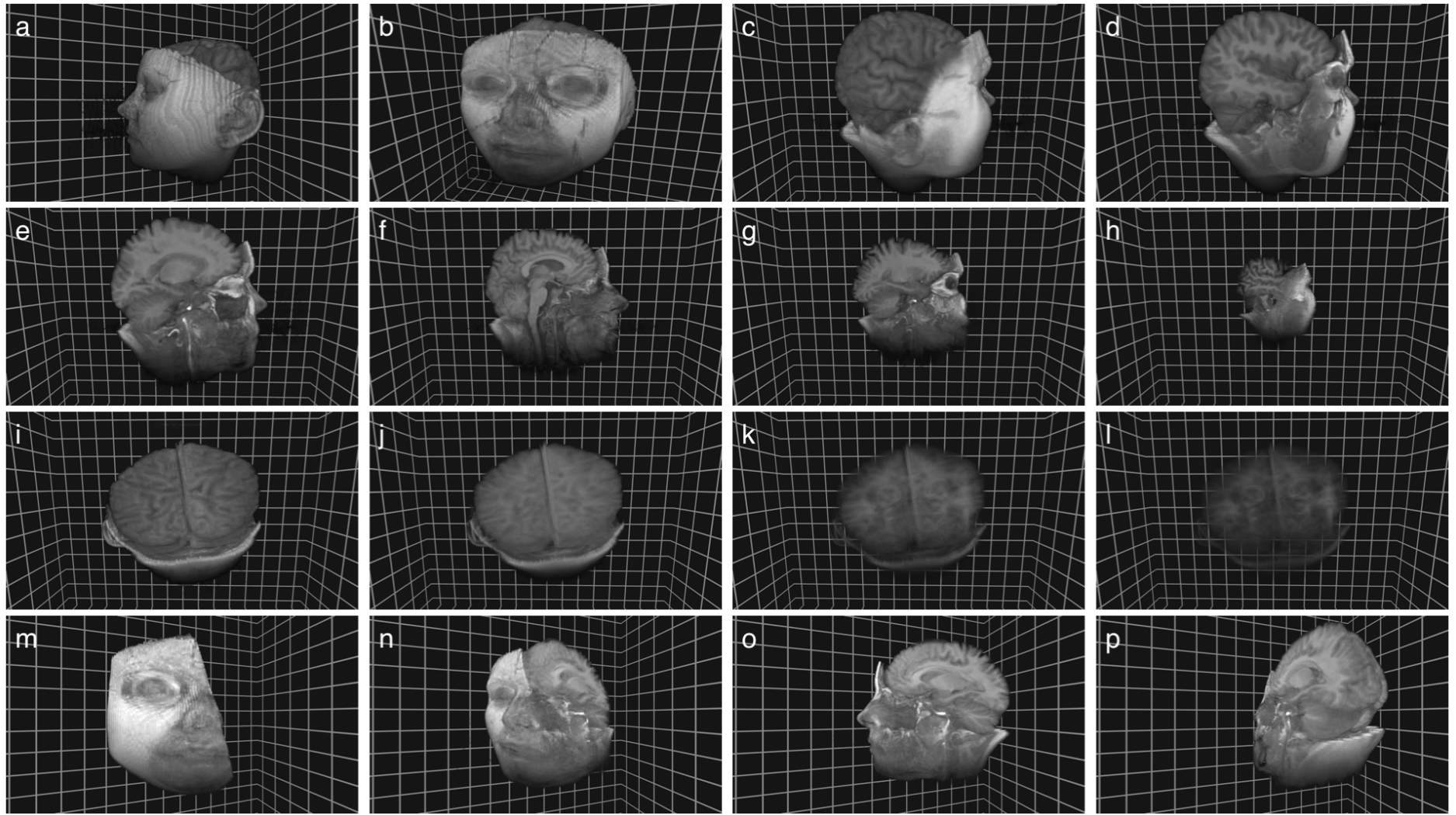


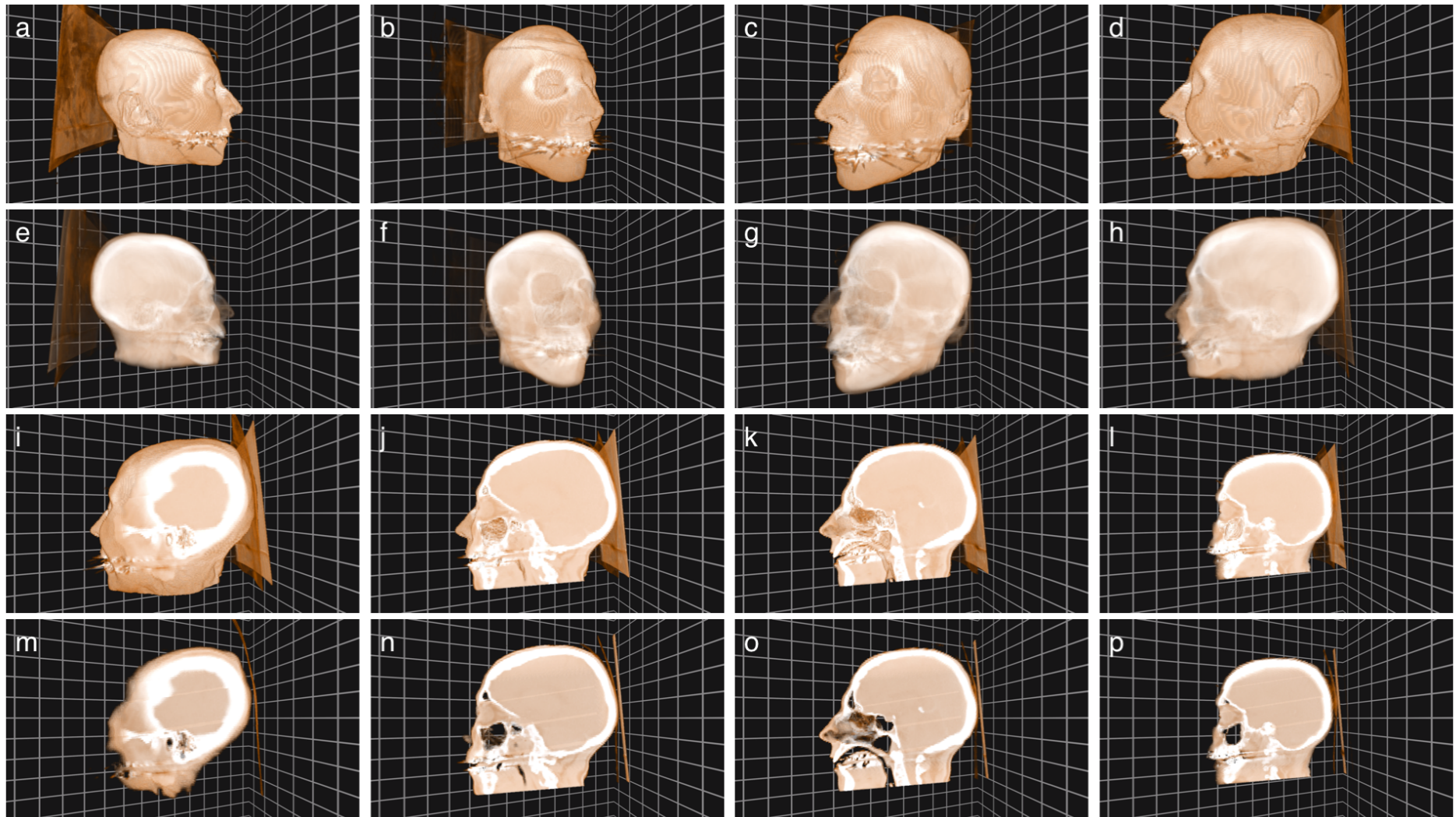
In the format provided by the authors and unedited.

# A new online tool for visualization of volumetric data

Marcus Fantham and Clemens F. Kaminski

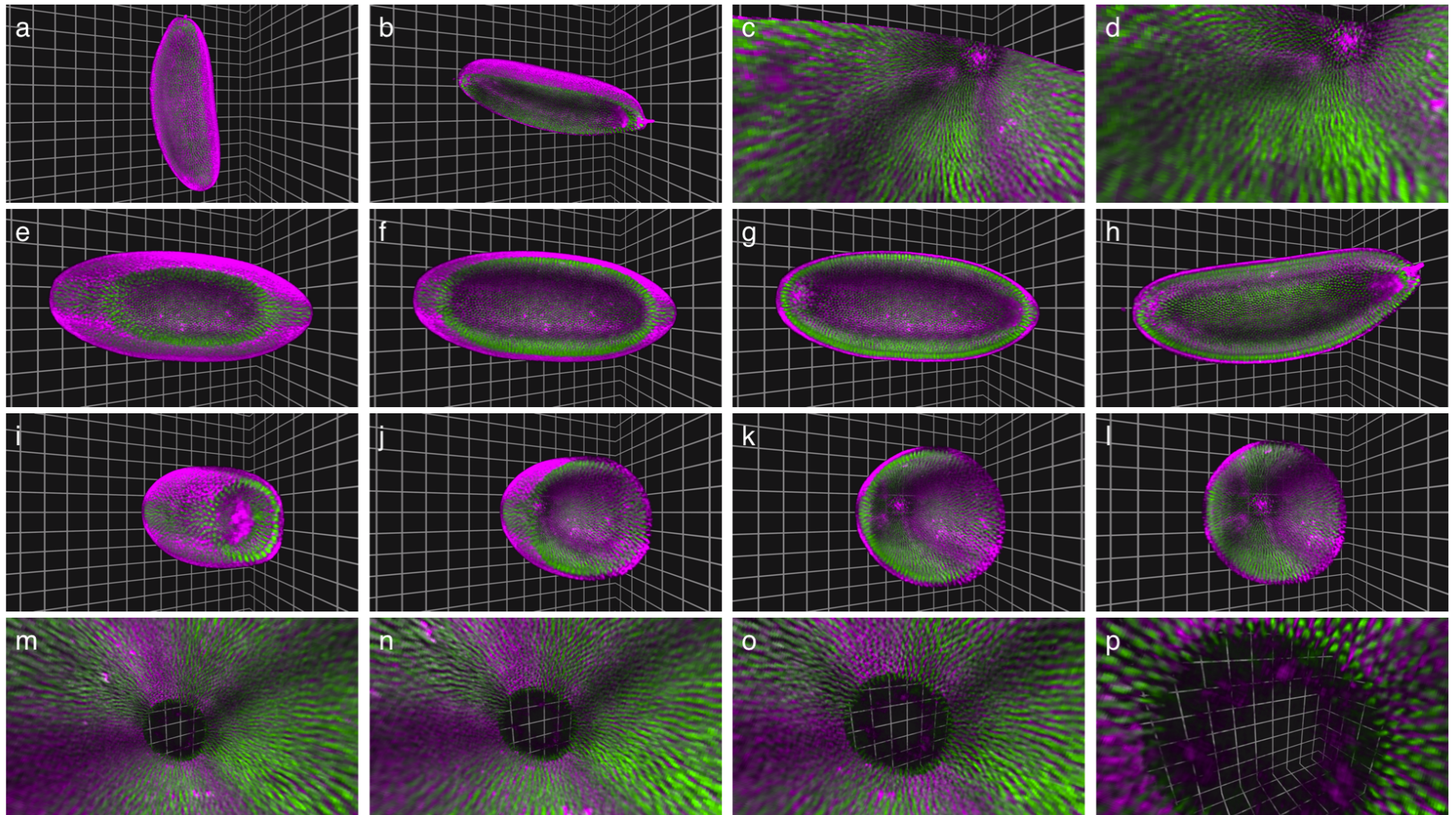


**Supplementary Figure 1** An example of FPBioimage rendering a Magnetic Resonance Imaging (MRI) scan of a human head, obtained from the Stanford Volume Archive [1]. Panels **a-c** show the head from different camera perspectives; **d-h** show the clipping plane progressing through the head to remove voxels; **i-l** show the head rendered with different levels of opacity; and **m-p** show a single cut, made using the clipping plane, observed from different rotations of the head.



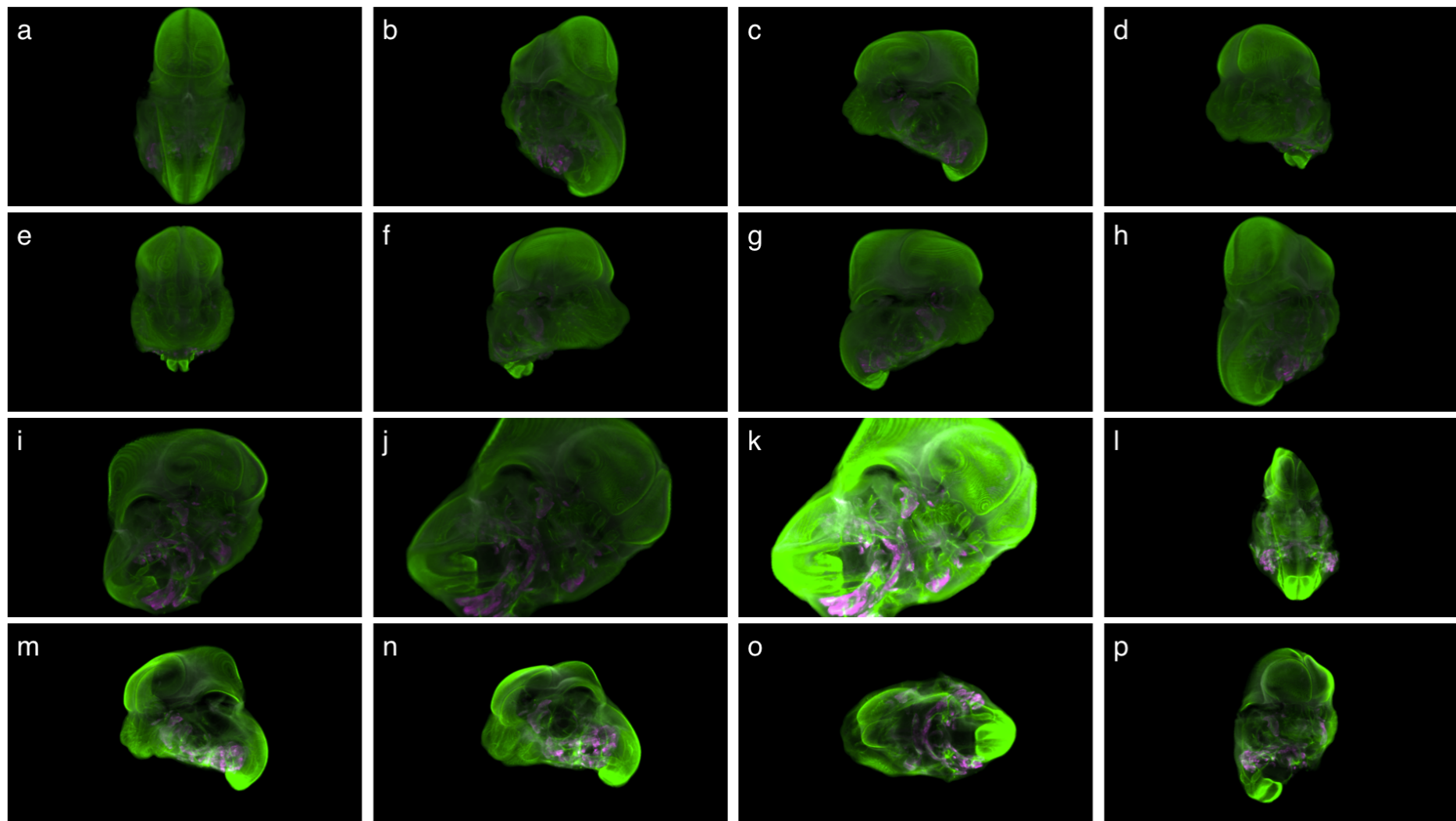
**Supplementary Figure 2** An example of FPBioimage rendering an X-ray Computed Tomography (CT) scan of a human head, obtained from the Stanford Volume Archive [1]. Panels **a-d** show the head at different rotations; **e-h** show the same rotations as a-d, but with a higher level of transparency; **i-l** show screenshots of the clipping plane progressing through the head; and **m-p** show the clipping plane at the same positions as i-l, but operating in slice mode, providing thin sections of the head.



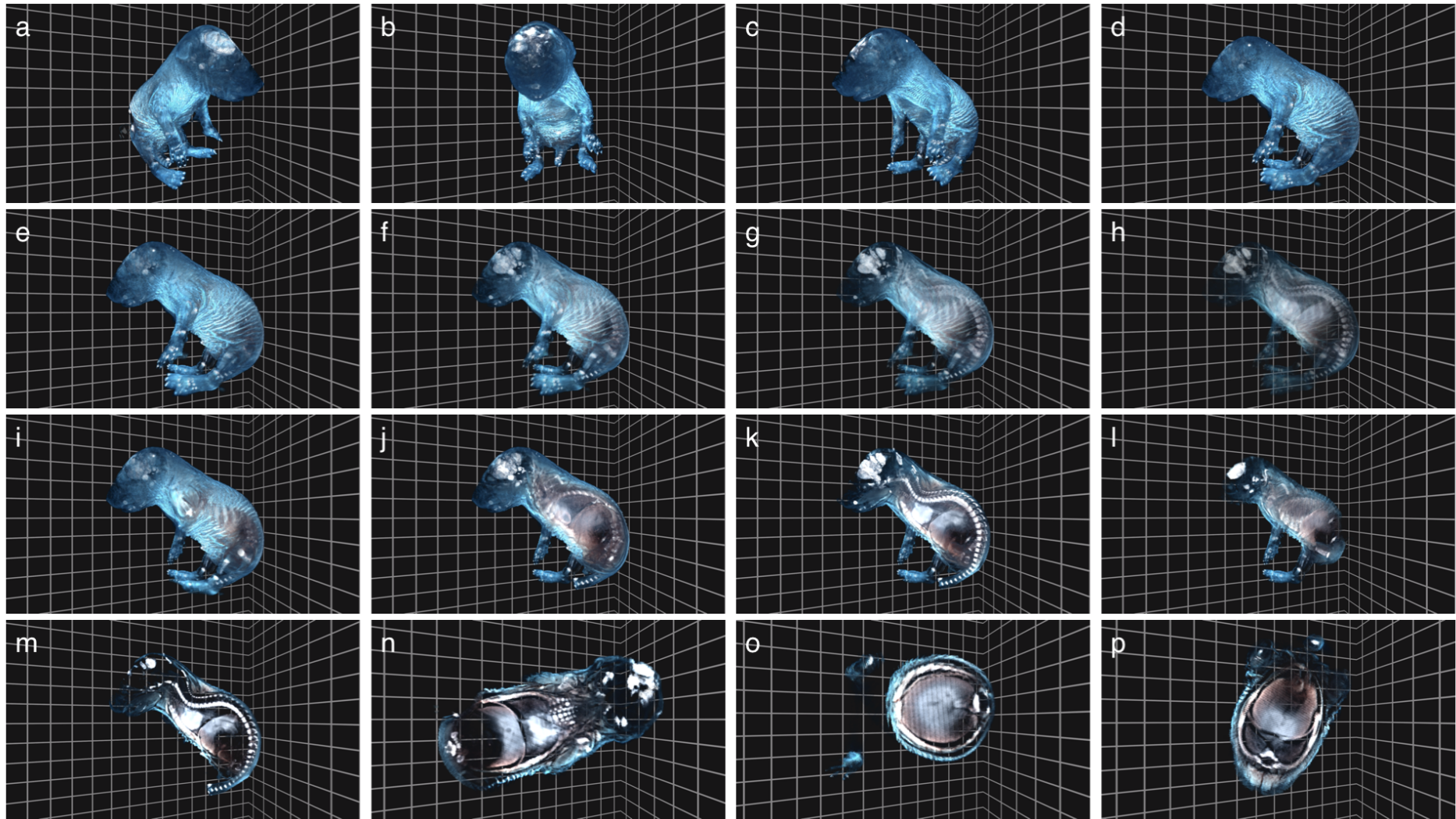


**Supplementary Figure 3** Light-sheet microscopy data of a blastoderm-stage drosophila embryo with the nucleus labelled in green and the membrane in magenta. Panel **a** shows the whole embryo; panels **b-d** show a cut made on the embryo using the clipping plane viewed from different camera perspectives. Panels **e-g** show the clipping plane cutting at progressively deeper planes into the 3D data, and panel **h** shows the embryo rotated through the clipping plane. Panels **i-l** show the clipping plane progressing into the embryo along the major axis, and panels **m-p** show the embryo with a cut viewed from the inside. Raw image data was kindly provided by Phillip Keller [2].

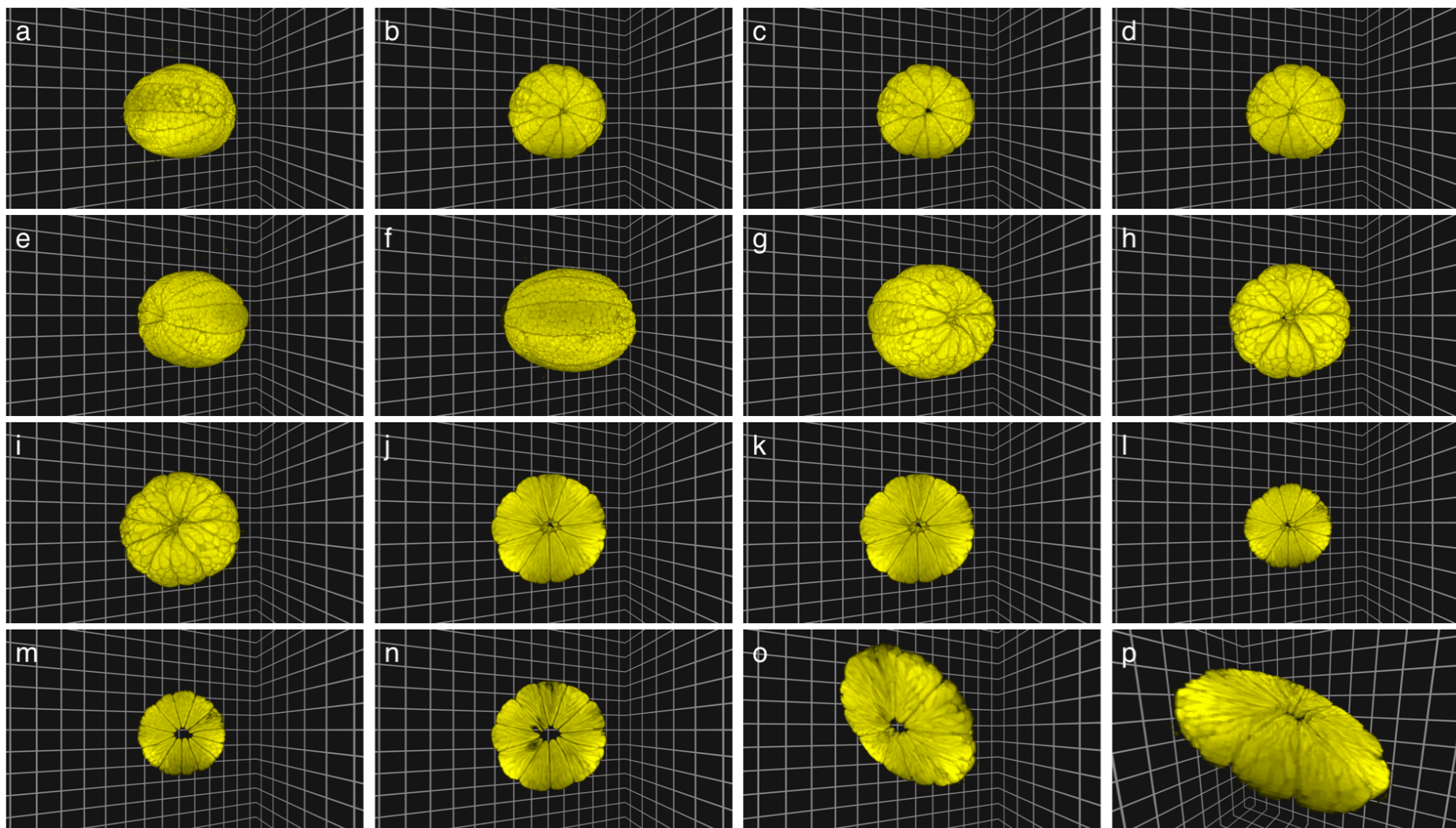




**Supplementary Figure 4** 'OPTiSPIM' data of a mouse embryo's head with muscles colored in magenta and neurons colored in green. Panels **a-h** show the data at 8 different rotations; **j** and **k** show the data from the same perspective, but with different levels for opacity and intensity; and panels **l-p** show the data with a cut, viewed from different perspectives. Note that in this figure the grid pattern has been hidden by pressing 'H' on the keyboard. Raw image data was kindly provided by Jim Swoger [3].

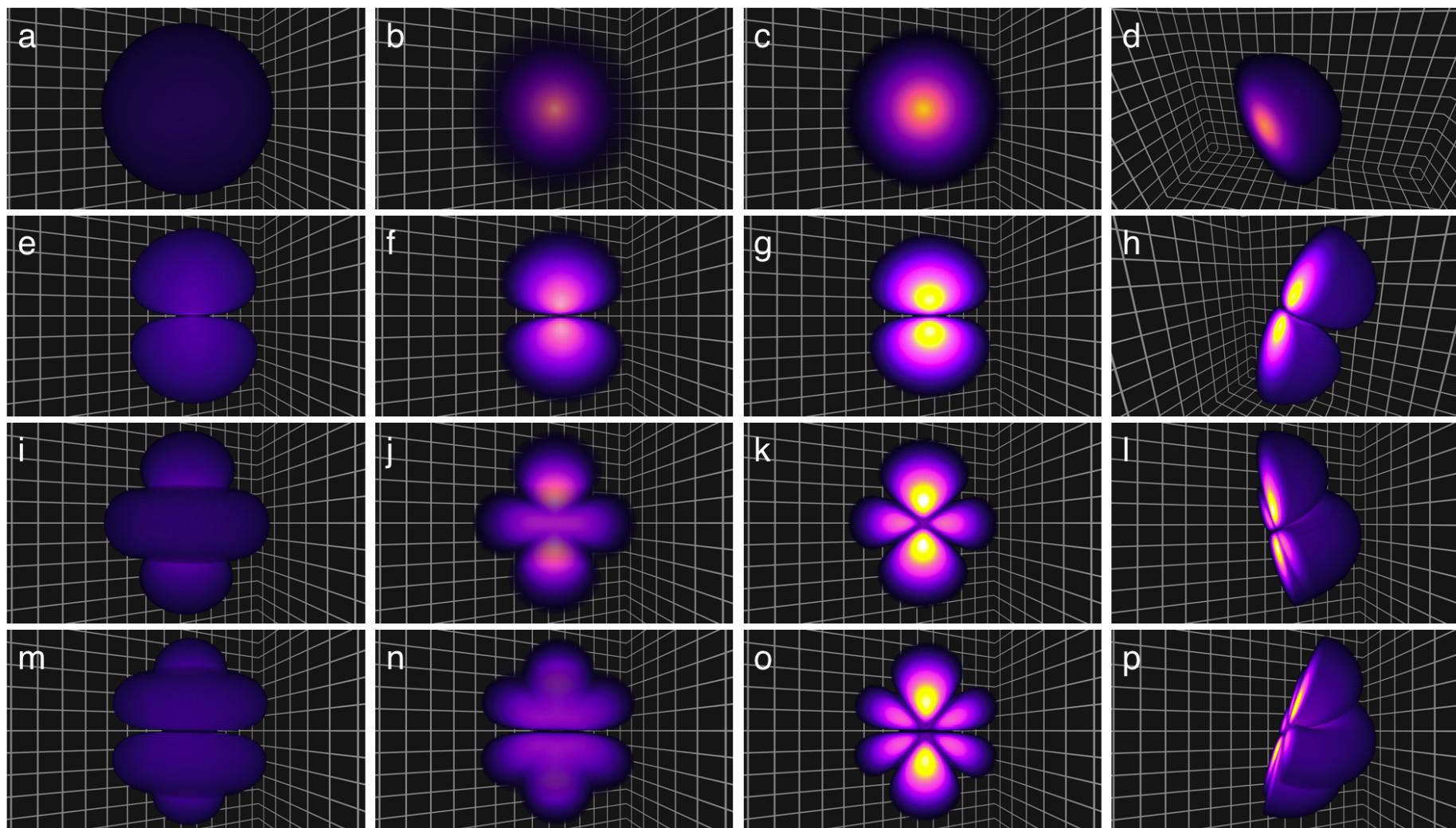


**Supplementary Figure 5** Optical Projection Tomography (OPT) data of a mouse embryo, kindly provided by James McGinty [4]. Panels **a-d** show the data viewed at different rotations; **e-h** show a single view rendered with different levels of opacity; **i-l** show the same view with the clipping plane used to cut away voxels; and **m-p** show various optical sections obtained by using the clipping plane in slice mode.

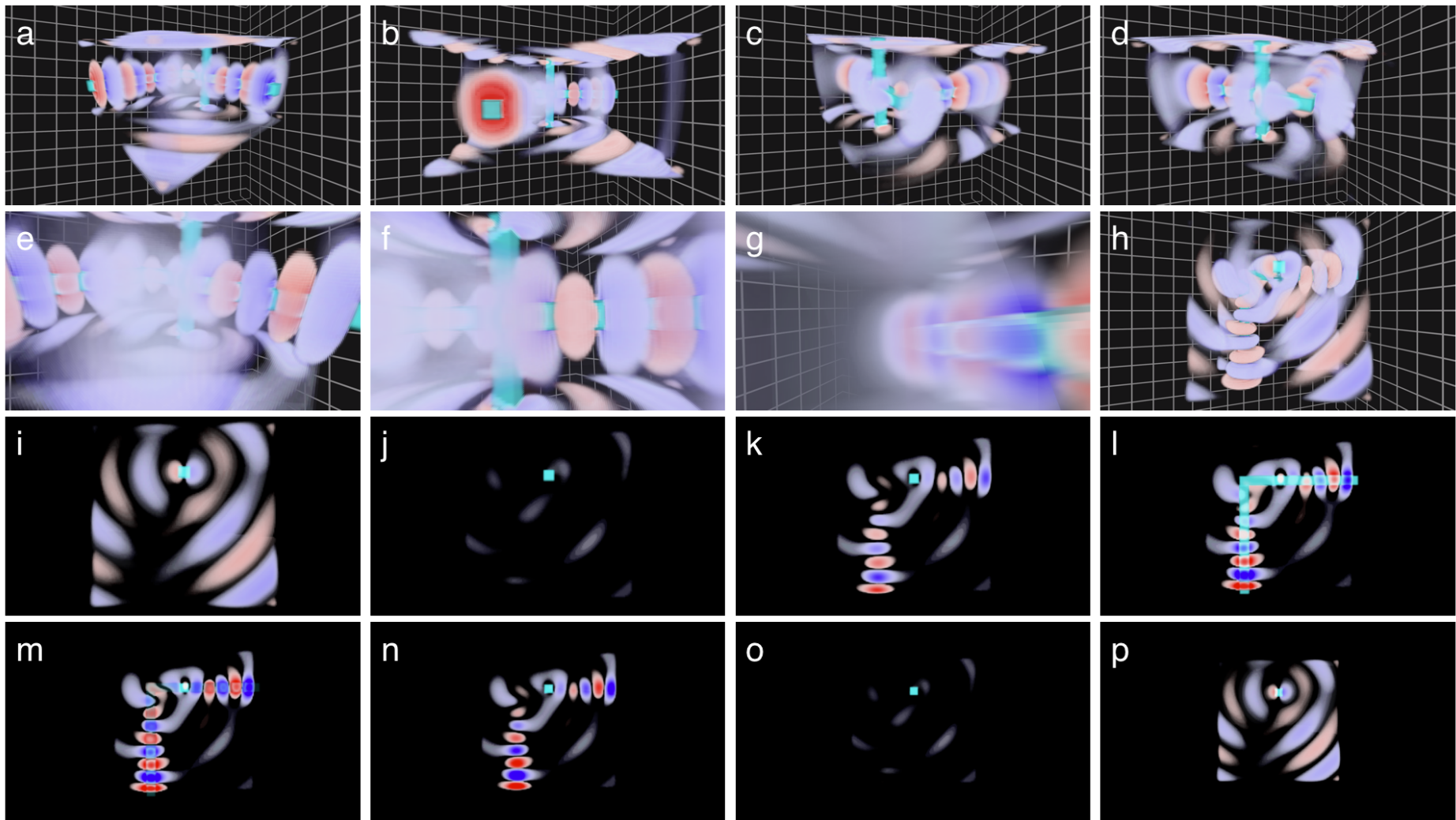


**Supplementary Figure 6** MRI data of a lemon. Panels **a-h** show the lemon at different rotations; **i-l** shows the lemon cut progressively deeper using the clipping plane; and **m-p** show the sectioning tool producing thin slices of lemon. Data captured by Adam Colbourne and kindly provided by Joe Cooper.

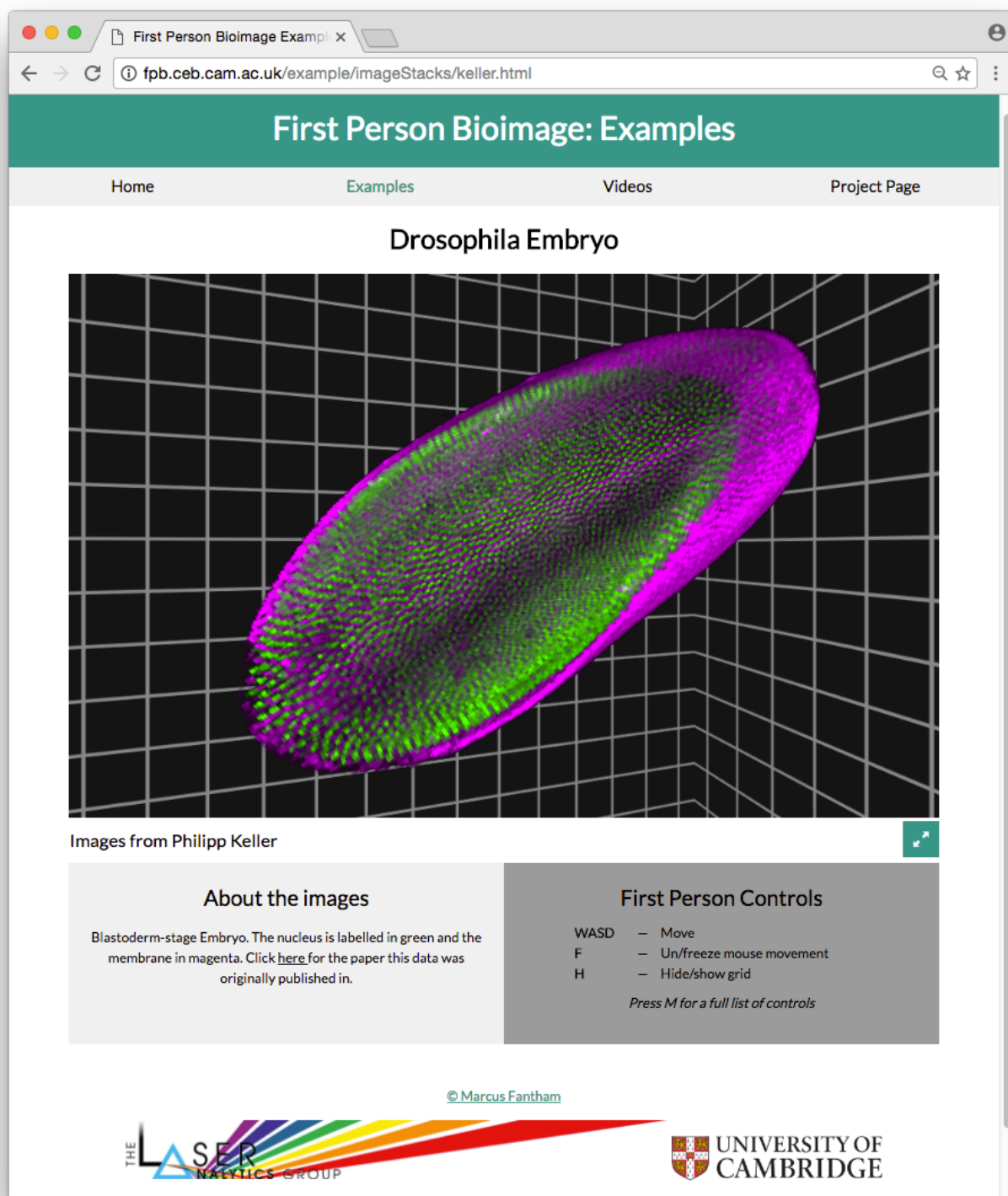




**Supplementary Figure 7:** Hydrogenic wavefunctions showing various electron orbitals of an atom. Panels **a-d** show the 1s-orbital, **e-h** the 2p-orbital, **i-l** the 3d-orbital and **m-p** the 4f-orbital. The first column shows a solid rendering of the orbitals; the second column applies transparency to the orbitals; the third column shows the orbitals with a clipping plane applied down the center; and the fourth column shows the orbitals with the same clipping plane viewed from a different perspective. Orbital data was generated in MATLAB.

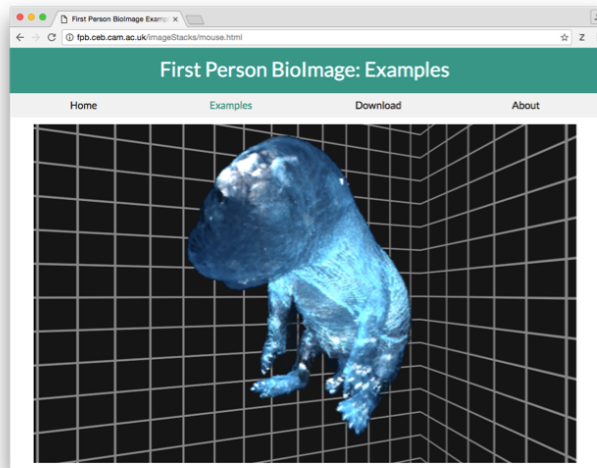


**Supplementary Figure 8:** A simulation of the electric field propagation of a point source through a three-dimensional waveguide. Panels **a-h** show the simulation volume from a variety of different perspectives, including from within the volume. Panels **i-p** show the waveguide from the same perspective as **h**, but with the clipping plane utilized in slice mode to visualize the electric field at various depths in the volume. The data was created using Meep [5], a freely available finite-difference time-domain (FDTD) software package.

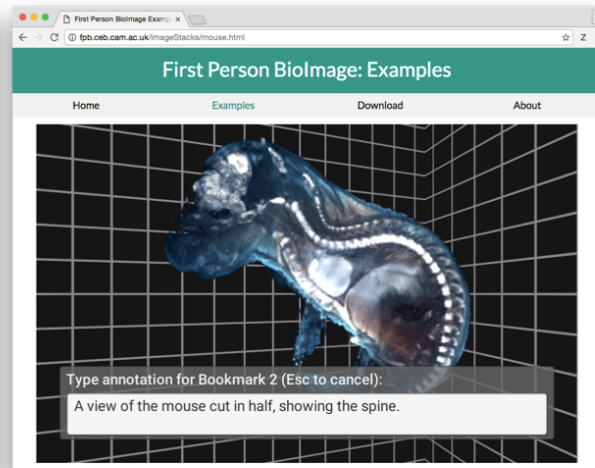


**Supplementary Figure 9** An example webpage demonstrating how FPBioimage can be integrated into a website for global sharing of volumetric imaging data [2]. See <http://fpb.ceb.cam.ac.uk/example/> for a live view of the example website.

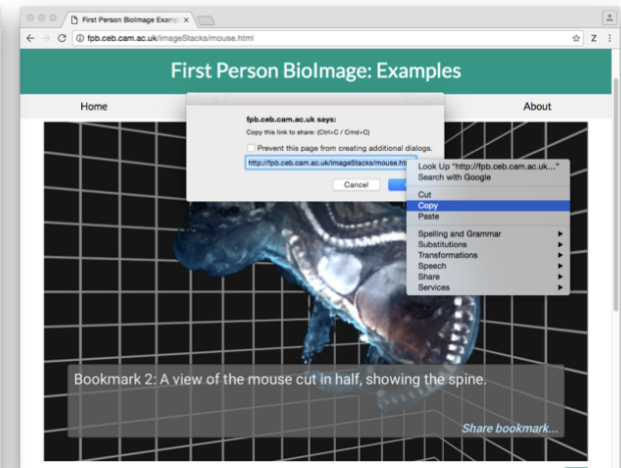




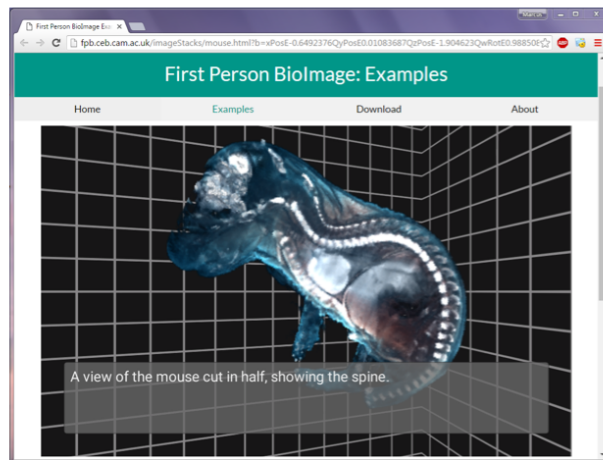
a



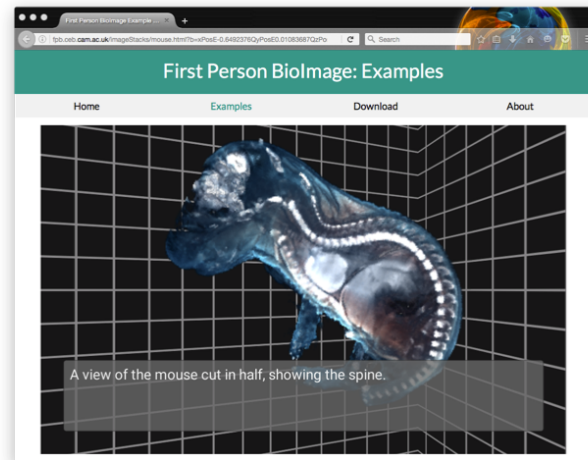
b



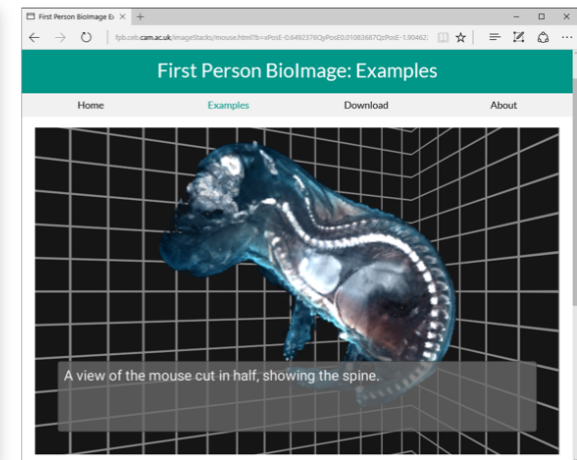
c



d



e



f

**Supplementary Figure 10** Panel **a** shows OPT data [4] displayed in the web browser; **b** shows the creation of a bookmark; and **c** shows how to create a link to share this bookmark with other researchers. Panels **d-f** show that this link can be opened to view the bookmarked data in any modern web browser, including in Google Chrome on Windows 7, Firefox on OSX, and Microsoft Edge on Windows 10 respectively.

## References

- [1] Levoy, M. "Display of surfaces from volume data." *IEEE Comput. Graph. Appl.* **8(3)**, 29-37 (1988).
- [2] Tomer, R., Khairy, K., Amat, F., & Keller, P. J. "Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy." *Nat. Methods*, **9(7)**, 755-763 (2012).
- [3] Mayer, J., Robert-Moreno, A., Danuser, R., Stein, J. V., Sharpe, J., & Swoger, J. "OPTiSPIM: integrating optical projection tomography in light sheet microscopy extends specimen characterization to nonfluorescent contrasts." *Opt. Lett.*, **39(4)**, 1053-1056 (2014).
- [4] Sharpe, James, Ulf Ahlgren, Paul Perry, Bill Hill, Allyson Ross, Jacob Hecksher-Sørensen, Richard Baldock, & Duncan Davidson. "Optical projection tomography as a tool for 3D microscopy and gene expression studies." *Science*, **296(5567)**, 541-545 (2002).
- [5] Ardavan F. Oskooi, David Roundy, Mihai Ibanescu, Peter Bermel, J. D. Joannopoulos, and Steven G. Johnson. "MEEP: A flexible free-software package for electromagnetic simulations by the FDTD method." *Computer Physics Communications* **181**, 687–702 (2010).

# First Person Bioimage: User Guide

## Introduction

This guide provides instructions on the use of FPBioimage to view and explore volumetric image data shared on the web. The guide is available online at <http://fpb.ceb.cam.ac.uk/userguide/> and is accompanied by an instructional video. If you need instructions on how to share your own imaging data, please see the **Sharing Guide**.

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## Overview of Controls

Control	Action
Click-and-drag	Rotate model
Arrow keys	Rotate model
Scroll wheel	Zoom
<i>Camera Movement</i>	
W,S	Forward, back
A, D	Left, right
E, Q	Up, down
F	Toggle <i>First Person</i> mode
Mouse	Camera direction (in <i>First Person</i> mode)
<i>Rendering Settings</i>	
H	Hide boundaries
<, >	Decrease/increase opacity
-, +	Decrease/increase intensity
{, }	Decrease/increase intensity cutoff
<i>Bookmarking</i>	
B	Create a bookmark
Number keys	Restore bookmark
<i>Clipping and Slicing</i>	
I, K	Move forward/backward ( <i>clipping plane</i> )
J, L	Rotate left/right ( <i>clipping plane</i> )
O, U	Tilt forward/backward ( <i>clipping plane</i> )
X	Freeze plane on model
Z	Reset plane
N	Toggle clip/slice mode



## Basic Controls

*These controls should be familiar to anyone who has used 3D software before, and intuitive to those new to 3D viewers.*

### Rotate model

The model can be rotated by click-and-drag operation of the mouse. Alternatively, the arrow keys will rotate the model about its centre.

### Zoom

The camera can be zoomed in and out of the model using the mouse scroll wheel. Alternatively, the W and S keys can be used if the mouse has no scroll wheel.

## Camera Movement

*To view the data from different perspectives, the position of the camera can be moved.*

### Forward/backward, left/right

The WASD keys are used to move the camera around the model, as they form the same shape as arrow keys. This key combination is commonly used in first-person perspective 3D games, and will be familiar to many.

### Up/down

The camera can be moved up and down using E and Q respectively.

### First Person mode

Pressing F toggles *First Person* mode. When enabled, the mouse is used to change the direction in which the camera is pointing. Again, this interface will be familiar to those who have played first-person perspective games, and provides an immersive view of 3D imaging data.

## Rendering Settings

*The rendering settings can be adjusted to ensure that different details in the data can be visualised optimally.*

### Hide boundaries

The boundaries provide a sense of perspective and also prevent the camera from getting 'lost' in the emptiness of 3D space. Their visibility can be switched off with H, which will show the image data against a black background.

### Opacity

The opacity of the data can be decreased and increased with the < and > keys respectively. Lowering the opacity allows voxels inside the volume to be viewed through the outer layers.

### Intensity

The intensity or brightness of the data can be increased or decreased with the + and – keys respectively.

### Cutoff threshold

In order to render the volume, the darkest voxels are not displayed, as determined by the cutoff threshold. If there is a dark haze around the model, the cutoff threshold should be increased with }. If there is detail missing in the model, however, the threshold can be lowered with {.

## Bookmarking

*The bookmarking feature built into FPBioimage allows the user to save views locally to return to at a later time. Bookmarks can also be shared with others to provide particular views of the data.*

### Creating a bookmark

The bookmark creation procedure is initialised by pressing B. This will prompt the user to choose a number key to save the bookmark under. Next, an annotation can be entered to describe the view. On pressing enter, the bookmark will be saved. Saving a bookmark under number 0 means that this view will be loaded next time the data is loaded.

### Restoring a bookmark

Pressing one of the number keys at any time will restore the bookmark saved under that number. Note that bookmarks are saved onto the user's computer as a browser cookie, and so can be restored between sessions.

### Sharing a bookmark

After creating or restoring a bookmark it can be shared by clicking the 'Share bookmark...' button. This will pop up a box with a link to the bookmark. This link can then be shared in an email, Twitter post or academic journal. The link contains all information about the bookmarked view, including the annotation, so can be long; users may wish to consider using a URL shortener such as [goo.gl](https://goo.gl) or [tinyurl.com](https://tinyurl.com) when sharing links.

## Clipping and Slicing

*The clipping and slicing feature provided by FPBioimage can provide unique views of the data, providing novel information which cannot be obtained from the raw image slices. The data can be sliced from any direction, and the cut locked in position on the model to view it from any perspective.*

### Moving the clipping plane

The clipping plane starts a short distance in front of the camera, and voxels between the camera and clipping plane are removed. As the camera approaches the model, deeper layers inside the model become exposed. The clipping plane can be moved further from or closer to the camera with I and K. It can also be rotated left and right with J and L, or tilted forward and backward with O and U respectively. Note that the IJKL keys are chosen to mimic the layout of arrow keys.

### Freezing the clipping plane

The clipping plane starts attached to the camera. This means that as the camera moves, the position of the clipping plane changes with it. The clipping plane can be locked to the model by pressing X. Pressing the Z key resets the clipping plane so that it is re-attached to the camera.

### Slice mode

The N key toggles between clipping mode and slicing mode. In clipping mode, voxels between the camera and the clipping plane are not rendered. In slice mode, just a thin slice around the slicing plane is shown.

## Software and Hardware Requirements

64-bit operating system (eg. Windows 7 64-bit, Windows 10 64-bit, Mac OS X 10.7+)

64-bit web browser (eg. Google Chrome 64-bit, Microsoft Edge, Safari, Firefox 64-bit)

2GB+ RAM (depending on the complexity of the dataset)

# First Person Bioimage: Sharing Guide

## Introduction

This guide provides instruction on sharing volumetric image data using the FPBioimage. This sharing guide is available online at <http://fpb.ceb.cam.ac.uk/sharingguide/> and is accompanied by an instructional video.

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## Installing the viewer on your web server

1. Download the software from <http://fpb.ceb.cam.ac.uk/downloads/>
2. Unzip the contents of the .zip file
3. Upload the folder 'FPBioimage' to your web server using your ftp client.

## Preparing images for the viewer

Images must be saved as a stack of PNG files.

The maximum resolution supported by FPBioimage is 500x500 pixels, and 500 images.

The smaller the image size, the shorter the loading time will be for those viewing your data.

To prepare volumetric imaging data for the viewer we recommend the use of [ImageJ](#), an open-source imaging processing package. Whilst these instructions explain how to prepare your images using ImageJ, any image processing software which will prepare images to the specification above can be used.

The *Scale* function in ImageJ can be used to adjust the image stack to an appropriate size.

Before saving in PNG format, the data must be rendered as an RGB image. Do this by selecting Image ► Color ► Stack to RGB. Uncheck "Keep Source", and click OK. The stack can then be saved as PNG slices using File ► Save As ► Image Sequence, and selecting PNG as the Format.



## Creating a simple webpage for the viewer

FPBioimage runs on a webpage. In order to display volumetric data correctly the webpage requires some information about the data, for example the location of the images and the voxel size.

### Modifying the provided website template

The simplest way to display your 3D data online is to modify the provided website template, which is included in the software package (Supplementary Information 3). You are free to modify and embellish the template webpage as you see fit for your website.

## Advanced webpage creation

### Creating your own webpage

To create your own FPBioimage webpage from scratch requires the definition of several Javascript variables, a Javascript file to be called, and a WebGL canvas which needs to be included in the body of the webpage.

The following Javascript variables need to be defined in the header of the webpage (i.e. between the <head> and </head> tags):

Variable Name	Type	Example	Description
pathToImages	string	imageStacks/lemon	Path to the image stack folder relative to the folder containing the FPBioimage folder.
uniqueName	string	Lemon	Defines a unique name. If two data sets share the same name on your website the bookmark behaviour may be unexpected.
numberOfImages	int	269	The number of images in the image stack.
imagePrefix	string	lemon_z	The images' file names before the incrementing number.
numberingFormat	string	0000	Defines the format of the incrementing number in the images' file names.
voxelSize	int[3]	[1,1,1]	The ratio of x:y:z voxel size.
pathToFPBioimage	string	../FPBioimage	Relative path from the webpage to the FPBioimage folder.

The FPBioimage viewer should be included in the body of the webpage using the following HTML snippet:

```
<canvas class="emscripten" id="canvas"
oncontextmenu="event.preventDefault()" height="600px"
width="960px"></canvas>
```

The height and width can be changed as required.

The last thing to be defined in the body of the webpage should be the code required to load FPBioimage into the viewer. This HTML snippet should come after all page elements, since it can take a few seconds to load and will halt loading of the rest of the webpage for this time. The required HTML is as follows:

```
<div id="scriptLoader"> </div>  
<script src="../../FPBioimage/FPBioimageLoader.js"></script>
```

Note that if you want to preview the webpage offline on your local computer (i.e. not on a web server) you must set up a [localhost server](#) for FPBioimage to work correctly.

## Further assistance

We are happy to provide further assistance to help you share your 3D data online. Email the developer at [fpBioimage@gmail.com](mailto:fpBioimage@gmail.com).

## **Software Developer Notes**

Use of FPBioimage requires a citation of the original publication of the software in Nature Photonics. If FPBioimage is used to share data published in an academic journal, the original publication must be acknowledged in the references section of the publication.

First Person Bioimage (FPBioimage) is distributed under the [Creative Commons Attribution-ShareAlike 4.0 International license](#). This means that, providing you credit the original software appropriately, you are free to copy, redistribute, remix, transform and build upon the software, as long as future contributions are distributed under the same license.

To allow you to make modifications to the software, it is released open-source on a github repository, at <https://github.com/fpbioimage/assets>.

FPBioimage has been developed using the Unity Game Engine, a 3D development platform designed for creation of computer games. If you wish to develop FPBioimage further, you will require a copy of Unity. A free version is available from <https://unity3d.com/>, although if you wish to redistribute FPBioimage without the Unity splash screen you will require a professional license.

When you have downloaded or cloned the git repository, import FPBioimage into your Unity project by clicking Assets > Import Package > Custom Package... and selecting the "FPBioimage.unpackage" file.

For further information, contact the developer at [fpbioimage@gmail.com](mailto:fpbioimage@gmail.com).