

Building a Scanning Fluorescence System Using Acton

Research Components and LabVIEW Software

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ABSTRACT

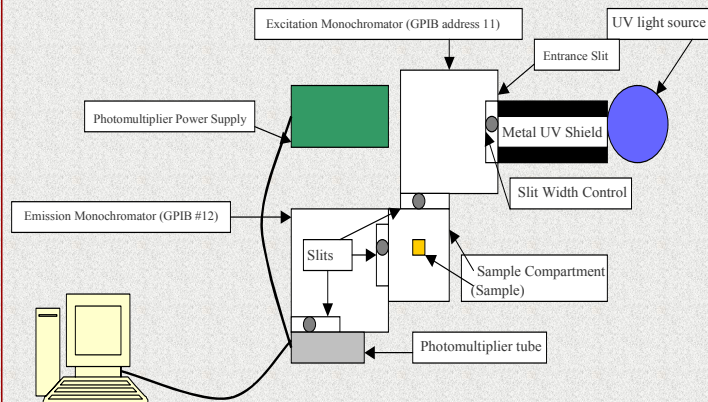
Fluorescing molecules emit light over a range of wavelengths. $I(\lambda_{em})$ describes the emission intensity spectrum. Emission at a particular wavelength is a function of the excitation wavelength. $I(\lambda_{ex})$ is the excitation spectrum.

Fluorescence detection is a popular analytical method mainly because other light-based techniques are restricted by high detection limits. Fluorescence spectrometers can accurately measure extremely low concentrations.

GOALS

- To obtain a working fluorescence spectrometer using Acton Research components (a permanent addition to the Earlham College Chemistry Department).
- To design computer programs for the system using LabVIEW software to control excitation and emission scans, to record PMT signals, to present 3-D plots of signal intensity vs. emission and excitation, and to store data in spreadsheet format.
- To test the sensitivity of the spectrofluorimeter using a known quinine fluorescence system.
- To study the fluorescence of GFP and GFP-UV+Serp, and compare and contrast the 3-D plots.
- To study the fluorescence of fluorotryptophan and tryptophan.

EXPERIMENTAL SETUP



SPECIFICATIONS

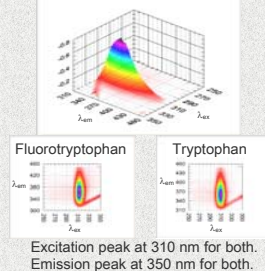
Acton Research Corporation Components:
 Excitation Monochromator (SpectraPro-150)
 Sample Chamber (Model SC-447)
 Emission Monochromator (SpectraPro-150)
 Photomultiplier Housing (Model PD-438)
 Programmable PMT High Voltage Power Supply (PHV-400)
 Photomultiplier Tube (Hamamatsu, R928)
 UV source: 250 Watt Xenon Arc Lamp

COMPUTER PROGRAMS

Excitation scan Dual scans for 3-D plots
 Emission scan Wavelength calibration

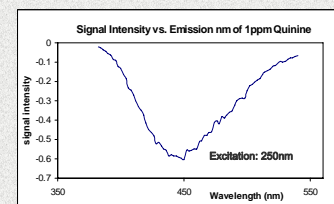
TRYPTOPHANS

Tryptophan Excitation-Emission map

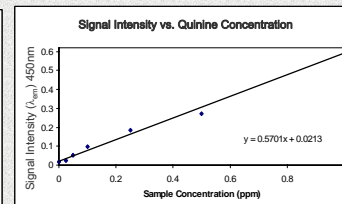


FLUORESCENCE SPECTROSCOPY OF QUININE

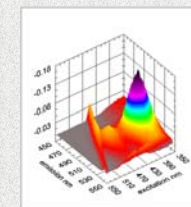
EXAMPLE OF EMISSION SPECTRUM



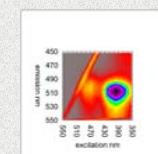
CALIBRATION CURVE



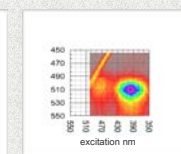
SPECTROSCOPY OF GFP* AND GFP-UV+SERPIN



Example of 3D graph for GFP



GFP



GFP-UV+SERPIN

For both graphs:
 Excitation peaks at 390 and 490 nm.
 Emission peak is at 509 nm.

*GFP purified using Bio-Rad Biotechnology Explorer™ Green Fluorescent Protein Purification Kit

CONCLUSIONS

- Constructed a scanning fluorescence spectrometer.
- Successfully developed several computer programs using LabVIEW software.
- Demonstrated good detection down 1 ppb for quinine.
- 3D graphs of GFP and GFP-UV+serpin show the same excitation and emission peaks, showing that serpin does not affect the fluorescence of GFP-UV. Therefore, it will be possible to observe serpin with this probe under a fluorescence microscope.
- Fluorescence spectra of tryptophan can help to provide a better understanding of tryptophan within Cytochrome *c* for future research on protein folding.
- The fluorine in fluorine-labeled tryptophan does not affect the fluorescence of tryptophan.

WHY GFP AND TRYPTOPHAN?

- GFP (Green Fluorescent Protein):** important because it glows bright green under short wavelength illumination at any time.
- Allows us to look directly into the inner workings of cells when attached as a tag to another protein.
- Professor Amy Mulnix, Earlham College, and Dr. Joshua Bornhorst, Howard Hughes Teaching Fellow, Earlham College, isolated serpin and attached it to GFP with a peptide linker.
- The excitation and emission spectra of GFP and GFP-UV+serpin were compared to see if the serpin affected the fluorescence of GFP.
- Tryptophan:** common amino acid found within proteins such as Cytochrome *c*, used as a probe for protein folding.

REFERENCES

- Rocheleau, J.V.; Edidin, M.; Piston, D.W. 2003. Intrasequence GFP in Class I MHC Molecules, a Rigid Probe for Fluorescence Anisotropy Measurements of the Membrane Environment. *Biophys. J.* 84:4078-4086
- Knight, A., Billinton, N. 2001. Distinguishing GFP from Cellular Autofluorescence. *J. Anal. Biochem.* 291:175-197
- Qui, L.; Pabit, S.A.; Roitberg, A.E.; Hagen, S.J. 2002. Smaller and Faster: The 20-residue Trp-Cage Protein Folds in 4 μ s. *J. Am. Chem. Soc.* 124:12952-12953

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FUTURE DEVELOPMENTS

- Studies of parallel to perpendicular polarization ratios
- Adapting the system to pulse fluorescence excitation studies.