# A Critical Evaluation of Emerging High Resolution Imaging Technologies for the Characterization of Complex Formulations

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## Introduction

Simultaneously acquiring physical and chemical microstructural information becomes challenging due to limitations posed by diffraction limit, a few hundred nanometers in most cases. With increasing numbers of complex formulation submissions to the Agency, of which many have nanoscale physical features, it is of critical importance to be able to characterize the physicochemical microstructure and to stay abreast of the technologies that enable such characterization. This work aims to offer a critical evaluation of two emerging technologies which offer promise to assist in the physicochemical characterization of complex formulations with nanoscale materials; in particular, Cytoviva® enhanced darkfield microscope with hyperspectral imaging and tip-enhanced Raman spectroscopy (TERS).

## Objectives

- Evaluate the Cytoviva® enhanced darkfield microscope for an ophthalmic emulsion and a nasal spray
  - Imaging capabilities
  - Hyperspectral mapping capabilities
- Evaluate tip-enhanced Raman spectroscopy (TERS) as both a sensing and hyperspectral imaging technique.
- Compare results to established techniques such as AFM, DLS and confocal Raman

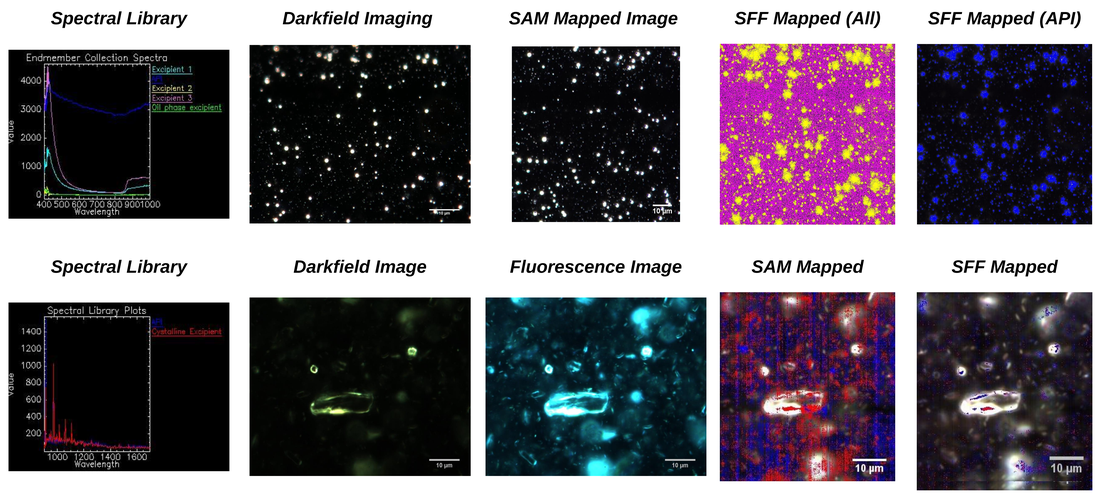
## Methods

**Cytoviva® Enhanced Darkfield Microscope with Hyperspectral mapping**

- Select marketed emulsions, nasal sprays and components were placed on a microscope slides with a coverslip.
- The CytoViva® microscope system was used to acquire darkfield microscopy images and hyperspectral plots using an enhanced darkfield transmission optical microscope (Olympus BX41) equipped with a CytoViva® unit (CytoViva®) and a hyperspectral imaging spectrophotometer unit (Headwall Photonics).
- CytoViva can measure small features that standard optical imaging techniques cannot by improving the alignment and focus of illumination and enhancing the signal-to-noise of nanoscale materials. The particle size distribution of the formulations was determined by ImageJ analysis of the captured optical images.
- This system is also capable of recording high quality spectra (high signal-to-noise ratio) in the visible and near infrared wavelength range (400-1000 nm). Hyperspectral images were acquired using a 0.5 s collection time and a halogen light source. Images were acquired using a 100x oil immersion lens.
- Qualitative hyperspectral analysis of the acquired images was performed using ENVI 4.8. Spectral libraries were built with known pure chemical components of the formulations and then applied to unmodified formulated products to obtain an image of their chemical microstructure.
- **Tip-Enhanced Raman Spectroscopy**
- Substrate (mica or Au on Si) was functionalized with poly-L-lysine
- Dilute (1:10) drug product was drop cast on substrate
- Gold coated or dielectric AFM tips were used for both point measurements and mapping

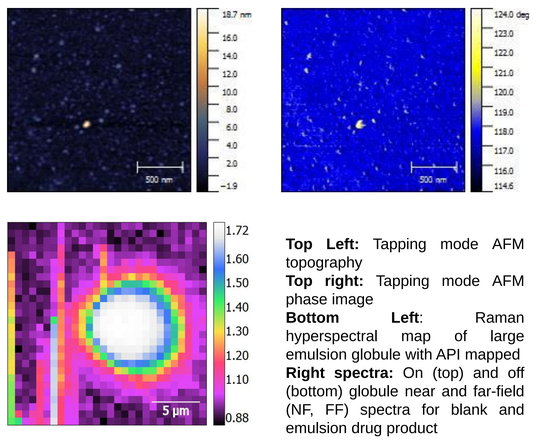
## Results

**Cytoviva® Enhanced Darkfield Microscope (Emulsion and Spray)**

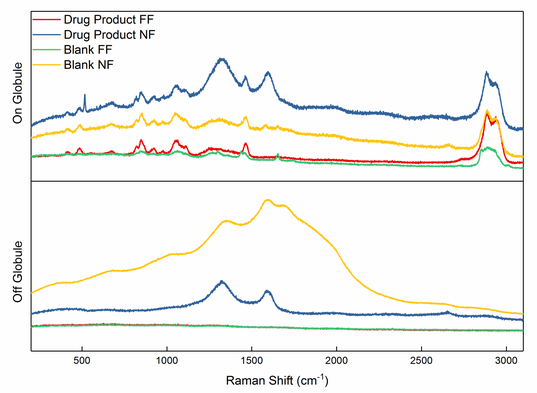



- Top Row: Darkfield microscopy of Ophthalmic Emulsions at various dilutions.
- Second Row: DLS Histograms corresponding to the dilutions in top row
- Third Row: Spectral library (VNIR) and associated darkfield and classified HSI Images for the ophthalmic emulsion
- Fourth Row: Spectral library(SWIR) and associated darkfield, fluorescence and classified images for nasal spray comprised of a crystalline API and excipient.

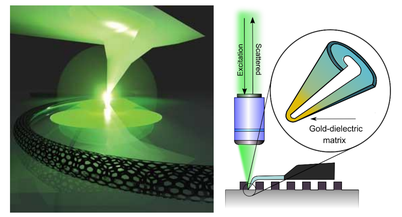
**Atomic Force Microscopy, Raman Spectroscopy and TERS (Emulsion)**




**Top Left:** Tapping mode AFM topography
**Top right:** Tapping mode AFM phase image
**Bottom Left**: Raman hyperspectral map of large emulsion globule with API mapped
**Right spectra:** On (top) and off (bottom) globule near and far-field (NF, FF) spectra for blank and emulsion drug product




## TERS Instrumentation




TERS combines AFM and Raman to obtain nearfield vibrational spectral information in an enhanced spot where the tip is interacting with the sample. Confocal or farfield is also collected.
**Left:** Beam bounce AFM with 60° illumination (Courtesy of Horiba Scientific)
**Right:** Tuning fork based AFM with 180° backscatter excitation.

## Discussion

The enhanced darkfield condenser allows for the visualization of nanoscale domains in complex formulations and provides complimentary information to sizing techniques such as DLS, such as the presence of agglomerates vs. discrete oil globules in the emulsion formulation. For materials with known fluorescence, an optional Hg lamp may provide additional visual information.
Spectral libraries may be built from reference materials which may assist in chemical identification, however, the mapping methodology and in some cases, such as the ophthalmic emulsion, are able to be validated with methods such as Raman spectroscopy. However, mapping methodology does not seem to be robust, as shown in the case of the nasal spray, with crystalline API and excipients, in which Raman hyperspectral mapping is not in good agreement with data provided by the Cytoviva® system.

The components of TERS, AFM and Raman have both been performed on an emulsion drug product, however only point spectroscopy using TERS has been performed due to the difficulty of the measurements. Point spectroscopy however shows some promise in TERS with a higher NF signal and enhancement of some Raman features relative to the FF.

## Conclusions

- The Cytoviva® enhanced darkfield microscope coupled to hyperspectral reflectance spectrometers allows for highly sensitive, yet diffraction limited, detection of nanoscale features within drug products containing emulsion micelles and globules, as well as crystalline materials.
- The Cytoviva® enhanced darkfield microscope coupled with a fluorescence source may offer higher sensitivity for visualizing certain excipients and APIs.
- Caution must be exercised when using hyperspectral mapping to assign chemical information with the Cytoviva system.
- TERS offers some promise for characterizing complex formulations as it combines strengths of AFM and Raman, however the current status is point measurements on and off areas of interest and is currently being developed for hyperspectral capabilities within liquid medium, such as emulsions.

## Acknowledgement and Disclaimer

- This project was supported in part by an appointment to the Research Participation Program at the Center for Drug Evaluation and Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.
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- ELW thanks A. R. Hight Walker at NIST and Horiba Scientific for instrument time