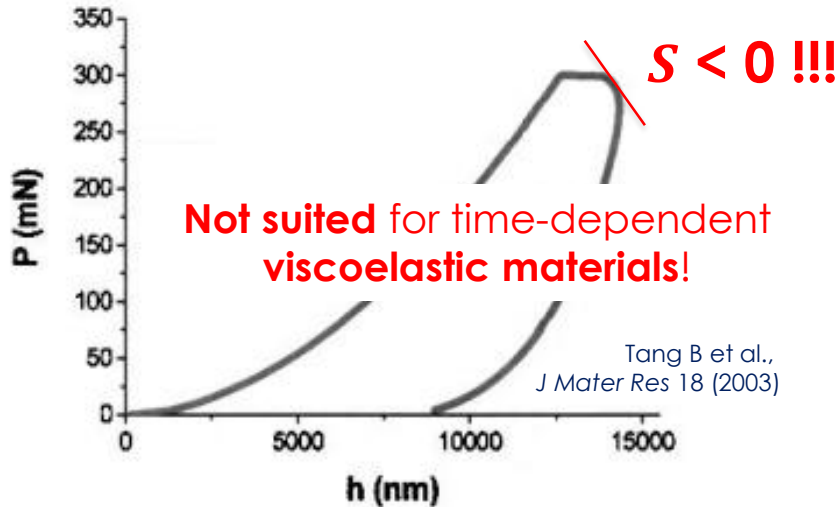
Viscoelastic parameters estimated using the nano- ϵM (est. value \pm standard error)

Parameter	PDMS		Gelatin	
	Maxwell SLS	GM2	Maxwell SLS	GM2
E_{inst} (kPa)	$1.74 \cdot 10^3 \pm 1.47 \cdot 10^1$	$1.74 \cdot 10^3 \pm 1.02 \cdot 10^2$	14.08 ± 0.58	$14.08 \pm 1.37 \cdot 10^3$
E_{eq} (kPa)	$8.82 \cdot 10^2 \pm 8.72 \cdot 10^{-1}$	$5.98 \cdot 10^2 \pm 7.14 \cdot 10^1$	1.84 ± 0.42	$4.07 \cdot 10^{-4} \pm 4.65 \cdot 10^2$
τ_1 (s)	$0.26 \pm 4.93 \cdot 10^{-3}$	$0.26 \pm 4.93 \cdot 10^{-3}$	6.90 ± 0.60	$14.78 \pm 2.36 \cdot 10^3$
τ_2 (s)	-	$1.04 \cdot 10^{12} \pm 3.68 \cdot 10^{11}$	-	$5.57 \pm 1.36 \cdot 10^3$
R^2	0.97	0.97	0.99	0.99

Values in red cannot be considered as significant since they are **almost meaningless** with **very large standard errors**, clearly indicating **GM2 model over-parameterisation**

Oliver-Pharr

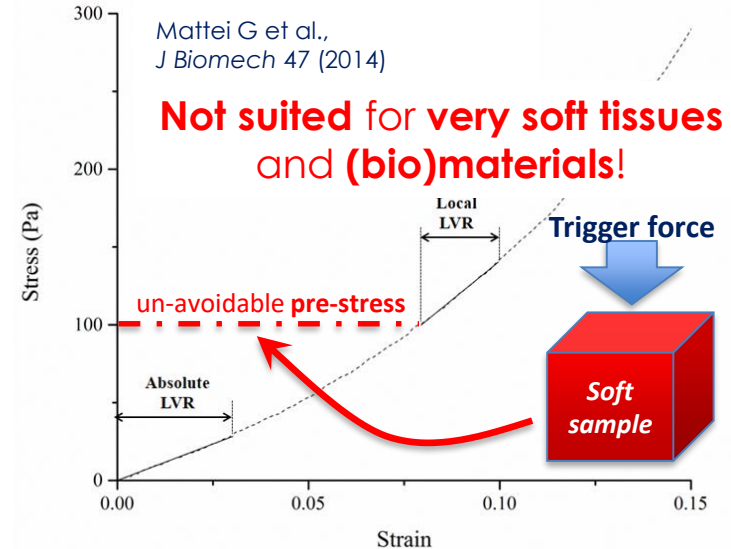
Oliver W, Pharr G, *J Mater Res* 7, 1564-83 (1992)



- **Elastic-plastic** contact model
- Analysis based on the **unloading curve** using P_{max} , h_{max} and **unloading slope S**
- **Characterises** material **elastic properties only**

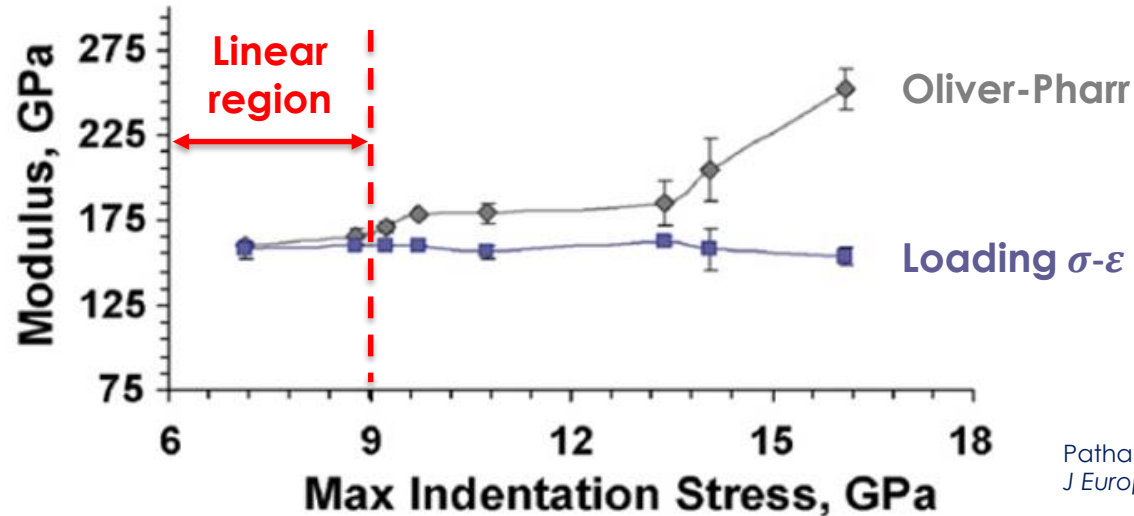
Nano-DMA

Mattei G et al., *J Biomech* 47 (2014)



- **Micro-scale equivalent** of the **bulk DMA**
- **CSM reduces** the **reliance on unloading curve** and **provide results** as a function of **indentation depth**
- **Need a small** but measurable **trigger force**

- **Mechanical properties** representative of the “**virgin**” material
- **Constant** derived (**visco**)**elastic parameters, regardless of the max load (or displacement)** chosen for the experiment



Pathak et al.,
J Europ Cer Soc 28 (2008)

- **During unloading** only the **elastic displacements** are recovered

Micro- vs. macro-scale results?

SLS viscoelastic parameters obtained at the micro- and macro-scale* (est. value \pm standard error)

* macro-scale values are taken from Tirella A et al., JBMR A 102 (2014)

Parameter	PDMS		Gelatin	
	Micro-scale (nano- $\dot{\epsilon}M$)	Macro-scale ($\dot{\epsilon}M$) ¹	Micro-scale (nano- $\dot{\epsilon}M$)	Macro-scale ($\dot{\epsilon}M$) ¹
E_{inst} (kPa)	$(1.74 \pm 0.01) \cdot 10^3$	< $(2.55 \pm 0.04) \cdot 10^3$	14.08 ± 0.58	23 ± 0.45
E_{eq} (kPa)	$(8.82 \pm 0.01) \cdot 10^2$	< $(2.14 \pm 0.01) \cdot 10^3$	1.84 ± 0.42	2.3 ± 0.10
τ_1 (s)	0.26 ± 0.01	< 0.66 ± 0.25	6.90 ± 0.60	> 0.19

- τ_1 **decrease** from macro- to **micro-scale** observed for **PDMS** is **consistent with literature**^{1,2,3}
- **Variations** between results at the **micro-** and **macro-scale** may be due to
 - **real differences** between the **bulk** and **surface** mechanical properties¹
 - nano-indentation σ_{ind} and ϵ_{ind} are **not the same** as engineering σ and ϵ



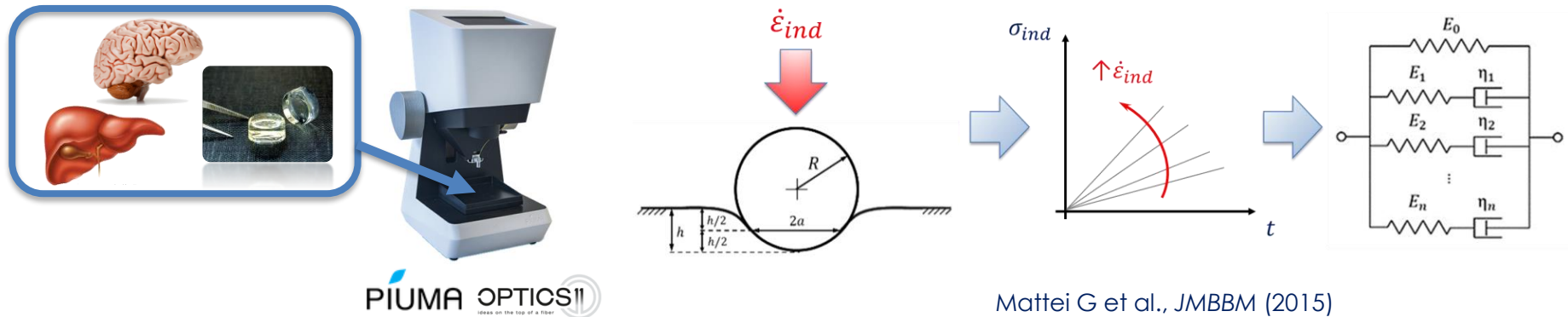
¹ Kaufman J et al., J Mater Res 23 (2008)

² Sasaki S et al., J Chem Phys 120 (2004)

³ Mak A et al., J Biomech (1987)

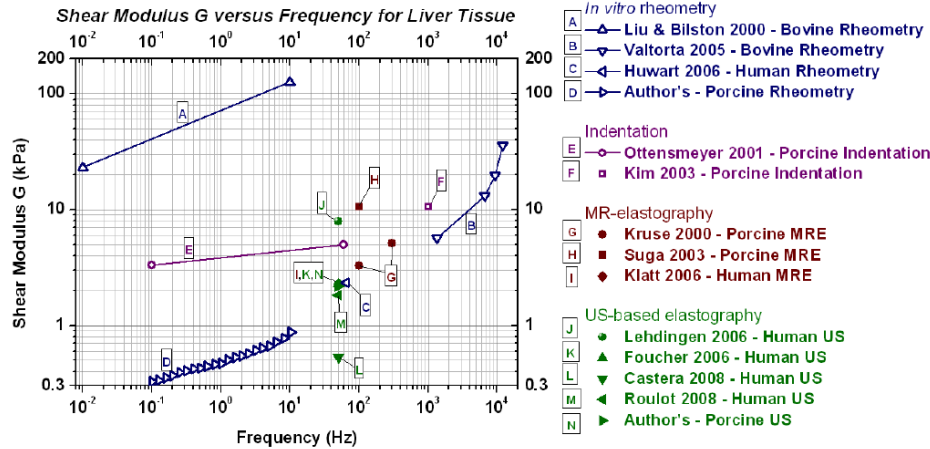
- The **nano- $\dot{\epsilon}M$**

- combines the **advantages** of the $\dot{\epsilon}M$ and **nano-indentation** techniques
- allows to **locally map** the **viscoelastic properties** of “**virgin**” materials in **absence** of **pre-stress**, being **advantageous over methods** based on the **unloading curve** or **requiring a force trigger**
- very **suited** for **soft biological tissues** and **biomaterials**
- can be **implemented** with **any displacement-controlled nano-indenter** (e.g. Optics 11 PIUMA)

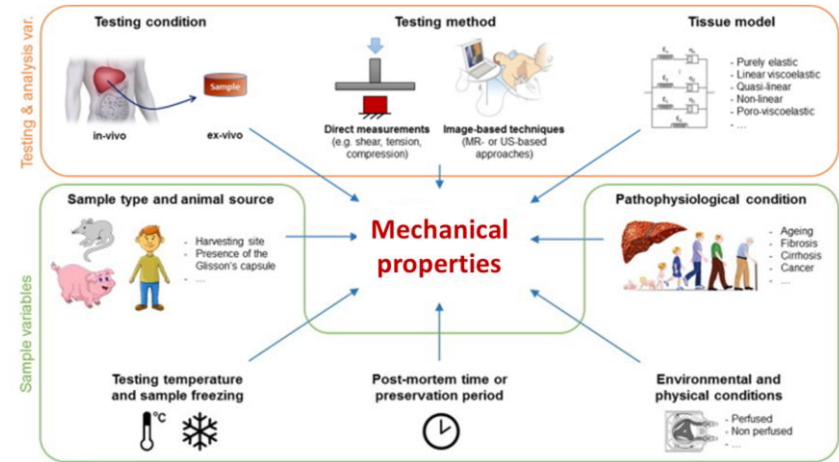


Does measuring in the frequency or strain-rate domain affect mechanical results?

- Little consensus in the literature

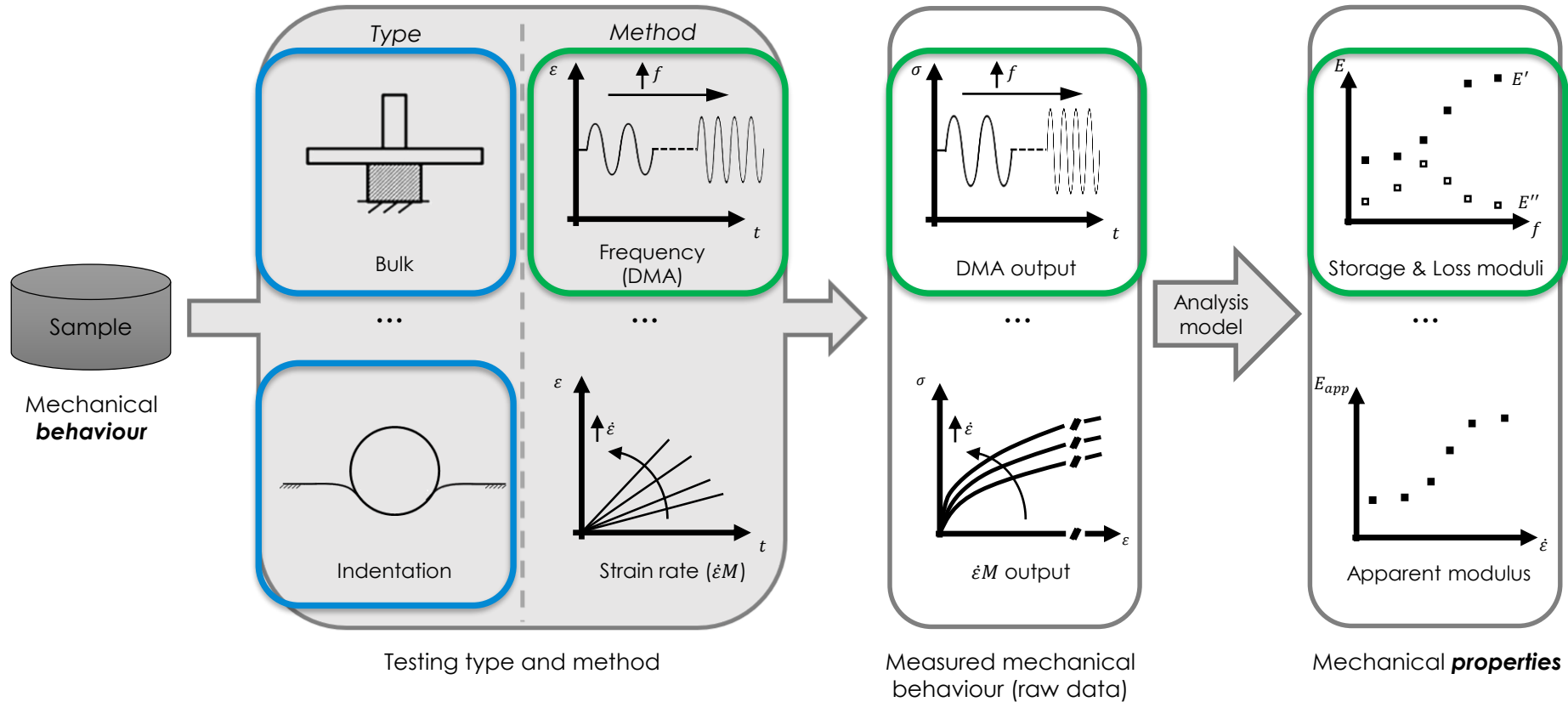


S Marchesseau et al, *Progr in Biophys and Mol Biol* 103:185-96 (2010)

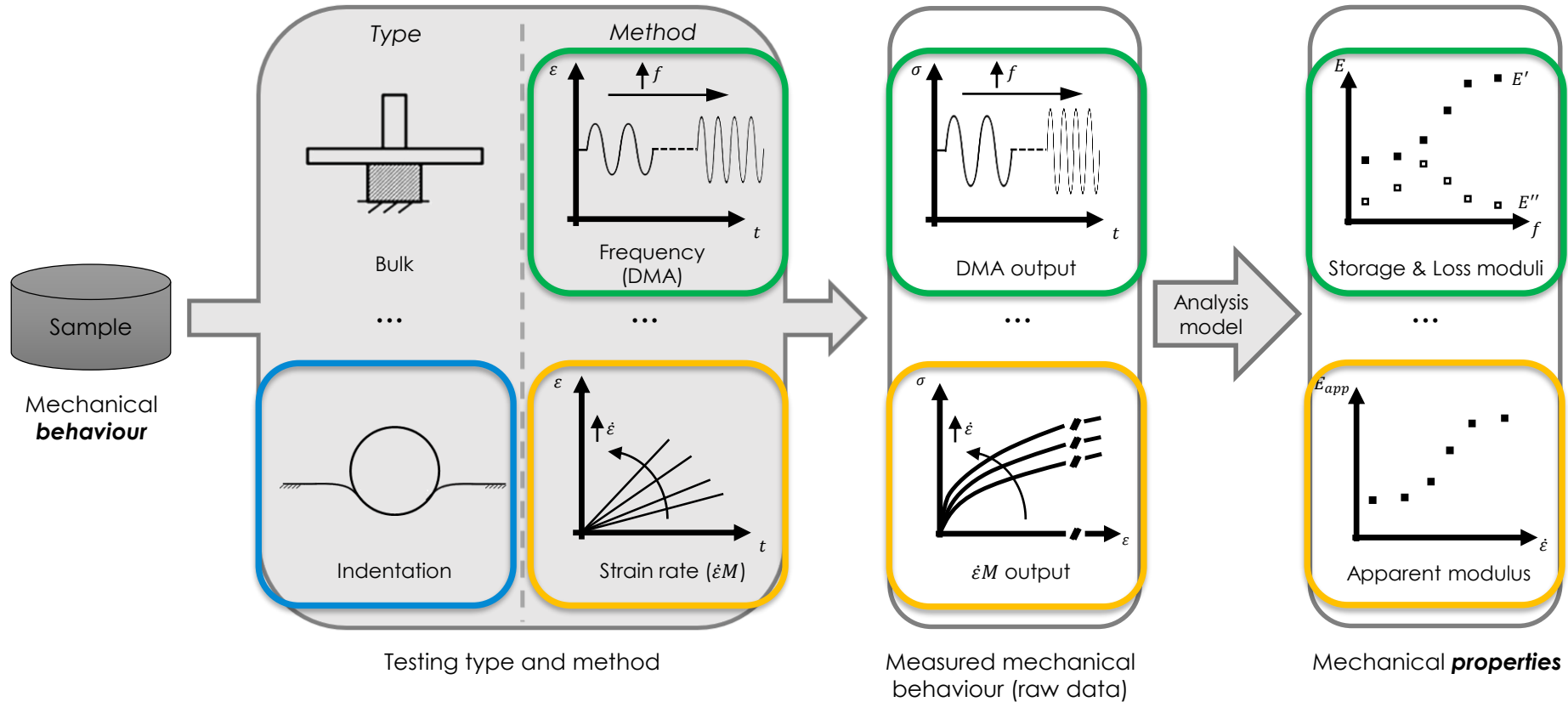


G Mattei and A Ahluwalia, *Acta Biom* 45:60-71 (2016)

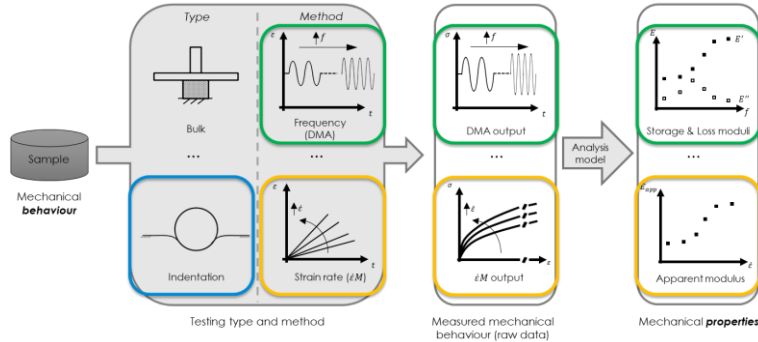
From sample mechanical *behaviour* to *properties*



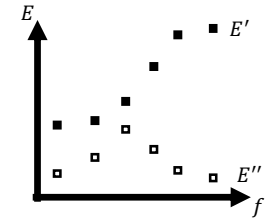
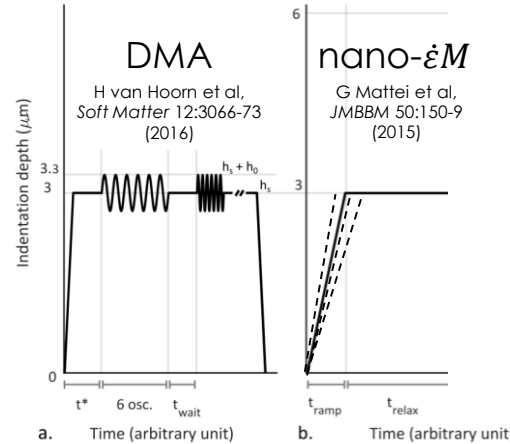
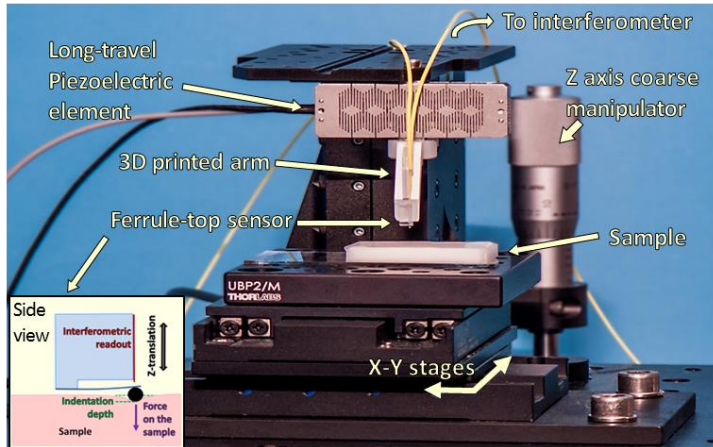
From sample mechanical behaviour to properties



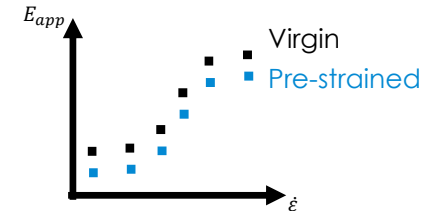
Aim and Strategy



Does measuring in the **frequency** or **strain-rate domain** affect **micro-mechanical properties**?

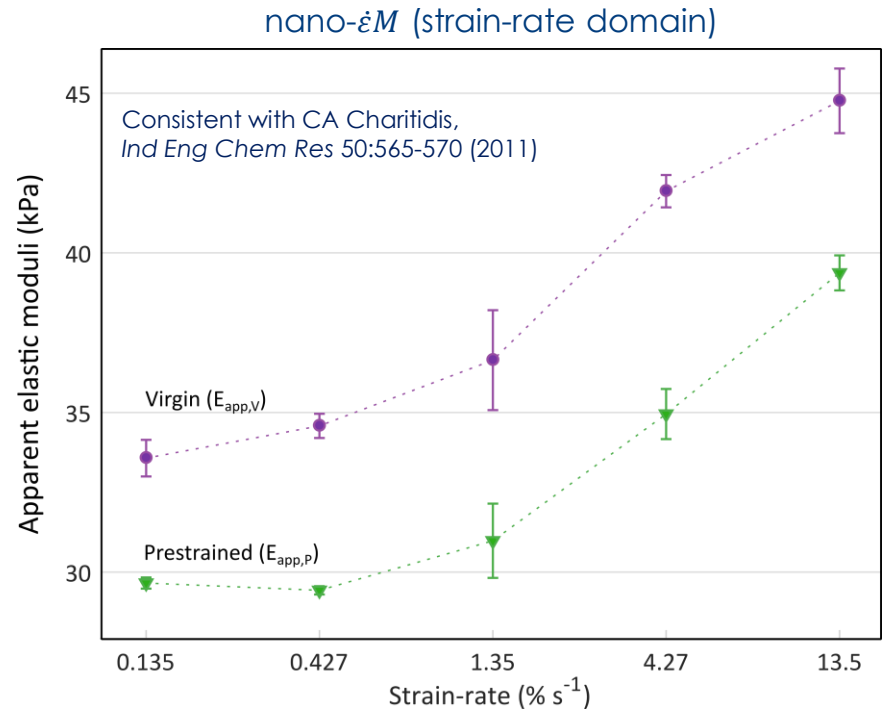
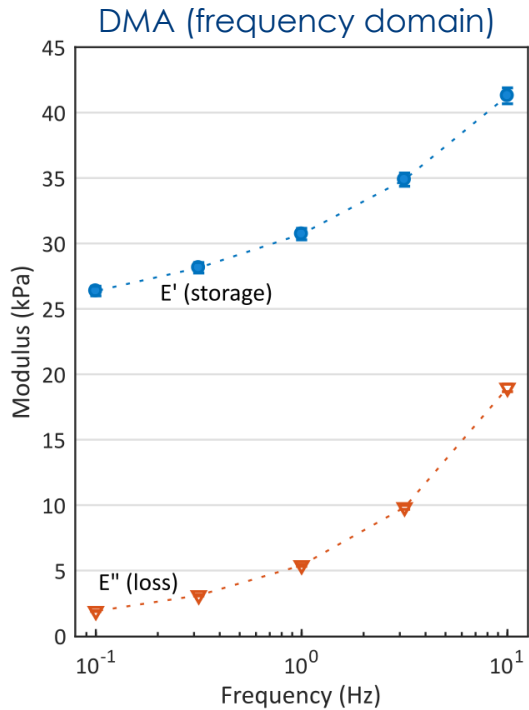


Storage & Loss moduli



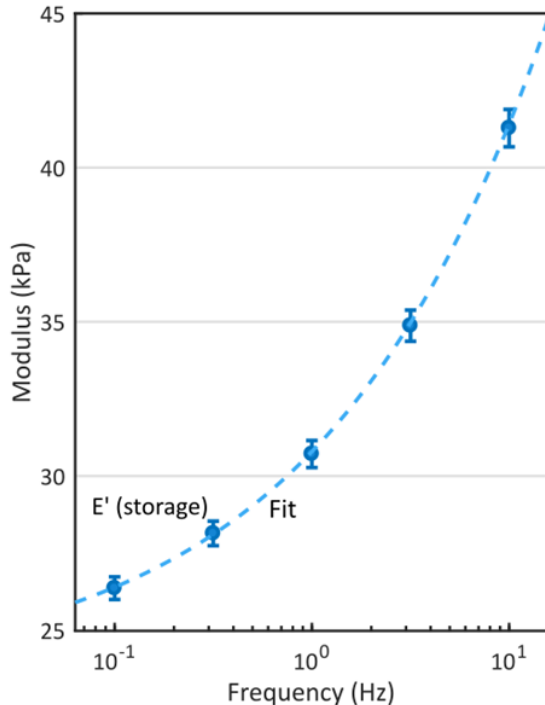
Apparent modulus

- **PDMS** (Sylgard 184, 50:1) samples tested at **25°C** with a **248 μm** radius probe



Comparing DMA and nano- ϵM results

- **Conversion from f to $\dot{\epsilon}$ domain** based on SE Zeltman et al, *Polymer* 101:1-6 (2016), assuming $1 f_c$ or τ



- 1) **Storage modulus master curve** from DMA data

$$E'(\omega) = a \cdot \tanh(b \cdot (\ln(\omega) + c)) + d \quad \omega = 2\pi f$$

- 2) $E'(\omega)$ converted into **time-domain relaxation modulus**

$$E(t) = \frac{2}{\pi} \int_0^{\infty} \frac{E'(\omega)}{\omega} \sin(\omega t) \partial \omega$$

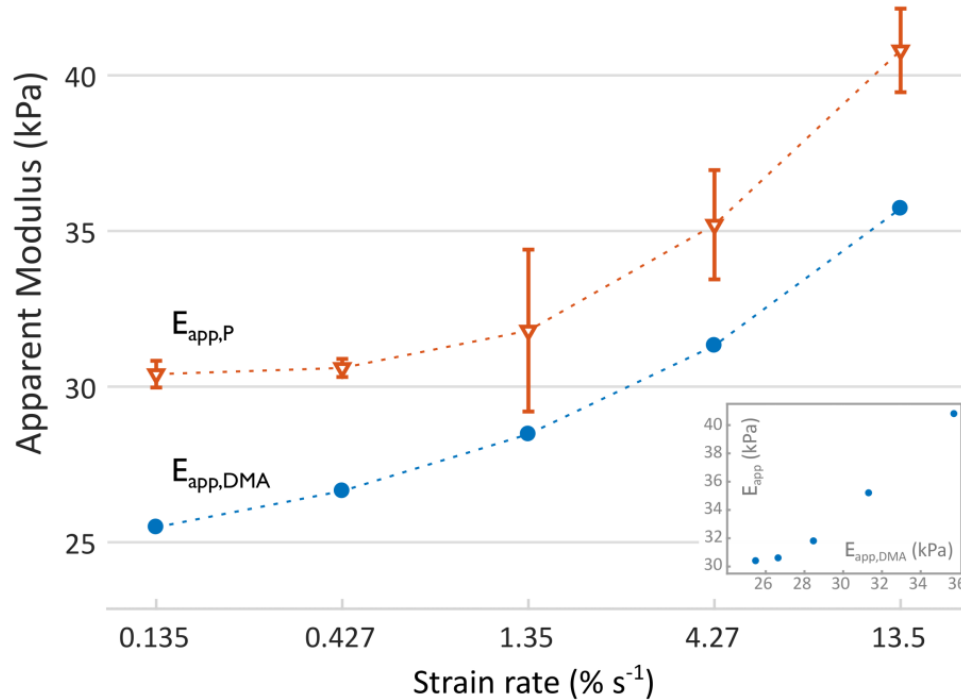
- 3) **Stress-time response** to a given strain history

$$\sigma(t) = E * \partial \epsilon = \int_{-\infty}^t E(t - \tau) \frac{\partial \epsilon(\tau)}{\partial \tau} \partial \tau \xrightarrow{\text{constant } \dot{\epsilon}} \sigma(t) = \dot{\epsilon} \int_0^t E(\tau) \partial \tau$$

- 4) **Stress-strain response** by linear transformation of $\epsilon = \dot{\epsilon} \cdot t$

$E_{app,DMA}(\dot{\epsilon})$ as **stress-strain slope** within LVR

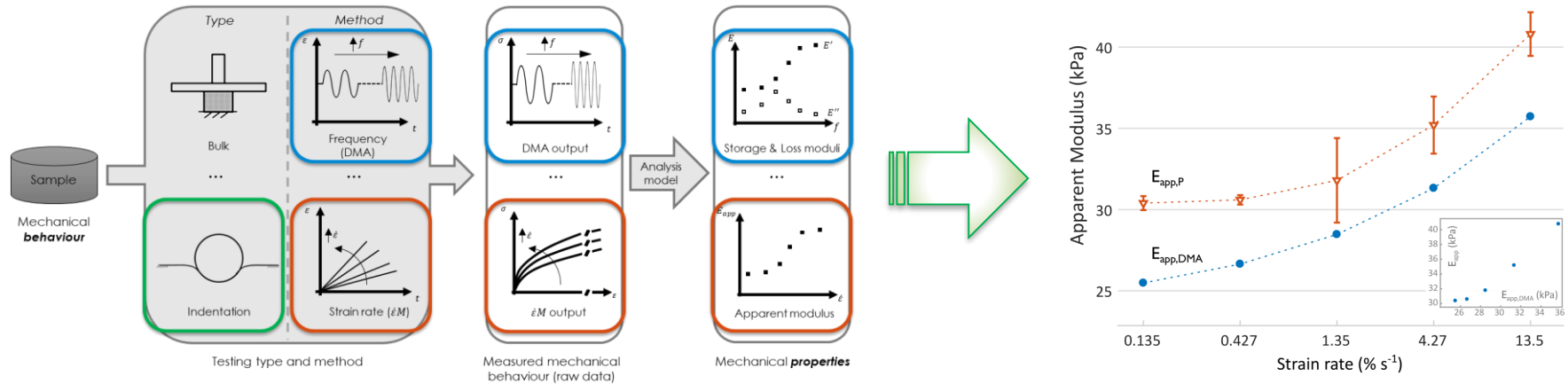
- DMA-derived apparent elastic moduli ($E_{app,DMA}$) versus nano- ϵM pre-strained ones ($E_{app,P}$)



- Increase** with **strain rate** (as expected)
- $E_{app,P} > E_{app,DMA}$, regardless of ϵ
- Optimal correlation** ($r = 0.99$) with almost **constant difference** between moduli (~10%) (consistent with SE Zeltman et al, *Polymer* 101:1-6, 2016)
 - Good agreement** between f and $\dot{\epsilon}$ results
 - Systematic error** possibly due to **narrow f range attainable** by our setup (0.1-10 Hz)

2% increase in d resulted in:
 - small master-curve transition upward
 - almost **perfect match** of the **moduli**

- **Frequency** and **strain-rate** domain results **directly compared**, **without** any **other source of variability**



The observed **compatibility** allows to **combine** these **methods** towards a **more comprehensive understanding** of **material viscoelastic** (time-dependent) **behaviour**, critical for several applications (biomechanics, tissue engineering, mechanobiology, ...)



Useful references

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