NanoBiophotonics
Department of Physics
University of Nevada, Reno
Outline

• A Journey into the Nano-cosmos
• Micro- and Nanolithography
• Near- and Middle-IR Spectroscopy
  – Principles, Instruments, Diagnostics and Applications
• Biomedical Applications of Liposomes
• Demonstration of Liposome Applications in Nanomedicine
• Acknowledgements
• Bibliography
A Journey into the Nano-cosmos
Size relationships

Picture: Flad & Flad Communication GmbH, Germany
Richard Feynman – the “Father of Nanotechnology”

1959: “There is plenty room at the bottom”
The three main property changes in the nano-world

Quantum-mechanical behaviour

Increased surface area

Molecular recognition

Technical physics
through changes in
• color, transparency
• hardness
• magnetism
• electrical conductivity

Chemical processes
through changes in
• melting and boiling point
• chemical reactivity
• catalytic yield

Bio-applications
through combination with
• self-organisation
• repair ability
• adaptability
• recognition

Pictures (from l. to r.): Institut fuer Phys. Chemie, Universitaet Hamburg, Germany; BASF AG, Germany; Siemens AG, Germany
Properties of different sized nano-particles

Schematic of a CdTe nano-particle with stabilizing shell

CdSe nano-particles in solution (1.5-4.0 nm)

Fluorescence depending on the particle size

Source and pictures: Institut fuer Phys. Chemie - Universitaet Hamburg, Germany
Properties of Photons

**Wavelength** \( \lambda = \frac{h}{p} = \frac{c}{\nu} \)

\( p \) = momentum, \( h \) = Planck constant, \( c \) = velocity of light in vacuum, \( \nu \) = frequency

**Free-space propagation (plane wave)**

\[
\vec{E} = \frac{1}{2} E_0 \left( e^{ik \cdot r - \omega t} + e^{ik \cdot r - \omega t} \right)
\]

\( k \) = wave vector, real quantity

\[
k = |k| = \frac{2\pi}{\lambda} \quad \omega = c|k|
\]
Properties of Photons

**Velocity:** \( c = c_0 / n \quad c_0 = 2.998 \times 10^8 \text{ ms}^{-1} \)
where \( n \) is the index of refraction of the material.

**Wavelength:** \( \lambda = c / \nu \quad [\text{nm}] \quad (1 \text{nm} = 10^{-9} \text{m}) \)
where \( \nu : \) frequency in [Hz] or [s\(^{-1}\)]

**Wave number:** \( \bar{\nu} = 1 / \lambda \quad [\text{cm}^{-1}] \)

**Wave vector:** \( \vec{k} \) in the direction of \( \vec{e} \)
\[
\vec{k} = (2 \pi / \lambda) \vec{e} \quad [\text{cm}^{-1}] 
\]
Properties of Photons

- **Energy:** \( E = h\omega = h\nu \)
  
  where \( h \) is Planck's constant.

  \[
  h = 6.626 \times 10^{-34} \text{ Js} \approx 4.1335 \times 10^{-15} \text{ eVs}
  \]

  \[
  1\text{ eV} \approx 1.60217646 \times 10^{-19} \text{ J}
  \]

<table>
<thead>
<tr>
<th>wavelength (nm)</th>
<th>energy (J)</th>
<th>energy (eV)</th>
<th>( \nu = 1/\lambda ) (cm(^{-1}))</th>
<th>( T ) (K)</th>
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<tr>
<td>10</td>
<td>1.99 \times 10^{-17}</td>
<td>124</td>
<td>1 000 000</td>
<td>28 977</td>
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<tr>
<td>200</td>
<td>9.93 \times 10^{-19}</td>
<td>6.20</td>
<td>50 000</td>
<td>14 488</td>
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<td>500</td>
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<td>2.48</td>
<td>20 000</td>
<td>5 795</td>
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<tr>
<td>1 \mu m</td>
<td>1.99 \times 10^{-19}</td>
<td>1.24</td>
<td>10 000</td>
<td>2 898</td>
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<tr>
<td>10 \mu m</td>
<td>1.99 \times 10^{-20}</td>
<td>0.124</td>
<td>1 000</td>
<td>290</td>
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</table>
Properties of Photons

- **Uncertainty of Position and Momentum:**
  \[ \Delta x \Delta p_x \geq \frac{h}{\pi} \quad \text{and} \quad \Delta y \Delta p_y \geq \frac{h}{\pi} \]

  Position angle uncertainty: \[ w_0 \theta \geq \frac{\lambda}{\pi} \]

Gaussian beam

2\(w_0\) is minimum beam waste
Properties of Photons

- **Energy-time uncertainty**: \( \Delta E \Delta t \geq \hbar \)
- **Frequency-time uncertainty**: \( \Delta \nu_{\text{FWHM}} \tau \geq 1/2\pi \)

where the frequency uncertainty \( \Delta \nu_{\text{FWHM}} \) is measured as the full width half maximum of the line shape function.
Photonic crystals

- Optical counterpart to an electronic semi-conductor with an optical gap in a defined wavelength range
- Can direct light beams, due to their special micro-structure
- Application areas: e.g. opto-electronics, fibre-optics, …

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Pictures: Institut fuer Physik - Universitaet Paderborn, Germany; MPI fuer Mikrostrukturphysik Halle, Germany
Nanoscale optical interactions

Nanoscale localization

  axial

  Evanescent wave

  Surface plasmon

  Total internal reflection

  Optical waveguide

lateral
Nano-biotechnology: Coupling of electronic and biological systems

A nerve cell (neuron) on a silicon semi-conductor structure enables the direct transmission of the nerve impulses of the cell to the semi-conductor in the form of electronic signals. Such test cells could help in the development and testing of new drugs.

Pictures: MPI fuer Biochemie, Germany(l.) and Infineon Technologies, Germany(r.)
The Electromagnetic Spectrum
Fraunhofer Diffraction

\[ \Delta x = \frac{\lambda}{2n \sin \alpha} \]
Extreme Ultra Violet (EUV) 14 nm Laser Plasma Source

1064nm, 400mJ, 10ps, 10Hz

Zr Filter (300nm)

ML Mirror for 13.9nm

Probe position

Counts

wavelength [nm]
Design of a Single Elliptical Focussing Capillary

![Diagram of elliptical capillary with dimensions and measurements]

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
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<tbody>
<tr>
<td>2a, mm</td>
<td>1000.003</td>
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<tr>
<td>2b, mm</td>
<td>3.31254</td>
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<tr>
<td>F1, mm</td>
<td>924</td>
</tr>
<tr>
<td>F2, mm</td>
<td>5</td>
</tr>
<tr>
<td>L, mm</td>
<td>70</td>
</tr>
<tr>
<td>Dhousing, mm</td>
<td>6</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.8</td>
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<tr>
<td>D2, mm</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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Focussing Capillary - Setup

Background due to direct transmission through capillary

In Focus

Low intensity in center

Out of Focus

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EUV Lithography
EUV Discharge Light Source in the 13-14nm wavelength region

Compact EUV Light Source: A microwave (c-band, 5.9-6.5 GHz) Xe EUV plasma source was designed and developed at LBNL.
Grazing Incidence Monochromator

The EUV Monochromator has been absolutely calibrated using a synchrotron beam line at Advanced Light Source (ALS), Berkley, CA.
Medium Resolution Xe EUV Spectrum for \( \lambda = 10-16 \) nm (Absolutely Calibrated)
Micro- and Nanolithography
Nanosphere Lithography

• Forming a close-packed monolayer of submicron size nanospheres (material could be i.e. a Polymer)

→ colloidal crystal mask

Schematic diagram of single-layer nanosphere mask
• Material (i.e. Silver or Gold) to be patterned is deposited on the substrate (through the interstitial holes between the nanospheres)

• Solvent wash to remove the polymer nanosphere → leaving behind a nanopatterned material
Comparison of Raman, MIR and NIR Spectroscopic Techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Raman</th>
<th>Mid-Infrared</th>
<th>Near-Infrared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fundamentals</strong></td>
<td>4000-50 cm⁻¹</td>
<td>4000-4000 cm⁻¹</td>
<td>12500-4000 cm⁻¹</td>
</tr>
<tr>
<td><strong>Scattering Technique</strong></td>
<td>Source: Monochromatic Radiation (Laser Vis-NIR)</td>
<td></td>
<td>Sources: (Dispersed) Polychromatic Radiation (Globar Tungsten)</td>
</tr>
<tr>
<td><strong>Information Contained in Scattered Radiation</strong></td>
<td>[ \Delta \mu / \Delta q = 0 ]</td>
<td></td>
<td>Information Contained in Absorbed Radiation</td>
</tr>
<tr>
<td><strong>Homonuclear Functionalities</strong></td>
<td>High Structural Selectivity</td>
<td></td>
<td>CH / OH / NH Functionalities</td>
</tr>
<tr>
<td><strong>Polar</strong></td>
<td></td>
<td>Low Structural Selectivity</td>
<td></td>
</tr>
<tr>
<td><strong>Beer's Law</strong></td>
<td>( \log_{10} \left( \frac{I}{I_0} \right) = A \cdot a \cdot b \cdot c )</td>
<td></td>
<td>(Beer's Law)</td>
</tr>
<tr>
<td><strong>Sample Preparation</strong></td>
<td>No Sample Preparation</td>
<td>Sample Preparation Required (Except ATR)</td>
<td>No Sample Preparation</td>
</tr>
<tr>
<td><strong>Sample Volume</strong></td>
<td>Small Sample Volume ((\mu l)) or Sample Thickness ((\mu m))</td>
<td></td>
<td>Large Sample Thickness (Up to cm)</td>
</tr>
<tr>
<td><strong>Optics</strong></td>
<td>Light-Fiber Optics (&gt; 100 m)</td>
<td>Limited</td>
<td>Light-Fiber Optics (&gt; 100 m)</td>
</tr>
</tbody>
</table>
Functional Principle of an Ultra-compact MicroMirror Spectrometer

• Entrance Slit
  – Illuminated by polychromatic radiation source

• Paraboloidal reflector
  – Makes the radiation bundle parallel
  – Reflection towards the micro mirror
  – Reflection towards the detection slit

• Micro mirror
  – Reflection to the diffraction grating
  – Reflection to the paraboloidal reflector
Functional Principle of an Ultra-compact MicroMirror Spectrometer

• Diffraction grating
  – Spectral Dispersion of radiation
  – First order of diffraction is coming back from the grating to the mirror

• Detector Slit
  – Monochromatic radiation affected by the sample interaction
Near Infrared Spectroscopy
Experimental Set Up
Comparison of Water and Nanoparticles solved in Water

![Graph showing transmission of water and nanoparticles](image.png)

Transmission (%)

Wavelength (um)

Water

Nanoparticle

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Fiber optical evanescent wave (FEW) Fourier Transform Infrared (FTIR) Spectroscopy for Biomedical Applications

(a) The evanescent wave phenomenon at plane interface

(b) Evanescent wave and interface phenomena at fiber boundary
FEW-FTIR Experimental Method
Classification of Human Skin Spectra In Vivo in the Amide I & II Range

Typical Human Skin Spectra In Vivo in the Vicinity of the Amide-I and Amide-II Bands
a) Absorption Spectra, b) Second Derivative of the Mean Spectrum
Melanoma Cancer Angular Diagnostics

Angular Plot of Normal Skin Tissue Eigenvectors and Melanoma Sample Vectors. Melanoma Vectors cover a Range from 13.8 to 19.4 Degrees.
Unmixing of FEW-FTIR Melanoma Tumor Spectra

Melanoma Amide I and II, Experimental Raw Data

Melanoma Amide I and II, Factor Analyzed Model Spectra Showing two Isolated Components A and B. C represents the sum of A and B.
Biochemical transformation from normal to malignant skin tissue

Normal Skin Tissue Mean(1), Normal Skin Tissue Hydrated Protein(1A), Normal Skin Tissue Lipoprotein(1B)

Melanoma Tumor Tissue Mean(1), Melanoma Tumor Tissue Hydrated Protein(1A), Melanoma Tumor Tissue Lipoprotein(1B)
Introduction To Ellipsometry

- Ellipsometry – a non-destructive tool to characterize thin surfaces e.g. Lipid layers
- Used to measure the thickness, refractive index, the order and conformation of the materials
- Principle based on polarization of light

Principle of Ellipsometry
Ellipsometric results on Lipid bilayers in the NIR and MIR range

OTS – Octadecyl Trichloro Silane

POPC - P1-Palmitoyl-2-Oleoyl-Sn-Glycero-3-Phosphocholine

tan Ψ is the Reflection Coefficient
Applications of NIR and MIR Spectroscopy

- Skin and body fluids analysis, biomedical diagnostics
- Skin cancer, in vivo tissue diagnostics
- Analysis of various biological systems, such as nanobiosensors and biochips,
- Bioimaging and gene sequencing techniques
- Metabolite, and blood monitoring, analysis of prediabetes
- In vivo lipid and cellular molecular interactions
- Foods, vegetables and beverages
- Nanocomposites
THE FLUID MOSAIC MODEL OF BIOLOGICAL MEMBRANES
Basic Lipid Structure

**Head Group**
- Polar, hydrophilic
- Group may vary in chemistry, charge, size
- Types: Choline, serine, ethanolamine, glycerol

**Tail Group**
- Nonpolar, hydrophobic
- Chain length varies
- Saturation varies
Lipid Bilayers (Liposomes)

- Properties of lipid aggregates
- Promising mechanisms for drug and substance delivery

Cross-section of a liposome nano structure

Acceptance of liposome into cell

Essential Phospholipid Lipid (EPL) nanoparticle interacting with the cell membrane
Liposomes contd...

- Bilayer Liposome model;
- Water soluble actives in the inner vesicle, proteins and enzymes in the bilayer membrane

- Monolayer Nanoemulsion model; Liposoluble actives in the inner vesicle
Process Of UV/Ozone Photolithography

Fluorescence image of a Texas Red labeled lipid sample (POPC)
Introducing QuSomes®
A Revolution in Product Efficacy
What is a QuSome®?

Typical Size Ranges: SLV: 20-50 nm – MVL: 100-1000 nm
QuSome® Encapsulation Study

Comparative encapsulation % profile of CoQ10 at room temperature.
CoQ10: Ubiquinone
## Liposomes vs. QuSomes®

<table>
<thead>
<tr>
<th>Property</th>
<th>Liposomes</th>
<th>QuSomes®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Starting Lipid-</strong></td>
<td>Phospholipids (PC)</td>
<td>PEG Lipids patented by BZL</td>
</tr>
<tr>
<td><strong>Skin Penetration-</strong></td>
<td>Good</td>
<td>Excellent</td>
</tr>
<tr>
<td><strong>Encapsulation-</strong></td>
<td>Good ~ 55%</td>
<td>Excellent~ 95%</td>
</tr>
<tr>
<td><strong>Manufacturing-</strong></td>
<td>Process Intensive, Expensive</td>
<td>Simple</td>
</tr>
<tr>
<td><strong>Stability-</strong></td>
<td>‘Kinetic Traps’</td>
<td>Thermodynamically Stable</td>
</tr>
</tbody>
</table>

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Efficiency of Substance Release

Comparative Release % profile of CoQ10.
LipoCeuticals vs. Conventional Vehicles for Skin Penetration

Your topical products benefit by utilizing liposomes when compared to “free actives” incorporated into conventional vehicles (ointments, creams, gels and lotions).
Aging Skin

- Visible characteristics of aging include:
  - Thickening
  - Redness
  - Wrinkling
  - Loss of firmness

- Biopsy shows sub-clinical inflammation

George Burns
1896-1996
Nanoxir®-The new Generation of Nanomedicine

The therapeutic effect of a nutrient is directly related to the Quantity and Rate at which the nutrient reaches the bloodstream (in our case 60s)
Transmucosal Delivery using Nanosprays

LipoSpray™

Penetration is greater with LipoSpray

Better penetration allows for decreased dose in some cases

Greater concentration in submucosal tissue

Higher concentration in the bloodstream

Traditional Spray

Mucosa

Submucosal Tissue

Connective Tissue

Bloodstream
LipoSpray® vs. Tablet
Bioavailability Model

The results show that LipoSpray® provides a faster onset of activity by approximately 30 minutes and provides twice the bioavailability.
Bioavailability of Vitamin B12

Bioavailability of Cyanocobalamin 1000mcg
Mean Plasma conc vs Time (N=12)

- LipoCap B12
- Immediate Release B12 Capsule
BioDrinks

- Nano delivery of nutrients
- Improved ingredient solubilization
- Enhanced bioavailability of ingredients
- Patents and patents pending
- Improved product performance
The End(s)
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