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# Liver capsule microstructure reorganization induced by biaxial loading assessed by multiphoton microscopy

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## Résumé :

*Le développement de modèles constitutifs basés sur la microstructure nécessite l'identification de paramètres microstructuraux, ainsi qu'une connaissance des mécanismes de réarrangement de la microstructure lorsqu'elle est soumise à un chargement mécanique. Dans cette étude, un protocole de chargement en gonflement equibiaxial et non equibiaxial sous microscope a été développé sur de la capsule de foie humaine. Ainsi, les changements dans l'organisation des fibres ont pu être suivis via imagerie par microscopie confocale multiphotonique. Les champs de déformations locaux ont aussi pu être calculés par suivi de marqueurs placés sur la structure. Les résultats indiquent que la réorientation des fibres de collagène, lorsqu'elle a lieu, intervient progressivement au cours du chargement. D'autre part, la déformation méridionale peut aussi être considérée comme un prédicteur de rupture fiable.*

## Abstract:

*The development of structural constitutive models relies on microstructural parameters and knowledge about the tissue microstructure behavior when submitted to mechanical loading. In this study, an in situ bulge test with varying loading conditions between equibiaxial and non-equibiaxial loading has been implemented on human liver capsule. Therefore, the changes of fibers arrangement can be monitored via imaging with multiphoton microscopy. The local strain fields can also be computed by following the displacements of markers on the structure. Results indicate that collagen reorientation, when it arises, takes place gradually all along mechanical loading. Meridional strain can also be considered as a valid rupture criterion.*

**Mots clefs: in situ elliptic bulge test; photobleaching; strain measurement; liver capsule; multiphoton microscopy; fibrous connective membrane**

# 1 Introduction

Studying the changes of microstructure organization with mechanical loading is an active research area since it is the starting point to develop structural constitutive models. Focus has been made on collagenous-based structure to understand how the fibers networks bear load, extend and break. Therefore in situ mechanical test have been developed such as uniaxial tensile test on rat tail tendon [1], human amnion [2], human liver capsule [3], but also biaxial stretch on bovine pericardium [4] or porcine coronary arteries [5].

At the macroscopic scale, bulge tests turned out to be a valuable method to investigate biological material behavior because it applies a stress state that is very close from what the tissues might experience in vivo [6], especially for the case of connective membranes encompassing pressurized organs. Liver capsule or Glisson's capsule is a connective fibrous membrane that encompasses hepatic parenchyma and as such, contributes greatly to liver mechanical behavior. It has been widely studied from a macroscopic point of view [7], [8] since it is involved in common livers injuries such as surface lacerations.

Thus, the aim of this study is to implement an in-situ bulge test under a two photon excitation microscope to assess liver capsule microstructure changes during inflation. The two photon excitation microscope allows observing the collagen and elastin fibers networks via second harmonic generation (SHG) and fluorescence emission respectively. Therefore, it can be used to quantify the reorientation of collagen fibers and the local strain at the apex of the bulge, following a method described in Jayyosi *et al.* [3].

## 2 Material and Methods

### 2.1 Experimental set up

Human livers were collected through the French voluntary corpse donation to Science program from the department of anatomy of the University of Rockefeller (DUAR, Lyon, France). Liver capsule was taken off and frozen at -20°C until test day. Samples were cut in three different shapes: circular (referred to as R1) and two elliptic shapes (R2 for a ratio 2 between major and minor axis and R4 for a ratio 4) to test various loading conditions between equibiaxial and non equibiaxial.

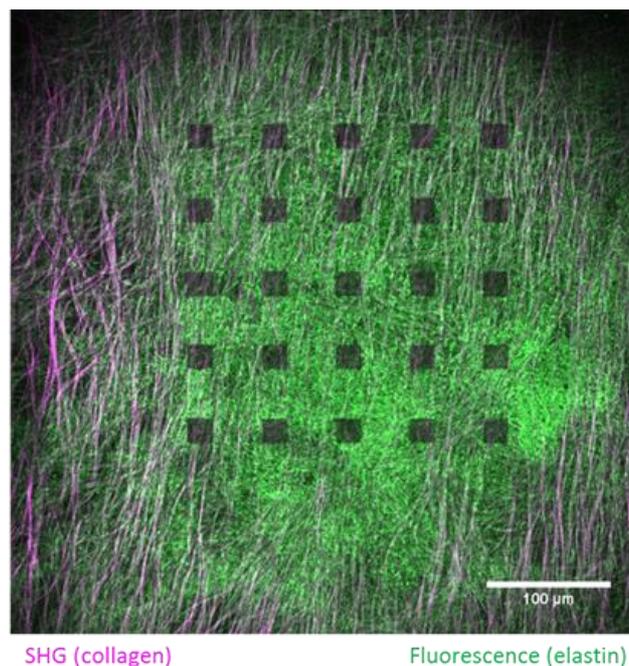
Loading was conducted with compressed air following steps of pressure increments of 0.1 bar until sample failure. Through the experiment, pressure and vertical displacement of the apex were acquired via a pressure sensor and the microscope objective displacement sensor. At each pressure step, the whole capsule thickness was imaged at the apex thanks to a two photon excitation microscope (NIKON, A1R MP PLUS®) at the IVTV platform (ANR-10-EQPX-06-01). Elastin fibers, that generate fluorescence signal, and collagen fibers that generate second harmonics were therefore imaged simultaneously on two separated channels. Total in plane field of view was 507 x 507  $\mu\text{m}^2$ , and one image every 0.4  $\mu\text{m}$  was taken in the thickness direction. Images settings were chosen as a good tradeoff between limited acquisition time, to limit creep effects, and a satisfactory image quality to allow easy image processing.

## 2.2 Strain and orientation measurements

Following the approach described in Jayyosi *et al.* [3], intrinsic markers were positioned on the fibrous structure by photobleaching before loading to create a grid that would be the base of a finite element mesh used to compute local strain. Therefore, 5 x 5 photobleached squares were made by increasing laser power locally resulting in a grid of 20 x 20  $\mu\text{m}^2$  equally spaced squares, as seen on figure 1.

The in plane position as well as out of plane positions of these squares were collected via image segmentation with ImageJ and used to calculate displacements. From them, we derived Green-Lagrange meridional and circumferential strain using the finite element interpolation in the fully integrated Belytschko-Tsay membrane elements in the software LS-DYNA®. The resolution of the images allowed computing strain with an accuracy of  $\pm 0.8\%$ .

The plugin OrientationJ of ImageJ and the method presented in Rezakhaniha *et al.* [9] was used to calculate orientation of collagen fibers along the stack. A Gaussian gradient structure tensor with a window size of 1 pixel was chosen to get the orientation distribution of every image of the stack at each pressure step. The orientation analysis is performed on the SHG (Second Harmonics Generation) images that show exclusively collagen fibers and on which photobleached squares can therefore not be seen.

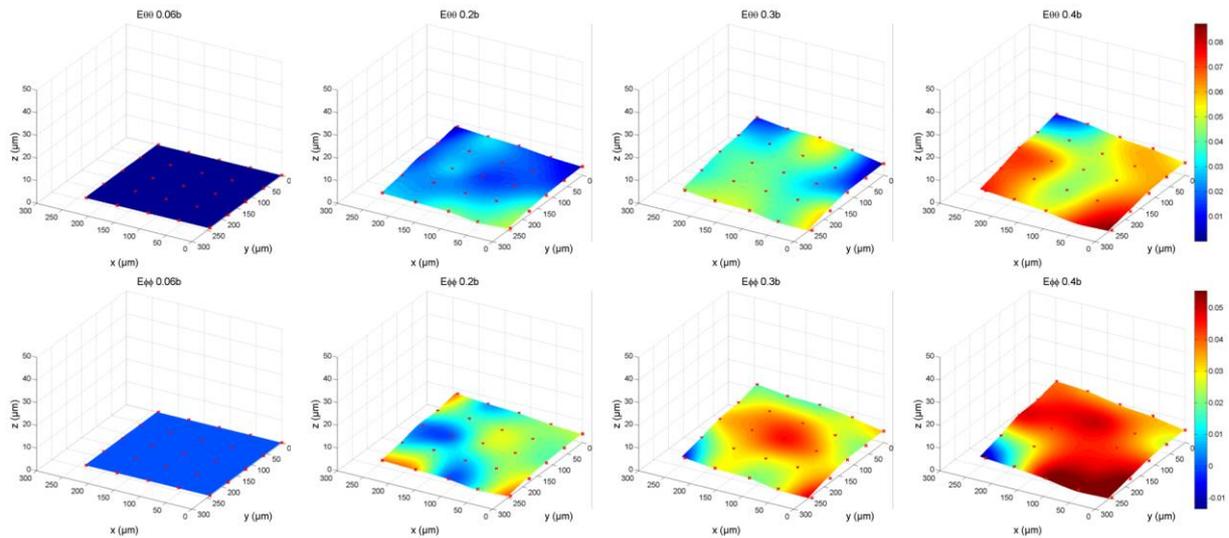


**Figure 1:** Human liver capsule observed with multiphoton microscopy at 0.1 bar at the apex. Green correspond to the fluorescence channel that shows the elastin fibers, while the Second Harmonic Generation (SHG) signal on which appear collagen fibers is shown in magenta. The photobleached grid, characterized by a local loss of fluorescence, is shown before loading.

### 3 Results and Discussion

#### 3.1 Local strain fields

Figure 2 presents an example of the meridional ( $E_{\theta\theta}$ ) and circumferential ( $E_{\varphi\varphi}$ ) strain fields evolution with loading. As seen, these strain fields presents some heterogeneity that comes from the local anisotropy of the liver capsule. However, they vary in the same range for all the samples tested. In fact, when we consider the values taken just before rupture on all samples, the meridional strain values present less variation than other parameters that could be used to predict rupture such as ultimate pressure or surface tension. These results might suggest that meridional strain could be a reliable rupture criterion for modeling purposes. As stresses can be difficult to assess on such structures, a criterion formulated in term of deformation is a good alternative to include in models of fibrous tissues.

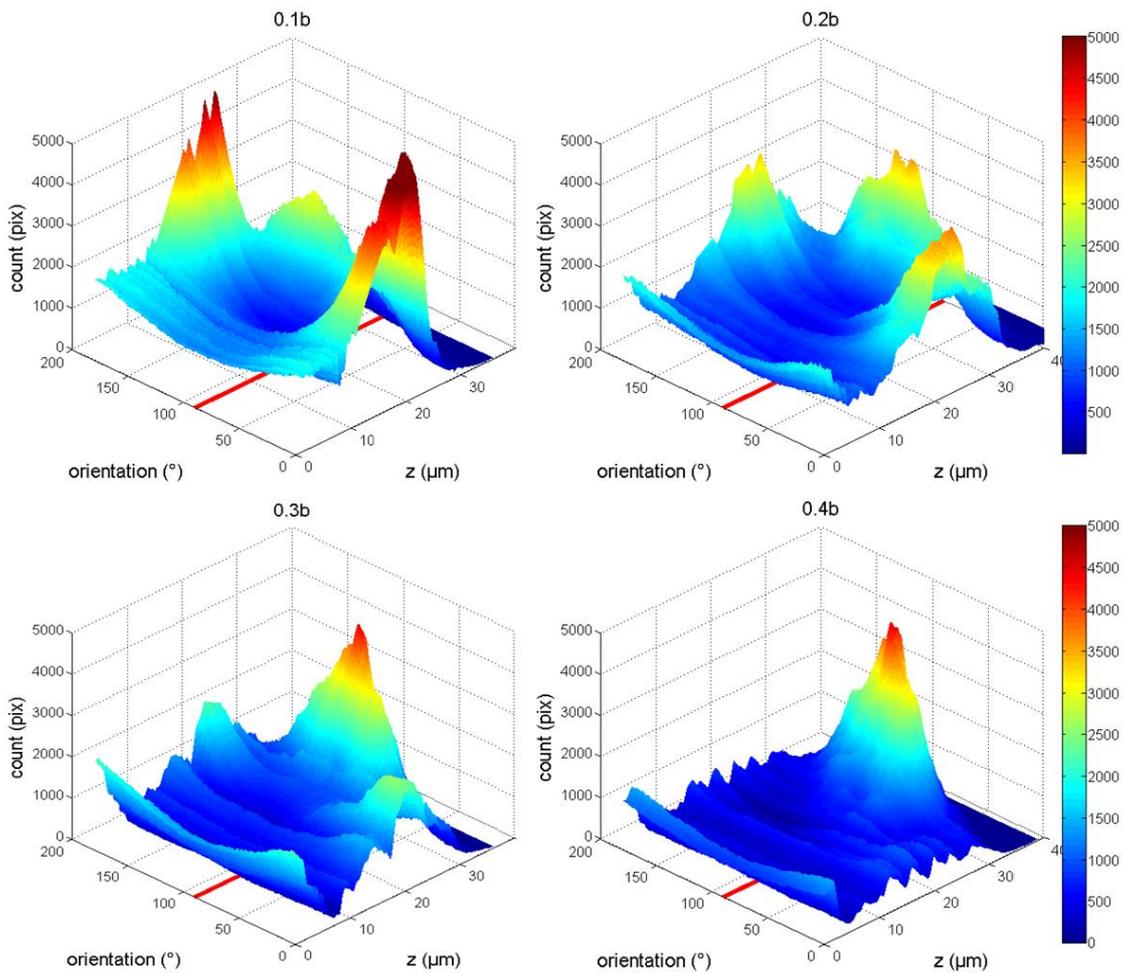


**Figure 2:** Example of Green-Lagrange meridional ( $E_{\theta\theta}$ ) and circumferential ( $E_{\varphi\varphi}$ ) strain field evolution during loading showing the heterogeneity of local strain in human liver capsule. Each image is associated to a level of pressure measured during image acquisition. The nodes of the finite element mesh that correspond to photobleached squares centers are indicated by red points.

#### 3.2 Fibers reorientation

As expected, the reorientation of fibers is not very important in the case of equibiaxial loading. For non equibiaxial loading, we noticed a reorientation of collagen fibers in the direction of the minor axis of the ellipse, which corresponds to the direction of maximal meridional strain. This trend was particularly emphasized on samples for which initial fibers main direction was way different from the main loading direction. Figure 3 illustrates the fiber reorientation phenomenon for the case of a non equibiaxial sample (R2) where the initial main fiber direction that was around  $0^\circ$  progressively fades away while fibers reorient at  $90^\circ$  which is the minor axis direction (indicated by the red line). Therefore, fibers with orientation close from the one of the minor axis reorient in that direction of maximal loading (it is the case for example for the plane located at  $30 \mu\text{m}$  on figure 3). Planes with

fibers oriented in a direction way different from the minor axis direction ( $z = 20 \mu\text{m}$  on figure 3 for instance) reorient as well in that direction but partially resulting in a layer with more or less isotropic property and no main orientation.



**Figure 3:** Change of collagen fibers orientation in the whole thickness with the increase of pressure in the case of a non-equibiaxial loading. The red line indicates the orientation of the minor axis of the elliptic sample that correspond with the direction of maximal meridional strain. The fibers gradually aligned with that principal direction

## 4 Conclusion

A new protocol allowing the observation of liver capsule microstructure under pressure has been developed. Therefore the kinematics and strain environment of the fibers networks have been assessed during a mechanical loading closer from in vivo conditions than uniaxial tensile test. Such local parameters as well as information on how the fiber network reacts to loading are crucial in order to develop constitutive based models that have many applications such as tissue engineering for instance.

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