Roboworm 3: Development of an automated drug screening platform for *Fasciola hepatica*.

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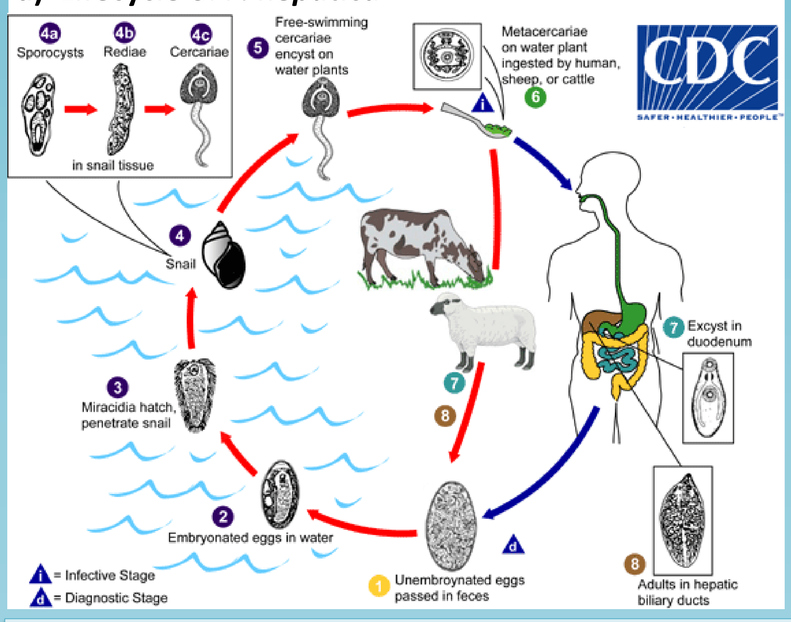
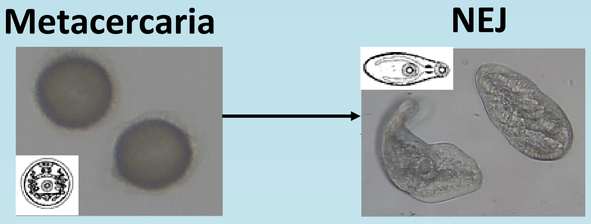
Parasitic helminths have a severe impact on both human and animal health. In humans, more than 2 billion people carry a helminth infection, causing morbidity, and a loss of more than 11 million disability adjusted life years (DALYs) globally. With respect to livestock, more then €99 M/annum is estimated to be lost due to helminth infection in the UK, with an additional €400M/annum being spent on treatment in the EU alone[1]. The Roboworm, high content (HC), high throughput screening (HTS) platform was initially developed for the larval schistosomula stage of parasitic helminth, *Schistosoma mansoni*. This project has utilised Roboworm to build an image analysis model based on the phenotype of the larval newly excysted juvenile (NEJ) stage of liver fluke, *Fasciola hepatica*. *F. hepatica* is responsible for causing the foodborne zoonotic disease, fascioliasis, affecting 2.4 million people globally[2], and is estimated to result in the loss of livestock equating to >645M/annum globally[3]. With limited development of anthelmintic therapies, and rising cases of anthelmintic resistance, development of new robust HTS techniques to discover new anthelmintic options are urgently needed[4]. It is hoped that the steps taken to develop Roboworm 3, described on this poster below, demonstrate the first steps taken to meet this need, and will eventually be applicable to other parasitic helminth species.

Excystment, culture and high content imaging of *F.hepatica* NEJs

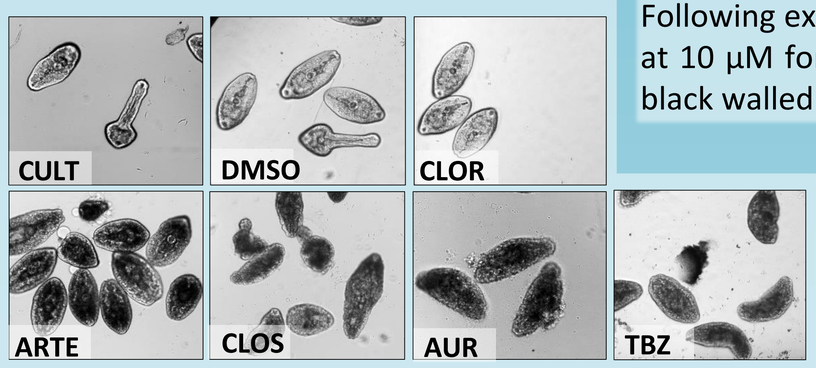
a) Lifecycle of *F. hepatica*



b) Excystment



c) 72hr drug treatment (10µM)

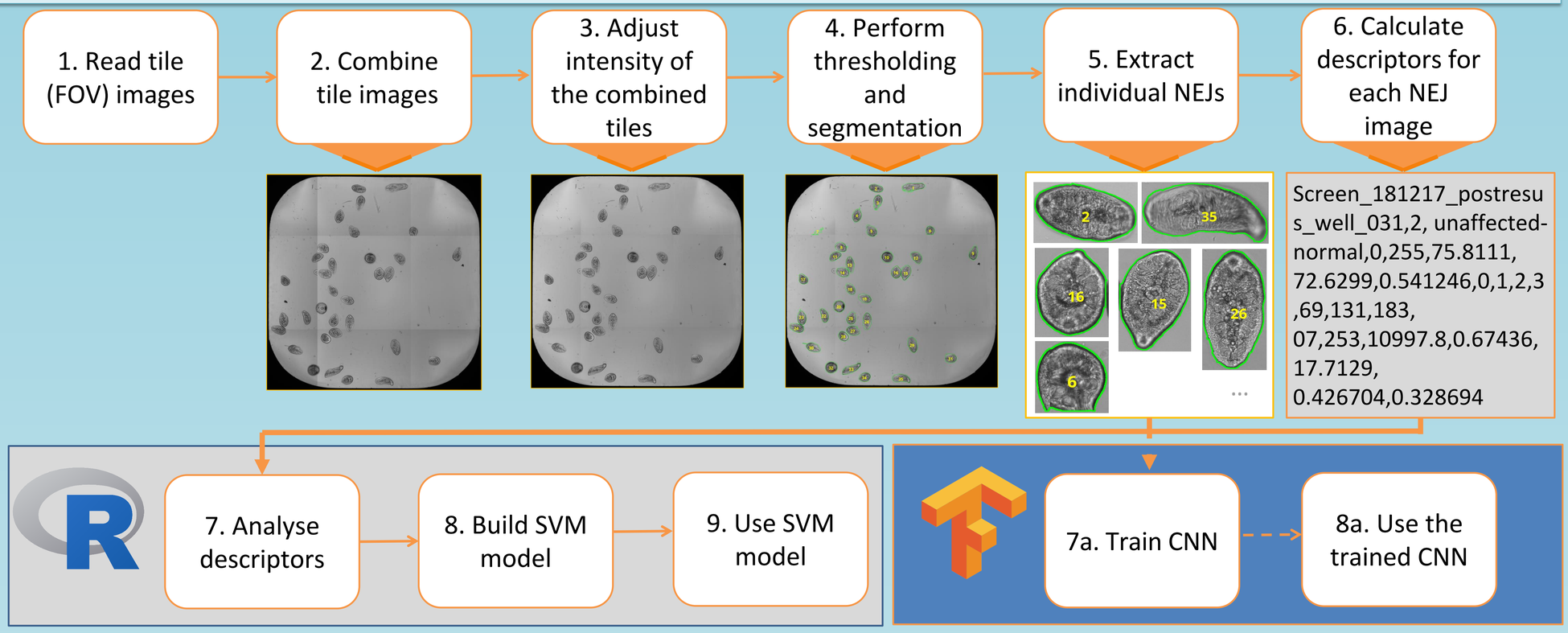


In order to collect HC images of 72 hour drug treated NEJs, the human metacercaria infective form of *F. hepatica* (a and b) were purchased from Ridgeway Research. NEJs were excysted using *in vitro* techniques mimicking digestion of the cyst and exposure to bile within the bile ducts of the definitive human/ animal host. Excystment efficiency ranged between 11-62%.
Following excystment, NEJs were cultured in each model treatment at 10 µM for 72 hours, with ~25 NEJs being cultured per well in a black walled 384 Perkin Elmer plate (c) and then imaged.

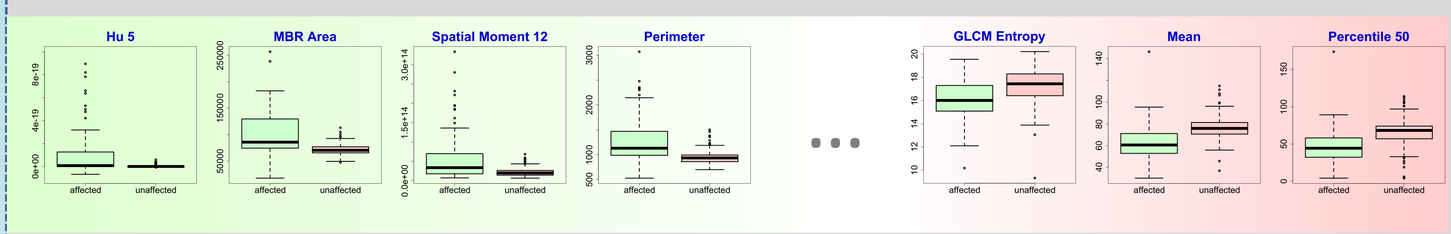
Treatments for model construction were based on current literature for treating Fascioliasis to try and achieve a range of severe phenotypes seen opposite (c). Culture (CULT), Clorsulon (CLOR), Artesunate (ARTE), Closantel (CLOS), Auranofin (AUR), Triclabendazole (TBZ).

Development of a biomedical image analysis gateway for *F.hepatica* NEJs by Informatics Unlimited Ltd.

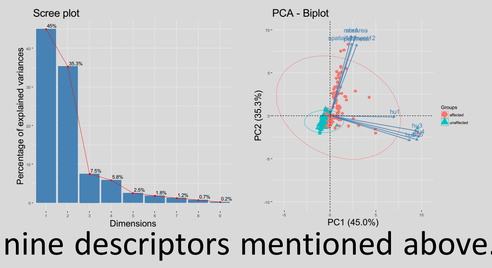
Following collection of images, several steps were taken to prepare the images for model construction (1-5). This was important in order to identify the best image descriptors to describe the phenotypes captured. Segmented NEJs were then categorised into affected and unaffected, and any segmented debris were ignored. Following categorisation image descriptors were applied to affected and unaffected NEJs to ascertain which image descriptors showed a significant difference between the two (6). A selection of the best image descriptors can be seen below (7-9)



Construction of the support vector machine (SVM) model (7-9):
76 texture-based, pixel intensity and morphological image descriptors calculated by a novel Biomedical Image Gateway system were sorted using a simple method based on their mean value and quartiles:



Top nine descriptors were chosen for further analysis: five Hu moments, spatial moment 12, the area and perimeter of the blob, and the area of the minimum bounding rectangle. PCA analysis demonstrated that first two principal components (Hu moments vs the remaining four features) can explain for 80% of variance.

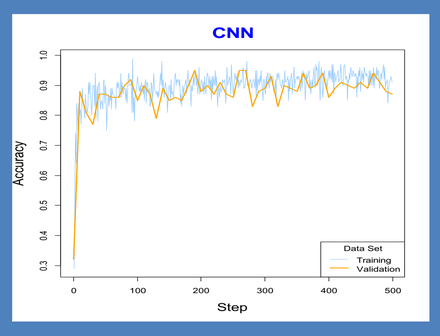


Next, a linear SVM model was trained using 80% of images and the nine descriptors mentioned above. It achieved 92.79% accuracy for the training data set and 84.31% for the test data set.
Increasing the number of used descriptors to 30 increased the accuracy to 96.63% and 90.2%, respectively:

9 Descriptors	Training		Test	
Prediction \ Reference	affected	unaffected	affected	unaffected
affected	72	3	16	3
unaffected	12	121	5	27
Accuracy		**0.9279**		**0.8431**

30 Descriptors	Training		Test	
Prediction \ Reference	affected	unaffected	affected	unaffected
affected	79	2	18	2
unaffected	5	122	3	28
Accuracy		**0.9663**		**0.902**

Application of an alternative modelling approach supports SVM findings (7a-8a):
As an alternative approach, an experiment in applying an existing CNN (Convolutional Neural Network) using the TensorFlow framework was performed.
The Inception-v3 model* trained for the ImageNet dataset was retrained using a subset of NEJ images and 500 training steps. Despite its a generic nature, after re-training, the Inception-v3 model achieved 91.50% accuracy for the training and 88.17% for the validation data set, respectively.



* https://arxiv.org/abs/1512.00567

Future work:
- Increase the number of parasites screened and treatment conditions for model construction.
- Improve thresholding methods to better account for uneven illumination.
- Better markers for watershed segmentation to tackle highly overlapping and out-of-focus blob
- Further progress in ML methods
- Create a user-friendly web-based interface to control the entire workflow and report results

References: BIOSCIENCE, E. 2016. Market assessment for an automated platform for compound screening against a range of helminth parasites[1], WORLD HEALTH ORGANISATION. 2018. *Foodborne trematode infections: Fascioliasis* [Online]. Available: http://www.who.int/foodborne_trematode_infections/fascioliasis/en/ [Accessed 25/03/18 2018][2], BIOSCIENCE, E. 2017. Position Paper Parasitic Flatworms for Aberystwyth University[3], COLES, G. 2001. The future of veterinary parasitology. *Veterinary parasitology,* 98, 31-39[4].