NUANCE BioCryo

Materials Ultramicrotomy, BioCryo's best kept secret

also,

Making the Cover, Crafting a Winning Image

Eric W. Roth
2019.07.17

WHO AM I?

Eric W. Roth
Electron Microscopy Specialist
NU*ANCE*, BioCryo Facility
Office: Silverman Hall East, B535
(basement across from the elevator)
847.467.4164
Pronouns: they, them, their /human

Microscopist > Scientist > Artist

Favorite food, *SPICY!*

WHAT I DO

- Training
- Service
- Collaboration
- Mentorship
- SEM, TEM, STEM
- EM sample prep
  - cryoTEM, cryoSEM, ultramicrotomy, cryo-ultramicrotomy, tissue processing,
  - Soft materials (polymers, etc.), biological materials, MOF's, Zeolites

"I'm here to support you and your research, and I really do care."

STRENGTHS

- CV literally says, “Extremely Steady Hands”
- Jack of all samples
- Graphic Arts
- Connected at NU, “If I don’t know how to get the data you need or how to do the technique, I probably know someone who does.”

WEAKNESS

- There's only one of me / calendar get's booked up fast!
- Aphasia… "sorry, I forget names and words sometimes..

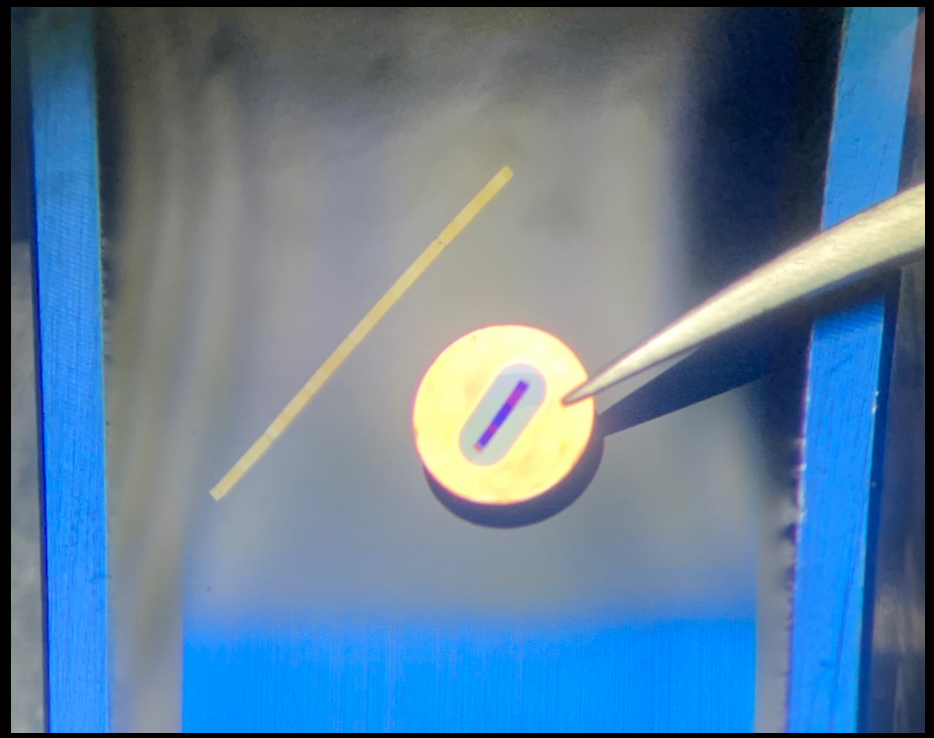
  (Yes, even if I've known you for years and years)."

MOTIVATIONS

- YOU! “sample prep and imaging is my art”
  - New samples = new challenges
  - Old samples, “mastery through endless repetition!”
- Training- “I want you to get high-quality data and quickly and painlessly as possible.”
- Humans, technology, biology, the environment, space exploration
- Art of the very small

ULTRAMICROTOMY

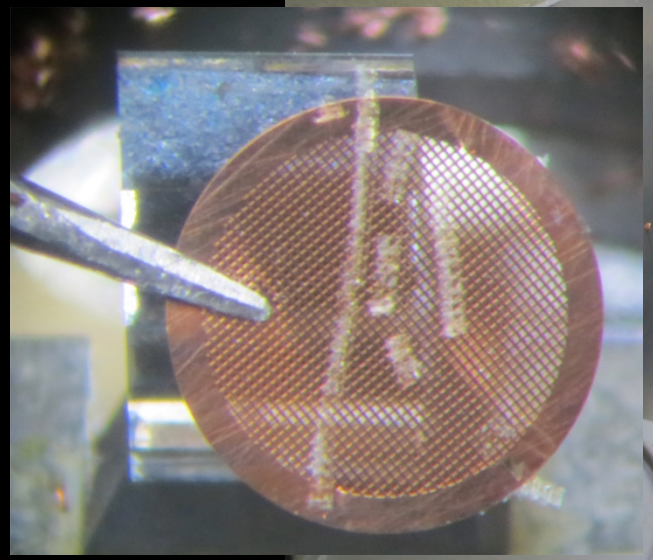
What is it



(alternative to FIB or Ion Milling, Dimpling, Polishing, etc.)

SO MANY TECHNIQUES

Room Temperature
Dry
Cryo
Warm Cryo with liquid media
Tokuyasu for Immuno Au Labeling
Ultra Sonic
En Face
Serial



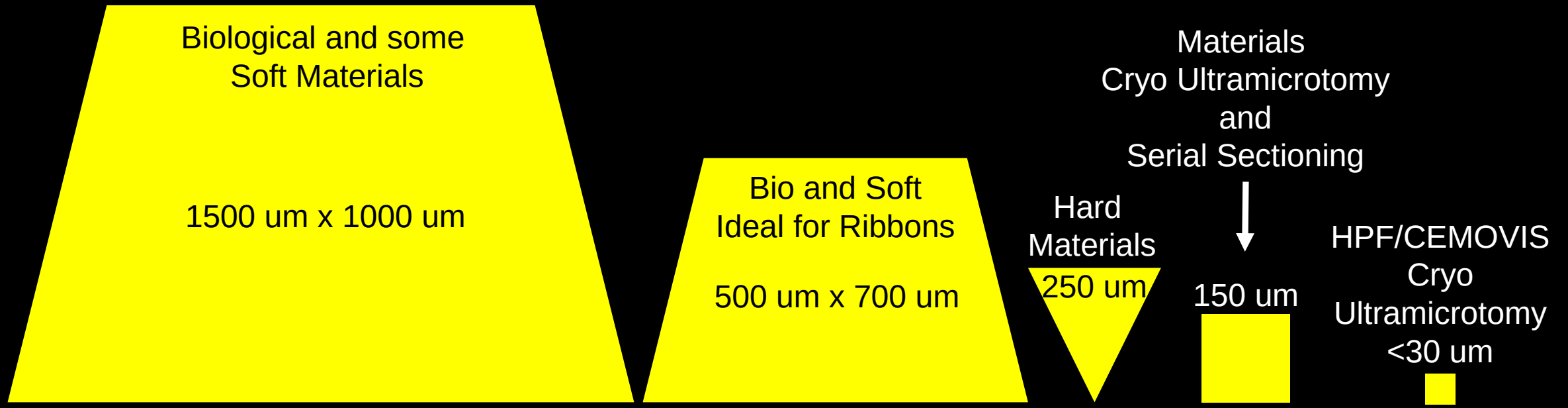
MATERIALS ULTRAMICROTOMY

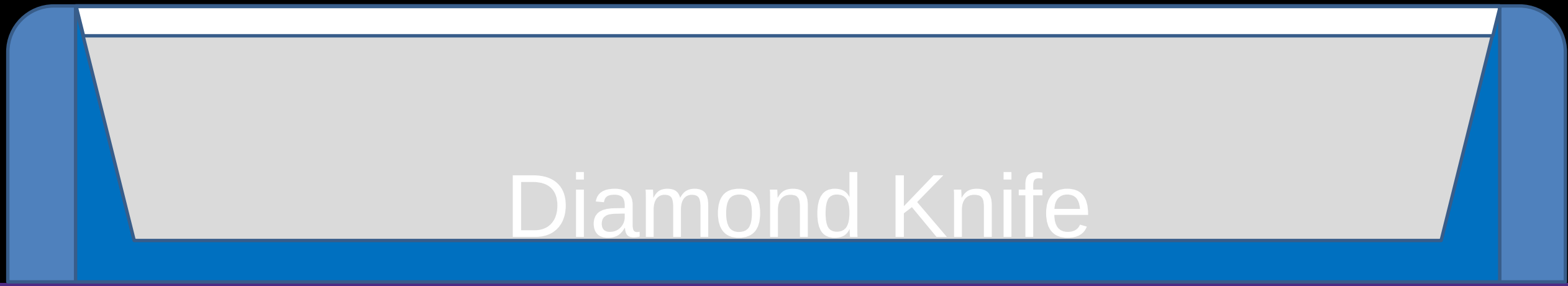
NOT JUST BIO, NOT JUST CRYO

- Polymers
- Paint
- MOF's/COF's
- Nano Particles/ Quantum Dots
- Zeolites
- Some Metals/Alloys
- Microfibers
- Silicates
- Sponges
- Hydrogels
- Aerogels
- 3D-Printed Materials

Sorry, no Silicon Wafers or Ceramics

BLOCK FACE SIZE / SHAPE



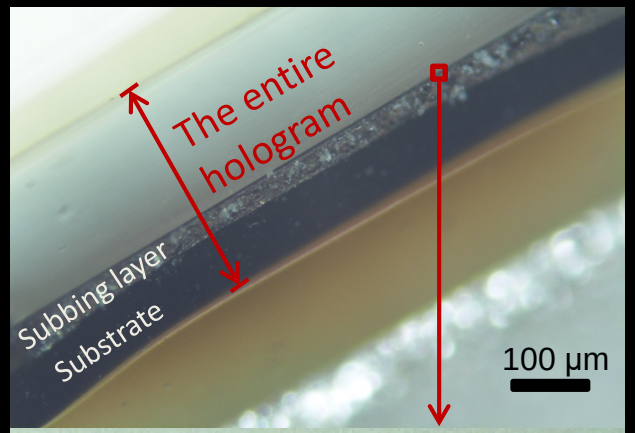


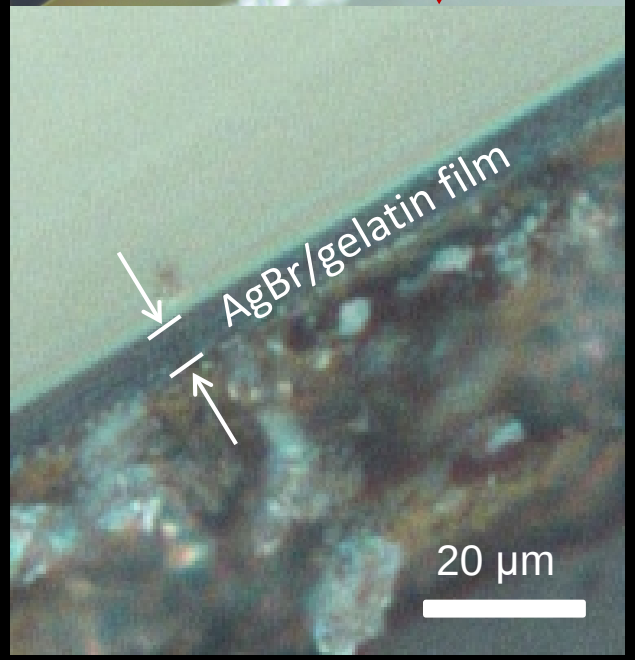
PARAMETERS

- Cutting Speed Vs. Thickness, Material, Block face size
- Sectioning Media (Water, DMSO, Mineral Oil, Dry(no media))
- Media Height Vs. Hydrophilic/Hydrophobic Materials
- Grid Choice, Composition (Cu, Ni, Au, Nylon, etc.)
- Grid Makeup (Slotted, 75 mesh, 400 mesh, square, hex, finder, etc.)
- Grid Substrate (Formvar, Carbon, Graphene Oxide, Lacey, None)

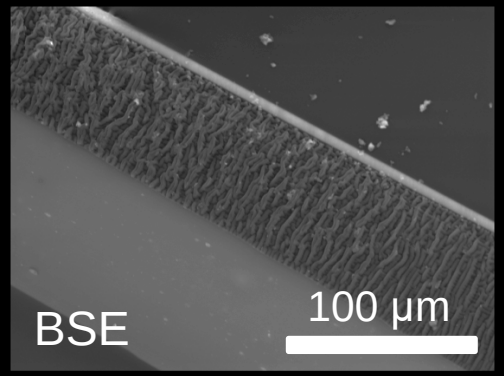
HOLOGRAPHIC FILM

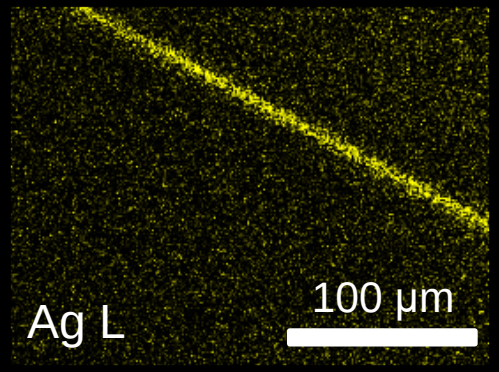
Pengxiao Hao, NU-ACCESS

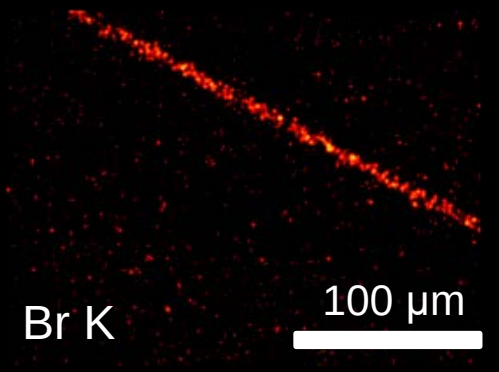




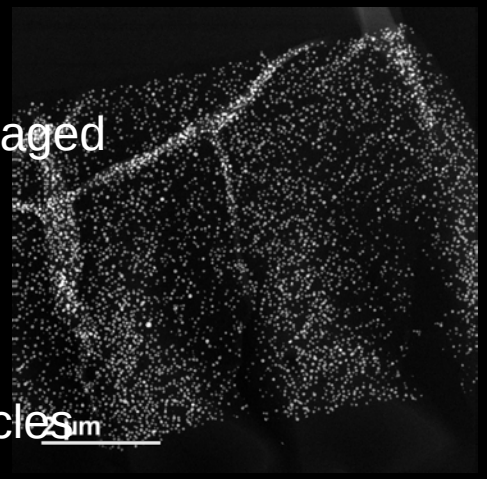
Block face Imaging and EDS in Hitachi 3400 SEM

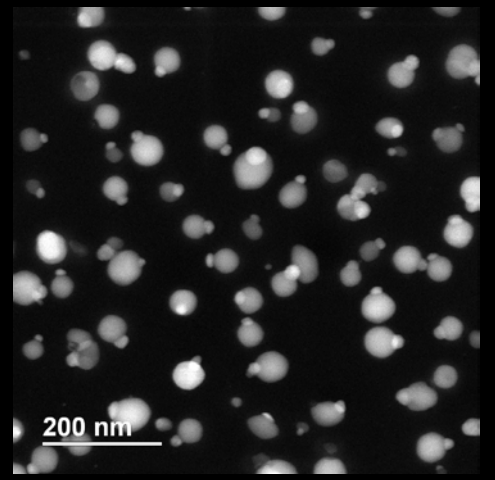


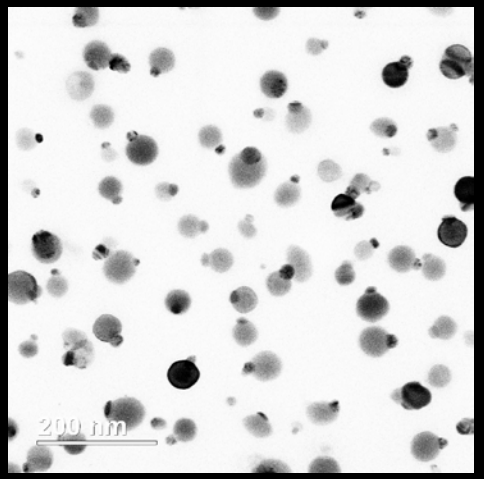




Ultrathin Section imaged in Hitachi HD2300 STEM revealing AgBr particles

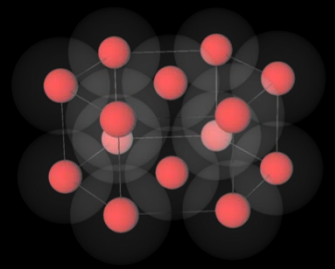




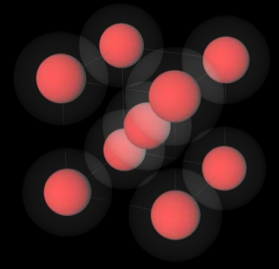


DNA-NANOPARTICLE CONJUGATES

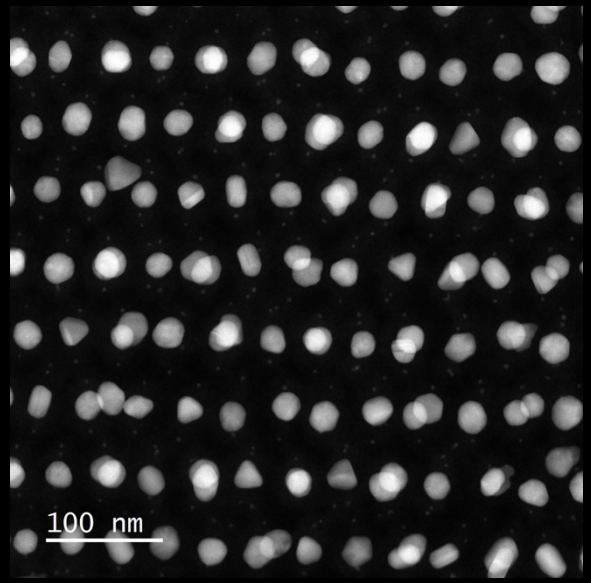
Shunzhi Wang, Mirkin Lab



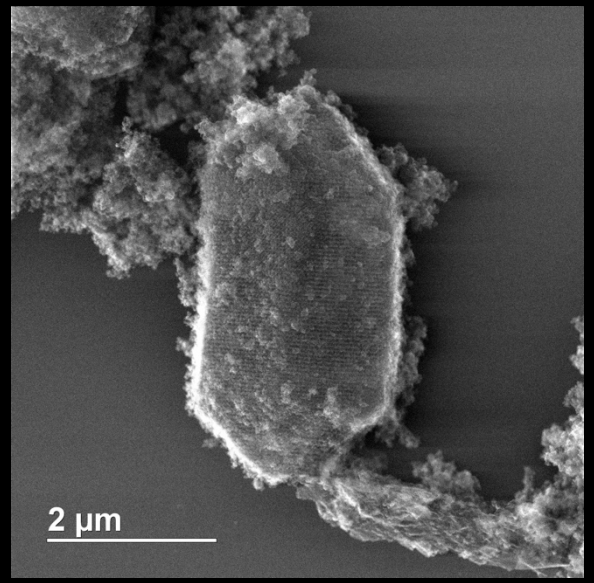
Simple hexagonal



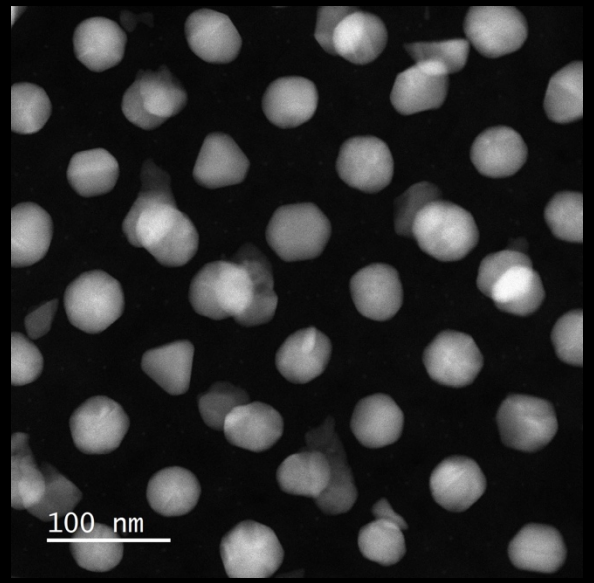
BCC



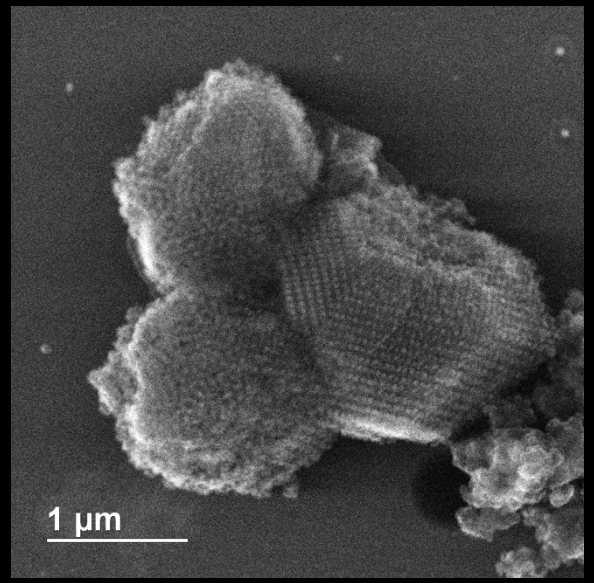
JEOL ARM300



Hitachi HD2300 STEM



JEOL ARM300

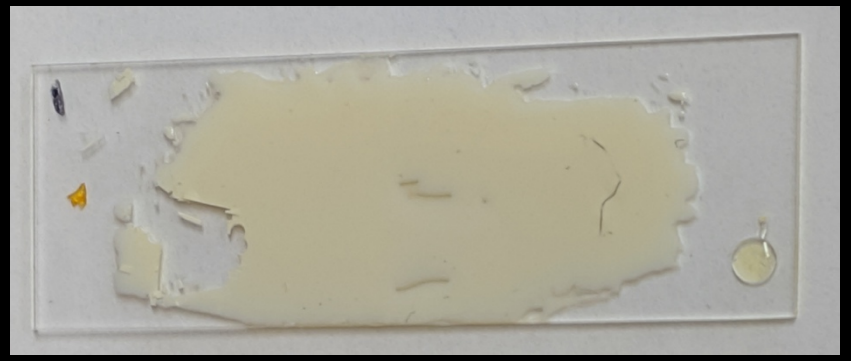


Hitachi HD2300 STEM

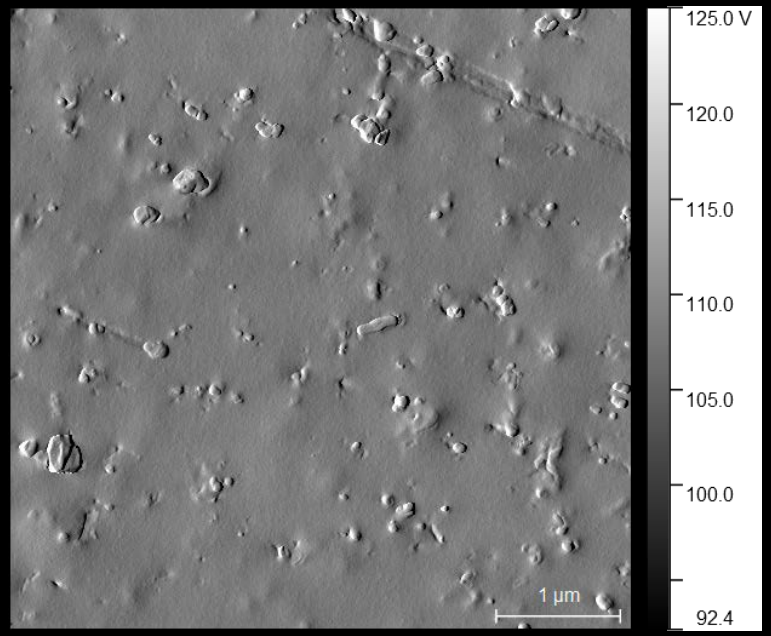
M. Girard,‡ S. Wang,‡ J.S. Du,‡ A. Das,‡ Z. Huang, V.P. Dravid, B. Lee, C.A. Mirkin, M. Olvera de la Cruz. "Particle Analogs of Electrons in Colloidal Crystals," *Science*, **2019**, *364*, 1174-1178.

PAINT FILMS

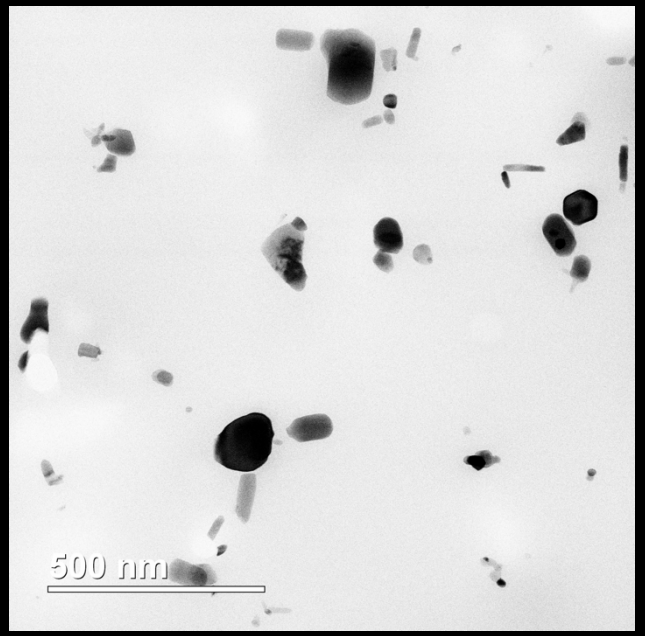
Stephanie Zaleski, NUACCESS

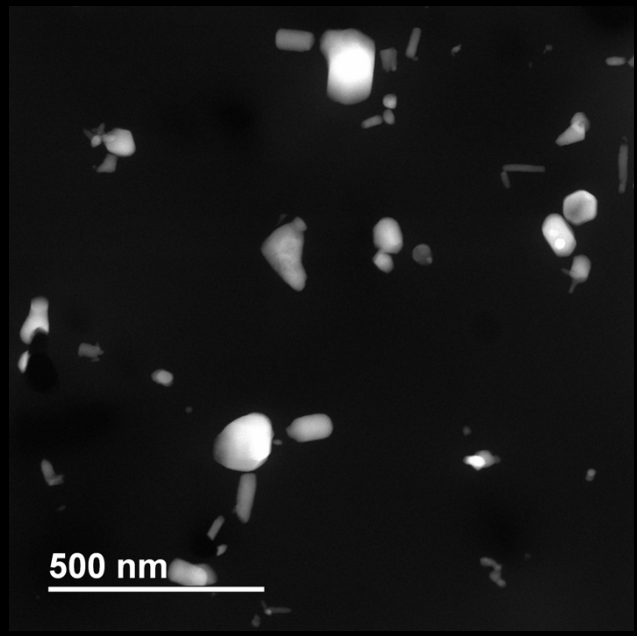


Sample embedded in
epoxy resin and sectioned
300 nm thick for AFM
90 nm thick for STEM



AFM: Amplitude image

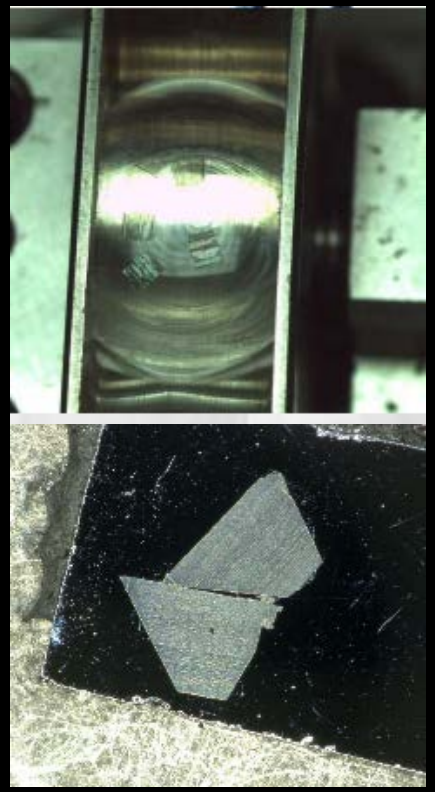




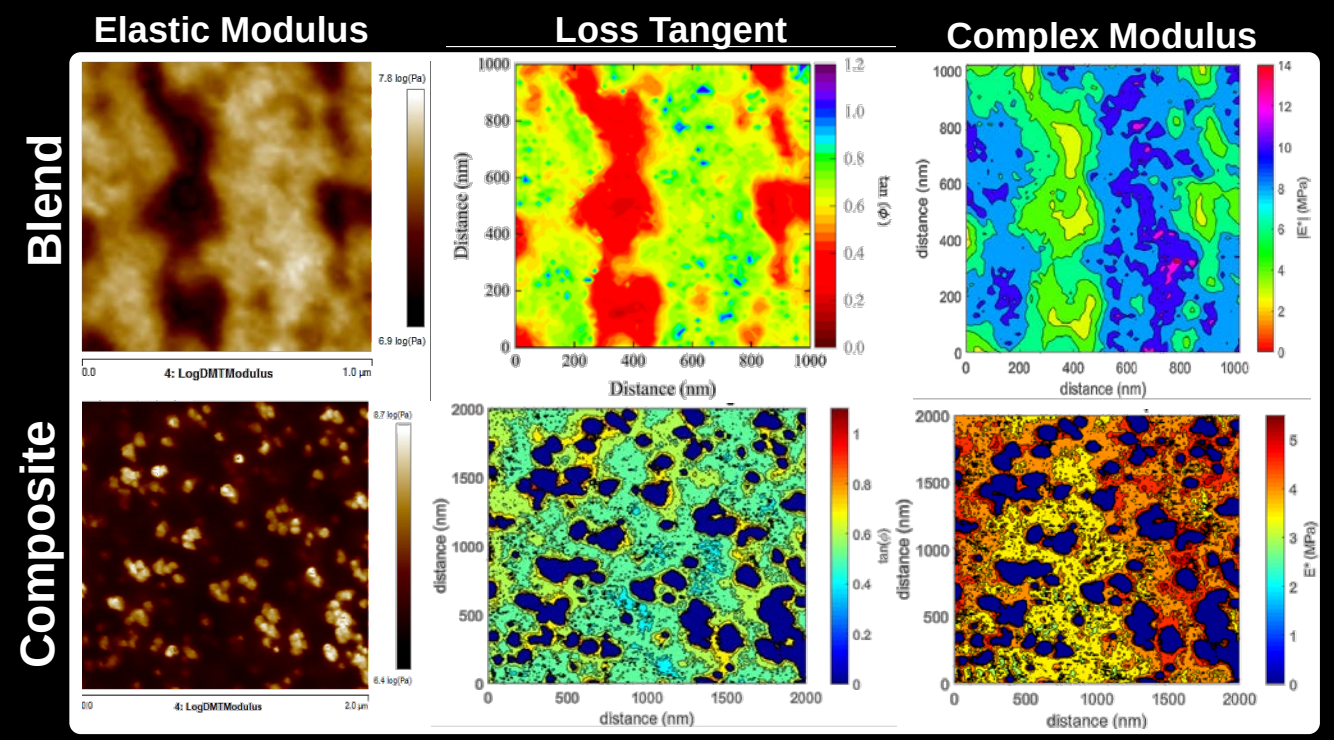
Hitachi HD2300 STEM TE Phase Contrast and HAADF

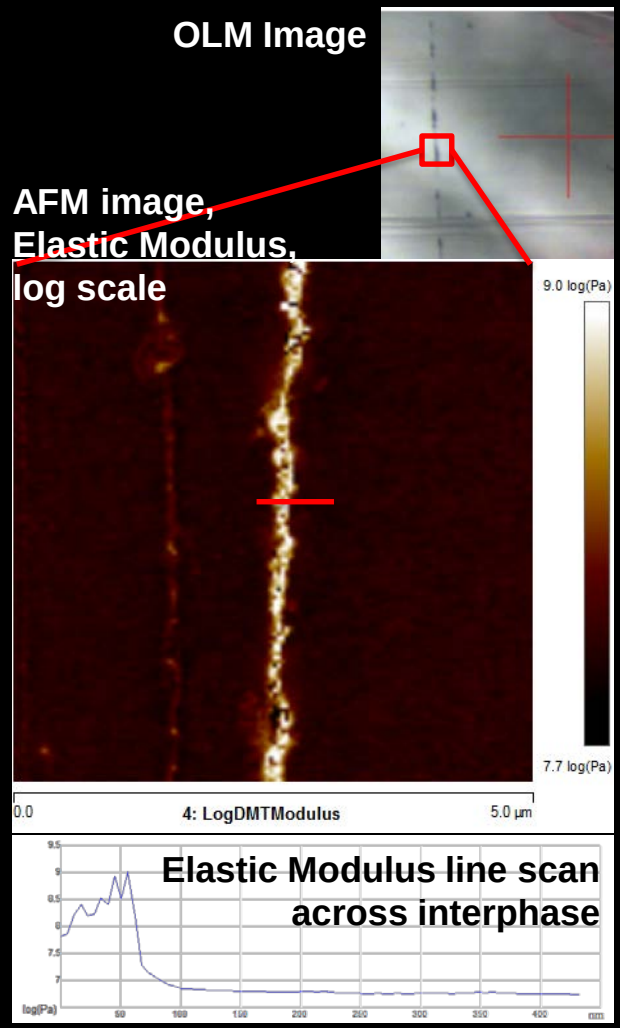
RUBBER BLENDS AND COMPOSITES

Matthew Eaton, Shull MSE



Cryo ultramicrotomy wet sectioning with DMSO:Water = 3:2



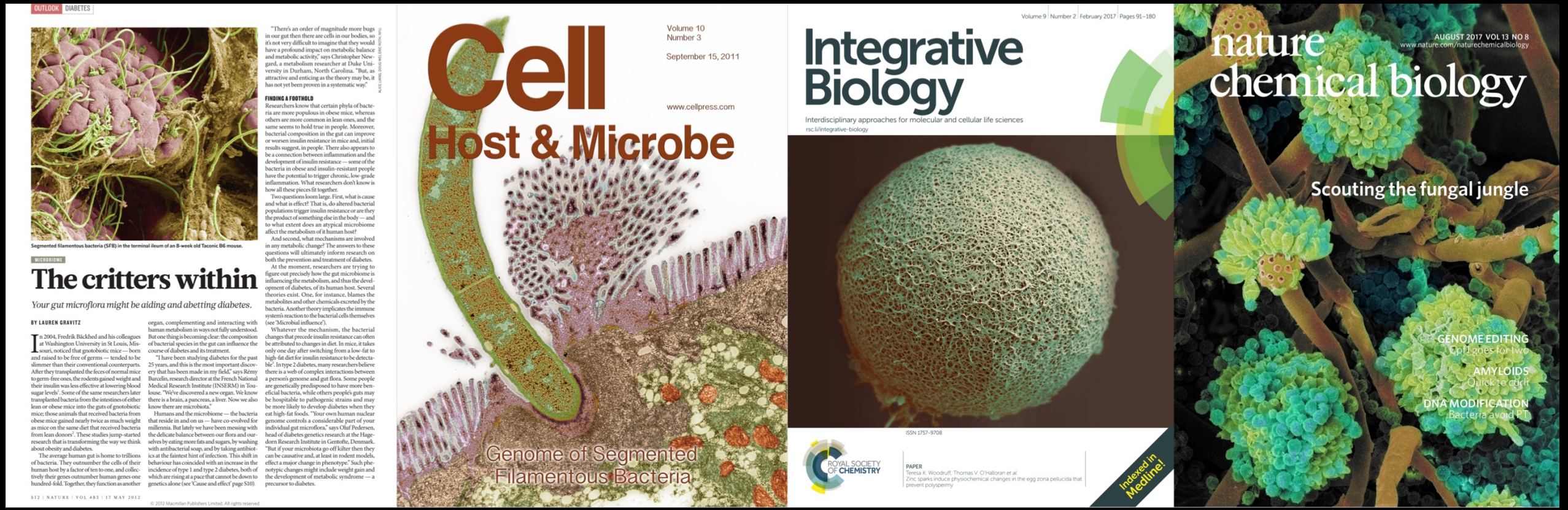


ULTRA SONIC KNIFE

NEW

- Alternate to cryo ultramicrotomy
- Ideal for biological or polymers
- Avoid compression artefacts

MAKING THE COVER



OUTLOOK DIABETES

Segmented filamentous bacteria (SFB) in the terminal ileum of an 8-week old Taconic B6 mouse.

MICROBIOME

The critters within

Your gut microflora might be aiding and abetting diabetes.

BY LAUREN GRAVITZ

In 2004, Fredrik Bäckhed and his colleagues at Washington University in St Louis, Missouri, noticed that gnotobiotic mice — born and raised to be free of germs — tended to be slimmer than their conventional counterparts. After they transplanted the feces of normal mice to germ-free ones, the rodents gained weight and their insulin was less effective at lowering blood sugar levels[1]. Some of the same researchers later transplanted bacteria from the intestines of either lean or obese mice into the guts of gnotobiotic mice; those animals that received bacteria from obese mice gained nearly twice as much weight as mice on the same diet that received bacteria from lean donors[2]. These studies jump-started research that is transforming the way we think about obesity and diabetes.

The average human gut is home to trillions of bacteria. They outnumber the cells of their human host by a factor of ten to one, and collectively their genes outnumber human genes one hundred-fold. Together, they function as another organ, complementing and interacting with human metabolism in ways not fully understood. But one thing is becoming clear: the composition of bacterial species in the gut can influence the course of diabetes and its treatment.

"I have been studying diabetes for the past 25 years, and this is the most important discovery that has been made in my field," says Rémy Burcelin, research director at the French National Medical Research Institute (INSERM) in Toulouse. "We've discovered a new organ. We know there is a brain, a pancreas, a liver. Now we also know there are microbiota."

Humans and the microbiome — the bacteria that reside in and on us — have co-evolved for millennia. But lately we have been messing with the delicate balance between our flora and ourselves by eating more fats and sugars, by washing with antibacterial soap, and by taking antibiotics at the faintest hint of infection. This shift in behaviour has coincided with an increase in the incidence of type 1 and type 2 diabetes, both of which are rising at a pace that cannot be down to genetics alone (see 'Cause and effect' page S10).

"There's an order of magnitude more bugs in our gut then there are cells in our bodies, so it's not very difficult to imagine that they would have a profound impact on metabolic balance and metabolic activity," says Christopher Newgard, a metabolism researcher at Duke University in Durham, North Carolina. "But, as attractive and enticing as the theory may be, it has not yet been proven in a systematic way."

ALICE LANG, DOUG WEI, ERIC ROTH, NYU

FINDING A FOOTHOLD

Researchers know that certain phyla of bacteria are more populous in obese mice, whereas others are more common in lean ones, and the same seems to hold true in people. Moreover, bacterial composition in the gut can improve or worsen insulin resistance in mice and, initial results suggest, in people. There also appears to be a connection between inflammation and the development of insulin resistance — some of the bacteria in obese and insulin-resistant people have the potential to trigger chronic, low-grade inflammation. What researchers don't know is how all these pieces fit together.

Two questions loom large. First, what is cause and what is effect? That is, do altered bacterial populations trigger insulin resistance or are they the product of something else in the body — and to what extent does an atypical microbiome affect the metabolism of it human host?

And second, what mechanisms are involved in any metabolic change? The answers to these questions will ultimately inform research on both the prevention and treatment of diabetes.

At the moment, researchers are trying to figure out precisely how the gut microbiome is influencing the metabolism, and thus the development of diabetes, of its human host. Several theories exist. One, for instance, blames the metabolites and other chemicals excreted by the bacteria. Another theory implicates the immune system's reaction to the bacterial cells themselves (see 'Microbial influence').

Whatever the mechanism, the bacterial changes that precede insulin resistance can often be attributed to changes in diet. In mice, it takes only one day after switching from a low-fat to high-fat diet for insulin resistance to be detectable[3]. In type 2 diabetes, many researchers believe there is a web of complex interactions between a person's genome and gut flora. Some people are genetically predisposed to have more beneficial bacteria, while others people's guts may be hospitable to pathogenic strains and may be more likely to develop diabetes when they eat high-fat foods. "Your own human nuclear genome controls a considerable part of your individual gut microflora," says Oluf Pedersen, head of diabetes genetics research at the Hagedorn Research Institute in Gentofte, Denmark. "But if your microbiota go off kilter then they can be causative and, at least in rodent models, effect a major change in phenotype." Such phenotypic changes might include weight gain and the development of metabolic syndrome — a precursor to diabetes.

Cell
Host & Microbe

Volume 10
Number 3

September 15, 2011

www.cellpress.com

Genome of Segmented Filamentous Bacteria

Integrative Biology

Volume 9 | Number 2 | February 2017 | Pages 91–180

Interdisciplinary approaches for molecular and cellular life sciences

rsc.li/integrative-biology

ISSN 1757-9708

ROYAL SOCIETY OF CHEMISTRY

PAPER

Teresa K. Woodruff, Thomas V. O'Halloran *et al.*

Zinc sparks induce physiochemical changes in the egg zona pellucida that prevent polyspermy

Indexed in Medline!

nature chemical biology

AUGUST 2017 VOL 13 NO 8

www.nature.com/naturechemicalbiology

Scouting the fungal jungle

GENOME EDITING

Opt for two

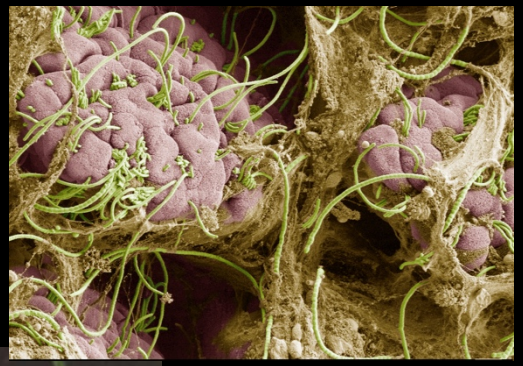
AMYLOIDS

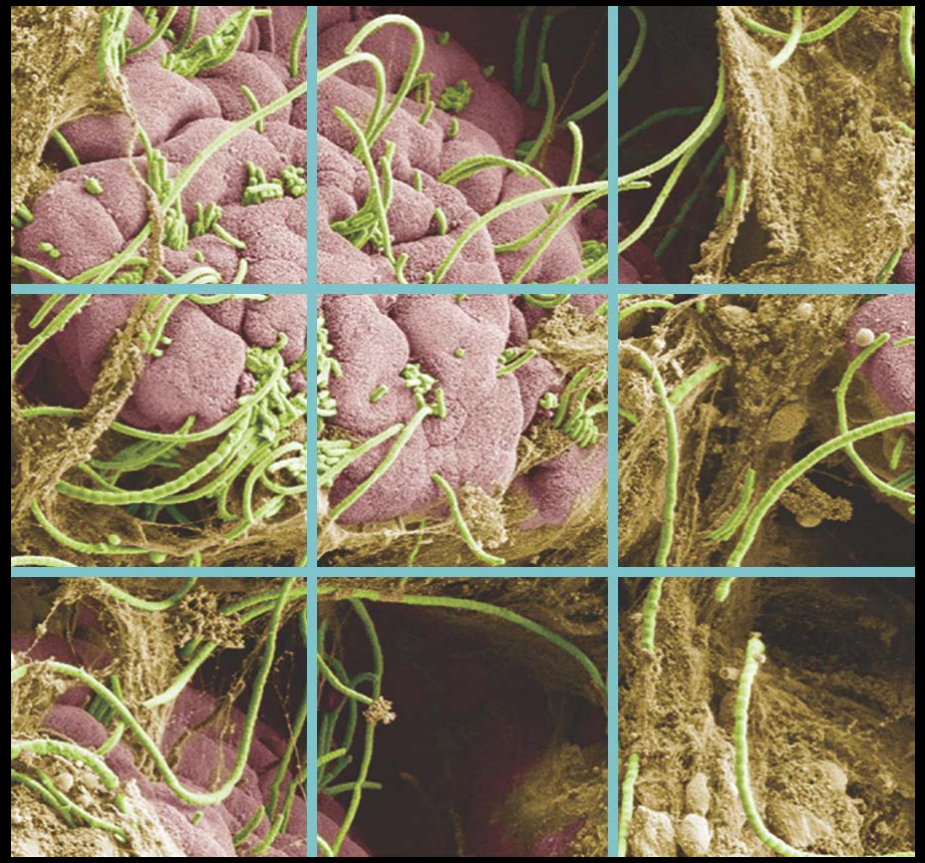
Quick to curl

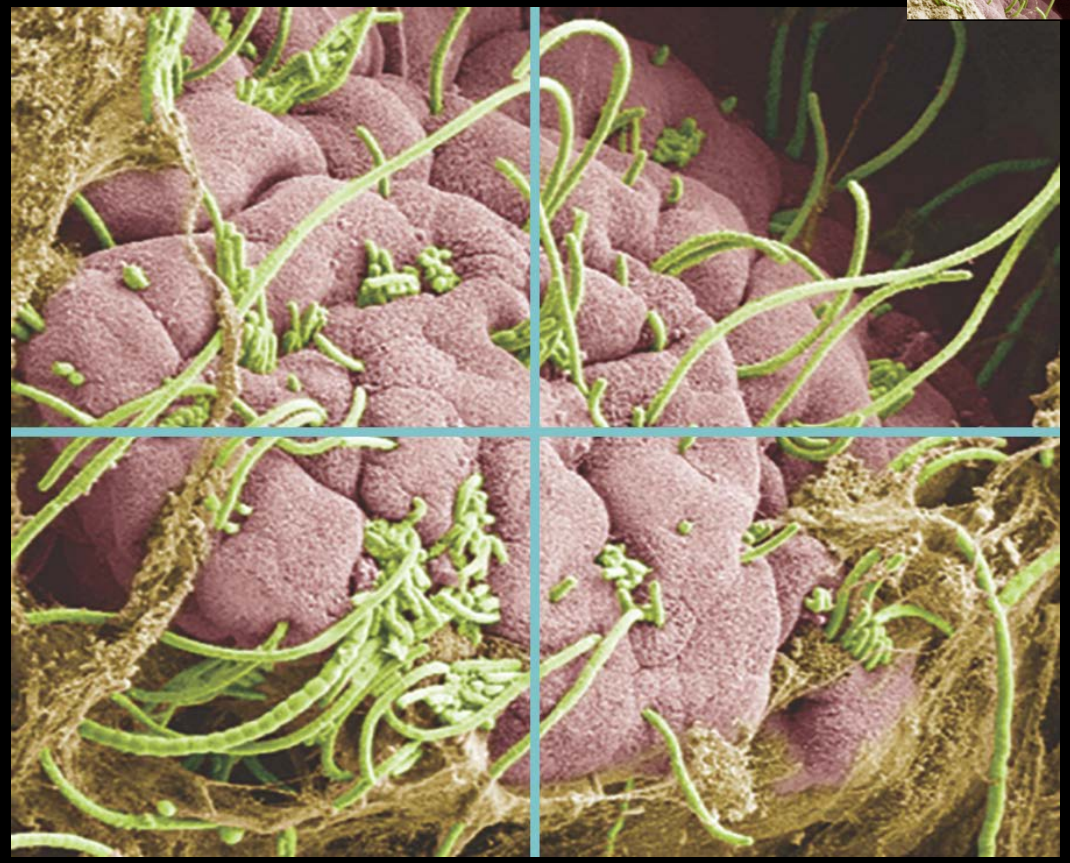
DNA MODIFICATION

Bacteria avoid PT

ATTRACTIVE ≠ USEFUL





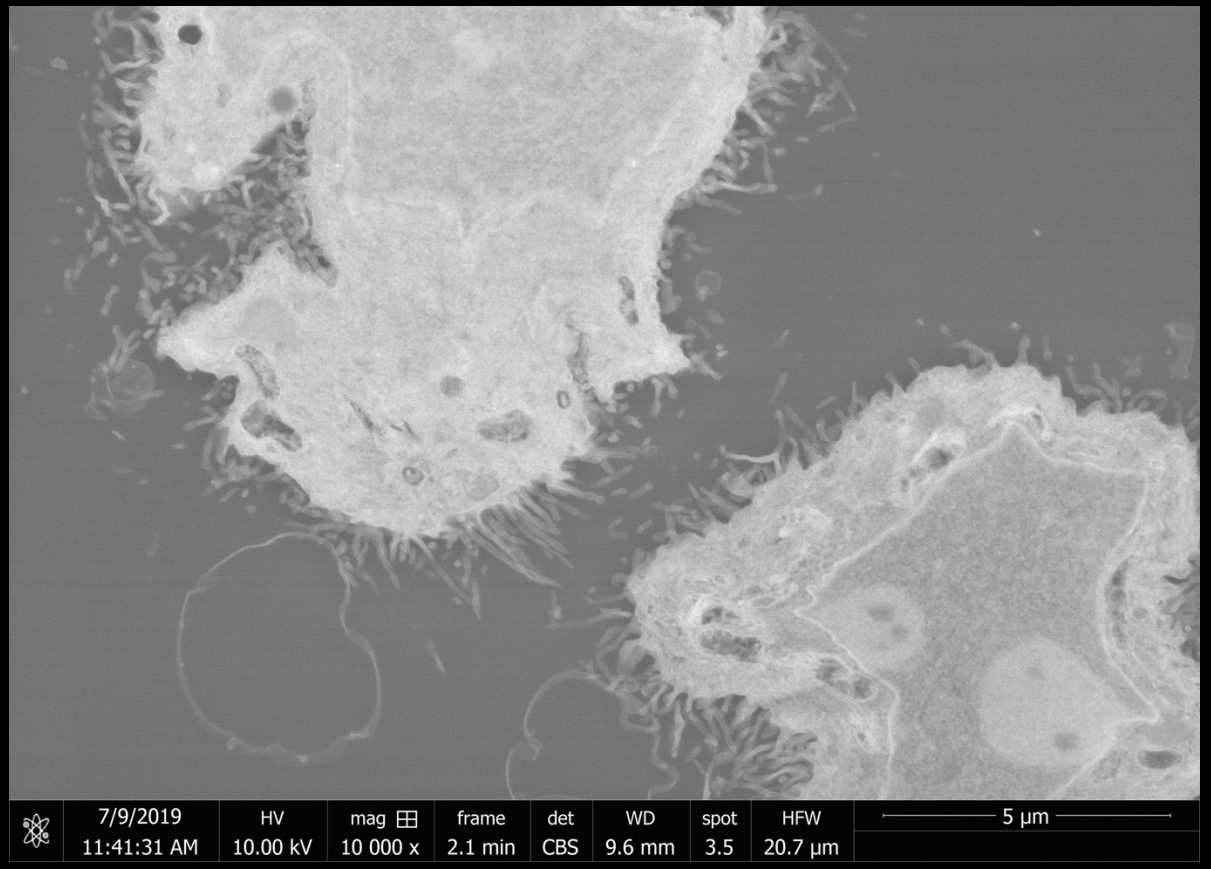


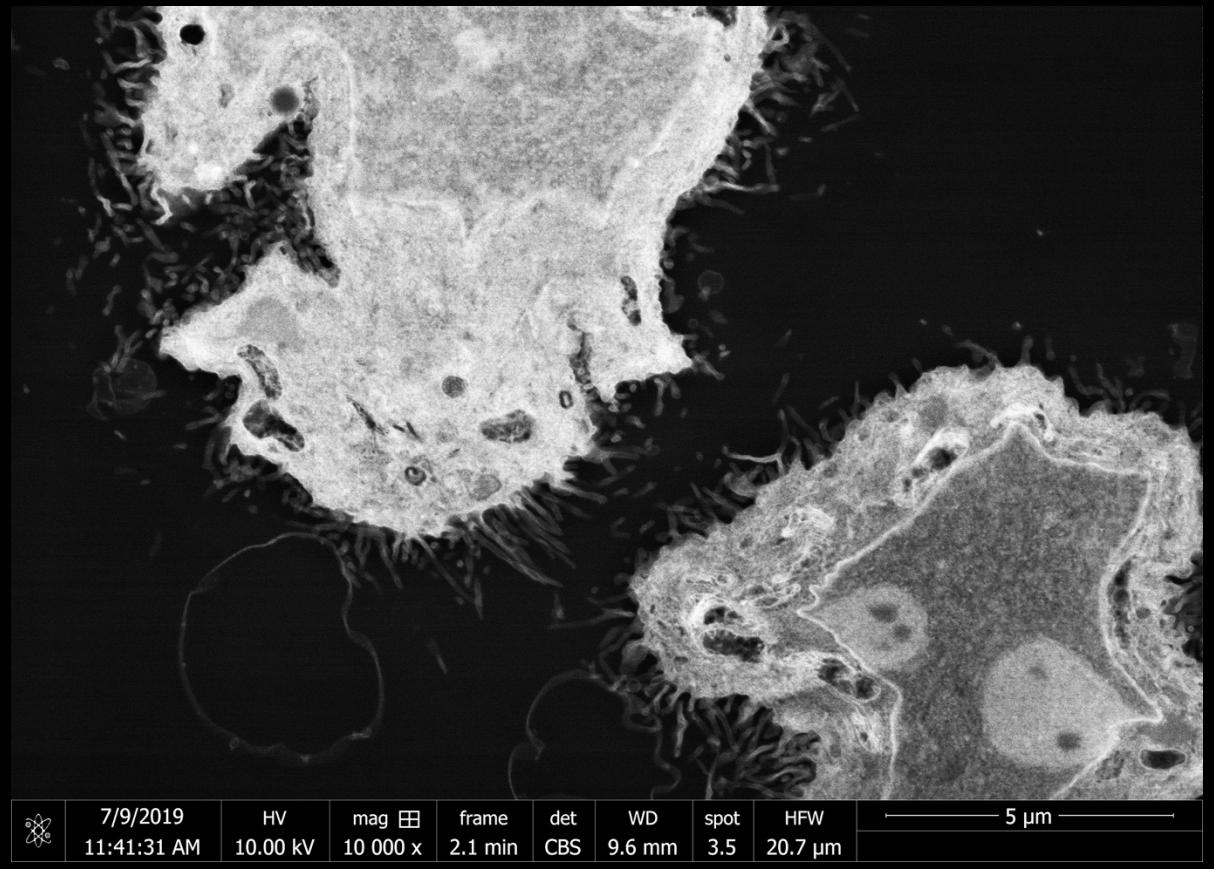
CROP

- Make new scale bar if necessary
- If asked for cover image, find out exact dimensions required by the publisher first! (300dpi, 9 x 12, etc.) Crop and scale image accordingly.

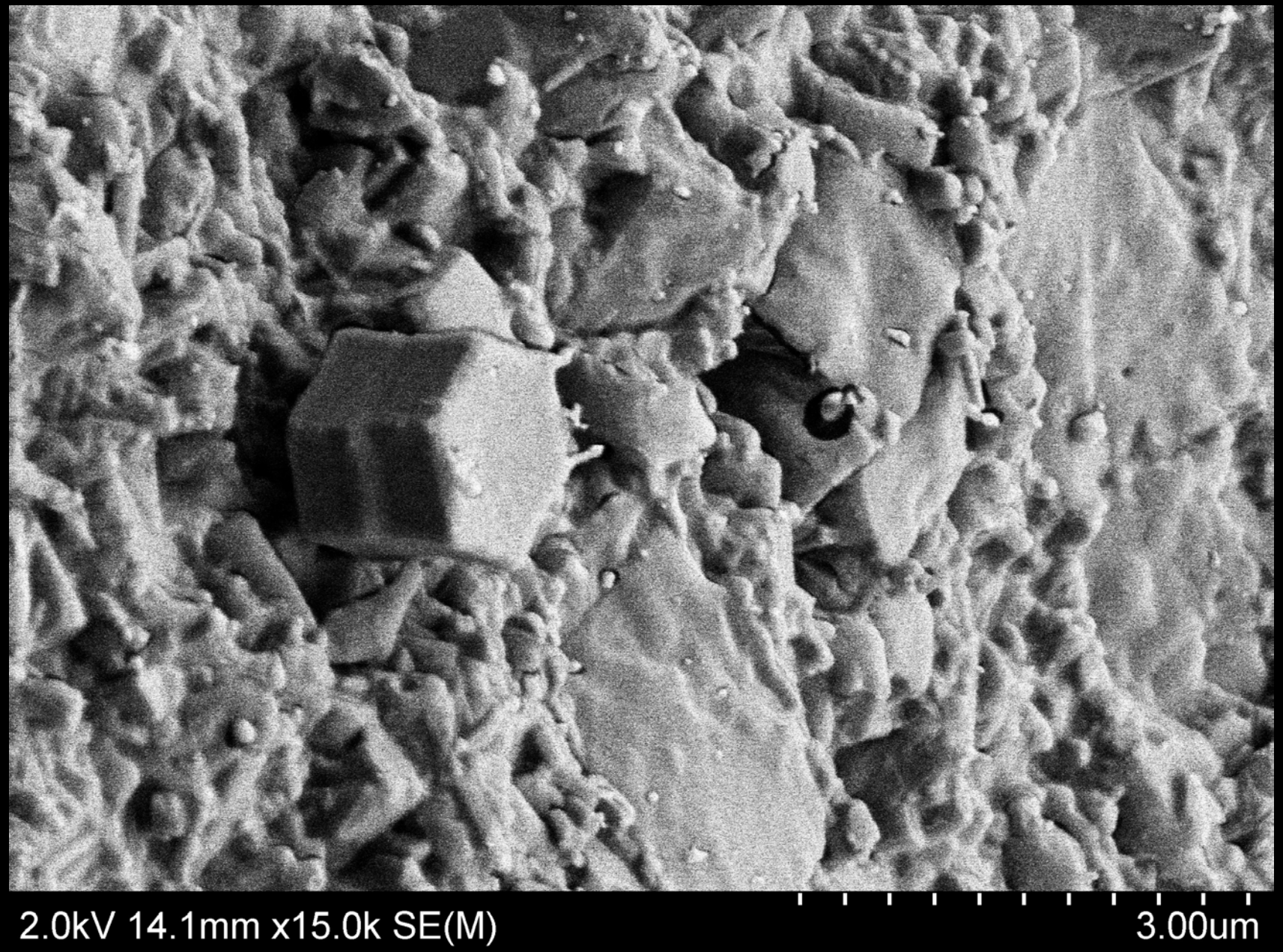
BRIGHTNESS / CONTRAST

Human Breast Cancer Cells, Vivian Shi, NFS REU 2019



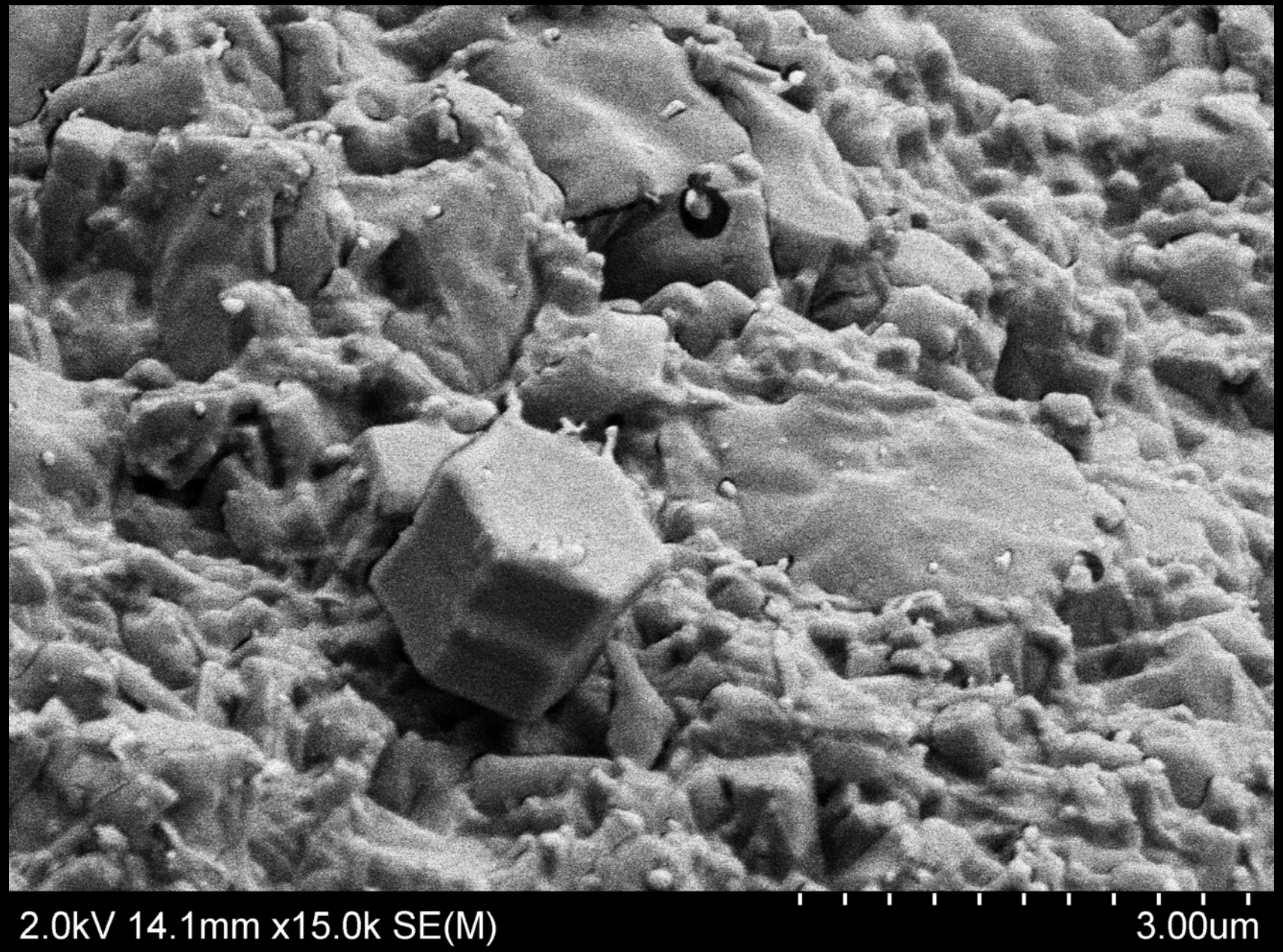


Auto Brightness Contrast (ABC) Vs. Adjusted



WHAT IS WRONG WITH THIS IMAGE?

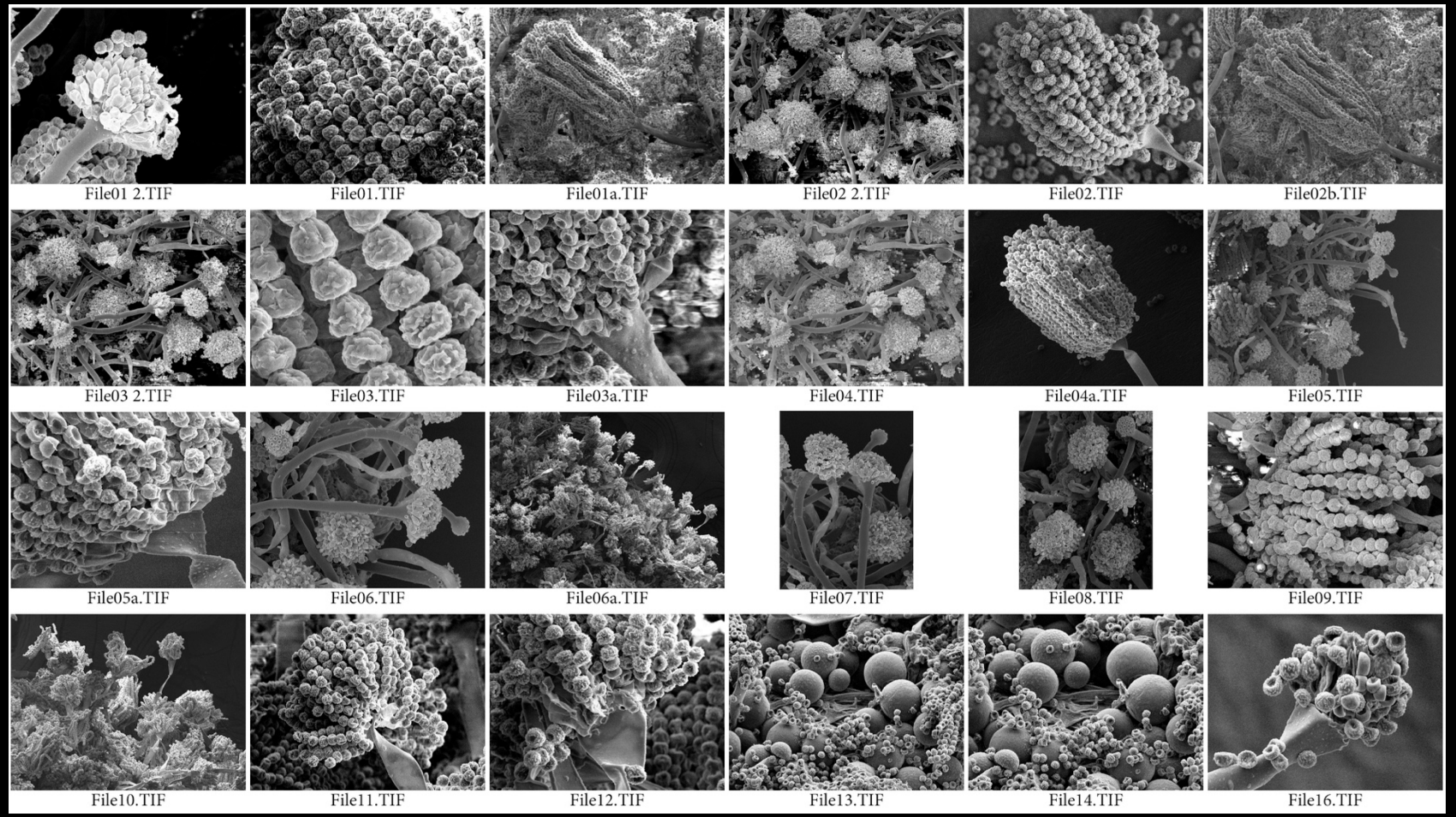
Sugar crystals in candy coating of chewing gum
CryoSEM, Hitachi S4800



RASTER ROTATION IS YOUR FRIEND!

Sugar crystals in candy coating of chewing gum
CryoSEM, Hitachi S4800

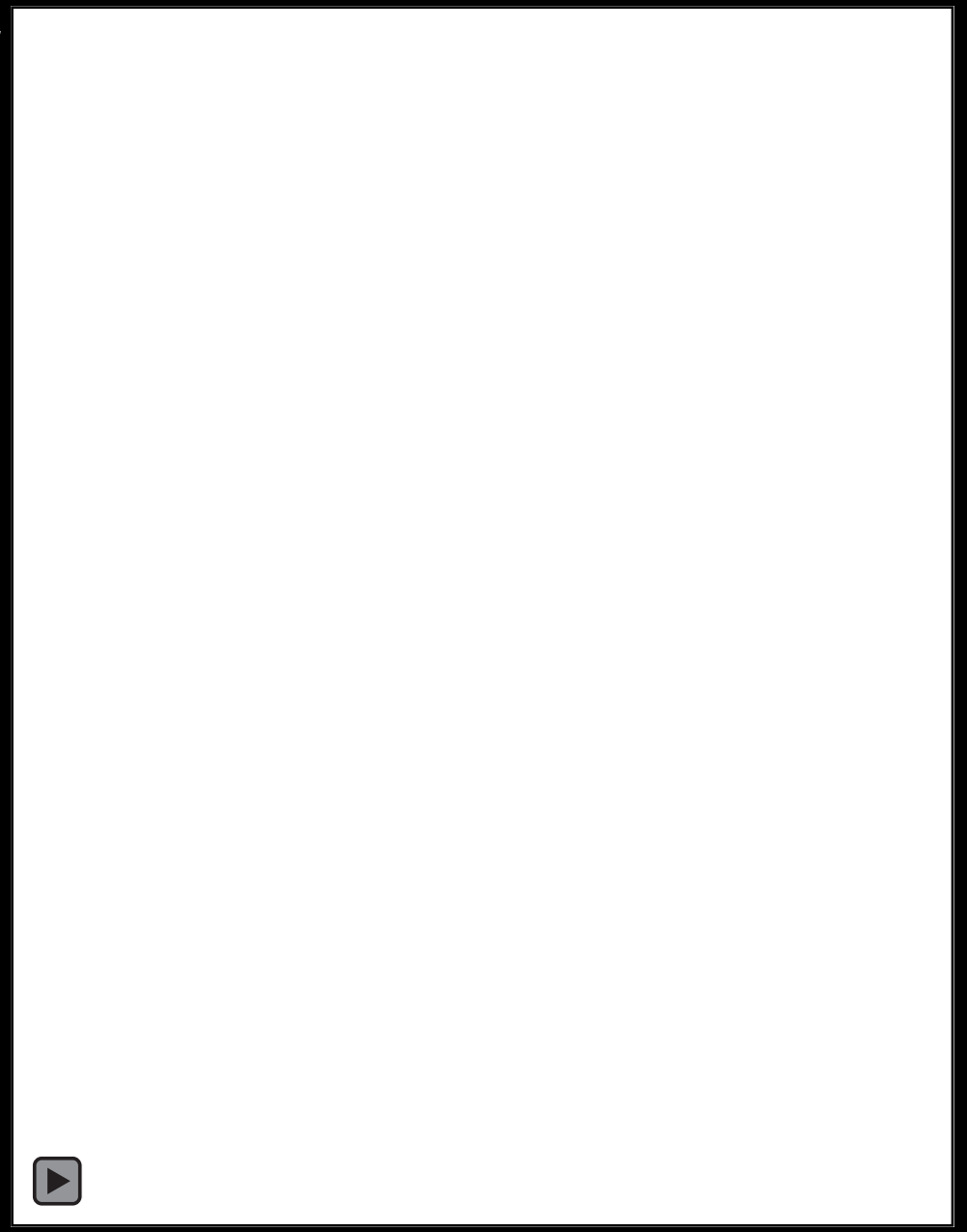
MANY, MANY, MANY IMAGES



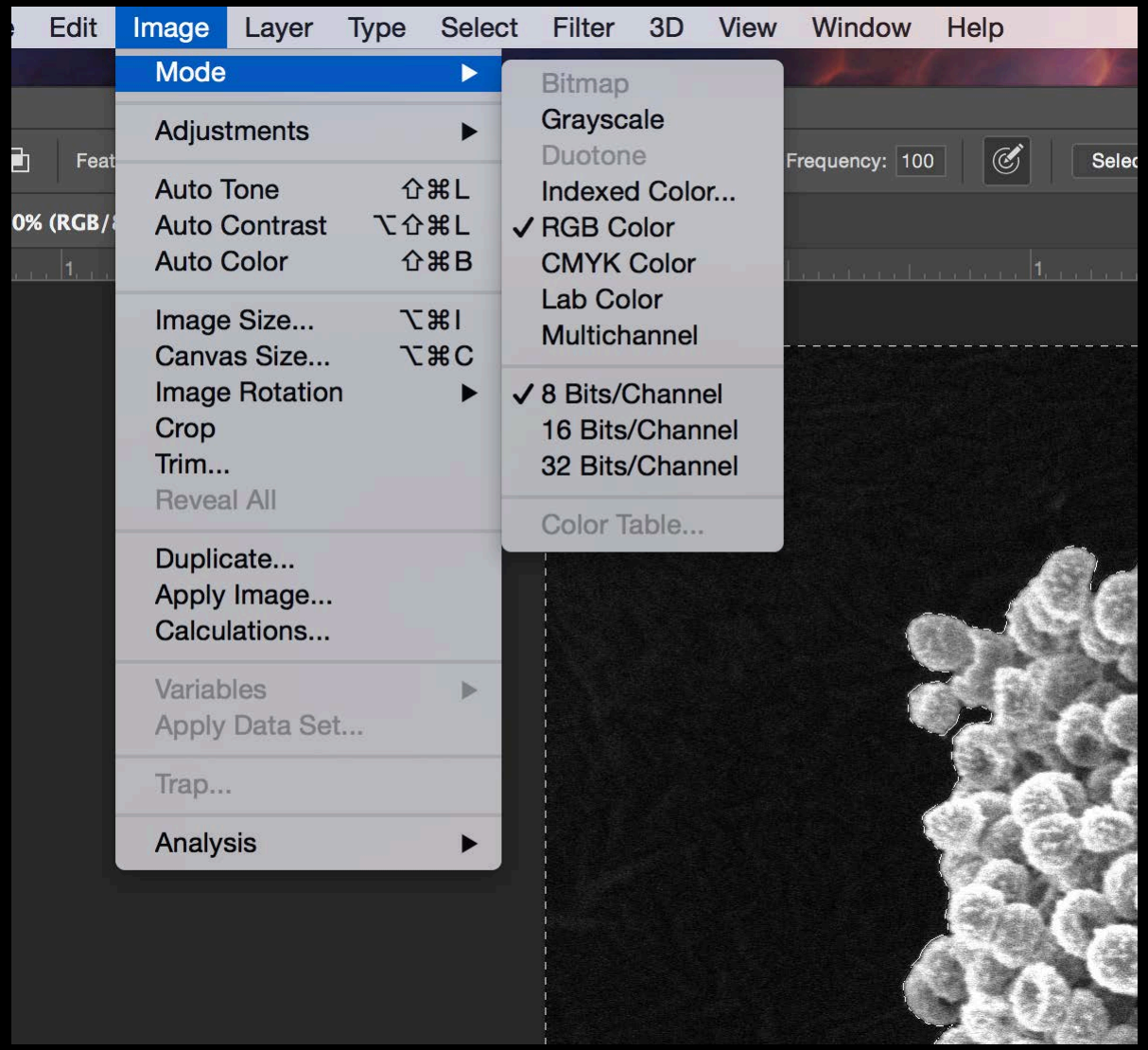
Hold Image Tournament

MAKE IT BEAU

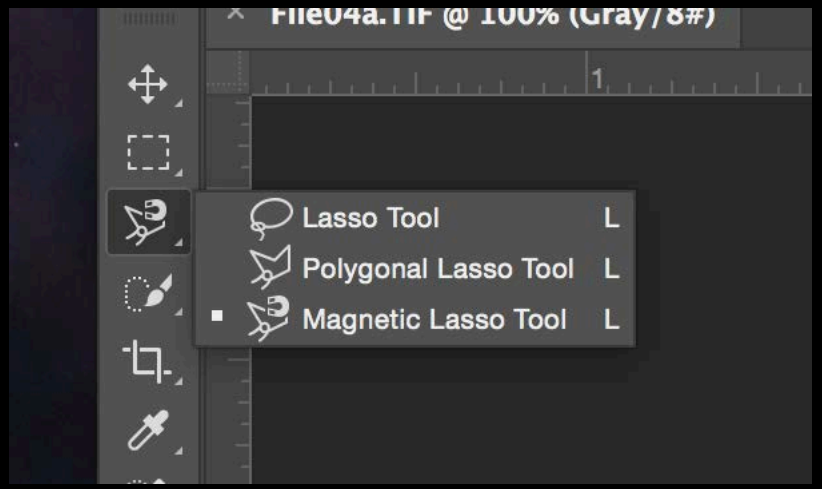
1. Image>Mode>RGB Color
2. Select AIO with magic wand, magnetic loop, etc.
3. Feather selection
4. Colorize selection
5. Invert selection
6. Colorize everything else
7. Select > Color Range
8. Feather selection
9. Modify color
10. Repeat 6-9 until happy

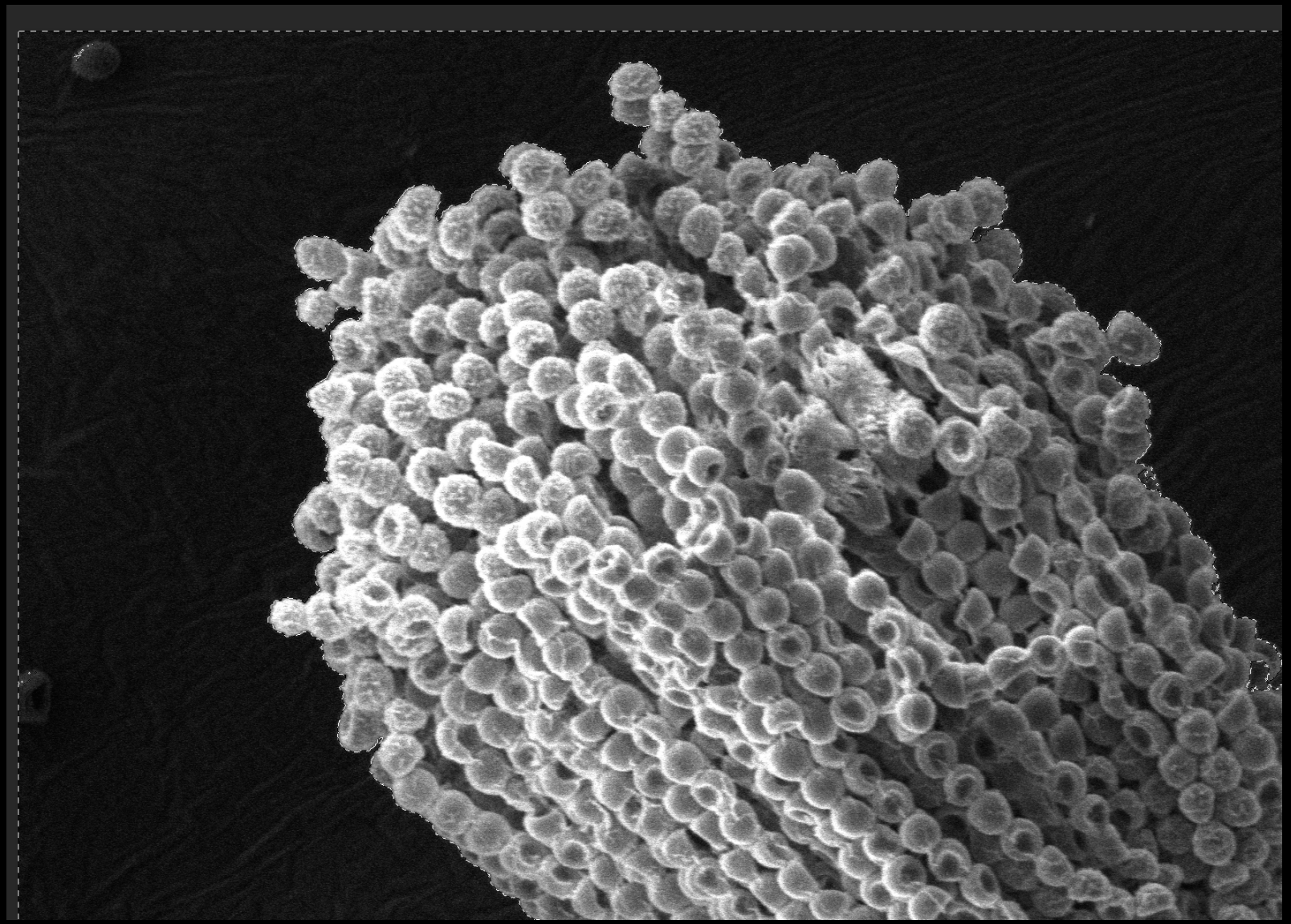


Change from Grayscale or Index Color to *RGB Color*

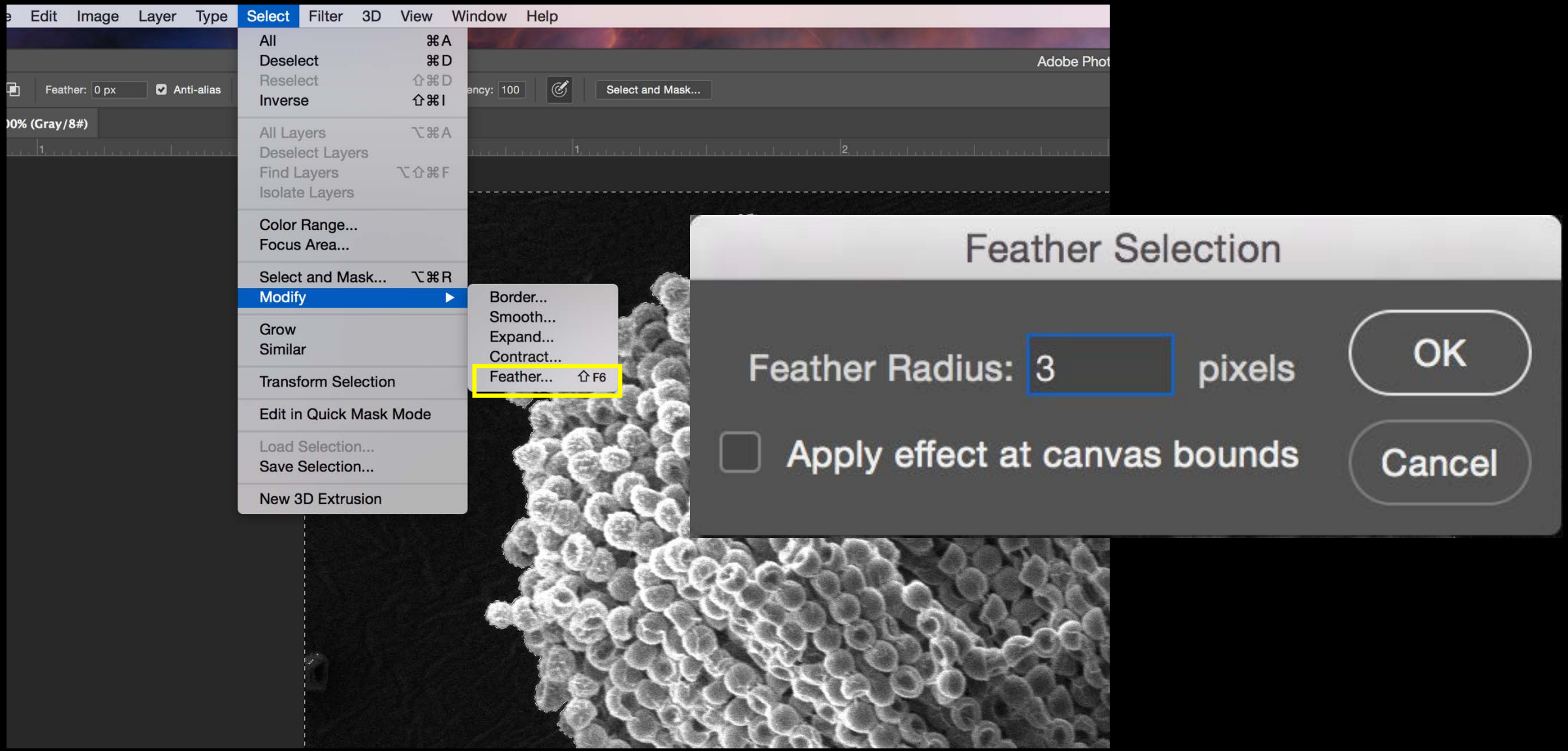


Select AIO with Magic Wand, Magnetic Loop, etc.

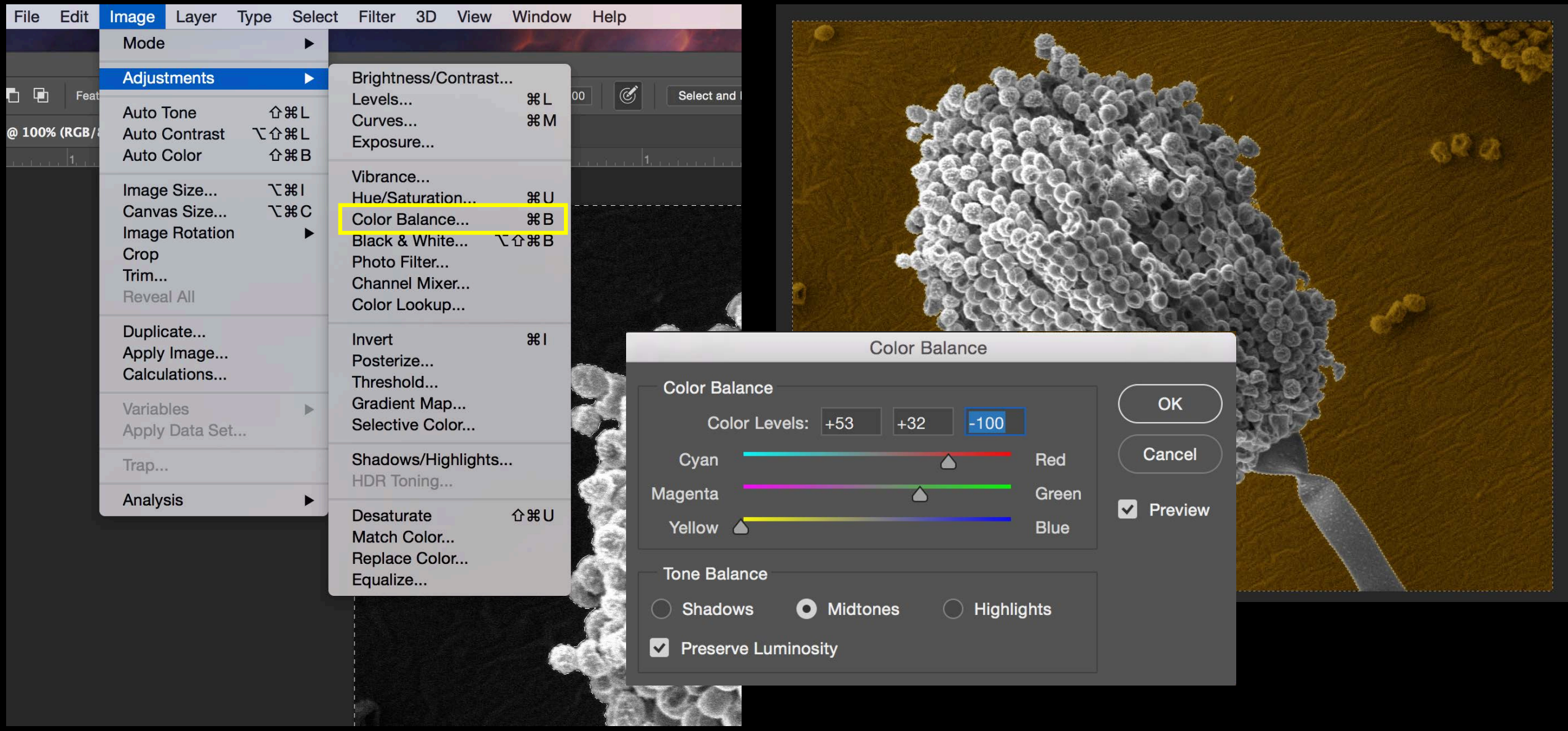




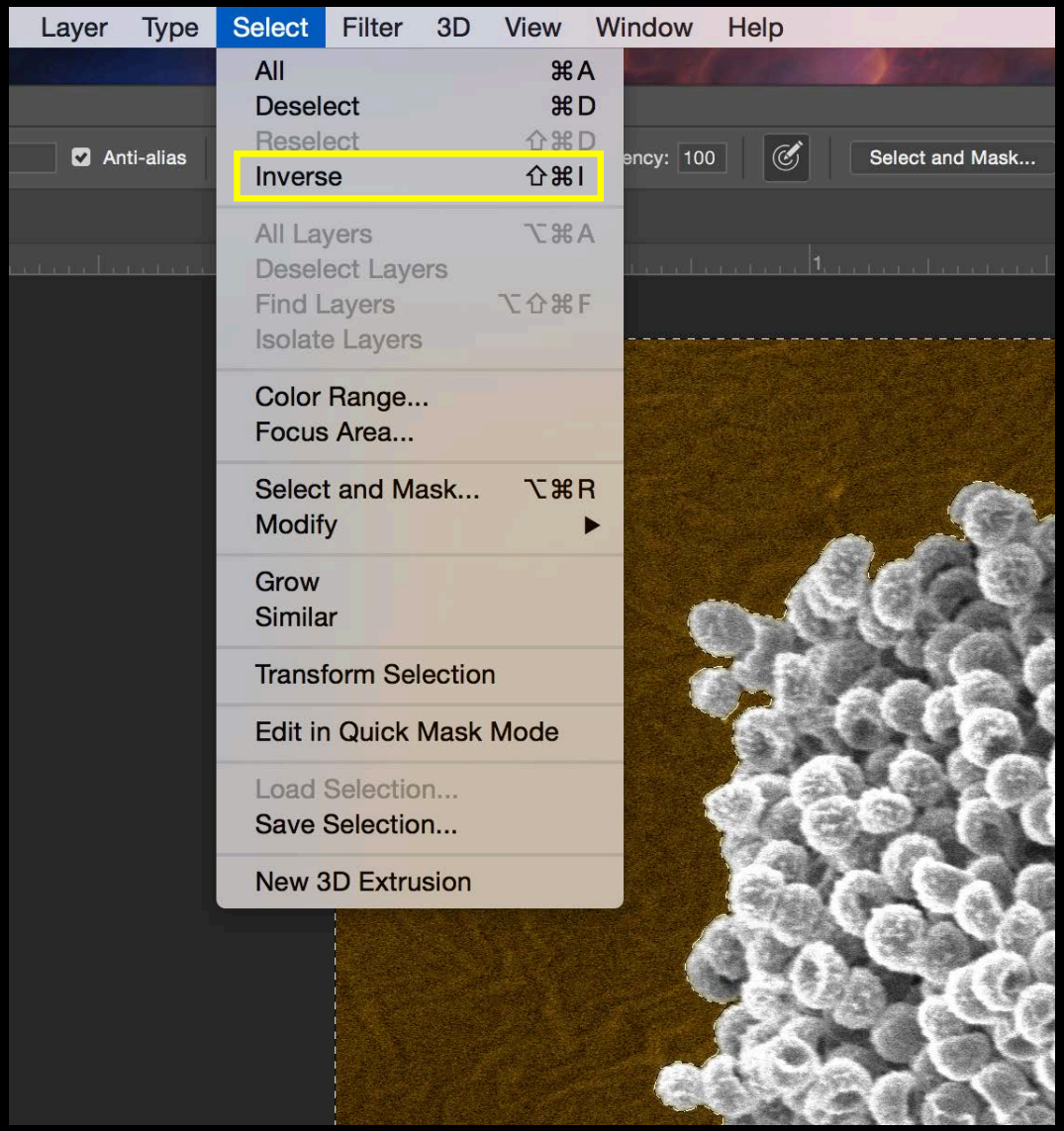
Feather Selection

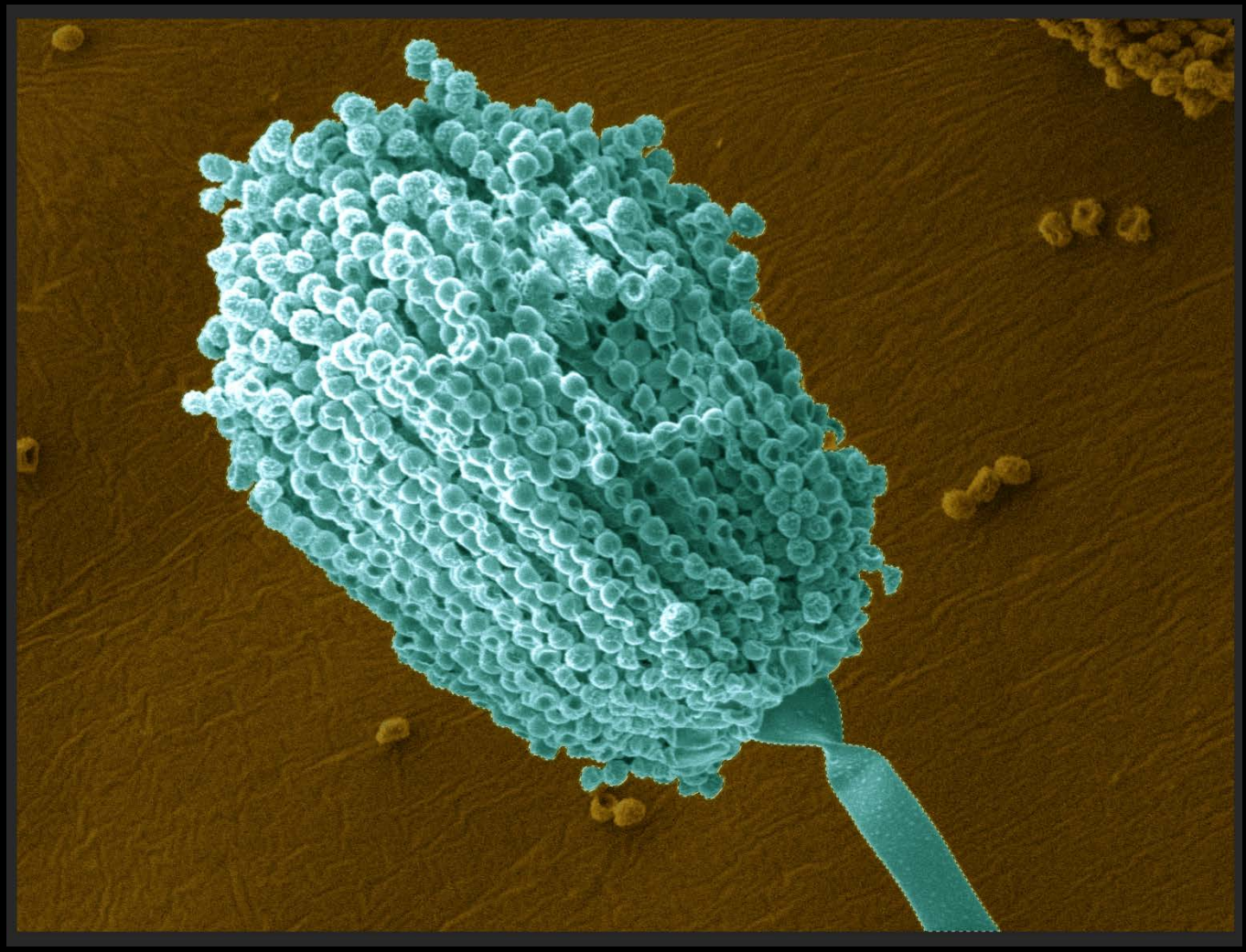


Colorize Selection

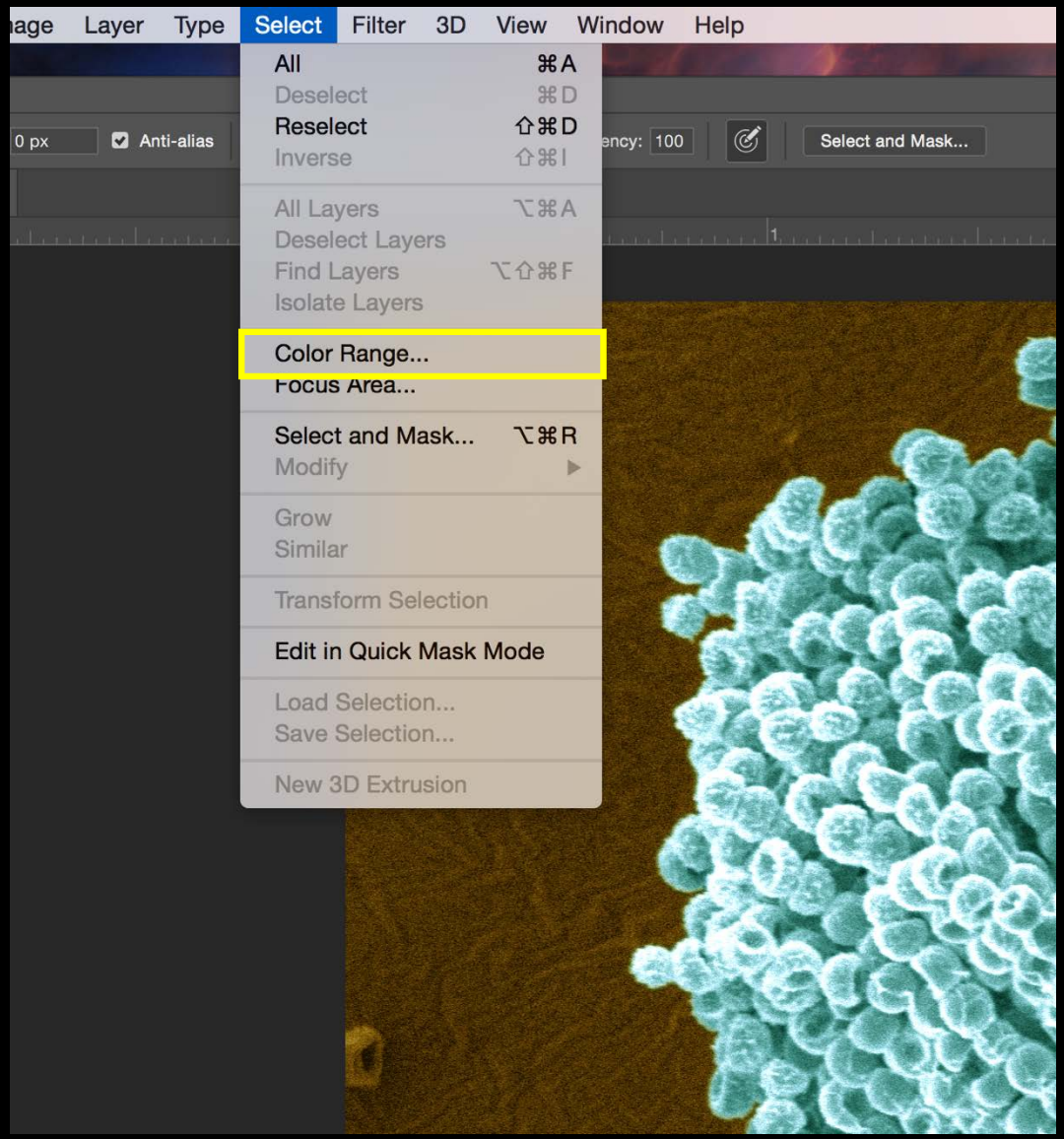


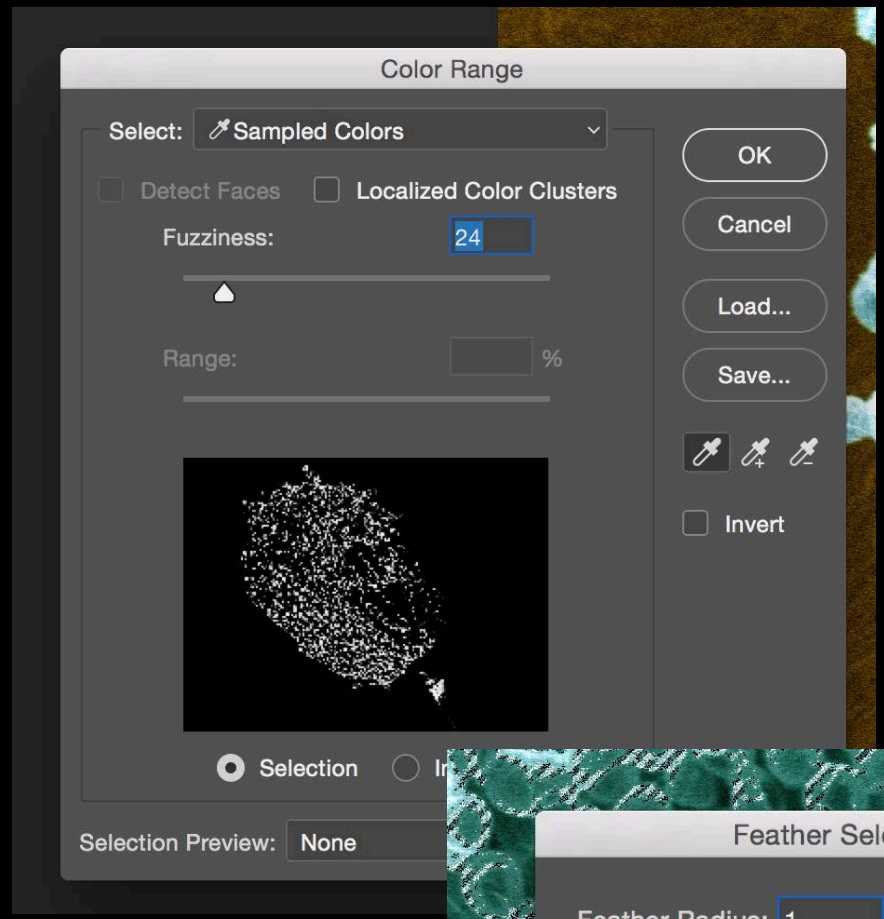
Invert Selection and Colorize

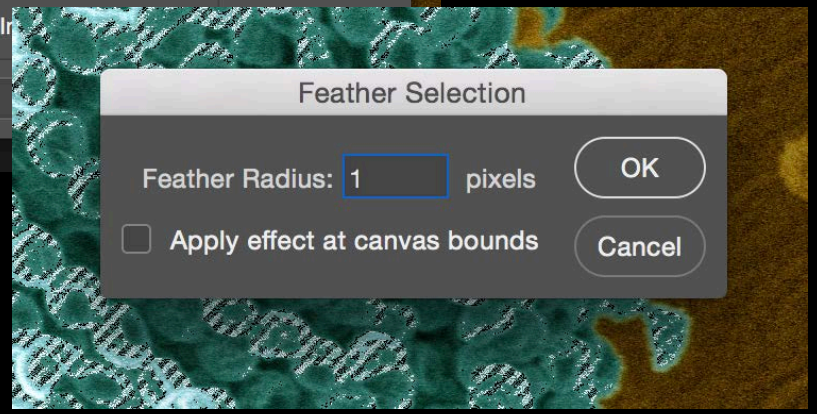




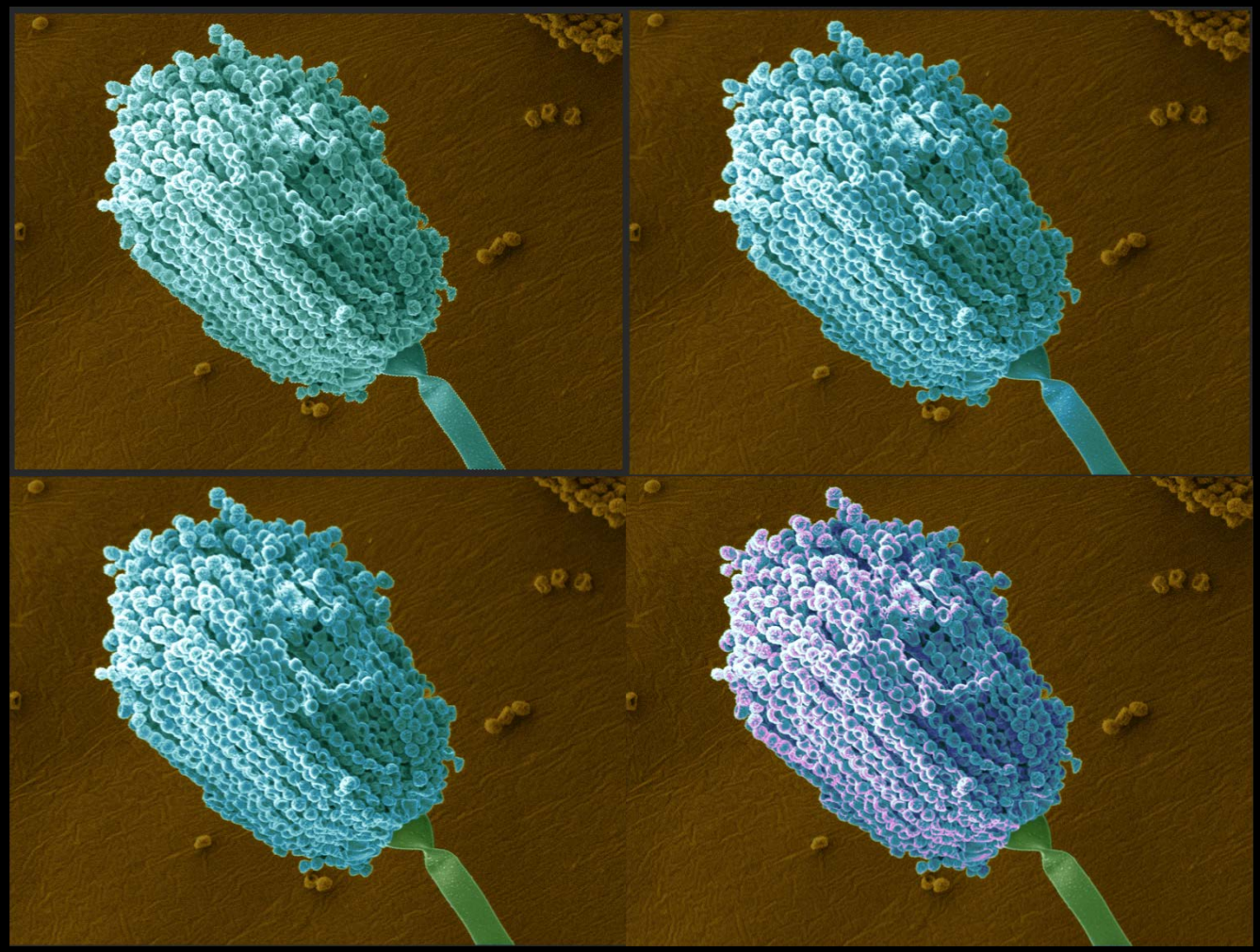
Select Color Range and Feather

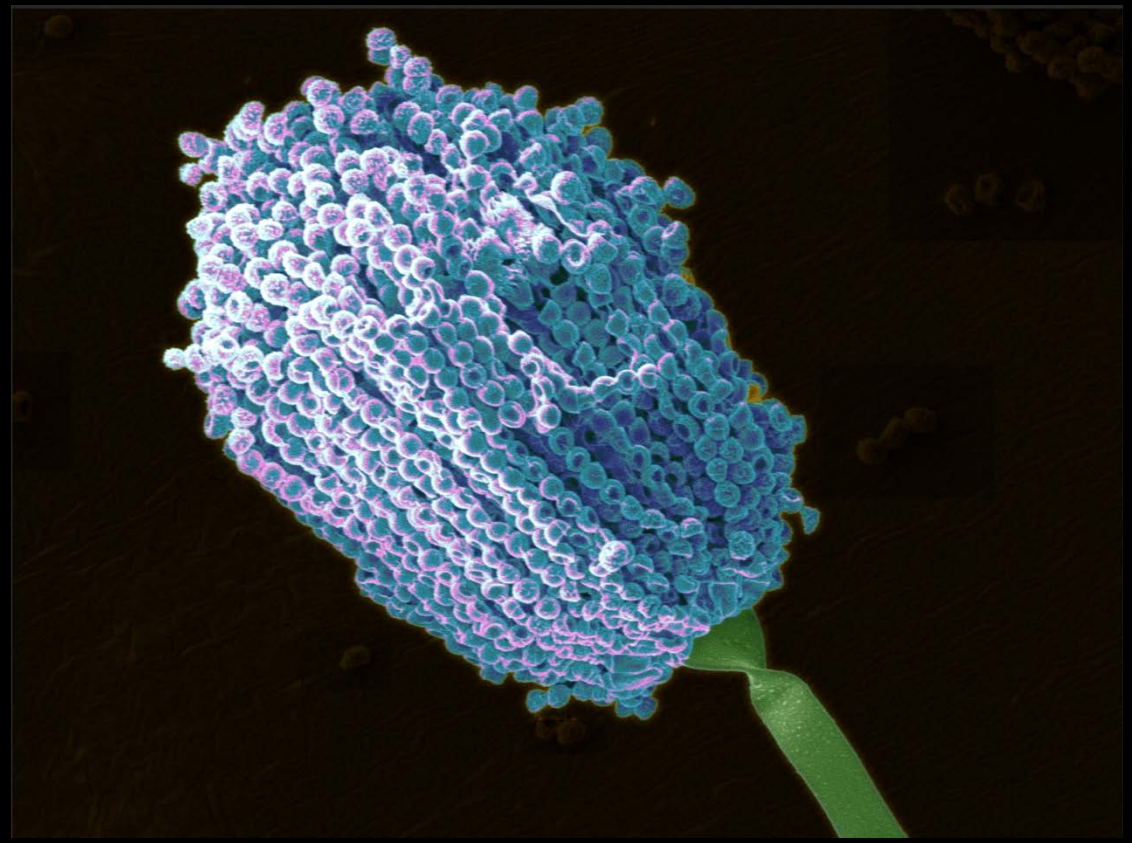






Repeat 6-9 Until Happy





TIPS AND TRICKS

- Remove scale bar when appropriate
- Don’t go overboard with color saturation. Less is more.
- One color does not usually impress
- Use complementary colors to create image harmony
- Attempt to use colors that reflect real life
- Don’t pass up interesting artefacts / anomalies
- SEM Raster Rotation is your friend!
  - shadows towards bottom, highlights from the top

PROMOTE YOURSELF!

- Image contests (Nikon, Cell, Nature, Science in Society (NU), etc.)
- Cover submissions
- Be careful
  - Make sure to clarify if publication allows for free use of, “your” image
  - Don’t scoop yourself
  - Observe Non-Disclosure Agreements

ACKNOWLEDGEMENTS

Vinayak Dravid, NU*ANCE*
Tom O'Halloran, CLP
Neil Kellerher, CLP
Kenneth Clevenger, Kellerher
Emily Que, O'Halloran

Reiner Bleher, BioCryo
Charlene Wilke, BioCryo
Katy Dean, NU*ANCE*
Tirzah Abbott, EPIC
Xiaobing Hu, EPIC
Paul Smeets, EPIC
Xinqi Chen, Keck-II
Gajendra Shekhawat, SPID
Ben Myers, SHyNE
Amy Morgan, NU*ANCE*
Elise Beck, NU*ANCE*
Chad Goeser, NU*ANCE*

Pengxio Hao, NUACCESS
Stephanie Zaleski, NUACCESS
Shunzhi Wang, Mirkin
Matthew Eaton, Shull
Stephanie Ribet, VPD
Vikas Nandwana, VPD
Roberto dos Reis, VPD
Yue Li, VPD/Backman
Vivian Shi, NFS REU SHyNE

Fengxia Liang, NYU
Dan Littman, NYU
Ivo Ivanov, NYU
Doug Wei, Zeiss

QUESTIONS..

ASK ME ANYTHING