

# Effect of limb rotation on radiographic alignment measurement in mal-aligned knees

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## Research Article

**Keywords:** Alignment measurement, Long-leg-radiography, Knee exion, Knee coronal deformity

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1 **Effect of limb rotation on radiographic alignment**  
2 **measurement in mal-aligned knees**

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4 the date of receipt and acceptance should be inserted later

5 **Abstract** Purpose. Long-leg-radiography (LLR) is commonly used for mea-  
6 surement of lower limb alignment. However, limb rotations during radiography  
7 may interfere with the alignment measurement. This study examines the ef-  
8 fect of limb rotation on the accuracy of measurements based on the mechanical  
9 and anatomical axes of the femur and tibia, with variations in knee flexion and  
10 coronal deformity.

11 Methods. Forty-five lower limbs of thirty patients were scanned with CT. Vir-  
12 tual LLRs simulating 5 rotational positions (neutral,  $\pm 10^\circ$ , and  $\pm 20^\circ$  internal  
13 rotation) were generated from the CT images. Changes in the hip-knee-ankle  
14 angle (HKA) and the femorotibial angle (FTA) were measured on each image  
15 with respect to neutral values. These changes were related to knee flexion and  
16 coronal deformity under both weight- and non-weight-bearing conditions.

17 Results. The measurement errors of the HKA and FTA derived from limb  
18 rotation were up to  $4.84 \pm 0.66^\circ$  and  $7.35 \pm 0.88^\circ$  respectively, and were corre-  
19 lated with knee flexion ( $p < 0.001$ ) and severe coronal deformity ( $p \leq 0.001$ ).  
20 Compared with non-weight-bearing position, coronal deformity measured in  
21 weight-bearing condition was  $2.62^\circ$  greater, the correlation coefficients between  
22 the coronal deformity and the deviation ranges of HKA and FTA were also  
23 greater.

24 Conclusion. Flexion and severe coronal deformity have significant influence  
25 on the measurement error of lower limb alignment. Errors can be amplified in  
26 the weight-bearing condition compared with the non-weight-bearing condition.  
27 The error of measurement of the anatomic axis is greater than the mechanical  
28 axis. Considering LLR is the gold standard image modality, attention should  
29 be paid to the measurement of knee alignment. Especially for the possible er-  
30 rors derives from weight-bearing long leg radiographs of patients with severe  
31 knee deformities.

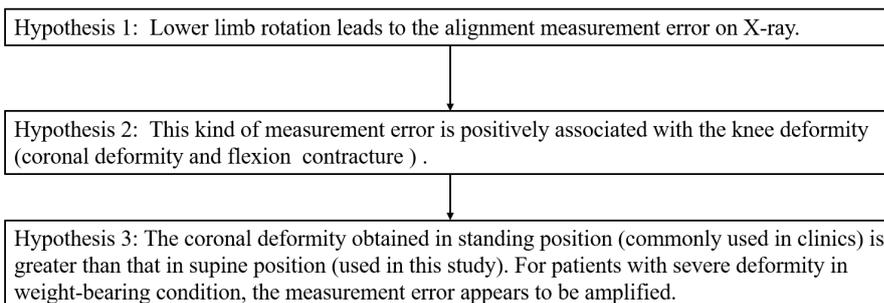
32 **Keywords** Alignment measurement · Long-leg-radiography · Knee flexion ·  
33 Knee coronal deformity

## 34 1 Introduction

35 The lower limb alignment is an important reference for clinical diagnosis and  
36 treatment of conditions affecting the knee. Malalignment of the lower extrem-  
37 ity is recognized as a critical component of the etiology of knee osteoarthri-  
38 tis[4][17][19]. Correct alignment is critical for the outcome of total knee arthro-  
39 plasty (TKA)[12], especially for personalized surgical design[16][21]. Lower  
40 limb alignment is commonly assessed using measurements of anatomical or  
41 mechanical (weight-bearing) axes of the femur and the tibia, as depicted on  
42 plain radiographs. Quantification of these axes usually requires identification  
43 of anatomical landmarks from hip to ankle. For evaluation of the knee align-  
44 ment, long leg radiographs (LLR) are considered as the gold standard image  
45 modality, with high inter- and intra-observer reliability.

46  
47 Correct and standardized patient positioning during imaging is the prereq-  
48 uisite for the accuracy of the lower limb alignment measurement while using  
49 LLR. However, considering patients with knee osteoarthritis or other joint  
50 disease are not able to fully extend the knee, it is reasonable to study the pos-  
51 sible alignment measurement errors of patients with severe deformity. Studies  
52 have reported that limb rotation or foot rotation could significantly affect the  
53 outcome [6][9][11][15]. The measurement error of hip-knee-ankle (HKA) angle  
54 could reach 4-10 degrees or even greater[14][15]. While the recommended HKA  
55 after TKA ranges from  $-3^\circ$  to  $3^\circ$ , which represents a proper knee alignment and  
56 prosthesis position. Therefore, the preoperative measurement error directly af-  
57 fects knee osteotomy. Previous studies have suggested that improved methods  
58 of measuring lower limb alignment are of greater importance to the advance-  
59 ment of knee osteotomy than the technical details of the surgical procedure  
60 itself [11][13][15].

61  
62 Although studies involving synthetic prosthesis[11][14], specimen of human  
63 lower limbs[3][20] and in-vivo images[7] all existed, the magnitude of these er-  
64 rors varies dramatically. Some researchers believed the long leg radiograph is  
65 a reliable method[1][10] and reported that the error caused by limb rotation  
66 was less than  $1^\circ$  within the acceptable range [18], whereas others found signif-  
67 icant errors in the measurements of severe varus knees [8][14][15] [18]. Coronal  
68 deformity, knee flexion contracture or some other anatomical features in pa-  
69 tients might be important factors that potentially affect the magnitude of  
70 measurement errors. Considering the in-vivo images derived from patients can  
71 better reflect real clinical application scenarios, the measurement error of knee  
72 alignment in patients with complex deformities should be investigated more  
73 in-depth. It is meaningful to quantify the magnitude of alignment measurement



**Fig. 1** This study attempts to prove these hypotheses.

74 errors in patients with different degrees of deformity.

75

76 The purposes of this study are: (1) to determine the influence of limb  
77 rotation on measurements of knee alignment derived from long-leg radiographs  
78 (2) to measure the additional influence of coronal deformity and knee flexion  
79 contracture; and (3) to determine whether weight-bearing affects the accuracy  
80 of the radiographic measurements. The significance of this study is to assist  
81 in reducing measurement errors of mechanical and anatomical leg axis. This  
82 study attempts to prove the following hypotheses(Fig.1).

## 83 2 Result

84 The median value of knee flexion was  $5.32^\circ$  ( $2.27^\circ$ ;  $8.38^\circ$ ). The median knee  
85 coronal deformity was  $7.37^\circ$  ( $4.25^\circ$ ;  $9.50^\circ$ ). Descriptive statistics of HKA and  
86 FTA deviation range were summarized in Table 3. The average deviation  
87 ranges of HKA and FTA were  $4.84 \pm 0.66^\circ$  (range: 0.56-25.03) and  $7.35 \pm 0.88^\circ$   
88 (range: 1.30- 30.42), respectively. As shown in Fig.4, the mean difference be-  
89 tween HKA and FTA deviation range was 2.50 ( $p < 0.01$ ). The intraclass cor-  
90 relation coefficients for HKA and FTA deviation range were perfect, 0.976  
91 ( $p < 0.05$ ) and 0.935 ( $p < 0.05$ ), respectively.

92

93 According to the two-way ANVOA: there were statistical differences in  
94 both HKA deviation range and FTA deviation range significantly for patient  
95 with varying flexion and coronal deformity ( $p < 0.05$ ); the interaction between  
96 flexion and coronal deformity also had a significant effect on HKA and FTA  
97 deviation range ( $p < 0.05$ ). Fig.5 illustrated the average HKA and FTA devia-  
98 tion range in patient with different flexion and coronal deformity.

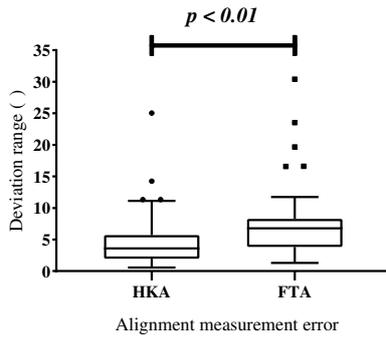
99

100 For flexion index, there were significant differences among the slight, mod-  
101 erate and severe groups in both HKA and FTA deviation range ( $p < 0.001$ )  
102 (Table 4). For coronal deformity index, there was no significant difference  
103 between the slight and moderate groups in both HKA and FTA deviation

**Table 1** HKA and FTA deviation range derived from varying degree of Flexion and Coronal deformity. Descriptive statistics

	HKA Deviation Range (M $\pm$ S.E.M.)	FTA Deviation Range (M $\pm$ S.E.M.)
Slight Flexion group	2.15 $\pm$ 0.26°	3.95 $\pm$ 0.45°
Moderate Flexion group	5.32 $\pm$ 0.39°	7.47 $\pm$ 0.43°
Severe Flexion group	12.81 $\pm$ 2.20°	18.19 $\pm$ 2.72°
Slight Coronal deformity group	4.57 $\pm$ 1.67°	6.28 $\pm$ 1.95°
Moderate Coronal deformity group	4.43 $\pm$ 0.64°	6.85 $\pm$ 0.87°
Severe Coronal deformity group	7.24 $\pm$ 1.29°	11.92 $\pm$ 2.34°
Total	4.84 $\pm$ 0.66°	7.35 $\pm$ 0.88°

Note: M = Mean; S.E.M = Standard Error of Mean;

**Fig. 2** Box-plot illustrating the average deviation ranges of HKA and FTA. P value<0.01, difference between HKA and FTA deviation range being significant.

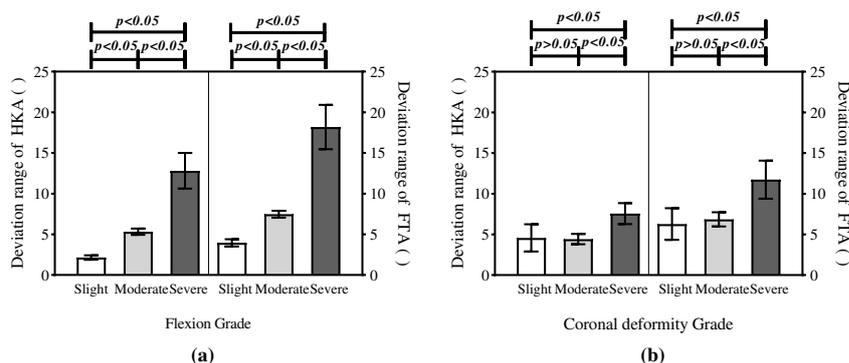
104 range ( $p > 0.05$ ). Yet the coronal deformity index of the severe group was sig-  
 105 nificantly different from that of the slight and moderate groups ( $p \leq 0.001$ )  
 106 (Table 5). Among the different degrees of coronal deformity, only the severe  
 107 group differed dramatically from the other two groups in HKA deviation range  
 108 ( $p \leq 0.001$ ) and in FTA deviation range ( $p < 0.001$ ). No significant difference was  
 109 found between the slight coronal deformity group and the moderate coronal  
 110 deformity group in HKA deviation range ( $p > 0.05$ ) and in FTA deviation range  
 111 ( $p > 0.05$ ).

**Table 2** Multiple Comparisons of Flexion group, the mean difference of HKA deviation range and the mean difference of FTA deviation range.

Flexion group	Mean Difference	HKA deviation			FTA deviation			
		S.E	Sig.	95% C.I.	Mean Difference	S.E	Sig.	95% C.I.
Slight- Moderate	-3.17*	0.49	<0.001	-4.16 ~ -2.17	-3.51*	0.80	<0.001	-5.14 ~ -1.89
Moderate-Severe	-8.04*	0.73	<0.001	-9.51 ~ -6.57	-11.98*	1.18	<0.001	-14.38 ~ -9.59
Slight - Severe	-11.20*	0.70	<0.001	-12.63 ~ -9.78	-15.50*	1.14	<0.001	-17.82 ~ -13.18

Note: S.E, stander error; Sig., significance probability; C.I., confidence interval.

\*: The mean difference is significant at the 0.05 level.



**Fig. 3** The deviation range of HKA and FTA in patient with different flexion and coronal deformity. P value<0.05 means that difference between the two corresponding groups is significant in 95% confidence level. (a) Participates were divided into slight, moderate and severe flexion group by the degree of flexion contracture. Left part showed the HKA deviation range in the slight, moderate and severe flexion group, and the right part showed the FTA deviation range in the slight, moderate and severe flexion group. (b) Participates were divided into slight, moderate and severe coronal deformity group by the degree of coronal deformity. Left part showed the HKA deviation range in the slight, moderate and severe coronal deformity group, and the right part showed the FTA deviation range in the slight, moderate and severe coronal deformity group.

**Table 3** Multiple Comparisons of Coronal deformity group, the mean difference of HKA deviation range and the mean difference of FTA deviation range.

Coronal deformity group	Mean Difference	HKA deviation			Mean Difference	FTA deviation		
		S.E	Sig.	95% C.I.		S.E	Sig.	95% C.I.
Slight- Moderate	0.14	0.51	0.783	-0.89~1.17	-0.57	0.83	0.493	-2.26~1.11
Moderate-Severe	-2.81*	0.69	<0.001	-4.22~-1.40	-5.06*	1.13	<0.001	-7.35~-2.78
Slight - Severe	-2.67*	0.75	0.001	-4.18~-1.16	-5.64*	1.21	<0.001	-8.10~-3.18

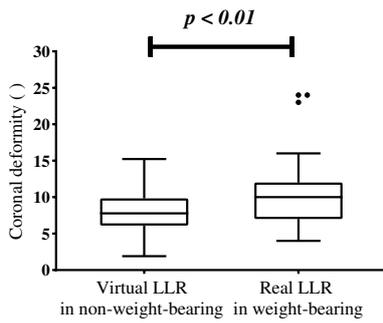
Note:S.E, stander error; Sig., significance probability; C.I., confidence interval.

\*: The mean difference is significant at the 0.05 level.

112 The deviation ranges of HKA and FTA were significantly correlated with  
 113 the knee flexion and coronal deformity (Table 6). In non-weight-bearing condi-  
 114 tion, the correlation coefficient between coronal deformity and HKA deviation  
 115 range was 0.307 ( $p < 0.05$ ); the correlation coefficient between coronal defor-  
 116 mity and with FTA deviation range was 0.504 ( $p < 0.01$ ). In weight-bearing  
 117 condition, the correlation coefficient between coronal deformity and HKA de-  
 118 viation range was 0.635 ( $p < 0.01$ ); the correlation coefficient between coronal  
 119 deformity and with FTA deviation range was 0.639 ( $p < 0.01$ ). Remarkable cor-  
 120 relations were found between the flexion angle and deviation ranges of both  
 121 HKA (0.933,  $p < 0.01$ ) and FTA (0.861,  $p < 0.01$ ).

122

123 The box-plot in Fig.6 illustrates the coronal deformity measured from  
 124 virtual LLR and real LLR. In neutral position, coronal deformity measured



**Fig. 4** Box-plot illustrating the coronal deformity measured in neutral position. Virtual LLR: virtual radiographs simulated from full-leg CT scan in non-weight-bearing condition. Real LLR: standing full-leg radiographs in weight-bearing condition.

125 on real LLR  $10.52 \pm 0.94^\circ$  was significantly greater than that on virtual LLR  
 126  $7.90 \pm 0.54^\circ$ . The mean difference of coronal deformity in non-weight-bearing  
 127 condition and weight-bearing condition was  $2.62 \pm 0.68^\circ$  ( $p < 0.01$ ).

### 128 3 Discussion

129 The lower limb alignment measurement is a crucial factor in the preoperative  
 130 plan and postoperative assessment of a knee replacement. Inconsistent posi-  
 131 tioning during the capture of X-ray and resulting projected image can bring  
 132 the measurement errors of the lower limb alignment. However, the argument  
 133 over whether these measurement errors are acceptable and related to the knee  
 134 deformity has not settled. This study quantitatively revealed that the influence  
 135 of limb rotation on measurements of knee alignment derived from long-leg ra-  
 136 diographs, the measurement errors corresponding to different deformed knees,  
 137 and the difference of radiographic measurements on weight-bearing and non-  
 138 weight-bearing conditions.

139 In this study, limb rotation caused the deviation ranges of HKA and FTA  
 140 measurement were greater than  $3^\circ$ . The degree of the anatomical axis varia-  
 141 tion was greater than that of the mechanical axis. Although tilted projections  
 142 can lead to the measurement errors of the lower limb alignment, the magni-  
 143 tude of these errors measured in different studies vary dramatically. Wright  
 144 JG[20] reported that the deviation range of FTA measurement was less than  
 145  $1^\circ$  under the limb rotation from  $20^\circ$  external rotation to  $20^\circ$  internal rotation,  
 146 whereas Swanson KE [18] found significant errors in severe deformity knees  
 147 up to  $7.8^\circ$ . This could be explained by the fact that the degree of deformity  
 148 of the lower limb would influence the measurement error of the alignment axis.  
 149

150 Our results demonstrated that the coronal deformity affected the align-  
 151 ment measurement errors greatly. Multiple comparisons of slight, moderate  
 152

153 and severe degrees of coronal deformity indicated that the severe group (coro-  
154 nal deformity $>10^\circ$ ) differed significantly from the other two groups in align-  
155 ment measurement errors. Compared with the non-weight-bearing condition,  
156 the coronal deformity of weight-bearing condition had greater influence on the  
157 measurement errors. However, the effects of coronal deformity on the assess-  
158 ment of alignment on LLR in the previous studies were controversial. Swanson  
159 et al.[18] discussed in a saw-bones research of alignment measurements in de-  
160 formed limbs, that the FTA measurement in limbs with severe valgus or varus  
161 deformity was more sensitive to the effect of rotation than in normally aligned  
162 limbs. This is in line with our results that both mechanical and anatomical  
163 axis measurement errors in the severe coronal deformity group were greater  
164 than that in the slight or the moderate group. Jud et al.[7] conclude that de-  
165 viations in mechanical leg axis measurements did not vary relevantly through  
166 the coronal deformity. This is an interesting conclusion that is consistent with  
167 the findings of the present study, because they only analyzed coronal deformity  
168 below 9 degrees. The coronal deformities of our research subjects ranged from  
169 0.62 to 19.55 degrees. No significant difference was found between the slight  
170 and the moderate coronal deformity group in alignment measurement errors.  
171 While the severe group (coronal deformity $>10^\circ$ ) had significant differences  
172 with the other two groups in alignment measurement errors. Additionally, this  
173 study measured both HKA and FTA deviation range of each subject. On the  
174 contrary, the study conducted by Kawakami et al.[8] reported no significant  
175 correlation between coronal deformity and measurement error. Kawakami et  
176 al. set different limb rotation ranges for different individuals to calculate the  
177 measurement error. In the present study, the limb rotation from internal  $20^\circ$   
178 to external  $20^\circ$  was performed uniformly for each subject. And the present  
179 study further collected coronal deformity in the weight-bearing condition to  
180 compare with the result obtained from the non-weight-bearing condition. The  
181 opposite conclusion was probably reached from these two aspects. Therefore,  
182 the hypothesis that coronal deformity and the measurement errors were sig-  
183 nificantly related is credible. The effect of coronal deformity on the assessment  
184 of alignment on real LLR may be more pronounced. The method of alignment  
185 measurement should vary from slight to severe patients of varus and valgus.

187 The knee flexion angle plays an important role in the potential error of  
188 the alignment measurement. Multiple comparison of the slight, moderate and  
189 severe flexion group showed significant differences for all combinations in align-  
190 ment measurement error. According to a study with synthetic model by Lon-  
191 ner et al.[11], the FTA deviation range of samples with flexion varied more  
192 than the control group. This statement was consistent with our study and  
193 a similar conclusion was reached in a radiographic cadaver study conducted  
194 by Brouwer et al. [3]. Differently, the present study recruited clinical patients  
195 instead of using synthetic models or specimen of human lower limbs. Both  
196 knee osteoarthritis and normal individuals under clinical condition were ob-  
197 served. Since the bony anatomy varies widely, the lower limbs alignment in  
198 vivo state can reflect the real morphology information. On the other hand, the

199 knee flexion of each subject was not preset by the researchers, but appeared  
200 naturally. Knee flexion angles were calculated by the self-developed program  
201 for quantitative analysis. The difference of the measured coronal deformity was  
202 significant between the weight-bearing condition and the non-weight-bearing  
203 condition. Brouwer et al.[2] performed a standing and a supine LLR in 20  
204 patients with varus deformity and found an average of 2 ° more varus devi-  
205 ation in the standing position than in the supine position. In our study, the  
206 virtual LLR reconstructed from CT scanning in the supine position was used  
207 to compare with the real LLR in the standing position. The average HKA  
208 measured on the virtual LLR is 2.83° less than that on the real LLR. This  
209 could be explained by the reduction of loading forces across the knee joint  
210 in the supine position. Although the clinical examination of standing LLR  
211 is different from the supine position LLR we used, it is not expected to alter  
212 the trends of above results observed from the virtual LLR in the present study.

213  
214 There are some limitations to this study. Firstly, the virtual LLR were  
215 simulated from supine CT scanning, which results in a slight difference from  
216 the clinical examinations. It will be more ideal to obtain the CT images in the  
217 weight-bearing condition. Consequently, this study compared the lower limb  
218 alignment measured on virtual LLR with the real LLR, as the complement of  
219 our results obtained in non-weight-bearing position. Secondly, when exploring  
220 the relation between knee coronal deformity and measurement errors in the  
221 weight-bearing condition, it is not rigorous enough to use HKA reflected on  
222 the real LLR to represent knee coronal deformity. However, this provides some  
223 clues to the relation between coronal deformity and the measurement errors  
224 in clinics. Thirdly, the gender-specific differences of coronal alignment in os-  
225 teoarthritic knees were not clear according to a review by Hess et al [5]. The  
226 participants of this study were mostly females (73%) and thus, we could not  
227 show a gender difference. Some studies proposed that assessing the lower limb  
228 alignment in subjects with an uneven gender ratio might be biased. The last  
229 limitation is associated with the low proportion of severe lower limb malfor-  
230 mation subjects. However, current results demonstrated a trend that the more  
231 severe the deformity, the greater the lower limb alignment measurement error  
232 might be.

## 233 4 Conclusions

234 The alignment measurement of lower limb on LLR can be affected by the  
235 limb rotation. However, the measurement errors caused by this effect vary  
236 individually. Both knee flexion and coronal deformity have significant effect  
237 on such measurement error. When comparing the varying coronal deformity  
238 group, the severe group (coronal deformity > 10°) differed significantly from  
239 the other two groups in HKA deviation range and in FTA deviation range.  
240 Moreover, compared with the non-weight-bearing condition, the influence of  
241 coronal deformity on measurement errors will be amplified in weight-bearing

**Table 4** Patient baseline demographics and image information collection in clinics

	Participants (n = 30)
Median age, years (median 25th; 75th quartiles)	67.0 (62.0;67.5)
Female, n (%)	22 (73.3%)
Bilateral knee, n (%)	15 (50%)
Non-weight-bearing CT scan, n (%)	30 (100%)
Weight-bearing LLR image, n (%)	21 (70%)

condition. In addition, the error of measuring the anatomic axis on LLR is greater than that of mechanical axis. Therefore, attention should be paid to the errors of measuring lower limb alignment on LLR, especially for patients with severe knee deformity in weight-bearing condition.

## 5 Materials and Methods

### 5.1 Subjects and Imaging Procedure

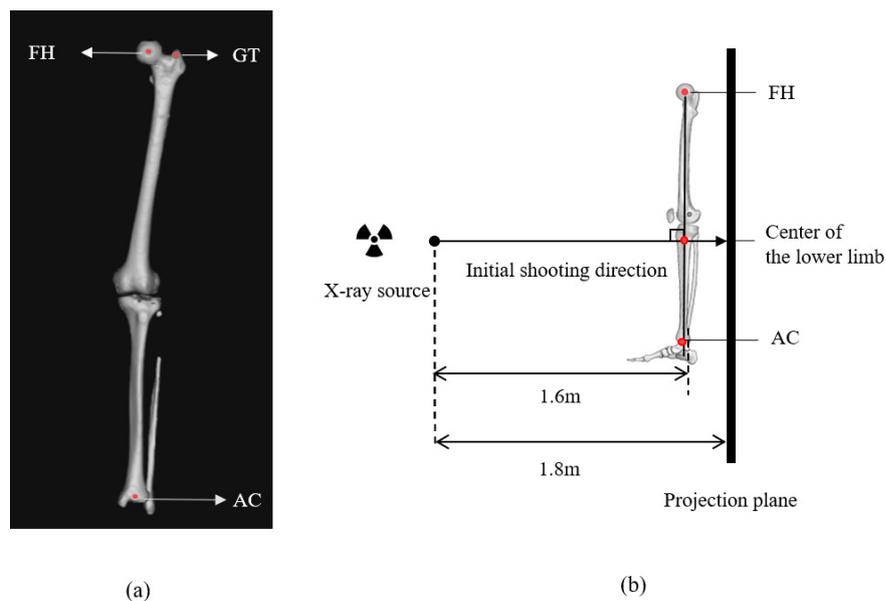
From May 2018 to November 2019, 30 patients (45 knees) with the median age of 67 (range from 48 to 94) were enrolled in this study (Table. 1). Twenty-two patients (33 knees) were female and eight patients (12 knees) were males. Patients with a history of bone loss, infection, tumor, congenital disease, and lower extremity surgery were excluded from participation. CT scans of all subjects (45 knees) and weight-bearing LLR for some patients (31 Knees) with knee arthritis were detected preoperatively. Radiographs were performed with 7-22 mAs and 70-90 kVp, depending on the body mass. CT scans (Siemens SOMATOM Definition Flash, Germany) of the lower extremities in the supine position were obtained using a 1.0 mm section thickness with 120KVp. The method of measuring the knee flexion and coronal deformity is shown in Supplementary Materials. The knee flexion in supine position and coronal deformity of each patient appeared naturally were calculated by a custom program. According to the degree of knee flexion and coronal deformity in non-weight-bearing condition, 45 knees were graded as shown in Table 2. The degree of knee flexion and coronal deformity is divided by three levels: 0-5 degrees; 5-10 degrees; more than 10 degrees.

### 5.2 Alignment measurement

The determination of anatomical planes depends on three landmarks from 3D models (Fig. 2 (a)): center of the femoral head (FH), center of ankle joint (AC), and tip of the greater trochanter (GT). To be specific, FH was defined as the well-fitting sphere center point of the femoral head; AC was defined as the diagonal intersection of the ankle joint surface; and GT was defined as the tip of the greater trochanter from the frontal forward direction. Then

**Table 5** According to the degree of knee flexion and coronal deformity, 45 knees were classified into the following levels.

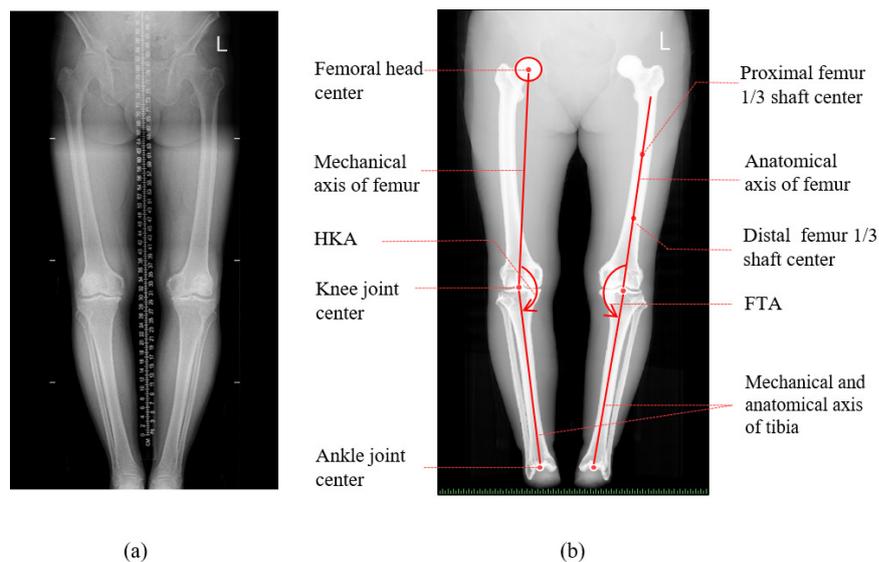
Grade	Flexion or Coronal deformity angle	Knee (N= 45)
Slight Flexion	0-5°	22 (48.9%)
Moderate Flexion	5-10°	17 (37.8%)
Severe Flexion	>10°	6 (13.3%)
Slight Coronal deformity	0-5°	14 (31.1%)
Moderate Coronal deformity	5-10°	25 (55.6%)
Severe Coronal deformity	>10°	6 (13.3%)



**Fig. 5** Applying the 3D model of the lower limb reconstructed from CT scanning to set parameters and geometric relations for virtual X - ray capturing. (a) Three landmarks to determine anatomical planes on 3D model: center of the femoral head (FH), center of ankle joint (AC), and tip of the greater trochanter (GT). (b) The schematic diagram of capturing virtual X - ray by using the lower limb 3D model. The center of the lower limb was defined as the midpoint of line FH-AC. The X-ray source was 1.6 and 1.8 meters away from the center of the lower limb and the projection plane, respectively. The initial shooting direction was perpendicular to line FH-AC, passing through the center of the lower limb.

272 coronal plane was defined as the plane passing through Points FH, AC, and  
 273 GT. Sagittal plane was defined as the plane perpendicular to coronal plane  
 274 and passing through line FH-AC.

275  
 276 To measure the axial alignment of non-weight-bearing conditions and compare  
 277 with weight-bearing LLRs, the virtual LLRs were reconstructed by a  
 278 Python program. To simulate the real LLR imaging, the X-ray source was po-



**Fig. 6** A real long-leg radiograph in clinics and a virtual long-leg radiograph in this study. (a) Real long-leg radiograph was captured in weight-bearing condition. (b) Virtual long-leg radiograph was captured in non-weight-bearing condition. Both mechanical axis and anatomical axis were measured on virtual LLR. The HKA was defined as the mechanical axial of the femur and tibia. The FTA was defined as the anatomical axial of the femur and tibia.

279 sitioned at 1.6 meters forward from midpoint of line FH-AC and the projection  
 280 plane was 1.8 meters from the X-ray source. The initial beam direction was  
 281 oriented perpendicular to the coronal plane. Fig. 2(b) shows the schematic of  
 282 capturing virtual LLR. The virtual LLR of each knee was reconstructed from  
 283 the projection of the 3D model by using a self-developed python program. Real  
 284 LLR in weight-bearing condition and virtual LLR in non-weight-bearing con-  
 285 dition were shown in Fig. 3. From the radiograph, the mechanical axis of femur  
 286 was defined as the line passing from the center of femoral head to the center  
 287 of the knee joint; the anatomical axis of femur was defined as the line passing  
 288 midpoint of the upper and lower 1/3 of the femoral medullary cavity; both the  
 289 mechanical and anatomical axis of tibia were defined as the line passing the  
 290 center of the knee and ankle joint. HKA was defined as the medial side angle of  
 291 mechanical axis; and FTA was defined as the medial side angle of anatomical  
 292 axis. Both HKA and FTA were measured on virtual LLR as shown in Fig. 3(a).  
 293

294 To simulate the clinical radiographic capture and quantify the variability  
 295 of HKAs and FTAs measured on LLR, the 3D model was rotated from the  
 296 neutral position. The rotation axis was defined as the line FH-AC. With the  
 297 rotation of the lower limb in  $10^\circ$  increments, ranging from  $20^\circ$  internally to  
 298  $20^\circ$  externally, five series of virtual LLRs were obtained. The range (maximum

minus minimum) of HKAs and FTAs in each group was defined as the HKA deviation and the FTA deviation, respectively.

### 5.3 Statistical Analysis

To assess the impact of knee coronal deformity and flexion angle on the deviation of HKA and FTA, two-way ANOVAs were calculated using SPSS 22.0 (SPSS Corp, Chicago, USA). The threshold for statistical significance was set at  $\alpha = 0.05$ . The normality test and uniformity test of error variance are carried out for the raw data. Following that, multiple comparison least significant difference (LSD) was run to compare the mean changes between each event. In addition, Spearman's correlation coefficients were accessed to analysis the relationship between variables. The method of measuring the knee coronal and flexion angle were shown in Supplementary Materials. The percentages for categorical variables were summarized. Means, standard deviations and the standard error of mean or medians with interquartile ranges were performed for continuous variables. The differences of coronal deformity between virtual LLR (non-weight-bearing condition) and real LLR (weight-bearing condition) were analyzed by a paired t-test. Intra-observer reliability was calculated by the intraclass correlation coefficient (ICC). The interpretation scales of ICC are classified according to following: 0–0.20 is slight, 0.21–0.40 is fair, 0.41–0.60 is moderate, 0.61–0.80 is substantial, and 0.81–1.00 is perfect.

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