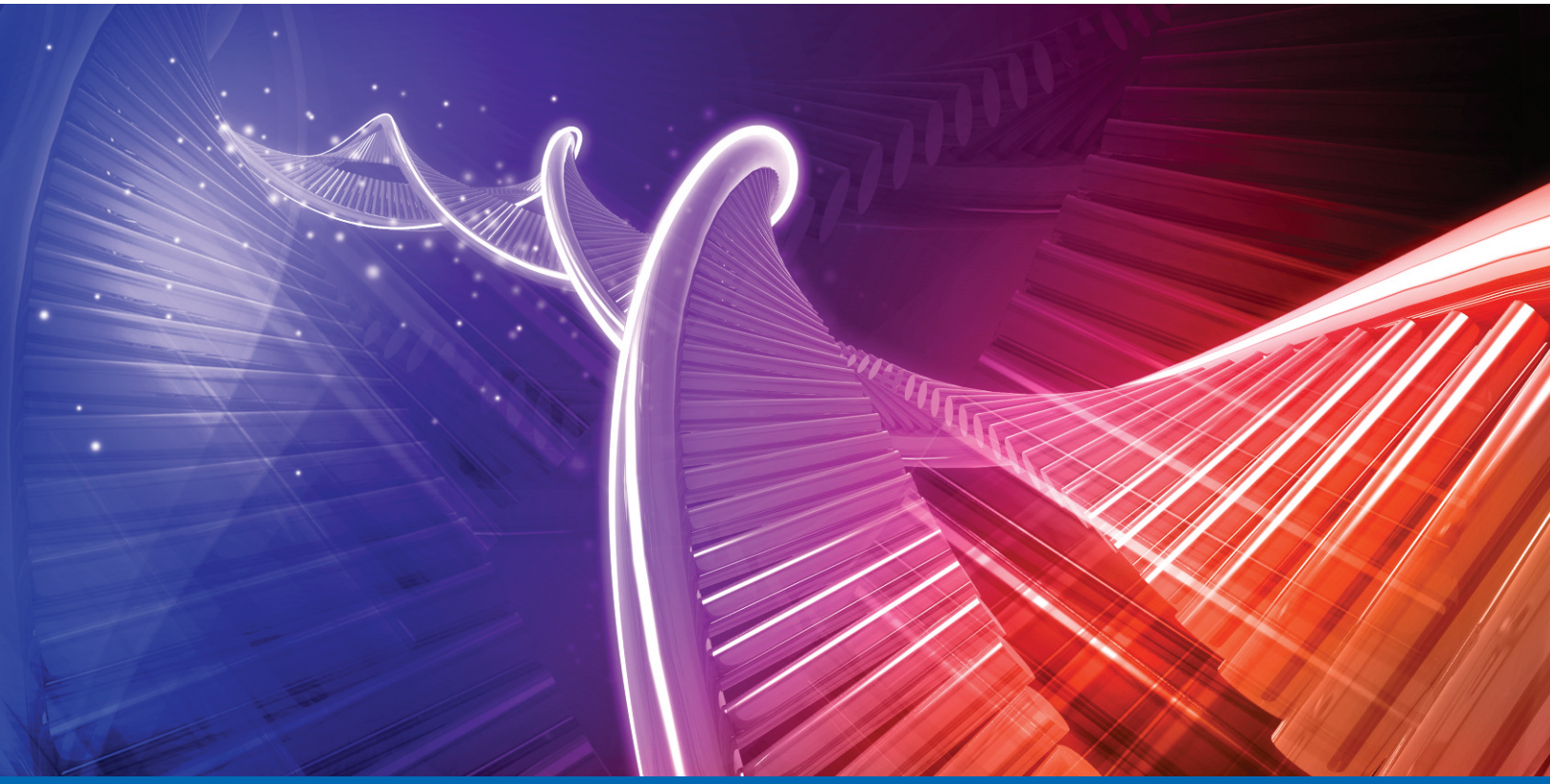
Biopharmaceutical Process and Quality Consortium

3nd Biopharmaceutical Summit 2014



MAY 27-30, 2014

May 29-30, 2014: Workshop on PAT and QbD in Biopharmaceutical Industry

May 27-28, 2014: Advanced Training on PAT and QbD Principles in Biopharmaceuticals

Hosted by Biopharmaceutical Process and Quality Consortium (BPQC), Massachusetts BioManufacturing Center (MBMC), University of Massachusetts Lowell, Mass Biologics (UMass Medical School)

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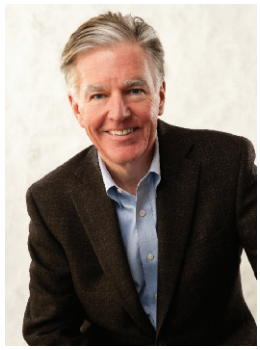
Learning with Purpose

University of Massachusetts Lowell

May 27, 2014



Dear Attendees,

On behalf of the entire faculty and staff at the University of Massachusetts Lowell, welcome to our campus.

We are thrilled to be hosting the Biopharmaceutical Process and Quality Consortium's **Third Annual Biopharmaceutical Summit.**

Over the next four days, you'll join scientists, engineers and industry representatives in discussing the most pressing and industrially relevant biopharmaceutical challenges of our times.

There will be workshops, in-depth skills training and keynote speeches by industry leaders from esteemed agencies like the FDA. And there will be plenty of opportunities for networking with professionals from major biopharmaceutical manufacturers and technology providers such as Biogen Idec, Genzyme, Pfizer, Momenta, Millipore and GE.

This Summit is truly a collaboration between academia and industry. We are confident—given the diverse backgrounds of those of you here this week—that by the end of your time at UMass Lowell, you will have collectively taken significant steps in providing a roadmap for the next-generation biopharmaceutical industry.

Thank you for joining us.

Sincerely,

Martin T. Meehan, '78
Chancellor

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SCHEDULE

Part I. Advanced Training on PAT/QbD in Biopharmaceuticals

May 27-28, 2014 (Tuesday and Wednesday), 8:00 AM - 5:00 PM

Module #	Description	Type	Instructor
Day 1	**Multivariate Batch Modeling**		
M1, 8 AM	Batch Evolution Modeling I (Modeling)	Lecture	NS
M2, 9 AM	Batch Evolution Modeling II (Validation)	Lecture	SY
M3, 10 AM	Case Study Demonstration	Tutorial	NS/SY
M4, 11 AM	Tutorial on Batch Evolution Modeling	Tutorial	NS/SY
12 PM	Lunch		
M5, 1 PM	Batch Level Modeling I	Lecture	NS
M6, 2 PM	Tutorial on Raw material and CC impact on CQA	Tutorial	NS/SY
M7, 3 PM	Batch Level Modeling II	Lecture	SY
M8, 4 PM	Tutorial on CQA Prediction	Tutorial	NS/SY
Day 2	**QbD and Design Space Assessment**		
M9, 8 AM	DOE Concepts	Lecture	SY
M10, 9 AM	Modeling, Optimization and Validation	Lecture	NS
M11, 10 AM	Tutorial on Modeling Building with Media/CQA	Tutorial	NS/SY
M12, 11 AM	Tutorial on Robustness and Design Space	Tutorial	NS/SY
12 PM	Lunch		
M13, 1 PM	Design Space	Lecture	SY
M14, 2 PM	Tutorial on Design Space Building	Tutorial	NS/SY
M15, 3 PM	CQA Specification	Lecture	NS
M16, 4 PM	Tutorial on CQA Specification	Tutorial	NS/SY

Notes: NS: Nirav Shah (Umetrics); SY: Seongkyu Yoon (UMass Lowell)

Part II. BPQC Business Meeting and Reception

May 28, 2014 (Wednesday), 1:00 PM - 6:30 PM

1:00 PM (Location TBD)	**Business meeting with consortium member companies.** For appointment, RSVP (bpqc@uml.edu or seongkyu_yoon@uml.edu, 978-934-4741)
6:30 PM (Location TBD)	**RECEPTION** (Trainees, BPQC members and all workshop participants are invited)

Part III. Workshop

May 29 (Thursday): 8:00 AM – 5:00 PM

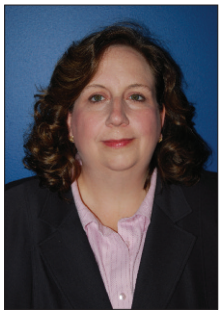
8:00 AM	**REGISTRATION**
8:30 AM	**WELCOME AND INTRODUCTION** (UMass Lowell and organizer)
8:40 AM	**Session I: PAT/QbD, Motivation and Perspectives** Morning Session Chair: Dr. Thomas Ryll, Sr. Director of Cell-Culture Development, Biogen Idec (8:40-8:50 AM) **PLENARY PRESENTATION:** Dr. Christine Moore, Program Director of FDA/CDER, **"Challenges and Opportunities for Continuous Pharmaceutical Manufacturing in QbD framework"** (8:50-9:30 AM) **"Application of PAT to support QbD in Bioprocessing"** Jeff Doyle, Manager of Process Analytical Sciences (9:30-10:00 AM)
10:00 AM	**BREAK**
10:20 AM	**"Real-time cell culture control in an integrated benchtop platform: implications for research"** Jean-Francois P Hamel, PhD, MIT/Chem Engineering (10:20-10:50 AM) **"Improved Measurements and Standards to Support the Development of Protein Therapeutics"** Michael Tarlov, PhD, Chief, Biomolecular Measurement Division, NIST (10:50-11:20 AM) **PANEL DISCUSSION** (11:20 -12:00 PM) Panelists: Dr. Ryll (BiogenIdec) and Speakers
12:00 PM	**NETWORKING LUNCH**
1:30 PM	**Session II: Challenges and Practices** Afternoon Session Chair: Michael Tarlov, PhD, Chief, Biomolecular Measurement Division, NIST **PLENARY PRESENTATION 2: "PAT, Future of Biotechnology"** Kurt Brorson, PhD, Head, Division of Monoclonal Antibody, FDA/CDER (1:30-2:00 PM) **"Rapid Development and Scale-up of Therapeutic Antibodies – A QbD Perspective with a Case Study for a Biosimilar Trastuzumab"** Kumar Dhanasekharan, PhD, Director of Process Development, Cook Pharmica LLC, (2:00-2:30) **"Biologics Advanced Process Control at BiogenIdec"** LiLong Huang, PhD, Sr Engineering, Manufacturing Sciences, BiogenIdec, (2:30-3:00 PM)
3:00 PM	**BREAK**
3:10 PM	**"Perspective in QbD in Drug development and Manufacturing"** Bert Frohlich, PhD, Director of Bioengineering, Shire, (3:10-3:40 PM) **"Using QbD and PAT to Develop Adaptive Manufacturing Processes"** Jose Gomes, PhD, Principal Engineer, Pfizer (3:40-4:10 PM) **"Development application and single use probes for building Design Space of live cell concentration by dielectric spectroscopy"** John Carvell, PhD, Managing Director, Aber Instrument (4:10-4:40 PM) **PANEL DISCUSSION** (4:40 – 5:20 PM) Panelists: Michael Tarlov and Speakers
5:20 PM	**RECEPTION AND NETWORKING POSTER SESSION** 20-30 posters will be prepared

May 30 (Friday): 8:00 AM – 5:00 PM

8:00 AM	**REGISTRATION**
8:20 AM	**Session III: Challenges and Practices 2** (8:20-8:30 AM) Morning Session Chair: Dr. Jack Prior, Sr. Director of Manufacturing Sciences and Technology, Genzyme **"QbD in Drug Product Formulation"** Andrew Cowen, CEO of of Biopharma Technology Ltd. (8:30-9:00 AM) **"Exploring the linkage between cell culture and downstream processing via statistical design"** Erik Read, PhD, Principal Scientist, Division of Monoclonal Antibody, CDER/FDA (9:00-9:30 AM) **"Raman spectroscopy applied to monitoring, modeling and control of biologics production"** John-Paul Smelko, Sr. Engineer, Cell-Culture Development, BiogenIdec, (9:30-10:00 AM) **"QbD in Continuous mAb production"** Tim Johnson, PhD, Associate Director, Commercial Cell-culture development (10:00-10:30)
10:30 AM	**BREAK**
10:40 AM	**Session IV: Biopharmaceutical Consortium Session – Driving for Future** Session Chair: Dr. Kevin Bittorf, VP of LivingProof Inc. (Advisory Board) (10:40-10:50 AM) **"Consortium Research Update",** Consortium Faculty (10:50-11:20 AM) **"Consortium Graduate Research Update",** Graduate Students (11:20-11:50 AM) **"Core Research Facility and Cleanroom Facility",** Graduate Students (11:50-12:00 PM)
12:00 PM	**NETWORKING LUNCH**
1:30 PM	**Session V: Path-forward** Session Chair: Dr. Kevin Bittorf, VP of LivingProof Inc. **"Systems Technology in Biologics QbD Implementation"** Richard Braatz, PhD, Professor, Chemical Engineering, MIT,(1:30-2:00 PM) **"Regulatory Perspective on Implementation of MSPC for Pharmaceutical Manufacturing"** Bogdan Kurtyka, PhD, Review Chemist, FDA/CDER/ONDQA (2:00-2:30 PM) **"Understanding the effects of process conditions on protein glycosylation and proteoglycan synthesis"** Susan Sharfstein, PhD, Associate Professor, Chemical Engineering, SUNY Albany, (2:30-3:00 PM) **"QbD in Continuous Manufacturing of mAb"** Maurizio Cattaneo, PhD, CEO of Biovolutions, (3:00-3:30 PM) **PANEL DISCUSSION** (3:30 – 4:00 PM) Panelists: Kevin Bittorf and Session V Speakers
4:00 PM	**CLOSING REMARKS** (Sadettin Ozturk, PhD, Mass Biologics)

For questions: bpqc@uml.edu, or Seongkyu Yoon (Seongkyu_yoon@uml.edu; 978-934-4741)

PRESENTERS



Christine Moore, Ph. D.
Program Director, FDA/CDER

Dr. Moore is currently acting as the Director of the Office of New Drug Quality Assessment in FDA's Center for Drug Evaluation and Research. ONDQA evaluates the chemistry, manufacturing and controls information for all investigational new drugs for small molecules, new drug applications and supplemental new drug applications. Christine started at the agency in 2004 as the Branch Chief of the newly created Manufacturing Science group and later moved into the Deputy Office Director Position. Christine has been actively involved in FDA's Quality by Design efforts. Prior to joining the FDA, she worked for 10 years in API process development, scale-up, and Process Analytical Technologies at Pfizer and Searle/Pharmacia for both small and large molecule development. Her background is in chemical, biochemical and biomedical engineering, with degrees from Northwestern and MIT.

Title: Challenges and Opportunities for Continuous Pharmaceutical Manufacturing Under QbD framework

Abstract: While continuous processing has been long used in the chemical and food process industry, it is an emerging technology for pharmaceutical manufacturing. For both batch and continuous manufacturing, the regulatory expectations for assurance of quality remain the same. However, traditional approaches may need to be modified for continuous manufacturing due to the differences in operation. This presentation will discuss scientific and regulatory considerations for implementation of continuous manufacturing for new and existing pharmaceuticals. Applicants are encouraged to communicate early and frequently with FDA to discuss their proposed approaches.

Jeff Doyle
Manager of Process Analytical Sciences, Pfizer

Based in Andover Massachusetts, and supporting the delivery of value driven Process Analytical Technology for the US based Pfizer Specialty Biotech sites, Jeff leads multiple cross-functional PAT project teams. Before joining the Process Analytical Sciences Group (PASG) in 2011, Jeff led advanced data analysis and manufacturing process monitoring at the Andover site where he ultimately championed PAT. Achievements within PASG include successful PAT implementations in the manufacture of both mAbs and vaccines. He holds a B.S. in Chemical Engineering from Clarkson University.

Title: Application of PAT to support QbD in Bioprocessing

Abstract: At Pfizer, the Process Analytical Sciences Group (PASG) is synonymous with Process Analytical Technology (PAT) but it's the close collaboration with Research and Development that makes application of innovative technologies possible and successful in clinical projects. Application of PAT is considered during new process development as part of a systematic Right First Time approach to QBD. This approach aims to both prioritize application of PAT prior to filling and allow for developing technology integration post filing.

Jean-François Hamel, Ph.D.
Research Engineer, Chemical Engineering, MIT

Jean-François teaches biological and bioprocessing engineering laboratory courses and is a research engineer in MIT's Chemical Engineering Department, and an industry consultant. He has studied original upstream and downstream problems in varied microbial and cell culture processes, at the bench (down to µL) and pilot scales (up to cubic meter), and integrated advanced analytical technologies for process improvement. In his teaching and research Jean-François has had the opportunity to beta-test or evaluate novel technologies, such as the first rock-bed single-use bioreactor and portable microbial flow cytometer, in-situ glucose and O2/CO2 optical sensors, auto-samplers, modular analyzers, expanded-bed chromatography, and computer simulation tools. His current projects focus on biofuels from microalgae and yeast, vaccine antigen and monoclonal antibody from mammalian cells, stem cells, and proteins from microbes, which have been studied in traditional or single-use bioreactors. Jean-François received a MS in Biochemical Engineering from MIT, and a Ph.D. from the Pierre and Marie Curie University (part of "Sorbonne Universités").

Title: Real-time cell culture control in an integrated benchtop platform: implications for research and training

Abstract: Real-time control in an integrated benchtop platform is both an effective research tool for process development and improvement, and useful for training students about process integration. Consisting of a bioreactor, an autosampler and filter, and an analyzer this platform was employed to familiarize college students with the concepts of cell culture, process control and analytics, during a research project. In this case the modules were connected using Openness, Productivity and Collaboration (OPC) communication protocols, and development environment tools (LabVIEW), both of which enabled nutrient feeding and control during the fermentation. Using a PAT framework, E. coli was cultured in traditional stirred-tank and in disposable air-lift bioreactors. Samples were withdrawn automatically and filtered in line, and cell-free supernatants were analyzed for glucose and serine (with a biochemical analyzer or HPLC, respectively).

The integrated benchtop platform was employed successfully for: 1) measuring glucose and serine throughout the fermentation, and 2) designing and implementing a feed strategy. The at-line nature of the autosampler, filter and analyzer also motivated the creation of a sterile barrier downstream of the bioreactor.

Lessons learned from this study will be presented in the dual context of the current research literature using PAT/QbD-based approaches for a) monitoring or controlling batch or continuous bioprocesses, and b) for teaching.

Michael J. Tarlov, Ph.D.
Chief, Biomolecular Measurement Division, NIST

Dr. Tarlov is the Chief of the Biomolecular Measurement Division of the Material Measurement Laboratory at the National Institute of Standards and Technology in Gaithersburg, Maryland. The Biomolecular Measurement Division develops the measurement science, standards, technology, and data required to support the nation's needs for determining the composition, structure, quantity, and function of biological molecules. These efforts underpin advances in the areas of biotechnology, DNA forensics, biomedical and bioscience research, and health care. He received a B.A. in Chemistry from Colgate University and a Ph.D. in Analytical Chemistry from the University of Minnesota. During his career at NIST he has authored or co-authored more than 80 publications in the areas of self-assembled monolayers, biochemical sensing, and bioprocess measurements. He also leads the NIST Biomanufacturing Program, which develops measurement science, standards, reference data, and technology to support the development, manufacturing, and regulatory approval of biologic medicines.

Title: Measurement Science and Standards to Support the Development of Safe and Effective Protein Therapeutics

Author: Michael J. Tarlov, Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-8315

Abstract: The Biomanufacturing Program at NIST is developing a suite of fundamental measurement science, standards, and reference data to enable more accurate and confident characterization of key attributes of protein therapeutics that are linked to their safety and efficacy. These measurement tools are expected to facilitate the development of innovative protein therapeutics and lower cost biosimilars, and more firmly underpin regulatory decisions. This talk will provide an overview of several activities including methods and reference materials for the measurement of protein particles and the development of a well-characterized monoclonal antibody reference material. In addition, how improved measurements and standards can support QbD approaches will be discussed.

Kurt Brorson, Ph.D.
Division Head of Monoclonal Antibody, FDA/CDER

Kurt Brorson, Ph.D. is a Research Biologist in CDER's Division of Monoclonal Antibodies, Office of Biotech Products. Kurt Brorson received a B.A. in biology from the University of Chicago (Chicago IL) in 1984 and Ph.D. in molecular biology from the California Institute of Technology (Pasadena CA) in 1990. After a 2-year postdoctoral fellowship at the NIH, he joined FDA as a fellow in 1992 and gradually rose through the ranks to senior investigator in 2012. In addition to review, inspection, training and policy activities, he conducts research on bioprocess monitoring and viral safety of biotechnology products. He has won numerous internal awards from FDA, CBER and CDER, as well as the Gordon R. Personeus Award from the Parenteral Drug Association in 2013. He is the author of more than 70 scientific journal articles and book chapters on various aspect of bioprocessing. He is a member of PDA, the American Chemical Society (BIOT), the American Association for the Advancement of Science and the American Society for Microbiology

Title: PAT and the Future of Biotechnology

Author: Kurt Brorson, Ph.D., Office of Biotechnology Products, CDER/FDA, 10903 New Hampshire Ave., Silver Spring MD 20903

Abstract: One of the important outcomes of CDER's 21st Century GMP initiative was the 2004 PAT guidance: "PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance."

Process Analytical Technology (PAT) is a system for designing, analyzing, and controlling manufacturing processes based on 1) an understanding of the scientific and engineering principals involved and 2) identification of the variables which affect product quality. The essence of PAT consists of real-time measurement of process intermediates and materials for feed-back control of a manufacturing process to maintain quality and consistency of the final product. In pharmaceutical world, PAT concepts were adopted early on by the small molecules industry; the progress in biotech community may be more incremental and gradual but optimistic. The PAT approach should give the biotech industry incentive to explore various pre-existing or novel analytical tools for measurements during, rather than at the end of, a process to get more information about the process and control it in real-time. With biopharmaceuticals, testing is more complex; using current technology, one cannot test for everything. However, we believe that targeted R & D can eventually evolve PAT approaches even for complex protein properties such as secondary structure and glycosylation patterns.

Implementation of PAT in bioprocessing is likely to follow the traditional incremental pattern of technology development in the bioprocess world. We envision that PAT will evolve from process control based on real-time measurement of (a) parameters that confirm that a unit operation/piece of equipment continues to be fit for purpose, move on to (b) those that directly correlate with a CQA, and then to finally (c) actual product (or raw material) critical quality attributes (CQAs, i.e. the traditional conception of PAT). As stated above, achievement of (c) requires surmounting significant technology barriers by intense and purposeful R & D.

PAT has the capacity to revolutionize the biopharmaceutical industry, but only if the opportunity is seized. The development and implementation of such technological advances have, and will continue to receive, strong support from the Food and Drug Administration. To speed up PAT implementation, it is vital that success stories be shared.

Kumar Dhanasekharan, Ph.D.
Director of Process Development, Cook Pharmica LLC.

Dr. Dhanasekharan is currently Director of Process Development at Cook Pharmica in Bloomington, Indiana and leads the cell culture, purification, analytical, and formulation development including lab operations and scale-up to meet client drug substance and drug product needs. Cook Pharmica is an integrated contract development and manufacturing organization providing the pharmaceutical and biopharmaceutical industries with biologics drug substance and parenteral manufacturing in vials and prefilled syringes. Prior to his current role, Kumar worked for 5.5 years at Genzyme in various roles including process development, process engineering and most recently served as the Associate Director of Process Sciences and Technology at Genzyme in Framingham, Massachusetts. He was responsible for manufacturing process development for both cell culture and protein purification for enzyme replacement therapies in driving a science and risk-based strategy for continuous process improvements including improved control strategies, cell culture productivity and downstream recovery improvements. In a previous role, he led the implementation of Quality by Design (QbD) principles in development and successfully led a QbD based Lyophilization scale-up and tech. transfer project for approval. He also led efforts in viral risk mitigation which resulted in an invention and patent on a UV-C based viral inactivation device. He also provided technical leadership for several projects related to Consent Decree remediation. Prior to joining Genzyme, Kumar led small-molecule process development focused on API crystallization at Bend Research Inc., Bend, OR. Prior to that he was group leader for consulting services at Fluent Inc. (Now ANSYS) with focus on both small-molecule and biologics development and manufacturing challenges across upstream, downstream, and Fill/ Finish operations. Kumar has a Ph.D. in Food Science from Rutgers University and a Bachelor's in Chemical Engineering from Indian Institute of Technology, Chennai, India. He has over 50 conference presentations and over 10 peer-reviewed publications

Title: Rapid Development and Scale-up of Therapeutic Antibodies—A QbD Perspective with a Case Study for a Biosimilar Trastuzumab

Author: Kumar Dhanasekharan, Ph.D., Director of Process Development, Cook Pharmica LLC

Abstract: Over the past decade, antibody based therapeutics has become mainstream for treating a broad range of life threatening conditions across auto-immune diseases to cancer therapies. As part of this maturity of the market for antibodies, there is increasing time pressure to bring a molecule to market as quickly and safely as possible. A systematic science-based platform approach to development is illustrated here for developing a scalable process for the production of a biosimilar. Platform approach begins with the development of a high producing CHO cell line (SELEXIS SUREtechnology™) expressing the desired target protein, followed by media screening and DOE based studies to identify the optimal combination media-feeds and optimize process conditions for product titer and quality. The process was successfully scaled up to a pilot scale setup for both upstream and downstream operations with comparable process and product attributes. Every step in the process was supported by detailed analytics methods such as N-Glycan analysis to demonstrate comparability with innovator molecule, SEC-HPLC and cEIF that allowed a holistic approach for process definition and scale-up. In summary, a process ready for tech-transfer to GMP clinical manufacturing was developed in an aggressive timeline that provided manufacturing-ready titers while meeting product quality attributes comparable to innovator molecule.

Lilong Huang, Ph.D.
Sr. Engineer, Manufacturing Sciences, BiogenIdec

Dr. Lilong Huang joined Biogen Idec in 2012 and is currently leading the global advanced process control (APC) initiative within the company. The mission of Biogen Idec's APC initiative is to transform research, development, and manufacturing data into better process understanding and eventually into actions to achieve improved product quality. Dr. Huang has worked in Chemical, Vaccine, and Biological industries for the past 9 years. Prior to joining Biogen Idec, he worked at Bayer, Merck, and Genzyme. His expertise includes manufacturing support, new facility startup, facility utilization optimization, data analytics and process monitoring/control.

Dr. Huang graduate from Beijing University of Chemical Technology in 1996 with a Bachelor of Science degree and from Tsinghua University in 1999 with a Master of Science degree both in Chemical Engineering. He received a Ph.D. in Chemical Engineering from Lehigh University in Bethlehem, PA in 2004.

Title: Biologics Advanced Process Control at Biogen-Idec

Authors: Lilong Huang, Joydeep Ganguly, Sarah Yuan, and Andre Walker

Abstract: With few exceptions, process control strategies in biologics manufacturing have not changed over the last 30 years adopting a linear approach to control individual unit operations. Principles established in the early 80s are still employed in today's Drug manufacturing.

At Biogen Idec, we are evaluating options to radically break with traditional control paradigms and significantly improve the process and product quality consistency of biologics products. To reach that goal, we shall explore sophisticated informatics to develop mata-models using available process knowledge and a wealth of development and manufacturing data to build end to end product quality predictive models.

In this talk, the authors will share the journey of Advanced Process Control in Biogen Idec, a case study, and our vision for the future of Biologics process control.

Bert Frohlich Ph.D.
Director of Bioengineering, Shire

Bert Frohlich is a Ph.D. Biochemical Engineer with 20+ years of experience in the biotechnology, pharmaceutical, and chemical industries in process/facility design and bioprocess development. He is currently Director of Bioengineering at Shire HGT developing cell culture-based processes for manufacture of recombinant proteinsprimarily for enzyme replacement therapies.

Title: Shire's Strategic Decision to Implement QbD for all Drug Development and Manufacturing

Abstract: Shire is embarking on a comprehensive implementation of QbD principles in biopharmaceutical drug development and manufacturing. An overview of the approach that Shire is taking will be presented including an outline of new business processes and the use of quality risk management (QRM) to arrive at an integrated control strategy. This strategy will be applied to both pipeline and legacy processes to better support life-cycle management while providing a high degree of assurance that the product quality specifications are met. Initial objectives, considerations, and challenges to design and implementation will also be discussed.

José M. Gomes
Principal Scientist, Culture Process Development, Pfizer, Inc.

José is a biochemist & biophysicist with considerable experience in cell culture, chemical engineering, and molecular biology including 17 years of industry experience. He joined Pfizer through the acquisition of Wyeth and currently holds the position of Principal Scientist within the Culture Process Development group in Biotherapeutics R&D. His work focuses on development and support of upstream processes for a wide gamut of projects from early-stage to late-stage clinical products, commercial products, and next-generation products. His particular areas of interest include understanding effects of process parameters on product quality, utilization of design of experiments & quality risk management, application of QbD, and understanding factors affecting process tech transfer & scale-up (including agitation and aeration effects). Prior to joining industry, José was involved in studying lipid and hormone metabolism at Tufts Medical School and the U.S.D.A.

Title: Using QbD and PAT to Develop Adaptive Manufacturing Processes

Abstract: As described in ICH Q8, product and process understanding, in combination with QRM, can be used to develop a manufacturing process so that variability can be compensated for in an adaptable manner to deliver consistent product quality.

Using this approach, an alternative manufacturing paradigm was developed where the variability of a cell culture process was less tightly constrained, while PAT and an adaptive process step was included to ensure consistent product quality.

Dr John Carvell
Aber Instruments Ltd., UK

John Carvell is the Managing Director of Aber Instruments and is based out of Aberystwyth, UK. He is a graduate in Biochemistry and received his Ph.D at Newcastle University, UK. John has held roles as Production Manager at a bakers yeast manufacturer and senior sale roles within engineering contractors before joining Aber Instruments Ltd in 1994. With the business over 90% export and split between both the Brewing and Biotechnology industry, he spends a large proportion of his time visiting companies involved in diverse range of applications for on-line biomass monitoring. John has presented papers and posters at many of the major biopharma and brewing conferences including the SIM (Society of Industrial Biotechnology), RAFT (Recent Advances in Fermentation Technology), ACS and at both the ASBC (American Society of Brewing Chemists)and MBBA (Master Brewers of America Association) annual meetings. He has also published various papers including a major review in Cytotechnology. When time permits John enjoys a number of activities including tennis and fly fishing.

Title: Recent developments in scaling down and using single use probes for measuring the live cell concentration by dielectric spectroscopy

Abstract: Real-time bioprocess monitoring is fundamental for maximizing yield, improving efficiency and process reproducibility, minimizing costs, optimizing product quality, and full understanding of how a system works. Bioreactors that are monitored continuously and in real-time offer the advantage of meeting current and future supply demands with biological product of the utmost quality and safety, achieved at the lowest overall cost and with least risk. This paper will focus on the latest developments in dielectric spectroscopy for live cell concentration measurement and how the technology has been scaled down allowing bioreactors with less than 100ml working volume to be monitored in real time. The presentation will also focus on how dielectric spectroscopy can also be applied to Single use Bioreactors in a cGMP environment and on samples down to 100 microlitre volume.

Andrew Cowen, Ph.D.
CEO, Biopharma Technology (UK)

Andrew Cowen is the Executive Chairman and co-proprietor of the Biopharma Group comprising Biopharma Process Systems, a distributor and service provider for three brands of freeze dryer and associated process equipment; Biopharma Technology , a producer of specialist lyophilisation instrumentation and provider of process development services across a range of stabilisation technologies and the Crowthorne Group, the UK's largest independent clean air validation and fumigation specialist. Andrew has had a varied career taking in spells in banking, venture capital and manufacturing and has a strong interest in process development. This has led to the investment in the development of QbD processes in Biopharma's core discipline of lyophilisation.

Title: Challenges for applying QbD approach to biopharmaceutical freeze-drying.

Abstact: The application of a QbD approach to the development of a freeze-dried pharmaceutical product is a complex science and still a matter of much debate. It certainly requires knowledge of a specific branch of formulation science, an appreciation of equipment limitations, and an understanding of the dynamic interplay between product and process during the various stages of the lyophilization process. This presentation will provide an overview of these issues, highlighting the importance of formulation characterisation, establishing where the main risks lie, and demonstrating how the construction of a design space (or a sequence of linked design spaces) might be approached, while also taking into account the economic constraints of the process.

John-Paul Smelko
Sr Engineer, Technical Development, BiogenIdec.

John-Paul Smelko is an eleven year veteran of developing and integrating new technologies into bioprocess platforms at Biogen Idec. John-Paul manages a team of eight scientists spanning five exciting cell culture technologies with the goal of advanced process control (APC). These technologies include Raman spectroscopy, di-electric spectroscopy, off-gas analysis, product quality at-line analytics, and advanced optical techniques. Prior to his current role, John-Paul has helped pioneer many new technologies at Biogen Idec such as developing and transferring the first centrifuge process into large scale manufacturing (LSM), spokesman and early adopter for the Millipore POD platform, development and integration of single use bags (SUBs) along with disposable sensor technology and the introduction and advancement of Raman spectroscopy. His expertise includes manufacturing and pilot lab operations, cell culture and centrifugation process development, bioreactor characterization, process transfer, disposable systems development and facility design, and new technology exploration, adoption and integration.

John-Paul Smelko graduated from University of Arkansas with a bachelor degree in Chemical Engineering and is a die-hard Razorback fan!

Title: "Raman Spectroscopy applied to monitoring, modeling, and control of biologics production"

Authors: John Paul Smelko, Justin Moretto, Brandon Berry, Thomas Matthews, An Zhang, Kelly Wiltberger, Thomas Ryll

Abstract: Over the past 30 years, cell culture monitoring and advanced process control strategies have not kept pace with the advancements in therapeutic protein production. What if today you were still calling your friends and family on a rotary phone like in the 80's instead of using your smartphone to communicate with others on Facebook, Twitter and LinkedIn as we know today. It would be a different world. I would argue this alternate world is not so much different than the Biotechnology industry today with respect to process monitoring and control. Other industries have shown as production levels go up process efficiencies must improve to create a business advantage. We are close to this tipping point with 1g/L/day volumetric throughputs within the next five to ten years. Having best in breed technology and using that information and hardware to create new and innovative processes that are more consistent, productive and meet the product quality demands of our patients is where we need to be. At Biogen Idec, we are exploring multiple options both upstream and downstream that can achieve this vision. Raman Spectroscopy is one such technology that has been explored for the past 5 years at Biogen Idec in cell culture development.

In this talk, the authors will share some insights and examples into the use of Raman spectroscopy to monitor key cell culture growth and metabolic parameters with specific focus on data curation, model development, and MFG implementation with adaptive control.

Timothy J. Johnson, Ph.D.
Associate Director
Commercial Cell Culture Development, Sanofi

Dr. Timothy Johnson is an Associate Director in the Commercial Cell Culture Development department at Sanofi, in Framingham MA. He received a Ph.D. in Chemical Engineering from the University of Washington, Seattle in 1999 and a B.S. in Chemical Engineering from the University of Minnesota in 1995. Tim's initial career focused on advancing microfluidic designs and applications while at The National Institute of Standards and Technology, Tecan group ltd., and BioProcessors Corp. Since 2007, at Genzyme Corp., Tim has been responsible for supporting, understanding, and improving the production of commercial cell culture processes. Most recently, he has been leading the upstream development efforts for Sanofi's continuous manufacturing platform for the universal production of biopharmaceuticals.

Title: Steady-State Continuous Bioprocessing And Time-Efficient Approaches Towards Process Characterization

Authors: Timothy Johnson, Jason Walther, Seul-A Bae, Neha Shah, Jon Wang, Kevin Brower, Rahul Godawat, Veena Warikoo, Chris Hwang, Konstantin Konstantinov

Late Stage Process Development, Genzyme, A Sanofi Co., Framingham, MA, USA

Abstract: The maturing of the bioprocessing industry is currently leading to projects centered around increased process understanding and control, process simplification, efficiency improvements, and increased facility flexibility. Encompassing these objectives, Genzyme, a Sanofi Co., over the last few years has been developing an integrated continuous biomanufacturing platform for the steady-state production of mammalian cell culture derived protein therapeutics ranging from recombinant monoclonal antibodies (mAbs) to complex enzymes.

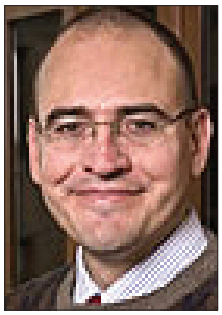
In leveraging the steady-state nature of the upstream perfusion process, we can now efficiently apply design of experiment (DoE) methodology to develop and characterize processes on timescales that are now comparable to more traditional fed-batch processes. This presentation will discuss the overall integrated continuous biomanufacturing platform and the results from a custom multivariate surface response experiment that covers the critical upstream operating space and the subsequent product quality impact. We will discuss how this knowledge can be leveraged to establish robust process control strategies and can facilitate integration with continuous downstream processes.

Erik Read
US Food and Drug Administration, Silver Spring, MD.

Erik K. Read is a Senior Staff Fellow at the US FDA where he is a product quality reviewer for the Division of Monoclonal Antibodies. He is also an Researcher in the FDA Bioprocessing Lab, and his current projects focuses on applying Process Analytical Technology to antibody producing mammalian cell culture unit operations. Erik received his Ph.D. in Molecular Microbial Genetics from the University of Illinois at Urbana-Champaign.

Title: Exploring the linkage between Cell culture abd Downstream Processing via statistical Design

Abstract: A pre-requisite to the application of Process Analytical Technology is a knowledge space populated with data that relates process parameters changes to the effects upon product quality attributes. In this presentation we explore the linkage between parameter changes during cell culture to effects on downstream process performance. To this end we have extended the analysis of a Plackett-Burman design of experiments (DoE) for an antibody- producing mammalian cell culture unit operation to include downstream chromatographic purification, bulk drug substance formulation, and stability testing. In combination with insights from formulation development DoE insights downstream processing was modified and the potential effects of PAT control opportunities were further defined.



Richard D. Braatz, Ph.D.
Professor Chemical Engineering, MIT

Richard D. Braatz is the Edwin R. Gilliland Professor at the Massachusetts Institute of Technology (MIT) where he does research in process systems engineering and its application to pharmaceutical, biopharmaceutical, and biological systems. He has consulted or collaborated with more than 20 companies including Merck, Pfizer, Novartis, Bristol Myers-Squibb, and Abbott Laboratories. His research in Process Analytical Technology and Quality by Design has been recognized by the Chemical Research Council Research Collaboration Award, the IEEE CSS Transition to Practice Award, the ISA Technical Innovation Award, the AIChE Excellence in Process Development Research Award, and the AIChE PD2M Award for Outstanding Contribution to QbD for Drug Substance.

Title: Systems Technology for Biologics Development and Production

Abstract: This talk provides an overview of systems technology that can be used in biologics development and production as a future bioprocessing technology. The different systems technologies discussed are (1) the construction of design spaces based on experimental data and mathematical models, (2) the use of dynamic process models to track disturbances as they propagate in production and to design control strategies for individual unit operations and the overall manufacturing facility, and (3) the relationship between quality assurance, sensitivity analysis, and the plant-wide control strategy.

Bogdan Kurtyka, PhD.
Review Chemist
FDA/CDER/ONDQA

Dr. Bogdan Kurtyka graduated from Georgetown University with a degree in Analytical Chemistry. For several years he worked for a manufacturer of NIR analyzers as a product manager responsible for software development and pharmaceutical applications. Since 2007 he has been working for the Food and Drug Administration as a CMC reviewer at the Office of New Drug Quality Assessment.

Abstract: Multivariate Statistical Process Control (MSPC) has been successfully used in many industries for quality control and early fault detection both in continuous processes and batch manufacturing. Process sensors measurements provide extensive real-time multivariate data that require statistical methods to facilitate data analysis and modeling. Recently constructive dialogue between industry and regulators was initiated to discuss approaches to ensuring drug products quality through the use of MSPC as an element of control strategy. Together with implementation of Quality by Design and Real Time Release Testing, MSPC has provided new opportunities and challenges to both the pharmaceutical industry and regulators regarding how to implement and evaluate the novel methods. The presentation will discuss scientific and regulatory aspects of MSPC for monitoring and control of pharmaceutical processes.

Susan Sharfstein, Ph.D.
Associate Professor, University at Albany, SUNY

Susan Sharfstein received her B.S. in chemical engineering with honors from Caltech in 1987 and her Ph.D. in chemical engineering from UC Berkeley in 1993, receiving graduate fellowships from the university and the National Science Foundation. She received a National Institutes of Health Individual Research Service Award to pursue postdoctoral studies, initially at UC Berkeley and subsequently at the UCLA Medical School. Dr. Sharfstein joined the faculty at the University of Toledo in Bioengineering in 1996. In 2000, she received a National Science Foundation POWRE award to study glycobiology at the New York State Department of Health Wadsworth Laboratories.

In 2001, she joined the Department of Chemical and Biological Engineering at Rensselaer Polytechnic Institute and in 2007 she received a dual appointment in Biology. In 2010, she joined the faculty at the College of Nanoscale Science and Engineering at the University at Albany as an Associate Professor of Nanobioscience. Professor Sharfstein received an NSF CAREER grant in 2000 for her work on hyperosmotic stress responses of hybridoma cells and the School of Engineering Education Excellence Award and the Class of 1951 Outstanding Teaching Award in 2007. She is the author of over 40 papers and book chapters in the field of mammalian cell biotechnology.

Title: Understanding the effects of process conditions on protein glycosylation and proteoglycan synthesis

Abstract: Chinese hamster ovary (CHO) cells are the workhorse of the biotechnology industry due to their ease of suspension culture, high levels of protein productivity and their ability to correctly fold, glycosylate, and secrete therapeutic glycoproteins. While many advances have been made in CHO cell culture, permitting routine titers of >1 g/L for monoclonal antibodies (and reported titers of >10 g/L), there is much to be understood about the effects of culture conditions on both the productivity and the quality of the molecules produced, particularly the glycan structures. This becomes of critical importance as molecules lose patent protection and manufacturers attempt to create biosimilar versions of therapeutic proteins.

In this presentation, I will discuss efforts in our laboratory to understand the role of culture conditions on the productivity and glycan structures for two glycoproteins, tissue plasminogen activator and secreted alkaline phosphatase. I will also describe our efforts to metabolically engineer CHO cells to produce heparin, a glycosaminoglycan that is used as a therapeutic carbohydrate. Metabolic engineering of CHO cells to produce heparin presents a new paradigm for the use of CHO cells to make non-protein therapeutics. It will also yield insight into the regulation of glycosylation pathways that may ultimately impact our understanding of glycosylation of therapeutic glycoproteins.

Maurizio Cattaneo, Ph.D.
CEO and President, BioVolution

Dr. Maurizio Cattaneo is a Certified Pharmaceutical Industry Professional (CPIP). He obtained his Bachelor of Applied Sciences Degree in Chemical Engineering from the University of Toronto and his MS and PhD degrees from McGill University (Canada). Dr. Cattaneo was a Research Officer at the Biotechnology Research Institute of the National Research Council of Canada where he specialized in the development of novel monoclonal antibodies using phage display. He consulted for many local companies including Percivia, LLC in the area of biosimilar development. Dr. Cattaneo has 6 patents in the field of biotechnology and was recently at MIT to develop single use systems for manufacturing monoclonal antibodies using cell culture. Dr. Maurizio Cattaneo is now the CEO of BioVolutions, Inc. a local CMO specializing in Innovative Biomanufacturing Solutions. He founded BioVolutions to offer therapeutic antibodies for Early Clinical Phases.

Title: QbD in Continuous Manufacturing of mAbs

Author: Maurizio Cattaneo, PhD, CEO BioVolutions Inc.

Abstract: Continuous manufacturing offers significant advantages over batch processes in terms of equipment footprint, batch to batch consistency, high productivities and reduced capital cost. A flexible and cost effective platform including both upstream as well as downstream processes for the production of mAbs, is presented. Equipment for the DOE study included a 2L Applikon bioreactor, an ATF-2 Perfusion system (Refine Technology) and a Quanta Sep 1000 (Sepragen Corporation) and controlled by Delta-V software (Finesse Corporation). By following a QbD approach, the identification of critical process parameters (CPPs) and critical quality attributes (CQAs) helped define a design space that meets both antibody titer as well as product quality attributes. Key process parameters of our DOE (using SAS JMP software-Taguchi Approach) included the type of media (X,Y,Z), cell culture duration (20-40 days), pH (6.8-7.2) and temperature (36.5-37.5 °C). Our optimal 34 days continuous perfusion run achieved steady state viable cell densities between 50 x 106 and 60 x 106 cell/mL (obtained after 4 days continuous perfusion -day 4-8), with perfusion rates of ≤1 vvd using daily monitoring of glucose (>1g/L) and lactate (<3g/L) and cell viabilities >90%. The pool harvest gave titers ≥0.8g/L resulting in overall quantities of antibody of at least 10X compared to Fed-Batch.

George Kachen
UMass Lowell ETIC Clean Rooms and BSL2 Facility for Industrial and Academic Research

Authors: Thomas Ferraguto, Teri Hamelin, George Kachen

Abstract: The Mark and Elisia Saab Emerging Technologies and Innovation Center at UMass Lowell is home to our 4,200-square-foot, Abbie Gregg-designed Class 100, 1,000 and 10,000 cleanrooms. The professionally managed laboratory is designed to service industrial and academic community researchers. Our sophisticated capabilities include e-beam lithography, thin film deposition, etching and lab metrology capabilities. Our infrastructure includes advanced gas delivery, abatement and toxic gas monitoring systems. In addition, there is a Class 10,000 BSL2 cleanroom with ESD controlled flooring and ionizers along with Baker BSL2 Hoods. Once training and service requirements are met, industrial use of these facilities is readily available. The benefits of these facilities to all users are low cost, easy/flexible setup and quick turn-around. Under certain circumstances, users can place their own equipment in clean room bays.

POSTERS

Novel Strategy for Media Formulation Development

Hemlata Hemlata and Seongkyu Yoon, University of Massachusette, Lowell

Abstract: Biosimiliar market is growing very fast. Media formulation is a critical step in biosimiliar development as the process is labor intensive and time consuming. The proposed strategy can reduce the time for media development significantly. The approach to develop the strategy includes media formulation for a particular cell line by using spent media analysis followed by regression modeling. The up or down regulation of genes associated with different metabolic processes as a function of various media components can be explained by performing gene expression analysis of cells grown in different media compositions. This strategy is supposed to decrease the time for media development significantly.

Multi-variate data Analysis for Protein A chromatography column

Ketki Behere[1], Rachel Wollacott[2], and Seongkyu Yoon[1], [1]University of Massachusetts Lowell; [2]MassBiologic

Abstract: The objective of this project was to build a regression model which could explain the variability in the batch column chromatography dataset and provide some predictability to the user. The first step was to build a Batch Evolution Model (BEM) which could explain each chromatography step in detail and provide predictability power to the loading and elution step. The eventual goal of this project was to give a direct comparison between the different batches which had different resin.

It was also required to identify the important response variables out of the given seven (Column height, column diameter, Asymmetry, HETP, resin capacity, loading and elution concentration) which provided significant contribution to the given dataset and thus can be extrapolated to batch chromatography in general. A validation of the BLM model was required to prove the efficiency of the model.
The BEM model gave considerable predictability to the loading and elution steps and confirmed that those process steps were most important in the entire batch run. The BLM model gave definite segregation between the batches with different resin. Three response variables were identified to show significant contribution to the dataset. Those were the column height, HETP and elution concentration.

Glycosylation Reaction Network Models

Sha Sha and Seongkyu Yoon, University of Massachusette, Lowell

Abstract: Tremendous efforts have been put into understanding and modification of glycosylation reaction pathways that result in uniform biopharmaceutical products. Mathematical modeling approaches are a paradigm recently emergingin research area striving toward this goal. One profound way to implement mathematical tools is the development of reaction network models which aims to describe simplified but the essence of glycosylation mechanism. This method theoretically relies on accurate simulation of glycosylation process in cells, therefore providing the capability for researchers to understand cell performance in regard of glycosylation output under differed culture conditions, and moreover serving a prediction tool of glycosylation profiles from early defined inputs, like cell nutrients feeding, cell lines, metabolites information, and so on.

A Calibration-free Application of Near Infrared Spectroscopy to the Determination of Viable Cell Density in Mmmalian Cells

Zhuangrong Huang and Seongkyu Yoon, University of Massachusette Lowell

Abstract: Near infrared spectroscopy (NIRS) is one of a number of potential in situ process analysis technologies (PATs) for application in cell culture processes. NIRS is simple to operate and well suited for the determination of the major components in cell culture, but their application requires significant calibration be made about the relationship with the NIR spectra. In this work a novel and simple calibration-free method for determination the viable cell density proposed. Multivariate curve resolution alternating least squares (MCR-ALS) is able to (i) extract from a complex spectral feature the number of involved components, (ii) attribute the resulting spectra to chemical compounds or structure responses, and (iii) quantify the individual spectral contributions with or without a priori knowledge. We have evaluated MCR-ALS for the routine analysis of viable cell density in mammalian cells. And then, the results were compared with those obtained from the most-used multivariate technique, partial least squares (PLS), as a representative of the group of regression methods, to evaluate the results of MCR-ALS.

Metabolomics for CHO-culture production

Seoyoung Park and Seongkyu Yoon, Department of chemical engineering, University of Massachusetts Lowell

Abstract: Metabolomics study enables the examination and identification of small molecules that revealing information on the target metabolic pathways in a cell. Metabolites are involved either directly or indirectly with every aspect of cell function, and thus metabolomics is to be a reflection of the phenotype of cell. Metabolomics analyses have many potential applications due to their inherent advantages. An important application that has recently emerged is to characterize cell cultures expressing protein therapeutics. Cell metabolomics consists of four sequential steps: (1) sample preparation and extraction to measure the intracellular metabolites in CHO cells, (2) metabolic profiles of low-weight metabolites based on mass spectroscopy (MS) or nuclear magnetic resonance (NMR) spectroscopy, (3) metabolites identification and (4) data analysis. Metabolomics requires special attention to describe two key steps in metabolomics study such as the metabolite extraction and metabolite measurement in order to detect as many metabolites as possible in given cell. As a result, these sequential steps provide insight about the cellular biochemical processes.

Challenges and Issues of Spectroscopy and Chemometrics Application in Protein Therapeutics Development and Manufacturing

Nicholas Trunfio and Seongkyu Yoon, University of Massachusetts Lowell

Abstract: Application of spectroscopic techniques has proved to be a powerful tool for development and manufacturing of mammalian cell culture, aiming at consistent therapeutic protein production. Since these techniques can provide fast, simple and non-invasive methods to obtain biochemical information about biopharmaceutical processes, it offers significant advantages over other traditional off-line analyzes. In the study, recent applications of various spectral technologies are addressed for raw material characterization, bioprocessing and product quantification and specification.

Effect of Media on Cellular Metabolism

Thomas Reimonn and Seongkyu Yoon, University of Massachusetts Lowell

Abstract: Manufacturers of biopharmaceutical medicines face an increasing need for more efficient production methods with tighter tolerances to meet emerging Quality by Design standards, increased generic and biosimilars competition, and novel drug design techniques. Using data from an FDA paper covering 12 monoclonal antibody-producing hybridoma bioreactor runs in supplemented growth media, principal component analysis is performed on metabolite concentrations to determine the factors that most affect production of monoclonal antibody. In addition, the use of metabolic flux analysis to determine how changes in supplementation strategy affect internal metabolic pathways leading to antibody production is discussed. Analysis of the growth data indicates that glucose supplementation in a fed-batch manner and aeration with a microsparger are the most significant factors affecting antibody production. The supplementation of amino acids that are depleted by the end of the trial increases yield, but has a smaller effect than aeration and feeding strategy.

The Search for MRI—a Novel Method to Automate and Control Bioreactor and Fermentor Feeding,

Greg Emmerson, Sam Watts (Stratophase Ltd, Romsey, Hanpshire, UK), George Barringer, (Stratophase, Groton, MA, USA)

Abstract: A novel system, the Ranger™, and method for in-situ, real time monitoring and control of nutrient feeding in upstream bioreactors and fermentors is described. MRI (Metabolic Rate Index) is a Process Variable that describes the overall state of the metabolic environment of the process under observation and is highly sensitive to any molecular level perturbation in the process media, such as occurs when a biological process is fed. The system is applicable to all scales of operation from process development to commercial production and is compatible with SUBs. This technology is in evaluation in microbial, fungal, and mammalian cultures.

Morphological Observations Using Image Cytometry for the Comparison of Trypan Blue and Fluorescence-based Cellular Viability

Leo L. Chan, Dmitry Kuksin, Daniel Laverty, Jean Qiu, and Bo Lin, Department of Technology R&D,
Nexcelom Bioscience LLC, Lawrence, MA 01843

Abstract: Determining cell viability is an essential component in many biological experiments ranging from standard cell culture to immunology and oncology research. The traditional method for cell viability measurement is the trypan blue exclusion assay, which utilizes a hemacytometer to manual enumerate trypan blue stained cells. However, trypan blue exclusion assays can be time-consuming, tedious, and have user-dependent variation. Studies have also shown that long incubation with trypan blue can induce cytotoxicity and artificially produce lower viability measurement. With the increase in the availability of fluorescence detection systems and fluorescent stains, numerous fluorescence-based viability measurement methods have been adopted, such as acridine orange (AO) and propidium iodide (PI), Calcein AM, DAPI, 7AAD, and CFDA. There have been numerous comparisons between trypan blue exclusion assays and fluorescence-based viability methods, where the results have shown that trypan blue method yielded higher viability measurement than fluorescent staining in a cell culture time course study. In this work, we compared cellular viability measured using trypan blue and AOPI. Similar results were obtained showing trypan blue produced higher viability measurements when viability decreased to below 70%. Utilizing image-based cytometry, morphological changes of trypan blue stained cells were observed, which may explain the differences between the two methods. Results showed that as cells begin to die in a cell culture, dying cells displayed large blue haze when stained with trypan blue that may be missed during manual cell count. In addition, multiple concentrations of trypan blue were tested that yielded similar results. At low trypan blue concentrations, cells were not properly stained, but at high trypan blue concentrations, many cells turned into large blue haze, which may generate artificially higher viability measurements. In conclusion, using image-based cytometry, we were able to observe morphological changes in the cells, which could have attributed to the differences between trypan blue and fluorescent staining. In addition, it allowed further characterization of trypan blue exclusion assay, which showed that the assay can potentially only function properly in a certain range of cell viabilities. Further studies can be performed to observe morphological changes of trypan blue stained cells at various incubation times.

Rapid Image Cytometry Method for Measuring Concentration and Viability of Primary Cells used in Cellular Therapy

Leo L. Chan, James Leeb, Dmitry Kuksin, and Jean Qiu, Department of Technology R&D,
Nexcelom Bioscience LLC, Lawrence, MA 01843, bAllCells, LLC, Alameda, CA 94502

Abstract: Cellular therapy has become a major clinical research field that creates tailor-made medical treatments for many human diseases. Primary cells obtained from patients and mouse models often contain nonspecific particles such as red blood cells (RBC), platelets, and cellular debris, which can make the cell sample analysis difficult. To remove nonspecific particles that can interfere with analysis, a ficoll gradient separation or RBC lysis is routinely performed. Measurements of concentration and viability of the cell sample are necessary for clinical researchers to qualify the collected patient samples for research and downstream processing. In this work, we validated a fluorescence-based image cytometry method using acridine orange (AO) and propidium iodide (PI) to rapidly measure concentration and viability without the need for tedious purification protocols. Using the Cellometer Vision instrument we performed the viability and concentration analysis and validated our results using the traditional hemacytometer method for samples including but not limited to peripheral blood mononuclear cells, mononuclear cells, Leuko Pac, bone marrow, cord blood, whole blood, bronchoaveolar lavage, and primary murine samples. This image-based cytometer method can increase clinical research efficiency by eliminating the need for purification steps and for manual counting. Furthermore, it can eliminate the user-to-user variation, thus improving the accuracy of the cell analysis.

Automation Method to Increase Efficiency in Cell Line Development

Sarah Kessela, Olivier Dérya, Leo L. Chana, Dmitry Kuksina, and Jean Qiu,
Department of Technology R&D, Nexcelom Bioscience LLC, Lawrence, MA 01843

Abstract: Development of monoclonal cell lines is essential in research and for the production of recombinant protein therapeutics. Due to the increasing number of biologic treatments, monoclonal antibody (mAb) producing cell lines are under increasing regulatory scrutiny, and therefore, ensuring monoclonality is becoming an essential step in the cell line development process. Some challenges in producing clones remain and relate to cell plating, identification and outgrowth of single cells for monoclonality. Researchers have used limiting dilution techniques and microscope visualization to ensure that populations of cells in a well were derived from a single cell. Moreover, transfected cells can exhibit slower growth rates to accommodate for the production of bio-products and require adjustments to selection reagents. Optimizing media formulations can increase the percentage of single cell clonal growth. In this study, using the CHOs cell line, the Celígo® Imaging Cytometer was used to optimize media, evaluate plating methods, monitor viability, identify single cells and track clone outgrowth to larger well plates. Automated and manual methods of plating cells in 384-well plates were compared. The data shows that using an automated liquid dispenser improved the plating reproducibility and decreased time for plating large number of limiting dilution plates when compared to manual methods. As isolated cells do not like to grow alone, Design of Experiment (DoE) methodologies and automated imaging were used for media formulation improvements to increase their growth and survival. Media supplements (B27, EGF, and bFGF) that have been shown to increase growth were tested with a full factorial DoE [3 levels, 3 factors, and single output response]. The results show that the use of B-27 positively promoted cell growth as compared to the control media. While the identification of single cells in brightfield is possible, using the fluorescent marker Cell Tracker Green to monitor the single cells was found advantageous and had no impact on cell viability. Wells growing a single cell were subsequently monitored for the formation of a colony, which also allowed for measurement of optimal passaging times of asynchronous clones. Overall, the Celígo Imaging Cytometer has demonstrated the utility of automation in the development and monitoring of new CHOs based cell lines for increasing efficiency in cell line development.

Scale Free Manufacturing Using Perfusion

Olivier Berteau[1], Lexan Lhu[2], David Sergeant[3], Seongkyu Yoon[4], [1]Global Product Manager Upstream Solutions at Charter Medical (France), [2]Application Engineer at Charter Medical (USA), [3]Founder and Scientist at Ipratech (Belgium), [4]UMass Lowell, Lowell, USA

Abstract: Perfusion, when it's well managed, offers many economical and performances advantages. Unfortunately, perfusion is often perceived as too complex, and perfusion devices thought to be too expensive and likely to clog; achieving higher productivity without a long, costly investment and optimization of the process and scale-up are seen as difficulties; Industry believe no process control can truly manage perfusion; and finally it is known that set up is too long, complex, and has too many risks of contamination if you are not a perfusion expert. This poster presentation is a breakthrough for cell culture experts who believe in perfusion model and scale-up of such model. From process development to production we are proposing a different model where scale-up and its management difficulties are by-passed with a SOP model: SCALE OUT PERFUSION Model Same equipment, same model, same process design from process development to continuous manufacturing at commercial scale will be explained. A case study using SOP is demonstrated based on performances obtained with a commercial media and a CHO commercial cell line. This innovative and unique method of perfusion control (SOP) leads to a new process development concept and manufacturing process design for mAbs and therapeutic proteins.

Characterizing of Raw Material Constituents on Cellular Flux rRates

Barbara Deschamp, University of Massacusetts Lowell

Abstract: This research addresses the characterization of metabolic pathways in the production of biopharmaceuticals. The effects of variability in the constituents of raw materials that drive the chemical reactions in the generation of end products are analyzed. An analysis of CHO cells in response to eleven different sets of wheat hydrolysate inputs is carried out using metabolic flux analysis. The input data includes a control set and six sets that have a range of composition variation. The input variation was analyzed to determine the variation of cellular flux rates. The stoichiometric matrix is formed by metabolites and their respective reactions A linear program was employed using biomass as the objective function to be maximized. The optimum intracellular metabolic fluxes are found with given constraints. The impact of raw material variability with respect to the calculated intra and extracellular flux rates is investigated.

Cell Line Qualifying Attributes for Prediction of Good Producers

Alessandro Mora, University of Massachusetts Lowell

Abstract: Early stage titer of IgG-expressing cell lines is not predictive of final titer in production fed-batch experiment, because of adaptation randomness from static to suspension culture and secondary because only static titer is used as selection criteria without the support of growth data. Despite the challenges in overcoming the latter, by employing a corrected density value, an analysis of the progression of growth and titer data has been conducted across 320 lines, from various genetic sets, in 3 different vessels from static to suspension conditions, in order to investigate patterns that define the best candidate cell line. Relationships were studied by multivariate analysis and a corrected specific productivity attribute in 96-well plate was showed to enhance the predictive power of titer in 96-well plate therefore capturing more top performers in the following production fed-batch experiment. This method might optimize the cell line screening process by reducing the workload and focusing only on the cell lines that retain the highest potential at the early stage.

Facile Biomolecular Conjugation with Chitosan-Poly(ethylene glycol) Microparticles via Strain-Promoted Alkyne-Azide Cycloaddition Reaction toward Rapid Bioprocess Monitoring Applications

Sukwon Jung and Hyunmin Yi*
Department of Chemical and Biological Engineering, Tufts University

Abstract: Rapid and reliable monitoring of biomacromolecules representing physiological events for facile bioprocess control is an unmet challenge. We strive to address this issue through development of facile fabrication and conjugation schemes for high capacity biosensing platforms that can be enlisted to capture direct variables such as mRNAs and proteins.

1. Fabrication of Chitosan-Poly(ethylene glycol) Microparticles via Replica Molding

Chitosan, a naturally derived amino-polysaccharide, has been widely studied as biomolecular conjugation platforms due to its high content of primary amines that offer covalent binding sites. However, some physical properties of chitosan have limited its utility in fabrication of biofunctionalized platforms. In this presentation, we demonstrate a facile scheme to fabricate chitosan-poly(ethylene glycol) (PEG) microparticles via replica molding, which offers a simple, robust, and scalable fabrication of polymeric microparticles with reliable duplication of complex 2D shapes. Fluorescent labeling and FTIR microscopy results indicate stable incorporation of chitosan within the microparticles as well as chemical reactivity toward anime-reactive chemistries.

2. Biomolecular Conjugation via Strain-Promoted Alkyne-Azide Cycloaddition Reaction

Traditional biomolecular conjugation schemes have some drawbacks such as limited selectivity, stability and biocompatibility. In this study, a bioorthogonal strain-promoted alkyne-azide cycloaddition (SPAAC) reaction was enlisted for selective conjugation of biomolecules with chitosan-PEG microparticles via stable triazole linkages under mild conjugation conditions. Fluorescence and confocal micrographs show that the biomolecules are selectively conjugated near the particle surfaces where mass transfer limitation is minimal. Results on biomolecular conjugation kinetics via the SPAAC reaction show multiple reaction regimes; rapid initial, intermediate, and steady final stage. Selective target protein capture with antibody conjugated microparticles show rapid binding kinetics, indicating the potential of our fabrication-conjugation approach for rapid and reliable monitoring of biomacromolecules in biopharmaceutical processes.

Evaluation of PBS 15 Air-Wheel® Single Use Bioreactor for a Fed-Batch Monoclonal Antibody Process

Tuhina Bhattacharya, Sadettin Ozturk, UMass Biologics, UMass Medical school, Worcestor, MA

Abstract: The Upstream Process Development team at MassBiologics has demonstrated successful cell culture process development utilizing MassBiologics's proprietary chemically defined media and feed platform in addition to optimized DASGIP bioreactor conditions to boost mAb expression and growth. In contrast to stirred tank bioreactors, the Air-Wheel® Single Use Bioreactor system utilizes rising gas bubbles and converts them into rotational mixing power, lowering the amount of shear stress on the cells. In this work we evaluated the PBS 15 Air-Wheel® using MassBiologics's optimized media and feed platform, pH control, dissolved oxygen control, temperature conditions, and a stable CHO cell line expressing an IgG1 mAb product. We compared its titer and growth patterns to concurrent DASGIP 1L bioreactors running under the same optimized conditions. All three 14 day fed-batch runs were conducted with one PBS 15 Air-Wheel®, two duplicate 1L DASGIP bioreactors with 15µm microspargers, and 100mL shake flask controls inoculated from each bioreactor system. However, the first experiment showed that evaporation for the Air-Wheel® culture was 43% higher than the DASGIP culture. As a result, the second experiment used diluted feed in order to compensate for the evaporating water calculated from the first experiment. Using the diluted feed reduced the evaporation rate to 27% when compared to DASGIP cultures without diluted feed. This led to the use of a lower Air-Wheel® agitation rate for the third run, which reduced the amount of gassing by 60%. Lower gassing reduced the evaporation rate further to 12%. All three experiments show that viable cell density, viability, titer, specific growth and death rates, productivity, and metabolism are comparable between the two bioreactor systems, and that the Air-Wheel® system provides improved CO2 stripping.

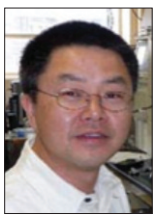
BPQC FACULTIES

Dr. Seongkyu Yoon is director of the Massachusetts BioManufacturing Center (MBMC), process system engineering and an assistant professor in the department of Chemical Engineering of the University of Massachusetts Lowell. His research area is Life Sciences Systems Engineering. Research covers Process Analytical Technology (PAT) and Quality by Design (QbD), Application of Design of Experiment (DoE) and MultiVariate Data Analysis (MVDA), supply chain management in biologics, and chemometrics in life sciences. Research aims at developing innovative systems technology with which one can improve drug development efficiency and manufacturing productivity, and developing innovative diagnostic systems and tools for selected diseases with chemometrics framework. He is currently developing system tools using a genomics and metabolic flux analysis approach to explain variability to productivity and quality of CHO (Chinese Hamster Ovary) mammalian cell-culture product. Integration of medical devices with multivariate statistical method is also being explored to develop practical diagnostic tools.

Dr. Yoon completed his Ph. D. in Chemical Engineering from McMaster University (Hamilton, Canada) under Prof. John F. MacGregor's supervision. Afterwards, he worked at Umetrics (Kinnelon, NJ) with Dr. Svante Wold and Nouna Kettaneh. He provided consulting and teaching on multivariate data analysis, experimental design, and batch analysis in various industries, pharmaceutical, biologics, semiconductor, petrochemical, and financial. Before joining UMass Lowell, Dr. Yoon worked at Biogen Idec Biopharmaceutical Inc. as process analytics group leader of manufacturing sciences. He implemented MSPC (Multivariate Statistical Process Control) to all unit operations of both commercial and clinical manufacturing. This pioneering work significantly improved manufacturing robustness and clarity. The MSPC system is now considered as an industry standard which most biopharmaceutical manufacturers adapted as common manufacturing system. He also worked at Hyundai Petrochemical (now LG Chemistry) as a process engineer and implemented Advanced Process Control and Real-time Optimizer to ethylene manufacturing process in early 1990.

Dr. Hyunmin Yi is currently an Assistant Professor at the Department of Chemical and Biological Engineering of Tufts University. He received his B.S. in Chemical Technology and M.S. in Biochemical Engineering from Seoul National University, and Ph.D. in Chemical Engineering from the University of Maryland at College Park. He has published over 30 research articles in peer-reviewed journals such as Analytical Chemistry, Journal of Materials Chemistry, Langmuir, Nano Letters, Biotechnology and Bioengineering, and Lab-on-a-Chip.

He has extensive service activities for the biochemical engineering community as a panelist at many NSF grant proposal review panels, reviewer for over 20 journals, and has been chair for several sessions at ACS and AIChE National Meetings. He is currently the lead-PI on two NSF research grants. Professor Hyunmin Yi's broad research interests are viral nanobiotechnology and biosensors. In the first area, his group utilizes genetically modified tobacco mosaic viruses (TMV) for readily controlled metal nanoparticle synthesis toward applications in environmental catalysis, organic synthesis and energy. In the second area, soft-lithographic techniques are enlisted for robust fabrication of polymeric hydrogel microparticles toward rapid and reliable in-situ bioprocess monitoring. The overarching theme in both of these areas is to understand and exploit the selective and programmable properties of biological and biochemical materials and interactions in facile fabrication and assembly of multifunctional materials.



Dr. Jin Xu is director of the Massachusetts BioManufacturing Center (MBMC) Protein Analysis and Characterization Laboratory and an assistant professor in the UMass Lowell Chemistry Department. He currently oversees and actively participates in protein structural/functional studies, protein product characterization and analytical development at MBMC. With his expertise in protein chemistry and biophysics, Dr. Xu also designs and conducts studies on the relationship between protein folding and protein productivity/quality.

Before joining UMass Lowell, Dr. Xu received his Ph.D in Biochemistry from the University of North Texas. Afterwards, he worked as Senior Research Scientist and Principal Scientist at Wyeth Pharmaceuticals for over five years, before most recently establishing and leading the protein chemistry group at Percivia, LLC.

Dr. Carl W. Lawton is director of the Massachusetts BioManufacturing Center (MBMC) and Associate Professor in the Department of Chemical Engineering at UMass Lowell. As director of the MBMC, Dr. Lawton is responsible for overseeing the coordination and completion of process development client services including expression development, fermentation and cell culture development, downstream processing, process optimization and characterization. He works closely with companies on the verge of biopharmaceutical production to give them the opportunity to utilize the Center's services to economically address staffing needs and learning curve constraints and to optimize time to market. Dr. Lawton creates and teaches customized training programs for biopharmaceutical manufacturing workforce as well as advising and teaching undergraduate and graduate students in the fields of chemical and biochemical engineering and others. He also is responsible for developing and maintaining an applied research program which focuses on technological advances to improve the quality, cost and productivity of large-scale biomanufacturing production. Before joining UMass Lowell and creating the MBMC, Dr. Lawton was a bioengineering process consultant to companies on both the east and west US coasts and in Canada.

Dr. Sanjeev Manohar is a Professor at the Department of Chemical Engineering at the University of Massachusetts Lowell and associate dean of the college of engineering. He holds Master's degrees in Chemistry from the University of Madras and in Organic Chemistry from Southern Illinois University and a Ph.D. in Organic/Polymer Chemistry from the University of Pennsylvania. His research is based on the synthesis and characterization of nanostructured materials for energy storage and medical applications; optically transparent, conducting films of carbon nanotubes on flexible substrates with performance that can rival commercial indium-tin-oxide conducting coatings; chemical warfare agent sensing using carbon nanotube coatings on flexible substrates; photocapacitors and batteries from dye-sensitized solar cells using a completely new design strategy involving plant extracts, and nanocarbons; green chemistry approaches to polymer/metal catalysts for fuel cells; synthesis and characterization of conducting polymer nanotubes/fibers and composites with noble metals; and controlled and targeted drug delivery across the blood-brain-barrier for treatment of Alzheimer's disease using nanoparticulate drug carriers.

Dr. Sadettin Ozturk is currently the Head of Process and Analytical Development, MassBiologics and Assistant Professor of Medicine, University of Massachusetts, Medical School. He has had a long career in cell culture process development, technology transfer, product licensing, and commercial manufacturing. His early contributions to the field focused on applying chemical engineering principles and process control strategies to the optimization and scale-up of cell culture processes. The scope of his work has expanded over the years, but it has always been focused on advancing cell technology. He was responsible for the development of numerous cell culture based processes and novel technologies that helped not only the companies that he worked for (Verax, Bayer, GlaxoSmithKline, and Johnson & Johnson), but contributed to the rest of the field through his numerous presentations and publications. Sadettin led process development activities and played a key role in the licensing and commercialization of two monoclonal antibodies, Stelera, and Simponi. In addition, he transferred and supported the commercial manufacturing of Kogenate and BeneFix. Sadettin has published numerous research articles, given presentations, delivered keynote lectures, and edited books. He is a member of several societies including ESACT, American Association for the Advancement of Science, New York Academy of Sciences, American Chemical Society, and American Institute of Chemical Engineering. Sadettin is involved in these scientific organizations and other community activities by serving on their Scientific Advisory Boards and organizing meetings and sessions. He has served Biochemical Technology (BIOT) division of American Chemical Society as the Division Chair, and then as a Councilor. He co-authored a well-respected book in the field entitled Cell Culture Technology for Pharmaceutical and Cellular Therapies. Sadettin also serves on Editorial and Review Boards for several journals and other publications.

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Hyunmin Yi, Associate Professor of Chemical and Biological Engineering, Tufts University
Sanjeev Manohar, Professor of Chemical Engineering, Univ. of Massachusetts Lowell
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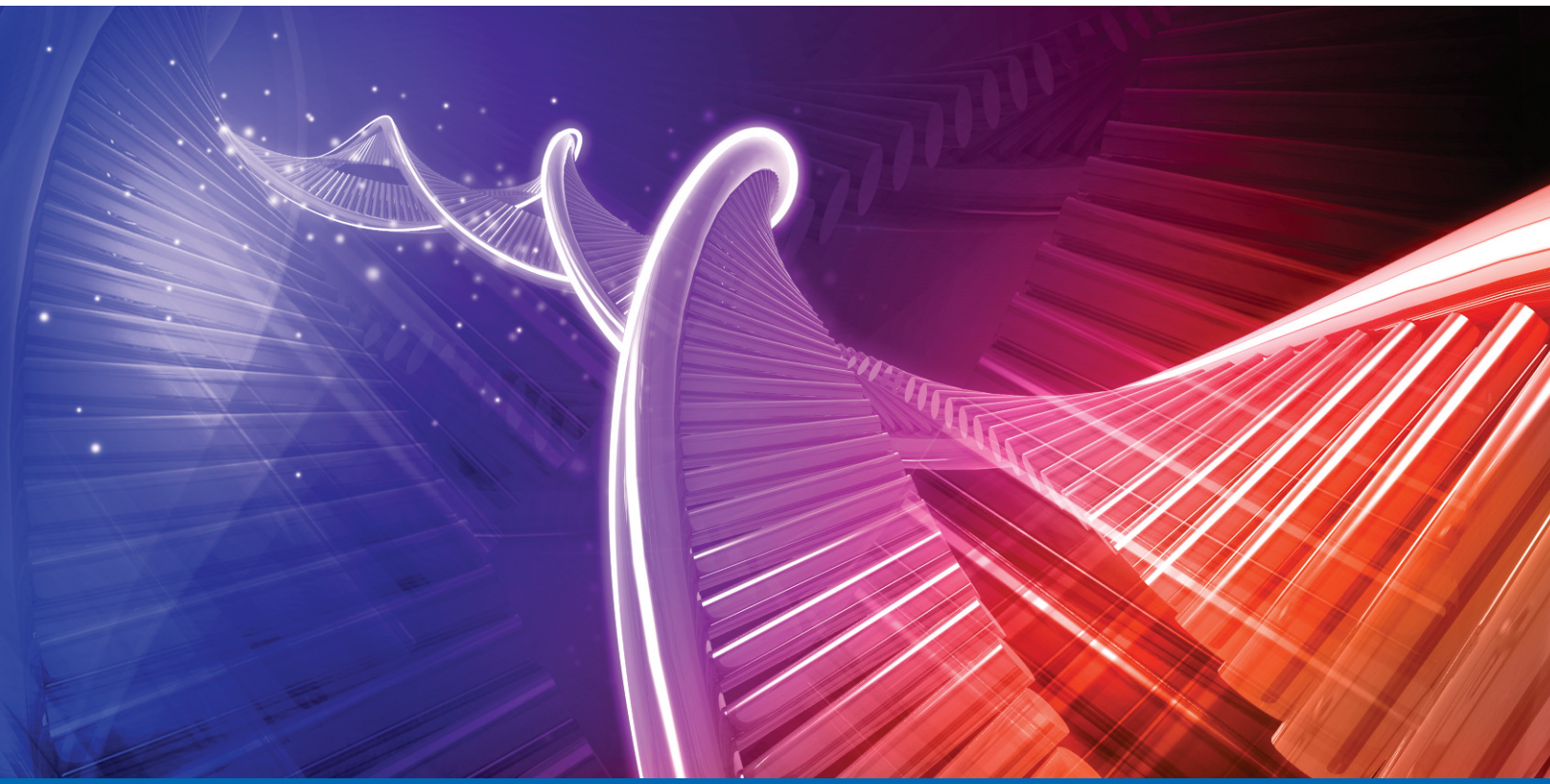

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