Fluorescence of Single-Walled Carbon Nanotubes: Applications in Physics, Chemistry, and Bio-medicine

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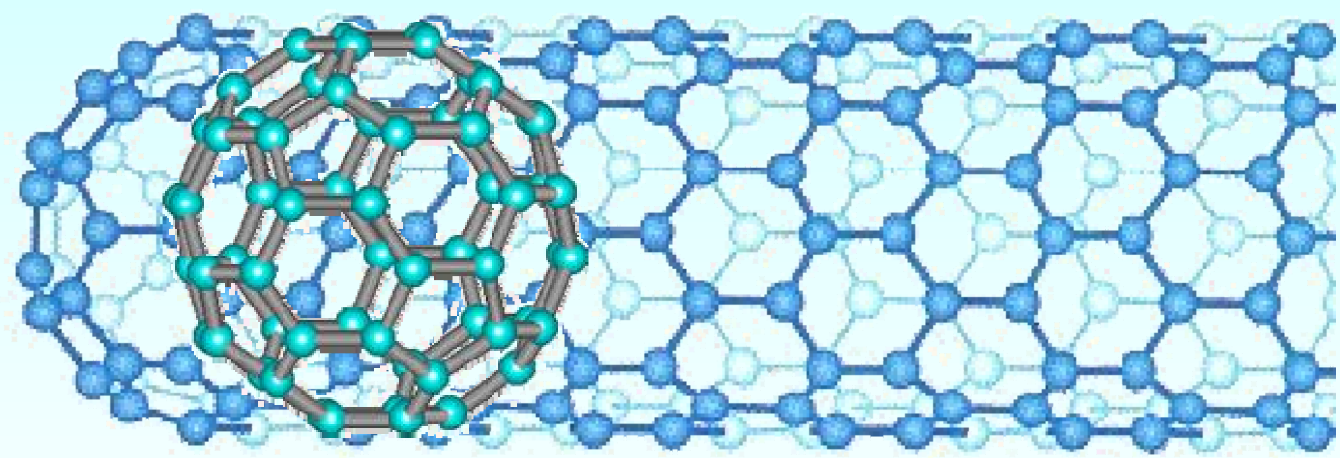
University of Tokyo February 18, 2010

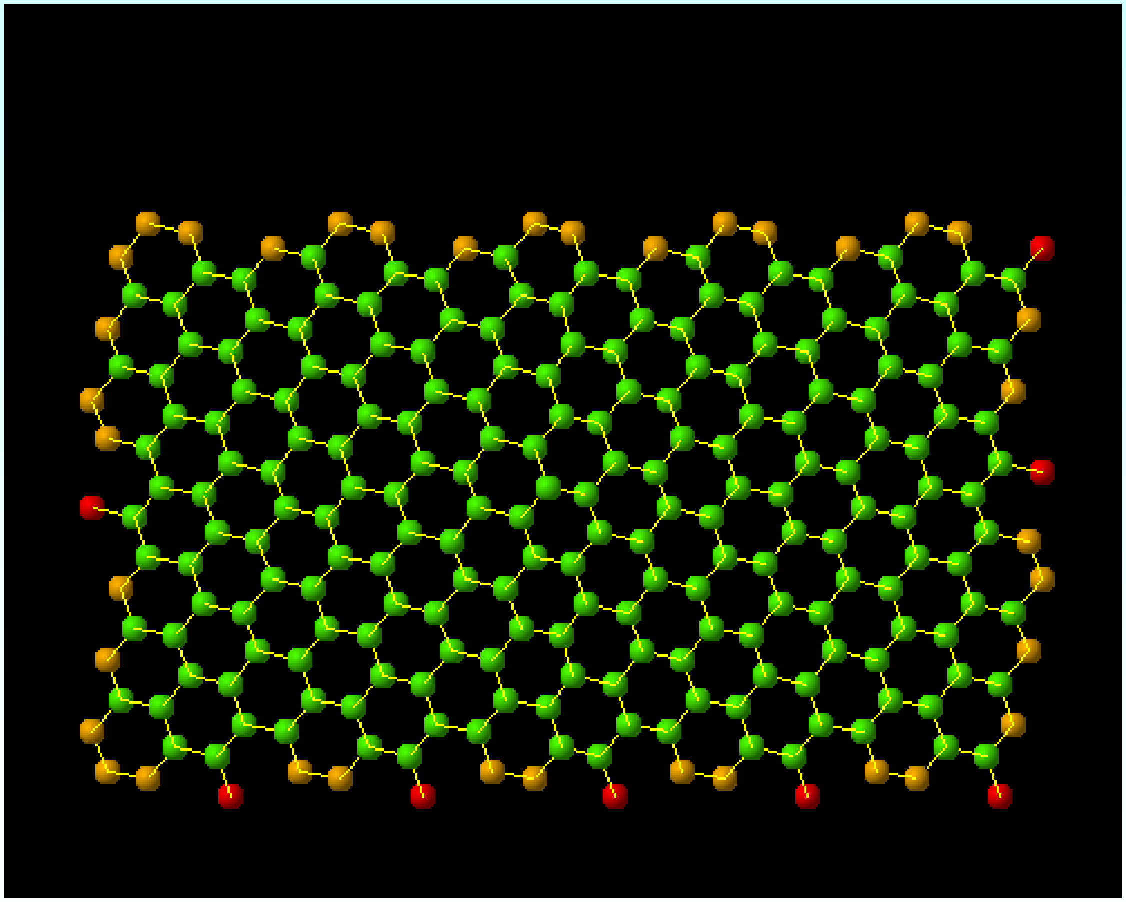
Carbon Nanostructures



C_{60}
(Buckminsterfullerene)

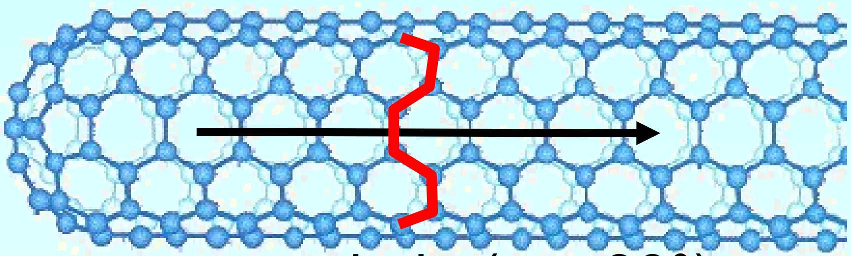
Single-walled Carbon Nanotube
(SWCNT)

Rolling up graphene to make a SWCNT

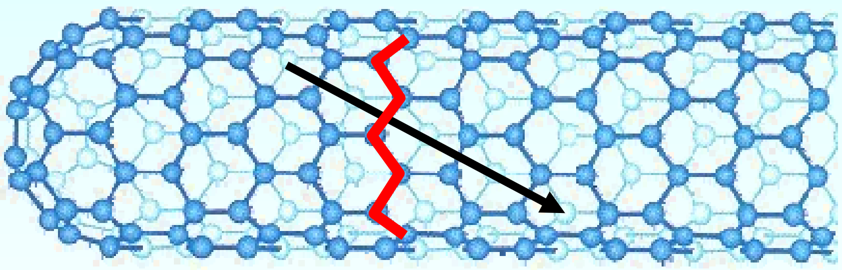


taken from http://www.photon.t.u-tokyo.ac.jp/~maruyama/wrapping.files/frame.html

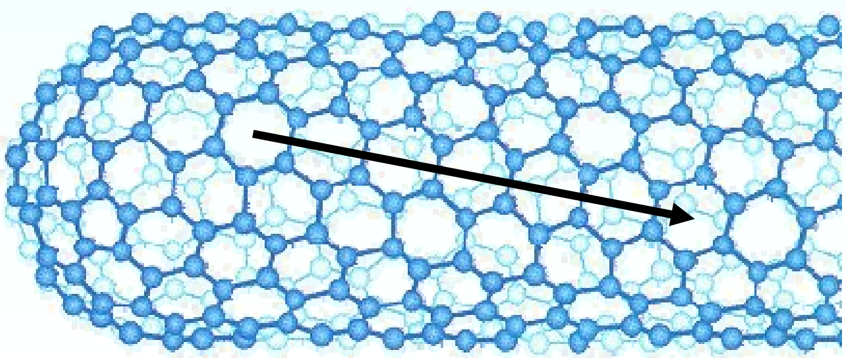
Many SWCNT structures exist
(different diameters and angles)



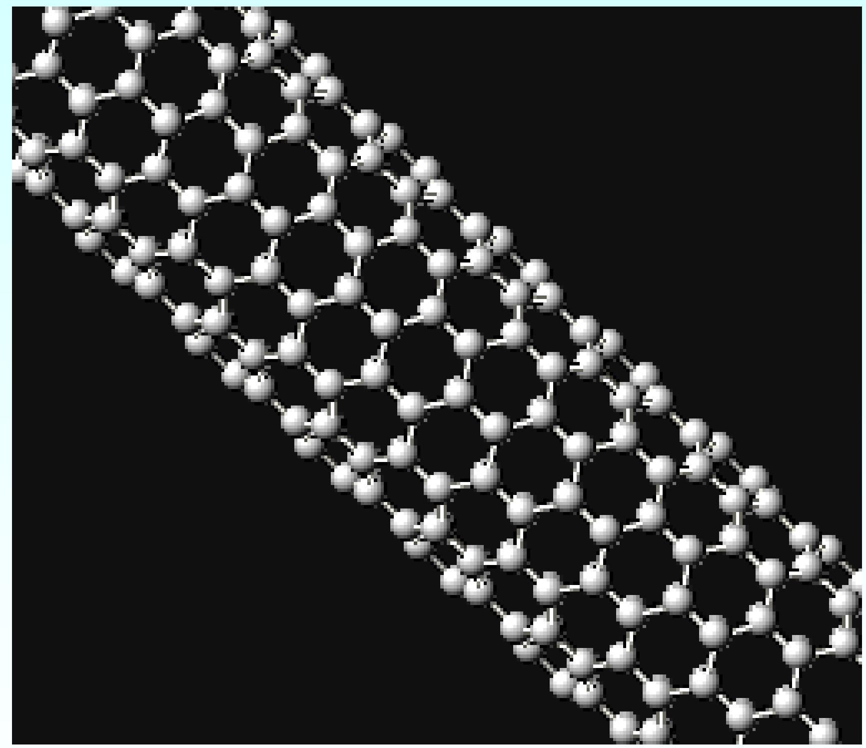
armchair ($\alpha = 30°$)



zigzag ($\alpha = 0°$)

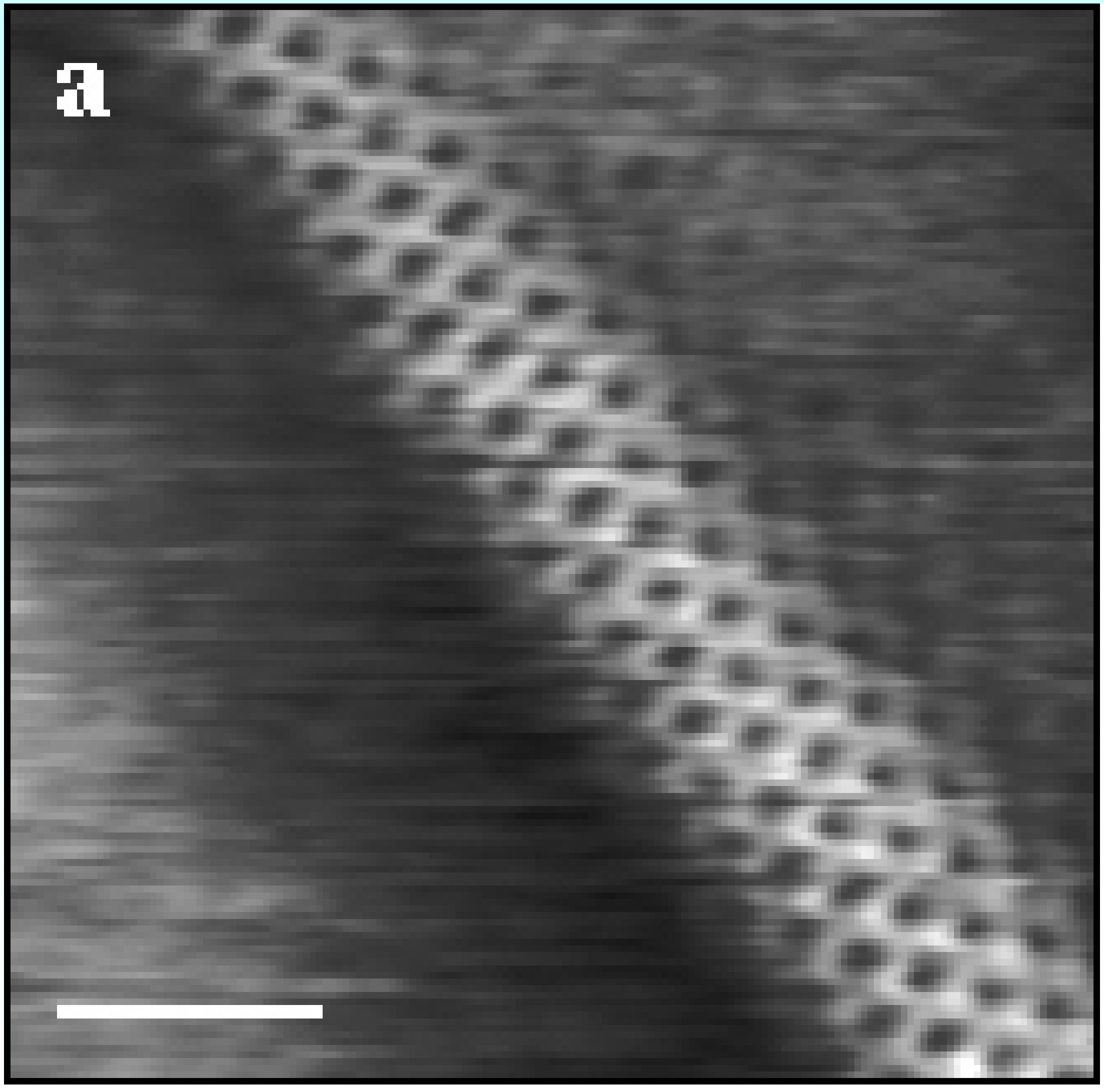


intermediate ($0 < \alpha < 30°$)



zigzag

STM Image of a Single-Walled Carbon Nanotube



Prof. C. Lieber, Harvard Univ.

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SWCNT Properties

Typical diameter: 0.6 – 3 nm

Typical lengths: 100 – 10,000 nm → large aspect ratios

Density: ~1.4 g / cm^3

Tensile strength: ~ 60 GPa → 50 x higher than steel

Persistence length: ~ 50 μm → very rigid

Surface area: > 1000 m^2 / g (every atom on surface)

Electrical transport: metallic or semiconducting

Optical spectra: intense π-π^* bands,
direct band-gap semiconductors

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Potential Uses of Carbon Nanotubes

Super-strong fibers

Light-weight electrical cables

High performance composite materials

Molecular scale computer circuitry

Chemical / biochemical sensors

Medical diagnosis and therapy

Field emission devices
(video display panels, X-ray sources)

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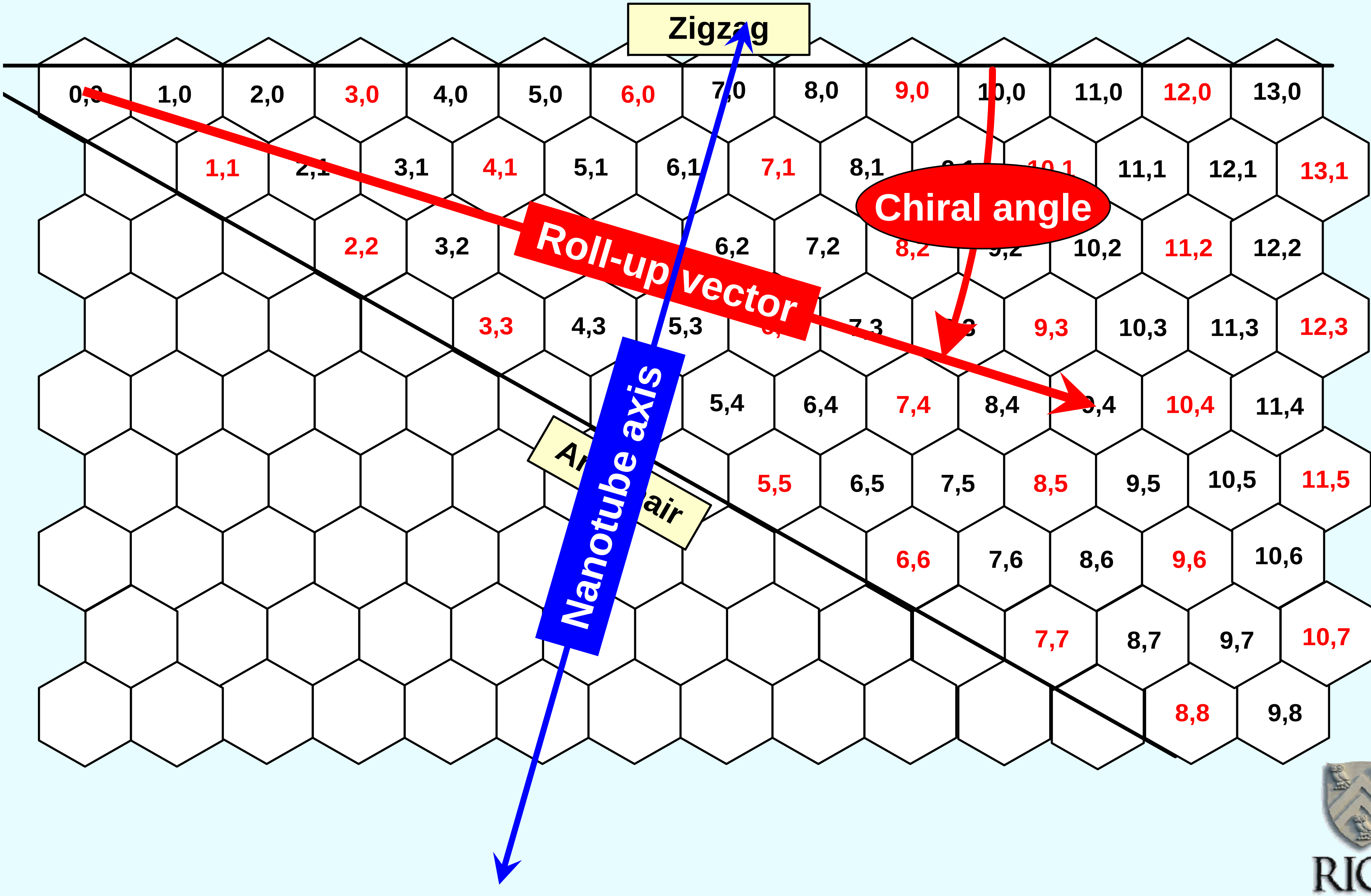
Nanotubes are produced as complex mixtures

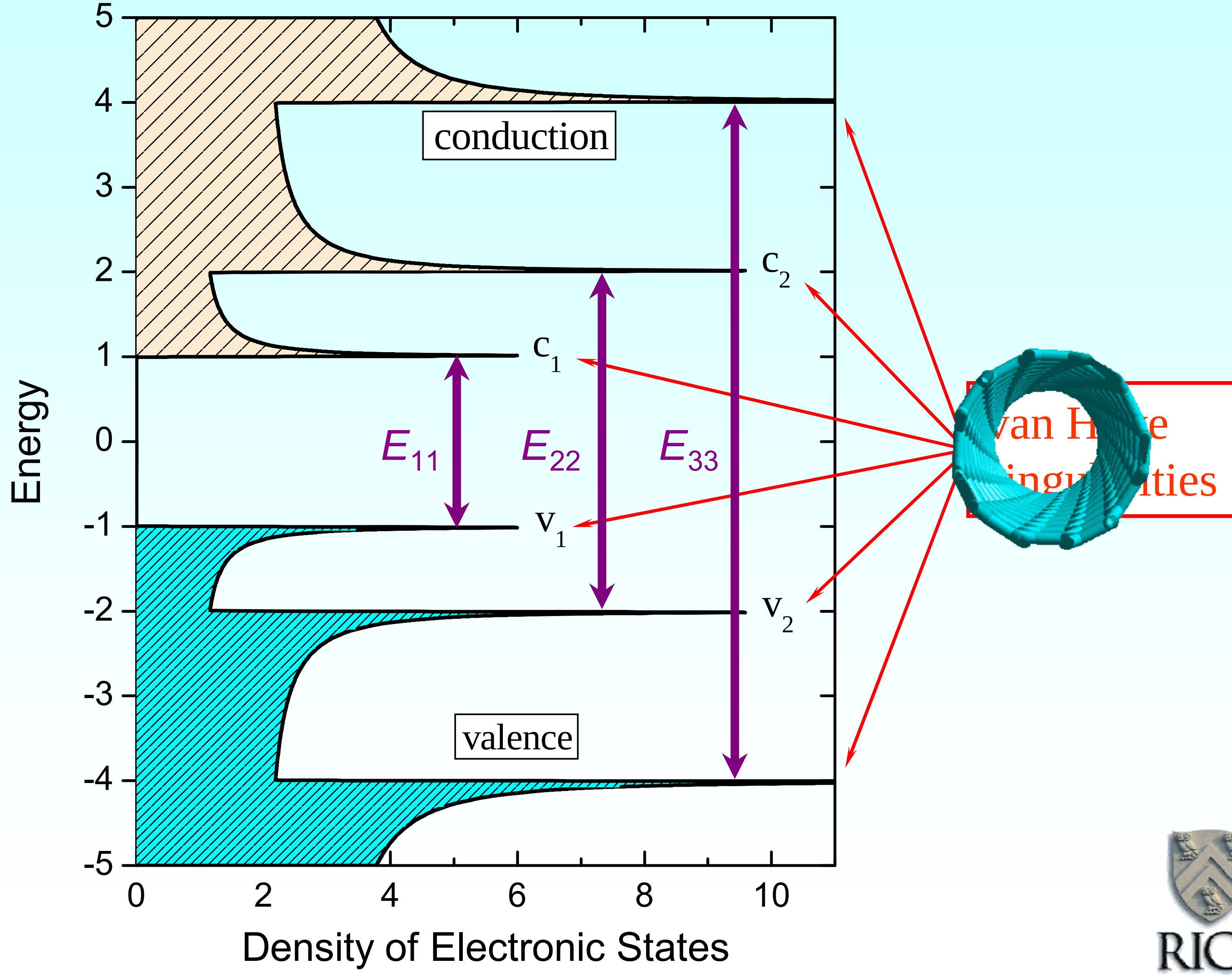
Even single-walled samples contain:

- many diameters
- many chiral angles
- many lengths (no effect on electronic structure)
- bundles of tubes bound by van der Waals forces
- impurities (residual catalyst, giant fullerenes,…)

Constructing nanotubes from a graphene sheet



Electronic states of a semiconducting SWCNT



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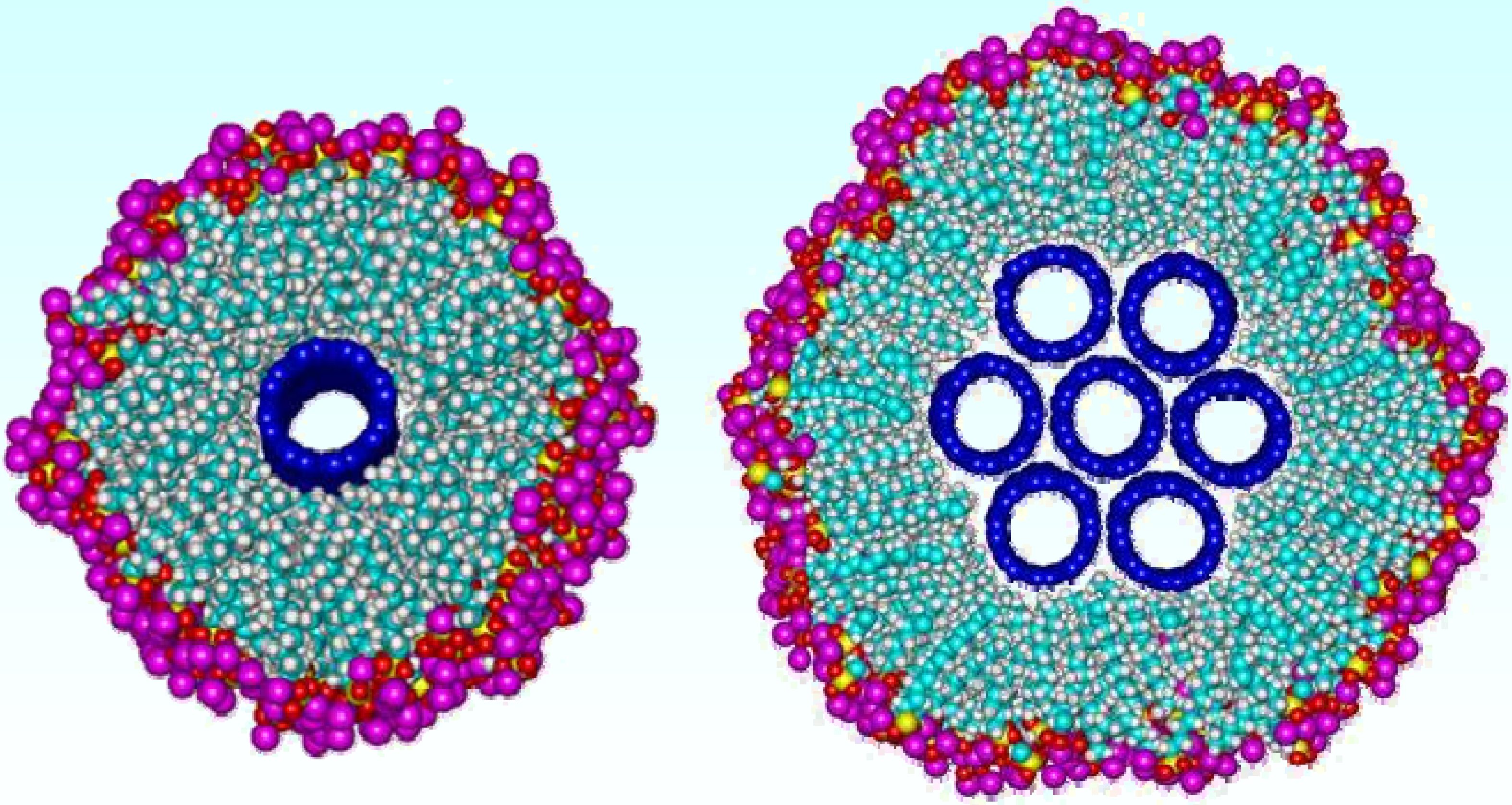
Surfactant coatings can suspend single-walled carbon nanotubes in water



Separate by centrifugation

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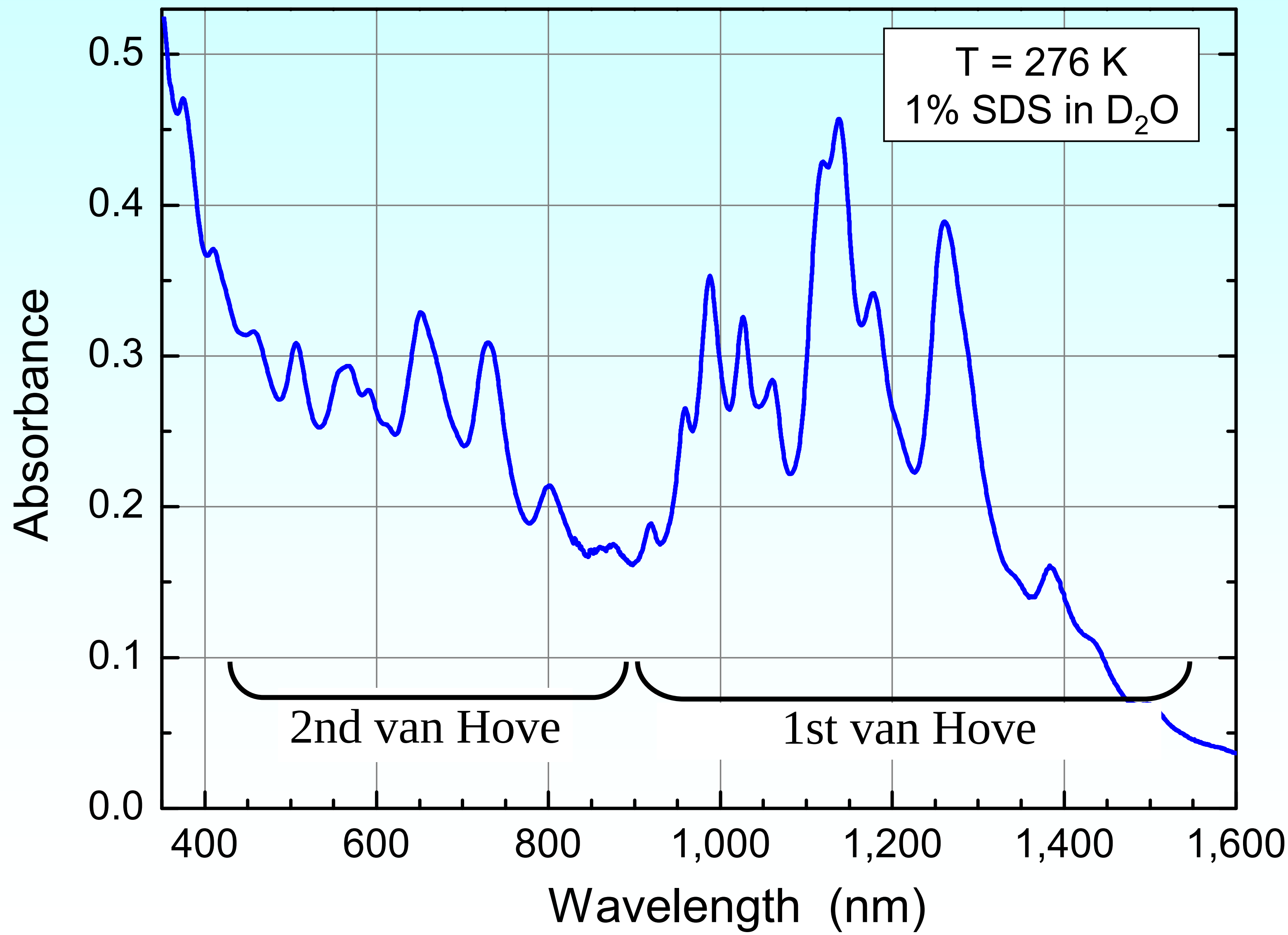
Absorption spectrum of SWCNTs in aqueous SDS suspension (after processing)



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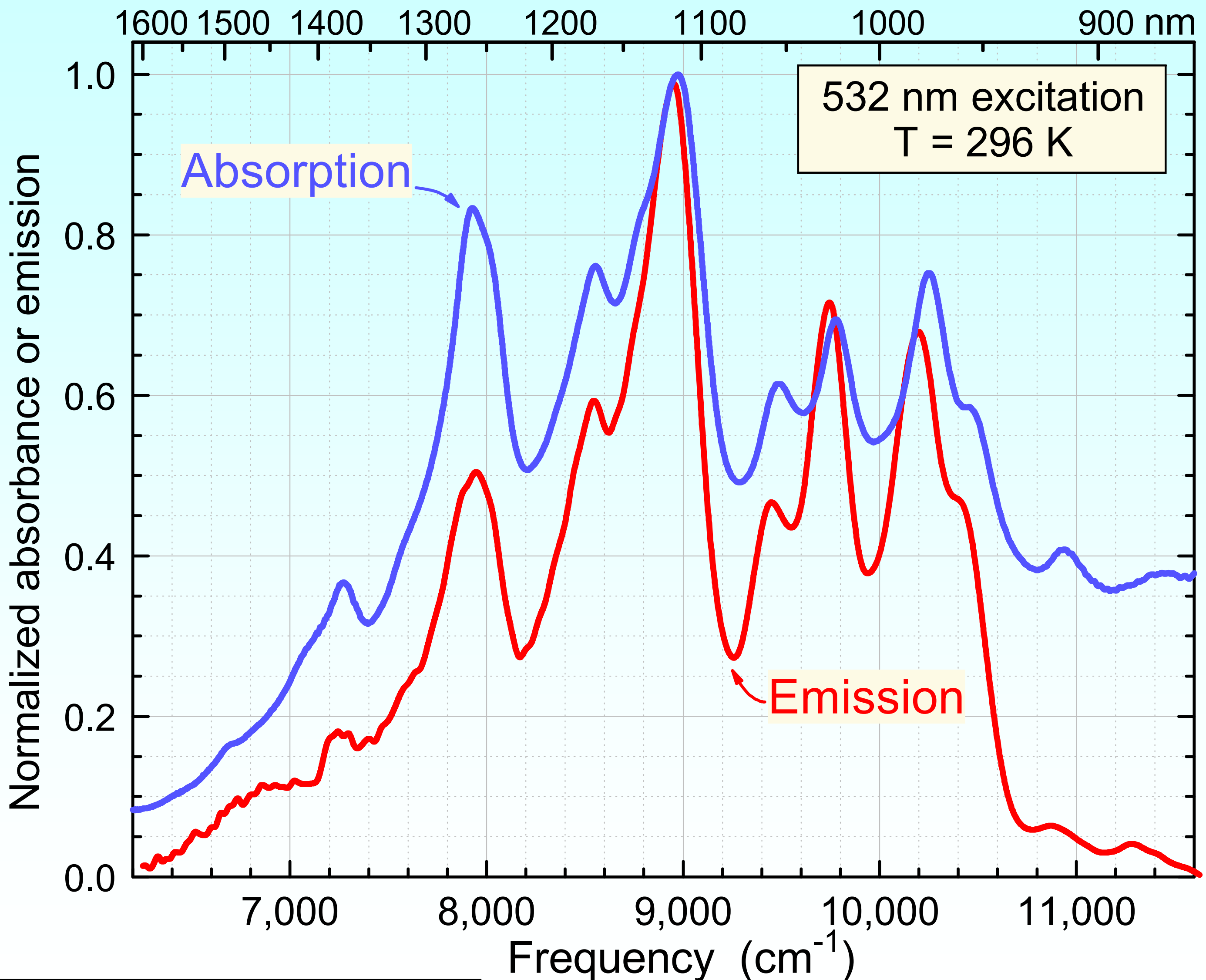
Near-infrared emission from SWCNTs in SDS



Science *297*, 593 (July 26, 2002)

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SWCNT Fluorescence Characteristics

Near-IR emission following visible excitation

Low average quantum yield (few % max)

Lifetime ~10^{-10} s

Highly photostable, blink-free

Strongly polarized parallel to tube axis

Quenched by bundling, chemical damage, close contact with SiO_2

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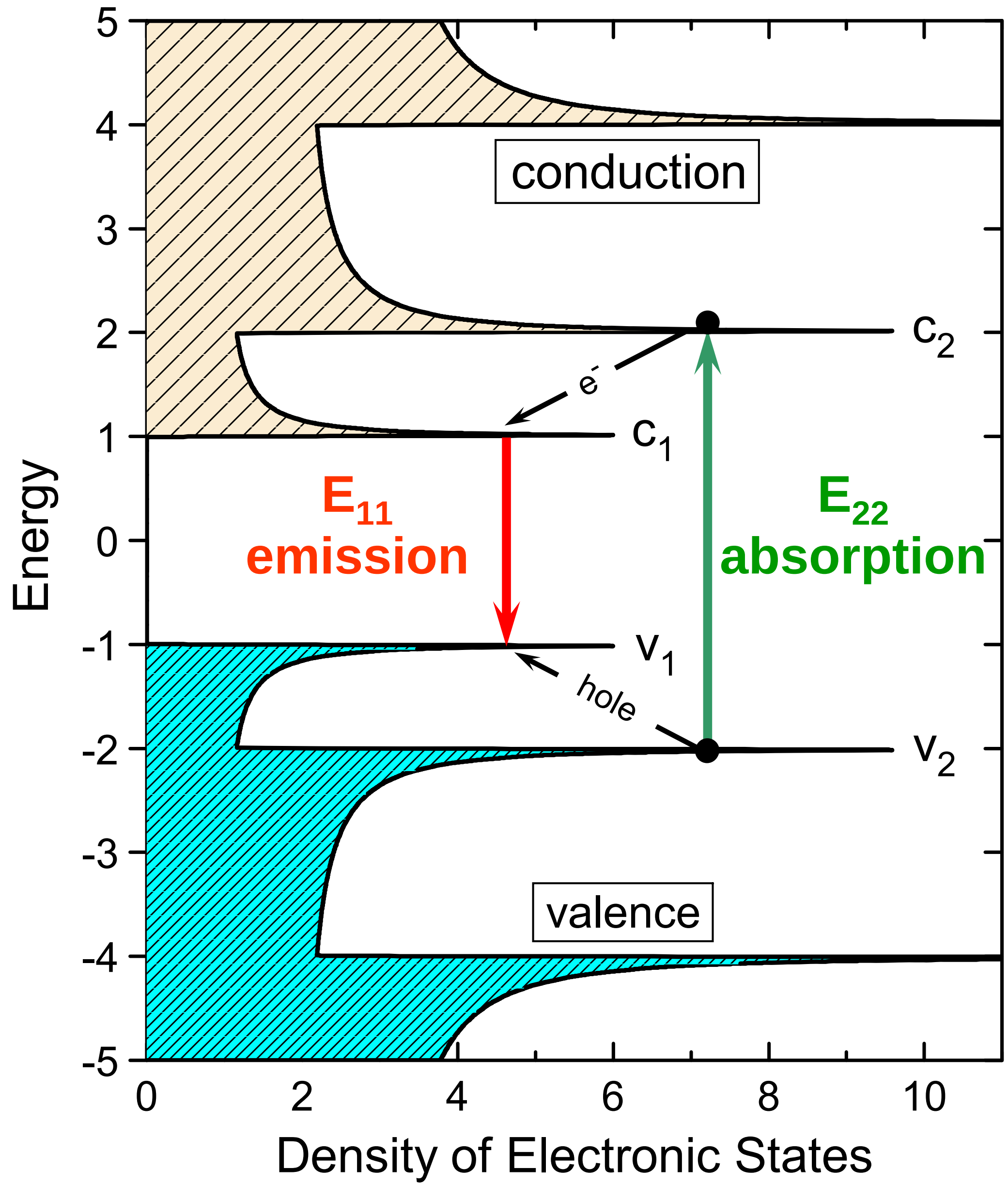
Spectrofluorimetry of semiconducting SWCNTs



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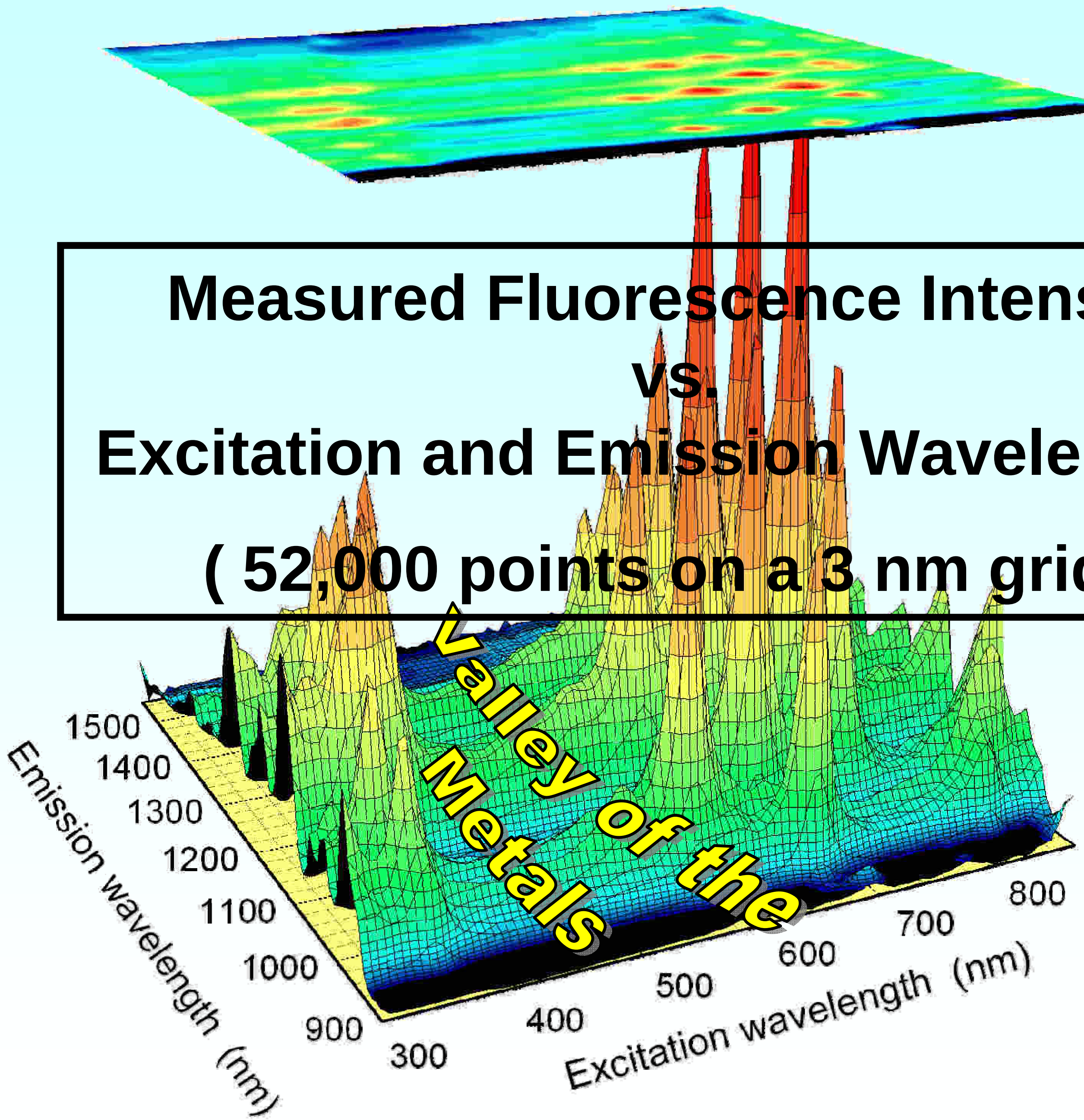
Measured Fluorescence Intensity
vs.
Excitation and Emission Wavelengths

(52,000 points on a 3 nm grid)



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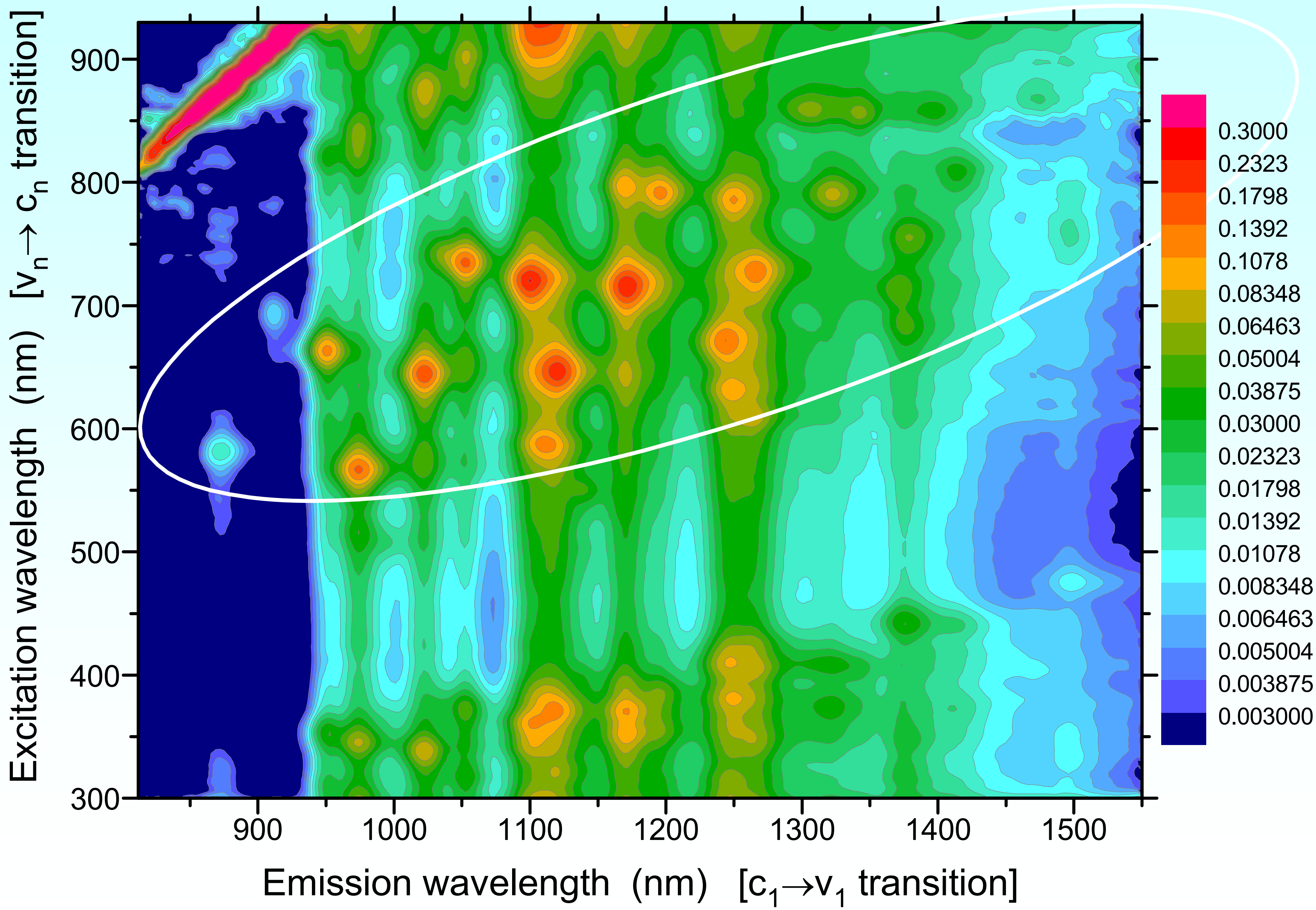
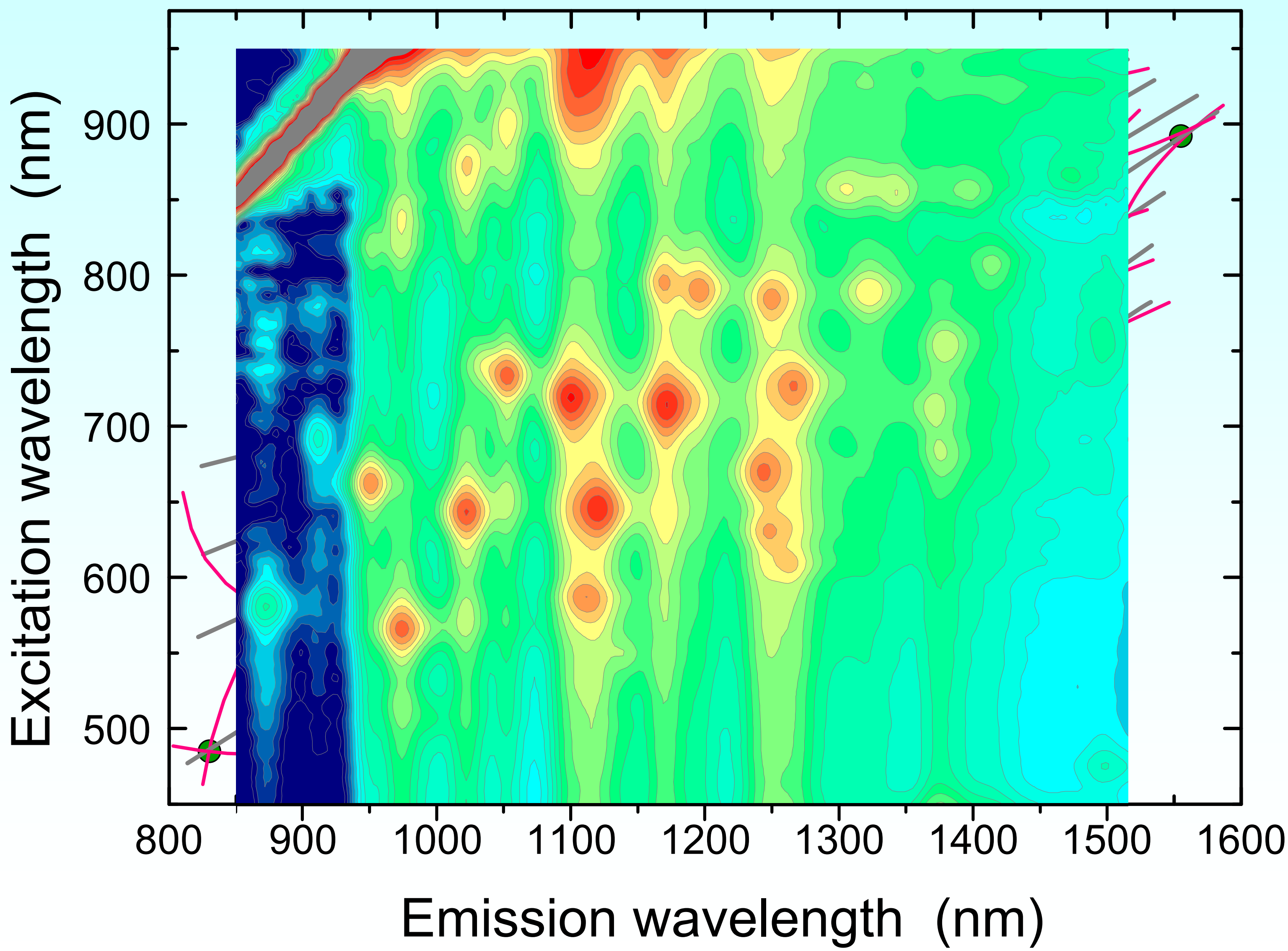
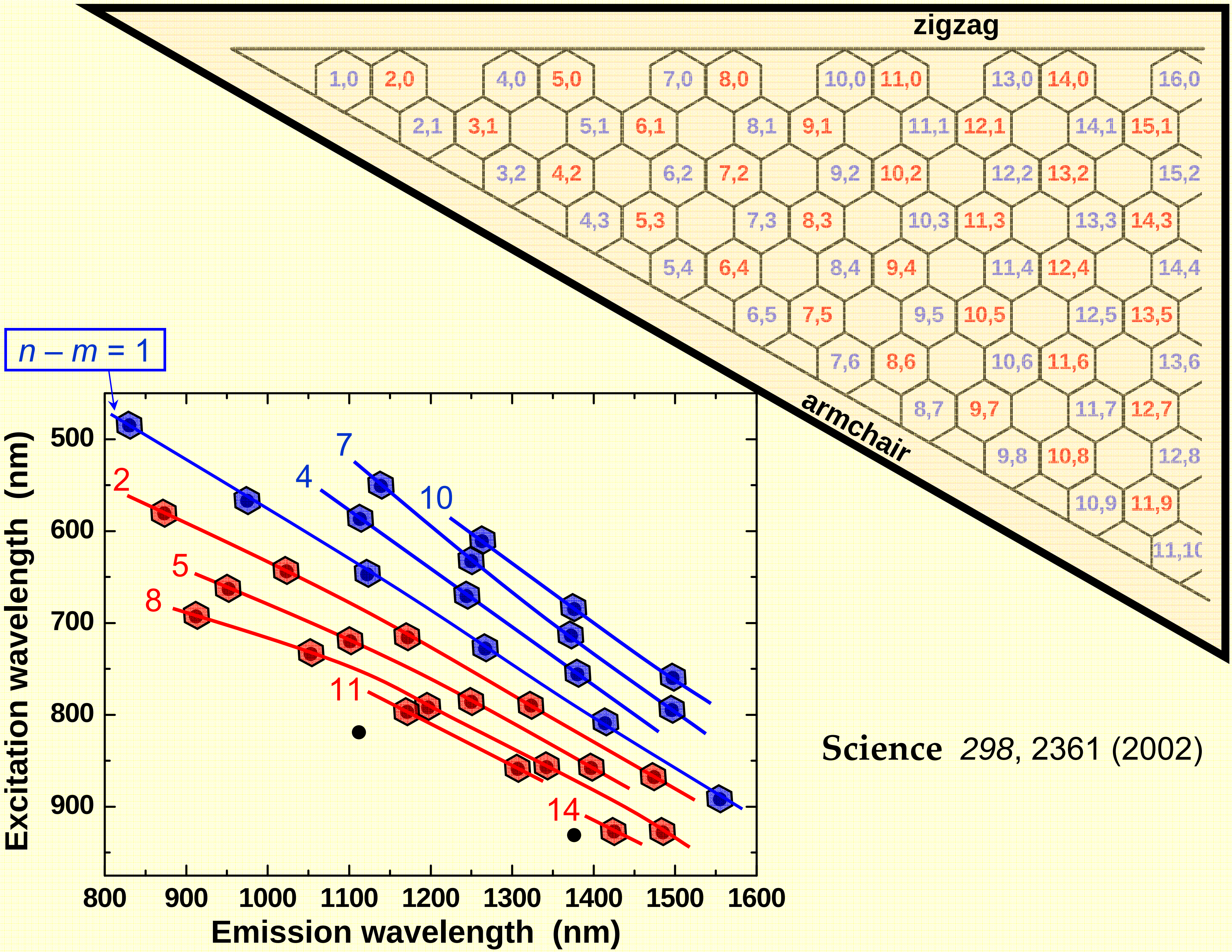
Contour plot of emission intensity



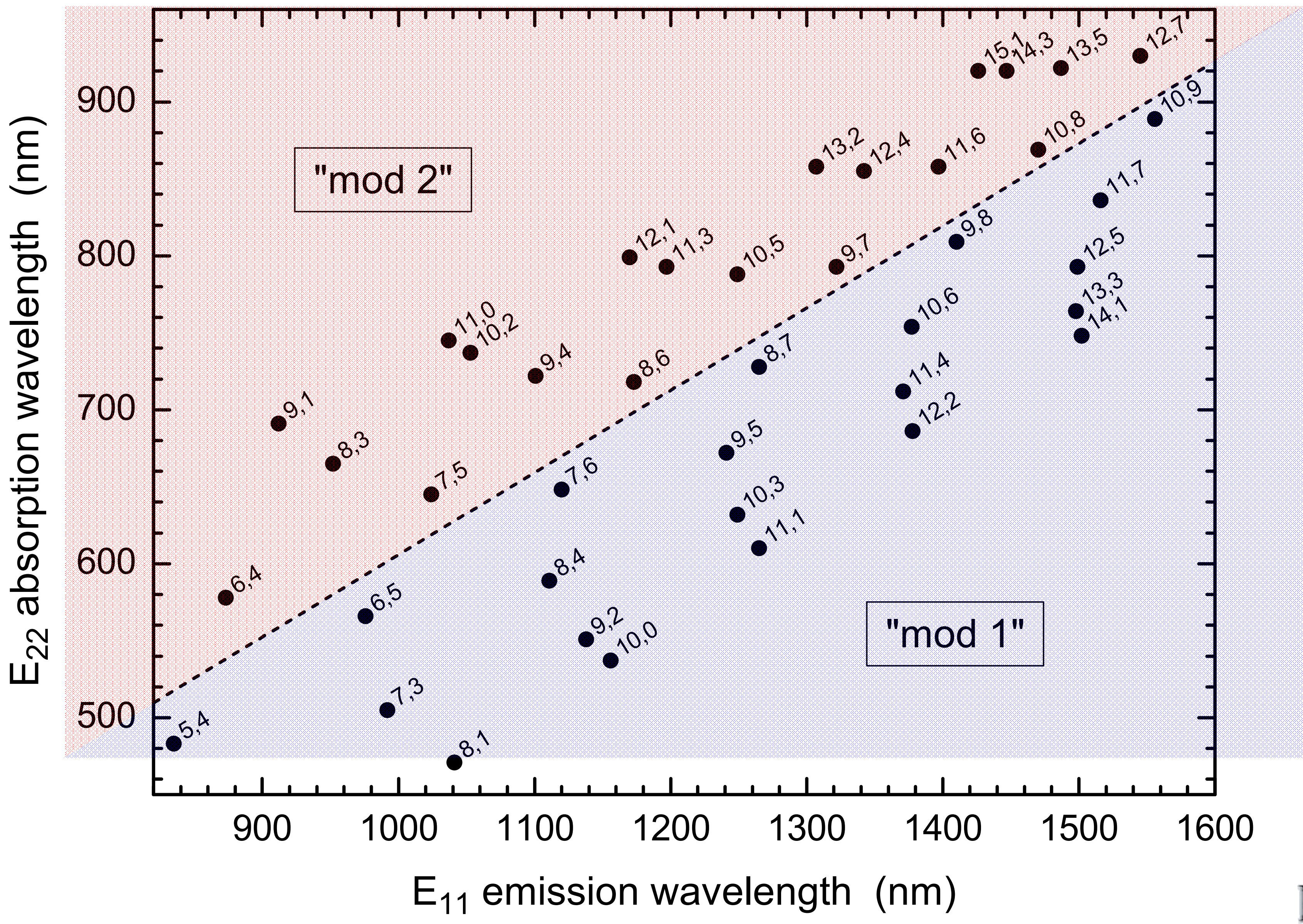
Patterns in the spectral data ?





Science *298*, 2361 (2002)

Spectral transitions mapped to structures



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Application

Analyzing SWCNT Mixtures

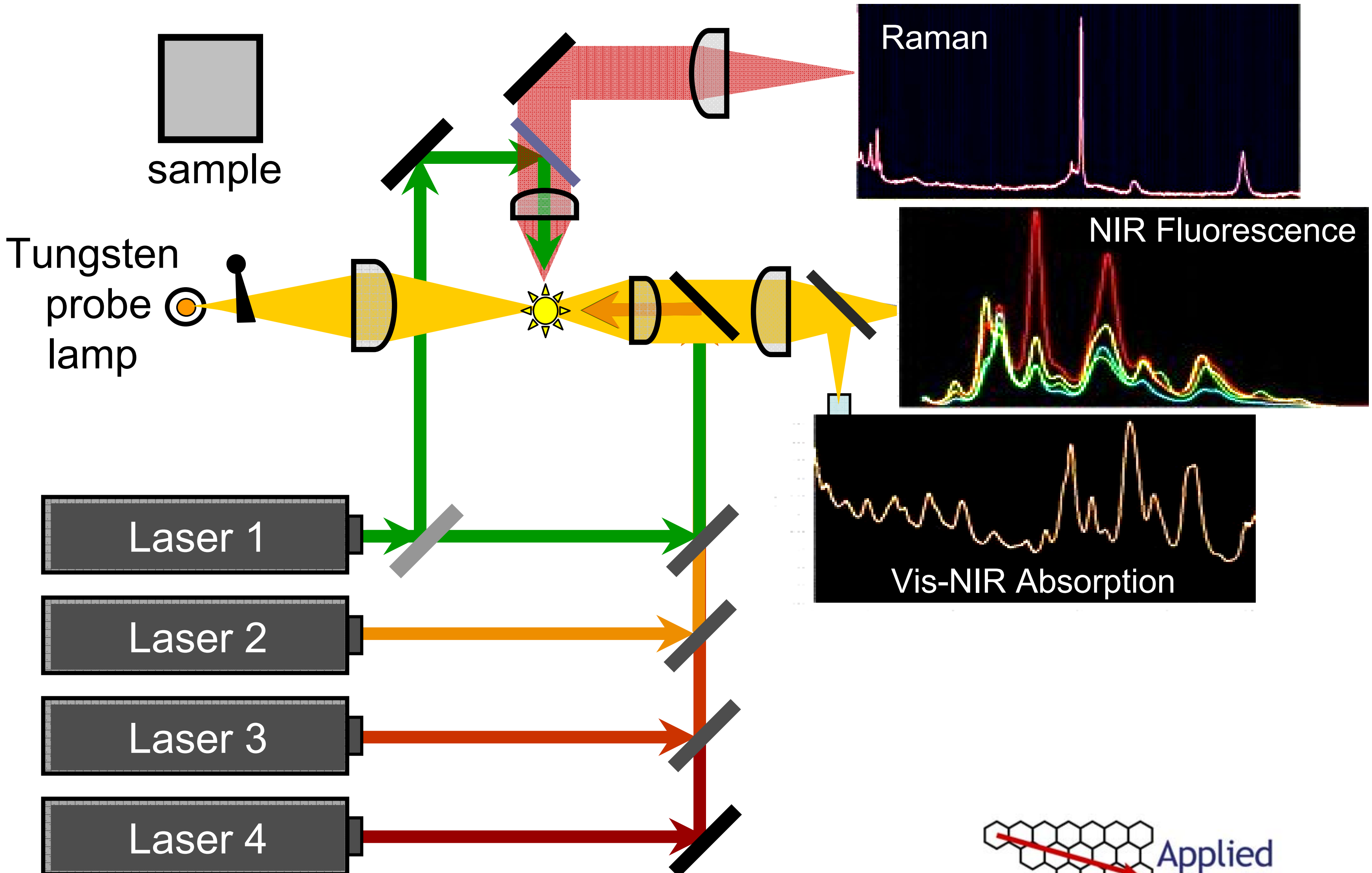
Benefits of Fluorimetric Analysis

- High sensitivity
- Few interferences
- Excellent (*n,m*) identification
- Broad (*n*,*m*) coverage with few excitation wavelengths
- High selectivity against impurities, imperfect tubes, bundles
- No background subtraction needed in analysis (unlike absorption methods)
- Relatively simple instrumentation

Goals for a turn-key SWCNT analyzer

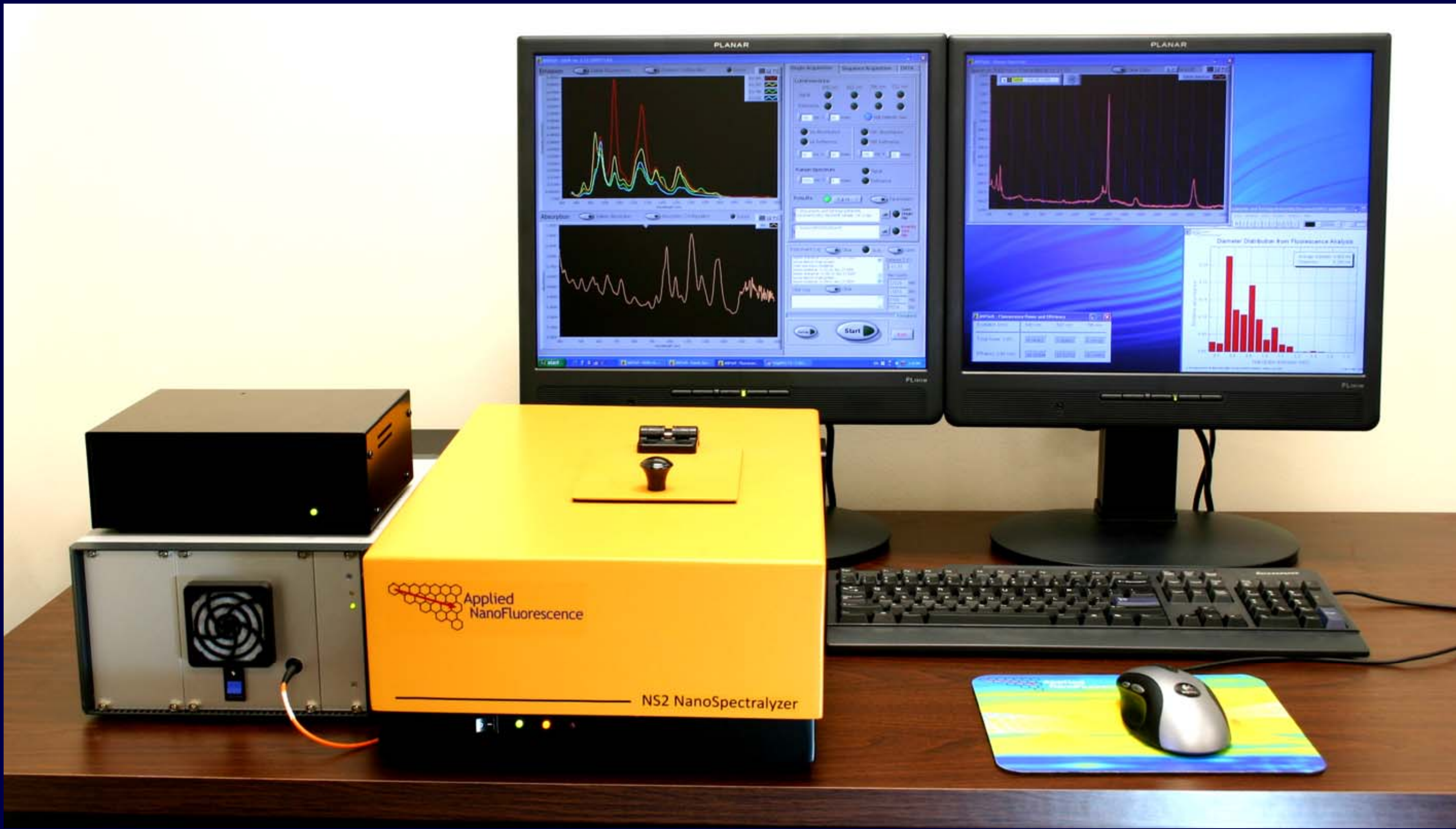
- Sensitive fluorescence detection
- Multi-mode spectroscopy
- Small sample volumes
- Simple sample preparation
- Fast measurements
- Automated data analysis
- Detailed analysis reports
- Quantitative analysis capability

NS2 NanoSpectralyzer

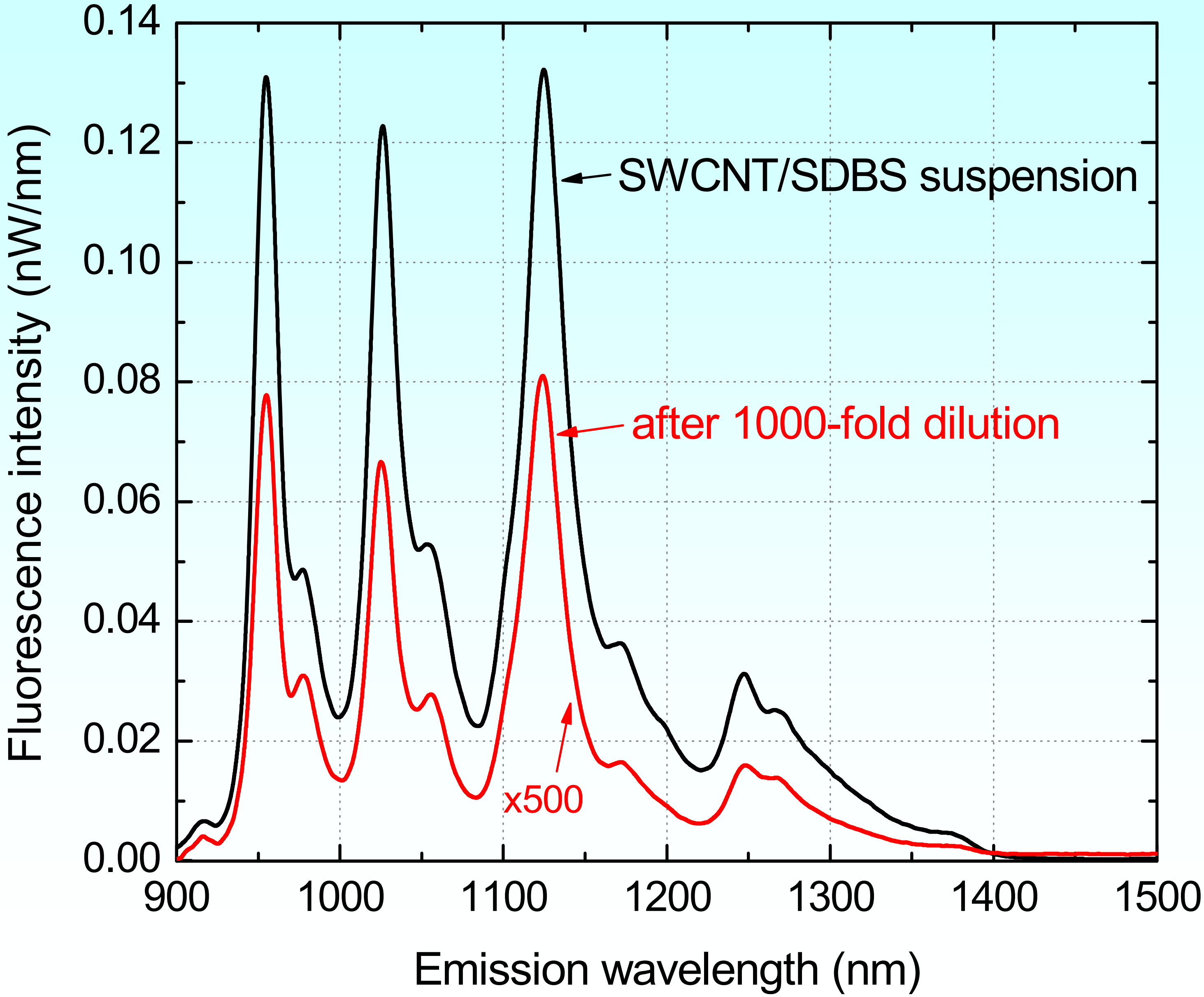


Applied NanoFluorescence

Model NS2 NanoSpectralyzer®



High sensitivity and dynamic range



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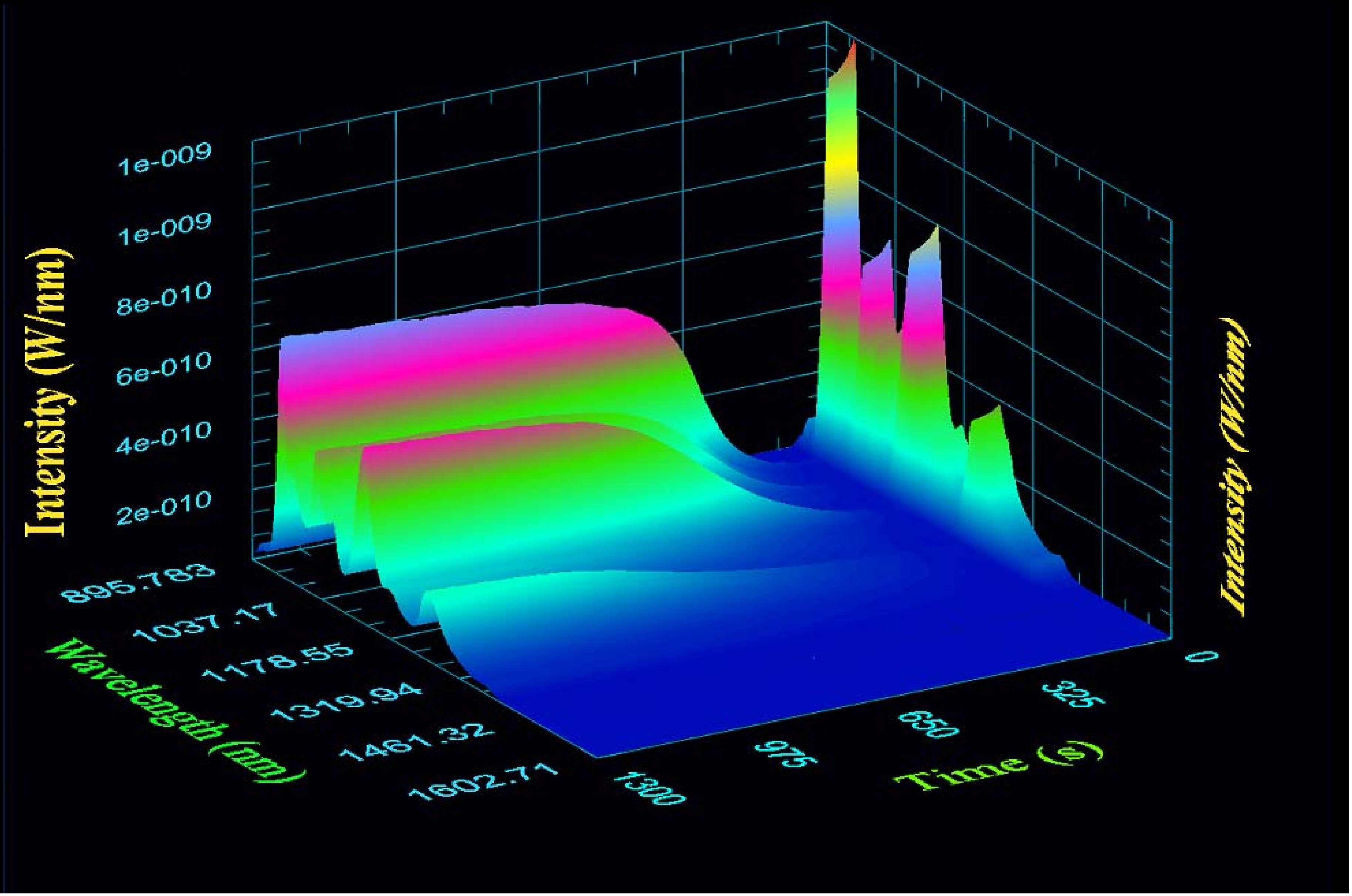
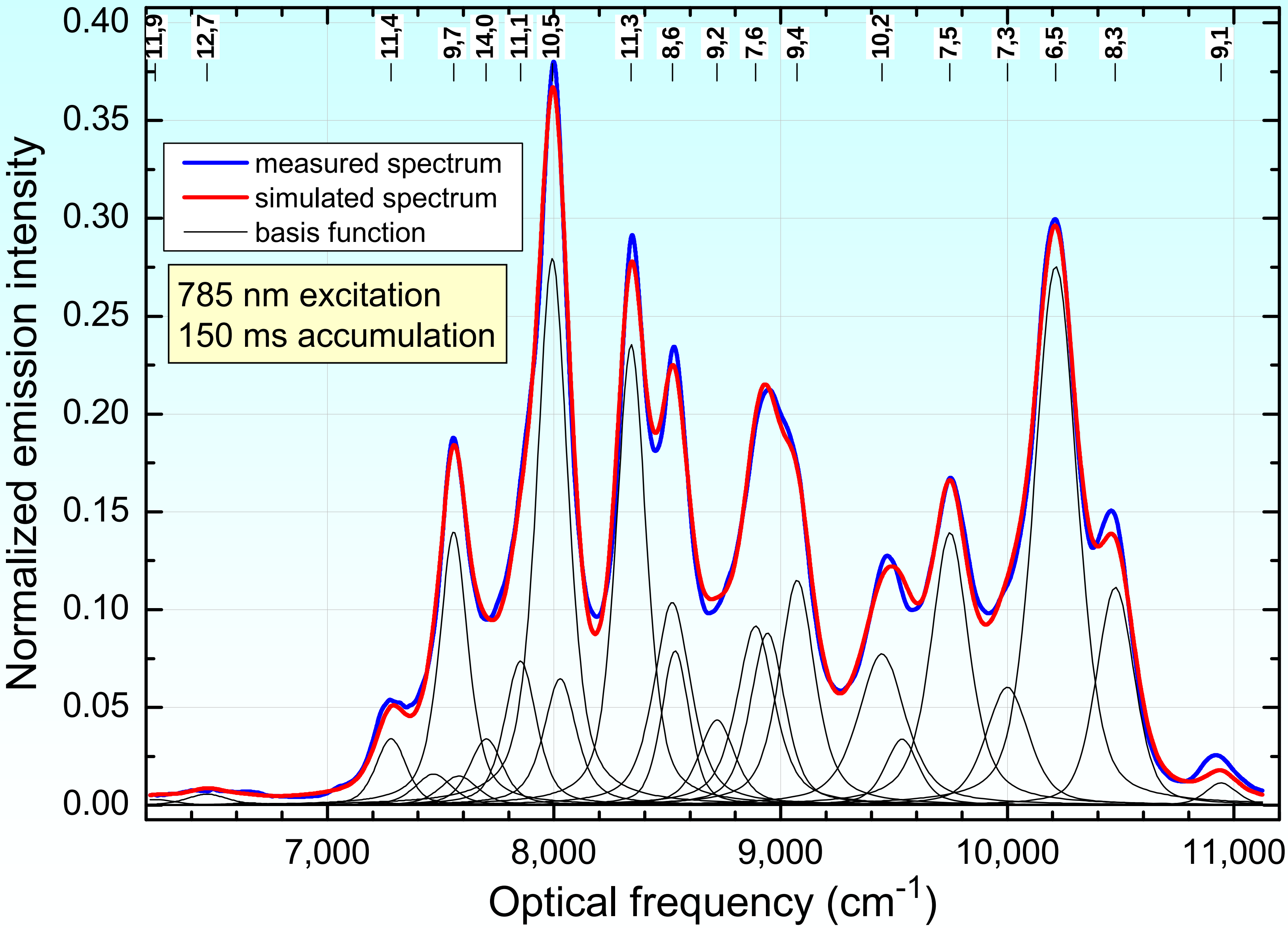
Sequence Mode for Kinetic Studies

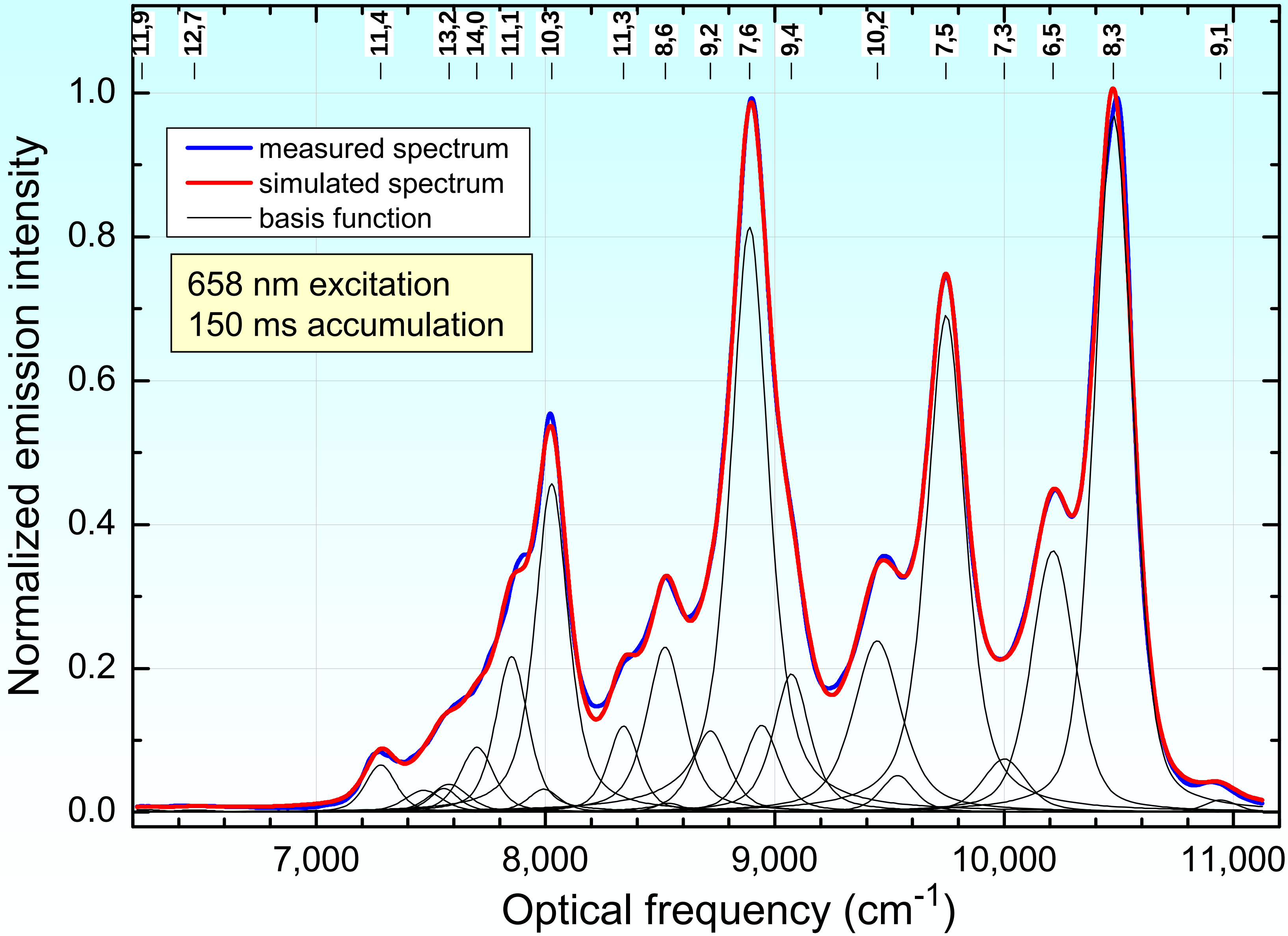
Record up to 20 spectra / second



Fluorescence spectrum with Global Fit simulation



Fluorescence spectrum with Global Fit simulation



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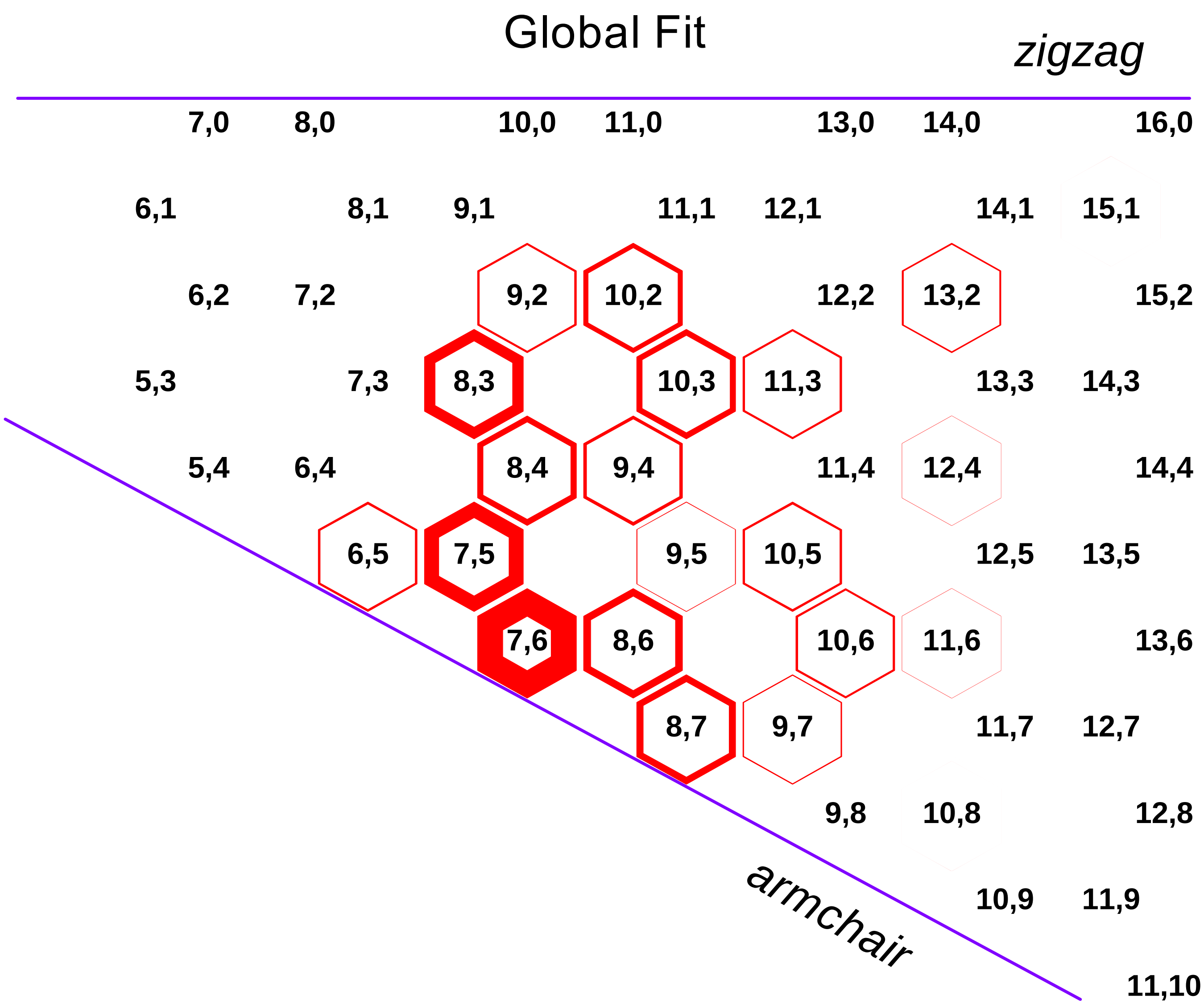
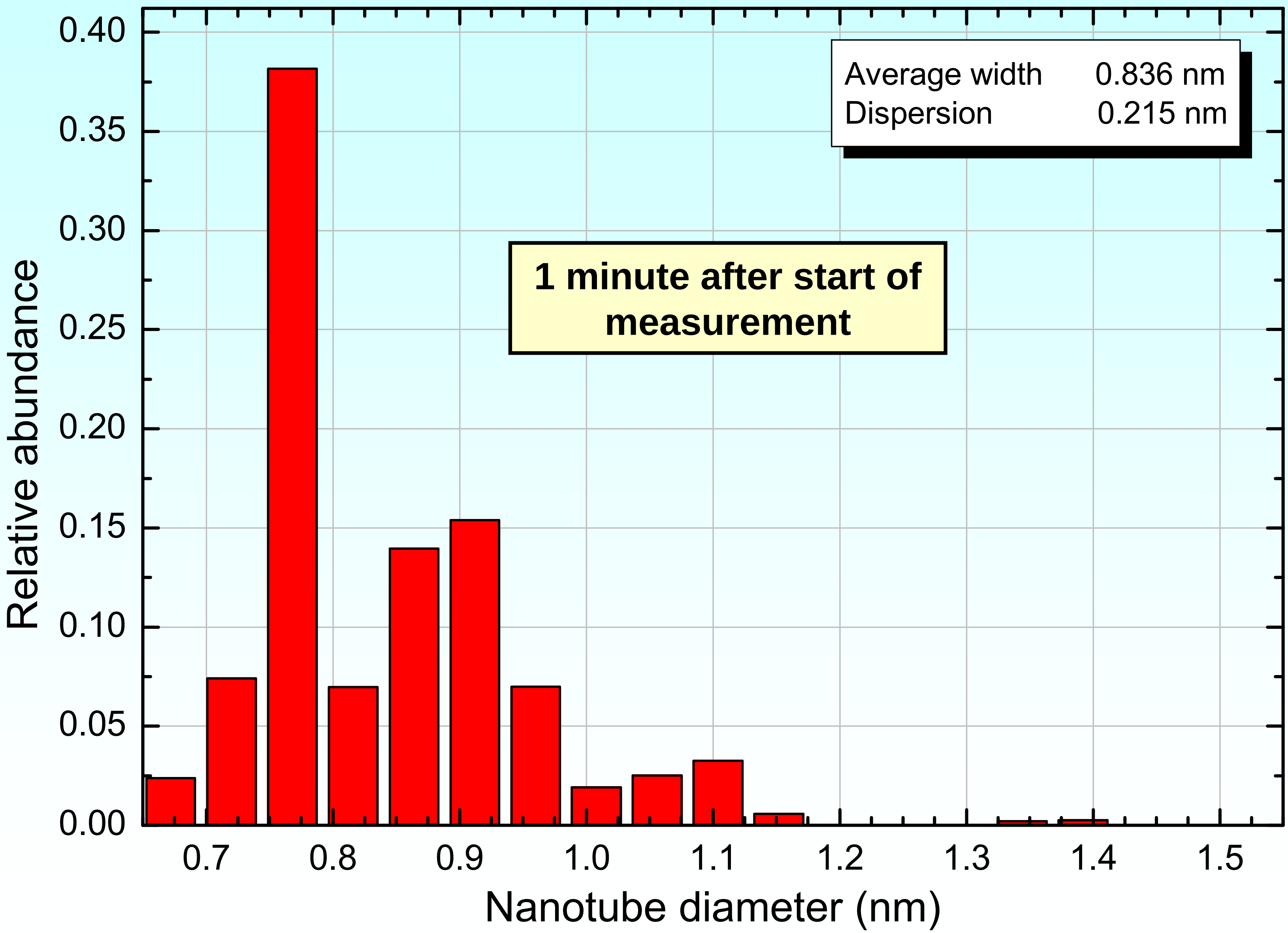
Automatically deduced (*n,m*) distribution



Automatically deduced diameter distribution



Fluorimetric (*n*,*m*) analysis of bulk SWCNT samples

Qualitative (*n*,*m*) analysis

OK

Quantitative (*n*,*m*) analysis

Still need to include sensitivity factors reflecting (*n*,*m*)-dependent fluorescence brightness

Factors controlling fluorescence brightness

Intrinsic: diameter
roll-up angle
mod 1 or *mod 2* identity

Extrinsic: structural defects
bundling
surfactant coating
quenching at ends
chemical processing

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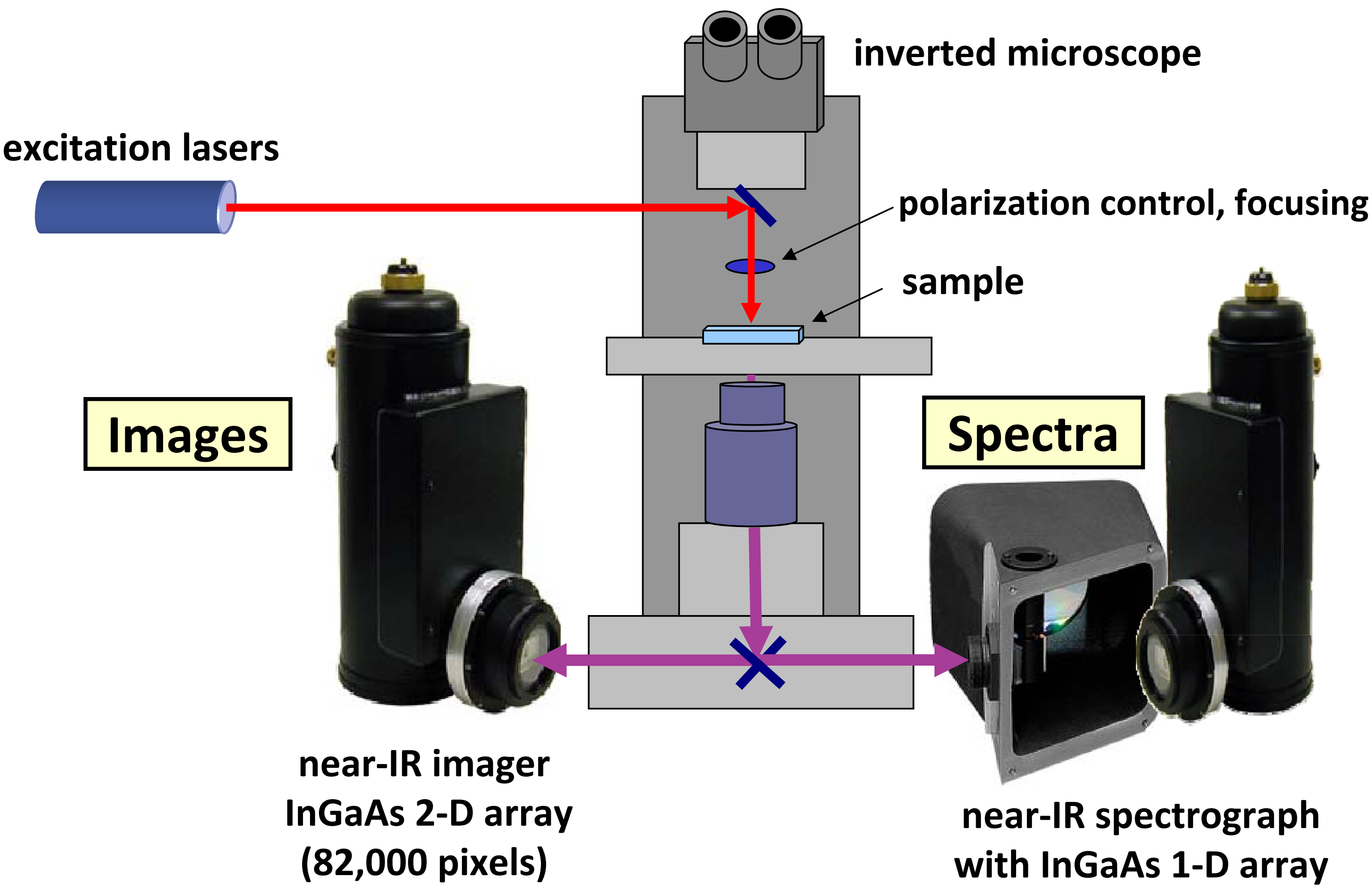
Intrinsic factors: Experimental plan

1. Prepare aqueous SDBS suspension with gentle processing
2. Find a bright, long SWCNT under the microscope
3. Identify its (*n,m*) from emission spectrum
4. Excite near its E_{22} resonance peak in linear intensity regime
5. Measure emission intensity per unit SWCNT length with calibrated excitation and detection

Apparatus for near-IR fluorescence microscopy

Tsyboulski, et al.
Nano Lett. 5, 975 (2005)



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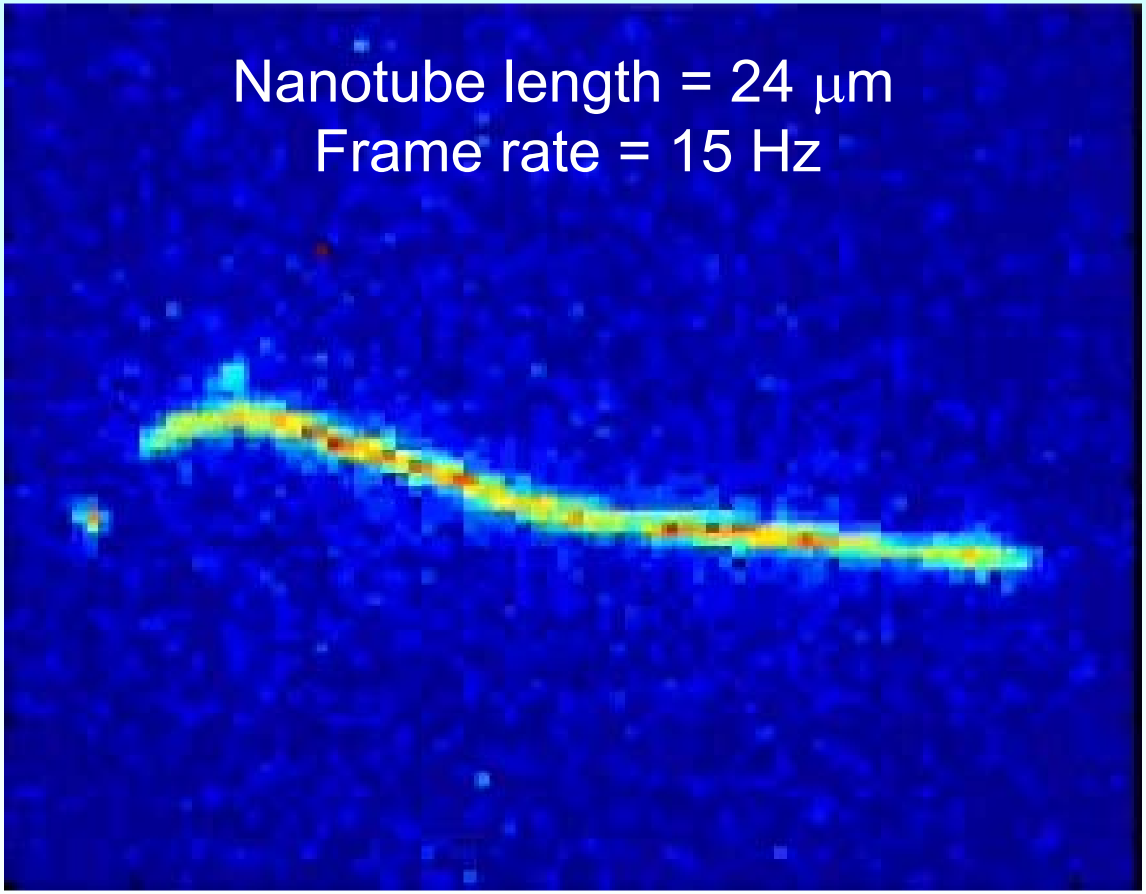
Fluorescence image of a free SWCNT in water suspension



Find: Persistence length varies as d^3

in-plane bending stiffness is ~650 N/m (> predicted)

N. Fakhri et al., *Proc. Natl. Acad. Sci. USA 106*, 14219 (2009)

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Measured emission flux =

excitation intensity x

$1 / h\nu_{22}$ x

$\sigma(\lambda_{22})$ x

Φ_{Fl} x

instrumental detection efficiency

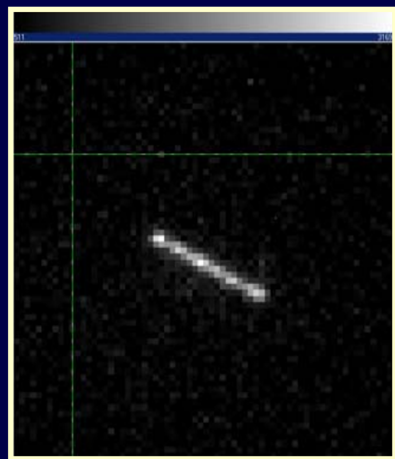
Obtain absolute values of $\sigma_{22}\ \Phi_{Fl}$

Standards for selecting SWCNTs

- Length > 3 μm
- Emission peak within 20 cm^{-1} of standard value (not bundled)
- Few or no dark regions along entire tube length
- Isolated and moving freely (not stuck)

SWCNT Selection

Good tubes



7.4 μm long (9,7)

6.3 μm long (10,2)

10.6 μm long (8,7)

Bad tube

Use the brightest segment

Tsyboulski, et al.
Nano Lett. 10, 3080 (**2007)**

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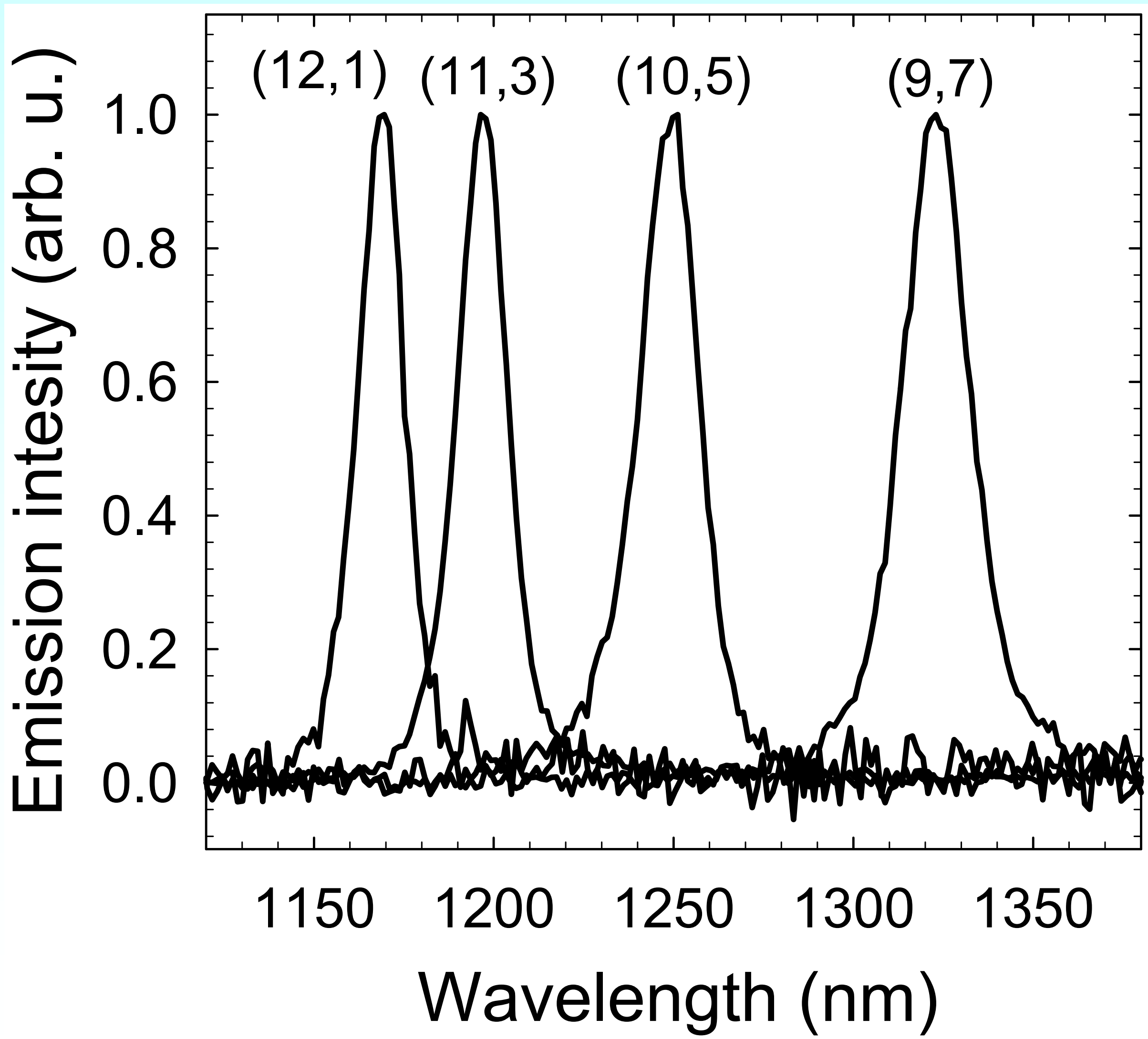
Emission spectra allow (*n*,*m*) identification of single SWNTs in aqueous suspension



New results for 31 different (*n*,*m*) structures in SDBS suspension

Findings

- Intrinsic fluorescence action cross-sections measured for 31 different (*n*,*m*) species
- Values decrease smoothly as E_{11} decreases below ~7500 cm^{-1}
- At higher E_{11} , *mod 2* tubes are brighter than *mod 1* , with differences greatest for small roll-up angles
- Zig-zag tubes seem unusually dim
- Brightest SWCNTs found so far are (10,2) and (12,1)
- Empirical model fits data with average error of 11%; can be used for extrapolation

Application

Improved (n,m) sorting of SWCNTs
using
Density Gradient Ultracentrifugation
and fluorescence analysis

Density Gradient Ultracentrifugation (DGU)

$$\mathrm{v} = \frac{d^2(\rho_p - \rho_l)g}{18\,\eta}$$

v = sedimentation speed

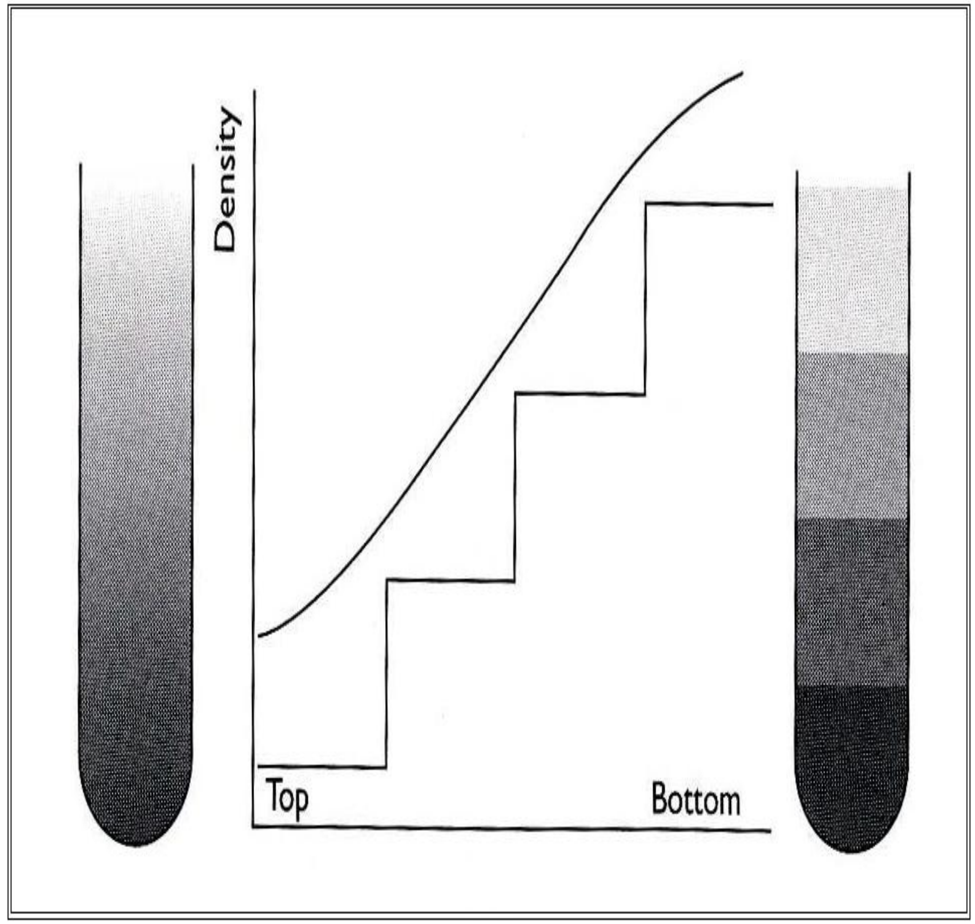
d = particle diameter

ρ_p = particle density

ρ_l = liquid density

η = liquid viscosity

g = centrifugal acceleration



Linear gradient

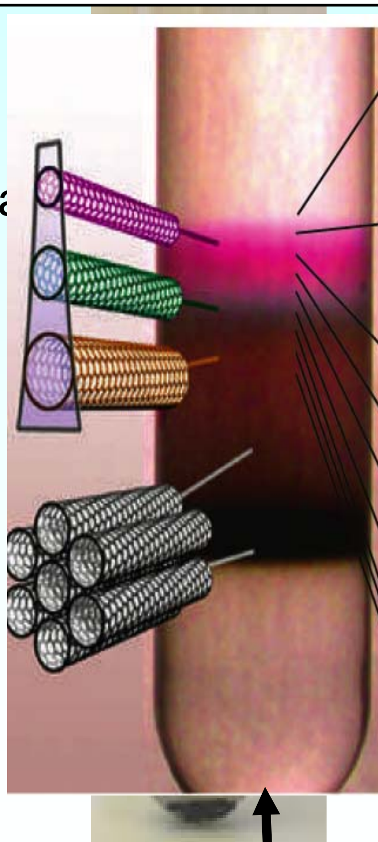
Step gradient

Ultracentrifugation processing of SWCNTs

N

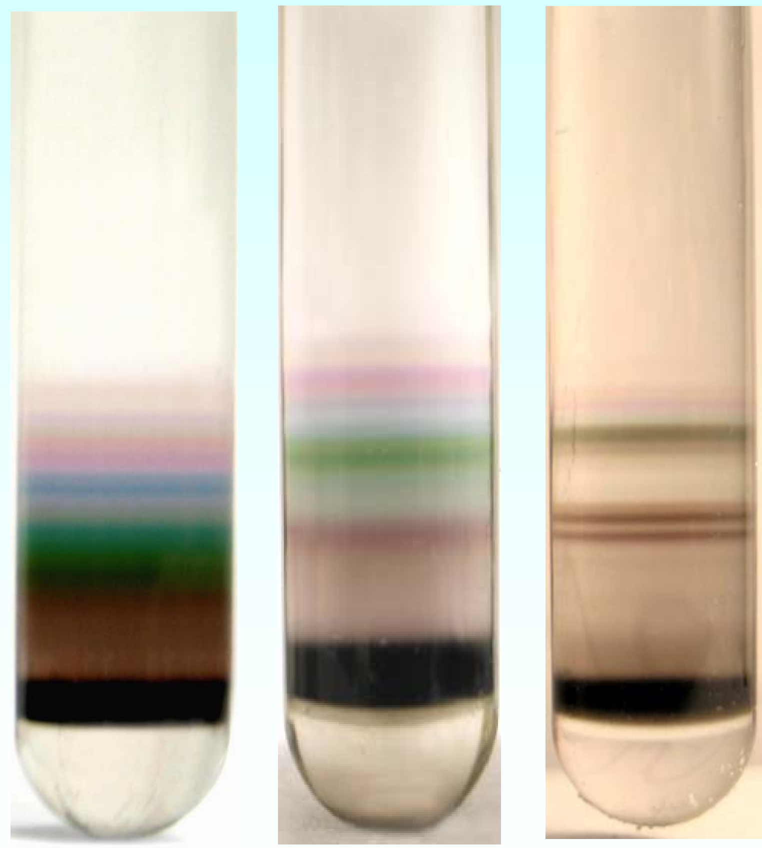
Density gradient (CoMoCAT)

SWCNT
supernat



Arnold et al., *Nature Nanotech*. *1*, 60 (2006)

Refined DGU (HiPco)



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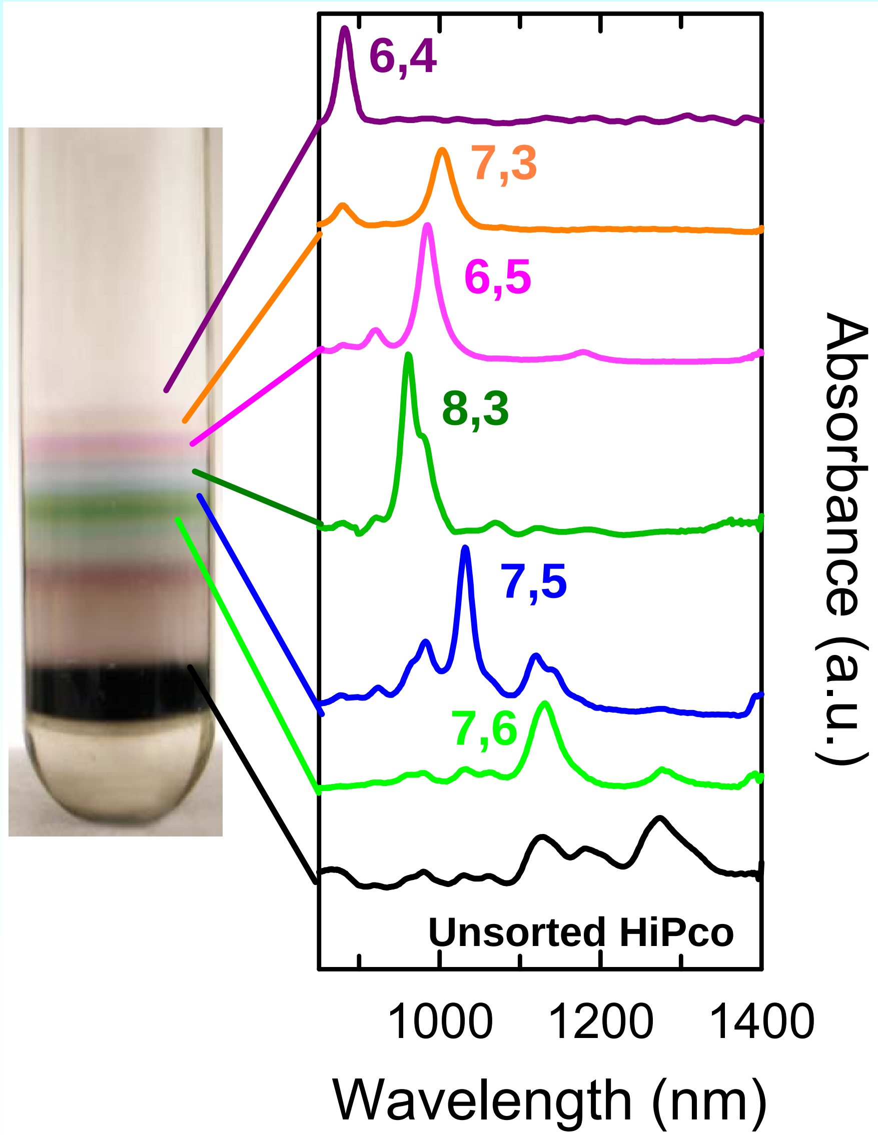
DGU spatially separates HiPco sample into (*n*,*m*) species



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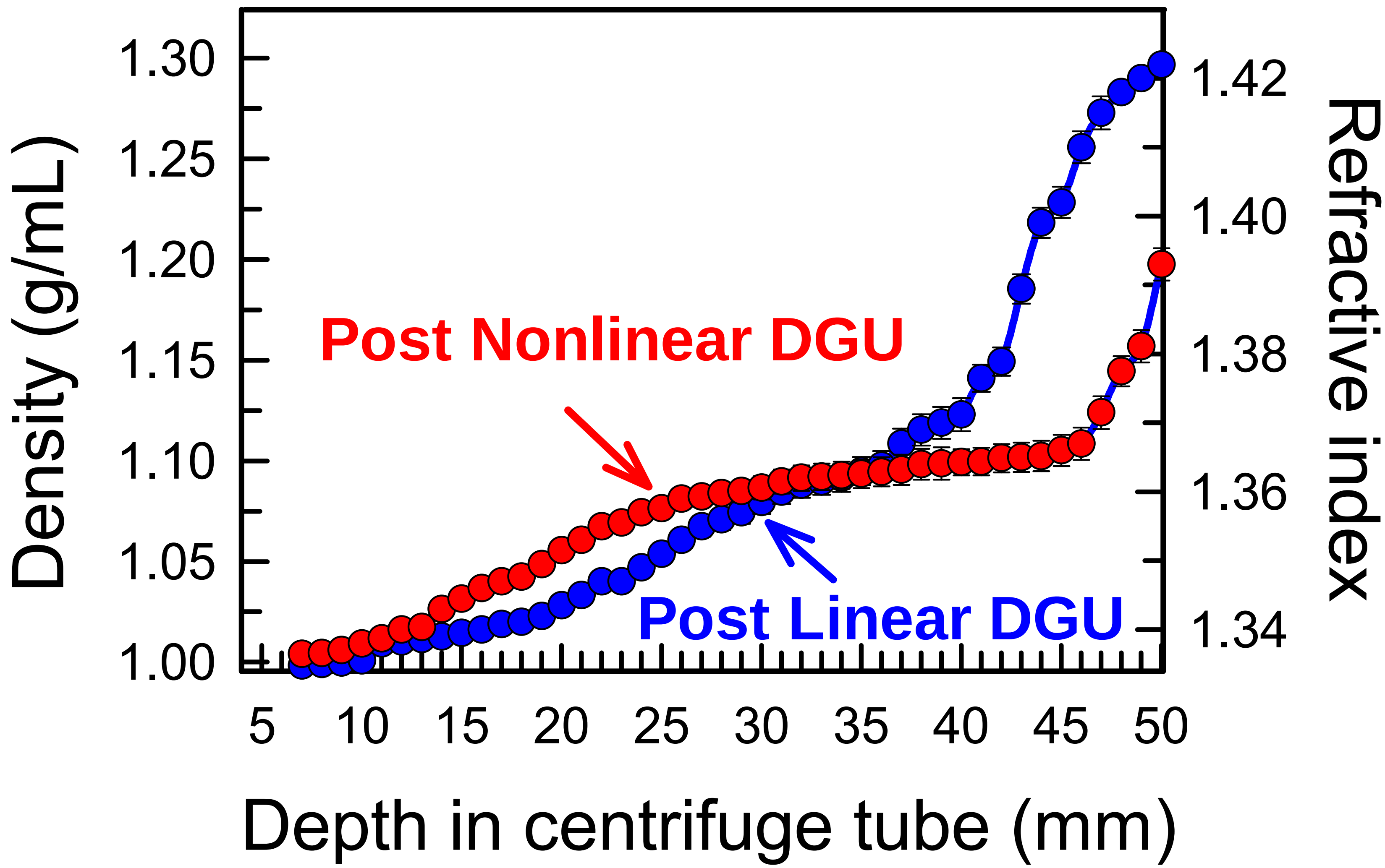
Comparison of density profiles after centrifugation



Factors in DGU separation of SWCNTs

- choice and concentration of surfactant
- prior sample processing
- sample loading (amount and position)
- form of density profile
- size and shape of centrifuge tube
- centrifugation speed and duration
- centrifugation temperature

Valuable new tool: *in situ* spectrometric analysis

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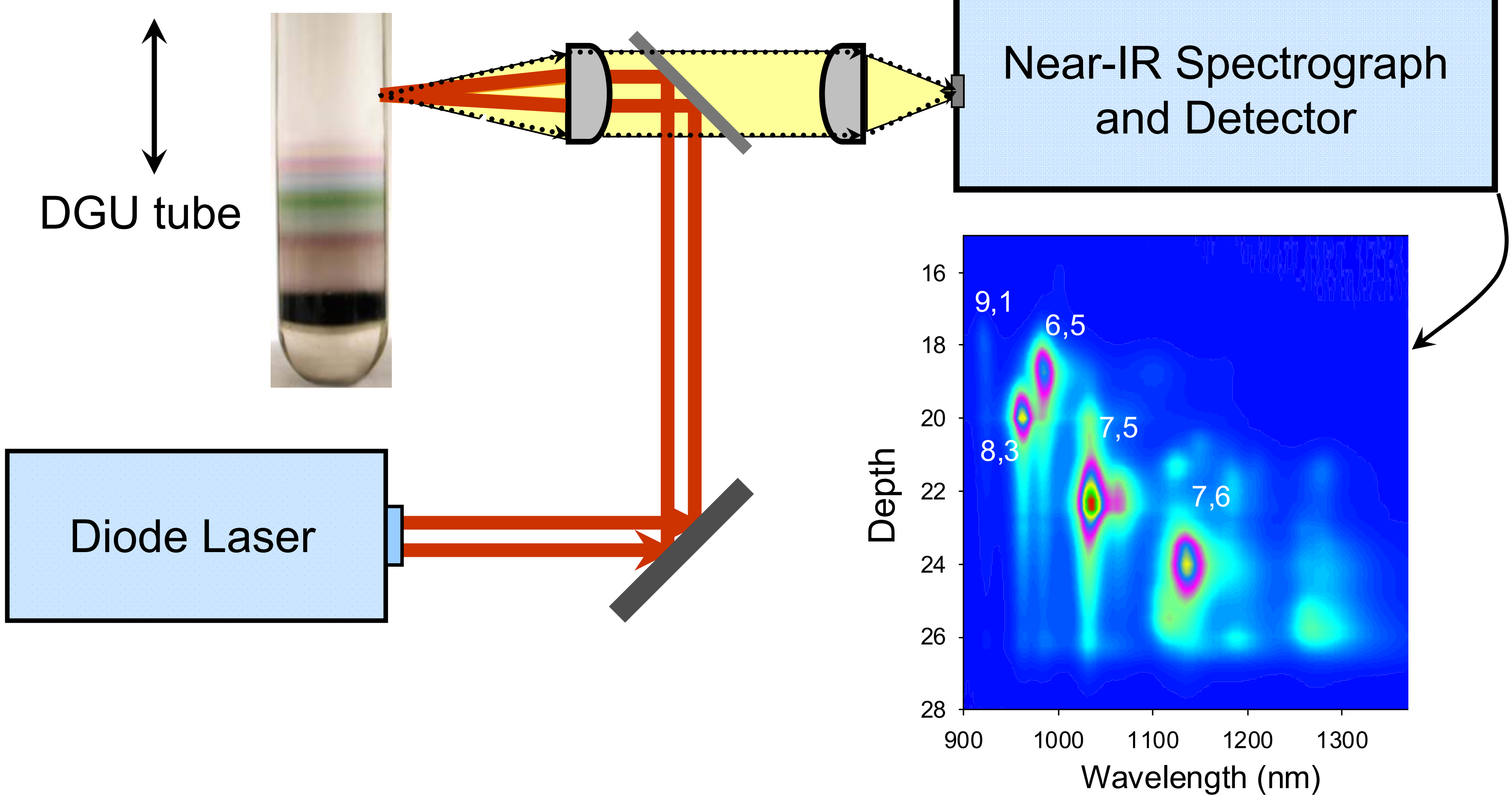
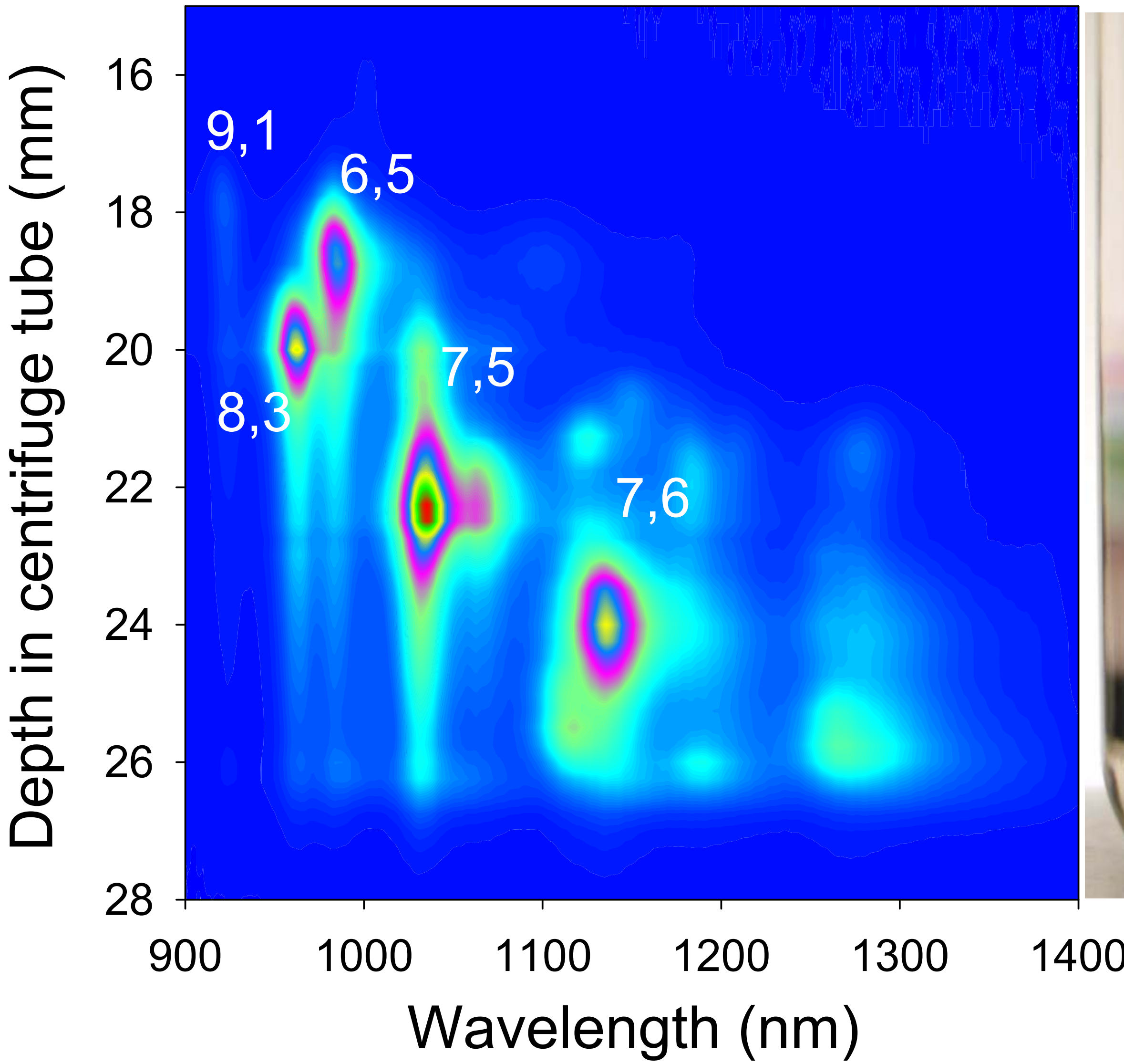
NanoSpectralyzer DGU Spectral Mapping Mode



Spatially resolved fluorescence spectra: *in situ* spectral map of DGU-processed centrifuge tube



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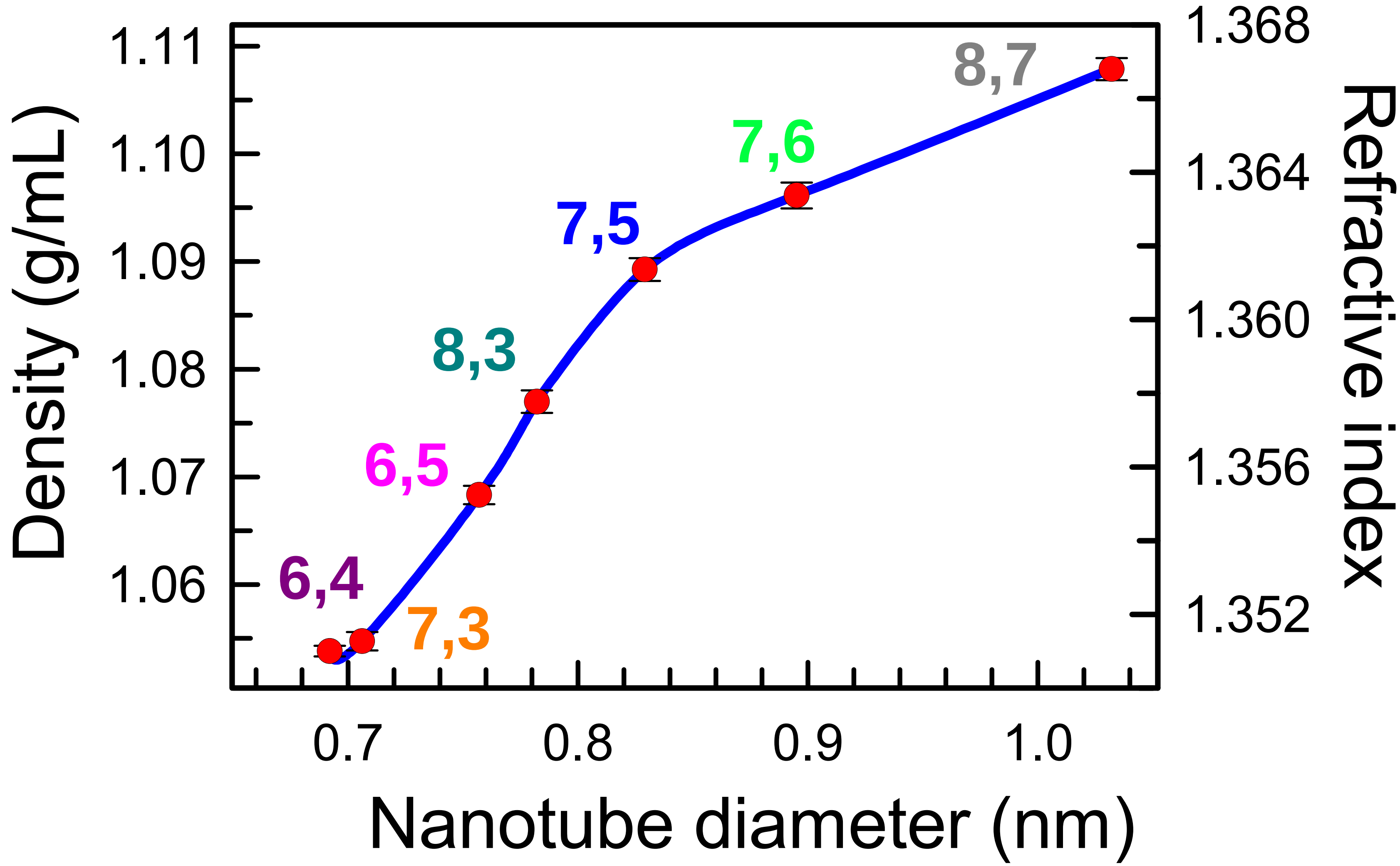
Densities of surfactant-suspended (*n*,*m*) species



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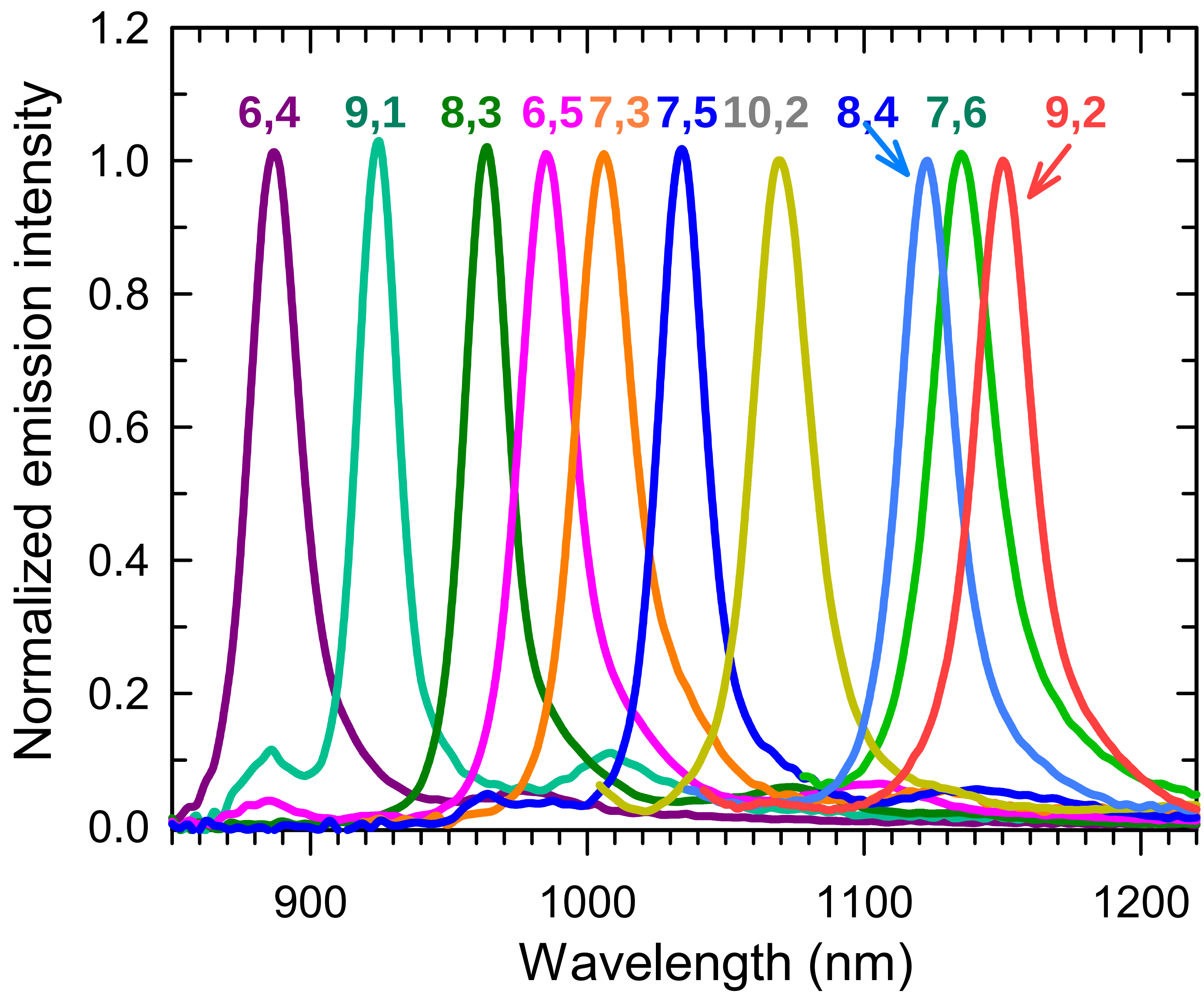
Fluorescence spectra of separated SWCNT fractions



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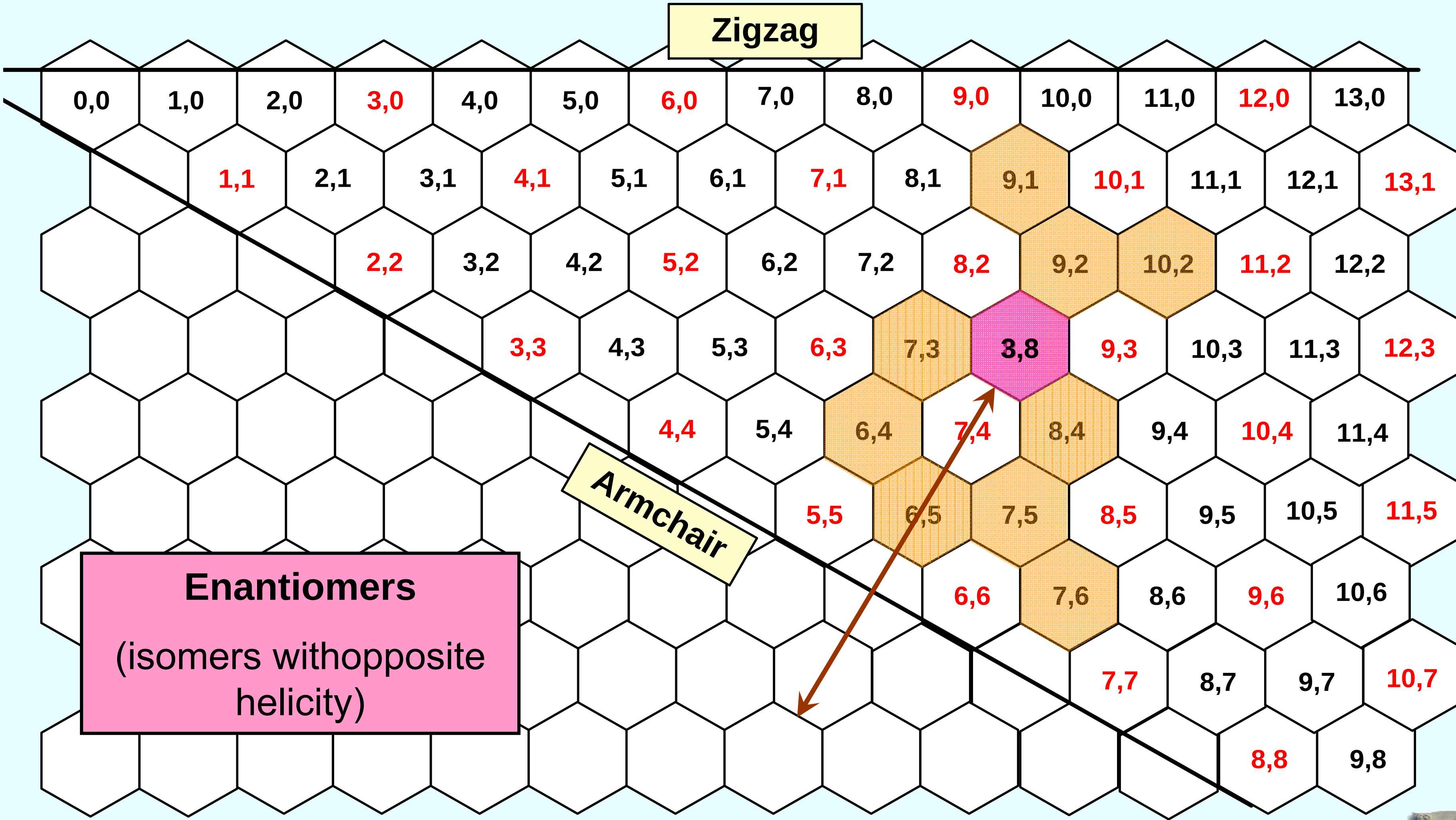
(*n*,*m*) species enriched from HiPco samples



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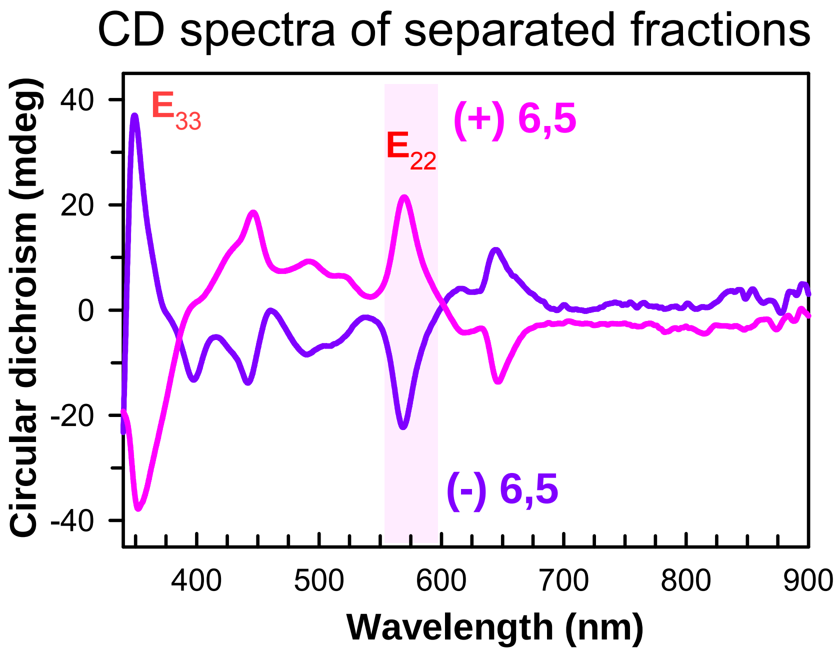
Separation of SWCNT enantiomers (left- and right-handed forms)



Application

Using near-IR fluorescence
to observe SWCNTs
in biomedical systems

Motivation

- **Toxicology**
  Study uptake, clearance, bio-distributions, and effects of SWCNTs in exposed organisms

- ***In vitro* cell biology**
  Monitor SWCNT behavior in cells
  Develop new fluorescent bio-markers as research tools

- **Medical diagnostics**
  Develop near-IR fluorescent contrast agents for non-invasive disease diagnosis

- **Medical therapeutics**
  Use targeted SWCNTs for drug delivery or thermal ablation therapy (near-IR absorptions)

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Near-infrared light is useful in biology

- Less absorption by tissues
- Less scattering by tissues
- Low "endogenous" fluorescence from natural compounds

But organic near-IR fluorophores

- rarely emit beyond ~800 nm
- have poor photo-stability

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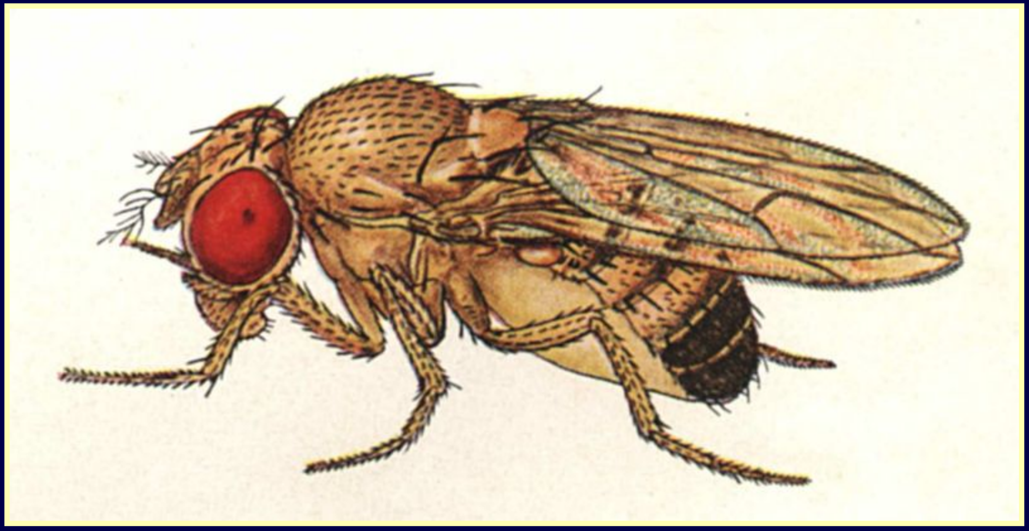
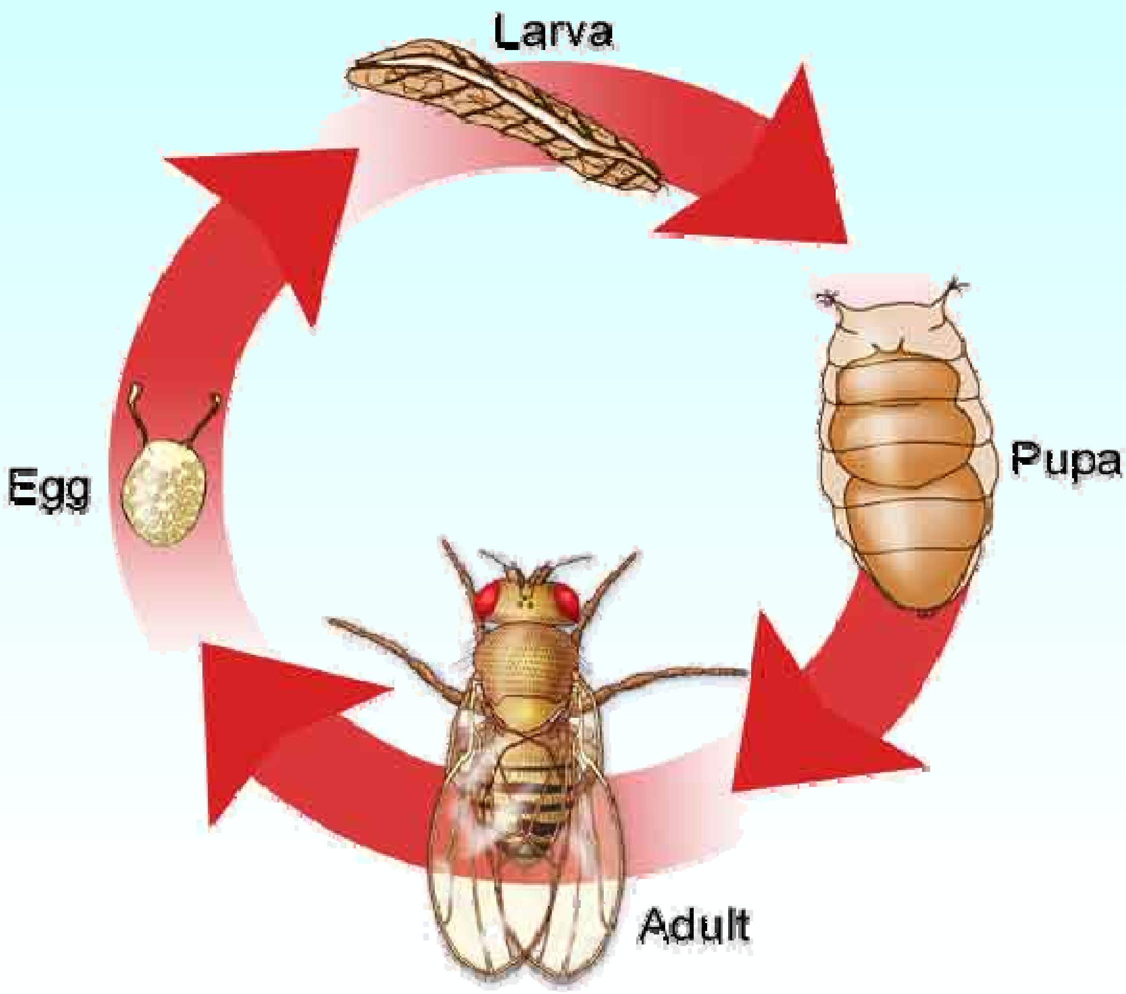
Fluorescence Studies of SWCNTs in Fruit Flies

(Drosophila melanogaster)



Drosophila Life Cycle



Drosophila study - Methods

- Prepare food containing yeast and SWCNTs in BSA suspension (9 ppm SWCNT by mass)
- Feed SWCNT food to fly larvae
- Observe development to adult flies
- Image SWCNT fluorescence from intact larvae
- Image SWCNT fluorescence from larval tissues
- Compare SWCNT content of different tissues

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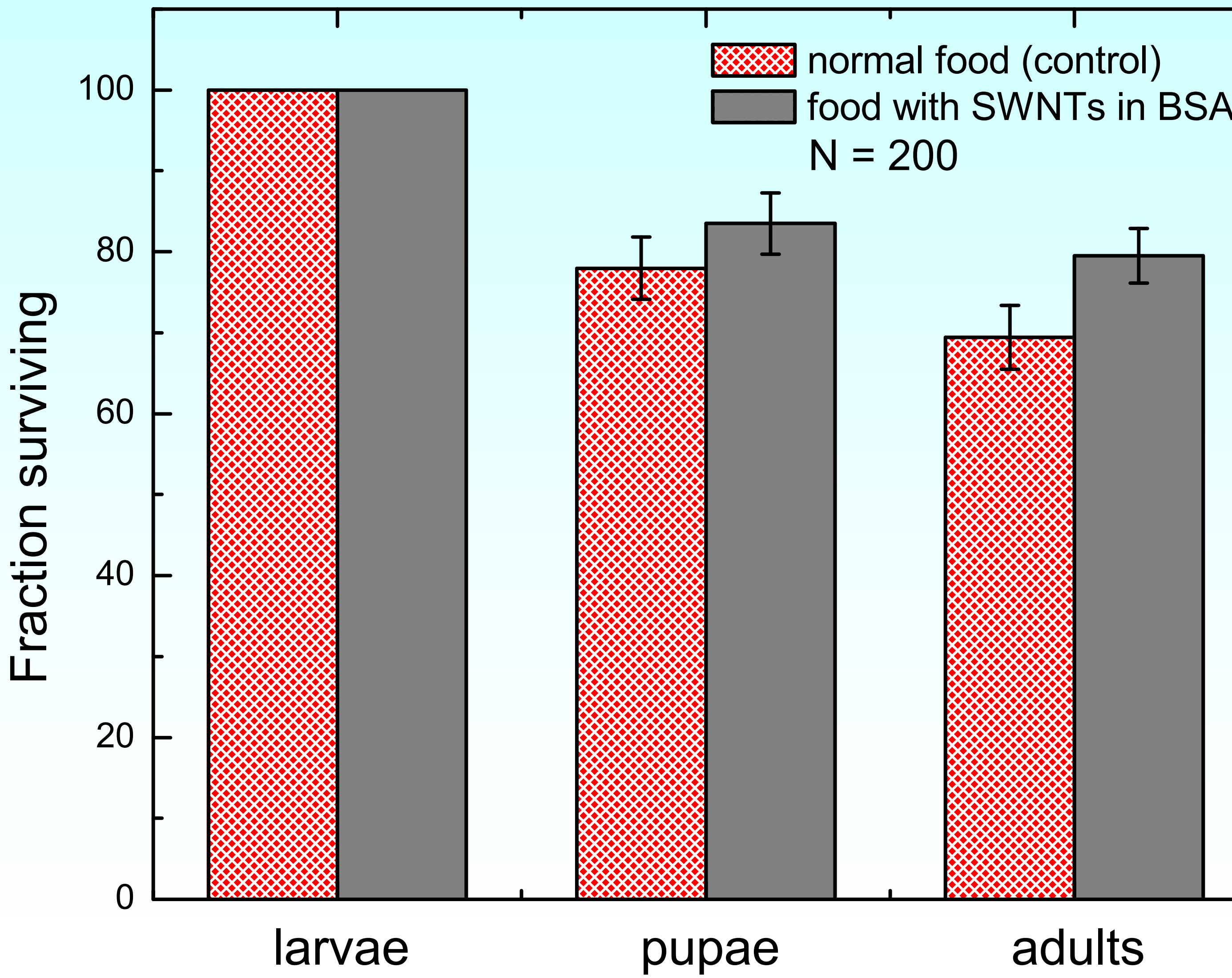
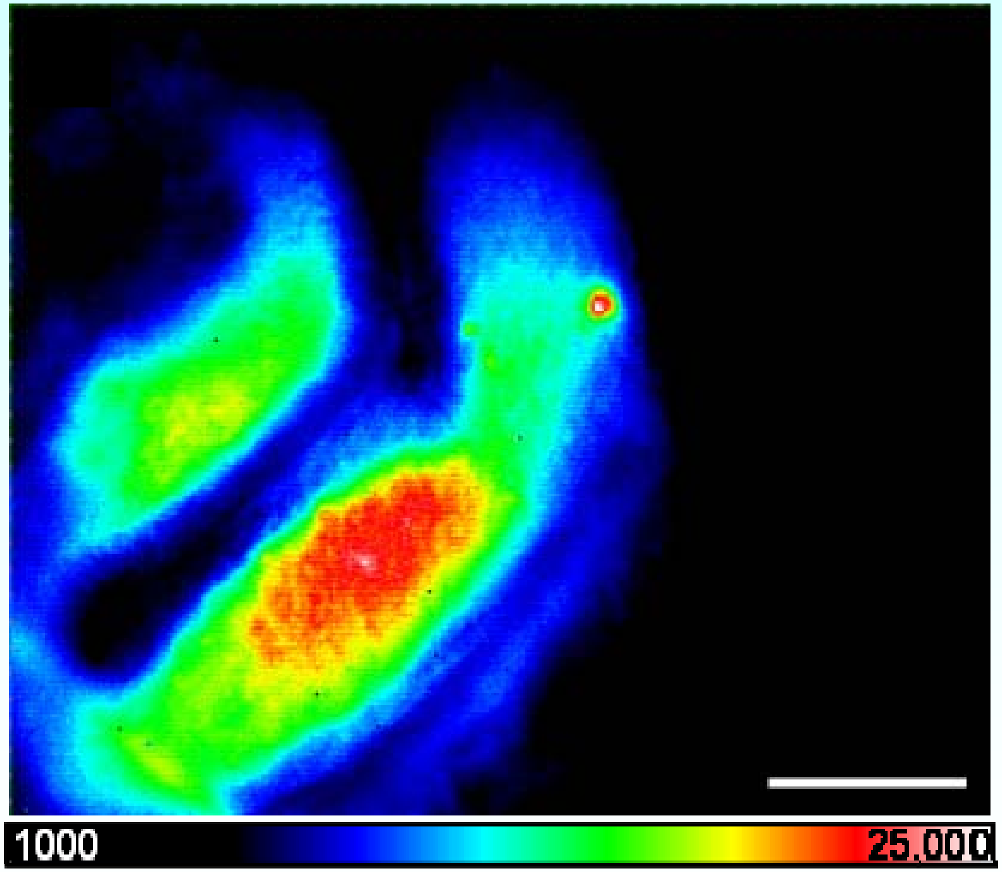
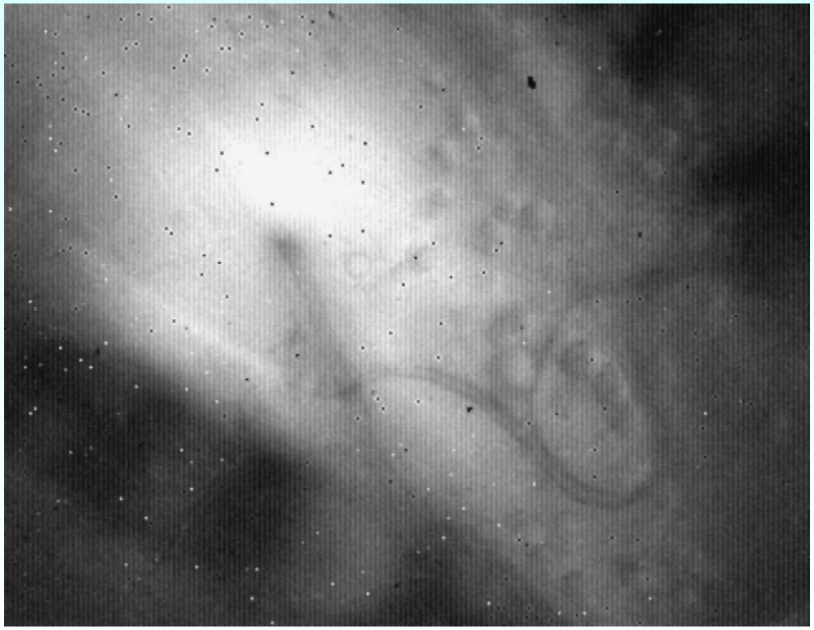
Drosophila viability not impaired by nanotubes in food

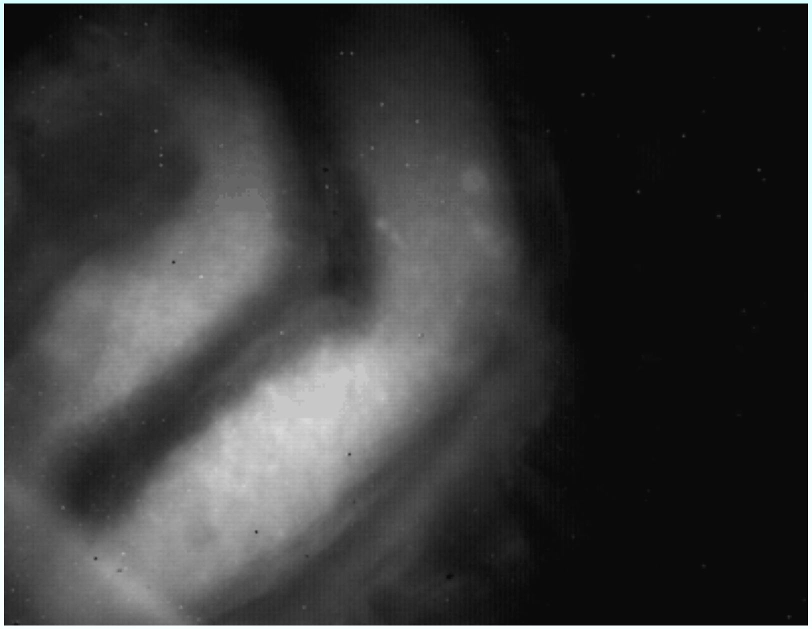


Fluorescence of SWCNTs inside gut of a living Drosophila larva fed with nanotube food

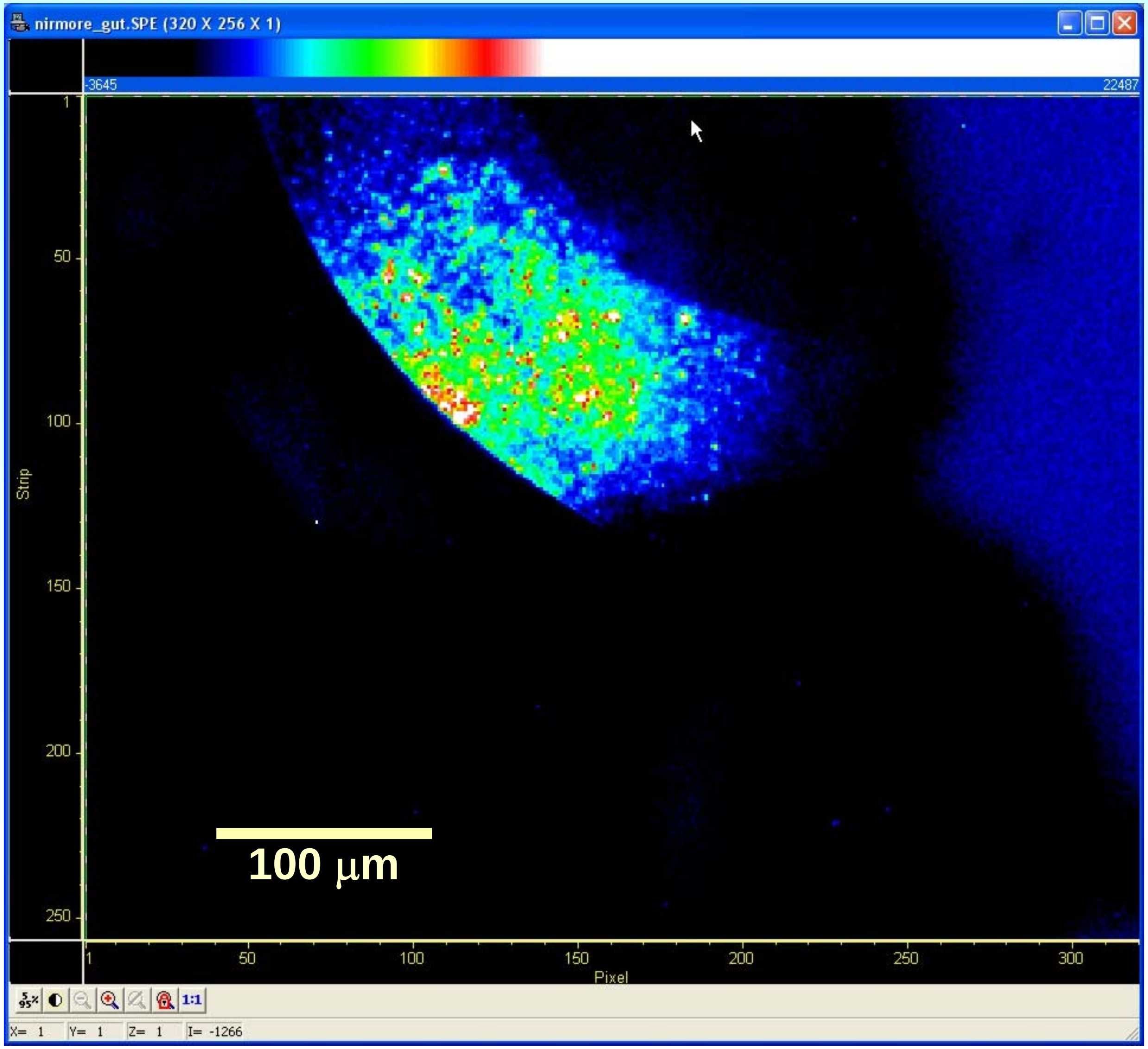


Fluorescence of SWCNTs inside gut of a living Drosophila larva





Dissected gut of Drosophila (fruit fly) larva fed with SWCNT-yeast paste



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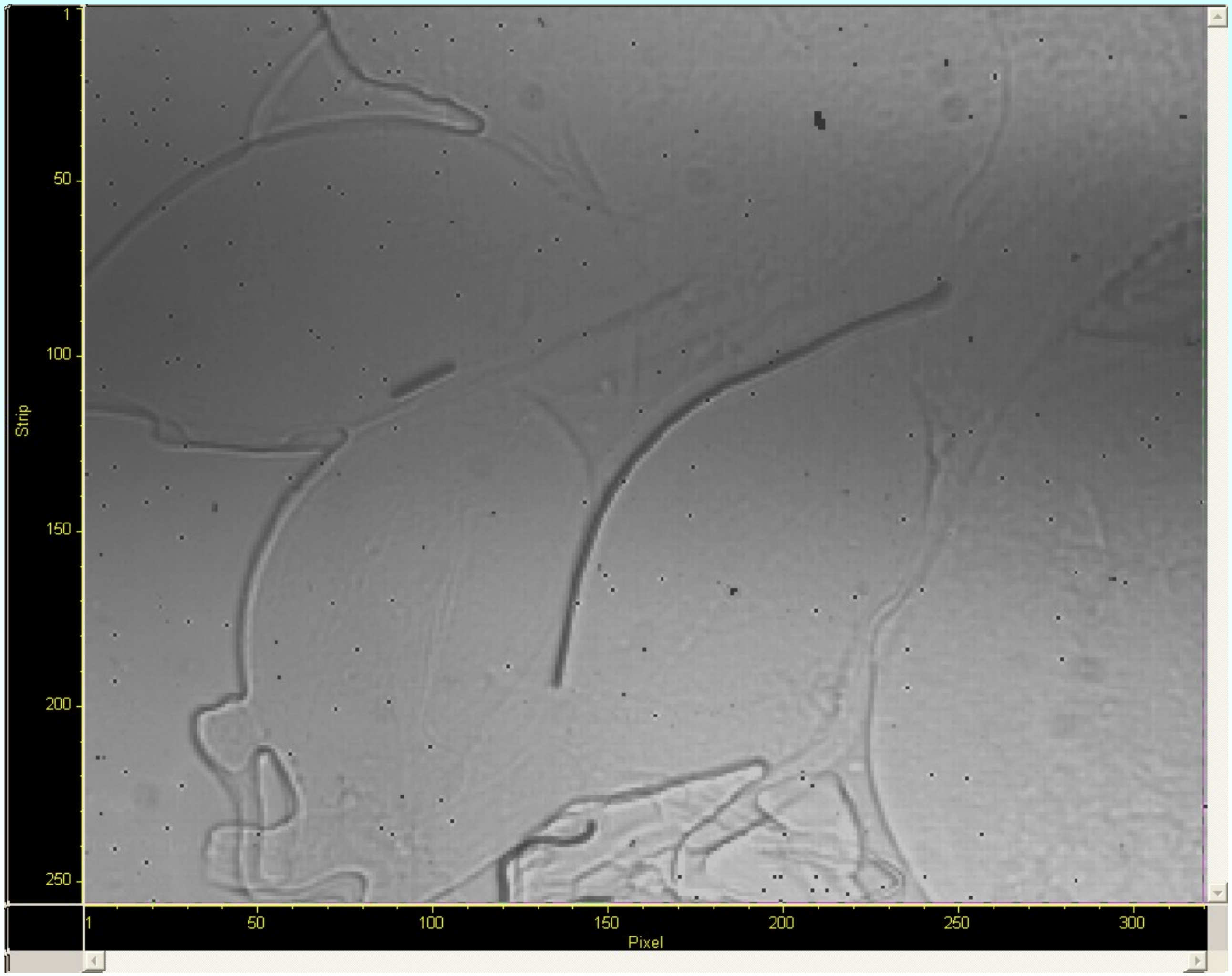
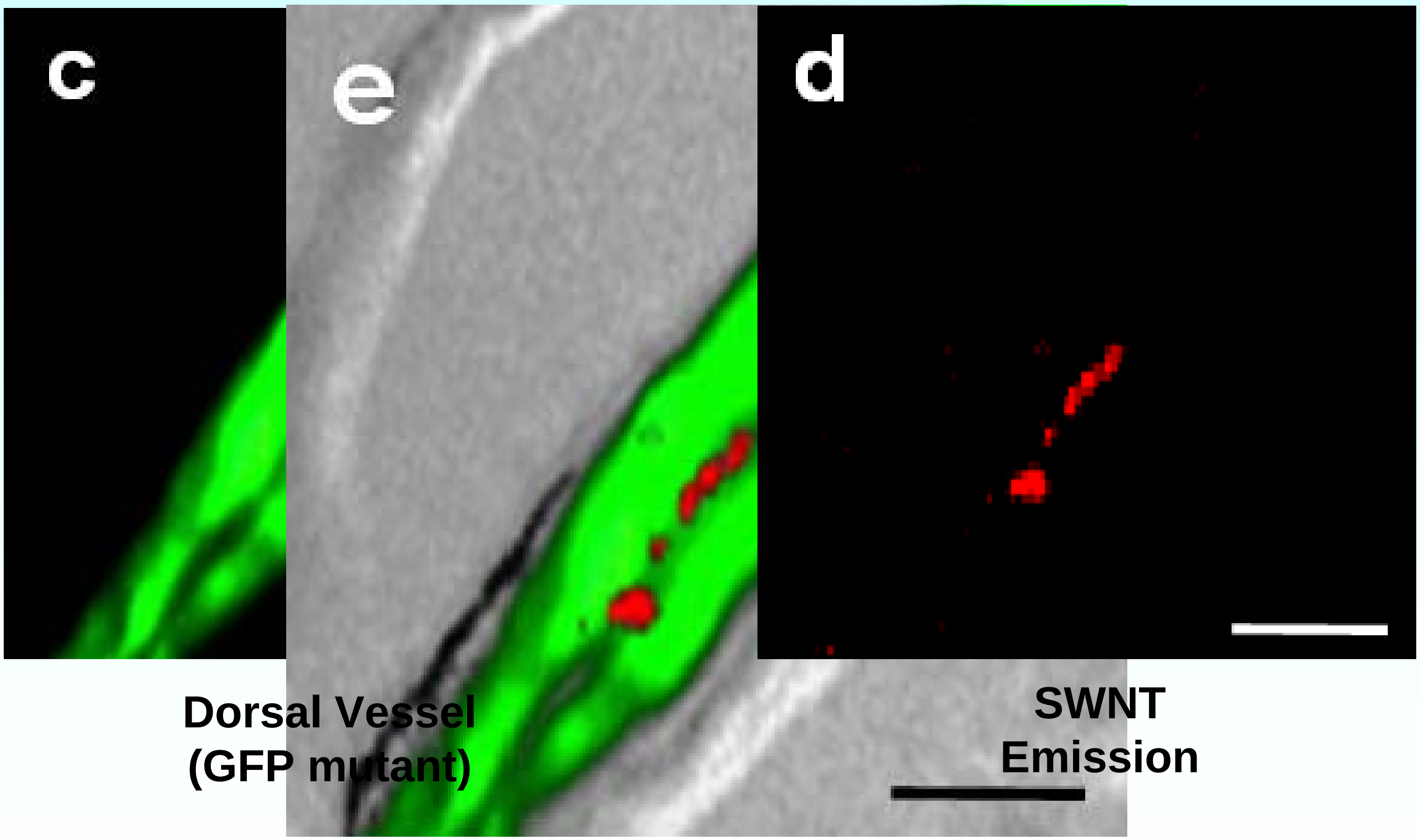
SWCNTs in the dorsal vessel of dissected Drosophila (fruit fly) larva after oral exposure



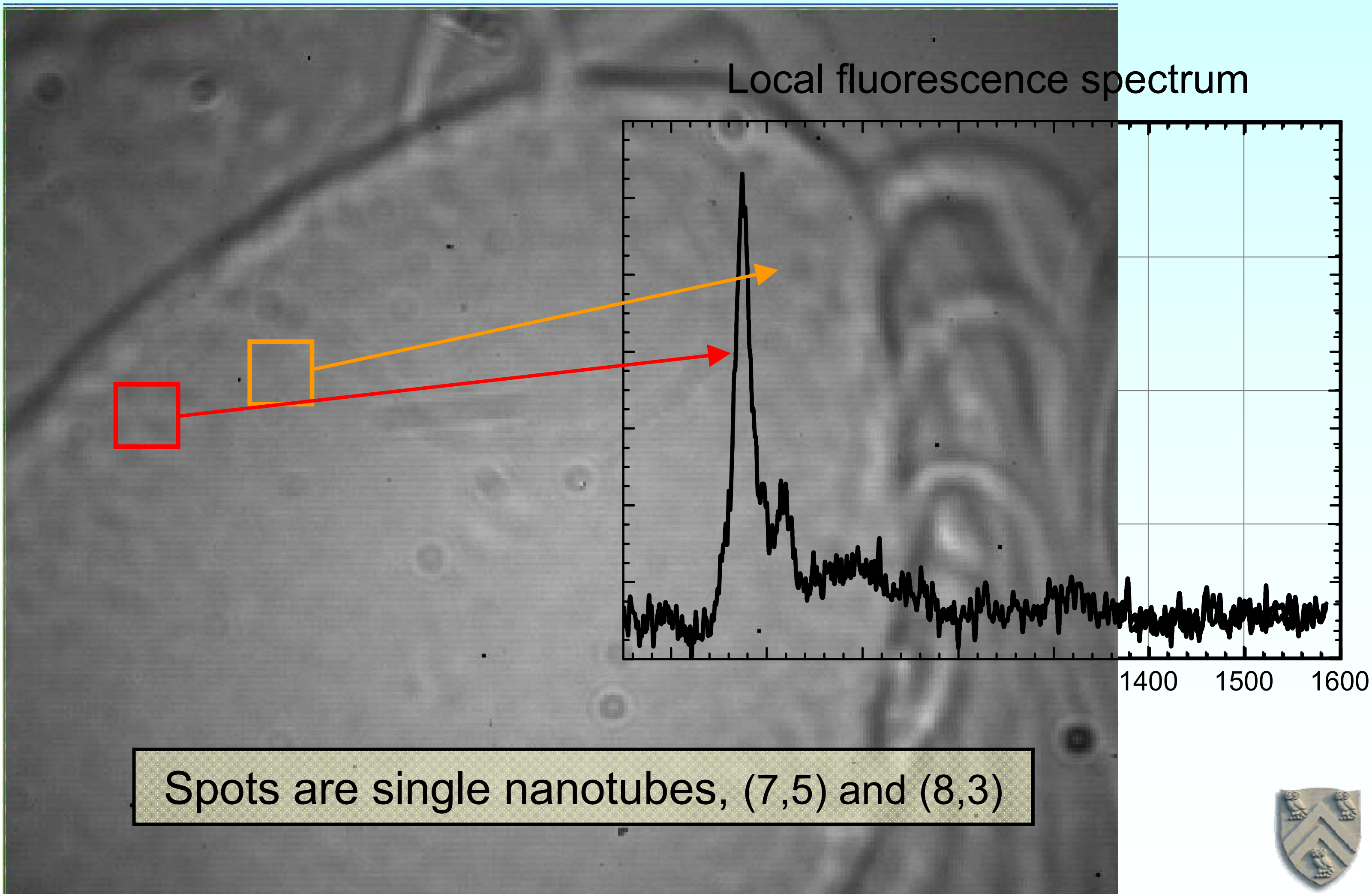
SWCNTs in the dorsal vessel



Dorsal Vessel
(GFP mutant)

SWNT
Emission

Dissected brain tissue of Drosophila larva fed with SWCNT-yeast paste



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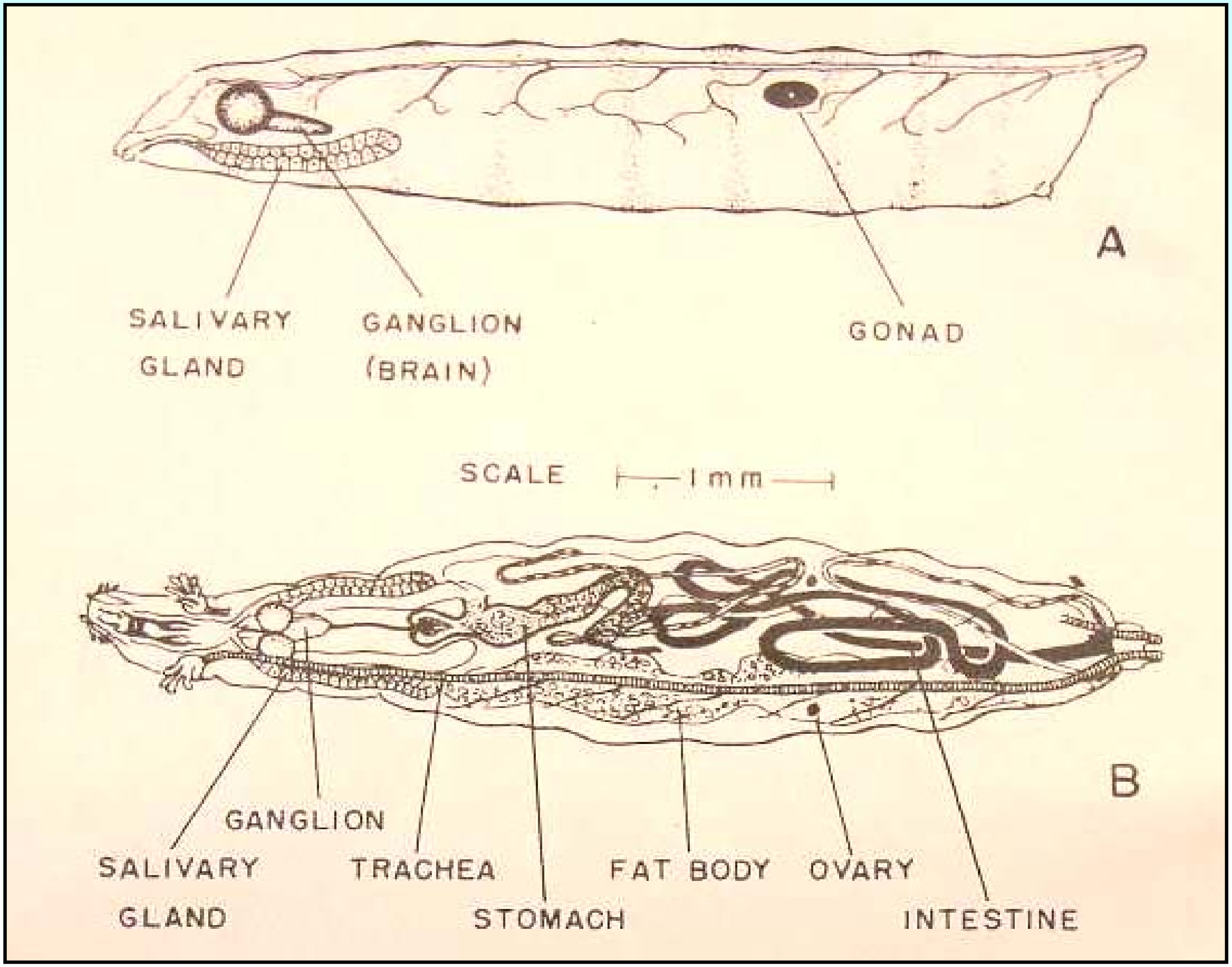
SWCNT biodistribution in Drosophila larvae after oral administration

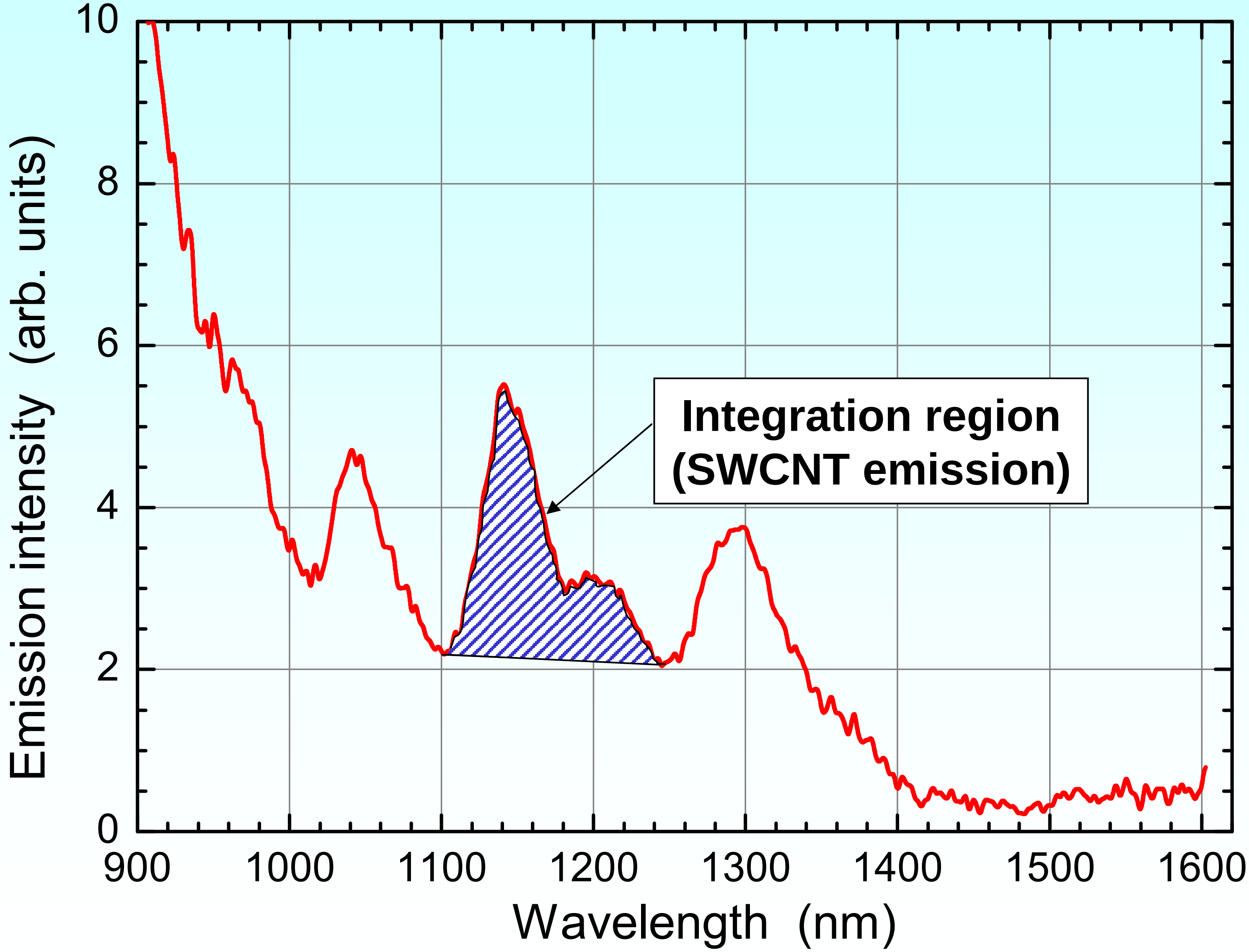


Fluorescence Studies of SWNTs in Rabbits

Rabbit study - Method

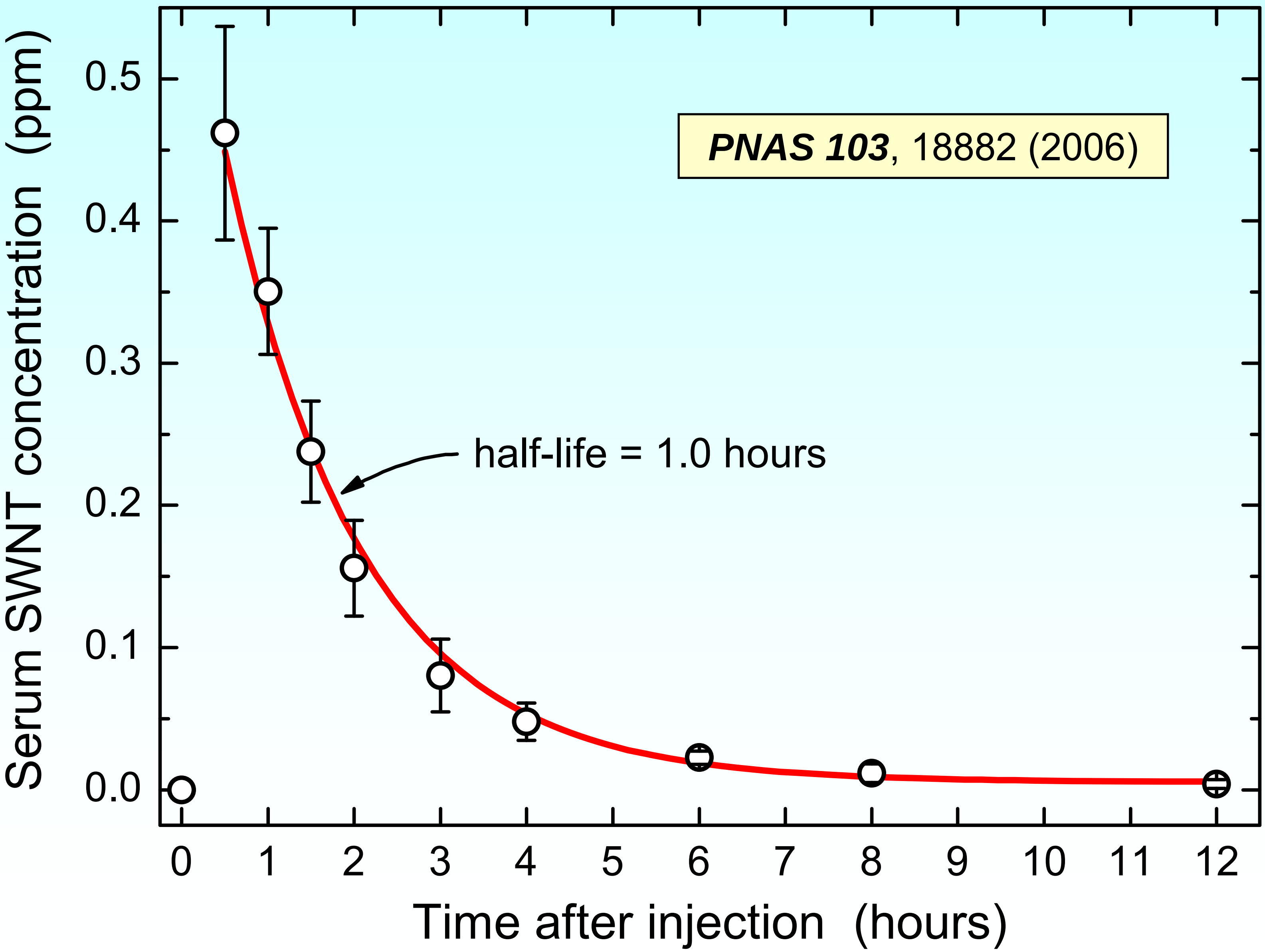
- Inject 7.5 mL of aqueous Pluronic suspension of pristine SWCNTs into jugular vein
  (initial SWCNT blood concentration = 0.75 ppm)
- Collect 1 mL blood samples at intervals over a 24 hour period
- Analyze serum fractions with NanoSpectralyzer® to find SWCNT concentrations
- Use near-IR fluorescence microscopy to examine tissue samples, get biodistribution information

SWCNT fluorescence spectrum from rabbit blood serum



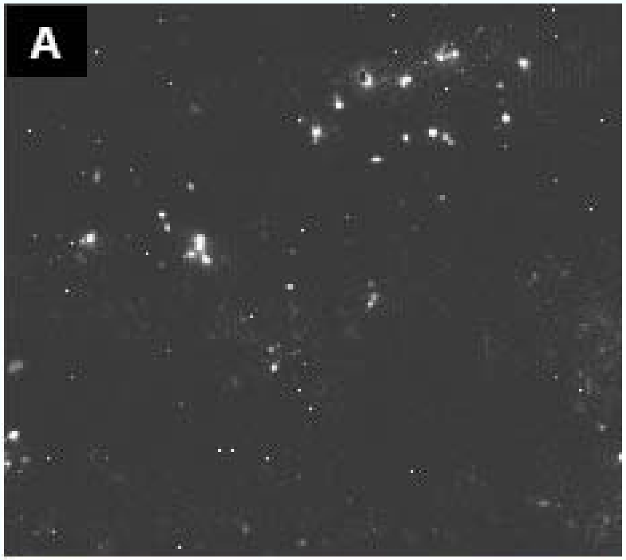
RICE

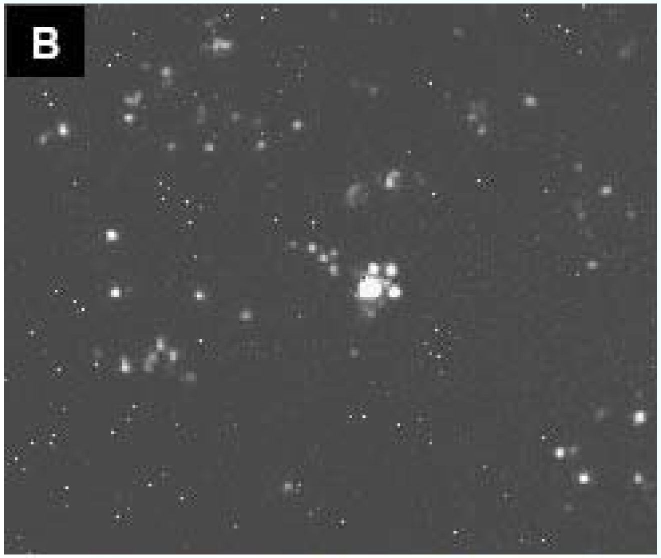
SWCNT elimination kinetics from rabbit blood circulation



RICE

Rabbit liver 24 hours after i.v. SWCNT administration





RICE

Rabbit study - Results

- Circulation half-life of pristine SWCNTs is 1 hour
- SWCNTs cause no acute toxic effects
- At 24 hours, liver had much higher SWCNT concentration than other tissues
- Virtually no SWCNTs found in kidney tissue

Summary

Recent review paper:
Weisman, *Analytical & Bioanalytical Chem. 396*, 1015 (2010)

- Near-IR fluorescence of SWCNTs allows structural identification, trace detection, and imaging (even of single tubes)
- Customized multi-mode spectrometric instruments are available
- Fluorescence brightness per carbon atom varies systematically with SWCNT structure; calibration factors are now measured
- Structurally sorted bulk samples are becoming available
- Fluorescence methods will help develop SWCNT biomedical applications

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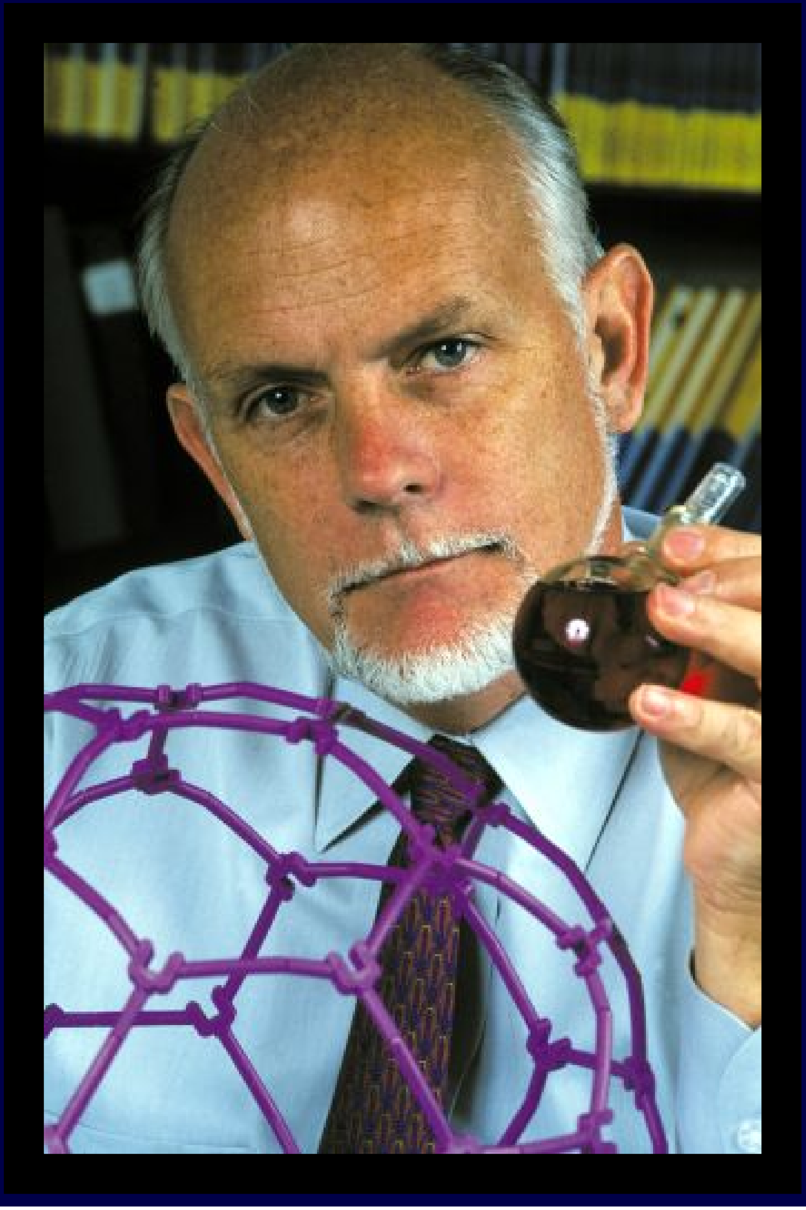
RICE

Richard E. Smalley
1943 - 2005



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