LUMICKS C-Trap training

University of California, San Francisco

Nastaran Hadizadeh| Lead Application Scientist

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LUMICKS

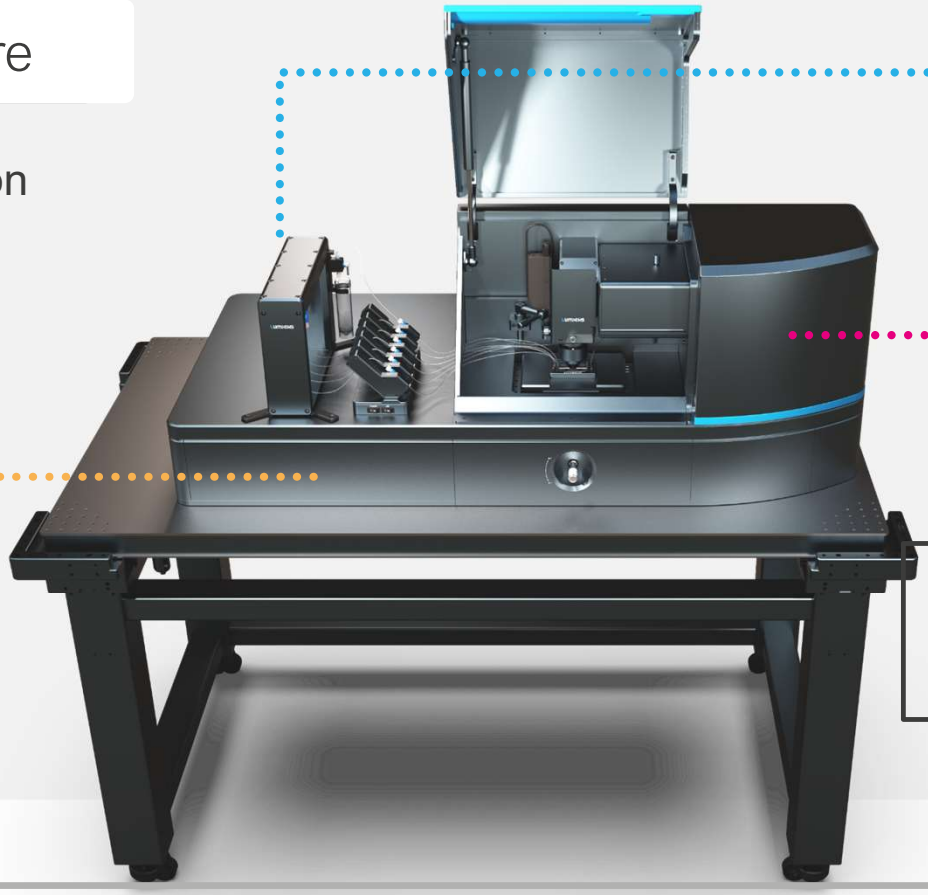
LUMICKS C-Trap is a dynamic single-molecule microscope combining optical tweezers and fluorescence imaging

Software

- Integrates everything on Bluelake

Microfluidics

- Manipulate
- Compartmentalize different conditions



Imaging

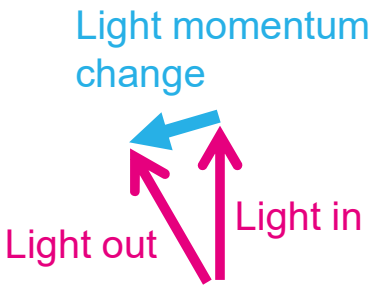
- Visualize

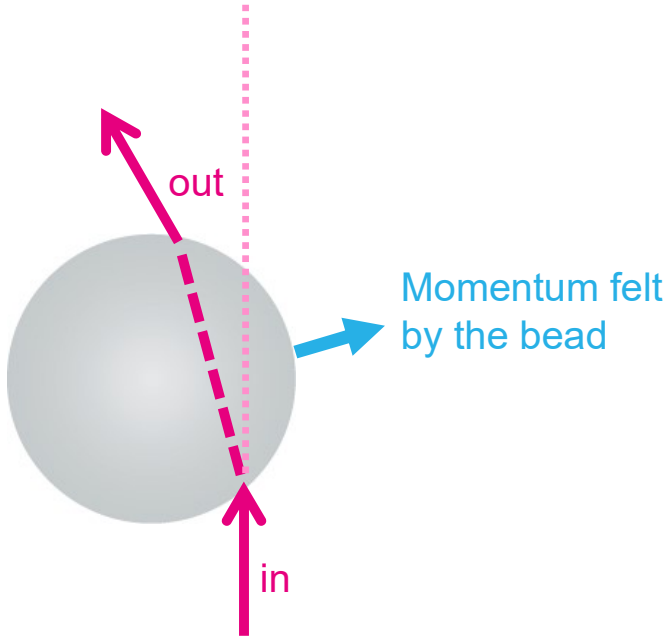
Optical Traps

- Manipulate
- Apply & measure forces

Optical traps – a change in light momentum applies forces to particles

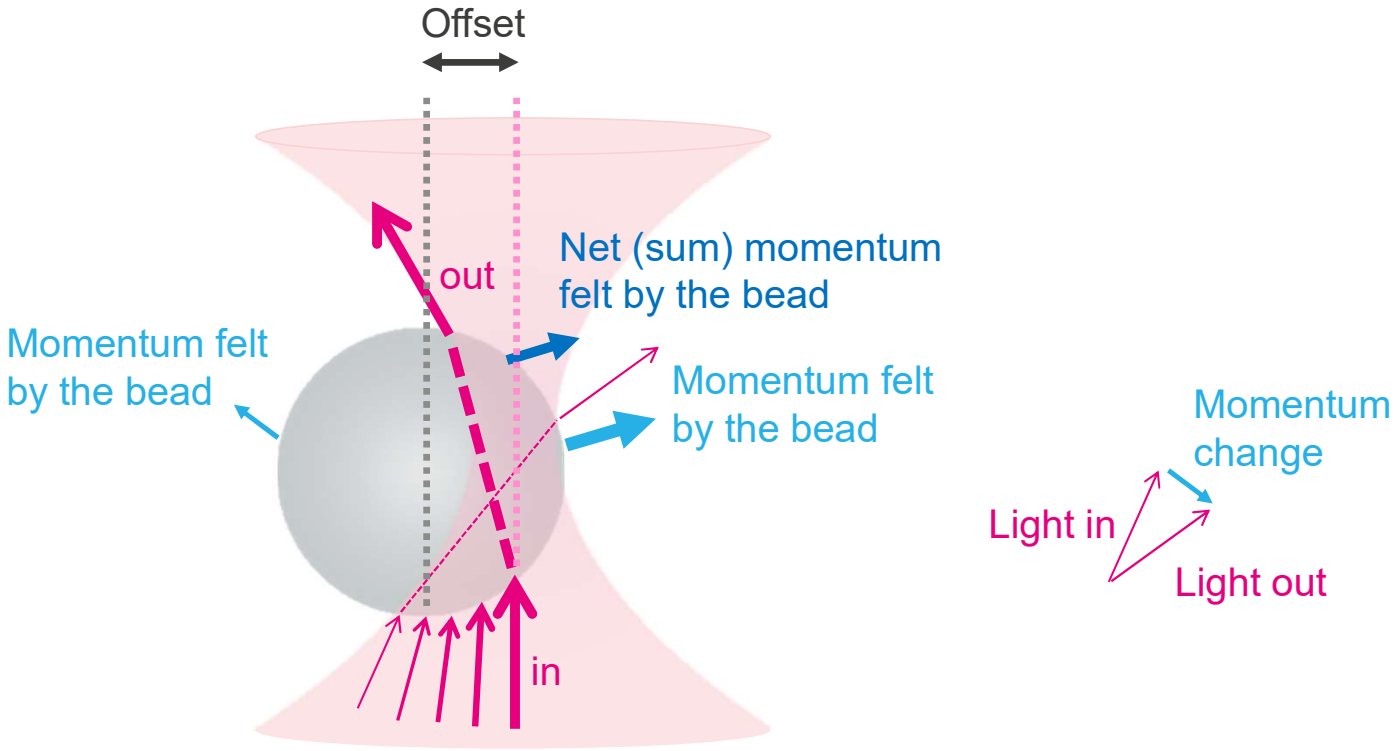
Light does not have mass
but has momentum





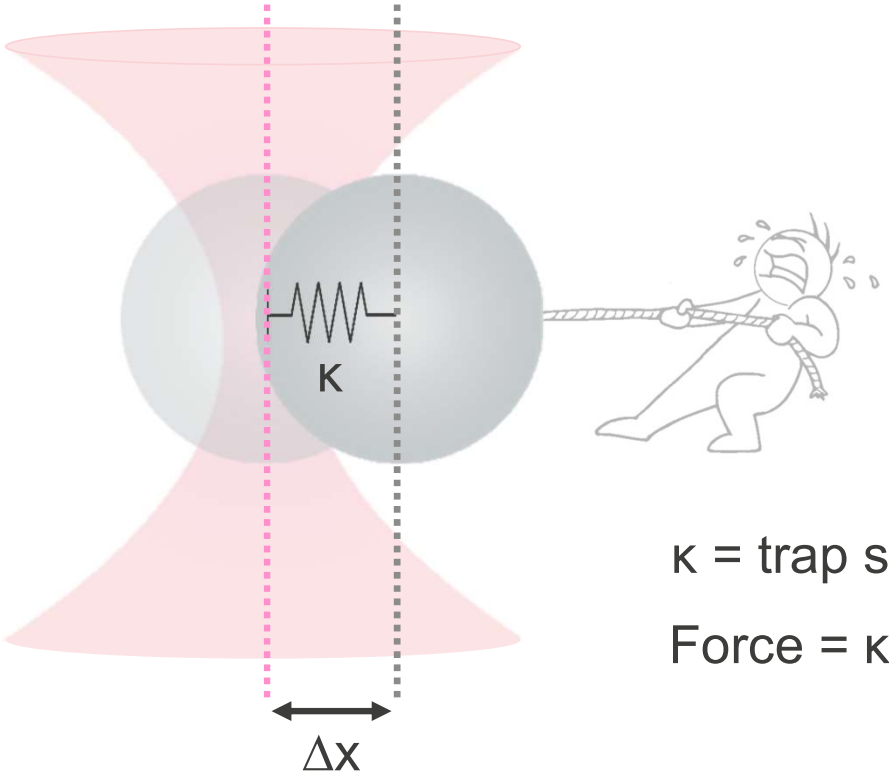
Conservation of momentum: the reaction on the bead must be equal and opposite (Newton's 3rd law)

Optical traps – a change in light momentum applies forces to particles



The sum of momentum felt by the bead across all photons in 3D result in a net force pulling the bead towards the center of the trap focus (and slightly up).

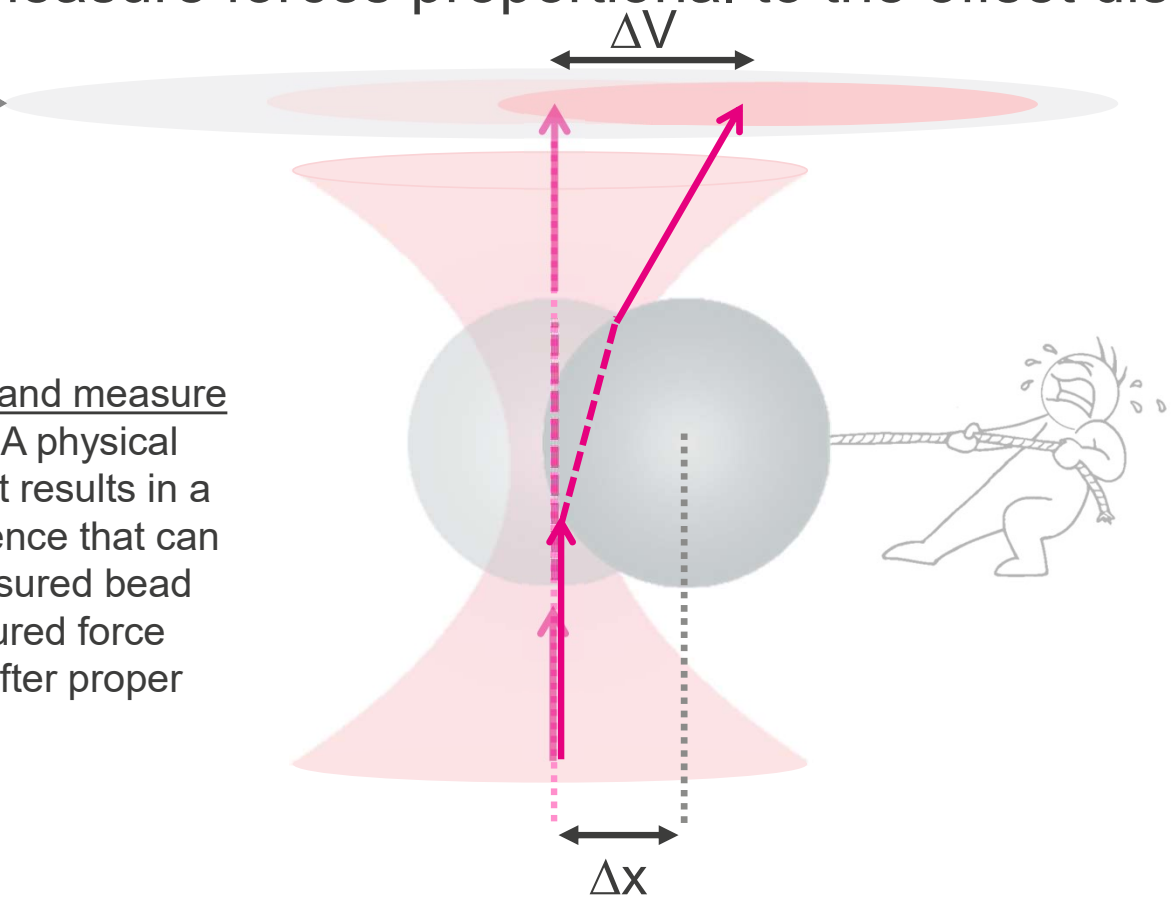
Optical traps – apply forces proportional to the offset displacement



κ = trap stiffness

Force = κΔx

Optical traps – measure forces proportional to the offset displacement

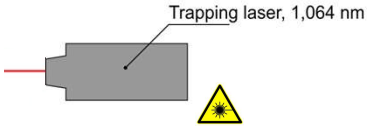


Optical traps both apply and measure forces at the same time. A physical bead offset displacement results in a measured voltage difference that can be converted into a measured bead displacement and measured force (with the trap stiffness) after proper calibration

Optical traps – optical laser power control

Trapping laser (0-100%)

Controls the output power of the IPG laser box. It is typically set at 100% for the best performances. Connected to the **interlock** for safety (20W at source and about ~3.5 W maximum at objective)



Optical traps – optical laser power control

Overall power (0-100%)

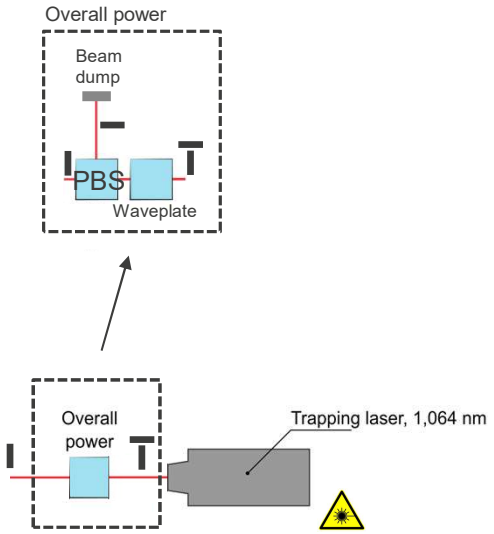
Controls the power of the trapping laser that continues in the optical path using a **waveplate**, combined with a **polarizing beam splitter (PBS)** and a beam dump

Waveplate = specifically polarizes the light, for example 50% vertical, 50% horizontal

PBS = specifically splits the light, based on polarization

Polarization

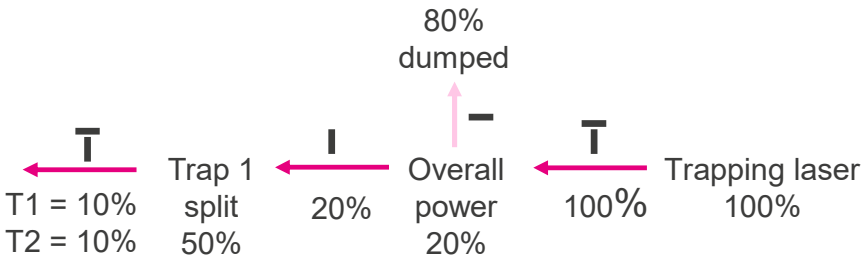
– = horizontal
I = vertical

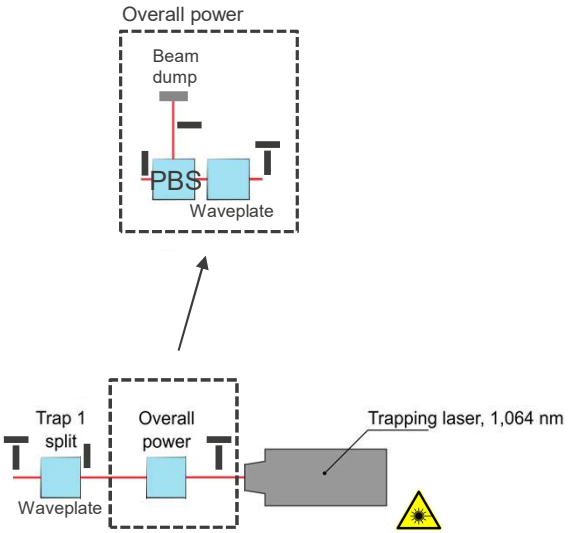


Optical traps – optical laser power control

Trap 1 split (0-100%)

Controls the distribution of the power on Trap 1 using a waveplate





Optical traps – Trap 1, Trap 2, Trap 1 + 2 steering

Trap 1+2 XY

Piezo mirror with a range of motion of 40x40 µm and an internal sensor. Mainly used as a quick manipulation tool

Trap 1+2 Z (telescope)

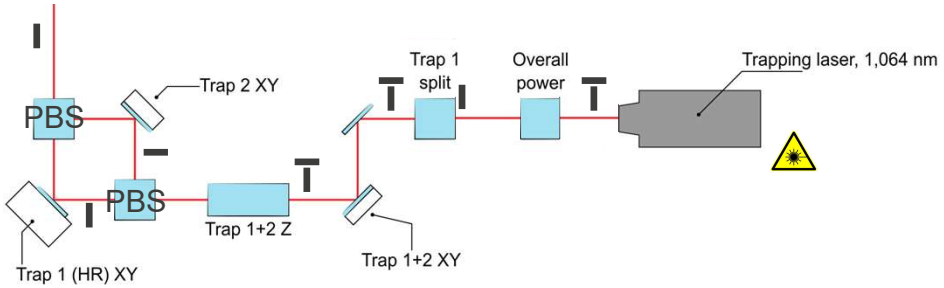
Piezo linear stage with an internal sensor. Mainly used to align the optical traps Z-plane with the imaging plane or for quick manipulation of the dumbbell in Z

Trap 1 XY (high resolution)

Piezo mirror with a range of motion of 27x38 µm and sub-nm step size. Used for very precise manipulation

Trap 2 XY

Piezo mirror with a range of motion of > 50x50 µm, 5-10 nm step size and no internal sensor. Mainly used as a static measurement probe



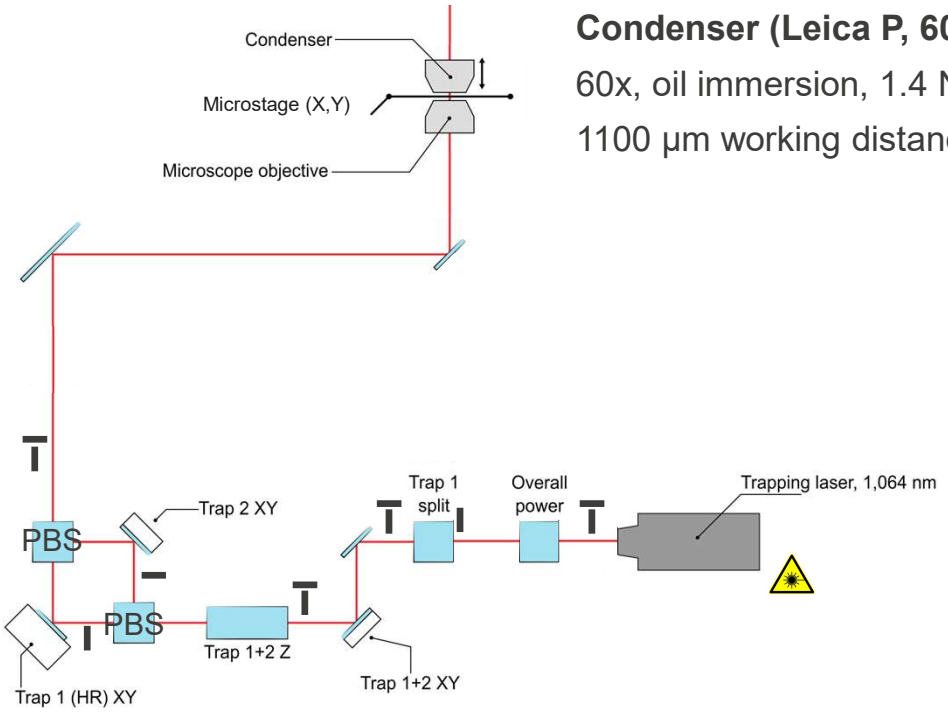
Optical traps – objective, stage and condenser

Water objective (Nikon CFI Plan Apo 60 X)

60x, water immersion, 1.2 NA, 270 µm working distance, correction collar for coverslip thickness between 0.15 – 0.18 mm (0.17 is standard, 0.15 is sometimes used to improve trapping of small beads, i.e. D < 1 µm)

Microstage XY

Stage with a max range of motion of >15 x 15 **mm** and a max displacement speed of 2 mm/s for macroscopic movements



Condenser (Leica P, 60x, 1.40)

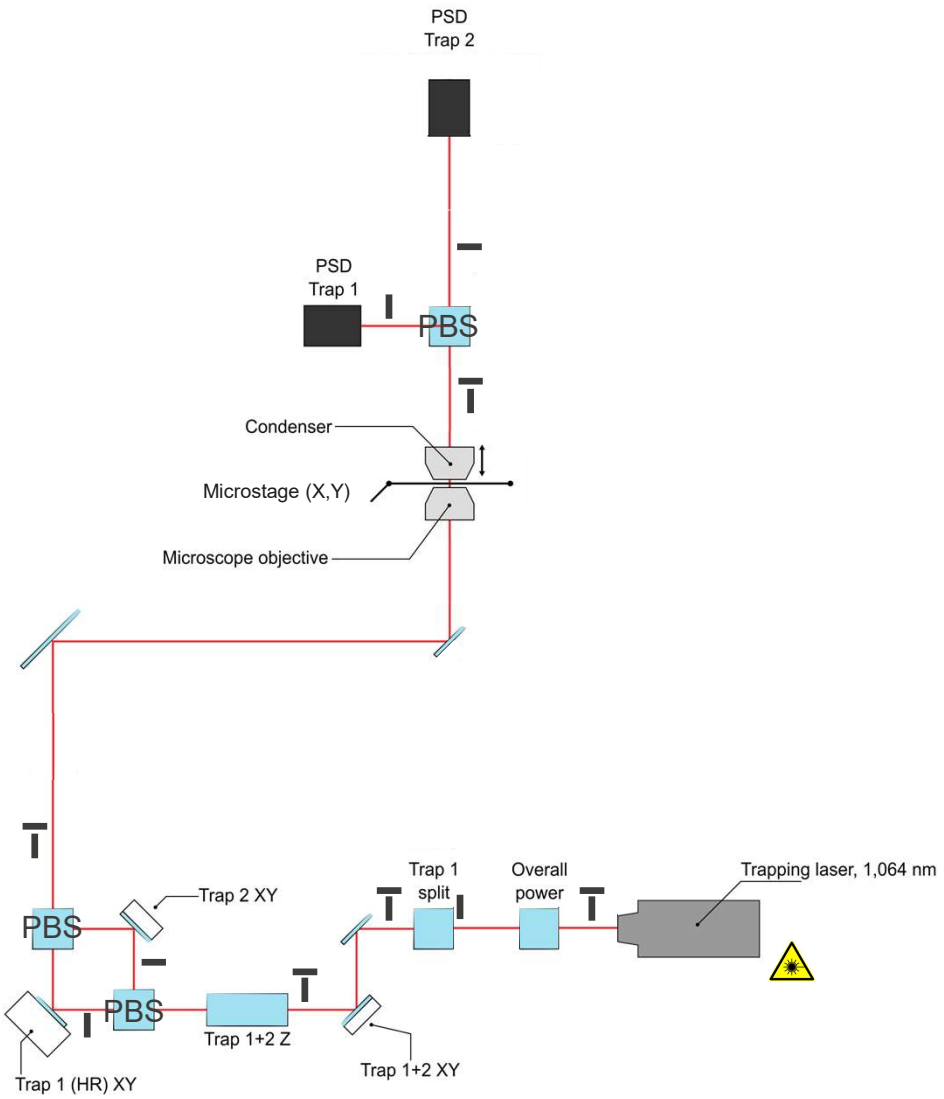
60x, oil immersion, 1.4 NA

1100 µm working distance

Optical traps – force detection

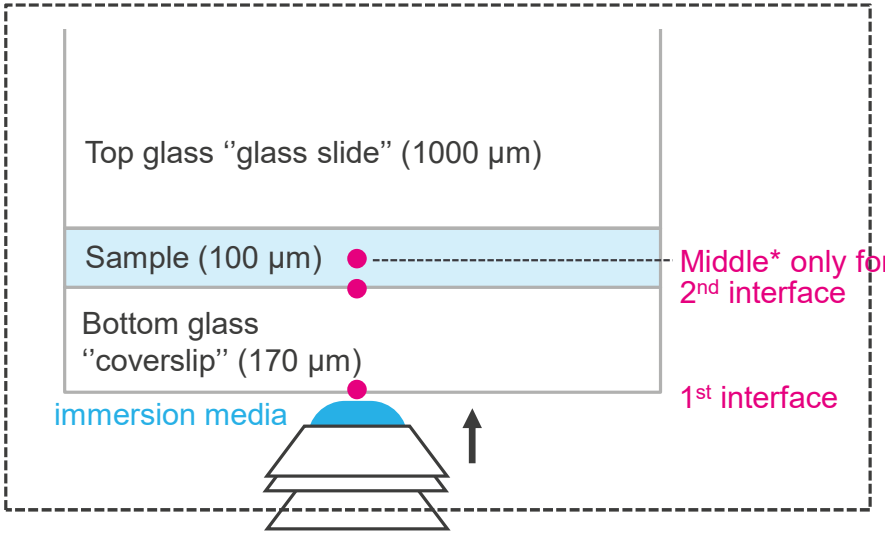
Position Sensitive Detector (PSD, 2x)

Detects the displacement of the bead off the optical trap laser center which **translates to accurate forces after calibration.** The data acquisition system allows the forces to be detected at 100 kHz.

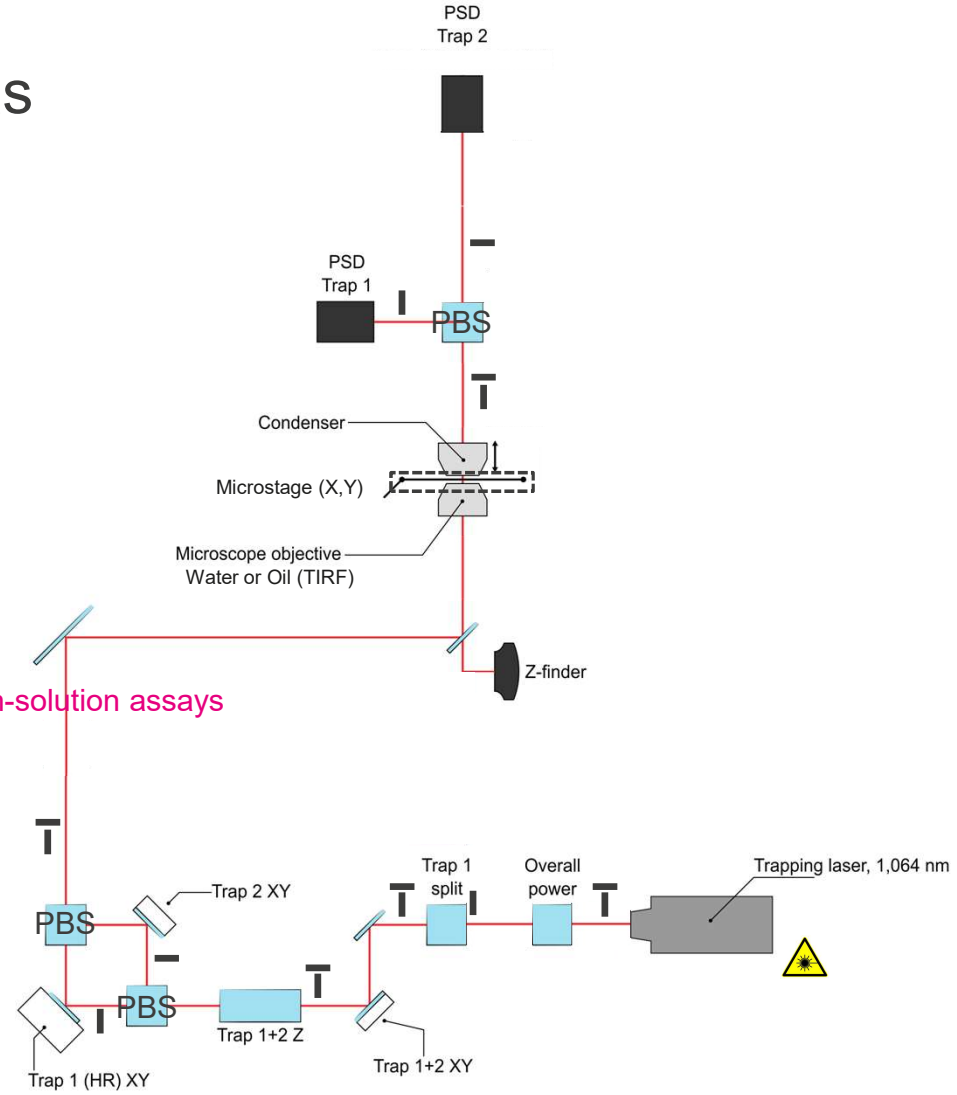


Optical traps – diagnostic cameras

Z-finder (CMOS)
Detects reflections from the 1064 nm optical trap laser at different medium-medium interface (for example, water-glass) to help set up the system

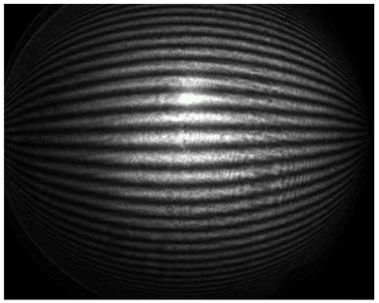


Exact coverslip, sample, and glass slide **thickness** can vary in custom made samples

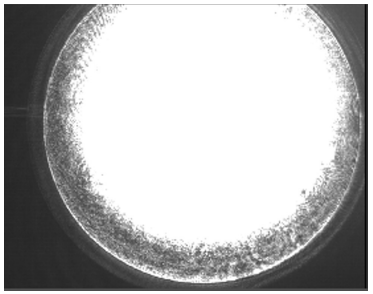


Optical traps – diagnostic cameras

Moon cam (CMOS)
Visualizes the back-focal plane to ensure proper collimation of the transmitted trapping laser light

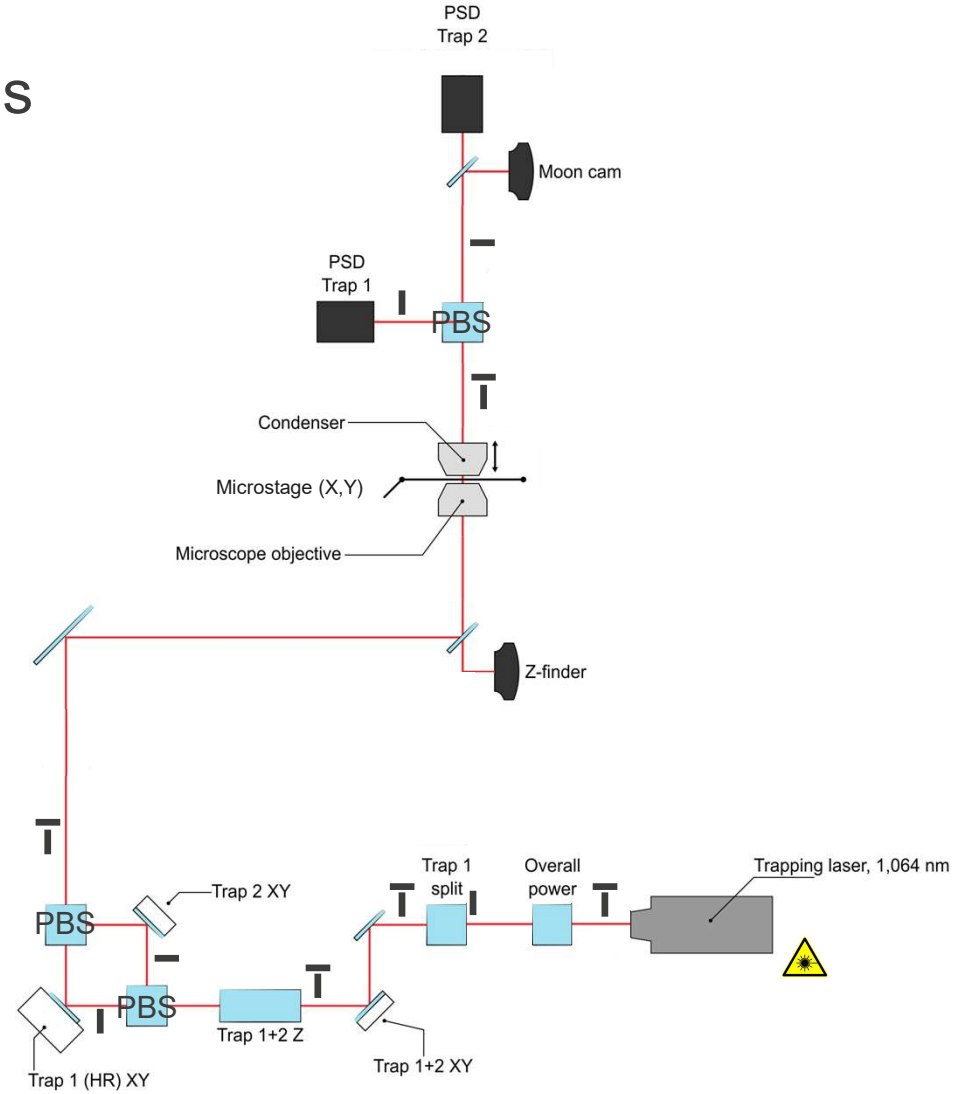


When checking the condenser position



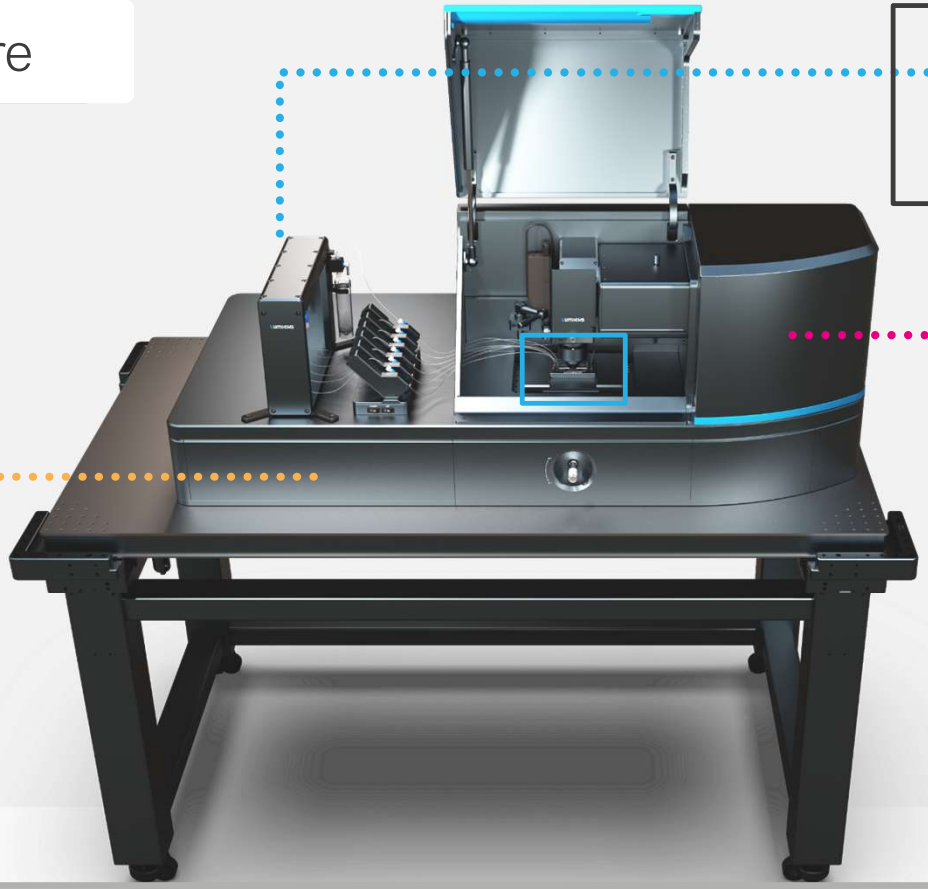
When a bead is trapped in trap 2

Just **illustrative** examples here, this varies between systems



Software

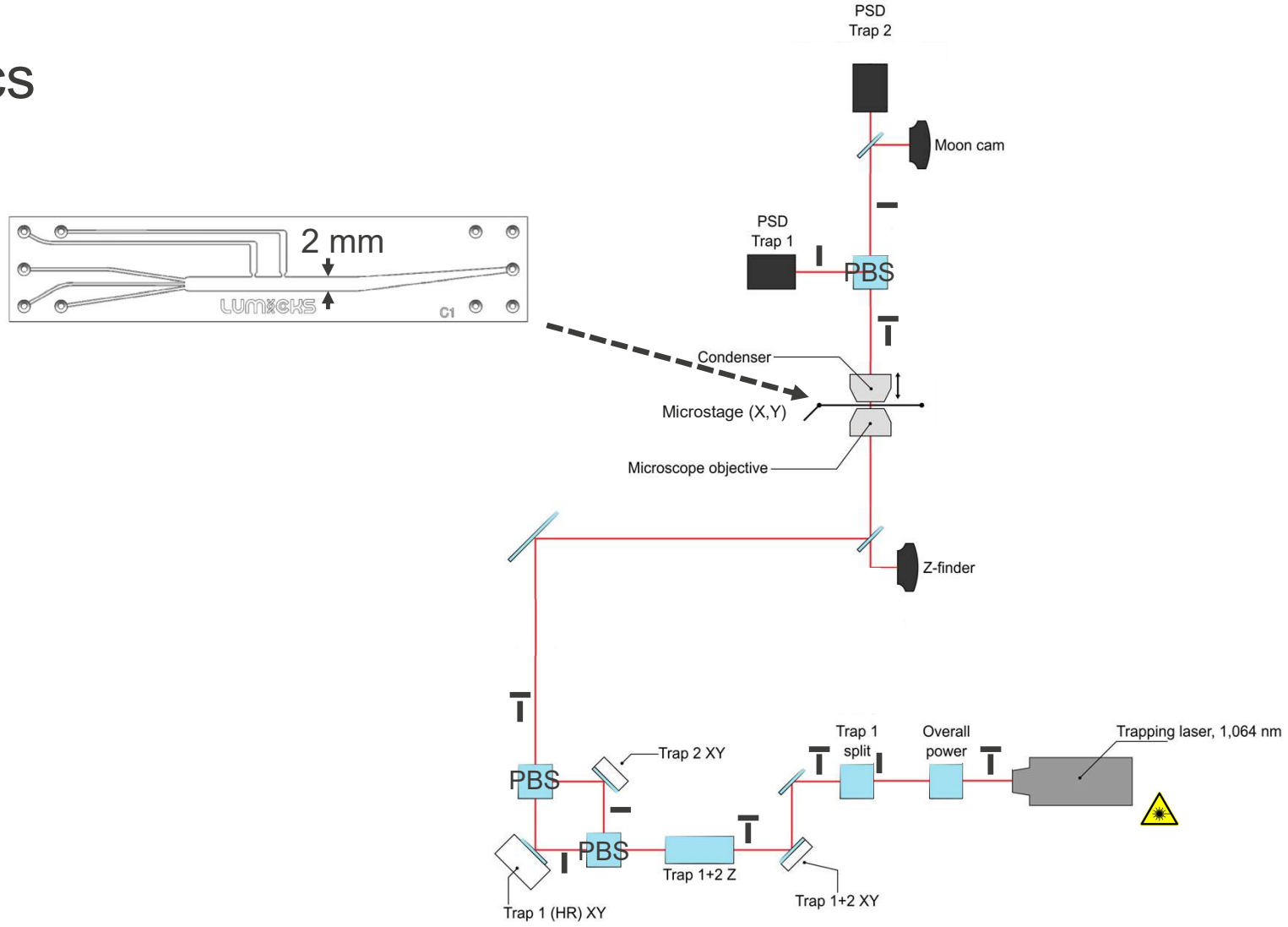
Microfluidics



Imaging

Optical Traps

Microfluidics



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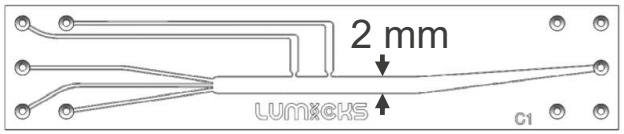
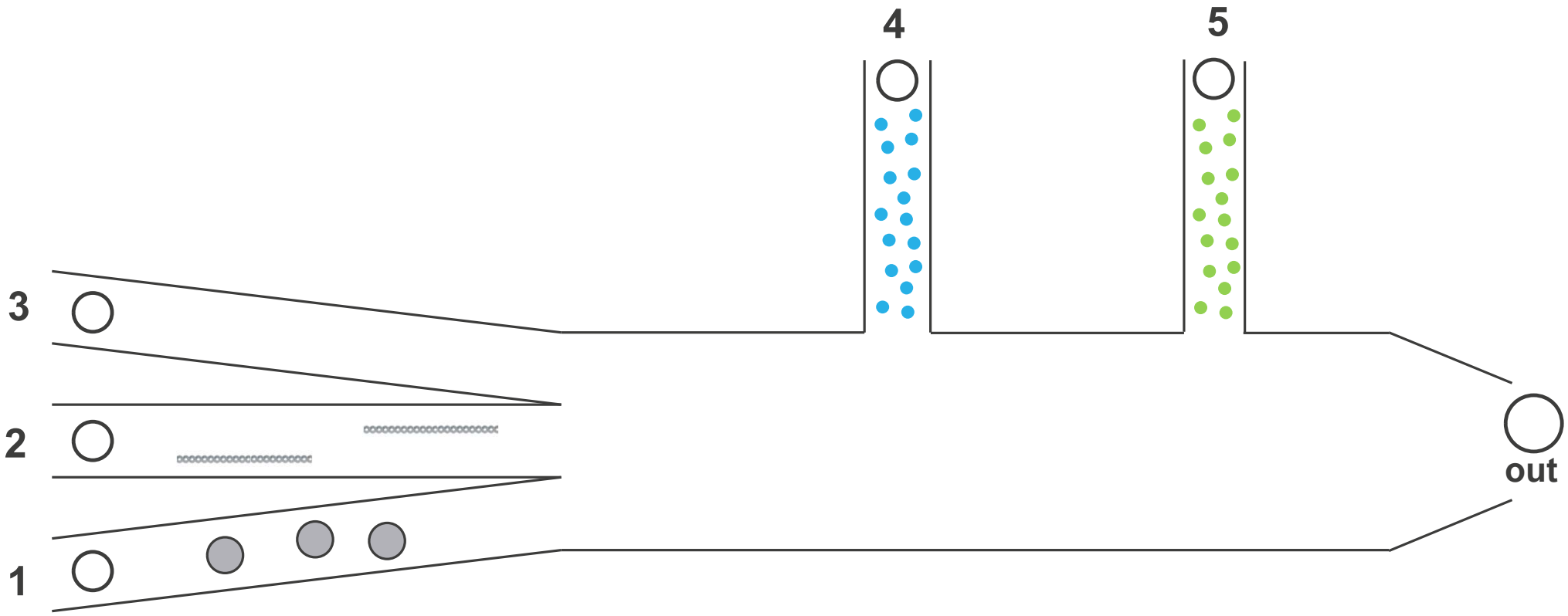


Flowcell volume ~12 µL
Min volume per syringe ~ 200 µL

Microfluidics



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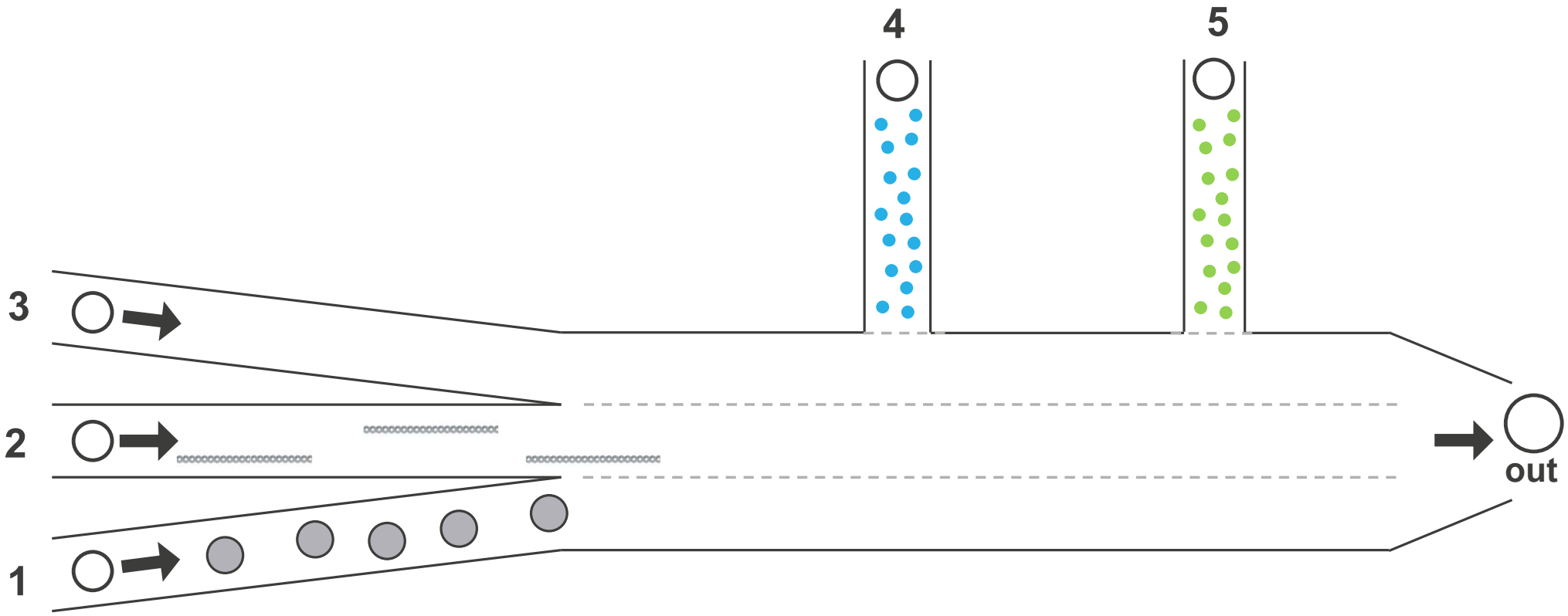

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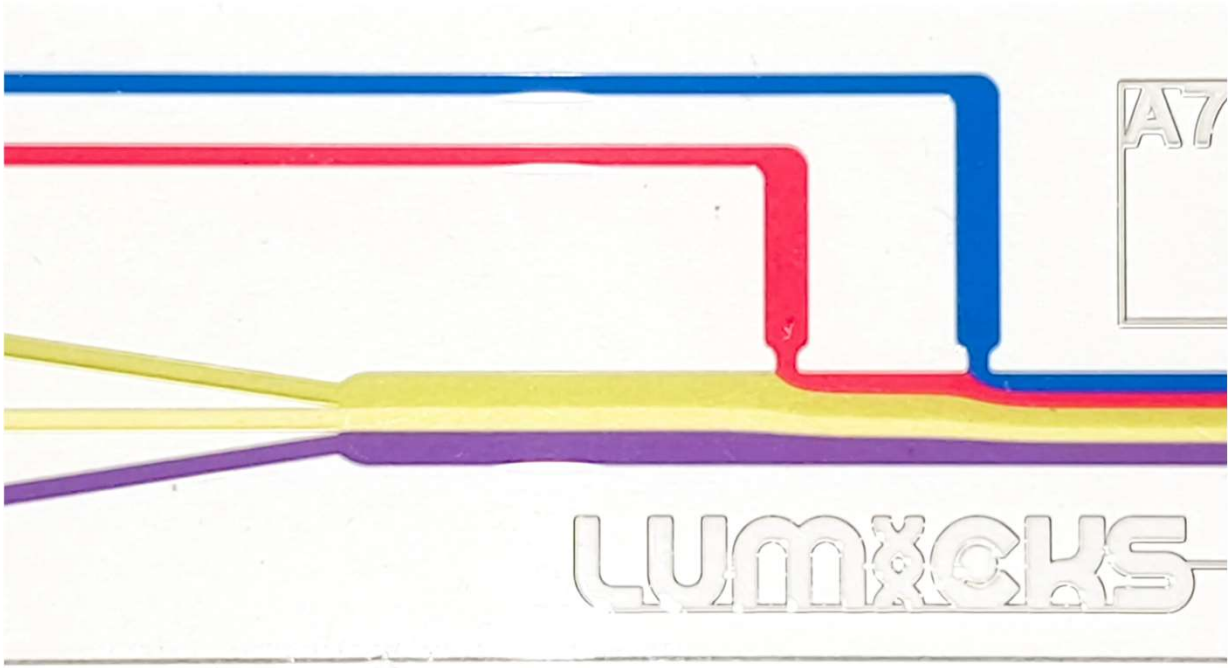

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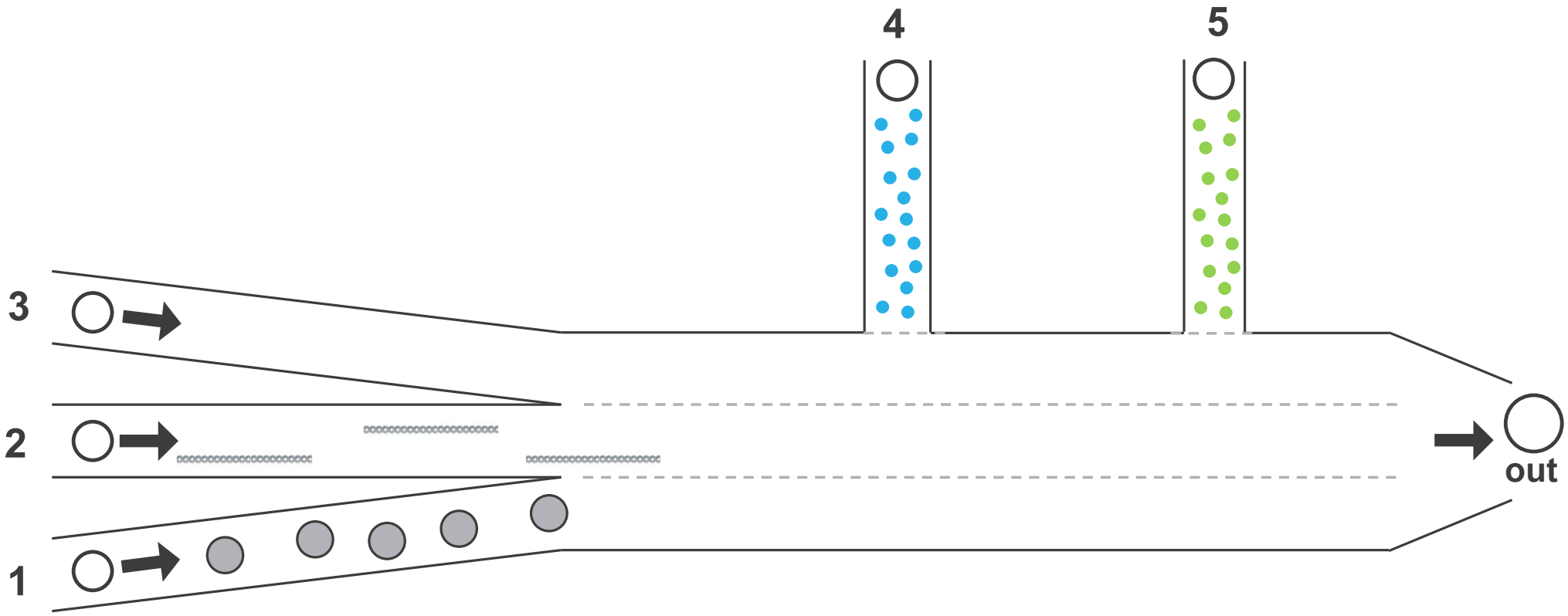

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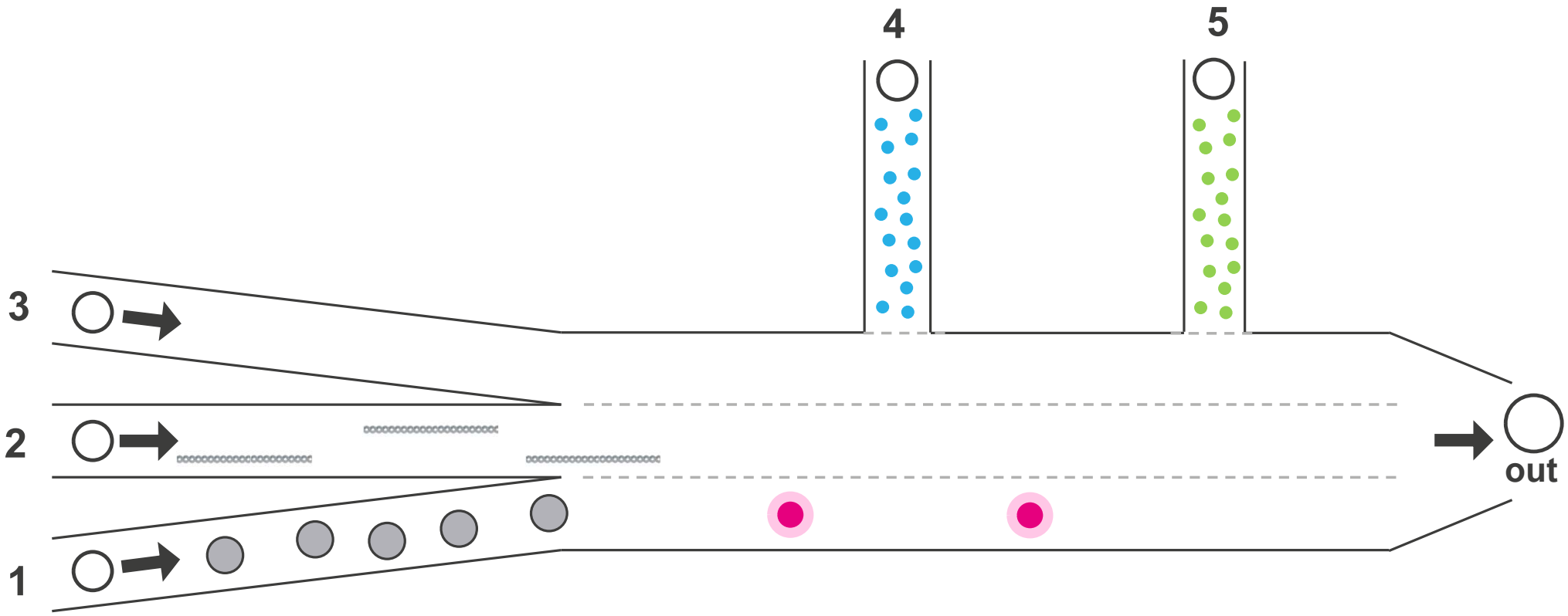

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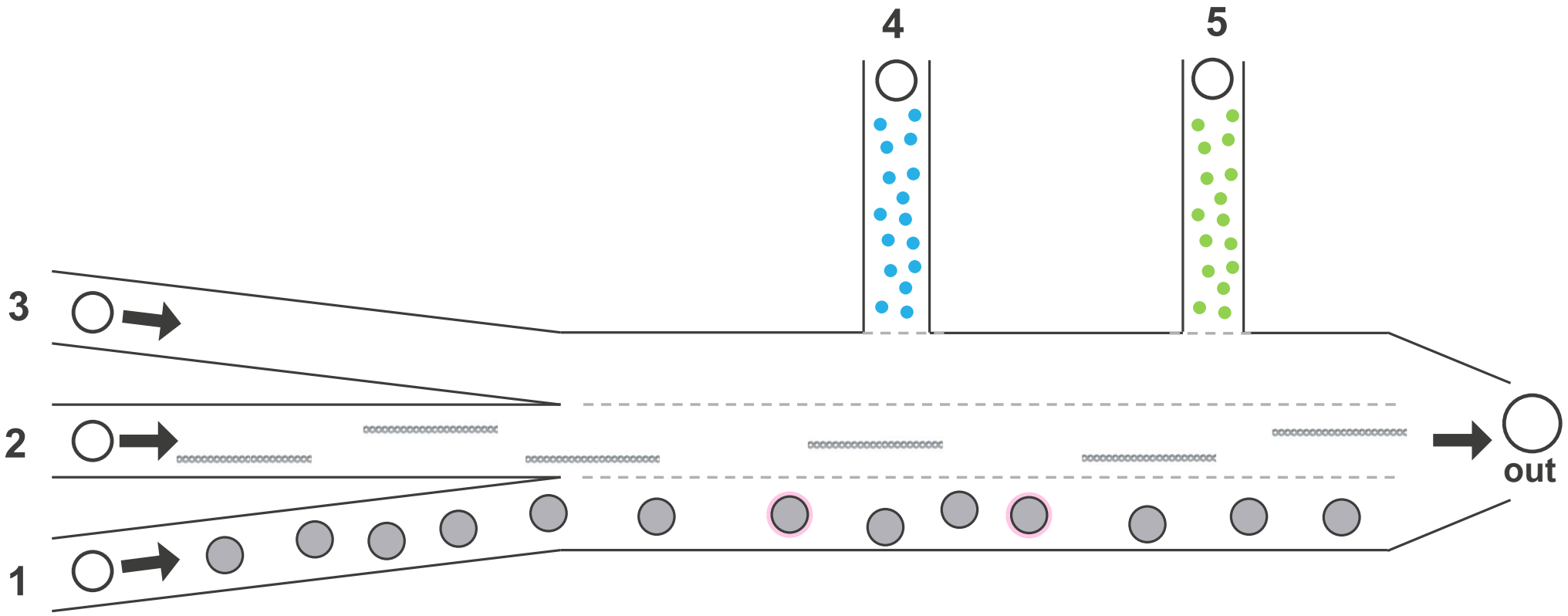

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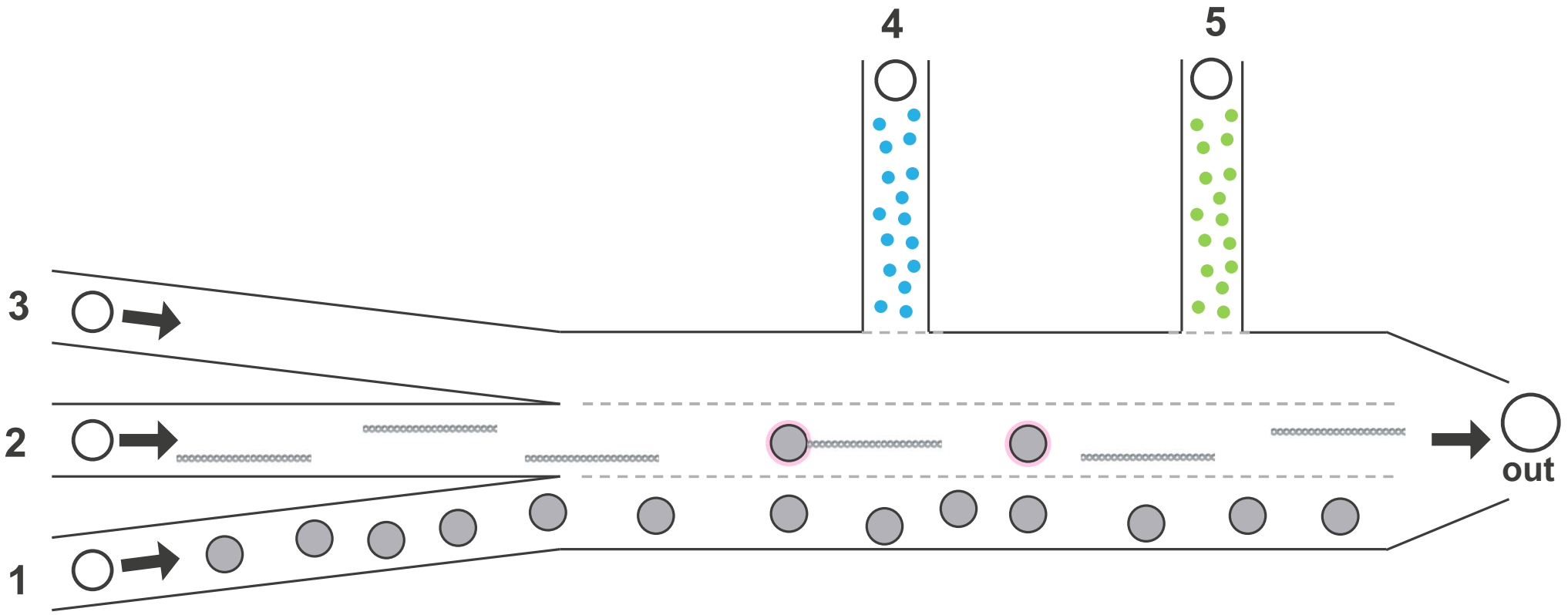

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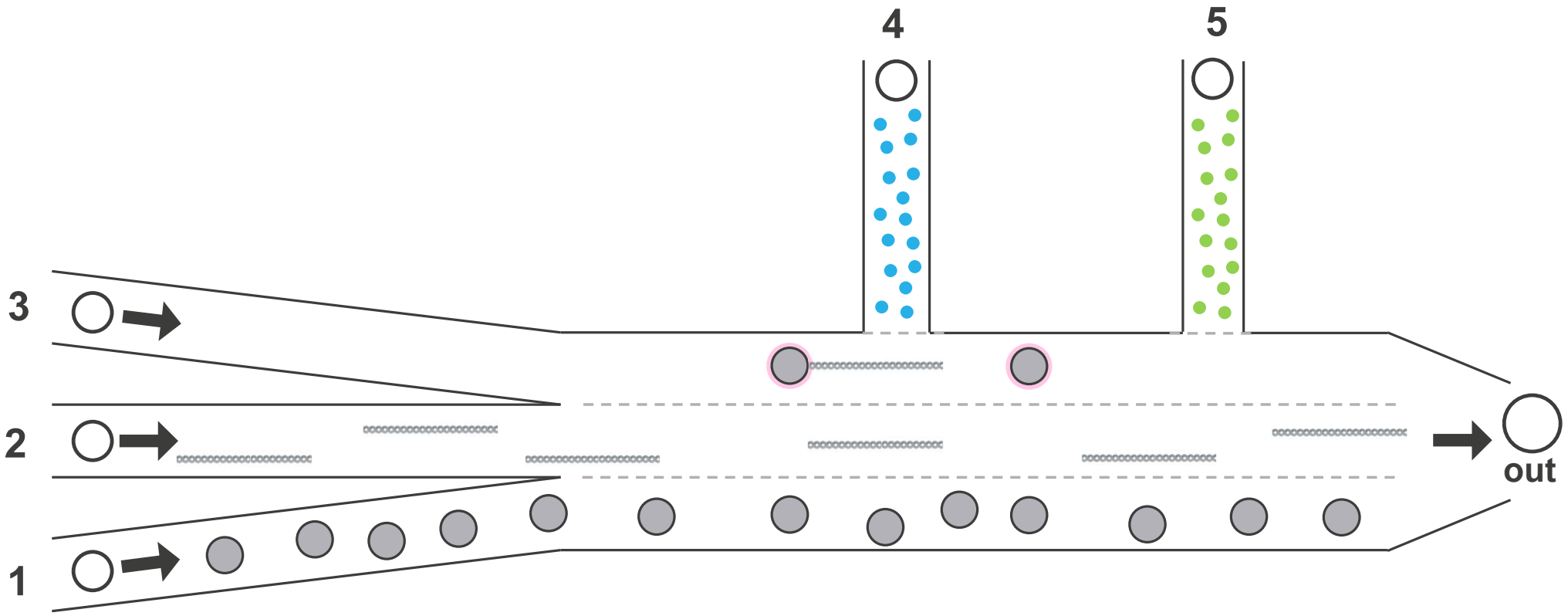

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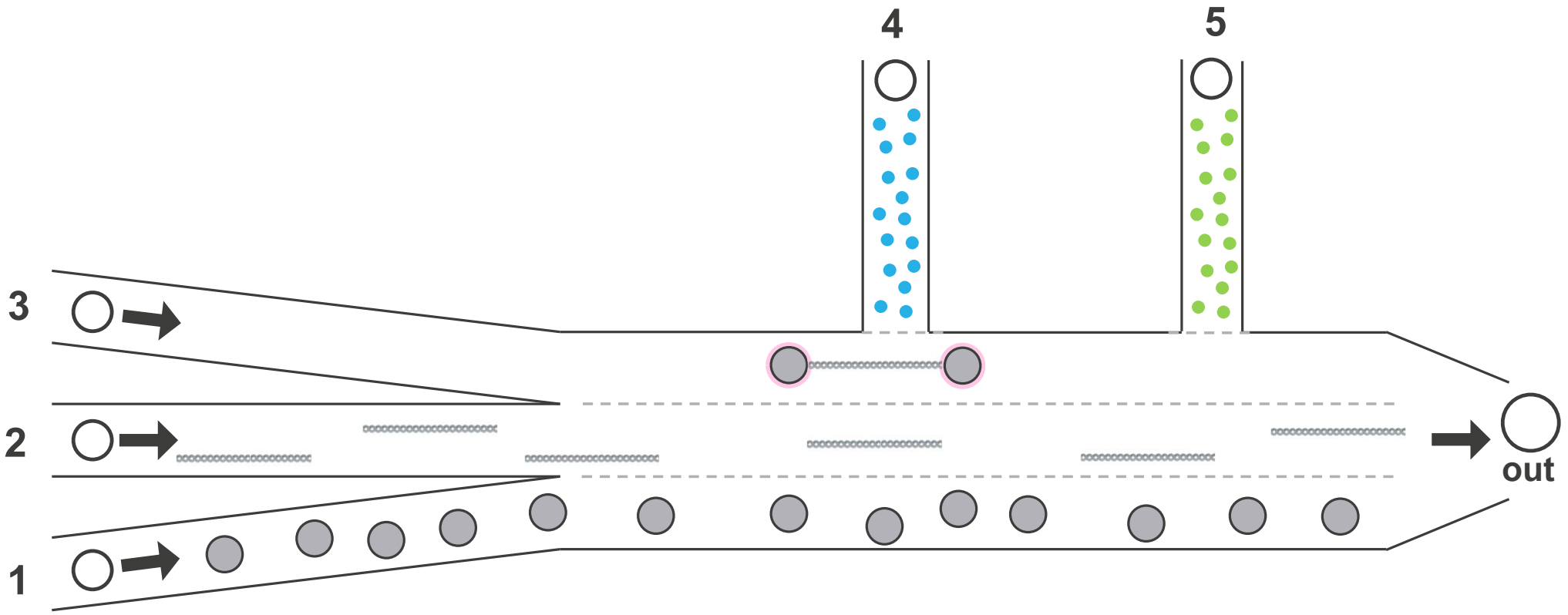

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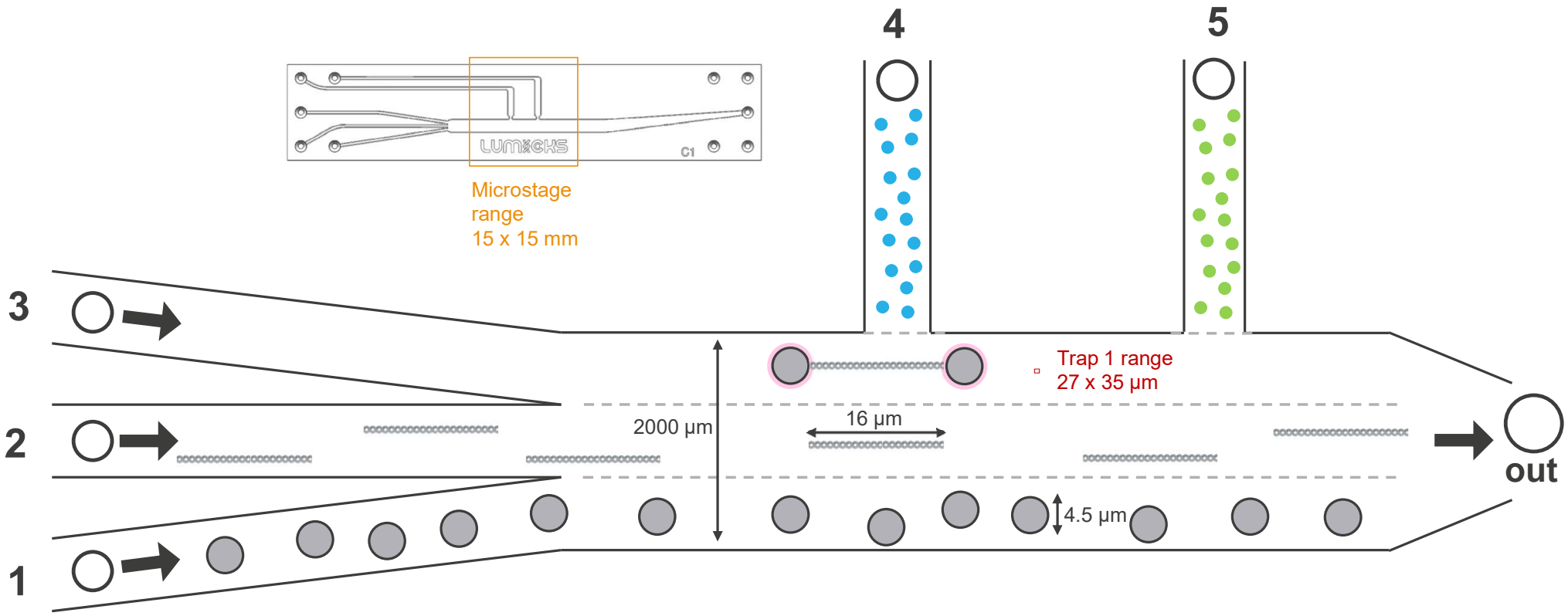

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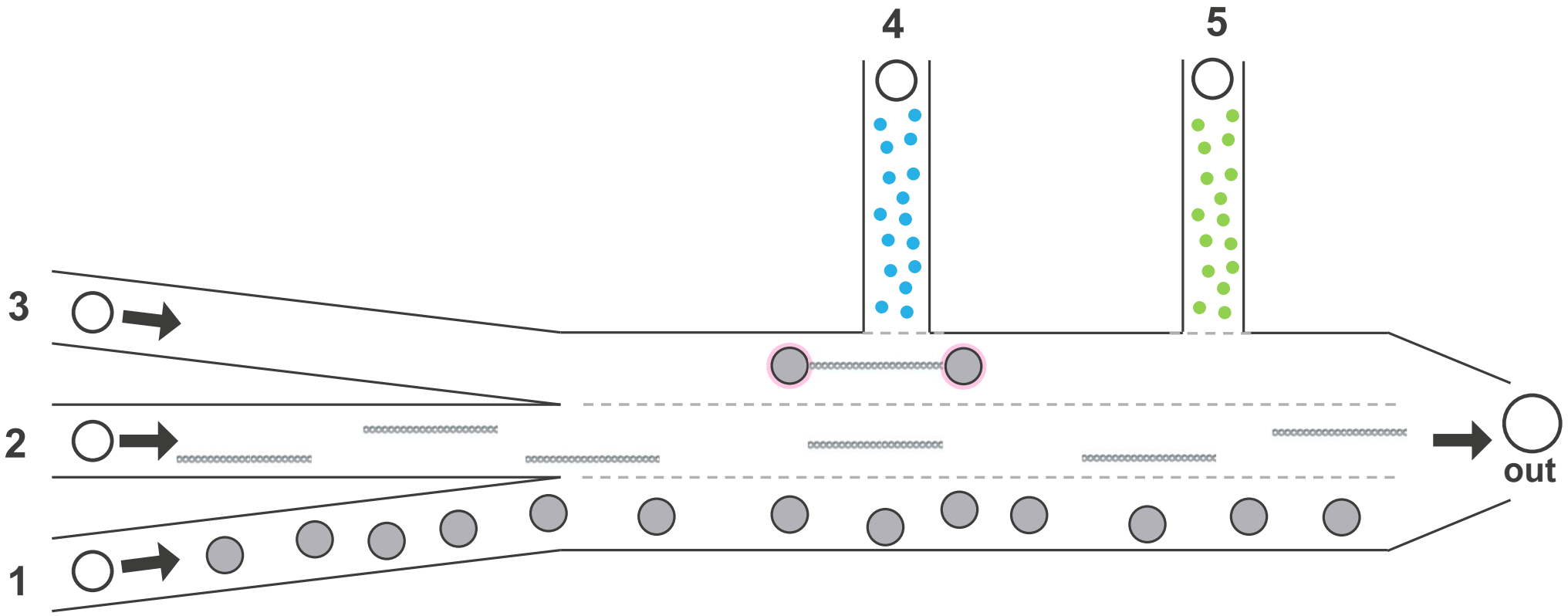

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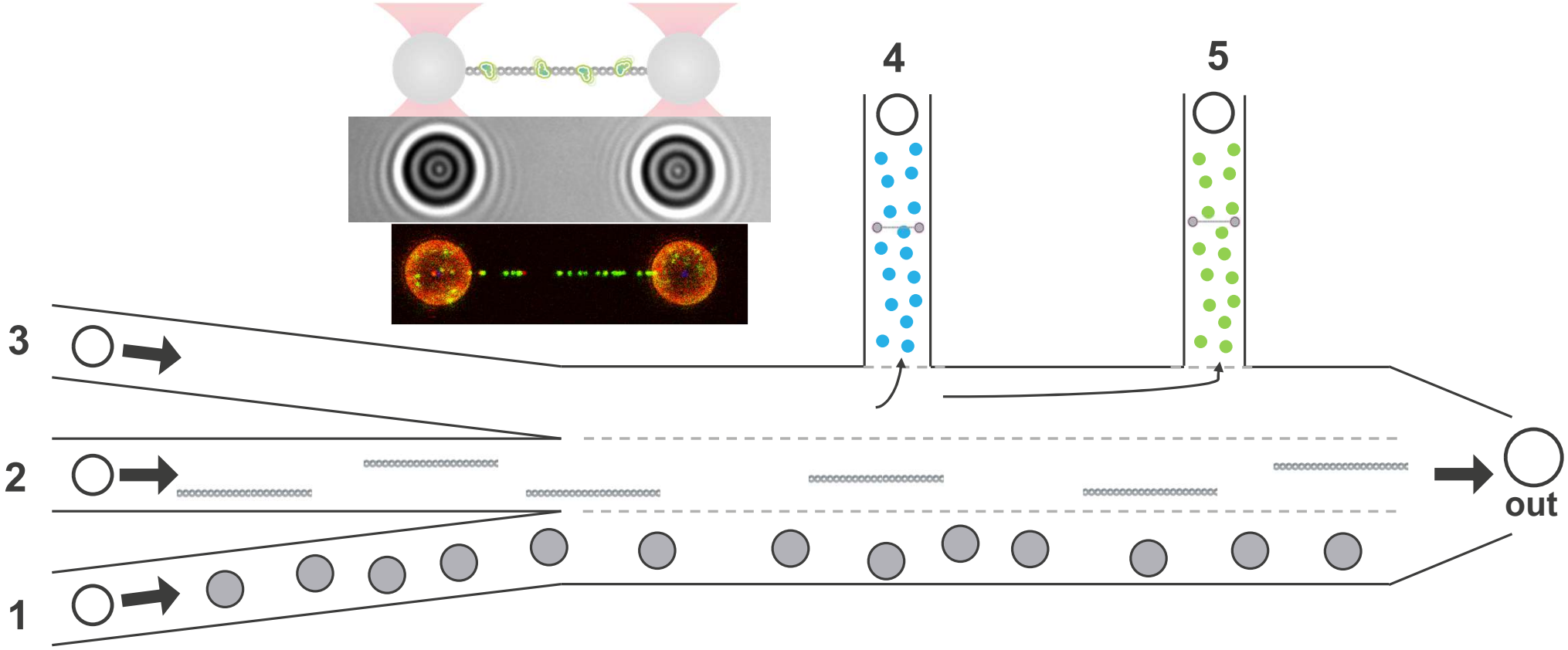


Standard protocols: Cleaning reagent protocol* (~75 mins without passivation)

*you may notice minor differences with other cleaning reagent protocols
Full protocol here: https://store.lumicks.com/wp-content/uploads/2022_uFlux-flow-cell-cleaning-and-passivation-protocol-1.pdf

1. Add 0.7 mL of cleaning reagent (<5% sodium hypochlorite), flush 0.5 mL (2 bar, ~10 mins)

   Note: do not leave cleaning reagent for extended periods (more than 24h). Instead, leave the system in 10x diluted cleaning reagent (after step 2) for up to several weeks/months
2. Remove the remaining 0.2 mL cleaning reagent, add 1 mL Milli-Q, flush 0.5 mL (2 bar, ~10 mins)
3. Remove the remaining 0.5 mL of diluted cleaning reagent
4. Add 2 ml of Milli-Q, add 100 µL of reducing agent on top, mix well in the syringe, flush 1.5 mL (2 bar, ~35 mins)
5. Remove the remaining 0.5 mL, add 1 mL Milli-Q, flush 0.5 mL (2 bar, ~10 mins)
6. Passivation (if desired): BSA + Pluronics (~65 mins) OR casein (~45 mins)

   Note: we recommend to use the casein passivation in combination with the Alkaline cleaning reagent cleaning protocol unless sodium hypochlorite is absolutely needed, e.g. to remove intercalators (more details in protocols)
7. Add your samples

Standard protocols: BSA + Pluronics passivation (~65 mins)

Full protocol here: https://store.lumicks.com/wp-content/uploads/2022_uFlux-flow-cell-cleaning-and-passivation-protocol-1.pdf

1. Add 0.5 mL 0.1 % BSA solution (10x dilution from stock) to channels 4 and 5, flush ~0.250 mL (0.3-0.4 bar, 20 mins)
   Note: always open channel 6, and flush channels 1-2-3 with blank buffer at the same time as channels 4 and 5 to maintain proper laminar flow
2. Remove the remaining BSA solution
3. Add 0.5 mL of 0.5% Pluronics solution (10x dilution from stock) to 4 and 5, flush ~0.250 mL (0.3-0.4 bar, 20 mins)
4. Remove the remaining Pluronics solution
5. Add 0.5 mL of working buffer to 4 and 5, flush ~0.250 mL (0.3-0.4 bar, ~20 mins)

Standard protocols: Alkaline cleaning reagent protocol (~45 mins without passivation)

Full protocol here: https://store.lumicks.com/wp-content/uploads/2023-Cleaning-and-passivation-protocol.pdf

1. Dilute 0.2 ml of alkaline cleaning reagent (ACR) with 9.8 ml of Milli-Q water (50x dilution)
2. Add 2 ml of diluted ACR in each syringe, flush at least 1 mL (2 bar, ~20 mins)

   Note: if it is the first time ACR is being used, a slightly more rigorous protocol is recommended (see link above)

   Note: leaving diluted ACR overnight will result in even more thorough cleaning. However, do not leave it in for more than 24h

   Note: leave the system in Milli-Q between experiments
3. Remove the remaining diluted ACR from all syringes and rinse the syringes once with 1 ml Milli-Q

   Note: Rinsing = add and remove, without flushing

   Note: in case intercalating dyes have been used, use additionally the cleaning reagent protocol with sodium hypochlorite
4. Add 2 ml of Milli-Q in each syringe, flush at least 1 mL (2 bar, 20 mins)
5. Passivation (if desired): BSA + Pluronics (~65 mins) OR casein (~45 mins)

   Note: we recommend to use the casein passivation in combination with the Alkaline cleaning reagent cleaning protocol unless sodium hypochlorite is absolutely needed, e.g. to remove intercalators (more details in protocols)
6. Add your samples

Standard protocols: Casein passivation* (~45 mins)

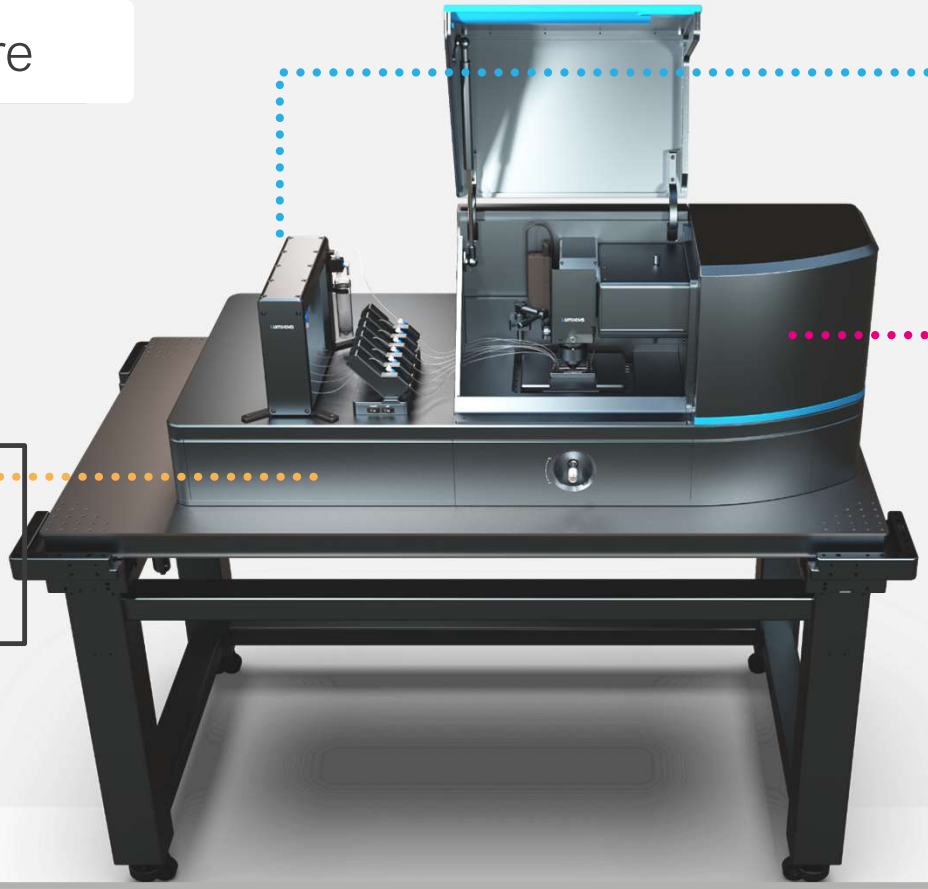
*works best in combination with the alkaline cleaning reagent protocol
Full protocol here: https://store.lumicks.com/wp-content/uploads/2023-Cleaning-and-passivation-protocol.pdf

1. Add 0.5 mL of 0.05% casein solution (20x dilution from stock, in PBS) in channel 4 and 5
2. Flush at 2 bar for 2 minutes, then, flush at 0.4 for 10 mins
3. Stop the flow, incubate the casein for 20 mins without flow
4. Remove the remaining casein solution from the syringe.
5. Add 1 ml of your experimental buffer or buffer of choice, apply a flow of 2 bar for 2 minutes, then 0.4 bar for 10 minutes

   Note: do not let the channels run dry

Software

Microfluidics



Imaging

Optical Traps

Imaging – bright-field

Bright-field illumination

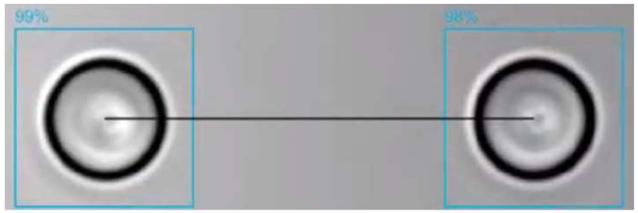
LED, 850 nm

Bright-field camera (CMOS)

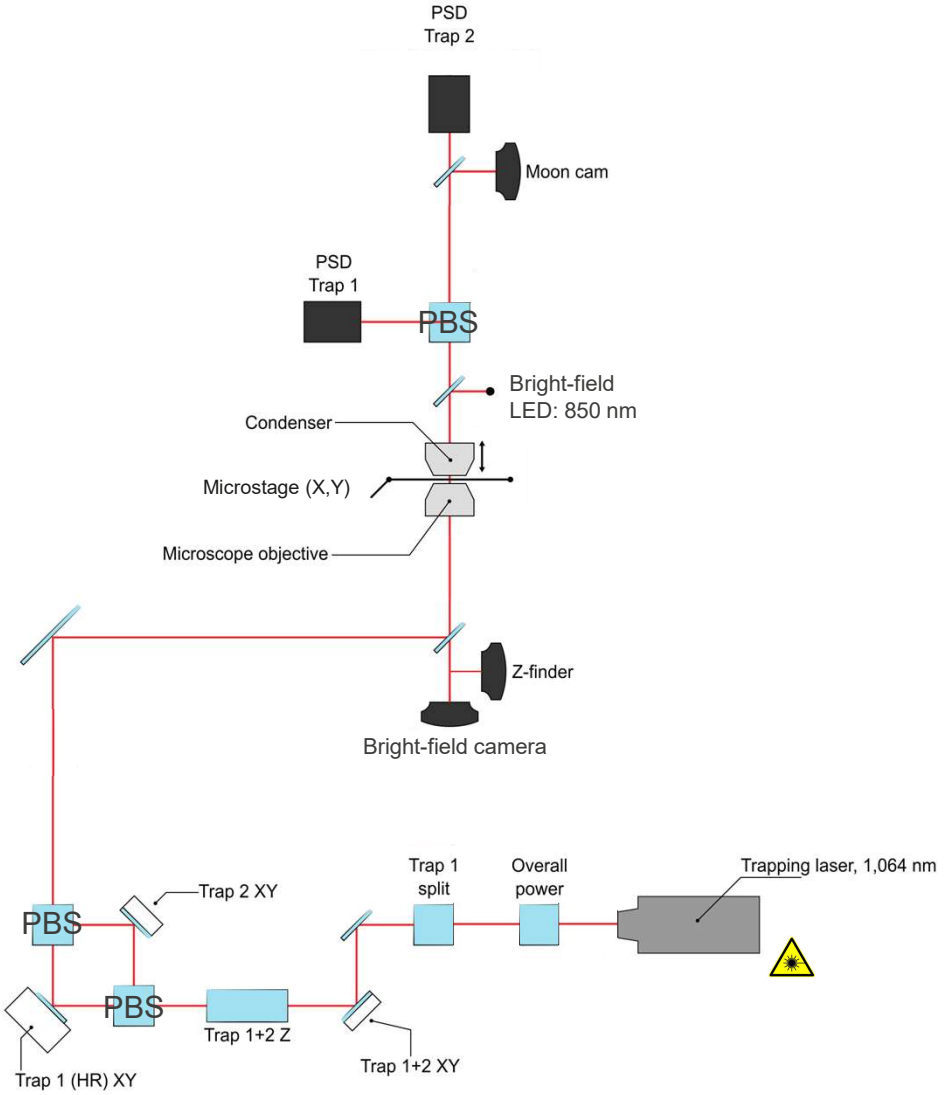
High speed camera to visualize the sample in bright-field and perform bead template tracking. The field of view is ~110x70 µm

20 µm

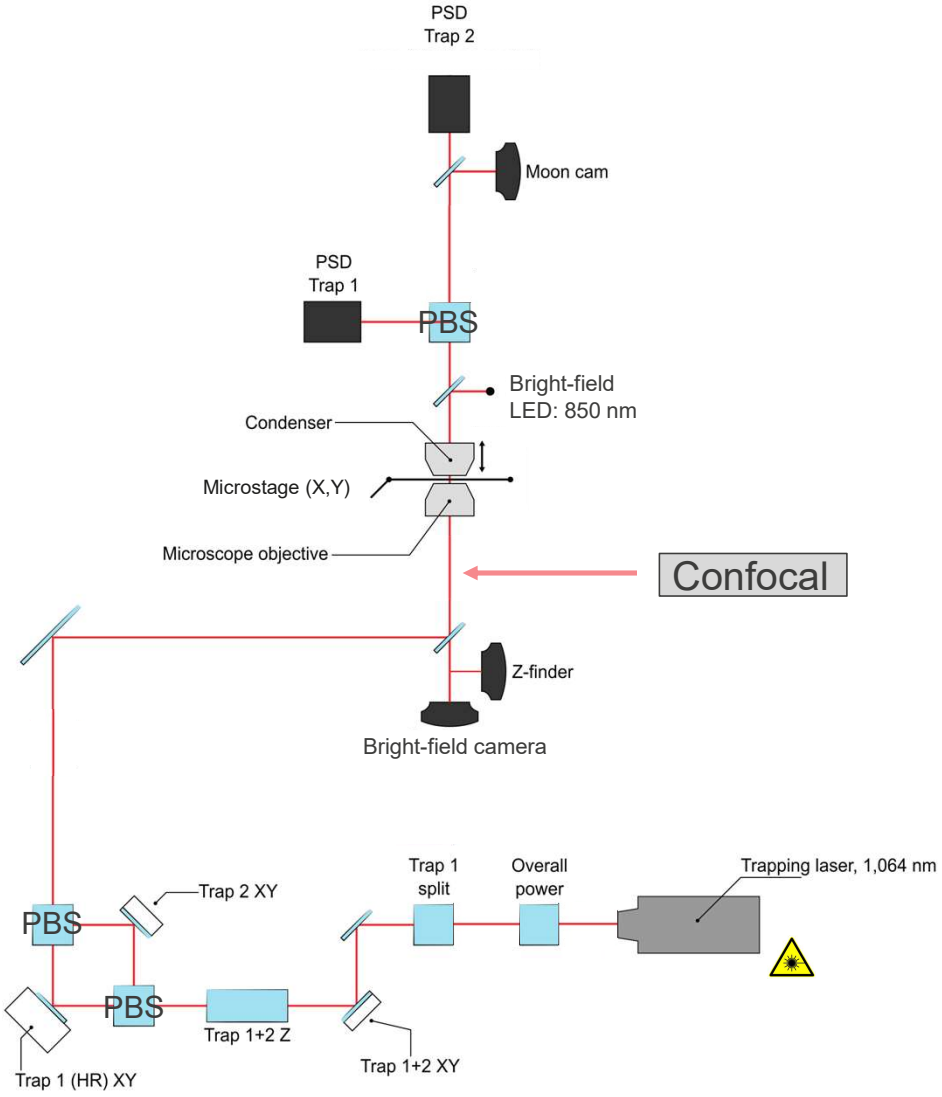
6 µm



For typical λ-DNA assay = 15-100 Hz



Imaging – confocal



Imaging – confocal excitation and detection

Avalanche Photodiode (APD)

Fluorescence detectors with single-photon sensitivity, 50-70% photon detection efficiency, low dark count noise (<250 counts/s), 45 ns dead time

Excitation lasers (0-100%)

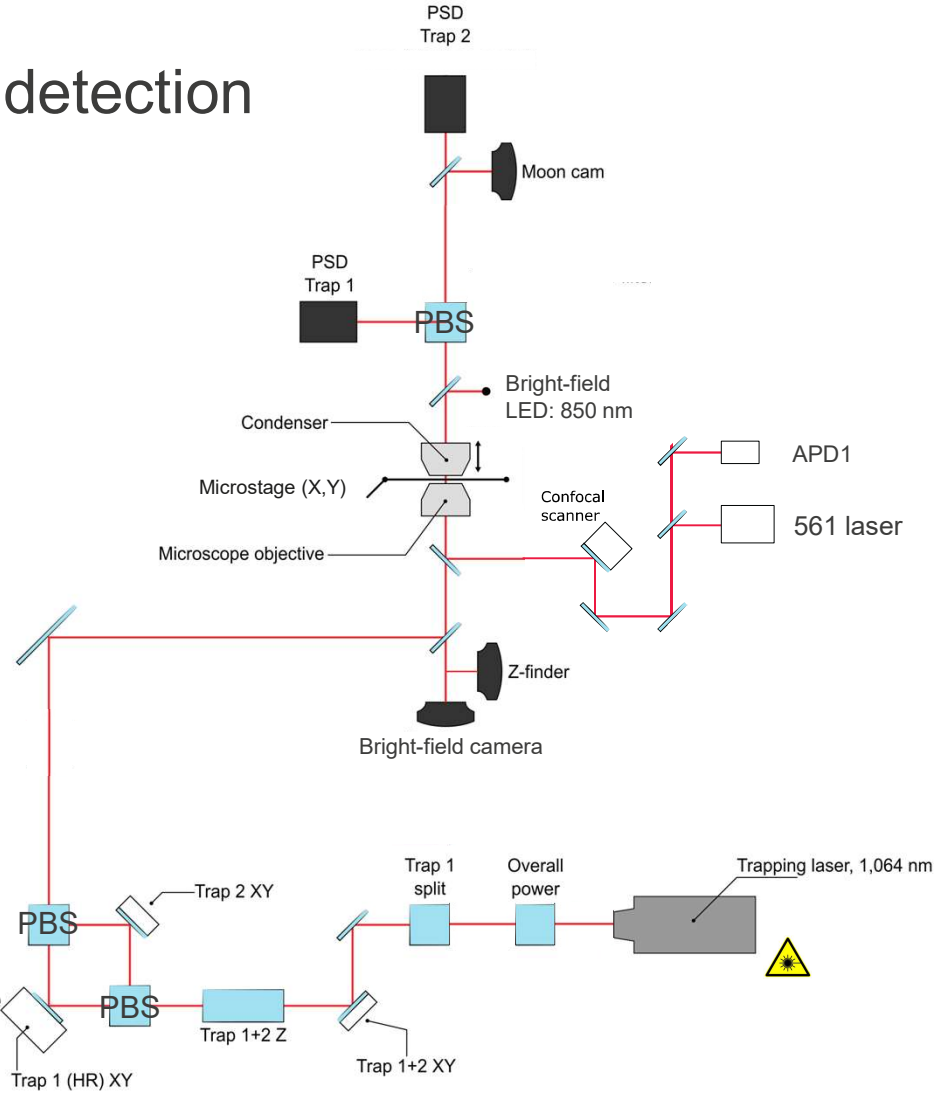
561 nm (“green”)

Emission filter

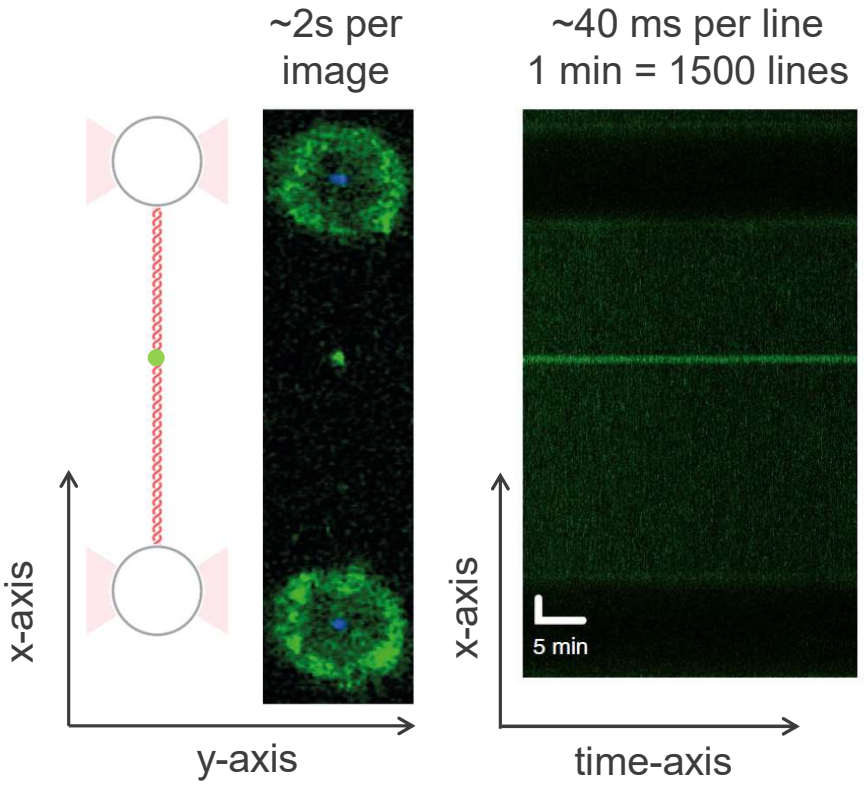
600/50

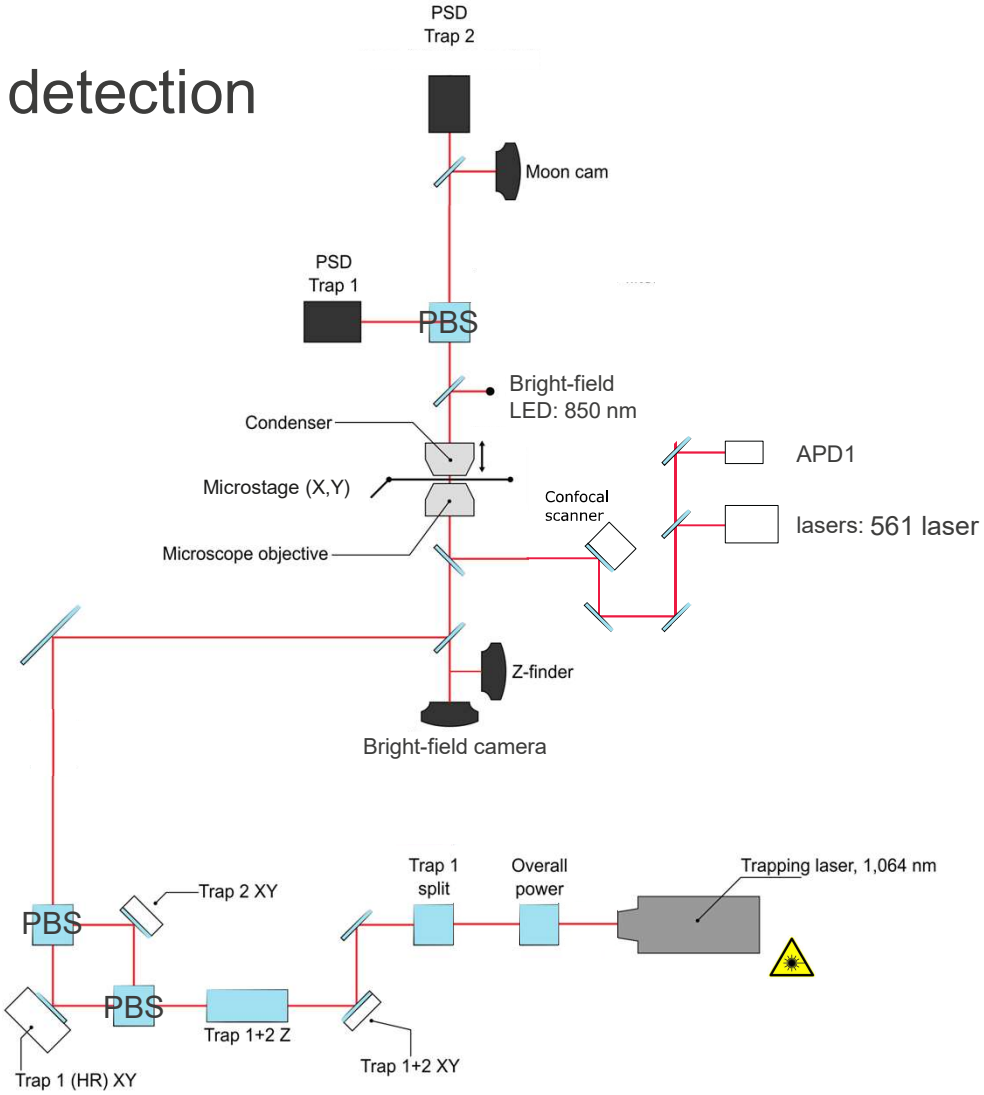
Confocal scanner

- 2-axis, closed-loop control, 50x35 um range
- Position resolution and repeatability <2 nm
- Scanning speed up to 200 Hz per line (1 axis)
- Typical 1-axis scans for **λ-DNA assay = 25 ms per line**
- Typical 2-axis scans for **λ-DNA assay = 1.5 s per image**
- Pinhole size (airy units): 1.5



Imaging – confocal excitation and detection





Newton et al. (2019) Nature Structural & Molecular Biology

Technical and Application Support

General questions

help.Lumicks.com

Application support/general questions

Nastaran Hadizadeh, PhD
n.hadizadeh@lumicks.com

Technical issues

LUMICKS support
with application scientist in cc:
support@lumicks.com