Electron Microscopy in the Age of “*Big Data*”

CCEM Summer School on Electron Microscopy

June 5, 2019 - Hamilton, ON

Joshua Taillon

joshua.taillon@nist.gov

NIST Disclaimer

Certain commercial equipment, instruments, materials, vendors, and software are identified in this talk for example purposes and to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

A Brief Introduction

- **Materials scientist by training**
- **Background in materials characterization**
  - TEM, FIB/SEM, EDS/EELS, etc.
- **Stumbled into the world of "advanced" data analysis during my Ph.D. and fell down the rabbit hole**
  - Not a full-time researcher; not IT; not a developer
  - Somewhere on the spectrum of researcher $\leftrightarrow$ data scientist
  - Active contributor to HyperSpy
- **NIST Office of Data and Informatics**
  - Not *actually* a microscopist...

About NIST

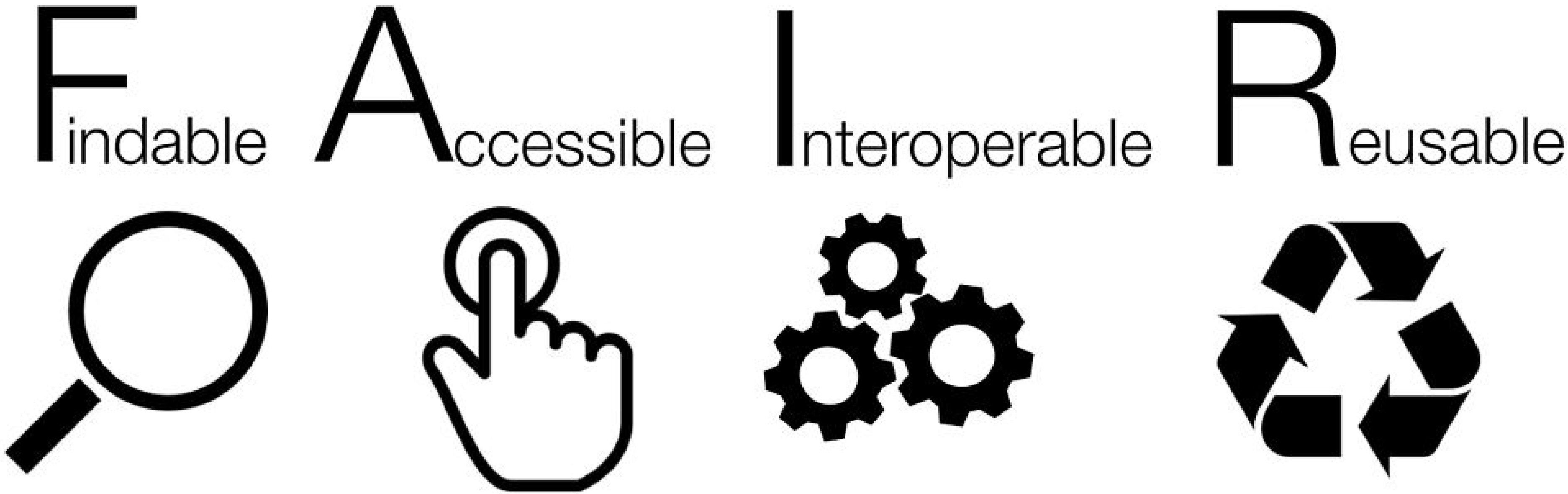
Gaithersburg, MD

Boulder, CO

Office of Data and Informatics

- Provides data expertise and resources to NIST researchers
- Develops best practices to optimize FAIR data products
- Supports research into advanced manipulation, visualization, and analysis of large data sets

Making EM data FAIR



Wilkinson *et al.*, *Scientific Data*, **3**, 160018, 2016 (*link*)
Image: Sangya Pundir - CC-BY-SA 4.0

A dream for EM data publication

- **Publishing data, workflow, and results as one package**
- **LIGO Gravitational Wave detection notebooks**
  - If Nobel Prize winners can do it, we can too!
- **Publishing includes a workflow along with results**
  - Trying to reproduce others' implementations is a waste of scientific (and financial) capital
  - A "journal article" should be able to be downloaded and the analysis reproduced
  - Like a supercharged "Methods" section

High-level Outline

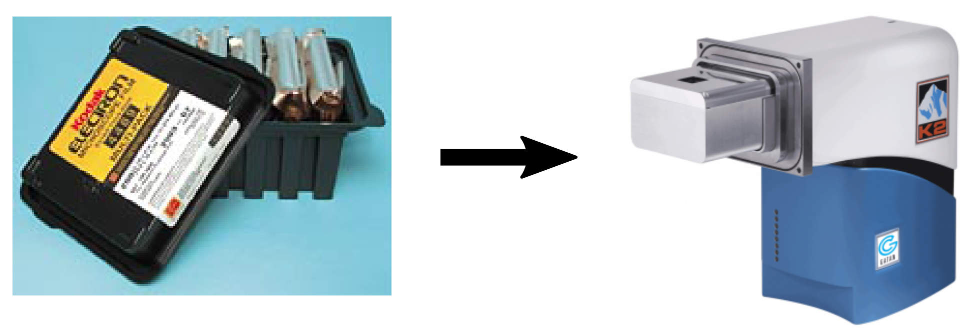
- **What is *computational microscopy*?**
- **Real-world examples of "big data" analysis in EM**
- **The advent of open tools**
  - *Now you can get the same result as your neighbor!*
- **Deeper dive into "signal separation"**
  - Methods, examples, *gotchas*, etc.

WHAT IS *COMPUTATIONAL MICROSCOPY*?

What *is not* CM?

This is personal opinion! Feel free to disagree...

- **Digitization**

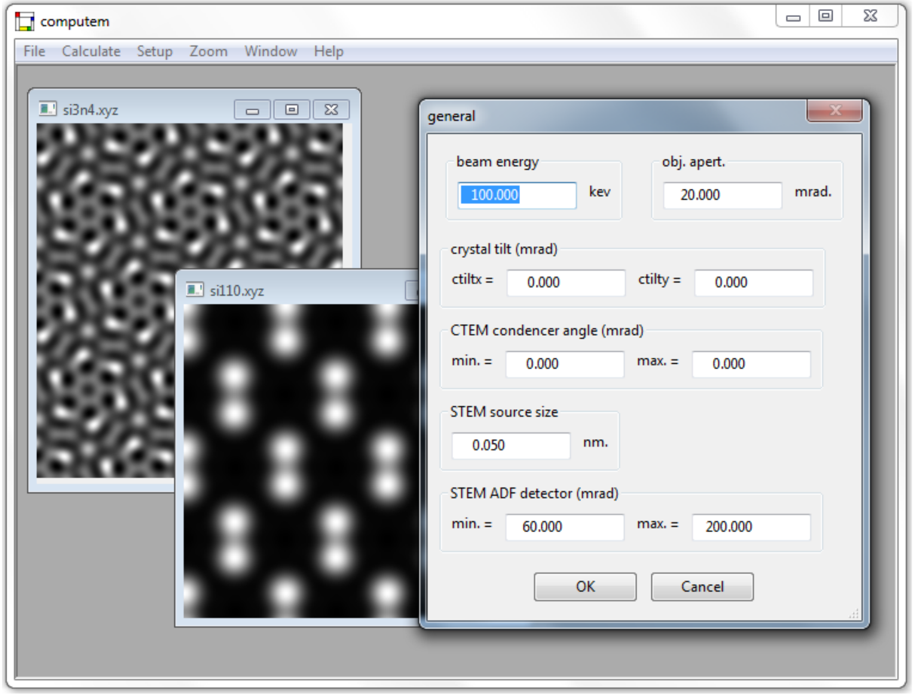


From film to CCD (Ted Pella; Gatan)

What *is not* CM?

This is personal opinion! Feel free to disagree...

- **Digitization**
- **Image simulation**

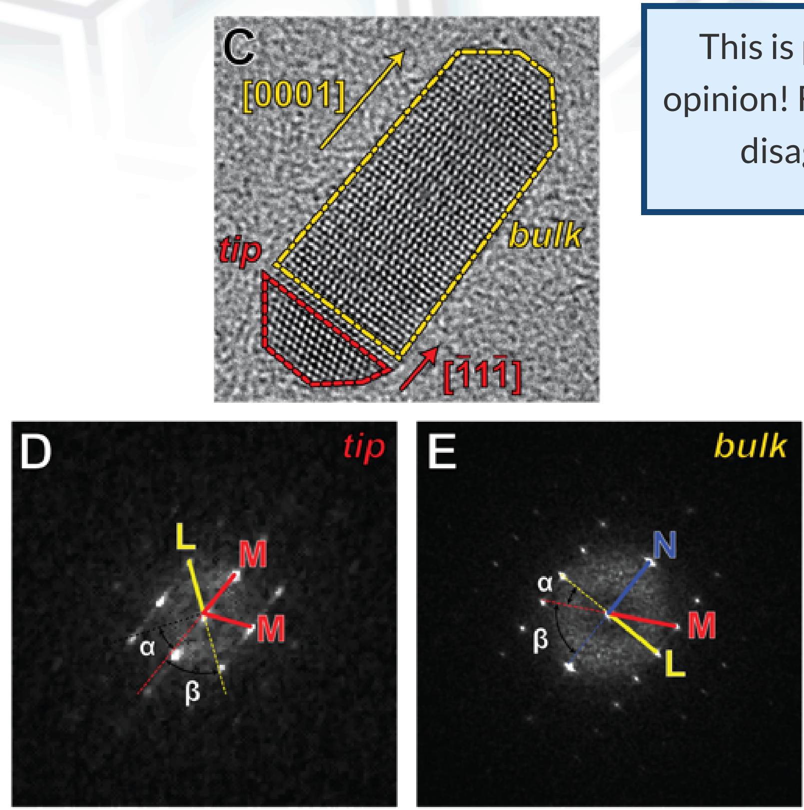


EJ Kirkland; Multislice TEM Simulation

What *is not* CM?

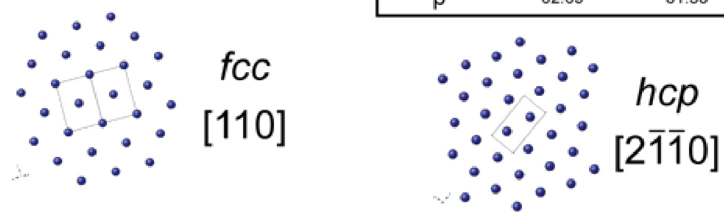
This is personal opinion! Feel free to disagree...

- **Digitization**
- **Image simulation**
- **"Traditional" image analysis**



Parameter	Measured	Expected
$^{L}/_{M}$	1.162	1.155
α	36.16°	35.26°
β	54.63°	54.74°

Parameter	Measured	Expected
$^{M}/_{L}$	1.144	1.139
$^{N}/_{L}$	1.09	1.09
α	28.62°	28.62°
β	62.69°	61.38°



HRTEM Fourier analysis

Attempting a definition

This is personal opinion! Feel free to disagree...

Microscopy directed by or collected primarily for computational processes (as opposed to by/for the user directly)

Attempting a definition

This is personal opinion! Feel free to disagree...

- **Relevant buzzwords:**
  - Machine learning
  - Artificial intelligence
  - Autonomous measurement
  - Dynamic sampling
  - Compressive sensing
  - Sparse imaging

Why computational microscopy?

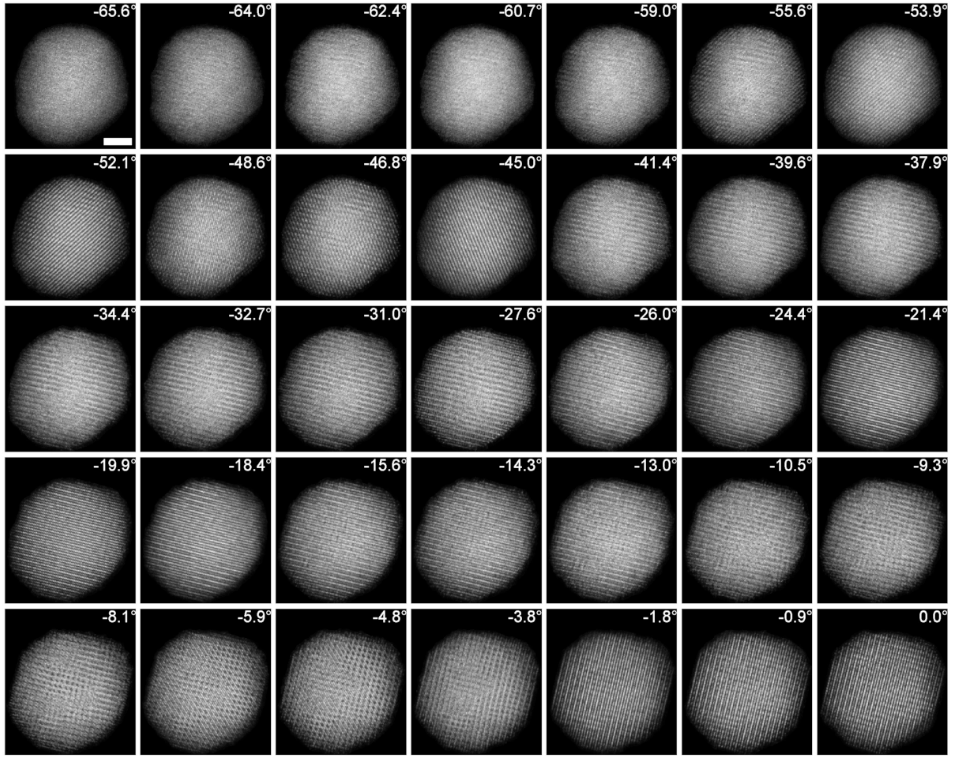
- **Statistical power**
  - Is your image actually *representative*?
- **Reproducibility is the default**
  - Computers only do what you tell them too (*we hope*)
- **Leverages massive advances in computational power**

Some real-world examples of computational microscopy and "big data"

(1/5) Electron Tomography

- Y. Yang, J. Miao, *et al.*, *Nature*, **542**, 75-79, 2017 ([link](#))

- 68 ADF-STEM images of FePt nanoparticle
- Located 6,569 Fe and 16,627 Pt atoms to 21.6 pm precision (correlated with multislice simulations)
- Calculated SROP to distinguish individual grains
- 3D information at the atomic scale



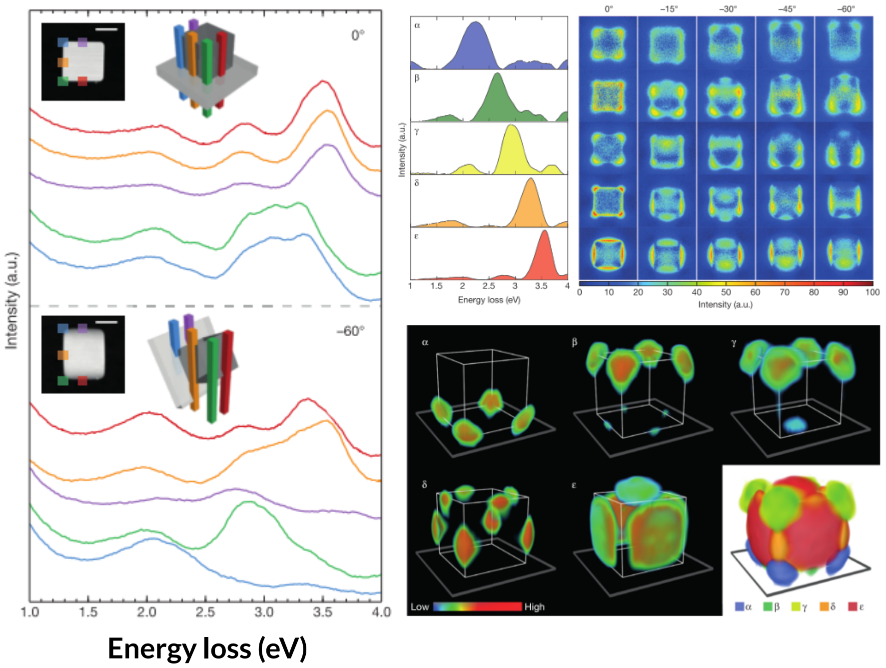
Selection of nanoparticle tilt images

(1/5) Electron Tomography

(2/5) ML for Factor Analysis

- O. Nicoletti, P. Midgley, *et al.*, *Nature*, **502**, 80-84, 2013 (link)

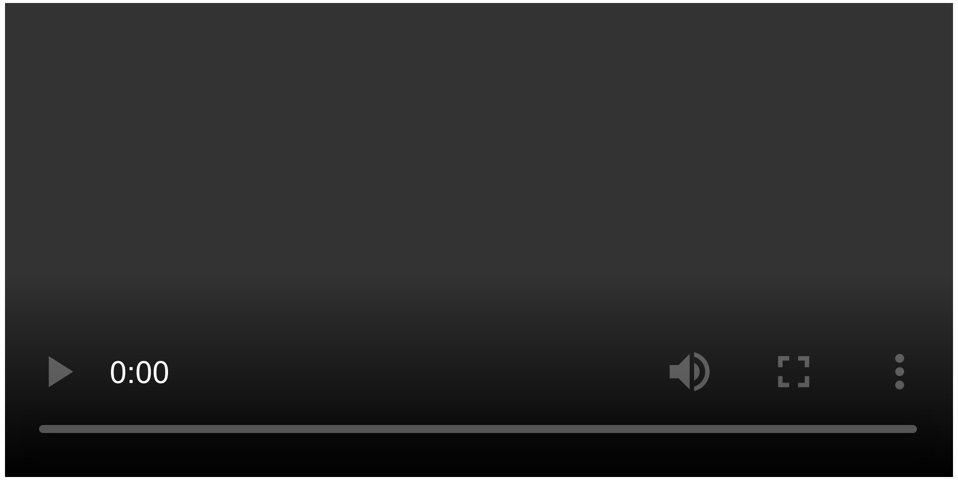
- Non-negative matrix factorization of EELS spectra
- Identifying meaningful spectral components in a sea of overlapping signals
- Combine with tilt-tomography for 3D information
- Identified nanoparticle plasmon resonances



(2/5) ML for Factor Analysis

- O. Nicoletti, P. Midgley, *et al.*, *Nature*, **502**, 80-84, 2013 (link)

- Non-negative matrix factorization of EELS spectra
- Identifying meaningful spectral components in a sea of overlapping signals
- Combine with tilt-tomography for 3D information
- Identified nanoparticle plasmon resonances



(3/5) Autonomous Metrology

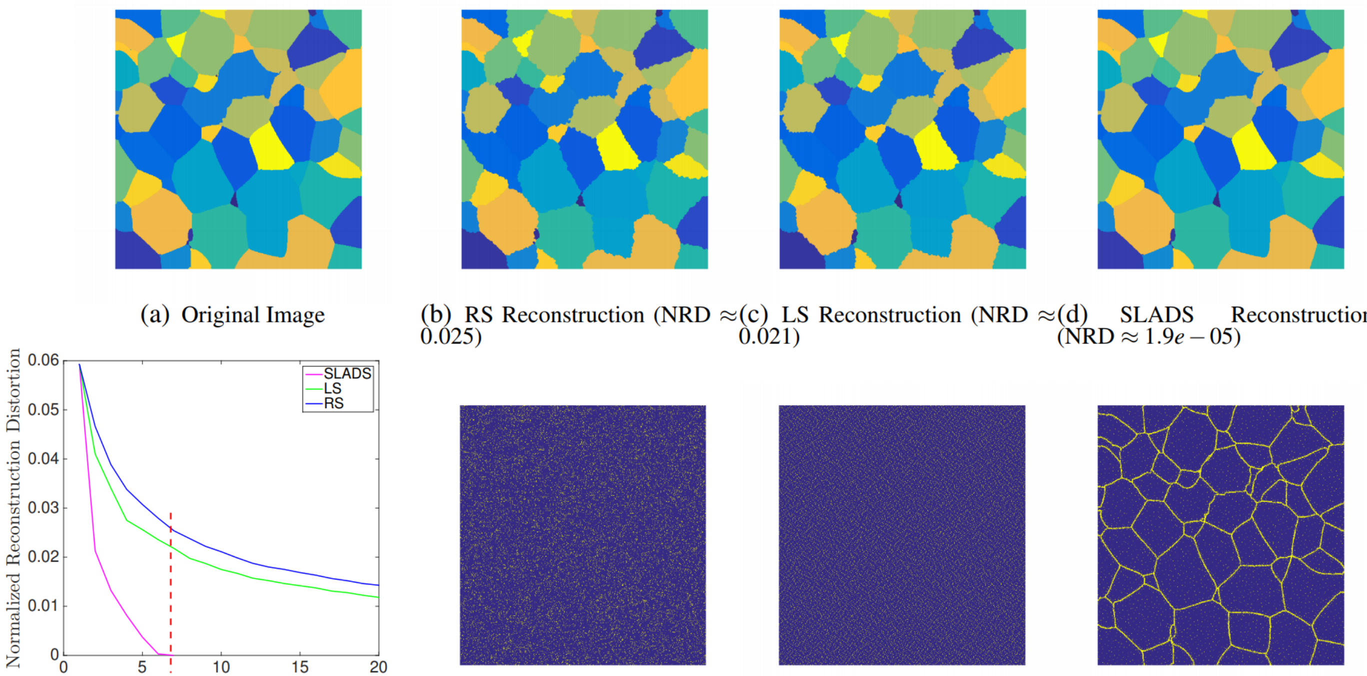
- A. Kusne, I. Takeuchi, *et al.*, *Nanotechnology*, **26**, 444002, 2015 (link)

- High-throughput XRD for combinatorial materials discovery
- Autonomous phase diagram mapping of composition spread wafer
- Phase diagram is estimated at each step based on collected data and physics-informed ML algorithms
- Unsupervised AI determines new composition to measure to best estimate phase diagram

(4/5) Dynamic Sampling in SEM

- G. Godaliyadda, C. Bouman, *et al.*, *Electronic Imaging*, **19**, 1-8, 2016 (link)

- Supervised Learning Approach to Dynamic Sampling (SLADS)
- Sparse imaging and weighted inpainting reconstruction
- Train algorithm offline to measure how much certain types of pixels reduce overall distortion
- Online, pick new pixels to reduce expected distortion in reconstruction



(a) Original Image (b) RS Reconstruction (NRD ≈ 0.025) (c) LS Reconstruction (NRD ≈ 0.021) (d) SLADS Reconstruction (NRD ≈ $1.9e-05$)

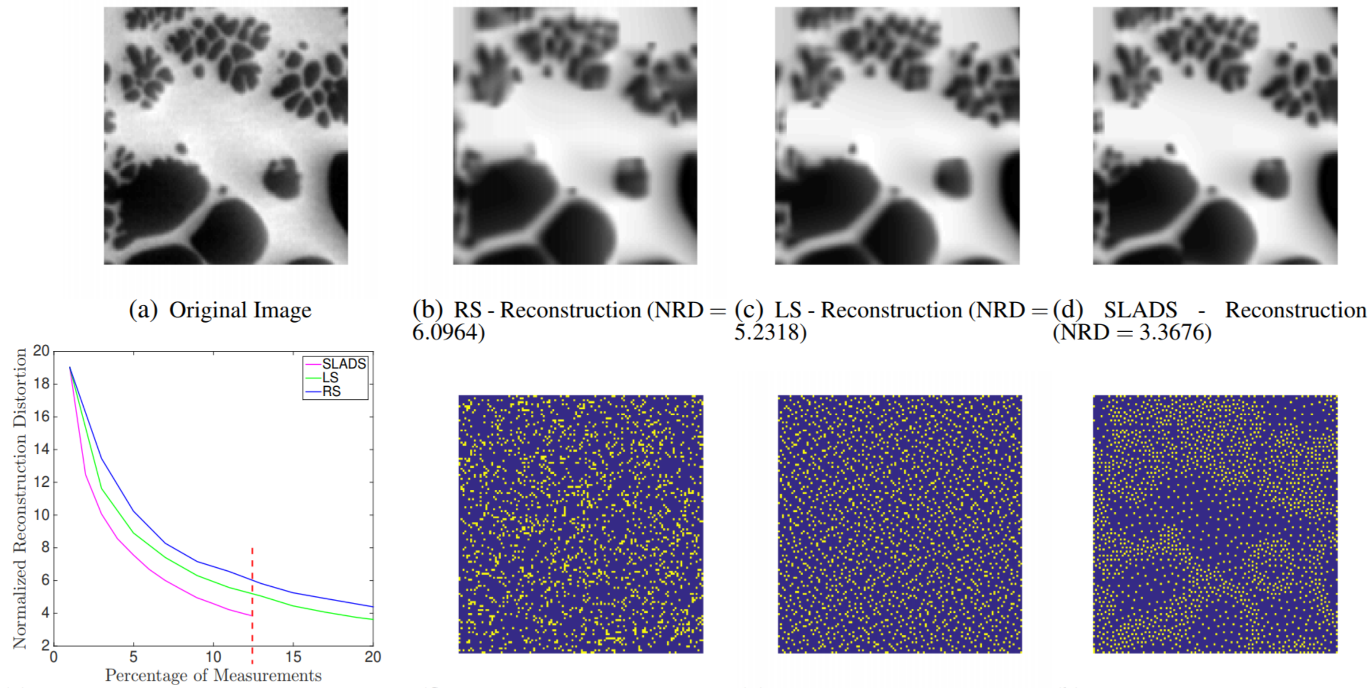
(e) Normalized reconstruction distortion (range 0-1) vs Percentage of samples (f) RS Measurement mask (6.94%) (g) LS Measurement mask (6.94%) (h) SLADS Measurement mask (6.94%)

Simulated EBSD patterns

(4/5) Dynamic Sampling in SEM

- G. Godaliyadda, C. Bouman, *et al.*, *Electronic Imaging*, **19**, 1-8, 2016 (link)

- Supervised Learning Approach to Dynamic Sampling (SLADS)
- Sparse imaging and weighted inpainting reconstruction
- Train algorithm offline to measure how much certain types of pixels reduce overall distortion
- Online, pick new pixels to reduce expected distortion in reconstruction



(a) Original Image (b) RS - Reconstruction (NRD = 6.0964) (c) LS - Reconstruction (NRD = 5.2318) (d) SLADS - Reconstruction (NRD = 3.3676)

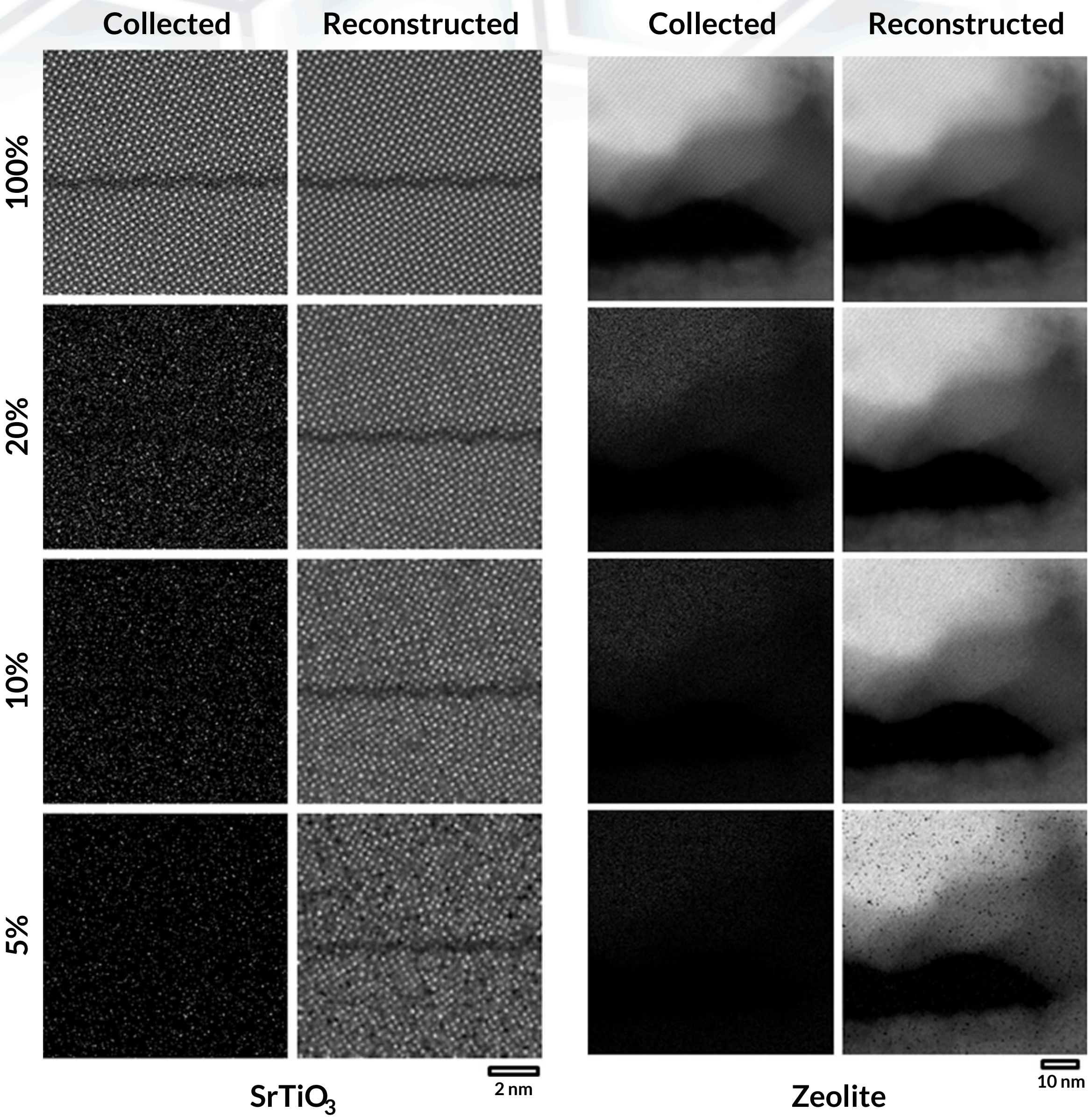
(e) Normalized reconstruction distortion (range 0-255) vs Percentage of samples (f) RS - Measurement mask (12.34%) (g) LS - Measurement mask (12.34%) (h) SLADS - Measurement mask (12.34%)

Experimental SEM images

(5/5) STEM CS

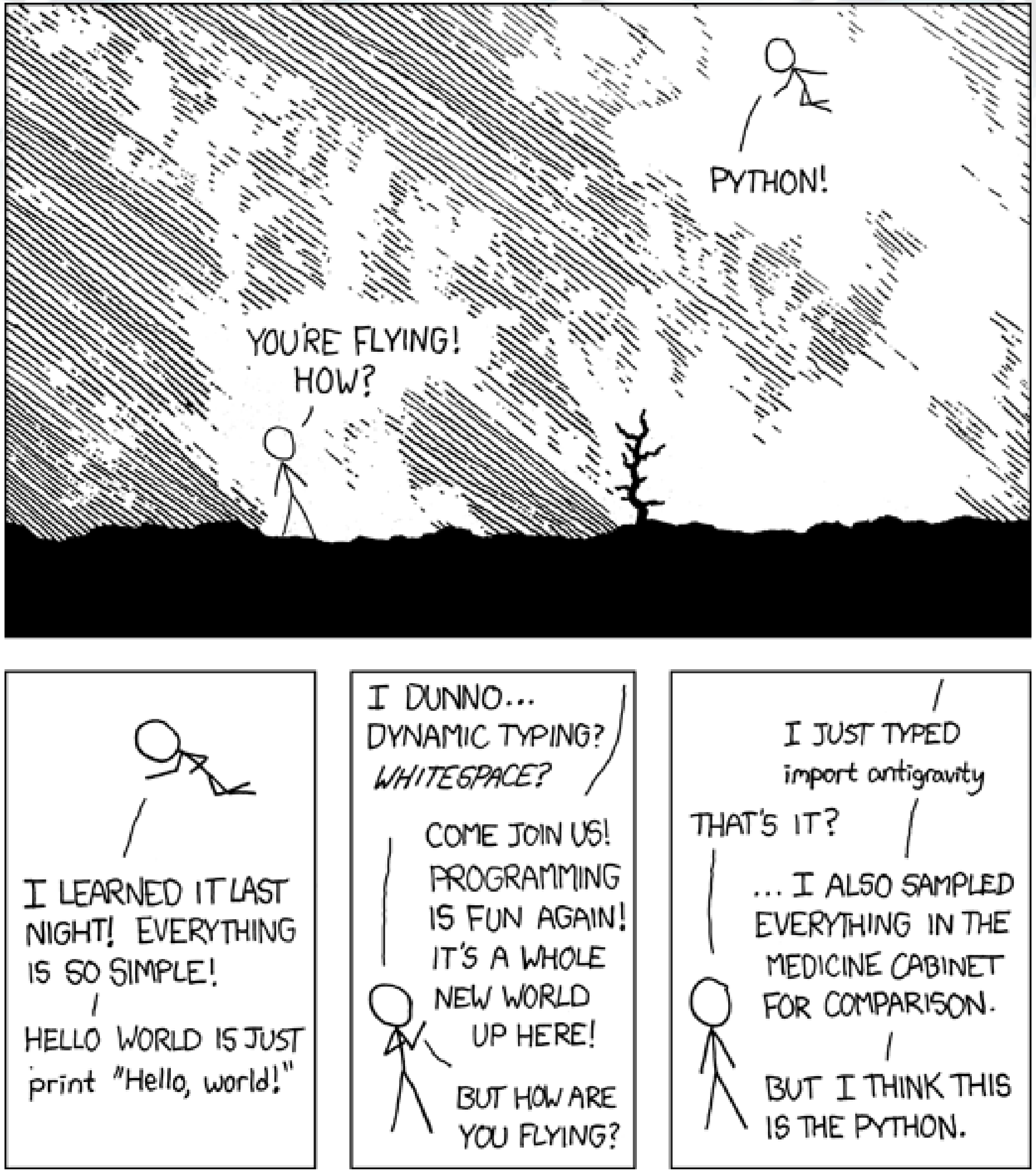
- A. Stevens, N. Browning, *et al.*, *Microscopy*, **63**, 41-51, 2014. (link)

- Intentionally acquire image at severe undersampling conditions
- Use ℓ_1-norm convex optimization to fill in the missing details
- An interesting means to get around the Nyquist-Shannon limit
- Demonstrated with random sampling in both STEM and SEM



Compressed Sensing STEM reconstructions

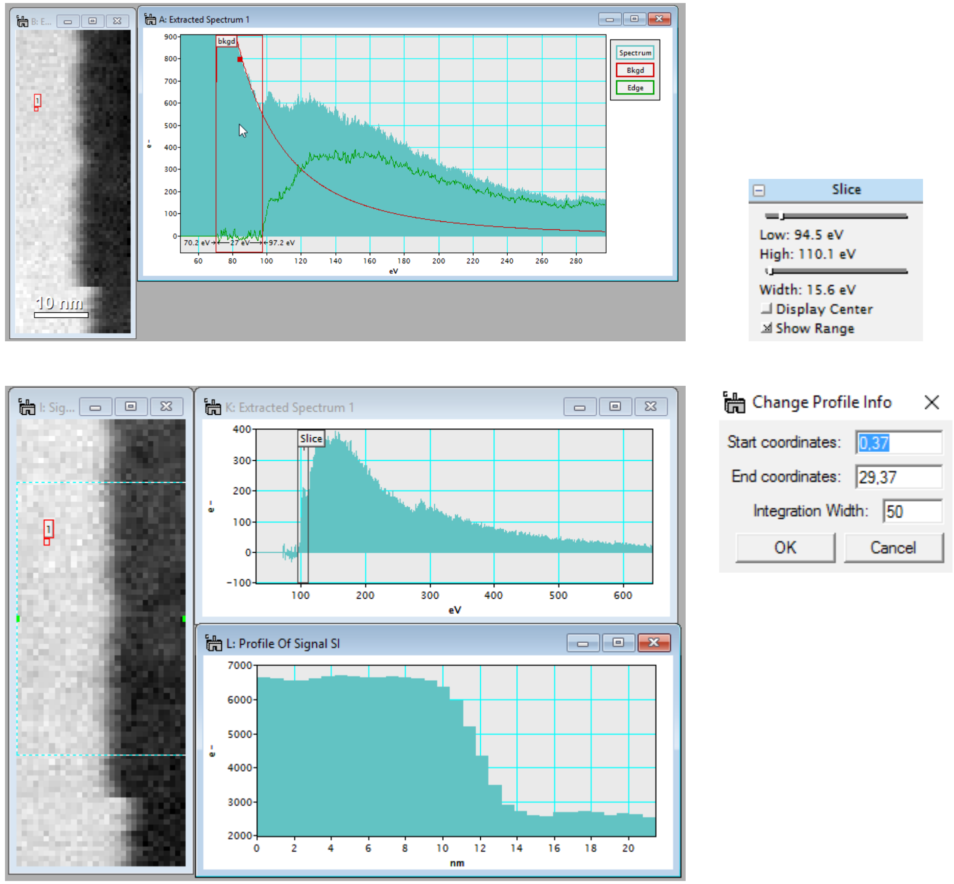
OPEN TOOLS FOR ELECTRON MICROSCOPY



Randall Munroe (2007) - [xkcd]

A "typical" EM analysis

- One or more software packages typically necessary
- Often vendor-provided
- GUI-driven with many options, sometimes “black-box”
- Typically, no log recorded
  - Hope you keep a good notebook!
  - Can you reproduce your post-doc's analysis?
- Tightly integrated with equipment/acquisition

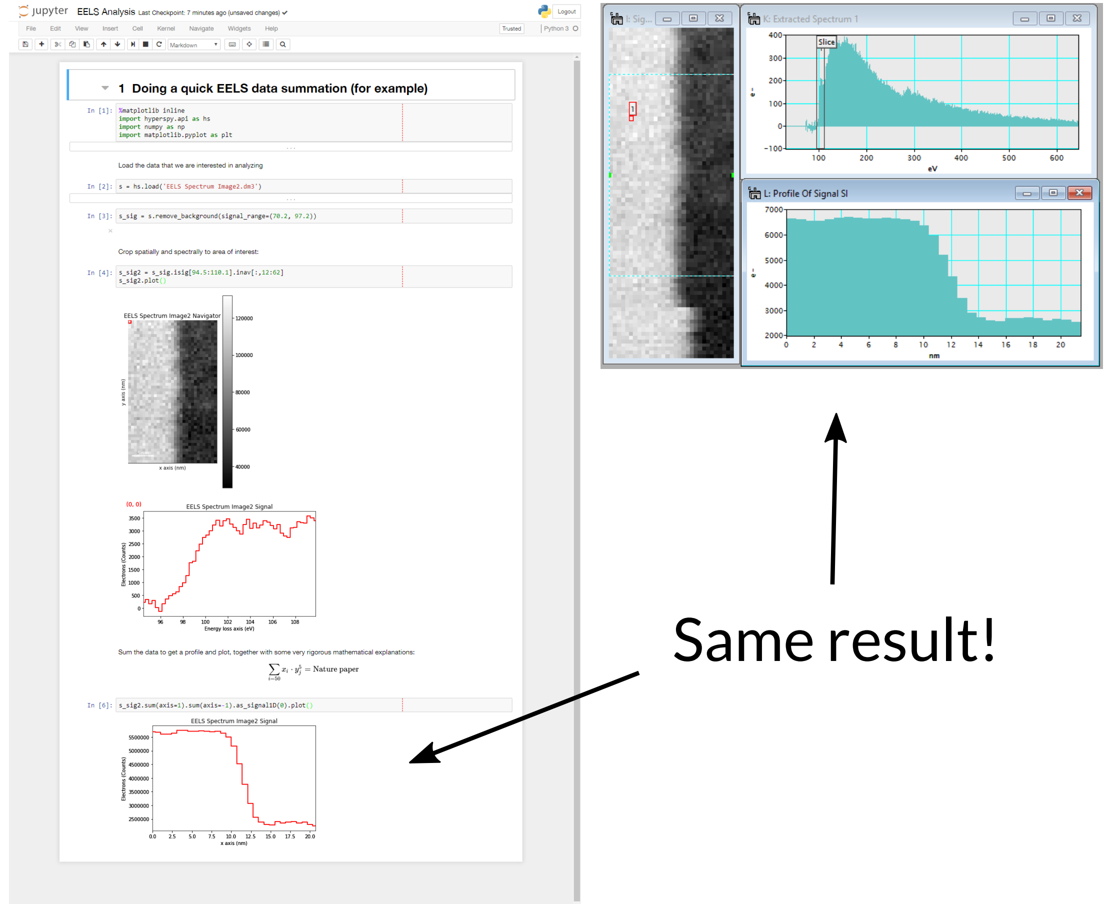


Extracting EELS intensity profile in Gatan Digital Micrograph

A better way?

- A "typical" EELS analysis, but totally reproducible

- Computation within a “notebook” environment
- Seamless mixing of notetaking, mathematics, and data analysis
- Notebook is rendered in any web browser
- Version controlled and exportable to PDF, HTML, Markdown, etc.



Notebook compared to GUI

A better way?

- **Borrowing data tools from other fields**
- **Notebook-based tools are used extensively in data science**
  - Jupyter Notebook
    - http://jupyter.org/ is open-source option
    - Works with Python, Julia, R, Scala, Matlab, Fortran, Ruby, Spark, Go, C, etc.
  - Proprietary options:
    - Mathematica, Maple, Matlab (sort of)
- **Other options**
  - GUI recorders and reporting
  - Data pipelines – Common Workflow Language (CWL)
- **Requires data interoperability**

A better way?

Please join one of the HyperSpy tutorials this week if you want to learn more!

```
import hyperspy.api as hs
s = hs.load('EELS Spectrum Image.dm3')
s_sig = s.remove_background(signal_range=(70.2, 97.2))
s_crop = s_sig.inav[:, 12:62].isig[94.5:110.1]
s_crop.sum(axis=(1,-1)).plot()
```

Only 5 lines of code!

A burgeoning ecosystem

General Purpose

HyperSpy	http://hyperspy.org/
Nion Swift	https://nionswift.readthedocs.io/en/stable/
pycroscopy	https://github.com/pycroscopy/pycroscopy

Others

PyQSTEM	https://github.com/jacobjma/PyQSTEM
HRTEMFringe Analyzer	https://github.com/ialxn/HRTEMFringeAnalyzer
Atomap	https://atomap.org/

Pixelated STEM

pyXem	https://pyxem.github.io/pyxem/
pixStem	https://pixstem.org/
LiberTEM	https://github.com/LiberTEM/LiberTEM
fpd	https://gitlab.com/fpdpy/fpd/

Tomography

tomopy	https://tomopy.readthedocs.io/en/latest/
tomotools	https://github.com/AndrewHerzing/tomotools
tomviz	https://tomviz.org/

And many others (all free!)...

All built on Python!

Click to visit each project

Unsupervised hyperspectral signal separation (spectral unmixing)

Overview

- **What techniques is this applicable to?**
- **What is unmixing (phase mapping)?**
- **Vendor options vs. open solutions**
- **Demonstration of a few different unmixing algorithms**

Applicable techniques

- **Raster-based scanning spectroscopic methods:**
  - Scanning transmission electron microscopy (STEM)
    - Electron energy-loss spectroscopy (EELS) and EDS
  - Scanning electron microscopy (SEM)
    - X-ray energy dispersive spectroscopy (EDS)
  - X-ray fluorescence spectroscopy mapping (XRF and µXRF)
  - Infrared spectroscopy mapping (FTIR)
- **Image-based methods**
  - Time series images of kinetic behaviors
  - Through-focal series in TEM (extracting true structure)

Applicable techniques

- **Raster-based scanning spectroscopic methods:**
  - Scanning transmission electron microscopy (STEM)
    - Electron energy-loss spectroscopy (EELS) and EDS
  - Scanning electron microscopy (SEM)
    - X-ray energy dispersive spectroscopy (EDS)
  - X-ray fluorescence spectroscopy mapping (XRF and µXRF)
  - Infrared spectroscopy mapping (FTIR)
- **Image-based methods**
  - Time series images of kinetic behaviors
  - Through-focal series in TEM (extracting true structure)

X-ray energy dispersive spectroscopy (EDS)

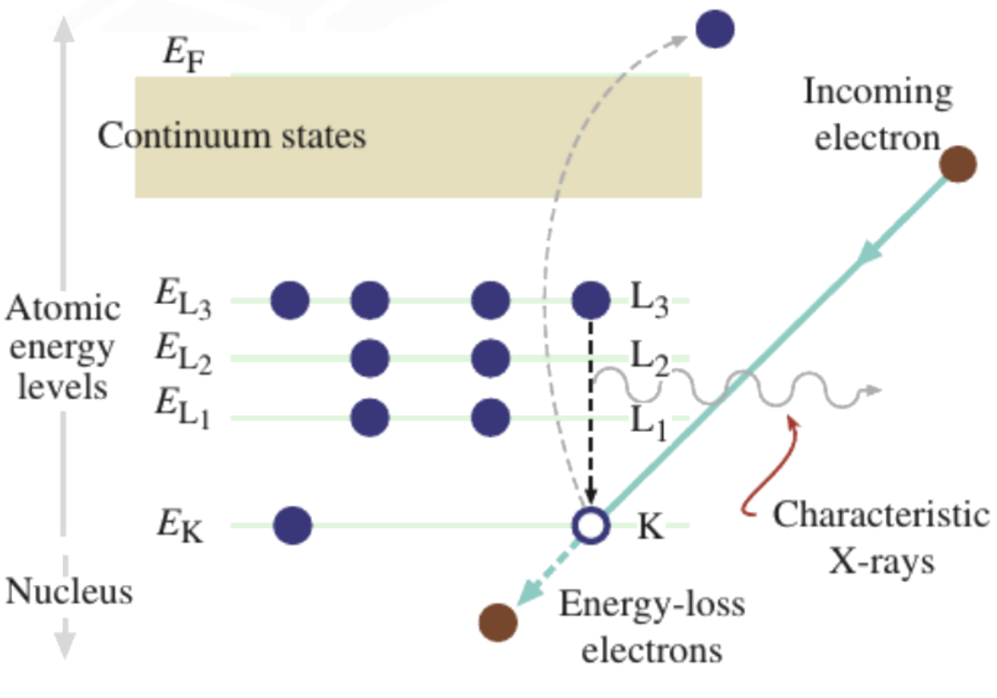
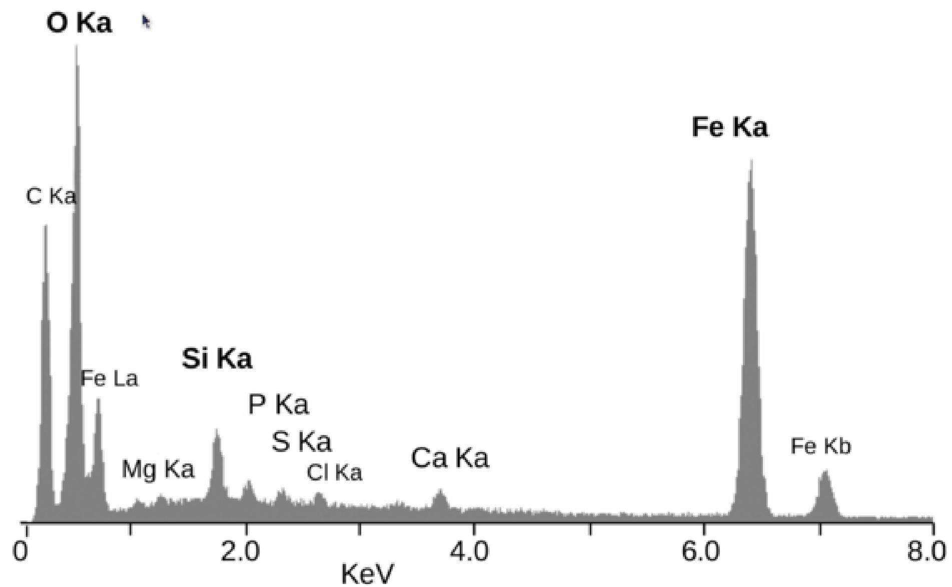


FIGURE 4.2. The ionization process. An inner (K) shell electron is ejected from the atom by a high-energy electron. When the hole in the K shell is filled by an electron from the L shell, characteristic (K_α) X-ray emission occurs. The beam electron loses energy but continues on through the specimen.

Williams and Carter, *Transmission Electron Microscopy*, p. 55 (2009)



EDS spectrum of the mineral crust of the vent shrimp *Rimicaris exoculata*. Most of these peaks are X-rays given off as electrons return to the K electron shell. (K-alpha and K-beta lines) One peak is from the L shell of iron. (source)

What *is* hyperspectral unmixing?

- **Start with some hyperspectral data:**

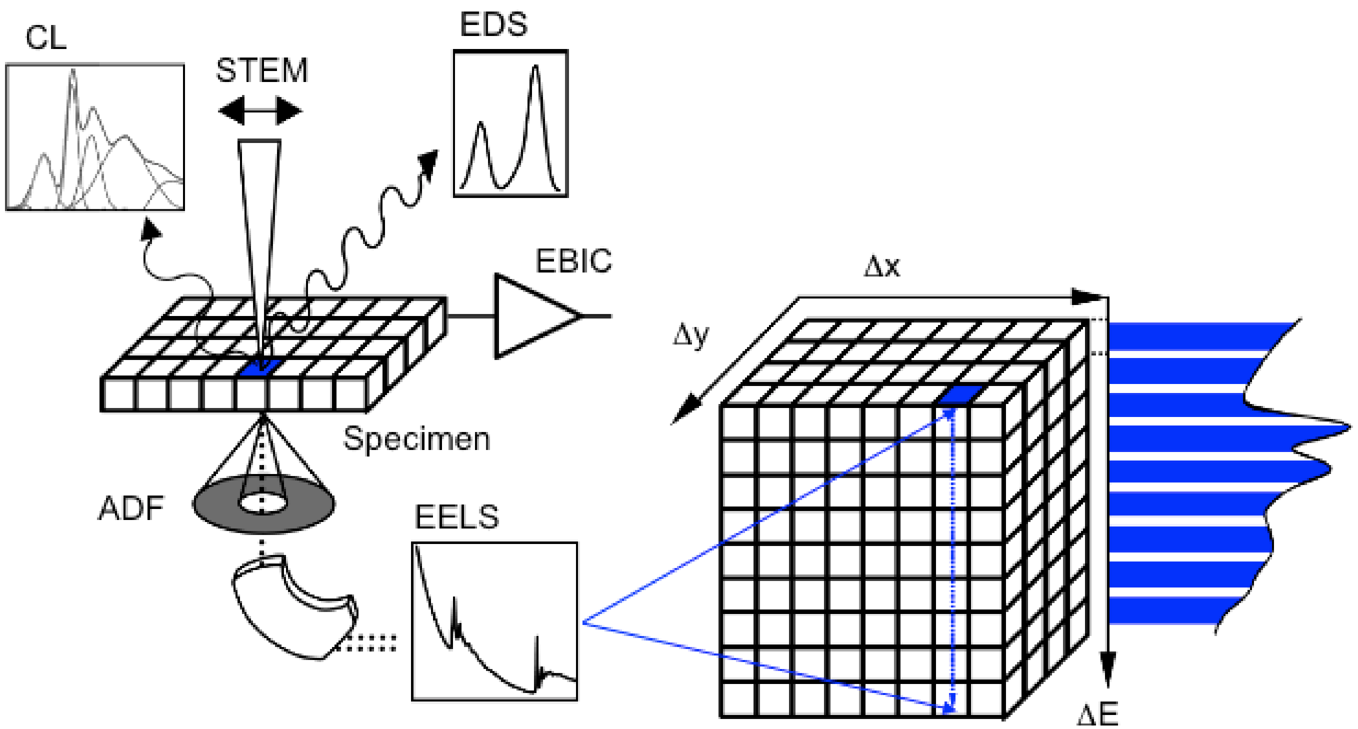


Image courtesy of Gatan, Inc.

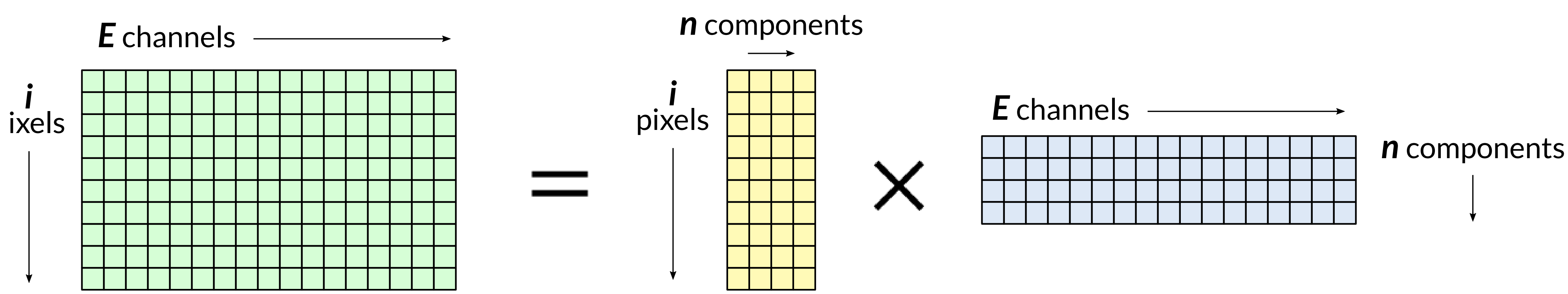
What *is* hyperspectral unmixing?

Collected spectrum image data = **Where is each signal located?** × **What does each signal look like?**

$$\mathbf{D}_{(x,y),E} = \mathbf{W}_{(x,y),n} \times \mathbf{S}_{n,E} + \epsilon_{(x,y),E}$$

$$\mathbf{D}_{i,E} = \mathbf{W}_{i,n} \times \mathbf{S}_{n,E} + \epsilon_{i,E}$$

What *is* hyperspectral unmixing?



$$(i \times E) \qquad (i \times n) \qquad (n \times E)$$

What do the vendors offer?

- **If you've used a modern EDS software package, you probably have done hyperspectral unmixing (they usually call it *phase mapping*)...**

What do the vendors offer?

- **If you've used a modern EDS software package, you probably have done hyperspectral unmixing (they usually call it *phase mapping*)...**

Strengths/challenges of *vendor* options

The Good

- **Simple point-and-click operation**
- **Tight integration**
  - Collection, visualization, reporting, etc.
- **Usually runs in real-time**
- **Integration with other data sources (*e.g.* EBSD)**
- **Generally "just works"**

The Not So Good

- **Extremely "black box"**
- **Reproducibility (!)**
  - Configurable options with little understanding of why
- **What are the uncertainties?**
- **Tied to software ($)**
- **Choice of vendor should not change the scientific result**

Strengths/challenges of *open source* options

The Not So Good

- **Usually not point-and-click**
- **(Can be) difficult to access raw data from the vendor software**
- **Generally only post-processing**
- **Learning curve can be substantial**
- **Can take a lot more fiddling**

The Good

- **You know what's happening**
- **Reproducibility (!)**
  - Anyone can recreate your analysis (including you!)
- **Uncertainty can be understood**
- **Usually free**
- **Results do not depend on vendor**

Offline "phase mapping"

- **Many algorithms exist to solve:** $\quad {}_{(x,y),E} = \quad {}_{(x,y)} \times \mathbf{S}_E$
  - Assumptions implicit in each affect their suitability for EDS, EELS, etc.
- **Primary methods:**
  - Principal component analysis (PCA) — finds non-physical spectra that describe the most variance in the datacube
  - Independent component analysis (ICA) — maximizes independence between spectral results
  - Multivariate curve resolution (MCR) and non-negative matrix factorization (NMF) — enforce positivity in spectral components and weights

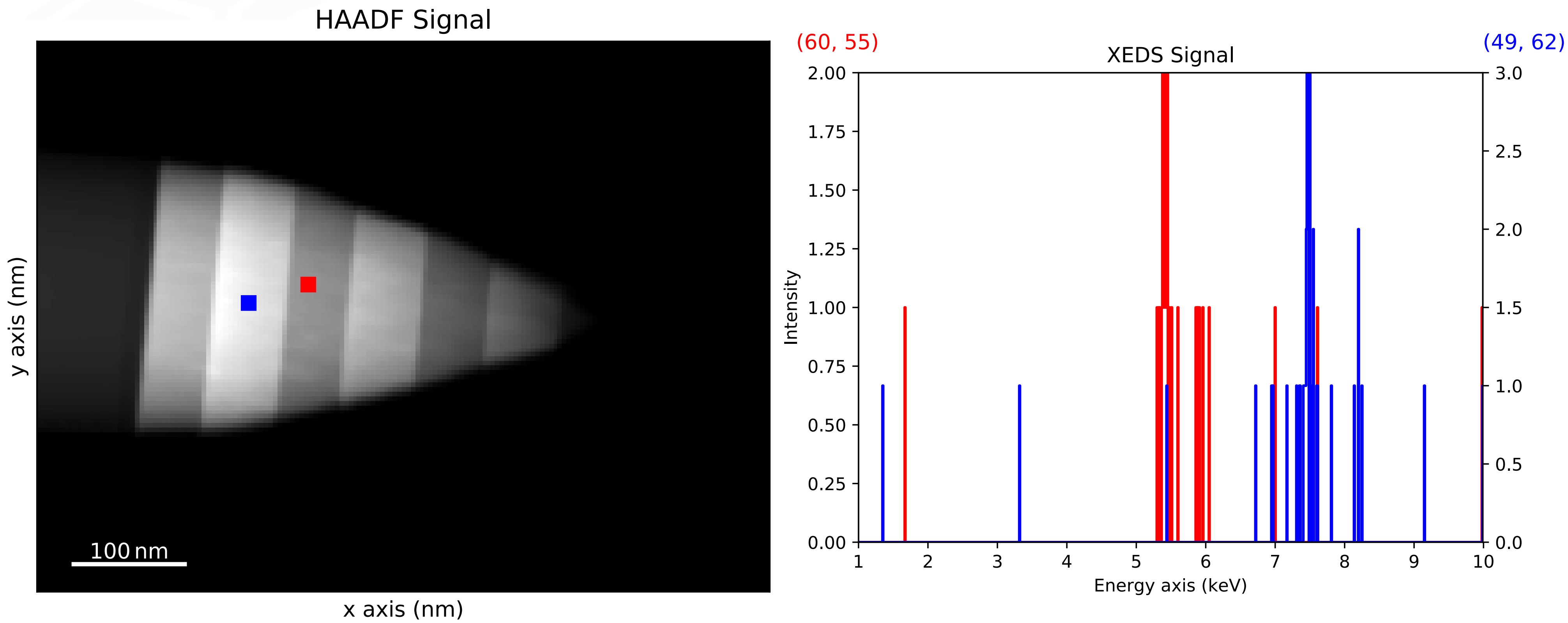
Offline "phase mapping"

- **Many algorithms exist to solve:** $\quad {}_{(x,y),E} = \quad {}_{(x,y)} \times \mathbf{S}_E$
  - Assumptions implicit in each affect their suitability for EDS, EELS, etc.
- **Other methods:**
  - Geometric methods — Vertex component analysis (VCA), Minimum volume simplex analysis (MVSA), and others...
  - Monte Carlo methods — Bayesian linear unmixing (BLU)
  - Clustering methods — k-means, Gaussian mixture modeling (GMM)

A simple case

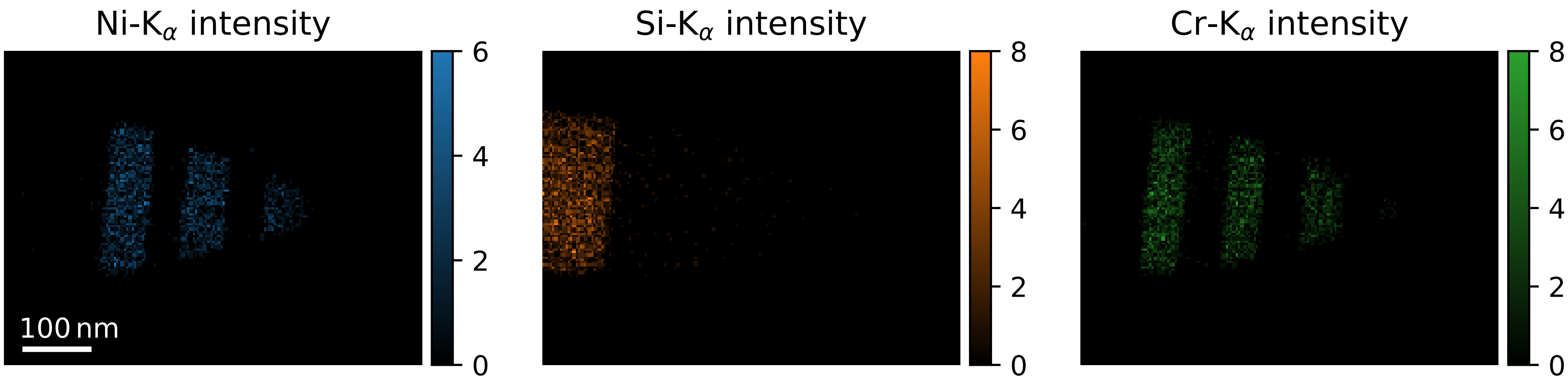
- **Signal separation enabling EDS tomography**
- **Atom probe specimen fabricated from NIST SRM 2135c**
  - Ni/Cr thin film depth profile standard (on Si substrate)
  - Layer thicknesses are approximately 56 nm
  - Data collected by Andrew Herzing (NIST)
- **Data collected from 0 to 360 degrees tilt in increments of 5 degrees**
  - Dataset is 165 x 124 x 73 x 900
- **HAADF and XEDS SI data collected simultaneously**

A simple case



Single pixel counts in the single digits
Cr and Ni visible, but noisy

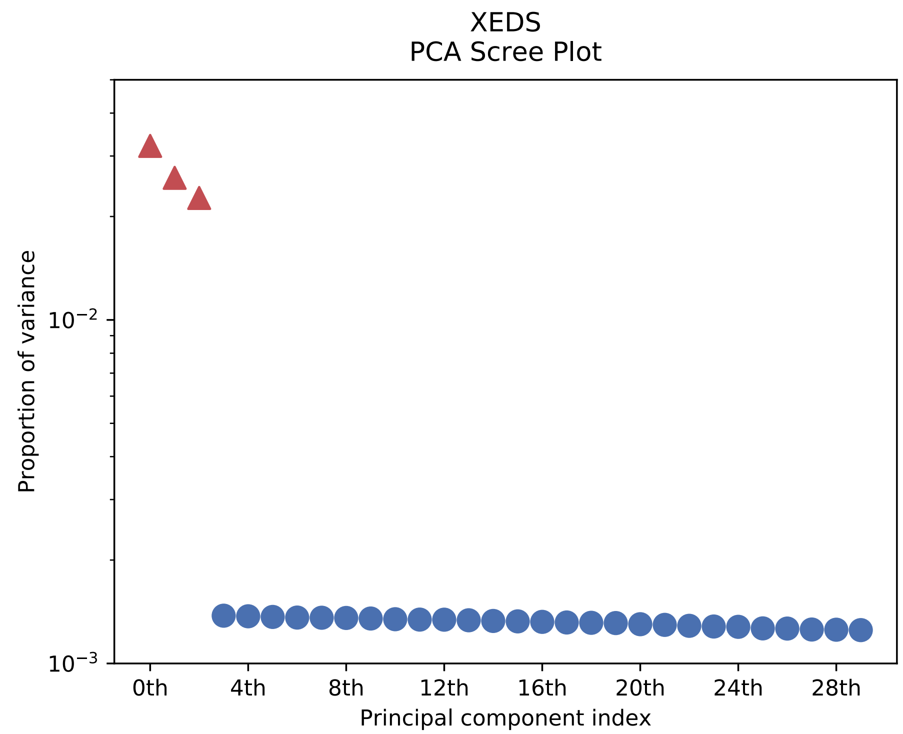
A simple case



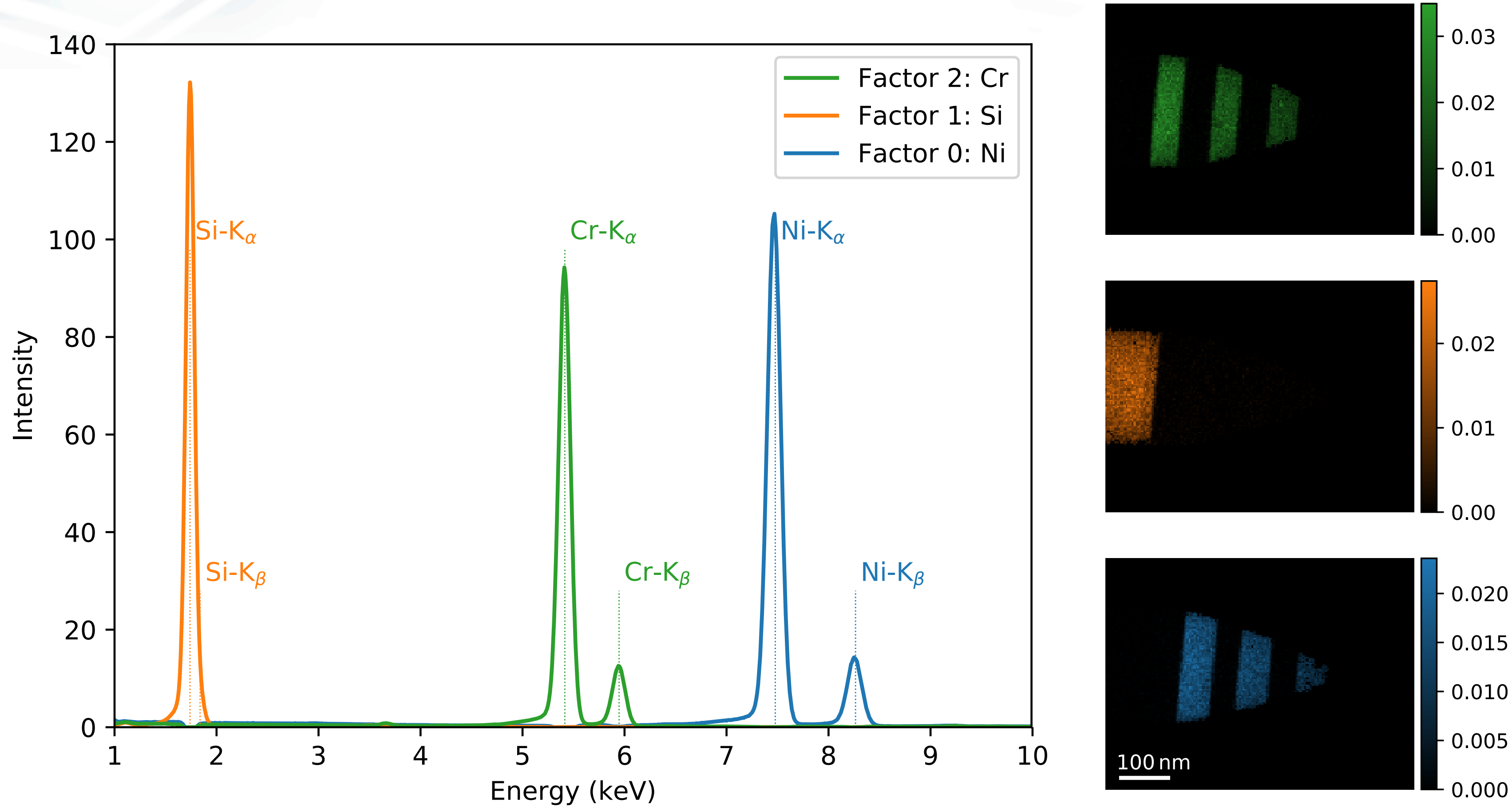
Single pixel counts in the single digits
Cr and Ni visible, but noisy

Number of components

- *a priori* we know there should be three components
- PCA orders components by "described variance"
- Three important components confirmed

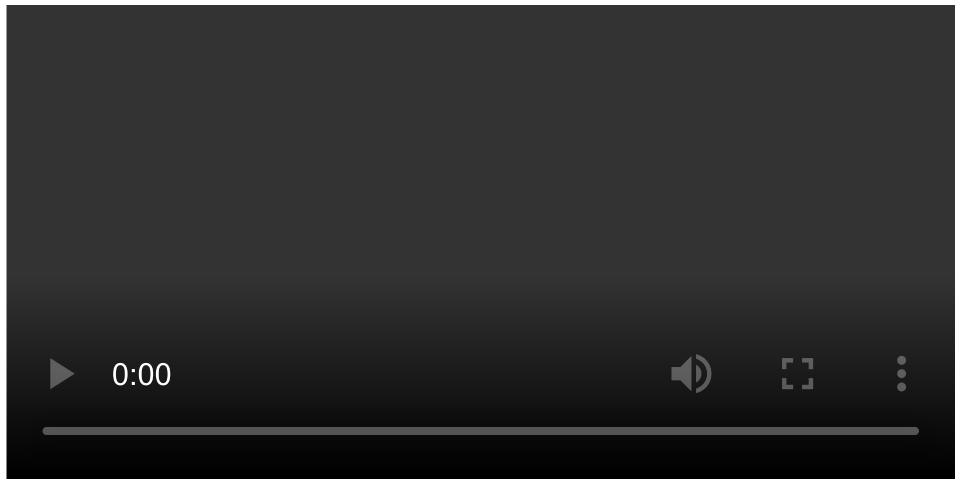


NMF with three components



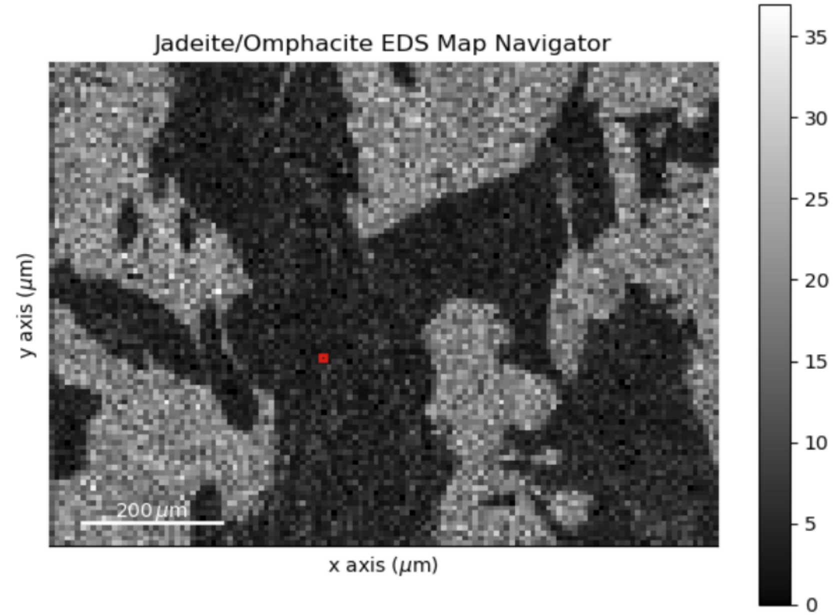
One component for each element (phase)
Drastically enhances S/N ratio in "loading" maps

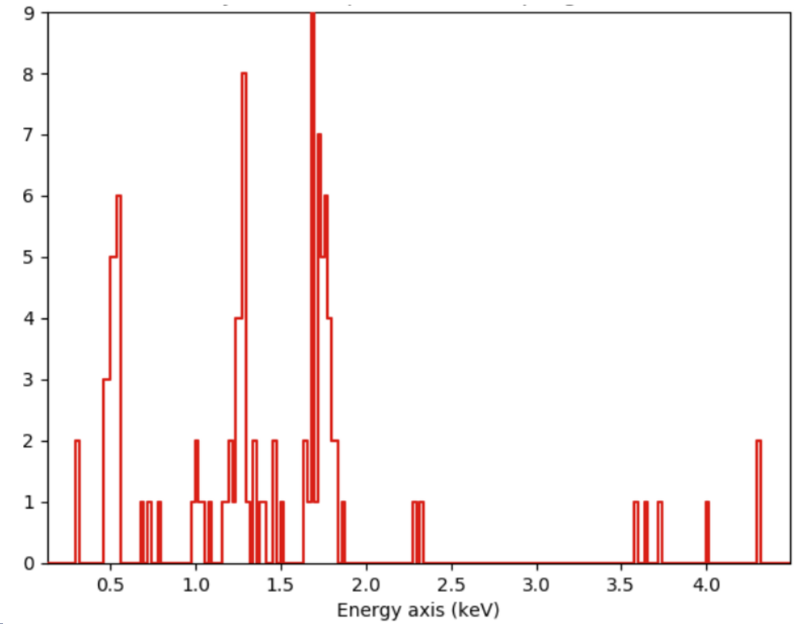
Tomography with NMF loadings



Another example

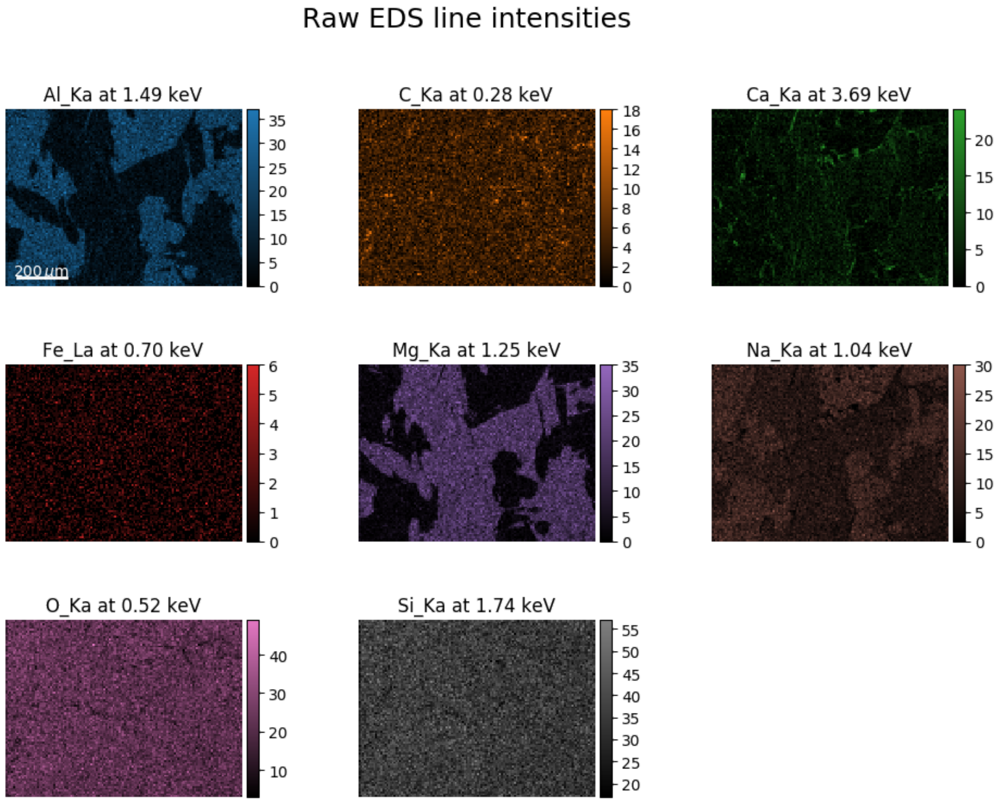
- **SEM-EDS mapping data**
- **Japan 1957 Research Specimen from Freer Gallery of Art**
  - Data from an ongoing research project at Smithsonian's Museum Conservation Institute
  - Courtesy of Thomas Lam and Edward P. Vicenzi
- **Map specifics:**
  - 30 keV primary beam
  - 512 x 384 pixels; 1564 spectral channels
  - Jadeite ($NaAlSi_2O_6$) and Omphacite ($(Ca,Na)(Mg,Fe^{2+},Al)Si_2O_6$)





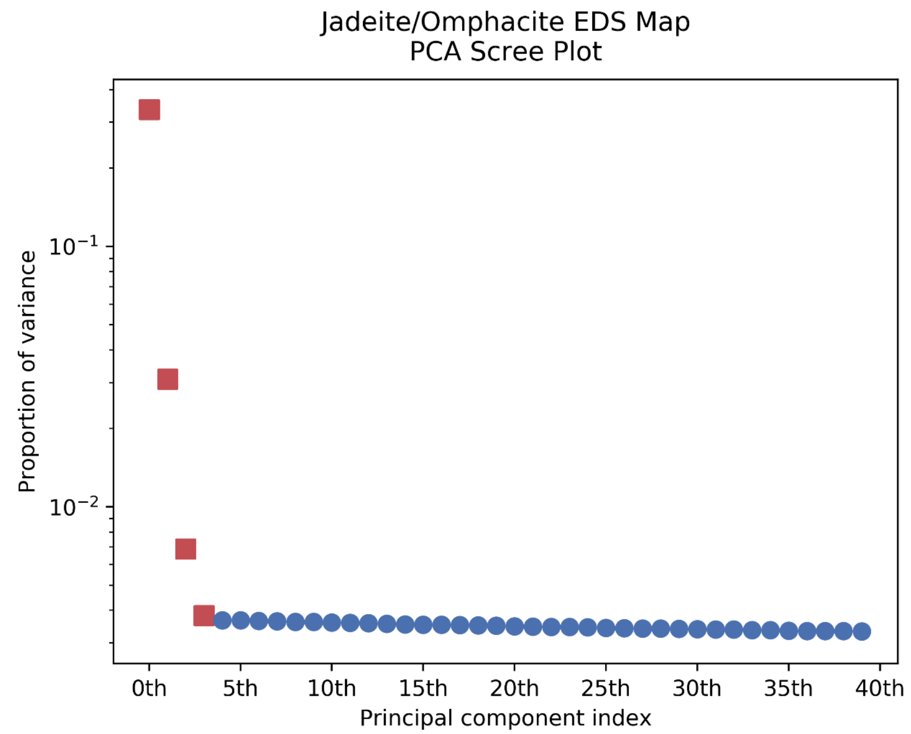
Another example

- **SEM-EDS mapping data**
- **Japan 1957 Research Specimen from Freer Gallery of Art**
  - Data from an ongoing research project at Smithsonian's Museum Conservation Institute
  - Courtesy of Thomas Lam and Edward P. Vicenzi
- **Map specifics:**
  - 30 keV primary beam
  - 512 x 384 pixels; 1564 spectral channels
  - Jadeite ($NaAlSi_2O_6$) and Omphacite ($(Ca,Na)(Mg,Fe^{2+},Al)Si_2O_6$)

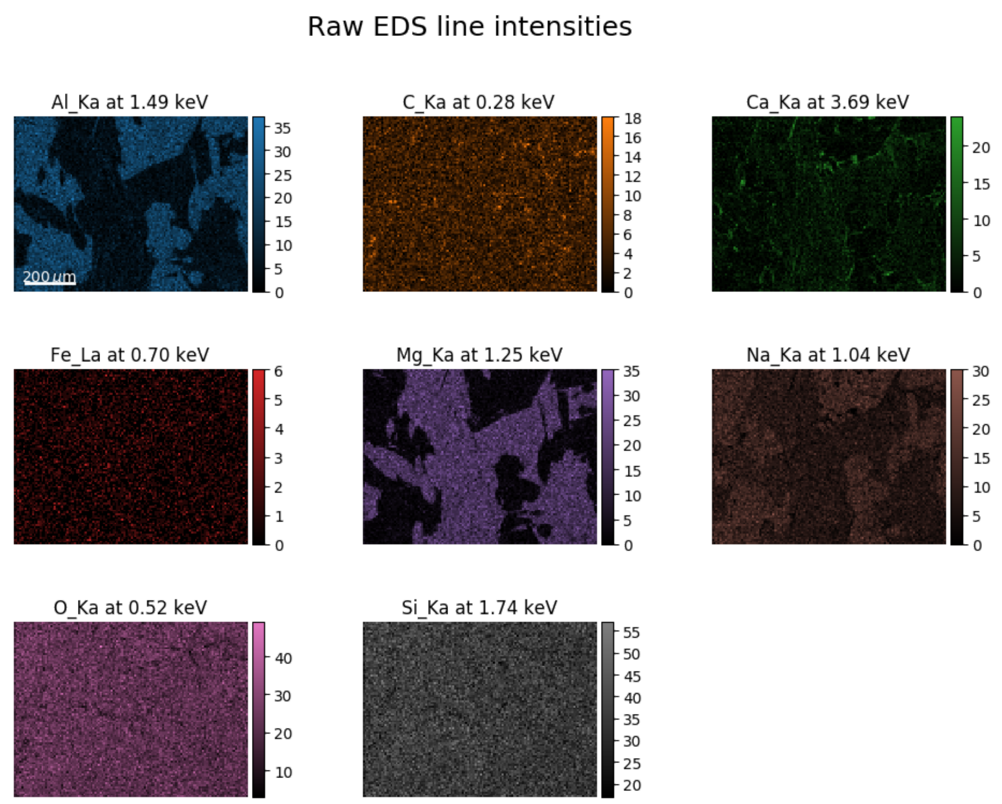


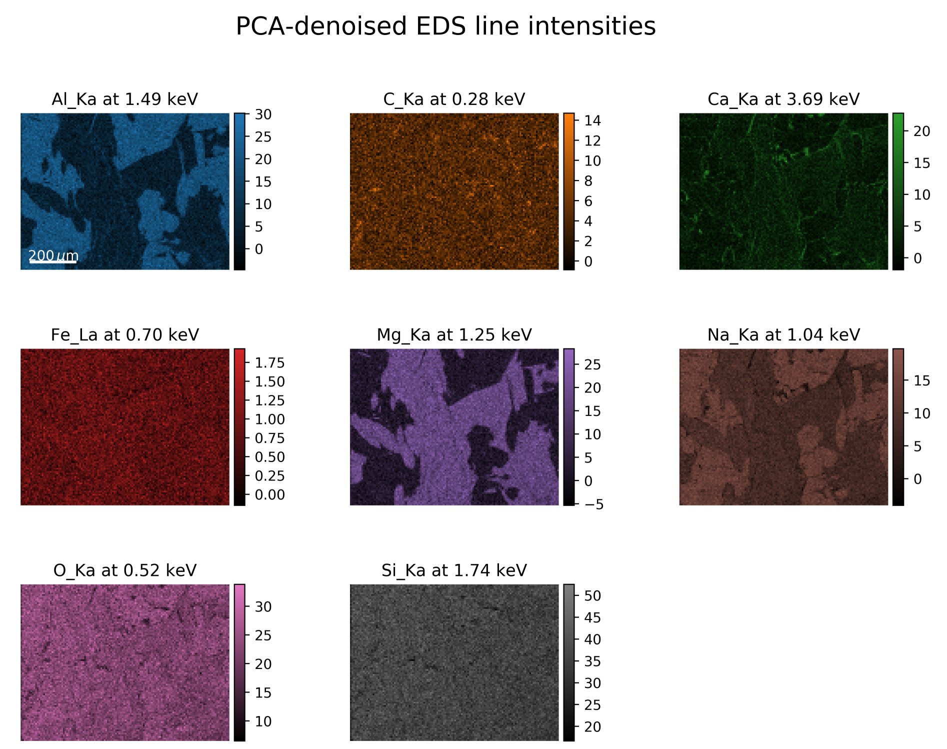
How many components are needed?

- PCA helps determine the answer with a scree plot
- Order the components by decreasing amount of contained variance on logarithmic scale
- "Correct" number of components generally at the discontinuity in the scree plot

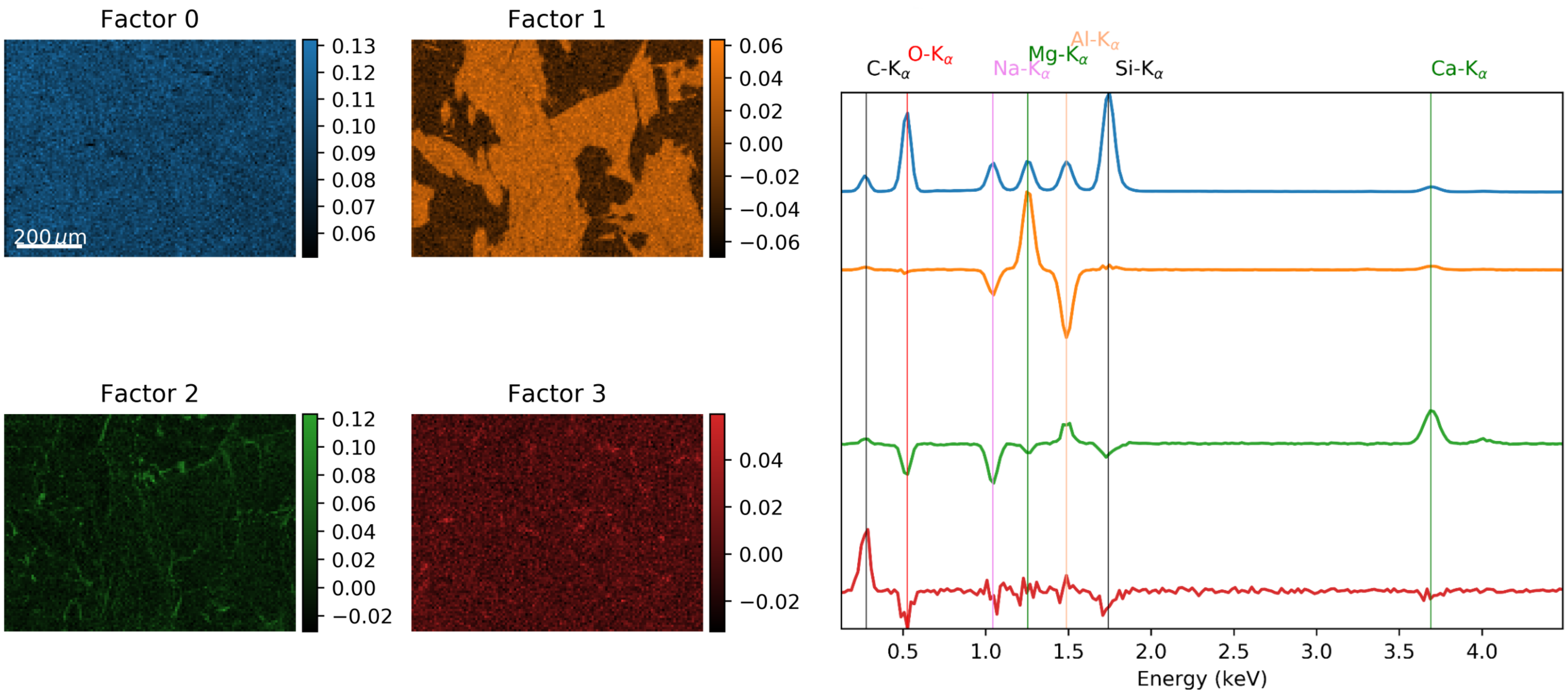


PCA Denoising $(n = 4)$

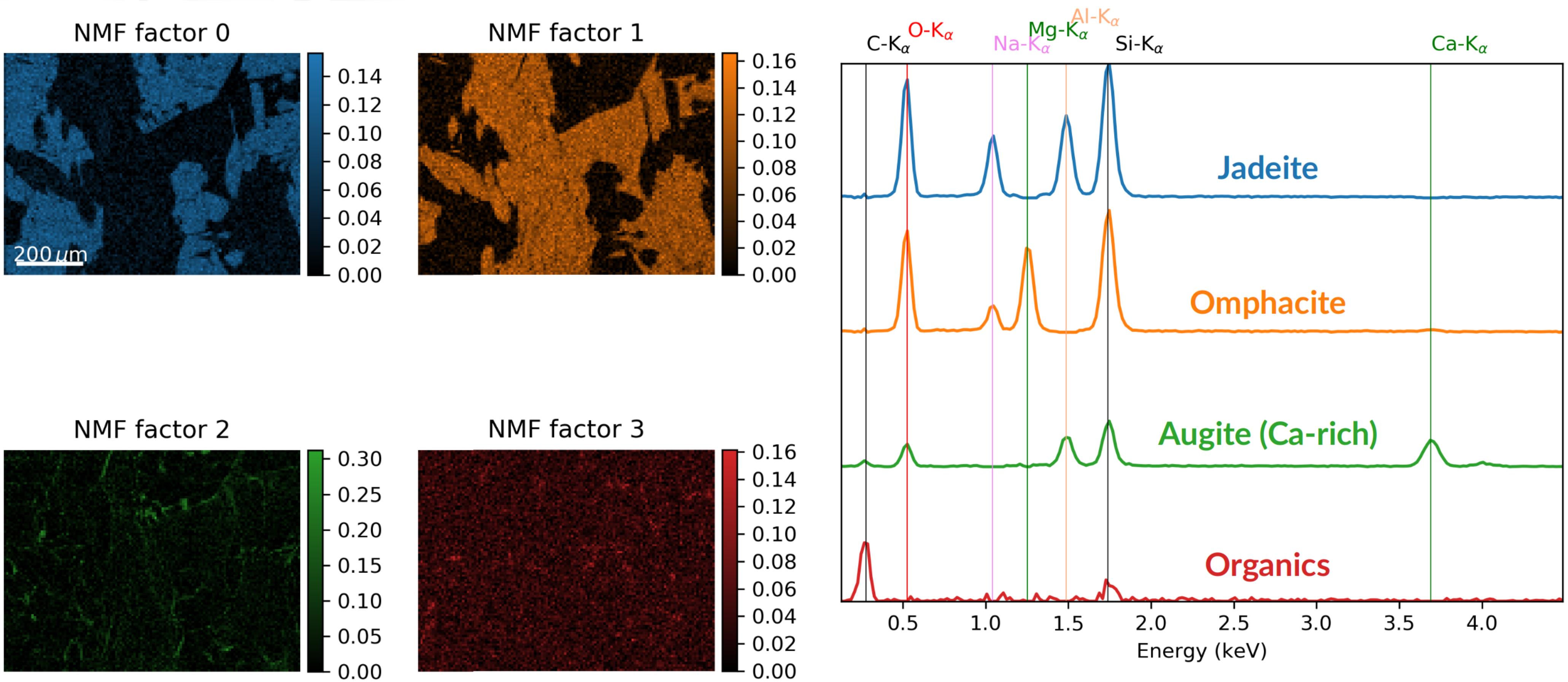




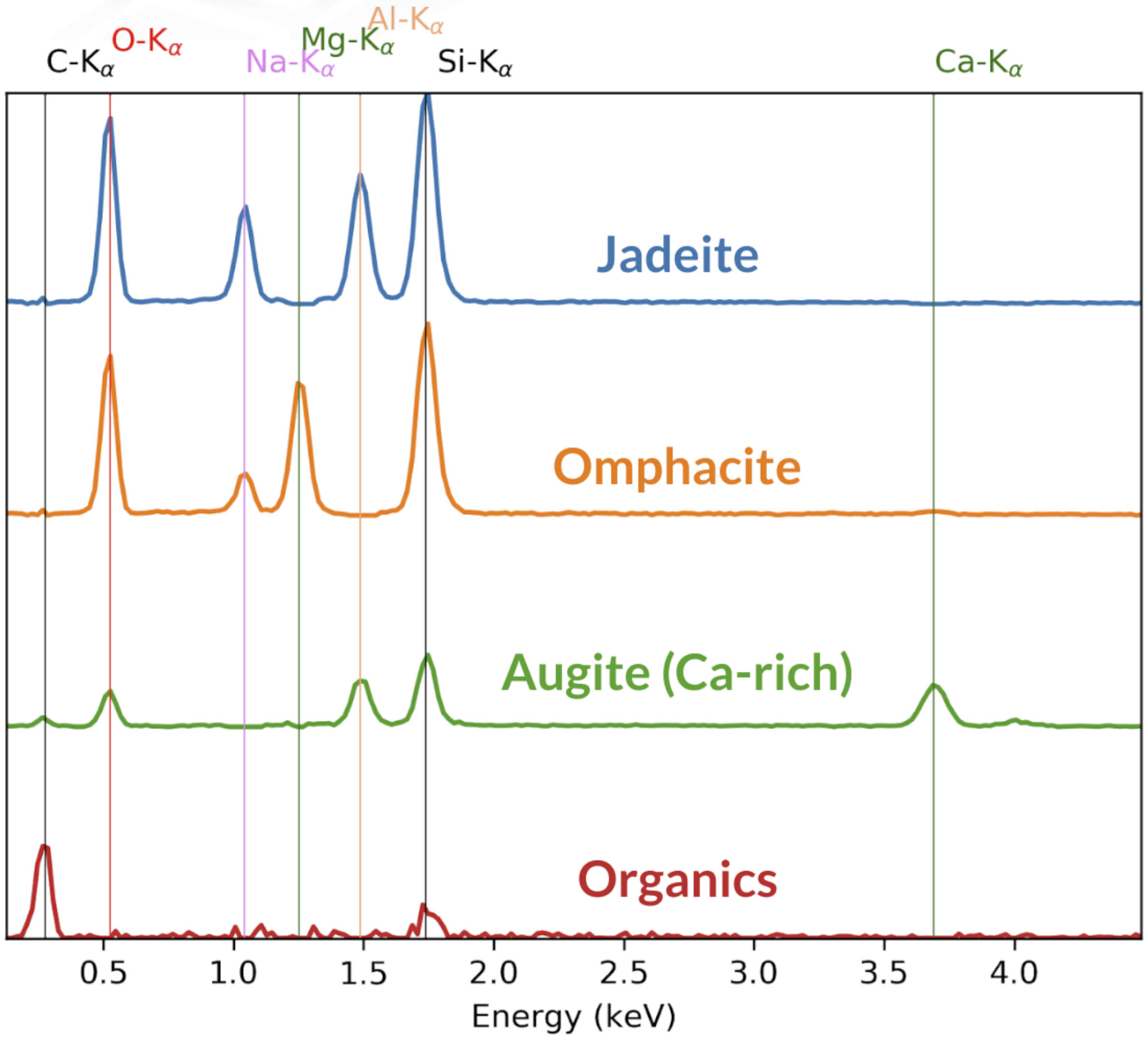
Principal component analysis ($n = 4$)



Non-negative Matrix Factorization (NMF)



A peculiar result



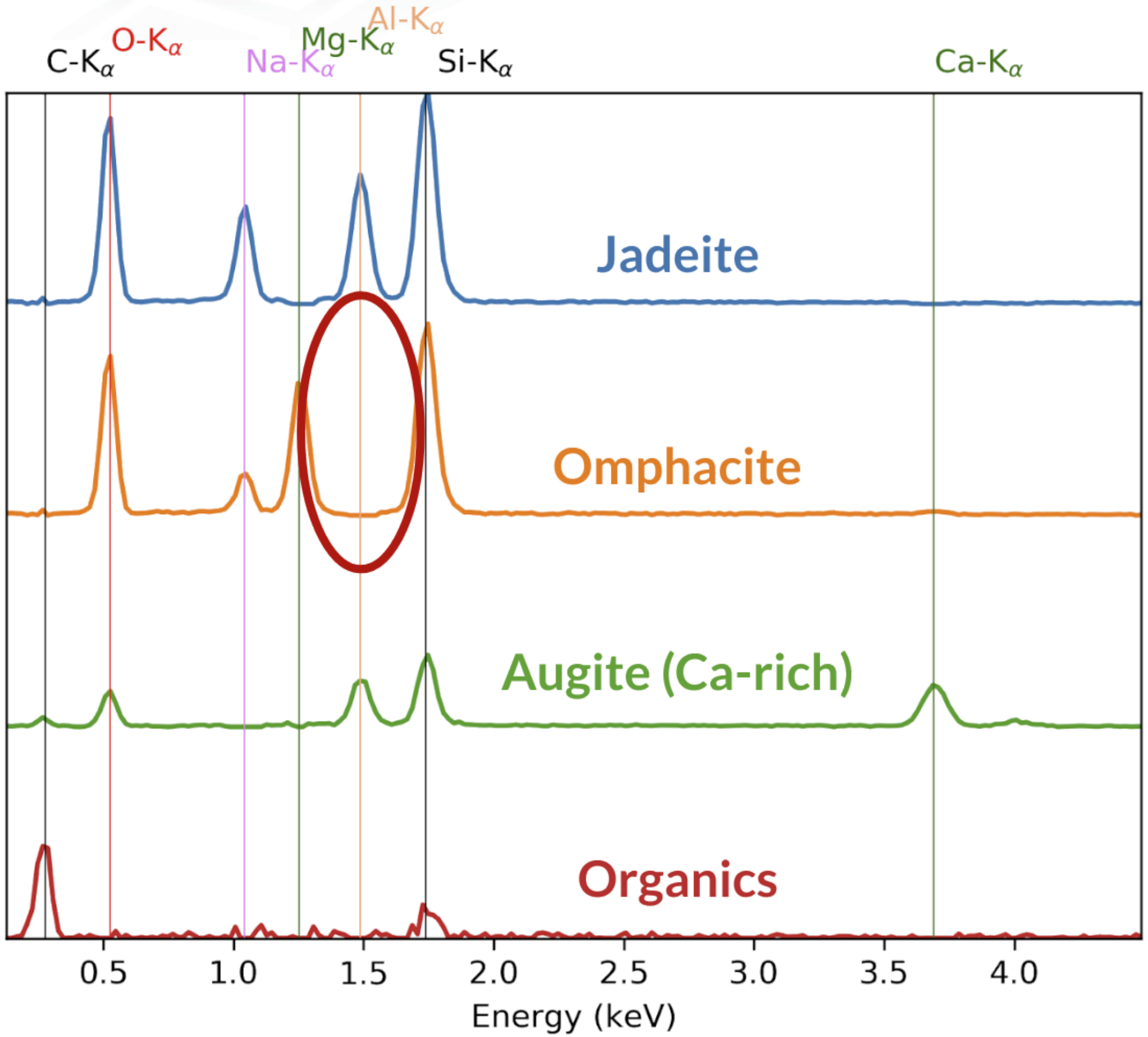
$Na(Al,Fe^{3+})Si_2O_6$

$(Ca,Na,Mg)(Mg,Fe^{2+}, Fe^{3+},Al)Si_2O_6$

$(Ca_xMg_yFe_z)(Mg_{y1}Fe_{z1})Si_2O_6$

Mostly carbon, some residual silicon signa

A peculiar result



$Na(Al,Fe^{3+})Si_2O_6$

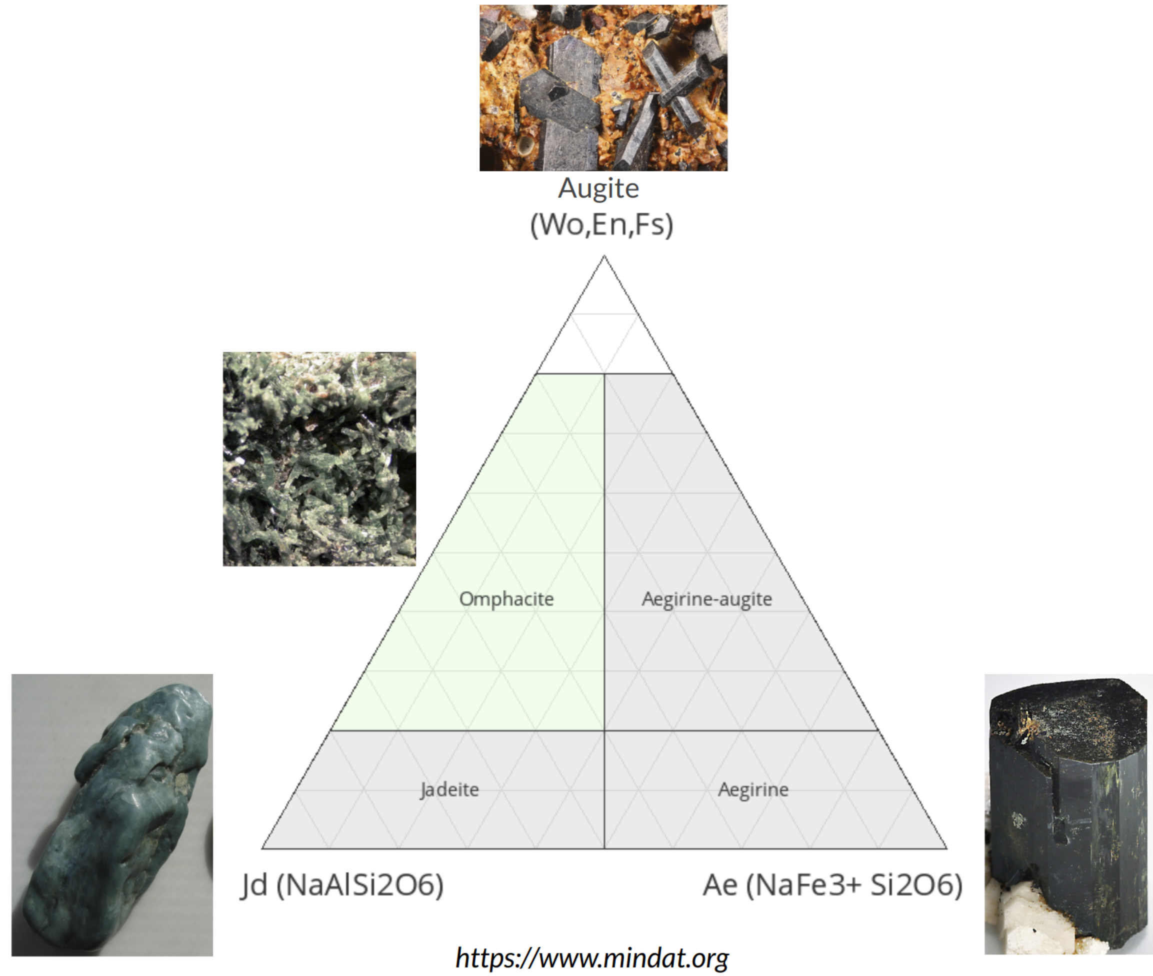
$(Ca,Na,Mg)(Mg,Fe^{2+}, Fe^{3+},Al)Si_2O_6$

$(Ca_xMg_yFe_z)(Mg_{y1}Fe_{z1})Si_2O_6$

Mostly carbon, some residual silicon signa

A solid solution explains the result!

- **Omphacite is solid solution of:**
  - Jadeite – $Na(Al,Fe^{3+})Si_2O_6$
  - Augite – $(Ca_xMg_yFe_z)(Mg_{y1}Fe_{z1})Si_2O_6$
  - Aegirine – $NaFe^{3+}Si_2O_6$



Remaining Questions in Signal Separation

- **Have to be careful!**
- **Still not really known what this means for more precise quantification**
  - Unmixing results are not always deterministic (depends on algorithm)
  - Dependent on constraints, assumptions, and signal quality (noise level)
- **Do count statistics, noise characteristics, etc. hold valid after signal separation?**
- **No/few rigorous studies of quantification/unmixing in EM spectroscopy**
- **Lots of opportunities for further research**

Conclusions

- **Computational microscopy is coming!**
- **With ever-growing data sizes and improving computational resources, we are at the very beginning of this field**
- **These methods are very powerful, but their implications and validity are still not well understood**
  - Uncertainties, artefacts, etc.
- **Machines will soon be better at this than we are**
  - Better to make sure you’re on the same team as them 😉

More reading for the interested

- **Reviews and microscopy-specific information:**
  - P.M. Voyles, “Informatics and data science in materials microscopy,” *Curr. Opin. Solid State Mater. Sci.*, **21**, 141–158, 2017. — especially Section 3.1 (doi:10.1016/j.cossms.2016.10.001)
  - P. Potapov, “Why Principal Component Analysis of STEM spectrum-images results in “abstract”, uninterpretable loadings?,” *Ultramicroscopy*, **160**, 197–212, 2016. (doi:10.1016/j.ultramic.2015.10.020)
  - R. Kannan, et al., “Deep data analysis via physically constrained linear unmixing: universal framework, domain examples, and a community-wide platform,” *Adv. Struct. Chem. Imaging.*, **4**, 6, 2018. (doi:10.1186/s40679-018-0055-8)

- **Example applications:**
  - D. Rossouw, et al., “Blind source separation aided characterization of the γ′ strengthening phase in an advanced nickel-based superalloy by spectroscopic 4D electron microscopy,” *Acta Mater.*, **107**, 229-238, 2016. (doi:10.1016/j.actamat.2016.01.042)
  - G. Lucas, P. Burdet, M. Cantoni, C. Hébert, “Multivariate statistical analysis as a tool for the segmentation of 3D spectral data,” *Micron.* **52–53**, 49-56, 2013. (doi:10.1016/j.micron.2013.08.005)

Thank you!

Joshua Taillon

joshua.taillon@nist.gov