

# Image-based Multi-scale Mechanical Analysis of Strain Amplification in Neurons Embedded in Collagen Gel

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## ARTICLE HISTORY

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## ABSTRACT

A general multi-scale strategy is presented for modeling the mechanical environment of a group of neurons that were embedded within a collagenous matrix. The results of the multi-scale simulation are used to estimate the local strains that arise in neurons when the extracellular matrix is deformed. The distribution of local strains was found to depend strongly on the configuration of the embedded neurons relative to the loading direction, reflecting the anisotropic mechanical behavior of the neurons. More importantly, the applied strain on the surrounding extracellular matrix is amplified in the neurons for all loading configurations that are considered. In the most severe case, the applied strain is amplified by at least a factor of 2 in 10% of the neurons by volume. The approach presented in this paper provides an extension to the capability of past methods by enabling the realistic representation of complex cell geometry into a multi-scale framework. The simulation results for the embedded neurons provide local strain information that is not accessible by current experimental techniques.

## 1. Introduction

In early studies of tissue mechanics (Fung 1993), the focus was on developing constitutive equations that described the mechanical behavior of a tissue as a continuous, homogeneous entity. Even the continuous tissue mechanics problem is extremely difficult, with issues such as viscoelasticity (Limbert and Middleton 2004; Peña et al. 2010), nonlinearity (Fung 1993), multiphasicity (Mow et al. 1980; Simon 1992), anisotropy (Fung 1993; Humphrey 2001; Ehret and Itskov 2009), active stress generation (Ambrosi and Pezzuto 2011), damage (Alastrué et al. 2007), and growth and remodeling (Humphrey and Rajagopal 2002; Menzel and Kuhl 2012) all coming into play under various circumstances. Much of the work in biomechanics has attempted to capture these different complexities at a continuum level given the macroscopic nature of humans and the apparent continuity of the tissue when viewed on the macroscopic scale.

More recently, however, it has been recognized that the essential unit that responds to a macroscopic load is in fact microscopic, such as a somatosensory nerve ending (Quindlen et al. 2016), a chondrocyte within articular cartilage (Guilak and Mow 2000), a cell remodeling its scaffold in an engineered blood vessel (Isenberg et al. 2006; Syedain et al. 2017), or - most relevant to the current work - a neurite undergoing physiological and excessive stretch in collagen constructs mimicking (spinal) ligaments (Sperry et al. 2017; Zhang et al. 2017a). In each of these cases, a macroscopic, tissue-scale loading condition is transferred to the microscopic, cell-scale stress, which in turn drives the cellular response. If we hope to understand the microscopic response to a macroscopic load, a scale-bridging strategy is needed.

The constrained-mixture approach (Humphrey and Rajagopal 2002) has been effective in predicting growth and remodeling at the continuum scale, but it does not account for the local cell stress that arises. As such, constrained-mixture models are not designed to address the effect of local strain field variations due to the presence of a cell, as observed experimentally in fibroblast-populated collagen gels (Pizzo 2005).

Inspired by classic work in the theory of composite materials (Hashin 1962), numerous studies have now been made where models are constructed of a cell surrounded by acellular material. In proposed cases, the approach employed is to place a cell of idealized shape (sphere or ellipsoid) in the center of the cubic domain made up of an acellular material. A notable extension is the multi-cell simulation study of Marquez, Genin, and Elson (Marquez et al. 2010), in which cylindrical or ellipsoidal cells were progressively added to the system, and an exponential increase in the tangent moduli of the extracellular matrix was observed when the cells percolated the domain. Other contributions (Lai et al. 2013; Zhang et al. 2013a,b) were the replacement of the closed-form constitutive equation with a multi-scale model that allows better representation of the extracellular matrix structure.

In spite of these recent advances, there is still more work to be done in pursuing a realistic representation of the cell's or tumor's mechanical environment under a given tissue load. Perhaps the most salient missing feature of current models is the fact that the microscopic entity is rarely spherical in shape. For example, cells spread and polarize within a collagen matrix, e.g., (Guido and Tranquillo 1993; Friedl et al. 1998), and a malignant tumor can have an extremely complex geometry (Cristini et al. 2003). There is a compelling need for methods that can account for the detailed microscale geometry when downscaling mechanical information.

Major barriers to a realistic representation of complex cell geometry include the generation for a model geometry from experimentally measured images and the subsequent mesh generation on that model geometry. Furthermore, the number of degrees of freedom required to properly represent the complex cell geometry is large, and when coupled with a multi-scale framework that is required to model the fibrous architecture of the extracellular matrix, the computational demand can become prohibitive. In this work, we addressed some of those challenges by developing a multi-scale model of a group of neurons embedded within a collagenous matrix. The objective in this work was to develop a general modeling strategy in which (1) confocal microscopy or another three-dimensional imaging modality is used to generate a realistic representation of a complex cell geometry, (2) that geometric model, along with the surrounding extracellular matrix, is converted to a finite-element mesh, (3) a multi-scale model is implemented on that mesh using a network microstructural model at each Gauss point (Chandran and Barocas 2007; Stylianopoulos and Barocas 2007), and (4) the solution of the mechanics problem is used to determine the distribution of strains within the cell, i.e., at a scale and with a resolution that is not accessible to direct microscopic

observations.

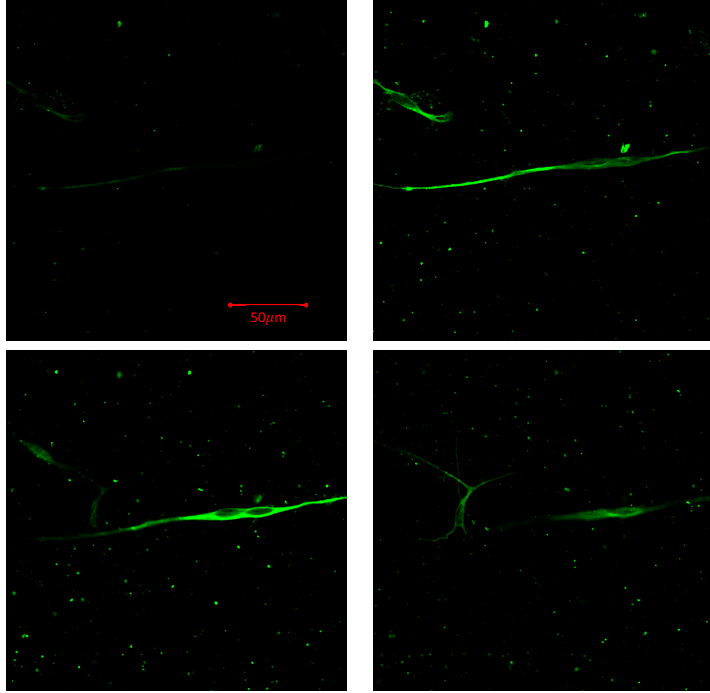
Although the approach described herein is general, it is focused on the specific experimental system in which dorsal root ganglion (DRG) neurons are embedded in a collagen gel. This model system and others like it (Zhang et al. 2016, 2017a,b), allow detailed study of the cellular mechanisms of neuronal injury in a more controllable environment than can be achieved in vivo. As such, it has the potential to provide insight into the mechanism of neuronal injury under mechanical load, with markers of neuronal injury being quantified in response to different macroscopic strains and/or strain rates (Zhang et al. 2016). To summarize the experimental system, neurons are embedded in collagen gels to mimic the innervation of ligamentous tissue, such as that found in the spinal facet capsular ligament (McLain and Pickar 1998; Kallakuri 2012) and the knee joint capsule (Schultz et al. 1984; Khalsa et al. 1996). This in vitro system has been utilized to study the effects of macroscopic and microscopic scale tissue mechanics on neuronal responses in the context of pain. Previous studies using this and similar neuron-collagen culture models suggest that neuronal activation and pain signaling can be modulated by the macroscopic strain and collagen organization of the surrounding extracellular matrix (Zhang et al. 2016, 2017a). However, the system does not at present enable experimental evaluation of detailed neuronal mechanics, which is important for understanding the local mechanisms of neuronal mechanosensing and/or injury due to tissue loading. Therefore, an image-based multi-scale model that complements the neuron-collagen in vitro system is developed to investigate the relationships between macroscopic tissue deformations and mechanical changes in neurons.

The complex geometry of the neurons is incorporated into our model by employing tools (Simmetrix 2017) that are capable of generating geometric models from experimental images (Klaas et al. 2014, 2013) and of meshing the resulting geometric model (Shephard 2000). Additionally, the large computational demands of a multi-scale model on the resulting mesh are accommodated by the adaptive multi-scale simulation infrastructure (AMSI) library (Delalondre et al. 2010; Tobin et al. in preparation), which was designed to handle massively parallel simulations simultaneously on multiple scales. From this model, we observe strong strain amplification in the neurons due primarily to their complex geometry. The results provide a relation between the applied far field and the neuronal strain leading to the onset of pain.

## 2. Representation of Complex Cell Geometry

A realistic representation of complex cell geometry was generated from three-dimensional (3D) voxelated data that originated from a stack of image slices. In this work, confocal Z-stack images were taken from an unloaded 3D neuron-collagen culture system. Briefly, DRG were harvested from embryonic day 18 rats, dissociated and embedded in a collagen Type I gel (2mg/ml; Corning Inc; Corning, NY), using methods described in prior studies (Cullen et al. 2012; Zhang et al. 2016, 2017a). The structure of neurons is visualized by fluorescence labeling of the cytoskeletal protein  $\beta$ III-tubulin (Abcam Cambridge, MA), as described previously (Zhang et al. 2016, 2017a). A Zeiss LSM 510 confocal microscope (Carl Zeiss Inc; Thornwood, NY) is used to image the neurons. Z-stack images (spanning  $2\mu\text{m}$  thickness with  $0.2\mu\text{m}$  steps) are taken at a resolution of  $0.22\mu\text{m}/\text{pixel}$  with a 40X objective. Five consecutive images, each representing a thickness of  $2\mu\text{m}$ , are used to reconstruct the neuronal structure in 3D. All images are segmented using *ImageJ* (Schneider et al. 2012) based on the positive labeling of  $\beta$ III-tubulin for model construction, and speckle noise in the background is

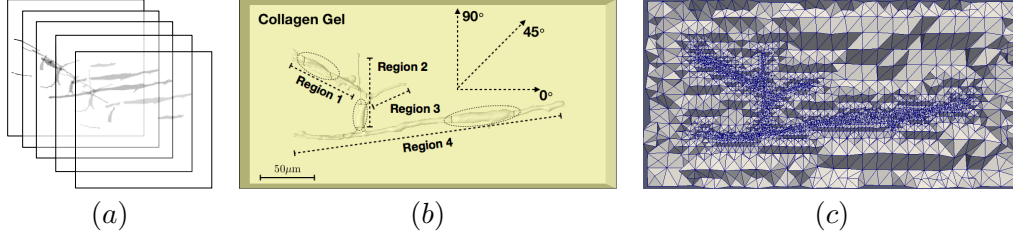
removed in Adobe Photoshop (Adobe Systems Incorporated; San Jose, CA). Sample raw confocal Z-stack images that are used in our model are shown in Fig. 1.



**Figure 1.** Sample of raw images taken from Zeiss LSM 510 confocal microscope that were used in our model. The scale bar in the top left image represents  $50 \mu\text{m}$  and applies to all images. The images were stacked from top to bottom in the following order: top left, top right, bottom left, and bottom right.

The confocal images were converted to a 3D voxelated data set using *ImageJ* (Schneider et al. 2012). Noise in the voxelated data due to the limited level of contrast in the imaging technique is removed using a combination of erosion/dilation, manual reassignment, small object removal, and smoothing operations via the *ImageToModel* tool (Klaas et al. 2013, 2014; Simmetrix 2017). Once processed, the voxelated data are converted into a discrete geometry via an initial triangulation that is based on voxel level operations where mesh coarsening is not performed until after the surface triangulation has been smoothed (Klaas et al. 2013). Quantization artifacts on the surface of the geometry due to the voxel nature of the data are removed using a smoothing algorithm that consists of three steps (Klaas et al. 2013): (1) calculate the surface normal of the desired surface geometry at each mesh face based on the normals of neighboring mesh faces; (2) smooth surface normals on the mesh to obtain the normals of desired surface geometry; (3) adjust the mesh vertex positions iteratively to create a surface that matches the smoothed surface normals.

Subsequently, the discrete geometry generated from the voxelated neuron-in-gel data is enclosed in a box that represents the domain of the collagen gel. The neuron-gel interface is treated as perfectly bonded. A graded mesh of 82,105 tetrahedral elements is generated on the non-manifold neuron-in-gel geometry using the SimModeler tool of Simmetrix Inc. (Shephard 2000; Simmetrix 2017). The neuron-in-gel voxelated data, discrete geometry, and corresponding mesh is shown in Fig. 2. The model contains three connected neurons and the cellular bodies are identified in Fig. 2(b) with ovals. The remaining neuronal structure represents axons and dendrites.



**Figure 2.** (a) Stack of confocal images of neurons from Fig. 1. (b) Geometric model of neurons that are embedded in a collagen gel. The region enclosing the neurons is the collagen gel. (c) Cut in neuron-in-gel model showing the generated mesh. In (b), the neuron cell bodies are marked with dashed ovals. The axes of load directions corresponding to 0°, 45°, and 90° and regions of neurons corresponding to different local axes are also schematically illustrated.

### 3. Multi-Scale Method for Modeling Collagen Gel

Collagen gels have been modeled in the past using a multi-scale formulation based on volume averaging of fiber-network representative volume elements (RVEs) (Chandran and Barocas 2007; Stylianopoulos and Barocas 2007; Lai et al. 2012; Lake et al. 2012; Zhang et al. 2013a,b). **In this study, we also employ the volume-averaging based multi-scale formulation. However, our model differs from those used in the past in several ways. The RVE definition is different, including the network architecture and the fact that the microscopic fiber forces are converted to the macroscopic Cauchy stress via a scaling factor that takes into account the difference between the physical size of the RVE and the realistic segment length of the network modeled. Furthermore, fiber forces are modeled using a piecewise function that transitions from nonlinear to linear when the fibers are compressed or stretched in tension beyond a certain strain value.** The multi-scale formulation consists of two scales: the collagen-network scale on which the network of collagen fibrils is represented explicitly, and the macroscopic scale that represents the continuum tissue level. Quantities corresponding to the collagen-network (or microscopic) and macroscopic scales are denoted with  $(m)$  and  $(M)$  superscripts, respectively.

The collagen-network scale is represented by a fiber network, which defines the RVE. Each RVE is generated from the Delaunay triangulation of randomly placed seed points - the edges of the triangulation represent the fibers of the network. Each fiber only carries an axial load, with the axial forces in tension and compression given by

$$T^{(m)} = \begin{cases} H_C \lambda_f + h_C & \text{if } \lambda_f < \lambda_C \\ \frac{E_f A_f}{B} \left[ \exp(0.5B(\lambda_f^2 - 1)) - 1 \right] & \text{if } \lambda_f \in [\lambda_C, \lambda_S] \\ H_S \lambda_f + h_S & \text{if } \lambda_f > \lambda_S \end{cases} \quad (1)$$

where

$E_f$  : elastic modulus of a collagen fiber.

$A_f$  : cross-sectional area of fiber.

$B$  : parameter that controls nonlinearity.

$\lambda_f \equiv \frac{l}{l_0}$  is the stretch, where  $l$  and  $l_0$  are the current and initial lengths of the fiber, respectively.

$\lambda_S$  : fiber stretch value at which the fiber relation transitions from nonlinear to linear.

$\lambda_C$  : fiber compression value at which the fiber relationship transitions from nonlinear to linear.

$H_n \equiv E_f A_f \lambda_n \exp(0.5B(\lambda_n^2 - 1))$  for  $n = S$  or  $C$ .

$h_n \equiv \frac{E_f A_f}{B} [\exp(0.5B(\lambda_n^2 - 1)) - 1] - H_n \lambda_n$  for  $n = S$  or  $C$ .

The fiber force vs. stretch in tension transitions from a nonlinear to linear relationship when the fiber is stretched beyond  $\lambda_S$ , while the fiber force in compression transitions from a nonlinear to linear relationship when the fiber is compressed below  $\lambda_C$ . The transition to a linear fiber force relationship for large fiber stretch in tension is consistent with experimental measurements of single collagen fibers (Eppell et al. 2006; Svensson et al. 2010).

Displacement boundary conditions are imposed in order to solve for network equilibrium in the RVE. The displacements at the corners of the RVE are dictated by the deformation state of the macroscopic scale via

$$u_i^{(c,m)} = (F_{ij}^{(M)} - \delta_{ij}) \frac{x_j^{(c,m)}}{L^{(m)}}, \quad (2)$$

where  $F_{ij}^{(M)}$  is the deformation gradient tensor of the macroscopic scale,  $u^{(c,m)}$  are the displacements at the corners of the RVE, and  $x^{(c,m)}/L^{(m)}$  are the coordinates of the corners of the RVE. In Eq. (2),  $x_j^{(c,m)}$  are coordinates of a dimensionless computational domain that is a cube spanning the domain defined by (-0.5,0.5) in each of the coordinate axes. The scale conversion,  $1/L^{(m)}$ , relates the unit lengths between the computational and physical domains. The scaling parameter,  $L^{(m)}$ , is determined by comparing the average fiber length in the RVEs to the average fiber length in the collagen gel of density 2 mg/mL used in (Zhang et al. 2016). The mean fiber length in such a network is 1.81  $\mu\text{m}$  (Lindström et al. 2013), while the mean segment length in the RVEs used in this work is 0.26. Based on these values  $L^{(m)} = 7 \mu\text{m}$ .

Equation (2) is valid because the center of the RVE is defined at the origin of the computational domain. We control the motion of all nodes on the surface of the RVE and allow the interior degrees of freedom to relax such to minimize the RVE potential energy. The displacements on each node lying on the surface of the RVE are determined via linear interpolation of  $x_j^{(c,m)}$ .

Upon solving for network equilibrium in each RVE, the volume-averaged Cauchy stress tensor of the collagen-network scale is computed from the forces acting on nodes that lie on the surface of the RVE (boundary cross-links)

$$s_{ij}^{(m)} = \frac{1}{V^{(m)}} \sum_{\text{boundary cross-links}} x_i^{(m)} T_j^{(m)}, \quad (3)$$

where  $V^{(m)}$  is the current volume of the RVE,  $T_j^{(m)}$  is the  $j^{\text{th}}$  component of the fiber force, and  $s_{ij}^{(m)}$  are elements of the microscopic stress tensor. **Since Eq. (3) is calculated from the dimensionless RVE computational domain,  $s_{ij}^{(m)}$  has units of force.** The macroscopic volume-averaged Cauchy stress tensor is determined from scaling  $s_{ij}^{(m)}$  by  $L^{(m)}$ :

$$\sigma_{ij}^{(M)} = s_{ij}^{(m)} \left( \frac{1}{L^{(m)}} \right)^2. \quad (4)$$

This scaling is needed due to the difference in length scales between the physical macroscopic domain and the non dimensional microscopic computational domain. The averaged Cauchy stress tensor of Eq. (4) is used to solve the volume-averaged macroscopic force balance, using a procedure similar to that described in (Chandran and Barocas 2007):

$$\sigma_{ij,i}^{(M)} = \frac{1}{V^{(m)}} \int_{\partial V^{(m)}} \left( s_{ij}^{(m)} - \sigma_{ij}^{(M)} \right) u_{k,i}^{(m)} n_k dA^{(m)}, \quad (5)$$

where  $u_k^{(m)}$  is the displacement of the RVE boundary on the microscale and  $n_k$  is the unit normal vector of the corresponding surface. Equation (5) arises from the integration of the microscopic-scale (RVE) Cauchy stress balance,  $\sigma_{ij,i}^{(m)} = 0$ . If the RVE did not deform, then the averaged equation would just be  $\sigma_{ij,i}^{(M)} = 0$ . When the RVE deforms, however, and that deformation is position-dependent, the average of the divergence of the stress ( $\sigma_{ij,i}^{(m)}$ ) is no longer equal to the divergence of the average stress ( $\sigma_{ij,i}^{(M)}$ ), and a correction term must be introduced, analogous to the corresponding term in the Reynolds transport theorem (Whitaker 1999). This term is expressed by the right-hand side of Eq. (5) and accounts for the correlation between RVE stress and boundary displacement.

#### 4. Constitutive Relationship for Neurons

Cross-linked axially-aligned microtubule bundles are a major structural feature of axons and give rise to macroscopic mechanical anisotropy (Peter and Mofrad 2012). To account for such mechanical anisotropy, the axons are modeled as a transversely isotropic hyperelastic material (Bonet and Burton 1998; Bonet and Wood 2008). The elements of the Cauchy stress tensor and spatial (Eulerian) elasticity tensor are expressed in terms of neo-Hookean (nh) and transversely isotropic (trns) components (Bonet and Burton 1998)

$$\sigma_{ij} = \sigma_{ij}^{\text{nh}} + \sigma_{ij}^{\text{trns}} \quad \text{and} \quad c_{ijkl} = c_{ijkl}^{\text{nh}} + c_{ijkl}^{\text{trns}}, \quad (6)$$

respectively, where

$$\begin{aligned}
\sigma_{ij}^{\text{nh}} &= \frac{\mu}{J}(b_{ij} - \delta_{ij}) + \lambda(J - 1)\delta_{ij} \\
\sigma_{ij}^{\text{trns}} &= \frac{2\beta}{J}(a_r a_r - 1)\delta_{ij} + \frac{2}{J}[\alpha + 2\beta \ln J + 2\gamma(a_r a_r - 1)]a_i a_j - \frac{\alpha}{J}(b_{is} a_s a_j + a_i b_{jr} a_r) \\
c_{ijkl}^{\text{nh}} &= \lambda(2J - 1)\delta_{ij}\delta_{kl} + \frac{2}{J}[\mu - \lambda J(2J - 1)]\delta_{ik}\delta_{jl} \\
c_{ijkl}^{\text{trns}} &= \frac{8\gamma}{J}a_i a_j a_k a_l + \frac{4\beta}{J}(a_i a_j \delta_{kl} + \delta_{ij} a_k a_l) - \frac{\alpha}{J}(a_i a_l b_{jk} + b_{ik} a_j a_l) - \frac{4\beta}{J}(a_r a_r - 1)\delta_{ik}\delta_{jl}.
\end{aligned} \tag{7}$$

In Eq. (7),  $b_{ij}$  are elements of the left Cauchy-Green strain tensor and  $a_i$  is a component of the mapping of the direction of anisotropy in the undeformed state,  $A_k$ , to the current state via the deformation gradient,  $F_{ik}$ , via  $a_i \equiv F_{ik}A_k$ .  $\lambda$  and  $\mu$  are the Lamé constants that define the material properties of the isotropic component, while  $\beta$ ,  $\gamma$ , and  $\alpha$  are parameters that define the material properties of the anisotropic component. The parameters of Eq. (7) can be written in terms of material constants as

$$\begin{aligned}
\lambda &= \frac{2\mu(\nu + n\nu^2)}{m} & \gamma &= \frac{E_A(1 - \nu)}{8m} - \frac{\lambda + 2\mu}{8} + \frac{\alpha}{2} - \beta \\
\alpha &= \mu - G_A & m &= 1 - \nu - 2n\nu^2 \\
\beta &= \frac{\mu\nu^2(1 - n)}{2m} & n &= \frac{E_A}{2\mu(1 + \nu)},
\end{aligned} \tag{8}$$

where  $\mu$  is the shear modulus,  $G_A$  is the axial shear modulus,  $\nu$  is the Poisson ratio, and  $E_A$  is axial Young's modulus. In our analysis, the shear modulus is set to be isotropic such that  $G_A = \mu$  and  $\alpha = 0$ . All the constants in Eq. (8) are set once all material constants ( $\mu$ ,  $G_A$ ,  $\nu$ , and  $E_A$ ) are specified.

## 5. Parameter Specifications

### 5.1. Parameters of Collagen Network

Collagen I gels identical to those in which the neurons are embedded are subjected to uniaxial tensile loading at 0.5mm/s up to 25% strain using an Instron 5865 (Instron, Norwood, MA), as described in Zhang et al. (2016). The measured stress-strain curve is shown in Fig. 3(a). This data is used to calibrate three parameters ( $E_f$ ,  $B$ , and  $\lambda_S$ ) of the constitutive behavior of fibers of the collagen network. The prediction of the model gel is obtained with an RVE containing a network of density identical to that of the physical gel. Table 1 shows the set of parameters used for the collagen network.

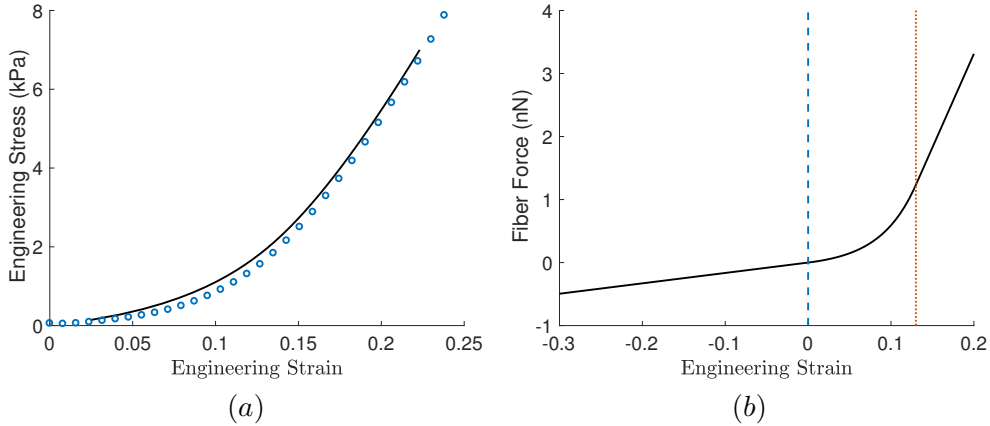
As seen in Fig. 3(a), a good fit to the experimental stress-strain curve is obtained using the parameters listed in Table 1. The value of  $A_f$  is chosen according to (Dutov et al. 2016) where the fiber radius of rat tail collagen I is measured to be 162 nm. The parameters  $E_f$  and  $B$  are adjusted to capture the stiffening in the stress-strain curve at approximately 11% strain observed experimentally, while  $\lambda_S$  is adjusted to capture the stiffness of the linear regime in the stress-strain curve at large strains. Since the fibers in this model only have axial forces, the value of  $\lambda_C$  is selected to give a stiff-



Parameter	Specification	Source
$E_f$	200 kPa	Fit to experimental data.
$A_f$	$0.0825 \mu\text{m}^2$	Based on Dutov et al. (2016)
$B$	20	Fit to experimental data.
$\lambda_S$	1.13	Fit to experimental data.
$\lambda_C$	1.0	Chosen by authors.
$H_S$	$297.25 \mu\text{N}$	Calculated from $E_f$ , $A_f$ , $B$ , and $\lambda_S$ .
$H_C$	$16.5 \mu\text{N}$	Calculated from $E_f$ , $A_f$ , $B$ , and $\lambda_C$ .
$h_S$	$-322.74 \mu\text{N}$	Calculated from $E_f$ , $A_f$ , $B$ , $\lambda_S$ , and $H_S$ .
$h_C$	$-15.67 \mu\text{N}$	Calculated from $E_f$ , $A_f$ , $B$ , $\lambda_C$ , and $H_C$ .
$L^{(m)}$	$7 \mu\text{m}$	Based on mean segment length of RVE reported in Section 3.

**Table 1.** Parameters of the constitutive behavior of the fiber network used to model the collagen gel.

ness in compression that is significantly smaller than that in tension. This represents the softening associated with buckling or off-axis loading of fibers in compression. By choosing  $\lambda_C = 1.0$ , the response in compression remains linear for all strains encountered in simulations. The fiber-force vs. strain curve for the parameters listed in Table 1 is plotted in Fig. 3(b).



**Figure 3.** Plots of (a) model network (solid black line) and experimental gel (blue circles) stress-strain, and (b) model collagen fiber force vs. fiber-strain curve for parameters listed in Table 1. In (b), the values of  $\lambda_C$  and  $\lambda_S$  are marked by blue dashed and red dotted vertical lines, respectively.

For the parameters in Table 1, fiber alignment was also examined. Although fibers in our simulations rearrange in 3D, a two-dimensional (2D) orientation index,  $P_2$ , is considered in order to make the connection with experimental observations which are performed in a planar view of the sample. The value of  $P_2$  is calculated from the angle  $\theta$  between the projection of the fiber in the plane of analysis and the direction of stretch and is given by

$$P_2 = 2 \langle \cos^2 \theta \rangle - 1, \quad (9)$$

where  $\langle x \rangle$  denotes the ensemble average of  $x$  over all fibers. The value of  $P_2$  ranges from 1, which corresponds to the case in which all fibers are aligned with the stretch direction, to -1, which corresponds to the case in which all fibers are orthogonal to the

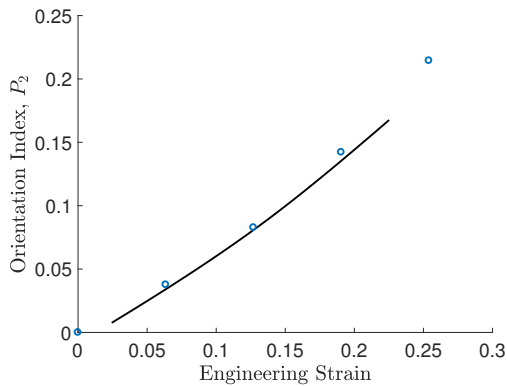
stretch direction.  $P_2 = 0$  corresponds to random orientation of fibers in the network.

Experimentally, the fiber alignment in innervated collagenous tissue is measured using quantitative polarized light imaging (QPLI) (Quinn and Winkelstein 2008, 2009) where alignment angle  $\alpha$  and retardation  $\delta$  are measured in each pixel. The customized QPLI system used in these experiments (Zhang et al. 2016) is comprised of a fiberoptic light source (Dolan-Jenner Industries Inc., Boxborough, MA), a motor-controlled linear polarizer (Edmund Optics, Barrington, NJ) that rotates at 750 rpm, and a circular analyzer mounted to a high-speed camera (Phantom-v9.1; Vision Research Inc, Wayne, NJ) (Zhang et al. 2016). The QPLI system is integrated with a mechanical testing device, and collagen I gels are placed between the rotating polarizer and the circular analyzer. Polarized light images are acquired during tensile loading at 500 fps with 14.5 pixel/mm resolution and processed using harmonic analysis to extract the alignment angle and retardation (Tower et al. 2002; Quinn and Winkelstein 2008). The orientation index  $P_2$  is calculated from  $\alpha$  and  $\delta$  of the QPLI method via (see Appendix A)

$$P_2 = 2 \int_0^\pi p(\theta) \cos^2 \theta d\theta - 1$$

$$p(\theta) = \frac{1}{N} \sum_{i=1}^N \left[ \frac{C - 2\delta_i}{\pi C} + \frac{4\delta_i}{\pi C} \cos^2(\theta - \alpha_i) \right], \quad (10)$$

where the probability distribution,  $p(\theta)$ , is a convenient approximation of the actual distribution of collagen orientation in the sample. In Eq. (10),  $\delta_i$  and  $\alpha_i$  are the retardation and alignment angle, respectively, of the  $i^{th}$  pixel. The proportionality constant  $C$  relates  $\delta_i$  to the difference in the eigenvalues of the orientation tensor. A method to infer  $C$  based on the experimental conditions is not available. Hence, we fit  $C$  to the experimental  $P_2$  vs. strain curve. When  $C = 0.5$ , good agreement between the experimental and simulated fiber orientation is achieved (Fig. 4). Such agreement between simulation and experimental results reinforces our choice of the RVE fiber network parameters listed in Table 1.



**Figure 4.** Fiber alignment metric  $P_2$  of simulation using parameters listed in Table 1 and of experimental results using  $C = 0.5$  for Eq. (10). Results from simulation are plotted using a black solid line, while experimentally measured data are plotted in blue dots.

## 5.2. Parameters of the Constitutive Description of the Neurons

The value of  $E_A$  in Eq. (8) is based on the study by Peter and Mofrad (Peter and Mofrad 2012), where the mechanical behavior of axonal microtubule bundles under tension was simulated with a discrete bead-spring model. To apply their results for microtubule bundles to our neuron model, we assume that only the microtubule bundles carry force in the axon and that the stress of the microtubule bundles can be redistributed over the cross-sectional area of the axon. Since the microtubules are the stiffest component of the cytoskeleton (Fletcher and Mullins 2010), the mechanical contribution from the actin filaments (membrane cortex) is ignored. Based on these assumptions, the axial stiffness of the axon can be related to the axial stiffness of the microtubule bundles,  $E_{MTb}$ , by

$$E_A = \frac{E_{MTb}A_{MTb}}{A_A}, \quad (11)$$

where  $A_{MTb}$  and  $A_A$  are the cross-sectional areas of the microtubule bundle and axon, respectively. Peter and Mofrad (Peter and Mofrad 2012) suggested that the stress-strain relationship of the microtubule bundle is well represented by a power-law fit that results in a strain-dependent elastic modulus. For this study,  $E_{MTb}$  is taken to be  $1 \times 10^5$  kPa, which corresponds to a low strain of less than 0.1%. The value of  $A_{MTb}$  is determined to be  $0.00933 \mu\text{m}^2$  based on the diameter of each microtubule and the number of microtubule cross sections within a microtubule bundle, see Ref. (Peter and Mofrad 2012) for schematic. We assume that the cross-section of the microtubule bundle contains 19 rows of microtubules, each with a diameter of 25 nm. Lastly, the value of  $A_A$  is determined to be  $13.3 \mu\text{m}^2$ , based on an average axon diameter of approximately  $4 \mu\text{m}$  in the neurons.  $E_A$  is determined from Eq. (11) to be 70 kPa.

The parameters used in Eq. (8) for modeling the neuron structure are listed in Table 2. The transverse stiffness of the axons and cell bodies was set to 1.5 kPa based on

Parameter	Specification	Source
$E_A$	70 kPa	Calculated from Eq. (11).
$E_T$	1.5 kPa	Based on Simon et al. (2016)
$\nu$	0.3	Chosen by authors.
$\mu$	0.577 kPa	Calculated from $E_T$ and $\nu$ .
$G_A(= \mu)$	0.577 kPa	Shear modulus assumed to be isotropic.
$t$	$30 \mu\text{m}$	From analysis in Section 7.
$n$	46.7	Calculated from $E_A$ , $\mu$ , and $\nu$ .
$m$	-7.7	Calculated from $\nu$ and $n$ .
$\lambda$	-0.67 kPa	Calculated from $\nu$ , $\mu$ , $n$ , and $m$ .
$\alpha$	0 kPa	Calculated from $\mu$ and $G_A$ .
$\beta$	0.154 kPa	Calculated from $\mu$ , $\nu$ , $n$ , and $m$ .
$\gamma$	-1.01 kPa	Calculated from $E_A$ , $\nu$ , $m$ , $\lambda$ , $\mu$ , $\alpha$ , and $\beta$ .

**Table 2.** Parameters for constitutive model of neuron.

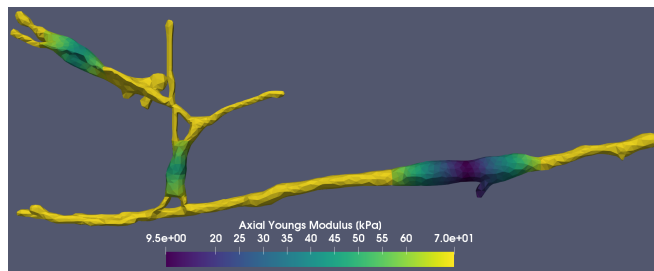
the measurements of Simon et al. (Simon et al. 2016). The six constants in Eq. (8) are all determined when the values listed in Table 2 are fully specified.

The axial stiffness of the cell body is chosen based on the relative alignment of the microtubules. The microtubules transition from an almost perfectly aligned state in the axon to a generally unaligned state within the cell body (Mofrad and Kamm

2006). Within the cell body, the microtubules become progressively less aligned with the center of the cell body having the least alignment. Consequently, the axial stiffness within the cell body decreases and is lowest at the center. The cell bodies are modeled as a transversely isotropic hyperelastic material of axial stiffness represented by a function of position within a cell, Eq. 12.

$$E_A^{cell} = \left(1 - \frac{D}{t}\right) E_A. \quad (12)$$

In Eq. (12)  $D$  is the distance from an interior point of the cell body to the closest axon, and  $t$  is a constant describing the rate of change of  $E_A^{cell}$ . The geometry shown in Fig. 2(b) indicates that the parameter  $D$  ranges from 9 to 25  $\mu\text{m}$ . We select a value of  $t = 30 \mu\text{m}$  for our calculations. The axial stiffness of the neuron structure evaluated based on these parameters is shown in Fig. 5. The variation of the axial stiffness within



**Figure 5.** Axial stiffness of neuron structure analyzed.

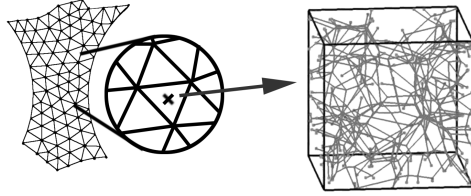
the cell body is controlled by parameter  $t$  and is assumed. To understand how this variation affects the final strain distribution in the neurons, the parameter  $t$  in Eq. (12) is adjusted. Values of  $t = 28 \mu\text{m}$ ,  $30 \mu\text{m}$ , and  $50 \mu\text{m}$  are considered (discussed in Appendix B), and the strain distribution within the neurons is found to be insensitive to the parameter  $t$ .

## 6. Implementation

### 6.1. Adaptive multi-scale simulation infrastructure

Our multi-scale model is implemented using the adaptive multi-scale simulation infrastructure (AMSI) (Tobin et al. in preperation), which is designed to support massively parallel simulations on multiple scales. AMSI accommodates the computational demands of the multi-scale simulation by combining existing single-scale analysis codes into multi-scale analyses. The macroscopic simulation code and the microscopic simulation codes are independent codes coupled through the AMSI libraries. AMSI manages the parallel execution space for each code, as well as the parallel communication responsible for the transmission of required data between them.

The macroscopic-scale domain is discretized using an unstructured mesh that is partitioned across the parallel execution space of the scale. Figure 6 depicts the relationship between the two scales with respect to the macroscopic-scale unstructured mesh, an individual numerical integration point on that mesh, and the RVE-level microscopic-scale simulation corresponding to that macroscopic-scale location.



**Figure 6.** Schematic showing the hierarchy of scales in the multi-scale simulation

AMSI manages the relationship between the domains of the macroscopic and microscopic scale problem. At each macroscopic-scale numerical integration point, the deformation gradient  $F_{ij}$  is calculated and provided to the AMSI coupling system, which uses a communication plan to provide the value to the collagen-network scale RVE simulation related to the integration point. This  $F_{ij}$  value is used by the collagen-network scale, as shown in Eq. (2), to establish the boundary conditions for the current state of the RVE problem. Once a solution has been reached for the collagen-network scale simulation, the macroscopic-scale volume-averaged Cauchy stress term  $\sigma_{ij}^{(M)}$  from Eq. (4) and stress derivative terms  $\sigma_{ij,k}^{(M)}$  are supplied to AMSI. These terms are communicated to the macroscopic scale and associated with the coupled integration point for use in assembling the macroscopic-scale linear algebraic system for the current Newton iteration.

Up- and down-scaling transformations of the coupling data currently occur in the user code after (or before) employing the communication plan. The down-scaling of the deformation gradient terms to generate the displacement on the corner of an RVE in Eq. 2 takes place at the collagen-network scale after the coupling communication. The up-scaling in Eq. 4 also takes place at the collagen-network scale, prior to communicating the stress terms to the macroscopic scale.

## 6.2. Computational Model Solved

The solution to the macroscopic boundary value problem in Eq. (5) is obtained using the Finite Element-method (FEM). The domain of the embedded neurons is discretized by a mesh consisting of 82,105 linear tetrahedral elements - 14,250 in the neurons and 67,855 in the collagen gel - giving rise to more than 985k degrees of freedom (DOF) on the macroscopic scale.

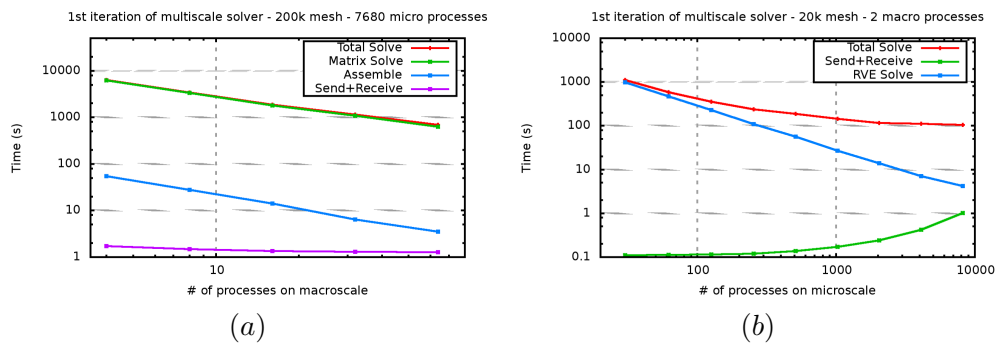
The computation demands are substantially greater for the collagen-network scale where each fiber network RVE consists of, on average, 284 fiber cross links. Three DOF exist for each fiber cross link, giving rise to 852 DOF per RVE. Since there is a single RVE for each numerical integration point of the macroscopic mesh of the collagen gel, the total DOF for the collagen-network scale is more than 57 million (852 DOF/RVE  $\times$  67,855 RVEs).

The large computational demands of the neuron-in-gel multi-scale model requires both the macroscopic and microscopic scale calculations to be performed in parallel. We employ the AMSI libraries to manage the parallel execution space for each scale. For our simulations, a total of 2048 cores were used on the AMOS BlueGene/Q main-

tained by the Center for Computational Innovations at RPI. Each node on the AMOS system has a 16-core 1.6GHz A2 processor with 16GB of DDR3 RAM, thus 128 nodes were allocated to the execution of this problem, with 128GB of memory. Of the assigned cores, 128 were devoted to the macroscale computation, while the remaining 1920 cores were used to compute the macroscale RVE simulations.

As the macroscale and macroscale codes are assigned separate partitions of the parallel execution space, the simulation never sees 100% parallel utilization due to the need of each scale to block while waiting for the other to compute. In order to achieve good performance given this constraint, we consider the computational demand of each scale and partition the simulation scales to prioritize process utilization and reduce process idle time.

The DOF count at each scale provides an initial estimate of the computational demand of the problem, however the complexity of the physics and software implementation of each scale also contributes a heuristic weight which, when applied to the DOF count, allows a more appropriate comparison of the computational demand at each scale. Particularly, this approach justifies the use of a core ratio between the macroscale and macroscale problems other than one derived simply from the DOF count, which would be approximately 1:57 macroscale cores to macroscale cores if only taking DOFs into account. Instead the ratio of 1:15 was heuristically determined to provide a better overall process utilization by minimizing core idle time.



**Figure 7.** Strong-scaling the (a) macroscale and (b) microscale parallel allocations while holding the other scale allocation constant. Measurements taken with the multi-scale code applied to a different problem.

As the allocation and processing of individual macroscale simulations is embarrassingly parallel, the allocation ratio can be scaled in favor of the macroscale to reduce overall time-to-solution (see figure 7b). This approach sacrifices macroscale process utilization when holding the macroscale allocation and problem size constant as it increases the macroscale idle time linearly since the time-to-solution of the macroscale is held constant. Increasing the allocation ratio in favor of the macroscale has less impact on the overall process utilization (see figure 7a) or time-to-solution as both of these are dominated by the work to be conducted at the macroscale.

Increasing the total parallel allocation while holding the problem size and allocation ratio constant reduces time-to-solution while retaining the same process utilization characteristics up to the point where the macroscale code begins to lose efficiency due to increasing communication overhead on a problem of constant size.

## 7. Results and Discussion

The embedded neuron model described above provides local strain information within the neuron structure that is inaccessible by current experimental procedures. Therefore, the neuron model serves as a computational microscope that can be used to probe the neurons' response to its mechanical environment under load. For this study, the collagen gel surrounding the neurons is subjected to grip strains of up to 16%, which corresponds to the threshold loading beyond which pain is reported to arise (Lee et al. 2004; Dong et al. 2012; Zhang et al. 2016; Ita et al. 2017; Zhang et al. 2017a).

The strain within the neurons is quantified by the complementary cumulative distribution function (ccdf) defined as

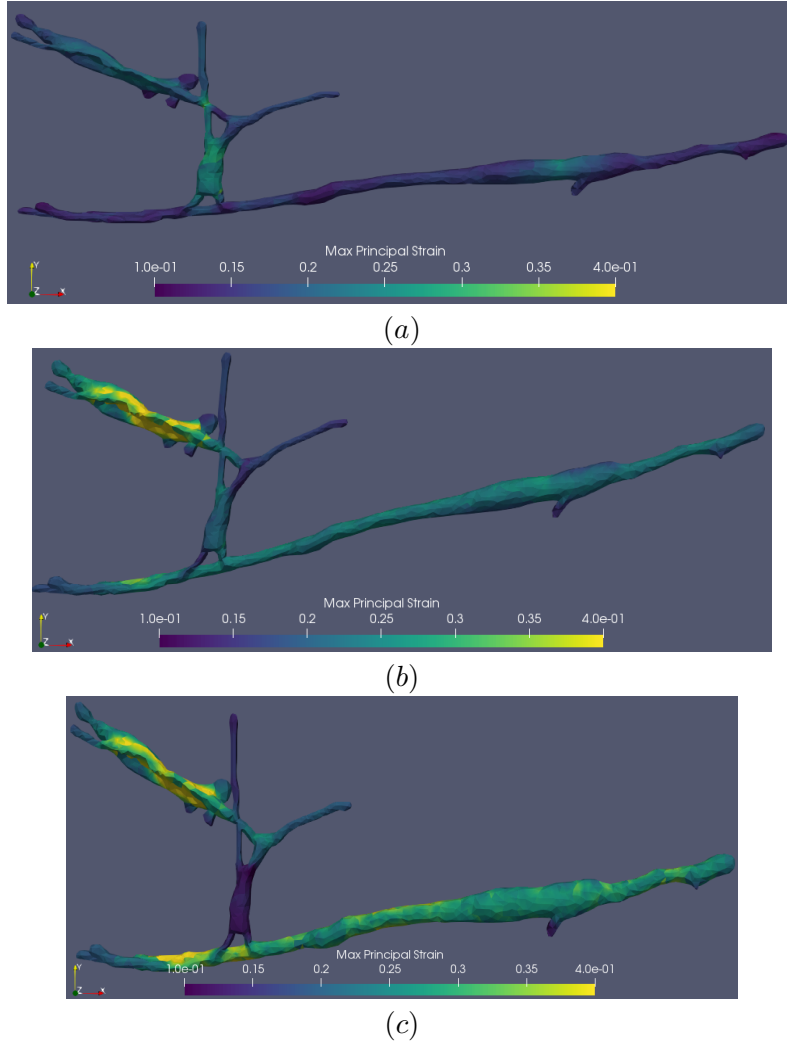
$$\text{ccdf}(X) = \int_X^{\infty} p_{\epsilon^*}(X) d\epsilon^* = \Pr[\epsilon^* \geq X], \quad (13)$$

where  $p_{\epsilon^*}(X)$  is the probability density function of  $\epsilon^*$ , and  $\epsilon^*$  is either the axial,  $\epsilon_{\text{ax}}$ , or transverse,  $\epsilon_{\text{trns}}$ , strain with respect to the local axon axis; the four regions corresponding to the different local axes in the neurons are shown schematically in Fig. 2(b). Equation 13 is the probability that an arbitrary point within a neuron experiences an axial or transverse strain *greater than or equal to*  $X$ . The value measured by Eq. (13) is approximately equal to the volume fraction of a neuron that experiences a strain greater than or equal to  $X$ . In the analysis below, the Green-Lagrange strain is used for measuring deformation.

### 7.1. Strain Distribution within Neuronal Bodies

In vivo, a patch of tissue can be loaded in various ways depending on the activity or event imposing the load. To gain an understanding of the strain in the neuronal bodies under arbitrary loading, we examined the axial and transverse strain distributions in the neurons for the three different loading conditions shown in Fig. 2(b) for  $t = 30 \mu\text{m}$ . The model is rotated relative to the far-field loading direction in-plane because the structure considered is approximately planar. Color maps of the maximum principal strain in the neurons for a grip strain of 16% (painful ligament loading) and for  $0^\circ$ ,  $45^\circ$ , and  $90^\circ$  loading are plotted in Fig. 8. For all angles of loading, large portions of the neurons experience strains that are greater than the 16% applied grip strain. This result indicates that the applied strains on the collagen gel are amplified in the neurons, which could lead to neuronal damage even when the surrounding tissue is moderately loaded.

To gain a more quantitative understanding of the strain distribution in the neurons, we focused on the normal strains along and transverse to the axon axis, and computed their ccdf for two levels of far field strain (10% and 16% corresponding to non-painful and painful ligament loading, respectively) and three loading directions (Fig. 2(b)). The resulting ccdf are shown in Fig. 9 for the entire domain corresponding to neuronal bodies and axons. **The insets show the ccdf corresponding to each region in the neurons when loaded at 16% strain.** The shapes of the strain distributions vary significantly for different applied strains and loading directions. The strong dependence of the strain distribution on loading configuration reflects the anisotropic nature of the embedded neurons, where the axial stiffness is more than one order of magnitude greater than the transverse stiffness. Relative to the stiffness of the surrounding collagen gel, the axial stiffness of the neurons is larger while the transverse stiffness of the neurons is



**Figure 8.** Color maps of maximum principal strain in neurons for grip strain of 16% for loading angles of (a)  $0^\circ$ , (b)  $45^\circ$ , and (c)  $90^\circ$ . The color bar ranges from 10% to 40% maximum principal strain.

smaller. This explains the shift of the means of the strain distributions in each neuron shown in the insets to Fig. 9 (considering that various neurons are oriented differently relative to the loading direction in each case). The mean of the distribution depends on the orientation of the respective neuron axis relative to the far field loading direction. However, the variance of the distribution of strains in each neuron is approximately the same. This shows that strain amplification caused by the intrinsic heterogeneity of the model takes place in all neurons, irrespective of their orientation.

In all cases, the strain magnitude within the neurons is larger for an applied strain of 16%, as expected; the black solid curve lies to the right (left) of the blue dashed curve for positive (negative) values of strain. Interestingly, aside from the axial strain case for  $0^\circ$  loading, the same fraction of the neuron volume experiences a strain that is greater than or equal to the applied grip strain, regardless of the strain magnitude or the loading direction; that is, the red dashed and solid lines intersect the same value on the ordinate of the ccdf. In the case of axial strain for  $0^\circ$  loading, the fraction of the neuron volume that amplifies the applied strain increases with grip strain.



The fraction of the neuron volume that experiences a value of strain that is greater than or equal to the far-field applied strain is listed in Table 3 for all cases plotted in Fig. 9. As seen in Table 3, the applied strain on the collagen gel is amplified in the

Loading Direction	axial strain		transverse strains	
	$\epsilon_{\text{grip}} = 10\%$	$\epsilon_{\text{grip}} = 16\%$	$\epsilon_{\text{grip}} = 10\%$	$\epsilon_{\text{grip}} = 16\%$
0°	19%	28%	9%	9%
45°	0%	0%	23%	23%
90°	13%	13%	67%	67%

**Table 3.** Fraction of the neuron volume that experiences a strain that is greater than or equal to the applied strain on surrounding collagen gel for different loading directions.

neurons predominantly along the axial direction for 0° loading and in the transverse direction for 45° and 90° loading. For 45° loading, less than 0.1% of the neuron volume experiences an axial strain that is greater than or equal to the applied strain.

The values listed in Table 3 are consistent with how the embedded neuron is oriented relative to the different loading directions. When loading at 0° a majority of the neuron volume (regions 1, 2, and 4 in Fig. 2(b)) is loaded predominantly in the axial direction causing a larger fraction of the neurons to be amplified in the axial direction. On the other hand, when loading at 90° a majority of the neuron volume (regions 1, 3, and 4 in Fig. 2(b)) are loaded predominantly in the transverse direction causing a larger fraction of the neurons to be amplified in the transverse direction. The amplification of the applied strain in the transverse direction is significantly greater because the neurons are much more compliant in the transverse direction relative to the axial direction. The neurons also experience a significant strain amplification in the transverse direction when loaded at 45° because the axial direction of region 1 lies perpendicular to the 45° loading direction.

It should be noted that the strain distribution can be predicted using a much simpler model in which an anisotropic body (a neuron) is embedded in a homogeneous matrix. In such case, the strain in the softer transverse direction would be larger than that in the axial direction. Such a model would predict that strain amplification cannot happen when the neuron is loaded along its axis. The results shown in the insets to Fig. 9 indicate that strain amplification occurs in all loading cases considered and hence that accounting for complex geometry is essential in this case. This justifies the development of models that capture the exact geometry of the sample and which can be used as a virtual microscope that complements lower resolution experimental observations.

Physiologically more important are the tails of the strain distributions, which represent the largest strains experienced by the neurons. The minimum strains experienced by the most-deformed 10% of the neurons (by volume fraction) are listed in Table 4 for each loading case. Consistent with above, the largest minimum strain experienced by the most-deformed 10% of the neurons occurs in the axial direction for 0° loading and in the transverse direction for 45° and 90° loading. When this minimum strain is greater than the applied grip strain, amplification arises. Loading cases in which amplification arises are marked with an asterisk in Table 4.

When the system is loaded at 90°, 10% of the neuron volume experiences a strain amplification of at least a factor of 2 in the transverse direction. When loaded at 45°, the same fraction of the neuron volume experiences an amplification of at least a factor of 1.7. When loaded at 0°, 10% of the neuron volume experiences a strain amplification

Loading Direction	Largest 10% of axial strain		Largest 10% of transverse strain	
	$\epsilon_{\text{grip}} = 10\%$	$\epsilon_{\text{grip}} = 16\%$	$\epsilon_{\text{grip}} = 10\%$	$\epsilon_{\text{grip}} = 16\%$
0°	11%*	19%*	4%	6%
45°	6%	10%	17%*	28%*
90°	5%	9%	22%*	37%*

**Table 4.** The minimum strain values experienced by the most-deformed 10% of the neurons. Asterisks indicate strain values that are greater than the applied strain, i.e., applied strain is amplified within the neurons.

of at least a factor of 1.1 in the axial direction. The smaller amplification in the axial direction is due to the larger stiffness of the neurons in the axial direction relative to the transverse direction.

In the above analysis, we used the strains along and transverse to the axons, as well as the maximum principal strain as measures of neuron deformation. However, the actual cause of neuronal injury remains unknown. There is still much work to be done in the experimental characterization of neuronal injury and identification of the mechanisms leading to pain.

## 8. Summary

We present a general modeling strategy in which (1) a realistic representation of a complex cell geometry is generated from confocal microscopy images of dorsal root ganglion neurons embedded in a collagen gel; (2) the geometric model is converted into a finite-element mesh; (3) a multi-scale model is implemented to describe the extracellular matrix (collagen gel) in the mesh; and (4) an analysis of the strain distributions within the neurons due to mechanical loading on the extracellular matrix is performed.

The large computational demands that arise from the unstructured mesh of a complex cell geometry coupled with a multi-scale model are accommodated through the AMSI library, which is used to manage the parallel execution spaces of the macroscopic and microscopic scale simulation codes and to plan and enact the parallel communication operations required to couple the two codes together into a multi-scale simulation.

For the multi-scale modeling of the extracellular matrix, we introduce a fiber RVE model that can be parameterized to match the mechanical behavior of collagen I, which surrounds the neurons; the modeling parameters for the fiber model are based on experimentally measured stress-strain and fiber-alignment results. The simulation results for the embedded neurons provide local strain information that is not accessible by current experimental techniques, thereby providing important insights about the mechanism of neuronal injury under mechanical loading. We find that the strain distribution in the neurons depends strongly on the configuration of the embedded neurons relative to the loading direction, which reflects the anisotropic mechanical properties of the neurons. More importantly, we observe that the applied strain on the surrounding environment is amplified within the neurons for all loading configurations considered. In the most severe cases, the applied strain is amplified by at least a factor of 2 in 10% of the neuron volume.

The work presented in this paper provides an extension to the capability of past modeling strategies by enabling the realistic representation of complex cell geometry into a multi-scale framework. Although several physiologically important mechanisms

for the embedded neuron system have been left out, e.g., chemical mediators, calcium signaling, and membrane viability, the results from the simplified system nonetheless provide meaningful results that can be built upon in future work.

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## Appendix A. Derivation of $P_2$ from $\alpha$ and $\delta$ of the QPLI method

Consider a distribution of orientation  $\theta$  for pixel  $i$  as

$$p_i(\theta) = q_i + r_i \cos^2(\theta - \alpha_i) \quad \text{for } \theta \in (0, \pi), \quad (\text{A1})$$

where  $\alpha_i$  is the alignment measured at pixel  $i$  by the QPLI method and  $q_i$  and  $r_i$  are constants for the  $i^{\text{th}}$  pixel. Applying the normalization

$$\int_0^\pi p_i(\theta) d\theta = 1, \quad (\text{A2})$$

a relationship between  $q_i$  and  $r_i$  is obtained:

$$\pi q_i + \frac{\pi}{2} r_i = 1. \quad (\text{A3})$$

Using the relationship in Eq. (A3), Eq. (A1) can be rewritten as

$$p_i(\theta) = q_i + \frac{2}{\pi}(1 - \pi q_i) \cos^2(\theta - \alpha_i) \quad (\text{A4})$$

The orientation tensor for each pixel can be written in terms of  $p_i(\theta)$  as

$$\mathbf{A}^i = \begin{bmatrix} \int_0^\pi p_i(\theta) \cos^2(\theta) d\theta & \int_0^\pi p_i(\theta) \sin(\theta) \cos(\theta) d\theta \\ \int_0^\pi p_i(\theta) \sin(\theta) \cos(\theta) d\theta & \int_0^\pi p_i(\theta) \sin^2(\theta) d\theta \end{bmatrix} = \frac{1 - \pi q_i}{4} \begin{bmatrix} \cos(2\alpha_i) & \sin(2\alpha_i) \\ \sin(2\alpha_i) & -\cos(2\alpha_i) \end{bmatrix} \quad (\text{A5})$$

where the eigenvalues of  $\mathbf{A}^i$  are

$$\Lambda_1 = \frac{1 + \pi q_i}{4} \quad \text{and} \quad \Lambda_2 = \frac{3 - \pi q_i}{4}. \quad (\text{A6})$$

The retardation at each pixel,  $\delta_i$ , is related to the the eigenvalues through a proportionality constant,  $C$ , by

$$\delta_i = C |\Lambda_2 - \Lambda_1| = C \left( \frac{1 - \pi q_i}{2} \right). \quad (\text{A7})$$

From Eq. (A7), the constant  $q_i$  can be written in terms of the retardation as

$$q_i = \frac{1}{\pi} \left( 1 - \frac{2\delta_i}{C} \right), \quad (\text{A8})$$

and the distribution at pixel  $i$  from Eq. (A4) can be rewritten as

$$p_i(\theta) = \frac{C - 2\delta_i}{\pi C} + \frac{4\delta_i}{\pi C} \cos^2(\theta - \alpha_i). \quad (\text{A9})$$

The distribution of orientation  $\theta$  for the entire image is simply the average over the distribution of each pixel

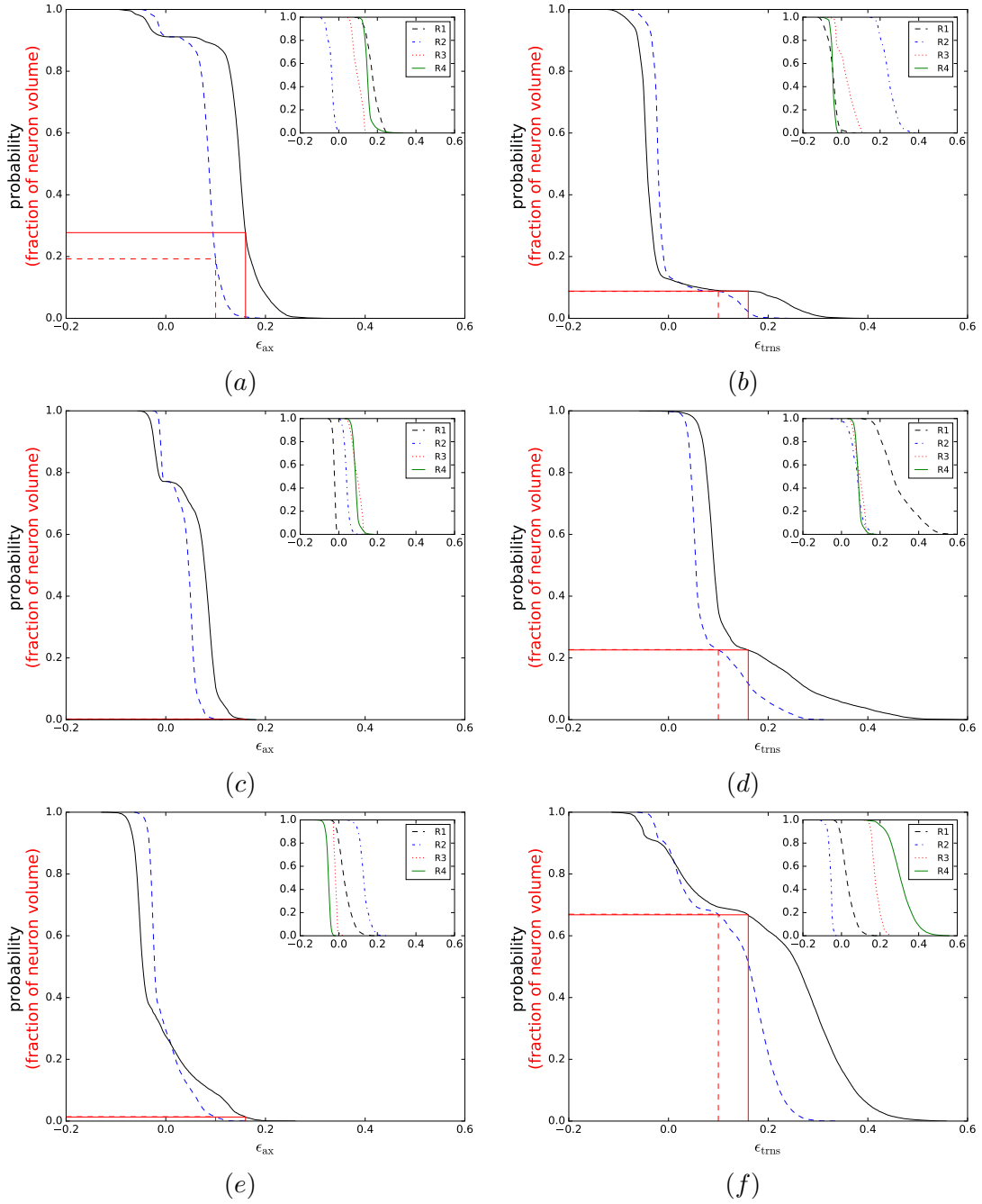
$$p(\theta) = \frac{1}{N} \sum_{i=1}^N p_i(\theta) = \frac{1}{N} \sum_{i=1}^N \left[ \frac{C - 2\delta_i}{\pi C} + \frac{4\delta_i}{\pi C} \cos^2(\theta - \alpha_i) \right], \quad (\text{A10})$$

which is the equation presented in Eq. (10).

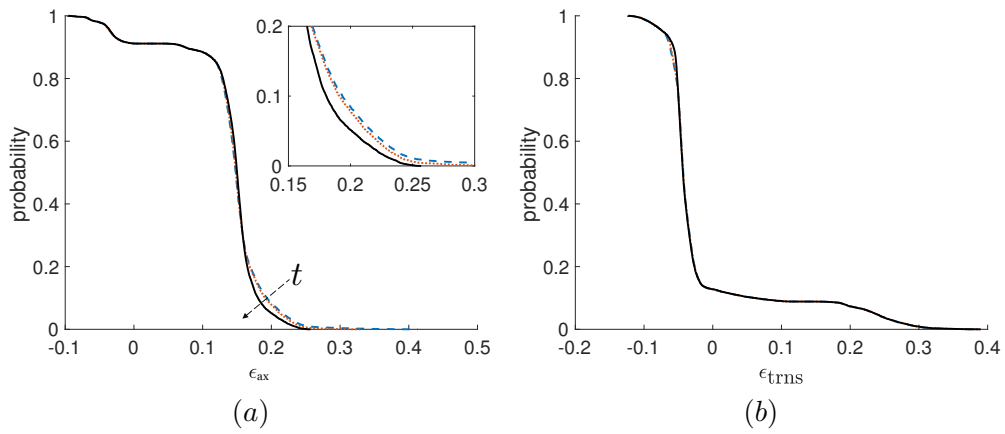
## **Appendix B. Varying Axial Stiffness ( $t$ ) in the Cell Body**

The complementary cumulative distribution function for different axial stiffnesses of the cell body (different values of  $t$ ) are plotted in Fig. B1 for the  $0^\circ$  loading case for a grip strain of 16% on the collagen gel. These results suggest that the strain distribution within the neurons is insensitive to the axial distribution of stiffness of the cell body. The negative values of strain seen in the distributions are due to lateral contraction that arises from the Poisson effect.





**Figure 9.** Complementary cumulative distribution functions for axial (left column) and transverse (right column) strains in the neurons for  $0^\circ$  (top row),  $45^\circ$  (middle row), and  $90^\circ$  (bottom row) loading. Distributions for 10% (blue dashed line) and 16% (black solid line) grip strains are plotted for all loading directions. The dashed and solid red lines indicate the approximate fraction of the neuron volume that experience a strain of greater than or equal to 10% and 16%, respectively. The insets show the cdf corresponding to each region of the neurons (indicated in Fig. 2(b)) when loaded at 16% strain.



**Figure B1.** Complimentary cumulative distribution functions for (a) axial and (b) transverse strains in the neuron due to a  $0^\circ$  load of 16% grip strain. The distributions are for axial cell-body stiffnesses corresponding to  $t=28 \mu\text{m}$  (blue dashed line),  $30 \mu\text{m}$  (red dotted line), and  $50 \mu\text{m}$  (black solid line). The dashed arrow in (a) indicates the direction of increasing  $t$  and the inset in (a) enlarges the heel of the distribution to aid visual interpretation.