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Novel methodology for characterizing regional variations in material properties of murine aortas

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KEYWORDS **DIGITAL IMAGE CORRELATION, CONSTITUTIVE RELATIONS, ANISOTROPY, AORTA, STRUCTURE-FUNCTION**

37

38 **ABSTRACT**

39 Many vascular disorders, including aortic aneurysms and dissections, are characterized by localized
40 changes in wall composition and structure. Notwithstanding the importance of histopathologic changes
41 that occur at the microstructural level, macroscopic manifestations ultimately dictate the mechanical
42 functionality and structural integrity of the aortic wall. Understanding structure–function relationships
43 locally is thus critical for gaining increased insight into conditions that render a vessel susceptible to
44 disease or failure. Given the scarcity of human data, mouse models are increasingly useful in this
45 regard. In this paper, we present a novel inverse characterization of regional, nonlinear, anisotropic
46 properties of the murine aorta. Full-field biaxial data are collected using a panoramic-digital image
47 correlation system and an inverse method, based on the principle of virtual power, is used to estimate
48 values of material parameters regionally for a microstructurally motivated constitutive relation. We
49 validate our experimental-computational approach by comparing results to those from standard biaxial
50 testing. Results for the non-diseased suprarenal abdominal aorta from apolipoprotein-E null mice
51 reveal material heterogeneities, with significant differences between dorsal and ventral as well as
52 between proximal and distal locations, which may arise in part due to differential perivascular support
53 and localized branches. Overall results were validated for both a membrane and a thick-wall model
54 that delineated medial and adventitial properties. Whereas full-field characterization can be useful in
55 the study of normal arteries, we submit that it will be particularly useful for studying complex lesions
56 such as aneurysms, which can now be pursued with confidence given the present validation.

57

58 **INTRODUCTION**

59 Aortic aneurysms and dissections are biologically and mechanically complex vascular
60 pathologies that are responsible for significant death and disability in industrialized nations. They are
61 characterized geometrically by complex fusiform dilatations and histopathologically by a non-uniform
62 fragmentation of elastic fibers, loss of smooth muscle cell functionality, and remodeling of fibrillar
63 collagen [1,2]. The ultimate fate of the aortic wall is dictated by the resulting structural integrity, for
64 dissection and rupture occur when local wall stress exceeds local wall strength. This vulnerable
65 situation can arise when the degradation of extracellular matrix outpaces deposition. Improvements
66 in medical imaging and computational methods have enabled the development of patient-specific
67 fluid-solid interaction models of aortic aneurysm and dissection biomechanics [3–5], but these
68 models are often based on assumptions of homogeneous material properties and uniform wall
69 thicknesses, both of which can render predictions of intramural stresses inaccurate.

70 Histopathological changes that occur at the microstructural level manifest at the macroscopic
71 level as altered mechanical functionality and structural integrity. Correlations between local wall
72 composition and mechanical properties can thus provide increased insight into conditions that

73 render a vessel susceptible to failure or disease. Whereas regional variations in microstructure are
74 quantified easily using standard histological and immunohistochemical methods, there is yet a
75 pressing need for methods suitable for quantifying spatial heterogeneities in material and structural
76 properties of aneurysmal and dissected lesions and to correlate these heterogeneities with the
77 underlying microstructure in order to gain increased insight into the mechanics of complex vascular
78 pathologies.

79 Toward this end, we develop and apply a novel inverse method, based on the principle of
80 virtual power, which can determine locally varying values of the constitutive parameters from full-
81 field data acquired using a biaxial panoramic-digital image correlation (p-DIC) method. By focusing
82 first on normal vessels, the associated results can be compared with those obtained via standard
83 biaxial extension-inflation testing and constitutive modeling [6]. Whereas both the p-DIC and the
84 biaxial extension-inflation techniques have been separately presented and validated in previous
85 papers [7–11], the scope of the current study is the development of a novel combination of the
86 inverse and p-DIC methods and its validation as a combined mechanical testing procedure designed
87 for local material characterization. Toward this end, we first detail the proposed combined method
88 and then present illustrative results for the suprarenal abdominal aorta from two young, non-
89 diseased, apolipoprotein-E null ($ApoE^{-/-}$) mice. Mean behaviors correspond well with those from
90 standard testing and analysis but, in addition, regional heterogeneities in material properties are
91 successfully reconstructed. We submit that this novel experimental-computational approach
92 represents another important step towards improving our ability to study complex vascular lesions as
93 it will enable one to correlate, for the first time, regional distributions of material properties with the
94 underlying microstructure.

95

96 MATERIALS AND METHODS

97 *Animal Model.* All animal protocols were approved by the Yale University Institutional Animal
98 Care and Use Committee and followed methods detailed previously [6,7]. Non-diseased control
99 samples, as opposed to dissecting aneurysm samples, were used given the goal of validating the new
100 inverse methodology. Briefly, two male $ApoE^{-/-}$ mice were euthanized at ~20 weeks of age (cf.
101 Supplemental Table 1) using an intraperitoneal injection of Beuthanasia-D and the abdominal aorta
102 was excised en bloc. The suprarenal segment (from the final pair of intercostal branches to the right
103 renal artery) was prepared for mechanical testing by removing excess perivascular tissue and ligating
104 all side branches using a single strand from braided 7-0 nylon suture.

105 *Standard Biaxial Mechanical Testing.* The excised samples (Sample A for mouse 1 and Sample
106 B for mouse 2) were cannulated with custom drawn glass pipets, secured using 6-0 silk sutures, and

107 placed within a validated computer-controlled testing system that allowed for now standard biaxial
108 inflation-extension testing [8]. Preconditioning consisted of 4 cycles of pressurization from 10 to 140
109 mmHg at the estimated in vivo length, following previous reports [6,7]. Next, samples were subjected
110 to three cyclic pressure-diameter (P - d) tests consisting of pressurization from 10 to 140 mmHg at
111 fixed axial stretches of $0.95\lambda^0$, λ^0 , and $1.05\lambda^0$, where λ^0 is the estimated in vivo axial stretch, and four
112 cyclic axial force-length (f - l) tests consisting of loading from 0 to 35 mN at fixed pressures of 10, 60,
113 100, and 140 mmHg. Note that, similar to previous reports [6,12], the estimated in vivo axial stretch,
114 λ^0 , was defined as the value that minimized variations in transducer measured axial load upon
115 pressurization. Tests were performed at room temperature in a Hanks buffered physiologic solution
116 (HBSS), which yields a fully passive mechanical behavior [9]. As previously demonstrated [13], there
117 are no discernible differences in the measured passive properties between room temperature (19-
118 21°C) and physiologic temperature (37°C).

119 **Panoramic - Digital Image Correlation System.** Following biaxial inflation-extension tests, the
120 samples were placed in a custom p-DIC system [10] to monitor full-field surface deformations at
121 multiple states of pressurization and axial stretch using a 45° concave conical mirror and known
122 calibration target (Fig. 1A,B). The samples were re-cannulated proximally and distally on a single
123 through-the-lumen blunt-ended needle composite with one fixed and one sliding end to allow both
124 pressurization and axial stretch (Fig. 1C). The specimens were air-brushed to generate a random
125 speckle pattern of black and white India ink, submerged in HBSS at room temperature, and placed
126 co-axially within the conical mirror to visualize the entire lateral surface when viewed from a single
127 vertically mounted digital camera (DALSA Falcon 4M30, cf. Fig. 1B). Eight rotationally symmetric
128 images about the central axis of the conical mirror were acquired at each quasi-statically loaded
129 configuration according to the loading protocol shown in Fig. 1D, then analyzed using custom Matlab
130 scripts to perform the cross-correlations between unwrapped images needed to compute full-field
131 surface deformations [11].

132 **Wall kinematics.** A global coordinate system was defined by an origin, located on the central
133 axis at the base of the conical mirror, and a Cartesian basis, defined by three vectors ($\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z$).
134 Vector \mathbf{e}_z was aligned along the long axis of the needle. A cylindrical coordinate system was also
135 defined by three locally orthogonal base vectors ($\mathbf{e}_r, \mathbf{e}_\theta, \mathbf{e}_z$). The reference configuration ($P(t = 0)$
136 and $\lambda_z(t = 0)$) was set at pressure $P = 80$ mmHg and axial stretch $\lambda_z = \lambda^0$. For any material point
137 represented by its position vector \mathbf{X} , the Cartesian coordinates in the reference configuration were
138 denoted both by the triplet (X, Y, Z) and the cylindrical coordinates (R, θ, Z) . Hence, in a deformed
139 configuration, the coordinates for the current position $\mathbf{x}(t)$ of the same material point were
140 $(x(t), y(t), z(t))$ and $(r(t), \theta(t), z(t))$; here t denotes subsequent configurations achieved quasi-

141 statically, not a dynamic process.

142 The outer wall surface of each artery S^o was meshed in the reference configuration with
143 >5,000 nodes for full-field deformation measurements, but parametrically re-meshed with 400 nodes
144 for parameter estimation within small local patches. The $Z \in [0, L]$ coordinate was divided into 20
145 segments (where L was the reference length of the sample) and the $\theta \in [0, 2\pi]$ coordinate was
146 divided in 20 angular sectors. For each node n defined in S^o , the Cartesian (X_n^o, Y_n^o, Z_n^o) and/or
147 cylindrical $(R_n^o, \theta_n^o, Z_n^o)$ coordinates were reconstructed using the calibration parameters of the p-DIC
148 system. Each material point in S^o was then tracked in all deformed configurations by applying a
149 custom serial correlation algorithm between neighboring configurations (i.e., all pressures $P(t)$ and
150 axial stretches $\lambda_z(t)$). For instance, while at λ^o , the result of the correlation between images at 60
151 and 70 mmHg was used to initiate the correlation between images at 70 and 80 mmHg, respectively.
152 In other words, the resulting correlated mesh was stored and taken as a reference to be correlated to
153 a neighboring deformed configuration (i.e., 80 to 90 mmHg, 60 to 50 mmHg, and so forth) until all
154 deformed configurations had been processed. In this way, data were collected at each node n of the
155 reconstructed p-DIC point cloud: $(x_n^o(t), y_n^o(t), z_n^o(t))$ and/or $(r_n^o(t), \theta_n^o(t), z_n^o(t))$ for every biaxially
156 loaded configuration (pressure $P(t)$ ranging from 10 to 140 mmHg in increments of 10 mmHg at axial
157 stretches of $\lambda_z(t) = 0.95\lambda^o, \lambda^o$ and $1.05\lambda^o$). Note, too, that the biaxial load at each configuration was
158 held for ~2 minutes to allow image acquisition, which is in contrast to the continuous cyclic loading of
159 standard biaxial tests.

160 Unit vectors normal to the outer surface, denoted respectively by $\mathbf{n}_n(t)$ and \mathbf{N}_n for each
161 node n in the current and reference configurations, were deduced from the geometrical
162 reconstruction of S^o based on p-DIC data. A local orthonormal basis $(\mathbf{G}_n^1, \mathbf{G}_n^2, \mathbf{N}_n)$ was defined in the
163 reference configuration, where \mathbf{G}_n^1 and \mathbf{G}_n^2 were aligned with directions of maximum and minimum
164 principal curvatures of S^o at node n , respectively. We let $(\mathbf{g}_n^1(t), \mathbf{g}_n^2(t), \mathbf{n}_n(t))$ denote the local
165 orthonormal basis, $\kappa_n^1(t)$ denote the maximum curvature, and $\kappa_n^2(t)$ denote the minimum curvature,
166 for every node n defined in each deformed configuration at time t .

167 Nodal positions across the wall $(x_n^w(t, \xi), y_n^w(t, \xi), z_n^w(t, \xi))$ were defined as

$$(x_n^o(t) - x_n^w(t, \xi))\mathbf{e}_x + (y_n^o(t) - y_n^w(t, \xi))\mathbf{e}_y + (z_n^o(t) - z_n^w(t, \xi))\mathbf{e}_z = (1 - \xi)h(t)\mathbf{n}_n(t) \quad (1)$$

168 for every node n in each configuration at each time t , where $\xi \in [0, 1]$ indicated the through-the-
169 thickness position between the inner ($\xi = 0$) and outer ($\xi = 1$) radii. Assuming a constant wall
170 volume at each loaded configuration (tissue incompressibility), a uniform thickness $h(t)$ was
171 deduced from the average thickness measured in the unloaded configuration, denoted H . The
172 average thickness in the unloaded configuration was measured using an optical coherence

173 tomography (OCT) system.

174 The deformation gradient tensor $\mathbf{F}_n^W(t, \xi)$ at the surface was written as follows (in 2D
175 summation notation)

$$\mathbf{F}_n^W(t, \xi) = F_{ij,n}^W(t, \xi) \mathbf{g}_n^i(t) \otimes \mathbf{G}_n^j + \frac{1}{\det(\mathbf{F}_n^W(t, \xi))} \mathbf{n}_n(t) \otimes \mathbf{N}_n, \quad (2)$$

176 where, at every node n and through-the-thickness position ξ in a deformed configuration at time t ,
177 the components of the deformation gradient tensor $F_{11,n}^W(t, \xi)$, $F_{22,n}^W(t, \xi)$, $F_{12,n}^W(t, \xi)$, and $F_{21,n}^W(t, \xi)$
178 were deduced from the set of current coordinates $(x_n^W(t, \xi), y_n^W(t, \xi), z_n^W(t, \xi))$ and reference
179 coordinates $(X_n^W(t, \xi), Y_n^W(t, \xi), Z_n^W(t, \xi))$ using a finite difference algorithm.

180 *Constitutive Relations.* Similar to prior work [14], the aortic wall was modeled as a
181 hyperelastic material with a strain energy function, defined per unit mass, of the form

$$W_n(t, \xi) = \phi^e(\xi) W_n^e(t, \xi) + \phi^m(\xi) W_n^m(t, \xi) + \phi^c(\xi) W_n^c(t, \xi) + \phi^a(\xi) W_n^a(t, \xi), \quad (3)$$

182 where $\phi^e(\xi)$ is the mass fraction of elastin, $\phi^m(\xi)$ is the mass fraction of circumferential collagen
183 fibers and smooth muscle cells, $\phi^c(\xi)$ is the mass fraction of diagonal collagen fibers, and $\phi^a(\xi)$ is
184 the mass fraction of axial collagen fibers. In particular, following previous histological reports of wall
185 composition in the murine suprarenal abdominal aorta [15], we assigned the layer-specific mass
186 fractions for each constituent to be

$$187 \quad \phi^e(\xi) = 0.49, \quad \phi^m(\xi) = 0.49, \quad \phi^c(\xi) = 0.01, \quad \phi^a(\xi) = 0.01, \quad \text{for } 0 \leq \xi \leq \frac{h_{media}(t)}{h(t)} \text{ (media)}$$

$$188 \quad \phi^e(\xi) = 0.04, \quad \phi^m(\xi) = 0.04, \quad \phi^c(\xi) = 0.80, \quad \phi^a(\xi) = 0.12, \quad \text{for } \frac{h_{media}(t)}{h(t)} \leq \xi \leq 1 \text{ (adventitia)}$$

189 where $h_{media}(t)/h(t) = 0.69$ in the unloaded reference configuration. The constitutive relations
190 used to describe the stored energy contribution of each constituent were (cf. [14])

$$W_n^e(t, \xi) = \frac{c_n^e}{2} [\text{tr}(\mathbf{C}_n^e(t, \xi)) - 3] \quad (4)$$

$$W_n^m(t, \xi) = \frac{c_n^m}{4k_n^m} \left[e^{k_n^m |[\lambda_n^m(t, \xi)]^2 - 1|_+^2} - 1 \right] + \alpha_n^m \left[e^{k_n^m |[\lambda_n^m(t, \xi)]^2 - 1|_-^2} - 1 \right] \quad (5)$$

$$W_n^c(t, \xi) = \frac{c_n^c}{2k_n^c} \left[\sum_{i=1}^2 \left[e^{k_n^c |[\lambda_n^{ci}(t, \xi)]^2 - 1|_+^2} - 1 \right] + \alpha_n^c \left[e^{k_n^c |[\lambda_n^{ci}(t, \xi)]^2 - 1|_-^2} - 1 \right] \right] \quad (6)$$

$$W_n^a(t, \xi) = \frac{c_n^a}{4k_n^a} \left[e^{k_n^a |[\lambda_n^a(t, \xi)]^2 - 1|_+^2} - 1 \right] + \alpha_n^a \left[e^{k_n^a |[\lambda_n^a(t, \xi)]^2 - 1|_-^2} - 1 \right] \quad (7)$$

191 where $c_n^e, c_n^m, c_n^c, c_n^a, k_n^m, k_n^c, k_n^a$ are material parameters and $\alpha_n^m, \alpha_n^c, \alpha_n^a$ are ratios that account for
192 the differential contribution of fibers in compression and tension. Thus, several additional material

193 parameters were defined as $c_n^{j,c} = \alpha_n^j c_n^j$ for $j = m, c, a$, with superscript c denoting compression.
 194 Here, the notation $|\bullet|_+$ indicates the contribution of fibers in tension, whereas $|\bullet|_-$ indicates the
 195 contribution of (laterally supported) fibers in compression.

196 Consistent with the concept that the arterial wall can be modeled as a constrained mixture
 197 consisting of multiple constituents that have different natural configurations and yet the same
 198 motions [14,16], the right Cauchy-Green tensors and associated fiber stretches for each constituent
 199 at node n assumed the following forms. The right Cauchy-Green tensor for elastin is

$$\mathbf{C}_n^e(t, \xi) = (\mathbf{F}_n^w(t, \xi) \mathbf{G}_n^e)^T \mathbf{F}_n^w(t, \xi) \mathbf{G}_n^e, \quad (8)$$

200 where \mathbf{G}_n^e is the unique deposition stretch tensor for elastin, namely

$$\mathbf{G}_n^e = G_n^{e1} \mathbf{G}_n^1 \otimes \mathbf{G}_n^1 + G_n^{e2} \mathbf{G}_n^2 \otimes \mathbf{G}_n^2 + \frac{1}{G_n^{e1} G_n^{e2}} \mathbf{N}_n \otimes \mathbf{N}_n. \quad (9)$$

201 G_n^{e1} is the deposition stretch of elastin in the circumferential direction and G_n^{e2} is the deposition
 202 stretch of elastin in the axial direction. Similarly, the stretch of the smooth muscle cells and
 203 associated circumferentially oriented collagen fibers $\lambda_n^m(t, \xi)$ is defined as

$$\lambda_n^m(t, \xi) = G_n^m \sqrt{\mathbf{C}_n^w(t, \xi) : (\mathbf{G}_n^1 \otimes \mathbf{G}_n^1)}, \quad (10)$$

204 where G_n^m is the deposition stretch of the smooth muscle cells / collagen fibers and

$$\mathbf{C}_n^w(t, \xi) = (\mathbf{F}_n^w(t, \xi))^T \mathbf{F}_n^w(t, \xi). \quad (11)$$

205 $\lambda_n^{ci}(t, \xi)$ is the stretch for the two symmetric diagonal collagen fiber families ($i = 1, 2$) defined as

$$\lambda_n^{ci}(t, \xi) = G_n^c \sqrt{\mathbf{C}_n^w(t, \xi) : (\mathbf{A}_n^i \otimes \mathbf{A}_n^i)}, \quad (12)$$

206 where G_n^c is the deposition stretch of each diagonal collagen fiber family and \mathbf{A}_n^i is the fiber direction
 207 in the reference configuration which was defined as

$$\mathbf{A}_n^i = \cos(\beta_n^c) \mathbf{G}_n^1 - (-1)^i \sin(\beta_n^c) \mathbf{G}_n^2, \quad (13)$$

208 where $\mp \beta_n^c$ represents an average angle towards the axial direction, with circumferential fibers at
 209 $\beta_n^c=0^\circ$ and axial fibers at $\beta_n^c=90^\circ$). Finally, $\lambda_n^a(t, \xi)$ is the average stretch of axial collagen fibers,
 210 namely

$$\lambda_n^a(t) = G_n^a \sqrt{\mathbf{C}_n^w(t) : (\mathbf{G}_n^2 \otimes \mathbf{G}_n^2)}, \quad (14)$$

211 where G_n^a is the deposition stretch of the axially-oriented collagen fibers.

212 *Computation of Intramural Stress.* The Cauchy stress tensor at every node n and through-the-
 213 thickness position ξ in a deformed configuration at time t (pressure $P(t)$ ranging from 10 to 140
 214 mmHg at fixed axial stretches of $\lambda_z(t)=0.95\lambda^0, \lambda^0$, and $1.05\lambda^0$) was generalized as follows

$$\boldsymbol{\sigma}_n^w(t, \xi) = -p_n^w(t, \xi)\mathbf{I} + \frac{2}{\det(\mathbf{F}_n^w(t, \xi))} \mathbf{F}_n^w(t, \xi) \frac{\partial W_n(t, \xi)}{\partial \mathbf{C}_n^w(t)} \mathbf{F}_n^w(t, \xi)^T. \quad (15)$$

215 Using Eqs. 2, 3, and 11, the expression for the Cauchy stress could be re-written such that

$$\begin{aligned} \boldsymbol{\sigma}_n^w(t, \xi) = & -p_n^w(t, \xi)\mathbf{I} + \phi^e(\xi)c_n^e \mathbf{B}_n^e(t, \xi) + \phi^m(\xi)c_n^m \Psi_n^m(t, \xi)(G_n^m)^2 \mathbf{g}_n^1(t) \otimes \mathbf{g}_n^1(t) + \\ & + \phi^a(\xi)c_n^a \Psi_n^a(t, \xi)(G_n^a)^2 \mathbf{g}_n^2(t) \otimes \mathbf{g}_n^2(t) + \sum_{i=1}^2 \phi^c(\xi)c_n^c \Psi_n^{ci}(t, \xi)(G_n^c)^2 \mathbf{a}_n^i(t, \xi) \otimes \mathbf{a}_n^i(t, \xi), \end{aligned} \quad (16)$$

216 where the left Cauchy-Green tensor for elastin is written as

$$\mathbf{B}_n^e(t, \xi) = \mathbf{F}_n^w(t, \xi) \mathbf{G}_n^e(\mathbf{F}_n^w(t, \xi) \mathbf{G}_n^e)^T, \quad (17)$$

217 and the Ψ_n terms for each constituent in the constrained mixture are

$$\Psi_n^m(t, \xi) = |[\lambda_n^m(t, \xi)]^2 - 1|_+ e^{k_n^m |[\lambda_n^m(t, \xi)]^2 - 1|_+} + \alpha_n^m |[\lambda_n^m(t, \xi)]^2 - 1|_- e^{k_n^m |[\lambda_n^m(t, \xi)]^2 - 1|_-}, \quad (18)$$

$$\Psi_n^a(t, \xi) = |[\lambda_n^a(t, \xi)]^2 - 1|_+ e^{k_n^a |[\lambda_n^a(t, \xi)]^2 - 1|_+} + \alpha_n^a |[\lambda_n^a(t, \xi)]^2 - 1|_- e^{k_n^a |[\lambda_n^a(t, \xi)]^2 - 1|_-}, \quad (19)$$

$$\Psi_n^{ci}(t, \xi) = |[\lambda_n^{ci}(t, \xi)]^2 - 1|_+ e^{k_n^c |[\lambda_n^{ci}(t, \xi)]^2 - 1|_+} + \alpha_n^c |[\lambda_n^{ci}(t, \xi)]^2 - 1|_- e^{k_n^c |[\lambda_n^{ci}(t, \xi)]^2 - 1|_-}, \quad (20)$$

218 whereas the collagen fiber directions in the current configuration \mathbf{a}_n^i (for $i = 1, 2$) are

$$\mathbf{a}_n^i(t, \xi) = \mathbf{F}_n^w(t, \xi) \mathbf{A}_n^i. \quad (21)$$

219 Note that $p_n^w(t, \xi)$ is a scalar function that enforces the kinematic constraint of no local changes of
220 volume.

221 In summary, the list of the 16 unknown material parameters to be identified was

- 222 - 7 elastic coefficients in tension/compression: $c_n^e, c_n^m, c_n^c, c_n^a, c_n^{m,c}, c_n^{c,c}, c_n^{a,c}$
- 223 - 3 exponential coefficients: k_n^m, k_n^c, k_n^a
- 224 - 5 deposition stretch parameters: $G_n^{e1}, G_n^{e2}, G_n^m, G_n^c, G_n^a$
- 225 - the average angle of diagonal fibers: β_n^c

226 **Fortunately, the values of many of these parameters are well bounded (e.g., tension/compression**
227 **ratios, deposition stretches, and fiber angles), which favors the estimation process.** Finally, note that
228 for all tensor components, fiber stretches, and material parameters, the subscript n indicates that
229 they could take a different value at each node n due to the possible regional variations of material
230 properties.

231 *The inverse method.* Our objective was to identify values of model parameters separately for
232 each node n (i.e., localized region). For this we employed an inverse method where we first defined a
233 cost function J involving the computed stress (Eq. 16) as well as the experimentally measured and

234 theoretically predicted pressures ($P^{exp}(t)$ and $P^{th}(t)$) and axial loads ($f^{exp}(t)$ and $f^{th}(t)$). The
 235 parameters to be identified were continuously updated until we found the minimum of the cost
 236 function J .

237 As the suprarenal abdominal aorta does not have a perfectly cylindrical shape, equations of
 238 thick-walled cylinders relating the theoretically predicted pressure $P^{th}(t)$ and the computed stresses
 239 could not be used directly. A more general equation was obtained using the virtual fields method
 240 [17–19], namely

$$P^{th}(t) = h(t) \int_0^1 \frac{\sigma_{11,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{1/\kappa_n^1(t) - (1 - \xi)h(t)} + \frac{\sigma_{22,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{1/\kappa_n^2(t) - (1 - \xi)h(t)} d\xi, \quad (22)$$

241 where,

$$\sigma_{11,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{g}_n^1(t) \otimes \mathbf{g}_n^1(t)), \quad (23)$$

$$\sigma_{22,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{g}_n^2(t) \otimes \mathbf{g}_n^2(t)), \quad (24)$$

$$\sigma_{33,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{n}_n(t) \otimes \mathbf{n}_n(t)). \quad (25)$$

242 Details of the derivation of Eq. 22 are given in the Appendix, Proof 1. It is an extension of the
 243 traditional equations for thick-walled cylinders, where both the local circumferential and axial
 244 curvatures of the artery are accounted for to ensure equilibrium. This difference is particularly
 245 important for mouse aortas as they may be curved in their traction-free configuration and may show
 246 axial bending effects during inflation-extension testing.

247 A second equation involving the axial load was necessary to close the system. Again using the
 248 virtual fields method (Appendix, Proof 2), the theoretically predicted axial load $f^{th}(t)$ could be
 249 related to the computed stresses using the following equation.

$$f^{th}(t) = \pi h(t) \int_0^1 [2\sigma_{zz,n}^w(t, \xi) - \sigma_{xx,n}^w(t, \xi) - \sigma_{yy,n}^w(t, \xi)] [r^o(t) - (1 - \xi)h(t)] d\xi \quad (26)$$

250 where $r^o(t)$ was the outer radius and,

$$\sigma_{xx,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{e}_x \otimes \mathbf{e}_x), \quad (27)$$

$$\sigma_{yy,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{e}_y \otimes \mathbf{e}_y), \quad (28)$$

$$\sigma_{zz,n}^w(t, \xi) = \boldsymbol{\sigma}_n^w(t, \xi) : (\mathbf{e}_z \otimes \mathbf{e}_z), \quad (29)$$

251 Finally, using Eqs. 22 and 26, we defined the following cost function at each node n :

$$J_n = \sum_{k=1}^K \left(\frac{P^{exp}(t_k) - P^{th}(t_k)}{\bar{P}^{exp}(t_k)} \right)^2 + \left(\frac{f^{exp}(t_k) - f^{th}(t_k)}{\bar{f}^{exp}(t_k)} \right)^2 \quad (30)$$

252 where K is the total number of experimentally measured configurations k , and the overbar notation
 253 denotes an average over all data points (e.g., $\bar{P}^{exp}(t_k) = \sum_{k=1}^K P^{exp}(t_k)/K$). It is important to note
 254 that, for the p-DIC data sets, pressure was measured directly in the device as it was varied
 255 incrementally in steps of 10 mmHg at each of 3 different axial stretches. In contrast, the associated
 256 axial force, for each prescribed pressure and axial stretch, was assumed to be the same as that
 257 measured in the standard biaxial test, which is why the same axial stretches were used. Hence, the
 258 standard biaxial tests not only provided an important comparative approach for parameter
 259 estimation, they also provided axial force data for the p-DIC data sets.

260 *Thin wall assumption.* Following many prior reports on the mechanical properties of murine
 261 aortas (cf. [7]), as a first approximation the wall may be modeled mechanically as a membrane (i.e.,
 262 transmurally homogenized) under physiologic loads. Such an approach is particularly useful for fluid-
 263 solid interaction implementations (cf. [20]) where it is the structural, not material, stiffness that is of
 264 most importance. Toward this end, one can replace $\mathbf{F}_n^w(t, \xi)$ by its average over the thickness and
 265 deduce $\mathbf{F}_n(t)$ using a simpler kinematic description. Values of stress $\sigma_n^w(t, \xi)$ in Eqs. 22 and 26 then
 266 become $\sigma_n(t)$ (with no thickness dependence). In this case, the expressions for the theoretically
 267 predicted pressure and axial load can be reduced to

$$p^{th,*}(t) = h(t) \left(\frac{(\sigma_{11,n}(t) - \sigma_{33,n}(t))}{1/\kappa_n^1(t) - h(t)/2} + \frac{(\sigma_{22,n}(t) - \sigma_{33,n}(t))}{1/\kappa_n^2(t) - h(t)/2} \right), \quad (31)$$

$$f^{th,*}(t) = \pi h(t) [r^o(t) - h(t)/2] \left(2\sigma_{zz,n}(t) - \sigma_{xx,n}(t) - \sigma_{yy,n}(t) \right). \quad (32)$$

268 Thus, minimization of a modified cost function required,

$$J_n^* = \sum_{k=1}^K \left(\frac{p^{exp}(t_k) - p^{th,*}(t_k)}{\bar{P}^{exp}(t_k)} \right)^2 + \left(\frac{f^{exp}(t_k) - f^{th,*}(t_k)}{\bar{f}^{exp}(t_k)} \right)^2. \quad (33)$$

269

270 *Resolution of the inverse problem.* The requisite minimization and material parameter
 271 identification is achieved in two steps, namely:

272 Step 1. minimize J_n (or J_n^*) with respect to the material parameters
 273 $(c_n^e, c_n^m, c_n^c, c_n^a, c_n^{m,c}, c_n^{c,c}, c_n^{a,c})$ using a non-negative linear least squares algorithm.

274 Step 2. minimize J_n (or J_n^*) with respect to the remaining parameters using a bounded genetic
 275 algorithm. The bounds that were used for the minimization procedure are consistent with
 276 [14] and are reported in Table 1.

277 The stopping criteria included both a time limit (30 s for each node n) and a tolerance (10^{-6}) for the
 278 improvement of the cost function from one iteration to the next. After the resolution, a coefficient of
 279 determination R_n^2 is computed using Eq. 22. In the case of the thin-wall assumption, R_n^{2*} is computed
 280 using Eq. 31 and defined as

$$R_n^{2*} = 1 - \frac{\sum_{k=1}^K (P^{th,*}(t_k) - P^{exp}(t_k))^2}{\sum_{k=1}^K (\bar{P}^{exp}(t_k) - P^{exp}(t_k))^2} \quad (35)$$

281 Table 1. Bounds used for the material parameter identification procedure.

Parameter	Lower bound	Upper bound
G_n^c, G_n^a, G_n^m	1.01	1.15
k_n^m, k_n^c, k_n^a	0.001	10
G_n^{e1}	2	2.4
G_n^{e2}	1.6	2
β_n^c	25°	70°

282
 283 *Suprarenal Branch Locations.* Because all suprarenal branches were ligated to enable
 284 pressurization during testing, several major branch locations could be identified on the reconstructed
 285 vessel surface as large deviations in local radius. Potential regions of influence around these branch
 286 locations were extracted using a modified branch splitting procedure in the open-source Vascular
 287 Modeling Toolkit (VMTK, www.vmtk.org). Briefly, seed points for centerline computations
 288 (*vmtkcenterlines*) were manually placed near the maximum local radius of each branch location.
 289 Branching centerline paths to each manually placed target were computed using a maximum
 290 inscribed sphere radius algorithm [21]. Additional VMTK subroutines were then used
 291 (*vmtkbranchextractor*, *vmtkbranchclipper*) to extract a region about each branch based on the
 292 locations of centerline bifurcations. Finally, the boundary of each clipped surface was extracted,
 293 converted into a cylindrical coordinate system, and overlaid on 2D representations of the full-field
 294 data, which facilitated regional comparisons. For example, Fig. 2 shows three to four potential
 295 regions of branch influence (dashed lines) located near (1) smaller suprarenal branches, (2) the celiac
 296 artery, (3) the superior mesenteric artery, and (4) the right renal artery. Following identification of
 297 several major branch locations, each reconstructed surface was rotated such that the average
 298 position of largest regions of branch influence (1 and 2) were aligned with an angle of 0° in cylindrical
 299 coordinates (positive x-axis).

300 **Statistical Analysis.** Notwithstanding the utility of computing quantities of interest in many

301 different regions, 400 in our case, for purposes of illustration and statistical ease, distributions of
302 identified material parameters, stored energy, and linearized stiffness were also divided into four
303 larger regions for comparison: ventral-top, dorsal-top, ventral-bottom, and dorsal-bottom, with $n =$
304 100 observations per region. Regional differences were assessed both within and between Samples
305 A and B. Namely, values on the dorsal and ventral halves at the same top or bottom position and for
306 the top and bottom halves on the same dorsal or ventral side within the same sample were
307 compared using a one-way ANOVA followed by a post-hoc Bonferroni correction. Values from the
308 same region for different samples were compared using a standard Student's t-test. For all
309 comparisons, a value of $P < 0.05$ was considered significant. Supplemental Table 2 summarizes all
310 identified values by region, with values expressed as mean \pm SEM and statistical significance
311 indicated when appropriate.

312

313 RESULTS

314 Morphometric information for the two mice and their respective aortic samples is given in
315 Supplemental Table 1. Figure 2 shows regional distributions (at 400 possible locations) of the
316 coefficients of determination for the inverse estimation for both samples based on the thin-walled
317 assumption. Associated distributions of representative best-fit parameters (c_n^e , c_n^m , c_n^c) and β_n^c) are
318 shown in Supplemental Figures 1 and 2, with mean values for the four larger regions (ventral-top,
319 etc.) listed in Supplemental Table 2. In particular, note that Figure 2 and Supplemental Figures 1 and
320 2 show values of either R_n^{2*} or the identified model parameters on both a reconstructed 3D surface
321 of the aorta in its reference configuration of 80 mmHg at λ^0 (left) and a 2D representation in a
322 parameterized (θ, z) space (right). All distributions suggest regional heterogeneities, albeit to varying
323 degrees. As revealed further by Figure 2, however, not all locations yielded reliable inverse
324 estimations; regions with $R_n^{2*} < 0.90$ are indicated with (transparent) white patches. Specifically, for
325 Sample A, only 89% of all patches had a coefficient of determination above the threshold,
326 with more than 72% of accepted patches having a value larger than 0.95. In contrast, results for
327 Sample B revealed a larger proportion of patches above threshold with 95% of all patches accepted
328 and 83% of accepted patches having $R_n^{2*} < 0.95$; in this case, discarded patches were often close to
329 the cannulation ligatures at the top and bottom of the sample, which may be a result of "end
330 effects".

331 There are two primary regions on the surface of Sample A (centered at $z = 4$ mm and $\theta =$
332 $\pm 90^\circ$) that can be identified with low coefficients of determination (Fig. 2A). These areas of low R^{2*}
333 tended to localize on the lateral sides of the regions of branch influence, in particular branch

334 locations 1 and 2. Indeed, it appeared that areas of low mean curvature (Fig. 2, solid lines) tended to
 335 co-localize with regions of low R^{2*} , suggesting that the local curvature of the reconstructed sample
 336 has a significant impact on the ability of the thin-walled model to fit data, as evidenced by the explicit
 337 dependence of $P^{th,*}(t)$ on the principal curvatures κ_n^1 and κ_n^2 (cf. Eq. 31). Although regions of low
 338 mean curvature may arise as a local effect of branch ligation, the ability of the p-DIC analysis to
 339 capture modes of bending upon pressurization at a fixed axial stretch may also contribute to regions
 340 of low R^{2*} .

341 Gross regional variations in material properties were analyzed by comparing distributions
 342 (which excluded non-identified patches) for the four non-overlapping regions: ventral-top, dorsal-
 343 top, ventral-bottom, and dorsal-bottom. Following alignment of major branch locations with the 0°
 344 circumferential coordinate, a straightforward definition of the ventral and dorsal halves of the
 345 sample was given by the ranges $\theta_v \in \left[-\frac{\pi}{2}, \frac{\pi}{2}\right]$ and $\theta_d \in \left[\frac{\pi}{2}, \frac{3\pi}{2}\right]$, respectively; top (proximal) and
 346 bottom (distal) halves were defined as all nodes above and below $Z = L/2$. Illustrative results for
 347 several identified parameters from both samples are shown in Fig. 3 using a histogram
 348 representation: black bars for Sample A and white bars for Sample B, with overlapping values
 349 indicated by grey shading. Note that patches containing a branch ostium are included in the
 350 histogram representation but we have verified that this does not adversely affect the results and
 351 statistical comparison between regions.

352 Significant differences in identified parameter values were found between regions on each
 353 sample. Specifically, for Sample A the elastin parameter c_n^e was significantly lower on the dorsal side
 354 independent of axial position (proximal to distal), whereas for Sample B the lowest values were
 355 found in the ventral-bottom quadrant. The circumferential collagen parameter c_n^m and the diagonal
 356 collagen parameter c_n^c were significantly higher on the bottom half of each sample, independent of
 357 the dorsal-ventral sides; the dorsal-top quadrant showed opposite trends in c_n^c between samples.
 358 The identified spatial distribution of the axial collagen parameter c_n^a was opposite across samples.
 359 Namely, c_n^a was highest on the bottom half of Sample A and on the top half of Sample B. Finally, for
 360 both samples, the fiber angle β_n^c was highest in the ventral-bottom quadrant. Despite several similar
 361 trends in significance, all identified elastic coefficients were found to be significantly larger in
 362 magnitude on Sample B as compared to Sample A, highlighting the utility of the inverse method in
 363 identifying distributions of sample specific properties. Although there are statistically significant
 364 differences in material parameters by region, the dispersions are wider than the average difference
 365 between the means; this is especially the case for c_n^m , c_n^c , and c_n^a , which may have been induced by a
 366 smaller sensitivity of the cost function to these parameters or the existence of inter-correlations
 367 between parameters. Indeed, the number of parameters for the material model is relatively large (16

368 total) and we note that full-field information was collected only on the outer (adventitial) surface.

369 Although individual material parameters are important, it is their collective contribution to
370 modeling the material properties that is most important. Among the properties of most importance
371 are the stored energy density W_n and the circumferential and axial material stiffness [6,22]. Regional
372 distributions (at up to 400 locations) of stored energy density were computed for two different
373 loaded configurations for both samples (Fig. 4A-D): pressures of $P(t)=80$ mmHg (left) and 140 mmHg
374 (right), at individual values of the in-vivo axial stretch $\lambda_z(t)=\lambda^0$. It is seen that, in contrast to
375 distributions of individual material parameters, the regional distributions in strain energy density are
376 relatively smooth. In addition, the dorsal halves of the samples store significantly less energy than
377 the ventral half upon pressurization to 140 mmHg (Sample A: 107.7 ± 2.2 kPa vs. 116.05 ± 2.2 kPa,
378 Sample B: 99.3 ± 1.3 kPa vs. 122.3 ± 2.3 kPa; $P < 0.05$). The removal of perivascular support needed to
379 enable in-vitro mechanical testing, in particular the dorsal support of the spine, likely contributed to
380 the measured differences in dorsal versus ventral energy storage capability under the action of a
381 uniform distending pressure. Similar to c_n^e , the stored energy also tended to be higher in the central
382 region (i.e., $z = 1.5 - 5.5$ mm), independent of pressure. That the distribution of W_n corresponds to
383 the distribution of c_n^e is consistent with the elastic fibers being the main contributor to energy
384 storage, the primary function of large arteries such as the aorta.

385 Figure 5 shows regional distributions (at up to 400 locations) of the circumferential (C_{1111} ,
386 left) and axial (C_{2222} , right) components of the linearized material stiffness for Samples A and B
387 computed at a loaded configuration of $P(t)=100$ mmHg and individual value of $\lambda_z(t)=\lambda^0$. Shown,
388 too, are histograms for the four larger regions. Circumferential stiffness was significantly lower in the
389 dorsal-top quadrants of both samples as compared to the other quadrants in their respective dorsal
390 or top halves. Axial stiffness, on the other hand, was significantly different on both the ventral and
391 bottom halves of each sample. Specifically, the ventral half had higher stiffness than the dorsal half
392 independent of top or bottom location (Sample A: 1.32 ± 0.03 MPa vs. 1.15 ± 0.02 MPa, Sample B:
393 1.67 ± 0.04 MPa vs. 1.49 ± 0.02 MPa; $P < 0.05$), and the bottom half had higher stiffness than the top
394 independent of the dorsal-ventral side (Sample A: 1.35 ± 0.03 MPa vs. 1.10 ± 0.02 MPa, Sample B:
395 1.61 ± 0.04 MPa vs. 1.55 ± 0.03 MPa; $P < 0.05$). One main structural difference between the four
396 quadrants, of course, is the location of the major suprarenal branches. Comparison of stiffness
397 distributions with branch sites for Samples A and B (dashed lines, Fig. 5A-D) suggests that higher
398 values of biaxial stiffness tend to co-localize with regions of branch influence. Specifically,
399 circumferential stiffness tends to be higher at the boundaries whereas axial stiffness is higher over
400 the entire branch area.

401 In standard biaxial inflation-extension tests, one typically measures on-line both the outer
402 diameter and the axial force in response to changes in pressure and axial stretch. Such data were

403 collected for both samples prior to the biaxial p-DIC testing, and values of the constitutive
 404 parameters were estimated using traditional nonlinear regression [6]. Representative values of the
 405 estimated material parameters (c^e , c^m , c^c and β^c) and associated scalar metrics of goodness of fit or
 406 material behavior (R^2 , W , C_{1111} and C_{2222}) are reported in Table 2. The best-fit parameter values
 407 are generally in good agreement with the local distributions that were obtained using the p-DIC data.
 408

409 Table 2. Best-fit model parameters and associated scalar metrics estimated using nonlinear
 410 regression of standard biaxial experimental data for Samples A and B.

Sample	c^e (kPa)	c^m (kPa)	c^c (kPa)	β^c	W (kPa) @80mmHg	W (kPa) 140mmHg	C_{1111} (MPa)	C_{2222} (MPa)	R^{2*}
A	71	385	959	51°	70	93	1.78	1.18	0.98
B	65	128	1470	46°	61	79	1.74	0.96	0.99

411
 412 Finally, as a qualitative comparison of approaches, we computed local pressure-radius curves
 413 and local circumferential stress-stretch curves for every node at which material parameters were
 414 identified (Fig. 6). Data from the standard (global) biaxial inflation-extension approach (black circles)
 415 were compared to the reconstructed pressure-radius (left) and stress-stretch (right) curves for
 416 Samples A and B. Although similar curves can be generated for all tested axial stretch values, data are
 417 shown only at $\lambda_z(t)=\lambda^0$, for clarity. The sets of gray curves represent the reconstructed behavior of
 418 the inverse method based on the locally identified material parameters from all patches above a
 419 given R_n^{2*} value. Namely, the light-gray and dark-gray sets of curves show the behavior for all patches
 420 with $R^{2*} > 0.95$ and $R^{2*} > 0.99$, respectively. For Sample A, the light-gray curves represent 72% of
 421 all identified patches (257/356 patches) and the dark-gray curves represent 6.5% of all identified
 422 patches (23/356 patches). Similarly, for the better fitting Sample B, the light-grey curves represent
 423 83% of all identified patches (316/380 patches) and the dark-grey curves represent 2.9% of all
 424 identified patches (11/380 patches). Overall, there is a very good agreement between the standard
 425 and new p-DIC based approaches. In particular, the mean global responses fall well within the
 426 expected dispersion of the identified local responses. This overall agreement highlights the both the
 427 general utility and added advantage of local measurements even in healthy aortic tissue.
 428

429 DISCUSSION

430 **Relevance of inverse methods.** Advances in medical imaging and computational biomechanics
 431 have enabled investigators to study patient-specific models of hemodynamics, wall stress, and even

432 fluid-solid interactions. The utility of such models depends, however, on the goodness of the
433 specified material properties and boundary conditions. With regard to the former, increasing
434 attention is appropriately being directed toward in vivo estimations of arterial [23,24] and
435 aneurysmal [25,26] properties. Such attempts are nevertheless complicated by the limited
436 information that is available via in vivo measurements as well as by the existence of regional
437 variations in properties both along the normal arterial tree and within diseased segments [15,27–30].
438 Indeed, because of the complexity of the constitutive behavior of the aorta in health and disease, it is
439 inconceivable that in vivo data alone could enable both the identification of appropriate functional
440 forms of the constitutive relations and the calculation of best-fit values of the associated material
441 parameters. Complementary in vitro tests are essential. For example, in vitro biaxial tests on planar
442 or cylindrical specimens allow one to perform the multiple protocols that are necessary to generate
443 the over-determined systems of equations that ensure robust parameter estimation. Such tests
444 enable much more, however. They also allow a more careful evaluation of the appropriateness of
445 specific functional forms of the constitutive relations and identification of ranges for the values of the
446 material parameters, which provides important constraints on the estimations [31,32]; they similarly
447 allow equal quantification of circumferential and axial behaviors and their coupling (e.g., [33,34]),
448 even if directional deformations are not equal in vivo. Given such information from in vitro tests, one
449 can then focus in vivo on parameter estimation alone.

450 Notwithstanding the advantages of standard biaxial testing, there is yet a need for more
451 advanced in vitro methods, including ones that can both delineate possible regional variations in
452 cases of disease and better correlate such variations with the underlying microscopic composition
453 and structure. For this reason, we developed a novel approach that combines in vitro biaxial
454 panoramic-digital image correlation (p-DIC) based mechanical testing with a nonlinear inverse
455 material characterization method. The former has been described in detail previously [10,35,36]. The
456 latter can be accomplished in multiple ways, but we employed a virtual fields method that has
457 proven useful in different applications [17,19]. This approach allows one to derive relatively simple
458 extensions of traditional relations for thick-walled cylindrical geometries [37,38] that yet allow one to
459 account for added complexities, including axial bending during testing and associated changes in
460 local curvatures.

461 *Choice of material model.* We used a four-fiber family model that has proven reliable in
462 describing murine arterial behavior in multiple studies [6,7,9,14,15,20]. Although motivated by
463 microstructural information, this model was developed primarily to capture phenomenologically the
464 anisotropic response of blood vessels subjected to extension-inflation tests, which ultimately
465 depends on constituent fractions, fiber orientations, cross-linking, physical entanglements, and so
466 forth. The four-fiber model is thought to allow complexities beyond just fiber orientations and has

467 been shown (in comparison to two- and six-) to represent a good compromise between model
468 complexity and goodness of fit when applied to 7 independent pressure-diameter (P - d) and axial
469 load-axial stretch (f - l) protocols obtained during standard biaxial extension-inflation testing [6]. In
470 particular, all of the parameters of the four-fiber family model contribute to the fitting of the data;
471 uncertainty in the best-fit values was estimated using a nonparametric bootstrap approach, and no
472 fundamental problem of identifiability was found [6]. When using a reduced number of protocols in
473 the material parameter identification (e.g., only the 3 P - d protocols, with force measurement as in
474 the current study, as opposed to the 3 P - d and 4 f - l protocols), the primary effect was a modest
475 change in the identified fiber angle that tended to increase the biaxial linearized stiffness.

476 One recent addition to the four-fiber family model is the incorporation of deposition
477 stretches, which permits convenient predictions of residual stresses while using an in vivo reference
478 configuration [14]. We showed that deposition stretch values should remain within a narrow range
479 to ensure reasonable model predictions. Hence, although their inclusion adds to the number of
480 parameters, they are well bounded and do not compromise the identification of the standard
481 parameters. This overall previous experience with extension-inflation tests of excised arteries
482 supports the use of the present model to simultaneously fit P - d - f data at different extensions.
483 Whereas all previous analyses based on this model (i.e., traditional analyses) were based on the
484 assumption of a perfectly cylindrical geometry, herein we extended this approach to local analyses,
485 for each position on the reconstructed surface of the blood vessel, based on full-field measurements
486 for every applied pressure and every applied axial stretch that yield the local surface deformation
487 gradient.

488 The principle of virtual power allows the model to locally adjust to data at every position
489 instead of global adjustment based only on the measured (P - d) and (f - l) curves. For every position,
490 the identification is based on $14 \times 3 \times 2 = 84$ independent data points (14 pressures \times 3 axial stretches \times
491 2 independent equations). Therefore, similar to the traditional approach, at every position defined
492 on the surface of the sample, the number of reconstructed data points is sufficient to ensure the
493 overdetermined number of equations needed to identify the parameters in the model. As in
494 traditional analyses, however, we note that one disadvantage of Fung-type exponential models is
495 that there are intrinsic correlations amongst the parameters c_n^i and k_n^i (cf. Eqs. 5-7). Hence, the cost
496 function can often be minimized equally well using different combinations of the c_n^i and k_n^i
497 parameters. This is one reason why we should focus more on the predicted material properties, such
498 as energy storage or material stiffness, rather than individual material parameters.

499 *Uncertainty of the inverse method.* The question of uncertainty for each material parameter
500 holds in all nonlinear models and associated testing. To evaluate this point, we repeated the
501 identification after adding noise to the experimental data. White noise with a standard deviation of

502 0.05 was added to each component of the deformation gradient. This standard deviation was chosen
503 to be larger than the measurement uncertainty (which was less than 0.01 for the deformation
504 gradient) to amplify its effects on the identification. After the new identification, similar distributions
505 of material parameters were reconstructed. Changes on the R_n^{2*} criterion were negligible, thus
506 suggesting that the goodness of fit depends more on the ability of the model to fit the data than on
507 random noise in the data. Similarly, we tested the sensitivity of the identification procedure to the
508 initial set of parameter values used in the nonlinear optimization. The identification was performed
509 several times with different sets of initial values that were drawn randomly from within the defined
510 bounds (cf. Table 1). Independent of initialization, the results again showed similar values of the
511 material parameters and R_n^{2*} distributions, and especially the derived material properties, thus
512 suggesting that the observed heterogeneities are deterministic in nature.

513 Results are also sensitive to other inevitable experimental uncertainties, which further
514 complicate parameter estimation. Among the many causes of uncertainty, consistent experimental
515 definition of a reference configuration, often taken to be the traction-free state, is challenging.
516 Potential effects of these errors can be assessed by repeating the estimations for different values
517 that define the reference configuration [38]. Our approach, using a near in vivo reference
518 configuration, naturally reduces the uncertainty in comparison with measurements of traction-free
519 configurations because of the extreme compliance of arteries at low loads.

520 *Regional variations in material properties.* Notwithstanding possible uncertainties in our
521 characterizations, heterogeneities appeared to manifest both locally (based on nodal values) and
522 regionally (based on the four regions so defined). In particular, steep variations appeared for some
523 parameters, such as those meant to model the collagen fibers, whereas mild variations arose for
524 other parameters, including those meant to model the elastic fibers. Considering the
525 phenomenological nature of the model, however, one should not try to over-interpret the underlying
526 reasons for these variations in parameter values, with or without knowledge of the underlying
527 histological structure. Rather, it is best to focus on metrics such as energy storage and material
528 stiffness (Figs. 4 and 5) when comparing material behaviors either regionally or from specimen-to-
529 specimen. Additionally, to distinguish between uncertainty and true regional variations in material
530 properties, regions should be defined with a significant number of data points (for local estimates) or
531 patches (for regional estimates) to have appropriate statistical power and anatomical relevance (for
532 instance, dorsal, ventral, top and bottom halves).

533 The suprarenal aorta has significant perivascular support in vivo, namely, the spine and
534 dorsal musculature. Interestingly, the energy stored upon pressurization in vitro was significantly
535 lower on the dorsal than the ventral side for both tested samples. It thus seems that the capability of
536 the dorsal side of the aorta to store energy, ultimately to be used to work on the blood during

537 diastole, is potentially reduced throughout development due to this increased perivascular support.
538 Values of circumferential and axial material stiffness tended to be higher near the axial boundaries
539 and on the ventral side of the sample. Higher stiffness near the top (proximal) and bottom (distal)
540 edge is likely an “end-effect” due to cannulation and pressurization of the sample at a fixed axial
541 stretch; in contrast, the increased ventral stiffness tends to co-localize with regions of branch
542 influence. The increased stiffness near branch locations could similarly result from the ligatures that
543 are needed to enable mechanical testing. In particular, circumferential stiffness is often increased on
544 the lateral sides of the branch region, consistent with the ligation at the branch ostium potentially
545 playing a role upon pressurization. Increased axial stiffness at branch locations is likely compounded
546 by the fact that axial stretch is influenced by the distance between the cannulation sutures. This
547 effect can necessarily vary over the surface of the sample and lead to a reduced relative stretch at
548 the branch locations and ultimately contribute to increasing the stiffness.

549 **Coefficients of determination.** Another fundamental question relates to the origin of regional
550 variations for the $R_n^{2*} < 0.90$ criterion, especially in Sample A. This goodness-of-fit is related to the
551 ability / inability of the model to capture the experimental response locally. Mismatches between
552 model and data could be a source of bias for the identified parameters, hence we discarded patches
553 where R_n^{2*} is lower than 0.90. Discrepancies often localized close to cannulation ligatures or near
554 branches (Fig. 2). Additionally, large central regions of discrepancy for Sample A likely resulted, in
555 part, from bending instabilities and associated changes in local mean curvature that occur in an
556 inflated vessel maintained at a constant axial stretch [39]. Bending was even triggered at low
557 pressures, for the suprarenal aorta is curved slightly upon excision. For this reason, the suprarenal
558 abdominal aorta is probably one of the most challenging case studies for our novel identification
559 method. Nevertheless, despite difficulties related to bending and the presence of branches, there
560 was very good agreement between the myriad identified local behaviors and the global response of
561 the same sample (Fig. 6). Moreover, strain energy and stiffness estimated from the standard biaxial
562 tests (Table 2) were consistent with the center value of the regional distributions.

563 Recall that the biaxial loading was maintained for several minutes at each state to allow p-
564 DIC image acquisition. Hence, in contrast to the continuous cyclic loading of the standard biaxial
565 tests, the loading was incremental in the p-DIC tests. Direct comparisons between continuous and
566 incremental loading protocols in separate standard biaxial tests on the same sample (not shown)
567 revealed no difference in the measured pressure-diameter behavior, hence the difference in loading
568 protocol did not appear to be a concern. We also checked whether a thick-wall model that delineates
569 medial and adventitial properties would improve the coefficients of determination when compared
570 to a membrane model. Again, however, this did not have a significant effect on the results (not
571 shown). This finding is somewhat consistent with the wall thickness being at least one order of

572 magnitude lower than radius upon pressurization (e.g., radius of 0.65 mm and thickness of 0.04 mm,
573 on average), but also because both methods rely on measuring deformations or diameters at the
574 outer surface and invoking incompressibility of the wall.

575

576 **CONCLUSIONS**

577 We submit that the present experimental-computational method for local arterial
578 characterization represents another important step toward the ultimate goal of understanding better
579 the structure-property relationships that underlie regional variations in material properties along the
580 arterial tree and especially within many arterial lesions, including aortic aneurysms. Combining such
581 findings with advances in growth and remodeling simulations (e.g., [40,41]) promises to improve our
582 ability to predict subsequent mechano-adaptations or disease progression. Many challenges yet
583 remain, however. Highly localized defects in wall structure, as, for example, localized deposits of
584 calcium or pools of mucoid material, may play significant roles in initiating local failure processes
585 including those that initiate intramural delamination [42,43]. Such defects could be difficult to
586 identify even with sophisticated methods such as p-DIC and will likely require additional transmural
587 imaging and layer specific inverse methods. The present validation study provides a firm foundation
588 upon which to build, however.

589

590

591 **CONFLICT OF INTEREST**

592 None.

593

594

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599

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- 719

720 **APPENDIX**

721 The principle of virtual power (PVP) is an integral expression of the equilibrium equations across a
 722 solid, which may be written quasi-statically as

$$-\underbrace{\int_{\omega(t)} \boldsymbol{\sigma} : (\nabla \otimes \boldsymbol{\xi}^*) d\omega}_{P_{int}^*} + \underbrace{\oint_{\partial\omega(t)} \mathbf{T} \cdot \boldsymbol{\xi}^* ds}_{P_{ext}^*} = 0 \quad (\text{A1})$$

723 where $\boldsymbol{\xi}^*$ is a virtual velocity field defined across the volume of the solid (denoted $\omega(t)$) and $\nabla \otimes \boldsymbol{\xi}^*$
 724 is the gradient of $\boldsymbol{\xi}^*$. \mathbf{T} are the tractions across the boundary (surface denoted $\partial\omega(t)$), P_{int}^* is the
 725 virtual power of internal forces and P_{ext}^* is the virtual power of external forces.

726 The PVP has been used for the identification of material properties since 1990 through the virtual
 727 fields method (VFM), which is an inverse method based on the use of full-field deformation data
 728 [17,18]. The VFM was recently applied to the identification of uniform material properties in arterial
 729 walls [19] and will be extended herein to consider regional variations of material properties. Hence,
 730 let us consider two virtual fields \mathbf{u}^* and \mathbf{v}^* defined across a given patch n

$$\mathbf{u}^*(\xi) = \left[\frac{(1/\kappa_n^1 - h)(1/\kappa_n^2 - h)(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{1/\kappa_n^1 - (1 - \xi)h} \frac{(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{1/\kappa_n^2 - (1 - \xi)h} \right] \mathbf{n}_n \quad (\text{A2})$$

$$\mathbf{v}^* = -\frac{x}{2} \mathbf{e}_x - \frac{y}{2} \mathbf{e}_y + z \mathbf{e}_z \quad (\text{A3})$$

731 where κ_n^1 and κ_n^2 are the average maximum and minimum principal curvatures, respectively.
 732 Therefore, $1/\kappa_n^1$ is the radius of curvature on the outer surface along the direction of the maximum
 733 principal curvature and $1/\kappa_n^2$ is the radius of curvature on the outer surface along the direction of the
 734 minimum principal curvature. The radii of curvature at any position ξ between the inner ($\xi = 0$) and
 735 outer ($\xi = 1$) surfaces for the inner surface are then $1/\kappa_n^1 - (1 - \xi)h$ and $1/\kappa_n^2 - (1 - \xi)h$.

736 In this appendix, we prove that Eq. A1 written with \mathbf{u}^* yields Eq. 22 (Proof 1) and similarly Eq. A1
 737 written with \mathbf{v}^* yields Eq. 26 (Proof 2).

738 *Proof 1:*

739 The gradient of \mathbf{u}^* may be written as follows

$$\begin{aligned} \nabla \otimes \mathbf{u}^* = & \frac{(1/\kappa_n^1 - h)(1/\kappa_n^2 - h)(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{(1/\kappa_n^2 - (1 - \xi)h)(1/\kappa_n^1 - (1 - \xi)h)^2} \mathbf{g}_n^1 \otimes \mathbf{g}_n^1 + \frac{(1/\kappa_n^1 - h)(1/\kappa_n^2 - h)(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{(1/\kappa_n^1 - (1 - \xi)h)(1/\kappa_n^2 - (1 - \xi)h)^2} \mathbf{g}_n^2 \otimes \mathbf{g}_n^2 \\ & - \left[\frac{(1/\kappa_n^1 - h)(1/\kappa_n^2 - h)(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{(1/\kappa_n^2 - (1 - \xi)h)(1/\kappa_n^1 - (1 - \xi)h)^2} + \frac{(1/\kappa_n^1 - h)(1/\kappa_n^2 - h)(1/\kappa_n^1 + 1/\kappa_n^2 - 2h)}{(1/\kappa_n^1 - (1 - \xi)h)(1/\kappa_n^2 - (1 - \xi)h)^2} \right] \mathbf{n}_n \otimes \mathbf{n}_n \end{aligned} \quad (\text{A4})$$

740 Plugging in and evaluating the integral expression for P_{int}^* (cf. Eq. A1)

$$\begin{aligned}
P_{int}^*(t) = & -h(t)(1/\kappa_n^1(t) - h(t))(1/\kappa_n^2(t) - h(t))(1/\kappa_n^1(t) + 1/\kappa_n^2(t)) \\
& - 2h(t) \int_0^1 \left(\frac{\sigma_{11,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^2(t) - (1 - \xi)h(t))(1/\kappa_n^1(t) - (1 - \xi)h(t))^2} \right. \\
& \left. + \frac{\sigma_{22,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^1(t) - (1 - \xi)h(t))(1/\kappa_n^2(t) - (1 - \xi)h(t))^2} \right) A_n(t, \xi) d\xi
\end{aligned} \tag{A5}$$

741

742 where $A_n(t, \xi)$ is the area of patch n at radial position ξ and may be written

$$A_n(t, \xi) = (1/\kappa_n^1(t) - (1 - \xi)h(t))(1/\kappa_n^2(t) - (1 - \xi)h(t))\Theta_n^1(t)\Theta_n^2(t)$$

743 where Θ_n^1 and Θ_n^2 are two angles defining the angular sector of patch n along the directions of the
744 maximum and minimum principal curvatures, respectively. Introducing the expression of $A_n(t, \xi)$
745 into Eq. A5, we obtain

$$\begin{aligned}
P_{int}^*(t) = & -h(t)(1/\kappa_n^1(t) - h(t))(1/\kappa_n^2(t) - h(t))(1/\kappa_n^1(t) + 1/\kappa_n^2(t)) \\
& - 2h(t)\Theta_n^1(t)\Theta_n^2(t) \int_0^1 \left(\frac{\sigma_{11,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^1(t) - (1 - \xi)h(t))} + \frac{\sigma_{22,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^2(t) - (1 - \xi)h(t))} \right) d\xi
\end{aligned} \tag{A6}$$

746 Regarding the virtual work on the boundaries, shear stresses are null so only the virtual work of the
747 internal pressure needs to be considered

$$\begin{aligned}
P_{ext}^*(t) = & P(t) (1/\kappa_n^1(t) - h(t)) (1/\kappa_n^2(t) - h(t)) \Theta_n^1(t)\Theta_n^2(t) (1/\kappa_n^1(t) + 1/\kappa_n^2(t)) \\
& - 2h(t)
\end{aligned} \tag{A7}$$

748 So, using Eq. A1, we have:

$$P(t) = h(t) \int_0^1 \left(\frac{\sigma_{11,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^1(t) - (1 - \xi)h(t))} + \frac{\sigma_{22,n}^w(t, \xi) - \sigma_{33,n}^w(t, \xi)}{(1/\kappa_n^2(t) - (1 - \xi)h(t))} \right) d\xi$$

749 *Proof 2:*

750 A second virtual field is required here. Indeed, even if the artery shape is not a perfect cylinder, κ_n^2 is
751 globally the curvature along the axis of the artery and may take very small values at most of the
752 patches. The result is that $\sigma_{zz,n}^w$ has little influence in Eq. 22, leading to almost no sensitivity to a
753 material parameter such as c_n^a . To address this issue, a second virtual field involving $\sigma_{zz,n}^w$ even in the
754 patches where $\kappa_n^2 \cong 0$ is proposed, and will involve the measured axial load $f(t)$. The simplest
755 virtual field both satisfying these requirements and zeroing the virtual work of the hydrostatic
756 pressure is given by \mathbf{v}^* (cf. Eq. A3). The gradient may be written as

$$\nabla \otimes \mathbf{v}^* = -\frac{1}{2} \mathbf{e}_x \otimes \mathbf{e}_x - \frac{1}{2} \mathbf{e}_y \otimes \mathbf{e}_y + \mathbf{e}_z \otimes \mathbf{e}_z \tag{A8}$$

757 We assume here that the same internal virtual work is shared along the circumferential direction.

$$P_{int}^*(t) = - \int_{\omega(t)} \boldsymbol{\sigma} : (\nabla \otimes \mathbf{v}^*) d\omega = -\frac{1}{K} \int_{\text{whole circumference}} \boldsymbol{\sigma} : (\nabla \otimes \mathbf{v}^*) d\omega$$

758 Where K is the number of patches along the circumferential direction.

759 Then we have

$$P_{int}^*(t) = -\frac{1}{K} \int_{z_n-b/2}^{z_n+b/2} \int_0^{2\pi} \int_{r^o-h}^{r^o} [\sigma_{zz,n}^w(t, \xi) - \sigma_{xx,n}^w(t, \xi)/2 - \sigma_{yy,n}^w(t, \xi)/2] dr r d\theta dz$$

$$P_{int}^*(t) = -\frac{\pi}{K} b(t) h(t) \int_0^1 [2\sigma_{zz,n}^w(t, \xi) - \sigma_{xx,n}^w(t, \xi) - \sigma_{yy,n}^w(t, \xi)] [r^o(t) - (1 - \xi)h(t)] d\xi \quad (A9)$$

760 Where r^o is the average radius of the cross section of the considered patch and b is the length of the
761 patch in the z direction.

762 Regarding the virtual work across the boundaries, shear stresses are again neglected and we have
763 only to consider the virtual work of the internal pressure on the inner surface and the virtual work of
764 the axial stress. We assume that the external virtual work is also shared along the circumferential
765 direction, yielding

$$P_{ext}^*(t) = \oint_{\partial\omega(t)} \mathbf{T} \cdot \mathbf{v}^* ds = \frac{1}{K} \oint_{\text{whole circumference}} \mathbf{T} \cdot \mathbf{v}^* ds$$

766 Then we have

$$P_{ext}^*(t) = \frac{1}{K} \left[-\frac{P(t)}{2} b(t) 2\pi [r^o(t) - h(t)]^2 + b(t) h(t) \int_0^1 \sigma_{zz,n}^w(t, \xi) 2\pi [r^o(t) - (1 - \xi)h(t)] d\xi \right]$$

$$P_{ext}^*(t) = \frac{b(t)}{K} \left[-P\pi [r_n^o(t) - h(t)]^2 + \left(2\pi h(t) \int_0^1 \sigma_{zz,n}^w(t, \xi) [r^o(t) - (1 - \xi)h(t)] d\xi \right) \right] \quad (A10)$$

767 We recognize in the bracket of Eq. A10 the formula of the axial load [37,38] and we eventually obtain

$$P_{ext}^*(t) = \frac{b(t)}{K} f(t) \quad (A11)$$

768 Thus, using Eq. A1, we have:

$$f(t) = \pi h(t) \int_0^1 [2\sigma_{zz,n}^w(t, \xi) - \sigma_{xx,n}^w(t, \xi) - \sigma_{yy,n}^w(t, \xi)] [r^o(t) - (1 - \xi)h(t)] d\xi$$

769

770

771

772 **Supplemental Table 1. Morphometric data and vessel geometry for Samples A and B**

773

	Sample A	Sample B
Age (wks)	18.3	21.0
Body Mass (g)	31.0	31.8
Unloaded Outer Diameter (μm)	784	966
Unloaded Wall Thickness (μm)	99	119
Unloaded Axial Length (mm)	5.37	6.11
<i>In-vivo</i> Axial Stretch	1.67	1.55

774 **Supplemental Table 2. Comparison of identified material parameters and scalar metrics in four**
775 **defined regions on Samples A and B. * P < 0.05 between dorsal and ventral sides on same top or**
776 **bottom half, †P < 0.05 between top and bottom halves on same dorsal or ventral side, # P < 0.05**
777 **between regions on Sample A and Sample B, §P < 0.05 between total patches on Sample A and**
778 **Sample B.**

Region	Sample A				
	Ventral, Top n 81	Dorsal, Top 91	Ventral, Bottom 92	Dorsal, Bottom 92	Total 356
R ^{2*}	0.961 ± 0.002	0.955 ± 0.003	0.964 ± 0.002	0.971 ± 0.002 †	0.963 ± 0.001
c ^e (MPa)	0.086 ± 0.002	0.074 ± 0.002 *	0.089 ± 0.002	0.077 ± 0.002 *	0.082 ± 0.001
c ^m (MPa)	0.112 ± 0.015	0.076 ± 0.010	0.195 ± 0.017 †	0.181 ± 0.014 †	0.142 ± 0.008
c ^c (MPa)	3.089 ± 0.196	1.942 ± 0.122 *	2.884 ± 0.162	3.282 ± 0.238 †	2.793 ± 0.096
c ^a (MPa)	0.334 ± 0.090	0.648 ± 0.098	1.240 ± 0.232	1.107 ± 0.154	0.848 ± 0.081
c ^{m,c} (MPa)	0.672 ± 0.021	0.566 ± 0.019 *	0.566 ± 0.013 †	0.488 ± 0.017 †,*	0.570 ± 0.009
c ^{c,c} (MPa)	0.720 ± 0.066	0.421 ± 0.115	0.966 ± 0.060	0.755 ± 0.073 †	0.716 ± 0.042
c ^{a,c} (MPa)	4.583 ± 3.041	1.235 ± 0.334	10.399 ± 9.072	374.43 ± 372.41	100.81 ± 96.27
β ^c (deg)	48.43 ± 0.34	49.81 ± 0.38	51.49 ± 0.55 †	49.21 ± 0.48	49.77 ± 0.23
G ^{e1}	2.192 ± 0.014	2.161 ± 0.013	2.185 ± 0.013	2.196 ± 0.014	2.183 ± 0.007
G ^{e2}	1.812 ± 0.011	1.786 ± 0.011	1.805 ± 0.011	1.815 ± 0.012	1.804 ± 0.006
G ^m	1.030 ± 0.002	1.029 ± 0.002	1.036 ± 0.003	1.030 ± 0.002	1.031 ± 0.001
G ^c	1.064 ± 0.005	1.091 ± 0.005 *	1.061 ± 0.005	1.069 ± 0.005 †	1.071 ± 0.003
G ^a	1.092 ± 0.005	1.088 ± 0.005	1.081 ± 0.005	1.093 ± 0.005	1.088 ± 0.002
k ^m	0.866 ± 0.080	0.662 ± 0.059	0.671 ± 0.067	0.466 ± 0.058	0.660 ± 0.034
k ^{c/a}	1.903 ± 0.102	1.137 ± 0.087 *	1.801 ± 0.085	1.263 ± 0.112	1.515 ± 0.051
W ₈₀ (kPa)	79.1 ± 1.9	66.2 ± 1.7 *	80.2 ± 1.7	71.4 ± 1.5 *	74.1 ± 0.9
W ₁₄₀ (kPa)	106.4 ± 2.6	105.8 ± 3.0	124.6 ± 3.1 †	109.5 ± 3.3 *	111.7 ± 1.6
C ₁₁₁₁ (MPa)	1.248 ± 0.038	0.937 ± 0.024 *	1.218 ± 0.026	1.242 ± 0.045 †	1.159 ± 0.018
C ₂₂₂₂ (MPa)	1.192 ± 0.028	1.026 ± 0.020	1.433 ± 0.052 †	1.275 ± 0.035 †	1.233 ± 0.020

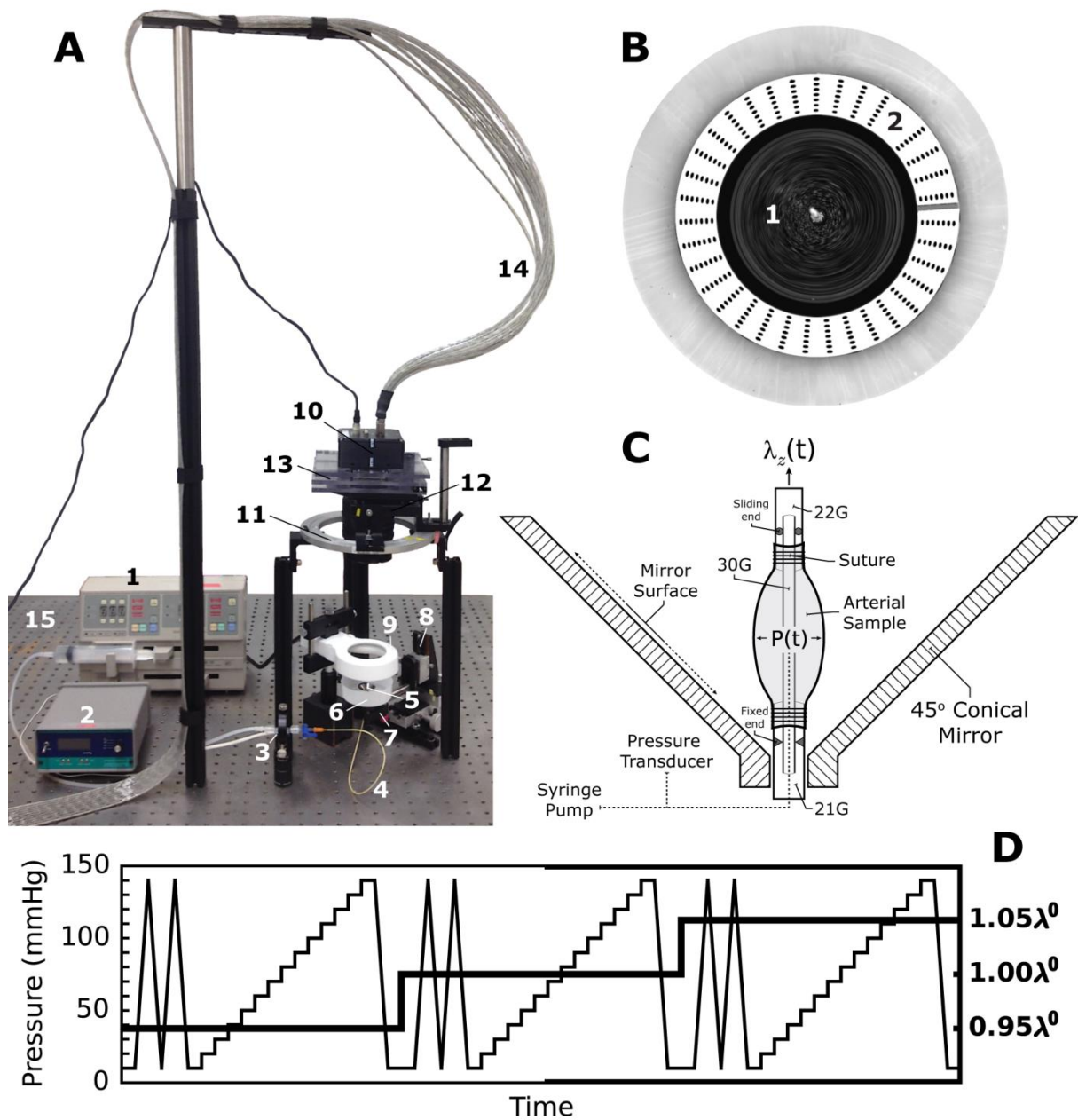
Region	Sample B				
	Ventral, Top n 98	Dorsal, Top 97	Ventral, Bottom 91	Dorsal, Bottom 94	Total 380
R ^{2*}	0.982 ± 0.001 #	0.961 ± 0.002 *	0.964 ± 0.002 †	0.968 ± 0.002	0.969 ± 0.001 §
c ^e (MPa)	0.093 ± 0.002	0.086 ± 0.002 #	0.080 ± 0.002 #,†	0.087 ± 0.002 #	0.086 ± 0.001 §
c ^m (MPa)	0.123 ± 0.014	0.158 ± 0.015 #	0.274 ± 0.026 #,†	0.174 ± 0.019 *	0.181 ± 0.01 §
c ^c (MPa)	2.362 ± 0.128	3.202 ± 0.146 #,*	3.703 ± 0.198 #,†	3.759 ± 0.122	3.243 ± 0.08 §
c ^a (MPa)	6.989 ± 0.392 #	3.761 ± 0.365 #,*	3.007 ± 0.407 #,†	3.131 ± 0.272 #	4.257 ± 0.199 §
c ^{m,c} (MPa)	0.265 ± 0.009 #	0.264 ± 0.013 #	0.237 ± 0.010 #	0.230 ± 0.011 #	0.249 ± 0.006 §
c ^{c,c} (MPa)	1.050 ± 0.026 #	1.152 ± 0.046 #	0.978 ± 0.076	1.230 ± 0.082 #	1.103 ± 0.031 §
c ^{a,c} (MPa)	0.171 ± 0.040	0.024 ± 0.024	0.659 ± 0.628	0.072 ± 0.061	0.226 ± 0.151
β ^c (deg)	44.59 ± 0.65 #	46.91 ± 0.71 #	51.61 ± 0.84 †	47.17 ± 0.72 *	47.50 ± 0.39 §
G ^{e1}	2.200 ± 0.013	2.201 ± 0.012	2.199 ± 0.014	2.219 ± 0.013	2.205 ± 0.006 §
G ^{e2}	1.818 ± 0.010	1.819 ± 0.010	1.818 ± 0.011	1.833 ± 0.011	1.822 ± 0.005 §
G ^m	1.110 ± 0.004 #	1.104 ± 0.004 #	1.110 ± 0.004 #	1.110 ± 0.004 #	1.109 ± 0.002 §
G ^c	1.039 ± 0.004 #	1.039 ± 0.004 #	1.035 ± 0.002 #	1.038 ± 0.003 #	1.038 ± 0.002 §
G ^a	1.021 ± 0.003 #	1.027 ± 0.003 #	1.046 ± 0.004 #,†	1.030 ± 0.003 #	1.031 ± 0.002 §
k ^m	1.348 ± 0.146 #	0.431 ± 0.060 #,*	0.804 ± 0.089 †	0.540 ± 0.071	0.784 ± 0.052 §
k ^{c/a}	2.076 ± 0.230	3.067 ± 0.218 *	1.446 ± 0.197	1.683 ± 0.196 †	2.081 ± 0.110 §
W ₈₀ (kPa)	84.0 ± 1.2	78.8 ± 1.1 #	73.2 ± 1.4 #,†	81.8 ± 1.9 #,*	79.5 ± 0.7 §
W ₁₄₀ (kPa)	141.7 ± 2.5 #	94.5 ± 1.5 *	101.5 ± 2.7 #,†	104.2 ± 2.2	110.8 ± 1.5
C ₁₁₁₁ (MPa)	1.650 ± 0.063 #	1.451 ± 0.027 #,*	1.568 ± 0.058 #	1.745 ± 0.038 #,†	1.603 ± 0.025 §
C ₂₂₂₂ (MPa)	1.620 ± 0.039 #	1.472 ± 0.036 #	1.718 ± 0.067 #	1.518 ± 0.034 #,*	1.580 ± 0.023 §

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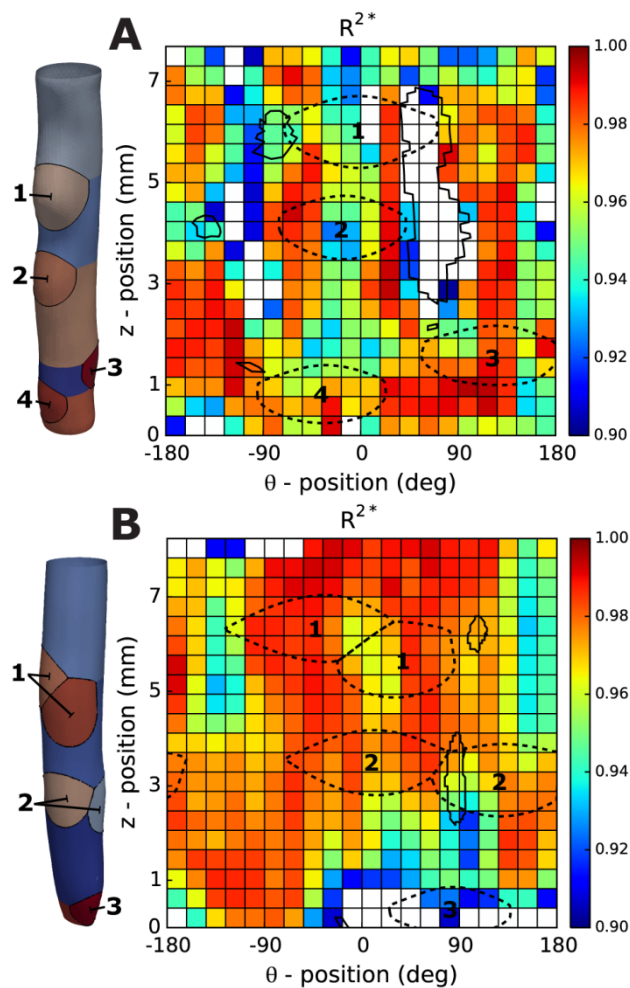


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785 *Figure 1. Overview of the p-DIC system. A.* Components include a syringe pump (1), pressure
786 monitor (2), pressure transducer (3), and tubing (4) for pressure control. A 45° conical mirror (5) is
787 located within a specimen bath (6) and mounted atop a small kinematic mount (7) that is attached to
788 a 3-axis translational stage (8). An annular light source (9) is used for illumination. The digital camera
789 (10) is mounted vertically above the sample on a rotational stage (11) via a large kinematic mount
790 (12) and custom translational stage (13). Images are acquired and sent to the computer for analysis
791 through a camera link cable (14), and the entire system is placed on a precision optical bench (15). **B.**

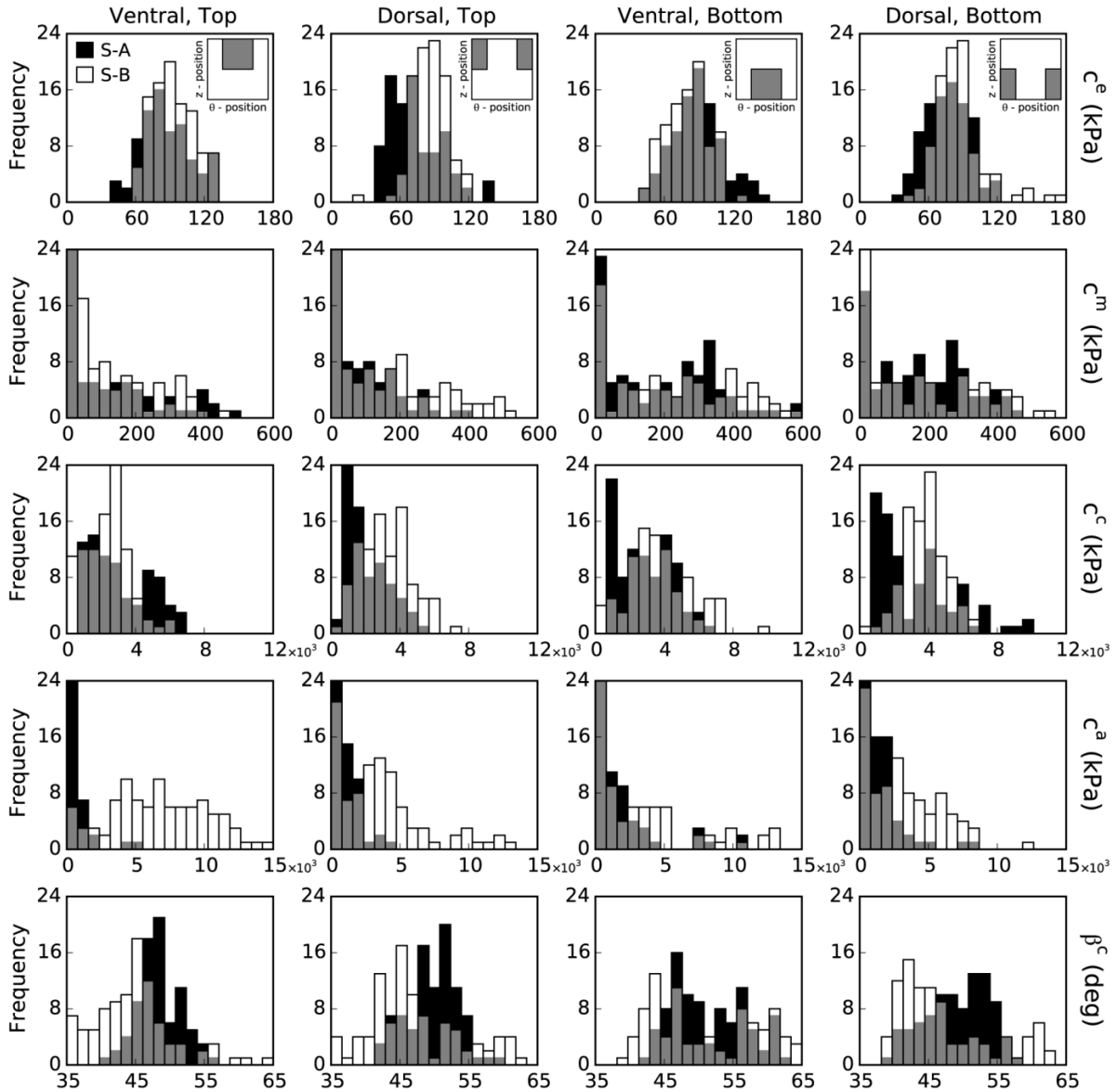
792 Top-view of 45° conical mirror inside of specimen bath showing the speckle pattern on the
 793 measurement surface (1) and the calibration target (2) used for 3D reconstruction. **C.** Schematic of
 794 the cannulation of a slightly aneurysmal specimen showing different gauge needles, locations of fixed
 795 and sliding ends, and methods to pressurize and axially stretch the specimen. **D.** Loading protocol
 796 used for mechanical testing: for each axial stretch (bold solid line), the sample underwent two cycles
 797 of preconditioning followed by a step-wise increase in pressure from 10 to 140 mmHg in 10 mmHg
 798 increments (thin solid line).
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 803 *Figure 2. Spatial distribution of the coefficients of determination. Goodness of fit for A. Specimen A*
 804 *and B. Specimen B. Both panels show outputs from a modified branch splitting algorithm to highlight*
 805 *regions of influence due to specimen branches (1-4). Results are shown in both a 3D (left) and 2D*
 806 *(right) representation over the entire surface of each sample. Boundaries of both low mean*
 807 *curvature (solid enclosed regions) and regions of branch influence (dashed ellipses) are overlaid in*
 808 *the 2D representation to show localization with regions of low R_n^{2*} .*

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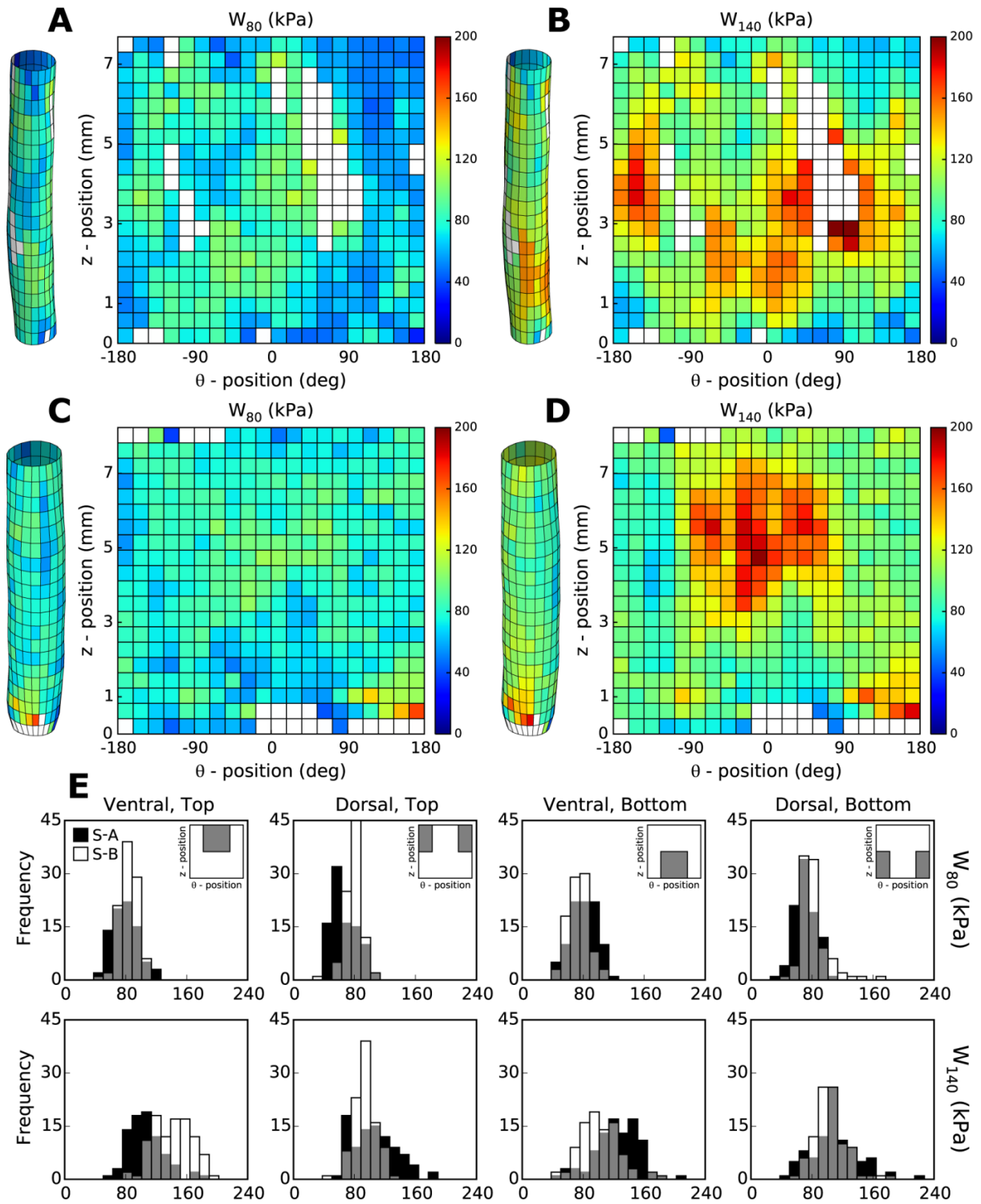
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813 *Figure 3. Histogram distributions of identified material parameters. Results from the identification*
814 *procedure are shown for c_n^e (first row), c_n^m (second row), c_n^c (third row), c_n^a (fourth row), and β_n^c (fifth*
815 *row) for both Sample A (S-A, black bars) and Sample B (S-B, white bars). All identified parameters are*
816 *heterogeneous and spatially varying. Results are also shown by region: ventral-top (first column),*
817 *dorsal-top (second column), ventral-bottom (third column), and dorsal-bottom (fourth column). The*
818 *grey bars indicate overlapping results for the two samples.*

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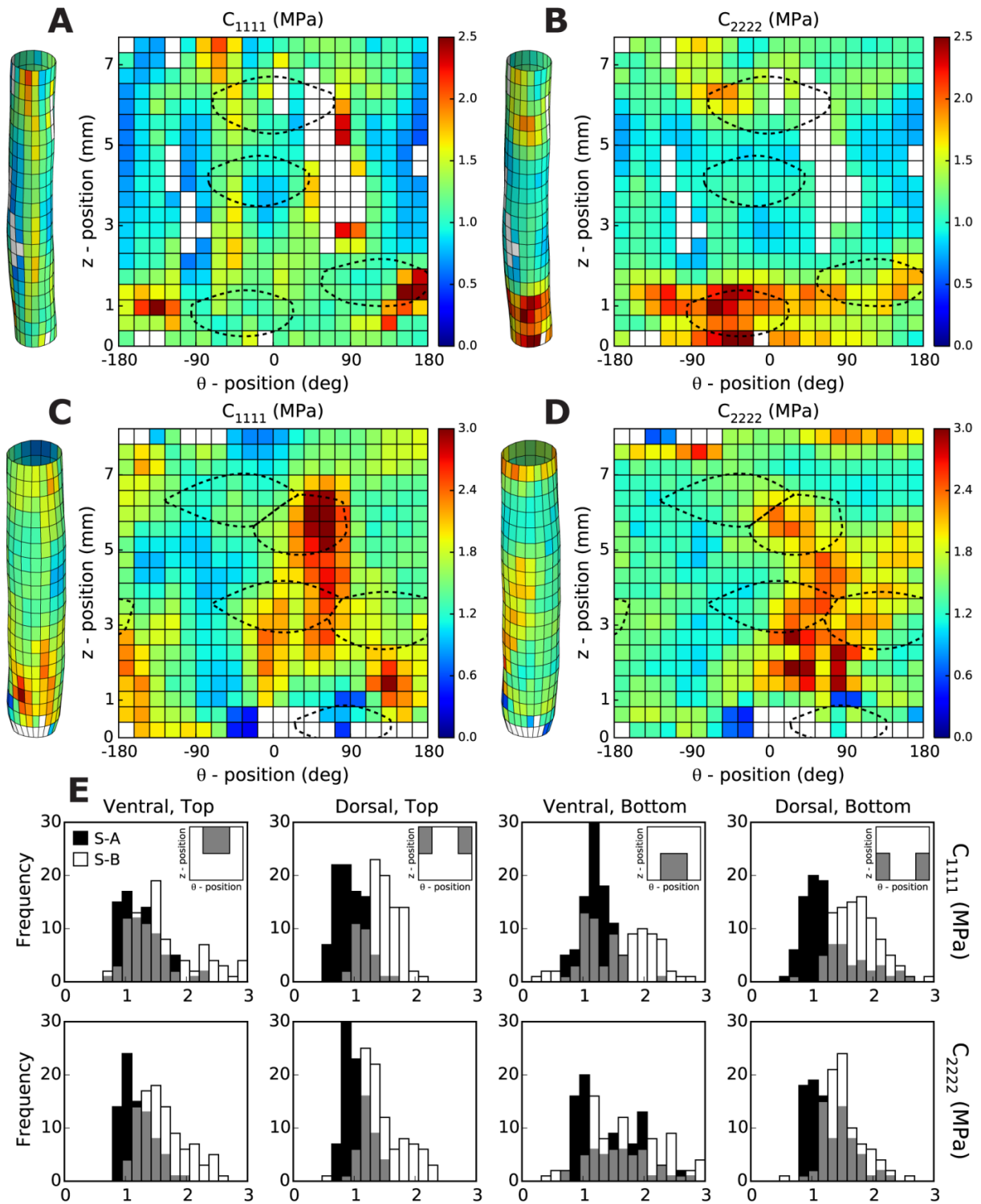
824 **Figure 4. Spatial distribution of strain energy.** The strain energy density was computed (Eq. 3) using

825 the identified material properties over the surface of A-B. Sample A and C-D. Sample B. Results are

826 shown for two loaded configurations: A,C. $P(t)=80$ mmHg at $\lambda_z(t) = \lambda^0$ and B,D. $P(t)=140$ mmHg at

827 $\lambda_z(t) = \lambda^0$. E. Histograms show spatial distributions in each quadrant for both samples (cf. Fig. 3).

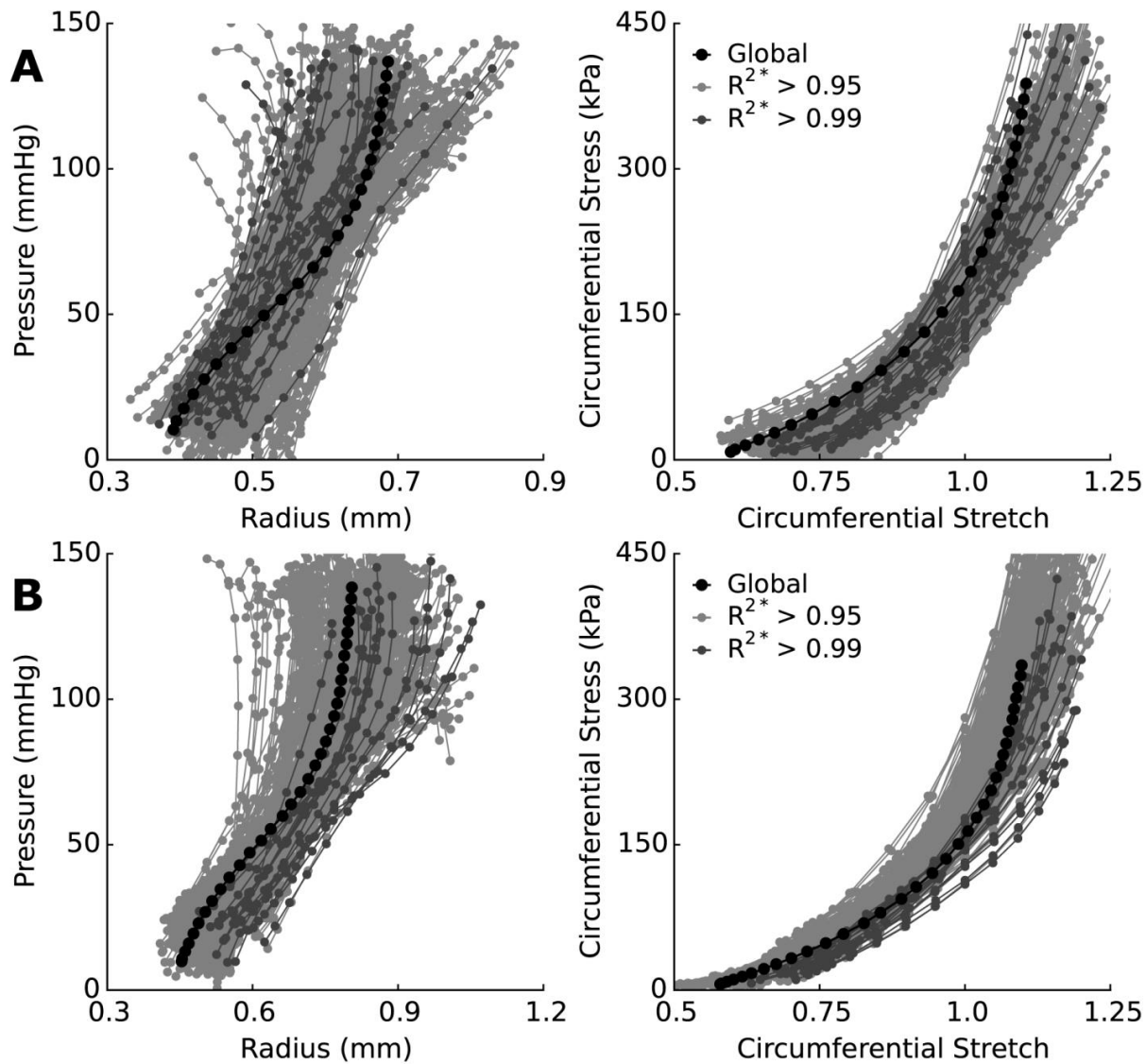
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830 **Figure 5. Spatial distribution of biaxial material stiffness.** The biaxial material stiffness was computed
 831 using the identified material parameters over the surface of **A-B**. Sample A and **C-D**. Sample B.
 832 Results are shown for **A,C**, circumferential (C_{1111}) and **B,D**, axial (C_{2222}) stiffness evaluated at a
 833 loaded configuration of $P(t)=100$ mmHg and $\lambda_z(t) = \lambda^0$. Regions of influence due to branches
 834 (dashed lines) are overlaid to show localization near regions of high stiffness. **E**. Histograms show
 835 spatial distributions in each quadrant for both samples (cf. Fig. 3).

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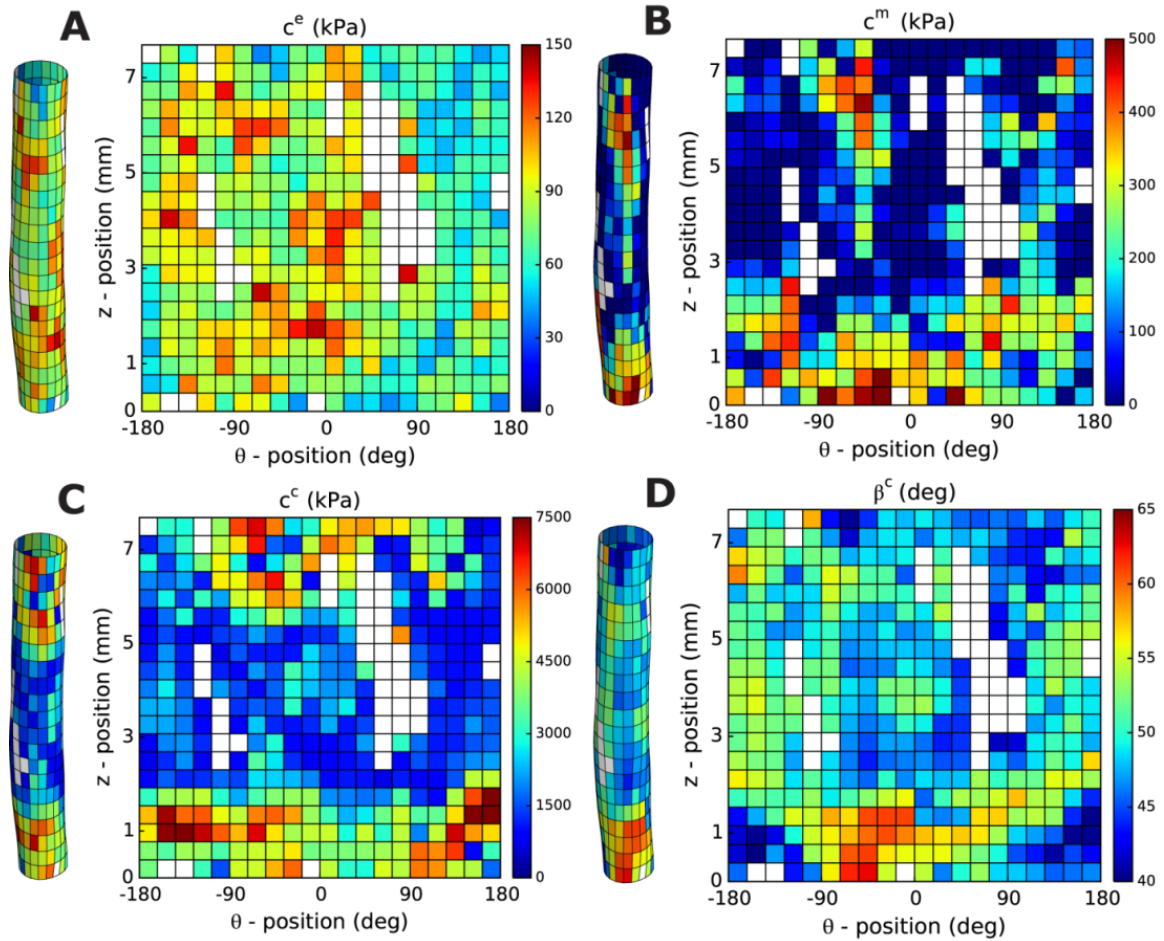


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842 *Figure 6. Comparison of p-DIC and Standard Biaxial Results.* The reconstructed pressure-radius (left)
843 and circumferential stress-stretch (right) behaviors for **A. Sample A** and **B. Sample B** were compared
844 to standard biaxial testing results (black circles). Local responses are compared for locations with an
845 R_n^{2*} value above 0.95 (light-gray) and 0.99 (dark-gray). Comparison is shown only for data collected at
846 $\lambda_z(t) = \lambda^0$, for clarity.

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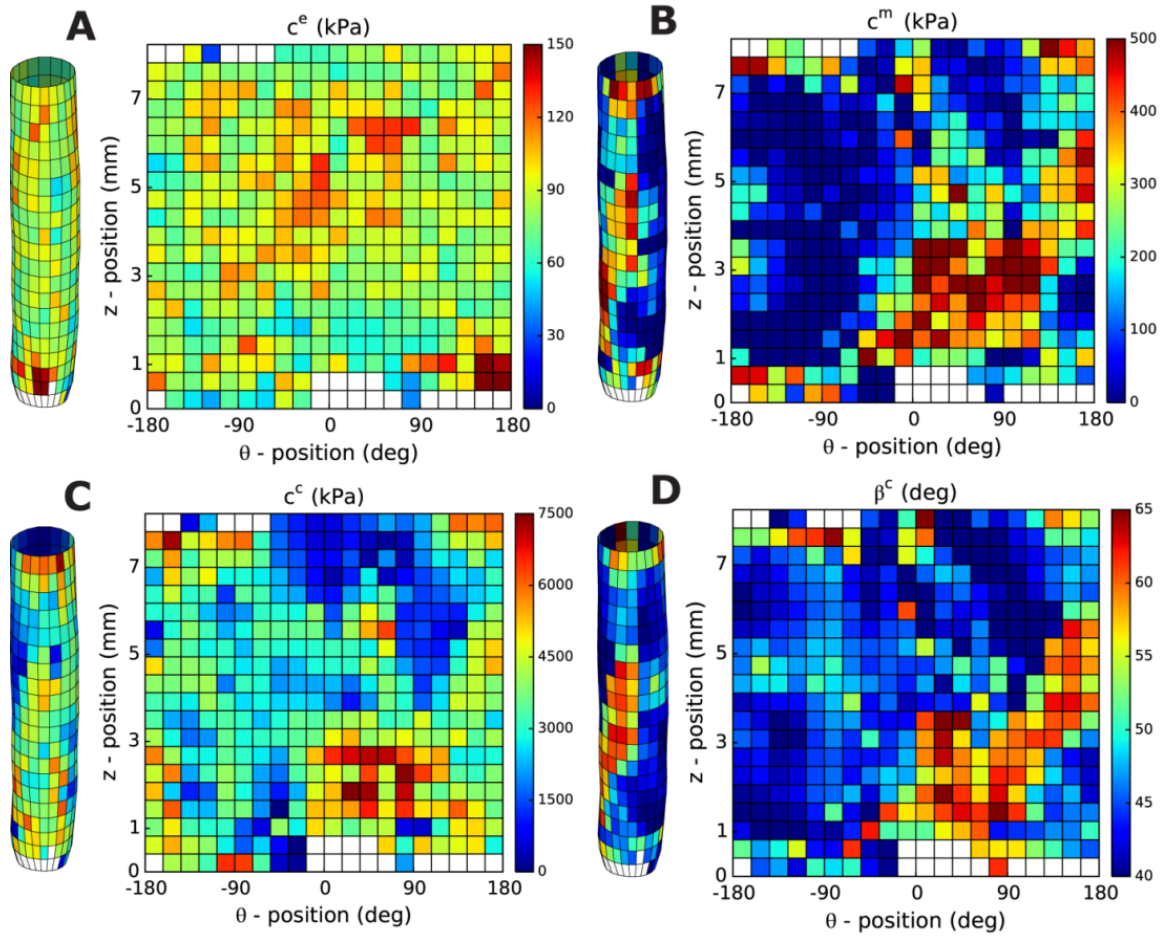
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Supplemental Figure 1. Regional distributions of identified material parameters for Sample A. Results are shown for c^e , c^m , c^c , and β^c . Transparent (white) regions show nodes where the coefficient of determination failed to reach 0.90.

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877 **Supplemental Figure 2. Regional distributions of identified material parameters for Sample B. Results**
878 **are shown for c^e , c^m , c^c , and β^c . Transparent (white) regions show nodes where the coefficient of**
879 **determination failed to reach 0.90.**