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REVIEW

Strategies for automated analysis of *C. elegans* locomotion

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Abstract Automated analysis of *C. elegans* behaviour is a rapidly developing field, offering the possibility of behaviour-based, high-throughput drug screens and systematic phenotyping. Standard methods for parameterizing worm shapes and movements are emerging, and progress has been made towards overcoming the difficulties introduced by interactions between worms, as well as worm coiling and omega turning. Current methods have facilitated the identification of subtle phenotypes and the characterisation of roles of neurones in forward locomotion and chemotaxis, as well as the quantitative characterisation of behaviour choice and circadian patterns of activity. Given the speed with which *C. elegans* has been deployed in genetic screens and chemical screens, it is to be hoped that wormtrackers may eventually provide similar rapidity in assaying behavioural phenotypes. However, considerable progress must be made before this can be accomplished. In the case of genome-wide RNAi screens, for example, the presence in the worm genome of some 19,000 genes means that even the minimal user intervention in an automatic phenotyping system will be very costly. Nonetheless, recent advances have shown that drug actions on large numbers of worms can be tracked, raising hopes that high-throughput behavioural screens may soon be available.

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Introduction

The free living, soil nematode, *Caenorhabditis elegans*, is a model genetic organism introduced by Sydney Brenner (Brenner, 1974), which has been used extensively for tackling fundamental questions in neuroscience (Chalasani et al., 2007; Ruvinsky et al., 2007; Von Stetina et al., 2007). Its nervous system is simple, consisting of only 302 neurons in the adult hermaphrodite (Sulston et al., 1983). Approximately 5000 chemical synapses and 600 gap junctions provide functional points of contact between the neurons (White et al., 1986). Although simple in its structure and neuronal connectivity, this nervous system controls a rich diversity of behaviours. These include locomotion, chemosensory and mechanosensory responses, as well as responses to temperature. Feeding, defecation, mating and egg laying behaviours are also well documented, and neurons controlling many of these behaviours have been identified (Chalasani et al., 2007; de Bono and Maricq, 2005; Hardaker et al., 2001; Kindt et al., 2007; Suzuki et al., 2008; Wakabayashi et al., 2004; Watts and Strogatz, 1998). The transparency of the worm and the invariant positions of neuronal cell bodies greatly facilitate the knocking out by laser of identifiable cells.

Over the last decade, exciting developments in electrophysiology on neurons and muscles in dissected preparations (Richmond and Jorgensen, 1999), as well as *in vivo* imaging of neuronal activity (Kerr and Schafer, 2006), have advanced considerably our understanding of the neurophysiological events underlying a particular behavioural response (Kindt et al., 2007; Suzuki et al., 2008). More recently, advances in the development of genetically targeted optical probes have allowed some experimenter control over the activity of neurones in the worm nervous system. For example, expressing light-activated chloride

Table 1 Some examples of nervous system and neuromuscular disorders modelled in *C. elegans*

Disease	Details	references
Alzheimer's Disease	Transgenic expression of human Aβ	(Drake et al., 2003; Link et al., 2003)
Alzheimer's Disease	Expression of modified tau	(Brandt et al., 2008)
Huntington's Disease	Polyglutamine repeats	(Faber et al., 2002; Satyal et al., 2000)
Spinal muscular atrophy	RNAi of SMN	(Burt et al., 2006; Miguel-Aliaga et al., 1999)
Parkinson's Disease	RNAi modifier screen of synuclein formations	(van Ham et al., 2008)

channels in identified neurones through a neurone-specific promoter allows spiking activity to be suppressed at controlled times (Zhang et al., 2007), and expressing the algal channelrhodopsin-2 protein in neurons or muscle allows cells to be excited by illumination (Nagel et al., 2005). The first animal genome to be sequenced was that of *C. elegans* (The *C. elegans* Sequencing Consortium, 1998), and the discovery of the RNA interference method for selective gene knockdown by Fire et al., (1998) and the ease of delivery of double-stranded RNA by feeding the worms with *E. coli* expressing siRNA encoding the target gene has since paved the way for genome-wide RNAi screens (Kamath et al., 2003). There are a number of *C. elegans* models of human disease, including Alzheimer's disease, Spinal Muscular Atrophy and Huntington's Disease (Table 1). These models lend themselves to high throughput screens using chemicals and RNAi to explore new routes to therapy and to test new chemical candidates in an inexpensive, *in vivo* disease model.

The current need for automated analysis of nematode locomotion

However, despite the utility of *C. elegans* models for genetic manipulations, their deployment in high throughput screens has been limited by labour-intensive manual assays used to score phenotypes. There is thus an urgent need for more rapid, and more consistent, methods for scoring phenotypes if the power of these models is to be fully utilized. Genome-wide approaches require rapid and trustworthy methods of assaying the phenotypic effects of genetic manipulations. Where mutations result in morphological phenotypes, large-scale screening presents little difficulties. In comparison, the effects of mutations on behaviour are more difficult to quantify, undermining some of the potential of this model organism as a tool for investigating the roles of genes and cells in generating behaviour.

The main sources of this difficulty are twofold. First, behavioural phenotypes are currently poorly defined and are designated using imprecise terminology. The "uncoordinated" phenotypes, for example, do not specify precisely which of the many aspects of the animal's locomotion, such as the amplitude of sinusoidal crawling, the frequency of turning or the speed of longitudinal wave progression, are uncoordinated. *Unc* terms such as "coiled", "loopy" or "sluggish", leave the experimenter exposed to the risk of subjectivity and inadvertent bias. The second source of difficulty is the paucity of methods for rapidly assaying *C. elegans* behaviour and representing behavioural phenotypes numerically for rigorous statistical analysis or for scoring in high-throughput screens. Over the past few years, the development of automated analysis of *C. elegans* locomotor behaviour using computer vision has begun to address this problem. Here we review the major approaches used in these analyses, illustrate how they have contributed to developing our understanding of *C. elegans* locomotion and locomotor phenotypes and assess their potential in large-scale screens.

Three strategies for automated analysis of *C. elegans* behaviour

Current computer vision programs for analysing *C. elegans* behaviour represent an attempt to increase the speed and precision with which worms can be phenotyped. In general, these "wormtracker" systems take one of three approaches. One of these is to measure the switching between specific, modular behaviours, such as crawling or egg laying. Another approach is to extract a discrete set of measurements from a limited aspect of a specific behaviour such as locomotion, foraging or egg-laying, in order to address a specific experimental question. A third, more complex and inductive approach, is to attempt a comprehensive description of all behaviours over a prolonged time period using a large list of numerical parameters. These large parameter datasets can then be used either in scoring a population of worms for a particular phenotype, as might be done in large scale chemical or genetic screens, or for quantifying phenotypes or even describing phenotypes hitherto thought of as being indistinguishable from wild type. This approach offers the possibility of providing numerically rigorous, comprehensive descriptions of worm behavioural phenotypes of breadth to match that of genetic approaches, but it is highly demanding of the programmer, and requires all the battery of current machine vision

expertise to be applied. Laboratories using this approach invariably achieve success with the help of experienced programmers, either in-house or by collaboration, and the willingness of these laboratories to share the fruits of these efforts is much valued by neuroscientists (Baek et al., 2002; Huang et al., 2007; Suzuki et al., 2008).

Tracking overall behaviour

In laboratory conditions, where worms are cultured on agar with or without *E. coli* as food, the behavioural repertoire of *C. elegans* is very limited, consisting mostly of locomotion, foraging (slow crawling with lateral head movements accompanied by pharyngeal pumping) and egg-laying. Locomotion has in turn been classified into four discrete activities: forward motion, backward motion, omega bends (changing direction of crawling by bending back on itself forming an omega-shape), and resting (Shingai, 2000). The modular character of this limited set of behaviours favours machine vision analysis approaches, as a small set of morphological or movement criteria can be established that reliably identifies each specific activity. The approach adopted in one such study (Hoshi and Shingai, 2006) was to classify worm movements over several minutes into each of the four locomotion states by first identifying the head and tail using a combination of four criteria (Fig. 1), permitting the identification of forward and backward movements. The remaining two locomotor states, resting and curling, could then be identified respectively as the absence of displacement and the failure by the system to identify the head. This program was used to determine the roles of identified neurones in

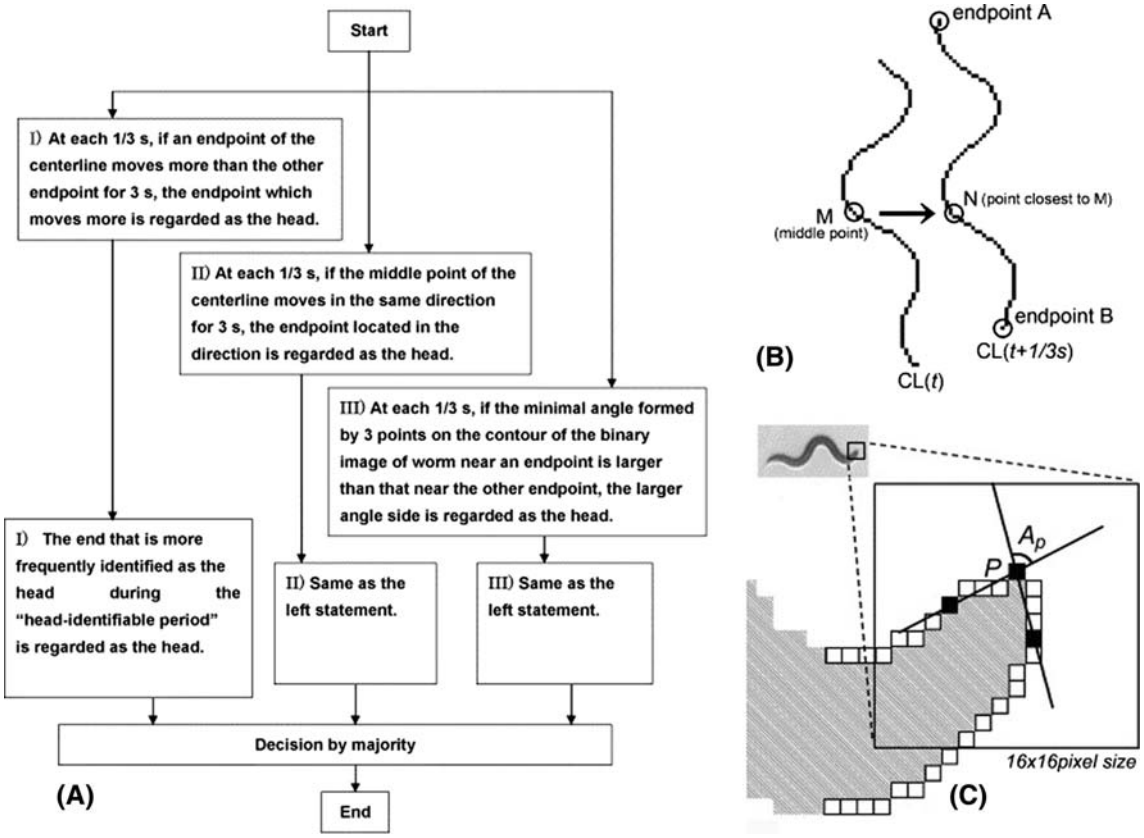

Fig. 1 The method used by (Hoshi and Shingai 2006) to classify worm behaviours using computer vision. **a** The decision tree for identifying the head, which is the first step in behaviour identification in this system. **b** Determination of the direction of movement, the second step in behaviour classification. Images of the centerlines at time t and t + 1/3 s, designated by CL(t) and CL(t + 1/3 s), are superimposed on the image frame. The middle point of CL(t) is denoted by M. The point N on CL(t + 1/3 s) which is closest to M is found by measuring the distance between M and every point on CL(t + 1/3 s). The direction of movement is determined by assessing distances, along CL(t + 1/3 s), from the point N to the endpoint A or B. In this figure, if N is closer to B than A, then the worm is moving in the direction of A. **c** Angle made by three points near the head in the contour of a binary image of a worm. From the binary image of a worm, two 16 × 16 pixel size squares whose centers are situated at endpoints of the centerline are extracted. In each square, a point P and two points that are 4 pixels apart along the borderline from P are taken on the borderline of the image. AP is the angle at P made by connecting the three points. By moving the three points on the borderline, the smallest angle APmin is obtained for the worm's image in each square. Adapted from Hoshi and Shingai (2006), with permission

locomotion by measuring the effects of laser ablating single neurones upon time-dependent switching between locomotor behaviours (Wakabayashi et al., 2004).

Because the analysis of the activities of individual worms can be computationally expensive and yet still in some cases require considerable human supervision, some wormtrackers have applied a coarser resolution of measurement. For example, a less precise measure of worm activity has been achieved by measuring the scatter of an infrared beam passing through a culture containing several worms, the degree of time-dependent scattering providing an index of the overall level of movement in the worms (Simonetta and Golombek, 2007). This application was used successfully to measure *C. elegans* circadian rhythms.

Detection and measurement of distinct behaviours

Automated analysis can also be accomplished by performing a discrete set of measurements on a specific behaviour, where the tracked behaviour is associated with a unique visual feature. Foraging, for example, involves a series of side-to-side movements of the head (Kindt et al., 2007), thus providing a visual cue for the automatic detection and analysis of foraging (Huang et al., 2008). A similar method was deployed to measure the control of egg-laying and turning events by serotonin (Hardaker et al., 2001). Another such system measured olfactory changes in swimming behaviour using an automated process that films swimming and counts the number of times the head/tail distance falls below a certain threshold value (Luo et al., 2008). Such approaches are limited to behaviours that are marked by a unique and distinctive visual feature, but are comparatively simple in computational terms.

Measuring the complete behavioural repertoire using large parameter sets

Some worm trackers are designed to track large number of worms, but avoid excessive computational demand by extracting a limited set of features from them. The "Parallel Worm Tracker" from the laboratory of Miriam Goodman extracts the positions of the worm centroids and from that determine the tracks of individual worms (Ramot et al., 2008). This system has the advantage over many similar ones in that several worms can be tracked simultaneously. However, although these tracks contain information that can be derived from them, such as speed and rate of reversals, considerable details about the conformation of the worms is lost, including the rate and amplitude of sinusoidal bending, both of which may provide clues to the biological significance of mutations. Nonetheless, this approach is computationally efficient and has proved useful in measuring the time-dependent induction of paralysis by levamisole (Ramot et al., 2008). Another wormtracker has been recently developed for analysing the thrashing behaviour of worms in a liquid medium (Tsechpenakis et al., 2008). So-called "Thrashing assays", which measure swimming in liquid medium, are widely used as a parameterized assay for the actions of drugs or the locomotor effects of mutations, but are time-consuming and liable to subjectivity. The availability of an automated swimming (thrashing) assay, particularly one that can analyse several worms simultaneously, will be of great value for high throughput screening.

Perhaps the most exciting developments are wormtrackers whose goal is to allow a comprehensive set of measurements to be performed on freely moving worms. William Schafer's lab have made significant contributions to developing this kind of computer vision approach to worm phenotyping. Using a system which employed a moving stage to follow freely moving worms, they measured 94 morphological and dynamic parameters from wild type and mutant worms (Baek et al., 2002). These parameters were then analysed using the "classification and regression tree" (CART) method, an established technique for discovering parameters that reliably distinguish populations. This approach allowed wild-type and 5 mutant strains to be automatically distinguished with about 90% accuracy. A similar system expanded to incorporate 253 parameters used natural clustering and principal components analysis to distinguish 8 distinct strains (Geng et al., 2003). Further refinements of the system allowed distinct behaviours, such as foraging, to be included in the parameter set (Feng et al., 2004). The release of these software systems as compiled packages (Cronin et al., 2005; Feng et al., 2004) provides a valuable resource for *C. elegans* neuroscientists.

Computational strategies in worm tracking

All automated vision programmes for worms follow a similar processing strategy which usually involves extraction of the worm from the background (segmentation) followed by reduction of the worm to a skeleton (Figure 2). This process then allows a large number of parameters to be extracted. This invariably begins with the problem of segmentation (separation of the worm from the background). The ease with which the human visual system distinguishes a target object from the background, a task performed unconsciously, conceals the difficulties of such a task. The problem is to identify features inherent in the picture that allows the machine to determine where the worm ends and the background begins. The difficulties can be compounded by the variety of visual contexts that the tracker can be presented with, notably differences in lighting conditions

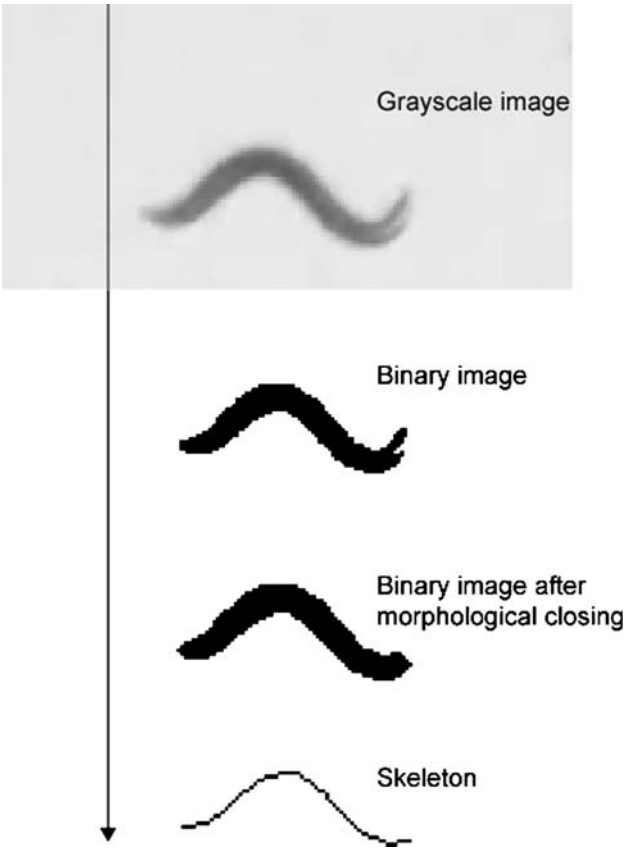

Fig. 2 Computer vision is a solution to the problem of abstracting numerical information from a sequence of images. Worms must first be segmented from the background. This can be done by several different methods, the simplest of which is to segment by intensity threshold. Pixels identified as being worm are then set to a value of 1 and the background to a value of 0 to produce a binary image. Segmentation is often imperfect and can leave holes inside the worm body and small irregularities in the outline. These can be removed using a standard closing operation (a morphological dilation followed by an erosion). The resulting silhouette can then be reduced with thinning algorithms to result in a skeleton, which is a 1 dimensional curve easily represented and analyzed numerically. In addition, the edge pixels can be identified to produce an outline of the worm

and the presence of clutter (worm tracks and eggs). Different lighting conditions can make a worm appear as light on a dark background or as dark on a light background, while in many lighting conditions some parts of the worm are of the same intensity as the background. The presence of clutter is even more challenging, as the worms leave sinusoidal tracks which strongly resemble worms. Some of these problems can be overcome by exercising care to film the worms under conditions that provide consistently high contrast between worm and background, in other words filming with the restrictions of the program in mind. Segmentation can be achieved by thresholding alone – attributing pixels to worm or background according to whether their intensity exceeds a certain threshold value. There are many well-developed algorithms for determining the threshold, the simplest (and least safe) being to calculate the value midway between the extremes. However, where segmentation relies entirely on threshold, it is preferable to allow the user to interactively set the threshold, by either using a slider or entering a value as a program parameter. Segmenting by intensity threshold is computationally simple and fast, but there are other methods of segmentation that are automatic and therefore better suited to fully automated systems such as those used in high throughput screens. The method deployed by Schafer's laboratory (Geng et al., 2003) is based on the standard deviation of regions in the image. A small window scans across the image, and if the mean intensity in the window is less than $0.7b$ (b is the intensity of the background level) or the standard deviation larger than 0.3 m (m is the mean intensity in the scanning window) then the pixel was considered to be a pixel of the worm body and allocated to the worm. This algorithm performs well because it is insensitive to regional (low frequency) alterations in background. However, it is very slow and has to be applied to every image in the sequence. Another approach is to segment worms according to texture. K-means segmentation separates an image by grouping regions into a predetermined set of clusters. This is a fast algorithm, but it is not always possible to determine in advance which cluster will represent the worm or how many clusters need to be chosen so that one of them will be worm. An additional approach is to use edge detection methods. The well used Sobel and Canny edge detectors offer the advantage of being relatively insensitive to slow changes in illumination and so are unaffected by uneven illumination of the scene, something which can make threshold and even K-means segmentation unworkable in certain situations.

Whichever method is chosen, segmentation results in a 2 dimensional binary (ie consisting of 1 s or 0 s) matrix, in which one value represents a pixel on foreground and the other represents pixels belonging to the background. Imperfections in segmentation can leave holes inside the worm object which are usually removed using an established opening operation. Some features can be readily extracted at this point, such as the area calculated by counting the 1 s and correcting for diagonal errors (the border passing at 45° through a pixel should be counted as ½, whereas simple pixel counting would return a 1 or 0 at this point). The bounding ellipse along with its orientation and eccentricity are also usually extracted. The boundary of the worm can easily be estimated by setting all "on" pixels without an "off" neighbour to "off", allowing the perimeter to be estimated from which features such as compactness and roundness can be derived.

For mathematical and statistical analysis, the shape of the worm must be parameterized (Fig 3). The numerical description of shape, however, is a complex topic. The

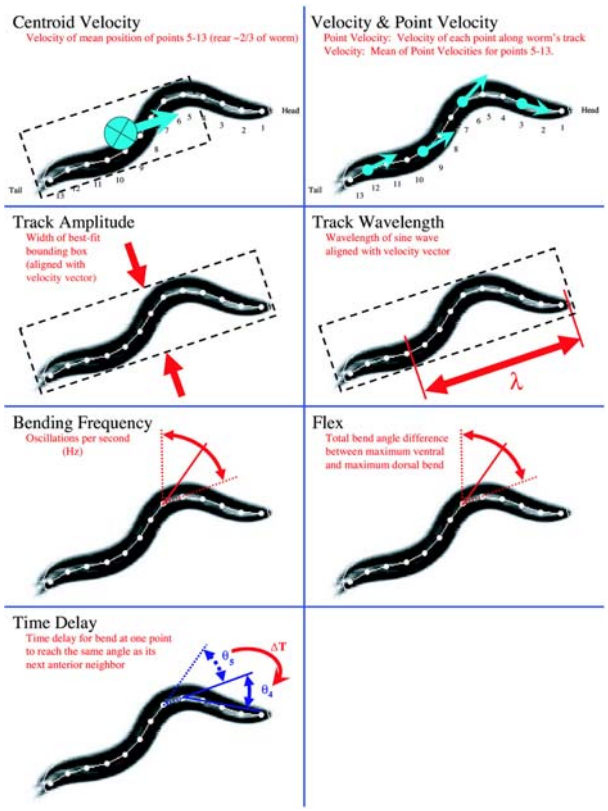

Fig. 3 An illustration of some parameters of worm movement that can be extracted from a skeletonised representation of a worm combined with its outline. Many trackers aim to collect as many parameters as possible, many of which will be redundant. Reproduced with permission from (Cronin et al. 2005)

problem of reducing a worm outline to a set of numbers is compounded by the range of possible shapes a worm may adopt, worms being highly deformable and non-rigid. Most analysis methods aim to reproduce shape abstractly and normalised for position, orientation and sometimes for scale, so that shapes can then be compared using standard methods such as least square differences. A common solution in automated methods is to reduce the worm to a skeleton (a one pixel-thick line through the centre of the shape) and the worm's outline. The resultant skeleton is a 1 dimensional shape and is therefore easier to parameterize as it can be considered mathematically as an arc function. The skeleton can also be represented as a polyline (a string of connected straight lines) and the angles between the segments measured. The process of skeletonization is liable to error as most algorithms are sensitive to small errors in the shape. A worm should ideally be skeletonized to a single curve, but small protuberances or holes left in the closing operation result in branches and loops, or even more complicated graphs. Skeletonization errors are even more severe when there are errors in the boundaries introduced by worm coiling or omega bends. A coiled or omega-bent worm will skeletonize to a circle (or a very short line if the central hole is filled by the closing operation). Few published computer vision programmes are capable of overcoming this – with the notable exception of one system (Roussel et al., 2007) which uses a completely different approach to determining the spine. Here, once the edges of the worm are identified using active contours, the edges are fit to a closed B-spline (a closed spline is one that forms a continuous loop) and dynamic programming is used to find a path between the edges that minimises the distance to either edge. This results in a spine with no branches as well as a width measurement at each point along the spine, these two datasets being enough to reconstruct the worm shape. This program also, attempts to resolve the problems that arise when worms coil or collide.

In addition to parameters derived from worm shapes on a per-frame basis, it is usually desirable to represent the movement of the worms, both in terms of the deformation and translation. The lowest level of inter-frame analysis is to describe the evolution of per-frame parameters over time. Thus, overall speed of individual worms is described as the rate of change of their respective centroids (the centroid is a point representing the visual "centre of gravity" of an object). This is essentially what the Goodman wormtracker provides (Ramot et al., 2008). More detail about the changes in deformation can be derived from changes in the conformation of the skeleton. In principle this can be done by representing the continual curvature of the spine changing continuously over time. This can be parameterized and therefore compared between worms and used as part of a strategy to identify phenotypes. However, this does not allow for a point along a worm's body to be correlated between frames, as the same point in the spine may represent slightly different points along the worm's length if the pixel-length of the worm changes between frames. The solution followed by many programs, including NEMO (Tsibidis and Tavernarakis, 2007), (Fig. 4) is to convert the spine into a string of connected straight segments of fixed number. Each of these segments therefore approximates to a segment of the worm body. Angles between segments can therefore be measured and their changes over time represented.

Current developments in worm trackers

Among those programs that track multiple worms, few attempt to resolve the problems that arise when worms interact or when individual worms coil up on themselves. Interaction presents difficulties both for segmenting a region into two separate worms and for preserving identity of the two worms during and after interactions. Most worm trackers simply stop the track at this point and resume them

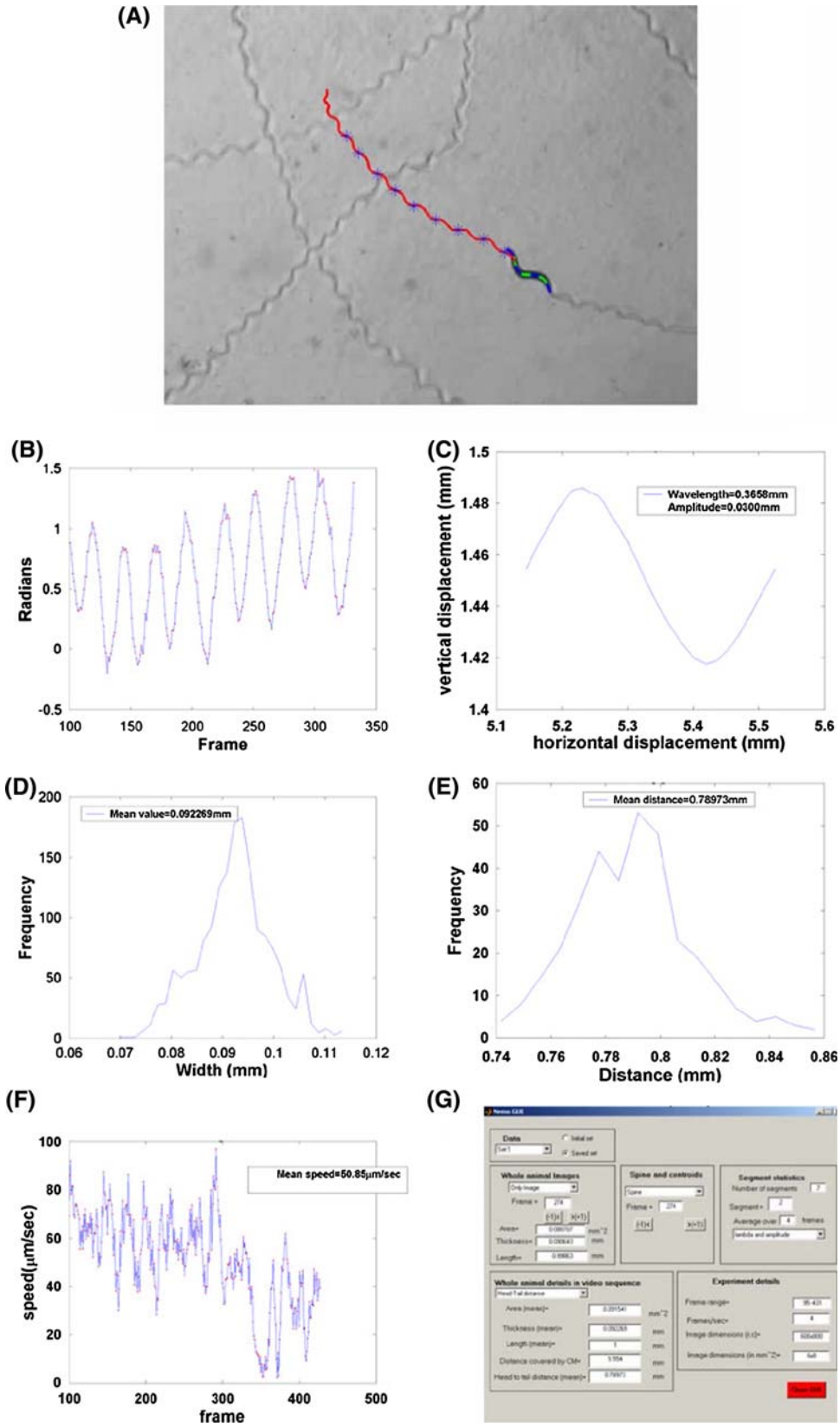

Fig. 4 Images and data plots from the NEMO wormtracker programme illustrate user-friendly approaches to extracting several parameters from movies of freely-moving worms. **a** Trajectory of animal locomotion, **b** angle evolution with respect to frame number, **c** waveform of the movement, **d** width histogram, **e** distance between head-tail, **f** speed series with respect to frame number, **g** Graphical user interface. Adapted from (Tsibidis and Tavernarakis 2007)

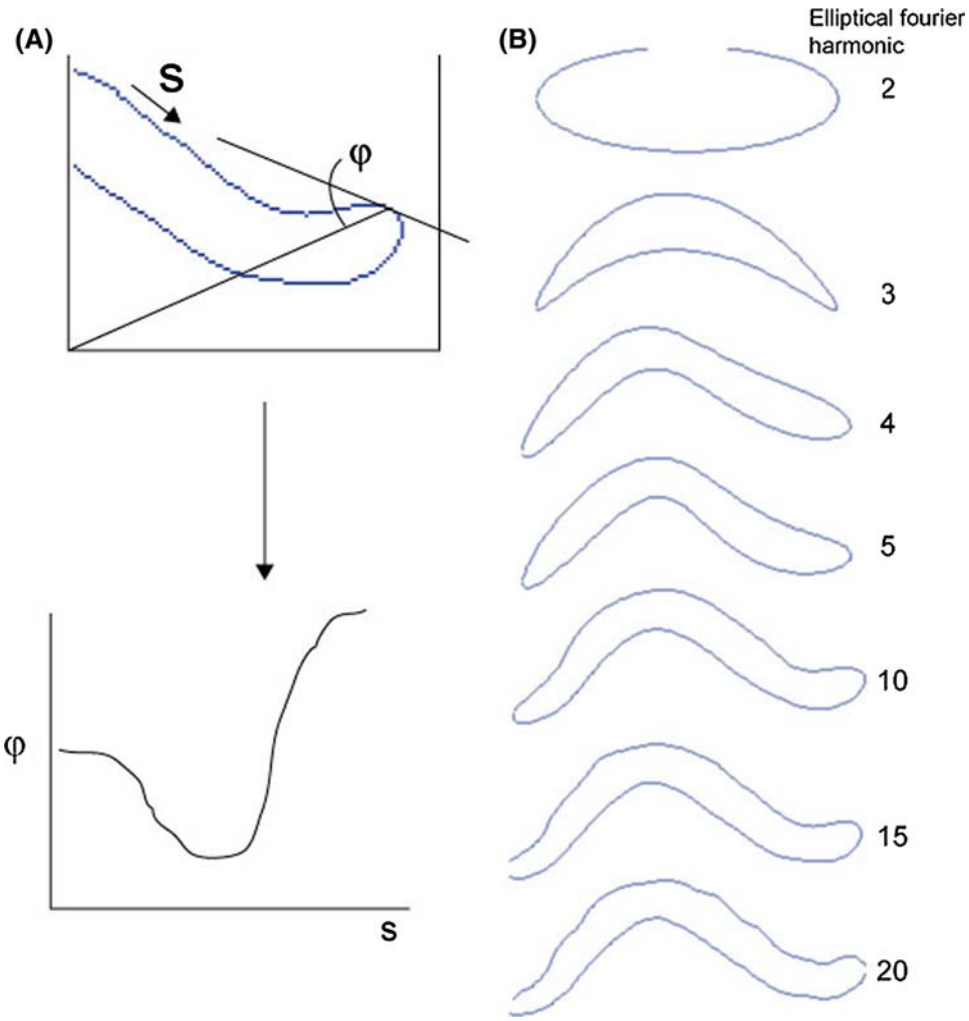

Fig. 5 Future developments in worm tracking might take advantage of several methods for parameterizing arbitrary closed shapes. **a** the Ψ-S function is derived from an outline by taking the angle, φ, formed at a *point S* on the outline between a *line* from the origin and the tangent at S. This descriptor is invariant for position but not for size or orientation. **b** Elliptical Fourier decomposition analyses any arbitrary shape in terms of a series of ellipses, analogous to the classical Fourier decomposition of a one-dimensional signal into sines and cosines. It provides a descriptor comprising a set of harmonics which progressively describe finer details of the shape. The minimum number of harmonics required to provide a satisfactory reconstruction of the shape must be determined experimentally. This method is invariant for position and can be made invariant for scale and orientation

as new tracks after interaction is finished. However, programs and algorithms are now being developed to address this problem (Huang et al., 2007). One computational model (Roussel et al., 2007) combines hypothesis tracking (following several possible hypotheses that interpret the image sequence and selecting the most likely) with an energy barrier approach which encapsulates the observation that when worms collide they usually prefer to pass side-by-side rather than to cross over. In another study, the Central Diffuse Kalman Filter, which has generally proved powerful in many computer vision applications to identify partly occluded shapes, was combined with worm models to allow interpretation of images of worms partly occluding each other (Fontaine et al., 2006). The application of these algorithms results in more robust measurements, and by allowing interacting worms to be tracked, it may be possible to capture the effects of mutations on social behaviours, as well as increase the range of parameter space over which new phenotypes can be identified.

In the wider field of computer vision, considerable improvements in performance of object recognition are achieved by providing the machine with a model of the target objects and this real-world knowledge is then used to map a 2D image to these models. These solutions perform considerably better than the purely bottom-up approaches used in machine vision of *C. elegans* (Fontaine et al., 2006). Given that there are constraints on the shapes that worms can adopt, as well as on the rate at which those shapes can change, a system in which model worms are matched to an image can be envisaged, and would be expected to perform better in situations where most existing processes fail, notably worm coiling, aggregates of worms and omega turns. This approach has been adopted. Worms were modelled as a series of rectangular blocks connected with springs, and the complexity of the matching reduced using dynamic programming (Huang et al., 2007). This system correctly tracked pairs of worms at over 75% accuracy even when worms were touching each other.

Future directions in tracking

Given that many of the parameters measured by worm trackers hinge upon the numerical description of shape, there remains much scope for exploiting the many well-established methods of representing 2 dimensional geometric forms in ways that are invariant for position, scale and rotation. A convenient way of representing shape is to take the Ψ-S relationship of the outline (Figure 5A). Because the outline is endless, the Ψ-S is therefore cyclic and so can be decomposed using Fourier methods. Alternatively, the first derivative of the chain-code describing the worm outline can be normalized to produce a scale, rotation and position invariant unique number describing that shape. The only disadvantage with this method is that it would require the length of the outline (the number of items in the chain code or the number of points describing the periphery) to be constant from worm to worm and from frame to frame. A more recent application is the use of elliptical Fourier decomposition (Figure 5B), which describes the shape in terms of a series of ellipses, similar to the way in which standard Fourier transforms describe a signal in terms of a series of sines and cosines. This approach has been used successfully to quantify morphology of primate jaws and brachiopod shells, and provides a numerical means of comparing shapes that is then amenable to statistical analysis. Furthermore, standard Fourier transforms of outlines and elliptical Fourier decompositions can be used to measure the rate at which shapes change form by taking the Euclidean distance between the harmonics. In principle, since the worms are presumably limited in the rates at which they can change shape, limitations in the Euclidean distance between respective harmonics between frames can be used to anticipate the position of the worm edge in subsequent frames which could improve the identification of worm outline in complex situations such as coiling, omega turns or aggregations.

The need for standardization

If the problems of machine vision are solved and machine vision is increasingly combined with genetics, it will become important for data from different studies to be readily comparable (Feng et al., 2004). However, although this might argue for standardization in the parameters used in describing worm locomotion, this should not be done at the cost of flexibility. The choice of parameters to be measured by any machine vision system is critically dependent upon the application. In cases where a specific behaviour, such as egg-laying, is addressed, or in screening for a known morphology, the choice will be determined as that which maximises the likelihood of correctly identifying a feature and minimises the likelihood of wrongly identifying one. In contrast, applications which focus on rigorous definition of known phenotypes or those requiring natural clustering of phenotypes will benefit from a wide range of parameters. In the latter case, if a biological interpretation is the ultimate goal, this will argue for parameters that are easily mapped to biological features. This would favour parameters that can easily be interpreted in terms of the sensory and motor systems of the worm over more abstract geometrical parameters such as Fourier descriptors. However, the fundamental advantage of the numerical approach inherent in worm trackers means that however difficult, there is likely to be a function that maps any pairs of parameters. However, it will be highly desirable for large datasets on diverse worm strains to be directly comparable, highlighting the eventual need for an agreed set of standards which, like comparable standards in gene expression data, must have the inbuilt flexibility to accommodate emerging needs.

Another exciting area of standardization includes placing worms in a standard particulate environment, such as "artificial dirt" that has recently been developed (Lockery et al., 2008)

Conclusions: state of the art and the potential for high-throughput genetic and chemical screening

Automated analysis of *C. elegans* behaviour is a rapidly developing field. Parameterization of worm outlines and skeletons has become a standard method, and attempts at overcoming the difficulties introduced by interactions between worms, as well as worm coiling and omega turning, are meeting with some success. Current methods have facilitated the identification of subtle phenotypes (Huang et al., 2006) and the characterisation of roles of neurones in forward locomotion (Shingai, 2000) and chemotaxis (Suzuki et al., 2008), as well as the quantitative characterisation of behaviour choice (Hoshi and Shingai, 2006) and circadian patterns of activity (Simonetta and Golombek, 2007). Given the speed with which *C. elegans* has been deployed in genetic screens and chemical screens, it is to be hoped that wormtrackers may eventually provide similar rapidity in assaying behavioural phenotypes. However, considerable progress must be made before this can be accomplished. In the case of genome-wide RNAi screens, for example, the presence in the worm genome of some 19,000 genes means that even the minimal user intervention in an automatic phenotyping system will be very costly. Nonetheless, recent advances have shown that drug actions on large numbers of worms can be tracked (Ramot et al., 2008; Tsechpenakis et al., 2008), raising hopes that high-throughput behaviour-based drug screens may soon be available.

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