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Experimental Methods notes

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GENERAL TUTORIAL SESSION #3

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EXPERIMENTAL TECHNIQUES

1. OPTICAL TWEEZERS - DR. MATTHEW J. LANG (MIT)

FROM MOLECULAR MECHANICS TO CELLULAR MACHINERY

FORCES ON OPTICAL TRAPS

- SCATTER ⇒ PUSH BEAD OUT OF FOCAL POINT
- GRADIENT ⇒ PUSH BEAD TOWARDS FOCAL POINT

STABLE TRAP: $F_{SCATTER} = F_{GRADIENT}$

TRAPPING LASERS ⇒ NEAR INFRA-RED (LESS DAMAGING TO BIOLOGICAL SPECIMENS)

CALIBRATIONS ⇒ CHARACTERIZE TRAP STIFFNESS

$$F_{TRAP} = K \Delta x$$

K = TRAP STIFFNESS (HOOKEAN)

Δx = DISPLACEMENT OF PARTICLE FROM CENTER OF TRAP

2. ATOMIC FORCE MICROSCOPY (AFM) AND MOLECULAR FORCE MICROSCOPY - DR KRYSTYN VAN VLIET (MIT)

AFM ⇒ "SEEING BY FEELING"

↳ CANTILEVER WITH PROBING TIP ⇒ MEASURES VERTICAL DISPLACEMTS / FORCES

★ FEEDBACK LOOP TO APPLY CONSTANT FORCE WHILE IMAGING. (FINE STRUCTURE)

HIGH RESOLUTION DUE TO ERROR BETWEEN CONSECUTIVE POINT SCANS

FOR FORCE SPECTROSCOPY (MOLECULAR) THE AFM CAN BE USE

TO "POKE" THE SPECIMEN AND THEN RETRACT THE PROBE AND LOOK FOR THE HYSTERISIS TO CHARACTERIZE THE TIP-SPECIMEN INTERACTION. THIS APPROACH CAN BE USED TO PROBE SINGLE MOLECULAR INTERACTIONS ⇒ DIFFICULTY IN OBTAINING THE RIGHT CHEMISTRY

3. MICRORHEOLOGY OF COMPLEX FLUIDS - DR. PETER SO (MIT)

- ELASTIC COMPONENT (G')
- LOSS COMPONENT (G'')

$G^*(\omega) = G'(\omega) + iG''(\omega)$

COMPLEX SHEAR MODULUS IS FREQUENCY DEPENDENT

(ACTUAL DEFORMATION)

- ACTIVE METHOD → MAGNETIC MICRORHEOMETER
- PASSIVE METHOD (THERMAL FORCES) → SINGE PARTICLE TRACKING, MULTIPLE PARTICLE TRACKING } HIGH SPEED

MAGNETIC MICRORHEOMETER

→ GENERATES MAGNETIC FIELD TO ATTRACT FERRO-MAGNETIC PARTICLE

→ FORCE DEPENDS ON FIELD GRADIENT AND PARTICLE VOLUME

→ BANDWITH LIMITED BY INDUCTANCE (~ KHz)

PROBLEMS:

→ SIZE OF PARTICLE

→ BEAD ROLLING ON SURFACE

SINGLE PARTICLE TRACKING

THERMAL FORCES: LANGEVIN EQUATION

$$\underbrace{m\dot{v}}_{\text{ACCELERATION}} = \underbrace{f(t)}_{\text{FORCE}} + \underbrace{\int_0^t \xi(t-t')\, v(t')\, dt'}_{\text{MEMORY FUNCTION}}$$

WHAT NEEDS TO BE MEASURED IS THE MEAN SQUARE DISPLACEMENT (MSD)

$$\langle \Delta R^2(\tau) \rangle = \langle (\vec{r}(t+\tau) - \vec{r}(t))^2 \rangle$$

$\vec{r}(t)$ COMES FROM POSITION TRACKING OF PARTICLE

- RESOLUTION DEPENDS ON AMOUNT OF PHOTONS REACHING THE DETECTOR.

<u>TWO- AND MULTIPLE PARTICLE TRACKING</u>

LOOKS AT CORRELATED MOTION BETWEEN PARTICLES

→ DOES NOT DEPEND ON PARTICLE SIZE BUT ON DISTANCE BETWEEN PARTICLES

→ MEASURES GLOBAL PROPERTIES INSTEAD OF LOCAL PROPERTIES

→ DRAWBACK: USE OF WIDE FIELD CAMERA INSTEAD OF PHOTODIODE ⇒ LOWER TIME RESOLUTION

4. MICROSCOPY - DR. PETER SO (MIT)

SEE SLIDES FOR RAY TRACING EXAMPLES AND MICROSCOPE MODEL.

DIFFRACTION AND INTERFERENCE DEVIATES IDEAL OPTICS INTO "REAL" OPTICS

POINT SPREAD FUNCTION (PSF) ⇒ IMAGE OF AN IDEAL POINT

λ ← LIGHT WAVELENGHT

FWHM $\tilde{}$ $\frac{\lambda}{2}$

IN REAL IMAGING WHAT WE SEE IS THE CONVOLUTION OF THE OBJECT WITH THE PSF ⇒ OBJECT LOOKS "FUZZY"

CONVOLUTION THEOREM: THE CONVOLUTION IN SPATIAL DOMAIN IS EQUIVALENT TO THE MULTIPLICATION IN THE FREQUENCY DOMAIN (FOURIER SPACE)

OTF ⇒ OPTICAL TRANSFE FUNCTION

↳ LOSS OF HIGH FREQUENCY COMPONENTS

HOW TO FIX THIS PROBLEM??

→ DECONVOLUTION

$$\tilde{I}(\vec{k}) = \tilde{O}(\vec{k}) \cdot OTF(\vec{k})$$

↓

DECONVOLUTION

$$\tilde{O}(\vec{k}) = \tilde{I}(\vec{k}) \cdot OTF(\vec{k})^{-1}$$

↓

$$O(\vec{r}) = \mathcal{F}^{-1}[\tilde{O}(\vec{k})]$$

$\mathcal{F}$ = FOURIER TRANSFOR

$\mathcal{F}^{-1}$ = INVERSE $\mathcal{F}$

CONFOCAL MICROSCOPY

ONLY THE LIGHT IN THE FOCAL PLANE IS AQUIRED, SO YOU CAN HAVE DEPTH DISCRIMINATION AND GENERATE 3D IMAGES.

MULTIPHOTON MICROSCOPY

- ONLY IMAGES AT HIGH PHOTON FLUX ⇒ FOCAL POINT
  ↳ 3D RESOLUTION
- USE IR ⇒ LESS DAMAGING TO BIOLOGICAL SPECIMENS
- LESS PHOTOTOXICITY

5. MICROPIPETTE ASPIRATION & MICRO FLUIDICS

DR. C.T. LIM (NATIONAL UNIVERSITY OF SINGAPORE)

MALARIA ⇒ STIFFENS THE CELL

USE μ-PIPETTE AND μ-FLUIDICS TO STUDY MECHANICAL PROPERTIES OF INFECTED RED BLOOD CELLS (RBC)

DUAL PIPETTE ASSAY ⇒ APPLY LOW FORCES TO SEPARATE ADHERENT CELLS OR PROTEIN COATED LIPOSOMES

MICROFLUIDICS ⇒ SMALL SAMPLE VOLUME

LOOK AT CELLS DEFORMING WHILE PASSING THROUGH A NARROW CHANNEL (~2μm)

6. MEMS BASED TOOLS -

LAST TWO LECTURES MISSING!!