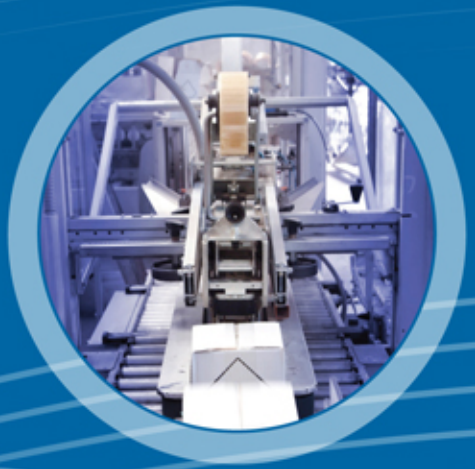
PDA
Parenteral Drug Association

Connecting People, Science and Regulation®

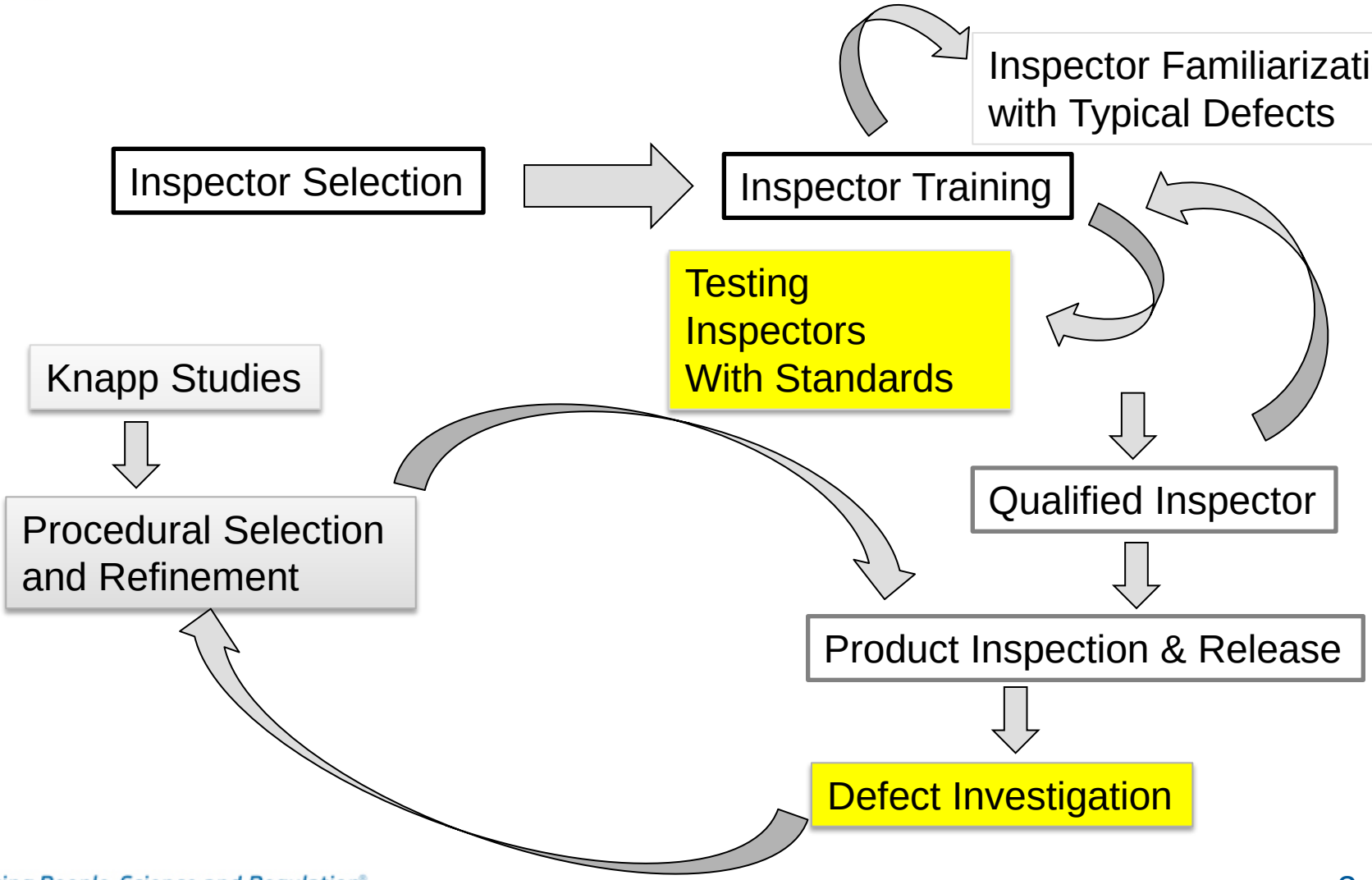
Microscopy Practices in Visible Particle Identification and USP Initiatives for Subvisibles



Outline

- Visual Inspection is a Program
- Microscopy Support is a Key Program Component of Inspection Practices
  - Verification of Standards
  - Verification of Rejects
  - Defect Charaterization
  - Sourcing
- The Microscopical Path is Essential for Full ID
- Identification Scemes
- USP Initiatives

Visual Inspection Program



Particle Lab as Nexus

Inspection Standards
- Generation
- Verification

Material Science
- Unknowns
- Excipient Evaluation
- Polymorphism
- Material Selection

Regulatory
- Responses
- Insert changes
- Registration Studies

Particle Lab Capabilities
- Inspection
- Microscopical Methods
  - Macro – Micro
  - Particle Manipulation
  - Microchemical Tests
  - Photography
  - PLM
  - Thermal
- Spectroscopy
- Particle Counting
- Elemental Analysis

QA Support
- AQL Rejects
- Complaints
- Recalls

R&D Support
- CCC studies
- Product Use Trials
  - Inserts
  - Labelling
- Alternate Methods

QC Release
788-1, 788-2

Production Support
- Process Capability
  - Component Prep
  - Consumables Integrity
  - Fixtures Wear
- Vendor Evaluation

The Microscopical Path

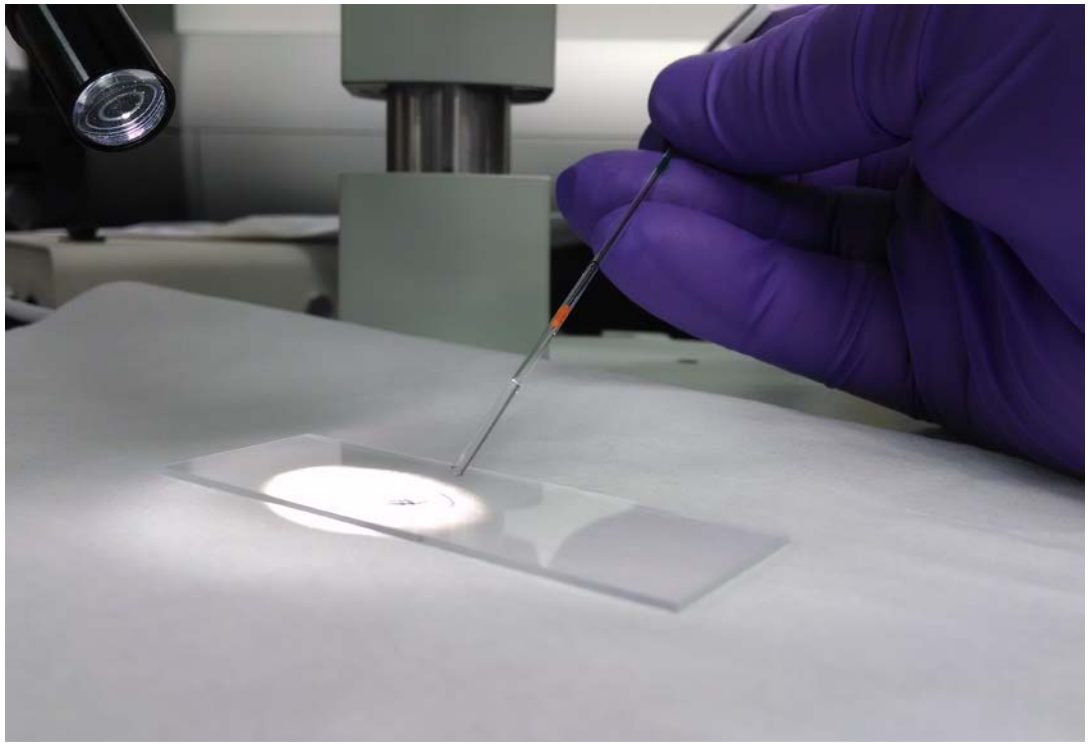
- Visual verification of reject
- Isolation of Particle(s)
- Microscopical Path
  - Begin simply
  - Observation without Destruction
  - Simple to complex analytical progression
- Light microscopical Evaluation
  - Visual-Low-High Progression
    - Stereomicroscopy
    - Polarized Light Microscopy
    - Electron Microscopy
    - Micro-Spectroscopy
- Determination of Nature
  - Association
  - Context
  - Particle Size, Number
  - Matrices, Layers
  - Size, shape, functional groups, solubility
  - Crystallinity
  - Immediate Recognition
- Electron Microscopy
  - Form, Association
  - Elemental analysis
- Microspectroscopy
  - *IR*
  - *Raman*
- Mass Spectrometry
  - EI, ToF-SIMS modes

Isolation

CAPILLARY WITHDRAWAL
FILTRATION

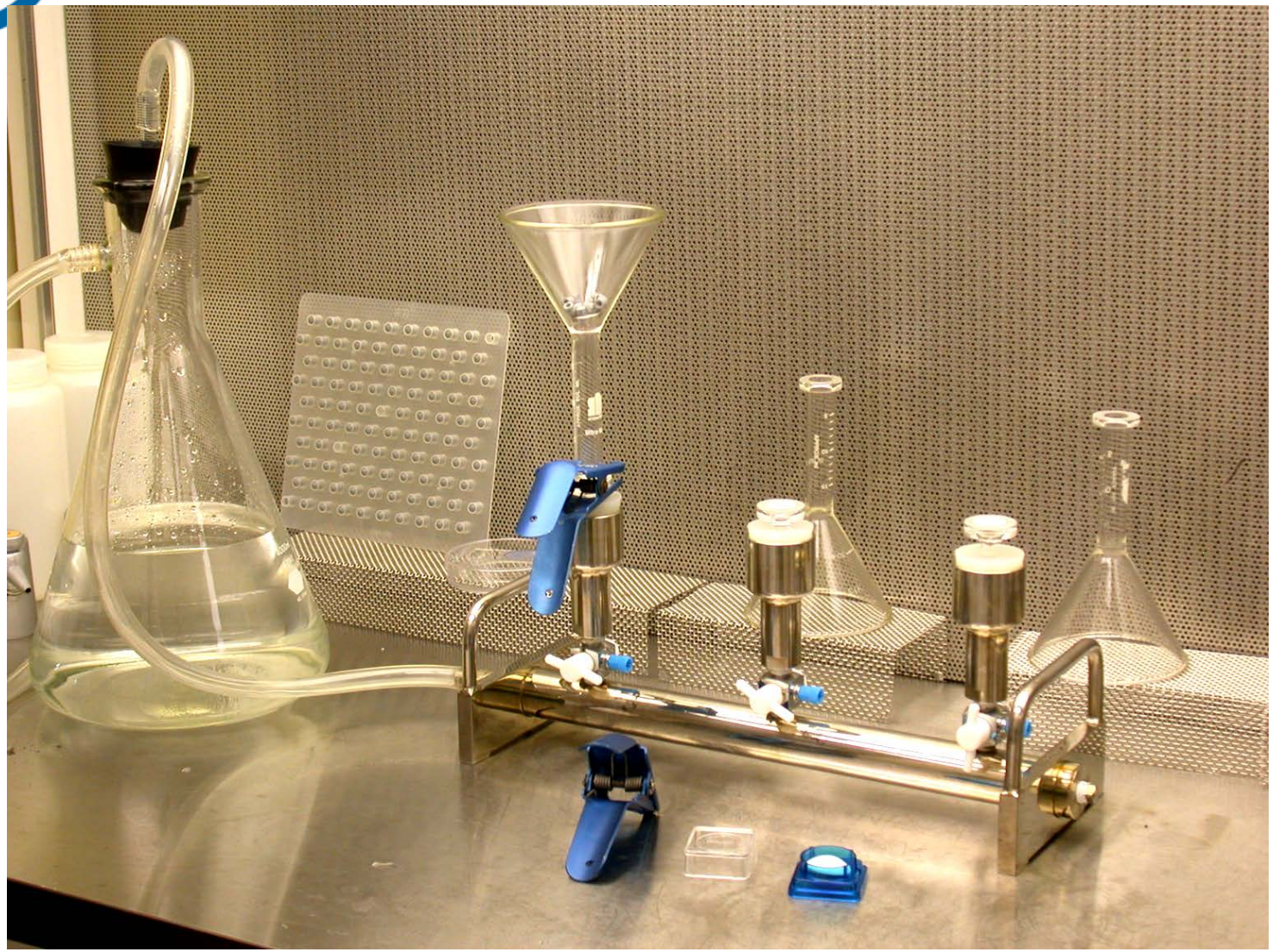
Withdrawal by Capillary

Observe - Remove



Isolation by Filtration

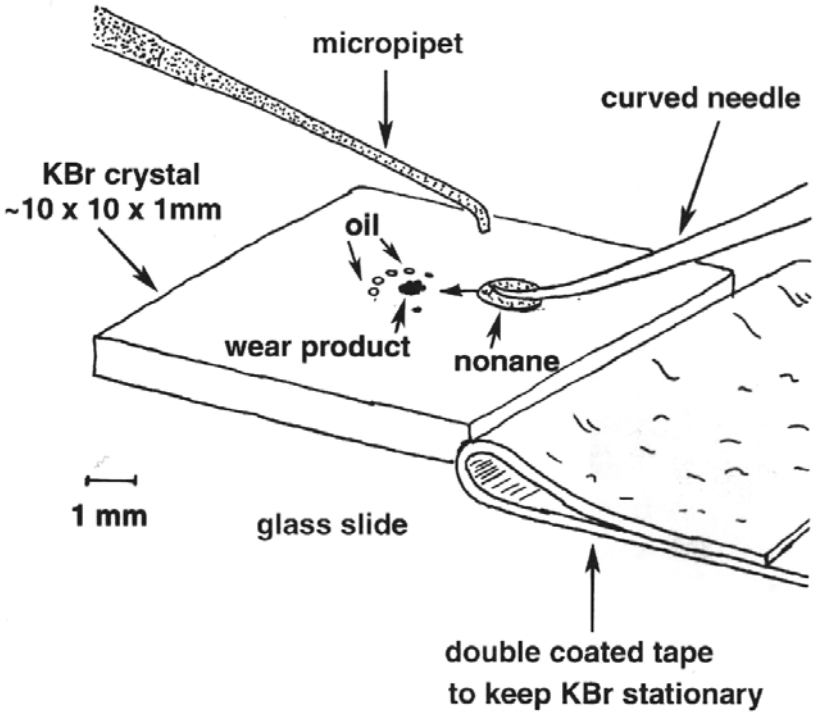
USP Membrane Microscopy Method 2



Selection of Observed Particles

Isolation and Manipulation Methods

- Direct removal, dry
  - Tungsten wire, 1-5µm tip
  - Cat's whisker
  - Fine scalpel, cleaver (MicroTool™)
  - Facilitate with water or adhesive
- Direct removal, wet
  - Capillary tube (Wiretrol)
  - Poly tube, drawn to fine tip
  - Swipe of a Membrane wedge
- Filtration
  - Membrane selection/prep
- Centrifugation
- Transfers, Concentration
  - Dried KBr
  - Cleaned filter paper
  - Capillary tube



Teetsov 1977

Microscopical Path

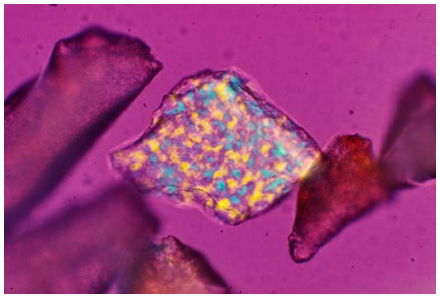
- First – Detection by Inspection
- Second – Verify, Isolate and Characterize
  - Isolate material causing rejection
    - Directly by capillary withdrawal
      - In inspection hood
      - On stereomicroscope stage
    - Membrane filtration
      - General PM isolation, rinsing
      - Locate target PM
  - Use Stereomicroscopy and Polarized Light Microscopy for Initial Observations
- Third - Characterization and Identification (?)
    - Size, Morphology, Physical character; reflectivity, opacity, color, softness, magneticity
    - PLM observation in wet mount
      - Birefringence
      - Extinction
      - Elongation
    - RI, Melt and Microchemical Tests

Microscopical Path

- Fourth - ***Identify***
  - Molecular character
    - Microspectroscopy
      - Mid-IR
      - Raman
      - UV-Vis
      - Mass spectrometry
  - Atomic character; Elemental composition
    - SEM-EDS
    - LIBS
    - TOF-SIMS

The Nature of Material

- Association
  - Singular
    - Liquid
    - Solid
    - Combinations
  - Multiple
    - Aggregate/Agglomerate
      - no distinct boundaries (matrix evident?)
      - boundaries?
      - with similar material, foreign material?
      - Groups of groups?
      - Homogeneous heterogeneity?
    - Polycrystalline
    - Microcrystalline
    - Cryptocrystalline
  - Layered
  - Coated



- Crystallinity
  - None Evident
    - Amorphous
      - Methods?
  - Evident -or- Continuum
    - “Liquid”: 2-D order
    - Solid: 3-D order
      - Isometric (1 ri)
      - Uniaxial (2 ri)
        - Tetragonal
        - Hexagonal (trigonal)
      - Biaxial (3 ri)
        - Orthorhombic
        - Monoclinic
        - Triclinic
  - Sub-optimal solid state

Particulate Matter Remediation

- Detection
- Isolation
- Characterization
- Identification
- Remediation/control
  - Investigation of Change
  - Process Improvements
  - Predictive Stability
    - Defined Stability Sets
      - To, T_1, T_2, T_3…..
    - Cyclical Analysis
      - Temperature
      - Light

Identification Schemes

- Fibers
  - RI, elongation, birefringence
- Particulate Matter
  - Color, reflectivity, transparency, RI....
- Metallic
  - Magneticity?
- Glassy
  - RI

Fiber Types

- Natural
  - Mineral – asbestiform
  - Biological
    - Plant
      - Seed hairs (cotton)
      - Bast (flax, hemp, ramie, straws, etc.)
      - Bark-Leaf (abaca, manila, palm, etc.)
      - Hull – Coir
      - Deciduous
      - Coniferous
      - Chitin (insect)
    - Animal
      - Hairs, Webs, Cocoons

Fiber Types

- Semi-Synthetic
  - Rayon, Viscose, Vinyon
  - Cellulose nitrate
  - Cellulose esters
    - Acetates, Ethylcellulose
  - Protein, Casein Soy
- Synthetic
  - Polyolefin
  - Polystyrene
  - Copolymers
  - Acrylic
  - Nylon
  - Orlon
  - Polyacrylate
  - Aramid
  - Teflon

Fiber Types

- Synthetic Inorganic
  - Glassy
    - Fiberglass
    - Rock Glass and Wool
    - Composites – HEPA media
  - Metallic
    - Wire
    - Steel wool
    - Tinsel

Identification Considerations

- Fibers
  - Length/Width
  - Cross-section
  - Scales? (Hairs) and shape
  - Biological? (Morphology)
  - Smooth?
  - Striations? Lobed?
  - Birefringence
  - Elongation
  - Principal R.I.’s

Fiber Tests

- Physical
  - R.I.
    - 1.52/1.55 Celluloses
    - 1.535/1.71 Polyester
    - 1.58/1.52 Nylon
    - 1.34/1.38 Teflon
    - 1.54/1.51 Hair
  - Sign of Elongation (R.I. orientation)
  - Birefringence
  - Melting Point
- Chemical
  - Solubility
  - Stains
    - Cellulose
      - Stahl Rg [No. 10] blue-violet
      - Lignin: Stahl Universal Rg [No. 21] yellow
    - Chitin: polymeric n-acetylglucosamine
      - boil in [KOH], rinse with 90% alcohol, add weak iodine = red-violet color

Thermal Behavior of Common Fibrous Materials

Fiber Type	Flame/residue	m.p.
Acetate	Burns with melting/brittle bead	230˚ C and Acel™ 260˚ C Arnel™288˚ C-302˚ C
Acrylic **Modacrylic**	Fuses away, burn-melt/ hard irregular black bead	Orlon™ = Indeterminate Dynel™ ~188˚ C Verel™ ~210˚ C
Aramid	Carbonizes above 800˚ F	Nonmelting
Modacrylic		210˚ C
Naturals: **Cotton** **Flax** **Silk** **hair**	 Does not fuse or shrink, burns Does not fuse or shrink, burns Fuses away, burn-melt/soft ash Fuses away, burns slowly, some melt/soft black ash	 Nonmelting Nonmelting Nonmelting Nonmelting
Nylon	Fuses, shrinks/hard gray bead	“6” 213˚ C “6-6” 250˚ C
Nytril		Darvan™ ~218˚ C
Olefin:	Fuses, shrinks/hard tan bead	Polyethylene 135˚ C Polypropylene 170˚ C
Polyester (polyethylene terphthalate)	Fuses, shrinks/hard black bead	Dacron™ 250˚ C Kodel™ 282˚ C Vycron™ 232˚ C
Rayon	No shrinkage/burns/no bead	Nonmelting
Saran	Fuses, shrinks/hard irregular black bead	168-177˚ C
Spandex: Lycra™, Vyrene™	Fuses, No shrinkage, burns-melts/soft ash	~230˚ C
Vinyon		140˚ C

Dupont and McCrone

Stains for Common Materials

- ***Herzberg no. 1, 2, 3 or Wilson Stain*** for Processed Celluloses
- ***Universal Reagent*** for common yet complex biological compounds [Stahl]
  1. Prepare 5mL of a lactic acid solution saturated with Sudan Red III or Sudan Red G and dilute to 30mL with lactic acid.
  2. Add 0.55gm aniline sulfate to 35mL water
  3. Add 0.55gm KI and 0.05gm iodine to 5mL water, add 5mL 96% ethanol
  - Combine 1-2-3 and add 2.5mL 37% HCl with stirring. Do not filter.
  - Add Rg drops, heat specimen:
    - Yellow = lignin
    - Red = lipids
    - Red-brown = cork
    - Blue-violet = starch

Herzberg Reagent varieties

No. 1: 100mL water + 22g KI + 2g crystalline I_2
No. 2: 100mL water + 6g KI + 1.5g crystalline I_2
No. 3:
solution A: 20g $ZnCl_2$ in 10mL water
solution B: 2.1g KI + 0.1g I_2 in 5mL water

Combine A + B, let stand 24 hr (to clearness) decant to amber airtight bottle, add a leaf of I_2.

Typical Herzberg color reactions:

Cellulose Type	Color
Linen (flax), cotton, hemp	Light to dark claret/purple
Esparto	Blue to claret/purple
Straw	Blue to violet
Chemical wood pulps	Blue
Manila	Olive green
Groundwood, Unbleached jute or straw	Yellow to colorless

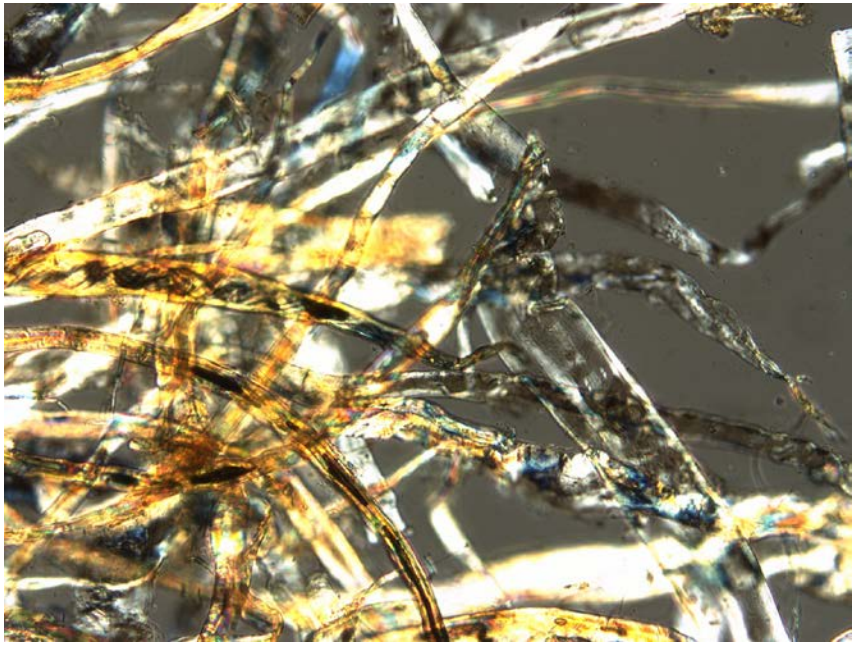
Wilson Fiber stain

180mL water + 15mL 37% formaldehyde, add 140g $Ca(NO_3)_2 – 4H_2$ 40g $CaCl_2 – 2.5\ H_2O$. Mix together, store in an amber airtight bottle

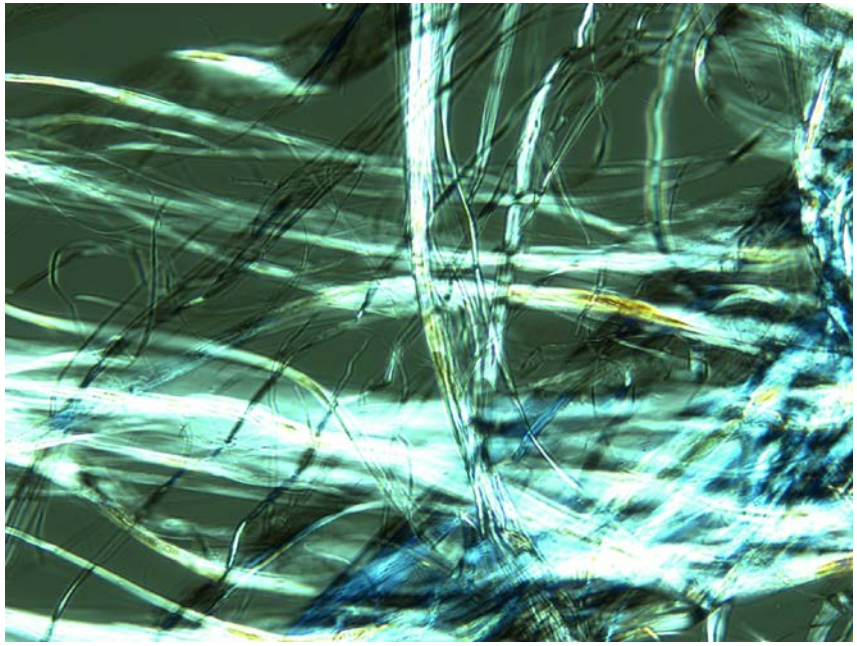
Typical Wilson color reactions:

Paper Process or Cellulose Type	Color
Soda	Dark claret/purple
Groundwood	Bright yellow
Bleached sulfite	Pale grey lavender
Sulfite	Colorless
Raw Kraft	Brown
Well-cooked Kraft	Grey
Bleached Kraft	Pale Blue
Raw straw	Green
Well-cooked straw	Blue
Cotton	Red
Linen (flax)	Pink

Extrinsic or Intrinsic?

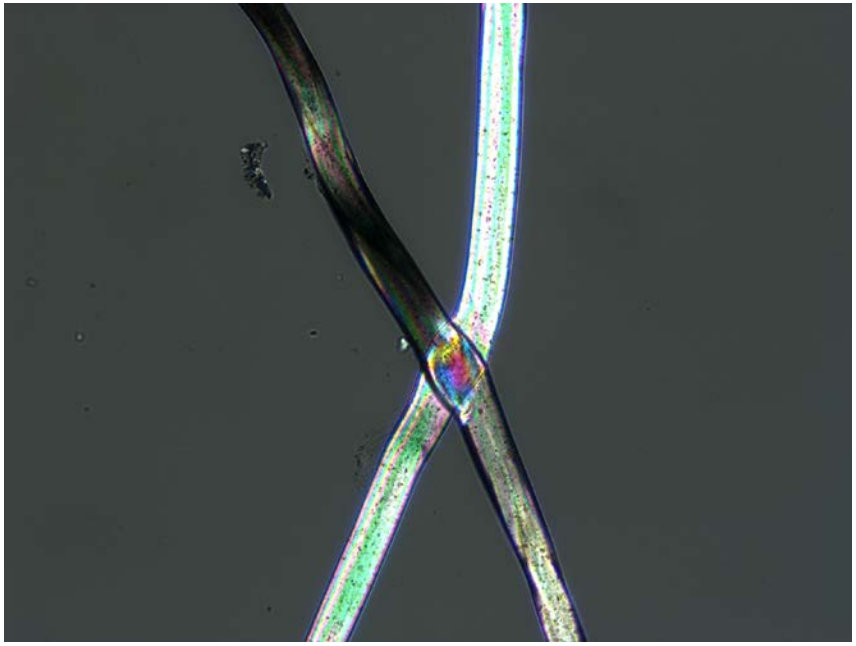


Cardboard

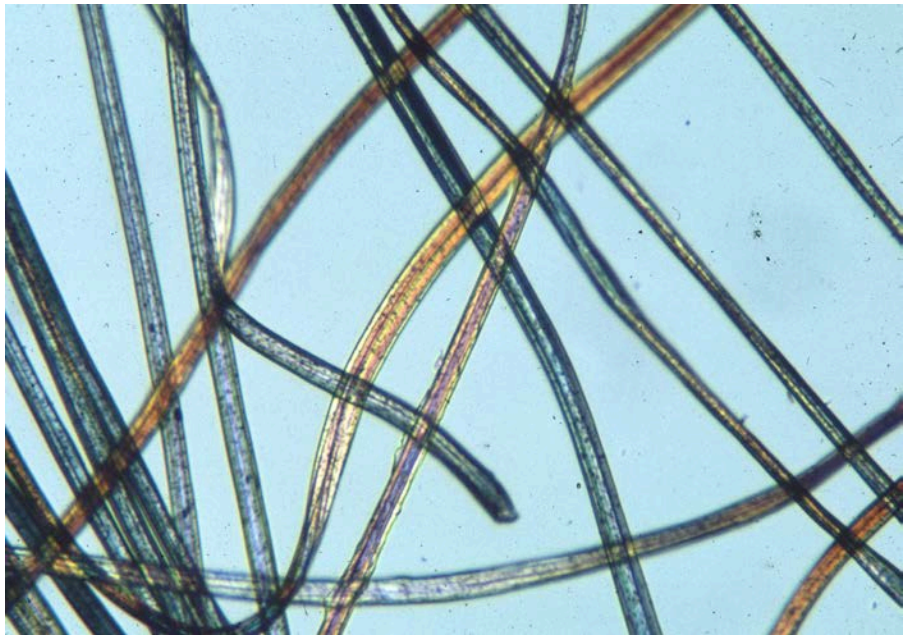


Tyvek

Synthetic Fibers

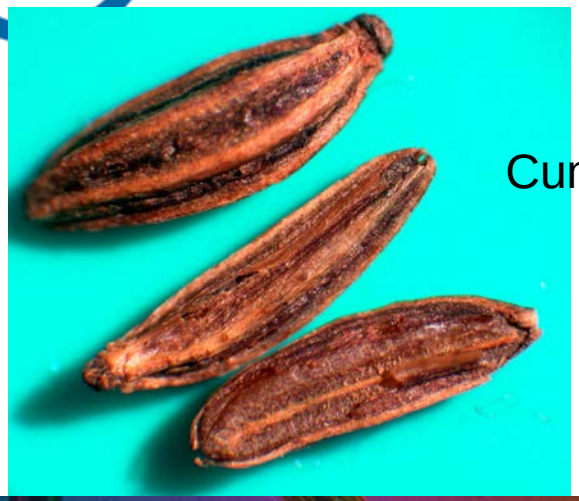


Polyester



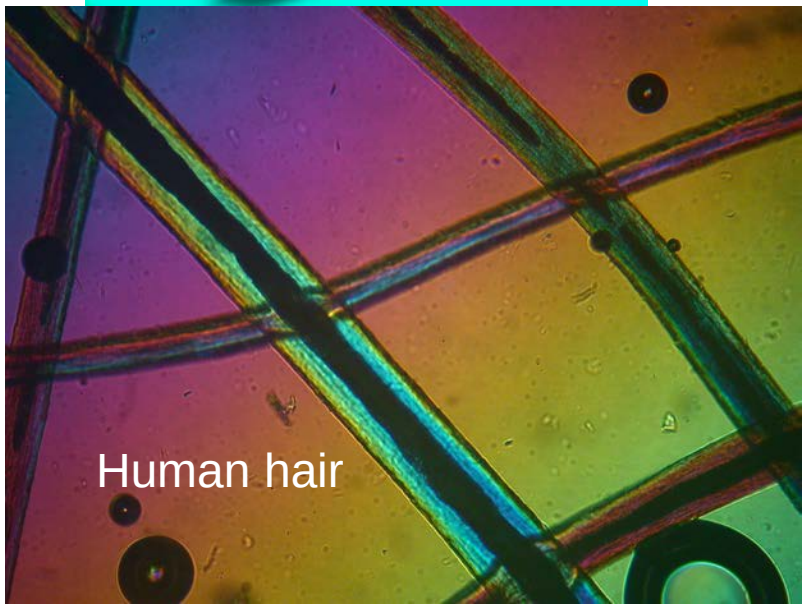
Rayon

Biologicals

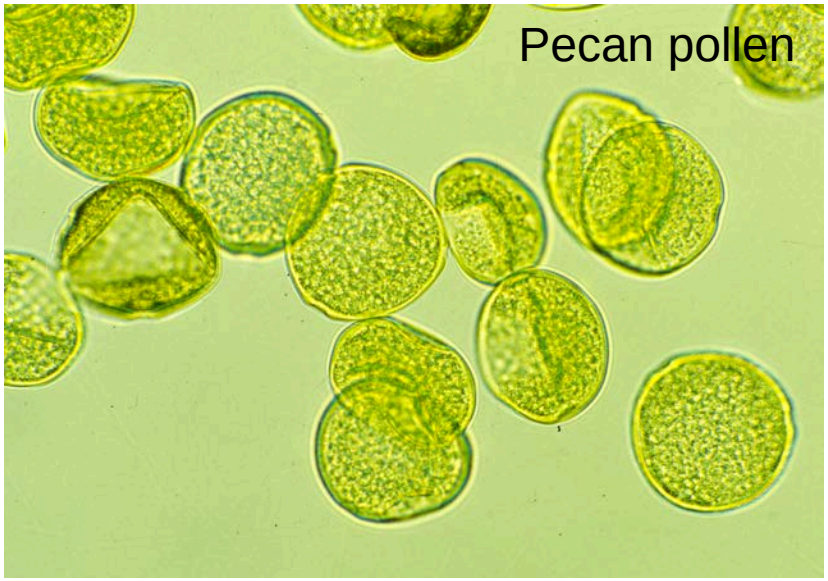


Cumin seed

Poppy seed



Human hair



Pecan pollen

Identification

- Metal
  - Size
  - Reflectivity
  - Magneticity
  - Microchemistry
  - Elemental Composition
    - SEM-EDX
    - LIBS
- Glass
  - Size
  - Color
  - R.I.
  - Dispersion of R.I.
    - Dispersion Staining
  - Elemental Composition
    - SEM-EDX
    - LIBS

Part II

USP INITIATIVES

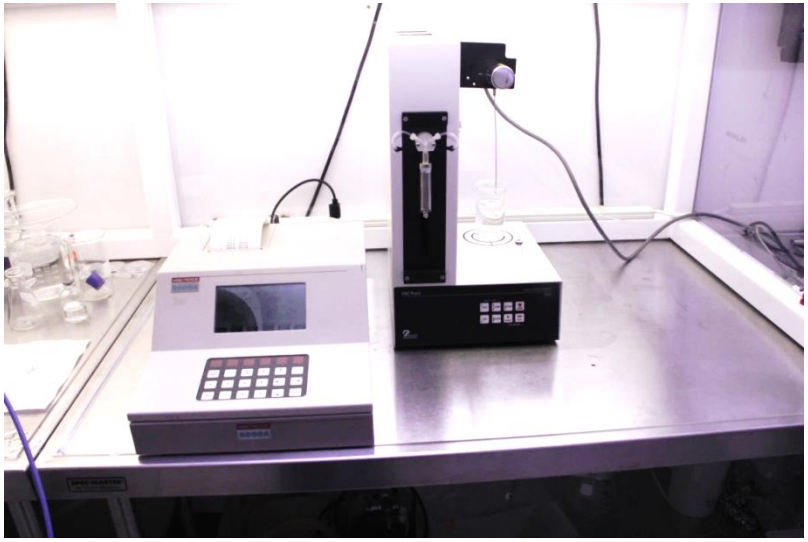
USP Guidance

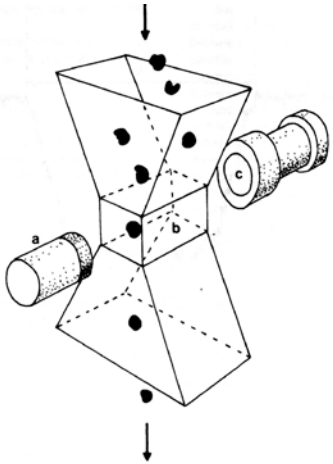
- Historical - Particles as contamination
- Current – particle categories
  - Extrinsic, external, contamination
  - Intrinsic
    - Internal, Process, Product-contact
      - Rubber, metal, polymers, consumables, container debris
      - Instability, growth, change
  - Inherent is product character

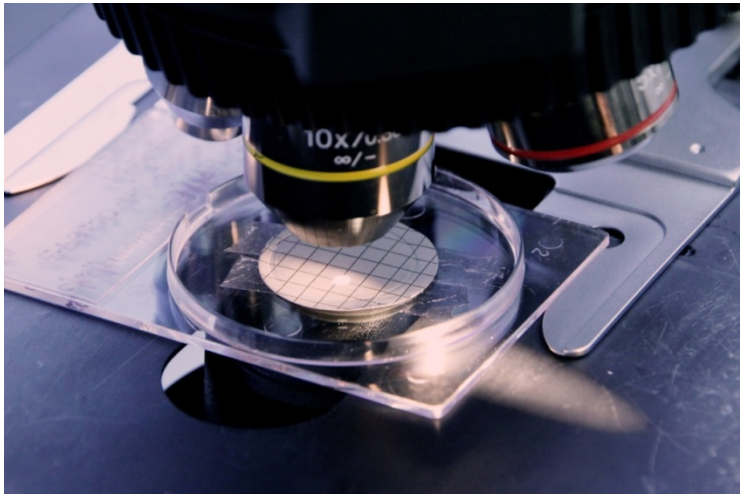
Particulate Matter Categories

Extrinsic	Intrinsic	Inherent
Wild, Outside the System	Inside the System	**Is** the System: Solution Micelles Emulsion Colloid Protein Assembly
Extremes are “Filth”	Product-contact	n/a
Microbial Vector	May have Microbial Content	Formulation-Relevant
Uncontrolled	Unplanned	Controlled, Expected
Additive	Additive or Changing	Stable Same TOR as EOS
Single to Many Particles	Various Physical States	Defined active ingredient
May be Considered Most Objectionable	Needs Planning & Control	Most Acceptable, Known

Historical Subvisible Particle Counting







USP Chapters Update

- Traditional <788> is Harmonized with Ph.Eur. and JP through the Pharmacopeal Discussion Group
  - Exploring addition of Flow Imaging Technique
- New <787> – improved <788> for protein formulae
- New <771> – Ophthalmic Products
- Particle Characterization
  - New <1787> – Array of Particle Characterization Techniques for 2-100µm Particles
  - Current <776> Optical Microscopy
  - Current IR <197>
  - Current Raman <1120>
  - Updated Scanning Electron Microscopy <1181>

More Detail…

- 787
  - De-aeration by vacuum
  - ≥10µm and ≥25µm limits
  - Individual containers, all volumes, allowed
  - No limits for sub-10µm
  - Total load at 6000/600 – 3000/300 and 25/3 – 12/2 for all volumes
- 1787
  - Particle Source definitions
  - Silicone oil discussion
  - Discussion regarding the sub-10µm population
  - Technique Sections
    - Size and Distribution
    - Size and Morphology
    - Characterization

USP 1787

- Particle Size & Distribution
  - Light Obscuration
  - Electrozone-Coulter
  - Laser Diffraction
- Particle Size & Morphology
  - Light Microscopy
  - Flow Imaging
  - Electron Microscopy
- Characterizion
  - Microspectroscopy
    - IR
    - Raman
  - EM-EDX
  - X-ray EELS
  - ToF-SIMS
  - Staining

Subvisible Particulate Matter Techniques

- **Compendial Methods USP/EP/JP <788> <789> <787>**
  - *Light Obscuration (LO)*
  - *Membrane Microscopy (MM)*
- **Alternate methods <1787>**
  - Electrozone (*Coulter)*
  - *Microscopy*
    - *Optical*
      - *IR*
      - *Raman*
    - *Electron*
    - *Image Analysis*
  - *Laser diffraction*
  - *Nephelometry*
  - *Flow Imaging*
  - *Photon Correlation Spectroscopy*

Review

- Compendial guidance provides the minimum benchmarks for visible and subvisible particle content
- Visual inspection is based upon Human Manual
- However, inspection is not just manual, semiautomatic or automatic processing – it consists of a comprehensive ***system*** of detection
- Subvisible determination methods are part of a ***system*** of particle content monitoring and investigation

Thank You

QUESTIONS?

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