

## MELANIN AUTOFLUORESCENCE IN CANCEROUS AND NORMAL MELANOCYTES

**Stephen Nighswander-Rempel, Peter Parsons** (Queensland Institute of Medical Research), **Halina Rubinsztein-Dunlop, and Paul Meredith.**

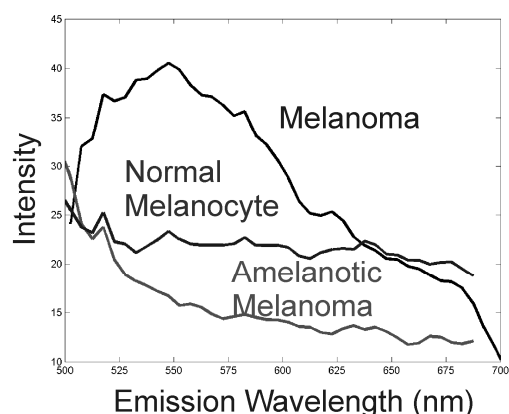
Centre for Biophotonics and Laser Science  
University of Queensland, Brisbane, Australia 4067  
Email: snighrem@physics.uq.edu.au

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Melanoma is a major health concern in Australia, but there is currently no rapid, non-invasive diagnostic for it. The key may lie in the melanin compound. We have shown that melanin does fluoresce, but with low quantum yield.[1] In this study, confocal fluorescence microscopy was used to image melanin fluorescence in cell lines and characterise melanin's emission spectra in normal and cancerous cells *in vivo*.

Samples of pigmented melanoma, amelanotic melanoma (cancerous, but lacking melanin), and normal melanocytes (skin cells) were fixed in methanol and mapped using CFM. Slides of synthetic melanin were prepared in an identical manner for comparison. All samples were excited at 476 nm in order to avoid exciting other natural fluorophores. Single images and wavelength series scans were acquired for each field of view. For single images, an emission bandwidth of 140 nm (510-650 nm) was used. For wavelength scans, fluorescence images were acquired at 5 nm intervals between 490 and 690 nm emission with a 5 nm detection bandwidth, providing full emission spectra of melanin within the cells.

Pigmented melanoma cells exhibited much more intense fluorescence within the cytoplasm than the other cell lines in most cases (see Figure). The emission profile of the amelanotic cell line strongly suggests that its spectrum is primarily due to scattering. Comparison of the melanoma emission spectrum with that of synthetic melanin indicates that its strong fluorescence is in fact likely due to melanin. The increased fluorescence intensity in melanoma cells over normal cells possibly suggests that something is quenching melanin fluorescence in the normal state, but this quenching is removed with the onset of cancer. Finally, fluorescence images of some melanoma cell lines exhibited areas of intense emission in the nuclei; no such features were observed in the nuclei of normal melanocytes. This difference suggests that melanin may also contribute to DNA damage in melanoma.



Differences in the intensity of fluorescence were observed between normal and cancerous melanocytes, and may provide a basis for the development of a rapid, non-invasive diagnostic tool for melanoma. Differences in melanin distribution also suggest that confocal fluorescence microscopy can provide valuable data on melanin structure and function *in vivo*.

[1] S.P. Nighswander-Rempel; J. Riesz, J. Gilmore, and P. Meredith, "A Quantum Yield Map for Synthetic Eumelanin," *J. Chem. Phys.* **123**: 194901 (2005).