From Microscope to Movies: 3D animations for teaching physiology

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INTRODUCTION

Recent literature [1] and the personal experience of the authors suggests that students today are relying less on printed textbooks and more on Google, Wikipedia and You-Tube. Students expect to be able to access educational content immediately and prefer online access. In addition, the CEO of John Smith (University Bookseller) has warned that "...the traditional bookstore model is not sustainable."

The detail of this general theme, and the move towards eBooks, is not the subject of this article but it sets the scene. If we accept that students have a desire for something more than the printed page then, as educators, we need to adapt. You-Tube is a fantastic resource for life sciences-related animations. A recent search for physiology animations returned almost 6000 movies. Whilst this represents a substantial educational resource, most of the animations have been created by artists and are not anatomically accurate at the (cellular) level of light microscope resolution (0.3 -1 µm).

A further internet search for the keyword 'medical animation company' returns several results which indicate that there is a growing market for animations of this kind. Looking at the professional 'showreels' it is clear that these high-end animation studios are capable of producing stunning, cinema-quality animations. Great care and attention is taken over the final rendering and the physics underlying the realistic movements are constantly improving. The animations generally take the user from the gross anatomical level down to the molecular level. The only criticism you could offer, for many of the commercially produced movies, is that at the cellular level the anatomy is not as accurate as it could be. As microscopists, we have an opportunity to fill this gap.

In this article we will describe a data flow we recently developed that enables us to create anatomically accurate 3D animations from microscope-based data. We will use vascular structure as the example in this article.

Figure 1 shows a conventional histological section of an artery. The staining shows the structure of the vascular wall and the image is typical of the type students are shown in undergraduate classes. From the 2D image we then instruct the students on the biology of the vascular wall and the interactions that occur between different cell types, autonomic nerves and the physical forces of blood flow and pressure.

We believe that complimenting the 2D histology with 3D animations will greatly enhance learning. We have previously shown that the resistance vasculature (small arteries that control blood pressure) is particularly well suited to study by fluorescence and confocal microscopy [2, 3]. This is due to the optical properties of the vessel wall and its thickness (60-100 µm, Figure 1) which permits good penetration of the confocal lasers. Until relatively recently, the fat surrounding virtually all blood vessels was not recognised as having a significant role in vascular biology. However, perivascular adipose tissue (PVAT) and its autonomic innervation represent one of the hottest topics in current vascular research [4]. Therefore, we will use the example of PVAT, autonomic nerves and resistance arteries to illustrate our method.

CONFOCAL (3D) MICROSCOPY

The first commercially available confocal laser scanning microscopes (CLSMs) emerged in the late 1980s and early 1990s, the first being the BioRad MRC 500 developed by Amos and White [5]. Our first microscope (purchased in 1993) was a NORAN Odyssey slit scanning confocal and provided us with the ability to study the 3D structure of the vascular wall [3, 6]. However, in the early 1990s computer architecture and software lagged well behind confocal microscopy and severely limited its impact due to the extremely high cost of the Silicon Graphics (SGI) workstations required to run such packages as VoxelView and IMARIS. Confocal data sets were stored on multiple floppy disks and the deconvolution and volumetric rendering process was measured in hours.

Moving forward 20 years we find that confocal microscopes have developed only a little (better lasers, better detectors, etc.). In contrast, computer hardware and software has undergone significant developments and can now fully exploit the CLSM-derived large datasets. Large-scale 3D image volumes can be manipulated with ease and 4D

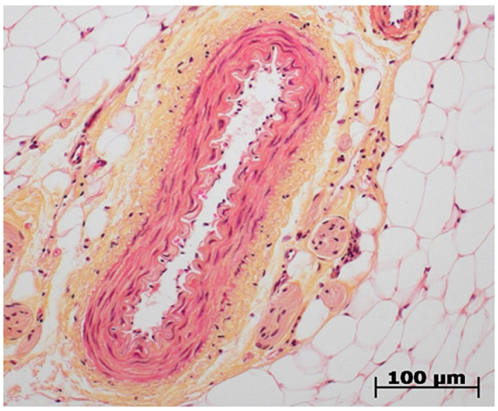

FIGURE 1
Mesenteric artery stained with haematoxylin (nuclei), eosin (cytoplasm), phloxine (red blood cells) and tartrazine (connective tissue) (HEPT). Adipocytes are arranged around the periphery of the vessel. The adventitial connective tissue shows up as a yellowish band around the vessel. The smooth muscle of the tunica media stains reddish-pink and appears to be 3-4 layers thick.

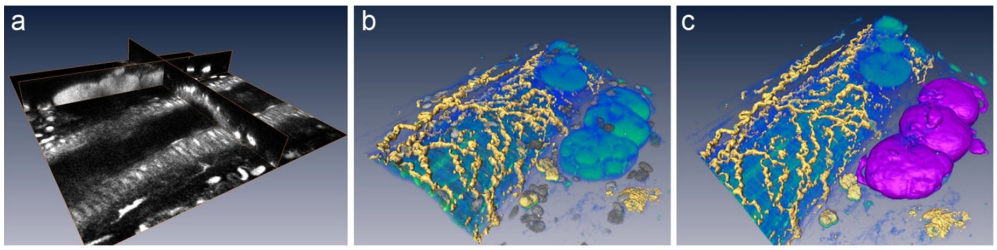

FIGURE 2
Standard data visualisation with AMIRA. (a) Orthogonal viewing of a small artery. The x-y plane shows the vascular wall in cross section; the dark area between the two walls is the lumen. (b) The data in (a) visualised as a volumetric rendering (voltex). Large adipocytes are shown in blue on the periphery of the vascular wall. The nerves in yellow are shown as an iso-surface. (c) Three of the adipocytes have been segmented and displayed as an iso-surface (magenta).

imaging (3D timelapse) is common. We believe the next stage is a creative one which is facilitated by the use of highly sophisticated animation software.

Normally, we would use relatively high power (x40-x60) high numerical aperture (NA 1.0-1.4) objectives for confocal work. This gives optimal resolution and good optical sectioning. If we follow the rules of good practice we would also oversample along the z-axis and collect serial sections at 0.15-0.3 µm spacing. This could produce an ideal image volume which could be deconvolved to produce an impressive rendering for rotation purposes.

However, the resulting data sets will be much larger than the animation software expects to work with. Therefore, we need to also collect z-series at lower magnifications and lower resolution. These low power scans give us the general shape of the tissue and structures which are used in some animation scenes where a high degree of detail is not required. Therefore, the building blocks of the animation are a collection of low- and high-magnification data sets which will become scenes in the movie. Low-resolution models are used for rotation and fly past and high-resolution models are used for mechanism of action (MoA) scenes. In some cases we may also duplicate structures to create a hypothetical region of the tissue which was not possible to scan.

CURRENT CONVENTIONS FOR DISPLAYING 3D VOLUMES OF CONFOCAL DATA

A number of free and commercially available products are available for reconstructing a confocal z-series. At the top end of the market are products like FEI-VSG-AMIRA (Figure 2) and Bitplane-IMARIS. Whilst both of these packages offer flexible visualisation options for confocal data (i.e. orthogonal viewing (Figure 2a), volume rendering (Figure 2b), surface generation (Figure 2c), etc., their animation capabilities are generally confined to zoom, pan, rotate and (limited) flythrough. For data visualisation purposes that is generally all that is required. However, if the generated surface meshes can be exported to a sophisticated animation package the possibilities are endless.

MOVIE MAKING

Wikipedia currently lists 30 different 3D animation packages. In this article we shall focus only on Autodesk Maya and Autodesk 3D Studio Max (3DSM). Both are available free as an 'education license' and offer a huge range of animation options. The data flow from microscope to MAYA is multistep process and at each step important aspects should be considered. Once you have collected a multichannel CLSM data set it can be saved in one of many formats; we find TIFF series to be particularly portable. Often we will run a data set through ImageJ for standard processing needs.

Our 3D visualisation platform of choice is AMIRA. We have found that the segmentation tools and iso-surface generation modules are particularly well suited to CLSM-derived data. Once suitable segmentation labels have been defined, a surface can be extracted and viewed. At this stage it is important to examine the number of geometric faces present within a surface. AMIRA tends to create very detailed surfaces often with 300,000, or more, geometric faces. These need to be reduced using the Simplifier module. The aim is to find the minimum number of faces that can accurately describe the structure (Figures 3a, 3b). Data structures are exported from AMIRA in .obj format which can be read by the animation packages (Figures 3c, 3d). A number of file formats can be used to import into MAYA or 3DSM, however we find the .obj to be the most reliable. Once the .obj file (mesh) is imported into the animation software it can be smoothed, manipulated, textured and lit as required (Figure 3d).

Once the structures are suitably textured, lit and animated the scene is rendered in a suitable production quality which can be anything from You-Tube (low resolution) to IMAX. At this point more realistic textures and materials can be used to enhance the scene (Figure 4). This can be a hugely time-consuming process measured in hours or days depending on the number of frames required and the rendering resolution. The individual frames must then be combined to make a movie file. Narration, soundtrack and text can be added using video compositing software such as Adobe After Effects.

The final movies that we produce are a combination of scenes depicting accurate anatomical structures with additional animated layers and objects (Figure 5). This lengthy movie making process prompts the question: "Is it worth it from an educational viewpoint and will undergraduate students benefit?"

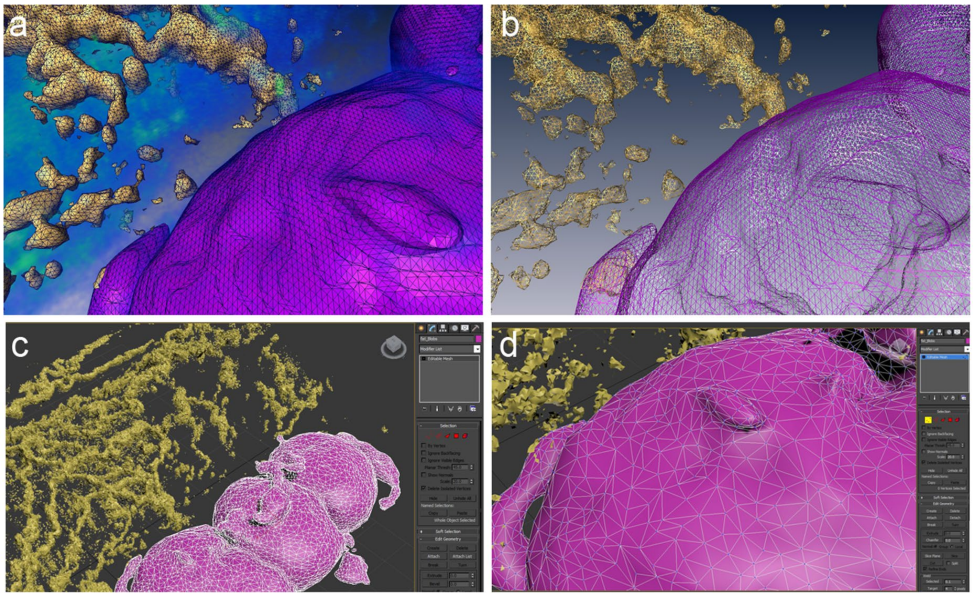

FIGURE 3
Data transfer from AMIRA to 3DS Max.
(a, b) Iso-surfaces are generated and displayed as wireframe structures comprising vertices and (triangular) faces.
(c) The wireframe mesh is imported into the animation software (3DS Max) in an .obj format.
(d) The mesh can be simplified, smoothed and manipulated for ease of animation and CPU processing.

EDUCATIONAL BENEFITS

The usefulness of animation in teaching is considered by Reed [7] who suggests that animations should "correspond to the desired structure" and should allow "close-ups, zooming, alternative perspectives and control of speed." 3D visualisation techniques and 3D animations are now commonplace in modern culture (i.e. films and special effects, video games, virtual reality). However, the use of interactive 3D technology has not been fully exploited for teaching life sciences.

Students often learn individual facts (elements) without necessarily connecting the elements to see the 'bigger picture'. High-element interactivity can be difficult to understand [8] and often difficult to teach. The 'elements' themselves can be complex and involve verbal or pictorial forms. This requires a recognition of different learning styles (visual, auditory, read-write and kinesthetic) when designing new instructional/teaching materials. In turn, this highlights the need for good 'instructional design' when considering the use of multimedia for teaching and learning.

Mayer's Multimedia Theory presents seven principles for the design of multimedia for instruction purposes [9]. A key principle is that narration is more effective than text within an animation and that narration with text can cause redundancy (i.e. reduced learning through increased cognitive load). Interestingly, Mayer proposes that

design effects will be more beneficial to low-knowledge, high-spatial learners. Overall, Mayer shows that cognitive load must be considered when designing multimedia [9].

Cognitive Load Theory (CLT) provides us with a framework in which we can describe, and understand, the process of learning [8]. A complicated concept will have intrinsic, extraneous and germane cognitive loads. The three cognitive loads are additive and can exceed cognitive ability (working memory) so care must be taken when designing animations or learning may be inhibited. A further consideration is an individual's ability to comprehend 3D space. This ability can be measured using a Purdue Spatial Visualisation Test (PSVT) with rotation [10]. Therefore, 3D animations should be used with caution as they could reinforce misconceptions if the scene is not accurate or is cognitively overloaded. However, anatomically accurate animation with the optimum cognitive load could be of benefit in teaching difficult concepts.

DISCUSSION

We have defined a relatively simple, but multistep, process for generating 3D movies from CLSM data. Mass media storage has enabled today's CLSM users to hoard terabytes of data which may never be used for research purposes. Perhaps some of those archived data could be used for teaching or public engagement through movie making. Some of the software packages we have cited have steep learning curves and it could be argued that this does not contribute to the science. However, we believe that in today's competitive research environment, funding bodies may look more favourably upon those groups that make an effort to engage students and the general public. We would encourage all 3D microscopists to look beyond the 'tilt and turn' 3D animation as an endpoint and start making real movies.

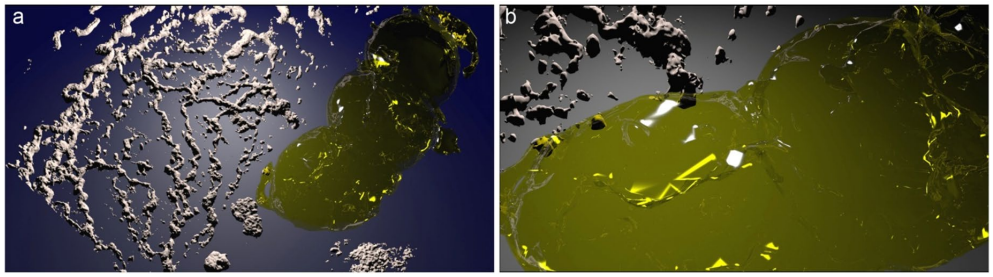

FIGURE 4
(a, b) The data in Figure 3 have been rendered at high resolution (HDTV) and the fat cells have been given a more realistic look using glassy materials and lighting.

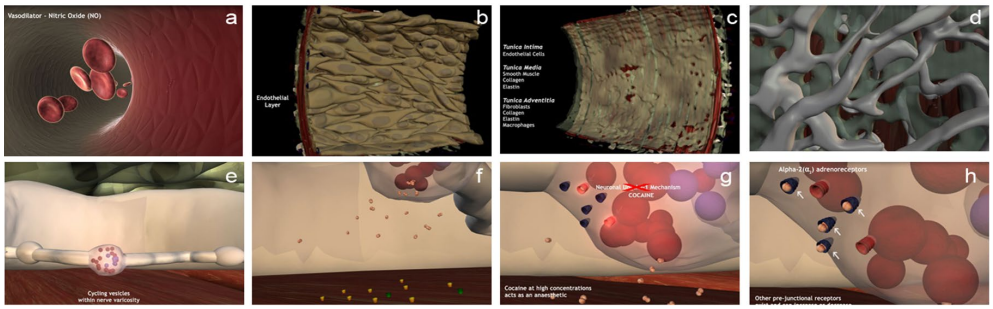

FIGURE 5
Screenshots from an animation describing the mechanism of action of sympathetic neurotransmission within the vascular wall. The red blood cells (a) in the first image were reconstructed from a 3D electron micrograph series. The top row images (b-d) of the wall are all derived directly from CLSM data. The lower panels (e-h) show additional animation layers of neurotransmitters and vesicle that have been added into to the CLSM-defined structures. The full movie can be viewed on YouTube via www.cardiovascular.org (news page).

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BIOGRAPHY

Craig Daly has a Masters and PhD from Glasgow University and is employed as a Research Fellow within the School of Life Sciences. Craig's main research interest is the autonomic control of the vasculature and more recently the influence of perivascular fat. Recent career developments have led to a more teaching-oriented role and so finding ways to combine research and teaching has become a major focus.

ABSTRACT

Confocal laser scanning microscopy (CLSM) provides highly detailed 3D structure of biological tissues at cellular and sub-cellular level. In this article we present a data flow which enables the import of CLSM data to sophisticated animation software, thus enabling the creation of anatomically accurate movies for educational and public engagement purposes. This requires consideration of multimedia and cognitive load theories.

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