

# Fluorescent Probes and Labels for Biomedical Applications

Leonid Patsenker<sup>1,2</sup>, Olga Kolosova<sup>1</sup>, Anatoliy Tatarskiy<sup>1</sup>, Iryna Fedyunayeva<sup>1</sup>, Yevgeniy Povrozin<sup>1</sup>, Inna Vermolenko<sup>1</sup>, Yuliya Kudryavtseva<sup>1</sup>, and Ewald Terpetschnig<sup>2</sup>

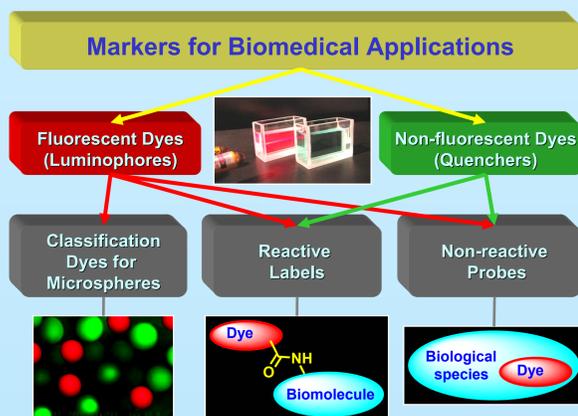


<sup>1</sup>State Scientific Institution "Institute for Single Crystals", NAS of Ukraine, 60 Lenin Ave., 61001 Kharkov, Ukraine, e-mail: patsenker@isc.kharkov.com, http://www.isc.kharkov.com/old

SETA BioMedicals

<sup>2</sup>SETA BioMedicals, LLC, 2014 Silver Ct East, Urbana, 61801, IL, USA, e-mail: info@setabiomedicals.com, http://www.setabiomedicals.com

Our dye repertoire can be subdivided into fluorescent dyes or **luminophores** and non-fluorescent dyes called **quenchers**. Both fluorescent and non-fluorescent dyes are used as **reactive labels** for covalent attachment to biomolecules, while fluorescent **probes** undergo non-covalent interactions with biomolecules. Fluorescent dyes are also utilized as **classification dyes** for single or multiple encoding of reactive microspheres (beads) in suspension arrays.

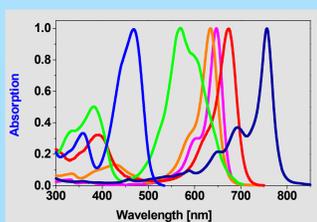


We have developed extremely bright and sensitive fluorescence dyes and real dark quenchers for use in biological and pharmaceutical research, clinical diagnostics, and high-throughput screening (HTS):

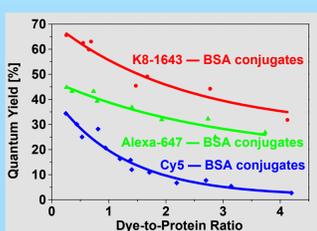
- **Reactive Red and Near-infrared (NIR) Fluorescent Labels** of the **Square** and **SETA** series for covalent attachment to biomolecules such as proteins, amino-acids, peptides, oligonucleotides, DNA, RNA, lipids and drugs
- **Fluorescent Probes** for proteins, lipids and cells
- **pH-Sensitive Probes and Labels**
- **Fluorescence Lifetime (FLT) Probes and Labels** of **SeTau** series for FLT and fluorescence polarization based applications
- **Dark quenchers** of the **SQ** series for Fluorescence Resonance Energy Transfer (FRET) applications
- **Classification Dyes** for single or multiple encoding of microspheres

## Wide Spectral Range

These dyes, probes and labels have several advantages as compared to other commercially available probes and labels: **Square** and **SETA** dyes absorb (300–850 nm) and emit (500–850 nm) in a wide spectral range. Unlike dyes of the **Cy** and **Alexa Fluor** series, these red and NIR emitting markers can be excited not only with the red, 635-nm and 670-nm diode lasers but also with the UV and blue diode lasers and light emitting diodes (LEDs) at 380-nm, 405-nm and 436-nm.

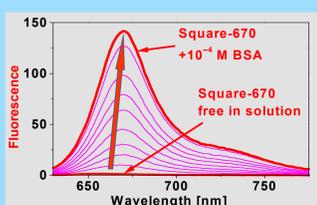


## Brightness



**Square** and **SETA** dyes of the **K8** series have high extinction coefficients (up to  $265,000 \text{ M}^{-1}\text{cm}^{-1}$ ). Protein conjugates of these dyes are extremely bright. **Quantum yields** (up to 70%) for our conjugates are unmatched by **Cy5** or **Alexa** dyes.

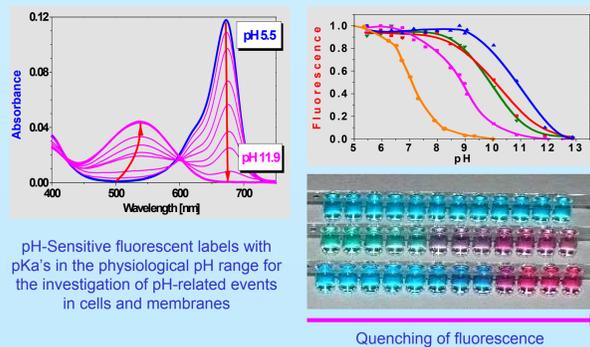
## Sensitivity to Microenvironment



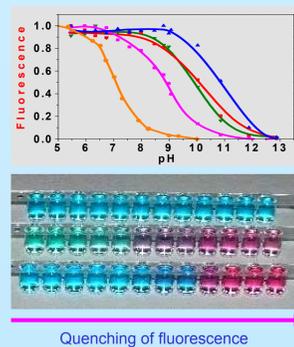
Red and near-IR probes and labels show noticeable increases of fluorescence intensity and longer fluorescence lifetimes in presence of large biomolecules such as proteins and lipids.

## pH-Sensitive Labels for Biological Applications

These are **pH-sensitive markers** with tuneable pKa's in the range from **5.5–10.5**:

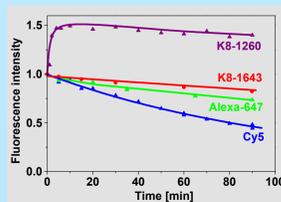


pH-Sensitive fluorescent labels with pKa's in the physiological pH range for the investigation of pH-related events in cells and membranes



Quenching of fluorescence

## High Photostability



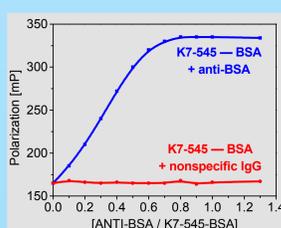
Normalized fluorescence intensity vs. exposition time for free dyes

**Square** and **SETA** dyes of the **K8** series are much more photostable compared to **Cy5** and **Alexa** cyanine dyes. This is particularly important in **biological imaging** applications. For additional information on this topic we refer you to our poster "Thio-squarylium dyes as highly photo-stable markers"

## Probes for Fluorescence Lifetime Imaging and Homogeneous Fluorescence Polarization Assays For High- Molecular-Weight Analytes

**SeTau** tracers show fluorescence in the blue and green spectral region and have **FLTs** up to 40 ns in water. **SeTau** dyes are perfectly suited for use in homogeneous fluorescence polarization assay of high molecular-weight antigens and substantial polarization increases are observed upon binding of the high molecular-weight tracers to the antibody (see below).

Red and near-IR microenvironment-sensitive probes exhibit lifetime increases from 500 ps up to 2–3 ns upon binding to biomolecules. These probes have high affinity for proteins, biomembranes and lipoproteins and are useful to detect and quantitate these analytes.

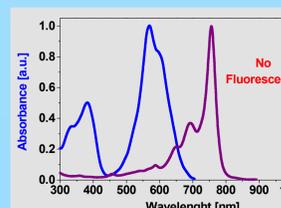


Changes in Fluorescence Polarization of **K7-545**-labeled BSA (MW ~ 65 kDa) upon titration with anti-BSA. The labeled BSA species has still a relatively low polarization of 165 mP and only upon addition of specific antibody the polarization increases gradually to its final value of 335 mP, which demonstrates the usefulness of this label for the measurement of high-molecular-weight antigens in a Fluorescence Polarization Immunoassay (FPA)

## Dark Quenchers

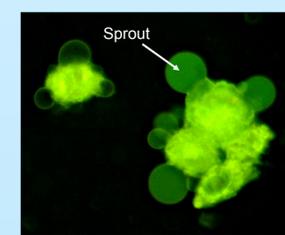
Our reactive **Super Quenchers** of the **SQ** series absorb in the **500–800 nm** spectral range. They exhibit no residual fluorescence and are perfectly suited for covalent labeling of proteins, peptides and oligo-nucleotides for use in FRET and real-time PCR based applications.

Number	Product Name	Absorption max. [nm]	Extinction Coefficient [ $\text{M}^{-1}\text{cm}^{-1}$ ]	Solubility
K8-1649	SQ740-mono-NHS	740	44,000	Water, Ethanol, DMF, DMSO
K8-2602	SQ755-di-NHS	698, 756	200,000	Water, Ethanol, DMF, DMSO

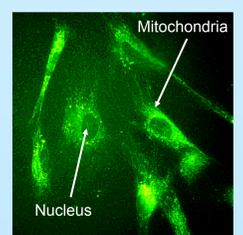


## Dyes for Biological Imaging

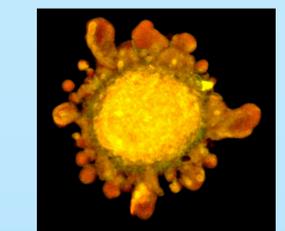
The higher photostability of **Square** and **SETA** probes and labels as compared to **Cy** or **Alexa Fluors** is an advantage in biological imaging where longer exposure times help to improve the image quality.



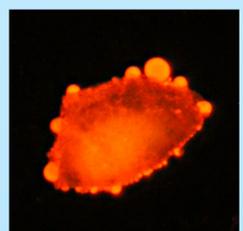
Human fibroblast cells of conjunctive tissue stained with dye **K8-3010** in aqueous media. Plasma membrane vesicles are green. Excitation wavelength: 470 nm



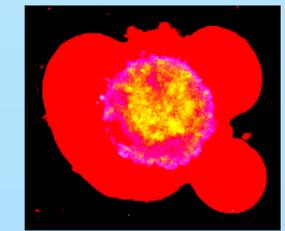
Human fibroblast cells on a glass slide in 3 days culture stained with dye **K8-3010**. Active mitochondria are brightly green while nucleus does not fluoresce. Excitation wavelength: 470 nm



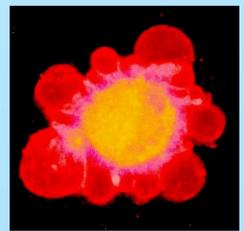
Human fibroblast cells stained with potential-sensitive dye **K5-1000**. Nucleus is orange, plasma membrane vesicles are orange-brown. Excitation wavelength: 470 nm



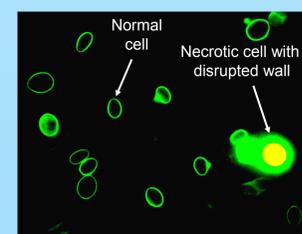
Human fibroblast cells stained with dye **K8-1355**. Plasma membrane vesicles are orange-red. Excitation wavelength: 580 nm



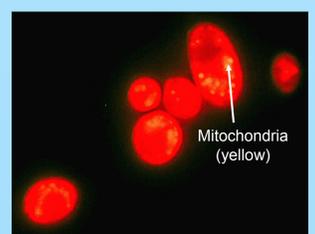
Human fibroblast cells stained with **K8-1400**. Nucleus is yellow-orange, plasma membrane vesicles are red. Excitation wavelength: 580 nm



Human fibroblast cells stained with **K8-1500**. Nucleus is yellow-orange, plasma membrane vesicles are red. Excitation wavelength: 580 nm



Yeast cells (*Saccharomyces cerevisiae*) stained with dye **K8-3010**. Cell walls are bright green. Necrotic cells with disrupted cell walls have bright green fluorescence from cell interior. Excitation wavelength: 470 nm



Yeast cells (*Saccharomyces cerevisiae*) stained with dye **K8-1400**. Nucleus does not fluoresce, cytoplasm is red, active mitochondria are yellow. Excitation wavelength: 580 nm

## Classification Dyes for Microspheres for a wide spectral range

