From Confocal Microscope to Virtual Reality and Computer Games; technical and educational considerations

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Abstract

Will it ever be possible to visualise a microscope-mounted live cell in virtual reality in real time? It is possible, and we may be closer than many think. The article outlines the workflow involved in taking confocal laser scanning microscopy (CLSM) data through to fully immersive virtual reality (VR) and interactive games. For the past 30 years, the size of standard CLSM data sets has generally exceeded the capabilities of standard desktop PCs and thus hindered the ability to process them in creative ways. Modern gaming PCs, high end graphics cards and VR headsets have changed all that. A moderately priced desktop PC can now extend the value of a CLSM data set through the use of 3D animation software and game engines. There are considerable technical processes that CLSM datasets need to pass through as there is no single piece of software that will cover the entire sequence. However, most of the required software components are freely available and this article serves as an introduction to these. Finally, the use of these new multimedia technologies is considered in an educational context. These are exciting technological times for data visualisation, and microscope users should be thinking about how to exploit their own archive of 3D image data.

Introduction

Confocal laser scanning microscopes (CLSMs) have been commercially available for over 30 years. Even the earliest of these instruments, of the mid-80s (eg BioRad MRC 500), were capable of collecting a perfectly aligned (z-axis) series of 2D images that would present significant 3D processing problems to the desktop computers of the time. Our own system, an Odyssey slit-scanning CLSM of the early 90s, could easily produce 40 x (512x512 pixel) 256k images of the vascular wall (Arribas et al., 1994). Each single z-series needed to be stored on one full box of 10 floppy disks. The PC used to drive the CLSM struggled to do much more than create a maximum intensity projection of the data and rotate it around the x or y axis. To achieve anything close to 3D rendering required the purchase of some fairly expensive software and hardware (IMARIS v1, VoxelView, FiRender and a Silicon Graphics Onyx workstation). Visualisation in 3D could cost around 50% of the cost of the CLSM itself. Translate that into today's costs for a standard CLSM and you have an idea of the scale of the problem. From about 1993-2002 computing power increased slowly but CLSM systems were producing more channels of data at ever increasing speeds and image resolution. So, file sizes continued to get larger.

In 2003 we were lucky enough to gain access to the Glasgow Science Centre Virtual Reality suite (figure 1). This was partly due to having a collection of 3D data sets that were in a suitable format. The resulting 3D (stereo projection) experience was less than impressive; but we felt like pioneers. Particularly since this facility was powered by something called an SGI Onyx Reality Monster, which cost about the same as a luxury penthouse.

In the following 10 years, desktop computing would catch up with the capability of CLSM systems. This was largely due to the popularity of PC gaming, lowering costs of RAM and the availability of extremely powerful graphics cards. On the software side, the growing popularity of digital 3D art, animation and modelling also provided tools that could be of significant use to CLSM users (Daly et al., 2014). The purpose of the present article is to introduce a selection of software tools and techniques that are routinely used by 3D animators and game designers but may not be obviously relevant to microscopists. The general aim is to encourage CLSM users, who archive vast quantities of 3D data, to think about how that information could be more fully exploited.

Thresholding & Segmentation

3D animation and game engine software require data that is in the form of a wire mesh. Processing and manipulation of the mesh is described in the sections that follow. The first stage in creating a mesh (or Iso-Surface) is thresholding and segmentation (T&S). The process of T&S requires the identification of specific structures within a volume. For CLSM data this will generally be based on intensity or colour of an object. There are many techniques for achieving T&S, some of which can be quite elaborate in their attempts to split touching objects (Luo et al. 1998) or to identify pathological regions within medical images (Malood et al., 2018). Most microscopy and image analysis users will be very familiar with these techniques and so this section will be brief and touch on a few utilities that the author has found useful.

IMARIS image analysis software (Bitplane) is a very powerful visualisation and analysis tool which has a simple user interface and a good automated T&S routine. At the time of writing the current version is 9.2.1, which was used for evaluation. The automatic T&S function works on-the-fly and is particularly good for datasets with strong contrast between objects. Once an iso-surface is created it can be exported in .wrl or .iv formats. The .wrl format can be read by MeshLab (see below)

AMIRA image analysis software (Thermo Scientific) is also a very advanced and powerful 3D visualisation and analysis package. At the time of writing the current version is 6.7, which was used for evaluation. The T&S module offers some very useful tools that include paint brushes, lasso, magic wand, contour, blow, top-hat, watershed and histogram for intensity (threshold) selections. These are particularly useful for 'difficult to segment' datasets. At the iso-surface generation stage, AMIRA also reports the dimensions (size) of the geometry and offers a mesh-reduction option. It is particularly useful to have this reduction facility within the AMIRA package as it can, in some cases, mean that one step can be missed prior to exporting to animation or games software.

There are a few freely available packages that can be extremely helpful. The most common is ImageJ/Fiji which most, if not all, microscopists will be aware of. The 3D plugins (3D viewer and Volume Viewer) are
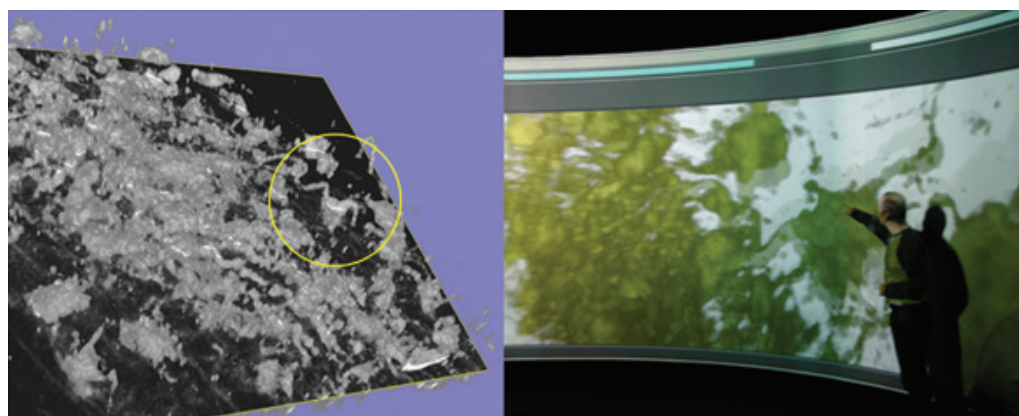

Figure 1. A confocal-derived volumetric rendering (from IMARIS software) of the adventitial surface of an artery and a peri-vascular nerve (circled, left). The author (right) in 2003 presenting the same data set in a VR suite that used stereo projection and 3D glasses on a curved screen to produce a 3D view of the data. The stereo (blurry) screen view was switched off for the purposes of photography.

useful for visualisation but limited in their 3D T&S capabilities.

'Seg3D' is a particularly good package for T&S, as the name suggests it provides a means of segmenting a 3D volume to create a surface. The surface can then be exported in a variety of formats (.vtk, .fac., .pts., .val and .stl).

3DSlicer is very powerful and free. This 3D medical imaging package is currently at v4.10 at the time of writing and whilst mainly focused on MRI & CT image data, it can be used successfully with microscope-based TIFF and PNG series. 3DSlicer has been continually developed over 20 years and now offers an impressive set of T&S tools alongside a growing suite of plugins which now includes a VR module. Although not extensively used by the author, it is anticipated that 3DSlicer would be more than capable of inserting into the workflow detailed in this article (Figure 2).

T&S are essential components of the workflow towards fully immersive interaction in virtual reality. Therefore, it is worth spending time evaluating each of the packages mentioned above with representative data sets of the type most likely to be used.

Working with Meshes

The overall success of the workflow described here rests largely on the quality of the iso-surfaces (meshes). The goal is always to produce a mesh which comprises the minimum amount of geometry and still accurately represents the original objects. In some cases, the surface mesh from a large single channel data set from a modern confocal microscope could comprise 40 million faces and be around 1.5Gb in size. Meshes of this size need to be reduced to facilitate manipulation by other software packages (Romaszewski et al., 2013). Figure 3 shows a typical first pass geometry reduction using AMIRA's 'simplify' option. The mesh is reduced by 33% before being exported to more specialised mesh editing software for further reduction, refinement and possible conversion to quads. Instant Meshes, Mesh Lab, Meshmixer and other applications provide tools to reduce and quadrate the triangular faces. Animation and modelling software (eg Maya, ZBrush
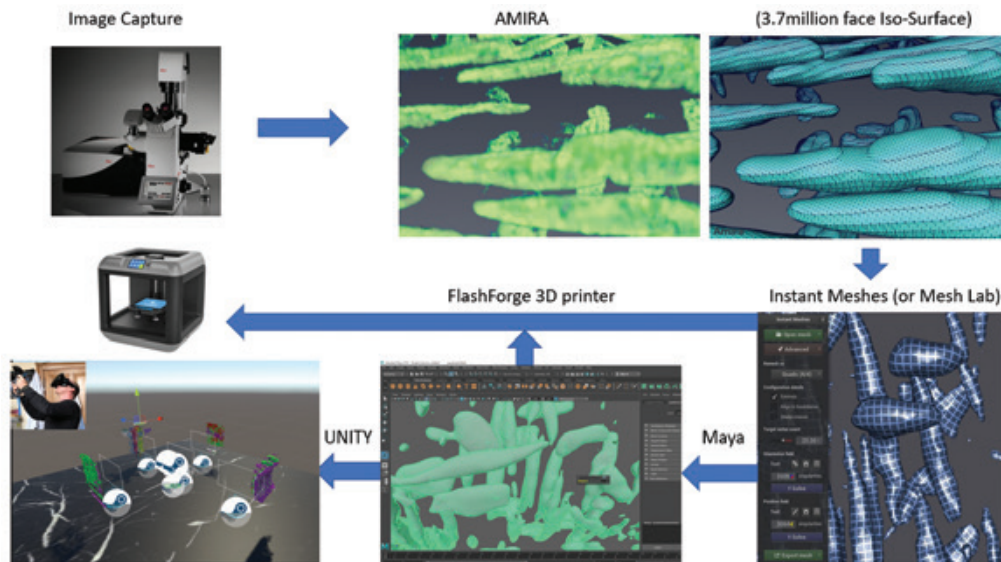
Figure 2. The data workflow from confocal microscope to 3D printing and virtual reality. Data (a multi-channel z-series) is collected via confocal microscopy and passed to the thresholding and segmentation package of choice. In this case it is AMIRA and the dataset is the tunica media of a mouse mesenteric artery. The objects are smooth muscle cell nuclei (typically 25um x 2um size). Thresholding and segmentation routines create an iso-surface made from vertices and faces (wireframe mesh). The resulting mesh is processed and sent to either a 3D printer or to animation software (eg Maya, 3Ds Max, Blender, Cinema 4D). The mesh is altered, textured, illuminated and animated at this stage. Finally, the modified mesh (including animation and textures) can be sent to UNITY for incorporation into interactive games or VR scenes.
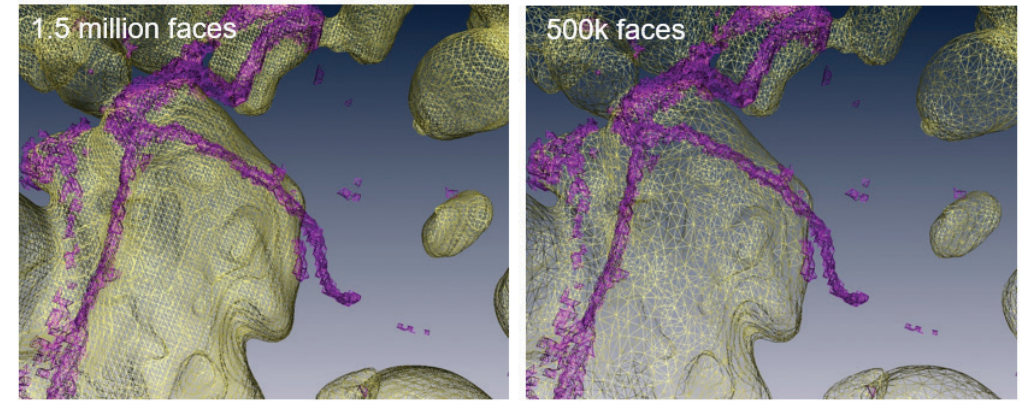
Figure 3. A sympathetic nerve fibre (purple) makes contact with a perivascular adipocyte (yellow). The original iso-surface, generated through thresholding and segmentation produced a mesh containing 1.5 million faces. Reducing the poly-count by 33% (500k faces) results in little, if any, loss of anatomical detail.

and Mudbox) can work with triangles or quads but the preference (among 3D artists) appears to be for quads for smoother subdivisions and manipulation. Internet forums are filled with debate on the topic. The choice of working with triangles or quads will depend on the task at hand. The author's preference is for a quad mesh where possible but it is not always essential. It should be noted though that most microscope or imaging-based software (i.e. AMIRA, IMARIS and 3DSlicer) will create a triangulated iso-surface mesh by default. This is perfectly acceptable for most applications. Figure 2 shows examples of smooth muscle cell nuclei as triangulated meshes in AMIRA but as quad meshes in both Instant Meshes and MAYA.

Passing through a 'mesh editing' stage offers the opportunity to 'fix' any minor defects. Sometimes a segmentation may not completely capture an entire structure, and this can result in broken edges or holes in a surface. It may be necessary to split two surfaces that are touching, if one will be textured differently or animated separately. Alternatively, two surfaces may need to be joined or smoothed. Often modelling or animation software may complain when a surface contains 'non-manifold' geometry. This could be a vector which has no face or two vectors that are missing a 3rd to complete a face. This can cause problems for some animation or 3D printing jobs. Fortunately, most mesh editors will have tools to address these and similar problems (figure 4). The

current version of MeshLab (v2016.12 at the time of writing, December 2018) offers an exhaustive list of tools for mesh editing and is an essential part of the author's workflow.

3D Rendering & Animation
Volume Rendering

3D gaming and design software has radically improved the capability of volume rendering. Previously, microscopists were limited to fairly basic volumetric rendering based on intensity and transparency of voxels. High end Graphics Processing Unit (GPU) cards and modern Computer Generated Imagery (CGI) techniques now offer real-time ray casting to create very realistic looking 3D scenes that can be rendered on-the-fly. Whilst a detailed discussion of ray tracing and ray casting is beyond the scope of this article, the technology seems poised to enter a new
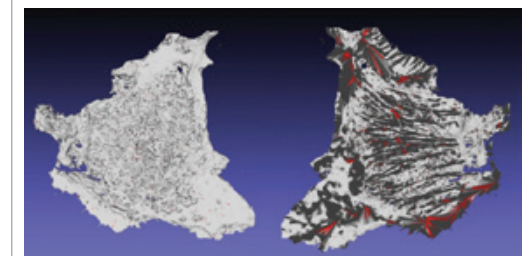
Figure 4. Two views of a single COS cell scanned using a Leica SP8 CLSM, segmented in AMIRA and passed to MeshLab for analysis of non-manifold geometry. The top surface of the cell (left) has no defects. The underside of the cell (right) displays non-manifold geometry (in red) which requires attention. The cell measures 65.8µm at its longest axis. Size bars are not shown as the 3D volumes can have slightly different tilt angles to facilitate visualisation of specific elements.

Figure 5. Comparison of voxel rendering with 'Arnold' photorealistic rendering. The image on the left is a 'voltex' rendering of a perfusion-fixed vascular segment (mouse mesentery artery). It is a 3-channel confocal fluorescence data set comprising cell nuclei (grey, syto 61) alpha1-adrenoceptor ligand (green, prazosin) and a beta-adrenoceptor ligand (orange, TMR-cgp12177). The volume rendering shows the 3 channels in differing opacities. Right image; the same data set has been rendered using Autodesk Maya Arnold' using photorealistic surfaces such as ceramic (white external elastic lamina, pink autonomic nerve), emeralds (adventitial cell nuclei) and glass (yellow perivascular adipocytes). Size bars are not shown due to the 3D nature of the volumes. For reference, the smooth muscle cell nuclei (grey) in the left image are ~25um x 2um in size.

phase that will bring a significant leap in both speed and quality of rendering. A top of the range gaming GPU card (eg NVIDIA GeForce RTX 2080) will cost around £750 (December 2018). The author's developments have relied on a cheaper NVIDIA GTX 1070 (~£350) in a PC with 16Gb of RAM. A recent development in the ophthalmology field has seen one group demonstrate real time immersive VR visualisation of Optical Coherence Tomography using two GPU cards (NVIDIA GTX 1080 & 670) for ray casting and additional graphics processing (Draelos et al., 2018). These authors explore the idea of real-time surgery where the surgeon has a VR view of the live tissue.

Surface Rendering

Animation software (eg Autodesk Maya) provides 3D rendering options in which real life textures can be used. This approach places a heavy load on the processor as each surface reflection and transparency must be calculated such that shadows fall in the correct place, thus giving materials a realistic look. Maya uses 'Arnold' rendering by default but several other plug-ins are available. A comparison between a standard AMIRA volume rendering and a Maya-Arnold rendering is shown in figure 5. The Arnold render has the appearance of a real life solid

object due to the use of familiar looking materials such as copper wire, ceramic and emeralds. It should be noted that a single view can take several minutes to render, depending on the complexity of light and texture. Therefore a simple 360 degree rotation could take hours to render out as a series of frames that need to then be composited to a movie file.

CLSM users will have their own preference for 3D processing software. The simplest platform is probably ImageJ/Fiji running 'Volume Viewer' or '3D Viewer'. Both of these ImageJ plugins are very good and offer a range of rendering options. ImageJ is extremely useful for creating z-projections (figure 6a), altering image depth (16-bit to 8-bit etc) and exporting data in a variety of formats and will therefore easily fit into any workflow.

The limitation of most (if not all) microscope-based 3D packages is that 'animation' generally refers only to the camera position and altering the visibility of volumes and surfaces. Typically, the user only has control over a single camera that can fly through and around a 3D volume. The output can then be saved as a movie. This is perfectly adequate for presenting the structure of a 3D model. However, what if you want to animate the model itself? You may have a beautiful 3D model of a cell, but you may want to animate its movement, contraction, release or uptake of substance etc. For that you need to convert the data into a form that could be read by dedicated 3D animation software (Daly et al., 2014). Autodesk

MAYA, 3DsMax, Cinema 4D and Blender, plus a host of others, offer full control over the 3D meshes and also provide physics-based tools such as particle flows and motion utilities. These capabilities can be combined to make some truly stunning animations (Pool 2016).

Animation

Software for the creation of 3D animations has developed at an incredible rate over the past 10 years. Every software update provides better physics engines and more realistic lighting and textures. The microscopy world has not yet fully embraced this type of software into its normal arsenal of tools and may be missing an opportunity. The software can be very complicated to use and learning curves are steep. However, with every updated version and the development of new packages, interfaces tend to become slightly simpler. It should be recognised though that even with a simplified user interface, the background knowledge required to produce professional output is significant. The author has chosen Autodesk Maya as a platform of choice for a number of reasons. 1. It is very widely used and there are countless YouTube tutorials. 2. The education licence is free through Autodesk University. 3. There are multiple related Autodesk products that are file-type (cross) compatible. 4. It is an extremely powerful, industry standard, platform. 5. Project files can be directly ported to the game engine UNITY (see following section).
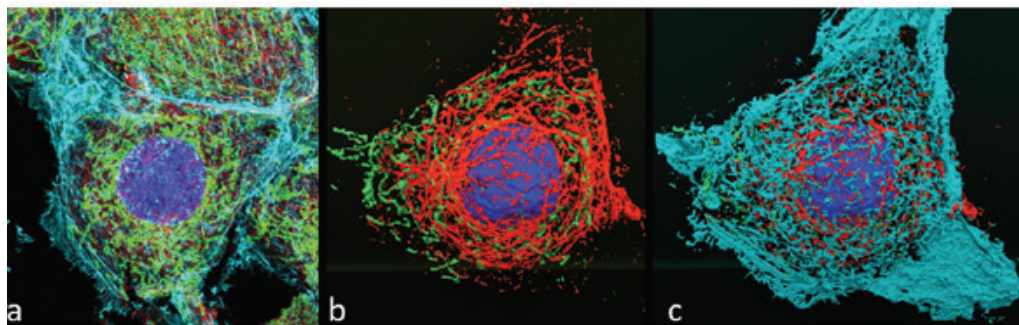

Figure 6. Comparison of ImageJ z-projection and Autodesk MAYA 'Arnold' rendering of extracted surfaces. a) 4 channel CLSM (imageJ) z-projection of a stained COS cell. Tubulin (cyan), Mitochondria (green), actin (red), nucleus (blue). b) 3D surface model, Arnold rendering of actin, mitochondria and nucleus. c) 3D surface model, Arnold rendering of the full data set. In both b) & c) the wireframe surface has been rotated and cropped to remove adjacent cell material. The cell measures 65.8μm at its longest axis. Size bars are not shown as the 3D volumes can have slightly different tilt angles to facilitate visualisation of specific elements.
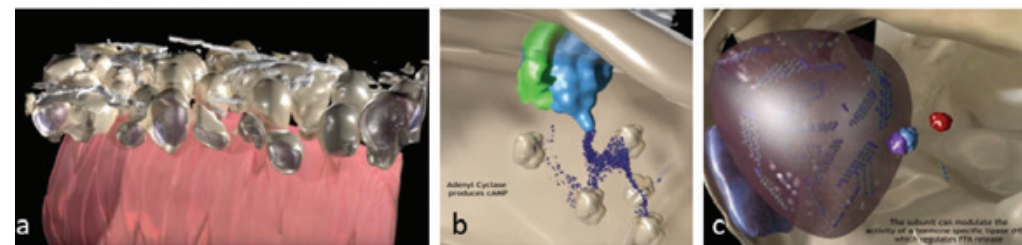

Figure 7. Combining confocal microscopy data, 3D protein structures, artistic components and particle flows in a single animation. The figure shows screenshots from a complete animation which can be viewed at the author's YouTube channel. [see www.cardiovascular.org for YouTube link] a) A confocal laser scanned data set of the mouse mesentery artery peri-vascular adipose tissue (PVAT, gold) with accompanying autonomic nerve supply (white). These structures are captured directly using CLSM. The pink smooth muscle cells (tunica media) are drawn and added for context. b) the inner surface of a PVAT cell (confocal data) with adenylate cyclase (blue) and a bound G-protein alpha-subunit (green) activates a particle flow of cAMP molecules (purple). The cAMP stream is modified by an invisible wind generator and virtual magnets are placed within the phosphodiesterase molecules (beige) to attract (and degrade) the cAMP. c) The large lipid blob containing the triglycerides has been added to the scene (CLSM-derived framework); the hormone specific lipase (blue and purple) structure was downloaded from the protein data bank (PDB) as was the catalytic subunit of protein kinase A (PKA, red). The full animation can be viewed at the author's YouTube channel.

Figure 8. Visualising volumetric CLSM data in VR using the application 'ConfocalVR' (www.confocalvr.com). The data being visualised by the author is a confocal volumetric reconstruction of a segment of vascular (arterial) wall. Slider controls shown on the screen on the left image enable alterations in brightness, contrast, image depth etc.

The importance of extracting a good wireframe mesh (or surface) has been discussed above. Animation software provides virtually unlimited scope for manipulating and texturing the extracted mesh. In addition, other 'actors' can be added to the scene. These could be in the form of neurotransmitters or cellular vesicles. Essentially, components of the scene that cannot be derived from microscopy or other means instead need to be created (drawn). Figure 7 provides several examples. In figure 7a, the gold coloured cells are derived from laser scanning microscopy but the pink blood vessel was created and added to provide a spatial context. In figure 7b the G-protein alpha-subunit, adenyl cyclase and phosphodiesterase molecules were all extracted as 3D models from the protein data bank. The purple stream of cAMP molecules has been created and is an artistic impression. Similarly, the triglycerides inside the lipid blob of the adipocyte (figure 7c) have also been created, as was the lipid blob itself. However, both of these characters in the animation are situated inside a cell that was collected using laser scanning microscopy. The combination of art and science is extremely powerful and holds enormous potential. However, it must be handled with care as the capacity to reinforce misconceptions in the

learner can be detrimental, particularly if the design of educational multimedia is not carefully considered (Daly et al., 2016). This is discussed below.

Virtual Reality & Gaming

The development of commercial gaming products has ushered in a new era for microscope-based data visualisations (Daly 2018). The HTC Vive (and Vive pro) and Oculus Rift are the main fully immersive Head Mounted Display (HMD) units available at the time of writing. However, the technology is advancing rapidly and other units will be available soon. True 'retina display' resolution is a while away but once it is achieved in VR the scenes will be indistinguishable from reality. That will be an interesting time for philosophers but for microscopists it holds the promise of being able to visualise very high resolution data sets that microscopes will no doubt be producing. At present the author is restricted to data sets around 512px x 512px x 60slices (in VR) but this can only increase.

It is a relatively simple process to visualise a confocal volumetric data set (i.e. not an iso-surface mesh) in VR using 'ConfocalVR' (figure 8). This application is written using the UNITY game engine and accepts a confocal (composite) data set that has been exported

as a .nfti format using imageJ (Figure 8). Whilst the current version does not allow extensive interaction with individual objects (within a volume) it does provide an outstanding view of a 3channel data set. Controls for brightness, contrast and image depth are also provided. At the time of writing the newest version now incorporates a collaborative option where multiple users at remote sites can examine the same dataset. This is a must-have application for anyone working with CLSM and VR.

Co-localisation studies, in mouse embryonic fibroblasts, have also been performed in VR using a fully immersive HMD. Although still in its early stages of development in user interaction, this type of work holds great promise (Theart et al., 2016).

A striking feature of examining CLSM data by walking through it and around it is the dramatic change in perspective. A data set that the user may be completely familiar with through examination on a flat screen takes on a truly different dimension when projected at a relative size of about 1.5 meters square in an interactive space. Involving the cerebellum in the learning process by physically moving around the data (for example as in Figure 8) may confer significant cognitive advantages (Buckner, 2013).

The use of game engine software for the analysis of

microscope-based data may not be obvious at first. What is a game? One definition may be; a series of task oriented movements that leads to an overall objective. The objective could be moving from A to B whilst collecting objects and/or information that will assist in the next game level. A game needs a scene or environment to provide the context and the player needs to be able to interact with the scene in order to complete a task. Game design software provides pre-fabricated tools to enable quick creation of a game.

Returning to wireframe meshes and surfaces; microscope-based 3D assets can be considered as game objects or characters in a game. One standard file format for 3D game objects is .obj and this file format can be derived using the methods described previously. Figure 9 shows a scene from a game that is currently under development by the author. Each of the surfaces shown in figure 6 have been imported into UNITY Game Engine. The game also incorporates a series of pre-fabricated tools that enable player movement, teleportation, interaction and visualisation in either VR or on a flat screen. Yellow platforms and blue teleportation pads provide a means of moving around the scene in VR whilst physically occupying a fairly restricted space (i.e. the constraints of a tethered VR-HMD). It should be noted that very little (if any) coding/scripting
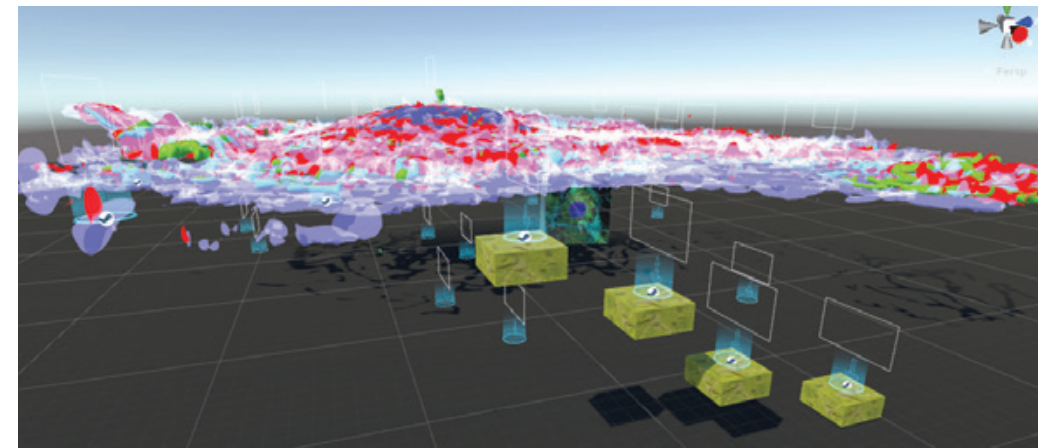

Figure 9. VR scene and/or 3D game using cellular structural data. The COS cell surfaces shown in figure 6b and 6c have been imported into UNITY 3D. The player can explore the scene using a fully immersive VR headset. The player can 'teleport', using a hand-held controller/pointer to any of the blue circular teleport pads. The yellow blocks enable the player to climb up onto the cell and walk across its surface. The scene can then be developed into a game by adding interactive objects and tasks. The game can be delivered as 3D platform style game using a flat screen or as a full VR game.

is required to build a basic game. It only requires assembling a series of software development toolkits (SDKs), prefabs and 3D assets.

Gamification in education is a growing field and 'serious games' are already well established although more research is still required to identify the educational effectiveness of the various game styles and components (Mayer 2019). There exists a huge potential to exploit the masses of data that undoubtedly exist on the hard drives and within the archives of most CLSM users. Data sets that may not have been pretty enough to make it to publication could perhaps serve as modifiable 3D assets for game development. With the inevitable increase in remote learning, and research funders' demands for greater public engagement and impact, game creation of scientific findings could be an attractive solution.

Educational Considerations

If considerable time is to be spent making 3D animations, VR scenes and educational games, it is essential that these are effective learning tools. Some guidance is available in the form of cognitive load theory (CLT, Pass et al., 2003) and multimedia theory (MMT, Mayer 2014). Briefly, CLT recognises three types of cognitive load; intrinsic, extraneous and germane. At its simplest level these three loads can be interpreted as object (or in this case 'scene') complexity, design and background knowledge respectively. This is a gross simplification but will suffice for this brief discussion as it gives us broad aspects to consider. CLSM-based data will have an inherent complexity of multiple structures and features (intrinsic load). The viewer of any educational content will have a particular level of prior knowledge that can be drawn upon by working memory to create new learning (germane load). The instructional design may incorporate additional material that places the information and tasks in context. That additional content can place an extraneous load on the learner if it is unnecessary. The three cognitive loads are additive and can lead to overload which will reduce learning. In general, for CLSM-related animations we can control all of the cognitive loading but reducing extraneous load may be the most effective when presenting a learner with a complex 3D scene.

Multimedia theory describes the use of pictures and sounds and the way in which these are integrated into the learning process. The basic idea is that (written) words and pictures are visual and so occupy the same mental processing channel. Thus it could be expected that an educational multimedia design would favour pictures and narration rather than pictures with on-screen text. Having two things to look at simultaneously would split attention. In a study conducted by the author looking at 3D-animation design preferences of a group of (36) undergraduate students the majority preferred to view an animation that had both on-screen text and narration (unpublished observations). This contravenes the 'redundancy principle' of multimedia theory and could be expected to confer an unnecessary (extraneous) cognitive load. A full discussion is outwith the scope of this article. However, suffice to say that current theories of learning and cognitive load may require to be revised to align with modern educational animation and VR content. Having a huge archive of 3D assets from laser scanning microscopy and access to complex software for constructing fabulous content is therefore not enough. It is essential that serious thought is given to the instructive design of this type of content.

Conclusion

This article aims to stimulate microscopy users to think about how they might utilise their archived bank of images (2D or 3D). Although the workflow described above focuses mainly on 3D there is a huge potential in creating sophisticated multimedia incorporating 2D images and using software such as Adobe After Effects or equivalent. As lecture capture and online learning grow in popularity there will come a time when lecturers (and others) will need to spend more time on content creation rather than content delivery.

This article concentrates on taking CLSM data sets through to VR visualisation but does not mention Augmented Reality (AR). At present VR is a mature product at the plateau of its technology life-cycle curve where hardware refinement and content creation is driving the market. AR is well behind the curve but can be expected to catch up. Quite how AR and microscopy will integrate is a matter of some speculation but a lot of what we learn on the VR journey should be applicable.

Overall, this is an exciting time for 3D (and 4D) microscopy. After a long lag, the computing power and visualisation technology can match the output of the microscopes. We are not yet at the stage of providing realtime VR visualisation of a live cell mounted on a scanning microscope, but that is surely only a matter of time.

Acknowledgements

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