Modelling Experimental Injection Profiles for HPLC Simulations

Tyler Brau and Dwight Stoll

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The use of simulations in studying the fundamentals of separations in high performance liquid chromatography (HPLC) has proven to be useful for modelling the non-ideal aspects of real-life chromatographic conditions. Simulations have also allowed for more detailed investigations into the affect various chromatographic parameters have on the quality of a separation, such as column length, void volume, flow rate, and mismatch between sample and mobile phase solvents. However, it has been shown that the use of real experimental injection profiles is necessary in order to obtain accurate results. This has limited the number of conditions that can be simulated, as each simulation must use an injection profile specific to the chromatographic conditions that has been measured experimentally. Measuring a large number of injection profiles is time-consuming, making the simulation of separations at an arbitrary set of conditions difficult. In this presentation we will describe recent work on the development of a new equation that can approximate the shape of experimental injection profiles, with parameters that are dependent upon the volume of the injection as well as the flow rate. We have developed a general procedure for estimating the parameters of this equation for a given injection profile, and a procedure for estimating the shape of an injection profile at a given injection volume and flow rate. These procedures enable accurate simulation of many chromatographic conditions based on a relatively small set of experimental measurements.

American Bison Mating and Behavior

Abby Carsten and Jon Grinnell

Gustavus Adolphus College

While the American Bison (*Bison bison*) was once a keystone species of the Great Plains, human activity devastated this species to the point where less than a thousand remained by the end of the nineteenth century. Maximizing genetic diversity and natural behavior are challenges of the utmost importance for the future of this majestic creature and the health of our prairies. The Samuel H. Ordway Prairie herd of about 300 bison was observed over mating season of 2006 (July-August) and mating behaviors were recorded for sexually mature males (age 4 and older). The following year, DNA parentage data was collected from the roughly 100 calves born that year. This data was used to compare the hours a bull spends "tending" (a behavior which entails following and guarding a female) to his mating success by number of calves fathered. Preliminary results show a trend of more time spent tending correlating to higher mating success except for the few most dominant bulls which spend less time tending but still have more successful matings. More in depth analysis with different parameters should be examined to determine a relationship. When this study is finished it may provide valuable information to herd managers for maximizing the genetic diversity of their bison.

The Impact of Acetyl-CoA Carboxylase 2 mutants on Prolyl Hydroxylase 3 Function

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In low nutrient condition or cellular stress, AMP-activated protein kinase (AMPK) phosphorylates acetyl-CoA carboxylase (ACC) at residue S212, which activates mitochondrial fatty acid oxidation (FAO) to boost ATP levels. In high nutrient condition, stress sensing enzymes prolyl hydroxylase 3 (PHD3) hydroxylates ACC2. ACC2 then activates the conversion of acetyl-CoA to malonyl-CoA, which inhibits the fatty acid transport protein CPT1 and thus represses FAO. Since ACC2 hydroxylation site (P450) is essential for PHD3-mediated FAO, we hypothesized that human ACC2 P450A or mouse ACC2 P440A mutant will inhibit the hydroxylation of ACC2 by PHD3 and thus activates FAO. In contrast, human ACC2 S222A or mouse ACC2 S212A mutant promotes the hydroxylation of ACC2 by PHD3 and represses FAO as a result. The goal of the study is to generate ACC2 S212A and P440A mutant in C2C12 cell line (mouse mesenchymal stem cell) by utilizing CRISPR/Cas9 system. We cut our target sites using 6 different single guide RNAs (sgRNAs) and established knock in mutant with single strand ODN (ssODN). sgRNA and ssODN were transfected using FuGENE 6 and colonies were selected to get one site mutated CRISPR cell lines. Genomic DNA was sequenced to confirm CRISPR mutants and the cell proliferation will be measured. The ultimate goal is to examine the regulation of fat oxidation by PHD3 and the role of P440 and S12 residue in ACC2 in mouse cell system for the first time, and can help identify new pathways in lipid metabolism.

Improving Compostable Plastics: Synthesis of a Substituted Glycolide Monomer from Citric Acid

Sarah Claessens and Thomas Hoye

University of Minnesota

Sustainable polymers have become increasingly more sought after. Polylactic acid (PLA) has proven to be compostable and sustainable in origin, however PLA is unsuitable for certain applications, such as film blowing, due to its low melt strength. To increase the melt strength, we synthesized a new monomer from citric acid to be copolymerized with PLA. This monomer was synthesized in three steps without using protecting groups. We developed a method to purify the compounds via recrystallization, distillation, and/or column chromatography. This potential copolymerization of this novel monomer with lactide is capable of inducing branching, a method known to increase its melt processing strength. Potential applications of this copolymer are varied, but most notably is the manufacturing of plastic grocery bags that degrade in a much shorter time frame than current materials.

Instability and Complexity of Polycyclic Musk Galaxolide

Amy Crawford and Dwight Stoll

Gustavus Adolphus College

Galaxolide is a member of a family of polycyclic musks used as a fragrance in many consumer products. There have been numerous reports describing detection of galaxolide in wastewater treatment plant (WWTP) effluents and surface waters in Minnesota. It has been found to interfere with the MDR/MDX defenses of aquatic life, ultimately affecting their ability to protect themselves from other toxins present the environment. It is also suspected to be an endocrine disrupting compound and been labeled as a contaminant of emerging concern by the Environmental Protection Agency.

Although there is a fair amount of work published on studies of the fate of galaxolide in the environment and wastewater treatment streams, in our own work we have encountered a number of difficulties that have not been reported previously, to the best of our knowledge. In this presentation we will describe the results of a number of lines of inquiry related to these peculiarities.

To the best of our knowledge, a highly pure analytical standard for galaxolide is not commercially available. We will describe results of different approaches to produce highly pure galaxolide on a semi-preparative scale. We also find that different species present in commercially available galaxolide materials exhibit very different detector responses when using liquid chromatography coupled with UV absorbance or electrospray ionization masss spectrometry. Finally, we find that highly purified galaxolide is prone to rapid (timescale of minutes) transformation when exposed to water.

Landslide Hazards and Impacts on Minnesota's Natural Environment

Emily Fischer, Alex Senjem, Laura Triplett, and Julie Bartley

Gustavus Adolphus College

An excessive amount of sediment in the Minnesota River has resulted in environmental sustainability issues that can affect water quality and the riparian ecosystem of the river. Most of these sediments are coming from bluff erosion and landslides that are adjacent to the stream. Landslides have also damaged infrastructure and endangered people's lives in MN. Understanding where these features are located within MN may help support policies where hazard areas are monitored more closely.

In this research, we will identify the distribution, frequency, and the failure types of landslides by using GIS (Geographical Information System) to digitize these features. In the end, we will have a geospatial database that documents the slide locations.

Detection of Atmospheric Gravity Waves through Wavelet Analysis

Espen Fredrick, Vatsala Adile, and Darsa Donelan

Gustavus Adolphus College

This study investigated the use of Morlet wavelet analysis in the detection of gravity wave structure in the atmosphere of terrestrial bodies, primarily Mars, Venus, and Titan. Atmospheric profiles from data collected by planetary probes and satellites were processed to generate 2D images of wave structure in each analyzed atmosphere. The analysis shows a correlation between vertical wave structure at altitudes and wavelengths to those previously found using other methods such as comparing temperature gradient profiles to the dry adiabatic lapse rate. This suggests the use of Morlet wavelet analysis as a viable alternative to previously used methods for detection of small-scale variability.

Photodegradation of the herbicide, Dicamba (3,6-Dichloro-2-methoxybenzoic acid), in Aqueous Solutions and on Plant Surfaces

Kaitlyn Gruber and Dr. Amanda Nienow

Gustavus Adolphus College

Dicamba (3,6-Dichloro-2-methoxybenzoic acid), a broadleaf herbicide, was exposed to UV radiation in aqueous solutions and on surfaces of epicuticular waxes of corn and soybean leaves to observe the photochemical degradation. HPLC analysis of the dicamba samples was done to track degradation and calculate the rate constants for quantitative comparisons. The irradiation of aqueous solutions of dicamba under different conditions were compared to observe the effect of environmental factors on the photodegradation. Aqueous solution rate constants were significantly slower in increased acidic conditions and increased NOM concentrations, but excess and reduced oxygen concentrations did not affect the kinetics rate constant. In addition, faster rates of photodegradation were observed in the aqueous solutions than the wax surfaces. Mass spectrometry was used to analyze aqueous solution samples of irradiated dicamba to determine possible photoproducts.

Directionality in Neural Network Avalanches

Kathryn Hagen and John Beggs

Indiana University

Incredible progress has been made concerning macroscopic study of connectivity between brain areas, but microscopic brain connectivity on the network scale remains little understood. Recent technical innovations have made it possible to analyze neural networks on the spatial and temporal scale of individual neurons and action potentials, allowing us to elucidate microscopic

network mechanisms. We used a novel 512-microelectrode array to record action potentials in a network of rat cortical tissue and examine avalanches, which are single propagating bursts of electrical activity. Statistical physics determined significant connections between individual neurons. We analyzed information flow in connectivity space, which filtered out insignificant connections, and sorted neurons by physical parameters to determine whether patterns exist or information propagates randomly. Frequency of action potentials, or firing rate, and number of outgoing connections seem to be significant parameters. On average, neurons fire to higher firing rate and lower outgoing connection neurons. Thus far, these results seem consistent with previous theories concerning information flow through different layers of the cortex. This unique view of neural networks in connectivity space may provide great insight to information flow in the brain.

Population-specific interactions of sex and developmental phenotypic plasticity on the thermal tolerance of a marine copepod.

Sydney Hedberg and Hans Dam

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Understanding how populations respond and adapt to temperature is important in predicting how ongoing climate change will impact organisms. Copepods, the most abundant animals in the oceans, link primary producers to fish and mediate biogeochemical cycles, yet, our understanding of the various factors that impact their thermal tolerance is still limited. Variation in thermal tolerance, the ability to withstand thermal stress, can result from both genetic adaptation and developmental phenotypic plasticity, but the effects of sex on thermal performance often go uncharacterized. This study aims to describe the sex-specific response of the ubiquitous copepod, *Acartia tonsa,* to increased temperatures, and the mechanisms that underlie this response. Common garden experiments were used to test for effects of local adaptation (Connecticut vs. Florida), developmental phenotypic plasticity ($18℃$ vs. $22℃$), and individual sex on the thermal performance of *A. tonsa*. Female thermal tolerance was significantly higher than that of males in both populations. While the thermal tolerance of Florida individuals was significantly higher than Connecticut individuals, developmental phenotypic plasticity was only observed in the Connecticut population. Lower male thermal tolerances suggest that sperm limitation may decrease copepod population size under future temperature increases, possibly having implications on the oceanic food web. Furthermore, the population comparisons imply that low latitude populations are more vulnerable to projected warming despite having a higher thermal tolerance, because they lack the ability to respond to rising temperatures through developmental phenotypic plasticity.

Drugging Protein-Protein Interactions: The KIX Domain

Kira Holton, Kati Williams, and Scott Bur

Gustavus Adolphus College

Creating drugs that can target protein-protein interactions is the next frontier for drug discovery. Drugs that can target protein-protein interactions would be extremely beneficial, especially for diseases such as Mixed Lineage Leukemia (MLL). However, the nature of the protein-protein interaction makes finding ligands much more difficult. New techniques are needed to make this route of drug discovery possible. Fragment-based ligand design and Protein- Observed fluorine (PrOF) NMR can circumvent some of the problems posed by the nature of the protein-protein interaction. Fragments were made to specifically target the interactions between MLL fusion proteins and the KIX domain of the CREB binding protein. The fragments of interest include GAMLE1, GAEEB1, and GAKEH1. PrOF NMR provided the location of binding of the synthesized ligand fragments, and displayed if the shifts were dose dependent.

The Simulation of Complex Liquid Chromatography Systems

Thomas Lauer, Tyler Brau, and Dwight Stoll

Gustavus Adolphus College

As the performance of liquid chromatography (LC) columns and instruments continues to improve through advances in particles, technology, design, and operation of various instrument components, it is becoming increasingly apparent that the use of computers and simulations is invaluable. The large amount of time and money that these systems save is valuable when compared to the cost of performing the same process in a laboratory. Recently, through our development of web applications (web apps), we are making the understanding of complex LC concepts more accessible to the general public. These concepts include the effect of extra-column dispersion on separation efficiency, and simulation of sample injection and analyte elution processes for liquid chromatography.

Multidimensional chromatographic approaches for the analysis of biopharmaceuticals

Gabriel Leme, Hayley Lhotka, Dwight Stoll

Gustavus Adolphus College

Recombinant therapeutic proteins (as monoclonal antibodies and their derivatives) represent the fastest growing category of biopharmaceutical proteins[1] and the largest market segment of current biopharmaceutical products. They have become the most promising class of therapeutics for many applications, particularly in cancer, immune disorders, and infectious diseases[2]. These

drugs are manufactured through recombinant DNA technology, custom-designed to target specific antigens and offer unique pharmacological advantages as specificity, selectivity, long half-life, and excellent safety profile. They have a large and complex chemical structure and can suffer a variety of modifications during the production process, for instance, variations in the primary sequence that can result in a series of charge variants[3], and heterogeneous glycosylation[4]. This structural complexity and heterogeneity imposes tremendous challenges - even for the most advanced analytical techniques, in order to ensure that the therapeutic protein meets predefined quality specifications. This work will summarize the recent advances achieved by our group for the analysis of biopharmaceuticals using two-dimensional liquid chromatography coupled to mass spectrometry (2DLC-MS) and middle-up approaches. The results obtained so far reinforce the power of the technique and suggest its use for the assessment of quality of biopharmaceuticals products.

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Increased resolving power and detection sensitivity of two-dimensional liquid chromatography for bottom-up analysis of therapeutic proteins

Hayley Lhotka, David C. Harmes, Benjamin Madigan, Gabriel M. Leme, Gregory O. Staples, and Dwight R. Stoll

Gustavus Adolphus College

The biopharmaceutical industry relies on separation, identification, and quantitation of peptides produced from enzymatic digestion of protein therapeutics to demonstrate product knowledge to regulatory agencies. Peptide-level characterization is implemented throughout the drug development process, including during optimization of cell culture conditions, and monitoring the stability of formulations. Mass spectrometry is an indispensable tool for these applications. Nevertheless, improvements in separation methods to simplify samples presented to the mass spectrometer generally improve the quality of identification and quantitation of results. Demand for increased separation power on shorter timescales is building for multiple reasons. Among these factors are the interest in using LC/MS for host-cell protein (HCP) characterization, the emerging multi-attribute method (MAM), and development of peptide-level process analytical

technologies. We have optimized a two-dimensional liquid chromatography (2D-LC) approach involving reversed-phase separations in both dimensions – the first at high pH and the second at low pH. We have explored a number of variables in search of optimal second dimension columns and conditions for this application, including second dimension cycle time (20-90 seconds), column length (20-50 mm), particle size (1.8 to 5.0 micron),particle morphology (superficially porous vs. fully porous), stationary phase chemistry, and second dimension flow rate (1.0-2.5 mL/min.). These experiments have enabled a deeper understanding of the compromises between separation peak capacity, analysis time, and detection sensitivity faced by analysts using 2D-LC. Furthermore, the deeper understanding gained by these experiments have allowed us to use our methods to tackle complex biological separations and work towards solutions for problems, like HCP contamination, facing the biopharmaceutical industry.

Diving Deep – Continued Studies of the Longitudinal Diffusion Coefficient in Liquid Chromatography

Devin Makey[1], Huiying Song[2], Monika Dittmann[3], Dwight Stoll[1], Gert Desmet[4], Deirdre Cabooter[2]

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Liquid chromatography is used for the separation of a mixture by passing it in solution through a medium in which the chemical constituents of the mixture move at different rates. Many modern applications of liquid chromatography require efficient separations with narrow peaks, however, peak broadening can cause separations to be less efficient. The longitudinal diffusion coefficient (i.e., the b-term in efficiency vs. flow rate relationships) is the only source of axial peak broadening in the absence of mobile phase flow through the column. A detailed understanding of the b-term is essential when studying mass transfer mechanisms in high performance liquid chromatography (HPLC). In spite of more than five decades of study of the b-term by hundreds of investigators, there are still important unanswered questions about this peak broadening mechanism. In this presentation we will describe recent efforts in our laboratories to refine and develop approaches that will yield deeper understanding on this topic.

Most recently we have been using a so-called peak parking approach to study the effects of chromatographically relevant variables on the b-term, including the test analyte, mobile phase composition, stationary phase chemistry, and particle pore size. Using these data we are making a first attempt is made to rationalize the parameters influencing the b-term in packed particle columns.

Availability and accessibility of community assets that could increase physical activity and nutritious eating among children of migrant farmworkers in Elysian, Minnesota

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University of Minnesota

Migrant farmworkers are people that migrate from their permanent home in order to find temporary work in the agricultural sector. The children of migrant and seasonal farmworkers have a high prevalence of overweight and obesity, with reports ranging from 31% to 73%. The purpose of this study was to identify and describe assets that could promote healthy eating and increase physical activity of migrant children going to school in the Elysian community. In person interviews were conducted with ten staff members from the Elysian Migrant Head Start; phone interviews were then conducted with businesses/organizations that were mentioned as assets during the in person interviews (2 businesses/organizations) and some that were not identified as assets (2 businesses). Responses were analyzed to create a community asset map and the strengths and barriers for the assets and recommendations they had for the community were noted. There were five types of assets that were identified (city parks, a state trail, a migrant Head Start, a library, and a bakery & deli). Recommendations for the Elysian community included building a grocery store, fostering partnerships between city governmental offices and organizations working with children, and increasing the availability of vegetarian and more nutritious options. Throughout the interviews, the information on the availability of other assets was strong. However, there was a lack of knowledge between the different assets on what resources or options they offered that could help children of migrant farmworkers live healthier lives.

Investigating the Role of LMO3 in Dopaminergic Neurons and Parkinson's Disease

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National Institute of Health

LMO3 is a transcriptional corregulator protein that is highly expressed in the same region of the brain that is more vulnerable to degeneration in Parkinson's disease (*substantia nigra pars compacta*). LMO3 could be a key molecule to understand this vulnerability, but its function is still relatively unknown. This project focused on verifying results from a yeast two-hybrid screen in HEK293T cells in an effort to increase understanding of what LMO3 interacts with in cells and give insights to its potential function.

Estimating invasive potential of Echinacea pallida and its hybrids (E. pallida x angustifolia) in a severely fragmented prairie habitat

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Echinacea pallida is a long-lived prairie forb that was recently introduced to a prairie restoration in Douglas County, Minnesota. It naturally forms hybrids with its native congener *Echinacea angustifolia* via vector-mediated pollination within and between prairie remnants. This study tests the ecophysiology and viability fitness of exotic basal *Echinacea pallida* and its hybrids (*pallida* x *angustifolia*) relative to *E. angustifolia*. To test this, four crosstypes (two conspecifics and two reciprocal crosstypes) were planted in a common garden in 2013, and survival and leaf morphology were measured in following years. In 2018, data on leaf thickness, photosynthetic rate (A_{max}) and water use efficiency (WUE) were also gathered. *Echinacea angustifolia* conspecific crosses had the lowest survivorship, A_{max}, WUE, leaf number, longest leaf length, and leaf thickness relative to hybrids and *E. pallida*. These results indicate that basal *E. angustifolia* have lower viability fitness than *Echinacea* hybrids and *E. pallida* in native *angustifolia* habitat. The potential for genetic swamping of *E. angustifolia* facilitated by vector-mediated pollination between remnants is high; proper management strategies need to be employed.

Investigating Class I Lanthipeptide Clusters Containing Isoaspartate Methyltransferases

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Lanthipeptides are a class of ribosomally synthesized and post-translationally modified peptides that are characterized by the thioether crosslinks that occur between cysteine and dehydrated serine or dehydrated threonine residues. These cyclized peptides display remarkable stability and bioactivity, with many lanthipeptides exhibiting antimicrobial properties. Genome mining efforts have been used to uncover potential sources of lanthipeptides, and genes for additional post-translational modifications seem to commonly occur with the genes necessary for lanthipeptide production. The most common gene that occurs in the lanthipeptide clusters is an isoaspartate methyltransferase-like gene, though its function in lanthipeptide synthesis is still under investigation. Four strains of *Actinobacteria* containing lanthipeptide and isoaspartate methyltransferase-like genes were investigated for potential lanthipeptide production. MALDI-TOF-MS data was utilized to study lanthipeptide production by the *Actinobacteria* strains, with some promising peaks being flagged for further study. The final stage of the experiment involved the cloning of the lanthipeptide gene cluster into *Escherichia coli* for further investigation, with three of the four genes being successfully inserted.

Modeling the consequences of increased kinetochore protein levels in cancer cells using yeast

Maicy Vossen and Laura Burrack

Gustavus Adolphus College

Cancer is a disease of uncontrolled cell division, which is caused by problems within the cell cycle. Factors that disrupt the cell cycle include DNA mutations and aneuploidy resulting in abnormal amounts of proteins in the cell. In certain types of cancer, there is an increased amount of a protein called CENP-A, and CENP-A overexpression correlates with poor patient prognosis. CENP-A is a protein in the kinetochore complex that is essential for microtubule attachment and chromosome segregation. To begin to understand why CENP-A overexpression has such severe consequences, a yeast model, *C. albicans*, was used to monitor growth differences using genetic and pharmacological approaches. We found that there was no significant difference between the growth rates of *C. albicans* with and without CENP-A overexpression. However, our results showed that alterations of the CENP-A protein levels changed how cells responded to cyclosporine, a proposed anti-cancer drug. Currently, we are characterizing the mechanism of how CENP-A overexpression may alter the effectiveness of cyclosporine.

Implications of dispersion in connecting capillaries for separation systems involving post-column flow splitting

Harrison Willenbring, Eli Larson, Thomas Lauer, Monika Dittmann, Dwight R. Stoll

High-performance liquid chromatography (HPLC) is a powerful analytical tool used to separate and identify the constituents of complex mixtures. In the process of HPLC a sample is injected into a solvent, called the mobile phase, that flows from the injection point to the separation device, called a column. Numerous microscopic particles coated in a stationary phase reside in the column, and as compounds from the sample pass through they are retained for different periods of time based on their individual affinities to the stationary phase. After these compounds exit, or elute from, the column they are detected by some type of detector, typically a UV/VIS diode-array detector (DAD) or mass spectrometer (MS). In either case, a signal is sent to the operating computer in the form of peaks that provide useful data regarding the separation. These peaks are subject to numerous changes based on the system configuration, including flow rate, connecting tubing dimensions, mobile phase composition and so on. In this work we specifically investigated the degree of broadening (i.e. increases in peak width) that occurs when using connecting tubing of different dimensions under conditions where part of the mobile phase flow exiting the column is split and diverted to waste – so-called 'flow splitting' conditions. We found that adding a post-column split will decrease the original variance of the peak by the split factor (e.g. a split factor of two will result in a peak with half of the original variance), which in turn makes the peak much more susceptible to broadening within connecting tubing. Our results act as a guideline to other chromatographers in selecting connecting tubes of appropriate dimensions that will help prevent this unwanted broadening effect under flow splitting conditions.

Cosmological Dependence of the First Order Power Spectrum Response

Xiaoqi Yu and Alexandre Barreira

Max Planck Institute of Astrophysics

We measure the cosmological dependence of the first order power spectrum response to a long wavelength isotropic perturbation using the separate universe simulation method. As a first step, we limit ourselves to explore the cosmological dependence on matter density parameters Ωm . By taking the amplitude of perturbation to be $\delta L = \pm 0.05$ for all cosmologies, we find that the value first order response depends on Ωm. On the scale of $k = 2.67$ h/Mpc, the response for matter density parameter on Planck's upper 95% confidence interval of $\Omega m = 0.3213$ falls 5% below the response for mean density parameter with $\Omega m = 0.3089$, and the response for density parameter on Planck's lower constrain $\Omega m = 0.2965$ is about 10% higher than that for the mean. These results also indicate the possible dependence of the responses on other cosmological parameters, which can be found using the same method as we employed here. The cosmological dependence of the responses, which has been neglected before, can be a concern when taking the response approach to measure the higher order statistics of the large scale structure.

NIR-NIR Upconversion Nanoparticles for Latent Fingerprint Development: Determination of Optimal Particle Size

Michaela Zachman and Stanley May

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Upconversion phosphors are materials that generate luminescence at shorter wavelengths relative to the excitation light. NIR-to-NIR upconversion nanoparticles (NIR-NIR UCNP), consisting of $NaYF_4$:Yb,Tm, show great promise for the development of latent fingerprints. NIR-NIR UCNP developed fingerprints can be imaged in ambient light and on substrates which have fluorescent properties or 'busy' backgrounds. An investigation into the effect of the size of the upconversion nanoparticles on the quality of the developed fingerprint was conducted. NIR-NIR UCNP covering a range of particle sizes were applied in powder form via two different techniques to develop latent fingerprints. The developed fingerprints were characterized using optical imaging and scanning electron microscopy. Image quality was assessed based on image intensity, resolution, and contrast. No significant size-dependent differences were found for the resolution or contrast.