Applications of AC electrokinetic methods to genomics and proteomics

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ORS Awards Scheme

Outline

- Introduction - electrokinetic particle movement
- Electrokinetics – dielectrophoresis (DEP) of submicron-sized dielectrically polarisable particle in non-uniform electric fields
- Polarisation mechanisms
- DEP basics & many particle movement
- Theoretical predictions/simulations
- Experimental measurements & quantification
- Examples:
  - Latex 216 nm diameter micro-spheres
  - 12 kilo-base pair (~ 4 μm) DNA
- Comparison between theory and experiments
- Applications in genomics/proteomics
- DEP cell separation & other AC electrokinetics

Introduction

- Motivation: controlled, non-contact, movement of sub-micron/nanoscale bio-particles

- AC electrokinetics $\rightarrow$ movement of sub-micron particles using Alternating Current (AC) electric fields

- Sub-micron particles: colloidal suspensions: latex beads (model colloids – can bio-conjugate), viruses, DNA, proteins

- Polarisable particles: exhibit surface charge, or charged groups

- Dielectrophoresis (DEP): dipole induced by non-uniform AC electric fields (Pohl, 1978) $\rightarrow$ movement of a particle

- AC potentials applied to electrode structures micro-fabricated on glass avoid hydrolysis $\rightarrow$ use non-uniform electric fields

- Electrokinetic movement $\rightarrow$ measure polarisability, applications in separation science, genomics & proteomics
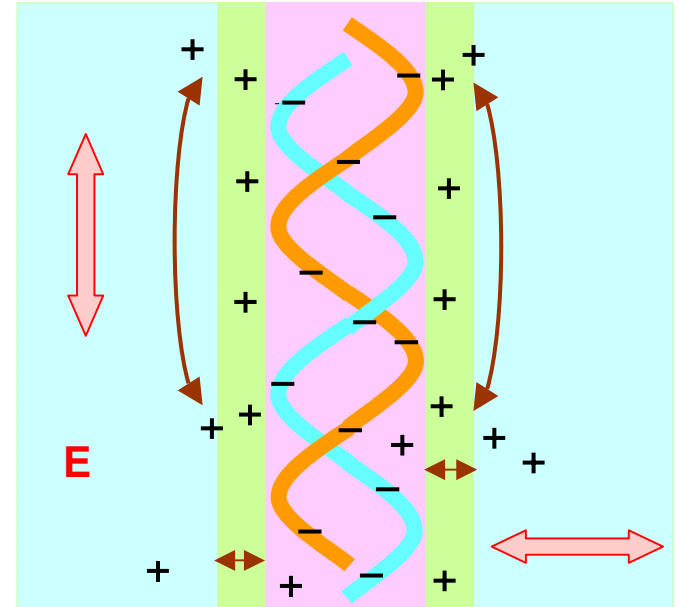
Polarisation mechanisms I

- polarisation – 'intention' of charges to move in response to externally applied electric field
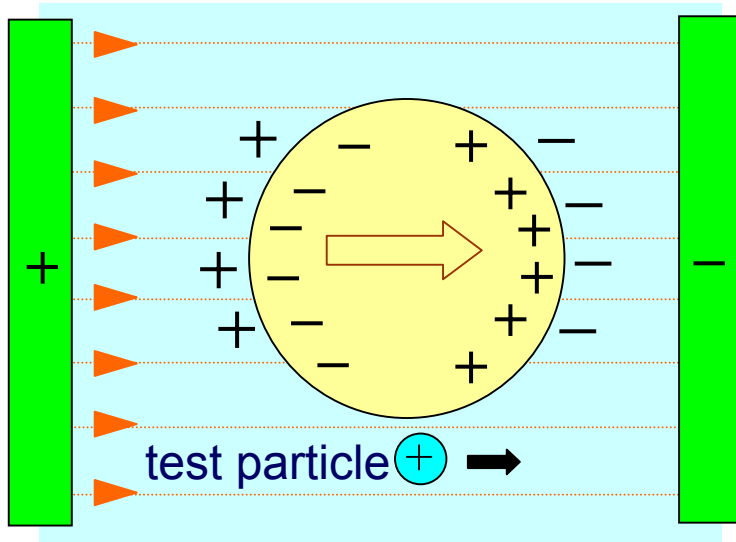
Maxwell-Wagner (M-W) interfacial polarisation

Charge accumulates at interface between dielectric particle & aqueous medium.
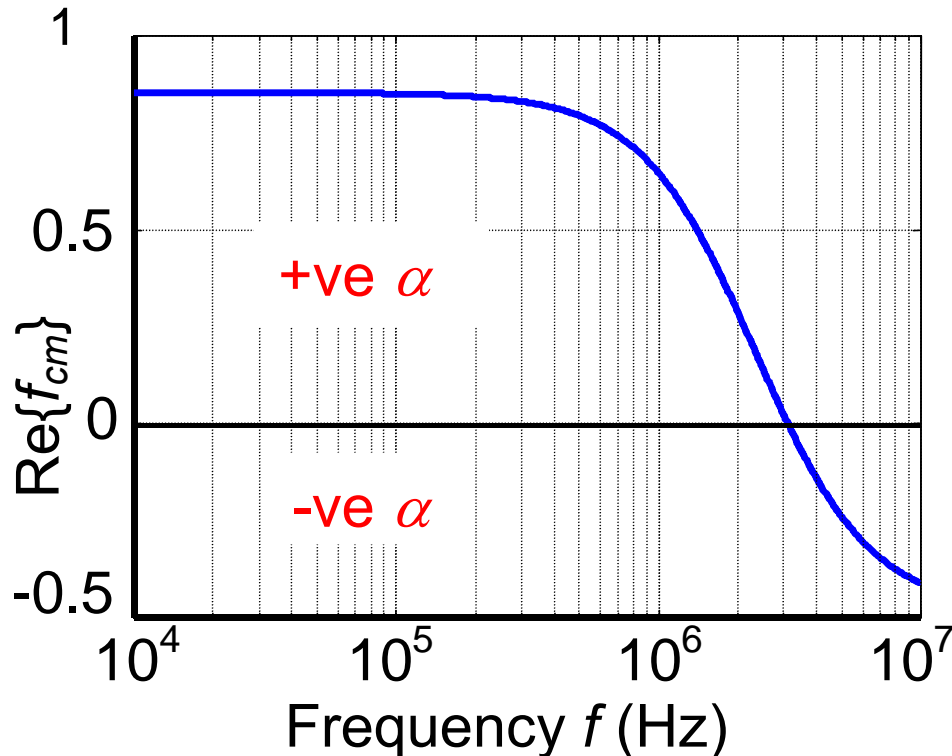
Counterion polarisation

Particle (e.g. DNA) attracts counterions. AC electric field causes tangential & radial movement of counterions.

Polarisation mechanisms II

- Maxwell-Wagner & counterion polarisation mechanisms depend on transit times of charges that respond to electric (E) field changes $\rightarrow$ frequency dependent effective polarisability, $\alpha(f)$

- M-W polarisability:

$$\alpha(\omega) = 3\varepsilon_m \, \mathrm{Re} \underbrace{\{(\underline{\varepsilon}_p^* - \underline{\varepsilon}_m^*)\big/(\underline{\varepsilon}_p^* + 2\underline{\varepsilon}_m^*)\}}_{\text{Claussius-Mossotti } f_{CM}}$$
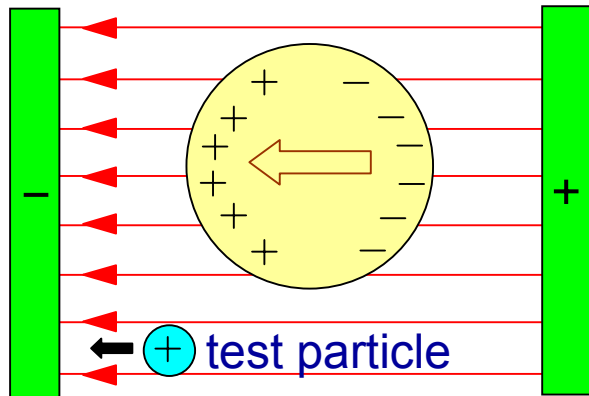
where Re = Real part,

$$\underline{\varepsilon}^* = \varepsilon - j\sigma/\omega, \; \varepsilon = \text{permittivity},$$

$$\sigma = conductivity, p = particle,$$

$$m = medium \; and \; \omega = 2\pi f$$
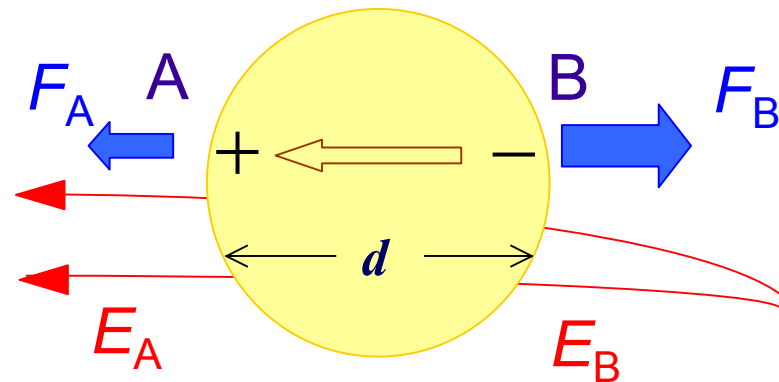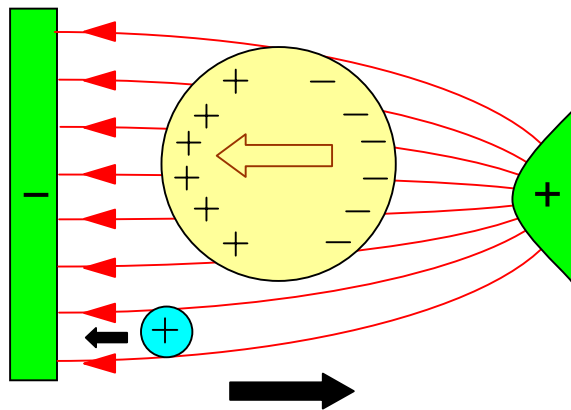

Re{f_{CM}} for 216 nm diameter latex spheres in low σ_m = 1.7 mS m^{-1} changes sign near 3 MHz

Dielectrophoresis (DEP) basics I

Uniform electric (E) field: neutral particle $\rightarrow$ polarises $\rightarrow$ Coulombic forces $\rightarrow$ zero net force on body

Non-uniform E field: Imbalance of Coulombic forces at A & B $\rightarrow$ neutral body moves ✓ (+ve DEP)

test particle

F_A A   B F_B

E_A E_B

d

induced (or effective) dipole moment (C m)

$$\vec{F}_{particle} = q\vec{E}_A - q\vec{E}_B = q\left(E_B - E_A\right) = q\left(E(x+d) - E(x)\right) \cong \overbrace{qd}\frac{dE}{dx}$$
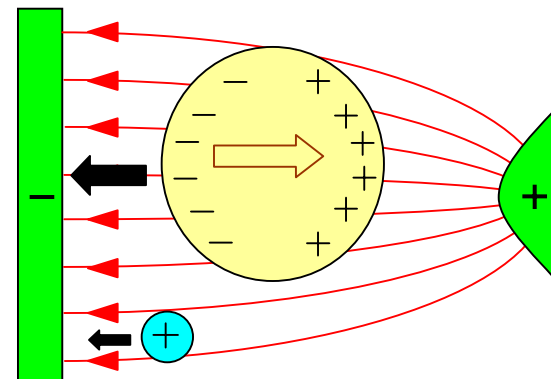
Dielectrophoresis (DEP) basics II

$$\vec{F}_{DEP}(\underline{x},t) = \left(\vec{p}(\underline{x},t) \cdot \vec{\nabla} \right) \vec{E}(\underline{x},t), \ \vec{p}(\underline{x},t) = \alpha v \vec{E}(\underline{x},t) \ \& \ \vec{\nabla} = \text{grad}$$

induced dipole moment p $\quad \alpha$ effective dipole moment per unit volume & E

- α (or induced polarisability) depends on dielectric properties of particle & medium: $\alpha = \pm \rightarrow$ can change direction of movement
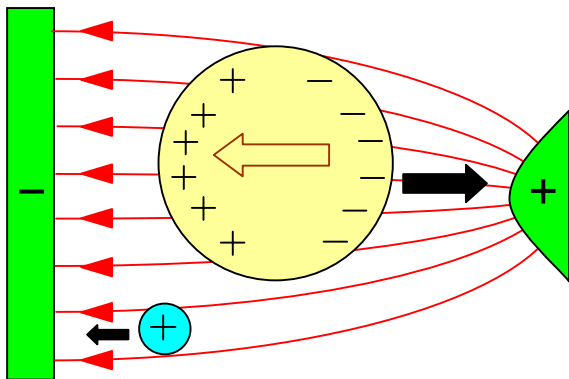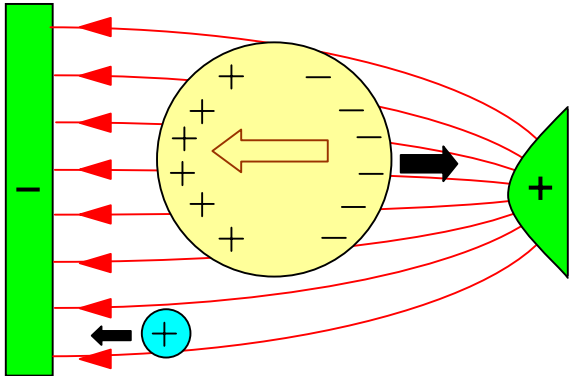

Positive DEP: movement towards high field regions
particle α **>** medium α

Negative DEP: movement away from high field regions
particle α **<** medium α

DEP basics III

- *E* field *induces* & *interacts* with dipole $\rightarrow E^2$

Non-uniform field $\rightarrow$ net force $\rightarrow$ movement to left (for +ve DEP)
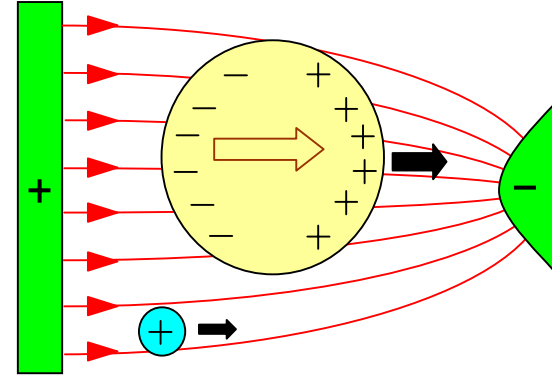
E field reversed – particle movement in same direction

$$\sim E \rightarrow \vec{F}_{DEP}(\underline{x}) = \left\langle \frac{1}{2}\alpha v \vec{\nabla}\left|\vec{E}(\underline{x})\right|^2 \cos^2(\omega t) \right\rangle_{\sim t_{\text{av.}}} = \frac{1}{4}\alpha v \vec{\nabla}\left|\vec{E}(\underline{x})\right|^2$$

- Small-time average movement $\propto E^2$ squared (i.e. *not* dependent of *E* field direction & electrode polarity) $\rightarrow$ use AC
- AC (> 10 kHz) – avoid hydrolysis – microchip application ✓

Modelling DEP particle movement

- Explore DEP particle movement - single particle or ensemble
- Sub-micron size particles (bio-conjugated colloids, DNA, proteins, viruses, etc) $\rightarrow$ Brownian motion (diffusion)
- Ignore inertia, gravity & buoyancy forces
- Modified diffusion (or Fokker Planck) equation (FPE) describes space-time evolution of particle concentration c

flux

Boltzmann temp. ✓

$$\frac{\partial c(\underline{x},t)}{\partial t} = -\vec{\nabla}.(\overbrace{\vec{J}_{DEP} + \vec{J}_{diff}}) = -\frac{1}{\zeta}\vec{\nabla}.\left(c(\underline{x},t)\vec{F}_{DEP}(\underline{x})\right) + \frac{\overbrace{k_B T}}{\zeta}\nabla^2 c(\underline{x},t) \quad (1)$$

Solve Laplace's eqn
$\rightarrow$ array geometry

Stokes' drag ✓

diffusion ✓

$$\vec{F}_{DEP}(\underline{x}) = \frac{1}{4}\alpha(\omega)v k(\underline{x})V_0^2 \quad (2) \qquad n_p(t) = \int\limits_{Vol} c(\underline{x},t)d\,\underline{x} \quad (3)$$

+ve ✓ ✓

- Solve (1) - (3) $\rightarrow$ predict time-dependent DEP collections

Positive DEP particle collections I
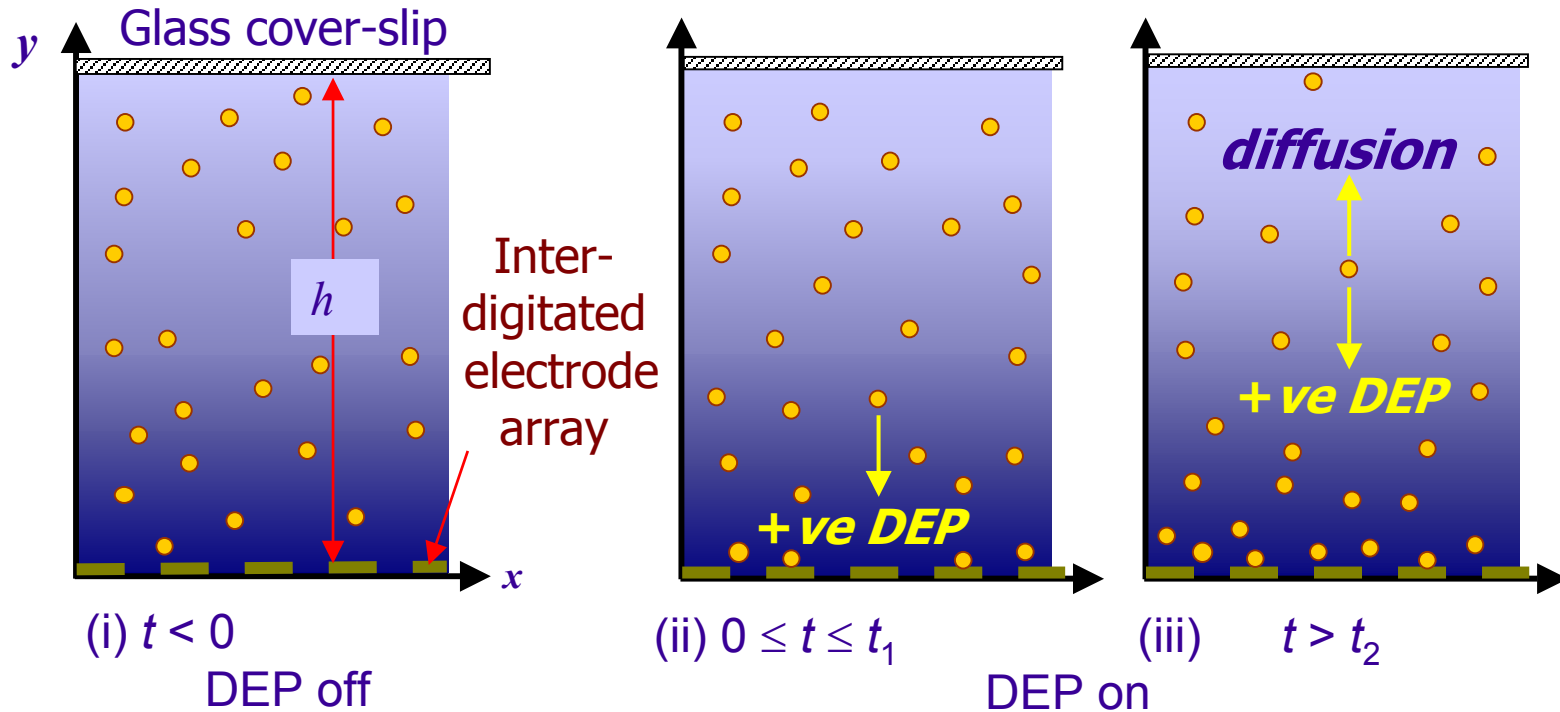
- **Key parameters for measuring +ve DEP particle collection**
  - initial DEP collection rate, d$n(0)$/dt - consider only DEP flux
  - initial to steady-state transition, $\Delta n = n(\infty) - n(0)$ - DEP & diffusion particle flux balance as $t \rightarrow \infty$
  - rise time τ

Positive DEP particle collections II

- Numerically solve (1) – (3) utilizing array geometric symmetry
- Time-dependent diffusion-limited DEP collections simulated predicted by FPE system

Particle number $n(t)$ (or fluorescence $F(t)$)

Initial DEP collection rate $\dot{F}(0)$

Fluorescence $F(t)$ essentially at steady state

Initial-to-steady state fluorescence change
$\Delta F = F(\infty) - F(0) \cong F(t >> t_2) - F(0)$

0 t_1 t_2 Time t (s)
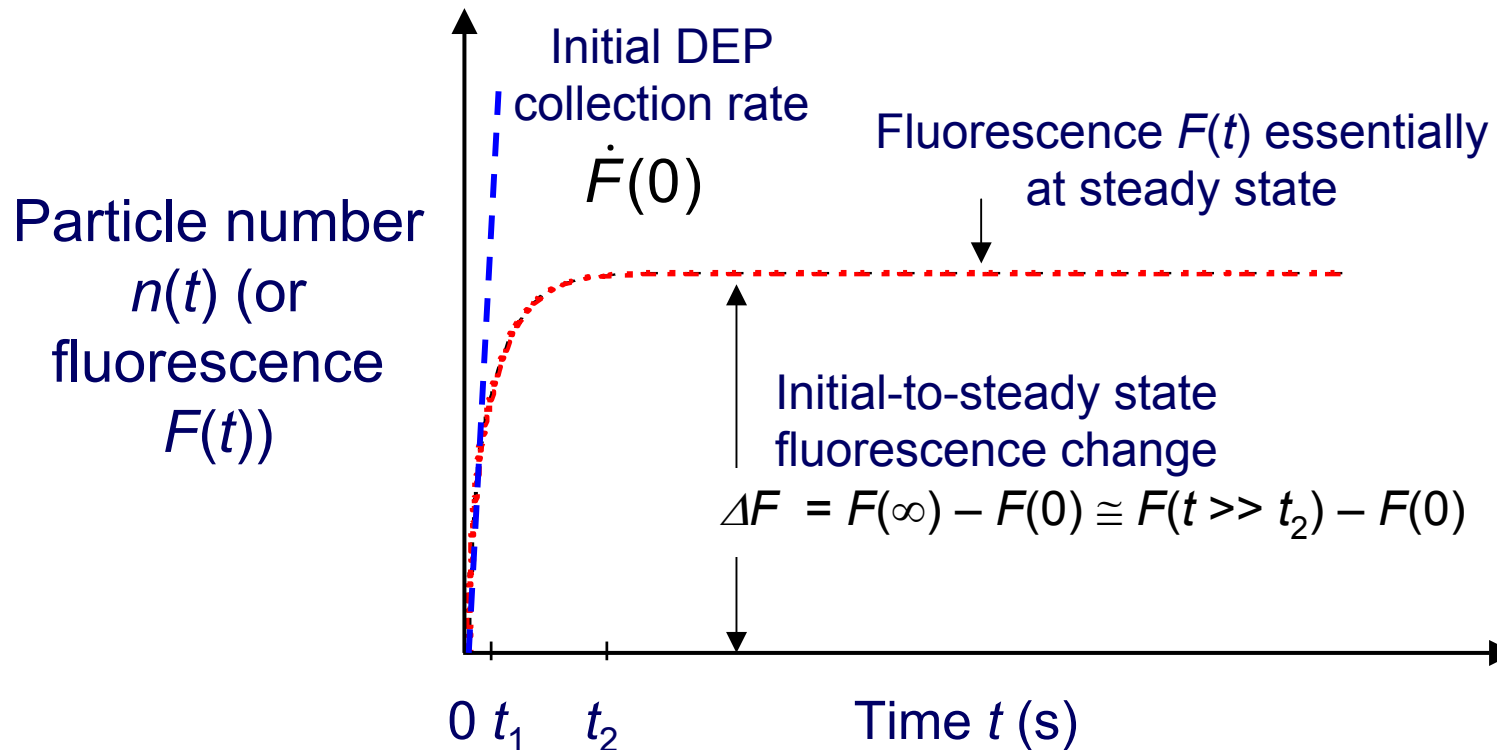
- Compile *predicted* collection time-profiles for 216 nm diameter latex beads for range of frequencies & voltages

Predicted +ve DEP collections

- Latex microspheres (beads) – model colloids e.g. 216 nm diameter
- can bio-conjugate – use for investigations in molecular biology

Trends:

$n_p \uparrow$ as $V_o \uparrow$

$n_p \downarrow$ as $f \uparrow$

or $(\alpha \downarrow)$

recall $\alpha \cong 0$

@ f = 3 MHz

Predicted particle collection n_p (a.u.)

Simulated DEP particle collection

Simulated particle relaxation by diffusion (DEP off)

V_o = 1.2 V, f = 2.0 MHz

V_o = 0.6 V, f = 500 kHz
V_o = 0.6 V, f = 1.0 MHz

V_o = 0.6 V, f = 2.0 MHz
V_o = 0.3 V, f = 2.0 MHz

Time t (s)
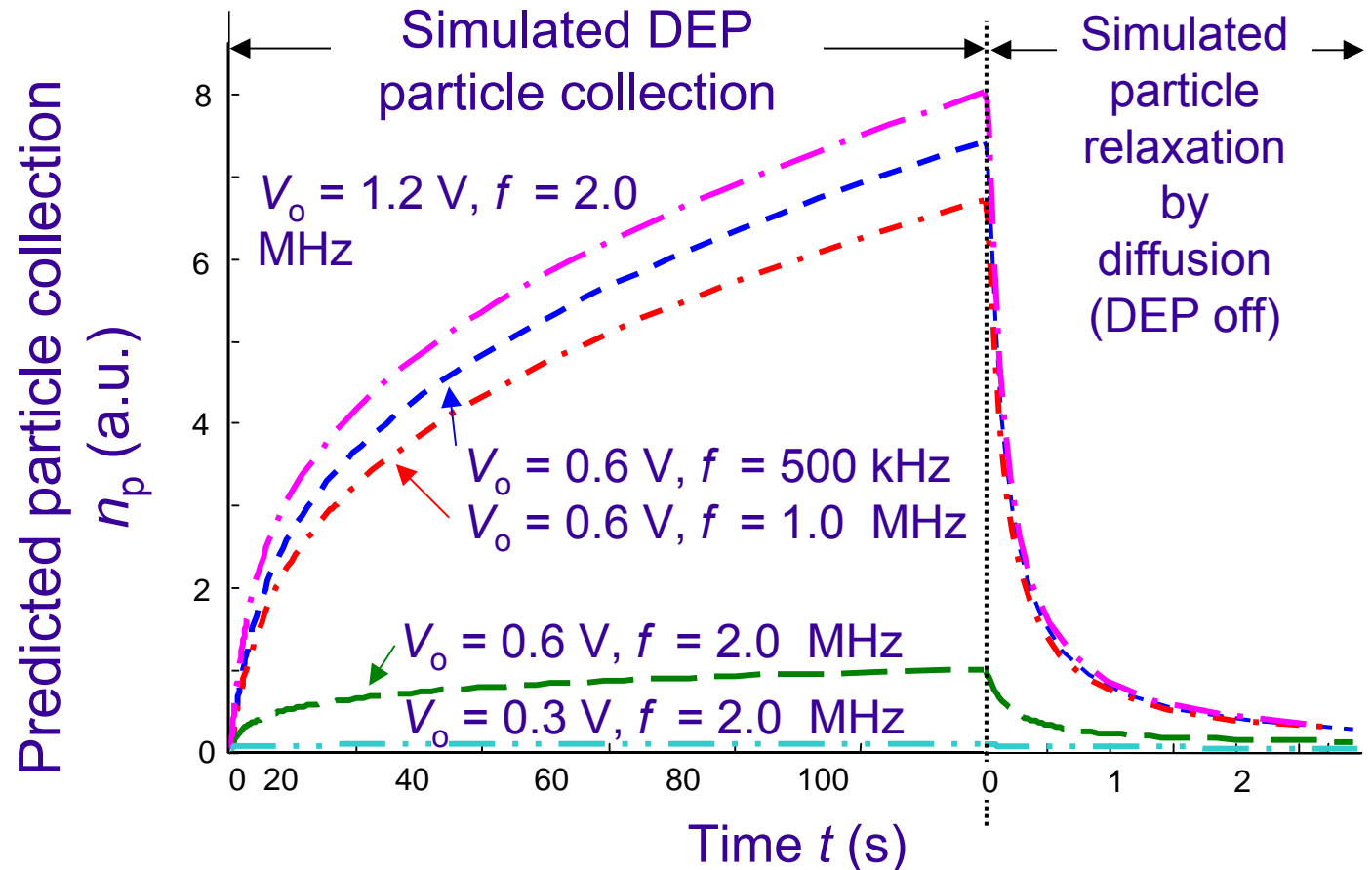
- Compare with *measured* time-dependent DEP collection/relaxation experiments using fluorescence microscopy

DEP experiments I

- DEP collection of DNA onto interdigitated electrodes microfabricated using standard photolithography

UV

Patterned mask

resist coating

Glass slide

Vertical, y

Transverse *x*

Longitudinal, z

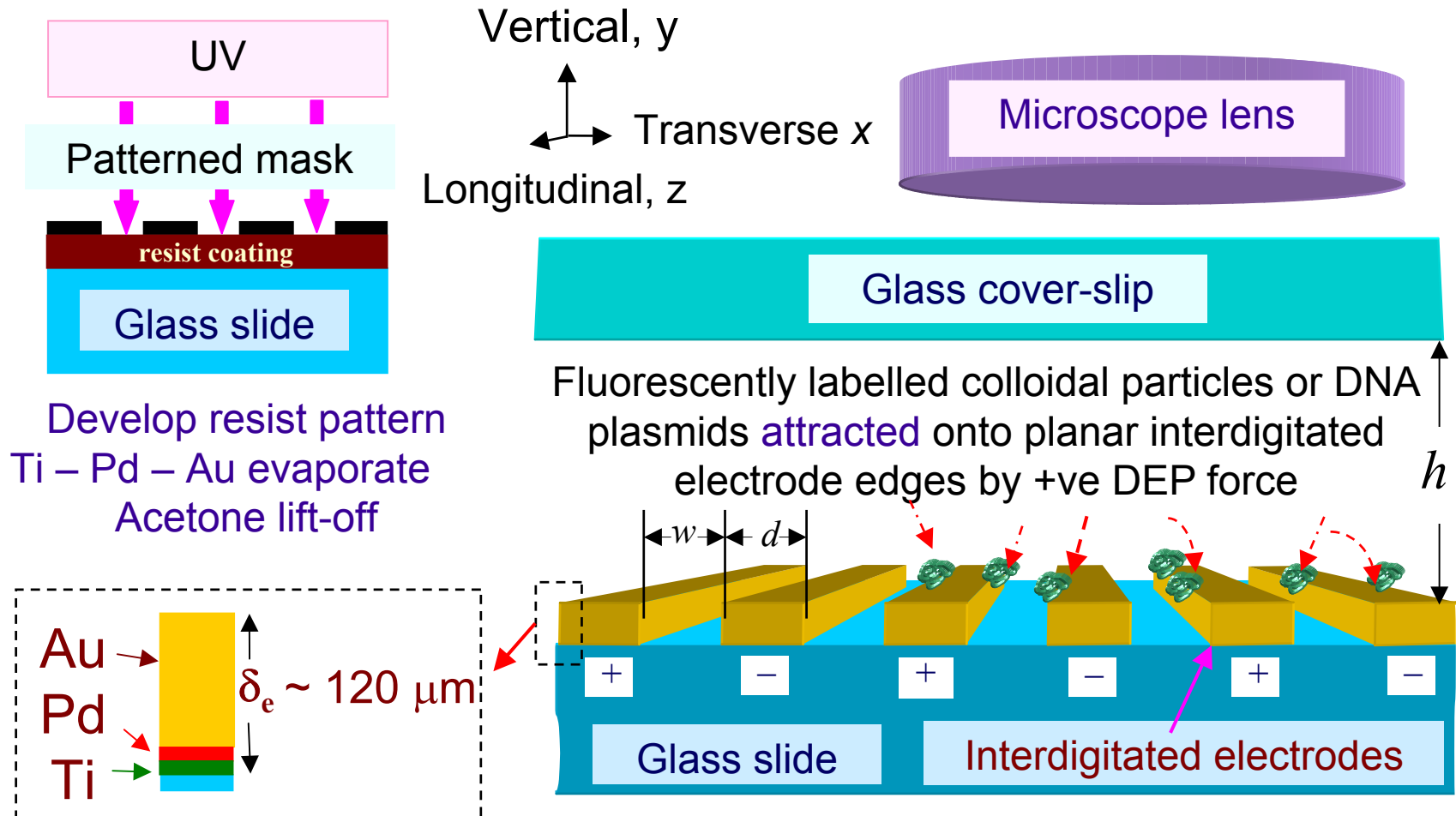Microscope lens

Glass cover-slip

Fluorescently labelled colloidal particles or DNA plasmids attracted onto planar interdigitated electrode edges by +ve DEP force

h

Develop resist pattern
Ti – Pd – Au evaporate
Acetone lift-off

Au
Pd
Ti

$\delta_e \sim 120\ \mu m$

w d

+   −   +   −   +   −

Glass slide

Interdigitated electrodes

DEP experiments II

AC signal generator & amplifier

Oscilloscope

VCR

VC

TV

Micro-scope

Microfabricated separately addressable interdigitated electrode array on PCB

~ 10 μl solution micro-pipetted on electrode

180°   0°
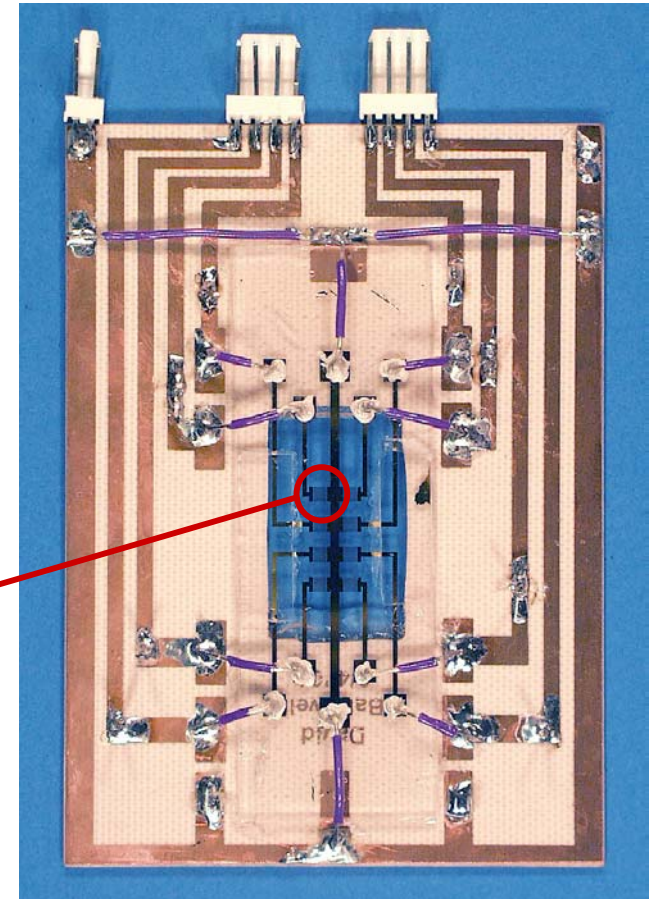
DEP experiments: latex bead example

(a) DEP off — 10 μm width, 216 diameter nm latex beads suspended in solution, Longitudinal, z, Transverse, x

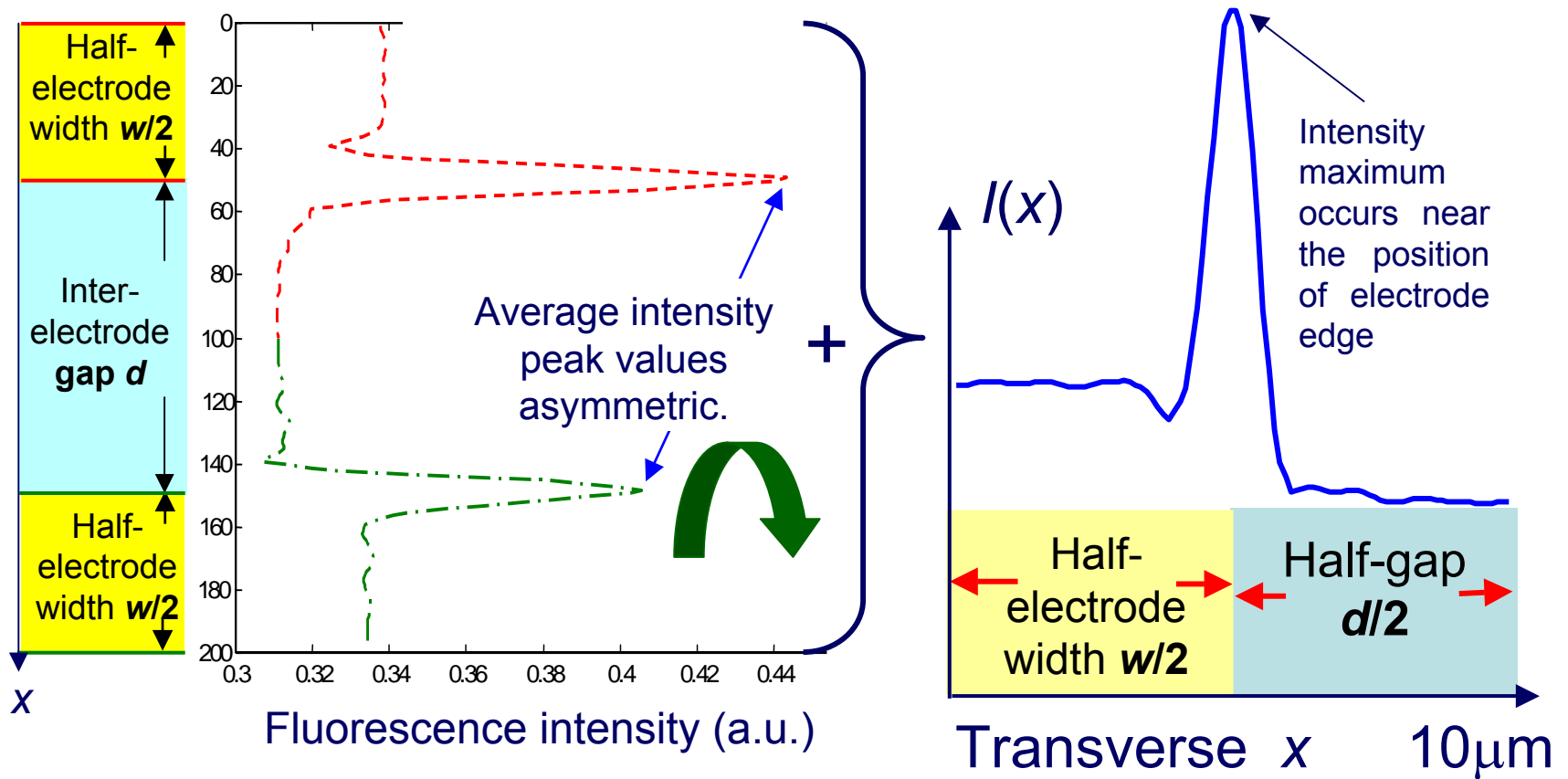**(b) DEP on** — 10 μm gap, 216 nm latex beads collect onto electrode edges, 50 μm

(c) $I(x)$, X
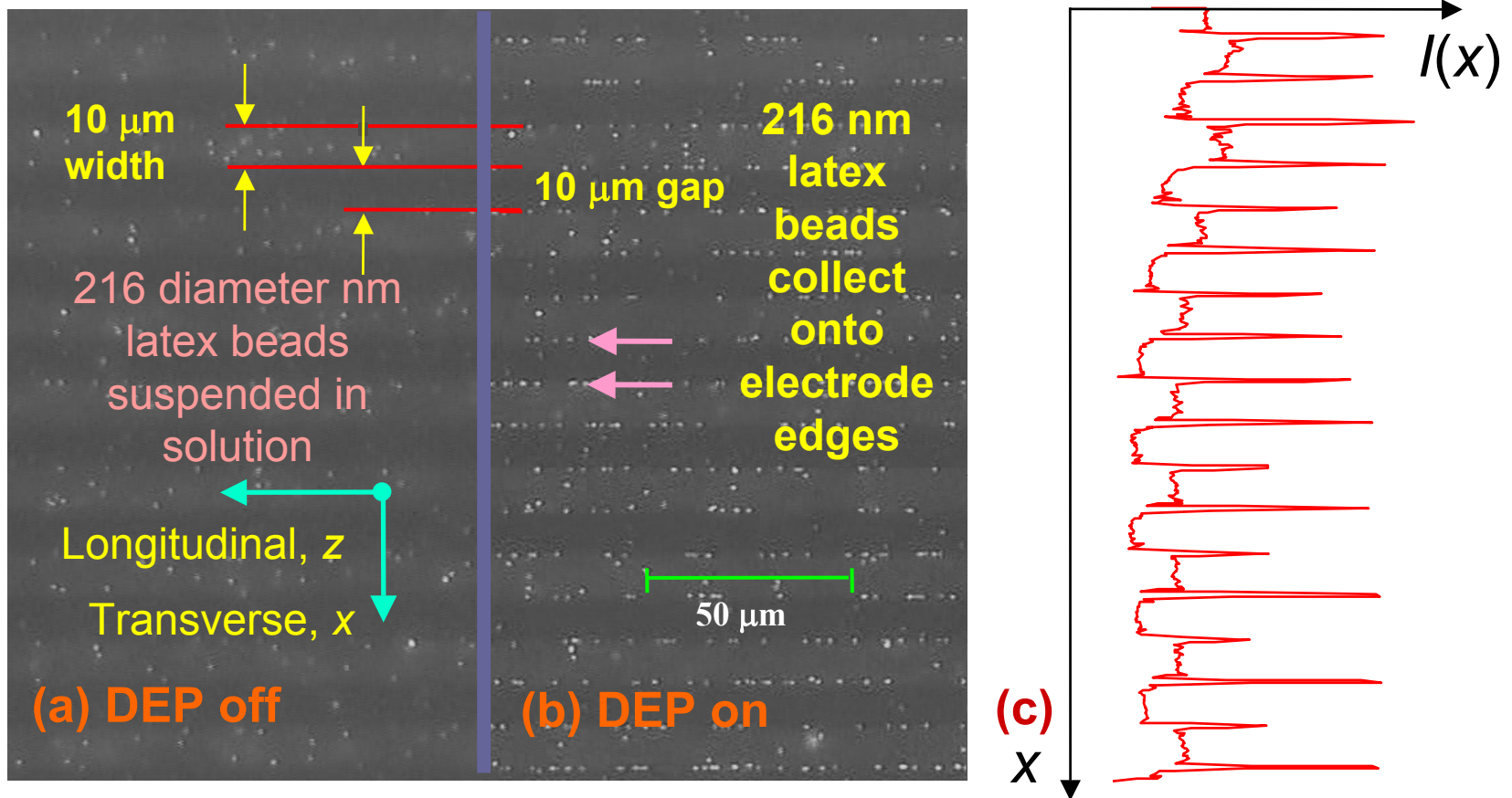
Positive DEP collection of 216 nm diameter latex beads onto $d = w = 10$ μm interdigitated electrodes (a) ~ 1 second before DEP force applied (b) ~ 5 seconds after DEP force applied

Typical fluorescence intensity $I(x)$ 'snapshot' of DEP collections – longitudinal average
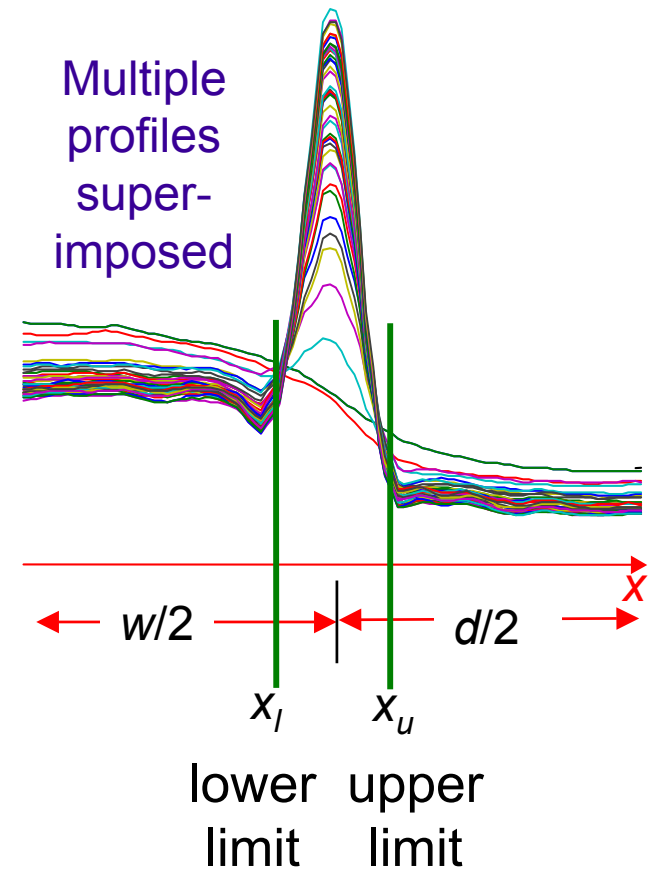
Quantifying DEP collections I

- Periodic average along transverse, x, $\rightarrow$ w/2 + d + w/2
- Reflection, + $\rightarrow$ 'characteristic' *w*/2 + *d*/2 intensity

Half-electrode width **w/2**

Inter-electrode **gap d**

Half-electrode width **w/2**

x

Fluorescence intensity (a.u.)

Average intensity peak values asymmetric.

+

$I(x)$

Intensity maximum occurs near the position of electrode edge

Half-electrode width **w/2**

Half-gap **d/2**

Transverse *x*   10µm

Quantifying DEP collections II

- Frame sequence of characteristic intensities $\rightarrow I(x,t)$
- Transverse integration between selected lower & upper limits $\rightarrow I_E(t)$
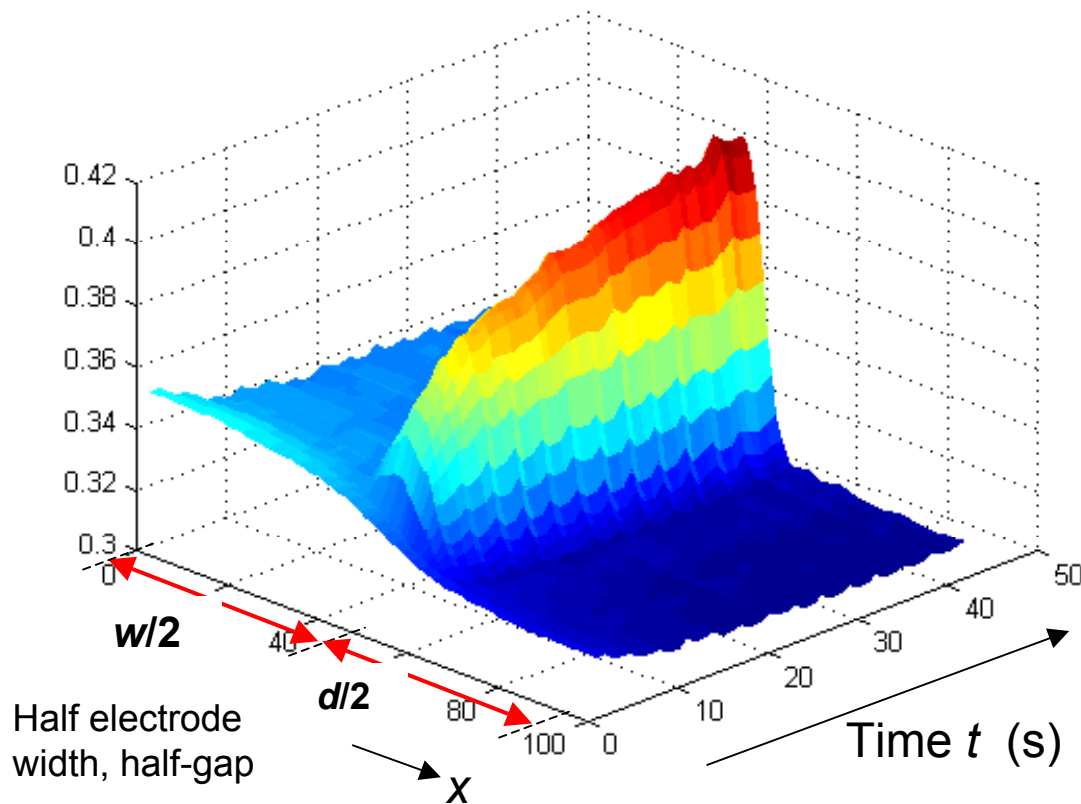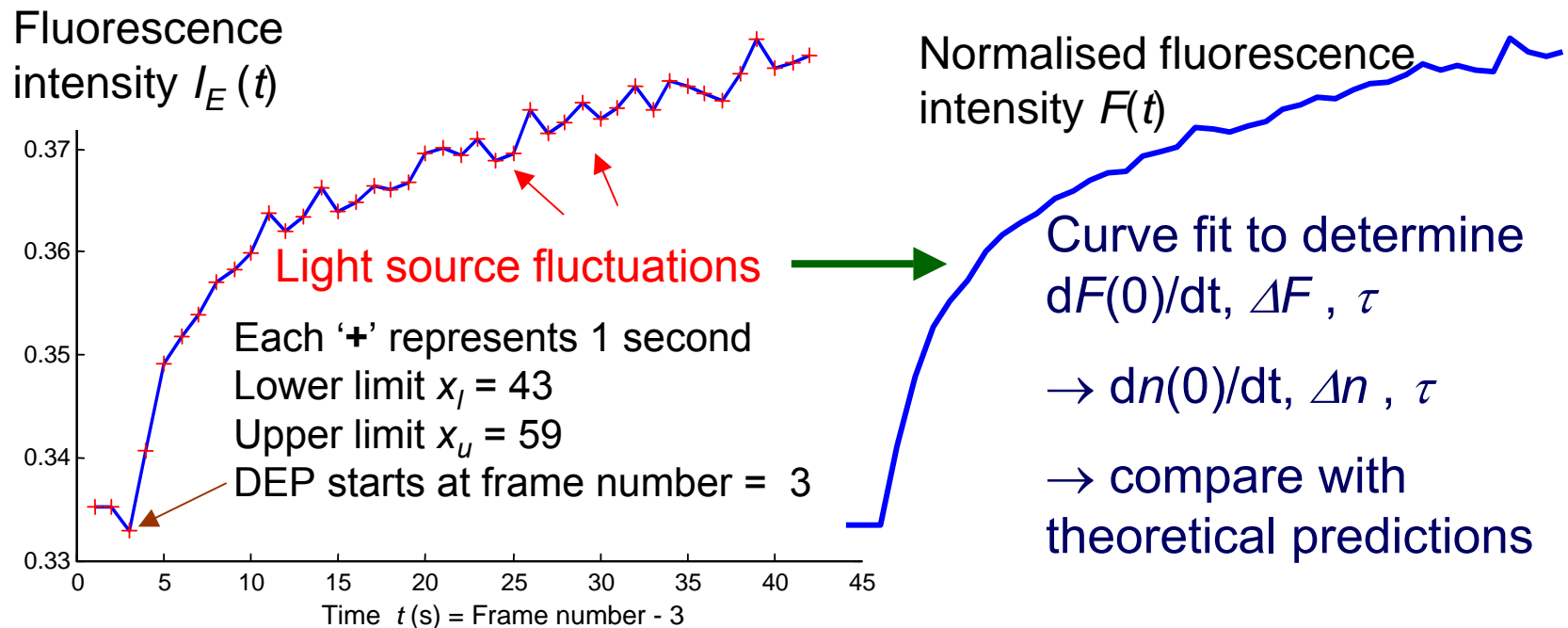
Quantifying DEP collections III

- Normalise $I_E(t)$ to smooth $\rightarrow F(t) = I_E(t)/I_T(t)$

Fluorescence intensity $I_E(t)$

Normalised fluorescence intensity $F(t)$

Light source fluctuations

Each '+' represents 1 second
Lower limit $x_l = 43$
Upper limit $x_u = 59$
DEP starts at frame number = 3

Time t (s) = Frame number - 3

Curve fit to determine $dF(0)/dt$, ΔF, τ

$\rightarrow dn(0)/dt$, Δn, τ
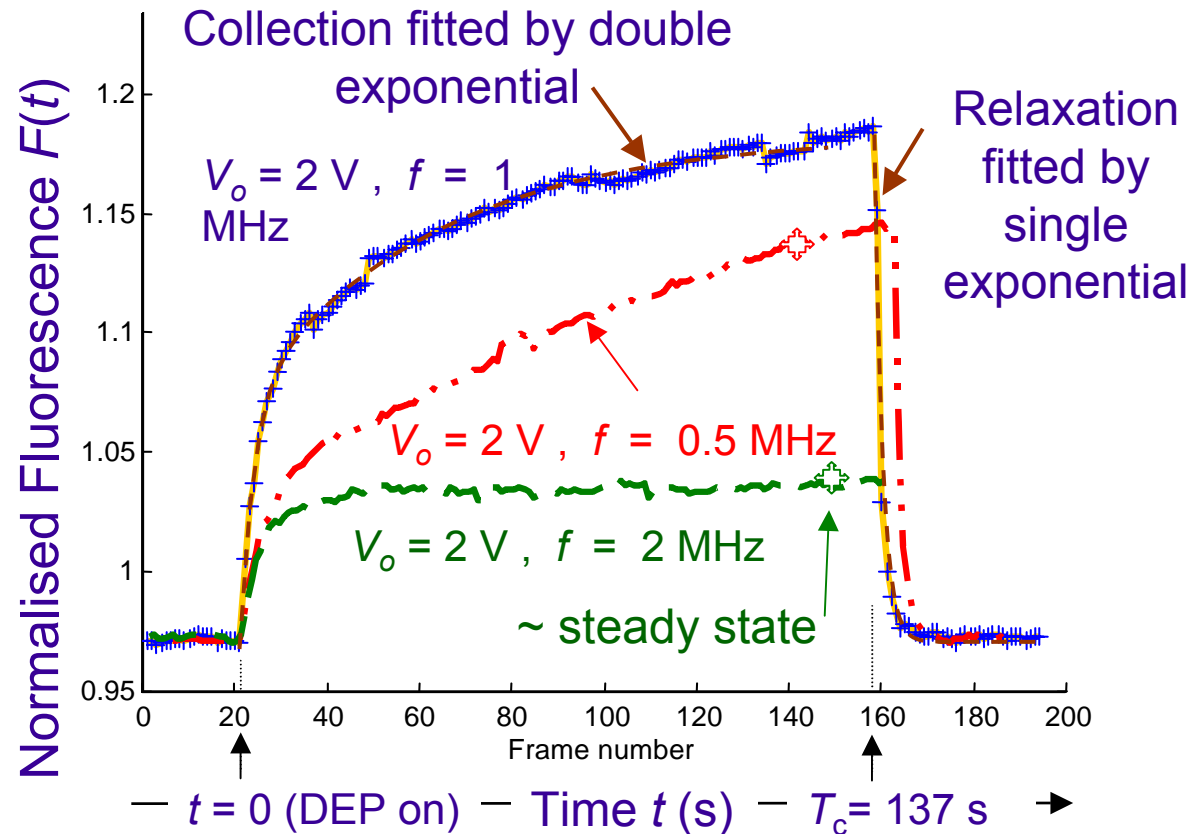
$\rightarrow$ compare with theoretical predictions

Details of image processing method based on fluorescence microscopy & using geometrical properties of interdigitated electrode arrays is given in:
Bakewell, D J & Morgan, H (2001) and (2004)

Quantifying DEP collections IV

- Collections & relaxation of 216 nm diameter microspheres

Collection profiles exhibit variation – need ≥ 3 replicates for each V_o & f

Collection fitted by double exponential

Relaxation fitted by single exponential

$V_o = 2$ V , $f = 1$ MHz

$V_o = 2$ V , $f = 0.5$ MHz

$V_o = 2$ V , $f = 2$ MHz

~ steady state

Normalised Fluorescence $F(t)$

Frame number

$t = 0$ (DEP on) — Time t (s) — $T_c = 137$ s

- To compare experiment with simulation: $\dot{F}(0)$ & ΔF $\rightarrow$ $\dfrac{\dot{n}_e(0)}{n_e(0)}$ & $\dfrac{\Delta n_e(120)}{n_e(0)}$

Latex microspheres I: theory vs experiment

f (MHz)	Experiment			2-D FPE simulation			
	V_{o_e} (V)	$\dfrac{\dot{n}_e(0)}{n_e(0)}$	$\dfrac{\Delta n_e(120)}{n_e(0)}$	V_o (V)	Re$\{f_{CM}\}$	$\dfrac{\dot{n}_p(0)}{n_p(0)}$	$\dfrac{\Delta n_p(120)}{n_p(0)}$
0.5	2	9.4	58	0.6	0.74	13	150
1	2	8.9	48	0.6	0.64	11	130
2	2	4.6	29	0.6	0.38	5.1	19
2	4	18	62	1.2	0.38	20	160
2	2	4.1	32	0.6	0.38	5.1	19
2	1	1.6	4.2	0.3	0.38	1.3	1.0

Comparisons between theory and experiment for DEP collections of 216 nm diameter latex beads
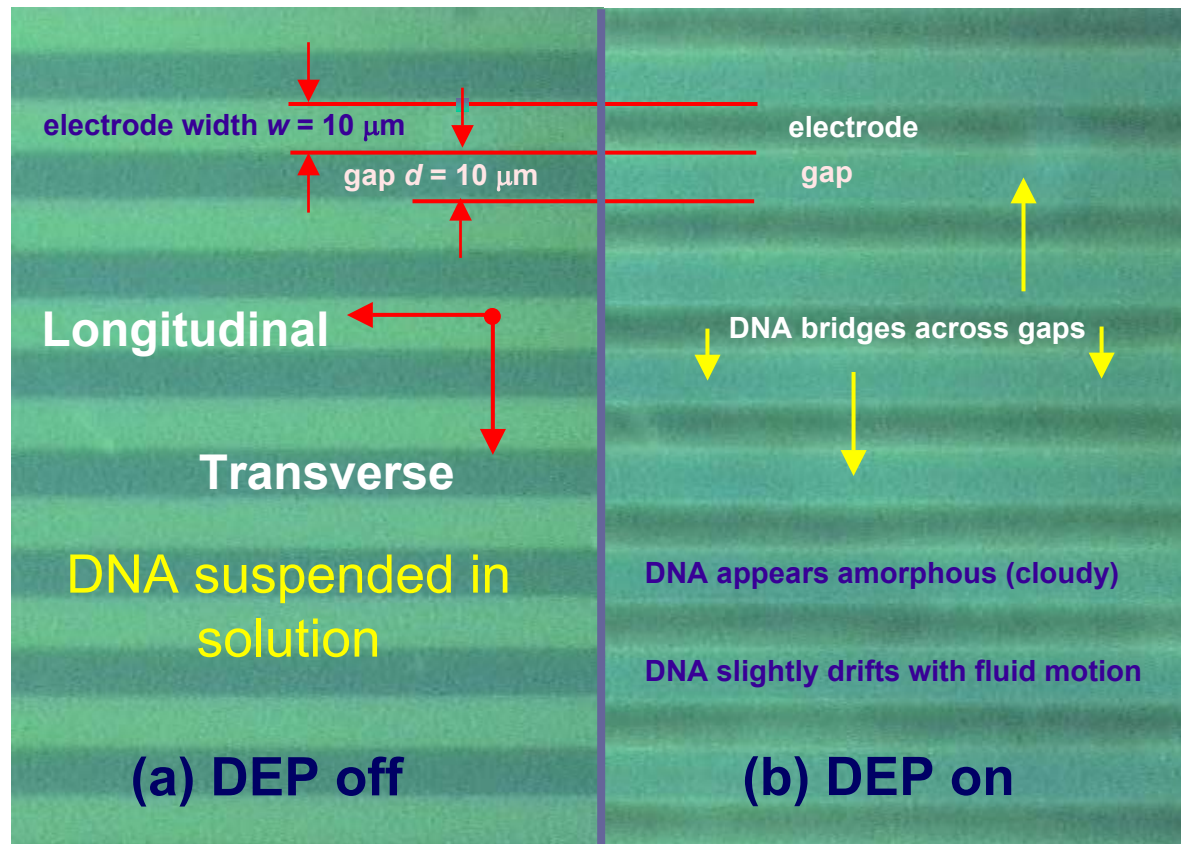
- Theory & experiment concur qualitatively
- Using 3 replicates: d$n(0)$/dt & $\Delta n \downarrow$ as $f \uparrow$ for FPE model and experiment $\Rightarrow$ polarisability $\downarrow$ - concurs with Re$\{f_{CM}\}$ trend
- Significantly lower V_o required in simulations than experiment
- Theory & experiment concur better for d$n(0)$/dt than Δn

Latex microspheres II: theory vs experiment
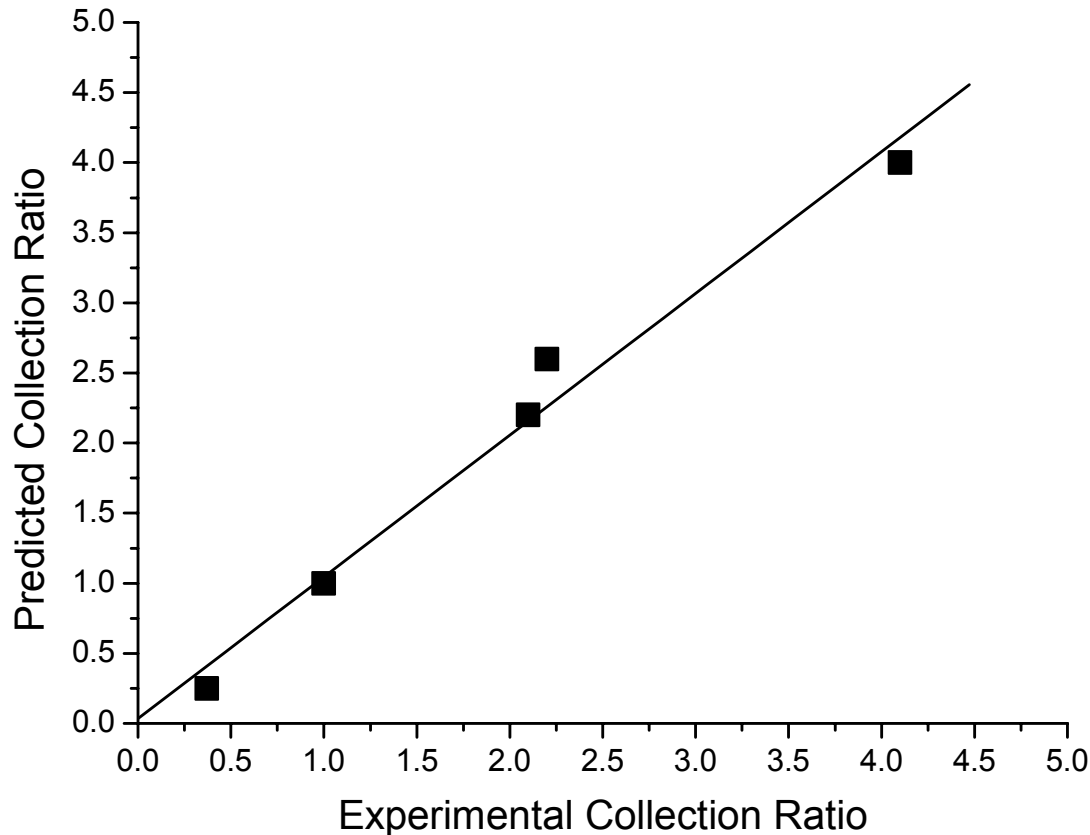
- ~ 1 correlation for $dn(0)/dt$ ratios between theory and experiment
- Fluid motion around (and above) electrode edges confounds DEP collections
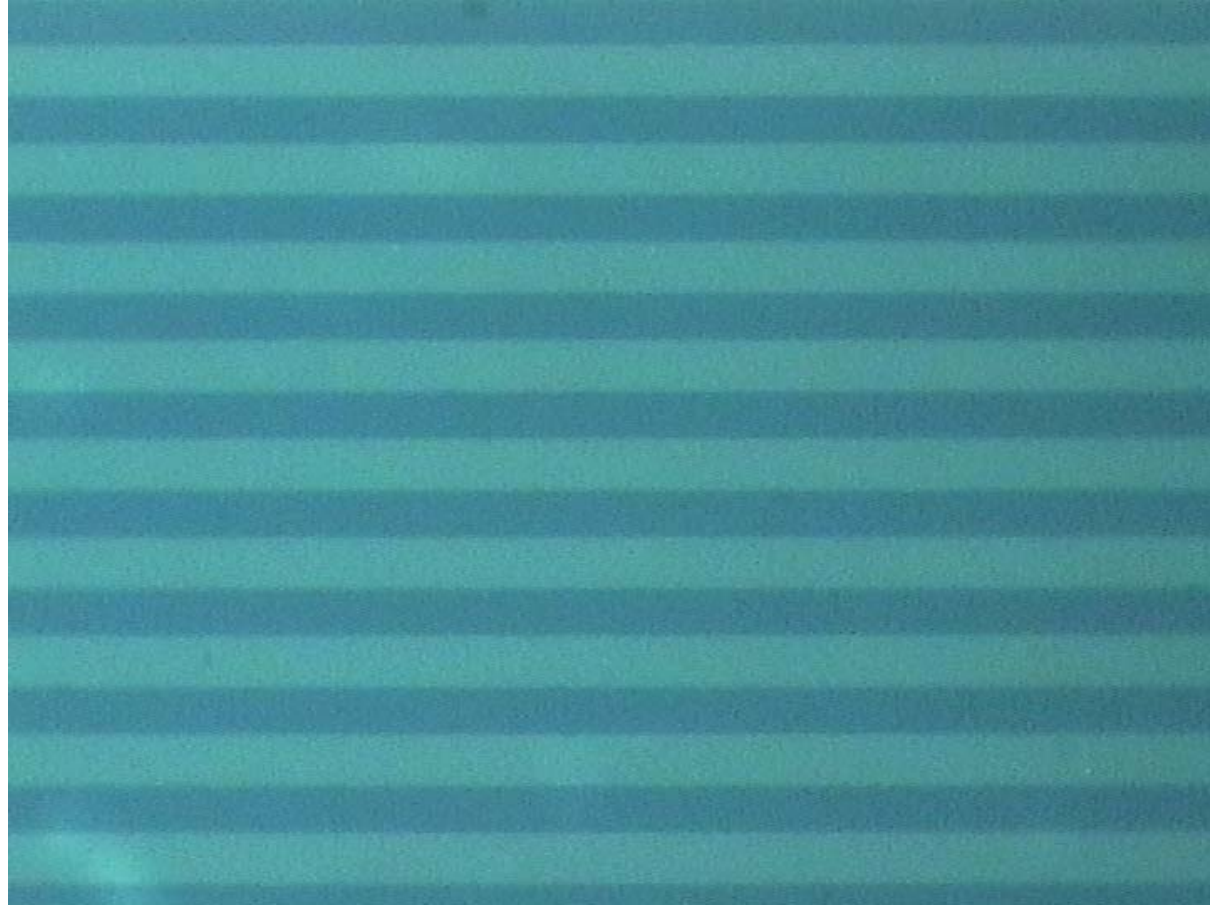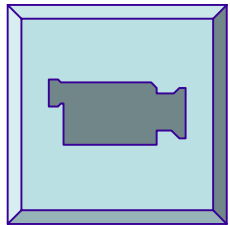
Positive DEP collection of DNA I

electrode width w = 10 μm

gap d = 10 μm

Longitudinal

Transverse

DNA suspended in solution

(a) DEP off

electrode

gap

DNA bridges across gaps

DNA appears amorphous (cloudy)

DNA slightly drifts with fluid motion

(b) DEP on

12 kbp plasmid DNA – inter-wound like a "twisted elastic band" (4 μm contour length, ~1 μm size)

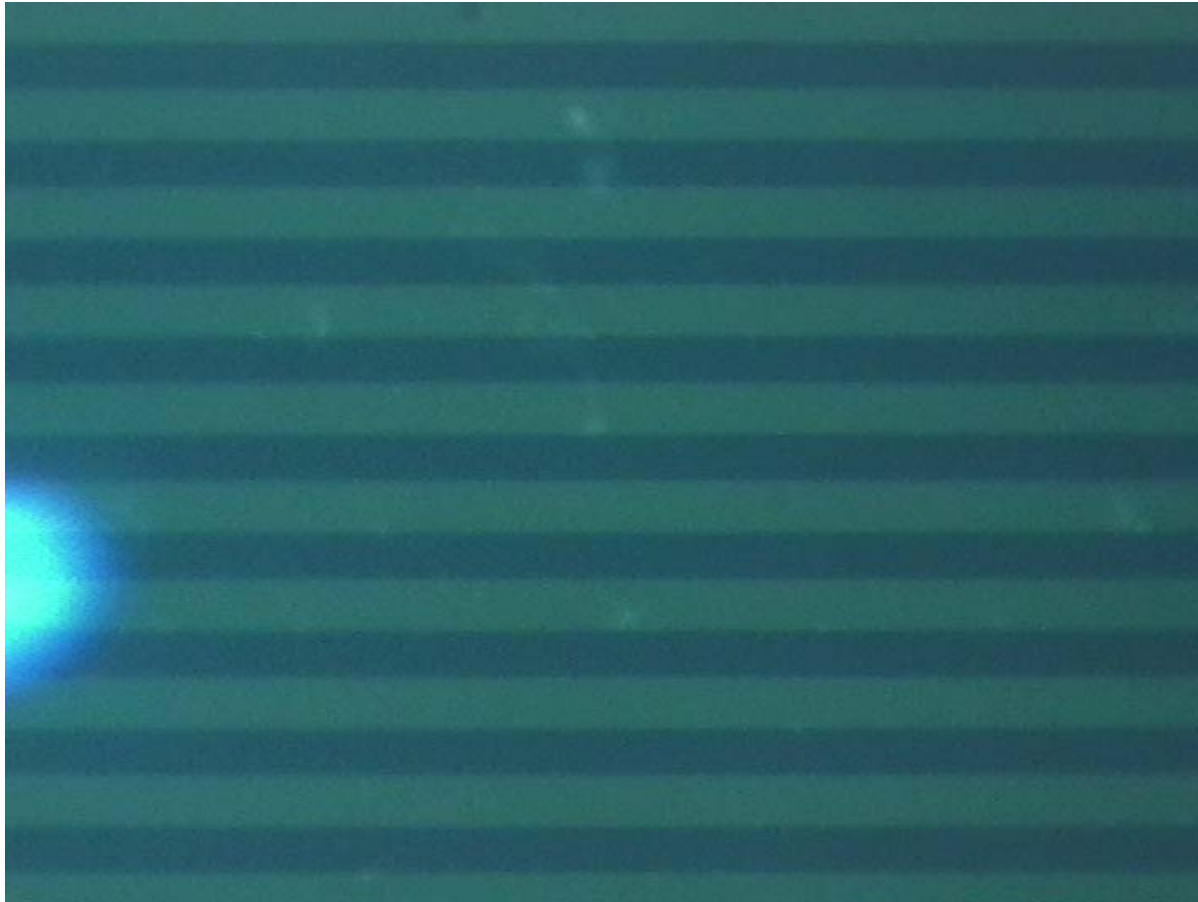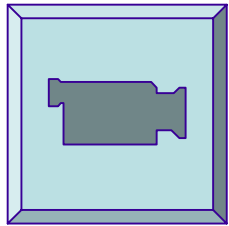+ve DEP → stable trapping of DNA between electrodes

DAPI fluorescent labelled DNA plasmid suspension – half-frame width video images: (a) before onset of DEP and (b) 4.2 seconds after onset of DEP for V_o = 4.5 V, f = 200 kHz, σ_m = 5 mS/m

Positive DEP collection of DNA IIa

- DAPI labelled DNA plasmid suspension: video off/on DEP V_o = 4.5 V, f = 200 kHz, σ_m = 5 mS/m

Positive DEP collection of DNA IIb

- DAPI labelled DNA plasmid suspension: video off/on DEP V_o = 4.5 V, f = 500 kHz
- Challenges for image processing (array movement, fluorescent debris, etc) !
- DEP response is less than for 200 kHz (α reduced)

Positive DEP collection of DNA III

- DNA collection – transverse average over gap $\rightarrow$ collection time 'profiles'
- Frequency dependent collection decreases as polarisability, $\alpha \downarrow$ (or as frequency $\uparrow$)
  - initial collection rate dF(0)/dt
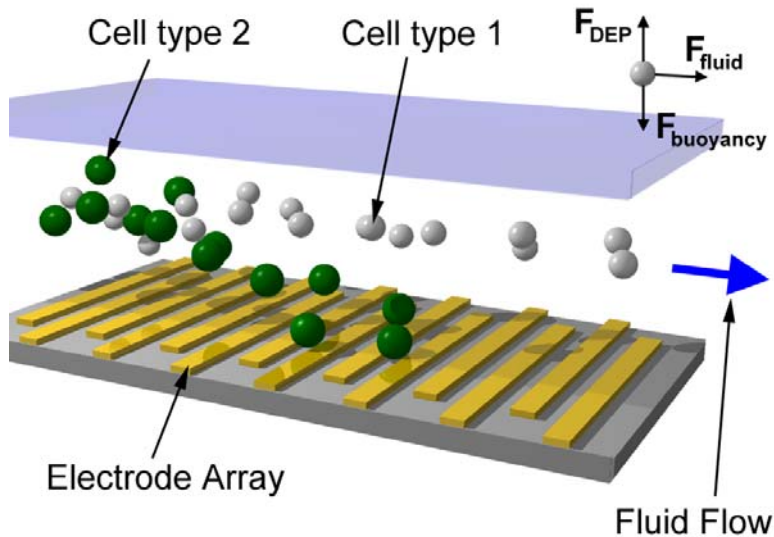 - initial to steady-state transition, Δn

Recent analysis of DNA

- initial collection rate dF(0)/dt & initial to steady-state transition, Δn, exhibit variations but can be distinguished for sufficient frequency differences
- Reproducibility within each experiment
- Dielectric spectroscopy – 3 dispersions 140 kHz, 2 MHz & 12 MHz $\rightarrow \alpha$
  see Bakewell et al (2000) Biochem. Biophys. Acta, **1493**, 151-158
- Comparison with theory – qualitative ✓ , quantitative ✗
- fluid motion confounds DEP collections
- details: Bakewell, D. J. & Morgan, H. Dielectrophoresis of DNA: time and frequency dependent collections on microelectrodes (submitted)

DEP & genomics/proteomics

- Low voltage ~ 10 V controlled DNA trap & release attractive for purifying & concentrating DNA prior to PCR amplification (Crippen et al 2000)

- Surface of latex beads can be chemically modified to attach DNA, enzymes etc → 'molecular surgery'

- Use positive and negative DEP for cell separation
  - applications in environment e.g. detection of bacteria in water
  - improve cell type homogeneity prior to micro-array gene expression analysis (Cheng, et al, 1998; Huang, et al, 2002)
  - DEP can alter gene expression but effects can be taken into account

- 'Indirect' application:
  - microfluidic circulation can improve oligonucleotide hybridisation efficiency for DNA microarrays (Yuen et al 2003)
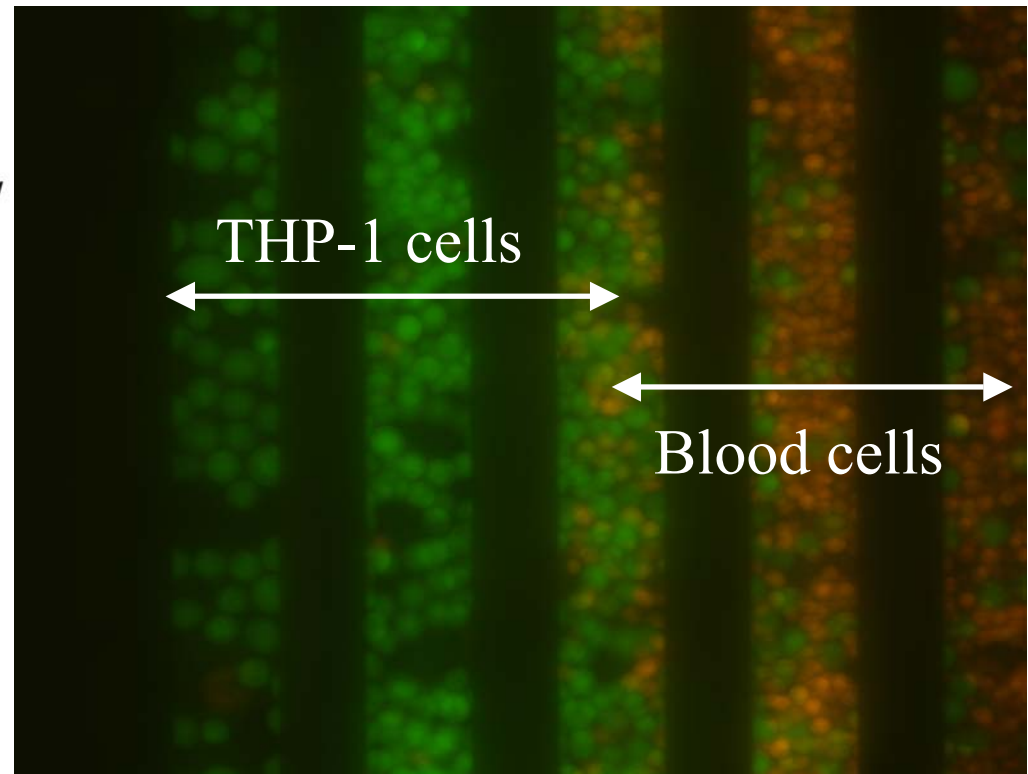 - assembly of micro-wires (Hermanson et al, 2001)

DEP – cell separation

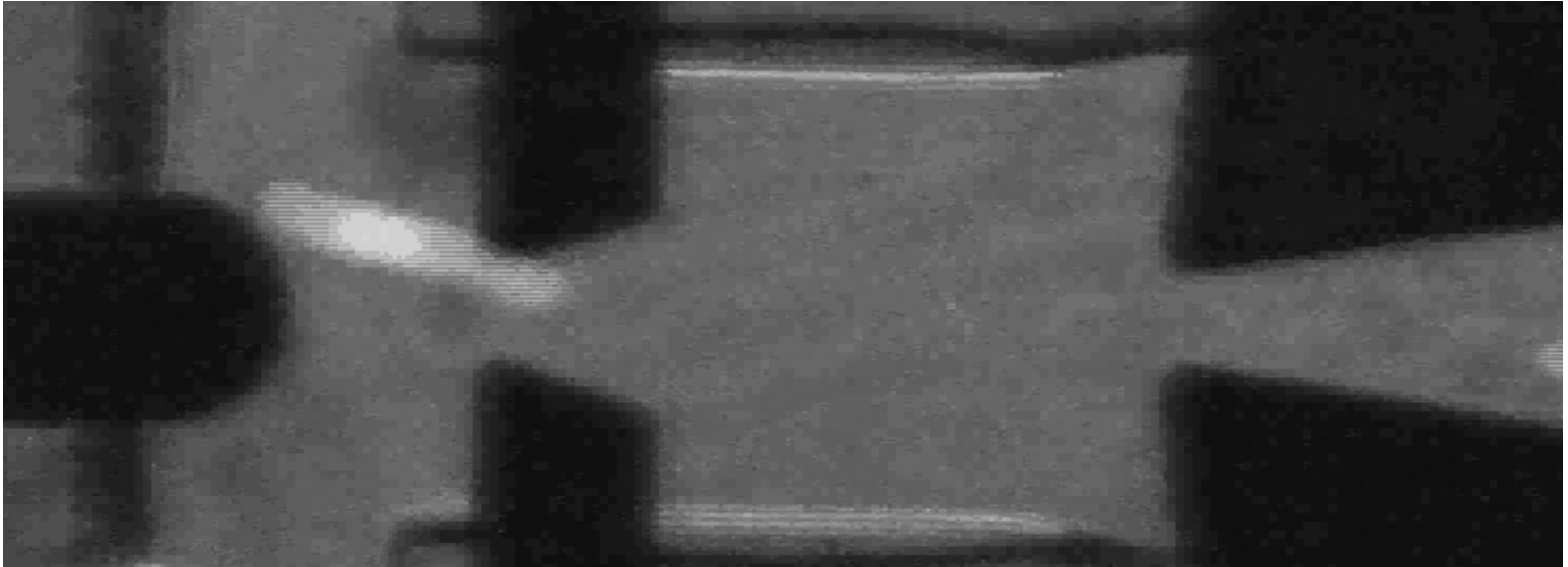Cell type 2   Cell type 1 F_{DEP} F_{fluid} $F_{buoyancy}$

Electrode Array

Fluid Flow

Cells are pre-focused
by –ve DEP

Direction of
fluid flow

Electrode widths = 40 μm

THP-1 cells

Blood cells

- Courtesy D. Holmes &
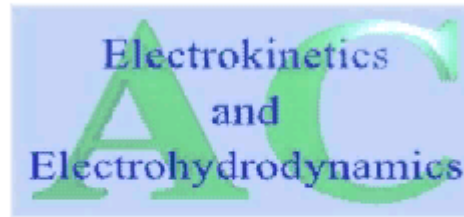 H. Morgan

DEP cell sorting principle

- 6 μm diameter latex microsphere

Other AC electrokinetics

- Electro-rotation - changing frequency reverses rotation of cell

University of Glasgow

Electrokinetics
and
Electrohydrodynamics

AC

Electrorotation
of cells

- Courtesy of N. Green & H. Morgan (Uni. of Southampton)

- Travelling wave dielectrophoresis

AC electrokinetic summary

- Polarisation & *E* induced polarisability
- focus on DEP aspect of AC electrokinetics
  - particle α **>** medium $\alpha \rightarrow$ +ve DEP (attracted to high *E* regions)
  - particle α **<** medium $\alpha \rightarrow$ -ve DEP (repelled from high *E* regions)
- DEP collections on to planar interdigitated electrode arrays
  - FPE modelling
  - experimental set-up using fluorescent microscopy
- Example particle collections onto planar interdigitated electrode arrays
  - +ve DEP collections of 216 nm diameter latex microspheres (beads)
  - +ve DEP collections of 12 kbp plasmid DNA
- Theory & experiment qualitatively concur ✓ quantitative ✗
- Discrepancies - electro-osmotic fluid – needs further investigation
- Demonstrate – collection characterisation & trapping of DNA & beads
- Apply same principles to proteins, mammalian cells, bacteria, etc
- Applications of DEP to genomics/proteomics include methods for DNA concentration, cell sorting, prior to microarray analysis, etc.

References

* *see* special issue *IEEE Eng. Med. Biol.*, **22**, 6 (2003) Micromedicine: sorting cells and finding bugs with micro- and nanoelectrokinetics.

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Acknowledgements

- Presentation preparation: Dr. K. Vass, Bioinformatics Centre, Beatson Laboratories CR-UK, Garscube Estate, Glasgow, UK.

- Supervision, advice and cell separation images: Prof. H. Morgan, Drs. D. Holmes, N. Green and I. Ermolina, School of Electronics and Computer Science, University of Southampton, UK.

- Microfabication & laboratory experiments: staff at Bioelectronics Research Centre, Department of Electronics & Electrical Engineering, University of Glasgow, UK.

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