

# Standard correlation settings

select a file, make sure to choose the „tinyTIFF“ fileformat!!!

pixel size should be  $400 \times 400 \text{ nm}^2$   
for our SPIM!

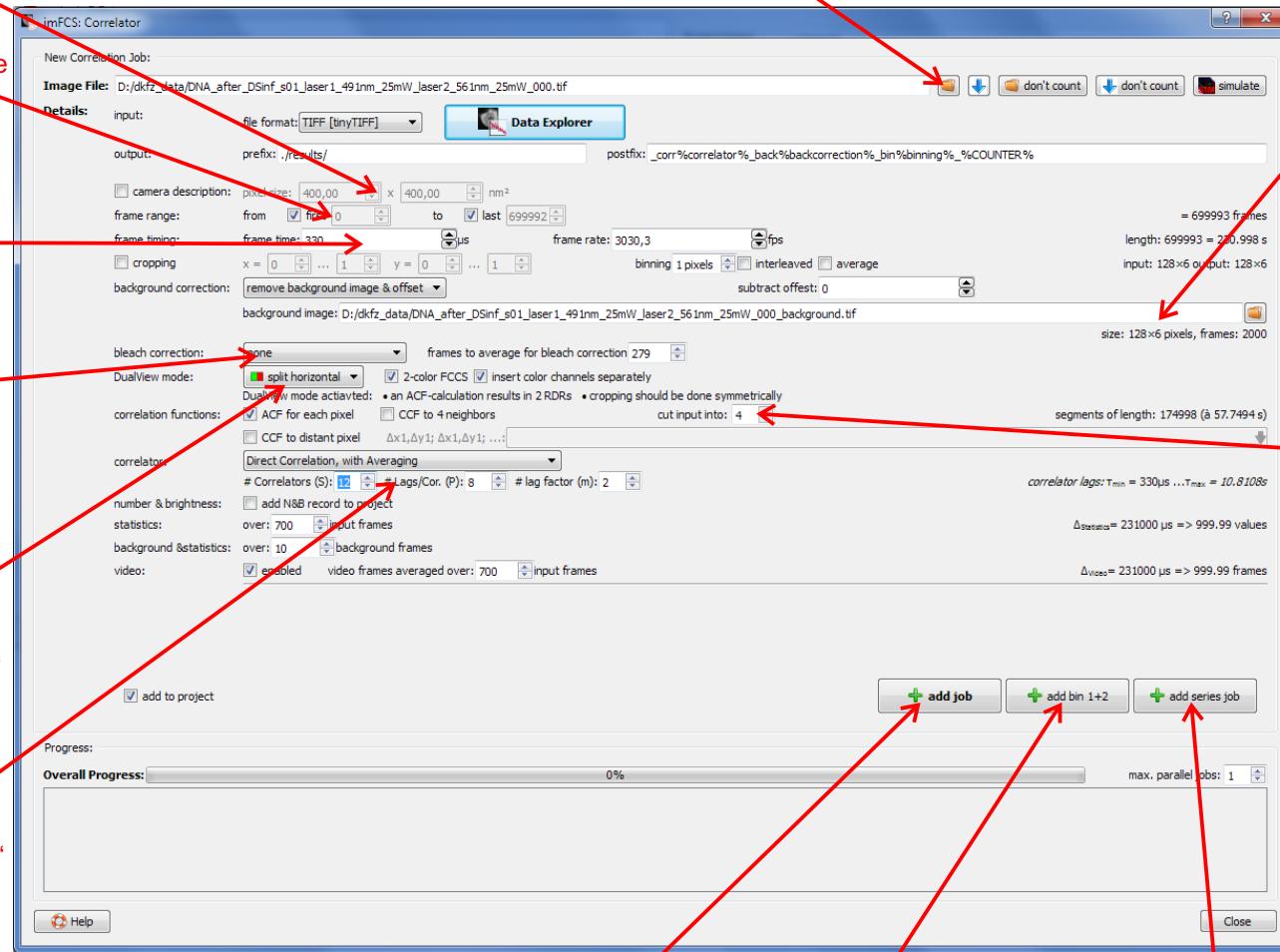
you can correlate only a subrange of the  
frames in the file, e.g. 0..74999 for the  
first half of a file with 150000 frames!

frame time should be between 300 $\mu\text{s}$   
and 1100 $\mu\text{s}$ , depending on the settings  
you used.

bleach correction:

- beads: NONE
- DNA...: „1-exponential LM-fit“
- cells: try „1-exponential LM-fit“ or  
„1-exponential (poly2) LM-fit“

for FCCS measurements:  
make sure DualView mode is „split hor.“



- use „Direct Correlation with Averaging“  
 $m=2$  and  $P=8$
- set  $S$ , so  $\tau_{\max} <$  segment length and  
 $\tau_{\max} \sim 5\text{-}10\text{s}$

add a coorelation  
job with the current  
settings

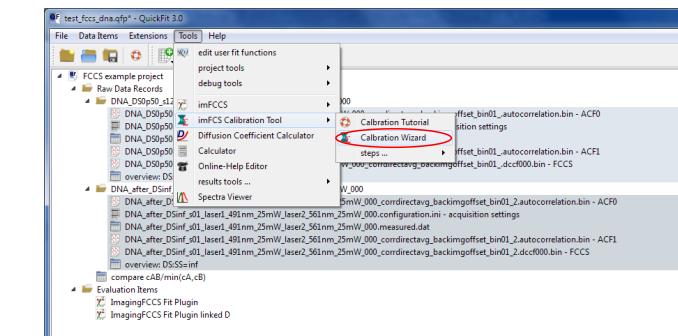
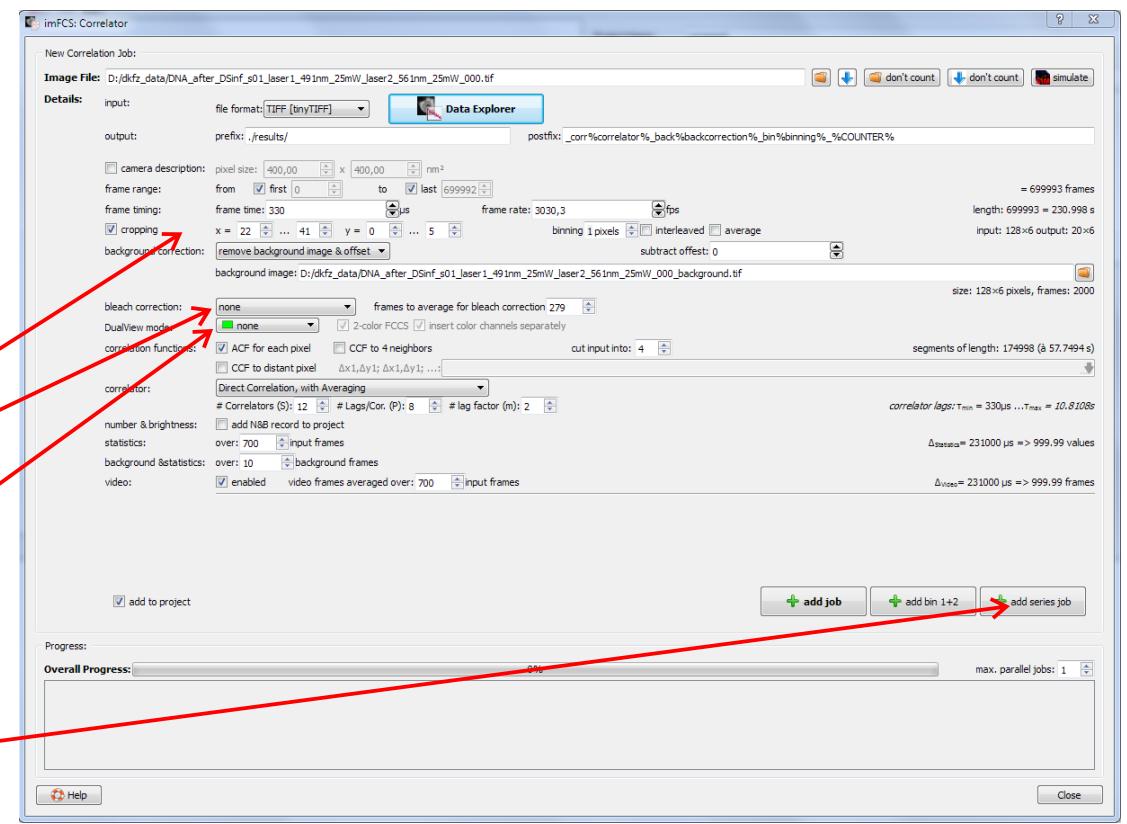
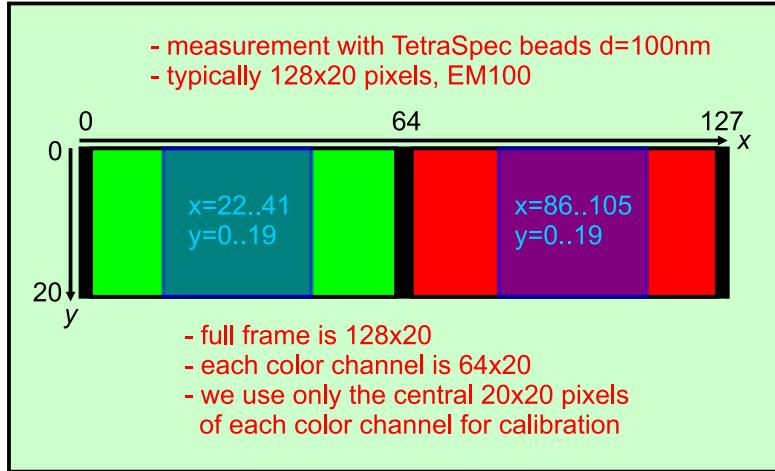
add two coorelation  
jobs with the current  
settings and no binning  
+ 2x2 binning

select a range of binning  
settings and add a correlation  
job for each!  
(use for calibration: bin 1-5)

if background image is corrupted  
(red error message), select another  
one, e.g. from the prev. or next  
dataset ... that's usually OK!

cut into 3-5 segments  
(segements shouldn't be  
much shorter than 15s)

# Correlation settings for Focal Volume Calibration

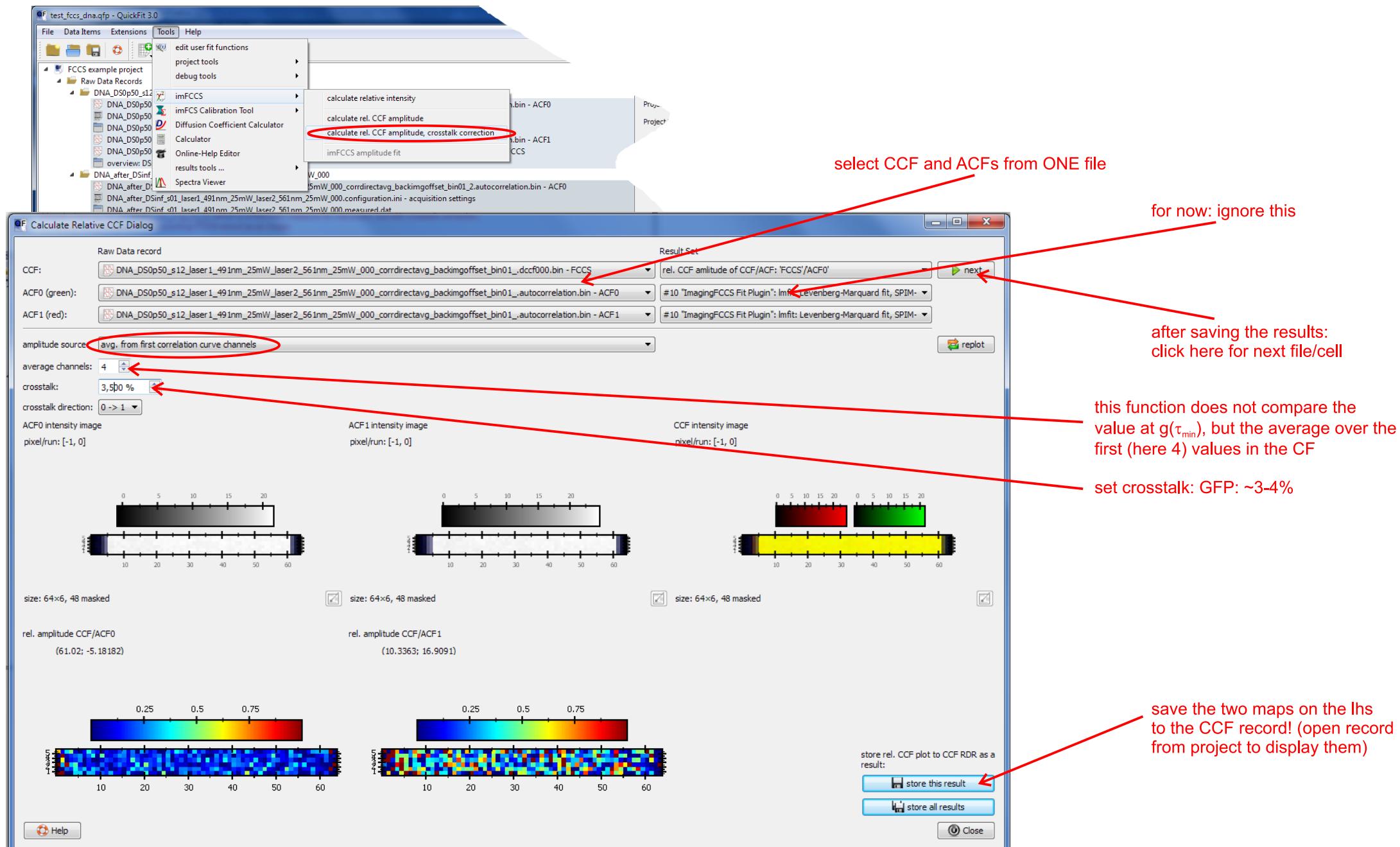


then follow steps in the imFCS Calibration wizard

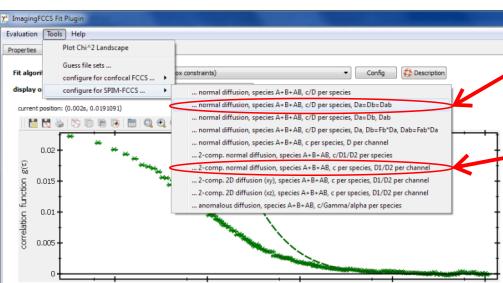
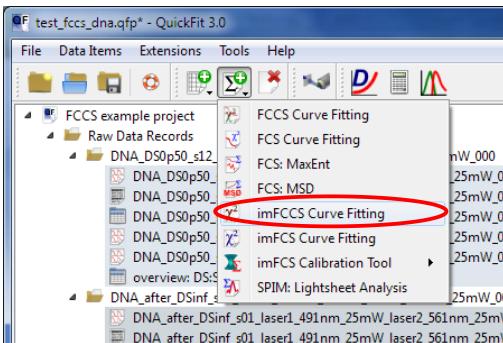
1. add fit objects, as the  $w_{xy}$  is typically  $\sim 600\text{nm}$  ( $1/e^2$ ) I choose test sizes of  $\sigma_{xy} = w_{xy}/2 = 200, 300, 400$  (now  $1/\sqrt{e}$  widths!!!, as model used for calibratio is typically  $1/\sqrt{e}$ )  
set  $\sigma_z = w_z/2$  to the value obtained from the bead scan (<http://www.dkfz.de/Macromol/quickfit/beadscan.html>)
2. perform fits ( $\sigma_{xy}$  and  $\sigma_z$  are fixed, D and c are free parameters) -> gives curves of D(a), pixel-width: a=initial\_pixel\_size\*binning
3. collect D-curves, select a D for further fits at high binning (usually a=2000nm=5\*400nm), curves should converge from above and below to an average D-value
4. add fit, now D and  $\sigma_z$  are fixed,  $\sigma_{xy}$  is free parameter
5. collect  $\sigma_{xy}$  data, typically I use the value of  $\sigma_{xy}$  at a=400nm, as this is the best estimate ... this value can be read from the table with thre results created in the project!

# Calculate crosstalk-corrected relative correlation amplitude

this calculates  $g_{CCF}(\tau_{min}) / \min(g_{green}(\tau_{min}), g_{red}(\tau_{min}))$ , which is a first basic measure for interaction, but with crosstalk correction, the results can be viewed in the according FCCS-record as an image



# Global imFCCS curve fitting



good for dimer/IRE/DNAS standard sample, as this assumes that all species (A,B,AB) have the same diffusion coefficient (i.e. approx. the same size)

good for more complex samples, as this assumes that there are 3 species (A,B,AB), but no assumptions on the diffusion coefficients is made, The model uses two-component diffusion for each channel separately, so the Ds can NOT be easily assigned to any specific species ... it's more like an effective D, when averaging over all available species!

if (for any reason) there is only one component in any channel, you can always set  $D_2=10$ ,  $r_2=0$  and fix both parameters, which will effectively result in a 1-component fit!

set  $1/e^2$  height of green focus here (from beadscan)

set  $1/e^2$  width of green focus here (from calibration)

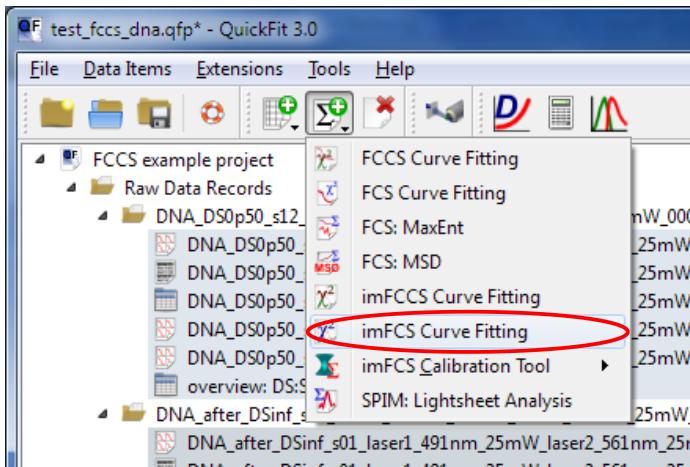
set crosstalk coefficient here (3% = 0.03)

set  $1/e^2$  height of red focus here (from beadscan)

set  $1/e^2$  width of red focus here (from calibration)

parameter	value	error	unit	fix	global	value	error	unit	fix	global	value	error	unit	fix	global
file	DNA_DS0p50_s12_laser1_491nm...autocorrelation.bin - ACF0				DNA_DS0p50_s12_laser1_491nm...autocorrelation.bin - ACF1					DNA_DS0p50_s12_laser1_491nm...et_bin01_dccf000.bin - FCCS					
fit model	SPIM-FCCS: 2-comp. normal di...nel, ACF green ( $1/e^{1/2}$ radii)				SPIM-FCCS: 2-comp. normal di...nnel, ACF red ( $1/e^{1/2}$ radii)					SPIM-FCCS: 2-comp. normal dif...r channel, CCF ( $1/e^{1/2}$ radii)					
$C_a$	10	0	nM	<input checked="" type="checkbox"/>	global #0	10	0	nM	<input checked="" type="checkbox"/>	global #0	10	0	nM	<input checked="" type="checkbox"/>	global #0
$C_{ab}$	5	0	nM	<input checked="" type="checkbox"/>	global #2	5	0	nM	<input checked="" type="checkbox"/>	global #2	5	0	nM	<input checked="" type="checkbox"/>	global #2
$D_1$	10	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>		10	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>		10	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>	
$D_2$	100	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>		100	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>		10	0	$\mu\text{m}^2/\text{s}$	<input checked="" type="checkbox"/>	
$r_2$	0.5	0		<input checked="" type="checkbox"/>		0.5	0		<input checked="" type="checkbox"/>		0	0		<input checked="" type="checkbox"/>	
$G_\infty$	0	0		<input checked="" type="checkbox"/>		0	0		<input checked="" type="checkbox"/>		0	0		<input checked="" type="checkbox"/>	
$z_g$	1140	0	nm	<input checked="" type="checkbox"/>	global #9	1140	0	nm	<input checked="" type="checkbox"/>	global #9	1140	0	nm	<input checked="" type="checkbox"/>	global #9
$w_g$	540	0	nm	<input checked="" type="checkbox"/>	global #7	540	0	nm	<input checked="" type="checkbox"/>	global #7	540	0	nm	<input checked="" type="checkbox"/>	global #7
$a$	400	0	nm	<input checked="" type="checkbox"/>	global #11	400	0	nm	<input checked="" type="checkbox"/>	global #11	400	0	nm	<input checked="" type="checkbox"/>	global #11
$V_{eff,g}$	0.5	0	fL			0.5	0	fL			0.5	0	fL		
$\langle F_g \rangle$	57429.842	1400	Hz			57429.842	1400	Hz			57429.842	1400	Hz		
$B_g$	0	0	Hz			0	0	Hz			0	0	Hz		
$\eta_g$	0.5	0	counts/nM			0.5	0	counts/nM			0.5	0	counts/nM		
$CPM_g$	0.5	0	cpm			0.5	0	cpm			0.5	0	cpm		
$C_b$						10	0	nM	<input checked="" type="checkbox"/>	global #1	10	0	nM	<input checked="" type="checkbox"/>	global #1
$\kappa$						0.03	0		<input checked="" type="checkbox"/>	global #3	0.03	0		<input checked="" type="checkbox"/>	global #3
$d_x$						0	0	nm	<input checked="" type="checkbox"/>	global #4	0	0	nm	<input checked="" type="checkbox"/>	global #4
$d_y$						0	0	nm	<input checked="" type="checkbox"/>	global #5	0	0	nm	<input checked="" type="checkbox"/>	global #5
$d_z$						0	0	nm	<input checked="" type="checkbox"/>	global #6	0	0	nm	<input checked="" type="checkbox"/>	global #6
$z_r$						1150	0	nm	<input checked="" type="checkbox"/>	global #10	1150	0	nm	<input checked="" type="checkbox"/>	global #10
$w_r$						550	0	nm	<input checked="" type="checkbox"/>	global #8	550	0	nm	<input checked="" type="checkbox"/>	global #8
$V_{eff,r}$						0.5	0	fL			0.5	0	fL		
$\langle F_r \rangle$						62047.642	1100	Hz			62047.642	1100	Hz		

# imFCS curve fitting (each channel separately)



sometimes the simulated annealing algorithm is better  
(but also VERY SLOW)

good model to start with: 1-3 component normal diffusion

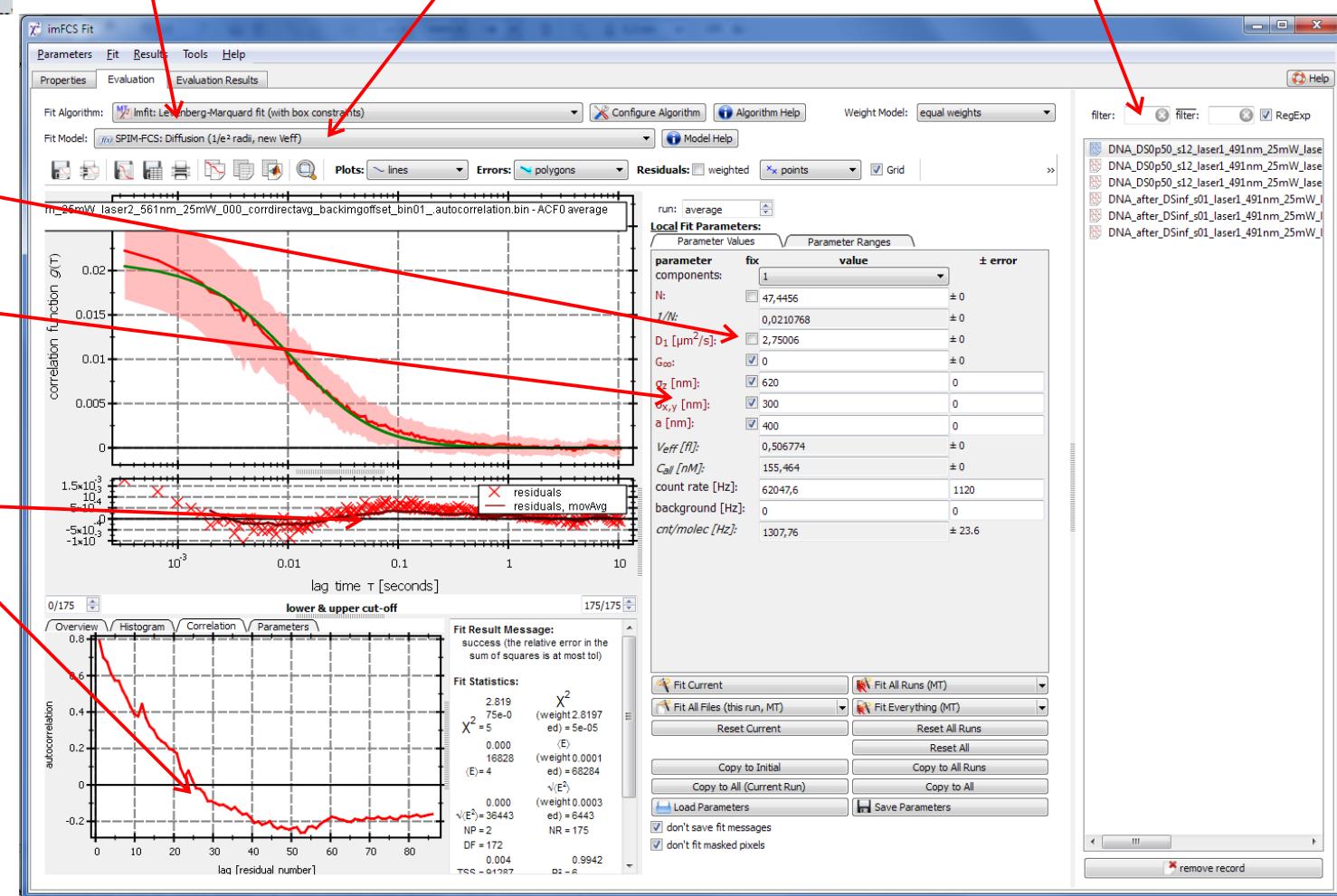
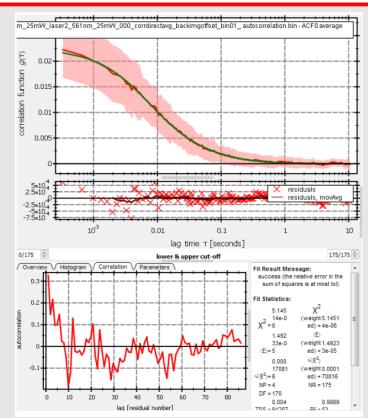
select number of components

put here the  $1/e^2$ -focus width (from SPIM-FCS calibration)  
and  $1/e^2$ -height (from beadscan) for the selected channel

**NOTE:** this should be  $w_z$ ,  $w_{xy}$  (will be fixed in  
next version), as the model is  $1/e^2$

S-shape of residuals: typically a second component  
is needed!

better results with  
2 components:

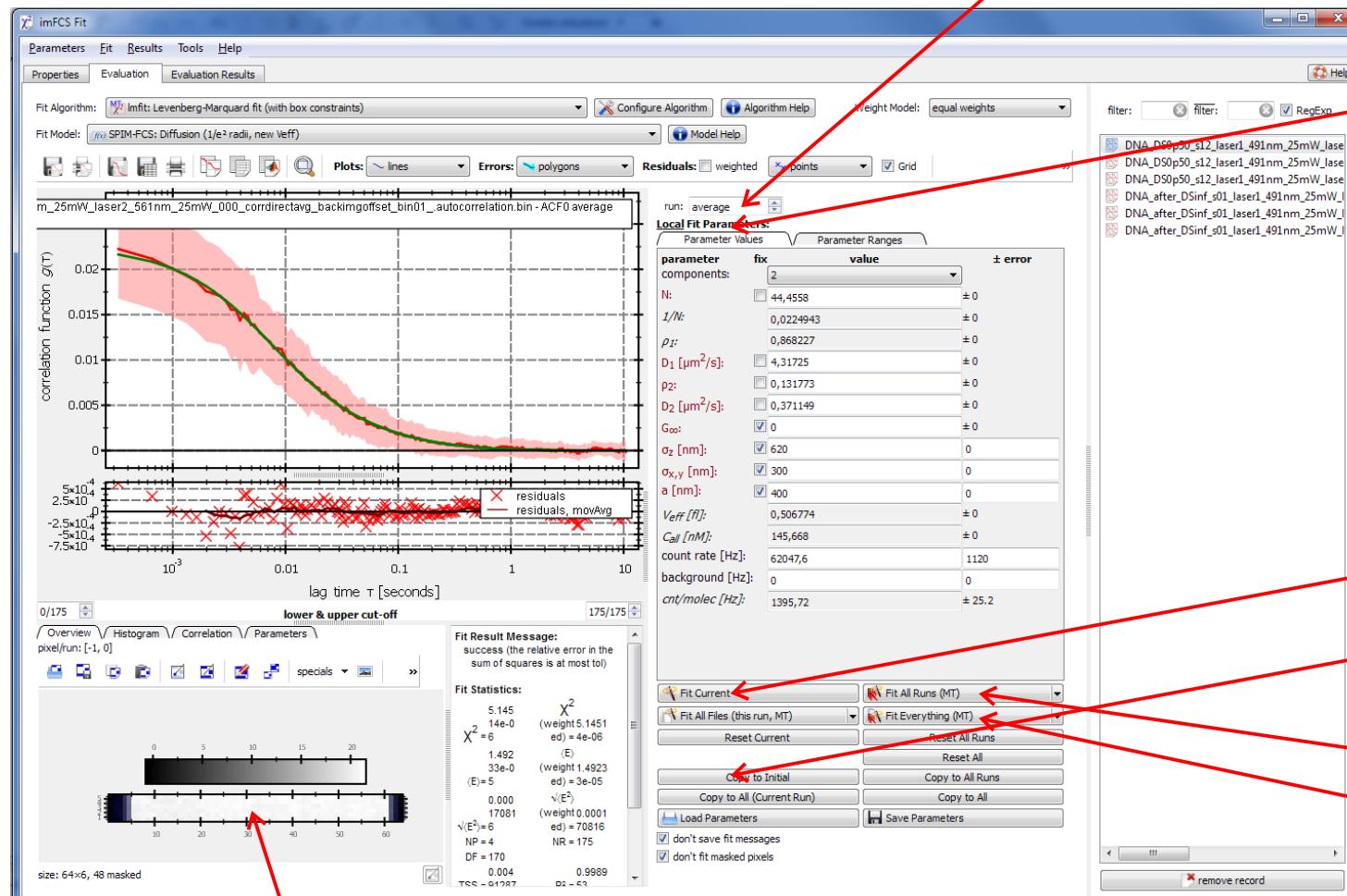


put here:

- „ACF0“: to filter list of files for green channel only
- „ACF1“: to filter for red channel
- „FCCS“: to filter for crosscorrelation functions only
- empty to switch off filtering (see image)

# imFCS curve fitting (each channel separately)

RUN = PIXEL !!! pixels are numbered (as runs) from bottom left, row-wise, run -1 = average over all non-masked pixels



global = initial values used for ALL pixels  
local = results after fit for the CURRENT pixel

click on any pixel to display the CF of this pixel

click to fit current pixel/currently displayed curve

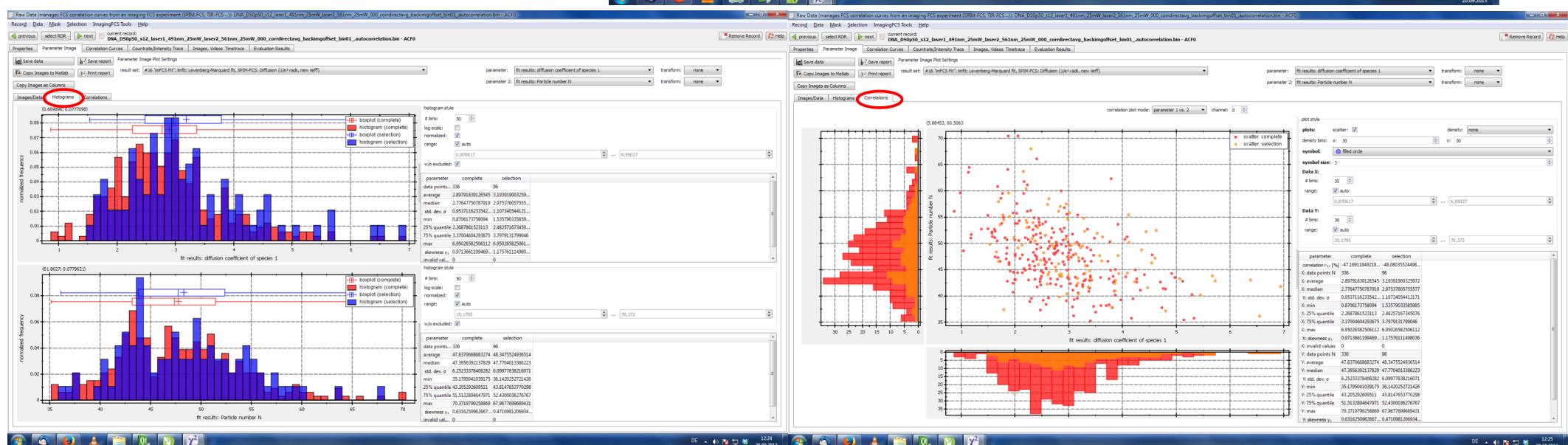
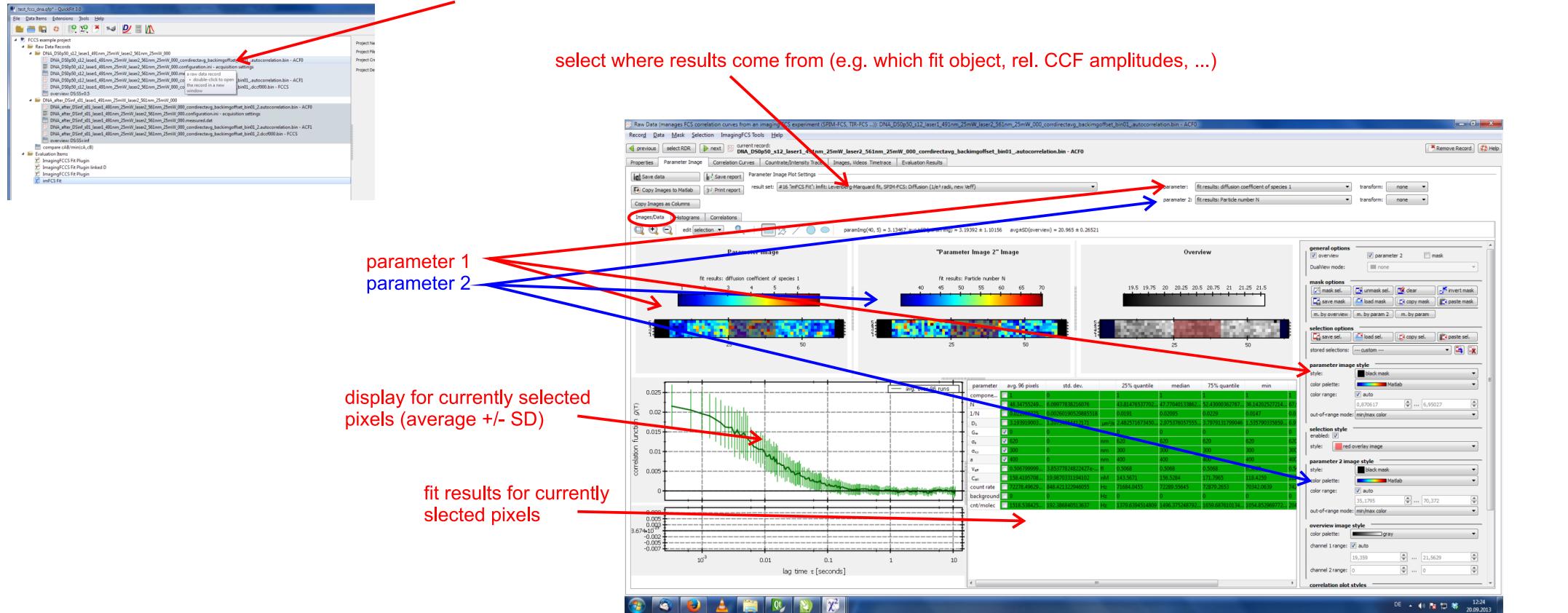
click to copy current results to the initial (global) values

fit all pixels in current file

fit all pixels in all files

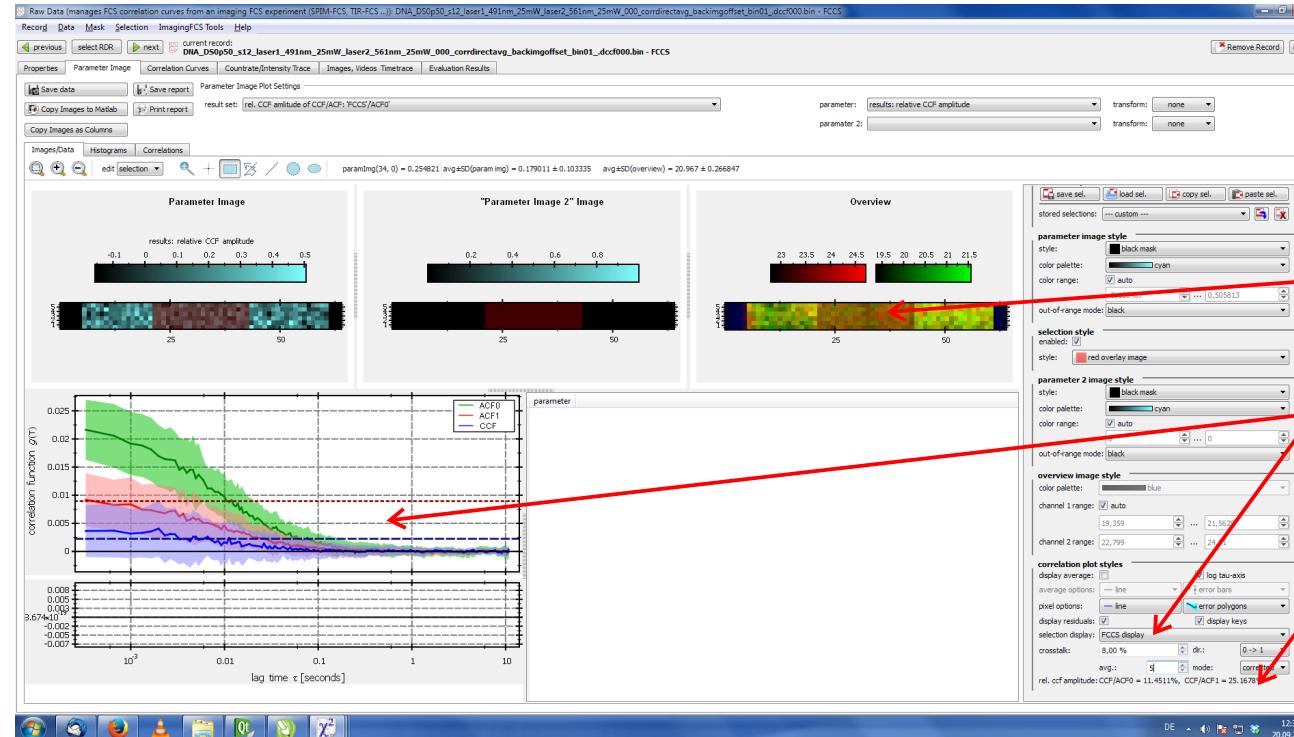
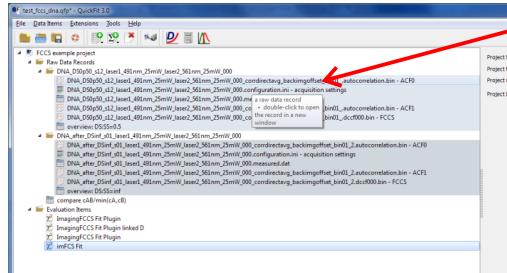
# displaying imFCS/imFCCS results as parameter images

double-click any imFCS record ( ) to open editor/display window



# fast overview of imFCCS results

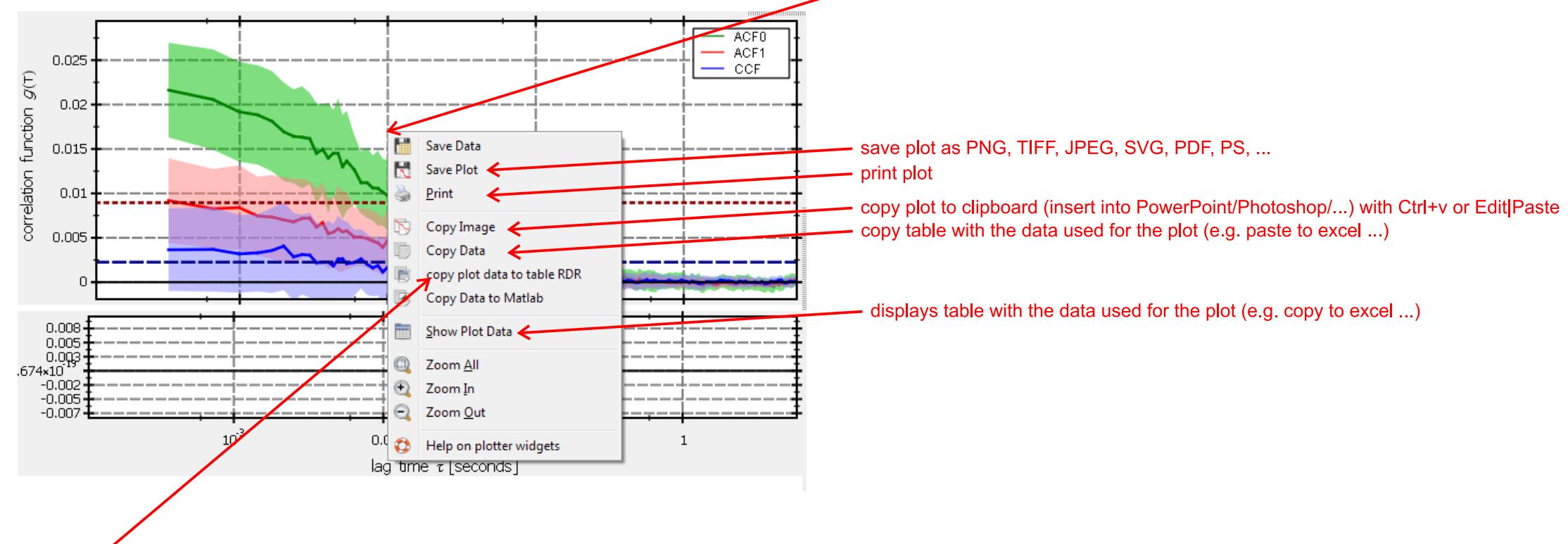
double-click a „.... - FCCS“ record ( ) to open editor/display window



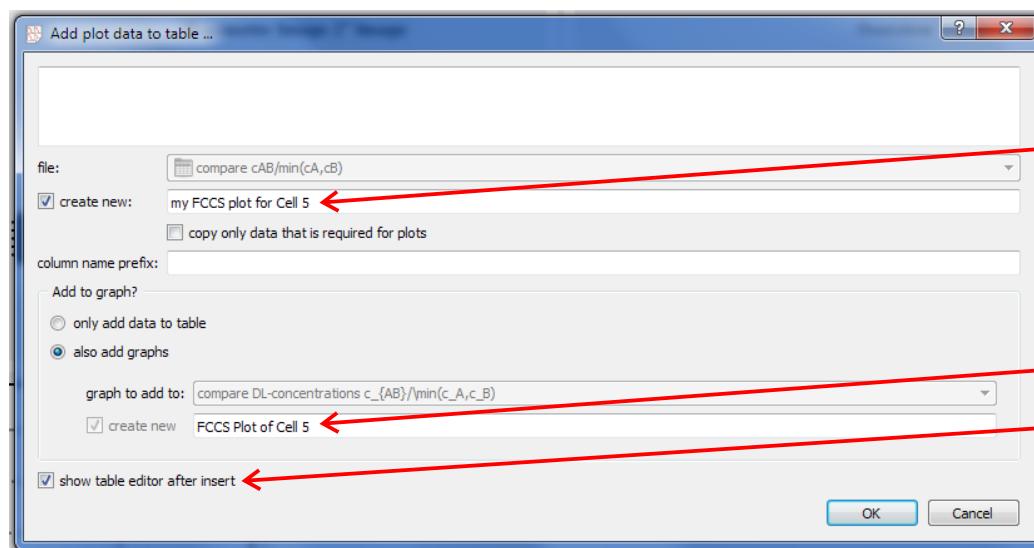
1. select a single or a range of pixels
2. set crosstalk and choose „FCCS display“ mode
3. plot shows ACFs + CCF and crosstalk-corrected CCF-amplitude as lines

4. numbers are given on bottom right

# Plots in QuickFit



1. add a new table-record to the current Qf3 project that contains the current plot (incl. a copy of the necessary data)
2. a new window will pop up:

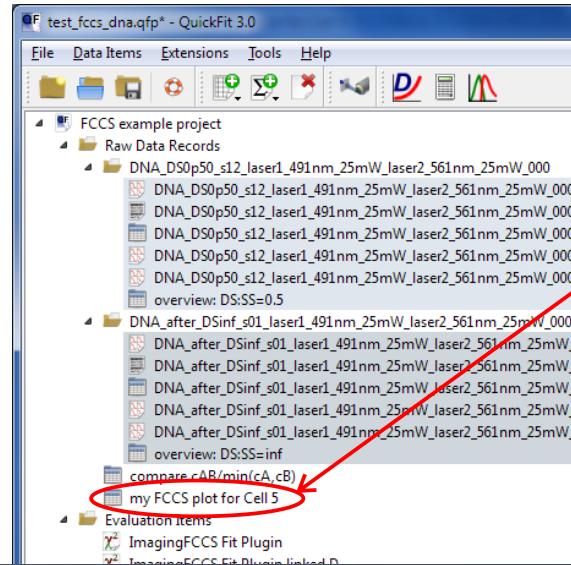


name for record, or select an existing record (data/plot will be added)

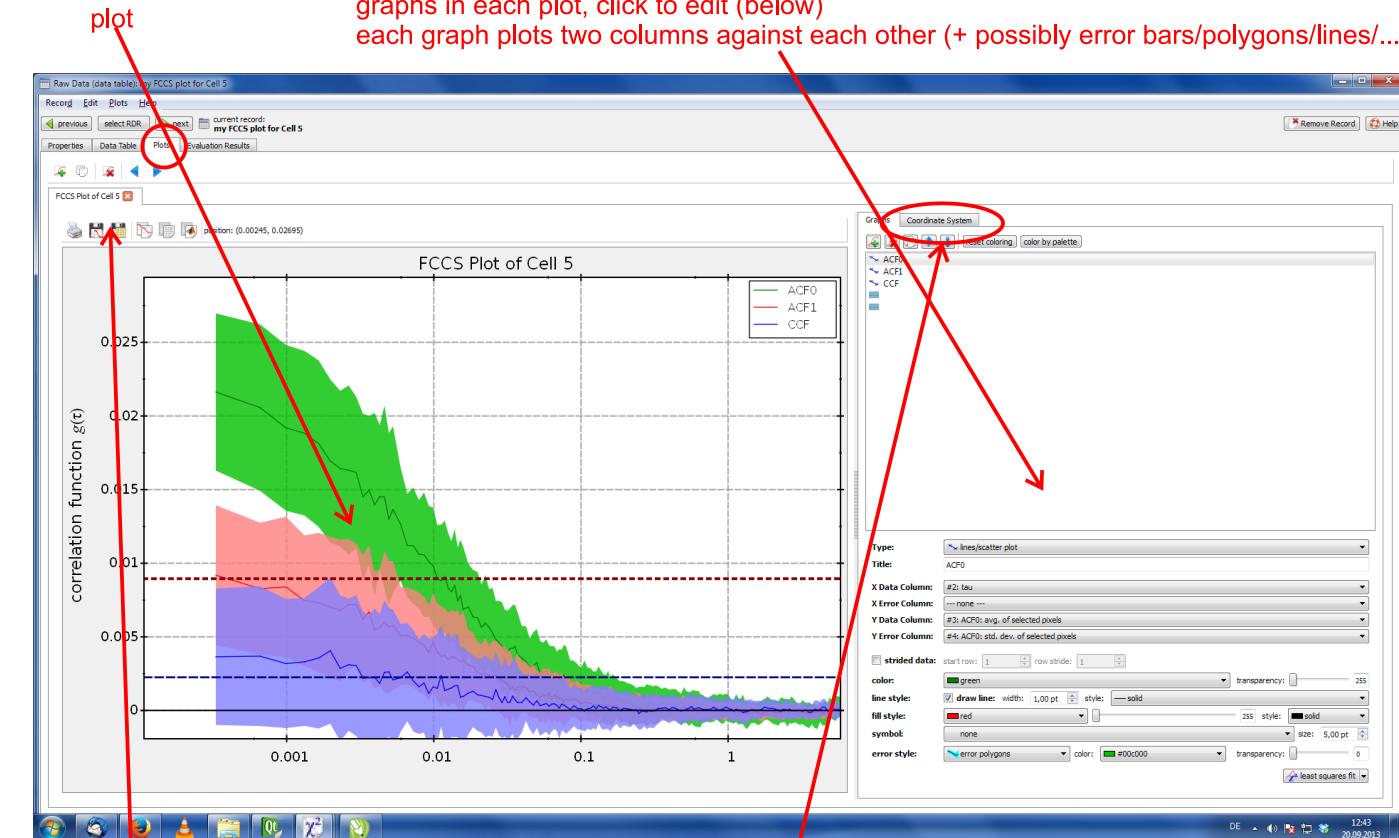
name for new plot, or select an existing plot (curves will be added to the selected plot)

if checked, immediately opens the editor for the new table/plot

# Plots in QuickFit



now the project contains a table-record for the plot  
(double-click to open)



save/print/copy plot

data table used for plot  
copy/paste to Excel should work

click to edit coordinate system/axis labels/...