Microgravity Experiment Case Studies
for the Student Spaceflight Experiments Program

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Revised: March 10, 2016

This document describes case studies of different microgravity experiments suitable for the Fluids Mixing Enclosure (FME) mini-laboratory, organized by science category. Note that the purpose of this document is just to give starting points for students to come up with their own ideas; the experiment possibilities are in no way limited by the descriptions below.

These case studies reflect experiments that can be conducted using a variety of FME configurations: Type 1 with a single experiment volume ("Main Volume"); Type 2 with two experiment volumes ("Volume 1" and "Volume 2"); and Type 3 with three experiment volumes ("Volume 1", "Volume 2", and "Volume 3"). Some experiments described below make use of a specific type of FME, but don't let that limit your thinking! Instead, each team is advised to come up with their own unique experiment, and use the type of FME that makes the most sense. To read about the different ways the FME can be configured and operated, visit the SSEP Mission 10 to ISS Mini-Laboratory Operation page
http://ssep.ncesse.org/?p=20162

1. Case Studies: Bacteria

Growth of Bacteria and Biofilms in Microgravity

There are many possible ways to study the growth of bacteria in microgravity:

- Investigate how microgravity affects the growth of different kinds of bacteria cultures compared with a control culture on Earth;
- Investigate how microgravity affects the formation of biofilms of bacteria such as *Bifidobacterium* on different kinds of surfaces (which have been sterilized before the experiment) compared with a control system on Earth. *E.g.,* are the biofilms thicker in one case, or are the bacteria distributed differently on the surfaces?
- Expose endospores (dormant forms of bacteria) of organisms such as *Bacillus subtilis* to microgravity, and compare the growth of the bacteria from reactivated endospores once they are brought back to Earth, with the growth of bacteria from endospores that were not exposed to microgravity.

These experiments could address many areas of science, from assessing whether organisms such as bacteria could be transported across space on the surfaces of meteoroids, to providing information on the survival and growth of bacteria inside spacecraft.

Antibiotic Resistance of Bacteria in Microgravity
Previous studies have suggested that antibiotics may not work as well in microgravity as on Earth for inhibiting bacterial growth. As an example of an experiment investigating this phenomenon, one could place dormant bacteria in one experiment volume of the FME. The bacteria are activated in orbit by bringing them into contact with an appropriate activating solution contained in another volume, and then exposed to an antibiotic substance contained in the third volume. By comparing the bacterial growth with a control experiment conducted on Earth, the possibility of enhanced resistance of bacteria to antibiotics in microgravity can be investigated. These studies may lead to better antibiotic materials and disinfectants for use in space and on Earth.

Bacteria experiments conducted during previous SSEP flight opportunities include: 1) *The Effect of Microgravity on the Ability of Ethanol to Kill E. coli* (STS-134); 2) *Effects of Microgravity on Lysozyme's Antibacterial Properties* (STS-134); 3) *The Effect of Microgravity on Biofilm Formation by E. coli on Polystyrene Particles* (STS-134); 4) *The Effect of Micro-gravity on the Viability of Lactobacillus* GG (STS-134); 5) *How Does Spaceflight Alter Mutation Rate, Growth Rate, Rate of Plasmid Uptake, and Ability to Withstand Subsequent Stressors in a Bacterial Strain?* (STS-134); 6) *The Growth Rate of Lactobacillus acidophilus in Microgravity* (STS-135); 7) *Effect of Microgravity on the Antibacterial Resistance of P. aeruginosa* (Mission 1 to ISS); 8) *Does Hay Bacillus Break Down Human Waste (Represented by Brown Egg) in Microgravity as Well as in Earth Gravity?* (Mission 1 to ISS); 9) *Effect of Microgravity on Reproduction of Curli Producing E. coli O157:H7 438950R* (Mission 1 to ISS); 10) *Effect of Arthrobacter on Polyethylene Decomposition Rate in Microgravity* (Mission 1 to ISS); and 11) *Escherichia coli in Microgravity* (Mission 1 to ISS). While some of these experiments were conducted during the Space Shuttle missions STS-134 and STS-135 using the MDA mini-laboratory, and so the exact experiment details may be somewhat different from the experiments conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. Descriptions of these SSEP experiments can be found on the Selected Experiments page at the SSEP Community Network Hubsite: http://ssep.ncesse.org/communities/experiments-selected-for-flight/

When designing bacteria experiments to be conducted aboard the International Space Station (ISS), it is important to consider the time constraints of the experiment: the time it takes to transport the experiment to the ISS, the length of the experiment, and the transportation of the experiment results back to the student team (see the SSEP Mission 10 to ISS Mini-Laboratory Operation page - http://ssep.ncesse.org/?p=20162 - for more details on these times.) The first of these considerations may affect the student team's choice of samples to use: since it will take several weeks for the FME to be transported to the ISS, it is likely that the bacteria used in the experiment will need to be in some dormant form (such as endospores or freeze-dried) that can be activated in orbit using an appropriate activating solution. Otherwise the bacteria may have reproduced and grown to the point where they will run out of resources, perish, and decay before the FME reaches the microgravity environment. The student teams have control over the second consideration – the duration of the experiment aboard the ISS – by deciding the best time to activate the experiment during the flight. The third consideration – the time it takes to transport the experiment results back to the experimenters – may lead to the student team deciding to terminate the experiment at a certain point before landing, since the transportation time may be

too long for the organisms to survive or for the effect of microgravity to remain detectable after returning to normal gravity conditions on Earth. As a result, the student team may want to use biological fixatives or growth inhibitors to stop or slow down the growth of the organisms and preserve them for analysis after landing. See the document *Using Biologicals in SSEP Experiments: Dormant Forms, Fixatives, and Growth Inhibitors* for more information on the approaches the student teams may want to consider when designing their biological experiment. The document can be found in the Document Library at the SSEP Website.

2. Case Studies: Cell Biology

Cell Growth in Microgravity

Comparing the growth and behavior of various types of cells when they are exposed to microgravity with a control experiment on Earth will make it possible to determine how different cells respond to microgravity. For example, studying the growth of collagen in a growth medium can provide important information on how bone cells produce collagen in microgravity, which may aid in developing strategies for the treatment of osteoporosis, among other possible applications. By comparing the rate of cell growth in microgravity and on Earth, one can investigate whether cell growth is slower in microgravity, as previous studies have suggested.

Cell Response to Chemical or Biological Agents in Microgravity

In this modification of the cell growth experiment in microgravity, a cell sample is placed in one of the volumes of the FME and a solution containing chemical or biological agents is placed in the other volume(s), and the two (or three) are brought into contact in orbit. The response of the cells to the chemical or biological agents under microgravity conditions can be compared with a control experiment conducted on Earth. For example, studying the response of cells to exposure to bacteria through phagocytosis (a process where infection-fighting cells engulf and destroy foreign materials) in microgravity can not only help evaluate the capability of these cells to fight infections during spaceflight, but also provide a better understanding of their behavior in general. By exposing cell cultures to various chemical agents it may be possible to determine ways to promote or inhibit cell growth in microgravity conditions through exposure to different environmental agents.

Cell growth and environmental response experiments conducted during previous SSEP flight opportunities include: 1) *Development of Prokaryotic Cell Walls in Microgravity* (STS-134); 2) *What is the Effect of Microgravity on the Growth Rate of Murine Myoblasts?* (STS-134); 3) *Microgravity's Effects on Morphagens in Common Species* (STS-134); 4) *All Mixed Up (Based on Gause's 1932 Experiment): The Effect of Microgravity on the Interaction of Paramecium bursaria and Paramecium caudatum in a Mixed Culture, using Yeast and Bacteria as a Food Source* (STS-135); 5) *The Effects of Microgravity on Oil Production in Salt-Stressed Chlamydomonas reinhardtii* (STS-135); 6) *Effects of Microgravity on Osteoblast Specialization and Bone Growth* (STS-135); 7) *How Does Parathyroid Hormone Affect Changes in Bone Mass in Microgravity?* (Mission 1 to ISS); 8) *Hepatocyte Development in Bioscaffolds Infused with TGFB3 in Microgravity* (Mission 1 to ISS); and 9) *Will Vitamin C Preserve Bone Density in Microgravity?* (Mission 1 to ISS). While some of these experiments were conducted during the Space Shuttle missions STS-134 and STS-135 using the MDA mini-laboratory, and so the exact experiment details may be somewhat different from the experiments conducted using the Fluids

Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. Descriptions of these SSEP experiments can be found on the Selected Experiments page at the SSEP Community Network Hubsite: http://ssep.ncesse.org/communities/experiments-selected-for-flight/

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3. Case Studies: Fish, Other Aquatic Life, and Animal Studies

Development of Organisms in Microgravity

By allowing fish (or other animal life) eggs to hatch and embryos to develop in a microgravity environment, and comparing the results with a control experiment conducted on Earth, it is possible to determine how the lack of gravity affects the development of these animals. The experiment can be done by using just one experiment volume of the FME to expose the contents to microgravity for several weeks, or by activating the development of the eggs and embryos by placing them in one volume of the mini-laboratory, suitable growth medium in the other, and allowing the two volumes to mix in orbit. A third volume could be used to provide additional resources (such as water, oxygen, and nutrients) to help the organisms survive the duration of the flight. Even if the organisms were to perish during the experiment, it is possible to design analysis techniques that will provide useful information on the behavior and growth of the biological samples by studying the remains of the organisms.

Growth of Aquatic Life in Microgravity

To determine if fish and other aquatic life such as shrimp might be a good food source during long-duration space flight, it is important to see how well they grow in microgravity. This could

be done by allowing eggs to hatch, or larvae to develop in microgravity and then seeing how much food they consume when compared with a control experiment conducted on Earth, or by comparing the size of the specimens grown in microgravity to those grown on Earth. For example, the protein-rich muscles of shrimp develop when they work against gravity while swimming. When in microgravity, the shrimp need to swim less, which could lead to a smaller appetite and smaller muscle growth, making the shrimp grown in microgravity a less valuable protein-rich food source.

Regeneration of the Planaria Worm in Microgravity
The experiment to see how the regeneration of the body parts of the Planaria worm may differ in microgravity from the process on the surface of Earth can be achieved by placing worms with missing segments in water, and comparing how the regeneration of lost body parts in microgravity may differ from a control experiment performed on Earth. Typically, a planaria worm that is split lengthwise will regenerate into two individual worms; does this occur in the same way in microgravity?

Animal life experiments conducted during previous SSEP flight opportunities include: 1) *Brine Shrimp Development* (STS-134); 2) *The Development of Fertilized Tilapia Fish Eggs in Space* (STS-134); 3) *Swimming Patterns and Development of Zebra Fish after Exposure to Microgravity* (STS-134); 4) *Will Microgravity Effect the Development of Goldfish?* (STS-135); 5) *How Does Microgravity Affect the Maximum Cell Size of Tardigrades?* (STS-135); 6) *Killifish in Space* (Mission 1 to ISS); 7) *The Physiological Effects of Microgravity and Increased Levels of Radiation on Wild-Type and Genetically Engineered Caenorhabditis elegans* (Mission 1 to ISS); and 8) *Spider Development and Gravity* (Mission 1 to ISS). While some of these experiments were conducted during the Space Shuttle missions STS-134 and STS-135 using the MDA mini-laboratory, and so the exact experiment details may be somewhat different from the experiments conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. Descriptions of these SSEP experiments can be found on the Selected Experiments page at the SSEP Community Network Hubsite:
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When designing aquatic life experiments to be conducted aboard the International Space Station (ISS), it is important to consider the time constraints of the experiment: the time it takes to transport the experiment to the ISS, the length of the experiment, and the transportation of the experiment results back to the student team (see the SSEP Mission 10 to ISS Mini-Laboratory Operation page - http://ssep.ncesse.org/?p=20162 - for more details on these times.) The first of these considerations may affect the student team's choice of samples to use: since it will take several weeks for the FME to be transported to the ISS, it is likely that the organisms to be used in the experiment, for example, will need to be in some dormant form (such as dormant eggs, cysts, or freeze-dried) that can be activated in orbit using an appropriate activating solution. Otherwise the organisms may have reproduced and grown to the point where they will run out of resources, perish, and decay before the FME reaches the microgravity environment. The student teams have control over the second consideration – the duration of the experiment aboard the ISS – by deciding the best time to activate the experiment during the flight. The third consideration – the time it takes to transport the experiment results back to the experimenters – may lead to the

student team deciding to terminate the experiment at a certain point before landing, since the transportation time may be too long for the organisms to survive or for the effect of microgravity to remain detectable after returning to normal gravity conditions on Earth. As a result, the student team may want to use biological fixatives to kill the organisms and preserve them for analysis after landing. See the document *Using Biologicals in SSEP Experiments: Dormant Forms, Fixatives, and Growth Inhibitors* for more information on the approaches the student teams may want to consider when designing their biological experiment. The document can be found in the Document Library at the SSEP Website.

4. Case Studies: Fluid Diffusion

A simple way to investigate fluid diffusion in microgravity is to place two fluids with different densities in two volumes of the FME and bring them into contact in orbit. The mixing of the two fluids could be examined by taking a series of photographs of or filming the interaction between the two fluids. Alternatively, if the two fluid volumes could be separated after the fluids have mixed for a certain period of time but before the FME is returned to Earth, comparing the level of mixing between the two fluids in the experiment conducted in microgravity and a control experiment performed on Earth can provide information of the effectiveness of fluid diffusion in microgravity. In a variation, particles could be placed in one of the fluids to see how they affect the mixing of the two fluids, and how the particles become distributed through the combined volume of the FME. The challenge of developing an effective fluid diffusion experiment for this flight opportunity comes from the basic design of the mini-laboratory. Since the FME is opaque, it is not possible to film or photograph the samples once they have been loaded, and so there is no way to observe the fluid diffusion process in orbit. Furthermore, while the two fluids contained in the separate volumes of the FME can be brought into contact in orbit, the fluid volumes cannot be separated again before the FME is returned to Earth and the effect of fluid diffusion that took place in microgravity is wiped out by the return to normal gravity conditions on Earth. The same limitations for observing the effect of fluid diffusion in microgravity applied to the previous SSEP flight opportunities; therefore, no SSEP experiments studying this process have been flown to date.

5. Case Studies: Food Products

Nutritional Value of Food Products Containing Probiotics in Microgravity

By placing a food product containing probiotics in the FME, it is possible to determine how well micro-organisms beneficial to humans survive in microgravity. After landing, samples of the food product containing probiotics can be cultured, and the level of growth of the bacteria compared with the growth of bacteria taken from samples of a control food product that remained on Earth.

Food Storage and Spoilage of Foods in Microgravity

The effectiveness of various food storage materials could be assessed by encasing foods in storage material in the FME, and comparing the product after exposure to the microgravity environment with a control product that remained on Earth. Alternatively, one could place a chemical or a harmful bacteria solution in the second volume of the FME, and bring it into contact with the food product in orbit. The level of spoilage of the food product during the flight

can then be compared with a control experiment conducted on Earth. These experiments address how effective food storage methods might be developed for human spaceflight missions.

Food product experiments conducted during previous SSEP flight opportunities include: 1) *Honey as a Preservative on Long Duration Space Flights* (STS-134); 2) *Microgravity Yeast Experiment* (STS-135)*;* 3) *Microgravity Wine* (Mission 1 to ISS); and 4) *Yeast in Space!* (Mission 1 to ISS). While some of these experiments were conducted during the Space Shuttle missions STS-134 and STS-135 using the MDA mini-laboratory, and so the exact experiment details may be somewhat different from the experiments conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. Descriptions of these SSEP experiments can be found on the Selected Experiments page at the SSEP Community Network Hubsite: http://ssep.ncesse.org/communities/experiments-selected-for-flight/

When designing food product experiments to be conducted aboard the International Space Station (ISS), it is important to consider the time constraints of the experiment: the time it takes to transport the experiment to the ISS, the length of the experiment, and the transportation of the experiment results back to the student team (see the SSEP Mission 10 to ISS Mini-Laboratory Operation page - http://ssep.ncesse.org/?p=20162 - for more details on these times.) The first of these considerations may affect the student team's choice of samples to use: since it will take several weeks for the FME to be transported to the ISS, the teams may want to use products that either can tolerate the long transportation time, or use some dormant form of the food products (such as dried) that can be activated in orbit using an appropriate activating solution (if desired). Otherwise the food products may have spoiled before the FME reaches the microgravity environment. The student teams have control over the second consideration – the duration of the experiment aboard the ISS – by deciding the best time to activate the experiment during the flight. The third consideration – the time it takes to transport the experiment results back to the experimenters – may be sufficiently short that the effect of microgravity on the food products is still clearly detectable once the samples are returned to the student team, but if the team is investigating situations where their experiment results may be affected as soon as the FME is returned to Earth, the team may want to consider ways to preserve the experiment results before the FME leaves the ISS. For bacteria and cell biology experiments, one way to preserve the samples is through the use of fixatives and biological inhibitors to kill the organisms and preserve the samples. While the use of these samples may be overkill for food products, the student teams may want to read through the document *Using Biologicals in SSEP Experiments: Dormant Forms, Fixatives, and Growth Inhibitors* to see if there are any approaches the team may want to adopt for their experiment. The document can be found in the Document Library at the SSEP Website.

6. Case Studies: Inorganic Crystal Growth

Growing Inorganic Crystals in Microgravity

One way to produce inorganic crystals in orbit is to create a crystallization solution (for example by mixing hot water into inorganic crystal chemicals, such as potassium aluminum sulfate or potassium chloride sulfate, and letting the solution cool to room temperature), then placing the solution in one volume of the FME, and placing a wicking substance such as cotton in the other

volume. Bringing the two volumes together in orbit allows moisture to be wicked away from the crystallization solution and growth of the crystals to be activated. The crystals grown in orbit can be compared with control crystals grown on Earth to see whether the crystals grown in microgravity are larger and purer, as previous studies have suggested.

One inorganic crystal growth experiment has been conducted during previous SSEP flight opportunities: *Deposition and Formation of Zinc Phosphate Crystals in Microgravity* (STS-135). While this experiment was conducted during the Space Shuttle mission STS-135 using the MDA mini-laboratory, and so the exact details of how the experiment was conducted are somewhat different from the experiments that will be conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. The description of this SSEP experiment can be found on the Selected Experiments page at the SSEP Community Network Hubsite:
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7. Case Studies: Microencapsulation

Creating Microcapsules in Microgravity

In a microencapsulation experiment, two immiscible fluids can be placed in the two volumes of the FME. One fluid contains the planned contents of the microcapsule, such as the drug ciprofloxacin, while the other includes the planned coating of the microcapsule, such as polyvinyl pyrollidone. The two fluids are brought together in microgravity, the solution is allowed to mix and evaporate, and after landing, the resulting microcapsules are analyzed to determine their shape and size. The results can be compared with those from a control experiment performed on Earth.

One microencapsulation experiment has been conducted during previous SSEP flight opportunities: *Efficiency of Microencapsulation in Microgravity as Compared to Gravity* (STS-134). While this experiment was conducted during the Space Shuttle mission STS-134 using the MDA mini-laboratory, and so the exact details of how the experiment was conducted are somewhat different from the experiments that will be conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. The description of this SSEP experiment can be found on the Selected Experiments page at the SSEP Community Network Hubsite:
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8. Case Studies: Protein Crystal Growth

Growing Protein Crystals in Microgravity

One way to create protein crystals in microgravity using the FME is to dissolve the protein in a medium such as a sodium acetate buffer, or a solution made of Tris HCl buffer and ammonium sulfate. The protein solution is placed in one volume of the FME, and a salt solution in the second. In orbit, when the two volumes are brought into contact, protein crystals start forming from the solution; this technique for protein crystal production is called “interfacial diffusion.” In a variation of this experiment called “step diffusion”, the protein is mixed in a low-salt solution and placed in the first volume, and when it comes into contact with a high-concentration salt

solution in the second volume, the solution becomes supersaturated and crystals start to nucleate at the interface of the two fluids. After a desired nucleation time, the salt concentration of the protein solution can be lowered by mixing in a low or intermediate concentration salt solution contained in the third volume of the FME. This makes it possible for the protein crystals to grow for the remainder of the experiment.

One protein crystal growth experiment has been conducted during previous SSEP flight opportunities: *Lysozyme Protein Crystal Growth in Microgravity* (STS-134). While this experiment was conducted during the Space Shuttle mission STS-134 using the MDA mini-laboratory, and so the exact details of how the experiment was conducted are somewhat different from the experiments that will be conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. The description of this SSEP experiment can be found on the Selected Experiments page at the SSEP Community Network Hubsite:
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9. Case Studies: Seeds & Plant Studies

Seed Germination and Plant Growth in Microgravity

There are numerous experiments that can be conducted with plants and seeds to determine how plants grow, and whether seeds can germinate and develop properly in microgravity. For example, seeds can be placed in different kinds of mixtures of water and soil (or another type of growth medium such as rockwool) in the main volume of the FME, and the germination of the seeds and the growth of the seedlings after exposure to several weeks of microgravity can be compared with a control experiment conducted on Earth. One variation of this experiment could have the germination activated in orbit by placing seeds and water in two separate volumes of the FME, and bringing the two volumes into contact in orbit. In yet another variation, an experiment could investigate how effective a chemical designed to promote plant growth is in microgravity when compared with a control experiment conducted on Earth.

Seed and plant study experiments conducted during previous SSEP flight opportunities include: 1) *Apples in Space* (STS-134); 2) *Does Radiation Exposure Effect Seed Germination without the Protection of the Ozone Layer?* (STS-134); 3) *Microgravity's Effect on Tomato Growth* (STS-135); 4) *Physiological effects of microgravity on germination and growth of Arabidopsis thaliana* (STS-135); 5) *Effects of Microgravity on Goodstreak Wheat* (STS-135)*;* 6) *The Effect of Microgravity on the Use of Cactus Mucilage for Water Purification* (Mission 1 to ISS); and 7) *The Effect of Microgravity on the Quality and Nutritional Value of the Seed Sprout of a Germinated 92M72 Genetically-Modified Soy Bean* (Mission 1 to ISS). While some of these experiments were conducted during the Space Shuttle missions STS-134 and STS-135 using the MDA mini-laboratory, and so the exact experiment details may be somewhat different from the experiments conducted using the Fluids Mixing Enclosures aboard the International Space Station, the basic approach to experiment design in this area of microgravity research is the same. Descriptions of these SSEP experiments can be found on the Selected Experiments page at the SSEP Community Network Hubsite:
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When designing plant study experiments to be conducted aboard the International Space Station (ISS), it is important to consider the time constraints of the experiment: the time it takes to transport the experiment to the ISS, the length of the experiment, and the transportation of the experiment results back to the student team (see the SSEP Mission 10 to ISS Mini-Laboratory Operation page - http://ssep.ncesse.org/?p=20162 - for more details on these times.) The first of these considerations may affect the student team's choice of basic experiment design: since it will take several weeks for the FME to be transported to the ISS, the teams may want to use seeds that either have germination times long enough not to have germinated before the experiment arrives at the ISS (if the seeds are placed in water inside the FME) or remain dry until they are activated in the ISS. The student teams have further control over this second consideration – the duration of the experiment aboard the ISS – by deciding the best time to activate the experiment during the flight. Still, the overall duration of the flight may direct the student teams to use seeds that have germination times short enough to make it more likely for germination to occur during the stay of the FME aboard the ISS, but not so short that the germination or plant growth process progresses past the point of what the team wants to investigate. The third consideration – the time it takes to transport the experiment results back to the experimenters – may be sufficiently short that the effect of microgravity on the plants and seeds is still clearly detectable once the samples are returned to the student team, but if the team is investigating situations where their experiment results may be affected as soon as the FME is returned to Earth, the team may want to consider ways to preserve the experiment results before the FME leaves the ISS. For bacteria and cell biology experiments, one way to preserve the samples is through the use of fixatives and biological inhibitors to kill the organisms and preserve the samples. While the use of these samples may be overkill for plant studies, the student teams may want to read through the document *Using Biologicals in SSEP Experiments: Dormant Forms, Fixatives, and Growth Inhibitors* to see if there are any approaches the team may want to adopt for their experiment. The document can be found in the Document Library at the SSEP Website.