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# 1 Calcified cartilage revealed in whole joint 2 by X-ray Phase contrast imaging

3  
4 Ludovic Broche<sup>1,2</sup>, Bertrand Favier<sup>3</sup>, H el ene Roug e-Labriet<sup>2,4</sup>, Sabine Drevet<sup>3,5</sup>, Bernard  
5 Lardy<sup>3,6</sup>, Emmanuel Brun<sup>2,\*</sup>, Benjamin Lemasson<sup>1</sup>

6  
7 <sup>1</sup>Univ. Grenoble Alpes, INSERM, U1216, Grenoble Institut Neurosciences, Grenoble, France

8 <sup>2</sup>University Grenoble Alpes, Inserm, UA7, STROBE Laboratory, Grenoble, 38000, France

9 <sup>3</sup>University Grenoble Alpes, GREPI EA7408, Grenoble, 38000, France

10 <sup>4</sup>Novitom, Grenoble, 38000, France

11 <sup>5</sup>University hospital A. Michallon, Orthogeriatric Unit, Geriatric Department Grenoble, 38000,  
12 France

13 <sup>6</sup>University hospital A. Michallon, Biochemical Enzymology and protein laboratory, Grenoble,  
14 38000, France

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16

## 17 Abstract

18 **Objective:** X-ray Phase Contrast Imaging (PCI) is an emerging modality that will be in the next few years  
19 available in a wider range of preclinical set-ups. In this study, we compare this imaging technique with  
20 conventional preclinical modalities in an osteoarthritis mouse model.

21 Method

22 Phase contrast technique was performed on 6 post-mortem, monoiodoacetate-induced osteoarthritis  
23 knees and 6 bilateral control knees. The mice knees were then imaged using magnetic resonance imaging  
24 and conventional micro computed tomography. Examples of imaging surrogate markers are reported:  
25 local distances within the articular space, cartilage surface roughness, calcified cartilage thickness,  
26 number, volume and locations of osteophytes.

27 **Results:** Thanks to PCI, we can show in 3D calcified cartilage without contrast agent by a non-invasive  
28 technique. The phase contrast images reveal more details than conventional imaging techniques,  
29 especially at smaller scales, with for instance a higher number of micro-calcifications detected (57, 314  
30 and 329 for MRI, conventional micro-CT and phase contrast imaging respectively). Calcified cartilage  
31 thickness was measured with a significant difference ( $p < 0.01$ ) between the control ( $23.4 \pm 17.2 \mu\text{m}$ ) and the  
32 osteoarthritis induced animal ( $46.9 \pm 19.0 \mu\text{m}$ ).

33 **Conclusions:** X-ray phase contrast imaging outperforms the conventional imaging modalities for  
34 assessing the different tissue types (soft and hard). This new imaging modality seems to bring new  
35 relevant surrogate markers for following-up small animal models even for low-grade osteoarthritis

36

## 37 Keywords

38 X-ray phase contrast Imaging, Calcified Cartilage; 3D markers

39

# 40 Introduction

41 Osteoarthritis (OA) represents the most widespread osteoarticular disease. It constitutes a growing public  
42 health problem, with our aging population and expanding obesity wave. Nowadays it represents the most  
43 common cause for disability with consecutive annual health care costs of up to 128 billion dollars in the  
44 US<sup>1,2</sup>. New therapeutic and symptomatic options for cartilage diseases have been tested in the past years  
45 in animal models and clinical, such as analgesic treatment<sup>3</sup>, injection of monoclonal antibody<sup>4-6</sup> or low  
46 dose radiotherapy<sup>7</sup>. Imaging modalities are becoming a gold standard to quantify the effectiveness of such  
47 therapies. Thus, it is crucial to develop, robust and reproducible joint imaging techniques with a high  
48 spatial resolution and sensitive to soft and hard tissues. Current clinical and preclinical imaging modalities  
49 have limitations in the detection of early cartilage and bony changes. Indeed, conventional X-ray  
50 absorption based Computed Tomography (CT) allows clear visualization of bone tissues but provides  
51 reduced sensitivity to soft tissues<sup>8</sup>. Cartilage is indeed poorly visible in conventional micro-CT and it  
52 mainly shows advanced and irreversible cartilage degradation resulting in joint space narrowing. Changes  
53 in the composition of joint cartilage or soft tissues are usually rather evaluated using Magnetic Resonance  
54 Imaging (MRI) using sequences such as ultrashort-TE<sup>9</sup>. Yet, images obtained by MRI struggle to render  
55 properly bony changes and microcalcifications<sup>10</sup>. Indeed, MRI cannot so far reach the few microns spatial  
56 resolution required to characterize properly microcalcifications. Fluorescence imaging is also used to  
57 quantify the distribution of specific chemical elements such as reactive oxygen species but is limited to  
58 imaging at the macro scale and in two dimensions<sup>11</sup>.

59 Using a wave approach with X-rays, the refraction index of light element materials can be a thousand  
60 times greater than its counterpart the absorption factor for the wavelength in radiology<sup>12</sup>. This induces a  
61 much greater contrast for soft tissues with X-ray imaging methods based on the detection of the light  
62 refraction or with a wave description the phase<sup>13</sup>. These methods, called Phase Contrast Imaging (PCI) in  
63 comparison to the conventional method based on absorption. PCI has a strong correlation with MRI in  
64 measuring cartilage thickness and with conventional CT in detecting subchondral bony changes<sup>14,15</sup>. It is  
65 indeed possible to visualize simultaneously and in details the high absorbing tissues like bone, and the  
66 low-absorbing tissues like cartilage and soft tissue (synovial membranes, muscles ...) as demonstrated by  
67 Horng<sup>16</sup>. Nevertheless, to our knowledge, only a few studies evaluated PCI for to study small animal  
68 models albeit being a fast 3D imaging technique, contrast agent independent, in-vivo compatible and with  
69 a high special resolution.

70 The objectives of this study are to present the very first results obtained using PCI on a murine OA model  
71 and to evaluate the PCI capability to depict soft and hard tissues of the knee compared to the established  
72 preclinical imaging modalities. We also propose several new 3D morphometric surrogate markers with a  
73 special focus on articular cartilage, calcified cartilage and osteophytes.

74

## 75 Methods

### 76 Overall Protocol

77 All imaging protocols were done post-mortem on six mice, in which we analyzed both knees ( $n_{control} = 6$ ,  
78  $n_{MIA} = 6$ ). The removed knees were formalin fixed, agarose imbedded, and synchrotron phase contrast  
79 imaging (PCI) was performed, followed by MRI and conventional x-ray microscopy. Then samples were  
80 then sliced for histological analysis.

### 81 Animal Manipulation

82 In accordance with the Directive 2010/63/EU, the experiments were performed in an agreed animal  
83 facility (C3851610006) evaluated by an Ethical Committee for Animal Welfare and authorized (APAFIS  
84 #13792-201802261434542 v3). OA pain model was induced by bilateral single intra-articular injection of  
85 monoiodoacetate (MIA-OA, Sigma, Saint Quentin-Fallavier, France) in the knee. Six 6 weeks old C57BL6

86 female mice (Janvier Labs, France) were anaesthetized by xylazine and ketamine injection (10 and 100  
87 mg/kg), a 0.5 mm skin incision was made over both knee, MIA (0.1 mg/ $\mu$ l, 5 $\mu$ l) or the same volume of  
88 phosphate buffer (PBS) for control animals was injected through the infra-patellar tendon perpendicular  
89 to the tibia using Hamilton syringe with 25 $\mu$ L capacity and dispensable 0.4 mm diameter needles. Mice  
90 received buprenorphine once (0.1mg/kg, sub-cutaneous) for pain management during recovery. Mice  
91 were monitored for 10 weeks for obvious locomotor disability by visual observation and Rotarod testing  
92 (Bioseb, Vitrolles, France) and then sacrificed. Knees were removed, fixated in 4% formaldehyde for 48h  
93 and embedded in PBS with 2 % agarose.

#### 94 **Synchrotron X-ray Imaging**

95 Image acquisition was performed at the biomedical beamline of the ESRF (Grenoble, France). A  
96 propagation-based PCI technique was used to enhance contrast in between small density changes by  
97 capitalizing on both x-ray absorption and refraction. This imaging technique uses highly coherent  
98 radiation, with a long sample to detector distance (11 m) to measure interferences between refracted  
99 radiations (see Figure 6). Synchrotron X-ray sources are particularly suited to provide such  
100 characteristics, allowing for an acquisition time of 8 min at an isotropic voxel size of 6.1 $\times$ 6.1 $\times$ 6.1 $\mu$ m<sup>3</sup>. A  
101 wiggler source was used to produce broad-spectrum synchrotron radiation. The continuous spectrum was  
102 filtered to a 52 keV narrow energy band by a double bent Silicon crystal monochromator. This relatively  
103 high energy was chosen as a compromise between contrast, loss of spatial resolution due to streak  
104 artefacts caused by phase shifts bigger than a pixel and the very long distance imposed by the beamline  
105 configuration at the time of the experiment. Images were recorded with a sCMos camera (PCO edge) with  
106 a 2560  $\times$  2160 pixels chip. The projections acquired 11 m away from the sample were brought into focus  
107 using the Paganin algorithm<sup>33</sup>, the  $\frac{\delta}{\beta}$  ratio was set to 1000 (the theoretical value of bone) knowing the  
108 setup geometry and the approximate elemental composition of the sample. A total of 2500 projections  
109 was acquired to cover the 360 degree.

#### 110 **Magnetic Resonance Imaging**

111 MRI was performed at 9.4T in a horizontal bore magnet (Bruker Biospec 94 / 20 Avance III-HD, Bruker  
112 BioSpec, Ettlingen, Germany) at the IRMaGe MRI facility (La Tronche, France) equipped with an helium  
113 cooled cryoprobe and a phased-array surface receive coils configuration. A 3D ultra-short echo time (UTE)  
114 pulse sequence was acquired (echo time = 0.00813 ms; repetition time = 4.0 ms; flip angle = 5 $^{\circ}$  ;  
115 acquisition matrix = 300  $\times$  300  $\times$  300, average = 4). Such acquisition allows acquiring a volume of  
116 15 $\times$ 15 $\times$ 15mm<sup>3</sup>, at a voxel size of 50 $\times$ 50 $\times$ 50 $\mu$ m<sup>3</sup> in 17 min.

#### 117 **Conventional X-ray microtomography**

118 Samples were imaged on a conventional microtomograph (EasyTom XL from RXSolution, Chavanod,  
119 France) at the SIMaP- GPM2 lab (Grenoble, France). A conventional microfocus X-ray tube was used, with  
120 a maximum high voltage set to 60 kVp coupled with a 127  $\mu$ m flat panel detector allowing for an  
121 acquisition time of 11 min for a 11.1  $\times$  11.1  $\times$  8.8 mm<sup>3</sup> volume at a voxel size of 6.06 $\mu$ m<sup>3</sup>. The source spot  
122 size was adjusted to be inferior to 5 $\mu$ m. The number of projections was 3600.

#### 123 **Histological Analysis**

124 Samples were paraffin embedded; 4 $\mu$ m slices were cut every 30 $\mu$ m and colored using the Safranin-O Fast-  
125 Green technique described by Glasson<sup>17</sup> except that the optimal decalcification time was 48h in 20 %  
126 EDTA. Histological images were acquired with a Zeiss Axio Scan slide scanner. The OARSI histological  
127 grading system guideline was used to grade the severity of OA<sup>17</sup>. This semi-quantitative scoring system  
128 ranged from 0 to 6 (the higher the more severe) and was applied to all four quadrants (score out of 24):  
129 medial femur, medial tibia, lateral femur, lateral tibia.

130

#### 131 **PCI Processing and Analysis**

132 Bone and calcified cartilage structure were segmented using a region-growing algorithm<sup>34</sup>. A  
133 morphological opening was then used to remove structures smaller than 3 voxels in diameter<sup>35</sup>. All  
134 segmented elements (bone and calcified cartilage), not connected to the tibia, femur, fibula, menisci and  
135 patella were labelled as calcifications, for which we reported their number and volumes. The marching

136 cube algorithm<sup>36</sup> was applied to every segmented structure to generate the polygonal surface meshes. A  
137 feature preserving mesh smoothing<sup>37</sup> algorithm was then applied to remove aliasing artifacts. Distance  
138 map was calculated from the tibia and femur segmented mesh with a fast-marching algorithm<sup>38</sup>. From the  
139 distance map, the surface of maximum distances (i.e. surface for which the tibia and femur are  
140 equidistant) was extracted to compute the mean and standard deviation of the interspace. Effective  
141 thickness of the dense cartilage structures was characterized for both the tibia and femur using a maximal  
142 included ball algorithm through the iMorph software<sup>39</sup>. Using the same regions of interest at the condyle  
143 positions, the segmented calcified cartilage were numerically filled with growing spheres to fill up the  
144 entire volume. The distribution of all the spheres diameters was then normalized to their total numbers  
145 ( $n_{sphere} \approx 300$ ). All the curvature point distribution were recorded with negative value for concave surfaces  
146 and positive value for convex surfaces roughness was computed with the VTK library<sup>40</sup>.

## 147 **Statistics**

148 Pairwise comparisons were performed by using independent Student-Newman-Keuls tests on each  
149 condyle interspace with the tibia, in between experimental conditions. When both condyles were included  
150 in one tested group a Kolmogorov-Smirnov test was applied to consider the distributions bimodality.  
151 Asymmetric aperture map distributions were pairwise tested in between conditions using a Man-Whitney  
152 test. Finally, a Levene's test was used to test pairwise comparison of the curvature map standard  
153 deviations. All statistical tests were carried out with a significance level of  $p < 0.01$  using the SciPy  
154 statistical library with the Python3.7 language<sup>41,42</sup>.

# 155 **Results**

## 156 **Comparison of the Phase Contrast Imaging technique to MRI, X-ray micro-tomography**

157 Figure 1 shows a representative slice, extracted from each 3D volumes, of a MIA-OA mice knee. MRI  
158 (Figure 1A) with a ultra-short echo time (UTE) sequence shows a readable contrast between bone and  
159 tissue structure as well as within the tissue structures (tendons, muscles, cartilages). But, the lack of  
160 image definition (pixel size of  $50\mu m^3$ ) gives a poor geometric representation of the overall bone, meniscus  
161 and tibial ligaments and underestimate the total number of calcifications ( $N_{calcification_{MRI}} = 57$ ,  
162  $N_{calcification_{CT}} = 314$  vs  $N_{calcification_{PhaseContrast}} = 329$ ). Due to poor contrast within the bone structure,  
163 calcified cartilage, cortical and trabecular bone appear homogeneous with the MRI technique.  
164 Conventional X-ray micro-tomography (Figure 1B) with its high resolution shows a precise morphology of  
165 the bone structure but completely lacks contrast within soft and bony tissues. PCI (Figure 1C) allies a high  
166 image precision good differentiation between all anatomical elements of the knee joint (muscle, tendon,  
167 calcified cartilage, articular cartilage, bone and calcifications). The insets shown in Figure 1D and E are  
168 situated in the joint space and show the same zone with adapted gray scales for enhancing either the  
169 articular cartilage and synovial fluid (Figure 1D) or the calcified cartilage (Figure 1E). On these example,  
170 one can clearly see, in the same imaging modality, all the element of the knee joint: cartilage, calcified  
171 cartilage and chondrocytes. All the imaging dataset for the leg imaged with the different modalities are  
172 available at doi://10.17605/OSF.IO/W4P6M.

173

## 174 **Validation of PCI results with histological images**

175 To validate our PCI data with the gold standard histology, we present in Figure 2, a comparison on a  
176 representative slice of a control (Figure 2A,C,E,G,H) and a MIA-OA (Figure 2B,D,F,I,J) mice knees acquired  
177 by PCI (Figure 2A-D) and safranin-O colored histological sections (Figure 2E-J) with a focus on the medial  
178 femoral condyle area (C,D,G-J). To highlight the unique capabilities of PCI to render such contrast, the PCI  
179 contrast window was set to highlight the density changes within the calcified structures. The articular  
180 cartilage is therefore too dark to be visible and we indicate by a red dotted line its boundaries found  
181 thanks to a segmentation based on another contrast windowing (see method section). The distinction  
182 between the subchondral bone (+) and the calcified cartilage (\*) can be visualized with PCI on both the  
183 control and the MIA slices. Dark rounded dots, visible within the calcified cartilage in PCI are ascribed to  
184 chondrocytes ( $\gamma$ ). In the knee histological sections, the purple safranin-O stains the cartilage  
185 peptidoglycans. The calcified cartilage can be found on these images by a reddish color that seem to be

186 correlated with the PCI images in both the control and MIA images. In the MIA-treated knee, osteoarthritis  
187 is assessed by disorganization and broadening of the cartilage, safranin-O negative areas at the surface  
188 (Figure 2I), clefts at the cartilage surface with dead cell ghosts (Figure 2J). These features can also be seen  
189 on the PCI slices, with a thicker calcified cartilage layer and with darker and larger chondrocytes.

190

### 191 3D histomorphometry analysis of two joints

192 Figure 3 and Figure 4 show representative examples of the different surrogate markers PCI may provide  
193 on both the control and MIA knee joints. After the segmentation of our images in bone and calcified  
194 cartilages zones we can measure virtual 3D histomorphometry quantities that are non-accessible with  
195 gold standard 2D histology.

196 The Figure 3 presents rapid and direct 3D measurements of features similar to those used in human X-ray  
197 imaging or in histological evaluation of OA. We calculated on our segmented bone structures the mean  
198 femur to tibia inter-space at the condyle positions in three dimensions (Figure 3A). The regions of interest  
199 where the mean distance and their standard deviation were calculated are indicated by the gray square. It  
200 is interesting to note that by averaging the mean inter-distance of both condyles no statistical differences  
201 appears in between the Control and the MIA joints ( $56.7 \pm 8.5 \mu\text{m}$  vs  $57.4 \pm 17.3 \mu\text{m}$ ) (Plain color bars in  
202 Figure 3D ). But the MIA-OA knee displays a destabilization between his two condyles with a thin medial  
203 femur/tibia interspace ( $39.4 \pm 7.1 \mu\text{m}$ ) and a large lateral femur/tibia interspace ( $70.3 \pm 6.5 \mu\text{m}$ ), while such  
204 asymmetry is not present on the control knee ( $54.2 \pm 8.5 \mu\text{m}$  vs  $58.2 \pm 8.5 \mu\text{m}$  ).

205 Thanks to a granulometry algorithm<sup>19</sup>, we can calculate in three dimensions the local thickness of the  
206 different tissues. The Figure 3B shows on a sagittal cross-section the distribution of the local thickness  
207 whilst the Figure 3C represents those values projected in 3D on the segmented surfaces. The full  
208 distribution of calcified cartilage thickness in both femur and tibia for each condition is shown in the  
209 lower panel E and F. The thickness analysis on the calcified cartilage shows an overall increased in  
210 thickness with the MIA-OA knee  $46.9 \pm 22.4 \mu\text{m}$  compared to the control  $23.4 \pm 17.2 \mu\text{m}$  thickness. Bone wise,  
211 the femur and tibia calcified cartilage mean thickness are  $47.6 \pm 15.7 \mu\text{m}$  and  $46.3 \pm 22.4 \mu\text{m}$  respectively  
212 on the MIA-OA knee, and  $28.7 \pm 18.6 \mu\text{m}$  and  $18.1 \pm 13.9 \mu\text{m}$  respectively in the control knee. No statistical  
213 differences were found in between the femur and tibia on each of the two conditions for this mouse. We  
214 can nevertheless note that the local thickness is the largest in the MIA joints within the condyles where  
215 the femur/tibia interspace is the smallest. The total volume of MIA calcified cartilage in the full joint is also  
216 largely superior with  $V_{MIA} = 9.42 \times 10^{-2} \text{ mm}^3$  vs  $V_{Ctrl} = 6.35 \times 10^{-2} \text{ mm}^3$

217 Additionally, we computed three-dimensional based measurements on the PCI images that are not  
218 accessible with histology. We first computed the surface roughness (estimated as the local curvature of a  
219 surface) of different interfaces: the calcified cartilage/articular cartilage interface and the calcified  
220 cartilage/ subchondral bone interface. The Figure 4A-D shows such measurements where the roughness  
221 is projected onto the surfaces (represented in false colors) of both the calcified cartilage (A and C) and  
222 subchondral bone (B and D) in an example of control (A and B) and MIA (C and D) joints. The subfigure  
223 4E-H, together with an exemplary image, shows the roughness distributions restricted to the condyles  
224 positions. Figure 4E and F is the measurement performed on the femur, and G and H on the tibia. Note that  
225 only the femur subchondral bone displays a significant increase in curvature variability on the MIA-OA  
226 model (curvature  $\sigma_{Curv_{OA}} = 0.143 \mu\text{m}^{-2}$ ;  $\sigma_{Curv_{Ctrl}} = 0.075 \mu\text{m}^{-2}$ ).

227 Figure 4I-L shows the 3D distribution of all the segmented osteophytes in both control (I) and MIA (J)  
228 where the osteophytes were colored. Osteophytes were defined as high density elements not connected to  
229 the three main bones or the menisci. The graph in Figure 4K and L, shows a 2D projections in sagittal and  
230 transverse coordinates where the volume of the osteophytes is schematized by a circle with the same  
231 volume. As one can see, the control knee presents some osteophytes probably due to the injection of PBS  
232 but the MIA-OA knee displays a significantly higher amount ( $n_{OA} = 31$  vs  $n_{Ctrl} = 13$  ) with a larger total  
233 volume ( $V_{OA} = 4.29 \times 10^2 \mu\text{L}$  vs  $V_{Ctrl} = 6.01 \times 10^3 \mu\text{L}$ ).

234

### 235 Quantification of the whole image database

236 All the computed metric results are recapitulated in table 1. Figure 5 displays the comparison of the  
237 morphological parameters computed in the same fashion of the above examples on the PCI images but for

238 the all the MIA injected legs ( $n = 6$ ) and their controlateral legs ( $n = 6$ ). Figure 5A presents the mean inter-  
239 space distance between femur and tibia taking into account both condyles (bars in plain color). The  
240 distance is significantly higher (p-value  $\leq 0.01$ ) for the control than for the MIA knees. The group displays  
241 the same trend as in the above example when measuring specifically the interspace in between condyles.  
242 The same strong destabilization appears in between the MIA medial and lateral condyle ( $25.6 \pm 6.8 \mu\text{m}$  vs  
243  $70.7 \pm 4.3 \mu\text{m}$ ) while in the control group such effect is statistically non relevant ( $55.6 \pm 9.1 \mu\text{m}$  vs  
244  $56.7 \pm 4.1 \mu\text{m}$ ).

245 The same trend can be observed when measuring the calcified cartilage thickness (Figure 5B and C). The  
246 MIA model animals display a significant higher thickness compared to the control for both the femurs  
247 ( $24.2 \pm 7.3 \mu\text{m}$  vs  $47.7 \pm 15.4 \mu\text{m}$ ) and the tibia ( $25.1 \pm 8.1 \mu\text{m}$  vs  $44.4 \pm 17.2 \mu\text{m}$ ).

248 The surfaces roughness at the calcified and articular cartilage interface shows a smooth surface along the  
249 control and the MIA group for both the femur and tibia (Figure 5D and F). On the other hand, as shown in  
250 Figure 5E and G, the roughness on the tidemark between the calcified cartilage and subchondral bone is  
251 significantly different in between the two measured groups with a larger variability in curvature surface  
252 for the MIA model for both and tibia.

253 The calcification distribution is significantly different between the model and the control ( $nb_{MIA} = 54.8 \pm$   
254  $21.5$  vs  $nb_{Ctr} = 12.1 \pm 4.21$ ) (Figure 5H). The volumes distribution seems bimodal in the MIA model with a  
255 total of  $V_{Ctr} = 2.77 \times 10^3 \pm 4.26 \times 10^2 \mu\text{L}$  vs  $V_{MIA} = 3.70 \times 10^4 \pm 6.84 \times 10^3 \mu\text{L}$ . As expected, the smallest  
256 calcifications are mainly distributed in the tightest spaces in between the two condyles.

257 The total volume of calcified cartilage was also higher in the MIA group compare to the control with a  
258 mean volume per animal of  $V_{MIA} = 11.0 \pm 2.45 \times 10^{-2} \text{ mm}^3$  vs  $V_{Ctr} = 5.84 \pm 0.86 \times 10^{-2} \text{ mm}^3$

259

## 260 Discussion

261 The main objective of this work was to study whether Phase Contrast Imaging is a suitable imaging  
262 modality for studying at high resolution a murine osteoarthritis model. We demonstrated that compared  
263 to conventional MRI and conventional micro CT images, PCI technique has a higher image sensibility and  
264 resolution. It allows in a single scan a full representation of the soft and calcified tissue structures at the  
265 same resolution than conventional micro-CT that is limited to bone visualization only. We showed for the  
266 first time the possibility to distinguish in 3D, using a non-invasive technique i.e. in complete unaltered  
267 mice knees, the articular and calcified cartilages as well as the subchondral bone and some clustered  
268 chondrocytes.

269 In this study, for sake of comparison with literature, we used the most characterized chemical-induced OA  
270 mouse model based on the injection into the rodent knee joint of mono-iodoacetate (MIA), an inhibitor of  
271 glyceraldehyde-3-phosphate dehydrogenase, a glycolysis enzyme<sup>20</sup>. Although not fully depicting the  
272 disease progression in human, this MIA-OA model induces chondrocytes cell death within a week, a  
273 rapidly evolving OA and formation of osteophytes in rodent<sup>21</sup>. The chemical agents (Mono-Iodo-Acetate  
274 (MIA), papain, collagenase) induce very serious lesions with complete and rapid destruction of the  
275 cartilage making it impossible to study the early phases of OA disease. Despite that, to depict the joints  
276 tissue elements with enough contrast at the micro scale in such a small model remain highly challenging  
277 with conventional pre-clinical imaging modalities. In this study the histological lesions are severe and  
278 confirm that an OA has been induced. Some samples are close to the maximum of the OARSI scoring  
279 system with large depletion of proteoglycan staining, while other have a lack of articular cartilage. Mice  
280 calcified cartilage is proportionally larger in mice than in human<sup>17</sup> and mice MIA-OA model is mildly  
281 affected by mechanical stress due to the animal weight<sup>20</sup>. We nevertheless found thickening of the  
282 calcified cartilage and a rougher surface of the tidemark in the OA-model. As expected on an MIA-OA  
283 model<sup>22</sup>, we also found a drastic increase in micro-calcification number and volume. Such metrics could  
284 potentially be a relevant biomarker to spot early stage of MIA-OA model. On the other hand, surface  
285 roughness of the subchondral bone and calcified cartilage tidemarks did not exhibit any pertinent

286 outcomes on that model. We also observed no sign of subchondral bone alteration such as osteophytes,  
287 vascular infiltration or osteoclasts. In a hypothetical clinical usage of the PCI technique for osteoarthritic  
288 joint detection, calcified cartilage thickening could potentially join the already existing diagnostic  
289 radiographic hallmarks.

290 The secondary objective of this study was to present some surrogate markers this 3D imaging modality  
291 may offer. Besides the measurements of features classically admitted as hallmarks of OA (cartilage  
292 thickness, distance between bones), we proposed new morphological biomarkers to grade cartilage  
293 alterations, calculated here in 3D. These markers were calculated on the calcified cartilage due to the lack  
294 of articular cartilage on some animals of the MIA group, but in principles all these morphological  
295 parameters could have been measured on other tissues. In summary, we have shown that synchrotron  
296 radiation phase contrast imaging is a promising technique to rapidly detect and quantify microstructures  
297 for the investigation of small animal models of osteoarthritis. For example, the mean distance between the  
298 tibial plate and the femur is not different between the control and the MIA group but a difference can be  
299 observed if one measure separately the condyles. As expected, the calcified cartilage is thicker than in the  
300 OA group even in a OA low grade knee. The number and volume of calcifications seem to be a good  
301 indicator of the disease. The roughness of the interface between the calcified cartilage and the  
302 subchondral bone seem to be discriminant as well contrary to the interface between articular and calcified  
303 cartilage.

304 The main limitation of this study is the low number of investigated samples (n=6). Future studies will be  
305 carried out in larger groups of animals and other mouse model (surgically induced for example) to further  
306 evaluate the usefulness of PCI discriminant markers in the OA diseases. This limitation is mainly due to  
307 the limited access to synchrotron. But as previously shown the PCI technique is perfectly compatible with  
308 the preclinical in-vivo applications<sup>23,24</sup>. The next steps will consist in performing such experiment *in-vivo*  
309 on laboratory systems at a reduced radiation dose. Indeed synchrotron limited access, does not allow to  
310 follow-up the pathologies or at the cost of using many animals; while thanks to recent development  
311 performing such experiment on conventional X-ray source seems feasible in relatively short future.  
312 Indeed, the technique does not need any sample preparation or chemical injection and the deposited  
313 radiation dose could drastically be reduced with iterative reconstruction algorithm<sup>25</sup>. Latest development  
314 in PCI techniques using grating interferometry<sup>26-28</sup> has proven the feasibility of performing phase  
315 contrast with clinical sources. Unfortunately, in these studies the application of PCI remains in 2D due to  
316 the additional optics used. A simple approach using a random modulator<sup>29-31</sup> has recently shown that PCI  
317 can be transferred on conventional source with short acquisition time and reduced radiation dose to the  
318 sample<sup>32</sup>.

319 To conclude, PCI provides in a single examination, a better and a more detailed depiction of the different  
320 tissues of small animal models. The sensitivity of PCI makes possible a diagnosis and staging tool of the  
321 joint deterioration to support the development of new pre-osteoarthritic treatments and could in the  
322 future be applied to clinical studies.

## 323 Author contributions

324 Study conception and design: LB, BF, HRL, SD, BLA, BLE, EB; Data acquisition: LB, BF, HRL, EB, BLE; Data  
325 analysis and interpretation: LB, BLE, EB; Drafting the article: LB, BF, HRL, SD, BLA, BLE, EB; Article final  
326 approval: LB, BF, HRL, SD, BLA, BLE, EB.

327

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## Conflict of Interest.

The authors declare no conflict of interests

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## References

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**Figure 1.** Representative images of one osteoarthritis knee sample observed by each imaging technique: A: MRI with a ultra-short echo time sequence (echo time = 0.00813 ms; repetition time = 4.0 ms; flip angle = 5°; voxel size =  $50 \times 50 \times 50 \mu m^3$ ), B: Conventional X-ray micro-tomography Image (Source voltage = 60 kVp; voxel size =  $6.06 \times 6.06 \times 6.06 \mu m^3$ ) and C: Synchrotron X-ray phase contrast imaging (source energy = 52 keV; voxel size =  $6.1 \times 6.1 \times 6.1 \mu m^3$ ), D,E: Phase contrast Image magnification with a soft ( D ) and hard ( E ) tissue contrast window.

**Figure 2.** Representative images acquired by PCI (A-D) and their corresponding histological slices (E-J) of the control (A,C,E,G,H) and the MIA-OA model (B,D,F,I,J). Magnified images of the lateral condyle (yellow and black square) highlight the subchondral bone ( $\dagger$ ), calcified cartilage (\*), uncalcified cartilage (red dotted line) and example of chondrocytes ( $\gamma$ )

**Figure 3.** Quantification of the knee joint interspace and calcified cartilage thickness. A and D : Computed inter-space between the condyles contact points and tibia (gray squares) on both control and MIA-OA model. Bars chart show the mean value and standard deviation of all calculated inter-spaces (\*:  $p < 0.01$ ). B and C: Computed calcified cartilage thickness for control and MIA-OA model, with B a representative cross section and C the 3D distribution of local thickness. E and F : distribution of all calculated thickness in on the femur (E) and the tibia (F) (\*:  $p < 0.01$ ).

**Figure 4.** Quantification of knees structures roughness and calcifications. A-H: Computed roughness of the surface linemark between the calcified cartilage and cartilage (A and C) and Subchondral bone and calcified cartilage (B and D). A and D: 3D mapping of the calculated roughness E-H: Roughness Distribution calculated in between the two condyles in the Femur (E and F) and Tibia (G and H) I-J: Quantification of all the labeled calcifications . I and J: 3D rendering of the detected calcification, on the control knee (I) and MIA (J). K-L: 2D representation of all the condrocite coronaly projected with surface area the volume of each condrocites.

**Figure 5.** Quantification of knees structures morphology. Comparison made on the MIA model (n=6) and the controlateral legs (n=6). A : Computed mean and standard deviation inter-space between the condyles contact points and tibia (\*:  $p < 0.01$ ). B and C Histogram of the thickness of the calcified cartilage for the femurs and the tibias. D, E, F, G: Computed surface roughness on the calcified/uncalcified cartilage tidemark (D and F) and subchondral bone/calcified cartilage tidemark (E and G). H: Representation of the location and the number of calcifications (up) with their volume distribution (bottom).

**Figure 6.** Experimental set up of the X-ray Phase Contrast Image A wiggler source produced a broad-spectrum synchrotron radiation which was filtered to a narrow energy band at 52 keV by a double bent Silicon crystal. Sample was 11 meters from the detector in order to broaden x-ray interference. A gadolinium oxysulfide phosphor screen was used as scintillator to convert the x-ray to visible light. Images in visible light were then recorded indirectly by a sCMOS camera coupled with an optical system giving a voxel size of  $6.1 \times 6.1 \times 6.1 \mu m^3$ .

A

500  $\mu\text{m}$ 

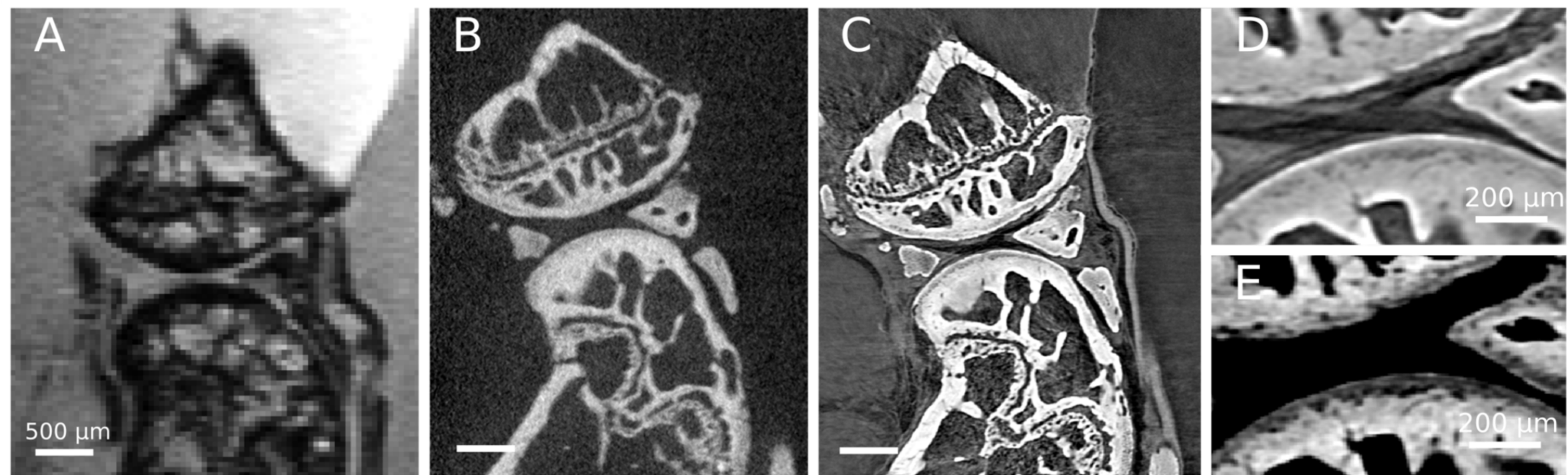
B

C

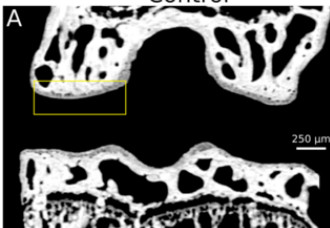
D

200  $\mu\text{m}$ 

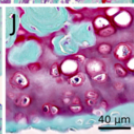
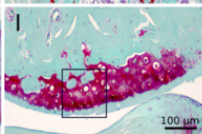
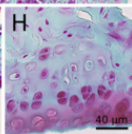
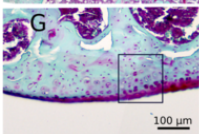
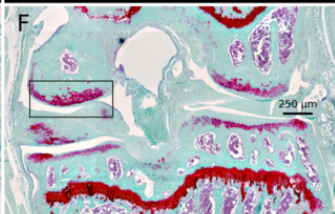
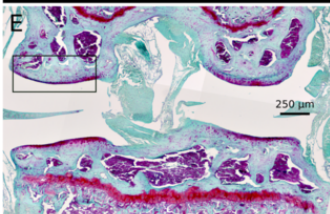
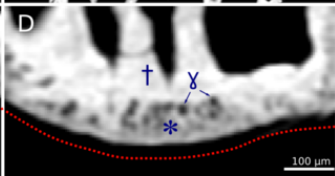
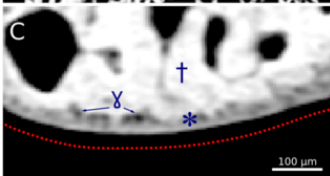
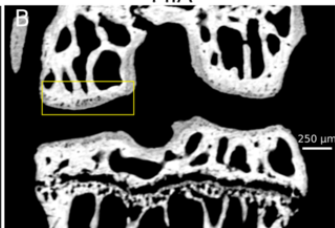
E

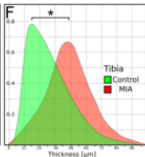
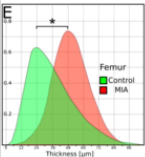
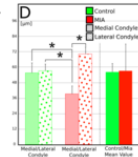
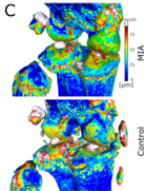
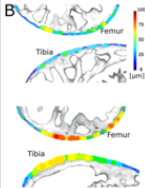
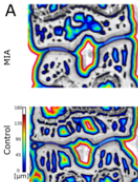
200  $\mu\text{m}$ 

Control

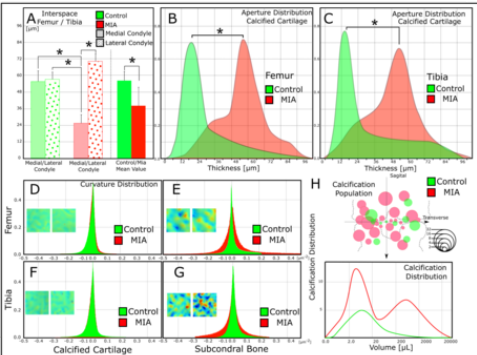


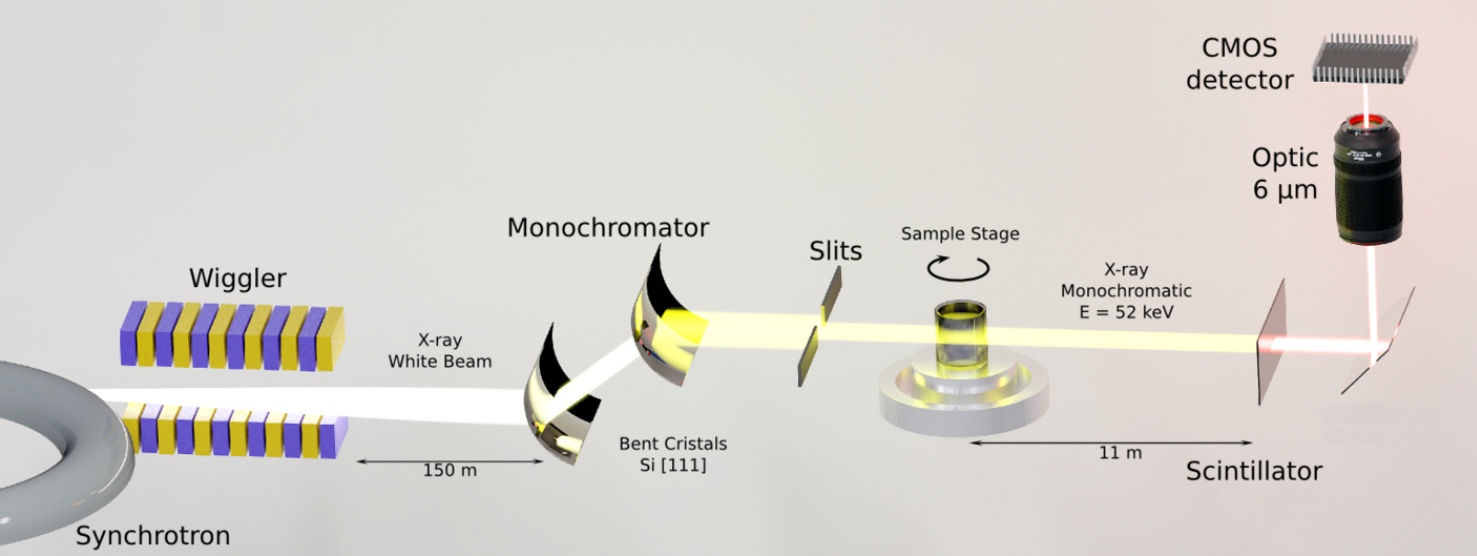
MIA











<b>Morphological Metrics</b>			
	Control (n=6)	MIA (n=6)	$p < 0.01$
$D_{BothCondyles} [\mu m]$	56.3 ± 6.6	37.4 ± 13.9	
$D_{MedialCondyle} [\mu m]$	55.6 ± 9.1	25.6 ± 6.8	***
$D_{LateralCondyle} [\mu m]$	56.7 ± 4.1	70.7 ± 4.3	
$T_{Femur} [\mu m]$	24.2 ± 7.3	47.7 ± 15.4	**
$T_{Tibia} [\mu m]$	25.1 ± 8.1	44.4 ± 17.2	
$V_{CalcifiedCartilage} [\mu m]$	5.84 ± 0.86	11.0 ± 2.45	*
$C_{Femur} [mm^2]_{CalcifiedCartilage}$	$4.32 \pm 1.09 \times 10^{-2}$	$4.57 \pm 1.72 \times 10^{-2}$	
$C_{Tibia} [mm^2]_{CalcifiedCartilage}$	$4.75 \pm 1.14 \times 10^{-2}$	$4.81 \pm 1.82 \times 10^{-2}$	**
$C_{Femur} [mm^2]_{SubcondralBone}$	$6.78 \pm 2.25 \times 10^{-2}$	$12.7 \pm 3.54 \times 10^{-2}$	
$C_{Tibia} [mm^2]_{SubcondralBone}$	$7.54 \pm 2.47 \times 10^{-2}$	$11.6 \pm 2.85 \times 10^{-2}$	
$N_{Calcification}$	12.1 ± 4.21	54.8 ± 21.5	*
$V_{Calcification} [\mu L]$	$2.77 \pm 0.462 \times 10^3$	$3.70 \pm 0.684 \times 10^4$	*

**Table 1.** All computed metrics on the knees database (control n= 6 and MIA n = 6). With  $D$ : Interspace the tibia and the femur;  $T$ : local thickness of the calcified cartilage;  $V$ : volume;  $C$ : Curvature of the specified surface;  $N$ : Number of calcification. Each \* a statistical difference in between the control and MIA group with  $p < 0.001$