

AUTONOMOUS DAYLIGHT DETECTION OF LIFE BY FLUORESCENCE IMAGING. S. Weinstein¹ and D. Pane¹, L.A. Ernst¹, E. Minkley¹, F. Lanni¹, D.S. Wettergreen², M. Wagner², S. Heys², J. Teza², A.S. Waggoner¹.
¹Molecular Biosensor and Imaging Center, Carnegie Mellon University, 4400 5th Ave, Pittsburgh, PA 15213;
²Robotics Institute, Carnegie Mellon University, Pittsburgh, PA 15213

Introduction: An integrated fluorescence imaging system was used to detect biomarkers from extant microbial colonies and biofilms during autonomous rover exploration. Intrinsic fluorescence arises from naturally occurring fluorophores, such as chlorophyll and phycobiliproteins of cyanobacteria and algae. Other biomarkers were visualized by applied fluorogenic probes which produce different color fluorescence signals upon association with extra-cellular polysaccharides (EPS), proteins, nucleic acids and lipids.

A serious issue confronting imaging biological fluorescence has been the high levels of background light levels. In sunny conditions, the background can reach three to four orders of magnitude greater than the levels of the fluorescence emitted from the target. We have overcome this limitation using an imager with a high-powered flashlamp as the light source synchronized with a gated CCD camera. This system provides greatly enhanced fluorescence signal over background and daylight fluorescence imaging has been accomplished with just the shading of the rover itself. The integration of multiple flashes has further increased the quality of the image.

The Biomarker Detection Imager: The imaging system employs a Perkin Elmer high-intensity Xenon flash lamp and a Roper Cool SNAP cooled CCD camera. Six optical interference filters can be automatically switched into the excitation flash path and another set of 10 emission filters are can be rotated in front of the imaging camera. The FI has a field-of-view of 10 cm X 10 cm, with a pixel resolution of 100 microns. The light from the flashlamp is split by a fiber bundle into four beams to provide more uniform illumination at the targeted surface. The camera is gated to acquire a fluorescence image for a 20 microsecond period during the flash. This 20 microsecond flash reduces sunlight exposure to the camera by a factor of over 10,000. In the flash mode two sequential images are obtained, the first with out a flash containing the ambient sunlight picked up by the camera and the second, obtained during the flash, containing the ambient reflected sunlight imaged plus the fluorescence image due to flash excitation. For highest sensitivity we obtain 50 images using this sequence. After subtraction, the fluorescence signal image becomes isolated from sunlight contributions. Quality RGB images can also be acquired for context

for the fluorescence images and also for geologic study.

The enhancement of intrinsic fluorescence by water is determined by imaging the chlorophyll fluorescence before and after automated spraying of the targeted region. Other biomarker signals are imaged before and after application of the fluorogenic probes to maximize detection sensitivity. The image sets are normalized for intensity and background to optimize feature detection. Algorithms included in the "Science on the fly" application have been used with these images.