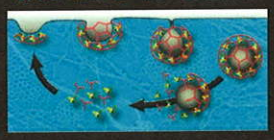
STARS
Basic Science Symposium
FRONTIERS OF BIOMEDICAL IMAGING

ZOOM IN
ZOOM IN
ZOOM IN
A LITTLE MORE

"How Microscopes Reveal the Life of Cells"



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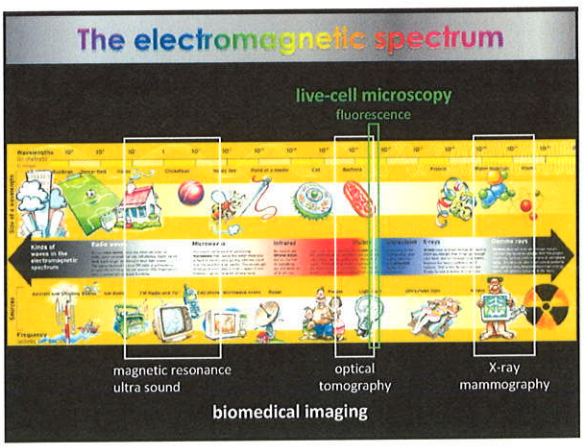
Dept. of Cell Biology

Biomedical imaging v. microscopy

- Non-invasive visualization of internal organs, tissue, etc.
- Image: 2D signal f(x,y), 3D f(x,y,z) or 4D f(x,y,z,t)
- Imaging techniques:
  - Projection X-ray (Radiography)
  - X-ray Computed Tomography (CT)
  - Nuclear Medicine (PET scan)
  - Ultrasound
  - Magnetic Resonance Imaging
  - …

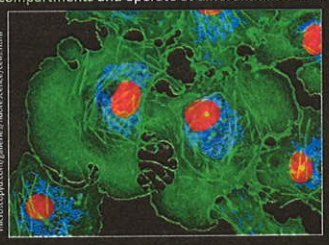
- Non-invasive visualization of intracellular organelles and structures…
- Image: 2D signal f(x,y), 3D f(x,y,z) or 4D f(x,y,z,t)
- Imaging techniques:
  - Wide-field epi-fluorescence
  - Confocal microscopy
  - Total internal reflection fluorescence microscopy (TIRFM)
  - multi-photon fluorescence
  - Photoactivated localization microscopy (PALM)
  - structured-illumination microscopy (SIM)
  - *stochastic optical reconstruction microscopy (STORM)*
  - *stimulated emission depletion (STED)*
  - …

The electromagnetic spectrum



Microscopy opens a window into the cell

"Protein functions are regulated in an integrated network, which results from dynamic protein transport, post-translational modifications and specific protein-protein interaction. These networks can span across different subcellular compartments and operate at different time scales..."

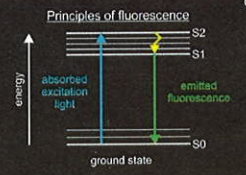


COS-7 cells
nuclei (DNA)
actin
mitochondria

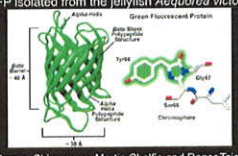
The spatio-temporal localization of a protein in the cell is the first step to understand its function and to integrate it in the complex cell network.

Fluorescent probes for live-cell imaging

Principles of fluorescence



GFP isolated from the jellyfish *Aequorea victoria*



Osamu Shimomura, Martin Chalfie and Roger Tsien
Nobel Prize 2008

eGFP variants

Live-cell imaging = high-content method

What information is present in a digital image obtained by fluorescence microscopy?

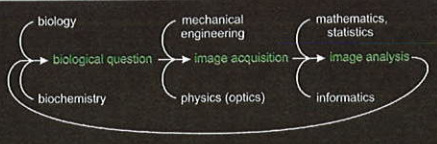
1.spatial: can be used to calculate distances, areas, velocities and protein-protein interactions.

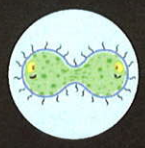
2.intensities: can be used to calculate the local concentration of fluorophores in a specimen.

3.temporal: can be used to determine the hierarchy of protein function.

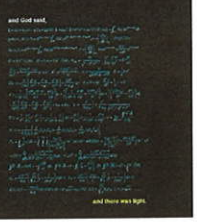
An image is not just a pretty picture – it also contains large amounts of scientific data

Quantitative live-cell imaging is a multi-disciplinary approach

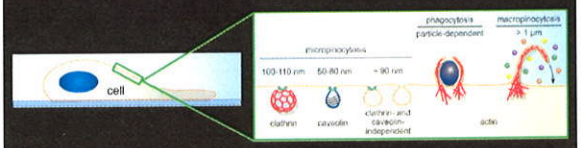




Biology – the only science where multiplication and division mean the same thing



Clathrin-mediated endocytosis (CME)



- At the physical barrier between the intra- and extracellular milieu, CME constitutes the major entry doorway into eukaryotic cells.

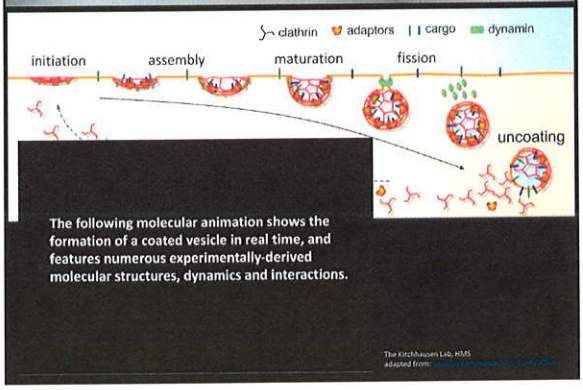
➡ nutrient uptake, signal transduction, control of cell surface composition, cell migration and division, lipid and protein homeostasis, immune response and neurotransmission

all tissues: wound-healing
brain: neurotransmission
kidneys: recapture of ultra-filtrated proteins, nephrotoxicity

intestine: uptake entero-patogenic bacteria, oral vaccines
thyroid: regulation of thyroid hormone production

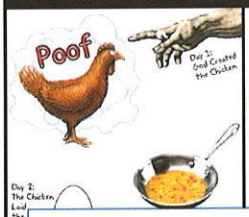
skeleton: bone resorption
liver: uptake and plasma clearance of cholesterol
bronchi: viruses, aerosolic vaccines

CCP maturation in multiple steps

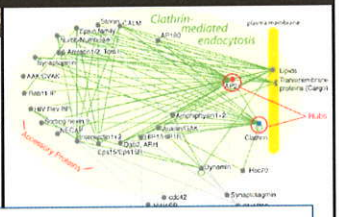


CME interactome

How it's illustrated

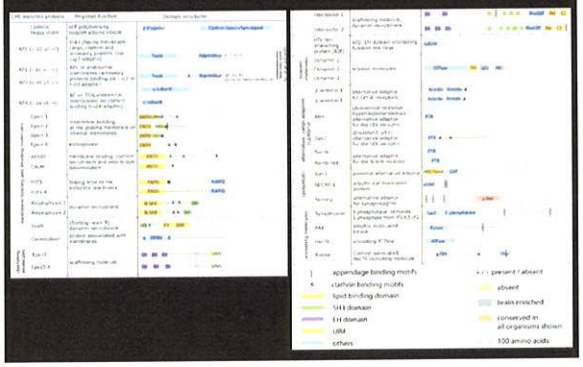


Reality

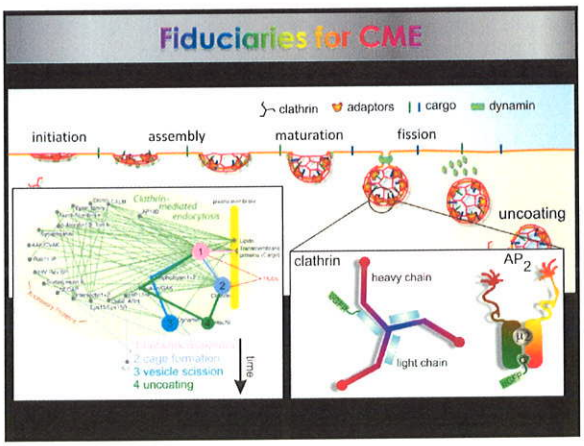


Although a large number of proteins has been involved in CME, little is known about the molecular mechanisms that regulate CME

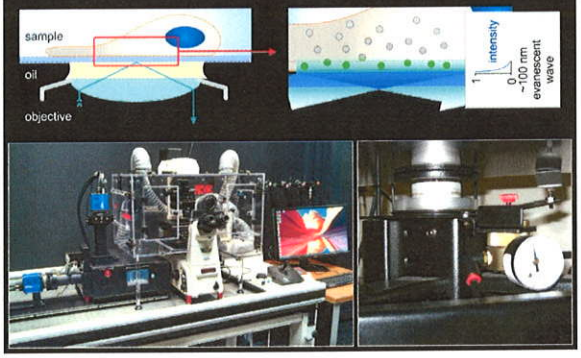
Interactome + redundancy = robustness



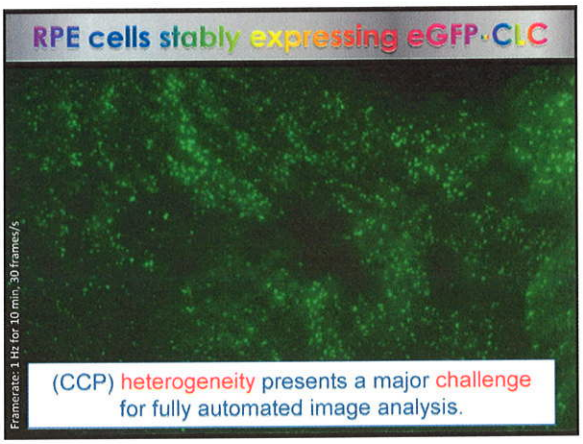
Fiduciaries for CME



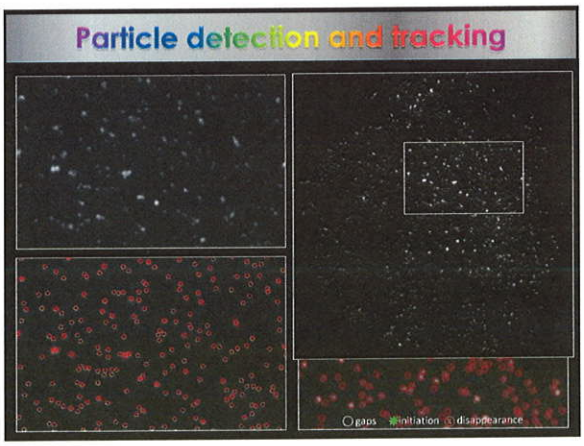
Total internal reflection-FM



RPE cells stably expressing eGFP-CLC



Particle detection and tracking



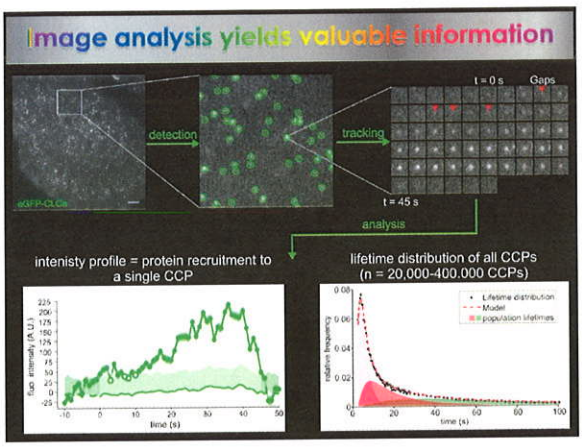
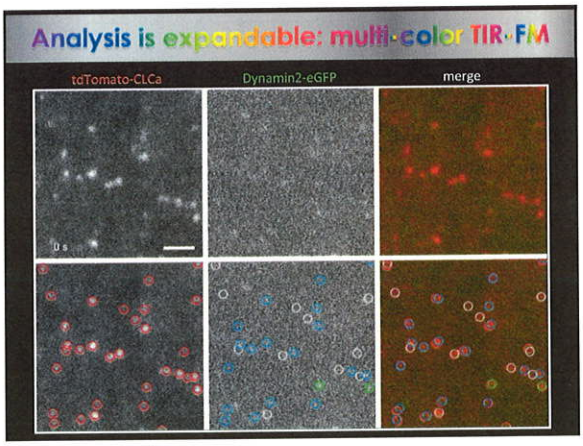
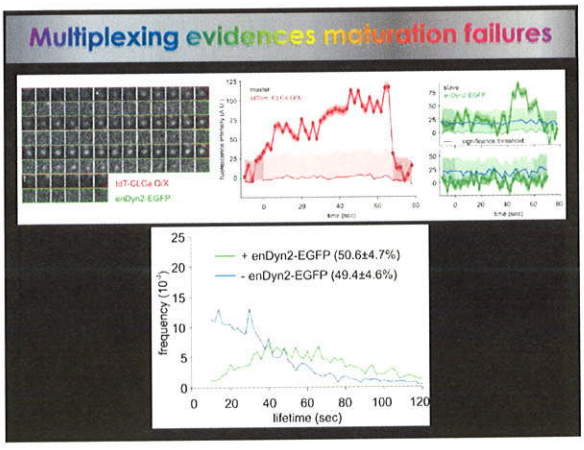
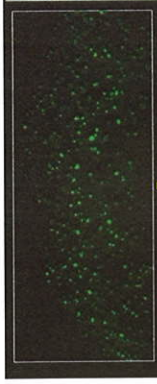


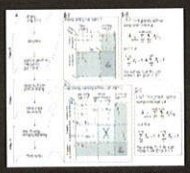
Image analysis yields valuable information
detection
tracking
t = 0 s
Gaps
t = 45 s
eGFP-CLCa
analysis
intenisty profile = protein recruitment to a single CCP
lifetime distribution of all CCPs (n = 20,000-400.000 CCPs)
Lifetime distribution
Model
Population lifetimes
time (s)


Analysis is expandable: multi-color TIR-FM
tdTomato-CLCa
Dynamin2-eGFP
merge
0 s


Multiplexing evidences maturation failures
tdT-CLCa Q/X
enDyn2-EGFP
+ enDyn2-EGFP (50.6±4.7%)
- enDyn2-EGFP (49.4±4.6%)
frequency (10⁻²)
lifetime (sec)

High-yield automated image analysis





Jaqaman et al., Nat. Methods; 2008

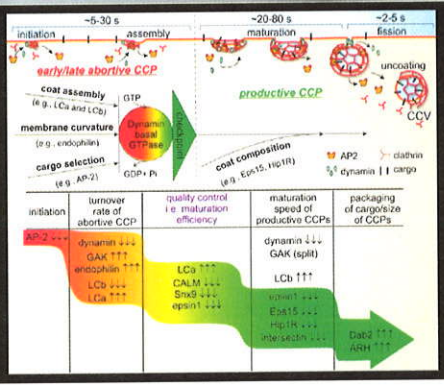
Single-color TIR-FM

- Protein recruitment profiles
- CCP lifetimes
- CCP densities
- Spatial organization
- Relative contribution of CCP subpopulations

Multi-color TIR-FM

- Temporal hierarchy of protein function
- Protein-protein

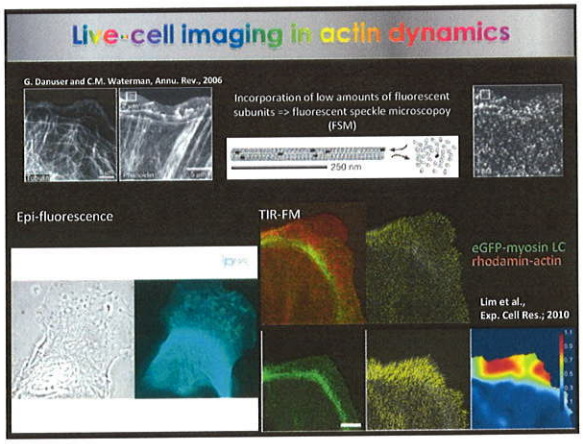
CME is gated by a checkpoint



Live-cell imaging in cell migration

Lim et al., Exp. Cell Res.; 2010

Live-cell imaging in actin dynamics



Take home message

Based on advances in imaging techniques and automated, computer-based image analysis, fluorescent live-cell imaging is increasingly used to quantify spatial and temporal relationships between molecules in biological specimens, thereby opening a window into the molecular regulation of life.