Ocean World Microhabitats 101

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SEM of a diatom from sediments found inside the Greenland Ice Sheet, Kangerlussuaq, Greenland.

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The search for life in the Ocean Worlds

What is the spatial distribution of life in the Deep Ice habitats of Ocean Worlds? How do we look for it?
Deep Ice in the Solar System

Ocean Worlds have very deep ice; the largest have ice convection

(All figures show log scale from surface)

Rocky bodies

- Earth: 2-3 km
- Mars: 2-3 km

Ocean Worlds

- Enceladus: 15-30 km
- Europa: 15-30 km
- Titan: 100 km
Life in ice on Earth

Microbes in liquid micropockets

Microbes from arctic sea ice in grain boundaries
Life in Deep Ice

*Herminiimonas glaciei UMB49* isolated from 3 km beneath the Greenland ice sheet GISP2 ice core, (264 K, 30 Mpa) 120,000 year old ice

Image credit (above): Reto Stöckli, NASA GSFC (via NASA Earth Observatory)

Deep Ice will be the first habitable environment encountered in Ocean World exploration.
Europa Deep Ice habitability

P, T conditions similar to 3 km beneath Greenland ice sheet

“Is it habitable?” → “Is it inhabited?”

Europa (side view)

Planococcus halocryophilis Or1
Growth at 258 K (-15 C), 0.1 MPa
Mykytczuk et al., ISME 7 (2013) 1211-1226.

Psychromonas kaikoae JT7304
Optimum growth 283 K, 50 MPa

Herminiimonas glaciei UMB49
GISP2, 3 km deep (264 K, 30 MPa)
Instrument testing and microhabitat “discovery”

Summit Station, Greenland Expedition
July, 2019
Drill-instrument field test to 100 m depth
Instrument testing to 107 m depth

Cross section of glacial drill

Drill power train

DUV instrument

Spectral map

microbe

Malaska et al., Astrobiology 20 (2020), 1185-1211 (open-access)
What we expected:
Organics in layers

What we saw:
Organics in spots

Image of GISP2 core near 1.837 km

PSTAR_WATSON fluorescence map at 93.8 m
Malaska et al., Astrobiology 20 (2020), 1185-1211 (open-access).
Microhabitats are chemically unique

Different colors mean different organic chromophores

Different colored spots, but same color across spot
→ Spots are uniform, but diverse

→ Each microenvironment is a tiny world

Malaska et al., in prep.

RGB fluorescence [412.9, 385.3, 313.7 nm]
Microhabitats from diverse analog environments

WAIS Antarctic ice core WDC06A, 455 m depth

DUV fluorescence (Rohde PhD thesis., 2004)

Lake Fryxyll, Antarctica top ice

Laser induced fluorescence (Sattler et al., 2010)

Deep lake sediments

Mass-spectral imaging (MSI) map (ratio of fatty acid types) (Obreht et al., 2000)

Microbial mat

Silver impression

$^{32}$S nano-SIMs (Fike et al., 2008)
Analog Ocean World environments with microhabitats

Deposition firn Summit
*Deep UV fluorescence*
(Rohde thesis, 2010; Malaska et al. unpublished)

Deposition glacial
WAIS, GISP2, Summit
*Deep UV fluorescence*
(Rohde thesis, 2010 Malaska et al., 2020, and unpublished)

Fumarole
*UV fluorescence-BONCAT/SEM*
(Marlow et al., 2020)

Microbial mat
*Nano-SIMS MS $^{32}\text{S} \text{ ratio}*
(Fike et al., 2009)

Deep ice lens
Lake Fryxell
*Laser fluorescence*
(Stattler et al., 2010)

Deep ice lens sediments
Refrozen supraglacial melt pond
*Deep UV fluorescence*
(Malaska et al., unpublished)

Sea ice
*UV fluorescence-DAPI/micro*
(Junge et al. 2001, 2004)

Bottom sediments
Ice age lake sediments
*Nano-SIMS MS $^{13}\text{C} \text{ ratio}*
(Obreht et al., 2020)
Organic hotspot lessons

Things clump into spots: ca. 0.1 mm to 1 mm across

Individual spots are uniform across spot

Spots can be diverse from each other

Observed in many Earth environments
How microhabitats form
Pure water ice freezes out - pushes impurities into liquid channels

Unfrozen liquids with concentrated salts, high acid
Lower freezing point
Can stay liquid longer
Liquid environment for microbes!!!

Nutrients concentrate in microhabitats during freezout

Data from GISP2 ice core at 146 m depth
Salts in GISP2 come from snow deposition

<table>
<thead>
<tr>
<th>ion</th>
<th>bulk</th>
<th>vein</th>
<th>Conc factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate</td>
<td>0.26 uM</td>
<td>101 mM</td>
<td>200,000</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.89 uM</td>
<td>53.6 mM</td>
<td>400,000</td>
</tr>
</tbody>
</table>

Huge increase in local ion concentrations in ice veins
Start point $\rightarrow$ liquid eutectic
Chemically concentrated microenvironments
10 – 100 uL volume per L of bulk ice volume (1 ppm)

Cell size matters
Small solids stay out of growing ice

Small clastic insoluble solids do not get stuck in ice.

Small grains pushed into veins; Big solids are stuck in ice
It is good to be small (< 5 microns)!

Microbes in ice micropockets will be small

Comparison of cell sizes

Deep Ice microbes are ultrasmall

Eukaryotes

Red blood cell (8 μm)

Bacteria

Escherichia coli (1.5 μm)

Staphylococcus aureus (0.9 μm)

Mycoplasma genitalium (0.4 μm)

Deep ice microbes (0.2 μm)

Image of microbe from GISP2 ice core 3 km deep
Miteva and Benchley, Appl and Env. Microbiology 71 (2005) 7806-7818.
Greenland Deep Ice microbes are ultrasmall

From filtration and culture of 3 km deep Greenland ice (GISP2)

There are over a billion times more microhabitats in the Greenland ice sheet than there are stars in the Milky Way.

Potentially more than 10x more ice microhabitats in Titan’s Deep Ice than stars in the Universe.
Targeting microhabitats for astrobiology

Microhabitat characteristics
Technology and techniques to sample
Microhabitat targeting workflow

Detect → acquire → analyze

- Image acquisition (automatic)
- Target analysis selection
- Analytical results for decision making
- Data transmission
- Sample analysis
- Fine Targeting
- Sample acquisition
- Sample shuttling
- Sample ingestion
First step: Microhabitat Detection

Fluorescence is a good technique for spot detection.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pro: sensitive</th>
<th>Pro: Deep molecular information</th>
<th>Pro: Morphology of target and substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep UV fluorescence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laser induced fluorescence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro-epifluorescence BONCAT DAPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NanoSIMS (silver transfer)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanning electron microscope SEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+ epifluorescence?)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Con: Added stains give info, but may be too Earth-specific
Con: technically complicated, requires atomic flat surface (development difficult)
Con: technically complicated, non-specific to biology may not be “easily” flight ready
Example size class images: ROI, micropocket, cells

ROI
300 microns – 10 mm

micropocket
10 – 300 microns

cells
<< 10 microns
Measured widths show three size classes: ROI, micropockets, and microbial cells.
“individual” (single individual)  
“houses” (many individuals together)  
“villages” (many houses together)

Bacterial cells  
Micropocket with cells  
ROI of connected micropockets
Excision diameter

How do we drill out a target sample?

Try to get a pure undiluted concentrated sample? or try to get maximal amount sample? or something in between?

Magenta circle is excision diameter

<table>
<thead>
<tr>
<th>options</th>
<th>Maximal pure</th>
<th>Some of it</th>
<th>Most of it</th>
<th>All of it</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% purity</td>
<td>90% purity</td>
<td>50% purity</td>
<td>30% purity</td>
</tr>
<tr>
<td></td>
<td>20% of avail amt.</td>
<td>30% of avail amt.</td>
<td>90% of avail amt.</td>
<td>100% of avail amt.</td>
</tr>
</tbody>
</table>

Green is stuff we want.
Excision diameter not same as feature diameter

Excision diameter is diameter needed to melt drill and acquire target

ROIs:  500 – 10,000 microns  
(0.5 – 10 mm)

micropockets:  20 – 300 microns

Single cell extraction:  2 – 30 micron diameter  
(melt needle?)

Image and excision circle not to scale

Precision targeting at micron scale will be an engineering challenge
Example: Excision sample targeting of 93.8 m ice core map
Excision diameter is 1.3 mm diameter circle

RGB fluorescence
[412.9, 385.3, 313.7 nm]

Excision cleanly acquires ROIs with different spectral characteristics
Selecting excision depth: How deep do we extract?
If too deep, risk dilution and cross-contamination with other spots

Excision depth to average half feature diameter
Excision depth to average full feature diameter
Excision depth to set column depth in target material
For ROI, maximum is <1 cm

Variable extraction depth will enable higher effective concentrations
**Microtargeting enables high concentrations**

Precision targeting give lower collected volumes – less dilution …but same amount of material → higher concentration for instruments!

<table>
<thead>
<tr>
<th>Excision volume (1 cm core)</th>
<th>Effective cell concentration [estimated cells per cm$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROIs</td>
<td>2 – 1000 microliters</td>
</tr>
<tr>
<td>micropockets</td>
<td>1 nanoliter – 1 microliter</td>
</tr>
<tr>
<td>cells</td>
<td>&lt; 1E-2 nanoliter</td>
</tr>
</tbody>
</table>

Concentrations can be 100x higher than older bulk melt sampling [1]

State-of-the art detection of microbes in ice is ca. 1E2 cells per cm$^3$

Need for micro- and nanoliter sample handling

New technologies needed for new scales (gray zone)

Small volume sample handling:

Single microliter to nanoliter manipulation

Plot courtesy Aaron Noell
Instrument needs
A new scale for planetary instrumentation

Detection – what is needed to identify targets?

<table>
<thead>
<tr>
<th>Feature type</th>
<th>FOV [mm²]</th>
<th>Pixel scale [microns per pixel]</th>
<th>Technique type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROIs</td>
<td>1 – 1000</td>
<td>100-500</td>
<td>DUV fluorescence, MALDI-TOF</td>
</tr>
<tr>
<td>micropockets</td>
<td>1E-4 – 1</td>
<td>0.5 – 20</td>
<td>Oil-immersion microscopy, Nano-SIMS, SEM</td>
</tr>
<tr>
<td>cells</td>
<td>1E-4 – 0.1</td>
<td>0.1 – 1</td>
<td>Epifluorescence (DAPI, BONCAT)</td>
</tr>
</tbody>
</table>

Acquisition – what do we need to acquire and analyze?

<table>
<thead>
<tr>
<th>Feature type</th>
<th>Excision diameter range [microns]</th>
<th>Excision cell counts range [cells]</th>
<th>Excision volumes range [microliters]</th>
<th>Excision effective cell concentration range [cells per cm³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROIs</td>
<td>500-10,000</td>
<td>100-20,000</td>
<td>2-1000</td>
<td>1E4-5E5</td>
</tr>
<tr>
<td>micropockets</td>
<td>20-300</td>
<td>0.1-20</td>
<td>1E-3 – 1</td>
<td>5E2-5E6</td>
</tr>
<tr>
<td>cells</td>
<td>2-30</td>
<td>1</td>
<td>1E-5-1E-2</td>
<td>1E5-5E7</td>
</tr>
</tbody>
</table>

Malaska, M.J., Carpenter, K., Hofmann, A.; Noell, A. “Targeting microhabitats for Ocean Worlds, manuscript in preparation for submission to Planetary Science Journal
Microhabitats summary
A new scale for planetary exploration

Life likes to clump

Deep Ice microenvironments will be first habitat we explore in the Ocean Worlds

Spatial distribution of ice microhabitats variable: ROIs, micropockets, cells

Significant advantage to target microenvironments

Adapt new techniques and instrumentation to small scale