Supplementary materials for

3D revisualisation: a new method to revisit segmented data

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This supplementary Information includes:

Supplementary Notes

Drishti v2.7

Installation

Drishti v2.7 is available to download from https://github.com/nci/*Drishti* under “Releases”.

When you click, it will lead to a version-specific webpage where the.zip file is located at the bottom of the page; click *Drishti* v2.7.zip to download *Drishti*.

Source code and detailed information about this new release are also available on GitHub. Please note that *Drishti* v2.7 can only run on the Windows operating system. However, users can compile and install *Drishti* v2.7 for CentOS/Ubuntu. Sample compilation script for *nix systems is provided to users with the source code.

Once the download is finished, unzip the .zip file. Go to the “bins” folder; then, you should be able to run *Drishti* v2.7. *Drishti* is designed as a portable application, thus making sure you know where the portable directory is stored on your computer.

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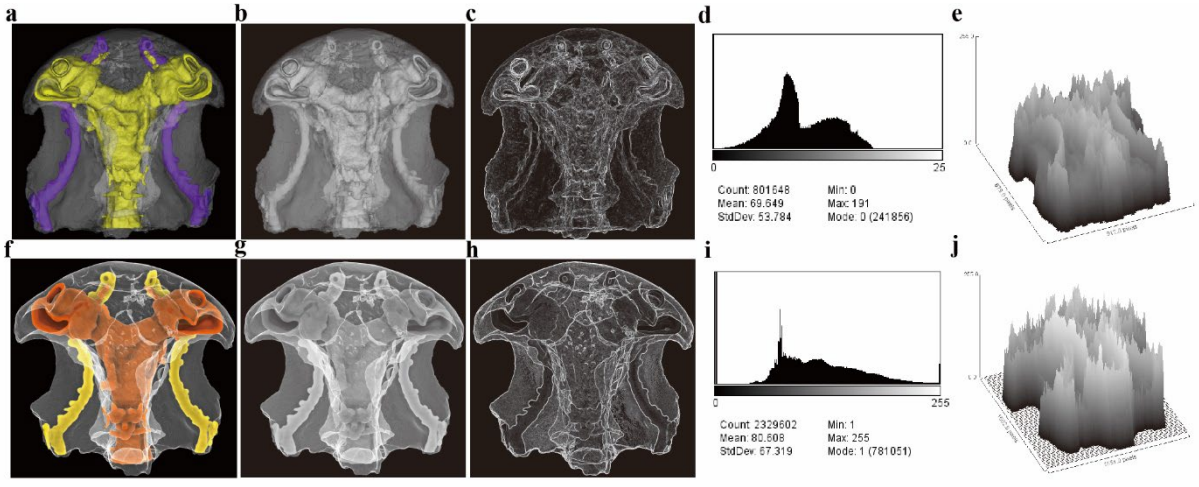
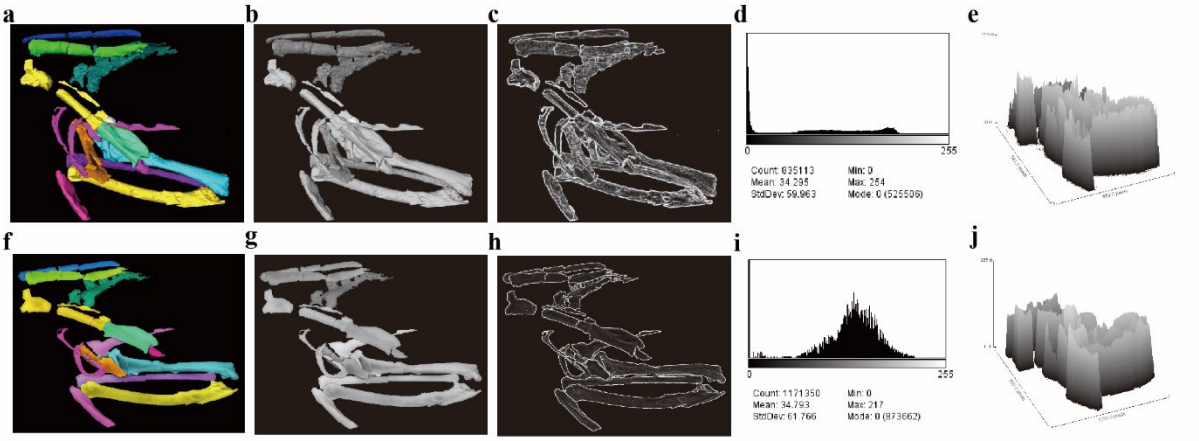
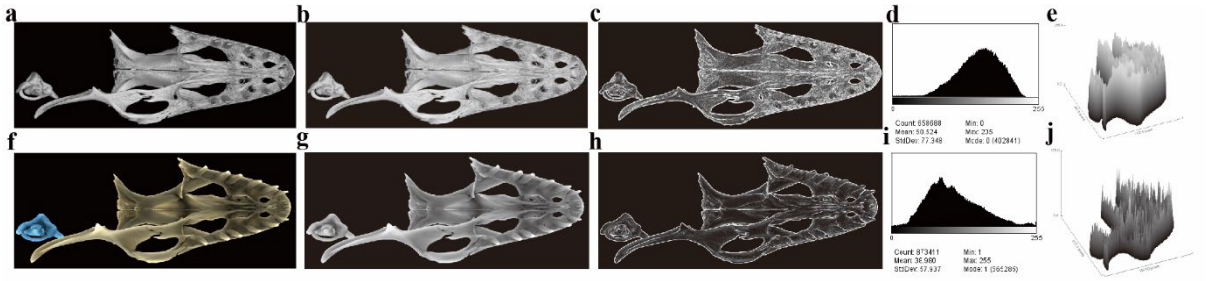
Supplementary Figures



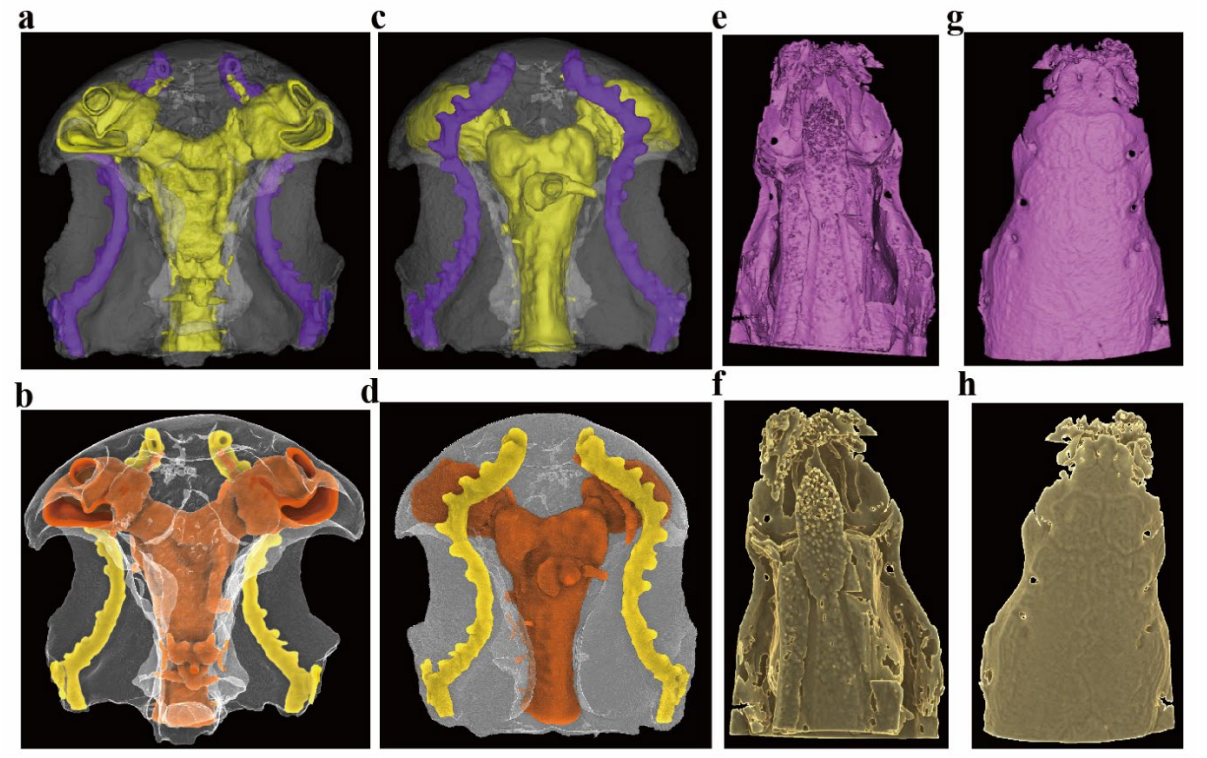
Supplementary Figure 1. Ventral view of the segmented braincase of fossil fish *Tungsenia* IVPP V10687. a-c, Extracted images from Mimics. **a**, Original image output from Mimics. **b**, 8-bits greyscale image of **a**. **c**, Detected edges of **b**. **d**, 2D histogram of **b**. **e**, the 3D surface plot of **b**. **f-h**, Extracted images from *Drishti* after revisualization. **f**, Original image output from *Drishti* in the same orientation and scale of **a**. **g**, Revisualized result corresponds to **b**. **h**, Revisualized result corresponds to **c**. **i**, 2D histogram of **g. j**, the 3D surface plot of **g**.



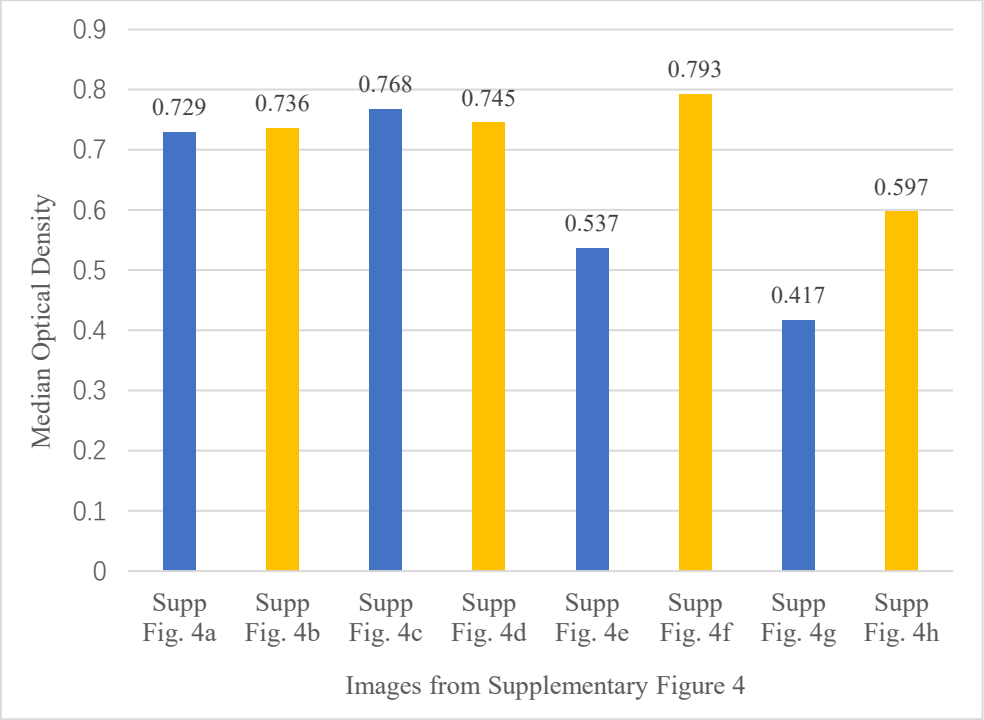
Supplementary Figure 2. Lateral view of segmented fossil bird *Linxiavis* IVPP V24116. a-c, Extracted images from Avizo. **a**, Original image output from Mimics. **b**, 8-bits greyscale image of **a**. **c**, Detected edges of **b**. **d**, 2D histogram of **b**. **e**, the 3D surface plot of **b**. **f-h**, Extracted images from *Drishti* after revisualization. **f**, Original image output from *Drishti* in the same orientation and scale of **a**. **g**, Revisualized result corresponds to **b**. **h**, Revisualized result corresponds to **c**. **i**, 2D histogram of **g. j**, the 3D surface plot of **g**.



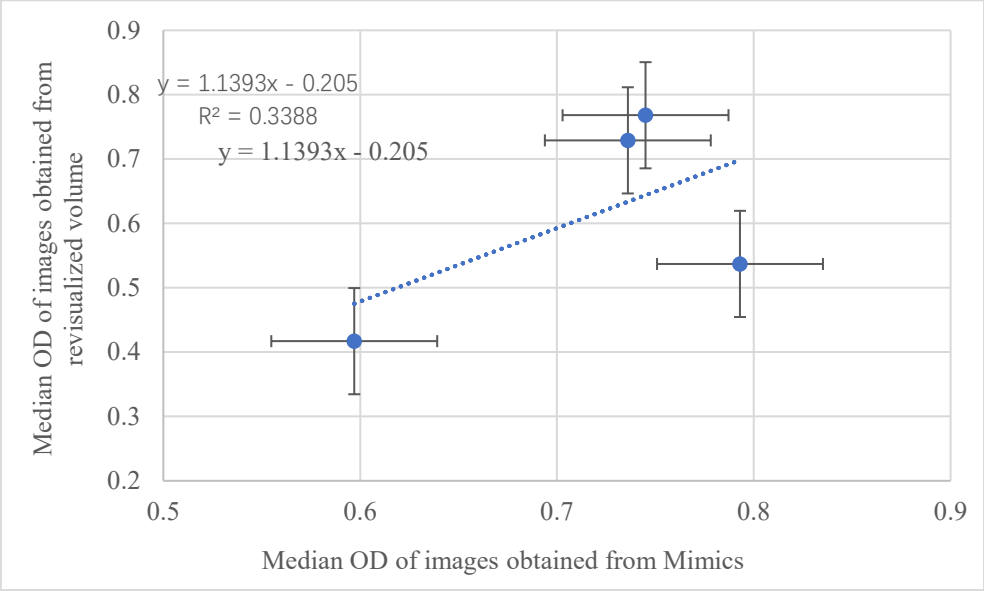
Supplementary Figure 3. Ventral view of the segmented upper jaw and inner ear of lizard Varanus indicus (AMNH R58389). a-c, Extracted images from Mimics. **a**, Original image output from Mimics. **b**, 8-bits greyscale image of **a**. **c**, Detected edges of **b**. **d**, 2D histogram of **b**. **e**, the 3D surface plot of **b**. **f-h**, Extracted images from *Drishti* after revisualization. **f**, Original image output from *Drishti* in the same orientation and scale of **a**. **g**, Revisualized result corresponds to **b**. **h**, Revisualized result corresponds to **c**. **i**, 2D histogram of **g. j**, the 3D surface plot of **g**.



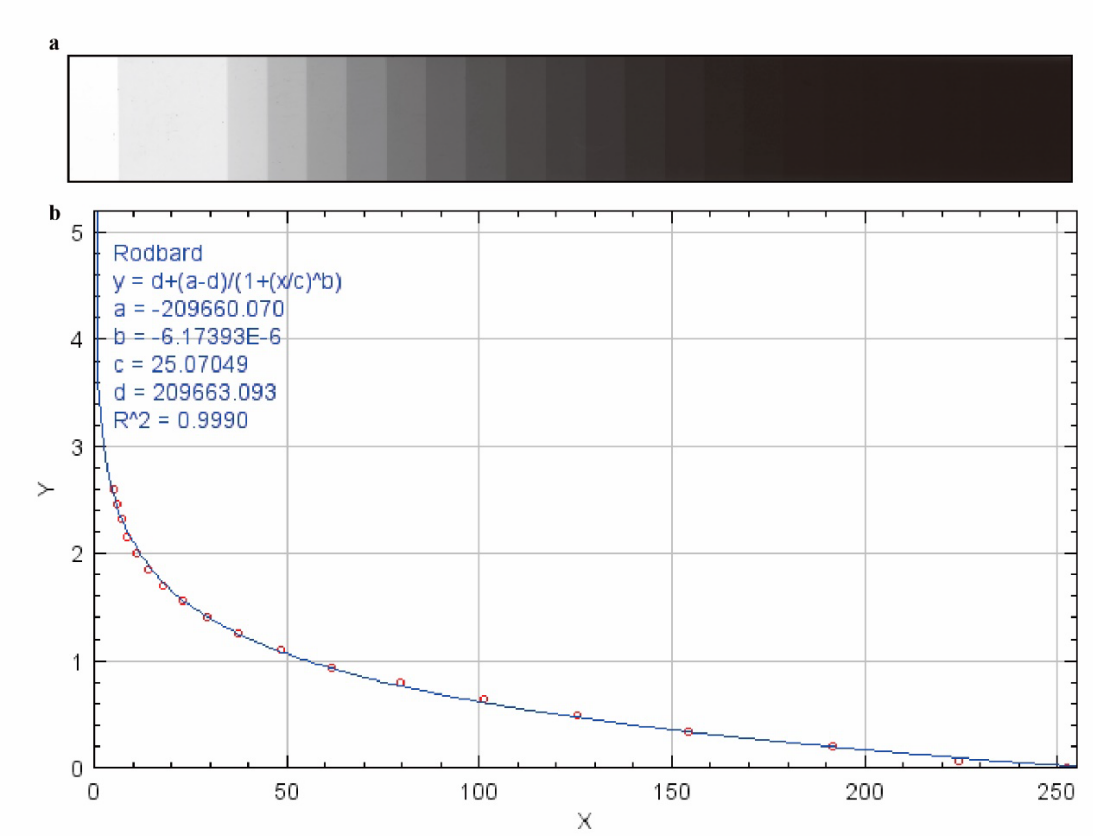
Supplementary Figure 4. Images used for optical density measurements. All images are from segmented volumes using Mimics and their corresponded revisualized segmented volume data using Drishti. **a-d**, Extracted images of *Tungsenia* IVPP V10687. **a-b**, Ventral view, from segmented volume (**a**) and revisualized segmented volume (**b**). **c-d**, Dorsal view, from segmented volume (**c**) and revisualized segmented volume (**d**). **e-h**, Extracted images of *Erofoichthys* IVPP OV2715. **e-f**, Ventral view, from segmented volume (**e**) and revisualized segmented volume (**f**). **g-h**, Dorsal view, from segmented volume (**g**) and revisualized segmented volume (**h**).



Supplementary Figure 5. The median optical density of images from Supplementary Fig.4. Calibration of optical density was completed using an 8-bits 21 steps-table using ImageJ. Data analysis was carried out in Microsoft Excel.



Supplementary Figure 6. Correlation between the median optical density of images in Supplementary Fig.4. The X-axis represents the median optical density of images obtained from the original segmented volume using Mimics (i.e. Supplementary Fig.4a,c,e,g). Y-axis represents the median optical density of images obtained from the revisualized volume using *Drishti* v2.7 (i.e. Supplementary Fig. 4b,d,f,h). Analysis was carried out in Microsoft Excel.



Supplementary Figure 7. Step table and calibration curve were used for this study. a, step-table. b, calibrated Optical Density curve with the correlation coefficient R^2 equals 0.9990.