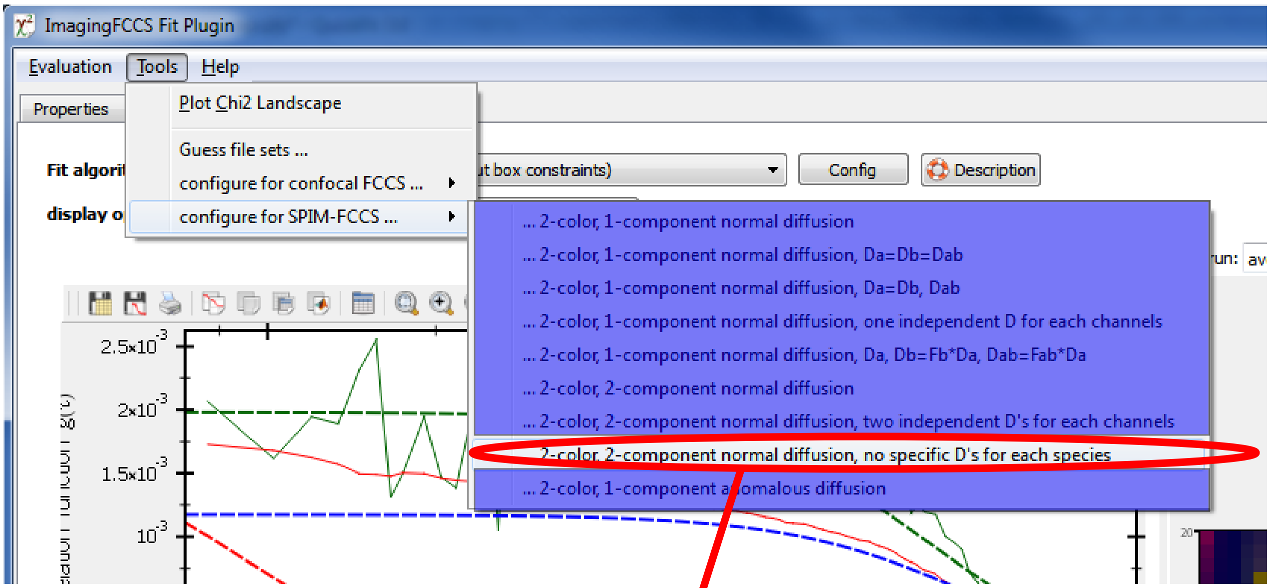
new imFCCS fit models



new models two D‘s per channel

all other models: one/two D‘s per species

old 1- or 2-component models:

The $G_{gr}^{X}(t;\, D_x,\, D_{x2})$ functions are the ACFs (rr/gg) or CCFs (gr) for a species X with 1 or two diffusing components. These functions contain the focus-shift terms, the different focal volumes (one for red obe fro green, one for green-red), the anomality (for anomalous models) ...

$$g_{gg}(\tau) = \frac{\eta_g^2 G_{gg}^{A}(\tau; D_A) + \eta_g^2 G_{gg}^{AB}(\tau; D_{AB})}{\eta_g^2 \cdot (c_A + c_{AB})^2} \qquad (1)$$

$$g_{rr}(\tau) = \frac{\eta_r^2 \cdot \left[G_{rr}^{B}(\tau; D_B) + G_{rr}^{AB}(\tau; D_{AB})\right] + \kappa_{gr}^2 \eta_g^2 \cdot \left[G_{gg}^{A}(\tau; D_A) + G_{gg}^{AB}(\tau; D_{AB})\right] + 2\kappa_{gr}\eta_r\eta_g G_{gr}^{AB}(\tau; D_{AB})}{(\kappa_{gr}\eta_g c_A + (\eta_r + \kappa_{gr}\eta_g) \cdot c_{AB} + \eta_r c_B)^2} \qquad (2)$$

$$g_{gr}(\tau) = g_{rg}(\tau) = \frac{\eta_g\eta_r G_{gr}^{AB}(\tau; D_{AB}) + \kappa_{gr}\eta_g\eta_r G_{gr}^{A}(\tau; D_A) + \kappa_{gr}\eta_g^2 \cdot G_{gg}^{AB}(\tau; D_{AB})}{(\eta_g c_A + \eta_g c_{AB}) \cdot (\kappa_{gr}\eta_g c_A + (\eta_r + \kappa_{gr}\eta_g) c_{AB} + \eta_r c_B)} \qquad (3)$$

new simplified model:

now there is only one correlation function $G_{gr}(t;\, D_1^{gr},\, D_2^{gr})$ for each channel (gg,rr,gr), which does NOT depend on any species, so D_1^{g}, D_2^{gr} are effectivediffusion coefficient for each channel, which can only indirectly be relatied to the species.

$$g_{gg}(\tau) = \frac{\eta_g^2 c_A + \eta_g^2 c_{AB}}{\eta_g^2 \cdot (c_A + c_{AB})^2} \cdot G_{gg}(\tau; D_1^{gg}, D_2^{gg}) \qquad (10)$$

$$g_{rr}(\tau) = \frac{\eta_r^2 \cdot \left[c_B + c_{AB}\right] + \kappa_{gr}^2\eta_g^2 \cdot \left[c_A + c_{AB}\right] + 2\kappa_{gr}\eta_r\eta_g c_{AB}}{\left(\kappa_{gr}\eta_g c_A + (\eta_r + \kappa_{gr}\eta_g) \cdot c_{AB} + \eta_r c_B\right)^2} \cdot G_{rr}(\tau; D_1^{rr}, D_2^{rr}) \qquad (11)$$

$$g_{gr}(\tau) = g_{rg}(\tau) = \frac{\eta_g\eta_r c_{AB} + \kappa_{gr}\eta_g\eta_r c_A + \kappa_{gr}\eta_g^2 \cdot c_{AB}}{(\eta_g c_A + \eta_g c_{AB}) \cdot (\kappa_{gr}\eta_g c_A + (\eta_r + \kappa_{gr}\eta_g) c_{AB} + \eta_r c_B)} \cdot G_{gr}(\tau; D_1^{gr}, D_2^{gr}) \qquad (12)$$

Foo etal. 2012:

COLORING:
* $1/(N_A V_x)$ is implicit in the $G_{xy}(t;D)$
* the red terms depend on V_r (G_{rr})
* the green terms depend on V_g (G_{gg})
* the blue terms depend on the apparent FCCS-volume V_{gr} (G_{gr})

=> crosstalk comes from the red / green-red volume (not the green, as above!)

$$g_G(0)=\frac{(q_g\eta_{g,G}+q_r\eta_{r,G})^2C_{gr}+\eta_{g,G}{}^2C_g+\eta_{r,G}{}^2C_r}{N_AV_G\left[(q_g\eta_{g,G}+q_r\eta_{r,G})C_{gr}+\eta_{g,G}C_g+\eta_{r,G}C_r+B_G/N_AV_G\right]^2}, \quad \text{(S8)}$$

$$g_R(0)=\frac{(q_g\eta_{g,R}+q_r\eta_{r,R})^2C_{gr}+\eta_{g,R}{}^2C_g+\eta_{r,R}{}^2C_r}{N_AV_R\left[(q_g\eta_{g,R}+q_r\eta_{r,R})C_{gr}+\eta_{g,R}C_g+\eta_{r,R}C_r+B_R/N_AV_R\right]^2}, \quad \text{(S9)}$$

$$g_x(0)=\frac{(q_g\eta_{g,G}+q_r\eta_{r,G})(q_g\eta_{g,R}+q_r\eta_{r,R})C_{gr}+\eta_{g,R}\eta_{g,R}C_g+\eta_{r,G}\eta_{r,R}C_r}{N_AV_{GR,app}\left[(q_g\eta_{g,G}+q_r\eta_{r,G})C_{gr}+\eta_{g,G}C_g+\eta_{r,G}C_r+B_G/N_AV_G\right]}. \quad \text{(S10)}$$
$$\times\left[(q_g\eta_{g,R}+q_r\eta_{r,R})C_{gr}+\eta_{g,R}C_g+\eta_{r,R}C_r+B_R/N_AV_R\right]^{-1}$$

my model (full)

$$g_{gg}(\tau)=\frac{\eta_g^2G_{gg}^A(\tau;D_A)+\eta_g^2G_{gg}^{AB}(\tau;D_{AB})}{\eta_g^2\cdot(c_A+c_{AB})^2} \quad (1)$$

$$g_{rr}(\tau)=\frac{\eta_r^2\cdot\left(G_{rr}^B(\tau;D_B)+G_{rr}^{AB}(\tau;D_{AB})\right)+\kappa_{gr}^2\eta_g^2\left[G_{gg}^A(\tau;D_A)+G_{gg}^{AB}(\tau;D_{AB})\right]+2\kappa_{gr}\eta_r\eta_gG_{gr}^{AB}(\tau;D_{AB})}{\left(\kappa_{gr}\eta_gc_A+(\eta_r+\kappa_{gr}\eta_g)\cdot c_{AB}+\eta_rc_B\right)^2} \quad (2)$$

$$g_{gr}(\tau)=g_{rg}(\tau)=\frac{\eta_g\eta_rG_{gr}^{AB}(\tau;D_{AB})+\kappa_{gr}\eta_g\eta_rG_{gr}^A(\tau;D_A)+\kappa_{gr}\eta_g^2\cdot G_{gg}^{AB}(\tau;D_{AB})}{(\eta_gc_A+\eta_gc_{AB})\cdot(\kappa_{gr}\eta_gc_A+(\eta_r+\kappa_{gr}\eta_g)c_{AB}+\eta_rc_B)} \quad (3)$$

=> crosstalk comes from the green volume!

=> crosstalk comes from the red / green-red volume (not the green, as above!)

my model (simplified):

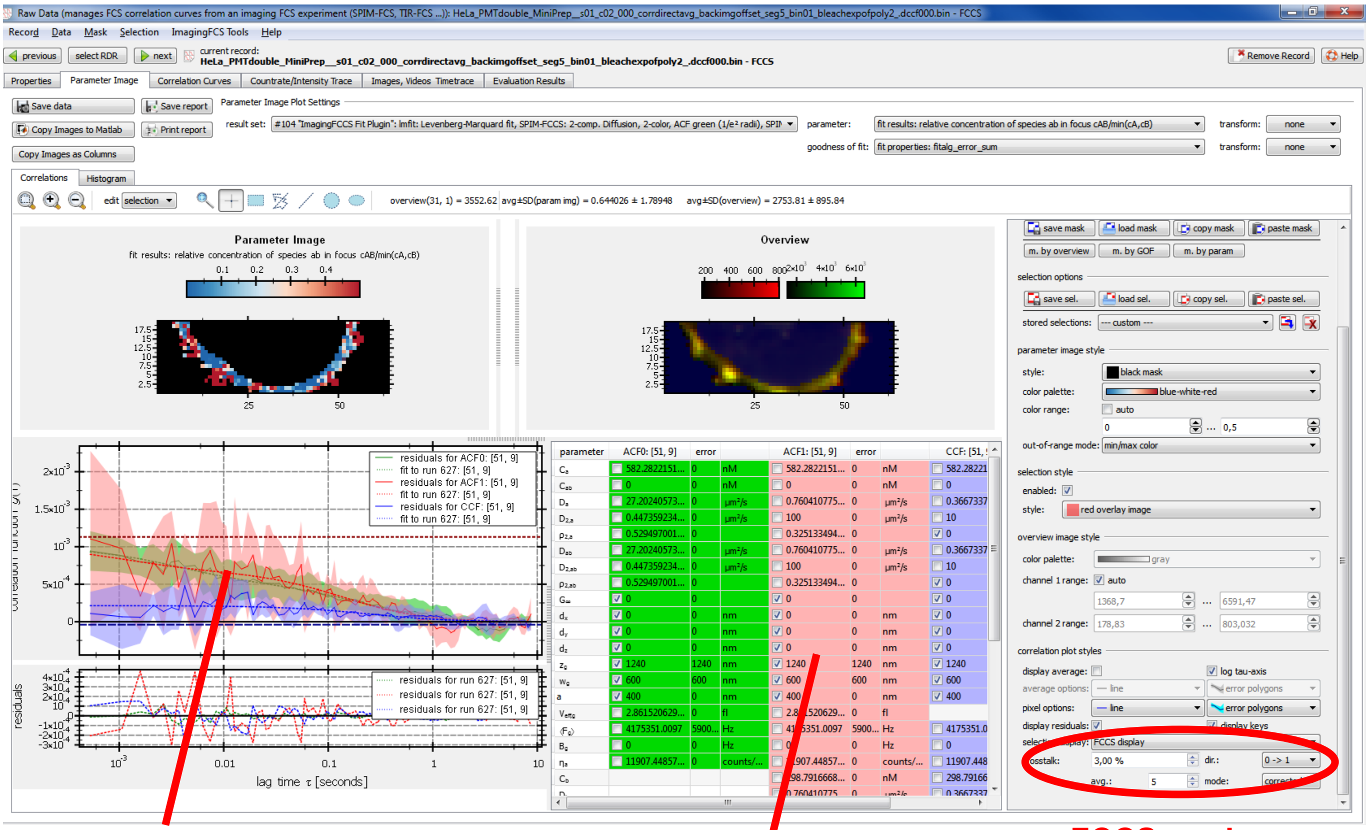
$$g_{gg}(\tau)=\frac{\eta_g^2c_A+\eta_g^2c_{AB}}{\eta_g^2\cdot(c_A+c_{AB})^2}\cdot G_{gg}(\tau;D_1^{gg},D_2^{gg}) \quad (10)$$

$$g_{rr}(\tau)=\frac{\eta_r^2\cdot[c_B+c_{AB}]+\kappa_{gr}^2\eta_g^2\cdot[c_A+c_{AB}]+2\kappa_{gr}\eta_r\eta_gc_{AB}}{\left(\kappa_{gr}\eta_gc_A+(\eta_r+\kappa_{gr}\eta_g)\cdot c_{AB}+\eta_rc_B\right)^2}\cdot G_{rr}(\tau;D_1^{rr},D_2^{rr}) \quad (11)$$

$$g_{gr}(\tau)=g_{rg}(\tau)=\frac{\eta_g\eta_rc_{AB}+\kappa_{gr}\eta_g\eta_rc_A+\kappa_{gr}\eta_g^2\cdot c_{AB}}{(\eta_gc_A+\eta_gc_{AB})\cdot(\kappa_{gr}\eta_gc_A+(\eta_r+\kappa_{gr}\eta_g)c_{AB}+\eta_rc_B)}\cdot G_{gr}(\tau;D_1^{gr},D_2^{gr}) \quad (12)$$

these are the same, up to
* the background-term B_x/N_AV_x which is already corrected for before correlation
* your's is written for the amplitudes and has an overall factor of $1/(N_AV_x)$, which is implicitly contained in my correlation functions $G_x(t; D_1,D_2)$
* you measured the intensities eta... separately, I estimate them from the measurement itself, by setting $eta_g=I_g/(c_A+c_{AB})$ and $eta_r=(I_r\text{-kappa}*I_g)/(c_B+c_{AB})$. So the remaining assumtion is that the green dye has the same brightness in the dimeric and monomeric form (so especially there is no FRET ...). The crosstalk factor kappa is determined separately here too.

FCCS-display mode in imagingFCS RDR



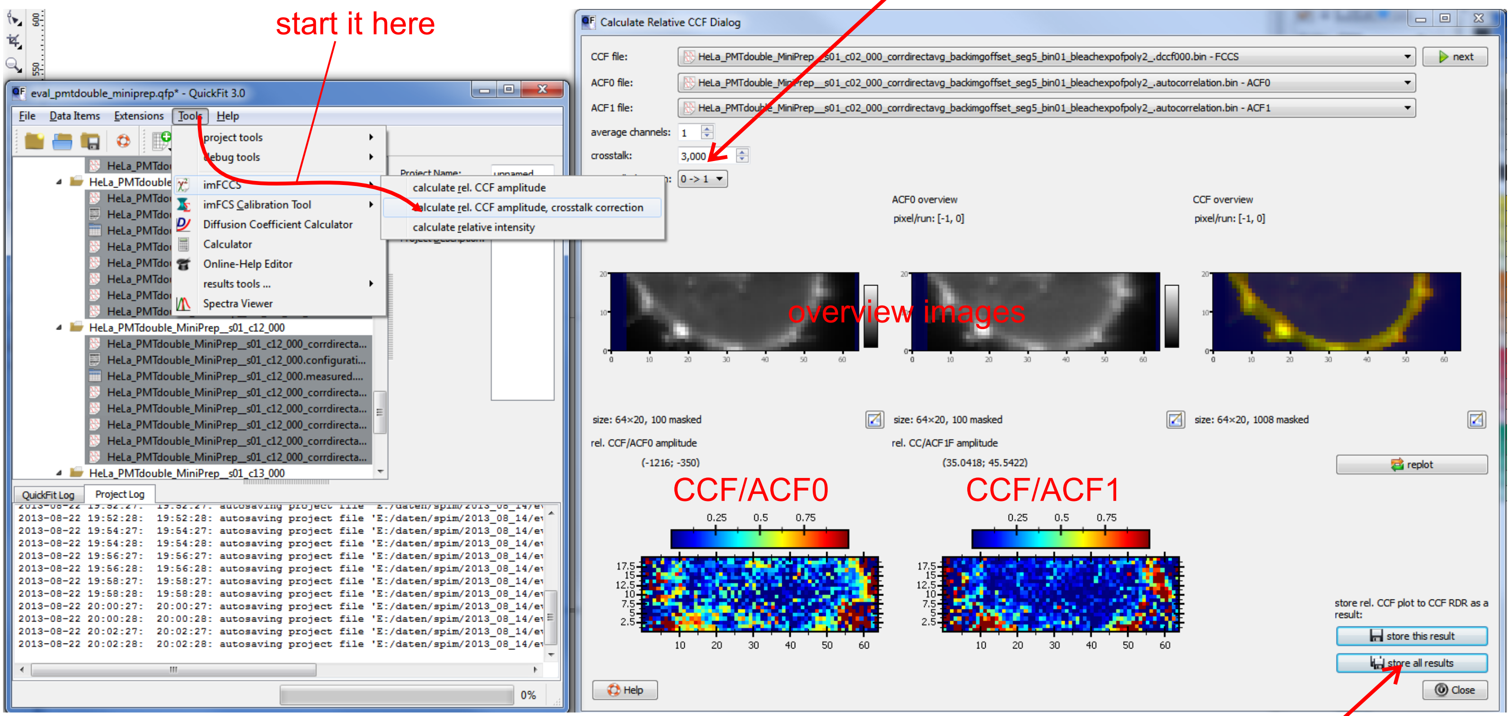
ACF0+ACF1+FCCS
hor. lines indicate crosstalk-corrected amplitudes of FCCS (blue) and ACF1 (red)

result statistics for
ACF0+ACF1+FCCS

FCCS mode

Tool for crosstalk-corrected relative CCF amplitudes

enter
- crosstalk
- CF channels to average for amplitude
- crosstalk direction



apply evaluation to all records AFTER the current record and store results.

The results are available in the imagingFCS RDR as their own result set! (as any other fit results!)

amplitudes are calculated as average over the first few CF channels!

equations for correction from:
Bacia, Petrasek, Schwille, CHemPhysChem 13(5), 2012

$$\hat{G}_g = G_g$$

$$\hat{G}_r = \frac{\kappa^2 F_g^2 G_g + F_r^2 G_r - 2\kappa F_g F_r X}{(F_r - \kappa F_g)^2}$$

$$\hat{X} = \frac{-\kappa F_g G_g + F_r X}{F_r - \kappa F_g}$$