

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.

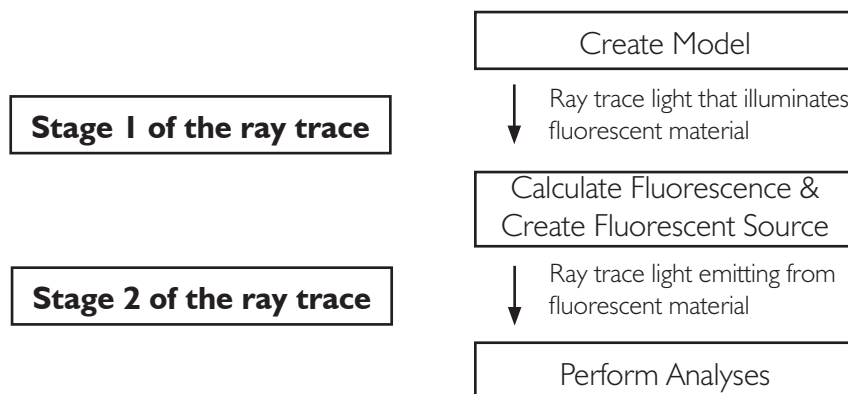


Figure 1. Fluorescence modeling sequence showing two stage ray trace

In the first stage of the ray trace, excitation rays illuminate the fluorescent material. The rays must be in the excitation portion of the fluorescent material 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 source files which contain rays generated by the fluorescing material. The second stage of the ray trace uses the newly created source 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 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 a devoted set of Fluorescence Properties in TracePro. This allows users 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 that you can define:

- 1) Quantum Efficiency value
- 2) Peak molar extinction value
- 3) In the Excitation Table:
 - a. Wavelength (this is added via the **Add** button)
 - b. Temperature (this is added via the **Add** button)
 - c. Relative Absorption (these values are directly editable)
 - d. Relative Excitation (these values are directly editable)
- 4) In the Emission Table:
 - a. Wavelength (this is added via the **Add** button)
 - b. Temperature (this is added via the **Add** button)
 - c. Relative Emission (these values are directly editable)

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.

Temperature (K)	Excitation Wavelength (um)	Relative Absorption	Relative Excitation
300	0.3	.126659975	.126659975
300	0.3005	.123870838	.123870838
300	0.301	.12080331	.12080331
300	0.3015	.117613769	.117613769
300	0.302	.114172525	.114172525
300	0.3025	.110539824	.110539824
300	0.303	.106732441	.106732441
300	0.3035	.102760119	.102760119
300	0.304	.098635954	.098635954

Figure 2. Example Absorption/Excitation Table.

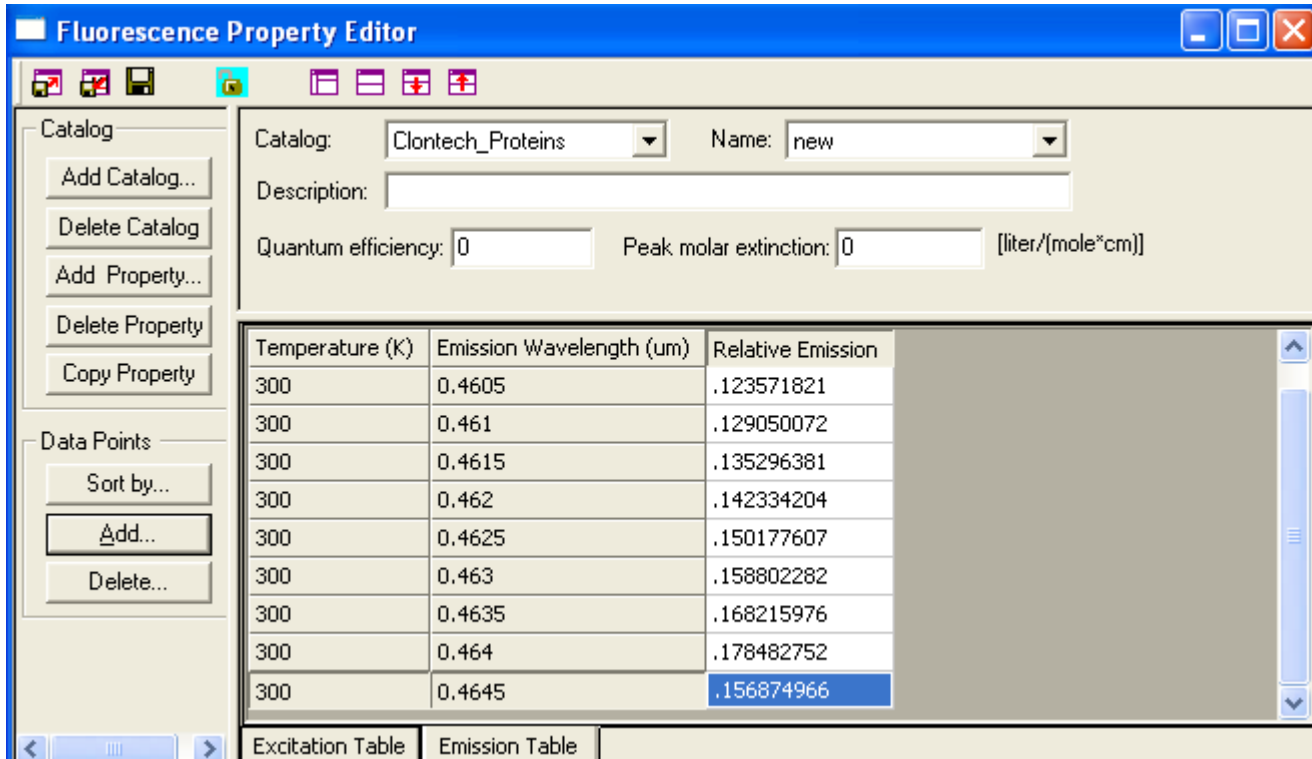


Figure 3. Example Fluorescence Emission Table

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

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

where K_{peak} is the peak molar extinction corresponding to the value of I 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, Options tab. Accompanying the Fluorescence checkbox is an **Insert File Source** checkbox and a dropdown list with the choices **Generate emission sources only** and **Immediately trace emission wavelengths**. The **Insert File Source** checkbox allows you to link the generated fluorescence source 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 **Discrete 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 be involved in the fluorescence calculation. The end result of this stage is that source files containing fluorescent ray data are created.

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

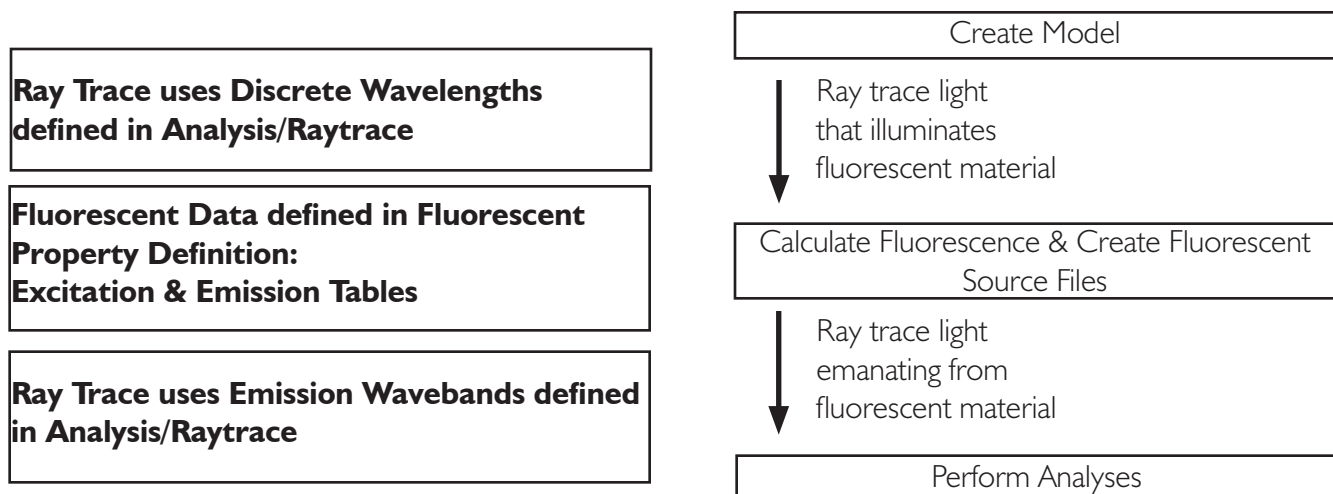


Figure 4. Fluorescence modeling sequences showing where data is drawn from each step in the process.

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 raytrace) 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.

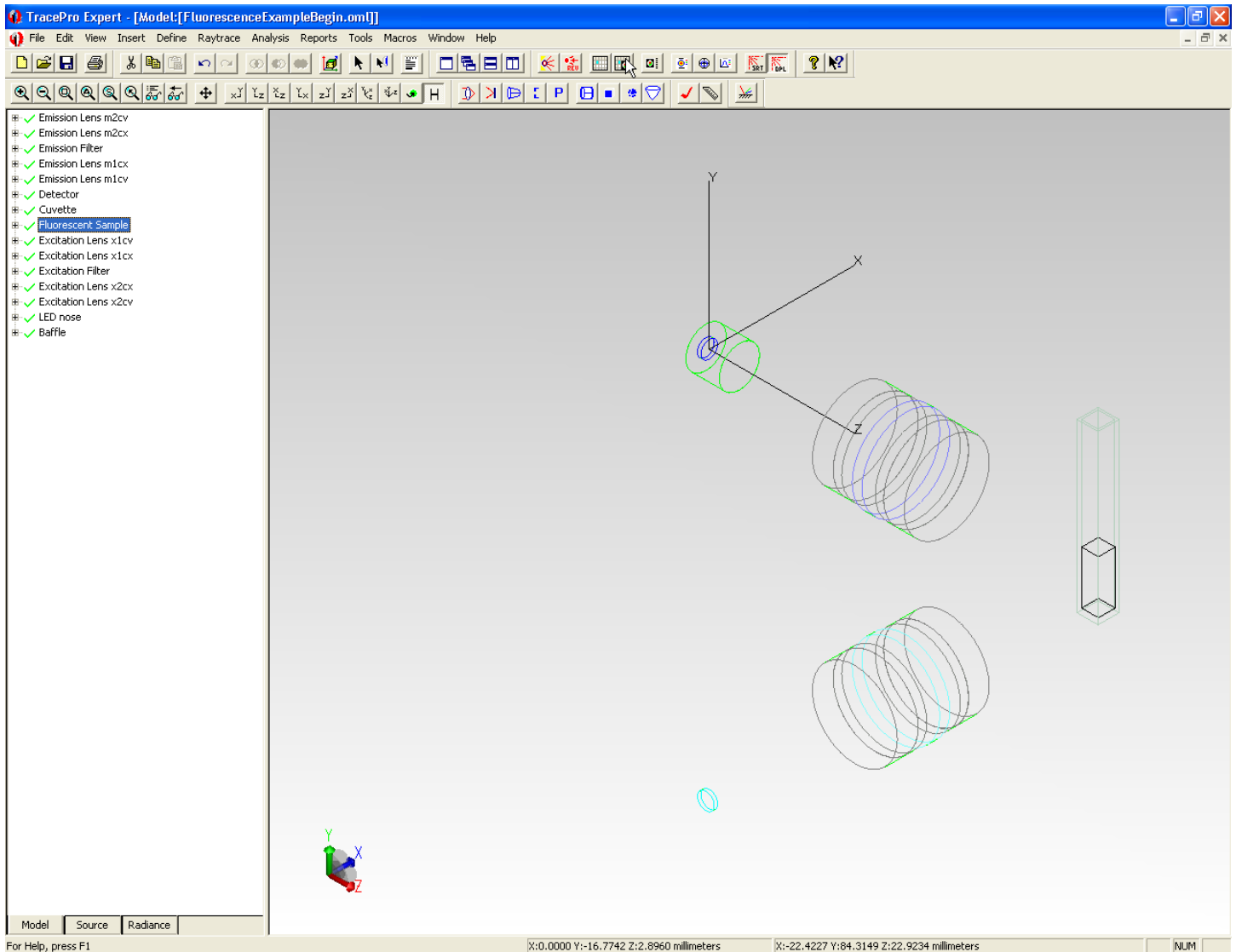
2) **Generate emission sources only**

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 source file(s) into the model (or any model you choose)
- b. adding the discrete wavelengths corresponding to the fluorescence wavebands to the Discrete wavelengths in the Wavelengths tab of the Raytrace Options dialog box
- c. removing the excitation wavelengths from the list of discrete wavelengths (optional: these would be absorbed by the fluorescent material, but you may want to keep them for other reasons)
- d. modifying any surface sources in the model to have zero rays, or removing the surface sources completely, or excluding individual surface sources from the TracePro source tree (optional: you may want to keep surface sources in the analysis)
- e. unchecking the Fluorescence option in the Raytrace Option dialog box
- f. initiating a ray trace making sure that Source Files are enabled in the trace

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 source files containing fluorescent rays. Open the model **FluorescenceExampleBegin.oml**, shown in isometric view below.



This model makes use of custom properties. To import the properties into your Properties Database, select Tools/Database/Import, and select the file "FluorescenceExampleProperties.txt". If prompted to replace existing properties, select "Yes to All".

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**, Material tab, and select a fluorescing property to apply. We will select the **Alexa Fluor® 488 Dye** from the **Invitrogen_OrganicDyes** as shown.

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

Enter the emission wavelength range from .493 to .588 um with 5 bands selected. Set the wavelength ranges from 0 to .493 and from .588 to INF to have 0 bands.

TracePro Expert - [Model:[FluorescenceExampleBegin.oml]]

File Edit View Insert Define Raytrace Analysis Reports Tools Macros Window Help

Model Tree:

- ✓ Emission Lens m2cv
- ✓ Emission Lens m2cx
- ✓ Emission Filter
- ✓ Emission Lens m1cx
- ✓ Emission Lens m1cv
- ✓ Detector
- ✓ Cuvette
- ✓ **Fluorescent Sample**
 - Surface 0
 - Surface 1
 - Surface 2
 - Surface 3
 - Surface 4
 - Surface 5
 - Entity 8
 - Material from IR
 - Material name WATER
 - Fluorescence from Invitrogen_OrganicDyes
 - Fluorescence name Alexa Fluor® 488 Dye
- ✓ Excitation Lens x1cv
- ✓ Excitation Lens x1cx
- ✓ Excitation Filter
- ✓ Excitation Lens x2cx
- ✓ Excitation Lens x2cv
- ✓ LED nose
- ✓ Baffle

Apply Properties

Importance Sampling | Exit Surface | Diffraction | Raytrace Flag

Material | Surface | Surface Source | Prescription | Color

Mueller Matrix | Gradient Index | Bulk Scatter | Temperature

Class and User Data | RepTile | Temperature Distribution | Fluorescence

Catalog: **Invitrogen_OrganicDyes**

Name: **Alexa Fluor® 488 Dye**

Description: Spectra represents conjugate prepared by coupling product to protein or other biomolecule.

Quantum Efficiency: 0.55

Peak Molar Extinction: 78000 liter/(mole*cm)

Molar Concentration: 1e-010 moles/liter

Wavelength: .588

From (µm)	To (µm)	# Inc.	Calc. Wavelength
0	0.493	0	0.2465
0.493	0.588	5	0.5025
			0.5215
			0.5405
			0.5595

Model Source Radiance

For Help, press F1 X:0.0000 Y:-16.7742 Z:2.8960 millimeters X:6.5861 Y:84.4803 Z:51.7669 millimeters NUM

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.

Fluorescence Property Editor

Catalog: Name:

Description:

Quantum efficiency: Peak molar extinction: [liter/(mole*cm)]

Temperature (K)	Excitation Wavelength (μm)	Relative Absorption	Relative Excitation
300	0.251	0.9407304	0.9407304
300	0.252	0.8822697	0.8822697
300	0.253	0.828492	0.828492
300	0.254	0.7823125	0.7823125
300	0.255	0.7441641	0.7441641
300	0.256	0.7132793	0.7132793
300	0.257	0.6901089	0.6901089
300	0.258	0.6698339	0.6698339
300	0.259	0.6511948	0.6511948
300	0.26	0.6340891	0.6340891
300	0.261	0.6199538	0.6199538
300	0.262	0.6054483	0.6054483
300	0.263	0.5943069	0.5943069
300	0.264	0.5830869	0.5830869
300	0.265	0.572851	0.572851
300	0.266	0.5671425	0.5671425
300	0.267	0.5657646	0.5657646

Excitation Table | Emission Table

Select the Emission Table tab in the editor and note that the material fluoresces between wavelengths of about 0.475 to 0.675 μm (a portion of the table is shown below).

Fluorescence Property Editor

Catalog: Name:

Description:

Quantum efficiency: Peak molar extinction: [liter/(mole*cm)]

Temperature (K)	Emission Wavelength (μm)	Relative Emission
300	0.659	0.008167662
300	0.66	0.007988073
300	0.661	0.007606689
300	0.662	0.00729558
300	0.663	0.007169916
300	0.664	0.007080365
300	0.665	0.006980078
300	0.666	0.006610407
300	0.667	0.006739731
300	0.668	0.006196326
300	0.669	0.006103847
300	0.67	0.006104335
300	0.671	0.006017713
300	0.672	0.00555727
300	0.673	0.005344008
300	0.674	0.005249089
300	0.675	0.005088044

Excitation Table | Emission Table

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 tab. The model is currently set to trace at a wavelength of .5461 um, but we want to trace an Excitation wavelength of .499 um. Select .5461 in the table and click the Delete button, then type .499 and click the Add button. Click Apply.

The screenshot shows the TracePro Expert interface with the 'Apply Properties' dialog box open for the 'Emitter' surface. The dialog is configured for Flux emission with 10000 rays and a wavelength of 0.499 micrometers. The 'Wavelengths' table is updated to reflect these changes.

Wavelength (µm)	Weight	Flux	# Rays
0.499	1	1	10000
Totals		1	10000

The 'Wavelengths' table in the dialog is as follows:

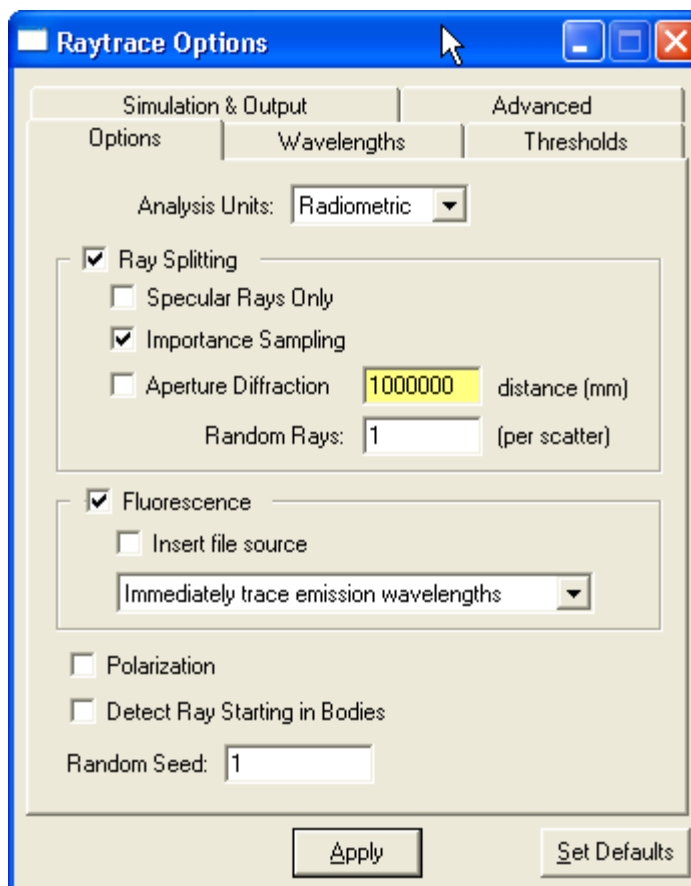
Type	Value	Action
Discrete wavelengths	.499	Add

The 'Wavelengths' table in the dialog is as follows:

Wavelength (µm)	Weight	Flux	# Rays
0.499	1	1	10000
Totals		1	10000

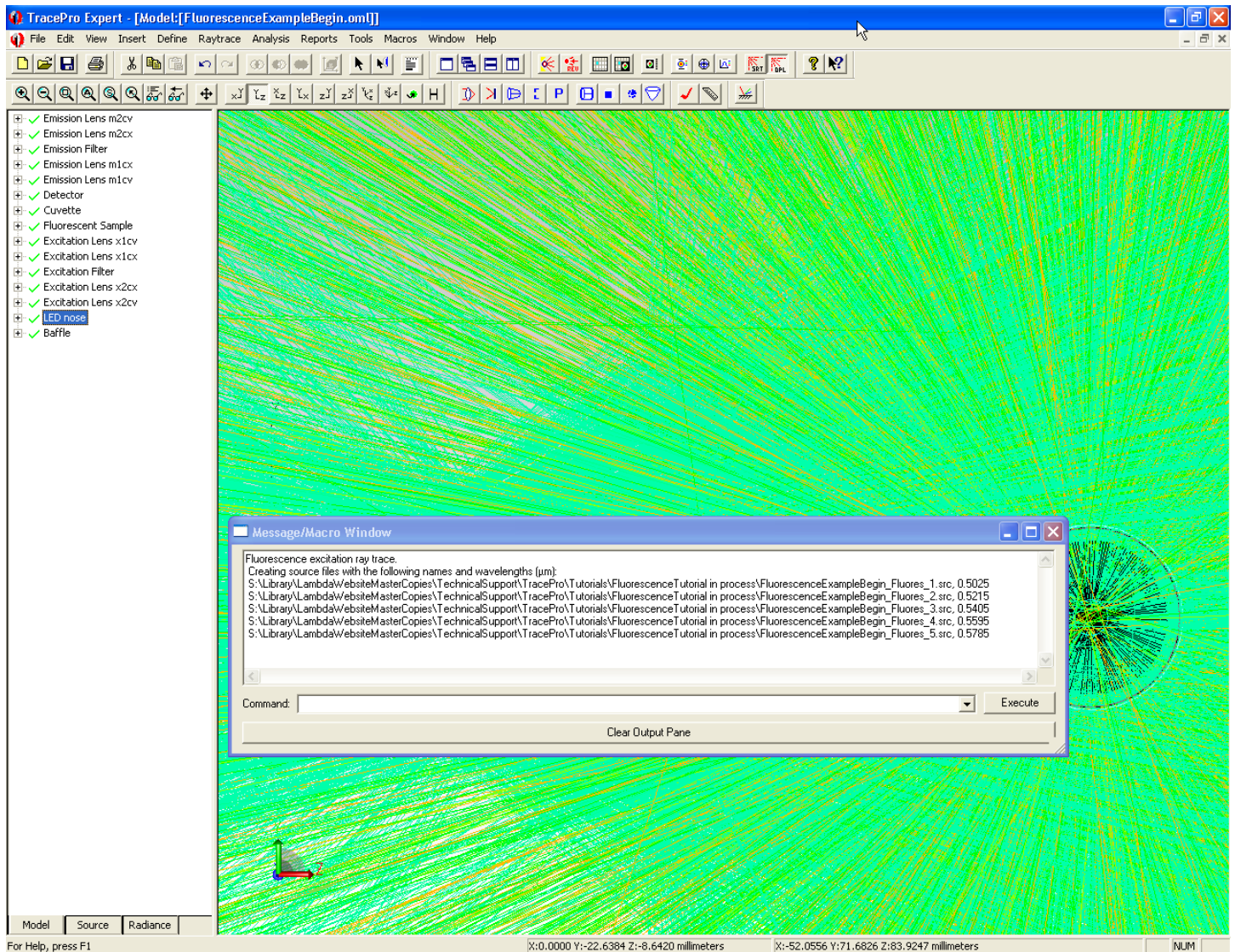
Select the **Options** tab of the **Raytrace Options** dialog box (Raytrace Menu) and click the **Fluorescence** checkbox. In the list under the checkbox, ignore the **Insert File Source** checkbox and select **Immediately trace emission wavelengths**, and click **Apply** to set the selections. The dialog 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.

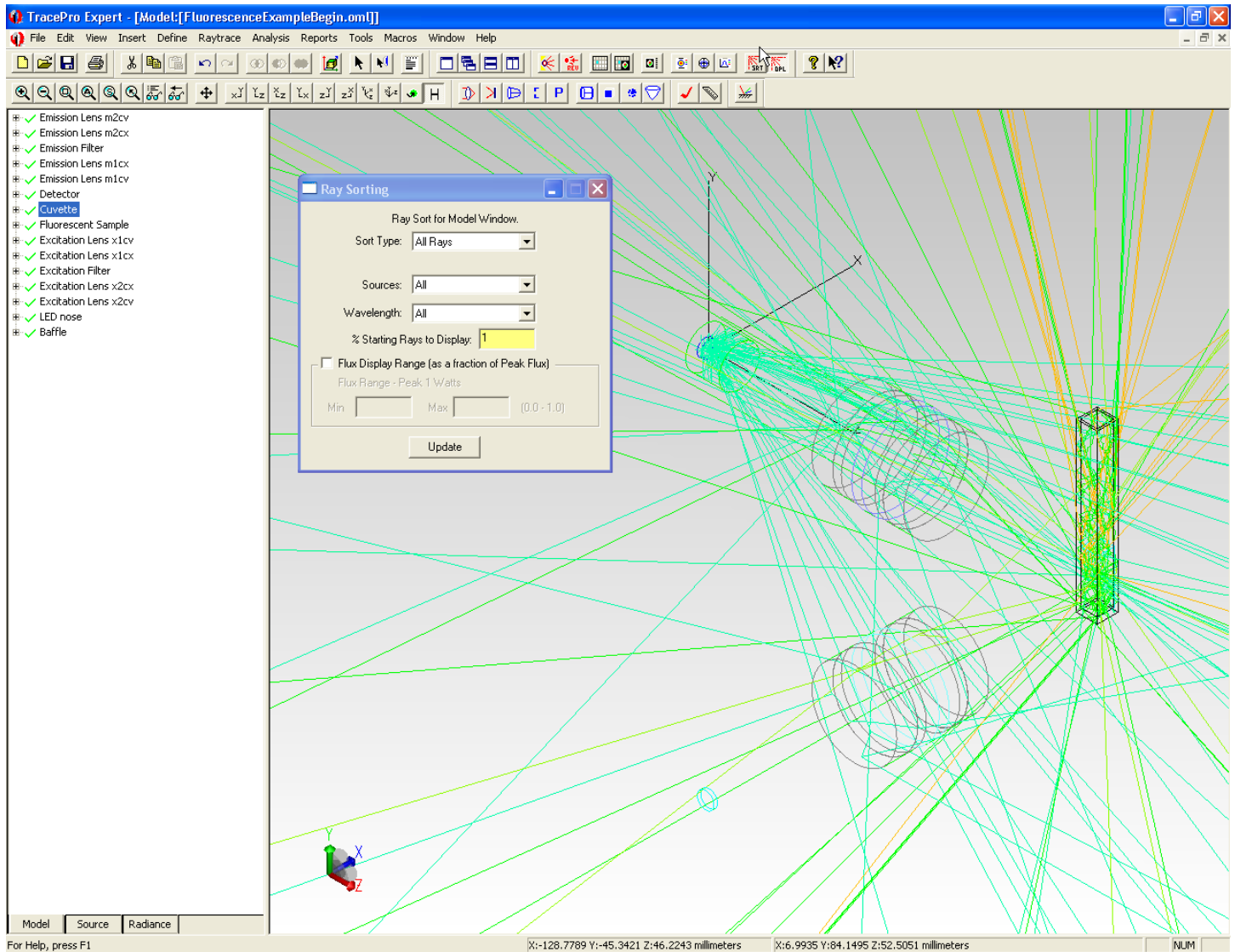


To begin the ray trace, select Raytrace/Trace Rays. TracePro traces the excitation rays and saves the source of the emitted fluorescent rays in binary source files with extension “*.src”. The Message/Macro Window indicates that the File Sources were created for the Fluorescence emission rays. In the second stage of the raytrace, rays are emitted from the Fluorescence Emission File Sources.

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.

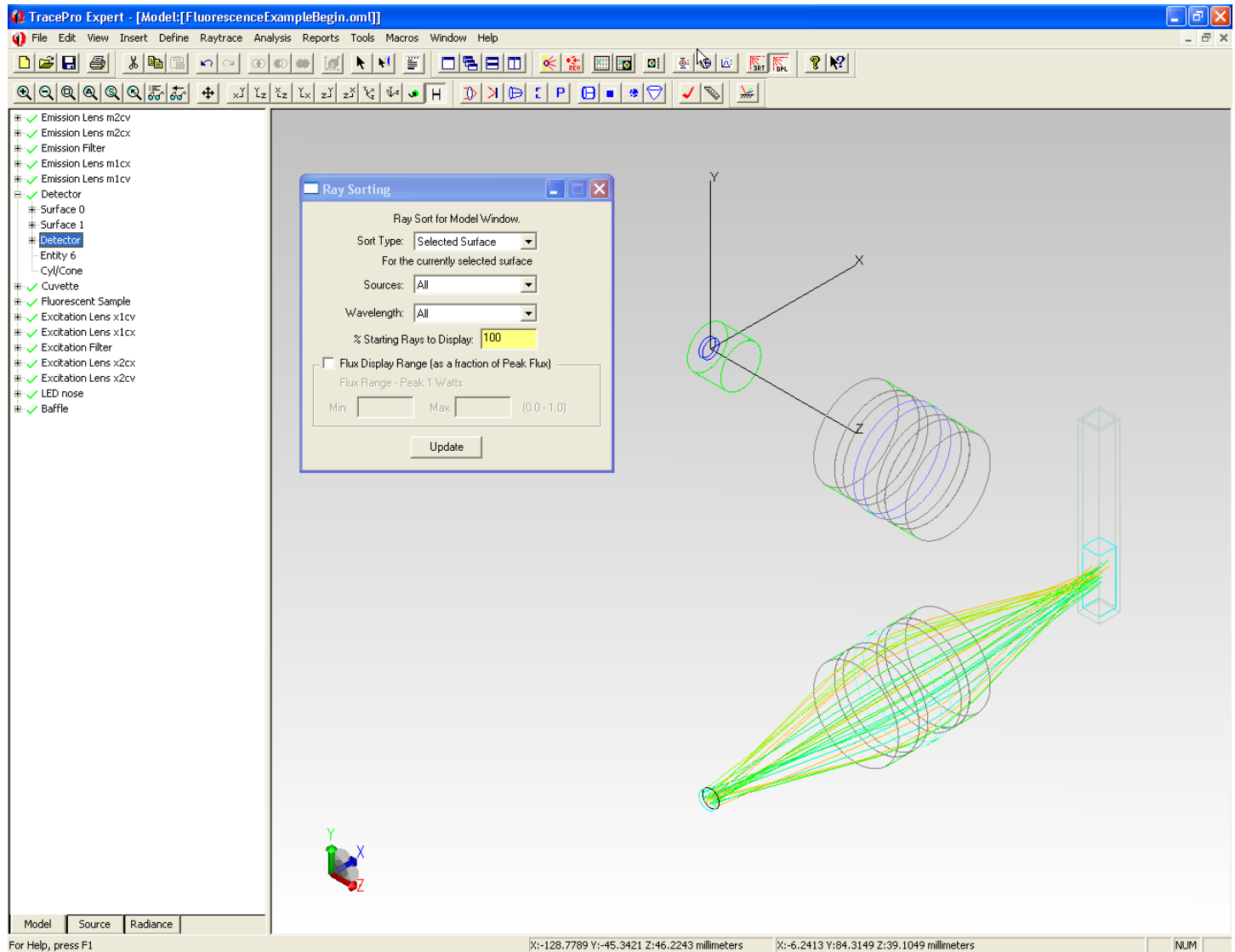


However, you can reduce the number of rays shown in the model by utilizing the ray Sorting dialog (Analysis/Ray Sorting). Setting the Starting Rays to Display to 1% will give you a model layout that looks like this.



Analysis

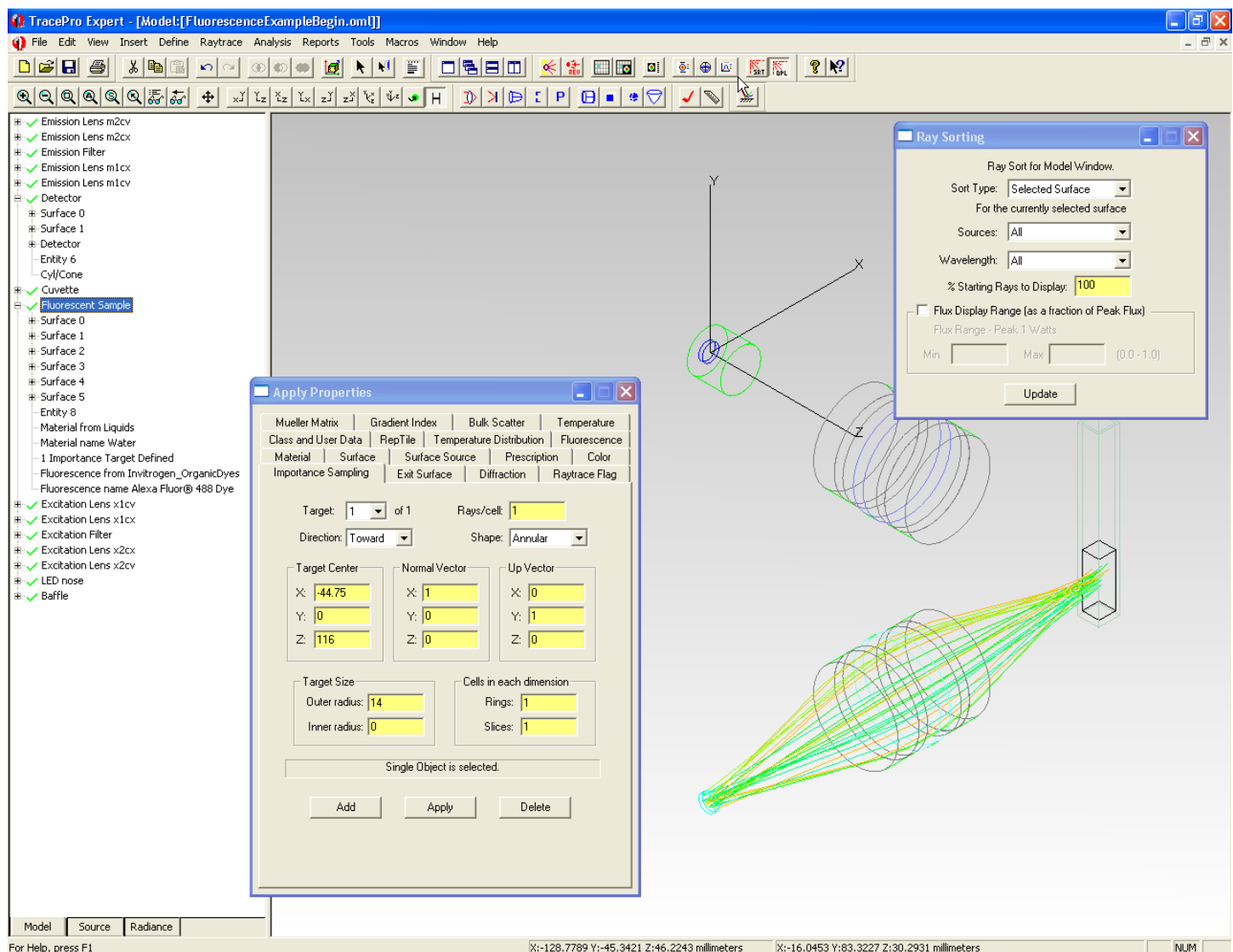
We are mostly interested in rays that hit the detector. We can show these rays by first selecting the Detector surface on the Detector object, then selecting **Analysis/Ray Sorting** to open the **Ray Sorting** dialog. For **Sort Type**, select **Selected Surface**, and then click **Update**. Make sure that the **% Starting Rays to Display** is set to 100. 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 (an irradiance map, flux report, incident ray table, ...etc), but at this point there are so few rays to display, it will not serve any purpose. Next we will concentrate on forcing more rays to reach the detector.

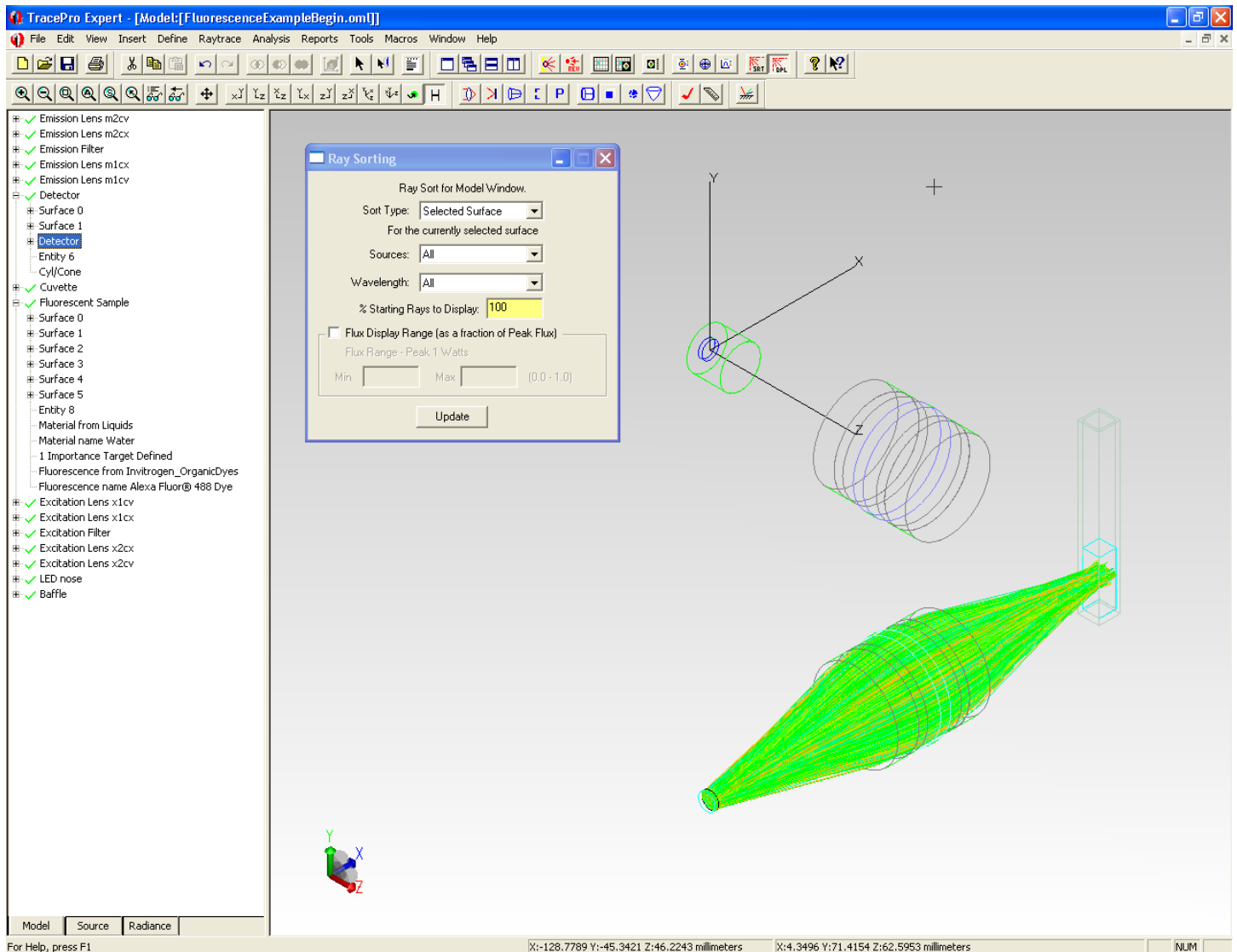
Adding importance sampling

We can increase the number of detected rays 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. To generate importance-sampled fluorescence emission rays, we must apply importance sampling to the **object*** that has the fluorescence property applied to it. To do this select the object named **Fluorescent Sample** in the Model Tree. Select **Define/Apply Properties, Importance Sampling** tab. We wish to importance sample towards the lenses leading to the detector, so enter the importance sampling target data shown in, 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 surface source ray trace by selecting **Raytrace/Trace Rays**. The ray trace will begin as before, and new file sources will be generated, over-writing the previous ones. Now select **Analysis/Ray Sorting** and sort rays on the detector surface, as before. The resulting Model Layout is shown. Many more rays now reach the detector.



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

