Fluorescence

Requirements

Models: FluorescenceExampleBegin.oml

Properties: FluorescenceExampleProperties.txt

Editions: TracePro Expert

Introduction

TracePro Expert is capable of modeling fluorescent material. Fluorescent material absorbs light in one wavelength band (excitation wavelengths) and emits light in another, longer, wavelength band (emission wavelengths). TracePro performs fluorescence modeling by using a combination of Fluorescence Properties and a specialized two stage ray tracing sequence.

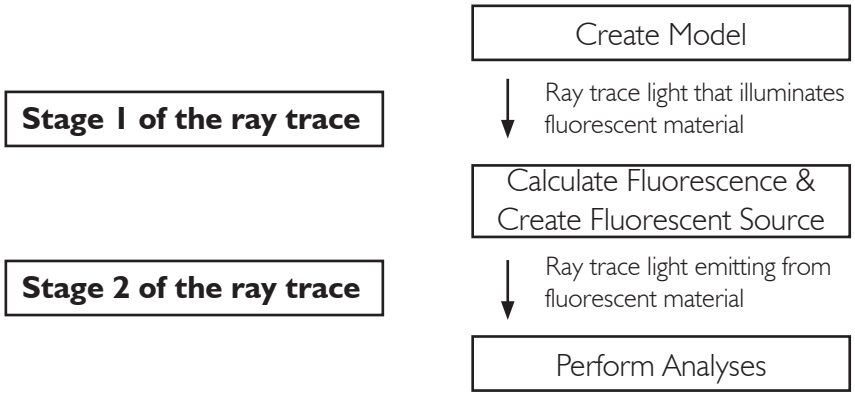


Figure 1. Fluorescence modeling sequence showing two stage ray trace

In the first stage of the ray trace, excitation rays illuminate the fluorescent material. The rays must be in a non-zero part of the excitation spectrum and the model geometry must allow the fluorescent material to be illuminated. A byproduct of this first stage of the ray trace is that TracePro automatically creates ray files that contain rays generated by the fluorescing material. The second stage of the ray trace uses the newly created ray files to trace the fluorescing rays themselves. TracePro allows you to choose whether the two stages of the ray trace should be performed together (where TracePro starts the second stage of the ray trace immediately after the first stage is complete) or separately (where the you can use the stored fluorescent source files to perform the second stage of the ray-trace at a later time).

Fluorescence Properties

Fluorescence characteristics are modeled using Fluorescence Properties in TracePro. This allows you to define fluorescence properties separately from material properties and then apply fluorescence properties in differing amounts to different materials. Since Fluorescence Properties are defined separately from Material Properties, fluorescence is defined in the Fluorescence Property Editor (**Define|Edit Property Data>Fluorescence Properties**). Clicking on the *Add Property* button creates a new fluorescence property with the following characteristics:

1. Conversion efficiency value
2. Peak molar extinction value
3. In the Excitation Table:
    a. Wavelength
    b. Temperature
    c. Relative Absorption
    d. Relative Excitation
4. In the Emission Table:
    a. Wavelength
    b. Temperature
    c. Relative Emission

NOTE: If you have any trouble editing these values, make sure that the property is unlocked for editing.

The relative absorption, relative excitation, and relative emission values are normalized. An example Absorption/Excitation tab is shown in Figure 2, and an example Fluorescence Emission Table is shown in Figure 3.

You can also use the Fluorescence Property Generator to more easily create Fluorescence Properties from measured data or manufacturer specifications.

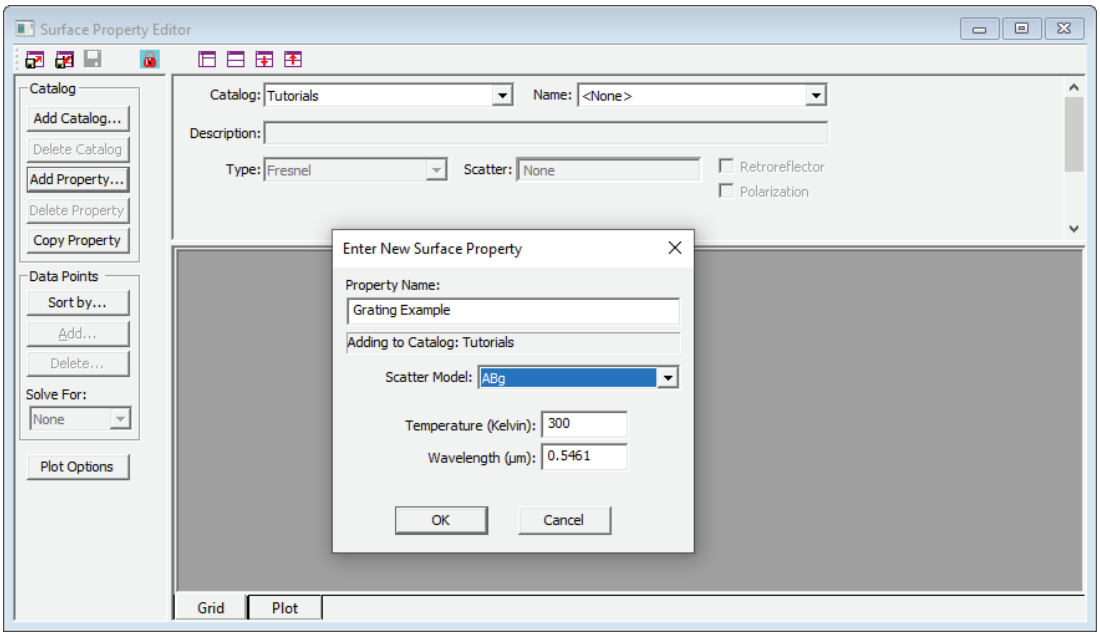


Figure 2. Example Absorption/Excitation Table.

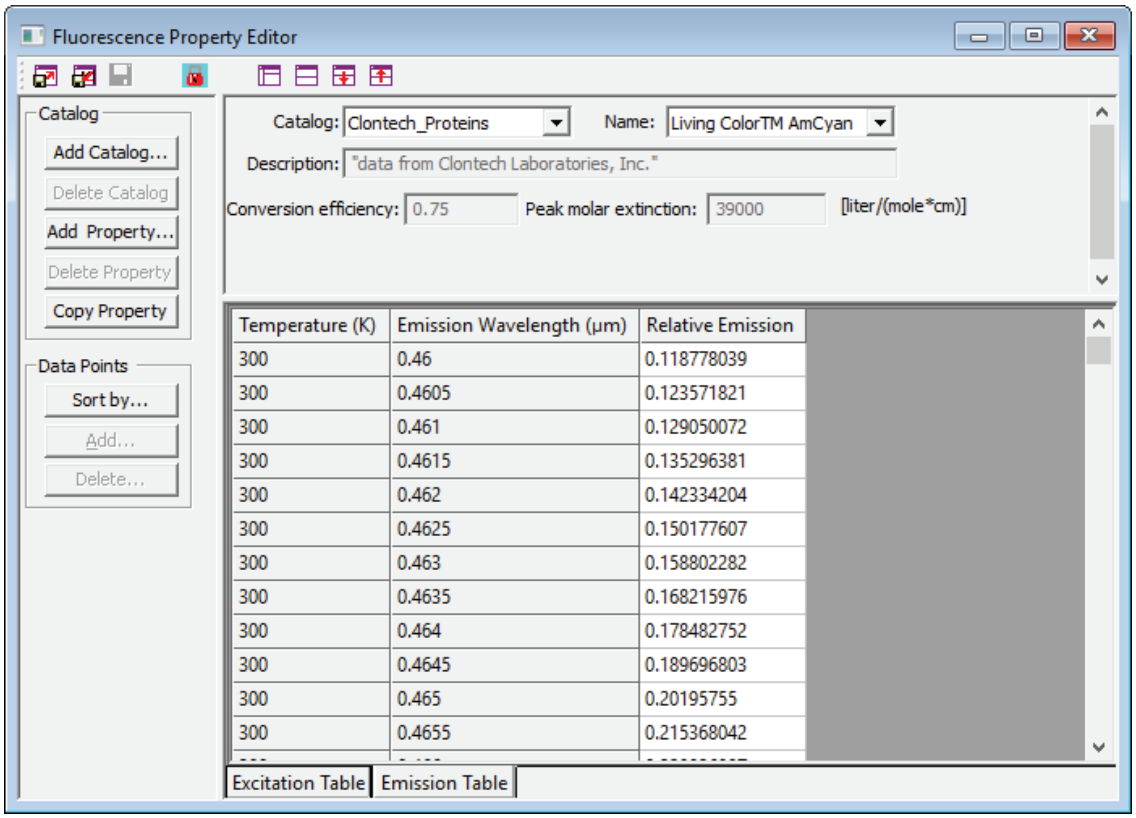


Figure 3. Example Fluorescence Emission Table.

It is customary, in measuring flourescence spectra, to express the peak molar extinction in base 10 rather than base e. The base 10 absorption coefficient is then

$$\mu_a^{10}(\lambda) = ab(\lambda)K_{peak}C_{molar}$$

where K_{peak} is the peak molar extinction corresponding to the value of 1 in the relative absorption $ab(\lambda)$, and C_{molar} is the molar concentration in the particular sample. The transmittance through a sample of thickness t is then

$$\tau = 10^{-\mu_a^{10}t}$$

The absorption coefficient used in a non-fluorescent material property in TracePro is related to the base 10 absorption coefficient by

$$\mu_a = \mu_a^{10} \ln 10.$$

This is used for Lambert/Beer Law absorption, in which the transmittance through a thickness t is

$$\tau = e^{-\mu_a t}$$

The optics absorption coefficient μ_a is computed internally in TracePro for use in the raytrace. The same applies to the relative excitation values.

Raytrace Options

The generation of fluorescence rays can be enabled via a checkbox in the **Raytrace Options** dialog box, *Options* tab. Accompanying the *Fluorescence* checkbox is an *Insert file source* checkbox and a dropdown list with the choices *Generate emission source only* and *Immediately trace emission wavelengths*. The *Insert file source* checkbox allows you to link the generated fluorescence ray files to your model; this link will be saved with the model. The choice in the drop-down list dictates whether the two stages of the fluorescence raytrace are performed separately or together.

Fluorescence ray trace

A fluorescence ray trace is done in two stages:

Stage 1: Initial rays are traced in the TracePro model. Any wavelengths defined in the Wavelength tab of the *Apply Properties | Surface Source* dialog box (that also happen to be within the excitation band of fluorescent materials in the model) will generate fluorescence rays. The end result of this stage is that ray files containing fluorescent ray data are created.

Stage 2: Fluorescence rays are traced from the previously generated ray files at the emission wavelengths defined in the *Apply Properties | Fluorescence* dialog box.

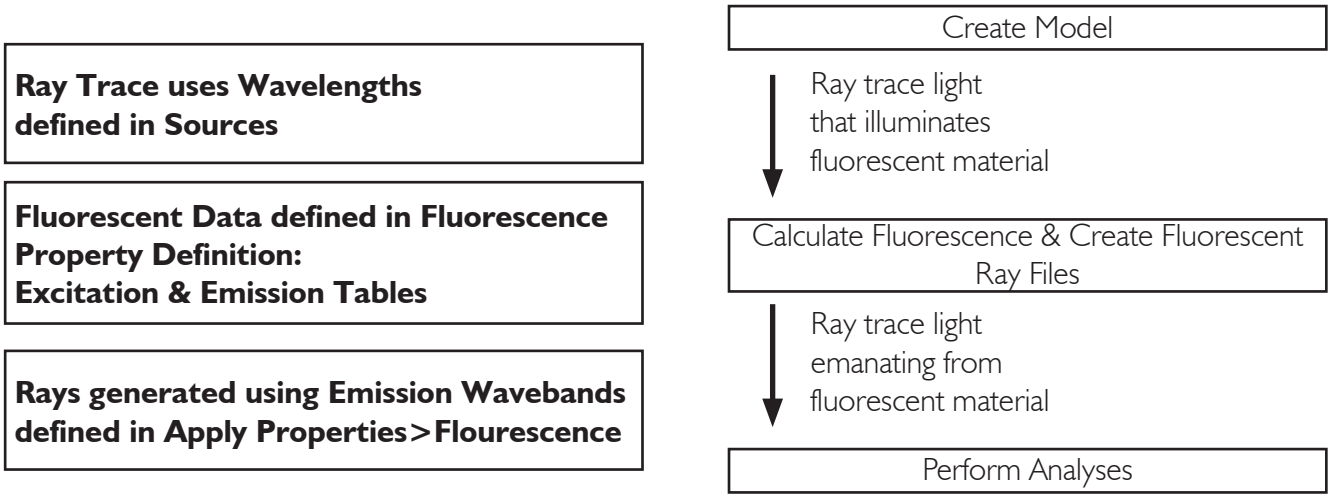


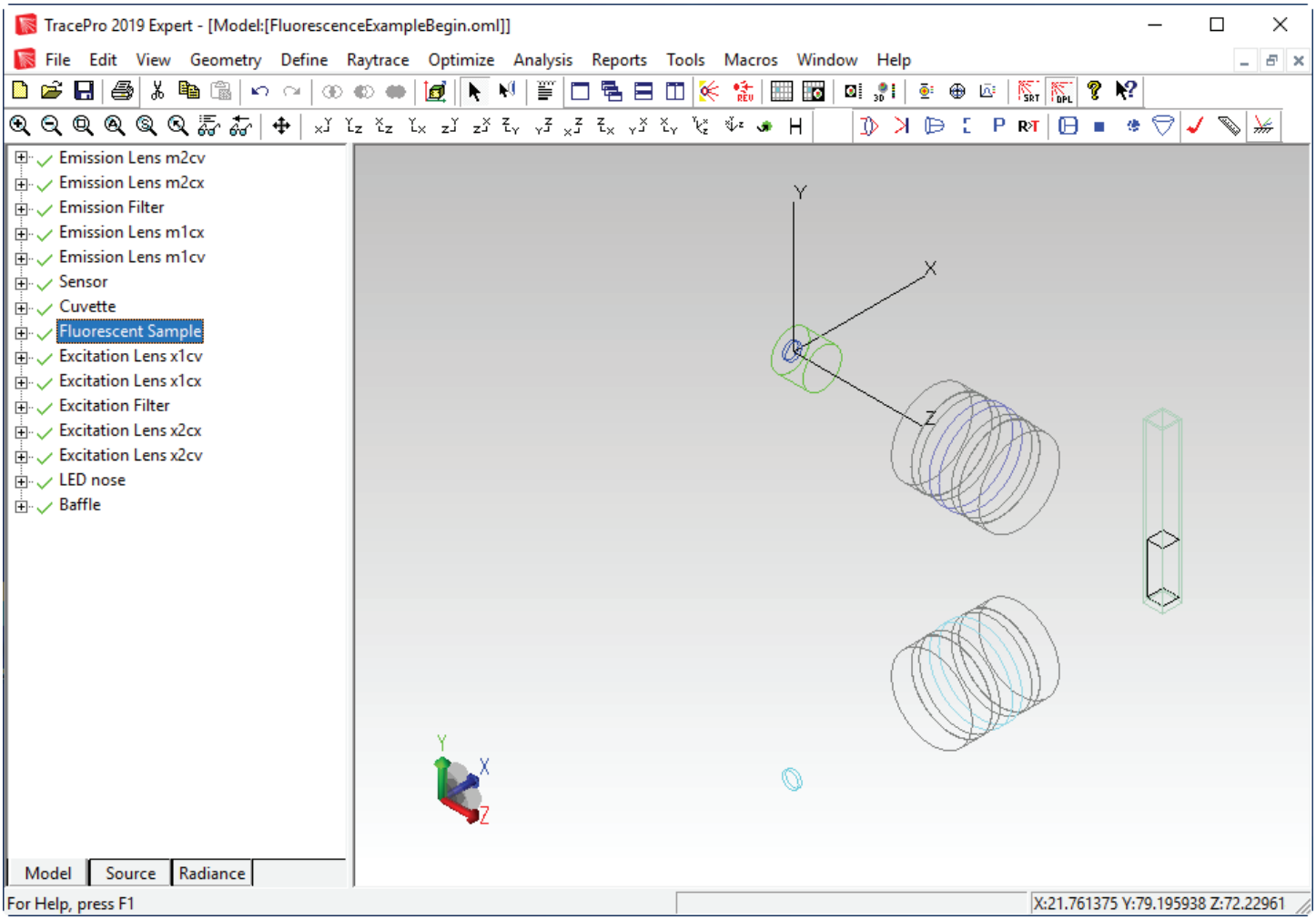
Figure 4. Fluorescence modeling sequences showing where data is drawn from each step in the process.

You can trace fluorescence rays in either of two ways:

1. Immediately trace emission wavelengths
   In this method, at the conclusion of the first stage of the raytrace (the excitation raytrace), the second stage (the emission ray-trace) will automatically begin, so that emission rays are "mixed in" with the excitation rays. All irradiance map features, candela plots, flux report, etc. report the fluorescence wavelength results along with the excitation wavelength results.
2. Generate emission sources only
   In this method, the emission ray files are generated, but the emission rays will not be traced. You can trace them later at your discretion, by:
   a. Inserting the emission ray file into the model as a File Source.
   b. Excluding individual sources from the TracePro source tree (optional: you may want to keep sources in the analysis).
   c. Unchecking the Fluorescence option in the **Raytrace Options** dialog box.
   d. Initiating a ray-trace, making sure that the fluorescence File Source is enabled in the trace.
   e. Exclude/include emission wavelengeths as desired using the Source/Wavelength Selector on the Raytrace menu.

Fluorescence modeling example

This simple model has a cuvette containing fluorescent material that is illuminated by light at excitation wavelengths of the material. The material then fluoresces, generating a ray file containing fluorescent rays. Open the model **FluorescenceExampleBegin.oml**, shown in isometric view below.

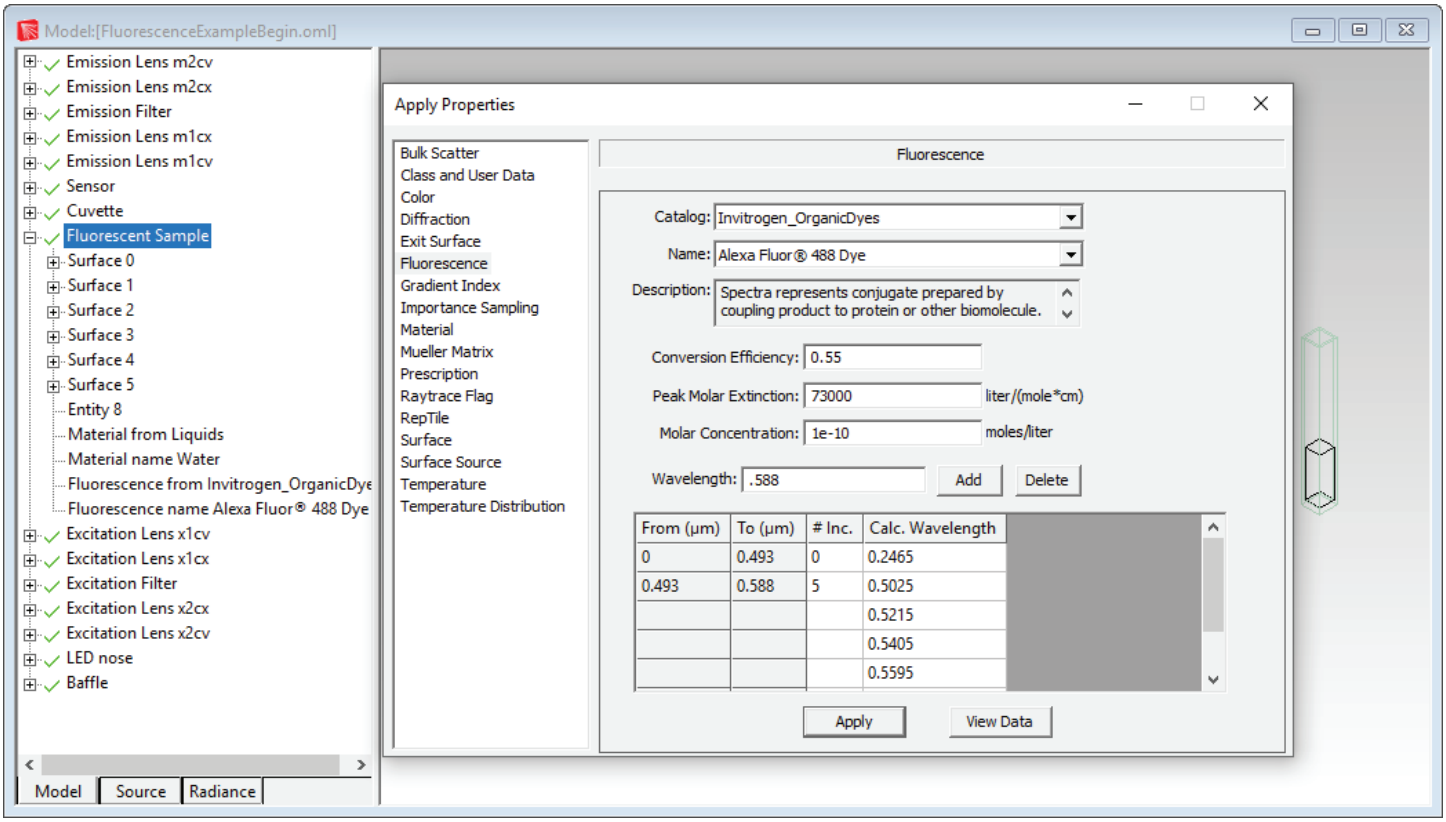


The cuvette consists of a glass envelope and water with a fluorescent dye inside. To add fluorescent properties to the contents of the cuvette, we first select the object Fluorescent Sample in the model tree, then select **Define|Apply Properties**, *Fluorescence* tab, and select a fluorescing property to apply. We will select the *Alexa Fluor® 488 Dye* property from the *Invitrogen_OrganicDyes* catalog as shown.

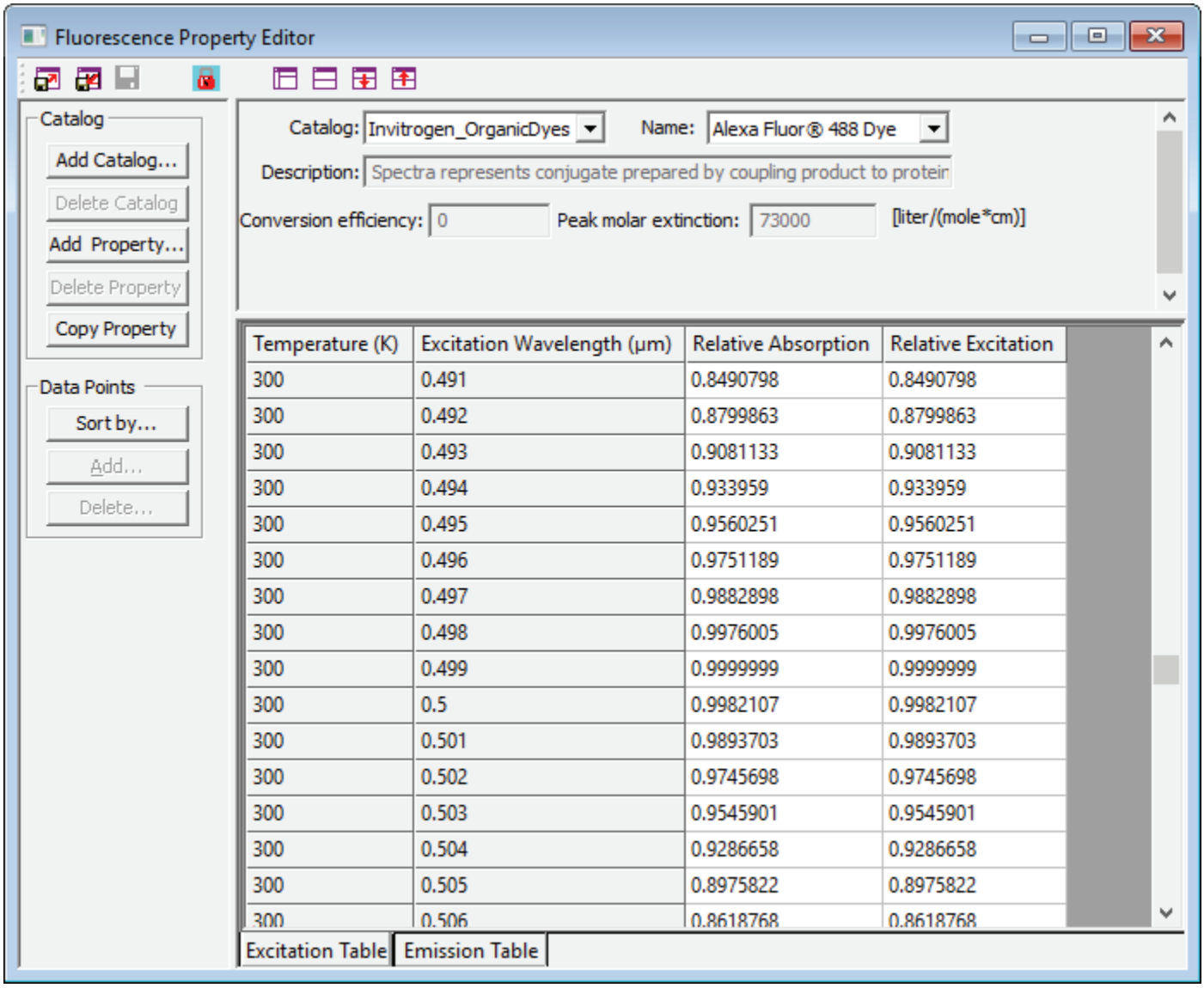
Enter the *Conversion Efficiency*, *Peak Molar Extinction* and *Molar Concentration* as shown in the dialog box.

Add wavelengths .493 and .588 um to the wavelength table. Set the emission wavelength range from .493 to .588 um to have 5 increments. Set the wavelength ranges from 0 to .493 and from .588 to INF to have 0 bands.

Click Apply to apply the fluorescence property.

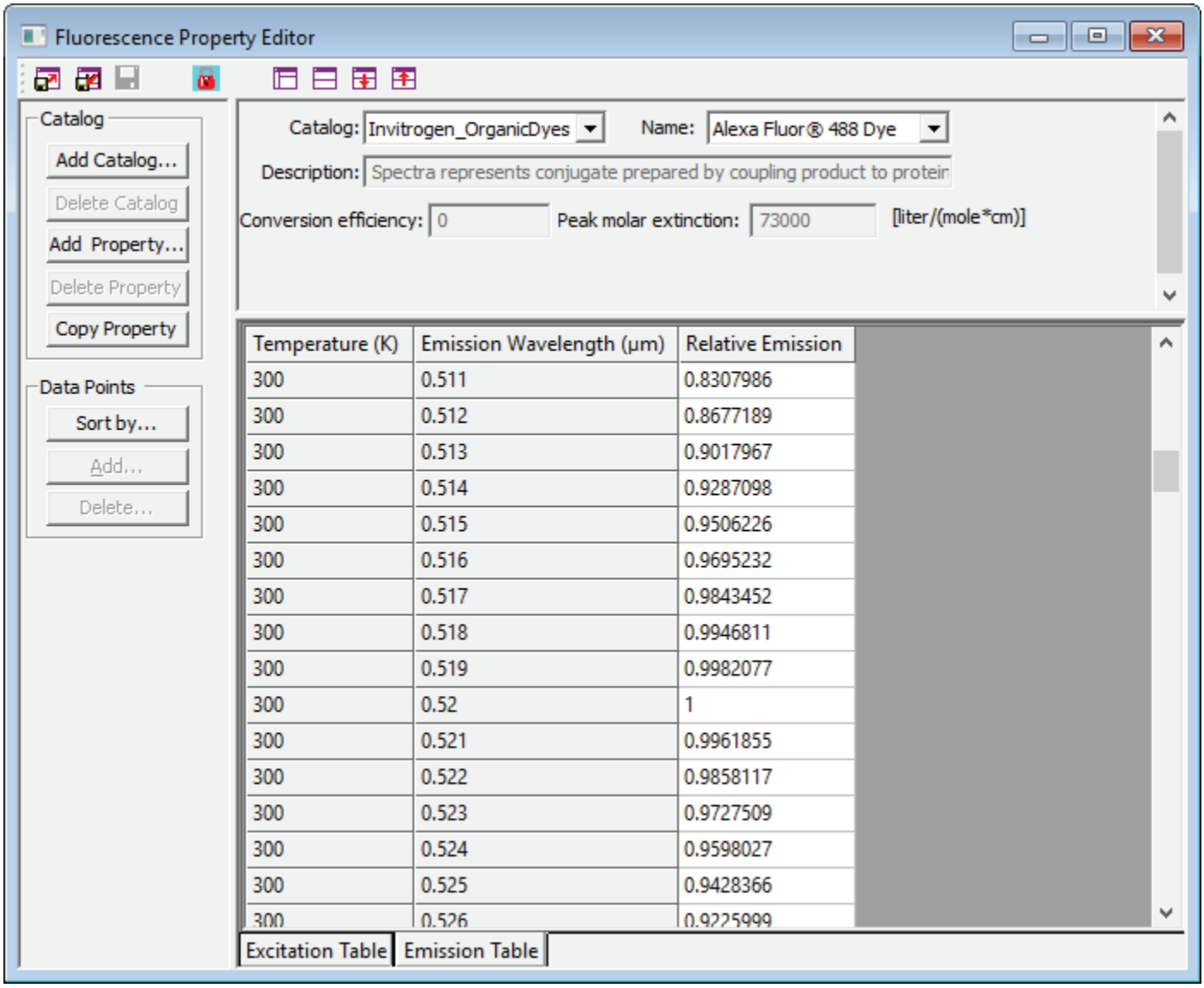


Before closing the *Apply Properties* dialog box, click the *View Data* button. This will open the *Fluorescence Property Editor* and display the properties of the *Alexa Fluor® 488 Dye*. Select the *Excitation Table* tab at the bottom of the editor. By scrolling down in the table you can see that the *Relative Excitation* of this material has its peak (a value of approximately 1.0) at a wavelength of 0.499 μm as shown. We will use this wavelength for the excitation ray trace.



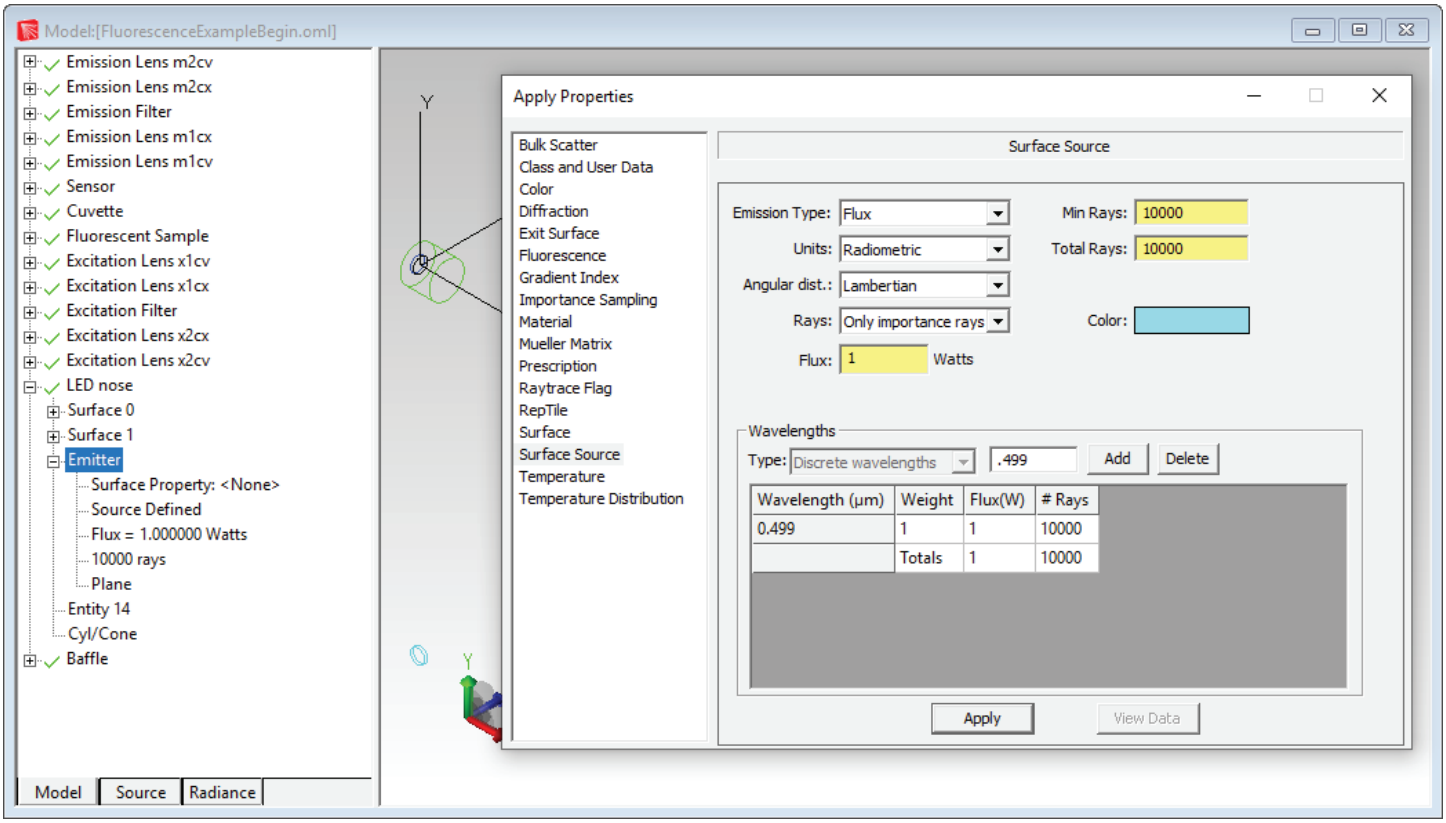
Temperature (K)	Excitation Wavelength (µm)	Relative Absorption	Relative Excitation
300	0.491	0.8490798	0.8490798
300	0.492	0.8799863	0.8799863
300	0.493	0.9081133	0.9081133
300	0.494	0.933959	0.933959
300	0.495	0.9560251	0.9560251
300	0.496	0.9751189	0.9751189
300	0.497	0.9882898	0.9882898
300	0.498	0.9976005	0.9976005
300	0.499	0.9999999	0.9999999
300	0.5	0.9982107	0.9982107
300	0.501	0.9893703	0.9893703
300	0.502	0.9745698	0.9745698
300	0.503	0.9545901	0.9545901
300	0.504	0.9286658	0.9286658
300	0.505	0.8975822	0.8975822
300	0.506	0.8618768	0.8618768

Select the *Emmision Tabl*e tab in the editor and note that the material fluoresces between wavelengths of about 0.475 to 0.675 mm (a portion of the table is shown below).



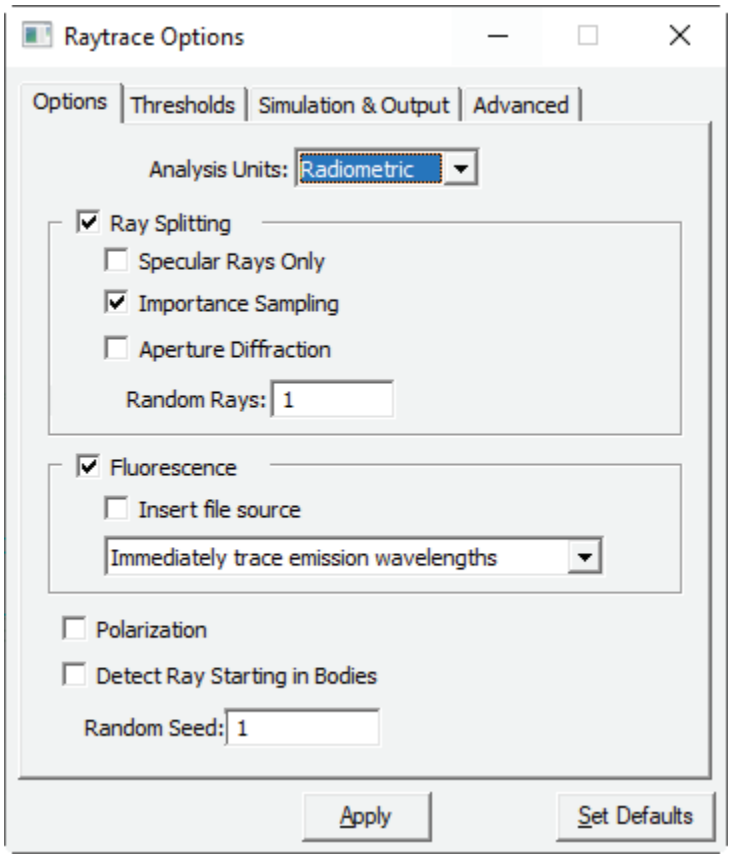
Temperature (K)	Emission Wavelength (µm)	Relative Emission
300	0.511	0.8307986
300	0.512	0.8677189
300	0.513	0.9017967
300	0.514	0.9287098
300	0.515	0.9506226
300	0.516	0.9695232
300	0.517	0.9843452
300	0.518	0.9946811
300	0.519	0.9982077
300	0.52	1
300	0.521	0.9961855
300	0.522	0.9858117
300	0.523	0.9727509
300	0.524	0.9598027
300	0.525	0.9428366
300	0.526	0.9225999

To enter the wavelength data for the excitation light and the fluorescence emission light, select the Emitter surface of the object named LED nose, right-click, choose **Properties**, and view the *Surface Source* page. The model is currently set to trace at a *Wavelength* of .5461 μm, but we want to trace an Excitation *Wavelength* of .499 μm. Select .5461 in the table and click the *Delete* button, then type .499 and click the *Add* button. Click *Apply*.



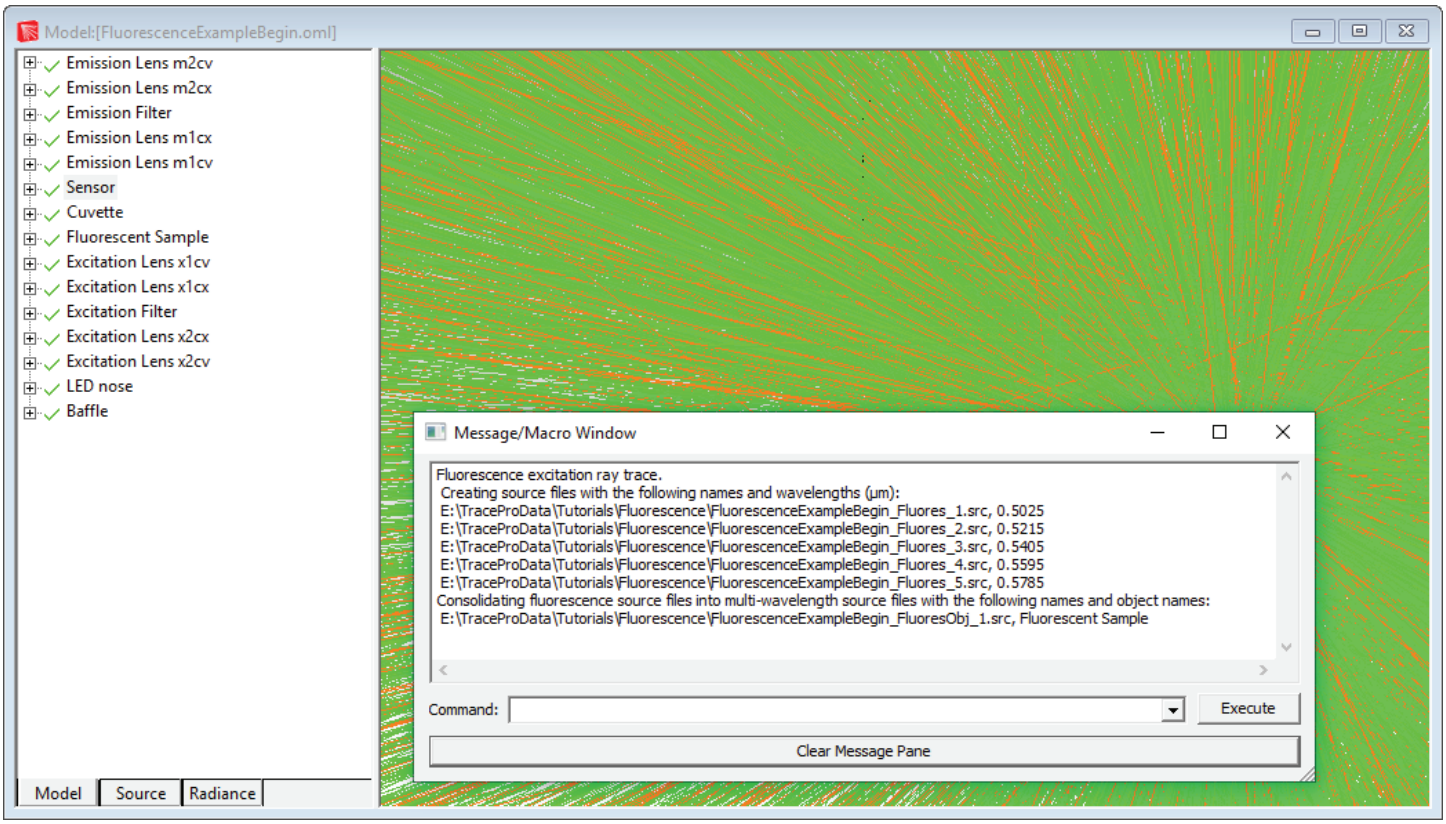
Select the *Options* tab of the *Raytrace Options* dialog box (Raytrace Menu) and click the *Fluorescence* checkbox. In the list under the checkbox, ignore the *Insert File Source* checkbox and select *Immediatley trace emission wavelengths*, and click *Apply* to set the selections. The dialog box should appear as below. By making this selection, we are choosing the two stages of the fluorescence ray trace to be run right after one another. We will trace rays at the excitation wavelength, TracePro will generate fluorescing sources corresponding to the data in the Fluorescence emission wavebands and then immediately perform a second ray trace to trace the fluorescing rays.

Now we are ready to do the Fluorescence ray-trace. Close all dialog boxes and windows if you have not already done so.

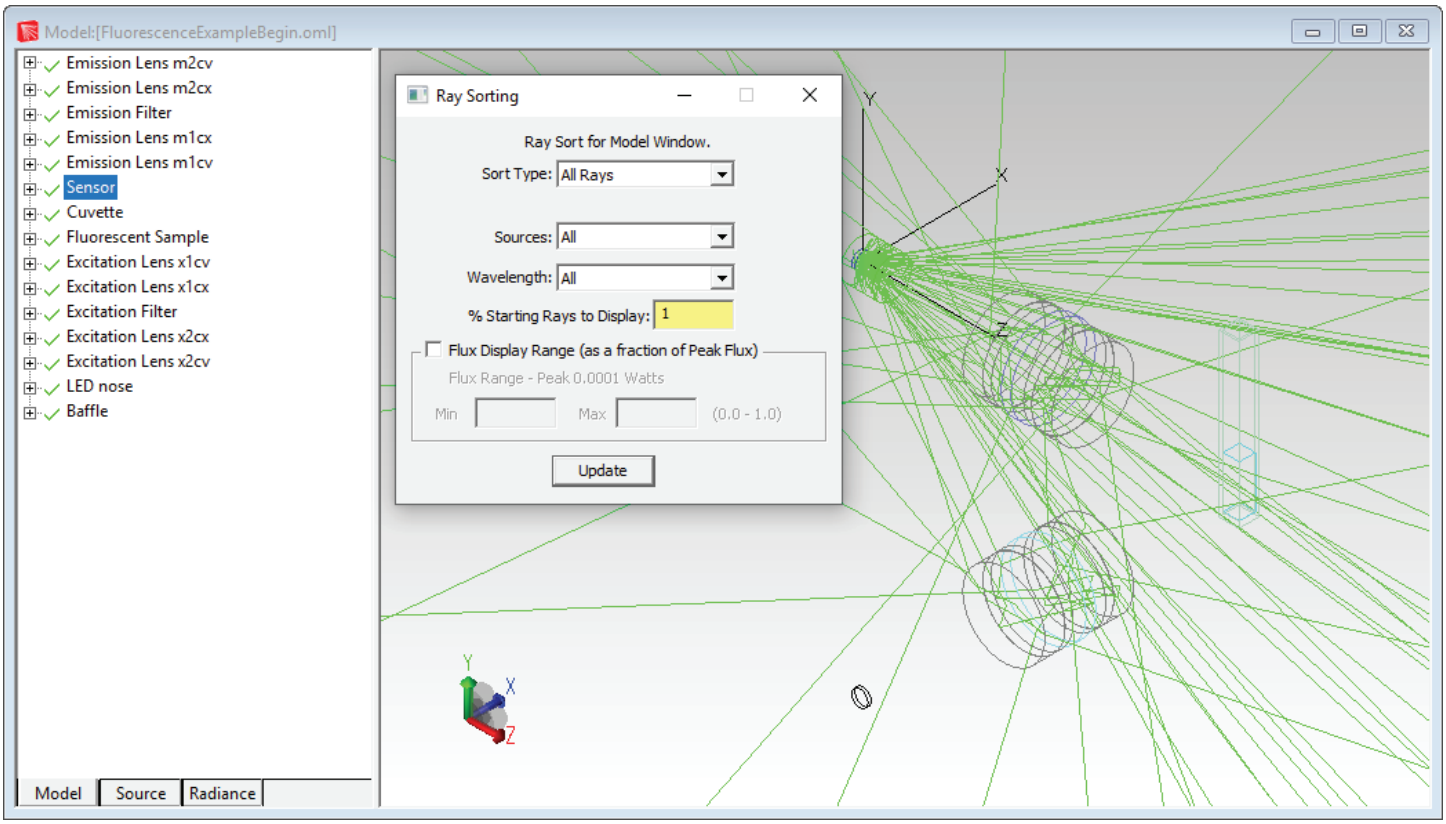


To begin the ray trace, select **Raytrace|Trace Rays**. TracePro traces the excitation rays and saves the source of the emitted fluorescent rays in a binary source file with extension *.src. The *Message/Macro Window* indicates that the ray file was created for the fluorescence emission rays. In the second stage of the raytrace, rays are emitted from the fluorescence emission ray file.

Viewing the model after the rays have been traced is not very useful. There are so many rays that the model is difficult to see.

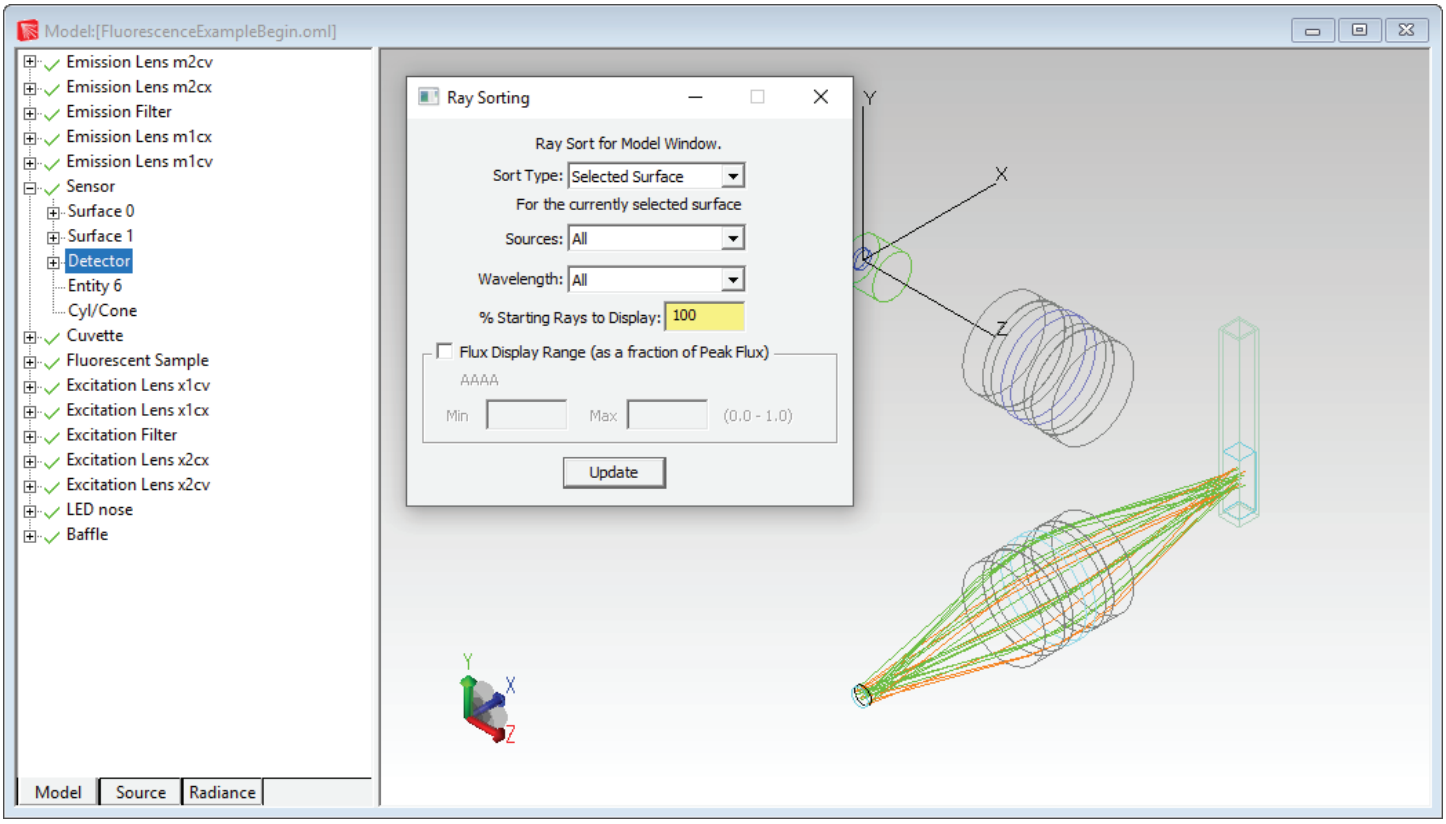


You can reduce the number of rays shown in the model using the *Ray Sorting* dialog box (**Analysis|Ray Sorting**). Setting the % *Starting Rays to Display* to 1% will give you a ray display that looks as shown below.



Analysis

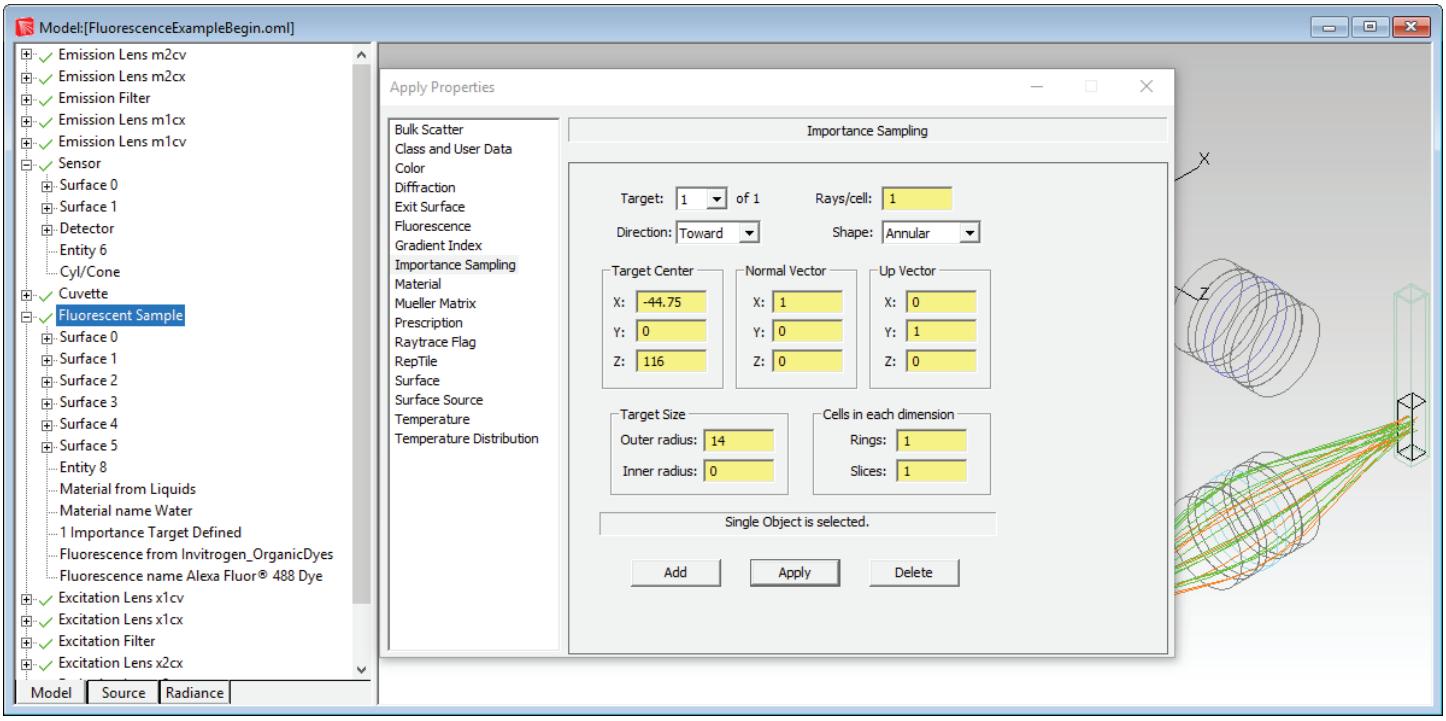
We are mostly interested in rays that hit the detector. We can show these rays by first selecting the Detector surface on the Sensor object, then selecting **Analysis|Ray Sorting** to open the *Ray Sorting* dialog. For *Sort Type*, select Selected Surface, set the % of rays to 100, and then click *Update*. The display will appear as in the figure below. We have traced only a few rays for this example, so only a few rays are shown hitting the detector. An accurate simulation would require many more rays.



We can display all output analyses in TracePro as for any other ray trace (irradiance map, flux report, incident ray table, ...etc), but at this point there are so few rays to display, it will not be useful. Next we will concentrate on making more rays reach the detector.

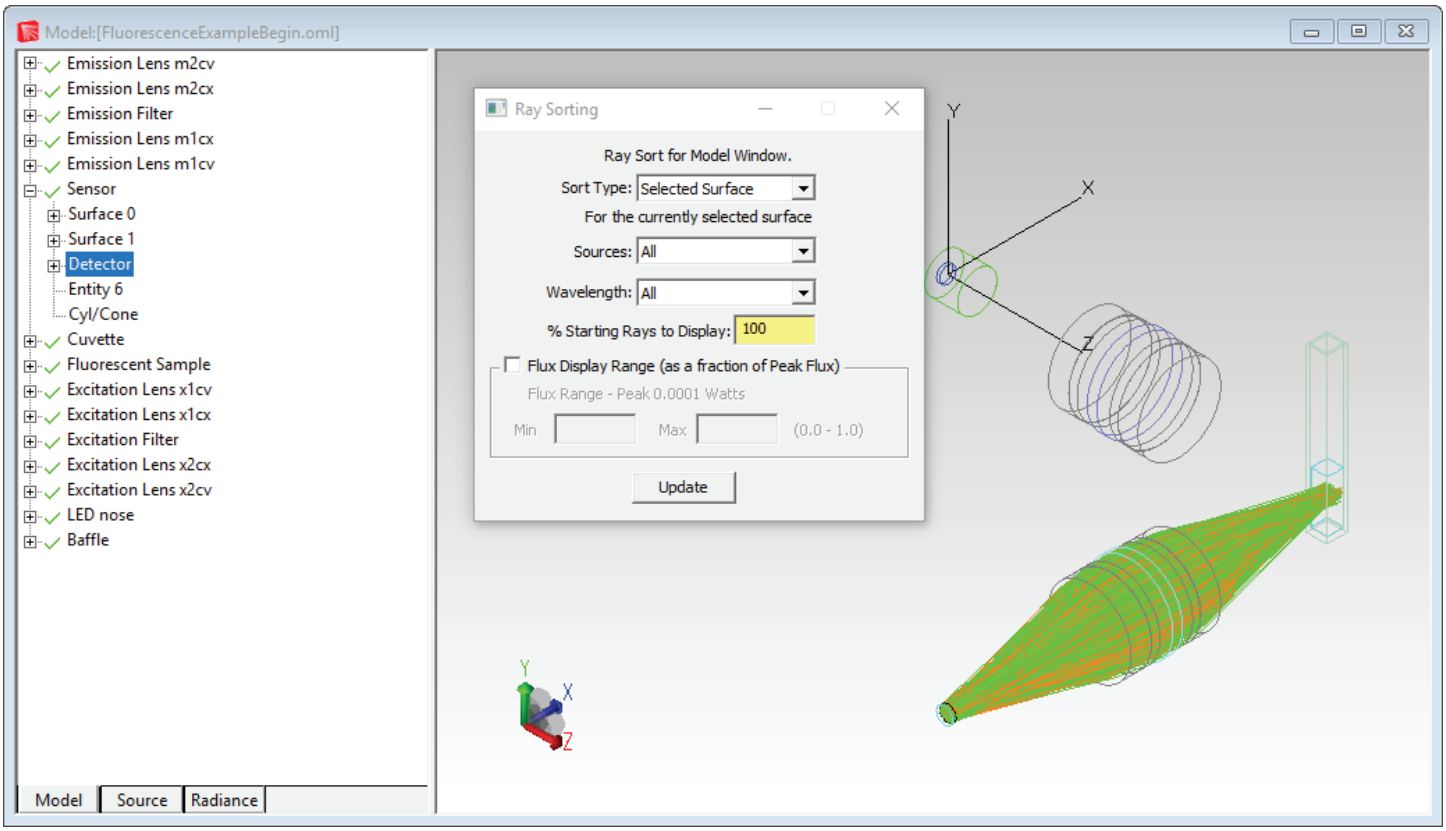
Adding importance sampling

We can increase the number of rays reaching the detector by simply tracing more rays in the excitation ray-trace, but we can also specify importance sampling for the fluorescence emission rays to get better sampling at the detector. This means that whenever a ray in the excitation ray-trace (stage 1 of the fluorescence ray trace) generates a fluorescence ray for a file source, one or more importance sampled rays will be generated also directed toward importance sampling targets. To generate importance-sampled fluorescence emission rays, we must apply an importance sampling target to the object* that has the fluorescence property applied to it. To do this, select the object named Fluorescent Sample in the system tree. Select **Define|Apply Properties**, *Importance Sampling* page. We wish to importance sample towards the lenses leading to the detector, so enter the importance sampling target data shown below, and click *Apply*.



* The importance sampling should be applied to the object itself (not just individual surfaces of the object) because fluorescence is an object (not a surface) property.

Perform a ray-trace by selecting **Raytrace|Trace Rays**. The ray-trace will begin as before, and a new ray file will be generated, over-writing the previous one. Now select **Analysis|Ray Sorting** and sort rays on the detector surface, as before. The resulting ray display is shown below. Many more rays now reach the detector.



A log scale irradiance map shows that over 1000 rays now reach the Detector surface of the Sensor object.