

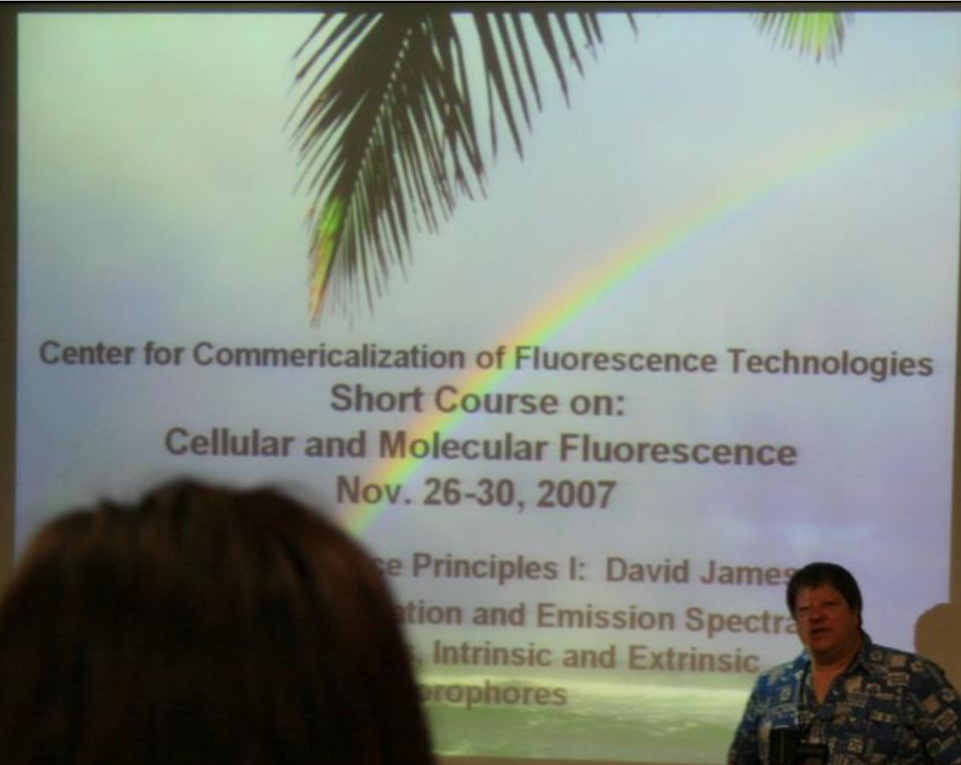
EXIT

Center for Commercialization of Fluorescence  
Short Course on  
Cellular and Molecular Fluorescence  
Nov. 26-30, 2007

Basic Fluorescence Principles  
Absorption, Excitation, and  
Quantum Yields, Intensity  
Fluorescence





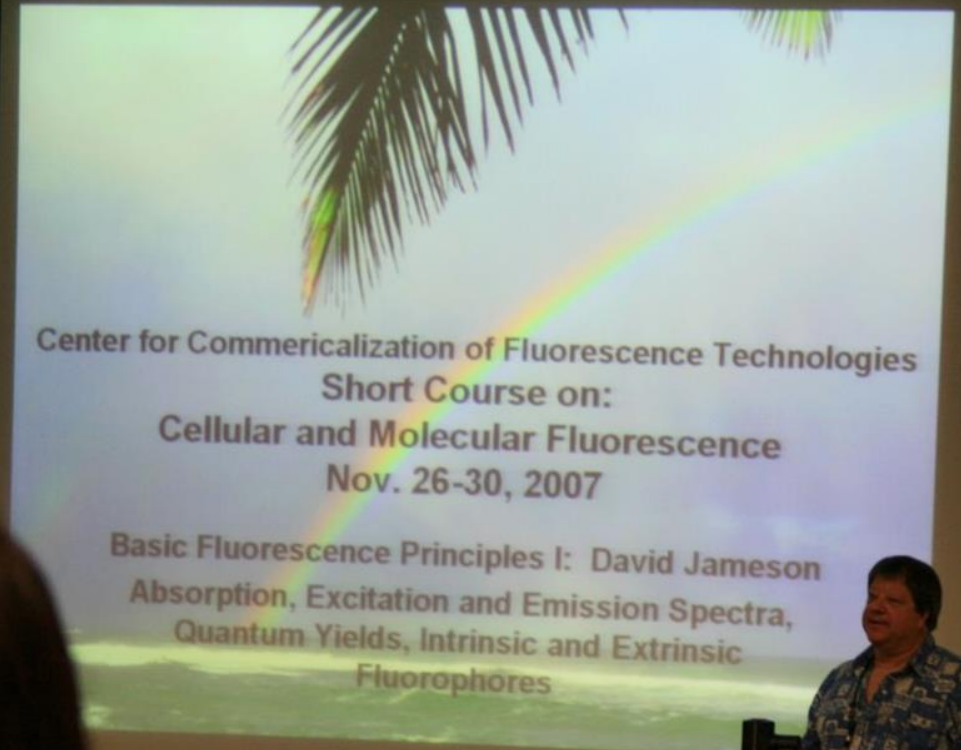


Center for Commercialization of Fluorescence Technologies  
Short Course on:  
Cellular and Molecular Fluorescence  
Nov. 26-30, 2007

... Principles I: David James  
... and Emission Spectra  
... Intrinsic and Extrinsic  
... Phosphores







Center for Commercialization of Fluorescence Technologies  
Short Course on:  
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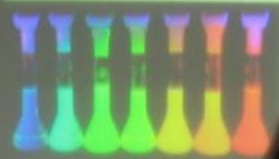
Basic Fluorescence Principles I: David Jameson  
Absorption, Excitation and Emission Spectra,  
Quantum Yields, Intrinsic and Extrinsic  
Fluorophores





### Why fluorescence?

- its pretty!
- it provides information on the molecular environment
- it provides information on dynamic processes on the nanosecond timescale



Fluorescence is essentially...  
m...  
sh...  
th...

EXIT

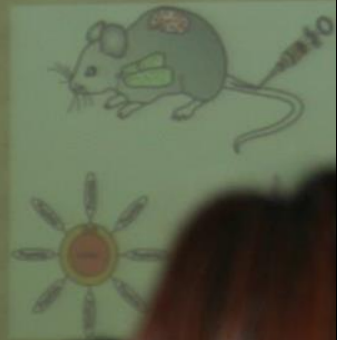


EXIT



# Nanocrystal

Blood vessels express molecular  
organs,  
Peptides that recognize these vasc  
atta



## ng in vivo

sh the vasculature of individual  
ES.  
e been identified, purified and

n of the peptides directed the  
s to the appropriate site in the  
showing that nanocrystals can  
argeted *in vivo* with an exquisite  
specificity.

1. Schematic representation of  
targeting. Intravenous delivery of  
into specific tissues of the mouse.  
s were coated with either peptides  
or with peptides and PEG. PEG  
the Qdots maintain solubility in  
in solvents and minimize  
specific binding.

5, 2003 | vol. 9 | no. 20 | 12657-52621

























ORTH TEXAS HEALTH SCIENCE CENTER AT FORT WORTH  
Center for BioHealth

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200  
201  
202  
213















































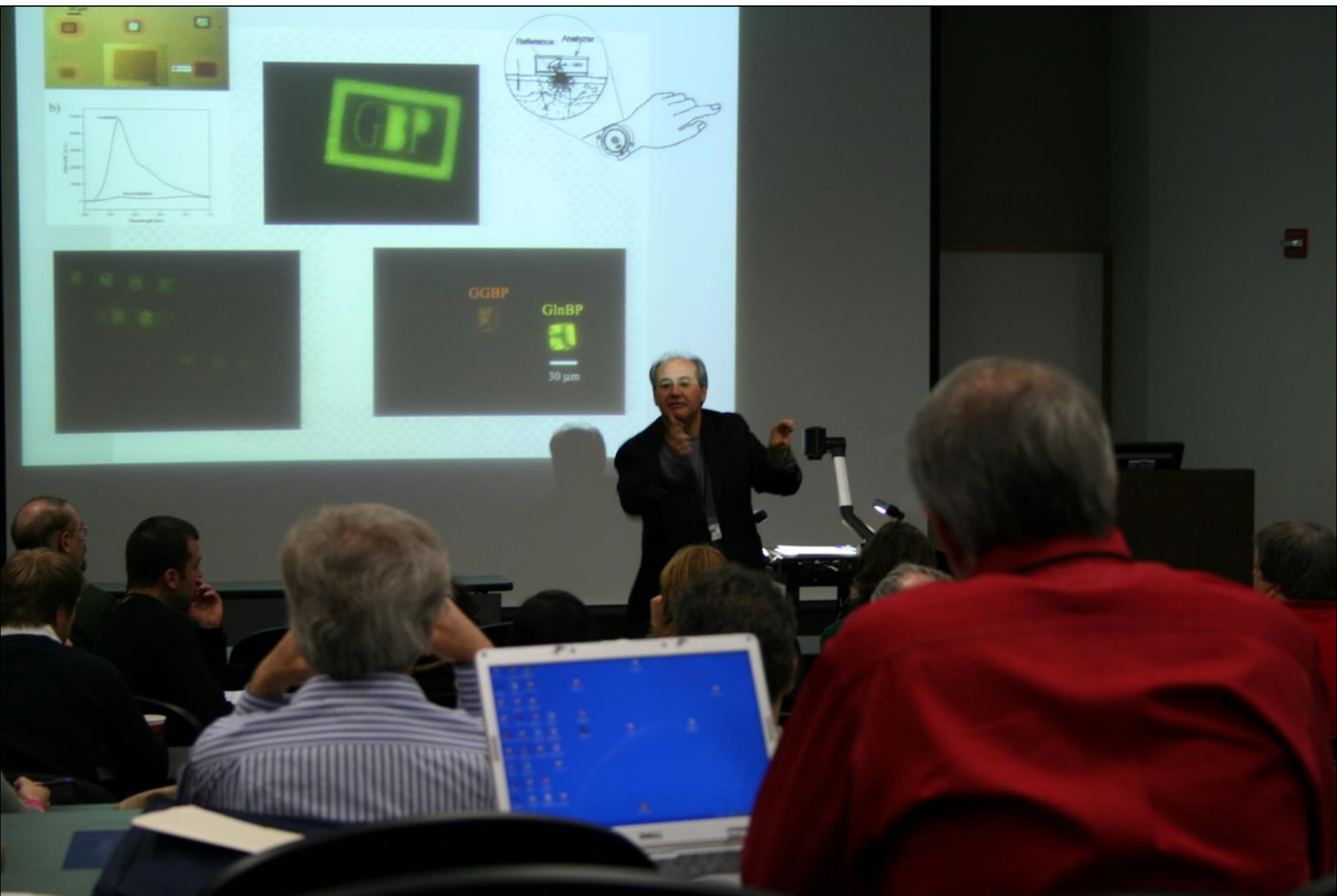




The binding protein family has a structure organized  
in two main domains  
that form the ligand binding site





























# Total Internal Reflection Fluorescence Microscopy

## Principles

### Excitation

1. Plane wave refraction/reflection at glass/water interface for TIR
2. Propagation, phase, polarization, and intensity of the evanescent electric field
3. Incidence angle and evanescent illumination penetration depth
4. Metal film at the glass/water interface and surface plasmon resonance

### Emission

1. Dipole emitter near and far-field
2. Plane wave expansion facilitating refraction/reflection at an interface
3. Manifestations of the near-field
4. Emission intensity vs polar angle
5. Emission intensity into a 1.45 NA objective vs dipole distance to interface









# Total Internal Reflection Fluorescence Microscopy

## Principles

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### Future Nanomedicine and Diagnostics

Combinatorial Nanomedicine: A New Paradigm for Drug Discovery and Delivery



Microfluidic, self-assembling, and programmable nanomedicine for targeted drug delivery and diagnostic applications.

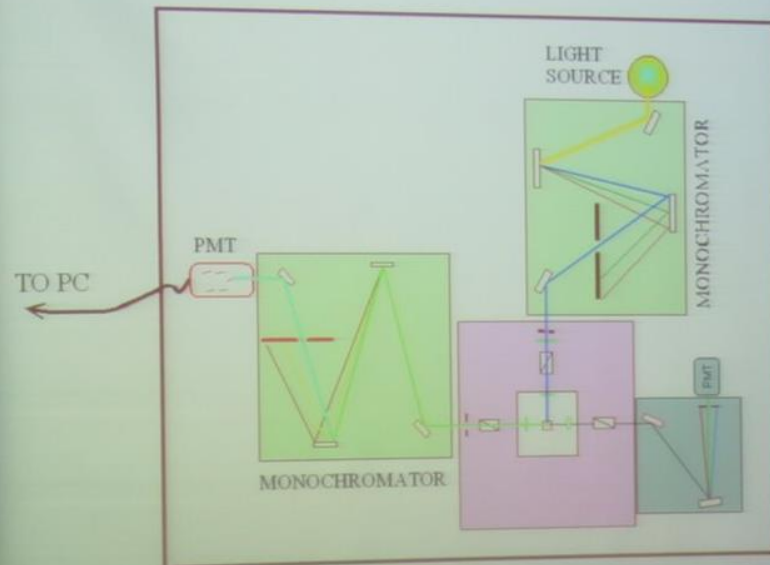
Combinatorial nanomedicine for drug discovery and delivery.

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# BLOCK SCHEMATIC OF SPECTROFLUOROMETER





# Multi-Photon Induced Fluorescence Spectroscopy

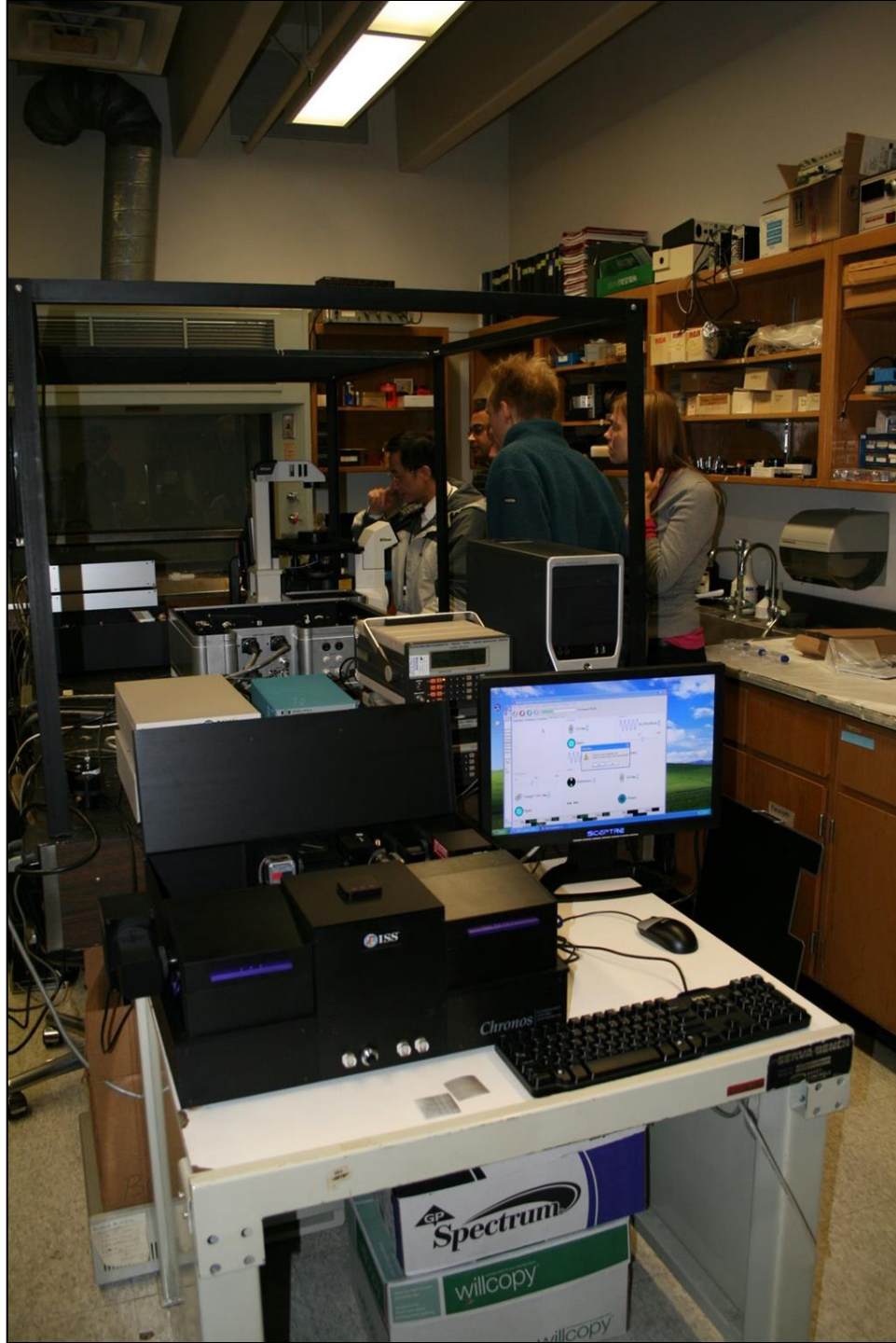
Ignacy Gryczynski

Center for Commercialization of Fluorescence Technologies



UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER  
FORT WORTH

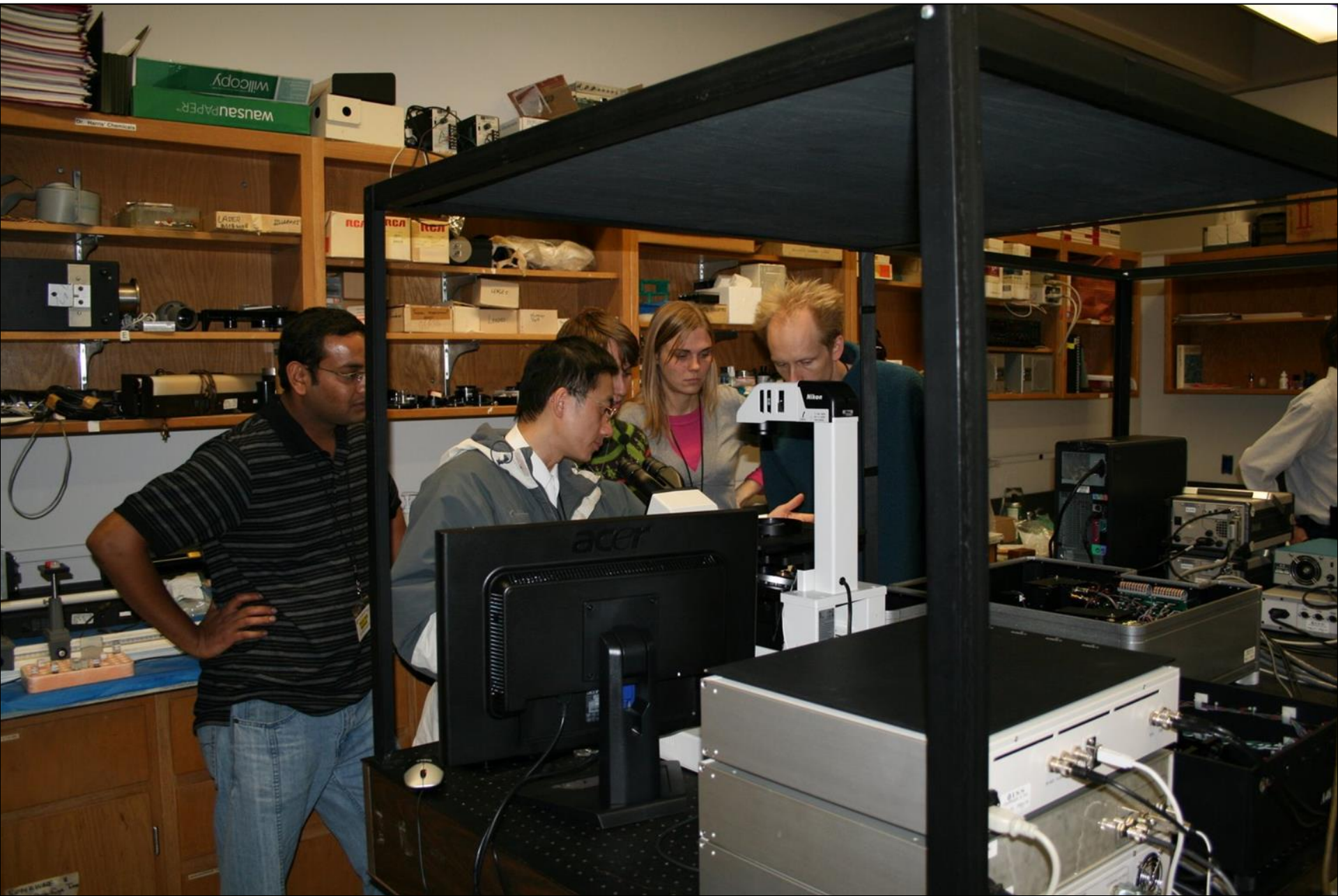














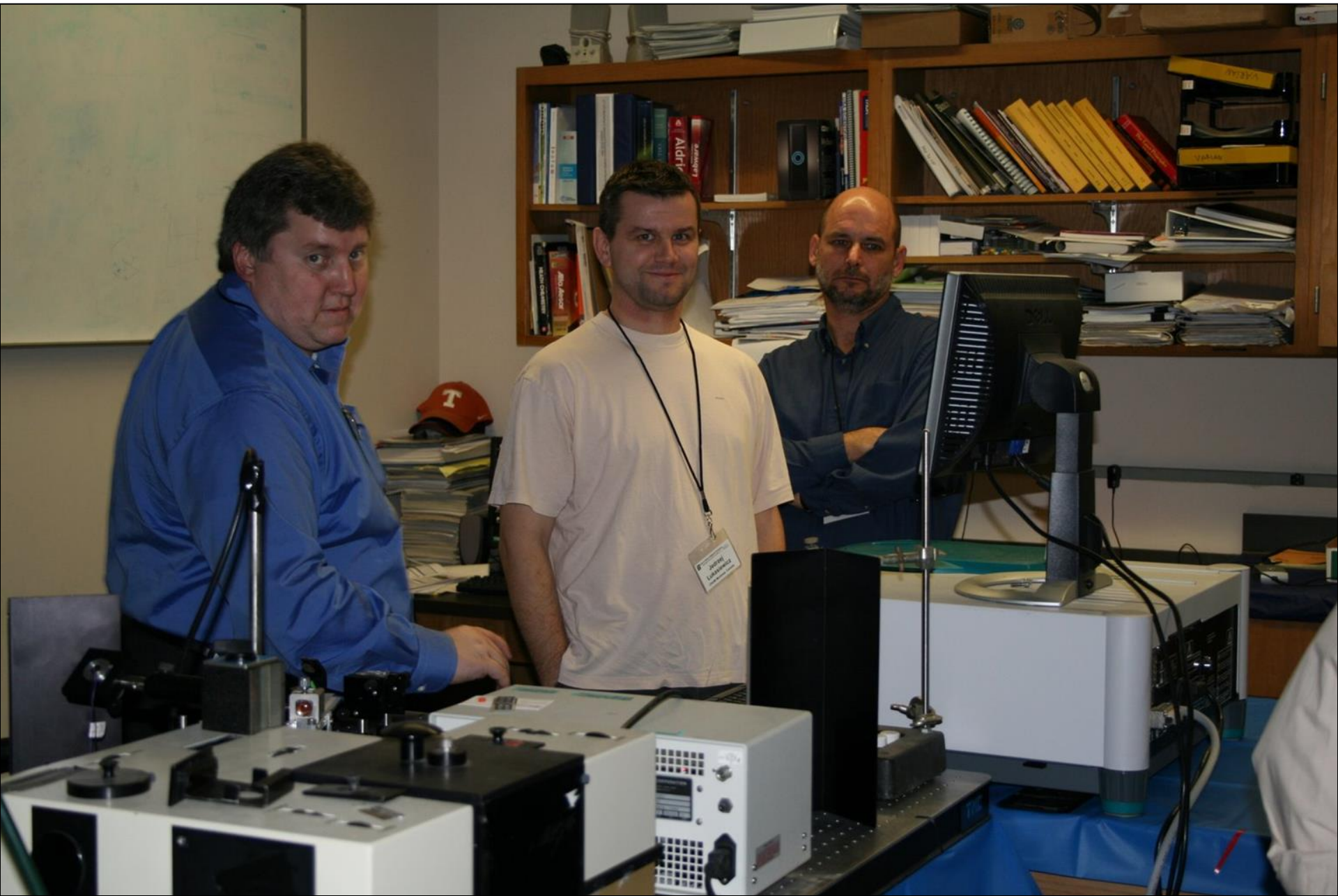




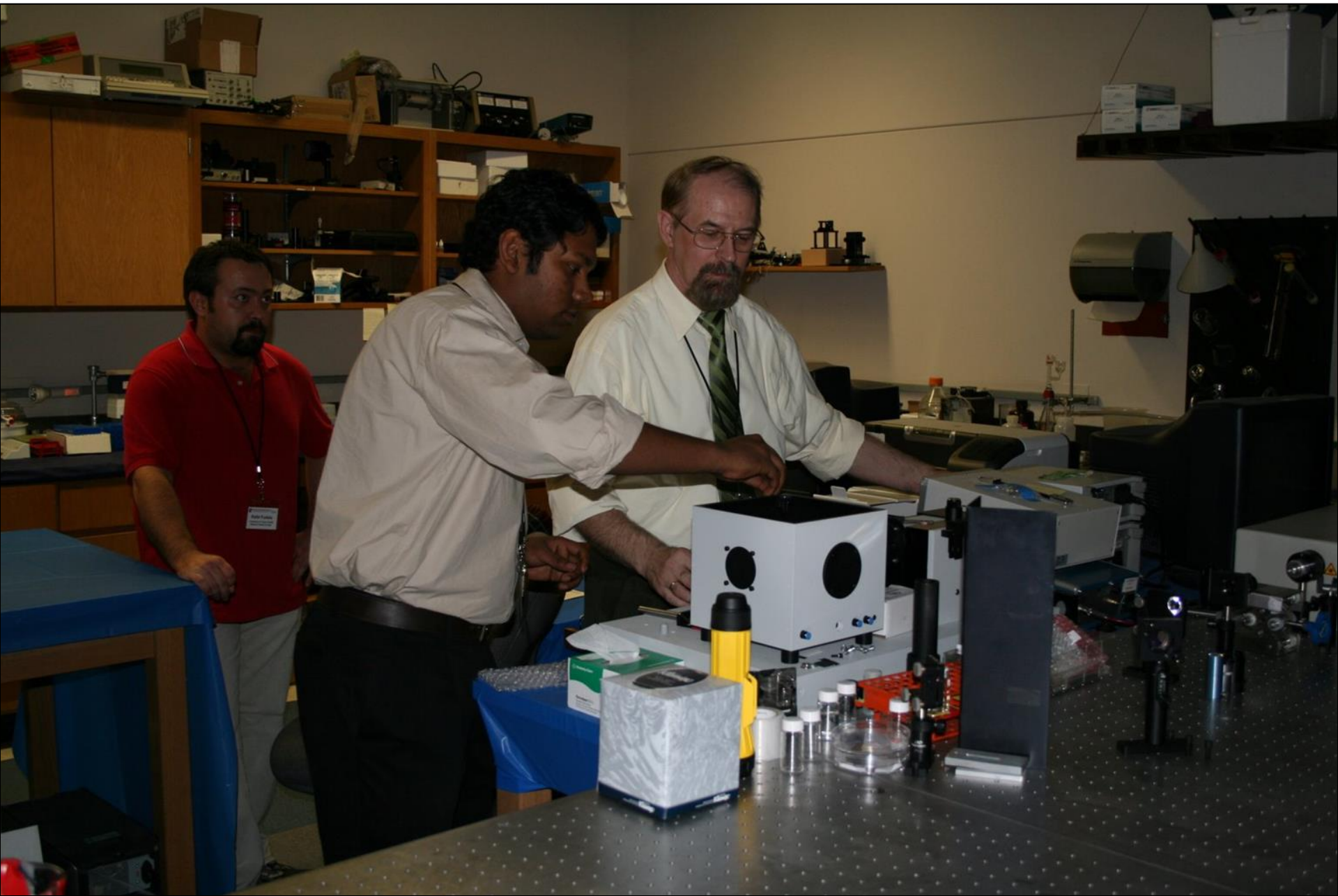








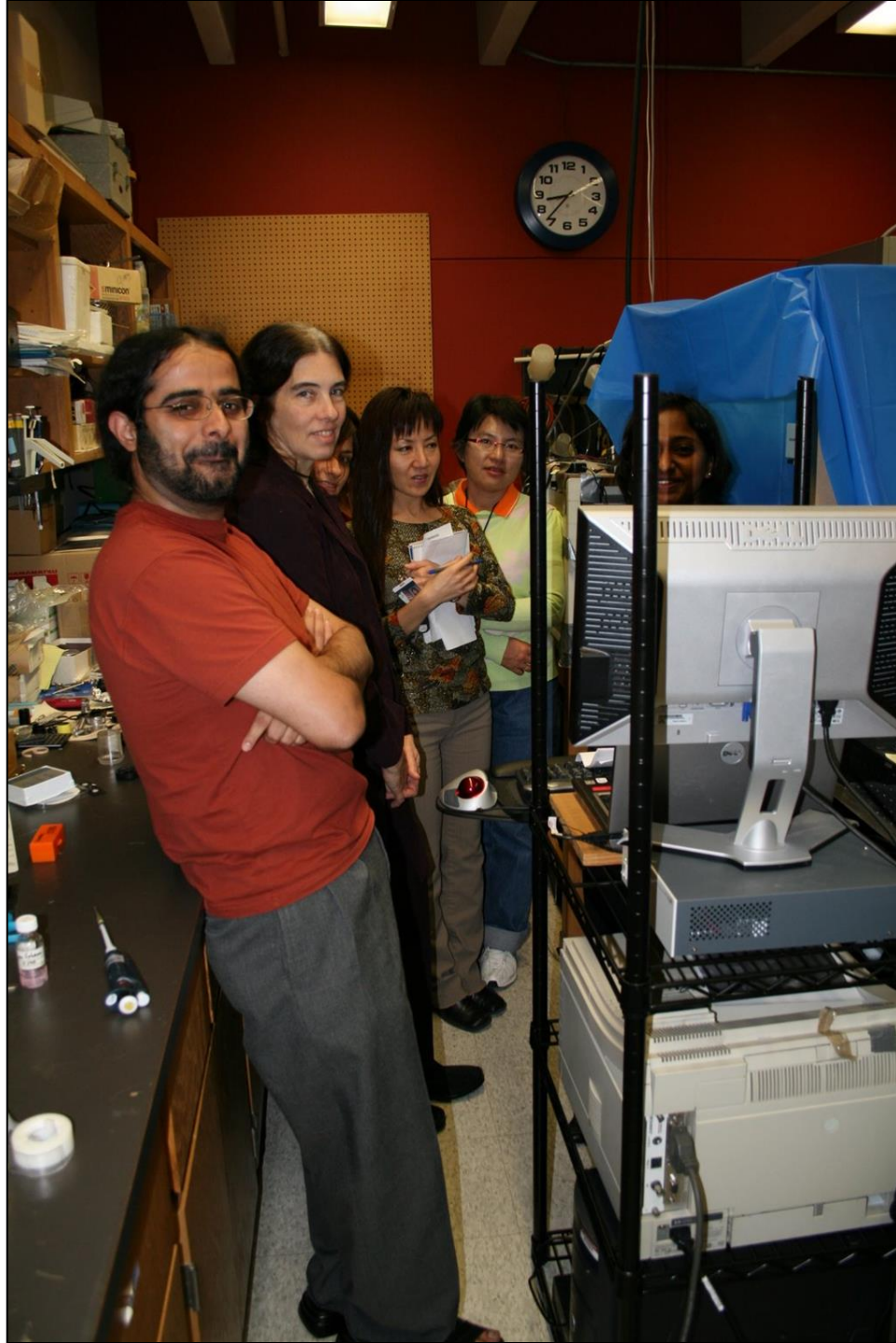


















































































## Outline

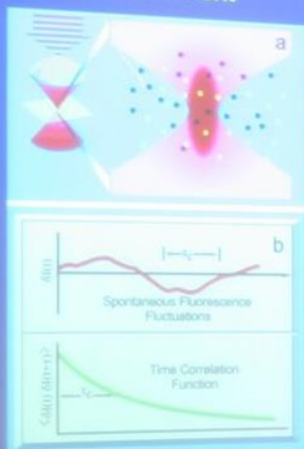
- Introduction—motivation and concept of FCS
- Measurement of dynamic properties by FCS
- Amplitude methods—PCH and FIDA
- Fluorescence photobleaching recovery and single molecule (particle) measurements
- Applications in solution and in cells







## Schematic of a Simple FCS Diffusion Measurement



Maiti, S., U. Haupts, et al. (1997). Proc Natl Acad Sci U.S.A 94(22): 11753-7.









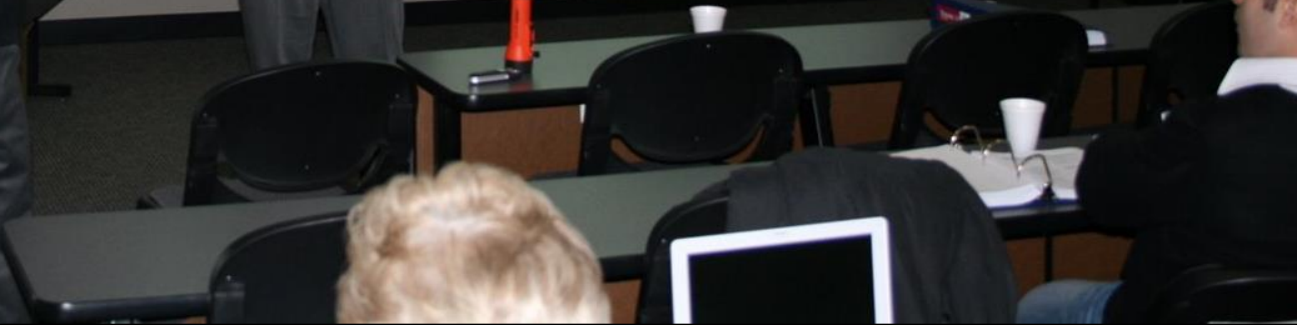








# Brightness and number of molecules can be measured independently



















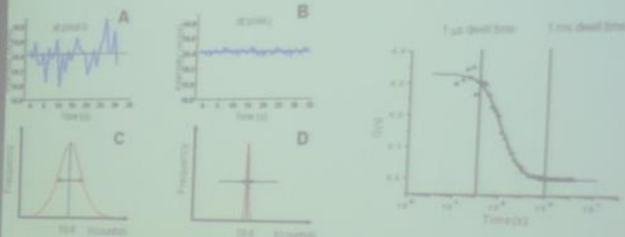








### The Basic Idea



Variance due to particle fluctuations

$$\sigma_n^2 = \epsilon^2 n$$

Variance due to detector read noise

$$\sigma_d^2 = m$$

Average intensity to use pixel

$$\langle k \rangle = \epsilon n$$

$$B = \frac{\sigma^2}{\langle k \rangle} = \epsilon + 1$$

$$N = \frac{\langle k \rangle^2}{\sigma^2} = \frac{m}{\epsilon + 1}$$















































































































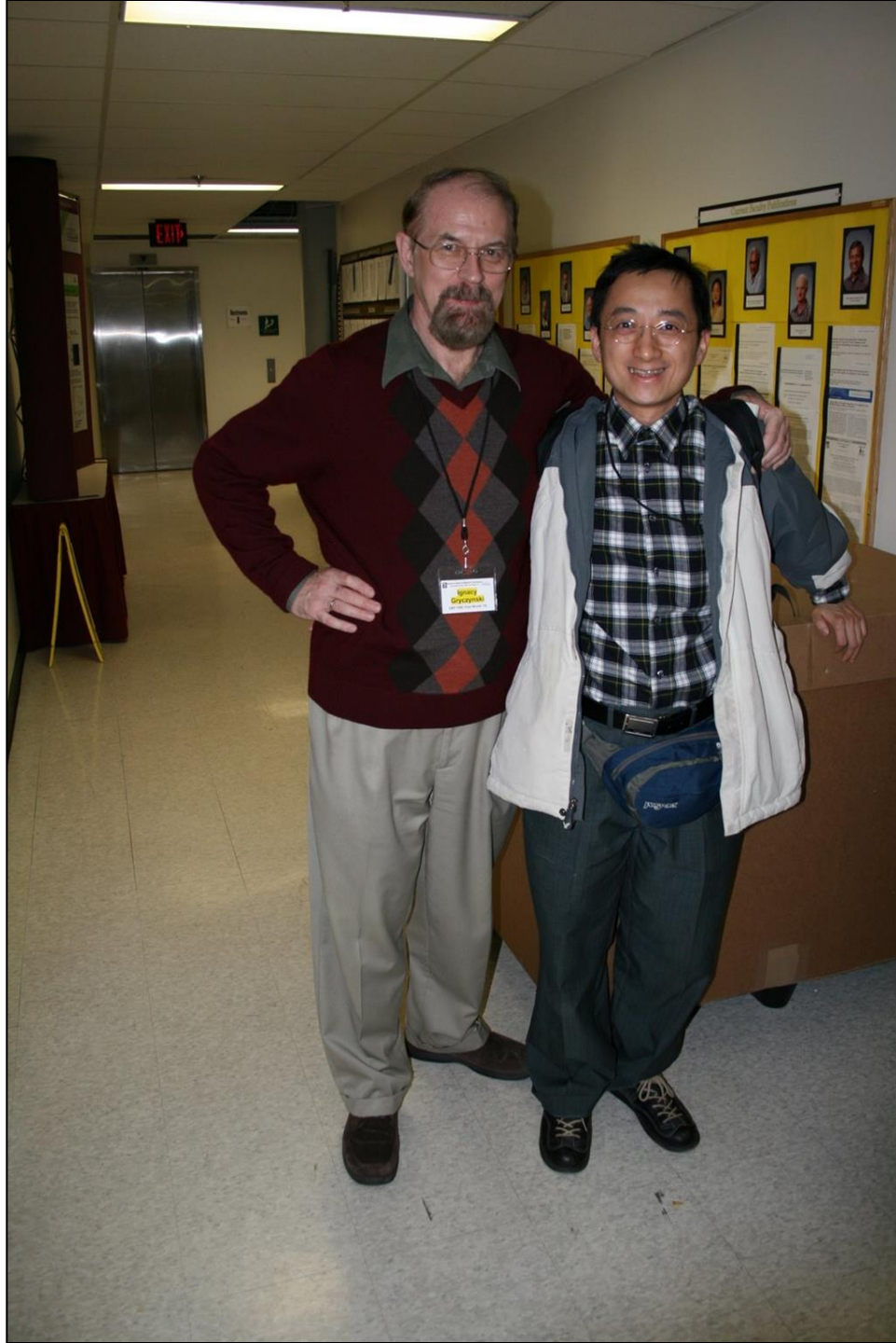




































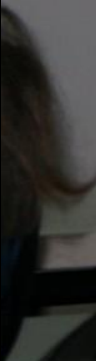








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## Outline

- Analytical ultracentrifugation
  - Description and applications
- Fluorescence detector design
- Applications
  - Normal Use Tracer Sedimentation (NUTS)
  - Biological On-Line Tracer Sedimentation (BOLTS)













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