

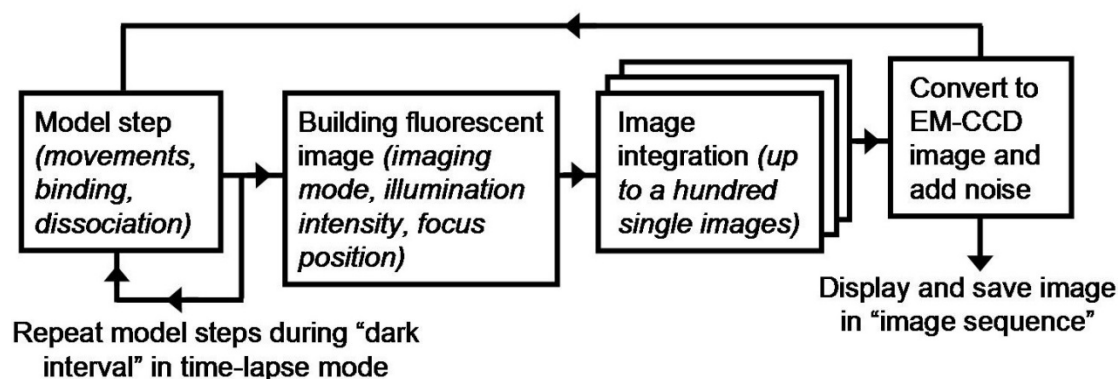
## GMcellModel Help

### Overview:

GMcellModel simulates single molecule dynamics in a virtual cell producing sequences of fluorescent images built according to the simulated imaging conditions (illumination and camera settings). The model contains arbitrary number of molecules belonging to 3 major classes:

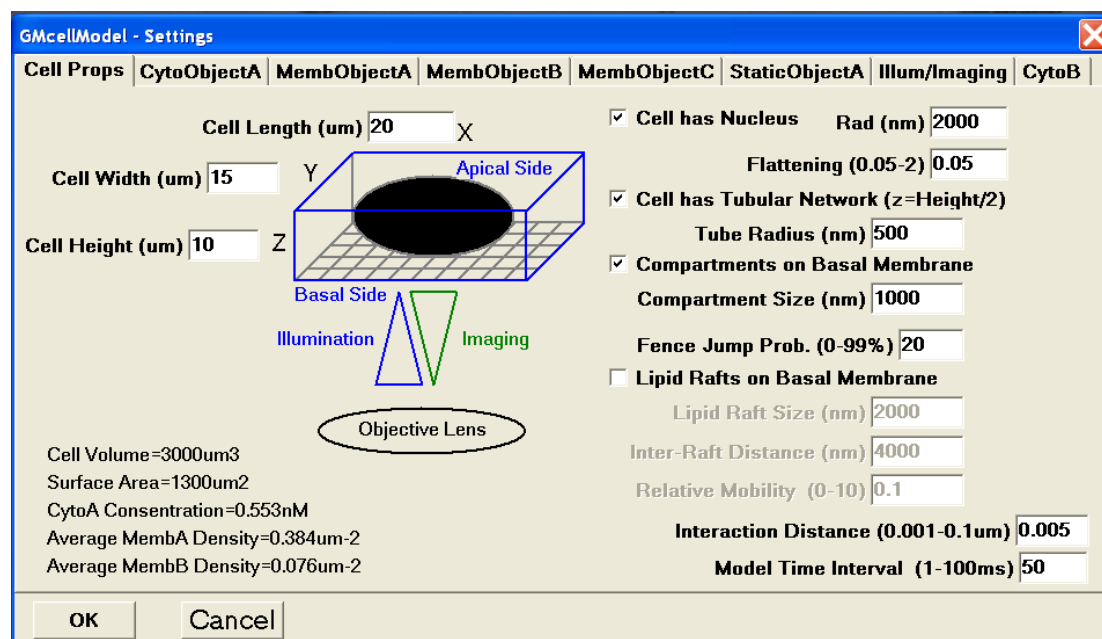
- Cytoplasmic molecules (“Cyto” class)
  - objects move by a 3-dimensional random walk inside the cell and can bind/unbind objects belonging to Memb or Stat classes. If binding occurs the pair move together with mobility determined by the slowest object.
- Membrane localised molecules (“Memb” class)
  - objects localised at the plasma membrane can move by a 2-dimensional random walk or can be immobile. They can bind/unbind Cyto and Memb class objects.
- Static molecules (“Stat” class)
  - located inside the cell as groups of immobile objects either forming fibres or randomly located over a fixed region (e.g. nuclear membrane). They can bind/unbind Cyto class objects.

Each object may have up to 4 fluorophores of green and red colours. At each time point (virtual video frame) all object positions are computed according to a random walk, calculated knowing the properties of each individual object. Object collisions are recorded and binding/unbinding events are computed based on Monte Carlo first order kinetics. The new object positions are then used to compute the number of photons emitted by each fluorophore, depending on the level of illumination at that particular point in the cell. All of the emitted photons are collected by a virtual CCD camera according to simple geometric optics so that a virtual image of the cell is formed by the emission from all active (non-bleached) fluorophores illuminated under the chosen imaging conditions (including counts due to additional noise). The sequences of images, generated during the model run, can be saved for future analysis as sequences of BMP files or as GMV files – a proprietary file format used in the partner software for single molecule detection and tracking “GMimPro” [www.nimr.mrc.ac.uk/gmimpro/](http://www.nimr.mrc.ac.uk/gmimpro/). Detailed information about this model is published in the Journal of Royal Society *Interface* (2014).



## Settings (F2):

The Settings dialog window contains several tabbed pages that are used to set the properties of each class of objects the virtual cell parameters and the simulated imaging conditions.



## Cell Props:

The virtual cell is a rectangular cuboid of a set **cell size** of length, width and height, (x, y, z) dimensions (in  $\mu\text{m}$ ). The lower or basal surface is composed of plasma membrane, is closest to the microscope objective and is set to be at the focal plane (defined as  $\zeta=0$ ). Membrane associated molecules (see **MembObjectA&B** below) move freely within, but are confined to, this plane and when they reach an edge they continue to move on the adjoining side of the parallelepiped.

“Cyto” objects (see below) can be excluded from an optional, ellipsoidal **Nucleus** the nucleus has a radius **Rad** (in nm) in x-y plane and an extended or flattened radius in the z plane, **Flattening** (scaling factor of 0.05 to 2). The user should avoid using nucleus sizes bigger than cell sizes.

“Cyto” objects can be retained inside a **Tubular Network** within the cell. A grid of interconnected tubes is placed at  $\zeta = (z / 2)$  with 5  $\mu\text{m}$  intervals.

*Tip: In order to visualise the above structures during the model run the user should run the model for a few hundred of frames to allow “Cyto” molecules to equilibrate across the cell volume then by pressing the “C” key “Cyto” objects are marked as white dots. It is recommended to use a short time steps (e.g. 1 ms) to model “CytoA” dynamics within the tubular structures and around the nucleus because molecular movements are limited in the narrow spaces. It is also advised to limit the cell height (z) so that the tubular network can be illuminated by the evanescent wave. By pressing “C” again the white dots are hidden.*

In order to simulate “hop diffusion” or membrane discontinuities, the plasma membrane can be divided into square [Compartments](#) of arbitrary [Size](#). Membrane molecules (see [MembObjectA/B](#)) can cross the simulated energy barriers (fences) if the probability of crossing is set above 0. Each time a moving molecule is about to move into a neighbouring compartment the Random Number Generator (RNG) is used to check the probability of crossing using a probability value set in [Fence Jump Prob.](#) Alternatively, the membrane can contain “compartments” (e.g. [Lipid Rafts](#)) which are simulated as square patches of membrane with altered viscosity. The user can adjust the [Size](#) and the [Distance](#) between the compartments as well as the [Relative Mobility](#) within the compartment. If the mobility inside the compartment is smaller (<1.0), than the surrounding membrane, molecules will become concentrated within the compartment. If mobility is higher (>1.0) molecules will concentrate outside the compartment.

The [Cell Props](#) page also contains inputs for [Interaction Distance](#), which should correspond to the sizes of reacting molecules (e.g. 3-5 nm for protein molecules).

The model [Time Step](#) can be set (in ms) (i.e. the Monte Carlo integration period, sometimes called delta t) for each model cycle. Note that if the model simulates fast moving molecules (e.g., diffusion rate  $2\text{-}20\text{ }\mu\text{m}^2\text{ s}^{-1}$ ) and frame rate is slow (<50 fps) it is advised to sum a few short time-steps (e.g., sum of 10 time-steps 5 ms each) to create one fluorescent image (see Illumination/Imaging page [N of Steps summed in One Image](#)). In the left corner of [Cell Props](#) page you can see some statistics: Cell volume and surface area (all 6 sides of the cell), concentration of CytoA objects, and density of membrane (A/B) objects.

### [CytoObjectA:](#)

This page sets properties of objects moving in cytoplasm: [Number](#) of CytoA objects, number of [Green Fluorophores](#) per CytoA object and [Fraction](#) of CytoA objects with active fluorescent tag. Note if an object has more than one green fluorophore per molecule and fraction is set to <100%, some CytoA objects will have different number of active fluorescent tags at the beginning of the model run. The same rule applies to the [Red Fluorophores](#) on CytoA objects (and to the [MembA&B](#) and [Stat](#) objects, see below). The user cannot alter the number of objects or fluorescent tags number in the middle of a model run (the model will restart) but can change the [Diffusion Rate](#). Normally Cyto objects diffuse outside the nucleus but if check-box [Place CytoA inside Nucleus](#) is checked, the [CytoA](#) objects will move only inside nucleus (if [Nucleus](#) tick-box on CellProps page is checked **before** the model run).

GMCellModel - Settings

Cell Props | **CytoObjectA** | MembObjectA | MembObjectB | MembObjectC | StaticObjectA | Illum/Imaging | CytoB

Number of Cyto Objects A:  CytoA Concentration=0.553nM

Green Fluorophores per ObjA:  Red Fluorophores per ObjA:

Green Fluorescent Fraction (%):  Red Fluorescent Fraction (%):

Diffusion Rate (um2/s):  Average Jump per Time Step=1000nm

☐ Place CytoA Objects inside Nucleus

\*\*\*\*\*

OK Cancel

## CytoB

This object class is used to simulate background fluorescence noise due to “unidentified flying objects”. They can be added to simulate background noise that adds in addition to sources of camera noise (see below)

GMCellModel - Settings

Cell Props | CytoObjectA | MembObjectA | MembObjectB | MembObjectC | StaticObjectA | Illum/Imaging | **CytoB**

Number of Cyto Objects B:

Diffusion Rate (um2/s):

OK Cancel

## MembObjectA/B

These two pages contain settings analogous to the CytoA page (see above) and also ComboBox with the selection of the initial distribution of MembA/B objects at cell membrane. In a simple case, when membrane molecules mobile, the molecules will be distributed randomly across all the membrane after some period of equilibration. However when molecules have limited mobility their initial distribution will be important for imaging and statistical analysis. Some specific initial distribution (Filopodia, Moving Filaments, and Exocytosis) will also affect the future movements of the molecules. There are also tick-boxes which control binding of Cyto and Membrane molecules to each other. MembA molecules also can form dimers. The binding and dissociation events are determined by [Binding Rate](#) and [Dissociation Rate](#) coefficients. For the binding events, the model checks the Average Interaction Time (AIT) for every eligible pair of molecules. AIT depends on the distance between molecules, their mobility, and [Interaction Distance](#) (set on CellProps page). The AIT is multiplied by Binding Rate and used as an input for RNG determining the outcome of binding test. The [Dissociation Rate](#) is used as a parameter in RNG testing possible dissociation event for every bound pair of molecules every time step. [Note *MembObjectC* is not currently used]

GMcellModel - Settings

Cell Props | CytoObjectA | **MembObjectA** | MembObjectB | MembObjectC | StaticObjectA | Illum/Imaging | CytoB

Number of Memb A objects: 500      Average MembA Density=0.384um-2

Green Fluorophores per MembA: 1      Red Fluorophores per MembA: 1

Fluorescent Fraction (%): 100      Fluorescent Fraction (%): 100

Site A Diffusion Rate (um2/s): 0.3      Average Jump per Time Step=244.948nm

Initial Distribution of Memb A: Random on Apical-Basal M. ▾

☒ Memb A can bind Cyto A

CytoA-MembA Binding Rate (s-1): 500000      CytoA-MembA Dissociation Rate (s-1): 0.2

☒ Memb A can form dimer

A-A Binding Rate (s-1): 100000      A-A Dissociation Rate (s-1): 1

\*\*\*\*\*

Release Rate (Filopodia mode) (s-1): 1      Molecules in Filament: 500

OK      Cancel

### StaticObjectsA:

Static objects remain stationary and cannot change position inside cell during the model run. The "StatA" objects can form "Straight Fibres", and "Curved Fibres" (laying in horizontal plane at  $\zeta=100\text{nm}$ ), "Fibres Curved in 3D", and "Decorate Nucleus Membrane". In the last case molecules randomly placed at the nucleus membrane (+/- 20 nm). The size and shape of nucleus is set on CellProps page. CytoA molecules bound to StatA filaments can move to the left along the fibres with the [Velocity](#) set on this page. Both tick-boxes [CytoA can Bind StatA](#) and [CytoA can Bind StatA](#) should be checked.

GMcellModel - Settings

Cell Props | CytoObjectA | MembObjectA | MembObjectB | MembObjectC | **StaticObjectA** | Illum/Imaging | CytoB

Number of Memb A objects: 500

Green Fluorophores per StaticA: 2      Red Fluorophores per StaticA: 0

Fluorescent Fraction (%): 100      Fluorescent Fraction (%): 100

Distribution of Static A Qbjects  
Strait Fibers in Cytoplasm (at z=100nm) ▾

Distance between Objects in Fiber (nm): 8

☒ CytoA Can Move to the right along Static A

Velocity (um/s): 5

☒ Static A can bind Cyto A

CytoA-StatA Binding Rate (s-1): 500000      CytoA-StatA Dissociation Rate (s-1): 0.1

OK      Cancel

### Illumination/Imaging:

This page contains a group of parameters. First group controls the emission of single fluorophores. Full Width at Half Maximum ([FWHM](#)) of a single

molecule image placed at the focal plane of an objective lens can be set close to the theoretical limit for given wavelength ( $\sim 1/2$  of the wavelength of emitted light). There is also [Emission Rates](#) and [Photobleaching Rates](#) for both Green and Red fluorophores. NOTE - these rates set for the fluorophores placed in the centre of the image at coordinates =  $(x/2, y/2, \zeta=0)$ ; where illumination is brightest. Fluorophores placed in other parts of the cell could receive much weaker illumination (depends on the illumination pattern) and as consequence emit proportionally smaller number of photons. The bleaching rate for Green and Red fluorophores is also set for the molecules in the centre of the image  $(x/2, y/2, \zeta=0)$ . It will be proportionally smaller for the weakly illuminated molecules. The image of single fluorophore (2-D Gaussian spot) placed above or below current focal plane will be increased (blurred), while the number of emitted photons remains the same. Ideally the relation between fluorophore FWHM and distance from the focal plane is described by simple hyperbolic function  $FWHM(z) = K_{obj} * (\zeta - \zeta_{focal})^2 + FWHM_{focal}$ , where  $\zeta$  is the z-position of a molecule (above coverslip level),  $\zeta_{focal}$  is a current position of focal plane of the objective lens (usually  $\zeta_{focus}$  is defined as 0),  $FWHM_{focal}$  is the size of a fluorophore image at the focal plane (see above). The measured relation between FWHM of single fluorescent molecule (Cy3B) and movements along the z-axis of the objective lens 100X1.45NA resulted in  $K_{obj} \approx 0.6 \mu m^{-1}$ .

GMcellModel - Settings

Cell Props | CytoObjectA | **MembObjectA** | MembObjectB | MembObjectC | StaticObjectA | **Illum/Imaging** | CytoB

Diffraction Limited Spot Size (FWHM)(nm)  Kobj for Z-axis (per um)

Green Emission Rate (photons/s)  Red Emission Rate (photons/s)

Green Photobleaching Rate (s-1)  Red Photobleaching Rate (s-1)

**Illumination**

Cell Profile (length) ->

Illumination Profile Size (FWHM, um)  TIR angle (o)

Iris Diaphragm Radius (um)

**Camera Settings**

N of Model Steps Summed in One Image  ☐ 63o / 68o TIRFM

N of Dark Time Steps (Time Lapse)  ☐ Alternating Illumination

Camera Noise (counts/pixel)  ☒ Split Image - Two colours

Camera Gain (EMCCD)  ☒ EMCCD Camera

OK Cancel

There are 3 modes of illumination used in this model: Epi-illumination, Total Internal Reflection Fluorescence Microscopy (TIRFM), and confocal imaging. It is assumed that in two first modes laser beam has a 2D Gaussian shape profile determined by its FWHM, The user can set [Laser Beam FWHM](#) to control the pattern of illumination. The FWHM can be increased (e.g.,  $\gg$  Cell Length and Width) to make the nearly-flat illumination pattern in x-y plane. The illuminated area can be shaped by a circular [iris Diaphragm](#). In "Epiillum" and "Confocal" modes the whole cell volume will be illuminated within the limits of the size of [Iris Diaphragm](#). The cell profile (along cell length) is shown next to these Edit Controls. The TIR angle parameter can be changed between 63 and 75 degrees to control the depth of TIR illumination. NOTE – It



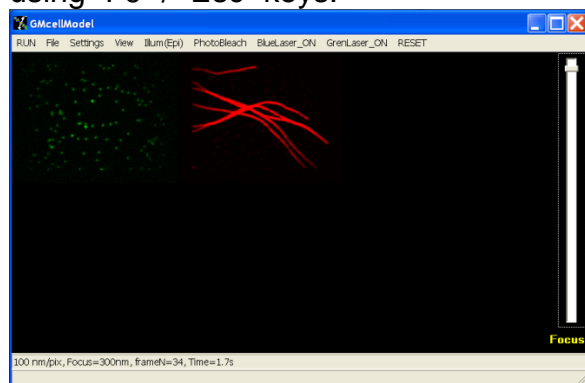
also depends on the wavelength of the illumination (488nm for Green fluorescence and 561 nm for Red fluorescence simulation).

### Camera Settings.

Few model steps can be summed to create one camera image. It gives realistic images of the fluorescent objects moving considerable distances during one image acquisition. For example if we set model time step (see above) to 5 ms and set N of [Model Steps summed in One Image](#) to 10 we will get camera rate of 20 fps which is achievable by many sensitive cameras. At this rate slow moving molecules will look like sharp spots of light, while fast randomly moving molecules look like dim clouds. If the [Number of Dark Time Steps](#) is set above zero, the model will run in a time-lapse mode when one illuminated image is followed by specified number of dark time-steps. For example if we use above settings (summing 10 images) and set [N of Dark Time-Steps](#) to 90, the model will display (and record) one fluorescent image (50ms exposure time) every 0.5 s giving 2fps (The photobleaching rate will be reduced accordingly). The model will add few “noise” counts to every pixel on the image which simulate camera and photon noise. The noise counts have Gaussian random distribution which sigma value is set as a [Camera Noise](#). EMCCD cameras multiply every registered photon by an average number determined by [Camera Gain](#). This multiplier has some degree of variation which, in this model, was set as a square root of pixel value. The EMCCD mode can be switched ON/OFF using [EMCCD camera](#) check-box. If two colours are used in the model run the results can be displayed / recorded in few modes – [Image Split](#) mode used to display Green and Red images side by side (NOTE - make sure that both lasers are switched ON in the main menu). [Alternating Illumination](#) mode is used to illuminate one frame with one colour and next one with another (Green-Red-Green-Red and so on). The results can be displayed and recorded as one image or as double if [Image Split](#) box is checked.

### The Main Window menus.

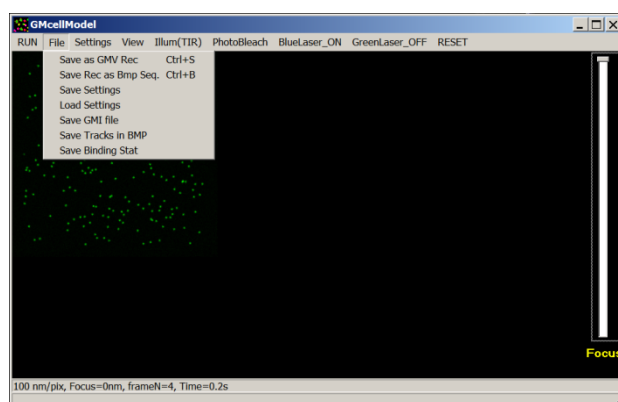
User can start and stop model run but pressing “[RUN/STOP](#)” menu item or by using “F9” / “Esc” keys.



### File submenu:

at any moment before or during model run user can start recording “sequence of fluorescent images” in the specific GMV file format used in Single Molecule Detection and Tracking software GMimPro (see separate GMimPro help files and recommended papers to learn this software). GMimPro can be used to

display, analyse, and save as sequences of BMP or binary files original GMV files generated by the model. GMimPro webpage ([www.nimr.mrc.ac.uk/gmimpro](http://www.nimr.mrc.ac.uk/gmimpro)) also contains ImageJ plug-ins for importing GMV files into ImageJ. The results also can be saved as 24-bit BMP files



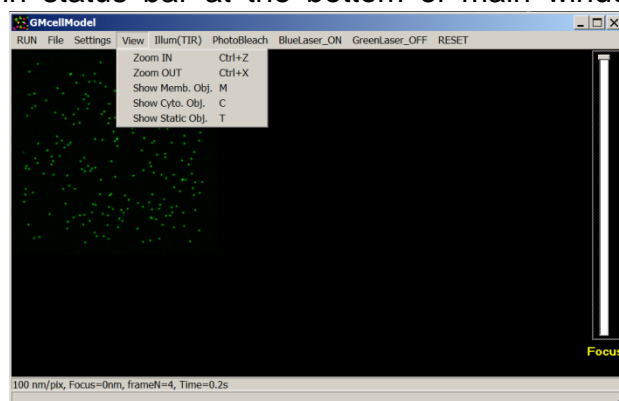
using **Save Rec. as BMP Seq.** Please specify the file name for the first frame. WARNING – file name should not have number or “.bmp” string at the end of the filename because the index specifying image number will be added to the filename automatically. It is strongly advised to create a separate folder for each BMP image sequence created at the model

run. In both record modes the recording is terminated by pressing ESC key. WARNING – make sure that the fluorescence (‘F’ key) and at least one of the lasers is switched ON before you start recording.

Most initial conditions in the Settings menu can be stored for future model runs using **Save Settings** option. The current settings will be stored in a data file with default extension “gms”. These settings can be loaded using **Load Settings** line in the File menu. NOTE – if the numbers of objects of any class or cell sizes loaded from settings file do not match the current settings, the model will start a new run using initial distributions of membrane and static objects stored in loaded file.

### **View submenu**

The user can change camera pixel sizes used in the model (default value 100 nm/pixel) using “Ctrl+Z” and “Ctrl+Z” key combinations (current scale is shown in status bar at the bottom of main window). NOTE – These changes will



affect the sizes and brightness of the images of fluorescent objects projected on camera CCD. Normally, objective lens x100 would increase image size 100 times to the size of CCD pixels (e.g., Andor iXon897BV has CCD pixel sizes 16  $\mu$ m, so an objective lens x100 would give camera scale 160 nm/pixel). The current XY positions of

“Cyto” objects can be shown on CCD image using ‘C’ key (white dots), “memb” objects using ‘M’ key (red/yellow dots), “stat” objects using ‘T’ key (blue dots).

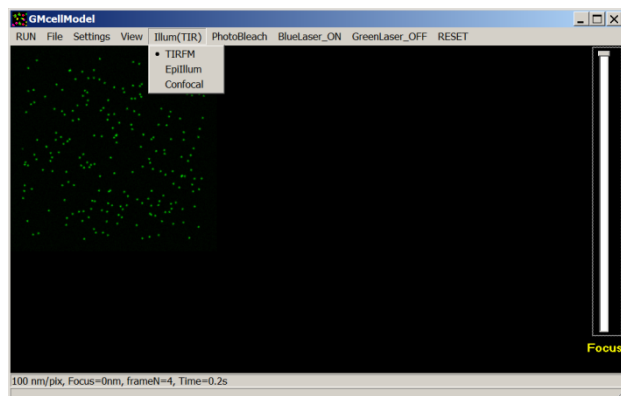
### **Illumination submenu**

**TIRFM** (Total Internal Reflection Fluorescence Microscopy) – cell is illuminated according to the rules of optics for Evanesce Wave propagation at the interface between mediums with high and low refractive indices (See



Axelrod et. al., 1992). The profile of illumination is determined by the colour of illumination, TIR angle, and laser beam profile settings (see Settings->Illum/Imaging menu).

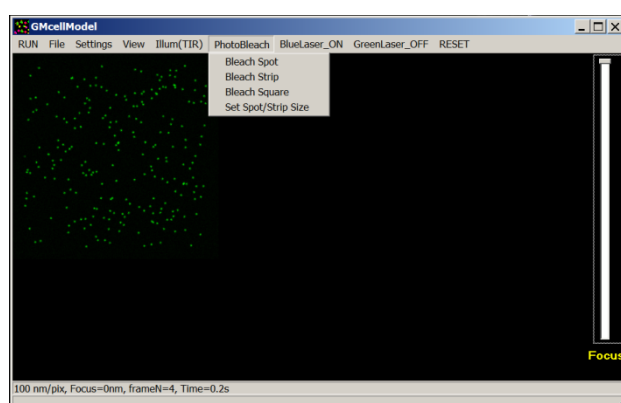
**Epillum.** – The laser beam penetrates the whole cell. Its XY intensity profile depends on the **Laser Beam FWHM** and **Iris Diaphragm** settings (see Settings Menu).



**Confocal** illumination – It is a simulation of scanning method of illumination / imaging where intensity of illumination is constant at any XY point in the current focal plane (laser beam focused in the focal plane of the objective lens). The intensity of illumination decreases dramatically above and below focal plane (outside the focused

laser beam) due to a sharp increase in the size of laser beam cone. It is also assumed that a “virtual pinhole” blocks most of the emission from the fluorescent objects placed above or below current focal plane.

**Photobleach:** The user can bleach all the fluorescent molecules within chosen spot (circle), square, or strip area. NOTE - If bleaching is done in TIRFM mode “Cyto” and “Stat” molecules will not be affected by the bleach



(only molecules at basal cell membrane (“Memb” objects)), but in the “Epillum” or “Confocal” modes molecules of all types will be bleached. Make sure that the one or both lasers (Blue, Green) are ON at the moment of bleaching to bleach one or both colours. The size/diameter of bleaching area can be set in **Set Spot/Strip Size** Edit control. The Round

Spot or Square is placed in the middle of the cell, while bleached Strip is placed at the right edge of the cell.

**BlueLaser** and **GreenLaser** menu items allow to switch Blue or Green illumination ON and OFF. These controls will not work if **Alternating Illumination** check-box is checked in the **Settings** menu. NOTE – make sure that Illumination is ON (key ‘F’) to bleach molecules.

**RESET** - click this menu item to reset the model – all the objects will be placed according to the initial distributions and all available fluorophores (see Settings) will be reactivated (made fluorescent again if they were bleached in the previous run).

## **List of Key Combinations / Shortcuts**

### **Start/Stop/Reset:**

1. F2 – Launch Settings dialog Window
2. F4 – Reset time ( $t=0s$ )
3. F9 – Run
4. Esc – stop run / stop recording
5. 'Space' make one model step. Note: more than one step may be required to form a new image on the screen.

### **Action Controls:**

6. Ctrl+'S' - Save GMV record
7. Ctrl+'B' – Save record as Sequence of BMP files
8. Ctrl+'Z' – Zoom In – Decrease size of CCD pixel
9. Ctrl+'X' – Zoom Out – Increase size of CCD pixel
10. Ctrl+'N' – Reset the model – go to initial state and distribution of present objects.
11. 'F' – Switch ON/OFF illumination. Note: make sure that one or both lasers are ON to be able to illuminate “the cell”.

### **Display Modifiers:**

12. 'C' – Show/Hide white dots marking x-y positions of CytoA objects
13. 'M' – Show/Hide red and yellow dots marking x-y positions of MembA and MembB objects
14. 'T' - Show/Hide blue dots marking x-y positions of StatA objects

PS You can contact Gregory Mashanov [gmashan@nimr.mrc.ac.uk](mailto:gmashan@nimr.mrc.ac.uk) if you have a specific query, but I advise you to read *Interface* (2014) publication and run the model using provided scenarios (gms) first.