QUANTITATIVE DARK-FIELD MICROSCOPY OF GOLD NANOSHELLS IN CELLS

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Gold nanoshells are biologically inert nanoparticles with unique optical properties. Biological molecules can be affixed to the exterior of gold nanoshells and thus transported into cells. Upon laser irradiation, the affixed molecules can be controllably released. This technique allows for the transport and controlled delivery of a variety of therapeutic molecules, such as DNA and proteins, and thus holds possibility for a variety of medical applications in disease treatment. However, the number of therapeutic molecules delivered depends upon the number of gold nanoshells that can be placed in cells, and this quantity remains unknown. We have constructed a transmission dark-field microscope for imaging and quantifying the number of nanoshells taken up within individual cells. Controlled by a custom-written LabView software program, this dark field-microscope collects a series of images which are then processed, resulting in a quantitative estimate of the number of gold nanoshells inside of a cell and a three dimensional representation of their location.

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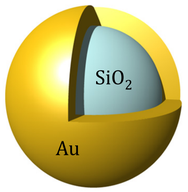
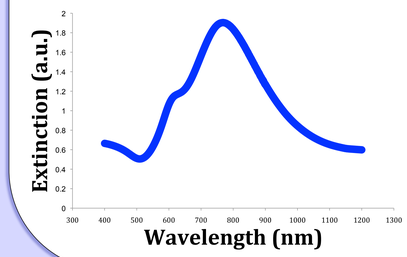
Introduction

Gold nanoshells are biologically inert molecules upon which therapeutic molecules, such as DNA and proteins, can be affixed. Once gold nanoshells are transported into cells, the affixed molecules can be controllably released by laser irradiation.



Gold Nanoshell

Nanoshell Extinction Spectra



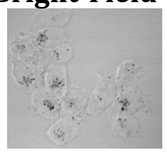
However, these treatments cannot be fully realized until the number gold nanoshells that can be placed into a cell is known. Current methods of quantification rely on ICP-MS (ion coupled plasma- mass spectrometry), which is both time consuming and costly.

Image Collection: Dark-Field Microscopy

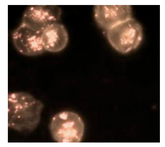
As opposed to bright-field microscopy, dark-field collects only light scattered by the sample. Thus making it ideal for imaging gold nanoshells which scatter light, at frequencies dependent upon the size of the nanoshells, when illuminated. A dark-field microscope was constructed that allows for stage movement in the z-direction.

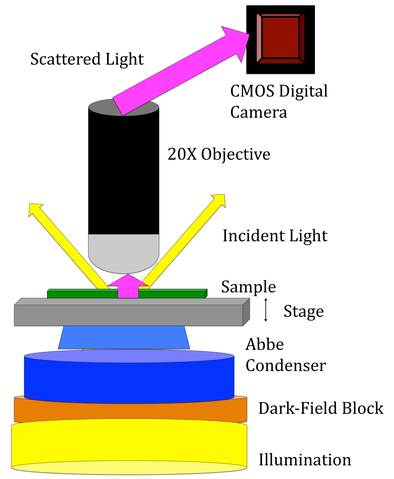
Gold nanoshells in H1299 lung cancer cells

Bright-Field



Dark-Field

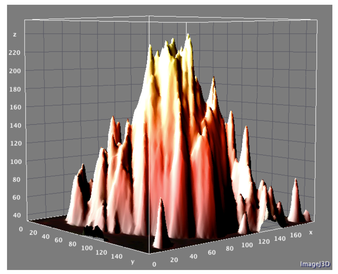




Dark-Field Microscope

Quantification

Approximately 650 gold nanoshells were found in the image of a single H1299 cell. ICP-MS analysis measurements suggest 200 gold nanoshells per cell, based upon mass averaging of gold mass per nanoparticle. Dark-field imaging however, is able to account for the exact location of the nanoparticles, thus nanoparticles both inside and outside the cell were counted.



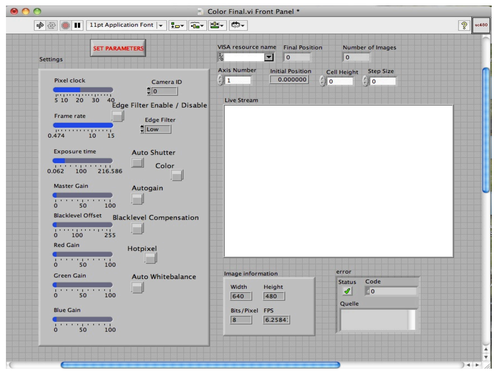
Three-dimensional representation of gold nanoshells within a cell

Cell Internalization of Nanoshells

H1299 lung cancer cells were used in nanoparticle uptake for their ease of culture and nanoshell uptake. The cells were incubated with commercially-produced gold nanoshells (150 nm diameter, 120 nm core, 15 nm shell) were imaged within for a period of approximately four hours.

Image Collection: Software Design

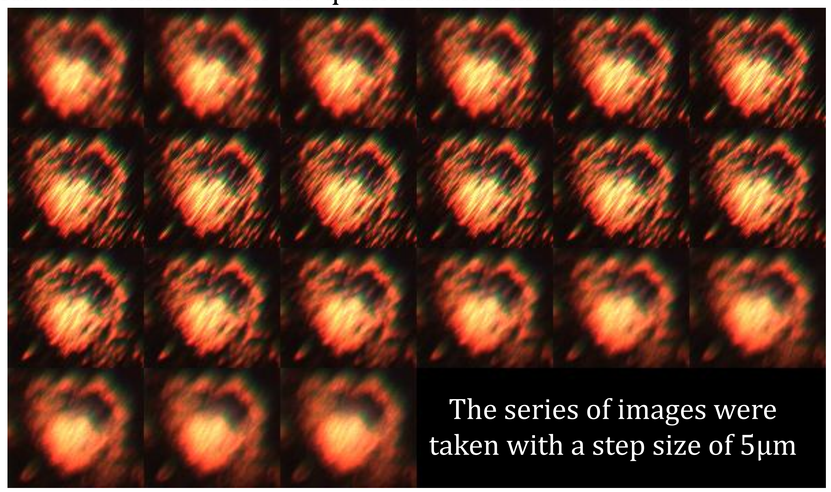
A custom-designed LabVIEW™ program was used to synchronize z-axis stage movement with the image-capturing of a 32-bit CMOS digital camera



Custom LabVIEW™ user interface displaying imaging controls

Image Processing: Identification of Nanoshells

The position of each nanoparticle is determined by scanning the z-stack for the maximum intensity of each bright spot. Images were processed with ImageJ™ software's 3D Viewer. Nanoparticles were found above and below the exterior of the cell and these were included in the quantification measurements.



Z-Stack Images

Conclusion

The number of gold nanoshells can fit into a H1299 lung cancer cells is 650. Dark-field spectroscopy provides a low-cost, fifteen minute method of gold nanoshell quantification. Using the known number of gold nanoshells that can be contained within a cell, developments can be made to deliver precise dosages of biological material using gold nanoshells and the light-triggered release method

Future Work

- Measure uptake response to a change in the size of gold nanoshells or cell type
- Observe quantitative change in cellular uptake due to varied incubation conditions

Acknowledgements

I would like to thank Dr. Surbhi Lal, Mr. Jared Day, and Mr. Michael McClain for their assistance in this project. Additionally, I would like to thank Dr. Junichiro Kono, Dr. Cheryl Matherly, and Ms. Sarah Phillips for the opportunity to conduct research and work alongside our Japanese colleagues in nanoscience.

This material is based upon work supported by the National Science Foundation's Partnerships for International Research & Education Program (OISE-0968405).