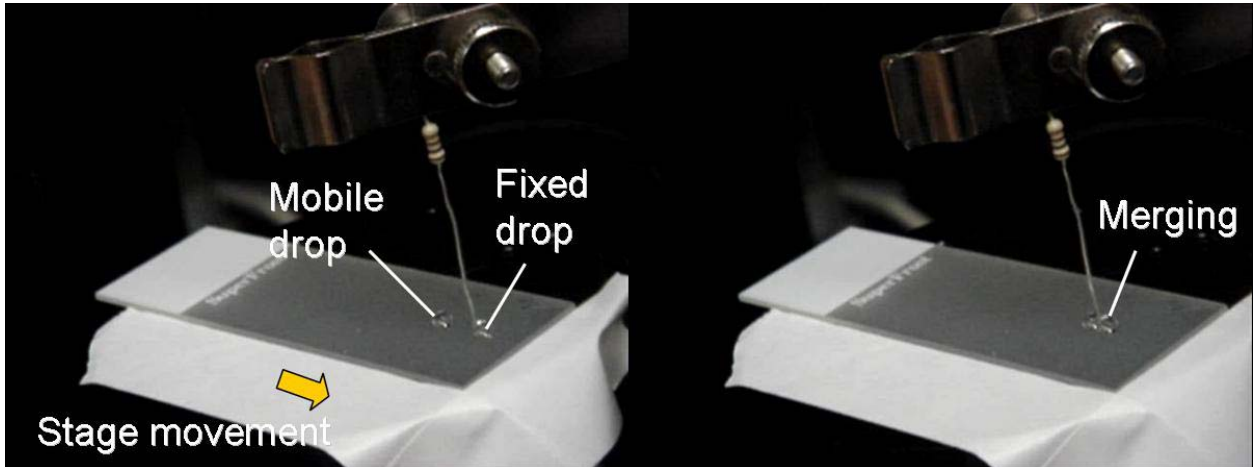
Droplet Manipulations on Superhydrophobic Surface for Latex Immunoagglutination Assays Using Backscattering Detection

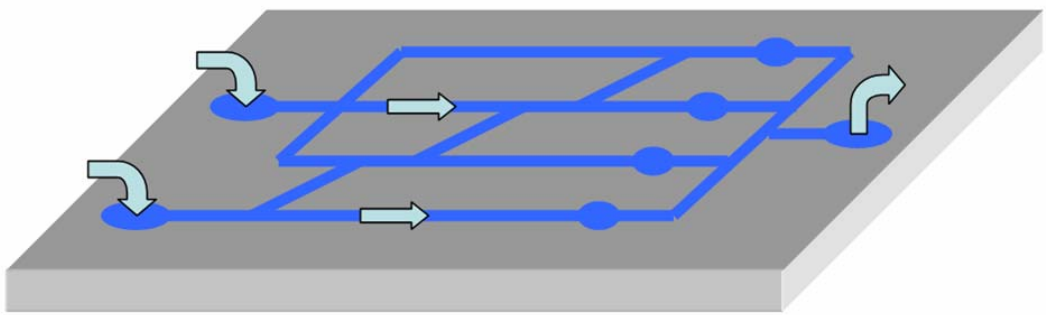
Jeong-Yeol Yoon, Ph.D.
Department of Agricultural & Biosystems Engineering
The University of Arizona, Tucson, Arizona 85721-0038, United States



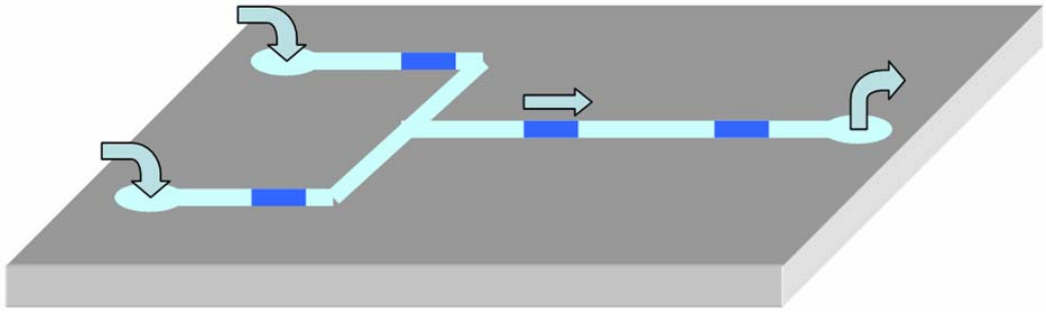
• Lab-on-a-chip (LOC)

- Chemical or biological “lab” on a semiconductor “chip”
- Biological fluids flow through a miniaturized circuit (= a network of microchannels)
- Requires: microfabrication + microfluidics
- Semiconductor chip: a set of instructions can be reprogrammed
- LOC: reprogram the reaction protocol with “moving” components (microvalves, micropumps, etc.)

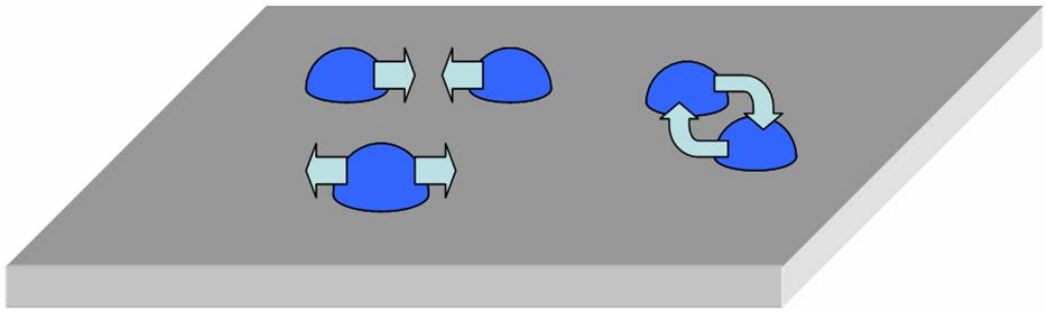
• Droplet microfluidics



Continuous flow
(for serial dilution)
No reprogrammability

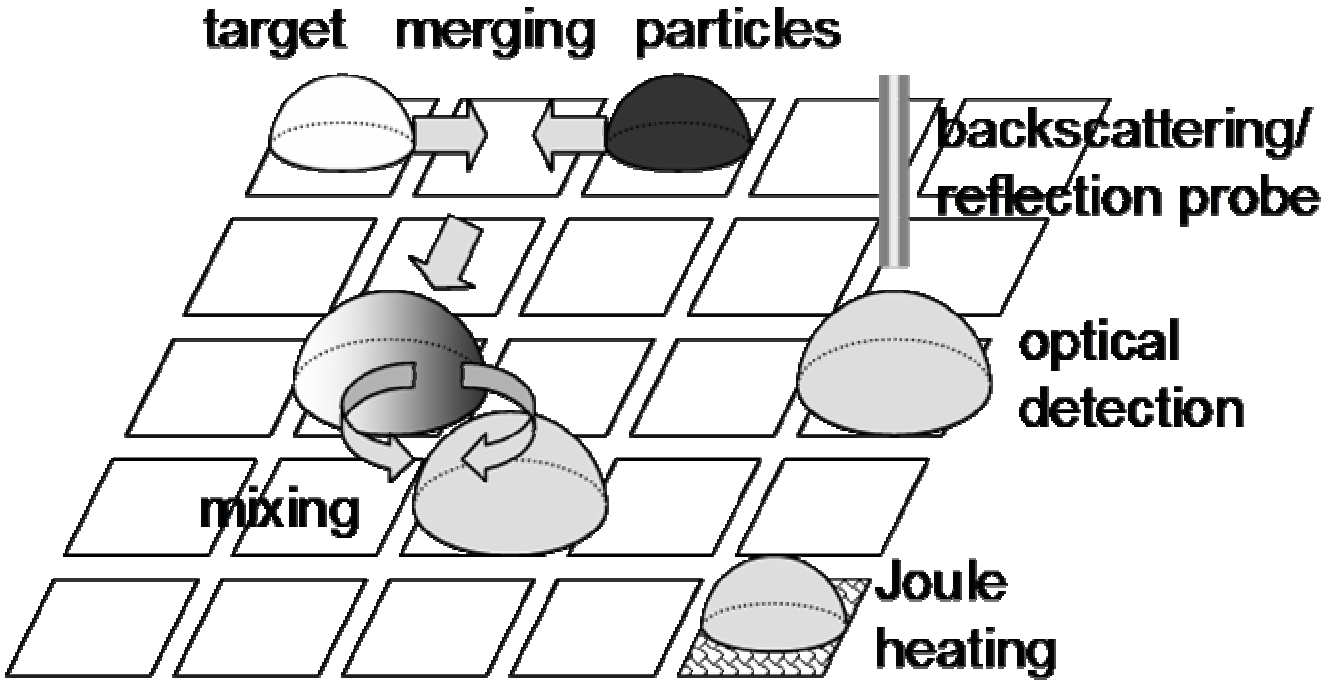


Discrete plugs (droplets)
within microchannels
Limited reprogrammability

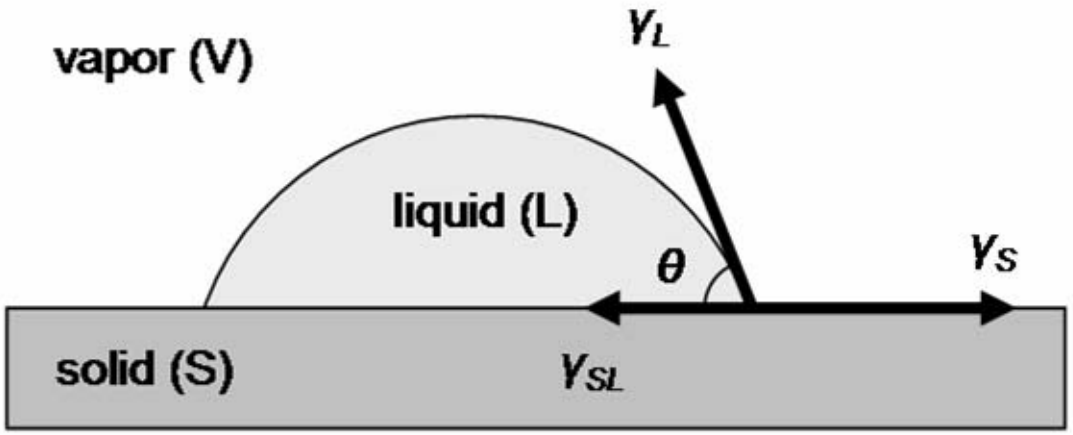


Droplets on an open surface
Merging, splitting & mixing
Maximum reprogrammability

- **Open-surface digital microfluidics**

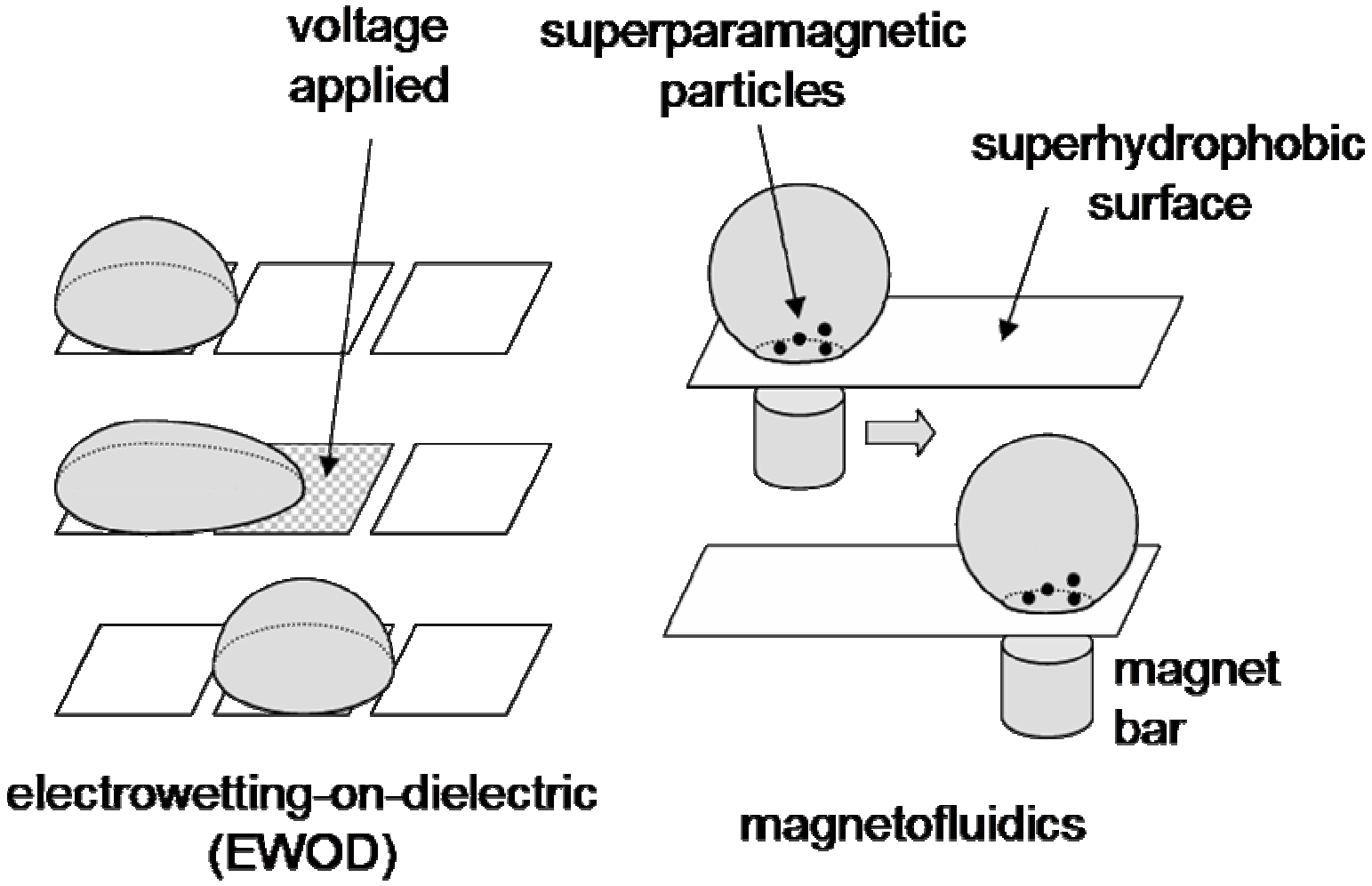


• Contact angle and surface tension



- Young's equation: $\gamma_{SL} = \gamma_S - \gamma_L \cos\theta$
- Dupré equation: $W_a = \gamma_S + \gamma_L - \gamma_{SL}$
- Young-Dupré equation: $W_a = \gamma_L (1 + \cos\theta)$

- **Two available methods: EWOD and magnetofluidics**



• Complications of EWOD and magnetofluidics

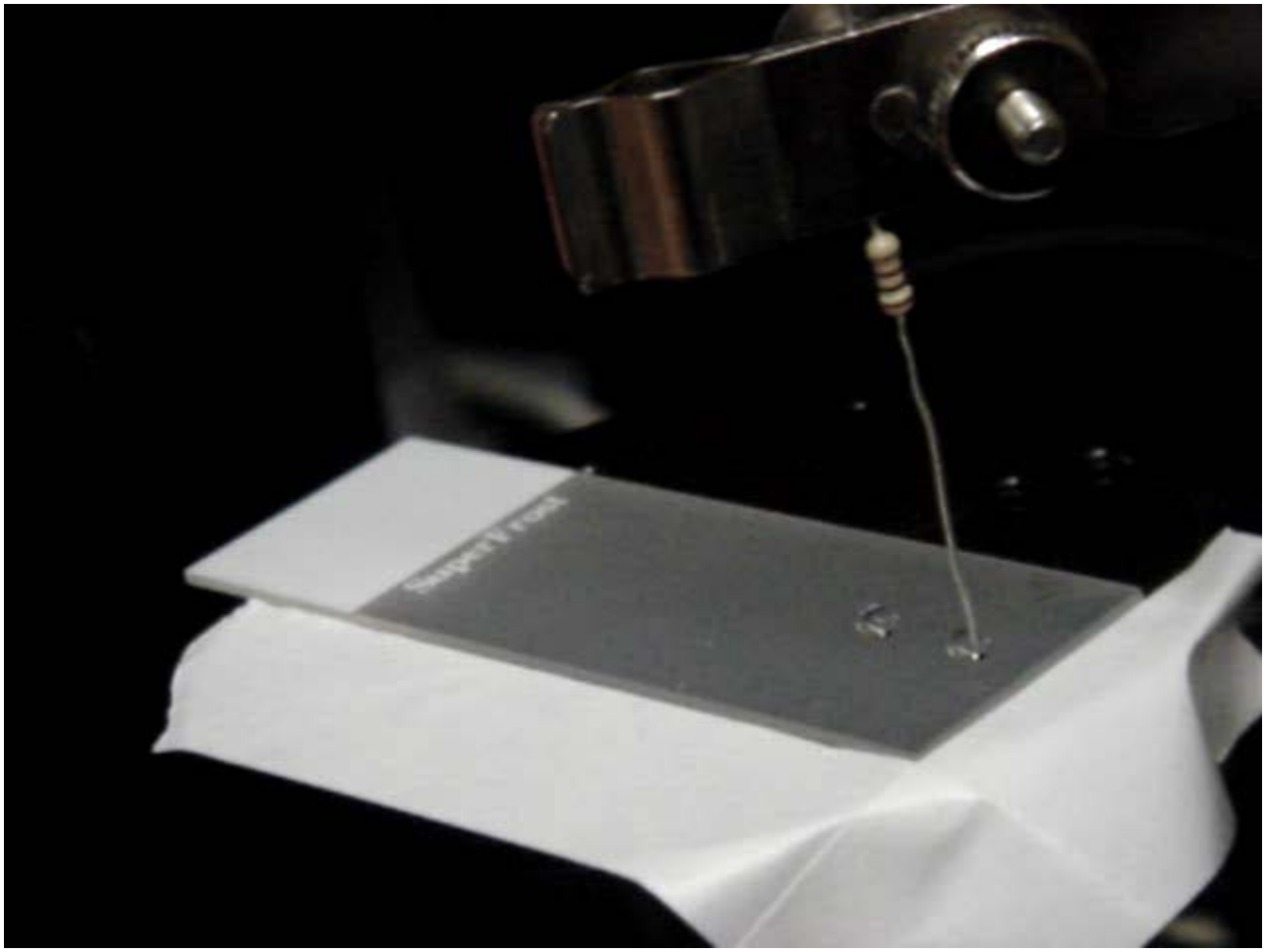
EWOD

- Contact angle saturation
- Electrolysis and/or dielectric breakdown
- Fabrication complexity
- Biofouling and/or contact line pinning
- Effect of electrical field

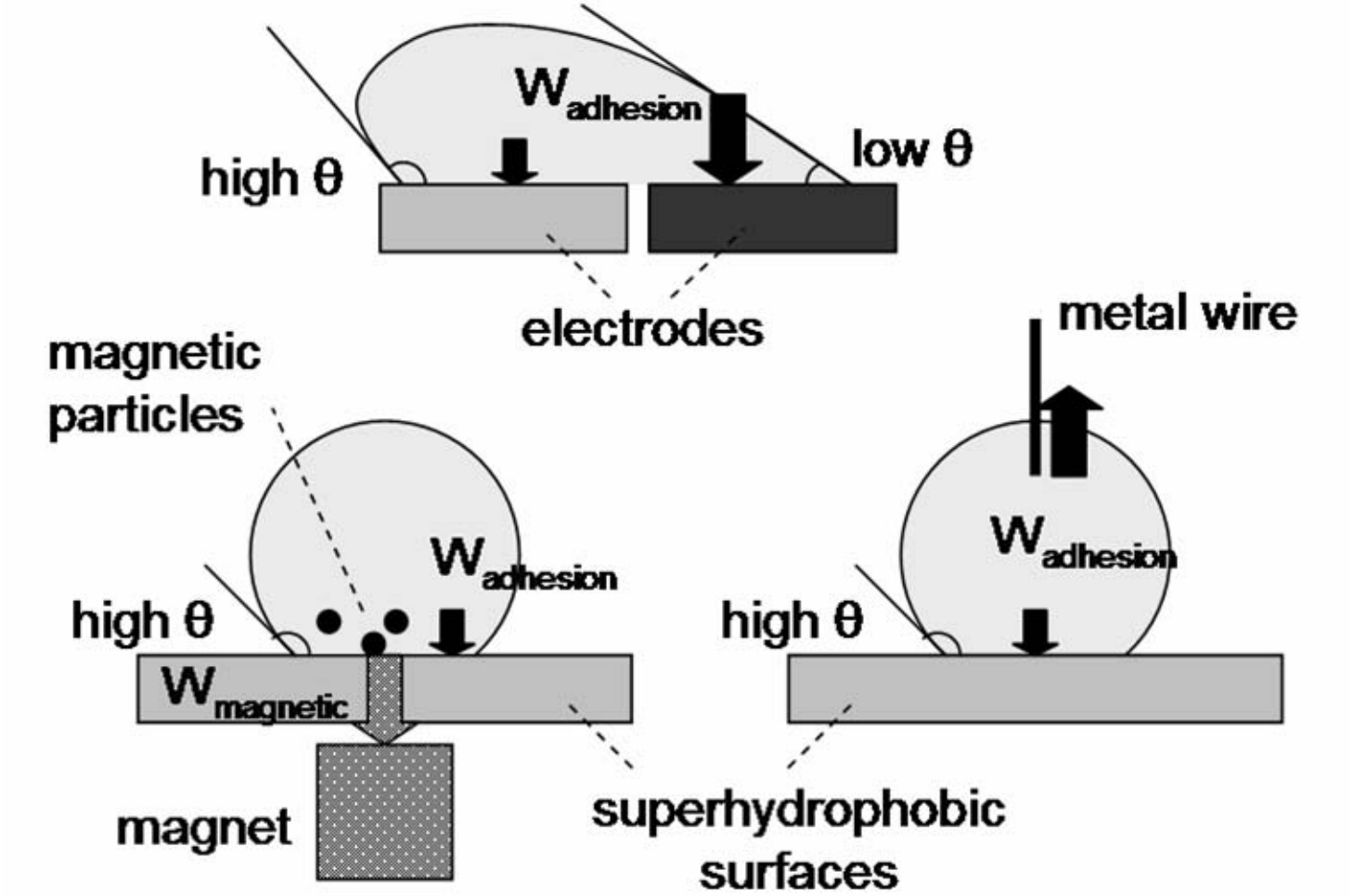
Magnetofluidics

- The use of magnetic particles
- Effect of magnetic field

- **New method: Wire-guide manipulation**



- **Work of adhesion study**



• Work of adhesion study: EWOD

- γ_L = 73 mN/m = 73 mJ/m^2 for water
- θ changes from 115° to 80°
- W_a = (73 mJ/m^2) (1 + cos 115°) = **42 mJ/m^2**
- W_a = (73 mJ/m^2) (1 + cos 80°) = **86 mJ/m^2** (ca. twice)
- A proper contact area to adjacent electrode can make the droplet movement

• Work of adhesion study: Wire-guide

Metal wire

- $\theta \approx 10°$ for typical conducting metal (metal wire of a resistor)
- 0.5 mm OD, inserted into a droplet by 2 mm = contact area = 0.39 mm^2
- (73 mJ/m^2) (1 + cos 10°) (0.39 mm^2) = **57 nJ**

Superhydrophobic surface

- $\theta = 145°$ for superhydrophobic surface (from Surface Innovations)
- Contact area = 6.9 mm^2 (for 10 µL drop; as measured by contact angle analyzer)
- (73 mJ/m^2) (1 + cos 145°) (6.9 mm^2) = **26 nJ** (so it follows a wire!)

Polystyrene surface (Petri dish)

- (73 mJ/m^2) (1 + cos 90°) (10.5 mm^2) = **750 nJ** (does not follow a wire)

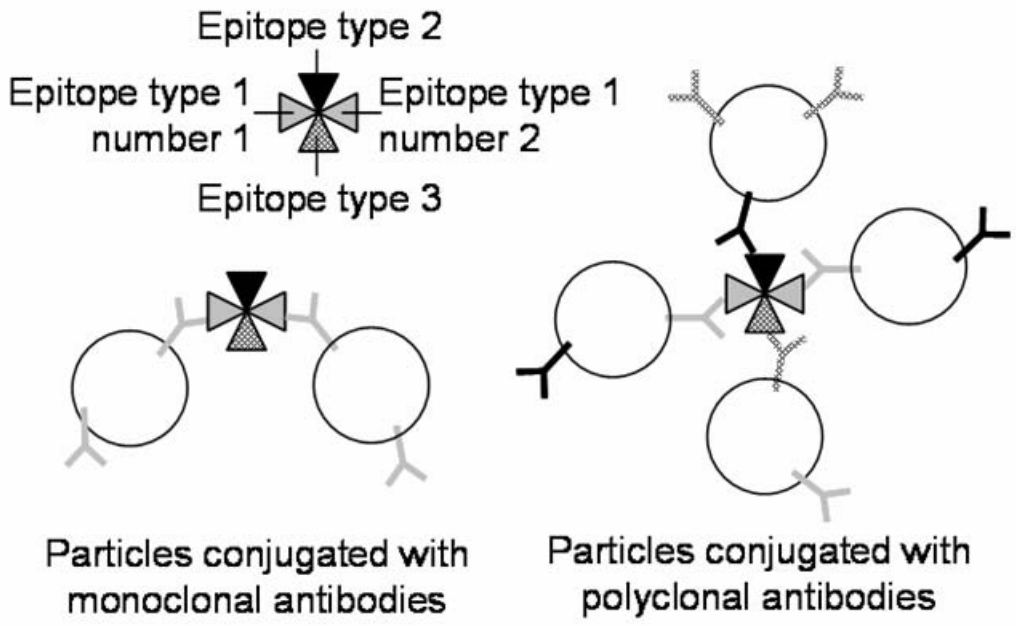
Teflon surface

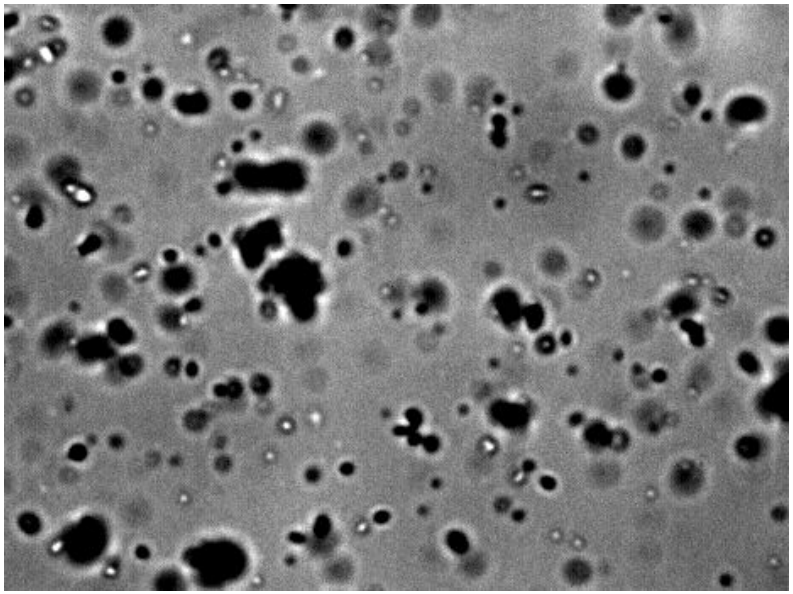
- (73 mJ/m^2) (1 + cos 115°) (6.9 mm^2) = **290 nJ** (does not follow a wire)

• Latex immunoagglutination assay (LIA)

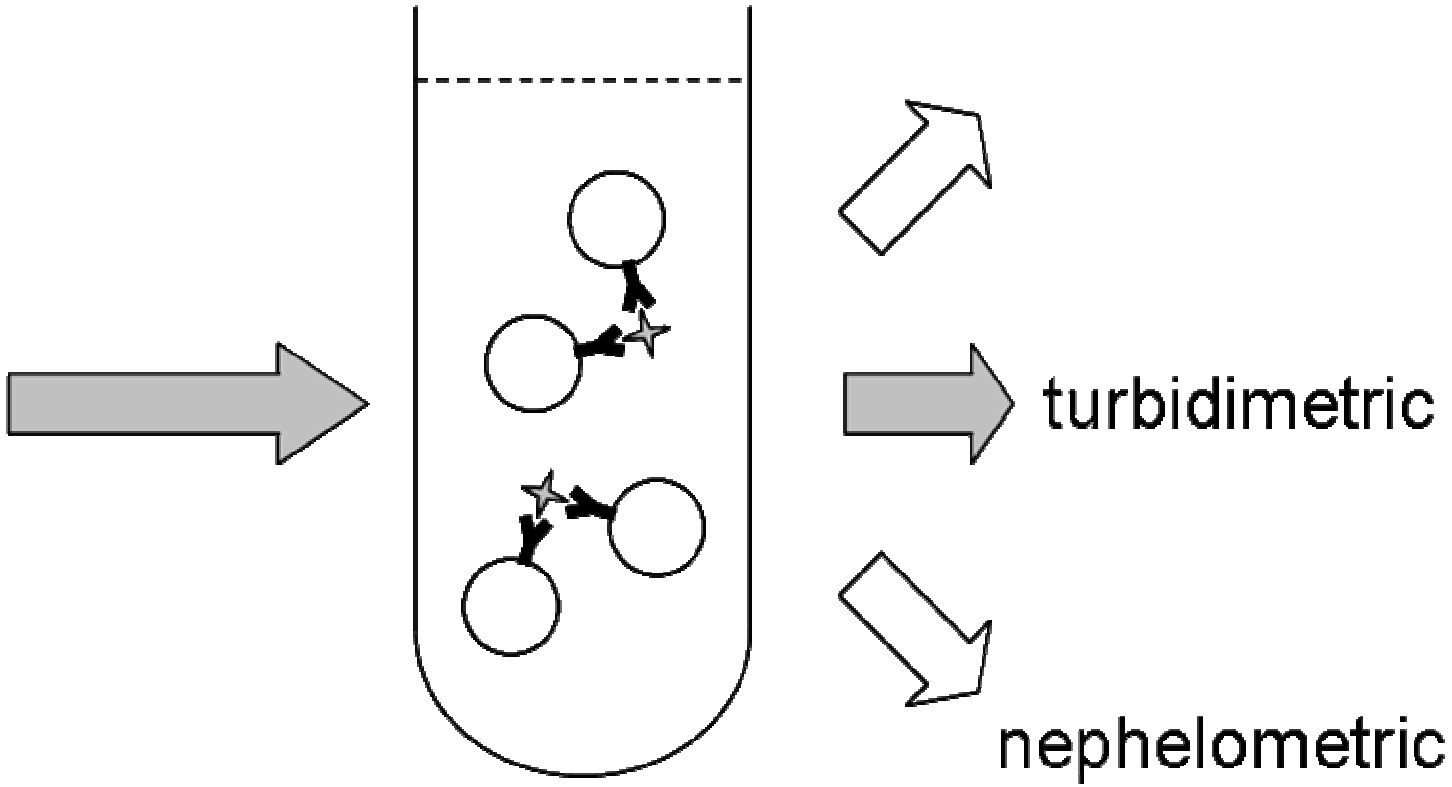
- LIA has NOT been demonstrated in LOC (diffusional mixing issue)
- First demonstrated by UA Biosensors Lab in 2006

  Han, Kim & Yoon, Anal. Chim. Acta 584: 252 (2007)

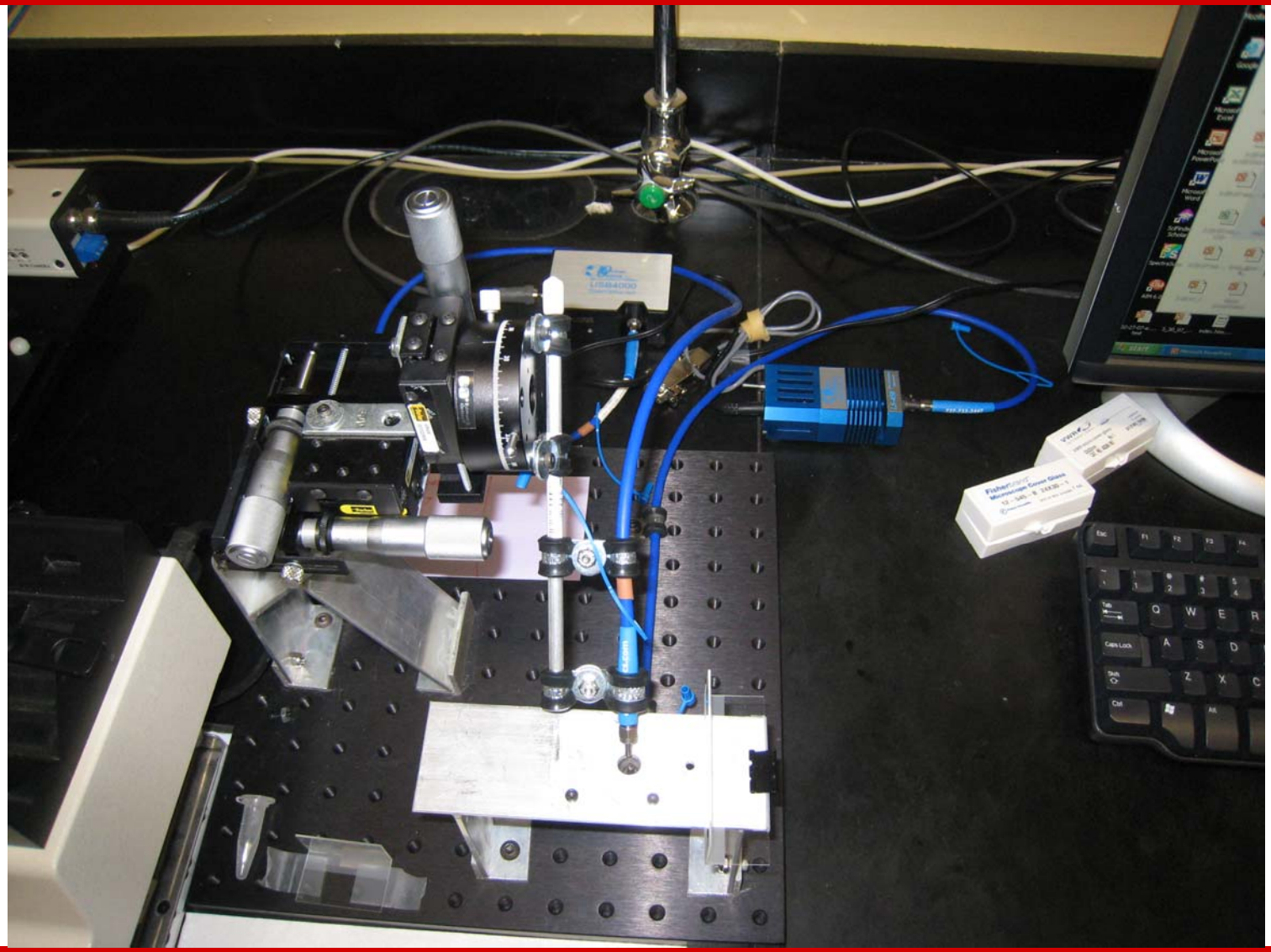




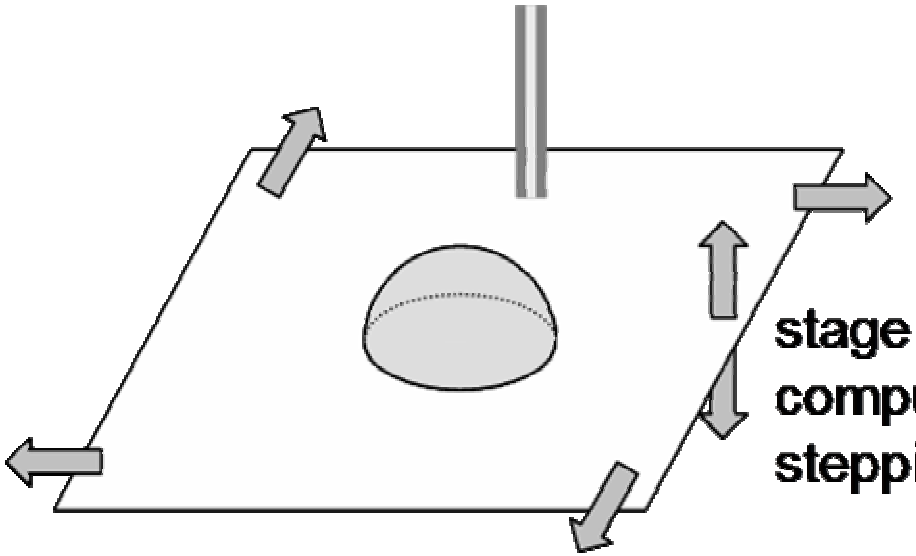
- **Light scattering detection of LIA**



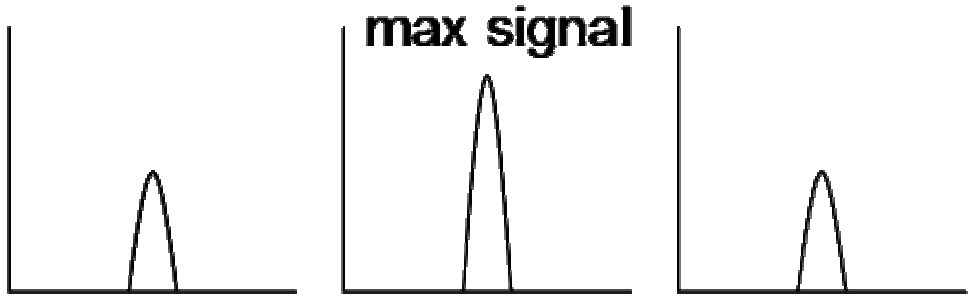
- **Light scattering detection of LIA on LOC**



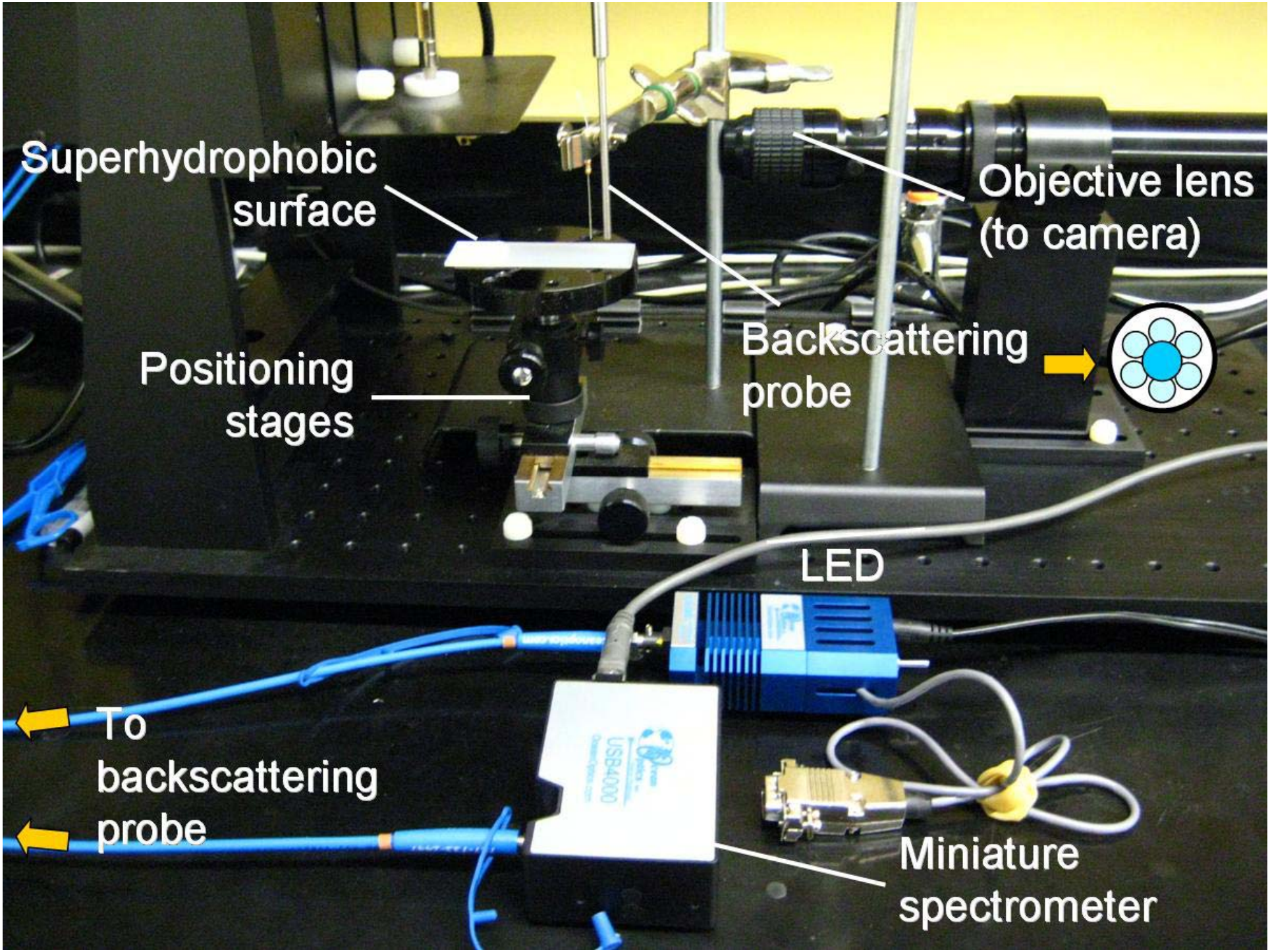
- **Backscattering detection**

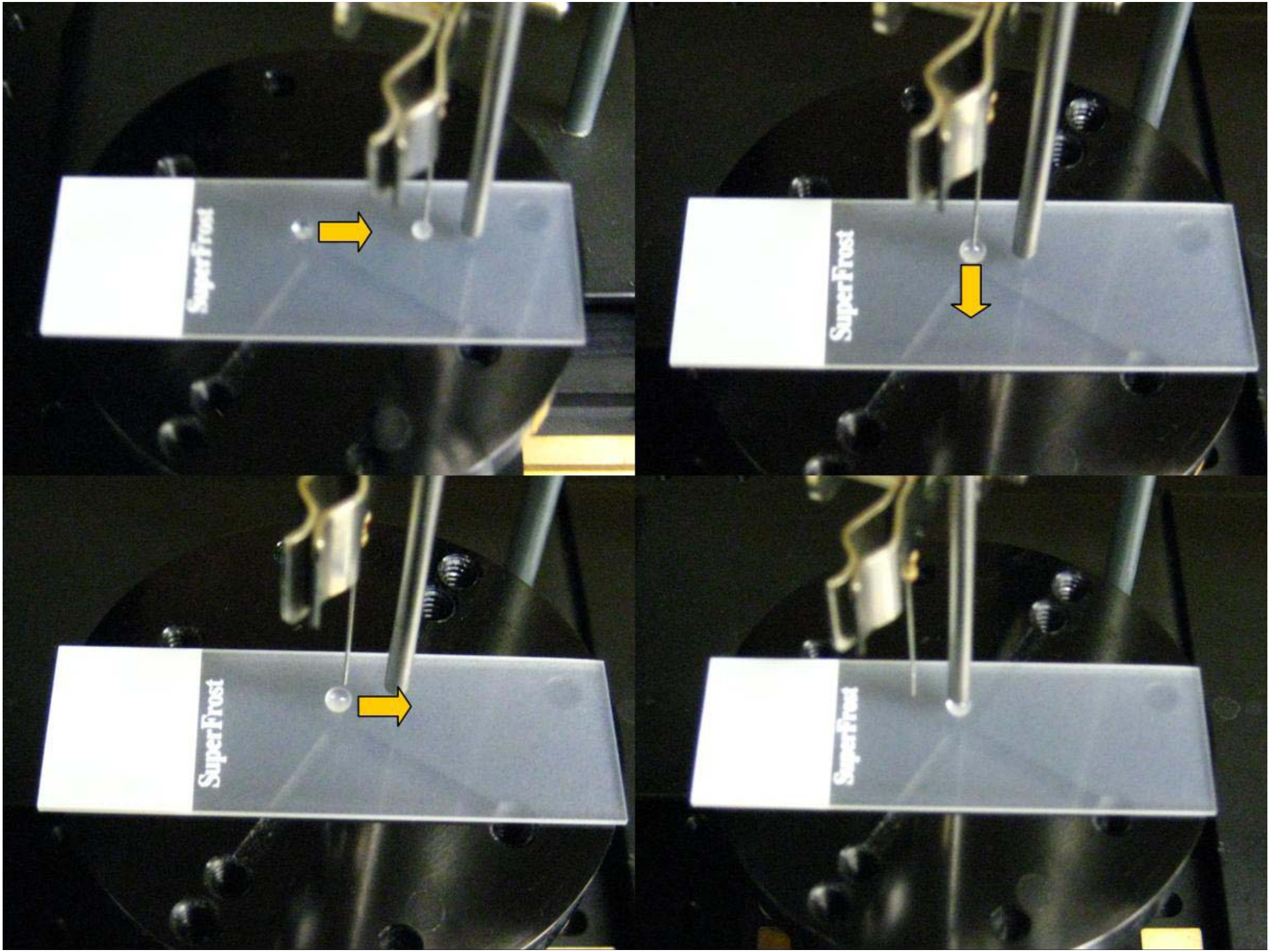


stage movements by computer-controlled stepping motors



spectra as calibrating droplet location over x-axis (repeated over y- and z-axis)


Superhydrophobic surface
Objective lens (to camera)
Backscattering probe
Positioning stages
LED
To backscattering probe
Miniature spectrometer


SuperFrost
SuperFrost
SuperFrost
SuperFrost

• Materials

mIgG (mouse immunoglobulin G)

- Dissolved in 10 mM pH 7.4 PBS
- Serially diluted

BVDV (bovine viral diarrhea virus)

- Cultured in Madin-Darby bovine kidney cells (MDBK) with tissue culture media, containing 5-10% fetal calf serum
- MDBK denatured and washed by centrifuging
- $TCID_{50}$/mL (tissue culture cell infectious dose 50%) was provided by the manufacturer
- Serially diluted

• Materials

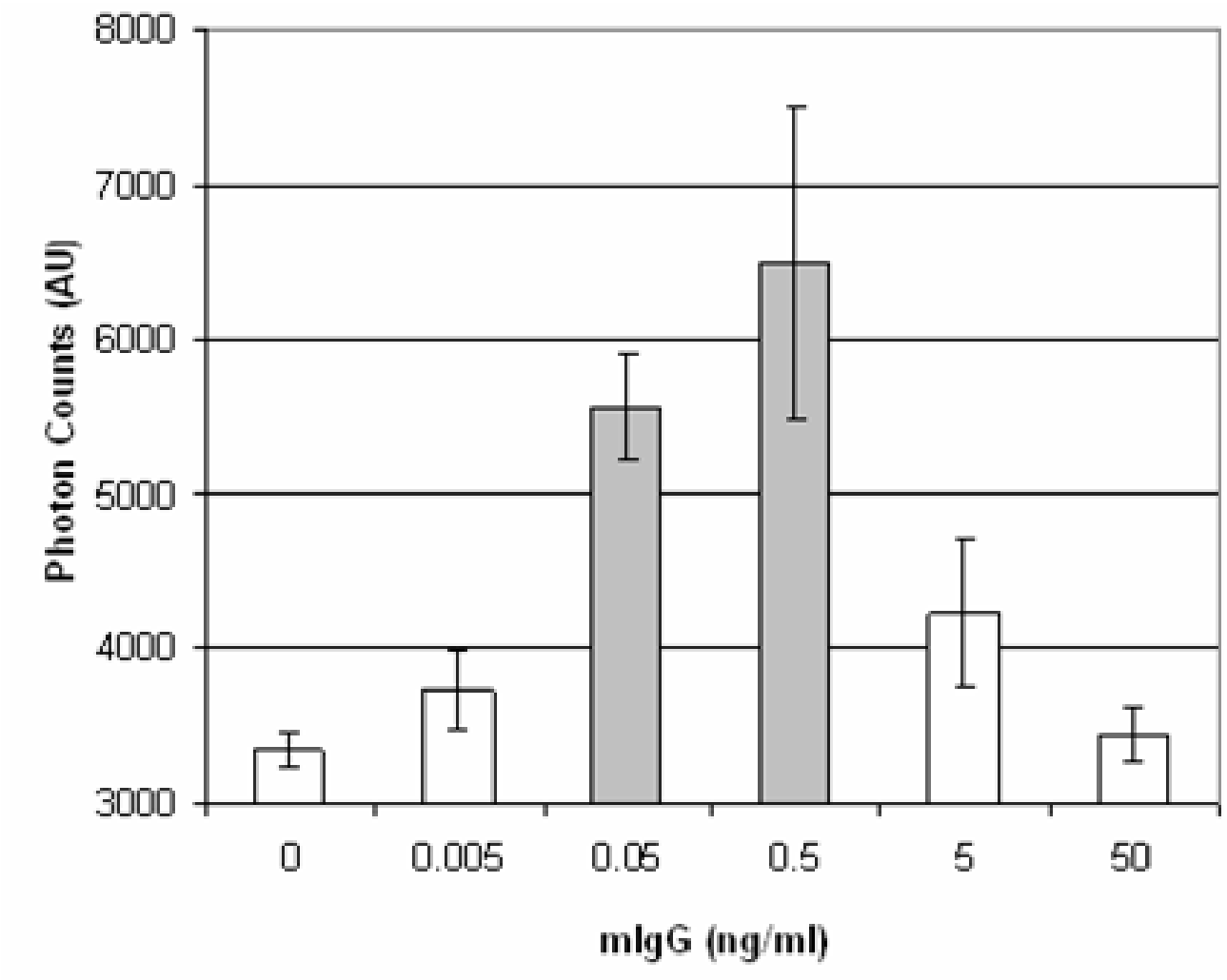
E. coli (*Escherichia coli*)

- Lyophilized E. coli K-12 powder
- Cultured in brain heart infusion broth at 37°C for 20 h
- Plated on eosin methylene blue agar, incubated at 37°C for 20 h
- Colony forming unit (CFU) was evaluated with a light microscope
- Serially diluted

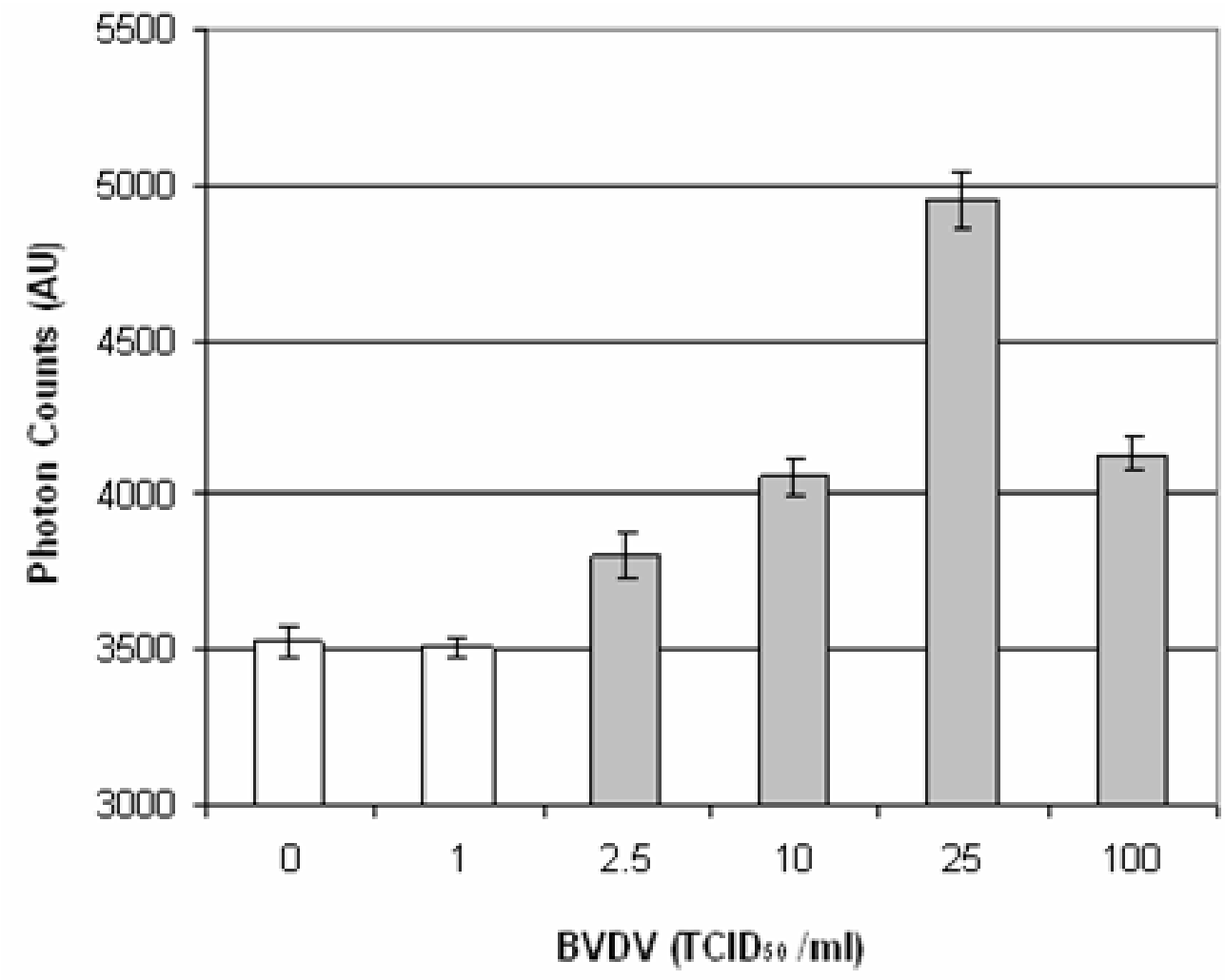
Antibody-conjugated particles

- 920-nm; 10.3 $Å^2$ parking area (highly carboxylated)
- Ab-conjugation: physical adsorption (~33% surface coverage)
- Followed by 2x washing (centrifuge-resuspension)

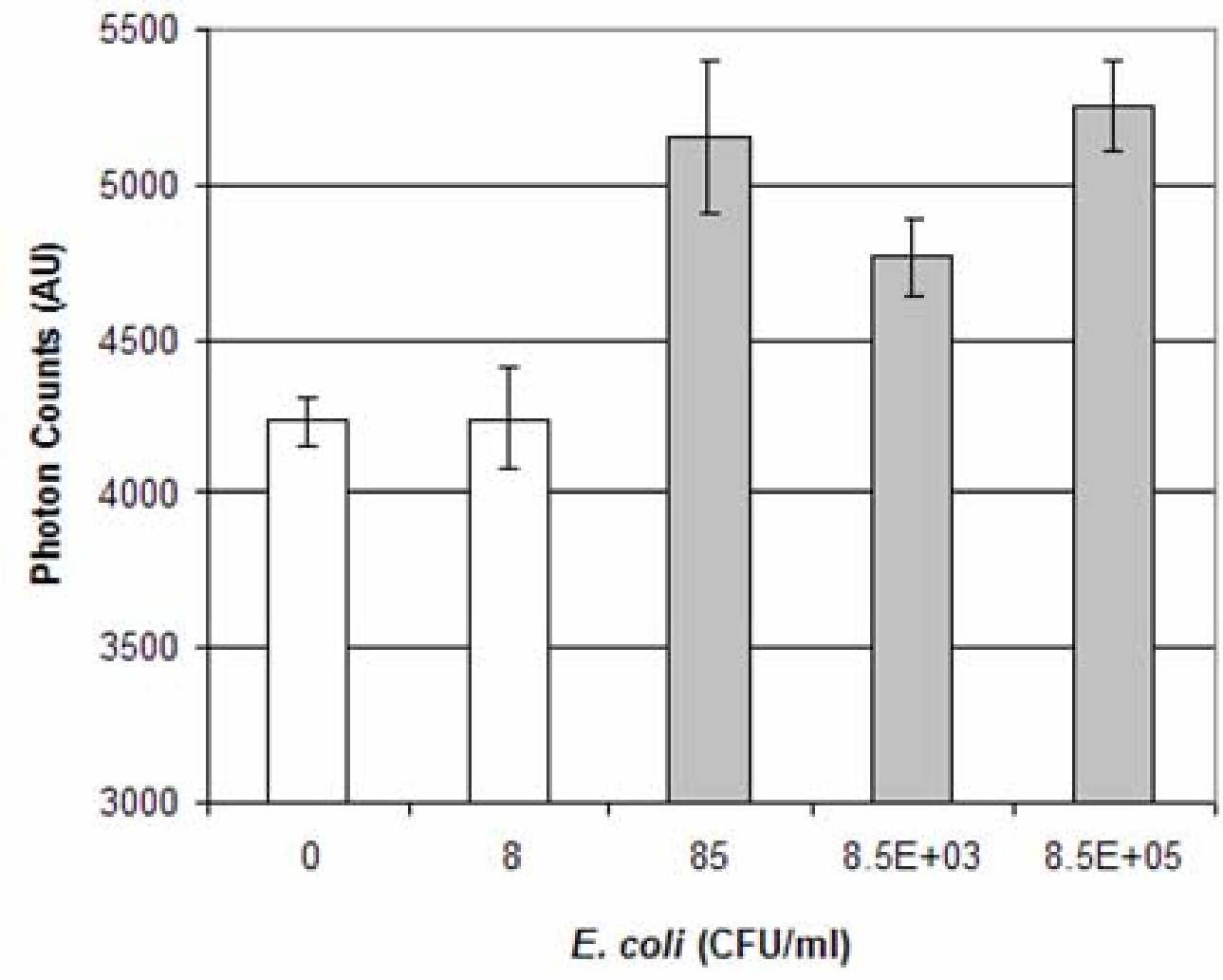
- **Model protein: mouse immunoglobulin G (mIgG)**



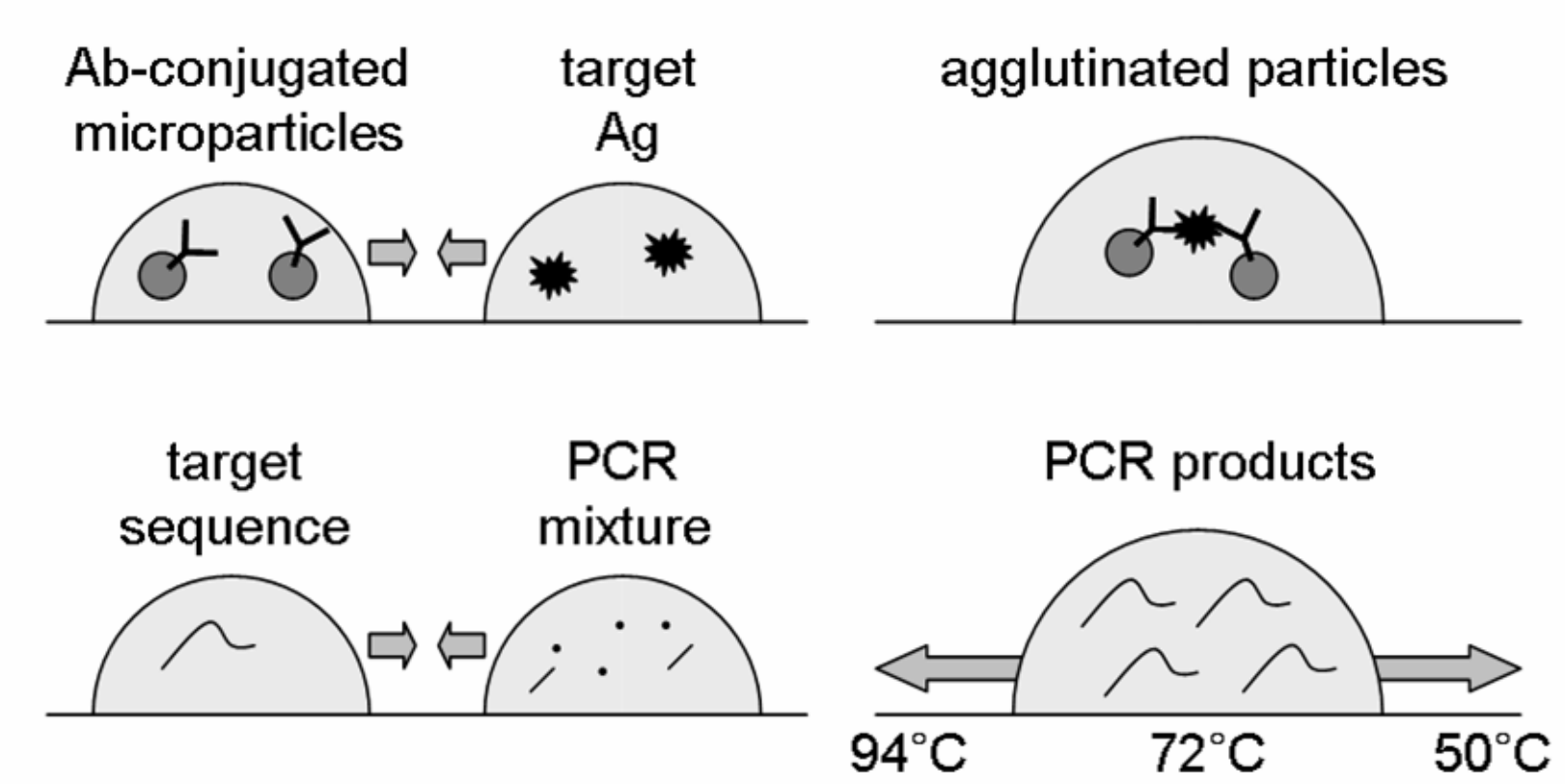
- **Model virus: bovine viral diarrhea virus (BVDV)**



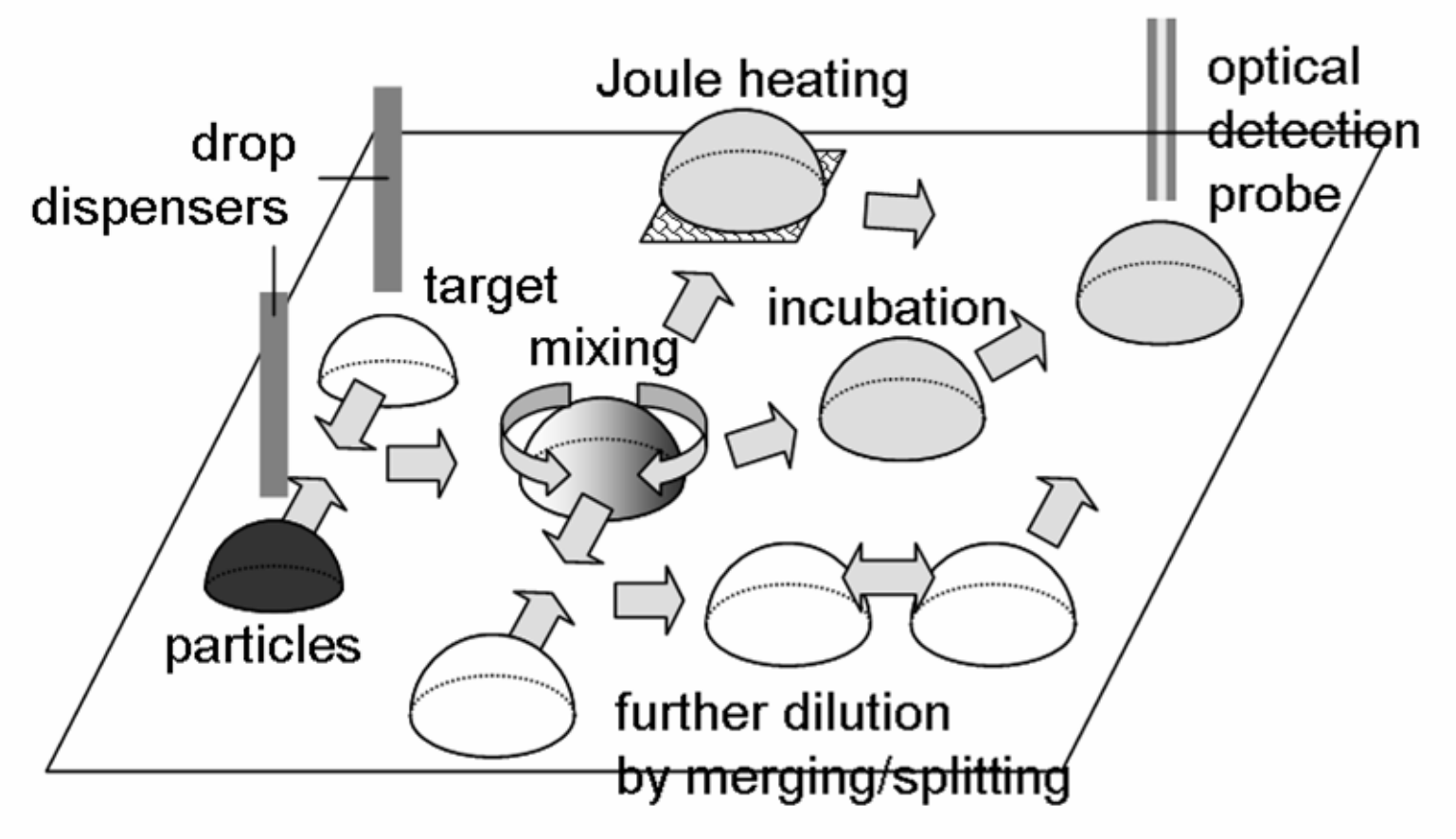
- **Model bacterium:** *Escherichia coli* (*E. coli*)



• Future research: LIA vs PCR



- **Future research: reprogrammable protocols**



• Conclusion

- A simple yet new method of droplet manipulation (or open-surface digital microfluidics) was demonstrated
- Light scattering detection of particle immunoassays were demonstrated on this new platform
- Detection limits are extremely low:

  50 pg/mL for mIgG (model protein)

  2.5 $TCID_{50}$/mL for BVDV (model virus)

  85 CFU/mL for *E. coli* (model bacterium)

• Acknowledgments

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