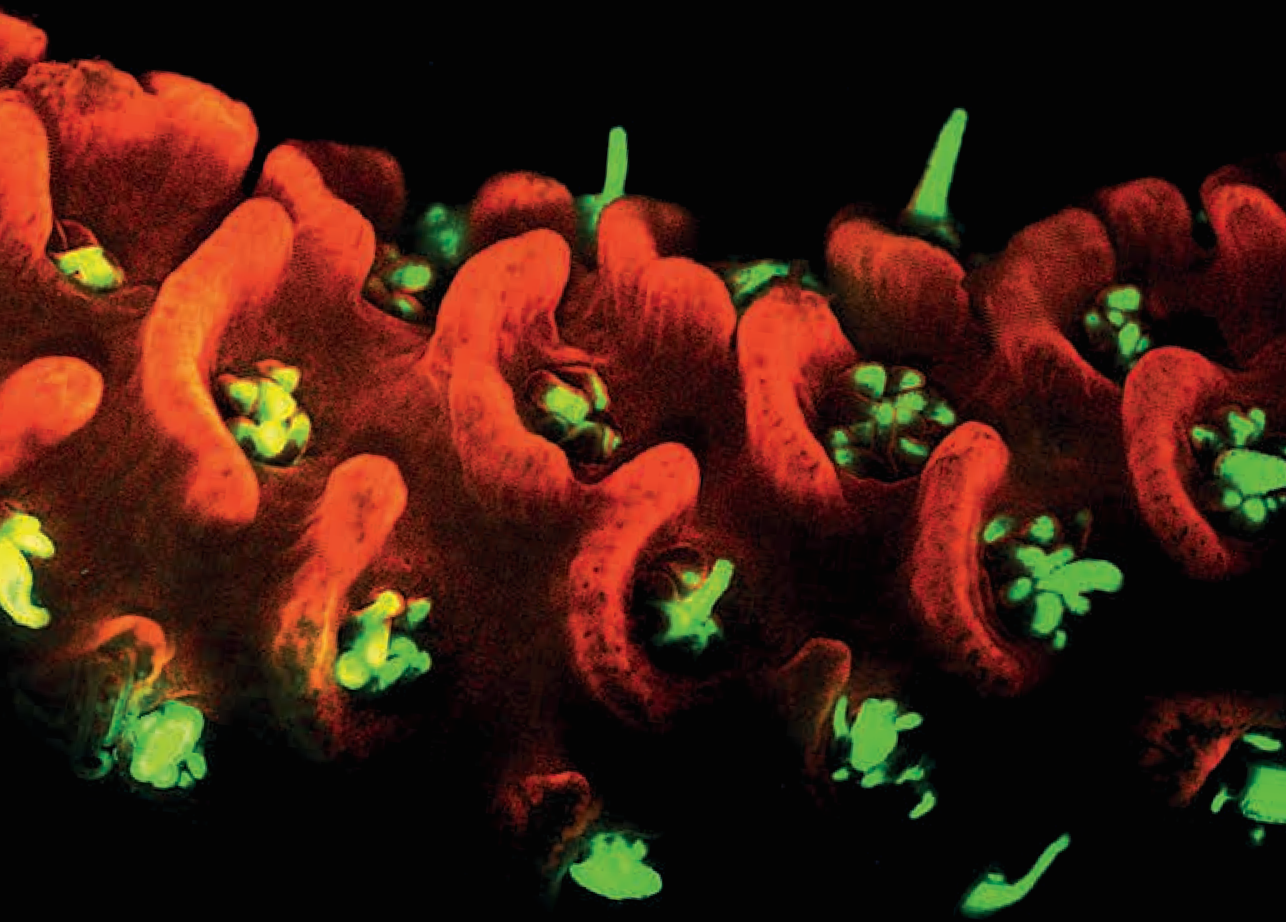
Ocean and Earth Science,
National Oceanography Centre
Southampton

UNIVERSITY OF
Southampton

# See the light.
# The science of coral fluorescence

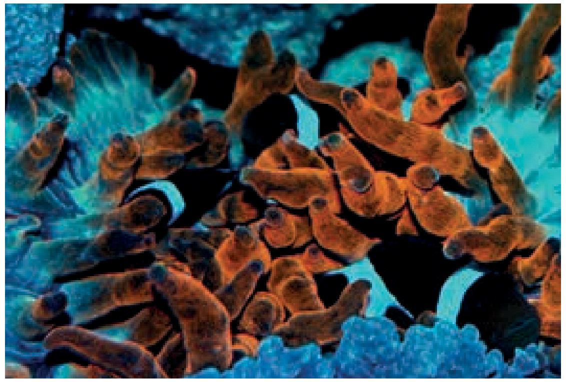


# What is fluorescence?

Fluorescence is a physical process during which light of a distinct colour is taken up by certain dye particles and subsequently re-emitted with a different, more red-shifted colour. Fluorescence can be best viewed when these pigments are excited with ultraviolet or blue light and the green or red fluorescence stands out from the blue background. Even in the presence of white light, strongly fluorescent dyes give a „neon glow“ to the coloured animals or objects. Everyday examples for fluorescent dyes can be found in marker pens or high visibility clothing.

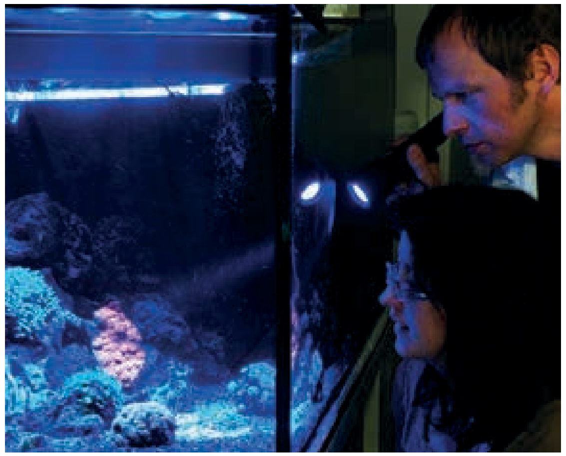
Not all pigments are fluorescent: The orange colour of clown fishes is produced by non-fluorescent dyes that are similar to those of the carrot. The orange colour of their host anemone is due to a red fluorescent pigment (Ref. 1).

Reference 1: Wiedenmann, J., Schenk, A., Rocker, C., Girod, A., Spindler, K.D., Nienhaus, G.U. 2002. A far-red fluorescent protein with fast maturation and reduced oligomerization tendency from Entacmaea quadricolor (Anthozoa, Actinaria). Proc. Natl. Acad. Sci. USA 99, 11646-11651.



Clownfishes (*Amphiprion percula*) in their host anemone *Entacmaea quadricolor*. Under white light both organisms look orange (upper image). Under blue light excitation only the orange fluorescent pigments of the anemone's tentacles are visible (lower image).

Fluorescence in action. Images show the fluorescence of high visibility clothing, marker pens and purified coral pigments under daylight (left) and under blue light (right).



Blue light can excite coral fluorescence. Here, blue light torches are used to stimulate coral fluorescence in the Coral Reef Laboratory of the University of Southampton.



# The Fluorescence Aquarium in the reception area of NOCS

The Coral Reef Tank in the NOCS reception area is a combination of display, research and teaching aquarium dedicated to demonstrating our commitment to public access and engagement about marine science. The system is maintained by the Coral Reef Laboratory and circulates more than 2200 litres of artificial sea water with the display tank holding 600 litres. The tank is illuminated applying an advanced lighting concept using AquaRay® LEDs.

The mission of the tank is:

- To provide a sustainable supply of corals for research purposes.
- To enhance the learning experience of students by providing a living display of organisms relevant to their courses.
- To contribute to the conservation of endangered species.

The tank is specifically designed to demonstrate the fluorescence of reef corals and other cnidarians. Fluorescence can be demonstrated by pressing the button next to the coral reef tank. This switches off the white light while the blue light stays on. Blue light–induced green and red fluorescence will become visible in some animals. Light conditions will return slowly to normal after the pressure on the button is released. This function is only available when the white lights are on.

**Reference**

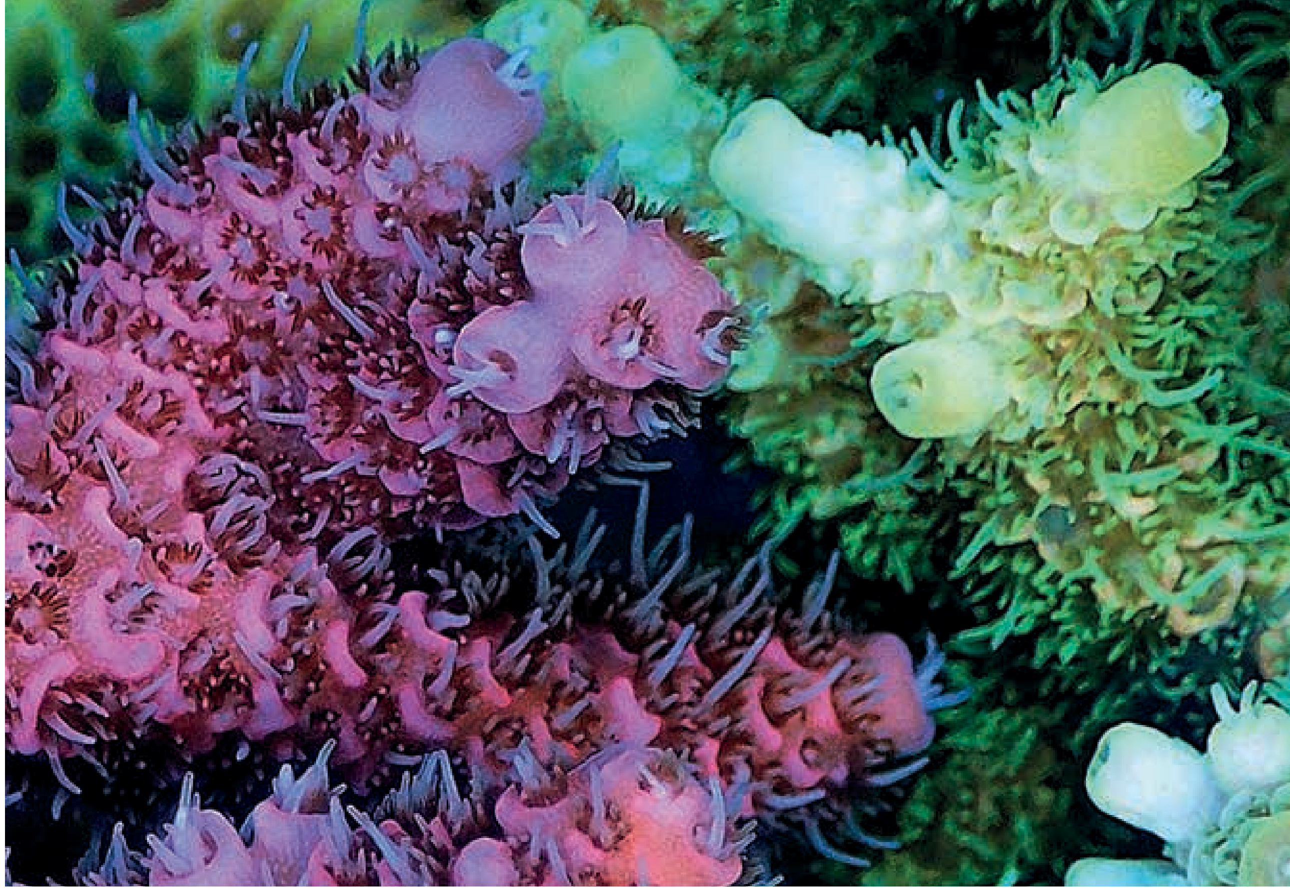
D'Angelo, C. & Wiedenmann, J. 2012. An experimental mesocosm for long-term studies of reef corals. J. Mar. Biol. Assoc. U.K. 92, 769-775.

**Acknowledgements**

The Coral Reef Tank at NOCS is sponsored by Tropical Marine Centre London (TMC) and Tropic Marin, Wartenberg.

The fluorescence tank of the Coral Reef Laboratory in the NOCS reception area

# Fluorescent proteins from stinging animals (Cnidaria)



Fluorescent pigments of corals and other cnidarians are proteins that can emit cyan, green, yellow or red light. Together they form the family of so-called green fluorescent protein (GFP) –like pigments. They are responsible for many of the bright colours of corals and other cnidarians such as sea anemones. In contrast, the brownish appearances of some of these animals under daylight is due to the photosynthetic pigments of the symbiotic algae that live in their tissue.

The corals benefit from the photosynthetic products of the algae. If corals loose their symbiotic algae due to stress, their white calcareous skeleton shines through their tissue, resulting in a bleached appearence of the coral (Ref. 2-5).

**Reference 2:** Oswald, F., Schmitt, F., Leutenegger, A., Ivanchenko, S., D'Angelo, C., et al., & Wiedenmann, J. 2007. Contributions of host and symbiont pigments to the coloration of reef corals. FEBS J. 274, 1102-1109.

**Reference 3:** D'Angelo, C., Denzel, A., Vogt, A., Matz, M.V., Oswald, F., Salih, A., Nienhaus, G.U., Wiedenmann, J. 2008. Blue light regulation of host pigment in reef-building corals. Mar. Ecol. Prog. Ser. 364, 97-106.

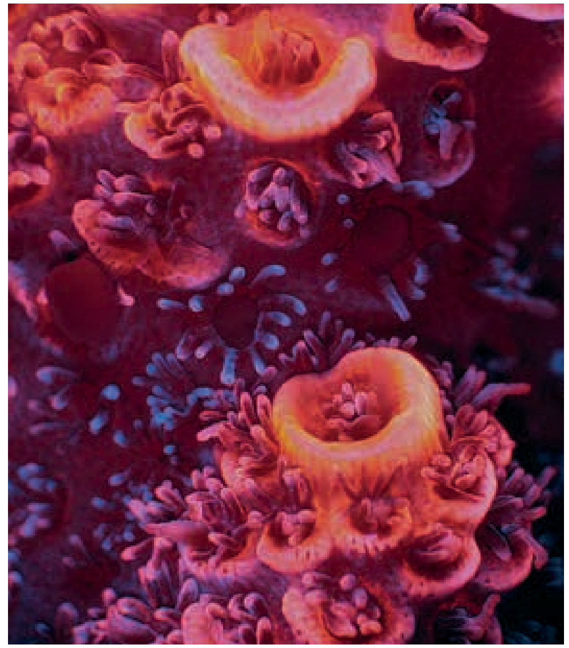
**Reference 4:** Wiedenmann, J., C. D'Angelo, E.G. Smith, A.N. Hunt, F.E. Legiret, A.D. Postle, et al. 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Clim. Change 3: 160-164.

Corals such as *Acropora palmata* (below) produce small amounts of fluorescent proteins and appear brownish due to the colour of the algal symbionts that shines throught the animal tissue. The loss of the algae results in coral bleaching (above) (Ref. 4).

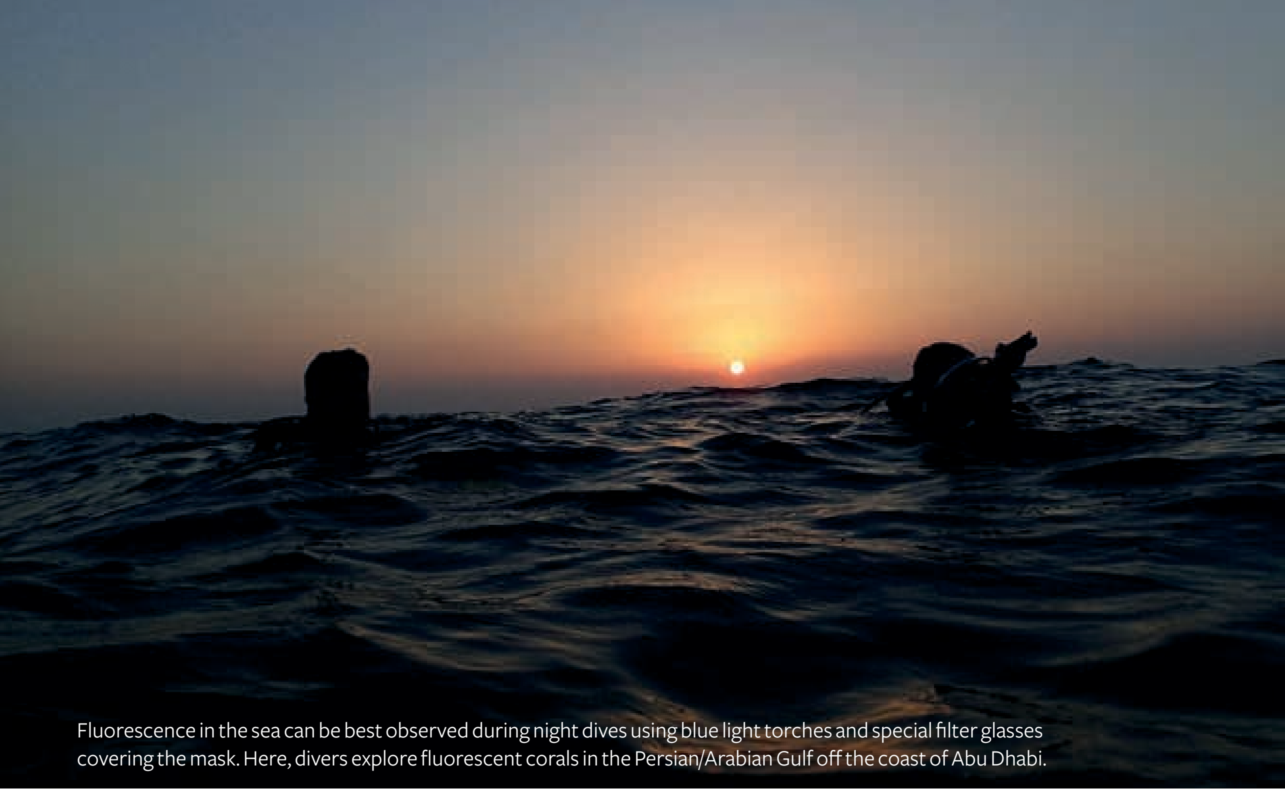
Under intense light, staghorn corals such as *Acropora millepora* (above) accumulate high amounts of green and red fluorescent proteins that dominate their visual appearance. The coral pigments mask the brown colour of the symbiotic algae in the coral tissue.

Corals use multiple copies of the same pigment gene to increase the production of the dye molecules. The colour of the corals depends on the number and type of active pigment genes (Ref. 3). So-called "colour morphs", representatives of the same species with different colouration can be caused by deviating numbers of active pigment genes (Ref. 5). Green and red colour morphs of the coral *Acropora millepora* are shown above.

**Reference 5:** Gittins, J.R., C. D'Angelo, F. Oswald, R.J. Edwards and J. Wiedenmann. 2015. Fluorescent protein-mediated colour polymorphism in reef corals: multicopy genes extend the adaptation/acclimatization potential to variable light environments. Mol. Ecol. 24: 453-465.

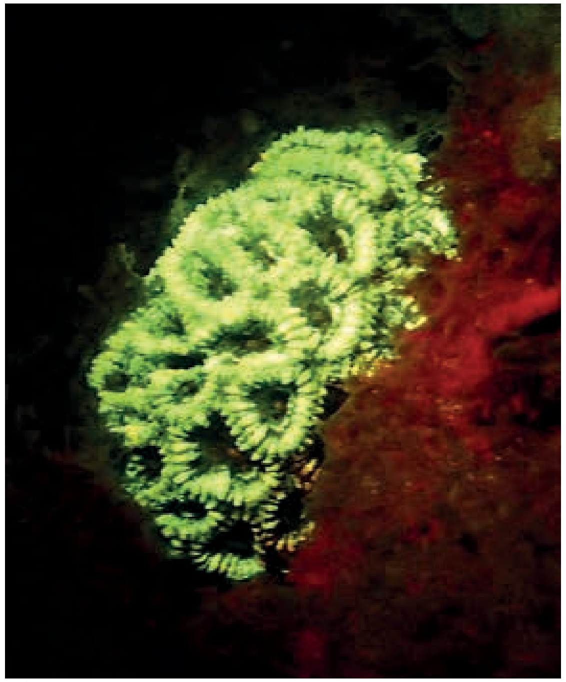


Fluorescence of a red colour morph of *Acropora millepora* photographed under the fluorescence microscope

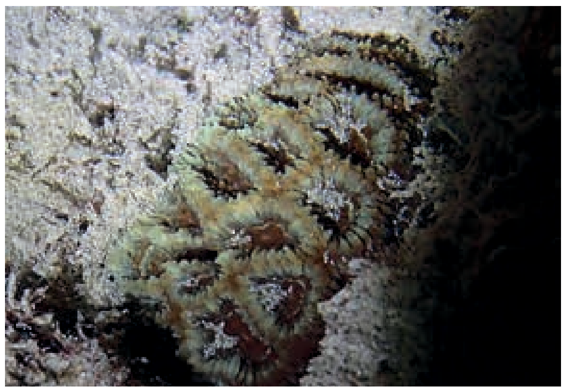


Fluorescence in the sea can be best observed during night dives using blue light torches and special filter glasses covering the mask. Here, divers explore fluorescent corals in the Persian/Arabian Gulf off the coast of Abu Dhabi.

# Fluorescence in the sea



A juvenile coral growing on a breakwater photographed under white light illumination (below) and it's fluorescence imaged under blue light (left) with a yellow long pass filter that prevents the excitation light from entering the camera. Such filters can be also used by divers in goggle-format. Since corals can be easily spotted with this equipment, fluorescence monitoring is an emerging technique for habitat mapping in coral reefs.



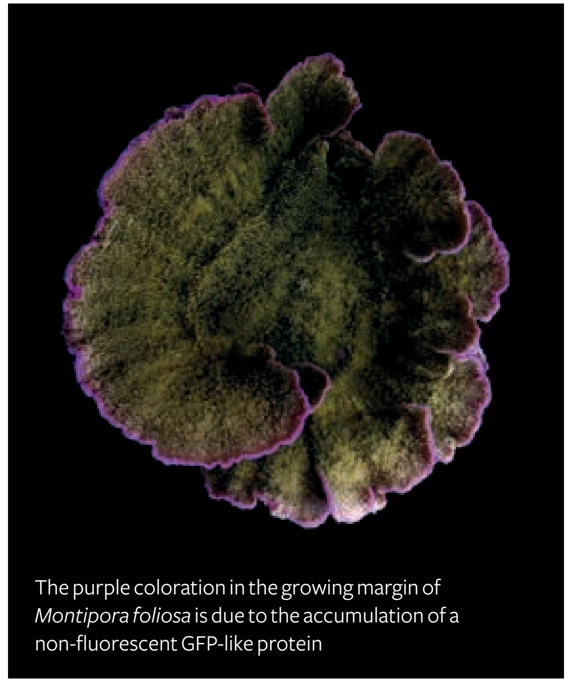


Pink, non-fluorescent chromoproteins in a *Pocillopora* coral in the Red Sea.

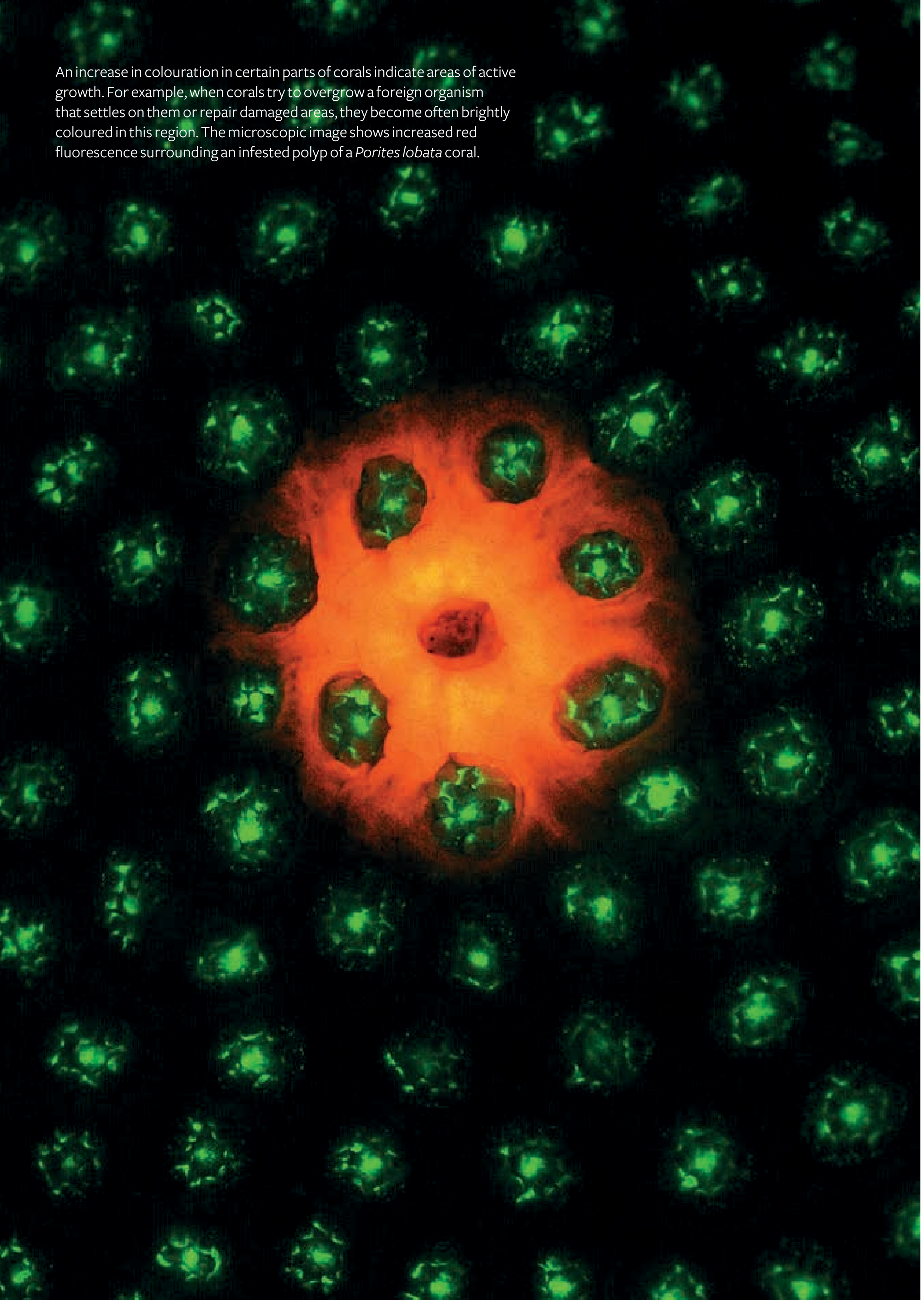
# Non-fluorescent GFP-like proteins

Not all members of the fluorescent protein family are strongly fluorescent. The purple, pink and blue pigments (chromoproteins, CPs) that are often found in the growing tips or margins of corals are biochemically very similar to the highly fluorescent proteins. However, a few amino acid exchanges leave them brightly coloured but essentially non-fluorescent (Ref. 6).

**Reference 6:** D'Angelo C., Smith E.G., Oswald F., Burt J., Tchernov D. and Wiedenmann J. 2012 Locally accelerated growth is part of the innate immune response and repair mechanisms in reefbuilding corals as detected by green fluorescent protein (GFP)-like pigments. Coral Reefs 31, 1045-1056.

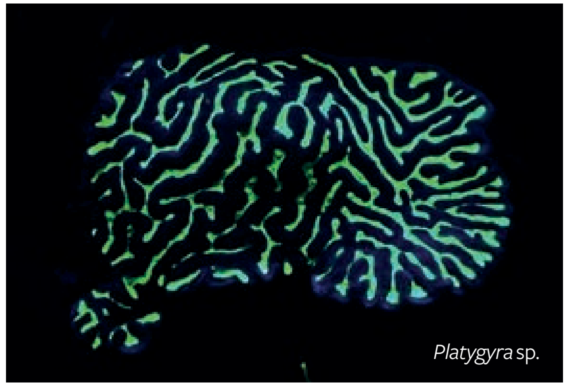


The purple coloration in the growing margin of *Montipora foliosa* is due to the accumulation of a non-fluorescent GFP-like protein



An increase in colouration in certain parts of corals indicate areas of active growth. For example, when corals try to overgrow a foreign organism that settles on them or repair damaged areas, they become often brightly coloured in this region. The microscopic image shows increased red fluorescence surrounding an infested polyp of a *Porites lobata* coral.

# Functions of fluorescent proteins and chromoproteins in corals



*Platygyra* sp.

Fluorescent proteins and their non-fluorescent counterparts can help the corals to optimise the light levels inside their tissue. In shallow sunlit waters, some of these fluorescent dyes and chemically related nonfluorescent chromoproteins shield the corals' symbiotic algae from excess light (Ref. 7). In deeper water, fluorescent proteins are thought to help the coral symbionts to make better use of the low amounts of bluish light that is available in their environment (Ref. 8).

**Reference 7:** Smith, E.G., D'Angelo, C., Salih, A., Wiedenmann, J. 2013. Screening by coral green fluorescent protein (GFP)-like chromoproteins supports a role in photoprotection of zooxanthellae. Coral Reefs 32, 463-474

**Reference 8:** Eyal, G., Wiedenmann, J., Grinblat, M., D'Angelo, et al. 2015. Spectral diversity and regulation of coral fluorescence in a mesophotic reef habitat in the Red Sea. PLoS ONE, 10, e0128697.

Some corals increase the production of photoprotective pigments from the fluorescent protein family when they are exposed to more intense sunlight. When humans get a sun tan, corals become more colourful!

Red fluorescence of a *Dipsastraea* (*Favia*) sp. coral from mesophotic reefs of the Red Sea

# Applications of fluorescent proteins in biomedical research

The information required to produce a fluorescent protein is encoded by a single gene. Using molecular biological techniques, the colour genes can be isolated from corals and transferred into cells of other organisms. There, they can be used as advanced imaging tools that allow biomedical researchers to understand how life works at the cellular level (Ref. 9-10). Common applications are the labeling and tracking of proteins or measuring of gene activity in living cells. Fluorescence proteins are also used for instance, to develop and test new cancer drugs.

**Reference 9:** Wiedenmann, J., Oswald, F., Nienhaus, G.U. 2009. Fluorescent proteins for live cell imaging: opportunities, limitations, and challenges. IUBMB Life 61, 1029-1042.

**Reference 10:** Kredel, S., Oswald, F., Nienhaus, K., Deuschle, K., Röcker, C., Wolff, M., Heilker, R., Nienhaus, G.U., Wiedenmann, J. 2009. mRuby, a bright monomeric red fluorescent protein for labeling of subcellular structures. PLoS ONE 4, e4391.

The Nobel Prize in Chemistry 2008 was awarded to the scientists Shimomura, Chalfie and Tsien for their discovery of the first green fluorescent protein from the jellyfish *Aequorea victoria* and its use as marker protein.

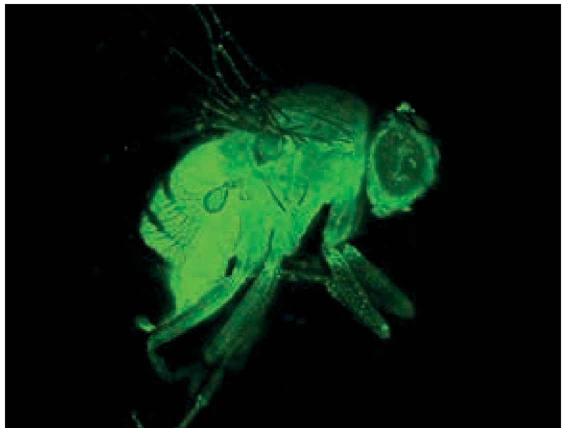
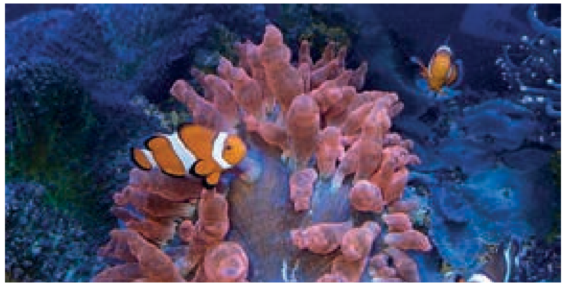
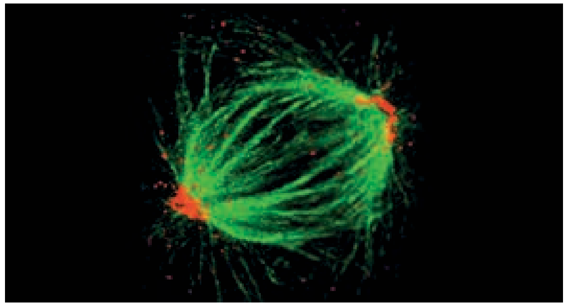


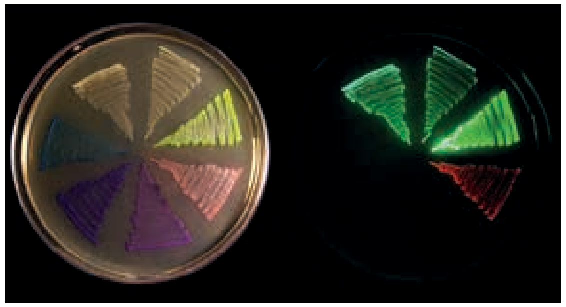
Image of the fruitfly (*Drosophila*) producing a green fluorescent protein which was isolated by the Wiedenmanngroup from the reef coral *Lobophyllia hemprichii* (Image: Dr. Franz Oswald, University of Ulm, Germany).



The sea anemone *Entacmaea quadricolor* in the NOCS Coral Reef Tank from which the precursor of the red fluorescent protein mRuby was isolated.



The optimised marker mRuby was generated by Wiedenmann and his team using protein engineering techniques. It can be used in combination with green fluorescent dyes in multicolour labelling experiments. The fluorescence microscopic image shows the equal partitioning of cellular organelles (peroxisomes, highlighted red by mRuby) into the daugther cells during cell division (Ref. 10). Parts of the cell skeleton are labeled with a green fluorescent marker.



Agar plates with streaks of genetically modified bacteria producing FPs and CPs from *Acropora millepora* (left: daylight; right: fluorescence). Bacterial expression facilitates the characterisation and optimisation of novel marker proteins.

Fluorescence of corals from mesophotic reefs of the Red Sea

Fluorescence of
*Lobophyllia hemprichii*
in the Red Sea

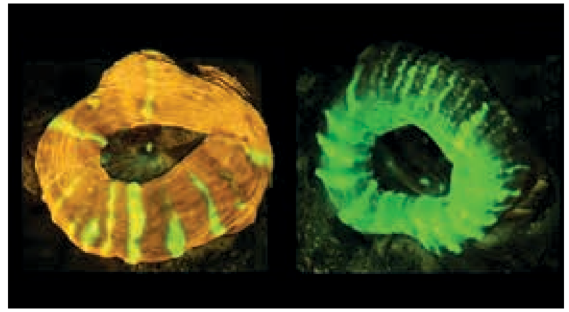
# Photoconvertible fluorescent proteins and their applications

The coral *Lobophyllia hemprichii*, shown in the figure on the right, produces a green fluorescent protein called „EosFP". This pigment turns red by a process termed photoconversion when it is exposed to violet light (Ref. 11). *L. hemprichii* changes its colour from green to red when it is exposed to sufficient violet light.

**Reference 11:** Wiedenmann, J., Ivanchenko, S., Oswald, F., Schmitt, F., Rocker, C., Salih, A., Spindler, K.D., Nienhaus, G.U. 2004. EosFP, a fluorescent marker protein with UV-inducible green-to-red fluorescence conversion. Proc. Natl. Acad. Sci. USA 101, 15905-15910.

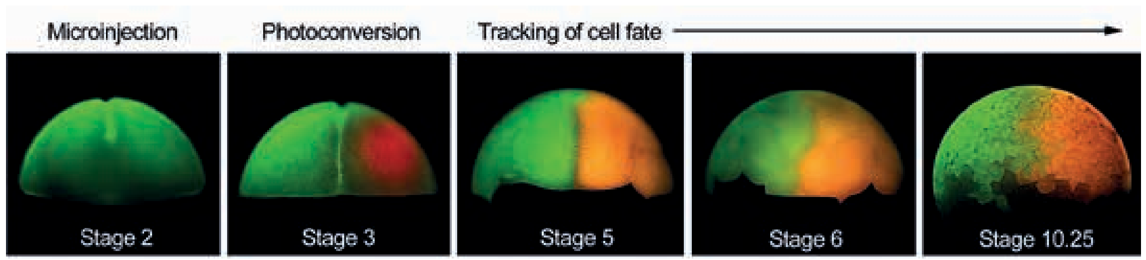
**Reference 12:** Wiedenmann, J., Gayda, S., Adam, V., Oswald, F., Nienhaus, K., Bourgeois, D., Nienhaus, G.U. 2011. From EosFP to mIrisFP: Structure-based development of advanced photoactivatable marker proteins of the GFP-family. J. Biophotonics 4, 377-390.

**Reference 13:** Wacker, S.A., Oswald, F., Wiedenmann, J., Knochel, W, 2007. A green to red photoconvertible protein as an analyzing tool for early vertebrate development. Dev. Dyn. 236, 473-



The fluorescence colour of the coral *Lobophyllia hemprichii* changes when grown without (left) or with violet light (right).

The green-to-red photoconvertible fluorescent protein EosFP can be used to track single protein molecules in living cells. It has helped to develop imaging concepts that improve the maximal resolution of optical microscopes by one order of magnitude (Ref. 12). The scientist Eric Betzig won the 2014 Nobel Prize in Chemistry for inventing this particular superresolution microscopy technique!




A developing frog egg (*Xenopus*) was labelled by microinjection with the green-to-red switchable fluorescent protein EosFP. A single cell was highlighted red under the microscope by a brief focussed illumination with violet light. The fate of this cell and its descendant daughter cells can be followed subsequently during the early development of the tadpole (Ref. 13).

SOES 2026 students isolating fluorescent coral pigments

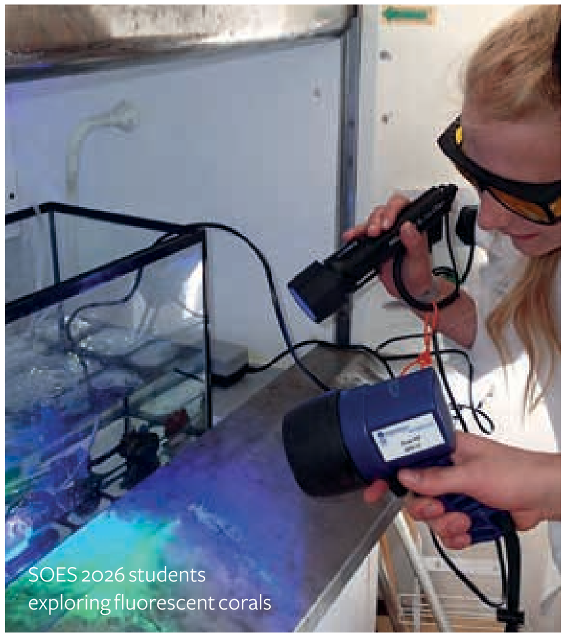
# Thinking about studying?

Learn more about coral fluorescence and biomedical applications of fluorescent proteins as part of the University of Southampton's Marine Biology Module SOES2026 "Introduction to Marine Molecular Biology"

Get involved and purify fluorescent proteins in the practical courses of SOES2026!

Hear from world-leading experts about their cutting-edge molecular research ranging from tropical coral reefs to the deep sea!

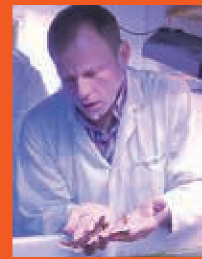
Molecular biology is an irreplaceble tool for modern Marine Biology. We will prepare you to use it!



SOES 2026 students exploring fluorescent corals



# Author info



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**The Coral Reef Laboratory**

The team of the Coral Reef Laboratory works on a broad range of aspects of coral reef biology. Their research contributes also new insights in the function of fluorescent proteins and provides novel imaging tools for biomedical applications.

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Frontcover: Green and red fluorescence of a red colour morph of the staghorn coral *Acropora millepora* photographed under the fluorescence microscope.

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