

Cross-cutting Applications (CCA)

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Table of Contents

1. [Description of Science and Objectives](#)
 - 1.1 Radiation Biology
 - 1.1.1 Irradiation Studies
 - 1.1.2 Low Dose Radiation Biology
 - 1.2 Underground Agriculture
 - 1.3 Algal Biomass
 - 1.4 Microelectronics in a Low Dose Environment

2. [Experiments](#)
 - 2.1 Initial Activities and Developmental Needs
 - 2.2 Facility Needs at Homestake
 - 2.3 Education and Outreach
 - 2.4 Risk Identification
 - 2.5 Management

3. [Activities and Budget Estimates](#)
 - 3.1 Current objectives for S4
 - 3.2 Current estimates for S5
 - 3.3 Schedule

4. [List of collaborators](#)

5. [Supplementary Budget Material](#)

6. [References](#)

1. Description of Science and Objectives

The unique underground environment of DUSEL will enable the study of long-standing problems of national and international import, like food and energy.

Imagine, for example, not only developing the next generation of the energy efficient lighting organic-LEDs (OLEDs), but using them to develop underground agriculture practices that are directly transferable to manned missions to the moon or Mars. Or of studying the effect of very low doses of radiation on biological systems in order to answer the long-standing health-physics question of whether there is a low dose threshold to chronic exposure.

Not only does going underground shield one from radiation, but it also isolates plant life from the myriad of life above ground, allowing plant biology studies without the fear of cross-pollination. This could allow the development underground of new pharmaceuticals or biofuels.

Beyond the physical environment underground, the sociological environment of DUSEL will provide a natural synergy for researchers in these multi-disciplinary and potentially transformational areas. The imagination is the limit to the uses of the underground in these areas, and in applications that haven't been dreamed of yet. They will certainly spark the curiosity of the next generation of scientists, engineers, homeowners, and astronauts.

The cross cutting applications working group (a.k.a. Effects of Energetic Particles) includes four areas of multidisciplinary research: Radiation Biology, Underground Agriculture, Algal Biomass, and Microelectronics in a Low Dose Environment. We address each of these individually below.

1.1 Radiation Biology

Radiation biology can be divided into two basic areas of interest: The irradiation of microbes already living in a low dose environment, and the exposure of surface organisms to "chronic" low doses of radiation. The radiation biology group will have broader impacts on the history of deep life, the development of new applications of native microbes (including biofuels), and the relationship between low levels of radiation and cancer rates.

Why study radiation biology at Homestake?

1. The microbes, extremophiles, thermophiles, and other lifeforms under study are native to Homestake.
2. Facilities for low dose radiation biology can be placed deeper than anywhere else.
3. It will have synergy with other working groups such as baseline characterization and especially the low background counting facility for the reduced-Radon air and shielding from the rock.
4. Low dose studies needed to assess cancer risks from occupational doses, nuclear power, space/air travel.
5. Radiation is important to the study of the early development of life, and may have applications such as selecting out hyper-producers of ethanol.
6. It will significantly enhance the multidisciplinary nature of DUSEL.

1.1.1 Irradiation Studies

What role did radiation play in life's initial development? If the Earth initially had an opaque, Venusian+ atmosphere, then no photosynthesis would have been possible. A transparent atmosphere with no ozone layer would have allowed too much ultraviolet radiation to reach the surface. Radioactivity underground from uranium, thorium, and potassium (among other elements) would have been higher than what exists today. At a certain level radiation may have favored the development of radiolysis (or other mechanisms) prior to photosynthesis for the extraction of energy from the environment, and said radiation levels may have fostered a steady rate of mutations necessary for early adaptations.

What role does radiation play today in cellular functions? Most microbial life at Homestake will not have seen high doses of radiation for millions if not billions of years. Will current deep life develop new mechanisms for radiation resistance or cellular repair? Will latent genes be reactivated? Is radiation necessary for life to develop underneath the present biosphere, or for that matter, normal cellular operation at the surface? What substances enhance or inhibit a response to radiation in life that hasn't been exposed to radiation? Such questions may be important for the treatment of cancer with radiation and/or pharmaceuticals. New ways of repairing radiation damage for long-term space flight may also arise from similar research.

Most of the microorganisms and multi-cellular microorganisms familiar and readily available are from the surface of the planet. Spontaneous mutations are deemed to be caused by background radiation from solar and cosmic sources, and from radioactivity of some chemical isotopes¹ causing damage to DNA, and physiologic events such as errors in DNA replication^{2,3}.

Induced mutations occur at a higher rate than spontaneous mutations, due to increased dose of mutagen (such as radioactivity) above background levels. DUSEL would provide a desirable location for mutation studies of both radiosensitive and radioresistant organisms. The mass of rock provides shielding that will eliminate most all radiation from solar and cosmic sources; while providing ready access to a variety of microorganisms from different depths in the mine, some of which have evolved with low radiation levels, but some of which may have evolved in the presence of elevated radiation levels. Recent findings supporting the production of hydrogen gas via radiolysis in the deep sea subsurface^{4, 5, 6} suggest that a similar situation could exist for microorganisms deep in the terrestrial subsurface that may be accessed by deep drilling at DUSEL.

Additional research over the last few years has revealed a variety of radiation-resistant microorganisms in a variety of Earth environments^{7, 8, 9, 10}. Some bacteria such as *Deinococcus* can survive massive radiation doses of ionizing radiation^{11, 12, 13}, and the mechanism of radiation resistance for these bacteria seems to lie with certain cell proteins that complex with manganese¹⁴. Several species of fungi have been shown to be surprisingly resistant to ionizing radiation^{15, 16, 17, 18} and certain fungi producing melanin pigments may actually obtain energy from interaction of ionizing radiation with their melanin¹⁹.

Radiation-resistant extremophiles would be significant both for their potential study in astrobiology, but also in their ability to survive efforts for sterilization of medical equipment. Some radiation-resistant microorganisms may have a variety of industrial uses²⁰, including the degradation of lignocellulose that can impact the production of biofuels like ethanol. Lignocellulose and other fibrous structures in plants have historically hindered the production of ethanol from feedstocks other than corn or sugar beets such as switchgrass or wood chips. Not only will it be important to understand how lignocellulosic-degrading microbes may be killed via sterilization by radiation (as opposed to heat that is commonly used), but the addition of these microbes before/after direct irradiation pretreatments of the feedstock itself may enhance our accessibility to the sugars from the lignocellulosic matrix of the plant. Furthermore, it may be possible to select out hyper-producers of ethanol via irradiation²¹.

1.1.2 *Low Dose Radiation Biology*

The biological effects of low doses of radiation are not entirely understood, but they are needed to predict the health effects of radiation for space travel, occupational doses in nuclear power, and radiation therapy. Furthermore, low doses of radiation may impact medical treatments since resistance to disease and additional radiation treatment may change after chronic low dose exposures.

Low dose radiation biology is currently supported by both DOE and NASA (see <http://www.lowdose.energy.gov>) at facilities such as WIPP, and initial development could occur and is occurring outside Homestake. For example, low dose studies on mammalian tissue cultures are currently underway at Brookhaven National Lab in the shielded room once used by Ray Davis (which reduces the cosmic ray flux by a factor of 10). However, the 4850 foot level at Homestake has a greater overburden and lower total flux of muons than any of these sites.

The latest US report of the effects of low levels of ionizing radiation (BEIR VII) ²², based to a large extent on the epidemiological data, has accepted a Linear Non-threshold Hypothesis (LNT), i.e. a linear relationship between the biological response to radiation and the radiation dose. However, contrary evidence in the report indicates that cells exposed to low levels of radiation are more resistant to subsequent radiation doses: Such behavior has been termed “hormesis”. Many health physicists and radiation biologists support a more thorough investigation and verification of the hormesis effect ²³. Generally speaking, a low dose of radiation is any dose that is below what we receive from natural background radiation (roughly 3.7 mGy), but alternate definitions place this threshold as high as 200 mGy ²⁴.

If the LNT hypothesis is correct, then cells grown in a low dose environment should show improved biological properties such as increased growth rates, decreased spontaneous mutation rates, and decreased levels of endogenous DNA damages. If hormesis occurs, ambient radiation levels should benefit the cell through induction of repair or toleration paths that could correct damage arising from DNA replication or other radiation challenges. Trials on the effects of “chronic” low dose exposures on yeast and hamster cells by the Gran Sasso collaboration “PULEX” appear to indicate an adaptive response to the low dose environment through reduced resistance to chemical and radiation treatment ^{25,26}.

1.2 Underground Agriculture

Culturing of green plants below ground at DUSEL would have utility at Homestake, elsewhere on the surface of the Earth, and at potential human settlements in the solar system ^{27,28,29,30}.

Why should underground agriculture be pursued?

1. Potential food and fiber production for extraterrestrial colonization of the solar system. Underground facilities would be most likely needed to provide protection from UV and ionizing radiation and establishment of a buffered environment.
2. Oxygenic photosynthesis for terraforming other planets.

3. Potential food and fiber production for human settlements that must exist below ground for extended periods in event of catastrophic events such as asteroid impacts.
4. Establish large terrestrial underground isolated growth rooms for isolation and growth of transgenic crops.
5. Terrestrial plants (multi-cellular; containing lignin in cell walls to rise up against gravity) versus algae (unicellular or multi-cellular; no lignin in cell walls; float on or in water column)...which works best in underground environments?
6. To culture green plants underground, for their use in radiation exposure experiments along with other organisms studied at different depths in DUSEL. Plants afford unique advantages over other organisms in mutation studies³¹.

Growing crops underground has been done at other facilities, such as the 60 acre former limestone quarry in Indiana operated by Controlled Pharming Ventures LLC, or the underground growth chamber run by SubTerra LLC in Michigan's White Pine Mine or other locations in Canada by Prairie Plant Systems, Inc. These tend to specialize in the production of regulated biopharmaceuticals and proteins, and benefit from the isolation of pollen, control of environmental conditions, and isolation from the diurnal cycle.

This begs the question, why pursue underground agriculture at DUSEL?

1. Transgenic crops and other plants would take advantage of the temperature gradient at the mine to optimize growth characteristics.
2. We have proposed that DUSEL could be a base of operations to develop, test, and study sustainable agricultural systems for lunar or martian colonization for 2020 and beyond. The reduction of cosmic rays, temperature control, and the availability of space at Homestake closer to the surface are important factors.
3. The wide variety of regolith and excavated rock available at Homestake will be useful for the production of synthetic soils, since the suitability of rock for the preparation of a soil off-world will not be measured until sites there are excavated. Plant-soil, soil-microbe, plant-air, plant-light, and many other interactions all happening at the same time make the engineering of a soil from excavated rock a dynamic study for both terrestrial and off-world applications.
4. Green plants in DUSEL can be used to keep DUSEL "green". Underground agriculture can help purify and recycle water in method akin to the "living machines" of Todd ³². Remediation of heavy metals is another potential application.
5. Both transgenic crops and sustainable agriculture studies could use many of the drifts at Homestake that would not otherwise be used for Physics, especially those above the

4850 foot level. The availability of underground space is important for attracting companies to the region.

6. To measure the processing of various elements in plants and soils, underground agriculture could be a user of the low background counting facility. For instance, neutron activation analysis could be used to trace the absorption of heavier metals.

1.3 Algal Biomass

What is algal biomass, and why make it?

Biomass from algae can have many uses depending on the species. The biomass contains lipids, carbohydrates, and proteins that can be used for fuels (biodiesel, ethanol, hydrogen), lubricants, feed, organic matter for soil, etc. Researchers have identified a species that produces calcium carbonate which may serve as a mortar for binding naturally occurring construction materials used for shelter and storage. The biomass produced may be used as feed stuff for aquatic animals (shrimp, oysters, fish, etc.). Algal biomass may also be dried and used as an emergency food source for people. Algal species have been shown to contain compounds that can be used as pharmaceuticals, nutraceuticals, and as dietary supplements. Biomass production will require nutrients such as carbon, sulfur, phosphorus, and nitrogen (ammonia). These nutrients can be provided by waste streams produced by human activities thus allowing the treatment and recycling of grey water. Carbon for biomass production can be obtained from carbon dioxide allowing for the removal of carbon dioxide from the air and replenishment of oxygen. Algae biomass may also support scale is difficult. Research will lead to coupling algal biomass production underground carbon sequestration strategies at Homestake.

Why produce algal biomass at Homestake?

1. Production of algae above ground and below ground will be compared. Removal of the diurnal cycle and control of environmental conditions may allow for more efficient production.
2. The effects of a significant reduction in cosmic rays on production and the mutation or metabolic rates of algae have not been assessed to a large extent. Algae cultures can be grown in conjunction with the low dose radiation biology group.
3. Homestake has several species of algae both above ground, near the surface, and in lighting fixtures. The properties of these algae and their use in industrial applications need to be investigated.

4. Algal biomass production at Homestake can take advantage of the upper levels of the mine which will not be utilized to full capacity by the Dark Matter and other basic physics experiments planned.
5. South Dakota is actively pursuing biofuels production. Algal biomass will complement and enhance on-going economic development in the Northern Plains region for biofuels. Energy companies may have interest in strains of algae produced for use in carbon sequestration strategies.
6. In conjunction with the underground agriculture group, algae can contribute to making DUSEL a “green” laboratory via the processing of grey water and other wastes, and remediation of heavy metals. Algal biomass can also be a source of nutrients for underground agriculture.

1.4 Microelectronics in a Low Dose Environment

In general, radiation testing of microelectronics is performed using an accelerator for ionizing radiation, a gamma irradiator for total dose effects, or a neutron generator. However, when system life testing in a non-accelerated environment is preferable, going underground to lower the dose from cosmic rays is required. In certain cases, lower dose effects can have a greater impact than higher doses on microelectronics. This impacts calibration methods, predicted lifetimes, and performance for many devices, including those used for defense or communication in space, if not data acquisition in a low dose environment at Homestake.

Should a radiation testing facility be built underground at Homestake, other uses are likely to surface. Lifetime performance studies for materials and components are also possible.

In principle, such a radiation testing facility can be implemented at many other underground laboratories. But the following statements favor installation of one at Homestake.

1. It would stimulate the local economy. Hi-tech companies based in South Dakota would have ready access to such a facility.
2. Devices produced underground at Homestake for use in industry or research would not be exposed to surface levels of cosmic rays.
3. With a facility both at the surface and at the 300 foot level, it would have ready access to incoming students and thus could be a center for many E&O activities.
4. It would provide a multidisciplinary service for a minimal cost.
5. It would provide excellent synergy with the low background counting group.

[Back to top.](#)

2. Experiments

We present here a current status of experimental needs and a projection of developmental activities for all subgroups in Cross-Cutting Applications. This may change in response to searches for external funding and new collaborators that are on-going.

2.1 Initial Activities and Experimental Needs

2.1.1 Irradiation Studies

Several irradiation studies have been planned to occur during the SUSEL era, and other similar studies will occur into the initial stages of DUSEL. We propose to perform initial baseline studies of the effects of irradiation on pure cultures of microorganisms already isolated from the Homestake Mine. Irradiation can take place at the Cobalt-60 sterilizer operated by the 3M Corporation in Brookings, South Dakota; and at the X-ray machine operated by the USDA in Columbia, MO. Kill rates for several samples can be obtained and the results compared between gamma and X-ray irradiations, and ethanologenic or methanogenic strains will be sought. Analyses will take place on the campus of South Dakota State University.

Data from this study will be used in future grant opportunities regarding irradiation of thermophiles, extremophiles, and other microbes at Homestake. Whole soil from drift areas of Homestake will be irradiated, and then cultivated to see which microbes survived the irradiation. Comparison will be made to isolates obtained from soils with and without irradiation. Percent of the initial population surviving irradiation will be assayed; and specific isolates will be assayed for their ability to break down lignocellulose. We will also correlate the ability of microbial strains to withstand stresses of temperature and desiccation with the ability to tolerate radiation exposure. An X-ray irradiator available to the baseline community would support studies of the role of radiation in the development of deep life.

2.1.2 Low Dose Radiation Biology

Two initial studies have been envisioned so far for low dose radiation biology. First, human cells would be cultured in Brookhaven, placed at the surface and at 4850 for several generations, and then delivered back to Brookhaven for analysis. Dr. Betsy Sutherland, director of the NASA Space Radiation Laboratory, would conduct the research. She is also serving as the liaison for the group with the DOE Low Dose Biology Program. Cell counts, rates of apoptosis, gene expression, and proteomics of the underground and surface cultures would be compared. Secondary studies would apply radiation (perhaps via an X-ray source or radiomimetic drug) and examine X-ray induced and spontaneous mutation rates, rates of survival, and rates of radiation repair.

Second, Dr. Doug McFarland of South Dakota State University will examine the impact of chronic low doses of radiation upon skeletal muscle cells and human cancer cells. The mortal cells to be used will be myogenic satellite cells derived from skeletal muscle and embryonic myoblasts, both of which are derived from avian sources. The cells have been cloned to produce pure cultures, uncontaminated with non-myogenic cells such as fibroblasts. The other group of cells to be used will be several lines of human cancer cells, which behave quite differently. For the skeletal muscle cells, magnitudes of growth factor signaling and rates of proliferation, differentiation, apoptosis, and metabolism will be studied. Cancer cell lines available from the ATCC (which includes colon adenocarcinoma, lung adenocarcinoma, ovarian adenocarcinoma, mammary gland cancer, human osteosarcoma, and human skin melanoma) will be studied for similar characteristics.

2.1.3 Underground Agriculture

The first experiments could occur in the SUSEL era at an SDSU greenhouse with rock excavated from Homestake and regular greenhouse lighting. These experiments will provide data for evaluation of the particle size of crushed parent-rock media for drainage and support of plant growth. They will examine a variety of genotypes representing grass and dicot species to find conditions that will allow rapid germination and plant growth. Plants in these experiments will be short growth duration annuals, allowing for several replications of an experiment in a year.

The second set of experiments will utilize plant materials selected from the above study. This set of experiments will involve manipulation of the parent-rock media for drainage, pH, plant nutrition, and monitor the accumulation of potentially toxic materials by plants. These experiments will be conducted from seed germination to senescence. Evaluations of soil development, soil microflora populations, plant roots, and root exudates will be to provide insights into the modification of nascent soil environments by biological organisms. Seedling vigor and plant growth will be concurrently evaluated as the nascent soil environments evolve.

The initial experiments will develop into a more thorough study at DUSEL which addresses the following objectives.

1. Development of a low input growth medium consisting of geological materials inherent to the geological strata of the underground facility.
 - a. This will require the development of a growth matrix that will physically support water, gas, and nutrient transport sufficient for plant root growth and development.
 - b. Develop a growth medium that will supply and buffer chemical constituents needed for plant uptake and growth.
 - c. The biological component of the growth media will also need to be developed to support nutrient cycling, decomposition of plant materials and associated biochemical energy conversions and flow within the soil. How much can native subsurface microbes provide needed partnerships with plant roots? Will surface soil microbes need to be introduced in large numbers to partner with roots and foster plant growth and development?
 - d. Monitor changes in microbial populations associated with the rock material and plant roots using denaturing gradient gel electrophoresis ^{33, 34}.
 - e. Same questions as above, addressed for algal growth
2. Understanding of impacts of the microbial-media-plant systems on the evolution of a medium environment that supports improved root growth and development.
 - a. Evaluate selected microbial populations on nutrient cycling, organic decomposition, and physio-chemical properties of the growth media.
 - i. Native microbial populations from the mine
 - ii. Introduced microbial populations from the surface
 - iii. Microbial populations that are internally associated with seeds and plant tissues in sterilized environments.
 - iv. Same questions as above, addressed for algal growth
3. Development a sustainable aerial physical environment that supports an economic yield (in terms of input-output analyses).
 - a. Development of LED high efficiency lighting.
 - i. Determination of energy and mass balance in the growing environment.
 - ii. Organic LEDs may provide either the entire solar spectrum or an optimal range of frequencies for plant growth.
 - b. Control of plant movement needed for optimum plant growth and development.

- c. Circadian rhythms
- d. Gaseous composition (N₂, O₂, CO₂, trace gases that impact plant growth and development)

2.1.4 Algal Biomass

Work crews entering the mine system have reported that algae appear to be in the mine. The algae likely have come from the surface around the mine area and may have adapted to live in the mine environment. Assaying the algal species in the mine will provide a baseline for algal studies and selection of algal species to be used in S5 research. The location of the algae (around lights and vents for example) will provide insight as to how the algae have traveled in the mine as well as to how they survive. The activity of the algae will be ascertained too (dormant, growing, basically surviving, etc.).

Enclosed Photobioreactor development has generally used either natural sunlight or artificial light. Algae can utilize light in the wavelengths of 400-500nm and 600-700nm for photosynthesis. Light with wavelengths in the range of 600-700nm has less energy (thereby requiring less energy to generate the photons) than the 400-500nm wavelengths. PBR efficiency will be significantly improved if solar energy can be utilized to the fullest extent possible through photovoltaics or direct piping of light. Efficient utilization of solar energy which has light wavelengths outside the PAR range will require dividing the spectrum into ranges that can be used efficiently for photosynthesis and photovoltaic systems to produce electricity. The electricity produced will be used to run LEDs or OLEDs that either supplement the solar energy used directly for photosynthesis or to provide light when solar energy is not available.

The efficient use of light in a PBR is also influenced by mixing in the PBR which brings the algae cells into and out of light and dark areas of the PBR. Mixing can be accomplished by bubbling air containing carbon dioxide into the PBR. The bubbled air also provided carbon dioxide to the algae thereby coupling light utilization with gas transfer. Sparger design and location with light penetration will impact PBR physical dimensions and operation.

Preliminary studies need to be performed prior to S5 that lead to the development of a Photobioreactor (PBR) that will be used to produce algal biomass underground and at the surface. Biomass production above ground will be compared to production underground during S5 so that the impact of underground production can be determined as well as establishing areas of underground production that require further research. Comparison studies conducted during S5 will also allow for studying the impact that cosmic radiation has on mutation rates of the algae.

Given the manpower and monies described in the budget section for year 1 only, the following is proposed for the first year of development.

Task 1: Conduct a literature review to determine desirable algal species to use, scale up process that have been used in the past that are applicable, lighting systems and intensities, and mixing systems used.

Task 2: Determine available solar energy at SDSU and at Homestake. Also evaluate cosmic radiation levels at both locations.

Task 3: Develop a modular PBR system that can be used at SDSU and Homestake which will be adaptable to different lighting systems and spargers.

Task 4: Develop a solar collection system, methods of separating the solar spectrum, photovoltaic systems that utilize different frequency bands of light energy, and LEDs and OLEDs to convert the electricity produced into the desired wavelength.

Task 5: Produce algal biomass at SDSU for baseline studies and to provide preliminary test data on PBR performance.

Task 6: From 3 and 4 above identify space needs above and underground for S5 research. An engineering study will be commissioned to determine feasibility, cost, and time to construct facilities.

Task 7: An assay of the algal species in the mine will be conducted to determine what species have adapted to life in the mine environment.

Month	1	2	3	4	5	6	7	8	9	10	11	12
Task 1	***	***	***	***								
Task 2		***	***	***	***	***						
Task 3	***	***	***	***	***	***	***	***	***			
Task 4	***	***	***	***	***	***	***	***	***			
Task 5										***	***	***
Task 6								***	***	***	***	***
Task 7		***	***	***	***	***	***	***	***	***		

Proposed timeline for the first year of algal biomass development.

2.1.6 Microelectronics in a Low Dose Environment

Initial contacts have been made with radiation testing experts, and discussions are in progress. While there could be many different users of such a facility to, we provide a couple of examples below. Devices such as LEDs and photovoltaics could also be tested to evaluate their

performance under different fluxes of cosmic rays, which has ramifications for energy efficiency of said devices.

Enhanced Low Dose Rate Effects in bipolar transistors

The radiation hardness community has observed that some types of linear integrated circuits constructed with bipolar integrated transistors degrade more rapidly when irradiated at a much lower dose rate than conventionally used during radiation hardness testing. Such effects are referred to as Enhanced Low Dose Rate Effects, or ELDRS.

The MIL-STD-883, Test Method 1019, standard condition dose rate of 50 to 300 rad(Si)/second is used primarily throughout the industry to baseline the radiation hardness level for devices that may be used in a radiation environment. These same devices, however, when subjected to dose rates below 50 rad(Si)/second, can exhibit significantly lower total dose hardness levels. An electronic system deployed in space can encounter ionizing dose rates of 0.001 rad(Si)/second, or less. This phenomenon is of particular concern because the design margins that enable circuits to tolerate radiation are often based on characterization data obtained at the standard 50 to 300 rad(Si)/second dose rate.

ELDRS effects are not well understood. A program to study radiation effects at very low dose is hampered by statistics and background. Such an experiment could run long term at the 300 ft level in Homestake in a controlled environment.

Alpha particle effects in packaging of chips

Chip manufacturers are concerned with screening packaging material used in the manufacturing process for potential alpha emission at very low levels. To this end, large area detectors are under development at IBM³⁵ and other companies. These measurements are limited by background and could be conducted in the low background environment of Homestake, in an area that is radon reduced. The natural alpha background of this facility would have to be carefully measured and controlled.

2.1.7 Organic LED Development

A preliminary work will be conducted to develop a future research project on low cost flexible organic light emitting diodes (OLED). The OLED will mimic sunlight spectrum to initiate photosynthesis processes. The OLED can generate either full-spectrum or any specific spectrum (e.g. blue or red) light needed for the growth of algae and other crops.

Currently the commercial greenhouse lighting fixtures for crops growth use inorganic materials. The lights typically have narrow blue-centered spectrum emission. To truly get full

spectrum light, the blue spectrum of metal halide must be blended with red spectrum for high pressure sodium. These lighting systems will be quite heavy and expensive. Fortunately in the last 20 years, OLED has become alternative to the inorganic lighting system. The beauty of OLED is that they are mechanically flexible and will be much less expensive than their traditional counterparts since they can be processed from solution processing techniques and therefore offer lower capital cost for mass production. These OLED techniques are compatible with printing techniques and roll processes for flexible manufacturing, and scalable to large substrates.

OLED Application in Underground Agriculture - Potential food and fiber production will be required for extraterrestrial colonization of the solar system. Underground facilities would be most likely needed to provide protection from ionizing radiation and establishment of a buffered environment. The OLED project will be to develop a flexible OLED system with variable spectrum to mimic the sunlight for the photosynthesis during the crop growth.

OLED Application in Algal Biomass Production - Photobioreactors (PBR) for algae growth will be tested lab scale and algal prototype scale both at SDSU and Homestake to evaluate the effects of underground production and evaluate changes to counter adverse effects found. Surface systems are exposed to UV light and some ionizing radiation which tends to damage algae produced in a PBR. The damage produced in the algae may lead to mutations in the algae, altering their composition making it necessary to restocking of the PBR with algae. Light energy is essential for algal biomass production and is one of the main factors that controls production and requires the most effort to obtain. A novel new lighting scheme is anticipated for the underground PBR. Efficient photosynthesis can be achieved with wavelengths around 680 – 700 nm. The OLED system with light emitting in the range of 680 – 700 nm will be fabricate with a capability to adjust the light intensity.

Preliminary work will be performed in the S4 project for future research in developing OLED systems for Underground Agriculture (UA) and Underground Algal Biomass Production (UABP) at Homestake. In order to achieve this goal, the following six tasks will be completed:

Task 1: Conduct a literature review: we will perform a thorough literature search on OLED for large scale application. The Strengths, Weaknesses, Opportunities, and Threats (SWOT) involved in OLED will be analyzed.

Task 2: Search for materials and facilities: We will search and locate the materials, equipment, and facilities needed for OLED development.

Task 3: Evaluate the area required underground at Homestake for OLED system. The room to start, the room to develop into, the levels to be better suited, the temperature requirement for OLED operation.

Task 4: Plans for OLED lab establishment. Locate the labs at SDSU for OLED material synthesis, device fabrication, device characterization and optimization.

Task 5: Make a research plan with objectives. The goal is to develop next-generation flexible OLEDs with longer lifetime using a new family of light emitting polymers and a novel device structure. The OLED will be used in UA and UABP at Homestake. An initial plan with several tasks will be outlined.

Task / Month	1	2	3	4	5	6	7	8	9	10	11	12
Task 1	***	***	***									
Task 2			***	***	***							
Task 3					***	***	***					
Task 4								***	***	***		
Task 5										***	***	***

Timeline for initial organic LED (OLED) Development

2.2 Facility Needs at Homestake

2.2.1 Radiation Biology

We recommend that a biofacility (shared among all biological interests at Homestake) be developed at the surface. Radiation biology would use this facility to compare samples at the surface and underground, and to process and prepare samples going into or out of the mine. We also recommend the establishment of a low dose biofacility at the 4850 foot level to take advantage of the reduced-Radon air and shielding provided by the low background counting facility. We encourage the development of a cell culture facility at both the surface and underground laboratories. Many different tools required for eukaryotic studies, such as incubators, biosafety cabinets, refrigeration and cryogenics, centrifuges, ventilation and air filtration, and other lab equipment, will also be needed by other biological interests.

The design of the surface and underground facilities will be the prime effort for radiation biology in S4. Limited preliminary studies, such as irradiating microbes that already have been isolated, will also occur.

Surface Facility	Underground Facility
4' Biological Safety Cabinets	4' Biological Safety Cabinets
CO2 Incubator	CO2 Incubator
Liquid N2 Tank	Liquid N2 Tank
10, 20, 40x Inverted Microscope	10, 20, 40x Inverted Microscope
Refrigerator	Refrigerator
Freezer	Freezer
Ultra-low freezer	Air + CO2 Tank
Autoclave	Tank regulator
UV-visible/fluorescent plate reader	Cultureware + other consumables
Computer	Pipettors
Air + 5% CO2 tank	Glassware
Tank regulator	Magnetic Stirrer
Cultureware + other consumables	Water Bath
Pipettors	Lab Cart
Glassware	
Magnetic stirrers	
Water Bath	
NanoPure Water System	

A list of equipment and supplies for both an underground and a surface cell culture facility are provided above. With regard to space, it has been estimated that a 16 foot by 25 foot space will be suitable for a cell culture facility with a 7 by 4 foot center island workspace. 110 Volt electrical service and sinks with distilled water will be needed.

An autoclave would be placed in a separate room to be shared with other microbiologists. CO2 could be purchased at 100% and diluted to 5% locally. An X-ray irradiator similar to one used for medical uses (250 Kvp) could also be placed accordingly to be shared among biological and material science interests.

Long-term, enhanced infrastructure for tracking mutations and DNA damage may also be desired at Homestake.

2.2.2 Underground Agriculture

Initial infrastructure at Homestake should have the following characteristics (We do not assume full implementation of light piping or organic LED systems below).

- Space
 - 2 sites: 1) moderate, and 2) deep in the mine
 - 6 m × 8 m with 3 m height
- Water
 - Access to potable water
 - Access to deionizer
 - Water storage and removal capabilities
- Temperature control
 - Ability to set temperature range within $\pm 2.5^{\circ}\text{C}$
 - Ability to set temperature changes for night and day regimes
 - Air-flow controls
- Electricity
 - 500 watts/ m²
 - Electric grid to provide power and attachment for lights
- Lights
 - LED or equivalent to provide 1000 micromoles m⁻² s⁻¹ PAR
- Microprocessor controllers to regulate environmental parameters
- Remote monitoring capabilities (internet access)
 - Sensors (humidity, temperature, light, water)
 - Tie in to low radiation monitoring facilities
- Processed parent rock material for soil matrix
- Access to laboratory facilities to test growth medium and to make supplements to nutritional components
- Access to an autoclave to process and sterilize media for plant culture

2.2.3 Algal Biomass

At present, it is not believed that ambient Radon levels within the mine will have any deleterious effect on algae growth. While background radiation levels should be monitored, we do not expect any great need for Radon mitigation techniques.

A proper location for both prototype photobioreactors and any built for industrial production needs to be determined. Light and power from the surface delivered underground will require design and planning, such as any installation of photovoltaic systems to reduce overall consumption. Systems that mix, store, and process algae will all require power.

In addition, the temperature can be critical for the optimal performance of algae, which may also be affected by the choice of depth in the mine. Other environmental factors such as humidity and water quality also need to be addressed.

2.2.4 Microelectronics in a Low Dose Environment

We propose the development of two electronics and materials laboratories at Homestake. The first will be at the 300 foot level, and will require a Radon-reduced environment and a clean room. The second will be an electronics and materials laboratory on the surface. Comparisons of effects at the surface and at the 300 foot level for devices and materials will be evaluated.

An X-ray irradiator for the cross-cutting applications group could be shared among microelectronics testing and radiation biology. A scanning electron microscope should also be included in one of the materials laboratories, and in fact one could be shared with biological interests at DUSEL. Glove boxes for manipulation of materials may also be needed.

Background radiation levels for alphas, gammas, and neutrons must be determined. This could be done in conjunction with the low background counting facility, but also could be done by undergraduates and teachers as part of our education and outreach efforts. Environmental controls for temperature and humidity will be needed for uniform testing conditions.

2.3 Education and Outreach

In S4, the cross-cutting applications working group seeks funding to develop an E&O workshop and associated curriculum in year 1, and execute said workshop in year 2. E&O will also be part of the collaboration workshop to be developed. Up to 24 high school teachers will be accepted into a 5-day workshop that will cover fundamentals of radiation science, biological effects of radiation and radiation safety, materials science, and effects of radiation on plants and microbes (and/or other topics as decided upon during the collaboration workshop). Teachers will receive a per diem for meals, housing, curriculum material, and a stipend for attending during the summer.

E&O opportunities will also become available in the cross-cutting applications group via public tours of facilities at the surface and the 300 foot level of Homestake, participation by students and teachers in the assay of background radiation of these sites, and participation by undergraduate and graduate students in research related to S4 and S5 activities. Lab activities suitable for inclusion for Science on the Move, a mobile science lab that visits different locations

in South Dakota by truck, can also be developed. Plant and soil science could also provide agricultural displays for the visitor's center.

2.4 Risk Identification

We discuss below possible risks to personnel, the laboratory, and other experiments at DUSEL.

2.4.1 *Radiation Biology*

Radiation biology should have a local source of ionizing radiation. An X-ray irradiator is preferred to a radiological source or an accelerator since it can be turned off and will not activate surrounding materials. Thus, there will be a need for a technician, radiation safety oversight and monitoring, and restricted access to the device.

Biological containment will be essential, especially when dealing with human cell cultures to avoid any possibility of transmitting any virus or other disease. Proper ventilation and the use of fume hoods and/or HEPA filtration will be desired. If low dose radiation biology is placed near the low background counting facility, any cross-contamination (fingerprints, chemicals, fluids) with the sensitive physics detectors must be avoided.

Radiation biology will also use compressed CO₂ and cryogenics with liquid nitrogen. Proper ventilation underground and storage and disposal should be considered.

2.4.2 *Underground Agriculture*

In general, the Radon levels present in the mine will not affect plant development. While those levels should be monitored, we do not expect a significant need for Radon mitigation or extra shielding. It is unclear how zero radiation would affect plant growth.

With regard to biopharmaceuticals the biggest challenge will be biological containment. Level 2 containment will be relatively easy to achieve and can be done on the surface, but level 3 containment would be much more expensive to do (and will be avoided initially).

For all agricultural applications, the processing of water and waste material will be required. Any such material or dust would need to be isolated from most if not all physics experiments.

2.4.3 Algal Biomass

We recommend that algal biomass and the other biology-related tasks be isolated from the Physics experiments. Processing of gases such as oxygen and carbon dioxide will occur, so proper storage and/or ventilation of said gases would be required. The proper disposal of and/or reprocessing of algae wastes needs to be developed. Algal biomass would need to wait for baseline characterization to perform a preliminary survey of any drift before deploying photobioreactors. In general the workspace should be kept clean to reduce dust that can travel elsewhere.

2.4.4 Microelectronics in a Low Dose Environment

If various chemicals and bottled gases are required for the electronics and materials laboratories, then proper storage and ventilation will be desired. Due to the location of this facility, it should not impact other experiments at DUSEL. A clean environment will be needed to prevent the transport of dust to other areas of the lab.

2.5 Management

Dr. Robert McTaggart from South Dakota State University in Brookings, South Dakota has been serving as the working group leader for cross-cutting applications, and will continue as program manager for the group. He will also be the point of contact regarding facility needs.

Dr. Betsy Sutherland from Brookhaven National Laboratory is serving as the liaison between cross-cutting applications and the DOE Low Dose Radiation Biology Program. Dr. Bruce Bleakley (SDSU) is leading activities for irradiation studies of native microbes.

Dr. Peggy McMahan from the Lawrence Berkeley National Laboratory is directing efforts for Microelectronics in a Low Dose Environment.

Neil Reese and Thomas Schumacher (SDSU) are leading the underground agriculture group.

Gary Anderson is leading the development for the algal biomass group.

[Back to top.](#)

3. Activities and Budget Estimates

3.1 Current objectives for S4

We discuss current estimates for initial developmental activities for the experiments in section 3.2. These costs are better suited for an external grant or implementation in S5 than S4 however. We propose the following activities to occur during the duration of S4.

Firstly, we would like to hold a collaboration workshop in Year 1. This will have several goals:

1. to identify areas for which resources can be shared within the subgroups
2. to develop engineering design needs
3. to identify external grant opportunities
4. to identify E&O opportunities
5. to attract new collaborators.

Secondly, we would like to develop and implement a summer high school teachers' workshop in Year 2 on topics to be agreed upon at the collaboration workshop. These may include topics such as the fundamentals of radiation; health physics and radiation safety; applications of radiation in materials, biology, or agriculture; and curriculum development.

Thirdly, engineering design of the experiments and integration with engineering at DUSEL will need to occur. It's unclear at this time how many engineers will be needed for the group. Microelectronics testing and radiation biology will require the most integration into DUSEL because of their requirements for low background, reduced radon, clean rooms, and ventilation. Underground agriculture and algal biomass will need more internal experimental development, such as the production of organic LED lighting; heating and cooling systems; systems to process water, nutrients, and wastes, etc.

Other costs include a month of summer salary per year to support management of the group, a travel budget, and indirect costs. The lead institution at the present time is South Dakota State University.

The current budget estimate in S4 for the cross-cutting applications group is found below.

S4 Budget

Workshops:

Collaboration workshop (Year 1)	\$25,000
E&O workshop (Year 2)	\$20,000
E&O development (Year 1 and 3)	\$15,000

Engineering Design:

Microelectronics	\$50,000
Radiation Biology	\$50,000
Underground Agriculture	\$50,000
Algal Biomass	\$50,000

Management:

1 month of summer salary plus benefits per year	\$25,000
Travel	\$20,000
Total Direct Costs	\$305,000
Indirect (0.45* Direct)	\$137,250
Total Costs	\$442,250

3.2 Current estimates for S5

Some estimates for initial activities were submitted to this report for one-year costs: In these cases the author has estimated costs for 3 years based on that information assuming 5% increase in salaries, and the most expensive year will be year 1. Furthermore, if initial development has been completed prior to S5, then these estimates will need to be replaced.

The group does not have an estimate currently for implementation of Level 2 containment for biopharmaceuticals, operation of the microelectronics testing laboratory, engineering required for developing agricultural systems suitable for use by NASA, or industrial processing of algal

biomass at Homestake. Other potential interests, such as NASA or the USD School of Medicine, may significantly change the desired level of activity at Homestake for S5.

We are awaiting an estimate on an X-ray irradiator. A glove box used at SDSU for materials processing costs roughly \$120K.

- Radiation Biology
 - Brookhaven/DUSEL study \$605 K
 - SDSU w/ cell culture at DUSEL \$791 K
 - Irradiation studies \$90 K
 - X-ray irradiator not included
- Underground Agriculture
 - Sustainable plant growth (includes LED study) \$728 K
 - Biopharmaceuticals not included
- Algal Biomass
 - Assay and photobioreactor \$1,352 K
- Microelectronics
 - Testing facilities not included
- **Current Estimate** **\$3.564 million**

3.3 Schedule

All years: continue search for external funding, collaboration partners; engineering design

Year 1:

- Collaboration workshop
- Development of E&O workshop
- Preliminary engineering design

Year 2:

- Summer E&O workshop for teachers
- Initial S5 activities and plan for S5 set
- Continued engineering design

Year 3:

- Facility plans for S5 finalized
- E&O development for next workshop outside S4

Year 4:

- Implementation of S5 plans

[Back to top.](#)

4. List of Collaborators

Collaborators for the Cross-cutting Applications group

Program Manager:

Robert McTaggart South Dakota State University

Radiation Biology:

Betsy Sutherland Brookhaven National Laboratory

Doug McFarland South Dakota State University

Bruce Bleakley South Dakota State University

Underground Agriculture:

Neil Reese South Dakota State University

Thomas Schumacher South Dakota State University

Xingyou Gu South Dakota State University

Arvid Boe South Dakota State University

Eugene Butler South Dakota State University

Howard Woodard South Dakota State University

Algal Biomass:

Gary Anderson South Dakota State University

Anil Kommarreddy South Dakota State University

Qiquan Qiao South Dakota State University

Michael Twedt South Dakota State University

Larry Browning South Dakota State University

Kurt Rosentrater USDA Insect Laboratory

Joel Cuello University of Arizona

Microelectronics in a Low Dose Environment:

Peggy McMahan Lawrence Berkeley National Laboratory

Haiping Hong South Dakota School of Mines and Technology

Contact has been made with NASA, the USD School of Medicine, and several radiation testing experts.

[Back to top.](#)

5. Supplementary Budget Information

Initial study of human cells cultured at Brookhaven, delivered to Homestake, and then returned to Brookhaven (assuming no cell culture facility at Homestake):

Salaries, Wages, and Fringe (Dr. Sutherland, Post-doc)	\$ 195,032
Supplies	\$ 61,818
Travel	\$ 44,200
Other	\$ 25,654
Indirect Costs	\$ 278,175
Total	\$ 604,880

Start-up of cell culture facilities and initial study of muscle cells and human cancer lines

	Year 1	Years 2&3
Equipment for above ground cell culture facility	\$149,932	\$35,800
Equipment for below ground cell culture facility	\$47,132	\$35,800
Post-doc	\$40,000	\$86,100
Fringe benefits/insurance (22.5%)	\$9,000	\$19,373
Total Direct Costs	\$246,064	\$177,073
Indirect (0.45*Total)	\$110,729	\$79,683
Total (Direct + Indirect)	\$356,759	\$433,829
	\$356,759 + \$433,829 = \$790,588	

Initial irradiation studies (with irradiation done elsewhere):

Money to amend stipend for Master's Student (\$6K peryear)	\$18,000
Undergraduate labor	\$ 6,000
O&M for reagents, labware	\$ 8,000
Contractual work for ID of isolates	\$ 2,500
Travel	\$ 5,000
1 mth summer salary plus benefits (~\$7,500 per mth)	\$22,500
Total direct costs	\$62,000
Indirect (0.45 X Direct Costs)	\$27,900
Total	\$89,900

Equipment and Supply Costs for initial cell culture facility start-up

Below ground		Equipment and Supply List	
ITEM	Amount	Price (each)	TOTAL PRICE
4' Biological Safety Cabinets	2	\$7,500	\$15,000
CO2 incubator	2	\$5,000	\$10,000
Liquid N2 Tank	1	\$1,800	\$1,800
10, 20, 40x Inverted Microscope	1	\$5,500	\$5,500
Refrigerator	1	\$450	\$450
Freezer	1	\$650	\$650
Air + CO2 tank	2	\$350	\$700
Tank Regulator	2	\$400	\$800
Cultureware + other consumables		\$7,900	\$7,900
Pipettors		\$2,000	\$2,000
Glassware		\$1,180	\$1,180
Magnetic Stirrer	1	\$237	\$237
Water Bath	1	\$615	\$615
Lab Cart	1	\$300	<u>\$300</u>
		total:	\$47,132

Above ground		Equipment and Supply List	
ITEM	Amount	Price (each)	TOTAL PRICE
4' Biological Safety Cabinet	2	\$7,500	\$15,000
CO2 Incubator	2	\$5,000	\$10,000
Liquid N2 tank	1	\$1,800	\$1,800
10, 20, 40x inverted microscope	1	\$5,500	\$5,500
Refrigerator	1	\$450	\$450
Freezer	1	\$650	\$650
Ultra-low freezer	1	\$12,900	\$12,900
Autoclave	1	\$40,000	\$40,000
uv-vis./fluorescent Plate reader	1	\$42,000	\$42,000
Computer	1	\$1,000	\$1,000
Air +5% CO2 tank	2	\$350	\$700
Tank Regulator	2	\$400	\$400
Cultureware + other consumables		\$7,900	\$7,900
Pipettors		\$2,000	\$2,000
Glassware		\$1,180	\$1,180
Magnetic stirrers	1	\$237	\$237
Water Bath	1	\$615	\$615
Lab Cart	1	\$300	\$300
NanoPure Water System	1	<u>\$7,300</u>	<u>\$7,300</u>
		total:	\$149,932

Underground Agriculture

	Year 1	Years 2&3
Funding for 1 year full-time technician with benefits	\$50,000	\$107,625
Hourly undergraduate labor (20 h/week, 50 weeks, \$10/h)	\$10,000	\$20,000
Transport of parent rock material and processing (Assuming rock extraction at no cost)	\$20,000	\$20,000
Greenhouse space and supplies	\$ 8,000	\$20,000
Sand-culture tanks and pumps	\$20,000	\$10,000
Lights	\$ 5,000	\$10,000
Laboratory supplies for soil and microbiology analyses	\$10,000	\$25,000
Monitoring equipment	\$26,000	\$10,000
Two incubators (Percival Scientific Inc.)	\$20,000	\$10,000
Organic LED Development	\$50,000	\$50,000
Total Direct Costs	\$219,000	\$282,625
Indirect Costs = 0.45*total direct costs	\$98,550	\$127,182
Total	\$317,550	\$409,807

$$\$317,550 + \$409,807 = \$727,357$$

Algal Biomass

	Year 1	Years 2&3
Three Graduate Students	\$ 65,000	\$139,913
Technicians (instrumentation/PBR construction)	\$ 30,000	\$64,575
Faculty	\$ 20,000	\$43,050
Materials to build PBR, instrumentation, computers	\$100,000	\$50,000
Engineering Study	\$ 50,000	\$50,000
Assaying algae (grad student, lab services, faculty)	\$ 50,000	\$107,625
Solar collector/Photovoltaic	\$ 70,000	\$70,000
Travel	\$ 7000	\$15,000
Total Direct Costs	\$392,000	\$540,163
Overhead	\$176,400	\$243,074
Total	\$568,400	\$783,237

$$\$568,400 + \$783,237 = \$1,351,637$$

E&O Workshop Budget

Meals:	$\$26/\text{day}/\text{participant} \times 24 \text{ participants} \times 5 \text{ days} =$	$\$ 3,120$
Housing:	$\$130/\text{week}/\text{participant} \times 24 \text{ participants} \times 1 \text{ week} =$	$\$ 3,120$
Stipends:	$\$90/\text{day}/\text{participant} \times 24 \text{ participants} \times 5 \text{ days} =$	$\$10,800$
Subtotal		$\$17,040$

Round-up to \$20K to include supplies, etc.

The total of these estimates, if all funded for three years, is \$3.564 million. This does not include an estimate for setting up a microelectronics testing laboratory at the surface and the 300 foot level, or several other items previously mentioned that could occur.

[Back to top.](#)

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