



**The Abdus Salam
International Centre for Theoretical Physics**



2037-5

Introduction to Optofluidics

1 - 5 June 2009

Use of high-speed imaging technique for applications in optical tweezers

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U.K.*

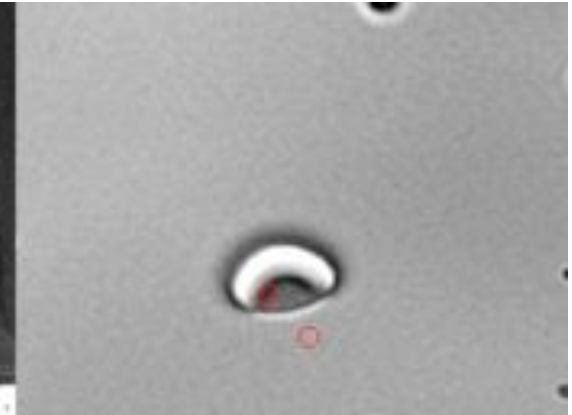
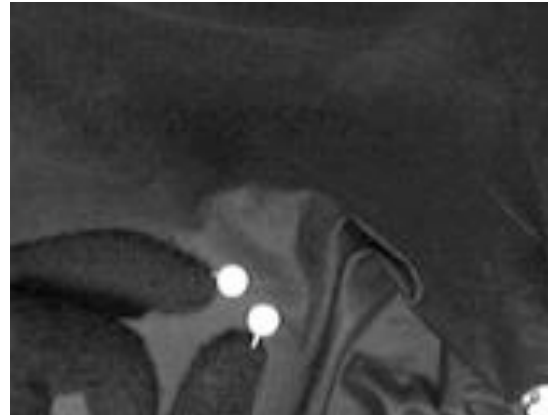
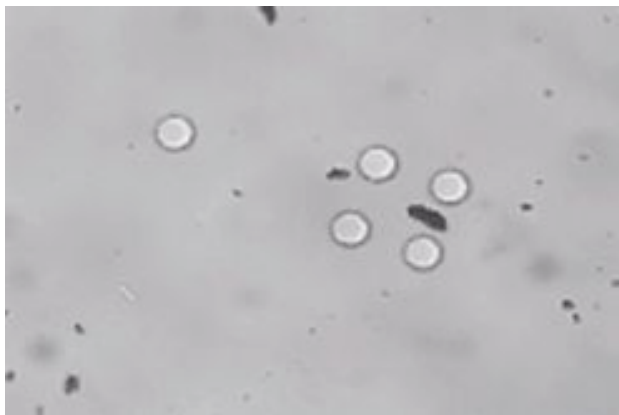
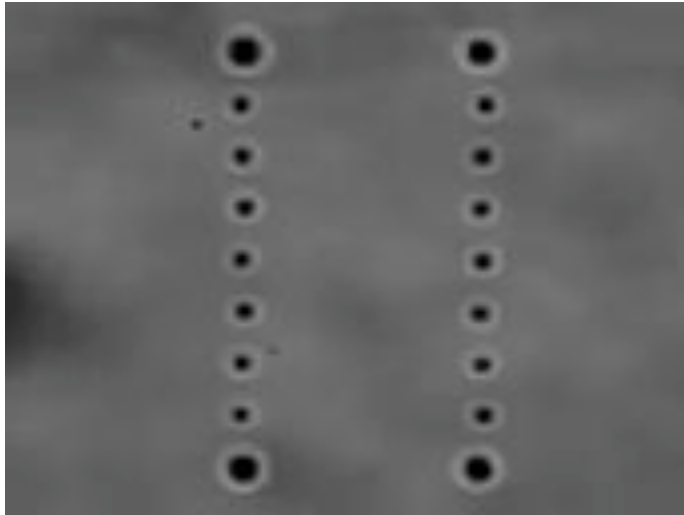
Use of high-speed imaging technique for applications in optical tweezers

Miles Padgett

Department of Physics and Astronomy



My last lecture – interfaces to holographic tweezers



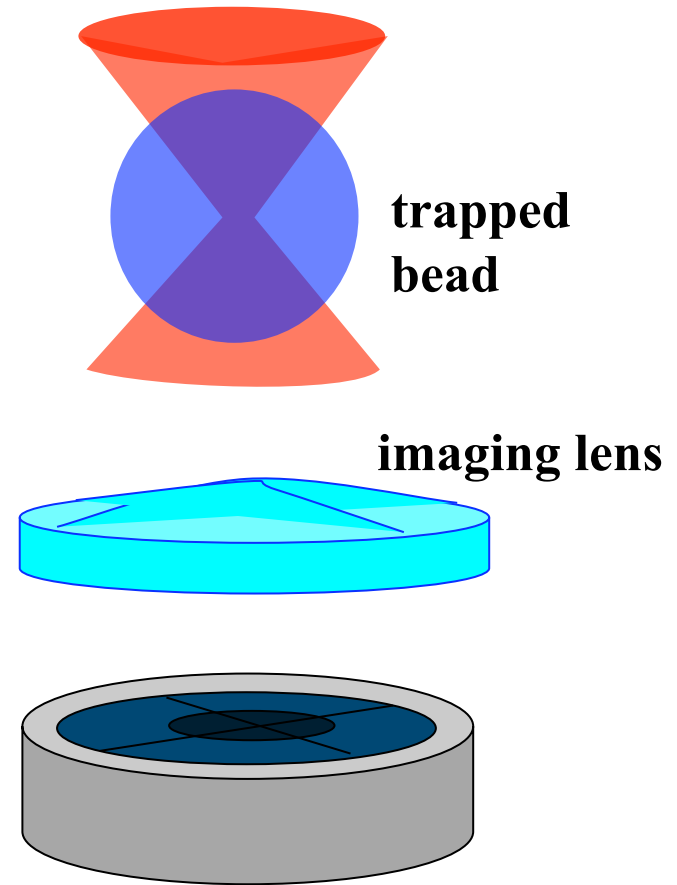
Part 2

- Methods and Applications of High-speed Imaging
 - Flow meter
 - Hydrodynamic coupling
 - Trap dynamics
 - Haptic feedback



Where's that particle?

- Particles shadow on a quadrant detector
 - Differential signal from quadrants gives $< \text{nm}$ precision!
 - $> 10 \text{kHz}$ response
 - Precision limit is thermal noise in amplifiers
 - Can use deflected laser light for more signal
- Video?
 - Limited by frame rate
 - Determine (sub pixel) particle position from “centre of mass”
 - Precision/speed limit by signal

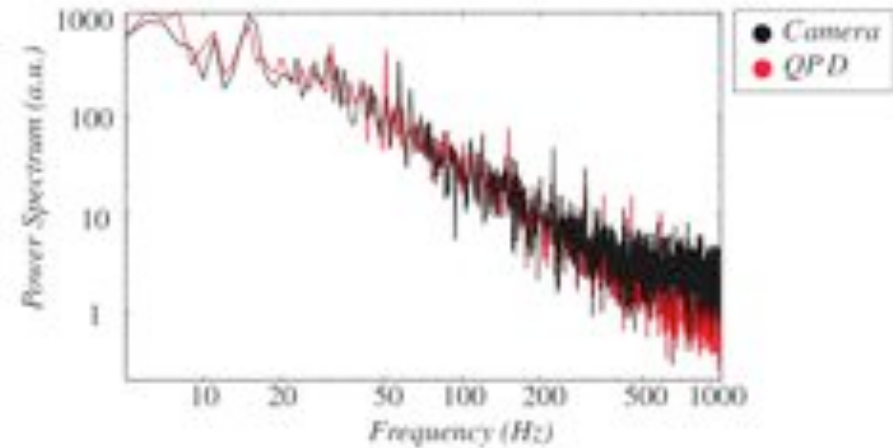
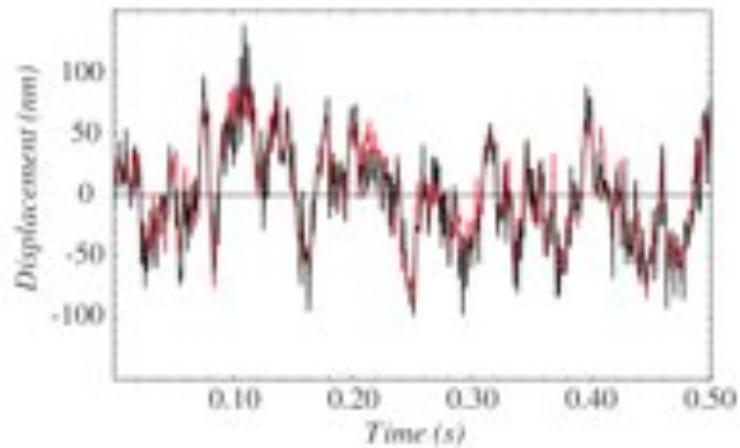


8-bit Firewire (images buffered on camera)

- CCD (1024x1024)
- 8-bit digitisation
- Full-field frame-rate 500Hz
- Reduced frame-rate upto 16kHz
 - “letter box” R.O.I
- Images stored in camera and subsequently downloaded.....
 - 4secs of data takes \approx 10mins to download



High speed video v.s. Quadrant Cell



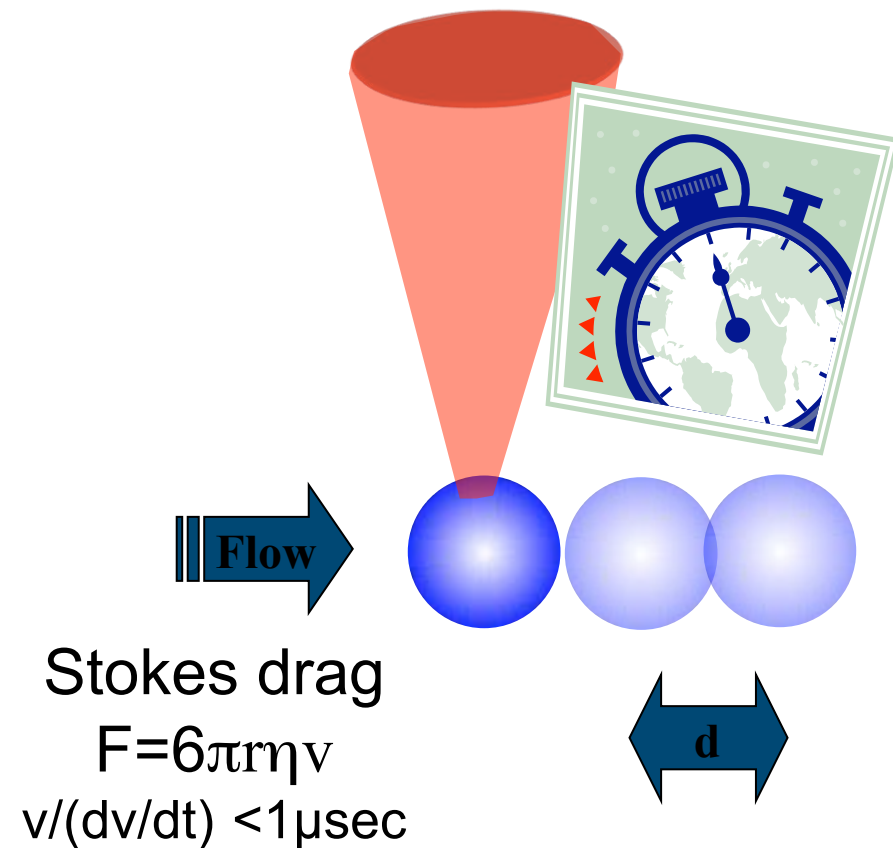
12-bit Firewire

- CCD (1392x1040)
- 12-bit digitisation
- Full-field frame-rate $\approx 20\text{Hz}$
- LabView drivers
- Controlable shutter speed



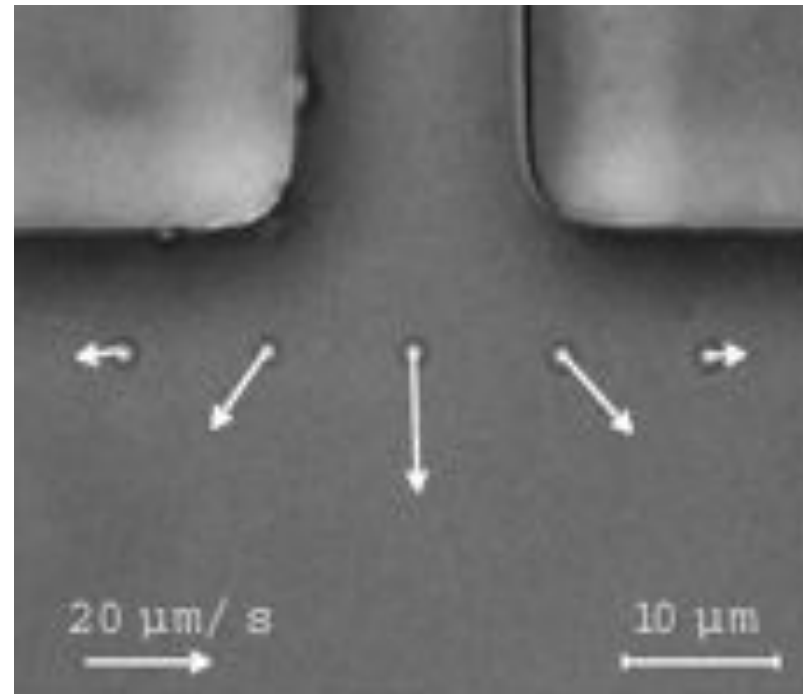
“Speed camera” for fluid flow

- Take two short exposure images with fixed time offset (10msec)
- Calibration independent of trap characteristics
- Flow rate 0.1-50 $\mu\text{m/s}$
- N.B. $v/(dv/dt) < 1\mu\text{sec}$



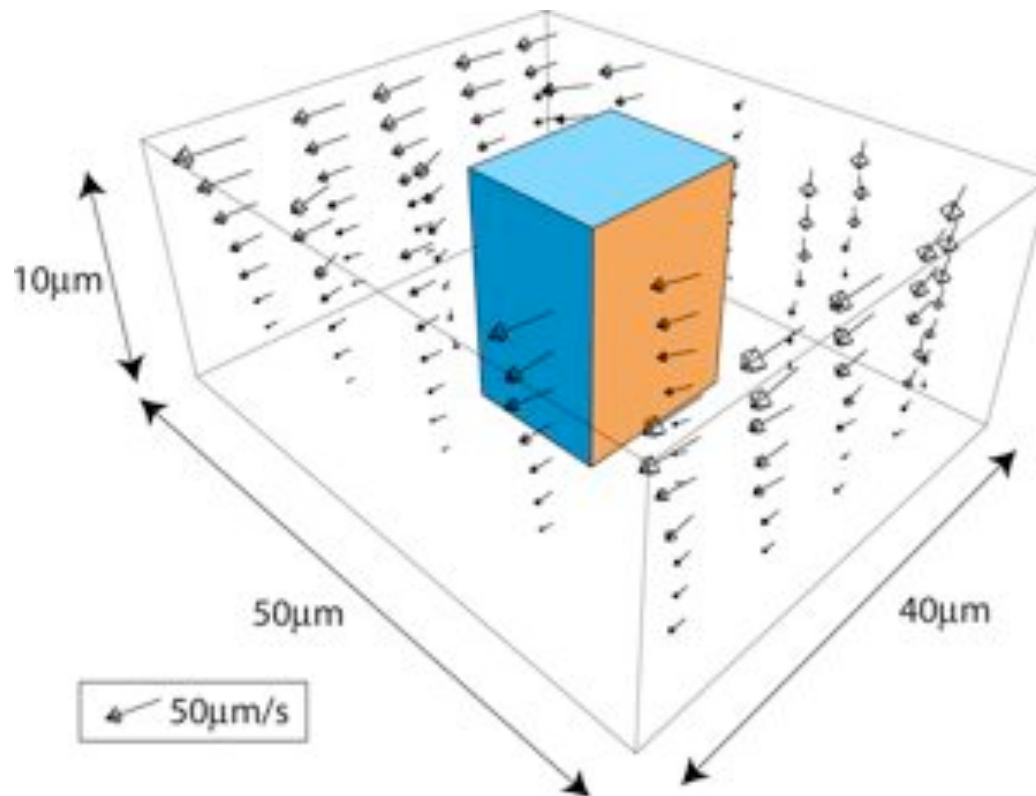
Measuring flow (2D) in micro-fluidic channel (3D)

- 15 μm wide channel in PDMS
 - e-beam mask
 - Expose photo resist on silicon wafer
 - Dry etch to leave channel negative (ridges)
 - Coat with PDMS
 - Peel off to give channel network
 - Seal with cover-slip



DiLeonardo *et al.* PRL, 96, 134502, 2006

Mapping of 3D fluid Flow



Mushfique *et al.* Proc. IMechE, 222, 829, 2006

Commercial (cheap) CMOS

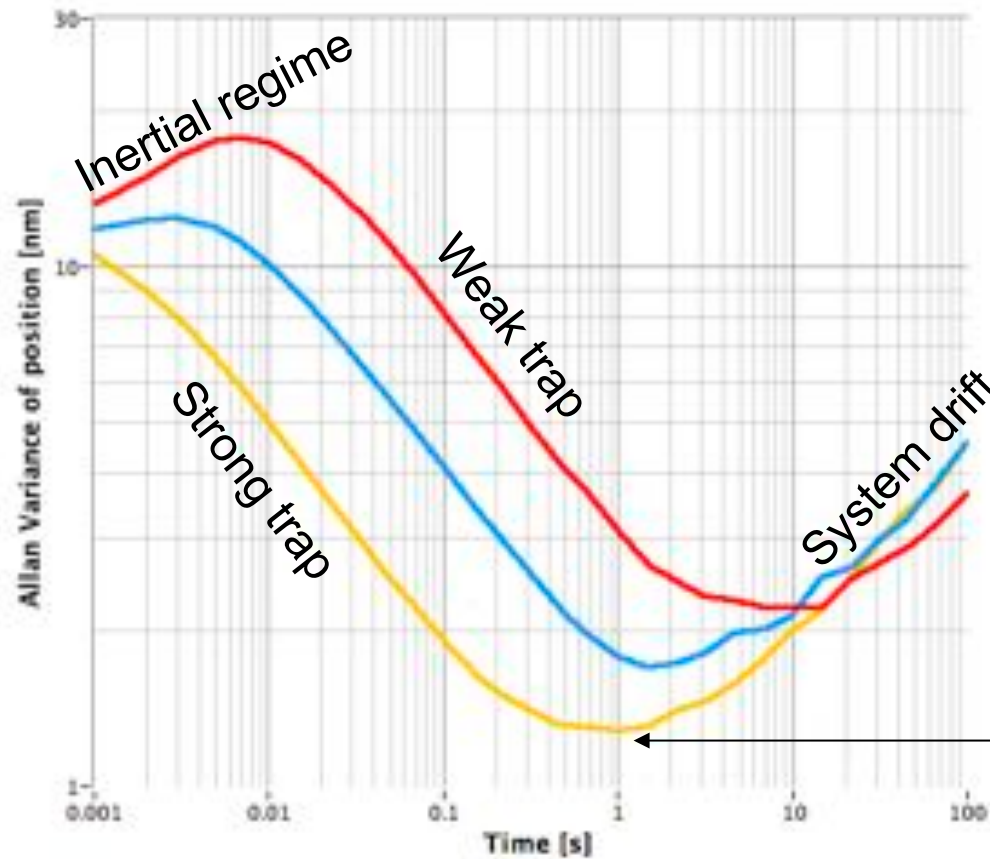
- CMOS (1280x1024)
- 8-bit digitisation
- Full-field, frame rate $\approx 30\text{Hz}$
- Fully selectable R.O.I (100x100).
 - Frame rate upto 1kHz
- LabView drivers
 - Can calculate centre-of-mass positions in real time



Limits on Particle Tracking

- The Thermal Limit (?)
 - What is the best (open-loop) accuracy we can get when measuring the force on a bead?
 - Brownian motion makes the particle wobble about its equilibrium position in the trap (uncertainty so uncertainty in force is approximately constant)
 - Camera noise makes the particle appear to wobble about its true position
 - We average over multiple measurements to find the equilibrium position in the trap (i.e. a force other than Brownian motion), so
 - Does higher bandwidth mean better accuracy?
 - No: measurements must be independent \Rightarrow Maximum useful frequency.

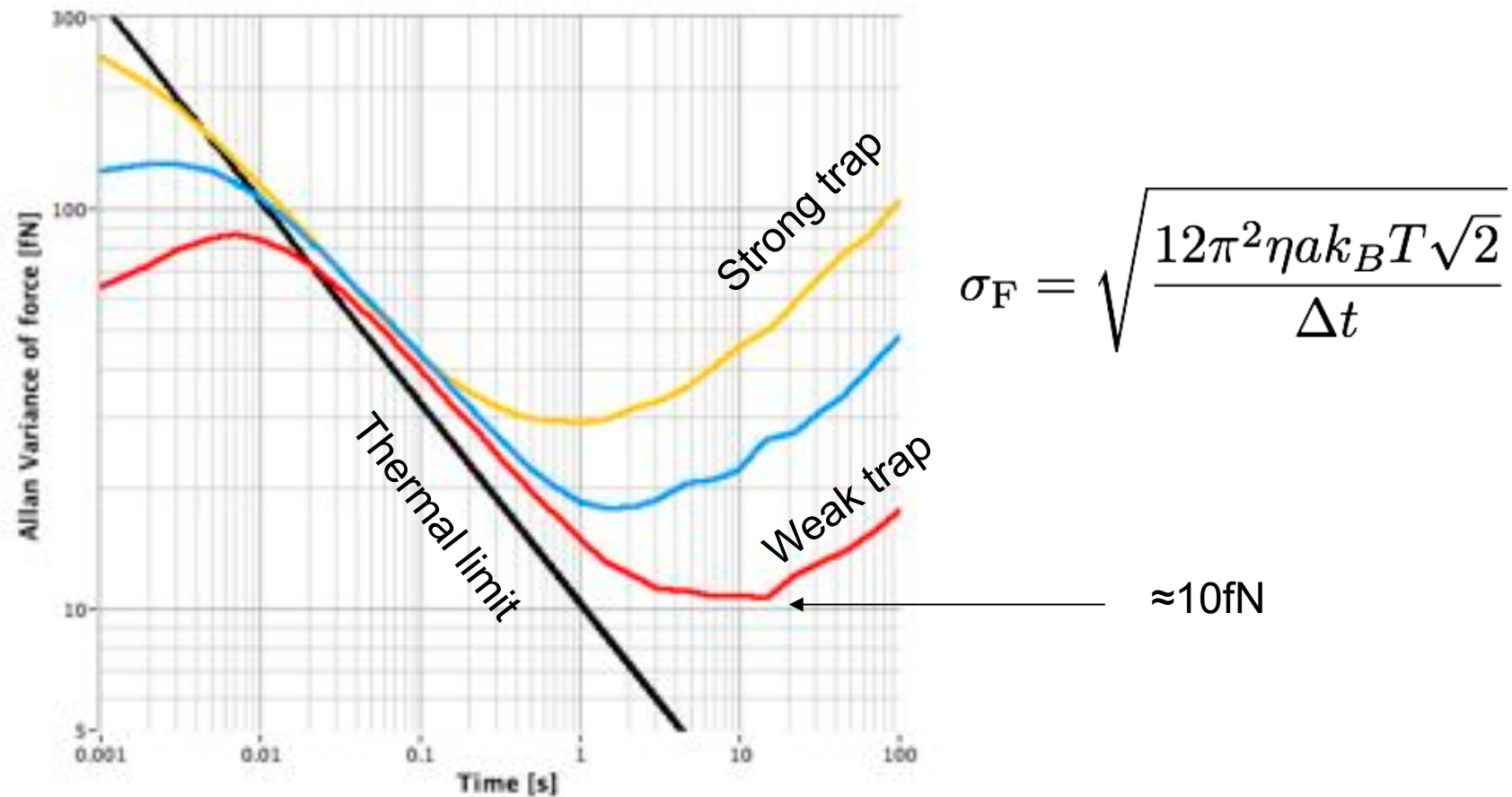
Allan Variance of particle position



$$\sigma_y(\tau) = \sqrt{\frac{1}{2} \langle (y_{n+1} - y_n)^2 \rangle}$$

≈2nm

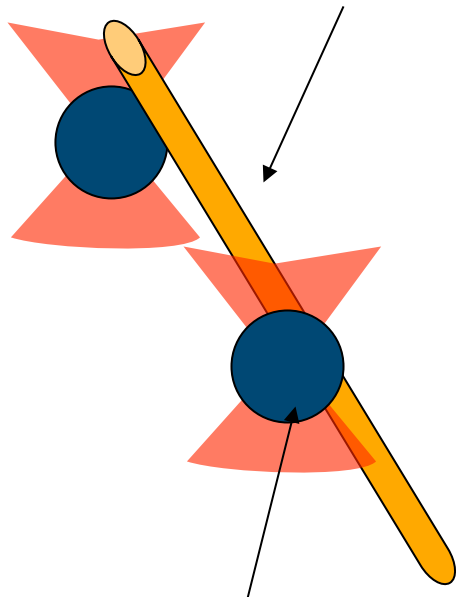
Allan Variance of (open loop) force measurement



Gibson *et al.* Opt. Exp, 14561, 2008

Tweezed probes (collab. Miles and Carberry Bristol)

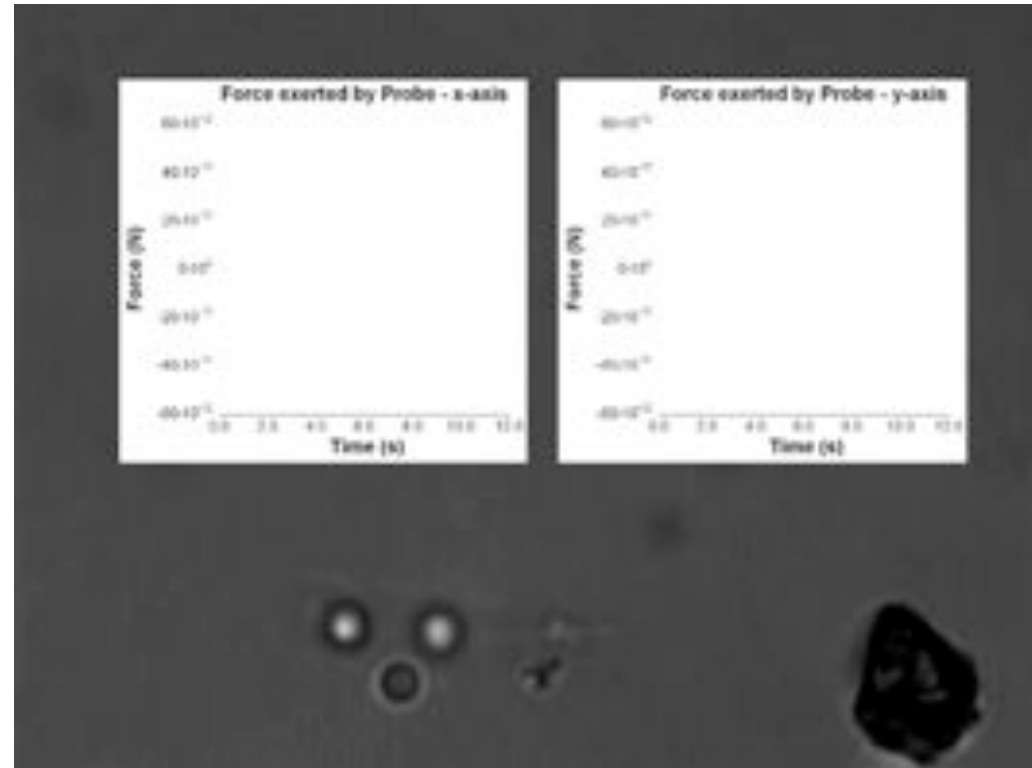
<100nm dia. CdS (biotin)



2µm dia silica (streptavidin)

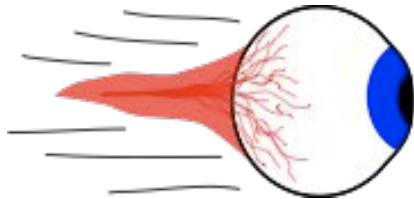
AFM nN-pN (@<nm)

Tweezers pN-fN (@>10 nm)

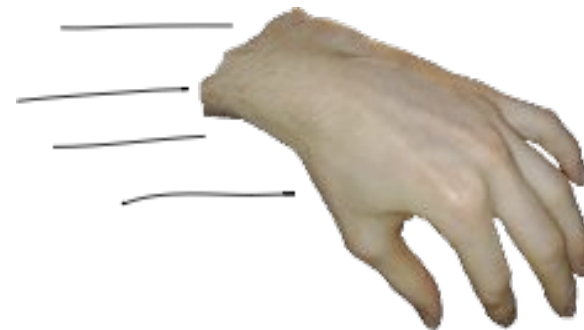


Motivation for Haptic Feedback

- The Hand is Faster than the Eye
 - We track movement visually with a bandwidth of about 24Hz



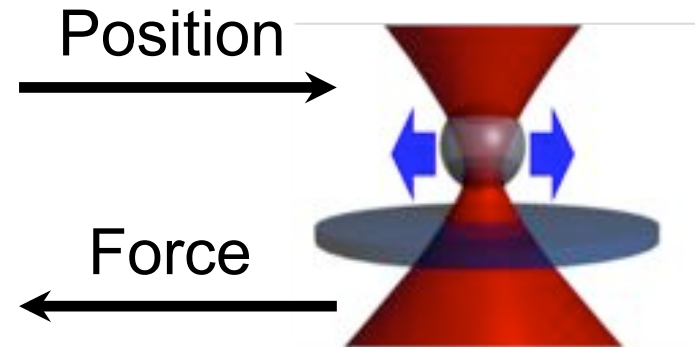
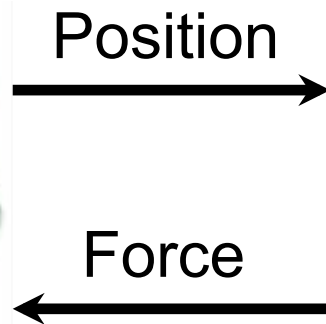
- We sense forces with a bandwidth of about 1000Hz
 - The hand is a natural force sensor



Haptic Feedback



Macroworld
(Haptic Controller)
Novint Falcon

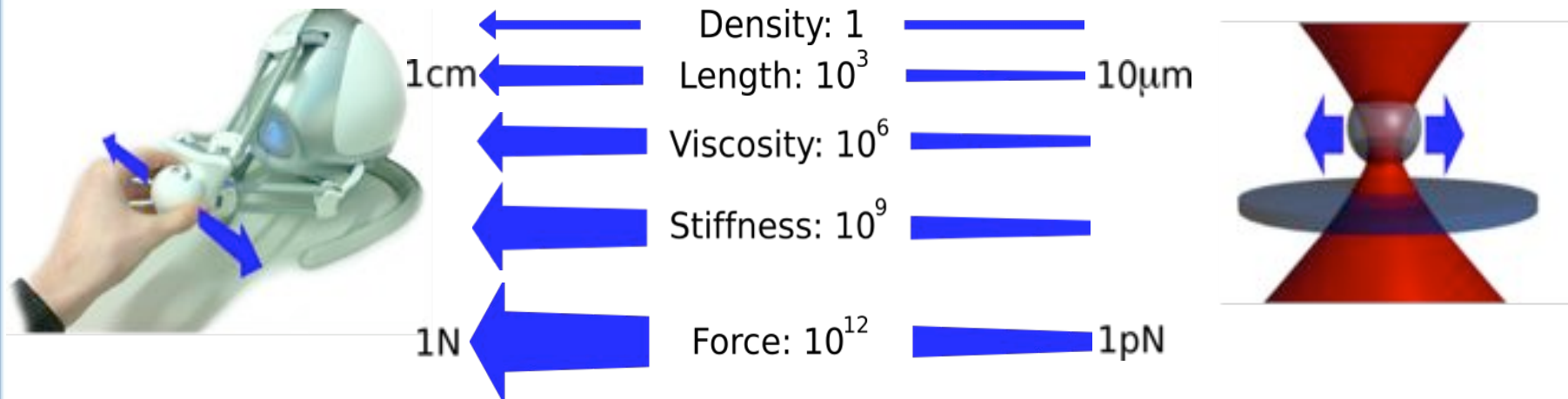


Microworld
(Optical
Tweezers)



Haptic Feedback

- Coupling the Microworld to the Macroworld
 - Microworld quantities are of very different magnitudes

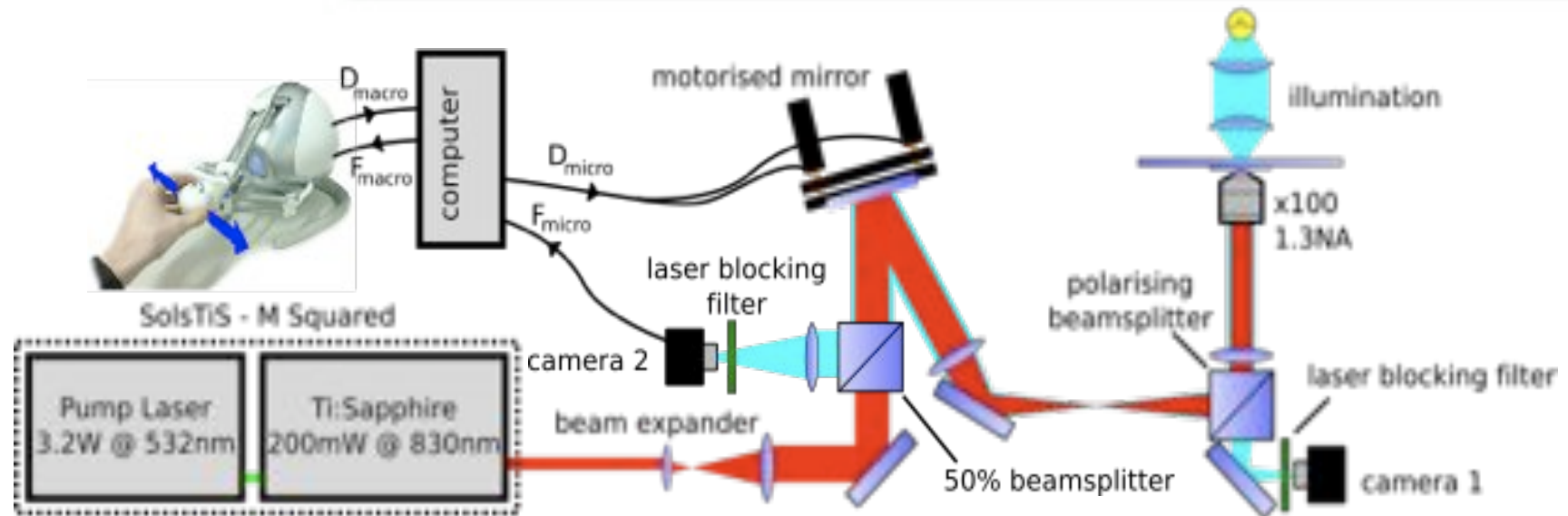


- Microworld dynamics are very different

– Microworld $F \propto \gamma \frac{dx}{dt} + F_{Brownian}$

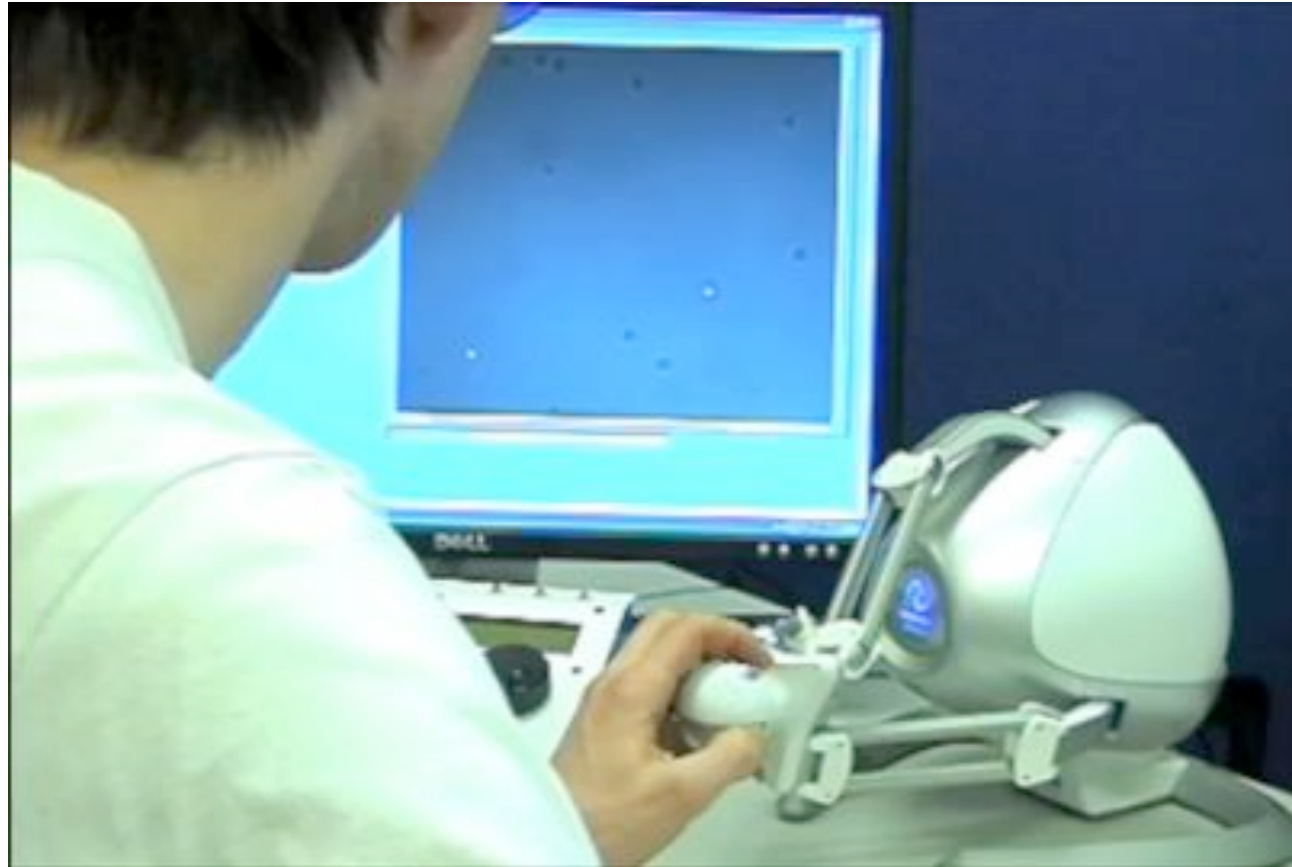
– Macroworld $F \propto m \frac{d^2x}{dt^2} + mg$

Implementing the System



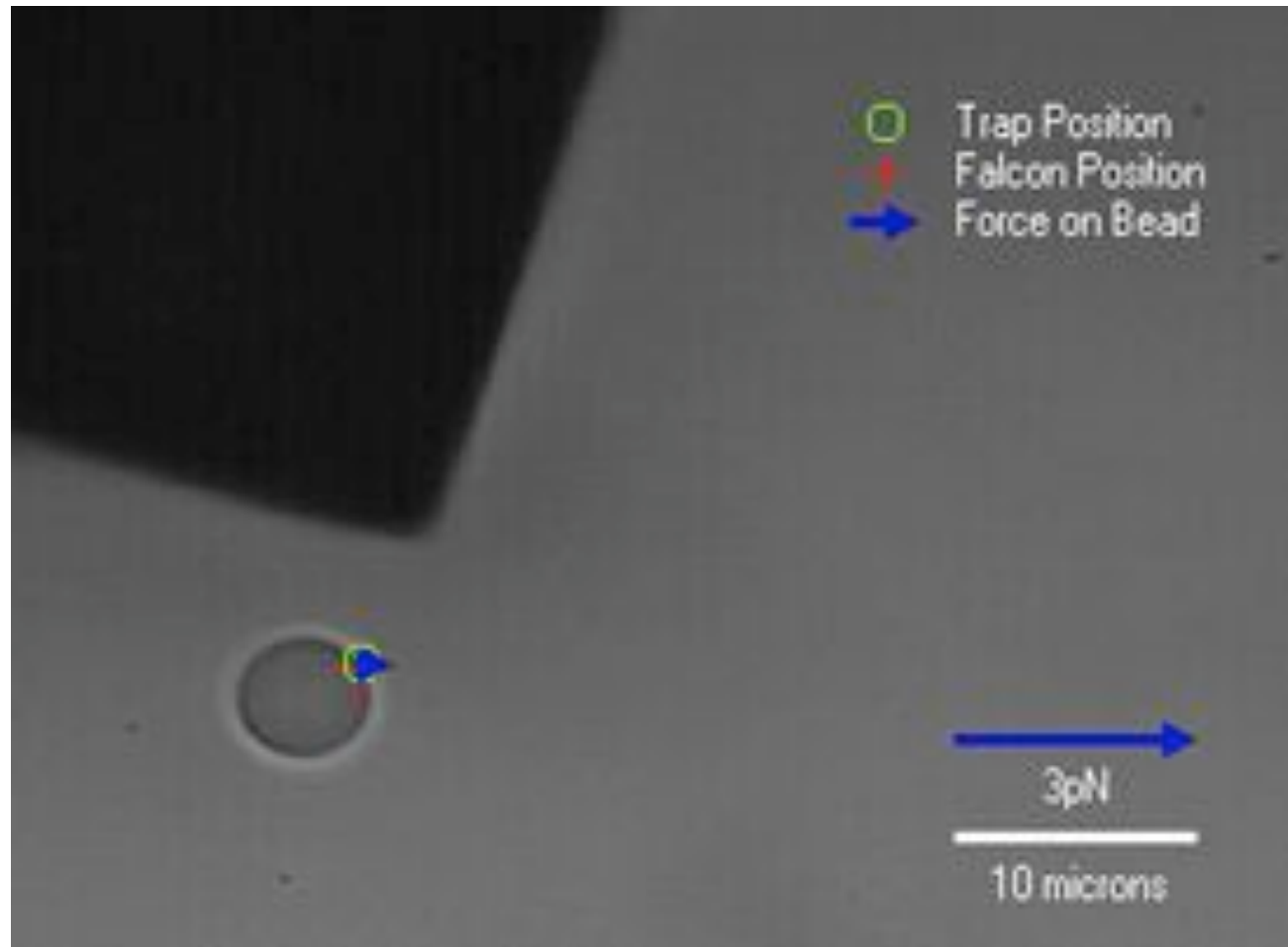
- “De-Scanned” Camera for Force Measurement
 - Image moves as trap is steered
 - Image of laser spot stays fixed
 - Makes it simple to measure
 - No need to track the spot
 - Insensitive to tracking errors/lag
 - ROI on camera can be small

Haptic Feedback in Action



Pacoret et al. in press Opt. Express 2009

Haptic Feedback in Action



Force Measurement

- Video Particle Tracking
 - Simple set-up
 - Calibration of pixel size can be done with a calibration slide
 - Calibrating the trap requires no assumptions about viscosity, particle size, etc.
 - Non-Spherical Particles
 - Image analysis can track more complex particles (e.g. cells)
 - Multiple Particles
 - Several particles can appear in each camera frame
 - Compatible with e.g. holographic techniques
 - High Speed
 - Image acquisition faster than 1kHz (with modern CMOS cameras)
 - Processing on-the-fly using a centre-of-mass algorithm on a standard PC

Cameras for Particle Tracking

• High Speed Cameras

- Buffer-based devices
 - Store images in a buffer at high-speed
 - Download images later and analyse offline
 - Limited recording length, not real-time



Redlake Digital Imaging Systems
<http://www.redlake.com>

- Smart Cameras
 - Hardware image analysis, send only coordinates of particles
 - Best bandwidth (several kHz)
 - Measure up to 16 particles

Durham Smart Imaging
<http://www.durhamsi.co.uk>

- CMOS Cameras
 - Reduced region of interest (ROI) to save bandwidth
 - Cheap and flexible
 - Image analysis of multiple particles on a desktop



Prosilica EC1280
<http://www.prosilica.com>

