Spectrally Resolved Fluorescence Correlation Spectroscopy Based on Global Analysis (SRFCS-G)

Michael J. R. Previte[1,2*], Serge Pelet[1,3], Ki Hean Kim[1,4], Christoph Buehler[1,5], and Peter T. C. So[1,6]

[1]Department of Biological Engineering, Massachusetts Institute of Technology, 77 Massachusetts Av., Cambridge, MA 02139
[2]University of Maryland Biotechnology Institute, 725 W. Lombard Street, Baltimore, MD 21202
[3]Institute of Biochemistry Swiss Federal Institute of Technology Zurich (ETH), Schafmattstr. 18 HPM G9.1, CH-8093 Zurich, Switzerland
[4]Wellman Center for Photomedicine, Massachusetts General Hospital, 50 Blossom St., BAR714, Boston, MA 02114 617-724-5532
[5]Novartis Institutes for BioMedical Research, Vienna, Innovative Screening Technologies, Brunner Strasse 59, A-1235 Vienna, Austria
[6]Department of Mechanical Engineering, Massachusetts Institute of Technology,77 Mass Ave, Cambridge, MA 02139

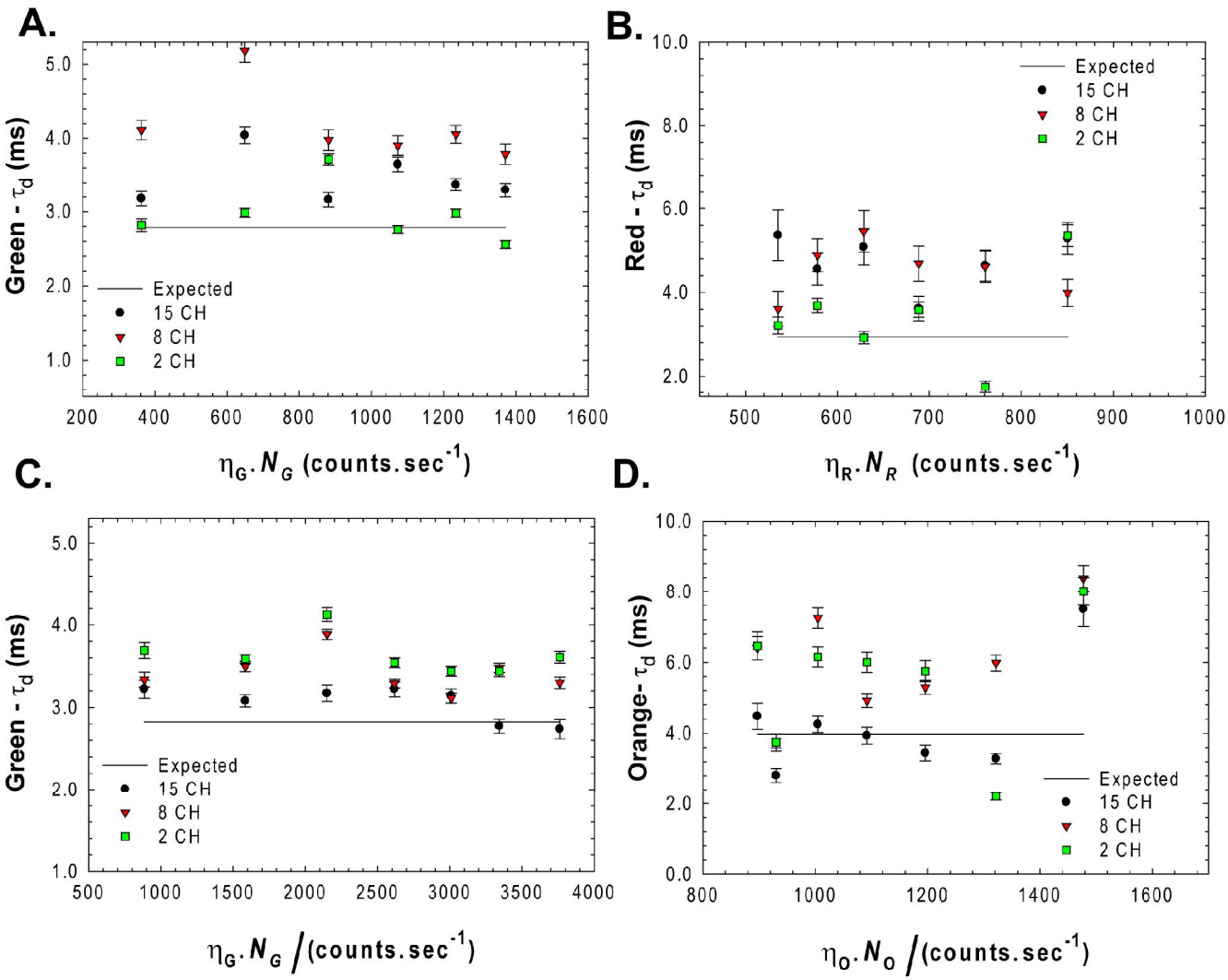
email: prev_31k@mit.edu

Title Running Head: Spectral Resolved Correlation Spectroscopy

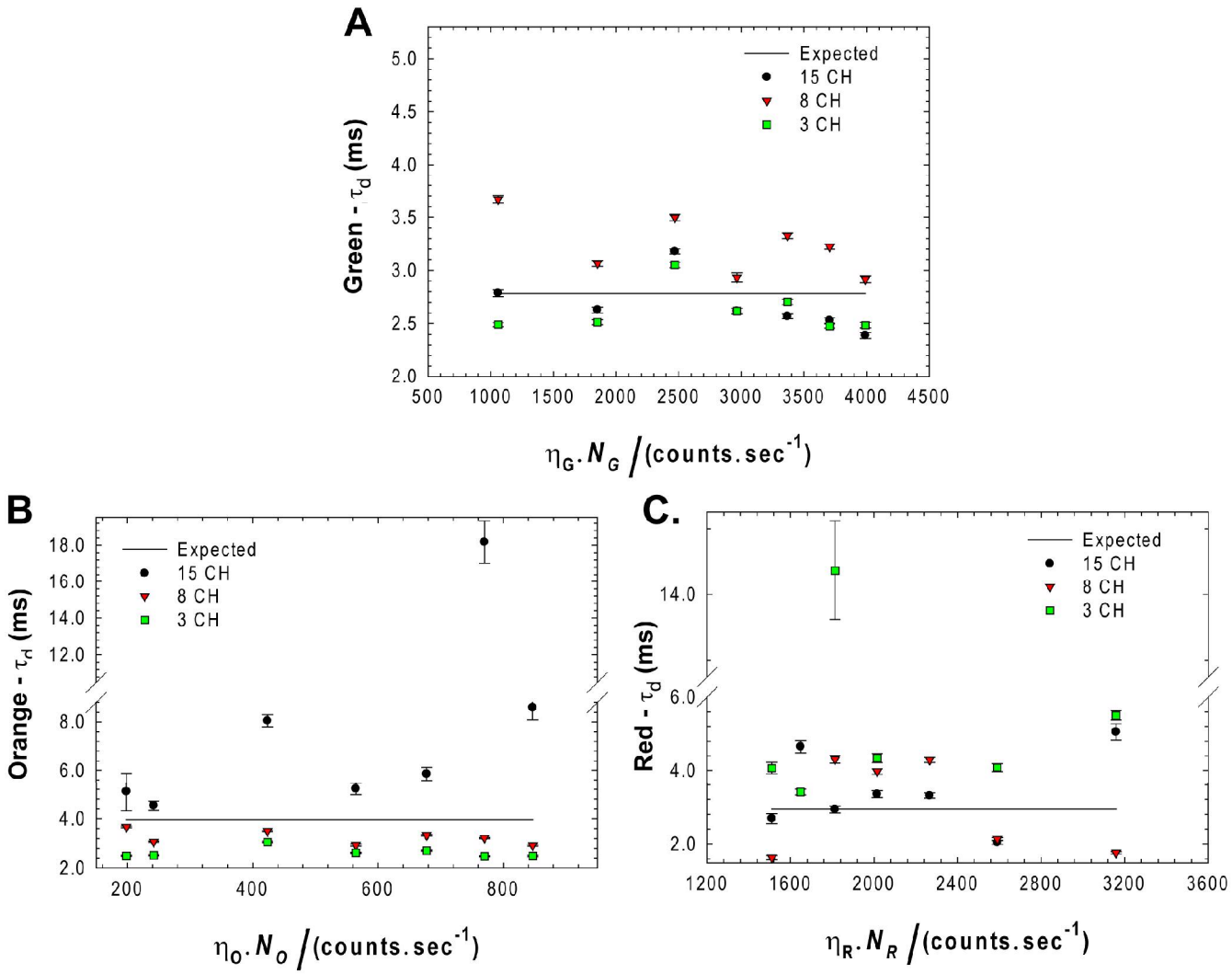
Contents:

Fitted Data	N:	σ_N	τ_d (ms)	$\sigma_{\tau d}$ (ms)
CH 3	17.2	2.06	0.231	0.47
CH 4	18.7	0.873	0.244	0.20
CH 5	19.3	1.14	0.250	0.26
CH 6	17.7	2.84	0.152	0.40
Sum (CH 1-15)	18.1	0.240	0.275	0.065

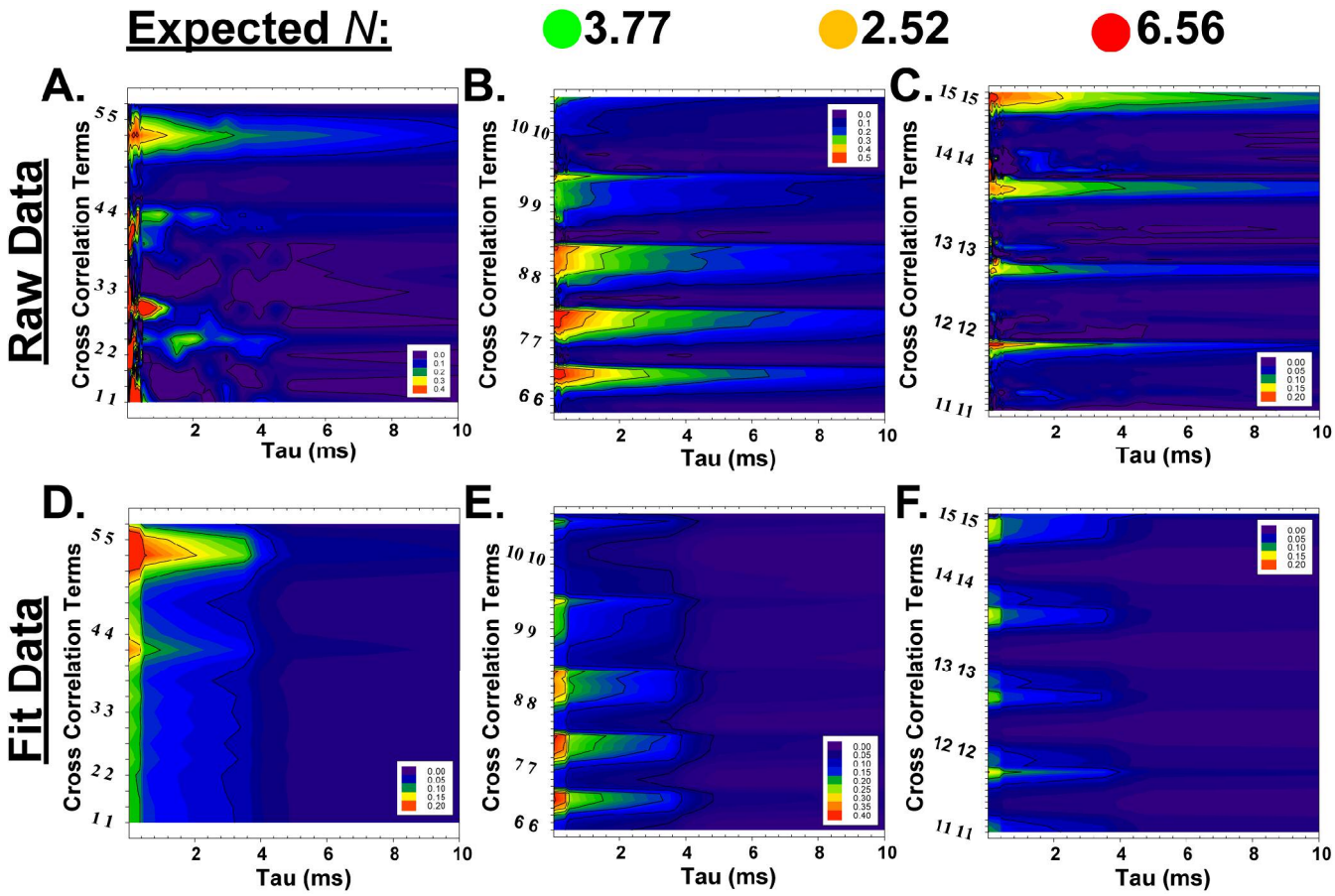
Supplementary Table 1: The number concentrations, diffusion times and respective standard deviations recovered from global fits of correlated data for 25 nM Alexa 488 in 50% glycerol recorded from channels 3-6 and summed channel (1-15) data.



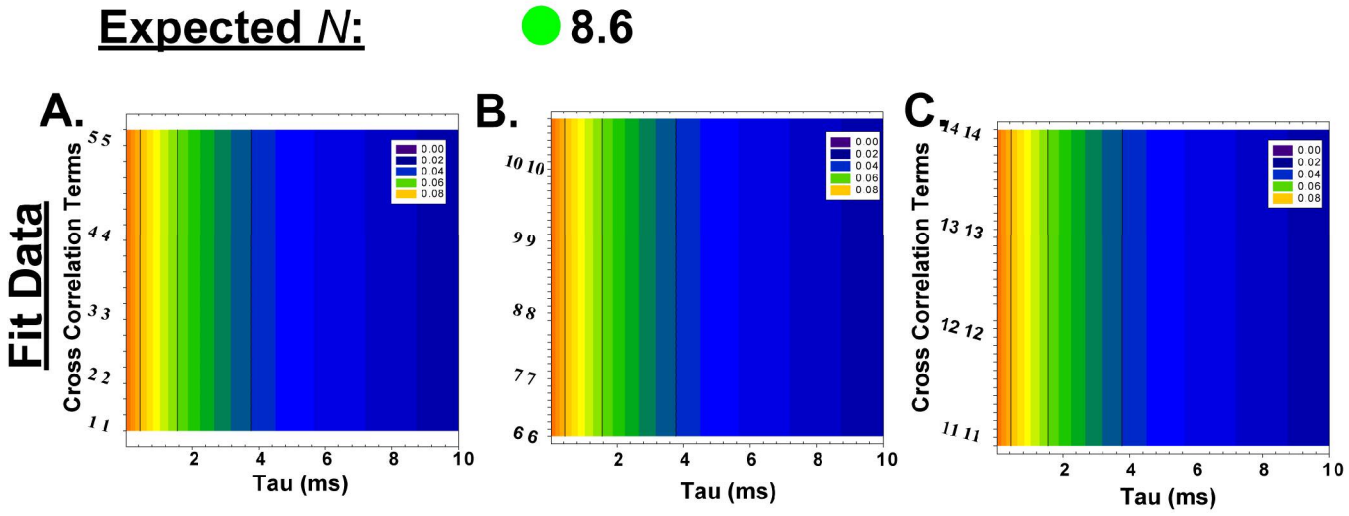
Supplementary Figure 1: Recovered diffusion times of **A)** green and **B)** red beads in the G:R mixtures and **C)** green and **D)** orange beads in the G:O mixtures plotted against the product of molecular brightness (η) and expected number of molecules (*N*). Data are fit using 15 channel SRFCS with global analysis (15 CH, circles); 8 channel SRFCS with global analysis with (8 CH, inverted triangles); and least square fitting of the autocorrelation curves from binned G and R or G and O data (2 CH, squares). Recovered diffusion times predicted from fits of single channel (G, O, or R) autocorrelation curves are shown for reference (expected, solid line).



Supplementary Figure 2: Recovered diffusion times of **A)** green and **B)** orange and **C)** red beads in three color mixtures versus the product of molecular brightness (η) and expected number of molecules (*N*). Data are fit using 15 channel SRFCS with global analysis (15 CH, circles); 8 channel SRFCS with global analysis with (8 CH, inverted triangles); and least square fitting of the autocorrelation curves from binned G, R, or O data (3 CH, squares). Recovered diffusion times predicted from fits of single channel (G, O, or R) autocorrelation curves are shown for reference (expected, solid line).



Supplementary Figure 3: Raw data surface plots of a 3 color mixture of beads that contains approximately 3.37 green beads; 2.62 orange beads; and 6.56 red beads. Cross correlation decays are shown for channels 1-5 in panel A; 6-10 in B; and 11-15 in C. Contour plots of the fit data from the same 3 color mixture. Cross correlation decays are shown for channels 1-5 in panel D; 6-10 in E; and 11-15 in F. The legend displays the color scale for the G(0) amplitudes (z axis data) and the y axis specifies the specific cross correlation term (see text for further description).



Supplementary Figure 4: Contour plots of the fit data from a bead sample that contains approximately 8.6 green beads. Cross correlation decays are shown for channels 1-5 in panel A; 6-10 in B; and 11-15 in C. The legend displays the color scale for the G(0) amplitudes (z axis data) and the y axis specifies the specific cross correlation term (see text for further description).