Measurement Assurance Case Study: Nanofiber Diameter

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Strategies for Achieving Measurement Assurance



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"



Automation

Increasing "n"

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

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Reference Materials

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Reference Materials are <u>homogeneous</u> & <u>stable</u> 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)





103 Synthetic Images

Reference Materials

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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





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







Orthogonal Measurements

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 (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

Sensitivity Testing

(Design of Exps.)

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- Straight vs curved
- Aligned vs disordered
- Multiple diameters



Multimodal Diameter Samples



(Failed on 10 Diameters)





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MATERIAL MEASUREMENT LABORATORY

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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:

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

Manufacturer's Fiber Dia. = 31.0 μm







Euclidian Distance Map

Raw Image

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

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)



Operator Training

Web Training Module

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

Web training where users download & analyze images with DiameterJ



Understanding Output from DiameterJ

Fill out the below information and begin the 20 question Quiz only AFTER reading the documents found at the links below.

* Required

Participant Number *
Save this number somewhere. DO NOT LOSE THIS NUMBER. You will need it later.
953128
*
Installation and Use of DiameterJ - https://goo.gl/4e5j0P
*
*
Output of DiameterJ - https://goo.gl/1er0Lc

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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 (Cause & Effect) Ishikawa to identify variability

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Ishikawa Diagram

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





Inter-Laboratory Comparison Study

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- *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



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



GitHub	This repository Search	Explore Fea	tures Enterprise Pricing		JISSEMI	
NHotalin	g / DiameterJ		⊙ Watch 1	Web	:	
mageJ or FIJI p	plugin for Analysis of images for fibers	🛇 0 releases	∰ 1 contributor	• h	http://imagej.net/[http://fiii.sc/Diame	
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DimageJ of SitHub, Inc	DiameterJ ^[1] is a free, open source plugin created for ImageJ, ImageJ 2, and FIJI developed at the National Institute of Standards and Technology. DiameterJ is a validated nanofiber diameter characterization tool. DiameterJ is able to analyze an image and find the diameter of nanofibers or microfibers at every pixel along a fibers axis and produces a histogram of these diameters. Included with this histogram are summary statistics such as mean fiber diameter and most occurring fiber diameter (mode). DiameterJ also bundles OrientationJ gt ^[2] for a complete analysis of fiber orientation within an image as well as the "Analyze Particles" function built into ImageJ/FIJI to analyze pore space within scaffolds and produce summary statistics for pores.		DiameterJ (ImageJ 1.48 or newer (including ImageJ 2.XX) and FIJI) Author Nathan Hotaling, Nathan Hotaling Maintainer Nathan Hotaling, Mathan Hotaling File ImageJ 1.48a to 2.XX - Download v. 1.014 @ FIJI any version - Download v. 1.014 @ Source Source Code @ Initial release Initial release February 2015 Latest version September 22 nd , 2015 Development wy 000 (finite melaned hot high)	Dataset for the va DiameterJ, an op diameter measur press.		
	Contents	← → X ff 🗅 imagej.n	Development V X.003 (tirst version released publicity) status tet/DiameterJ			
	[hide] • 1 DiameterJ • 1.1 Overview • 1.1.1 Citation/Reference I • 1.2 Download Link • 1.3 How DiameterJ Works • 1.3 Sementation		Discussion DiameterJ DiameterJ	Read Viet	Create account Search	
 1.3.1 Segmentation 1.3.2 Super Pixel Diame 1.3.3 Fiber Diameter His 1.3.4 Mesh Hole Analysi 1.3.5 Fiber Orientation 1.4 How to Use Diameter J 1.4.1 Image Segmentation 		Welcome Downloads Contact and Help ✓ Leam Introduction User Guide Tutonals Plugins Techniques > Develop ► Tools	Diameters ^[11] is a free, open source plugin created for ImageJ, ImageJ 2, and FLI dev Institute of Standards and Technology. DiameterJ is a validated nanofiber diameter ch DiameterJ is able to analyze an image and find the diameter of nanofibers or microfibe fibers axis and produces a histogram of these diameters. Included with this histogram as mean fiber diameter and most occurring fiber diameter (mode). DiameterJ also bun compilet analysis of fiber orientation within an image as well as the "Analyze Particles" imageJiFLJI to analyze por space within scaffolds and produce summary statistics for Contents [hide]	eloped at the National aracterization tool. rs at every pixel along a are summary statistics such dies OrientationJ d ²²¹ for a function built into pores.	Diameter J (Image J 1.48 or newer (including Image J 2.XX) and FJJI) Author Nathan Hotaling, Nathan Hotaling, Maintainer Nathan Hotaling, Image J 1.48 to 2.XX - Download v. 1.014.gr File Image J 1.48 to 2.XX - Download v. 1.014.gr Till any version - Download v. 1.014.gr Source Source Code @r	
NI	ട	F TOOIS	1 DiameterJ 1.1 Overview 1.1.1 Citation/Reference Information 1.2 Download Link 1.3 How DiameterJ Works 1.3.1 Segmentation 1.3.2 Segment Pixel Diameter		Initial release February 2015 Latest version September 22 nd , 2015 Development v X 003 (first version released publicly) status Category Plugins Analysis	

ssemination

- magej.net/DiameterJ
- ii.sc/DiameterJ
- github.com/NHotaling/DiameterJ
- g NA, et al. (2015) DiameterJ: a ed open source nanofiber diameter rement tool. Biomaterials 61, 327-338
- a & Images: Hotaling NA et al. (2015) et for the validation and use of terJ, an open source nanofiber er measurement tool. Data in Brief, in

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BORATORY



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!