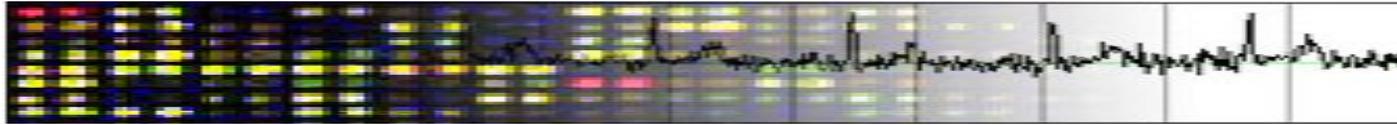


Biomedical Information Technology

2.771J BEH.453J HST.958J Spring 2005

Lecture 21 April 2005

Biological Image Information I: A Description of the Modalities



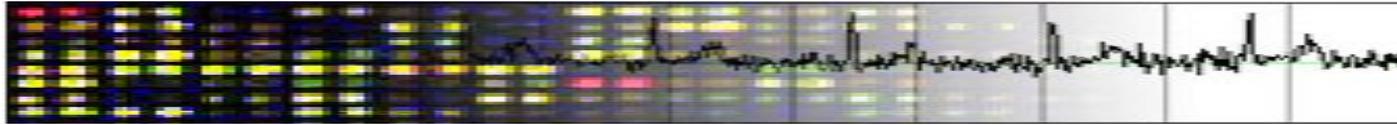
The Modalities

- ❖ Direct photography
- ❖ SEM and TEM
- ❖ Cryo-EM
- ❖ Two-photon microscopy
- ❖ Confocal microscopy
- ❖ Fluorescence microscopy
- ❖ Common requirements



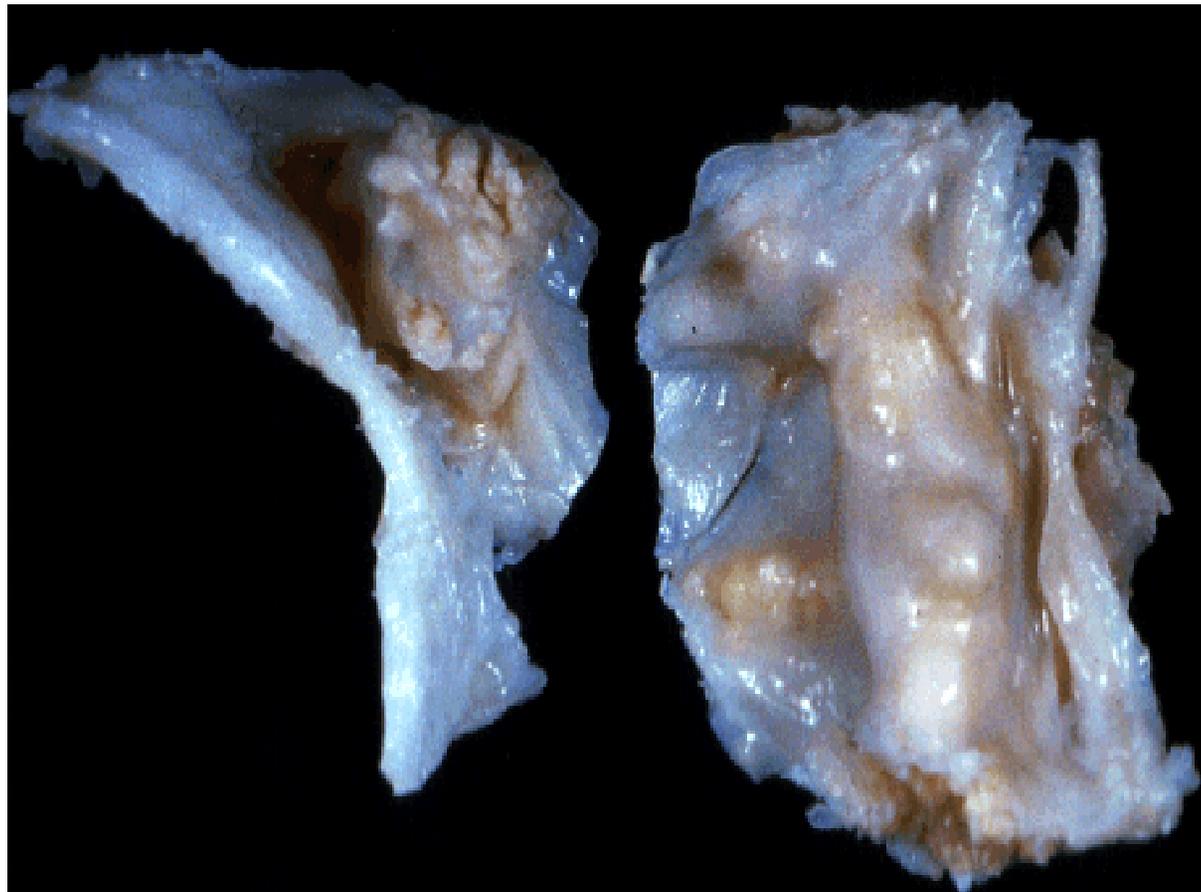
Consider your personal camera

- *Camera type*
- *Lens type and focal length*
- *Shutter speed*
- *Exposure time*
- *Focal distance*
- *Resolution*
- *Compression*
- *Flash settings*
- *White balance*
- *Special effects (e.g. incandescent)*



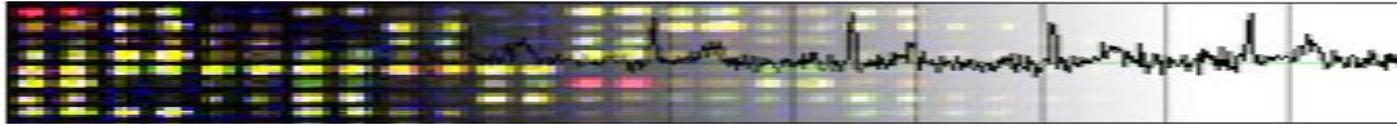
Direct photography for macroscopic specimens

Human Carotid Bifurcation



Ref: Dr. John Fallon, Mass. General Hosp. © cfdewey 2005

Courtesy of Dr. John Fallon. Used with permission.



Conventional wide-field microscopy

- ❖ Light source
 - Mercury arc lamp
 - Laser
 - Inverted for fluorescence
- ❖ Detector
 - CCD array
 - Cooled -10C
 - Background light
 - Dark current

Image removed for copyright reasons.

Fig.: www.microscopyu.com

Confocal microscopy

- ❖ Scanning confocal

Image removed for copyright reasons.

Fig.: www.microscopyu.com

- ❖ Spinning disk confocal

Confocal microscopy information flow

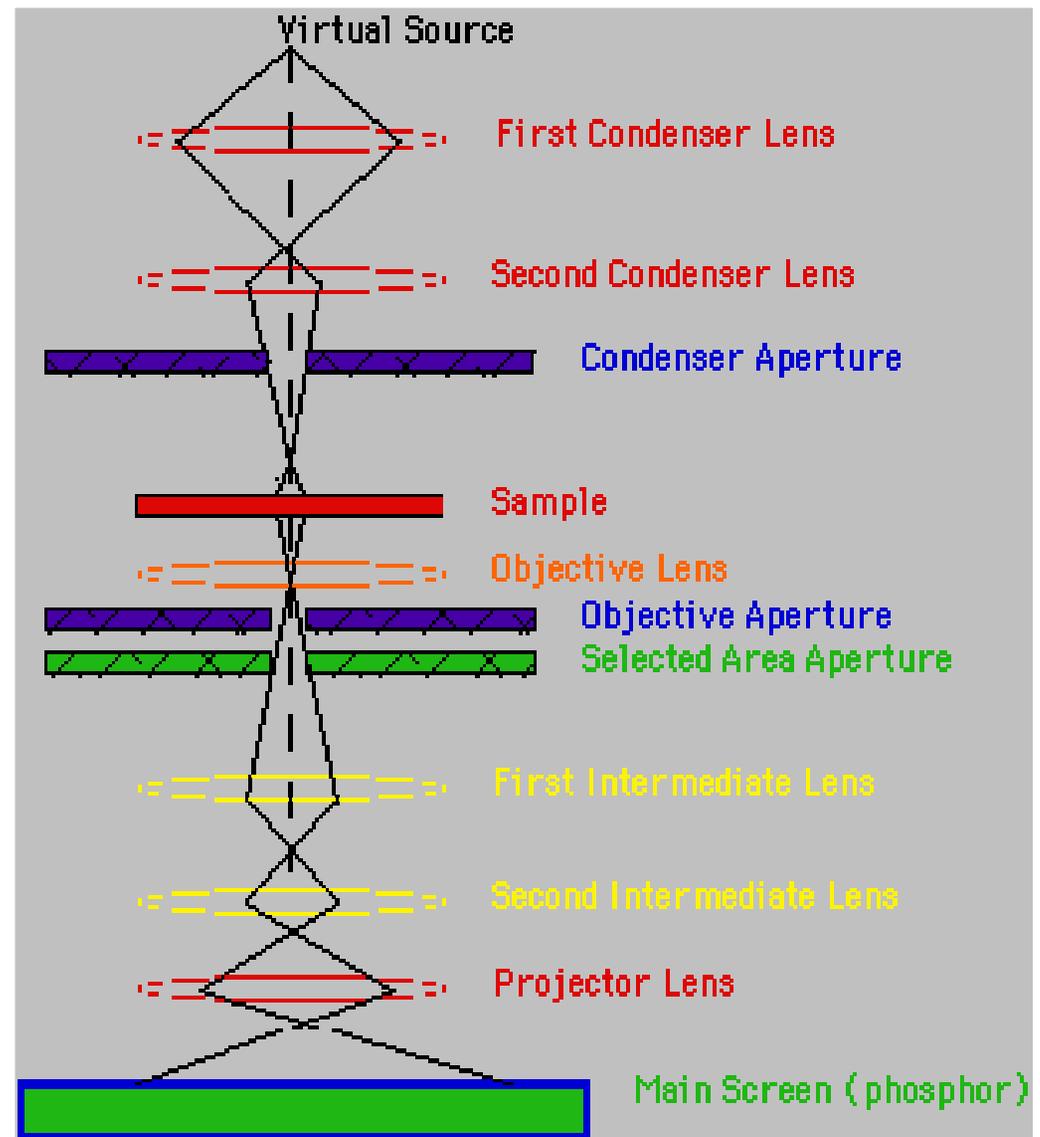
Image removed for copyright reasons.

Fig.: www.microscopyu.com



SEM and TEM

- ❖ SEM: Scanning Electron Microscopy
- ❖ TEM: Transmission Electron Microscopy



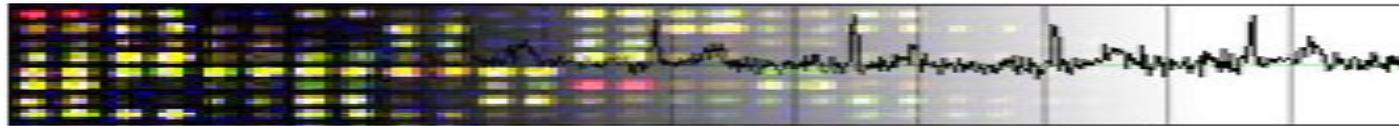
Ref: <http://www.unl.edu/CMRAcfem/temoptic.htm>

Unaligned cytoskeleton

0.2 μm |

Image removed for copyright reasons.

S-1 Label, unoriented. From R. Satcher et al., Microcirculation (1995)



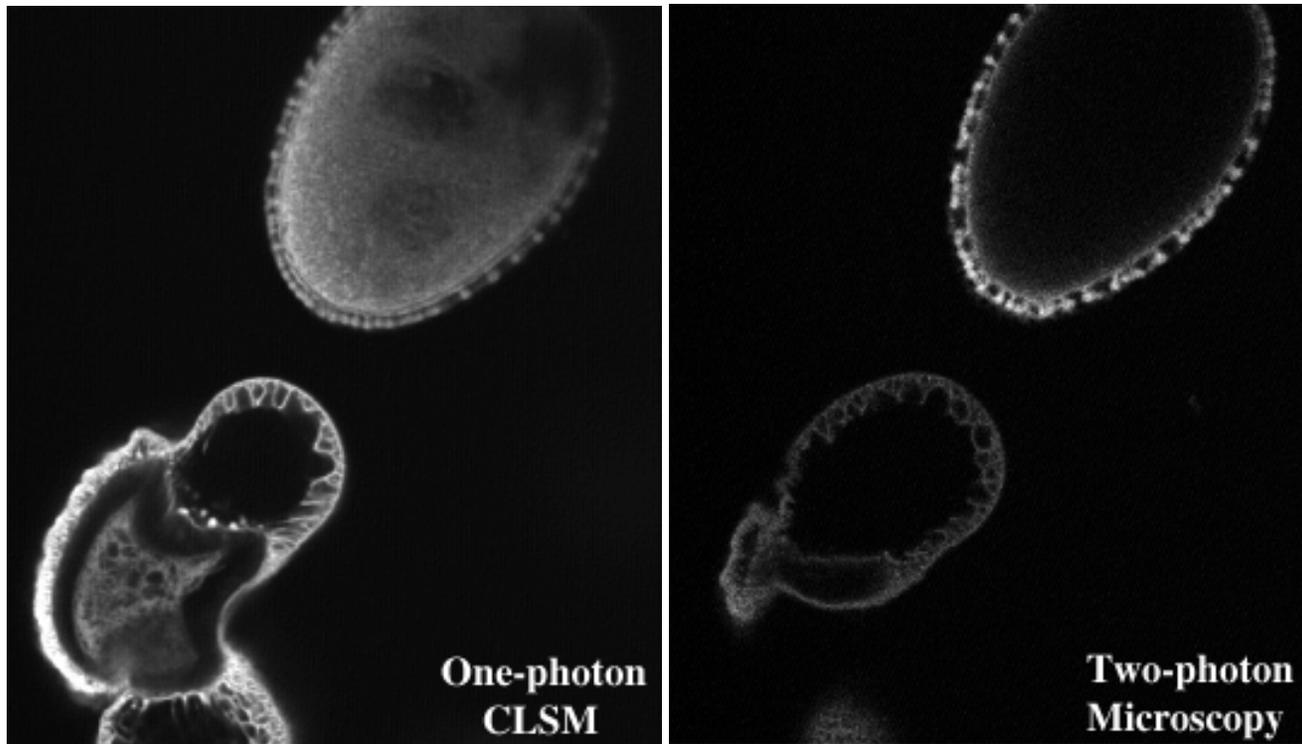
Cryo-EM

- ❖ Used to get crystal structure, esp. of proteins
- ❖ Many angles of images recorded
- ❖ Cryo techniques to stop thermal motion and reduce damage during irradiation. Can also prepare very thin biological samples without shadowing artifacts.
- ❖ Resolution down to ~ 0.2 nm in common systems.

*For a general tutorial and list of references, see
<http://em-outreach.sdsc.edu/web-course/toccontents.html>*

Two-photon microscopy

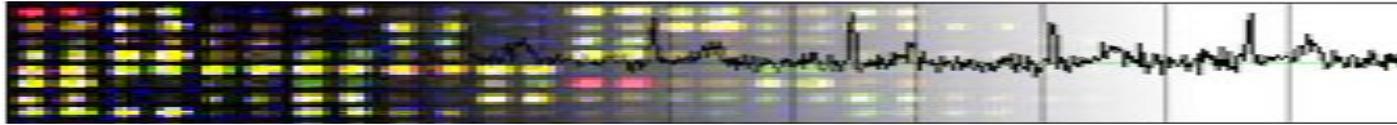
Two-photon excitation of fluorescence is based on the principle that two photons of longer wavelength light are simultaneously absorbed by a fluorochrome which would normally be excited by a single photon with a shorter wavelength. The images below, of pollen granules, clearly show the differences. The two-photon image was generated using 704nm excitation light while the one-photon image was generated using 488nm.



Ref: http://www.cbit.uchc.edu/microscopy_nv/two_photon.html

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Courtesy of Paul Campagnola. Used with permission.



Fluorescence microscopy

- ❖ Quantitative analysis of content
- ❖ Spatial distribution of proteins
- ❖ Study dynamic changes of cell content
- ❖ Total internal reflection methods
- ❖ Issues:
 - Characterization of dye and filters for detection
 - Bleaching and activation methods
 - Calibration of absolute intensity
 - Signal to noise for small samples

Use of alternative fluorescent dyes *(Osborn)*

live

fix → detergent extract

Six photos removed for copyright reasons.

EGFP-actin

A546-phalloidin

merge

Use of multiple fluorescent dyes

Dr. Mario E. Lacouture Chicago
Human osteosarcoma cells (800x)
<http://www.microscopyu.com/smallworld/gallery/contests/2002/honorablelarge06.html>

**Photo removed for
copyright reasons.**

Prof. Paul Campagnolo U Conn
SHG/TPEF Imaging of Pregnant Worm
http://www.cbit.uhc.edu/faculty_nv/campagnola/shg.html

**Photo removed for
copyright reasons.**

Use of multiple fluorescent dyes

Dr. Thomas J. Deerinck

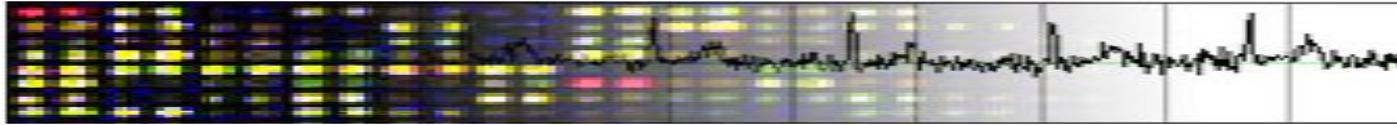
National Center for Microscopy & Imaging Research

UCSD La Jolla, California, USA.

Sagittal section of mouse cerebellum (40x) Fluorescence and Confocal

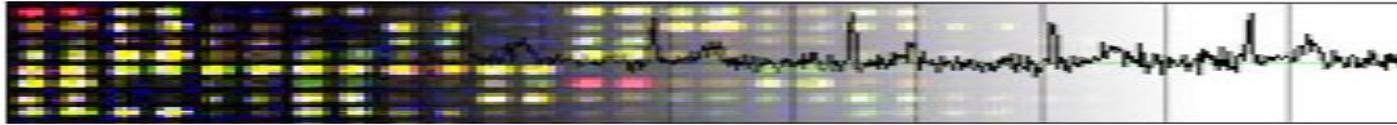
<http://www.microscopyu.com/smallworld/gallery/contests/2002/1stverylarge2002.html>

**Photo removed for
copyright reasons.**



Total internal reflectance microscopy

Image removed for copyright reasons.



Fluorescent tagging of molecules

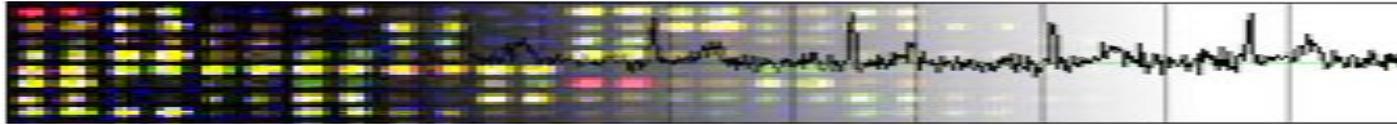
❖ PAF:

PhotoActivated Fluorescence

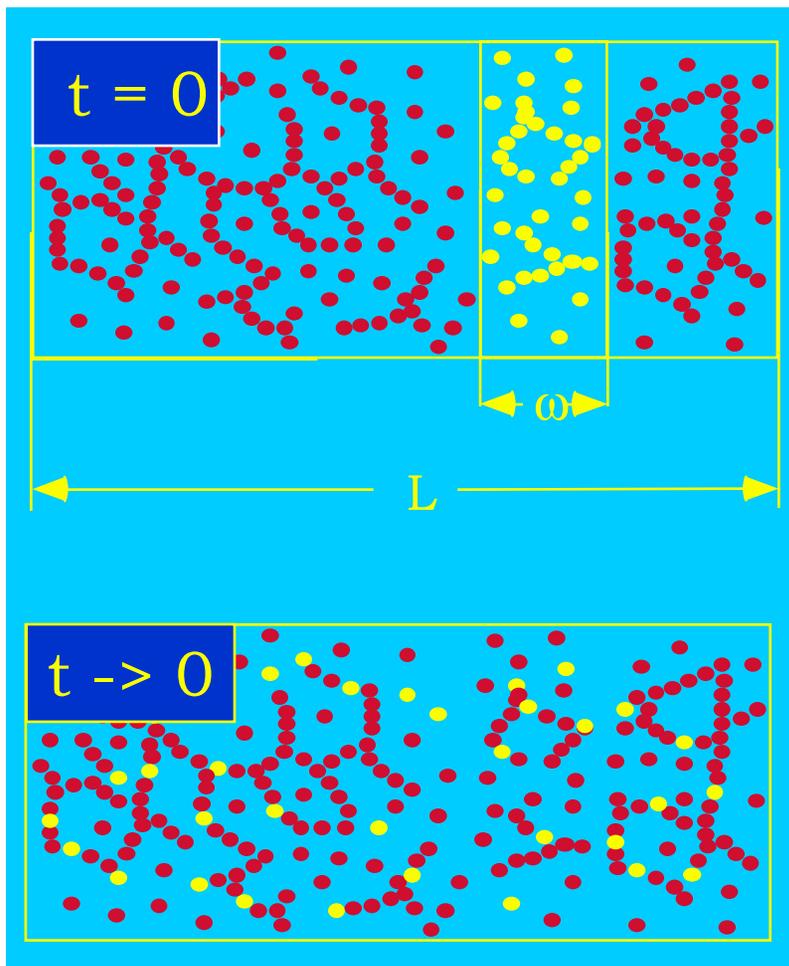
❖ FRAP:

Fluorescence Recovery After Photobleaching

❖ Advantages and limitations of the methods

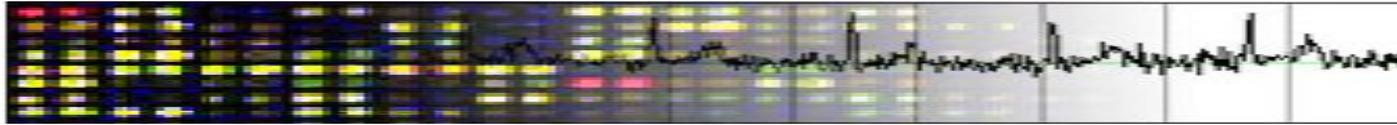


Interpreting PAF/FRAP



Idealizations:

- 1) Geometry
- 2) Uniform actin distributions and dynamics
- 3) No filament diffusion



Interpreting PAF/FRAP

Monomer transport

$$\frac{\partial C_m^*}{\partial t^*} = \frac{\partial^2 C_m^*}{\partial x^{*2}} + \gamma\beta(C_f^* - C_m^*)$$

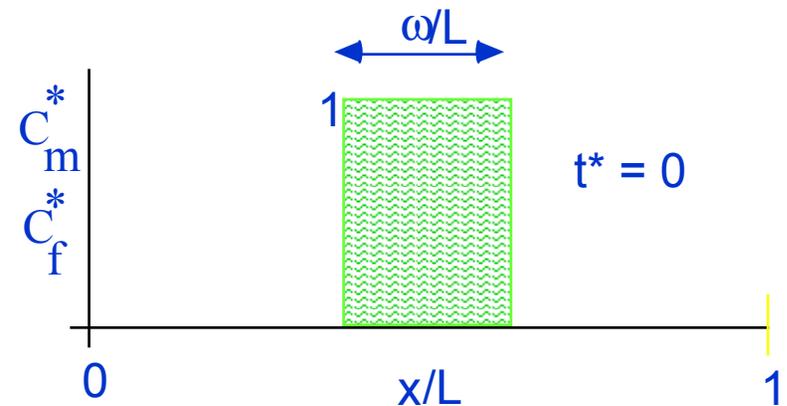
Polymer transport

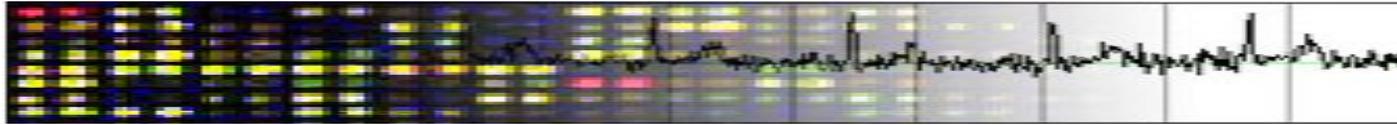
$$\frac{\partial C_f^*}{\partial t^*} = -\beta(C_f^* - C_m^*)$$

Boundary Conditions

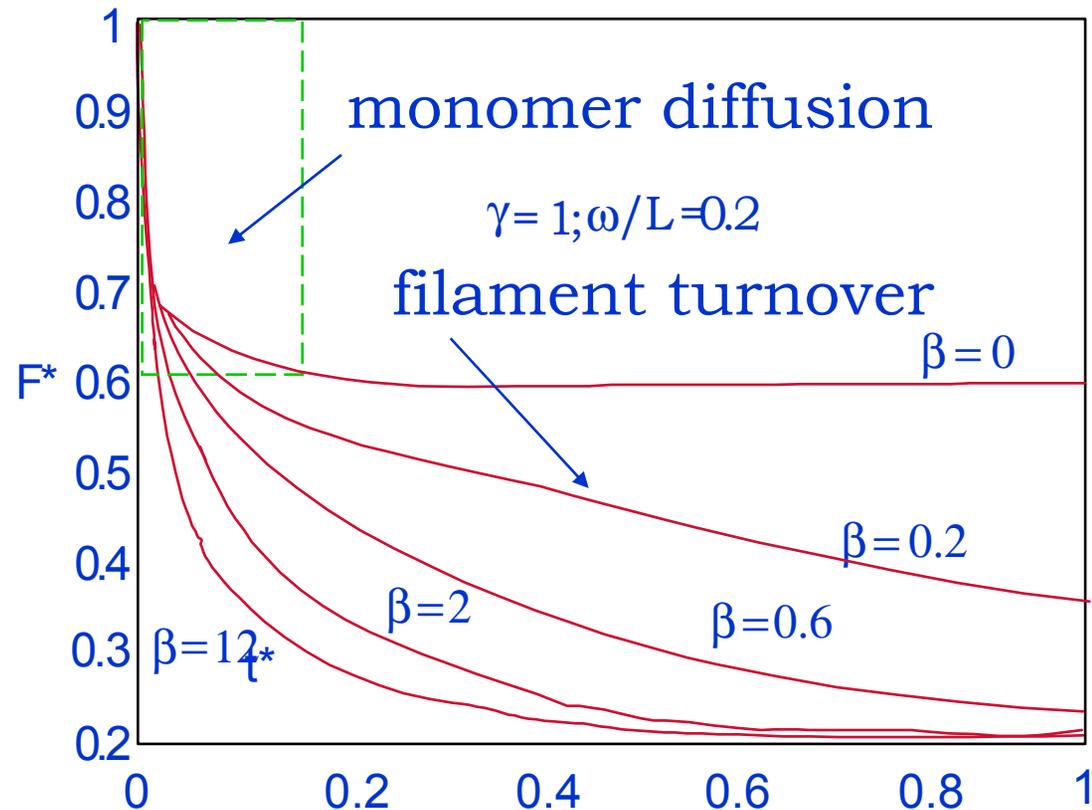
$$\left. \frac{\partial C_m^*}{\partial x^*} \right|_{x^*=0} = 0, \quad \left. \frac{\partial C_m^*}{\partial x^*} \right|_{x^*=1} = 0$$

Initial Conditions



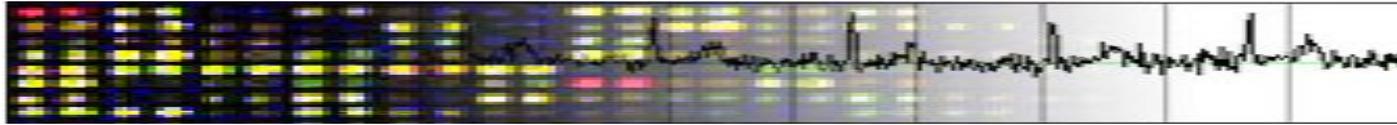


Interpreting PAF

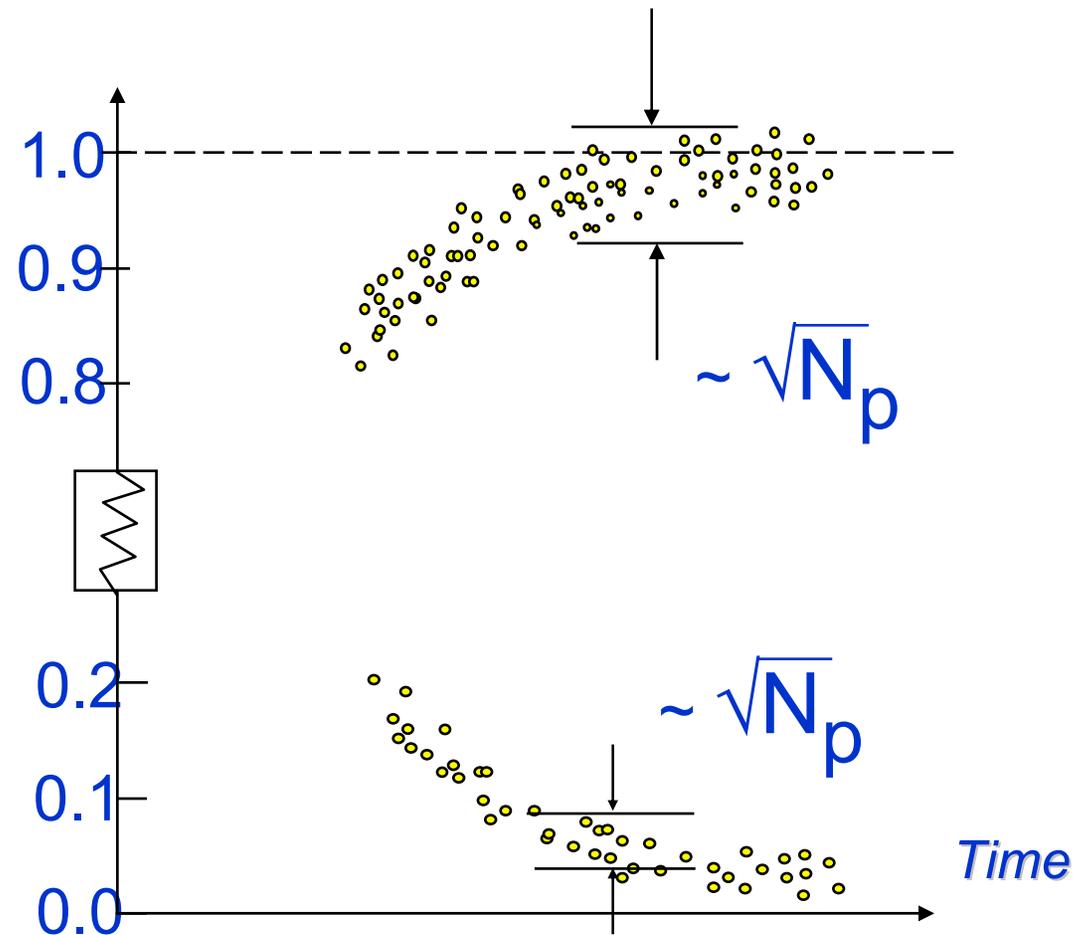


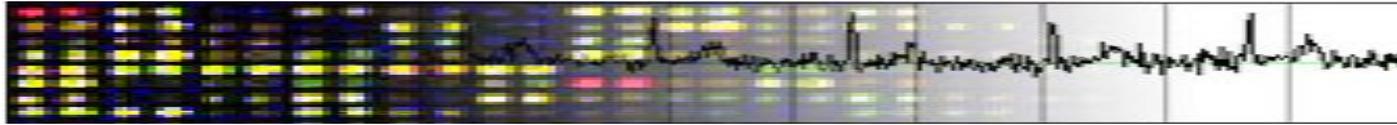
$$\beta = \frac{\omega^2}{D \tau}$$

$\beta \gg 1$ implies diffusion slower than turnover
 $\beta \ll 1$ implies turnover slower than diffusion



Experimental issues: signal – to- noise





Conclusions re image information objects

- ❖ Images can be large:
 - 2-D and 3 MB
 - 3-D and 40 MB
 - 4-D and ~ 1TB
- ❖ Dynamic changes of content:
 - Serial pictures, need **Study** and **Series**
- ❖ Large variation in parameters for each experimental method. Maybe 100 parameters for each method
- ❖ Alignment and normalization issues with sequential images. Lots of metadata.