High throughput imaging and analysis for studying feeding and digestion in a marine invertebrate model

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The "Multiscope" (also known as the Rapid Access Parallel microscope) system enables multiple fields of view to be relayed to the same detector providing applications in high-throughput imaging. The Multiscope is the product of a collaboration between the groups of Dr Alex Corbett (Exeter University) and Prof Gil Bub (McGill University) [1]. In this project the Multiscope has been implemented as a multi-well plate reader to analyse the movements of microscopic (~200µm) larvae of the marine worm *Platynereis dumerilii*.

Platynereis is an excellent model for studying the regulation of feeding and digestion as it is easy to keep in the lab and the small size and transparency of *Platynereis* larvae and juveniles mean that the entire digestive process can be imaged in live, freely moving individuals [2]. A variety of neuropeptides can be used to modify appetite, food search behaviour and gut motility in *Platynereis*. The neuropeptide complement largely overlaps with that of other organisms [3], including mammals, allowing us to study the function and evolution of specific conserved neuropeptides in the regulation of animal digestive systems. Modulations in the behaviour of *Platynereis* were identified by analysing Multiscope images of the larvae in different feeding environments.

Aim

The aim of the project was to use bright field images of the *Platynereis* larvae captured with the Multiscope to identify changes in feeding behaviour by extracting parameters such as speed and trajectory of each larva. Imaging multiple fields of view in parallel improves reproducibility, which is a recognised issue in life sciences [5].

Method

Throughout the project, videos of freely-moving larvae were generated using the Multiscope in the presence or absence of food. The Multiscope can image multiple spatially separated samples at high-resolution without movement of the sample or the imaging system, allowing us to image nine wells occupied by the larvae in parallel. Initially, ~18 larvae were placed in each well filled with sea water and covered with a cover slip to avoid a meniscus. The larvae were imaged, and later fed with algae to be imaged again, so as to compare the difference in larval behaviour in the presence and absence of food. 900 images were taken before, and 900 after feeding (100 images per well) at a rate of 1 frame per second (fps).

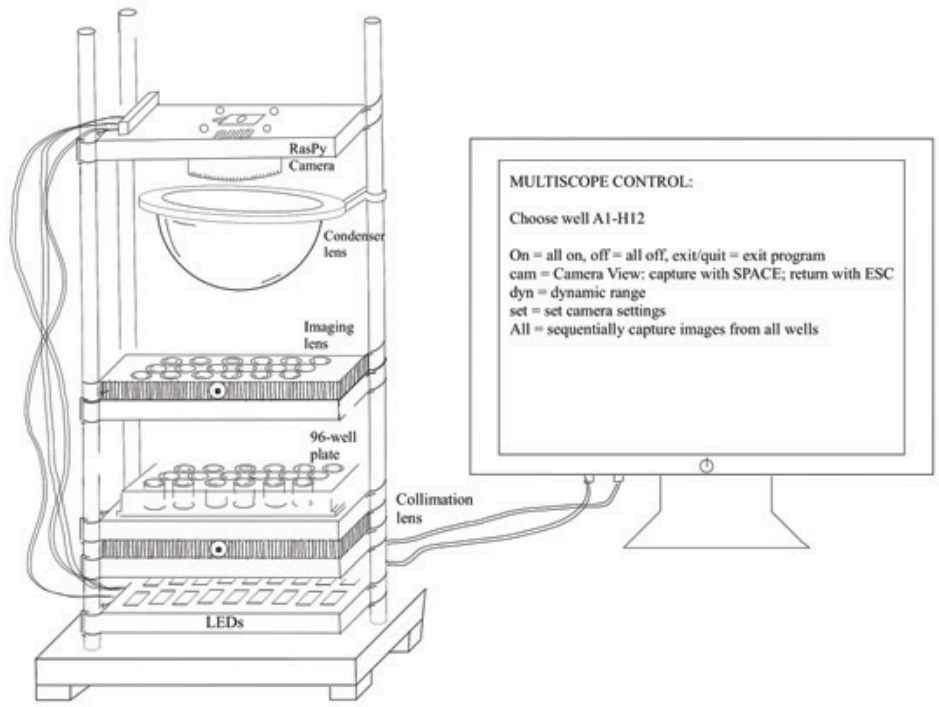

Figure 1. An illustration of the Multiscope (left) used to provide high throughput screening by imaging multiple wells in parallel. Multiscope illumination and frame capture are controlled using a Raspberry Pi at the base of the Multiscope.

The nine fields of view were first stitched together into a video file using a custom Python script. Automated visual tracking software, 'TRex' [4], was then used to further process the videos to extract features of interest, such as speeds, trajectories and visual fields. As an alternative to TRex, the 'OpenCv' Python module was used for analysis as it contains all of the main image and video processing functionality. In the comparison, TRex was favoured due to its additional features such as estimating the visual fields of each larva based on midline orientation.

Results and Discussion

Initial experiments indicated limitations in continuous tracking when using a restricted field of view as tracking would be lost when larvae move out of the frame. The Multiscope optics was modified to reduce the system magnification and increase the field of view to ~80% of the well area. The next experiments focused on validating the method of using tracking to measure changes in larvae behaviour by looking at changes before and after the addition of food (algae). Following data analysis, it could be seen that the speeds of the larvae increased post-feeding as expected (Figure 4), validating the approach. The next step would be to monitor the influence of different neuropeptides on behaviour, but time constraints meant that these experiments were beyond the scope of this study.

Analysing the obtained data using the tracking software turned out to be more challenging than anticipated – initially, the larvae frequently moved in and out of the field of view, causing problems with identity tracking and therefore making it difficult to extract any features of interest. This was improved by enlarging the field of view and decreasing the number of larvae in the well, though this did not fully resolve the issue of identity tracking. It was

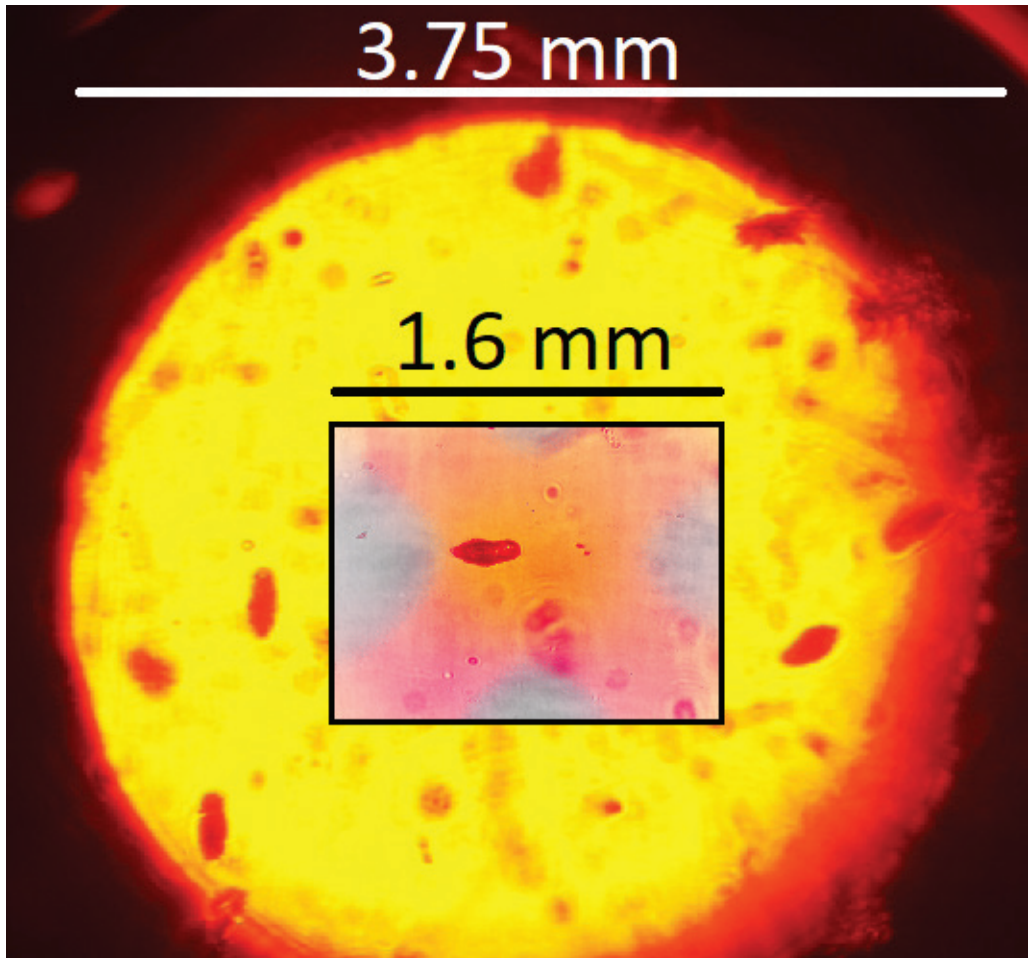

Figure 2. An image of Platynereis dumerilii captured by the Multiscope, showing the larger, low magnification field of view. The smaller field of view from the high magnification system is shown inset.

often the case that TRex failed to track the larvae, so the identities of the larvae in each frame had to be edited manually and the data had to be extracted after this time-consuming process. Manually editing identities also introduced a bias towards selecting and tracking larvae which remained relatively still. Putting fewer larvae in each well (e.g. nine larvae compared to the previous 18) proved to make tracking and the subsequent analysis much easier. Additionally, it was identified that the number of frames per second (fps) was low for accurate tracking. The rate of 1 fps meant that each well was imaged with a nine-second interval. As a result, there was significant movement of larvae between some frames, which hindered the tracking abilities of the software.

Overall, by refining the optics and image processing we were able to successfully identify, process and analyse more larvae tracking data throughout the experiment.

Reflections

Without the support from the Royal Microscopical Society and Dr Alex Corbett, I would not have had the opportunity to learn more about microscopy, image processing techniques and computational analysis used in research. I enjoyed working in fine detail while investigating complex biological systems and gained a deeper insight into how optical instruments function, both theoretically and experimentally. Having had little experience with practical physics and laboratory work due to the pandemic, the project gave me an opportunity to appreciate the experimental aspect of physics more while encouraging a deeper interest in optics. Additionally, the project developed my programming skills while working with new Python modules for image processing and tracking. Using the openly available software such as 'TRex' allowed me to work with some of the more complex tools available for scientific research.

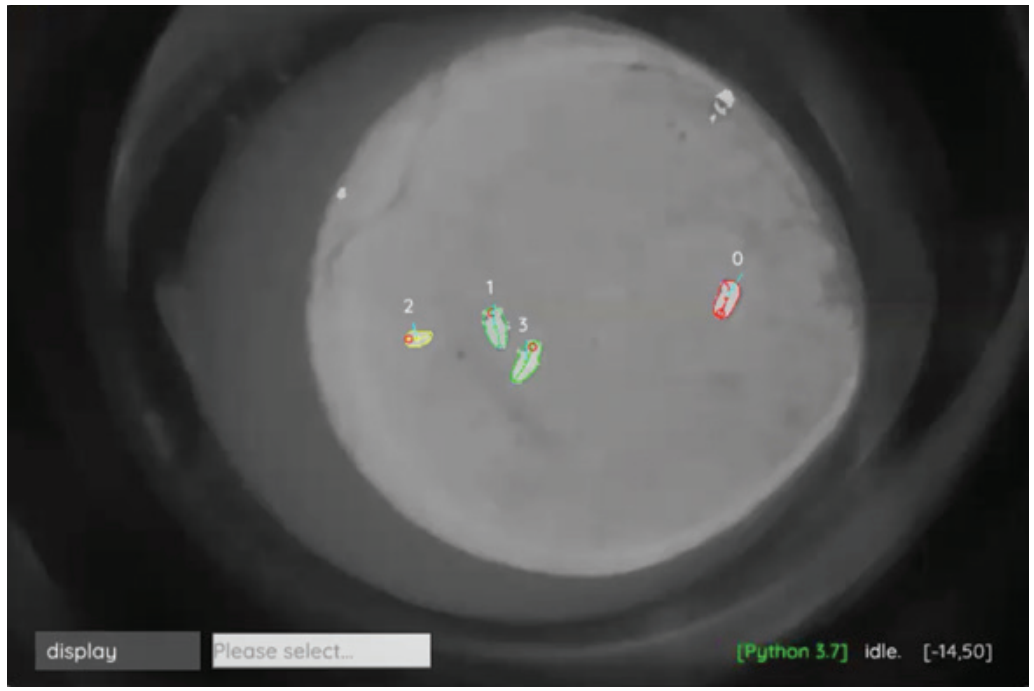

Figure 3. Using 'TRex' to analyse the obtained data.

One of the main highlights of my time in Exeter was becoming a part of the research community. I was given an opportunity to work with leading researchers at an institution I had never previously visited. Attending talks, visiting laboratories and reading relevant research papers gave an insight into how physics is developed and applied beyond lecture theatres, teaching me some of the skills necessary to become a researcher.

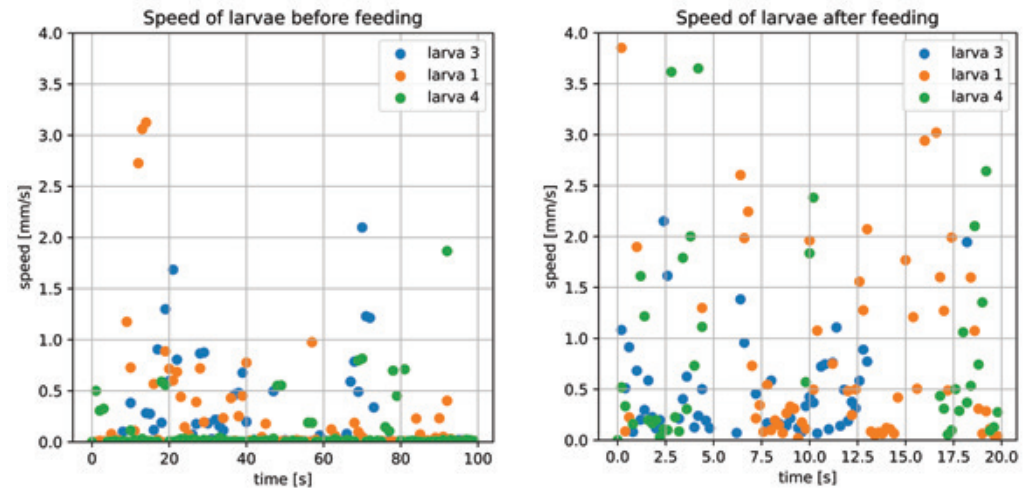

Figure 4. Speed distribution of manually selected larvae pre and post feeding.

References

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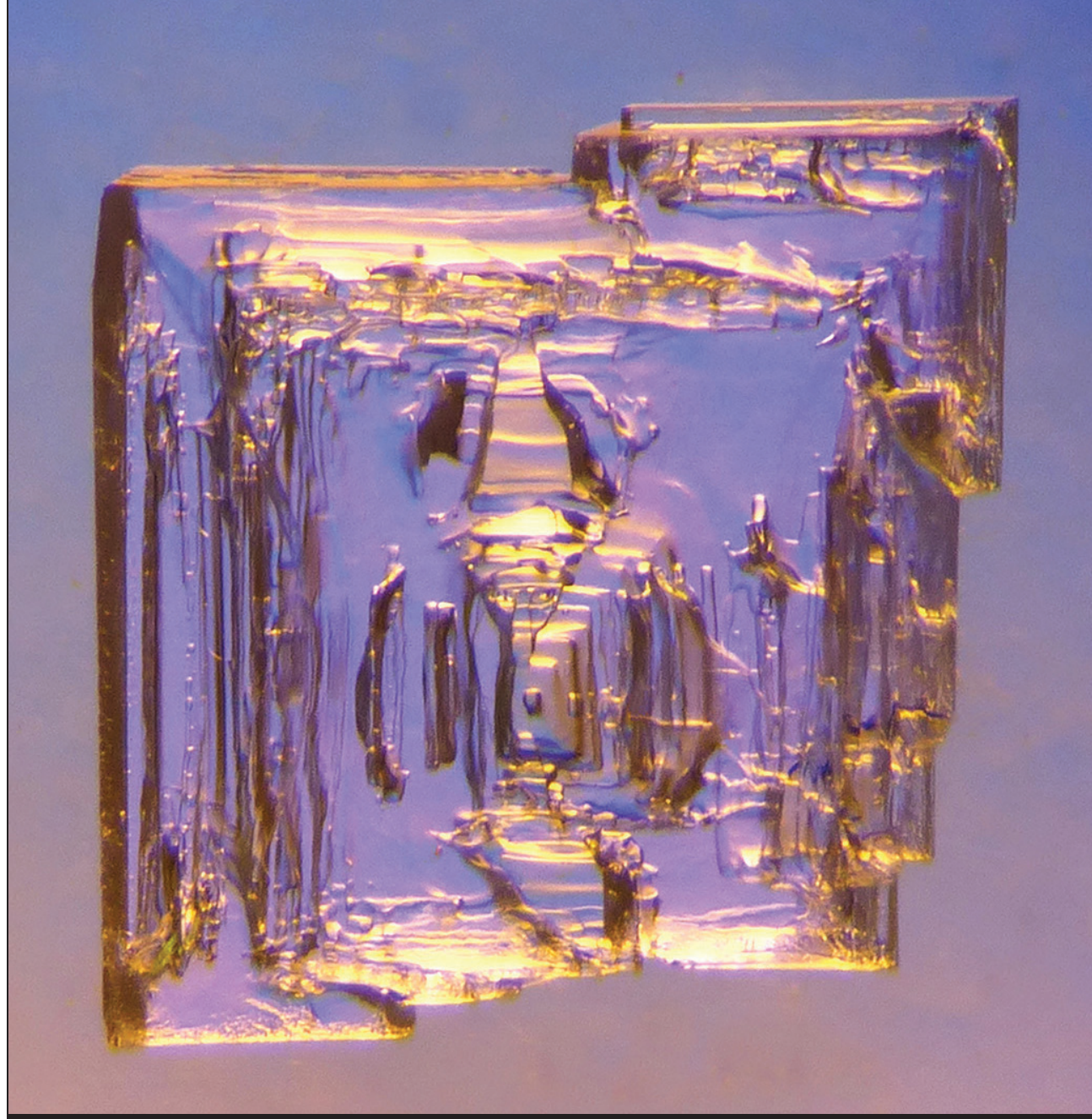
Future plans

Cross-fertilisation between biology, physics and mathematics allows researchers to interrogate biological complexity at all scales, from studying cells to populations and organisms, and during the next few years I hope to further pursue studies in biological and soft matter physics through theoretical, computational and experimental tools.

Imaging of biological systems and structures plays a crucial role in driving biophysics research and I am grateful to the Royal Microscopical Society for funding this opportunity and Dr Corbett for his support and guidance. I have gained a deeper understanding of the topic that will most likely be a major part of my studies in the upcoming years.



Crystal of low-sodium salt (sodium-potassium chloride), magnified 25x

Revital Katznelson

Chosen as the December image for the 2021 RMS Digital Calendar.