

***In vitro* Standard Probe for Fluorescence Cross-correlation Spectroscopy (FCCS)**

A comprehensive manual

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488/633 standard: order# 5-0000-504
488/543 standard: order# 5-0000-604

1. Introduction

1.1 General Considerations

Fluorescence correlation spectroscopy (FCS) gives information about processes, which generate temporal fluctuations of the measured fluorescence in a sample of fluorescent molecules. In the easiest case, fluctuations are caused by the Brownian motion of fluorescent molecules in the observation volume giving information about translational diffusion coefficients.

Since the introduction of FCS in the 1970s⁽¹⁻³⁾ it has also been used to determine rotational diffusion coefficients, kinetic rate constants, aggregation and molecular weights⁽⁴⁻¹²⁾. With the implementation of confocal optics and the development of commercial devices, FCS has found a broad practice in biological questions like binding states of biomolecules, enzyme activities up to cellular applications. Unfortunately, detecting binding events of biomolecules by autocorrelation analysis lacks high sensitivity due to dependence on decelerated translational diffusion by increase of the molecular mass.

A significant extension of FCS is the dual-colour cross-correlation analysis (FCCS)⁽¹³⁻²⁰⁾, which is based on the simultaneous detection of two spectrally separable colours by splitting the emitted fluorescence into two distinct detection channels using adequate dichroic mirrors and filters.

Cross-correlation analysis implies the time correlation of the fluorescent signals in the two detection channels with each other delivering information about molecules which carry both fluorescent dyes. FCCS has been used in various binding studies of biomolecules *in vitro* and *in vivo*.

With the IBA *In Vitro* Standard Probe for Fluorescence Cross-correlation Spectroscopy it is now possible to verify the daily adjustment of the optical setup for fluorescence cross-correlation measurements.

Necessary Materials for Measurements

2. Starting point methods

2.1. General considerations

The instructions given below represent typical protocols that were applied successfully *in vitro*, in solution using different setups for fluorescence cross-correlation spectroscopy. Optimal conditions depend essentially on accurate adjustment of the confocal detection system. Thus, an optimum may be found in overlap of the laser foci and the respective detection foci, and additionally overlap of the two distinct detection foci. It may be helpful to adjust the optical setup for maximal cross-talk to the second detection channel using the solely green labelled sample excited only by the green laser, at the expense of the overlap of laser and detection foci of the second channel (especially recommended for older Zeiss Confocor systems).

2.2 Standard protocols for *in vitro* cross-correlation measurements

Materials and important notes

- 1 vial containing 50 μl double labeled Standard
- 2 vials containing 50 μl single labeled standard (1st and 2nd channel)
- Choose appropriate beam path for the FCCS 488/633 or 488/543 standard probe with excitation wavelengths 488/633 or 488/543, respectively.
- Vortex the cross-correlation standard probe before use.
- Store the cross-correlation standard probe at 4°C or on ice.
- Do not reuse aliquots of the standard probe after cross-correlation measurements.
- Verify the obtained cross-correlation signal by checking the channel separation of the optical setup for cross-talk using the single labelled samples obtained by IBA.

Standard protocol for cross-correlation measurements *in vitro*

1. Adjust the optical setup for optimal overlap of the two detection foci.
2. Dilute the cross-correlation standard probe 1:10 with d.d. water, place 20 μl on a cover slip and leave it for 1 min.
3. Perform cross-correlation measurements.
4. Adjustment of the optical setup for detection of cross-correlation may be disordered due to temperature variations. Check the proper adjustment by performing cross-correlation measurements using a new aliquot of the cross-correlation standard probe every few hours.

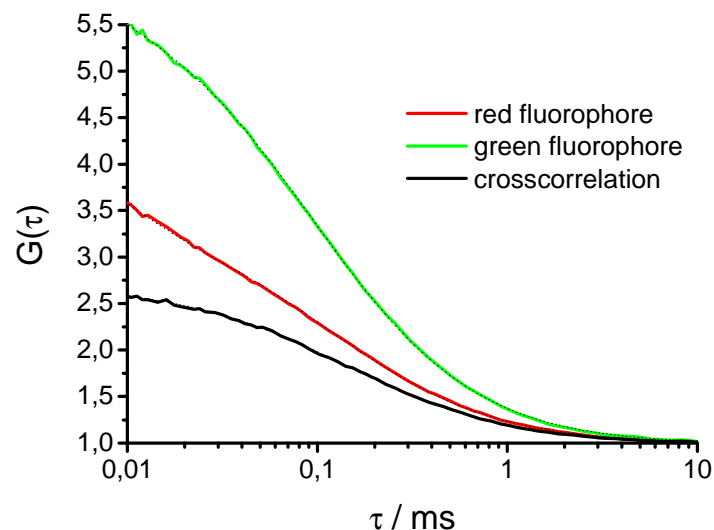


Diagram 1: Exemplary cross-correlation measurement using the IBA *In Vitro* FCCS Standard Probe 488/633. The cross-correlation signal mainly depends on proper adjustment of the optical setup.

Related products

***In Vivo* Standard Probe for Fluorescence Cross-correlation Spectroscopy**

488/633 in vivo standard: order# 5-0000-505

488/543 in vivo standard: order# 5-0000-605

References

1. Madge, D., Elson, E.L. and Webb, W.W. (1972). Thermodynamic fluctuations in a reacting system-measurement by fluorescence correlation spectroscopy. *Phys. Rev. Lett.* 29:705-708.
2. Elson, E.L. and Madge, D. (1974). Fluorescence correlation spectroscopy I. Conceptual basics and theory. *Biopolymers* 13:1-27.
3. Madge, D., Elson, E.L. and Webb, W.W. (1974). Fluorescence correlation spectroscopy. II. An experimental realization. *Biopolymers* 13: 29-61.
4. Brock, R. and Jovin, T.M. (2001). Fluorescence correlation microscopy (FCM): Fluorescence correlation spectroscopy (FCS) in cell biology. *Fluorescence Correlation Spectroscopy: Theory and Application* 65:132-161.
5. Elson, E.L. (2001). Fluorescence correlation spectroscopy measures molecular transport in cells [Review]. *Traffic* 2(11):789-796.
6. Williams, R.M., Zipfel, W.R. and Webb, W.W. (2001). Multiphoton microscopy in biological research [Review]. *Curr. Opin. Chem. Biol.* 5(5):603-608.
7. Hess, S.T., Huang, S.H., Heikal, A.A. and Webb W.W. (2002). Biological and chemical applications of fluorescence correlation spectroscopy: A review [Review]. *Biochem.* 41(3):697-705.
8. Thompson, N.L., Lieto, A.M. and Allen, N.W. (2002). Recent advances in fluorescence correlation spectroscopy [Review]. *Curr. Opinion Struct. Biol.* 12(5):634-641.
9. Hink, M.A. Borst, J.W. and Visser, A.J.W.G. (2003). Fluorescence correlation spectroscopy of GFP fusion proteins in living plant cells [Review]. *Biophotonics* 361:93-112.
10. Muller JD. Chen Y. Gratton E. (2003). Fluorescence correlation spectroscopy [Review]. *Biophotonics* 361:69-92.
11. Gosch M. and Rigler R. (2005). Fluorescence correlation spectroscopy of molecular motions and kinetics [Review]. *Advanced Drug Delivery Reviews.* 57(1):169-190.
12. Vukojevic, V., Pramanik, A., Yakovleva, T., Rigler, R., Terenius, L. and Bakalkin, G. (2005). Study of molecular events in cells by fluorescence correlation spectroscopy [Review]. *Cell Mol. Life Sci.* 62(5):535-550.
13. Eigen, M. and Rigler, R. (1994). Sorting single molecules: Application to diagnostics and evolutionary biotechnology. *Proc. Natl. Acad. Sci. USA* 91: 5740-5747.
14. Kettling, U., Koltermann, A., Schwille, P. and Eigen, M. (1998). Real-time enzyme kinetics monitored by dual-color fluorescence cross-correlation spectroscopy. *Proc. Natl. Acad. Sci. USA* 95:1416-1420.
15. Schwille P. (2001). Cross-correlation analysis in FCS. *Fluorescence Correlation Spectroscopy: Theory and Application* 65:360-378.
16. Weidemann, T., Wachsmuth, M., Tewes, M., Rippe, K. and Langowski, J. (2002). Analysis of Ligand Binding by Two-Colour Fluorescence Cross-Correlation Spectroscopy. *Single Mol.* 3:49-61.
17. Berland, K.M. (2004). Detection of specific DNA sequences using dual-color two-photon fluorescence correlation spectroscopy. *J. Biotech.* 108(2):127-136.
18. Elson, E.L. (2004). Quick tour of fluorescence correlation spectroscopy from its inception. *J. Biomed. Opt.* 9(5):857-864.
19. Thews, E., Gerken, M., Eckert, R., Zapfel, J., Tietz, C. and Wrachtrup, J. (2005). Cross talk free fluorescence cross correlation spectroscopy in live cells. *Biophys. J.* 89(3):2069-2076.
20. Gosch, M., Blom, H., Anderegg, S., Korn, K., Thyberg, P., Wells, M., Lasser, T. and Rigler, R. (2005). Parallel dual-color fluorescence cross-correlation spectroscopy using diffractive optical elements. *J. Biomed. Opt.* 10(5):54008.