Particle Size Determination and Raman Spectroscopic Evaluation of a Semi-solid Vaginal Dosage Form

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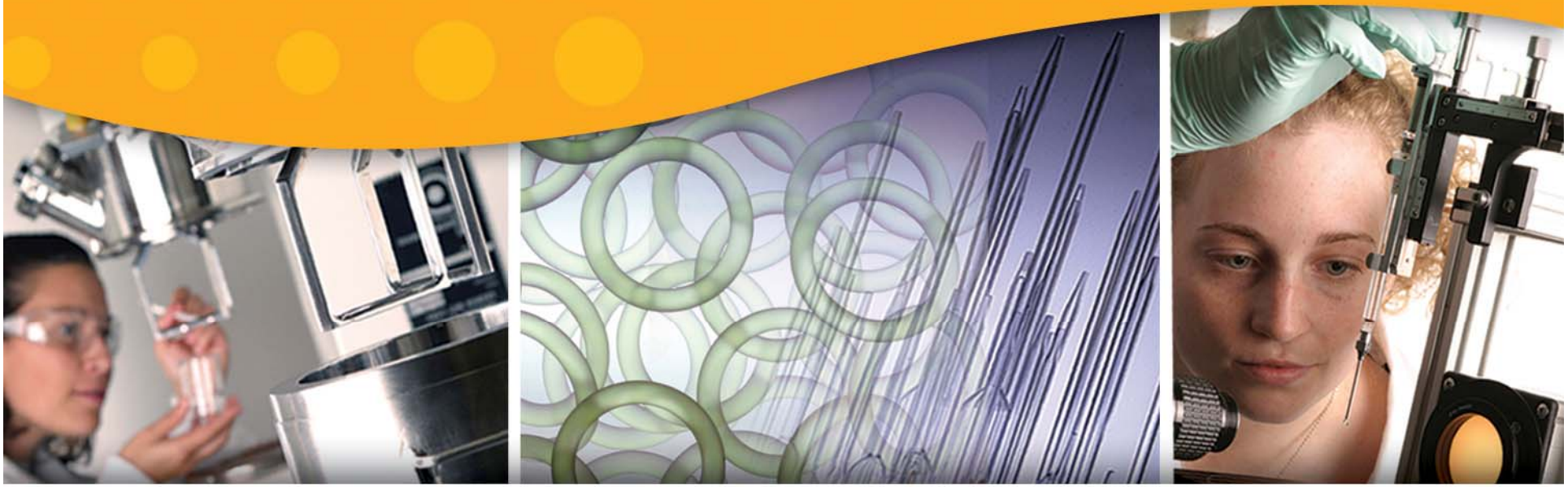

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What We Do

- Complete post-discovery CMC development
  - World leader in BCS II formulation based on data driven systematic approach
- API characterization
- Analytical methods development
- Drug product formulation
  - Leveraging physicochemical properties against array of drug delivery technologies across all dosage forms and routes of administration
- Preclinical and clinical trial material manufacture
  - Highly potent, sterile and non-sterile
- cGLP/cGMP analytic and bioanalytic support
- Full ICH compliant stability programs

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Corporate

- Founded in 1991, privately held, profitable
- Bethlehem, PA, 25,000 sqft, 40+ employees
- State-of-the-art cGLP/cGMP analytical/ bioanalytical and physical characterization
- Sterile, non-sterile and high potency cGMP production suites
- Full ICH compliant stability programs
- Dedicated highly potent compound handling infrastructure
- DEA licensed, FDA registered

- CRO servicing Pharmaceutical and Biotech companies

Staff

- Decades of experience
- Zero turnover of senior management over last 6 years other than additions
- Advanced degrees ranging from Medicine to Peptide Chemistry to Colloid and Polymer Sciences
- Previous Director level oversight of analytical, quality, formulation, clinical and cGMP production groups
- Experience in international, large/small/startup pharma, biotech, government and not-for-profit
- Multiple commercialized technologies
- > 100 patents pending / issued ranging from nanoparticles to NCE's to medical devices, well published

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Approach

- Provide our clients with the best possible solutions
- We do this by consolidating technologies across disciplines and industries
- PSI-originated and in-licensed technologies
- Partnerships with industry leading equipment manufacturers
- Partnerships with API suppliers
- Partnerships with industry leading key excipient suppliers

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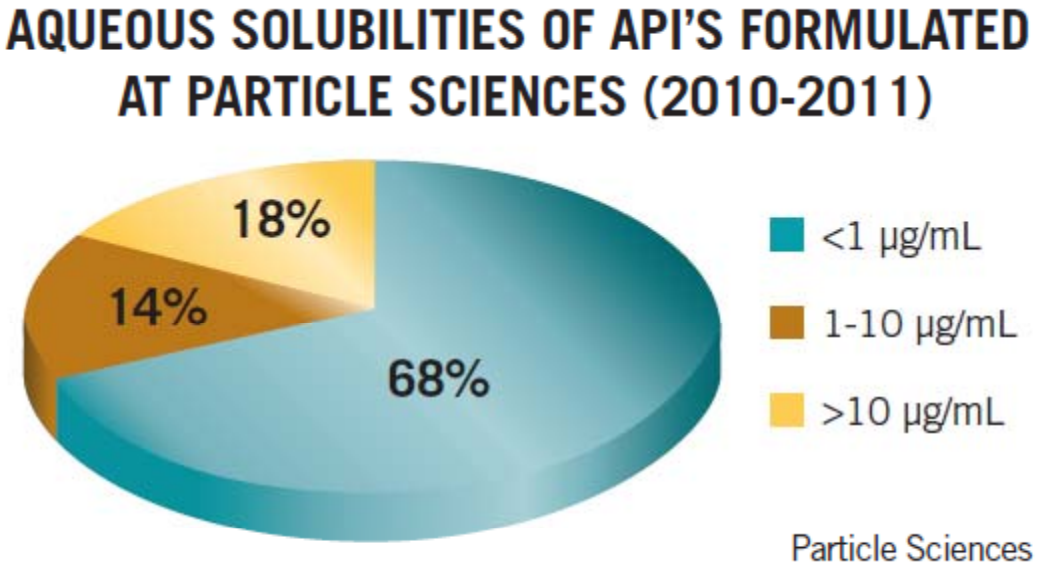
Our Work

- 40% parenteral
- 30% mucosal
- 10% ocular
- 20% oral

- 80% small molecules
- 20% large molecules
- 40% high potency

- 70% preclinical
- 30% clinical
- 30% sterile/aseptic



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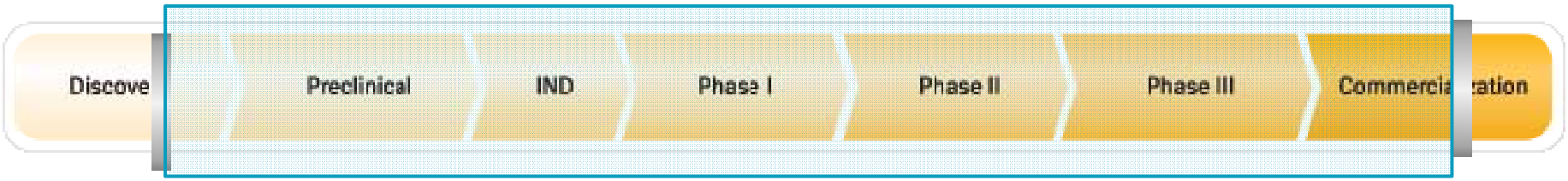
Our Role

- Post-discovery through clinical development
- Moving into commercial production with our clients
- Routine work through true innovation & invention



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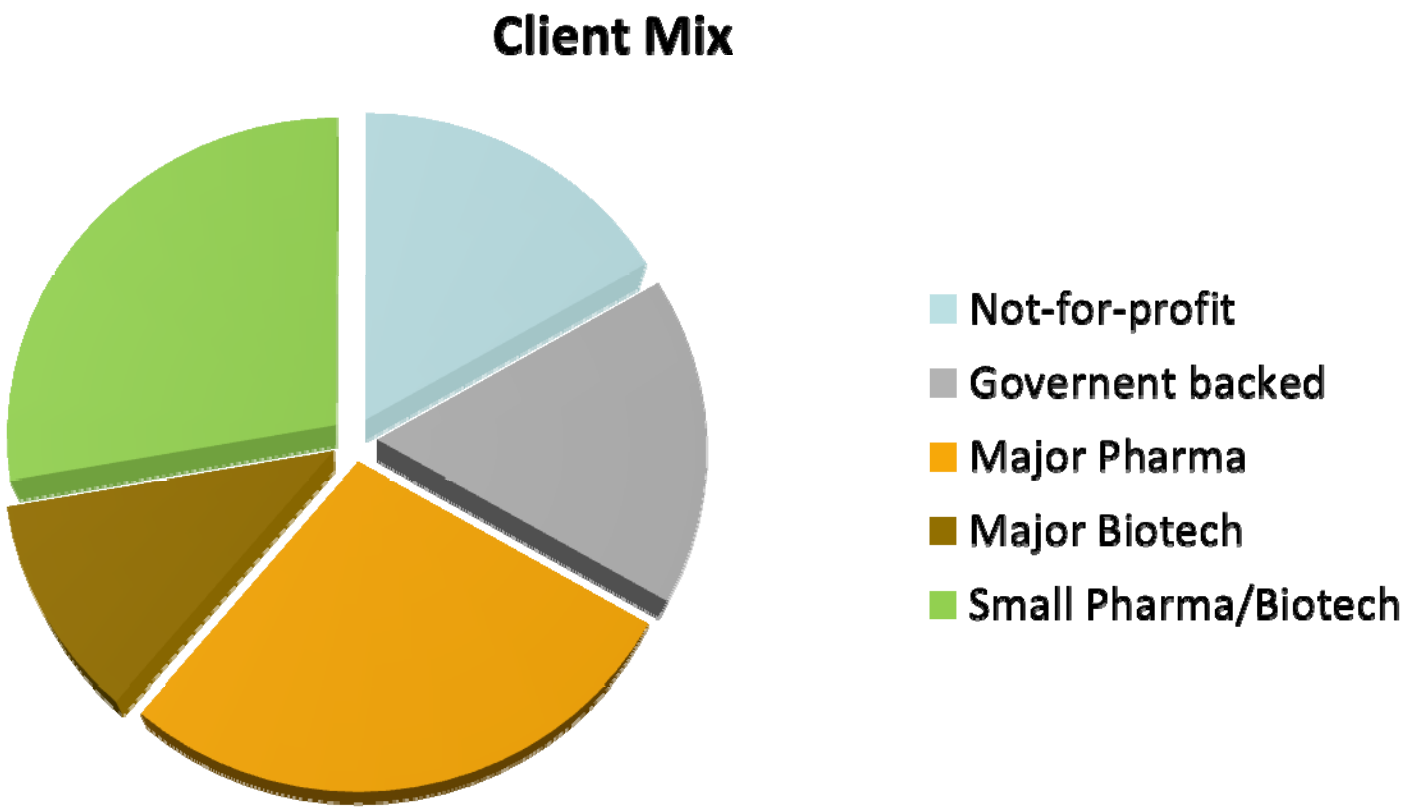
Our Clients



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Special Expertise

- Drug Delivery across variety of dosage forms
  - Micro / Nano-particulates
  - Solid solutions
  - Solvent systems
  - Drug/device combinations
- Analysis, characterization & cGMP production of particulate systems
- Analysis, characterization & cGMP production of combination (drug/device) products
- Highly potent compounds

- DEA licensed with full containment for high potency compounds

Industry leading

- cGLP/cGMP analytic and bioanalytic services
- cGLP/cGMP physical characterization
- Clinical trial manufacturing
  - Sterile and non-sterile products
- Formulation development
- Particle size reduction
- ICH compatible stability programs
- Handling of highly potent compounds

- Particle Sciences analyzed over 50,000
- samples in the past two years alone

Process Equipment

- Injection molders (lab and pilot scale)
- Hot Melt Extrusion: Compounder / Extruders / Pelletizer (lab and pilot scale)
- Class 100K, 10K, and 100 clean rooms
- Dedicated potent compound Class 100K clean room
- Laminar flow hoods
- Laboratory homogenizers, Admixer
- Microfluidics® high pressure homogenizers
- Microfluidics PureNano™ Continuous Crystallizer system
- Three ultrasonic dispersers / Homogenizers with in-line capability
- High energy media mills
- Two 1.5 liter jacketed double planetary mixer with vacuum
- Two mini-spray dryers with organic solvent capability

Extensively equipped to ensure process viability

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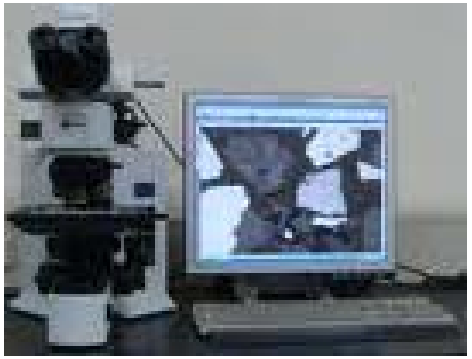
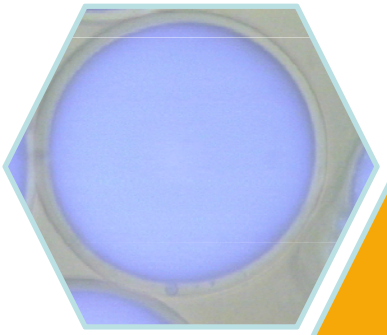
Analytical Tools



- Multiple HPLC, UPLC
- LC/MS Ion Trap
- Multiple LC/MS Triple Quads
- Dried blood spot analysis
- NMR, SEM, XRPD by third parties
- Raman imaging with particle size/morphology integration
- Percutaneous absorption (IVRT)
- Detectors include Ultraviolet, Photo Diode Array, Refractive Index, Evaporative Light Scattering
- FTIR
- DSC / TGA
- Microscopic image analysis
- Fluorophotometer
- Gel electrophoresis
- USP dissolution apparatus
- Karl Fischer – Volumetric and Coulometric



State-of-the-art separation and detection techniques

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Physical Chacterization

- Particle Sizing
  - Dynamic light scattering (DLS)
  - Fraunhofer laser diffraction (wet/dry)
  - X-ray and photo disc centrifuge sedimentometry
  - Optical counting (SPOS)
  - Image analysis
- Zeta Potential
  - Electrophoretic light scattering
  - Phase analysis light scattering
  - Streaming potential
- Rheology
  - Oscillating rheometer
  - Rotational viscometer
  - Tack & compressive force
- Raman with particle size/morphology analysis
- Advanced Instruments 3320 Osmometer
- Kruss Contact Angle/Surface Energy Measuring
- Turbiscan sedimentation-stability analyzer

Full validation of characterization techniques

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Intravaginal Dosage Forms

- **Intravaginal Rings (IVRs)**: drug-eluting polymeric rings
- Creams: semi-solid emulsions typically applied topically to skin
- Pessaries: vaginal suppositories
- Ovules : oval vaginal suppositories that are applied using an applicator
- Inserts: tablet vaginal suppositories that are applied using an applicator
- Strips: dissolvable filmstrips
- **GELS**: semi-solid, jelly-like materials which exhibit no flow on standing

Microbicidal Gels for Prevention of HIV

- Gels represent a viable approach
  - Allows women to protect themselves
  - Can be used without the knowledge of their partner
  - Aqueous-based vehicle that can contain one or more active pharmaceutical ingredients (API's)
    - Some API's are not water soluble and are present as particulate suspension in gel
    - Need to be able to measure particle size distribution of API at release and on stability

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Particle Sizing of Particulate API in Gels

- Two methods evaluated
  - Horiba LA-950V2 light scattering particle size analyzer using paste cell
    - Robust, fast
    - Ensemble technique – samples millions of particles
  - Light microscopy with Clemex image analysis
    - Gives data on particle size and morphology
    - Count technique – limited number of particles sampled (i.e., 10,000)
    - “Gold” standard
  - Both methods demonstrated to be suitable and validatable

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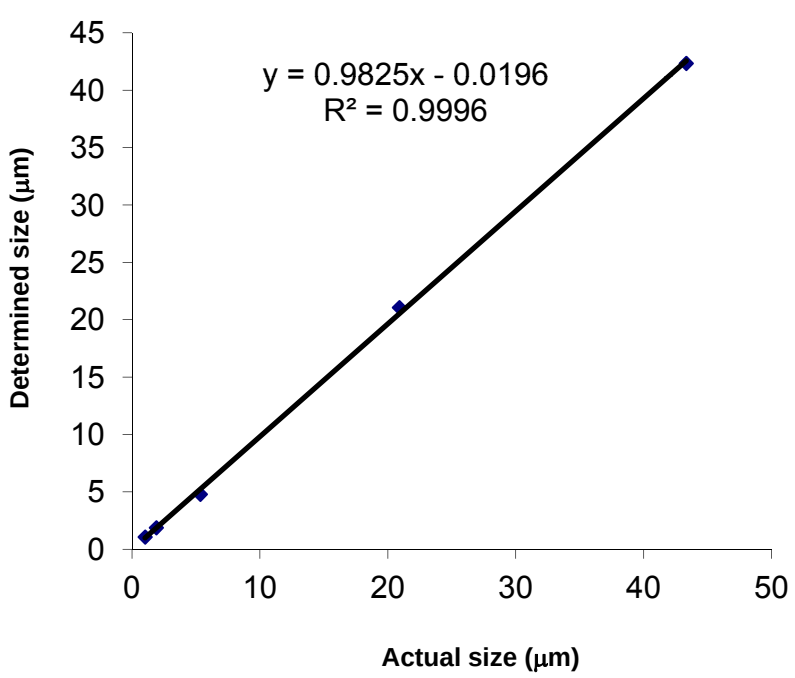
Horiba LA-950V2

- Method development and assessment using polystyrene standards
  - Method details
    - Performed using the paste cell
    - Polystyrene (PS) particles (1 – 40 μ) spiked into Placebo Gels at ~0.1% (v/v)
    - Sample measurement immediately following placebo blank. Sample held to the placebo blank thickness
    - Sample thickness generally between 100 and 250 μ with 90% Transmittance
    - $D_v(50)$ used in assessment
    - Bubbles, bubbles, bubbles!!!
  - 2 Gel Types: A – clear, B – translucent, hazy

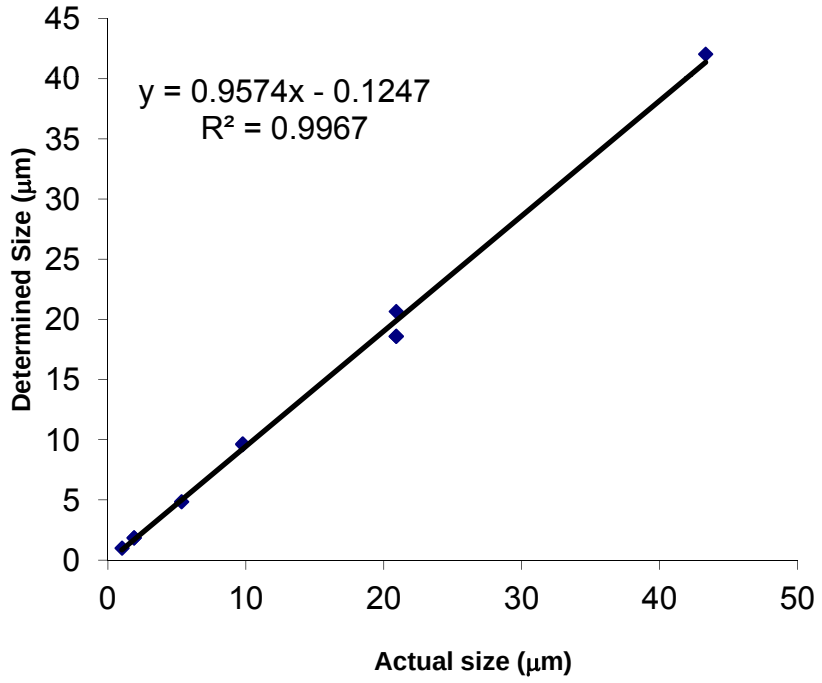
Horiba LA-950V2

- Good Linearity Demonstrated for Both Gel Types

Placebo A with PS Latex Particles



Placebo B with PS Latex Particles



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Horiba LA-950V2

Precision Study

Gel	Expected	Replicate	RSD
Gel A	5 μ	3	0.7%
Gel B	2 μ	4	0.4%
	20 μ	3	2.4%

Accuracy Study

Gel	Expected	% Accuracy
Gel A	1 μ	103.9
	2 μ	98.6
	5 μ	89.7
	20 μ	100.8
	40 μ	97.7
Gel B	1 μ	97.4
	2 μ	97.4
	5 μ	90.8
	10 μ	98.5
	20 μ	92.3
	40 μ	97.0

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Horiba LA-950V2

- Conclusions
- Particle size determination of aqueous gels using laser diffraction is possible
- Sample preparation critical – need to eliminate/minimize bubbles
- Linear response for a range of polystyrene standards demonstrated
- Excellent precision can be obtained
- Good to excellent accuracy can be obtained

Particle Size Distribution via Light Microscopy

- Particle sizing method development
  - Two API's in an aqueous gel formulation
    - API-1 soluble
    - API-2 insoluble
    - Developed to monitor particle size on stability
  - Clemex Image Analysis
    - 400X count routine using multilevel grab with combined grayscale and contrast thresholding
    - All objects less than 5 x 5 pixels rejected (~1.1 μm square)
    - Automated, 300 field pattern loaded and allowed to acquire 10,000 counted objects. (Overkill in the case of PS standards)
    - Sample thickness held to a 25 μ thickness
    - Assessed using $D_n(50)$ and $D_v(50)$

Particle Size Distribution via Light Microscopy

- Linearity (Average n = 3)

Standard Added	Number Mean	Number Median ($D_n(50)$)	Volume Mean	Volume Median ($D_v(50)$)
3 µ (3.005)	3.11 µ	3.10 µ	3.19 µ	3.11 µ
5 µ (4.987)	4.92 µ	5.06 µ	5.28 µ	5.07 µ
12 µ (12.01)	11.22 µ	11.96 µ	12.13 µ	11.98 µ
25 µ (25.09)	21.10 µ	24.93 µ	26.65 µ	25.07 µ

- Accuracy (Average n = 3)

Standard Added	Number Mean	Number Median ($D_n(50)$)	Volume Mean	Volume Median ($D_v(50)$)
3 µ (3.005)	103.4%	103.1%	106.3%	103.3%
5 µ (4.987)	98.7%	101.4%	105.8%	101.7%
12 µ (12.01)	93.4%	99.6%	101.0%	99.7%
25 µ (25.09)	84.1%	99.4%	106.2%	99.9%

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Particle Size Distribution via Light Microscopy

- Precision (RSD of n=3)

Standard Added	Number Mean	Number Median ($D_n(50)$)	Volume Mean	Volume Median ($D_v(50)$)
3 µ (3.005)	0.56%	0.59%	0.30%	0.61%
5 µ (4.987)	0.47%	0.86%	4.00%	0.93%
12 µ (12.01)	0.09%	0.19%	0.21%	0.93%
25 µ (25.09)	2.53%	0.20%	0.90%	0.22%

- Conclusions
  - Excellent Linearity (all r^2 > 0.9970)
  - Good precision – No relative standard deviation of greater than 4%
  - Good accuracy using either volume or number weighted medians
    - Both volume and number means exhibit greater deviation

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Particle Size Distribution via Light Microscopy

- Sample Determination

Gel Sample	Number Mean	Number Median ($D_n(50)$)	Volume Mean	Volume Median ($D_v(50)$)
Gel A	2.73 µ	2.65 µ	5.41 µ	3.29 µ
Gel B	7.54 µ	5.99 µ	20.87 µ	19.87 µ
Gel C	7.90 µ	9.14 µ	11.26 µ	11.06 µ
Gel D	4.59 µ	3.89 µ	9.24 µ	8.73 µ

- Sample Precision (n = 6)

Sample	Number Mean	Number Median ($D_n(50)$)	Volume Mean	Volume Median ($D_v(50)$)
Gel D	0.41%	0.66%	3.61%	1.23%

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Light Microscopy with Image Analysis and Laser Diffraction Comparison

- Spiked Whitehouse glass standards into placebo gel and measured using Clemex image analysis package and Horiba LA-950V2
  - Mixed Whitehouse Scientific PS 192 (polydisperse 1-10 μ) standard at @ 0.1% v/v (full 0.1 g vial) with Placebo A
  - Sample analyzed via Clemex image analysis
    - Particle size distribution (by number) imported into Excel
  - Sample analyzed via Horiba using paste cell
    - Particle size distribution (by number) imported into Excel (150 iterations)

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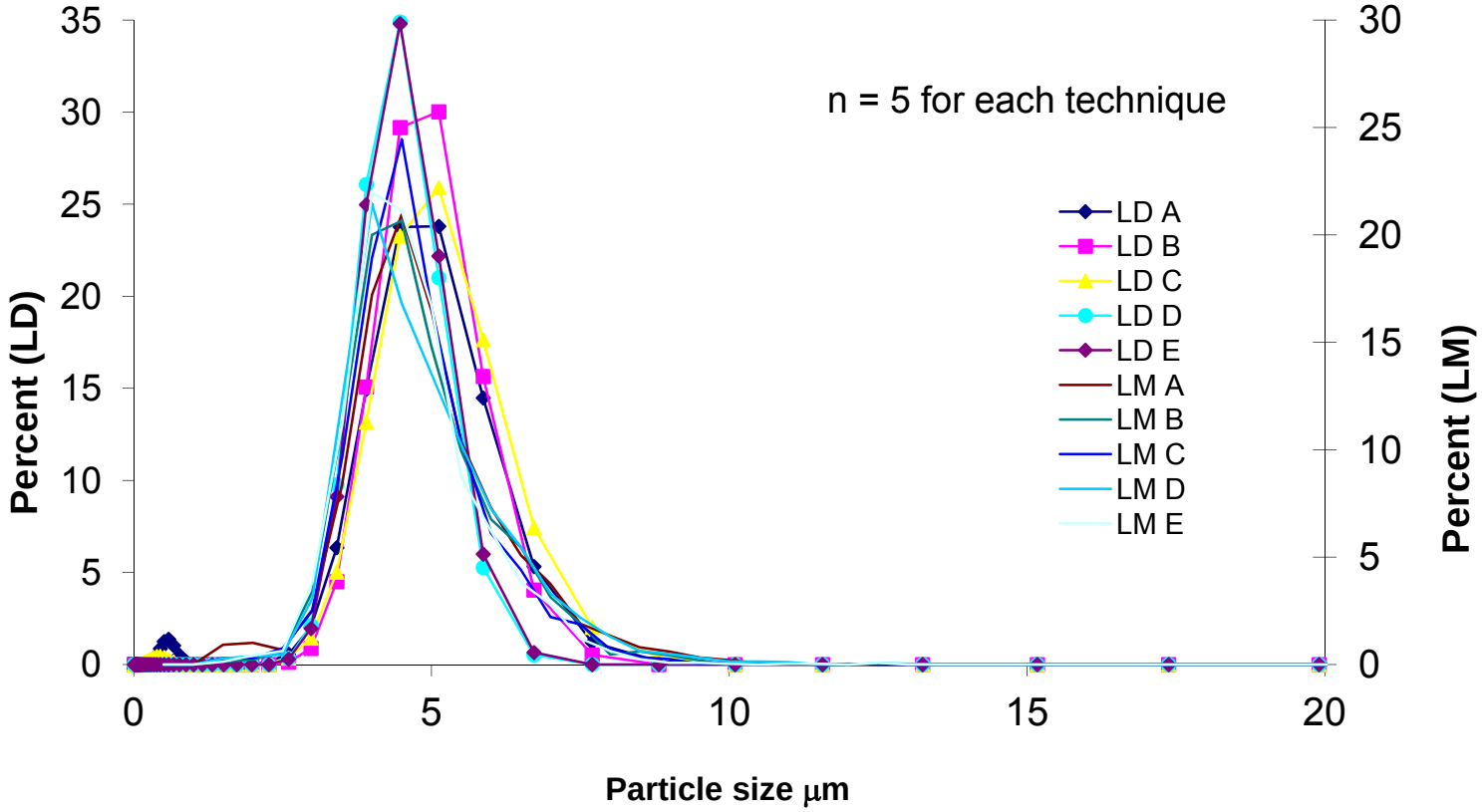
Light Microscopy with Image Analysis and Laser Diffraction Comparison

Number Distributions for the Two Methods



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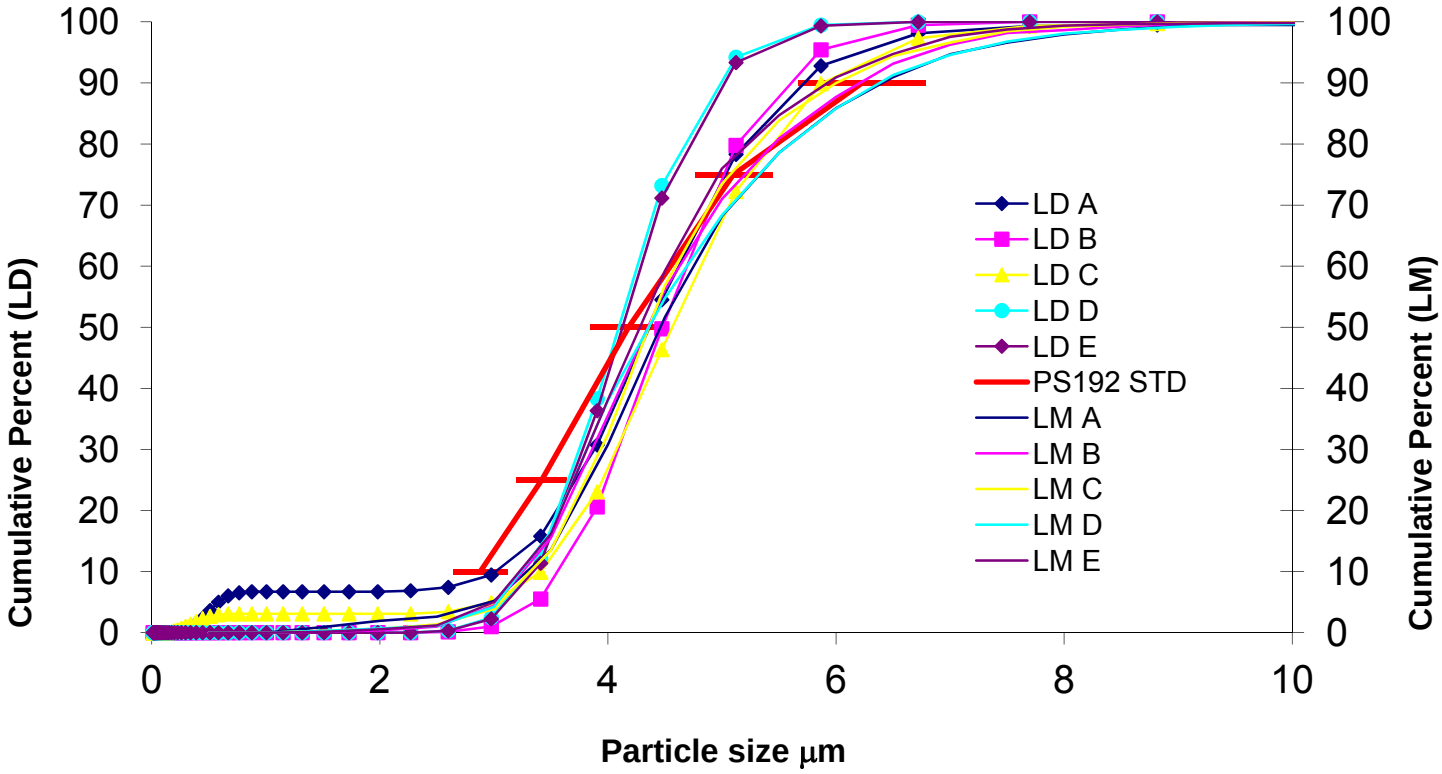
Light Microscopy with Image Analysis and Laser Diffraction Comparison

Cumulative Distributions Overlaid with Published Error in Distribution of Standard



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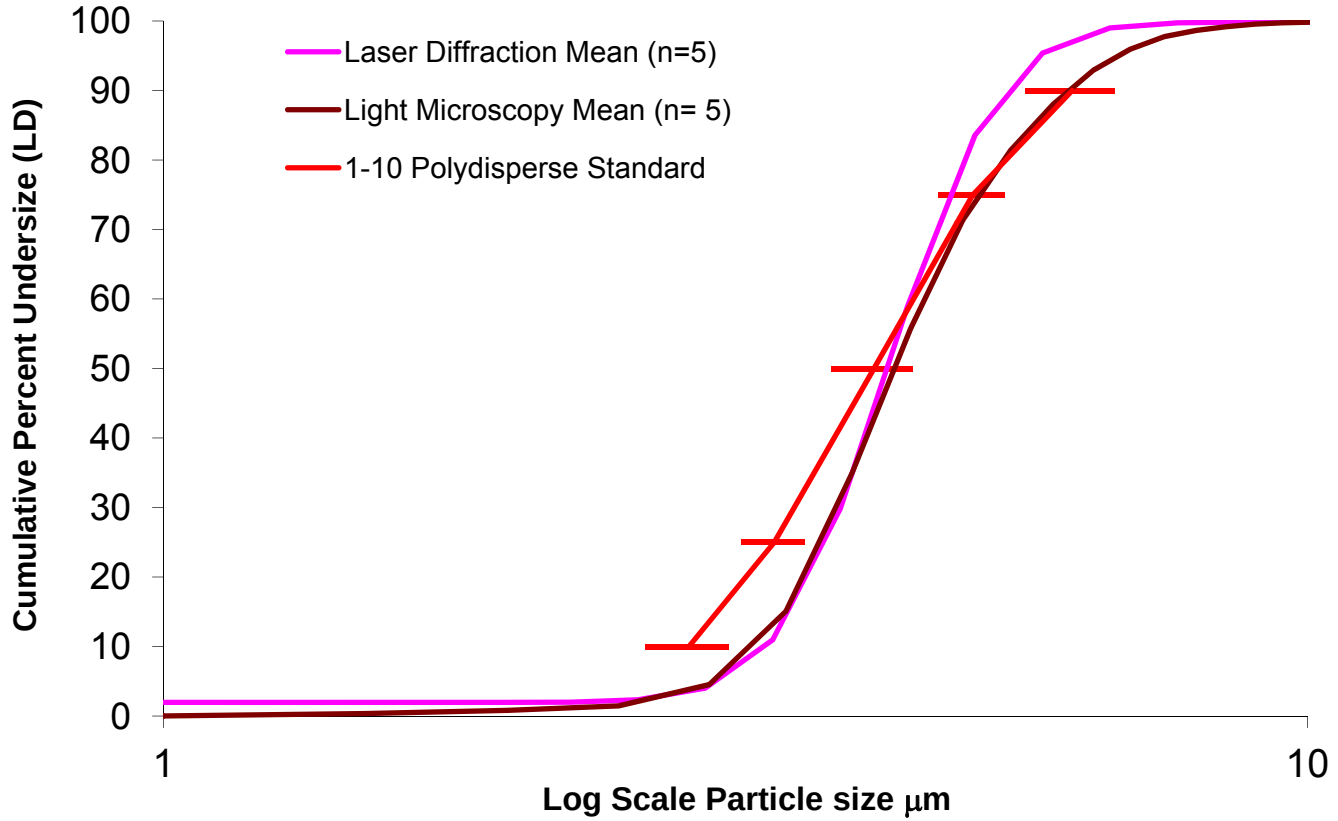
Light Microscopy with Image Analysis and Laser Diffraction Comparison

- Average Cumulative Distributions Overlaid



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Light Microscopy with Image Analysis and Laser Diffraction Comparison

That was nice for standards, but what about unknowns?

Gel Type	Light Microscopy ($D_v(50)$)	Horiba ($D_v(50)$)	Aspect Ratio
Gel A	3.3 μ	3.4 μ	1.5
Gel B	19.9 μ	49.9 μ	2.4
Gel C	11.1 μ	10.2 μ	1.7
Gel D	8.7 μ	5.3 μ	1.6

Determinations spread possibly determined by particle shape factors. Aspect ratio mildly indicative.

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Conclusions

- Both Horiba LA-950V2 and Light Microscopy with Clemex Image Analysis:
  - Are suitable for measuring particle size of particulates in gels
  - Give comparable particle size distributions – both volume-based and number-based
  - Are capable of being validated

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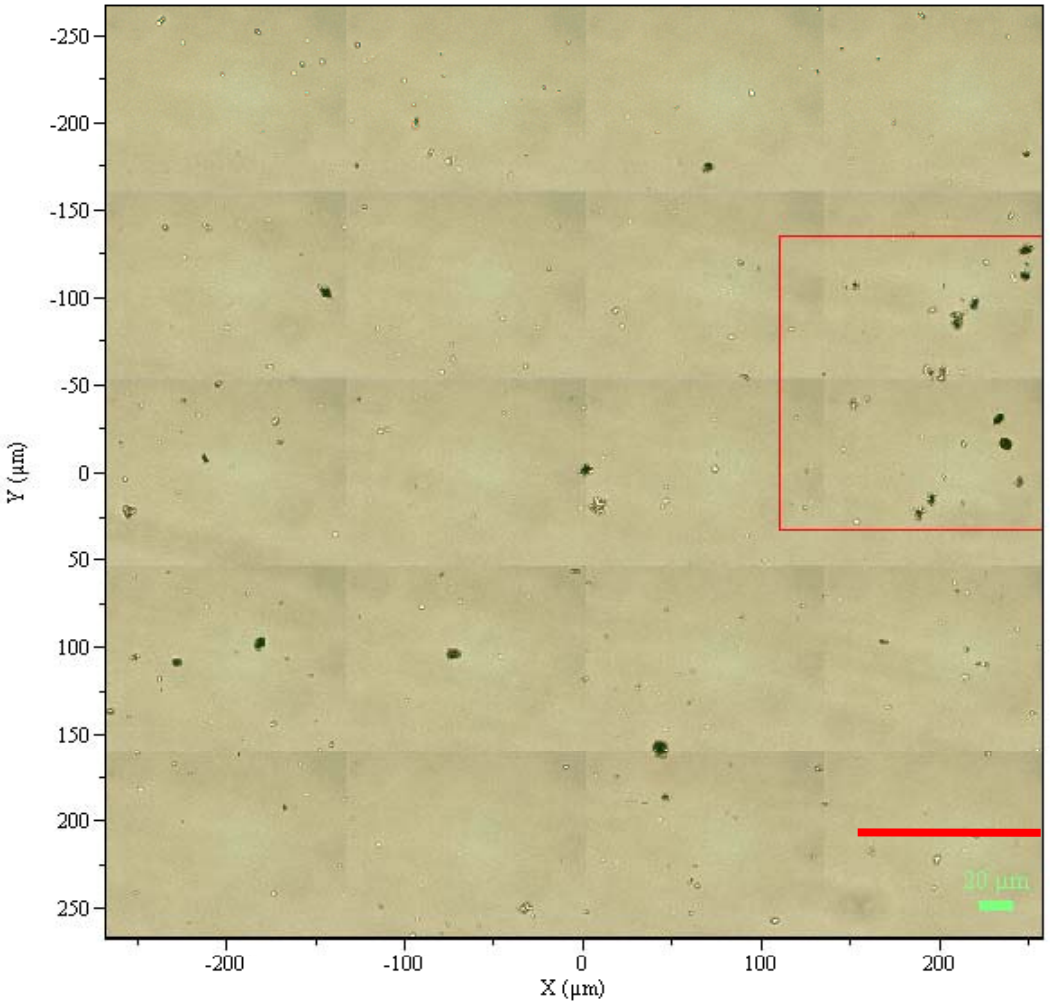
Raman Analysis – Gel



Step 1: Record a BF photomicrograph
Mosaic for a large area

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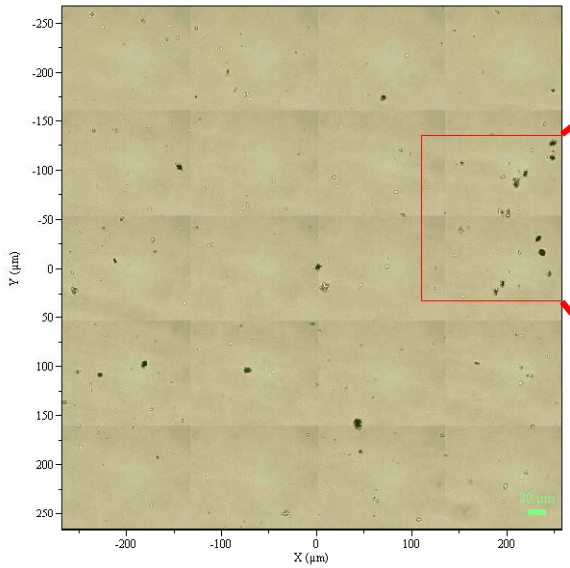
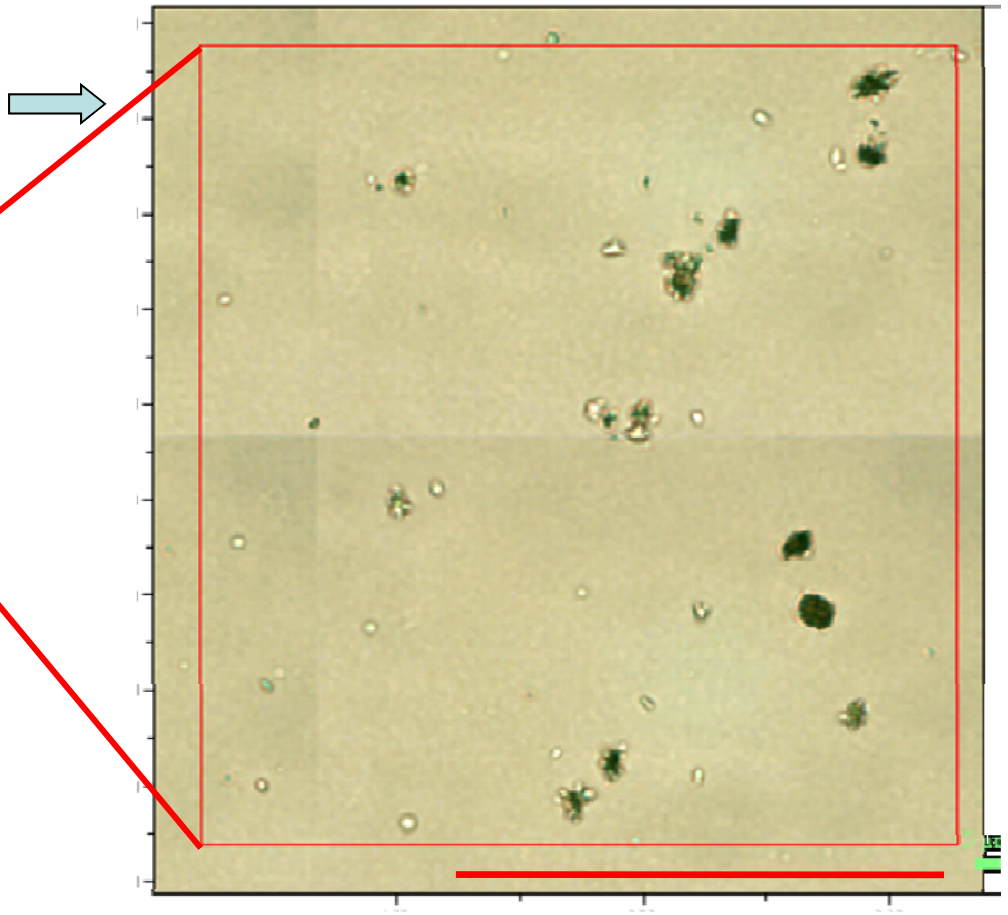
Raman Analysis – Gel

Step 2: Select a region of Interest (ROI)





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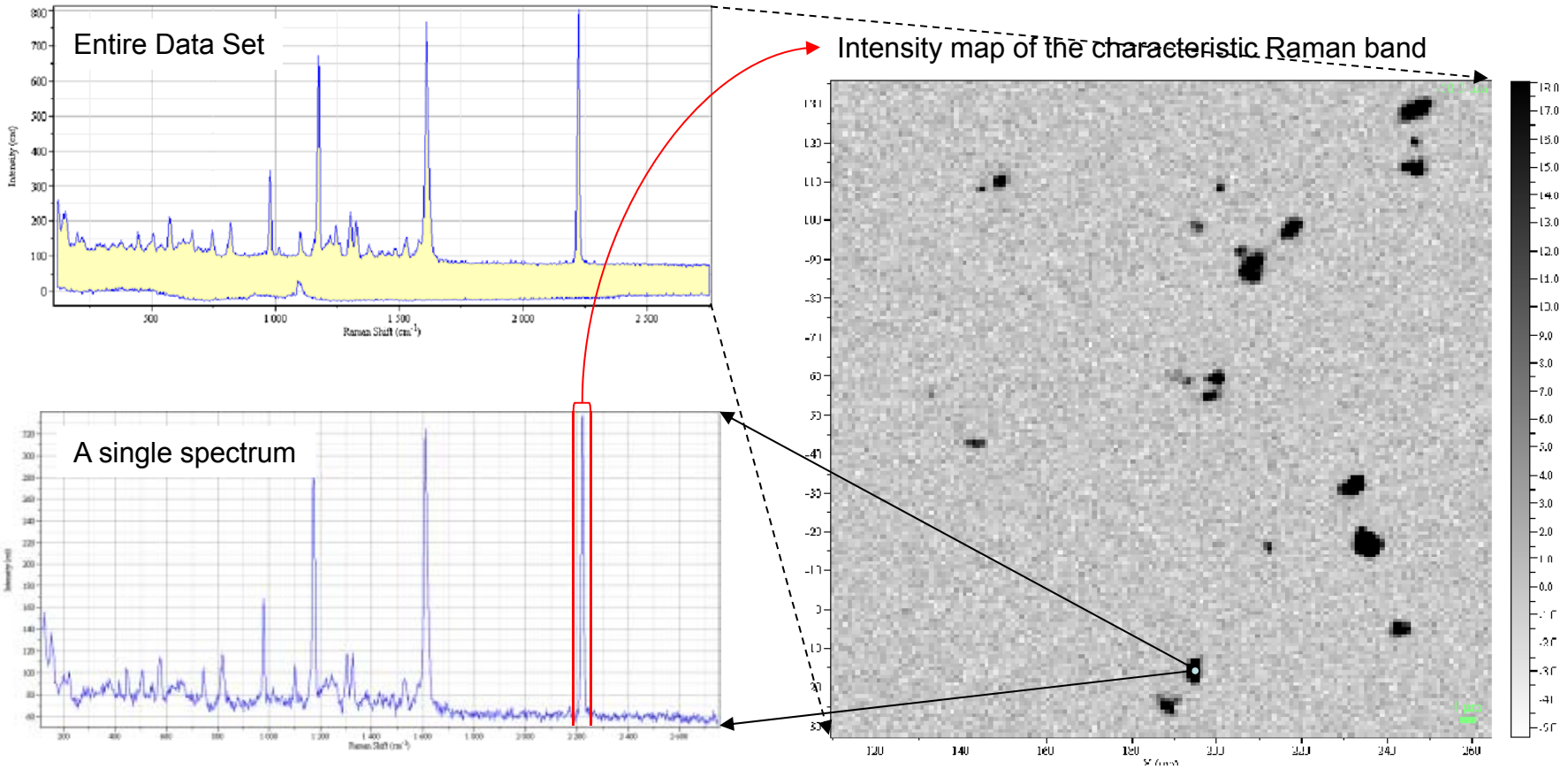
Raman Analysis - Gel

Step 3: Measure a Raman spectrum every 1 µm in a XY map
Step 4: Select the characteristic Raman band and map its intensity



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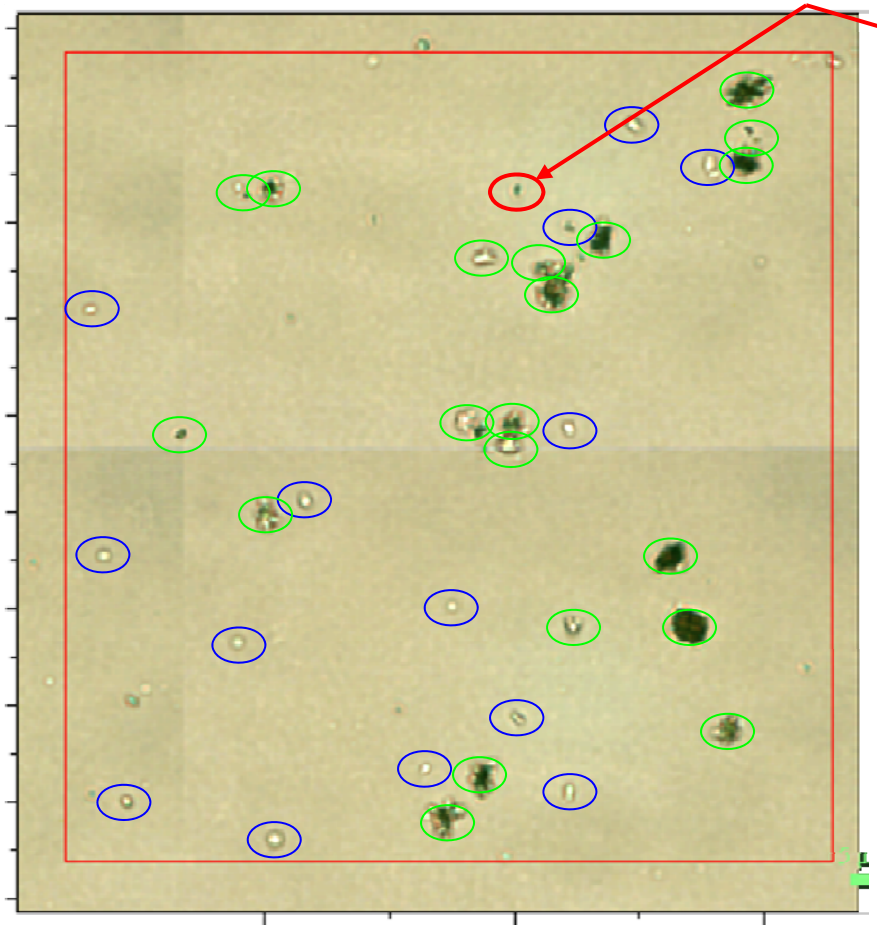
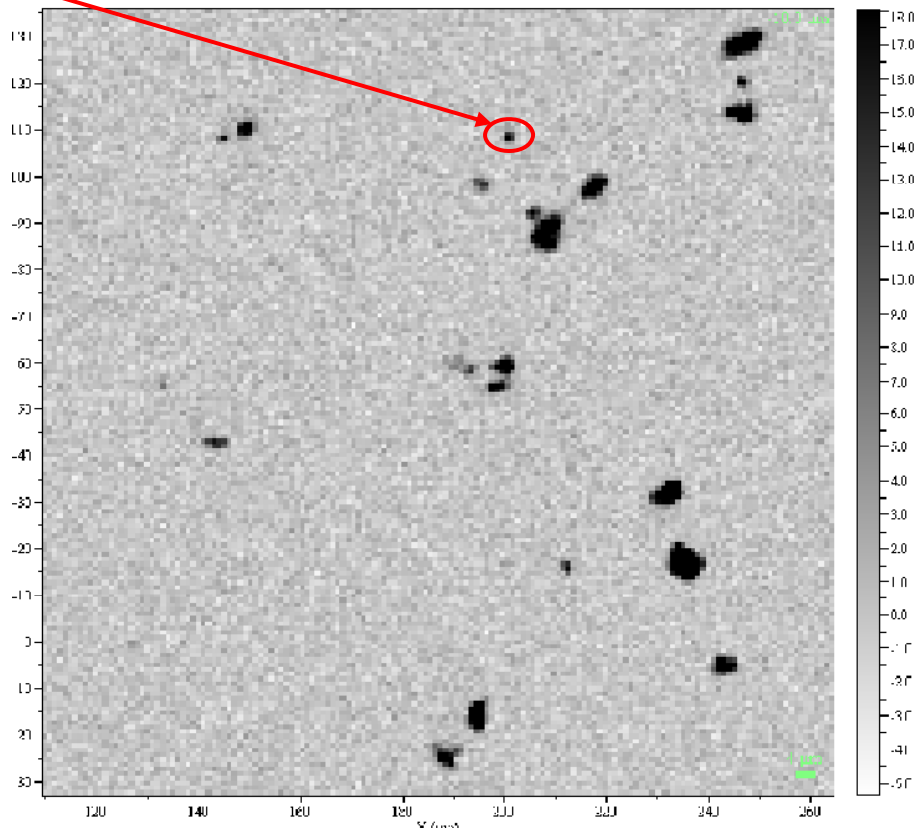
Raman Analysis – Gel Advantage

Objects selected by image analysis and confirmed by Raman as NOT API

Objects selected by particle analysis as API and confirmed by Raman as API

Object NOT selected by particle analysis as API but proved to be API by Raman.





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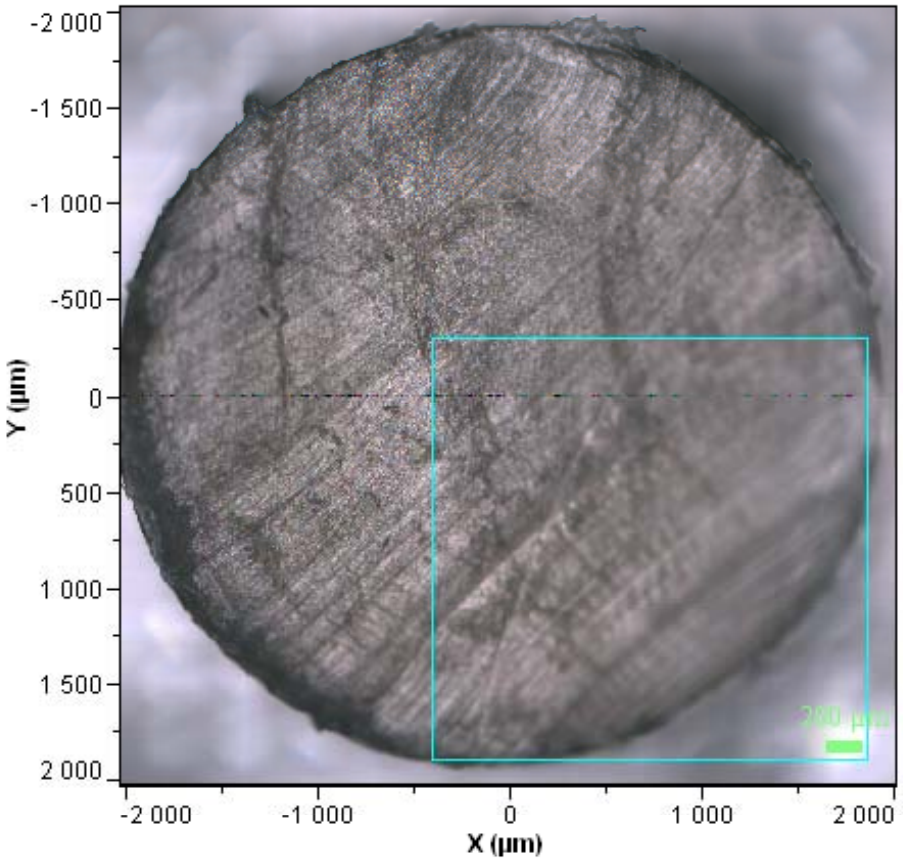
Raman Analysis – Intravaginal Ring



EVA IVR cross section

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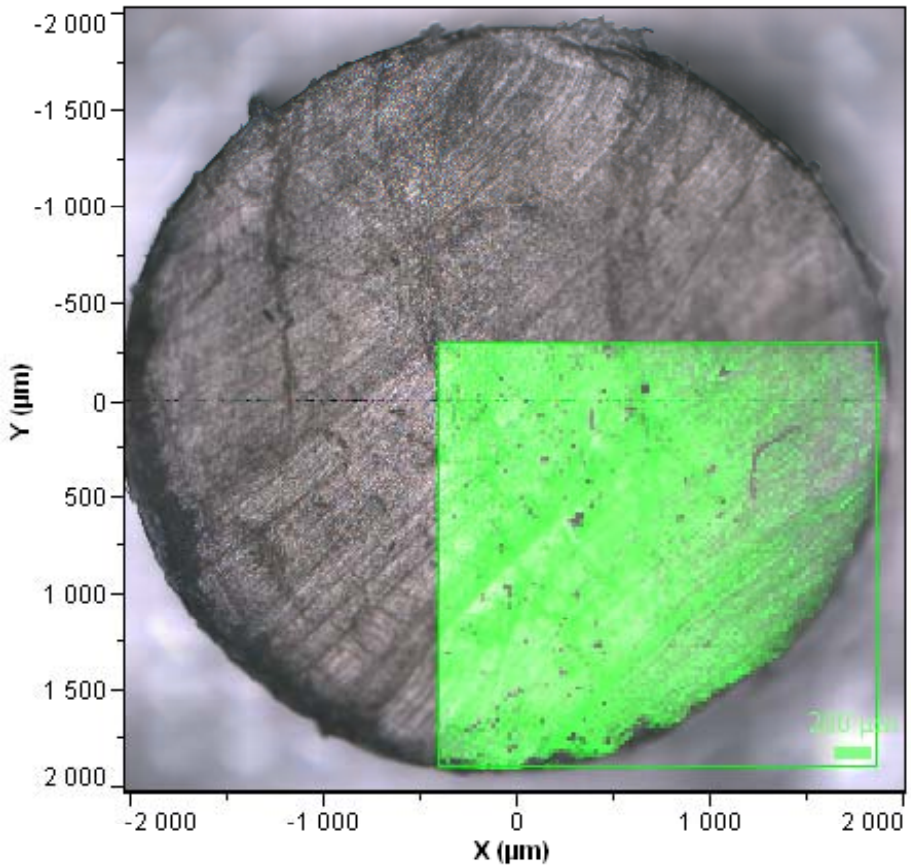
Raman Analysis – Intravaginal Ring



EVA IVR cross section
EVA Raman signal

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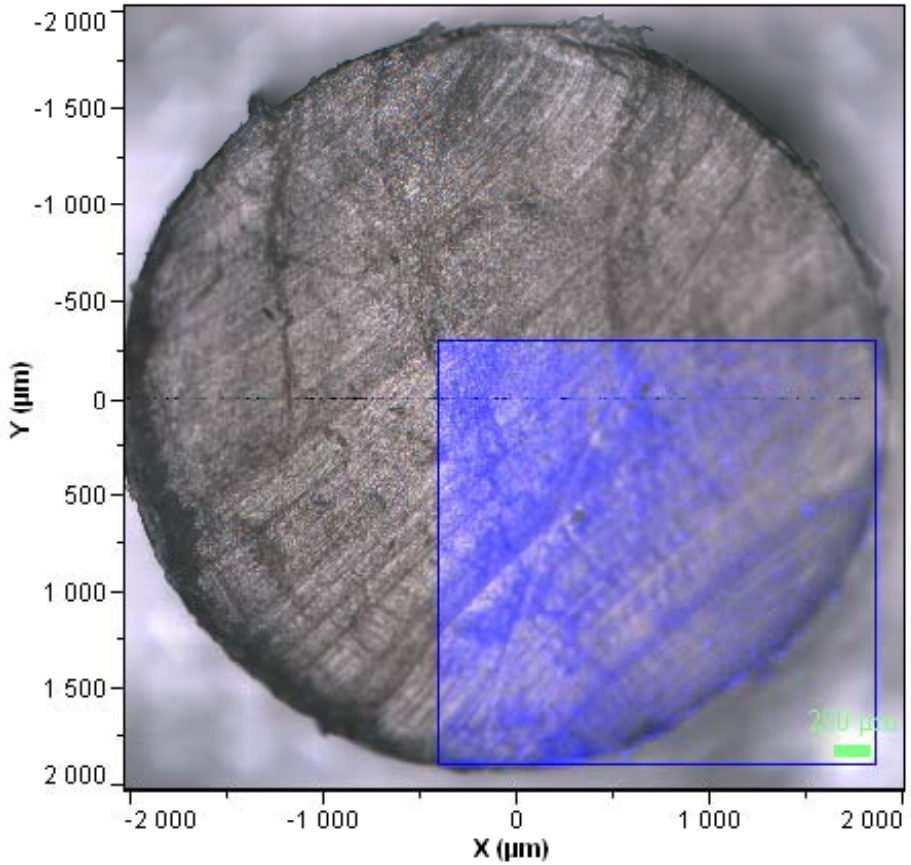
Raman Analysis – Intravaginal Ring



EVA IVR cross section
API Raman signal

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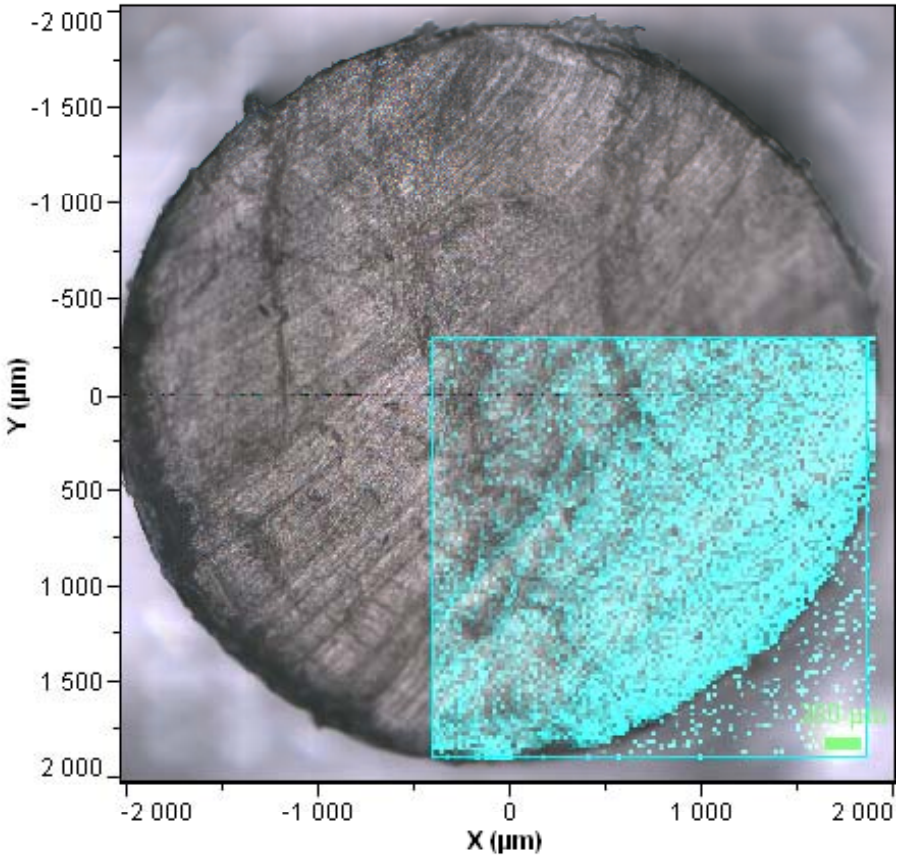
Raman Analysis – Intravaginal Ring



EVA IVR cross section
EVA / API signal ratio

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