

Chemical and Biological Defense Test and Evaluation (T&E) Future Challenges

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National Defense University

July 2012

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE JUL 2012		2. REPORT TYPE		3. DATES COVERED 00-00-2012 to 00-00-2012	
4. TITLE AND SUBTITLE Chemical and Biological Defense Test and Evaluation (T&E) Future Challenges				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) National Defense University, Center for Technology and National Security Policy, 300 Fifth Avenue, Fort Lesley J. McNair, Washington, DC, 20319				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 13	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

The views expressed in this article are those of the authors and do not reflect the official policy or position of the National Defense University, Department of Defense, or U.S. Government. All information and sources for this paper were drawn from unclassified materials.

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OBJECTIVES

The objective of this study was to identify emerging technical, methodological, and infrastructure challenges for future Chemical and Biological Defense (CBD) Test and Evaluation (T&E) investment, and to serve as a prequel to the development of a full strategic T&E Roadmap. The Center for Technology and National Security Policy (CTNSP) of the National Defense University (NDU) led the study.

BASIS OF THE ANALYSIS

The CTNSP study group conducted a five-point analysis of the CBD program. The group reviewed requirements documents, the relevant science and technology (S&T) literature, existing CBD strategic plans, the current S&T investment portfolio, and current commodity programs. The Joint S&T Office (JSTO) Focused Innovative Thrusts and legacy S&T portfolio, the JSTO Strategic Science Roadmap, the Joint Requirements Office (JRO) requirements, and the Joint Program Executive Office (JPEO) programs represented the reviewed materials.

RATIONALE FOR THE STUDY

Science and technology has been moving at an exponential pace, especially within disciplines critical to the CBD program. These primarily include the biological sciences, especially systems and synthetic biology and structural biology; information science, particularly bioinformatics; nanotechnology, specifically the ability to tailor nanomaterials from “the ground up,” and to design and fabricate devices on the nanoscale; and combinatorial chemistry. These rapid developments underpin both next generation threats and new countermeasure technologies that must be subjected to rigorous T&E before fielding.

The T&E community has been confronted with numerous challenges when new technology is developed. The classic example is polymerase chain reaction (PCR), which permits the high fidelity amplification of DNA. The development of rapid PCR assays led to the ability to unambiguously detect and identify biological threat agents present at vanishingly small levels, but the vulnerabilities of the assays were not originally well understood by either the S&T or the T&E communities.

Issues such as sample contaminants (e.g., chelating agents that reduce the availability of the PCR Mg^{+2} cofactor or excess salts and other soil component inhibitors found on test ranges or in operational environments) that do not affect the older, immunoassay-based detection technologies were serious interferents in the PCR formats. This led to erroneous false negative conclusions when samples contaminated with interferents failed to indicate the presence of DNA because the PCR reaction was inhibited. However, available PCR kits overcome inhibitory effects of test range soil components that become entrained in reaction mixtures as shown by recent work at Dugway Proving Ground (Soil Fluorescent

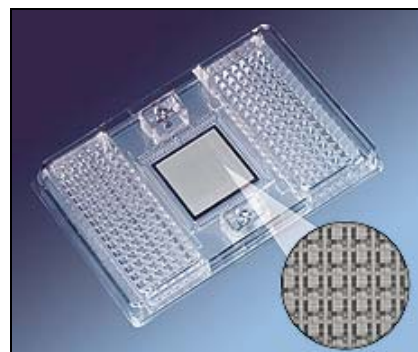


Figure 1. The Fluidigm Dynamic Array IFC

Background Study, Phase I Report, Dugway proving Ground, 15 March 2011)¹. A more ubiquitous issue related to T&E of biological recognition elements (BRE) (i.e., the moiety that recognizes and permits the detection of the threat agent by binding to it) is quality control and quality assurance. The affinity and specificity of antibodies tend to vary from one lot to another; different laboratories make antibodies for a particular threat agent that binds to different epitopes on the agent; and the manufacturing processes for next generation BREs, such as molecular imprints, have not been standardized. This can lead to highly variable results and lack of data comparability between research, development, test, and evaluation (RDT&E) organizations. Finally, the physico-chemical properties of new agents can be radically different than traditional agents, posing unforeseen problems when analyzed by existing procedures. This was the case for the unique properties of next generation chemical agents and is likely to be an even bigger issue with the advent of nanomaterials, which tend to have radically different properties at the nano-scale than they do at the meso- or macro-scales.

Three additional issues for the T&E community are: (1) understanding likely operational conditions and replicating them under T&E scenarios; (2) presenting the challenge to the reference systems in the appropriate phase (e.g., vapor, droplet, particulates); and (3) overcoming problems of simulants accurately representing systems performance. Many T&E technologies work well under more or less pristine testing conditions, but are subject to interference from the background “noise” inherent in the real world. On the battlefield, smoke, dust, and other particulates can have deleterious effects on detectors, and under even the best of conditions, the background chemical contamination and biological flora and fauna will make up the preponderance of the total measured, resulting in a tricky signal-to-noise problem that must be resolved.

It should be noted that changes in the nature of the threat, and indeed the technology and other issues discussed below, may actually surface at times outside their “expected” (near, mid, far) terms. Thus, something currently considered near term may emerge in the mid or far term due to both technical and/or operational considerations. For example, while chimeric organisms, which comprise genetic material, metabolic pathways, and capabilities of two or more organisms, may be available in the near term, appropriate methodologies for effectively disseminating them may not be available. Other issues may be connected to the scale of use, ranging from large areas in military attacks to smaller disseminations in urban areas.

CHANGING NATURE OF THE THREAT

For the purposes of this study, the temporal dimension was divided into Near Term (1–5 years), Mid Term (5–10 years), and Far Term (10–15 years). This analysis was performed as part of a Strategic Science Roadmapping effort for JSTO and the subject matter experts (SME) included Service lab, academic, and commercial scientists, as well as operational personnel. The analyses were performed in individual 2-day mini-workshops.

In the Near Term, one can expect to see chimeric organisms; stealth pathogens; non-traditional chemical agents (NTA), which include both chemicals and biochemicals by the Office of the Secretary of Defense (OSD) definition; chemically impregnated particulates; and other novel chemical agents. Most of these are current realities, and the time frame refers primarily to when

¹ Jeff Mohr, Bob McGhin, Jim Cornette. SAIC. “Dugway Proving Ground Soil Fluorescent Background Study,” 15 March 2011.

they would become a concern to T&E efforts. Chimeric organisms are those that have been genetically manipulated to include genes or entire metabolic pathways that confer novel capabilities on the original organism. The simplest example is inserting genes into expression systems, such as *E coli*, which then enable the production of a protein product, such as an enzyme. These are common technologies used in the biomanufacturing industry, but could be subverted to yield stealth pathogens, or organisms which appear to be harmless background biota but are, in fact, capable of causing illness. Even in the absence of intentional genetic engineering, on average, we see one new emerging disease per year just as a result of natural recombination or mutation, as well as the increasing frequency of antibiotic resistant microbes. Modifications to increase their transmissibility, ability to jump species, or to avoid detection or treatment are already possible. The NTAs and chemically impregnated particulates (e.g., dusty agents) currently exist, as do novel chemical agents defined here as industrial chemicals put to a new use or new synthetic molecules beyond the NTAs. These may be made either chemically or biologically, and may vary from relatively small molecules (e.g., nerve or mustard agents) to more complex substances (e.g., hormones, toxins). Taken together, these chemical agents have in common the fact that they often present novel physico-chemical properties that must be taken into consideration when designing T&E methodologies and facilities.

The Mid Term will be marked by the development in biology of synthetic molecular systems and the more ubiquitous use of nanomaterials with unique properties. Synthetic molecular systems refer to the construction of artificial structures which mimic the functionality of living system but are themselves not alive. Such systems could be constructed of many different sorts of materials, including those designed from non-canonical amino acids, and would present enormous difficulties for detection and standardization of T&E procedures. Nanomaterials, commonly referred to as approximately 1–100 nanometers along at least one dimension, present critical safety considerations as we learn more about these materials. Their small size permits them to at once have toxic characteristics not evident at larger scales, while penetrating protective systems and exacerbating decontamination of test equipment. Materials with these dimensions are of sufficient concern that a Memorandum for the Heads of Executive Departments and Agencies on Policy Principles for the U.S. Decision-Making Concerning Regulation and Oversight of Applications of Nanotechnology and Nanomaterials was approved by the Director, Office of Science and Technology Policy; Administrator, Office of Information and Regulatory Affairs, Office of Management and Budget; and Chief, Agricultural Negotiator, Office of the United States Trade Representative, on June 09, 2011.²

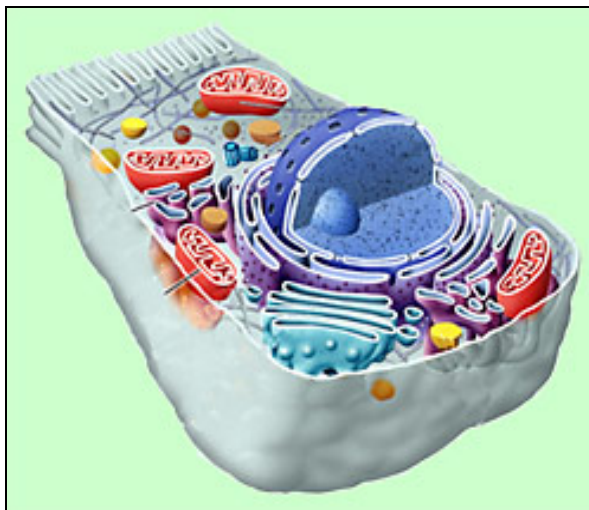


Figure 2. Synthetic Molecular Systems

² John J. Young, Jr. Under Secretary of Defense (AT&L) Memorandum, "Environmental, Safety and Occupational Health (ESOH) Risks from Engineered Nanomaterials," 13 May 2008.

The Far Term is speculative, but certainly includes the development of completely de novo organisms with characteristics that cannot be predicted a priori. The ability to mix and match artificial components, a.k.a. “biobricks,” and the standardization of the parts and design rules will permit the design of almost any new organism with tailored characteristics, further challenging T&E methodologies. These advances will require complete rethinking of CBD T&E capabilities and methodologies as biological threats take a more leading role after a 90-year focus on chemicals.

EMERGING TECHNOLOGIES

Technology is accelerating at an exponential pace, and this is no truer than in the biological and information sciences. Also included is nanotechnology, which we consider to be chemistry at a very small scale. The key concept is that of “convergence,” that is, two or more disciplines converge at a point in time where they catalyze the emergence of an entirely new area of investigation. An older example is the development and fielding of a remote passive sensor, enabled by the advent of microprocessors coupled with infrared spectroscopy. A more recent example was the convergence of genomics and information technology (IT), which resulted in the relatively quick sequencing of the complete human genome. Since convergence is best seen retrospectively, it is difficult to predict new trends in science, but a few areas merit attention. Again, these analyses were conducted with SMEs from Service, academic, and commercial laboratories in a series of 2-day mini-workshops.

In the Near Term, nanofluidic devices, next generation electronic sequencing, and adaptable



Figure 3. Mini Implantable Biosensor

algorithms will be significant issues for both biological and chemical detection. Nanofluidic devices, with channels an order of magnitude smaller than a human hair, have been developed and present significant challenges to sample acquisition, preparation, and handling; ease of handling of the devices themselves; and device standardization. These advantages may be partially offset by the technical challenges of maintaining clear channels when nanofluidic devices are used under operational conditions that involve dusts and other potential contaminants as described in Section 6a. Next generation sequencing on electronic chips raises the issue of data comparisons using different sequencing technologies. Adaptable algorithms, which will be integrated within the chip along with the sample acquisition and detector components, present the problem of independently testing the subcomponents when trouble shooting detector technologies.

In the Mid Term, the enormous data sets generated by complete “panomics” will present problems for data analysis. Panomics refers to genomics, transcriptomics, proteomics, metabolomics, and regulomics, that is, the complete set of biomarkers that will be accessible in the near future. Such biological complexity and large, mostly noisy data sets will present significant analytical problems such as signal-to-noise and the ability to compare results across different technologies which may be using different data or data analytical algorithms. In short, is what is purported to be measured actually what is being measured? However, based on the last

two decades of advancement in the “omics” realm, little doubt exists that the scientific and technical community will be up to the task of handling and manipulating massive data sets.

The Far Term is, as with the threat assessment, very speculative, but it is likely that adaptive, self-programming materials will be designed and incorporated into detectors and protective equipment. These will present a moving target as they adapt to the T&E environments and will require innovative approaches to standardized testing. The recent publication of the *National Strategy for CBRNE Standards* by the National Science and Technology Council, Subcommittee on Standards, May 2011, will do much to establish an enduring framework for standardized CBD testing in the future.³

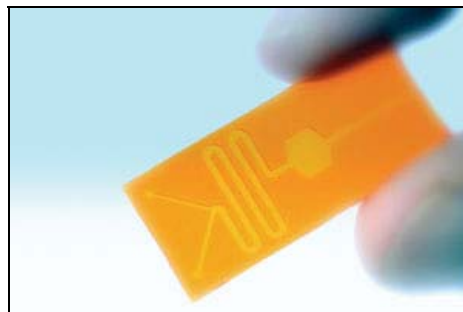


Figure 4. Nanofluidic Laboratory on a Chip

FUTURE T&E CHALLENGES

a) Equipment Challenges:

The study group identified six significant challenges that can be classified as equipment or materiel related. These are decreased size scales of nanodevices, mimicking realistic operational conditions, testing systems rather than components, sensors for manikins, detection of NTA droplets, and aerosol sensors. As devices become smaller, sample handling and preparation become of paramount importance. Current nano-scale devices exist that are an order of magnitude smaller than a human hair, and such devices will require a great deal of sample clean-up in order to prevent fouling, maintain flow, and account for turbulence and mass action effects within the device. Integrating these tiny components seamlessly into a system, as in “lab on a chip” formats, will make it very difficult to test and troubleshoot individual components. In addition, simply handling such miniscule devices will present problems, such as aerosol presentation within test chambers, and the large numbers of devices that could be integrated into a system will result in challenges for data collection and analysis.

One consistent theme within the interviewed T&E community was the need to develop realistic test conditions in the sense of mimicking operational conditions. Everything works well under pristine laboratory conditions, but real-world conditions often introduce uncontrolled variables that can skew results in favor of one technology over another. It will be necessary to identify the key variables and to develop test conditions that replicate operational environments likely to be encountered. This is also true of testing devices. Subsystems can (and usually do) function in an inter-dependent manner, and it is difficult to extrapolate test results of individual subsystems to the integrated whole. This is the opposite issue to that cited above, namely, being able to attribute performance issues to particular subcomponents when they are completely integrated into a unitary device.

³ Executive Office of the President, “Policy Principles for the U.S. Decision-making Concerning Regulation and Oversight of Applications of Nanotechnology and Nanomaterials,” 9 June 2011.

A more specific set of issues was identified with respect to testing protective clothing on manikins. Sensors under the clothing are not ideal, and increasing sophistication and decreasing size of sensors, as with modern crash test dummies, for example, will lead to the integration of thousands of sensors into test manikins. Further, there is a need to develop sensors that are designed to work with aerosols, in addition to vapor sensors.

b) Methodology Challenges:

A large number of identified issues could be grouped under the rubric “methods development.” These can roughly be grouped into three categories: data analysis; agent/simulant preparation and systems performance correlations; and field measurement issues. Under the data analysis category, the enormous amount of data that will be available as a result of panomics measurements and the proliferation of micro- and nano-scale sensors will pose a serious challenge to data collection and analysis for both fielded and test reference equipment. This is due to both the sheer volume of data, much of which will be noise, and to the lack of standardization among very complex and often incomplete data sets. Algorithms will need to be developed, annotated, and standardized for peta computing (1 petabyte = 1,000 terabytes), and more robust algorithms for agent/simulant correlation will need to be developed.

Agent and simulant sample preparation has been an ongoing issue and is likely to be exacerbated as new agents and, therefore, new simulants are developed. Organisms grown under slightly different conditions of nutrients, the number of “passes” before harvesting, and spray or freeze drying techniques can have radically different characteristics. Agents that have been prepared for weaponization are typically in a starved state and can be significantly different, physically and biochemically, than their healthy counterparts. As synthetic molecular systems and even artificial organisms are developed, the issue of standardization will either become critical, or will be replaced with a more generic set of detection parameters based on the particular detection technologies being developed. Considerable debate also exists on the need to be able to collect intact (i.e., viable) samples, suggesting a need for standard requirements regarding viability (i.e., does viable mean alive, able to reproduce, or in the case of a toxin, active, and to what extent?).

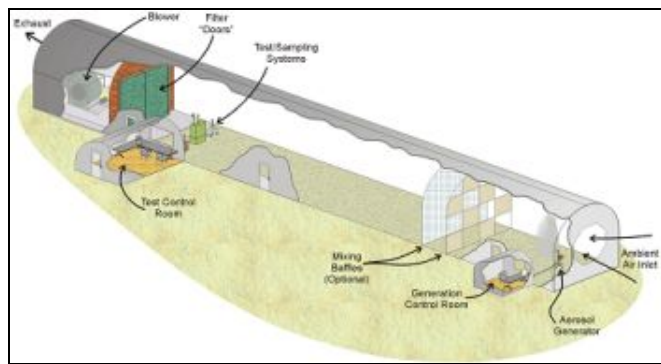


Figure 5. Battelle's Ambient Breeze Tunnel

The T&E community universally recognized pragmatic issues related to field testing. The overall rubric could be summed up as obtaining “ground truth” within the testing environment. A major concern was reference measurements, and whether point measurements—even when they are numerous—accurately reflect conditions within the test volume of the chamber or ambient breeze tunnel. This issue was reflected in comments that current test chambers are “under instrumented,” and this is going to be a bigger problem in the future as the need to collect detailed molecular-level information

becomes integral to the detection technology being assessed. Finally, there is a need for improved field calibration of aerosols and standardization of wet slurry vs. dry powdered dissemination.

c) Threat Agent Challenges:

Next generation biological and chemical threats are going to pose major challenges for the development of adequate simulants and standards. The incredible potential of synthetic biology to create new structures and “organisms” not found in nature and the sheer number of possibilities will complicate both counter-measures and T&E of detection technologies. Beyond that, two critical T&E issues have considerable health and safety impact. First, containment and protection of personnel from next generation threats will be complicated by their physico-chemical characteristics such as the ability to penetrate protective materials, adhere to surfaces, and remain viable under harsh decontamination conditions. Second, verification of decontamination of toxins, next generation chemical agents, and proteinaceous toxins will require the development of new decontamination materials, new and more sensitive measurement techniques, and possibly new protective barrier materials.

A FEW NEW TECHNOLOGIES

A review of the literature identified five new technologies at TRL-7 or better readiness level and which could be quickly exploited for chemical and biological defense. Much as the emergence of PCR-based assays caused T&E problems that were not well understood until after conclusions had been drawn from the data, these and other new technologies may have similar impacts. (1) Super conducting quantum interference device (SQUID) is essentially a magnetometer used to measure very weak magnetic fields (see Figure 6). These are beginning to have research uses in biology, could be used to detect very small numbers of organisms, but are subject to interference from even very weak magnetic fields. In addition, a signal-to-noise issue must be accounted for during both the test and data analysis. SQUIDs also require cooling to very low temperatures, presenting a development challenge. (2) Bio micro-electro-mechanical systems (Bio-MEMS) of demonstrated utility for both biological and chemical detection and sometimes known as “lab on a chip” have been discussed previously and present handling, sample preparation, and troubleshooting issues that will concern T&E personnel. (3) Digital Array Integrated Fluidics Circuits (DAIFC) present many of the same applications and issues as Bio-MEMS as well as those of PCR, and their ability to process physiological fluids will present safety concerns. (4) Quantum cascade lasers (QCL) operate in the mid to far infrared red part of the spectrum, permitting laser operation in materials with poor optical properties. Devices constructed of such materials may have unique characteristics that will require methods development. (5) Giant MagnetoResistivity (GMR) is based on very large resistance changes in alternating metallic layers, and devices constructed of such materials will require special handling and test procedures. (See Appendix A)

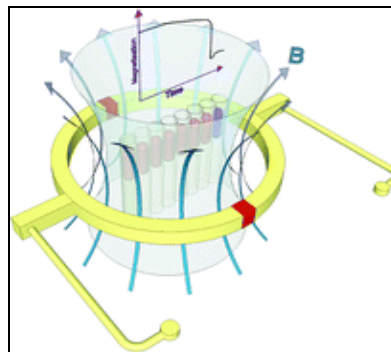


Figure 6. SQUID

GENERAL THEMES

Overall, the study group concluded that five major themes capture the primary challenges the T&E community will face as new technologies are developed. These are as follows:

1. Nano-scale operations that exploit the advances being made through the U.S. nanotechnology initiative as well as from international efforts
2. Enhanced requirements for safe handling of 21st century threat agents that include chemicals, biochemicals, and natural as well as modified microorganisms
3. Large, complex, possibly incomplete data sets that require rapid, real-time analysis
4. Creation of operationally realistic test scenarios and conditions for testing unknowns and scenarios in which original attack location cannot be determined; includes modeling and simulation (M&S) for evolving threats and unknowns, and forensics-based analysis to post-identify attack
5. Development of simulants and associated methodologies through which system performance-based agent-simulant correlations can be characterized

RECOMMENDATIONS

While it is recognized that the challenges are growing and far reaching for infrastructure viability and maintaining technological advantage in economic constraint, the scope of this study focused solely on technological challenges.

1. Deputy Undersecretary of the Army, Test and Evaluation Office (DUSA-TE) and Program Managers (PM) engage SMEs for both existing and next generation programs for which (in the latter case) specific technologies have not yet been selected. These SMEs can be drawn largely from Service laboratory personnel who are familiar with operational requirements, are networked with industry and academia, and as a practical matter are generally less expensive to engage. They also provide a high degree of continuity not available from other SMEs whose length of engagement depends on specific project funding. A strong, dynamic technology watch is essential, however this can be achieved.
2. Develop a formal strategic roadmap to identify and prioritize infrastructure, methodological, and threat agent challenges. The roadmap should recognize the shift toward biological and biosurveillance of emerging pathogens. The roadmap would be used to develop Program Office Management (POM) strategy, explain and defend programs, optimize investments, and ensure anticipation of future shock.

THE ROADMAPPING PROCESS

The roadmapping process is a formal procedure that follows a disciplined approach to mapping a desired end state. Various types of roadmaps exist, ranging from simple product roadmaps focused on a single commodity, to overarching strategic research roadmaps that cover a wide range of technology areas. In addition, roadmaps can be developed with unique characteristics suited for either sponsors or performers. Strategic roadmaps have a dual use: they lend clarity to

planning and investment; and they are able to map investments to products, hence defend programs. The Albright Strategy Group, LLC developed the graphic shown in Figure 7.

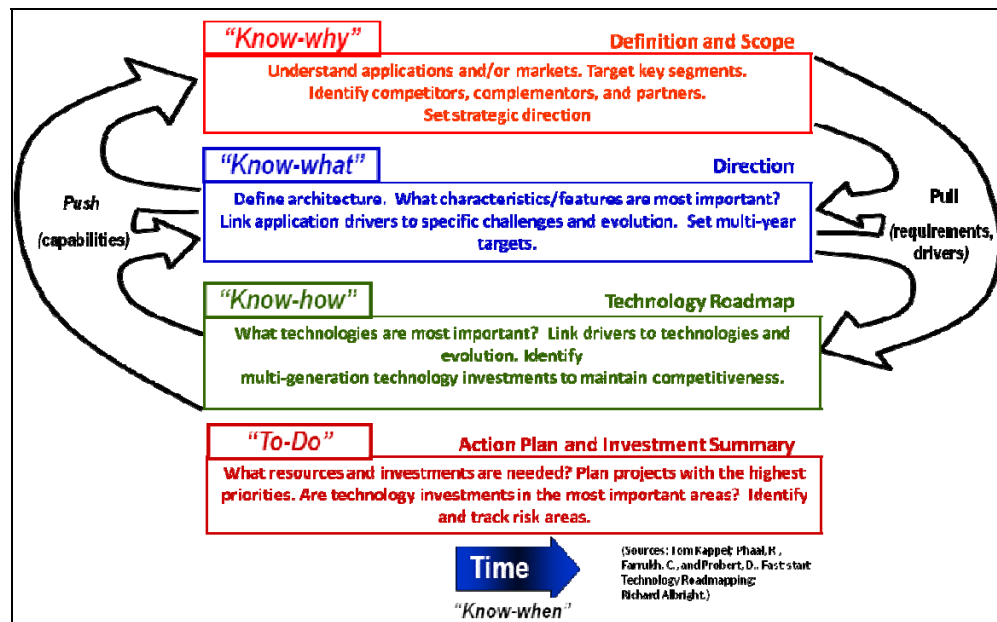


Figure 7. Roadmap Planning in Four Steps

APPENDIX A: TECHNOLOGY LIST

1) Spectral

- a) *Wavelengths*: Ultraviolet (UV); vis ; near infrared (NIR) (0.7–5 microns) ; short-wave infrared (SWIR) (2–5 microns); long-wave infrared (LWIR) (8–12 microns); far infrared (IR) (20–100 microns); TeraHertz (THz); and microwave.
- b) *Methods*: Miniaturized spectrometers; folded path length cells; photoacoustic spectrometers; Raman; surface-enhanced Raman spectroscopy (SERS); coherent anti-Stokes Raman scattering (CARS); resonance Raman; breakdown spectroscopy (laser-induced breakdown spectroscopy [LIBS] and spark-induced breakdown spectroscopy [SIBS]); fluorescence; dynamic light scattering; fiber optics; femtosecond lasers; and hyperspectral imaging.

2) Other Methods

- a) *Technologies*: Photoionization; lab on a chip (microfluidics, electrokinetics); functionalized microcantilevers; quantum dots (gene and aptimer arrays); conducting polymers; miniaturized mass spectrometer (MS); nuclear magnetic resonance (NMR) and nuclear quadrupole resonance (NQR); porous organic polymers and molecularly imprinted polymers (MIP); dendrimers; liquid crystals; flow cytometry; new enzymes; nanotechnology (nanomaterials, giant magnetoresistance [GMR], SQUIDs, gold nanoparticles/metal-oxide-semiconductor [MOS] junctions); ionic liquid electrolytes; solid phase microextraction (SPME/GC); ion mobility spectrometry (IMS)/mass spectrometry (MS); lipid diffraction gratings by dipping pen nanolithography; molecular computing; and molecular motors and machines.
- b) *Data processing*: Artificial intelligence (AI) and neural networks; game theory; swarm theory; massive parallel processing; role-based decisionmaking; and fuzzy logic.