Quantification of Bubble Defects in Candidate Reference Material Glasses: **Bubble Trouble Part II**

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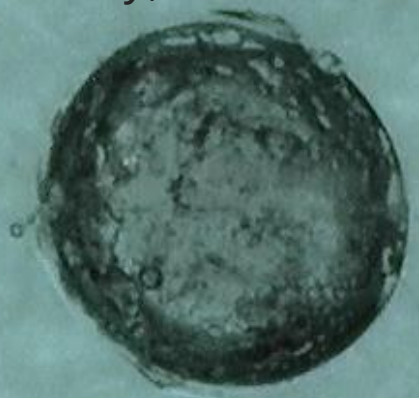


Outline

- Glass in forensic investigations
- Instruments/methods in glass analysis
- Sources of variability
- Limitations of current standards
- Quantification of defects in candidate microanalysis standards

Glass in Forensic Investigations

Float Glass

- Most common type of trace evidence found at crime scenes
  - windshields, windows, tv screens

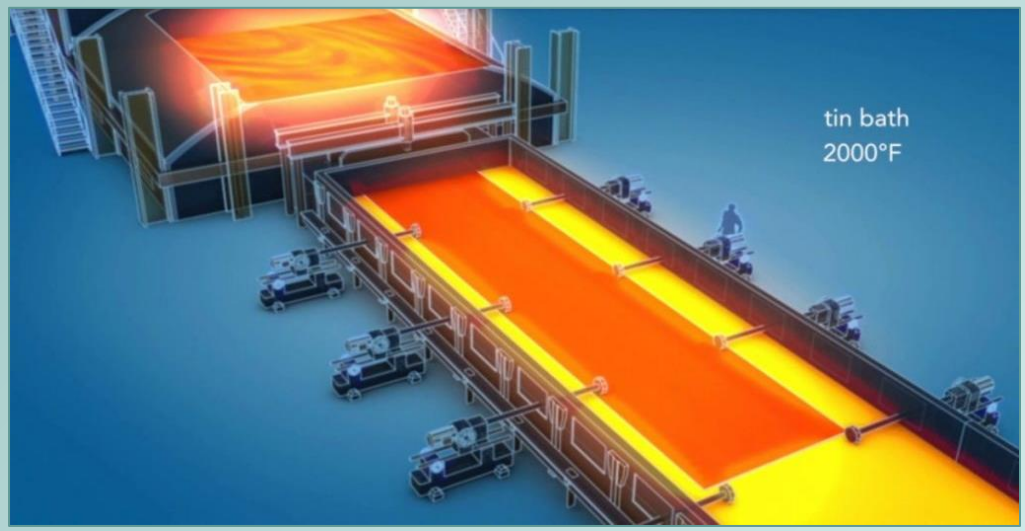


Image from VisionTIR; Temperature control in float glass manufacturing
Temperature control in float glass manufacturing | VisionTIR

Forensic Examination

- Elemental quantification
- Homogeneity of the sample
- Identify source of unknowns

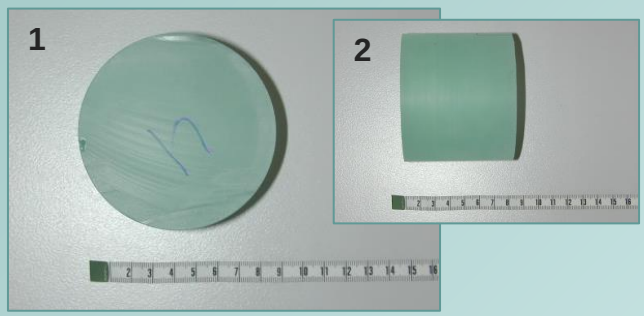
Image from Crime Museum; Glass analysis
https://www.crimemuseum.org/crime-library/forensic-investigation/glass-analysis/

Glass in Forensic Investigations

Workflow of evidence testing

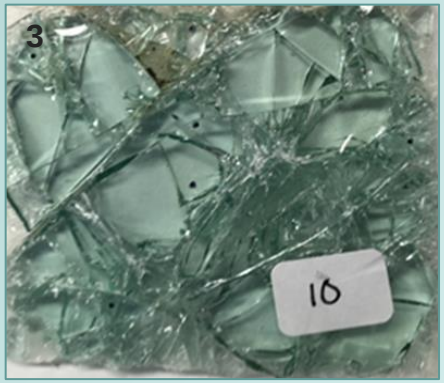
Calibrate

Standard Reference Materials (SRMs) to **increase confidence in results**



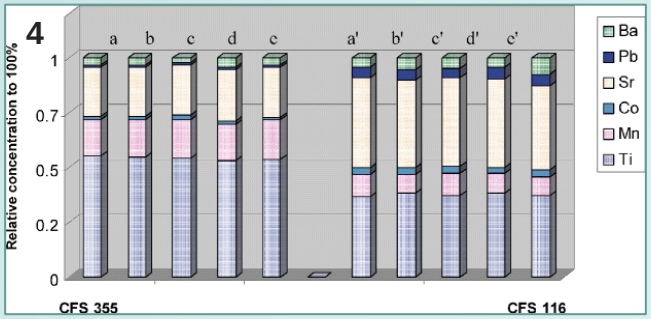
Measure

Test sample unknowns and knowns



Analyze

Associated or non-associated based on elemental similarity



Repeatability assessment 5 replicate measurements of two different glass samples analyzed by LA-ICP-MS

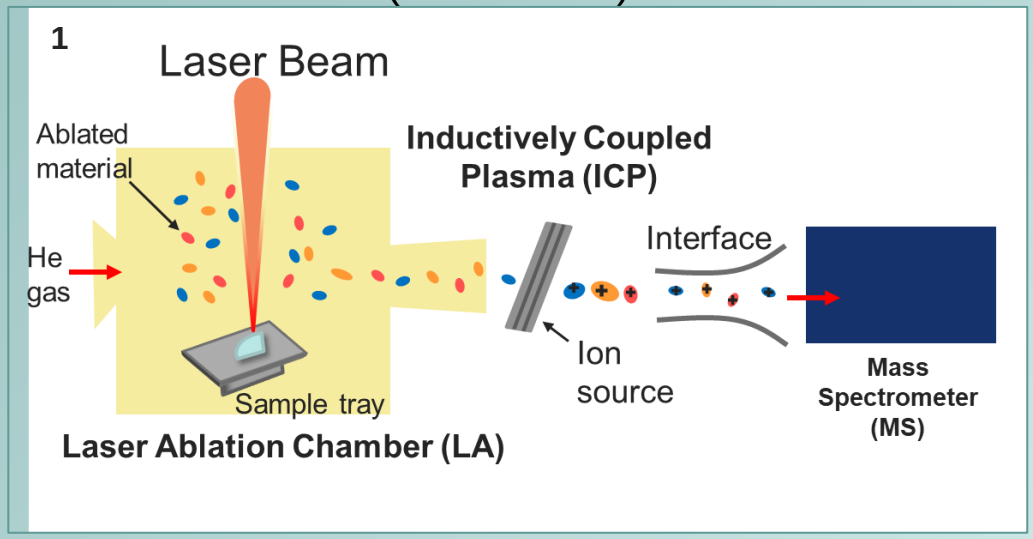
1,2. Becker Stefan, Laser Ablation ICP-MS in Forensic Glass Analysis: A Decade of Experience (2007)

3. Glass Evidence Analysis | NIST

4. Trejos, T., Montero, S. & Almirall, J.R. Analysis and comparison of glass fragments by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and ICP-MS. Anal Bioanal Chem 376, 1255–1264 (2003). https://doi.org/10.1007/s00216-003-1968-0

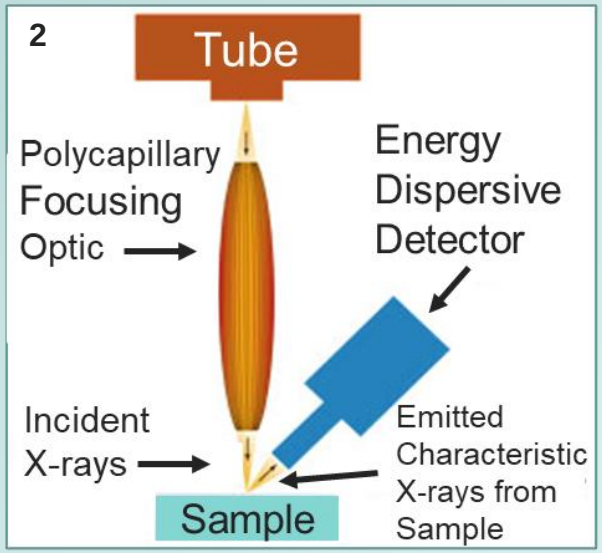
Typical Glass Analysis Techniques Require Careful Calibration

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)



- Low detection limits for most of periodic table
- Minimal sample prep
- Minimally destructive
- ★ Sensitive to defects
- Spot size of 50 µm - 100 µm

Micro-X-ray Fluorescence (µ-XRF)

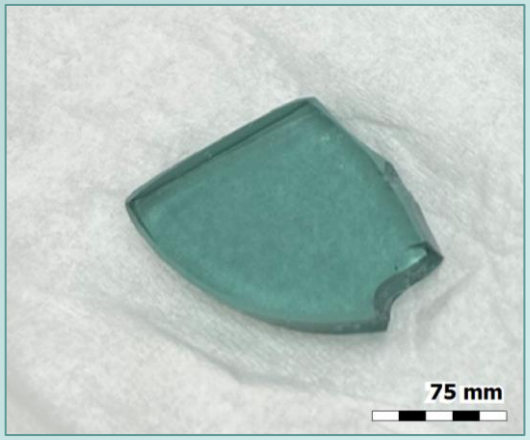


- Non-Destructive
- Homogeneity analysis
- Less sensitive to defects
- Spot size of 20 µm - 1000 µm

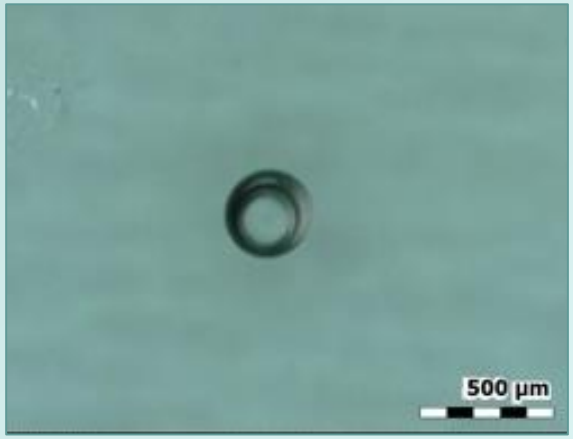
1. Weiskirchen, S.; Kim, P.; Weiskirchen, R. Laser Ablation Inductively Coupled Plasma Spectrometry: Metal Imaging in Experimental and Clinical Wilson Disease. *Inorganics* **2019**, *7*, 54.
2. Micro X-ray Fluorescence (µXRF) - XOS

Defects Hinder Interpretability of Glass Analysis

- Current NIST SRM 610 is not certified for microanalysis
  - (e.g., LA-ICP-MS, µ-XRF)



- New candidate SRMs representative of modern float glass elemental composition are being evaluated
  - Characterization of the new candidate SRMs includes evaluation of defects capable of altering calibration accuracy

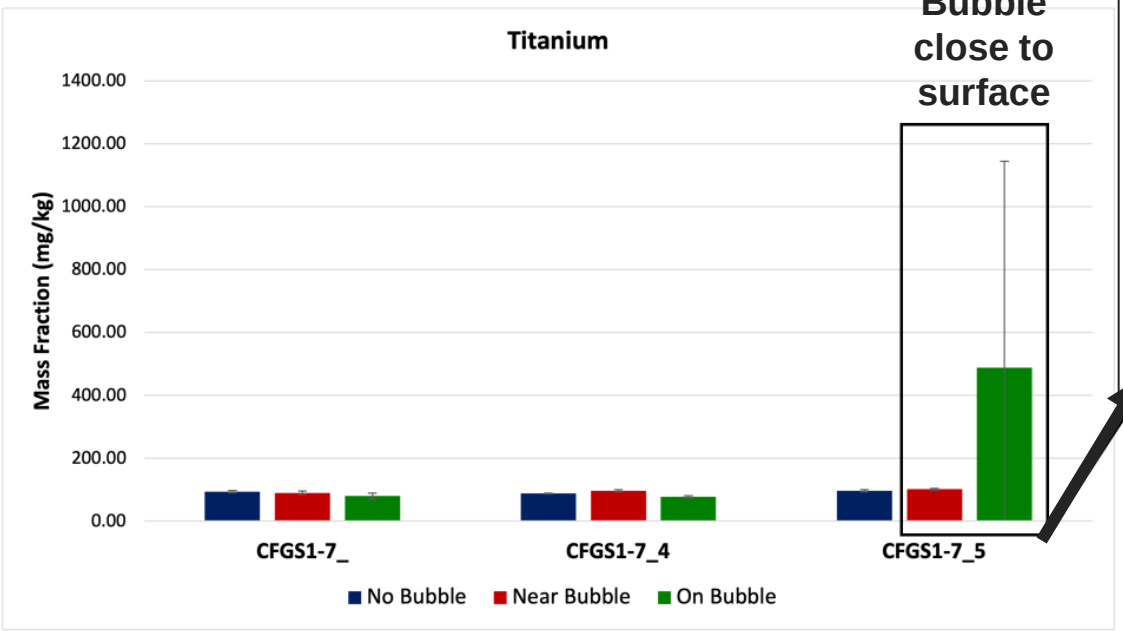


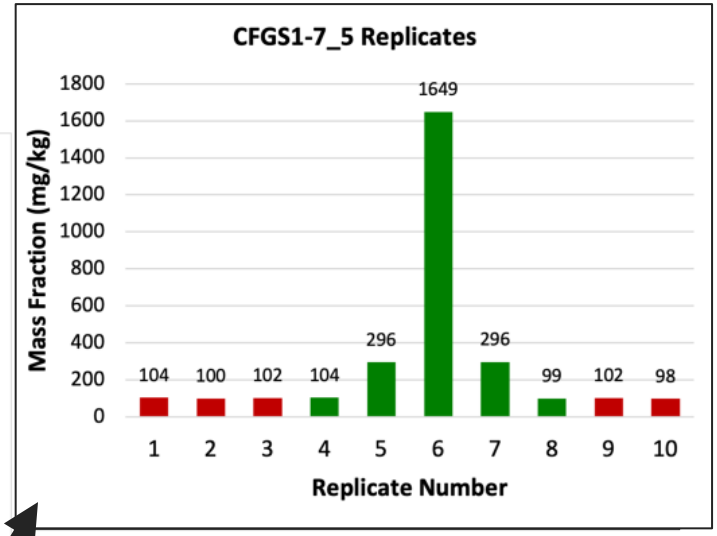
Defects Hinder Interpretability of Glass Analysis

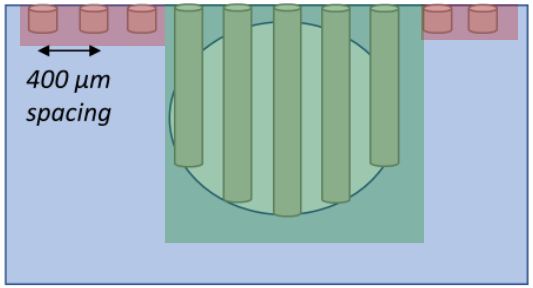
Ruthmara Corzo: CFGS LA-ICP-MS Analysis Summary (Corning Forensic Glass Standard (CFGS))

Effect of distance from bubble on LA-ICP-MS elemental mass fraction (mg/kg)

Titanium and Tin







Characterizing and accounting for defects in SRMs may result in better accuracy and improved quantitative comparisons

Methods for Characterizing Bubble Defects

IMAGING

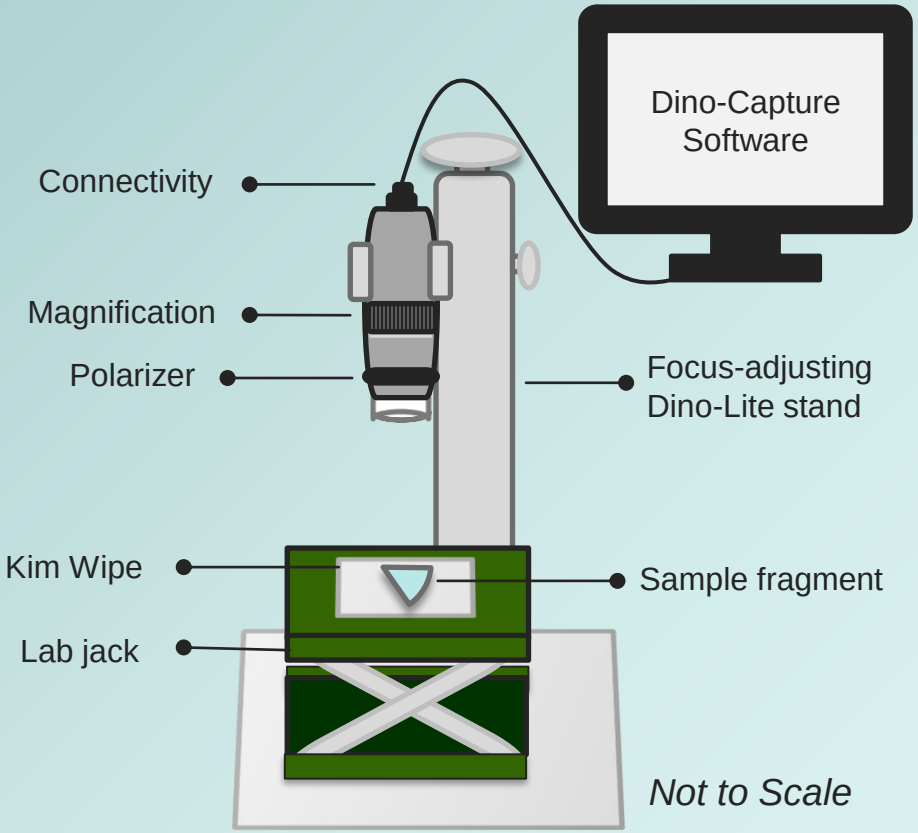
- AM4113ZTL Dino-Lite Digital Microscope Version 1.5.50
  - Resolution 22 µm

MEASURING

- Dino-Capture 2.0 imaging software
- Digital Surf, Mountains (®, MDS) image processing software Version 10

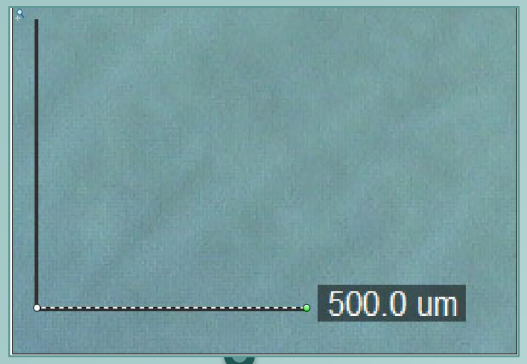
COMPARISON

- Antonio, Raine: Bubble Trouble (Part 1) (2023) CFGS1 data



Dino-Lite Digital Microscope setup

Digital Surf Automation: Selected Operations



Individual results

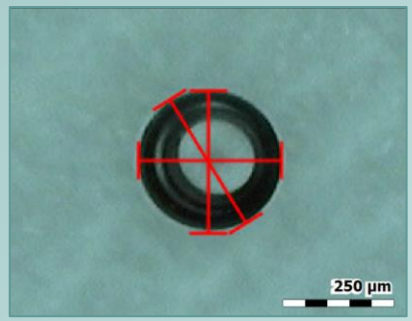
Parameters	Equivalent radius	Equivalent diameter	Mean diameter
Unit	mm	mm	mm
Particle #745	0.1483	0.2967	0.3233
Particle #746	0.00349	0.00698	0.004684
Particle #747	0.002015	0.00403	0.002032
Particle #748	0.00403	0.00806	0.005456
Particle #749	0.002015	0.00403	0.002032

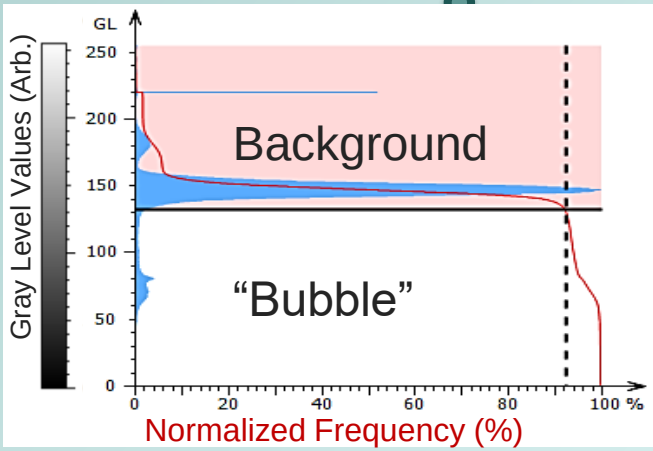
Scale Image

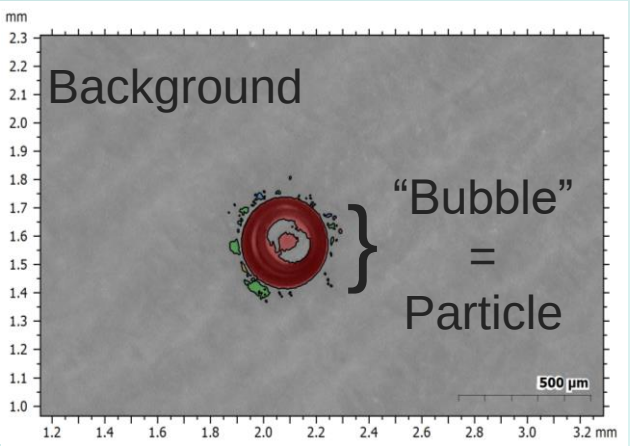
Particle Analysis: Threshold

Particle Analysis: Select Parameters

Dino-Capture: Manual Measurements



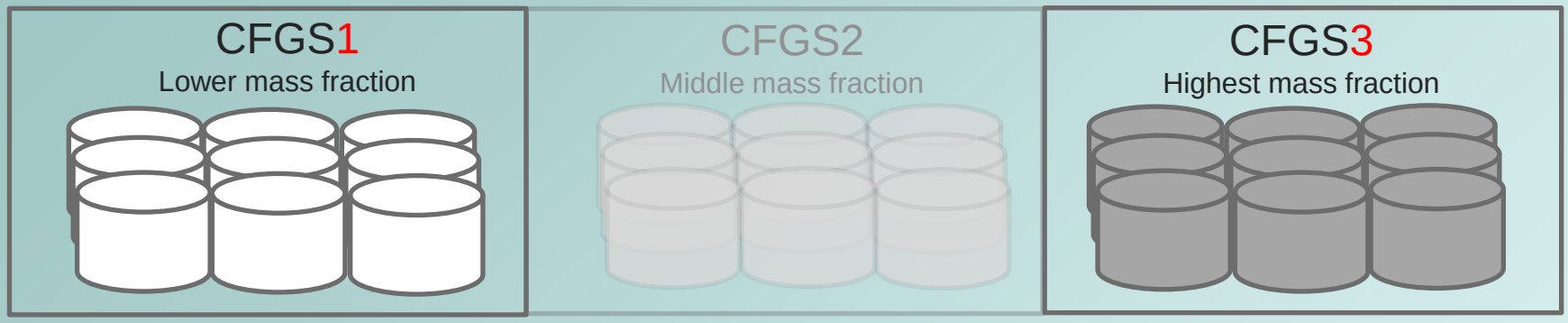


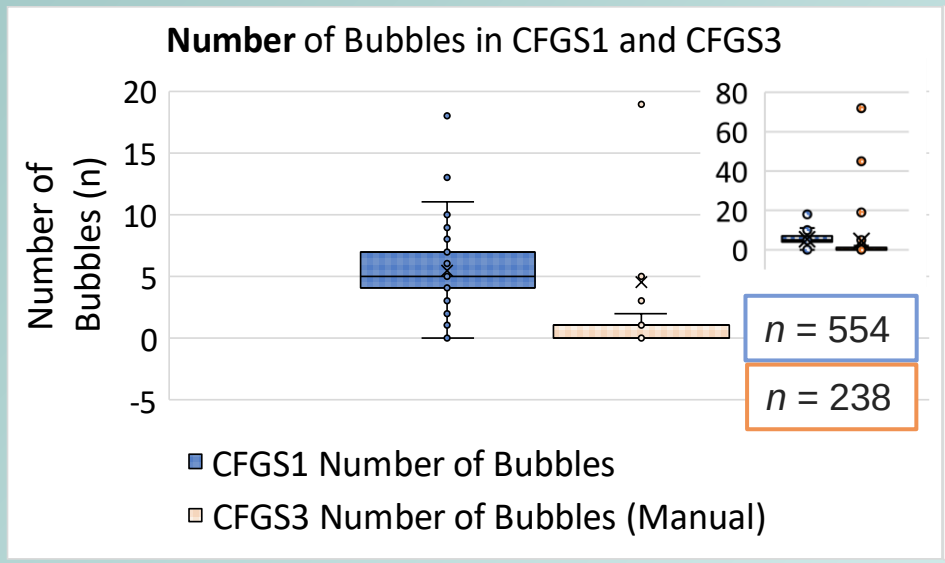


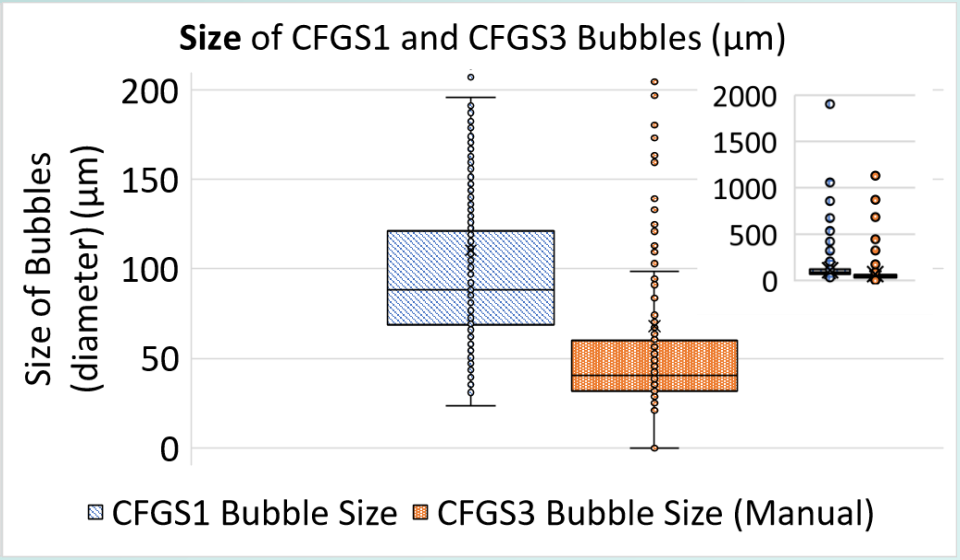
Thresholding: way to identify features by assigning them to 2 ≤ grayscale values

Trends in Candidate SRMs

- We are studying three candidate SRMs whose properties reflect current float glass elemental makeup



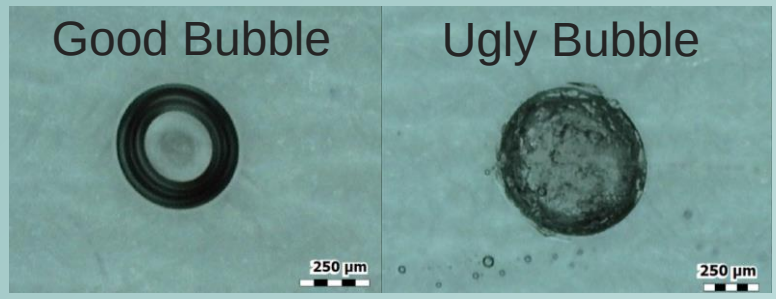




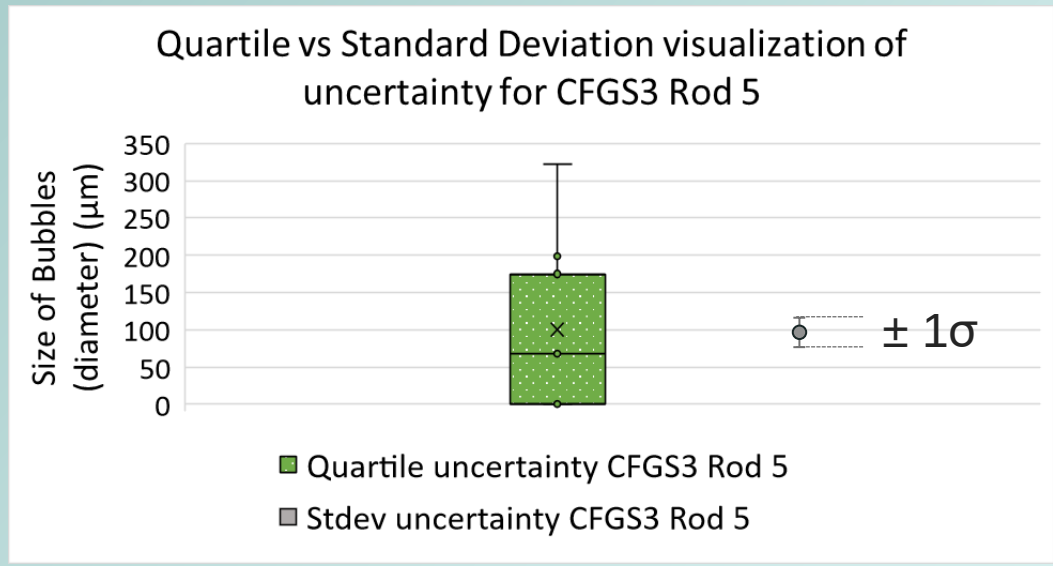
Size Uncertainties and Data Variability

Types of uncertainty

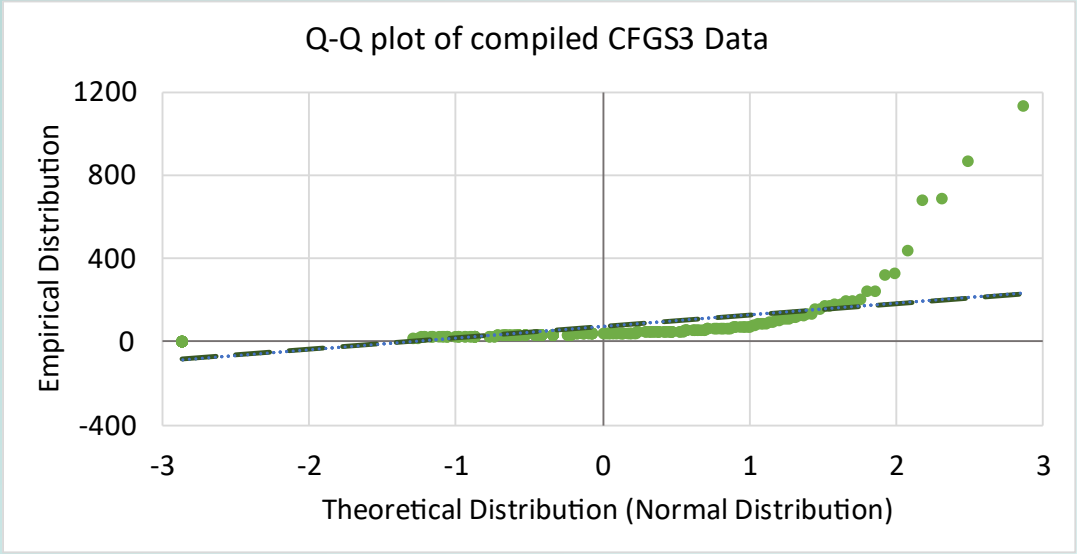
- Instrument resolution
- Sample population uncertainty



CFGS3 example defects

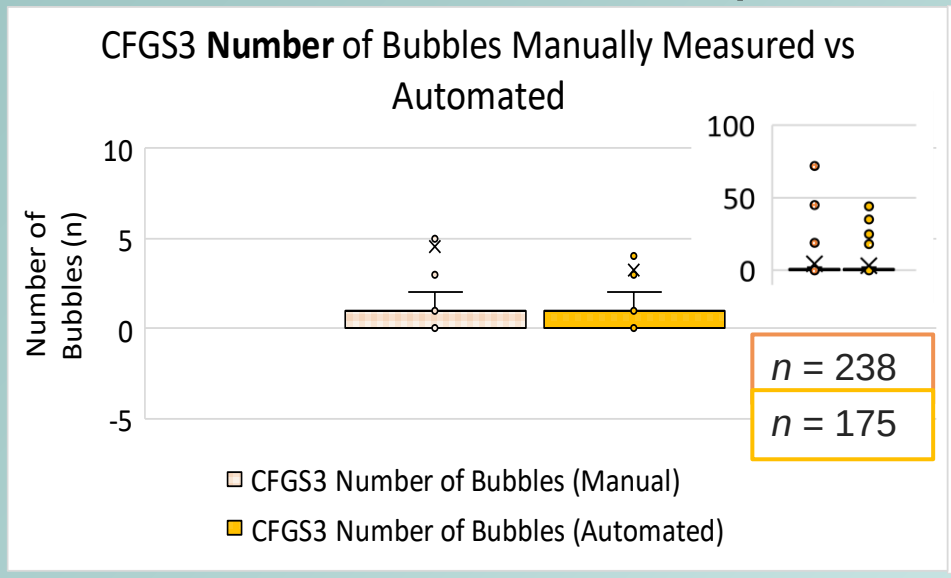


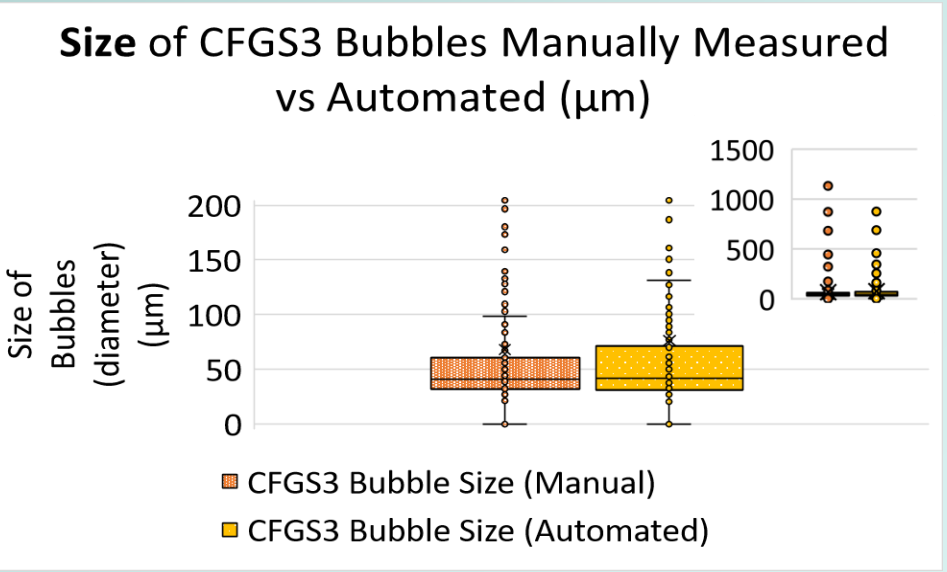
- Quartile
  - Median centered
- Standard deviation (Stdev)
  - Mean centered
- Quantile-Quantile (Q-Q) plot
  - Visualize if data follows probability distribution

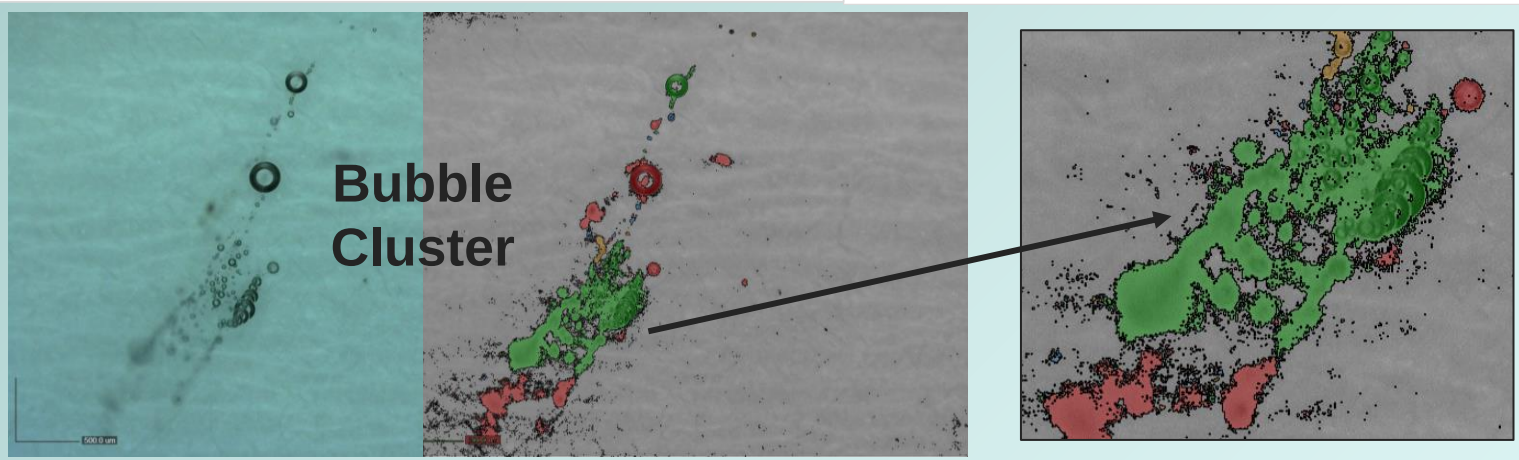


Assessment of Automated Image processing

- ☺ Eliminates uncertainties from user-to-user variation
- ☺ Rapid batch analysis
- ☹ Limited in detection of particle clusters







Future Steps

- Investigate outlier CFGS3 fragments and determine frequency
- Improve automated template for bubble measurements
- Complete imaging and measurements for CFGS2
- Develop a suggested protocol for SRM use to improve accuracy of measurements (avoid defects)

Special Thanks To

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Leland Harringer

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NIST FORENSIC SCIENCES

Questions?

1. Claudia Martinez: Homogeneity assessment of the elemental composition of windshield glass by µ-XRF, LIBS, and LA-ICP-MS analysis (2022). https://doi.org/10.1016/j.forc.2021.100384
2. Douglas C. Duckworth: Forensic glass analysis by ICP-MS: a multi-element assessment of discriminating power *via* analysis of variance and pairwise comparisons (2002). https://doi.org/10.1039/B201575G
3. Jose Almirall: Validation of Novel Statistical Approaches for the Interpretation of Trace Evidence; Glass Analysis using LA-ICP-MS (2022).
4. ASTM International (2024) *E2927-23 - Standard Test Method for Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons* (ASTM International, West Conshohocken, PA). https://doi.org/10.1520/E2927-16E01
5. ASTM International (2022) *E2926-17 – Standard Test Method for Comparison of Glass Using Micro X-ray Fluorescence (µ-XRF) Spectrometry* (ASTM International, West Conshohocken, PA). https://doi.org/10.1520/E2926-17
6. Pete Bankhead: Introduction to Bioimage Analysis (2022). https://bioimagebook.github.io/chapters/2-processing/3-thresholding/thresholding.html
7. Mehmet Sezgin: Survey over image thresholding techniques and quantitative performance evaluation (2004) http://dx.doi.org/10.1117/1.1631315

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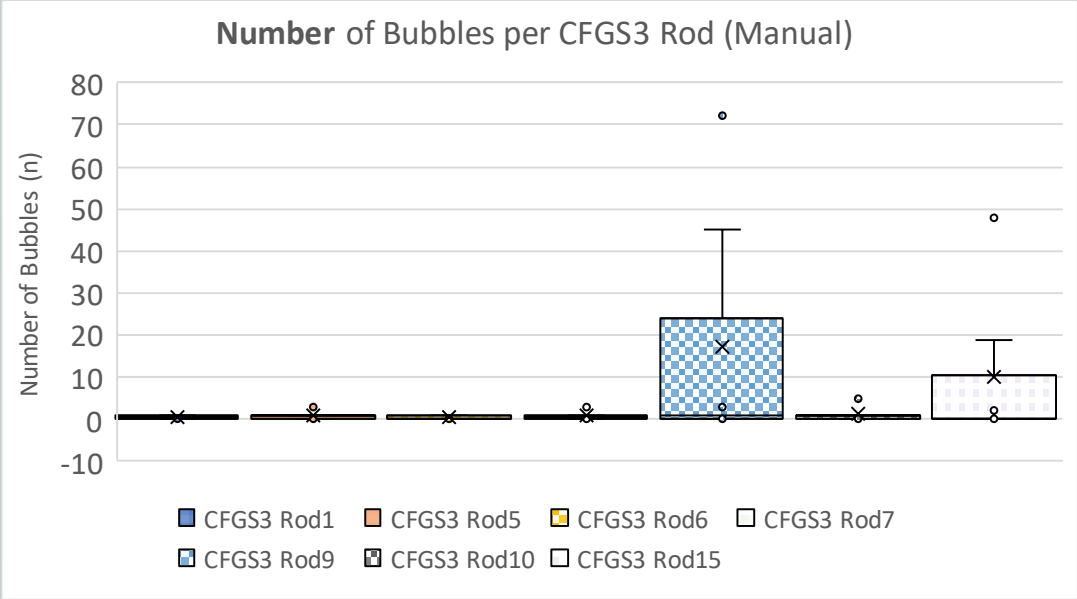
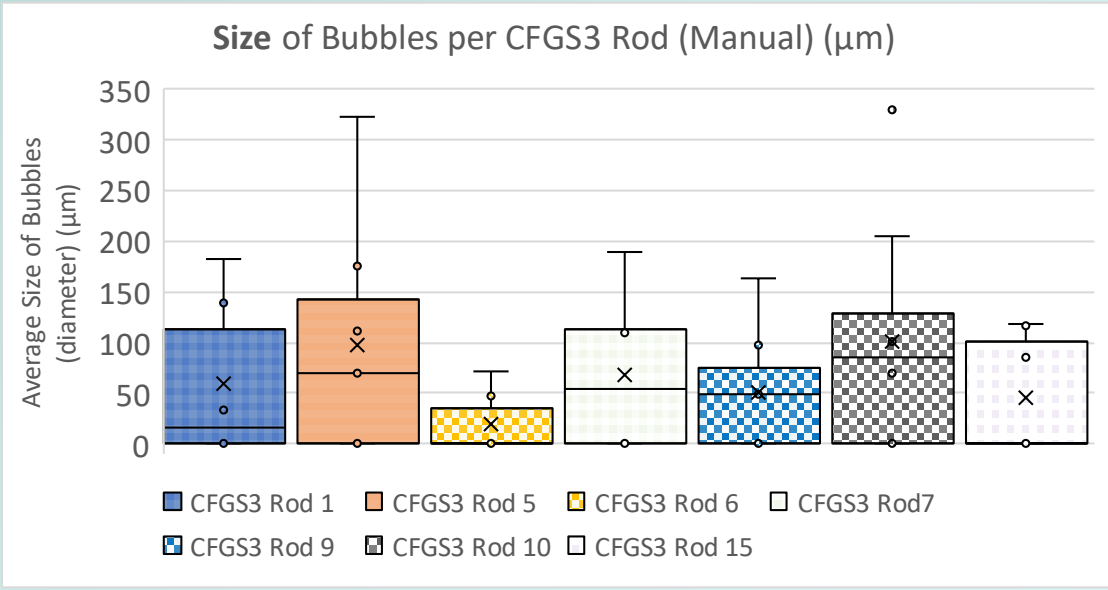
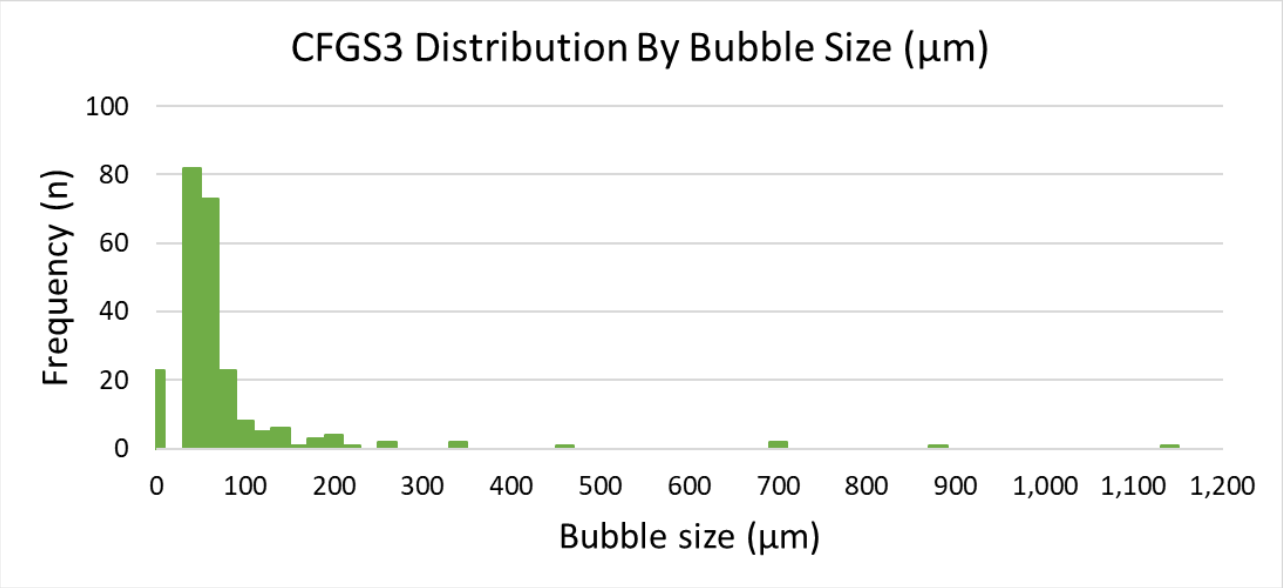
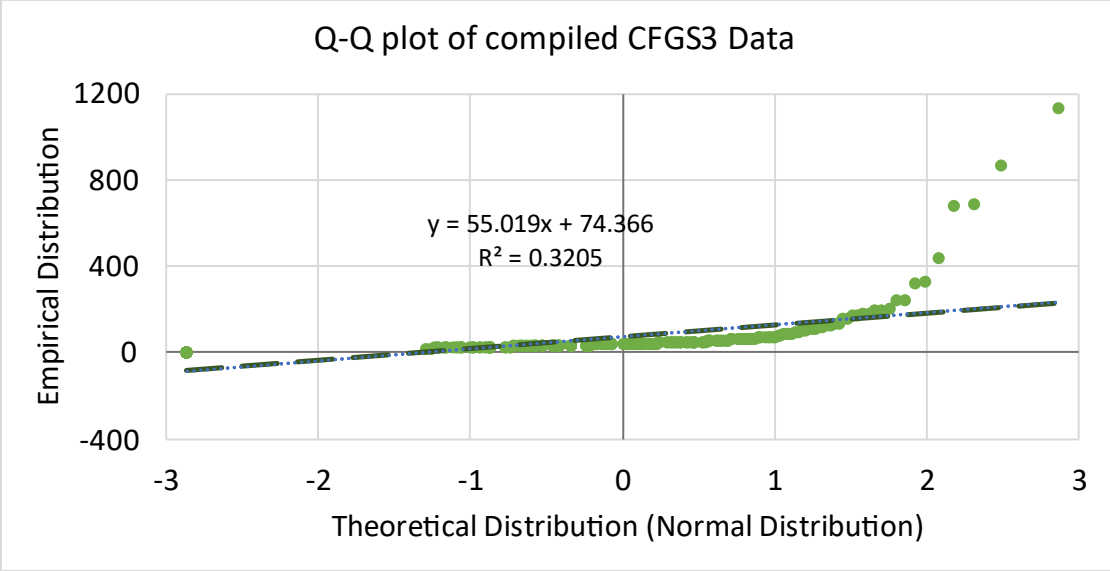
CFGS3 Variability Data



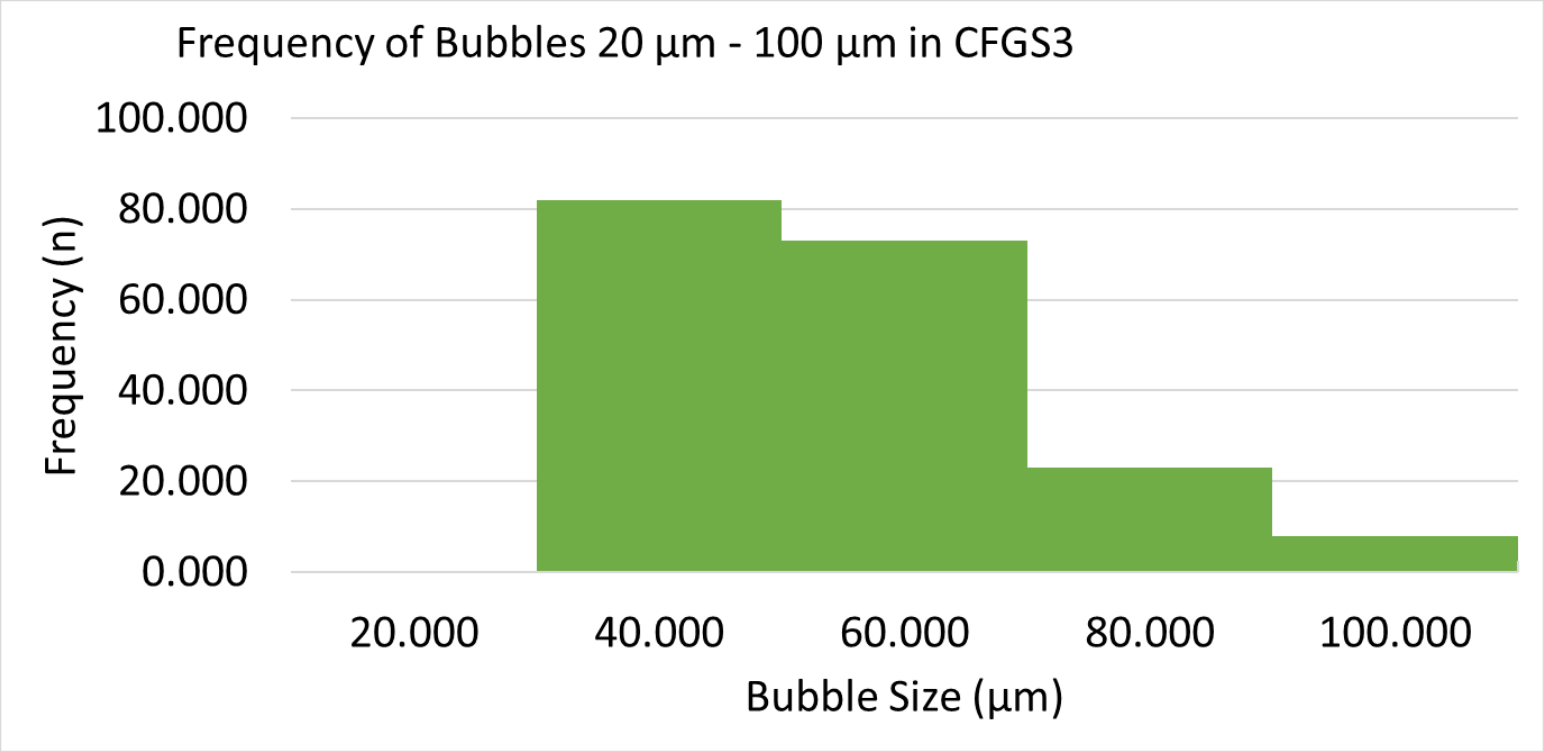


Visualization of CFGS3 non-normal distribution



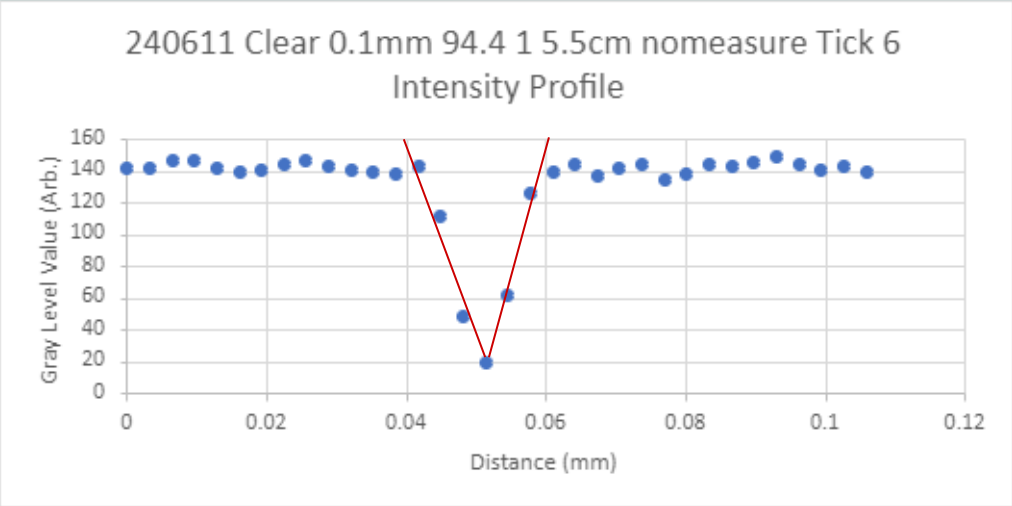


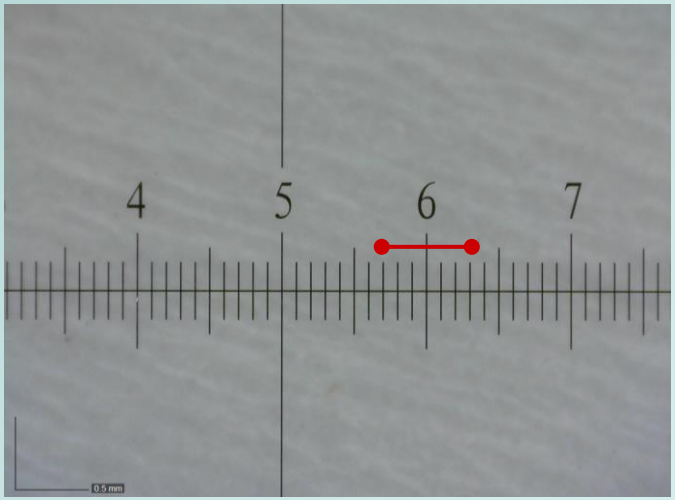
Defect sizes Within Range of Laser-Ablation Spot Size



- Total number of measurements in CFGS3: 238 (n)
- Total number of measurements in CFGS3 between 20 µm - 100 µm: 186 (n)
- 78.15 % of all CFGS3 bubbles are below 100 µm
- 44.09 % of small bubbles (20 µm - 100 µm) are between 20 µm - 40 µm

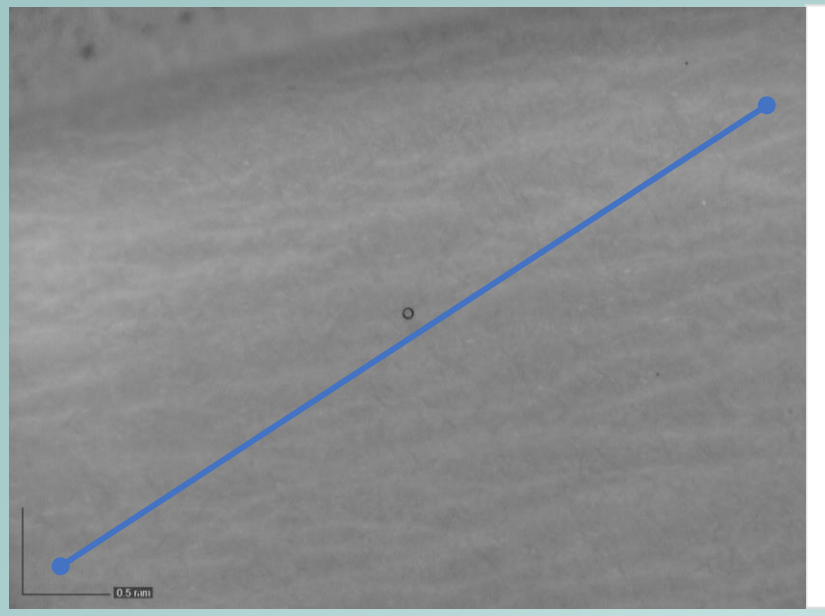
Determining Image Resolution

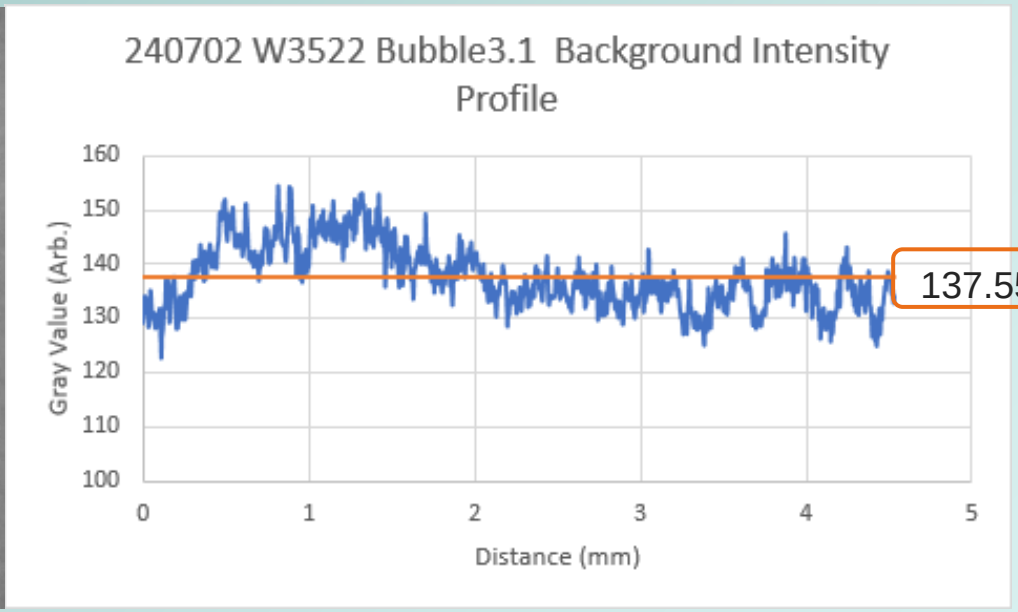




- Resolution of 1 pixel
- Width of 1 pixel = 0.006 mm
  - 22 µm

Automated Measurements; Determining Gray Value Threshold





- Overall average Background GL: 140.84
- Attempted lower thresholds of 1 sigma, 2 sigma, and 3 sigma
  - Lower sigma selected to identify gray values below threshold as “particles” (darker gray values)