NEWSCORNER

Mycorrhiza movies

Mycorrhiza enables authors to post electronic supplementary material on the World Wide Web in association with text articles appearing in the journal. One example of such supplementary material is movie sequences of biological events captured with video in real time.

In our laboratory we use video-microscopy to study hyphae of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. We are investigating the roles of the hyphal vacuolar network in nutrient transport and metabolic processing. Although static images adequately show the general organization of the vacuolar system (Fig. 1), it is only with real-time video that one can appreciate its astonishing motility and potential as a long-distance transport conduit. You can see two of our movies of this phenomenon at **http://link.springer.de/link/service/journals/00572/supp/list97.htm.** Here we describe how to view these movies, and how to make them.

How to view QuickTime and MPEG movies

Mycorrhiza currently accepts video sequences in two digital video formats: MPEG and QuickTime (.MOV). Video for Windows (.AVI) is not used by *Mycorrhiza*. Movies in either MPEG or QuickTime format can be viewed on both Macs and PCs. However, MPEG movies are very demanding of hardware and software, and users with slow computers will see a degraded version.

To view QuickTime movies, it is best to get the latest version of QuickTime (2.5 for Mac; 2.1.2 for all Windows flavors). Mac systems ship with QuickTime already installed, but updating will help. Windows software does not include QuickTime, but it can be downloaded for free for both Mac and PC systems from **http://quicktime.apple.com/sw/sw3.html**. Make sure to get any add-on that will work with your system while you are at this site.

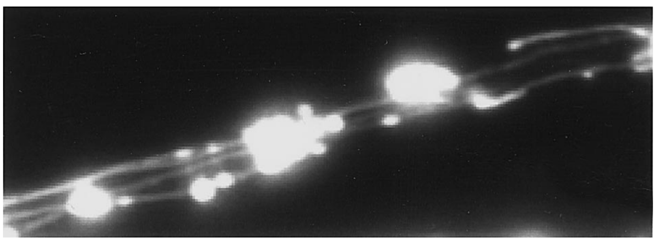

Fig. 1 Tubular and spherical vacuoles in a fluorescently-loaded apical hyphal cell of *Pisolithus tinctorius*. To see them in action, watch the video sequences at http://link.springer.de/link/service/journals/00572/supp/list97.htm

Now, use your browser to access the *Mycorrhiza* Web site, and click on the item linked to our QuickTime video sequence. One of several things may happen, depending on which Web browser you use and how it is configured. One possibility is that the movie will download and automatically appear in a window on your screen. In other cases, the movie will download to your hard disk but will not be launched; active intervention on your part is required. Mac and Windows 95/NT users need to find the downloaded file and click on it, thus launching QuickTime. Windows 3.x users must run their QuickTime application, then open the movie file. Another possibility is that your browser will prompt you to download some (usually free) software from the Web that will help you to view the movie.

Similarly to QuickTime movies, MPEG movies that you select in a browser window will either be automatically launched by the browser, or will require your intervention once downloaded. For Mac users with a PowerPC processor, the easiest solution for viewing MPEG movies is to use the free QuickTime MPEG browser plug-in available at **http://quicktime.apple.com/sw/qtmac.html**. Users of non-PowerPC Macs can use the stand-alone freeware program Sparkle from **http://www.midcoast.com.au/pub/tucows/mac/multimedia.html**. There are many free MPEG players for PCs. See the page **http://www.mpeg.org/index.html/MPEG-videoplayer.html** for a list and instructions for use with your browser. This site also includes links to many commercial MPEG software players that, together with a powerful computer, are usually necessary if you want to see MPEG movies at their best without using a special MPEG hardware card.

Soon, QuickTime version 3.0 should soon be available for free at the Apple QuickTime site listed above. This version should greatly simplify video viewing for many users because it can play QuickTime, MPEG, and Video for Windows files on any Mac running System 7.0 or above, and any PC running Windows 95 or NT.

Image acquisition for producing Web movies

Producing Web movies involves two steps: image acquisition and image editing and formatting. Real-time image acquisition is too complex to fully describe here, so we focus on considerations involved in choosing a camera, computer components, an input-output device, and image acquisition software, while always balancing image quality against limitations of data transfer rates.

Cameras have one of two types of light sensor: vacuum tubes or solid-state chips with a rectangular array of individual pixel sensors. Vacuum tube cameras still represent the best value in resolution, but are less robust, shorter lived and less suited to quantitative light measurements than their solid-state successors. With solid-state cameras, a 500 ′ 500 pixel array will provide both good video and useable still images. At the other extreme, 1000 ′ 1000 pixel arrays are currently the maximum that can output data at video rate. Comparably priced vacuum tube cameras, however, can provide video output at double this resolution.

In solid state cameras, digital output is higher quality than analog output, but is more expensive and is slower than analog output, so one must make sure that it can occur at video rates. If using analog output, "RGB" provides slightly better color accuracy and image quality than "composite video." One should buy a black-and-white camera, however, unless color is necessary. Solid-state color cameras either must use three sensor arrays (with corresponding high price and tripled file sizes), or must cover alternate rows of pixels with red, green, and blue filters, thereby reducing output resolution to one-third.

Camera sensitivity and dynamic range are also important, especially for fluorescence microscopy. Unfortunately, very high sensitivity favors detection of random noise fluctuations. This problem can be reduced by electronic "Peltier cooling" (of solid-state cameras), frame integration (by digital-output cameras only), or by hardware- or software-based frame averaging. Poor sensitivity can be partially overcome with the addition of an image intensifier device. Solid-state camera ability to handle great variation in intensity within an image depends on "bit depth." Fourteen bit cameras have greater dynamic range than eight-bit ones, for example, but they also produce a great volume of data and must be capable of doing so at video rates.

Video acquisition, processing and editing place great demands on computers. A critical "bottleneck" has been internal data transfers. PCI-bus-based systems allow video rate transfer of much larger frame sizes than earlier systems. RAM memory is also important because many acquisition programs first place data in RAM. On our Mac we have 300 MB of RAM. Moreover, if video files are in the 100-MB size range, a large hard disk or suitable data writer (e.g., CD-ROM) is needed.

Analog camera output is converted to computer digital data by hardware devices called "frame grabbers." Frame grabbers and digital signal input-output devices must be able to cope with video rates of data transfer. Sophisticated devices can perform desirable data processing functions such as frame averaging.

There are hundreds of image acquisition/analysis programs from which to choose. Make sure that your software can control your input-output device. Start with free public domain software such as NIH Image until you are sure what you need.

Publishing Web movies: shrink to fit!

The main consideration in Web video production is balancing image quality against file size. *Mycorrhiza* does not limit the maximum size of movies, but about 1 MB will not eternally lock up the computer of anyone downloading your movie. One megabyte is not much, however, for a medium as data-intensive as digital video. Fortunately, many tricks can help squeeze a movie into a megabyte. Because users with slow computers or modems will have difficulty viewing MPEG movies, we suggest QuickTime as the format for *Mycorrhiza* contributors. If you wish, you may publish copies in each format, as allowed by *Mycorrhiza*. We focus on QuickTime movie production.

The simplest way to compact your QuickTime movie is to keep it short. The largest number of readers seeing the main point will maximize its impact; just a few patient ones seeing elaborate detail will not. Basic editing can be done on Macs with the QuickTime MoviePlayer program. If you don't wish to delete entire movie sections, you can try reducing the number of frames per action sequence. Although you may have captured your movie at 25–30 frames per second, it may still view adequately after deletion of intermediate frames. QuickTime does not permit systematic frame deletion, but some acquisition programs that store the movie as a stack of separate images do. A program with this feature that we use is NIH Image, developed and continually maintained by the U.S. National Institutes of Health. A free Mac version can be downloaded at **http://rsb.info.nih.gov/nih-image/**, and a free Windows 95 version (called ImagePC) at **ftp://codon.nih.gov/pub/nih-image-spin-offs/**. Acquisition programs often offer other compaction options such as batch cropping and scaling. Recent versions of Adobe Photoshop, for example, allow batch processing of file stacks. (Photoshop can also produce title frames that can be pasted into a movie after saving them in TIFF format.) Mac users can use the relatively inexpensive Movie Cleaner Pro to crop and scale frames of compiled QuickTime movies. Adobe's Premiere offers professional-quality editing on Macs and PCs, but is expensive and requires a powerful computer.

The most powerful way to shrink a movie is by data compression. Movie compression differs from other file compression in that some data are discarded. Many movies can be considerably compacted without serious loss of quality, however, because often only limited new information is introduced from frame to frame. Instead of saving each frame, compression algorithms periodically store a reference frame and then save only the information that differs in intervening frames. The most powerful compression algorithms (e.g., MPEG) can compress movies 200-fold, but sophisticated decompression is needed for viewing the movie. Cinepak, the compression-decompression algorithm, or codec, almost universally used for Web QuickTime publishing, can compress movies by a factor of three to ten. Cinepak can usually be selected from among codecs in a Save menu sub menu of acquisition programs.

Additional choices when saving QuickTime movies that influence file size and image quality are: data transfer rate (do not exceed 150 kBps to accommodate slow systems), spacing of reference frames (experiment with 4–15 frame spacing), spatial quality (start with Medium), color or black and white (as appropriate), and frame rate (which determines playback speed). A full discussion of these and other QuickTime issues can be found at **http://www.QuickTimeFAQ.org**.

Finally, Mac users need to make their QuickTime movies viewable on other computers. To accomplish this, a completed QuickTime movie can be opened with MoviePlayer and resaved, ticking the boxes marked "Make movie self-contained" and "Playable on non-Apple computers." QuickTime movies saved on a PC do not need extra attention to be viewable on Macs.

We look forward to seeing your movies on the *Mycorrhiza* Web site!

Geoff Hyde, Louise Cole, Anne Ashford
School of Biological Science
University of New South Wales, Sydney, Australia

NB: The second clip consists of four different parts, which were copied and pasted together on a Mac. In the current versions of quicktime, therefore the clip may not run correctly under other systems than Mac.