

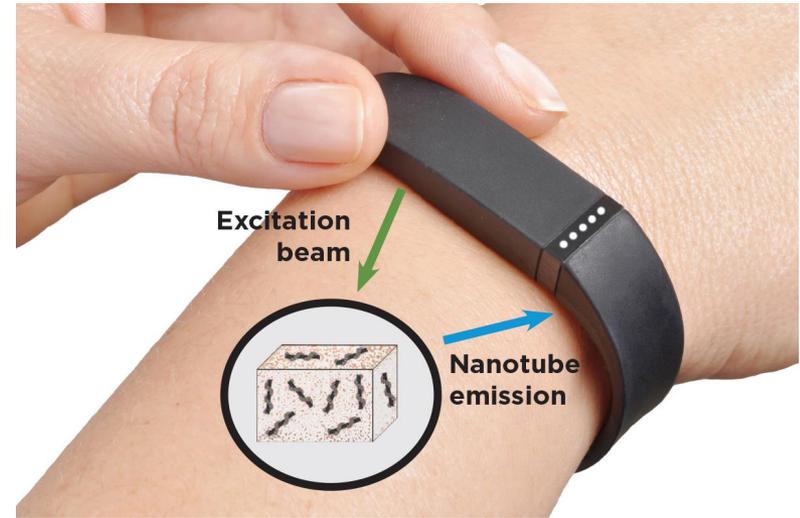
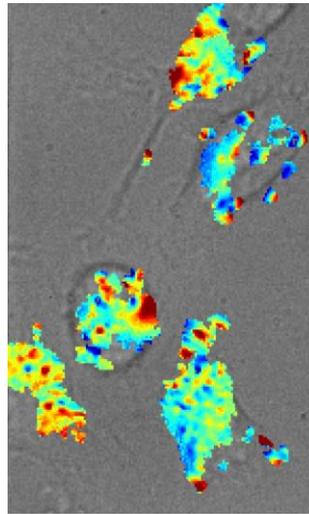
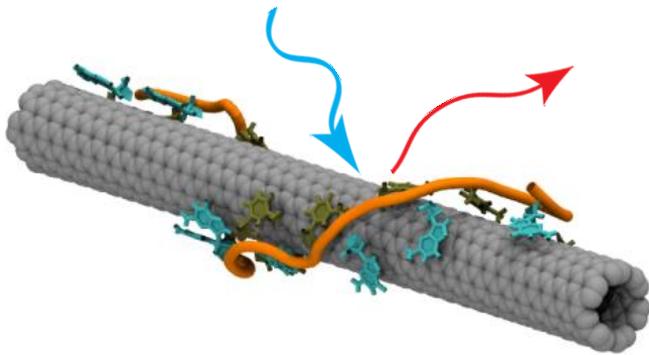
Carbon Nanotube Optical Sensors for *In Vivo* Measurement of Disease States

Daniel A. Heller, PhD

Head, Cancer Nanomedicine Laboratory

Associate Member, Memorial Sloan Kettering Cancer Center

Associate Professor, Weill Cornell Medicine, Cornell University



A Nanoscience/Bioengineering Lab at... Memorial Sloan Kettering Cancer Center

Sloan Kettering Institute



Memorial Sloan-Kettering
Cancer Center



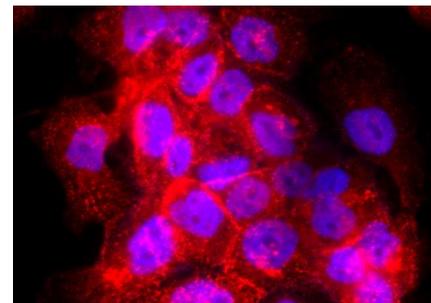
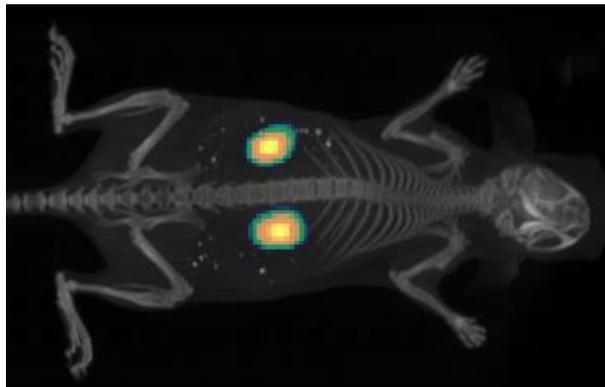
Weill Cornell Medicine
Graduate School of
Medical Sciences

A partnership with the Sloan Kettering Institute

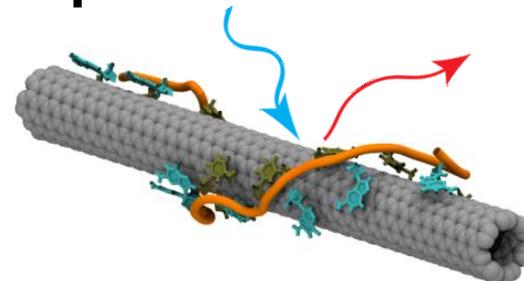
Cancer Nanomedicine Laboratory

Localized Detection in Living Systems

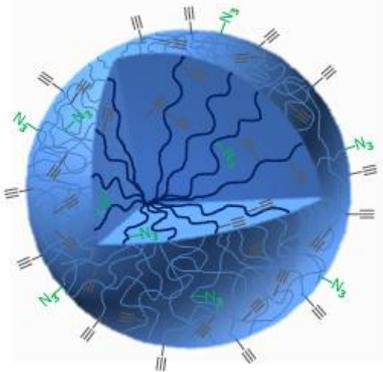
Targeted Drug Delivery



Nanoprobes/Nanosensors



Targeted Nanoparticles



- Williams, et. al, *Nano Lett* (2015)
- Shamay, et. al. *Sci Transl Med* (2016)
- Mizrachi, et. al. *Nat Commun* (2017)
- Williams, et. al. *Hypertension* (2017)
- Shamay, et. al. *Nat Mater* (2018)

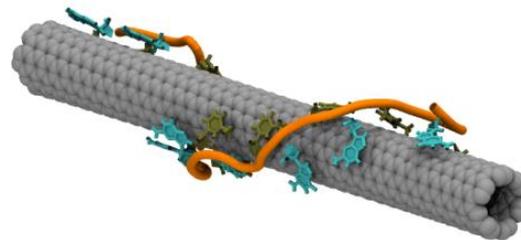
- Budhathoki-Uprety, et al. *JACS* (2014)
- Roxbury, et al. *Sci Rep* (2015)
- Roxbury, et al. *ACS Nano* (2016)
- Jena, et al. *Carbon* (2016)
- Galassi, et al. *Anal Chem* (2017)
- Budhathoki-Uprety, et al. *ACS Nano* (2017)
- Budhathoki-Uprety, et al. *J Mater Chem B* (2017)
- Jena, et al. *ACS Nano* (2017)
- Harvey, et al. *ACS Appl Mater Interfaces* (2017)
- Harvey, et al. *Nat Biomed Eng* (2017)
- Williams, et al. *Science Advances* (2018)

Nanoscale Tools Can Enable New Biology, Drug Discovery, and Diagnosis

Biologists



Research
Tools
←
Drug Screening
Assays



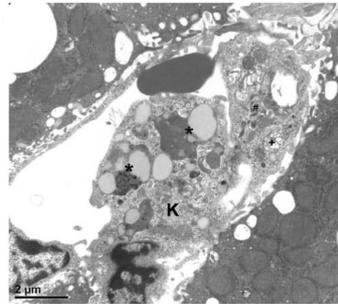
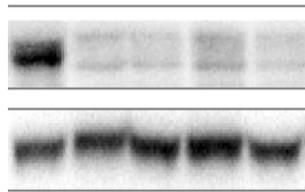
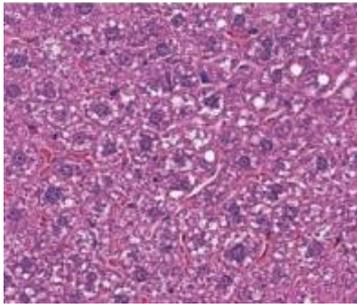
↓
Diagnostics
Therapies

Clinicians

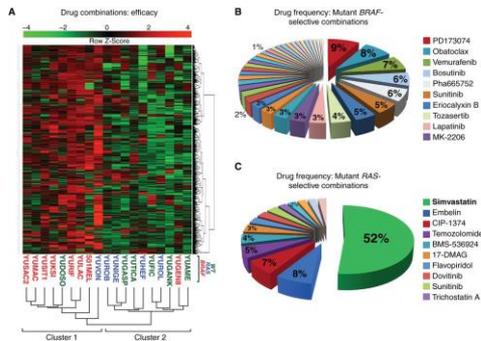
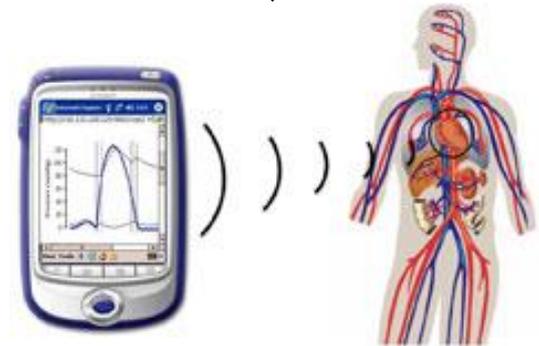
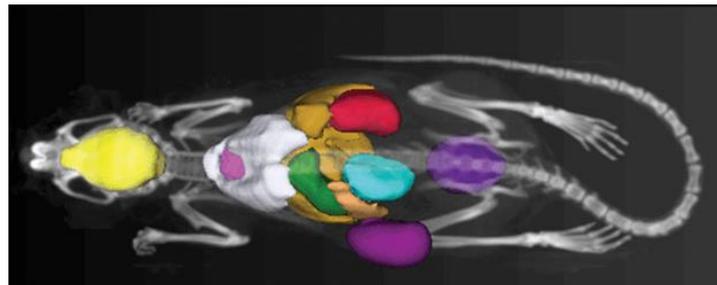
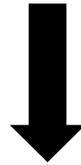


We Need Better Methods to Detect Analytes in Living Systems

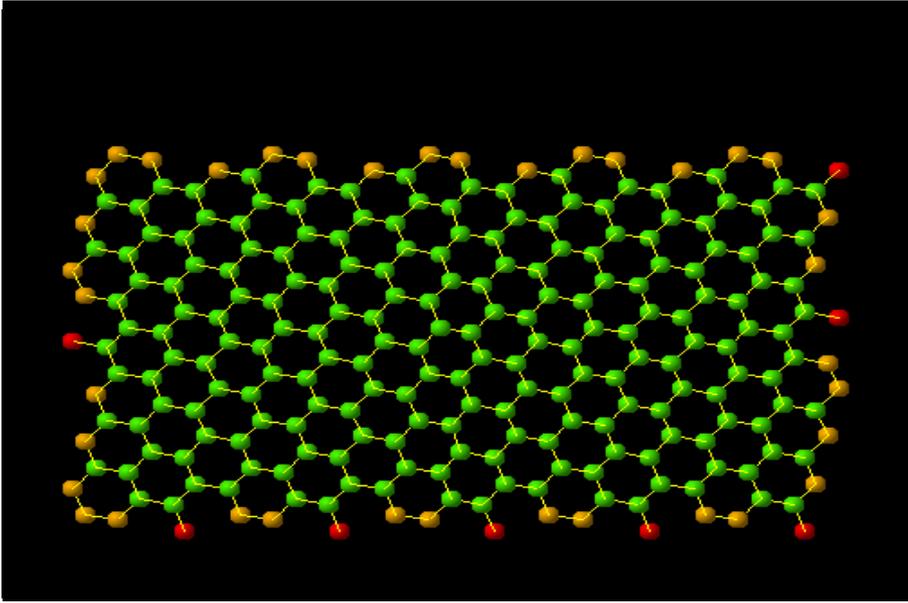
Quantitative Tools for:
Biological Research & Drug Development



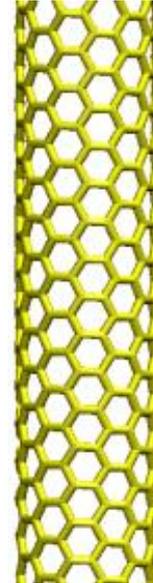
Disease
Diagnosis



Carbon Nanotubes



(6,5)



(7,6)

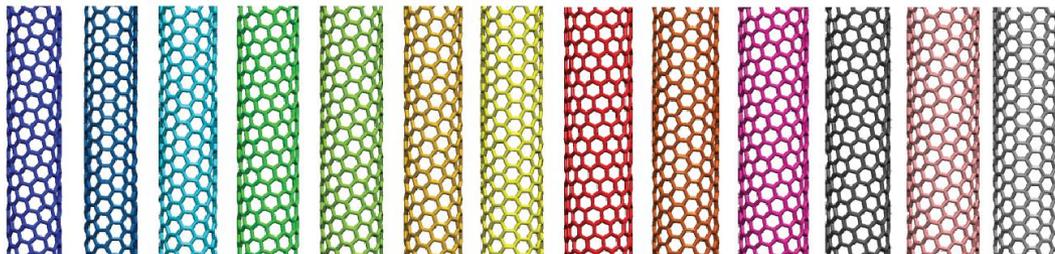


(10,3)

>50 different nanotube structures

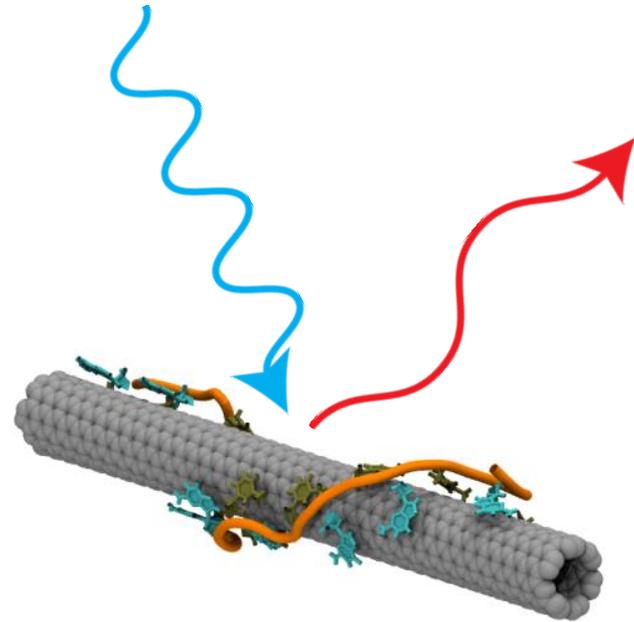
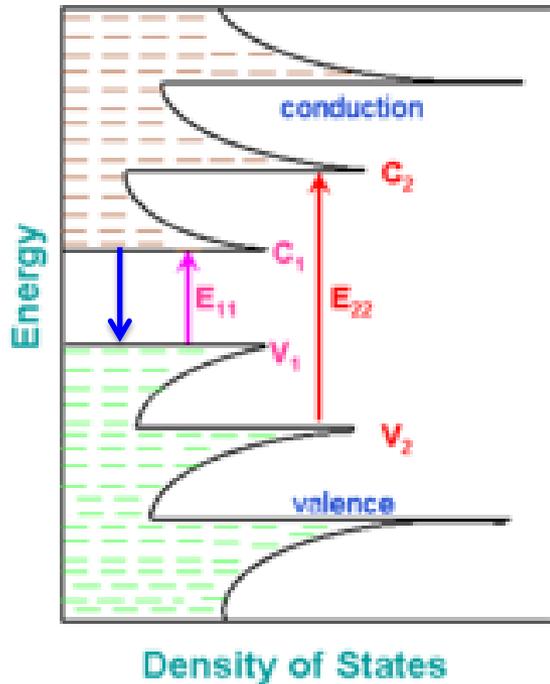
Diameters: 0.5 - 2nm

Lengths: 100-1000 nm



(8,3) (6,5) (7,5) (10,2) (9,4) (8,4) (7,6) (12,1) (8,6) (11,3) (10,3) (10,5) (8,7)

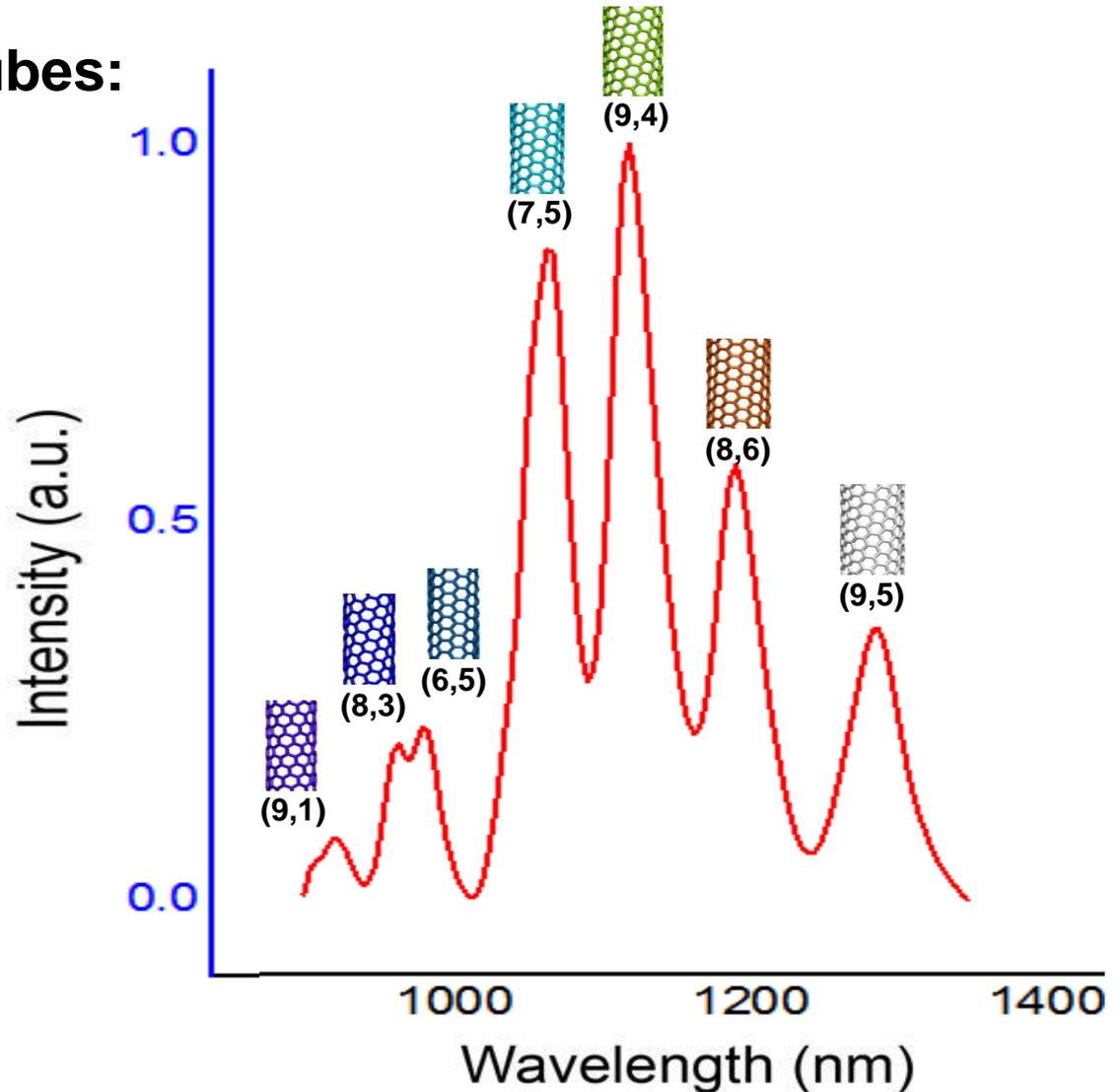
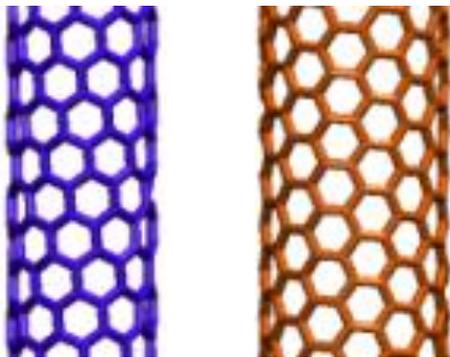
Semiconducting Carbon Nanotubes Emit Near-IR Bandgap Photoluminescence (Fluorescence)



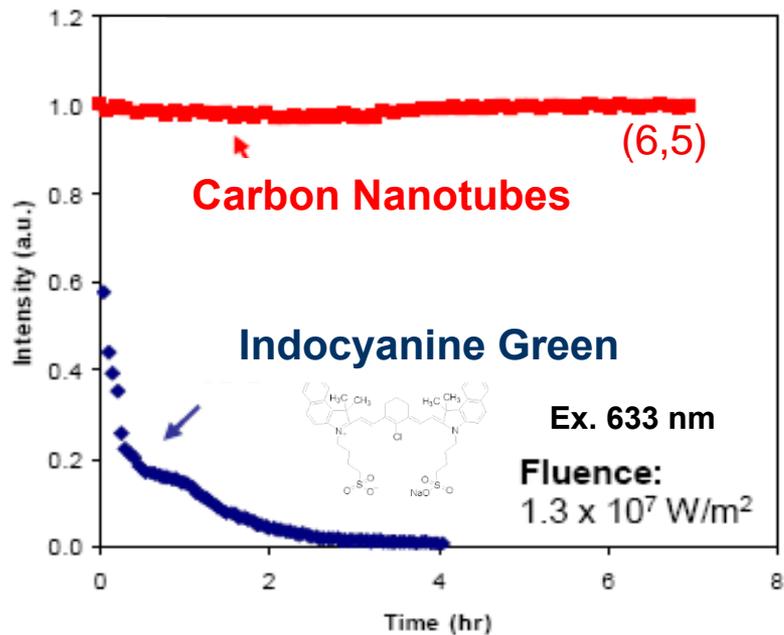
Carbon Nanotube Fluorescence is Structure-Dependent

Semiconducting Nanotubes:
Near-Infrared Emission
900-1600 nm

Narrow Band Emission
Structure/Diameter-
Dependent



Nanotube Emission is Photostable and in the Tissue-Penetrating Near-Infrared Region

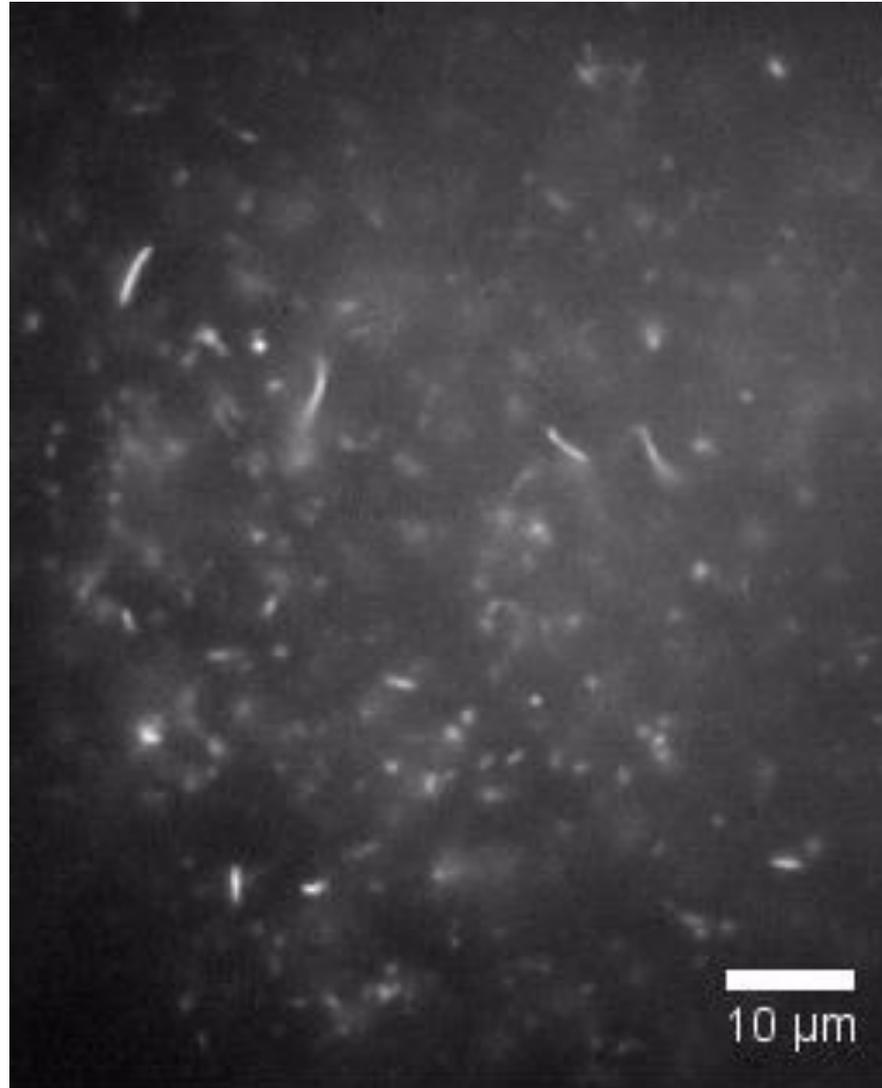


Single-Nanotubes can be Imaged with a Fluorescence Microscope

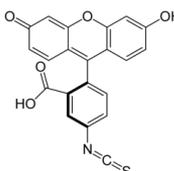
Standard
epifluorescence
microscope

730 nm excitation

InGaAs (SWIR)
array detector



Nanotube Fluorescence Overcomes Certain Constraints of Dyes

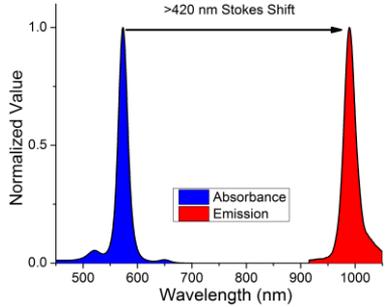
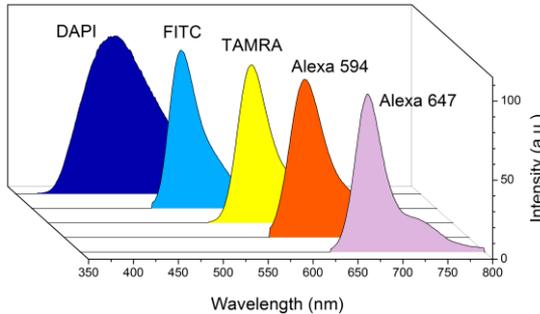


Organic Fluorophores

Semiconducting SWCNTs

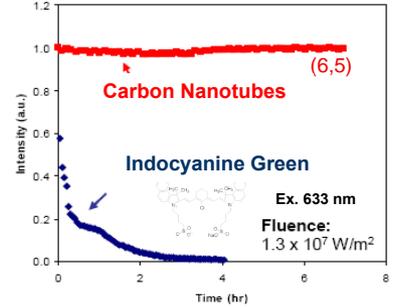
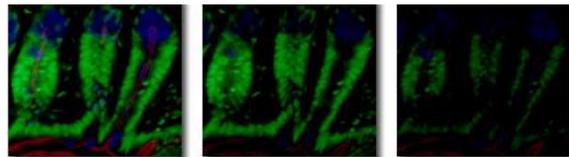


Large Bandwidth + Small Stokes Shift



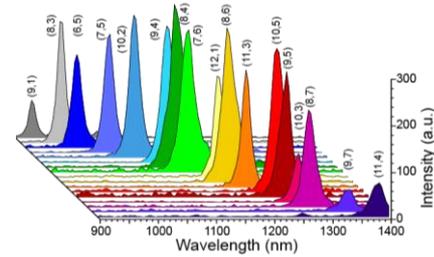
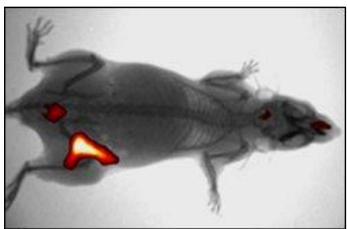
~20 nm FWHM
~400 nm Stokes shift

Low Photostability



Minimal photobleaching

Few stable NIR probes



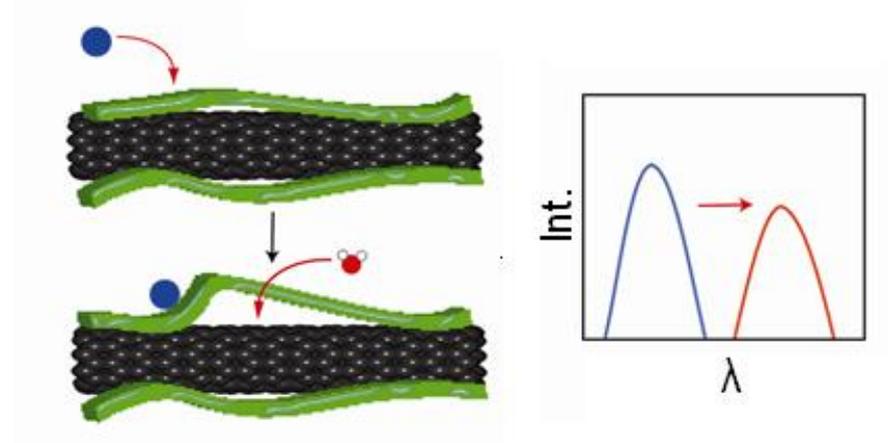
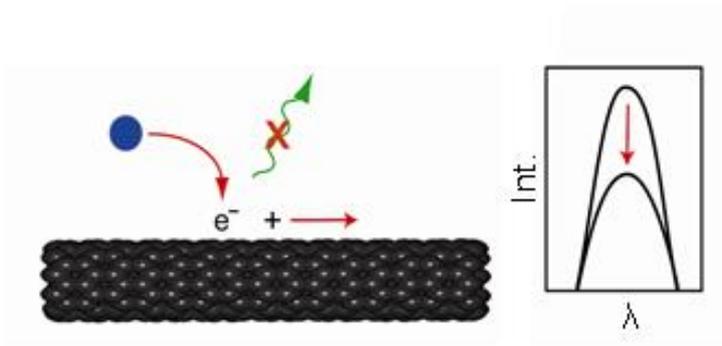
>17 stable NIR probes

Nanotube Emission is Sensitive to the Immediate Environment

1: Intensity



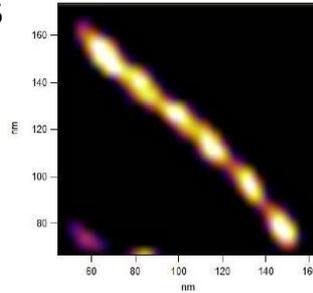
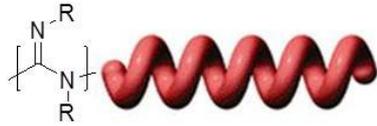
2: Wavelength



Additional Developments Required for Application

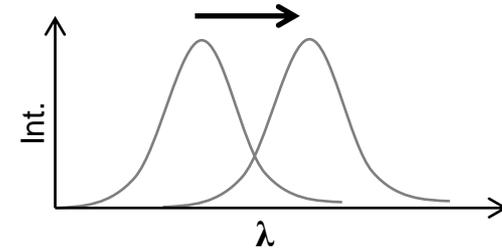
Polymer Functionalization

Helical polycarbodiimides



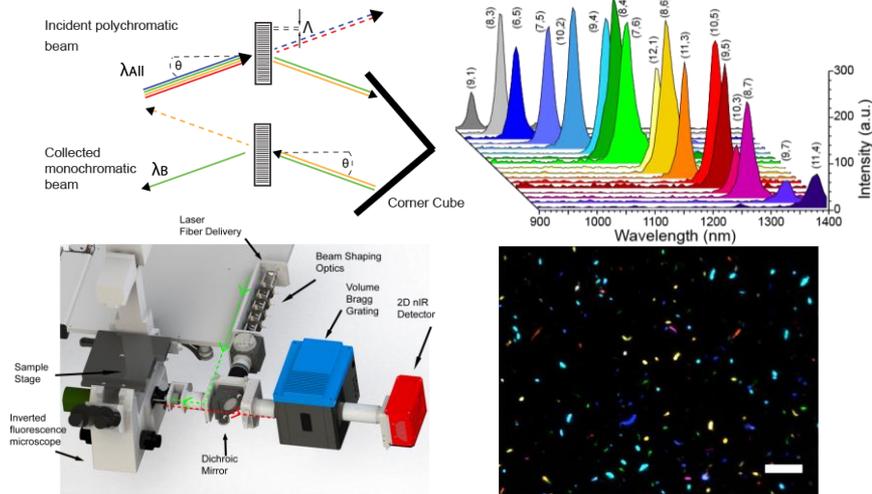
Budhathoki-Uprety, et. al., *JACS* (2014)

Understanding Spectral Response



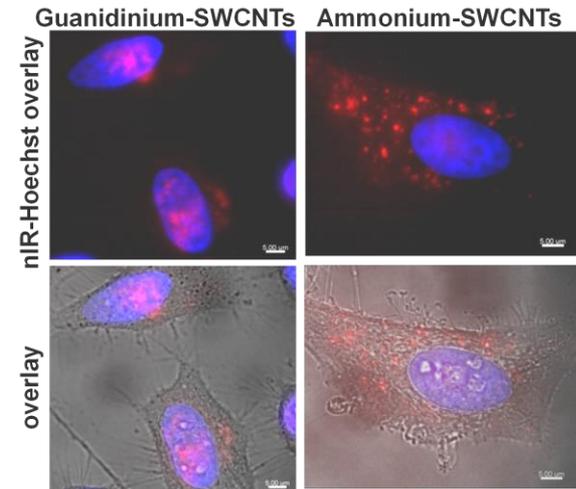
Roxbury, Horoszko, et. al.,
ACS Nano (2016)

Hyperspectral Imaging Platform



Roxbury, et. al., *Sci Rep.* (2015)

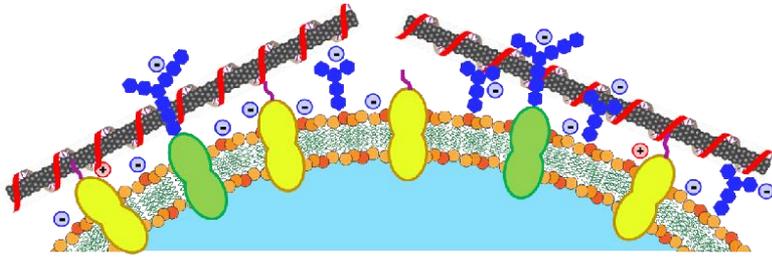
Sub-Cellular Localization



Budhathoki-Uprety, Langenbacher, et. al.,
J Mater Chem B (2017)

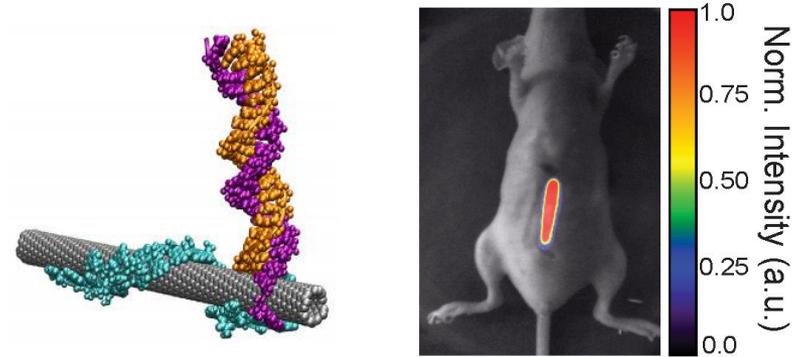
In Situ Measurements

Cell Surface Electrostatic Charge



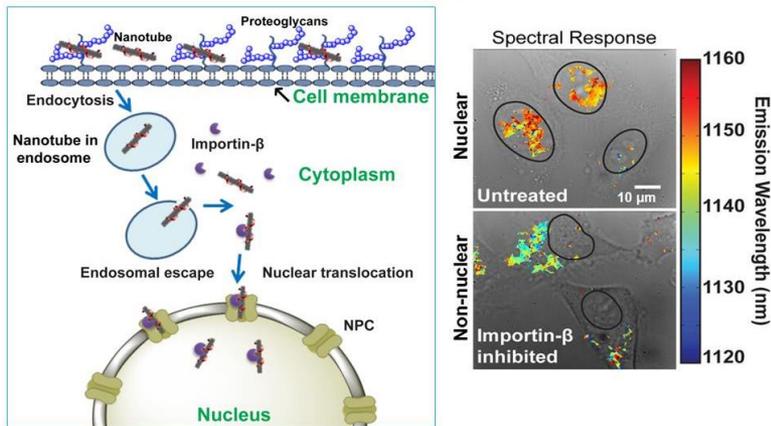
Roxbury, et. al., *ACS Nano* (2016)

microRNA In Vivo



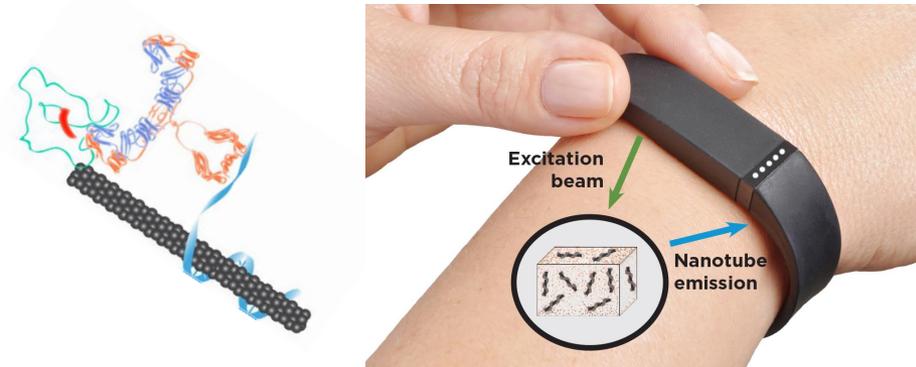
Harvey, et. al., *Nat Biomed Eng* (2017)

Nuclear Pore Transport



Budhathoki-Uprety, et. al. *ACS Nano* (2017)

Ovarian Cancer Biomarker HE4



Williams, et al., *Sci Adv* (2018)

Motivation: Lipid Accumulation in the Lysosomes is Implicated in Diverse Pathologies

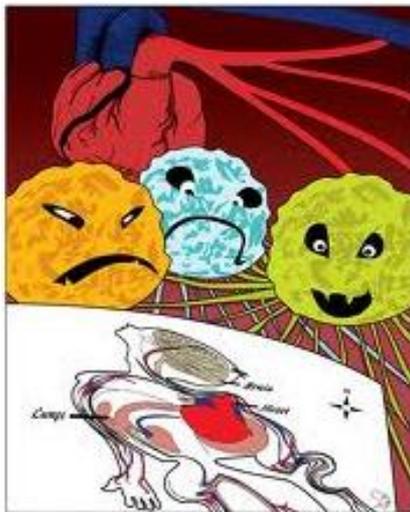
Atherosclerosis



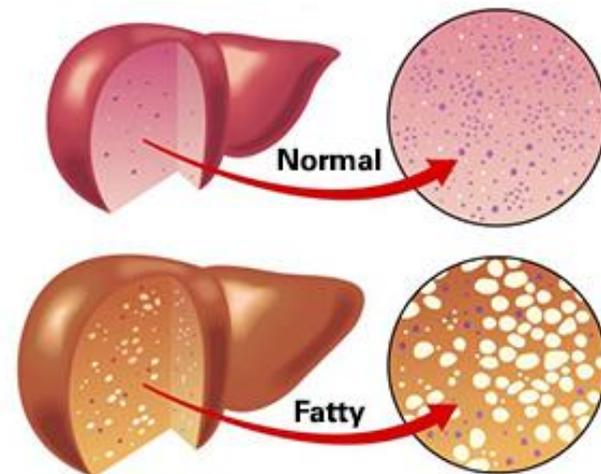
Neurodegenerative Diseases



Cancer

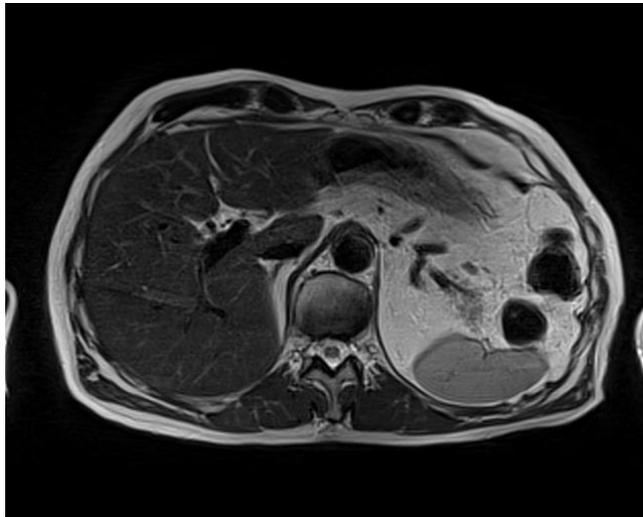


Fatty Liver Disease

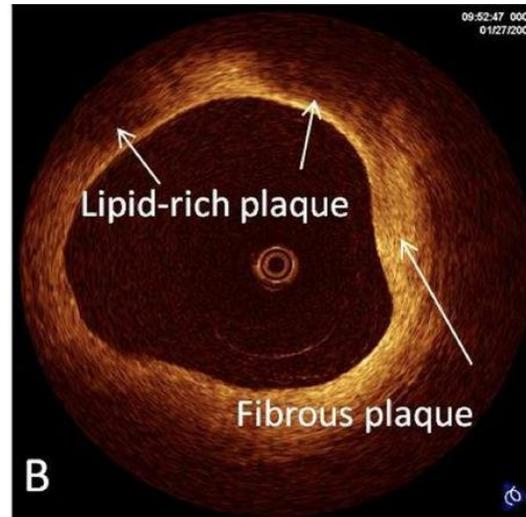


Current Techniques are Unable to Specifically Detect Endolysosomal Lipid Accumulation in Live Cells/In Vivo

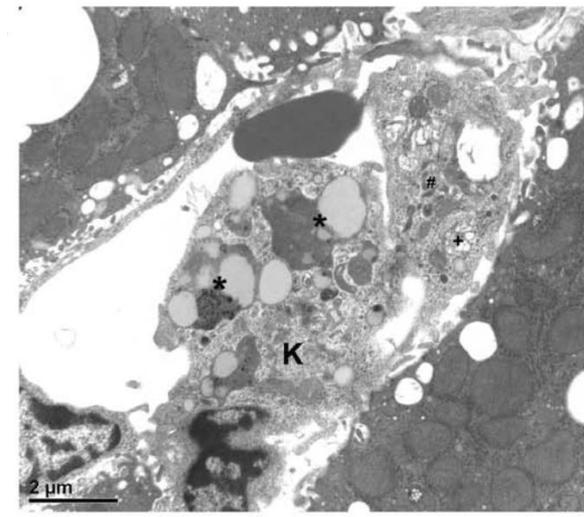
Magnetic Resonance Imaging



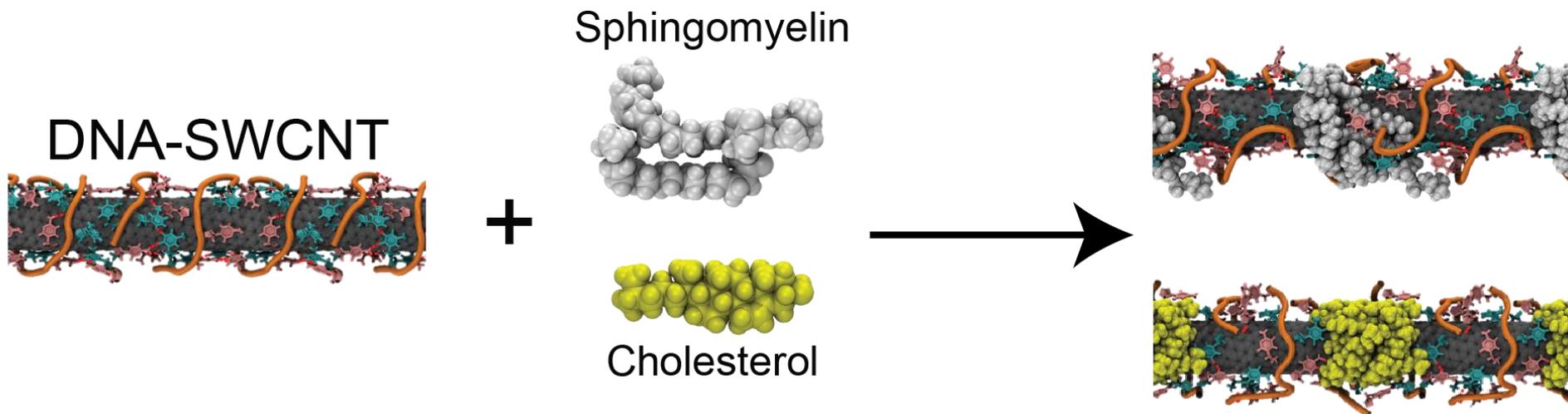
Optical Coherence Tomography



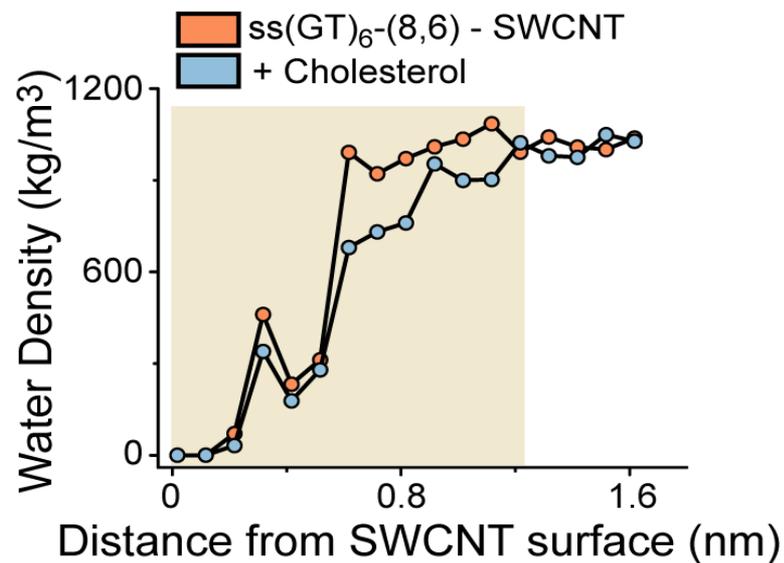
Transmission Electron Microscopy



Molecular Dynamics Suggests Lipids Will Displace Water on SWCNT Surface, Modulate Dielectric

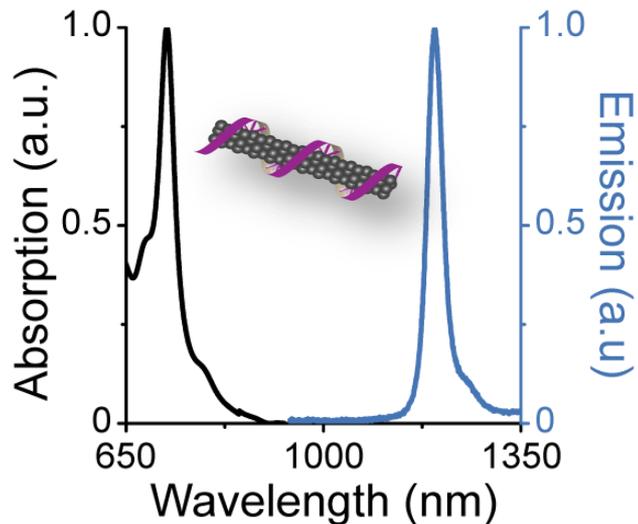


All-atom replica
exchange molecular
dynamics simulations

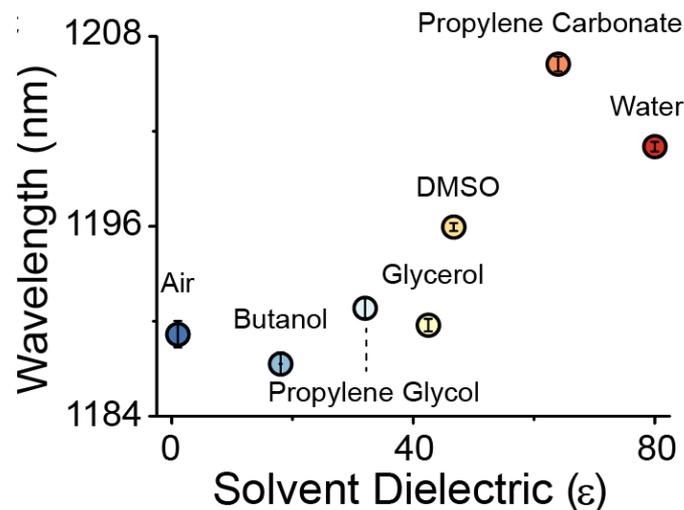


Nanotube Emission Responds to Lipophilicity

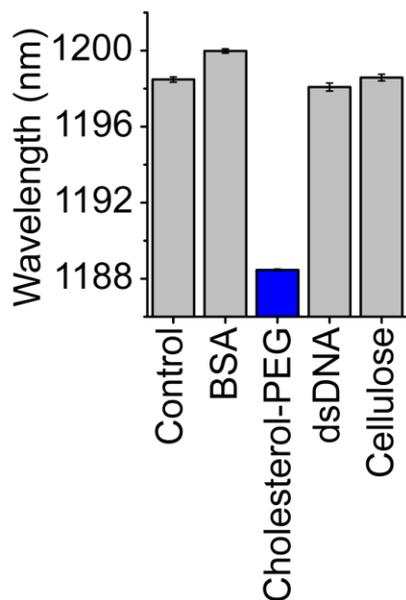
Separated DNA-SWCNT (IEX or ATPE)



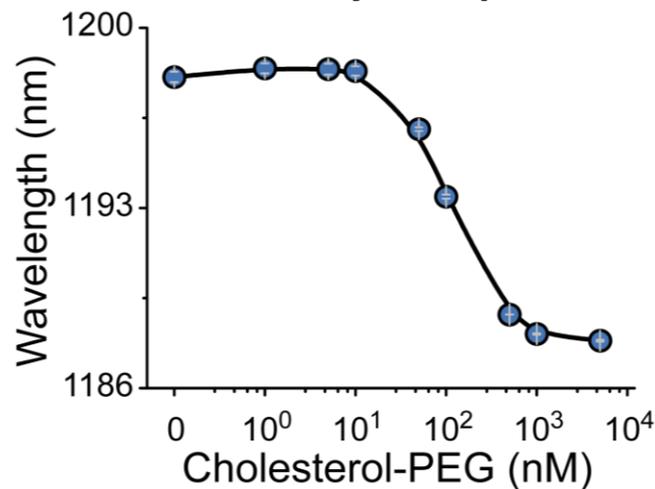
Response to Solvent Dielectric



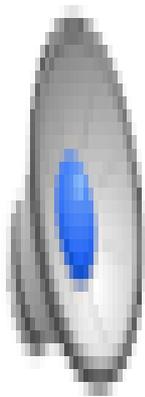
Selectivity to Lipids



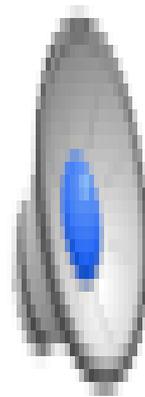
Sensitivity to Lipids



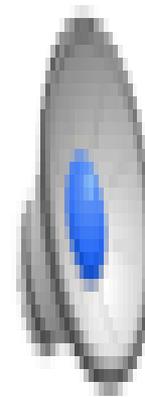
Reporter Localizes in the Lysosomal Lumen



6 hours after uptake

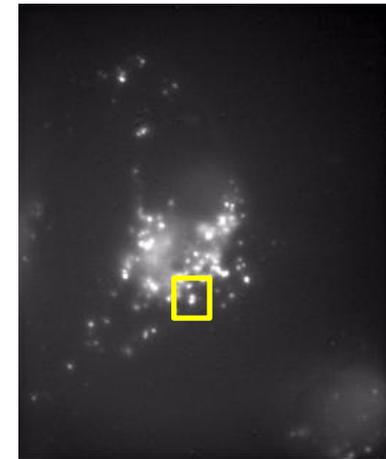
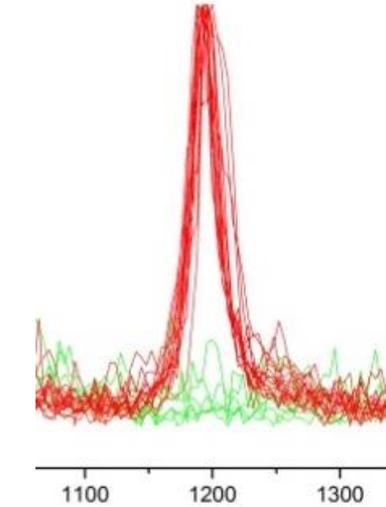
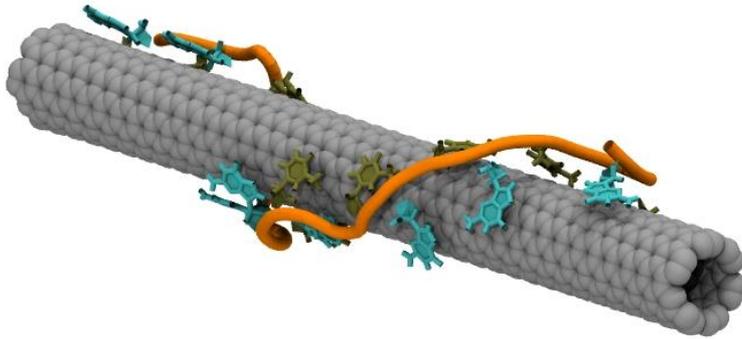


24 hours after uptake



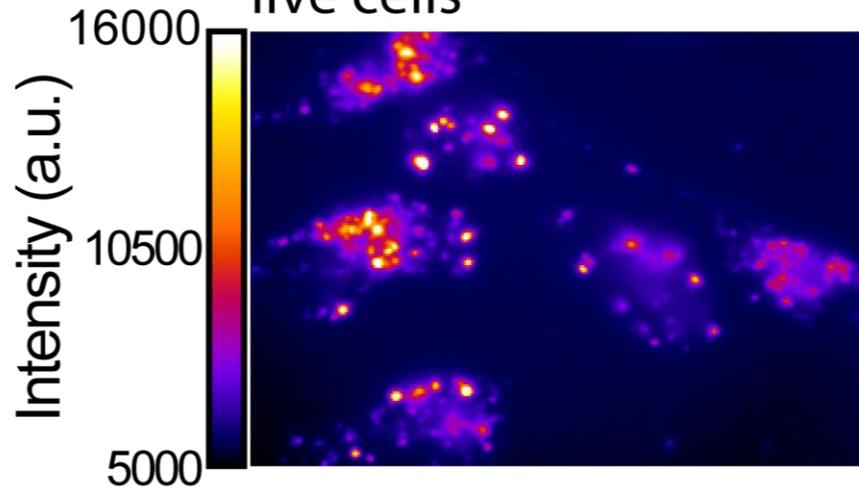
48 hours after uptake

Lipid Reporter Introduced to Cells, Spectral Imaging

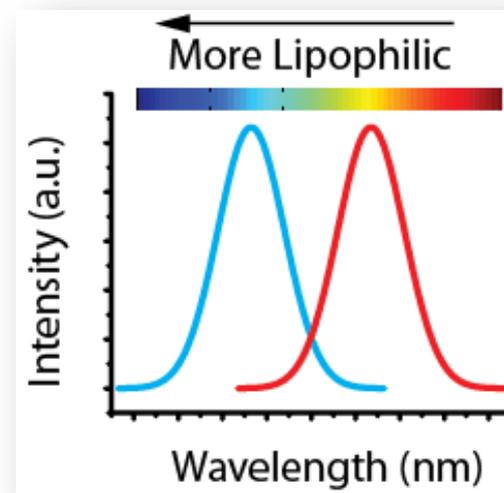
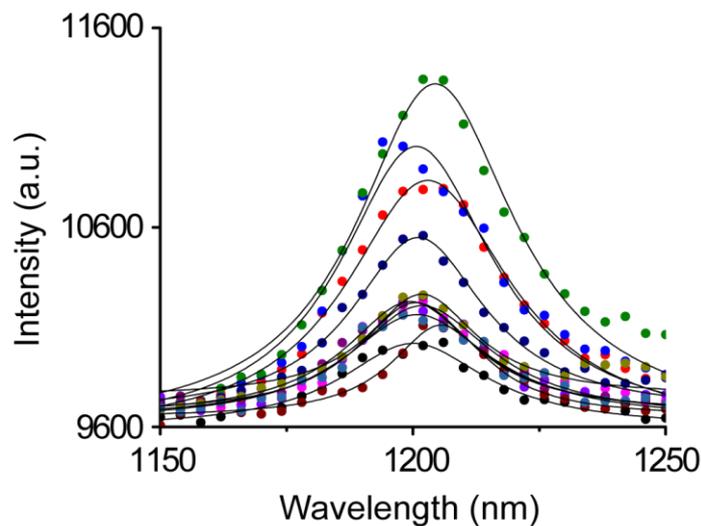
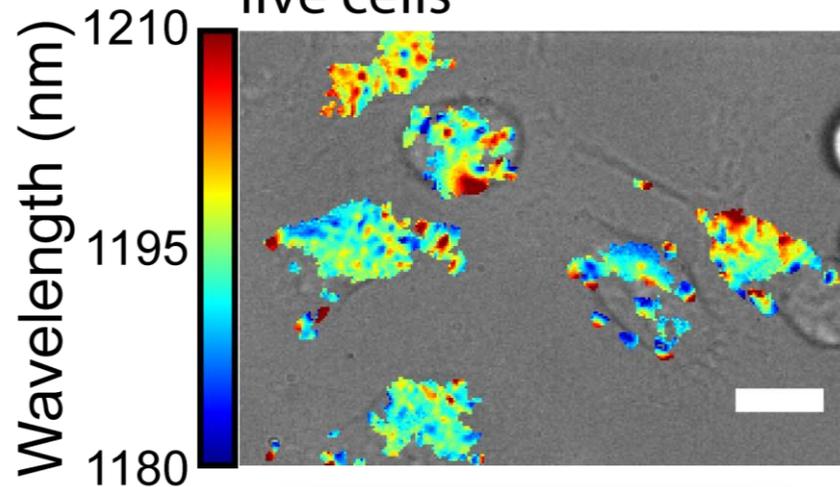


Hyperspectral Images of Nanotubes in the Lysosomes of Live Cells

nIR fluorescence in
live cells

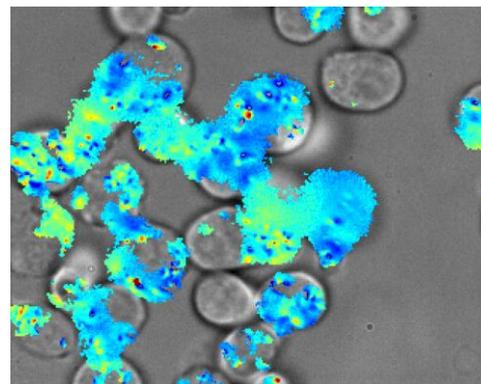
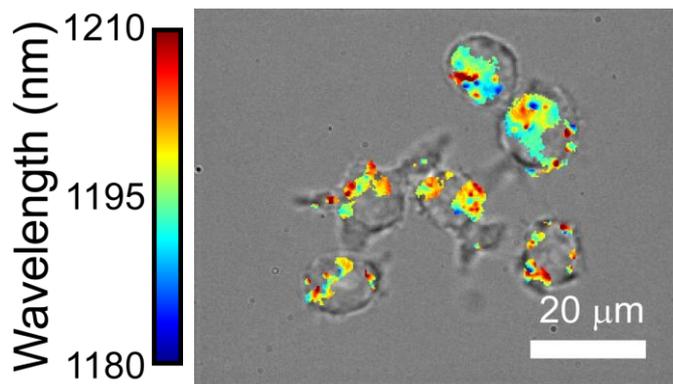
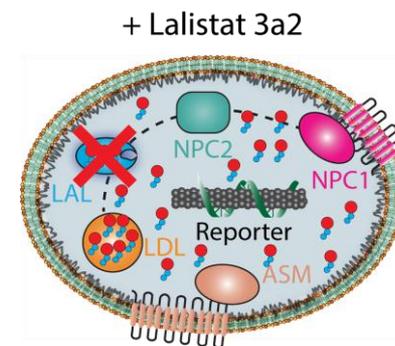
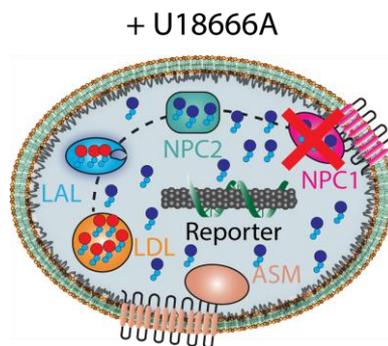
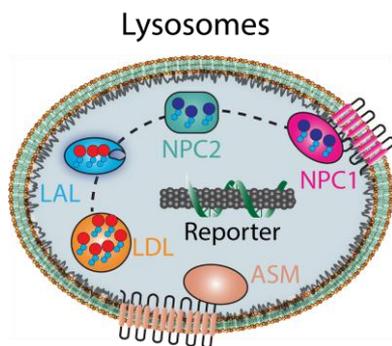


Lysosomal lipid map in
live cells

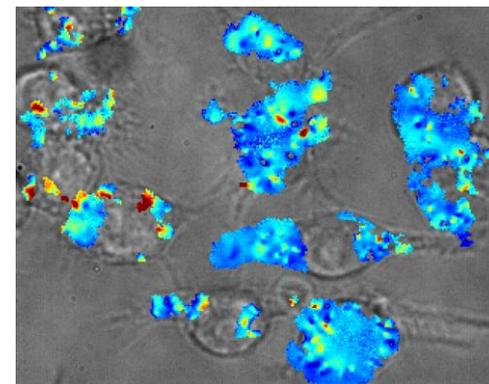


HS Imaging of Reporter Maps Lipid Accumulation

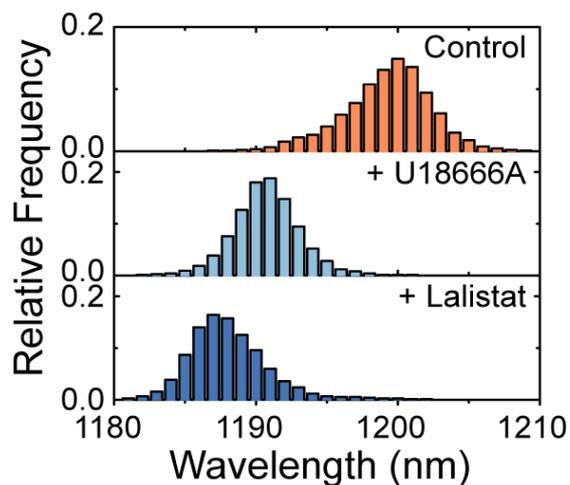
RAW 264.7
macrophages



Free cholesterol



Esterified cholesterol

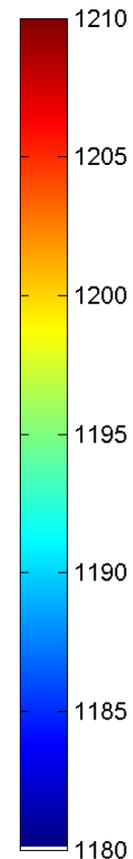
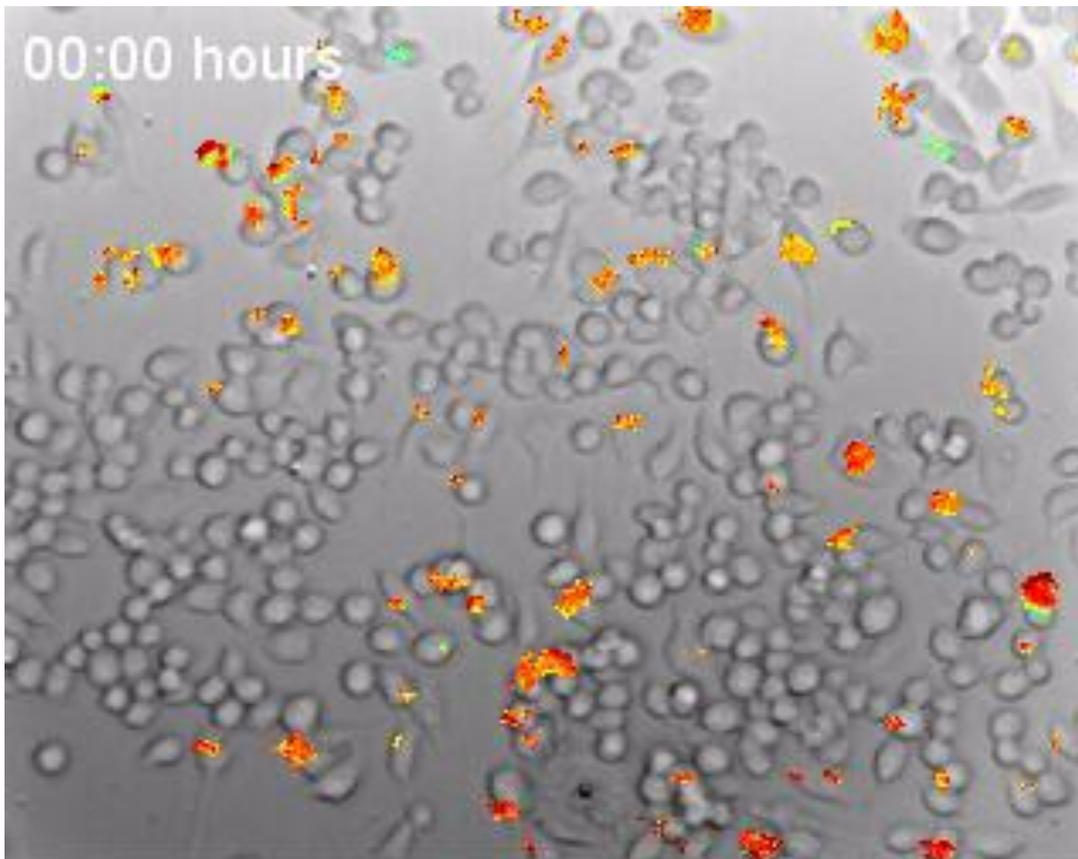


Blue shift due to general
lysosomal lipid accumulation

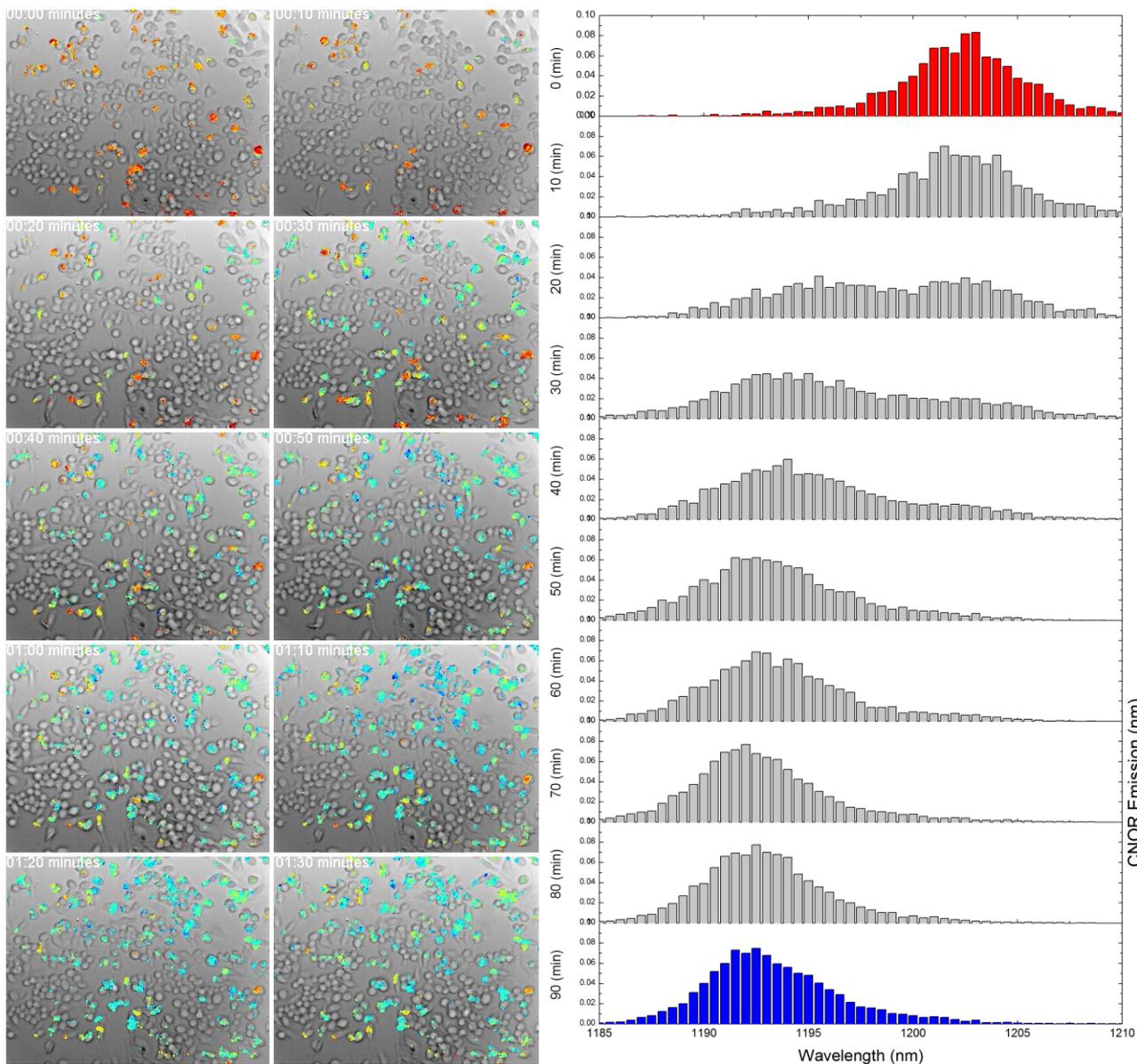
Nanotube Reporter Measures Single-Cell Cholesterol Accumulation

Nanotubes in cells for 120 minutes.

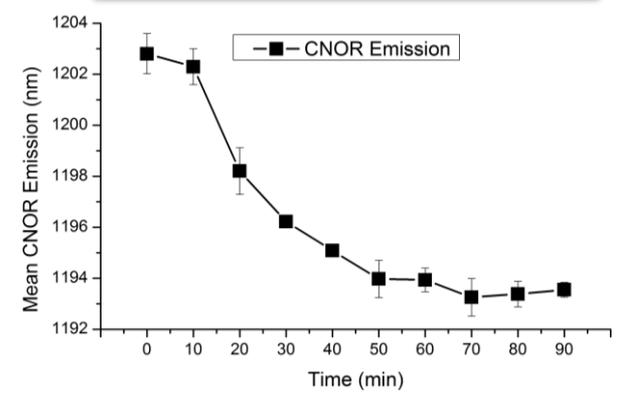
Addition of acLDL + U18666A to induce cholesterol accumulation



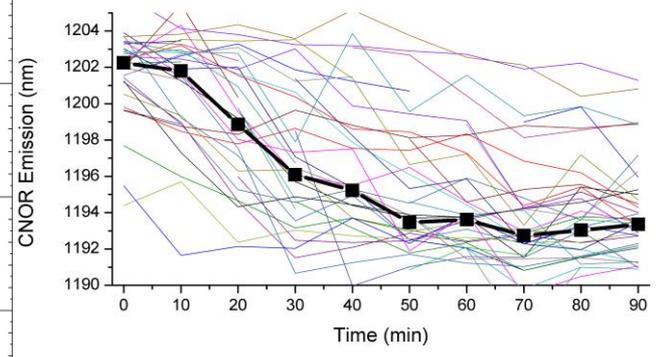
Reporter Measures Single Cell Kinetics



Equilibrium ~ 50 minutes

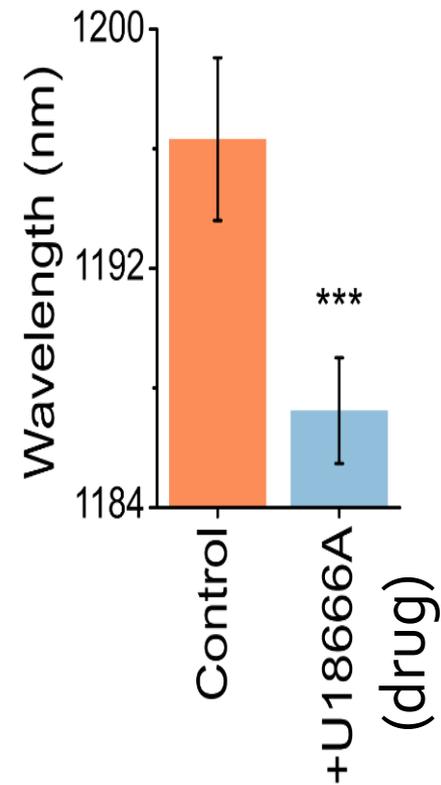
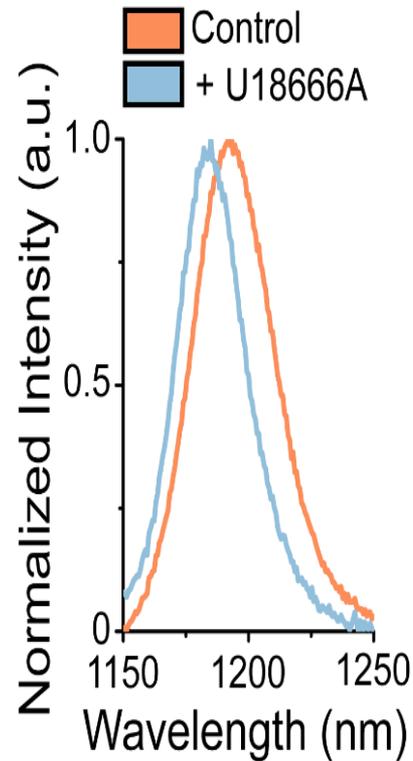
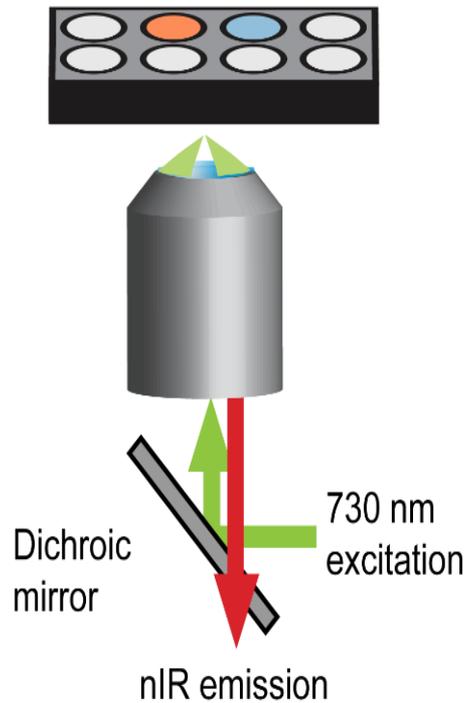


Single Cell Kinetics



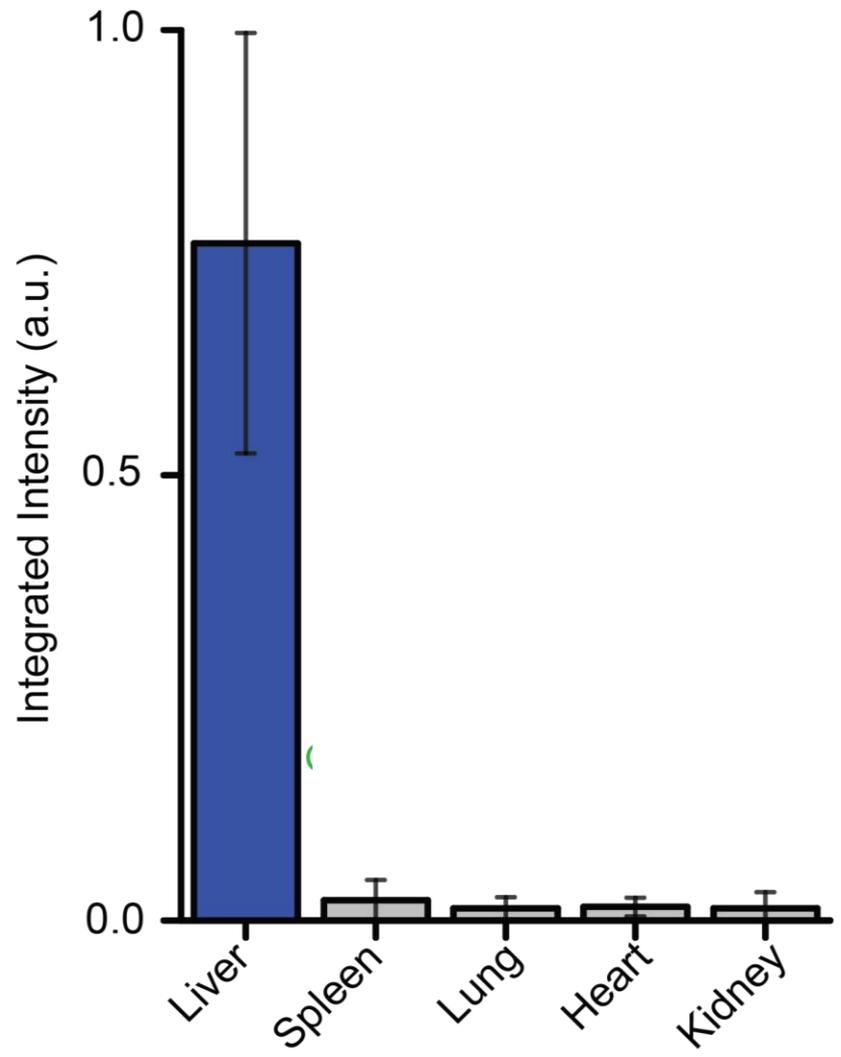
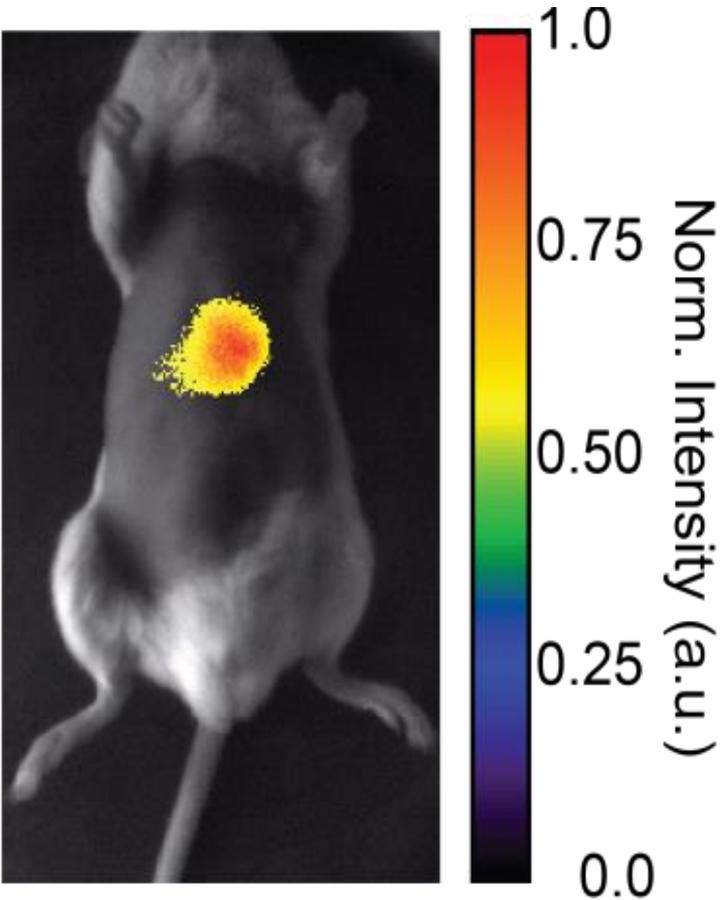
Measurements Can be Done in High-Throughput

Live RAW 264.7 cells
in 96-well plate



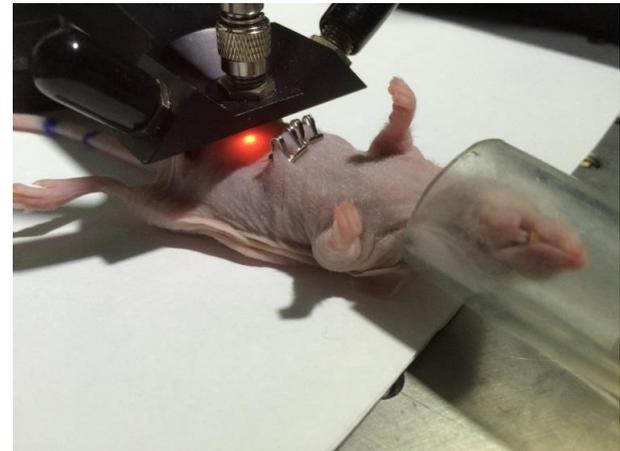
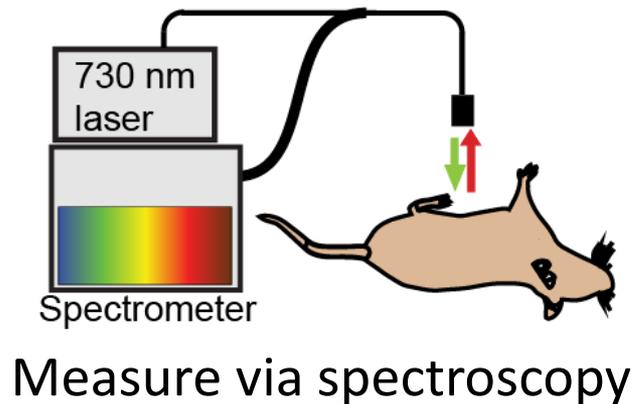
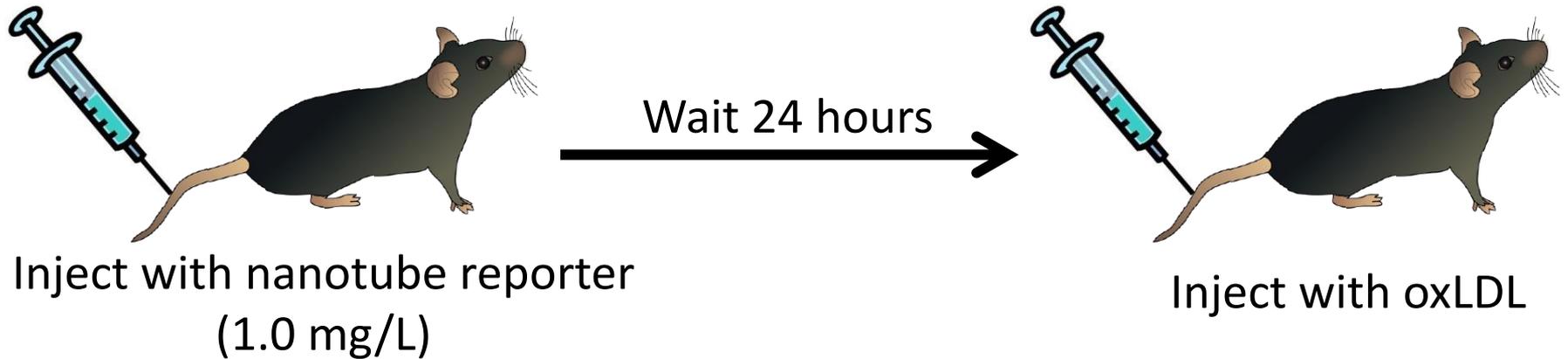
High-throughput drug screening assay

Nanotube Reporter Localizes to Liver Kupffer Cells when Injected Intravenously



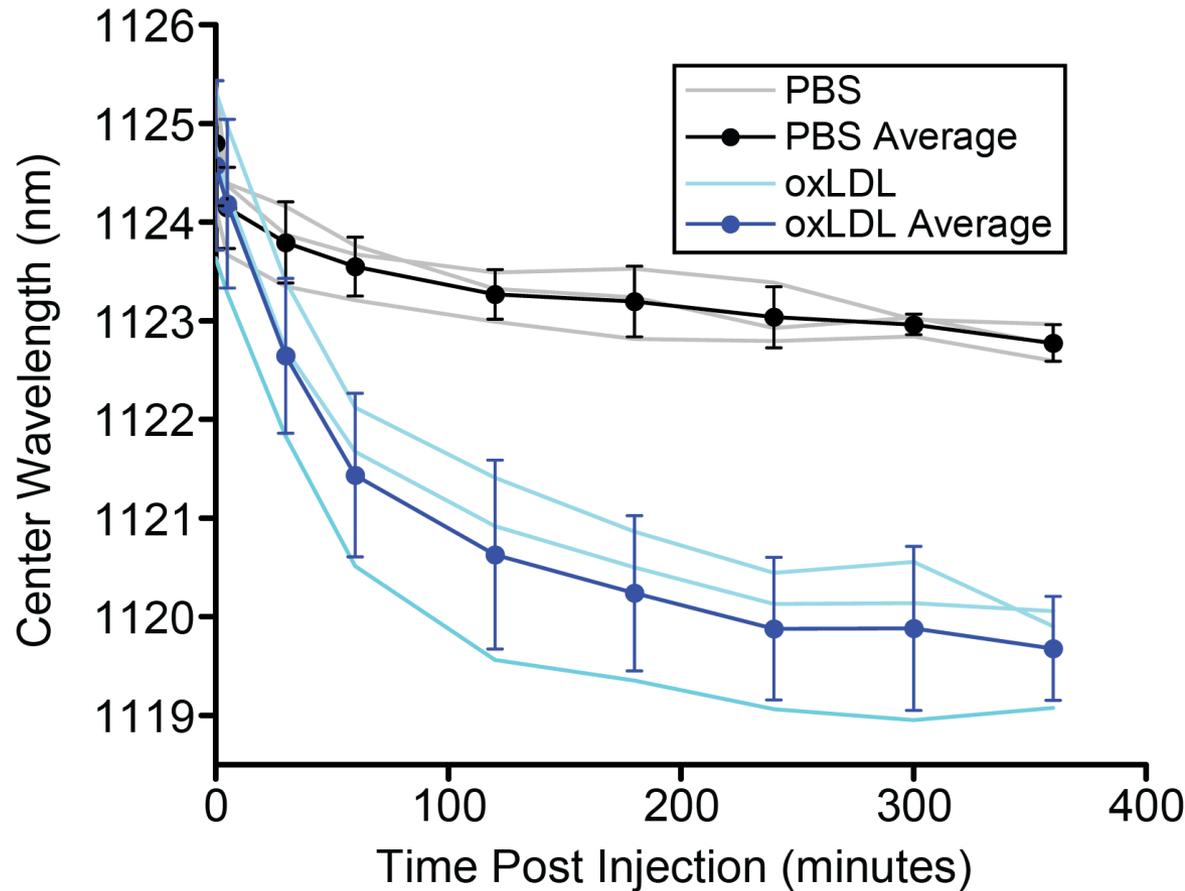
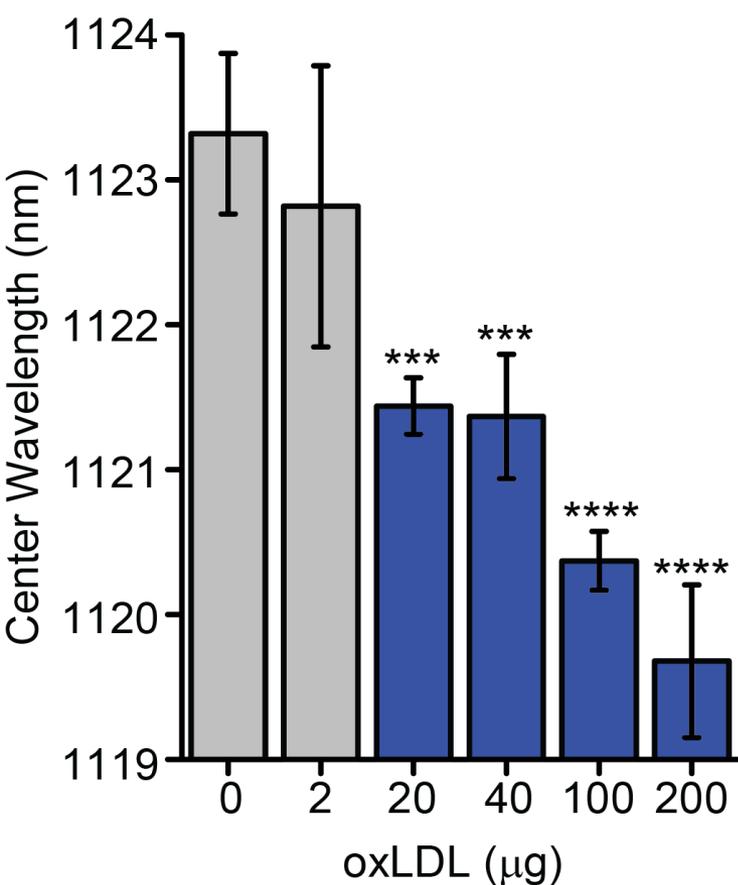
Experiment: Measure Uptake and Accumulation of oxLDL in the Liver

The uptake and accumulation of oxLDL in Kupffer cells has been implicated in atherosclerosis and non-alcoholic fatty liver disease.

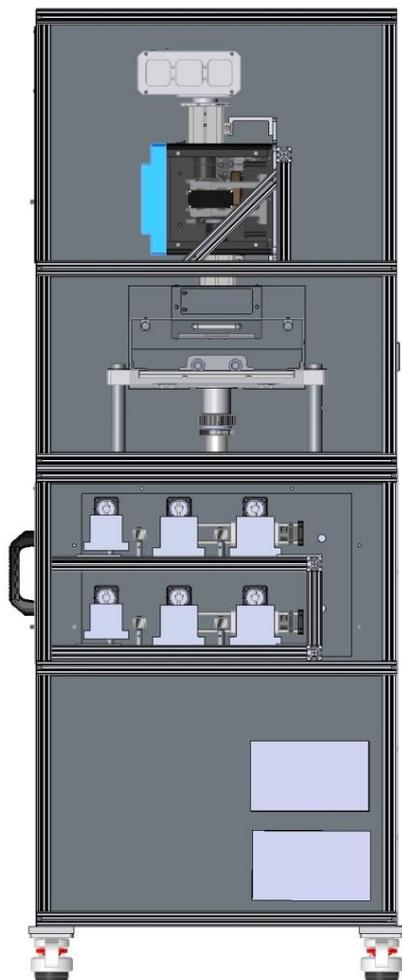


The Reporter Detected the Uptake of oxLDL In Vivo

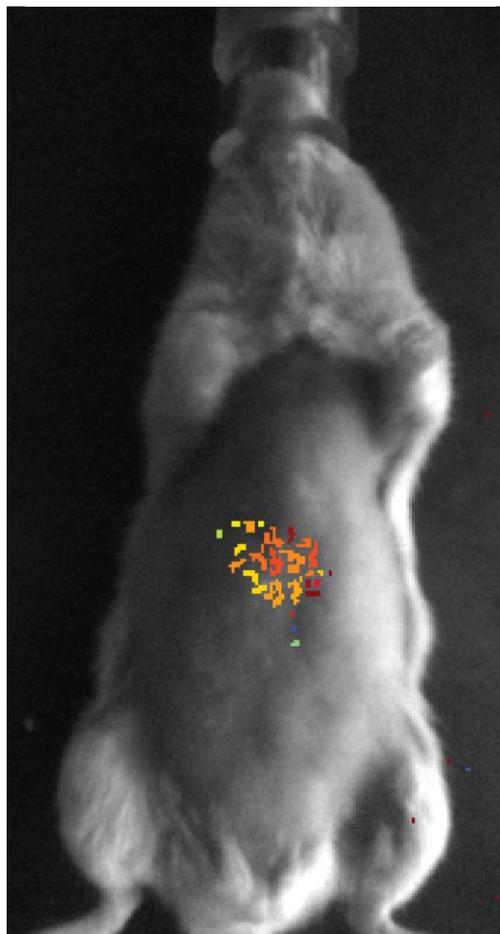
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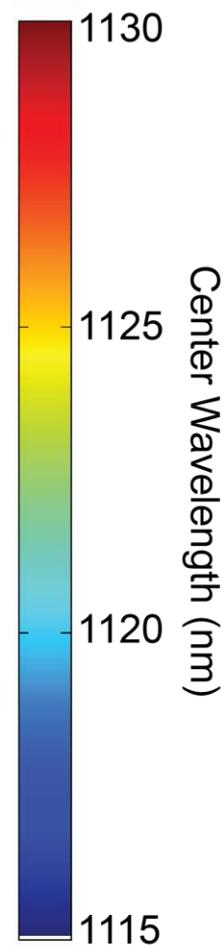
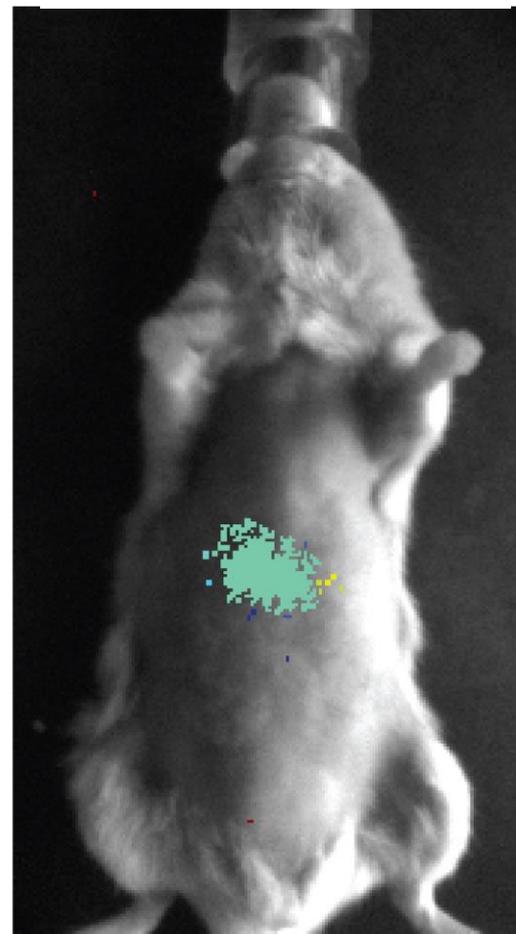
Hyperspectral Imaging Non-Invasively Maps Lipids In Vivo



Lipid Poor

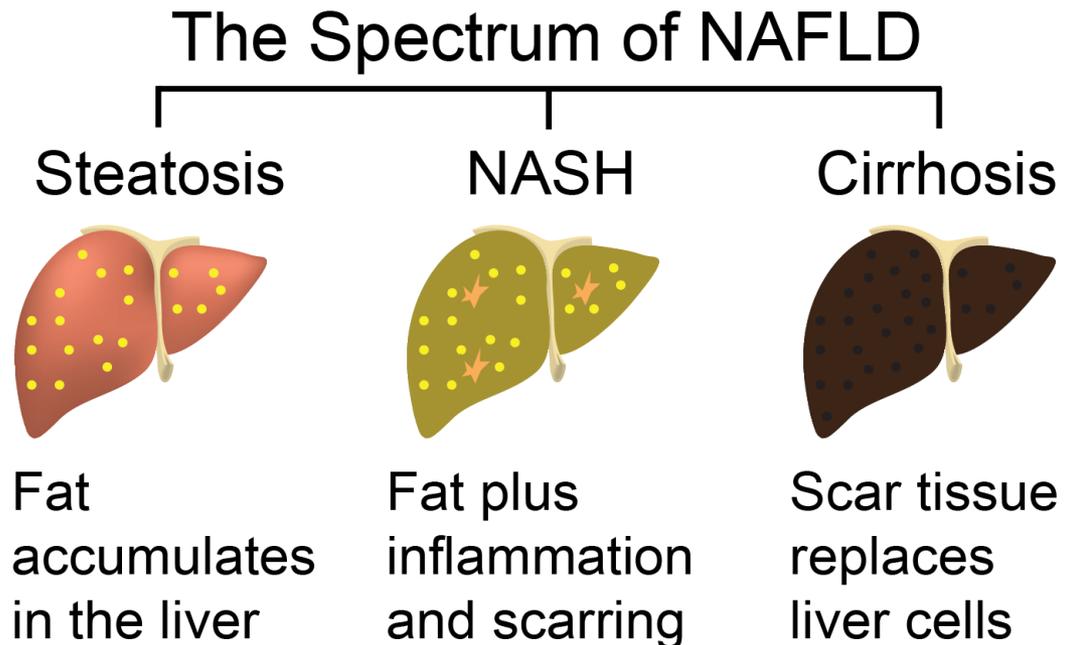


Lipid Rich



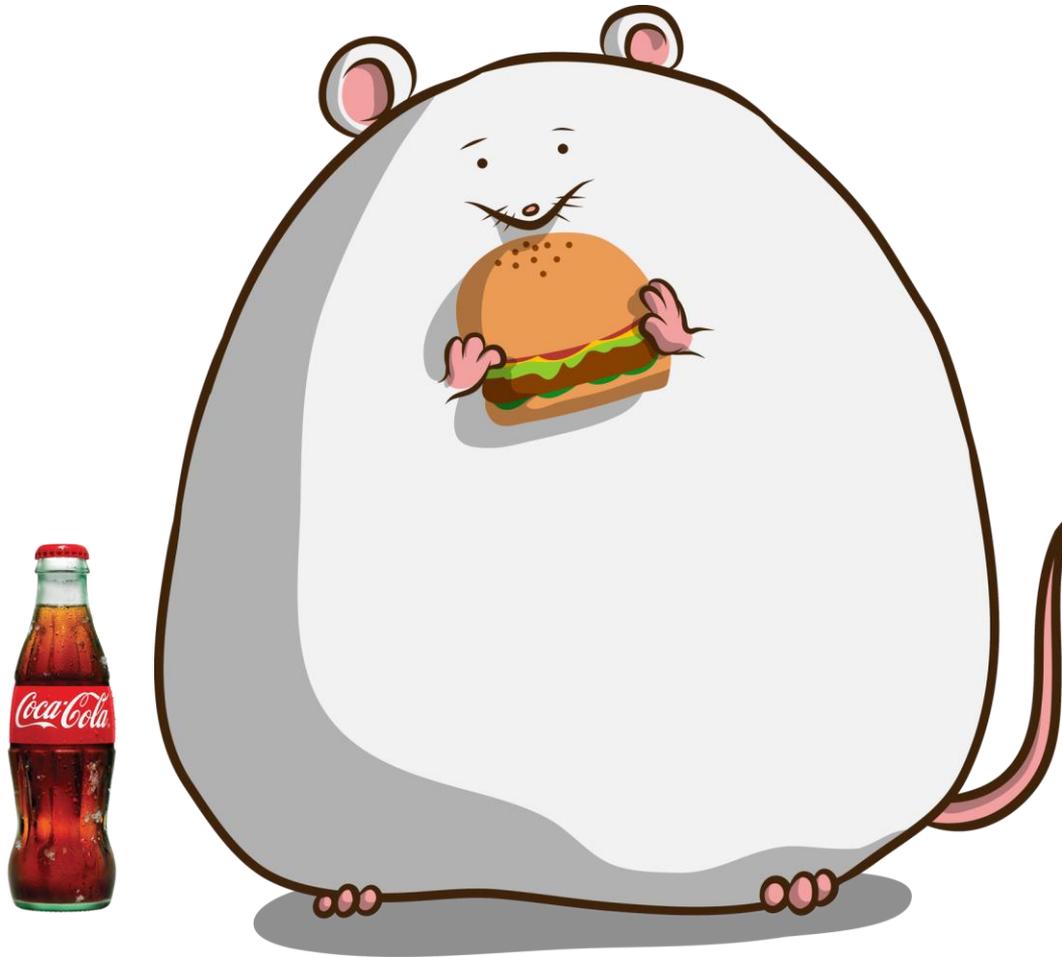
NAFLD is a Spectrum of Disorders

- Non-alcoholic fatty liver disease (NAFLD) affects over 30% of the general population
- It is unknown why NAFLD progresses to NASH
- The only way to diagnose NASH is with a liver biopsy



Experiment: Non-Alcoholic Fatty Liver Disease (NAFLD)

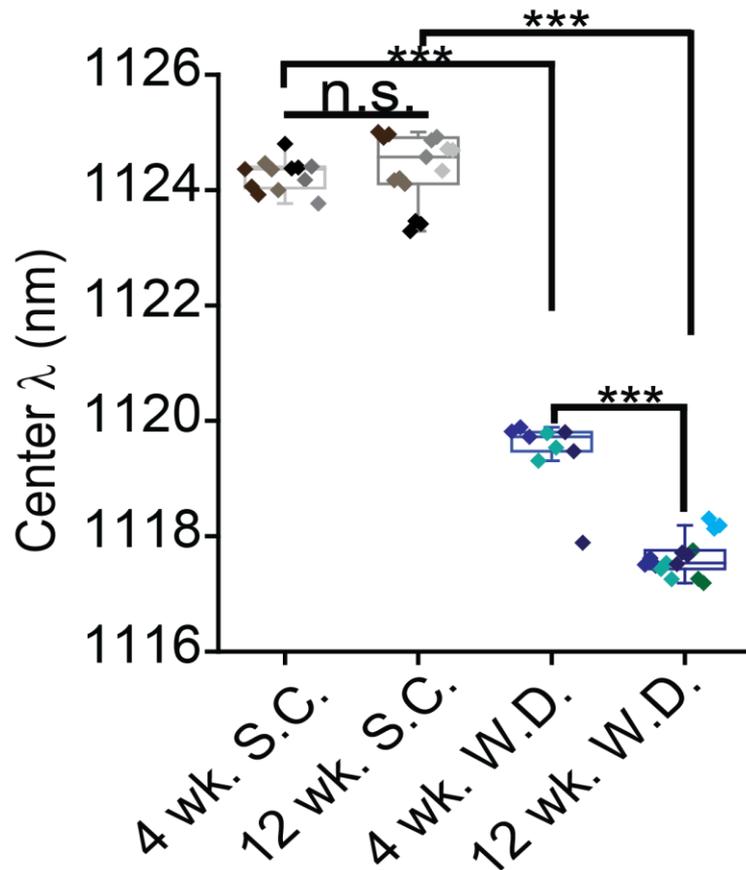
NAFLD was induced in male C57BL/6 mice by feeding with a Western diet with water supplemented with high fructose corn syrup equivalent



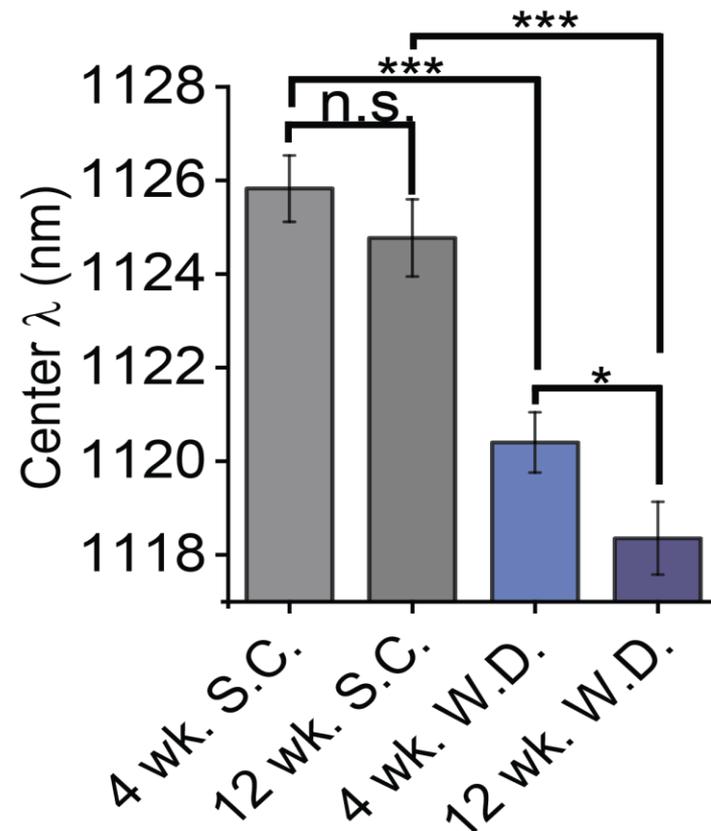
The Reporter Non-Invasively Detects NAFLD In Vivo

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In vivo emission λ



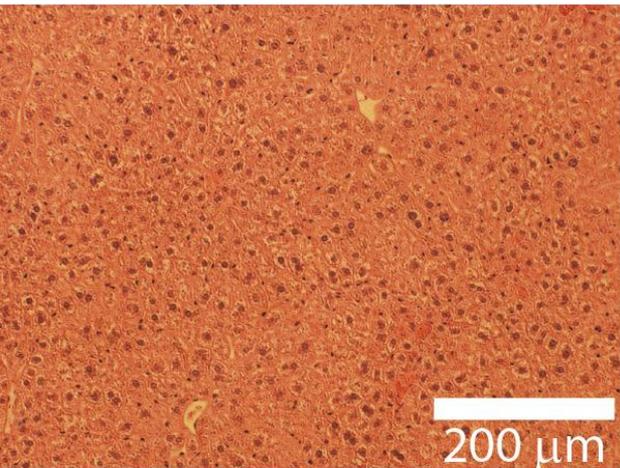
Ex vivo emission λ



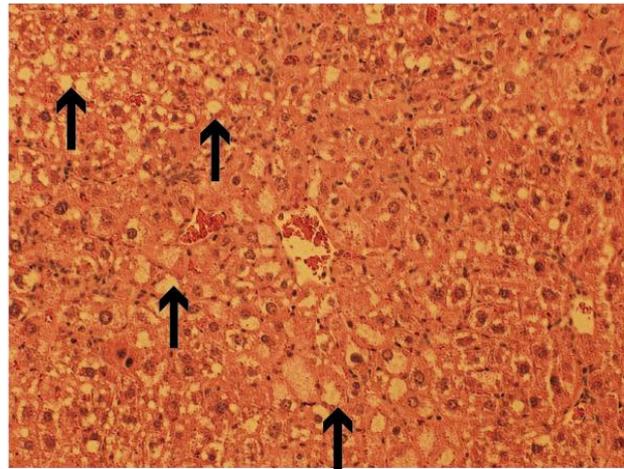
The Reporter Non-Invasively Detects NAFLD In Vivo

The Western Diet caused hepatic steatosis and the presence of inflammatory foci indicating that mice were suffering from a progressive form of NAFLD

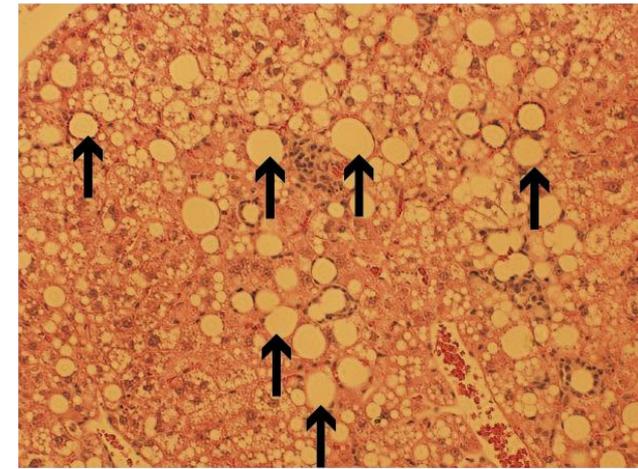
Standard Chow



Western Diet (4 Weeks)



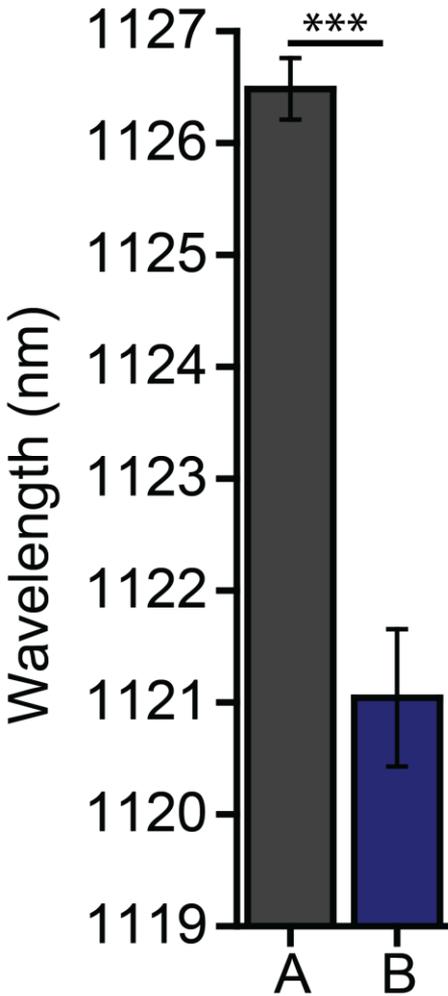
Western Diet (12 Weeks)



↑ = Steatosis

The reporter was able to non-invasively monitor NAFLD progression *in vivo*

Reporter Detects Early-Stage NAFLD In Vivo

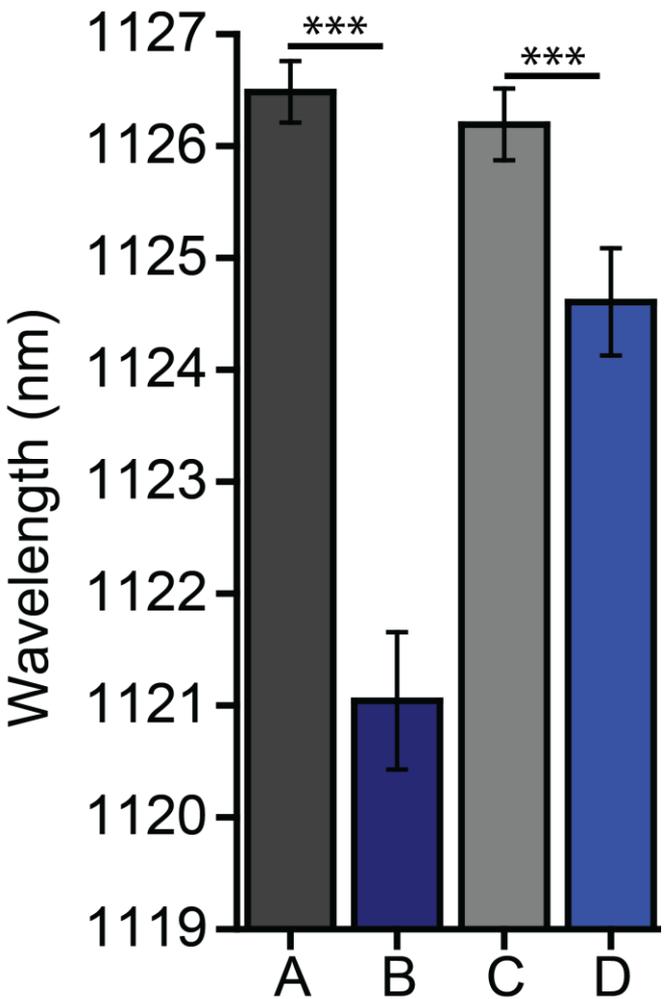


Group Details

A: S.C. for 2 weeks

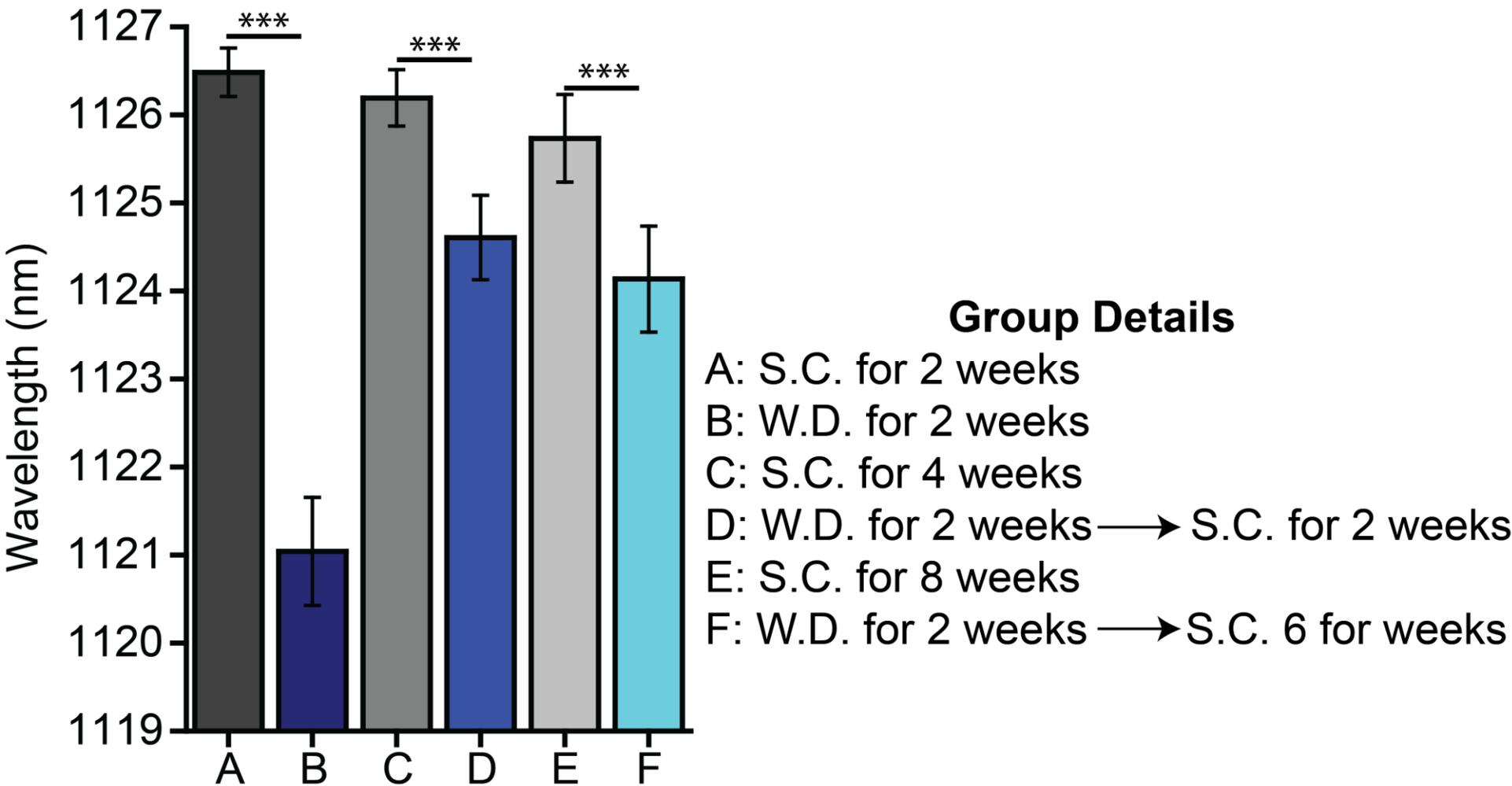
B: W.D. for 2 weeks

A Short-Term Change in Diet Can Have Long-Lasting Effects on Kupffer Cells



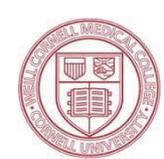
Group Details
A: S.C. for 2 weeks
B: W.D. for 2 weeks
C: S.C. for 4 weeks
D: W.D. for 2 weeks → S.C. for 2 weeks

A Short-Term Change in Diet Can Have Long-Lasting Effects on Kupffer Cells



Summary and Going Forward

- Carbon nanotubes can be applied for basic biology investigations, drug screening/development, and diagnosis.
- A carbon nanotube optical reporter measures endolysosomal lipid accumulation.
- Reporter also measures lipids in Kupffer cells non-invasively in vivo, and in a progressive NAFLD/NASH model (Western Diet).
- Short-term changes in diet have long-term consequences on Kupffer cells.
- Toxicity of properly functionalized carbon nanotubes is minimal and is most relevant in this application for determining how the material may modulate the measurement.
- Bad dietary choices are more toxic than carbon nanotubes.



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Cancer Center™



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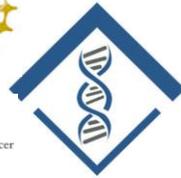
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PERSHING SQUARE SOHN
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Imaging and Radiation Sciences
Metastasis and Tumor Ecosystems Center
Center for Mol. Imaging and Nanotechnology

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Daniel A. Heller, PhD

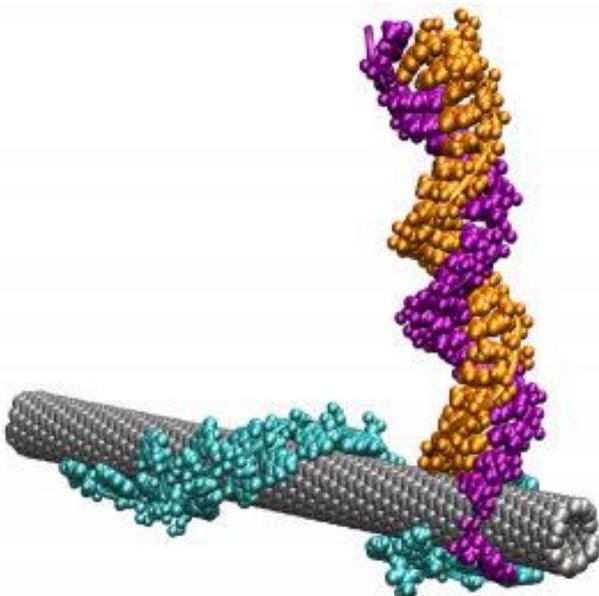
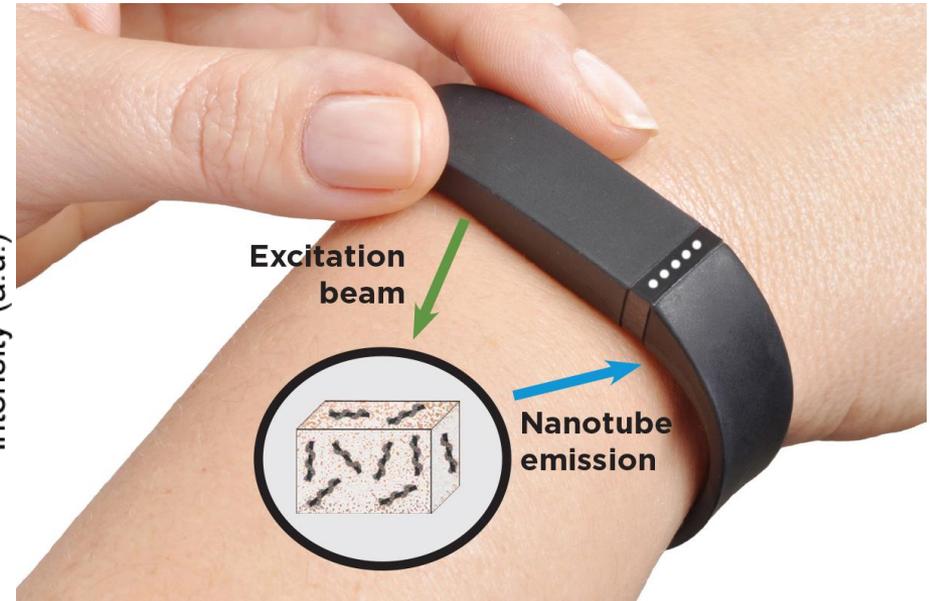
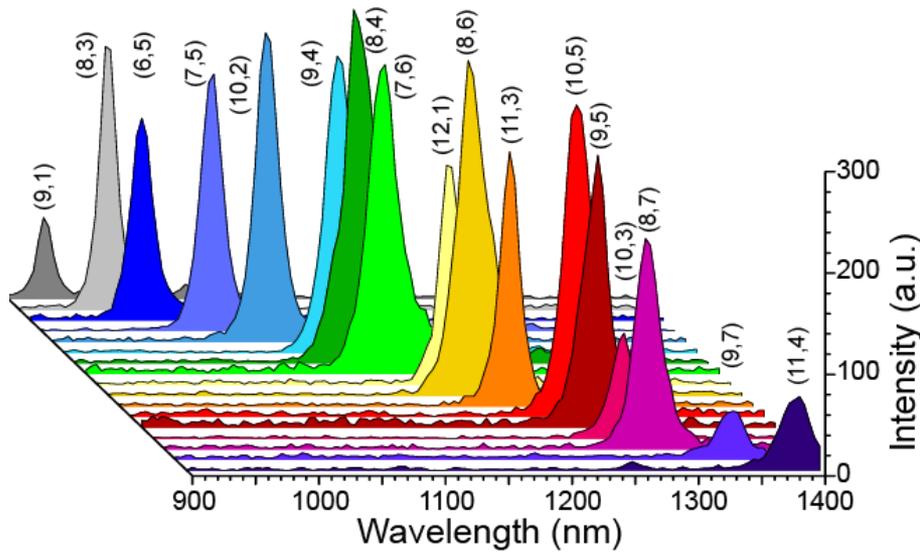
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