UNIVERSITÀ DI PISA

Centro E. Piaggio
bioengineering and robotics research center

INGEGNERIA DEI TESSUTI BIOLOGICI:

DYNAMIC MECHANICAL ANALYSIS (DMA)

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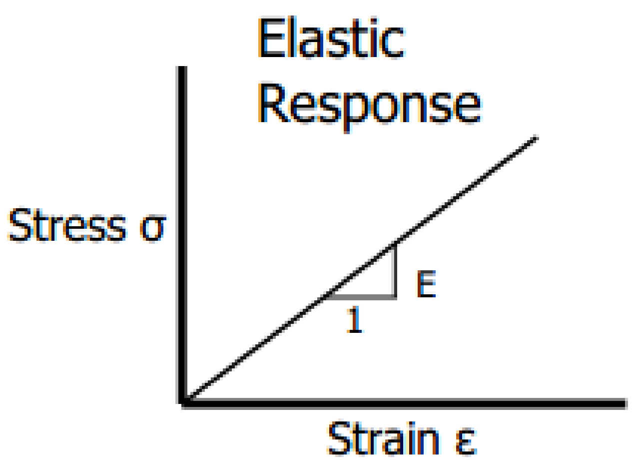
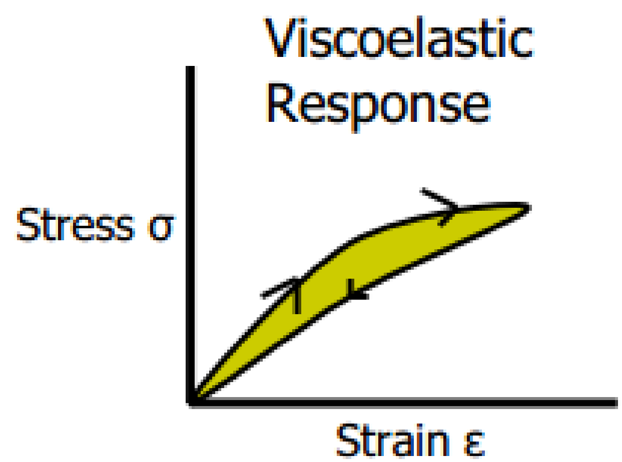
Viscoelasticity

- **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





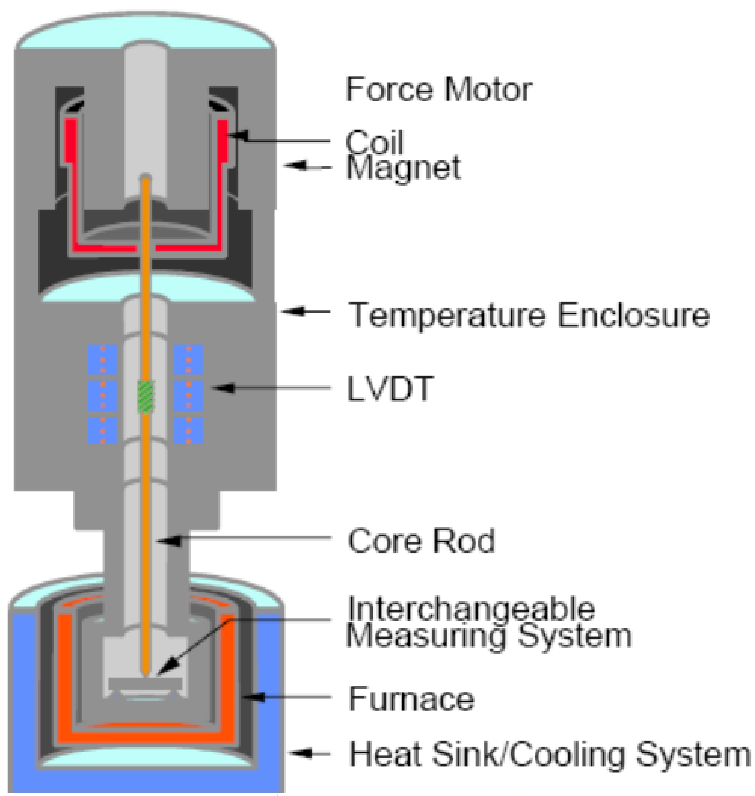
Methods to characterise viscoelasticity

- **Time** domain
  - Creep response
  - Stress relaxation
  - Epsilon dot Method ($\dot{\varepsilon}M$, Tirella A. et al., JBMR 2013)

- **Frequency** domain
  - Dynamic mechanical analysis (DMA)
  - Dynamic mechanical thermal analysis (DMTA)

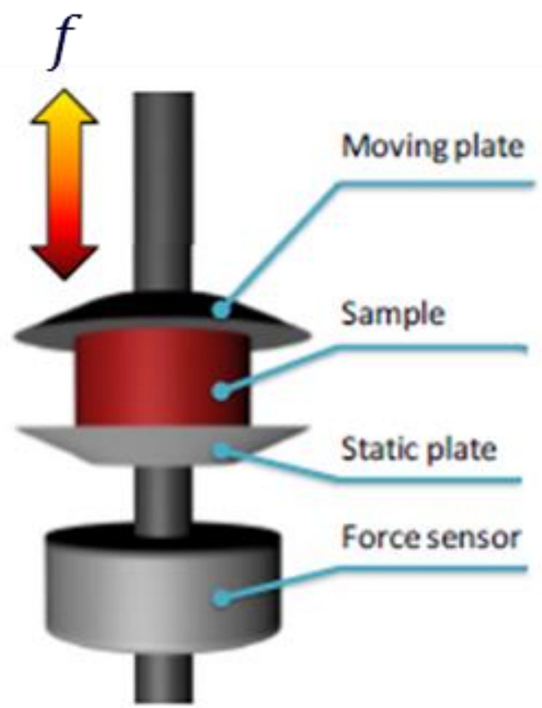
DMA overview

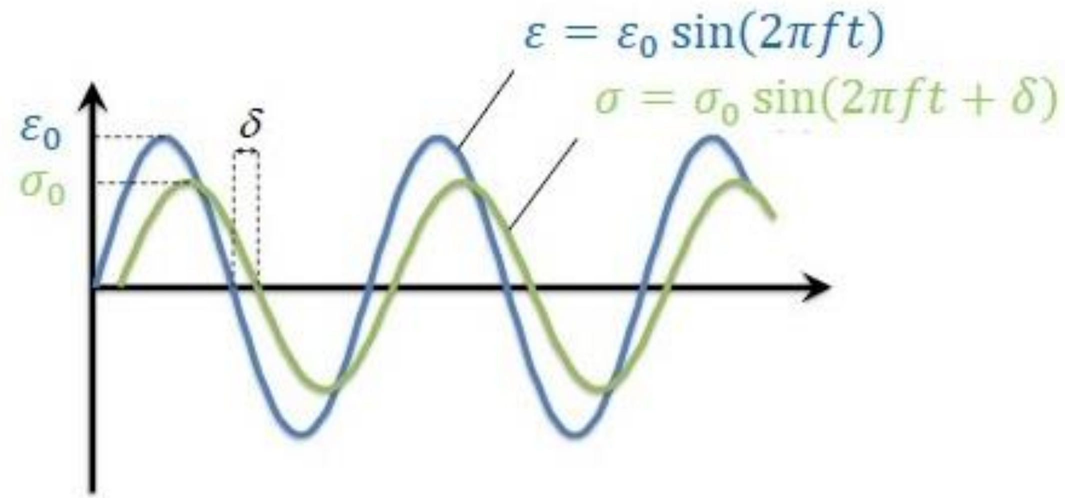
- Dynamic mechanical analysis (DMA) is a standard **force-triggered method** to **determine viscoelastic properties** of materials by **applying a small amplitude cyclic strain** on a sample and **measuring the resultant cyclic stress response**.



DMA overview

- 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.

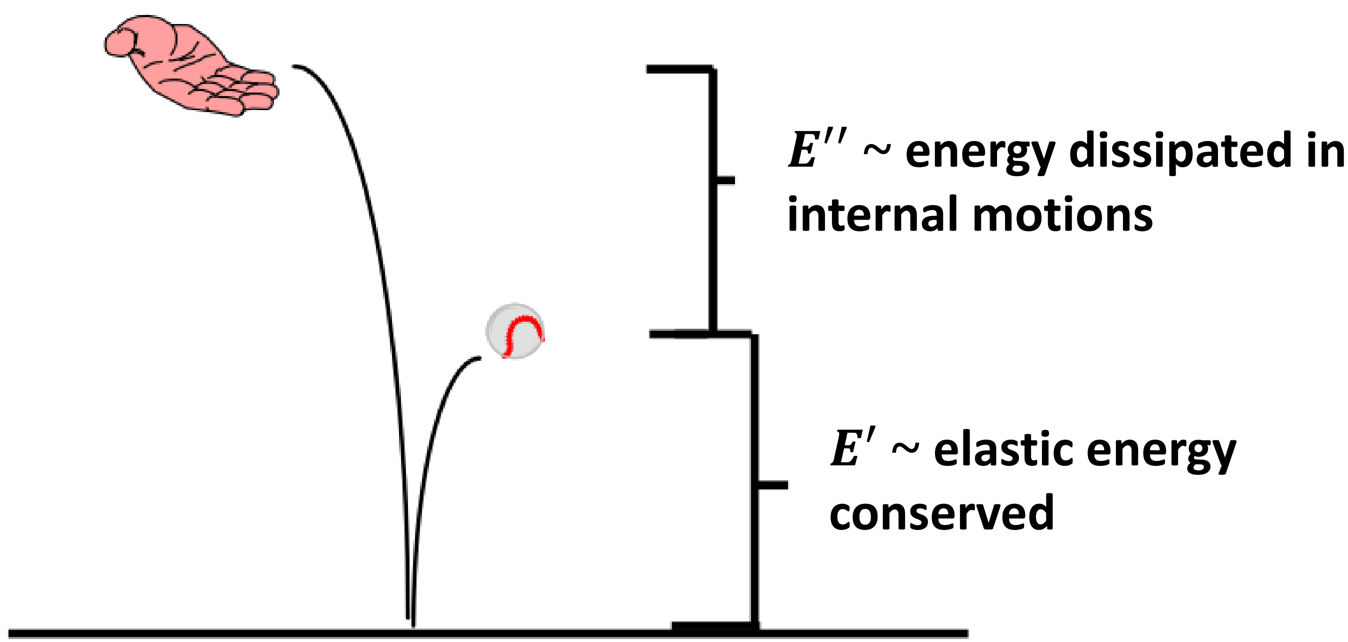




Viscoelastic material response is characterised by a **phase lag** (**δ**) between the strain input and the stress response, which is comprised **between 0°** (**purely elastic**) and **90°** (**purely viscous**). This phase lag is **due to** the excess **time necessary for molecular motions and relaxations**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

Complex, storage and loss modulus

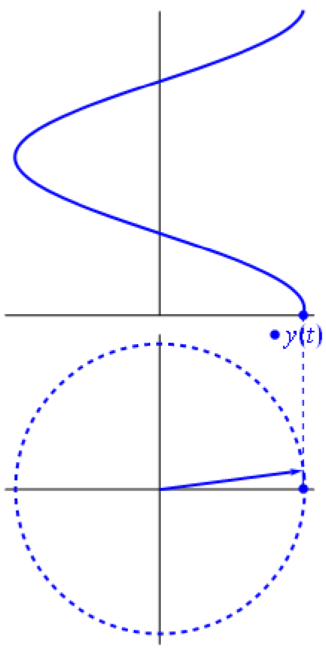
- The dynamic mechanical properties are quantified with the **complex modulus** ($\boldsymbol{E^*}$), which can be thought as an **overall resistance** to deformation under dynamic loading. The complex modulus is composed of the **storage** ($\boldsymbol{E'}$, elastic component) and the **loss** ($\boldsymbol{E''}$,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

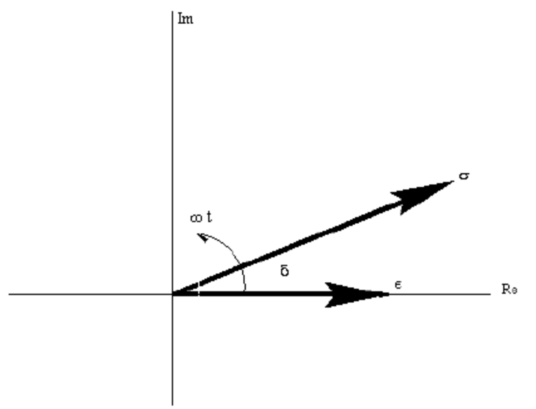


Definitions

- 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**.

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





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

Rotating vector representation of harmonic stress and strain

$$\boldsymbol{E}^* = \frac{\sigma}{\varepsilon} = \frac{\sigma_0}{\varepsilon_0} e^{i\delta} =$$

$$= \frac{\sigma_0}{\varepsilon_0} (\cos\delta + i \sin\delta) =$$

$$= \boldsymbol{E}' + \boldsymbol{iE}''$$

Storage modulus

$E' = E^* \, cos(\delta)$

Loss modulus

$E'' = E^* \, sin(\delta)$

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

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

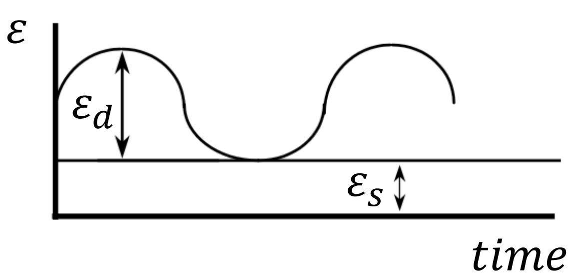
Test modes

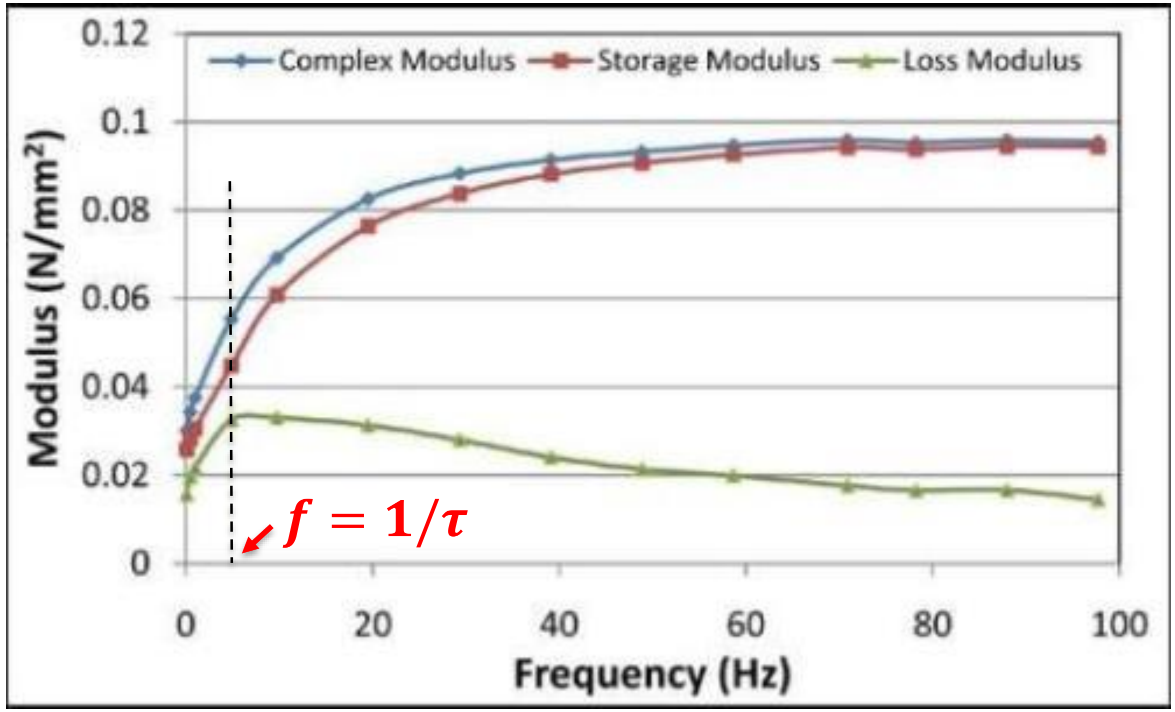
- **Temperature sweep**: Modulus and damping are recorded as the sample is heated
- **Frequency sweep**: Modulus and damping are recorded as the sample is loaded at increasing (or decreasing) frequencies
- **Stress amplitude sweep**: Modulus and damping are recorded as the sample stress is increased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

Frequency sweep tests

- 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)

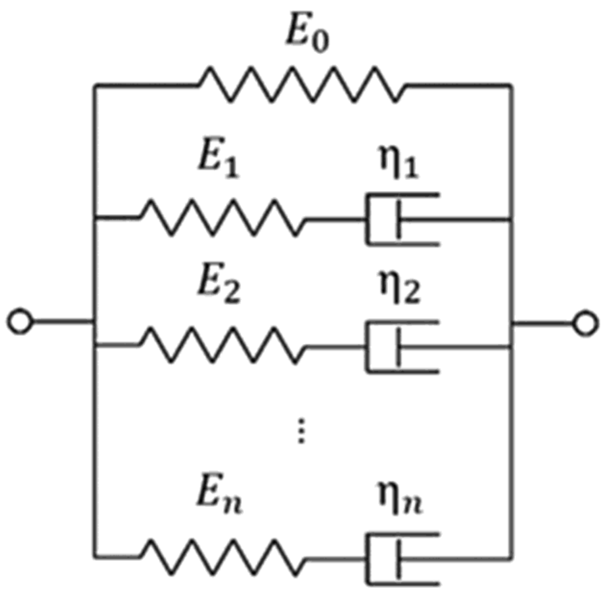




- **Peaks** in $\mathbf{tan(\delta)}$ or $\boldsymbol{E''}$ with respect to frequency identify the **characteristic relaxation frequencies** of the viscoelastic sample under testing, defined as $\boldsymbol{f = 1/\tau}$, where $\boldsymbol{\tau}$ is the **characteristic relaxation time)**

Lumped models to describe material linear viscoelastic response

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



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

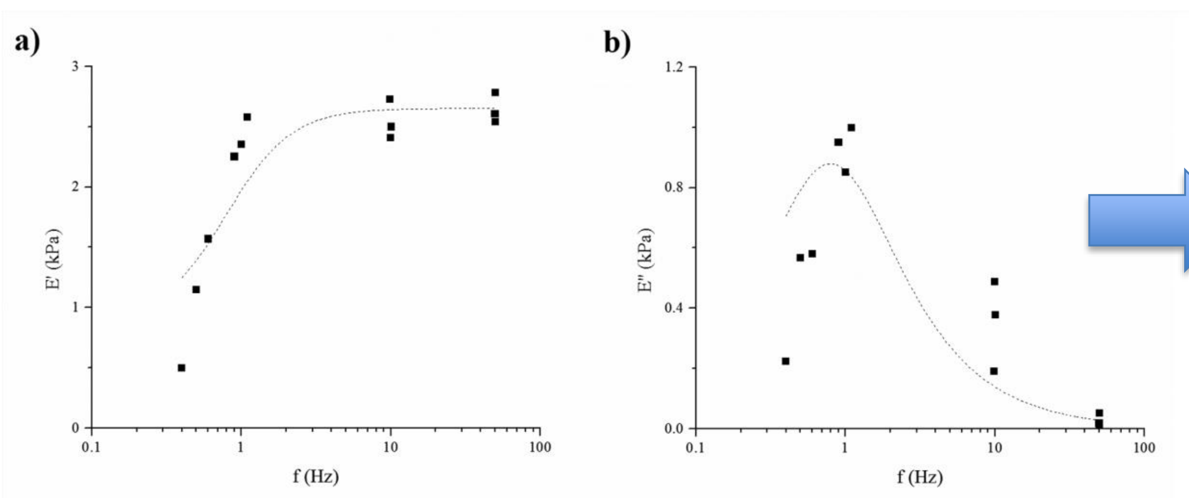
GM model transfer function in the Laplace domain

Lumped parameters derivation from frequency sweep tests

- Calculate the **complex conjugate of the GM modulus** ($\boldsymbol{E^*_{GM}}$) 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

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

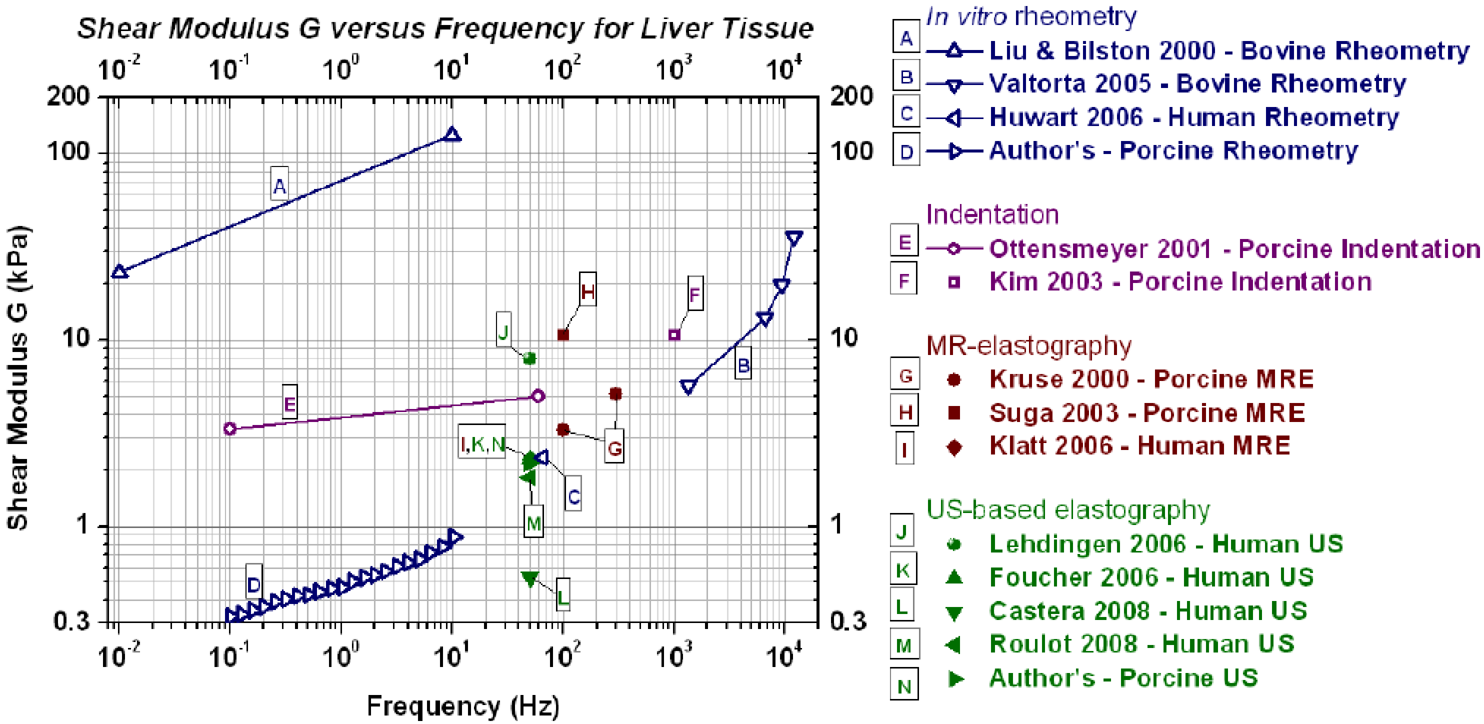
- **Global fitting** with **shared parameters** (χ^2 minimisation)



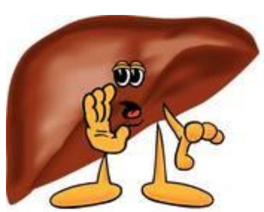
E_i, η_i

CASE OF STUDY:
THE LIVER

SoA: a myriad of different results



Source: S. Marchesseau et al., *Progress in biophysics and molecular biology*, 103:2–3, pp. 185–96, 2010



Many **variables and factors affect** measured **liver mechanical properties,** leading to a **lack of consensus and unique properties**, which are **critical for developing appropriate viscoelastic models**

Typical variability factors

- **Testing condition**
  - ***in-vivo***: *tissue in its* ***natural state****, but many* ***testing limitations***
  - ***ex-vivo:*** *better for* ***developing testing devices****,* ***protocols*** *and* ***tissue models***
- **Testing method and experimental setup**
  - **Direct measurements** or **image-based techniques**
  - **Time, strain rate** or **frequency range** considered
- **Tissue sample**
  - **Type and source:** animal source, presence of Glisson's capsule
  - **Status:** environmental testing parameters, physical conditions, post-mortem time, preservation period, pathophysiological state, preload

From this multifaceted research area emerges **the need to:**

1. **clearly identify the parameters of interest**
2. **develop suitable experimental testing setup and protocols** for the **unique identification** of **liver viscoelastic parameters**

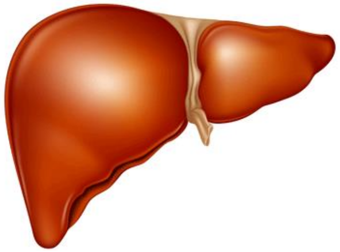
Aim and strategy

AIM: establishing an **experimental testing and analysis framework** to **unequivocally characterise** the **liver viscoelastic behaviour** in the **LVR** (linear viscoelastic region)

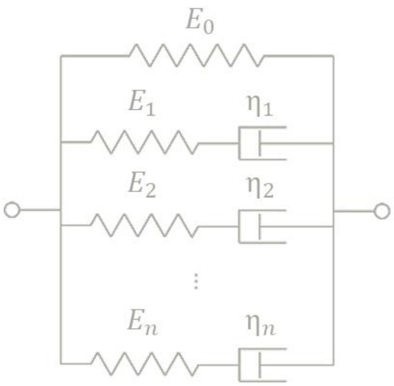
STRATEGY: ***ex-vivo*** measurements in **unconfined compression** using **common testing apparatus** and **2 different testing methods**

- $\dot{\varepsilon}M$, **a solution** to **avoid major drawbacks** of force- or strain-triggered methods **in testing floppy samples** (e.g. **long test duration** and significant **sample pre-load**)
- ***step-reconstructed*** **DMA**, a modification of a **widely used technique** for viscoelastic characterisation of materials

Sample preparation → Mechanical tests → Modelling viscoelastic behaviour → Lumped parameter estimation

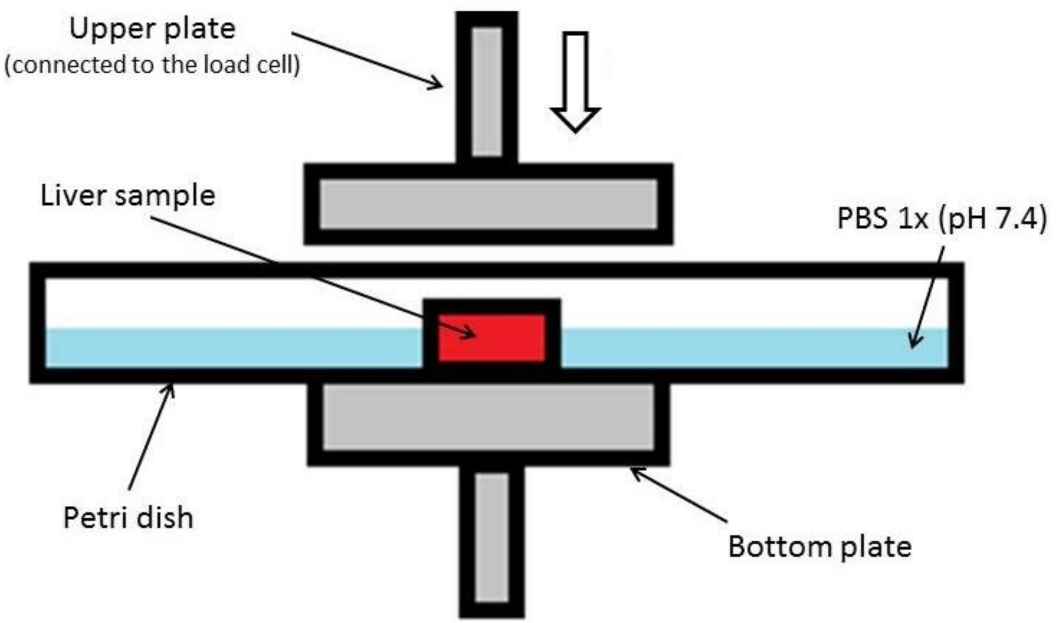


Sample preparation

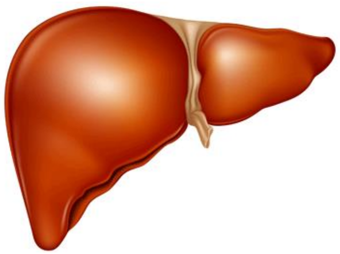


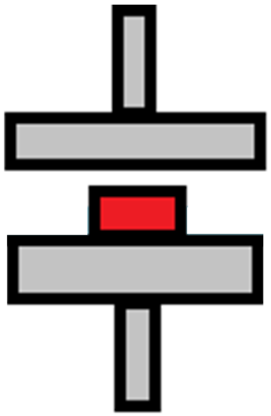
Sample preparation and testing configuration

- **Cylindrical liver samples** (14 mm diameter, 3 mm thickness) collected from **1 year old healthy pigs avoiding Glisson's capsule** and **macroscopic vasculature**
- **Repeatable testing condition** → samples **equilibrium swollen in PBS 1x at 4°C**, then **brought to room T** and **carefully measured** prior testing

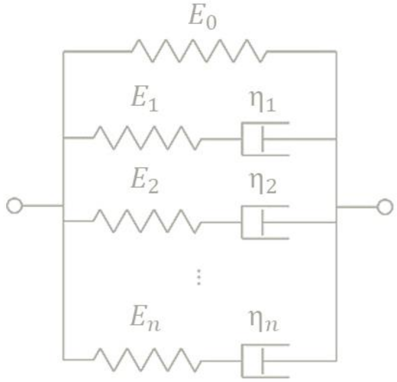


Testing configuration





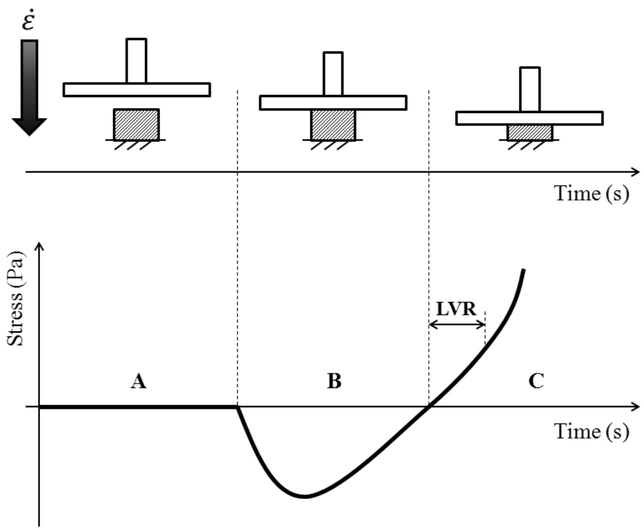
Mechanical tests



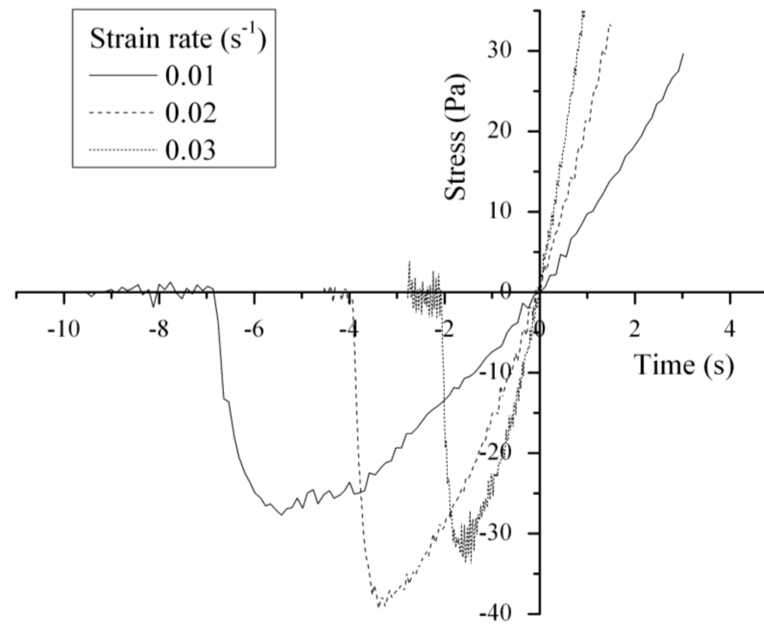
$\dot{\varepsilon}M$: short test with no pre-load

A. Tirella, G. Mattei, A. Ahluwalia, JBMR Part A (2013)

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



- ✓ Implementable with **all uniaxial testing devices**
- ✓ **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**
- x Need **preliminary tests** or an ***a priori*** **knowledge of the material relaxation behaviour** to **choose $\dot{\varepsilon}$**



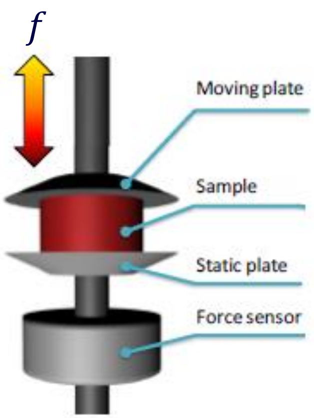
Experimental stress-time data at various $\dot{\varepsilon}$
(only **LVR values** are shown in zone C)

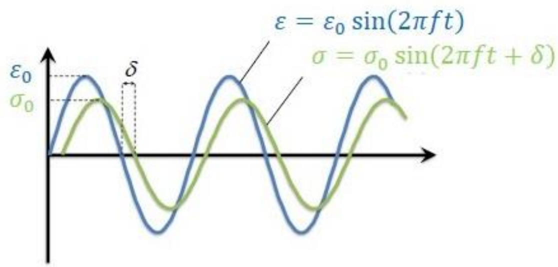
Zwick/Roell Z005, 10N load cell

3 samples x *3 $\dot{\varepsilon}$ = 9 samples*

DMA: a widely accepted method

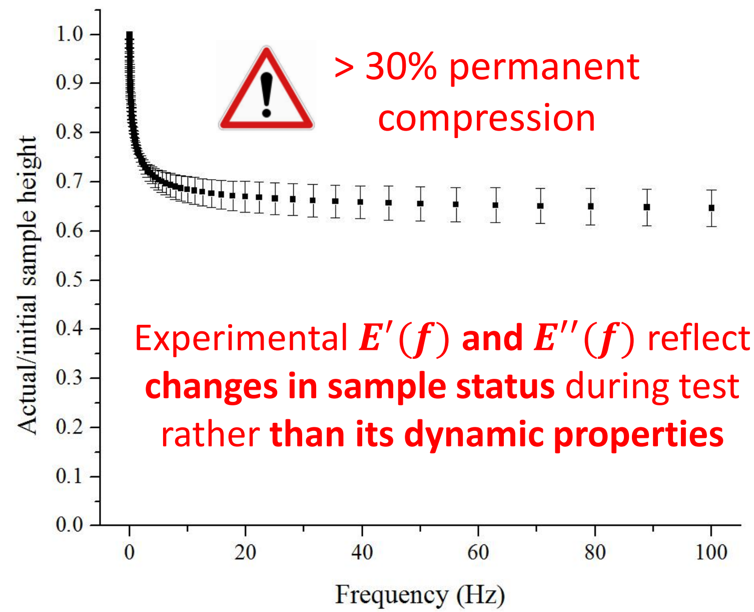
DMA paradigm: characterise viscoelastic behaviour testing samples at **different frequencies** (f), then **analysing** $E'(f)$ **and** $E''(f)$





$$E' = \frac{\sigma_0}{\varepsilon_0}\cos(\delta) \qquad E'' = \frac{\sigma_0}{\varepsilon_0}\sin(\delta) \qquad E^* = E' + iE''$$

- ✓ **Largely accepted** for **viscoelastic characterisation**
- ✓ Wide **frequency sweep** tests **simplify testing set-up** avoiding preliminary tests or any *a priori* knowledge
- x **Long testing time** may **degrade the sample**
- x **Trigger force** may **significantly pre-load samples**
- x **Preliminary strain-sweep tests** to **derive the LVR**



Permanent deformation during a 0.05 – 100 Hz frequency sweep test (~ **1.5 h**)

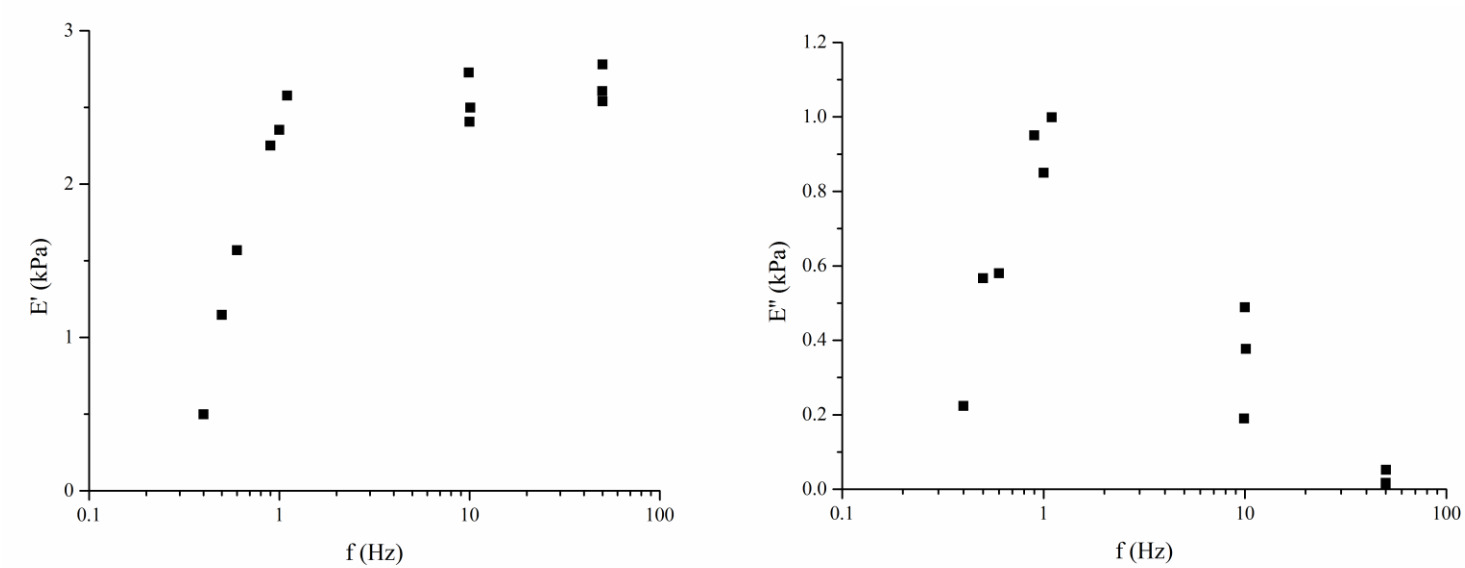
*GABO Eplexor 150N, **10mN** trigger force*

3 samples

step-reconstructed (SRDMA)

G. Mattei, A. Tirella, G. Gallone, A. Ahluwalia, *submitted*

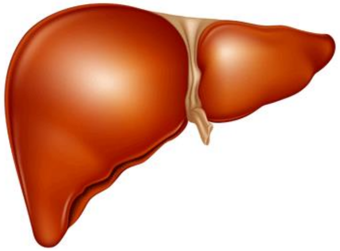
SRDMA paradigm: perform **DMA measurements around specific *f*,** then **reconstruct $E'(f)$ and $E''(f)$ over** the whole **frequency range of interest**

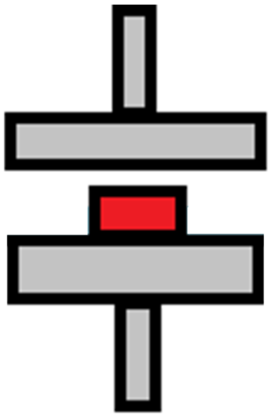


Storage (E') and loss (E'') moduli measured around *f* = 0.5, 1, 10 and 50 Hz (*f* - 0.1 Hz, *f*, *f* + 0.1 Hz)

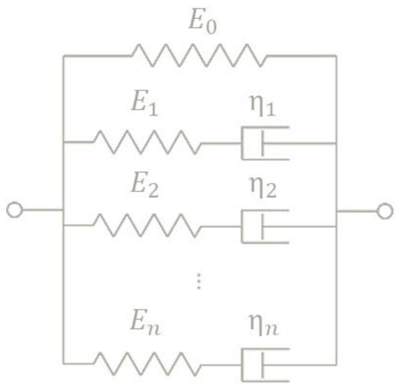
GABO Eplexor 150N, ***10mN*** *trigger force*
3 samples x *4 f = 12 samples*

- ✓ **Short testing time →** **no sample deterioration** (**< 2 %** permanent compression in the *worst* case, i.e. *f* = 0.5 Hz)
- x **Trigger force →** **sample pre-load**
- x Need **preliminary tests** or an ***a priori*** **knowledge of the material relaxation behaviour** to **choose *f***

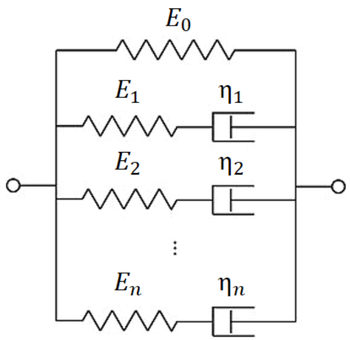




Modelling viscoelastic behaviour



Generalised Maxwell (GM) model

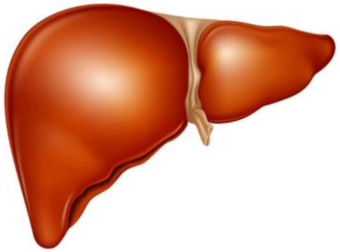


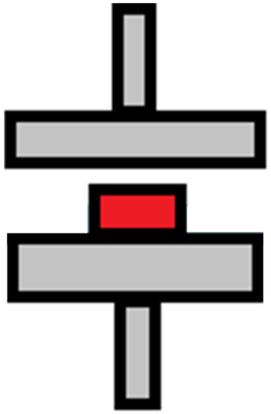
$\tau_i = \eta_i / E_i$ $\quad$ i^{th} relaxation time

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

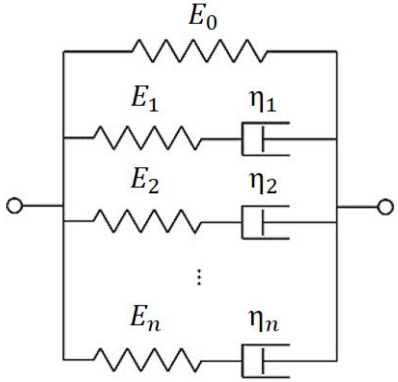
Transfer function in the **Laplace** domain

	$\dot{\varepsilon}M$ needs $\sigma(t)$ response to a fixed $\dot{\varepsilon}$	**SRDMA** needs $E'(f)$ and $E''(f)$
General form	$\bar{\sigma} = H_{GM}(s) \cdot \frac{\lvert\dot{\varepsilon}\rvert}{s^2}$ — **Laplace transform** of a **constant $\dot{\varepsilon}$ input** with **amplitude** $\lvert\dot{\varepsilon}\rvert$ **Inverse Laplace** transformation	$E^*_{GM}(f) = \underbrace{\left(E_0 + \sum_{i=i}^{n} \frac{4\, E_i\, \eta_i^2\, f^2\, \pi^2}{E_i^2 \;+\; 4\, \eta_i^2 f^2 \pi^2}\right)}_{E'(f)} + i \underbrace{\left(\sum_{i=i}^{n} \frac{2\, E_i^2\, \eta_i\, f\, \pi}{E_i^2 \;+\; 4\, \eta_i^2 f^2 \pi^2}\right)}_{E''(f)}$
Max SLS (n = 1)	$\sigma(t) = \dot{\varepsilon}\left[E_0 t + \eta_1\left(1 - e^{-\frac{E_1}{\eta_1}t}\right)\right]$	substitute $\boldsymbol{n}$ **= 1** in the general equation
GM2 (n = 2)	$\sigma(t) = \dot{\varepsilon}\left[E_0 t + \eta_1\left(1 - e^{-\frac{E_1}{\eta_1}t}\right) + \eta_2\left(1 - e^{-\frac{E_2}{\eta_2}t}\right)\right]$	substitute $\boldsymbol{n}$ **= 2** in the general equation





Lumped parameter estimation



Global fitting with shared parameters

$\dot{\varepsilon}M$	SRDMA

1. Choose a **lumped parameter model**

2. Calculate $\sigma(t)$ response to a **fixed $\dot{\varepsilon}$**	**2. Calculate $E'(f)$** and **$E''(f)$**

3. Build a **unique dataset** for the **global fit** and **share** the **viscoelastic parameters**

4. Fix $\dot{\varepsilon}$ in the fitting equation of **each experimental $\sigma(t)$** to the **applied $\dot{\varepsilon}$**	**4. Associate exp. data** to the **modelled expressions** of $E'(f)$ and $E''(f)$

5. Global fit performing χ^2 **minimisation** in a **combined parameter space**

Annealing scheme
to avoid most of the local minima

Viscoelastic constants (*E_i*, *η_i*) for the **chosen model**

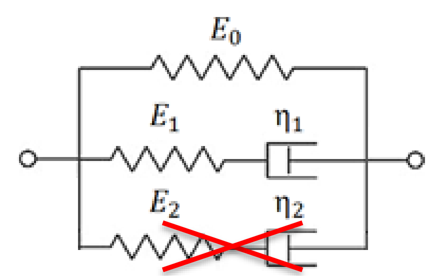
Global fitting results

Porcine liver viscoelastic parameters (estimated value ± standard error)

	Maxwell SLS		GM2	
Parameter	$\dot{\varepsilon}M$	**SRDMA**	$\dot{\varepsilon}M$	**SRDMA**
E_{inst} (kPa)	2.04 ± 0.01	2.65 ± 0.30	2.04 ± ($3.21 \cdot 10^2$) *n.s.*	2.65 ± ($3.61 \cdot 10^5$) *n.s*
E_{eq} (kPa)	0.91 ± 0.01	0.89 ± 0.22	0.91 ± 0.01	0.89 ± 0.56
τ_1 (s)	1.10 ± 0.02	0.20 ± 0.06	1.10 ± ($3.05 \cdot 10^3$) *n.s.*	0.20 ± ($1.14 \cdot 10^5$) *n.s.*
τ_2 (s)	-	-	1.10 ± ($3.05 \cdot 10^3$) *n.s.*	0.20 ± ($0.65 \cdot 10^5$) *n.s.*
R^2	0.97	0.92	0.97	0.92

n.s. → non significant estimate

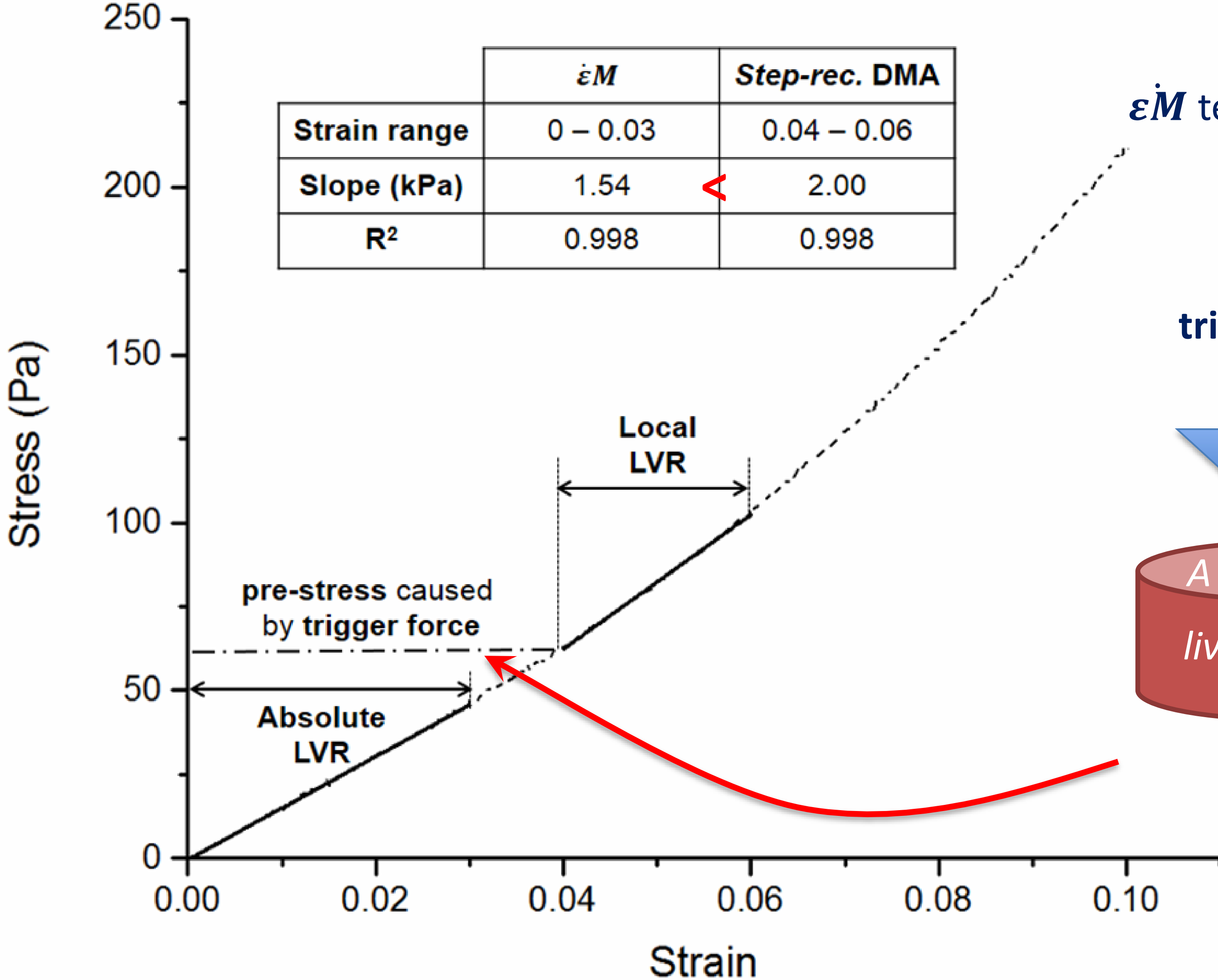
- ✓ **Maxwell SLS** model **is sufficient** whatever the method
- ✓ **GM2 → over-parameterisation** of liver viscoelastic behaviour

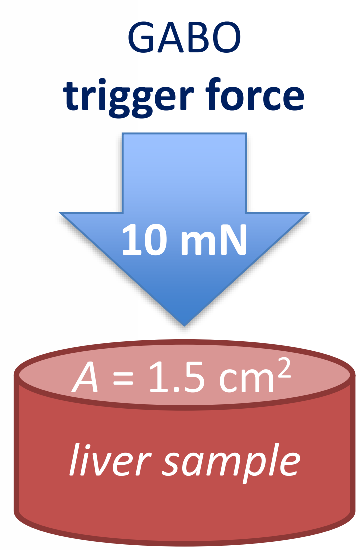


$\dot{\varepsilon}M$ and **SRDMA results** are **significantly different** (*t-test*, $p < 0.05$)

Absolute vs local LVR

	$\dot{\varepsilon}M$	*Step-rec.* DMA
Strain range	0 – 0.03	0.04 – 0.06
Slope (kPa)	1.54 <	2.00
R^2	0.998	0.998





Testing very soft tissues: conclusion

Long test
F or **strain trigger**

sample status changes
conventional DMA

Short test
F or **strain trigger**

local LVR
step-rec. DMA

Short test
No trigger

actual properties
$\varepsilon\dot{M}$

- $\varepsilon\dot{M}$ gives a **good estimation** of **liver viscoelastic parameters in the LVR**
- A **wider range of** $\dot{\varepsilon}$ should be considered for a **more accurate estimation of** τ
- Caution in **over-interpreting *ex-vivo* data** (sample **status** is generally **different than *in-vivo*** and **dependent on many factors**, such as T, preservation period)

Contacts

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