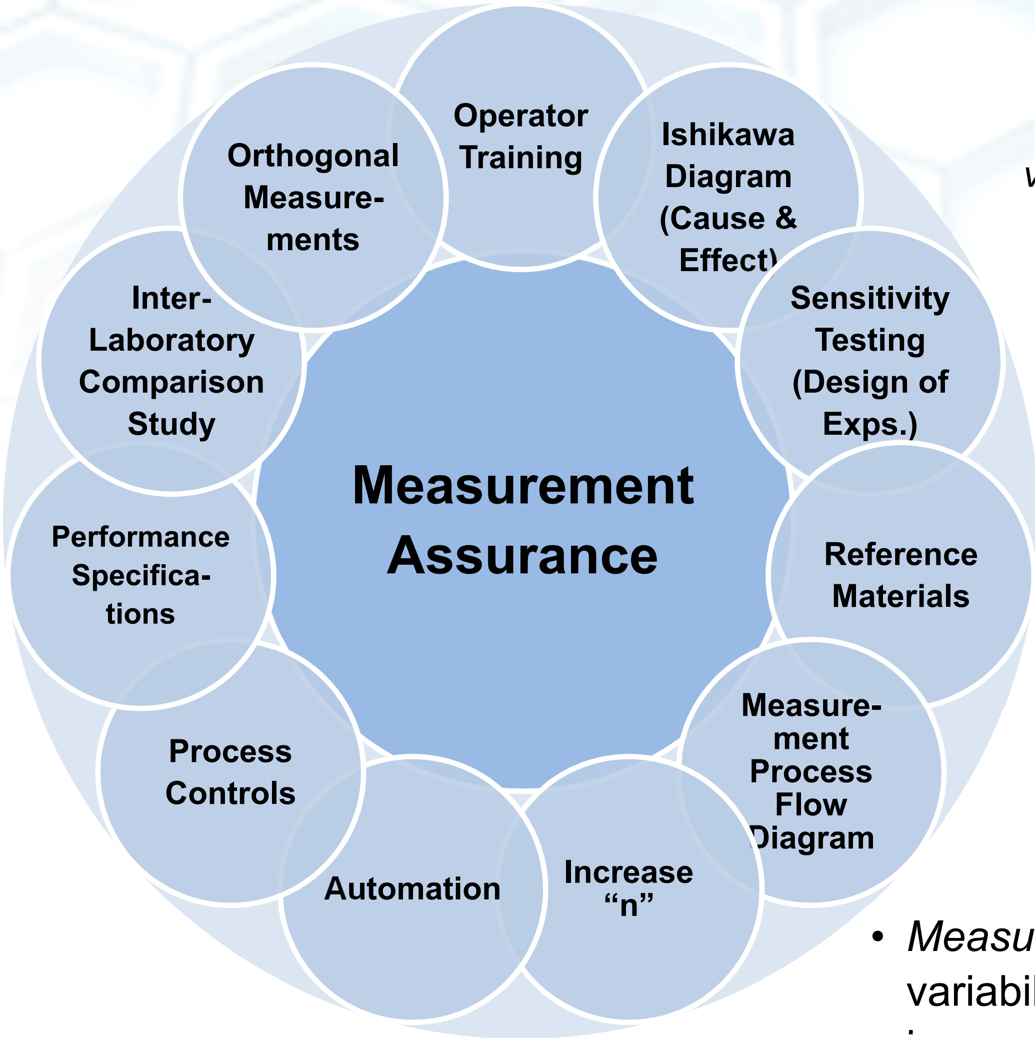
Measurement Assurance Case Study: Nanofiber Diameter

Carl G. Simon, Jr., Ph.D.
Biosystems & Biomaterials Division
National Institute of Standards & Technology
Gaithersburg, MD, U.S.A.
carl.simon@nist.gov

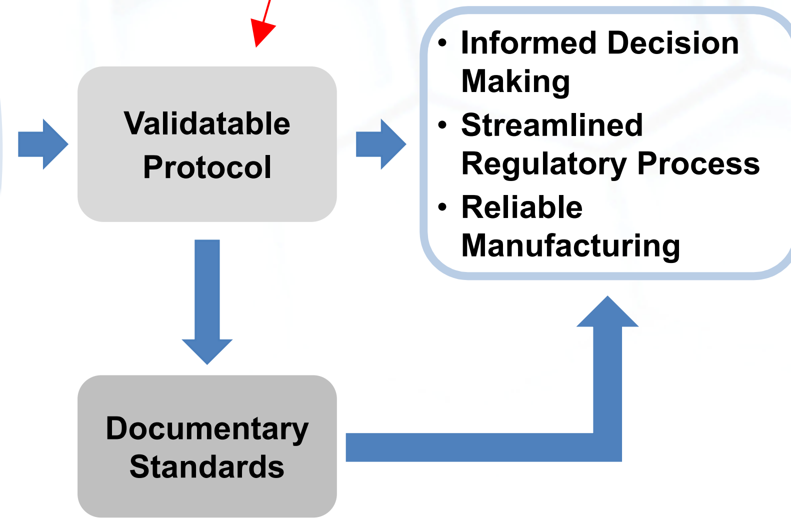
Contributors:

Nathan Hotaling, Kapil Bharti, Haydn Kriel

Strategies for Achieving Measurement Assurance

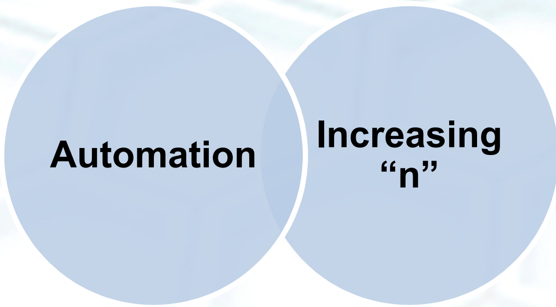


“Validatable” not “validated”, since validation is a continuous process that must be established be each lab that implements the measurement

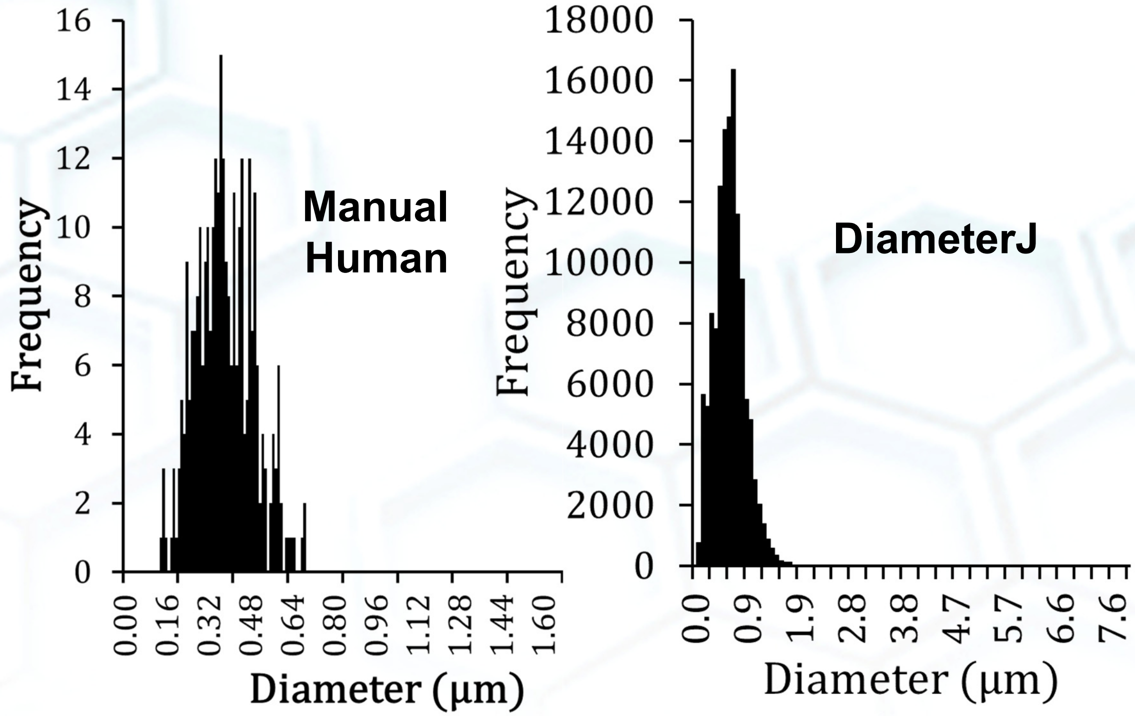


- *Measurement Assurance:* Evaluating & reducing variability in a measurement to improve confidence in results for use in decision-making
- Underpins development of documentary standards

- Measurement assurance strategies for measuring nanofiber diameter
- 2013 ASTM Workshop on Scaffold Standards & Measurements (Indianapolis, IN, USA): #1 need identified was “better measurements for scaffold structure”



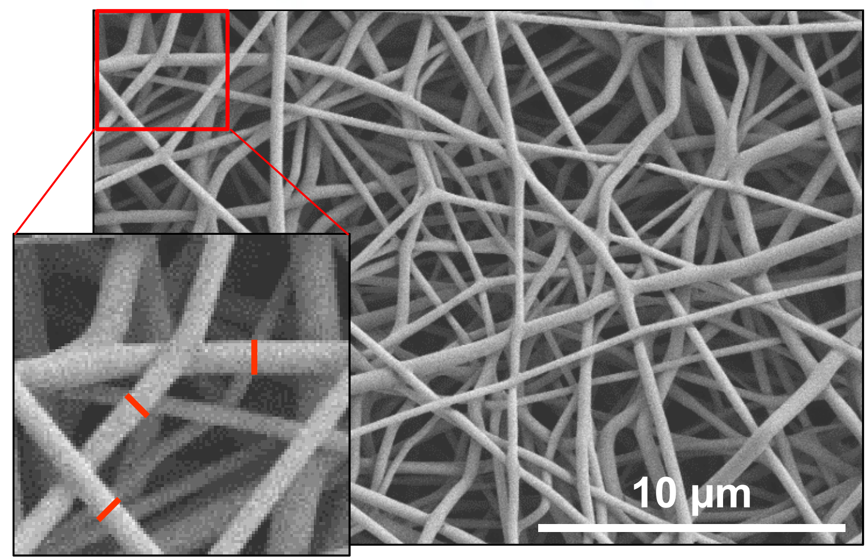
DiameterJ: Automated Image Analysis



- Current practice is manual measurement using a line tool in imaging software (ImageJ)
  - Slow (10 min/image) & low n
  - Human bias

Increasing "n" (number of measurements) enables better statistics & better modeling of the probability distribution function (histogram)

Automation increases the number of measurements & reduces human bias

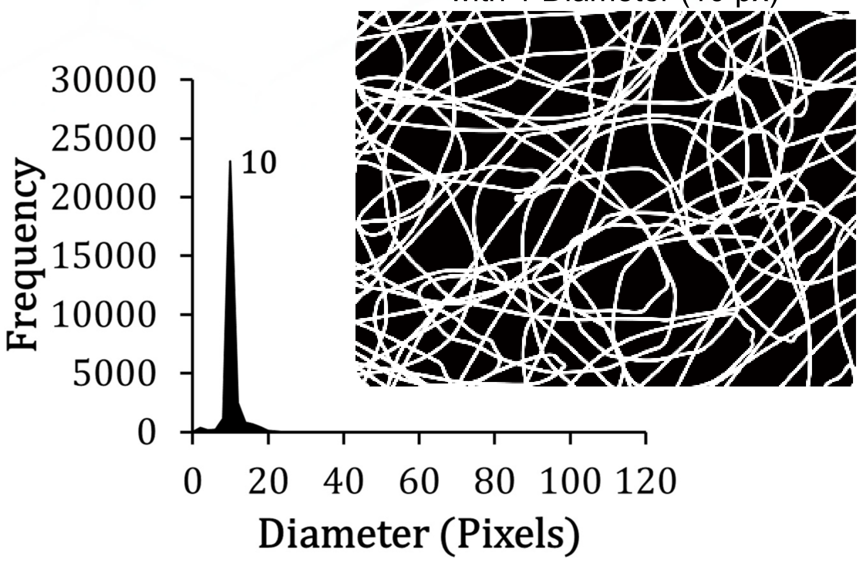


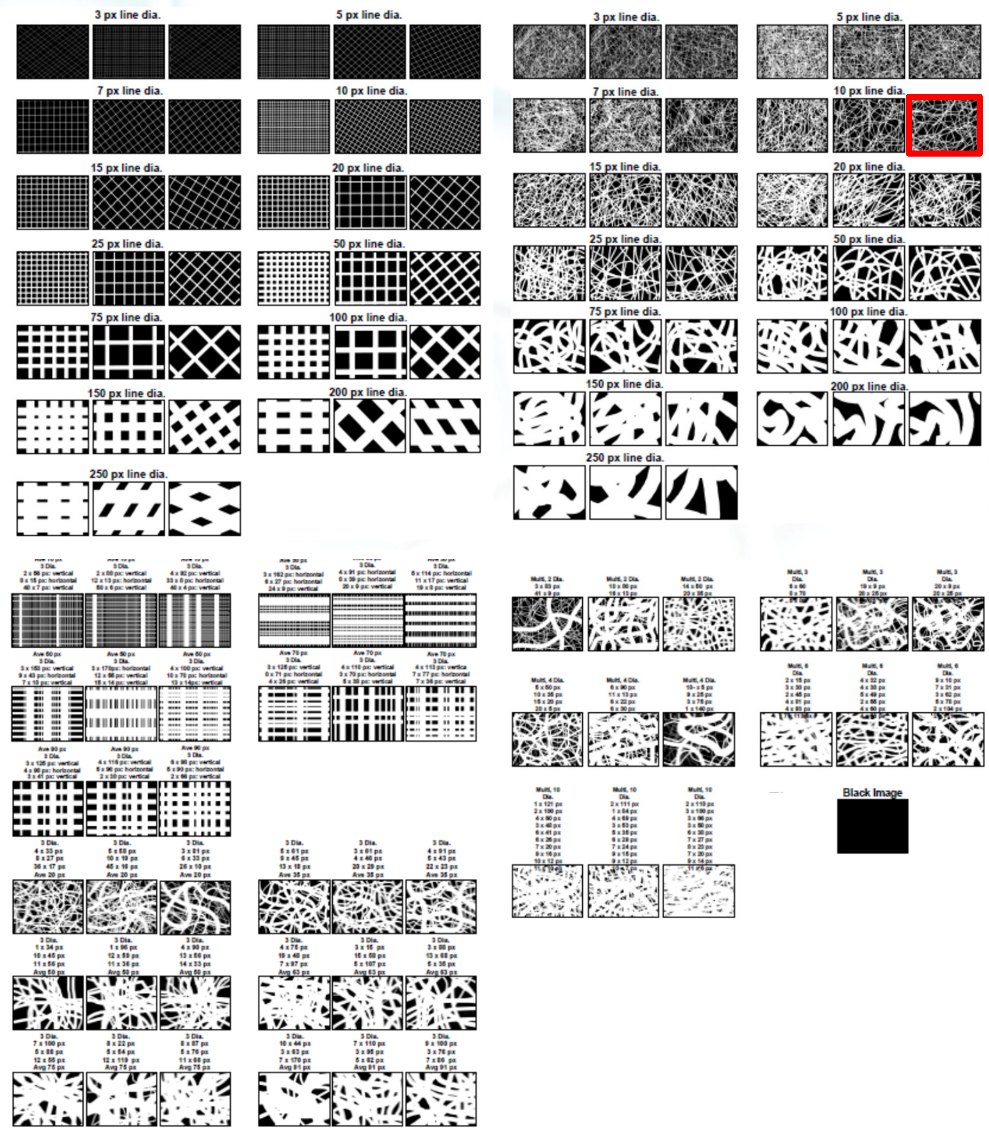
103 Synthetic Images

Reference Materials

Reference Materials are homogeneous & stable in regard to specified properties for use in calibration, to serve as a control or to serve as a reference point for comparability (ISO Guide 35)

Digital Synthetic Image with 1 Diameter (10 px)

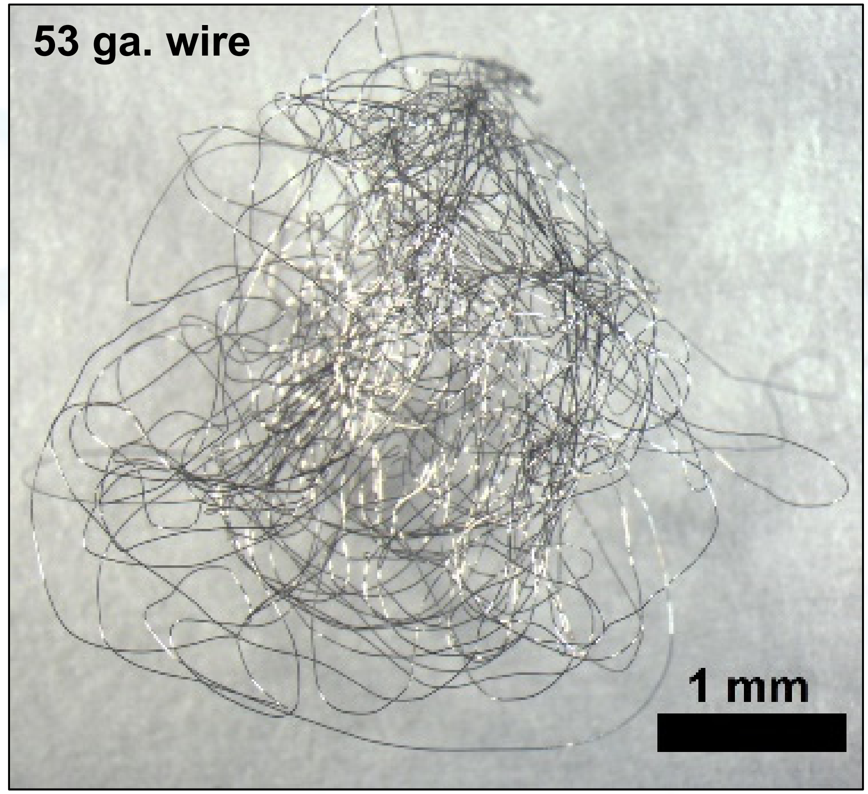




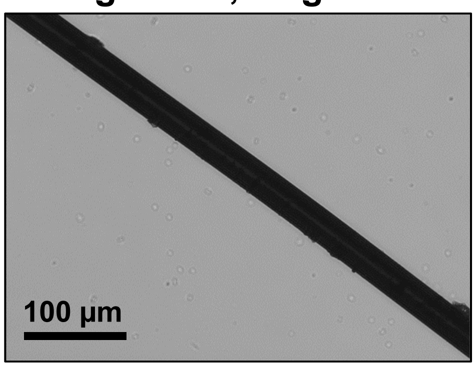
Reference Materials

Steel Wire with Known Diameter

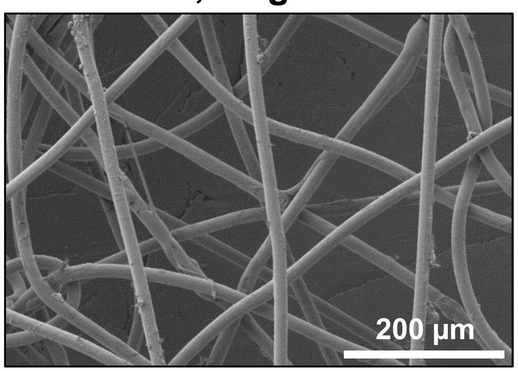
- Narrow gauge stainless steel wire (HSM Wire)
- Manufacturer measures dia. with resistivity & calipers
- Wire dia. verified with light microscopy & human manual segmentation in SEM



Brightfield, 48 ga. wire



SEM, 53 ga. wire

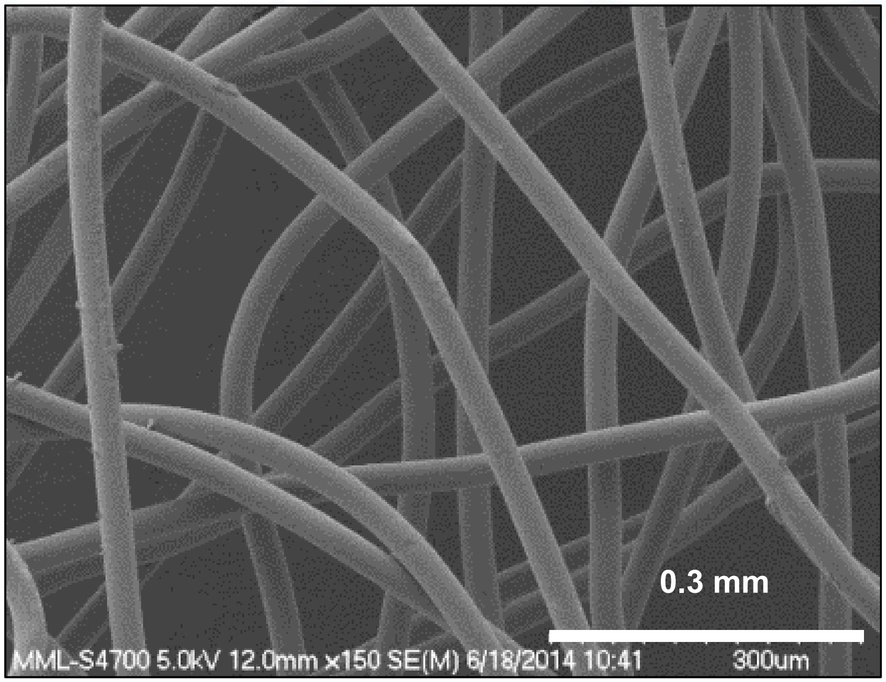


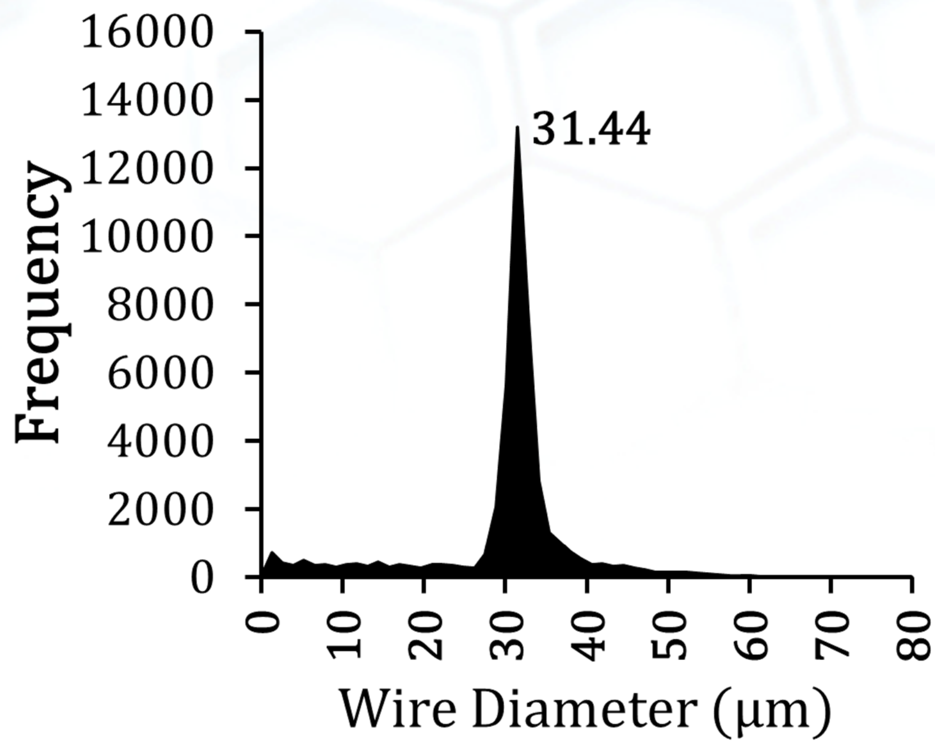
Wire Gauge	Manufacturer Reported Dia. (µm)	Light Microscopy Dia. (µm)
48	31.0	31.1 (0.1)
50	25.5	25.6 (0.1)
53	16.75	16.7 (0.1)

Reference Materials

Steel Wire with Known Diameter

48 Gauge Steel Wire





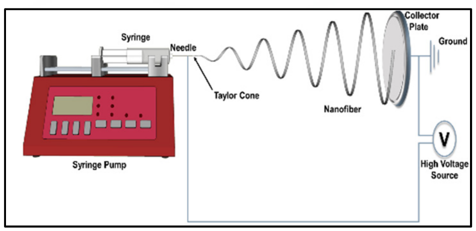
Measurement Process Flow Diagram

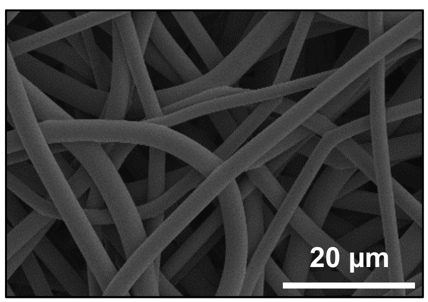
Flow Diagram is a tool to formally map a measurement process so that each step can be considered for its contribution to measurement uncertainty

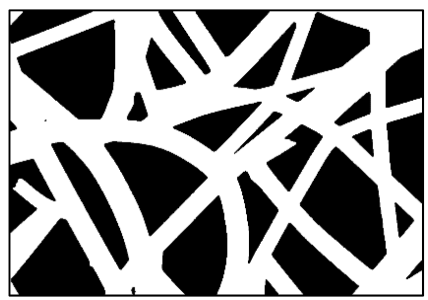
Electrospin Fibers (Sample Preparation) → **Image in Scanning Electron Microscope** → **Segment Images** → **Measure Fiber Diameter in Segmented Images** → **Analyze Data, Determine Fiber Diameter Distribution**

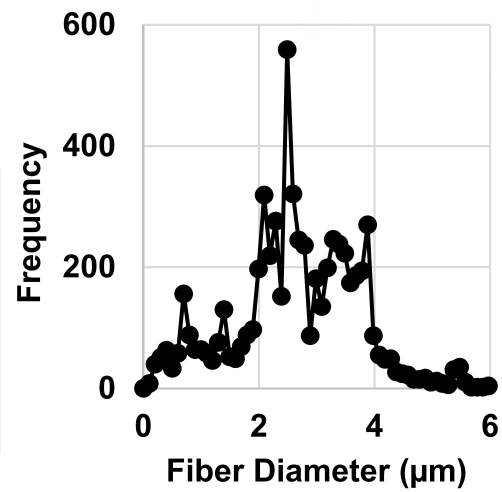
Synthetic Images

Steel Reference Wire









Orthogonal Measure-ments

Orthogonal Measurements

Orthogonal Measurements: Confidence in a measurement result is enhanced when multiple measurement methods give a similar value of a material property

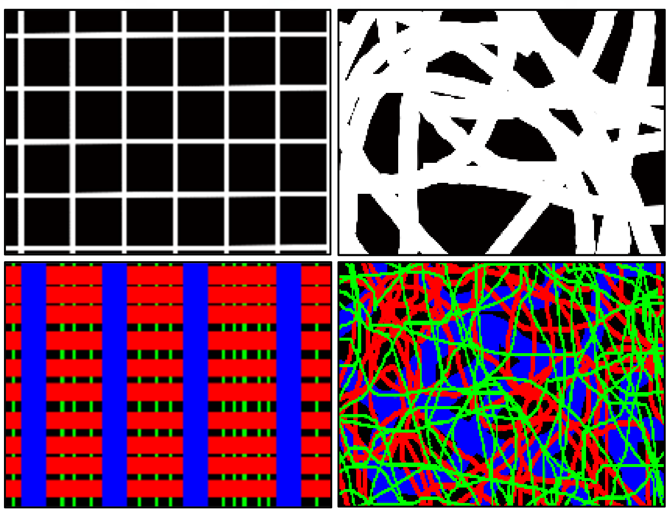
- Orthogonal Measurements
  - More precise than the measurement that you are trying to assure (slower, expensive, harder)
  - Based on a different physical principle
- Synthetic images
  - Counted pixels by hand (very IMPORTANT, MSPaint didn't work)
- Steel reference wires
  - Manufacturer measured resistivity
  - Manufacture measured with calipers
  - Optical imaging of fibers
  - Human manual measurement with ImageJ line tool in SEMs
- Electrospun polymer fibers
  - Human manual measurement with ImageJ line tool in SEMs

Sensitivity Testing (Design of Exps.)

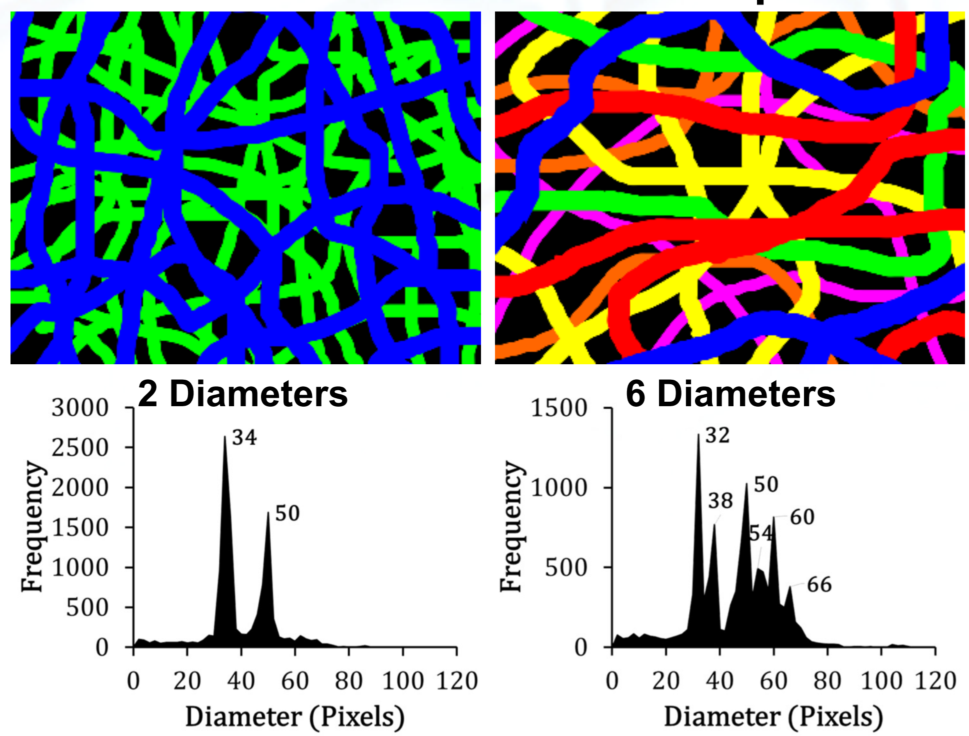
Sensitivity Testing (Ruggedness Testing) (Design of Experiments)

Sensitivity Testing can identify key measurement parameters that must be controlled to make the measurement more reliable

103 synthetic images:
- Different diameters
- Straight vs curved
- Aligned vs disordered
- Multiple diameters



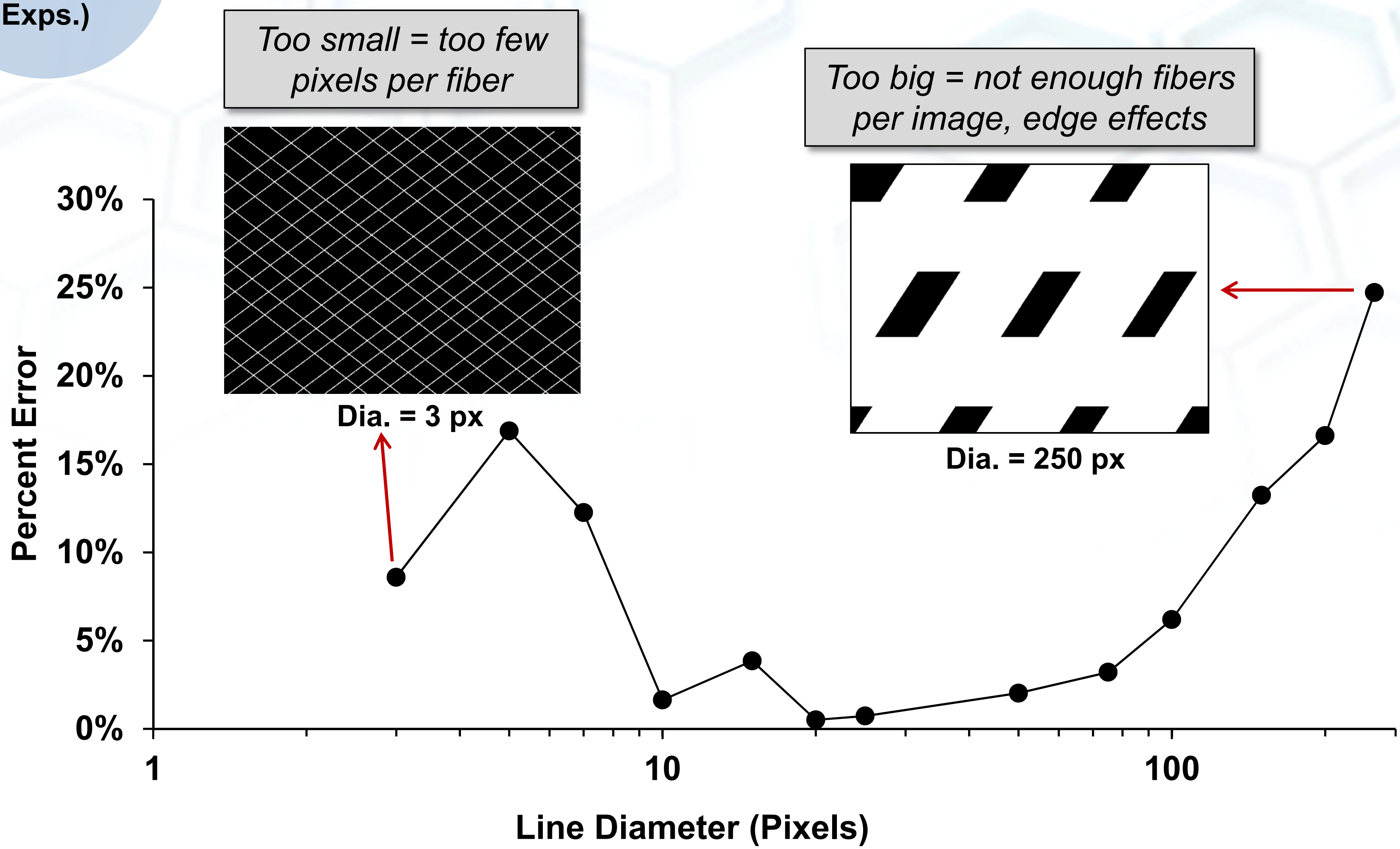
Multimodal Diameter Samples



(Failed on 10 Diameters)

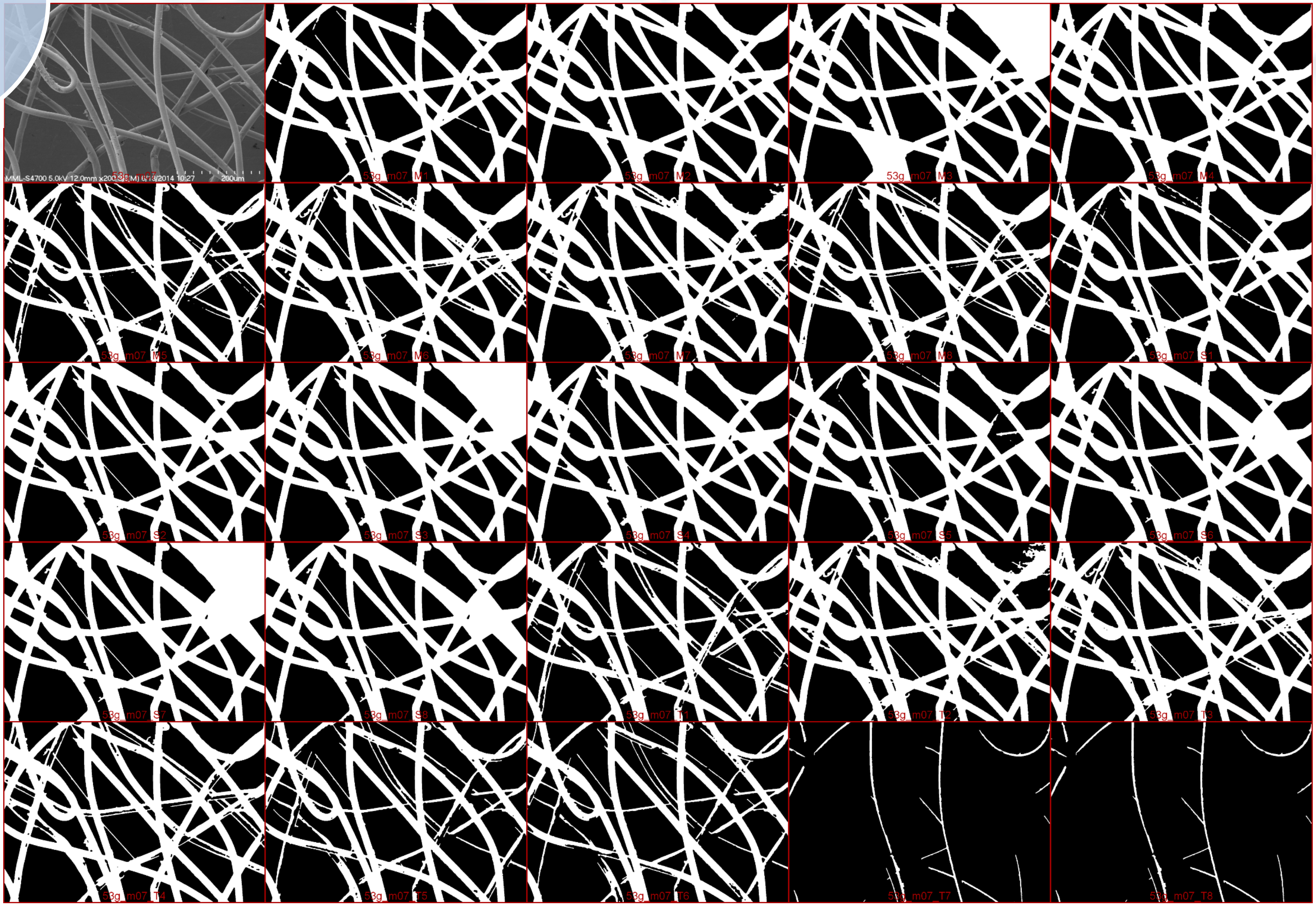
Sensitivity Testing (Design of Exps.)

Establishing Fiber Diameter Measurement Range



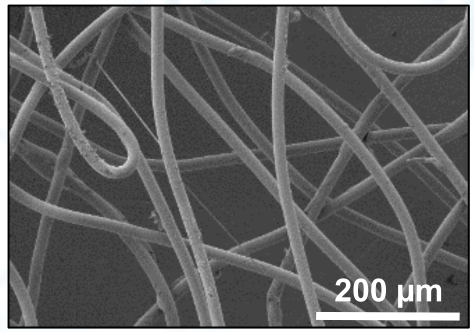
Effect of Segmentation (24 Algorithms Tested)

Sensitivity Testing (Design of Exps.)

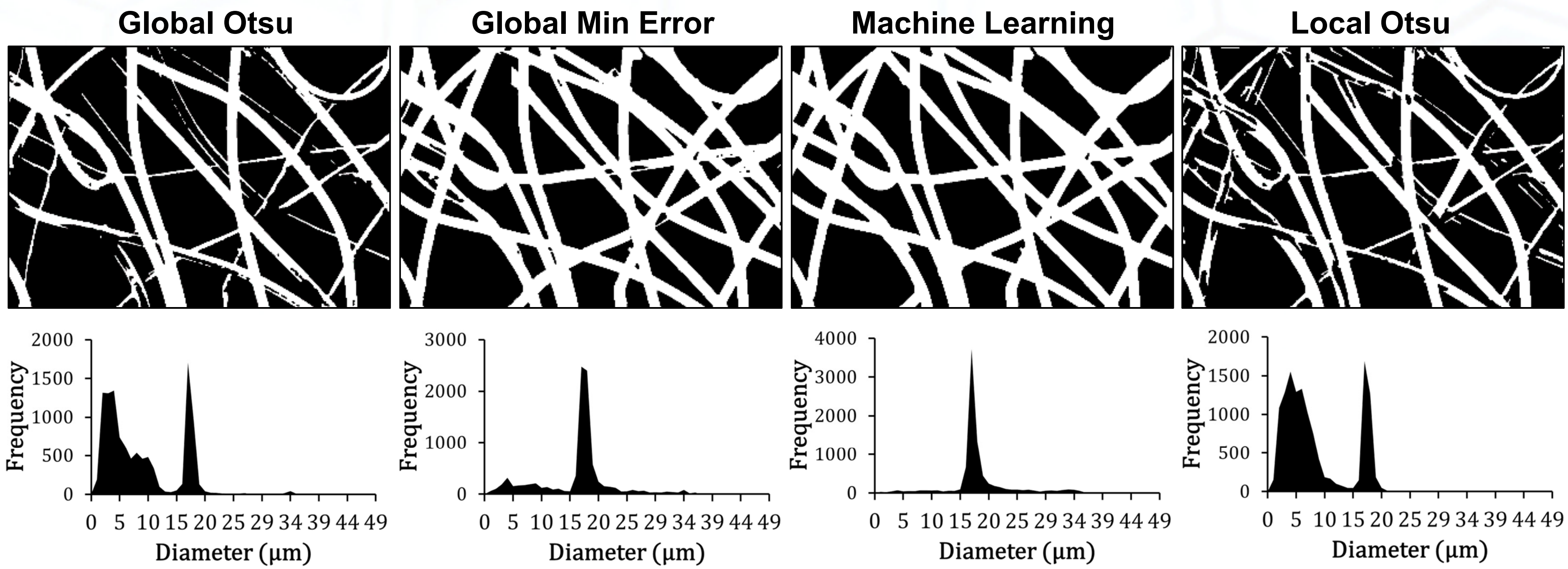


Sensitivity Testing (Design of Exps.)

Effect of Segmentation on DiameterJ Results



Raw SEM micrograph of
53 ga, steel reference wire
(fiber dia. 16.7 µm)



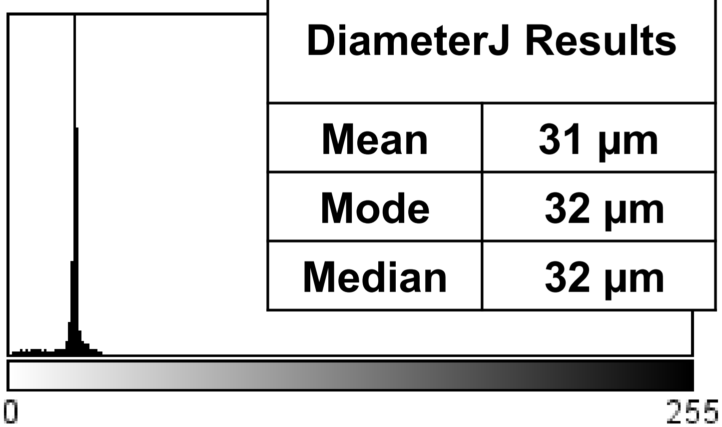
Process Controls

Process Controls

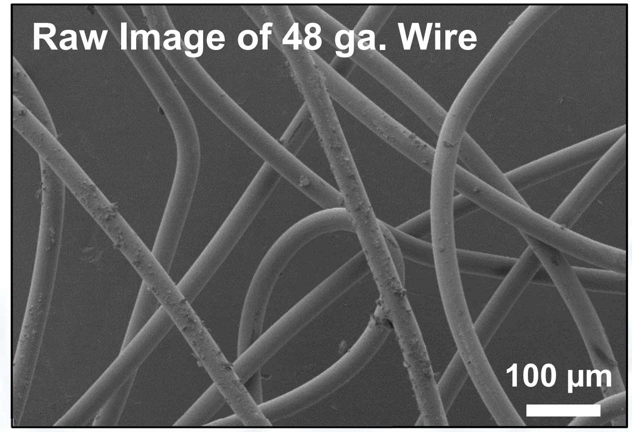
Process Controls are procedures to monitor critical points in a measurement process to check that steps are performing according to specifications

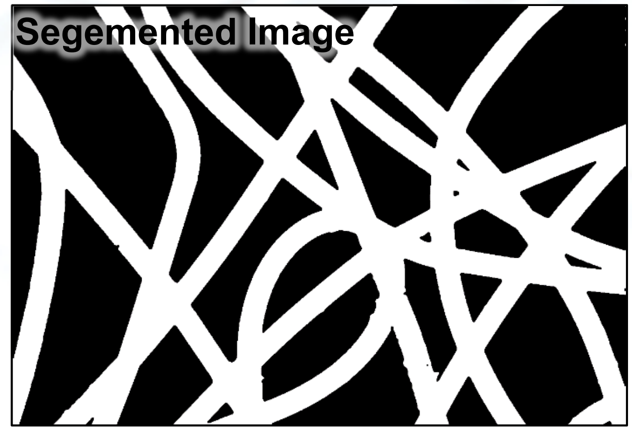
Visually compare raw image with:

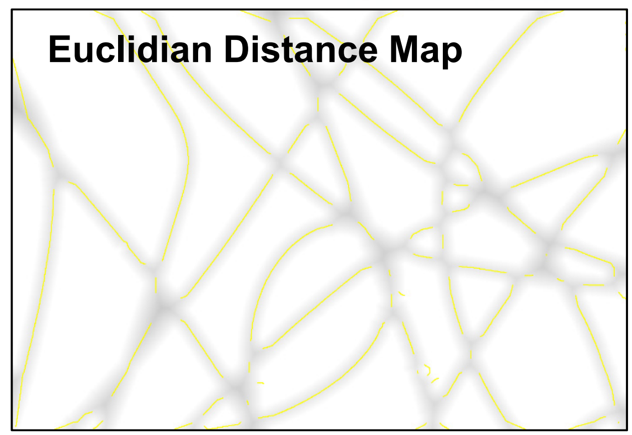
- Segmentations
- Euclidian distance map
- Histogram (bimodal?)
- DiameterJ results



Manufacturer’s Fiber Dia. = 31.0 µm	
DiameterJ Results	
Mean	**31 µm**
Mode	**32 µm**
Median	**32 µm**



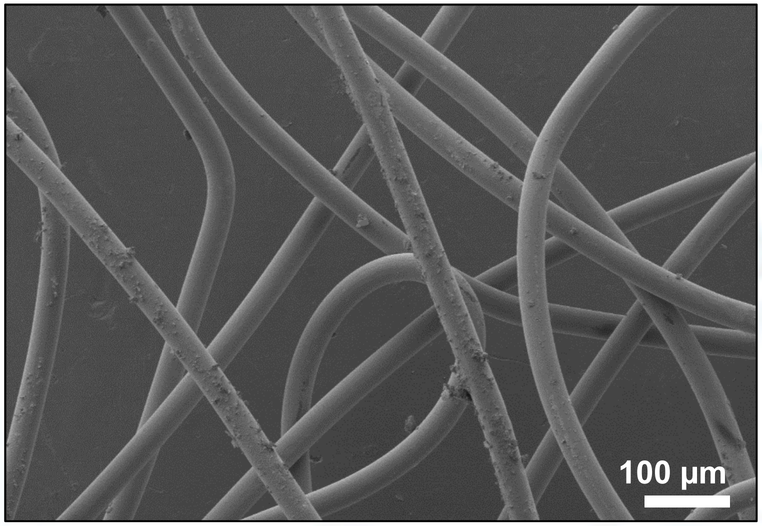




Process Controls

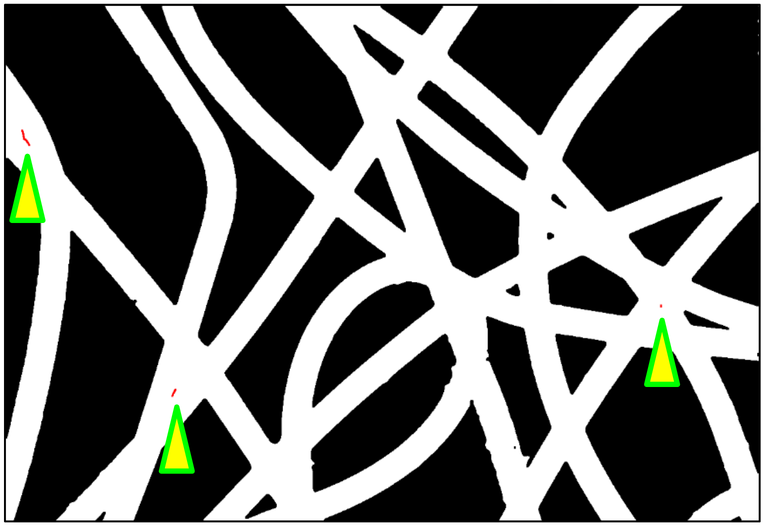
- To help identify errors, DiameterJ has a locator tool which labels locations where fibers diameters of a given range were found
- Fibers along image edge, poor segmentation or fiber overlap can yield errant measurements

Raw Image



Red Lines = 1 px to 255 px

Red Lines = 40 px to 255 px



Performance Specifications

Performance Specifications

Performance Specifications are established by the user from sensitivity testing & charting process control data; if test specifications are not met, then results can not be used in decision-making

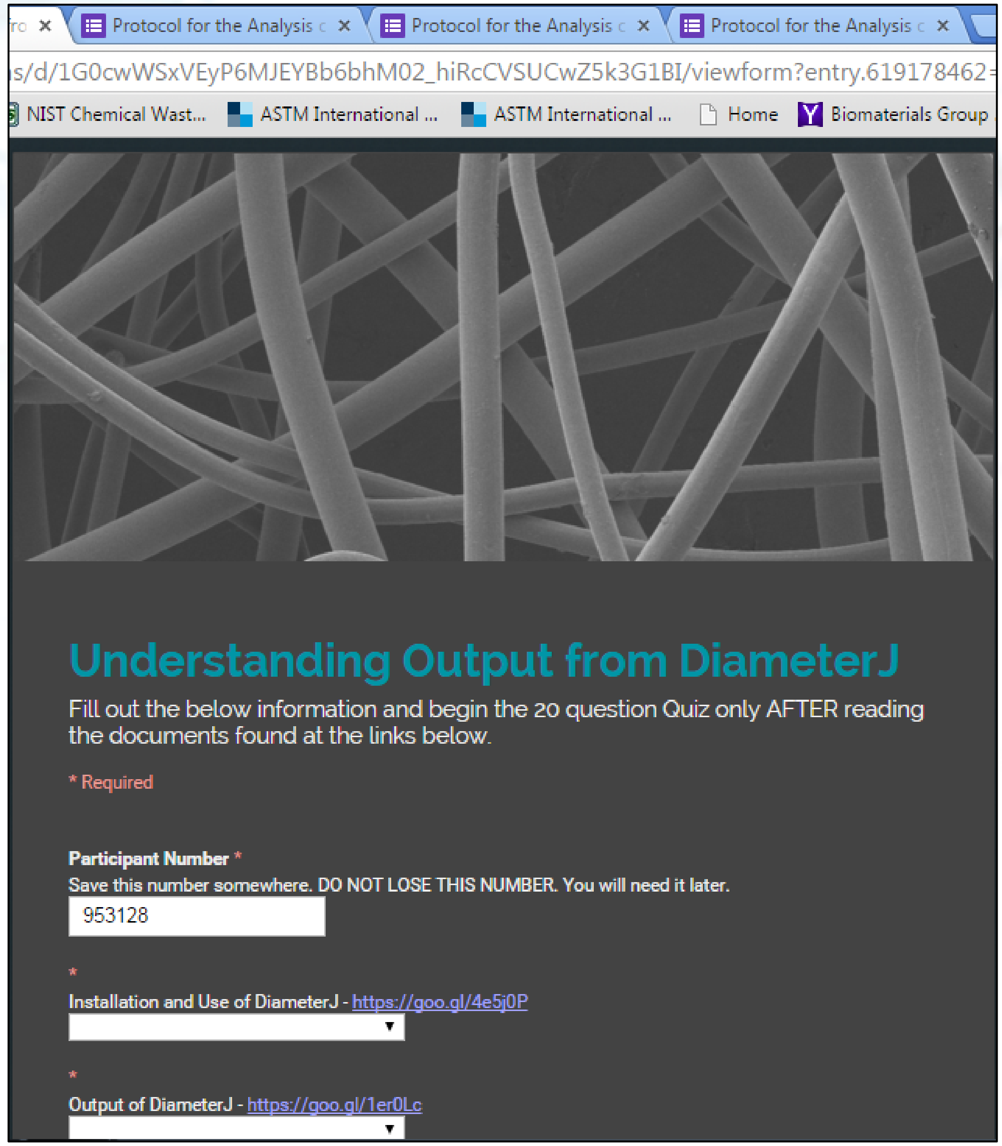
- Fibers must be at least 10 px in diameter
- Fibers should not be greater than 10% of the smallest dimension of the image
  - Example: SEM imaging of 500 nm fibers should be conducted at a magnification between 1500X and 10000X for a 1280 px by 960 px image capture
- Visual Examination: Fiber diameters in raw images qualitatively agree with segmentations & DiameterJ results
- For multimodal distributions, modes must be separated by more than 3 px
- In the system tested, 6 fiber dia. peaks is maximum # of peaks for 1 image
- If you don't meet these specifications…then test result should be questioned (possibly rejected)

Web Training Module

Operator Training

Operator Training improves measurement precision to improve comparability between different operators & labs

Web training where users download & analyze images with DiameterJ



Inter-Laboratory Comparison Study

Comparing Operator Performance Before & After Training

Inter-Laboratory Comparisons asses the robustness of an assay across different labs & results are used to refine the protocol

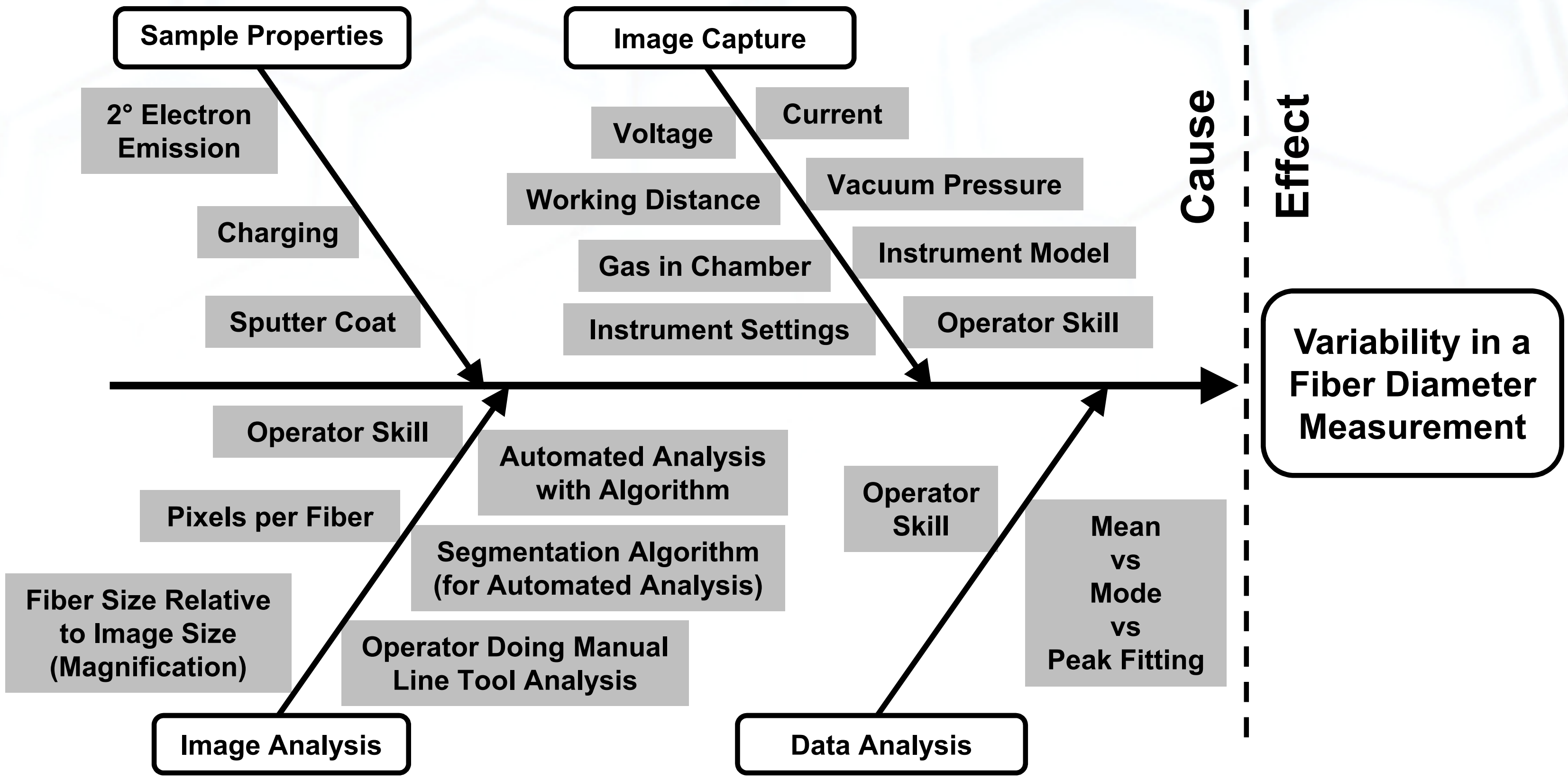
- *IN PROGRESS: Intra*-lab comparability with Matt Becker Lab (Univ. of Akron, USA), 17 students analyzing images before/after training to assess improvement
- Test images of reference wires (48 ga. & 50 ga.)
- Protocol Refinement: Keep magnification constant

Ishikawa Diagram

Ishikawa Diagram (Cause & Effect)

Ishikawa Diagram is graphical tool to identify potential sources of variability in a process

Developed in 1960s by Kaoru Ishikawa who pioneered quality management processes in the Kawasaki shipyards

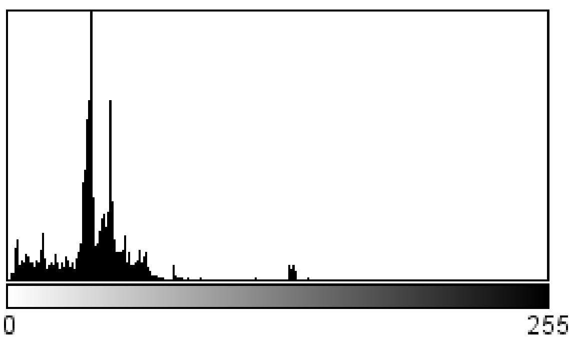


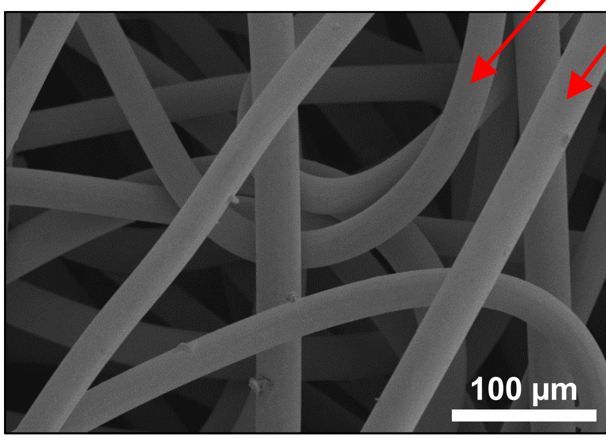
Comparing Operator Performance Before & After Training

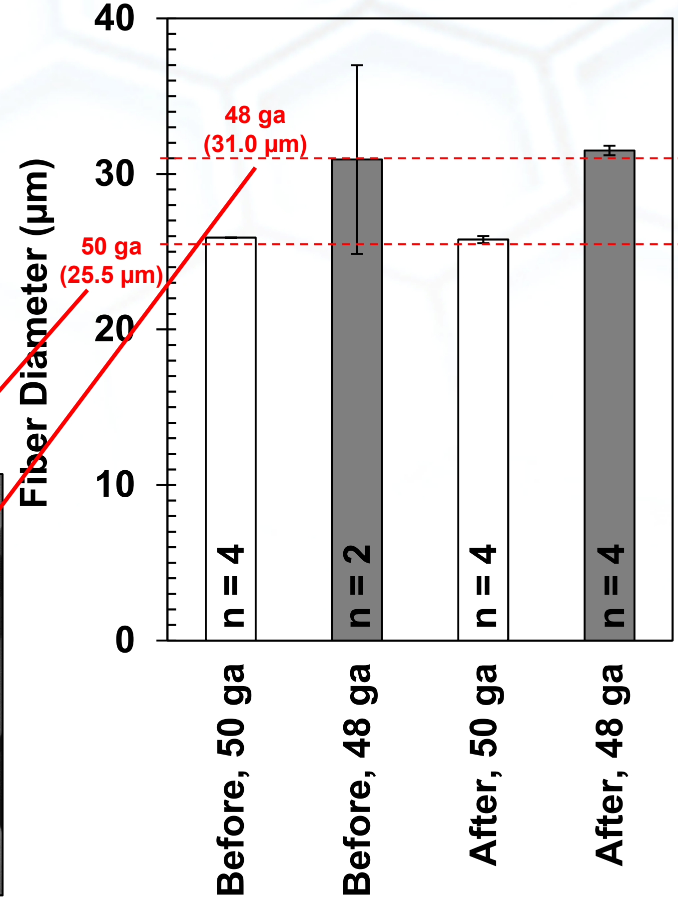
Inter-Laboratory Comparisons asses the robustness of an assay across different labs & results are used to refine the protocol

- **Before Training:** 2 of 4 operators identified the bimodal distribution
- **After Training:** 4 of 4 operators identified the bimodal distribution

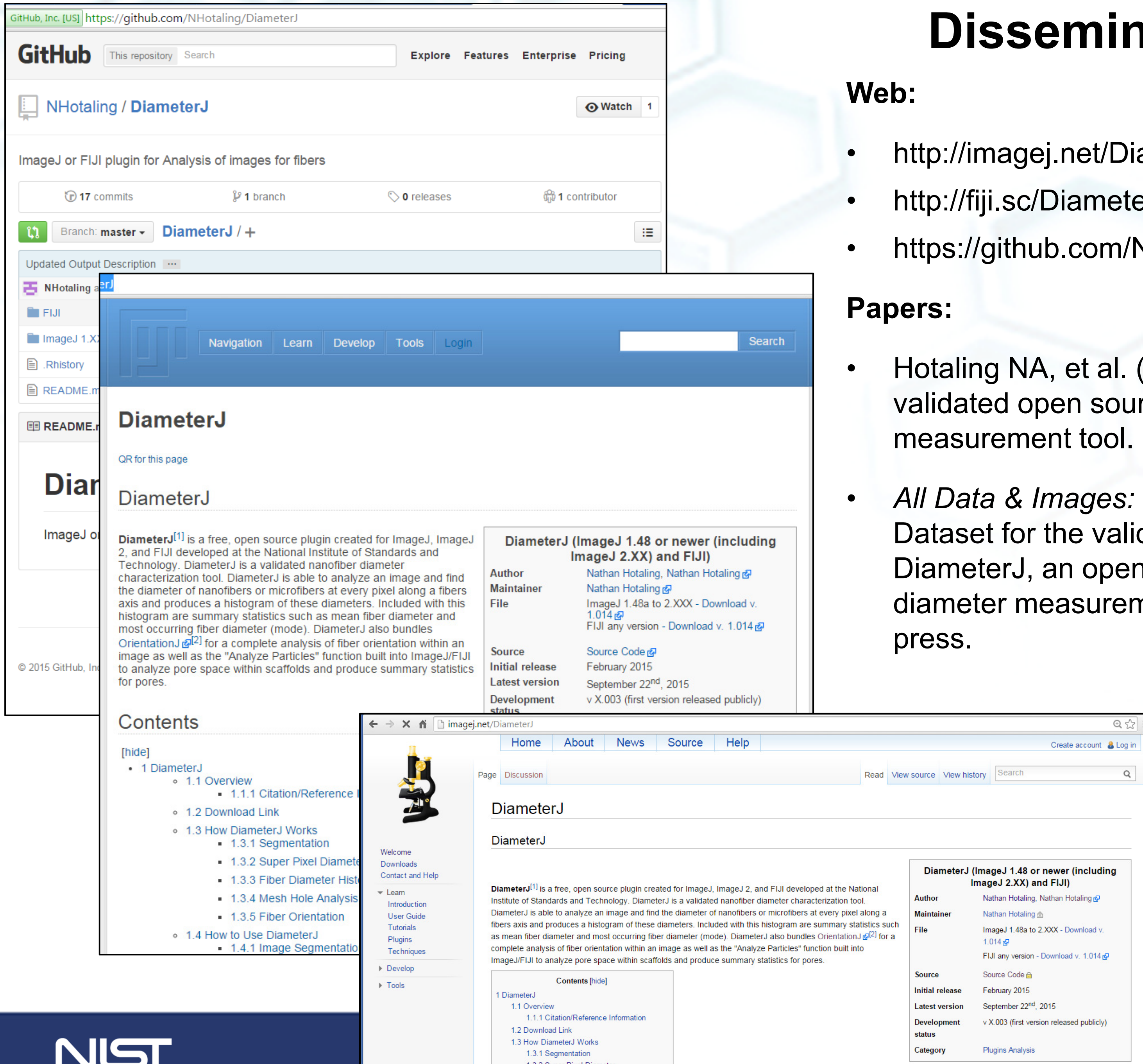
- *IN PROGRESS: Intra*-lab comparability study where participants analyze images before/after training to assess improvement
- Test images are mixture of 48 ga. & 50 ga. Wire
- NEW SPECIFICATION: Use constant magnification







Dissemination



Web:

- http://imagej.net/DiameterJ
- http://fiji.sc/DiameterJ
- https://github.com/NHotaling/DiameterJ

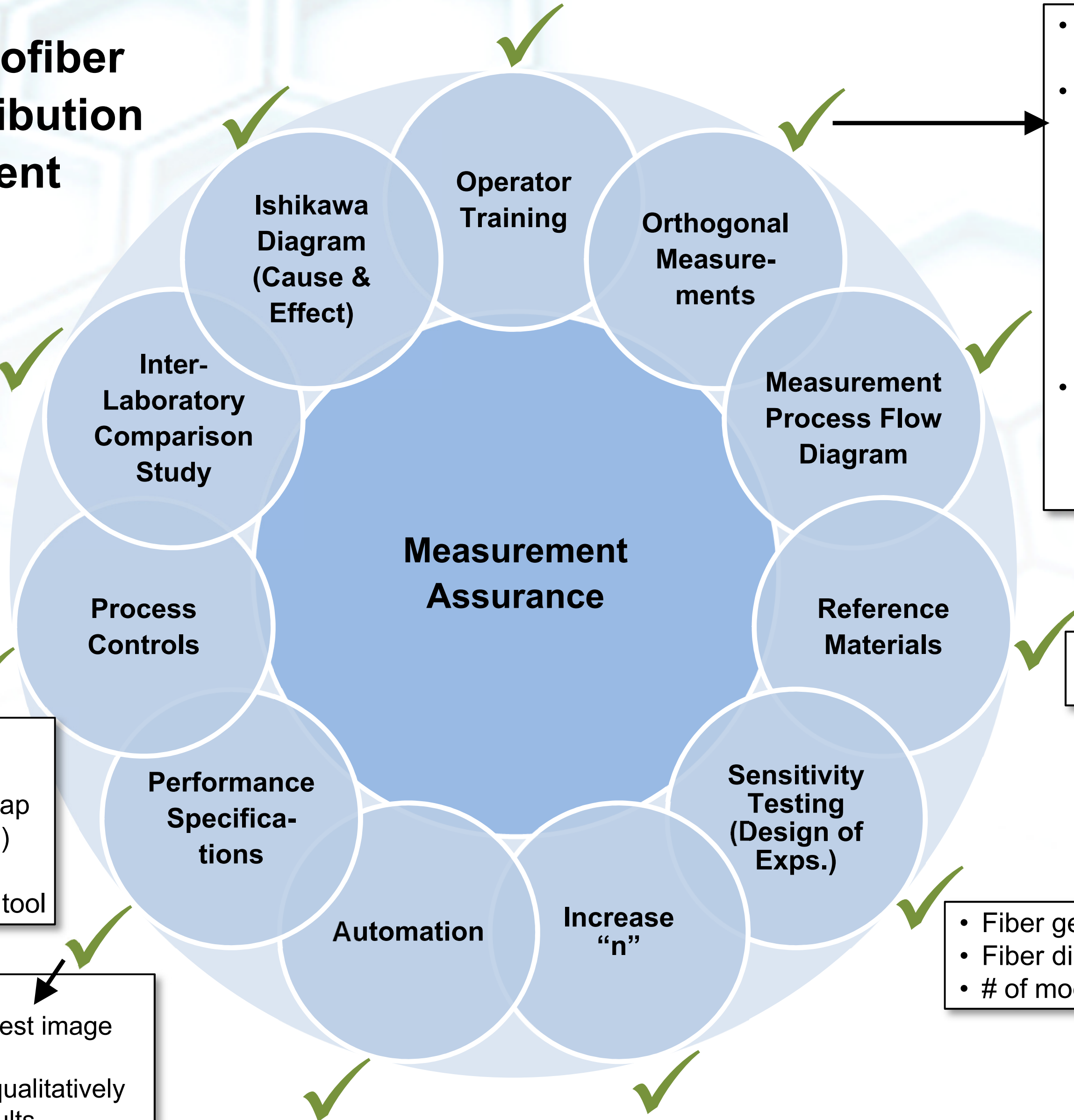
Papers:

- Hotaling NA, et al. (2015) DiameterJ: a validated open source nanofiber diameter measurement tool. Biomaterials 61, 327-338
- *All Data & Images:* Hotaling NA et al. (2015) Dataset for the validation and use of DiameterJ, an open source nanofiber diameter measurement tool. Data in Brief, in press.

Assuring Nanofiber Diameter Distribution Measurement

- Synthetic images: Counted pixels by hand
- Steel reference wires
  - Human manual measurement with ImageJ line tool
  - Optical imaging of fibers
  - Manufacturer resistivity measurement
  - Manufacturer caliper measurement
- Electrospun PLGA fibers
  - Human manual measurement with ImageJ line tool

“Intra”-lab underway (DiamterJ training module on the web)



- Synthetic images
- Reference wire

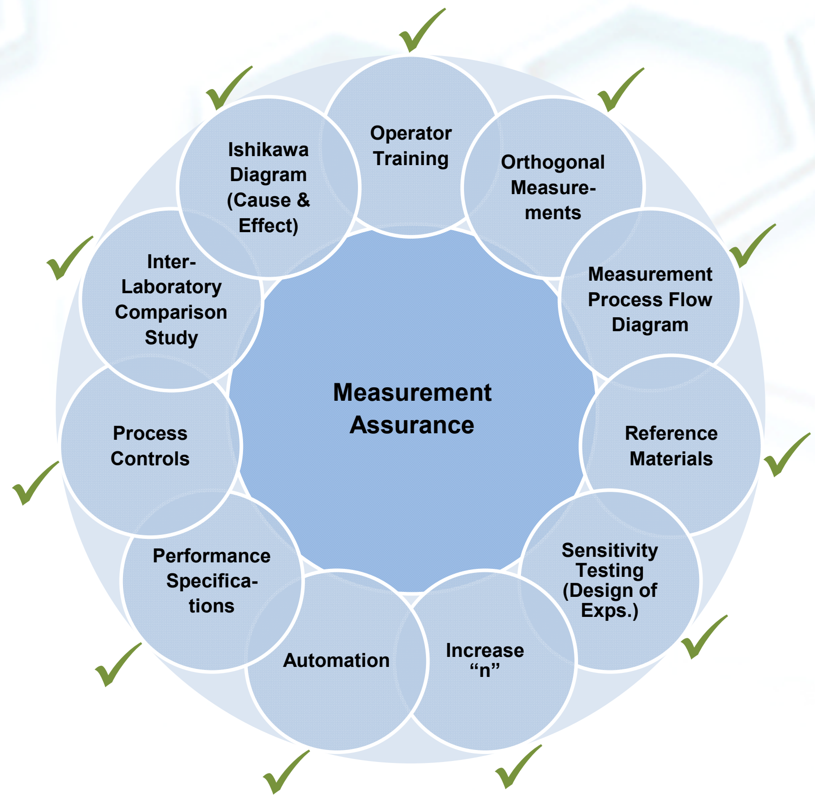
- Compare raw image to:
  - Segmentations
  - Euclidian distance map
  - Histogram (bimodal?)
  - DiameterJ results
- Identify errors w/ locator tool

- Fiber geometry
- Fiber dia. range
- # of modes

- Fibers >10 px in dia.
- Fibers < 10% of the smallest image dimension
- Fiber dia. in raw images qualitatively agree with DiameterJ results
- Segmentations are of good quality when visually compared to raw image
- Multimodes separated by >3 px
- 6 fiber dia. peaks is maximum

Summary

- "*product consistency & lack of standards is possibly the single greatest challenge facing the field*"
- Approach measurement process as a manufacturing process
- *Measurement Assurance:* Evaluate & reduce variability in order to improve confidence results to support decision-making (before writing a standard)



Thank you!