FluoroFit32 program does the lifetime fit of the fluorescence data using the following two models:

1) $\mathrm{M_i} = e^{\frac{-\mathrm{i}}{\tau}}$ (no convolution)

The tail part of the fluorescence decay should be selected for the proper fit.

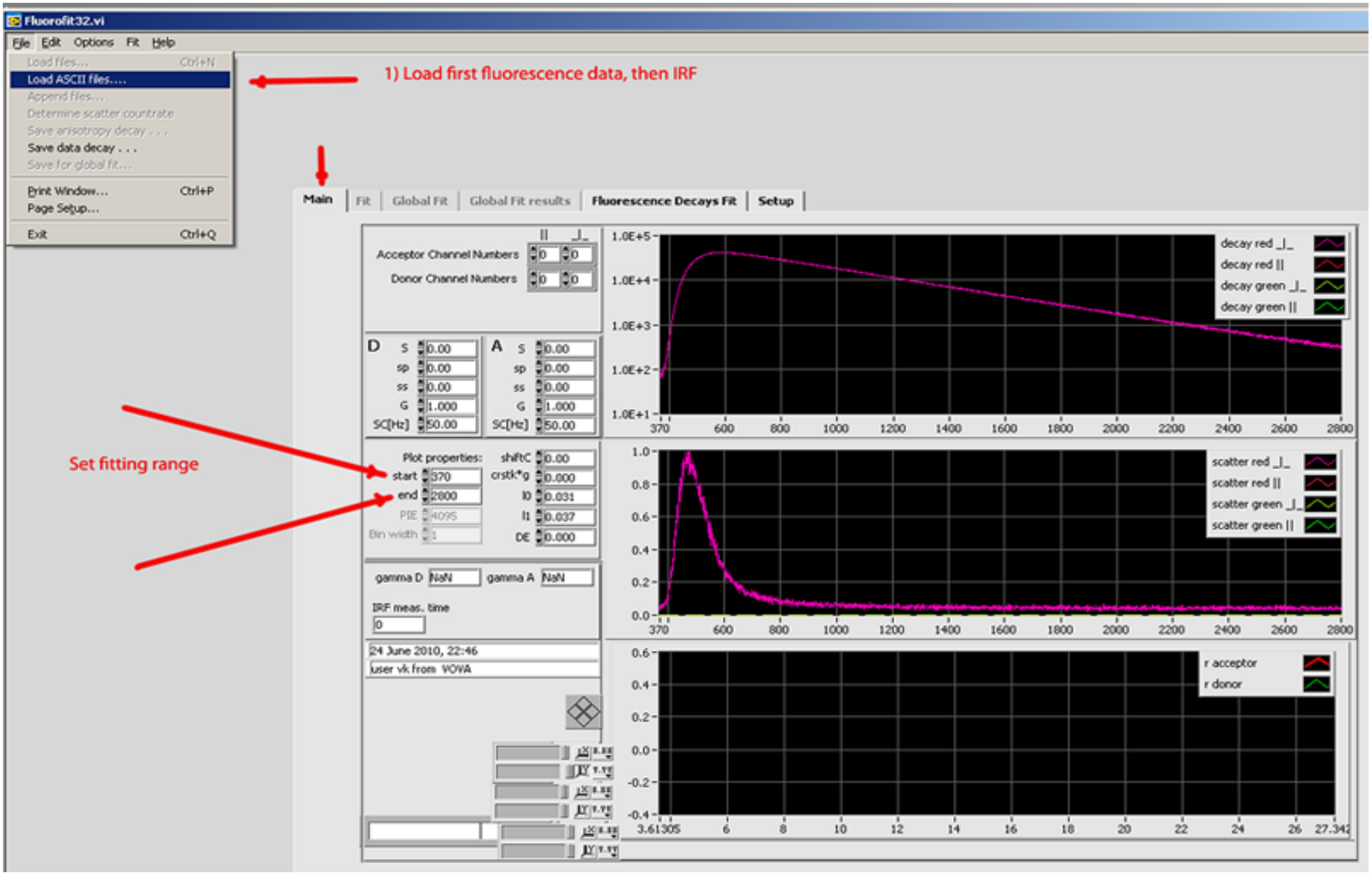
2) $M_i^{\cdot} = (1-\gamma) * IRF_i^{cut} \otimes e^{\frac{-\mathrm{i}}{\tau}} + \gamma * IRF$,

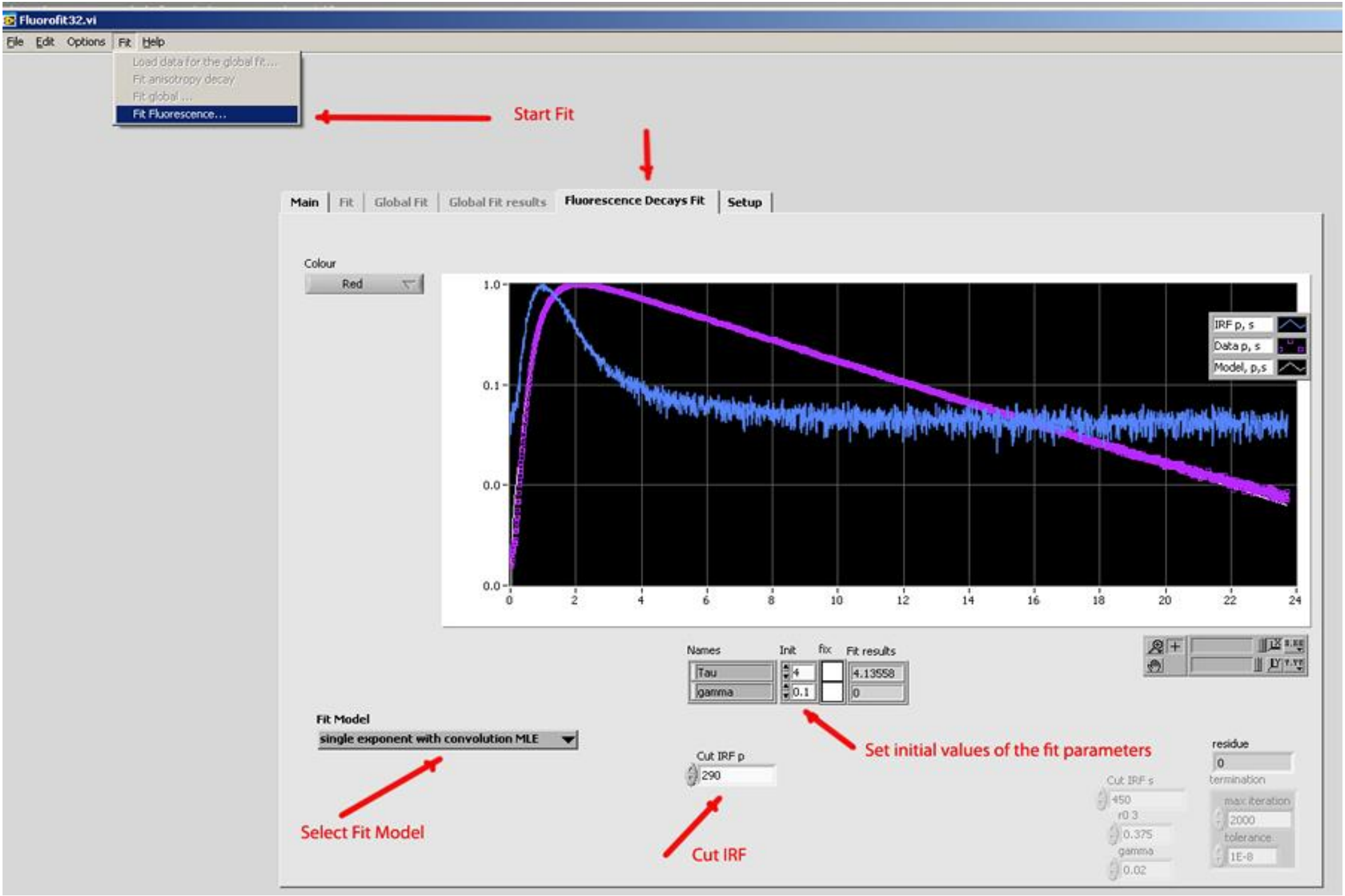
where IRF is the instrument response function, γ defines contribution of the scattered light into the fluorescence decay, τ - fluorescence lifetime.

Recording of the accurate IRF is a rather difficult task. The excitation wavelength is usually blocked by a filter that cannot be easily removed. The recorded laser pulse profile can contain a noticeable fraction of the dark counts of the detector, resulting in a flat offset, and other artifacts (reflections, etc.). Therefore it does not represent the true excitation profile of the pulse.

The flat dark count offset of the recorded IRF can be cut out at certain position (use "Cut IRF p" control). Cut IRF^{cut} is used for the convolution.

The relevant controls are described below.


Fluorofit32.vi
File Edit Options Fit Help
Load files... Ctrl+N
Load ASCII files....
Append files...
Determine scatter countrate
Save anisotropy decay . . .
Save data decay . . .
Save for global fit....
Print Window... Ctrl+P
Page Setup...
Exit Ctrl+Q
1) Load first fluorescence data, then IRF
Main
Fit
Global Fit
Global Fit results
Fluorescence Decays Fit
Setup
Acceptor Channel Numbers
Donor Channel Numbers
D
A
S
sp
ss
G
SC[Hz]
Plot properties:
start
end
PIE
Bin width
shiftC
crstk*g
l0
l1
DE
gamma D
gamma A
IRF meas. time
24 June 2010, 22:46
user vk from VOVA
Set fitting range
decay red _|_
decay red ||
decay green _|_
decay green ||
scatter red _|_
scatter red ||
scatter green _|_
scatter green ||
r acceptor
r donor


Fluorofit32.vi
File Edit Options Fit Help
Load data for the global fit...
Fit anisotropy decay
Fit global ...
Fit Fluorescence...
Start Fit
Main
Fit
Global Fit
Global Fit results
Fluorescence Decays Fit
Setup
Colour
Red
IRF p, s
Data p, s
Model, p,s
Names
Init
fix
Fit results
Tau
4
4.13558
gamma
0.1
0
Fit Model
single exponent with convolution MLE
Cut IRF p
290
Set initial values of the fit parameters
Select Fit Model
Cut IRF
residue
0
termination
Cut IRF s
450
r0 3
0.375
gamma
0.02
max iteration
2000
tolerance
1E-8