ELASTOMERIC MICROPILLAR ARRAYS FOR THE STUDY OF PROTRUSIVE FORCES IN HYPHAL INVASION

V. Nock[1], **A. Tayagui**[2] and A. Garrill[2]

[1] Biomolecular Interaction Centre, Department of Electrical and Computer Engineering, University of Canterbury, NEW ZEALAND
[2] School of Biological Sciences, University of Canterbury, NEW ZEALAND

Biomolecular Interaction Centre

UC UNIVERSITY OF CANTERBURY Te Whare Wānanga o Waitaha CHRISTCHURCH NEW ZEALAND

Abstract

- Fungi and Oomycetes are microorganisms that can be pathogenic and grow invasively causing significant economic losses and diseases[1].
- These organisms grow by extending the cell at the tip. This involves turgor pressure, cell wall yielding and a dynamic cytoskeleton, giving rise to a protrusive force[2,3].
- A Lab-on-a-Chip platform, with integrated force sensor based on elastomeric micro-pillars, is allowing us to study the molecular mechanisms which enable the generation of protrusive force at the tip of invasively-growing hyphae.
- A maximum force of 16 µN was measured for the oomycete *Achlya bisexualis* cultured on the chip.

Materials and Methods

For protrusive force measurement with elastomeric micro-pillars, we have developed a PDMS-based platform for use with the oomycete *Achlya bisexualis* (Fig. 1a).

The design included chamber outlines, measurement pillars (15 µm diameter; 30 µm height) and a 5 µm spacer-layer to enable free movement of the pillar tops (Fig. 1b).

The PDMS chips were coated with PYG broth and then a plug of the *Achlya* culture was transferred onto them.

Hyphae were left to grow from the seeding area into the pillar arrays for approximately 12 h.

The deflection of the pillars was recorded using a microscope-based imaging setup (Nikon Eclipse 80i)[4,5].

The convertion of the measured pillars deflection into force values was obtained by a custom image-processing algorithm implemented in MATLAB in conjuction with pre-calibrated mechanical pillar properties[6].

a)

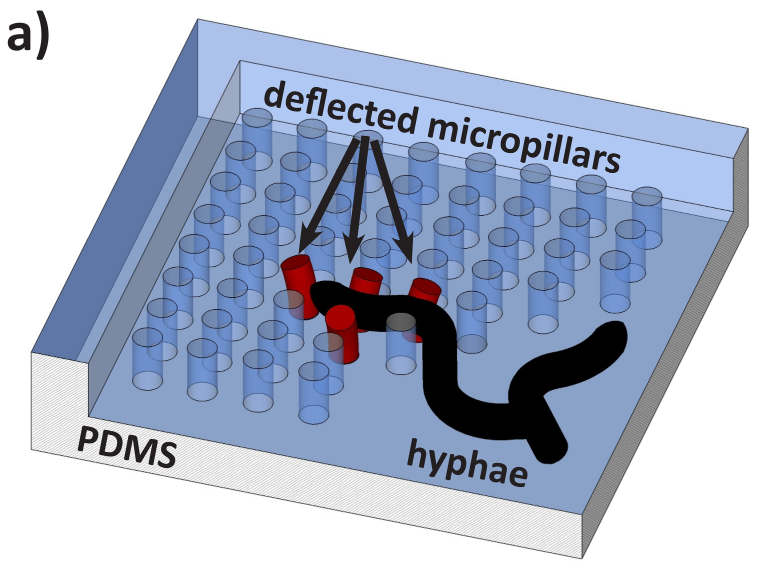
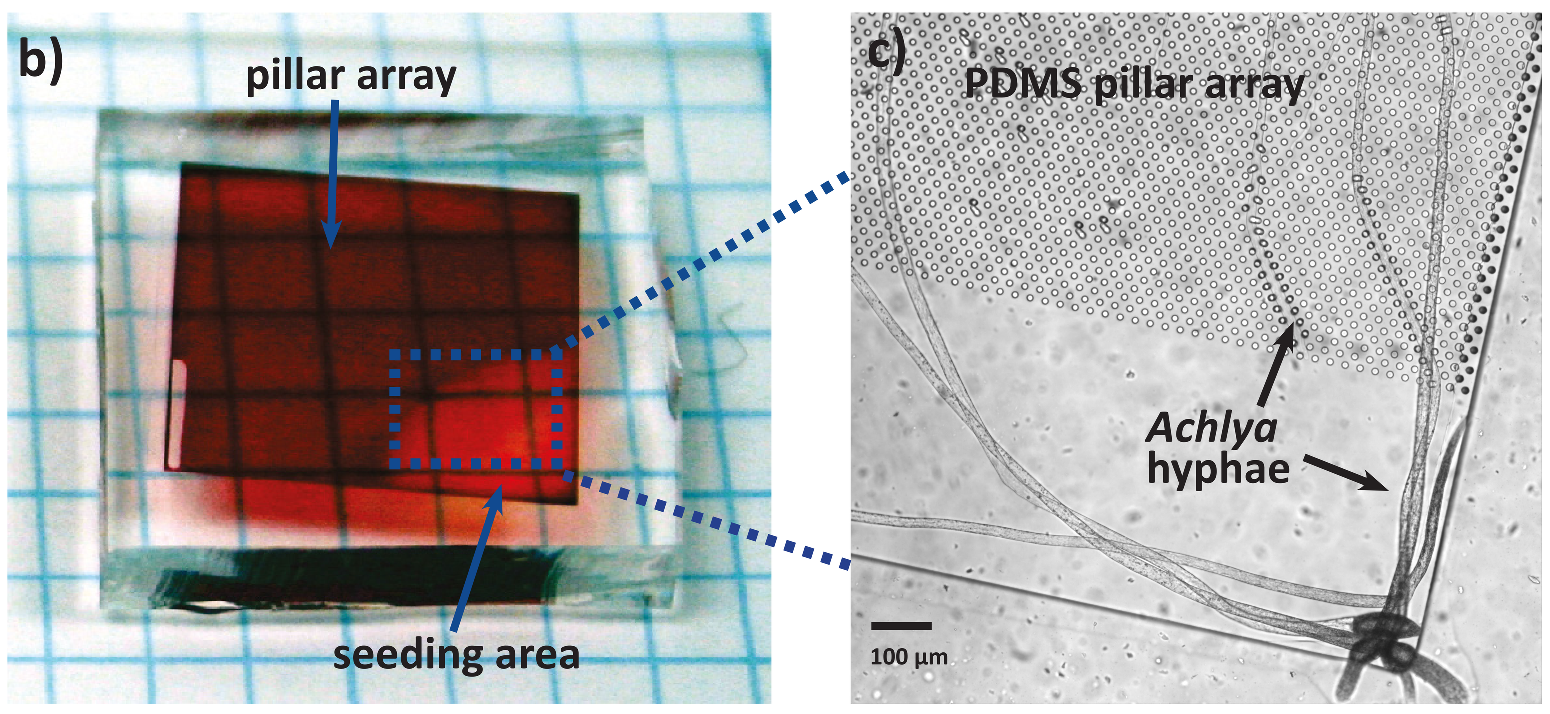


Figure 1: Experimental setup: (a) Schematic of the measurement setup showing fungal hyphae growing into an array of micro-pillars. Pillar deflection is recorded using an imaging setup and converted into force magnitude and direction. (b) Photograph of the fabricated PDMS chip containing an array of sensing pillars and a seeding area (red-colored water used for visualization). (c) Optical micrograph of *Achlya* hyphae extending from a mycelial plug into the micro-pillar array.

b) c)



Results and Discussion

Seeded hyphae have been observed to grow from and through both aqueous and agar-containing media on the chips.

Since hyphae of *Achlya* can grow to a width of more than 30 µm, the spacing between the pillars and the pillar dimensions are crucial to successfully measure protrusive forces.

The measurement of forces of a single hypha from a mycelial plug of *Achlya* on the chip was possible (Fig. 2a).

An example of a hyphal force pattern measured using the device is shown in Fig. 2b. Both force magnitudes and directional components can be extracted from each measurement pillar. Directions plotted in Fig. 2b indicate that the hypha shown in Fig 2a was undergoing contraction during this particular measurement, a behavior difficult to determine using other force sensing designs.

Total force magnitudes up to a maximum of 16 µN were recorded.

A future device design will allow us, in a similar fashion, to guide a hyphal tip onto a single pillar.

a) b)

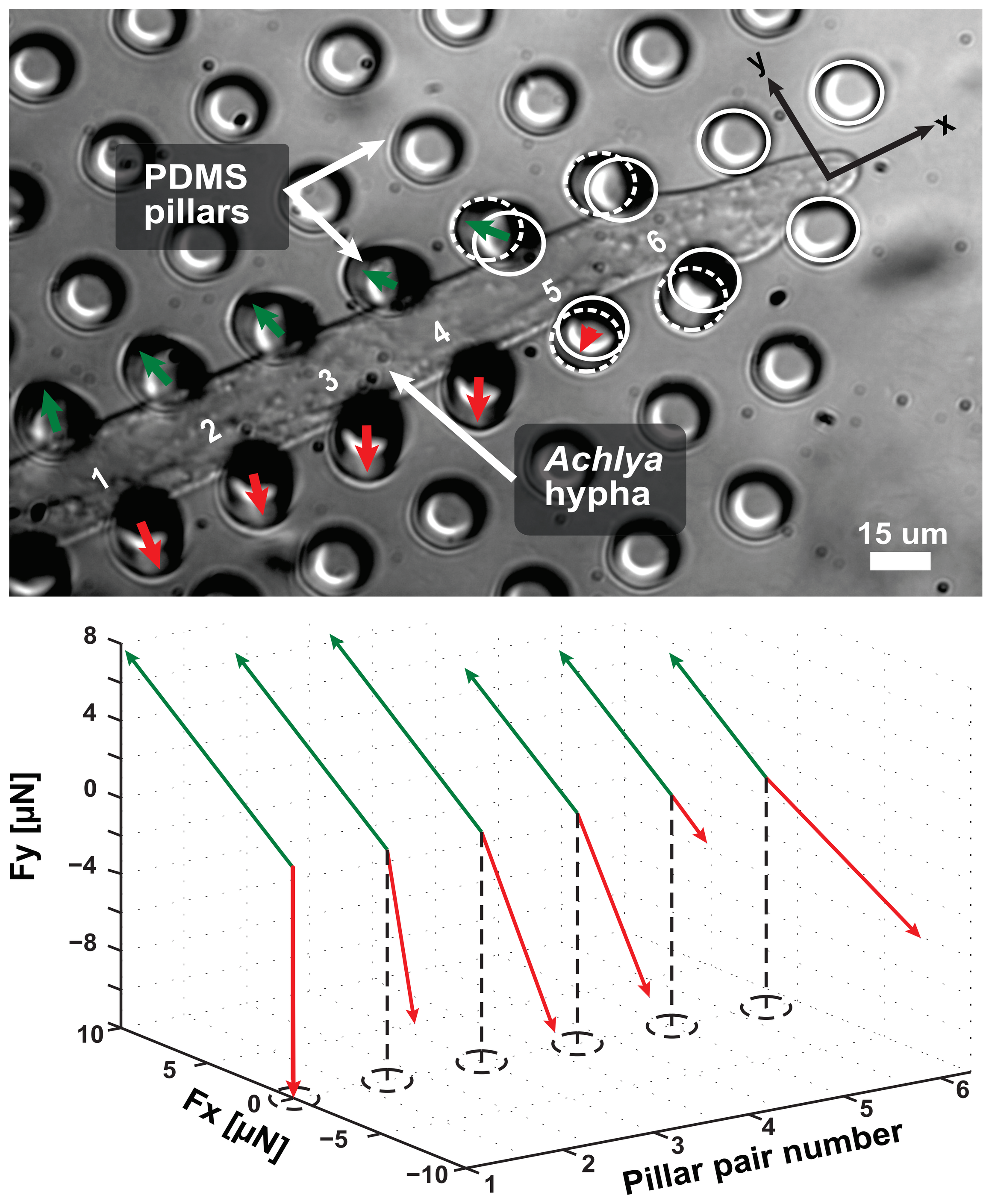


Figure 2: Force sensing on living hyphae. (a) Micrograph of *Achlya* hyphae growing through an elastomeric micro-pillar array. Full and dashed circles indicate tracking of initial and final positions of pillar tops, respectively. Red and green arrows show the derived force magnitudes and directions from pillar pairs 1 to 6. (b) Plot of force magnitudes and directions along the length of the hyphae as function of pillar pair number. Green arrows correspond to top, red to bottom pillar row.

Conclusions

We have demonstrated the use of elastomeric micro-pillar arrays for the study of protrusive forces in hyphal invasion.

Oomycete growth was monitored on-chip and hyphae were observed to grow into micro-pillar arrays.

Force measurements were performed by recording pillar deflection and the magnitude and force direction were compared to existing methods. A maximum total force of 16 µN was measured.

The current platform provides a useful tool to study the molecular mechanisms enabling protrusive force and may help to address the many diseases and infections that occur due to invasive fungal and oomycete growth[7].

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