Phenotyping Earthworm by Image Analysis

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Abstract—Non-destructive phenotyping of earthworms by digital imaging and image analysis is the novel concept being proposed and explored in this paper. Earthworms are very important component of plant soil interaction via rhizosphere. Although a lot of research resources have been applied to phenotyping roots by image analysis, there has been practically insignificant work on phenotyping earthworms by image analysis. We put together some tailor made image analysis techniques (segmentation, medial axis thinning) along with a mathematical model for earthworms, to compute the volume, surface area and length of earthworms. We developed a novel radius versus length plot to identify the mouth-end, clitellum, and anus-end of earthworms by machine vision. We then compare the results of the phenotyping measurement obtained by our approach to those of the intercept principle. Intercept principle has been commonly used for phenotyping roots. Further more we propose a novel colour signature for blobs obtained by segmenting earthworms for colour analysis of the earthworms. It is expected that the colour information of earthworms can give clues on bioavailability of nutrients in soil or/and for earthworm species recognition. Both by qualitative and quantitative analysis we show that the segmentation and phenotype computation are better than the conventional approach of intercept principle.

I. INTRODUCTION

Phenotyping earthworms by image analysis is the novel idea proposed and explored in this paper. Earthworms play a vital role in soil ecosystems by directly and indirectly affecting the growth and distribution of root system architecture [1]. Earthworms are also important for growth and distribution of micro-organisms thus playing a vital role in improving the quality of soil and healthy interaction between plant and soil [2]. Earthworms are an essential part of the soil fauna [3], they represent a significant proportion of the soil biomass and therefore regarded as a useful indicator of soil health [4]. Earthworms are also ideal organisms for monitoring the bioavailabilities of nutrients and contaminants in soils because they are widely distributed and large enough to be handled easily [5].

There have been several applications of digital imaging and image analysis for modelling soil [6], and also for modelling earthworm and soil interactions [7], [8]. In [6] Sanjeev et al. present a method of using digital images and their transformation to model the hydric conductivity of soil. A platform for tracking multiple worms on food was presented in [9]. There have been quantitative image analysis studies on how earthworm displaces soil by its movements [7]. This paper showed successful use of image analysis tool Particle Image Velocimetry (PIV) on quantification of the distance and direction of soil particle displacement by earthworm locomotion. They studied the movement behaviour of two species A. longa and L. terrestris. The quiver plots and quantitative analysis of soil displacement could easily distinguish the behaviour of two species. In [8] Capwiz et al. used image analysis and mathematical morphology to characterise the 3-dimensional (D) structure of earthworm burrows. Expensive 2-D and 3-D x-ray computed tomography were employed to generate 3D reconstruction of the burrow system using a specifically developed segmentation algorithm.

There has been several imaging and image analysis systems developed for phenotyping root system architecture (RSA) [10]. Machine learning techniques has also been applied for improving the accuracy of root phenotyping by digital image processing [11], [12], [13]. In situ 3-D reconstruction system for RSA has been presented in [14] for phenotyping roots. Computer Vision and pattern recognition has also been used for phenotyping and identification of moths, bees and insects, [15], [16], [17] to aid entomologist and agriculturists in their studies and research endeavours. Earthworm phenotyping will have long term impact on the research of plant functional genomics, vermiculture and studies relating plant soil interactions.

II. THE PROPOSED METHOD

The earthworms after removal from soil are washed and placed collectively or individually on a tray for scanning using a flat bed scanner. In our initial attempts we had water in the scanning tray similar to the method used for scanning roots, however during experiments we discovered that the movements of the earthworms are more languid in absence of water. The images were scanned as colour RGB images at 600 dpi, however higher resolution can be used to get more detailed phenotypes at the expense of more processing time. The scanned images were processed with the segmentation algorithm explained in II-B. Segmentation results after connected component analysis are processed by medial axis thinning.
to obtain the central axis of the earthworms. The image features were processed along with mathematical parameters to get phenotypes of the earthworm. Colour image along with segmentation mask was used to generate the cluster signature of the earthworm for quantification of the colour phenotype of the worm.

A. Outline and organization

Figure 1 shows the schematic block diagram of the system developed for imaging and processing the images to extract earthworm phenotypes.

Rest of the paper is organized as follows: In Section II-B, we briefly describe the segmentation processes and the need for a novel earthworm segmentation methodology. In Section III, we introduce a mathematical model for earthworm and relate them to imaging and image analysis parameters for computing the shape phenotypes. We briefly describe the line intercept principle in section IV, which is commonly used for root phenotyping and compare our results to them in the result section VI. The cluster signature for quantification of the colour information of earthworm is introduced and briefly described in Section V. Finally, in Section VII we give a summary appraisal of our results and outline some future ideas of research.

B. Segmentation of earthworms

Segmentation is an important preprocessing step in image analysis problems. Being a precursor the end result of the image analysis solution depends heavily on the quality of the segmentation results. In our attempt to segment the earthworms in their scanned images we found shadows to be of significant problem. Shadow detection and removal is also an important problem in digital image analysis and is also well researched [18], [19]. However, usually scanned images don’t have the problem of shadow formation, but due to non-negligible thickness of earthworms, they do cast shadow in their scanned images. Figure 2(a) shows an example of earthworm image with its shadow. Figures 2(b), (c), (d) show segmentation results using Otsu’s thresholding [20], adaptive thresholding [21] and colour space transformation [18] and then thresholding. This experience from our work confirms the notion that off the shelf algorithms are not going to give the desired result in all applications. Computer vision and image analysis systems have to be designed specifically for particular application [22]. To solve the problem of segmentation for this particular case we decided to use the approach of supervised classification. We chose this method as we expected the with few examples of manually labelled images for earthworm and background we will be able to segment most of the other images. Each pixel in a digital image has to be classified as earthworm pixel or non-earthworm, background pixel. For training of the supervised classification algorithm some of the sample earthworm images are manually labelled.

Given a manually labelled image the statistics of pixels belonging to earthworm and to the background are modelled as a Gaussian mixture model (GMM). The GMMs are then treated as class conditional density distributions to build the Bayes classification rule using the Neyman Pearson lemma. A similar classification approach was used for root tip detection in [13].

C. GMM density estimation

The GMM density estimation problem can be defined as follows: given a set of $Q$ feature vectors in a $D$--dimensional space, $\mathbf{z}_1, \ldots, \mathbf{z}_q, \ldots, \mathbf{z}_Q$, find the GMM probability density function $f(\mathbf{z}; \phi)$ that is most likely to generate the given set of $Q$ feature vectors, where $\phi$ are the set of parameters.

For future reference, $\mathbf{z}_q = J(x, y)$ is a vector of the three components $RGB$ of a pixel and $Q$ represents either the total number of pixels of the category earthworm or background. Here, $f(\mathbf{z}; \phi)$ will be assumed to have the form:

$$f(\mathbf{z}; \phi) = \sum_{k=1}^{K} w_k g(\mathbf{z}; \mu_k, \Sigma_k)$$

and

$$g(\mathbf{z}; \mu_k, \Sigma_k) = \frac{1}{\sqrt{2\pi|\Sigma_k|}} e^{-\frac{1}{2}(\mathbf{z}-\mu_k)^T \Sigma_k^{-1}(\mathbf{z}-\mu_k)}.$$  

The different Gaussian members of the GMM are identified by the different values of the parameter $\phi = (\phi_1, \ldots, \phi_k, \ldots, \phi_K)$, and $\phi_k = (w_k, \mu_k, \Sigma_k)$, where $w_k$ is the weight of the $k^{th}$ Gaussian; $\mu_k$ and $\Sigma_k$ are its mean and standard deviation, respectively. In posing the density
estimation problem, one asks given a set of values of the feature vector \( \mathbf{Z} = \{ \mathbf{z}_1, \ldots, \mathbf{z}_q, \ldots, \mathbf{z}_Q \} \) and a specified \( K \), find the \( \phi \) that would have most likely generated the \( \mathbf{Z} \). The meaning of “most likely” is determined by a function \( \Lambda(\mathbf{Z}, \phi) \) that measures the likelihood of a particular model given the set of feature vectors \( \{ \mathbf{z}_1, \ldots, \mathbf{z}_q, \ldots, \mathbf{z}_Q \} \). The likelihood function for GMM is

\[
\Lambda(\mathbf{Z}: \phi) = \prod_{q=1}^{Q} \sum_{k=1}^{K} w_k g(\mathbf{z}; \mu_k, \Sigma_k) \tag{3}
\]

and the log likelihood is

\[
\lambda(\mathbf{Z}: \phi) = \sum_{q=1}^{Q} \log \left( \sum_{k=1}^{K} w_k g(\mathbf{z}; \mu_k, \Sigma_k) \right) \tag{4}
\]

To find the local maximum of \( \lambda \) (or, equivalently, of \( \Lambda \)) the derivative of \( \lambda \) is computed with respect to \( \mu_k, \Sigma_k \), and \( w_k \) and set to zero. This gives a solution for \( \mu_k, \Sigma_k \), and \( w_k \). The EM algorithm starts with an initial estimate of \( \{ \mu_k(0), \Sigma_k(0) \} \) and iterates in an E-step and an M-step until convergence is obtained. The E- and M-steps involve the following computations:

- **E step**

  \[
  w^{(i)}(k/q) = \frac{w^{(i)}_k g(\mathbf{z}; \mu^{(i)}_k, \Sigma^{(i)}_k)}{\sum_{k=1}^{K} w^{(i)}_k g(\mathbf{z}; \mu^{(i)}_k, \Sigma^{(i)}_k)} \tag{5}
  \]

- **M step**

  \[
  \mu_k^{(i+1)} = \frac{\sum_{q=1}^{Q} w^{(i)}(k/q) \mathbf{z}_q}{\sum_{q=1}^{Q} w^{(i)}(k/q)} \tag{6}
  \]

  \[
  \Sigma_k^{(i+1)} = \frac{1}{D} \sum_{q=1}^{Q} w^{(i)}(k/q) (\mathbf{z}_q - \mu_k^{(i+1)}) (\mathbf{z}_q - \mu_k^{(i+1)})^T \tag{7}
  \]

  \[
  w^{(i+1)}_k = \frac{1}{\mathcal{Q}} \sum_{q=1}^{Q} w^{(i)}(k/q) \tag{8}
  \]

As experienced in previous studies, the EM algorithm did not converge on some occasions. To avoid the problem of convergence we used the mean-shift algorithm to cluster our data into different clusters using a fixed bandwidth. The bandwidth was empirically determined. Each cluster is then modelled as a Gaussian distribution and the weight of each Gaussian is computed by data to cluster association result. This method of estimating GMM ameliorates the problem of estimating the number of Gaussians in the GMM, although we are left with the new problem of estimating the optimal bandwidth.

**D. Bayes classification and Neyman Pearson criteria**

Using the training data, the class conditional density \( p(\mathbf{z}_q | c) \), the density distribution of pixel vector \( \mathbf{z}_q \) of class earthworm \( c \), is learnt. Similarly, the class conditional density \( p(\mathbf{z}_q | b) \) for background class, \( b \), is learnt.

\[
p(\mathbf{z}_q | c) = \sum_{k=1}^{K} w^c_k g(\mathbf{z}_q; \mu^c_k, \Sigma^c_k) \tag{9}
\]

\[
p(\mathbf{z}_q | b) = \sum_{i=1}^{L} w^b_i g(\mathbf{z}_q; \mu^b_i, \Sigma^b_i) \tag{9}
\]

Generally, the number of Gaussians used to represent the earthworm pixels will be different from the number employed to represent the background, i.e., \( K \neq L \). At present, it is difficult to a priori decide the optimal numbers of Gaussians, \( K \) and \( L \). However, the use of the mean-shift based clustering and GMM modelling does help solve the problem for this application. For the purpose of classifying a pixel \( J(x, y) \) its feature vector \( \mathbf{z}_q \) is analysed as follows. Using Bayes rule, the density and likelihood of class earthworm pixel given the pixel feature is obtained according to the expression,

\[
p(c | \mathbf{z}_q) = \frac{p(\mathbf{z}_q | c)p(c)}{p(\mathbf{z}_q)} \tag{10}
\]

Similarly, using Bayes rule, the density and likelihood of class background given the pixel feature vector \( \mathbf{z}_q \) is given by,

\[
p(b | \mathbf{z}_q) = \frac{p(\mathbf{z}_q | b)p(b)}{p(\mathbf{z}_q)} \tag{11}
\]

We used Neyman-Pearson Lemma as it asserts that a best critical region can be found by finding the \( D \)-dimensional points in the sample space for which the likelihood ratio is smaller than some constant. A variable \( \eta \) is defined as

\[
\eta = \frac{p(c | \mathbf{z}_q)}{p(b | \mathbf{z}_q)} = \frac{p(\mathbf{z}_q | c)p(c)}{p(\mathbf{z}_q | b)p(b)} \tag{12}
\]

For a given training data set, \( p(c) = 1 - p(b) \) is fixed and hence \( p(c)/p(b) \) can be treated as a constant, which can be moved to the left hand side of the equation (12), resulting in a new variable,

\[
\eta^* = \frac{p(b)}{p(c)} = \frac{p(\mathbf{z}_q | c)}{p(\mathbf{z}_q | b)} \tag{13}
\]

If \( \eta^* \) is greater than an empirical threshold, \( \eta_{th} \), then the pixel \( J(x, y) \) is classified as a earthworm pixel, otherwise it is classified as a background pixel. In a future work we will use Receiver Operating Characteristic (ROC) curves as a means of determining \( \eta_{th} \). At present, it is set by perceptually evaluating the segmentation results. After initial classification, connected component analysis and filtering of small size blobs and morphological operation is applied to get the segmentation results of the worms.

**E. Thinning and medial axis extraction**

Thinning algorithms transform an object to a set of digital arcs, which lie along the medial axes. The thinned structure obtained is not influenced by small contour inflections that may be present in the segmented region. The approach is to delete from the segmented blob border points that have more than one neighbour and whose deletion does not locally disconnect the blob. Here we have used an implementation of the thinning algorithm given in [23]. Figure 3 shows an example of result obtained by the segmentation and thinning algorithm. The conventional thinning algorithm causes removal of pixels at the edge of the contours. Therefore a modification of the conventional algorithm is needed to more accurately capture the phenotypic informations of the worm. The contours obtained by conventional thinning are extended by using the slope of thin edge pixels to the boundary of the segmented blob.
of an earthworm from its image then becomes
\[ \text{VOL} = \sum_{i=1}^{l} \pi \times (d_i/2)^2 \times \delta h_i \] (16)
and surface area \( \text{SUF}_A \),
\[ \text{SUF}_A = \sum_{i=1}^{l} \pi \times d_i \times \delta h_i \] (17)
and length \( L = \sum_{i=1}^{l} \delta h_i \).

IV. LINE INTERCEPT PRINCIPLE

Line intercept principle is generally used to estimate the length versus diameter distribution of scanned roots [24]. After segmentation a bi-level image of the root and background is obtained. The bi-level image is then thinned for calculation of the length of the roots. One pixel boundary of the bi-level image is deleted and root length is calculated again. The boundary pixel deletion and root length calculation cycle is repeated until no root pixel is left in the image. The number of single pixel boundary deletion is equivalent to the intercept length. The root length of the residual image after each boundary pixel removal is regarded as the length of the root with diameter greater than the calculated diameter. The intercept length \( I \) is equal to twice the number, \( n \), of boundary pixels deletion cycle, \( I = 2n \). Figure 4(a) and 4(b) shows and example of vertical and horizontal intercept lengths. The diameter \( D \) for horizontal intercept is related to the intercept length \( I \) by
\[ D = I \times \cos(\Theta), \]
where \( \Theta \) is angle between \( D \) and \( I \). The computation of \( \Theta \) is a crumble-some exercise, therefore and estimate of \( D \), \( D_{est} \) is computed instead using a constant multiplier \( k \)
\[ D_{est} = I \times k; \]
Assuming and even distribution of lines
\[ k = \frac{1}{(\int_{0}^{\pi/4} \cos(\Theta) \, d\Theta) / (\pi/4))} \]
\[ = 0.891 \]
thus \( D_{est} = 2 \times n \times 0.891 \).
The diameter \( D \) for vertical intercept is related to intercept length by
\[ D = I \times \cos(\pi/2 - \Theta) \]
The integral of \( \cos(\pi/2 - \Theta) \) provides the same value of \( k \) in the range \([0 - \pi/4]\) as above. The estimated diameter \( D_{est} \) when compared to actual diameter is in the range from 89.1 – 126.0% as \( \Theta \) is increased. The estimated error ranges from \(-10.9 - 26\%\), with overestimation being larger than the underestimation. Since there is an inherent error in the estimation of diameter by the intercept method, we expect to have better results by our above proposed method, in computation of the phenotypes such as volume and surface area.
V. PHENOTYPING BY COLOUR INFORMATION

For phenotyping earthworm by their colour information we propose the use of cluster signature. Cluster signature has been used for image retrieval [25] and for tracking of targets in [26]. Cluster signatures are better than colour histogram for the following reason:

- Transcends the problem of quantisation and binning effects associated with histograms.
- Usually are more space efficient than histograms. 3D histogram of RGB colour space requires more storage space than cluster signature of an earthworm in RGB space.

The colour phenotype of an earthworm is quantified and represented by a cluster signature which consists of a cluster centre \( f_c \) and its weight \( w_c \) sorted in descending order of the weight.

\[
S = \{s_c\}_{c=1}^{C} \quad \text{where} \quad s_c = \{f_c, w_c\} \quad \text{and} \quad w_c > w_d \quad \text{for} \quad c < d \quad \text{and} \quad \sum_{c=1}^{C} w_c = 1.
\]

Figure 5 (a) (b) show two sample image of earthworms and their cluster signature in a pictographic representation in 5 (c) (d). The colour properties of earthworms are species dependent and thus the cluster signature can be used for species identification. The subtle colour variations within species can also be attributed to the trace elements and metals in the soil and can thus be used for various abiotic and biotic stress conditions such as metal content studies of soil [3].

VI. RESULTS AND DISCUSSION

Examples of improved segmentation by the proposed method compared to other traditional and latest methods are already shown in the Figures 2 and 3. Figure 6(b) shows a plot of radius verses length plot of an earthworm image shown in 6(a). The mouth end of the worm has a steeper taper than the anus end and the clitellum portion is characterised by the plateau somewhere in the middle and closure to the mouth end. Table shows the values of volume, surface area and length computed by the proposed approach and the intercept principle. Figure 7 shows a plot of the weight verses volume of the earthworms by the proposed approach and the intercept principle. The \( R^2 \) for the plot of volume computed by the proposed approach is 0.9276 and for the plot of volume computed by the intercept principle is 0.8952. In terms of

\[\begin{array}{|c|c|c|c|}
\hline
\text{Method} & \text{Volume} & \text{Surface area} & \text{Length} \\
\hline
\text{Intercept} & 2.7873e-06m^3 & 4.5428e-04m^2 & 0.06370m \\
\text{Proposed} & 4.4094e-07m^3 & 1.0416e-04m^2 & 0.06599m \\
\hline
\end{array}\]

TABLE I

<table>
<thead>
<tr>
<th>Method</th>
<th>Volume</th>
<th>Surface area</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.7873e-06m^3</td>
<td>4.5428e-04m^2</td>
<td>0.06370m</td>
</tr>
<tr>
<td>Proposed</td>
<td>4.4094e-07m^3</td>
<td>1.0416e-04m^2</td>
<td>0.06599m</td>
</tr>
</tbody>
</table>

\( R^2 \) the plots don’t differ so much but the volume estimated by intercept is significantly over estimate. The results in table I shows similar trend of intercept principle based estimates of surface area and volume which are based on diameter estimates. This is some what what aspect expected as the intercept method does overestimate in its initial design and theory. Furthermore the standard deviation of proposed method is 1.5036e-08 and that of the intercept method is higher 1.5967e-07, there by indicating the that proposed method is more reliable. We assume that the actual weight of earthworm will be linearly related to its volume, which is obvious only when the density of earthworms are assumed to be constant.
In this paper we have proposed and developed a novel phenotyping platform for earthworms. The development involved generation of some tailor made image analysis algorithms. We have compared the supervised segmentation algorithm to other traditional and more sophisticated algorithms and the results are better than the other approaches. A mathematical model of earthworm is used to generate the phenotypes like volume (analogous to biomass), surface area and length. The obtained phenotypes are better and more reliable when compared to ground truth weight measurements. The detection of mouth and anus ends can be further improved by using the colour information along with radius versus length plot as the mouth-ends are usually more reddish than the anus end. A novel cluster signature representation for colour phenotype is proposed. This cluster signature will be very useful in recognizing the species of earthworm and also for trace metal analysis of soil by earthworm studies. In future works we will conduct experiments with larger number of earthworms of known species to validate our hypothesis of species identification of earthworm by their colour cluster signature. The greatest advantage of the phenotyping platform is that it is non-destructive and the earthworms imaged can be reused for further experimentation or returned back to the ecosystem.

VII. CONCLUSION

REFERENCES


