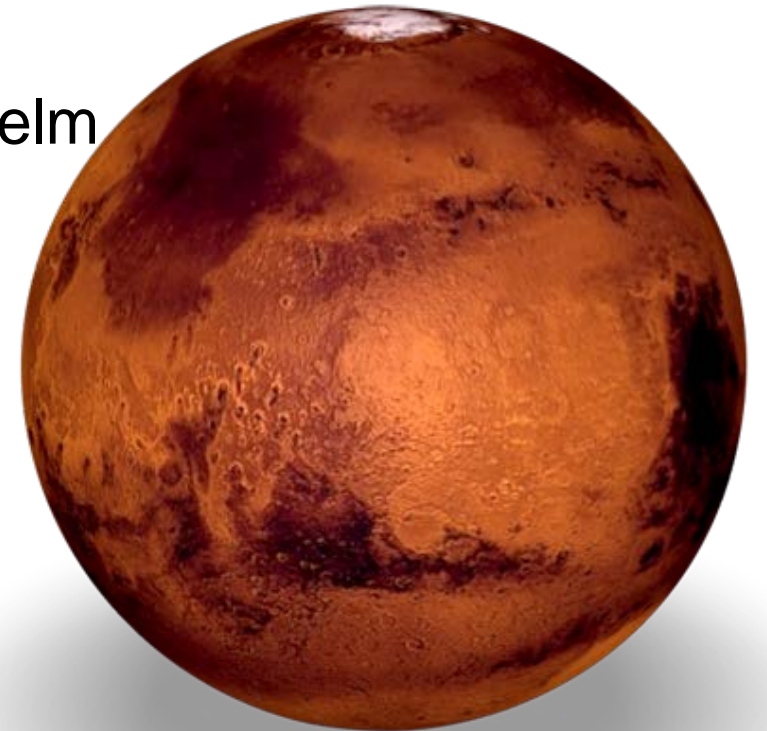


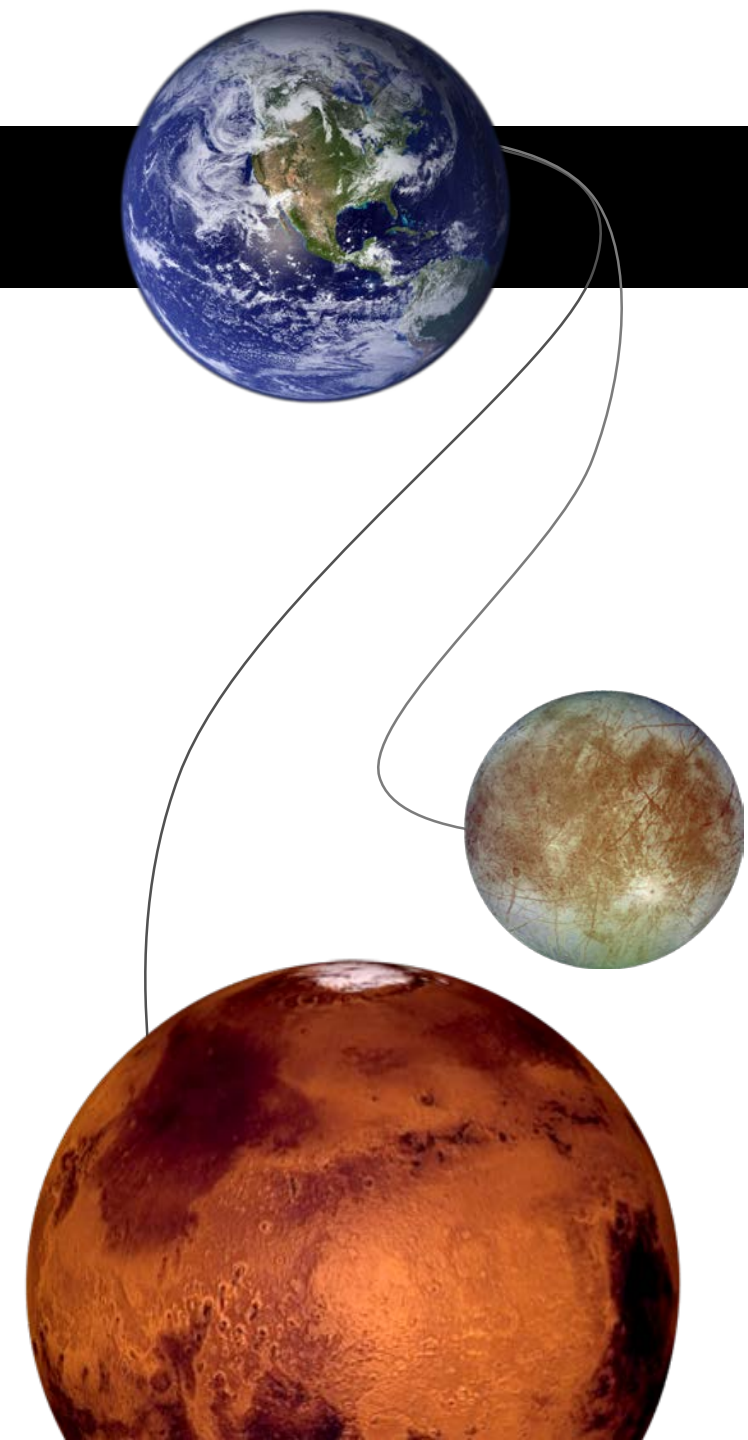
# Lipid decontamination procedures for life detection missions

Denise Buckner, Mary Beth Wilhelm



# Research Overview

1. Life detection search techniques
  - Biomarkers and lipids
  - Analytical instrumentation
2. ExCALiBR instrument concept
3. Contamination control
  - Current techniques
  - Experimental techniques
  - Effect on lipids
  - Instrument compatibility
4. Proposed solutions



# Biomarkers

- What are biomarkers?
  - Molecules form **biotically**
  - Precursors can form **abiotically**
  - Chemically unique
  - Essential for life as we know it
  - Possible evidence for extraterrestrial life
- Lipids
  - Universal biomarkers
  - Protect cells from water
  - Can survive for billions of years in rock
  - Found in meteorites, etc



Figure 1: Murchison Meteorite

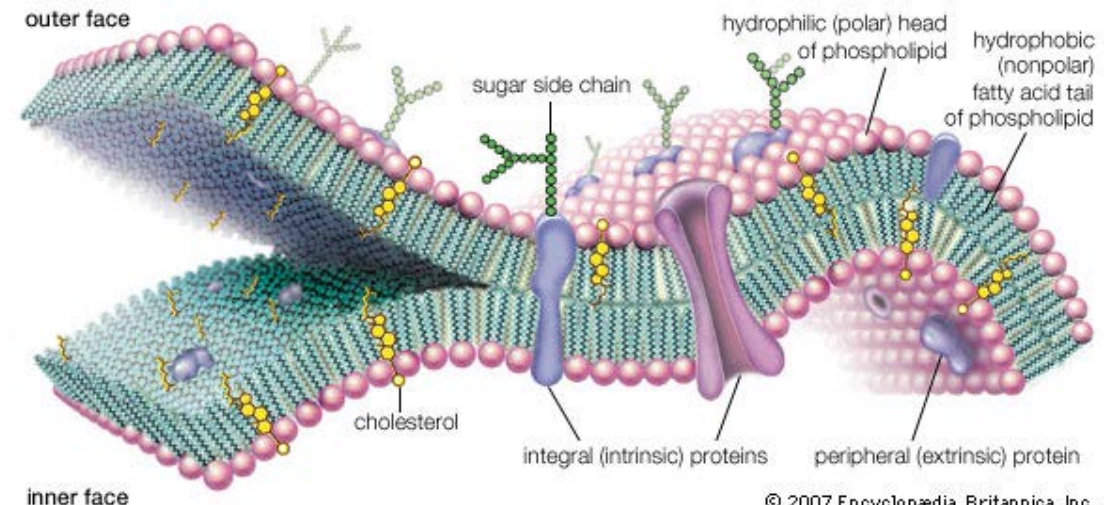


Figure 2: Cell membrane



# Searching for biomarkers: ExCALiBR

- Extractor for Chemical Analysis of Lipid Biomarkers in Regolith
- Mars, Titan, Europa, Enceladus, etc
- **Instrument capabilities**
  - *Extracting* lipids
  - *Automating* laboratory processes
  - *Streamlining* sample handling
  - *Detecting* low concentrations
  - *Validating* results
- DECONTAMINATION IS KEY!

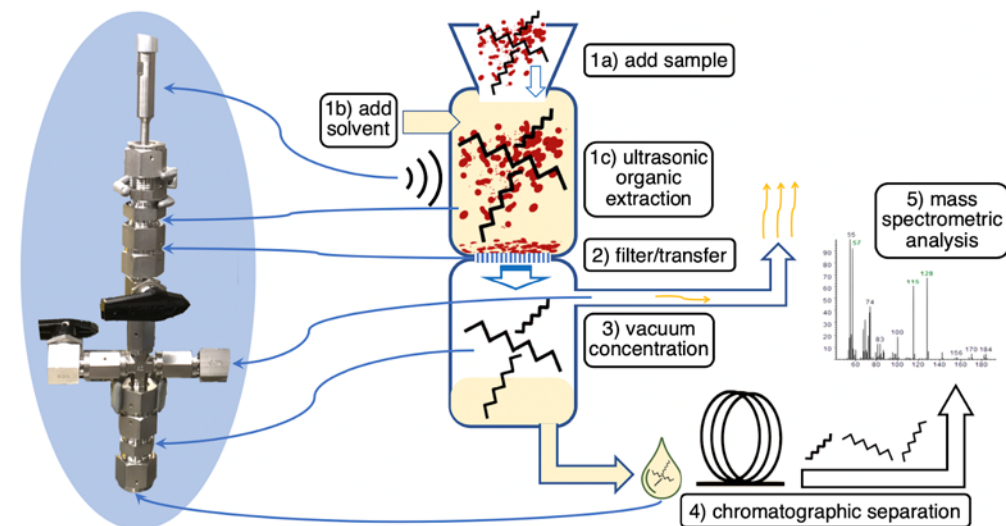


Figure 4: ExCALiBR prototype

# Contamination in spacecraft cleanrooms

- Planetary Protection (PP) vs Contamination Control (CC)

- <30 total surface spores

- Controlled, sterile environment

- Recent research: **9-70x** more microbes found

- Measured **viable** microbes:

- 10<sup>6</sup> cells/m<sup>2</sup> (spacecraft surfaces)
  - 4\*10<sup>3</sup> gene copies/5 g (embedded)

- HOWEVER, **4-8x** as many dead cells as alive

- Still meets planetary protection limits, but not clean enough to validate life detection results!*

**Figure 5: new species found**

Detected by:

- PhyloChip
- Cloning
- Both
- No detectable bacterial families
- Unclassified family
- \* Family that does not have cultivable organism (Representative GenBank # are given in parentheses)
- OTUs were categorized according to their first available class/OTU taxonomic level

# Current decontamination methods

- **NASA-approved methods:**

- Dry heat microbial reduction
  - *Kills* microbes
- Vapor phase H<sub>2</sub>O<sub>2</sub>
  - *Kills* microbes

- **Cleanroom methods:**

- Protective clothing
  - *Limits incoming* contaminants
- HEPA air filters:
  - *Removes airborne* contaminants
- Wiping with water, detergent, alcohol:
  - *Removes surface* contamination

- **Laboratory techniques:**

- Flushing with organic solvents
  - *Removes* contamination
- Bake at 500° C
  - *Destroys* organic molecules

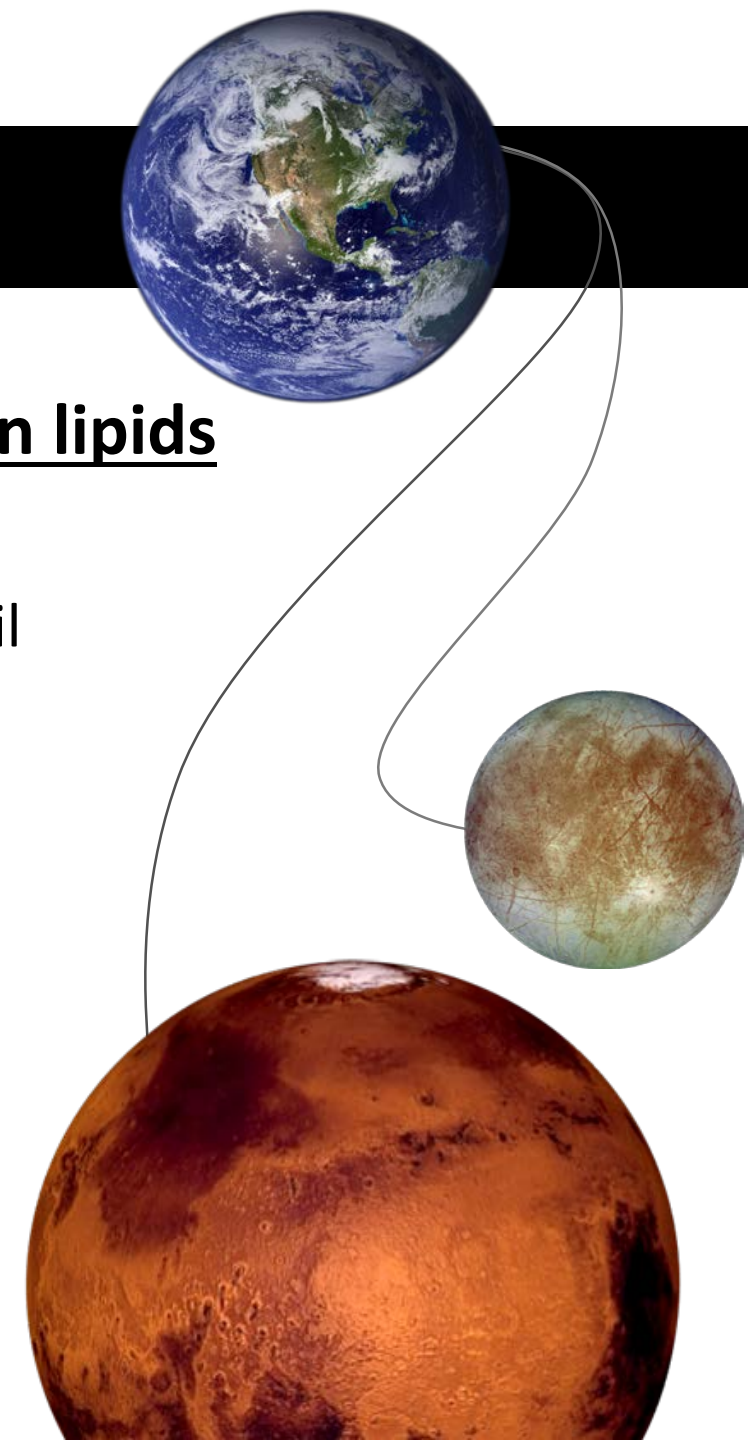


Figure 6:  
Spacecraft  
assembly  
cleanroom



# Implications for lipid detection

- Living cells, dead cells, and pieces of cells **all contain lipids**
- Encapsulated contamination
  - Drawn out by the solvents used to extract lipids from soil
  - Can be released during atmospheric entry
- Ultra-low limit of detection
- Important to decontaminate **whole instrument**  
**after assembly** in the cleanroom
- *Killing microbes and washing contamination away is not enough- instead, break the molecules*



# Proposed Solution: Electron Beam Irradiation

- **Concentrated dose** of **high energy electrons**
- Used for food, medical, and wastewater CC
- Energetic enough to:
  - Kill microbes
  - Break down lipids
- Safe for many materials
- Machine generated: (SAFE, tunable, controllable dose, cheap, no radioactive material needed)

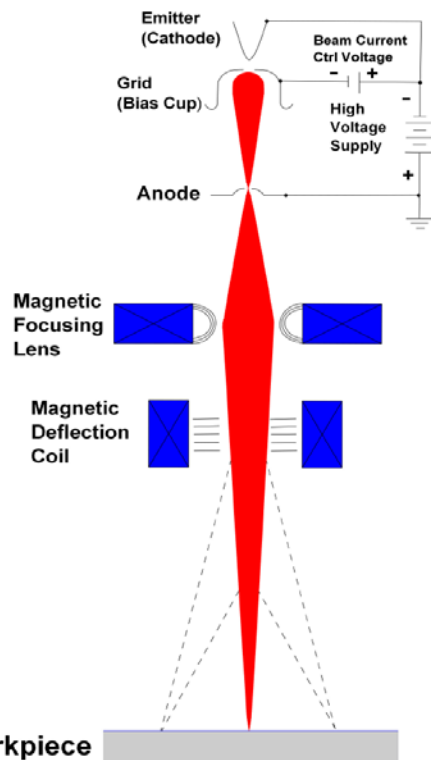


Figure 7: EBI

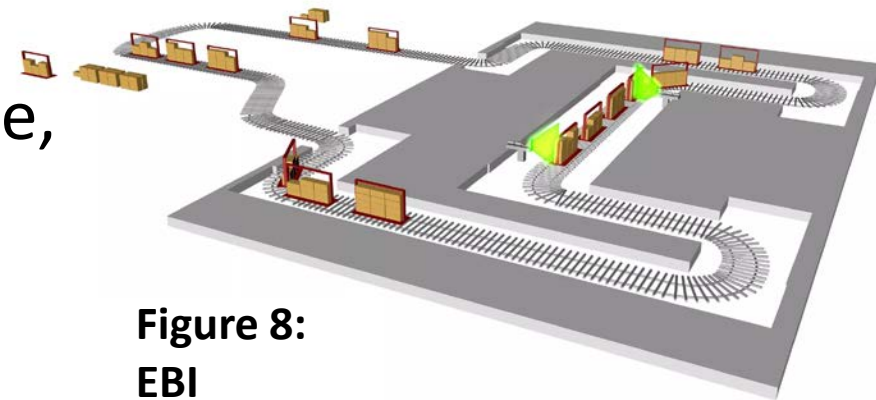


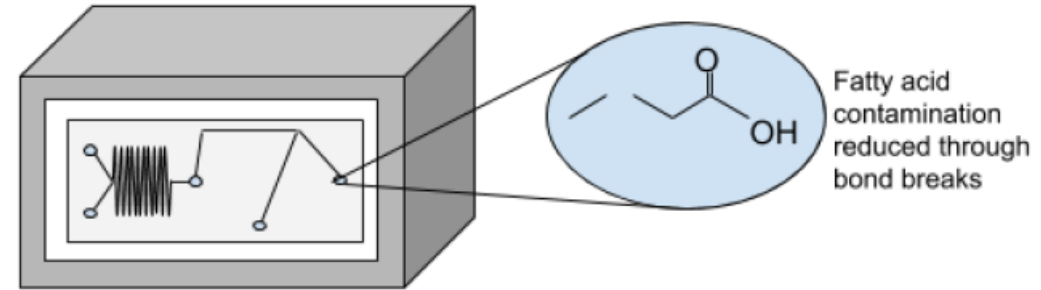
Figure 8:  
EBI  
facility



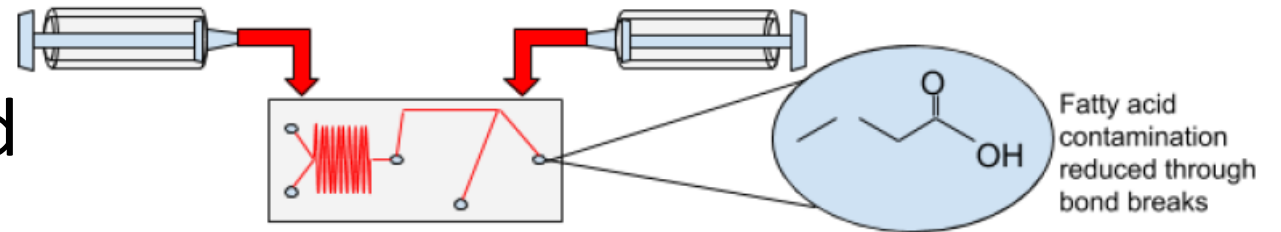
# Goal

- Goal: Develop a whole-instrument decontamination plan for ExCALiBR that **eliminates lipid contamination** through molecular bond breaks instead of mechanically removing or flushing away contamination

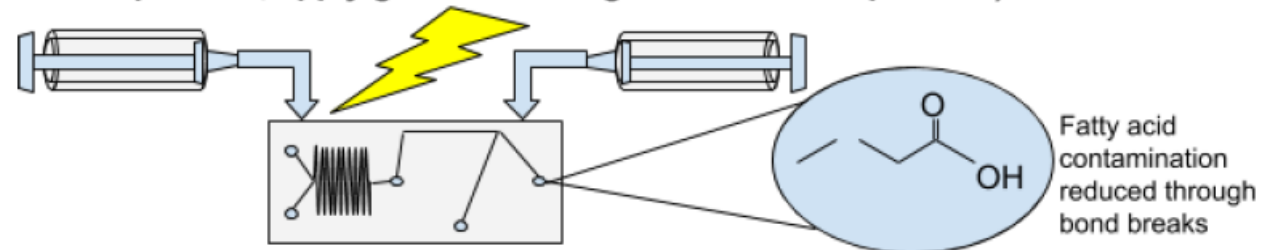
Step 1: Bake individual instrument components at highest temperature allowed



Step 2: Initial post-fabrication decontamination (e.g. flushing with acid, organic solvents)

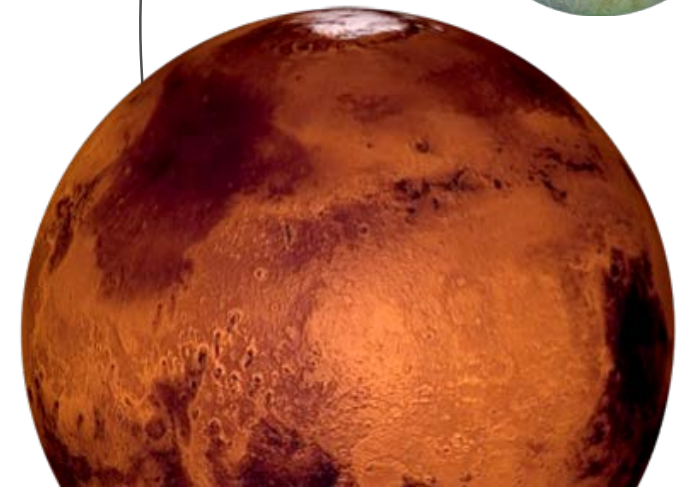
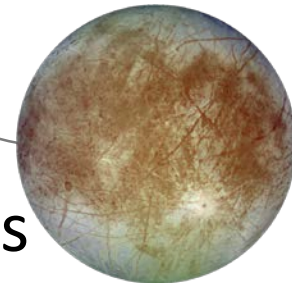


Step 3: Final post-fabrication modular cleaning (E beam hardy components, apply gentler cleaning to delicate components)



# Next steps

1. Quantify effects of NASA-approved, cleanroom, and lab decontamination techniques on lipids
2. Quantify effects of EBI on lipids
3. Determine material compatibility between EBI and ExCALiBR materials
4. Develop a whole- system CC plan to eliminate lipid contaminants by breaking molecular bonds



# Thanks, References, Figures

- Blue Marble Space Institute of Science
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- Dr. James Casler
- David Wilson

- Bauermeister, A. *et al.* Quantification of Encapsulated Bioburden in Spacecraft Polymer Materials by Cultivation-Dependent and Molecular Methods. *PLoS ONE*9,(2014).
- Duc, M. T. L. *et al.* Comprehensive Census of Bacteria in Clean Rooms by Using DNA Microarray and Cloning Methods. *Applied and Environmental Microbiology*75,6559–6567 (2009).
- Ho, J., Stanley, N. J. & Kuehn, T. H. Feasibility of using real-time optical methods for detecting the presence of viable bacteria aerosols at low concentrations in clean room environments. *Aerobiologia*27,163–172 (2010).
- Koskinen, K. *et al.* Microbial biodiversity assessment of the European Space Agency's ExoMars 2016 mission. *Microbiome*5,(2017).
- Kwan, K. *et al.* Evaluation of Procedures for the Collection, Processing, and Analysis of Biomolecules from Low-Biomass Surfaces. *Applied and Environmental Microbiology*77,2943–2953 (2011).
- <https://www.thermofisher.com/blog/proteomics/microfluidics-and-mass-spectrometry-based-proteomics/>
- <http://www.artancient.com/antiquities-for-sale/cultures/fossils-tribal-art-pre-columbian-art-sale/murchison-meteorite-cm2-carbonaceous-chondrite.html>
- <https://www.britannica.com/science/membrane-biology>
- Duc, M. T. L. *et al.* Comprehensive Census of Bacteria in Clean Rooms by Using DNA Microarray and Cloning Methods. *Applied and Environmental Microbiology*75,6559–6567 (2009).
- [https://microbewiki.kenyon.edu/index.php/Spacecraft\\_Assembly\\_Cleanrooms](https://microbewiki.kenyon.edu/index.php/Spacecraft_Assembly_Cleanrooms)
- [https://en.wikipedia.org/wiki/Electron\\_beam\\_processing](https://en.wikipedia.org/wiki/Electron_beam_processing)
- <https://phys.org/news/2012-02-electron-beam-irradiation-virus-related-health-lettuce.html>
- <https://steri-tek.com/e-beam/>

