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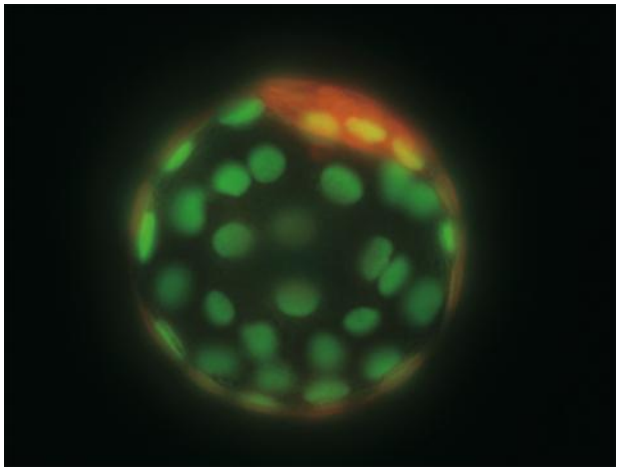
New, lower-cost approach to fluorescence imaging

February 13, 2012 – Fluorescence imaging is a foundational technology in modern cell and molecular biology, making it possible to label specific molecules and analyze their structural and functional properties. This imaging technique is not, however, without its disadvantages. The fluorescent light used to obtain images, for example, can cause significant damage to living cells and many of the dyes used lose their color quickly after exposure. Cost can also be an issue, as expensive equipment such as mercury lamps and lasers are need to generate the light needed to excite fluorescent molecules into their light-emitting state.

Now, Kazuo Yamagata and others in the Laboratory for Genomic Reprogramming (Teruhiko Wakayama, Team Leader) have showed that halogen lamps can serve as a cheaper, lower-intensity light source for use in fluorescence imaging studies in living cells. The report of this new technological development is published in *PLoS ONE*. Yamagata has since moved to take a position at Osaka University.



Mouse embryo imaged by fluorescence microscopy using an upright optical microscope equipped with halogen lamps and an excitation filter. Inner cell mass (red) and trophectoderm cells (green) are shown.

The demand for an alternative light source was first brought home to the team through their frequent interactions with labs in developing countries in Asia, for whom mercury lamps and lasers can be prohibitively expensive. “We knew that the motivation levels are very high in these labs, but their limited financial resources make it difficult to acquire advanced microscopes, which inevitably deepens the ‘research divide’ between developed and developing nations,” says Wakayama. “Fluorescence imaging traditionally requires powerful light sources, but with the advent of newer molecules, we thought it might be possible to achieve similar results even with weaker lamps.”

Imaging of this sort requires the use of excitation filters, which exclude light of wavelengths other than those needed to charge target fluorescent molecules into an excited, light-emitting state. In collaboration with Olympus, Yamagata et al. developed an adaptor to allow ordinary upright and inverted microscopes using only halogen lamps to be equipped with such filters, as well as filters to absorb light other than that emitted from a fluorescing sample. When they tested this new equipment on fluorescence-labeled mouse embryos, they found the results to be remarkably vivid, comparable to those typical of a traditional fluorescence microscope.

Fluorescence imaging normally produces images on a dark field, due to the filtering of light at visible wavelengths, but this can prove disadvantageous when scientists desire to observe cellular features in a brighter context. By modifying their adaptors, however, the team was able to create filters smaller than the optical path with “leaky” diaphragms that can be opened to allow light around the periphery of the image area to escape filtration, thereby making it possible to obtain bright field and fluorescence images simultaneously.

Length of exposure to intense light is a critical factor in determining cellular damage during imaging. To compare the efficiency of their halogen lamp system with that of traditional mercury lamps, the

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team injected oocytes with phycoerythrin-labeled antibody against histone (or fluorescent dye for the nucleus) and observed them using each approach. Those observed using a conventional fluorescence microscope faded within around 30 seconds, but the same sample tested using the halogen light system remained continuously visible for over 10 min. This lower-power approach may have real biological consequences – early embryos imaged using conventional technology show reduced developmental viability on implant in surrogate mothers, but those visualized using the new system showed no such effect.

“One of the biggest advantages of this approach is its low cost, which should put it within reach for many labs in economically developing parts of the world,” says Wakayama. “Of course, there are many different types of halogen lamp, so we will need to keep studying the optimum intensity, but we are hopeful that the adaptors we have developed will make it possible to attach fluorescence filters to ordinary optic microscopes at much lower cost.”

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