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Monitoring Embryonic Development in Eggs Through Time-Domain Diffuse Optical Spectroscopy

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Abstract: Time-domain diffuse optical spectroscopy was used to determine optical properties of chicken eggs during incubation. Results show the integrating sphere effect, heterogeneity, and embryonic development as challenges in the development of optical techniques for eggs.

1. Introduction

Optical techniques have attained a central role in high-throughput quality assessment in table egg production and process monitoring in hatcheries. Examples include the detection of blood spots and cracks in table eggs, and the determination of embryonic viability in hatching eggs. These applications are, however, challenged by the complex optical properties of avian eggs. Eggs are highly heterogeneous, containing the absorbing and scattering yolk (rich in water, carotenoids and (phospho-)lipids), suspended in the optically clear albumen (egg white) and enclosed by the highly scattering eggshell, which is also absorbing below 700 nm in brown-shelled eggs.

Our recent work using time-domain diffuse optical spectroscopy (TD-DOS) revealed that the optical properties of the eggshell, regardless of its color, result in integrating sphere-like behavior (see Fig. 1A), extending photon path lengths within the egg beyond one meter. [1] Despite the contribution of the integrating sphere effect to the time-of-flight distributions (DTOFs), we found that bulk absorption (μ_a) and scattering (μ_s') parameters of the egg can still be approximated from the inverse diffusion approximation (DA) with appropriate boundary conditions. This work explores the application of TD-DOS for the optical characterization of incubated chicken eggs, and the effect of embryological development on the egg's bulk optical properties during the first ten days of incubation.

2. Materials and methodology

TD-DOS was performed using a supercontinuum laser (SuperK Extreme, NKT Photonics) providing 100 ps pulses at 40 MHz repetition rate, dispersed by a Pellin Broca prism on a rotary stage, and coupled via a variable attenuator into a 62/135 µm graduated index fiber put in contact with the sample surface. Collection of diffuse photons was performed using a 1000/1035 µm step-index fiber, coupled to a silicon photomultiplier (S10362-11-050C, Hamamatsu) interface with a single photon counting module (MultiHarp 150 8N, PicoQuant). Time-of-flight distributions (DTOFs) were obtained throughout the 560-1100 nm range at 20 nm intervals. [2]

White-shelled eggs of the DeKalb White strain were selected for 43-44 mm diameter and incubated at 37.8 °C and 55 %RH with hourly tilting. Measurements were performed on 20 eggs before the start of incubation and on days 3-8 and 10 of embryonic development (E0, E3-8, E10), after which fertility was verified. All measurements were performed in transmittance geometry over the short vertical axis of the egg, positioning the long axis of the egg horizontal. Absorption (μ_a) and reduced scattering (μ_s') coefficients were determined via inversion of the DA for a homogeneous medium (n=1.35) with infinite medium boundary conditions and source-detector distance matching the diameter of each egg. [1,3]

3. Results

Visual inspection of the DTOFs and fitted DA at 700 nm (Fig. 1A) reveals the impact of the integrating sphere effect, which superimposes an additional exponential decay term on the conventional DTOFs, and thereby affects both the ingrowth and decay rate of the DTOFs. This makes fitting the DA (dashed lines) especially challenging for μ_s', which is mostly determined from the shape of the DTOF peak, while leaving μ_a mostly unaffected. [1]

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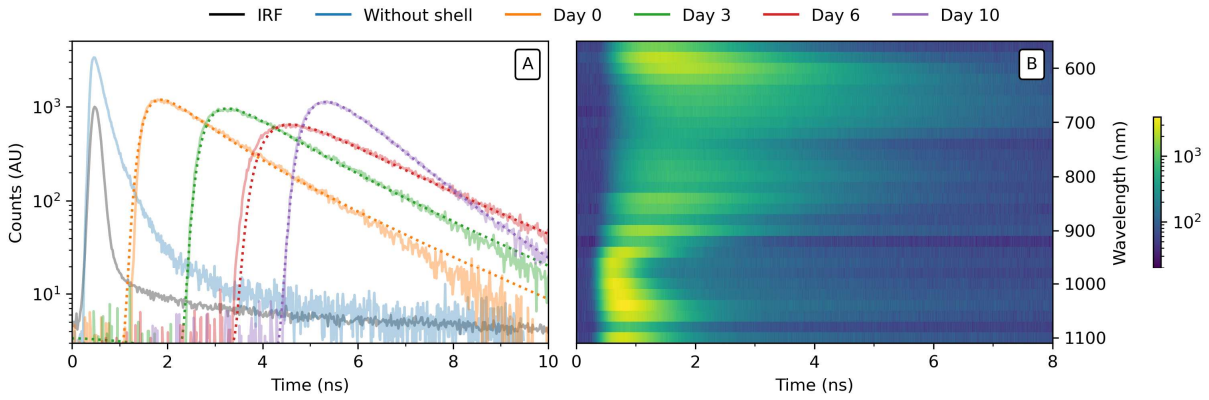

Fig. 1. A: typical DTOFs observed at 700 nm after removal of the eggshell in 5% hydrochloric acid, demonstrating the contribution of the integrating sphere effect, and for a single egg as incubation progresses, dashed lines present the fitted DA for μ_a and μ_s'. Note that the observed offset is artificial. B: heatmap of the DTOF intensity per wavelength for a typical egg on E6, clearly showing the shorter TOF due to absorption by hemoglobin and water (<600 and 920-1040 nm) and diffuse peaks in the scattering dominant region (600-900 nm).

Fitting of the DA to the individual DTOFs spanning 560-1100 nm (Fig. 1B) provides the μ_a- and μ_s'-spectra depicted in Fig. 2A-B. Considerable coupling between μ_a and μ_s' is however observed in μ_s' in regions where the dominant scattering assumption ($\mu_s' \gg \mu_a$) underlying the DA is violated, i.e. at absorption bands of hemoglobin (<600 nm) and water (920-1040 nm). This non-physical phenomenon becomes especially apparent at the water band, which shows a 50% decrease in μ_a during the first 6 days of incubation, contradicting the established 12% weight loss due to evaporation of water over the first 18 days of incubation. [4] To compensate for the observed coupling, the wavelength dependence of μ_s' in the 700-920 nm range was modelled using the Mie power law ($\hat{\mu}_s' \approx a\lambda^{-b}$) and extrapolated to the 560-1100 nm interval. Approximation of $\mu_{a,M}$ given $\hat{\mu}_s'$ (Fig. 2C) shows this approach forcefully prevents coupling at the hemoglobin and water bands, and mostly removes the non-physical variation in μ_a for the hemoglobin and water bands.

On E0, the average μ_a-spectrum reveals the presence of carotenoids and phospholipids in the yolk as the tail below 700 nm and the band at 850 nm superimposed on the water spectrum. Presence of scatterers in the yolk results in considerable Mie-type scattering ($\mu_s' \approx 2$ cm^{-1}, $b \approx 0.75$). Following the onset of hemoglobin synthesis by the end of E2, an appreciable increase of μ_a is observed below 800 nm during the subsequent days of incubation.

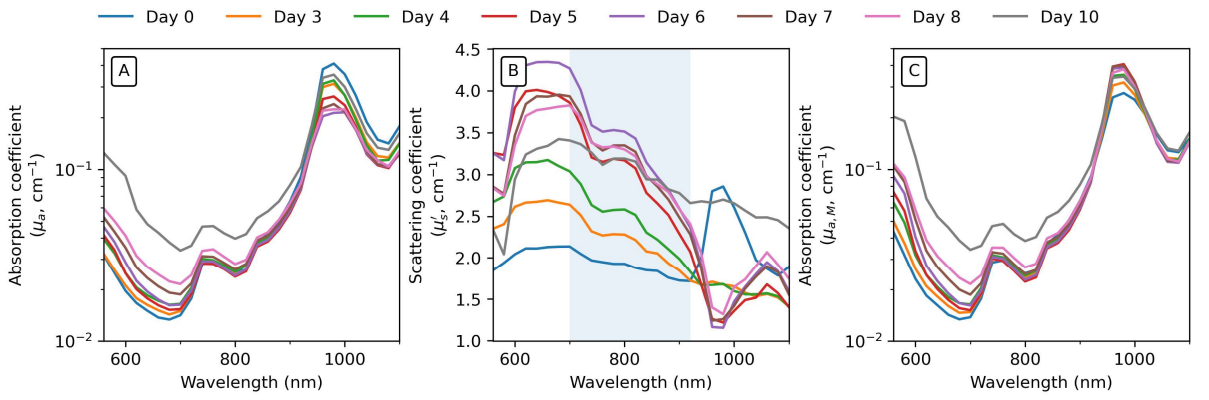

Fig. 2. A-B: average μ_a- and μ_s'-spectra obtained for each day of incubation, both retrieved via inversion of the DA. The shaded area in B marks scatter-dominant range used for fitting the Mie power law to μ_s' to give $\hat{\mu}_s'$ (not shown). C: average $\mu_{a,M}$-spectrum for each day of incubation, approximated with μ_s' constrained to $\hat{\mu}_s'$.

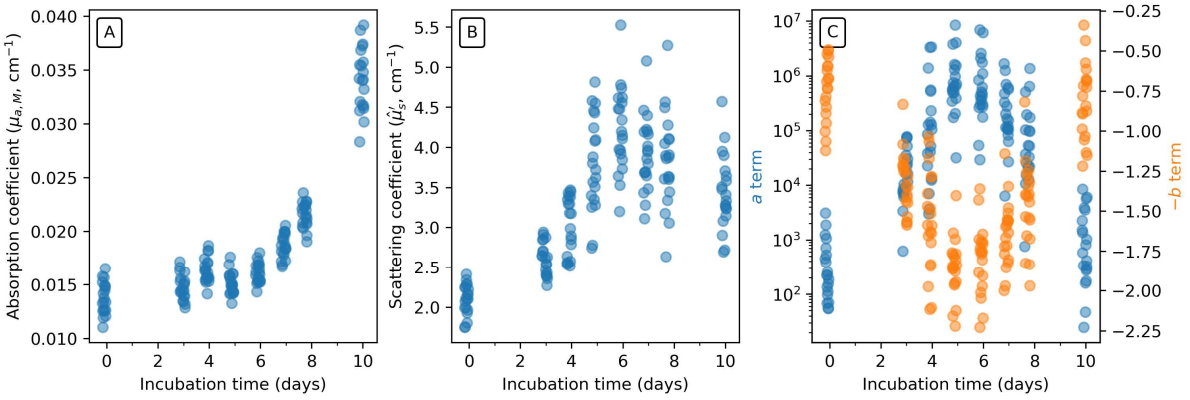

Fig. 3. A: absorption coefficient ($\mu_{a,M}$) at 700 nm over incubation time, approximated from the DA with μ'_s constrained to $\hat{\mu}'_s$. B: reduced scattering coefficient $\hat{\mu}'_s$ at 700 nm over incubation time, approximated by fitting the Mie power law to μ'_s in the 700-900 nm interval. C: evolution of the a- (left y-axis) and b-term of the Mie power law over incubation time.

The following development of (extra-)embryonic tissue and membranes results in an increase of $\hat{\mu}'_s$ from 2.5 cm^{-1} at E0 to a maximum of 4 cm^{-1} on E6, before reducing to 3.5 cm^{-1} by E10. A more elaborate trend is present in $\hat{\mu}'_s$, as both the a- and b-terms of the Mie power law follow the same trend (Fig. 3C), suggesting the number density of scatters (a-term) increases, while the size of scatters decreases (b-term). Aside from the formation of scattering (extra-)embryonic tissues and membranes, this relationship between incubation time and $\hat{\mu}'_s$ can be explained by physical changes inside the yolk. From E2, the egg yolk is rapidly hydrated by the albumen, giving rise to the sub-embryonic fluid (SEF), which reaches a maximum volume on E6. From E6 onwards, the SEF regresses to yield to the rapidly growing embryonic vesicles, notably including the optically clear allantois, explaining the observed reduction of $\hat{\mu}'_s$ by E10. [5]

4. Conclusion

The application of TD-DOS on incubated chicken eggs was demonstrated during the first ten days of incubation. Preliminary analysis of the retrieved optical properties reveals elaborate trends in μ'_s, following physical changes to the yolk, resulting in the temporary formation and regression of the SEF, in addition to the development of embryonic tissues. Although the temporal trends in optical properties with incubation can be explained by known biological processes, these results emphasize the challenges faced in the application of optical techniques for eggs. These findings especially emphasize the heterogenic and dynamic nature of incubated eggs, prompting careful consideration of the complex photon migration mechanisms at play at different stages of incubation.

5. Acknowledgements

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