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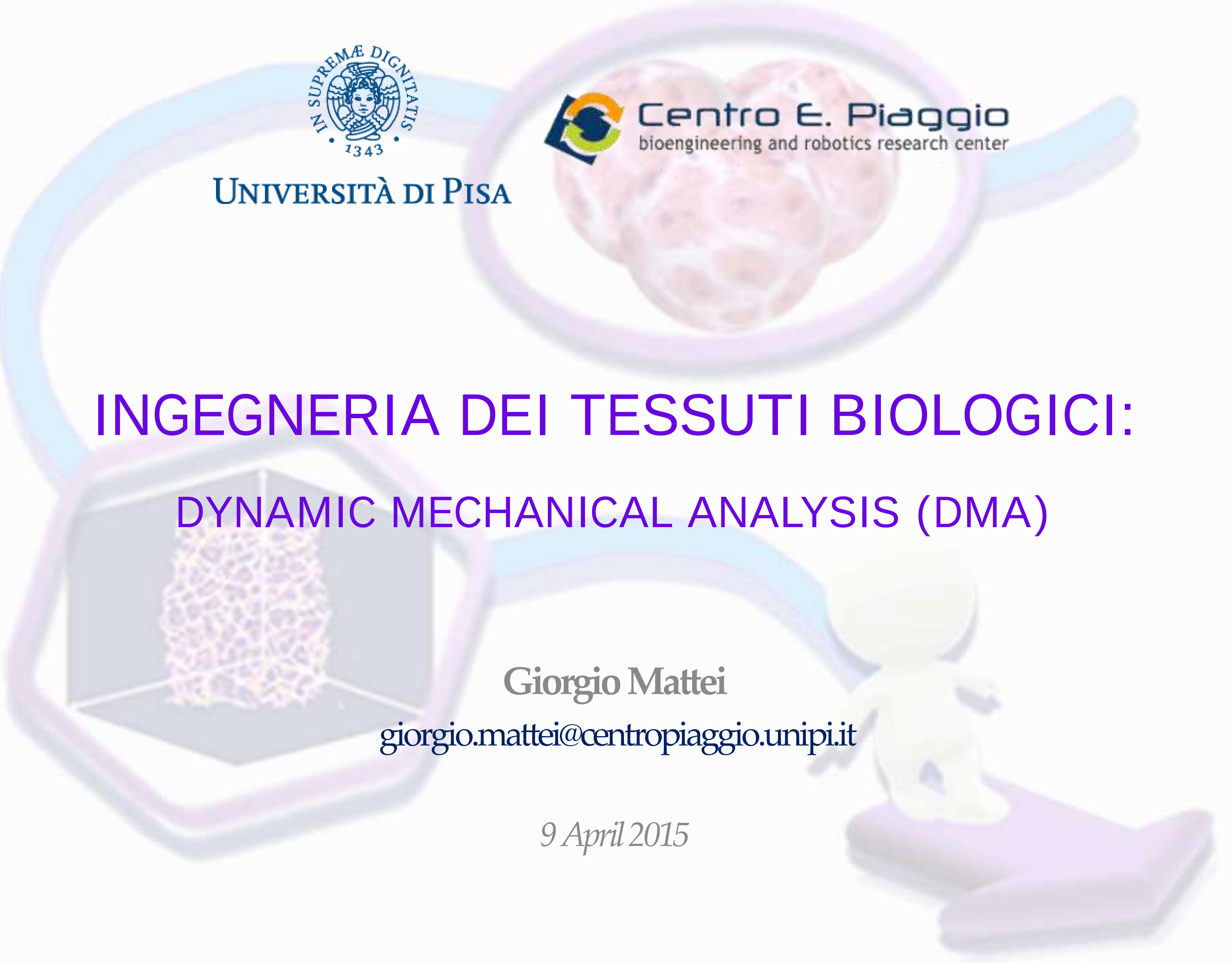

# INGEGNERIA DEI TESSUTI BIOLOGICI:

## DYNAMIC MECHANICAL ANALYSIS (DMA)

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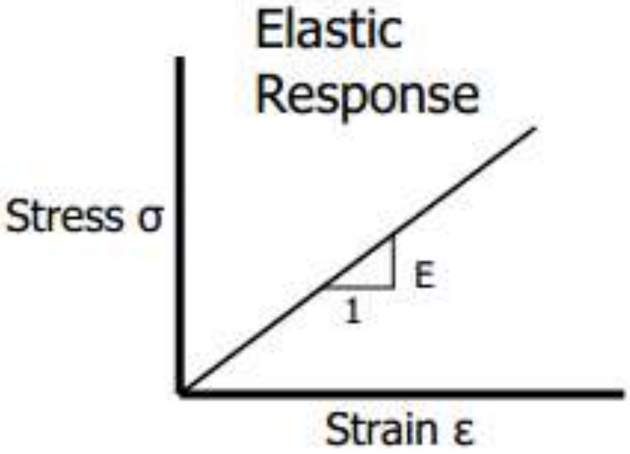
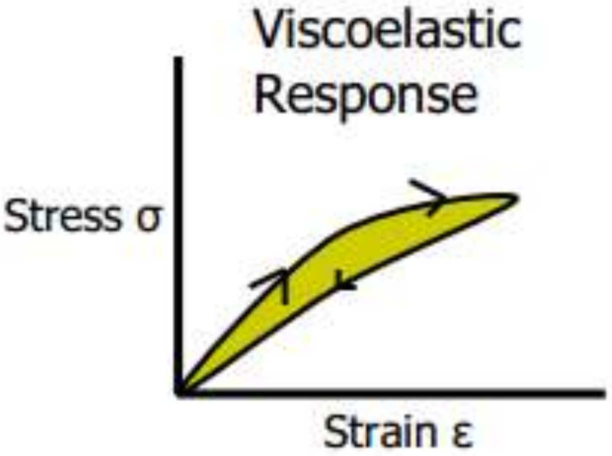
9 April 2015

# 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





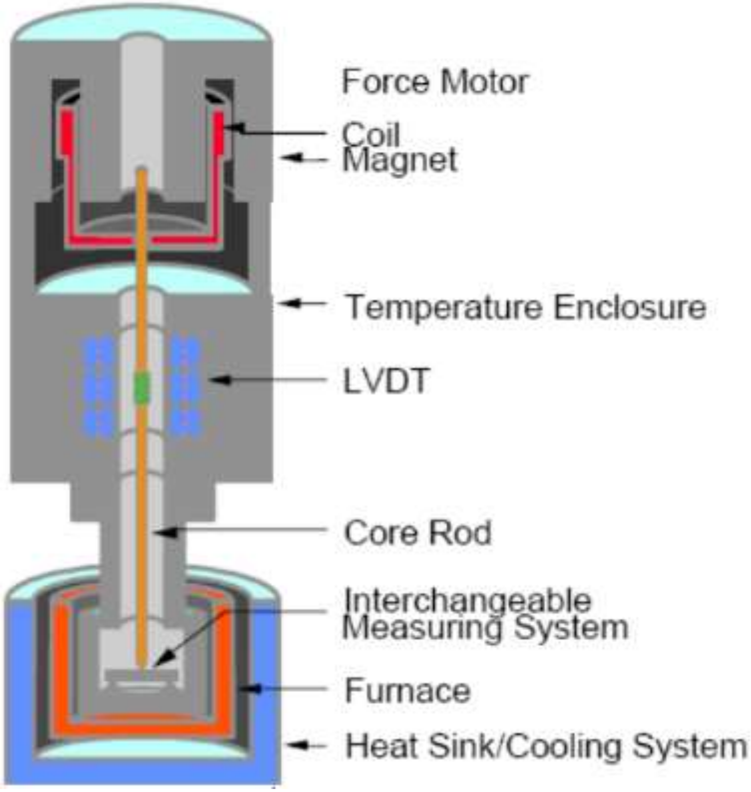
# Methods to characterise viscoelasticity

- **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)

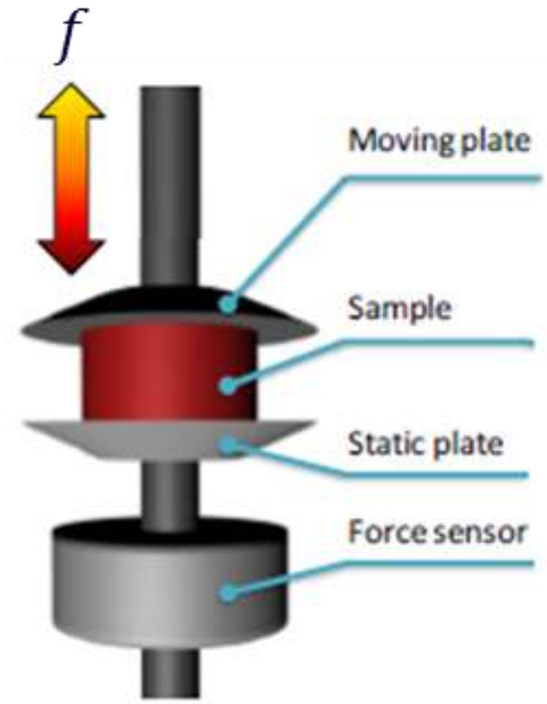
# DMA overview

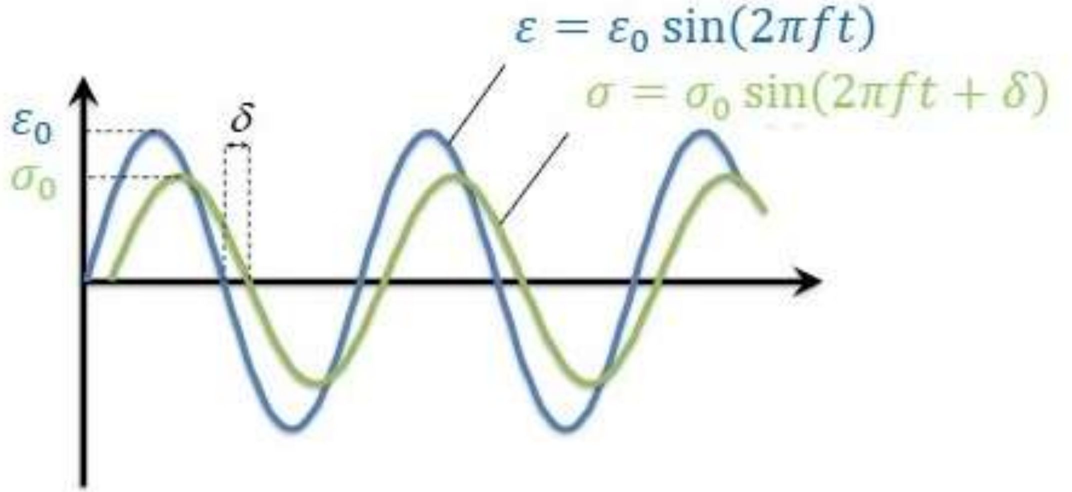
- Dynamic mechanical analysis (DMA) is a standard **force-triggered method** to **determine viscoelastic properties** of materials by **applying a small amplitude cyclic strain** on a sample and **measuring the resultant cyclic stress response**.



# 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.

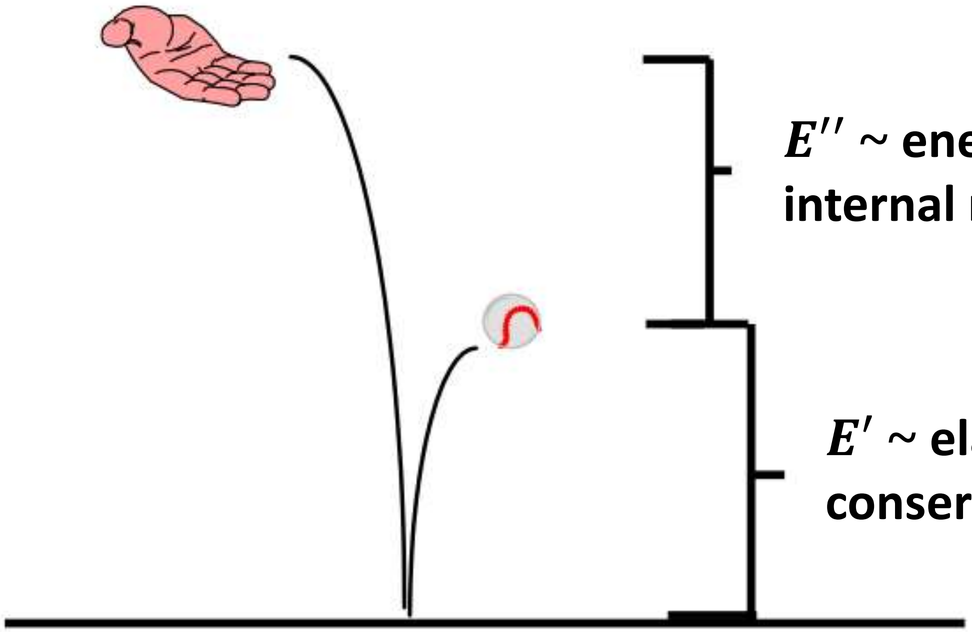




Viscoelastic material response is characterised by a **phase lag** (**δ**) between the strain input and the stress response, which is comprised **between 0°** (**purely elastic**) and **90°** (**purely viscous**). This phase lag is **due to** the excess **time necessary for molecular motions and relaxations** 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** ($\boldsymbol{E}^* = \boldsymbol{E}' + \boldsymbol{i}\boldsymbol{E}''$).



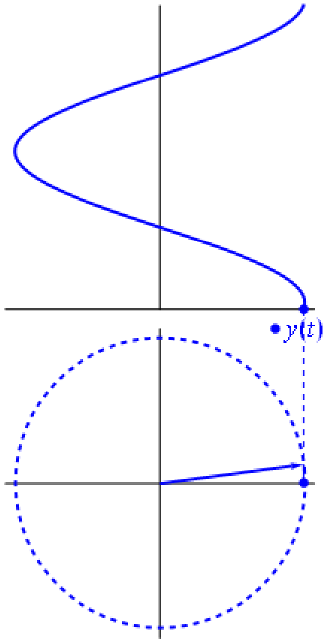
**$\boldsymbol{E}''$ ~ energy dissipated in internal motions**

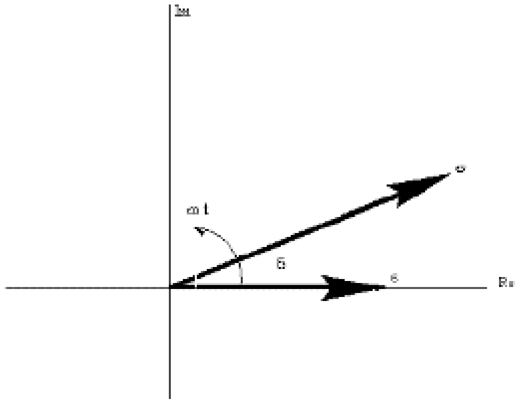
**$\boldsymbol{E}'$ ~ elastic energy conserved**

# 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 $\sigma$ and $\varepsilon$ 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

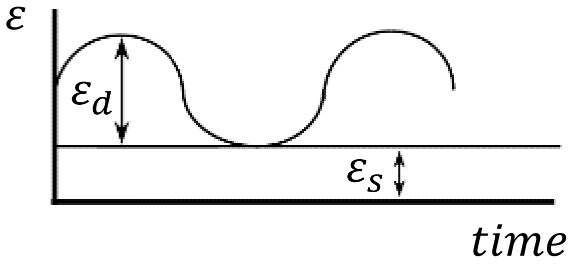
- **Temperature sweep**: Modulus and damping are recorded as the sample is heated
- **Frequency sweep**: Modulus and damping are recorded as the sample is loaded at increasing (or decreasing) frequencies
- **Stress amplitude sweep**: Modulus and damping are recorded as the sample stress is increased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

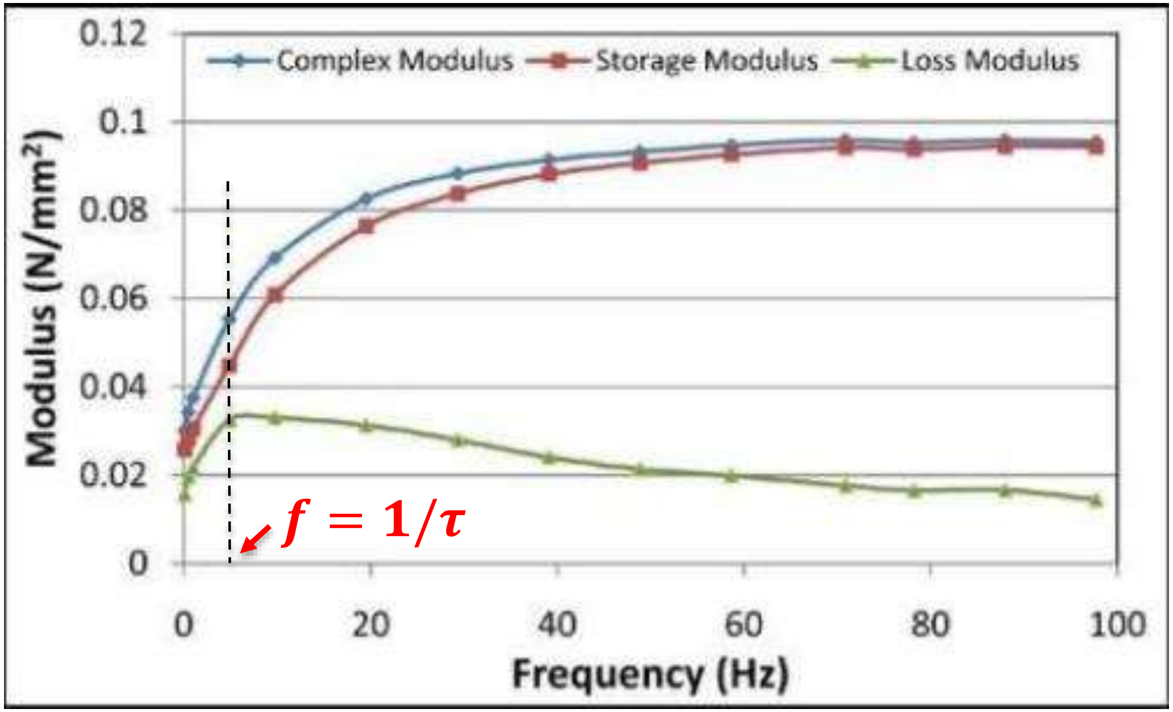
# Frequency sweep tests

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

**Test parameters**:

- Temperature (T)
- Frequency range (*f*)
- Static strain ($\varepsilon_s$)
- Dynamic strain ($\varepsilon_d$)

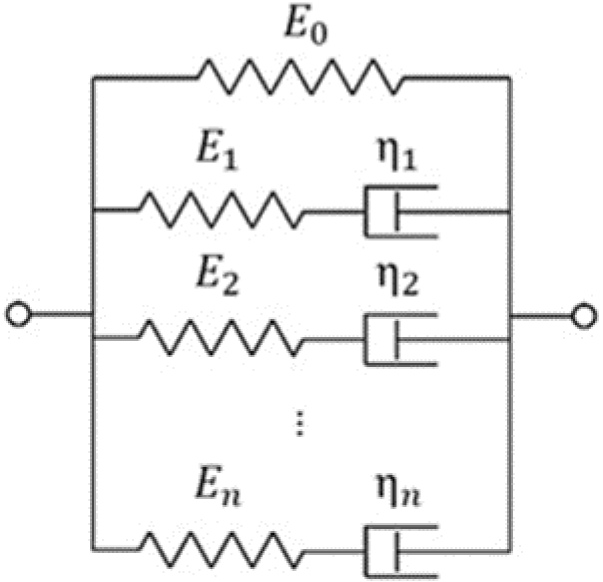




- **Peaks** in $\mathbf{tan(\delta)}$ or $\boldsymbol{E''}$ with respect to frequency identify the **characteristic relaxation frequencies** of the viscoelastic sample under testing, defined as $\boldsymbol{f = 1/\tau}$**,** where $\boldsymbol{\tau}$ is the **characteristic relaxation time)**

# 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 $\eta_i$) assembled **in parallel**, thus defining a set of $\boldsymbol{n}$ **different characteristic relaxation times** (i.e. $\boldsymbol{\tau_i = \eta_i / E_i}$)



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

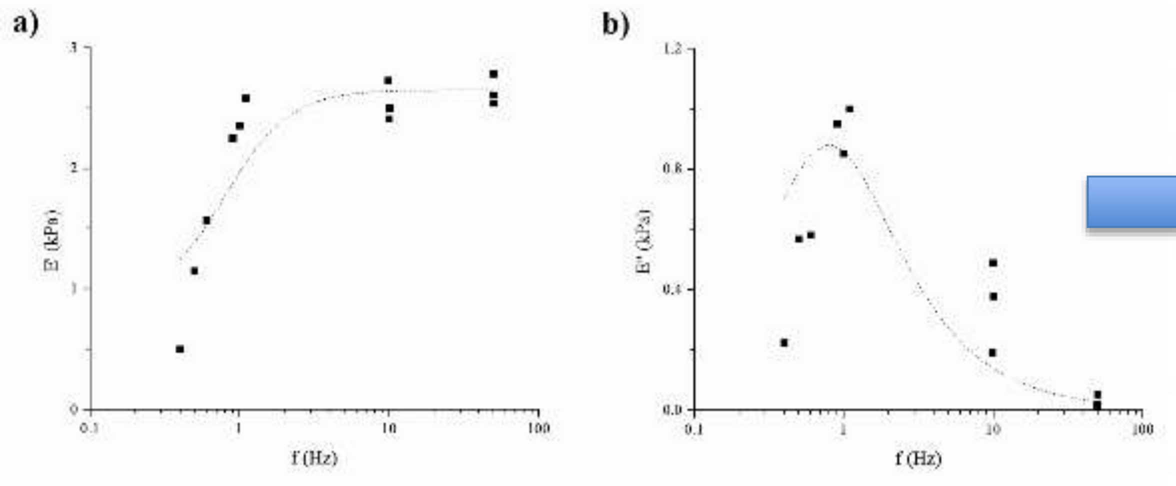
GM model transfer function in the Laplace domain

# 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

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

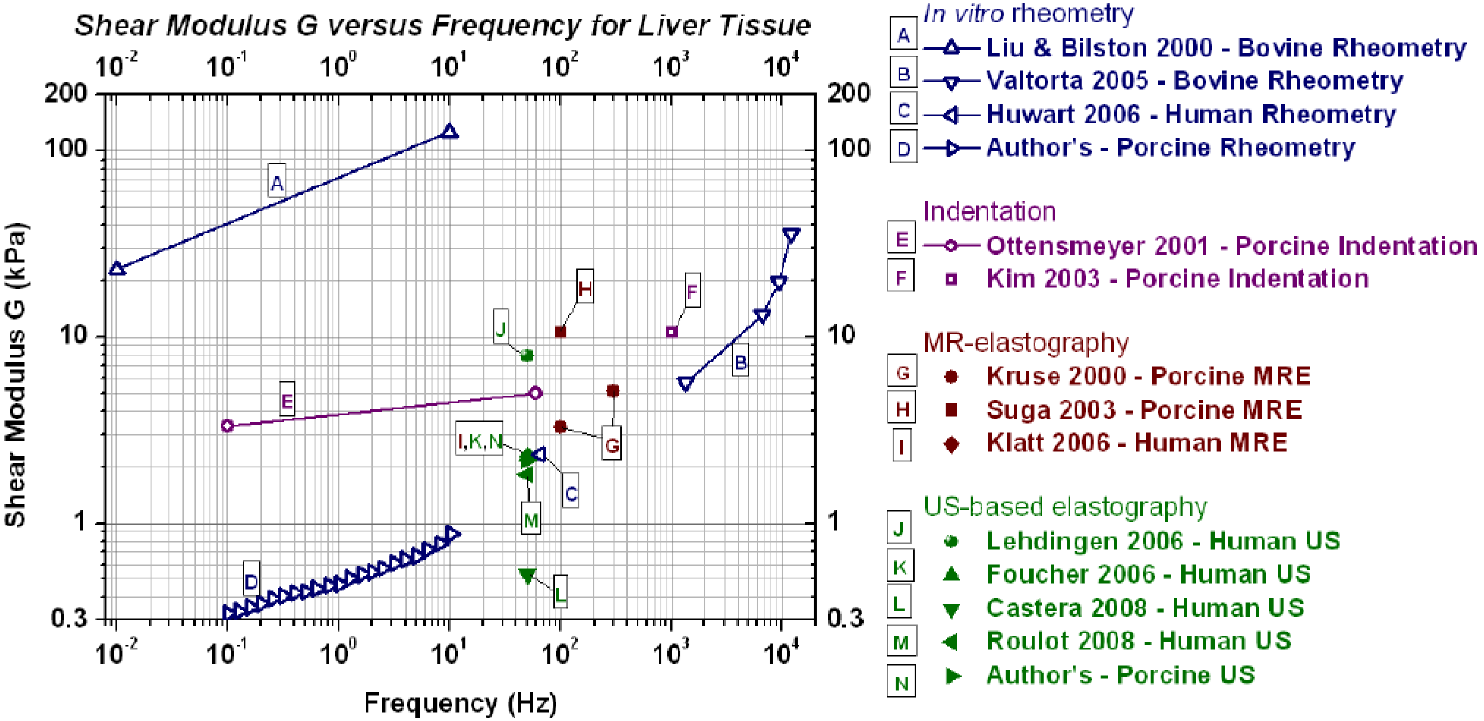
- **Global fitting** with **shared parameters** ($\chi^2$ minimisation)



$E_i, \eta_i$

# 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:**

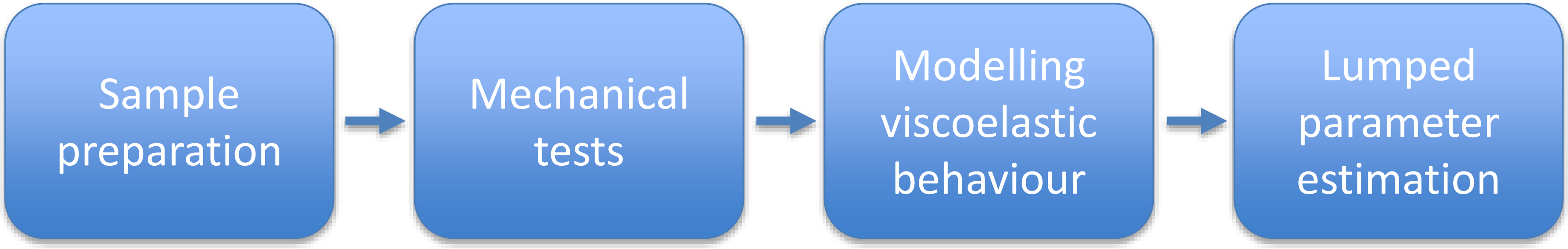
1. **clearly identify the parameters of interest**
2. **develop suitable experimental testing setup and protocols** for the **unique identification** of **liver viscoelastic parameters**

# Aim and strategy

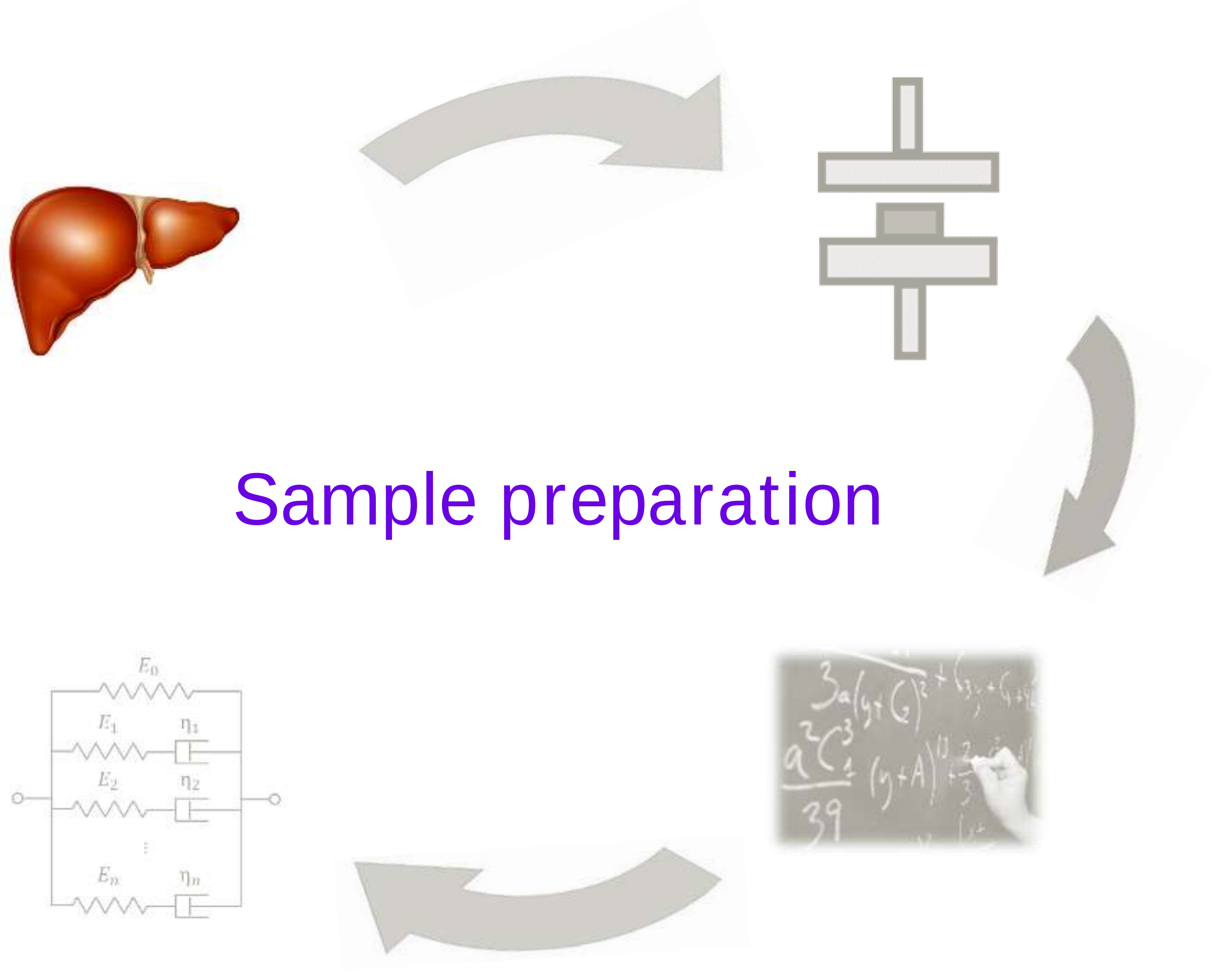
**AIM**: establishing an **experimental testing and analysis framework** to **unequivocally** **characterise** the **liver viscoelastic behaviour** in the **LVR** (linear viscoelastic region)

**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

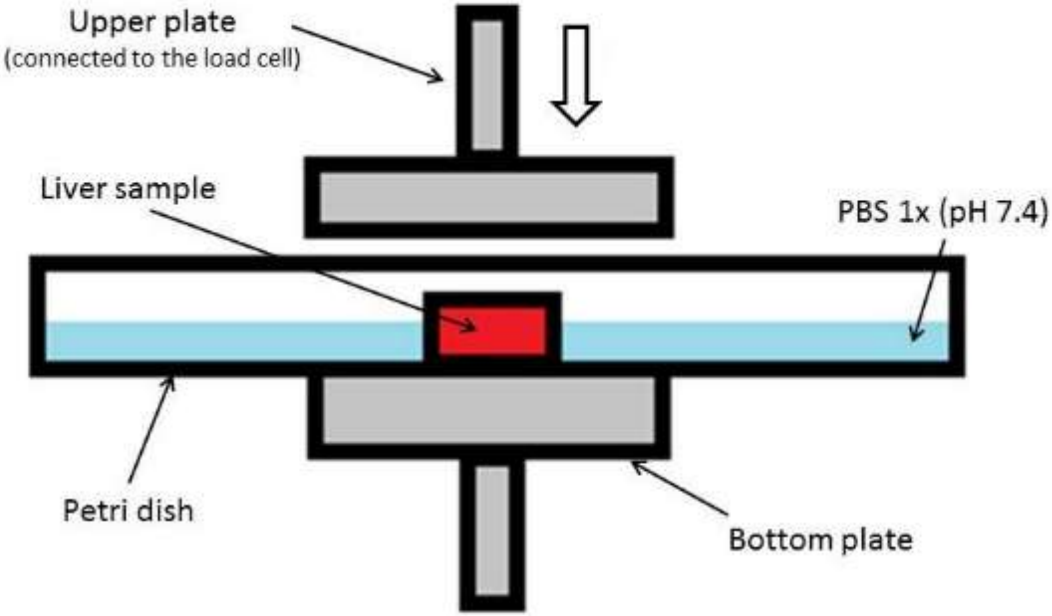


# Sample preparation

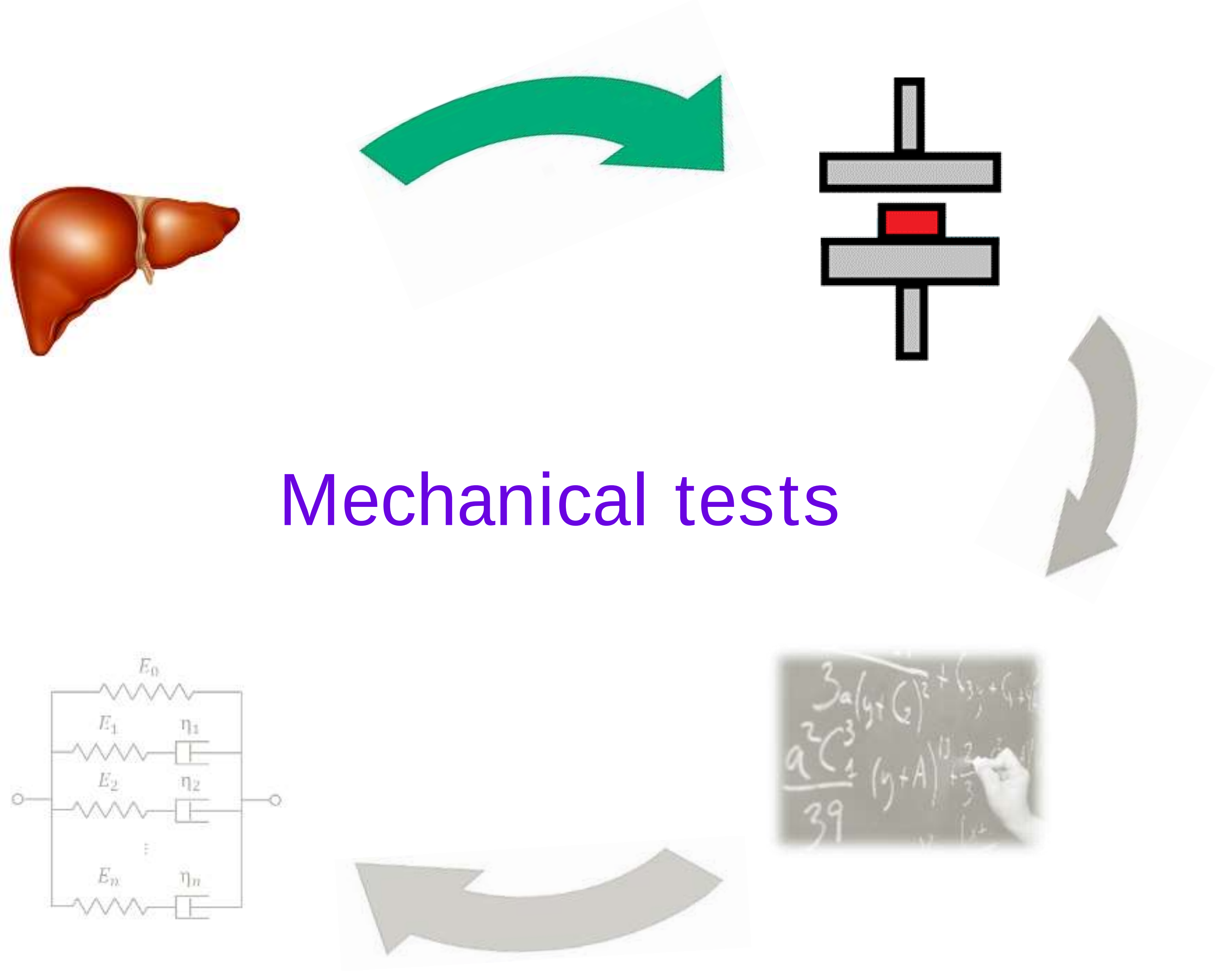


# Sample preparation and testing configuration

- **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**

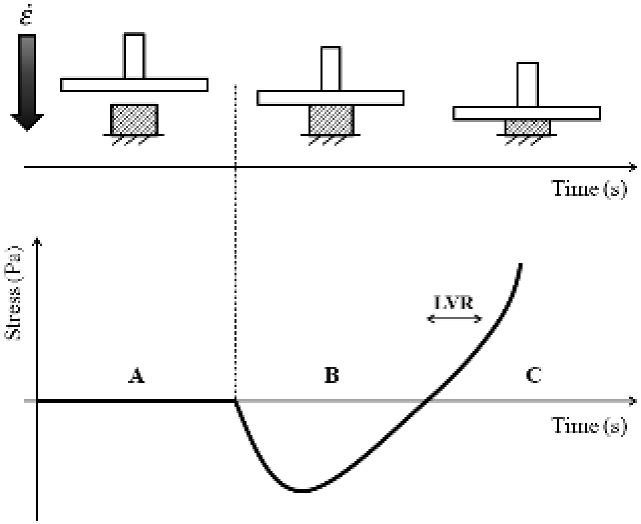


Mechanical tests

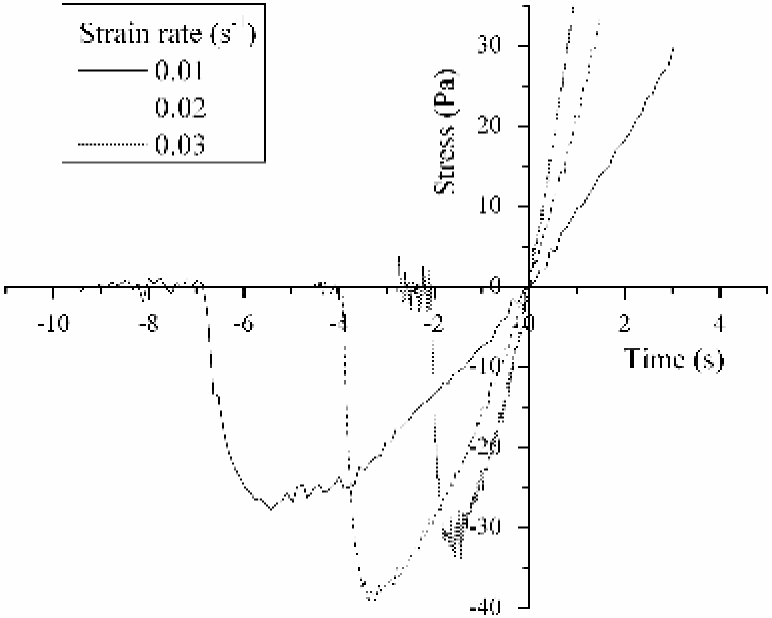
# $\dot{\varepsilon}M$: **short test** with **no pre-load**

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 $\sigma$-$\varepsilon$ 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 $\dot{\varepsilon}$**

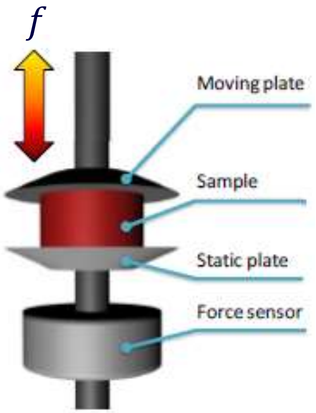
(only **LVR values** are shown in zone C)

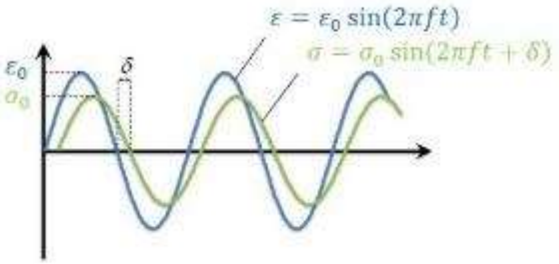
*Zwick/Roell Z005, 10N load cell*

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

# DMA: a **widely accepted** method

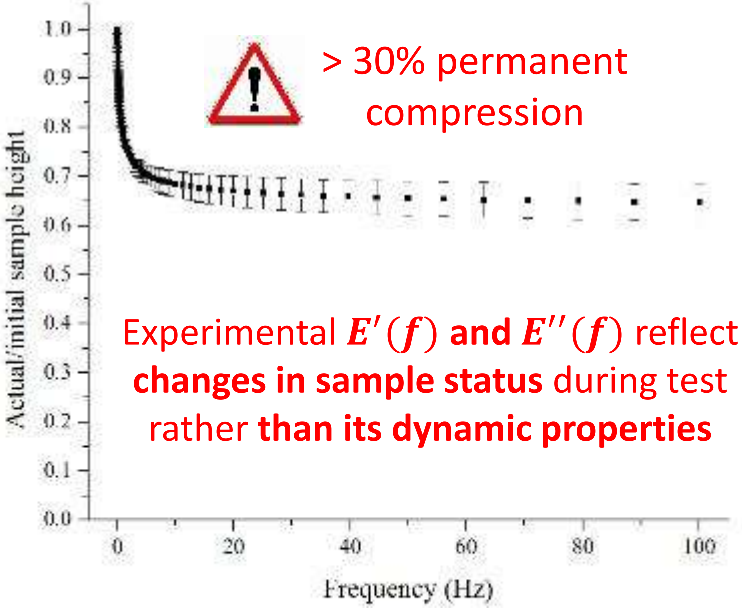
**DMA paradigm:** characterise viscoelastic behaviour testing samples at **different frequencies** ($f$), then **analysing** $E'(f)$ **and** $E''(f)$





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

- ✓ **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**



> 30% permanent compression

Experimental $E'(f)$ **and** $E''(f)$ reflect **changes in sample status** during test rather **than its dynamic properties**

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

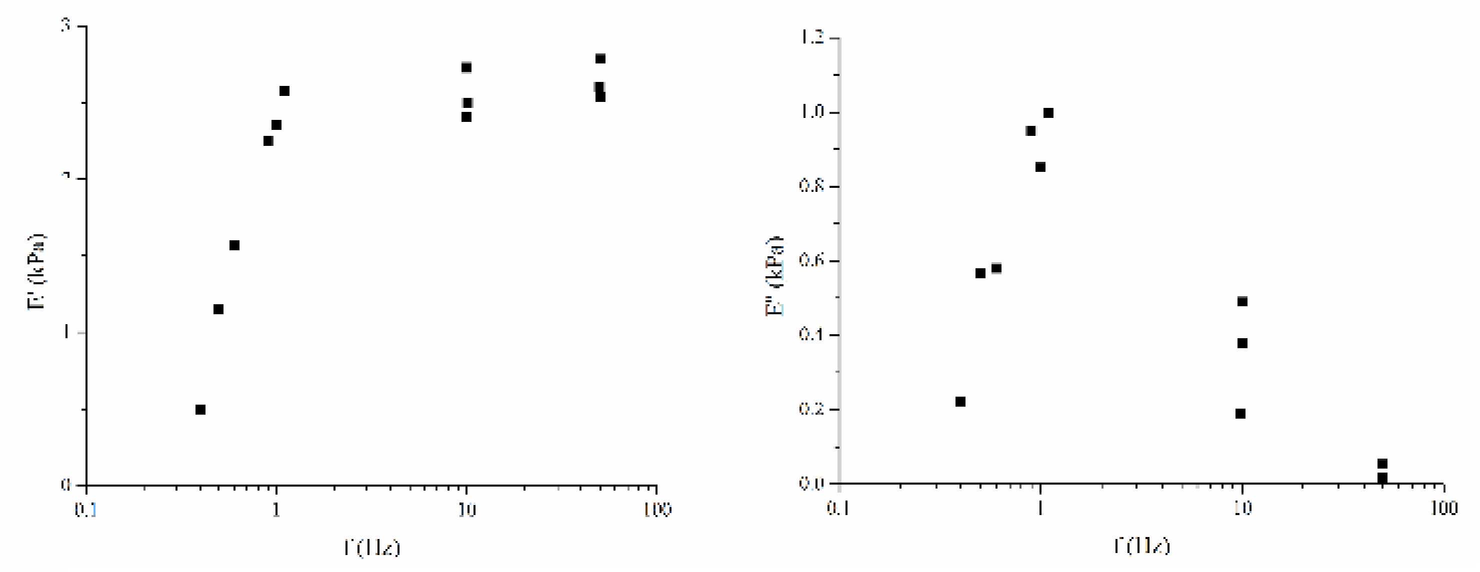
*GABO Eplexor 150N, **10mN** trigger force*

*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**



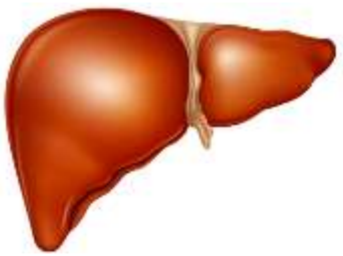
**Storage (E') and loss (E'') moduli measured around *f* = 0.5, 1, 10 and 50 Hz (*f* - 0.1 Hz, *f*, *f* + 0.1 Hz)**

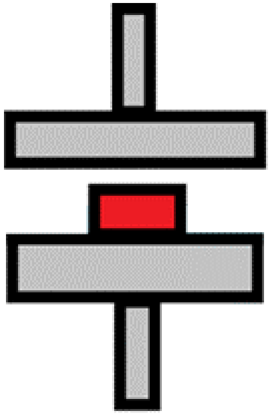
*GABO Eplexor 150N, **10mN** trigger force*
*3 samples* x *4 f = 12 samples*

✓ **Short testing time →** **no sample deterioration** (**< 2 %** permanent compression in the *worst* case, i.e. *f* = 0.5 Hz)

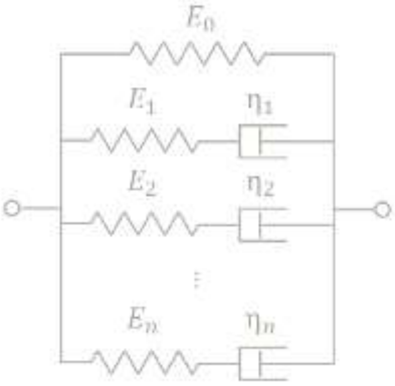
x **Trigger force →** **sample pre-load**

x Need **preliminary tests** or an ***a priori* knowledge of the material relaxation behaviour** to **choose *f***

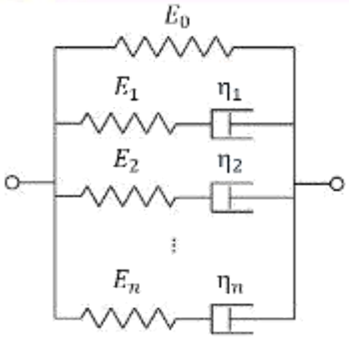




# Modelling viscoelastic behaviour



# Generalised Maxwell (GM) model

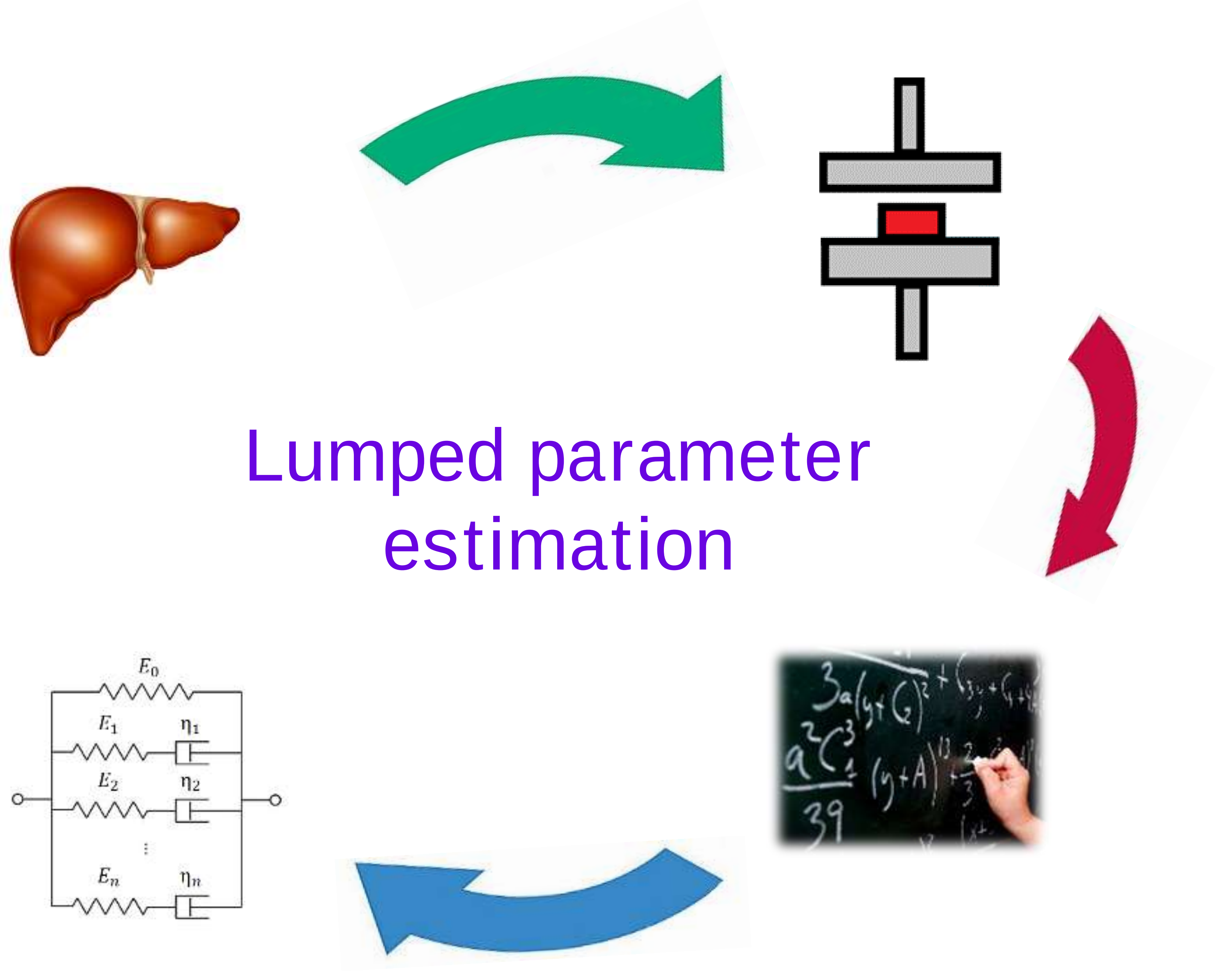


$\tau_i = \eta_i / E_i$ $i^{th}$ relaxation time

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

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** | $\bar{\sigma} = H_{GM}(s) \cdot \boxed{\frac{\lvert\dot{\varepsilon}\rvert}{s^2}}$ ← **Laplace transform** of a **constant $\dot{\varepsilon}$ input** with **amplitude** $\lvert\dot{\varepsilon}\rvert$<br>**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 $n$ **= 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 $n$ **= 2** in the general equation |



# Lumped parameter estimation

# **Global fitting** with **shared parameters**

| $\dot{\varepsilon}M$ | SRDMA |
|---|---|

**1. Choose** a **lumped parameter model**

| $\dot{\varepsilon}M$ | SRDMA |
|---|---|
| **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**

| $\dot{\varepsilon}M$ | SRDMA |
|---|---|
| **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 $\chi^2$ **minimisation** in a **combined parameter space**

**Annealing scheme**
to avoid most of the local minima

**Viscoelastic constants (*$E_i$*, *$\eta_i$*)** for the **chosen model**

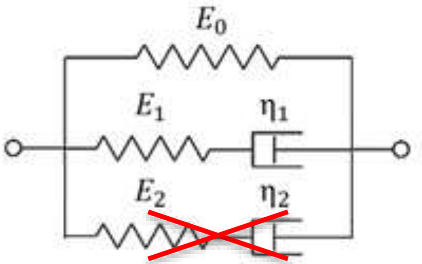
# Global fitting results

**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 |
| $\tau_1$ (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.* |
| $\tau_2$ (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 |

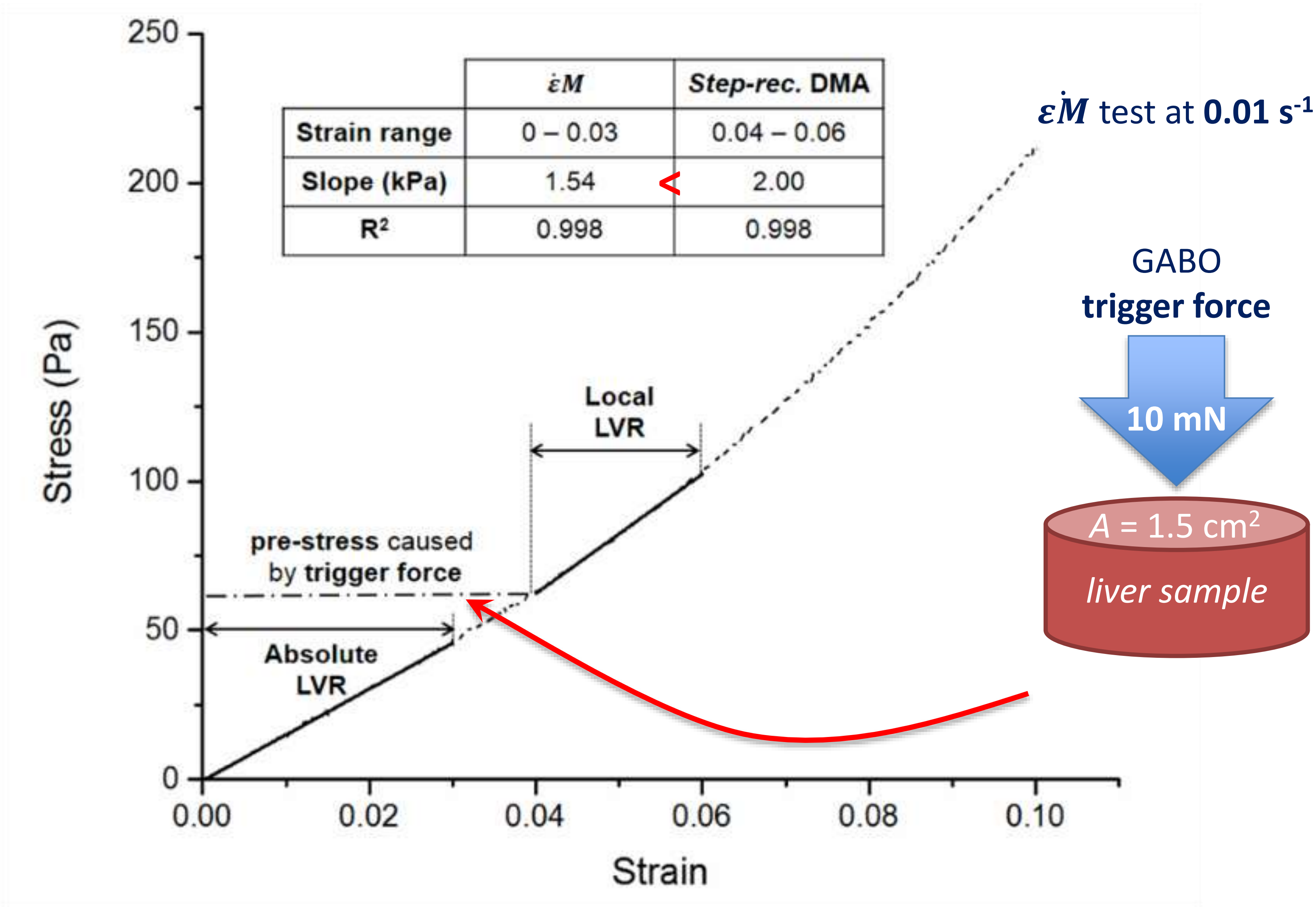
*n.s.* → *non significant estimate*

- ✓ **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 |
| **R²** | 0.998 | 0.998 |

$\dot{\varepsilon}M$ test at **0.01 s$^{-1}$**

GABO
**trigger force**

**10 mN**

$A$ = 1.5 cm$^2$
*liver sample*

# Testing **very soft tissues**: conclusion

| **Long test**<br>**F** or **strain trigger** | **Short test**<br>**F** or **strain trigger** | **Short test**<br>**No trigger** |
|---|---|---|
| ✗ | ! | ✓ |
| **sample status changes**<br>***conventional DMA*** | local LVR<br>*step-rec. DMA* | **actual properties**<br>$\boldsymbol{\varepsilon\dot{M}}$ |

- $\boldsymbol{\varepsilon\dot{M}}$ 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** $\boldsymbol{\tau}$
- 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)

# Practical exp: hair mechanical test

**Aula A210** – Dip. Ingegneria dell'Informazione (polo A)

- 15 Apr 2015 – 11.30-14.30
- 22 Apr 2015 – 11.30-14.30

# Contacts

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