A Computational Model of Light-Sheet Fluorescence Microscopy using Physically-based Rendering

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Abstract

We present a physically-based computational model of the light sheet fluorescence microscope (LSFM). Based on Monte Carlo ray tracing and geometric optics, our method simulates the operational aspects and image formation process of the LSFM. An extension for previous fluorescence models is developed to account for the intrinsic characteristics of fluorescent dyes in order to accurately simulate light interaction with fluorescent-tagged biological specimen. This extension was quantitatively validated against the fluorescence brightness equation and experimental spectra of different dyes. We demonstrate first results of our rendering pipeline to a simplified brain tissue model reconstructed from the somatosensory cortex of a young rat.

Categories and Subject Descriptors (according to ACM CCS): I.3.3 [Computer Graphics]: Picture/Image Generation—Rendering

1. Context

Light sheet fluorescence microscope (LSFM) is of growing importance for neuroscience research [AOR*13]. While this imaging data enables quantitative analysis of brain activity in vivo, it is also very attractive to use this type of data for quantitative comparison with computational brain tissue models for validation. Here, we present first steps for building a computational model of the LSFM to generate physically-plausible fluorescent images adhering to the energy conservation law, which allows to image a previously reconstructed brain tissue model *in silico*.

2. Main Contributions

1. Unbiased physically-plausible simulation of the LSFM imaging pipeline including illumination and acquisition systems, and the light interaction with fluorescent-tagged volumetric models of the specimen.
2. Extension of previous models [Gla95, CS04, GSMA08], of simulating fluorescence within participating media to account for the fundamental spectral properties of fluorescent dyes including their *exact* emission and excitation spectral profiles, quantum yield, molecular absorption coefficient and concentration.
3. Quantitative validation of the rendered fluorescent images against the fluorescence brightness equation and experimental spectral profiles of various fluorescent dyes.

3. Model Description: In Silico LSFM

Our model uses Monte Carlo ray tracing to compute a fluorescent image that can accurately reflect the optical path of the LSFM. This modelling include its optical setup and the operational aspects as well. Figure 1 shows a high-level diagram of the main components of the LSFM and their setup.

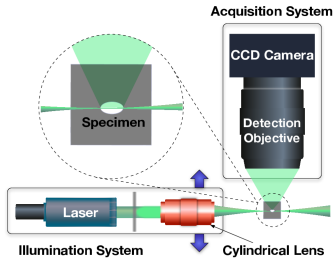

Figure 1: *Diagram of the LSFM units and optical setup.*

Fluorescence and Light Specimen Interaction In contrary to the fluorescence models proposed before, our model can additionally account for the dependence of the fluorescence emission on the wavelength of the exciting illumination and the exact spectral profiles of the dye. This formulation is crucial to yield accurate emission spectra in terms of the number of emitted photons for a given excitation wavelength. The fluorescence term in our extension models the transfer

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of energy from the excitation wavelength λ_{ex} to the emission wavelength λ_{em} given by the relative contribution of the excitation spectrum at λ_{ex} that gives rise to the emission at λ_{em} scaled by the spectral power density (SPD) of the emission spectrum at λ_{em} and the quantum yield of the material. For practical computational feasibility, the model does not consider secondary fluorescence, quenching nor photobleaching.

Illumination System The simulation of the entire illumination system on an element-by-element basis is computationally expensive. However, a valid model of the resulting light beam from the series of optical elements in this system can be approximated by a rectangular light profile. Accordingly, we have modelled the entire illumination setup by a rectangular directional area light with uniform illumination profile and single excitation wavelength. This model, however simplified, preserves the geometrical and spectral characteristics of the system and is highly plausible because the lateral dimension of the specimen is relatively small in the majority of the cases.

Acquisition System This stage is composed of a lens system, set of filters and a charge-coupled device (CCD) camera. Ignoring wave effects and magnification, the lens system is modelled with a thin lens camera with finite aperture [PH10]. The lens is always sampled at high rates to reduce the artifacts due to Monte Carlo noise. The spectral filters are modelled with a transparent layer of the same dimensions as the film, placed in front of the camera. The acquisition stage is synchronized with the illumination model to focus on the specimen where the illumination sheet is applied.

Implementation Our rendering pipeline was implemented on top of the physically-based rendering toolkit (PBRT) [PH10]. An annotated fluorescent volume grid was implemented to add the capability of rendering heterogeneous fluorescent specimens models. This grid stores per voxel the concentration of the dye and an index referring to the intrinsic characteristics of this fluorescent dye. A spectral validation framework was integrated into PBRT to quantitatively measure the emitted power spectrum in the scene and the detected spectral radiance arriving at the film.

4. Results, Conclusion and Future Work

Figure 2 demonstrates our results on a simplified brain model reconstructed from the somatosensory cortex of a young rat. This model was converted to a fluorescent volume using solid voxelization, tagging the interior structures of the volume with fluorescent labels with uniform fluorescence diffusion. Figure 2 shows the rendering of the same optical section of two volumes tagged with green fluorescence protein (GFP) and red fluorescence protein (RFP). To evaluate our fluorescence model, the wavelength of the exciting light sheet was varied from 355 to 561 nm and the resulting images were comparatively analyzed. Figure 3 shows

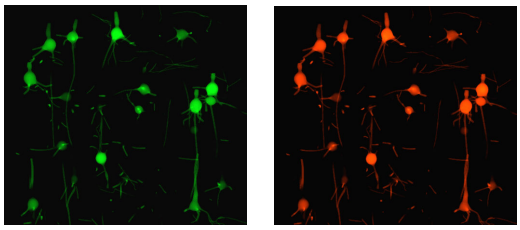

Figure 2: *Simulated LSFM optical sections for a cortical rat model tagged with GFP and RFP lit at their maximum excitation wavelengths(488 and 561 nm).*

the SPDs measured at the image plane for GFP and RFP before their conversion to RGB colors. The profiles of the measured SPDs and their relative amplitudes as a function of the excitation wavelength match the emission spectra of the fluorescent materials measured in the laboratory. The total detected fluorescence intensity from the simulation was validated against the brightness equation that evaluates the fluorescence produced by a fluorescent molecule in terms of its molecular absorption cross-section, its quantum yield and the flux of the incident light beam. The extension of the our LSFM model is still in progress. The current simplified brain model does not allow direct comparison between a realistic image and synthetic one, but the performance of the entire system will be evaluated relying on fluorescent beads with defined geometric shape. The thin lens model will be also replaced by a realistic camera model.

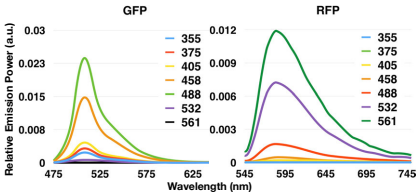

Figure 3: *Normalized measured SPDs at the camera film at different excitation wavelengths for GPF and RFP.*

References

[AOR*13] AHRENS M. B., ORGER M. B., ROBSON D. N., LI J. M., KELLER P. J.: Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nature Methods 10*, 5 (05 2013), 413–420. 1

[CS04] CEREZO B. E., SERON F. J.: Rendering natural waters taking fluorescence into account. *Computer Animation and Virtual Worlds 15*, 5 (2004), 471–484. 1

[Gla95] GLASSNER A.: A model for fluorescence and phosphorescence. In *Photorealistic Rendering Techniques*, Sakas G., Müller S., Shirley P., (Eds.), Focus on Computer Graphics. Springer Berlin Heidelberg, 1995, pp. 60–70. 1

[GSMA08] GUTIERREZ D., SERON F. J., MUNOZ A., ANSON O.: Visualizing underwater ocean optics. In *Computer Graphics Forum* (2008), vol. 27, Wiley Online Library, pp. 547–556. 1

[PH10] PHARR M., HUMPHREYS G.: *Physically Based Rendering, Second Edition: From Theory To Implementation*. Morgan Kaufmann Publishers Inc., San Francisco, CA, USA, 2010. 2