Contract No:

This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-08SR22470 with the U.S. Department of Energy (DOE) Office of Environmental Management (EM).

Disclaimer:

Development of Bio-Compatible Fluorescent Sensor

Project highlight. SRNL is looking to develop a next generation bio-compatible fluorescent dye that can be excreted through the urine. These materials would provide a better method than current practices used to track wild boar at the Savannah River Site.

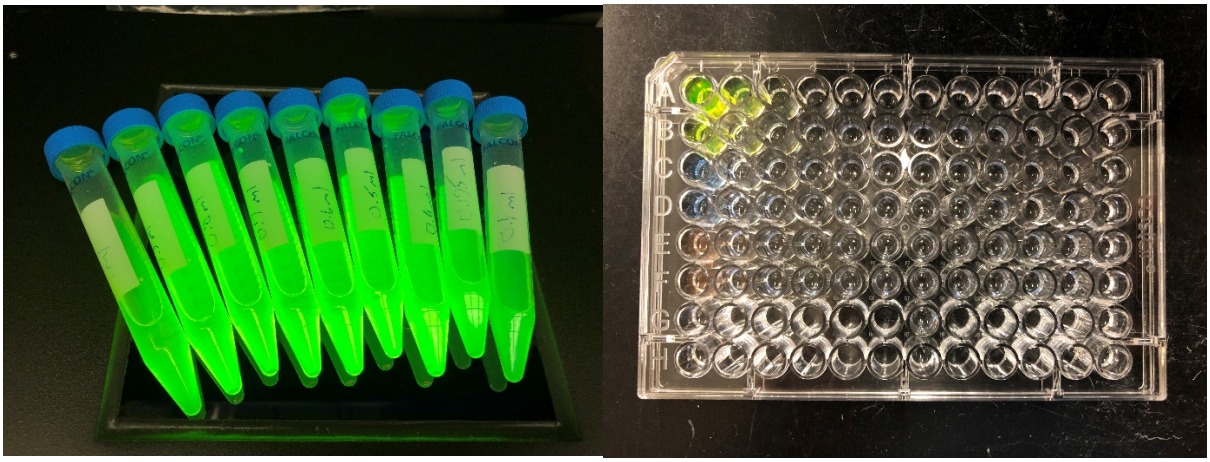


Figure 1. (left) Fluorescein dye at various concentrations in water. (right) Several dyes including fluorescein, Evans Blue, and IR-1061 dissolved in simulant Urine.

Awards and Recognition

Not Applicable

Intellectual Property Review

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

Signature **Date**

Development of Bio-Compatible Fluorescent Sensor

Project Team: Aaron L. Washington, II, John Bobbitt, III, LTC Jeremy Goodin

Subcontractor: N/A

Project Type: Seedling

Project Start Date: February 20, 2020
Project End Date: August 10, 2020

The development for biocompatible fluorescent sensors is used to understand the movement and tracking of wild boar at SRS. Current tracking methods for wild boar include physical tagging, camera monitoring, and foot tracks in the soil. None of these methods are highly effective in determining the path and habits of the animals as it depends on live imaging, soil consistency (lack of rain), and potentially a gps system is tagged properly. This system allows tracking using an additive digested by the animal and released in the urine that will fluoresce when excited with a particular laser. This method helps determine the actual movements of the animals and even provide a potential prediction model based on their routine. We partnered with Ft. Gordon to utilize their animal testing expertise in determining the best material and pathway for these materials to be excreted.

FY2020 Objectives

- Identify material with fluorescence in the optimal region to avoid animal concern that is also safe for ingestion
- Procure reagent dyes at both Ft. Gordon and SRNL
- Determine imaging capabilities at SRNL for determining optical clarity
- Image samples in solutions of water and simulant urine to determine minimum detection limits
- Identify requirements needed in a biocompatible compound that include fluorescence intensity, optional wavelengths, and excitation sources for routine imaging capabilities and detector systems.

Introduction

Imagine the difficulty in tracking wildlife over 310 square miles of densely wooded forest. You can place cameras, add tracking devices for the animals you can catch, or try to monitor footprints for travel patterns. But there's a lot of difficulty in determining the natural movements, habits, and habitable areas for an particular animal on a nuclear site. Even if you tracked and tagged a few using cameras and GPS, you may not get a complete picture of their movements. This project has developed a fluorescent dye that's excretable through the animal's urine that can be track over a long distance and intended to be visible up 100 yards unimpeded.

Our focus in this task was to determine the best material that's capable of fluorescing in an optimal energy range that's outside of the normal visible range to avoid spooking the animals or causing them to disturb the trails that they or others left behind. We determined that near infrared dyes that functioned mostly

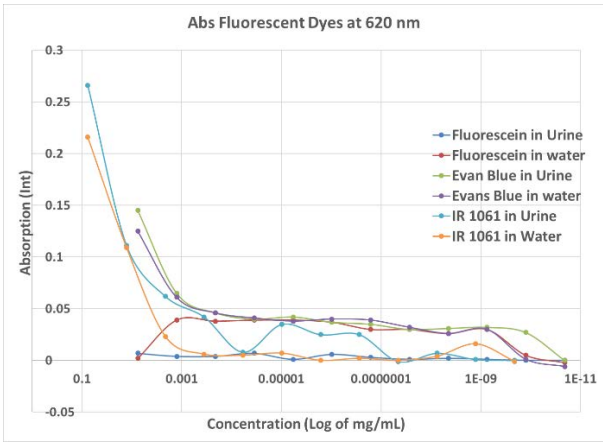
in the 900-1400 nm range worked best in avoiding the animal visual acquity. There are only a few biocompatible dyes that function well in this region. Additionally, we had to acquire the equipment necessary to both excite these dyes and be able to image them in light and dark environments.

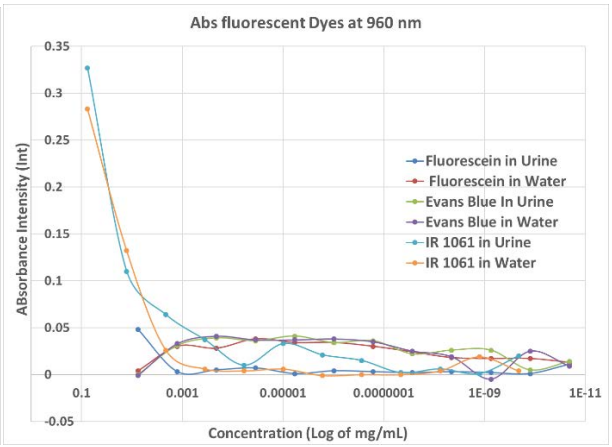
Approach

Wild boar are currently tracked and monitored for the motion and habitats across the Savannah River Site. Current methods focus on traditional tracking systems that typically fail atThe project focused on determining whether there was a COTS material available that could be excreted out of an animal throught their urinary track. Developing and testing a material that was not only bio-compatible but also fluorescent in a non-visible region to avoid scaring or altering the pattern of the animal.
SRNL additionally approached the project looking to determine the most effective method of dye delivery to the animal. The most common methods for drug delivery include: oral, intravenously, and subcutaneously. Ft Gordon DDEAMC routinely deals with testing and evaluating animal responses to various drugs and treatments. SRNL partnered with Ft Gordon staff and researchers to find the best material and testing method.

Results/Discussion

SRNL and DDEAMC have procured several fluorescent dyes that are bio-compatible and non-toxic at various dose levels. We evaluated them both visually with a black light illuminator and with a UV/Vis sprectometer/fluorimeter. The samples we suspended in both water and urine simulants for the visual and analytical analysis. The experiment has 3 different dyes (fluorescein, Evans Blue, and IR 1061) in serial dilution from concentrations of 0.077 mg/mL down to 2.1 x 10^{-11} mg/mL to show the limit of detection in both absorbance and fluorescence with concentration. The instrument, a BioTEK Synergy 2, was only able to anaylze at certain wavelengths namely: 200, 352, 620, 790, 860, and 962 nm.





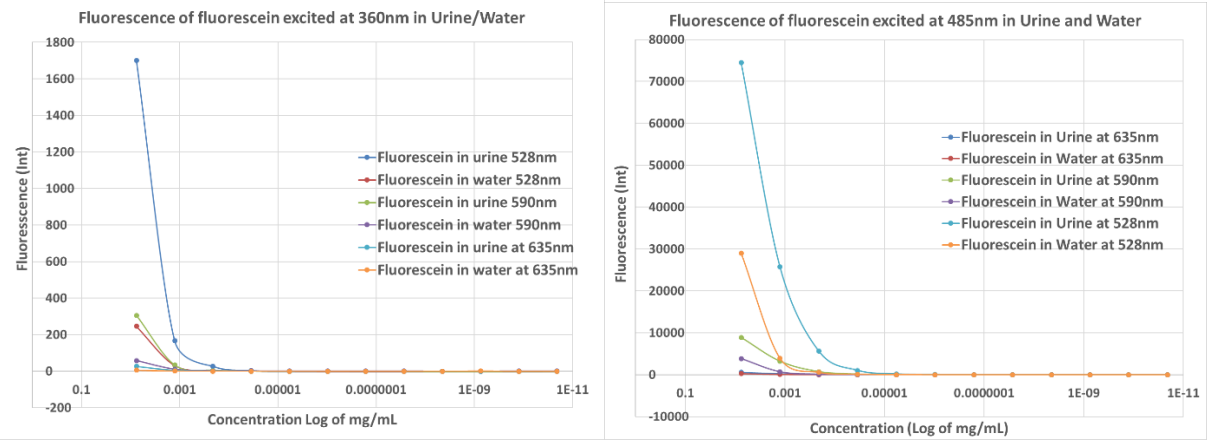


Figure 2: A) Absorption data of Fluorescein, Evans Blue, and IR 1061 suspended in simulated urine and water at various concentrations measured at 620 nm and B) 960 nm. C) Fluoresce of fluorescein in both water and urine excited at 360 and D) 485 nm with the filter set at either 528nm, 590nm, or 635nm.

There are some important takeaways from our experiment with these dyes in both a urine simulant and water. We now have confirmed a both the complete solubility of the dyes in different solutions, optical detection limits for both absorption and fluorescence, and optical absorption range of the dyes. This information was all acquired with collaboration with FT Gordon DDEAMC team.

There were some interesting observations and conclusions made about the samples. First, the samples in the Urine have higher absorption and fluorescence intensities than the water. This is a very positive response. In that manner we know that the samples will all be brighter in our actual bio samples. So forward testing should allow us to use only water as a conservative measure and assume this to be a baseline. Additionally, there is no non-excited luminescence observed in this material.

FY2020 Accomplishments

Brief descriptions of accomplishments to date in bullet form. Whenever possible, accomplishments should be stated quantitatively, as in the examples below, and indicate the contribution to meeting the objectives as well as the magnitude of the improvement over past work.

- Discovered that hydrogen storage capacities on HWCVD generated nano-crystalline graphite could be enhanced by the presence of iron nanoparticles. Preliminary measurements indicated an un-optimized capacity between 1 and 3 wt%.
- Compressor design optimization has reduced maximum speed from 4800 rpm to 3200 rpm with no increase in volume.

Future Directions

There are several future directions for this project.

- Identify potential surface surfactants/encapsulation substrates to be evaluated for enhanced biological stability
- Investigate reaction mechanisms for in-vivo activation of fluorescent properties for suitable excretion and lifetime analysis.
- Develop animal protocol to test materials in vivo using multiple methods of delivery

FY 2020 Peer-reviewed/Non-peer reviewed Publications

N/A

Presentations

N/A

References

Acronyms

DDEAMC – Dwight D. Eisenhower Army Medical Center
NIR – Near Infrared
SRNL - Savannah River National Laboratory
SRS - Savannah River Site
List all acronyms used in the report and explain what they stand for.

Intellectual Property

Patent Applicaton Submitted

Total Number of Post-Doctoral Researchers

N/A. No postdoctoral researchers.

Total Number of Student Researchers

N/A. No student researchers on this project.

External Collaborators (Universities, etc.)

Fort Gordon Dwight D. Eisenhower Army Medical Center