

# Video Capture and Editing as a Tool for the Storage, Distribution, and Illustration of Morphological Characters of Nematodes

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**Abstract:** Morphological identification and detailed observation of nematodes usually requires permanent slides, but these are never truly permanent and often prevent the same specimens to be used for other purposes. To efficiently record the morphology of nematodes in a format that allows easy archiving, editing, and distribution, we have assembled two micrographic video capture and editing (VCE) configurations. These assemblies allow production of short video clips that mimic multifocal observation of nematode specimens through a light microscope. Images so obtained can be used for training, management, and online access of “virtual voucher specimens” in taxonomic collections, routine screening of fixed or unfixed specimens, recording of ephemeral staining patterns, or recording of freshly dissected internal organs prior to their decomposition. We provide an overview of the components and operation of both of our systems and evaluate their efficiency and image quality. We conclude that VCE is a highly versatile approach that is likely to become widely used in nematology research and teaching.

**Key words:** computer applications, database, image editing, methods, microscopy, morphology, type collections, video, World Wide Web.

Despite the proliferation of various ultrastructural and molecular techniques, the light microscope (LM) continues to be the first-line tool for nematode identification. Unfortunately, many situations occur in which a specimen's micro-morphological characters are lost, distorted, or obscured. Thus, type specimens in taxonomic collections inevitably deteriorate or are accidentally lost, while teaching material is easily destroyed by inexperienced students. Specimens prepared for histochemical studies or for scanning electron microscopy (SEM) become opaque during staining or coating protocols, and often deteriorate quickly. Observations of some features, such as the cellular architecture of the female gonad, can be made only on freshly dissected specimens, which usually plasmolyse and decompose quickly. Other techniques such as transmission electron microscopy (TEM) and molecular analysis require destruction of the specimen.

Degradation of a specimen invariably interferes with subsequent verification of previous identifications and observations. Line drawings and still photographs can be used to record, illustrate, and distribute observations but have important limitations: they depend on subjective personal skills (especially so for line drawings) and simplify or omit three-dimensional topology of structures (especially so for still photos). Eisenback (1988) presented a simple method for superimposing several focal planes in one photomicrograph, but this method is applicable only to a maximum of five focal planes because it reduces contrast per focal plane and discards all topological information along the focal axis.

Alternative approaches for recording morphological observations on nematodes would be exceptionally useful to nematology. We assembled two non-automated video capture and editing (VCE) systems for multifocal recording of nematode morphology, as simplified derivations from the four-dimensional microscopes used in comparative embryology of nematodes (Schnabel et al., 1997; Thomas et al., 1996). Protocols for the recording, optimization, and distribution of multifocal images of nematode specimens were developed and tested. The resulting video files can easily be exchanged on disk or through networks, and viewed with various widely distributed digitized video player programs.

In this paper we briefly describe the components and protocols used for our VCE systems, as well as some of their relevant applications. Full details, examples, and links to manufacturers are available online at <http://faculty.ucr.edu/~pdeley/vce.html>. We conclude that VCE has a wide spectrum of uses in nematological teaching and research, and that it is in many ways complementary to digital still photography.

## MATERIALS AND METHODS

**Equipment and software:** We have assembled two systems: a basic VCE configuration (further referred to as Configuration 1), costing about \$20,000, and a somewhat more expensive VCE system with some more versatile or unusual parts (Configuration 2), costing about \$31,000 (Table 1). In both cases, all the included computer components are marketed to the advanced home computer user, manufactured by market leaders in their respective areas, readily available from many electronics suppliers, and compatible with many other models and brands. However, we have not tested these alternative brands and do not wish to claim or imply any preference for the tested models. The principles described here apply regardless of specific hardware and software choices. Although our configurations were based on IBM®-compatible computer components, various Macintosh®-compatible counterparts op-

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TABLE 1. Main components of the two tested Video Capture and Editing configurations.

	Configuration 1	Configuration 2
Optics	Olympus® BX50 microscope with 5-position universal condenser, mechanical stage, 6-position DIC nosepiece Objectives Plan Semi-Apochromat 4×, 10×, 20×, 40×, 100×/NA1.30  DIC optics including standard prism for 20×, 40×, and 100× objectives 1× video adapter + C-mount camera adapter Phototube with PE2.5× projection lens + L-adapter camera mount 35WH10× focusing eyepiece for phototube	Olympus® BX51 microscope with 8-position universal condenser, mechanical stage, 7-position DIC nosepiece Objectives Plan Semi-Apochromat 4×, 10×, 20×; Plan Apochromat 60×/NA1.40; water immersion Plan 100×/NA1.0 I-IR  DIC optics including HR prism for 20× and 60× objectives; HC prism for 100× objective 1× video adapter + C-mount camera adapter 0.35× CCD camera adapter (interchangeable with 1× adapter) Magnification changer 1×/1.25×/1.6×/2×
Cameras/ monitor	JVC® TK-C1381EG ½" single CCD color video camera	Olympus® OLY-200 ⅓" single CCD color video camera
Computer hardware	Olympus® SC35 single-lens reflex camera Advanced Micro Devices® K7 550-MHz with 64MB SDRAM  13.6-Gb 7200-rpm hard drive 50× CD-ROM drive 4×4×24× CD-RW drive Pinnacle Systems® DC30plus video capture card Daewoo® 17" .25mm monitor	Sony® S-M035 14" RGB monitor Advanced Micro Devices® Athlon 1.1 GHz with 128MB SDRAM  Fixed 20-Gb 7200-rpm hard drive 50× CD-ROM drive 8×4×32× CD-RW drive Pinnacle Systems® DV500 video capture card Amptron® 17" .25mm monitor Removable 30-Gb ATA/100 7200-rpm hard drive in ATA/100 caddy
Computer software	Microsoft Windows® 98 SE (includes Windows Media Player 6.01.05.0217) Adobe Premiere® 5.1c (included with DC30 plus) Apple QuickTime Player® 4.1.1 Pinnacle Systems® miroVideo Capture (included with DC30plus)	Microsoft Windows® 98 SE (includes Windows Media Player 6.4.07.1028) Adobe Premiere® 6.0 (included with DV500) Apple QuickTime Player® 4.1.1

erate along identical principles, and the finished VCE clips can be observed on either platform.

*Protocols:* Experimentation with both configurations led to a general protocol (Fig. 1). The first major stage consists of capturing each multifocal series of images, which involves two separate steps: initialization of the equipment and software (to ensure correct transmission to the capture card, conversion by the capture software, and storage in the proper folders of the hard disk), and the sequential capturing itself while manually focusing through one properly positioned part of a specimen at a time. Capturing and positioning can be repeated as many times as necessary to record entire body regions, or even to record all parts of a complete specimen at the highest magnification.

The second major stage consists of editing and compression of the captured video clips. Each clip often uses much more disk space and memory than is necessary, typically because it includes frames that are out of focus, adjacent frames that are virtually identical, and background areas that remain empty throughout the clip. Such redundancy can be minimized using Adobe® Premiere 5.1 or 6.0 to optimize file size and to mathematically compress clips. It is also possible to add text, scale bars, and arrows to one or more individual frames within a clip, so as to highlight important or indistinct structures in those levels of focus where they occur.

In the third and final major stage, the edited clip is tested and verified on various video player programs

(e.g., QuickTime Movie Player® and Windows Media Player®) to ensure that end users will be able to open it correctly regardless of their particular hardware and software. Such software typically includes a slider button that allows users to move forward and backward through a clip. In a multifocal VCE clip, this movement effectively simulates the focusing action of the microscope. Finally, for specimens captured as a series of clips, all VCE files can be linked to a clickable image map in a World Wide Web page, providing an overview that is accessible online or on disk through any Web browser.

## RESULTS

*Overall performance:* Both systems proved to be versatile and easy to use. The time required for a complete VCE procedure ranged from 5 minutes to an entire day, depending on the size of the specimen, number of files captured, and addition of text and labels of varying complexity. Some limitations were evident in both the optical and electronic components. For example, with Configuration 1 it was sometimes difficult to fit larger nematodes or bulkier dissections into the video display area. If neither the width nor the length fit within the display area at ×100, as when recording dissected gonads from *Meloidogyne* spp., resolution had to be compromised by choosing a lower magnification. Such limi-

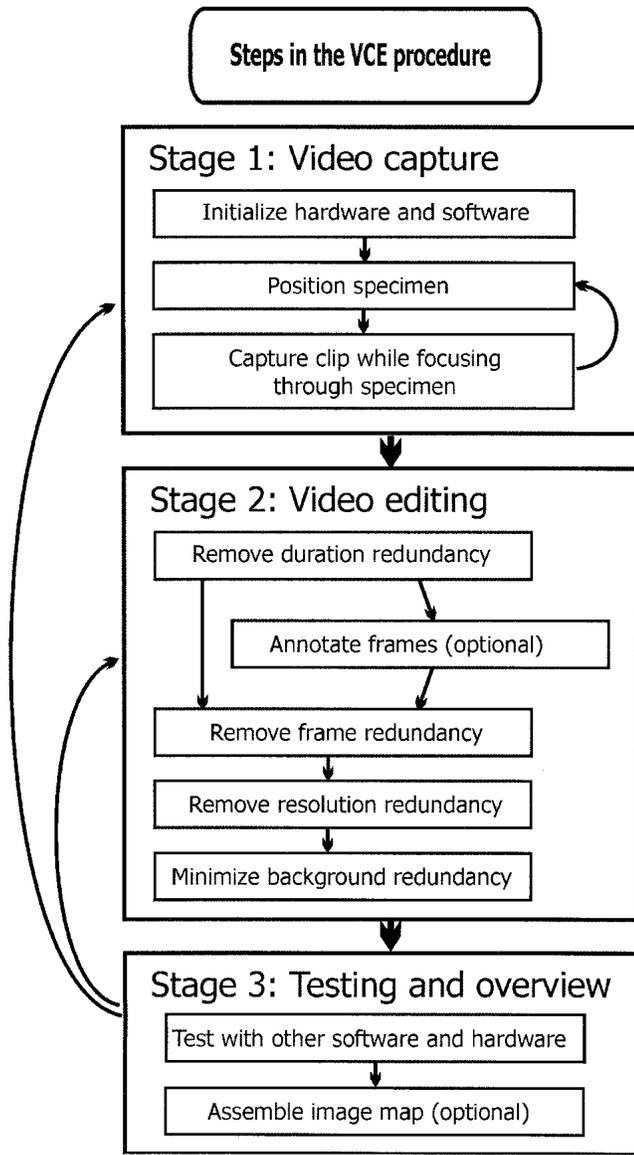


FIG. 1. Overview of the main steps in the VCE procedure.

tations were largely overcome by the various components of Configuration 2, providing greater flexibility in magnification.

The resolution of both video cameras tested was clearly limited in comparison to the actual resolution of the microscope optics, and did not always resolve cuticular punctations, nerve endings, or minute openings that were visible through the eyepieces of the LM. In dissections the precise appearance of cell membranes was also resolved more poorly in the video clips relative to the LM; nevertheless, resolution was quite sufficient for many illustrative purposes. Compared to still 35-mm film photographs, VCE frames were somewhat poorer in contrast but slightly better in resolution (Figs. 2,3). With photographs, it was also much more difficult to obtain a good multifocal series of a freshly dissected gonad in temporary mount due to muscular contractions and plasmolytic movements. Both our configura-

tions were sensitive to vibrations caused by other laboratory equipment, but this was much less problematic for video capture than for 35-mm photography, especially after mounting the microscopes on a wooden board resting on various kinds of elastic material.

*Examples of some applications:* Based on the developed procedures, we produced VCE files for a range of different purposes:

a) *Recording for Web sites:* VCE files can be easily incorporated into Web pages, but current limits to modem speeds make it impractical for the general public to access larger files. It is therefore better to minimize file size by reducing resolution to  $360 \times 270$  pixels and by applying more size-efficient compression algorithms such as Sorenson® Video, especially with the help of dedicated compression programs like Media Cleaner® 5.

b) *Recording demonstration material for disk access:* VCE clips greatly improve three-dimensional perception for students and other beginning microscopists by mimicking the focusing action as an integral part of observation. However, to provide recordings that are as detailed as possible, maximal resolution is required (in our case,  $720 \times 540$  pixels on PAL systems and  $720 \times 480$  pixels on NTSC systems). For maximal playback speed, such VCE files are best distributed on CD-ROM or DVD and copied to hard disk for playback. Also, transfer of knowledge is greatly improved by using the titling facilities of Adobe® Premiere to annotate all interesting structures *in those frames where they occur only* (Fig. 4). Titling is usually the most time-consuming step in the entire VCE procedure, but it allows the inexperienced viewer to unambiguously locate each structure and learn its technical name.

c) *Archiving large numbers of specimen and slides:* Many databasing and surveying applications benefit from the ability to link images to text records. VCE clips of the most important body parts of each observed specimen (usually lip region, cardia, vulval or spicular region, and tail) can easily be linked to records of various popular database programs. Depending on space constraints, it may be advisable to restrict file size of archival clips in the same way as for Web-site files, but continued growth in speed and capacity of computer hardware implies that processing time is usually a greater constraint than capacity. For maximal throughput, archival VCE clips can therefore save time by skipping the size optimization and titling steps. The  $\times 100$  water immersion objective of Configuration 2 allows VCE of heat-killed nematodes without the need for any fixation or a coverslip. In trial runs this objective was found to provide images of sufficient detail to allow rapid screening and recording of disposable specimens, thus bypassing the health risks of fixation and the time required for transfer to glycerin and mounting in permanent slides.

d) *Recording type material as "virtual voucher specimens":* In this case, the goal is to store an entire voucher specimen digitally at maximal resolution and at different

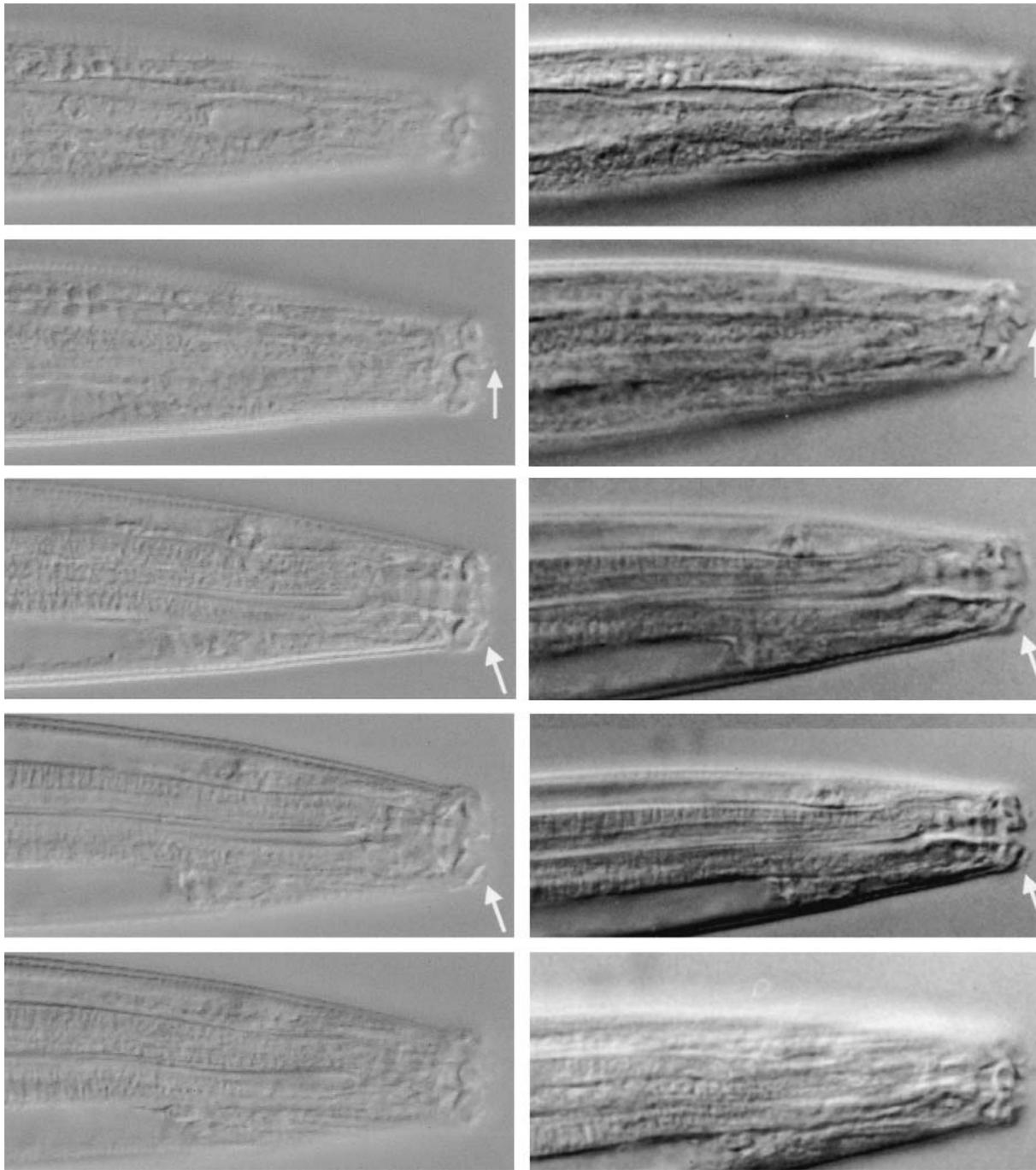


FIG. 2. Comparison of image quality obtained by VCE Configuration 1 (left column) vs. 35-mm still photography through the same optics (right column) of different focusing levels of the lip region of a formalin-fixed female *Panagrobelus stammeri* mounted in glycerin (Stock et al., 2002). Note the occurrence of setiform processes on the lips (arrows), which are resolved slightly better by VCE than with 35-mm film.

magnifications to produce a set of reference files that can, for most purposes, replace the physical specimen. Such “virtual voucher specimens” can be made accessible online or distributed on disk, thereby eliminating the risks of sending type slides by mail. They can also be used to completely record the morphology of important specimens before they suffer the effects of deteriorating preservation, or before they are used for SEM, TEM, sections, or *en face* mounts. In our experience,

complete VCE processing of smaller nematodes requires about 20 clips and takes 90 to 240 minutes per specimen, depending mainly on whether or not scale bars and a descriptive title are included. In larger nematodes like Longidoridae, the number of files required for complete capture is prohibitive, and an acceptable compromise is to capture an incomplete series of multifocal files by omitting those parts of the body that are irrelevant to diagnosis (e.g., where the body

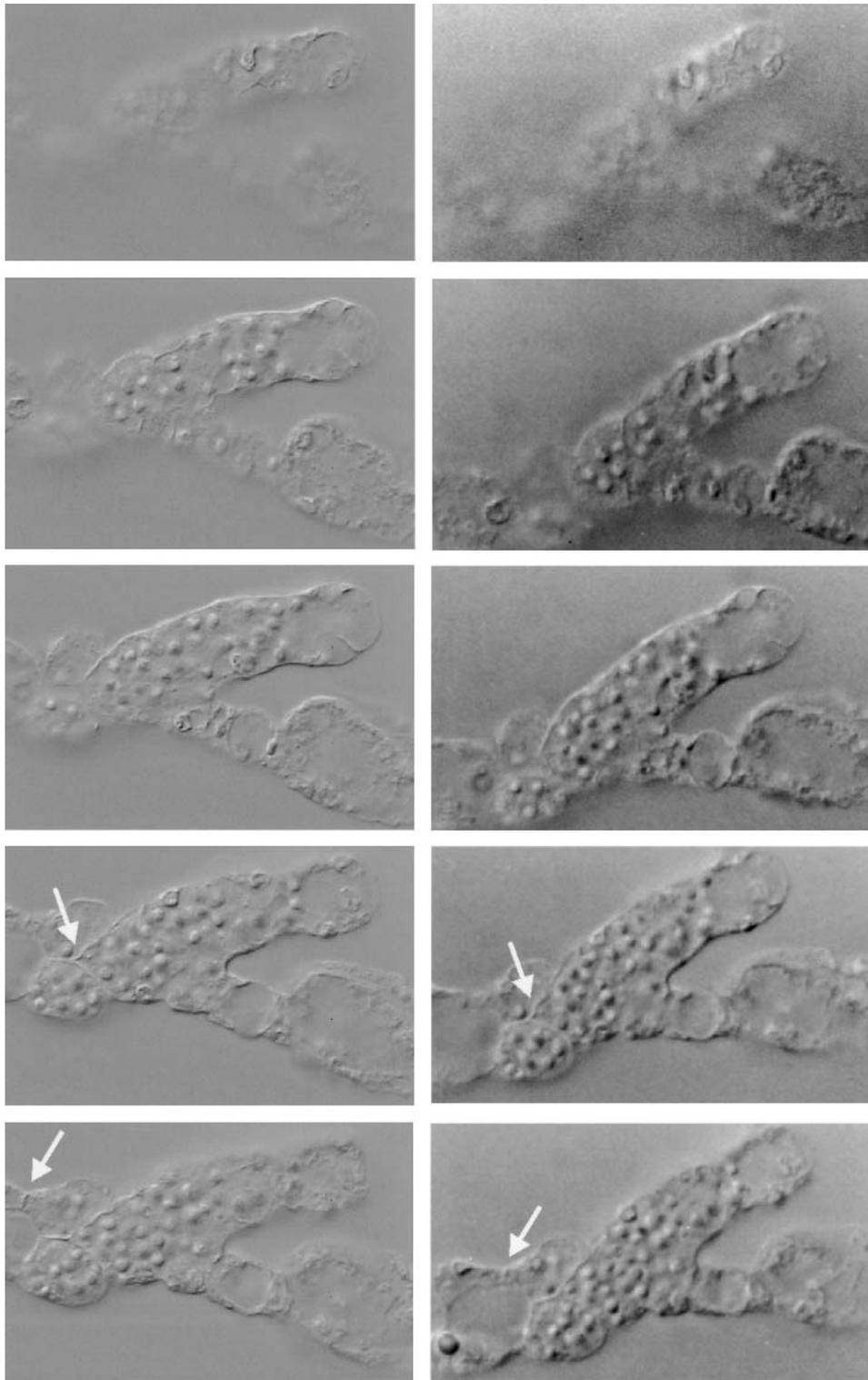


FIG. 3. Comparison of image quality obtained by VCE Configuration 1 (left column) vs. 35-mm still photography through the same optics (right column) of the freshly dissected spermatheca of *Coslenchus* sp. Note cell membranes (arrows) resolved better by VCE than 35-mm film.

contains only intestine). A clickable image map provides an easy overview of all recorded parts of such a complete or incomplete “virtual voucher specimen,” but its construction requires several hours’ additional work and is therefore more appropriate for holotypes than for less important reference specimens.

*e) Recording and documenting parts of dissected organs:* Dissected nematode organs often cannot be transferred to permanent mounts without drastic reduction of quality, and usually require a three-dimensional overview. VCE provides an inexpensive and fast tool for fulfilling both these demands, and can even be used to

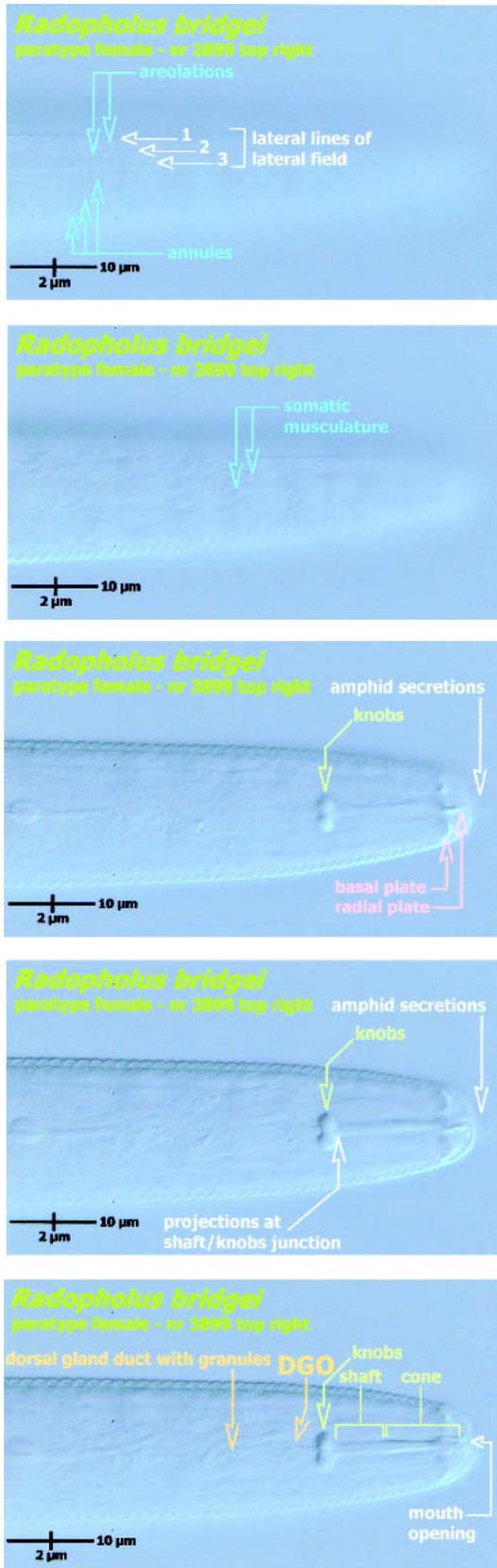


FIG. 4. Several frames from an annotated VCE clip of a female paratype of *Radopholus bridgei*, produced with VCE Configuration 1. Text and arrows are embedded in one frame, or in a series of subsequent frames, as appropriate for the various structures visible at the corresponding levels of focus. Note the small projections just anterior to the stylet knobs, which were not previously described in this species but are nevertheless resolved by the video camera.

record a dissected structure at different angles of view.

f) *Recording the staining of specific cells:* Histological stains are rarely permanent, and a VCE system allows the observer to capture stained cells or structures in their three-dimensional context. For example, we modified the regressive staining technique of Khrustalev and Hoberg (1996) and tested it on *Panagrolaimus rigidus*. We mounted stained nematodes directly in the destaining solution, and captured a section of the intestine at several time intervals during the destaining process. Afterward, we could easily select the VCE clip with the best contrast between nuclei and background, and store this as a permanent record.

## DISCUSSION

VCE is just one in a range of computer-based imaging applications, some of which we will briefly discuss. Compared to digital still photography, video capture may be limited in terms of the resolution attainable with existing analog cameras, but it allows much easier multifocal recording in one quick operation and results in a single file of comparable size to a single high-resolution digital still image. As a result, VCE and digital still photography are complementary rather than competing approaches. The latter is to be preferred when resolution is critical; the former is more appropriate for reproducing the focusing action of a microscope. At present, this gives each method a different niche among nematological techniques, although the distinction may narrow considerably with the development of increasingly affordable high-resolution digital video cameras.

Another relevant technological innovation is extended depth of field (EDF) (Tucker et al., 1999), i.e. image capture and digital enhancement resulting in still images with a much greater range of focus than permitted by standard microscope optics. We are not aware of any applications as yet to nematodes, so it remains to be seen how this technique will handle heavily sclerotized structures such as buccal capsules and stylets. Unlike VCE clips, an EDF image presumably does not preserve all structural clues to angle of view, which could mean that an EDF image of a nematode lip region would not preserve any distinction between a dorso-sublateral and a ventro-sublateral angle of view. Practical application to nematode specimens will clarify these issues and demonstrate to what extent EDF can be combined with other types of microscopy imaging.

Finally, comparison also needs to be made with Confocal Laser Scanning Microscopy (CLSM) (Czymmek et al., 1994; Donaldson and Lausberg, 1998). This approach has many advantages over VCE in that it significantly enhances resolution and relies on software capable of measuring distances as well as constructing three-dimensional models. However, CLSM systems are

significantly more expensive and alter the optical properties of the microscope in ways that make its use rather different from routine practice in nematology. By comparison, adding the various components of a VCE system can be done at a much lower cost without modifying the basic properties of the microscope. We have not yet tested our configurations for automated biometric analyses, as the emphasis here is on the basic recording and representation of morphology itself. Nevertheless, it is presumably possible to expand our hardware and software to incorporate three-dimensional imaging functions (Omasa and Kouda, 1998).

#### CONCLUSIONS

Video capture and editing allows the production of video clips that mimic the act of observing nematode specimens through LM. These clips can be modified and shared in a number of ways, and the optical quality of the obtained images is sufficient to supplement or replace direct observations through the microscope, allowing for a wide range of novel applications at the interface between preservation, illustration, and distribution of actual nematode specimens. Apart from the compound microscope itself, the required equipment is comparatively cheap and highly interchangeable, and involves no special expertise beyond the routine needs of a nematology laboratory. The obtained file sizes are well within the operational limits of speed and capacity of current generations of computers, and future developments will allow for further increases in efficiency. We therefore predict that VCE will quickly become widespread in nematology, and that many further uses will be developed in the process. Other applications that we wish to investigate include capture of images

from immobilized live nematodes as a means of combining high-magnification microscopy with molecular analyses of individual specimens. In the longer term, we hope that it may become possible to omit the need for production of permanent slides, with its concomitant hazards of handling and storing hazardous fixatives. We therefore continue to experiment with other accessories to further expand the versatility of VCE.

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