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1 **Quantification of stiffness measurement errors in resonant ultrasound spectroscopy**
2 **of human cortical bone**

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Resonant ultrasound spectroscopy (RUS) is the state-of-the-art method used to investigate the elastic properties of anisotropic solids. Recently, RUS was applied to measure human cortical bone, an anisotropic material with low Q-factor (20), which is challenging due to the difficulty in retrieving resonant frequencies. Determining the precision of the estimated stiffness constants is not straightforward because RUS is an indirect method involving minimizing the distance between measured and calculated resonant frequencies using a model. This work was motivated by the need to quantify the errors on stiffness constants due to different error sources in RUS, including uncertainties on the resonant frequencies and specimen dimensions and imperfect rectangular parallelepiped (RP) specimen geometry. The errors were firstly investigated using Monte-Carlo simulations with typical uncertainty values of experimentally measured resonant frequencies and dimensions assuming a perfect RP geometry. Secondly, the exact specimen geometry of a set of bone specimens were recorded by synchrotron radiation micro-computed tomography. Then, a 'virtual' RUS experiment is proposed to quantify the errors induced by imperfect geometry. Results show that for a bone specimen of $\sim 1^\circ$ perpendicularity and parallelism errors, an accuracy of a few percent ($< 6.2\%$) for all the stiffness constants and engineering moduli is achievable.

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14 I. INTRODUCTION

15 Bone adaptation in response to mechanical loading and the subsequent optimization of
16 bone strength are regulated by mechanosensitive osteocytes, which are capable of sensing
17 strain¹. For a given load, bone stiffness determines the local strain, hence investigating bone
18 stiffness in detail should allow gaining insight into bone functional adaptation mechanisms
19 and bone strength.

20 As the structure of human cortical bone, like many natural materials, is hierarchical², it
21 is necessary to investigate it at different scales. In particular, cortical bone elastic properties
22 at the mesoscale (millimeter-scale) are of special interest as they depend on tissue properties
23 at all the smaller length scales and have a direct impact on the mechanical behavior of bone
24 at the macroscale^{3,4}. In addition, this is the level at which cortical bone functions, in concert
25 with the overall gross shape of a bone in resisting functional loads⁵. The mesoscopic level
26 is also appropriate to investigate the regional variations of the elastic properties within a
27 bone⁶, which is necessary to refine finite element models to predict patterns of stress and
28 strain. In this context, precise and practical measurement methods for assessing cortical
29 bone elasticity at the mesoscale are needed.

30 In general, bone material can be considered as a transversely isotropic or orthotropic
31 material, hence engineering moduli such as Young's moduli, shear moduli, and Poisson's
32 ratio can be derived from the components of the stiffness tensor. Ultrasonic techniques
33 are well suited to probe the anisotropic elastic properties of bone. The most widely used
34 ultrasonic measurement method, which was introduced by Lang⁷ and used by many research
35 groups⁸⁻¹⁴, consists in measuring the ultrasonic wave velocity (UWV). Despite its apparent
36 simplicity, UWV measurements present several pitfalls that must be carefully considered.
37 The final result can be affected by some factors, including the size of the measured specimen
38 compared to the wavelength, the presence of heterogeneities, or the signal processing required
39 to estimate the time of flight to calculate velocity^{15,16}.

40 Resonant ultrasound spectroscopy (RUS) has been recently introduced as an alternative
41 technique to the measurement of human cortical bone stiffness¹⁷. RUS has been extensively
42 used since 1990's to investigate the elastic properties of solids as diverse as piezoelectric
43 materials¹⁸, metallic alloys¹⁹, metallic glasses²⁰ and composites²¹, hard polymers²², wood²³,
44 and mineralized tissues^{17,24,25} for applications ranging from theoretical physics to industrial

45 problems. The main advantage of RUS, compared to other techniques such as UWV mea-
 46 surements and mechanical testing, is that the full set of the elastic tensor can be assessed
 47 non-destructively from a single measurement^{26,27}. Briefly, in a RUS experiment, resonant
 48 frequencies of a free vibrating specimen are retrieved from the resonant spectrum measured
 49 by a pair of ultrasonic transducers. Then, the stiffness constants are adjusted using an
 50 iterative numerical procedure (inverse problem) until the calculated eigenfrequencies of a
 51 free vibration object (forward problem) match with the experimentally measured resonant
 52 frequencies.

53 Determining the precision of the different stiffness constants measured by RUS is not
 54 straightforward because RUS is an indirect method to obtain stiffness constants, involving
 55 the minimization of the distance between measured and calculated frequencies. Essentially,
 56 elasticity estimation errors arise from two sources^{19,26} (1) the imperfectly measured resonant
 57 frequencies; and (2) inadequate geometry of the forward model. The latter is caused by
 58 possible shape imperfections (i.e., non perfectly parallel or perpendicular surfaces) not taken
 59 into account in the model, and metrological errors in the measurement of the specimen's
 60 dimensions.

61 The effects of RUS measurement errors have been addressed to some extent in several
 62 studies in the case of perfectly rectangular parallelepiped (RP) shaped specimen geome-
 63 try^{26,28-30}. Regarding the first source of error (imperfectly measured resonant frequencies),
 64 the uncertainties on the determined stiffness constants have been estimated using the per-
 65 turbation theory (assuming perfect RP specimen geometry). By determining the sensitivity
 66 of the resonant frequencies to the stiffness constants, the uncertainties of the stiffness con-
 67 stants can be quantified as a function of the relative root mean square error (RMSE) σ_f
 68 expressing the misfit between the measured and calculated resonant frequencies^{26,29}. For
 69 instance, Sedlack et al.³⁰ quantified the typical uncertainties measured on a silicon carbide
 70 ceramics parallelepiped specimen and found relative measurement errors of less than 0.35%,
 71 0.80% and 2.80% for shear, longitudinal and off-diagonal stiffness constants respectively, for
 72 $\sigma_f = 0.25$ %. Regarding the second source of error (imperfect geometry), on an empirical
 73 basis, Migliori et al.^{26,31} recommended that shape errors in parallelism and perpendicularity
 74 between faces should be limited to 0.1% in order to keep errors on stiffness constants within
 75 acceptable bounds, that is, close to 1%. However, there is no data in the open literature to
 76 support these numbers, as far as we know.

77 When measuring bone elasticity using RUS, errors on the measured resonant frequencies
78 are larger compared to the case of other materials. This is related to the high viscoelastic
79 damping of the material (Q-factor ~ 20) resulting in resonant peaks overlapping and a
80 lower accuracy of the measured frequencies compared to the case of high-Q materials^{22,32}.
81 In many RUS applications only a few specimens are measured, and much time is devoted to
82 specimen's preparation in order to achieve an excellent geometrical quality. In contrast, the
83 high variability of elastic properties in biological materials, in particular within a bone³³,
84 implies that several tens of specimens should be measured in order to obtain representative
85 values of stiffness. As a result, polishing each bone specimen in successive steps³¹ to obtain
86 a very high geometrical quality is not practicable. Hence, the question arises of the accuracy
87 of the measured elasticity after a relatively simple preparation with a precision saw. To the
88 best of our knowledge, no systematic study has been conducted about neither the effects
89 of an imperfect specimen geometry on the elastic properties of cortical bone measured by
90 RUS, nor the combined effects when resonant frequencies uncertainties are also considered.

91 The objective of this study is to quantify the experimental errors when measuring cortical
92 bone elasticity with RUS. We take advantage of recent advances in RUS inverse problem
93 to quantify sources of errors using Monte Carlo simulations. Namely, the step consisting in
94 pairing measured frequencies and their calculated counterparts in the forward problem, pre-
95 viously achieved by an expert user with a trial-and-error method, was recently automated³⁴.
96 This allows an automated processing of RUS spectra which is a necessary condition for
97 Monte Carlo analyses of error propagation. The following error sources are considered:
98 (1) uncertainties on the measurement of frequencies; (2) uncertainties on the measurement
99 of dimensions (assuming a perfect RP shape); (3) imperfect specimen geometry (deviation
100 from a perfect RP). Although our primary focus is the application of RUS to measure bone,
101 the methodology introduced in this work and the quantified errors are of general interest for
102 the discussion of the precision and accuracy of RUS measurements of various materials.

103 Section II briefly recalls the theory of RUS, then Section III presents the specimens in-
104 cluded in this study and their experimental measurements. Firstly, their elasticity is assessed
105 by RUS and secondly, the geometry of the specimens is obtained from synchrotron radiation
106 micro-computed tomography (SR- μ CT) images. In Section IV, the effects of measurement
107 uncertainties caused by both specimen dimensions and frequency errors, are investigated by
108 Monte Carlo simulations. Section V investigates the errors associated to the deviation of

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109 the specimens's shape from a perfect RP. Here, the finite element method (FEM) is used
110 to calculate resonant frequencies accounting for the actual shape of the specimen. Finally,
111 results are discussed in Section VI.

112 II. RUS THEORY

113 RUS method is extensively described elsewhere^{26,27}. Here we summarize the process as
114 implemented in the present work. The determination of stiffness constants of the material
115 constitutive of a specimen of RP shape consists of the following steps: (1) the resonant
116 frequencies \mathbf{f}^{exp} of the specimen are measured; (2) using \mathbf{f}^{exp} , the stiffness constants \mathbf{C}_{ij}
117 ($ij = 11, 33, 13, 44, 66$) are determined by solving an optimization problem, i.e., minimizing
118 the objective function (Eq. (1))²⁶:

$$F(\mathbf{C}_{ij}) = \sum_k \left(\frac{f_k^{exp} - f_k^{mod}(\mathbf{C}_{ij})}{f_k^{exp}} \right)^2 \quad (1)$$

119 where \mathbf{f}^{mod} are simulated eigenfrequencies of a model of the specimen (forward problem)
120 and k is the index of the eigenfrequency. In the optimization, the mass is assumed known,
121 and the shape is assumed to be a perfect RP of known dimensions, collected in vector
122 **dim**. Frequencies \mathbf{f}^{mod} are calculated with the Rayleigh-Ritz method (RRM), which is a
123 semi-analytical method that yields the result in a fraction of a second on a modern desktop
124 computer. In Eq. (1), the experimental and simulated frequencies are assumed to be paired.
125 In the present work, pairing is done automatically in a Bayesian optimization strategy³⁴.

126 III. MEASUREMENTS

127 A. Specimens

128 Cortical bone specimens were harvested from the left femur of 18 human cadavers. The fe-
129 murs were provided by the Département Universitaire d'Anatomie Rockefeller (Lyon, France)
130 through the French program on voluntary corpse donation to science. The tissue donors or
131 their legal guardians provided informed written consent to give their tissue for investiga-
132 tions, in accord with legal clauses stated in the French Code of Public Health. Among the

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133 18 donors, 11 were females and 7 were males (50 – 95 years old, 77 ± 12.3 , mean \pm SD). The
134 fresh material was frozen and stored at -20°C .

135 The samples were slowly thawed and then, for each femur, approximately a 10 mm thick
136 cross section was cut perpendicular to the bone axis from the mid-diaphysis. The cross
137 section was then cut into 4 pieces (Fig. 1a). Two of these pieces (lateral and medial) were
138 then used to prepare a RP specimen. They were fixed on a stainless steel block (Fig. 1b)
139 that has three mutually perpendicular faces. Without unmounting the specimen, the steel
140 block was successively positioned on each of these three faces on a reference stage in order
141 to cut with a water-cooled low-speed diamond wire saw (Model 3241, Well, Lyon, France)
142 in three mutually perpendicular planes. From each donor, one or two RP shaped specimens
143 were prepared, which led to a set of 23 specimens. The nominal specimen size was $3 \times 4 \times 5$
144 mm^3 in radial (axis 1), circumferential (axis 2) and axial direction (axis 3), respectively,
145 defined by the anatomic shape of the femoral diaphysis. All specimens were kept hydrated
146 during sample preparation. The dimensions (dim^{exp}) and mass (m^{exp}) of each specimen
147 were measured by a digital caliper (precision ± 0.01 mm) and a balance (precision ± 0.1
148 mg), respectively.

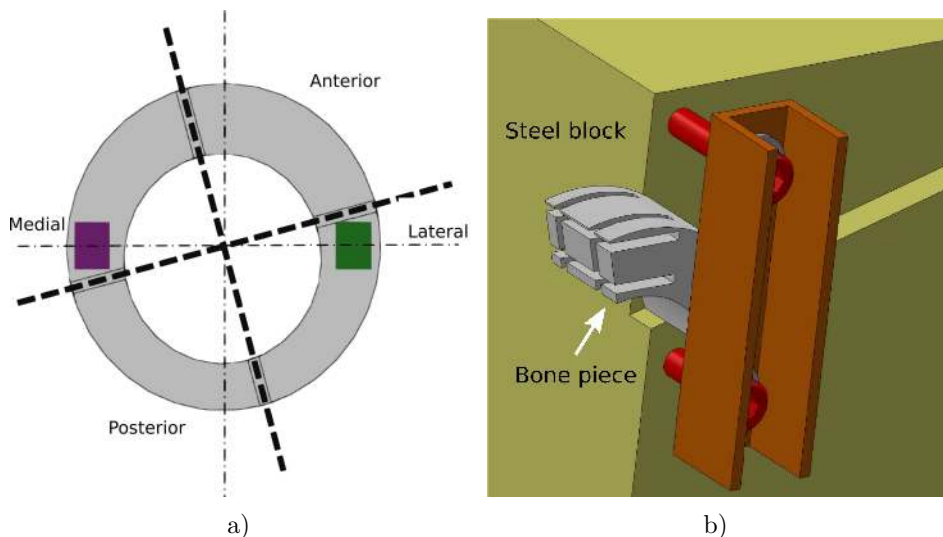


Figure 1. a) the cross section of a femur was cut into 4 pieces according to the anatomical locations: lateral, medial, posterior and anterior; b) the steel block on which a bone piece was fixed for being cut by a diamond wire saw to retrieve a cuboid specimen. Two pairs of perpendicular cuts were realized by successively positioning the block on a reference stage with two mutually perpendicular faces.

149 **B. Bone elasticity measurements by RUS**

150 The experiments to measure the resonant frequencies and the numerical inversion to
 151 calculate the stiffness constants were performed following the RUS methodology specially
 152 adapted for bone and extensively presented elsewhere^{17,34}. The procedure is briefly described
 153 as below. The bone specimen was placed on two opposite corners between two ultrasonic
 154 transducer (V154RM, Panametrics, Waltham, MA), one for emission and one for reception,
 155 to achieve a free boundary condition for vibration (Fig. 2).

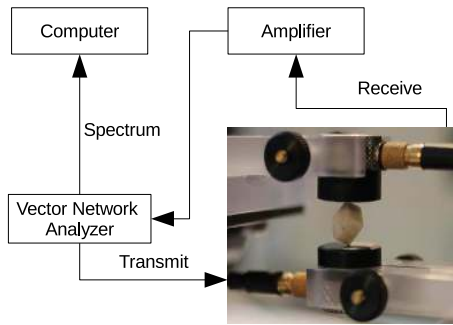


Figure 2. The RUS setup used in this study. A bone specimen is placed between two ultrasonic transducers at the two opposite corners to achieve a free boundary condition for vibration.

156 The frequency response of the vibration in a specified bandwidth, tuned so as to measure
 157 the 20-30 first resonant frequencies, was amplified by a broadband charge amplifier (HQA-
 158 15 M-10T, Femto Messtechnik GmbH, Berlin, Germany) and then recorded by a vector
 159 network analyzer (Bode 100, Omicron Electronics GmbH, Klaus, Austria). Six consecutive
 160 spectrum acquisitions were performed on each specimen at different orientations in order
 161 to maximize the number of detectable resonant frequencies. Then, the resonant frequencies
 162 were extracted from the spectra using the method dedicated to highly attenuative material³²
 163 (Fig. 3).

164 Finally, assuming a transversely isotropic symmetry^{12,35}, the stiffness constants \mathbf{C}_{ij}^{exp} ,
 165 were automatically calculated by solving the inverse problem formulated in a Bayesian
 166 framework³⁴(Sec. II). The prior information of the distribution of the stiffness constants,
 167 required for the Bayesian analysis, was taken from a previous study¹³. In the elastic tensor,
 168 $C_{12} = C_{11} - 2C_{66}$ and (1 - 2) is the isotropy plane; C_{11} and C_{33} are the longitudinal stiffness
 169 constants, C_{12} and C_{13} are the off-diagonal stiffness constants and C_{44} and C_{66} represent the
 170 shear stiffness constants.

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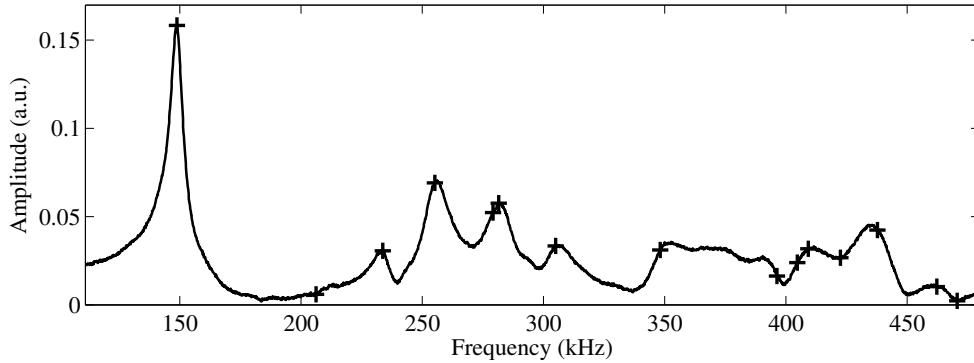


Figure 3. A typical resonant spectrum measured on a bone specimen. The plus signs (+) represent the extracted resonant frequencies.

171 C. Specimen geometry

172 The exact shape of the specimens and thus, deviation from the ideal RP shape was
173 obtained using SR- μ CT 3-D imaging, which was performed on the beamline ID19 at the
174 European Synchrotron Radiation Facility (ESRF, Grenoble, France). This SR- μ CT setup
175 is based on a 3D parallel beam geometry acquisition^{36,37}. The beam energy was tuned to
176 26 keV by using a (Si111) double crystal monochromator. A full set of 2D radiographic
177 images were recorded using a CDD detector (Gadox scintillator, optic lenses, 2048 \times 2048
178 Frelon Camera) by rotating the specimen in 1999 steps within a 360° range of rotation. The
179 detector system was fixed to get a pixel size of 6.5 μ m in the recorded images in which a
180 region of interest of 1400x940 pixels was selected to fit the specimen.

181 For each specimen, the SR- μ CT image (Fig. 4a) was reconstructed and binarized to
182 get the bone phase. In RUS, the material of the measured specimen is considered as a
183 homogeneous material. Here, the specimen is much larger than the representative volume
184 element of continuum mechanics⁴. Accordingly, the vascular pores that are visible in the 3D
185 image were filled up (Fig. 4b) using mathematical morphology operations to obtain a mask
186 of each slice. Then the convex envelope of the bone masks was calculated and considered to
187 be the exact shape of the specimen.

188 The quality of the geometry of the specimen was analyzed based on the reconstructed SR-
189 μ CT volume. The coordinates of the cloud of points of each specimen's face were collected
190 and the equation of the planes fitting each face in the least-square sense were determined.
191 The angles α and β between the normal of the planes were used to quantify the quality of
192 the specimen's geometry compared to a perfect RP (Fig. 5). The perpendicularity errors

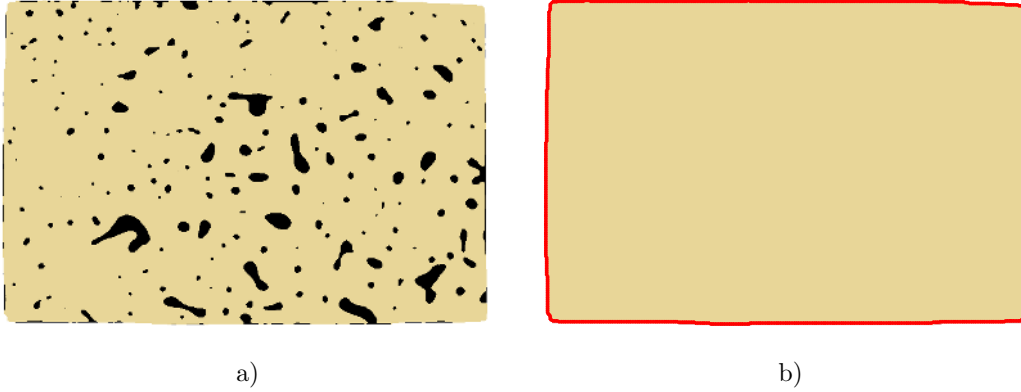


Figure 4. a) A slice of the binarized image of bone structure. b) The mask of the bone slice after filling up the pores (the black parts in a)) and the contours (red color) detected from the mask. The contours of all the masks determine the external envelope of the specimen which was used to quantify its perpendicularity and parallelism quality.

193 between adjacent faces were quantified by $\delta\alpha = 90^\circ - \alpha$. The parallelism errors between
 194 opposite faces were quantified by $\delta\beta = 180^\circ - \beta$. The values of the angle errors for the 23
 195 specimens (12 $\delta\alpha$ and 3 $\delta\beta$ per specimen) are collected in Fig. 6. The deviations (mean \pm std)
 196 from ideal perpendicularity and parallelism were $-0.07^\circ \pm 0.85^\circ$ and $0.30^\circ \pm 0.78^\circ$, respectively.

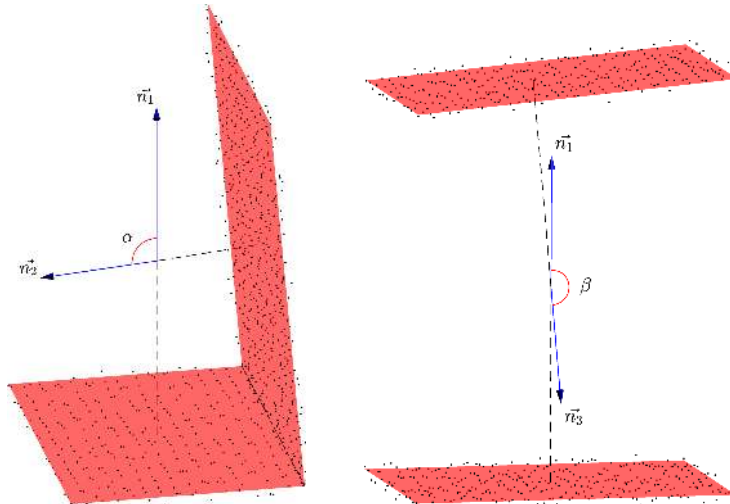


Figure 5. The angle α is defined by the angle between the normal (\vec{n}_1 and \vec{n}_2) of two adjacent faces which are found by fitting the cloud of points (the dots in the figures) with the equation of the best plane in the least-square sense; accordingly, β is defined by the angle between the normal (\vec{n}_1 and \vec{n}_3) of two opposite faces. For a perfect RP, α and β should equal to 90° and 180° , respectively.

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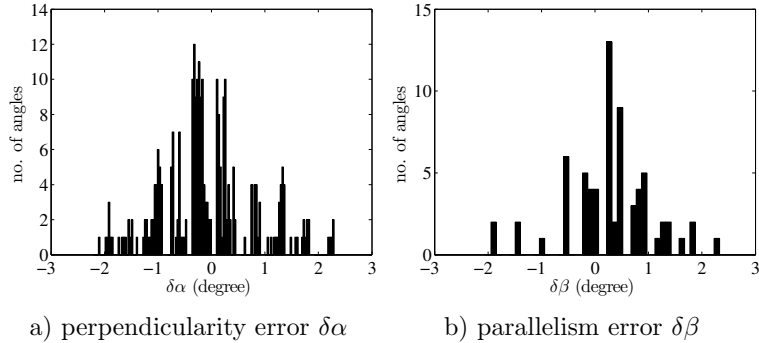


Figure 6. The distributions of the perpendicularity error $\delta\alpha$ and parallelism error $\delta\beta$ of the 23 specimens.

197 IV. SIMULATION OF THE ERRORS DUE TO UNCERTAINTIES ON 198 RESONANT FREQUENCIES AND DIMENSIONS

199 A. Method

200 We consider a perfect RP specimen as a reference, characterized by the dimensions \mathbf{dim}^0 ,
201 mass m^0 and stiffness constants \mathbf{C}_{ij}^0 shown in Table I. In Table I, the values of \mathbf{dim}^0 are
202 the mean values of the dimensions of the specimens used in this work. The value of m^0 was
203 calculated assuming a typical mass density value of 1.87 mg/mm^3 taken as the mean value
204 from a former study about human femoral cortical bone¹³. The values of \mathbf{C}_{ij}^0 correspond to
205 the mean values of the stiffness of human femoral cortical bone at the mid-diaphysis¹³. The
206 first 40 eigenfrequencies \mathbf{f}^0 of the reference specimen were calculated using the RRM. This
207 number of frequencies was chosen according to the experimental frequency bandwidth in RUS
208 measurements on human cortical bone specimens, which in practice contains approximately
209 40 resonant frequencies.

Table I. Properties of the reference RP bone specimen. The eigenfrequencies \mathbf{f}^0 of the reference specimens are associated to the parameters in this table.

\mathbf{dim}^0 (mm)	m^0 (mg)	\mathbf{C}_{ij}^0 (GPa)
$3 \times 4 \times 5$	112.2	19.58 29.04 11.74 5.83 4.28

210 In this section, Monte-Carlo simulations³⁸ were performed to quantify the propagation of
211 the errors due to uncertainties on resonant frequencies and specimen dimensions. Repeated

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212 calculations of the stiffness constants were performed, each time randomly varying the in-
213 put data (dimensions or/and resonant frequencies) within their stated limits of precision.
214 Then we quantified the variability of each stiffness constant caused by dimension errors, by
215 frequency errors, and by the association of both dimension and frequency errors.

216 The order of magnitude of the dimension error to be used in Monte-Carlo simulations
217 was obtained comparing, for each specimen, the SR- μ CT image with the dimensions \mathbf{dim}^{exp}
218 measured with the caliper. Specimen's dimensions obtained from the SR- μ CT image are
219 considered as a reference based on which the uncertainty of \mathbf{dim}^{exp} can be estimated. In
220 order to obtain a representative value ϵ of the dimension error, we compared, for each
221 specimen the volume of the bone SR- μ CT images and the volume of a hypothetical RP
222 of dimensions $\mathbf{dim}^{exp} \pm \epsilon$. By equating these volumes for each of the 23 specimens and
223 solving the equations, we obtained a series of values of ϵ shown in Fig. (7). The specimen's
224 dimensions obtained from the SR- μ CT image were found to be systematically smaller than
225 \mathbf{dim}^{exp} . We choose the mean value of $\epsilon \approx 0.04$ mm as a conservative value to represent the
226 accuracy of the dimensions measured by caliper. Accordingly, the uncertainty of \mathbf{dim}^{exp} was
227 set to 0.04 mm.

228 The standard error on the measured resonant frequencies used in Monte-Carlo simula-
229 tions was chosen to be 0.5%, which is typically the repeatability of the measured resonant
230 frequencies in bones¹⁷.

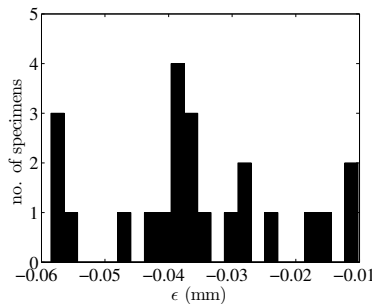


Figure 7. The distribution of the dimension error ϵ obtained by comparing for each specimen the volume of the bone SR- μ CT reconstruction and the volume of a hypothetical RP of dimensions $\mathbf{dim}^{exp} \pm \epsilon$.

231 **1. Effects of uncertainties on dimension**

232 To quantify the effects of imprecise dimension measurements, 1000 random realizations of
 233 dimensions were generated from independent normal distributions centered on \mathbf{dim}^0 with a
 234 standard deviation of 0.04 mm, $\mathbf{dim}^p \sim N(\mathbf{dim}^0, 0.04^2)$. The number of random realizations
 235 was chosen following preliminary convergence tests. For each realization p , the stiffness con-
 236 stants \mathbf{C}_{ij}^p , were obtained by solving the inverse problem using \mathbf{f}^0 as proxy for experimental
 237 frequencies, and the frequencies \mathbf{f}^p calculated for the inadequate forward model: specimen
 238 of perfect RP shape with uncertain dimensions \mathbf{dim}^p . The mass used in the forward model
 239 is that of the reference RP specimen (Table I). The inverse problem uses the objective
 240 function defined in Eq. (2). The stated input parameters for the simulation are summarized
 241 in Fig. 8 (block D).

$$F(\mathbf{C}_{ij}^p) = \sum_k \left(\frac{f_k^0 - f_k^p(\mathbf{C}_{ij}^p)}{f_k^0} \right)^2 \quad (2)$$

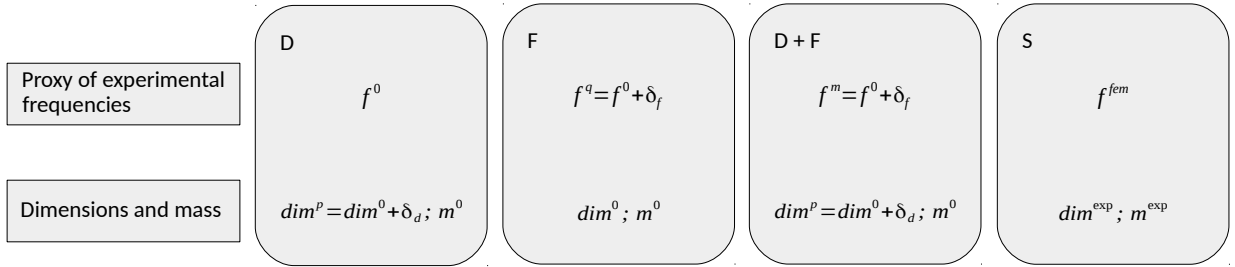


Figure 8. The input parameters for the simulations detailed in Secs. IV and V for quantifying stiffness estimation errors due to the experimental error sources: dimensions imprecision (block D), frequencies imprecision (block F), dimensions and frequencies imprecision (block D + F) and the imperfect specimen geometry (block S). δ_d and δ_f represent the deviations from the reference values dim^0 and f^0 , respectively, that are randomly generated for each realization. Given dimension and mass are the constants used for the forward model.

242 **2. Effects of uncertainties on frequencies**

243 In a similar way, for the analysis of frequency imprecision, 1000 random realizations of
 244 frequencies from a normal distribution centered on \mathbf{f}^0 were generated assuming a relative
 245 standard deviation of 0.5%, $\mathbf{f}^q \sim N(\mathbf{f}^0, (0.005\mathbf{f}^0)^2)$. The number of random realizations
 246 was chosen following preliminary convergence tests. The stiffness constants \mathbf{C}_{ij}^q , were then

247 obtained by solving the inverse problem based on the objective function (Eq. (3)) using \mathbf{f}^q
 248 as proxy for experimental frequency values with an error and \mathbf{f}^r calculated using \mathbf{dim}^0 , m^0
 249 and assuming a perfect RP specimen (Table I). The input parameters are summarized in
 250 Fig. 8 (block F).

$$F(\mathbf{C}_{ij}^q) = \sum_k \left(\frac{f_k^q - f_k^r(\mathbf{C}_{ij}^q)}{f_k^q} \right)^2 \quad (3)$$

251 3. Effects of uncertainties on dimension and frequencies

252 Finally, the effects of the association of dimension and frequency errors were analyzed
 253 together. Assuming the uncertainties on frequency and dimension are 0.5% and 0.04 mm,
 254 respectively, 200 independent frequency realizations and 200 independent realizations of di-
 255 mensions were generated from normal distributions, $\mathbf{f}^m \sim N(\mathbf{f}^0, (0.005\mathbf{f}^0)^2)$ and $\mathbf{dim}^n \sim$
 256 $N(\mathbf{dim}^0, 0.04^2)$. The number of random realizations was chosen following preliminary con-
 257 vergence tests. The stiffness constants \mathbf{C}_{ij}^{mn} were then obtained by solving the inverse prob-
 258 lem using \mathbf{f}^m as proxy for experimental frequencies with errors and \mathbf{f}^n calculated for the
 259 inadequate forward model : specimen of perfect RP shape with uncertain dimensions \mathbf{dim}^n .
 260 The mass used in the forward model is m^0 (Table I). Precisely, this is done using the ob-
 261 jective function defined in Eq. (4). The input parameters are summarized in Fig. 8 (block
 262 D+F).

$$F(\mathbf{C}_{ij}^{mn}) = \sum_k \left(\frac{f_k^m - f_k^n(\mathbf{C}_{ij}^{mn})}{f_k^m} \right)^2 \quad (4)$$

263 4. Data Analysis

264 For the three cases described above, the error $\delta\mathbf{C}_{ij}^{est}$ is calculated for each realization of
 265 the determined stiffness constants as

$$\delta\mathbf{C}_{ij}^{est} = \frac{\mathbf{C}_{ij}^{est} - \mathbf{C}_{ij}^0}{\mathbf{C}_{ij}^0} \times 100\% \quad (5)$$

266 where $\mathbf{C}_{ij}^{est} = (\mathbf{C}_{ij}^p, \mathbf{C}_{ij}^q, \mathbf{C}_{ij}^{mn})$ and \mathbf{C}_{ij}^0 is the elasticity of the reference specimen.

267 **B. Results**

268 The normality of the distribution of each $\delta\mathbf{C}_{ij}^{est}$ was verified using Shapiro-Wilk's test
 269 ($p < 0.05$). Table II summarizes the distribution of $\delta\mathbf{C}_{ij}^{est}$ (Eq. (5)) and the root-mean-
 270 square error σ_f representing the quality of the frequency fit at the minimum of the objective
 271 function. The engineering moduli, including the Young's moduli (E_1 and E_3) and the
 272 Poisson's ratio (ν_{23} , ν_{31} and ν_{21}), were also compared to the reference values (obtained from
 273 \mathbf{C}_{ij}^0 in Table I). The errors are summarized in Table II. The 95% confidence intervals
 274 (CIs) of the errors were evaluated (Fig. 9). For case (D), (F) and (D+F), the 95% CIs
 275 were calculated as $\text{mean} \pm 2 \times \text{SD}$. The values of the errors indicated in the following text
 276 correspond to the larger absolute value of the 95% CI bounds, unless otherwise stated.

Table II. The errors (mean \pm SD in %) on stiffness constants (Eq. (5)) and the engineering moduli due to four sources of error: uncertainties on dimension (D), on frequencies (F), on dimension and frequencies together (D+F) and imperfect specimen geometry (S) detailed in Sec. V.

Error source	D	F	D+F	S
δC_{11}	-0.41 \pm 1.51	-0.12 \pm 1.41	-0.52 \pm 1.70	3.56 \pm 1.61
δC_{33}	0.16 \pm 2.68	0.00 \pm 1.44	0.13 \pm 2.52	-2.18 \pm 1.51
δC_{13}	-0.21 \pm 1.38	-0.09 \pm 2.60	-0.36 \pm 2.16	2.22 \pm 2.08
δC_{44}	0.02 \pm 1.27	0.00 \pm 0.53	0.07 \pm 1.41	-0.52 \pm 0.91
δC_{66}	-0.01 \pm 1.10	-0.03 \pm 0.48	-0.05 \pm 1.18	0.85 \pm 0.68
δE_1	-0.16 \pm 1.08	-0.07 \pm 0.46	-0.22 \pm 1.09	1.55 \pm 0.82
δE_3	0.17 \pm 2.62	-0.00 \pm 1.09	0.20 \pm 2.67	-3.19 \pm 1.10
$\delta \nu_{23}$	0.03 \pm 1.92	-0.01 \pm 1.94	-0.06 \pm 2.68	2.78 \pm 1.15
$\delta \nu_{31}$	0.34 \pm 2.26	0.05 \pm 1.79	0.32 \pm 2.24	-2.01 \pm 1.53
$\delta \nu_{21}$	-0.50 \pm 2.06	-0.14 \pm 1.89	-0.55 \pm 2.24	2.42 \pm 1.49
σ_f	0.35 \pm 0.23	0.43 \pm 0.06	0.58 \pm 0.16	0.29 \pm 0.09

277 The errors caused by dimension imprecision (case (D)) and both dimension and frequency
 278 imprecision (case (D+F)) are comparable, i.e., less than 5.5% for C_{11} , C_{33} and C_{13} , less than

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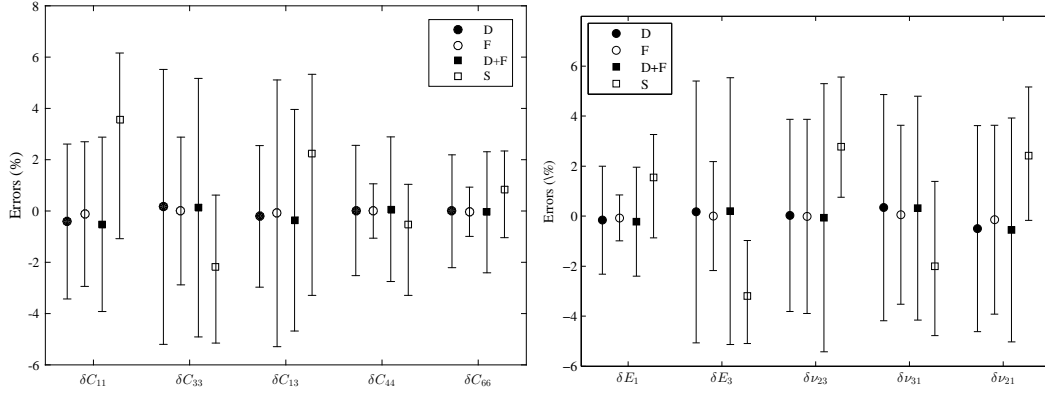


Figure 9. The mean and 95% confidence intervals of the errors on stiffness constants and engineering moduli corresponding to case (D), (F), (D+F) and (S). The error bars show the upper and lower bounds of the intervals and the mean values are represented at the center of the errorbars by the 'circle' or 'square' makers. For case (D), (F) and (D+F) the intervals were estimated as $\text{mean} \pm 2 \times \text{SD}$, for case (S) they were evaluated by fitting the cumulative distribution functions of the errors using kernel density estimators.

279 2.9% for C_{44} and C_{66} . Similar observation also applies to the engineering moduli for which
 280 the errors are less than 2.4% for E_1 , 5.5% for E_3 and 5.4% for the Poisson's ratios. Errors
 281 caused by frequency imprecision alone are less than 1.1% for C_{44} and C_{66} , 2.9% for C_{11} and
 282 C_{33} and 5.3% for C_{13} , which agrees well with the sensitivities of resonant frequencies to the
 283 stiffness constants²⁹. The error δC_{13} is larger when frequencies are imprecise compared to
 284 when dimensions are imprecise. The errors on shear stiffness constants (δC_{44} and δC_{66}) are
 285 smaller than the errors on longitudinal (δC_{11} and δC_{33}) and off-diagonal stiffness constants
 286 (δC_{13}) in all the 3 cases (D, F, and D+F). Overall, δE_3 is two times larger than δE_1 and
 287 the accuracy associated to Young's moduli E_1 and E_3 are similar to that associated to
 288 C_{11} and C_{33} . For all the stiffness constants, σ_f are around 0.35%, 0.43% and 0.58% when
 289 dimension imprecision, frequency imprecision and both dimension and frequency imprecision
 290 are considered, respectively.

291 **V. SIMULATION OF THE ERRORS DUE TO IMPERFECT SPECIMEN**
 292 **GEOMETRY**

293 **A. Method**

294 In RUS, the inverse problem to determine stiffness constants is solved assuming that
 295 the specimen is a perfect RP. In this section, we investigate the uncertainty on stiffness
 296 associated to this assumption resorting to a 'virtual' RUS experiment (Fig. 8 (block S) and
 297 Fig. 10):

298 (1) For each of the 23 bone specimens, the resonant frequencies \mathbf{f}^{fem} were calculated using
 299 the finite element method considering the actual specimen's geometry derived from SR- μ CT
 300 images, measured mass m^{exp} and specimen's stiffness \mathbf{C}_{ij}^{exp} determined in the usual manner
 301 assuming a perfect RP shape. Details on the finite element implementation are given in
 302 appendix (Appendix A).

303 (2) The stiffness constants \mathbf{C}_{ij}^{fem} of each specimen were estimated solving the inverse
 304 problem defined by the frequencies \mathbf{f}^{fem} (the first 40 frequencies) considered as measurements
 305 and a forward model characterized by a perfect RP geometry (dimensions \mathbf{dim}^{exp}) and
 306 specimen's mass (m^{exp}) (Sec. III).

307 These resulting \mathbf{C}_{ij}^{fem} are the stiffness constants of a RP bone specimen that would exhibit
 308 the same resonant frequencies as the imperfect shape bone specimens with stiffness constants
 309 \mathbf{C}_{ij}^{exp} . Constants \mathbf{C}_{ij}^{fem} are biased by imperfect specimen geometry and are compared to the
 310 true stiffness constants of the specimen used in the FEM model (\mathbf{C}_{ij}^{exp}). Namely, we calculate
 311 the errors $\delta\mathbf{C}_{ij}^{fem} = \frac{\mathbf{C}_{ij}^{fem} - \mathbf{C}_{ij}^{exp}}{\mathbf{C}_{ij}^{exp}} \times 100\%$.

312 **B. Results**

313 The errors on stiffness constants and the engineering moduli due to imperfect geometry
 314 of the specimens are summarized in Table II (last column). As only 23 specimens were
 315 included and the errors were not normally distributed, the 95% CIs of the errors (Table III
 316 and Fig. 9) were evaluated by fitting the cumulative distribution functions of the errors
 317 using kernel density estimators. For all the stiffness constants, there is a bias, i.e. the
 318 mean value of the errors is not zero and it can be positive or negative depending on the
 319 constant (the mean values vary from -3.19% to 3.56%). The SD of the errors varies from

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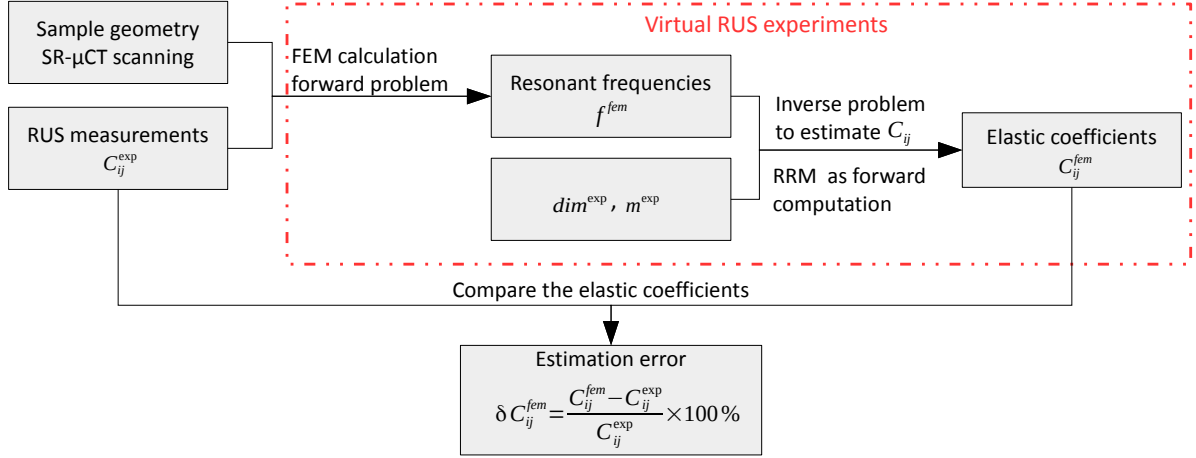


Figure 10. Diagram of the FEM simulation for quantifying the bias caused by imperfect specimen geometry. C_{ij}^{exp} are bone stiffness constants measured by RUS (Section III B), f^{fem} are the resonant frequencies calculated from the actual specimen geometry, \mathbf{dim}^{exp} are the dimensions of the specimens measured by caliper, C_{ij}^{fem} are the stiffness constants calculated by solving the inverse problem, and δC_{ij}^{fem} represent the estimation errors.

320 0.68% to 2.08%. In particular, the errors on shear stiffness constants present a smaller
 321 variation than longitudinal and off-diagonal ones (see the 95% CIs in Table III) and the errors
 322 on Young’s moduli present slightly less variability compared to the longitudinal stiffness
 323 constants (Table II and III).

Table III. The 95% CIs (in %) of the errors on stiffness constants and the engineering moduli due to imperfect specimen geometry.

	δC_{11}	δC_{33}	δC_{13}	δC_{44}	δC_{66}
95% CI	[-1.08, 6.16]	[-5.15, 0.62]	[-3.29, 5.33]	[-3.29, 1.04]	[-1.04, 2.34]
	δE_1	δE_3	$\delta \nu_{23}$	$\delta \nu_{31}$	$\delta \nu_{21}$
95% CI	[-0.87, 3.27]	[-5.09, -0.98]	[0.75, 5.56]	[-4.78, 1.39]	[-0.17, 5.17]

324 VI. DISCUSSION AND CONCLUSION

325 In this study, we performed simulations to quantify the errors on the stiffness constants
 326 determined from RUS measurements. We used typical elasticity values of human cortical
 327 bone as reference and studied the effects of errors due to (1) uncertainties on the mea-

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328 surement of frequencies; (2) uncertainties on the measurement of dimensions (assuming a
329 perfect RP shape); (3) imperfect specimen's geometry (deviation from a perfect RP). The
330 first two points were addressed with a calculation of error propagation with Monte-Carlo
331 simulations which require a statistical model of the quantities investigated. For dimensions
332 (of an assumed perfect RP) and frequencies, it is reasonable to assume normal distributions
333 around the reference values. The third source of error is the deviation of the shape from a
334 perfect RP. In that case we do not have a statistical model for the shape alterations, i.e.,
335 Monte-Carlo simulations cannot be used. Hence, the third point was addressed using actual
336 experimental data on a collection of 23 bone specimens. The main parameters of the Monte-
337 Carlo simulations were the assumed level of error on experimentally determined resonant
338 frequencies, set to 0.5%, and experimentally determined specimen's dimensions, set to 0.04
339 mm ($\sim 1\%$). The choice of these values is consistent with our experience of using RUS to
340 measure bone specimens¹⁷.

341 Using micro-CT, we could quantify the range of geometrical errors associated to a simple
342 specimen's preparation procedure. We found that perpendicularity and parallelism errors
343 were in average less than 1° and always less than 2° (Fig. 6).

344 Overall, we found errors on elasticity values of a few percents, or less than one percent,
345 depending on the considered stiffness constant. Note that we discuss the accuracy errors
346 reporting the 95% CIs of the error. Consistent with the findings of several previous stud-
347 ies^{26,29}, we found that the off-diagonal stiffness constants presented the highest errors and
348 shear constants the smallest ones. This is related to the higher sensitivity of RUS to shear
349 stiffness constants. Comparing the uncertainties of the sources of error (dimensions and
350 frequencies) and the uncertainties of the errors on shear stiffness constants (the most pre-
351 cisely determined ones), comparable values were observed (Table II), i.e., 0.04 mm ($\sim 1\%$)
352 uncertainty on dimensions and 0.5% uncertainty on frequencies leads to $\sim 1.2\%$ and $\sim 0.5\%$
353 uncertainties on the errors of shear stiffness constants, respectively. Additional Monte-Carlo
354 simulations, following the same routine in Section IV A showed that increasing the error level
355 of dimensions and frequencies by 20%, i.e., the uncertainties of dimensions and frequencies
356 became 0.05 mm and 0.6%, respectively, will increase the CIs of the error on shear stiffness
357 by $\sim 16\%$ to 25%, approximately.

358 For all the stiffness constants but C_{13} , dimension uncertainties lead to larger errors in
359 elasticity compared to the case where only frequency uncertainties are considered (Table II

360 and Fig. 9). For C_{13} , the largest error is observed for frequency uncertainties, suggesting that
 361 C_{13} may be less sensitive to dimension imperfections than to resonant frequencies in current
 362 simulation conditions. Interestingly, dimension uncertainties or a coupling of frequency and
 363 dimension uncertainties caused similar levels of errors on the stiffness constants (Table II
 364 and Fig. 9).

365 Deviation of the actual specimen's shape from a perfect RP affects the accuracy of the
 366 stiffness constants measured with RUS. This is because the forward model used to solve the
 367 inverse problem, assuming a perfect RP geometry, is not correct. The approach introduced
 368 in Section V aimed at simulating the effect of this source of error. It is important to
 369 note that, in general (when a micro-CT scan of the specimen is not available), only the
 370 mass can be accurately measured as opposed to the dimensions (because the geometry is
 371 in general not perfect). This is the reason why mass (m^{exp}) but not mass density was used
 372 in the simulations in Secs. IV and V. The uncertainty of the mass was about 0.1% since
 373 the precision of the balance is ± 0.1 mg and the mass of the bone specimens are around
 374 100 mg. A linear relationship exists between mass and stiffness constants, consequently,
 375 for given dimensions and resonant frequencies²⁶, a mass uncertainty of 0.1% will cause the
 376 same uncertainty (0.1%) on the stiffness constants, which is negligible compared to the error
 377 levels caused by other factors. Accordingly, the uncertainty of mass was not considered in
 378 this work. We have observed that most of the caliper-measured volumes were overestimated
 379 of approximately 3% in average compared to the volumes deduced from SR- μ CT images.
 380 Accordingly, the quantified elasticity errors are a result of both overestimated dimensions
 381 and irregularity of the RP shape. The elasticity errors due to an imperfect RP geometry
 382 (Table III and Fig 9) were between 2.3%~6.2%. The comparison of the contribution of the
 383 three sources of errors to the precision of RUS measurements shows that errors due to an
 384 imperfect geometry are found to be of the same order as the errors calculated by Monte-Carlo
 385 simulations caused by frequency or dimension uncertainties in our specific case.

386 It is noteworthy that the values of σ_f obtained from simulations in the present study
 387 ($\sigma_f \approx 0.58\%$ with Monte-Carlo simulations and $\sigma_f \approx 0.29\%$ using FEM simulations with
 388 the imperfect shape) are similar to values reported for actual RUS measurements of bone
 389 and other attenuative materials^{17,22} where σ_f is typically in the range 0.25-0.40%. This
 390 suggests that the simulations accurately reproduce the experimental error characteristic of
 391 RUS measurements. The level of errors quantified in the present study are consistent with

392 the reported precision of RUS for human cortical bone application (3%, 5% and 0.4% for
 393 longitudinal, off-diagonal and shear stiffness constants, respectively)¹⁷, estimated from the
 394 RMSE σ_f .

395 This study has introduced an original methodology to quantify errors in RUS measure-
 396 ments. The method was applied to bone but could be used to assess the accuracy for RUS
 397 measurements of various materials. Note that it has been possible to implement Monte-
 398 Carlo simulations only because an automated pairing of frequencies (for the calculation of
 399 the objective function) was possible. This automated pairing was initially developed to
 400 process spectra of attenuative materials where several resonant peaks can not be retrieved³⁴
 401 and it is also efficient to process synthetic resonant frequencies as in the present study where
 402 no peak is missing.

403 In RUS measurements, specimens are assumed to be homogeneous, although cortical
 404 bone specimens are inhomogeneous to some extent. When the wavelength is much greater
 405 than the length scale of the inhomogeneity, the material can be regarded as a homogeneous
 406 material. A conservative estimation of acceptable inhomogeneity in RUS was suggested by
 407 Ulrich et.al.²⁸. The maximum size of an inhomogeneity should be smaller than a threshold
 408 $\xi \leq 2l/n$, where l is the smallest dimension of the sample and n can be taken as the number
 409 of the considered resonant frequencies. Here with $l = 3 \text{ mm}$ and $n = 40$, the threshold
 410 is $\xi = 150 \text{ }\mu\text{m}$, which is larger than the diameter of the pores in human cortical bone
 411 (Haversian canals diameter is typically in the range of $20 - 100 \text{ }\mu\text{m}$). According to this
 412 criterion, bone specimens in the present work may be considered as homogeneous.

413 Aside of the uncertainties in the values of the inputs in RRM, including mass, dimensions
 414 and stiffness constants, the RRM has a limited accuracy associated to the truncation to M -
 415 th order of the polynomial approximation of the displacement field. Resonant frequencies
 416 calculated with RRM are more accurate with increasing values of M but as a counterpart,
 417 the computing time increases. In the present work, this was a critical issue because large
 418 numbers of iterations were involved to solve the inverse problem in the Bayesian framework.
 419 In practice, $M = 10$ used in this study, following the suggestion by Migliori and Sarrao²⁶, is a
 420 good compromise between accuracy and computing time if the first 50 resonant frequencies
 421 are considered. A preliminary test showed that the root-mean-square-error between the
 422 RRM-yielded frequencies when $M = 10$ and $M = 20$ is close to 0.07% for the first 40
 423 frequencies, which is negligible compared to the magnitude of other sources of error that we

424 handled with in this work.

425 This study has some limitations. We used simulated resonant frequencies as proxy for
 426 RUS data as input to the inverse problem. Precisely, the eigenfrequencies of the first forty
 427 vibration modes were used. In actual RUS experiments to measure bone, a maximum of
 428 fifteen to twenty frequencies among the first forty can actually be retrieved due to peak
 429 overlapping²². In theory, taking into account more frequencies should improve the precision
 430 of the determination of stiffness constants because more information is used for the inverse
 431 problem. However, in practice, the achievable precision also depends on the quality of the
 432 frequency measurement which decreases in the higher frequency range due to the increased
 433 modal density and peak overlapping. Since the resonant frequencies are much more sensitive
 434 to shear stiffness constants³⁹, it is expected that using less frequencies than in the present
 435 study would essentially decrease the precision of constants C_{11} , C_{33} and C_{13} but would have
 436 little impact on the precision of the shear stiffness constants. The results of the simulation
 437 in Sec. V critically rely on the actual pixel size in SR- μ CT experiments, because the exact
 438 shape of the specimens were used to compute the 'true' resonant frequencies for the inverse
 439 problem. However, we did not perform calibration for identifying the actual pixel size during
 440 SR- μ CT experiments. This could partly affect or bias the results. Another limitation is
 441 that we did not simulate the error on stiffness constants due to a combination of frequency
 442 uncertainty and imperfect RP geometry. In view of the results of Sec. IV, we expect that
 443 elasticity errors would only be slightly larger. Furthermore, some sources of errors in RUS
 444 have not been considered such as the effect of imperfect boundary conditions⁴⁰ and the
 445 uncertainty on the measurement of specimen's mass.

446 The validation of the measurement of bone elasticity with RUS relies (1) on the successful
 447 measurement of a reference transverse isotropic material with a Q-factor similar as bone's
 448 Q-factor²²; (2) on the comparison of the stiffness constants obtained with RUS and from
 449 the independent measurement of the time-of-flight of shear and longitudinal waves in bone
 450 specimens^{16,17}; and (3) on the results of the present study focused on the quantification of
 451 accuracy errors. The latter suggest that despite the typical non-perfect geometry of bone
 452 specimens and despite the relatively large uncertainty in the measurement of the bone reso-
 453 nance frequencies (due to attenuation), the stiffness constants are obtained with a maximum
 454 error of a few percents. A very conservative accuracy value can be quantified by the larger
 455 absolute value of the (non symmetric) 95% CI bounds; accuracy defined like this was 6.2%

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456 for longitudinal stiffness and 3.3% for shear stiffness, 5.1% for Young's moduli and 5.6% for
457 Poisson's ratios (Table III).

458 To further enhance the accuracy of bone RUS measurement, possible paths would be
459 (1) using a specific implementation of the Rayleigh-Ritz method for nonrectangular par-
460 allelepiped specimen²⁹, provided that the angles between the specimen's surfaces can be
461 measured; (2) decreasing the frequency uncertainty by improving the signal processing of
462 RUS spectra.

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473 Appendix A: Calculation of resonant frequencies using Finite element 474 modeling (FEM)

475 Bone was modeled as a homogeneous transversely isotropic material. The bone volumes
476 obtained from the SR- μ CT were discretized into about 3 million quadratic tetrahedral el-
477 ements. This corresponded to a maximum element size of 0.12 mm, which was chosen
478 after a convergence study and ensures at least 10 elements per smallest wavelength in the
479 investigated frequency bandwidth. A modal analysis was conducted to calculate the eigen-
480 frequencies. We used the software Code-Aster (ver 12.5, EDF R&D, France, license GNU
481 GPL, <http://www.code-aster.org>).

482 The accuracy of the finite element model was evaluated by comparing the first 40 FEM
483 eigenfrequencies to eigenfrequencies calculated with the Rayleigh-Ritz method for a perfect

484 RP bone specimen (Table I). The RMSE σ_f between eigenfrequencies calculated by the two
485 methods was $\sim 0.06\%$. After solving the inverse problem using FEM eigenfrequencies, the
486 errors in the stiffness constants were $\sim 0.05\%$, 0.60% and 0.30% on shear, longitudinal and
487 off-diagonal stiffness constants. These errors are at least one order of magnitude smaller
488 than the errors related to shape imperfections (Sec. V).

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