BurstML and ColorML

BurstML and **ColorML** are maximum likelihood (ML) methods developed to analyze photon bursts from diffusing molecules undergoing conformational transitions. The code below is specifically written for a molecule with two interconverting states.

ColorML gives the FRET efficiencies of the states and transition rates. It is based on a reduced likelihood function without fluorescence intensities and does not involve burst selection criteria.

BurstML gives brightness, diffusion times, FRET efficiencies of the states, and transition rates. It optimizes a likelihood function that explicitly incorporates translational diffusion and conformational dynamics.

Input: a list of photon bursts; each burst is a list $\{\{t_1,c_1\},\{t_2,c_2\},...\}$ with arrival time t_i and color c_i (donor or acceptor) for *i*th photon.

Further details can be found in I .V. Gopich, J.M. Louis, and H.S. Chung, “Maximum Likelihood Analysis of Diffusing Molecules with Conformational Dynamics in Single-Molecule FRET”.

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BurstML

BurstML1

BurstML1 uses an isotropic laser spot approximation. The extracted diffusion time taud = a^2/D needs to be converted to the diffusion time in the elongated laser spot.

Input parameters: *bursts* - list of bursts with photon arrival times and colors, *bA* and *bD* - acceptor and donor background count rates, *nTh* - threshold number of photons , *tTh* - threshold time, *qMax* - maximum dimensionless coordinate, *jMax* - the number of discrete points.

matrices1Dyn2st[n0List_,eList_,taudList_,kList_,bA_,bD_,qMax_,jMax_] -- calculates matrices N_A, N_D, $L+K-N_A-N_D$, and vector p_{eq} for 2-state system

logLBurstML1Dyn2st[bursts_,{n0List_,eList_,taudList_,kList_},bA_,bD_,nTh_,tTh_,qMax_,jMax_] -- log-likelihood of several bursts for given values of photon count rates *n0List*={n01,n02}, FRET efficiencies *eList*={e1,e2}, diffusion times *taudList*={taud1, taud2}, and transition rates *kList*={k1,k2}

findMaxLBurstML1Dyn2st[bursts_,{n0IniList_,eIniList_,taudIniList_,kIniList_},bA_,bD_,nTh_,tTh_,qMax_,jMax_] -- finds burstML1 parameters {{n01,n02},{e1,e2},{taud1,taud2},{k1,k2}} starting from the initial values *n0IniList* = {$n01_{ini}$, $n02_{ini}$}, *eIniList* = {$e1_{ini}$, $e2_{ini}$}, *taudIniList* = {$taud1_{ini}$, $taud2_{ini}$}, and *kIniList* = {$k1_{ini}$, $k2_{ini}$}.

stdBurstML1Dyn2stk1k2[bursts_,mlPar_,bA_,bD_,nTh_,tTh_,qMax_,jMax_]-- standard deviations of the burstML1 parameters {{n01,n02},{e1,e2},{taud1,taud2},{k1,k2}} estimated near the maximum *mlPar* = {{$n01^{ml}$, $n02^{ml}$}, {$e1^{ml}$, $e2^{ml}$}, {$taud1^{ml}$, $taud2^{ml}$}, {$k1^{ml}$, $k2^{ml}$}}.

stdBurstML1Dyn2stkp[bursts_,mlPar_,bA_,bD_,nTh_,tTh_,qMax_,jMax_]-- standard deviations of the burstML1 parameters {{n01,n02},{e1,e2},{taud1,taud2},{k,p1}} estimated near the maximum *mlPar* = {{$n01^{ml}$, $n02^{ml}$}, {$e1^{ml}$, $e2^{ml}$}, {$taud1^{ml}$, $taud2^{ml}$}, {$k1^{ml}$, $k2^{ml}$}}; k=k1+k2; p1=k2/k

corrPhi[w_]-- correction factor $a^2/a^2{}_{xy}$ to convert extracted diffusion time a^2/D in the isotropic laser spot to the diffusion time $\tau_d = a^2{}_{xy}/D$ in the elongated spot with the axial ratio *w*=az/axy .

BurstML2

BurstML2 finds diffusion time in the elongated spot $\tau_d = a^2{}_{xy}/D$ with the count rates, FRET efficiencies, and transition rates taken from burstML1. Aspect ratio *w*=az/axy.

matrices2Dyn2st[n0List_,eList_,taudList_,kList_,w_,bA_,bD_,qMax_,jMax_] -- calculates matrices N_A, N_D, $L + K - N_A - N_D$, and vector p_{eq}; 2 discretized coordinates

logLBurstML2Dyn2st[bursts_,{n0List_,eList_,taudList_,kList_},w_,bA_,bD_,nTh_,tTh_,qMax_,jMax_] -- log-likelihood of several bursts for given values of photon count rates *n0List*={n01,n02}, FRET efficiencies *eList*={e1,e2}, diffusion times *taudList*={taud1, taud2}, and transition rates *kList*={k1,k2} ; 2 discretized coordinates

findMaxLDBurstML2Dyn2st[bursts_,{n0List_,eList_,taudIniList_,kList_},w_,bA_,bD_,nTh_,tTh_,qMax_,jMax_] -- finds burstML2 diffusion times {taud1, taud2} in the elongated laser spot starting from the initial values *taudIniList* = {$taud1_{ini}$, $taud2_{ini}$} with other parameters fixed to previously found values *n0List* = {n01, n02}, *eList* = {e1, e2}, and *kList* = {k1, k2}.

stdDBurstML2Dyn2st[bursts_,mlPar_,w_,bA_,bD_,nTh_,tTh_,qMax_,jMax_]-- standard deviations of the burstML2 diffusion times {taud1,taud2} estimated near the maximum *mlPar* = {{$n01^{ml}$, $n02^{ml}$}, {$e1^{ml}$, $e2^{ml}$}, {$taud1^{ml}$, $taud2^{ml}$}, {$k1^{ml}$, $k2^{ml}$}}.

ColorML

logLColorMLDyn2st[bursts_,{e_List,k_List}] -- log-likelihood of several *bursts* for given values of FRET efficiencies *e_List*={e1,e2} and transition rates *k_List*={k1,k2}

findMaxLColorMLDyn2st[bursts_,{eIniList_,kIniList_}] -- finds colorML parameters {{e1,e2},{k1,k2}} starting from the initial values *eIniList* = {$e1_{ini}$, $e2_{ini}$} and *kIniList* = {$k1_{ini}$, $k2_{ini}$}

stdColorMLDyn2stk1k2[bursts_,mlPar_]-- standard deviation of the colorML parameters {{e1,e2},{k1,k2}} estimated near the maximum *mlPar* = $\{\{e1^{ml}, e2^{ml}\}, \{k1^{ml}, k2^{ml}\}\}$

stdColorMLDyn2stkp[bursts_,mlPar_]-- standard deviation of the colorML parameters {{e1,e2},{k,p1}}, k=k1+k2, p1=k2/(k1+k2) estimated near the maximum *mlPar* = $\{\{e1^{ml}, e2^{ml}\}, \{k1^{ml}, k2^{ml}\}\}$

eBNCorr[eApp_, bA_, bD_, nAv_] -- correction of the apparent FRET efficiency *eApp* for background noise (BN) with acceptor and donor count rates *bA* and *bD*; *nAv* - average photon count rate including BN.

Burst Fractions

When transitions are slow, the population of states in colorML is related to the fraction of bursts emitted by a molecule in corresponding state. This fraction can be estimated when count rates and diffusion coefficients are known and used to correct the colorML population when the states have unequal brightness and diffusivity.

matricesStatic[n0_,e_,taud_,bA_,bD_,qMax_,jMax_] -- calculates matrices N_A, N_D, $L - N_A - N_D$, and vector p_0; isotropic laser spot approximation (1 discretized coordinate)

z[n0_, taud_, w_,b_, nTh_, tTh_, qMax_, jMax_] - normalization factor *Z* ;

pb1[p1_,{n01_,n02_},{taud1_,taud2_},w_,b_,nTh_,tTh_,qMax_,jMax_]-- burst fraction for species 1

p1Corr[pb1_, {n01_, n02_}, {taud1_, taud2_}, w_,b_, nTh_, tTh_, qMax_, jMax_]-- corrected burst fraction

Photon Burst Simulation

Input model parameters:
n0List={n01,n02} - total (donor and acceptor) photon count rate in the center of the laser spot,
dList={D1,D2} - diffusion coefficients of the two states,
kList={k1,k2} - transition rates 1->2 and 2->2,
eList={e1,e2} - FRET efficiencies,
axy and *az* - lateral and axial laser spot sizes,
bA and *bD* - background count rates,
nTh - threshold number of photons,
tTh - threshold time.

photonTrajDyn2st[time_,n0List_,dList_,kList_,axy_,az_,r0_,qOut_,timeStep_]--generates a list of photon arrival times and corresponding molecule's states. The molecule starts from r0={x0,y0,z0} and diffuses for *time* (with time increment *timeStep*) in a region with reflecting boundary at dimensionless coordinate *qOut*=rOut/axy=zOut/az.

addColorDyn[photonStateTraj_,eList_]-- adds color labels according to FRET efficiencies *eList*={e1,e2} to the list of arrival times and states *photonStateTraj*

addBN[photonTraj_, bA_, bD_] -- adds background noise with count rates *bA* and *bD* to the photon trajectory *photonTraj* (a list of photon arrival times and colors {ti,ci})

selectBursts[photonTraj_, nTh_,tTh_] -- selects bursts from the list of photon arrival times and colors *photonTraj* using threshold time *tTh* and applies the threshold for the number of photons in a burst, *nTh*.

Examples

Parameters (in ms an μm)

```
axy := 0.5(*lateral spot size 0.5 μm*)
az := 1.5(*axial spot size 1.5 μm*)
nTh := 30(*theshold number of photons*)
tTh := 0.2(*threshold time 200 μs*)
qMax := 4. (*max dimensionless discretized coordinate q*)
jMax := 50(*max number of discret points (1 discretized coordinate)*)
jMax2 := 20(*max number of discret points (2 discretized coordinates)*)
qOut := 4.(*max dimensionless coordinate in photon trajectory simulations*)
timeStep := 0.0001 (*time step in photon trajectory simulations is 0.1 μs*)
n01 := 400. (*total photon count rate at the center of the spot *)
n02 := 200.
d1 := 0.1(*diffusion coefficient *)
d2 := 0.2
e1 := 0.3(*FRET efficiencies*)
e2 := 0.7
bA := 2.(*acceptor BN count rate*)
bD := 1.(*donor BN count rate*)
k1 := 1.(*transition rate 1→2*)
k2 := 1.(*transition rate 2→1*)
```

Example 1: Generate short photon sequences

generate a photon sequence of 200 ms with arrival times and states; show the first 20 photons

add color labels

add background noise

select bursts; the first photon in a burst has arrival time 0.

Example 2: Generate longer photon sequences

Generate bursts from nTraj=8 photon trajectories of duration 7 s each; unequal count rates and diffusion coefficients of the states

The same for a molecule with equal brightness and diffusivity in two states.

Example 3: BurstML

In[ ]:=
```
(*unequal count rates  and diffusion coefficients of the two states*)
n01 := 400. (*total photon count rate at the center of the spot *)
n02 := 200.
d1 := 0.1(*diffusion coefficient *)
d2 := 0.2
```

get generated bursts

In[ ]:=
```
SetDirectory[];
bursts = Get["burstsSampleUnEqual"];
```

find burstML1 parameters from 200 bursts

find standard deviations of {{n01, n02}, {e1, e2}, {td1, td2}, {k1, k2}} in burstML1:

find standard deviations of {{n01, n02}, {e1, e2}, {td1, td2}, {k,p1}} in burstML1:

correct the burstML1 isotropic diffusion time a^2 / D to get $a_{xy}{}^2 / D$

standard deviations of {td1, td2} corrected for the elongated spot

get diffusion coefficients from burstML1

find burstML2 diffusion times from 200 bursts

get diffusion coefficients from burstML2

find standard deviations of diffusion times in burstML2

Example 4: ColorML equal count rates and diffusion coefficients

In[ ]:=
```
(*equal count rates  and diffusion coefficients of the two states*)
n01 := 200. (*total photon count rate at the center of the spot *)
n02 := 200.
d1 := 0.2(*diffusion coefficient *)
d2 := 0.2
```

Get generated bursts

Find colorML parameters {{e1,e2},{k1,k2}} starting search from {e1,e2}={0.4,0.6} and {k1,k2}={1.2,1.5}

Find standard deviations of the colorML parameters {{e1,e2},{k1,k2}} from the ML values parsColorML.

Instead of the relaxation rates k1 and k2, the relaxation rate, k=k1+k2,and the population of the first state, p1=k2/(k1+k2), can be estimated. The colorML k and p1 are

The standard deviations of k and p1

FRET efficiencies in colorML are influenced by background noise. Uncorrected (apparent) FRET efficiencies are

Corrected FRET efficiencies depend on the mean photon count rates nAv defined as the total number of photons in all bursts divided by the total duration of all bursts

With the calculated nAv, the BN corrected FRET efficiencies are

Example 5: ColorML unequal count rates and diffusion coefficients

In[ ]:=
```
(*unequal count rates  and diffusion coefficients of the two states*)
n01 := 400. (*total photon count rate at the center of the spot *)
n02 := 200.
d1 := 0.1(*diffusion coefficient *)
d2 := 0.2
```

Get generated bursts

Find colorML parameters {{e1,e2},{k1,k2}} starting search from {e1,e2}={0.4,0.6} and {k1,k2}={1.2,1.5}

Find standard deviations of the colorML parameters {{e1,e2},{k1,k2}} from the ML values parsColorML.

Instead of the relaxation rates k1 and k2, the relaxation rate, k=k1+k2,and the population of the first state, p1=k2/(k1+k2), can be estimated. The colorML k and p1 are

The standard deviations of k and p1

FRET efficiencies in colorML are influenced by background noise. Uncorrected (apparent) FRET efficiencies are

Corrected FRET efficiencies depend on the mean photon count rates nAv defined as the total number of photons in all bursts divided by the total duration of all bursts

Corrected FRET efficiencies

colorML population can be interpreted as a burst fraction when transitions are slow

ColorML population can be corrected for unequal brightness and diffusivity using estimates for the count rates and diffusion coefficients (exact values in this case):