

BioEd<sup>SM</sup>



# Plants in Space



## TEACHER'S GUIDE

Gregory L. Vogt, Ed.D.

Nancy P. Moreno, Ph.D.

Stefanie Countryman, M.B.A.

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# Plants in Space

An Experiment Aboard the International Space Station

*by*

**Gregory L. Vogt, Ed.D.**

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**Stefanie Countryman, M.B.A.**

## RESOURCES

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# Contents

1. Introduction	1
2. Seeds and Germination	3
3. A Closer Look at Roots and Stems	4
4. Plants in Space Investigation: <i>Brassica rapa</i>	6
5. Preparing the Plant Growth Media	7
6. Constructing the Experiment Chamber	9
7. Planting the <i>Brassica rapa</i> Seeds	10
8. How Does Gravity Affect Root Growth?	11
9. How Does Microgravity Affect Plant Growth?	13
STS-134 Protocol: <i>Brassica rapa</i>	15
Teaming with Benefits – NASA and the NSBRI	16

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# 1



Gardening in space has been part of the International Space Station (ISS) from the beginning. Understanding photosynthesis and plant development is a critical component of future long-duration space missions. By generating oxygen, removing carbon dioxide and purifying water, living plants could help maintain a healthy spacecraft atmosphere and reduce the costs of air and water resupply. Plant research also will have direct application to future production of crops that the ISS crew could eat.

Shown above, astronaut Peggy A. Whitson, Expedition 5 NASA ISS Science Officer, holds the Advanced Astroculture soybean plant growth experiment in the Destiny laboratory on the ISS. Photo courtesy of NASA.

## IMAGE SOURCES

Complete image citations, including URLs, are available at the front of this guide.

# Introduction

On May 16, 2011, Space Shuttle *Endeavour* began its final mission, a trip to the International Space Station (ISS). In addition to its primary payload, the Shuttle carried two small-scale investigations that invite student participation. The first investigation involved the behavior of orb-weaving spiders, *Nephila clavipes*, in microgravity. The second examines plant root growth in space. This investigator's manual describes the plant root growth investigation and provides the details necessary for students and teachers to collect and analyze data while conducting their own parallel investigations.

Any classroom or individual around the world is invited to participate in this project. Each participant (or group) must set up an Earth-based growing chamber with plants to compare to those growing on the ISS. Once the investigation begins in the fall of 2011, a steady stream of ISS plant images will be made available for viewing on the BioEd Online ([www.bioedonline.org](http://www.bioedonline.org)) and K8 Science ([www.k8science.org](http://www.k8science.org)) websites. These images will provide many opportunities for creative studies that compare root growth in normal gravity with growth in microgravity.

This manual begins with a primer on plant roots and plant tropisms (growth movements in response to a stimulus). Later sections provide full details on setting up a ground chamber and growing the plants.

The guide does not present a formal research plan. This investigation allows—and requires—participants to

ask their own questions about plant root growth in microgravity and on Earth, and to collect the data needed to answer their questions.

## PREREQUISITES

While anyone can participate in the investigation, it is suggested that prior to beginning, each investigator become familiar with fundamental aspects of the microgravity environment of space and with basic research techniques. The following supplemental guides, available free of charge on BioEd Online and K8 Science, offer useful background information.

- *Designing Your Investigation*
- *Keeping a Naturalist Journal*
- *Microgravity*
- *Scientific Image Processing*

## PLANTS ON EARTH

Plants are found virtually everywhere on Earth's surface, from deserts to tropical rainforests to high mountains. Scientists have identified about 300,000 different species of plants, which are among the most adaptable of Earth's organisms. Plants can range in size from microscopic to the largest known living things. Like other living organisms, plants need energy, nutrients, air and water. They produce offspring, are made of cells, react to their surroundings, grow and die.

Plants' characteristic green color comes from the pigment, chlorophyll, which also is found in algae (close relatives of plants). Chlorophyll enables plants to capture light energy and convert it into chemical energy

through a process called photosynthesis. Photosynthetic organisms (green plants and their relatives) are Earth's primary recycling system. During photosynthesis, leaves extract carbon dioxide gas from the atmosphere and use it to store energy that enables plants to live and grow. At the same time, plants release the oxygen that enables our atmosphere to sustain life. In addition, plants are the first link in almost all food chains, upon which all animals and other consumers depend. They also are an important source of fiber, fuels and many medicines.

Land plants include tiny mosses, ferns, pines and flowering plants. Of these, flowering plants, or Angiosperms, are most numerous, with close to 250,000 species. Angiosperms typically are made up of roots, stems, leaves and flowers. Roots anchor the plant and absorb essential nutrients and water. Stems provide support, raising leaves and flowers above the ground, and serve as conduits through which nutrients, food molecules and water travel between roots, leaves and other parts. Leaves expand a plant's green surface area to maximize the capture of solar energy. Tiny pores in leaves enable the exchange of gases, particularly oxygen and carbon dioxide, between plants and the atmosphere.

Flowers contain the reproductive parts of a plant, the anthers (produce pollen) and the pistil or carpel (contains ovules, which become seeds after fertilization). Some flowers have showy petals or fragrance, which serve to attract animal pollinators, such as

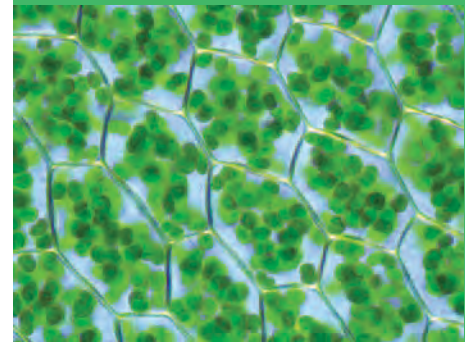
insects or birds. Plants with small, inconspicuous flowers, such as those found in grasses, typically rely on wind to carry pollen from one flower to another.

Successful pollination leads to seed formation inside the ovary or base of the pistil or pistils. After pollination, the ovary expands and becomes fleshy or hard, and begins to form the fruit. Sometimes, other flower parts become part of the fruit as well. In non-technical usage, "fruit" means a fleshy, sweet, edible seed-containing structure, such as an apple, orange, grape, etc. However, biologists consider any seed-containing plant structure to be a fruit. There are many kinds of fruits: pea pods, acorns, tomatoes and even corn kernels are just a few examples. Fruits serve important roles in seed dispersal. Some, such as coconuts, float to new environments; others, such as berries, are eaten along with their seeds, which are transported by animals to new locations.

Plants' atmospheric recycling and food production properties make them very important to planners of space missions. Voyages to the planets will require continuous replenishment of food, water and atmosphere. Plants could provide the basis for a closed, self-sustaining system that requires only the input of solar energy. ■



**A leaf is an above-ground plant organ specialized for the process of photosynthesis. The internal organization of most leaves has evolved to maximize exposure of chloroplasts to light, and to increase the absorption of carbon dioxide.** Photo © Jon Sullivan, released into the public domain on Wikipedia.



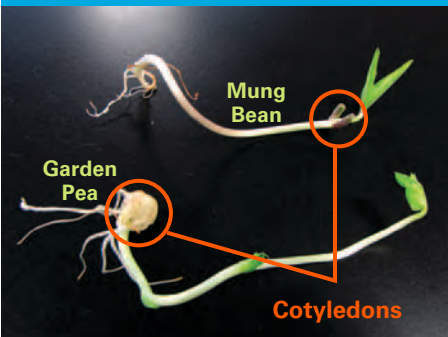
**This microscopic image of *Plagiomnium affine*, (a Many-fruited Thyme-moss), reveals its chloroplasts. Chloroplasts in plants capture radiant light energy from the sun and convert it into chemical energy. Oxygen is released as a waste product.** Photo © Kristian Peters, Wikipedia Creative Commons 3.0.

# 2



Sunflower seedlings, photographed three days after planting. Notice the initial curvature of the seedling stem, the two cotyledons emerging from the remnants of each seed coat, and the hairs on the developing primary root.

Photo © Bluemoose, Wikipedia Creative Commons 2.0.



Cotyledons may be carried above ground during germination or remain underground. The mung bean seedling (top) shows cotyledons that have been carried above ground. The green pea seedling's cotyledons remained encased in its seed coat, which acted as an underground storage organ.

Photo © Annkatrin Rose, Ph.D.

## Seeds and Germination

The seeds of flowering plants consist of a protective coat, an embryo and stored food. The embryo, which is a tiny new plant, remains dormant and protected until favorable conditions arise. One end of the embryo, the radicle, develops into the plant's root system. The other end of the embryo, called the hypocotyle, forms the initial stem and leaves. Most seeds also contain stored food to fuel development until the young plant begins to produce its own food through photosynthesis. Sometimes, the food is contained within the seed leaves or cotyledons. In other cases, the food surrounds the embryo as a starch reserve, known as endosperm.

When external conditions are satisfactory, the seed and embryo take in water. In a process called germination, the tiny new plant consumes its food reserves and begins to grow. Sometimes, germination also requires an additional environmental signal, such as light of the correct wavelengths or a series of days at a particular temperature.

During germination, the young plant sends out a single root, the radicle, to begin capturing water and serve as an anchor. Eventually, the growing radicle becomes the primary root. The primary roots of all land plants look much alike, but later development differentiates them. For example, carrots and radishes form fat taproots, consisting of the primary root with many thin, lateral branching roots. In other plants, such as grasses, the primary root is short-lived and is replaced by a new, fibrous root system that originates near the base of the stem.

Shortly after the radicle emerges, the shoot pushes through the seed coat. Often, the embryo stem curves and pushes through the soil as a hook to avoid damaging the delicate shoot tip. In some cases, the cotyledons emerge through the soil. In other cases, such as in pea plants, the cotyledons remain buried; only the new shoot tip is visible above ground.

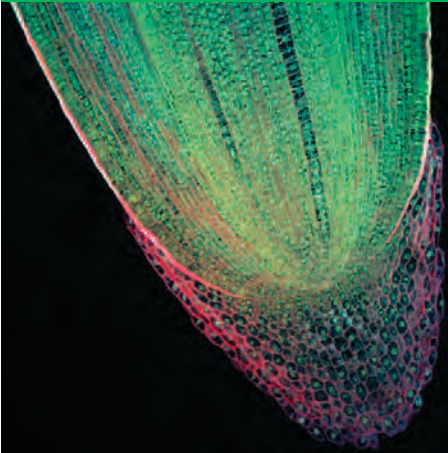


Cucumber seedling showing two oval-shaped cotyledons, a leaf (top) and emerging new leaves (center). Photo © Peter Chastain, Wikipedia Creative Commons 3.0.

The early leaves expand and begin the process of photosynthesis. The number of cotyledons present is a characteristic used to distinguish between the two major groups of flowering plants. Monocotyledonous plants ("monocots") have one seed leaf and dicotyledonous plants ("dicots") have two. Grasses are monocots. Beans and mustard plants (such as the Wisconsin Fast Plants® *Brassica rapa*, used for the Plants in Space investigation) are dicots. Similar to animal development, plant germination, growth, reproduction, and responses to the external environment are regulated by internal signaling pathways and hormones.

# 3

## A Closer Look at Roots and Stems



This microscopic photo of the tip of a maize root reveals the structure of its protective root cap. Photo by Jim Haseloff © Wellcome Images.



*Brassica rapa* seedlings shown with stems growing toward a light source. Photo by Travis Kelleher © Baylor College of Medicine.

**R**oots can do more than anchor a plant in soil and absorb water and nutrients. Thick roots, such as those of beets and carrots, are modified to store food supplies. Others, particularly those of legumes (beans, peanuts and their relatives), house bacteria that take in nitrogen from air and make it available in a different chemical form for use by the host plant.

A plant's first root, usually called the primary root, originates with the embryo. In dicots and gymnosperms (pine trees and their relatives), the primary root grows downward and forms a large taproot with lateral branches. In monocots, such as grasses, the primary root usually disappears and is replaced by a fibrous network of roots that form at the base of the stem.

Most roots grow continuously and follow the path of least resistance through the soil. The availability of oxygen (contained in spaces between soil particles), water and nutrients also influences the direction and proliferation of roots. Roots grow by adding cells at their tips. A layer of cells, collectively called the root cap, protects the rapidly dividing and expanding cells of the root tip (see image, upper left). As the root pushes its way through soil, cells on the outer surface of the root cap are sloughed off and replaced.

New and growing roots absorb water and nutrients through cellular tubes, called "root hairs," located just behind the root tip. These tiny hairs greatly increase the amount of surface area through which water and dissolved nutrients can pass into the root system.

Water and nutrients are transported efficiently throughout the rest of plant through the vascular system. Unlike vertebrate animals, which have a single closed circulatory system, plants have one network of tubules (called xylem) to transport water and mineral nutrients, and a separate set of conduits (called phloem) to carry products of photosynthesis.

Not all stems serve as plant support structures. Some stems, such as the underground tubers we call potatoes, are important for food storage. In other plants, stems are modified to facilitate climbing or twining (vines) and water storage (succulents, such as cacti).

The stems of many trees and woody shrubs are reinforced over time through the development of wood and bark. Known as secondary growth, this process enables plants to survive and grow for many years, and it leads to a gradual increase in the diameter and strength of stems, branches and roots.

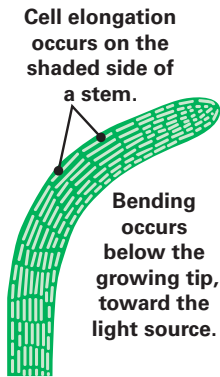
### ORIENTING STEMS AND ROOTS

Generally, leaves and stems grow upward, toward light sources, while roots grow downward. But plants do not have nervous systems or sensory organs—no eyes, ears, or vestibular system like animals have. So, how do plants "know" which way is up?

Plants sense and respond to their environments in a number of ways. Receptor molecules within plant cells perceive changes in external conditions, such as light, and initiate internal signaling pathways that enable the plant to react. Communication inside plants occurs

## PHOTOTROPISM

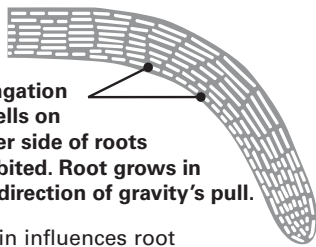
Auxins are plant hormones with important roles in plant growth and development. In light responses (phototropism), auxin causes cells on the shaded side of a stem to elongate more than cells on the lighted side, thereby bending the stem toward the light source. Illustration by M.S. Young



© Baylor College of Medicine.

## GRAVITROPISM

**Elongation of cells on lower side of roots inhibited. Root grows in the direction of gravity's pull.**



Auxin influences root orientation in response to gravity (gravitropism). If a plant is turned on its side, elongation of cells on the lower side of roots is inhibited, thereby bending the growing root downward. Illustration by M.S. Young

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through hormones, chemical substances produced in one part of the plant that have a developmental or physiological effect elsewhere in the plant. There are seven major kinds of plant hormones, and one, auxin, is primarily responsible for directional growth responses.

Light is important for plant development, including flowering and seed germination. It also is essential for photosynthesis, and can stimulate plant growth in a particular direction (toward or away from a certain wavelength of light). A plant's growth response to light is called phototropism, from the Greek words *trope* (for "turn") and *photo* (for "light"). A phototropic response involves the detection of a light wavelength by receptor molecules in plant cells, and transduction (i.e., conversion) of that signal into biochemical responses that lead to altered growth patterns.

Charles Darwin, the great evolutionary biologist, investigated grass seedlings' growth responses to blue light (about 460 nanometers in wavelength) as early as 1881. He already knew that growing plants would bend toward light coming from a single direction. However, he found that when he covered the tips of grass seedlings with a foil cap, the seedlings no longer tilted toward the light source. Normal bending occurred when he covered the seedling tips with a glass tube and when he covered the stem below the tip with an opaque collar. Darwin and his coinvestigator son, Francis, proposed that the seedlings were bending toward light in response to an "influence" that was transported down the stem from the growing tip.

In 1926, Fritz Went, a Dutch scientist, identified the chemical messenger that causes cells on the shaded side of a shoot to elongate and grow faster than cells on the lighted side, thereby bending the stem toward the light source.

He called this messenger hormone auxin. Today, synthetic auxins play important roles in agriculture as weed killers, and in preventing fruit from dropping off trees and bushes before it can be harvested.

Because stems grow toward a source of blue or white light (which, of course, contains wavelengths of light in the blue range), they are said to have a "positive" phototropic response. Conversely, roots have a weak response in the opposite direction. Because they grow away from a source of blue or white light, roots are said to have a "negative" phototropic response.

Plants also respond to red light, which can stimulate or inhibit seed germination, and sometimes has a role in the timing of flowering. These responses involve different receptor and signaling pathways than those related to phototropism. The roots of some plant species show a positive phototropic response to red light. Phototropism is an area of active investigation, with *Arabidopsis thaliana* mustard plants being studied in experiments on Earth and the International Space Station.

Gravity provides a much stronger stimulus than light does for root orientation, and also influences the direction of stem growth. If you place a plant seedling on its side in the dark, the stem still will curve upward and the roots will bend downward. This response to gravity is called gravitropism. Stems are negatively gravitropic and roots are positively gravitropic. Like phototropism, gravitropism involves auxin and different rates of cell elongation on the sides of the root or shoot. Special starch-containing structures, called amyloplasts, are believed to have a role in detecting gravity. Amyloplasts inside cells sink toward the direction of gravity's pull. ■

# 4



Wisconsin Fast Plants®, also known as a “rapid cycling *Brassica*,” are especially fast-growing, easily maintained varieties of *Brassica rapa* (field mustard, rapeseed, canola). While the species has long been cultivated for its oil, these varieties, which grow from seed to seed in just one month under constant illumination, are extensively used in schools to demonstrate flowering plant life cycles, Mendelian genetics and plant physiology. These flowers (above) were photographed on a two-week old plant. Photo © Robert A. Klips, Ph.D.

Find out more about Wisconsin Fast Plants® at [www.fastplants.org](http://www.fastplants.org). The site includes growing tips, downloadable explanations of the Fast Plants™ life cycle, and teaching resources and lessons for all grade levels.

## Plants in Space Investigation: *Brassica rapa*

The Plants in Space investigation will focus on root growth in *Brassica rapa*, a member of the crucifer, or mustard, family of plants (which also includes cabbage, turnips, and broccoli). *Brassica rapa*, also known as Wisconsin Fast Plants® or rapid cycling *Brassica*, were developed over 30 years at the University of Wisconsin. This is an ideal plant for student study, and for an investigation of plant root growth in microgravity. First, its complete life cycle, starting with germination of a seed and ending with the production of new seeds, takes approximately 30 days. Substantial root growth occurs in just a few days. Second, other than continuous light and water, little care is needed to grow these plants through a complete life cycle.

To send astronauts to distant locations in space, we must be able to grow plants to produce food and oxygen, and to process waste. The experiment onboard the International Space Station (ISS) will include 72 *Brassica rapa* plants, started 18 at a time, in a total of four planting sessions. For each session of the flight investigation, seeds will be germinated in a clear gel and allowed to grow for five days before being replaced by new seeds. The investigation will conclude after 28 days. The gel, a variant of agar, will provide moisture for seed germination and the production of roots. Plants both in microgravity and on Earth will be provided with artificial lighting (blue-enriched white light) and will be germinated in the same manner.

The primary variable in the investigation will be the effects of gravity. In space, plants will not sense the direction of gravity, and therefore, will not be impacted by gravitropism. Plants on Earth, however, will show typical gravitropic responses (roots growing in the direction of gravitational pull). What will happen to plants grown in space aboard the ISS, where the effects of gravity are greatly reduced? How will the roots grown in microgravity compare with those of the same type of seeds in normal gravity on Earth? Will the lights in the plants’ growing chambers help roots to grow and orient themselves normally?

Students and other investigators will be able to download daily images from the ISS, showing primary and secondary root growth for comparison and study. Because these images will be available permanently on the BioEd Online website ([www.bioedonline.org](http://www.bioedonline.org)), teachers and students will have the option of delaying the start of their classroom investigations until a convenient point in the school year. The investigation does not depend upon calendar-coordinated observations, or even being conducted while the plants are on ISS. In fact, it is possible to use this module at any time. As long as images of the space plants are paired with those of Earth-based plants at the same elapsed growth time, the comparison and activities will be successful. ■

# 5

## Preparing the Plant Growth Media



Germination flask containing two different densities of media, ready for delivery to NASA for transport to the International Space Station (ISS). The higher density layer of growth media used in the ISS experiment is visible about halfway down the flask.

Each flask is labeled with a serial number (S/N) and a color coded label to indicate different treatments. Gridlines on the flask are in 1/8-inch increments. Photo courtesy of BioServe Space Technologies.

The Plants in Space experiment on the International Space Station (ISS) repeats four times, with each session running seven days. Images downloaded daily from the ISS are made available for student investigators, who will be growing one or more Earth-based plants for comparison. After each seven-day session, new seeds will be started. The investigation will end after all four sessions have been completed. In total, the experiment aboard the ISS will use 72 plants, started in four groups of 18 each. Students should consider how many times they will repeat their Earth-based experiments, and how many seeds they will include in each repetition. All photographs of the experiments will be labeled by date and time, and made available on BioEd Online ([www.bioedonline.org](http://www.bioedonline.org)).

### MATERIALS

- 30-ml, flat-sided flasks (available from science supply companies) OR clear, 8-dram pill bottles, (available from pharmacies), one per plant
- Clear gelatin (available from grocery stores) OR agar agar powder or flakes (found in the Asian foods section of grocery stores, some health food stores, or online at [www.edenfoods.com](http://www.edenfoods.com), [www.amazon.com](http://www.amazon.com), etc.)
- 1,000 ml beaker
- Cooking thermometer
- Hotplate
- Oven or heat-resistant gloves
- Safety goggles
- Water

### SAFETY ISSUES

Be sure that anyone preparing or working with hot media solution wears eye protection and handles the beaker with heat-resistant gloves or oven mitts to prevent scalding of the skin. Clean work areas with disinfectant before and after any lab activity.

### PROCEDURE

Both agar agar and clear gelatin make excellent growing media for root study. When prepared, both media form clear gels that permit observation of the root formation process. Seeds inserted into these media will germinate using water locked within the gel. Neither material provides nutrients for extended plant growth, but each session of the ISS flight experiment will last only five days. Both agar agar and gelatin provide satisfactory conditions for that length of time, so there will be no need to add other nutrients.

Prepare the media according to the instructions on their packages. This will produce enough growth medium for several plants. Add agar agar flakes or gelatin powder to boiling water and allow them to dissolve completely.

The recipe can be changed to modify growth medium density (see details in the next section) to match the media used on the ISS. Half of the flasks used on the ISS will hold two layers of growth medium. If students plan to match this component of the flight experiment, some of the flasks/medicine bottles used in classroom investigations should be

**AEROPONICS.** Successful long-term missions into deep space will require crews to grow some of their own food during flight. Plants can provide fresh oxygen and clean drinking water. But this is about more than a breath of fresh air or taking a quick shower. Each ounce of food and water produced aboard a spacecraft reduces payload weight, thereby allowing the spacecraft to carry other cargo that can't be produced onboard.

Experiments conducted on the Space Shuttle and International Space Station (ISS) have grown plants in an air/mist environment that requires no soil and very little water. In this process, called aeroponics, plants are started from either cuttings or seeds, and then suspended mid-air in a growing chamber. The developing root systems grow in an enclosed, air-based environment that is regularly misted with a fine, nutrient-rich spray.



Aeroponic growing systems provide clean, efficient and rapid food production. Aeroponic crops can be planted and harvested year-round without interruption, and without contamination from soil, pesticides or residue. The clean and sterile growing environment greatly reduces the chances of spreading common plant diseases in a contained environment, such as the ISS or other spacecraft. Source: NASA. Photo courtesy of NASA and AgriHouse, Inc.

layered the same way. Add the denser medium to the flask or medicine bottle first, and pour the less dense medium on top. To limit blending between the two densities, allow the first layer to cool before adding the second. Likewise, let the second liquid cool a bit before adding.

After your agar agar or gelatin cools, cap the containers until they are needed. With careful, antiseptic handling, the media will remain usable for two to three weeks.

**Note:** Flasks on the ISS will contain a professional grade, agar-like growth medium called gellan gum, sold commercially as Phytigel™ (not to be confused with beauty products with similar names). Phytigel™ is available primarily in large quantities and is expensive, but regular agar agar and gelatin make suitable low-cost substitutes. ■

### GROWTH MEDIUM INSTRUCTIONS

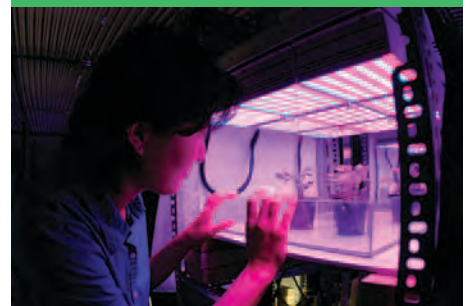
Add agar agar or gelatin mix to water that is being heated to a boil. When the water begins to boil, reduce the heat and simmer. Stir occasionally, until the flakes or powder are completely dissolved (about 5 minutes). Allow the solution to cool for a few minutes before transferring it to the flasks or pill bottles.

To create high-density agar agar, combine one level tablespoon of agar agar flakes with one cup of boiling water. For low-density agar agar, combine two level teaspoons of agar agar flakes with one cup of boiling water. For a high-density gelatin medium, combine four packets of dry gelatin with one cup of hot water. For low-density gelatin, combine two packages of dry gelatin to one cup of hot water. ■



**HYDROPONICS.** NASA researchers experiment on plants using different types of growth media. In a hydroponic plant growth chamber at NASA Kennedy Space Center (above), plant physiologist Ray Wheeler checks onions grown using a mineral nutrient solution in water instead of soil. Bibb lettuce is growing to his left and radishes are growing to his right.

Photo courtesy of NASA.



**LIGHTING.** In addition to testing different growth media, NASA scientists also conduct research to investigate different CO<sub>2</sub> concentrations and temperatures. Pictured above, Dr. Hyeon-Hye Kim checks plants in a growth chamber designed to test various light conditions. Such research will be crucial to long-term habitation of space by humans. Photo courtesy of NASA.

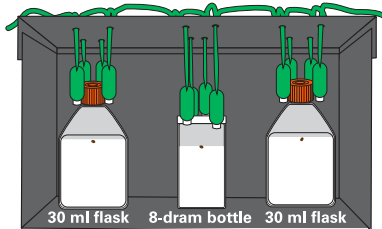
# 6

## Constructing the Experiment Chamber



Photo of flask viewed through an easy access “window” opening in a growth chamber created from a shoebox and white LED holiday lights. Photo by Travis Kelleher © Baylor College of Medicine.

### EXPERIMENT CHAMBER



Chamber interior, shown with one side of shoebox and one side of lid removed.



Closed chamber with viewing access door open. Not to scale.

Illustrations by G.L. Vogt and M.S. Young © Baylor College of Medicine.

The chamber on the International Space Station (ISS) that houses the flasks and seedlings exposes the developing plants to white light enriched with blue light (blue wavelengths range from 400–490 nm). Similar conditions can be created on Earth by keeping the flasks and plants in a container that provides an internal source of white-blue light and blocks all external light. White holiday LED lights are suitable, low-cost light sources for students’ ground-based experiment chambers. The experiment chambers themselves can be constructed of a variety of materials.

There is no single blueprint for the experiment chamber, so students should design and construct chambers according to their own plans. However, for the investigation to work, all experiment chambers must provide the following.

- Dark enclosure that blocks all outside light from reaching the plants in their growth containers. (Plants should be exposed to outside light only very briefly, and only during observation and data collection. Observations should be made in dim light or red light to minimize the effects on the outcomes of the experiment.)
- Easy access window through which to examine the plants and collect data related to the growth of roots. (Complete these steps as quickly as possible to minimize the amount of light entering the container.)
- Mechanism to hold/support four LED lights clustered near each seed container or flask.

Illustrations of a sample chamber using a shoebox and white LED holiday lights are provided to the left. (If using clear

soft drink bottles, the bottles’ exterior will need to be covered with a black box.)

### MATERIALS

#### See Setup

- Standard cardboard shoe box with separate lid
- Black tape (to hold lights in place and cover holes made for the lights)
- Knife or scissors
- White LED holiday light string (see “Safety Issues”)

### SETUP

Boxes should have a dark interior surface. If they do not, cover the interior with black construction paper or black paint.

### SAFETY ISSUES

Be sure to use LED holiday lights, not incandescent holiday bulbs. Incandescent lights produce heat that may become a fire hazard, injure the growing plants and/or soften the agar agar or gelatin. LED lights do not produce heat and are safe for the plants.

### PROCEDURE

1. Using the knife or scissors, poke 12 holes in the lid of the shoe box to accommodate 4 led lights per flask (see illustrations, left).
2. From one side of the box, cut out an easy access window, leaving a flap to close the window.
2. Insert LED lights into holes.
3. Cover shoe box with its lid until ready for planting seeds.
4. Set experiment chambers near an electrical outlet and, if possible, away from windows. ■

# 7

## Planting the *Brassica rapa* Seeds



Demonstration of how an astronaut on the ISS will insert a balsa wood strip with attached seeds into a prepared flask. Photo courtesy of BioServe Space Technologies.



Gently push the seed into the medium (about 1–2 mm). Illustration by G.L. Vogt © Baylor College of Medicine.

Once students have planned their investigations, constructed their experiment chambers and prepared germination flasks, it is time to plant seeds and begin the experiments. Wisconsin Fast Plants® germinate within 24 hours, and students should time their investigations accordingly. (For example, planting on a Monday might be advisable, unless students have access to their experiments over the weekend.)

Plant seeds do not have to be oriented in any particular direction for student-designed experiments on Earth. Due to gravitropism, roots of plants in the classroom setups will grow downward and stems will grow upward.

Orientation of the seeds is very important for the plants grown on the International Space Station (ISS), because gravity, and thus, gravitropism, will not be at work up there. Three seeds will be glued to a small strip of balsa wood (see photo, upper left). The strip will be pushed slightly into the growth medium so the seeds can begin germination. Seeds on strip A will be oriented “down.” The seeds on strip B will be oriented to the side, and on strip C, the seeds will be oriented “up.” Seeds on strip D will be oriented down. Furthermore, flask D will have two different densities of Phytogel™, with the denser layer on the bottom.

### MATERIALS

- Wisconsin Fast Plants® standard *Brassica rapa* seeds, one per flask (available from Carolina Biological Supply Company, Nasco Science or other biological supply company)
- Prepared flasks (see “Preparing the Plant Growth Media,” page 7)

- Prepared chambers (see “Constructing the Experiment Chamber,” page 9)
- Logbook
- Marker pen
- Metric ruler (mm)
- Pencil with dull tip
- Petri dish or shallow container
- Tweezers or forceps

### SAFETY ISSUES

Have students wash hands before and after any lab activity.

### PROCEDURE

1. Using a marker pen, give each seed flask its own number or other code. Record this identifying number or code in a logbook, along with the date and time the seed was planted, and the density of the growing medium in the container.
2. Open the seed packet and gently pour some of the seeds into a Petri dish or other shallow container. Avoid touching the seeds with your fingers.
2. Carefully pick up a seed with your tweezers and place it, centered and on top of the growth medium in a flask (see illustration, lower left).
3. Using the dull pencil tip, gently press the seed one or two millimeters into the growth medium.
4. Place the closed flask inside the experiment chamber. Securely cover the chamber with its lid, close the “window” flap and turn on the LED lights.
5. Begin recording observations. Make and record observations once or twice per day for five days (or more). ■

# 8



Auxin caused the roots to grow in the direction of the pull of gravity (gravitropism). Photo © University of Wisconsin Plant Teaching Collection, <http://botit.botany.wisc.edu>.



Supplementary educational materials about a variety of topics, such as Scientific Image Processing, Designing Your Investigation, and Naturalist Journals, are available for free download from [www.bioedonline.org](http://www.bioedonline.org) and [www.k8science.org](http://www.k8science.org).

## How Does Gravity Affect Root Growth?

To investigate the effects of gravity on growing plants, have students create a germination chamber from a locking-type plastic sandwich bag and a moistened paper towel. Each student should place a large seed (e.g., corn or bean) inside the sandwich bag/chamber, along with the paper towel. Then, have students seal the bag and tape it to a piece of cardboard. As the first root (radicle) begins to form, rotate the cardboard 90 degrees and observe what happens to the root growth.

### MATERIALS

#### Per student or group

- 1–2 large seeds, such as corn or bean
- Resealable sandwich bag
- Cardboard square, cut slightly larger than the sandwich bag
- One sheet of white paper toweling
- Clear tape
- Metric ruler
- Pair of scissors
- Water

### SAFETY ISSUES

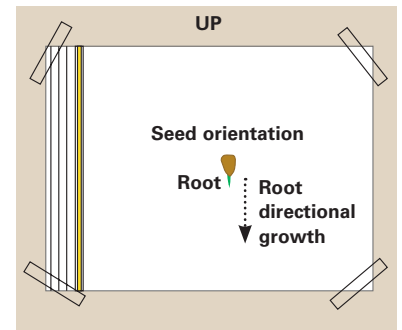
Have students wash hands before and after any lab activity. Clean work areas with disinfectant.

### PROCEDURE

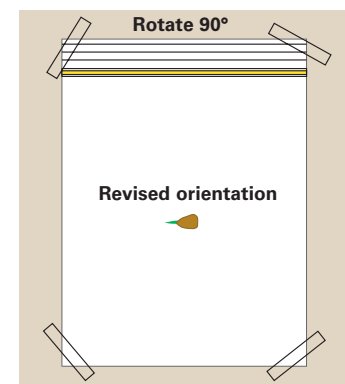
1. Fold a piece of paper towel to fit inside the sandwich bag.
2. Moisten the paper towel until it is uniformly damp. Empty any excess water from the towel and place the towel in the bag.
3. Position one or two seeds on top of and in the center of, the moistened

towel. The seeds should be visible through the bag. Seal the bag.

4. Position the bag in the center of the cardboard, and secure the corners with cellophane tape. Stretch the bag tightly to prevent sagging, and to help hold the seeds in place. Stand the cardboard upright on its side and lean it against a wall.



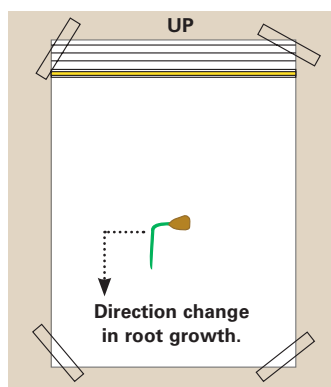
5. Observe the seed and record its appearance over the next few days.
6. When the first root has formed and grown one to two centimeters long, turn the cardboard 90 degrees, as shown below.



7. Continue observing and recording the root growth for several days.

## QUESTIONS TO DISCUSS

1. In which direction did the root begin growing?
2. What happened to the root growth when the cardboard was rotated?
3. Based on these observations, would you say that gravity affects the direction of root growth? If so, how?
4. Do you think the roots would grow in the same way on the International Space Station, where gravity's effects are not felt? What differences might there be, and why?
5. If a stem formed during your experiment, in which direction did it grow?
6. What happened to the stem when the cardboard was rotated?



## WHAT'S HAPPENING

Plants respond directly to Earth's gravitational attraction, and also to light. Stems grow upward, or away from the center of Earth, and towards light. Roots grow downward, or towards the center of Earth, and away from light. These responses to external stimuli are called tropisms. Plants' growth response to gravity is known as gravitropism; the growth response to light is phototropism. Both tropisms are controlled by plant growth hormones.

Indoleacetic acid, or auxin, is a plant

hormone that, in high concentrations, stimulates growth and elongation of cells in stems, while retarding the growth of root cells. When auxin is distributed uniformly throughout a stem, all sides of the stem grow at the same rate, thereby enabling the plant to grow toward light and away from gravity (see illustration on page 5). If the plant is tipped over on its side, auxin concentrates on the lower side of the stem, causing the cells on the lower side of the stem to elongate. This process turns the stem so that it once again grows upward, presumably toward the light.

Roots also will change direction when a plant is tipped on its side. Auxin concentrates on the lower sides of the roots and inhibits the elongation of root cells. As a result, root cells on the upper side of the root grow longer, turning the roots downward into soil and away from the light. Roots also will change direction when they encounter a dense object, such as a rock. In these cases, auxin concentrates on the lower side of the roots, enabling the roots to change direction and find a way around the rock so that normal growth can resume. ■



To find which plants are more suitable for life in a contained environment in outer space, NASA scientists experiment to learn how plants react to different kinds of lighting, carbon dioxide levels and temperature levels. Plants can provide people who live and work in space with food, a reliable source of oxygen, carbon dioxide removal, and water purification.

During a 418-day experiment at Kennedy Space Center, potato crops grown in the Biomass Production Chamber provided the equivalent of a continuous supply of oxygen for one astronaut, plus 55% of that astronaut's food requirements, and enough purified water for four astronauts, while also absorbing their expelled CO<sub>2</sub>. Photo courtesy of NASA.

# 9



An 8-dram pill bottle containing one *Brassica rapa* seed showing root growth. Photo by Travis Kelleher © Baylor College of Medicine.



ImageJ is a free scientific image processing program used by scientists to enhance and understand image data collected for analysis. The software may be used to determine the diameter of a root, angles between primary and secondary roots, and so forth. To learn more, download "Scientific Image Processing," at [www.bioedonline.org](http://www.bioedonline.org) or [www.k8science.org](http://www.k8science.org).

## How Does Microgravity Affect Plant Growth?

Scientists use clinostats to simulate the growth of plants in microgravity. A clinostat continually rotates plants through 360 degrees to eliminate a set direction for gravity. In this activity, students will construct a clinostat to investigate the effects of near microgravity on growing plants.

### MATERIALS

- Clinostat: Programmable robotic set, such as Lego® Mindstorms® NXT ([www.mindstorms.lego.com](http://www.mindstorms.lego.com)), shown upper left; or VEX® Robotics Design Systems ([www.vexrobotics.com](http://www.vexrobotics.com))
- Wisconsin Fast Plants® *Brassica rapa* seeds, one seed per bottle/flask (available from Carolina Biological Supply Company, Nasco Science or other biological supply company)
- Prepared germination bottles/flasks (See "Preparing the Plant Growth Media," page 7, and "Planting *Brassica rapa* Seeds," page 10, for details.)
  - 1,000 ml beaker
  - Clear, 8-dram pill bottles (available from pharmacies) OR 30-ml, flat-sided flasks (available from science supply companies), one per plant
  - Clear gelatin (available from grocery stores) OR agar agar powder or flakes (found in the Asian foods section of grocery stores, some health food stores or online at [edenfoods.com](http://edenfoods.com), [amazon.com](http://amazon.com), etc.)
  - Cooking thermometer
  - Hotplate
  - Logbook
  - Marker pen
  - Metric ruler (mm)
  - Oven or heat-resistant gloves

- Pencil with dull tip
- Petri dish or shallow container
- Safety goggles
- Tweezers or forceps
- Water

### SAFETY ISSUES

Be sure that anyone preparing or working with hot media solution wears eye protection and handles the beaker with heat-resistant gloves or oven mitts to prevent scalding of the skin. Clean work areas with disinfectant before and after any lab activity.

### PROCEDURE

1. Explain the purpose of a clinostat.
2. Challenge students to design their own clinostat using a classroom robotic system. The clinostat must be able to support one or more plants and rotate them automatically through 360 degrees. (See "Robotic System Computers," page 13.)
3. Use tape to mount the prepared flasks to the clinostat.
4. Start the clinostat rotating and begin plant observations. If using *Brassica rapa*, the seeds should germinate within 24 hours.

### WHAT'S HAPPENING?

Gravity's effects are nullified by the plants' rotation on the clinostat, which also prevents the hormone, auxin, from accumulating on one side of the stems or roots. As a result, the clinostat may cause stems and roots to demonstrate unusual growth behaviors. Roots may grow toward stems, and stems may grow horizontally, rather than upwards.

## CLINOSTATS

Clinostats are gravity compensation devices that subject plants to conditions that mimic microgravity. They have been used since 1879, when German botanist, Julius von Sachs, invented the clinostat to measure the effects of light and gravity on the movement of growing plants. Sachs was a contemporary of Charles Darwin and corresponded extensively with him on these issues. Darwin and his son, Francis, also used clinostats to investigate plant growth.

Clinostats are moving platforms that rotate plants to prevent their growth mechanisms from sensing the direction of gravity. The rotational speed is kept low—usually from one to four revolutions per minute (rpm)—to avoid any significant centrifugal acceleration effects on the subjects. Some clinostats are tilted; others are able to move three axes of rotation to provide complete randomization of gravity effects. Adjusting rotation speed and inclination of the rotation plane can subject plants to conditions that simulate a wide range of reduced gravity levels.

Clinostats trigger the same changes that occur when plants are in the microgravity of Earth orbit, and would occur during transit to the Moon and Mars. Microgravity impacts cellular operations, causing cellular components to move randomly within the plant cell and become more mixed within the cytoplasm. Many scientists are interested in this research topic because it opens new doors in understanding how plants will grow, develop, and behave when under different gravitational conditions.

## ALTERNATE CLINOSTAT

Classroom robot construction systems are common in many schools. If your

school does not have access to such systems, clinostats can be constructed from other materials. A 110 VAC motor with a rotation rate of one to four RPM can be used (check with an electronics store for suitable motors). The picture below shows a clinostat built with such a motor. The plant is grown in the bottom half of a plastic pill bottle. The support stand is made from plastic, but can also be made from wood.

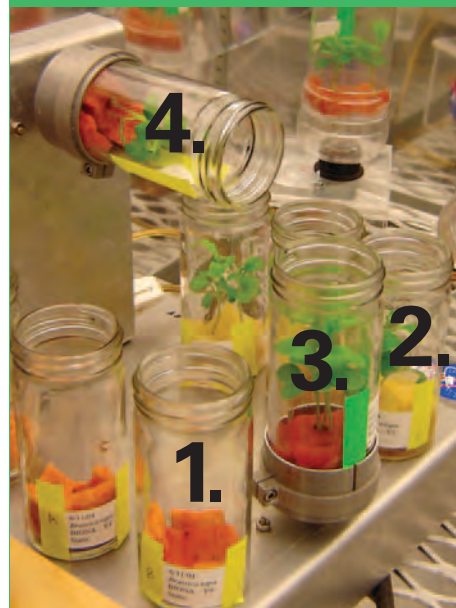
## ROBOTIC SYSTEM COMPUTERS

Depending upon the robotic system's programming capabilities, it may be necessary to use intermittent rotation. Lego® Mindstorms® NXT robot motors rotate too quickly for effective clinostat operations. However, they can be programmed to rotate through one-half turn every 30 or 60 seconds. This effectively masks the direction of gravity (see <http://mindstorms.lego.com>; or [www.ortop.org/NXT\\_Tutorial/index.html](http://www.ortop.org/NXT_Tutorial/index.html) for an online tutorial).



Clinostat made using Lego® Mindstorms® NXT robotic system. Photo by Travis Kelleher © Baylor College of Medicine.

Be sure to determine battery life. If a charge lasts for 12 hours, set up a system to switch out batteries before they are completely drained of energy. ■



Clinostats in NASA JSC's Biomass Production Chamber were used to test the effects of simulated microgravity upon the growth of *Brassica rapa*. The experiment had four variables.

1. Static control
2. Static experimental
3. Vertical experimental
4. Horizontal experimental 90° to gravity.

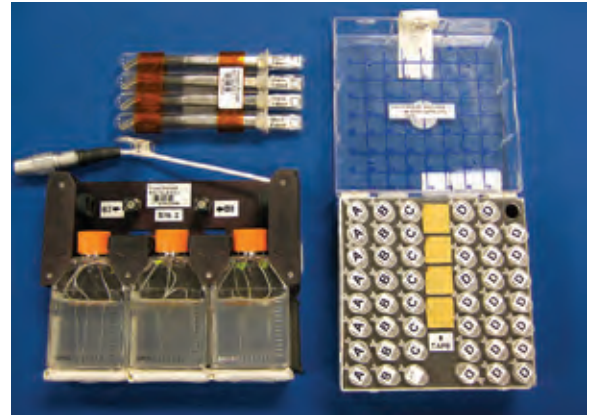
Photo by Thomas Vaughn, courtesy of NASA.

# STS-134 Protocol for *Brassica rapa*

*Brassica rapa*, or Wisconsin Fast Plants® are flowering plants that belong to the mustard family. These plants have a very quick life cycle of about a month, and in Earth's gravity environment, germination typically takes place after 1 to 2 days. By day 4, the stem will begin to experience significant growth toward the source of lighting, while the roots grow in the opposite direction to anchor the plant. Flowering of the plant takes place around day 14. Around day 35 (5 weeks), the plant begins to wilt and die.

The science objective of this mission is to examine the growth of *Brassica roots* in microgravity when grown under continuous white light (phototropism) and when the seeds are intentionally planted in different orientations.

- Preflight:** A seed box holding 27 balsa wood seed sticks mounted with *Brassica rapa* seeds (3 seeds per stick) inserted into individual seed stick tubes, two flask brackets to hold germination flasks (3 flasks per bracket), a storage box of tweezers, light barrier and stow bag insert will be assembled into flight configuration and shipped to NASA Kennedy Space Center (KSC) prior to launch.
- Five days prior to launch:** Phytigel™ (water-based medium) will be prepared and poured into seed germination flasks. The flasks will contain labels on the face, 1/8-inch gridlines and serial numbers which will either be black on clear ("light" condition) or white on clear "dark" condition) for visibility in both lighting conditions. Final assembly of the germination flasks will take place at KSC.
- Launch and Delivery:** The stow bag will be handed over 36 hours prior to launch of STS-134, for delivery and transfer of experiment components to the International Space Station (ISS).
- Aboard the ISS:** Temperature on the ISS will be about 25°C. *Brassica* growth is at its highest in a moist environment. Due to conditions on the ISS, the *Brassica* will be kept in an environment with 50% humidity.
- Lighting:** Proper lighting is crucial to this experiment. The bracket to hold the germination flasks is equipped to provide white light and infrared (IR) light to each individual flask from two separate downward angles, as well as backdrop lighting near the top of the flask. The "light" condition will utilize only the white lights. The "dark" configuration will utilize the IR lights, which are turned on only when photos are taken. Half of the plants will experience only the dark conditions. The other half will experience 24 hours of light.
- Planting:** A total of four separate plantings will occur. *Brassica* seed sticks will be inserted into flasks pre-filled with Phytigel™.
  - Installation and Seed Planting** A crewmember transfers 1 seed stick into each germination flask, inserts the flasks into the two brackets and installs both brackets and two camera modules into the CGBA Science Insert. Historical video will be captured of the planting activity for documentation.
  - Seed Planting 2–4:** A crewmember replaces the germination flasks with new seed sticks and germination flasks, and inserts flasks into the brackets.
- Measurements and observations:** Daily observations will be made of each flask during the experiment and



Sample of experiment components. Photo courtesy of BioServe Space Technologies.

results documented.

- Plant growth will be measured via units placed on the growth container at 1/8-inch increments. Images will be taken every 30 minutes during all 24 hours of each day. For each time lapse between images taken, the growth can be estimated to obtain a growth rate of both the roots and stems of the *Brassica* plant.
- As the experiment progresses, the Phytigel™ will begin to warp and decrease in volume as nutrients are consumed by the plants. Measuring the change in height of the Phytigel™ over time will help determine if this change is correlated with growth rates in the plants. Notes can also be made about whether the Phytigel™ has begun to pull away from the sides of the flask and if it is breaking up into small pieces.
- Other observations can be made, such as whether contamination has occurred within the plant flask, and the stage that the plant is currently in (germination, flowering, etc.). These observations may be key when comparing a generally accepted life cycle for *Brassica* plants with outcomes in microgravity. ■

# Teaming with Benefits

by Jeffrey P. Sutton, M.D., Ph.D., Director, National Space Biomedical Research Institute (NSBRI)

Space is a challenging environment for the human body. With long-duration missions, the physical and psychological stresses and risks to astronauts are significant. Finding answers to these health concerns is at the heart of the National Space Biomedical Research Institute's program. In turn, the Institute's research is helping to enhance medical care on Earth.



Dr. Jeffrey P. Sutton

NSBRI, a unique partnership between NASA and the academic and industrial communities, is advancing biomedical research with the goal of ensuring a safe and productive long-term human presence in space. By developing new approaches and countermeasures to prevent, minimize and reverse critical risks to health, the Institute plays an essential, enabling role for NASA. NSBRI bridges the research, technological and clinical expertise of the biomedical community with the scientific, engineering and operational expertise of NASA.

With nearly 60 science, technology and education projects, NSBRI engages investigators at leading institutions across the nation to conduct goal-directed, peer-reviewed research in a team approach. Key working relationships have been established with end users, including astronauts and flight surgeons at Johnson Space Center, NASA scientists and engineers, other federal agencies, industry and international partners. The value of these collaborations and revolutionary research advances that result from them is enormous and

unprecedented, with substantial benefits for both the space program and the American people.

Through our strategic plan, NSBRI takes a leadership role in countermeasure development and space life sciences education. The results-oriented research and development program is integrated and implemented using focused teams, with scientific and management directives that are innovative and dynamic. An active Board of Directors, External Advisory Council, Board of Scientific Counselors, User Panel, Industry Forum and Academic Consortium help guide NSBRI in achieving its goals and objectives.

It will become necessary to perform more investigations in the unique environment of space. The vision of using extended exposure to microgravity as a laboratory for discovery and exploration builds upon the legacy of NASA and our quest to push the frontier of human understanding about nature and ourselves.

NSBRI is maturing in an era of unparalleled scientific and technological advancement and opportunity. We are excited by the challenges confronting us, and by our collective ability to enhance human health and well-being in space, and on Earth. ■

## NSBRI RESEARCH AREAS

### CARDIOVASCULAR PROBLEMS

The amount of blood in the body is reduced when astronauts are in microgravity. The heart grows smaller and weaker, which makes astronauts feel dizzy and weak when they return to Earth. Heart failure and diabetes, experienced by many people on Earth, lead to similar problems.

### HUMAN FACTORS AND PERFORMANCE

Many factors can impact an astronaut's ability to work well in space or on the lunar surface. NSBRI is studying ways to improve daily living and keep crew members healthy, productive and safe during exploration missions. Efforts focus on reducing performance errors, improving nutrition, examining ways to improve sleep and scheduling of work shifts, and studying how specific types of lighting in the craft and habitat can improve alertness and performance.

### MUSCLE AND BONE LOSS

When muscles and bones do not have to work against gravity, they weaken and begin to waste away. Special exercises and other strategies to help astronauts' bones and muscles stay strong in space also may help older and bedridden people, who experience similar problems on Earth, as well as people whose work requires intense physical exertion, like firefighters and construction workers.

### NEUROBEHAVIORAL AND STRESS FACTORS

To ensure astronaut readiness for space flight, preflight prevention programs are being developed to avoid as many risks as possible to individual and

group behavioral health during flight and post flight. People on Earth can benefit from relevant assessment tests, monitoring and intervention.

### RADIATION EFFECTS AND CANCER

Exploration missions will expose astronauts to greater levels and more varied types of radiation. Radiation exposure can lead to many health problems, including acute effects such as nausea, vomiting, fatigue, skin injury and changes to white blood cell counts and the immune system. Longer-term effects include damage to the eyes, gastrointestinal system, lungs and central nervous system, and increased cancer risk. Learning how to keep astronauts safe from radiation may improve cancer treatments for people on Earth.

### SENSORIMOTOR AND BALANCE ISSUES

During their first days in space, astronauts can become dizzy and nauseous. Eventually they adjust, but once they return to Earth, they have a hard time walking and standing upright. Finding ways to counteract these effects could benefit millions of people with balance disorders.

### SMART MEDICAL SYSTEMS AND TECHNOLOGY

Since astronauts on long-duration missions will not be able to return quickly to Earth, new methods of remote medical diagnosis and treatment are necessary. These systems must be small, low-power, noninvasive and versatile. Portable medical care systems that monitor, diagnose and treat major illness and trauma during flight will have immediate benefits to medical care on Earth.

For current, in-depth information on NSBRI's cutting-edge research and innovative technologies, visit [www.nsbri.org](http://www.nsbri.org).