Spectroscopy Systems for Biodetection

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The Future of Biodetection Systems, Santa Fe, NM, September 26-27, 2006

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Presentation overview

- Overview of Spectroscopy Methods
- Chemical Markers
- Physicochemical Markers
- Recent Advances
- Example: Uv-vis Spectroscopy
- Vision for the Future

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Applications of Spectrometer Systems for Biodetection

- **Detection of Chemical Markers**
  - **Invasive (molecular)**
  - **Non-invasive (molecular-phenotype)**
- **Physicochemical Markers**
  - **Invasive (labelling)**
  - **Non-invasive (phenotype)**
- **Culture Methods (amplification)**
- **Non-Culture Methods (direct)**
- **Laboratory Applications**
- **Field (in-situ) Applications**

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Detection of Chemical Markers

- **GC-MS**
  - **Carbohydrates**
  - **Polysaccharides, Lipolysacharides**
  - **Fatty Acids**
- **FT-IR**
  - **Total chemical and biochemical composition**
- **UV Resonance Raman Spectroscopy**
  - **Aminoacids, proteins, nucleic acids, pigments**
- **Multidimensional Fluorescence**
  - **Natural Fluorescence**
  - **Fluorescence Labelling**
- **Fluorescence Lifetimes**
- **UV-Vis Spectroscopy**

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Detection of Chemical Markers

- **Advantages:**
  - **High Sensitivity and Specificity to the identified markers (Analytical Chemistry)**
  - **Trace Analysis Possible**
  - **Profiling of : carbohydrates, sugars, fatty acids, etc.**
- **Issues:**
  - **Marker Identification**
  - **Sampling (cultures, environmental, clinical)**
  - **Sample preparation (derivatization, amplification)**
  - **Interferences: require fractionation GC, HPLC**

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Detection of Physicochemical Markers

- **Angular Light Scattering**
  - **Static LS**
  - **Dynamic LS**
- **Microscopy**
  - **Fluorescence**
  - **FT-IR**
- **Imaging Methods**
- **Hybrid Methods**
  - **UV-Vis Spectroscopy**

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Detection of Physicochemical Markers

- **Advantages:**
  - **High Sensitivity**
  - **Portable Instrumentation**
  - **Non-invasive**
- **Issues:**
  - **Marker Identification (size, shape, polarization)**
  - **Non-Specific markers**
  - **Sampling**
  - **Sample preparation**
  - **Interferences: require fractionation (Flow Cytometry)**

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Analytical/Interpretation Methods

- **Theoretical**
  - **Band assignment**
  - **Quantification**
    - **Linear Models (Beer-Lambert)**
    - **Non-Linear Models (Mie theory)**
- **Statistical**
  - **Multivariate Statistical Models**
    - **Factor Analysis**
    - **Principal Components**
    - **Genetic Algorithms**
  - **Classification Algorithms**
    - **Pattern Recognition**

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Recent Advances

- **Miniaturized Instrumentation**
  - **Detectors (MS, IR, Uv-vis, Raman)**
  - **Sampling and Sample preparation systems**
    - **Microfluidics**
    - **Nano-technology**
- **Interpretation-Analysis**
  - **Improved Scattering Algorithms (T-Matrix)**
  - **Enhanced Computation Capabilities**
    - **Improved Multivariate Statistical Models**
    - **Factor Analysis**
    - **Partial Least Squares**
    - **Pattern Recognition**

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Biophotonics
Analyzer Requirements

- **Portable laboratories**
  - **Autonomous**
  - **Miniaturized**
- **Sophisticated sensors & instrumentation**
- **Multipurpose or multi-sensor capabilities**
- **Immobilized reagent technologies**
- **Rapid screening & diagnosis capabilities**
- **Efficient communication subsystems**
- **Local high performance processing capability**

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Implications of Miniaturized Fiber-Optics Spectrometry

- Measurements (spectrometer systems) can be taken to the process
- Sensors (fiber-optics) can be configured
- Sensors (fiber-distal ends) can be designed for specific applications (polymer & sol-gel immobilized reagents)
- Detectors (spectrometers) can be configured
- Design sampling-measurement systems with the objective of increasing the information content of a measurement (detector and sensor bundles)

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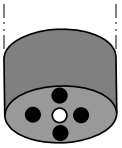
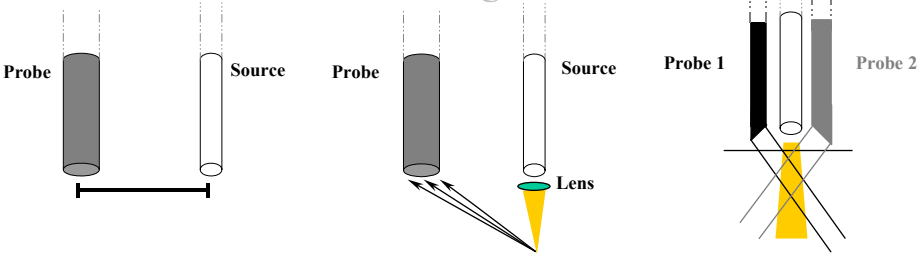
Reflectance/Backscattering

Probe



○ Source
● Detectors

Probe Configurations



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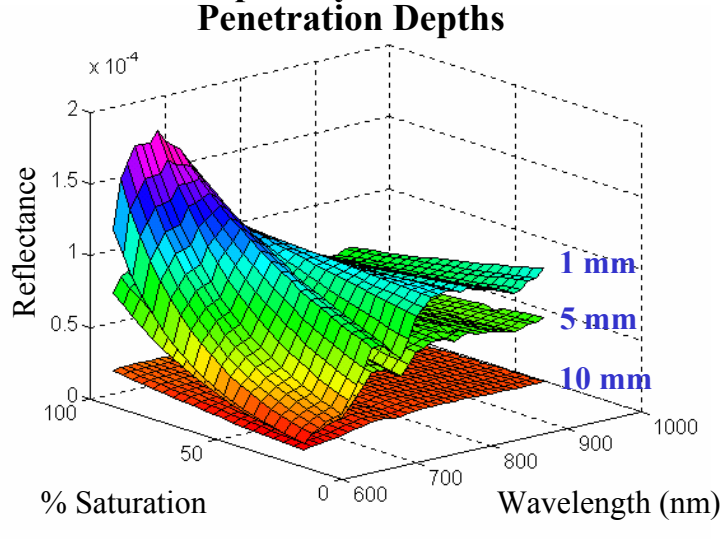
Reflectance of Optically Dense Media for Three Penetration Depths



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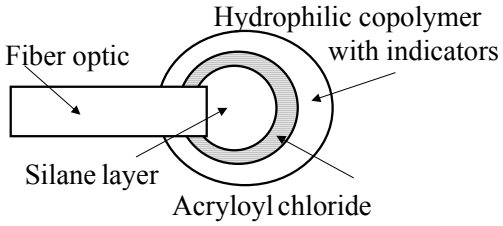
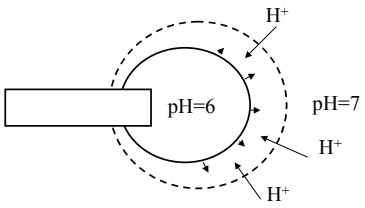
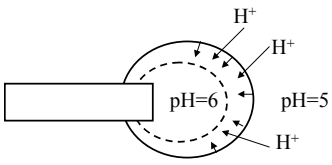
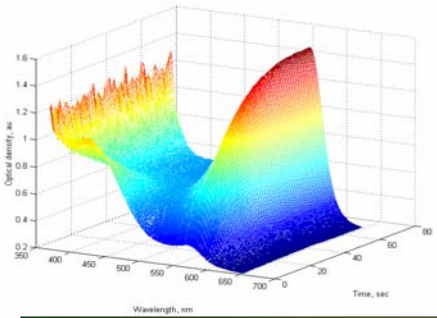
Fiber Optic Configuration









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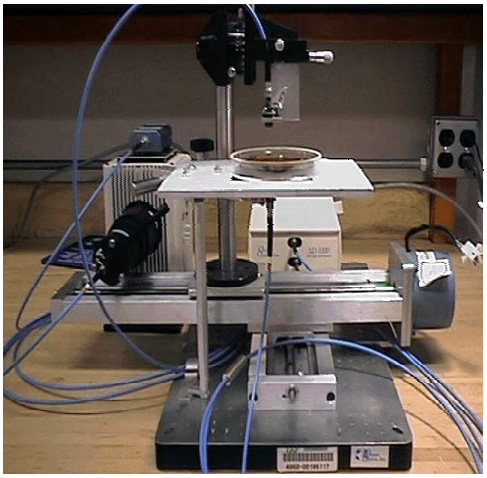
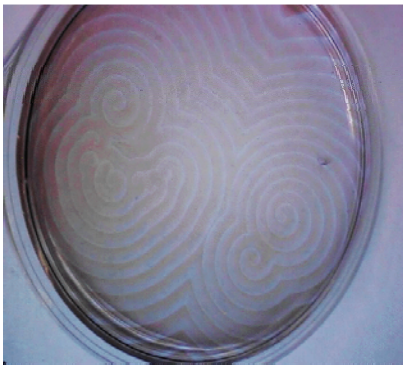
Spatial Patterns: the Belousov-Zhabotinsky Reaction





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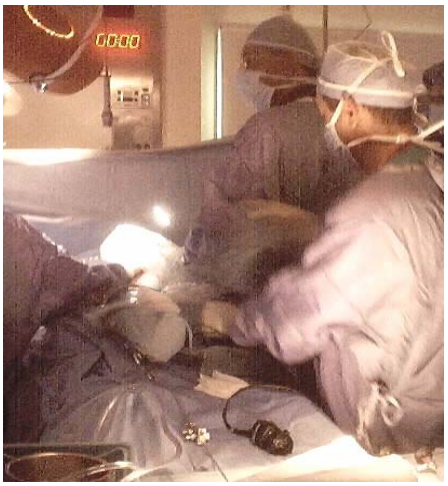
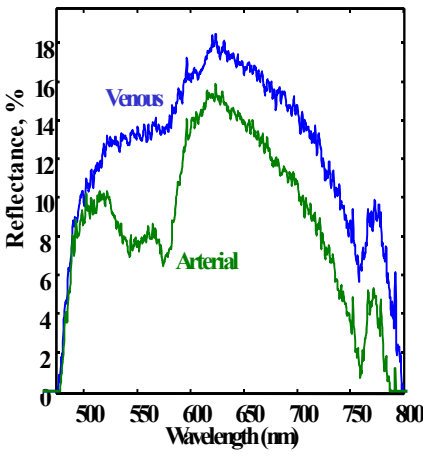
Endoscopy: application to fetal surgery





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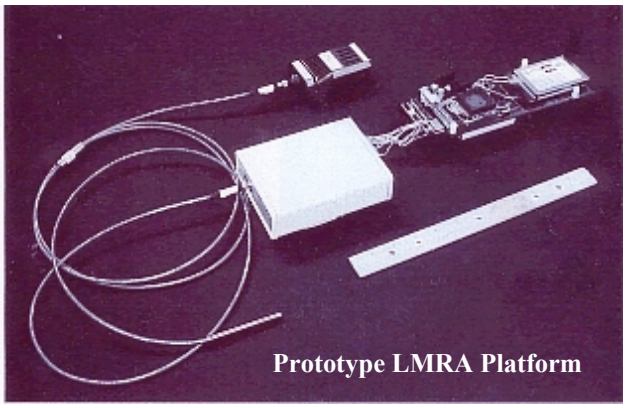
Measurement Platform

Subsystems

Detector-Sensor Array
Electronics Pack
Communication Unit
Global Positioning System



Prototype LMRA Platform

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- **Physicochemical Markers**
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- **Vision for the Future**

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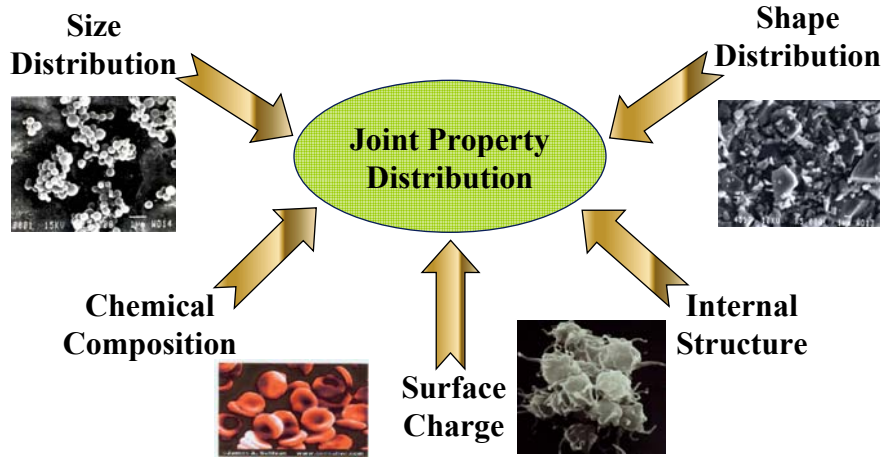
Particles Are Characterized by a Joint Property Distribution



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Issues in Bio-particle Characterization

Dilute Dispersions

- Enumeration
- Shape
- Composition
- Internal Structure
- Size
- Charge
- Orientation
- Mobility
- Optical properties
- Identification
- Limits of Detection

Concentrated Dispersions

Dense Media: skin, Suspensions, blood, cell cultures

- Fluid structure
  - Particle-Particle Interactions
  - Hydrodynamic Interactions (colony forming)
- Sampling
  - Sample Integrity
  - Representative Sampling
- Background
  - Growth Media
  - Detection Limits (contrast)

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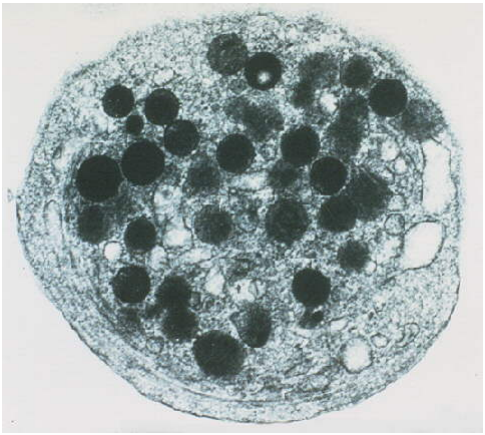
Spectrometry of Complex Particles: *Platelets*



Dense Bodies:
ADP/ATP (~1 M total)
Calcium (2 M)
Serotonin (0.065 M)
Pyrophosphate (0.3 M)

Granule Diameter: 20 to 30 nm
Platelet Diameter: 1.5 to 2.5 um

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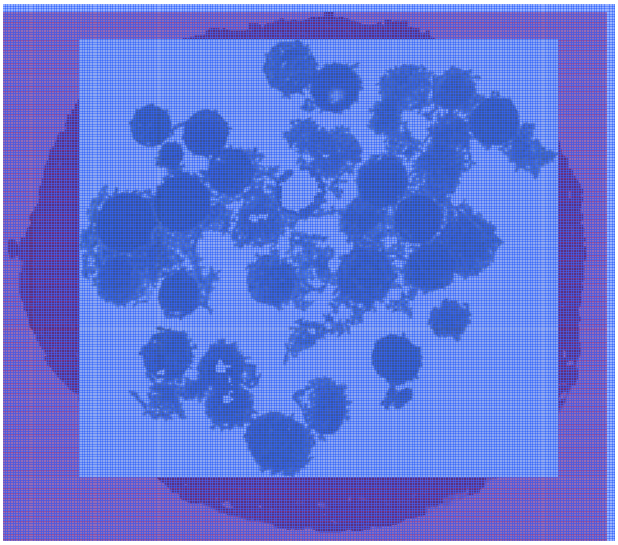

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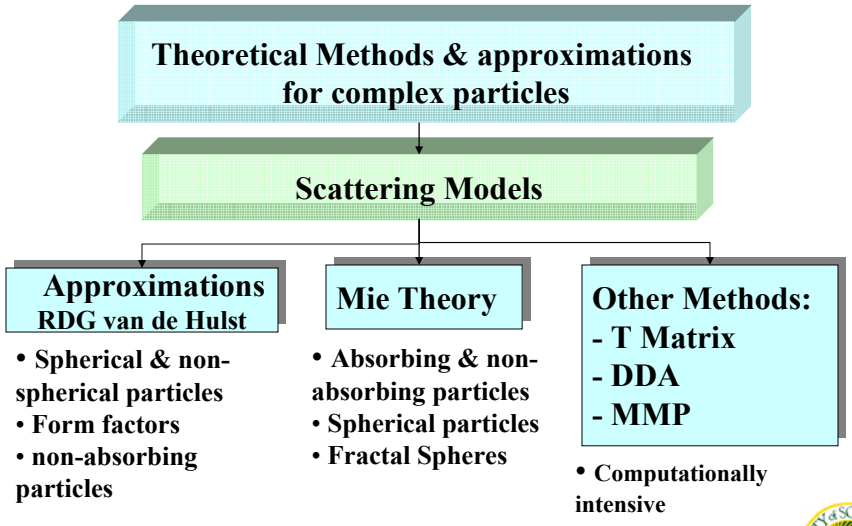
Advantages of Multiwavelength Spectroscopy

- large dynamic range (resolution: α/λ)
- composition is accessible through absorption
- real time measurements (sample times < 0.01 sec)
- large signal/noise
- suitable for in situ, at-line and in-line applications

Interpretation of spectral data



Single Scattering Models (Mie)

Optical Properties

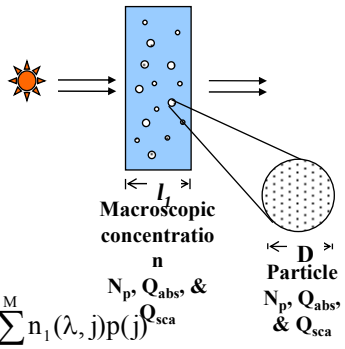
Particle Size Distribution

$$\tau(\lambda_o) = \ell N_p \frac{\pi}{4}\int_0^\infty Q\ (m,\alpha) D^2 f(D) dD$$

$$m(\lambda) = \frac{n_1(\lambda)}{n_o} + i\frac{k_1(\lambda)}{n_o} \qquad \alpha = \frac{\pi D}{\lambda}$$

$$\varepsilon(\lambda)_{obs} = \sum_j^M \frac{4\pi k_1(\lambda, j)}{\lambda} p(j): \qquad n_1(\lambda)_{obs} = \sum_j^M n_1(\lambda, j) p(j)$$

$$n(\omega) - 1 = \frac{2}{\pi}\int_0^\infty \frac{\Omega k(\Omega)}{\Omega^2 - \omega^2} d\Omega: \qquad k(\omega) = \frac{-2\omega}{\pi}\int_0^\infty \frac{n(\Omega)}{\Omega^2 - \omega^2} d\Omega$$



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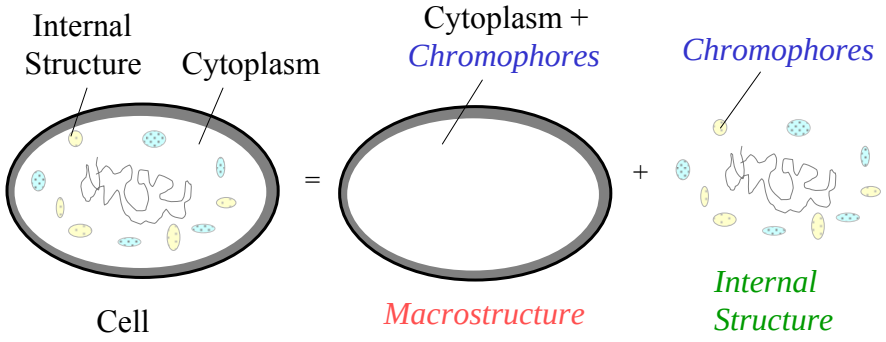
Theoretical interpretation model



$$\tau(\lambda_0) = N_p \ell \left(\frac{\pi}{4}\right)\int_0^\infty Q_{ext}(m(\lambda_0), D) D^2 f(D) dD$$

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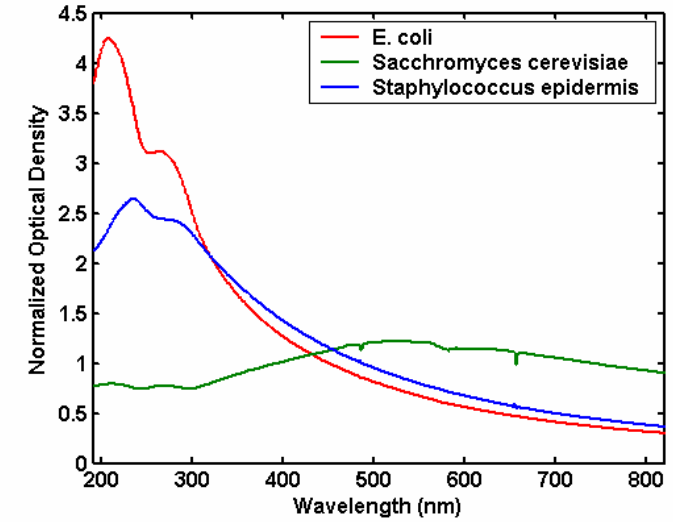
Typical Normalized Optical Density Spectra Measured from Suspensions of *Microorganisms*



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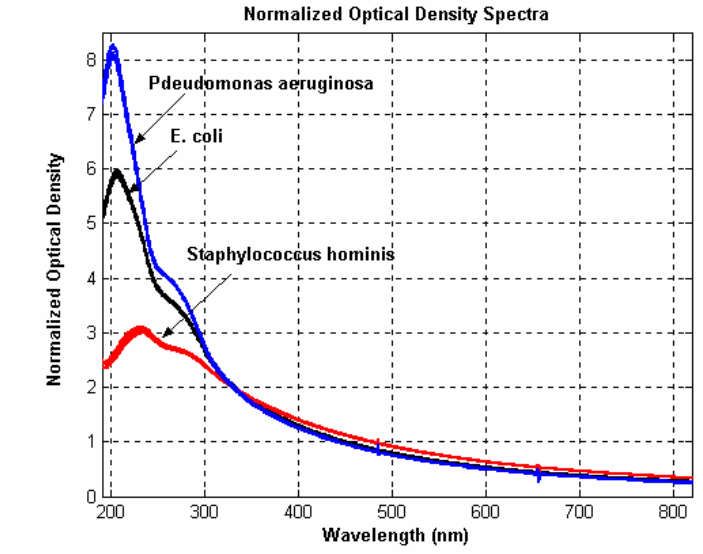
Comparison of Vegetative Cells



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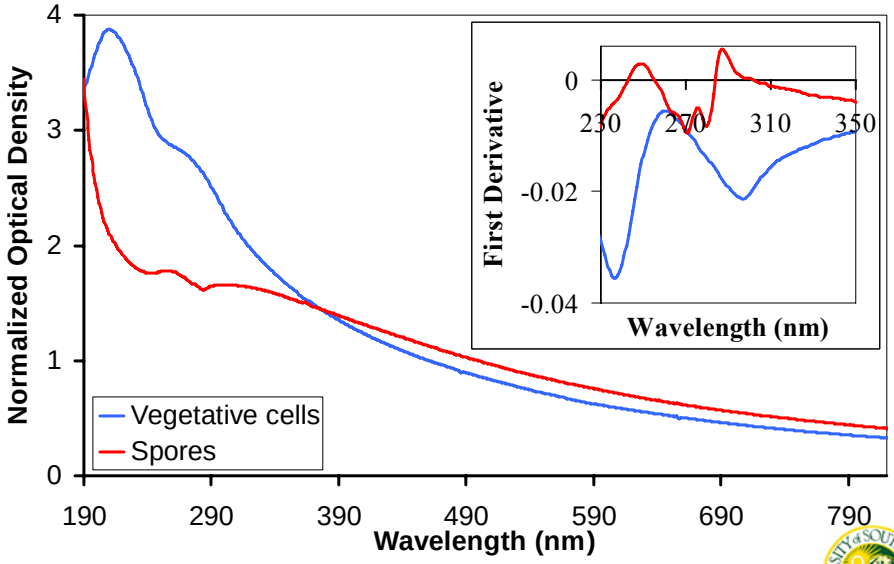
Measured Spectra: *B. globigii*



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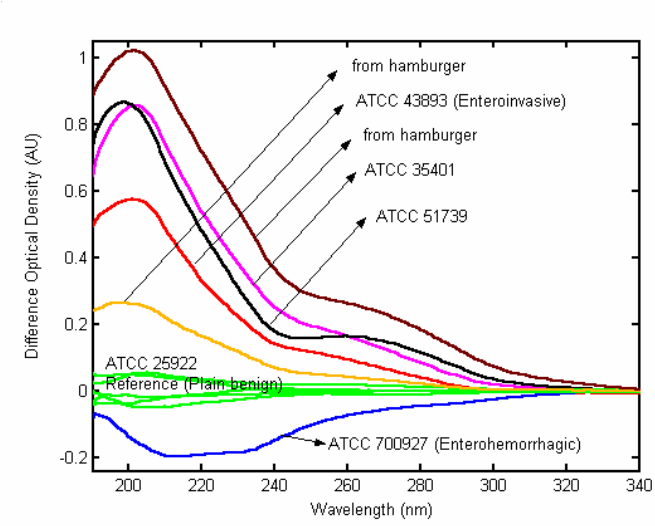
E. Coli - Difference of Optical Density



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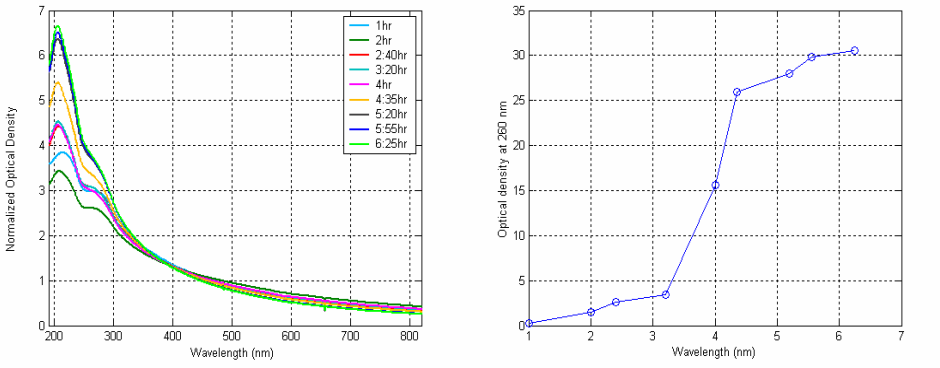
Growth behavior: *E. Coli*



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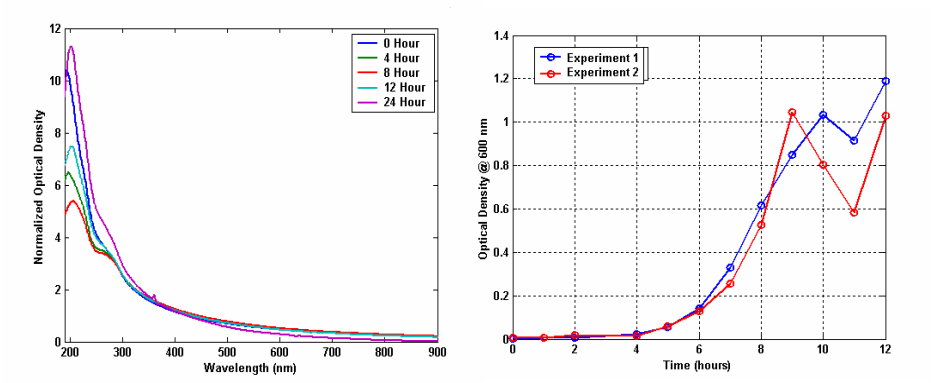
Growth Behavior: *Pantoea agglomerans* (Yersinia Pestis)



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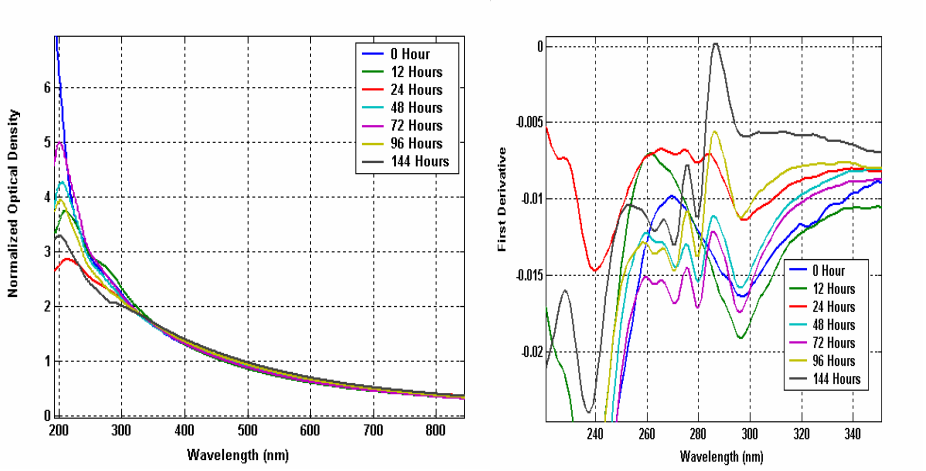
Sporulation Media: B. Subtilis



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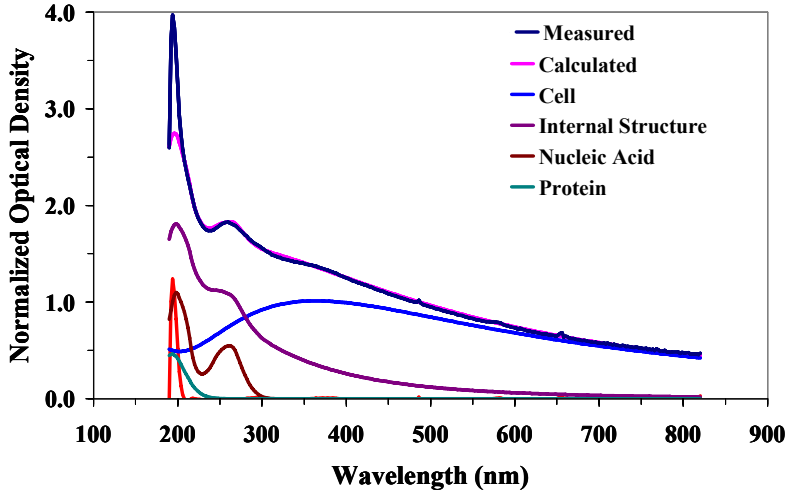
Deconvolution of the Cryptosporidium Transmission Spectrum



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Quantitative Interpretation of the Transmission Spectrum for Cryptosporidium

Microorganism

Average Size	**= 4.07 um**
Particle No	**= 341,000 per mL**

Corpuscles/Organelles (internal structure)

Hypochromicity	**= 0**
Average Size	**= 114 nm**
Volume Fraction	**= 0.4**
Fraction DNA+RNA+Nucleotides	**= 0.06**
Corpuscle Concentration	**= 5.21 ug/mL suspension**
Corpuscle Enumeration	**= 6.46 billion/mL suspension**
Max. No. of Corpuscles/cell	**= 46,481**
Estimated No corpuscles/cell	**= 18,950**
DNA+RNA+Nucleotides	**= 0.94 pg/cell**

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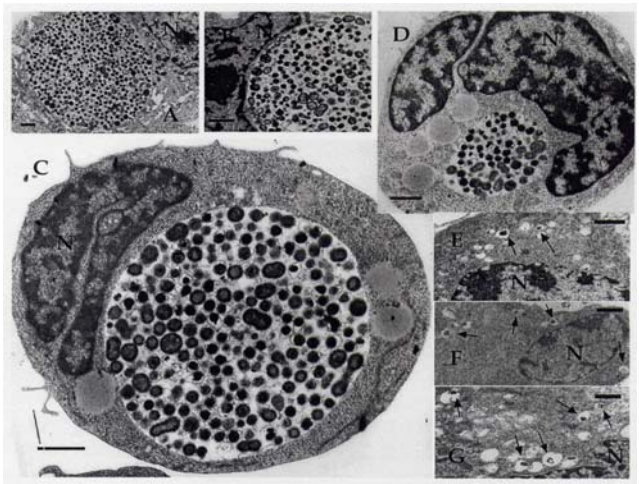
Chlamydia pneumoniae infected cells: HEp-2 (A); monocyte THP-1 (B, E); lymphoid Molt (C, F; lymphoid P3HR1 (D,G). Infected with 10 bacteria/cell after 72 Hrs of incubation. From *Y. Yamamoto et al. private Comm. October 2002*.



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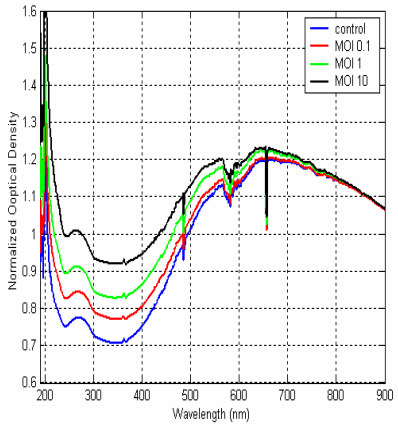
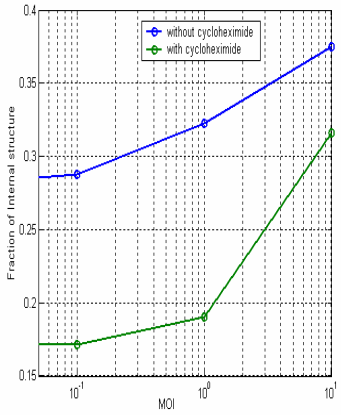
Representative data from the normalized optical density spectra of *THP-1 Cells (human monocyte cell)* infected with *C. pneumoniae*





MOI = Chlamydia cells/Host cells, Efficiency of infection = 1% (Yamamoto)

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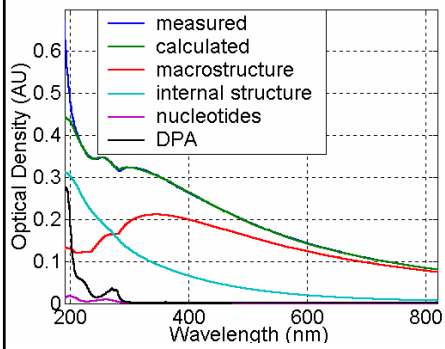
Quantitative Deconvolution

Bacillus globigii (spores)



Volume, μm^3 Literature values	0.782 <1
Equivalent diameter, µm	1.14
DNA+RNA, g/cell	1.52×10^{-14}
DPA, % (% dry cell (Murrell, 1969))	6.22 (5.6-13.55)
Fraction of internal structure, %	41.47
Average size internal structure, nm	132

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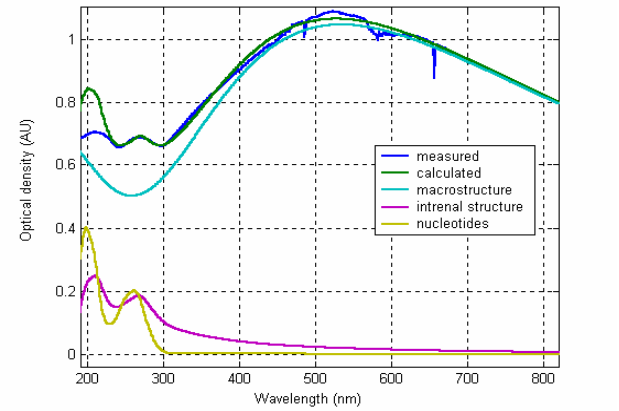
Quantitative Deconvolution of the Spectral Fingerprint of *Saccharomyces Cerevisiae (Baker's Yeast)*



	Literature		Quantitative Deconvolution
	Diploid	Haploid	
Size (um)	4	5 x 6	5.011
DNA+RNA Content (pg/cell)	1.217	1.934	1.85

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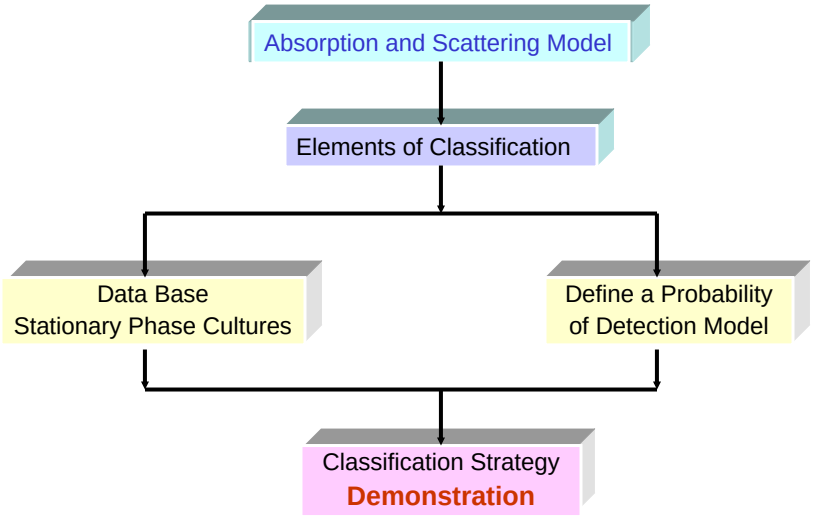
Schematic Representation for Classification Strategy



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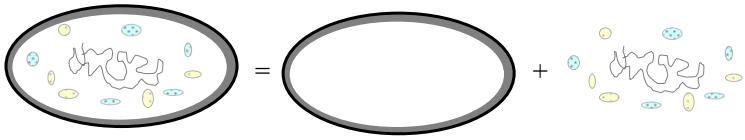
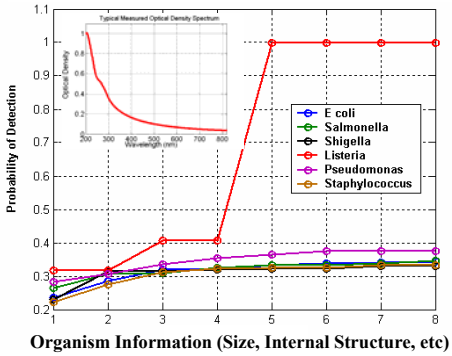
PROPOSED MODEL

Internal Structure | Cytoplasm | Cytoplasm + *Chromophores* | *Chromophores*



Cell | *Macrostructure* | *Internal Structure*



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Data base

Microorganisms:

1. *Bacterium:*
   - *Gram negative*
   - *Gram positive*
2. *Intracellular Parasites*
3. *Yeast*
4. *Protozoan Parasites*
5. *Viruses*
6. *Algae*

Measurement Matrix

- *Water*
- *Blood*
- *Menses*
- *Platelets*
- *Wash water*

Growth Media

- *Standard Growth Media*
- *Blood*
- *Menses*
- *Platelet rich Plasma*
- *Sporulation Media*

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Pathogens in data base

	Genus Name	Species Name	Strains	Comments
Gram Negative Bacteria	*Escherichia* **+2**	*coli*	10	**+2**
	Pantoea +	*agglomerans*	1	+
	Salmonella	*choleraesuis*	7	
	Shigella	*sonnei*	2	
	Shigella	*boydii*	1	
	Shigella	*flexneri*	1	
	Pseudomonas +	*aeruginosa*	1	+
	Pseudomonas	*fluorescens*	1	
Gram Positive Bacteria	*Enterococus*	*faecalis*	1	
	Staphylococcus	*aureus*	6	
	Staphylococcus	*hominis*	1	
	Staphylococcus **+2**	*epidermidis*	1	**+2**
	Listeria	*monocytogenes*	3	
	Listeria	*seeligeri*	1	
	Bacillus +*	*subtilis*	1	+*
	Bacillus +*	*anthrasis sterne*	1	+*
Intercellular Parasites	*Chlamydia* **1**	*pneumoniae*	1	**1**
Yeast	*Candida* **1,3**	*albicans*	1	**1,3**
Protozoan Parasites	*Cryptosporidium*	*parvum*	1	
	Giardia	*muris*	1	
	Giardia	*lamblia*	1	
	*Plasmodium***1**	*vivax*	1	**1**
	*Plasmodium***1**	*falciparum*	1	**1**
Viruses	*Enterovirus*	*poliovirus*	1	
	Group B Arbovirus	*dengue*	1	
Algae	*Synechecoccus*	*sp.*	1	
	Karenia	*brevis*	1	

+ denotes strains with vegetative growth curves
*** denotes strains with spore formation over time**
1. denotes strains grown in blood
2. denotes strains grown in platelets
3. denotes strains grown in menses

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Presentation overview

- **Overview of Spectroscopy Methods**
- **Chemical Markers**
- **Physicochemical Markers**
- **Recent Advances**
- **Example: Uv-vis Spectroscopy**
- **Vision for the Future**

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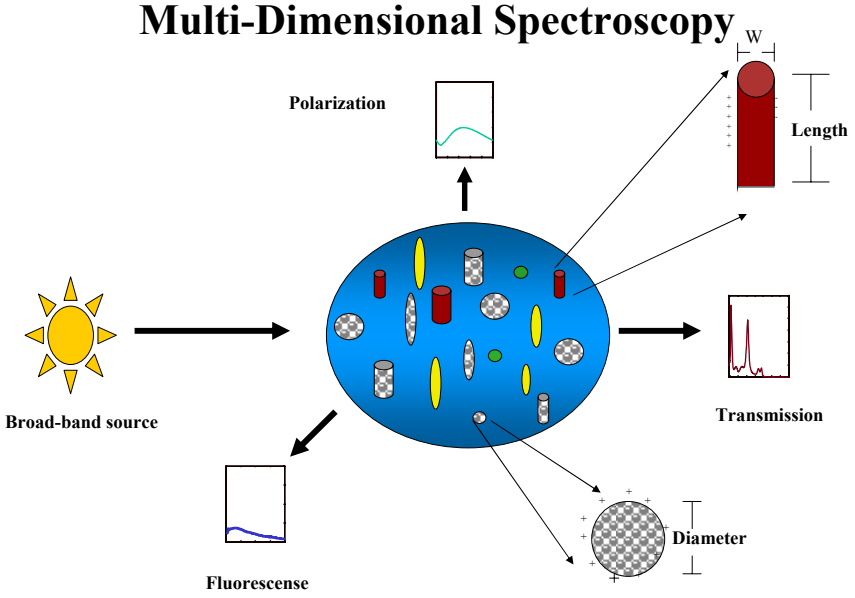
Multi-Dimensional Spectroscopy



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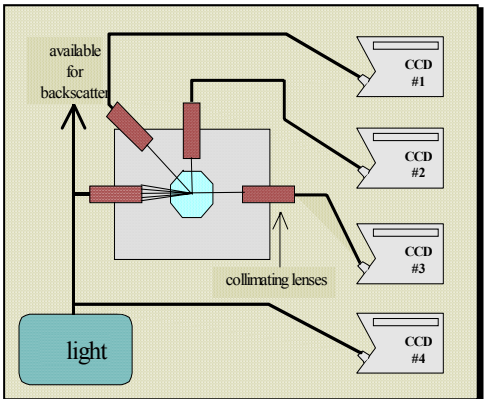
Multidimensional spectrometer concept

Features:

- Flexibility in angular measurements
- Additional angles easily added
- Backscattering measurements (bifurcated fibers)
- Polarized/unpolarized light
- Superimposed fields easily applied
- Suitable for on-line applications
- Parallel and converging beam
- Enhanced backscattering capability



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MAMW Prototype LANL

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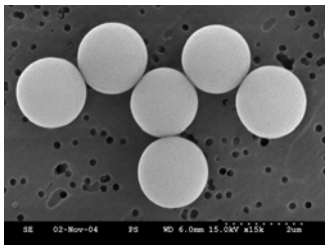
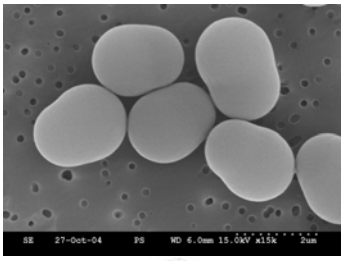
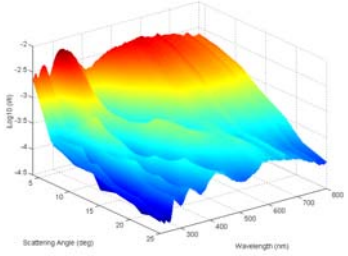
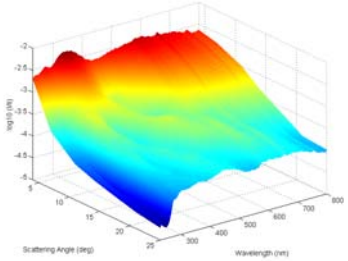
UV-VIS MAMW RESPONSES: Particle Shape









Spherical Particles: D= 1.9 µm

Peanut Shape Particles: 1.87 µm

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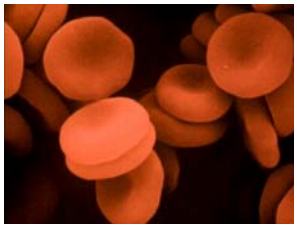
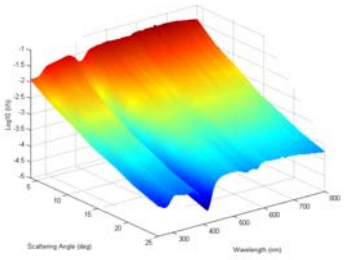
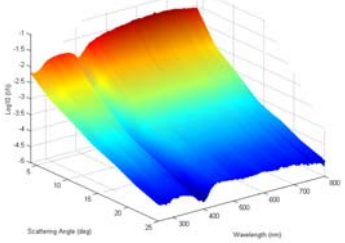
UV-VIS MAMW RESPONSES: NORMAL & SICKLED RBC's







Normal whole blood sample

Sickled whole blood sample

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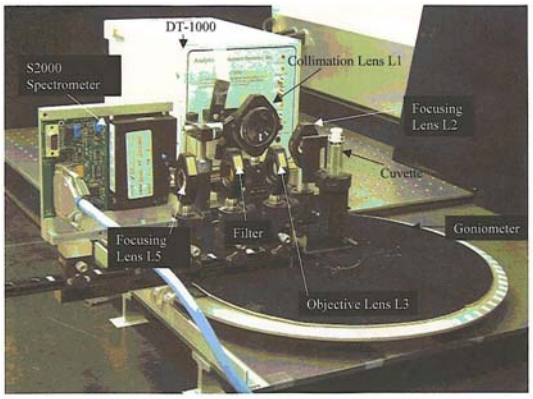
Photograph of the Fluorescence Measurement set up for the Multidimensional Spectrometer



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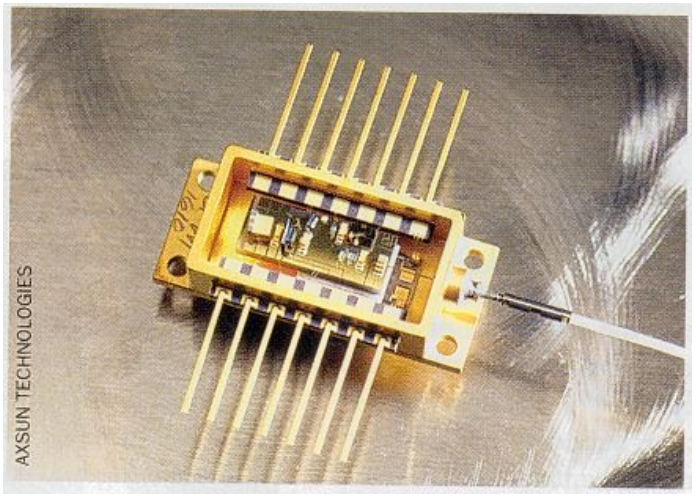
Miniaturized Spectrometers



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