Fluorescence Imager Performance

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Alan Waggoner, Shmuel Weinstein
MBIC, Carnegie Mellon University
Objective

Deploy an imager that detects fluorescence signals from sparse microorganisms and biofilms during autonomous rover exploration in daylight:

- naturally occurring chromophores, such as chlorophyll of cyanobacteria and lichens;
- fluorescent probes applied to soil and rocks;
- also, quality RGB of field-of-view.
Outline

- The 2004 Embodiment of the Fluorescence Imager (FI)
- Operation and Control of the FI on Zoe
- Fluorescent Dyes
- Mineral fluorescence
- Conclusion
Acknowledgments

The men who built it......

Stu Hayes
Chris Williams
Jim Teza
Alan Luders
Francisco Calderon
Dave Pane
Mike Wagner
The 2004 Embodiment of the FI

Schematic without Housing

Flashlamp

Fiber bundle light delivery (1 of 4)

6 Position Excitation filter wheel

10 Position Emission filter wheel

Camera

Side View

Front View
The 2004 Embodiment of the FI

Mounted on Zoe as Seen from Beneath (stowed position)
The 2004 Embodiment of the FI

Specifications

• Roper CoolSNAP cooled CCD camera, 12-bit, 1392 x 1040 array (used center 1024 x 1024 array), 6.45 μm pixels;
• Schneider Xenoplan 1.4/17 lens;
• FOV 10 cm x 10 cm, feature resolution 180 μm;
• PerkinElmer FX4400 flashlamp, 10 μsec/flash, 1 J/flash
• Housing size 21 cm x 24 cm x 45 cm,
• Power budget: camera 110 W, flash 35 W, controller 8.6 W, x-axis motor 10 W, z-axis motor 10 W, z-axis break 6 W, filter steppers 4 W. **Most power demand is during flash periods 153.6 W**
## The 2004 Embodiment of the FI

### Specifications

<table>
<thead>
<tr>
<th>Position</th>
<th>Wheel</th>
<th>Excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>ND2</td>
<td>-blank-</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>350/50 (325 nm – 375 nm)</td>
<td>R 630/60 (600 nm – 660 nm)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>450/50 (425 nm – 475 nm)</td>
<td>G 535/40 (515 nm – 555 nm)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>545/30 (530 nm – 560 nm)</td>
<td>B 470/40 (450 nm – 490 nm)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>665/45 (643 nm – 688 nm)</td>
<td>740/140 (670 nm – 810 nm)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>ND1</td>
<td>460/50 (435 nm – 485 nm)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>--</td>
<td>510/50 (485 nm – 535 nm)</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>--</td>
<td>620/60 (590 nm – 650 nm)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>--</td>
<td>825/50 (800 nm – 850 nm)</td>
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<tr>
<td>9</td>
<td></td>
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<td>-blank-</td>
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</tbody>
</table>
Operation and Control of the FI on Zoe

Extent of Movement of FI: X: 67.75 cm; Z: 25.5 cm; Y: Zoe travel.

Features:
• Auto-focus,
• Auto-exposure,
• Every RGB Image acquired with flash,
• Fluorescence images acquired by flash- no flash subtraction,
• Integration with a series of flash exposures,
• Flexibility of excitation and emission filter combinations.

Imaging protocol: RGB, chlorophyll (=blue excitation), chlorophyll check (=green excitation), [water spray], RGB, chlorophyll, chlorophyll check, [vinegar spray], RGB, chlorophyll, chlorophyll check, DNA, Protein, [fluorescent probes spray], DNA, Protein.
Fluorescent Imager Control

Fluorescent Imager Software Diagram

- Planner
- Interprocess Communication (IPC)
- Instrument Manager
- Interprocess Communication (IPC)
- Fluorescent Imager Controller
- Serial Interface to motion controller
- PVCam Interface to Photometrics
- CoolSnapHQ, Cooled CCD Camera
Operation and Control of the F1 on Zoe

Some of the actual configurations in 2004:

• Every fluorescent image consisted of 50 20μsec background subtracted sequences for a total of 1 min per image
• Auto-exposure on red emission filter
• Manual spraying of dyes and preps
• 3 position row image acquisition request performed in sequence rather than parallel.

Considerations:
- Spray overlap from sprayer and wind
- Inability to reposition imager precisely
- Time needed to complete the request
Operation and Control of the FI on Zoe

Results from the 2004 Field Operations

- Quality and high resolution RGB and fluorescent images
- Transitioned from “stick and rudder” to Instrument Manager control
- Reduced the data volume for the science team (scaled, later sub-sampled)
- Reliable auto-focus (over 95% samples in focus)
- Improved reliability throughout field season
- Power consumption reduction achieved by controlled shut-off during traverse and then during incubation time
Operation and Control of the FI on Zoe

Results from the 2004 Field Operations

Issues in the field:

- Filter position reliability.
- Sun interferes with early and late day imaging (resulting in image overflow)
- RGB color calibration not perfect
- Imager lockup – achieved some fault recovery but need more
- Movement during acquisition due to: a) rover creep b) wind
- Failed auto-focus when rover wheels higher than imaged area.
Operation and Control of the Fl on Zoe

Issues for the 2005 Field Season

• Error detection (focus, filter, imager lockup) and correction
• Focus position reproducibility and stage logging
• Compressing the volume of Fl Image data
• Immediate field team analysis - Can this improve ground truth?
• Improve RGB image to precisely represents the sample area color
• Improve reliability and speed of auto-focus
• Automated spray application and status
• Software for improved science team analysis
• continue automatic control of imager power on/of
# Fluorescent Dyes

## Dye Selection and Characterization

<table>
<thead>
<tr>
<th>Biomarkers:</th>
<th>DNA</th>
<th>proteins</th>
<th>lipids</th>
<th>carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell location:</td>
<td>inside</td>
<td>inside/surface</td>
<td>surface</td>
<td>EPS</td>
</tr>
<tr>
<td>Dyes:</td>
<td>Syto B/C</td>
<td>Sypro Red</td>
<td>PGH 1</td>
<td>Calcofluor</td>
</tr>
<tr>
<td>Exc/Em:</td>
<td>blue/green</td>
<td>green/red</td>
<td>red/NIR</td>
<td>UV/blue</td>
</tr>
<tr>
<td>Δ Fluor:</td>
<td>&gt;1000</td>
<td>&gt;100</td>
<td>&gt;50</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>
Fluorescent Dyes

Fluorescence Spectra for Four Dyes

Fluor. Intensity

Wavelength (nm)
The Dyes Used in the Field

DNA dye, Syto BC, absorption curve (Blue)
DNA dye, Syto BC, emission curve (Green)

Protein dye, Sypro Red, absorption curve (Orange)
Protein dye, Sypro Red, emission curve (Red)

Bacterial Excitation Filter
DNA Emission Filter
Green Excitation Filter
Protein Emission Filter
Chlorophyll Emission Filter
Fluorescent Dyes

Results from the 2004 Field Operations

• Two out of four dyes implemented in the field
• Dyes appeared to work in soil bacteria
• Dyes had difficulties in penetrating lichens
# Fluorescent Dyes

## Issues to Resolve

<table>
<thead>
<tr>
<th><strong>Challenge</strong></th>
<th><strong>Approach</strong></th>
</tr>
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<tbody>
<tr>
<td>Dye accessibility to biomarker</td>
<td>Treat target with dilute acetic acid (bio-compatibility, no sample manipulation)</td>
</tr>
<tr>
<td>Solubility of dyes for spraying</td>
<td>Use Pluronic non-ionic surfactant (adjust structures, no effect on action)</td>
</tr>
<tr>
<td>Sensitivity of detecting biota</td>
<td>SytoB/C shows ~100 bacteria/ 1mm spot (optimize [dye], spray volume &amp; time)</td>
</tr>
<tr>
<td>Improved EPS detection</td>
<td>Identify alternative fluorescent indicators</td>
</tr>
<tr>
<td>Mineral background signal</td>
<td>Alcian Blue analog (red / far red)</td>
</tr>
<tr>
<td></td>
<td>Boronic acid derivatives (variations in EPS of biofilms &amp; lichens)</td>
</tr>
<tr>
<td></td>
<td>Survey rock samples with imager/ filters (to what extent is this a problem?)</td>
</tr>
</tbody>
</table>
Mineral Fluorescence

Some Ideas on How to Deal with the Issue
Four fluorescent mineral
Four fluorescent minerals
Profile of excitation flash
Intensity profiles – Flash, rock, reflector
Enhancement bio-fluor / mineral lum

10 microsecond signal acquisition

1000 microsecond signal acquisition

\( \tau \) lum decay

Signal

time
Enhancement bio-fluor / mineral lum

Total signal = \( N \times \varepsilon \times \phi \times \text{Integral} \left( e^{-t/\tau} \right) \)

Integrate from \( t = 0 \) to end of acquisition
Reduction of mineral fluorescence

![Graph showing the reduction of mineral fluorescence over time and integration time.](image)
Tough trip!
The Atacama barber
Conclusion

• The FI performed remarkably well, delivering quality RGB and fluorescence images
• Both chlorophyll and fluorescent probes were detected
• Dyes showed some successes, and work is ongoing
• Improvements on the way