Microscopy, particle tracking, and applications

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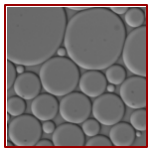
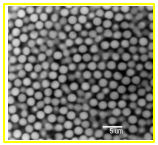
http://www.physics.emory.edu/~weeks/lab/



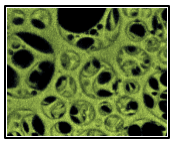
My work:

Microscopy to relate microscopic properties of soft materials to their macroscopic properties; especially colloidal pastes



colloidal paste

colloidal gel



emulsion

What is Microscopy?

Typically, use visible light, take pictures of samples.

Often, take many pictures: "video microscopy"

colloidal gel

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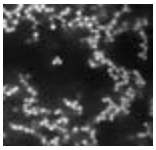
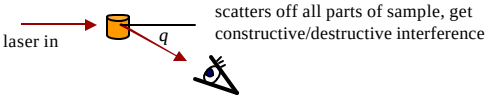


same colloidal gel

picture & movie from Gianguido Cianci (Emory)

What is light scattering?

Note: I am not an expert; Simon Mochrie coming soon



Warning: do not stare into laser with your eye.

What is light scattering?

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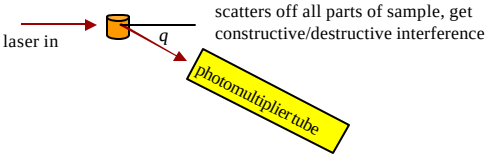

By observing at different angles, get information about Fourier Transform of structure of sample

What is light scattering?

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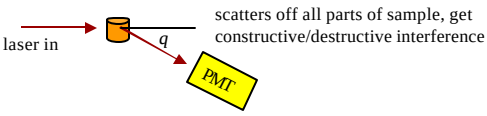


At fixed angle, observe intensity over time $I(t)$; like video microscopy, learn how things move.

If particles move ~ λ on average, signal $I(t)$ decorrelates.

Microscopy –vs– scattering

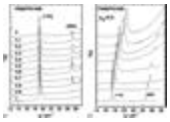
Scattering: average information from many parts of the sample
- Low noise
- Can see fast time scales $O(1\ \mu s)$
- Like an ensemble average
- Can use neutrons to get smaller length scales

Microscopy: detailed information from small part of sample
- More noise
- Slower time scales $O(30\ ms)$
- Longer time scales $O(hours)$
- See what goes into the average!

Microscopy –vs– scattering: Example 1

Scattering: average information from many parts of the sample
- Quantify crystal structure of sample

(from Wette, Schope, Palberg JCP 122, 144901 [2005])



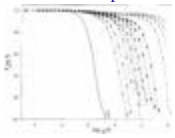
Microscopy: detailed information from small part of sample
- See shape of crystal nucleation site as it grows

(from Wette, Schope, Palberg JCP 123, 174902 [2005])

Microscopy –vs– scattering: Example 2

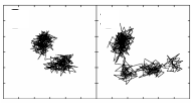
Scattering: average information from many parts of the sample
- Average motion in glassy samples

(from van Megen et al., PRE 58, 6073 [1998])



Microscopy: detailed information from small part of sample
- See motion of individual particles

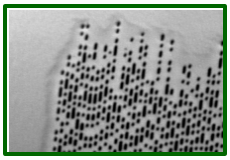
(from Weeks & Weitz., Chem Phys 284, 361 [2002])



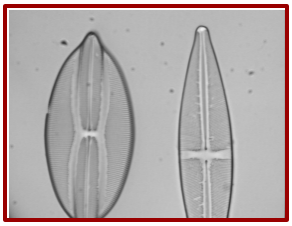
Types of microscopy: Brightfield

Basic optical microscopy

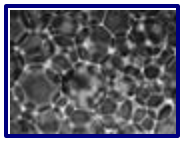
Advantages: cheap, easy
Disadvantage: requires "easy" sample



what is this?



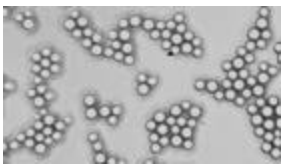
diatoms (biological critters)



emulsion

What is an easy sample?

Microscopy (and scattering) work because some part of the sample is a different index of refraction from the rest.
Example: colloidal particles, emulsion droplets



colloidal particles on coverslip (Hetal Patel, Emory)

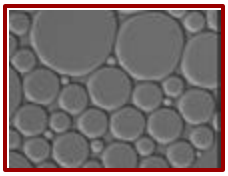
Easy: $Dn > 0.1$, low concentration of objects

Hard: $Dn \approx 0.01$ and/or high concentration of objects

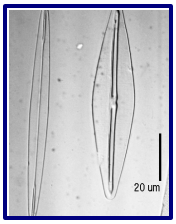
Types of microscopy: DIC

Differential Interference Contrast

Advantages: enhances subtle *Dn*; thin optical section, cool pictures
Disadvantage: costs ~ $10k, asymmetric images



emulsion

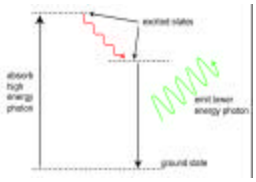


diatoms

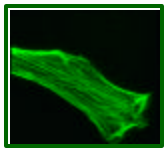
Types of microscopy: Fluorescence

very common in biology labs

Advantages: ignores *Dn*; can target dye, can use multiple dyes
Disadvantage: costs ~ $10k, requires dye, dye can bleach

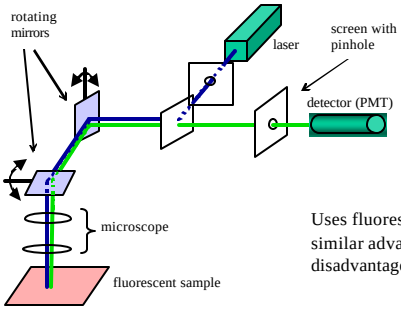


(from Semwogerere & Weeks, 2005)



actin filaments, Megan Valentine & Heather Rose
http://www.deas.harvard.edu/projects/weitzlab/coolpic16.html

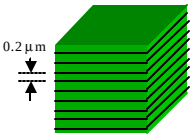
Types of microscopy: Confocal Microscopy

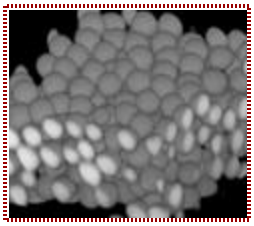


Uses fluorescence, has similar advantages & disadvantages

Confocal microscopy for 3D pictures

Scan many slices, reconstruct 3D image





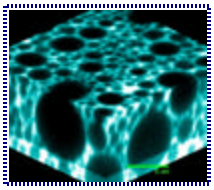
2.3 µm diameter PMMA particles

3D tracking article: Dinsmore et al., App. Optics **40**, 4152 (2001)

Types of microscopy: Confocal

advantages & disadvantages similar to fluorescence, plus…

Advantages: 3D pictures, can look through dense samples
Disadvantage: costs ~ $100-500k + maintenance



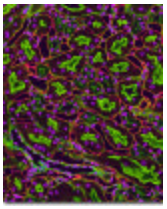
Emulsion
(Eric Weeks & Suliana Manley)

Emulsion
(Lotti Hollinger & Eric Weeks)

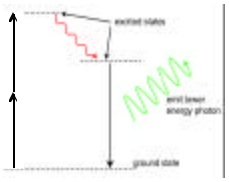
Types of microscopy: 2-photon

(or multi-photon)

Advantages: same as confocal, but less bleaching
Disadvantage: costs ~ $300-500k ??



absorb 2 low-energy photons simultaneously



Quantum dot fluorescence image of mouse kidney section
Thomas J. Deerinck , http://www.microscopyu.com/smallworld/gallery/

Types of microscopy: many others

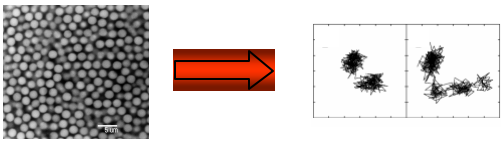
Darkfield
Phase contrast (often used for vesicles, biological samples)
Polarization

Near-field scanning optical microscopy (NSOM)
Total internal reflection microscopy (TIRM)
Coherent anti-Stokes Raman scattering (CARS) microscopy
4Pi microscopy
STED microscopy (see articles by Stefan W. Hell)

Atomic force microscopy (typically limited to surfaces)
Electron microscopy (motionless samples only?)

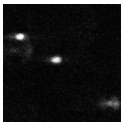
Particle Tracking

How to do it, and why

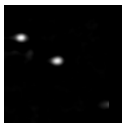


Free software at: http://www.physics.emory.edu/~weeks/idl/
See also Crocker & Grier, J. Colloid Interf. Sci. **179**, 298 (1996)

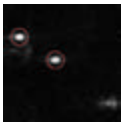
Particle tracking: start with image analysis to find the particles



1. Raw image



2. Spatial bandpass filter – remove high frequency noise, low frequency illumination variations

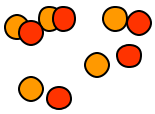


3. Find local maxima

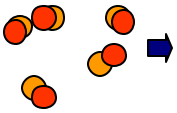
Free software at: http://www.physics.emory.edu/~weeks/idl/
See also Crocker & Grier, J. Colloid Interf. Sci. **179**, 298 (1996)

Track particle positions

Key idea: between each image, particles need to move less than interparticle spacing



If there is an overall flow, subtract it before tracking



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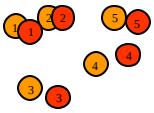


Software assigns each particle a unique ID number

Free software at: http://www.physics.emory.edu/~weeks/idl/
See also Crocker & Grier, J. Colloid Interf. Sci. **179**, 298 (1996)

Final results

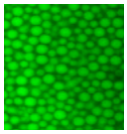
(x, y, t): up to 1000's of particles, 1000's of time steps
limited by image resolution, hard drive size

Software can do higher dimensions

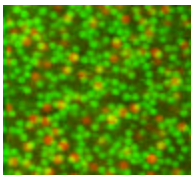
Software works with IDL, Matlab, LabView, or stand-alone

Free software at: http://www.physics.emory.edu/~weeks/idl/
See also Crocker & Grier, J. Colloid Interf. Sci. **179**, 298 (1996)

Microscopy tip



Tricky to get software to distinguish two particle sizes…



…so use two dyes to tell apart two species

(for fluorescence or confocal microscopy)

Confocal microscopy and 3D tracking

Microscopy:

- 30 images/s (512×480 pixels, 2D)
- one 3D " chunk" per 1 – 15 s depending on microscope
- $67 \times 63 \times 20\ \mu m^3$
- 100× oil / 1.4 N.A. objective
- Identify particles within 0.03µm (*xy*), 0.05 µm (*z*)

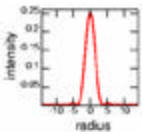
Particle tracking:

- Follow 3000-5000 particles, in 3D
- 200-1000 time steps = hours to days
- ≈ 4 GB of images per experiment

3D tracking article: Dinsmore et al., App. Optics **40**, 4152 (2001)

Resolution, Errors, Limitations

Image of point source of light is blurred by diffraction – get Airy disk



Optical resolution: (100× oil / 1.4 NA lens)
~0.2 µm in *x,y*
~0.5 µm in *z*

Ability to locate the position:
~0.02 µm, maybe better
depends on size of image of spot in pixels

Note: magnification less important than resolution
See www.physics.emory.edu/~weeks/confocal/resolution.html

"Application" #0: Brownian Motion in dilute samples

Leads to normal diffusion:

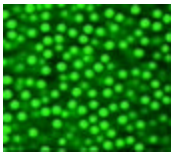
$$\langle \Delta x^2 \rangle = 2Dt$$

$$D = \frac{k_B T}{6\pi\eta a}$$

viscosity η

particle size *a*

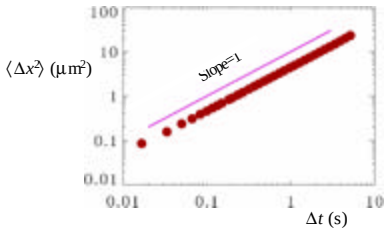
2 µm dia particles



5 µm

Diffusion: **dilute** samples

Mean square displacement:



Influence of noise

even a motionless particle …

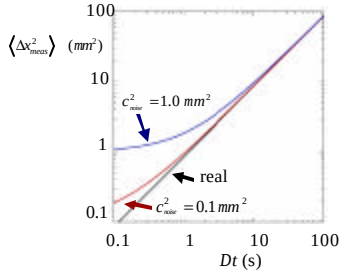
… looks like it's moving

$$\left\langle \Delta x^2_{measured} \right\rangle = \left\langle \Delta x^2_{real} \right\rangle + c^2_{noise}$$

(functions of *Dt*)

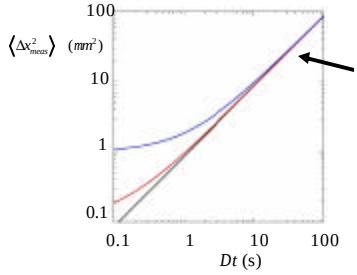
(not function of *Dt*)

Influence of noise: $\langle \Delta x^2_{measured} \rangle = \langle \Delta x^2_{real} \rangle + c^2_{noise}$



simulated diffusion of 1.0 µm diameter particles

Influence of noise: $\langle \Delta x^2_{measured} \rangle = \langle \Delta x^2_{real} \rangle + c^2_{noise}$



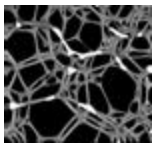
in limit $Dt \rightarrow \infty$ converges to true answer

simulated diffusion of 1.0 µm diameter particles

Application #1: drainage in foams

(with Stephan Koehler, Emory U.)

Foam has
* bubbles
* faces where 2 bubbles meet
* channels between 3 bubbles
* nodes where channels come together (between four bubbles)



Are channels rigid pipes or slippery pipes?

SA Koehler, S Hilgenfeldt, ER Weeks, and HA Stone, 2002, 2004

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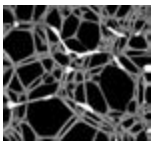

Are channels rigid pipes or slippery pipes?

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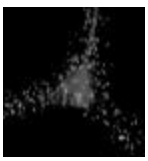
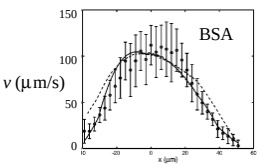
Foam drainage on microscale

tracking polystyrene tracers



g

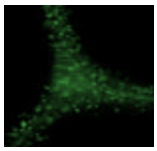


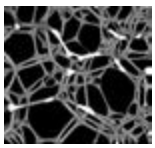
BSA protein as surfactant: like rigid wall, no-slip boundary (above)

SDS: acts as slippery boundary, flow more plug-like (not shown)

SA Koehler, S Hilgenfeldt, ER Weeks, and HA Stone, 2002, 2004

Microscopy tip



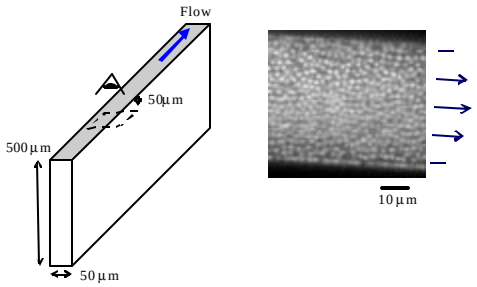


Water/air interfaces act like lenses

This works because we are at low liquid volume fraction – would be difficult/impossible for "wet foam"

Application #2: flow of suspensions through microchannels

(with Denis Semwogerere & Martin Frank at Emory, Jeff Morris at CCNY)

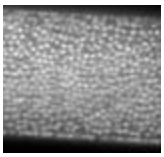


M Frank, D Anderson, ER Weeks, JF Morris, *J. Fluid Mech.* **493**, 363 (2003)

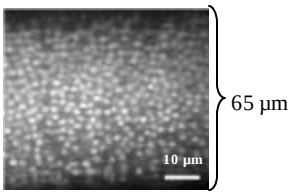
Experimental details

Suspension: 2.3 µm dia PMMA hard spheres
+ density-matched organic solvent
volume fraction f: 0.05 - 0.34

Imaging: High-speed confocal microscopy (up to 300 images per second)



Fast confocal microscopy crucial



We can image with:
- -scan speeds > 120 frames/second
- -particle speeds > 8000 µm/s
- -100× magnification

Movies: fast confocal microscopy

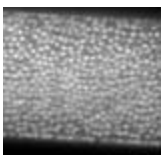
← *fast*	— *faster*	▬ *fastest*
		
v = 350 µm/s Colloids in view for ~ 90 ms	v = 1500 µm/s Colloids in view for ~ 20 ms	v = 6000 µm/s Colloids in view for ~ 5 ms

Taken with VT-eye, 160 frames/s (can go 2× faster than this)
~ 6 ms per frame, images are 30 µm wide

Microscopy tip

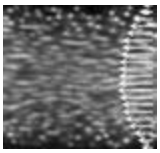
Crucial to match index of refraction for solvent, particles

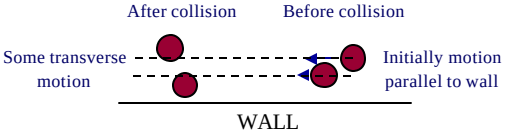
In my (limited) experience, some larger particles have a polydispersity of index of refraction: nearly impossible to see deep inside sample



Mechanism of particle migration

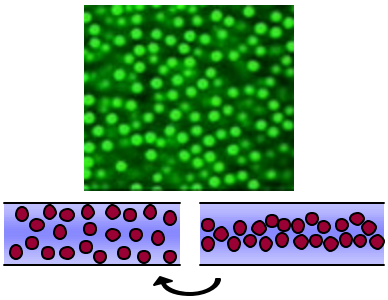
Time-lapse image of flowing particles





Phillips et al., *Phys. Fluids A* **4**, 30 (1992)

Effect of Brownian motion



homogenizes particle distribution

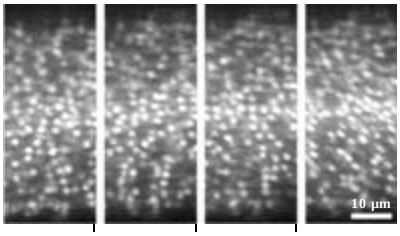
Control Parameter: Peclet number

$$Pe = \frac{t_d}{t_s} = \frac{3ph\,a^2 u}{k_B T}$$

Low *Pe* → Brownian effects strong
High *Pe* → Brownian effects weak

t : time to move own size
a: particle radius
?: viscosity
T: temperature

Experimental results: see migration



$Pe = 69$ | $Pe = 140$ | $Pe = 280$ | $Pe = 550$

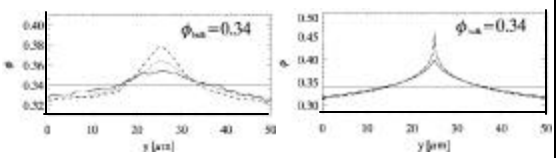
$125 \frac{\mu m}{s}$ | $250 \frac{\mu m}{s}$ | $500 \frac{\mu m}{s}$ | $1000 \frac{\mu m}{s}$

M Frank, D Anderson, ER Weeks, JF Morris, *J. Fluid Mech.* **493**, 363 (2003)

Experimental results: migration increases as *Pe* increases

Measure velocity profile, concentration profile as a function of Peclet number

Experiment *f(y)* | Model *f(y)*



M Frank, D Anderson, ER Weeks, JF Morris, *J. Fluid Mech.* **493**, 363 (2003)

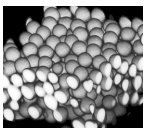
Summary

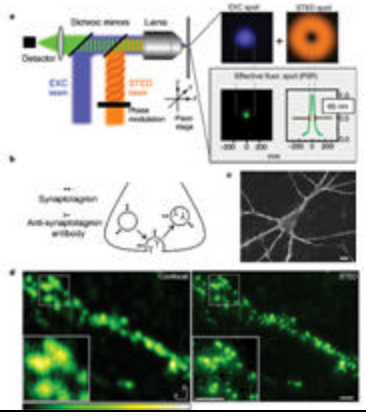
Microscopy tradeoffs:
cost, optical properties of sample, speed, 2D/3D, …

Particle tracking:
good for flow visualization
(can also use PIV: particle image velocimetry)
more to come in my next two talks

Movies, reprints, & free particle tracking software:
www.physics.emory.edu/~weeks/lab/

See especially: "Video microscopy of colloidal suspensions and colloidal crystals, " P Habdas & ER Weeks, Curr. Opinion in Coll. & Interf. Sci. (2002)





STED microscopy

Vesicles at synapse

S. Hell, Nature, April 2006

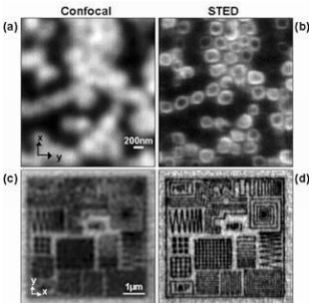
STED microscopy

STimulated Emission/Depletion
invented by Stefan Hell

~40 nm resolution

Image (d) enhanced with deconvolution

From:
www.physorg.com/news4359.html



References

Microscopy:

- P Habdas & ER Weeks, Curr. Opinion in Coll. & Interf. Sci. (2002)
- "Confocal Microscopy", D Semwogerere & ER Weeks, in *Encyclopedia of Biomaterials & Biomedical Engineering* (Taylor & Francis, 2005)
- (can download these at www.physics.emory.edu/~weeks/lab/)

Particle Tracking:

- JC Crocker & DG Grier, J. Colloid Interf. Sci. **179**, 298 (1996)
- AD Dinsmore et al., App. Optics **40**, 4152 (2001)
- free software: www.physics.emory.edu/~weeks/idl/

Applications:

- Foam drainage: SA Koehler et al., PRE **66**, 040601 (2002)
- Migration: M Frank et al., JFM **493**, 363 (2003)