Lab 3: Tissue Mechanics

October 20, 2011

Objective. In this lab, you will probe the mechanical properties of rubber, chicken skin, and cow stomach (tripe) using a simple self-built tensile stress measurement setup that takes advantage of gravity. You will use your measurements of the mechanical properties of these samples to investigate the validity of Hooke's law, to learn basics about stress and strain and their relationship for different regimes. We will dive a bit into the world of viscoelasticity and squishy materials, learn about not-so-common fluids and introduce spring-dashpot models. Furthermore, we want you to think about the question whether rubber could potentially be used as artificial skin and which changes occur in the mechanical properties of your skin as you age – the latter, of course, should be backed up by your experimental results in class.

Safety Measures At all times, rubber gloves must be worn when handling skin and stomach samples to decrease the risk of a salmonella infection. Make sure you wash your hands before you leave class.

Preliminary Questions

Preliminary questions are due at the beginning of the lab! Total: 4 pts

1. The units of the Young's modulus are Pa. Express Pa in other units and show how you can deduce it from equation 1. (0.5 pts)
2. Using equations 1 and 2, get the relationship between Y and k. (1 pt)
3. It has been shown in worms and mice that a low calorie diet can extend longevity and delay aging. Can you think of reasons why this may be the case? (1 pt; write half a page max)
4. Design an experiment which measures the forces cells exert on a substrate when they move using your knowledge of Hooke's law (1.5 pts; write half a page max, basic ideas are sufficient).

1 Introduction

When you pull the skin on your hand, you will notice that it snaps right back. If you would try out the same thing with your grandmother's hand, you may notice that the skin does not react as fast anymore. Why is that? As we age, the mechanical properties of our body change, and that is the most obvious with skin. Over the course of time our skin looses its elasticity and this process is sped up by frequent exposure to sunlight, UV rays, harsh weather, bad diet, lack of sleep, and lack of moisture. The appearance of wrinkles with increasing age is a direct indicator of the change in tissue properties.
The major components which are responsible for the elasticity of our skin are collagen and elastin, and as we age the production of them decreases, changing the properties of the skin. Further, dead skin

cells shed slower and the turnover of new skin cells may decrease. While these biological changes in our tissues begin in our 20s, the signs of aging begin at first unnoticed, such as the first gray hair, thin facial lines, dry skin, etc.

In this lab module, we will measure the elastic properties of fresh chicken skin and cow stomach samples, compare them to a non living material like rubber, and finally artificially age the skin samples to see how the aging process changes their elastic properties.

2 Theoretical Background

The mechanical response of a material under a tensile load is best analyzed by the so called uniaxial tension test. In this test, one end of the specimen is fixed while the other end is allowed to move under the application of a known force (independent variable). The resulting elongation (dependent variable) of the specimen is measured. The uniaxial tension test is a standard procedure in the material sciences and thus specific machines exist that can perform this test in a highly accurate manner. However, these machines are also very expensive and if one does not need such high accuracy, it makes much more sense to build oneself a simple and cheap setup to carry out those measurements. (This is what we will do and is described in detail a little later.) The characteristic response of a material is obtained by repeating the uniaxial tension test for a large number of specimens of the same material, but with altered parameters, such as changing the lengths, widths, cross-sectional areas, and under different magnitudes of tensile forces. The results of the uniaxial tension tests can be used to obtain a plot representing the relationship between the average stress (applied load/area) and the average strain (amount of elongation/total length), which represents the corresponding characteristic material deformation. An elastic material whose stress-strain diagram is a straight line is called a linearly elastic material or Hookean material. The slope of the line will give you directly the elastic modulus or Young's modulus (Y) of the material (units are Pa), which represents the stiffness of the material.

$$\sigma(t) = Y\varepsilon(t) \tag{1}$$

The larger the elastic modulus, the stiffer the material. For example, glass has an elastic modulus of 50–90 GPa, steel 200 GPa, wood 11 GPa and rubber 0.01–0.1 GPa. In contrast, individual cells have an elastic modulus of 10-1000Pa.

It is important to note that the definition of the elastic modulus in equation 1 requires a linear stress-strain relationship and material isotropy, i.e. the material has the same physical properties in all directions. Most materials, however, will only show a linear stress strain relationship for a small range of forces, then they will react non linearly for larger forces. Thus, in these cases, one needs to make sure that one is either in the linear regime or uses a different model if one wants to calculate the elastic modulus. An example for a complicated stress strain curve of a ductile material is given in Fig.1. Here, "ductile" refers to the extent that a given material can resist a deformation plastically without fracture. A ductile material will show "necking" (getting thinner) in the region where the fracture will occur, whereas a "brittle" material just breaks without necking. As you can imagine, ductility is an interesting parameter in the metal industry. Here, the material's ability to deform under tensile stress or compressive stress (called "malleability") determine how it can be manipulated using metal forming processes, such as hammering, rolling, and drawing. You may have seen Hooke's law a bit different previously in the following form:

$$F = kx \tag{2}$$

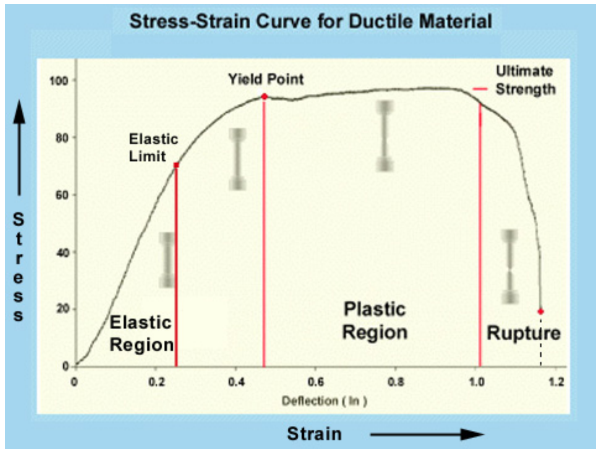


Figure 1: Stress-strain curves for a ductile material.

Equation 1 is just a more general way of expressing Hooke's law. The relationship between the spring constant k and the Young's modulus Y can be easily derived (and this is your preliminary question 2). For some materials, the stress-strain properties depend on how rapidly a material is stressed or strained. Materials which exhibit such a time-dependence are called **viscoelastic materials**. A viscoelastic material is a material that reacts like an elastic solid under some circumstances and like a viscous fluid under others. Embryonic tissues and certain cancers, for example, have this kind of property and can be characterized on long time scales as viscous fluids. Tissues can be altered on the molecular level (e.g. protein expression levels) and the change in mechanical properties can be measured quantitatively on a macroscopic scale (Young's modulus, viscosity, surface tension). Those changes lead to a change in

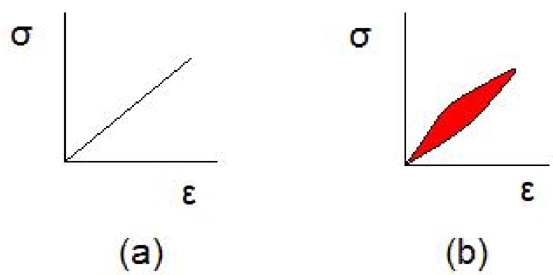


Figure 2: Stress-strain curves for (a) a Hookean elastic solid and (b) a viscoelastic material. Figure taken from wiki.org

behavior of the tissues, e.g. leading to a metastatic cancer from a previously localized cancer (see e.g. Reference [1] on the mechanics of embryonic tissues).
Purely elastic materials do not dissipate energy (heat) when a load is applied, then removed. However, a viscoelastic substance loses energy when a load is applied and then removed. This results in hysteresis in the stress strain curve as shown in Fig.2. The area of the loop is equal to the energy lost during the loading cycle. An everyday example for a viscoelastic material is toothpaste.

Thinking about toothpaste and other "weird" fluids from our everyday lives, such as shaving cream, ketchup or mayonnaise, you can already see that in the lab about the Reynolds number we were a bit simplistic when we introduced the fluid viscosity as the proportionality constant between the applied stress and the strain rate. Not all fluids act linearly with applied stress; some exhibit a non-linear or plastic response. Ordinary liquids, such as the glycerol you used previously, display a linear relationship between stress and strain rate, and thus have a fixed viscosity (at a given temperature), and are commonly called "Newtonian fluids". If the material exhibits a non-linear response, i.e. the viscosity is not a constant, it is categorized as a Non-Newtonian fluid. Ketchup, et al. are fluids of this kind. In fact, Ketchup and shaving cream are what is termed "Bingham liquids": They do not flow at low forces at all, but once a certain yield stress is overcome (e.g. you hit the back of the bottle), they flow easily and the viscosity is constant with mixing rate at strong forces. Mayonnaise is what is called a "Shear-thinning liquid"; its viscosity decreases with the mixing rate.

2.1 Viscoelasticity

The complex response of a viscoelastic material to an applied stress or strain can be modeled by mechanical systems composed of elastic and viscous elements. The elastic behavior of the material is hereby modeled by Hookean springs and the viscous part by damping elements (dashpots)[4, 3]. The spring reacts instantaneous and proportional to an applied stress σ. As discussed previously, for small deformations, Hooke's law is used to describe the spring's behavior:

$$\sigma(t) = Y\varepsilon(t) \tag{3}$$

After stress removal, the spring jumps instantaneously back to its relaxed state (Fig.3, model A). The

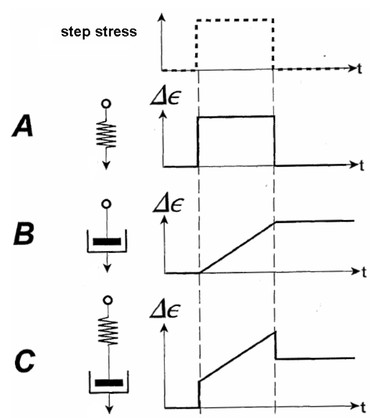


Figure 3: Mechanical models to describe elastic, viscous and viscoelastic materials. A-C illustrate different mechanical elements together with their strain response curve to an applied step stress shown in the upper most panel. (A) is a Hookean spring, (B) a dashpot element and (C) a Maxwell element. Illustration modified from [4].

dashpot behaves as a Newtonian fluid, i.e. the viscosity is independent of the strain rate, γ, and has a delayed response to the applied stress. It slowly elongates with a constant velocity. Upon force removal, the dashpot does not go back to its original state, but remains at its new elongated position (Fig.3, model B). The stress - strain rate relationship of the dashpot is given by [3]:

$$\sigma(t) = \eta\gamma(t) \tag{4}$$

As you already know from lab 1, the unit of viscosity is Pa·s or *Poise* (P), with 1 P=0.1 Pas.

The simplest viscoelastic model is the **Maxwell model**, where a Hookean spring and a viscous Newtonian element (dashpot) are combined in series. A Maxwell material is a kind of fluid (Fig.3, model C). If we would connect the spring and dashpot in parallel, we would get what is called a "Kelvin-Voigt material". As you can imagine, you can then connect these elements in series or parallel with each other to make more complicated structures. For the purpose of this lab, we will just consider the Maxwell model.
When a step stress is applied to a Maxwell element, the spring elongates instantaneously, followed by a second continuous and slower elongation of the dashpot. Upon force removal, the spring relaxes, but the dashpot remains in position, leading to a higher strain state than the original one (Fig.3, model C) [4], that's why its often referred to as a Maxwell fluid. The dynamics of the Maxwell system can be described by the following differential equation:

$$\dot{u} = \frac{\dot{\sigma}}{Y} + \frac{\sigma}{\eta} \tag{5}$$

with u the total extension of the system, i.e. the extension of the spring and the dashpot. The dot denotes differentiation with respect to time [4,3]. EQ.5 can also be written as:

$$\left(1 + \tau \frac{d}{dt}\right) \sigma = \eta \frac{du}{dt} \tag{6}$$

Here, τ is the relaxation time of the Maxwell system:

$$\tau = \frac{\eta}{Y} \tag{7}$$

EQ.7 shows that the elastic modulus and the viscosity are directly linked by the relaxation time of the system.

We will now solve EQ.6 for the case of a stress relaxation experiment (i.e. look at $\sigma(t)$ under constant deformation), where a step strain is applied to the Maxwell element. The initial condition for the Maxwell system is that the strain applied at $t = 0$ affects the spring, but not the dashpot:

$$u(0) = \frac{\sigma(0)}{Y} \tag{8}$$

The deformation $u(t)$ of the sample is given by the constant deformation u times the unit step function $\Theta(t)$:

$$u(t) = u\Theta(t) \tag{9}$$

with:

$$\Theta(t) = \left\{ \begin{array}{cc} 0 & \text{for } t < 0 \\ 1/2 & \text{for } t = 0 \\ 1 & \text{for } t > 0 \end{array} \right\} \tag{10}$$

The solution to the differential equation EQ.6 is thus given by a single exponential with relaxation time τ and constant deformation u:

$$\sigma(t) = \frac{\eta}{\tau} u e^{-t/\tau} = Y u e^{-t/\tau} \tag{11}$$

While the spring represents the elastic or energetic component of the response of such a viscoelastic system, the dashpot represents the conformational or entropic component. The magnitude of the spring constant is related to the fraction of mechanical energy stored reversibly as strain energy, whereas the dashpot visualizes the loss of energy due to dissipation [4]. When the Maxwell material is subjected to periodic oscillations, one can determine the complex shear modulus $G^*(\omega)$, which characterizes the frequency-dependent behavior of a material. The complex shear modulus of the Maxwell system is given by:

$$G^*(\omega) = i\omega \left(\frac{i\omega}{G} + \frac{1}{\eta} \right)^{-1} \tag{12}$$

Here, G is the plateau shear modulus. G^* can be written in terms of the loss modulus (viscous part), $G''(\omega)$, and storage modulus (elastic part), $G'(\omega)$, which correspond to the dissipated and stored parts of the mechanical energy in the system:

$$G^*(\omega) = G'(\omega) + iG''(\omega) \tag{13}$$

$$G'(\omega) = \frac{\sigma_0}{\varepsilon_0} \cdot cos(\delta) \tag{14}$$

$$G''(\omega) = \frac{\sigma_0}{\varepsilon_0} \cdot sin(\delta) \tag{15}$$

Here σ_0 and ε_0 are the amplitudes of stress and strain and δ is the phase shift between them. As you can see, for a purely elastic material, the shear modulus is always real. While purely elastic materials have stress and strain in phase ($\delta = 0$; no time-lag in the response!), purely viscous materials have a 90 degree phase lag between stress and strain. Viscoelastic materials exhibit behavior somewhere in between.
The shear modulus and the Young's modulus are related via:

$$Y = 2G(1 + \nu) \tag{16}$$

Here, ν denotes the Poisson's ratio. This ratio describes how much a material will expand/shrink in the direction perpendicular to an applied compressive/tensile force. The values of ν for an isotropic, linear

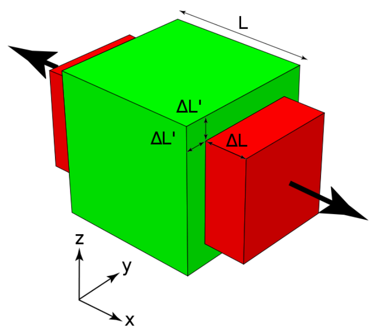


Figure 4: Illustration of the reactions of an isotropic linearly elastic cube to an applied external force. The green cube is unstressed, the red cube is the deformed green cube when a uniaxial tensile stress is applied in the direction of the arrows. ΔL and ΔL' denote these changes. $\nu \sim \frac{\Delta L'}{\Delta L}$ for small deformations. Image taken from wikipedia.

elastic material are within [-1.0, 0.5]; this is due to the requirement that the moduli (Y, G) have positive values. For rubber, $\nu \sim 0.5$; and we will assume the same for tissues. $\nu = 0.5$ is a special case as it describes incompressible materials. For incompressible materials, there is no volume change, but the shape of the material changes greatly upon the applied force. Thus, strictly speaking, the area, A, to which the tensile force is applied to, changes over time as we discuss at the beginning of the lab in week

1. The true stress, σ_{true} is therefore larger than the stress given by equation EQ1, σ_0. To compensate for this change in shape, we ask you to calculate the stress and strain for your lab reports also according to the following two equations (and compare with your results obtained for the case where you do not compensate). For the true stress:

$$\sigma_{true} = (1 + \epsilon_0)\sigma_0 \tag{17}$$

and for the true strain:

$$\epsilon_{true} = ln(1 + \epsilon_0) \tag{18}$$

Examples for the Poisson ratios for other materials are: Cork ($\sim$ 0), glass (0.29), aluminium (0.33). Note that the smaller the Poisson ratio, the larger the volume change the material experiences upon an applied force; for incompressible materials the volume change is zero and the shape change is large. In the case of exotic materials with a negative Poisson ratio (there was an interesting foam paper in Science magazine in 1987 about such a material: Science magazine 235, pp1038-1040), the material would actually get wider perpendicular to the direction in which you stretch it!

In order to determine which model describes the properties of a given viscoelastic material best, the material characteristics must be determined experimentally by a creep experiment, a stress-relaxation experiment or a dynamic loading experiment (application of a sinusoidal stress). In a creep experiment, a constant tensile stress is applied to the material and the strain curve is recorded as a function of time. In a stress-relaxation experiment, a constant strain is applied and the stress relaxation over time is recorded. In a dynamic loading experiment, a periodic oscillation is applied to determine the complex shear modulus.

3 Day I: Measuring Mechanical Properties

Today you will build your uniaxial tensile stress measurement setup and measure the elongation of rubber, chicken skin and stomach.

Materials Rubber; chicken skin; ruler; camera; lens; 2 boom stands; wire; falcon tubes; beads; clips; scissors; permanent marker; Labview; balance; cutting boards;

Sample Preparation Each group should prepare at least 2 samples of rubber and 2 samples of chicken skin.

1. **Rubber Preparation:**
   - With scissors cut the rubber band approximately 2-5 cm long. We have rubber bands of various width, so you can try out different ratios. Get at least 2 samples.
   - Using the ruler, record the length and width of each respective sample in cm. Use the caliper to measure the thickness of the rubber bands.
2. **Chicken Skin Preparation:**
   - Put on gloves.
   - Carefully cut at least 2 samples of chicken skin anywhere between 2-5 cm long and 1-2cm wide with the razor blade. Make sure to cut always away from your hands.

- Make sure you cut the chicken skin in the same general orientation; either length-wise or width-wise. This makes for a nice experiment in the end when you have time: how does the orientation of the chicken skin change your results?
- Using a ruler, record the length and width of each respective sample in cm. The thickness is estimated to be 1-2 mm (We won't measure this with the caliper to not get the latter all dirty).

Measurement Apparatus We will take advantage of gravity and build our measurement apparatus vertically. Fig.5 shows you how to mount the camera, the sample and the white screen for better contrast. You may also want to use the additional light source to get better contrast.

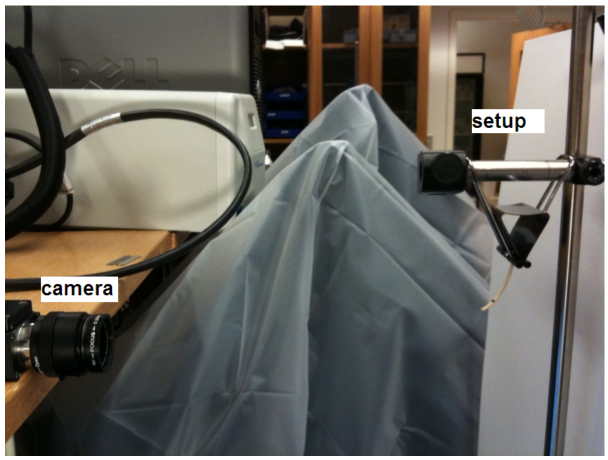


Figure 5: Setup for stress–strain measurements.

1. Attach a binder clip to one of the boom stands (in biology also called ring stand); this will be your fixed end.
2. Make sure to draw two horizontal lines close to the ends of the rubber band (they should end up close to the point where the binder clips attach to the sample later) with a permanent marker before you mount the band (see Fig.6). This line will be your reference for all your elongation measurements. Attach the rubber band or chicken skin band to the clip. Make sure it is positioned straight in the horizontal position. Put it into the binder clip far enough so it cannot slide out easily and that your upper reference line is still visible, but close to the clip.
3. Put the other binder clip on the free end. Make sure you get close to the second reference, but you can still see it clearly. You will hang weights from the clip using a 50mL falcon tube that you will fill with various numbers of beads.
4. Lay the camera on the shelf opposite of your setup in horizontal position (rotate it so that the longer side of the rectangular field of view is in the direction of your elongation) so you can see the sample once it is mounted.
5. Use the Labview program snapshot, found at

   `D:\Labview2007\Snapshot`

to capture images.

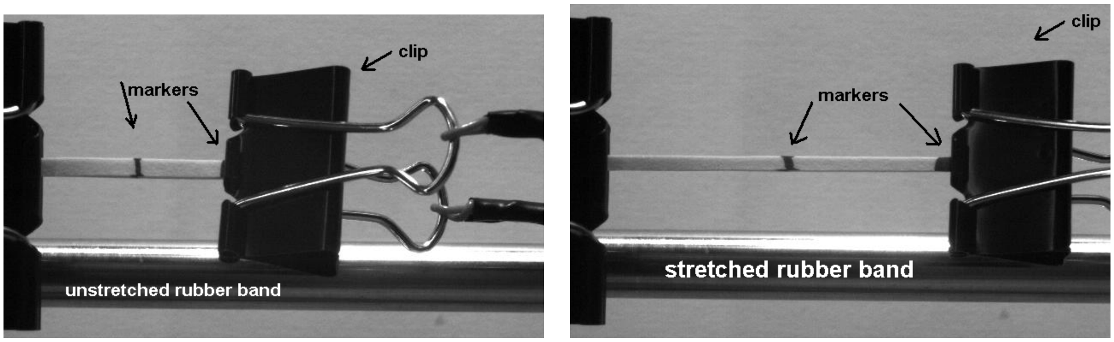
6. Weigh a bead on the balance so you can calculate how much weight you have in your falcon tube as a function of the number of beads you are adding. Each group has 200 beads; that's enough to fully fill the falcon tube. Be careful to not drop and lose beads. Use the provided funnel or build a funnel out of paper to fill them in the tube when you do your experiments.
7. It is best to start with the rubber first and then measure the chicken skin samples as the latter is a bit more squishy and slimy and thus harder to handle. You can wrap the ends of the chicken skin in a thin one-layer coast of kimwipe and thus make it harder for it to slip out of the paperclip.

We will show you an example setup during the demo at the beginning of the class.

Experimental Procedure

1. When everything is set up, you can start your measurements. Start with the rubber bands first.
2. Hang a rubber band from the clip as described in the previous section. Make sure it hangs straight. Take a picture with snap shot. This is your zero force, zero elongation baseline.
3. Make sure to take a picture of a ruler next to the band to get a pixel to mm conversion.
4. Now start hanging different weights from the rubber band. Start out with the paper clip/empty falcon tube first (don't forget to measure its weight on the electro balance).
5. Work your way up from smaller to larger weights. Start adding beads in 10 bead increments for the first 5 measurements, the continue in 20 bead increments until you have used all 200 beads. Make sure to let the system equilibrate before you take pictures. The rubber band should hang still and straight. Do not forget to take a picture every time before adding more beads!
6. Check for hysteresis in your measurements by also taking pictures as you remove the beads in the same increments given above.
7. Make sure you have calculated all your weights. Use the same weights for your other measurements with the other specimen.
8. Do the other rubber band(s). Once you have finished measuring the rubber, measure the chicken skins. Keep all your samples. You will need them again later!
9. When you have finished all your measurements, make sure you clean everything with ethanol.
10. When you are done cleaning up, give your samples to an AI.
11. The AI will put half of your samples in an oven overnight to dry them out and the other half in 4% paraformaldehyde (PFA), which is a fixative that crosslinks amino acids. PFA forms in aqueous formaldehyde (also called formalin) as a white precipitate and is thus essentially solidified formaldehyde, made up of connected formaldehyde molecules. It has the same uses as formaldehyde and is often used interchangeably in the laboratory.

    Next week you will test what effect these procedures have on the mechanical properties of your samples. We will call it "artificial aging".
12. Once you have finished your experiments with the rubber and skin samples, you can start taking measurements on the tripe samples (again, do 2).If you don't have enough time this week, you can do it next week as we won't age these samples.



(a) rubber band (b) stretched rubber band

Figure 6: Samples

4 Day II: The Effects of Aging on Tissue Elasticity

Today you will finish experiments from day 1 with the rubber if needed and test the properties of your samples that we tried to artificially age since last week by putting them either in 4% paraformaldehyde (PFA) or drying out in an oven.

Materials Rubber; chicken skin; ruler; camera; lens; 2 boom stands; wire; falcon tubes; beads; clips; scissors; permanent marker; Labview; balance; plastic garbage bags for specimen.

Experimental procedure

1. Take your aged samples and repeat the measurements from last week. Make sure to wear gloves for all samples as PFA is nasty! Do the samples behave the same or differently? Analyze as on day 1 and compare the results.

2. Remember to take a picture of a ruler next to the sample to get a pixel to mm conversion.

3. If you haven't already done so on day 1, measure one piece of fresh chicken skin in both orientations and compare the results.

4. We have some commercial skin lotion around in the lab. Test whether applying lotion to your dried out chicken skin samples will make them a bit more elastic again. You are also welcome to bring your own favorite lotion and test it. You need to test at least one lotion on your samples and discuss the results in your lab report.

5. If you haven't done so last week, measure the cow stomach samples.Does the stomach behave different than the skin since it is 2-dimensional and has structure? Explore.

6. When you are finished with your measurements, make sure to clean your spot with ethanol and discard the samples in the labeled plastic bags we left at your bench.

Additional questions: When you are done with your experiments, answer the following questions (their discussion is required in your lab report):

1. Up to which deformation are chicken skin and rubber linearly elastic? – Make sure that you only fit the elastic regions of your stress-strain curve!
2. Could you detect hysteresis in the system (show in your plots and discuss)?
3. How do the elastic moduli compare which you can extract from the linear region?
4. Do the materials behave ductile or brittle? – Explain your answer.
5. How does aging affect the properties of the materials? Which type of aging has a stronger effect, chemical or temperature based? What was the effect of applying lotion to your samples?
6. Does changing the orientation of chicken skin change the results? Elaborate your answer.
7. Do you think the properties of your skin are the same everywhere on your body? (Think of the soles of your feet versus your belly for example).....elaborate a bit on this idea and why things may be a certain way. Read the paper we posted on Blackboard by Chang et al. Try to summarize the major finding in 1-2 sentences. Are you surprised by the results? Why or why not?

5 Post-lab Writeup:

As usual, the write-up should be 5 pages or less, is due on Day 2 of the next lab, and should include the following sections:

- Results from your load-elongation data for both days. Make sure you use error propagation as applicable.
- Compare the results for the Young's moduli of chicken skin, rubber band, and cow stomach.
- Calculate the shear modulus for the chicken and rubber samples.
- Include your MATLAB figures of the stress-strain relationships and the calculation of the Young's modulus (appendix if necessary).
- Discuss your results on the aged samples; compare to the fresh ones. Implications?
- Discuss the questions we pose at the end of section day 2.
- Do not forget to discuss possible sources of error and experimental difficulties.

6 References:

1. Foty, R. A., Forgacs, G., Pfleger, C. M. and Steinberg, M. S. *Liquid properties of embryonic tissues: Measurement of interfacial tensions.*(1994) PRL 72, pp. 2298–2301.
2. H.Y. Chang, J.-T. Chi, S. Dudoit, C. Bondre, M.v.d. Rijn, D. Botstein and P.O. Brown *Diversity, topographic differentiation, and positional memory in human fibroblasts.*(2002) PNAS 99, pp. 1287712882
3. Fung, Y. *Biomechanics. Mechanical Properties of Living Tissues* (1993) Springer.

4. Glaser, R. *Biophysics* (1999) Springer.

5. Image 1 is taken from

   `http://invsee.asu.edu/srinivas/stress-strain/phase.html`

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