Emission Wavelength Dependence of Fluorescence Lifetimes of Bacteriological Spores

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Abstract

Concern about biological terror has increased greatly in the 21st century, and correspondingly, so has the need for accurate detection and identification of biological hazards, such as *B. anthracis*. Optical techniques have been shown to be useful for this purpose[1,2,3,4]. Use of fluorescence lifetimes as a function of emission wavelength for different materials using point detection methods appears to be an additional viable option. Although the lifetimes range only between 2 and 6 nanoseconds, most materials that were tested were distinguishable. A preliminary database has been compiled for use in a possible future detection system; single-blind tests of unknown materials confirmed the usefulness of the database.

1. Procedure and Equipment

A Laser Science nitrogen laser with a wavelength of 337 nm and duration of 4 nanoseconds was used as the excitation source. The laser light passed through a beam splitter, traveling to a trigger detector and to the sample stage. The samples were placed in a tantalum pan; conventional quartz containers produced fluorescence of about the same strength as the weakest of sample signals. The light was then gathered through a set of two lenses and a chosen wavelength was observed through a Spex 1680 0.22m double spectrometer. A Hamamatsu R7400U photomultiplier tube turned the light signal into an electrical signal, allowing the data to be digitized by a HP 54120B oscilloscope. The data were then analyzed by a commercially available software package FluoFit in order to find the lifetime for each individual wavelength. Despite the expectation that the decay curves are combinations of lifetimes from several materials present in the samples, only single lifetime fits were possible with this apparatus.

Slight variations in the initial conditions set for the fitting program Fluofit often resulted in variations of the lifetime fits of up to ± 0.3 nS. Figure 1 shows test data taken to estimate the repeatability of lifetime measurements. The curves are separate lifetime spectra taken months apart and the error bars represent the standard deviations of fits for ten separate measurements at three emission

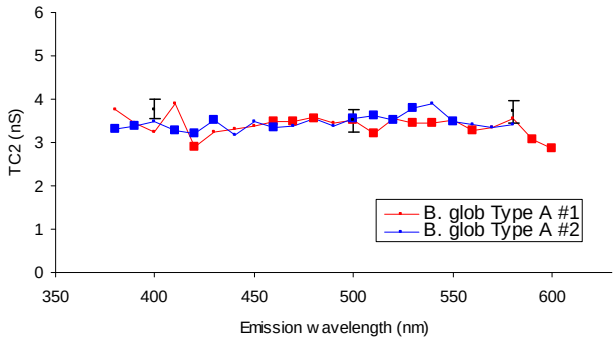

Figure 1: *B. globigii* Type A Repeatability Test

[1] Pinnick, Ronald G., Steven C. Hill, Stanley Niles, Dennis Garvey, Yong-Le Pan, Stephen Holler, Richard Chang, Jerold Bottiger and Burt V. Bronk, 'Real-time measurements of fluorescence spectra from single airborne biological particles,' *Field Anal. Chem and Tech.* **3** (1999) p221.
[2] Méjean, G., J. Kasparian, J. Yu, S. Frey, E. Salmon, and J.-P. Wolf, 'Remote detection and identification of biological aerosols using a femto-second tera-watt lidar system,' *Applied Physics B* **78** (2004) p535.
[3] Thomason, Jay, 'Spectroscopy takes security into the field,' *Photonics Spectra* **38**, 4 (2004) p82.
[4] Gaughan, Richard, 'Spectroscopy poised to seek terror agents,' *Photonics Spectra* **36**, 12 (2002) p23.

wavelengths. Because fluorescence lifetimes are generally expected to change slowly with emission wavelength, it appears that the trends of the lifetime spectra measured will be useful identifying factors.

The materials tested included vegetative cells of bacteria such as *B. globigii*, *B. atrophaeus B. subtilis var. niger*, *B. subtilis spiz*., and *E. coli*, which were of greater than 99% purity. Other materials were tested, but the results are not reported here. Materials were measured at emission wavelengths from 380 to 640 nanometers at 10 nm intervals.

In addition, time-integrated emission spectra were recorded. For these measurements, the PMT was replaced with a slower, but more sensitive Hamamatsu R928 PMT and the signal was recorded with a PAR 150 boxcar integrator which was set to capture all of the fluorescence emission. The data were fed to the computer through an external analog-to-digital interface.

2. Data

A database of the lifetime for each material at each emission wavelength was developed. On the following pages are shown the decay spectra for the tested materials. The bacteria, in general, each exhibited a particular decay lifetime spectrum. *B. globigii*, being the prototypical simulant for *B. anthracis*, was investigated the most thoroughly. As shown in Figure 2, *B. globigii* was tested in the forms of vegetative cells, spores, and a combination of vegetative cells, spores, and nutrient. Certain attributes of the graphs can be clearly observed, including constant, but slightly different lifetimes. Type A (tentatively identified as spores) and Type B (vegetative cells) are relatively indistinguishable within the uncertainty, yet *B. globigii* with nutrient seems to be distinguishable from the pure samples by the average decay lifetime of 4.5 nS compared to about 3.2 nS. A further look at the time-integrated emission spectrum of these three types of *B. globigii* indicates that Type A and Type B are indistinguishable (not shown here), while *B. globigii* with nutrient can easily be distinguished, as shown in Figure 3. The emission spectra were corrected for the PMT response, but were not corrected for the monochromator grating, since their primary use was for further comparison between the materials tested.

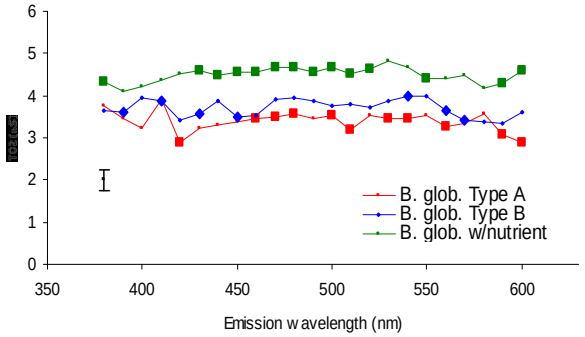

Figure 2: *B. globigii* Fluorescence Decay Lifetimes

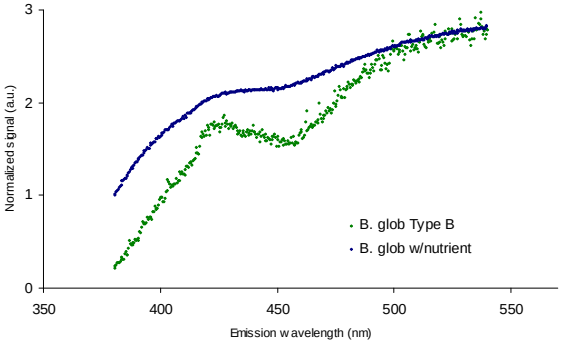

Figure 3: Emission spectrums of *B. globigii Type B* and *B. globigii with Nutrient*

A comparison of the other four bacteria yields promising results. From the decay lifetimes in Figure 4, *B. atrophaeus* and *B. sub. spiz.* are easily distinguishable. *B. atrophaeus* exhibits a general increase in lifetime from 3 nS to 5 nS in the 400 to 500 nm region, then remains constant, while *B. sub. spiz.* decreases from a lifetime of about 5.5 nS to 4.3 nS, then continues with that constant value. Contrarily, *E coli* and *B. sub. var. niger* seem to be extremely similar in both average decay lifetime and general trend throughout the wavelengths. In this case, the time integrated emission spectrum is necessary to distinguish these materials.

As earlier with *B. globigii*, the emission spectra of *E. coli* and *B. sub. var.* nig*er* are identical. Yet, the emission spectra of *B. atrophaeus* and *B. sub. spiz.* are also very similar, suggesting that both the fluorescence decay lifetime and the time-integrated

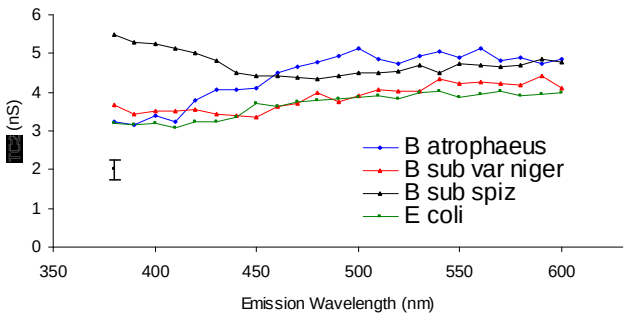

Figure 4: Fluorescence Lifetimes of Bacteria including *B. atrophaeus, B. sub. var. niger, B. sub. spiz,* and *E. coli.*

emission spectra are needed for accurate comparisons. Shown in Figure 5, *B. atrophaeus* exhibits slightly more fluorescence around 400 nm, but is otherwise identical with *B. sub. spiz.*, which also exhibits the same trends.

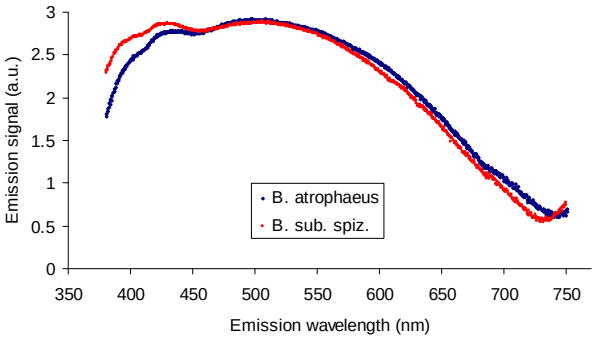

Figure 5: Emission Spectra of *B. atrophaeus* and *B. sub spiz*

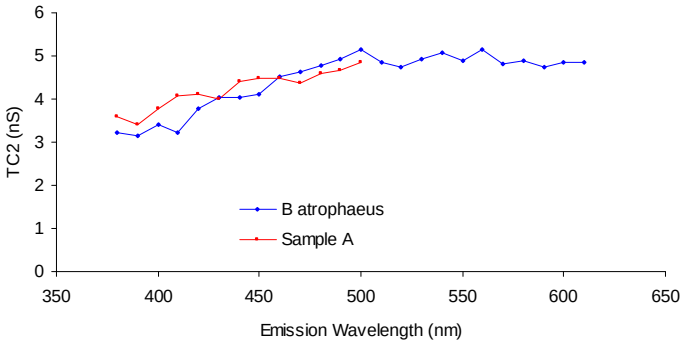

Figure 6: Sample A Comparison Test

3. Comparison Test

Four single-blind comparison tests for the bacteria were taken to check the database of decay lifetimes. Each used the fluorescence system to measure the lifetimes of the 'unknown' material, comparing the sample's data to that in the database. As shown in Figure 6, Sample A follows the same trend as *B. atrophaeus* and was easily identified correctly as *B. atrophaeus.*

The same is true for Sample B (Figure 7), which resembles very closely the data gathered for *B. sub. spiz.* The lines are almost indistinguishable. This led to the correct analysis that Sample B is actually *B. sub. spiz.*

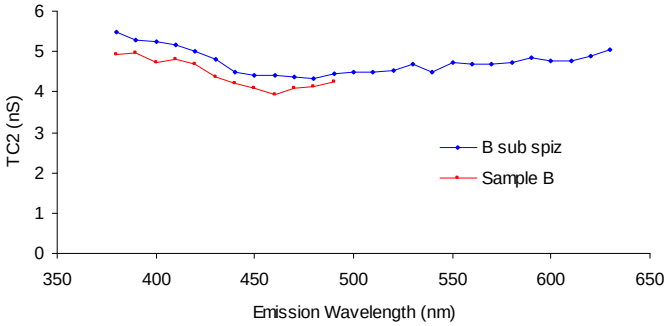

Figure 7: Sample B Comparison Test

Another example of comparison is among *B. sub. var. niger*, *E. coli*, and sample D. The relative pattern of each is that of a slight increase throughout the 380 nm – 600 nm range from approximately 2.7 nS to 4.0 nS. In this case, the lifetime spectrum (Figure 8) is insufficient for identifying Sample D; the time-integrated emission spectrum is also necessary for contrast between these materials. However, from this emission spectrum (Figure 9), Sample D is indistinguishable from *E coli* or *B. sub. var. niger.*

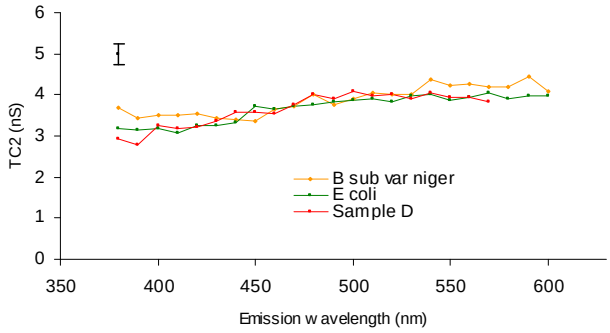

Figure 8: Sample D Comparison Lifetime with *B. sub. var. niger*, and *E. coli*

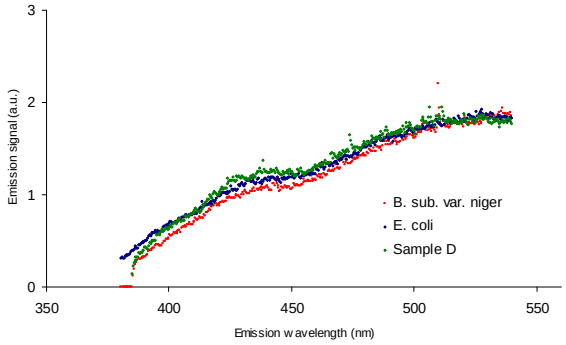

Figure 9: Emission Spectra for *B. sub var. niger, E. coli,* and Sample D

Sample E, as shown in Figure 10, has a fluorescence lifetime that is constant throughout the 400 nm to 600 nm range at about 3.7 nS. All of the different types of *B. globigii* exhibit a constant lifetime throughout this range. *B. globigii* with nutrient, at about 4.3 nS, has a lifetime greater than Sample E, while *B. globigii* Types A and B seem to be between 3.2 nS and 3.8 nS. Hence, from the fluorescence lifetime spectrum, Sample E is *B. globigii* Type A or B, which as indicated earlier were indistinguishable. A further comparison (Figure 11) with the emission spectra confirms that Sample E is not *B. globigii* with nutrient.

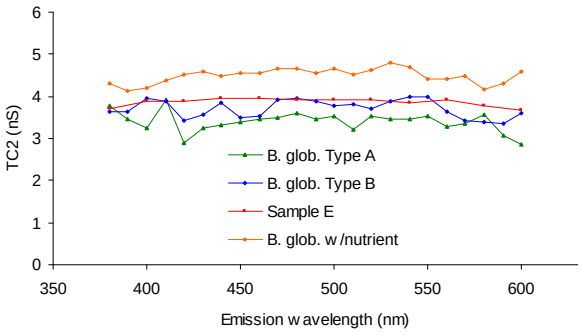

Figure 10: Sample E Comparison Lifetime with *B. globigii*

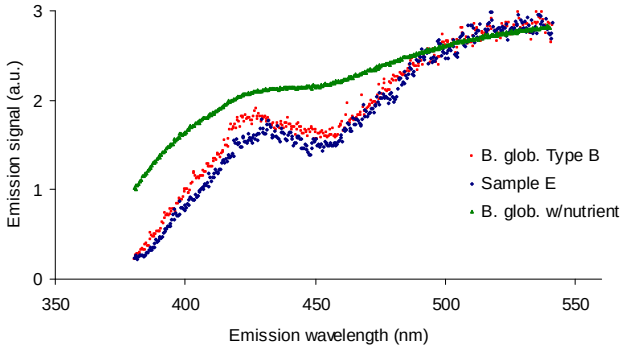

Figure 11: Emission Spectra for Sample D compared to *B. globigii.*

4. Conclusion

This experiment has lead to a preliminary database for the fluorescence lifetime decay spectra of five species of bacteria that possess at least passing similarities to *B. anthracis*. Sufficient differences are evident among four of these bacteria such that identification can be made with confidence. Time integrated emission spectra add an additional component of information that may be used to reduce any ambiguities. However, *E. coli* and *B. sub var. niger* are indistinguishable with either method. The four comparison tests seem to support the possibility that this system will be useful in field biological analysis. Further research will include further expansion of the database.

4. Acknowledgements

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