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Invited Review

Automated, high-throughput, motility analysis in *Caenorhabditis elegans* and parasitic nematodes: Applications in the search for new anthelmintics

Steven D. Buckingham, Frederick A. Partridge, David B. Sattelle *

Wolfson Institute for Biomedical Research, Division of Medicine, University College London, Cruciform Building, Gower Street, London WC1E 6BT, United Kingdom

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ABSTRACT

The scale of the damage worldwide to human health, animal health and agricultural crops resulting from parasitic nematodes, together with the paucity of treatments and the threat of developing resistance to the limited set of widely-deployed chemical tools, underlines the urgent need to develop novel drugs and chemicals to control nematode parasites. Robust chemical screens which can be automated are a key part of that discovery process. Hitherto, the successful automation of nematode behaviours has been a bottleneck in the chemical discovery process. As the measurement of nematode motility can provide a direct scalar readout of the activity of the neuromuscular system and an indirect measure of the health of the animal, this omission is acute. Motility offers a useful assay for high-throughput, phenotypic drug/chemical screening and several recent developments have helped realise, at least in part, the potential of nematode-based drug screening. Here we review the challenges encountered in automating nematode motility and some important developments in the application of machine vision, statistical imaging and tracking approaches which enable the automated characterisation of nematode movement. Such developments facilitate automated screening for new drugs and chemicals aimed at controlling human and animal nematode parasites (anthelmintics) and plant nematode parasites (nematicides).

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* Corresponding author at: Wolfson Institute for Biomedical Research, Division of Medicine, University College London, Cruciform Building, Gower Street, London WC1E 6BT, United Kingdom. Tel.: +44 (0) 2076796613.
E-mail address: d.sattelle@ucl.ac.uk (D.B. Sattelle).

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1. Introduction

Parasitic nematode worms present major challenges to human health, the health and productivity of farm livestock as well as crop plant productivity. Human parasitic nematodes infect about a third of the world's population, causing significant morbidity (Hotez et al., 2008). The scale of the veterinary anti-parasitic drug market (Evans and Chapple, 2002) and the annual harvest damage resulting from plant parasitic nematodes (Nicol et al., 2011) mean that new control chemicals are urgently required, a situation exacerbated in the case of nematodes that infest farm animals, where growing resistance to existing treatments is a major problem (Sutherland and Leathwick, 2011).

The shortage of new drugs and chemicals to combat nematode parasites and the threat of resistance are driving the search for new chemical leads. The most direct route to discovery is via nematode phenotypic screening and for some species the large number of worms that can be obtained facilitates such approaches. Although current trends in drug discovery have shifted towards target-based screening (Eder et al., 2014), phenotypic screens remain very successful (Swinney and Anthony, 2011 ; Eder et al., 2014). However, nematode phenotypic screening for new anthelmintic leads is currently severely limited by the rate at which the chemical impact on nematode phenotype can be scored. Large-scale screens require a simple readout and parameters of interest include animal death, paralysis, impaired motility and failure at some crucial point in the life cycle such as egg laying, hatching or moulting. Without automated phenotyping, the researcher has no option but to score manually. Even when scoring is straightforward, as with counting moving versus immobile animals, the task is slow, tedious and repetitive and is therefore subject to errors due to experimenter fatigue. Even the most accurately performed manual scoring systems may still discard a lot of data, making antiparasitic actions which affect behaviour without killing or totally paralysing the worms impractical for drug discovery screens. The same arguments apply to another area of drug discovery based on nematode (*Caenorhabditis elegans*) models of human disease where genetic models can be generated in a few weeks and the worms handled using standard liquid handling techniques, including microfluidics (Culetto and Sattelle, 2000; Kaletta and Hengartner, 2006). Once again the promise of large-scale, chemical screening of such models is limited by the rate at which their (often subtle) phenotypes can be assayed. Several approaches have been pursued in attempts to deliver robust, automated assays of nematode behaviour. In this review we first consider the challenges faced, then address recent progress in the field of motility automation and finally suggest a roadmap for the development of industry-standard, automated, nematode phenotyping.

2. The scale and urgency of the challenge to discover new anthelmintics

Almost one third of the world's population are infected with helminth parasites (Hotez et al., 2008). Helminth infections such as ascariasis, trichuriasis, strongyloidiasis, hookworm, liver fluke and intestinal flukes are among the most important gastrointestinal infections of humans with over 1 billion estimated to be suffering as a result of infection by one or more of these parasites (McCarty et al., 2014). Precise estimates of the adverse effects of nematodes on humans are hard to obtain, partly because of the difficulties in agreeing upon a robust and meaningful measure. However, a statistic known as Disability-Adjusted Life Years (DALYs), the estimated number of healthy years lost to a disease, has recently gained acceptance (Murray et al., 2012). The 2010 Global Burden of Disease (GBD) study reported 807–1221 million people suffer *Ascaris lumbricoides* infection, 604–795 million have *Trichuris trichiura* infection, and 576–740 million have hookworm (*Necator americanus*, *Ancylostoma duodenale*) infection (Vos et al., 2012). Although relatively few deaths were attributable to each species, the loss of 5.6 million DALYs globally was attributable to these infestations. The majority of DALYs were in Southeast Asia (47%) and sub-Saharan Africa (23%). Despite the acknowledged limitations of recording the impact of human parasitic nematodes in this way, the fact remains that such numbers, coupled with the indirect costs to agricultural productivity, amount to a serious human health and agricultural problem. There is considerable commercial incentive for developing new anthelmintics: the human health antiparasitic drug market is around $0.5 billion/annum. Despite these humanitarian and commercial incentives, it remains the case that nearly all anthelmintics currently used to treat humans were first developed as veterinary products.

The veterinary economic burden resulting from internal and external parasites is reflected in the scale of the global animal health drug market - approximately $11 billion/annum (Evans and Chapple, 2002). The problem of drug resistance in nematodes of veterinary importance is also considerable (Kaplan, 2004). Crop production can be severely compromised by nematode infections, which result in an estimated $118 billion annual loss (Atkinson et al., 2012). Such losses are disproportionately high in tropical and sub-tropical climates, often in countries least able to cope with major crop damage. For example, total crop production losses attributable to nematodes have been estimated at 14.6% in developing countries compared to 8.8% in developed countries (Nicol et al., 2011).

3. The problem of resistance

The considerable challenges arising from human and animal nematode parasites are compounded by the rise of drug/chemical resistance, which has been recognised for many years (Waller, 1999), and has now developed to the point where there are currently no major livestock-producing areas in which either levamisole or the benzimidazoles can be considered highly effective anthelmintics. Even more recently-introduced compounds such as moxidectin are largely ineffective for certain species in all such areas (Kaplan, 2004). There are also cases of resistance to monepantel, the most recently introduced anthelmintic, appearing in less than 2 years of it being used on a New Zealand farm (Scott et al., 2013).

Because resistance often applies to other members of the same chemical family, we urgently need to discover new classes of chemistry with potent anthelmintic actions. The quickest way to find new drugs and, simultaneously, new targets, is through phenotypic screening.

4. High-throughput phenotypic screening for new anthelmintics

In the search for novel human therapeutics, phenotypic screening is usually the domain of cell-based studies. This is justifiable due to the ability to screen extremely large chemical libraries relatively quickly. In contrast, *whole organism* screening, though desirable, is normally considered impractical, owing to high costs, unacceptable animal numbers required and lengthy generation times. When it comes to anthelmintics, target-based screening using cell lines is a relatively new field, though undoubtedly one that will grow (Woods et al., 2011). However large-scale, *whole organism* screening is not an obstacle when it comes to drug screening using nematodes. *C. elegans*, is a small (1 mm), self-fertilising, free-living roundworm and its fast generation time – 3 days from

embryo to fertile adult – and ease of laboratory maintenance lends itself well to the requirements of *whole organism* drug screening. Not all, but a number of important human, animal and plant parasitic nematodes can also be accessed in large enough numbers to permit library-scale chemical screening. These include *Toxocara canis* (the dog roundworm) (Ponce-Macotela et al., 2011), *A. lumbricoides* (Fairbairn et al., 1961), *Globodera rostochiensis* (Byrne et al., 2001), *Ascaris suum* (Urban and Douvres, 1981), *Trichuris muris* (Fahmy, 1954), *Ancylostoma ceylanicum* (Reiss et al., 2007) and *Haemonchus contortus* (Hubert and Kerboeuf, 1992). The use of *whole organisms* in antiparasitic drug screening confers important advantages. For example, cell-based assays do not fully replicate many complex physiological systems while *whole organism* screens have the benefit of self-selecting compounds with favourable pharmacokinetic parameters.

4.1. Lessons from using nematodes for human drug discovery

Although *C. elegans* is a free-living nematode, its similarity to parasitic species suggests that it can be used as an approximate model of parasitic worms. After all, it has been used successfully to model many human diseases (Culetto and Sattelle, 2000; Kaletta and Hengartner, 2006). The costs to a discovery pipeline caused by the differences between free-living and parasitic worms (false positives and negatives) are offset by the remarkable convenience of using this species. When placed in a liquid, *C. elegans* "swim" using a stereotyped, rhythmic, oscillating body motion. This motion is a direct readout of the neuromuscular system and an indirect assay of the health of the animal. Conveniently, worms continue to swim in liquid for long periods (indeed, worms can be maintained in liquid culture), although potential changes in swimming rate over time should be accounted for in using this as a scored phenotype. Swimming therefore offers a useful measurement, well-suited for the readily-scorable, phenotypic readout required for a large-scale screen, as it is quantified as a simple scalar number – the number of body bends per minute. The rate of swimming (thrashing) can be measured manually, either using a microscope and stopwatch or by observing films of swimming worms but in both cases this is laborious and error prone. Clearly, this approach does not lend itself to large-scale screening – the measurements need to be automated.

4.2. Parasitic nematodes and phenotypic screening: some biological considerations

Endoparasitic nematodes are adapted to the environment inside their host, and this often poses a challenge to maintaining them in the laboratory. It would be impractical to list all the many protocols for accomplishing this, but they include methods for hatching *T. canis* (the dog roundworm) (Ponce-Macotela et al., 2011), *A. lumbricoides* (Fairbairn et al., 1961) and *G. rostochiensis* (Byrne et al., 2001). Protocols for the maintenance of *A. suum* from third to fourth stage larvae (Urban and Douvres, 1981); as well as for isolating larvae of *T. muris* (Fahmy, 1954) are available as are techniques for culturing larvae of *A. ceylanicum* (Reiss et al., 2007) and *H. contortus* (Hubert and Kerboeuf, 1992). Thus, some parasitic nematodes can be maintained in conditions necessary for *whole organism* assays of drugs.

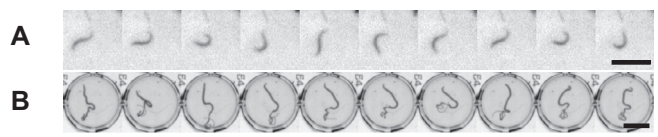

Fig. 1. Nematodes range in size and have diverse and complex behaviours. Movie frames showing (A) rhythmic swimming (thrashing) movements seen in *Caenorhabditis elegans* (scale indicates 1 mm) and (B) more complex movements recorded from *Trichuris muris* (scale indicates 3 mm).

4.3. Specifications for automated phenotyping and the challenge in meeting them

Anthelmintic drug discovery assays need to rapidly report compounds that kill worms, impair motility, or prevent the life cycle from continuing. Only a small proportion of drugs will have any such effect and the hit rate from even large chemical libraries can be notoriously small. Such an assay must therefore be fast enough to survey large chemical libraries in a very short time. Once a hit has been obtained, a further optimisation step must then be implemented in which a new chemical library is tested in order to explore the chemical space around the original hit molecule. All this highlights the demand for rapid, high-throughput assays. Clearly, manual assays such as staining with Lugol's iodine solution and counting the number of eggs that have hatched, or scoring worms manually for their motility, are not practical at such a scale of throughput. Thus, the discovery of new anthelmintic drugs requires some sort of automation. There are two aspects of assays that could be automated, first the detection of the drug's actions on the worm and secondly the automated handling of the nematodes. The challenges are compounded by the range of size and diverse activity of parasitic nematodes, many of which show complex patterns of movement quite distinct from that seen in *C. elegans* (Fig. 1).

4.4. Drug assay automation

An effective drug for treating nematode infestation is one that reduces, or eliminates, the worm burden on the host. There are several ways in which this can be done, but most drugs either halt the progression of the life cycle, or kill/paralyse worms. Although some parasitic worms can be maintained outside the host, often this is difficult, placing severe limitations on any automated method. This means that automated assays are limited, in practice, to finding drugs that either kill worms, or at least immobilize them. In addition, the experimenter usually has a limited choice as to which stages of the parasite can be conveniently studied in the laboratory. So the task of automating drug discovery in this context is often reduced to finding compounds that kill or immobilize a specific stage of the worm's life cycle.

4.5. Can computers tell when a drug is affecting a worm?

There are several possible approaches to automation, and a good introduction to the field is to be found in Husson et al. (2013). An obvious strategy is to try to emulate the way the assay is performed manually. For example, WR Schafer's lab have attempted to automate egg laying assays for the nematode *C. elegans* (Geng et al., 2005) using a computer vision approach. The algorithm detected the characteristic outline formed by an egg emerging from the body wall of an adult worm. Using similar approaches, several laboratories have over the past decade reported methods based on computer vision for identifying nematodes in images or even quantifying and identifying their behaviour (for review see Buckingham and Sattelle, 2008). Nearly all of these studies were aimed at *C. elegans* because of the scientific interest and medical applications as potential human disease models. We will re-examine them here as potential starting points for extending such approaches to parasitic nematodes and as case studies to assess the promise, and pitfalls, in attempting to redeploy algorithms developed for a free-living genetic model organism in assays using parasitic nematodes. In this context, we pose the question, "Can the

algorithms that work on *C. elegans* be applied to parasitic nematodes and are they sufficiently fast and robust to work in high-throughput screens?"

The promise of *C. elegans* as a model of human disease and as a model of metazoan nervous system function in general is hampered by a bottleneck in phenotyping. Mutants or knockouts of almost every gene in the *C. elegans* genome are available by order from repositories, and this opens up many exciting opportunities for mapping genes to function, as well as identifying new targets for human disease or novel anthelmintics (we have argued above that the potential for speed of discovery in the free living *C. elegans* is expected to compensate, at least partly, for its limitations as a model of parasitic species). In contrast, behavioural phenotyping is no faster and may even be slower, than that seen in studies of mice, and usually consists of subjective descriptors, with no rigorous definitions of such terms whose meaning is enshrined in community consensus. Although other scorable behavioural phenotypic assays exist, such as pharyngeal pumping (Jadhav and Rajini, 2009), the swimming assay (see above) remains the most widely accepted phenotypic scoring method.

Several laboratories, some from an engineering background and others from a "worm" background, have tackled the problem of automating worm (*C. elegans*) phenotyping. Most use well-established, machine-vision approaches (foreground segmentation, binarization, skeletonisation, etc.), often coupled with machine-learning algorithms that use supervised or unsupervised (e.g. clustering) methods to quantify behaviour. One such example is Nemo, which takes moving images of worms on plates coated with an agar-based growth medium and calculates several parameters of worm shape. Nemo has a graphical user interface and yields high resolution data (Tsibidis and Tavernarakis, 2007). Cronin et al. (2005) report another system following similar principles.

A major breakthrough in automation has been the development of low-cost, automated tracking microscopes, allowing the assembly of a behavioural database for 305 *C. elegans* strains by WR Schafer and colleagues (Yemini et al., 2013). This includes 76 mutants with no previously described phenotype. The database consists of 9203 short videos of individual worms segmented to extract behavioural and morphological features. The resource is accessible on line and data are available for further analysis. The authors have included summary statistics for 702 measures with statistical comparisons to wild-type controls so that phenotypes can be identified and understood by users. Among the many possible beneficial outcomes of this work is the development of new algorithms for feature measurements, some of which could greatly enhance drug screens.

Recently, the factors influencing the two kinds of *C. elegans* foraging behaviours (roaming and dwelling) have been explored by filming for 90 min at 3 frames/s worms on agar plates seeded with bacteria (Flavell et al., 2013). Worm trajectories were extracted from videos using custom scripts that calculated the speed and angular speed of each animal. Averaging over 10 s intervals, easily separated roaming and dwelling intervals (Ben Arous et al., 2009) allowing a description of an animal's trajectory as a sequence of roaming and dwelling intervals.

Some systems deserve particular mention for their innovative nature. Standard, flatbed scanners have been used to develop an automated lifespan assay (Stroustrup et al., 2013). The authors placed 16 petri dishes, each with adult worms, in each scanner, and the images were recorded over time online with a Linux command-line script. Worm shapes were identified using an unsupervised classifier that learns from 65 features and by defining dead worms as those worm-shapes that are not moving, lifespan data can be generated. It is clear that these high-granular approaches to phenotyping can be used to generate biologically meaningful results. For example, individual worms of many different genotypes have been tracked on agar plates and their movement defined using 161 behavioural parameters (Zheng et al., 2012). The results were analysed using unsupervised clustering (K-means), which revealed clusters of behavioural genes and many genes present in the same cluster were also in the same biochemical pathway.

A common feature of many of these systems is the provision of a detailed description of the evolution of some parameters of worm shape or position over time. To capture worm movements adequately, many parameters are often needed to specify each posture. One can imagine the change in shape over time being represented by a trajectory through a set of multidimensional coordinates, each dimension representing one of those shape parameters. Such a dataset can then be analysed using well-established pattern-recognition principles such as unsupervised clustering and the effects of chemicals can therefore be quantified. This has exciting potential, because a compound could be effective as an anthelmintic by affecting some aspect of behaviour that prevents the worm from being reproductively successful, without immediately killing the worm. However, this approach could only be of use if it can be deployed robustly in high-throughput screens. Later on, we address whether this is feasible.

Another distinct advantage offered by *C. elegans* is the ease of culture in liquid and manipulation using fluid handling techniques. This opens up the possibilities of applying microfluidic techniques to developing worm assays, with the prospect of partly overcoming the limitations of existing methods when applied to high-throughput assays. A COPAS sorter was used to seed worms and later count the adults resulting in an assay for worm reproductive capacity (Boyd et al., 2010). In this case, all handling was automated. A microfluidics approach has been adopted, raising cohorts of 30–50 worms in each of eight separate chambers on a single "WormFarm" polydimethylsiloxane chip throughout their lifespan (Xian et al., 2013). Standard computer algorithms detect worm death as well as behavioural and morphological features.

4.6. Are the algorithms developed for automation sufficiently robust for automated drug/chemical screening?

The simplest approach for a screen is to score the phenotypic effects of each compound in a library and follow up those with a score above a statistical threshold – the "primary hits." This approach is plagued with false positives, a problem that worsens as the size of the library increases. However, when an automated scoring algorithm is fast enough, very large (>100,000 compound) libraries can be screened allowing more sophisticated aggregated readouts such as a detailed structure-activity study. In addition, fast screens make it easier to run several replicates, again minimising the influence of false positives. However, several factors have limited the successful use of these algorithms in high-throughput screens. Most of them are labour intensive, requiring filming of worms on individual plates – indeed most of them require individual worms to be filmed or tracked, meaning individual adults have to be manually picked out and deposited. Even if this could be automated, the machine vision algorithms used are rarely robust against variations in the quality of image acquisition. For instance, many algorithms attempt to separate worm from background using thresholding. If the illumination is uneven, errors in this step are almost unavoidable. Most algorithms also perform skeletonisation – reducing the worm profile to a single, one-pixel wide curve. Again, this step is notoriously sensitive to the quality of its input, which in turn is sensitive to binarization, although this can be partly overcome by using techniques like Gaussian mixture models to track the evolution of worm shape and location (Mitchell et al., 2010; Sznitman et al., 2010) or using measures of curvature in edge-filtered images (Restif et al., 2014). The problems with these

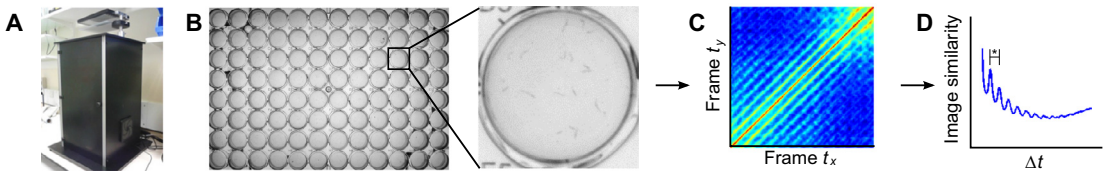

Fig. 2. Automated measurement of nematode swimming (thrashing) in 96-well plates using a covariance-based statistical approach enables motility measurement suitable for use in high-throughput drug/chemical screens. (A) The device (Wormwatcher) analyses movement by (B) simultaneously recording movies of worms movement in all wells of a 96-well plate. The movie is analysed by generating (C) a covariance matrix for each individual well showing the similarity between frames *tx* and *ty*. (D) The time dependence of similarity between pairs of images is used to estimate the time interval between rhythmic worm movements (*), and hence the thrashing rate.

approaches that we have listed can be minimised, but in practice the user finds that a lot of adjustments of parameters are needed to obtain reasonable results.

4.7. Statistical imaging approaches: one way forward for automated phenotypic screening

In our lab, we attempted to side-step some of the problems involved in image segmentation that we have highlighted above by replacing machine vision with an image statistics approach (Buckingham and Sattelle, 2009). Taking the well-established swimming assay as a model, we found that simply taking the covariance of a series of movie frames as an index of image similarity enabled the computer to count the number of frames that separate similar images (Fig. 2). This is effectively a crude measure of autocorrelation, and is remarkably robust against poor image quality and, critically, the number of worms in the image. We (Buckingham and Sattelle, 2009) found it yielded very similar results to manual-scoring (the R2 correlation between the machine readout and those of 2 independent manual scorers was greater than 0.9) but took only seconds to perform for a 96-well plate. The algorithm, developed using *C. elegans*, worked (without adjustment) for the parasitic nematode, *H. contortus* (Buckingham and Sattelle, 2009), and was able to generate concentration-inhibition curves demonstrating the effects of the anthelmintic, levamisole, on worm motility. While the swimming assay is a popular and convenient way to score motility in *C. elegans*, crude worm movement is inadequate as an indicator of worm killing in screens for anthelmintics. Although immobility does not necessarily imply death (there are chemicals that paralyse worms without killing them), a simple approach to determining how many worms are alive in a high-throughput context would be to decide where, and how much, movement is occurring in a suspension of worms. This can be done by comparing pairs of images taken at a fixed time interval, and counting the number of pixels that appear to have changed. The findings can be normalised for the number of worms in the well, or, more simply, can be normalised to the pixel count in each well before compounds have been added.

By filming *T. muris* for 10–20 s and using our own, in-house software, we calculated the changes in pixels resulting from worm movement. Results were reproducible between replicates and the speed of measurements permitted small-scale drug testing (Hurst et al., 2014) and its potential for high-throughput screening of chemical libraries is currently being explored. The idea of movement detection has been taken a step further, using the Lucas–Kanade algorithm (an established computer-vision method of providing movement vectors from movie images), and this has proven successful for drug screening studies on larger (macro) parasites (Marcellino et al., 2014; Storey et al., 2014). WormAssay is a 96-well, plate-based, visual imaging system that uses software to analyse drug actions on such worms. The authors have made available their open source software (Marcellino et al., 2014). The application and source code are available for free use, modification and redistribution under the terms of the GNU Public Licence, version 2 or later; see http://www.gnu.org/licences/gpl-2.0.html for details. The application and its source code can be downloaded from http://code.google.com/p/wormassay/ (see Table 1).

Table 1
Approaches to automated phenotyping of nematodes and the techniques deployed.

Automation approach		Techniques	References
(A) Tracking	Tracking overall behaviour	Classify discrete worm movements using machine vision	Shingai (2000) Hoshi and Shingai (2006)
	Beam interruption	Scattering of infra-red beam	Simonetta and Golombek (2007)
	NEMO	Skeletonisation, digitization and angle measurements using machine vision	Tsibidis and Tavernarakis (2007)
(B) Targeting discrete behaviours	Automation of foraging	Tracking side-to-side head movements	Ramot et al. (2008)
	Automation of egg laying		Hardaker et al. (2001)
	Swimming/thrashing	Plate-based covariance method	Buckingham and Sattelle (2009) Hurst et al. (2014)
(C) Microfluidics	Dead worm counting	COPAS sorter	Boyd et al. (2010)
		Microfluidics	Xian et al. (2013)
(D) Monitoring complete behavioural repertoire	Worm trackers	Single worm tracking: multiple morphological and dynamic characteristics	Baek et al. (2002) Geng et al. (2003) Feng et al. (2004) Cronin et al. (2005) Yemini et al. (2013)
		Multiple worm trackers	Stroustrup et al. (2013)
		Dark-field imaging and Lukas–Kanade tracking	Marcellino et al. (2014)
	Texture factor modelling (TFM)	Texture-based models for phenotyping in complex environments	Greenblum et al. (2014)

5. Conclusions

Rapid progress is being made in automating the analysis of nematode movement. Much of this effort has yielded computer programs that produce impressive results in identifying aspects of nematode movement and the roles played by genes and gene clusters in worm behaviour. However, for the most part, these research programmes have not had the requirements of high-throughput drug/chemical discovery in their sights. They are necessarily labour-intensive and can be very susceptible to inconsistencies in the experimental setup (such as changes in lighting).

How might these advances help us use automated phenotypic screens to find new anthelmintics? Large-scale, rapid phenotypic screening needs three elements to be in place. First, it needs model material (worms) that can be handled in large volumes by machines. Secondly, it requires an automatic readout that is simple (either scalar or low-dimensional vector) to interpret. Thirdly, it should be robust against variability in recording, since the need for intense manual curation would undermine speed. The first is met by *C. elegans*, a free-living nematode and genetic model organism as well as by some cultured or harvested parasitic worms, such as *H. contortus*, which are small and tough enough to be handled by fluidics or even microfluidics. The second and third are being met by algorithms that do not attempt to capture high-dimensional, fine-grained features of worm movement, but rather adopt proximal measures (such as a summary of movement vectors or the statistical comparison of image frames) that are largely orthogonal to the varying features of the image itself – the Lucas–Kanade algorithm, for example, would be little affected by changing the light intensity between images, and our covariance approach is completely unaffected by having a noisy background.

These considerations point to a possible roadmap for the development of automated, behavioural screens that can be used to speed up anthelmintic discovery by an order of magnitude. This roadmap has two branches. First, algorithms for extracting simple measures of worm motility from moving images (recorded or live) need to be developed. The most promising appear to be measures that infer the average magnitude of movement (Lucas–Kanade, number of pixel changes) or those that provide a more general statistical measure of the differences between successive images. Secondly, methods for the automated handling of worms need to be developed. Microfluidics is used routinely with cells and could be used, as is, with smaller worms but adaptations to the appropriate scale needed for the handling of larger nematodes are still required.

Conflict of interest

The authors declared that there is no conflict of interest.

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