

# TITLE PAGE

## Citation Format:

Vamshi Damagatla, Lennard van den Tweel, Ilaria Bargigia, Freek Ariese, Antonio Pifferi, "Exploring the optics of chicken eggs using time-domain diffuse optics", Proc. SPIE 13935, Diffuse Optical Spectroscopy and Imaging X, 139350X (December 18, 2025); <https://doi.org/10.1117/12.3098380>

## Abstract link:

Vamshi Damagatla, Lennard van den Tweel, Ilaria Bargigia, Freek Ariese, Antonio Pifferi, "Exploring the optics of chicken eggs using time-domain diffuse optics", Proc. Of SPIE-Optica 13935, Diffuse Optical Spectroscopy and Imaging X, 139350X (December 18, 2025); <https://doi.org/10.1117/12.3098380>

## Copyright notice:

Copyright 2025 Society of Photo-Optical Instrumentation Engineers. One print or electronic copy may be made for personal use only. Systematic reproduction and distribution, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper are prohibited.

# Exploring the optics of chicken eggs using time-domain diffuse optics

Vamshi Damagatla,<sup>1,\*</sup> Lennard van den Tweel,<sup>2,3,4,\*</sup> Ilaria Bargigia,<sup>1,5,\*</sup> Freek Ariese,<sup>3</sup> and Antonio Pifferi,<sup>1,6</sup>

<sup>1</sup> Politecnico di Milano, Dipartimento di Fisica, Milan, 20133, Italy

<sup>2</sup> Adaptation Physiology Group, Wageningen University Research, 6700 AH Wageningen, The Netherlands

<sup>3</sup> LaserLaB, Department of Physics and Astronomy, Vrije Universiteit Amsterdam, 1081 HV, The Netherlands

<sup>4</sup> Research Department, HatchTech B.V., Innovatielaan 3, 6745 XW De Klomp, The Netherlands

<sup>5</sup> Center for Nano Science and Technology, Istituto Italiano di Tecnologia@Polimi, Milan, 20134, Italy

<sup>6</sup> Istituto di Fotonica e nanotecnologie, Consiglio Nazionale delle Ricerche, 20133, Milan, Italy

\*saivamshi.damagatla@polimi.it

\*All three authors contributed equally to the work.

**Abstract:** We explore light propagation in chicken eggs using time-domain diffuse optical spectroscopy and Monte Carlo simulations which reveal the eggshell's role as an integrating sphere, and demonstrate the ability to distinguish incubated from unincubated eggs.  
© 2025 The Author(s)

## 1. Introduction

Optical technologies, including visible and near-infrared spectroscopy, are widely used for high-throughput food inspection. One particular industry in need of scientific assistance is that of chicken hatcheries, where infertile eggs occupy valuable incubation space, and underdeveloped chicks are culled, an unethical and unsustainable practice. Early, non-invasive identification of infertile eggs could improve efficiency and sustainability. With the chicken eggshell being known to be highly scattering in nature, the albumen relatively transparent, while the yolk has a medium scattering and absorption. To enable truly non-invasive measurements on eggs, an understanding of photon migration in eggs and the effects of the various components is required. In this work, we show the first time-domain diffuse optical spectroscopy (TD-DOS) [1] measurements to study light propagation in chicken eggs, supported by Monte Carlo simulations. We retrieve their optical properties and finally, also differentiate between an incubated and unincubated egg.

## 2. Materials and methodology

Measurements were performed with a state-of-the-art TD-DOS system [2]. A supercontinuum laser (SuperK Extreme, NKT Photonics) generated picosecond supercontinuum pulses at 40MHz, which were spatially dispersed using a Pellin-Broca prism. The prism was mounted on a precision rotating stage to allow wavelength selection, thus enabling scanning across the spectral range of 550–1100 nm. The light was coupled into the sample through a 62  $\mu\text{m}$  core fiber, through a variable attenuator to regulate the input power. Photons were collected using a 1 mm core diameter fiber and detected with a silicon photomultiplier (SiPM) with a 1 mm<sup>2</sup> active area. The SiPM converts detected single photons into voltage pulses, which were processed by a time-correlated single-photon counting (TCSPC) board (SPC-130, Becker Hickl) to generate a timing histogram of photon arrival times — henceforth referred to as the distribution of time-of-flight (DTOF) curve.

## 3. The integrating sphere effect

The first measurements (Fig. 1(a)) yielded DTOFs with unexpected, curiously long tails with a maximum time-of-flight (MTOF: which we define as the TOF when the photon counts are at 1% of the DTOF peak) reaching upto 6-8 ns, as shown in Fig. 1(c), for both brown and white eggs. Initial speculation that these DTOFs tails were due to fluorescence arising from Protoporphyrin IX (PPIX), which is present in brown and (to a lesser extent) in white eggshells was put to rest by the use of long pass and bandpass filters in the range off the emission spectrum of PPIX. The presence of long tails irrespective of the filters applied showed us that the DTOFs being detected were indeed at the excitation wavelength only. Fig. 1(d) shows a temporal map – the photon counts (normalised) versus the time for each injected/excitation wavelength – showing the presence of these long tails upto  $\approx 850$  nm. To understand better the origin of the signal, we treated a white egg with mild acid to dissolve the eggshell to obtain an egg with its contents held together only by its cell membranes (as shown in Fig. 1(b)) and performed

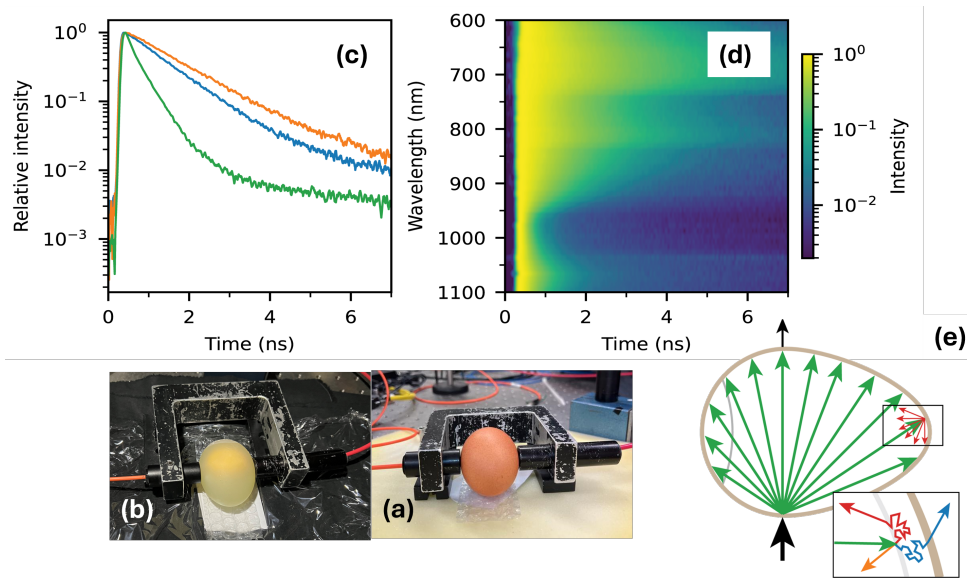


Fig. 1. (a) A brown egg being measured, (b) A white egg being measured after removing the eggshell using a mild acid, (c) The DTOFs obtained from the intact white and brown eggs, and from the acid treated egg (in orange, blue and green respectively), (d) A temporal map showing the detected intensity versus time while varying the injection wavelength from 600-1100 nm, (e) A depiction of the proposed integrating sphere effect due to the eggshell.

measurements on it. As shown in Fig. 1(c), we obtain a DTOF with an MTOF of around 2 ns, as is expected from a diffusive sample of similar dimensions. Finally we performed measurements on an 'egg phantom' – an empty eggshell which was drained of its contents – and we obtained back the long DTOFs with MTOFs  $> 6$  ns, but now extending over the entire wavelength range.

These results led to an exciting discovery that the eggshell is behaving as a natural 'integrating sphere'. It is known that shell has a reduced scattering of  $800\text{--}1000\text{ cm}^{-1}$  [3] and this can lead to photons being redirected back into volume of the egg either directly due to a diffuse reflectance at its inner surface or after having undergone multiple scattering events inside the shell as shown in Fig. 1(e). Considering that the shell is predominantly white with the brown eggs only having an external pigmented layer, photons are not absorbed largely inside the shell. This hypothesis was also verified using the gold standard of Monte Carlo (MC) simulations using Monte Carlo Extreme (MCX) [4]. We simulated a spherical egg with a homogeneous inner volume and shell with  $\mu'_s = 1000\text{ cm}^{-1}$  and were able to show the fluence being trapped inside the volume for greater than 6 ns. Further, by varying the scattering of the shell from  $5\text{--}1000\text{ cm}^{-1}$ , we noticed a reduction of the MTOF with reducing scattering values, showing the integrating sphere effect arising due to the high reduced scattering of the shell as shown in Fig. 2(b). Further, we were able to extend this applicability to other time-domain techniques and demonstrated the effect of the integrating sphere of the shell also on time-resolved Raman and fluorescence techniques experimentally.

#### 4. Probing the inside of the egg

To understand the light propagation better, measurements were performed by changing the measurement geometry with various combinations of source-detector combinations as shown in Fig. 2(c) and the corresponding DTOFs are shown in 2(d). Of the various combinations, only two combinations of S1-D1 and S3-D1 yield diffuse peaks while the others give us ballistic peaks. While the long tails due to the integrating sphere effect remain the same, the differences in the peaks can be explained due to the egg yolk being a semi free-floating structure in the more dense, transparent albumen, conveniently positioning it always on the top. Since the albumen lack scatterers, only the geometries S1-D1 and S3-D1 yield diffusive peaks as the early photons have to pass through the diffusive yolk to reach the detector, whereas in the other scenarios, photon detection is possible after a few direct reflections due to the eggshell, thus creating ballistic peaks with sharp rising edges. This functioning of the yolk similar to a baffle helps us to decide on the appropriate geometry to ensure that we probe the yolk sufficiently even with the early photons.

We then attempted to measure incubated eggs (not shown) and were able to show that the  $\mu'_s$  increases upon

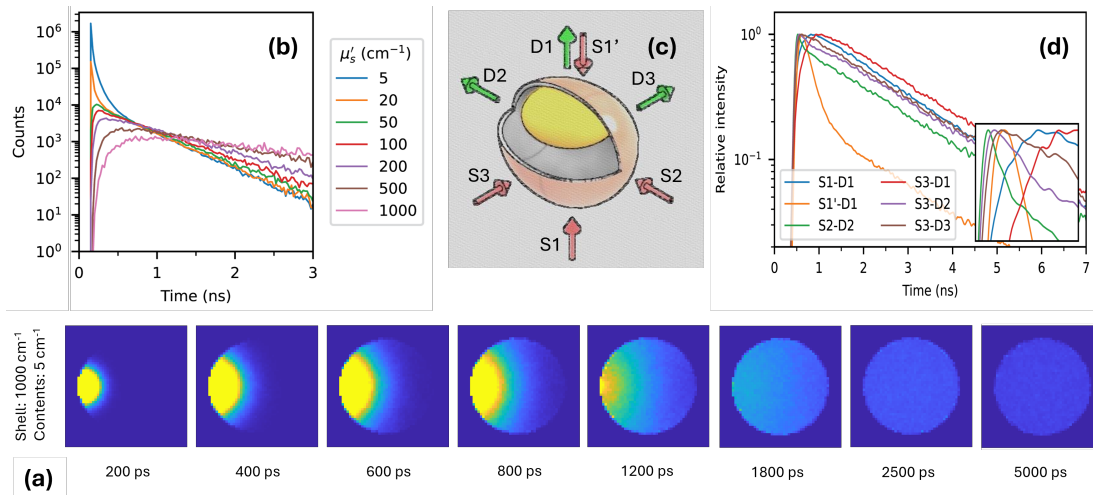


Fig. 2. (a) MC simulation showing the spatial fluence distribution with time, (b) MC DTOFs from a transmission simulation obtained by varying the  $\mu'_s$  of the shell, (c) Orientation of various measurement geometries, (d) DTOFs obtained with different geometries with an inset of a their peaks

incubation and can be attributed to the growing size of the embryo, the sub-embryonic fluid and the yolk sac. Coupled with the fact that we use assume the egg as a homogeneous medium for analysis purposes, the reduction of the transparent albumen changes the average composition of the egg. With regards to the absorption spectra, their shape follow that of the water spectrum with the characteristic peak at 980 nm, in line with expectations since a large percentage of the egg is made of water. However, we do have higher absorption below 700 nm and this is initially due to the presence of carotenoids in the yolk which give it its yellowish-orange colour. Upon incubation though, we clearly see an increase in  $\mu_a$  below 900 nm, and the lineshape of this difference between the spectra of an incubated egg and unincubated egg was found to follow the absorption of hemoglobin, clearly indicating the vasculature of the growing embryo.

## 5. Conclusions

We presented the first measurements with TD-DOS on intact eggs and were able to prove the remarkable discovery that the chicken eggshell behaves as a natural integrating sphere. Further, we studied the effects of geometry and also briefly the appropriate analysis model and were able to differentiate incubated from unincubated eggs. Further efforts are underway to perform statistically significant experimental campaigns to perform component analysis and quantify other biomarkers for industrial applications.

## Acknowledgments

The authors acknowledge funding from (i) Horizon 2020 LASERLAB-EUROPE (grant no. 871124, PID 28630), (ii) Horizon 2020 MSCA actions PHAST-ETN (ID: 860185); and (iii) Next Generation EU I-PHOQS (R0000016, ID D2B8D520, CUP B53C22001750006).

## References

1. A Pifferi, "New frontiers in time-domain diffuse optics, a review," J. of biomed. opt.**21**(9), 091310–091310 (2016).
2. Bossi, A., et. al. "Optical signatures of thermal damage on ex- vivo brain, lung and heart tissues using time-domain diffuse optical spectroscopy," Bio. Opt. Exp. 15(4), 2481–2497 (2024)
3. Athanasiadou, D. et al. "Nanostructure, osteopontin, and mechanical properties of calcitic avian eggshell". Sci Adv 4, eaar3219 (2018).
4. Q. Fang, D. A. Boas. "Monte Carlo simulation of photon migration in 3D turbid media accelerated by graphics processing units." Optics express 17.22 (2009): 20178-20190.