

## Flow field measurements during microorganism locomotion using MicroPIV and dynamic masking

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### ABSTRACT

Microorganism locomotion is often the focus of biologically inspired propulsion studies, as these organisms move through water in a very effective fashion. The locomotion effectiveness is required both in order to conserve energy, and to generate a minimum flow disturbance to avoid detection by predators. In this work we applied time-resolved imaging and MicroPIV while a uniflagellate microorganism (*Euglena Gracilis*) is swimming in water. *Euglena Gracilis* is known to use a thin whip-like structure (flagellum) to pull itself through water, in combination with rolling, stretching and contracting its flexible body. Time-resolved MicroPIV experiments have been performed to capture both the detailed time history of the organism's location and to compute time-resolved flow fields around the organism. In biological flows, pulsed laser illumination is often not preferred as this can simply disable the organism. For this reason, LED-based pulsed illumination was used in transmission mode. The shadow displacements of 1 $\mu$ m seeding particles were analyzed using an Adaptive PIV algorithm with refinement steps, outlier detection and deforming windows. A dynamic masking technique was also applied since the moving organism contributes to the cross-correlation function during PIV processing. In the current study, we generated dynamic masks of the *Euglena Gracilis* from the original image ensemble using a number of image processing functions. Subsequently these were used for both image masking and for vector masking. The flow field around the organism confirms that the flagellum is at the leading edge, pulling strokes backwards.

**Keywords:** Time-resolved MicroPIV, dynamic masking, bio-microfluidics

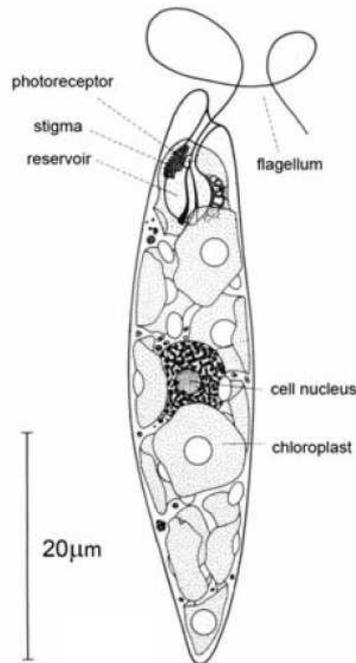
### INTRODUCTION

Microorganisms develop effective locomotion techniques in order to conserve energy and minimize flow disturbances to avoid detection by predators. Experimental investigation of locomotion techniques of real micro-swimmers provides significant input for research on biologically inspired propulsion studies. This experimental input must be characterized to quantify the hydrodynamics of microorganism locomotion. Recently different parameters have been proposed to quantify the hydrodynamics and energetics of copepods [1]. These parameters include spatial and temporal decay of flow disturbance, energy dissipation, energy budget, and propulsion efficiency. These parameters require the accurate measurement of the flow field structure in a time resolved fashion. Time-resolved MicroPIV has great potential in quantifying flow fields around microorganisms and identifying their swimming technique.

MicroPIV and MicroPTV have been used to quantify the flow fields and disturbances generated by the propulsion mechanism of some organisms [1-5]. A few challenges still exist in such measurements: First of all, the object in question is most likely present in the flow field during the measurements. Since we are only interested in the flow field information, the successful removal (masking) of the object from the raw particle images is desired, without removing any useful information from the flow field. Second challenge is about the illumination. Most time-resolved MicroPIV systems use a powerful, pulsed laser as illumination. This type of illumination can easily disable the organism or alter its normal locomotion behavior. A third challenge is related to repeatability of experiments to obtain reliable results. Performing measurements around biological objects means that there is very little control on the locomotion direction, and recapturing of certain dataset is extremely difficult, if not impossible. This requires very accurate PIV algorithms to calculate vector fields from single image pairs. A fourth challenge is related to the timing of the experiment; it is quite difficult to control

when the microorganism will swim through the field of view. Therefore experiments often require special hardware with ring image buffers with post trigger functionality.

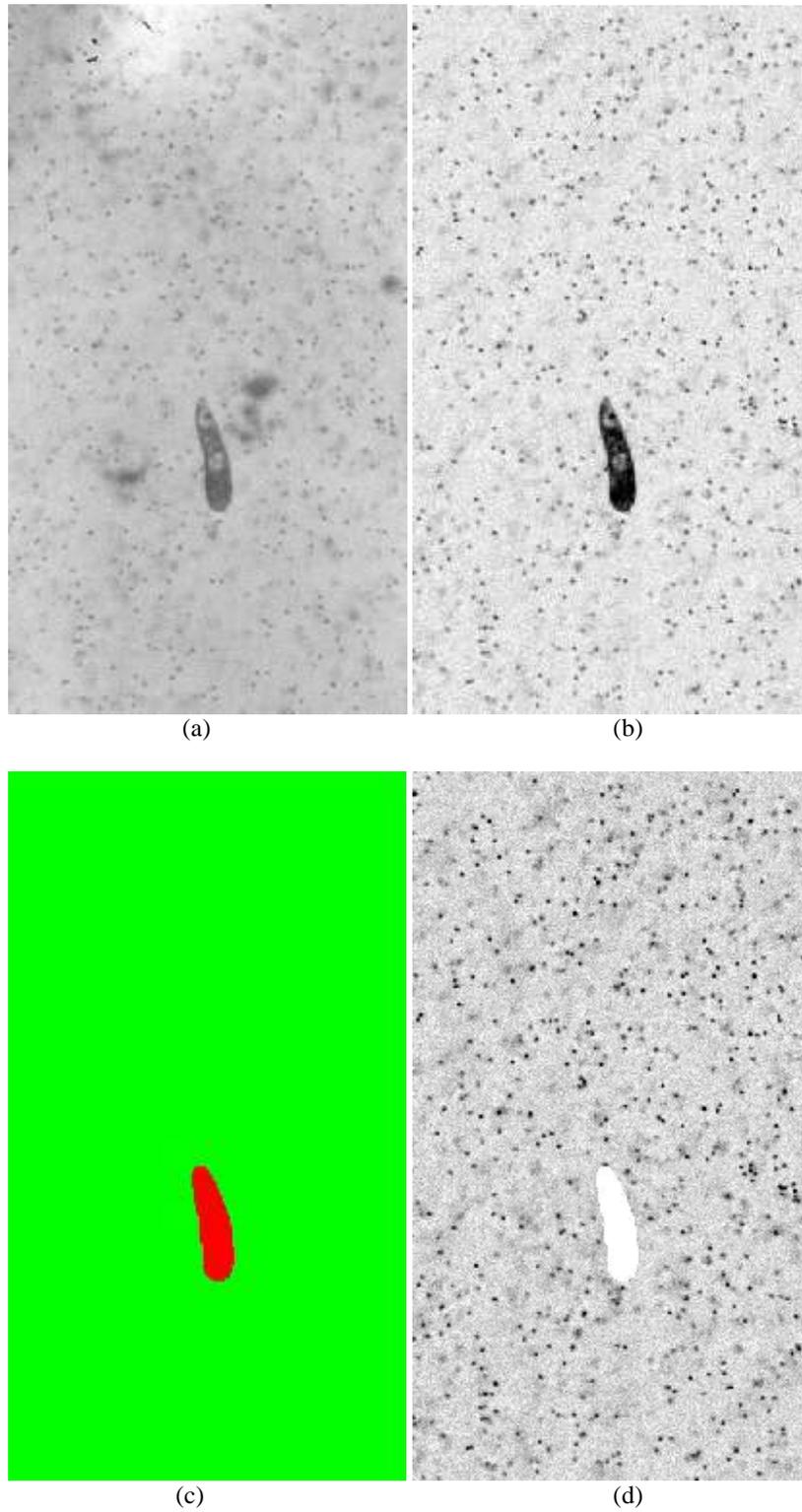
*Euglena Gracilis* is known to use a single whip-like structure called flagellum, in combination with rolling, stretching and contracting its flexible body. A schematic of this uniflagellate is shown in Fig. 1. Body length without the flagellum can vary between 20 $\mu\text{m}$  and 100 $\mu\text{m}$ . The flagellum is located at the end close to the photoreceptor, and the cell nucleus and chloroplast are centrally located in its body. Although imaging the flagellum is challenging, the photoreceptor and the chloroplast can be detected easily under a microscope (Fig. 2a).



**Figure 1** Schematic of *Euglena Gracilis*.

## EXPERIMENTAL SETUP

A culture of uniflagellate *Euglena Gracilis* were transferred to a 700 $\mu\text{m}$ -deep test channel containing filtered seawater. The measurement setup was a MicroPIV system manufactured by Dantec Dynamics. The system components included an inverted fluorescence Microscope (HiPerformance), a sensitive CMOS detector (SpeedSense M310), a synchronization device (80N77 Timer Box), and a pulsed LED illumination and electronics (Microstrobe). In biological flows, high-power pulsed laser illumination is often not preferred as this can disable, or affect the normal locomotion behavior of the organism. For this reason, a lower-power LED-based pulsed illumination was used in transmission mode. 1 $\mu\text{m}$ -diameter fluorescent seeding particles were introduced in small quantities until a sufficient seeding density was achieved for PIV. The seeding density was kept at a low level in order to avoid a potential change in the normal swimming behavior. The particle images were recorded at a frame rate of 12.5 fps, at a resolution of 1280x800 pixels. The images were acquired using a 40x magnification objective, producing a 0.4 mm x 0.64mm field of view (FoV). Later a smaller region of interest (ROI) was extracted with a resolution of 323x529 pixels, corresponding to 160 $\mu\text{m}$  x 265 $\mu\text{m}$  in the object space. Single frame image acquisition was performed with a constant time difference of 80ms. Since the full frame FoV was only approximately 0,25mm<sup>2</sup>, the images were stored in a ring buffer and a manual trigger stopped the acquisition after the organism had passed through the FoV. Consecutive frames were analyzed for PIV processing - a typical procedure for time-resolved PIV measurements: 83 frames were analyzed to produce 82 flow field measurements. Total recording time was 6.56s. Image recording, subsequent image processing and PIV analysis was performed using DynamicStudio (Dantec Dynamics, Skovlunde, Denmark). Image processing steps included ROI extraction, pixel inversion, background subtraction, image masking, PIV computation and vector masking.

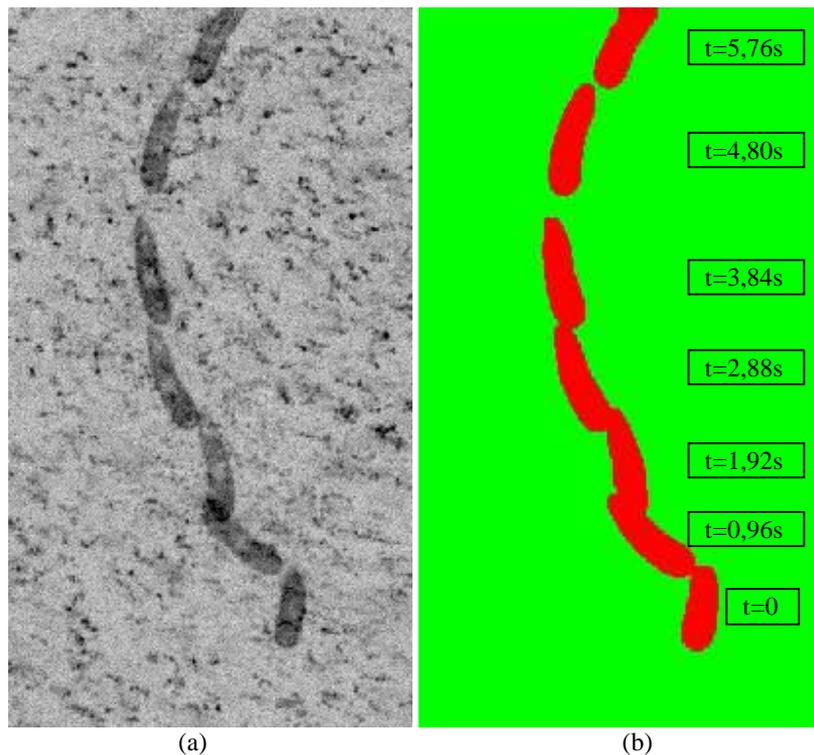


**Figure 2** *Euglena Gracilis* swimming in seawater with 1- $\mu\text{m}$  diameter seeding particles at  $t=1.92$  s. (a) Raw image before background subtraction (b) after background subtraction (c) dynamic mask (d) particle image after image masking

One of the raw images (at  $t=1.92$  s) is shown in Fig. 2a, where a  $47,5\mu\text{m}$ -tall *Euglena Gracilis* is visible in the sparsely seeded flow. In the acquired ensemble, *Euglena Gracilis* meanders upwards through filtered water; covering a distance of approximately  $250\mu\text{m}$  (Fig. 3). As a first step, pixel inversion is performed to work with positive particle images instead of particle shadows. Although we are working with positive particle images during processing, we choose to show shadow particle images in the rest of this text for better visibility. In the second step, a background subtraction is performed using the minimum pixel value found in the original ensemble (Fig 2b). Next, a dynamic mask is produced using the ensemble in the second step (Fig. 2c) and finally image masking is performed (Fig. 2d).

## DYNAMIC MASKING

Objects and surfaces often appear in PIV images. Unless masked, these contribute to the cross correlation function and as a result introduce an uncertainty in the PIV calculation. It is quite common and straightforward to use static masks to remove stationary objects, as these can be defined manually. However, it is not as trivial to mask moving objects or surfaces and literature on dynamic masking is quite limited. In practice, it is often possible to create dynamic masks of moving objects by applying a number of image processing functions to the original image ensemble. The idea behind dynamic masking is to get 0 pixel value on the object that is to be masked and retain the pixel value information everywhere else. This can be achieved in a two-step procedure. In step one; a new image ensemble is produced by filtering, thresholding etc. to obtain a pixel value of 0 on the object and 1 everywhere else. In step two, each time step of the new image ensemble is multiplied by the corresponding time step of the original ensemble. If the original image background pixel value is nonzero, this results in a sharp pixel value difference between the mask and the image background (Fig. 2d). As a remedy, background subtraction techniques can be used to obtain a 0 pixel value in the masked ensemble's background. Finally, the mask ensemble can be applied to mask either the raw images (image masking) and/or the PIV results (vector masking).



**Figure 3** Quality of dynamic masking (a) Recorded position of *Euglena Gracilis* every 0.96s. (b) Produced dynamic mask at these time steps.

In the current study, the following image processing chain produced an acceptable dynamic mask: a  $9 \times 9$  median filter, a closing filter with 10 iterations, thresholding (min:125 max:4096), pixel inversion, thresholding (min:3970 max:4096), erosion filter with 2 iterations, thresholding (min:0 max:1). Although the details of these steps are outside the scope of this text, the steps have been spelled out to show the level of complexity to achieve proper dynamic masking. In order to

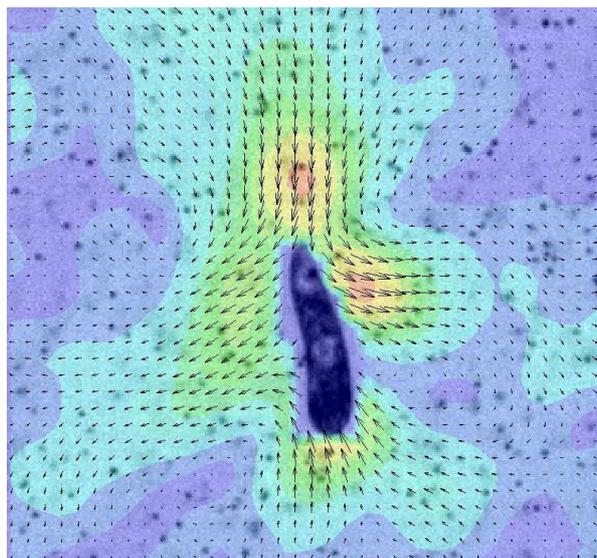
demonstrate the quality of the dynamic mask, position of *Euglena Gracilis* (Fig. 3a) and the used mask (Fig. 3b) are shown side by side at selected time steps. One immediate observation is that the mask is slightly larger than the organism. This is intentional and in order to keep a small margin around the masked object. Another observation is that the used mask may produce non-ideal results around image boundaries (see for example the top of Fig. 3b). Apart from these the mask captures the position and the shape of *Euglena Gracilis* in a successful fashion.

## ADAPTIVE PIV PROCESSING

After performing image masking, the particle displacements were computed using an Adaptive PIV algorithm with refinement steps, vector validation and deforming windows. The Adaptive PIV is an advanced and iterative cross-correlation based displacement estimator, where final interrogation windows of 32x32 pixel are used with 75% overlap. The vectors were considered valid if the peak height ratio is larger than 1.25. In other words, the displacement calculation was considered reliable when the highest peak (the assumed signal peak) is larger than 1.25 times the second highest peak (assumed to be noise) in the cross-correlation function. This is certainly not the only vector validation method, but it is one of the oldest. A threshold value of 1.2 is often used in the literature, so in this respect our threshold value of 1.25 is more conservative. The subpixel positioning accuracy of the Adaptive PIV algorithm was reported as 0.055 pixels with 95% confidence [6]. This corresponds to a 27.5nm displacement in the object space, and the velocity uncertainty is estimated as 0.34 $\mu$ m/s. An average filter in a 5x5 neighborhood and vector masking was applied after Adaptive PIV computations.

## RESULTS

A close up of the flow field around *Euglena Gracilis* at  $t=1.92s$  is shown in Fig. 4. In this figure vectors represent the  $u$  and  $v$  components of the flow field and colors represent the magnitude of local velocity. The maximum velocity is observed in red areas as 12 $\mu$ m/s and blue areas represent stagnant flow regions. It is immediately apparent that the fluid is drawn towards the organism upstream and downstream, and fluid is expelled from the organism on the sides. The downstream flow field can be explained as the wake in the aft of the swimmer, and the upstream flow field is produced most likely by the flagellum pulling a stroke, the source of propulsion. Due to continuity around the organism, the fluid is expelled outwards from the sides. This flow field also produces four small vortices, one at each corner of the image, i.e. due SW, SE, NW and NE of the organism. A similar flow field is observed also at  $t=3.52s$ ,  $4.32s$  and  $5.12s$ , exactly every 0.8s (corresponding to 1.25Hz). The time difference between the 1.92s and 3.52s is 1.6s, corresponding to half the above-mentioned frequency. It is not conclusive whether 1.25Hz is the beating frequency for the flagellum, because (i) the measurements are performed in a plane and the organism can rotate its flagellum, (ii) the number of data points is not sufficient to make this conclusion and further experimentation is necessary.



**Figure 4** Flow field around *Euglena Gracilis* at  $t=1.92s$ . Max velocity 12 $\mu$ m/s.

## CONCLUSIONS & FUTURE WORK

Several important conclusions can be drawn from this experiment: Time-resolved long-distance MicroPIV is a powerful technique for investigation of flows around microorganisms if a suitable illumination source is used. Lower-power pulsed-LED illumination proves to be an effective illumination mode, as it does not disable or affect the normal locomotion behavior of the organism. Furthermore, a dynamic masking approach is used successfully. The importance of image pre-processing and dynamic masking must be stressed as it can reveal important details about the flow field. Using these, we were able to reconfirm that the flagellum is at the leading edge, pulling strokes backwards. As a final remark, we can conclude that there is no single dynamic masking recipe that works globally. Dynamic masking strategies must be custom-made for each image ensemble. Future work includes the investigation of automatic post-triggering options to stop image acquisition in order to avoid long waiting times during the experiment. We also plan to perform PTV on the same dataset and conduct new experiments around microscopic swimmers using a time-resolved Stereoscopic MicroPIV system for 2D3C measurements.

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