Jet Propulsion Laboratory
California Institute of Technology

Ocean World Microhabitats 101

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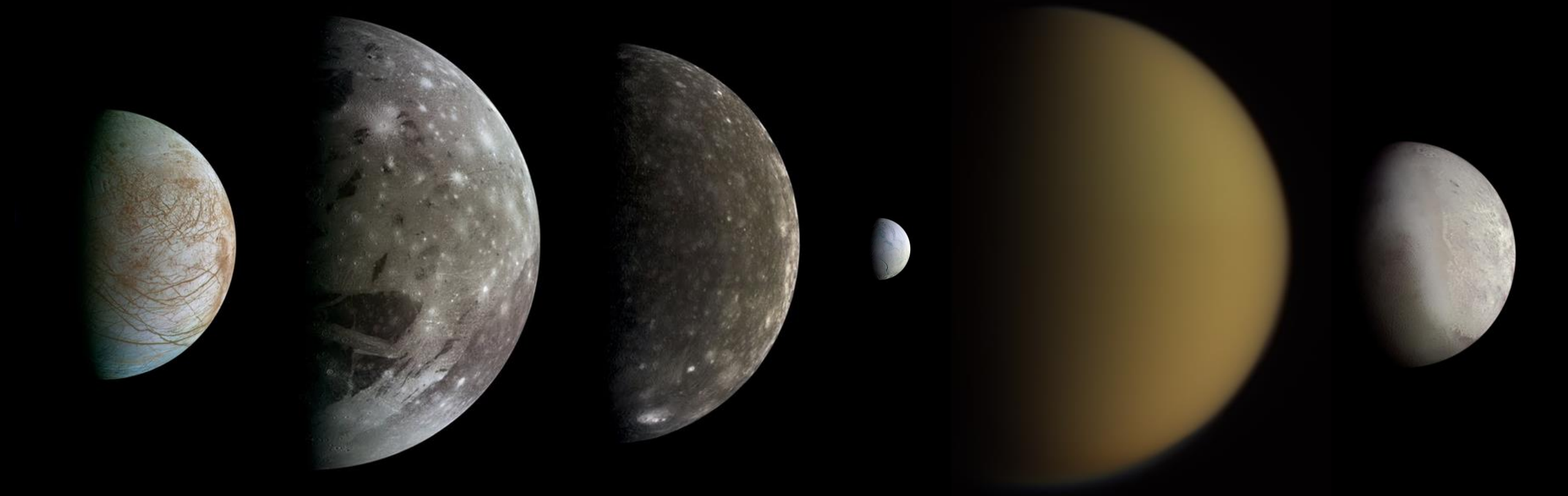
SEM of a diatom from sediments found inside the Greenland Ice Sheet, Kangerlussuaq, Greenland.

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?

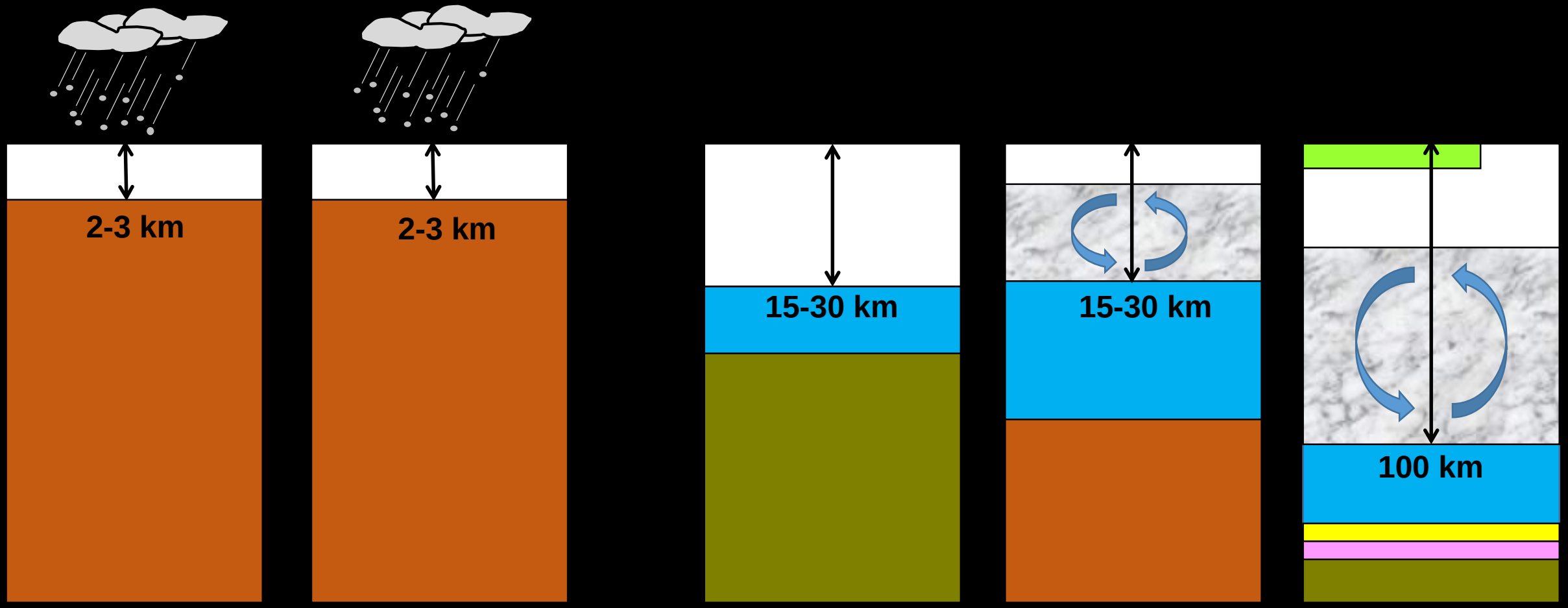


Original montage by Michelle Bannister, modified by Mike Malaska

Deep Ice in the Solar System

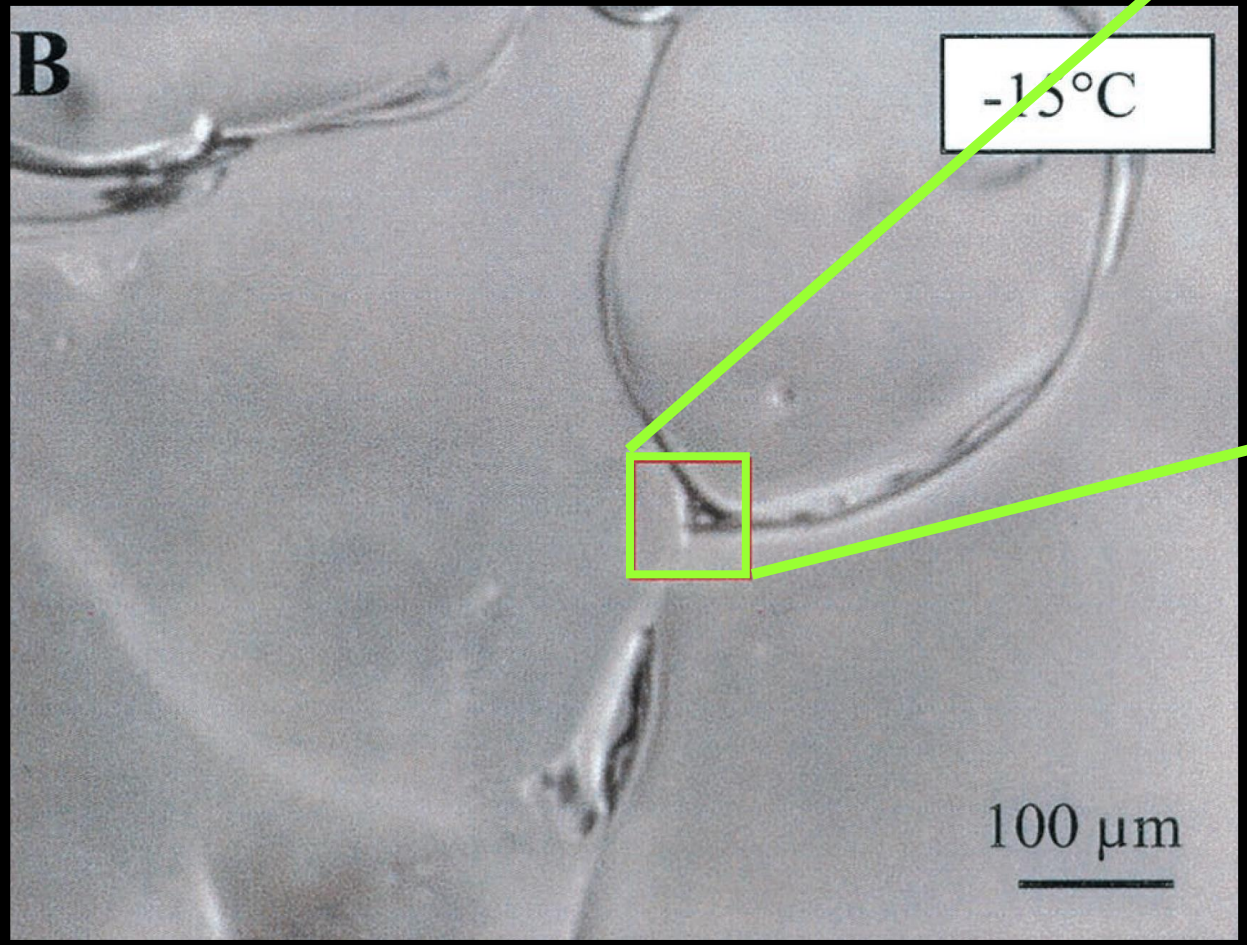
Ocean Worlds have very deep ice; the largest have ice convection

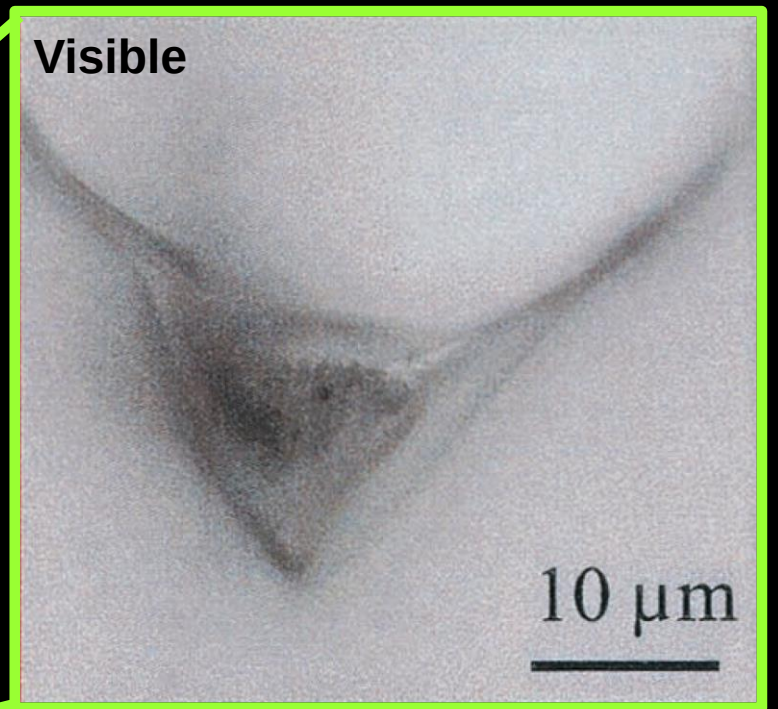
(All figures show log scale from surface)

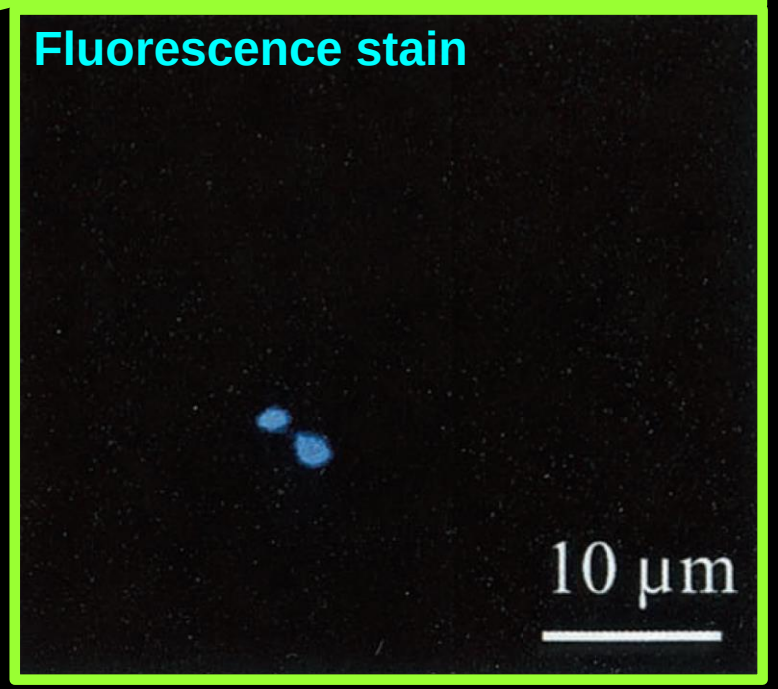


Life in ice on Earth

Microbes in liquid micropockets

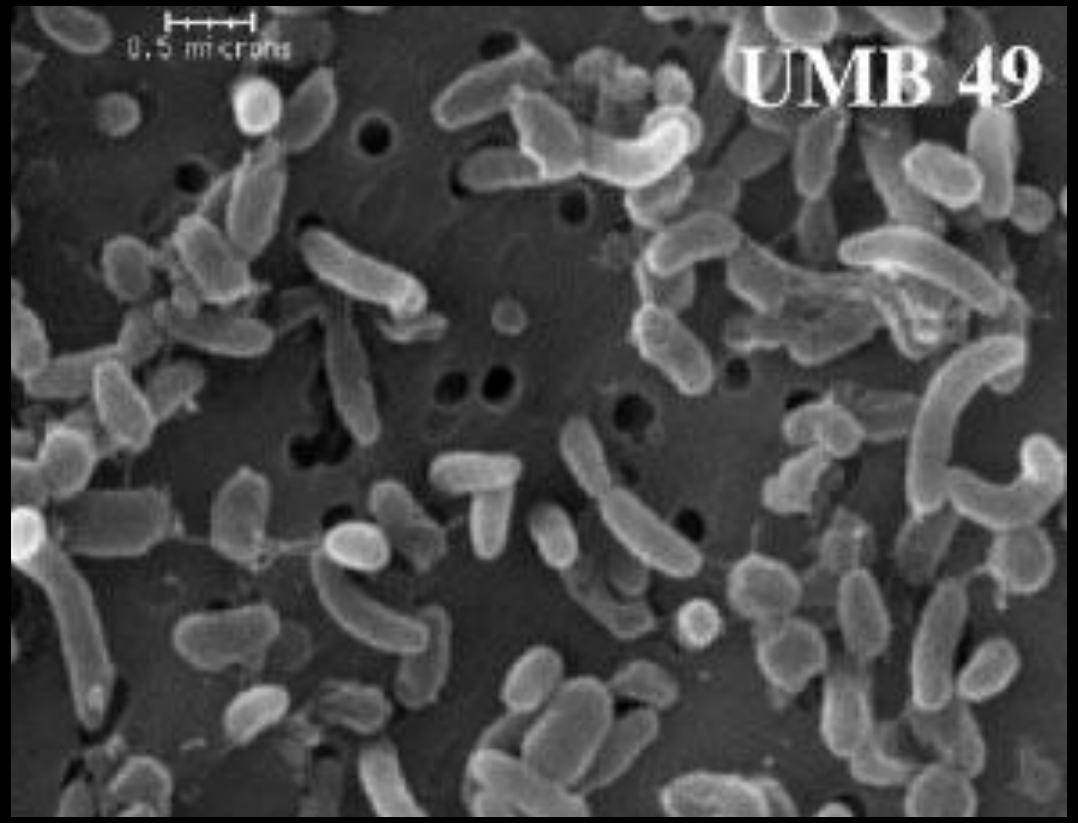






Microbes from arctic sea ice in grain boundaries
Junge et al., Appl. Env. Microbiol 70 (2004), 550-557.

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**

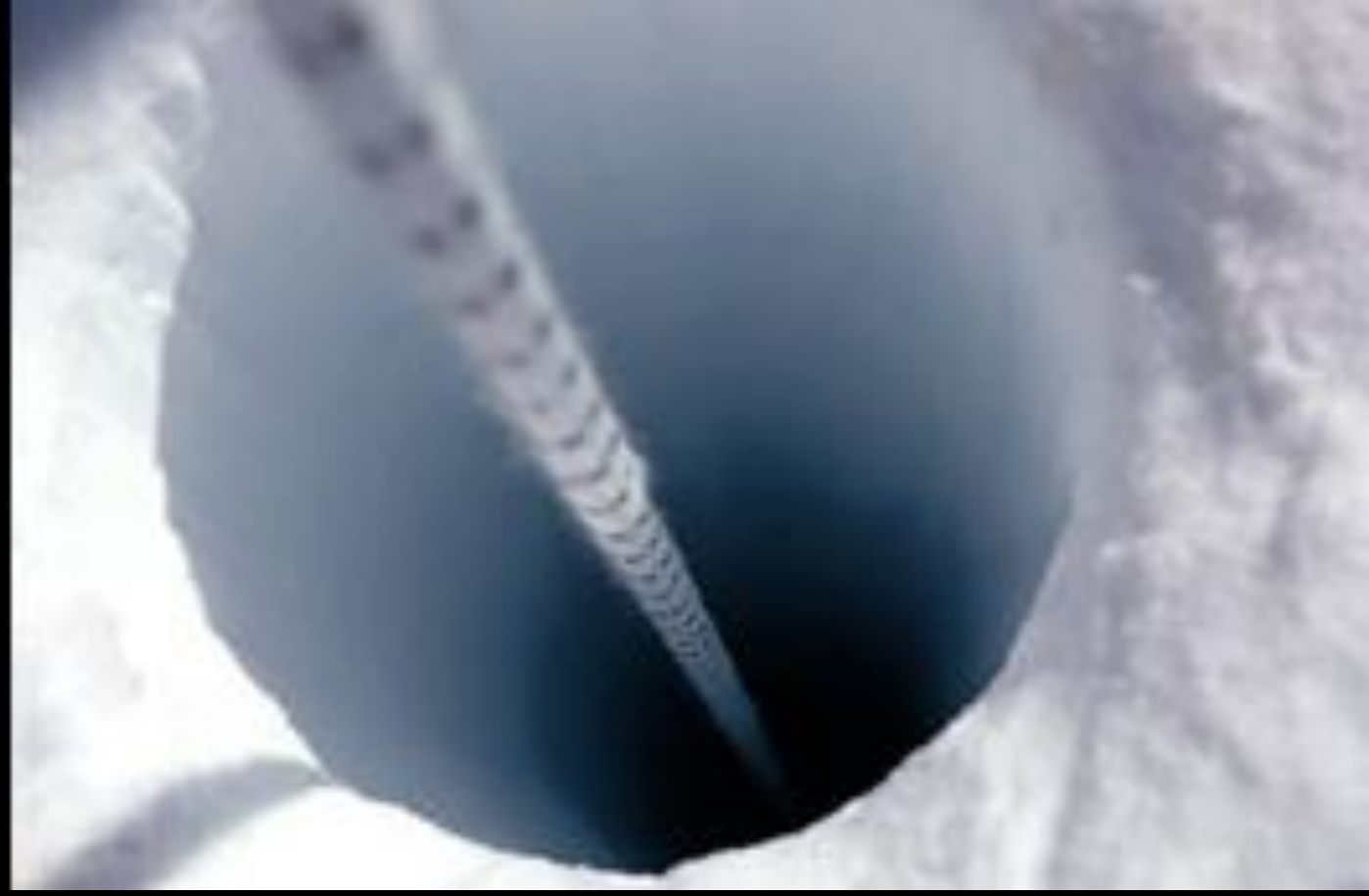


Image credit (above): Reto Stöckli, NASA GSFC (via NASA Earth Observatory)

Reference and left image credit:
Miteva and Benchley (2005). "Detection and isolation of ultrasmall micro-organisms from a 120,000-year-old Greenland glacier ice core". ***App. Env. Microbio. 71,*** **7806-7818.**

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

Europa Drill Concept

Convective ice

Potential deep ice habitat

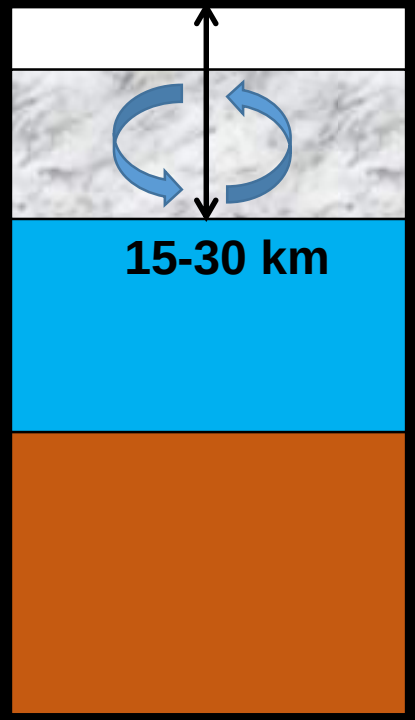
Rock-ocean interface?

Potential subsurface ocean habitat

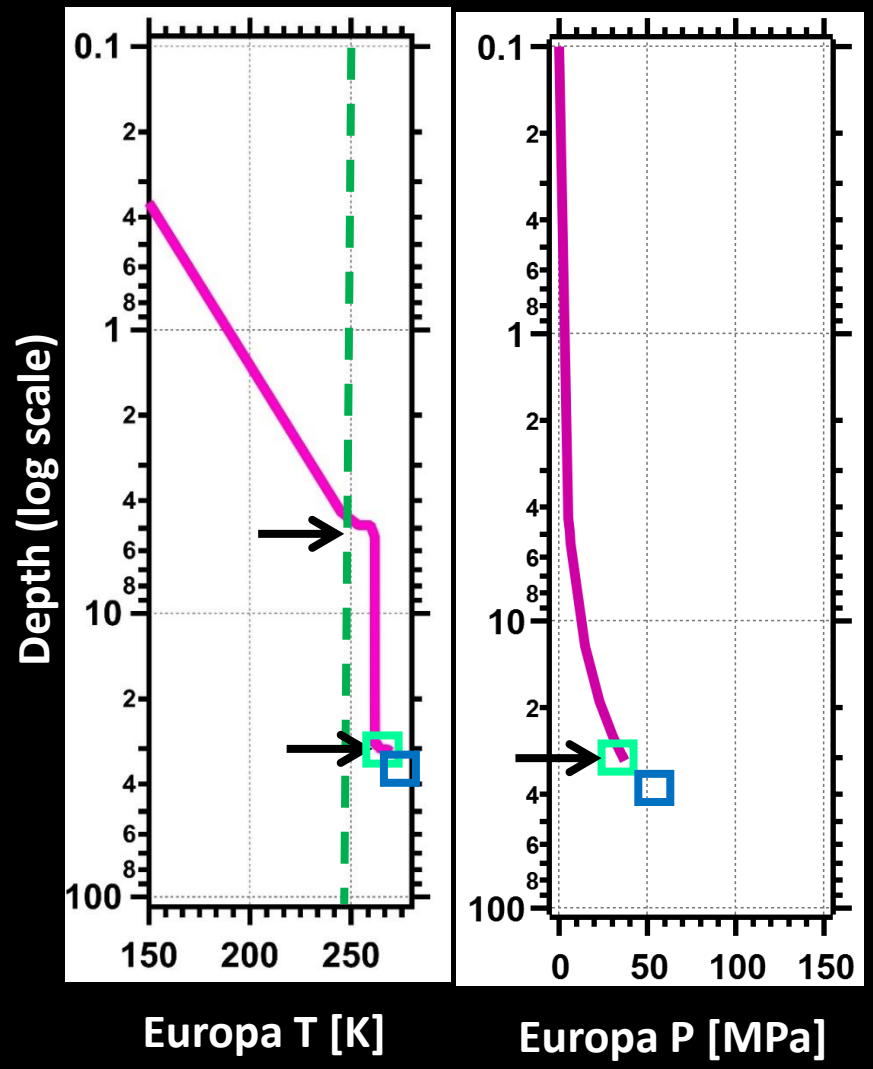
Europa Deep Ice habitability

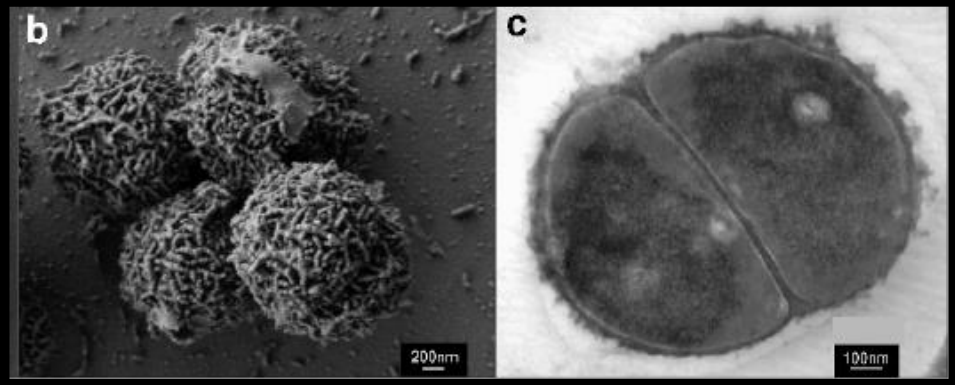
P, T conditions similar to 3 km beneath Greenland ice sheet

~~"Is it habitable?"~~ → "Is it inhabited?"

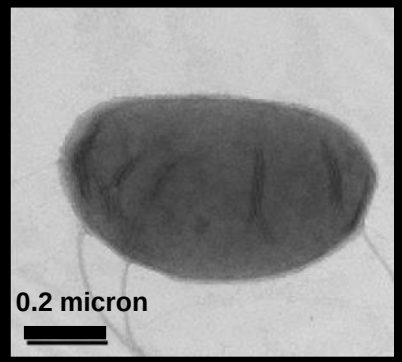


Europa
(side view)

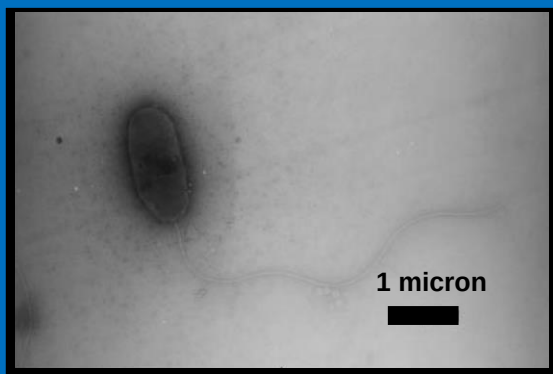




Planococcus halocryophilis Or1
Growth at 258 K (-15 C), 0.1 MPa
Mykytczuk et al., ISME 7 (2013) 1211-1226.



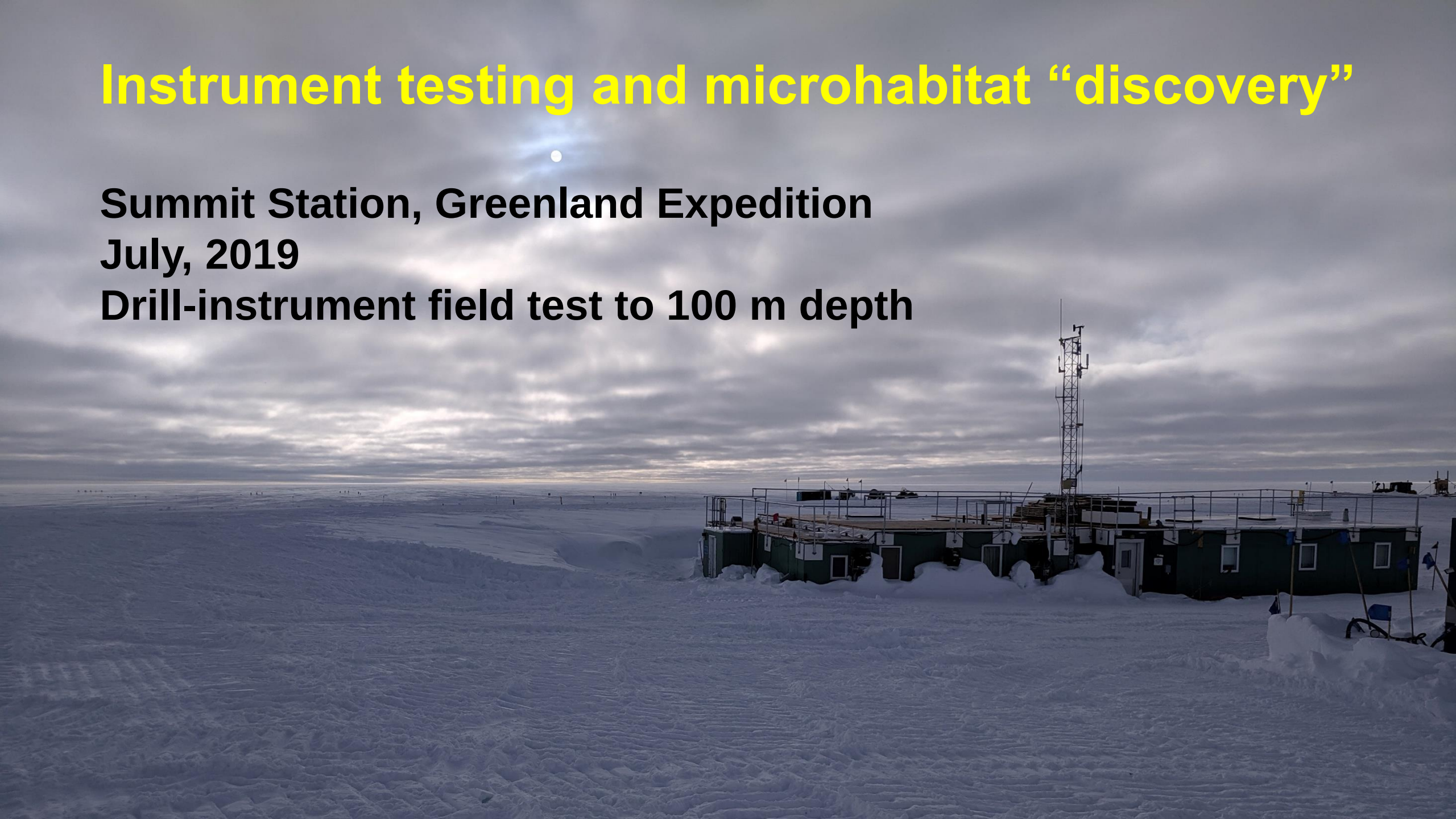
Herminiimonas glaciei UMB49
GISP2, 3 km deep (264 K, 30 MPa)
Loveland-Curtze et al., Int. J. Syst. Evol. Microbiol. 59 (2009), 1272-1277

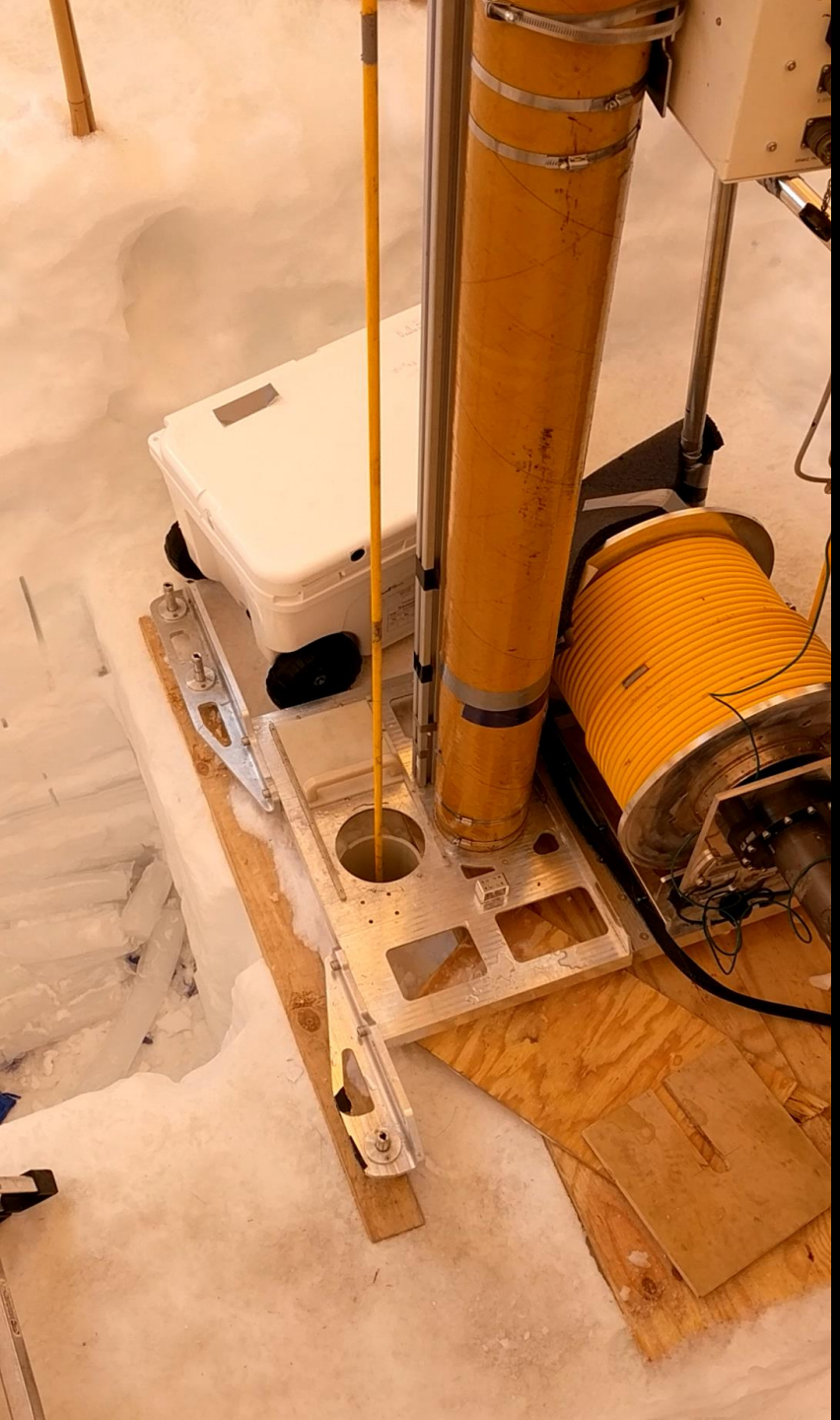


Psychromonas kaikoae JT7304
Optimum growth 283 K, 50 MPa
Nogi et al., Int. J. Syst. Evol. Microbiol. 52 (2002), 1527-1532.

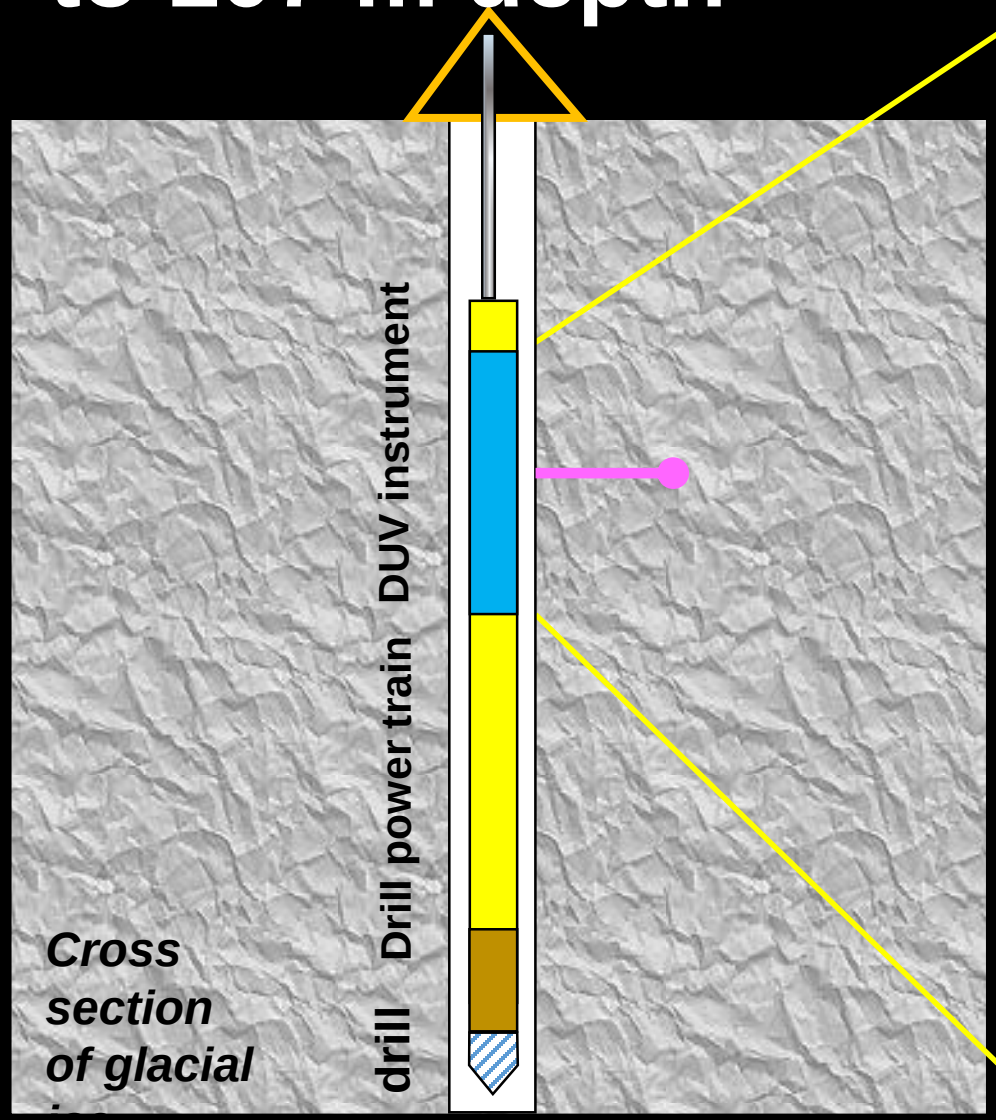
Instrument testing and microhabitat “discovery”

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

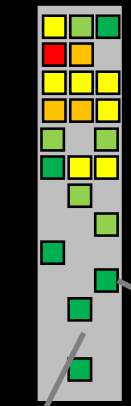


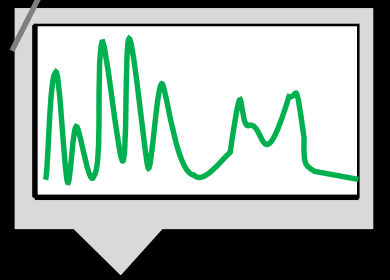


Instrument testing to 107 m depth



Spectral map

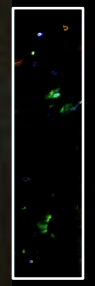




microbe

Malaska et al., Astrobiology 20 (2020), 1185-1211 (open-access)

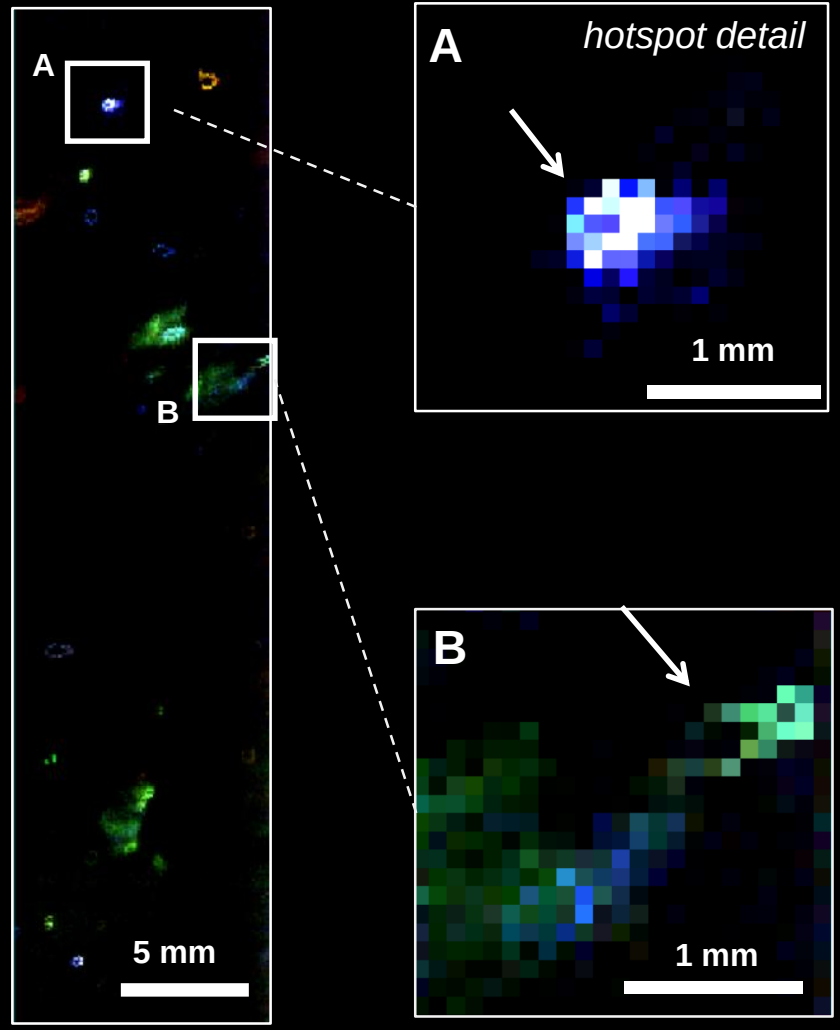
What we expected: Organics in layers



Scale comparison

Image of GISP2 core near 1.837 km

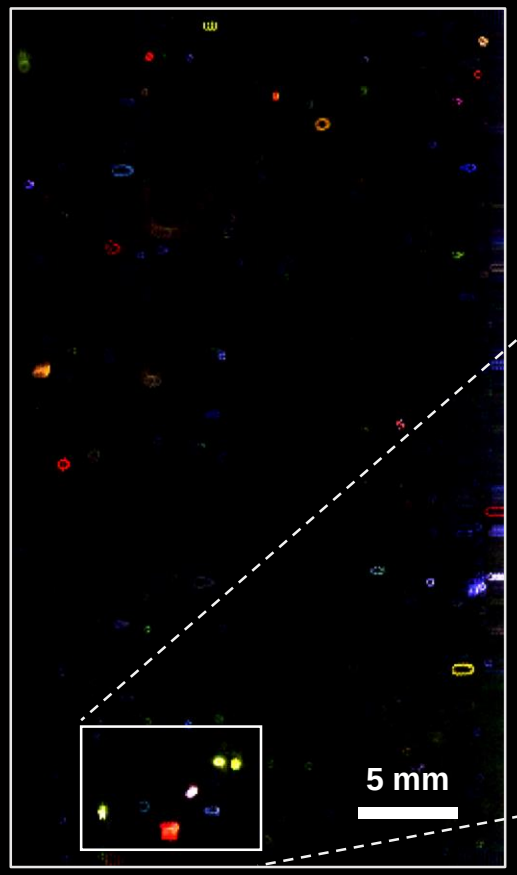
What we saw : Organics in spots



PSTAR_WATSON fluorescence map at 93.8 m

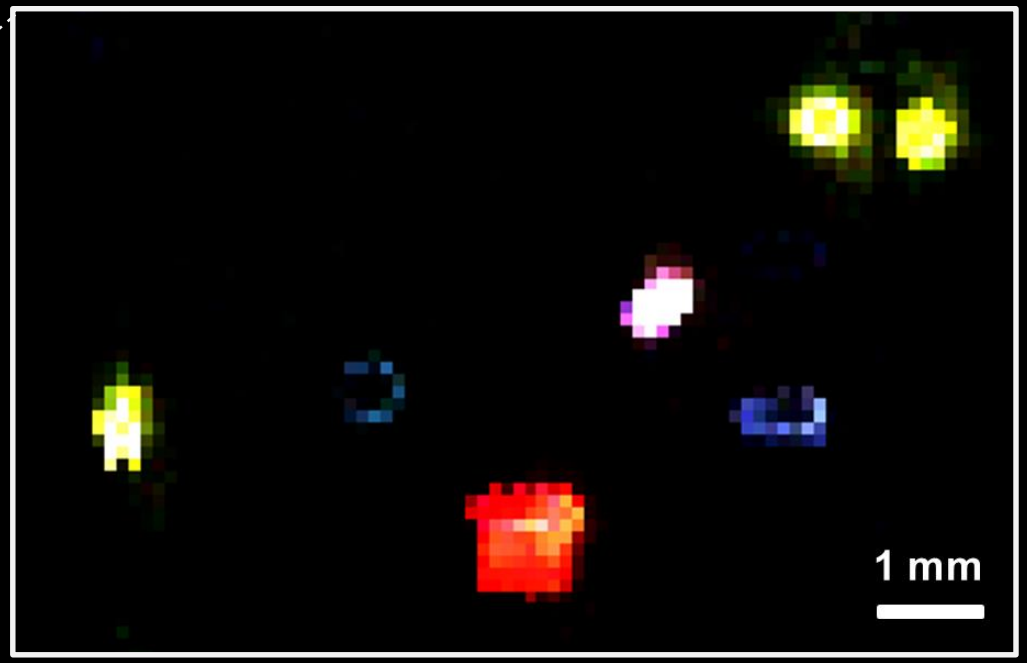
Malaska et al., Astrobiology 20 (2020), 1185-1211(open-access).

Microhabitats are chemically unique



106. 7 m depth

RGB fluorescence
[412.9, 385.3, 313.7 nm]
Malaska et al., in prep.

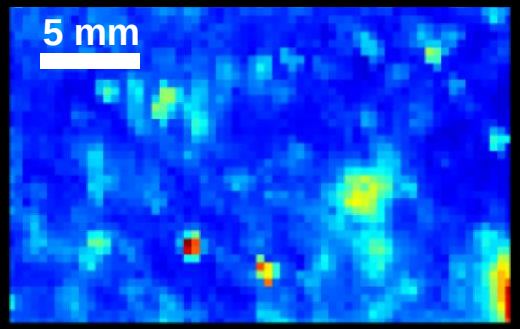


Different colors mean different organic chromophores

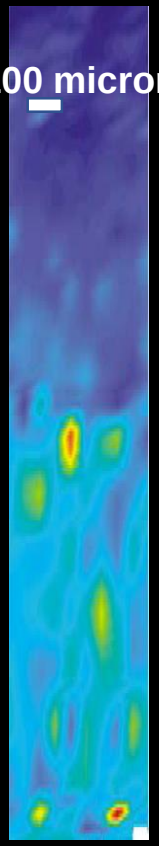
Different colored spots, but same color across spot
→ Spots are uniform, but diverse

→ Each microenvironment is a tiny world

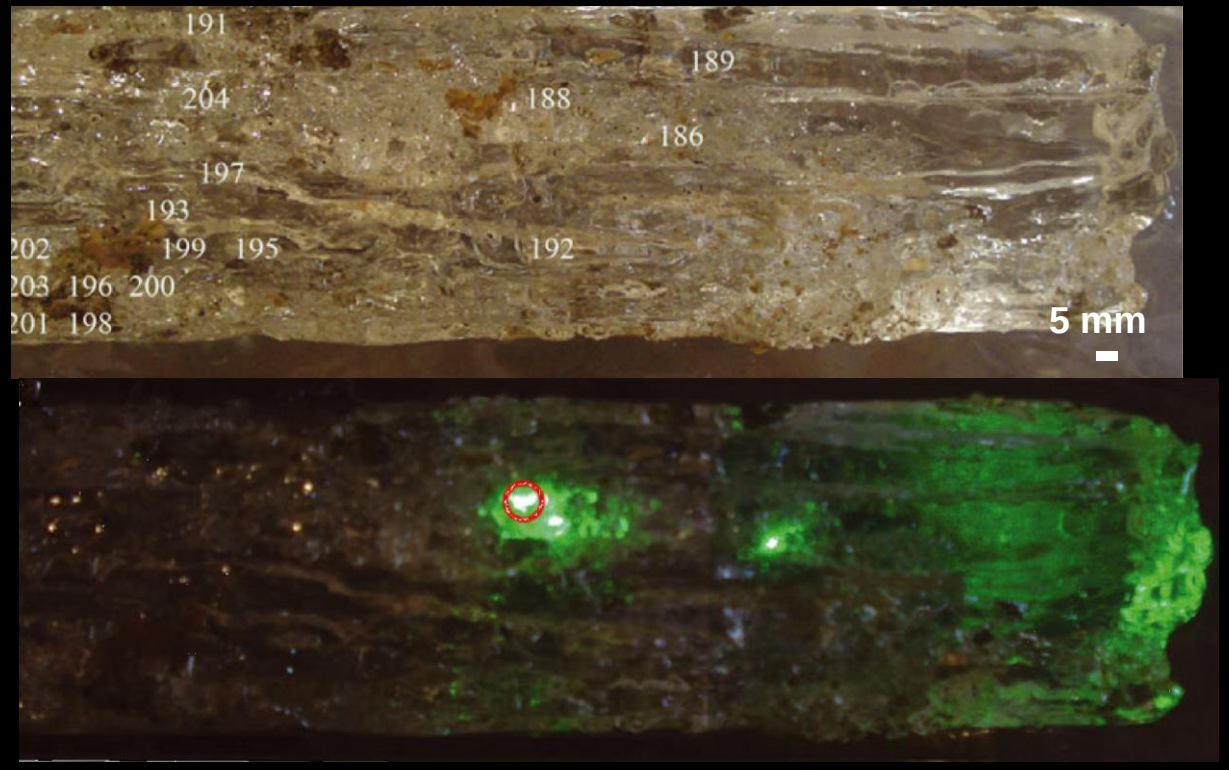
Microhabitats from diverse analog environments



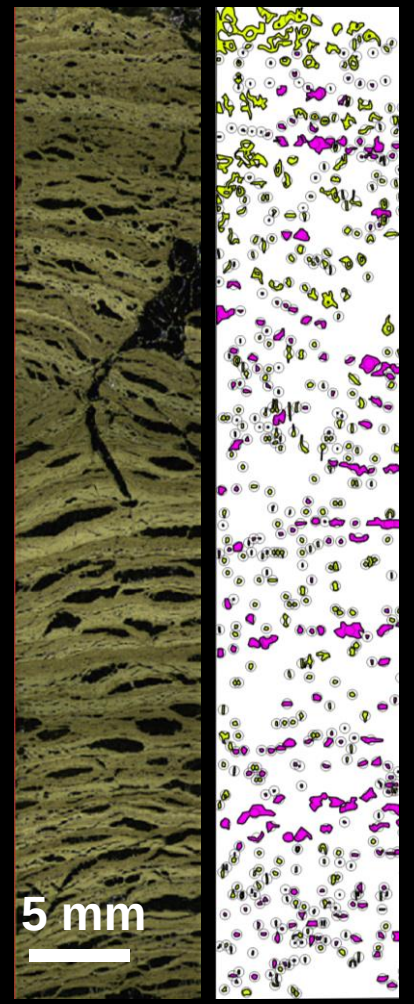
WAIS Antarctic ice core WDC06A, 455 m depth
DUV fluorescence
(Rohde PhD thesis., 2004)



Microbial mat
Silver impression
^{32}S nano-SIMs
(Fike et al., 2008)



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)

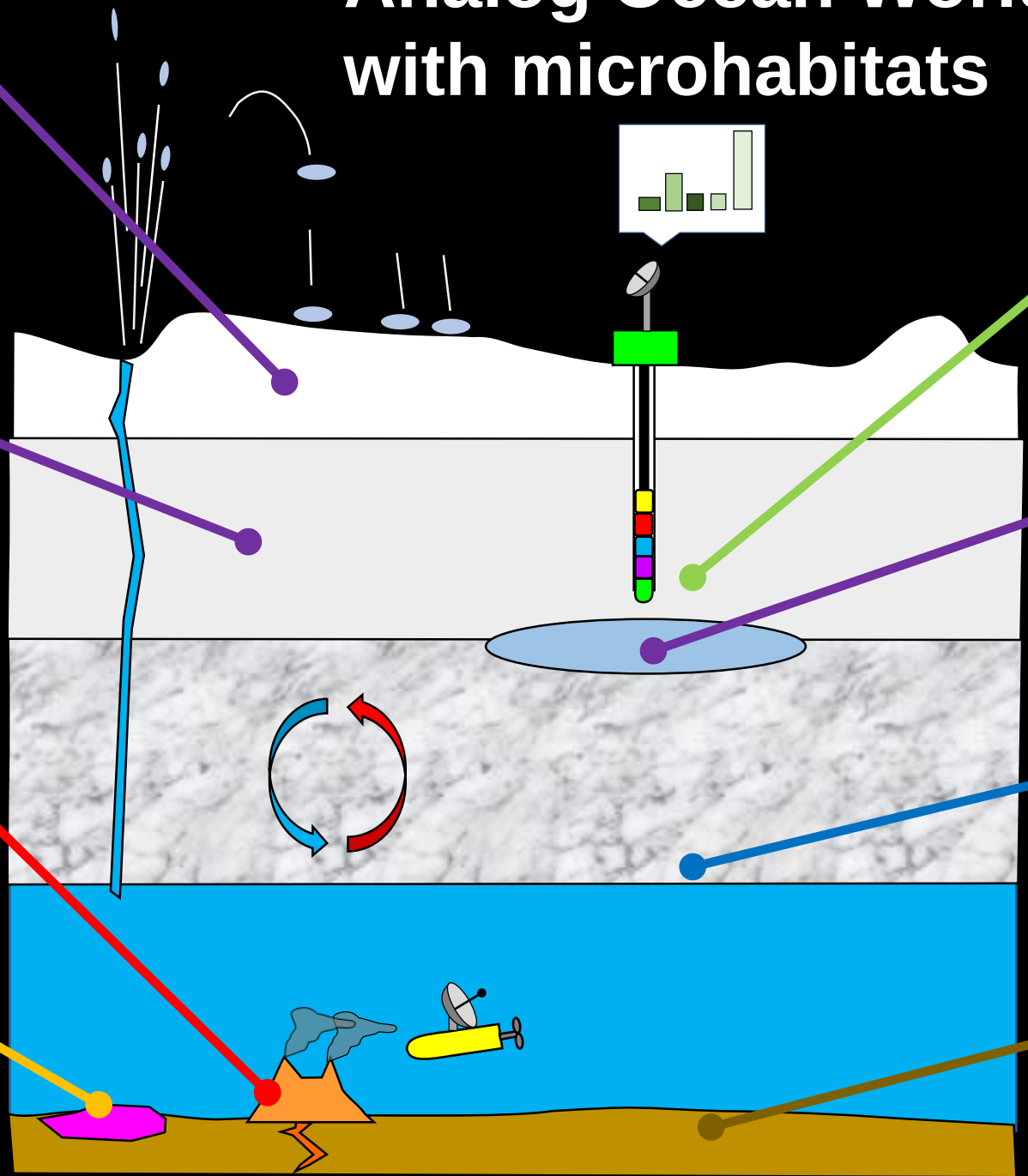
Analog Ocean World environments with microhabitats

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}S ratio
(Fike et al., 2009)



Deep ice lens
Lake Fryxyll
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}C 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

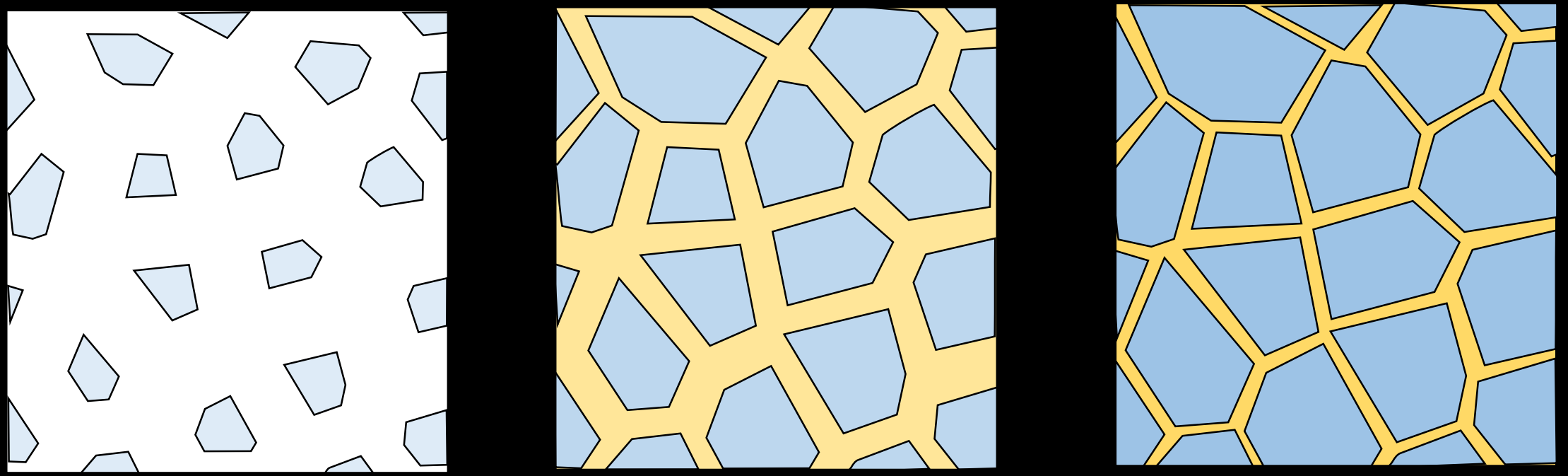
Spots can be diverse from each other

Observed in many Earth environments

How microhabitats form

Pure water ice freezes out - pushes impurities into liquid channels

time ⟶



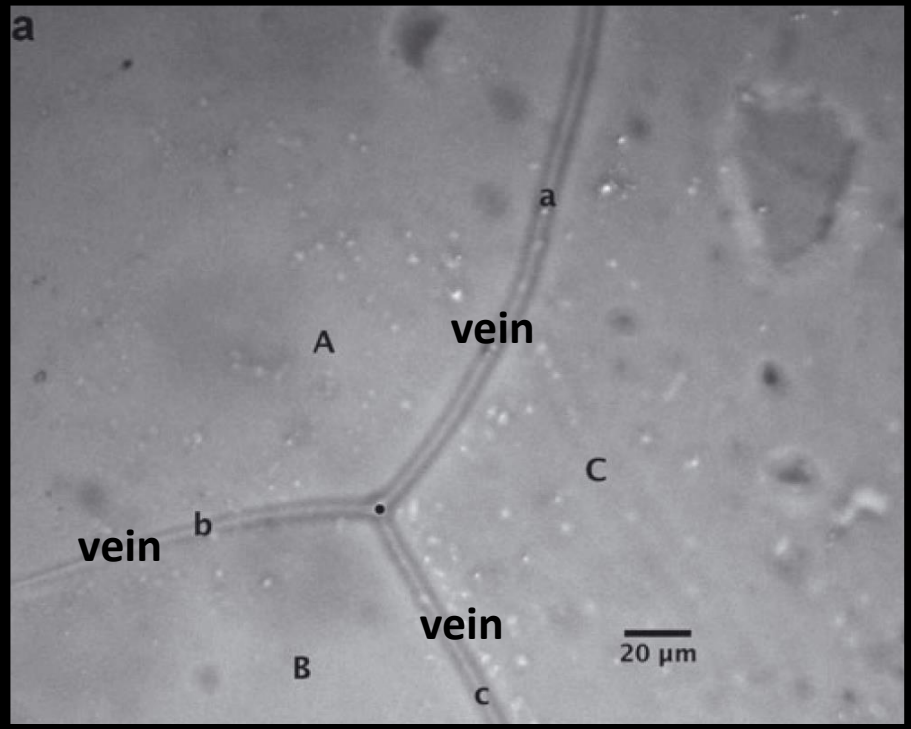
Unfrozen liquids with concentrated salts, high acid
Lower freezing point
Can stay liquid longer
Liquid environment for microbes!!!

Mader et al., Geology 34 (2006) 169-172.

Nutrients concentrate in microhabitats during freezout

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

ion	bulk	vein	Conc factor
Sulfate	0.26 uM	101 mM	200,000
Nitrate	0.89 uM	53.6 mM	400,000



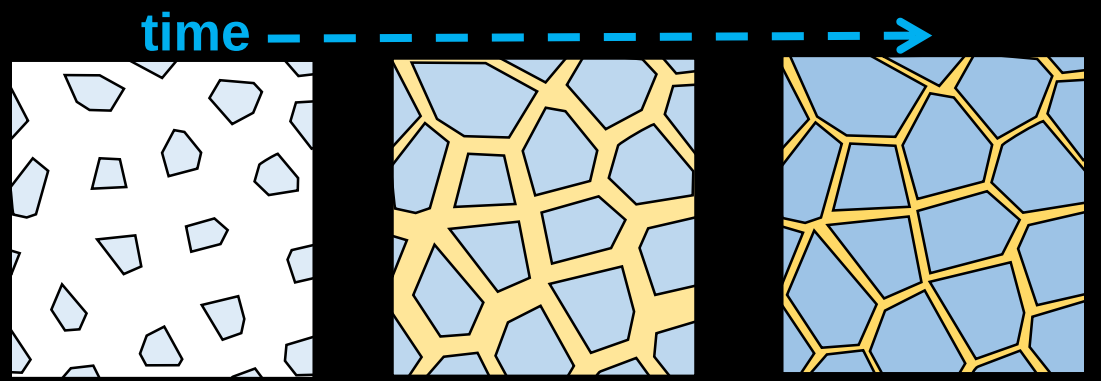
GISP2 ice core 146 m depth image
Vein structures shown

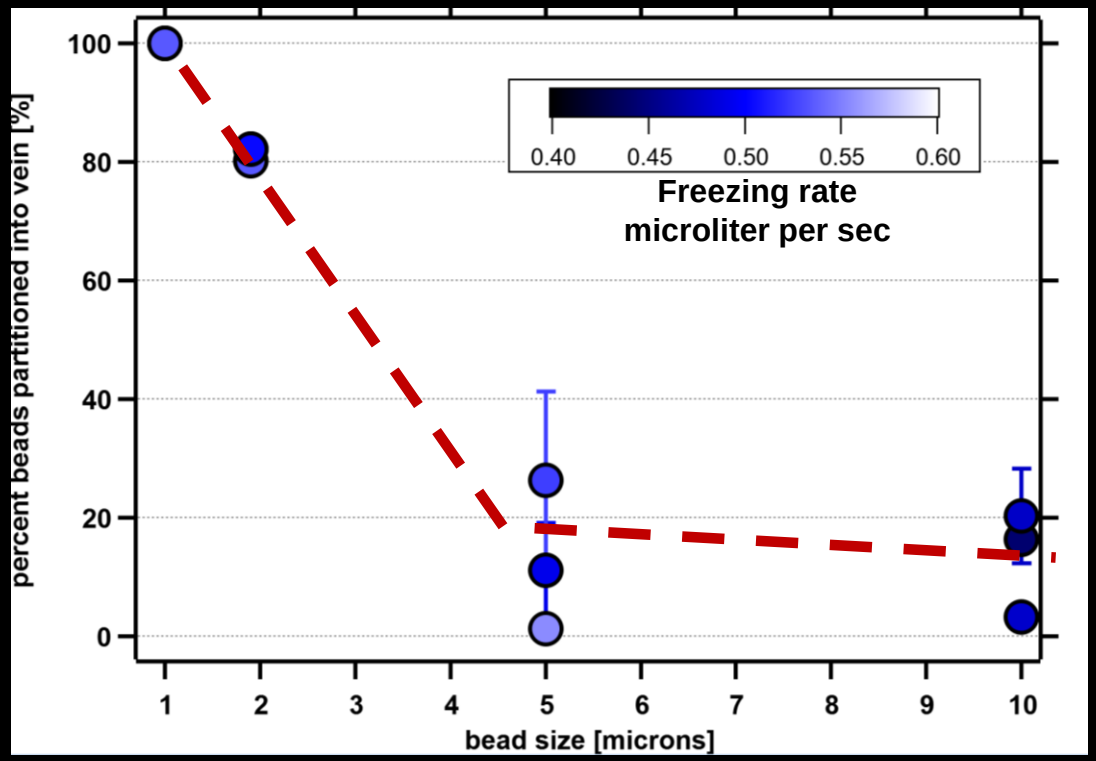
Huge increase in local ion concentrations in ice veins
Start point → liquid eutectic
Chemically concentrated microenvironments
10 – 100 uL volume per L of bulk ice volume (1 ppm)

Barletta et al., Journal of Glaciology 58 (2012) 1109 – 1118.

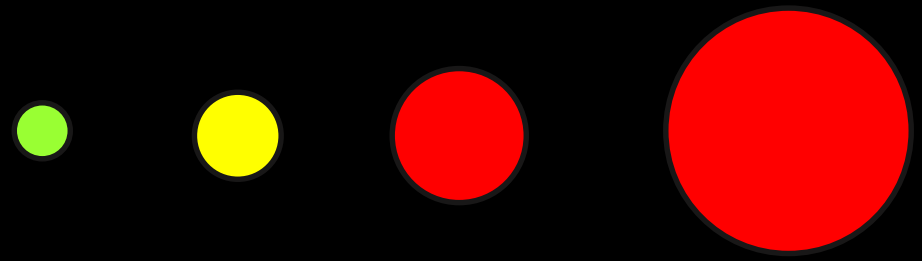
Cell size matters

Small solids stay out of growing ice





1 micron 2 micron 5 micron 10 micron



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

Mader et al., Geology 34 (2006) 169-172.

Comparison of cell sizes

Deep Ice microbes are ultrasmall

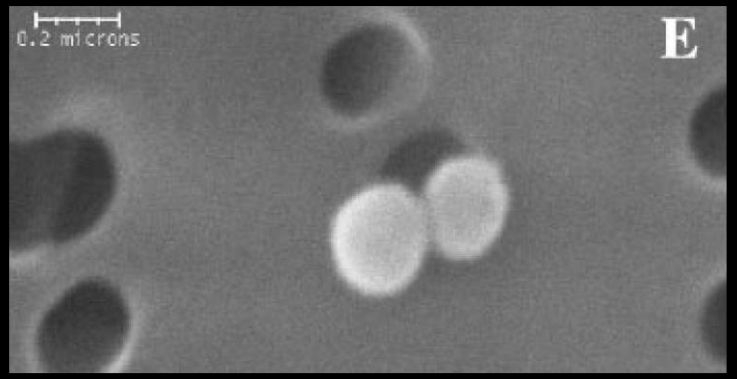


Image of microbe from GISP2 ice core 3 km deep
Miteva and Benchley, Appl and Env. Microbiology 71 (2005) 7806-7818.

Eukaryotes

Red blood cell (8 μm)

Bacteria

Escherichia coli (1.5 μm)

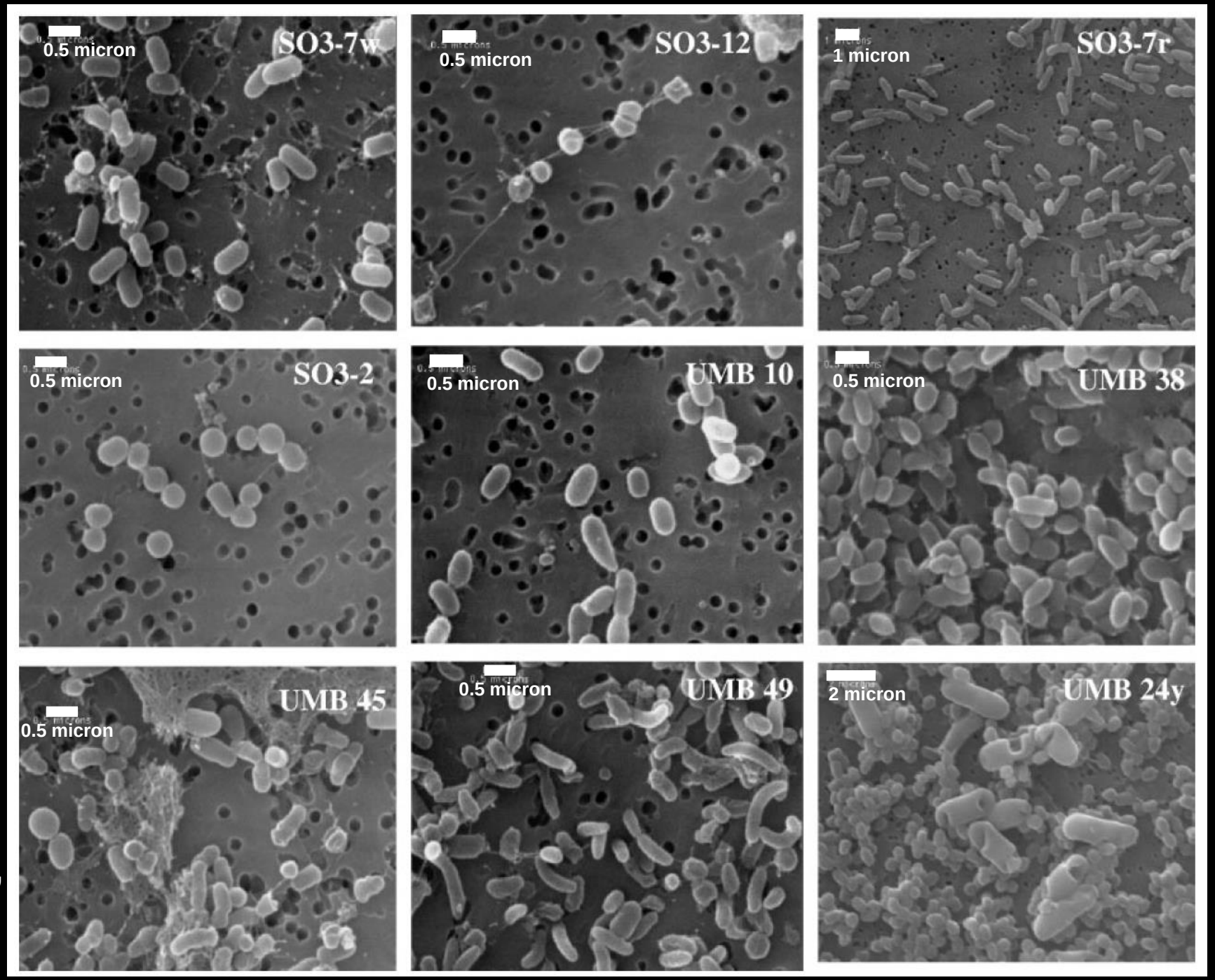
Staphylococcus aureus (0.9 μm)

Deep ice microbes (0.2 μm)

Mycoplasma genitalium (0.4 μm)

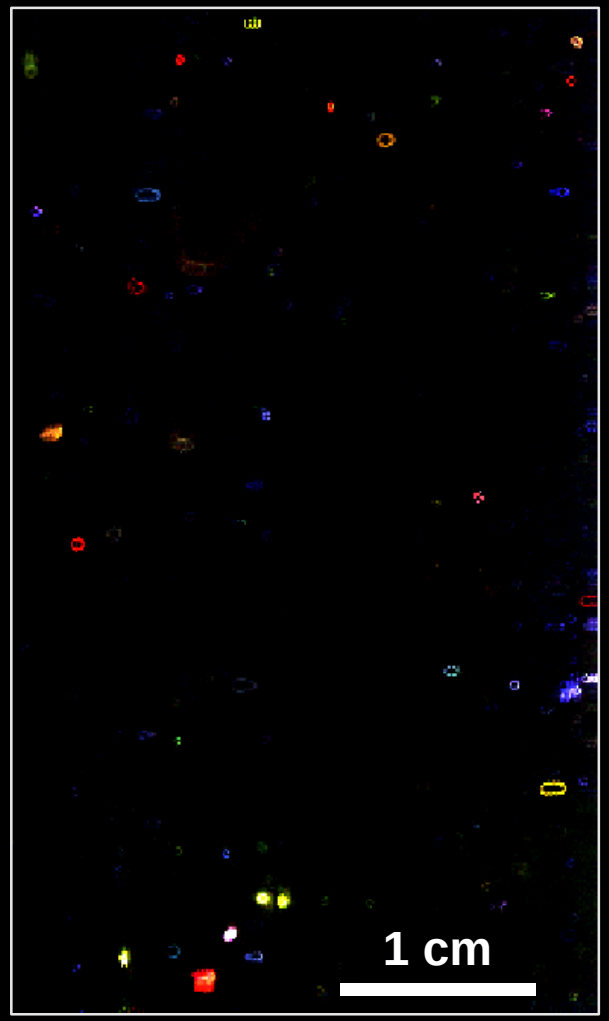
Greenland Deep Ice microbes are ultrasmall

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



Reference: Miteva and Brenchley, Appl and Env. Microbiology 71 (2005) 7806-7818.

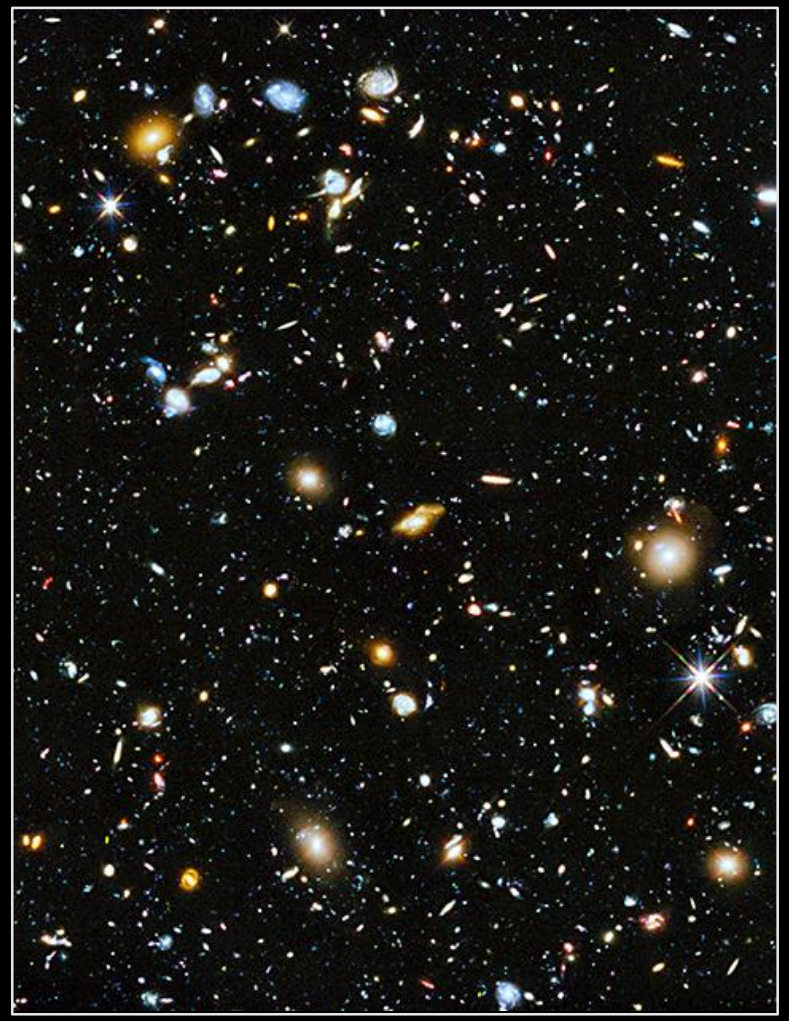
Many many many microhabitats in ice



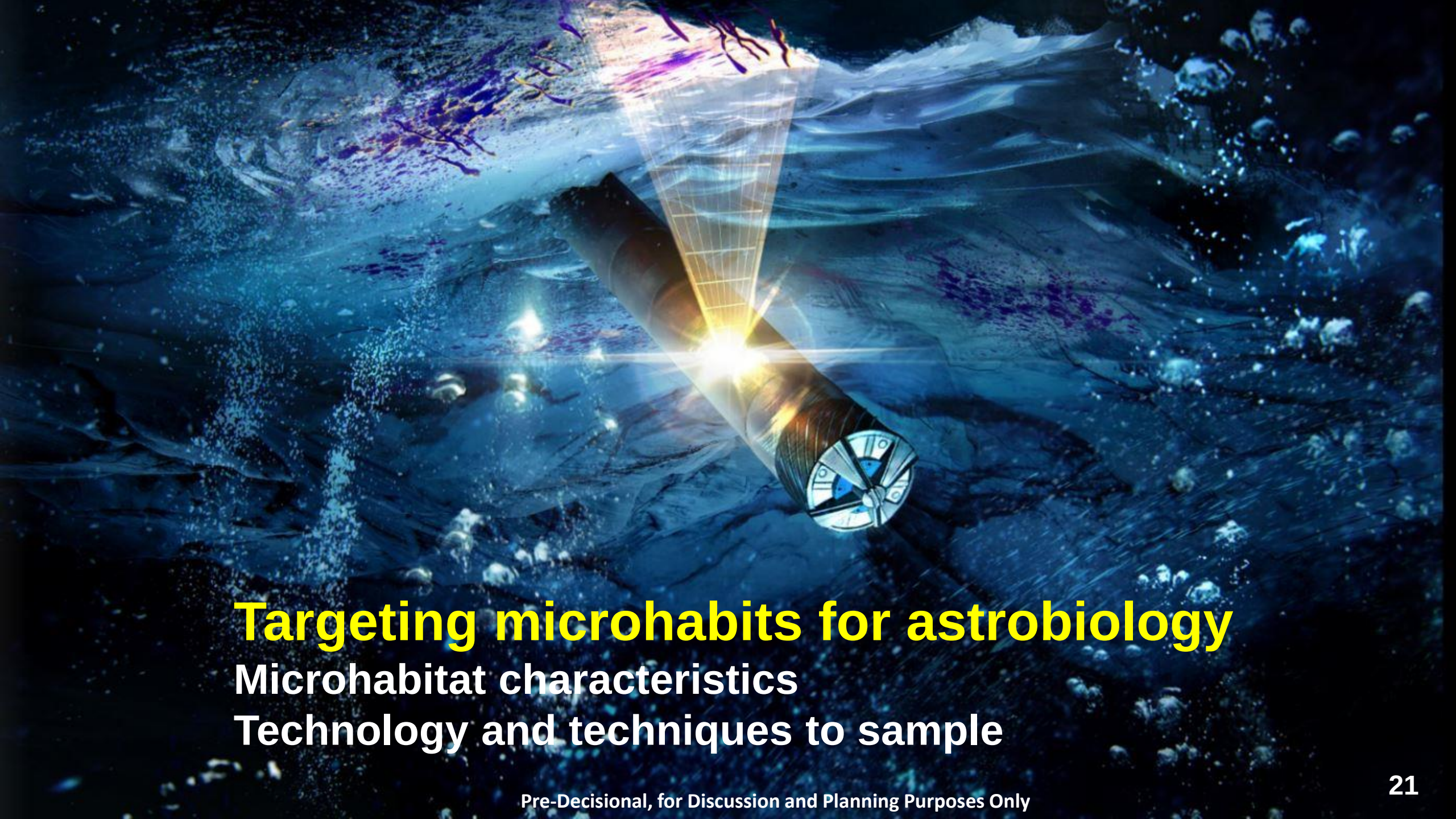
2.6 cm x 4 cm fluorescence map
7E21 microhabitats in Greenland ice
Estimated 4E25 microhabitats
in Titan's Deep Ice

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



Hubble Deep Field
Estimated 1E24 stars in Universe



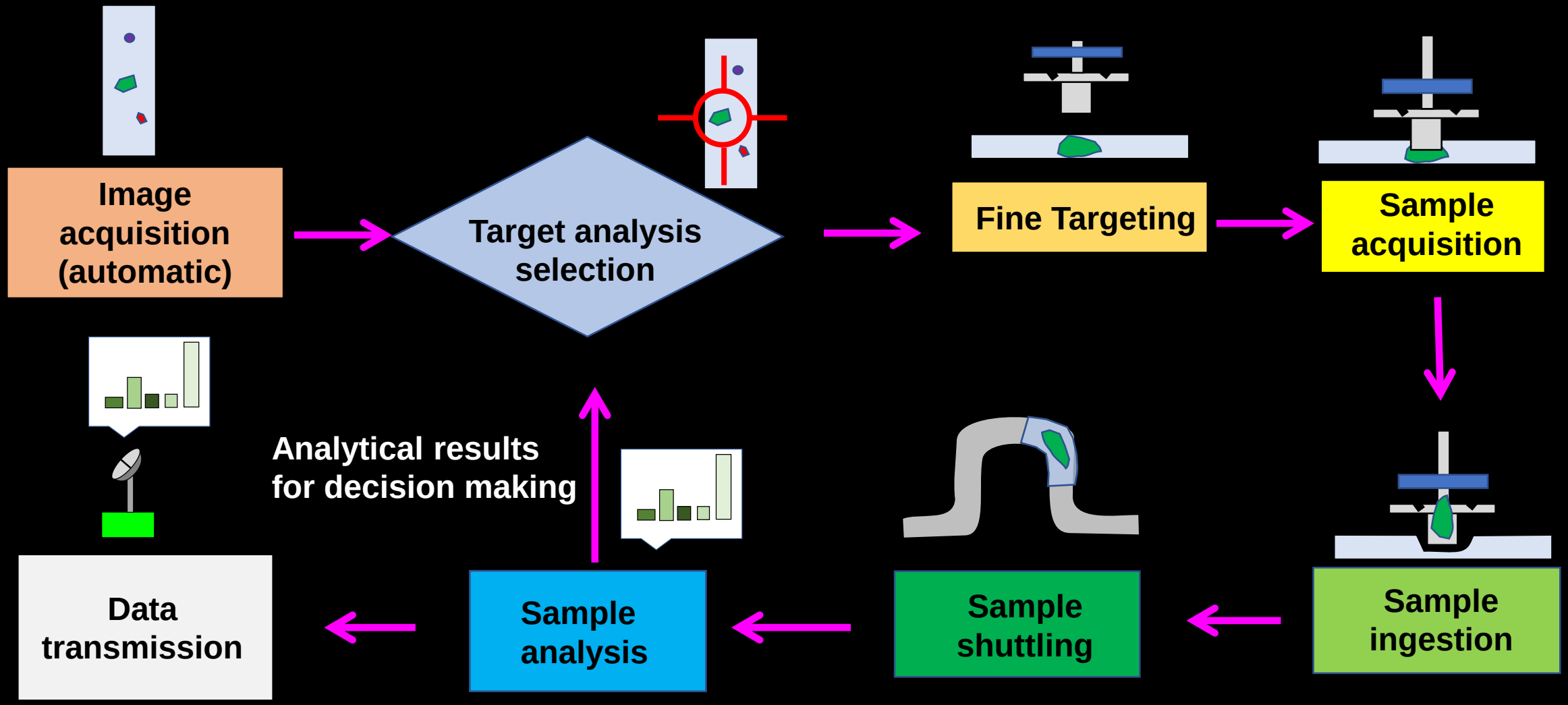
Targeting microhabits for astrobiology

Microhabitat characteristics

Technology and techniques to sample

Microhabitat targeting workflow

Detect→acquire→analyze



First step: Microhabitat Detection
Fluorescence is good technique for spot detection

Deep UV fluorescence

Laser induced fluorescence

Micro-epifluorescence
BONCAT
DAPI

Pro: sensitive
Flight-adaptable

Con: Added stains give info, but may be too Earth-specific

NanoSIMS

NanoSIMS (silver transfer)

Pro: Deep molecular information

Con: technically complicated, requires atomic flat surface (development difficult)

Scanning electron microscope SEM

(+ epifluorescence?)

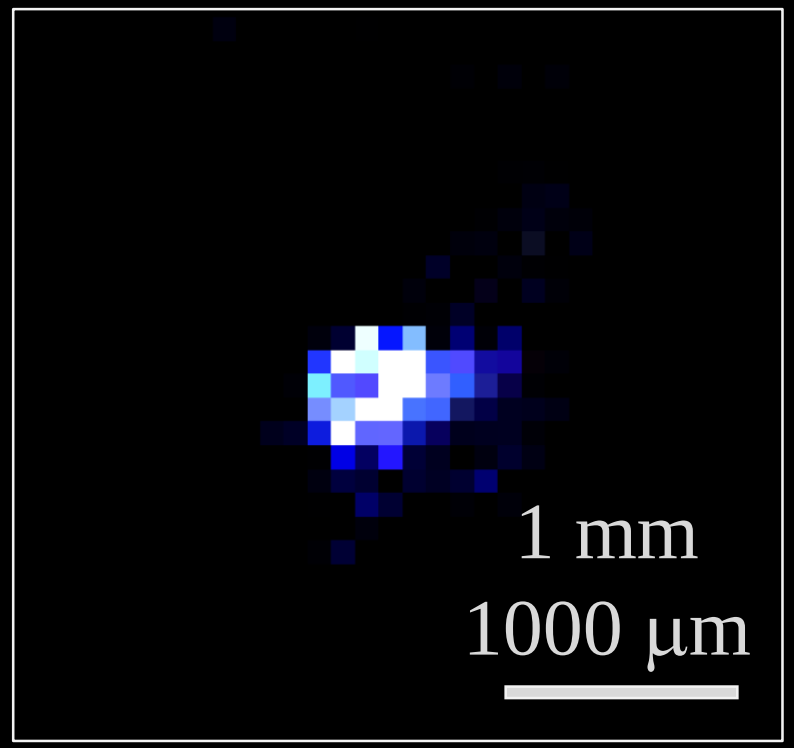
Pro: Morphology of target and substrate

Con: technically complicated, non-specific to biology may not be “easily” flight ready

Example size class images: ROI, micropocket, cells

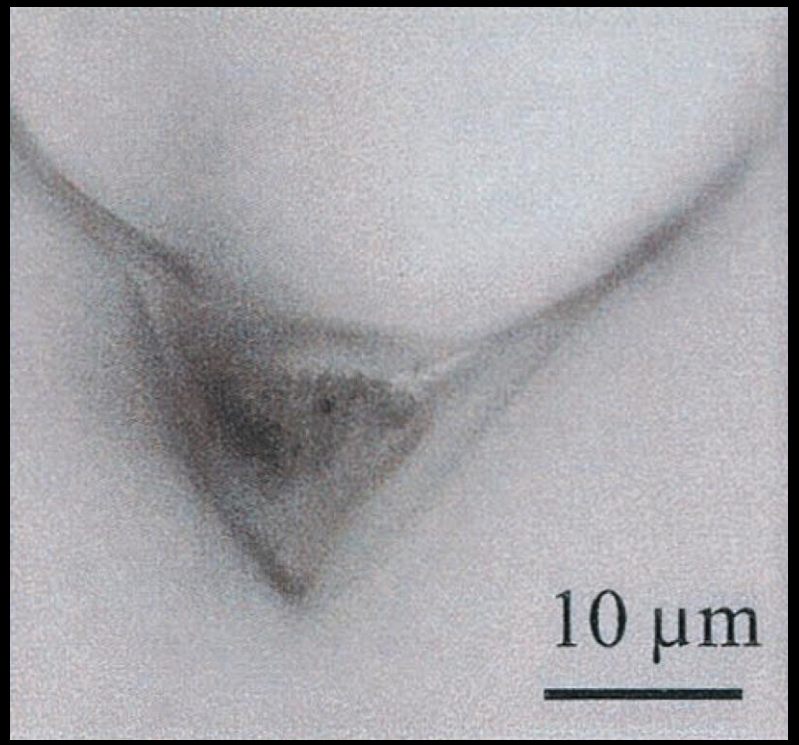
ROI

300 microns – 10 mm



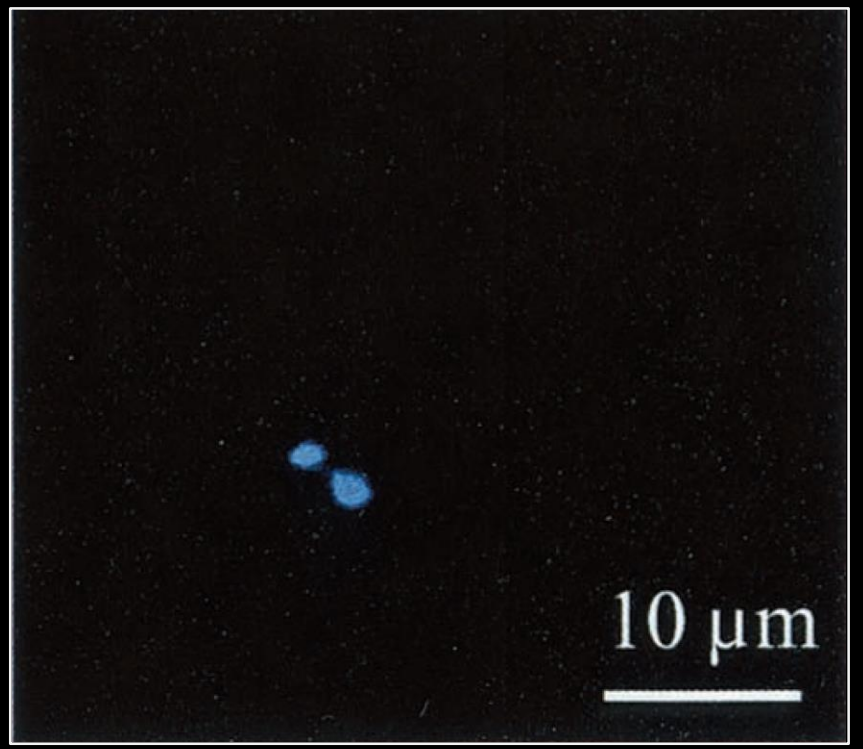
micropocket

10 – 300 microns

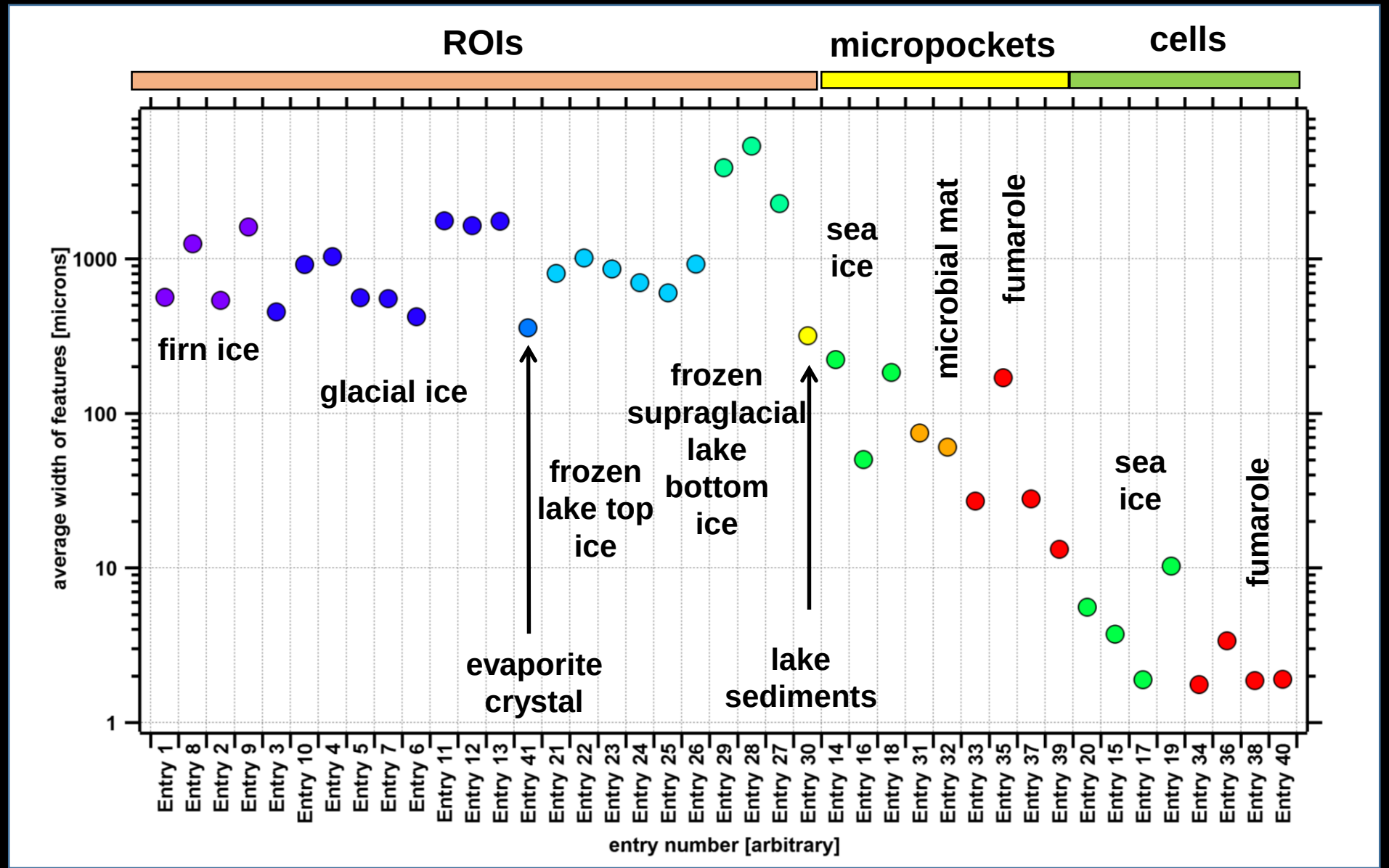


cells

<< 10 microns



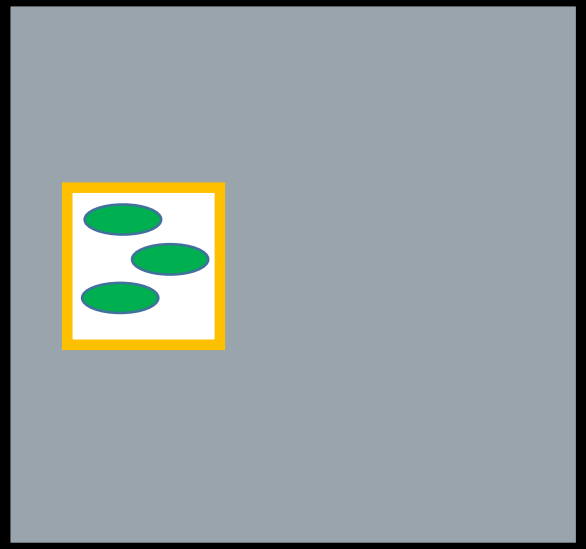
Measured widths show three size classes: ROI, micropockets, and microbial cells

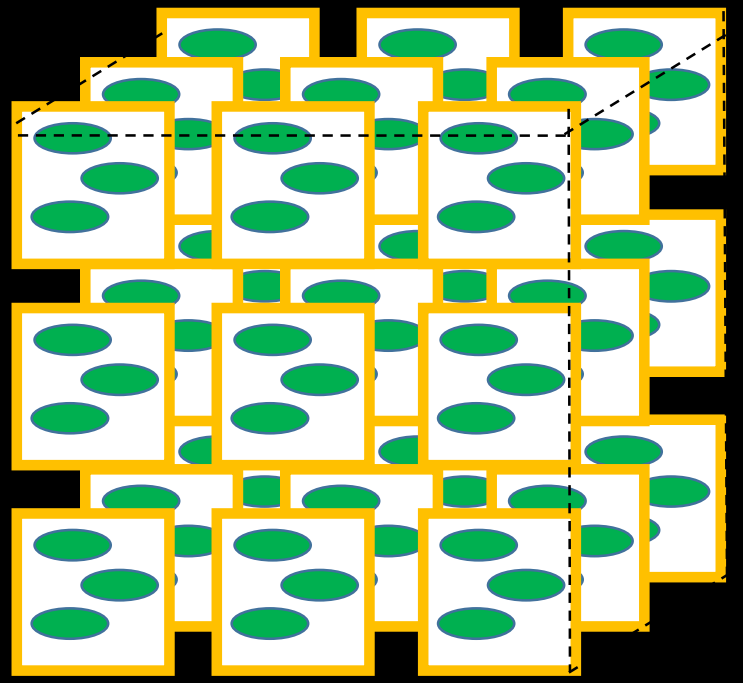


“individual”
(single individual)

“houses”
(many individuals together)

“villages”
(many houses together)





Bacterial cells

Micropocket with cells

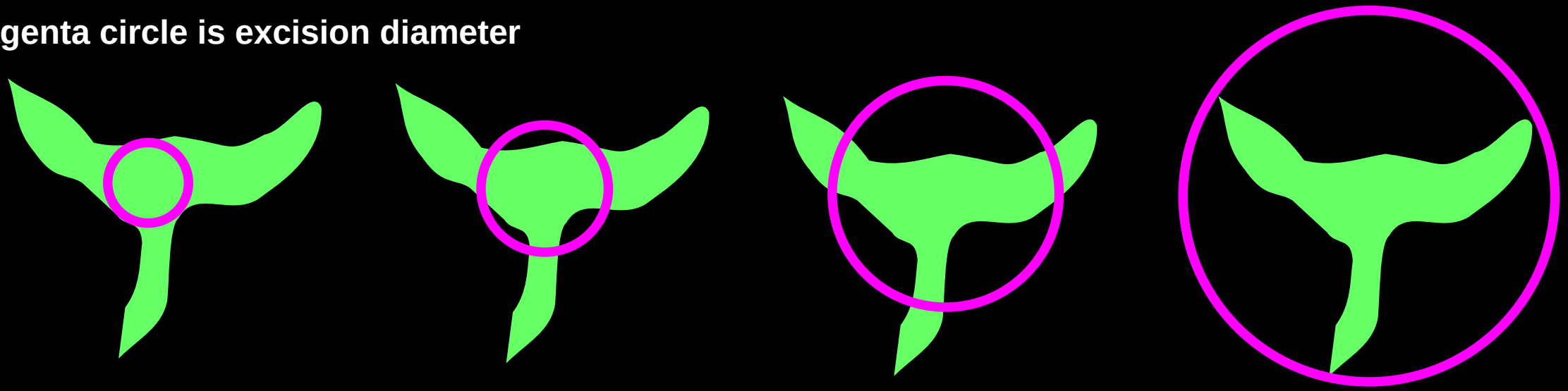
ROI of connected micropockets

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?

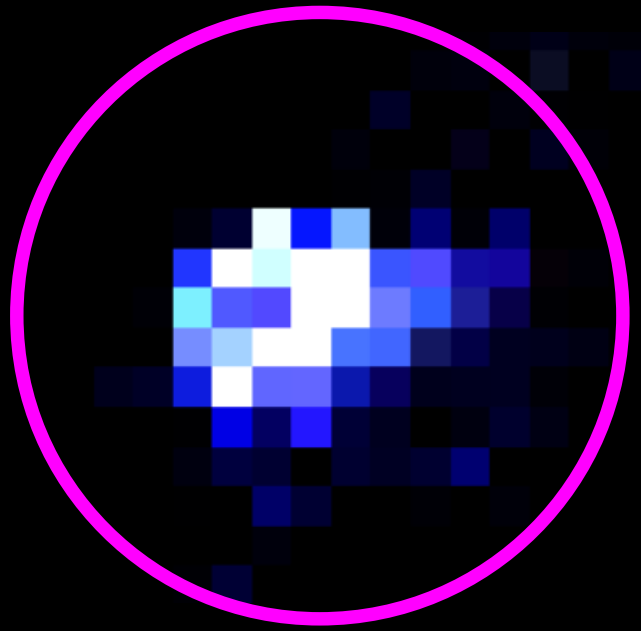
Magenta circle is excision diameter



options	**Maximal pure**	**Some of it**	**Most of it**	**All of it**
	100% purity	90% purity	50% purity	30% purity
	20% of avail amt.	30% of avail amt.	90% of avail amt.	100% of avail amt.

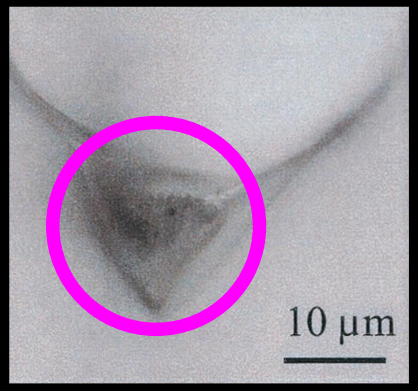
Excision diameter not same as feature diameter

Excision diameter is diameter needed to melt drill and acquire target

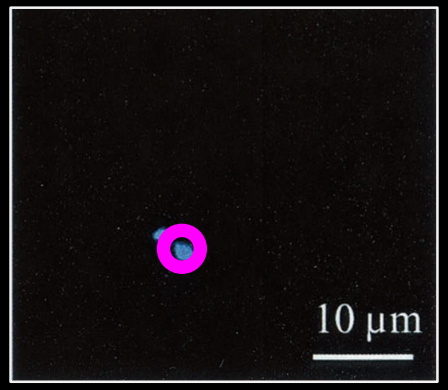


ROIs:
500 – 10,000 microns
(0.5 – 10 mm)

Image and excision circle not to scale



micropockets:
20 – 300 microns

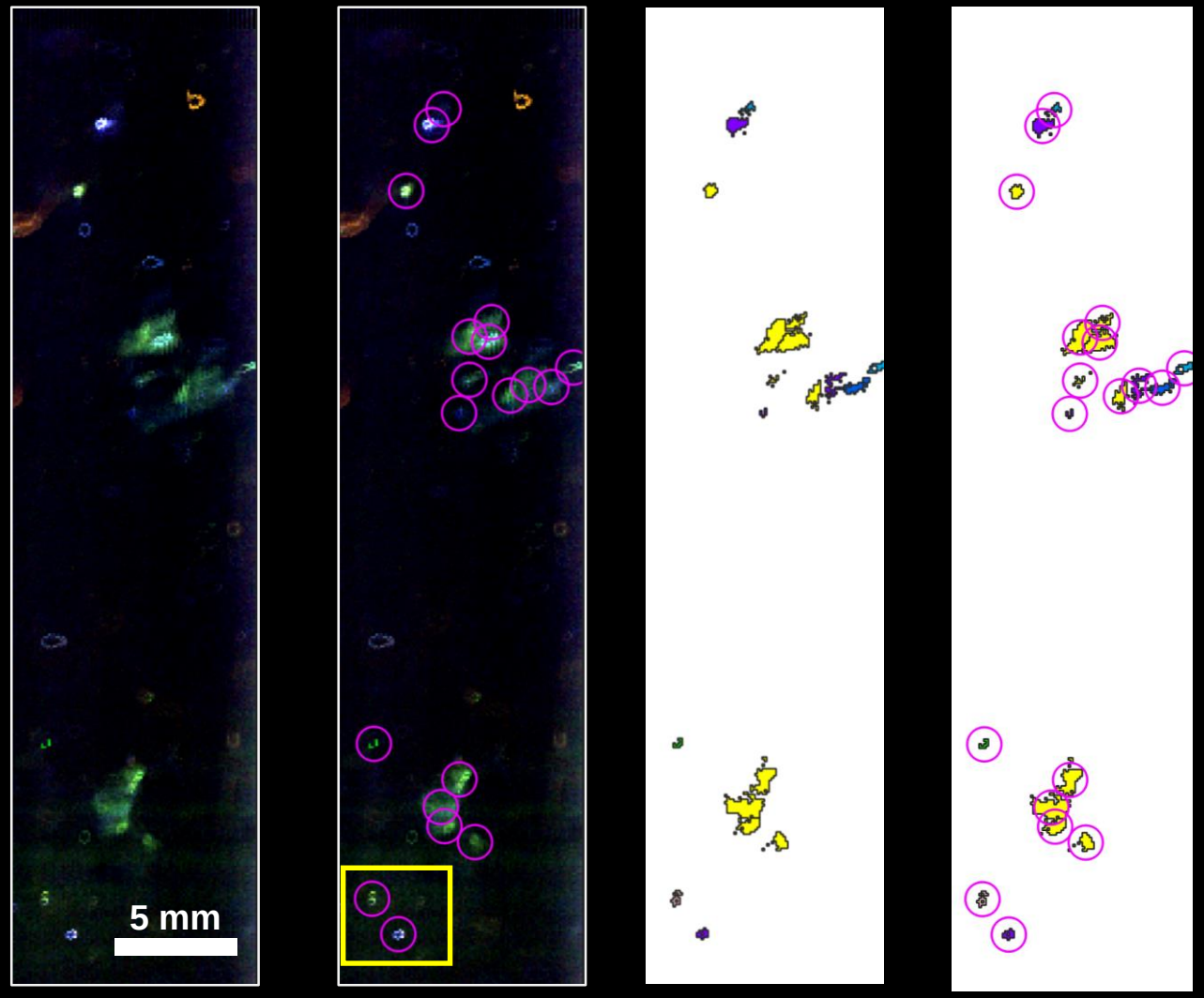


Single cell extraction:
2 – 30 micron diameter
(melt needle?)

Precision targeting at micron scale will be an engineering challenge

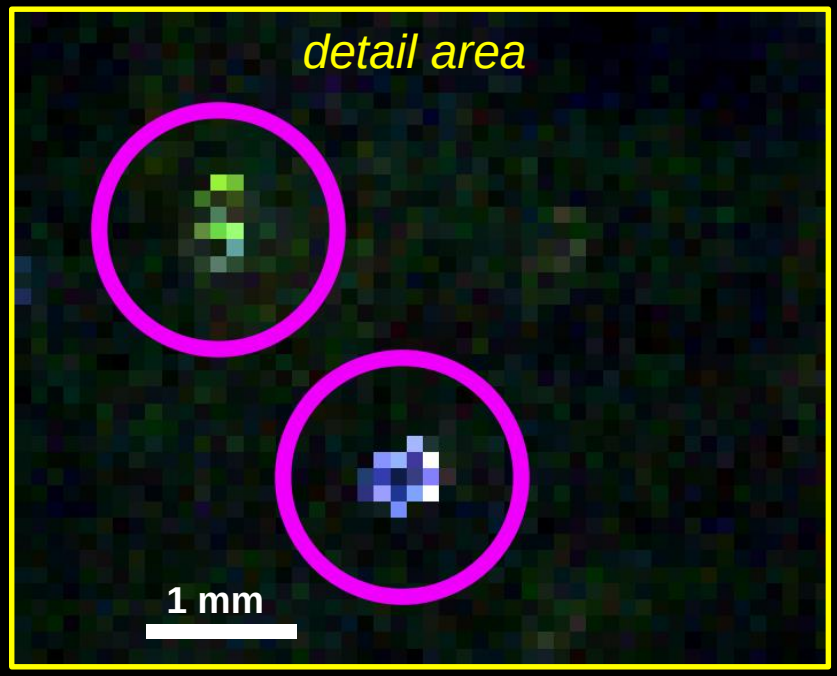
Example: Excision sample targeting of 93.8 m ice core map

Excision diameter is 1.3 mm diameter circle



RGB fluorescence
[412.9, 385.3, 313.7 nm]

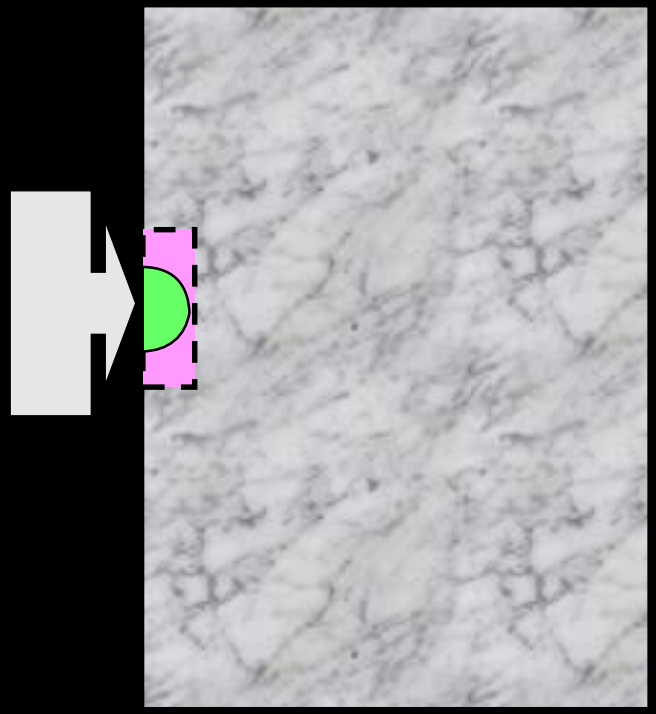
annotated



Excision cleanly acquires ROIs with different spectral characteristics

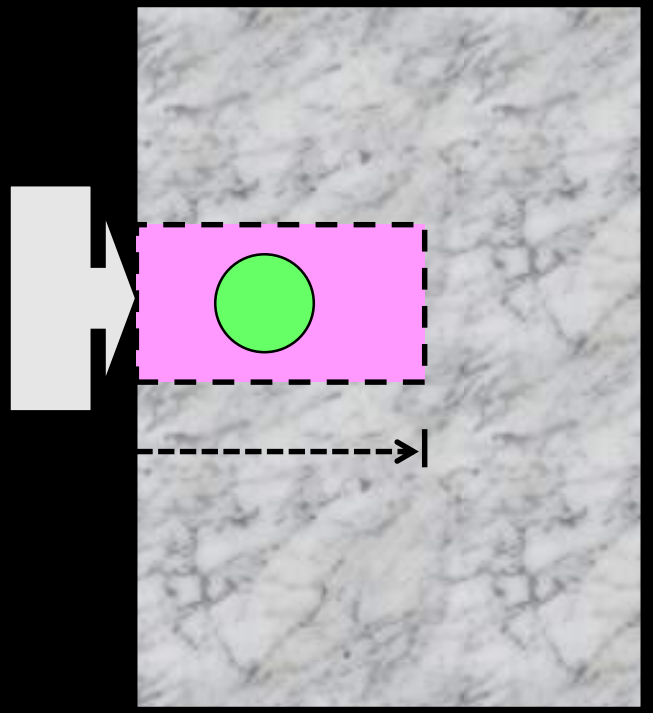
Selecting excision depth: How deep do we extract?

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

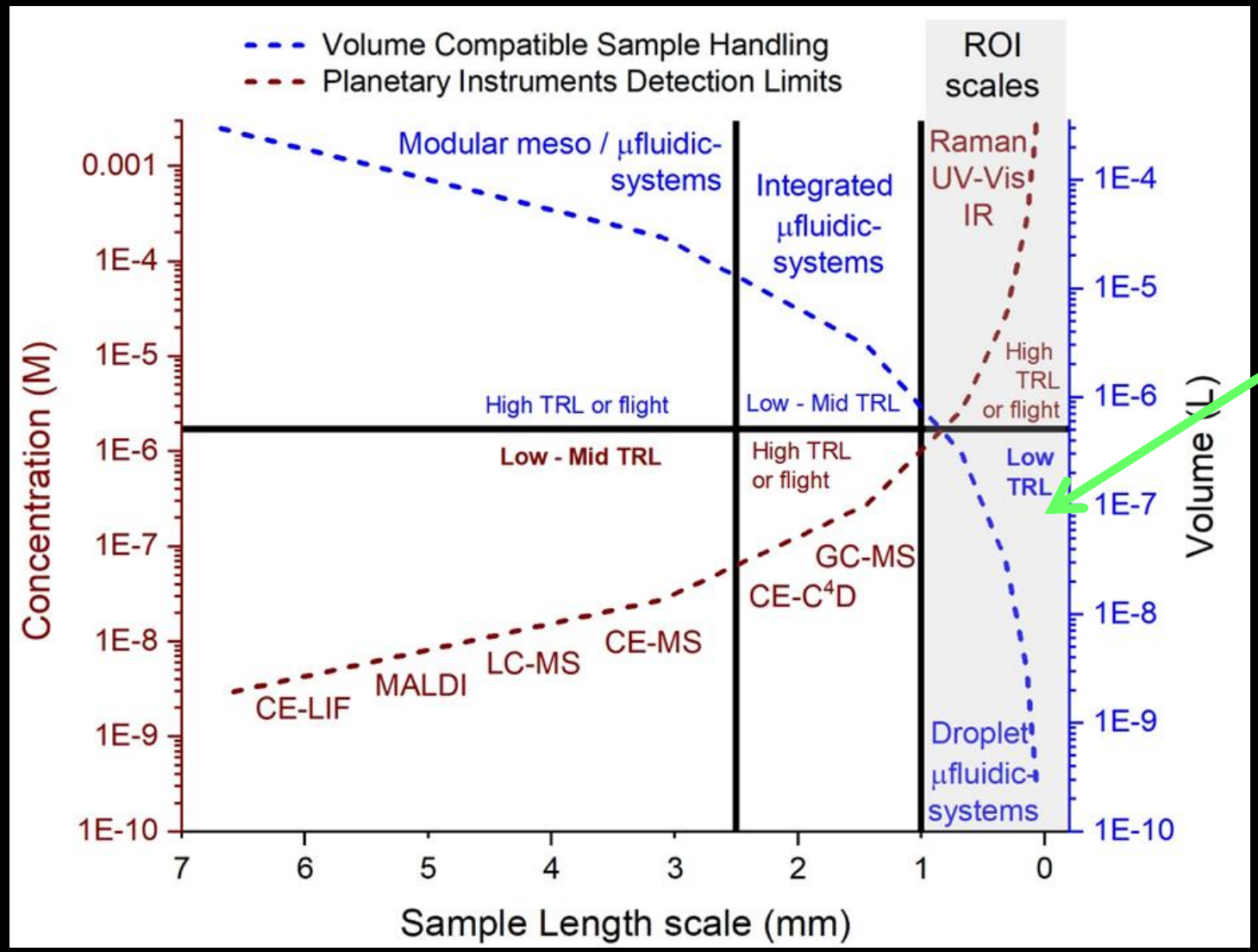
…but same amount of material → higher concentration for instruments!

	Excision volume (1 cm core)	Effective cell concentration [estimated cells per cm^3]
ROIs	2 – 1000 microliters	1E4 – 5E5
micropockets	1 nanoliter – 1 microliter	5E2 – 5E6
cells	< 1E-2 nanoliter	1E5 – 5E7

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

[1] Malaska et al., Astrobiology 20 (2020), 1185-1211 (open-access).

Need for micro- and nanoliter sample handling



New technologies needed for new scales (gray zone)

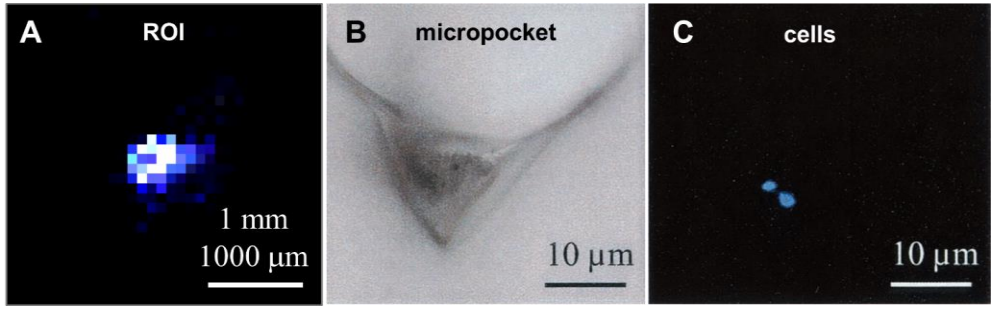
Small volume sample handling:

Single microliter to nanoliter manipulation

Plot courtesy Aaron Noell

Instrument needs

A new scale for planetary instrumentation



Detection – what is needed to identify targets?

Feature type	FOV [mm^2]	Pixel scale [microns per pixel]	Technique type
ROIs	1 – 1000	100-500	DUV fluorescence, MALDI-TOF
micropockets	1E-4 – 1	0.5 – 20	Oil-immersion microscopy, Nano-SIMS, SEM
cells	1E-4 – 0.1	0.1 – 1	Epifluorescence (DAPI, BONCAT)

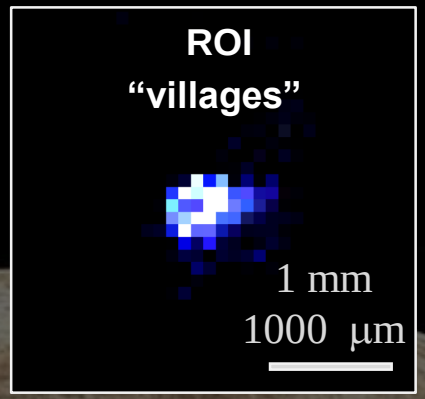
Acquisition – what do we need to acquire and analyze?

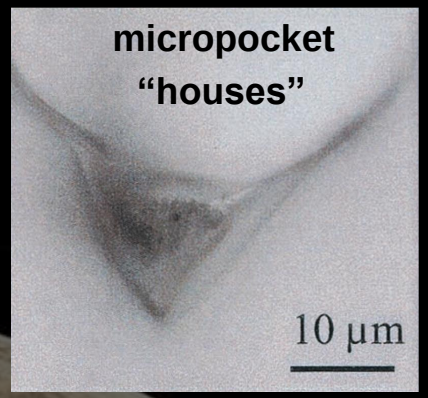
Feature type	Excision diameter range [microns]	Excision cell counts range [cells]	Excision volumes range [microliters]	Excision effective cell concentration range [cells per cm^3]
ROIs	500-10,000	100-20,000	2-1000	1E4-5E5
micropockets	20-300	0.1-20	1E-3 – 1	5E2-5E6
cells	2-30	1	1E-5-1E-2	1E5-5E7

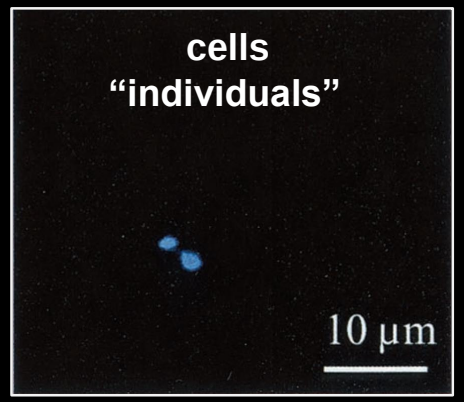
Malaska, M.J., Carpenter, K., Hofmann, A.; Noell, A. “Targeting microhabitats for Ocean Worlds, manuscript in preparation for submission to Planetary Science Journal

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 microhabits variable: ROIs, micropockets, cells

Significant advantage to target microenviroments

Adapt new techniques and instrumentation to small scale