NANOPARTICLES FOR FLIM-BASED MULTIPLEXED ANALYSIS AND IMAGING

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An important field of bioanalytical research, especially in the context of meeting current security and health concerns, is the increase of information content from bioassays. This can be achieved by robust and efficient strategies for the detection of multiple analytes or targets in a single measurement using detection methods which provide different analyte-specific parameters like fluorescence or Raman spectroscopy.

One of the most simple and cost efficient multiplexing technique presents fluorometry. An attractive alternative to well-established spectral or color multiplexing using spectrally distinguishable fluorescent labels presents lifetime multiplexing, which relies on fluorophores excitable at the same wavelength and sufficiently differing in their emission lifetime. [1] This enables distinction between spectrally rather similar fluorophores in the same detection channel. Lifetime multiplexing can be also combined with spectral multiplexing, thereby increasing the number of fluorophores / analytes detectable in parallel.

Here we report on a new multiplexing approach based on novel nanometer-sized particle labels doped with different NIR-emitting organic dyes of large Stokes shift. [2] Their excitation with visible light leads to emission in the near-infrared spectral region, thereby minimizing unspecific background signals like autofluorescence from endogeneous fluorophores in tissue, or scattering of excitation light. Moreover, with regard to bioimaging, the absorption of water, blood, and other tissue components, is minimum in the NIR. This enhances the penetration depth for fluorescence imaging applications and the detection sensitivity in bioassays.

To assess this strategy for bioanalysis, we present examples for a first proof-of-principle of the lifetime-based distinction between mixtures of NIR-emitting nanoparticles displaying very similar absorption and emission spectra, yet different fluorescence decay kinetics. [3] Fluorescence lifetime imaging microscopy (FLIM) measurements of 3T3 fibroblasts and J774 macrophages underline the potential of nanoparticle-based fluorescence lifetime multiplexing and imaging as a powerful tool in the life sciences and bioanalysis.