Chemical Mapping and Imaging by Spectroscopic Techniques

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Chemical Mapping/Imaging

What is Chemical Mapping/Imaging?

- The fusion of spectroscopy with microscopy or macroscopic imaging technologies to create a visual image of the distribution of chemical species (a chemical map) in a user defined area.

- A powerful analytical tool for chemical identification, solids characterization, and analysis of complex heterogeneous samples such as formulated drug products or devices.

- Enables a scientist to obtain spatial and spectral information quickly and accurately in minutes.

- Answers questions like, “what components are in my sample”, “how much of each is present”, and “where is each located?”
Chemical Mapping/Imaging

**Where is Chemical Mapping/Imaging applied?**

- **Pharmaceuticals**
  - API, Excipients, Process blends, Drug products (tablets, granules, microspheres, stents, lyophile cakes)

- **Biology/Biotechnology**
  - Tissue samples, Proteins

- **Agriculture**
  - Grain crops, Plants, Soil, Animal feed, Fertilizers

- **Food Science**
  - Meat, Dairy products, Cereal, Flour, Eggs

- **Industry**
  - Textiles, Polymers

- **Medicine/Biomedicine**
  - Medical devices, Blood glucose monitoring strips
Chemical Mapping/Imaging

• Is Chemical Mapping Different from Chemical Imaging?
  ➢ Yes!
  ➢ Chemical mapping is related to chemical imaging, but the difference is based on the approach to the measurement.
  ➢ In chemical mapping, a chemical image is generated by collecting data point-by-point while rastering across a 2-D map area.
    • Example: Renishaw inVia Raman Microscope
  ➢ In chemical, or hyperspectral imaging, a chemical image is generated by simultaneously collecting data across a 2-D map area. Each ‘sub-image’ represents the sample response for each spectral bandpass. The ‘sub-images’ are combined to form a three dimensional hyperspectral cube, or chemical image.
    • Example: Malvern Sapphire NIR Chemical Imaging System
Chemical Mapping

- The chemical image is created by rastering the laser beam across a predefined two-dimensional area
  - Step mode: step in known x,y increments
  - Streamline mode: defocus laser into a line, bin along the y-axis, step in x-axis

- x-direction: horizontal spatial position, y-direction: vertical spatial position
Chemical Imaging

- The chemical image is the foundation of any mapping or imaging approach
  - A chemical image dataset is represented by a three-dimensional cube, referred to as a hyperspectral cube:

  - **x-direction**: horizontal spatial position
  - **y-direction**: vertical spatial position
  - **z-direction**: spectral wavelength
Chemical Mapping/Imaging

- Chemical Mapping/Imaging can be performed by numerous techniques
  - Molecular Spectroscopy:
    - IR
    - NIR
    - Raman
    - ToF-SIMS
    - XRPD (new)
  - Elemental Spectroscopy
    - EDS
  - We have access to all these techniques!
Chemical Images

IR

NIR

ToF-SIMS

Raman
Chemical Imaging

• Selectivity
  ➢ Any form of chemical imaging/mapping requires selectivity
  • Diagnostic response (peak/series of peaks) for the components of interest (analyte)
Chemical Imaging

• Selectivity
  - Monitor the intensity of a diagnostic response (peak/series of peaks) for generation of the chemical image

- this particular $\lambda$ may be diagnostic for component #1

- this particular $\lambda$ may be diagnostic for component #2
Chemical Imaging

• Sample Preparation
  ➢ Requirements for sample preparation depend upon the objective of your study
    • Counterfeit Analysis (Packaging)
      ➢ No preparation is typically necessary (examining inks)
    • Drug-coated stent
      ➢ No preparation is typically necessary but require proper positioning under the spectrometer
    • Monitoring polymorphic interconversion during wet granulation
      ➢ Microtomy performed on a granule
    • Formulation reverse engineering
      ➢ Microtomy performed on a microsphere
Chemical Imaging

• Sample Preparation
  ➢ None – powder analysis:

  ➢ Microtomy – microsphere analysis:
Chemical Imaging

• Sample Preparation
  ➢ Microtomy
Chemical Imaging

• Data collection
  ➢ Requirements for spectral collection depend upon the objective of your study
    • Considerations:
      ➢ Spatial resolution
        » NIR: 10-, 40-, 80-micrometers
        » ToF-SIMS: 1-2 micrometers
        » Raman: 2 micrometers
        » IR: 10 micrometers
        » EDS: nanometer
        » XRPD: varies with angle, ~50-200 micrometers
      ➢ Spectral range (specificity)
      ➢ Exposure time/accumulations (S/N, sensitivity)
      ➢ Beam intensity (S/N, sensitivity)
    • Data collection times can vary from minutes to hours
Chemical Imaging

• Spectral Processing
  - After collection of the spectral data (typically 1000s), spatially dependent analytical responses are used to generate a chemical image
  - Univariate Analysis
    • Chemical image is based upon one variable:
      - Diagnostic peak height:
      - Diagnostic peak area:
Chemical Imaging

• Spectral Processing
  - After collection of the spectral data (typically 1000s), spatially dependent analytical responses are used to generate a chemical image
  - Multivariate Analysis
    - Chemical image is based upon statistical analysis of the multiple variables in a spectrum – Chemometrics
    - Unsupervised Analysis (Unknown system)
      - Principal Component Analysis (PCA)
      - Cluster Analysis
    - Supervised Analysis (Known system)
      - Direct Classical Least Squares (DCLS)
      - Partial Least Squares (PLS), also known as Projection of Latent Structures
Examples
Chemical Imaging

• Content Uniformity Application
  ➢ Performance testing of the solid oral dosage form showed variability in results
  ➢ Lot-to-lot variability that may correlate to manufacturing issues?

  ➢ Multi-component solid oral dosage form (tablets with API & excipients)
  ➢ Sample preparation: microtomy via steel blade to expose core
  ➢ Raman (Streamline™ mapping, 785 nm laser)
  ➢ ~1 hour map, 40.8 µm/pixel spatial resolution
  ➢ ~75,000 spectra/chemical image
Chemical Imaging

- Content Uniformity Application

  - Univariate generated image (503.9 cm⁻¹)
Chemical Imaging

- Content Uniformity Application
  - Univariate generated image (503.9 cm⁻¹)

<table>
<thead>
<tr>
<th>Run</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1170.7</td>
<td>1074.9</td>
<td>1271.5</td>
<td>1249.4</td>
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<tr>
<td>Std. Dev.</td>
<td>393.9</td>
<td>479.0</td>
<td>526.1</td>
<td>491.0</td>
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</tbody>
</table>
Chemical Imaging

• Component Identification Application
  ➢ Many recent advances in the field of formulation design
    • layered microspheres (core, interior layer, exterior enteric coating, etc.)
      ➢ location of components
      ➢ consistency of layers
      ➢ thickness of layers
    • transdermal patches (API, matrix, prevention of crystallization)
    • amorphous API formulation (API, stabilizers)
    • granule design
      ➢ multi-component granules – identification of components
      ➢ proximity of one component to another (stabilization)
  ➢ Development of a solid-oral dosage form
    • Is formulation performance related to proximity of components to each other?
  ➢ Multivariate approach – PLS2 Classification
    • Determining the spatial position of different components within a tablet
Chemical Imaging

• Component Identification Application
  ➢ Multivariate approach – PLS2 Classification
    • Determining the spatial position of different components within a tablet

![API Image](image1)
![Excipient #1 Image](image2)
![Excipient #2 Image](image3)
Chemical Imaging

- Component Identification Application
  - Multivariate approach – PLS2 Classification
    - Use of binary images and subsequent RGB overlays

- API binary image
  - thresholded based upon loading

- Excipient #1 binary image
  - thresholded based upon loading

- Excipient #2 binary image
  - thresholded based upon loading
Chemical Imaging

- Component Identification Application
  - Multivariate approach – PLS2 Classification
    - RGB overlay #1

- API represented as red pixels
- Excipient #1 represented as blue pixels
- Co-occupancy of a pixel position by both components is indicated by magenta pixels
Chemical Imaging

- Component Identification Application
  - Multivariate approach – PLS2 Classification
    - RGB overlay #2

- API represented as red pixels
- Excipient #2 represented as green pixels
- Co-occupancy of a pixel position by both components is indicated by yellow pixels
Chemical Imaging

- Domain Size Analysis Application
  - Determination of domain size ("particle size") for a component within a tablet
    - investigating the domain size of the API within a tablet
    - chemical image created by specific approach
    - threshold the original chemical image to create a binary image
    - thresholding based upon 7% API loading
Chemical Imaging

- Domain Size Analysis Application
  - API domain size

![Image of chemical imaging data with a grid and particle statistics table.](image_url)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Units</th>
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<tbody>
<tr>
<td>Number of Included Particles</td>
<td>151 particles</td>
<td></td>
</tr>
<tr>
<td>Percentage Area Covered</td>
<td>6.894%</td>
<td></td>
</tr>
<tr>
<td>Mean Area</td>
<td>$6.0015 \times 10^4$ square ( \mu \text{m} )</td>
<td></td>
</tr>
<tr>
<td>Area STD</td>
<td>$8.0309 \times 10^4$ square ( \mu \text{m} )</td>
<td></td>
</tr>
<tr>
<td>Mean Equivalent Diameter</td>
<td>229.5469 ( \mu \text{m} )</td>
<td></td>
</tr>
<tr>
<td>Diameter STD</td>
<td>154.5320 ( \mu \text{m} )</td>
<td></td>
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</table>
Chemical Imaging

• Counterfeit Analysis
  - World Health Organization
    • A counterfeit medicine is one which is deliberately and fraudulently mislabeled with respect to identity and/or source
    • Counterfeiting can apply to both branded and generic products
    • Counterfeit products may include:
      - products with the correct ingredients
      - products with the wrong ingredients
      - products without active ingredients
      - products with insufficient active ingredient
      - products with fake packaging
  - Globally, every 20th drug is a fake, >$30 billion market
    • Market penetration
    • Europe, USA, 2%, South Africa, 20%, Mexico, 25-40%
    • Third World, 70-80%
    • 8% of imported drugs are fake
    • 7% of world market is substandard
Chemical Imaging

• **Counterfeit Analysis**
  - Univariate approach (peak height)
  - Investigating pharmaceutical packaging
    - Optical images
      - Left: suspect packaging
      - Right: authentic packaging
Chemical Imaging

- Counterfeit Analysis
  - Univariate approach (peak height)
  - Investigating pharmaceutical packaging
    - Raman chemical images
      - Consistent red pixels indicate same type of ink on authentic package
      - The green pixels show different ink added to an authentic “3”
Volume Reconstruction

• Volume reconstruction consists of
  - x, y, z coordinates (3-D)
  - identification of a particular component

• Volume reconstruction permits:
  - content uniformity analysis in the drug product (API and/or excipients)
  - Domain (particle) size determination in the drug product (API and/or excipients)
  - phase purity analysis in the drug product
Volume Reconstruction

- Generate chemical images from each two-dimensional surface
- Absolute referencing necessary so surfaces can be stacked together for volume reconstruction
Volume Reconstruction

• Volume presentation:
Chemical Imaging

• Chemical Mapping/Imaging
  ➢ Exciting, relatively new field
  ➢ Fantastic application to pharma
    • Counterfeit analysis
    • Lot-to-lot variability
    • Content uniformity
    • Layer thickness
    • Formulation performance
    • Impurity analysis
    • Domain size (particle size)
    • Reverse engineering
Thank you for your attention.

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