PETERSBURG NUCLEAR PHYSICS INSTITUTE NAMED BY B.P. KONSTANTINOV
OF NATIONAL RESEARCH CENTER «KURCHATOV INSTITUTE»

The search for LIFE in the subglacial Lake Vostok in Antarctica

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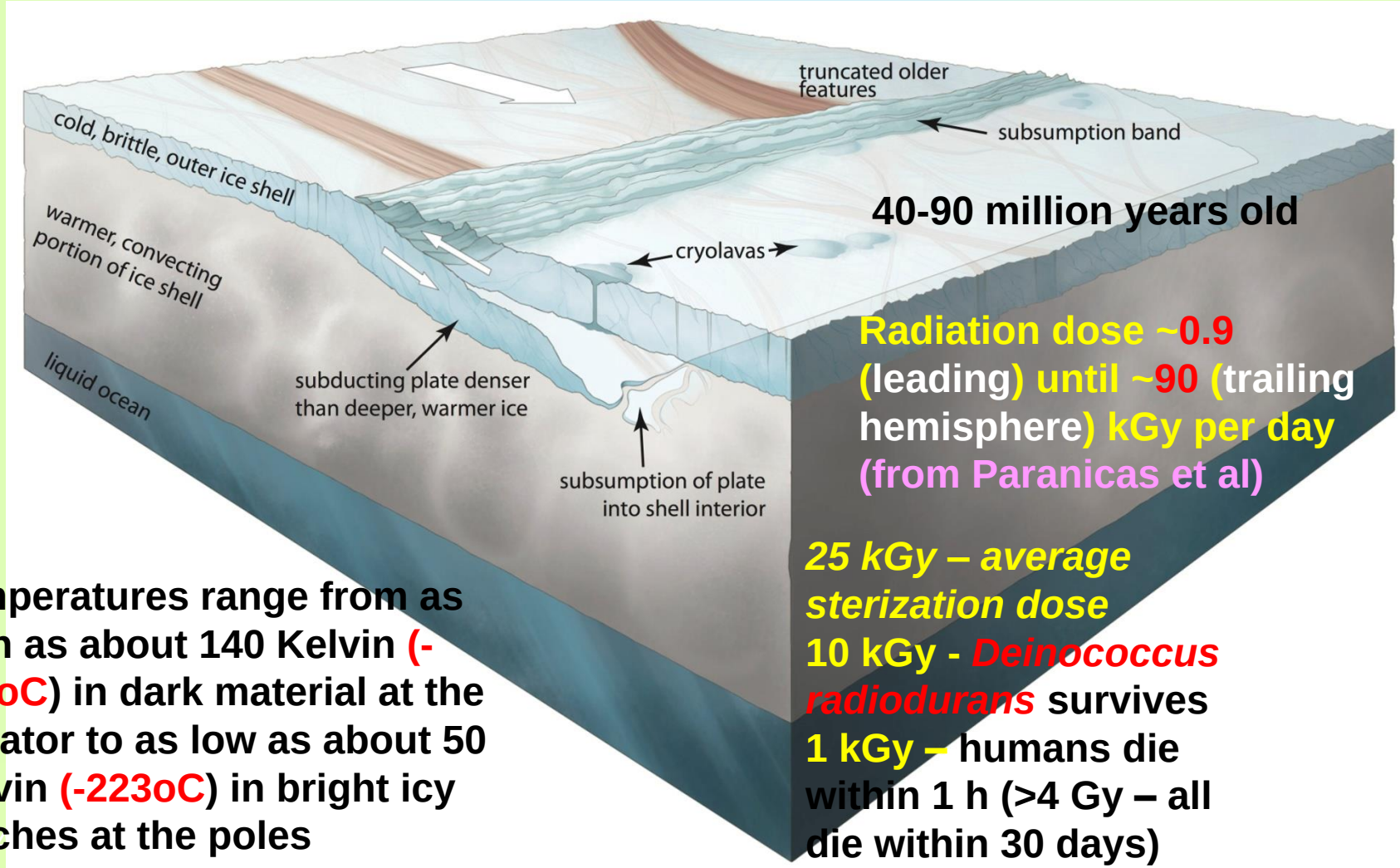
Life around us?

Searching for extraterrestrial life is one of the aims of astrobiology, and there are several potentially habitable worlds in the Solar System, in addition to Earth. Two of Jupiter's moons (Ganymede and Europa) and two of Saturn's moons (Titan and Enceladus) harbour **liquid-water oceans**. And Mars probably had vast liquid-water environments during much of its early history.

Lazcano 2012 Nature 488: 160-1

'Diving' Tectonic Plates on Jupiter's Moon Europa



40-90 million years old

Radiation dose ~0.9 (leading) until ~90 (trailing hemisphere) kGy per day (from Paranicas et al)

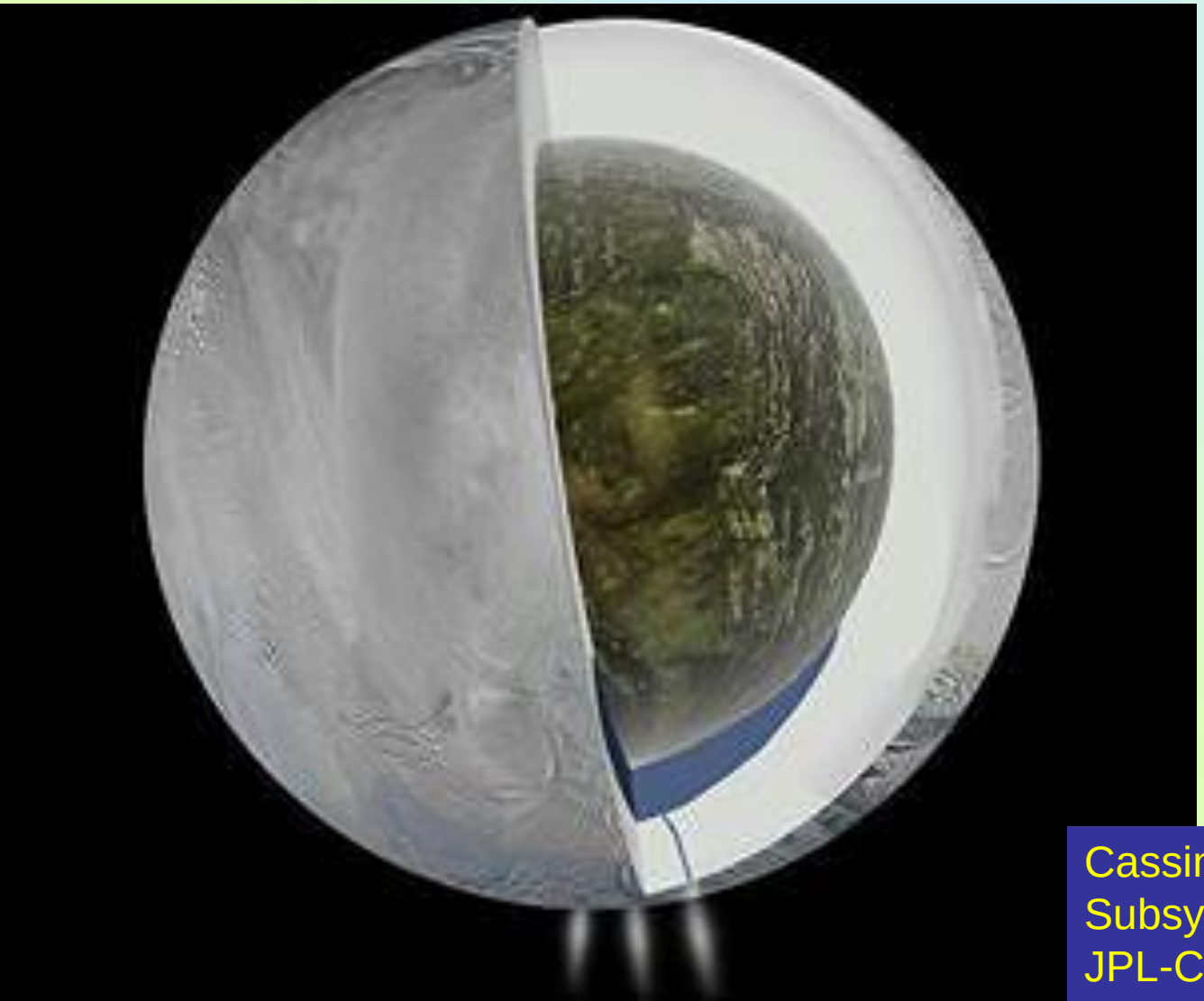
25 kGy – average sterization dose

10 kGy - *Deinococcus radiodurans* survives

1 kGy – humans die within 1 h (>4 Gy – all die within 30 days)

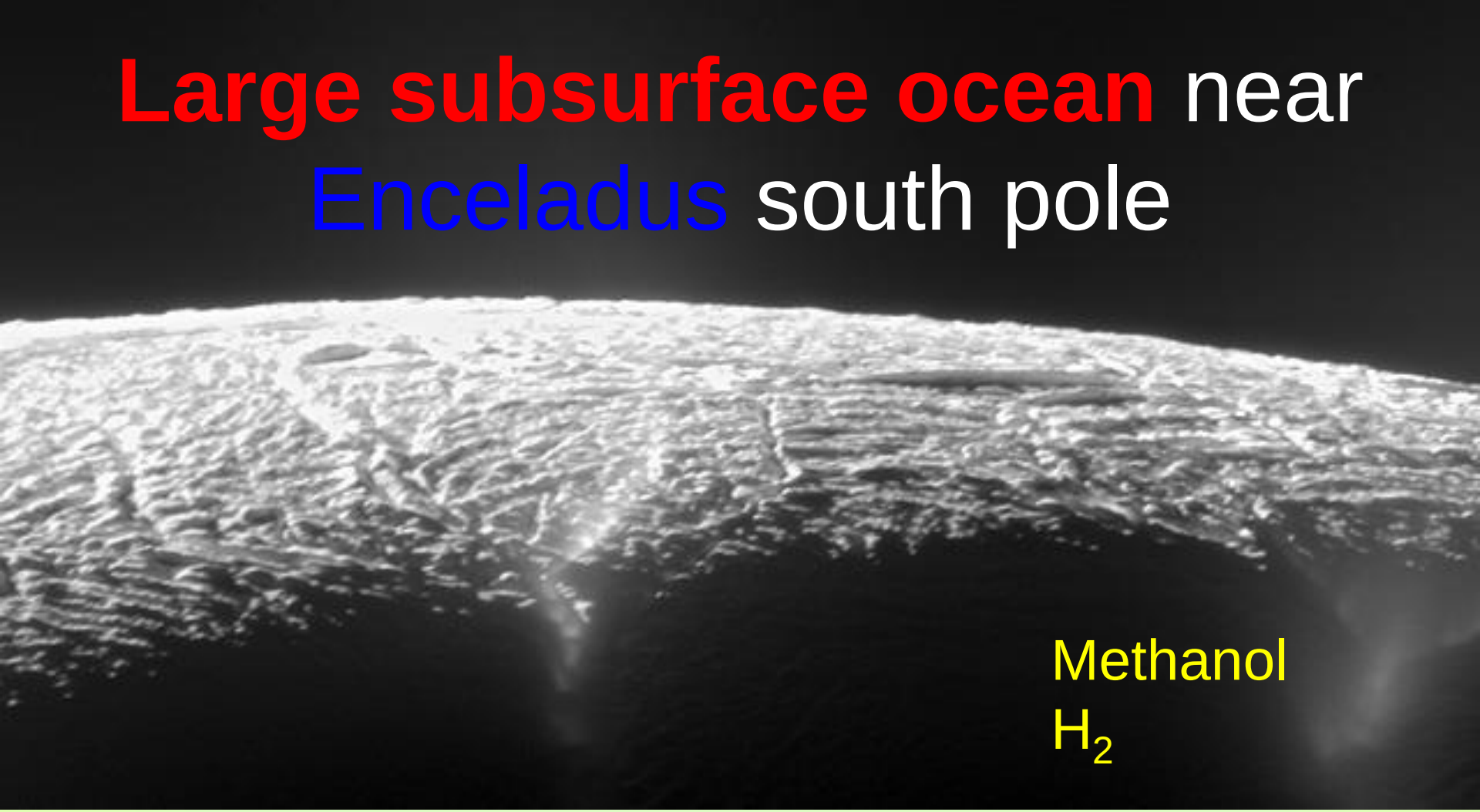
Temperatures range from as high as about 140 Kelvin (-133oC) in dark material at the equator to as low as about 50 Kelvin (-223oC) in bright icy patches at the poles

Large subsurface ocean near Enceladus south pole

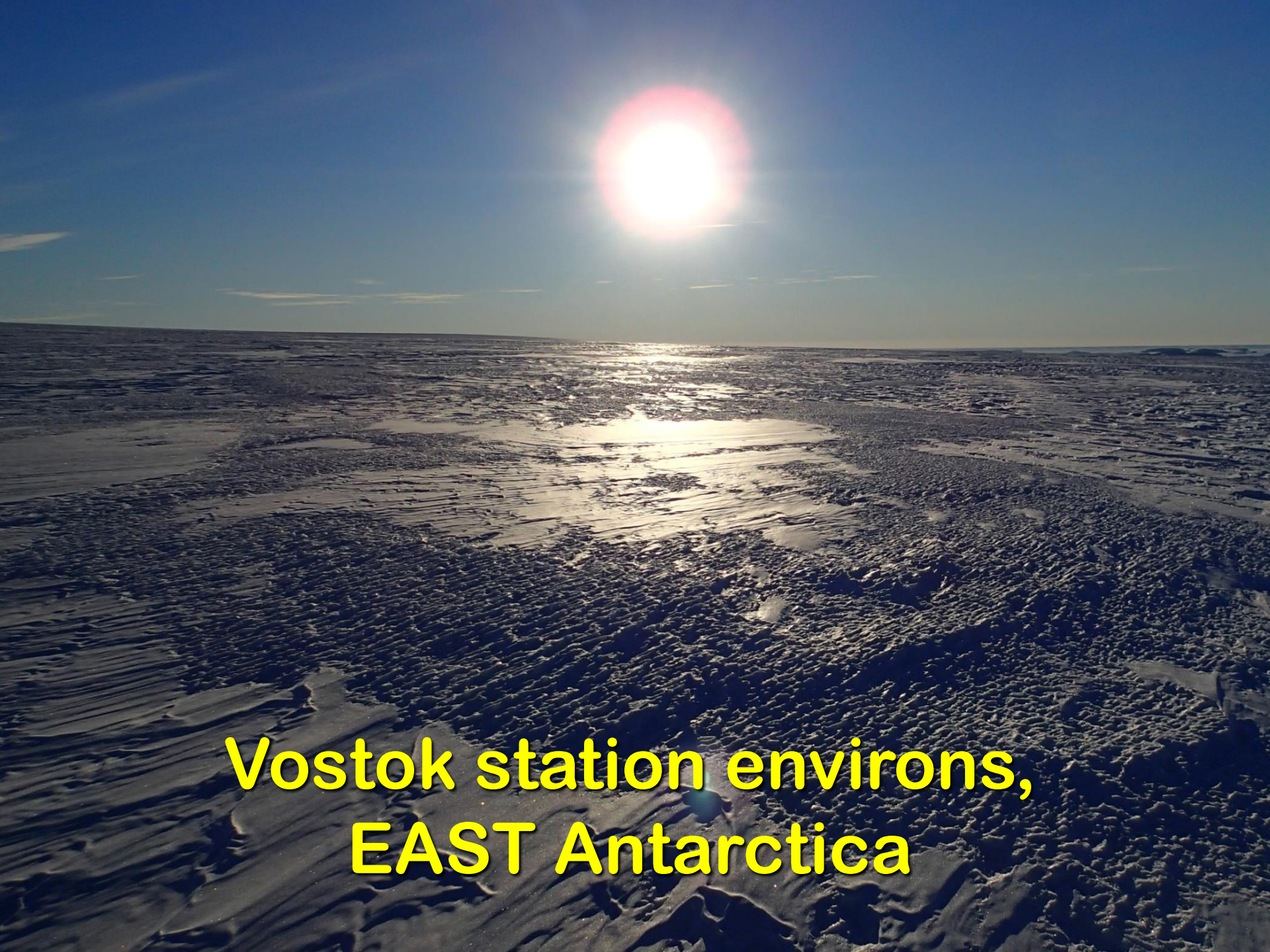


Cassini Imaging Science Subsystem –courtesy NASA-JPL-Caltech

Large subsurface ocean near Enceladus south pole

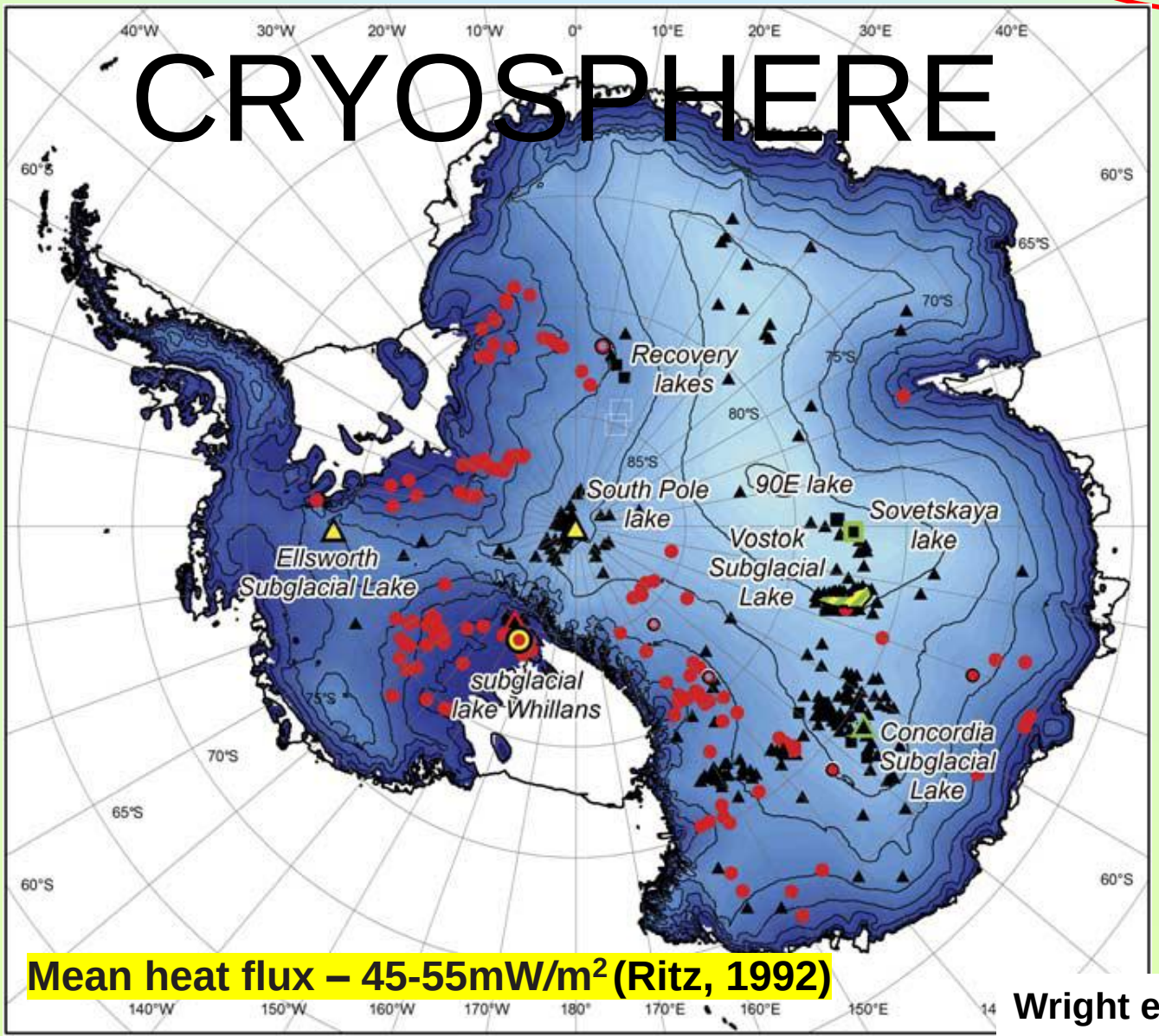


Saturn's moon Enceladus spews water vapor and ice particles from fractures in the ice. Scientists have found about 100 of these geysers.
Credit: NASA/JPL-Caltech/SSI

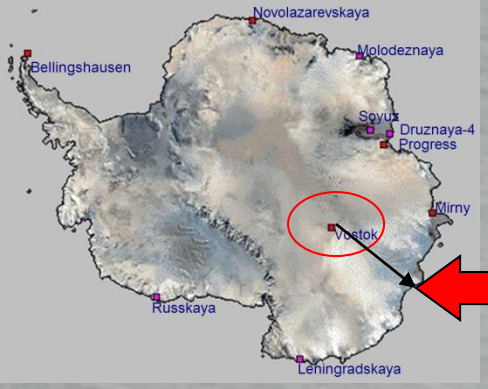

Vostok station environs,
EAST Antarctica

402 'lakes' – 2016 (Siegert, pers. comm.)

SALE Subglacial Antarctic Lake Environments - 379



Wright et Siegert , 2012



Vostok station (+3488m)

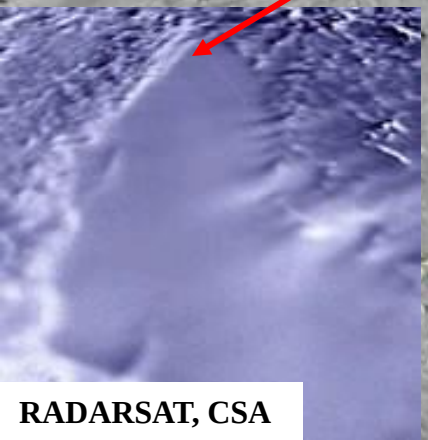
(78°S, 106°E) (since dec 1957)

67 kPa – 67% oxygen

1260 km from the coast

21 July 1983

Absolute minimum -89.2°C
Average ann. Temp. -55.1°C



RADARSAT, CSA

Lake Vostok (-3663m)

Lake Vostok Discovery

First reported at the **23rd session of SCAR in Rome** in **1994**

Published in ***Nature***
(**Kapitsa A.P. et al., 1996**)

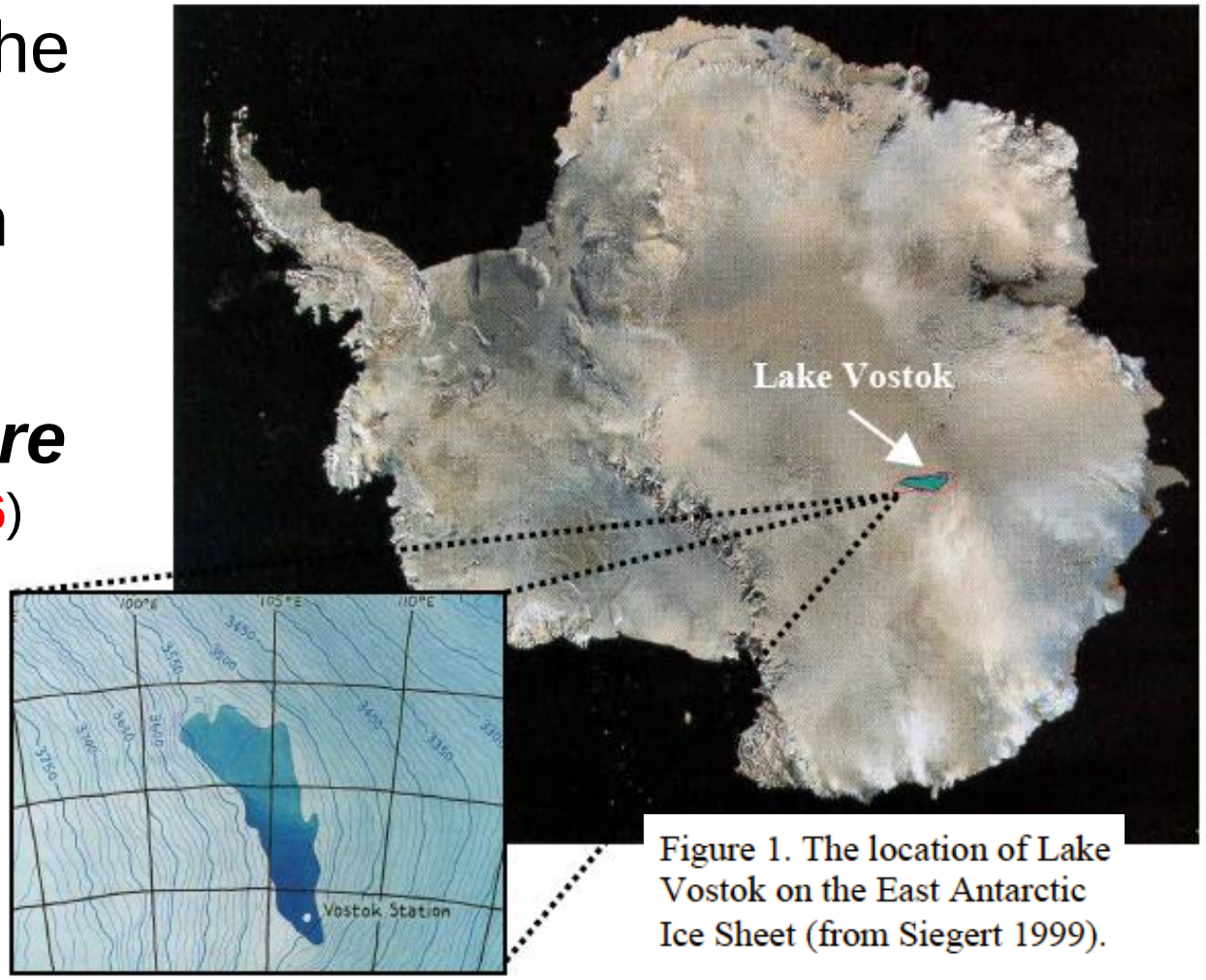
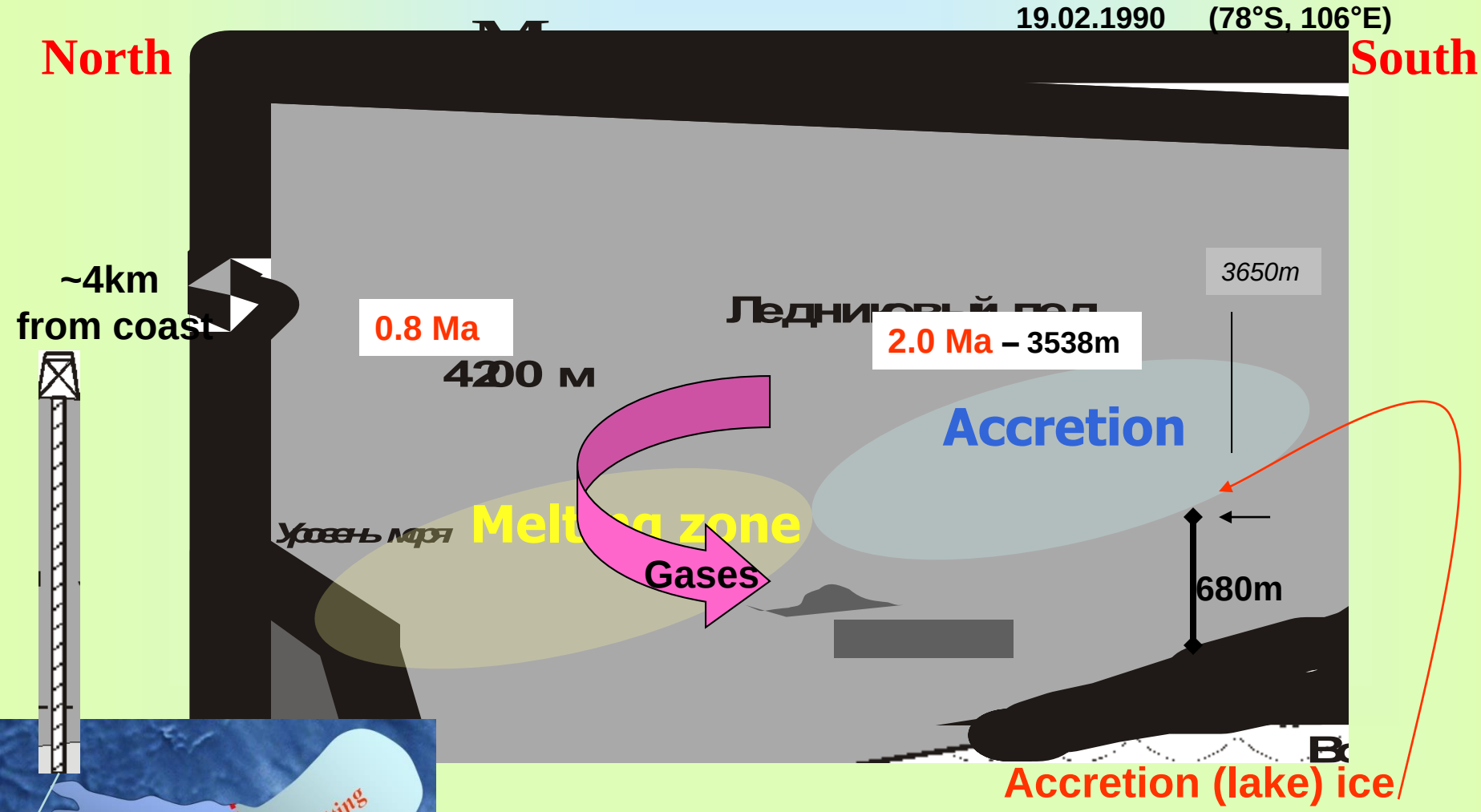
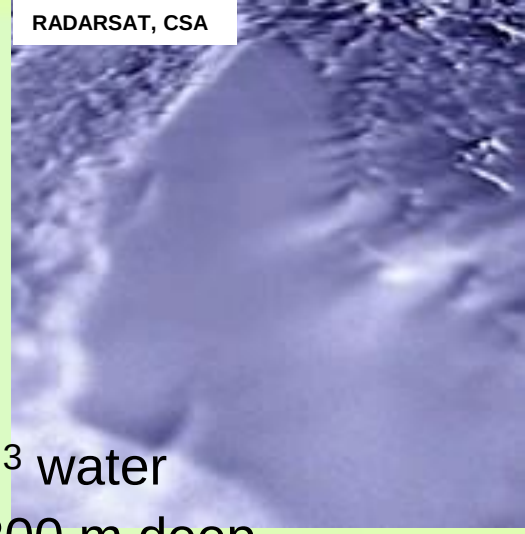


Figure 1. The location of Lake Vostok on the East Antarctic Ice Sheet (from Siegert 1999).

Kapitsa, A. P. & Sorochtin, O. G. *Sov. Antark. Eksped.* Info. Bull. **51,** 299–302 (1965)

Lake Vostok et ice core

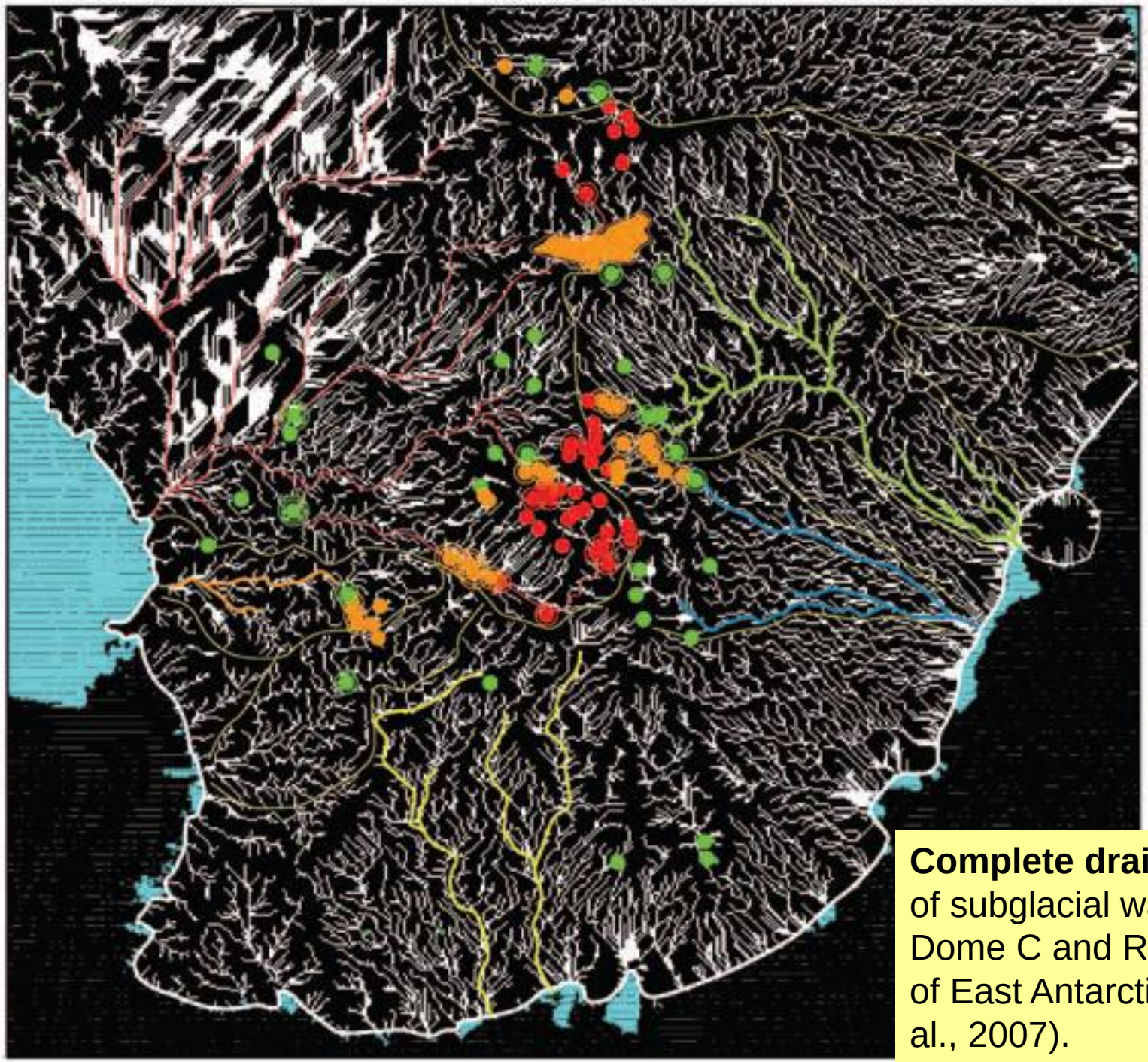




Lake Vostok settings

- **Huge** subglacial lake (*Popov et al., 2011*)
  - **270 x 70** km in size; **15 800** km^2 area; **6 100** km^3 water
  - **Two main basins** with the ridge between 150-200 m deep
  - Average depth - **400** m; max depth – ~**1220** m (south basin)
  - Water renewal – **80-100** Kyr
- **Buried** beneath 2.0-0.8 Myr old **3750-4200** m thick ice sheet
- **Isolated** from surface biota for >**14** Ma
  - Age of water (melted ice) - ~**1 Ma**
- **No hydrological links to other lakes**

2.0-0.8 Ma years old



Complete drainage pathways of subglacial water around the Dome C and Ridge B regions of East Antarctica (Siegert et al., 2007).


RADARSAT, CSA

Lake Vostok known since 1994

Published in ***Nature*** (**Kapitsa et al., 1996**)

Friendly environment?

FREE (LIQUID) WATER!

- Deeply ice buried (in dark) – **4 km**
- High pressure – **337-377** bar
- Permanently BUT not very cold – **-2.65**°C
- Likely **oxygen supersaturated** - **800 mg/L** (upper bound **700-1300** mg/L) – **320 mg/L**

(V Lipenkov – estimate from frozen water)

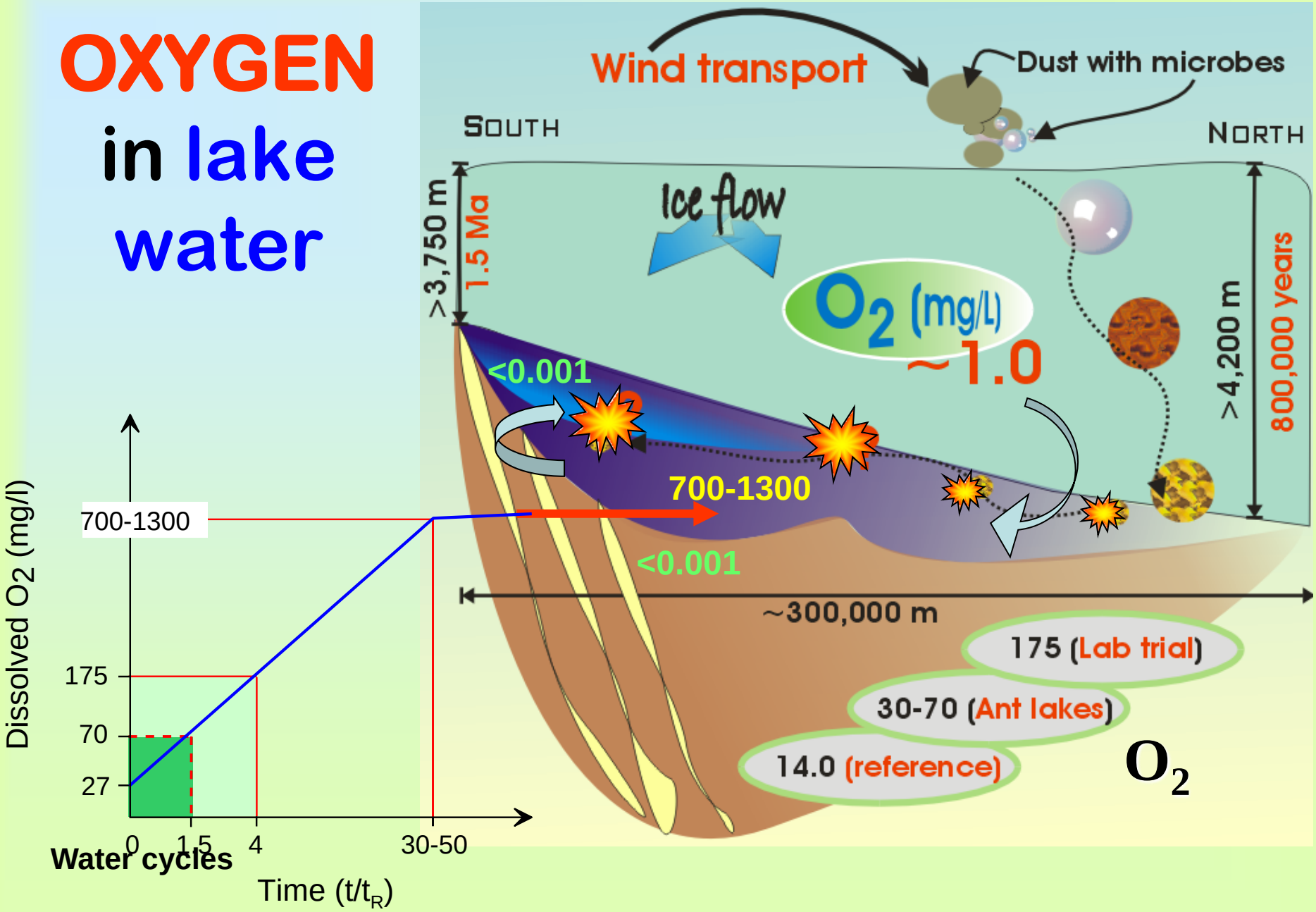
Low temperature limit for terrestrial life (Archaea, Bacteria, Eukarya)

Cell division by terrestrial microbes has not been reported below **-18°C** (255 K)

Cellular metabolic activity has not been demonstrated below **-33°C** (240 K), although some biophysical processes may be functional at lower temperatures

Rummel et al 2014 Astrobiology 14(11)_887-968

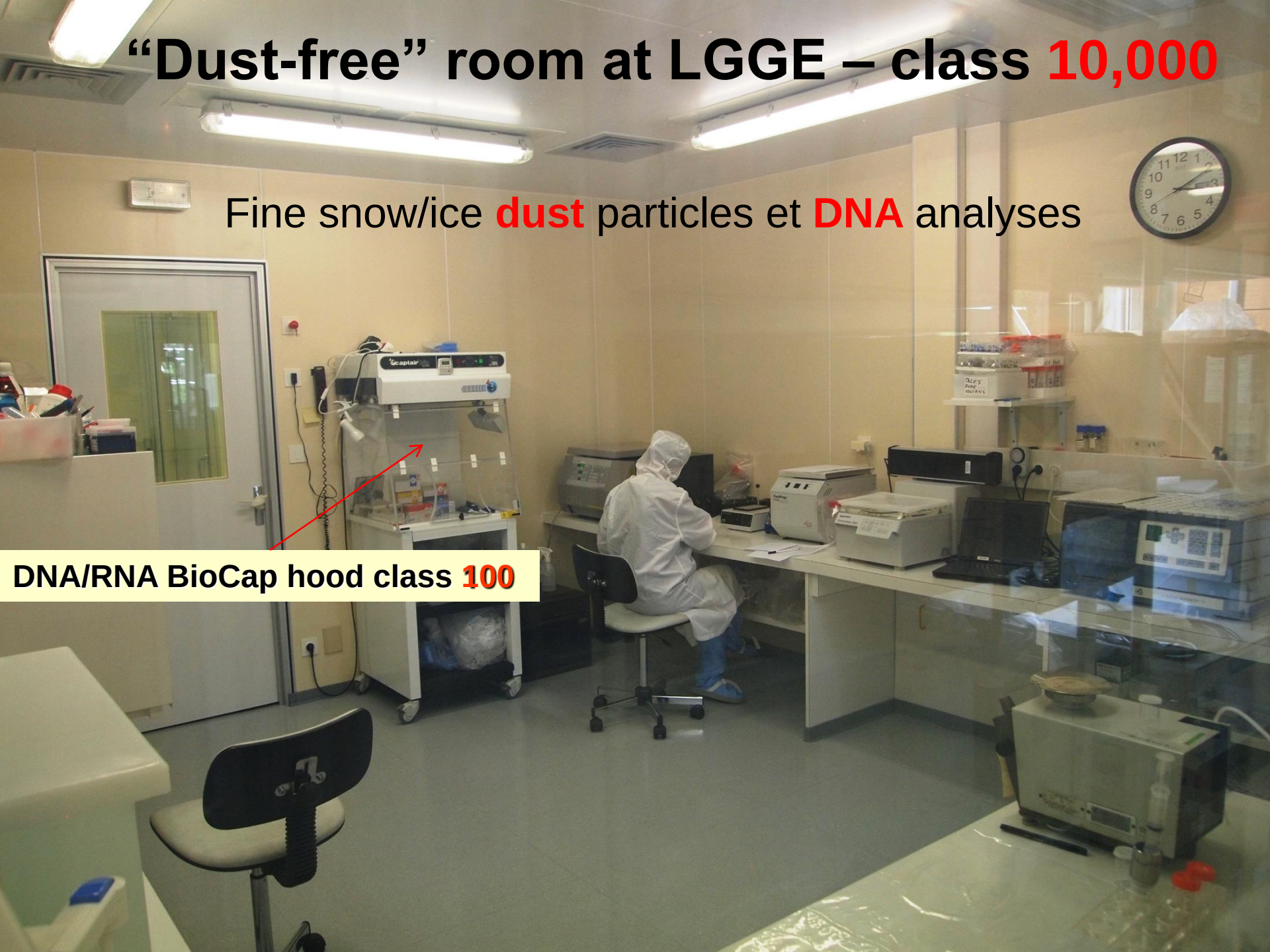
OXYGEN in lake water



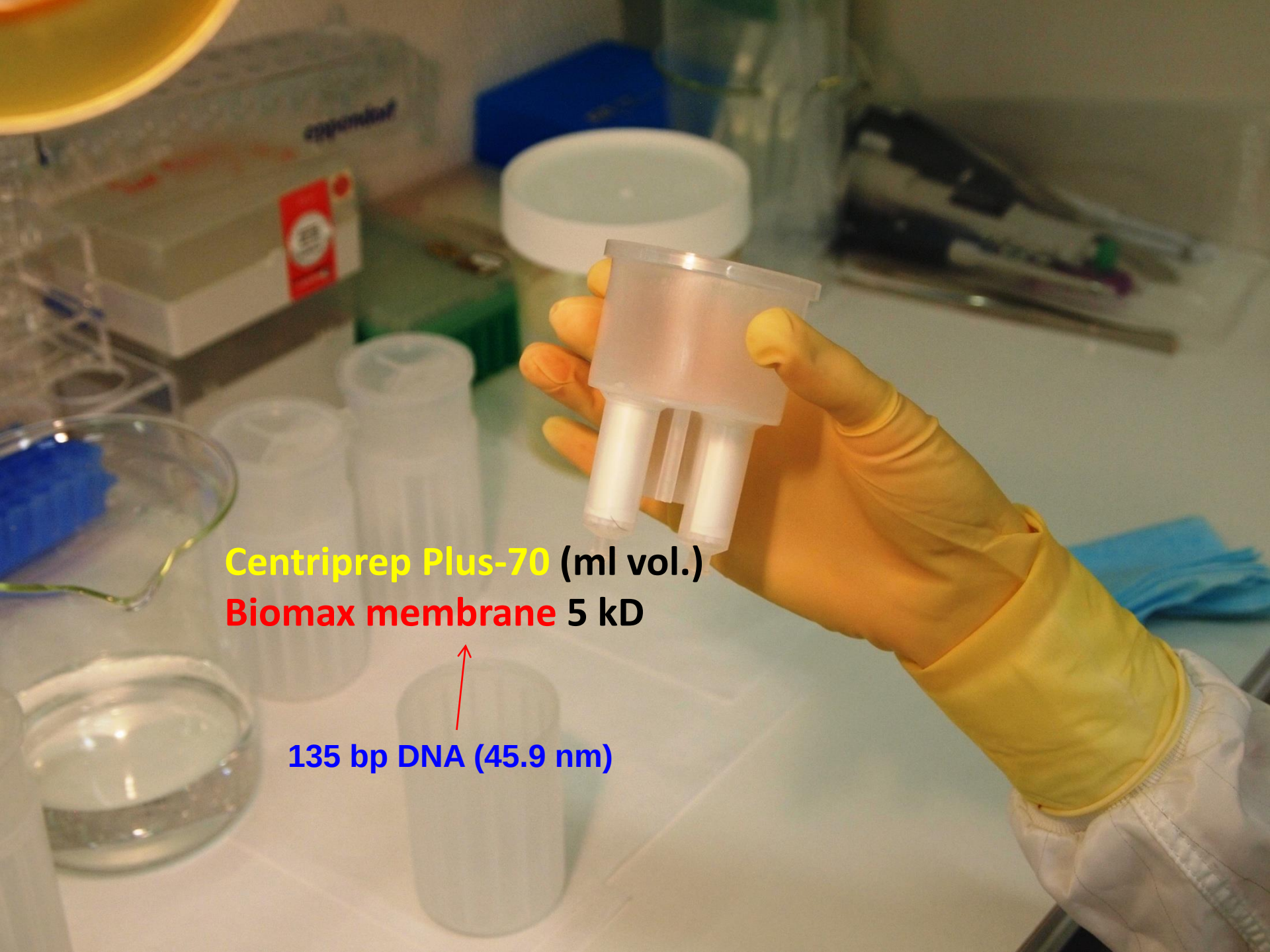
Cold room at LGGE

“Dust-free” room at LGGE – class 10,000

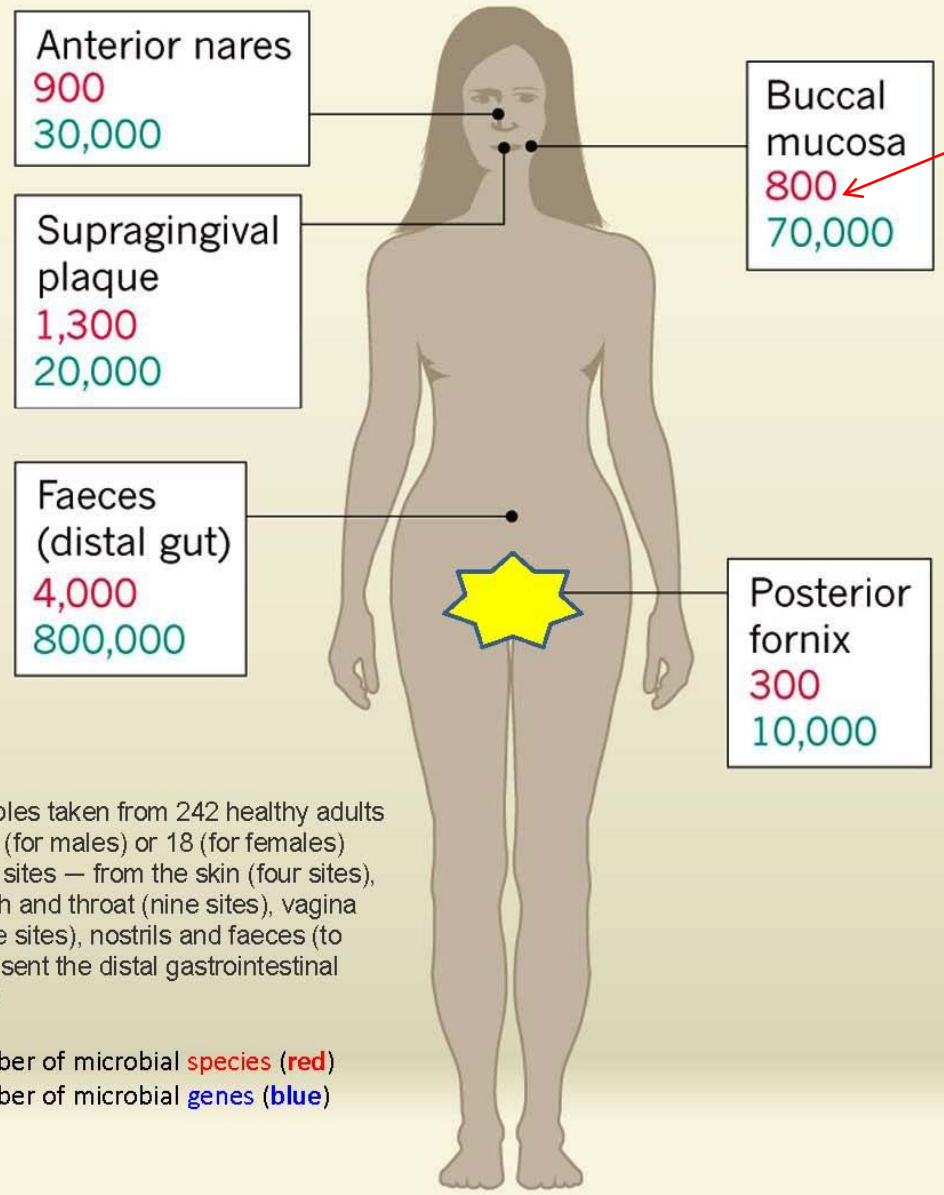
Fine snow/ice **dust** particles et **DNA** analyses



DNA/RNA BioCap hood class 100


Centriprep Plus-70 (ml vol.)
Biomax membrane 5 kD
135 bp DNA (45.9 nm)

Variation in diversity



Samples taken from 242 healthy adults at 15 (for males) or 18 (for females) body sites — from the skin (four sites), mouth and throat (nine sites), vagina (three sites), nostrils and faeces (to represent the distal gastrointestinal tract)

Number of microbial species (**red**)
Number of microbial genes (**blue**)

Relman 2012 Nature 486_194-5 - Microbiology: Learning about who we are

Number of microbial species (red)
Number of microbial genes (blue-cyan)

~~**Microbial inhabitants outnumber our body's own cells by about ten to one**~~

Vostok ice for Biology

REQUIREMENTS:

- Comprehensive **Biological controls**
  - Sham/mock DNA extraction
  - Negative PCR
  - Ice core wash water
  - Lab Environment (dust)
  - Vostok drill fluid
  - Outer-core (optionally)
  - **Repeatability**

309 seqs **July 2017**

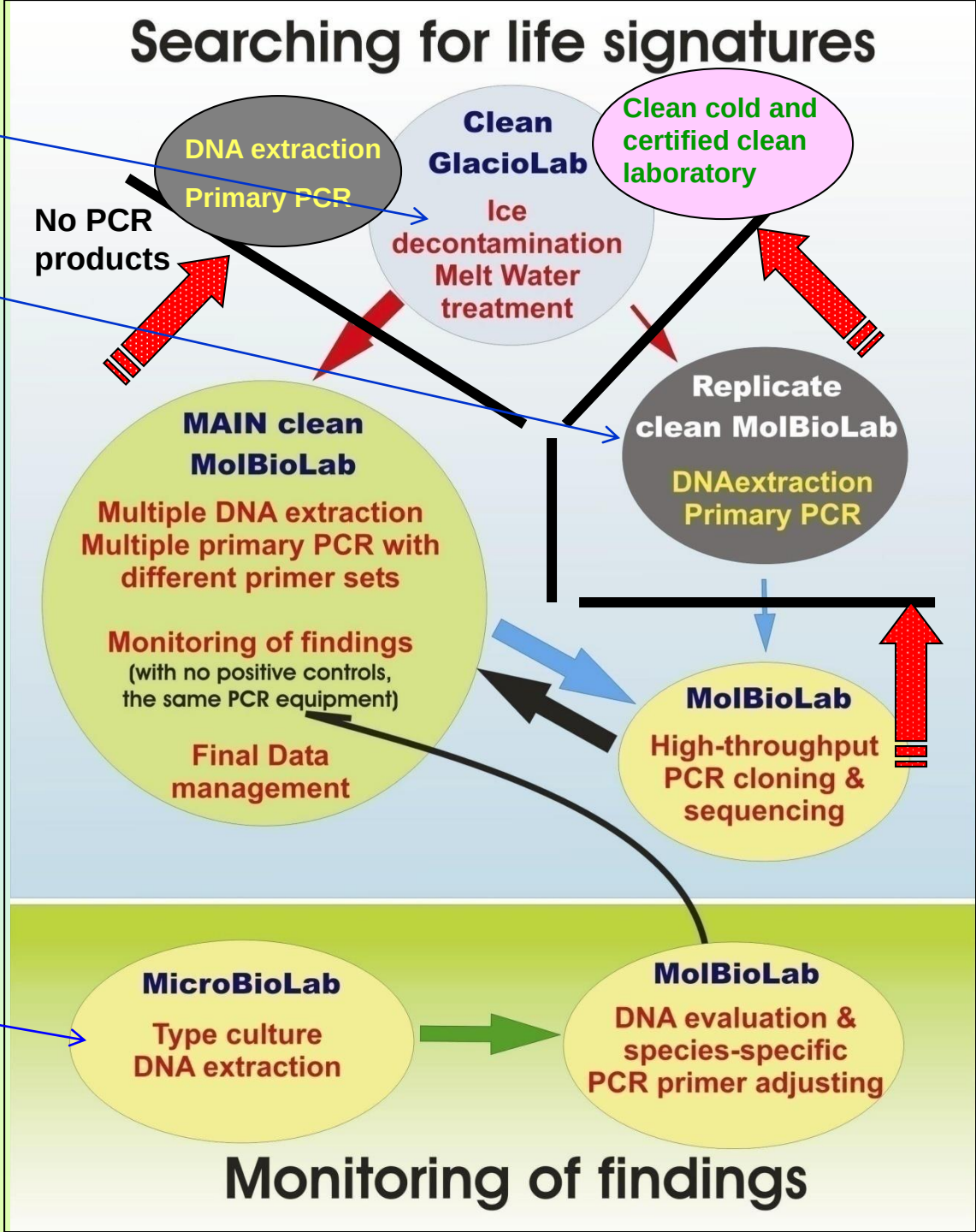
Contaminant databases

Grenoble – IGE/**LGGE** UGA

Lyon – l'ENS

A **framework** for biological studies adopted for Lake Vostok

Grenoble – IGE/**LTHE** UGA



We believe

Whatever we find

It's going to be special stuff, unique stuff, life forms we've never encountered before

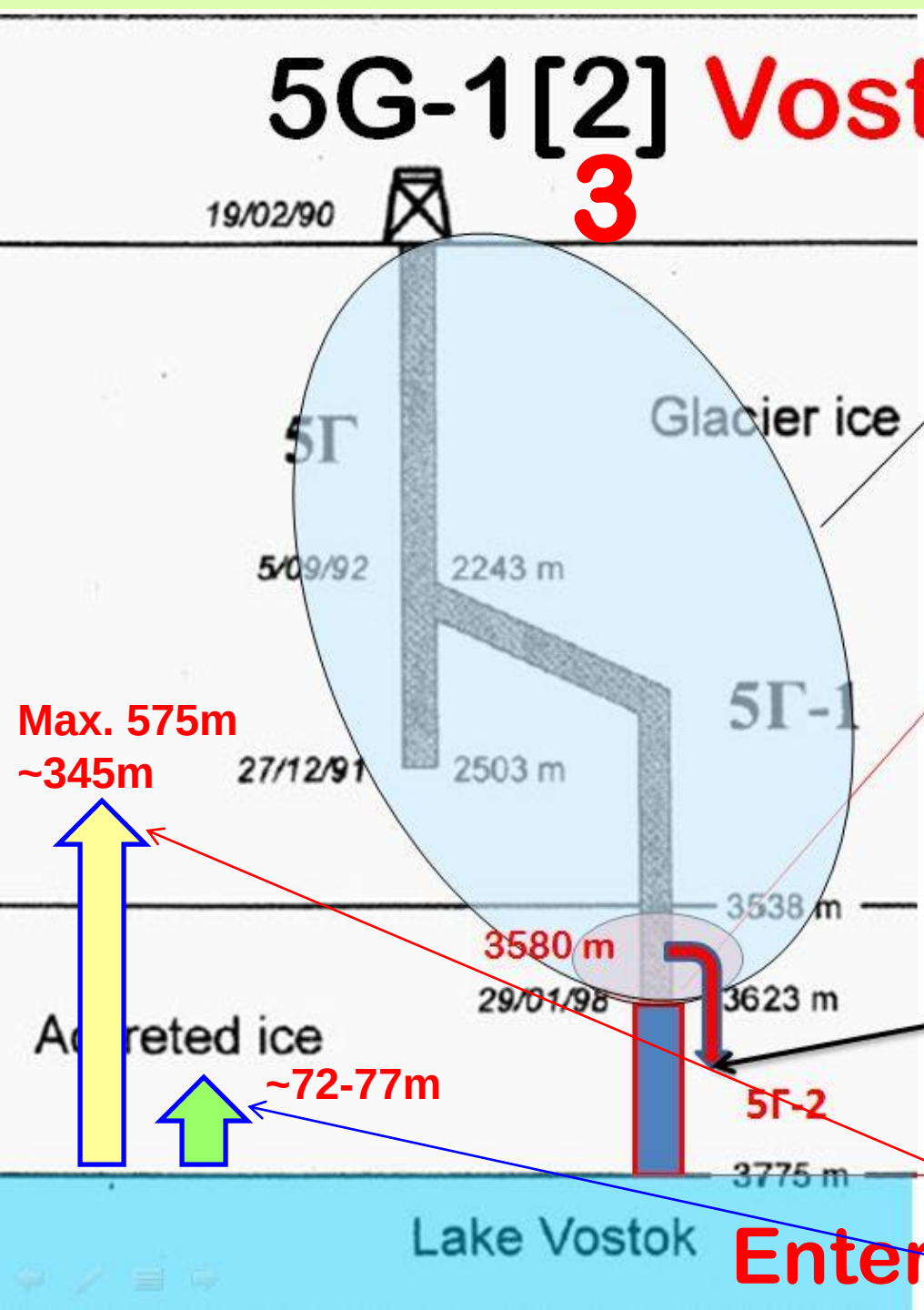
Oxygen-loving?

Extra-terrestrial?

RESULTS

Bacteria in Vostok drill-bit et borehole frozen water

5G-1[2] Vostok borehole



Russian-French-US collaborative program of Deep Drilling and Ice Core Study at Vostok station

Each party obtained 1/3 part of core

... **stopped for almost 8 years**

3650 m – 2005/06

3659 m – 2006/07

3667 m – 2007/08

3599 m – 2008/09

3650 m – 2009/10

~80m

3720 m - Jan 21, 2011

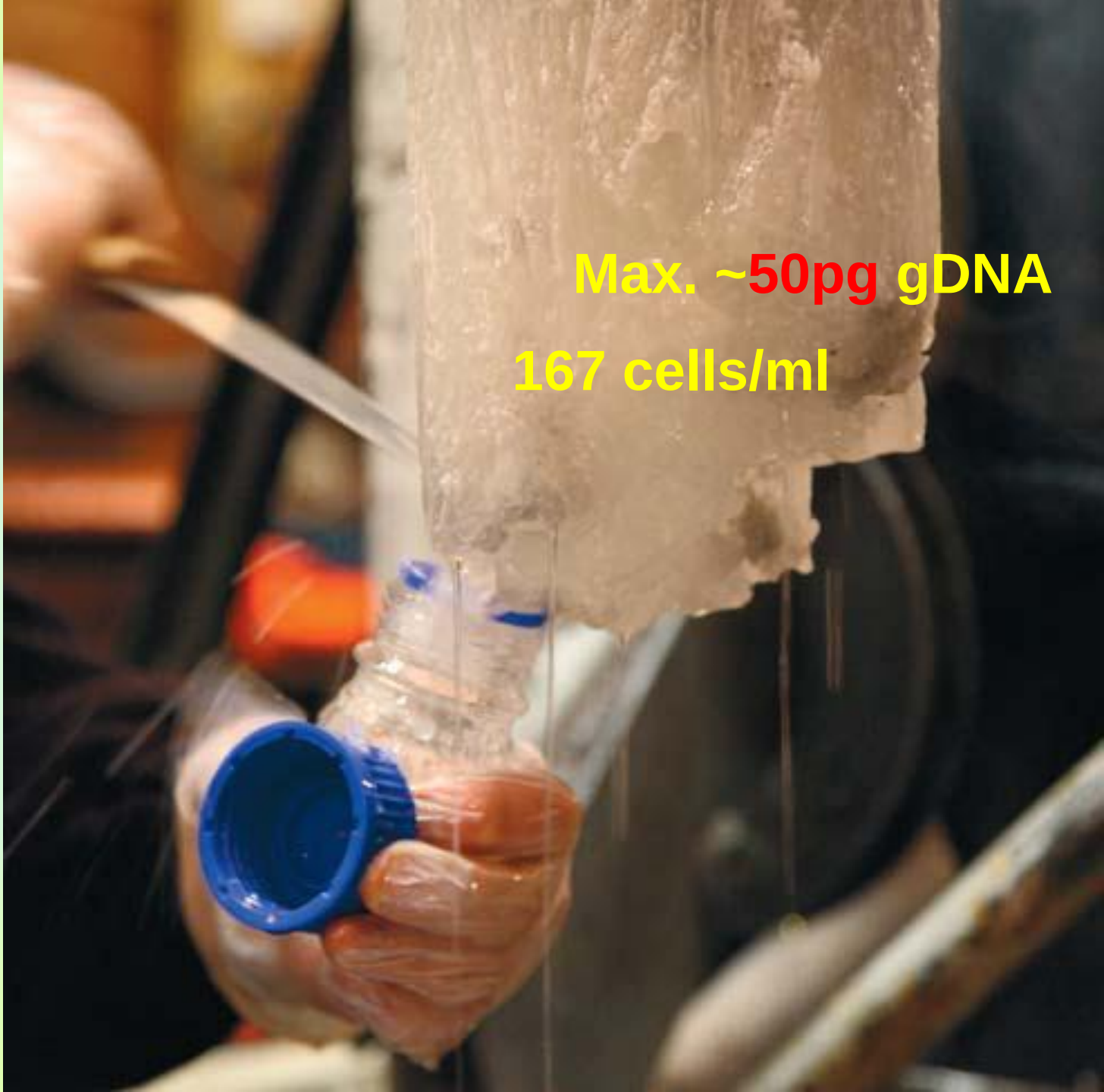
3750 m – ice-water boundary

1st - 3769.3 m – Fev 05, 2012

2nd - 3769.15 m – Jan 25, 2015

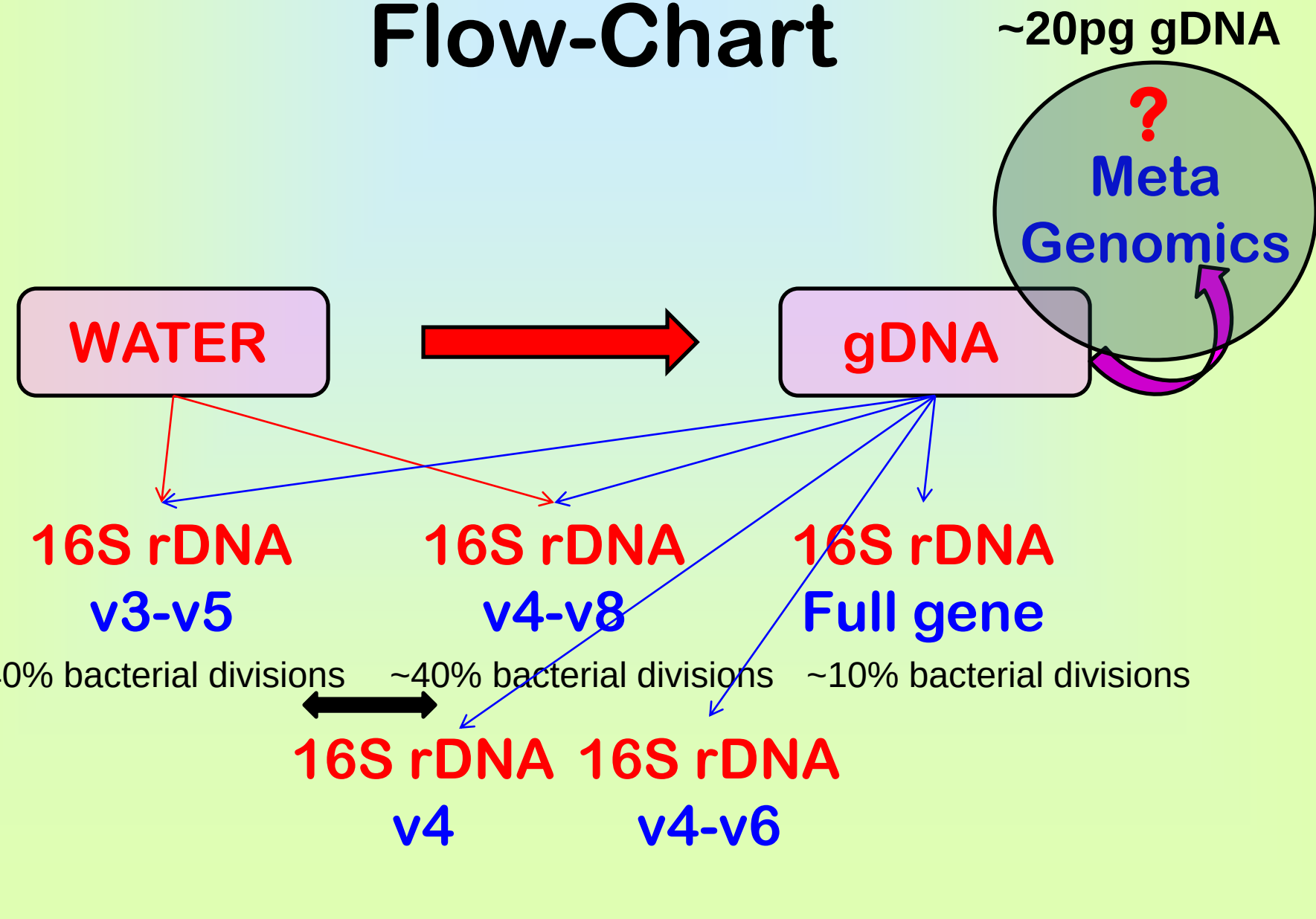
Lake Vostok 1st entry – feb 05, 2012

Drill-bit frozen water sample


Max. ~50pg gDNA
167 cells/ml

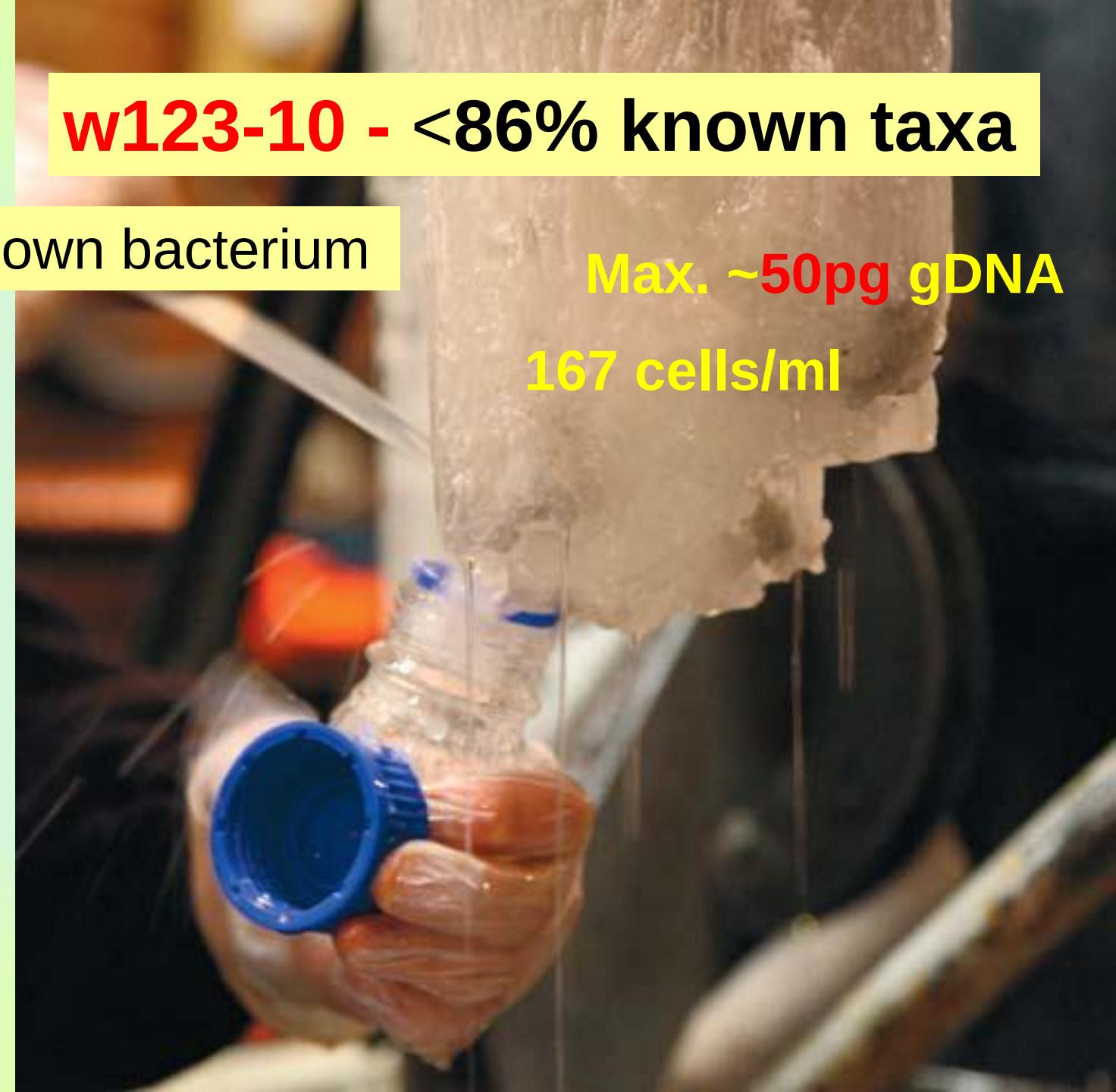
Water-DNA/PCR

Flow-Chart



Vostok frozen water (2 entries) – **50 (47 – 1st entry)** contaminant phylotypes

Clone	Phylotype
Proteobacteria	
Alpha-	8
Beta-	7
Gamma-	**21**
Actinobacteria	6
Firmicutes	7
Bacteroidetes	1



w123-10 - <86% known taxa

Unknown bacterium

Max. ~50pg gDNA

167 cells/ml

SEVEN DAYS *The news in brief*

RESEARCH

Life in Lake Vostok

Russian scientists say that they have found a hitherto-unknown type of bacterium in Antarctica's largest subglacial lake. Samples retrieved last year from Lake Vostok contain an "unclassified" bacterium whose DNA is less than 86% similar to known bacteria, Sergey Bulat of the Petersburg Nuclear Physics Institute in Gatchina announced at a meeting last week in Moscow. More tests and cleaner samples are needed to establish the physiology and biochemistry of the microbe, says Bulat. See go.nature.com/ydcmw4 for more.

CIRO DE LUCA/INFOPHOTO/PHOTOSHOT

Rebuilding Naples' City of Science

The Italian government has pledged €20 million (US$26 million) to help rebuild the City of Science (*Città della Scienza*), an iconic 12,000-square-metre exhibition centre and science museum in Naples that was destroyed by fire on 4 March (pictured). Italy's research minister, Francesco Profumo, said that the government would work with local authorities on a plan to rebuild the museum in just 18 months. The museum's management is also seeking voluntary donations. Investigators had not announced an official cause for the fire as *Nature* went to press, but they were reported by the Italian media to suspect arson.

Carbon spike

Atmospheric carbon dioxide concentrations rose by 2.59 parts per million (p.p.m.) in 2012, marking the sharpest increase since 1998, according to data from the US National Oceanic and Atmospheric Administration. Atmospheric CO_2 concentrations reflect rising global emissions, driven by developing countries, as well as variations in carbon uptake by plants and the oceans. The global average, calculated from measurements at the Mauna Loa Observatory in Hawaii and other locations, exceeded 395 p.p.m. in January, representing an increase of more than 70% from pre-industrial levels.

Anti-HIV trial

A clinical trial of the preventive powers of the anti-HIV drug tenofovir may have failed because women were not actually taking the drug. In 2011, the VOICE study, in 5,029 HIV-negative women in South Africa, Zimbabwe and Uganda, suggested that neither a vaginal gel nor tablets containing the drug could prevent HIV infection (see *Nature* 480, 10–11; 2011). Even though little unused product was returned, tenofovir was present in fewer than one-third of biological samples from participants assigned to receive it, study leaders revealed last week. Too few women took the drug to assess whether it did prevent HIV infection, although earlier studies have suggested it can work.

Elephant poaching

A surge in ivory poaching has driven the population of African forest elephants down to less than 10% of what could be supported by the available range. Researchers calculate that this species (*Loxodonta africana cyclotis*) lost 30% of its range and 62% of its population between 2002 and 2011 (F. Maisels *et al. PLoS ONE* **8**, e59469; 2013). The study was released as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) met in Bangkok; many scientists have urged CITES to clamp down on poaching and habitat destruction and to tackle demand for ivory (see *Nature* 494, 411–412; 2013).

Standard Higgs

The new particle discovered last year at CERN's Large Hadron Collider outside Geneva continues to behave just like the Higgs boson predicted by the standard model of particle physics, according to results presented last week at a conference in La Thuile, Italy. The latest data indicate that the boson decays into τ leptons as predicted, and also dampen earlier hints that the boson decays into pairs of photons more often than the standard model allows. No evidence yet points to theories beyond the standard model, such as supersymmetry (see *Nature* 491, 505–506; 2012).

POLICY

Trading species

Polar bears (*Ursus maritimus*) will not be given increased protection under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), but it looks increasingly likely that several species of shark will. Delegates meeting in Bangkok for the sixteenth CITES conference, which governs the trade in many animals and plants, voted against banning the trading of polar-bear parts.

RESEARCH

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Vostok **cells**/**phylotypes** (16S rRNA - v2-v5 reg.) - **SUMMARY**

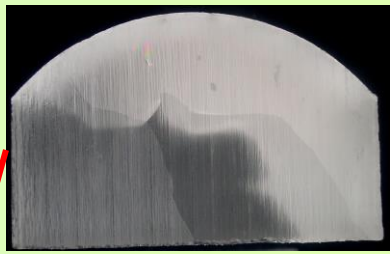
Ice type	Sample (m)	Cells/ml	(% Similarity with closest sequence in GenBank)
Snow (50 yr)	4.0-4.3	0–0.02	Contaminants
Glacier	122	1.9	
4.5 - 760 kyr	2005	2.4	
	2054	3–24	
	3471	1-4	
	3489	0	
	3504	1-5	
	3519	0-1	Contaminants
Accretion I	3547	0	Contaminants
30 – 40 kyr	3548	1	Contaminants
	3561	4–9	Contaminants + thermophile
	3607	ND	Thermophile (92%) Uncultured bacterium (410 bp, AF532061)
	3607-re	1	
	3608BK-re	ND	(95%) *Ilumatobacter fluminis* (526 bp, NR_041633) *Actino*
	5G2-3608	0	Contaminants
Accretion II	3613	3	
0 – 30 kyr	3621	2	
	3622	0.6	
	3635	4.7	
	5G2-3646	0	Contaminants
No more water pockets	3650	3.1	
	3650	4777	→ untreated surface
	3659	12	
	5G2-3714	0	
	5G2-3764	0	
Lake water	5G2-water* w2ori	167	Refer to below

99-100% - *thermophile Hydrogenophilus thermoluteolus* β-Proteo

?

99% - glacier sediment

Sulfate-reducing **bacteria**
Hydrogen-oxidizing bacteria
Methanogenic archaea?
Sulfur-oxidizing **bacteria**
Iron-oxidizing bacteria



•Lake water frozen on a drill bit

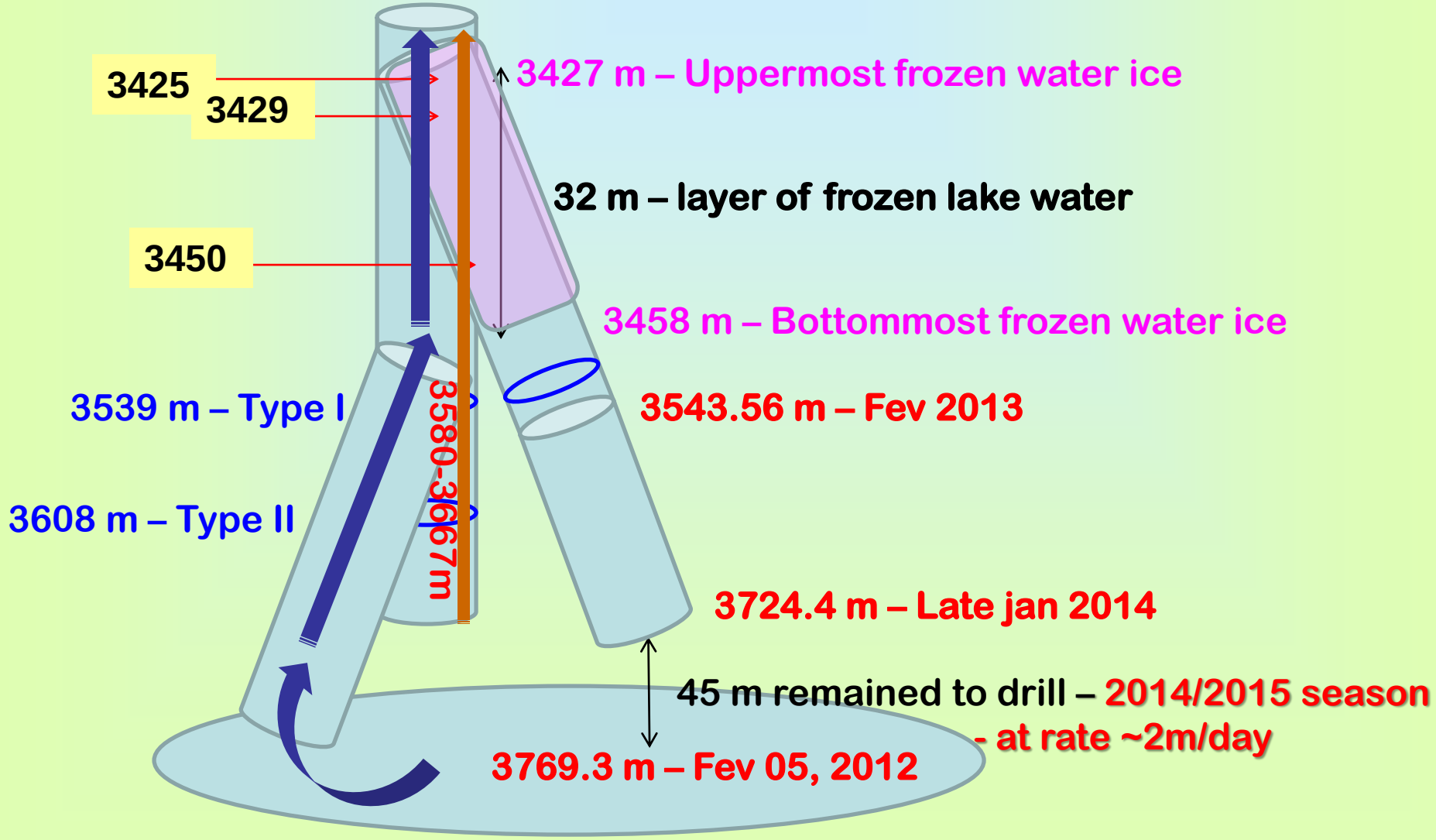
Bulat et al., 2004; 2012

Lake Vostok 1st entry – feb 05, 2012

Borehole frozen water samples

re-corred one year later

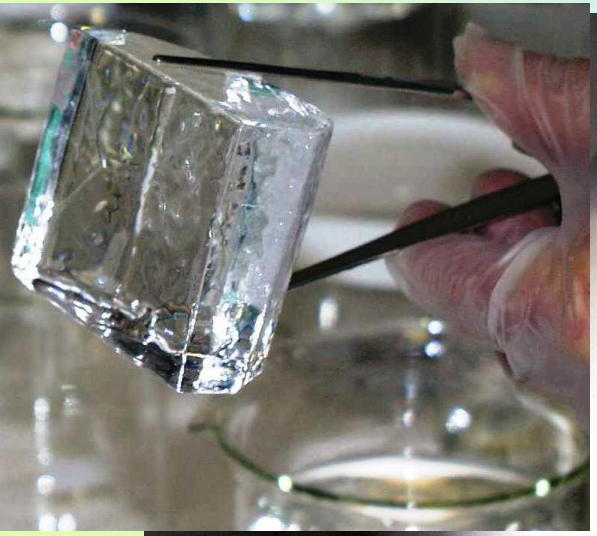
5G-2[3] Vostok borehole

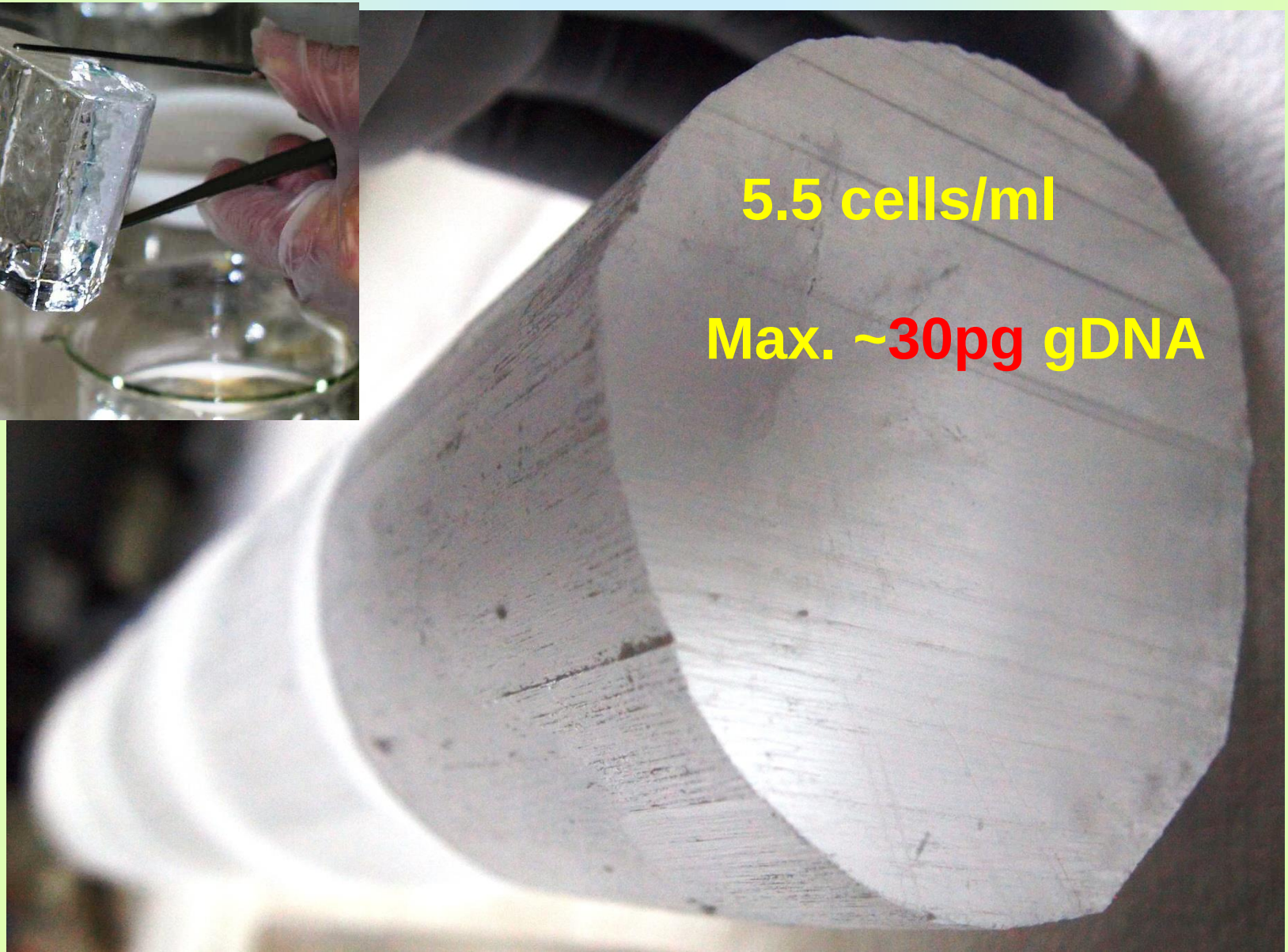


5G-1N-3425 Vostok water ice

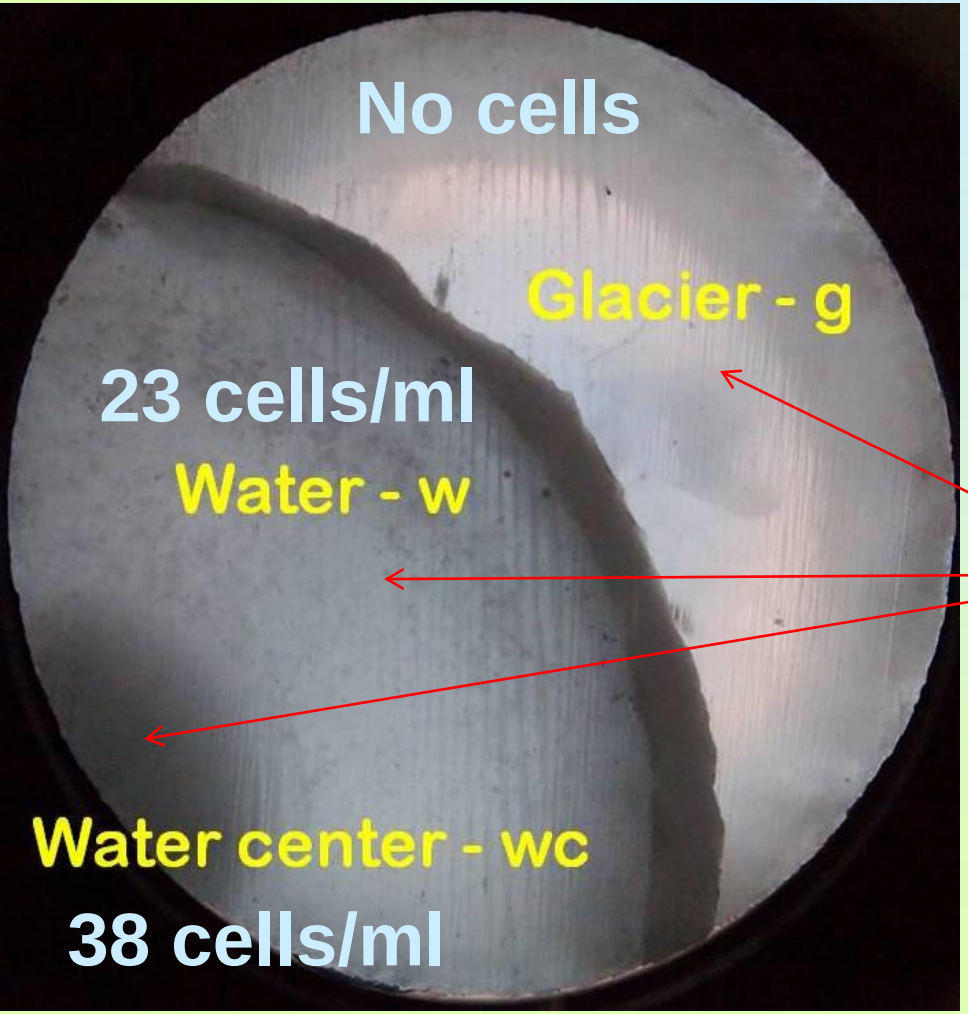
Bulat17 Sept-26

5G-1N-3429 Vostok water ice





5G-1N-3450 Vostok water ice



Ongoing analyses

Dust/microparticles

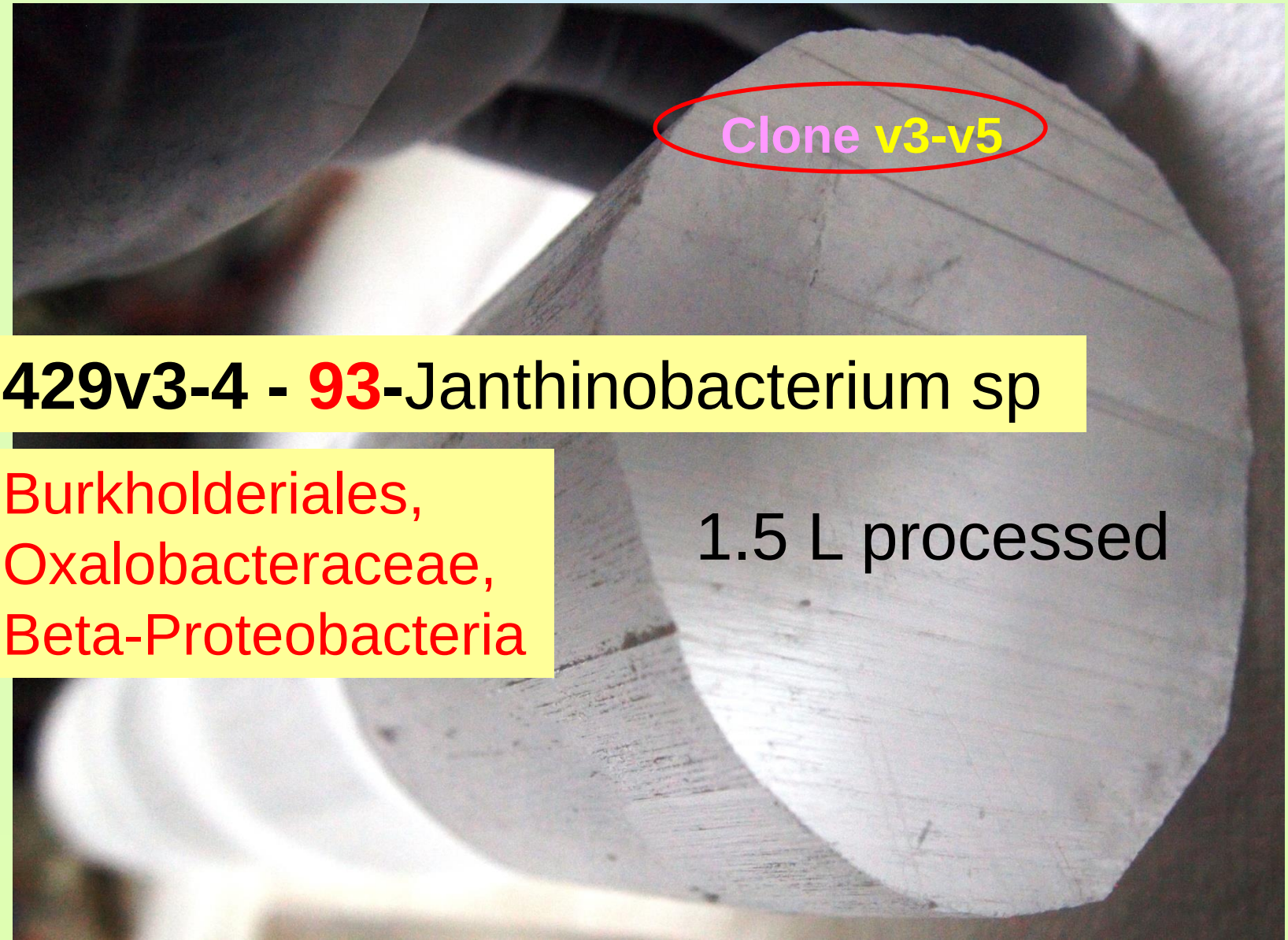
Analytical chemistry

Cell concentrations

gDNA → 16S rRNA gene amplifications

w123-10 specific PCR assay

5G-1N-3429 16S rRNA gene



3429v3-4 - 93-Janthinobacterium sp

Burkholderiales,
Oxalobacteraceae,
Beta-Proteobacteria

1.5 L processed

Vostok frozen water (2 entries) – **50 (47 – 1st entry)** contaminant phylotypes

Clone	Phylotype
Proteobacteria	
Alpha-	8
Beta-	7
Gamma-	**21**
Actinobacteria	6
Firmicutes	7
Bacteroidetes	1

CONCLUSION

It seems there is **UNKNOWN**
very tiny LIFE **(w123-10 population)**
in the **uppermost layer** of the
Lake Vostok water body

w123-10 - <**86**% known taxa

3429v3-4 – 93% - Janthinobacterium sp

Papers

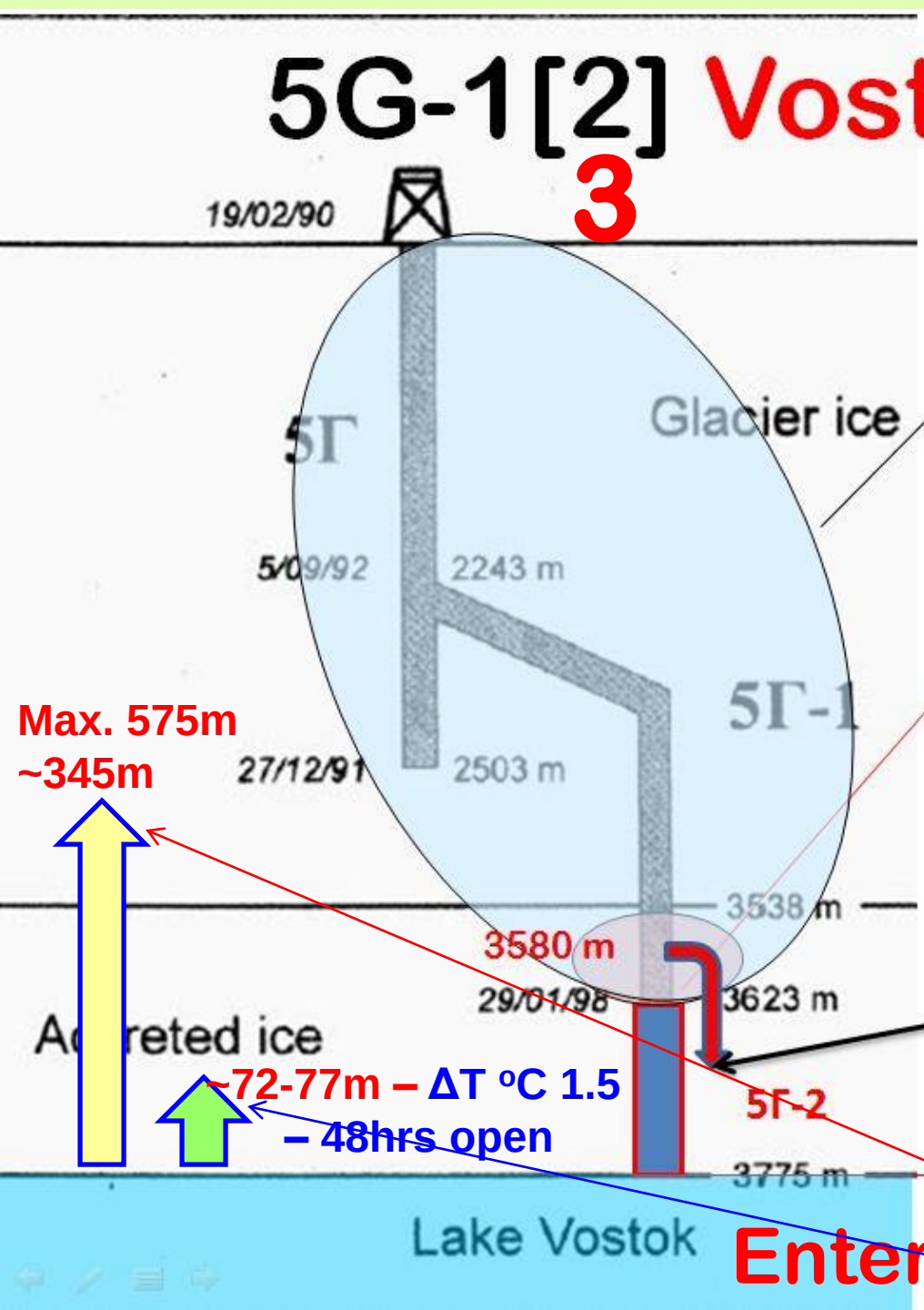
- **Bulat** S., J.R. Petit (2015) Vostok, Subglacial Lake. In Gargaud M, Irvine W (eds.) **Encyclopedia of Astrobiology**, Vol. 1, pp. 1-6 (Ch. 1765-2), Springer Heidelberg New York Dordrecht London

- **Bulat** S.A. (2016) Microbiology of the subglacial Lake Vostok: first results of borehole-frozen lake water analysis and prospects for searching lake inhabitants. ***Phil. Trans. R. Soc. A*** **374**: 20140292. http://dx.doi.org/10.1098/rsta.2014.0292

Lake Vostok 2nd entry – jan 25, 2015

Borehole frozen water samples
re-corred in **a few days**

5G-1[2] Vostok borehole



3

Russian-French-US collaborative program of Deep Drilling and Ice Core Study at Vostok station

Each party obtained 1/3 part of core

Max. 575m
~345m

~72-77m – ΔT °C 1.5
– 48hrs open

... stopped for almost 8 years

3650 m – 2005/06

3659 m – 2006/07

3667 m – 2007/08

3599 m – 2008/09

3650 m – 2009/10

~80m

3720 m - Jan 21, 2011

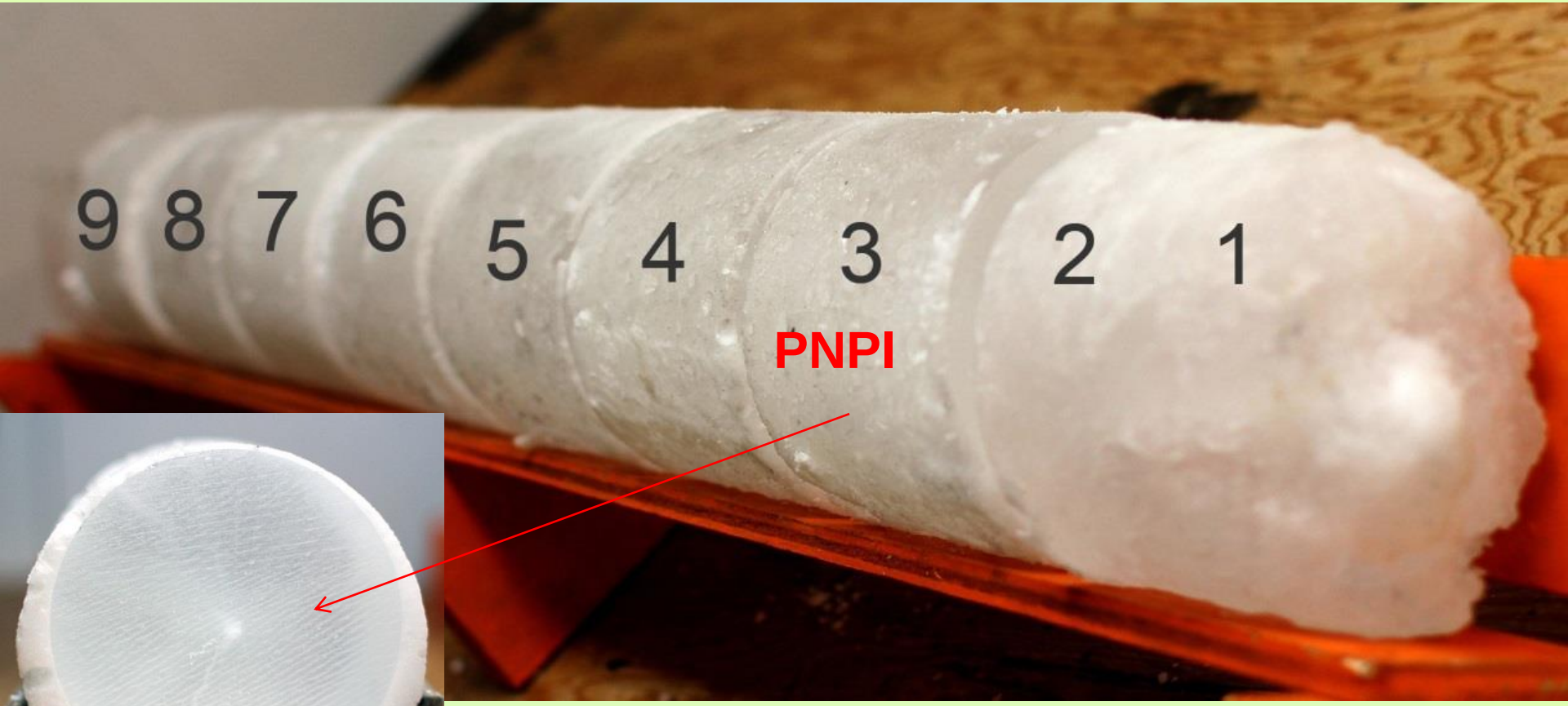
3750 m – ice-water boundary

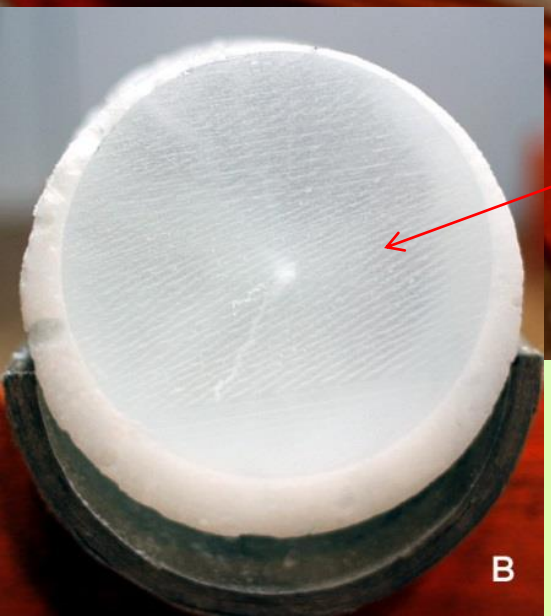
1st - 3769.3 m – Fev 05, 2012

2nd - 3769.15 m – Jan 25, 2015

Enter

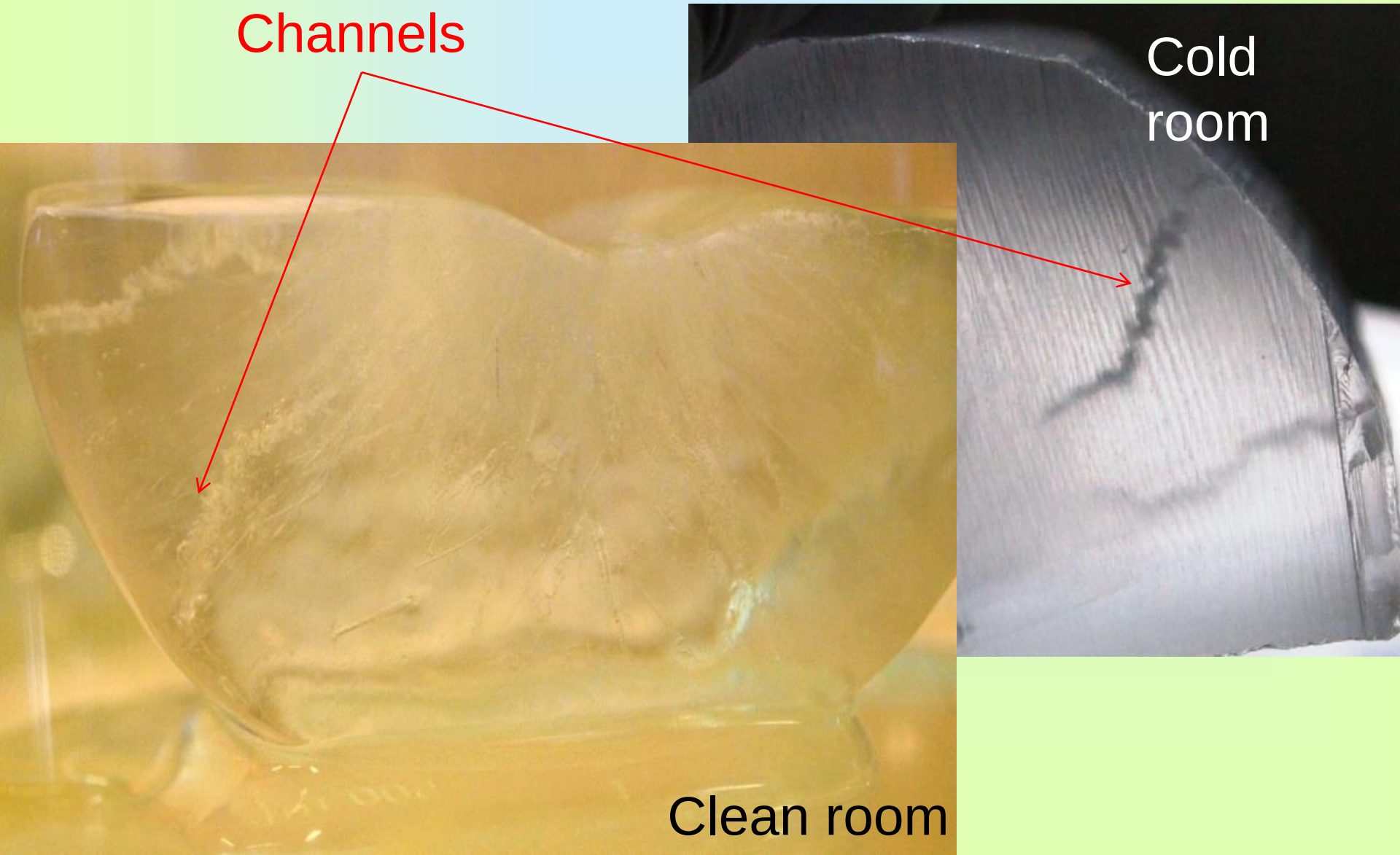
New 5G-3N borehole frozen lake water (3708.12-3708.94)



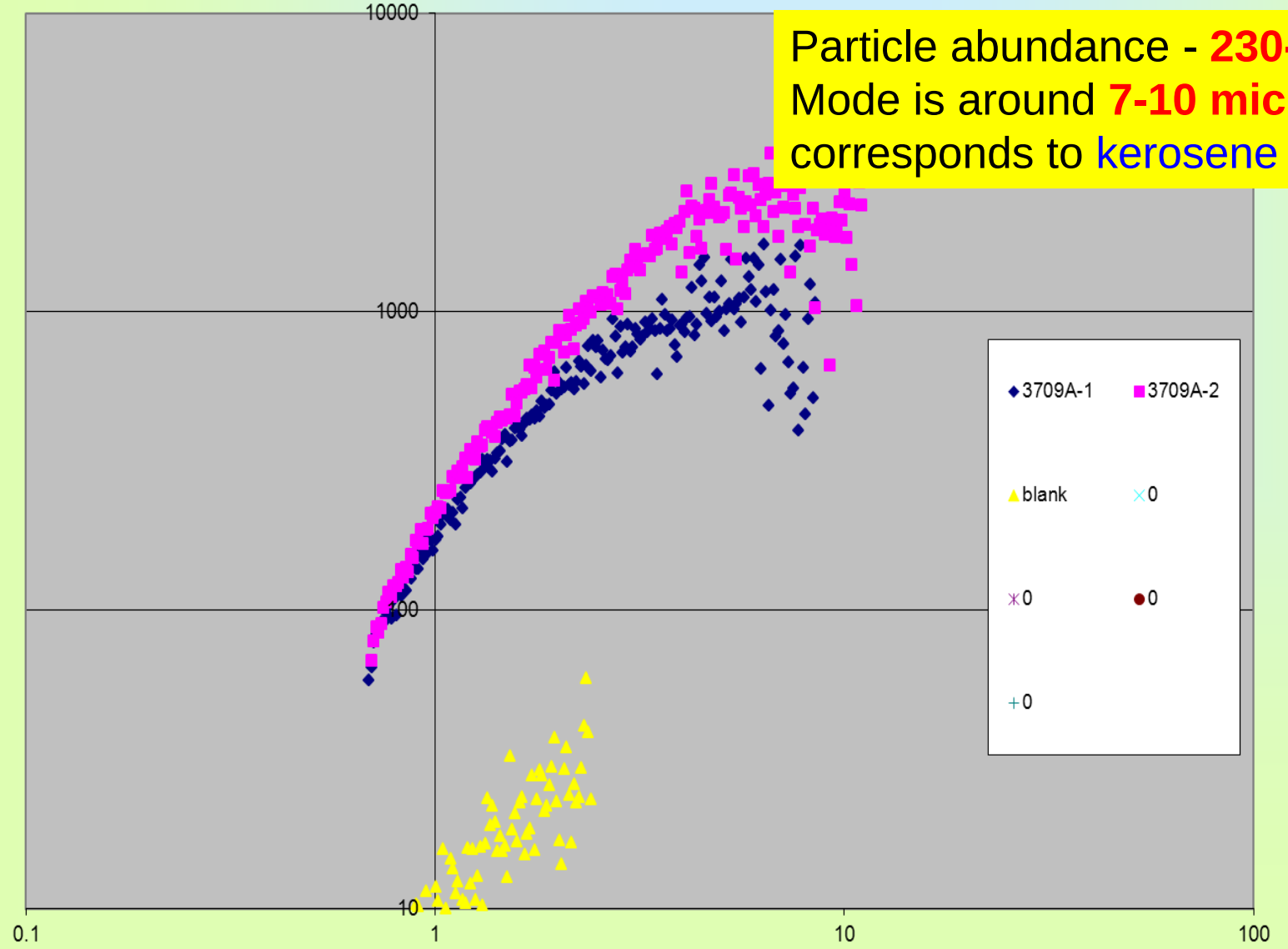


Astro-Art, Croatia

5G-3N-3709A 1-3 treatment



3709A 1-3 – Counter Coulter



Particle abundance - **230-500 ppb**
Mode is around **7-10 microns** that corresponds to kerosene micro-droplets

3709A 1-3 – **amino acid** content

- Metrohm ion chromatograph using a C4 cation and an aSupp5 anion columns
  - By Carlo Barbante
    - Institute for the Dynamics of Environmental Processes – CNR, Venice, and Department of Environmental Sciences, Informatics and Statistics, Ca'Foscari University of Venice, Venice 30123, Italy

All tested amino acids demonstrated values below of detection limits

3709A 1-3 – **cell concentrations**

- Flow cytofluorimetry using the SYBR Green-I dye and a BD FACSAria device
  - By Dominique Marie
    - Station Biologique de Roscoff
      Place Georges Teissier
      29682 ROSCOFF Cedex

16-29 cells/ml

No real populations

3709A 1-3 – rRNA gene amplicons

- Clean room condition PCR for 16S rRNA gene regions (cloning and sequencing):
  - **V3-v5 – weak signal → 4 phylotypes (contaminants)**
  - **V4-v8 – signal → 4 phylotypes (contaminants)**
  - **V4-v6 –** v weak signal
  - **V4 –** v weak signal
  - **'v3-v5' (w123-10 phylotype specific) – no signal**
  - **'v3-v5' (Hydrogenophilus-specific) – no signal**

Pseudomonas yamanorum (gamma-)
99.5-Stenotrophomonas maltophilia (gamma-)
Serratia myotis (gamma-)
99.9-Acinetobacter johnsonii (gamma-)

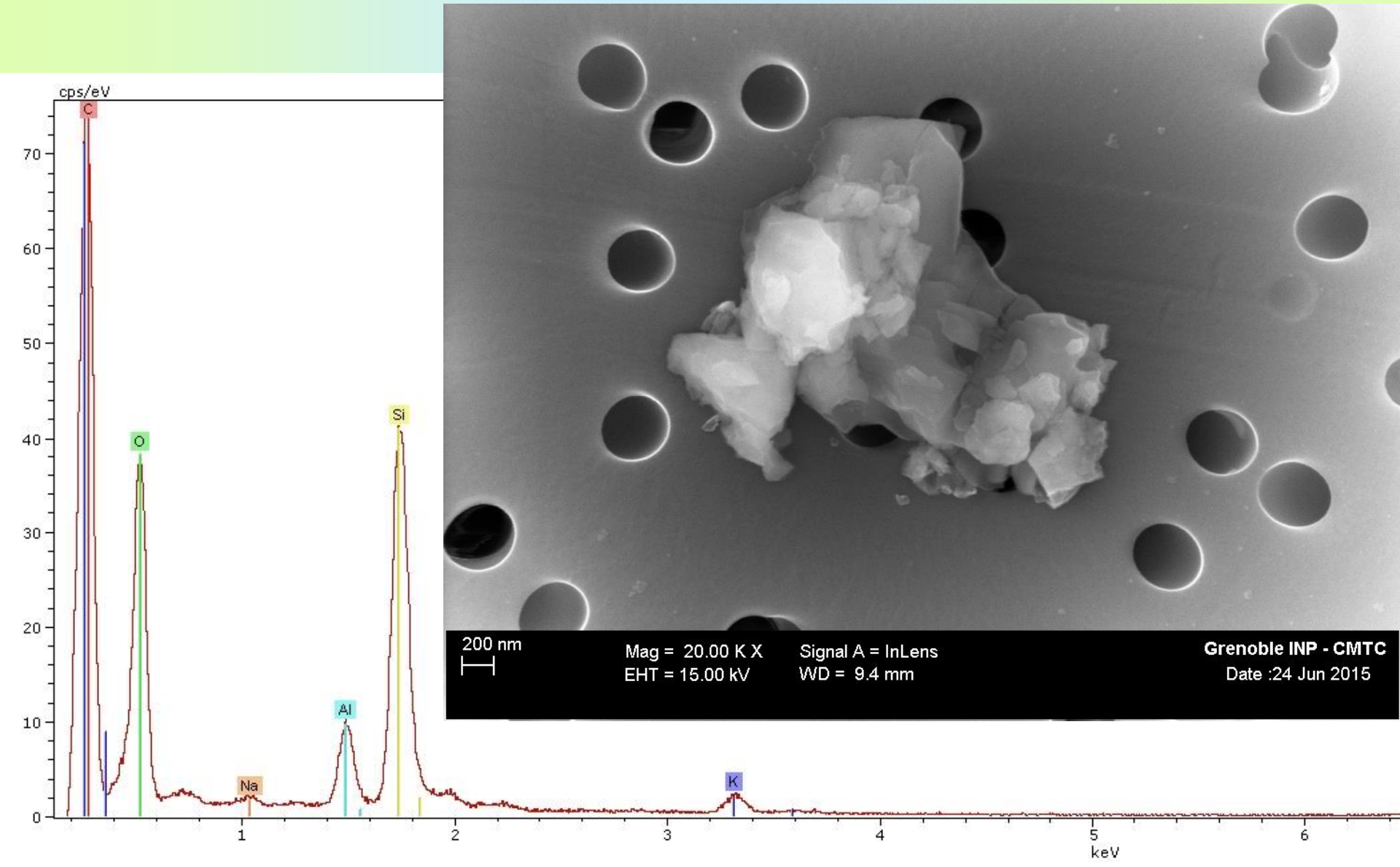
99.8-Arthrobacter russicus (actino)
99.8-Microbacterium ginsengisoli (actino)
Undibacterium oligocarboniphilum (beta-)
Escherichia coli (gamma-)

SEM-EDX analyses

SEM Zeiss Ultra 55 with Bruker 127eV - resolution at 15kV is 1nm.

3709A 1-3 frozen water (2nd entry)

Si-O-Al-[C] – 6/12 particles



Lake Vostok 3rd entry – feb 03, 2015

Borehole frozen water samples
re-corred **two years later**

5G-3N-**3698** et **3715** treatment

Freezing center

Cold room - top view

3698m

3715m

5G-3N-**3715**

Crystal ‘feathers’
and channels

5G-3N-**3698** et **3715** studies

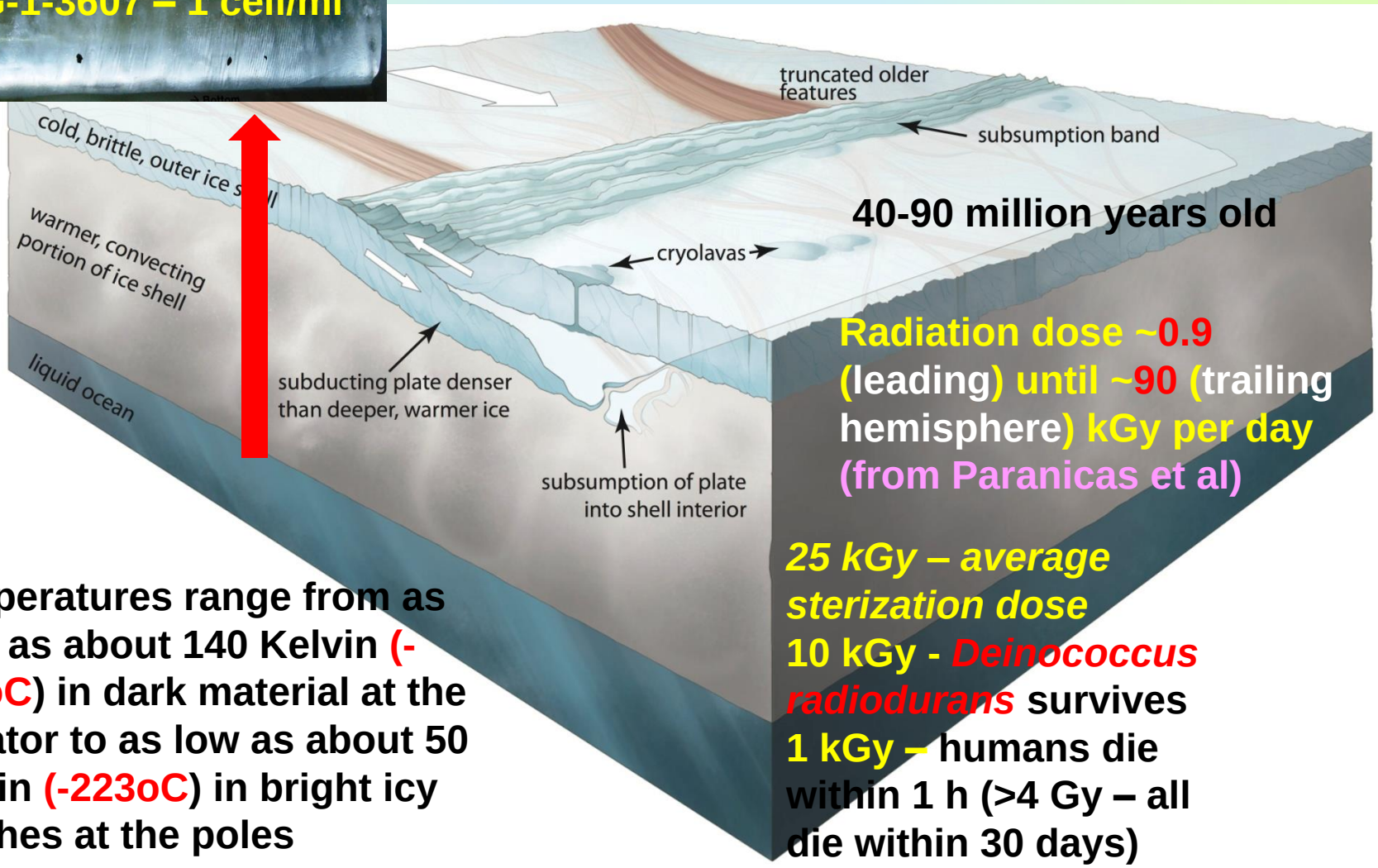
- Meltwater – strong **smell of kerosene**
  - No particles
  - No chemistry
  - Cell concentrations by flow cytometry
  - DNA analyses (16S rRNA gene v4-v6)

Ongoing since juin 23, 2017

Exciting results seem coming soon?

We surely need cleanly collected (with no DRILL FLUID) lake water

'Diving' Tectonic Plates on Jupiter's Moon Europa



40-90 million years old

Radiation dose ~0.9 (leading) until ~90 (trailing hemisphere) kGy per day (from Paranicas et al)

25 kGy – average sterization dose

10 kGy - *Deinococcus radiodurans* survives

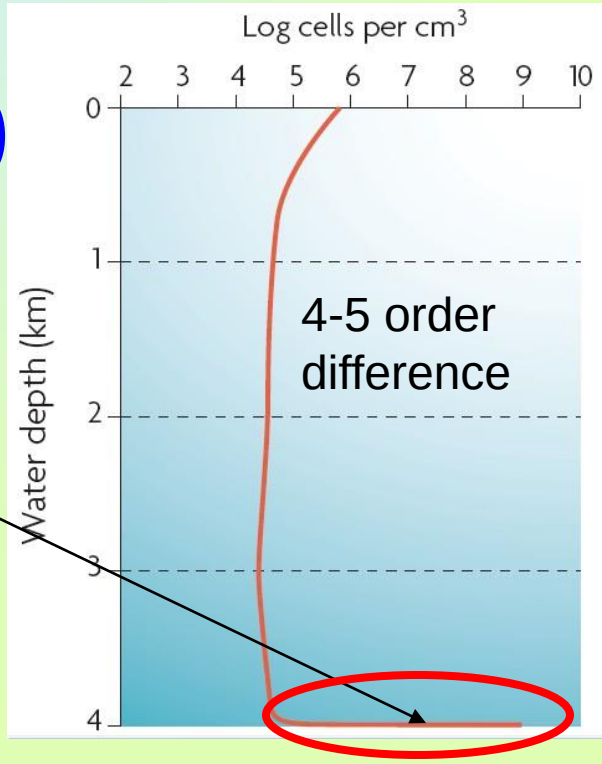
1 kGy – humans die within 1 h (>4 Gy – all die within 30 days)

Temperatures range from as high as about 140 Kelvin (-133oC) in dark material at the equator to as low as about 50 Kelvin (-223oC) in bright icy patches at the poles

10 years FUTURE DIRECTIONS

- **Rapidly frozen lake water** (within borehole) – since 2012

- Lake **water column (680m)**
- Lake **sediments (>300m)**



Microbial biomass at BIONTRANS
(*Jorgensen and Boetius 2007*)

Acknowlegements

- LGGE CNRS-UJF, Grenoble, FR
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  - Dominique Marie
- AARI, St Petersburg, RU
  - Vladimir Lipenkov, Alexey Ekaykin – ice cores
  - Valery Lukin – logistics
- PNPI Cryoastrobiol labo, St Petersburg-Gatchina, RU
  - D Karlov, M Doronin, M Khilchenko, G Pavlov, O Belova

Subglacial Lake Entry on the Horizon in Antarctica

- Subglacial Lake Vostok, the largest known subglacial lake on earth (**3769.3m-1.5km**)
- Subglacial environments beneath West Antarctic Whillans ice stream (**800m- 2m**)
- Subglacial Lake Ellsworth, West Antarctica (**3200m-160m**)

- **Fev 05, 2012 – Russia**
- **Jan 27, 2013 – US**
- **>2018 - UK**