

Table 1 (cont.)

	1 000	4.60	(29.23)	0.691	0.365	7.3
	L.S.D. (1 %)	1.276		0.009	0.005	
	L.S.D. (5 %)	0.842		0.006	0.003	
N-pinky	0	6.20	—	0.450	0.215	—
	10	5.50	(11.29)	0.520	0.242	104.1
	100	4.60	(25.80)	0.565	0.270	36.0
	1 000	4.10	(33.87)	0.625	0.325	9.4
	L.S.D. (1 %)	0.584		0.010	0.005	
	L.S.D. (5 %)	0.386		0.006	0.003	
N-tropic	0	5.70	—	0.365	0.178	—
	10	4.70	(17.54)	0.410	0.198	140.3
	100	3.60	(36.84)	0.455	0.220	49.9
	1 000	3.00	(47.36)	0.500	0.268	10.9
	L.S.D. (1 %)	0.201		0.007	0.003	
	L.S.D. (5 %)	0.133		0.004	0.002	
Sioux	0	6.30	—	0.435	0.212	—
	10	5.55	(11.90)	0.503	0.237	108.8
	100	4.65	(26.19)	0.548	0.265	36.2
	1 000	4.15	(34.12)	0.596	0.322	9.4
	L.S.D. (1 %)	0.259		0.009	0.003	
	L.S.D. (5 %)	0.170		0.006	0.002	
Trope GRO-7	0	5.80	—	0.400	0.193	—
	10	4.90	(15.51)	0.455	0.216	125.1
	100	3.80	(34.48)	0.500	0.240	43.6
	1 000	3.30	(43.10)	0.550	0.293	10.3
	L.S.D. (1 %)	0.279		0.007	0.006	
	L.S.D. (5 %)	0.184		0.004	0.004	

Each value is a mean of five replicates.

In parenthesis are given per cent reduction over control.

R = Reproduction factor of the nematode, Pf = Final population, Pi = Initial Population, O.D. = O-dihydroxy phenols.

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## THE POTENTIAL OF HIGH RESOLUTION VIDEO-ENHANCED CONTRAST MICROSCOPY IN NEMATOLOGICAL RESEARCH

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High power interference contrast ciné-microscopy is an important tool for studying nematode behaviour and is, perhaps, indispensable for detailed analysis of func-

tional morphology (e.g. Robertson & Wyss, 1979; Seymour, 1983a, b). Video recordings are very useful for an immediate analysis, provided the resolution is of suf-

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ficient standard. Seymour, Minter and Doncaster (1978) used a low-light remote recording system to study nematode feeding behaviour at very low light levels. Great advances in video recording techniques were made by Allen, Allen and Travis (1981) who optimised visual and photographic contrast in human and animal

cells with their video-enhanced contrast, differential interference contrast (AVEC-DIC) microscopy. For our observations on nematodes, we applied a less sophisticated and therefore less expensive system which still gives excellent results and may be considered a full substitute for cine-micrography when used for analytical purposes.

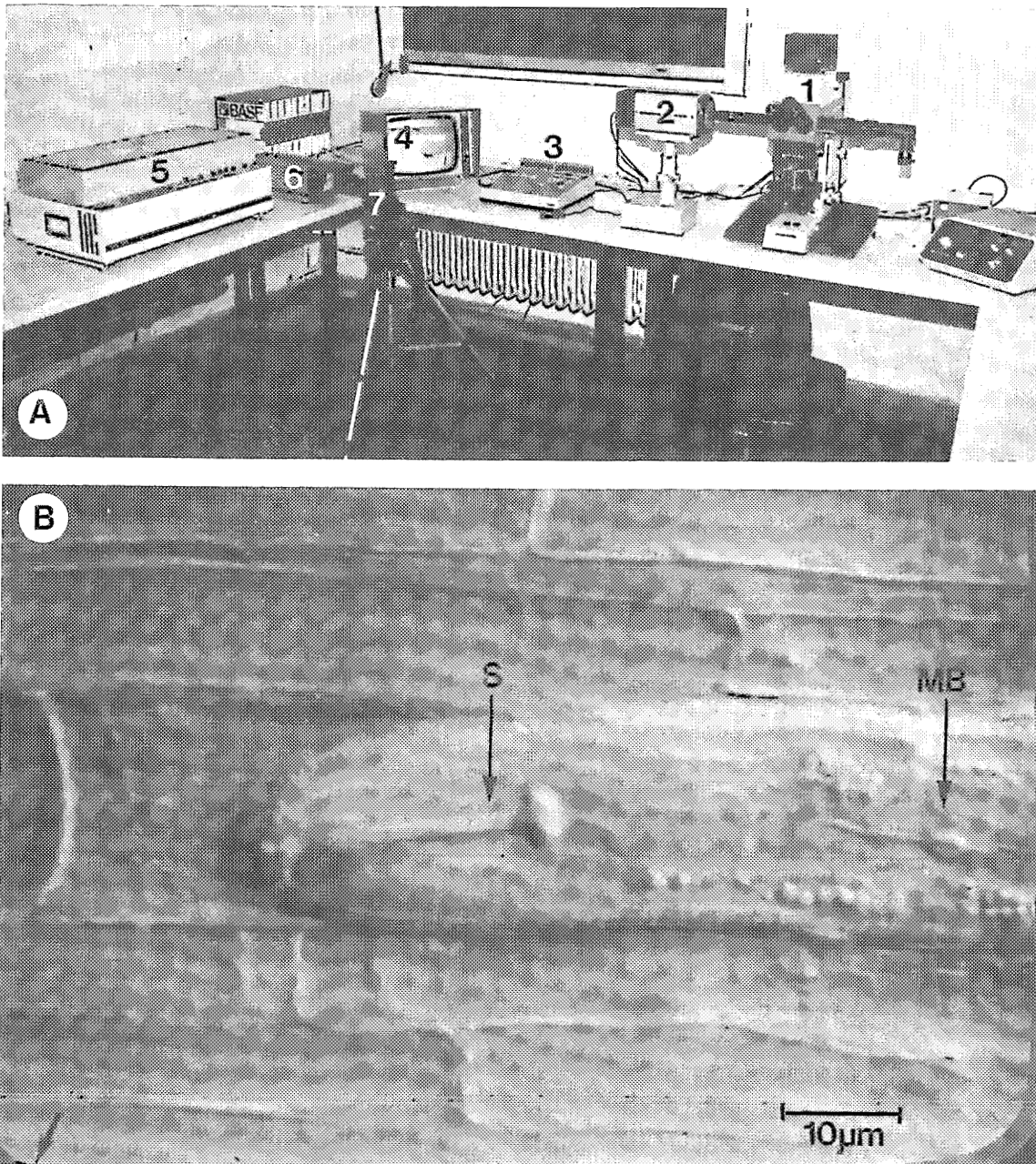


Fig. 1 A. Components of the high resolution video-enhanced microscopy system (see text). B: An infective J2 juvenile of *Heterodera schachtii* on its migration through a cortical cell, three cell layers beneath the root surface. S : stylet; MB : median oesophageal bulb; arrow points to the border of the picture monitor screen (Nr. 6 in Fig. 1 A) from which the picture was taken.

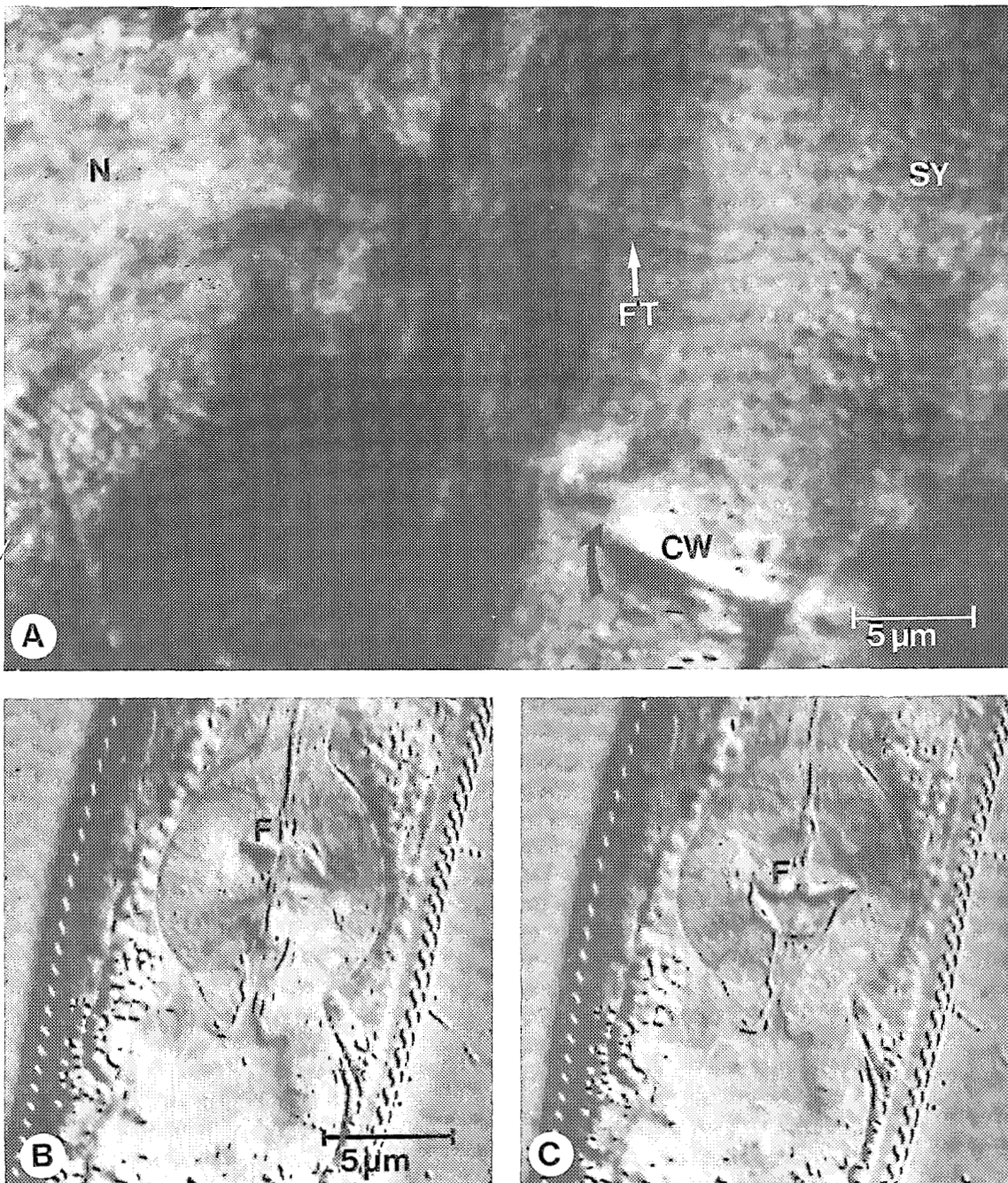


Fig. 2 A. *In vivo* observation of cytoplasm dynamics around the feeding tube (FT) of a female J3 *Heterodera schachtii* within the initial syncytial cell (SY). Note the modified zone of cytoplasm free of organelles around the feeding tube. CW : stub of a partially dissolved cell wall; N : head of the nematode; arrow points to flow of cytoplasm past the cell wall stub. B-C : The posterior oesophageal bulb of an *Acrobeles* species during food ingestion. C : Bulb flaps (F) start to invert, 6 frames (0.12 sec.) after B.

In addition, it enables a careful study of nematode behaviour and cell responses deep inside roots. Events that otherwise remain obscure when viewed through the differential interference contrast microscope, become well defined by video contrast enhancement. The system we use consists of the following components (Fig. 1A) :

- 1) Reichert differential interference contrast microscope "Polyvar" equipped with 100 × /1.32 N.A. and 40 × /1.00 N.A. planapochromatic oil immersion objectives and optovar 0.8 × /1 × /1.25 × 12 ×<sup>2</sup>.
- 2) Monochrome camera system Grundig FA 76 I, converted to 875 lines/50 Hz vertical frequency. Equipped with a 1" Newvicon pickup tube (light sensitivity 0.1 lx) and switch to select with/without line interlace to permit magnetic single frame recording.
- 3) Remote control Grundig FB 400 E to control all functions. With pulse generator fitted to set recording and playback sequence (1... 300 sec. in 11 stages). Single frame release is either manually or from an external source.
- 4) Picture monitor Grundig BG 330 (screen diagonal 31 cm), converted to 875 lines/50 Hz vertical frequency.
- 5) Professional 2.5 cm video recorder Grundig BK 411 HE, 10 MHz resolution, converted to 875 lines/50 Hz. 110 min. playing time (2.5 cm chromium dioxide BASF tapes CV 26 R). With electronic editing to assemble and insert various scenes; slow motion forward and reverse and additional module to record individual frames with stationary magnetic tape.
- 6) Picture monitor Grundig BG 12 (screen diagonal 12.8 cm), converted to 875 lines/50 Hz vertical frequency.

Relevant scenes from tape recordings are analysed by single frame release (50 pictures/sec.). For documentation, photographs (1 sec. exposure) are taken with a camera (7) from the small picture monitor (6).

Figures 1 B and 2 A-C illustrate the potential of this high resolution video-enhanced contrast system for nematological research. Figure 1 B shows an infective *Heterodera schachtii* juvenile during its intracellular migration through cortical cells towards its final feeding site within the vascular cylinder. The photograph was taken after the juvenile had reached the third layer of cortical cells in roots of rape-seed (*Brassica napus* var. *oleifera*) seedlings grown in sterile nutrient agar culture.

Figure 2 A shows, at about 3 800 × magnification, the feeding tube (FT) of a female fourth stage *H. schachtii* juvenile in the initial syncytial cell within the vascular cylinder of an oil radish (*Raphanus sativus* var. *oleiformis*) root. The *in vivo* formation and detection of these tubes can only be seen through the microscope by video contrast enhancement. In an electron microscopic study,

a zone of modified cytoplasm free of organelles was found to surround the feeding tubes of *H. schachtii* in initial syncytial cells of the same host (Wyss, Stender & Lehmann, 1984). The *in vivo* observations confirm this finding and support the assumption that feeding tube material may help to "predigest" cytoplasm for an efficient withdrawal of nutrients from the dense cytoplasm. For Figures 1 B and 2 A, observation chambers were used as described by Wyss (1973).

Figures 2 B and 2 C show the potential of the system to evaluate functional morphology, here within the posterior bulb of an *Acrobelles* species during food ingestion. In Figure 2 C the bulb flaps start to invert, six pictures (0.12 sec.) after 2 B. Single frames (438 lines) from the video tape are close to the quality of 16 mm ciné film recordings.

Apart from the immediate analysis of motion studies, important scenes of tape recordings can be filmed directly from the small picture monitor with a 35 mm ciné film camera, equipped with a phase shift adaptor. In this way a 16 mm research film\* on the behaviour of *H. schachtii* inside roots of host seedlings was produced (Wyss, Zunke & Inst. wiss. Film, 1985) which shows intracellular migration of J 2 juvenile, induction and maintenance of syncytia within the vascular cylinder as well as characteristic events during salivation and ingestion.

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\* The film (see reference) has an English commentary and can be ordered from : Institut für den wissenschaftlichen Film, Nonnenstieg 72, D-3400 Göttingen, W. Germany.