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Corresponding Author:	Satoshi Oki, M.D., Ph.D. Keio University School of Medicine Shinjuku, Tokyo JAPAN
Corresponding Author's Institution:	Keio University School of Medicine
Corresponding Author E-Mail:	satoshiohki@gmail.com
Order of Authors:	Satoshi Oki, M.D., Ph.D.
	Kazuya Kaneda
	Yoshitake Yamada
	Minoru Yamada
	Yutaro Morishige
	Kengo Harato
	Noboru Matsumura
	Takeo Nagura
	Masahiro Jinzaki
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TITLE:
Four-Dimensional CT Analysis Using Sequential 3D-3D Registration

AUTHORS AND AFFILIATIONS:
Satoshi Oki[1,2], Kazuya Kaneda[1], Yoshitake Yamada[3], Minoru Yamada[3], Yutaro Morishige[1], Kengo Harato[1], Noboru Matsumura[1], Takeo Nagura[1], Masahiro Jinzaki[3]

[1]Department of Orthopedic Surgery, Keio University School of Medicine, Tokyo, Japan
[2]Department of Orthopedic Surgery, Saiseikai Utsunomiya Hospital, Tochigi, Japan
[3]Department of Diagnostic Radiology, Keio University School of Medicine, Tokyo, Japan

Corresponding Author:
Satoshi Oki (satoshiohki@gmail.com)

Email Addresses of Co-authors:
Kazuya Kaneda (kazpleasure@gmail.com)
Yoshitake Yamada (yamada@rad.med.keio.ac.jp)
Minoru Yamada (yamada@z8.keio.jp)
Yutaro Morishige (yuyumorishige@gmail.com)
Kengo Harato (harato@keio.jp)
Noboru Matsumura (noboru18@gmail.com)
Takeo Nagura (nagura@keio.jp)
Masahiro Jinzaki (jinzaki@rad.med.keio.ac.jp)

KEYWORDS:
4DCT, image analysis, motion analysis, computed tomography, joint kinematics, surface registration

SUMMARY:
We analyzed joint kinematics from four-dimensional computed tomography data. The sequential 3D-3D registration method semiautomatically provides the kinematics of the moving bone with respect to the subject bone from four-dimensional computed tomography data.

ABSTRACT:
Four-dimensional computed tomography (4DCT) provides a series of volume data and visualizes joint motions. However, numerical analysis of 4DCT data remains difficult because segmentation in all volumetric frames is time-consuming. We aimed to analyze joint kinematics using a sequential 3D-3D registration technique to provide the kinematics of the moving bone with respect to the fixed bone semiautomatically using 4DCT DICOM data and existing software. Surface data of the source bones are reconstructed from 3DCT. The trimmed surface data are respectively matched with surface data from the first frame in 4DCT. These trimmed surfaces are sequentially matched until the last frame. These processes provide positional information for target bones in all frames of the 4DCT. Once the coordinate systems of the target bones are decided, translation and rotation angles between any two bones can be calculated. This 4DCT analysis offers advantages in kinematic analyses of complex structures such as carpal or tarsal bones. However, fast or large-scale motions cannot be traced because of motion artifacts.

INTRODUCTION:

Joint kinematics have been described using a number of methodologies, such as motion capture sensors, 2D-3D registration, and cadaveric studies. Each method has specific advantages and disadvantages. For example, motion capture sensors can measure fast, large-scale motions using infrared cameras with or without sensors on the subject[1,2]. However, these methods measure skin motion to infer joint kinematics, and therefore contain skin motion errors[3].

Cadaveric studies have been used to evaluate ranges of motion, instability, and contact areas[4-6]. This approach can measure small changes in small joints using CT or optical sensors attached directly to bone using pins or screws. Cadaveric models can mainly evaluate passive motions, although multiple actuators have been used to apply external forces to tendons to simulate dynamic motion[7]. Active joint motion can be measured by 2D-3D registration techniques, matching 3DCT images to 2D fluoroscopy images. Although the accuracy of the registration process remains controversial, the reported accuracy is generally high enough for large joint kinematics[8,9]. However, this method cannot be applied to small bones or multiple bones in narrow spaces.

In contrast, 4DCT is a dynamic CT method that obtains a series of volumetric data. Active joint motions can be analyzed using this approach[10]. This technology provides precise 3D positional data of all substances inside the CT gantry. The 3D joint motions are clearly visualized in a viewer. However, describing joint kinematics from such a series of volume data is still difficult, because all the bones are moving and no landmarks can be traced during the active motions in vivo.

We developed a method for 4DCT analysis that provides the in vivo joint kinematics of the whole bones around the joint during active motions. The aim of this article is to present our method, the sequential 3D-3D registration technique for 4DCT analysis, and show representative results obtained using this method.

PROTOCOL:

All methods described here have been approved by the Institutional Review Board of Keio University School of Medicine.

NOTE: Joint kinematics are measured by reconstructing the motion of a moving bone around a fixed bone. For knee joint kinematics, the femur is defined as the fixed bone and the tibia is defined as the moving bone.

1. CT imaging protocol

1.1. Set up the CT machine. Acquire CT examinations with a 320-detector-row CT system to allow for multiple phases of 3D volume data with 160 mm craniocaudal coverage. For example, in the analysis of knee kinematics, the image acquisition consists of 51 volume scans with a rotation time of 0.275 s, and all images are reconstructed using half reconstruction, so that the temporal resolution is approximately 0.16 s.

1.2. Use the following scanning parameters: peak tube voltage = 100 kVp; tube current = 40 mA; scan coverage = 160 mm; matrix size= 512 x 512 pixels; and reconstruction section thickness and section interval = 0.5 mm.

1.3. Place the target joint of the subject inside the CT gantry in the starting position of the 4DCT exam (**Figure 1**).

1.4. Before the CT exam, rehearse movements of the joint from the start position to the end position within the required examination time. Ask the subject to move the joint during the 10.275 s scan time and obtain a series of volume data. Store the sequential volume data in DICOM format.

1.5. Perform static 3DCT of all the target bones and store the data in DICOM format.

2. Surface reconstruction

2.1. Perform semiautomatic segmentation of 3DCT data (**Figure 2A**).

2.1.1. Load CT DICOM data by selecting all DICOM files of the static 3DCT data.

2.1.2. Open the label field by clicking **Edit New Label Field** and check which threshold CT attenuation value is appropriate to extract cortical bone from the source bone. Select materials with CT attenuation values above the threshold. For example, the bone cortex threshold for a young subject is set as **250**. Check the label for **Bone Cortex Selection** and manually modify the demarcation using an editing tool for consistency with the shape of the bone.

2.1.3. Generate the surface data (triangle meshes) from the labeled bone cortex position data (point cloud in the software). Store the surface data by exporting data in Standard Triangulated Language (STL) format.

2.1.4. Click **Generate Surface|Apply** on the label of the cortical bone. Click **File|Export Data As|STL Binary Little Endian** to save the surface data in STL format.

2.2. Perform automatic segmentation of 4DCT volume data (**Figure 2B**).

NOTE: Each frame of the DICOM data includes the distribution of the CT attenuation values in the CT gantry.

2.2.1. Set the threshold of the bone cortex as in static CT, and extract geometric data showing CT attenuation values above the threshold from all 51 frames of the 4DCT data using the DICOM reading module in the programming software. Adjust the threshold according to the bone density of the source bone. For example, for the osteoporotic bone, set the threshold lower.

2.2.2. Translate all positional data that have already been obtained in the previous step into a format that can be interpreted by image processing software (e.g., Avizo). In the image processing software, reconstruct all surface data of the point cloud with higher CT attenuation values than the threshold for all 4DCT frames using a batch processing script. The image processing software contains the function to read the script and export the surface data from the DICOM series data automatically. The batch script is shown in the **Supplemental Coding File**.

3. Image registration

NOTE: In this step, reconstruct the motions of the moving bone with respect to the fixed bone from the raw 4DCT DICOM data.

3.1. Perform surface registration from static 3DCT to the first frame of the 4DCT.

3.1.1. Trim the bones in a static 3DCT into partial segment data that are included in all frames of 4DCT for use with the iterative closest point (ICP) algorithm[11] in the 3D mesh editing software using the **Selecting Face** function (**Figure 3A**) by referring 4DCT movie data. The surface data from 4DCT are only partial segments that are included in each volume image because surface registration requires that one surface data point is included in another surface.

3.1.2. Pick three landmarks in the fixed and moving bones that can be easily identified from the trimmed 3DCT surface and the surface data of the first frame of 4DCT in the 3D mesh editing software using the **PickPoints** function (**Figure 3B**).

3.1.3. Match the partial fixed and moving bones roughly on the first frame of the 4DCT surface data (**Figure 3C**) according to the picked landmarks in 3.1.2. Next, perform surface registration using the ICP algorithm[11] using the open source software (e.g., VTK).

NOTE: This process provides homogeneous transformation matrices of the fixed and moving bones from the static 3DCT to the first frame of 4DCT (**Figure 3D**). These matrices are 4 x 4 matrices consisting of rotation and translation, as shown in **Figure 4**. The transformation matrix causing the reverse action can also be calculated.

3.2. Perform sequential surface registration (**Figure 5**).

3.2.1. Match the partial surfaces of the fixed and moving bone in the first 4DCT frame onto the surface data of the second frame. Next, match the partial surfaces of the i^{th} frame onto the $(i + 1)^{th}$ frame of 4DCT sequentially. Repeat this process until the last frame of the 4DCT by programming with use of the ICP module in the open source software.

3.3. Calculate transformation matrices from the static 3DCT to all frames in 4DCT according to the results of 3.1 and 3.2.

3.4. Reconstruct moving bone motion with respect to the fixed bone (**Figure 6**).

3.4.1. Reconstruct the kinematics of the moving bone with respect to the fixed bone from the matrices that represent the transformation from the static 3DCT to each 4DCT frame. Define the coordinate systems of the fixed and moving bones when the rotation parameters are measured (e.g., flexion angle or rotation angle calculated by the Euler/Cardan angle)[12-14].

REPRESENTATIVE RESULTS:

We describe the motion of the tibia during knee extension. The knee joint was positioned in the CT gantry. A triangle pillow was used to support the femur at the starting position. The knee was extended to a straight position over the course of 10 s. Radiation exposure was measured. In addition to 4DCT, static 3DCT of the whole femur, tibia, and patella was performed. Surface data of the whole femur and tibia were reconstructed. The threshold for HU numbers of the bone cortex was set as 250 HU and the surface data of all 51 frames were reconstructed.

The femur and tibia were trimmed into partial surface data that were included in all 4DCT frames by visually checking 4DCT movie data, which is created in the preset 4DCT software. In the static 3DCT surfaces and the first frame of the 4DCT, the landmarks of each segment were plotted. On the femur, the medial and lateral epicondyles and intercondylar notch were identified. On the tibia, the medial and lateral ends of the joint surface and tibial tuberosity were also identified as corresponding landmarks. Partial surface data of the femur and tibia were roughly matched with the first frame of the 4DCT data according to these three landmarks. These surfaces were then completely matched using the ICP algorithm.

Partial segments of the femur and tibia of the first frame were matched with the whole surface in the second frame. Partial fragments in the i^{th} frame were thus matched with the whole surface data of the $(i + 1)^{th}$ frame sequentially. In the ICP algorithm, the convergence criteria for mean distance between iterations was set as 0.01 mm.

The femur was defined as the fixed bone and the tibia as the moving bone. The 4 x 4 matrix that describes translation and rotation from the global coordinate system in the original CT DICOM data to the local coordinate system of the fixed bone is calculated. The coordinate system of the femur and tibia were defined in accordance with a previous report[15]. We calculated the motion of the tibia from the Euler/Cardan angles in ‘zxy’ order, meaning flexion, varus, and internal rotation, in that order[14].

Our method depends on the accuracy of image registration from the partial segments onto the whole surface data. We validated the accuracy of partial surface registration by decreasing the length of the femur and tibia incrementally by 1% along the long axis from 20%–1%. Surface registration of partial segments to the whole bones was performed for the entire set of lengths of the femur and tibia, and errors for rotation and translation from the parameters calculated from the whole bones were evaluated.

Results showed that the varus angle of the tibia gradually decreased as the tibia was extended (**Figure 7**). Tibial external rotation increased at the end of the extension. This external rotation corresponds with the “screw home movement” of the knee in previous reports[16,17].

The effective dose estimate for this CT protocol was 0.075 mSv, as determined by the dose length product measurement (187.5 mGy·cm) and appropriate normalized coefficients (0.0004) as reported in the literature[18].

In validation, graphs of the error for translation and rotation show that the error was tolerable for femur lengths longer than 9% of the whole length and tibia lengths longer than 7% of the whole length (**Figure 8**). At 10% of the length of the femur and 8% of the length of the tibia, errors were 0.02° for varus/valgus rotation, 0.02° for internal/external rotation, 0.01° for extension/flexion rotation, 0.10 mm for anterior/posterior translation, 0.14 mm for proximal/distal translation, and 0.11 mm lateral/medial translation. These translation errors are considered negligible because CT slice thickness is 0.5 mm and exceeds the error size. Internal and external rotation errors tended to fluctuate. This was thought to be caused by local minimum fit for iterative rotation along the long axis due to the symmetrical shape of the tibial joint surface.

As additional data, patellar kinematics were also calculated using the same method. We demonstrated the lateral tilt of the patella by tracking the norm of the patellar surface corresponding to the knee flexion angle as calculated from analysis of the tibia (**Supplement Figure 1**).

FIGURE AND TABLE LEGENDS:

Figure 1: Acquisition of 4DCT. The 4DCT examination for knee extension. The subject is instructed to lie down and position the knee in the CT gantry. At the starting position, the knee is set in the flexed position and extended within 10 s after the examination starts. In this figure, the subject extends the knee from 60° of flexion to maximum extension in 10 s.

Figure 2: Reconstruction of surface data. (**A**) Surface data of the whole femur (fixed bone) and whole tibia (moving bone) are reconstructed. (**B**) Using DICOM data from 4DCT, the positional data of the bone cortex showing CT attenuation values above the threshold are extracted in each frame. These positional data are input into the software and the surface data of all frames are reconstructed. The femur also moves (green arrow) with respect to the tibia (blue arrow).

Figure 3: Surface registration. (**A**) Surface data of the fixed and moving bones from 3DCT are trimmed into partial segments that are included in all 4DCT frames because the surface data from 4DCT are only partial segments, which are included in the CT gantry. (**B**) Three landmarks are picked in the partial segments of the static 3DCT and the first frame of the 4DCT. (**C**) The partial segments are matched with the first frame according to the landmarks. (**D**) The iterative closest point (ICP) algorithm is applied to match surface data.

Figure 4: Transformation matrix is calculated from surface registration. (**A**) Translation and rotation of surface data can be described in a 4 x 4 matrix (homogeneous transformation matrix). **Mref** represents the matrix of the fixed bone and **Mobj** represents the matrix of the moving bone. The lower right value represents the starting position and the upper left value represents the target position. For example, $^{1}\mathbf{Mref}_{s}$ translates the fixed bone in the static 3DCT position to the fixed bone in the first frame of 4DCT. (**B**) The rotation matrix is a 4 x

4 matrix. $\mathbf{R^3}$ is a 3 x 3 matrix that defines rotation and **d** is a 1 x 3 matrix that defines translation. $^{\mathbf{t}}\mathbf{R_3}$ is a transverse matrix of $\mathbf{R_3}$. (**C**) The upper right "**inv**" means the reverse action matrix.

Figure 5: Steps of sequential surface registration of all frames. The difference between i^{th} and $(i + 1)^{th}$ frames is very small. Partial segments of the i^{th} frame can be matched with whole surface data of the $(i + 1)^{th}$ frame only by the ICP algorithm. Surface registration is repeated sequentially until the last frame. The transformation matrix from the static 3DCT to each frame ($^{\mathbf{i}}\mathbf{M_s}$) is calculated.

Figure 6: Rotation angles are calculated using the defined coordinate systems of the fixed and moving bones. (**A**) The coordinate system of the fixed bone is defined[15]. The rotation matrices from static 3DCT to the local coordinate system of the fixed bone ($^{\mathbf{L}}\mathbf{Mref_S}$) are calculated. (**B**) The coordinate system of the moving bone is defined and drawn over the fixed bone in its local coordinate system[15]. Rotation matrices from the local moving bone to the local coordinate system of the fixed bone are calculated (**Mi**). From these matrices, angles of the moving bone with respect to the fixed bone are calculated using the Euler/Cardan angle.

Figure 7: Representative results show kinematics of the tibia during knee extension. (**A**) Extension of the tibia. From the starting frame, the tibia is extended almost constantly and extension speed increases around the end frame. (**B**) Tibial internal rotation. The transverse axis is the tibial extension angle. The tibia rotates internally to 10° of flexion and rotates externally until the end frame. (**C**) The valgus angles increase constantly during all frames of knee extension.

Figure 8: Validation of surface registration of the partial segment onto the whole bone. The lengths of the femur and tibia are decreased incrementally by 1% along the long axis from 20%–1%. Surface registration of partial segments to whole bones are performed for all sets of lengths of the femur and tibia, and errors for rotation and translation from the parameters calculated from the whole bones are evaluated. Perturbation analysis is shown in **Supplemental Figure 2**.

Supplement Figure 1: Patellar kinematics during knee extension. Patellar kinematics are also calculated using the same method. (**A**) A surface was fit on the surface data of the patella. The norm of the surface pointing anteriorly is calculated. The lateral tilt is defined as the lateral tilting angle of the norm in the coordinate system of the femur. (**B**) The patellar lateral tilt during knee extension is plotted corresponding to knee extension as calculated from tibial kinematics.

Supplemental Figure 2: Perturbation analysis.

DISCUSSION:

Our method allows visualization and quantification of the motions of whole bones and provides numerical positional data of the moving bone with respect to the fixed bone from 4DCT data. Many tools have been suggested for measuring joint kinematics. Motion skin markers can analyze total body motions over a long time. However, this method contains skin motion errors[3]. Joint kinematics should be estimated from the motion of adjacent bones. The 2D-3D registration method uses fluoroscopy and infers 3D kinematics from sequential 2D images. Translational errors are still present, although the analysis software has evolved to account for this. Many cadaveric studies have measured joint kinematics by taking CT images in different cadaver positions[19]. However, these represent passive motions from sequential static 3D images, and thus differ qualitatively from active motions.

There are several critical steps in this protocol. The surface data from 3DCT should be created precisely because this quality affects the accuracy of the initial surface registration to the first frame of 4DCT. Around the joint area, the threshold for the bone cortex may be different from the bone shaft. Threshold adjustment will be needed when the border of the bone cortex is unclear. Once the surface registration of all frames is finished, reconstructed motion should be checked. If surface registration for one frame fails, automated surface registration can be restarted from the next frame by picking the landmarks in the next frame and repeating the protocol.

The 4DCT method provides sequential volume data with accuracy almost as high as static 3DCT because CT DICOM data contain absolute coordinate values of all tissues in the CT gantry. Several studies have used 4DCT for investigations of joint kinematics[20,21]. However, in most, the observers picked landmarks from several frames and calculated the parameters (e.g., angles, translation). These data analysis processes contain human error that leads to measurement error. Our method of surface registration provides high accuracy image matching. Once plotted, the landmarks for the parameters can be traced according to the shape of the surface in each frame. Theoretically, manual surface segmentation for all 4DCT frames provides the most accurate data, but this process is far too time intensive. Recently, 4DCT has been used for motion analysis for wrist joints because the carpal bones are small and overlapped structures[22]. There have been several reports about automated bone tracing[23,24]. Goto et al. analyzed finger motions using normalized correlation coefficients that detect the similarity between two images[25]. We used surface registration because the position of the bone cortex surface is the most important landmark to describe joint kinematics.

We used an iterative closest point algorithm to trace the motion of the surface data in all frames. An iterative closest point algorithm matches two groups of point clouds or surface data to minimize surface-to-surface distance[11] but has several drawbacks. This algorithm is generally used to match two close surfaces. Therefore, when the two surfaces are located distant to each other, registration would occur in the ‘local minimum’ position, not the true matched position[26]. We overcome this drawback by taking three landmarks in each bone at first. The two surfaces are roughly matched according to these three landmarks. From these two positions, the ICP serves as the closest position. The frame rate of 4DCT is very short (0.2 s), so the surface position in the current frame is close to the surface position in the next frame. In cases of slow joint motion, the rough match step will not be needed for further frame-to-frame sequential surface registration. In addition, the relationship between the entirety of the two bones is reproduced by matching the entire static 3DCT surface data onto the partial 4DCT frame surface data. Generally, the coordinate system of the bone is defined from its entirety[12,27]. Reconstruction of the whole bone motion thus contributes to the description of joint angles. This accuracy largely depends on surface registration of the partial surface onto the whole surface data. In the representative data, we demonstrated that the availability of over 10% of the segments provides sufficient accuracy for the knee joint.

CT data provide all positional data included within the CT gantry area. The quality of the data depends solely on the quality of the CT machine. This method can thus be applied to small bones or multiple bones such as the carpal bones, which are difficult to trace by 2D-3D registration.

Several limitations must be mentioned. First, ICP depends on the shape of the partial segment. ICP is more accurate when the surface has geometric features such as bone spurs or cortical edges. On the other hand, when the surface shape is symmetrical, such as the radial head or sesamoid, ICP will provide a wrong rotation of the original surface. In addition, ICP also depends on the quality of the surface data. In case of osteoporotic bones, surface reconstruction largely depends on manual segmentation. That may lead to interobserver errors. Recently, computerized tissue segmentation on CT slices has been developed. However, human manual segmentation is still considered more reliable when identifying specific tissues[28,29]. Although the quality of the CT image cannot be changed, other limitations can be overcome by manual surface segmentation and registration. Second, when the joint motion is too fast, this method cannot trace the bone motions, because the CT images become blurry[30]. Frame-to-frame surface registration then fails because the two surfaces are too distant. Tolerable velocity depends on the target joint, because the joint morphology affects the success rate of surface registration. Studies of velocity tolerance for each joint will be needed in the future. In addition, joint motion should be performed inside the CT gantry. Therefore, for analysis of loading kinematics, optic sensors or 2D-2D registration are best.

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This study was approved by the Institutional Review Board of our institution (approval number: 20150128).

DISCLOSURES:

The authors have no competing financial interests.

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Figure 1

Figure 2

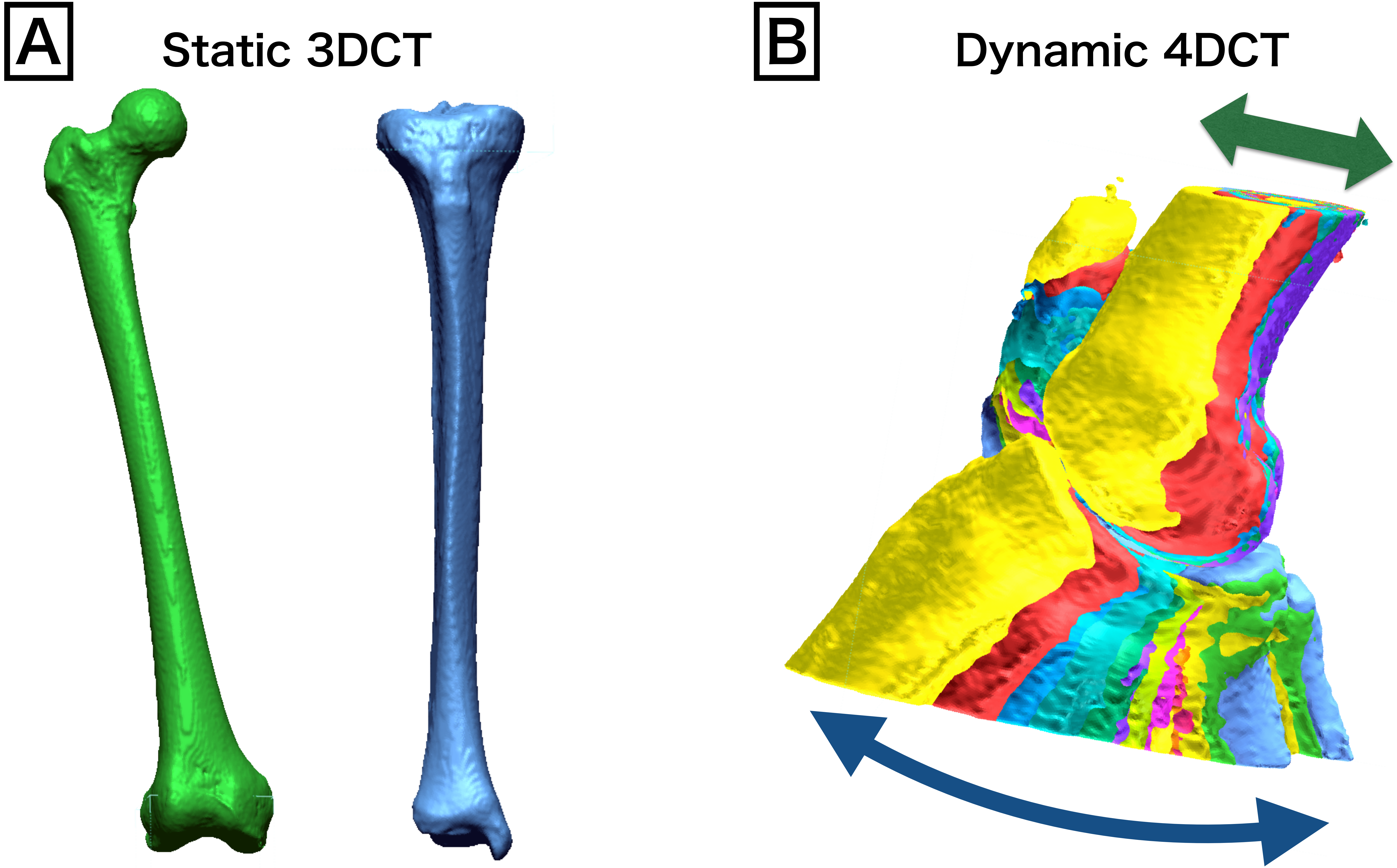


Figure 3

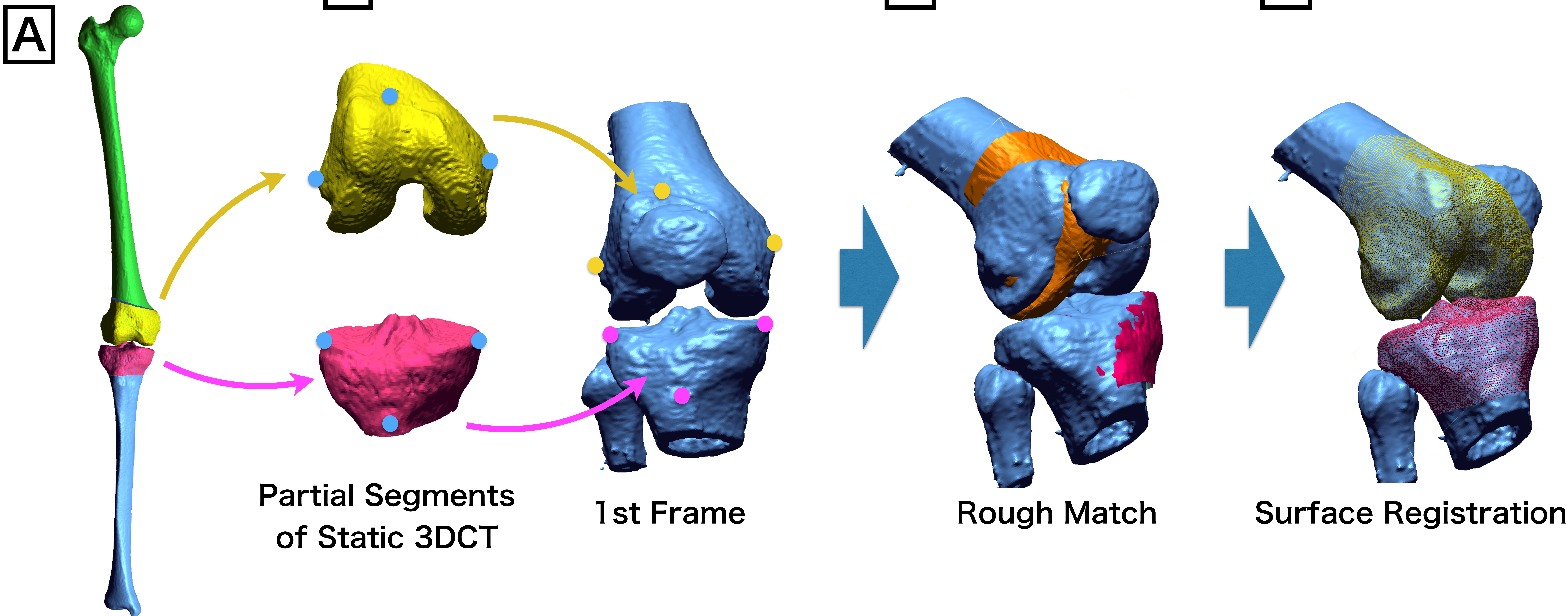
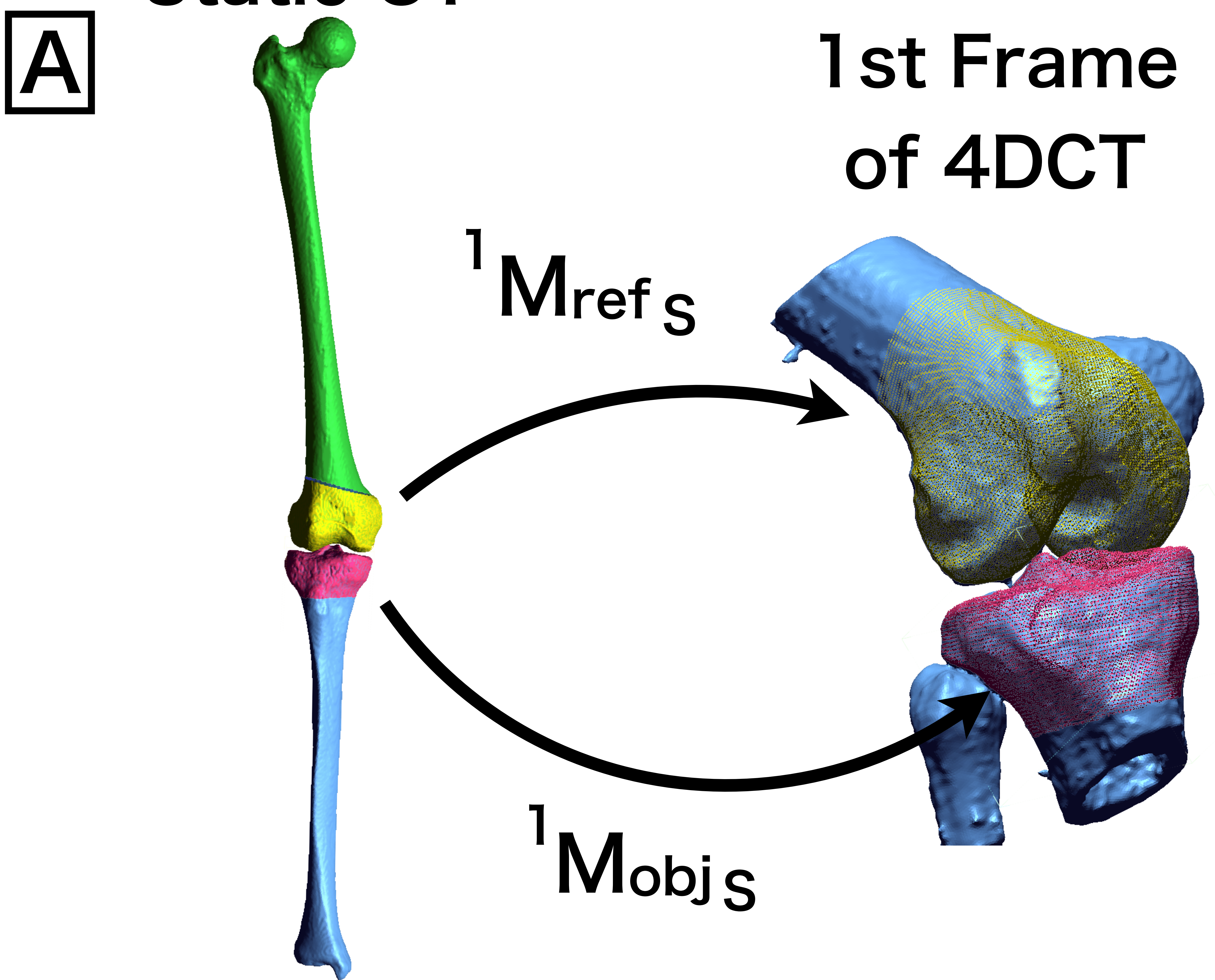

A
B
C
D
Partial Segments
of Static 3DCT
1st Frame
Rough Match
Surface Registration

Figure 4



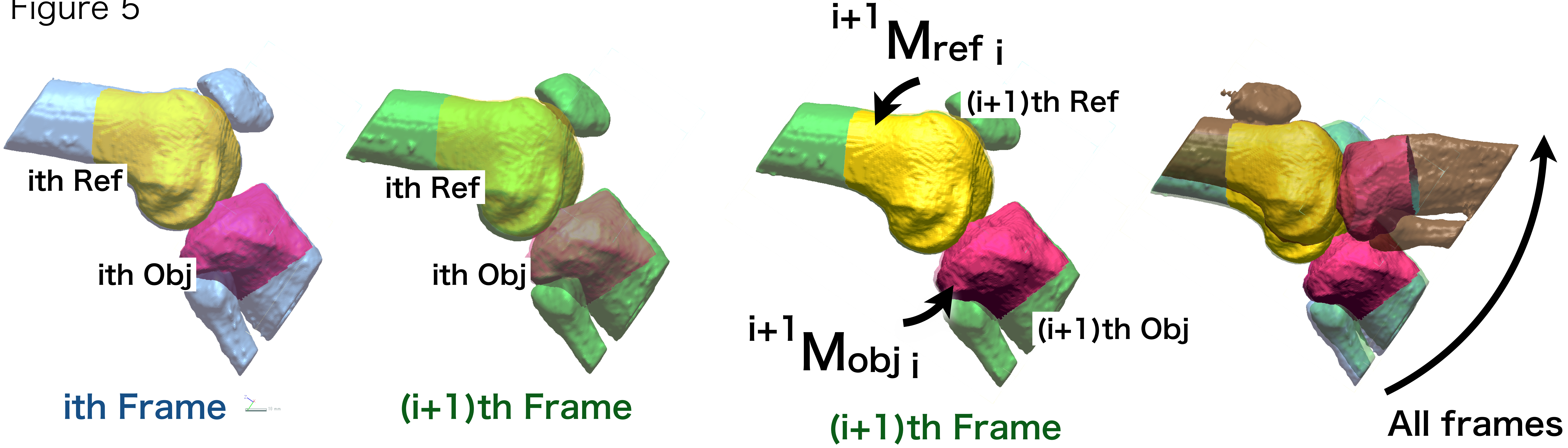
B

$$M = \left(\begin{array}{ccc|c} & R^3 & & d \\ \hline 0 & 0 & 0 & 1 \end{array}\right)$$

C

$$M^{inv} = \left(\begin{array}{ccc|c} & {}^{t}R^3 & & -{}^{t}R^3 \cdot d \\ \hline 0 & 0 & 0 & 1 \end{array}\right)$$

Figure 5



$$^{i}M_{ref\,S} = {}^{i}M_{ref\,i-1} \cdot {}^{i-1}M_{ref\,i-2} \cdots {}^{2}M_{ref\,1} \cdot {}^{1}M_{ref\,S}$$

$$^{i}M_{obj\,S} = {}^{i}M_{obj\,i-1} \cdot {}^{i-1}M_{obj\,i-2} \cdots {}^{2}M_{obj\,1} \cdot {}^{1}M_{obj\,S}$$

Figure 6

A

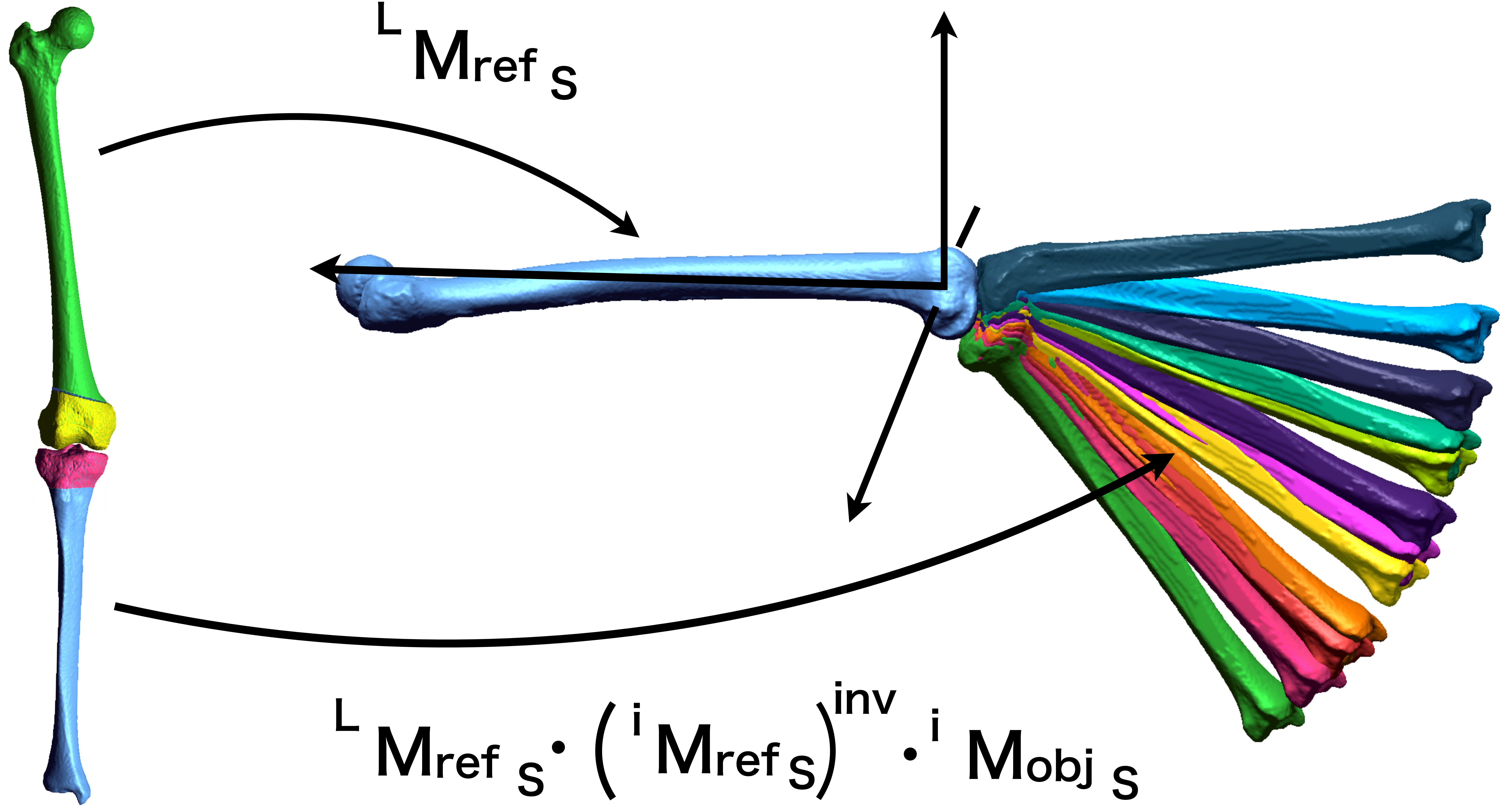
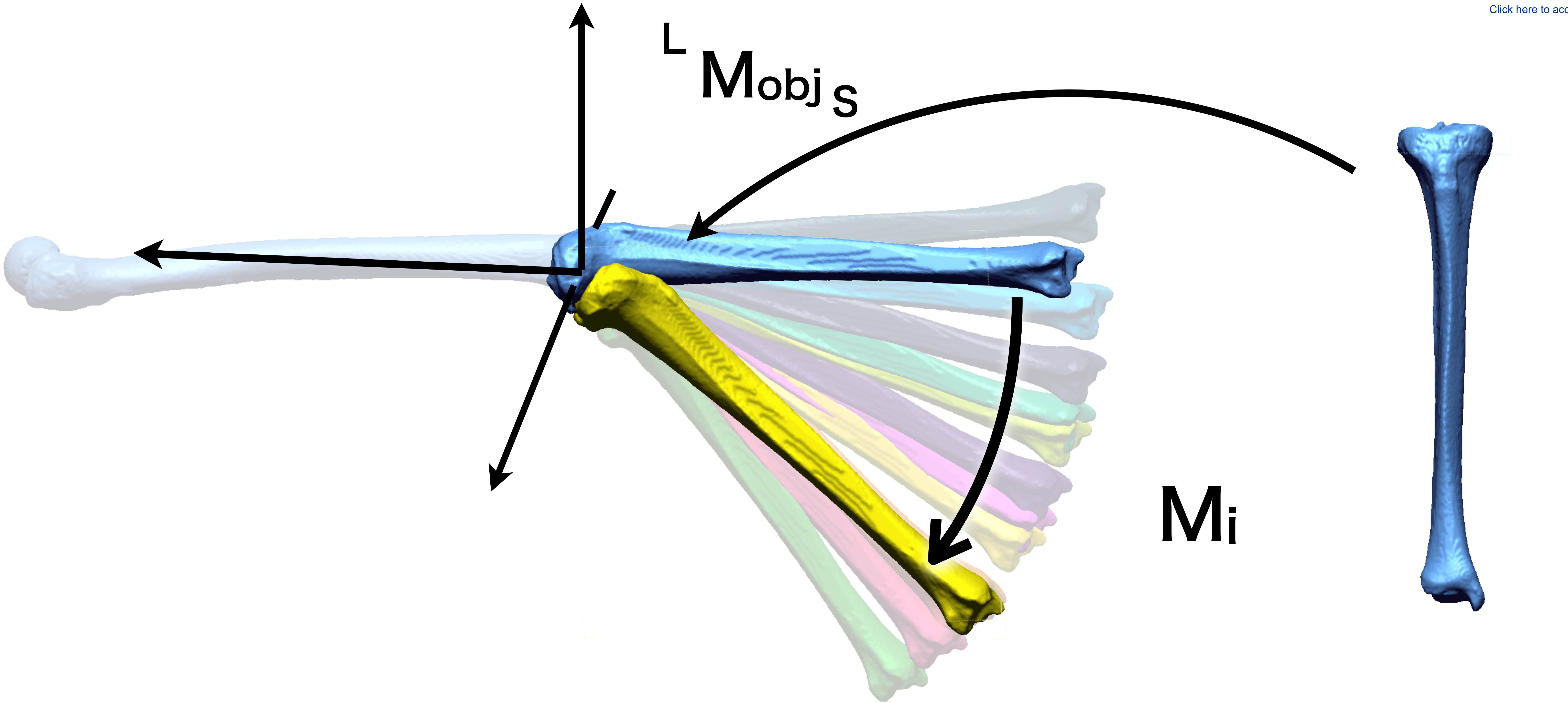


Figure 6

B



$$M_i = {}^{L}M_{ref\,S} \cdot \left({}^{i}M_{ref\,S}\right)^{inv} \cdot {}^{i}M_{obj\,S} \cdot \left({}^{L}M_{obj\,S}\right)^{inv}$$

Figure 7

Figure 7

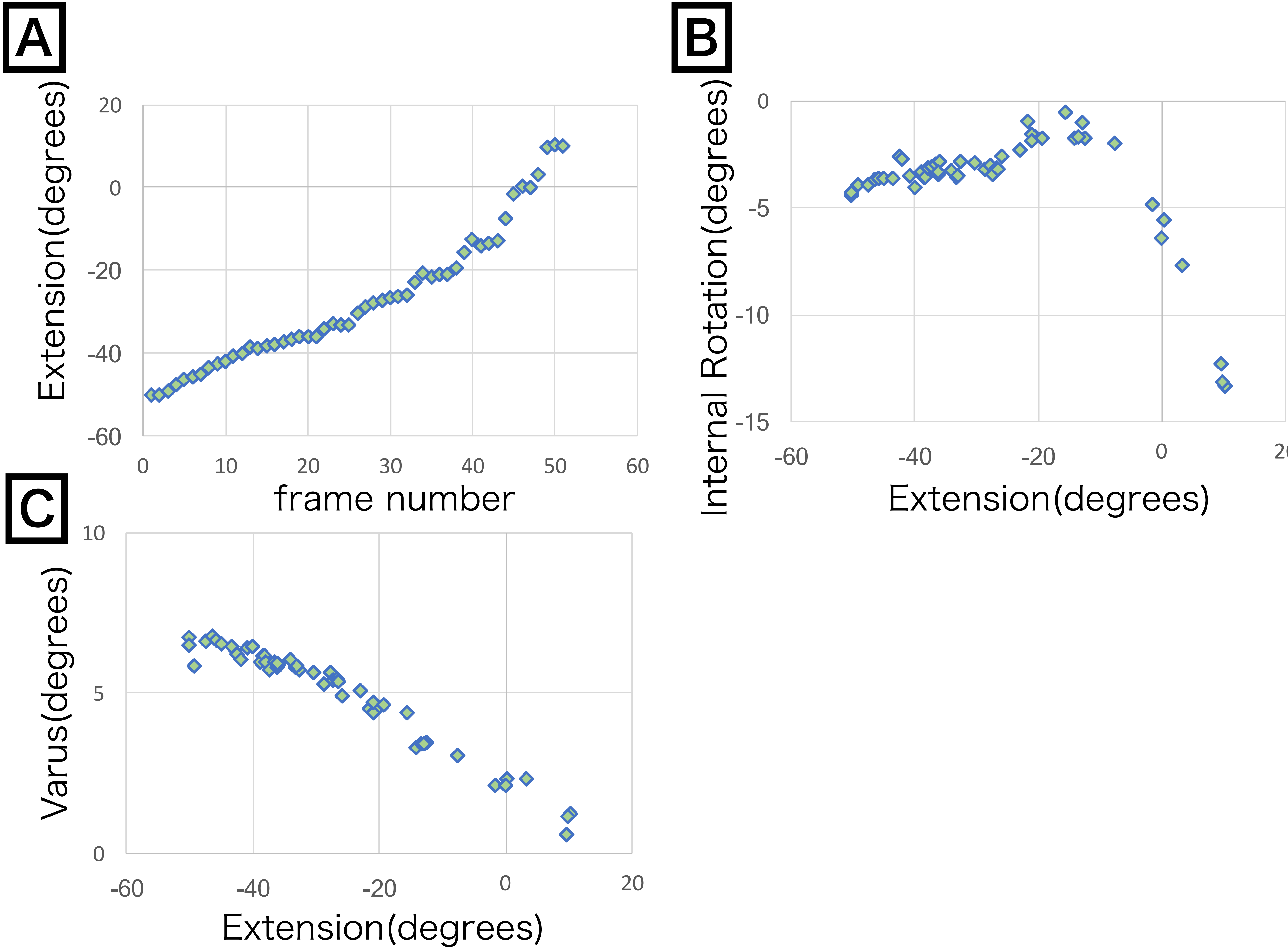
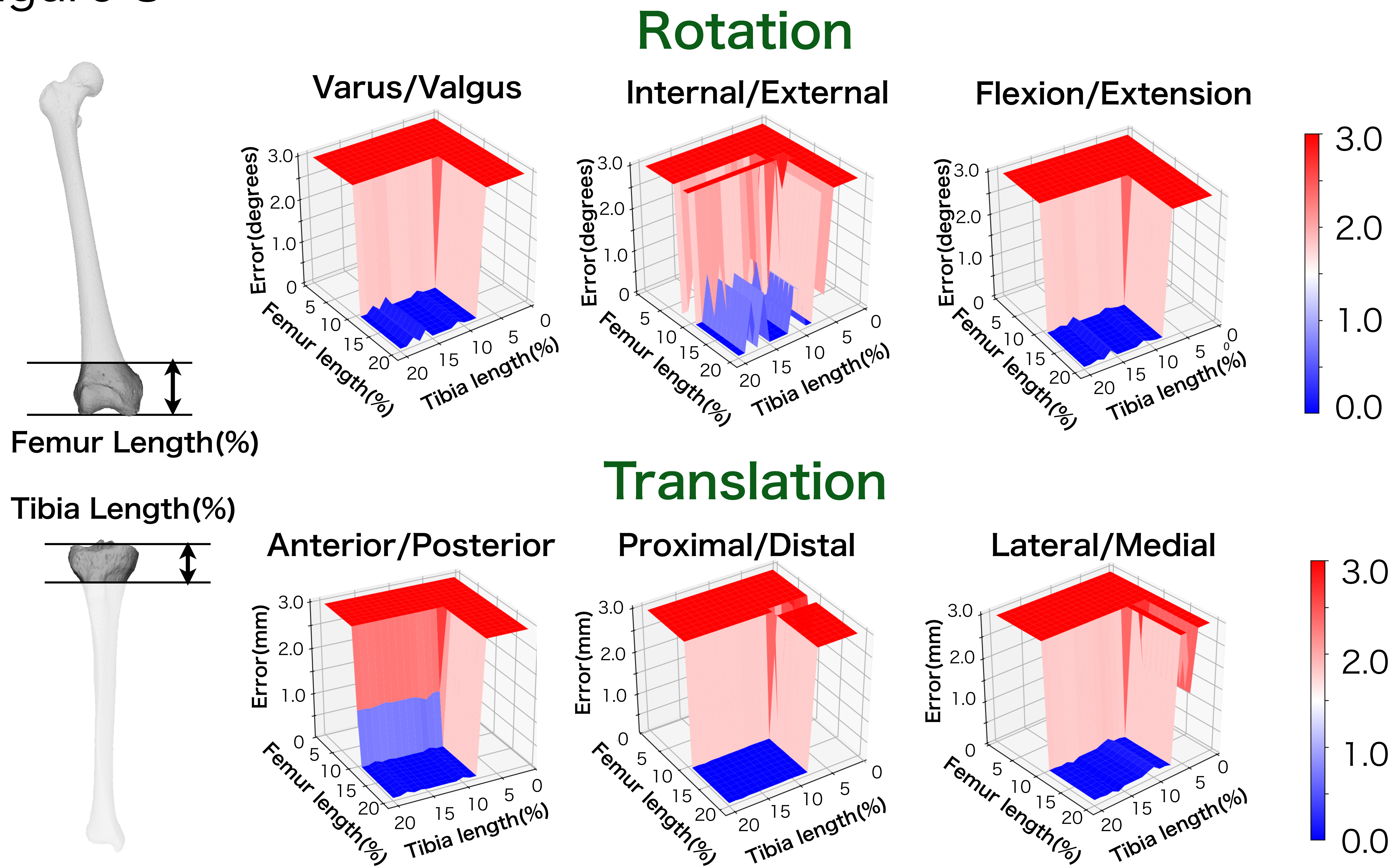


Figure 8


Rotation
Varus/Valgus
Internal/External
Flexion/Extension
Error(degrees)
3.0
2.0
1.0
0
Femur length(%)
Tibia length(%)
0
5
10
15
20
Femur Length(%)
Tibia Length(%)
Translation
Anterior/Posterior
Proximal/Distal
Lateral/Medial
Error(mm)
3.0
2.0
1.0
0.0

Name of Reagent/ Equipment	Company	Catalog Number
4DCT scanner	Canon medical systems (Tochigi, Japan)	N/A
AVIZO(9.3.0)*	Thermo Fisher Scientific (OR, USA)	N/A
Meshlab**	ISTI (Pisa, Italy)	N/A
VTK(6.3.0)***	Kitware (New York, USA)	N/A
Python(3.6.1)	Python Software Foundation	N/A

* Ryan, T. M. & Walker, A. Trabecular bone structure in the humeral and femo
293 (4), 719-729, doi:10.1002/ar.21139, (2010).

** MeshLab: an Open-Source Mesh Processing Tool. Sixth Eurographics Italian
P. Cignoni, M. Callieri, M. Corsini, M. Dellepiane, F. Ganovelli, G. Ranzuglia
*** https://vtk.org

Comments/Description

4DCT scan, Static 3DCT scan

Image processing software.

Surface reconstruction from CT DICOM data and point cloud data.

Surface trimming and landmark picking

Iterative Closest Points algorithm. Used in python language programming.

DICOM file processing to extract the point cloud from the bone cortex('dicom.py' module).

Calculation of the rotation matrices.(Numpy module)

Sequential image regestration using ICP algorithm

ral heads of anthropoid primates. Anat Rec (Hoboken).

Chapter Conference, page 129-136, 2008

We really appreciate reviewer's comments. Our article has become sophisticated by various insightful comments from the 5 reviewers. We revised the documents to integrate all comments and avoid contradiction.

Reviewers' comments:

Reviewer #4:

Review of "Four-dimensional CT analysis using iterative 3D-3D registration", by Satoshi Oki et al.

The authors have done a good job in improving the manuscript. For example, the steps in the registration procedure are better describe. I understand the general concept of the overall, but keep having problems in understanding and appreciating the details.

Major concerns

The authors describe the registration procedure that provides the 4x4 matrix (M) to In the description of the protocol, the authors refer to functions in a software package (e.g. Edit new label field/Generate surface->Apply/etc). However, there is no reference to that software package, nor is it clear what these functions exactly do.

This software has been used in many previous studies. We added the articles which used the same software for the similar application in the Material Excel document.

*Ryan, T. M. & Walker, A. Trabecular bone structure in the humeral and femoral heads of anthropoid primates. *Anat Rec (Hoboken)*. **293** (4), 719-729, doi:10.1002/ar.21139, (2010).

transform an object (out of CT) the a target frame in 4DCT (clearly described by Fig. 6). However, they also define a coordinate system (CS) for every surface mesh that was segmented out of the 3DCT and each frame of the 4DCT. This is confusing for two reasons:

1) From their explanation I understand that this is required to quantify rotations from one frame to another.

That is correct.

However, by defining a local CS in the CT world of reference, and by visualizing the "relative" motion (moving object with respect to fixed object), in that frame of reference, every frame-to-frame motion (translations and rotations) can be visualized and quantified with respect to the single local CS in the CT frame of reference.

As the reviewer pointed, it is possible to describe **change** of rotation angles and translation from the one certain frame (typically the starting frame) using coordinate system of the reference bone. However, starting position differs among subjects. To describe absolute values(rotation and position) of the starting position, we should define the coordinate system of the moving bone also. For example, in our representative result, Figure demonstrated the femur and tibia in their own local coordinate systems which represent 0° of external rotation, flexion and valus. However, in the first frame of 4DCT analysis, the knee extended from 50 of flexion. This starting angle can be calculated from two local coordinate systems. To avoid confusion, we added the reference of ISB recommendation.

Line 196-197

Define the coordinate systems of the fixed and moving bones when the rotation parameters are measured (e.g., flexion angle or rotation angle calculated by Euler/Cardan angle)[12-14].

12 Wu, G. et al. ISB recommendation on definitions of joint coordinate system of various joints for the reporting of human joint motion--part I: ankle, hip, and spine. International Society of Biomechanics. Journal of Biomechanics. 35 (4), 543-548, doi:10.1016/s0021-9290(01)00222-6, (2002).
13 Wu, G. et al. ISB recommendation on definitions of joint coordinate systems of various joints for the reporting of human joint motion--Part II: shoulder, elbow, wrist and hand. Journal of Biomechanics. 38 (5), 981-992, doi:10.1016/j.jbiomech.2004.05.042, (2005).

2) The matrix M can be obtained from the acquired "local coordinate systems" of a bone, in CT and frames in 4DCT, as well (without registration). M is basically

equivalent to a CS-to-CS transformation. It is therefore unclear to me why both methods (registration and all CSs) are required.

As the equation represents, calculation of the moving bone with respect to the reference bone require 4 matrices because the rotation angles in the local coordinate system are different from those in the global coordinate system. Two matrices are coordinate systems of the moving and reference bones and two are the results of surface registration of these segments.

- *Winter, D. A. Biomechanics and Motor Control of Human Movement. 4th edn, 176-200 (2009)*
- *Wu, G. et al. ISB recommendation on definitions of joint coordinate system of various joints for the reporting of human joint motion--part I: ankle, hip, and spine. International Society of Biomechanics. Journal of Biomechanics.* ***35*** *(4), 543-548, doi:10.1016/s0021-9290(01)00222-6, (2002).*
- *Wu, G. et al. ISB recommendation on definitions of joint coordinate systems of various joints for the reporting of human joint motion--Part II: shoulder, elbow, wrist and hand. Journal of Biomechanics.* ***38*** *(5), 981-992, doi:10.1016/j.jbiomech.2004.05.042, (2005)*

It is not explained how relevant motion parameters are obtained given the local coordinate systems.

Rotation angles are obtained from Euler/Cardan angles which was calculated from the 4×4 matrices. We added the reference.

> Line 196-197
>
> Define the coordinate systems of the fixed and moving bones when the rotation parameters are measured (e.g., flexion angle or rotation angle calculated by Euler/Cardan angle)[12-14].
>
> *14 Crawford, N. R., Yamaguchi, G. T. & Dickman, C. A. A new technique for determining 3-D joint angles: the tilt/twist method. Clinical Biomechanics (Bristol, Avon).* ***14*** *(3), 153-165 (1999).*

Although I find the method cumbersome, confusing and complex it is not necessarily unsound. I leave further judgement to the editor.

The authors added text explaining a method to evaluate the accuracy of registration. Details about the method are missing. For example, it is unclear to me what the gold standard position was in their experiments. The reported error values (especially for the rotation error) seem extremely small to me.

Based on the purpose of this journal, we added the detailed error analysis only in the comment to the Reviewers. In the first revision and second revision, we described the method of validation of the registration accuracy. In these validation procedures, we performed the validation about each bone segment. Thus the position does not matter with the accuracy. The ICP is very accurate method to match two surfaces of the same morphology. Unless local minimum error does not happen, the accuracy is very high because the segment is the same surface in static 3DCT and 4DCT.

-First Revision-

line 173 - have you verified that the registration solution is unique? I.e. as ICP is an optimization technique, it is sensitive to local minima. Perturbation analysis should be performed to assess whether the registration solution is unique. This is particularly necessary to mitigate any propagation of error from the serial way the registrations were determined/combined (i.e. frame 2 depends on registration of frame 1). Additionally, parameters for the ICP algorithm (rms convergence criterion) should be specified.

As reviewer pointed, local minimum convergence is a concern for ICP algorithm. We performed perturbation analysis with 10% length of the femur, 10% length of the tibia and whole surface of the patella. The surface data of these segment moved by 4×4 matrices which translates (every 1mm from 0 to 100 mm) or rotate the surface data (every 1° from -90° to +90°). Then the moved surface data were matched with the original data by ICP algorithm. Rotation angles and translation magnitude were calculated from the results of the ICP process.

The femur and the tibia showed high accuracy of registration under 60 degrees of rotation and 100mm of translation in any direction. Generally all perturbation showed error of 0.02mm in lateral translation. However, as we pointed in

DISCUSSION, patellar showed 2 degrees of error for rotation around X-axis because its shape is point symmetry around its anterior/posterior axis.

Minor concerns

Line 45: fixed bones. It seems to me that only one bone is the fixed bone.

The expression was corrected.

Line 45-46 moving bone with respect to fixed bone

Line 53: this method depends on CT quality -> of motion artifacts.

The expression was corrected

Line 53-54 However, fast or large-scale motions cannot be traced because of motion artifacts

Line 67: …simulate dynamic motion. Please add a reference.

We added a reference.

Line 67-68

although multiple actuators have been used to apply external forces to tendons in an attempt to simulate dynamic motion[7].

7. Omid, R. et al. Biomechanical analysis of latissimus dorsi tendon transfer with and without superior capsule reconstruction using dermal allograft. Journal of Shoulder and Elbow Surgery. ***28*** *(8), 1523-1530, doi:10.1016/j.jse.2019.01.016, (2019).*

Line 77: no bones are fixed. This is confusing because you chose one bone as the fixed bone.

We changed the expression.

Line 78-79

because all bones are moving and no landmarks can be traced during the active motions in vivo.

Line 99: If the rotation time is 0.275s and half a rotation is needed for a reconstruction, shouldn't the temperal resolution be 0.1375s?

We had similar comment from Reviewer #1 in the previous revision. We attach the discussion.

- Reviwer 1

Address why the reconstruction interval requires nearly an entire gantry rotation? There should be sufficient information in 180 degrees plus fan angle, meaning the temporal resolution can be improved beyond what is stated. Existing literature in wrist suggests temporal resolution of 75ms in a dual source system, so 150-170 is likely achievable.

Thank you for your insightful comments. We have added the following sentence regarding temporal resolution.

Line 100-101

…all images are reconstructed using half reconstruction, so that the temporal resolution is approximately 0.16 s. In addition, the reconstruction interval can be set as 0.1s. However, if we reduce the reconstruction interval from 0.2 s to 0.1s, the volume of data would double and the positions of segments would not change during the short interval. We therefore set the reconstruction interval as 0.2 s.

Line 14: entire moving and fixed. These words are confusing because the bones are not moving in the CT. I suggeset removing these words.

Thank you for your comment. We removed these words.

Line 115

Perform static 3DCT of the entire target bones and store the data in DICOM format also.

Line 139. This cannot be automatic software since 2.2.1 explains that a thresholds may need to be adjusted. Also, how does the software otherwise differentiate in the tibia and femur? Please change to semi-automatic or explain the procedure in more detail.

Segmentation process from 3DCT is semi-automatic. However, segmentation of the surface data from all frames in 4DCT is performed by automatically process (batch process) because it takes too much time to segment target bones in all frames. So, this is an 'automatic' process.

3.1 describes the Initial surface registration, but actually describes landmark-based registration (this seems initial registration to me), AND ICP registration. The latters seems like the final registration to me. Is the title therefore correct?

Thank you for your comment. “Initial” means surface registration to the first frame. This word would be confusion. We removed it.

Line 161

3.1. Surface registration from static 3DCT to first frame of the 4DCT

3.1.3

Line 150: Translate positional data that have already been obtained in the previous step. Please explain what positional data you refer to (in which step?).

Positional data of CT attenuation values above the threshold. We corrected the sentences.

Line 148-150

Positional data that have already been obtained in the previous step are translated into a format that can be interpreted by the image processing software.

Line 168: the CT gantry -> in each volume image

We corrected the sentence.

Line 165-166

…the surface data from 4DCT are only partial segments which are included in each volume image**.**

3.1.2/3.1.3. It is unclear to me why landmark-based (3.1.2) and moving bones roughly (3.1.3) are both necessary in an initialization step. Please clarify.

3.1.2 just explains picking landmarks and 3.1.3 explains rough match by these landmarks. We added the explanation

Line 173-174

Match the partial fixed and moving bones roughly on the first frame of the 4DCT surface data according to the picked landmarks in 3.1.2

Lines 186-7: N should be i.

We corrected the sentence.

Line 185-186

Next, match the partial surfaces of the ith frame onto the (i+1)th frame of 4DCT sequentially.

Line 190: Provide details about the open-source software.

The software name cannot be written in the manuscript. The material file includes the open source software VTK.

Instruction for Authors

Avoid the use of commercial language, including ™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the Table of Materials instead.

			Iterative Closest Points algorithm.
VTK(6.3.0)	Kitware (New York, USA)	N/A	Used in python language programming.

Line 200: See major concern above.

Line 221/2: I suggest removing Partial segments…in the second frame.

It is true that content is included in the next sentence. We removed the first sentence.

Lines 225-230: It is unclear in this section where (3DCT/4DCT) the global coordinate system is defined. Please explain.

The global coordinate system is the coordinate system of the original CT data. The positional data in global axis means the coordinates value in the CT DICOM data. We added the explanation.

Line 221-222

the global coordinate system in the original CT DICOM data

Line 229: The xyz axes seem inappropriate to me. Normally the z-axis of the CT is toward the center of the gantry. With this definition, internal rotation should be about the z-axis, etc.

It is true that Z-axis pointing the center of the gantry along with the CT bed, but in the field of biomechanics, it is common to define x-axis as antero-posterior axis, y-axis as the long axis of the segment and z-axis as the medial-lateral axis.

- *Baker, R. ISB recommendation on definition of joint coordinate systems for the reporting of human joint motion-part I: ankle, hip and spine. Journal of Biomechanics.* ***36*** *(2), 300-302; author reply 303-304 (2003).*
- *Wu, G. et al. ISB recommendation on definitions of joint coordinate system of various joints for the reporting of human joint motion--part I: ankle, hip, and spine. International Society of Biomechanics. Journal of Biomechanics.* ***35*** *(4), 543-548, doi:10.1016/s0021-9290(01)00222-6, (2002).*

Line 248-256. I recommend adding these results right after line 237 (the method).

Thank you for your insightful comment. We moved this paragraph right after the method section.

Line 274 fixed surface -> femur

We corrected the phrase.

Line 275: in addition to the moving surface -> with respect to the tibia

We corrected the sentence.

> Line 266-267 The femur also moves (green arrow) with respect to the tibia (blue arrow).

Line 278: As commented above, It is unclear to me why landmark-based registration (b), rough segmentation (c) are both required as an initial registration step. Please explain.

Figure 3B shows that the landmarks are picked in the software. Figure 3C shows rough match based on these three marks.

Line 283: Rotation matrix -> Transformation matrix

We corrected the word.

> **Line 278**
>
> **Transformation matrix is calculated from surface registration.**

Line 289: decides ->defines.

We changed the expression.

> Line 284-285

3 x 3 matrix that defines rotation and **d** is a 1 x 3 matrix that defines translation

Line 299: subject ->fixed

We corrected the word.

Line 294-295

Rotation angles are calculated using the defined coordinate systems of the fixed and moving bones.

Line 300 Please explain L, which is not defined, in LMrefs.

L measn local coordinate system. So $^{L}\mathbf{M\_s}$ means the transformation matrices which aligns the bone in the static 3DCT to its local coordinate system.

Line 296-297

Rotation matrices from static 3DCT to the local coordinate system of the fixed bone ($^{L}\mathbf{Mref}_{S}$) are calculated.

Reviewer #5:

Four-dimensional CT analysis using sequential 3D-3D registration for Journal of Visualized Experiments.

It is an important and topical area to quantify joint kinematics using four-dimensional computed tomography (4D CT). This paper addresses a much needed and important aspect of medical imaging. It appears that the technique authors describe can be extrapolated into many areas of research and clinical application. I wish to congratulate the authors on their effort on quantification of joint motion using 4D CT.

However, this paper does need revisions and there are few concerns.

In summary, the strength of the paper is that the technique authors use to quantify joint motion, in principle, is technically correct. However, the validity of the system in a scientific context can be questionable as the authors have performed this on only one case of knee motion and the method of validation is not clearly described.

Also, there are areas where more details can be added to increase clarity of the communication. (This is highlighted under relevant sections)

While this appears to be an interesting and useful technique, clarity of presentation, scientific accuracy, and compliance with research standards, and technical quality can further be improved.

We are doing research by comparing the healthy subjects and affected subject (e.g. patellar dislocation or anterior cruciate ligament rupture). However, the scope of this journal to present the methodology, and we presented just one case with instructing the registration technique.

Abstract

Quantitative analysis of dynamic computed tomography, if done accurately, will answer many questions on joint kinematics. While the aim of the study is clear in the abstract, more details on methodology can improve clarity of communication.

In the abstract, it is not very clear how this technique quantifies motion of bones. It would have been more informative to mention whether this is done using as existing software, custom made one or an inbuilt application that comes with the scanner.

Thank you for your insightful comment. We analyzed the 4DCT DICOM data using existing surface rendering software. We added the explanation.

> Line 44-46
>
> We aimed to analyze joint kinematics using a sequential 3D-3D registration technique to provide the kinematics of the moving bone with respect to fixed bone semi-automatically using 4DCT DICOM data and existing software.

How and whether the aim is achieved is not clearly mentioned. Precise details on what bones/ joints have been assessed and comments on the results, whether they have been acceptable, would improve the quality of this communication.

This method can be applied to any joint which can be moved inside the CT gantry. The analysis of the knee joint is just a representative result.

Details about whether this technique has been validated will also be of interest. More information on why and how it is mentioned that this is not suitable for large and rapid motion would enhance the clarity of the paper.

As we answered to the Reviewer #1 in the previous revision, CT artifact is caused by multiple factor. In 4DCT, motion speed, bone quality and position of the bone segment with respect to CT beam possibly influence the CT artifact. We have added the comment in the discussion.

Reviewer 1

Discussion: Refers to limitations when joint motion is 'too fast'. How fast is 'too fast'? Was any attempt made to ascertain the angular velocity of the joint at which the method is insufficient?

This depends on the morphological characteristic of the segment. A segment with larger size, less symmetricity and more landmarks such as bone spurs will demonstrate a higher success rate of tracing. Although 30°/s seems too fast for knee, wrist and finger motion analysis, we have not performed detailed analyses of velocity limitations. We have added mention of this problem as a future task in the Discussion.

Line 397-399

Tolerable velocity depends of the target joint, because the joint morphology affects the success rate of surface registration. Studies of velocity tolerance for each joint will be needed in the future.

Line 43- Suggest rewording as "numerical analysis of joint kinematics using 4D CT data". This is different to numerical analysis of soft tissue motion using the same imaging modality. The problems encountered and success rates are different. Hence, I would recommend that the authors should be specific on their aims.

Thank you for your comment. We reworded the sentence.

Line 43-44

However, numerical analysis of 4DCT data remains difficult because segmentation in all volumetric frame is time consuming.

Line 43- "However, numerical analysis of 4DCT data remains difficult because of the massive volume of data involved."-Accuracy of this statement is questionable. The numerical analysis of joint kinematics using 4 dimensional CT is difficult not because of the large volume of data involved. This is because of technical difficulty in developing universally acceptable algorithm to register images containing bones. Segmentation is time consuming, automatic segmentation needs a specialised software program. The volume of data is a concern, but this has been largely overcome in the last 10 years by good computer hardware/ workstations and introduction of segmentation and surface based modelling.

As the reviewer pointed, data volume is not a great concern. We paraphrase and specify the cause of difficulty.

Line 43-44

However, numerical analysis of 4DCT data remains difficult because segmentation in all volumetric frame is time consuming.

Line 46- Why would you need to 'trim' surface data (I think this should be 'trimmed 3D surface data to match the length of bones on 4D volumes' as 4D CT can only use the gantry width but 3 D CT can image the whole length of the bone). It would be good to explain this.

Thank you for your comment. Trimming step is required for ICP because ICP requires that one surface is included in another surface. The word number in abstract is limited, so we added the comment in the METHOD section.

Line 163-164

Trim the bones in a static 3DCT into partial segment data that are included in all frames of 4DCT for ICP

Line 50- "source bones"- suggest Rewording. Are they the bones segmented from 3 D CT?

These are the target bones which the researcher want to trace during 4DCT. As the reviewer pointed, these target bones should be reconstructed from 3DCT.

We reworded the word.

Line 50-52

Once the coordinate systems of the <u>target bones</u> are decided, translation and rotation angles between any two bones can be calculated.

Line 50-suggest Rewording "positional data such as translation and rotation" to "translation and rotation" because in a rigid body in motion, the only positional data you would calculate is translation and rotation. (These two words summarises all the 6 degrees of freedom)

Line 51/52- suggest rewording

That is correct. We reworded the sentence.

Line 50-52

Once the coordinate systems of the target bones are decided, translation and rotation angles between any two bones can be calculated.

Line 53- Can you quantify that and give an indication as to how large and how fast

As we answered to the Reviewer #1 in the previous revision, CT artifact is caused by multiple factor. In 4DCT, motion speed, bone quality and position of the bone segment with respect to CT beam possibly influence the CT artifact. We have added the comment in the discussion.

Reviewer 1

Discussion: Refers to limitations when joint motion is 'too fast'. How fast is 'too fast'? Was any attempt made to ascertain the angular velocity of the joint at which the method is insufficient?

This depends on the morphological characteristic of the segment. A segment with larger size, less symmetricity and more landmarks such as bone spurs will demonstrate a higher success rate of tracing. Although 30°/s seems too fast for knee, wrist and finger motion analysis, we have not performed detailed analyses of velocity limitations. We have added mention of this problem as a future task in the Discussion.

Line 397-399

Tolerable velocity depends of the target joint, because the joint morphology affects the success rate of surface registration. Studies of velocity tolerance for each joint will be needed in the future.

Introduction

Clearly identifies and explains the aims and the utility.

Line 64- suggest rewording "tiny"

We reworded the word.

Line 65

This approach can measure small motions in the small joints

Line 65- suggest rewording "of course"

We changed the expression.

Line 66-68

Cadaveric models can mainly evaluate passive motions, although multiple actuators have been used to apply external forces to tendons in an attempt to simulate dynamic motion.

Protocol

It would be informative to mention the software that has been used to do this. The steps may vary according to the software.

As the reviewer pointed, detailed steps may vary in other software. Information about the software is written in Material excel file according to the “Instruction to Authors”.

Avoid the use of commercial language, including ™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the Table of Materials instead.

Line 109- How would you ascertain that total motion take place during 10.25 s and why would you do that.

Total motion should be finished ‘in’ 10.25 s. So the subject rehearsal the motion beforehand with the count call of 10 seconds.

Line 115- suggest delete "also"

We removed the word.

Line 115

Perform static 3DCT of the entire target bones and store the data in DICOM format.

Line 147- Do you have a reference for 'to set the threshold for cortical bone lower in osteoporosis"? Can you make a numerical recommendation on this? Have you noted this in your sample- there is only one case described here, hence I would think this recommendation is not based on this study.

It depends on the bone density and CT data quality. Unfortunately, manual segmentation with referring to the static 3DCT is the best way to determine the threshold, although new technology such as artifitial intelligence or machine learning will be able to segment the bone cortex in future. As the reviewer pointed, it is confusing that we described this sentence in the 4DCT section. We moved this paragraph in the 3DCT segmentation section.

Line 150- The step is not clear. Please rephrase.

This process is done by Python module ‘dicom.py’. We wrote the usage of this module in material excel because we cannot write the name of the package in the manuscript.

DICOM file processing to extract the point cloud from the bone cortex('dicom.py' module).

Avoid the use of commercial language, including ™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the Table of Materials instead.

Line 151- It is good to mention the image processing software.

Thank you for your comment. We used a software named “AVIZO” as in the material excel file. We cannot describe detail because of Instruction for Authors.

AVIZO(9.3.0)	Thermo Fisher Scientific (OR, USA)	N/A	Image processing software. Surface reconstruction from CT DICOM data and point cloud data.

Line 161- How would you make sure that the fixed bone remains fixed throughout the arc of motion and avoid errors due to undesirable movement of the fixed bone.

As the reviewer pointed, this sentence was confusing. Actually, the bone itself is not fixed. After 4DCT, we reconstruct the image data as the motion of the moving bone with respect to the fixed bone using image registration described in **Section 3**. To avoid confusion, we paraphrase the sentence.

Line 158-159

In this step, reconstruct the motions of the moving bone with respect to the fixed bone from the raw 4DCT DICOM data.

Line 167- Not clear. Please reword. Why would be partial segments in 4D? I think you trim the 3D CT segment to match the length of the bone covered in 4DCT.

As the reviewer pointed, surface registration requires that the one surface is included in another surface. We added the explanation.

Line 166-167

because surface registration requires that one surface data is included in another surface.

Line 170-How would you manually clearly identify the landmarks mentioned. When the mark is identified it refers to one single point in the surface model. But the landmarks you have chosen involves relatively a wide area, for instance lateral epicondyle of the femur. How would you manually make sure that you pick exactly the same spot on the lateral femoral condyle in both images

This is just a rough registration of two surfaces. It is not necessary that these landmarks exactly coincide. After rough registration by manually picked landmarks, ICP does the fine registration. Rough registration is required to avoid local minimum problem in the latter ICP process.

Line 174- what is meant by 'partial fixed bones' and 'roughly'

"partial fixed and moving bones" are the trimmed bone in step 3.1.1. Roughly means that these surface are matched only according to the picked landmarks. As the reviewer pointed, these landmarks do not coincide exactly, this process is just a 'rough match'.

Line 176- What is the open source software, do you have a reference for that.

We used VTK as in the material excel file. As the "Instruction for Authors" says, we wrote the name of the software in the material list.

VTK(6.3.0)	Kitware (New York, USA)	N/A	Iterative Closest Points algorithm. Used in python language programming.

Line 185- Is this automatic

Once the surface registration to the first frame is done, sequential registration process until the last frame will be done automatically.

Line 200- How would you define the coordinate system

There are a number of coordinate systems(e.g. definition by International Society of Biomechanics(ISB)) to describes for each joint (e.g. for knee joint in the representative results). The examiners can apply any coordinate systems which well describe what they want to calculate in their 4DCT analyses.

- Baker, R. ISB recommendation on definition of joint coordinate systems for the reporting of human joint motion-part I: ankle, hip and spine. *Journal of Biomechanics.* **36** (2), 300-302; author reply 303-304 (2003).
- Wu, G. *et al.* ISB recommendation on definitions of joint coordinate system of various joints for the reporting of human joint motion--part I: ankle, hip, and spine. International Society of Biomechanics. *Journal of Biomechanics.* **35** (4), 543-548, doi:10.1016/s0021-9290(01)00222-6, (2002).

Representative results

One limitation of this paper is the fact that you have studied only one knee joint. I would expect it will be difficult to generalise the methodology and findings to more knees, and other joints. There will be questions on how to compare joint motion in two or multiple patients, what would be the reference etc.

As the reviewer pointed, it would be more reliable if we show more sample data. Actually, we are doing analysis by comparing the healthy volunteers and the patients with patellar dislocation or ligament injuries, and we apply this method to forearms or finger joints. However, it does not sound the scope of this article as in the instruction, we just show a single data.

- *Oki, S. et al. The relationship between the morphological axis and the kinematic axis of the proximal radius. Surgical and Radiologic Anatomy.* ***41*** *(4), 423-429, doi:10.1007/s00276-018-2131-0, (2019).*

Also, it will be interesting to know how you validated your results.
With segmentation, it will be important to state the accuracy of segmentation.
(Jacquard index, dice coefficient etc.) Have they been in acceptable?

Line 212- Section about trimming is not clear

We visually checked the included surface and trim the whole 3DCT.

We added the explanation.

> Line 208-209
>
> by visually checking 4DCT movie data which is created in the preset 4DCT software

Line 213-can you please specify the landmarks, and how you could pick the exact point on epicondyle, intercondylar notch etc.?

As we answered, it is not necessary that the examiner pick up the exactly same anatomical landmark, because this process is just a rough match.

Line 229- Can you please elaborate this.

We added the reference how to calculate the Euler/Cardan angles.

> Line 223-225
>
> We calculated the motion of the tibia from Euler/Cardan angles in ‘zxy’ order, meaning flexion, varus, and internal rotation, in that order[14]
>
> 14: Crawford, N. R., Yamaguchi, G. T. & Dickman, C. A. A new echnique for determining 3-D joint angles: the tilt/twist method. *Clinical Biomechanics (Bristol, Avon).* **14** (3), 153-165 (1999).

Line 234- This is not clear; How do you validate this? What is the absolute variable that you have compared your data with, or have you measured repeatability or reproducibility?

I do not think you can validate this on the same sample, done once.

Compared with the data which were calculated using the whole length of surface data. Reproducibility is not needed because the algorithm is a mathematical and automatic. As the reviewer pointed that the repeatability is a concern because landmarks would be different among examiners. However, perturbation analysis in the previous revise-comment proves differences in landmark pickup do not affect the result of ICP.

Line 236- Suggest elaborate, how you do this when 16 cm length is available for 4D CT

This length is enough to analyze the knee joint kinematics. However, as the reviewer pointed, the examiner should be careful to keep the knee joint in the CT gantry. That is the reason why we used triangle pillow and keep the femur in the same position during knee extension.

Line 248 to 256- With what have you made the comparison

As in the previous explanation. We compared these results with those using whole length of the femur, tibia and patella.

Also, with 0.5 mm axial slices, can you have an accuracy of 0.11 mm in translation. I would think it is limited by the slice thickness of CT.

As the reviewer pointed, Slice thickness of CT exceeds the accuracy of surface registration. We added the explanation.

> Line 244-246
>
> These translation errors are thought to be negligible because CT slice thickness is 0.5mm and exceeds these errors.

Figure and Table legends

Line 270-271-"whole bone of the whole femur"- suggest rewording as 'whole femur'

We corrected the sentence.

Line 277-278- Its not clear what is meant by "Surface data of the fixed and moving bones are trimmed into partial segments that are included in all 4DCT frames.

We added the explanation.

> Line 270-272
>
> Surface data of the fixed and moving bones from 3DCT are trimmed into partial segments that are included in all 4DCT frames because the surface data from 4DCT are only partial segments which are included in CT gantry.

Line 314-318- It is not clear how you would validate the method by reducing the length of bones registered. While it may give an idea whether trimmed surface models are good for this, compared to full length, I do not think it adds validity to the technique you describe.

Combined with the perturbation analysis, it proves the repeatability and tolerability for limited view of interest. However, as the reviewer pointed, it contains several limitation such as motion speeds and quality of the bone. These problems for each joint and each condition of CT condition should be evaluated in the future.

We added this limitation in DISCUSSION.

> Line 396-399
>
> Frame-to-frame surface registration then fails because the two surfaces are too distant. Tolerable velocity depends of the target joint, because the joint morphology affects the success rate of surface registration. Studies of velocity tolerance for each joint will be needed in the future.

Discussion

Line 330- 337- Repeats what is said in the introduction. Suggest inclusion in either section.

We removed the sentences.

Line 339-340- "The surface data from 3DCT should be created precisely because this quality affects the accuracy of the initial surface registration to the first frame of 4DCT". It would be good to elaborate measures that you have taken to ensure that, this criteria is met. Factors that reflect the quality of segmentation can be included.

As the reviewer pointed, we included the factors which include the quality of segmentation. We did the error analysis using surface data from 3DCT. To create rough surface data for surface registration error analysis is too subjective, so we did not perform.

> Line 388-391
>
> In addition, ICP also depends on the quality of the surface data. In case of osteoporotic bones, surface reconstruction largely depends on the manual segmentation. That may lead to interobserver errors.

Line 345- It would be useful to indicate how accurate is the selection of land marks manually, and what is the inter and intra observer variability when asked to mark "lateral femoral epicondyle" etc.

As we explained, it is not necessary to pick up landmarks so exactly.

Line 348-349- "Accuracy of 4 D CT" - Do you have a reference for this please.

Line 351-352- "This technique thus contains human error far, exceeding the accuracy CT itself." Do you have a reference for this?

Because CT DICOM data include absolute coordinate values. These data are literally "absolute". Measurement error is caused by data analysis process. We reworded the sentences.

Line 345-350

Four-dimensional CT provides sequential volume data with accuracy almost as high as static 3DCT because CT DICOM data contain absolute coordinate values of all tissues in the CT gantry. Several studies have used 4DCT for investigations of joint kinematics [20,21]. However, in most, the observers picked landmarks from several frames and calculated the parameters (e.g., angles, translation). These data analysis processes contain human error which lead to measurement error.

Line 354-355- "Theoretically, manual surface segmentation for all 4D CT frames provides the most accurate data" Do you have a reference for this please.

As the reviewer concerns, recently, computer segmentation such as convolutional neural network or deep learning achieve similar accuracy as manual segmentation by orthopedic surgeons. However, manual segmentation is still thought as gold standard to demarcate specific tissues in the CT slices. We added this comment in DISCUSSION and the references.

Line 391-393

Recently, computer oriented tissue segmentation on the CT slices have developed, however, human oriented manual segmentation is still thought as gold standard to identify specific tissues[28,29].

Line 363- It will be good to explain the registration parameters used.

We added the registration parameter in REPRESENTATIVE RESULT.

Line 218-219

In ICP algorithm, the convergence criteria for mean distance between iterations was set as 0.01 mm.

Line 370-372- In the abstract it is mentioned that this system is not suitable for rapid and large motions due to CT quality. But here, the idea is that it is due to the ICP algorithm not being able to register 2 models far apart from each other. This is contradictory.

As the reviewer pointed, these two sentences are contradictory. Both of frame-to-frame distance and CT quality will affect the traceability in 4DCT analysis. We added the phrase which consistent with the DISCUSSION.

> Line 53-54
>
> However, fast or large-scale motions cannot be traced because of motion artifacts and limited frame rates.

Line 383-384- Do you have any reference for this. With your study in one case of large bones can you come to that conclusion. Semiautomated segmentation of small bones which are close to each other, with overlap throughout, poses a different set of problems. It is not convincing that we can extrapolate this directly into small bones.

We published the research of forearm motion.

- *Oki, S. et al. The relationship between the morphological axis and the kinematic axis of the proximal radius. Surgical and Radiologic Anatomy.* ***41*** *(4), 423-429, doi:10.1007/s00276-018-2131-0, (2019).*

And there are some other studies which also showed its advantage to measure carpal bones.

- *Troupis, J. M. & Amis, B. Four-dimensional computed tomography and trigger lunate syndrome. Journal of Computer Assisted Tomography.* ***37*** *(4), 639-643, doi:10.1097/RCT.0b013e31828b68ec, (2013).*
- *Kakar, S. et al. The Role of Dynamic (4D) CT in the Detection of Scapholunate Ligament Injury. J Wrist Surg.* ***5*** *(4), 306-310, doi:10.1055/s-0035-1570463, (2016).*

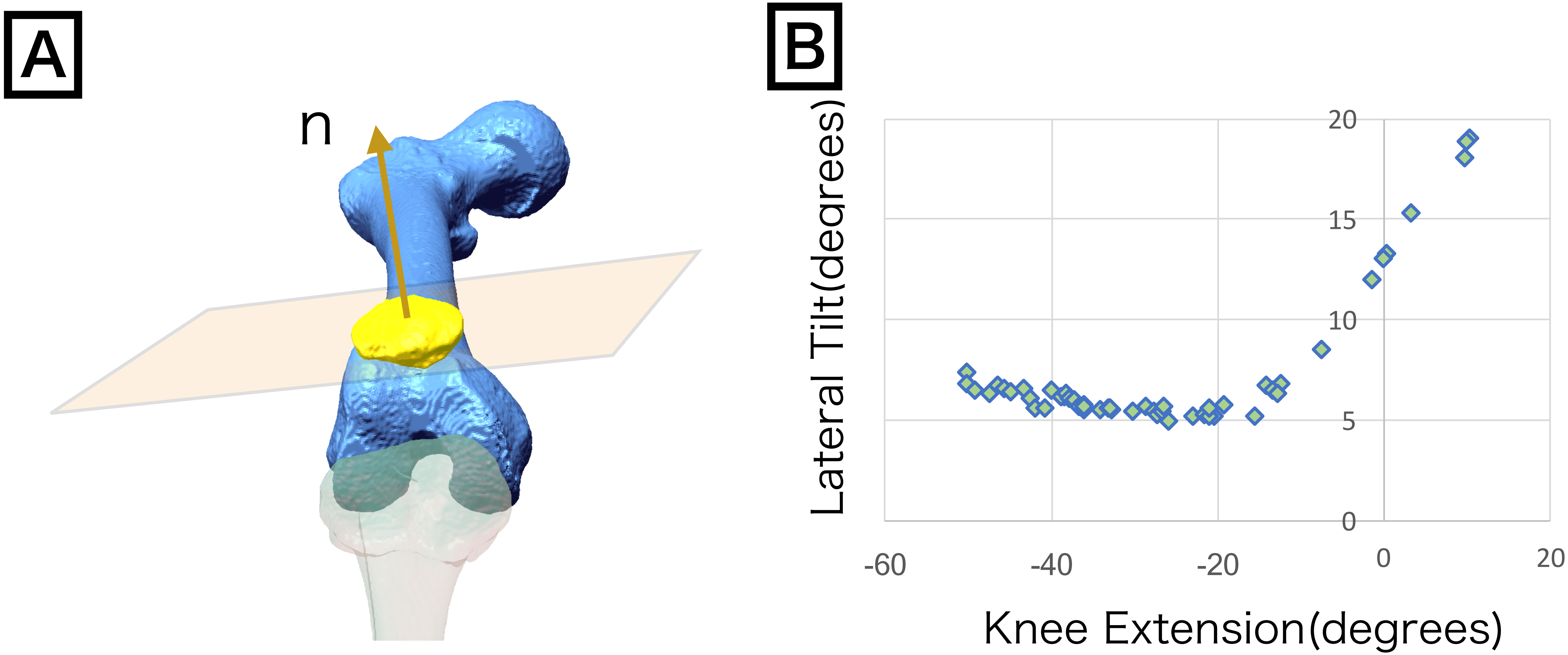
These are in the REFERENCES.

Line 394-please see comment for line 370-372.

References

None.

supplement 1

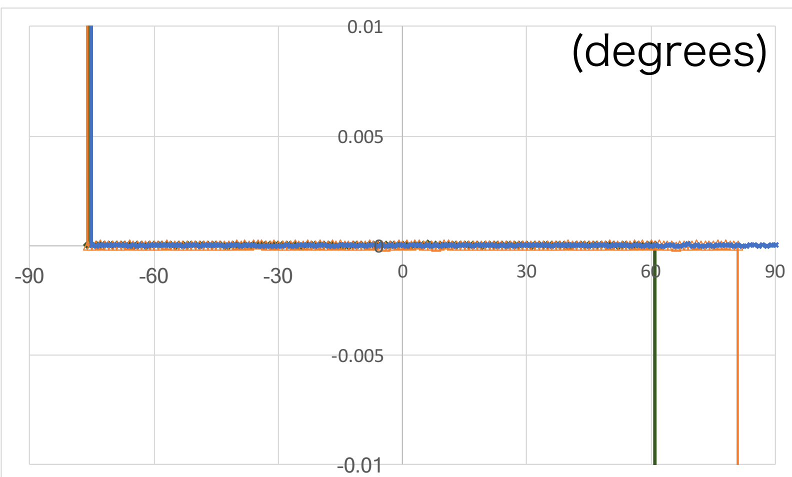


supplement2

Perturbation Analysis

Femur

Rotation



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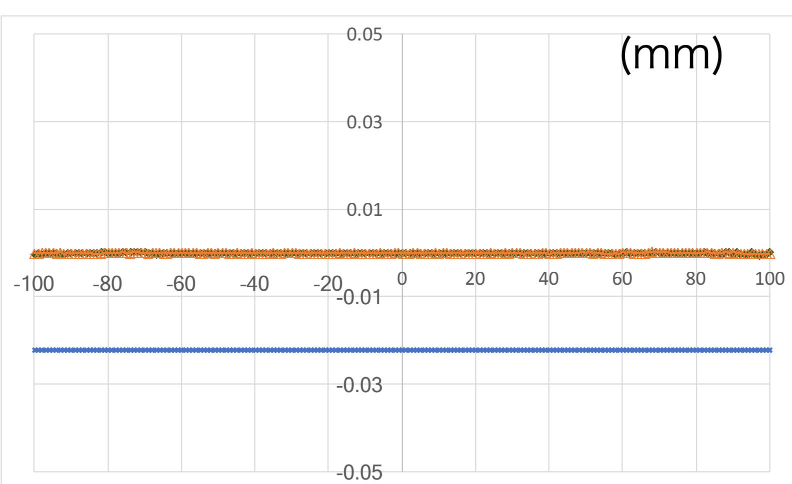
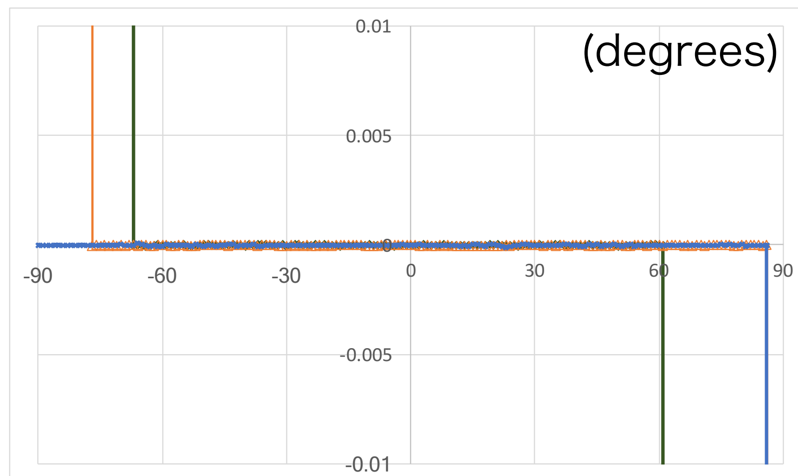


Tibia

Rotation



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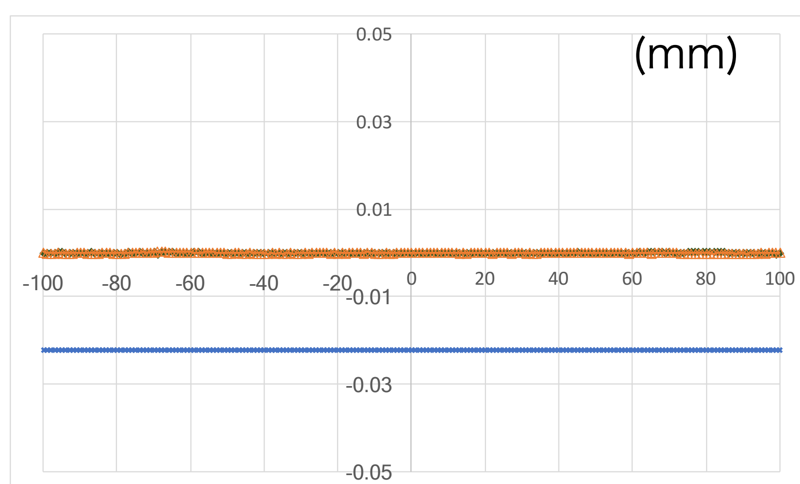
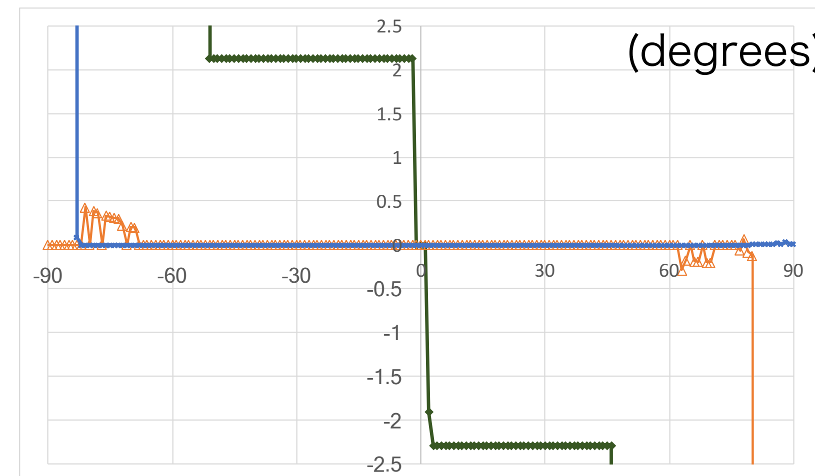
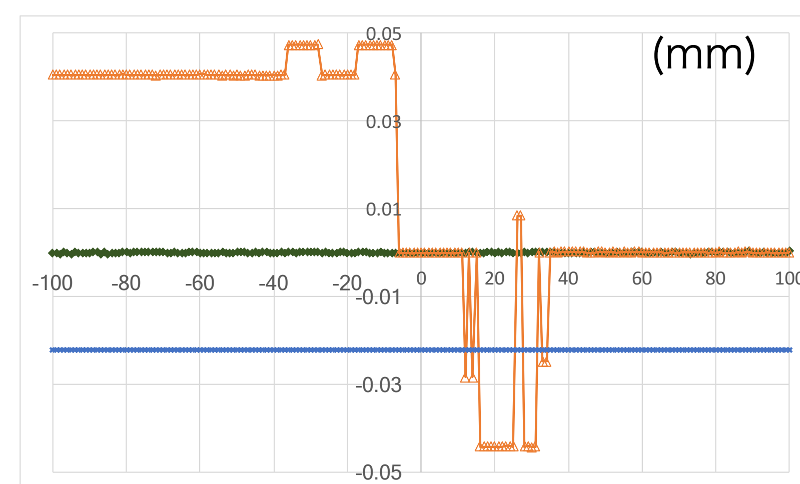


Patella

Rotation



Translation



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Author(s): **Satoshi Oki, Kazuya Kaneda, Yoshitake Yamada, Minoru Yamada, Yutaro Morishige, Kengo Harato, Noboru Matsumura, Takeo Nagura, Masahiro Jinzaki**

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Name:	Satoshi Oki
Department:	Department of Orthopedics
Institution:	Keio University School of Medicine
Title:	M.D., Ph.D.

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