Supplementary Information 1: Construction of a Lego Calibration Object

Supplement to: B.J. Knörlein, D.B. Baier, S.M. Gatesy, J.D. Laurence-Chasen and E.L. Brainerd. Validation of XMALab Software for Marker-based XROMM.

In this document we describe and provide assembly instructions for a 64-point X-ray calibration object made from Lego bricks with 5.0 mm steel spheres press-fit into select bricks as radio-opaque marker points (Figs. S1-S3). Lego bricks have been validated in the past for calibration of external view cameras (Baronti et al., 2010), and here we extend this use to X-ray cameras.

In prior XROMM work we used a custom-machined calibration object made from acrylic sheets with machined spacers and holes drilled for the steel spheres (Brainerd et al., 2010). Building an acrylic object requires access to high-precision machine tools, and the assembled object can warp and marker beads can move over time. As an alternative, to maximize precision and simplify construction, we developed an X-ray calibration object made out of Lego bricks.

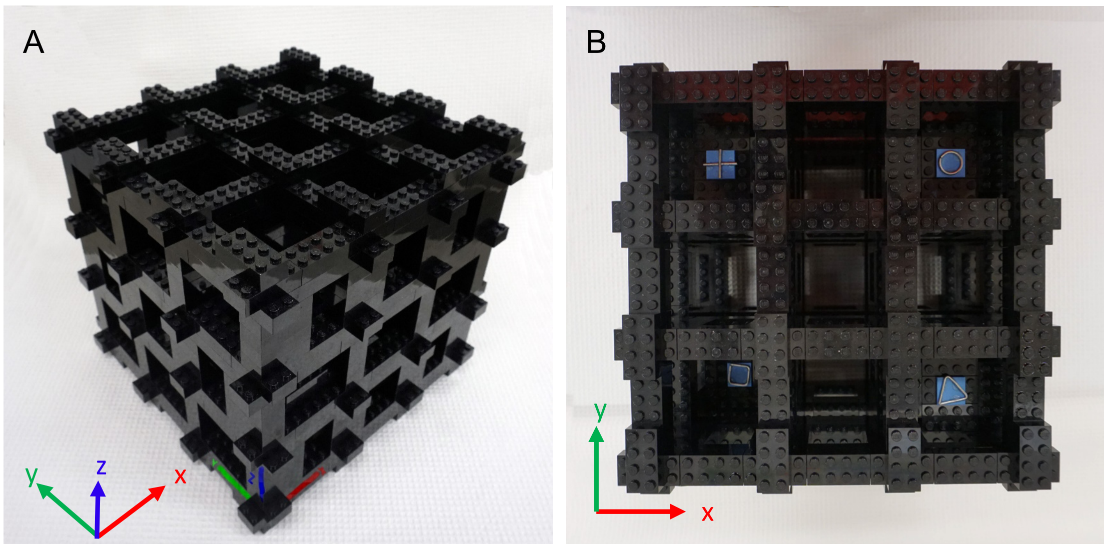


Figure S1. External views of a 64-point Lego calibration cube. (A) Oblique view. (B) Top views showing lead solder shapes used as reference points for determining the pose of the object.

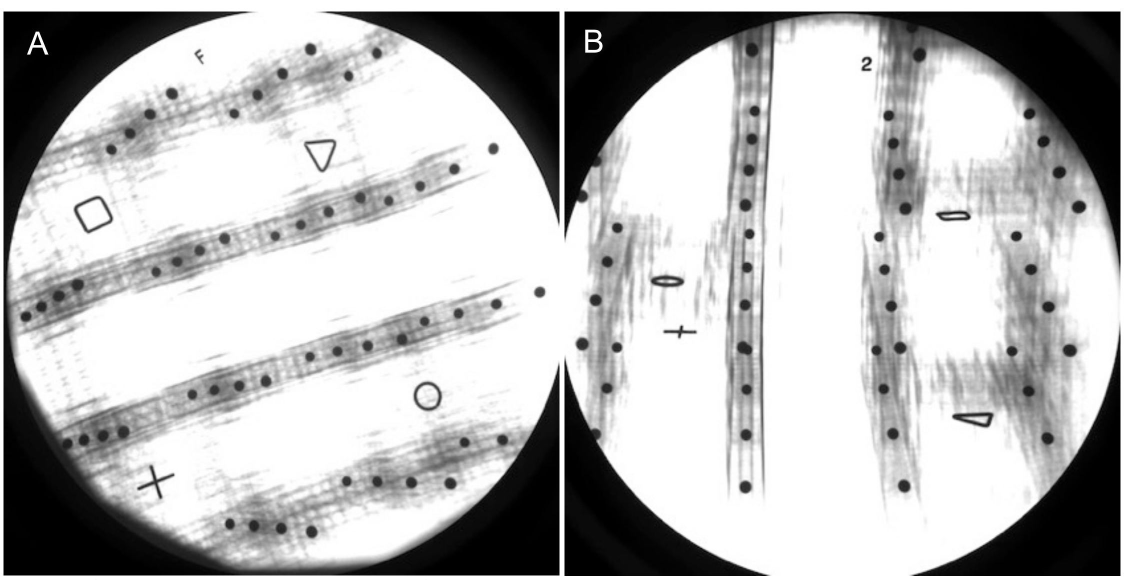


Figure S2. Biplanar X-ray images of a 64-point Lego calibration cube showing steel marker balls and lead solder reference shapes (square, circle, triangle, cross) for determining the pose of the object. The stainless steel balls are press-fit into selected Lego bricks to create a 4x4x4 lattice with 64 mm spacing. (A) Camera 1. (B) Camera 2.

The stainless steel balls in this calibration cube form a 4x4x4 lattice with 64 mm (6.4 cm) spacing between the beads (Fig. S2). The overall size of the marker lattice is 192x192x192 mm, and the overall size of the Lego calibration object is 224 mm x 224 mm x 211.2 mm. This is a large cube and typically not all of the points will appear in the X-ray images (Fig. S2). Construction of a smaller calibration object would be necessary for smaller fields of view. In the future, designs for other Lego calibration objects will be posted on the XMALab Bitbucket site (bitbucket.org/xromm/xmalab/).

Lego bricks are manufactured with high precision (tolerance 0.002 mm, from: http://cache.lego.com/downloads/aboutus/LEGO_company_profile_UK.pdf), as they must fit together firmly, yet be easily disassembled. These consistent dimensions make it possible to construct a robust Lego frame that holds steel balls at easily calculated and highly accurate positions. It is also convenient that Lego bricks have internal cylinders into which the 5.0 mm diameter steel spheres can be press-fit tightly (Fig. S3). The pegs of an interlocking brick fit around the cylinders, so the bricks still interlock tightly with the steel balls in place.

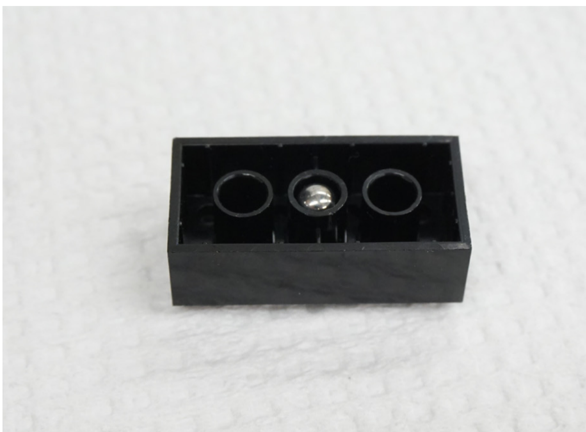


Figure S3. 2x4 Lego brick with a 5.0 mm stainless steel ball inserted into the center cylinder. Consistent depth is ensured by pressing ball in with brick inverted against a flat surface.

Below we describe the assembly process of the Lego cube and the framespec (Table S1) and reference (Table S2) files required for XMALab calibration. The frame specification file contains the xyz coordinates of the steel balls (Table S1). In the past we have expressed marker positions in framespec files in centimeters, but starting with this Lego object, we recommend writing all framespec files for XROMM in millimeters. In the past we have also placed the lead solder shapes around existing markers in the cube. Here we give them their own locations as points 65-68 in the framespec file (Table S1). The references file is a space-separated text file with the extension .ref that contains just the marker numbers and names of the shapes (Table S2). The user can also select reference points within the XMALab interface if some of the shapes are not visible but other points are known.

Required Parts:

- 64 5.0 mm spherical stainless steel balls (McMaster-Carr Part # 1598K26)
- 1.5 mm lead solder
- 492 2x4 Lego bricks Part #3001
- 104 2x6 Lego bricks Part #2456
- 4 2x2 Lego tiles Part #3068a

First, each bead was inserted into a 2x4 brick (Fig. S3). To ensure the beads were placed at identical depths, each bead was first fit partway into the center cylinder, then the brick was firmly pressed against a flat surface until the bottom of the bead was flush with the bottom of the brick.

Table S1. Lego cube frame specification file values and format (comma separated values; framespec.csv) for the 64-point Lego cube with 64 mm spacing between the beads, expressed in centimeters because cm units are more convenient for subsequent XROMM animation in Autodesk Maya.

x,y,z	
0,0,0	0,0,11.52
6.4,0,0	6.4,0,11.52
12.8,0,0	12.8,0,11.52
19.2,0,0	19.2,0,11.52
0,6.4,0	0,6.4,11.52
6.4,6.4,0	6.4,6.4,11.52
12.8,6.4,0	12.8,6.4,11.52
19.2,6.4,0	19.2,6.4,11.52
0,12.8,0	0,12.8,11.52
6.4,12.8,0	6.4,12.8,11.52
12.8,12.8,0	12.8,12.8,11.52
19.2,12.8,0	19.2,12.8,11.52
0,19.2,0	0,19.2,11.52
6.4,19.2,0	6.4,19.2,11.52
12.8,19.2,0	12.8,19.2,11.52
19.2,19.2,0	19.2,19.2,11.52
0,0,5.76	0,0,17.28
6.4,0,5.76	6.4,0,17.28
12.8,0,5.76	12.8,0,17.28
19.2,0,5.76	19.2,0,17.28
0,6.4,5.76	0,6.4,17.28
6.4,6.4,5.76	6.4,6.4,17.28
12.8,6.4,5.76	12.8,6.4,17.28
19.2,6.4,5.76	19.2,6.4,17.28
0,12.8,5.76	0,12.8,17.28
6.4,12.8,5.76	6.4,12.8,17.28
12.8,12.8,5.76	12.8,12.8,17.28
19.2,12.8,5.76	19.2,12.8,17.28
0,19.2,5.76	0,19.2,17.28
6.4,19.2,5.76	6.4,19.2,17.28
12.8,19.2,5.76	12.8,19.2,17.28
19.2,19.2,5.76	19.2,19.2,17.28
	3.2,3.2,9.76
	16,16,9.76
	16,3.2,15.52
	3.2,16,15.52

*the last 4 points are points 65-68 and contain the coordinates of the reference points used to determine calibration object orientation in XMALab (see Table S2).

Table S2. Lego cube references file values and format (space separated values, with file extension .ref, such as references.ref).

65 Square
66 Circle
67 Triangle
68 Cross

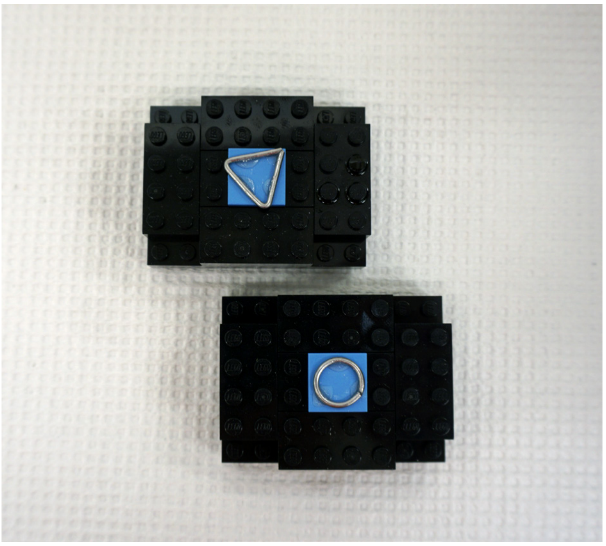


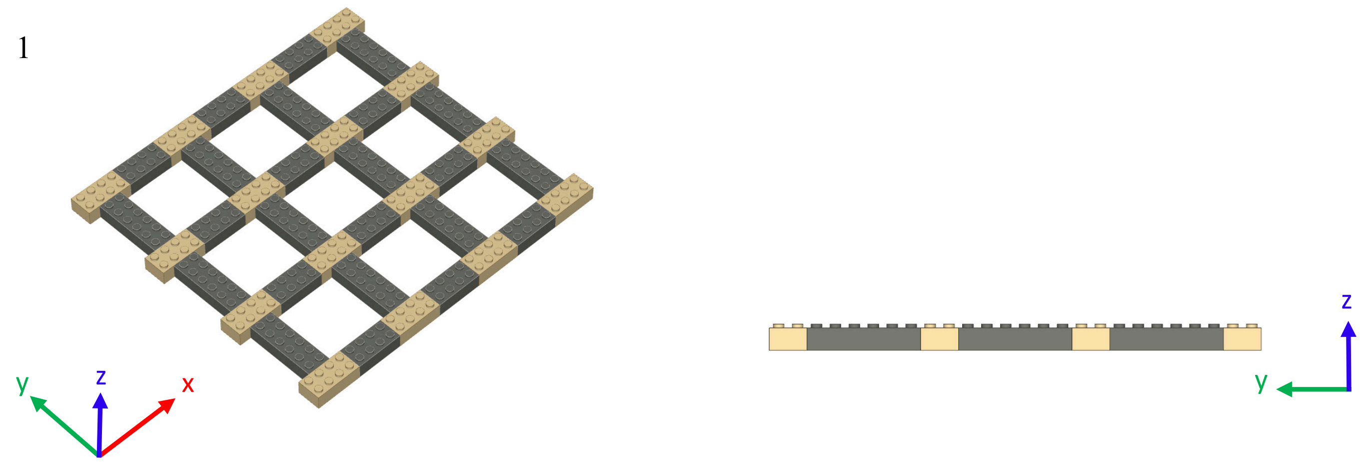
Figure S4: Shapes made of 1.5 mm lead solder were attached to 2x2 Lego tiles and serve as reference points for XMALab calibration.

To create the reference shapes, 1.5 mm lead solder was cut and bent into a square, circle, cross, and triangle, each the approximate size of a 2x2 Lego tile (Fig. S4). The shapes were laid flat on the tiles and secured in place with superglue. Once in place, the shapes provide a visual indication of the orientation of the cube.

Once all 64 beads were fit into the bricks and the lead solder shapes were made, we began assembly of the cube itself. Figure S5 on the following pages is a step-by-step construction guide, created with the open-source software Bricksmith (Allen Smith). While the bricks in the actual cube can be any color, in the guide, bricks that contain steel beads are beige. Steps 6-10 detail the assembly of the segments that support the four reference shapes that are used for automatic calibration in XMALab. However, we found it helpful to add a set of external axes labels, to make it easy to quickly determine the cube's orientation (Fig S1).

Figure S5. Lego cube assembly steps. Beige bricks contain a 5.0 mm lead bead.

1



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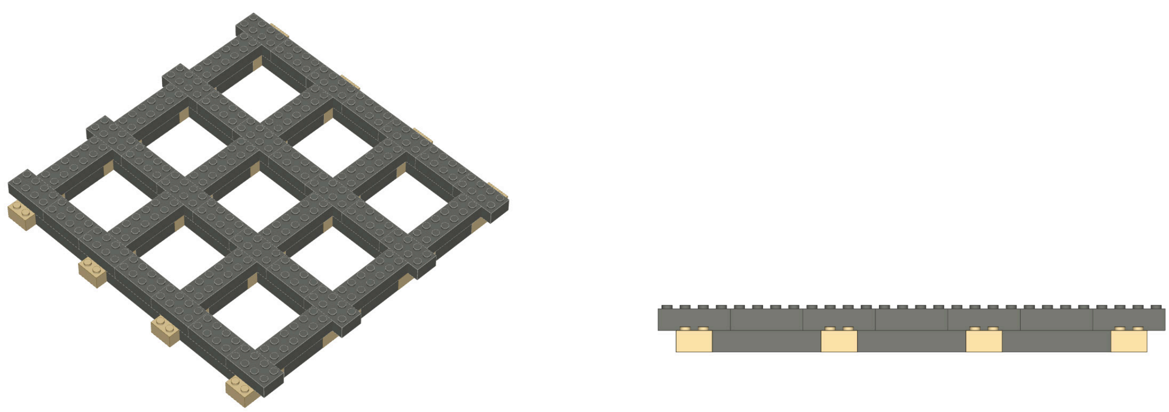

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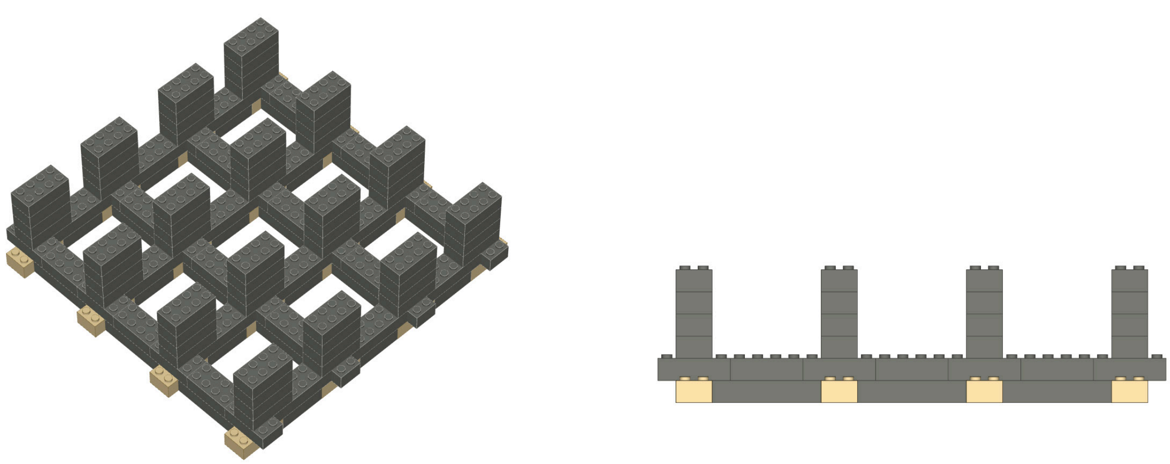

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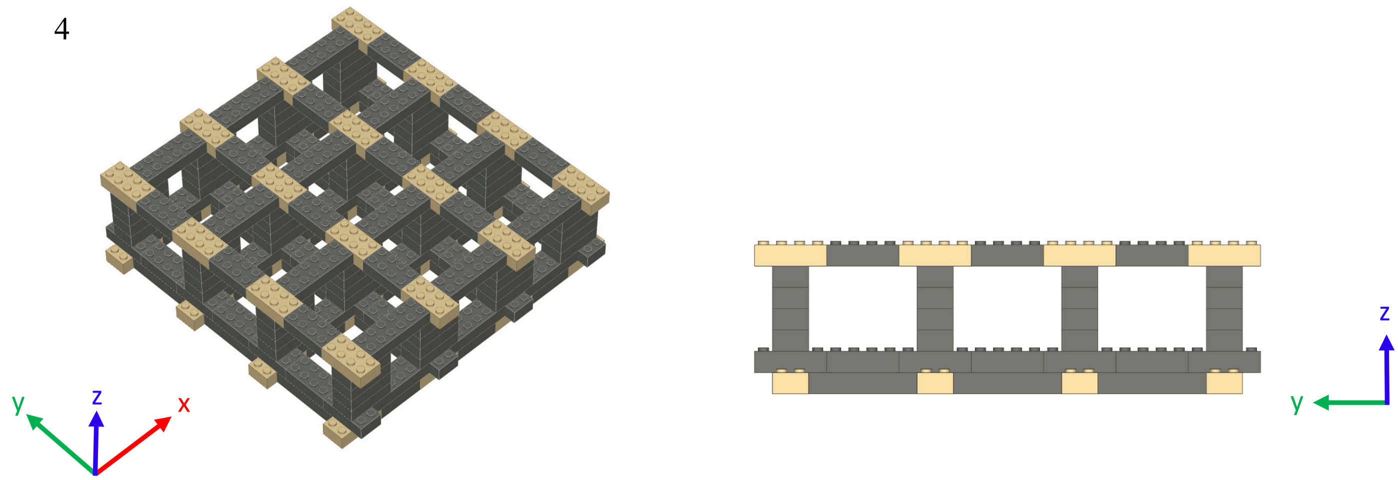

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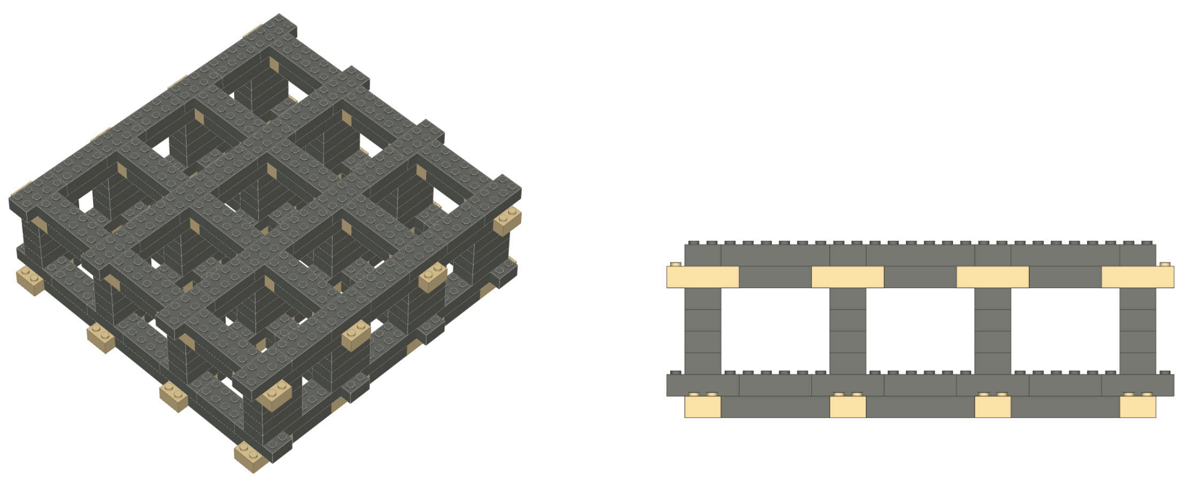

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*Steps 6-10 repeated four times, one for each lead solder shape: Square, circle, cross, and triangle. Shapes attached to tiles with superglue.

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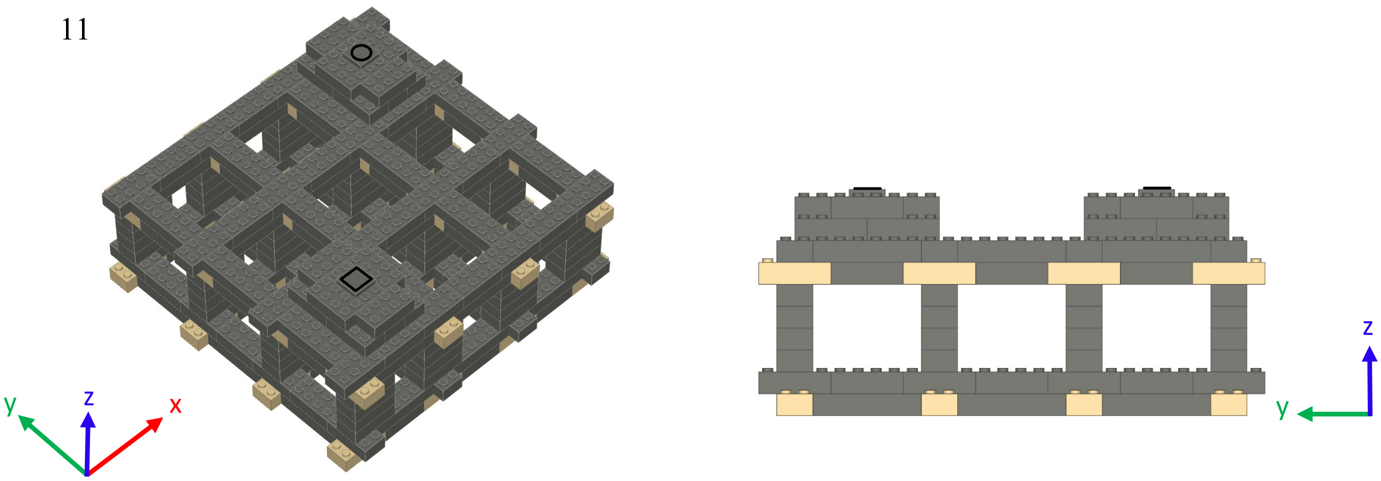

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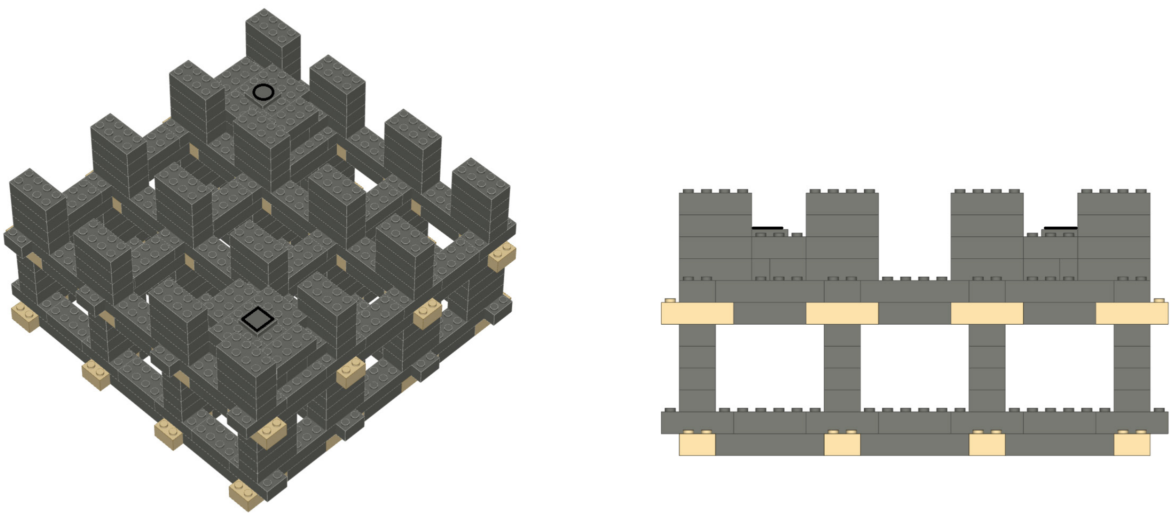

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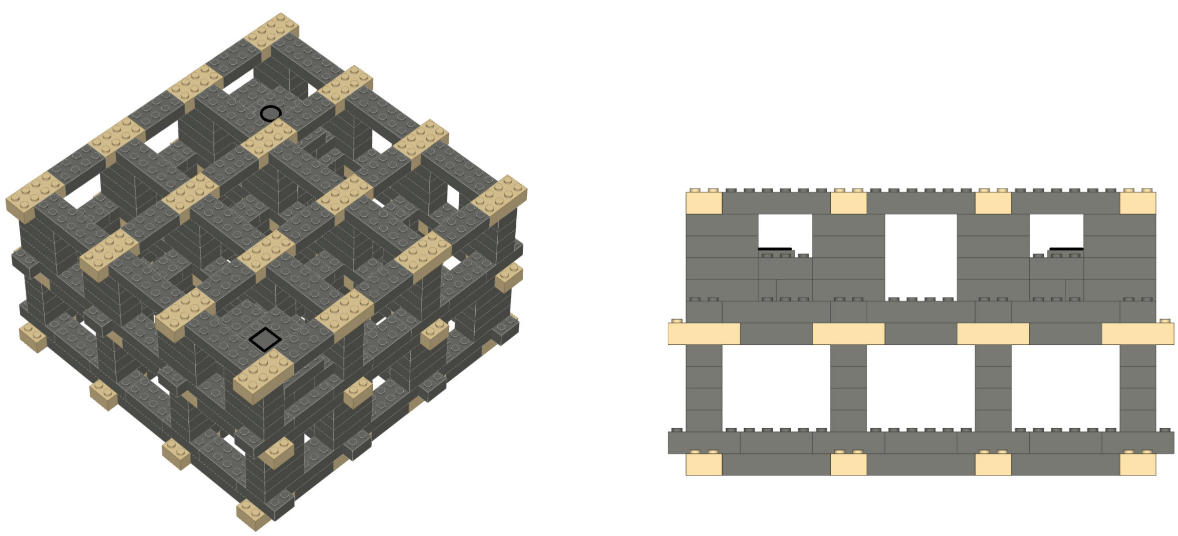

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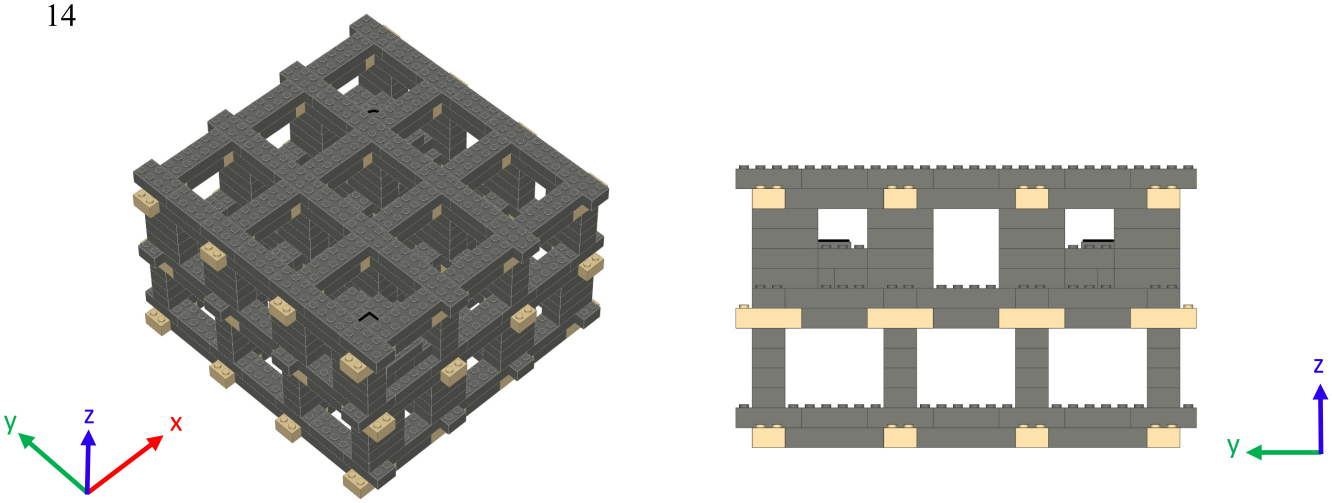

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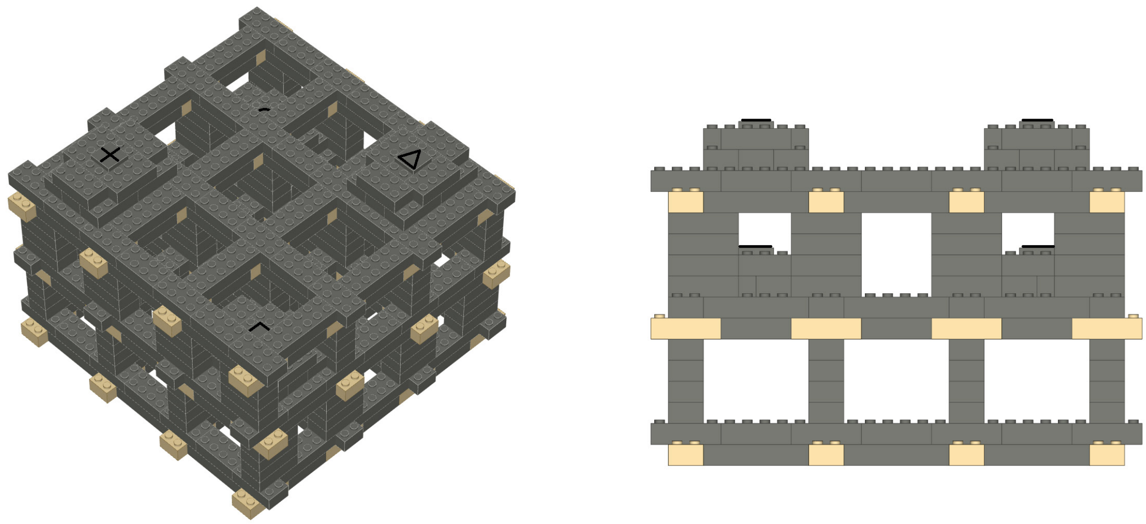

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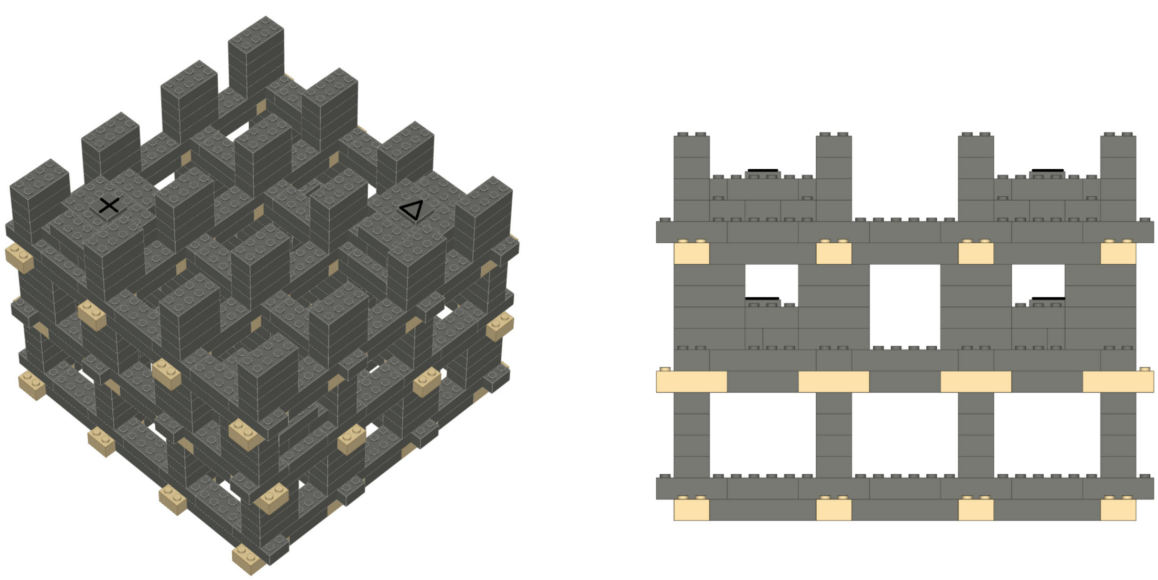

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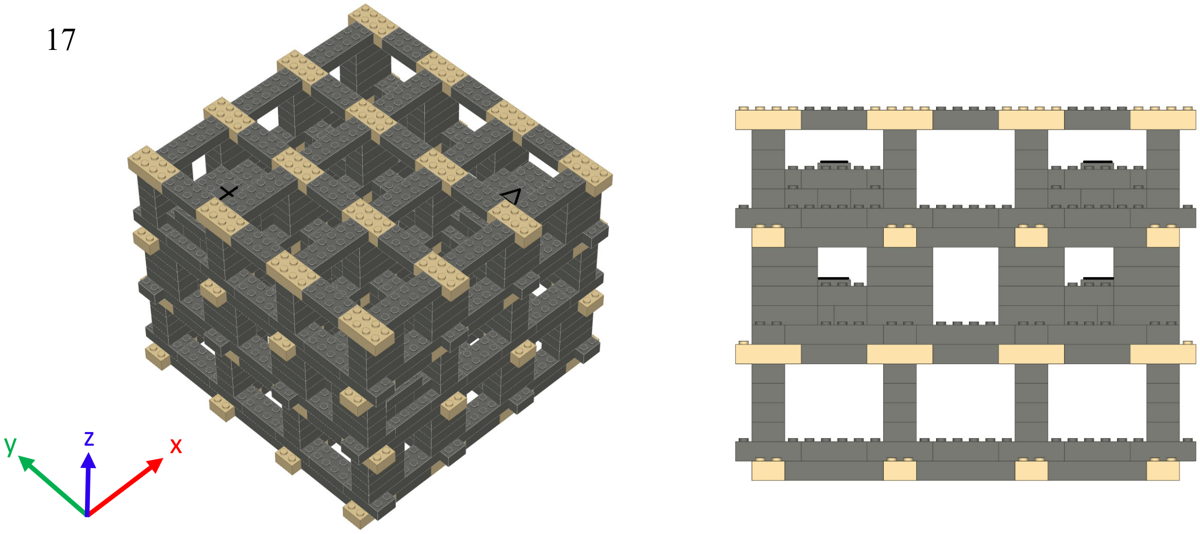

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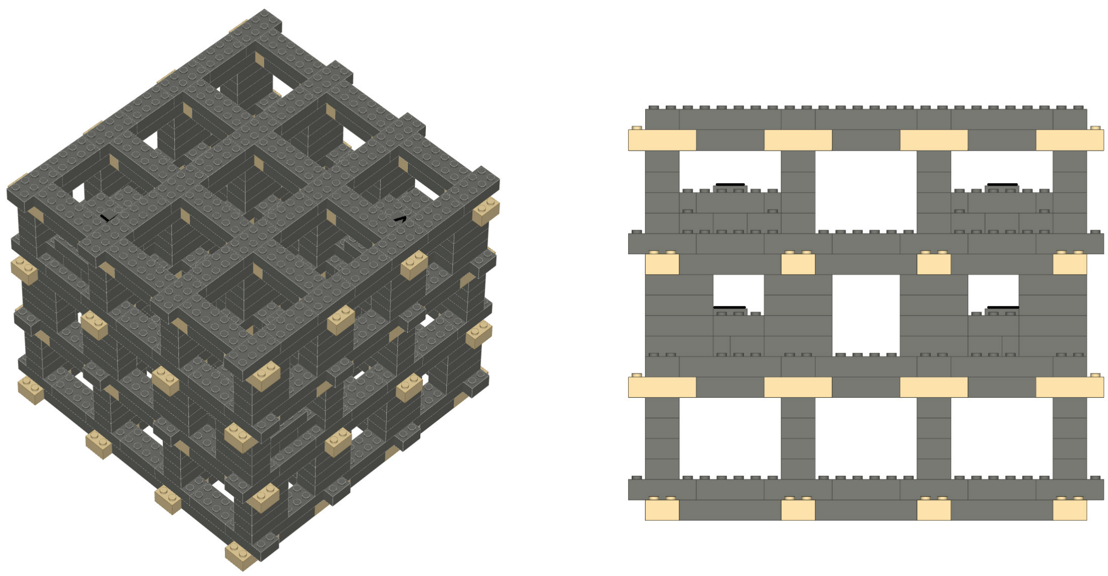

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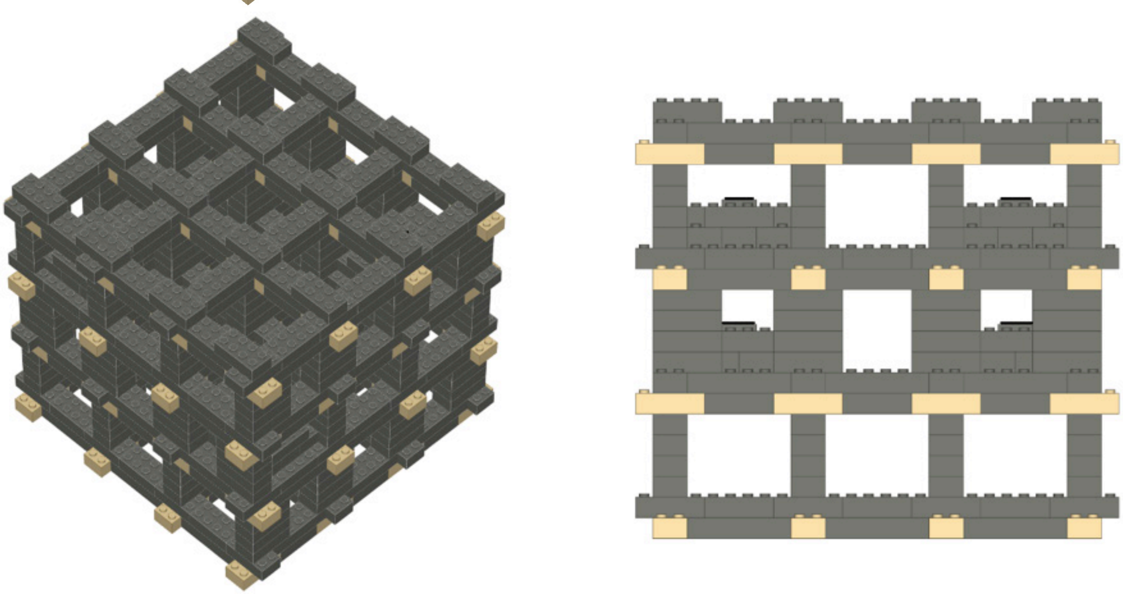

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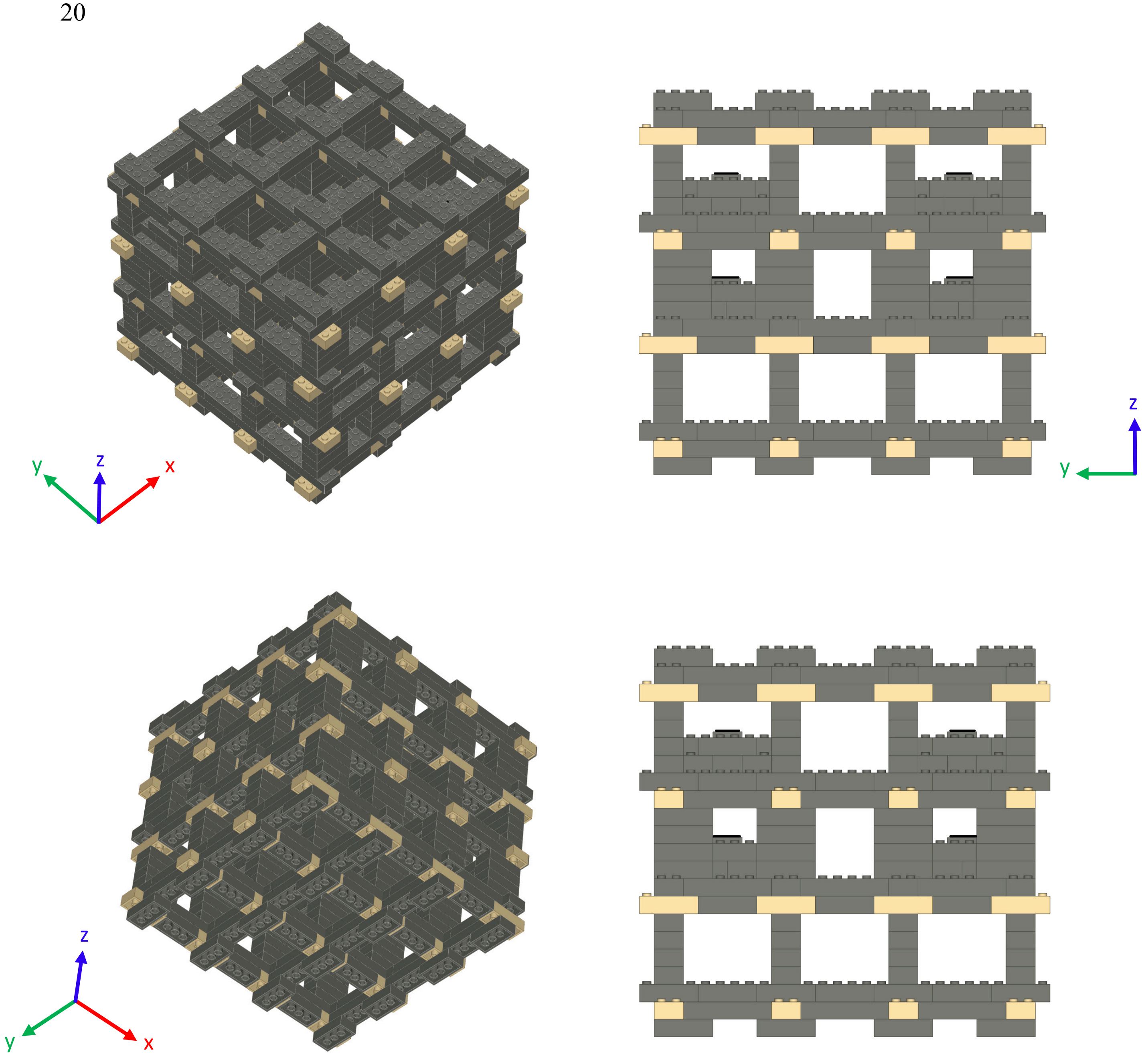

Baronti, L., Dellepiane, M. and Scopigno, R. (2010). Using Lego Pieces for Camera Calibration: a Preliminary Study. In *Eurographics 2010 - Short Papers*, eds. H. P. A. Lensch and S. Seipel): The Eurographics Association.

Brainerd, E. L., Baier, D. B., Gatesy, S. M., Hedrick, T. L., Metzger, K. A., Gilbert, S. L. and Crisco, J. (2010). X-ray Reconstruction of Moving Morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. *J. Exp. Zool.* **313A**, 262-279.

Supplementary Information 2: Repeatability of Rigid Body Motions Across Users

In this supplementary document we report repeatability results for all six degrees of freedom from the JCS analysis for nine users. To compare reproducibility among users for the rigid body transformations we imported the minipig cranium and lower jaw models into Autodesk Maya and placed a joint coordinate system (JCS) at the temporomandibular joint. We calculated the six-degree-of-freedom motions from the JCS for all nine users and then calculated the deviation from the mean for each frame for the nine users, and took the mean and standard deviation of all of those values across 435 frames.

The means and standard deviations and results of statistical tests comparing XMALab to XrayProject are presented in Table S3. Graphs of the data for nine users for the six degrees of freedom are presented in Figures S6-S11. The graphs include a precision threshold determined from a frozen specimen test (Menegaz et al., 2015). The threshold values are also available in Table S4.

Table S3. Repeatability XMALab and XrayProject for rigid body motions measured with a mandibular JCS relative to the mean value of all users (**** $p < 0.0001$)

	One-way repeated measures ANOVA F(1,15)	One-tailed paired t-test t(8)	Mean Deviation[1] (in mm or deg)		SD (in mm or deg)	
			XMALab	XrayProj.	XMALab	XrayProj.
Translation X	256.7 ****	15.541 ****	0.004	0.024	0.00081	0.0038
Translation Y	85.33 ****	8.4162 ****	0.011	0.061	0.0043	0.016
Translation Z	57.98 ****	7.1563 ****	0.022	0.082	0.0074	0.023
Rotation X	93.75 ****	8.8012 ****	0.012	0.055	0.0041	0.013
Rotation Y	85.13 ****	8.5546 ****	0.013	0.047	0.0042	0.011
Rotation Z	419.2 ****	18.226 ****	0.0064	0.041	0.0026	0.0043

[1]Mean deviation for all users across the 435 frames in the test sequence

Table S4. Precision thresholds for XMALab and XrayProject

	XMALab	XrayProject[1]
Translation X	0.043	0.06
Translation Y	0.13	0.26
Translation Z	0.19	0.44
Rotation X	0.089	0.26
Rotation Y	0.082	0.21
Rotation Z	0.067	0.13

1 from Menegaz et al., 2015

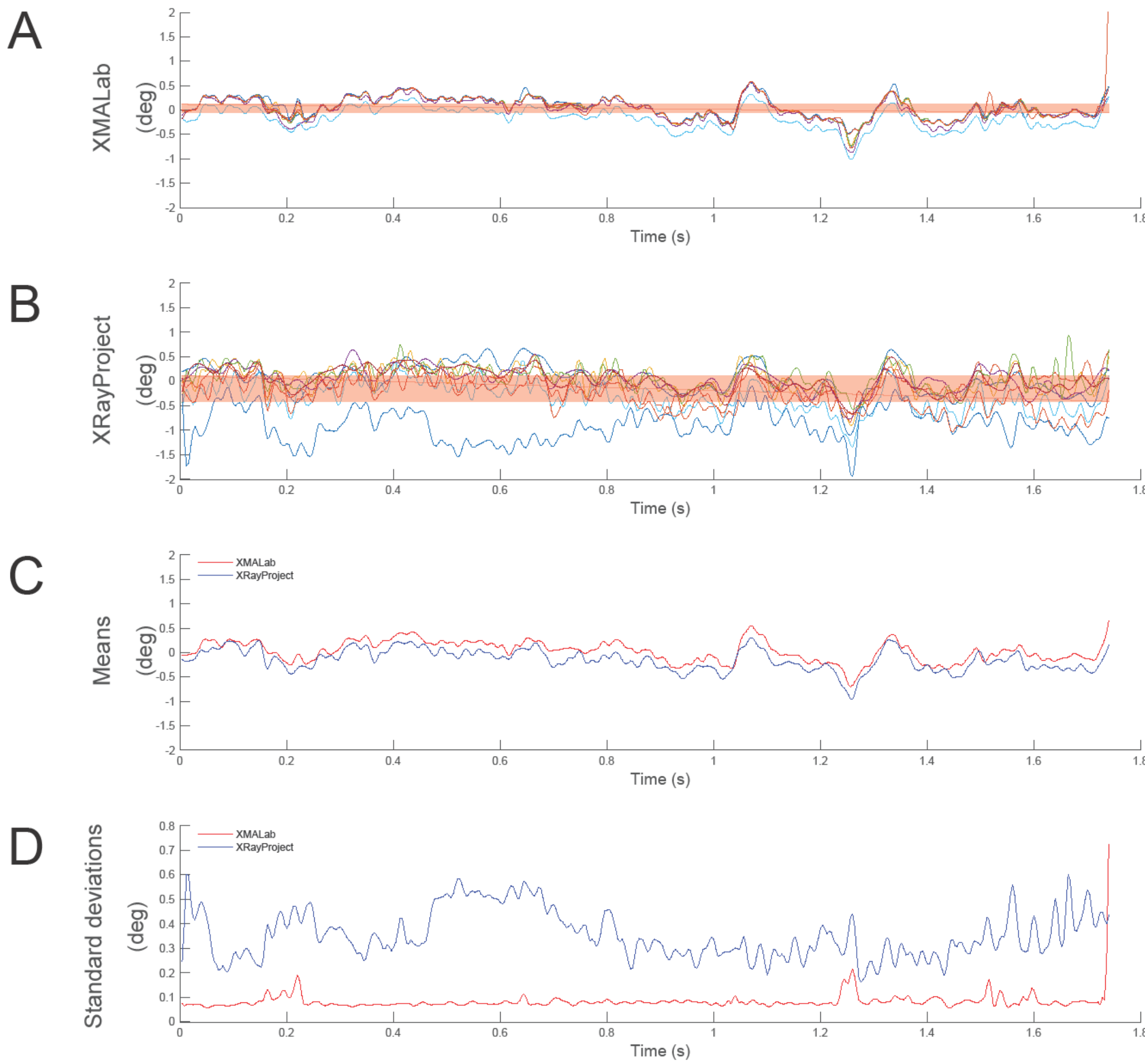

Figure S6. Variation among users in x-axis rotation (in degrees) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

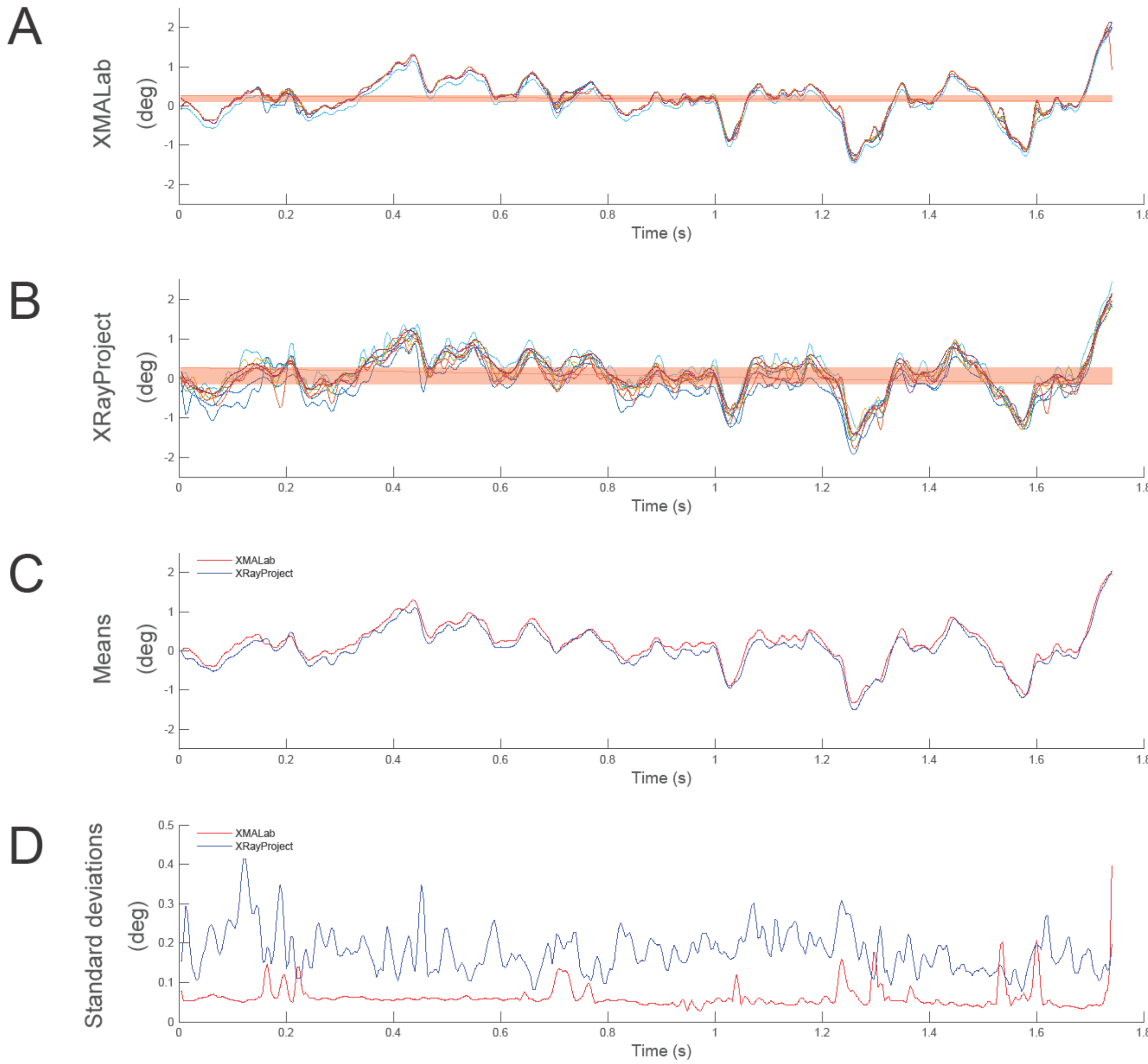

Figure S7. Variation among users in y-axis rotation (in degrees) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

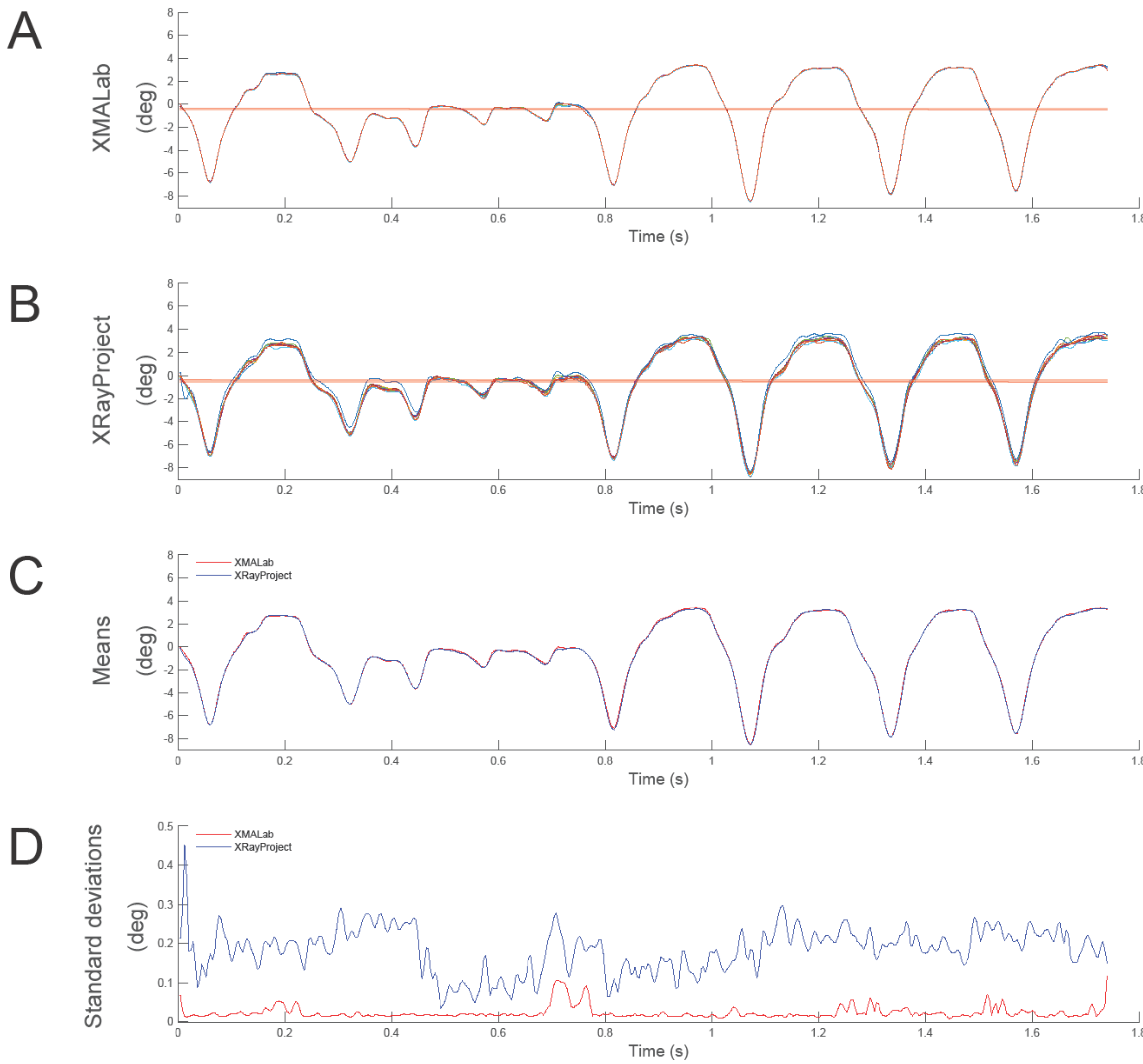

Figure S8. Variation among users in z-axis rotation (in degrees) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

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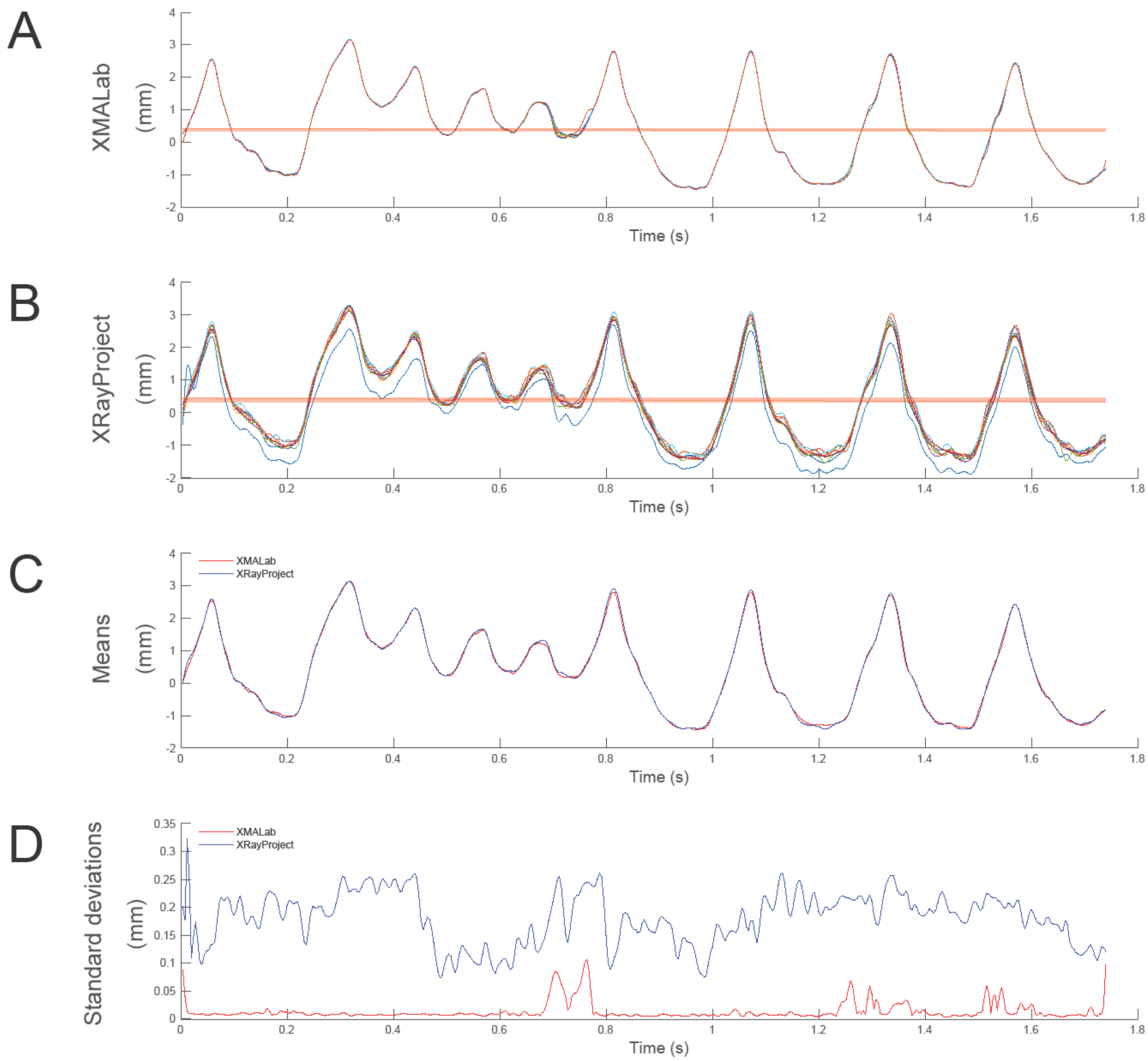

Figure S9. Variation among users in x-axis translation (in mm) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

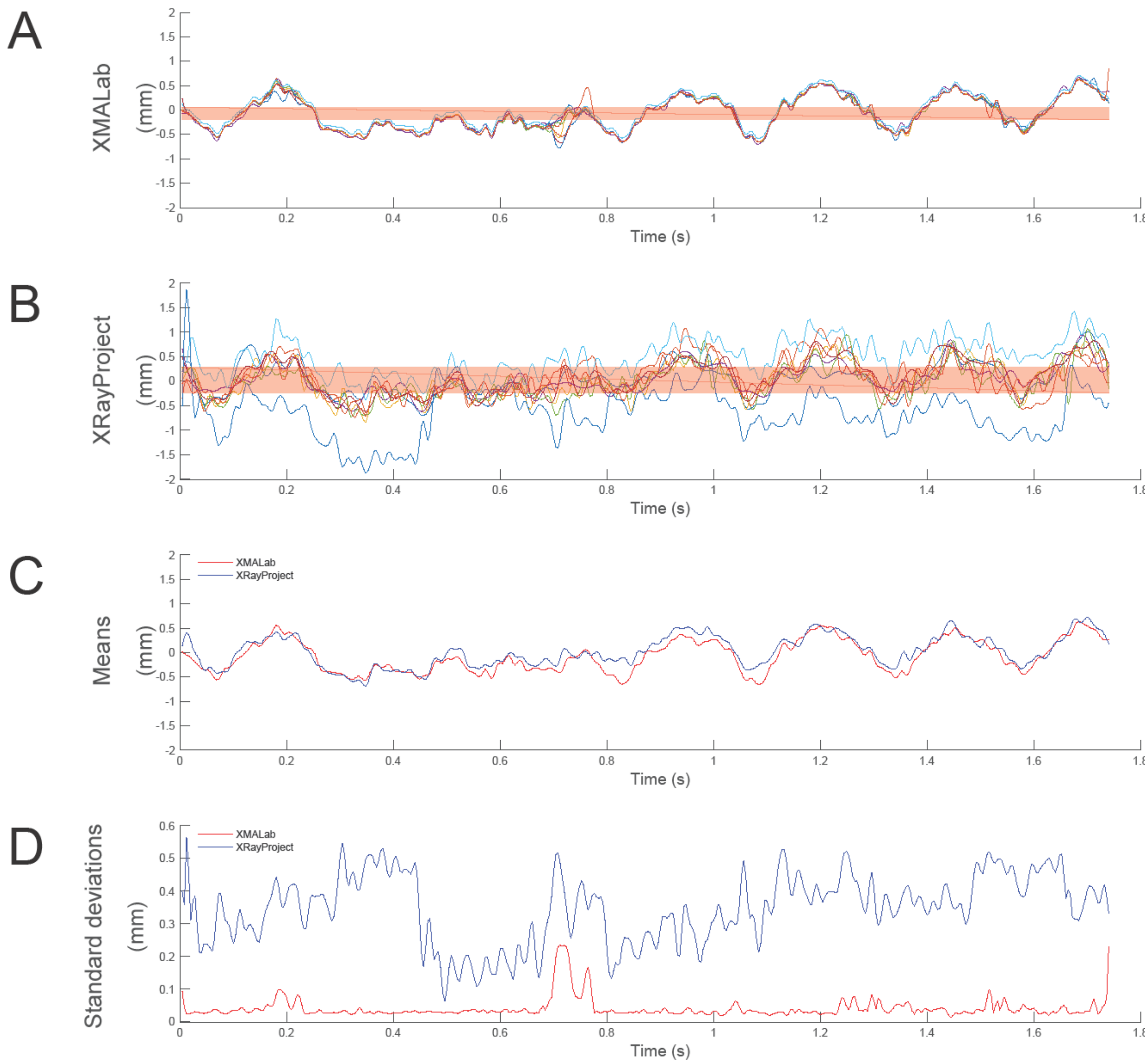

Figure S10. Variation among users in y-axis translation (in mm) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

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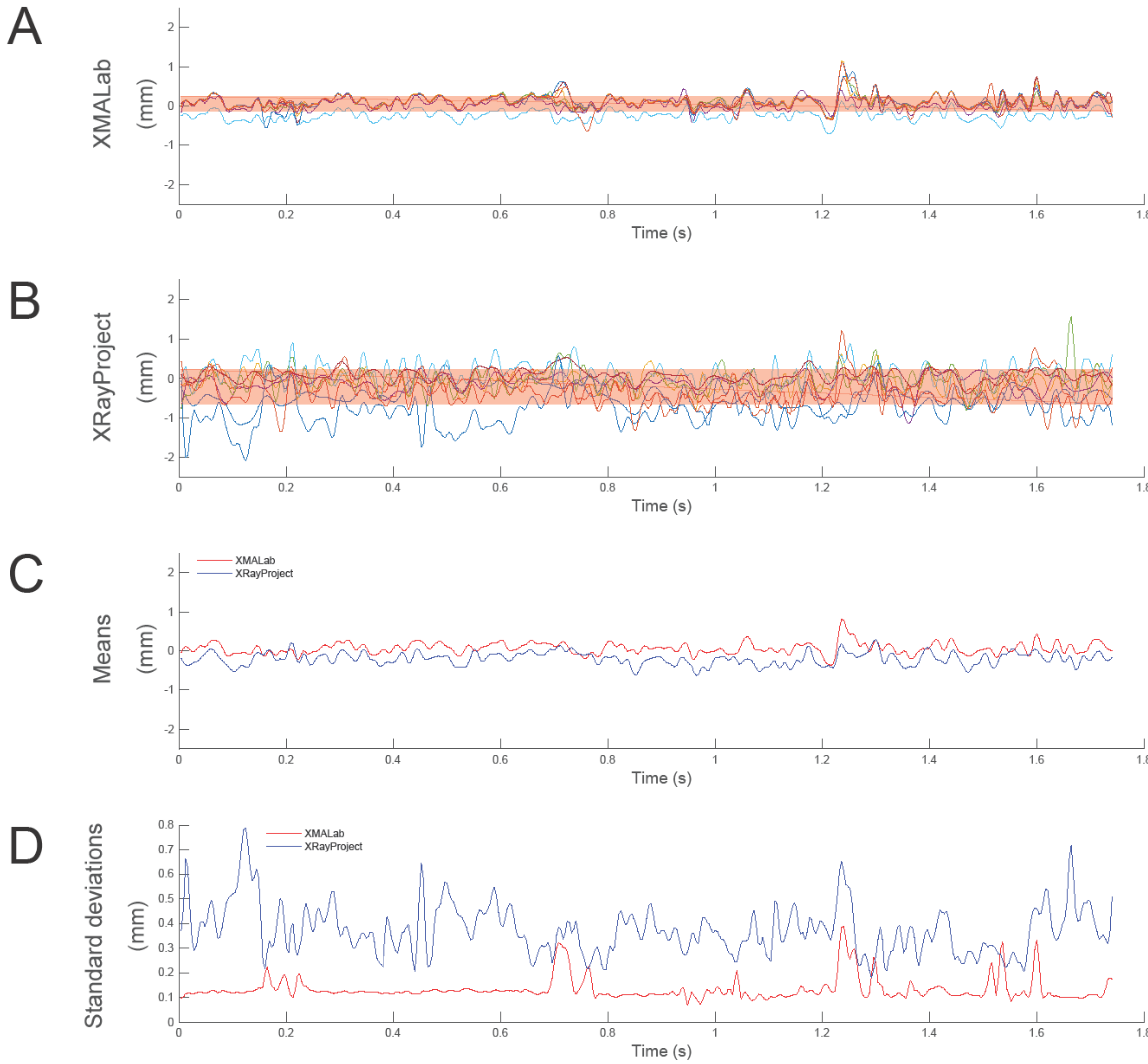

Figure S11. Variation among users in z-axis translation (in mm) from a joint coordinate system measuring motion of the mandible relative to the cranium in a miniature pig (Menegaz et al., 2015). (A) Results from XMALab for nine users. Line for each user is a different color. The precision threshold from a frozen specimen test for this degree of freedom is shown as a salmon-colored bar. (B) Results from XrayProject for nine users. Line for each user is a different color. Salmon-colored bar shows precision threshold. (C) Mean result for all users for the two programs; XMALab in red and XrayProject in blue. (D) Standard deviation for each time point among users; XMALab in red and XrayProject in blue.

Menegaz, R. A., Baier, D. B., Metzger, K. A., Herring, S. W. and Brainerd, E. L. (2015). XROMM analysis of tooth occlusion and temporomandibular joint kinematics during feeding in juvenile miniature pigs. *J Exp Biol* **218**, 2573-84.