How Does a Firefly Flash?

This report features the work of Yueh-Lin Tsai, Chia-Wei Li, Yeu-Kuang Hwu, and their co-workers published in Phys. Rev. Lett. ***113****, 258103 (2014).*

In actuating their internal lanterns on summer nights, fireflies create one of the most beautiful scenes in the world. In addition to its application to attract a mate, a bioluminescent signal enables light-emitting insects to communicate. How that bioluminescence works is an issue that still puzzles scientists. The biochemical mechanisms used by fireflies for flashing have been recently clarified as bioluminescence produced by chemical reactions inside their lanterns. The reaction compositions include calcium, adenosine triphosphate, luciferin and enzyme luciferase that are triggered by a sufficient oxygen flux,[1] but the biophysical mechanisms, such as a mechanism of fuel supply, have not been demonstrated. In 2014, Yueh-Lin Tsai and Chia-Wei Li from Institute of Molecular and Cellular Biology in National Tsing Hua University with Yeu-Kuang Hwu from Academia Sinica have resolved this mystery on combining two sophisticated imaging techniques at the TLS: synchrotron phase-contrast microtomography (PCμT) and a transmission X-ray microscope (TXM).[2] From the use of these techniques at high resolution, they accumulated convincing evidence to explain how the supply of oxygen works in firefly lanterns.

Two possible switching mechanisms of firefly luminescence have been proposed. The first mechanism

assumes that nitrogen oxide (NO) inhibits the mitochondrial activity that enables a sufficient flux of oxygen to reach the photocytes (light-emitting cells).[3] The second mechanism involves the presence of the tracheole fluid that can modulate the oxygen flux to provide an extra amount for flashing.[4] To test these two hypotheses, the greatest challenge was to reconstruct quantitatively the complicated tracheal structures inside firefly lanterns that help to estimate the flux of oxygen actually supplied through the tracheal system to the luminescent cells. The researchers used two advanced imaging techniques with X-rays from the synchrotron to reveal quantitatively the entire tracheal structures in three dimensions (3D). The first was PCμT at **BL01A1** to observe features on a micrometer scale with a field of view a few mm^2; the spatial resolution of the PCμT is about 1 μm. The second was TXM at **BL01B1** to observe features on a nanometer scale with a field of view a few $μm^2$; the spatial resolution of TXM is about 30–60 nm. Both techniques yield 3D tomographic images of the specimens. The average diameters of the tracheal tubes from the spiracle to the smallest terminal branches typically range from 120 μm to less than 300 nm; combining these two techniques enabled coverage of the full range of these scales. Both techniques provide phase-contrast images that are crucial to distinguish organic structures inside tissues without labeling with any absorption-enhanced reagents for X-rays.

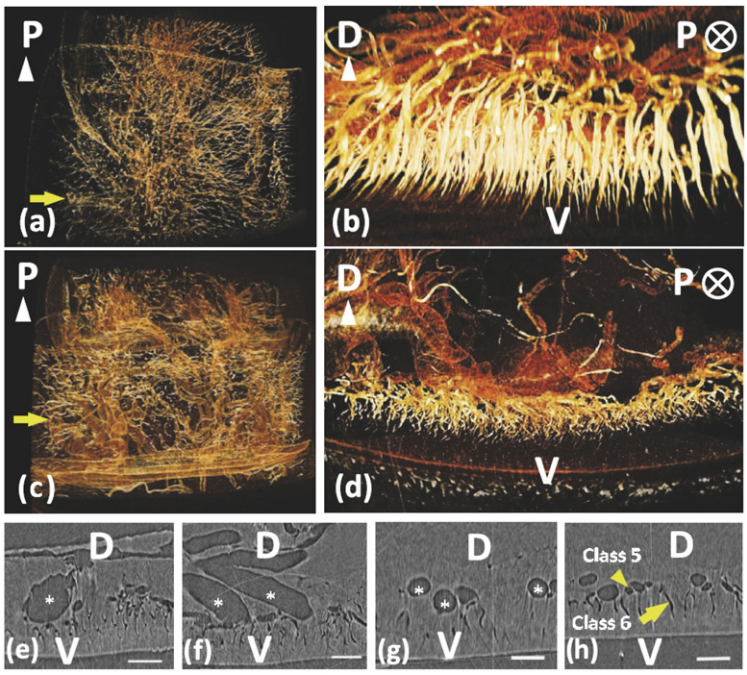

Fig. 1: 3D tomographic images of the tracheal system in *L. terminalis* (a)–(b) and *L. cerata* (c)–(d). (e)–(h) Cross-sectional images of tracheae reconstructed from X-ray tomography with various diameters. Scale bars: 100 μm. (Reproduced from Ref. 2)

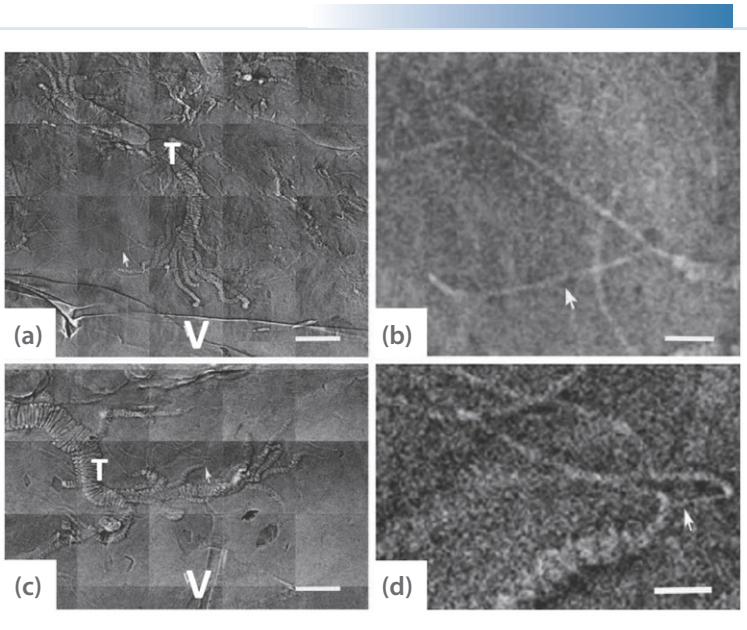

Fig. 2: TXM images of tracheoles for *L. terminalis* (a)–(b) and *L. cerata* (c)–(d). Scale bars: 10 μm [(a) and (c)], 2 μm [(b) and (d)]. (Reproduced from Ref. 2)

Figure 1 shows a 3D tomographic image of the complicated tracheal systems in *L. terminalis* (Figs. 1(a)-(b)) and *L. cerata* (Figs. 1(c)-(d)), which were reconstructed through use of the PCμT without labeling. Figures 1(e)-(h) show the cross sections of the tracheal tubes of various diameters. According to these geometric data,

the diameter, length and volume of the tracheal tubes, and also their density, were quantitatively measured.

The most important aspect is the terminal region of the tracheal system, which exhibits tracheal tubes, known as tracheoles, of the greatest density and hence the smallest average diameter, typically within 300 nm. The tracheoles play a critical role in gas exchange, such as directly supplying oxygen to the mitochondria or photocytes. Figure 2 shows X-ray images of tracheoles using TXM with ultrahigh spatial resolution. Quantitative analysis of the tiny tracheolar geometry enables one to estimate the limit of oxygen flux diffusing into the mitochondria or photocytes through the tracheoles.

The scientists observed that the consumption of oxygen corresponding to mitochondrial functions exceeds the maximum rate of oxygen diffusion from the tracheal system to the photocytes. In contrast, the maximum rate of diffusion of oxygen through the tracheoles is near the rate of oxygen consumption of photocytes for flashing. These conclusions support the first hypothesis of the luminescent mechanism of fireflies—that bioluminescence can be generated only on inhibiting the activities of mitochondria in the firefly lanterns. A sufficient portion of oxygen was thus able to diffuse into the luminescent cells to enable flashing. Based on the similarity of the rate of oxygen diffusion supplied through tracheoles and the rate of consumption of photocytes for flashing, the second hypothesis must be rejected. No extra amount of oxygen can thereby be wasted by the tracheole fluid, which fills inside the tracheoles. This work provides substantial evidence to solve the key open question about the biophysical mechanism of firefly flashing. (Reported by Chun-Chieh Wang)

References

1. L. Pinto da Silva, R. Simkovitch, D. Huppert, and J. C. G. Esteves da Silva, Chem. Phys. Chem. **14**, 2711 (2013).
2. Y.-L. Tsai, C.-W. Li, T.-M. Hong, J.-Z. Ho, E.-C. Yang, W.-Y. Wu, G. Margaritondo, S.-T. Hsu, E. B. L. Ong, and Y. Hwu, Phys. Rev. Lett. **113**, 258103 (2014).
3. B. A. Trimmer, J. R. Aprille, D. M. Dudzinski, C. J. Lagace, S. M. Lewis, T. Michel, S. Qazi, and R. M. Zayas, Science **292**, 2486 (2001).
4. G. S. Timmins, F. J. Robb, C. M. Wilmot, S. K. Jackson, and H. M. Swartz, J. Exp. Biol. **204**, 2795 (2001).