Science Robotics is a unique journal created to help advance the research and development of robotics for all environments. Science Robotics will provide a much-needed central forum to share the latest technological discoveries and to discuss the field’s critical issues.
Will your recent PhD graduate win?

Winners will have their essay published by *Science*, earn up to 30,000 USD and receive a prize in Stockholm in December. Find out more: www.sciencemag.org/prizes/scilifelab

Cell and Molecular Biology | Ecology and Environment | Genomics and Proteomics | Translational Medicine
Measuring Immunological Synapses Using Amnis® Imaging Flow Cytometers

Abstract

Interactions between antigen-specific T cells and the cognate ligand of antigen presenting cells (APC) involve cytoskeleton reorganization and recruitment of adhesive and signaling molecules to the site of contact. Sustained adhesion of T cells to APCs and formation of an immunological synapse are required for T cell stimulation and the antigen-specific response.

Immunological synapse formation may be measured by fluorescently labeling molecules recruited to the synapse and imaging via confocal or conventional fluorescence microscopy. However, immunological synapses are often rare and therefore difficult to analyze objectively and statistically by traditional microscopy methods. We used Amnis® imaging flow cytometers to collect imagery of a large number of cells to:

1. Identify cell conjugates and assess the percentage of T cells involved in an organized immunological synapse.
2. Investigate recruitment of the LFA-1 adhesion molecule and Lck signaling molecule to the synaptic complex.
3. Assess the degree of T cell activation by measuring the translocation of NFkB from the cytoplasm to the nucleus in the T cell in an objective and statistically significant manner.

Identifying T cell–APCs conjugates

Raji B cells were loaded with Staphylococcal enterotoxin B (SEB) to make APCs. SEB-loaded APCs were incubated with human T cells purified from peripheral blood. After incubation, cells were fixed, permeabilized and labeled for CD3 (T cells, orange) and CD19 (Raji B cells, yellow).

Figure 1 shows images from the ImageStream® Mark II (ISX) imaging flow cytometer (60x objective). A FlowSight® (FS) platform may also be used for this purpose. Conjugates containing one T cell and one APC were identified and isolated using Area and Aspect Ratio features on different image channels of the cell using IDEAS® image analysis software.

Figure 1. Gating strategy to isolate conjugates for immunological synapse analysis.

Step 1: Plot the Aspect Ratio vs Area of the brightfield (BF) image to identify doublets.

Step 2: Find doublets positive for both CD3 and CD19 using Intensity. [Not shown]

Step 3: Find CD3+CD19+ doublets with one CD19 cell using Aspect Ratio vs Area of the CD19 image. [Not shown]

Step 4: Find doublets with one CD3+ cell using Aspect Ratio vs Area of the CD3 image.

This final population consists of doublets with only one CD19+ Raji B cell and one CD3+ T cell. In each study shown, this method was used to identify the T cell–APC conjugates, which were then used for further analysis.
Identifying organized immunological synapses in the conjugates

Polymerization and concentration of actin at the immunological synapse results in high local pixel intensity (Figure 2A). The number of T cells with an organized immunological synapse was calculated by plotting the Bright Detail Intensity of actin in the interface (ISX) or valley (FS) vs the Bright Detail Similarity of CD3 and CD19 (Figure 2B). The percent of T cells in the Immune Synapse gate was 6.9% for the SEB-treated sample vs 0.49% for the control (no SEB) sample. FS imaging flow cytometer yielded similar results: 8.4% for the SEB-treated sample vs 1.6% for the no-SEB control.

NFkB translocation in T cells conjugated to APCs

Stimulating the TCR with cognate ligand/MHC complexes presented by APCs results in T cell activation and nuclear translocation of NFkB. Within the same SEB-treated sample, we quantitatively compared NFkB translocation in T cells that were in contact with SEB-loaded APCs (T cell–APC conjugates) vs single T cells. IDEAS® software was used to calculate a Similarity score of the T cell NFkB signal to T cell DAPI nuclear signal. The median Similarity score for the T cells in T cell–APC conjugates was 0.98, vs 0.29 for single T cells (Figure 3).

Conclusions

Imaging flow cytometry combines the quantitative power of large sample sizes common to flow cytometry with the information content of microscopy. This study used Amnis® imaging flow cytometers and its companion IDEAS® data analysis software to demonstrate in an objective and statistically robust manner that there is a significant increase in the number of cell conjugates with an organized immunological synapse when APCs have been loaded with SEB, and there is an increase in NFkB translocation when T cells are in contact with APCs.
Large Capacity Centrifuge
The HiCen XL large-scale high-speed centrifuge comes from the Herolab Red Line series, which is packed with a range of features. The unit has a capacity of 6 mL × 1,000 mL and offers 24 different rotors: 18 angle rotors, 4 swing-out rotors, and 2 vertical rotors. This microprocessor-controlled unit can be set to speeds between 500 rpm and 21,000 rpm with a g-force of 50,743 × g. What sets the HiCen XL apart from most centrifuges on the market is its low running noise when operated at full speed. All Herolab centrifuges feature maintenance-free brushless induction drives equipped with an overspeed detection device and an imbalance detector that shuts the unit down in the unlikely event of a problem. The upgraded control panel is easy to use and gives the operator all the information necessary to ensure proper running conditions.

Herolab
For info: +49-(0)-6222-5802-0
www.herolab.de/index.php/en

Field-Flow Fractionation System
The CF2000 Centrifugal Field-Flow Fractionation system sets a new standard for high-resolution separation and fractionation of nanoparticles. The Postnova CF2000 employs a centrifugal field as its driving force. Particles affected by this field are separated by dynamic diffusion on the basis of both particle size and density, allowing separation of different particulate materials having the same particle size. Separations on the CF2000 can be further optimized by using different eluents and temperature programs. The system is an ideal tool in areas including agriculture, cosmetics, ecology, food, and nanomaterials. Samples can be injected directly without filtration, allowing the characterization of even complex particulate materials without alteration and damage. Various add-on modules and detectors are available, including refractive index, multi-angle light scattering, and dynamic light scattering.

Postnova
For info: +44-(0)-1885-475007
www.postnova.com

Confocal Laser Scanning Microscope
The fast module for ZEISS LSM 880 with Airyscan enables parallel excitation and detection of four image pixels. This upgrade enables confocal superresolution imaging with four times the speed and 1.5X resolution improvement. The gain in imaging speed allows researchers to enter the domain of classic resonant-scanning systems, but with a much better signal-to-noise ratio. ZEISS LSM 880 with Airyscan can be used for single and multiphoton experiments. Released in 2014, the Airyscan detector quickly established itself as a new standard in confocal live-cell imaging. Since then, scientists have already used Airyscan’s increased resolution in all spatial dimensions, as well as its high sensitivity, to publish exciting new data in leading scientific journals. It has collected multiple awards, including the R&D 100 Special Recognition Award, the Scientist’s Choice Award for best new life science product, and the Innovation Award of the State of Thuringia.

ZEISS
For info: 800-233-2343
www.zeiss.com

Taq DNA Polymerase
The 2X At Taq Master Mix is an optimized, ready-to-use, 2X concentrated DNA amplification mixture containing At Taq DNA Polymerase, reaction buffer, deoxynucleotide triphosphates (dNTPs), and MgCl₂. At Taq DNA Polymerase is a complex of specific anti-Taq (At) monoclonal antibodies with top-quality thermostable Taq DNA Polymerase for automatic “Hot Start” amplification, resulting in greatly improved amplification specificity, sensitivity, and yield. 2X At Taq Master Mix is suitable for all routine DNA amplification applications, and provides reduced artifacts such as primer-dimer formation and mispriming in multiplex amplification. Its main features include time efficiency, decreased contamination due to the reduced number of pipetting steps, and stability for up to 6 months at 4°C, which allows immediate reaction without time-consuming thawing of reagents. Samples are available upon request, but there are only a limited number, so act fast to contact your local Vivantis distributor.

Vivantis
For info: +44-(0)-1223-515440
www.vivantechnologies.com

Ultra-Low Temperature Freezers
A new range of ultra-low temperature freezers, has been designed to offer laboratories a green solution: less power consumption, less noise, and high efficiency without compromising the integrity of samples. Thermo Scientific TSX ultra-low temperature freezers are available in two sizes. TSX freezers use up to 50% less energy than conventional-refrigerant ultra-low freezers, and deliver temperature uniformity that continuously adapts to a laboratory’s environment. Conventional ultra-low temperature freezers use single-speed compressors that continually cycle on and off, resulting in poor temperature recovery following door openings. TSX freezers are equipped with our unique V-drive technology. When doors are opened frequently or when samples are added to the freezer, the control system detects the activity and increases the drive speed to bring temperatures quickly back to the set point. The freezers also use water-blow foam insulation, which eliminates the off-gassing typical of urethane-insulated freezers.

Thermo Fisher Scientific
For info: 800-955-6288
www.thermofisher.com

Microscope Stage for Microplates
The H139 is a motorized microscope stage designed to enable life scientists to accurately position, move, and image up to two microplates or nine microscope slides. Offering precise movement (0.7-μm repeatability) in the X and Y axis (280 mm x 80 mm), it is the perfect tool for labs tasked with meticulous microscopic examination of large numbers of microplates or microscope slides, and gives users a uniquely powerful combination of precision, speed, and convenience. Designed for use with the leading inverted microscope models, it allows users to conduct two live-cell imaging experiments in microplates simultaneously, allowing more efficient use of imaging-center resources. Using Prior’s ProScan III control system, the H139 can be automatically adjusted to an exact position, quickly shift across and between samples, and automate imaging routines; the ProScan III also helps reduce strain from repeated manual operations.

Prior Scientific
For info: +44-(0)-1223-881711
www.prior-scientific.co.uk

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/about/new-products-section for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by Science or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.
CALL FOR PAPERS

2017 MRS® FALL MEETING & EXHIBIT
November 26–December 1, 2017 | Boston, Massachusetts

Abstract Submission Opens
May 15, 2017

Abstract Submission Deadline
June 15, 2017

BROADER IMPACT
B11 Community College and University Partnerships as Catalysts for Promoting Materials Science Education
B12 Materials Innovation for Sustainable Agriculture and Energy

BIOMATERIALS AND SOFT MATERIALS
BM1 Multiscale Mechanobiology and Biomechanics—Theory, Experiments, Computations
BM2 Multiphase Fluids for Materials Science—Droplets, Bubbles and Emulsions
BM3 Biological and Bioinspired Materials for Photonics and Electronics—From Living Organisms to Devices
BM4 Biomaterials for Regenerative Engineering
BM5 Polymer Gels in Materials Science—3D/4D Printing, Fundamentals and Applications
BM6 2D Nanomaterials in Health Care
BM7 Emerging Materials and Devices for Engineering Biological Function and Dynamics
BM8 Materials Design for Neural Interfaces
BM9 Stretchable Bioelectronics—From Sensor Skins to Implants and Soft Robots
BM10 Bioinspired Interfacial Materials with Superwettability
BM11 Modeling, Characterization, Fabrication and Applications of Advanced Biopolymers—Where Form Meets Function
BM12 Biomolecular Self-Assembly for Materials Design

ELECTRONICS, Magnetics AND PHOTONICS
EM1 Organic Semiconductors—Surface, Interface, Bulk Doping and Charge Transport
EM2 Multiferroics and Magnetoelectrics
EM3 Novel Materials and Architectures for Plasmonics—From the Ultraviolet to the Terahertz
EM4 Wide- and Ultra-Wide-Bandgap Materials and Devices
EM5 Oxide Interfaces—Lattice and Electronic Defect Interactions
EM6 Diamond Electronics, Sensors and Biotechnology—Fundamentals to Applications
EM7 Materials, Devices and Architectures for Neuromorphic Engineering and Brain-Inspired Computing
EM8 Emerging Materials for Quantum Information
EM9 Electronic and Ionic Dynamics at Solid-Liquid Interfaces
EM10 Solution-Processed Inorganics for Electronic and Photonic Device Applications

ENERGY AND SUSTAINABILITY
ES1 Perovskite Materials and Devices—Progress and Challenges
ES2 On the Way to Sustainable Solar Fuels—New Concepts, Materials and System Integration
ES3 Earth Abundant Metal Oxides, Sulphides and Selenides for Energy Systems and Devices
ES4 Interfaces in Electrochemical Energy Storage
ES5 Materials and Design for Resilient Energy Storage
ES6 Alkali Solid Electrolytes and Solid-State Batteries
ES7 Chromogenic Materials and Devices
ES8 Advanced Nuclear Materials—Design, Development and Deployment
ES9 Thermal Energy—Transfer, Conversion and Storage
ES10 Materials Efficiency to Enable a Circular Materials Economy
ES11 Silicon for Photovoltaics

NANOMATERIALS
NM1 Carbon Quantum Dots—Emerging Science and Technology
NM2 Anisotropic Carbon Nanomaterials—Frontiers in Basic and Applied Research
NM3 Progress in Developing and Applications of Functional One-Dimensional Nanostructures
NM4 Atomically Thin, Layered and 2D Non-Carbon Materials and Systems
NM5 Nanomaterials, Nanoparticles and Nanostructures Produced by Plasmas—Synthesis, Characterization and Applications
NM6 Semiconductor Nanocrystals, Plasmonic Nanoparticles and Metal-Hybrid Structures
NM7 Nanostructure-Based Optical Bioprobes—Advances, Trends and Challenges in Optical and Multimodal Bioimaging and Sensing
NM8 Defect-Induced Phenomena and New States of Matter at the Nanoscale

PROCESSING AND MANUFACTURING
PM1 Explore New Frontiers in Materials Design Using Plasmas—Synthesis, Processing and Characterization
PM2 Advances and Upcoming Research Strategies in Reactive Materials
PM3 Interfaces and Interface Engineering in Inorganic Materials
PM4 Micro-Assembly Technologies—Fundamentals to Applications

THEORY, CHARACTERIZATION AND MODELING
TC1 Multifunctional and Multifrequency Scanning Probe Microscopy
TC2 In Situ Studies of Materials Transformations
TC3 Emerging Prospects in Capabilities in Ion Beam Technology and Applications
TC4 Advanced Atomic Models in Materials Science
TC5 Uncertainty Quantification in Multiscale Materials Simulation
TC6 Mechanical Behavior at the Micro and Nanoscale—Bridging Between Computer Simulations and Experiments
TC7 Design, Control and Advanced Characterization of Functional Defects in Materials

CALL FOR PAPERS

Meeting Chairs
Ike Arslan Pacific Northwest National Laboratory
Jason A. Burdick University of Pennsylvania
Tao Deng Shanghai Jiao Tong University
James B. Hannon IBM T.J. Watson Research Center
Sanjay Mathur University of Cologne

www.mrs.org/fall2017

2017 iMatSci Innovator Showcase
CALL FOR EARLY-STAGE STARTUPS
Submission Site Opens: June 1, 2017

www.mrs.org/imatsci

MRS MATERIALS RESEARCH SOCIETY®
Advancing materials. Improving the quality of life.
506 Keystone Drive • Warrendale, PA 15086-7573
Tel 724.779.3003 • Fax 724.779.8313
info@mrs.org • www.mrs.org
NO REACTION
NO PROGRESS

We can’t simply hope that reason will prevail, we have to stand together and act.

Join the American Association for the Advancement of Science.