METROLOGY for DRUG DELIVERY

µ-PIV of ultra low flows using holography

Kerstin Thiemann (Hahn Schickard e.V.)

Workshop
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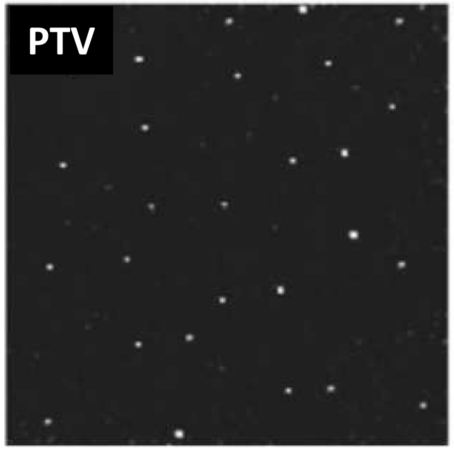
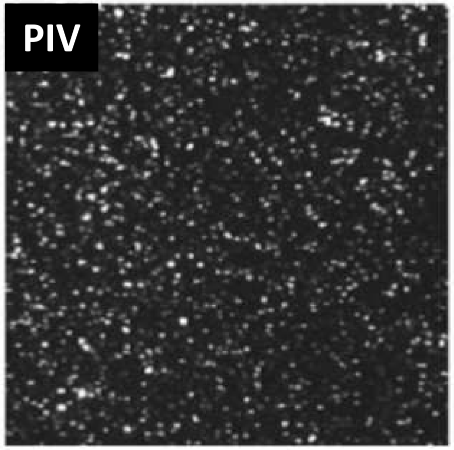
Flow visualization methods

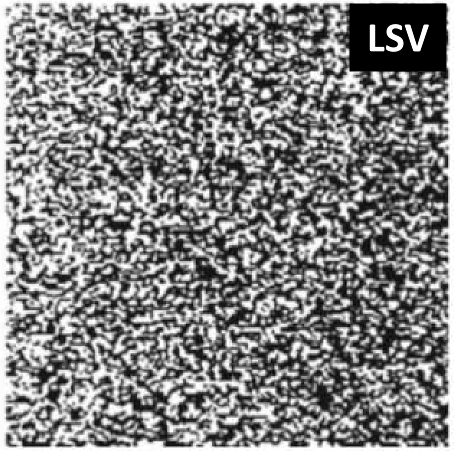
- Non-intrusive optical method of flow visualization
- Used to obtain instantaneous velocity measurements

Particle tracer methods

- Particle tracking velocimetry (PTV)
  - Low particle density
  - Measures velocity at a point
  - Path of a single particle is traced
- Particle imaging velocimetry (PIV)
  - Medium particle density
  - 2D or 3D velocity vector fields are produced
  - Cross-correlation is used
- Laser speckle velocimetry (LSV)
  - High particle density
  - Generated speckle is traced





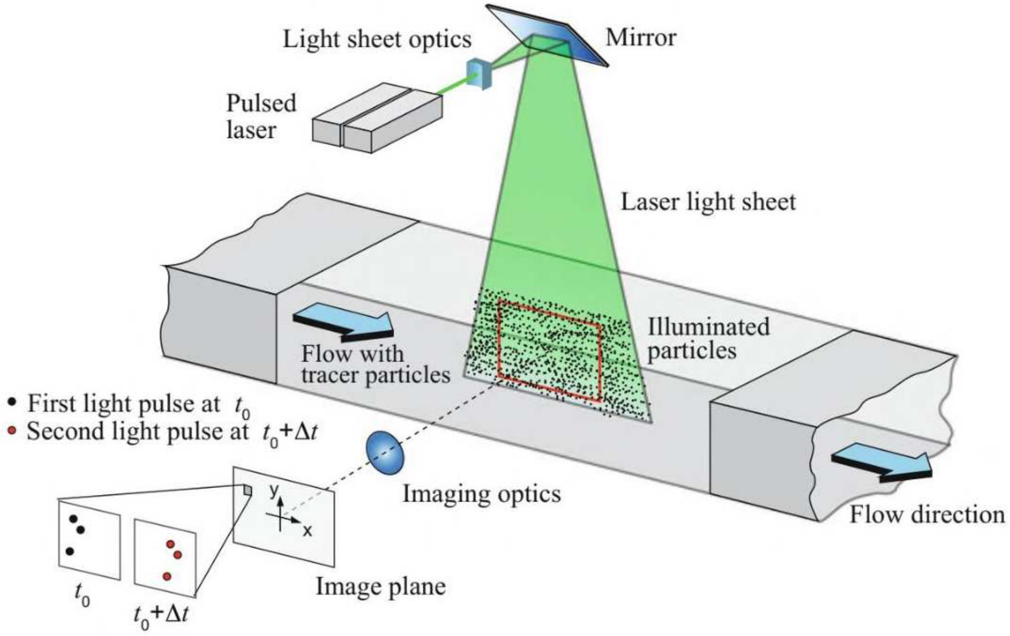


M. Raffel "Particle Image Velocimetry: A Practical Guide" Springer 2018

Particle Imaging Velocimetry (PIV)

Principle

- Developed by Ludwig Prandtl early 20th century
- Setup:
  - Optics for illumination
  - Channel
  - Imaging optics
  - Algorithm
- Particles are seeded in fluid
  - Particles are assumed to flow the flow dynamics
  - High particle density -> correlation
- Flow is illuminates
- Motion of particles are used to calculate the fluid behavior



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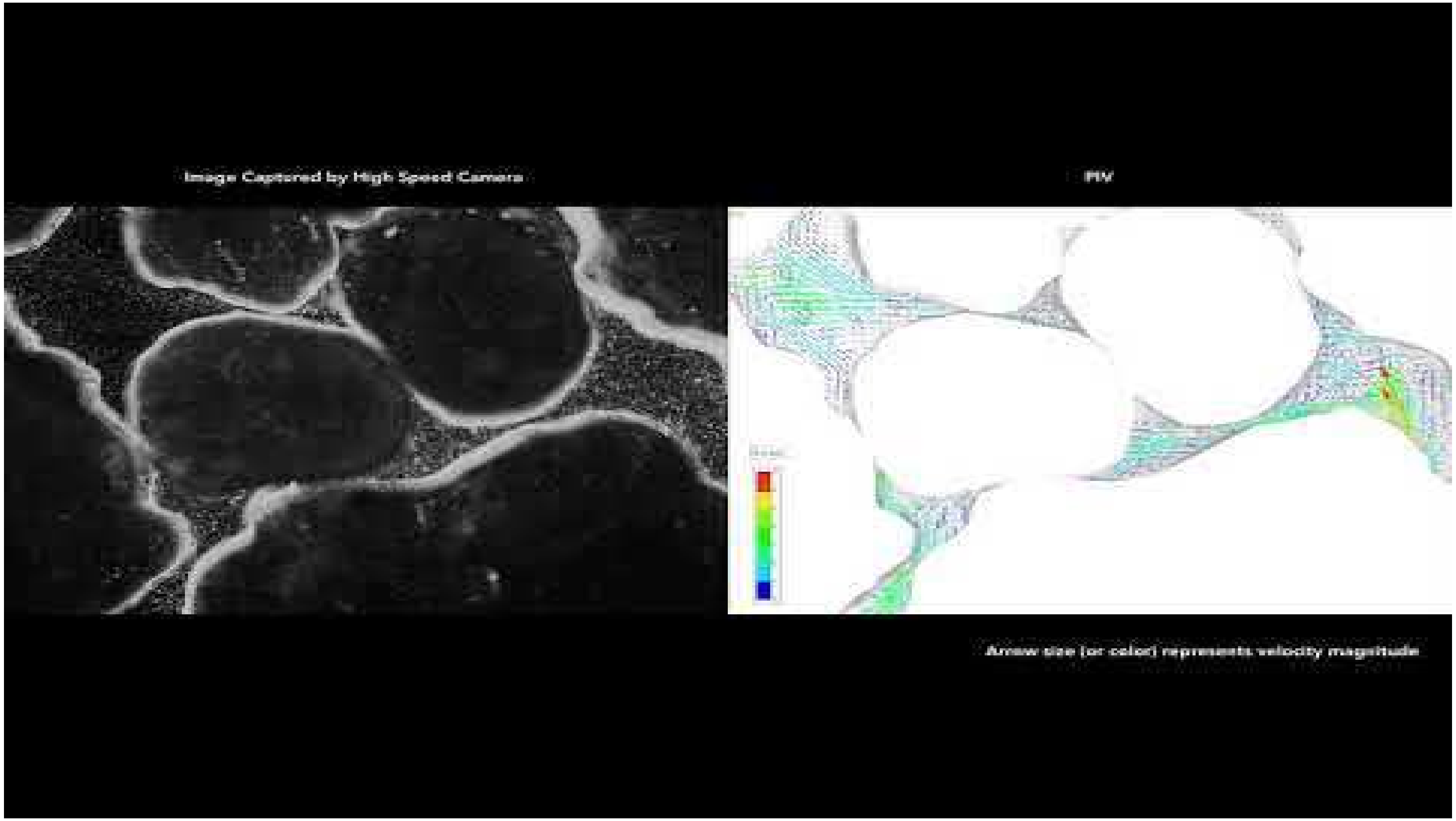
PIV – Current application



https://www.youtube.com/watch?v=WbW6r8kIOsg

Holography – Lensless imaging

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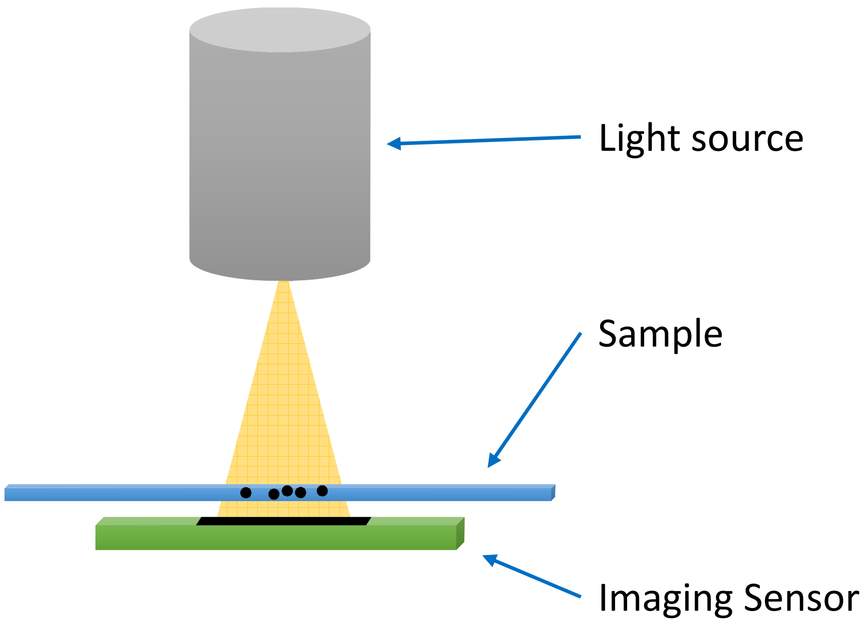
Holography

- Invented by Gabor (1948)
- "Science of making holograms"
- Hologram = photographic recording of a light field
- Light field can be reconstructed three dimensional

→ Recording and reconstruction of optical waves

- Set up is simple and lensless
- Basic components:
  - ➢ Light source: LED, Laser,…
  - ➢ Sample
  - ➢ Imaging Sensor: CMOS chip, CCD chip,…



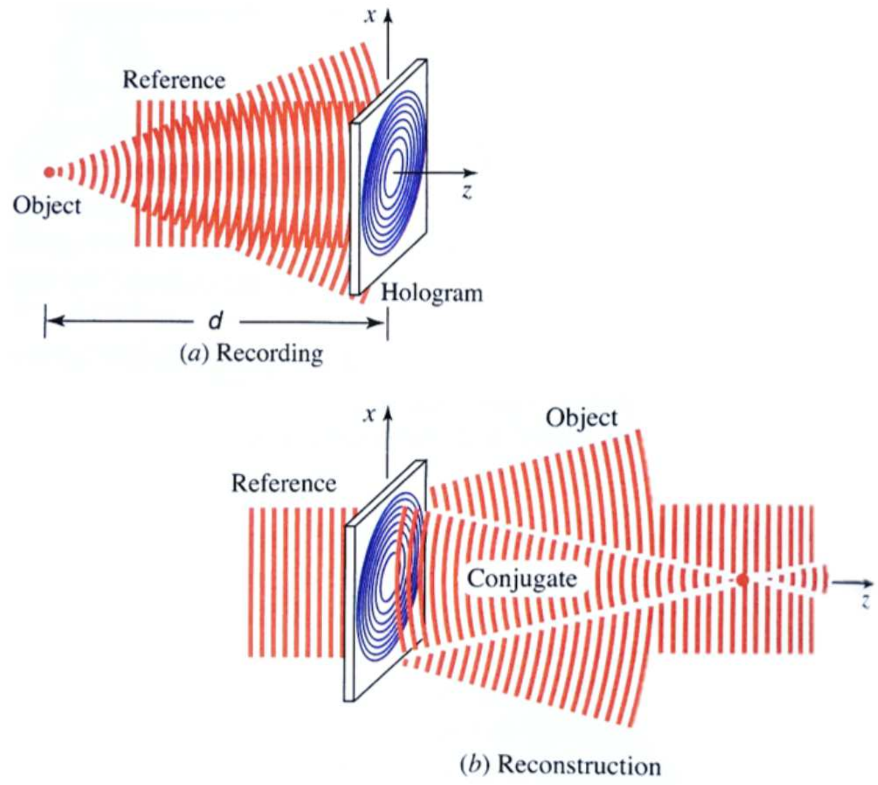
Holography – Introduction

Recording

- Object and reference wave form interference pattern
- Interference pattern contains information about the amplitude and phase of the incoming wave

Reconstruction

- Re-illuminating the hologram (optically)
- Fresnel-Kirchhoff integral (numerically)



Saleh et al. *Fundamentals of Photonics*. John Wiley & Sons, 2013.

Holography – Introduction

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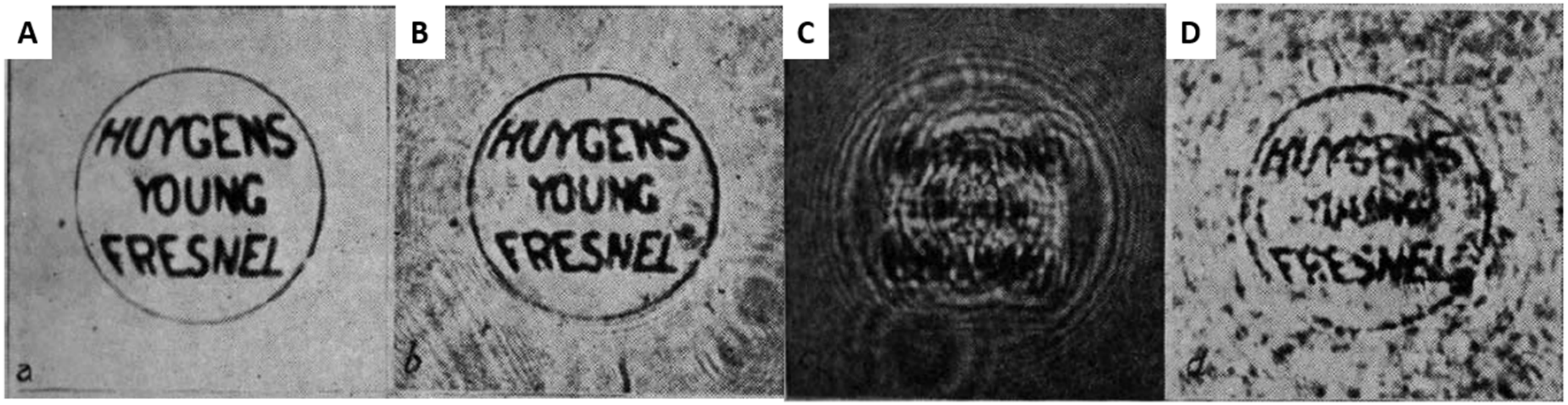
Original images of the work of Gabor in 1948

Image on micrograph | Image photographed through holography setup | Hologram | Reconstruction

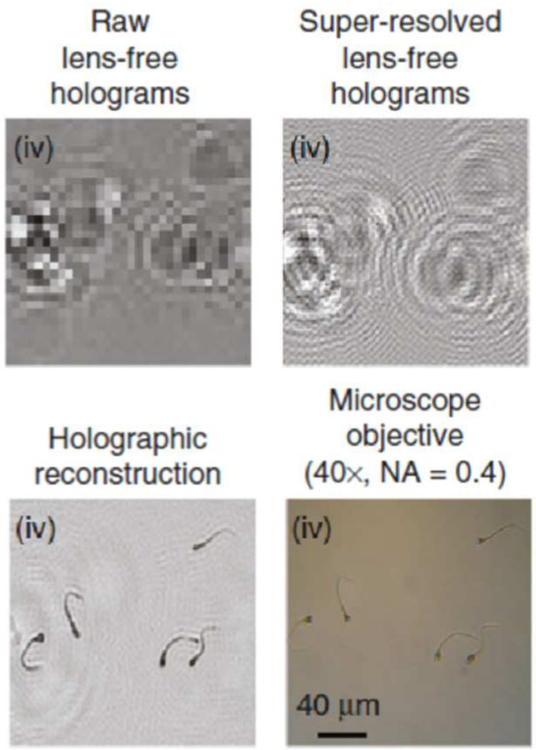


Dennis Gábor. *Nature*, 1948.

Holography – Current research

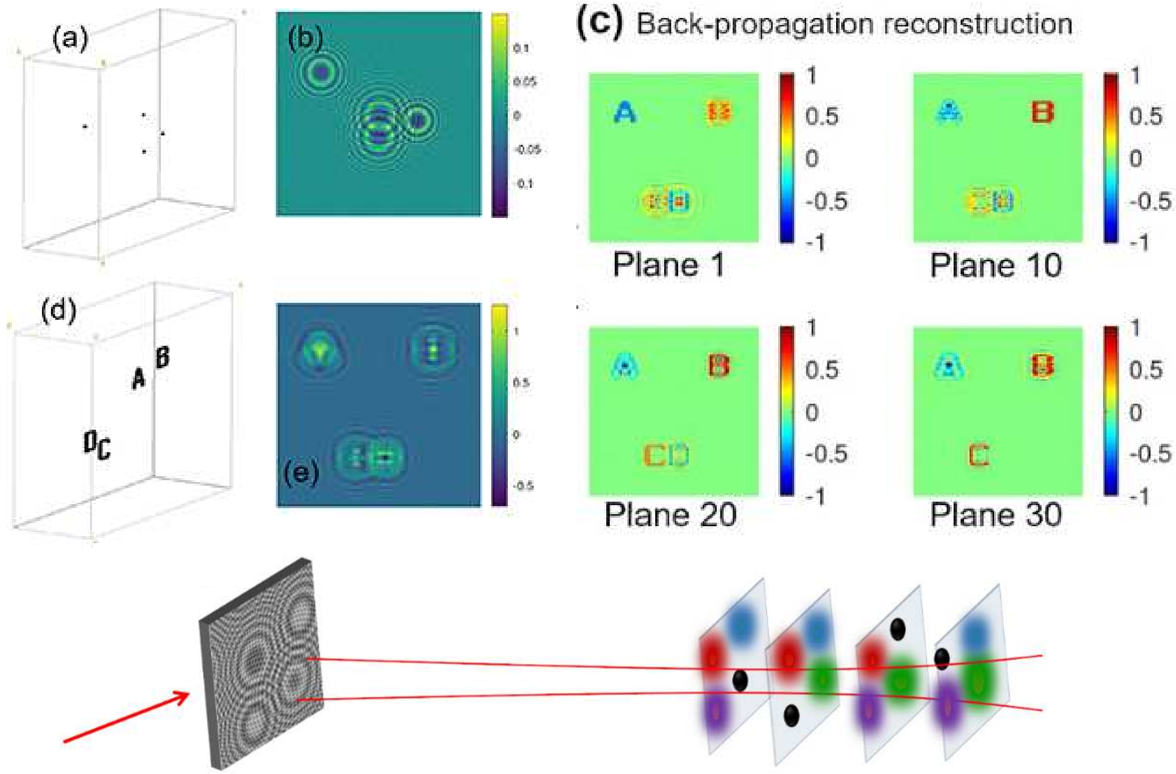
Imaging of sperm

(head 5 x 3 µm, tail 50 µm)



Alon Greenbaum, et al. *Nature methods*, 2012.

True 3D reconstruction



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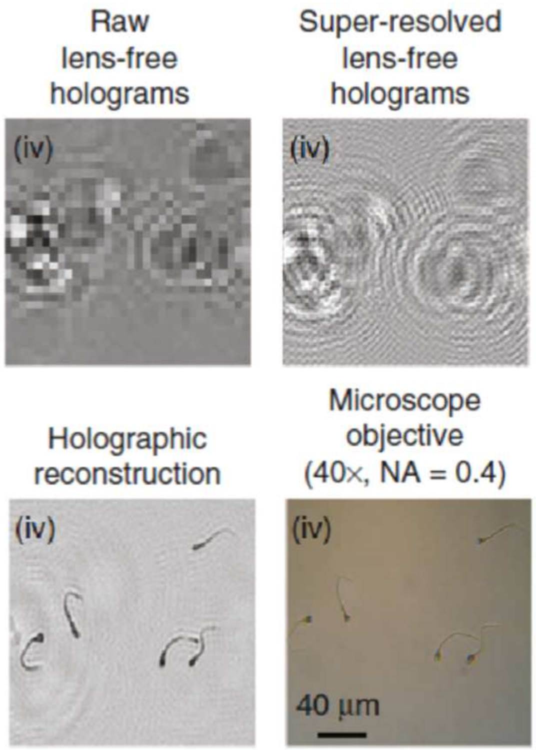

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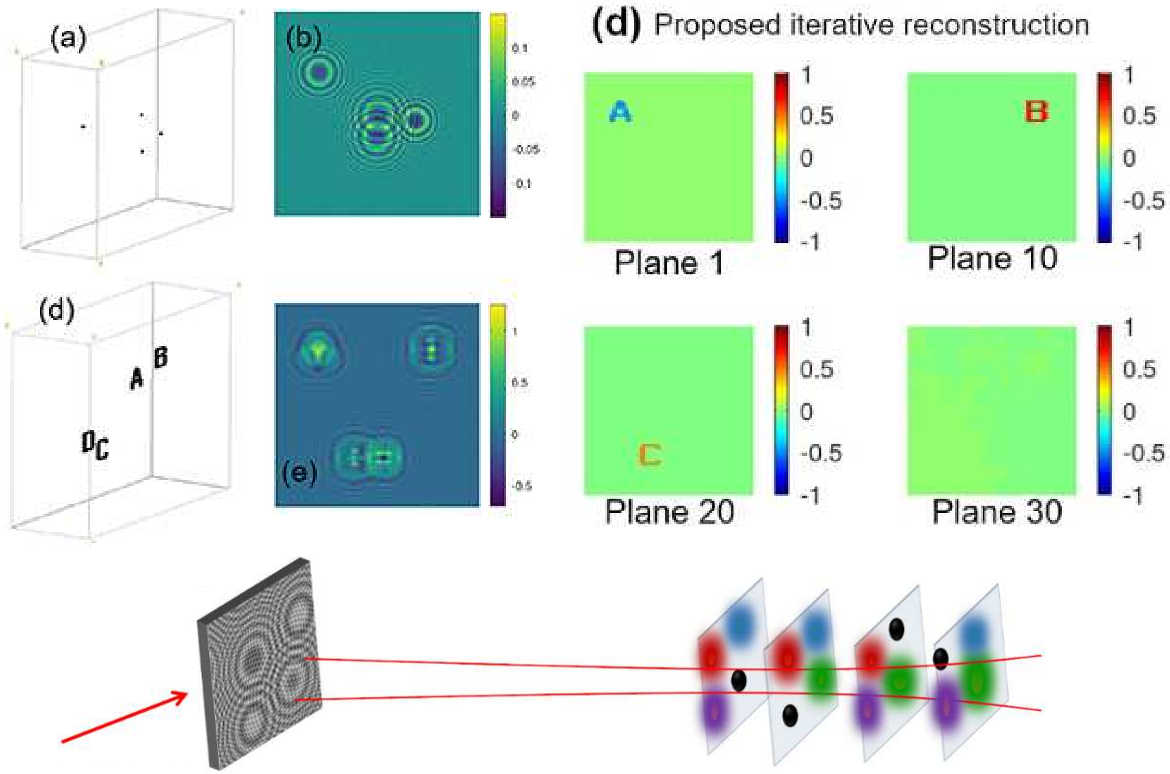
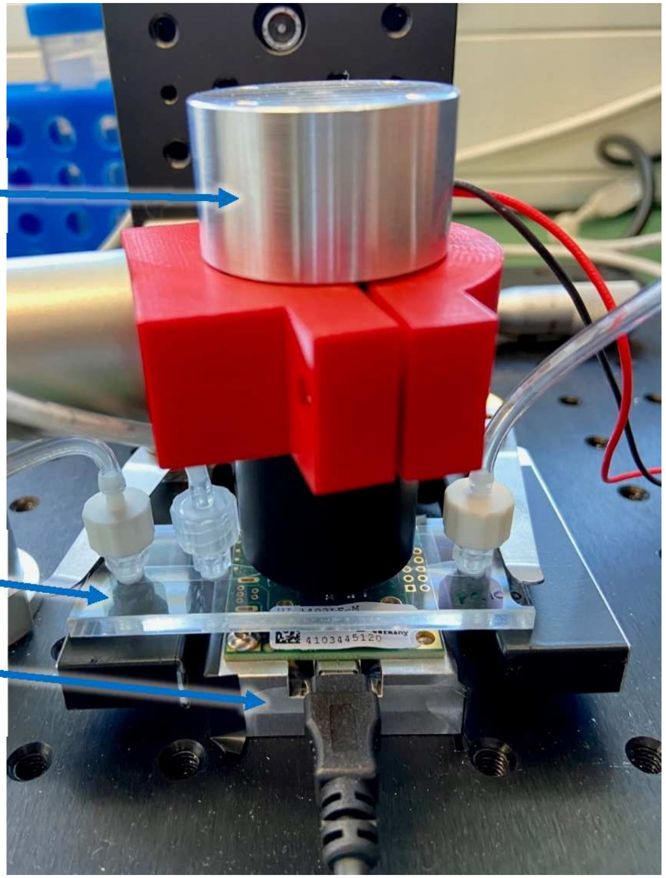


Jasleen Birdi, et al. *Jphys Photonics*, 2020.

Holography – Setup

- neMESYS pump (cetoni GmbH)
  - Flowrates > 1.1 nL/min
- Light source
  - LED
    - Wavelength: 455 nm
    - Power: 549 mW
  - Pinhole
    - Diameter: 50 +/- 3 µm
- Sample carrier
  - Custom made channel: 25 x 0.6 x 0.1 mm
- Imaging Sensor (CMOS)
  - UI-1492LE
    - Optical area: 6.413 x 4.589 mm
    - Pixel size: 1.67 µm
    - Frame rate: 3.2 fps

Desired flow range:
5 – 100 nL/min

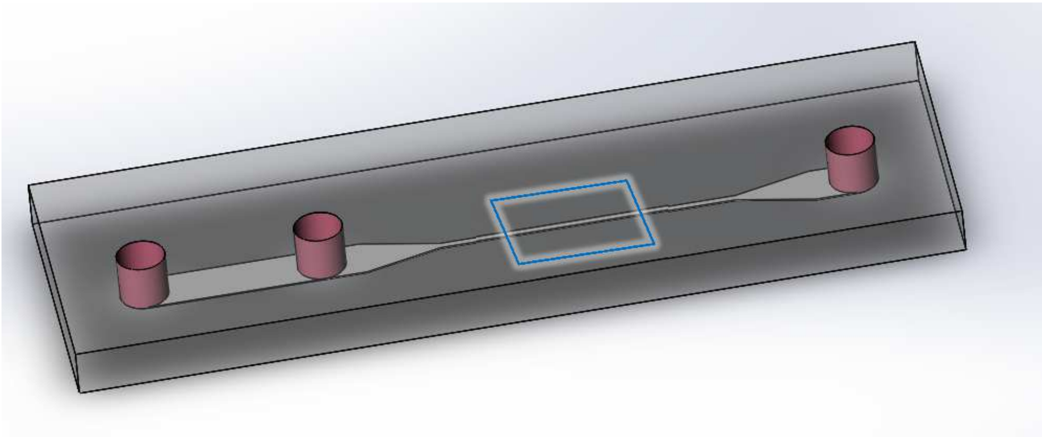


Holography – Custom made channel

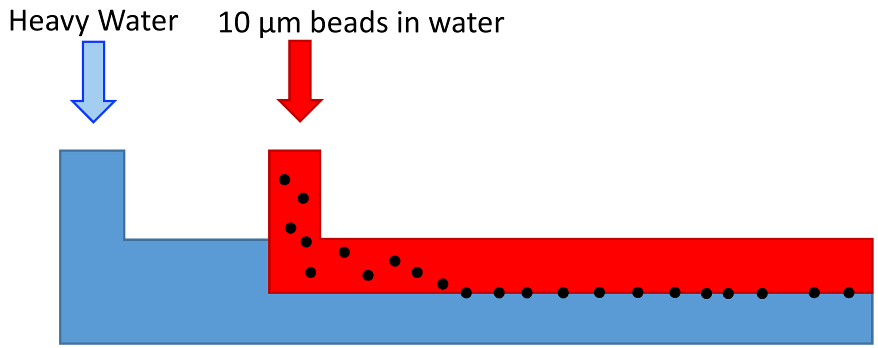
- Material: PMMA
- Double inlet system
  - Creates sheath flow
  - One inlet for heavy water, one for water seeded with particles
- Inlets contain UNF threads for Lure connectors
- Channel
  - Width: 600 µm
  - Two different channel heights

    Version 1: 100 µm

    Version 2: 50 µm
- Channel is milled and needs to be polished
- Channel is sealed with 3M foil



Double inlet system to create sheath flow



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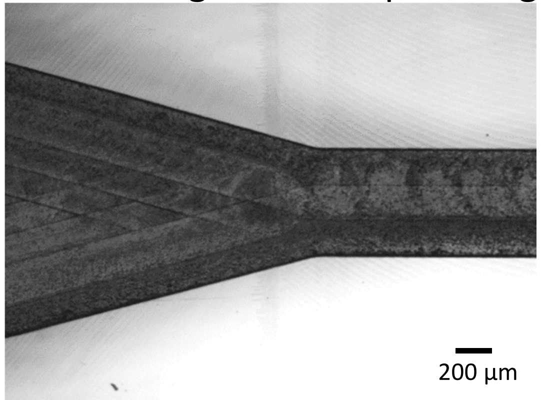
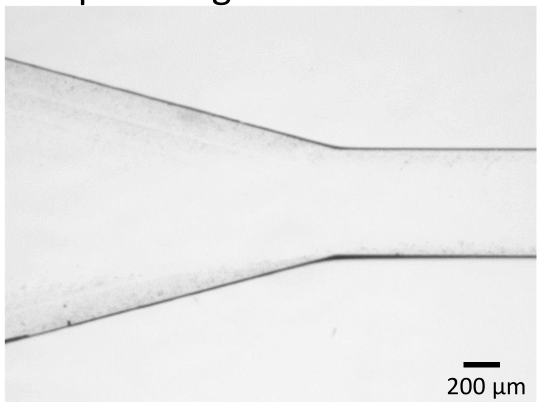
After milling - without polishing



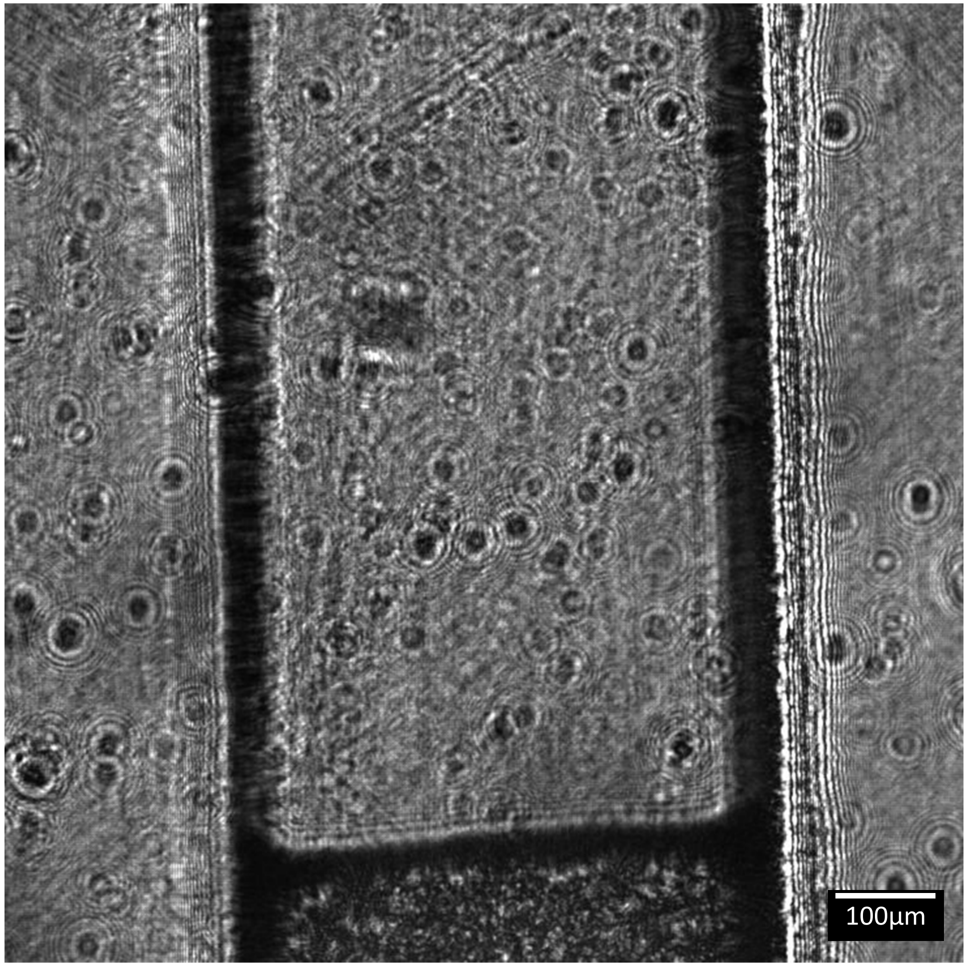
After polishing



*Images acquired with microscope

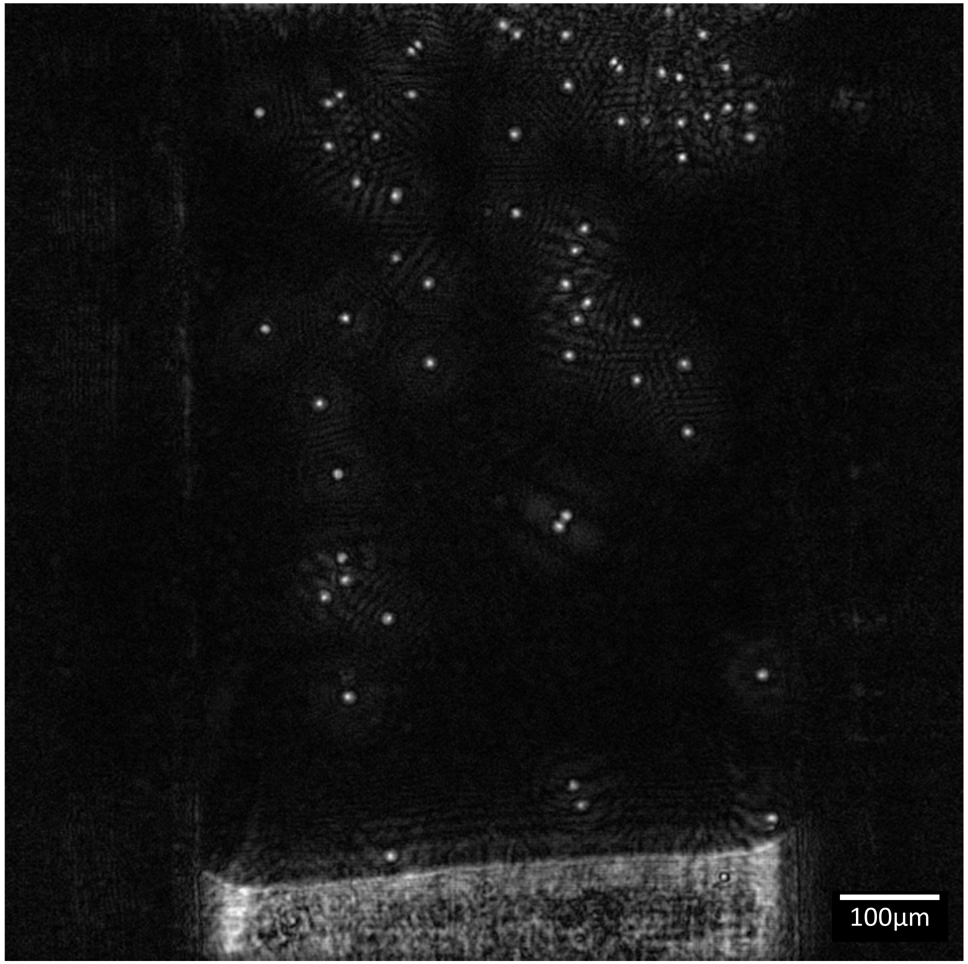
Holography – Acquired hologram

- Flow is generated via Hydrostatic pressure
  - flow rate ~ 400 nL/min
- Channel characteristics:
  - Height: 100 µm
  - Width: 600 µm
  - Milling patterns are polished away
  - Sealed with 3M foil
- Channel is filled with water and seeded with 10 µm PPS particles
- Small black particles outside of channel are dust/dirt



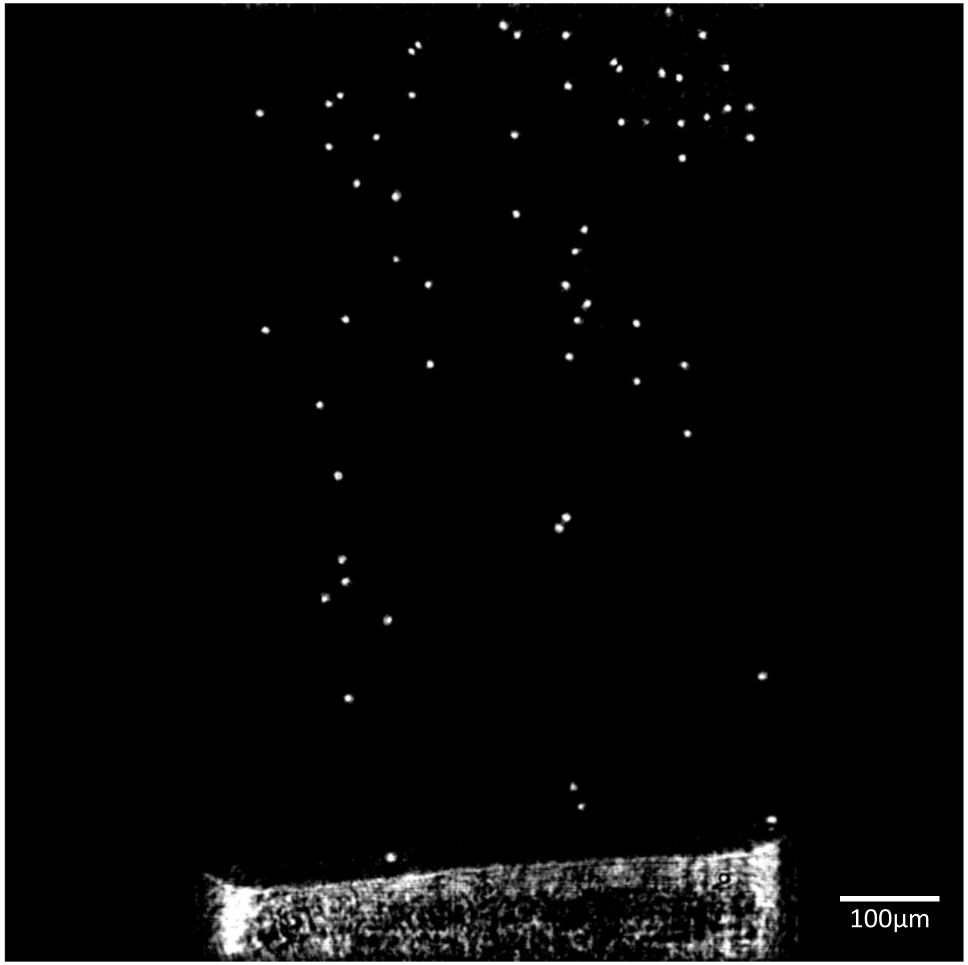
Holography – Reconstructed image

- Flow is generated via Hydrostatic pressure
  - flow rate ~ 400 nL/min
- Channel characteristics:
  - Height: 100 µm
  - Width: 600 µm
  - Milling patterns are polished away
  - Sealed with 3M foil
- Channel is filled with water and seeded with 10 µm PPS particles
- Image is reconstructed using the Holopy package for python
- Background subtraction removes irrelevant particles



Holography – PIV preparation

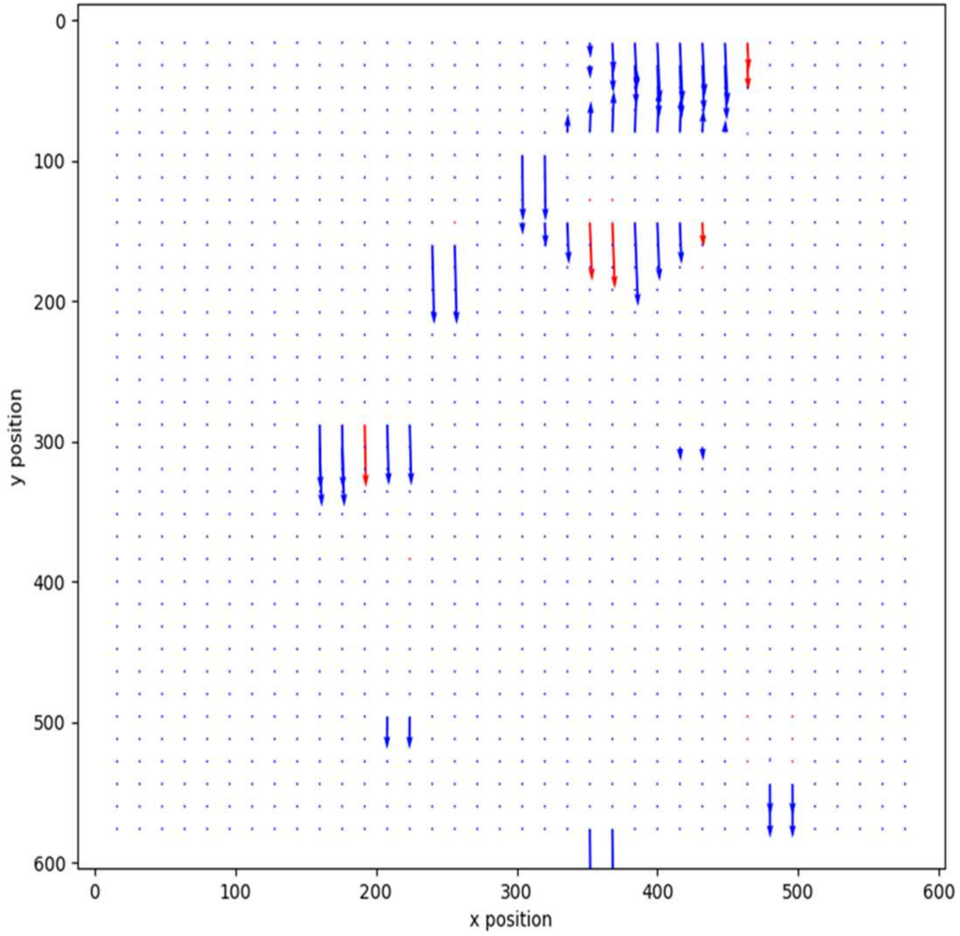
- Flow is generated via Hydrostatic pressure
  - flow rate ~ 400 nL/min
- Channel characteristics:
  - Height: 100 µm
  - Width: 600 µm
  - Milling patterns are polished away
  - Sealed with 3M foil
- Channel is filled with water and seeded with 10 µm PPS particles
- Image is reconstructed using the Holopy package for python
- Background subtraction removes irrelevant particles
- Threshold filter provides an almost binary image



PIV

- Generated Flow ~ 400 nL/min
- Channel characteristics:
  - Height: 100 µm
  - Width: 600 µm
- Channel is filled with water and seeded with 10 µm PPS particles
- PIV is performed using the OpenPiv package for python
- An interrogation window of 32 px is used

  - Too few particles
  - Only two images in comparison, not a batch process



PIV – Result

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- Generated Flow +/- 400 nL/min
- Channel characteristics:
  - Height: 100 µm
  - Width: 600 µm
- Channel is filled with water and seeded with 10 µm PPS particles
- PIV is performed using the OpenPiv package for python
- An interrogation window of 32 px is used

  - Too few particles
  - Only two images in comparison, not a batch process
  - Av. measured flow rate ~ 460 nL/min

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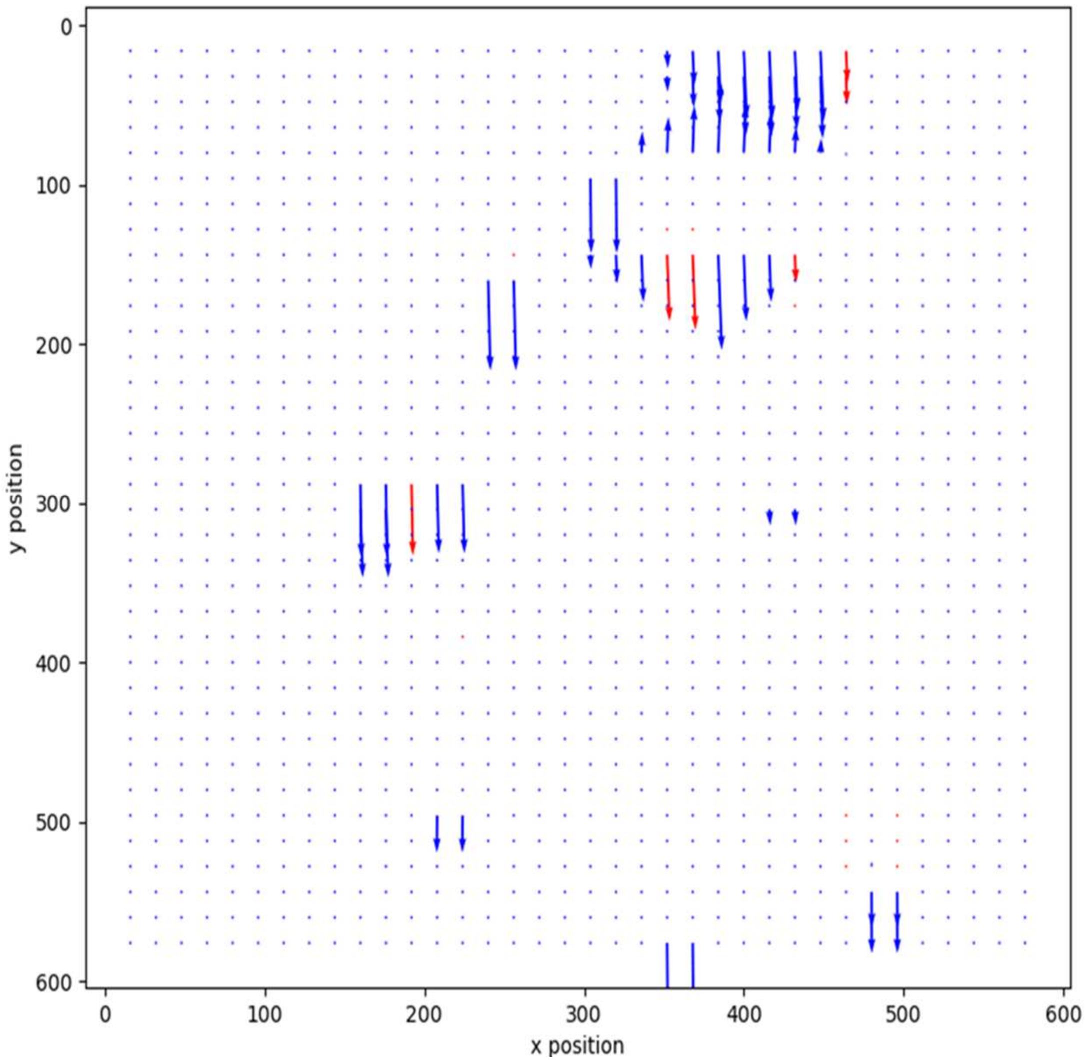
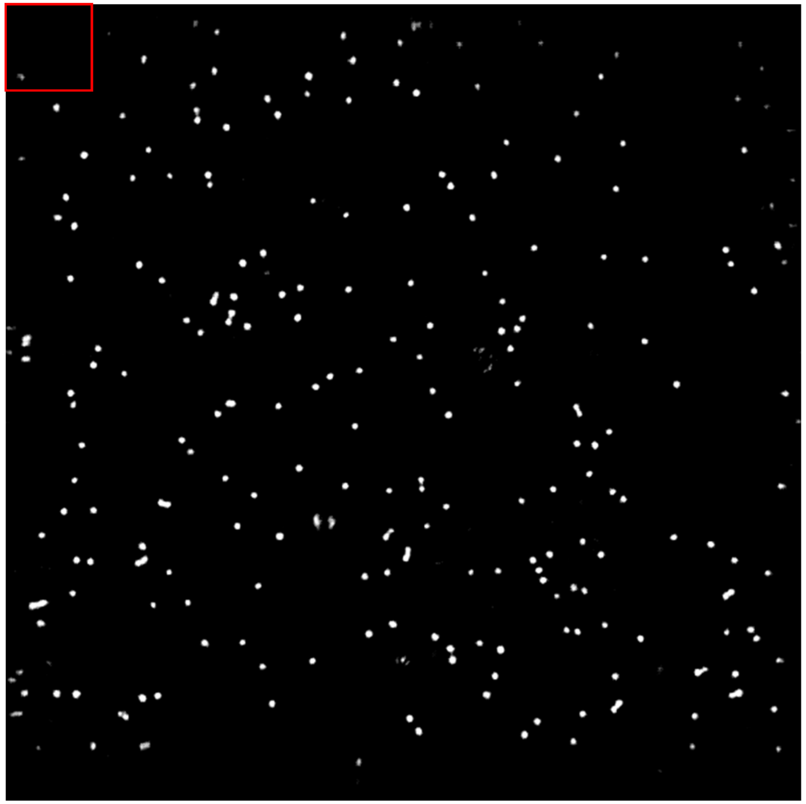
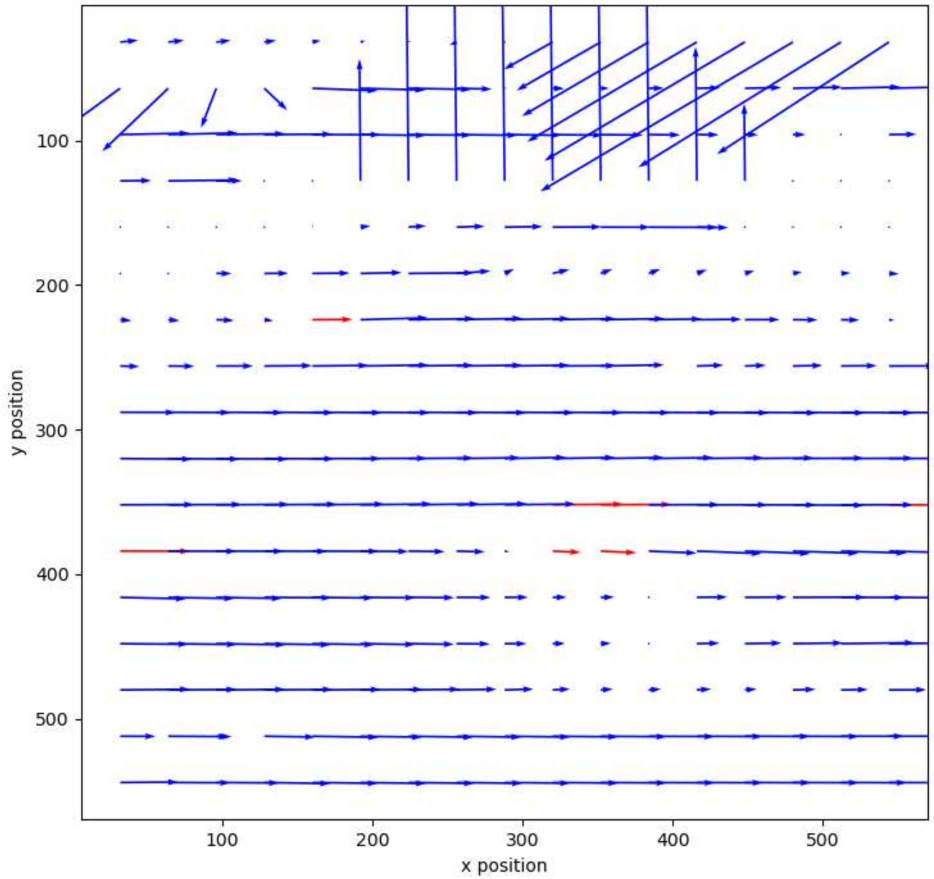


PIV– Aim

*Ibidi Chip – Flow rate: ~ 275 nL/min



Interrogation window = 64px



PIV – uncertainty budget: Concept

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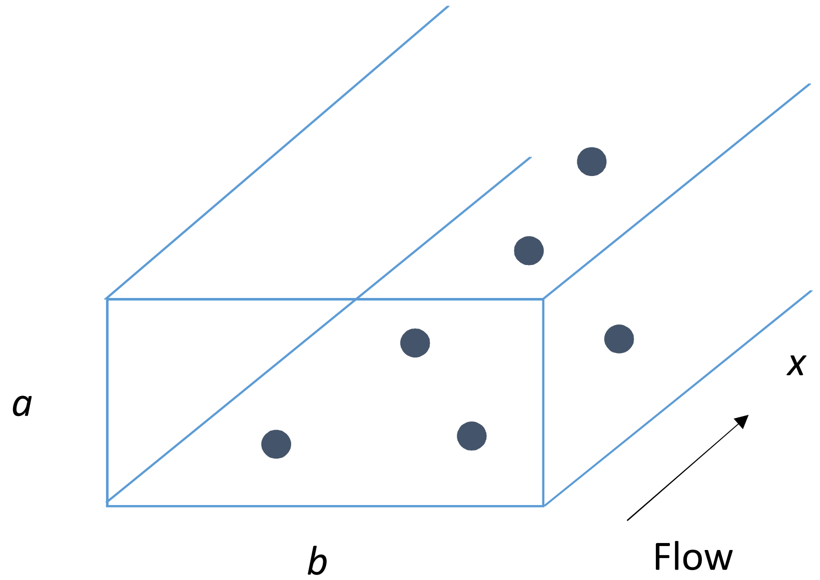
- Puropse: identify all uncertainties and conclude a summed total error that can be expected
- System: Channels featuring rectangular cross-section

$$Q_{vol} = v * a * b = \frac{x}{t} * a * b$$

- ➢ Particles are imaged with camera system featuring optical magnification M

$$Q_{vol} = v * a * b = \frac{1}{M} * \frac{x}{t} * a * b$$



PIV – uncertainty budget: Neglectable influences

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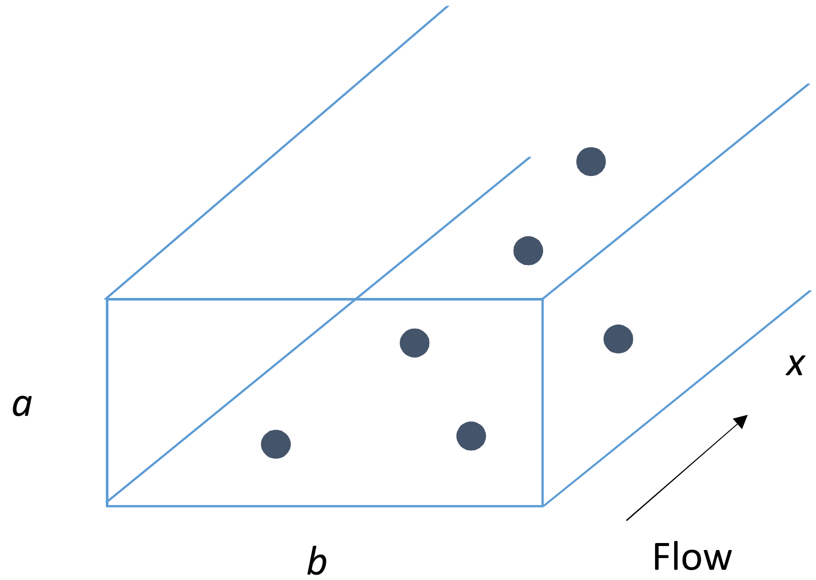
- Neglectable sources of error change the true position of particles by a margin that is far below resolution limit

$$Q_{vol} = v * a * b = \frac{x}{t} * a * b$$

- Neglectable error sources originate mainly from position or time inaccuracies
  - Brownian motion
  - Time stamp inaccuracies of recorded images
  - Peak locking (for particles smaller than pixel size)
  - Motion blur due to long exposure times



PIV – uncertainty budget: Main influences

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- Main influencing error sources
  - Fabrication error of micro channel

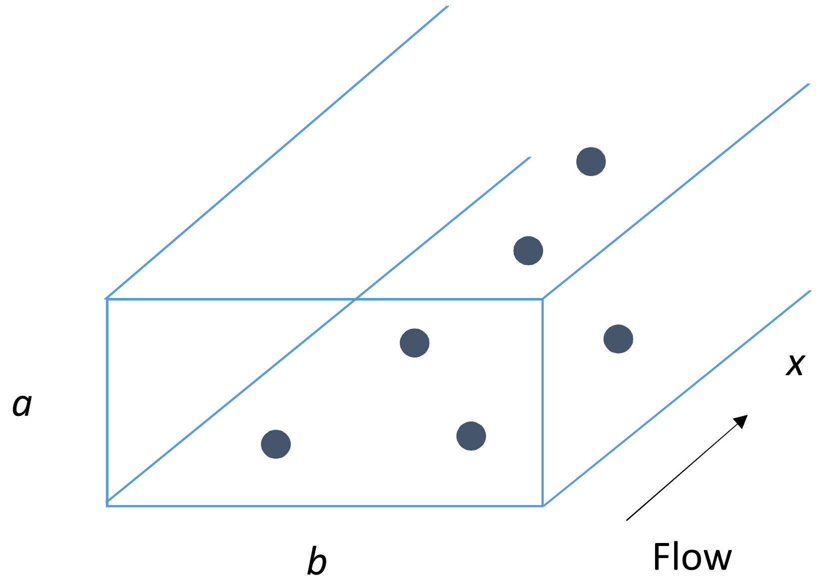
    ± 5 µm estimated for *a* and *b*
  - Angular deviation of camera and substrate from normal (90°)

    Maximum error amounts to ± 2 µm in *x*
- Total error calculation of independent errors

$$\frac{\Delta Q}{Q} = \sqrt{\left(\frac{\Delta x}{x}\right)^2 + \left(\frac{\Delta a}{a}\right)^2 + \left(\frac{\Delta b}{b}\right)^2}$$

$$= \sqrt{\left(\frac{2\ \mu m}{x}\right)^2 + \left(\frac{5\ \mu m}{a}\right)^2 + \left(\frac{5\ \mu m}{b}\right)^2}$$

$$\Delta Q = Q * \sqrt{\left(\frac{2\ \mu m}{x}\right)^2 + \left(\frac{5\ \mu m}{a}\right)^2 + \left(\frac{5\ \mu m}{b}\right)^2}$$



PIV – uncertainty budget: Results

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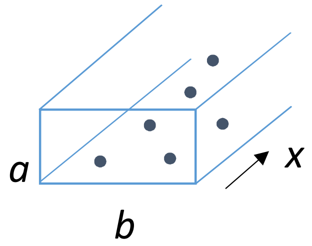


- Fabrication error of micro channel
  - ± 5 µm estimated for *a* and *b*
  - *b* = 600 µm
  - *a* = 100 µm or 50 µm
- Angular deviation of camera and substrate from normal (90°)
  - Maximum error amounts to ± 2 µm in *x*
  - *x* = 600 µm → FOV
- Desired flow rate:
  - *Q* = 5 – 100 nL/min
- Total error calculation of independent errors

$$\Delta Q = Q * \sqrt{\left(\frac{2\ \mu m}{x}\right)^2 + \left(\frac{5\ \mu m}{a}\right)^2 + \left(\frac{5\ \mu m}{b}\right)^2}$$

Table: *ΔQ* for different flow rates at different channel heights *a*

Flow rate (*Q*) in nL/min	*a* = 100 µm	*a* = 50 µm
460	23.367	46.185
400	20.319	40.161
275	13.969	27.611
100	5.079	10.040
10	0.508	1.004
5	0.254	0.502

→ **~5 % error** **~10 % error**

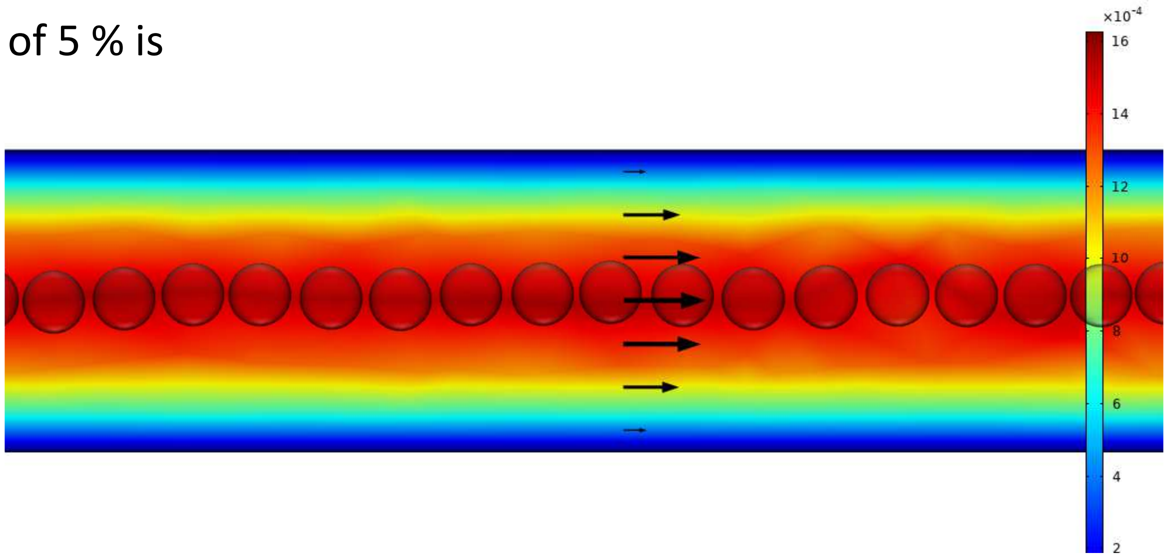
Conclusion and Outlook

Conclusion

- Digital in-line Holography setup is build
- Holograms with traced particles are recoded
- Reconstruction is done via Holopy
- PIV is done via OpenPIV
- Flows in the range of 400 nL/min are evaluated
- With the larger channels an error of 5 % is achieved

Outlook

- Flow rate will be further reduced with the neMESYS pump (cetoni GmbH)
  - To achieve 5 – 100 nL/min
- Realize sheath flow due to custom made channels
- PIV will be advanced to a batch process



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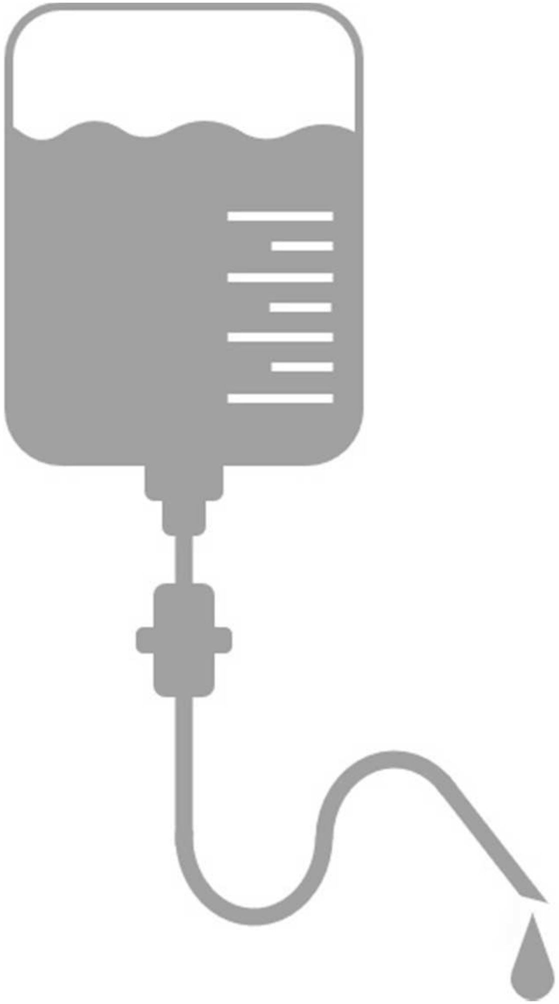

THANK YOU

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Project Team

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IPQ

METAS

UMC Utrecht

INESC MN
Microsystems and Nanotechnologies

FACH HOCHSCHULE LÜBECK
University of Applied Sciences

CZECH METROLOGY INSTITUTE

TÜV SÜD
NEL

CETIAT
ensemble, innover et valider

DANISH TECHNOLOGICAL INSTITUTE

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