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Imaging the rotational mobility by frequency domain time-resolved fluorescence anisotropy

Gilad Yahav, Shweta Pawar, Yitzchak Weber, Bar Atuar, Hamootal Duadi and Dror Fixler

Faculty of Engineering and the Institute of Nanotechnology and Advanced Materials, Bar Ilan University, Ramat Gan, 5290002, Israel

Measurements of the fluorescence anisotropy (FA) decay can reveal variation in the rotational mobility of the fluorophore, which is sensitive to the microviscosity or other factors influencing the diffusional motion of the fluorophores.

We present the frequency domain (FD) time-resolved FA imaging (FD TR-FAIM) system that enables the wide-field measurement of:

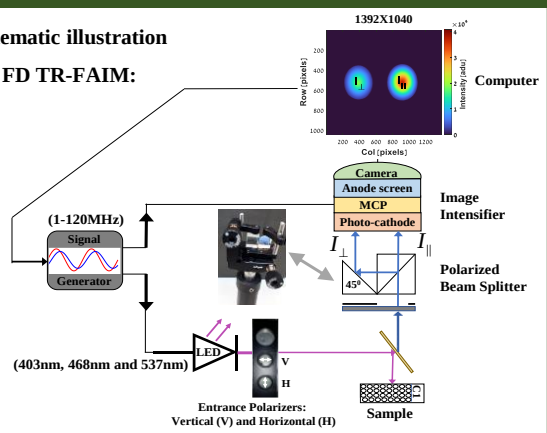
- The steady-state fluorescence intensity (FI) and the FI decay
- The steady-state FA and the FA decay

on a pixel-by-pixel basis

- ✓ **FI Imaging**
- ✓ **Fluorescence lifetime (FLT) imaging (FLIM)**
- ✓ **Steady-state FA imaging**
- ✓ **Rotational correlation time imaging**

- The FA decay analyses were integrated with existing FD FLIM technology by mainly introducing
  - **a vertical linear polarizer in the excitation path.**
  - **a polarized beam splitter in the emission path.**
- The phase delay and intensity ratios (AC and DC) between the polarized components of the fluorescence emission are recorded
- Leading to value estimations of
  - **Steady-state FA (*r*)**
  - **Rotational correlation time (θ)**
  - **Fundamental FA (r_0)**
- For FLT measurements the polarized beam splitter is replaced by a linear polarizer oriented 54.7^0 from the vertical (known as magic angle setting).

- **A Schematic illustration of the FD TR-FAIM:**



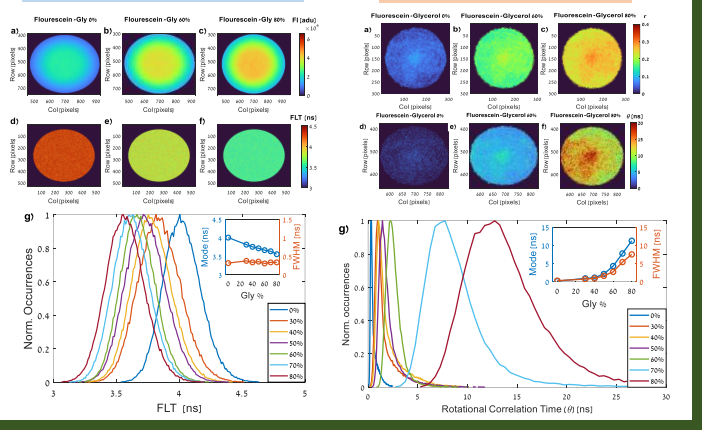
- The combined FD FLIM/ FD TR-FAIM was validated on 7 fluorescein solutions with increasing viscosity (increasing glycerol concentration between 0-80%)

Fluorescein in phosphate buffer saline

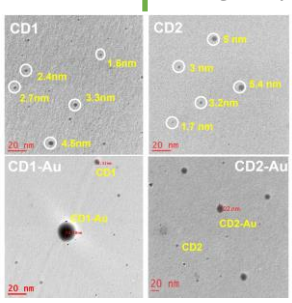
Glycerol [%]

FI and FLT Imaging

r and θ Imaging



- The integrated system was then applied for studies of two type of familiar carbon dots
  - PEI-CA-CDs (CD1)
  - PEI-p-PD-CDs (CD2)
  - ✚
  - CD1-AuNP
  - CD2-AuNP



FI Imaging | **FLT Imaging** | ***r* Imaging** | **θ Imaging**

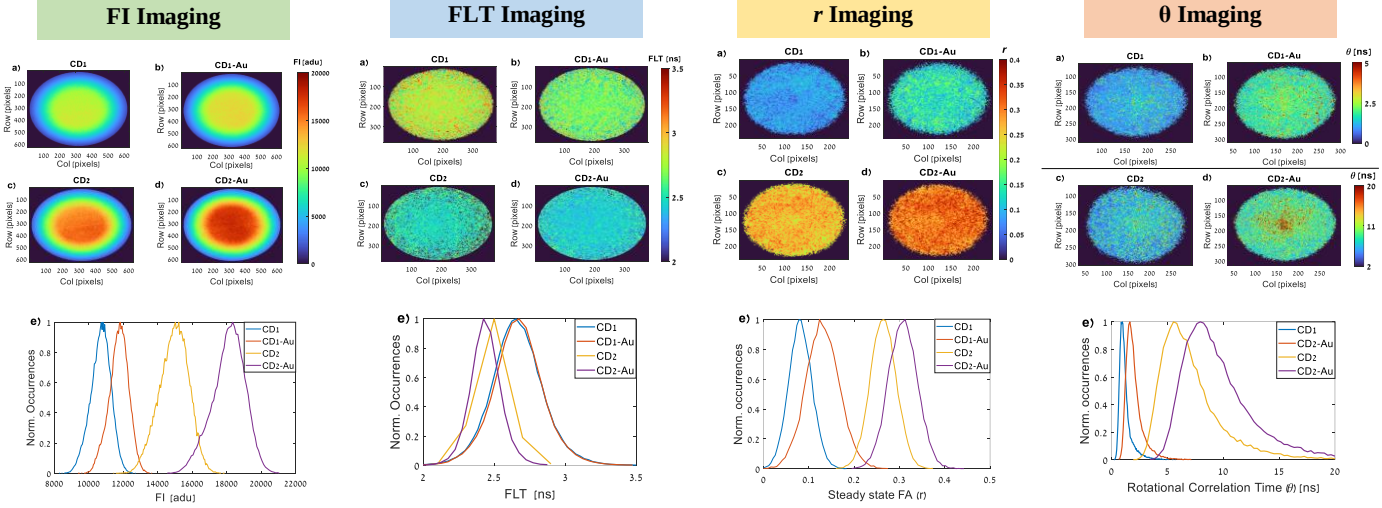


Table 1. Characterization of FI and FI decays of the four CDs.

	FI [adu]		FLT [ns]	
Sample	Mode	FWHM	Mode	FWHM
CD1	10,825±620	1,125±570	2.66±0.10	0.36±0.02
CD1-Au	11,825±570	1,100±850	2.62±0.05	0.33±0.02
CD2	15,225±625	2,000±400	2.47±0.19	0.20±0.05
CD2-Au	18,350±1070	2,000±400	2.42±0.06	0.21±0.04

Table 2 Characterization of steady-state FA and FA decays of the four CDs.

	r		*θ [ns]*		r_0
Sample	Mode	FWHM	Mode	FWHM	Mode
CD1	0.100±0.011	0.075±0.011	0.98±0.13	0.66±0.01	0.16±0.01
CD1-Au	0.150±0.013	0.065±0.005	1.65±0.20	1.10±0.13	0.23±0.05
CD2	0.280±0.008	0.078±0.009	5.55±1.08	4.26±0.47	0.36±0.01
CD2-Au	0.310±0.004	0.055±0.008	7.95±0.97	5.30±0.68	0.40±0.01

Generally, each of the main fluorescence characteristics (FI, FLT, *r* and θ) sheds its own light on the investigated samples. Nevertheless, the latter was the most favorable and beneficial either by probing the spatial changes in the rotational mobility across each sample and either by the evident variation in both the peak and the FWHM of each sample's θ distribution.

Gilad.yahav@gmail.com ***http://www.eng.biu.ac.il/fixlerd/***