Unattended Remote Electrobiotic
Toxic Agent Sensor

JPL Task Plan No. 80-6204 Rev A

A research and development project for the

Defense Advanced Research Projects Agency

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Quarterly Report
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A. EXECUTIVE SUMMARY

FUNDS: Total Received: $908.2K = FY ‘01 $248.2K + FY ‘02 $615.0K Obligated as of 02/28/02
$570.2K: Funds Exhaustion 214 days

Program Deliverables:

- Identify behavioral metrics response characteristics to specific chemicals
  Adapt multi-node computer model of *C. elegans* from lab to APS (9/01 – 5/02)
  Demo agent dose-rate correlation w/ lab motion detection software (2/01 – 9/02)
  Produce *C. elegans* genetically eng./screened for specific hypersensitivity (2/02 – 09/02)
  Test *C. elegans* response to a panel of agents (10/02 – 2/03) Report results (2/03)

- Investigate *C. elegans* for hypersensitivity to specific agent
  Design (genetically engineer/screen) *C. elegans* for hypersensitivity to one (or more) of a panel of selected specific agents (6/02 - 9/02) Report results (9/02)

- Demonstration of *C. elegans* imaging readout with APS electronic biocoupling
  Investigate biocoupling by shadow time differencing (9/01 – 11/02)
  Establish readout patterns from APS CMOS chip (10/01 – 6/02)
  Design, fab prototype biocoupling interface electronics test chip (6/02 – 01/03)
  Demonstrate *C. elegans* imaging readout (1/03 – 02/03) Report results (02/03)

Progress For This 2nd Quarterly Report Period:

1) Substantially accelerated *C. elegans* toxin hypersensitivity studies throughput at Caltech’s lab automated motion system – New S/W acceleration efforts as well as efforts directed at a complete digitization of Caltech’s automated tracking motion system were initiated and are continuing. This stage of acceleration now allows 5-6 times the dose response data to be generated in the same time so that statistically significant candidate mutant *C. elegans* data can be more easily and rapidly screened and identified for further genetic engineering. Efforts directed at initiating the parallel processing multiple tracking images of *C elegans* and other potential sentinel models (brine shrimp) were also conducted to further explore/improve higher speed screening to support the toxin hypersensitivity studies. Pre-processing algorithms were developed and applied to reduce the variability due to lighting variations and old nematode tracks for large field of view multiple tracking video files via development of JPL real-time, normalized, thresholded, and median filtered S/W codes as input to a largest connected component multiple nematode-tracking module. The digitized videos reduced in both spatial and temporal resolution by sub-sampling to simulate the APS output continued to produce clear response metric feature signals. (2/02) Ahead of Schedule

2) Achieved a reduced variability/spread in both velocity and frequency toxin response feature data at each arsenite toxin dose level (vs. aldicarb toxin studies) for wild *C elegans* dose-response curve with new laboratory automated motion-detection system and improved protocols. The accelerated Caltech automated lab system was used for higher throughput video monitoring and analysis of *C. elegans* dose response under more closely controlled test environmental parameters. (2/02) Ahead of Schedule

**Generated real-time motion files of multiple nematodes on-an-APS-chip for a variety of fields of view.** Programmed VIDI digital APS all parallel-out, electronic readout and parallel control code. Captured lensless refractogram of on-chip wild type nematode swimming motion and tracked image windows of single and multiple nematodes w/ and w/o intervening media. Achieved ~20 fps readout rate with a 50 x 50 pixel readout window using a new digital parallel-out wired VIDI 512 X 512 APS in the “rolling shutter” mode w/high speed acquisition & wireless ASICs ready for fab. *(2/02) Ahead of Schedule*

### B. Technical Progress

Robert Stirbl and Paul Sternberg gave a briefing at Dr. Rudolph’s DARPA TBB/ADT/AD conference in Miami Beach, FL on February 21, 2002.

That briefing presentation file was supplied to Dr. Rudolph after the meeting and is also attached to the same email as this second quarterly FY ‘02 DARPA status report (dated February 28, 2002).

**Summary of February 21st Presentation:** Rob Stirbl first briefly reviewed the top-level goals, technical approaches, challenges, task schedules, milestones and achievements to date for this 36-month program (outlined for the reader’s easy reference in the Appendix: Program Background).

Progress in improved toxin response data, an increase throughput in the *C. elegans* trials and substantially higher speeds for APS wireless image sensor technology was reported on.

Paul Sternberg then gave a more detailed briefing of the Caltech efforts aimed at developing a stable experimental baseline protocol for the automated motion metric that would allow a larger number of wild *C. elegans* baseline experiments to be conducted as a precursor to the mutant strain hypersensitivity trials.

Petri plate assay conditions for uniform bacterial lawn were covered for NGM Plates poured with compound NGM= [0.3 % NaCl; 1.7 % agar; 2.5 % peptone; 0.005% cholesterol; 1 mM CaCl2; 1 mM MgSo4; 25 mM K-PO4 pH 6], Plates aged >1 day. Bacterial lawn (fresh overnight E. coli OP50) spread and dried for 45 min until a slurry with the staged worms incubated on plate with incubation and observation at 20°C ± 0.5C. This protocol resulted in no observed change in controls’ movement over course of observation.

Paul then discussed the *C. elegans* aldicarb toxin dose response studies (acetylcholinesterase inhibitor) with >3 animals recorded at each condition and a three minute observation run.

Approximately 1100 observations were conducted and analyzed relative to confidence level measures using the SAS statistical analysis S/W techniques. Post Hoc, Tukey-
Kramer Comparisons with the commercial SAS statistical analysis program (analyzed for significant differences in variation) with 15 worms which were studied at each concentration (e.g., 0.000, 0.125 0.250 0.500 1.000 and 2.000 mM).

Concentration Significance of 0.05 was explored for parameter values of 0.000 to 0.250 and above, 0.125 to 0.500 and above, and 0.500 to 1.000 but not 2.000. Tests were based on mean positive motion.

The arsenite trials (Figures 1 and 2) provided tighter data distribution in the toxin response (compared to aldicarb trials - Figure 3) and appear to provide a clearer dose response separation for a factor of 4-8 increase in mMolar toxin dose. Data temporal sampling methods were employed similar to the approach used in sperm motility characterization software packages.

**Figure 1.**

![Graph showing the distribution of instantaneous velocity for arsenite response](image)

Wild-type *C. elegans* Distribution of Instantaneous Velocity for Arsenite Response—t=180 minutes—2/19/02
Figure 2.

Figure 3.

Wild-type C. elegans Mean Positive Velocity for Aldicarb Response (10/23/01 - 11/16/01)
Paul reviewed the program’s approach to the genetic engineering of *C. elegans* as two-pronged and achievable through genetically engineering increased toxin sensitivity into selected candidate mutant *C. elegans*. He described gene construction induced via the transcription of control regions (e.g., pan neuronal or specific neuronal cell types) and the creation of genetically modified organisms through insertion of a transgene into the *C. elegans* genome. He described current and planned work with neurological mutants, expected to exhibit increased sensitivity to toxins (e.g., channels, G-proteins, second messenger metabolism and exploration of modifications of transporter proteins that decrease resistance to toxins, e.g., P-glycoproteins - multi-drug resistance genes). Both standard and random selection of nematode strains which are to be carried out through exposing them to agents and automatically screening them for their apparent hypersensitivity or quantifiable change in their monitored motion vs. the background population was covered. Paul also reviewed the combinatorial advantage to engineering *C. elegans* via bipartite expression systems, which work together in order to augment the response to agents.

The Caltech laboratory setup (Figure 4 a-d) for automated motion sensing S/W of individual nematode tracking was described in terms of velocity, direction, as well as amplitude, phase angle and frequency features between segments. New laboratory H/W and S/W modifications that have increased throughput (Table 1) significantly for *C. elegans* screening were described in terms of improvements in conducting a number of systematic exposures to aldicarb and arsenite. The engineering of other types of nematodes, such as dauer larvae and anhydrobionic nematodes for conducting transgenesis, were options reviewed that could address extended *C. elegans* storage.

Efforts on automated multiple tracking of alternate model toxin dose response initiated for brine shrimp (Figure 5) were reported, as well as a proposed concept for “self-maintaining”, a cell based, toxin optical trigger sensed on an APS platform (with evaporated multi-layer interference filter). The optical signal would be generated by genetically engineering an existing cell in dauer *C. elegans*, which normally senses changes in the supply of nutrients in the nematode’s environment.

Rob Stirbl then continued the presentation covering JPL and Caltech nematode viability experiments in two different types of axenic media formulations (Figure 6), important in the consideration of fielded systems operations. Rob discussed JPL’s improved APS chip interface H/W and processing S/W (Figure 7) developed by Curtis Padgett’s team to extract quantifiable nematode behavior for multiple worms. In particular, Rob reported on these new methods that were developed based on the automated motion-sensing S/W from video records taken at the Caltech laboratory. These digitized preprocessed videos were not only reduced in resolution spatially but also reduced in resolution temporally by
sub sampling the original lab derived video sequence in order to simulate the output that would be derived from the APS imager.

He then covered the new JPL interface S/W and H/W, which substantially improved nematode image capture from 2 Hz serial digital output to > 15 Hz parallel digital output for a 100 x 100 pixel FOV. Plans for higher rates, ~1000 Hz, that would allow larger field-of-view outputs to be captured for multiple track processing by a new APS array that will be made possible with a new field programmable gate array under design were also covered. Figure 9 (a-e) shows various APS frame rates achieved for nematode contact imaging of swimming motion without agar (Figure 9 a & b) and with a 125 um intervening agar sheet (Figure 9 c-e).

Reduction in scene variability for large field-of-view, multiple nematode track, real-time processing was reviewed. Histograms were run to determine the threshold value that would separate out worms from the background images. Thresholding the images generates a binary Segmented Image where certain pixels are identified as potential worms. The segmented image may contain more than one object besides the worm; e.g., spurious blobs from worm trails, or material on the dish, or noise. The worms are found by using a simple clustering technique on the segmented images, which identifies the Largest Connected Component (Region) as the worm. JPL preprocessing of these new large FOV images eliminated illumination asymmetries and old nematode tracks (Figure 7). These preprocessed frames are then input into a largest connected component, multiple nematode-tracking S/W.
Figure 4 - Caltech laboratory setup for automated motion sensing

- a) computer controlled x,y tracking
- b) computer track file analysis displays
- c) track file acquisition display
- d) Frame segmentation data display

Video: recording of worm(s) on Petri plate or glass sandwich
Recognition: image analysis to recognize worm
Processing: rendering worm posture to x-y coordinates of 13 points
Metrics: extracting movement parameters from x-y in time
Table 1 – Caltech individual nematode automated motion sensing throughput past, current and planned improvement.

<table>
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<th>Old</th>
<th>Current</th>
<th>Planned</th>
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<td>February 2002</td>
<td>March 2002</td>
</tr>
<tr>
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<td>30 min</td>
<td>30 min</td>
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<tr>
<td>Total</td>
<td>420 min</td>
<td>85 min</td>
<td>50 min</td>
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</tbody>
</table>

Video: recording of worm(s) on Petri plate or glass sandwich
Recognition: image analysis to recognize worm
Processing: rendering worm posture to x-y coordinates of 13 points

Automated Brine Shrimp
Dose Response Motion Analysis

Figure 5 – Video of Caltech laboratory setup for automated motion sensing of brine shrimp.

Brine Shrimp in Aldicarb dosed agar taken with Caltech’s Automated Motion Lab system and under motion processing at JPL
A skim milk-supplemented axenic medium to support development and reproduction of *C. elegans*. Reproductive Hazards Laboratory, U.S. Army Center for Environmental Health Research, Bldg. 568, Fort Detrick, MD 21702
Hugh F LaPenotiere, Deborah Y French, Maria Szilagyi, Eric D Clegg
Worm Breeder’s Gazette 17(1): 33 (October 1, 2001)

(Eggs were prepared by bleach and allowed to hatch in media, and develop. After 36 hours the worms are 0.17 mm, about 1/5 the adult length. Swimming motion appears normal in both cases. Tests were conducted in tissue culture well plates on a rotary shaker for oxygen transfer).

486 66 MHz Processor (running dos)
Tracking multiple nematodes

Figure 8. Wide-field of view, digitized behavior videos used to monitor the simultaneous behavior of the N2-wild

Caltech lab recorded motion image sent to JPL

JPL post-processed image file: normalized, thresholded, median filtered, but pre-largest connected component track processed

Figure 9 (a-e) shows various APS frame rates achieved for nematode contact imaging of swimming motion without agar (a and b) and with a 125um

a) *C elegans* in fluid directly on chip (~7 fps, 12 um pixels, 80 x 80 RO)

b) *C elegans* in fluid directly on chip (~15 fps, 12 um pixels, 50 x 50 RO)

c) *C elegans* on 0.125 mm agar film on APS in fluid, (~7 fps, 12 um pixels, 80 x 80 RO)

d) *C elegans* - on 0.12 mm agar film on chip in fluid, (~8 fps, 125 um pixels, 70 x 70 RO)

e) *C elegans* - on 0.125 mm agar film on chip in fluid, (~9 fps, 12 um pixels, 60 x 60 RO)

On-chip fluid

Or nutrient
Rob concluded by discussing NASA’s interest in water contaminant monitoring as well as AMES Research Center John Hines interest in JPL teaming with his program associates on employing the filter modified, VIDI APS CMOS imager technology as a fluorescent sensor micro platform to monitor and report any changes in bioprocesses of yeast and other model organisms during on-orbit flights planned in FY ’03 on small free flying microsats. He also referred to the fact that Caltech has already adapted and demonstrated a prototype VIDI CMOS APS imaging system modified with an evaporated 31 layer Bragg filter to allow quantitative transverse flow optical fluorescence and absorption monitoring in the microfluidic channels. That microfluidics effort was supported under the DARPA funded Biofluidic Chips program to Caltech (Scherer, Quake, Adams, et. al.- Figures 10-13on pgs 16,17) and a joint Caltech/JPL Presidents Fund effort to quantitatively monitor fluorescence and absorption changes via contact/lensless microfluidic channel imaging using JPL’s high performance CMOS APS focal plane array.

Rob encouraged the TBB/ADT/AD community present to contact him and invited them to visit JPL and explore teaming relationships between the labs and companies to leverage and exploit the JPL/NASA/Caltech developed technology’s unique high performance sensor platform capabilities.

Based on the suggestion of Dr. Rudolph and other biologists he referred, a search and analysis has been initiated into the applicability of motion feature metrics and statistical tools employed in the motion metric studies in general and applied in the study of other biological entities (i.e., mammalian sperm, Dictyostelium, bacteria, Rotifers, alge, diatom, amoeba, chlamydomonas, brine shrimp, etc.). The sperm efforts to handle variations generally employ indices of percent motility, linear velocity measures normalized by complete track distance covered and utilize net vector velocities at longer acquisition periods to assess sperm viability vs. short term instantaneous changes in velocity.

C. Milestone Report on Program Deliverables:
Identify behavioral metrics response characteristics to specific chemicals
Adapt motion model of *C. elegans* from lab to APS (9/01 – 5/02) ONGOING
Demo agent dose-rate correlation w/ lab motion detection S/W (2/01 – 9/02) MET
Produce *C. elegans* gen. eng for specific hypersensitivity (2/02 – 09/02) ONGOING
Test *C. elegans* response to a panel of agents (10/02 – 2/03) FY03 Report results (10/02)

ONGOING

Investigate *C. elegans* for hypersensitivity to specific agent
Design (genetically engineer/screen) *C. elegans* for hypersensivity to one (or more) of a panel of selected specific agents (6/02 – 9/02) ONGOING Report results (02/03) FY03

Demonstration of *C. elegans* imaging readout with APS electronic biocoupling
Investigate biocoupling by shadow time differencing (9/01 – 11/02) MET
Establish readout patterns from APS CMOS chip (10/01 – 6/02) ONGOING
Design, fab prototype biocoupling interface test chip (6/02 -01/03) ONGOING
Demonstrate *C. elegans* imaging readout (1/03 – 02/03) ONGOING Report results (02/03) FY03
Progress For This Report Period:

FUNDS: Total Received: $908.2K = FY’01 $248.2K + FY’02 $615.K Expended as of 02/28/02 $570.2K: Funds Exhaustion 214 days

1) Substantially accelerated C. elegans toxin hypersensitivity studies throughput at Caltech’s lab automated motion system – New S/W acceleration efforts as well as efforts directed at a complete digitization of Caltech’s automated tracking motion system were initiated and are continuing. This stage of acceleration now allows 5-6 times the dose response data to be generated in the same time so that statistically significant candidate mutant C. elegans data can be more easily and rapidly screened and identified for further genetic engineering. Efforts directed at initiating the parallel processing multiple tracking images of C. elegans and other potential sentinel models (brine shrimp) were also conducted to further explore/improve higher speed screening to support the toxin hypersensitivity studies. Pre-processing algorithms were developed and applied to reduce the variability due to lighting variations and old nematode tracks for large field of view multiple tracking video files via development of JPL real-time, normalized, thresholded, and median filtered S/W codes as input to a largest connected component multiple nematode-tracking module. The digitized videos reduced in both spatial and temporal resolution by sub-sampling to simulate the APS output continued to produce clear response metric feature signals. (2/02) Ahead of Schedule

2) Achieved a reduced variability/spread in both velocity and frequency toxin response feature data at each arsenite toxin dose level (vs. aldicarb toxin studies) for wild C. elegans dose-response curve with new laboratory automated motion-detection system and improved protocols. The accelerated Caltech automated lab system was used for higher throughput video monitoring and analysis of C. elegans dose response under more closely controlled test environmental parameters. (2/02) Ahead of Schedule

3) Demonstrated improved APS electronic biocoupling. Built new higher speed digital, parallel-out, laptop APS data acquisition and wireless setup. Generated real-time motion files of multiple nematodes on-an-APS-chip for a variety of fields of view. Programmed VIDI digital APS all parallel-out, electronic readout and parallel control code. Captured lensless refractogram of on-chip wild type nematode swimming motion and tracked image windows of single and multiple nematodes w/ and w/o intervening media. Achieved ~20 fps readout rate with a 50 x 50 pixel readout window using a new digital parallel-out wired VIDI 512 X 512 APS in the “rolling shutter” mode w/high speed acquisition & wireless ASICs ready for fab. (2/02) Ahead of Schedule
D. Future Issues and Technology Transitions

Dr. Darrell Jan became the new JPL/NASA Program Office Manager for the current DARPA Unattended, Remote Electrobiotic Toxic Agent Sensor contract. Dr. Jan, who is the Deputy Director of JPL’s NASA Fundamental Biology Program, and chairs the board of NASA’s Advanced Environmental Monitoring & Control Project (AEMC), had previously connected Rob with John Hines who was interested in the APS technology for flight mission applications. After contact and discussions with John Hines, he invited R. Stirbl to visit NASA AMES and join in presenting two days of talks and seminars on the APS and DARPA’s Program.

Potential Approaches to improving signal-to-noise or reducing toxin response spread:

• Automatically follow more than 3 or 4 worms to provide reasonable estimates of dose, and/or

• Modify Caltech’s automated lab methodology for the better/faster approach to a field instrument by tracking toxin response within a selected *C elegans* candidate (longitudinal study) vs. worm acclimation prior to measurements.

We are in the process of exploring both options to improve signal-to-noise (substantially reduce dose response spread).

Contacts:

At the DARPA TBB/ADT/AD PI conference Rob gave a poster session, “Unattended, Remote Electrobiotic Toxic Agent Sensor - Preliminary Results”. The poster presentation, which included a laptop PowerPoint briefing, was shown for three hours and had a large number of government agency and academic personnel interested in exploring collaborations with JPL/Caltech. At Dr. Rudolph’s and Darrell Jan’s suggestion, Rob also attended sessions and spoke with researchers attending the DARPA Joint Bioflips/Simbiosys conference. John Hines, the NASA Ames director of Fundamental Biology programs, encouraged Rob to meet with DARPA BIOFLIPS PM, Mike Krihak, about the benefits of additional effort support to assist in developing an ultra-low power miniature VIDI APS fluorescent sensing platform for monitoring model systems on the ground and in space. Mike appeared open to receiving more information as a possible add-on to Dr. Scherer’s and Dr. Quake’s existing Bioflips effort at Caltech.

R. Stirbl met again with Dr. Alfredo Rayms-Keller, the Biotechnology Group Leader (Code B51), at the Chemical Biological Systems Technology Division of the Naval Surface Warfare Center, Dahlgren Division. In telephone discussions after the conference, Dr. Rayms-Keller again invited JPL/Caltech to work together with their DARPA-funded mosquito larvae toxin sensing group to test toxic agent challenges with the portable automated motion sensing system for *C. elegans*. An early April visit of JPL personnel to Dr. Rayms-Keller’s lab at Dahlgren is being discussed, and he will be
sending mosquito eggs to Wayne Schubert to hatch to try on the Caltech/JPL automated toxin metric sensing system.

Several efforts were discussed at the conference with Dr. Hines to have Ames-, JPL- and the DARPA-funded and microfluidics researchers at Caltech (Dr. Steve Quake, et. al.) work together on developing a micromonitoring microfluidics platform for space effects on GFP modified yeast and other model entities currently being explored under NASA and DARPA funding by Gregory Kovacs of Stanford University. JPL is currently working with Caltech President Fund support on incorporating an evaporated multilayer interference filter on top of a special purpose APS to permit on-chip laser-induced fluorescence studies.

R. Stirbl had discussions with Dr. Yosishacham-Diamand, Director of Nano Science and Technology Projects at Tel-Aviv University on joint studies using JPL’s high performance CMOS imager to capture a better green fluorescent protein signal than is possible with commercially available products for monitoring bacteria, yeast, and human cells.

R. Stirbl also met and had discussions with Dr. John Oprandy, Sci & Medical advisor for DTRA’s Chem/Bio Defense, Dr. Roy Thompson of US Army APGEC, and Dr. Bruce Harper, Life Sciences Division, Dugway Proving Grounds, about test service agreements for bio and chem agent test trials. Discussions covered working together on surety testing of real agents, as well as providing expert technical assistance and procedural review of simulant sensitivity protocols. Rob followed up with Dr. Harper after the conference to discuss the type of trials that could be conducted at Dugway. These included system, detector and collector/concentrator tests with live and inactive bio-agents and bio-agent simulants. Complete toxin monitoring systems are most appropriately tested in joint field trials. Laboratory trials were generally used for agent detector tests and agent activity assessment. Chambers are most appropriate to determine collector/concentrator efficacy.

The issue of micro concentrators was discussed, and Rob was referred to Micronics as a corporation that had received funding to develop micro concentrators/impingers. Rob contacted Dr. Weigi at Micronics and is reviewing information he received on their microfluidic concentrator and assessing its applicability to the current DARPA ADT effort.

At Janet Jensen’s and Doty Paterno’s (US Army, APGEC) pre-meeting suggestions, Rob also met with Eli Greenbaum, Oakridge National Laboratories and will be exploring the fluorescent platform being developed at Caltech for Dr. Greenbaum’s algae sensing efforts.

Another potential area of collaboration that was discussed at the conference was with Dr. Wikswo from Vanderbuilt University, who is assessing changes in metabolic function in cells and other model systems with the development of a MicroPhysiometer as an indicator to activity detection in nanoliter microfluidic channels provided by Caltech.
Other researchers interested in exploring collaborations to use the APS-based fluorescent sensing platform included Dr. Danny Dhanasekran from Temple University, doing work with modifying yeasts olfactory signal transduction systems, as well as Phil McFadden and Frank Chaplen of the University of Oregon to explore its use in their work on living cell chromatophore sensors for toxic agent sensing.

Rob also had discussions with Guenter Gross of UNT about obtaining the information that he briefed on existing toxins and sensing level targets.

Figure 10 – Caltech Fundamental System Approach
from “Microfluidic and Optoelectronic Integration for Biological Analysis” by Mark Adams
Figure 12 - Bromophenol Blue spread from 7.5mM to 30uM. Illuminated by pure white LED and directly readout lenslessly from an VIDI APS from “Microfluidic and Optoelectronic Integration for Biological Analysis” by Mark Adams

Figure 13 - Applications: Vis. Abs. Spectroscopy – Examples and Results-Bromophenol Blue from “Microfluidic and Optoelectronic Integration for Biological Analysis” by Mark Adams

Measurements performed at 588nm. Peak absorption wavelength for Bromophenol Blue is at 591nm
F. Appendix: Program Background

**GOAL:** The Jet Propulsion Laboratory (JPL), and Caltech (CIT), with the support of DARPA, will conduct a research and development program to produce a prototype of a revolutionary, quantitative, ultra-sensitive sensor capable of toxic-agent detection and classification. This low-cost, long-life, wireless detector will be designed to be packagable in a highly integrated, miniaturized enclosure ruggedized for high-impact deployment.

This advanced detector concept integrates a newly developed, automated quantitative metric of the response of the sub-millimeter nematode worm *C. elegans* to known external stimuli that Caltech has already demonstrated for altered gene function. The essence of this task is to transfer these demonstrated metrics into on-chip image processing for autonomous readout and to produce new signature metrics for agents of specific Department of Defense (DOD) interest by genetically engineering *C. elegans*. The proposed effort will employ JPL techniques to insert this proven metric for analyzing *C. elegans* movements onto a miniaturized sensing platform using an inexpensive, ultra-low power, “smart” complementary metal oxide semiconductor (CMOS) imager chip as a contact on-chip microscope that replaces the macro-scale, large laboratory microscope and processing computer. This biotechno-symbiosis sensor comprising a biological entity will respond predictably to a specific toxic environment and will act, in effect, as a quantitative, wireless “canary-on-a-chip” activity detector. For this proposed effort *C. elegans* nematodes will be engineered or selected for their improved agent sensitivity. They will then be plated in an appropriate nutrient medium onto the electronic biocoupling/processing detector array system that senses and reports any quantized reaction to that specific agent. Biocoupling is accomplished by the processing of lensless contact images of this selected bioentity’s motion. When movements characteristic of a given toxic signature are detected via the on-chip processing, the processor autonomously takes action to transmit the signal. Activity detection of signatures from unknown nerve agents could also induce a response resulting in a transmitted signal.

**BENEFIT:** Unlike conventional methods, this approach does not rely upon classical large-scale sensible parameters coupled with a separate external computer-based algorithm to deduce the presence of toxic agents. Toxicity is determined directly by a sensed metric of nematode movements in response to the agent(s) to which they were bred to react.

This innovation, as discussed, is anticipated to lead to the demonstration of a compact, highly sensitive, wireless detection system capable of functioning covertly in an unattended environment. Because this sensor uses a sub-millimeter biological entity as its first line of sensitivity and capitalizes upon the widely available and power-efficient CMOS-compatible integrated microimager electronics, its reliability is very high and it’s cost very low.

**TECHNICAL APPROACH:** The essence of this effort (see schedule in figures 1 & 2) is the following: 1) quantification of dose response to agent simulants in a laboratory set-up at Caltech, 2) proof-of-principle investigation and demonstration of on-chip *C. elegans* imaging with off-chip processing for autonomous readout, followed by 3) a transfer of these demonstrated agent dose software metrics into chip-level hardware to produce a new signature metric agent-activity assay of specific DOD interest via 4) genetically engineering the *C. elegans* for increased sensitivity and agent specificity, and 5) conducting actual agent tests at government field test facilities (i.e., Dalhgren, Dugway, USAMRIID, Battelle Labs, etc.). The effort employs Caltech expertise in adapting the existing metrics for on-chip worms, and JPL techniques to insert this proven metric software for analyzing *C. elegans* movements onto a miniaturized sensing platform using an inexpensive, ultra-low power, “smart” CMOS imager chip as a contact microscope with on-chip and hybridized processor interframe feature detection that replaces the macro-scale, large laboratory microscope and processing computer. This biotechno-symbiosis sensor comprising a biological entity will respond predictably to a specific toxic environment and will act, in effect, as a quantitative, wireless “canary-on-a-chip” activity detector. For this proposed effort, a strain of mutated or genetically engineered bioentity, *C. elegans* nematodes, will be selected for their improved agent sensitivity.
Key Milestones

The milestones and anticipated deliverables from this task are the following:

**Phase I:**

- Demonstration of *C. elegans* imaging readout with APS electronic biocoupling
- Report of agent-simulation dose-rate correlation with laboratory automated motion software
- Demonstration of a genetically engineered *C. elegans* hypersensitivity to one (or more) of a panel of selected agents.
- Phase I interim report

**Phase II:**

- Demonstration of software-to-hardware transfer of motion-metrics functions
- Demonstration of a prototype of a complete electrobioptic toxic-agent sensor
- Design of an integrated sensor system to include the motion-metrics ASIC, the APS biocoupler, and a wireless transmitter
- Final report

*C. elegans* with desired properties can either be engineered using transgenic and recombinant DNA technology, or identified by screening mutagenized populations of worms for genetic variants with the desired properties. Caltech has carried out hundreds of such genetic screens in other projects.

To extend the range of response or alter the sensitivity to agents, *C. elegans* can be partially “humanized” by constructing transgenic strains expressing human genes in addition to, or instead of, nematode genes. Another potential issue is the sensitivity of *C. elegans* to a particular compound. Significantly higher concentrations of agents are typically needed to exert effects on *C. elegans* than on mammalian tissue culture cells. However, some strains with altered cuticle (e.g., *cat-4*) generally lower doses are needed, indicating that the cuticle might be engineered to allow higher sensitivity. Moreover, the ability to genetically alter *C. elegans* should allow hypersensitivity to specific agents to be programmed, overriding any problems in permeability. Another approach to the permeability problem is to engineer sensory neurons that ultimately control motility through olfactory, mechanosensory, and chemosensory modes to respond to the agent of interest. Specific promoters for such cells exist, and the metrics developed by Caltech are expected to be sufficient as readout of these neurons.

In Phase I as we are calibrating the imaged readout of the *C. elegans* on an APS chip, it will be necessary to compare the responses measured by the APS electronic biocoupler with those obtained under the microscope. This specific task will involve the comparison with metrics measured on Petri plates in bacterial lawns with respect to known genetic alterations affecting muscle contractile force generation; general neural transmission; and specific neural transmitters. These metrics include amplitude of sinusoidal wave of muscle contraction, rate of wave propagation along the worm's body, and frequency of muscle contraction. We will adapt demonstrated quantifiable metrics of the *C. elegans* movements to respond to specific agent simulates of DoD interest. The existing multi-node *C. elegans* computer model will be modified to reflect the new response signatures, and responses of known genetic alterations will be quantified. This task will help define the requirements for genetically engineered *C. elegans* that are hypersensitive to specific agents. *C. elegans* responsive to a specific panel of toxic agents of strategic DoD interest will be designed during this phase. In addition Phase I efforts will produce *C. elegans* designed for specific agent hypersensitivity by well-understood genetic engineering techniques. The *C. elegans* response to a panel of agents will be tested to determine characteristic response signatures, and the movement metrics quantifying the new signatures will be refined. This task will involve the demonstration of a specialized *C. elegans* made hypersensitive to a specific agent or agent class via screening techniques and genetic engineering, where these engineering efforts seek to make the nematodes sensitive to agents not typically affecting them (e.g., “humanizing” the nematode by insertion of mammalian genes into its genome).
Phase II of this program will take place over the following eighteen months during which we will produce *C. elegans* designed for a broader or expanded class of agent hypersensitivity by well-understood genetic engineering techniques. The *C. elegans* response to an extended panel of agents will be tested to determine characteristic response signatures, and the movement metrics quantifying the new signatures will be refined. This task will involve the demonstration of a specialized *C. elegans* made hypersensitively responsive to both new specific and broader agent classes via screening techniques and genetic engineering, as performed in Phase I. Other genetic engineering efforts will control permeability of nematodes to exogenous agents. Screening efforts will proceed in parallel to include “irrational” or serendipitously harvested “design” principles, in addition to the genetic engineering approach. In the meantime, the circuit design of the FPGA-development breadboard biocoupling electronics will be integrated into a test chip and characterized for different bias conditions and temperatures.

A second task in Phase II will also involve integrating the motion-metric analytic functions into hardware. As with the biocoupler, the motion-metric electronics will first be configured as a FPGA-development breadboard for ease of component changes to facilitate optimization. The software functions will then be modeled into their respective hardware elements, integrated with the APS biocoupler, and tested first for functionality and then with the new *C. elegans* and agent simulant panels, as well as real agent panel tests at government field test facilities (i.e., Dugway, USAMRIID, Battelle Labs, etc.). The ultimate goal is to design and fabricate a miniaturized, agent-activity relevant, application specific integrated circuit (ASIC) embodying the operations of the motion-metric FPGA-development breadboard hardware and software.

The final task in Phase II is to design and produce the integrated system architecture to include the new motion-metrics ASIC and the APS biocoupler with a wireless communication system. An associated laboratory setup for a full-system array will be characterized to demonstrate the capabilities of integrating both proven and new design approaches. The final product of this effort is expected to be a prototype of a highly-integrated, ultra-low power, Electrobiotic Toxic-Agent, Dose Response Sensitive, Activity Sensor that is anticipated to substantially improve sensitivity for such applications as advanced mission support, treaty compliance, and stand-off situational awareness.

**Meeting Activity Detection Technology (ADT) Program Goals**

This work is directly in line with DARPA’s ADT programmatic goals of using biological systems for the detection of chemical, biological, and other toxins of interest to DOD needs. Although this effort primarily pertains to the Assay Development and the Chip Interface Engineering and Design areas of research identified in BAA 01 05, throughout the course of developing an electrobiotic sensor we will address, to some degree, each of the remaining four research areas. For example, the “shelf-life” of the *C. elegans* is an obvious concern, which falls under Longevity and Stability; how we extract useful information from the sensor relates to Computational Design, Statistical Analysis and Data Mining Tools; Sample Collection and Preparation must be addressed; and System Modeling and Integration, specifically the integration, must be dealt with to assure proper interconnection among the various critical components. The electrobiotic sensor brings in the full-system viewpoint of a biocoupled means for the detection and classification of toxins.
Figure 1. (above) A two-phase program enables discreetly identifiable development stages.

Figure 2. Phase I 18 month program – Detailed from Fig. 1

2nd Qtr Progress: All ahead of schedule, Hypersensitivity Trials Underway