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reports:

Xiaokun Shu, Antoine Royant, Michael Z. Lin, Todd A. Aguilera, Varda Lev-Ram, Paul A. Steinbach, and Roger Y. Tsien
Mammalian Expression of Infrared Fluorescent Proteins Engineered from a Bacterial Phytochrome

Science 2009; 324: 804-807 [\[Abstract\]](#) [\[Full text\]](#) [\[PDF\]](#)

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PUBLISHED E-LETTER RESPONSES:

▼ **Mouse Liver Fluorescence Is Shown with GFP**
 Sheldon Penman, Robert M. Hoffman (17 August 2009)

Mouse Liver Fluorescence Is Shown with GFP 17 August 2009 ▲

Sheldon Penman,
 Professor of
 Surgery Emeritus
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 Institute of
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 Cambridge, MA,
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 Robert M.
 Hoffman

Roger Tsien's laboratory recently reported the development of a long wavelength protein chromophore, with potential advantages for non-invasive imaging of internal organs by fluorescence in living animals ("Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome," by X. Shu *et al.*, Reports, 8 May 2009, p. 804). However, this otherwise impressive report erroneously claims that employing green fluorescent protein (GFP) yields no detectable external fluorescence from the liver, thereby ignoring an extensive and well-documented literature describing non-invasive imaging of internal GFP

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fluorescence in living animals (Figure 3). The claim is much more than merely an incorrect datum. External imaging of internal fluorescence is a relatively new technique and is supported by several hundred published peer-reviewed papers, including approximately 70 from the Hoffman group (for instance, 1, 2). For those entering the field, the stability and high quantum efficiency of GFP requires only inexpensive equipment for such experiments. Unfortunately, there are a surprising number of unsubstantiated claims of GFP failure. In this case, an apparently similar claim by a prestigious scientist in a leading scientific journal may unnecessarily retard progress in this field.

In contrast to the Shu *et al.* Report, panels C and D in Figure 1 of M. Yang *et al.* present non-invasive images of interior GFP fluorescence (3). Other images appear in numerous peer-reviewed publications, such as M. T. Henman *et al.* in *Nature* (4). Shu *et al.*'s data in the panel A of Figure 3 fail to show GFP fluorescence from a labeled mouse liver. This apparent contradiction is most probably due to the presence of intense keratin autofluorescence in the remnant hairs bordering the shaved area. This strong autofluorescence very likely punches through the filter channel, saturating the imaging instrument. Successful experiments imaging GFP use either nude mice or wild-type mice which are shaved to remove the hair.

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