

# Investigating biological processes at the nanoscale using CathodoLuminescence-Activated Imaging by Resonant Energy transfer (CLAIRE)

Clarice Aiello, Connor Bischak, Rebecca Wai  
& Naomi Ginsberg

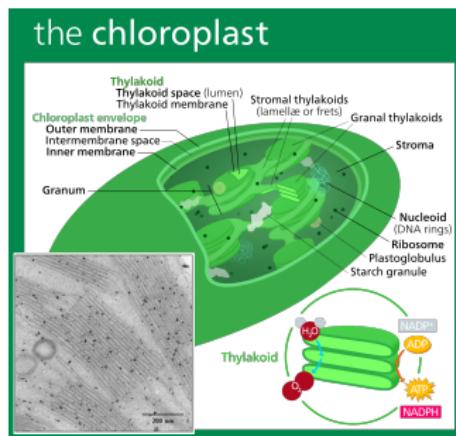
Molecular Biophysics & Integrated Bioimaging Retreat, 11/03/16

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is very relevant...

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Ex: investigating photosynthesis at the nanoscale, as it occurs



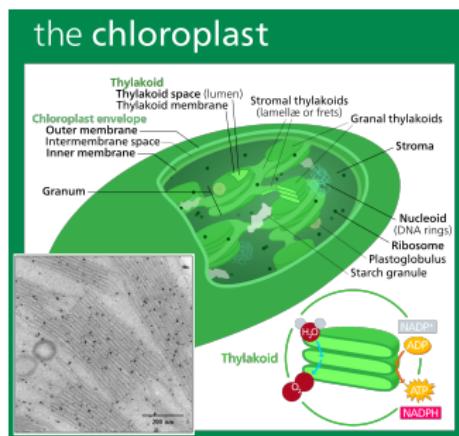
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TEM image of grana Yamamoto et al., Front. Plant Sci. 4, 433 (2013)

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- ▶ delicate samples



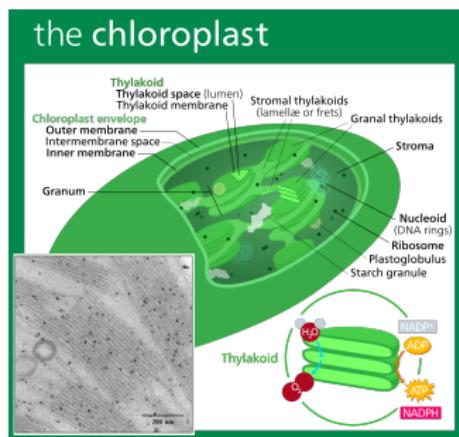
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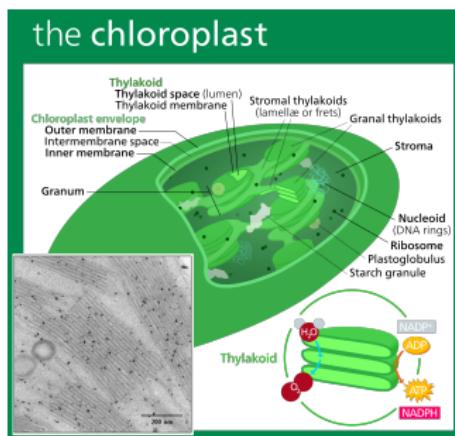
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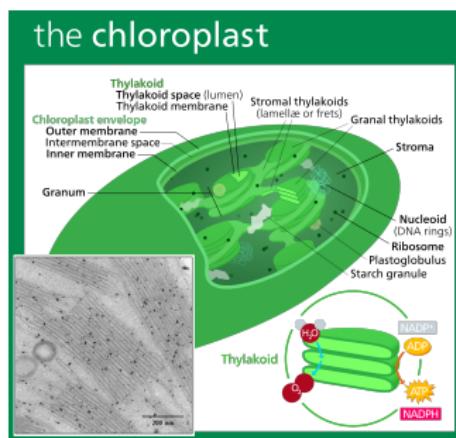
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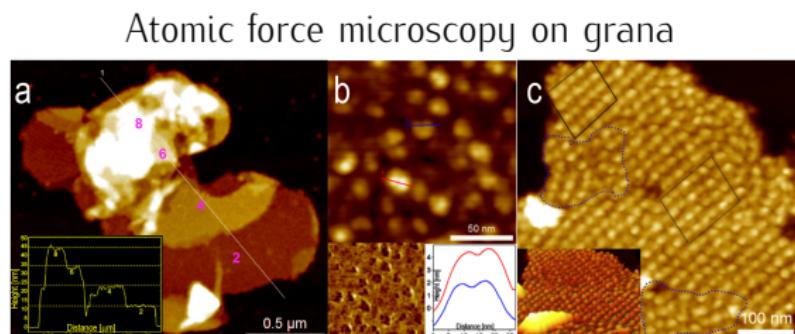
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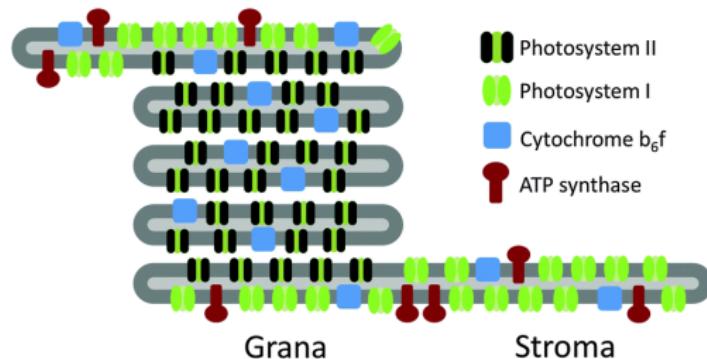
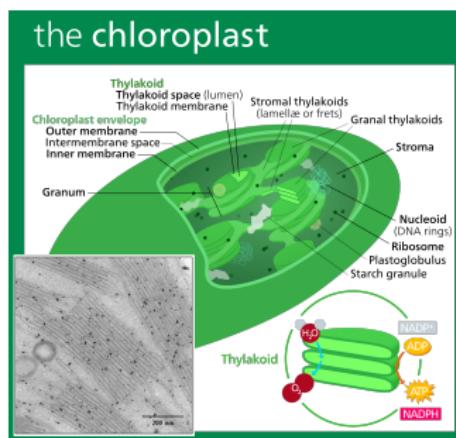
Ono et al., PLoS ONE 9, e101470 (2014)

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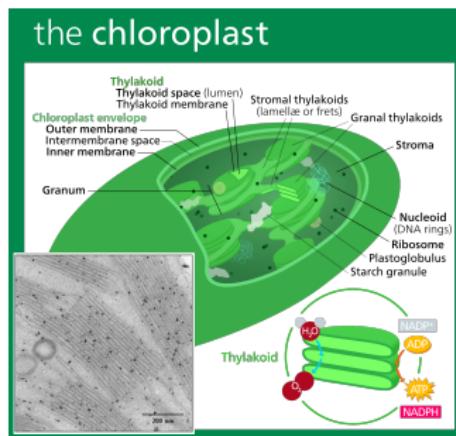
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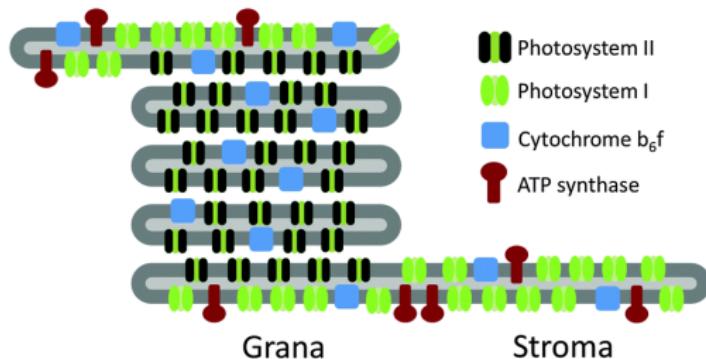
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- ▶ delicate samples → control damage
- ▶ tiny, with heterogeneities → super-resolution
- ▶ fixed samples may preclude study of full *in vivo* dynamics  
→ imaging under physiological conditions



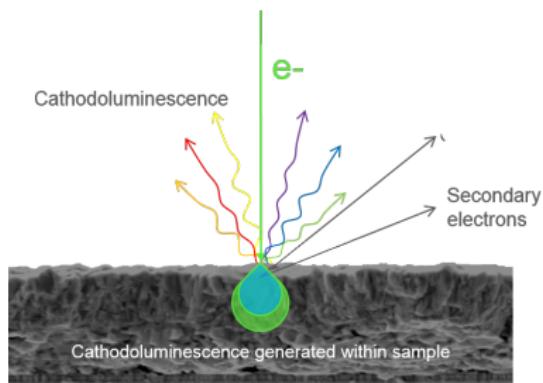
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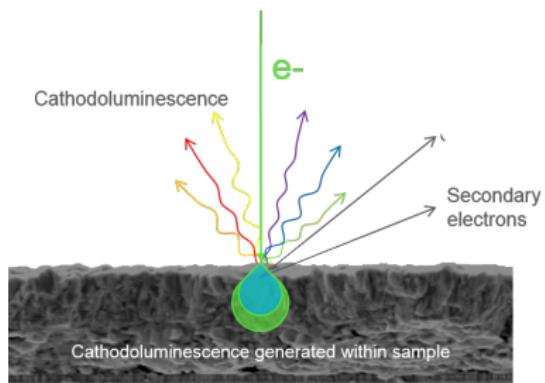
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We're electron microscopists with benefits:  
we also study cathodoluminescence



Adapted from Gatan Inc.

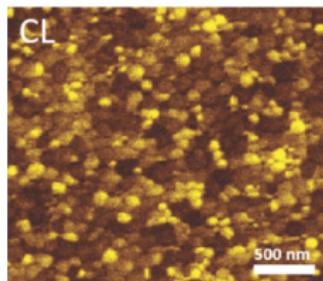
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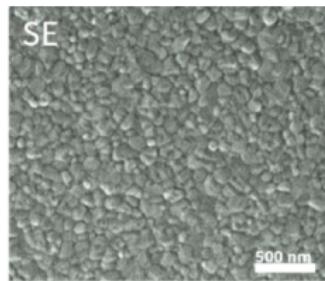
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# Learning more about the sample by adding contrast mechanisms: cathodoluminescence

Cathodoluminescence signal:  
contrast  $\leftrightarrow$  wavelength, intensity, lifetime



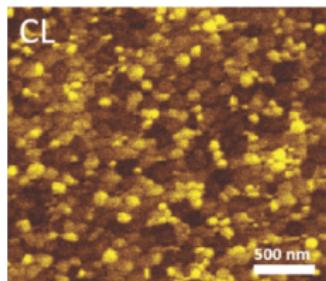
Electron signal:  
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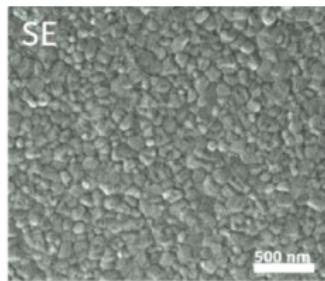
Bischak et al., Nano Letters 15, 4799 (2015)

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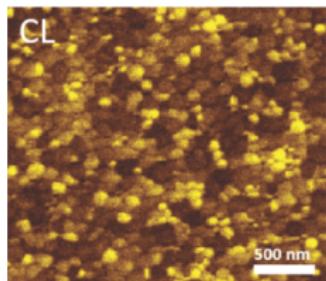


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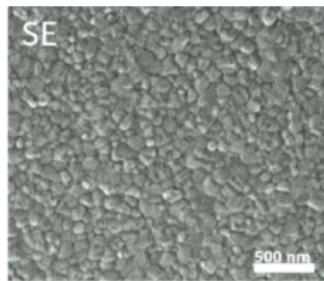
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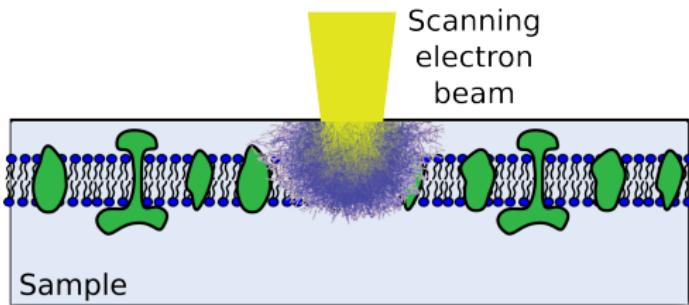
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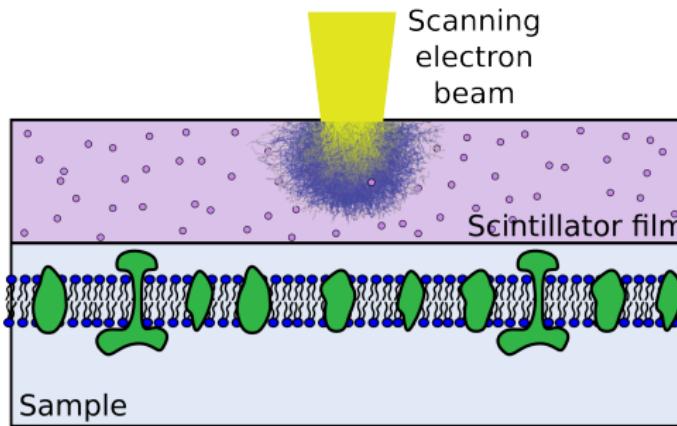
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(If sample damage is still bearable)  
(Most times it's not)

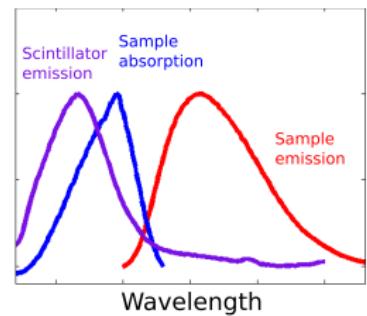
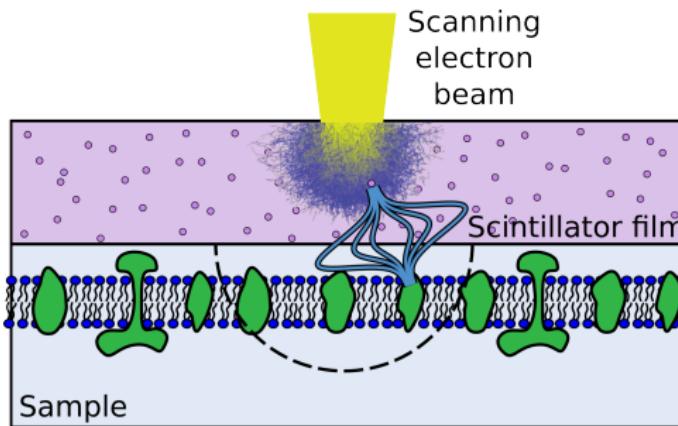
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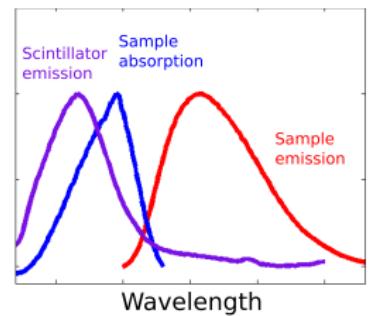
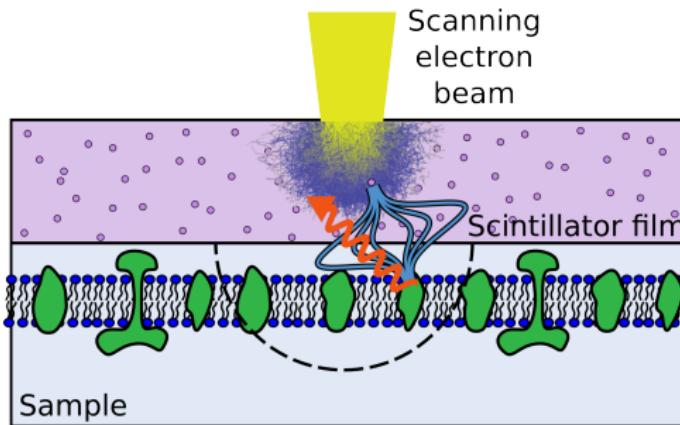


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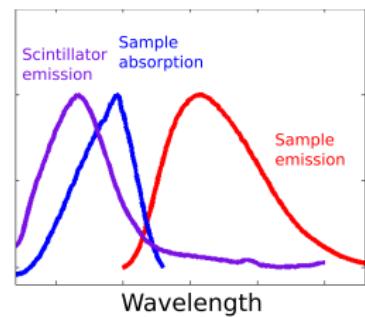
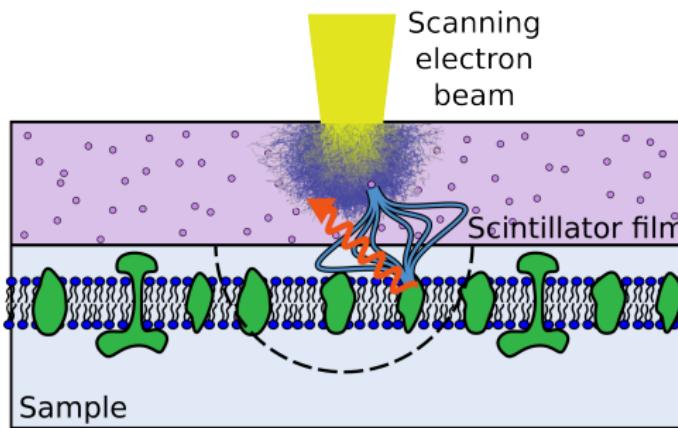
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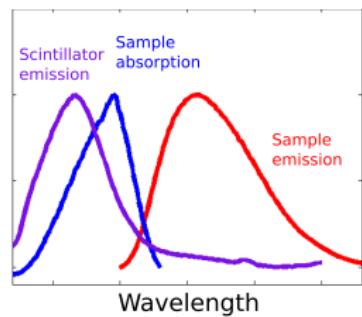
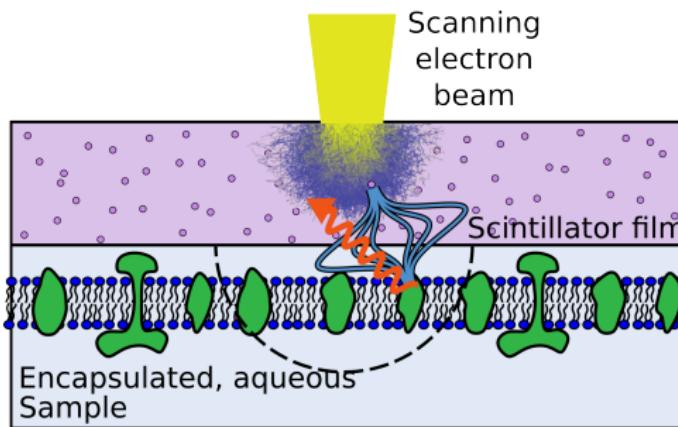
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- ▶ resolution: electron beam imprint ✓
- ▶ sample is protected from harmful radiation ✓

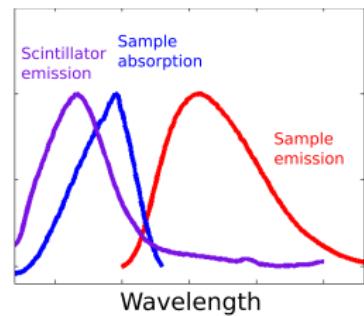
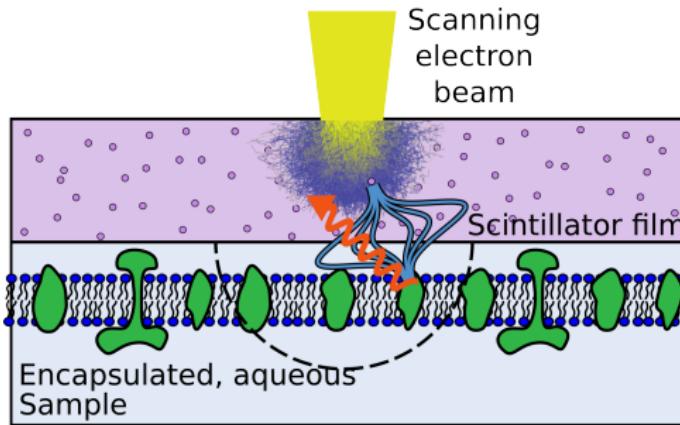
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...under physiological conditions

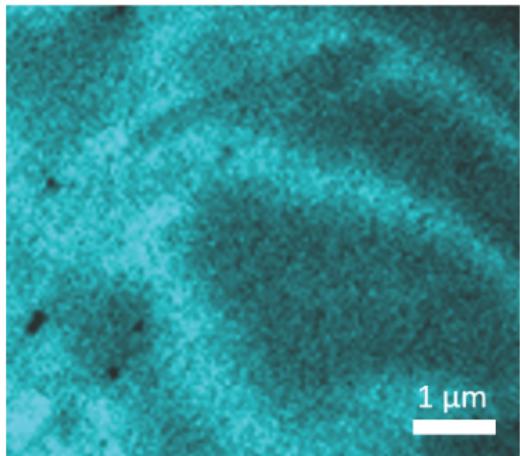


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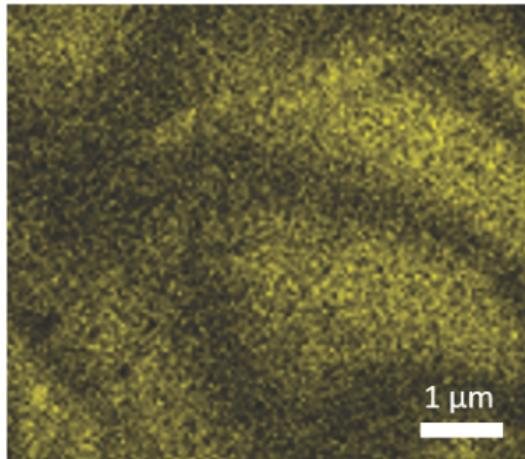
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- ▶ sample is protected from harmful radiation ✓
- ▶ sample can be observed dynamically ✓

# Evidence for CLAIRE already observed in soft materials

Scintillator photon channel



Soft polymer photon channel



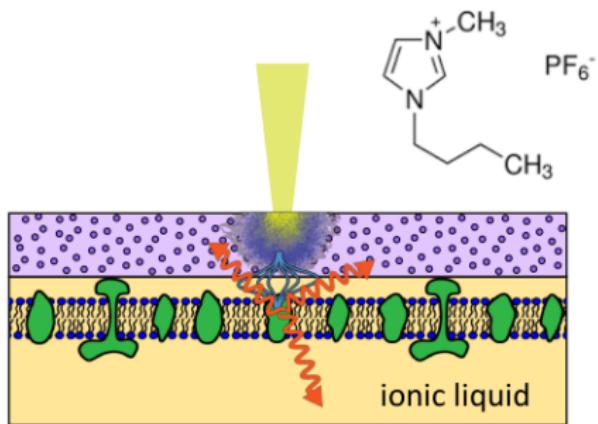
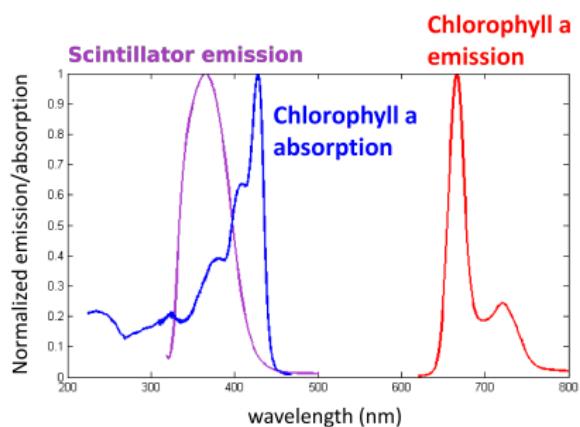
Bischak et al., Nano Letters 15, 3383 (2015)

anti-correlation in the scintillator and sample photon channels



effective resonant energy transfer

# Grana membranes are an ideal target for CLAIRE imaging

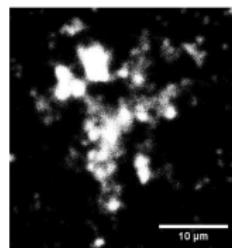
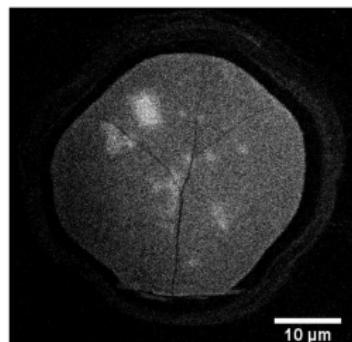


- ▶ ionic liquid matches sample's index of refraction

# First fluorescence signals from grana samples under CLAIRE geometry

- ▶ matches location of grana mapped with confocal microscopy
- ▶ matches chlorophyll A emission band

SEM fluorescence channel      Confocal microscope



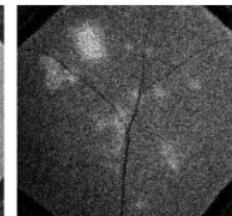
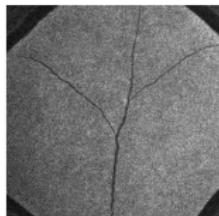
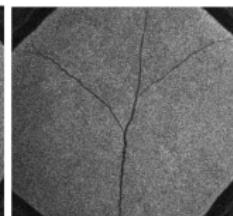
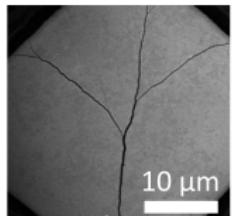
370-509 nm

485-535 nm

530-590 nm

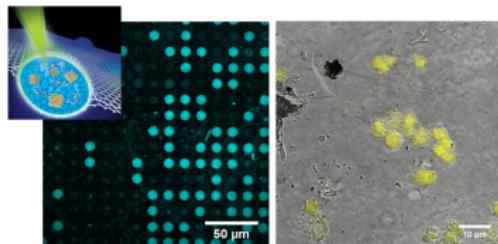
600-650 nm

650-720 nm

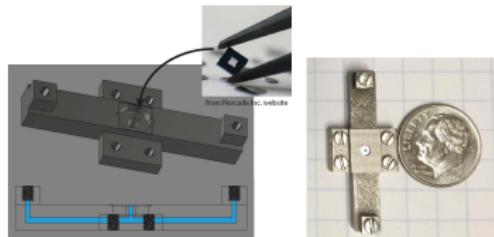


# Integration of liquid cell with imaging chip is underway

## Microscopic graphene liquid cells



## Macroscopic machined liquid cells

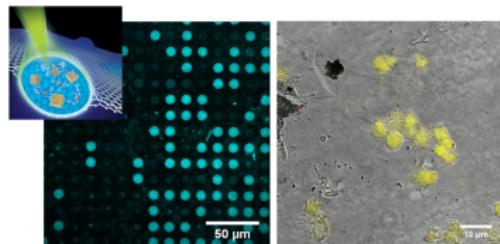


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- Yuk et al., Science 336, 61 (2012)
- ▶ deals well  
with scintillator breakage
- ▶ liquid (< 1 $\mu$ L) confirmed  
with solvatochromatic dye
- ▶ experimenting with  
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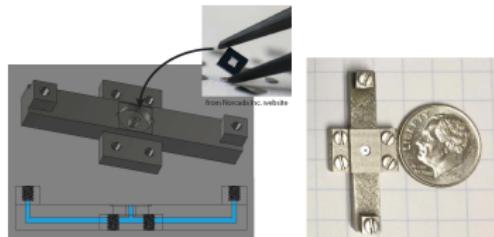
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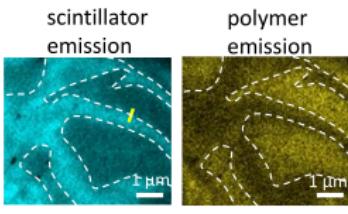
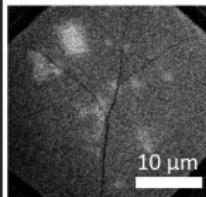
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→ goal is to study dynamics *in vivo*

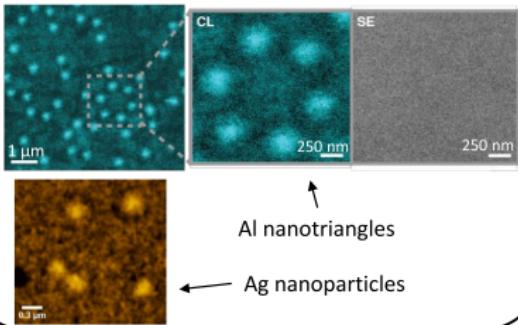
CLAIRe geometry has been used to image a myriad of samples, with various contrast mechanisms

#### endogenous fluorescence

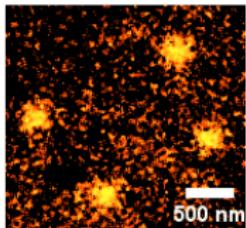


photosynthetic membranes

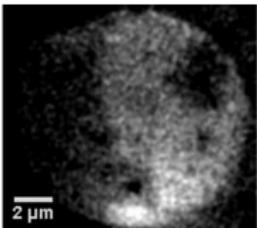
#### plasmonic enhancement



#### dynamics processes

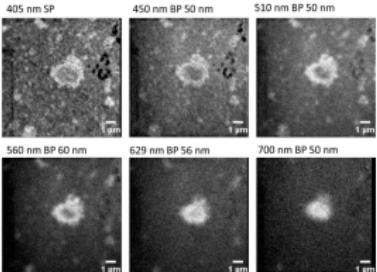


Ag nanoparticles in ionic liquids

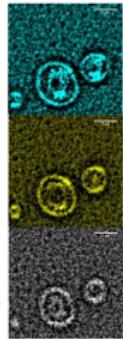


luminescent polymer nanoparticles in oil

#### label-free imaging



thylakoid clumps      polystyrene beads



# Ginsberg group & collaborators



Current members: Ben Cotts, Lucas Ginsberg,

**Connor Bischak**, Brendan Folie, Dannielle McCarthy,

**Clarice Aiello**, **Rebecca Wai**, Milan Delor, Trevor Roberts

Alums: **David Kaz**, Hannah Howard, **Craig Hetherington**,

**Hao Wu**, Jake Precht, **Claire Stachelrodt**, Sam Penwell,

Rodrigo Noriega

Funding: Sloan Foundation, Packard Foundation, DOE,

NSF, DARPA, Kavli Energy Nanosciences Institute, Dow

Chemical, NSERC, UCB College of Chemistry,

Agilent Technologies

Molecular Foundry, LBNL:

Frank Ogletree, Shaul Aloni

Schlom Lab, Cornell: Carolina Adamo, Zhe Wang

Martin & Ramesh Labs, UCB:

James Clarkson, Everton Bonturim

Marvell Nanofabrication Facility, UCB:

Richelieu Hemphill, Ryan Rivers

Niyogi Lab, UCB/LBNL:

Masa Iwai, Daniel Westcott, Alex Hertle, Matt Brooks

Bustamante Lab, UCB/LBNL: Bibiana Onoa