4D motility tracking of biological cells by digital holographic microscopy

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➢Outline

- Motivation & introduction
  - Motility of biological cells
  - Digital holographic microscopy (DHM)
- DHM setup
- Applications on 4D cellular motility tracking
- Conclusion

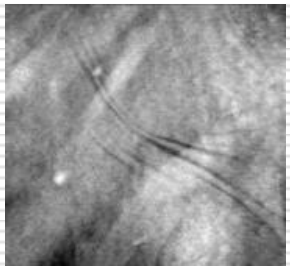
➢Motivation

Motility of biological cells

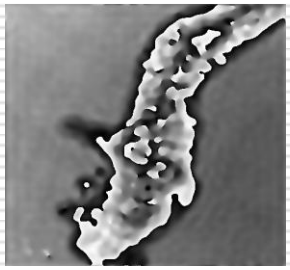
Cellular & Intracellular

Cell-substrate interactions

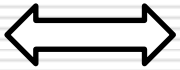
Traction forces



Intracellular fluctuation



Free-swimming cells



Profiling and tracking in 3D volume and time

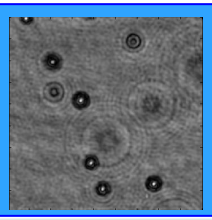
- Microspheres
- Microfibers
- Swimming cells
- Cells & microfibers interaction

Digital holographic microscopy (DHM)

Quantitative phase microscopy by digital holography (DH-QPM)

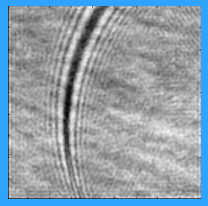
➢ Introduction

3D profiling of suspended microspheres



3D profiling

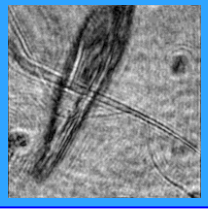
curved and random-oriented microfibers



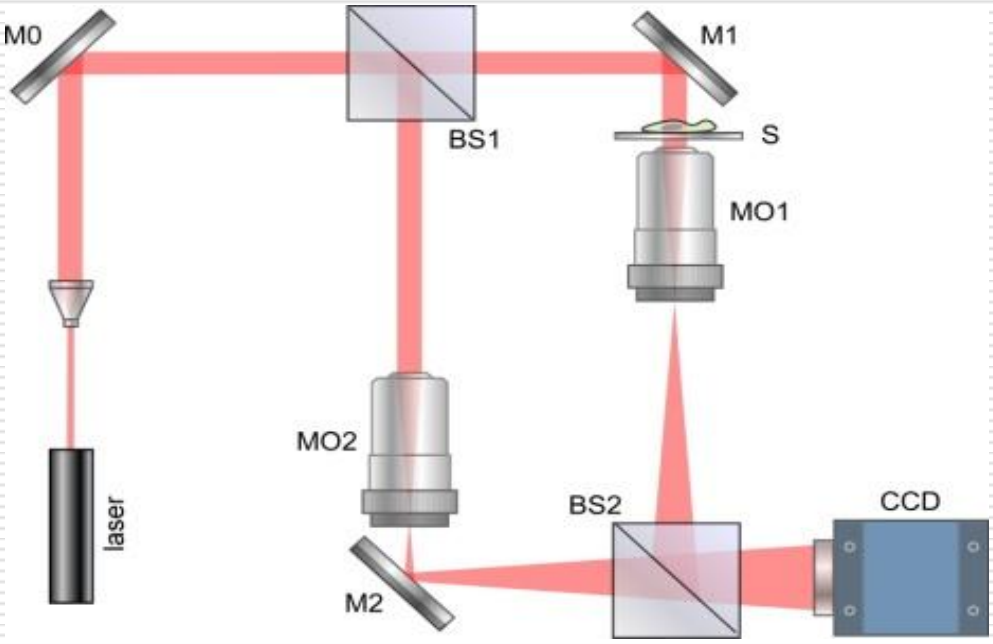
4D tracking

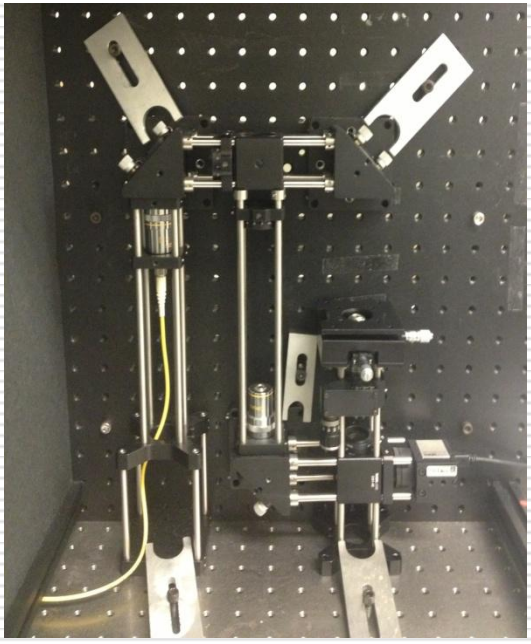
free-swimming cells

4D tracking of interactions between **swimming cells and microfibers**



➢DHM setup

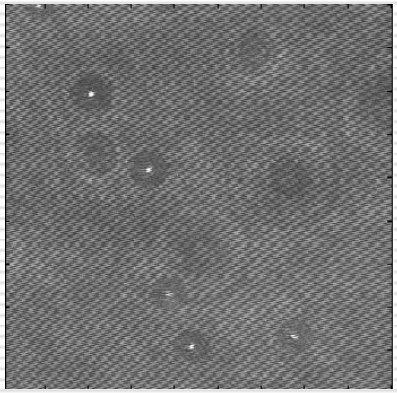




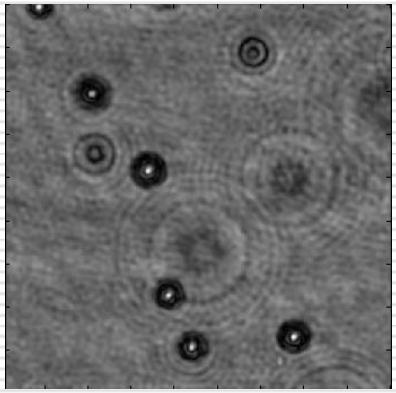
M's: mirrors; BS's: beam splitters; MO's: microscope objectives; S: sample object

➢3D profiles of suspended microspheres

Hologram

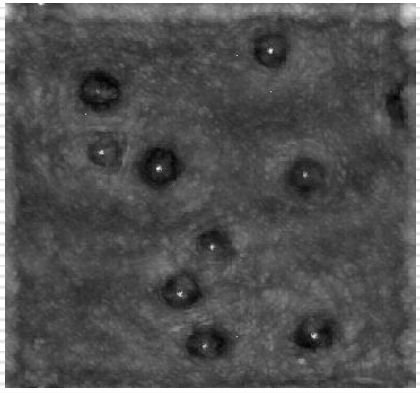


Amplitude

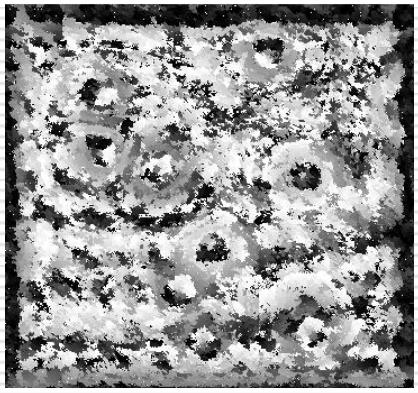


FOV 90×90 μm^2 with 464×464 pixels

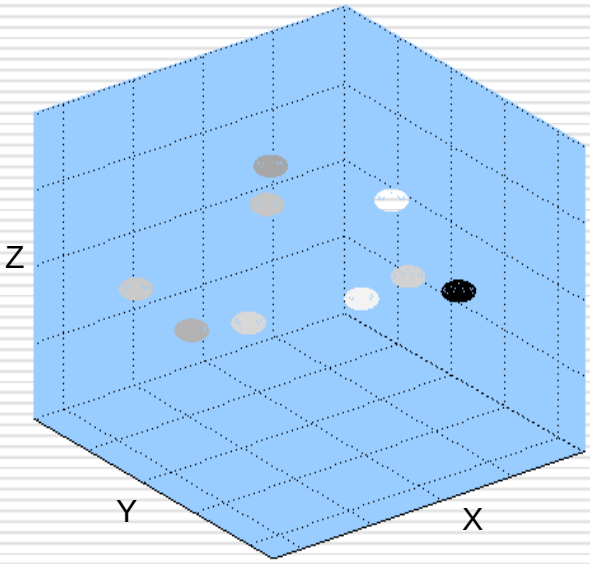
All-in-focus intensity profile



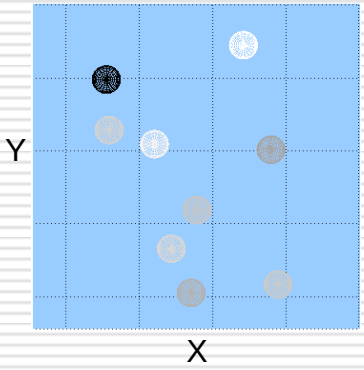
Depth position profile



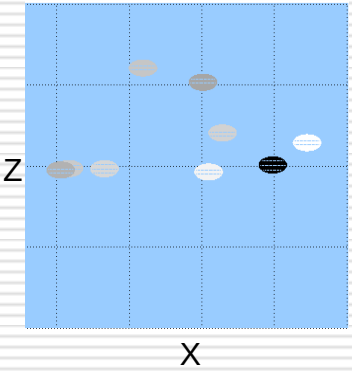
➢3D profiles of microspheres



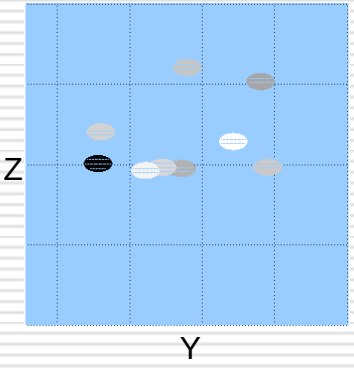
$90 \times 90 \times 200\ \mu m^3$



$90 \times 90\ \mu m^2$



$90 \times 200\ \mu m^2$

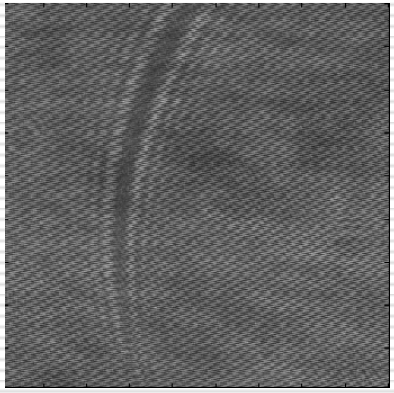


$90 \times 200\ \mu m^2$

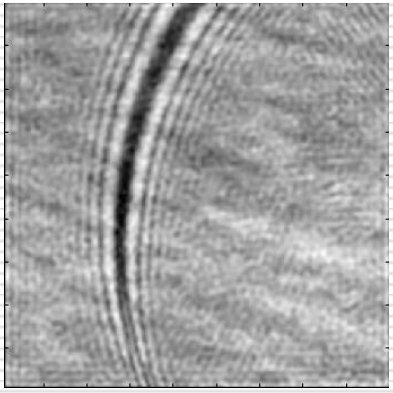
microsphere		1	2	3	4	5	6	7	8	9
centroid	x(μm)	57	53	23	10	34	81	7	51	13
	y(μm)	22	35	39	45	47	60	67	68	70
	z(μm)	20	-4	-2	-3	60	14	-6	51	-2
	Intensity($\times 10^3$)	1.97	2.16	2.01	1.81	1.91	2.23	1.49	1.74	1.93

➢3D profiles of curved microfibers

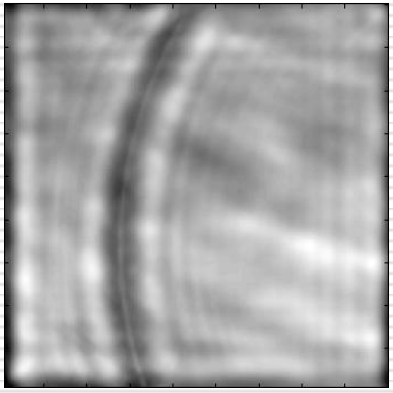
Hologram



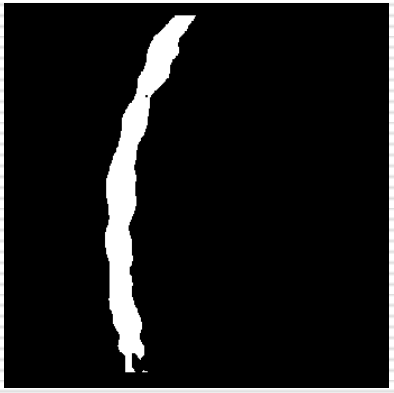
Amplitude



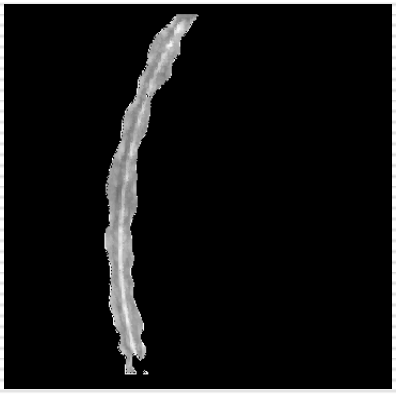
Axial projection



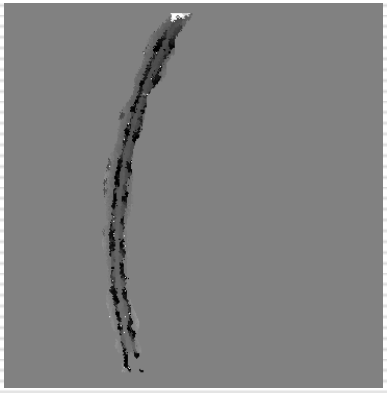
Binary image



All-in-focus intensity profile

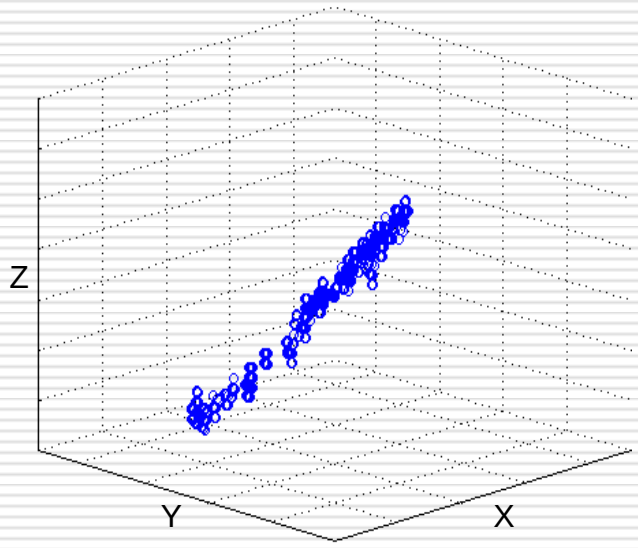


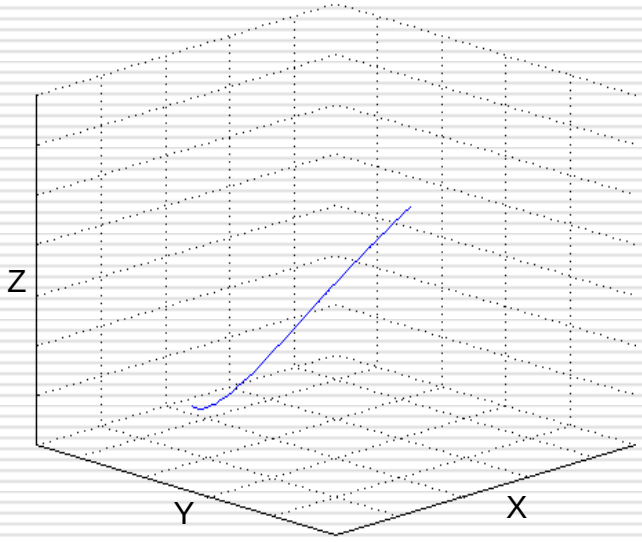
Depth position profile



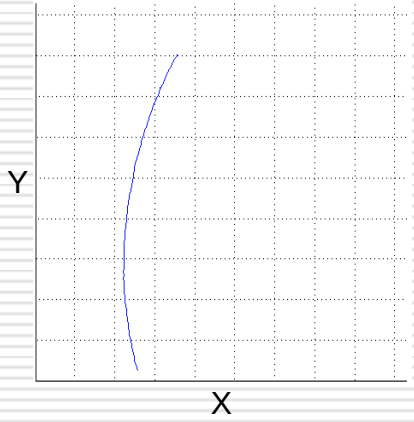
$90 \times 90\ \mu m^2$ with 464×464 pixels

➢3D profiles of curved microfibers

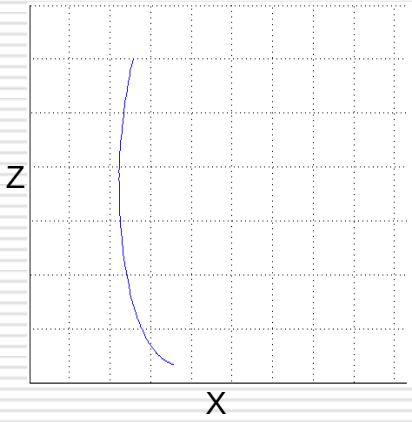




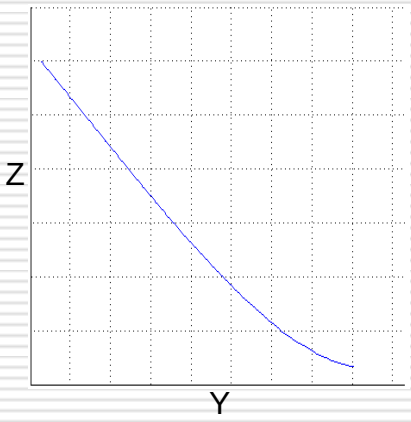
$90 \times 90 \times 35\ \mu m^3$



$90 \times 90\ \mu m^2$



$90 \times 35\ \mu m^2$

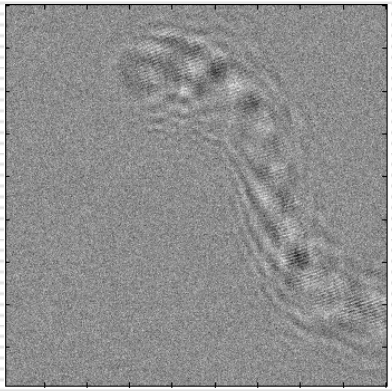


$90 \times 35\ \mu m^2$

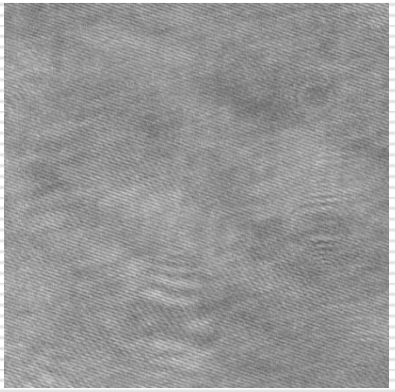
Length=126 μm

➢ 4D motility tracking of chilomonas

(H1-H2)+(H3-H4)+…+(H13-H14)

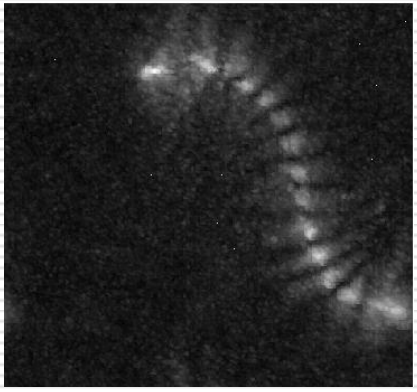


Amplitude

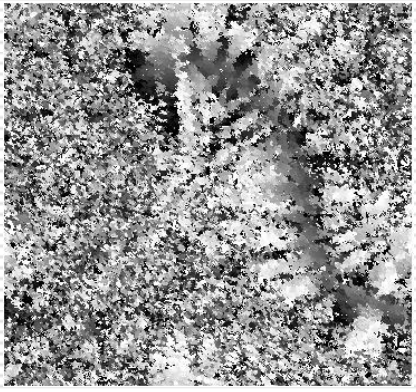


30fps

All-in-focus intensity profile

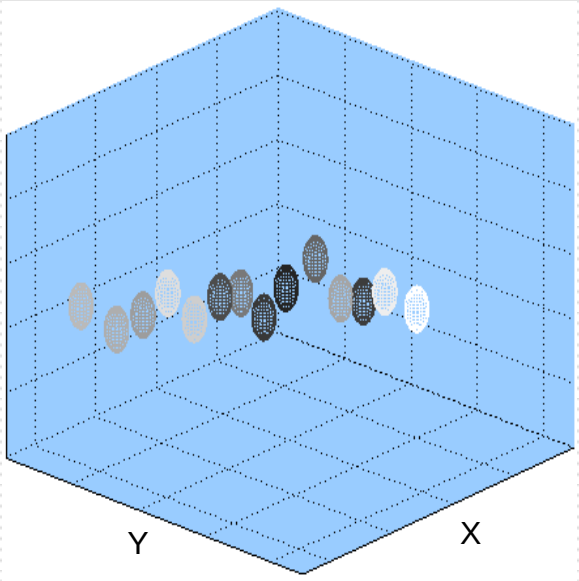


Depth position profile

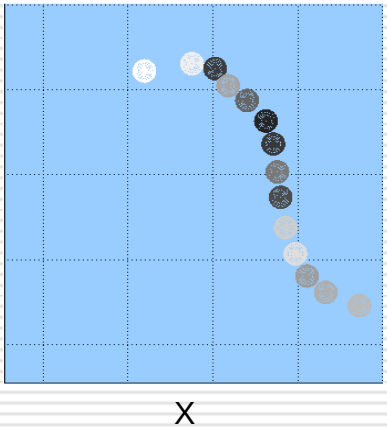


FOV 90×90 μm^2 with 464×464 pixels

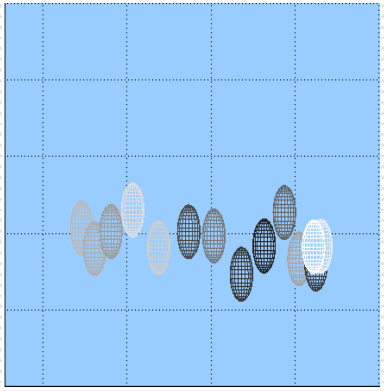
➢ 4D motility tracking of chilomonas



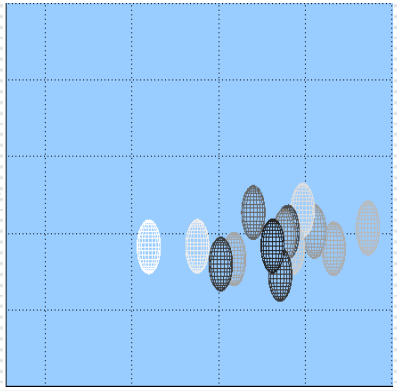
$90 \times 90 \times 200\ \mu m^3$



$90 \times 90\ \mu m^2$



$90 \times 200\ \mu m^2$



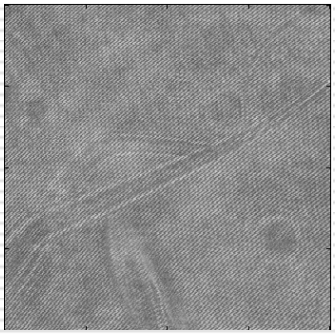
$90 \times 200\ \mu m^2$

Total path length=93 μm

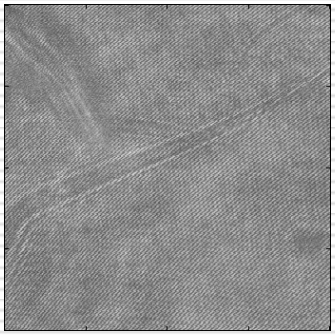
Velocity=198 μm/s

➢ 3D displacement of microfiber by paramecium

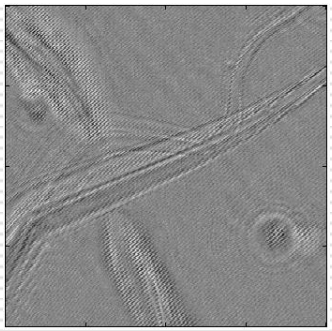
h1

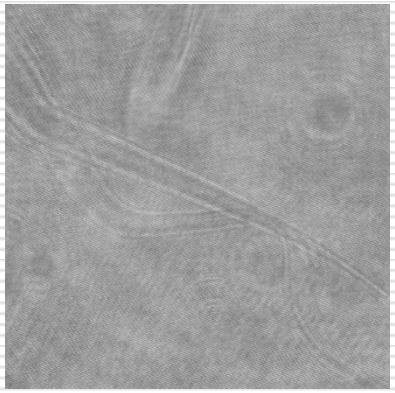


h2



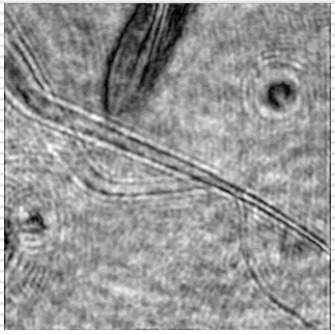
h1-h2



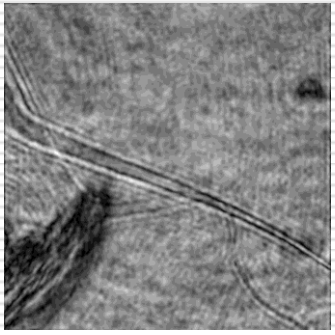


17fps

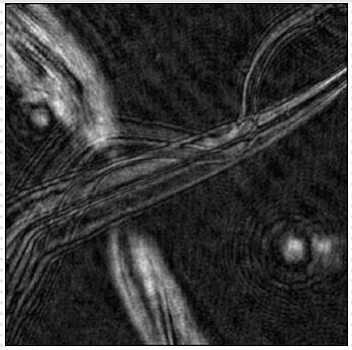
Z=0



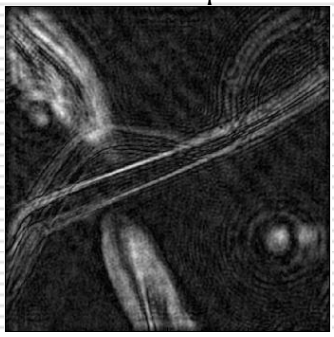
Z=0



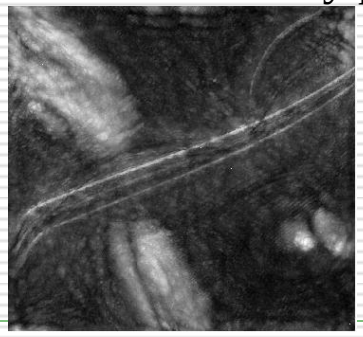
Z=0



Z=-8 μm



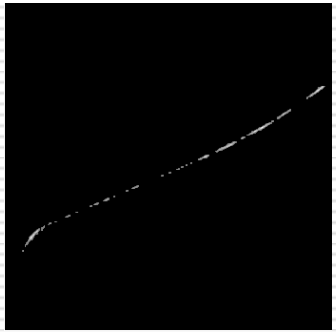
All-in-focus intensity profile

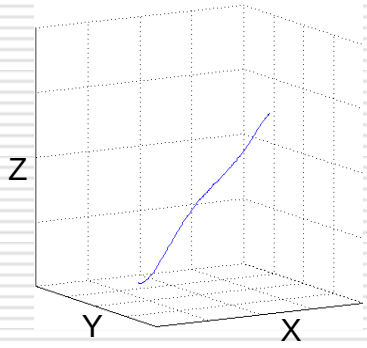


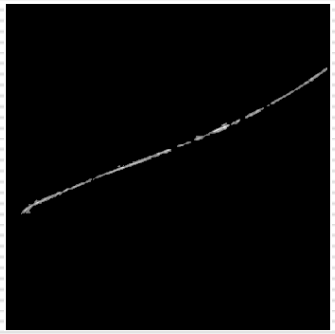
Depth position profile

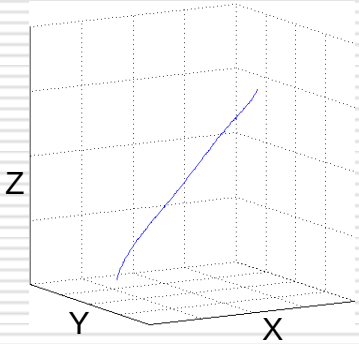
FOV 200×200 μm^2
with 768×768 pixels

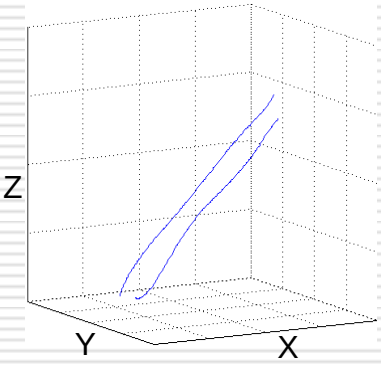
➢ 3D displacement of microfiber by paramecium



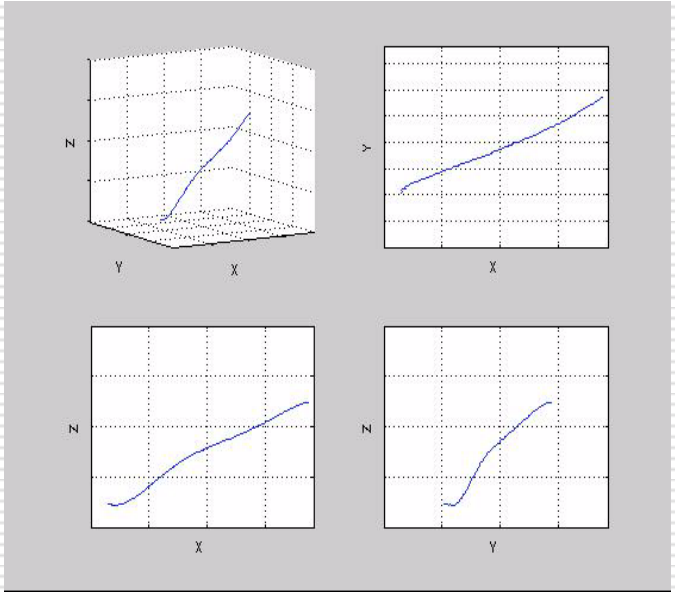




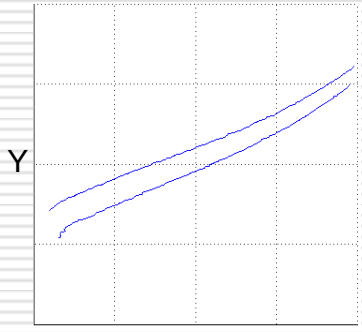




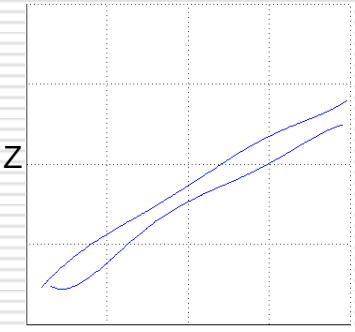
90×90×20 μm^3



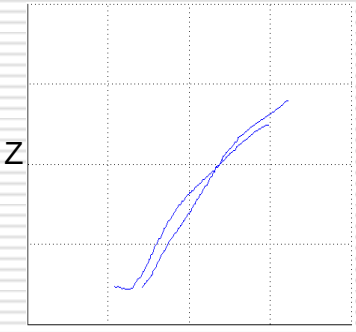
0.5s period



90×90 μm^2



90×20 μm^2



90×20 μm^2

Length=210.1 μm

Displacement=11.1μm

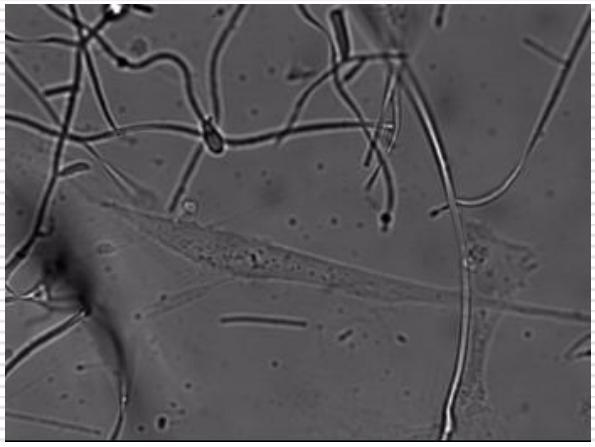
➢ Conclusion

4D motility tracking of biological cells by DHM

- Simplicity of apparatus
- Simplicity of sample preparation
- Reduction in time and data amount
- 3D distribution and moving trajectory in real-time
  - particles, living cells and fibers
  - size (few to hundred micrometer)
  - conditions (static, suspended and swim-through)
- DHM is shown to be an effective approach to study motility of biological cells with temporal and spatial resolution at the subsecond and micrometer level.

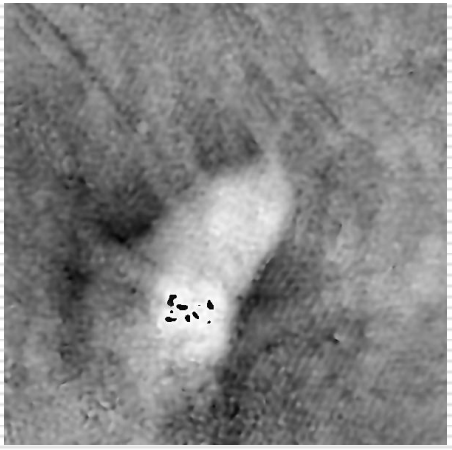
➢ Future work

- 3D adhesive cells & microfiber jungle interaction



  - Traction force of fibroblasts
  - Physical properties (stiffness) of microfibers

- 3D morphology change of biological cells based on phase profiles



Thank you!