

Spectrum: Fluorescence Imaging on the International Space Station

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The explosion of available fluorescent probes for cell structure and function offers an unprecedented range of possibilities for the monitoring of cellular activities in real time. Indeed, fluorescence imaging has revolutionized how cell biologists approach the analysis of protein dynamics, patterns of gene expression and even the spatial and temporal dynamics of signaling processes *in situ*, in the living, functioning cell. These kinds of fluorescence measurements are also highly applicable to monitoring biological function in the spaceflight environment, such as on the International Space Station (ISS). Thus, the resolution offered by such fluorescence-based imaging systems, coupled with the non-invasive nature of their measurements present a powerful approach to following biological responses. Further, the possibilities for remote operation during the experiment and storage of the resulting imaging data for later download and analyses are well suited to the practical limitations imposed on research designs by spaceflight. A driver for wanting to make such measurements is that the ISS provides a unique laboratory where researchers can perform experiments that are impossible on Earth. For example, assessing the biological responses to the long-term exposure to reduced gravity is currently only possible during spaceflight. Yet, such analyses are providing unique insights into how gravity impacts on the biology that evolved on Earth against a constant 1 x gravity.

However, spaceflight imposes some important constraints on the engineering and operation of microscope systems. For example, the lack of convective cooling in spaceflight means that temperature control becomes a significant concern. Similarly, the limited crew time available to tend the multitude of experiments running simultaneously on orbit means automation becomes a key feature. The cumulative effect of these constraints means that a fluorescence imaging system designed to work on Earth will likely perform poorly in the space. There is a fluorescence imaging system currently available on orbit, the Light Microscopy Module (LMM). Thus, the LMM does support fluorescence imaging and indeed has allowed plant specimens expressing green fluorescent protein markers to be successfully viewed on the ISS as part of both the CARA and APEX-03 and -05 experiments (e.g., Ferl & Paul, 2016). However, this equipment was designed with physical science experiments in mind and so has a range of features that impose limitations on experimental design for biologists. These features include limited environmental control and capacity for maintaining biological samples *in situ* and a lengthy access protocol designed around safety considerations related to its primary role in monitoring explosive combustion processes in spaceflight. Therefore, the National Aeronautics and Space Administration (NASA) has developed a new fluorescence imager called Spectrum that is specifically developed to operate on the ISS and to be well suited to a range of biological and physical science-related experimentation.

Thus, the Spectrum Imager is designed to essentially fulfill the requirements of a dissecting fluorescence microscope on Earth, offering the possibility for extended, time-lapse fluorescence imaging from biological and physical specimens during spaceflight. The key capabilities that biologists are likely to take advantage of are:

- A wide range of fluorescence imaging wavelengths, allowing for multiple fluorescent probes to be imaged in a single sample.
- A controlled environmental chamber for samples, allowing sample installation and maintenance for extended (multiple day) experimental runs.
- Chamber lighting that allows photosynthetic organisms to grow within the sample chamber.

This latter capability is especially important for plant biology experimentation as it offers the unique possibility to follow plant samples from seed germination to a growing plant while continuously being imaged. However, even without the benefit of the chamber lighting, Spectrum offers potential for experiments such as continuous following of yeast and bacterial colony growth along with monitoring fluorescent reporters in these microbial cells. The attendant possibilities of assessing the responses of mutants in these organisms to spaceflight in real time as a mission proceeds opens a new realm of potential insight into dynamic adaptation of biology to the spaceflight environment.

We have monitored two classes of biological samples in ground-based studies, plants and microbes, in order to define growth and imaging parameters during short-term and extended experiments within the Spectrum Imager. For plant biologists, the combination of fluorescence imaging capabilities and being able to monitor their subject plants as they germinate and grow within Spectrum offers an exciting range of new possibilities for exploring how plants dynamically respond to spaceflight. However, as with all new growth equipment, initial characterization of effects on the biology being studied is proving critical to successful usage on orbit. At a hardware level, issues such as the development of condensation on the faces of the plates being imaged has had to be overcome by imposing thermal gradients within the equipment. At the biological level, information such as how fast plants grow in Spectrum (which impacts on time course analyses), how healthy the seedlings are, whether they exhibit any stress responses and especially assessing the unit's fluorescence sensitivity and resolution have proven critical in defining its possible uses on orbit.

References

Ferl RJ, Paul AL. 2016. The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. *NPJ Microgravity* **2**: 15023.