

# FLUORESCENCE LIFETIME IMAGING OF CORAL FLUORESCENT PROTEINS

Guy Cox, Anya Salih  
Electron Microscope Unit;  
University of Sydney, NSW 2006, Australia.  
guy@emu.usyd.edu.au

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## 1. INTRODUCTION

Corals, like many other coelenterates, contain fluorescent pigments which show considerable homology with the well known Green Fluorescent Protein of the jellyfish *Aequoria* [1]. In corals, unlike jellyfish, multiple proteins are present and the range of excitations and emissions suggest the possibility of energy transfer [2]. The occurrence of FRET between fluorescent proteins in corals has already been reported and time-resolved spectra have shown the effect on fluorescent lifetime, but without any spatial resolution [3]. Lifetime confocal microscopy offers lower time resolution but excellent spatial resolution This study set out to determine the extent of FRET between pigments in two corals which differ in the arrangement of their pigments and hence possibly in pigment function.

## 2. MATERIALS AND METHODS

Living zooxanthellae and polyp tissue from aquarium-grown *Euphyllia ancora* and RNAlater® (Ambion Inc, Austin, Texas USA) treated *Acropora millepora* were examined in seawater. Purified, cloned FPs from *A. millepora* were provided by M. Matz, University of Florida and were examined either in solution or dried down on a slide. Samples were examined with a Nikon C1 confocal microscope attached to a Nikon TE2000 inverted microscope and equipped with a Nikon (Europe) LIMO lifetime imaging attachment [4]. Some samples were also examined in a Leica TCS SP2 AOBS confocal microscope fitted with a Becker & Hickl lifetime imaging module.

## 3. RESULTS

Lifetimes of the isolated *A. millepora* pigments were 2.8-2.9ns and 2.9ns. In the coral sample, imaging the entire emission spectrum from 420nm, the mean lifetime was reduced to 1.5ns, implying that FRET was occurring. Looking just at the fluorescence from FRET donors the lifetime was even shorter, at 1.3ns, supporting this interpretation. On the other hand, in *Euphyllia*, even though spectra show the presence of two or three fluorescent pigments, lifetime was 2.4ns, suggesting that no FRET took place.

## REFERENCES

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