

Cytosolic expression of Green Fluorescent Protein (GFP) and its derivatives in the yeast *Saccharomyces cerevisiae*: Detection in vivo using the Agilent Cary Eclipse

Application Note

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Introduction

Since first isolated in 1994 by Chalfie and colleagues, green fluorescent protein (GFP) has proven to be an immensely popular and powerful tool for the non-invasive monitoring of cellular events *in vivo*¹. GFP is a 238 amino acid protein isolated from the fluorescent jellyfish, *Aequoria victoria*. The protein itself has an elegant β -barrel structure measuring 30 x 40 Å, which shields an internal tripeptide fluorophore (Figure 1).

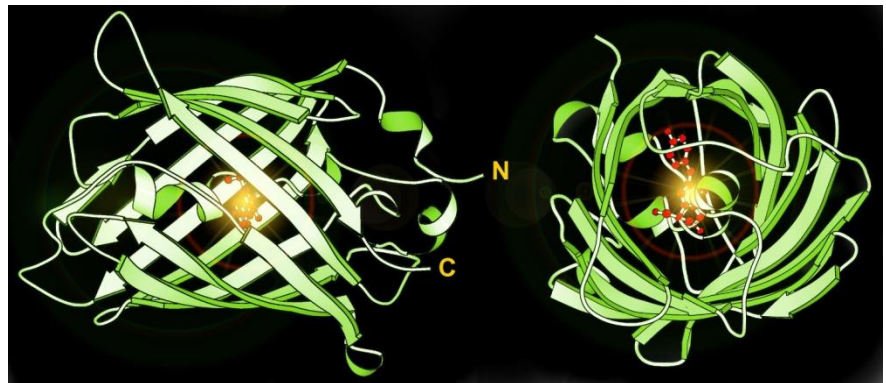


Figure 1. Schematic representation of green fluorescent protein. Tripeptide fluorophore in red

Upon excitation with light of 395 nm (peak excitation) or 475 nm, the GFP fluorophore emits bright green fluorescence at 509 nm. This can be detected using a variety of instruments such as fluorescence microscopes, fluorescence-activated cell sorters and fluorometers.



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GFP is an extremely attractive tool for today's life scientist, mainly because the protein matures without the need for any additional substrates or co-factors. This allows expression of GFP in an array of living organisms and cell systems, resulting in autocatalytic maturation and a fluorescent signal. In other words, GFP allows cells to produce their own fluorescent marker.

This omits the need for conventional fluorescence imaging procedures, which often damage or kill cells, and are not generally feasible for *in vivo* studies. GFP has allowed the real time study of cellular events in live, intact cells and organisms, allowing a better understanding of biological mechanisms in physiological models.

Fused to a protein of interest, one of the first applications of GFP was as a reporter of gene expression and protein localization. It was used to illuminate cellular organelles, the cytoskeleton, and the secretory pathway (reviewed by Gerdes and Kaether²). Since then, a rapidly increasing variety of applications for GFP and its derivatives have been developed. Mutational studies of the chromophore and surrounding amino acids have produced new GFPs with altered excitation and emission spectra. These include blue (BFP), cyan (CFP), and yellow (YFP) fluorescent proteins, all with enhanced maturation rates and fluorescent intensities³. The color spectrum was recently extended by Matz and co-workers⁴, who isolated a red fluorescent protein from the *Discosoma* coral (DsRed) with 30% sequence homology to *Aequoria* GFP. This wide variety of spectrally distinct fluorescent proteins provides distinguishable markers for the simultaneous study of multiple cellular events, as well as allowing more advanced applications such as the study of protein-protein interactions using the phenomena of fluorescence resonance energy transfer (FRET).

The fluorescent properties of the GFP family vary in terms of spectral profiles, intensity and susceptibility to photobleaching. Therefore it is important to optimize detection and measurement protocols for GFP and its

derivatives prior to experimental analysis in each experimental model. The present study aimed to detect and characterise GFPs expressed in the cytosol of live yeast cells (*Saccharomyces cerevisiae*) using the Agilent Cary Eclipse fluorescence spectrophotometer.

Materials and methods

(For part numbers see Reference 5)

Equipment

- Agilent Cary Eclipse fluorescence spectrophotometer
- Peltier-thermostatted multicell holder (with electromagnetic stirring)
- Temperature controller
- Temperature probes
- Magnetic stirrer bars
- Quartz cuvettes (4)

Yeast strains

YRD15 (*MAT α* , *his3*, *ura3*, *leu2*, *p⁺*) of the yeast *S. cerevisiae* was the parental strain used in this study. Genes encoding the GFP derivatives (green, blue, cyan, yellow) and red fluorescent protein (DsRED) were cloned into the yeast expression plasmid pAS1N and transformed into the yeast strain YRD15 as previously described⁶. Transformants were plated out on yeast minimal medium (0.75% yeast minimal medium w/o amino acids, 2% glucose, 1.5% agar) with selective markers as required and grown at 28 °C for 3–5 days.

Protocol

Yeast cells were washed twice in 1ml MilliQ water to remove contaminating media before being resuspended in MilliQ to a final optical density of 0.55 Abs at 650 nm. Cell suspensions (2 ml) were placed in disposable fluorescence cuvettes (Sarstedt) in the multicell holder, which was positioned in the sample compartment of the Cary Eclipse fluorescence spectrophotometer. The temperature within the cuvettes was set to 25 °C. Using the 'Scan' application, cell suspensions were excited with light of the appropriate wavelength for each specific fluorescent protein (See the Clontech Web

Site³ for a comprehensive list of excitation/emission maxima of fluorescent proteins.) Emission scans were recorded for GFP, BFP, CFP, YFP and DsRed. Operating parameters for scans of DsRed are given in Figure 2.

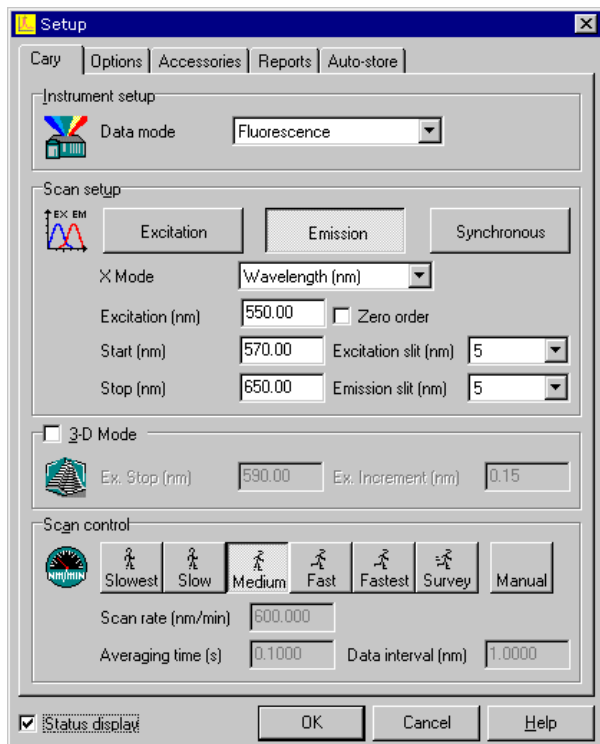


Figure 2. Instrumental parameters for detecting DsRed emission spectra (Emission maxima 583 nm) following excitation with 550 nm (peak excitation 558 nm)

Results

Emission spectra for each of the fluorescent proteins are shown in Figure 3.

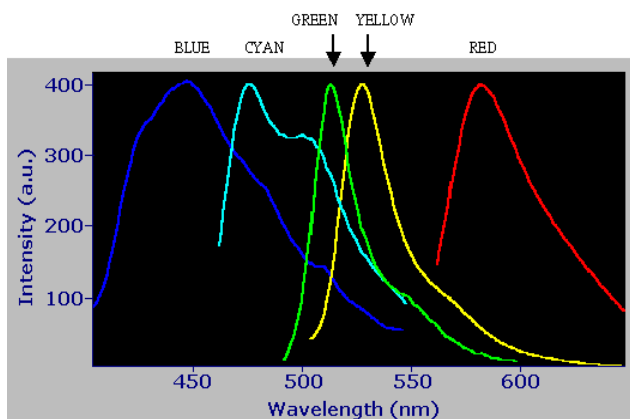


Figure 3. Intensity vs emission for the full spectrum of fluorescent proteins

Discussion

Spectra from all fluorescent proteins expressed in the cytosol of the yeast cells were easily recorded and correspond to expected characteristics. Noise produced by cellular autofluorescence and scatter was negligible, mainly due to internal filters fitted to both excitation and emission monochromators on the Agilent Cary Eclipse. Reducing autofluorescence (from either sample of interest or media) is of paramount importance when using fluorescent proteins that have either low fluorescent intensity, or are excited by high energy UV light (such as BFP). UV light initiates a strong fluorescent response from many components of the cellular environment and must be minimized to prevent masking of emission from target fluorescent proteins, especially when subtle protein interactions are being monitored⁷.

Conclusion

The Agilent Cary Eclipse with multicell and temperature control accessories provides a simple, rapid and reproducible assembly with which to measure and characterise different fluorescent proteins expressed in whole cells. The opportunity now exists to specifically tag proteins of interest within cells using different GFPs and monitor interactions simultaneously using multiwavelength readings— which allow automated excitation and reading of emission at up to six different fluorescent probes— *in vivo*.

References

1. Chalfie M, et al (1994) Science, 263; 802
2. Gerdes, H-H and Kaether, C. Green fluorescent protein: applications in cell biology, FEBS Letters, 389, 44-47, (1996).
3. Clontech Web Site: www.clontech.com
4. Matz, M. V., et al (1999) Nature Biotech. 17, 969–973.

5. Part numbers:

Product	Part Number
Agilent Cary Eclipse fluorescence spectrophotometer	00 100752 00
Peltier-thermostatted multicell holder	00 100755 00
Temperature controller for peltier	00 100386 00
Magnetic stirrer bars	66 100189 00
Temperature probe accessory	60 100408 00
Quartz cuvettes (rectangular, stoppered)	66 100012 00
Kit door for thermostatted accessories	99 101032 00
Cary Eclipse Scan software 85 101772 00	85 101772 00
Country kit (98 1002 XXXX) 9000 (AUS); 9100 (US); 9200 (Europe)	

6. Prescott, M., et al (1994), *Biochem. Biophys. Res. Commun.* 207, 943-949.

7. Lakowicz, J.R. (1999) Ch. 16: Protein Fluorescence. *Principles of Fluorescence Spectroscopy*, 2nd Edn. Kluwer Academic/Plenum Press, New York.

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