April 2011

Using Encapsulated Fluorescent Bioprobes to Detect Explosive Materials in Soil

Clint Smith  
*U.S. Army ERDC*

Joel Tabb  
*Agave Biosystems*

Follow this and additional works at: [https://commons.lib.jmu.edu/cisr-journal](https://commons.lib.jmu.edu/cisr-journal)  
Part of the *Other Public Affairs, Public Policy and Public Administration Commons*, and the *Peace and Conflict Studies Commons*

**Recommended Citation**  
Available at: [https://commons.lib.jmu.edu/cisr-journal/vol15/iss1/22](https://commons.lib.jmu.edu/cisr-journal/vol15/iss1/22)

This Article is brought to you for free and open access by the Center for International Stabilization and Recovery at JMU Scholarly Commons. It has been accepted for inclusion in Journal of Conventional Weapons Destruction by an authorized editor of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.
A range of industries. APOPO presently is taking Scent Tracing applications, including to develop a variety of useful operational Research and Development challenges and develop a workable system. It is, however, premature to assume that this is the case. In our opinion, the best way forward is to focus generally on the variables that affect odor detection by animals while endeavoring to train animals to detect explosive devices, i.e., changes in soil pH. In addition to the technical aspects of plants may one day provide a range of bio- and nanotechnologies for detection of soil contamination. Soil leaves a bright glow when containing free TNT. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.

This article examines the methods involved in using fluorescent bioprobes to detect explosive devices within soil. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.

This article examines the methods involved in using fluorescent bioprobes to detect explosive devices within soil. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.

This article examines the methods involved in using fluorescent bioprobes to detect explosive devices within soil. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.
maximum output from the fluorescence emissions obtained from the bioprobe’s interaction with the explosive materials.

**Methodology**

BRITE MINE, or Bioprobe Immuno-encapsulated Microorganisms with Nanoparticle-based Energetic Materials, is a hybrid organic and inorganic approach to detecting explosive agents within the environment. The advantages of this design are that the materials are not associated with recombinant DNA or genetic alterations, and are based on an environmental immune response (known as a humoral response). The silica microspheres (Millipore, USA) are non-toxic and inactive with the environment after use. Tested for TNT, the bioprobe detector has shown high sensitivity and specificity. This biologically-based detection probe has disadvantages involving the necessary stabilization of antibodies in the environment. In other words, this technology requires proper storage prior to application and does have a shelf life; however, that is not uncommon with organic-based approaches. Expanding the shelf life of this approach, the inorganic silica-based microspheres containing nano-silanized pores are used for antibody encapsulation and protection. In addition, there are potential signal-to-noise problems, part of the investigation continues in later stages of the research with use of fluorescent detector amplification or enhancement techniques illustrating that the “ON switch” would need to be used with a green-colored emission of the fluorescent tag when visualized by the operator. By using Dansyl-X (Sigma-Aldrich, USA) rather than the Blue/Green Alexa Fluor 488 nm (Invitrogen, USA), the tag could potentially amplify the distances at which great stand-off distances could be seen.

To visualize landmine or TNT detection in contaminated soil samples, using an airborne imaging system is necessary to sufficiently detect and measure fluorescence from the dispersed bioprobe on soil surfaces contaminated with landmines.

**Bioprobe Soil Testing**

Preliminary experiments demonstrated that the fluorescent bioprobes could readily detect low (0.2 ppm) to high (100 ppm) TNT levels dissolved in 99.9% spectral grade acetone (Sigma-Aldrich, USA) in soil samples at room temperature using a Tecnol spectrofluorometer (See Figure 1 on previous page). To further characterize the TNT detection in soil, additional experiments were conducted using standard topsoil from a local garden-supply center as a test matrix.

For these studies, topsoil was placed into 100-mm black Petri dishes or plastic tubs. To keep the soil moist and the bioprobes from being drawn into the soil matrix during TNT detection, samples were mixed with 10% (weight/volume) water prior to being placed in containers. Tap water was used for mixing with the soils. Soil samples were then compressed into the containers using 15–30 lbs of weight for 15 min. This compression flattened the soil surface, making photography and bioprobe detection easier and more consistent.

To reduce the amount of Alexa Fluor 488 nm-labeled bioprobes used in each experiment and to increase consistency between sprayed samples, additional soil tests were conducted using 1 kg (2.2-lb) plastic tubes. In these experiments, soil samples with known TNT concentrations were placed into the tubes and compacted with 3.6 kg (80 lbs) of weight for 15 min. Alexa Fluor 488 nm bioprobes were incubated with BHQ2-TNB [Tetranitrobenzoic] quencher first. This mixture was then sprayed over the soil surface for TNT detection with a 15-min minimum incubation time. BHQ2 quencher was acquired from Biosearch Technologies, USA and then synthesized with TNB to produce the BHQ2-TNB complex after a 1-h incubation which was completed in the laboratory. Figure 2 (above) and Figure 3 (next page) show the experiment’s results. Figure 2 shows spectral plots of the 1-kg plastic tube 15 min after the bioprobe application.

Several points related to the plots shown in Figure 2 need addressing. First, in the absence of TNT (soil control), fluorescence intensity is near to photon counts per second due to the lack of TNT and activated bioprobes. Second, without activated components and TNT measuring 6 ppm, the addition of bioprobes had an increase in the fluorescence spectral signature from natural AlexaFluor 488 nm that was unquenched. This minimal fluorescent yield can be reduced with further experimental conditions that down the fluorescence spectral signal, this is related to the bioprobe amplification or enhancement techniques utilized during the research with use of fluorescence detector amplification or enhancement techniques. Finally, the increases in bioprobe fluorescence were readily apparent 15 min after application. Bioprobes were comprised of the fully activated components in the presence of 100 ppm TNT. The maximum time at which the highest fluorescence was observed was about 20 min. The fluorescent magnitude of the fully activated bioprobes is greater than the bioprobes background fluorescence without full activation or the added quencher.

The results shown in Figures 2 and 3 demonstrate that the fluorescent bioprobes were capable of detecting TNT presence in soil samples. Currently, the shelf life of the bioprobe is at least one year stored at 4 C in a phosphate buffered saline solution. The detection system used for these experiments is a tripled Nd:YAG Lasers (Neoelded-doped Yttrium Aluminum Garnet) laser-induced fluorescence imaging system (Big Sky Laser Technologies, USA) was used to excite (355 nm) the bioprobes at a 1 m detection distance. As such, this project’s objectives were achievable within the controlled soil conditions. Even with a greater magnitude order, this increase of fluorescent emission may not be enough for making a positive identification when investigated at stand-off distances greater than 100 m. Future efforts will focus on the investigation of the utility of retroreflecting particles. These particle types would aid in amplifying the detection mechanism for better TNT signal output, thereby providing a better detection visual for the end user.

**Conclusions**

The results presented above demonstrate that novel inorganic and organic approaches can work for the stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies. Incorporating biopores into future minefield exercises may come about from these results; however, more research must be completed to enable the technology to be operationally field-ready. Future endeavors need to focus on the scale-up of materials for attempting experiments at larger ranges, the adoption of long stand-off detection, and development of materials or material modifications to aid in enhancement. Furthermore, research efforts such as this may lead to more novel studies involving explosive-detection applications for identification of post-conflict landmine proliferation.

---

Figure 2: Soil-based detection of TNT using fluorescent bioprobe. Samples were excited with a HORIBA Jobin Yvon Spectrofluorometer with the fiber optic attachment positioned nadir or 90-degree geometry at 355 nm Excitation. The 495 nm fluorescence emission is viewed in this data plot. Graphic courtesy of Joel Tabb, Ph.D.

Figure 3: Experimental setup with spectrofluorometer (HORIBA, Jobin Yvon) measurements with fiber optic attachment. 1 kg sample soils (not shown) were placed in this larger tub (shown) to obtain spectral measurements of non-active and active bioprobe with and without TNT. Graphic courtesy of Joel Tabb, Ph.D.

---

Joel Tabb, Ph.D.
Research Scientist
Agave Biosystems Inc.
401 East State Street, Suite 200
Wheaton, IL 60187 / USA
Tel: +1 630 272 0002
Fax: +1 630 272 0080
E-mail: jtabb@agavebio.com
Website: http://agavebio.com

Dr. Clint Smith is a Research Scientist for U.S. Army Engineer R&D Center. He has more than 25 years’ experience as a scientist for R&D basic and applied research efforts based on non-explosive and explosive technologies.

Dr. Clint Smith, Ph.D.
Research Scientist
U.S. Army ERDC
7701 Telegraph Road
Alexandria, VA 22315 / USA
Tel: +1 703 428 8200
Fax: +1 703 428 3732
E-mail: clint.b.smith@usace.army.mil

E-mail: jtabb@agavebio.com
Website: http://agavebio.com

---

See endnotes page 83

https://journals.lww.com/journalofewd/volume36/issue1/22researchanddevelopment-
TheJournalofEWandminexaction/spring2013/15.1

15.1 | spring 2013 | the Journal of EW and mine action | research and development