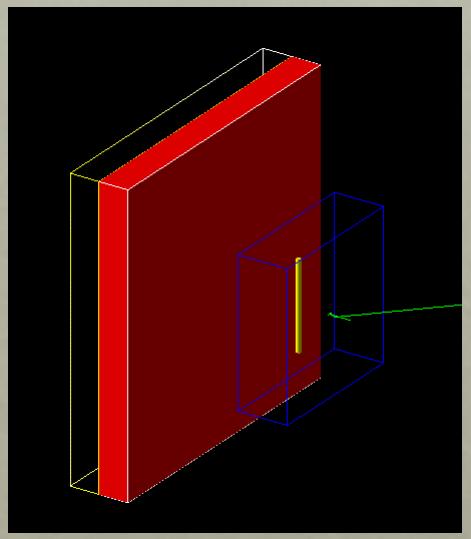
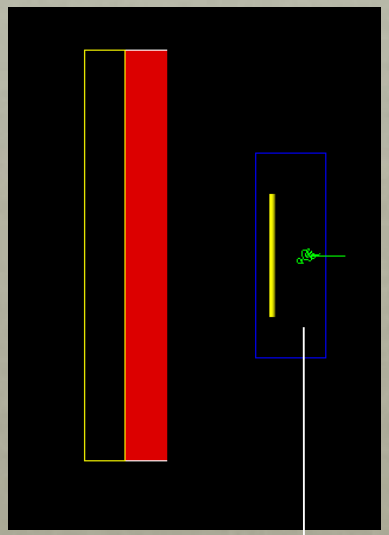
Optical Imaging Module Status

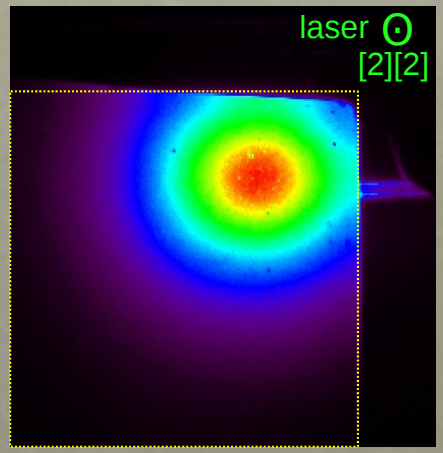
vesna

8th June 2012

Fluorescence Data versus Monte-Carlo Simulation



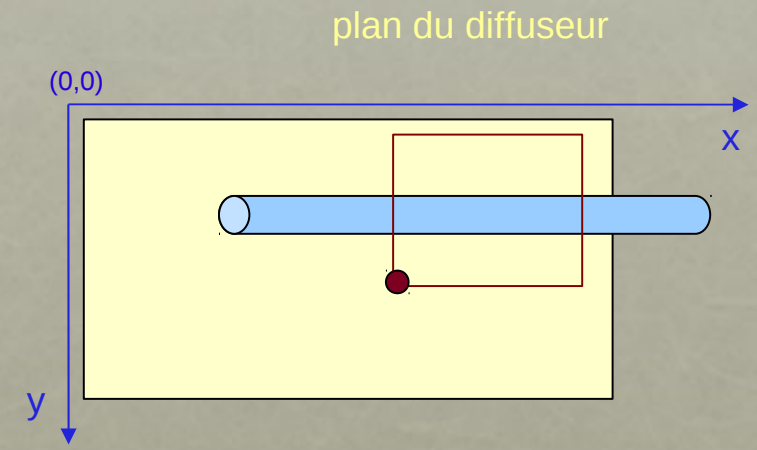




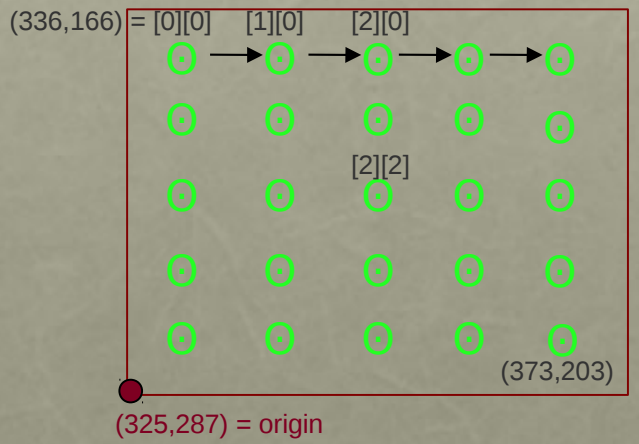
n = 1.47

$\mu_A = 0.2cm^{-1}$

$\mu_s' = 15cm^{-1}$



Grandissement du plan du diffuseur(cm/pixel) : 0.021127



TECHNICAL DATA SHEET

Fluorescent Imaging Agent

Caution: For Laboratory Use. A product for research purposes only.

HypoxiSense 680

Product Number: NEV11070

DESCRIPTION: *HypoxiSense 680* is a Carbonic Anhydrase IX (CAIX) targeted fluorescent *in vivo* imaging agent that can be used to image CAIX overexpression in tumors in response to regional tumor hypoxia.

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MATERIAL: ***(Needs to be reconstituted)***

CONTENTS: Each vial contains 24 nmol of *HypoxiSense 680* in dry solid form. Reconstitute *HypoxiSense 680* with 1.2 mL of 1 x PBS before injecting into animals. Each vial of packaged material provides sufficient reagent for imaging approximately 10 mice (weighing ~25 grams each) when using the recommended dose of 2 nmol (100 µL) of *HypoxiSense 680* per mouse.

PROPERTIES: The physical characteristics of *HypoxiSense 680* can be found in **Table 1 and Figure 1.**

Table 1. *HypoxiSense 680 Characteristics*

Property	Specification
MW	~1500 g mol^{-1}
Fluorescence[1]	
• Excitation	670 nm
• Emission	685 nm
Absorbance[1]	670 nm
Purity[2]	>90%
Appearance	Blue solid

1. Absorbance and fluorescence maxima of *HypoxiSense 680* in 1x PBS.
2. As determined by RP-HPLC and measuring absorbance at 670 nm.

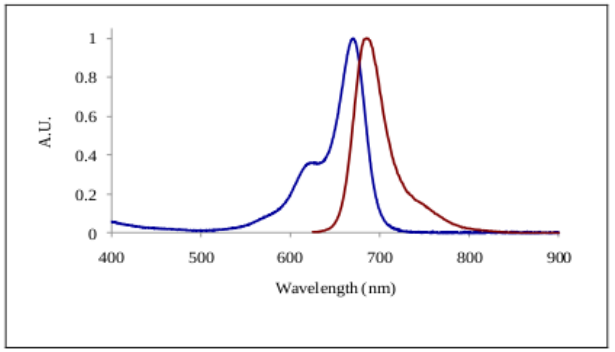
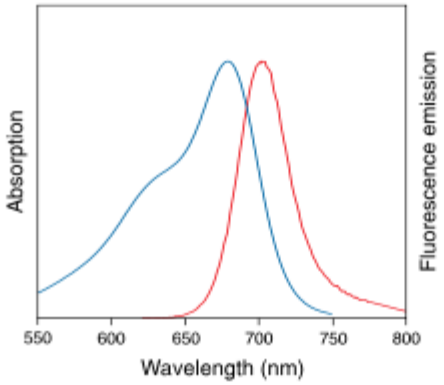


Figure 1. Normalized absorbance (blue) and fluorescence emission (red) spectra of *HypoxiSense 680* in 1x PBS.



Similarity with:

Alexa Fluor® 680 dye

Abs/Em maxima: 679/702 nm

Molecular weight: ~1150

Extinction coefficient: 184,000 $cm^{-1} M^{-1} = cm^{-1} mol L^{-1}$

Flow cytometer laser line: 633

Notes: compatible with ProLong® Gold and *Slowfade*® Gold antifade reagents

Spectrally similar dyes: Cy5.5 dye, allophycocyanin (APC)

Near-infrared fluorescence

Results using TITANE @ CCRT (gendsv)

400 Million of optical photons are generated : 250 processors (30min/proc)

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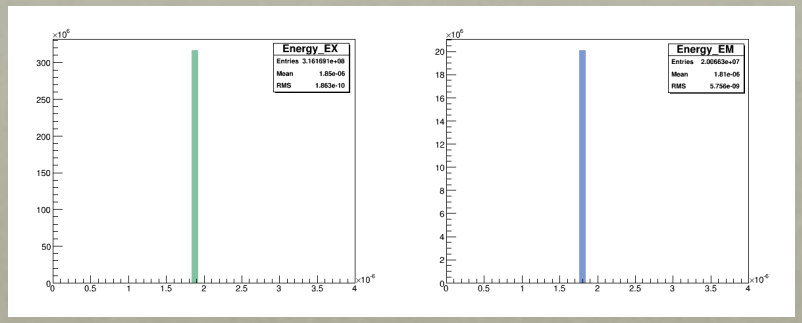
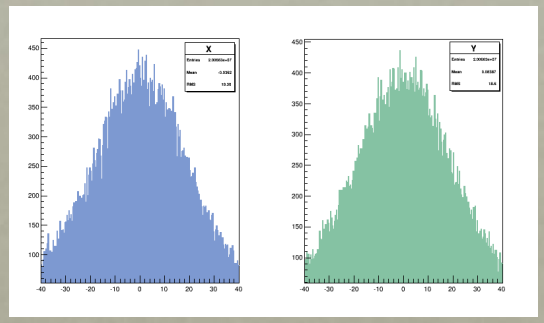
HypoxiSense 680
EX @ 670nm (1.85eV)



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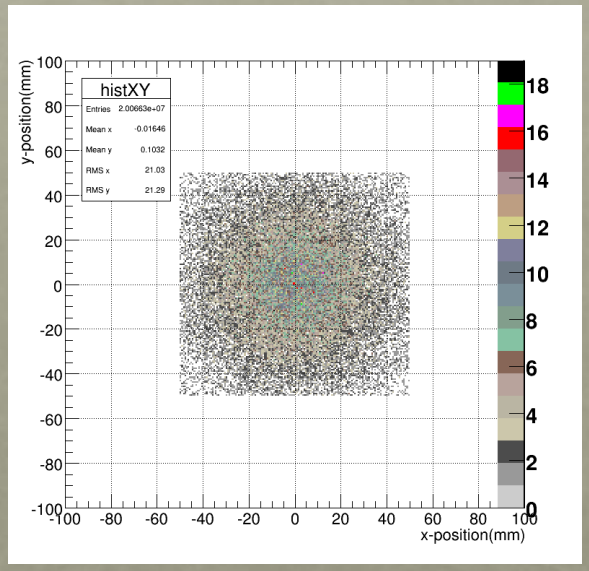
HypoxiSense 680
EM @ 685nm (1.81eV)



~320M EX photons
$1.84eV < E_{EX} < 1.86eV$

~20M EM photons
$1.80eV < E_{EM} < 1.82eV$

Relation between absorption coefficient, extinction coefficient and concentration

2.4.0.4 Sample Preparation

The phantom consist of tissue-mimicking mixture of homogeneous disperse of scatterers (micro sized uniform silica micro-spheres, polystyrene beads, Bangs Laboratories, Inc.) and absorbers (Protoporphyrin IX, Sigma R). For larger phantoms, a cheaper substitute Intralipid^{TM} 20% FRESENIUS KABI is used. The phantoms are placed in quartz cuvettes and semispherical glass bowl.

2.4.0.5 μ_a, μ_s Calculations

The scattering coefficient and anisotropy factor of the polystyrene beads is calculated through Mie-theory [25]. Absorption coefficient of absorber (Protoporphyrin IX) is calculated through experiment. Solutions of absorbers with different concentrations are placed in the cuvette and intensities are collected at the transmission mode. Now, Beer-Lambert law is applied to calculate the molar extinction coefficient at different concentrations by noting (I/I_0). According to Beer-Lambert law,

$$\frac{I}{I_0} = e^{-\mu_a x} = e^{-\epsilon C x} \tag{2.36}$$

$$\text{or,} \quad ln\left(\frac{I}{I_0}\right) = -\epsilon C x \tag{2.37}$$

Now, molar extinction coefficients can be calculated at desired concentration by interpolating the data plotted in figure (2.3), using above equation, and absorption coefficients is directly comes by the relation $\mu_a = \epsilon C$.

$$\mu_A = \varepsilon \times C$$

absorption coefficient

molar extinction coefficient

absorber concentration

$\mu_A = 184000\ cm^{-1}\ M^{-1} \times 0.5\ 10^{-6}\ M$

$= 0.092\ cm^{-1}$

$L_A = 10.87\ cm$