SoCal Flow SUMMIT 2013

March 25-26, 2013
Beckman Center,
University of California, Irvine

SoCal Flow SUMMIT 2013

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Spherotech Let the Possibilities Flow...

Southern California Flow Cytometry Association

Presents

SoCal Flow SUMMIT 2013

March 25-26, 2013

Hosted by the Sue & Bill Gross CIRM Stem Cell Research Center
University of California, Irvine
Beckman Center

Researchers from the Southland's regional research centers, clinical laboratories and biomedical industries will join together to share their contributions to Cytometry, network and learn of resources in the region.

SUMMIT 2013 Day One
March 25, 2013
9:00am - 5:30pm

Core Managers Dinner Meeting
March 25, 2013
5:30pm - 8:30pm
Everyone is welcome
Happy Hour by **Tonbo Biosciences**

SUMMIT 2013 Day Two
March 26, 2013
8:00am – 4:30pm

Pre– and Post– Summit Courses by Expert Cytometry
Post-Summit Seminar by Flowjo

PROGRAM

Scientific Presentations:

David Hedley MD PhD - Emerging Applications of Flow Cytometry in Molecular Oncology

Pratip K. Chattopadhyay PhD - The Twists and Turns of High Dimension HIV Research

Julie E. Lang MD FACS - Isolation and Molecular Profiling of Circulating Tumor Cells in Breast Cancer

Bartek Rajwa PhD – Signal Formation and Generalized Compensation Theory for Multispectral and Hyperspectral Flow Cytometry Systems

Peter J. Burke PhD – Nanofluidic Based Assays of Mitochondria

Alexander D. Boiko PhD – Targeting CD271+ Melanoma Tumor Initiating Cells Synergizes with CD47 Blockade to Prevent Multiple Organ Metastasis In-Vivo

Vendor Presentations from Event Sponsors

ADDITIONAL ATTRACTIONS –

ExCyte Cytometry will conduct Flow Cytometry Training March 24 and March 27
(Separate registration required)
Flow Cytometry Bootcamp on Sunday, March 24th, 2013
Advanced Course on Fluorescent Proteins, DNA Cell Cycle and Apoptosis on Wednesday, March 27th, 2013
Location: Sue & Bill Stem Cell Research Center, Gross Hall, 4th floor

Flowjo: Flow Cytometry Data Analysis Seminar March 28th, 2013
10am-11am Version 10 Live Demo
11am-12pm Compensation, Transformation Version 10
and Fluorish Live Demo
FREE, RSVP to seminarscrc@gmail.com
Location: Sue & Bill Stem Cell Research Center, Gross Hall, 4th floor

Welcome to SoCal Flow SUMMIT 2013

The Program Committee of the Southern California Flow Cytometry Association is pleased to present its second event, SoCal Flow SUMMIT 2013, on March 25-26, 2013, held at the Beckman Center, University of California at Irvine. All flow cytometry users from Southern California's regional research centers, clinical laboratories and leading biomedical industries can join together at this exciting event for networking, sharing and education with Flow Cytometry colleagues from across the Southern California counties.

We hope that you all will enjoy this exciting two-day meeting which will include scientific presentations from invited faculty, vendor presentations, a poster session, and a core manager dinner meeting open to all attendees. The scientific program includes six (6) hours of CEU made available by FloCyte Associates.

Southern California Flow Cytometry Association Program Committee

Lora Barsky, Chairperson
University of Southern California

Lucy Brown, Vice Chairperson
Beckman Research Institute /
City of Hope Medical Center

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About SoCal Flow

The Southern California Flow Cytometry Association (SoCal Flow) brings together research, clinical and biomedical scientists from Los Angeles, Kern, San Luis Obispo, Santa Barbara, San Bernardino, Riverside, Orange County, San Diego and Imperial counties. The Association reaches out to members to enhance collaborations, for brainstorming ideas, asking questions and communicating with each other via its message board. SoCal Flow provides opportunities to further the education of its members by hosting scientific talks, workshops and training courses to keep members abreast of recent developments and advances in the field of cytometry. The inaugural November 2011 SUMMIT at USC-HSC campus was made possible by generous vendor support. The success of SUMMIT 2011 made possible SUMMIT 2013. The Southern California Flow Cytometry Association is now incorporated in the state of California as a tax-exempt corporation, and is waiting to hear from the IRS on its application as a non-profit 501(c)6 trade association. The Association has been accepted as an affiliate of the International Society for the Advancement of Cytometry (ISAC).

ISAC
International Society for Advancement of Cytometry

By attending this meeting all registered attendees will automatically become members of the Southern California Flow Cytometry Association unless they choose to opt-out. The committee encourages all SUMMIT 2013 attendees to partake of this association and become active networking members of the Southern California Flow Cytometry Community. During the SUMMIT, there will be a fifteen minute annual meeting of the Association. We hope that you will participate in this meeting and feel free to give feedback on what you would like to see the Association presenting.

SoCal Flow
Cytometry Association

Corporate Scholarship Winners

SoCal Flow Executive Committee is pleased to award these SoCal Flow members a training scholarship for the associated training sessions offered by ExCyte Expert Cytometry. Applications were judged for merit and ranked by committee. Please help us congratulate these winners.

Salvador Garcia

Graduate Student in Biology at CSU San Bernadino, works at CHLA with Tracy Grikscheit, MD

Project: Successful growth of tissue-engineered small intestine (TESI) may someday be a treatment for children with short bowel syndrome (SBS). Present-day limitations of TESI include the volume of tissue generated and the poorly understood mechanism of TESI growth. Currently we are investigating the interaction between amniotic fluid stem cells (AFSC) and intestinal organoid units (OUs; intestinal multicellular aggregates with intestinal stem cells) in the formation of TESI. In preliminary experiments we have observed that AFSCs isolated by magnetic-activated cell sorting via CD117 antibodies have shown some expression of Lgr5 (Lgr5-GFP transgenic mice) an intestinal stem cell marker in their pluripotent state. Furthermore, we have observed that CD117+ cells can integrate within the OUs and sustain there for an indefinite time. It is still unknown if the dual expressing CD117 and Lgr5 cells integrate into the OUs, therefore it will be of great interest to isolate pure populations and examine their interactions.

Aditi Bauskar

Graduate Student in Cell and Neuobiology at University of Southern California, works for M. Elizabeth Fini, PhD

Project: My project (PI: M. Elizabeth Fini) involves isolating side population cells (SPC's) from the corneal epithelium (eye tissue) of mouse eyes. The SPCs may play an important factor in corneal regeneration and wound healing in eye injury. Our research hypothesis is that the numbers of side population cells in the cornea are different in different mouse strains. To test this idea, I will be isolating total corneal epithelial cells from different strains of mice by dispase and trypsin treatments of isolated cornea. I will then perform a side population discrimination assay to detect stem cells based on the dye efflux properties of ABC transporter (ATP-Binding Cassette Transporter protein) and count the SPC's using FACS sorting. These SPCs will represent the stem cell population of the corneal epithelium. The goal is to see whether the stem cell number of the corneal epithelium is affected or is different in different mice strains, and if so, we will further try to link the stains' genotypes (for example, single nucleotide polymorphism) and the SPC numbers by examining mice over 100 different strains.

Nathaniel Magilnick

Graduate Student in Molecular and Cellular Biology at City of Hope, works with Mark Boldin PhD

Project: I will be using flow cytometry to analyze the hematopoietic cell lineages in several microRNA knockout mouse models. One project involves the further analysis of the negative regulation of the immune system by a particular microRNA. We will be using flow cytometry to analyze Bone Marrow and Splenocytes to help define the cellular targets of this microRNA and its role in modulating the immune response.

Kaniel Cassady

Graduate Student at City of Hope, works for Defu Zeng and Art Riggs

Project: Our lab studies Graft versus Host Disease, a commonly fatal side-effect of Bone Marrow Transplantation. My project utilizes FACs to assess the effects of pre-conditioning our animal BMT recipients with anti-CD3. This regimen has been shown by our lab to prevent GVHD in our animal model--however, the mechanism is still unclear. Currently, I am investigating the tolerogenic modulation of Host dendritic cells and T-cells--an experiment that relies heavily on flow techniques.

Yaqiong Chen

Graduate Student at City of Hope, works for Ravi Bhatia

My current project is to study the function of mesenchymal stem cells in the leukemic bone marrow microenvironment. Previous study in our lab has shown that the leukemic bone marrow environment shows altered interaction with normal and leukemic hematopoietic stem cells. Preliminary data has obtained, in which the distribution of specific mesenchymal subpopulations is altered in leukemic BM. I utilize flow cytometry to isolate specific mesenchymal progenitor populations from normal and leukemic marrow, then establish culture assays including 3-dimensional cultures to study their differentiation (by RT-PCR, flow cytometry and immunohistostaining), their ability to support hematopoietic stem cells (cell counting and staining with FACS), and their gene expression patterns.

Gregory Cherryholmes

Graduate Student in Cancer Immunotherapy and Tumor Immunology at City of Hope, works for Hua Yu

My work involves looking at cancer stem cell phenotypes, but my project is moving into looking at cell cycle analysis and proliferation. In addition, I will be looking at chemoresistance and would like to combine efflux of innately -fluorescent chemotherapies with apoptotic analysis via flow cytometry. Therefore, deep understanding of these advanced techniques will help my future steps of my project.

SoCal Flow SUMMIT 2013 Program

Monday, March 25, 2013

9:00 - 9:45	Registration Continental Breakfast with coffee service
9:45 - 10:00	Opening Remarks
10:00 - 10:30	Raffi Manoukian:Cell SignalingTechnology: *"Cellular Signaling Pathway Analysis by Flow Cytometry and Immunofluorescence"*
10:30 - 11:20	**David Hedley, Ontario Cancer Institute** *"Emerging Applications of Flow Cytometry for Molecular Oncology"*
11:25 - 11:40	Rebecca LaFond: Miltenyi Biotec: *"Isolation and Analysis of Rare Cell Populations"*
11:45 - 12:00	Carol Oxford: ExCyte: "From antibodies to answers: ExCyting training to maximize your flow potential"
12:00 - 13:30	Buffet Lunch – Please visit the posters and vendor tables
13:30 - 14:20	**Pratip Chattopadhyay, NIH** *"The Twists and Turns of HIV Research"*
14:25 -- 14:55	Mervi Reunanen: BD Bioscience: *"New tools for multicolor flow cytometry from BD Biosciences"*
15:00 - 15:15	Jerry Barnhart: Sony Biotech: *"Sony Biotechnology - Simplicity through True Innovation"*
15:15 - 15:45	Coffee break Please visit the Posters and Vendor tables
15:50 - 16:40	**Julie E. Lang, USC** *"Isolation and Molecular Profiling of Circulating Tumor Cells in Breast Cancer"*
16:45 - 17:00	Lisa Nichols: Cytek: *"Upgrading your cytometer to keep pace with today's fluorochromes"*
17:00 - 17:30	Mirja Gunthart Wine: Beckman Coutler: "Simple Instracellular Staining: FoxP3 and Beyond"
17:30 - 20:30	Buffet DINNER & CORE MANAGER'S MEETING Happy Hour sponsored by Tonbo Scientific

SoCal Flow SUMMIT 2013 Program

Tuesday, March 26, 2013

8:00 - 8:30	Registration Continental Breakfast with coffee service
8:30 - 9:00	Wendell Smith: eBioscience: *"Getting the most out of your multicolor cytokine & transcription factor flow cytometry data"*
9:05 - 9:55	**Bartek Rajwa, Purdue** *"Signal formation and generalized compensation theory for multispectral and hyperspectral flow cytometry systems"*
10:00 - 10:30	Coffee break Please visit the Posters and Vendor tables
10:30 - 10:45	SoCal Flow Business Meeting
10:50 - 11:05	Tanya Tolmachoff: DeNovo Software: *"Top 10 Reasons why you need to use FCS Express for your analysis"*
11:10 - 11:40	Melissa Ma: Bio-Rad: *"The S3 ProDrop TM System, a Novel Approach to Automated Drop Delay"*
11:45 - 12:00	Shervin Javadi: Stratedigm: *"Applications in Flow Cytometry, Micro Particle Analysis and High Throughput Flow Cytometry Using Stratedigm's S1000 Analyzer"*
12:00 - 13:30	Buffet Lunch Please visit the Posters and Vendor tables
13:30 - 13:45	Ken Lau: Biolegend: *"Web Tools for Untangling Flow Cytometry"*
13:50 - 14:40	**Peter Burke, UCI** *"Nanofluidic Based Assays of Mitochondria"*
14:40 - 15:10	Coffee break Please visit the Posters and Vendor tables
15:15 - 15:30	Mike Roth, UCLA Geffen School of Med: *"Imaging Flow Cytometry reveals both Intracellular and Extracellular forms of the Cannabinoid Type 2 (CB2) Receptor"* sponsored by Millipore/Amnis
15:35 - 16:25	**Alexander Boiko, UCI** *"Targeting CD271+ Melanoma Tumor Initiating Cells Synergizes with CD47 Blockade to Prevent Multiple Organ metastasis In-Vivo"*
16:25 - 16:30	Adjourn (take down posters)

Speakers and Abstracts:

David Hedley MD PhD

Senior Scientist and Senior Staff Physician, Medical Oncology Department and Ontario Cancer Institute, Princess Margaret Hospital, University of Toronto, Canada.

Emerging Applications of Flow Cytometry in Molecular Oncology

The clinical landscape of molecular cancer therapeutics is shifting. Expectations that dramatic responses to signal transduction inhibitors, similar to those seen in CML patients treated with imatinib, have not been realized. Since it is likely that most oncogenic targets have now been identified, with effective inhibitors available for clinical testing, the emphasis is shifting towards combinations of targeted agents and the challenge is patient selection for specific drug combinations (recognizing that all of these agents have side effects). Despite early optimism, a deluge of genome sequencing data has failed to identify significant numbers of previously unknown druggable targets. These genomics studies have also rediscovered the analytical complexity of tumor heterogeneity, which is what flow is all about! On the other hand, there is major interest in the areas of epigenetic and metabolic regulation of cancers, which might be more tractable in terms of novel treatments, and those fields are currently wide open for translational research. So it is timely to review the potential for flow cytometry in the field of molecular oncology. In this talk I will overview some practical issues, particularly the development and optimization of novel flow techniques that can be applied to patient samples linked to clinical trials of targeted agents. I will emphasize applications where I believe that flow has a competitive edge relative to alternative approaches.

Pratip K. Chattopadhyay PhD

ImmunoTechnology Section, Vaccine Research Center, NIAID, NIH

The Twists and Turns of High Dimension HIV Research

The immune system is remarkably complex, consisting of hundreds of molecules that govern the differentiation and function of leukocytes. In combination, these markers can define an enormous number of cell subsets, complicating efforts to understand the most important cell types involved in an immune response. Nevertheless, this understanding is fundamental to our vaccine and treatment efforts in HIV. In this talk, I will describe our recent research into HIV immunity, and the lessons learned from it that our relevant to the design and analysis of polychromatic flow cytometry experiments. I will highlight new tools we have developed for automated analysis of data sets, and how these tools are able to reduce the complexity of immune analysis, and reveal the most important cell subsets involved in disease.

Julie E. Lang MD FACS
Associate Professor of Surgery, University of Southern California, Div.of Breast and Soft Tissue Surgery & Principal Investigator, Breast Surgical Oncology Translational Research Laboratory, Norris Comprehensive Cancer Center

Isolation and Molecular Profiling of Circulating Tumor Cells in Breast Cancer

Metastasis is responsible for virtually all breast cancer related deaths. Detection of circulating tumor cells (CTCs) has been demonstrated to correlate with worse prognosis in both metastatic and non-metastatic breast cancer (MBC) patients. CTCs, unlike most of the cancerous cells in primary tumors, have the ability to leave the primary tumor, enter the bloodstream, and may explain disease recurrence even after a prolonged disease free interval. Our team has developed a novel assay that captures rare CTCs from peripheral blood via a simple blood draw. Small populations of pure CTCs can be isolated for downstream molecular analyses. To our knowledge, our group is the first to develop a method for the isolation and very detailed molecular profiling of pure populations of CTCs in breast cancer.

Bartek Rajwa PhD
Research Assistant Professor Bindley Bioscience Center, Purdue University

Signal formation and generalized compensation theory for multispectral and hyperspectral flow cytometry systems

The process of spectral unmixing is routinely employed in flow cytometry (under the name of "compensation") and in image cytometry (automated microscopy and high-content-screening). In the broadest sense, unmixing is a procedure that allows for identifying individual signal constituents as well as the abundances in which they appear in single multispectral measurements (single cells in flow, or single pixels in imaging). In flow cytometry (FC) these measurements, which involve acquisition of fluorescence from labels attached to biomarkers, are traditionally represented mathematically as linear mixtures of pure signals contaminated by noise. Technically, spectral unmixing can be seen as a simple case of the inverse problem that estimates abundance of the labels from noise-contaminated observations of their fluorescence signals. During the unmixing step every measured data vector (describing a single cell, or an individual pixel in imaging) is decomposed into a set of spectral signatures (mixing matrix) and a set of corresponding abundances. With the introduction of multispectral flow cytometry using a number of channels larger than the number of labels, a rigorous approach to unmixing incorporating a realistic noise model became possible. This presentation will discuss a physics-based model in which FC measurements are approximated by Poisson or Gamma processes, and unmixing (compensation) is performed using a GLM framework. This approach allows recovery of true biomarker concentrations, improves the resolution, and avoids generation of artifactually spread or negative populations following the compensation. The presentation will discuss in depth the theoretical aspects of compensation, starting with the two-color model proposed by Loken (1977), via the multicolor compensation proposed by Bagwell and Adams (1993), to generalized multispectral cytometry unmixing conceptualized by Rajwa and Novo (2012). The talk does not require a thorough background in mathematics, physics, or statistics. However, a basic understanding of fluorescence-signal formation and college-level algebra will be assumed.

Peter J. Burke PhD
Professor, Department of EECS, Department of Biomedical Engineering, Department of Chemical Engineering and Materials Science, University of California, Irvine

Nanofluidic Based Assays of Mitochondria

In this talk we will present our recent work on combining nanotechnology, electrophysiology, metabolomics, and cancer research. These concepts all converge in the interrogation mitochondria, organelles that traditionally have been associated only with the production of ATP from pyruvate. Recent studies have shown they have a critical role in calcium signaling and regulation, as well as regulation of cell death. In our experiments, we use nanochannels to trap and interrogate optically the response of individual mitochondria isolated from cells to various chemical environments, including substrates and inhibitors of the electron transport chain as well as calcium induced depolarization of the membrane potential, the point of no return in apoptosis. This will be contrasted with the use of traditional flow cytometry to assay properties of sub-cellular organelles.

Alexander D. Boiko PhD
Molecular Biology & Biochemistry, University of California, Irvine

Targeting CD271+ Melanoma Tumor Initiating Cells Synergizes with CD47 Blockade to Prevent Multiple Organ Metastasis In-Vivo

Melanoma is one of the deadliest types of tumors and accounts for 79% of skin cancer deaths. The five-year survival rate for people whose melanoma is detected and treated before it spreads to the lymph nodes is around 90%. However, patients diagnosed with more advanced stages of the disease have a grim outlook with 5 year survival rate down to 65% for regional metastatic disease and 15% for distant metastasis. Despite significant effort, no long term effective therapies exist for advanced metastatic melanoma. Our laboratory has recently identified melanoma tumor initiating cells (MTICs) as CD271+ population from patient surgical samples (1). We have now established robust in-vivo model demonstrating that tumors arising from CD271+ population are capable of forming regional lymph node metastasis as well as colonize distant organs such as lungs and liver. We show that in addition to CD271 many metastatic melanomas express CD47. As was recently discovered by our lab, CD47 appears to be a key cell surface signal whose overexpression protects hematologic tumor cells from being eaten and eliminated by the cells of the immune system, macrophages (3, 4). Using in-vivo metastatic melanoma model we demonstrate that selective targeting of MTICs with CD271-Saporin conjugated antibody alone and in combination with CD47 blocking antibody dramatically reduces and in some cases completely eliminates regional and distant metastasis in NSG mice transplanted with aggressive melanoma cells isolated from patient sample. By analyzing publicly available melanoma array datasets we also reveal significant correlation between expression of CD47 and decreased survival of melanoma patients diagnosed with advanced stages of this disease.

Vendor Presentations from Event Sponsors:

Raffi Manoukian, Cell Signaling Technology	Cellular Signaling Pathway Analysis by Flow Cytometry and Immunofluorescence
Rebecca LaFond, Miltenyi Biotec	Isolation and Analysis of Rare Cell Populations
Carol Oxford, ExCyte Expert Cytometry	From antibodies to answers: ExCyting training to maximize your flow potential
Mervi Reunanen, BD Biosciences	New tools for multicolor flow cytometry from BD Biosciences
Jerry Barnhart, Sony Biotechnology	Simplicity through True Innovation
Lisa Nichols, Cytek Development	Upgrading your cytometer to keep pace with today's fluorochromes
Mirja Wine, Beckman Coulter	Simple Intracellular Staining: Fox P3 and Beyond
Wendell Smith, eBioscience	Getting the most out of your multicolor cytokine & transcription factor flow cytometry data
Tanya Tolmachoff, DeNovo Software	Top 10 Reasons why you need to use FCS Express for your analysis
Melissa Ma, BioRad	The S3 ProDropTM System, a Novel Approach to Automated Drop Delay
Shervin Javadi, Stratedigm	Applications in Flow Cytometry, Micro Particle Analysis and High Throughput Flow Cytometry using Stratedigm's S1000 Analyzer
Ken Lau, Biolegend	Web Tools for Untangling Flow Cytometry
Mike Roth MD, UCLA Geffen School of Med; for Millipore/Amnis	Imaging Flow Cytometry reveals both Intracellular and Extracellular forms of the Cannabinoid Type 2 (CB2) Receptor

NOTES

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Please be sure to thank our sponsors. The SoCal Flow Summit would not be possible without their generous support!

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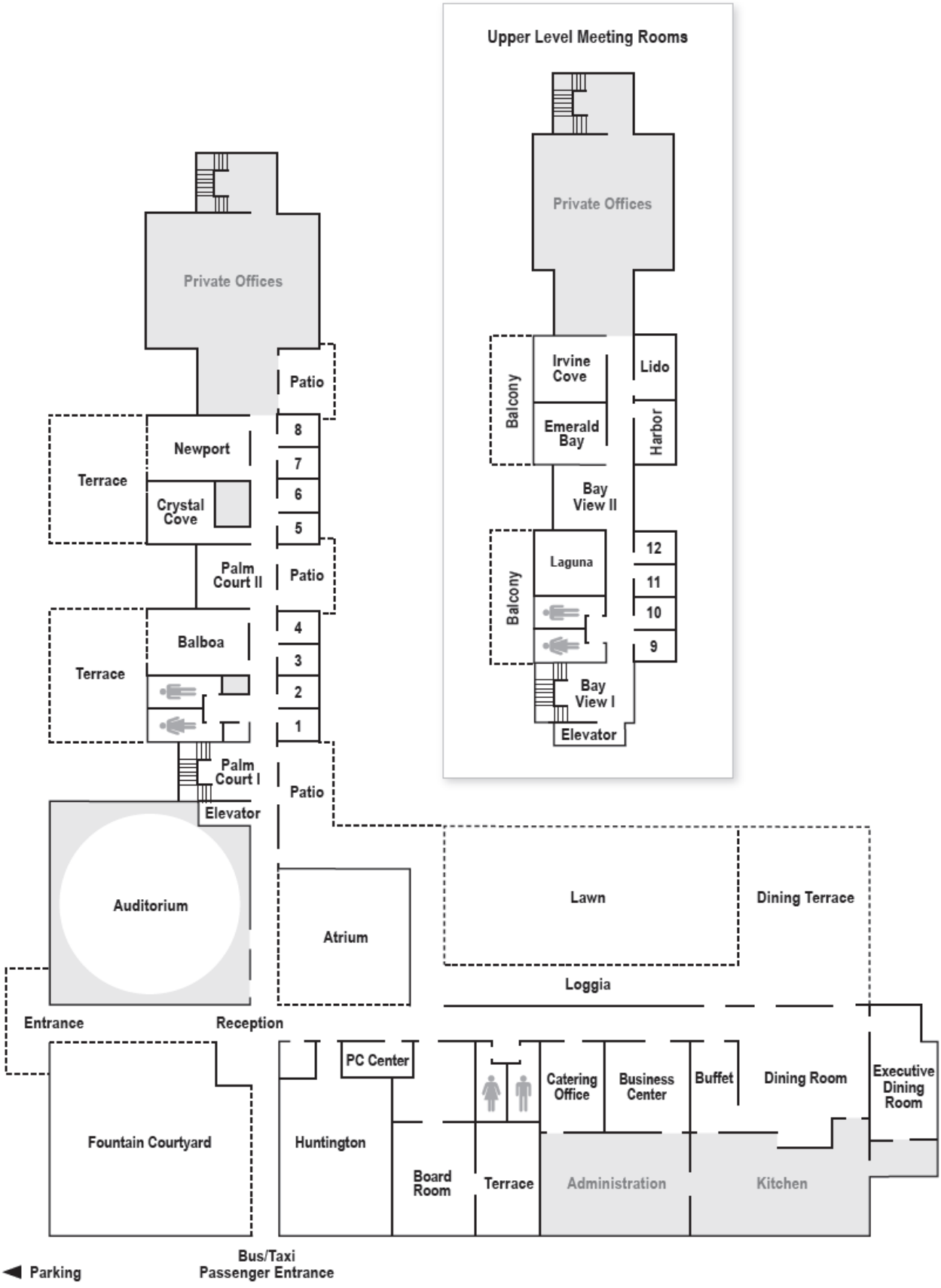

Beckman Center Floor Plan

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