

MCell And DReAMM Simulations Of Catecholamine Release Detection Using a Patch Clamp Technique

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OUTLINE

- ▶ Background :
 - Catecholamines
 - Patch Clamp
- ▶ Overview:
 - Blender
 - Mcell
 - DReAMM
- ▶ Results
- ▶ Difficulties
- ▶ Benefits

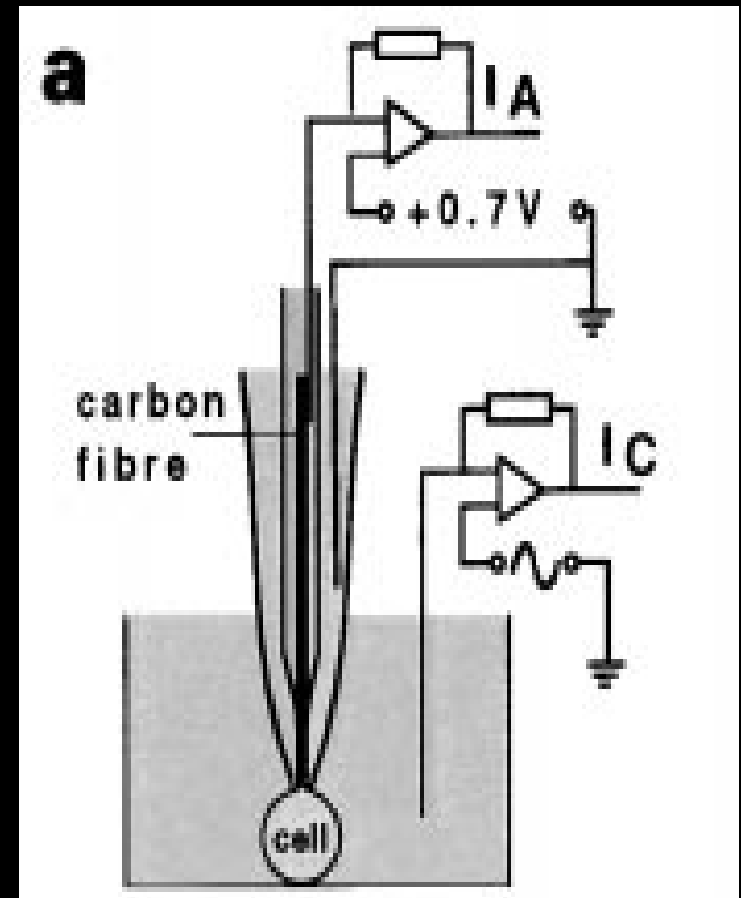


WHAT ARE CATECHOLAMINES?

- ▶ charged neurotransmitters
- ▶ released outside through a narrow fusion pore of a neuroendocrine cell 'chromaffin'
- ▶ 1 catecholamine out = 2 electrons
- ▶ requires a charge compensation by other ions such as Ca^{2+} to maintain an osmotic balance of charges

PATCH CLAMP:

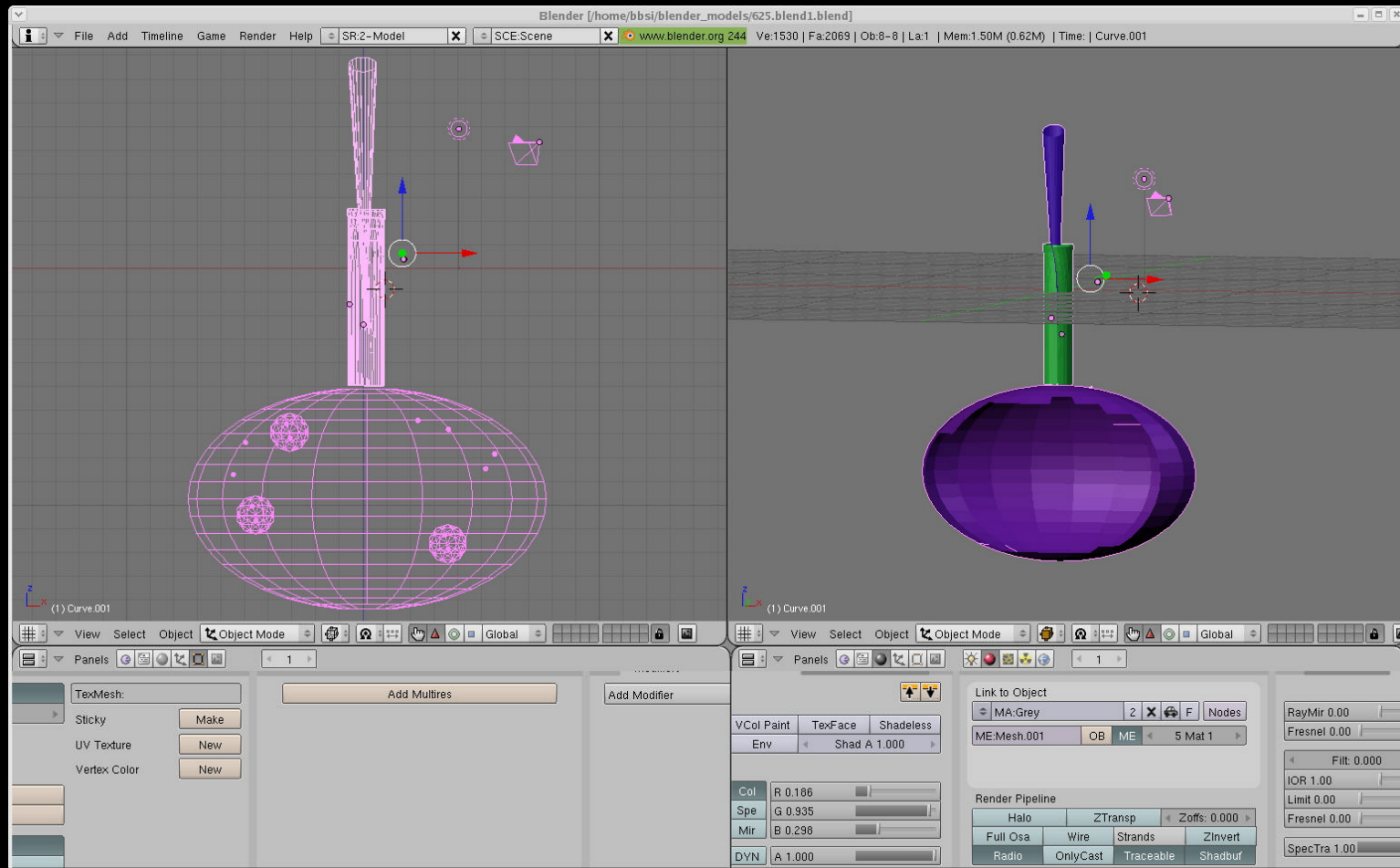
- ▶ A glass pipette with tip attached to surface of cell
- ▶ Contains a Carbon Fiber Electrode
- ▶ An external voltage
- ▶ Vesicles fuse into the Electrode



BLENDER

- ▶ an open source, free of charge tool for 3D visualization
- ▶ creation of basic shapes– cell, vesicle, patch clamp, carbon fiber electrode– for mesh generation
- ▶ Varying the geometry of meshes
 - Exclusive for structural editing

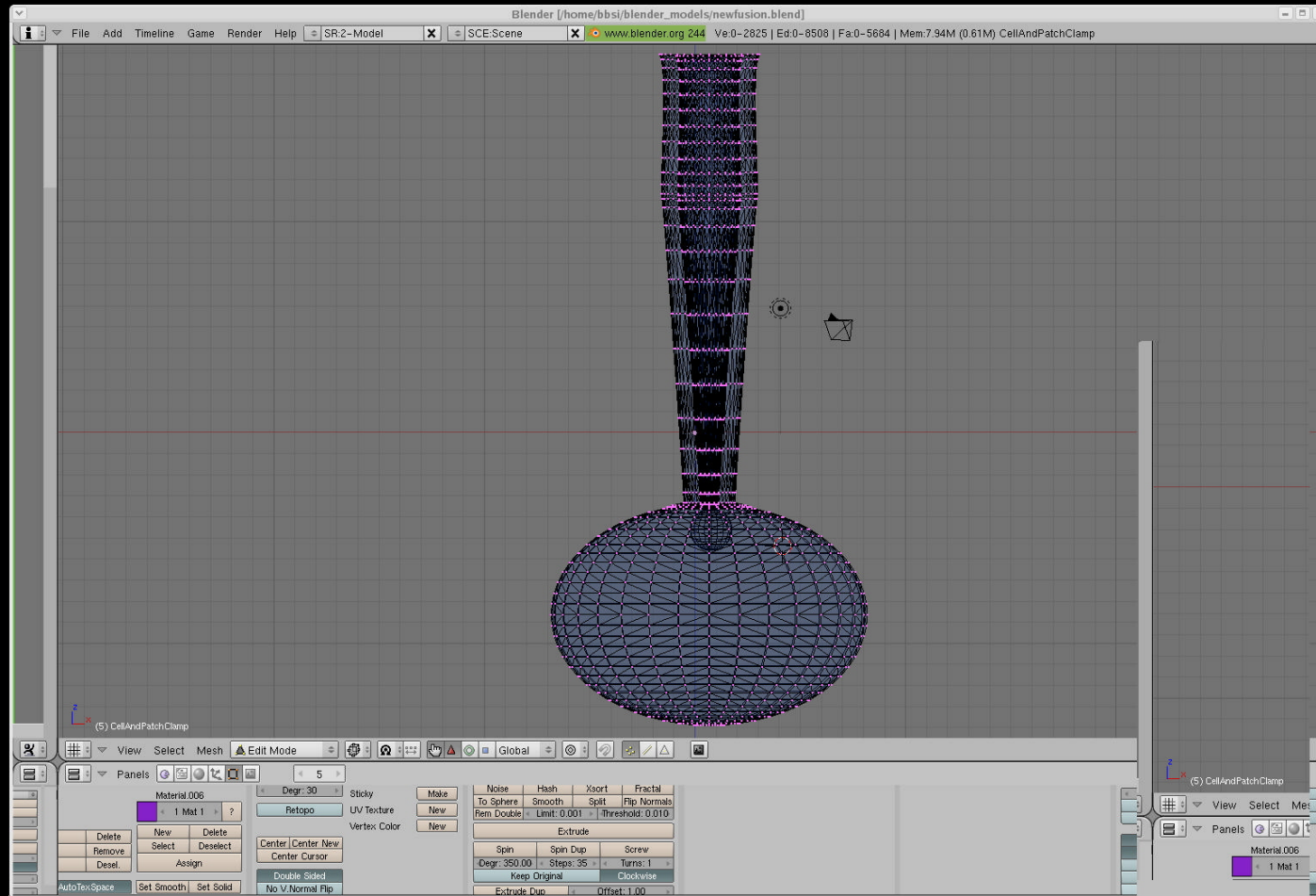
Early Blender Model



Understanding Blender

- ▶ Object Mode
 - objects are added, animation/ shape keys are added
- ▶ Edit Mode
 - vertices and faces of objects are edited (selected, erased...)
- ▶ Visual Example

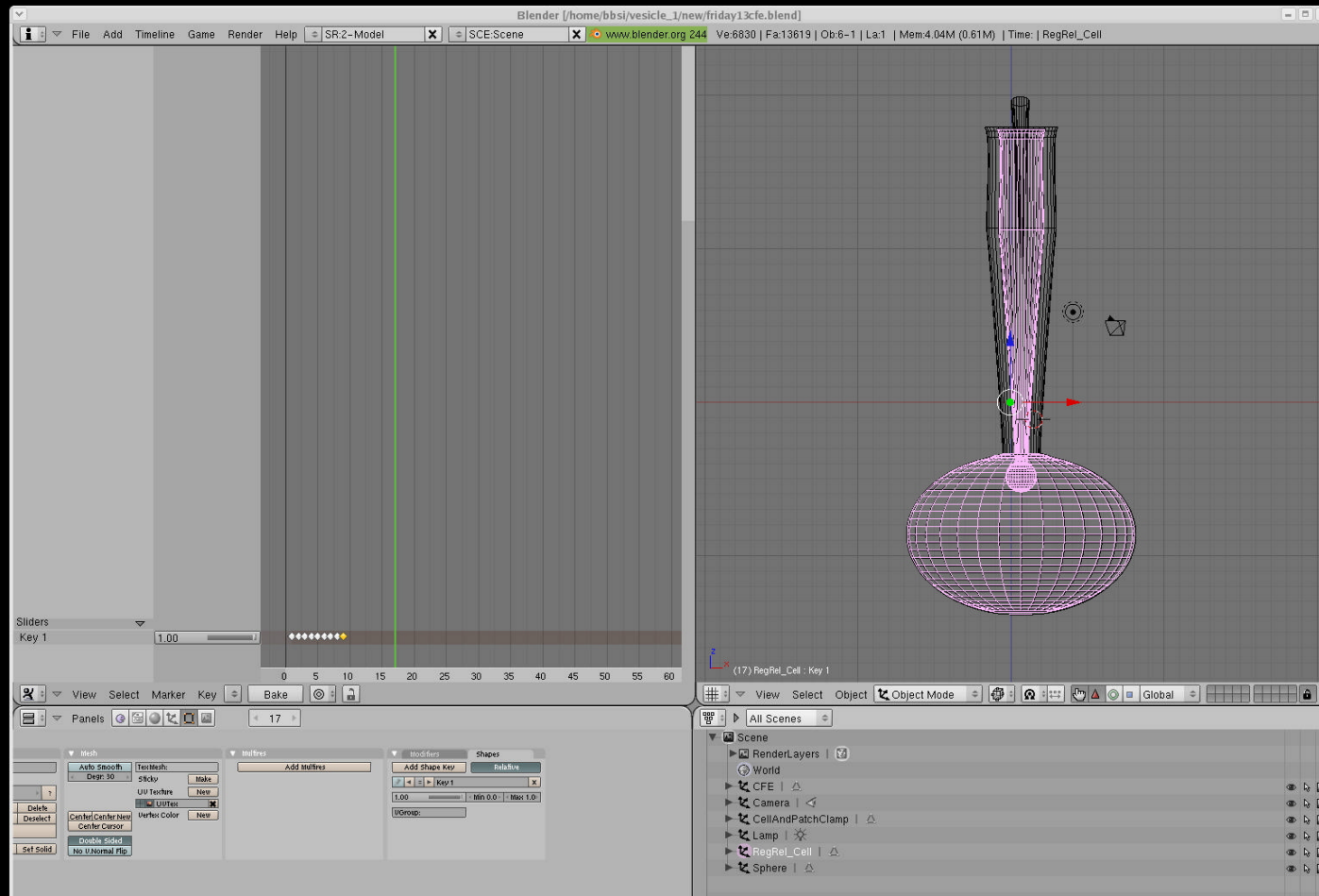
Later blender model



▶ Regions / Objects created:

- Cell
- Patch Clamp
- Carbon Fiber Electrode
- Vesicle
- Sphere
- Inner Patch Clamp
- Inner Cell

Creation of animation



Animation

- ▶ Action Editor
- ▶ Shape Keys defined in Object Mode
- ▶ manual shifting of objects and widening of fusion pore from the initial to the final position
- ▶ Use frames
- ▶ Run animation in Object Mode as well

Exporting in Blender

▶ Export to MCell

- Filename.blend → Filename.mdl
- Animations :
 - Define number of frame files else by default : 250

▶ Export to DReAMM

- Filename.blend → Filename.dx

MCell3 (Monte Carlo Cell)

- ▶ Simulations run in Mcell by reading MDL files
- ▶ Specialized Monte Carlo Algorithms used for simulation
- ▶ Separate MDL files created for each frame (total: 16) used in the animation

An MDL File

- Beginning of an MDL File
- Length of file can vary ~20,000 lines
- Iterations, molecules, sizes defined

```
iterations = 1000
ITERATIONS = iterations

TIME_STEP = 1E-6

CHECKPOINT_OUTFILE = "chkpt"
CHECKPOINT_INFILE = "chkpt"
CHECKPOINT_ITERATIONS = 1

DEFINE_MOLECULES{
  A(DIFFUSION_CONSTANT_3D = 1E-6)
}
DEFINE_SURFACE_CLASSES {
  transp_A (TRANSPARENT = A)
}

CellAndPatchClamp OBJECT {
CellAndPatchClamp POLYGON_LIST {
  VERTEX_LIST {
    [ 2.305919, -1.025761, 17.870571 ]
    [ 2.209323, -1.025761, 17.635550 ]
    [ 2.145248, -1.025761, 17.222996 ]
    [ 2.108607, -1.025761, 16.667757 ]
    [ 2.094315, -1.025761, 16.004677 ]
    [ 2.097285, -1.025761, 15.268599 ]
    [ 2.112432, -1.025761, 14.494371 ]
    [ 2.134670, -1.025761, 13.716839 ]
    [ 2.158911, -1.025761, 12.970846 ]
    [ 2.180070, -1.025761, 12.291239 ]
    [ 2.193062, -1.025761, 11.712862 ]
    [ 2.192799, -1.025761, 11.270562 ]
    [ 2.174196, -1.025761, 10.999183 ]
    [ 2.129092, -1.025761, 10.588499 ]
    [ 2.056683, -1.025762, 9.759933 ]
    [ 1.962921, -1.025762, 8.595798 ]
    [ 1.853758, -1.025762, 7.178411 ]
    [ 1.735148, -1.025762, 5.590085 ]
    [ 1.613040, -1.025762, 3.913136 ]
    [ 1.493389, -1.025762, 2.229879 ]
    [ 1.382145, -1.025762, 0.622630 ]
    [ 1.285261, -1.025762, -0.826298 ]
    [ 1.208689, -1.025762, -2.034589 ]
    [ 1.158381, -1.025763, -2.919928 ]
    [ 1.140289, -1.025763, -3.400000 ]
    [ 1.920157, -1.025763, -3.317909 ]
    [ 1.938249, -1.025763, -2.837837 ]
```

```
MCell: world bounding box in microns =
[ -6.811044 -6.63711 -13.874858 ] [ 8.188946 4.612887 17.952663 ]
Creating 3375 subvolumes (15,15,15 per axis)
MCell: checkpoint sequence number 1 begins at elapsed time 0 seconds
MCell: executing 1 iterations starting at iteration number 0.
Running...
Running simulation.
Iterations: 0 of 1
Iterations: 1 of 1
MCell: time = 1, writing to checkpoint file chkpt
Exiting run loop.
iterations = 1 ; elapsed time = 1e-06 seconds
Total number of random number use: 0
Total number of ray-subvolume intersection tests: 0
Total number of ray-polygon intersection tests: 0
Total number of ray-polygon intersections: 0
Total number of molecule-molecule collisions: 0
Total CPU time = 0.329949 (user) and 0.030995 (system)
Total wall clock time = 0 seconds
Done running.
[bbsi@dhcp61i new]$ mcell13 22.mdl.blend

MCell 3.001 (build (build date/CVS version date goes here))
Running on dhcp61i.psc.edu at Fri Jul 20 16:17:39 2007

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The Salk Institute for Biological Studies

MCell initializing simulation...
MCell[0]: random sequence 1
Defining molecules with the following diffusion constants:
  l_r_bar=0.0225675833 um for A
  l_r_bar=0.0225675833 um for B

Reaction probabilities generated for the following reactions:
  Probability 1.4716e-131 (s) set for A[l] @ transp_A[0] -> (TRANSPARENT)
  Probability 1.4716e-131 (s) set for B[l] @ transp_B[0] -> (TRANSPARENT)

MCell: world bounding box in microns =
[ -6.811044 -6.63711 -13.874858 ] [ 8.188946 4.612887 17.952663 ]
Creating 3375 subvolumes (15,15,15 per axis)
MCell: reading from checkpoint file chkpt
Checkpoint file was created with MCell Version 3.001
MCell: checkpoint sequence number 2 begins at elapsed time 1e-06 seconds
MCell: executing 1 iterations starting at iteration number 1.
Running...
Running simulation.
Iterations: 2 of 2
MCell: time = 2, writing to checkpoint file chkpt
Exiting run loop.
iterations = 2 ; elapsed time = 2e-06 seconds
Total number of random number use: 0
Total number of ray-subvolume intersection tests: 0
Total number of ray-polygon intersection tests: 0
Total number of ray-polygon intersections: 0
Total number of molecule-molecule collisions: 0
Total CPU time = 0.331949 (user) and 0.023996 (system)
Total wall clock time = 0 seconds
Done running.
[bbsi@dhcp61i new]$
```

MDL Commands

```
DEFINE_MOLECULES {  
    A {DIFFUSION_CONSTANT_3D = 1E-6}  
}
```

```
DEFINE_SURFACE_CLASSES {  
    transp_A {TRANSPARENT = A}  
}
```

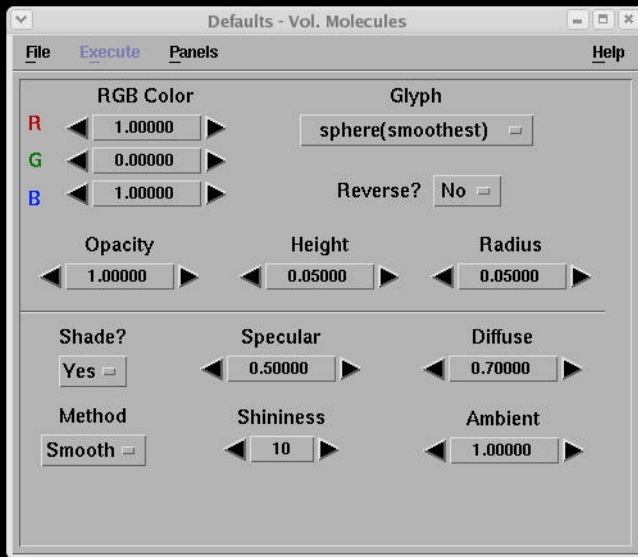
```
A_RELEASE_SITE  
{  
SHAPE = world.Sphere [whole_mesh]  
MOLECULE = A  
NUMBER_TO_RELEASE = 200  
}
```

Varying numbers in an MDL file

- ▶ Iterations
- ▶ Checkpoints
- ▶ Region Release
- ▶ Surface Classes
- ▶ Diffusion Constants

DReAMM (Design, Render & Animate Mcell Models)

- ▶ A computer aided design software
- ▶ Final visualization of Mcell simulations
 - Imports variety of mesh objects
- ▶ Variables such as opacity, shape, size can be altered
- ▶ Different 3D rotations and views (x, y, z axis)

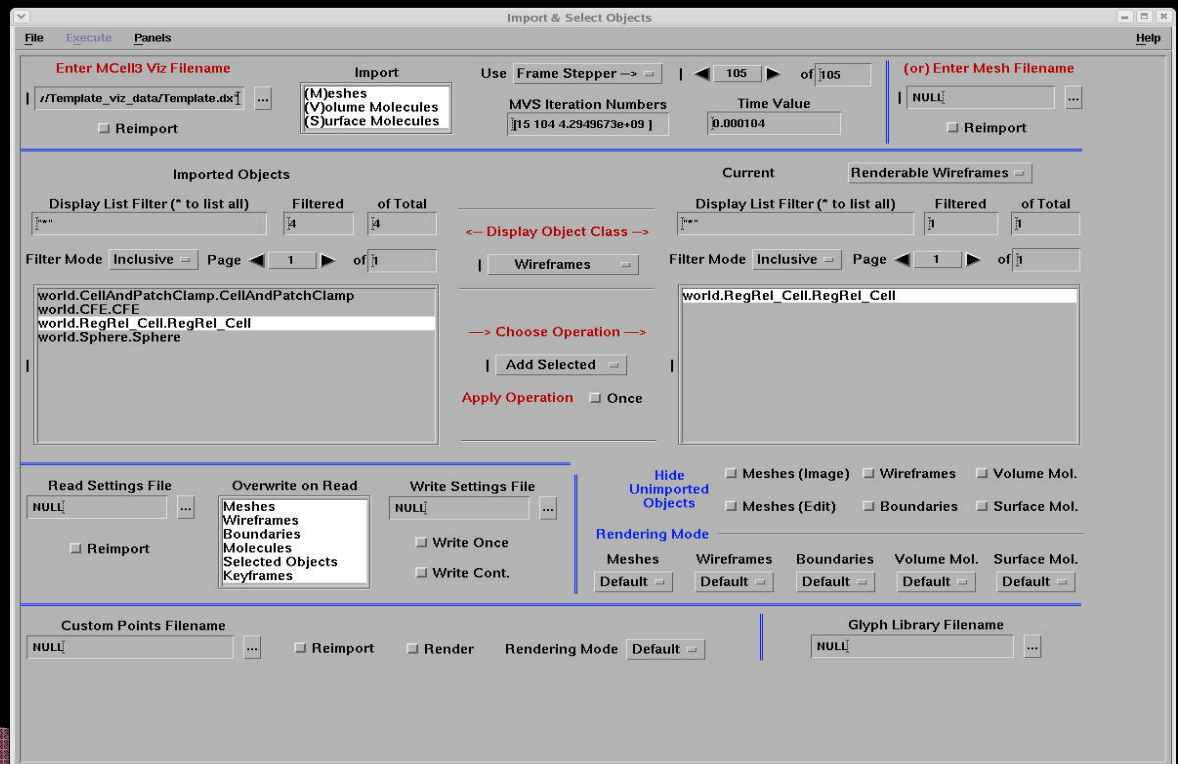


Import & Select Objects Menu

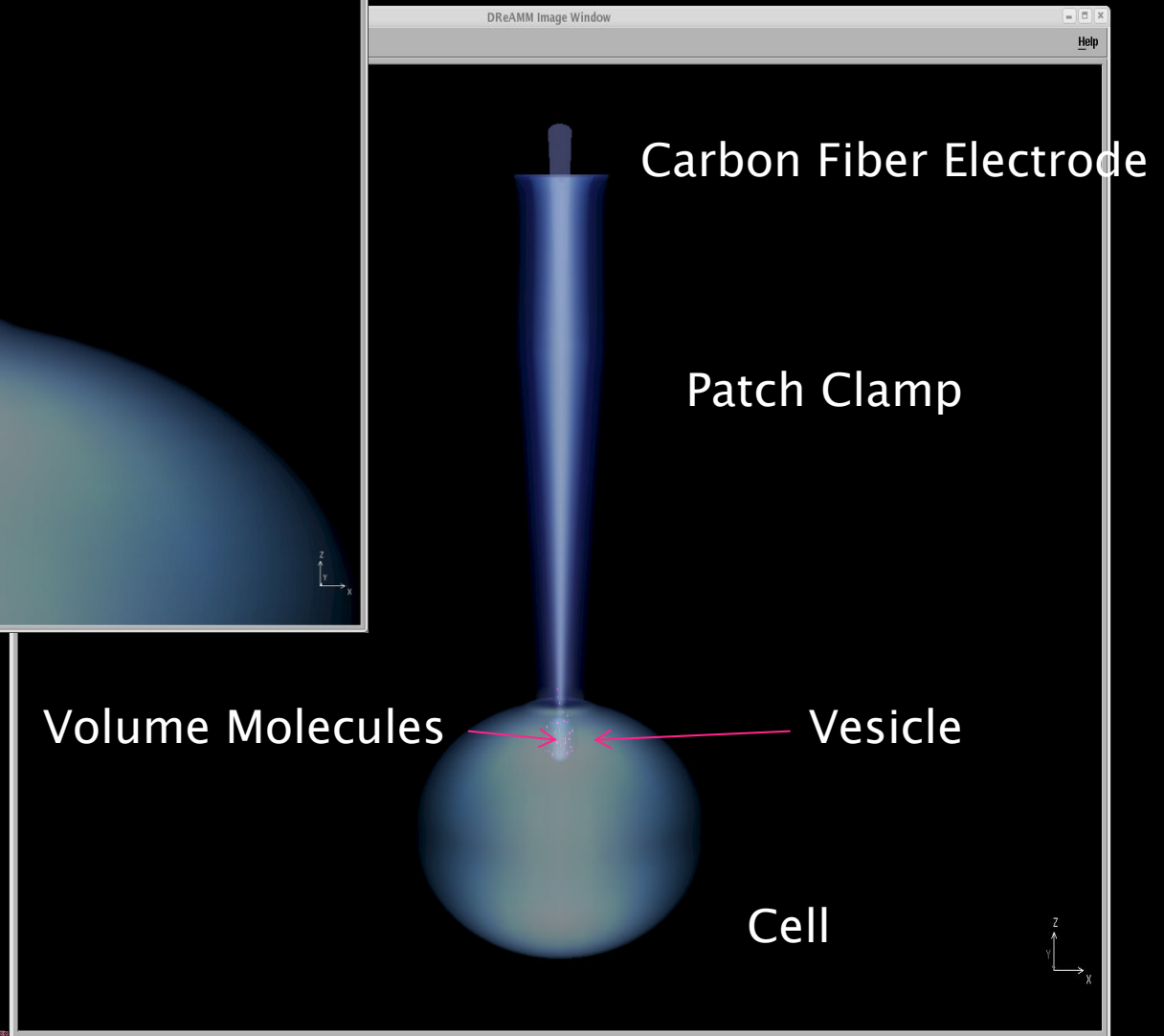
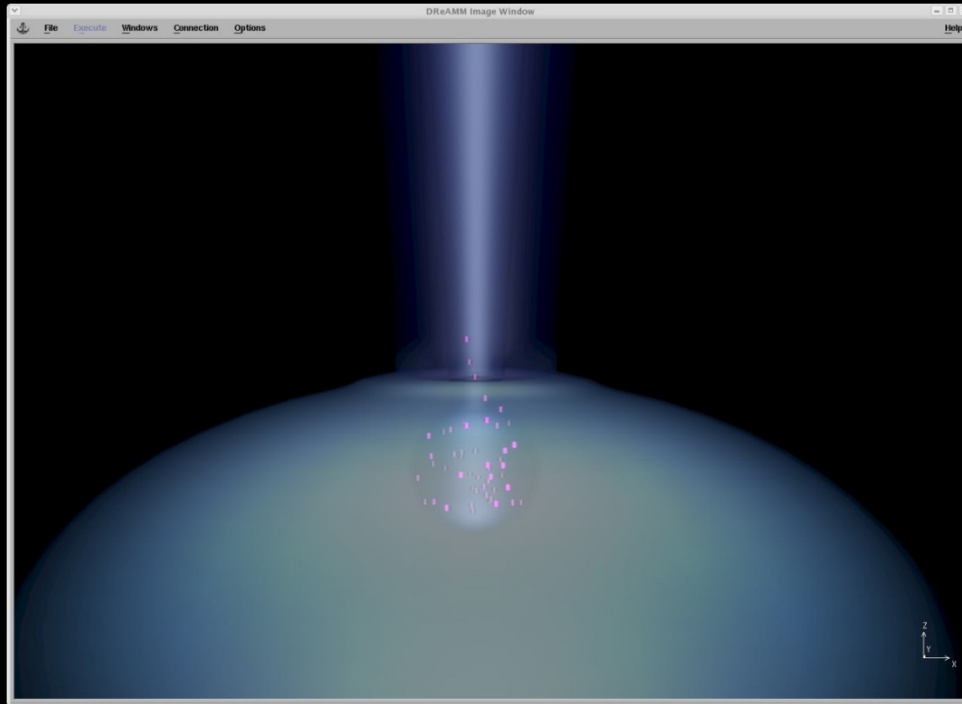
- Import viz data
- Select objects for editing
- Step through frames
- Edit meshes

Editing Volume Molecules

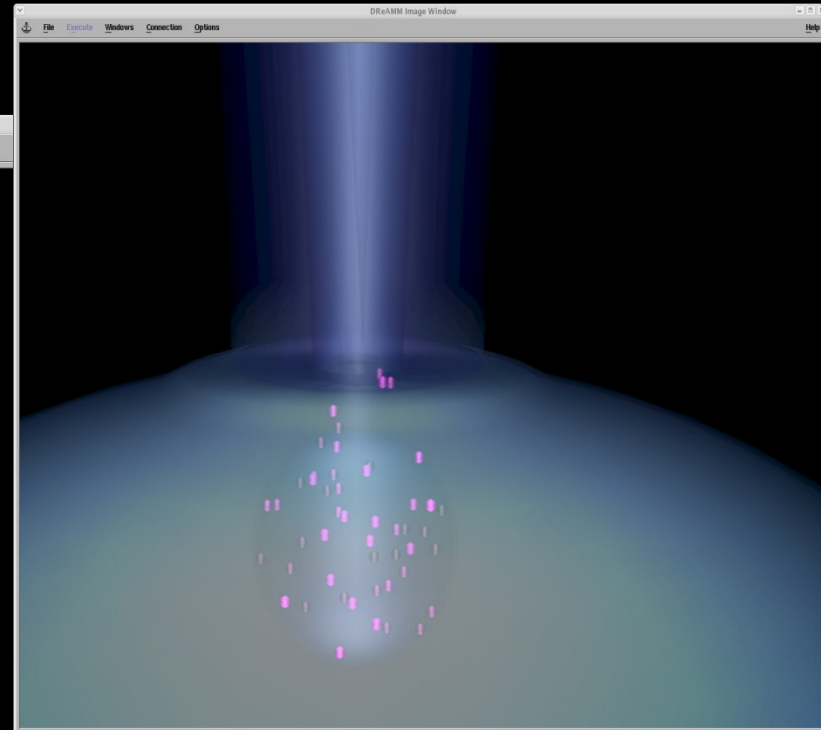
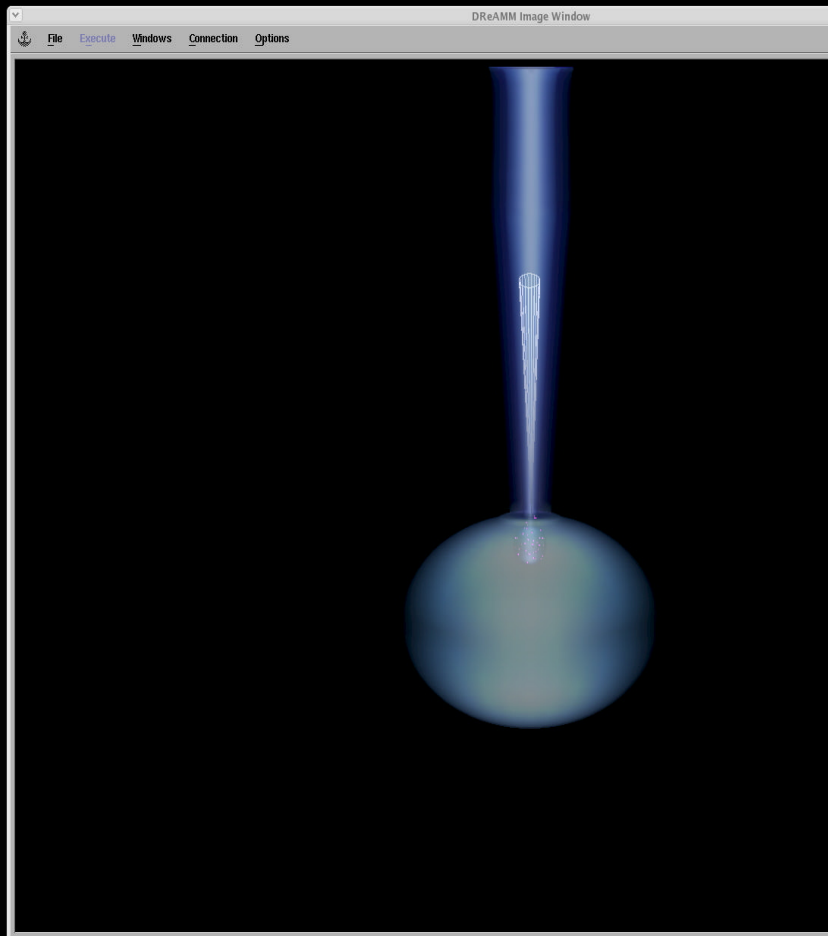
- color (red, blue, green)
- Shape
- Size
- opacity



Catecholamine Detection

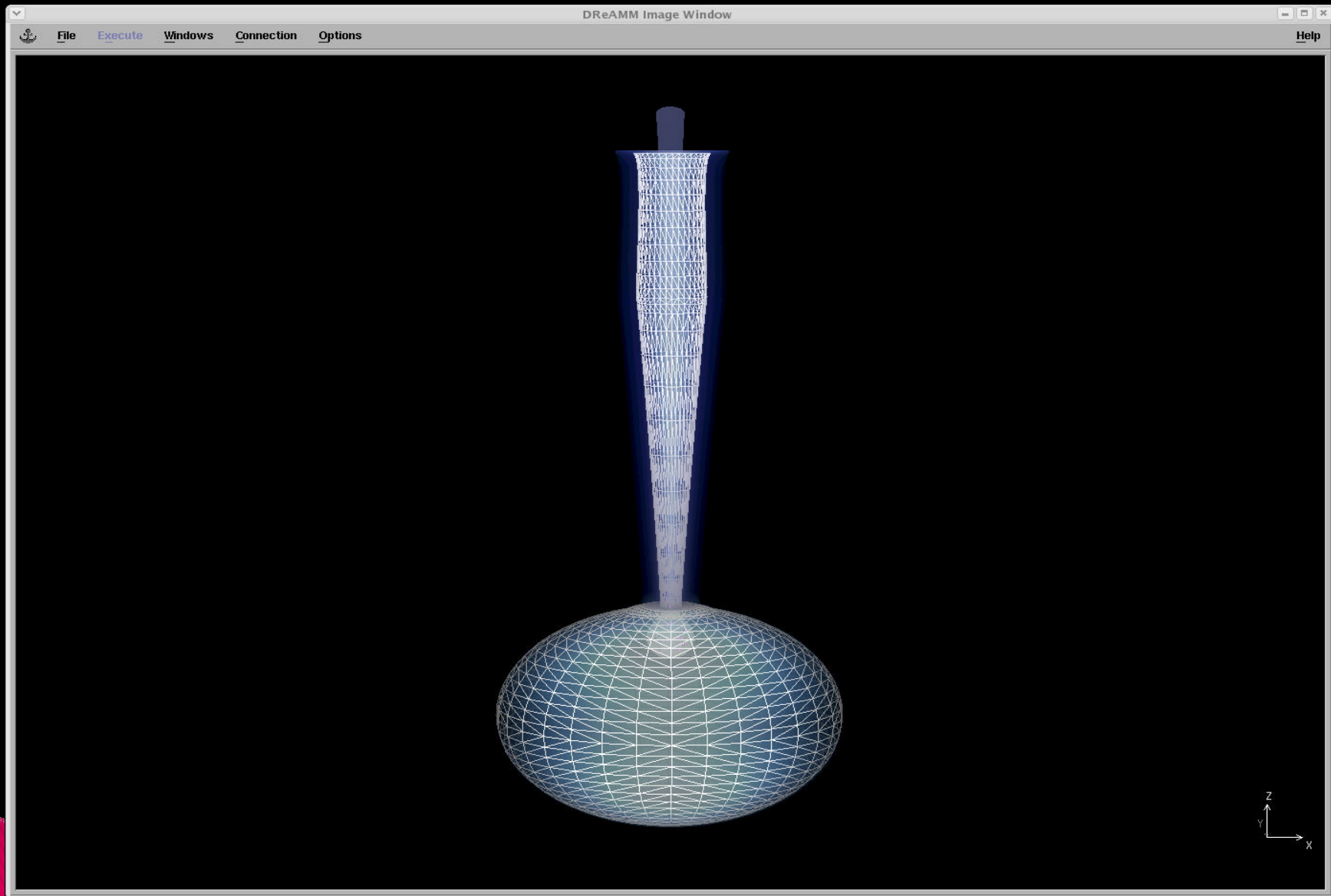


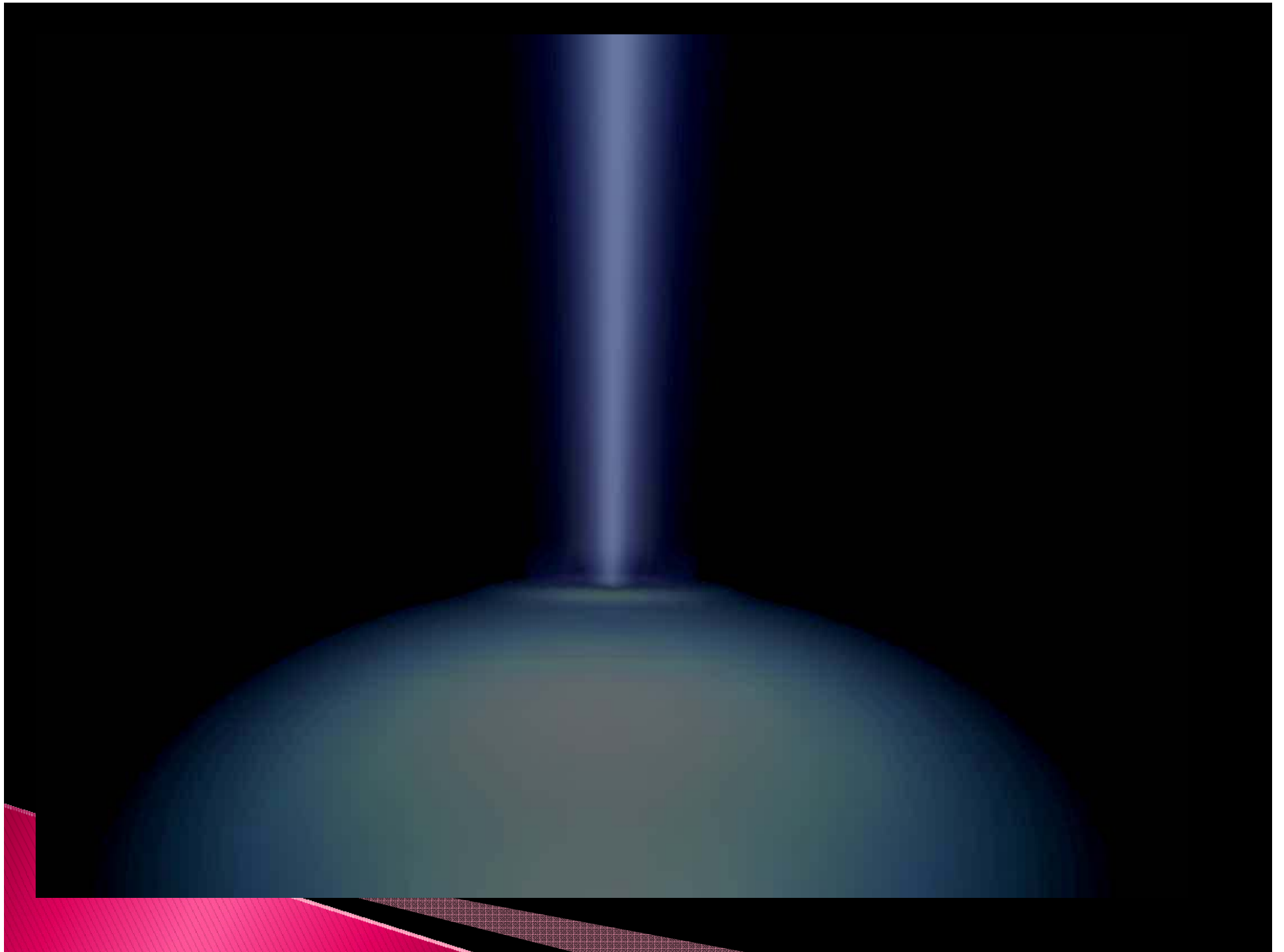
Carbon Fiber Electrode Varied



Number of molecules detected increases when the carbon fiber electrode is closer to membrane

Wireframes





Difficulties

▶ Blender/MCell

- Releasing on an unclosed region
- Syntax Errors
- Experimenting with number of iterations
- Undefined objects
- CANNOT join meshes once animation is defined
- EXTREMELY tedious

▶ DReAMM

- If errors in MDL file, no images displayed

Benefits

- ▶ Familiarity with the concept of patch clamp amperometry
- ▶ Knowledge of UNIX expanded
- ▶ Learned to work with the three new softwares: Blender, Mcell and DReAMM
- ▶ Used MDL which is similar to 'Object Oriented Programming'
- ▶ Acquired the art of having Patience !!!

Thank You!

- ▶ Acknowledgements:
 - Joel Stiles (MD/PhD)
 - Marcus Dittrich (PhD)
 - Jacob Czech

- ▶ Pittsburgh Supercomputing Center
(Carnegie Mellon University, University of Pittsburgh)