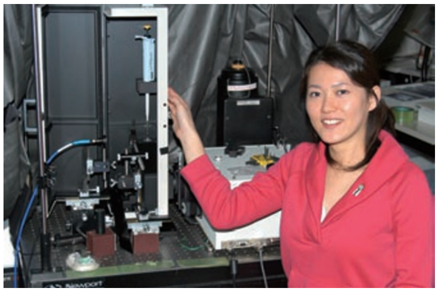
Glow in the dark

The ability of living organisms to generate light by bioluminescence is a fascinating phenomenon. *Nature Photonics* spoke to Yoriko Ando from the University of Tokyo about her recent quantitative analysis of the effect in fireflies.

What is firefly bioluminescence and why is it important?
It is a chemiluminescence reaction that takes place in fireflies, producing a distinctive yellow-green emission. A substance called luciferin is oxidized using the catalyst enzyme luciferase, magnesium ions and adenosine triphosphate (ATP) to produce oxyluciferin and energy in the form of light. Although there are many bioluminescent organisms in the world, the firefly is one of the most famous owing to the extremely high efficiency of its bioluminescence reaction. Its bioluminescence system is now widely used in medical, pharmaceutical, food-safety and environmental applications, and in research. Gene-expression analysis using luciferase is already used for biological research. Luciferase can be attached next to the promoter of a gene so that when the gene is expressed, luciferase is expressed at the same time. The gene expression can be monitored by measuring the light intensity from the luciferin–luciferase bioluminescence reaction. Food-safety examiners can determine the presence of bacteria by measuring the bioluminescence intensity from the luciferin–luciferase reaction, which reflects the amount of ATP in bacteria. Its potential in bio-imaging is another hot topic. For example, cancer cells can be labelled with a bioluminescent marker to determine which cells need to be treated.

What was the motivation for studying fireflies?
Firefly bioluminescence has attracted a lot of attention because of its extremely high efficiency — a quantum yield of 88%, as reported by Seliger and McElroy in 1959. However, two years later, the same group pointed out that a re-examination of this value was needed. Yet, no further investigations have been made. There is also the mystery of the exact mechanism that is responsible for the pH-dependent colour change of firefly bioluminescence between yellow-green and red emission. It has conventionally been modelled as a chemical equilibrium between the two states, but other models are also under discussion. We recently developed a quantitative measurement system based on a total-photon-flux spectrometer and decided to use it to analyse firefly bioluminescence with the aim of answering these questions.



In the future, Yoriko Ando plans to do further work on the quantitative bioluminescence measurement system and to study different types of firefly luciferase.

What did your results show?
We found that the quantum yield is actually only 41.0%, less than half the previously reported value. This value is, however, still higher than all other bioluminescence systems. We also studied the colour-change mechanism by decomposing the measured bioluminescence spectra into three gaussian components. We found that only the green emission peaking at 2.2 eV changes with the pH level. There is no intensity conversion between the yellow-green and red emission, which raises questions regarding the long-standing interpretation of the colour-change mechanism based on the concept of chemical equilibrium.

Tell me about your measurement system.
We have developed a total-photon-flux spectrometer, which consists of a grating spectrometer and a CCD camera with light-collection optics. The absolute sensitivity and the light-collection efficiency of this system are calibrated to a high level of accuracy. This spectrometer directly measures quantitative luminescence spectra scaled by the absolute number of photons in the total light flux from the bioluminescence, and can thus determine the quantum yields. The luciferase we used is a commercial product of purified *Photinus pyralis* luciferase.

What will be the impact of these findings?
Considering that fireflies are one of the most important bioluminescence systems, the correction of the quantum yield from 88% to 41.0% will have an enormous impact on bioluminescence science and related technologies. Most of the existing colour determination models are built based on the assumption of the chemical equilibrium between yellow-green and red emission. These models may need to be reconsidered to account for our findings. In addition, most current bioluminescence measurements are based on commercially available instruments that are not calibrated and thus only provide arbitrary units for the strength of the light emission. This makes it very hard for researchers to compare results between different systems. We think that the absolute measurements from our quantitative luminescence measurement system are important not only for scientific research but also for the applications of bioluminescence.

What are your future plans?
We are now collaborating with a bio-company to commercialize the quantitative luminescence measurement system. Measuring the quantum yield and quantitative spectra of different types of firefly luciferase and its mutants to investigate the colour-change mechanism is another subject that we want to tackle. We are also interested in measuring the efficiency of the chemical reaction and the fluorescence quantum yield of oxyluciferin, which will provide more clues to the bioluminescence reaction. Lastly, we intend to evaluate the activity of the luciferase during gene-expression reporting so that a more quantitative measurement of gene expression can be obtained.

Interview by Rachel Won.

Ando and her co-workers have a letter on firefly bioluminescence on p44 of this issue.