Developing Tools for Using the Live Cell as a ‘Laboratory for Fluorescence Dynamics’

Gerd Ulrich Nienhaus
Department of Biophysics, University of Ulm, Germany
Department of Physics, University of Illinois at Urbana-Champaign
Exploring the Molecular Basis of Life at the Cellular Level

Cellular Information Networks

in vitro → in vivo
Fluorescence Microscopy

- minimally invasive
- allows studies of living cells
- enormous technological progress
  - microscope design
    - Confocal microscopy
    - Multi-photon microscopy
    - Total internal reflection microscopy
    - 4Pi microscopy
    - STED microscopy
  - components
    - Detectors (cameras, APDs)
    - Lasers
    - Beam Scanners
    - Computers
  - fluorescence markers
    - Synthetic dyes
    - Quantum dots
    - Fluorescent proteins
Fluorescent Markers

Quantum dots

Organic dyes (Cy dyes, Alexa, TMR …)

Fluorescent proteins (GFP, eqFP611, EosFP FP499, FP512, DsRed, …)
Fluorescent Proteins from the GFP family

- protein-protein interactions
- protein localization
- developmental biology
- gene regulation
- drug screening (HTS, HCS)
- pH sensing
- Ca^{2+} sensing
- single-molecule assays

FPs can be expressed in many different organisms
Biomedical FP Application: High Content Screening (HCS)

Fusion construct:

IGF-1

hIR

Ras

PI3K

Inhibitors

PI-3,4-P2

PI-3,4,5-P3

PI-4,5-P2

AKT1-FP

Phosphatidyl inositol 3 kinase (PI3K) assay

Wolff et al., Combinatorial Chemistry and High Throughput Screening, in press.
AKT1-FP Translocation Kinetics

CHO-K1 cells transfected with hIR and AKT1-EGFP + IGF-1
Fluorescent Proteins from Anthozoa

Sea Anemone

Coral

Tube Anemone
Fluorescent Protein Research in Ulm

- isolation from natural sources
  J. Wiedenmann

- biophysical characterization
  - spectroscopy, crystallography, cellular imaging

- optimization by protein engineering
  - monomerization (important for fusion markers)
  - expression yield / temperature
  - far-red emitting proteins (eqFP611, FP630)
  - photo-switchable emission
Photoconverting Fluorescent Protein EosFP

Lobophyllia hemprichii

UV Photoconversion

![Graph showing UV photoconversion of EosFP WT green and red forms.](image)

- **Absorbance** and **Emission**
- **Wavelength (nm)**: 300 to 700
- **Action spectrum**

---

**Graph details:**
- EosFP WT green
- EosFP WT red form
- Wavelength range: 300 to 700 nm
- UV Photoconversion:
  - 400 nm
  - 516 nm to 581 nm
Crystal Structure of EosFP at 1.8 Å Resolution

Photo-induced Backbone Cleavage

UV

Phe61

Gly64

Tyr63

green chromophore

Phe61

Gly64

Tyr63

red chromophore
Single Molecule Studies

EosFP immobilized on a BSA-biotin surface via streptavidin

Exc. 4.3 µW @ 488nm (Ar ion)

Exc. 4.3 µW @ 488nm + 3 µW @ 400nm (TiSa)
Single Molecule Studies

**EosFP** variants immobilized on a BSA-biotin surface
488 + 400 nm excitation

**wt-EosFP**

- Efficient FRET in the tetramer

**Monomeric mutant (V123T-T158H)**

- One-step conversion/bleaching → monomer

Application: Dual-Color Images of Cell Division

HEK 293 co-expressing GFP-KX (tubulin associated) and RBP2-d2EosFP (chromatin associated)

Application: Tracking Proteins in Mitochondria

- HeLa cells expressing mEosFP with mitochondrial localization signal
- Excitation with Ar ion laser @ 488 nm, conversion with TiSa @ 404 nm
- Field size $36 \times 36 \, \mu m^2$
- Total duration $\sim 2 \, h$

Application: Developmental Biology

- Injection of *capped* mRNA at 2-cell stage of *Xenopus laevis*
- Local conversion at blastoporus
4Pi Fluorescence Microscopy

Confocal

4Pi

4Pi Deconvoluted

E. coli

Dil stain

500 nm

500 nm

110 nm
Actin Network (Confocal)

HeLa cell  Rhodamin-Phalloidin  514 nm excitation
Actin Network in 4Pi Resolution

HeLa cell    Rhodamin-Phalloidin    870 nm 2-Photon 4Pi excitation
Jurkat Cell – Colocalization of Nef with Lipid Rafts

pEYFP-C1 NL43nef
(HIV-1 accessory protein)

Lipid raft marker (Alexa Fluor 594)

4Pi excitation 900 nm

5 µm
Plasticity of Excitatory Synapses

Composition, structure, size of PSD (disk-shaped protein assembly)
4Pi Imaging of Synapses

Hippocampus neuron 7d

Postsynaptic: EGFP-Prosap2
Presynaptic: Bassoon-Texas Red
Protein Art with EosFP

Happy 60th Birthday, Enrico!
Acknowledgments

General Zoology and Endocrinology
Jörg Wiedenmann
Florian Schmitt

Biophysics
Stefanie Bacher
Vladimir Breus
Karen Deuschle
Stephanus Fengler
Jochen Fuchs
Sylvia Glaschick
Colin Heyes
Sergey Ivanchenko
Andrei Kobitski
Daniel Mark
Karin Nienhaus
Robert Rieger
Franz Oswald
Carlheinz Röcker
Michael Schmitz
Kyrylo Tron
Jörg Wiedenmann

Internal Medicine I
Franz Oswald

Virology
Frank Kirchhoff
Michael Schindler
Anke Specht

Anatomy and Cell Biology
Tobias Böckers
Andreas Grabrucker

Beatrice Vallone
Fabiana Renzi

Ralf Heilker
Herbert Nar