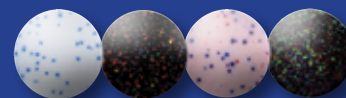# MULTIPLEXING ELISPOT
2-DAY WORKSHOP

CELLULAR TECHNOLOGY LIMITED
20521 CHAGRIN BOULEVARD, SHAKER HEIGHTS, CLEVELAND, OH USA

— · PROGRAM · —

DAY 1

9:00 A.M. – 9:30 A.M.

Welcome, Tour of Building
presented by Prof. Dr. Paul V. Lehmann

9:30 A.M. – 10:30 A.M.   LECTURE

The unique features of ELISPOT for immune monitoring:
General and T cells
presented by Prof. Dr. Paul V. Lehmann

10:30 A.M. – 10:40 A.M   COFFEE BREAK

10:40 A.M. – 11:00 A.M.   LECTURE

Analyzing T cell ELISPOT assay results:
SmartCount™ — Statistics-based automated size gating of T cell ELISPOTs permits obtaining objective, user-independent spot counts
presented by Dr. Alexey Karulin

11:00 A.M. – 12:00 NOON   LIVE DEMO / TUTORIAL

Analysis of single-color enzymatic T cell assays
presented by Tameem Ansari
- Scanning, single-color visible light
- Selecting spot category
- Automatic background corrections, SmartWell™
- Areal background corrections: Background balance, edge filter
- AutoGate™
- QC — Verify gate
- Multi-parameter AutoCount™

12:00 NOON – 12:30 P.M   LUNCH BREAK

12:30 P.M. – 1:30 P.M.   HANDS-ON TRAINING / Q&A

Analysis of single-color enzymatic T cell assays
presented by Tameem Ansari, Jodi Hanson, Richard Caspell & Villian Naeem
- Scanning, single-color visible light
- Selecting spot category
- Automatic background corrections, SmartWell™
- Areal background corrections: Background balance, edge filter
- AutoGate™
- QC — Verify gate
- Multi-parameter AutoCount™

1:30 P.M. – 2:30 P.M.   LECTURE

The unique features of B cell ELISPOT for immune monitoring
presented by Prof. Dr. Paul V. Lehmann
- Detection of B cell memory vs. active B cell response (plasmablasts)
- Why B cell ELISPOT #1 — Sensitivity: *HCMV exposure revealed by B cell ELISPOT that serodiagnostic failed to detect*
- Why B cell ELISPOT #2 — Diagnostic: *Detecting B cell autoimmunity in Multiple Sclerosis permitting diagnosis of the disease and monitoring of its activity*

2:30 P.M. – 3:00 P.M.   LECTURE

Novelty: ELISPOT for rapid assessment of the humoral immune response's affinity spectrum. Morphology and kinetics of B cell ELISPOT formation permits measuring the affinity for antigen of the individual antibody-secreting B cells
presented by Dr. Alexey Karulin

3:00 P.M. – 3:15 P.M   COFFEE BREAK

3:15 P.M. – 4:00 P.M.   LIVE DEMO / TUTORIAL

Features of the ImmunoSpot® Software
presented by Tameem Ansari
- Manual gating
- FCS output (Size vs. Density plots for affinity measurements)
- Audit trails
- Export and Presentation features

4:00 P.M. – 4:30 P.M.   HANDS-ON TRAINING / Q&A

Features of the ImmunoSpot® Software
presented by Tameem Ansari, Jodi Hanson, Richard Caspell & Villian Naeem
- Manual gating
- FCS output (Size vs. Density plots for affinity measurements)
- Audit trails
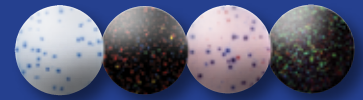- Export and Presentation features

4:30 P.M. – 4:45 P.M.   LECTURE

Double-color enzymatic T cell ELISPOT
presented by Prof. Dr. Paul V. Lehmann

4:45 P.M. – 5:30 P.M.   LIVE DEMO / Q&A

Double-color enzymatic T cell ELISPOT analysis
presented by Jodi Hanson

Continued

—·PROGRAM·—

DAY 2

9:00 A.M. –9:30 A.M.   LECTURE

Multi-color fluorescent ELISPOT — Theory and practice
presented by Dr. Alexey Karulin

9:30 A.M. – 10:00 A.M.   LIVE DEMO / TUTORIAL

Three and four-color fluorescent ELISPOT scanning and analysis
presented by Richard Caspell & Jodi Hanson
- Four-color scanning
- No cross-bleeding of CTL fluorochromes
- Four-color analysis of B cell assay (no coexpression possible)
- Three-color analysis of T cell assay (coexpression expected)

10:00 A.M. – 10:15 A.M.   COFFEE BREAK

10:15 A.M. – 11:00 A.M.   HANDS-ON TRAINING / Q&A

Three and four-color fluorescent ELISPOT scanning and analysis
presented by Richard Caspell, Jodi Hanson, Tameem Ansari & Villian Naeem
- Four-color scanning
- No cross-bleeding of CTL fluorochromes
- Four-color analysis of B cell assay (no coexpression possible)
- Three-color analysis of T cell assay (coexpression expected)
- Linearity for verification of coexpression

11:00 A.M. – 11:20 A.M.   LECTURE

Cut-offs for positive ELISPOT results: Statistics and how to experimentally increase the chance for the reliable detection of rare cells
presented by Dr. Alexey Karulin

11:20 A.M. –11:45 A.M.   LECTURE

ELISPOT assays in 384-well format
presented by Jodi Hanson

11:45 A.M. – 12:30 P.M.   LIVE DEMO / TUTORIAL / HANDS-ON

ELISPOT assays in 384-well format
presented by Jodi Hanson & Tameem Ansari
- 384-well scanning and analysis
- Data integration: SpotMap®
- Soliciting CTL support: Annotation tool

12:30 P.M. – 1:30 P.M   LUNCH BREAK

1:30 P.M. – 2:00 P.M.   LECTURE

Freezing-thawing of primary cells (PBMC, splenocytes, etc.)
presented by Prof. Dr. Paul V. Lehmann

2:00 P.M. – 2:30 P.M.   LECTURE

Cell Counting
presented by Dr. Srividya Sundararaman
- Single-cell imaging with fluorescent ImmunoSpot® Readers
- Live/Dead/Apoptotic cell counting
- Pre- and post-LDA counting for PBMC functionality

2:30 P.M. – 3:00 P.M.   LIVE DEMO / TUTORIAL / HANDS-ON

Demo and practice of LDA scanning and analysis
presented by Dr. Srividya Sundararaman

3:00 P.M. – 3:20 P.M.   LECTURE

Natural Killer Assay
presented by Dr. Srividya Sundararaman

3:20 P.M. – 3:50 P.M.   LIVE DEMO / TUTORIAL / HANDS-ON

Demo and practice of NK Assay scanning and analysis
presented by Dr. Srividya Sundararaman & Villian Naeem

3:50 P.M. – 4:15 P.M.   Q&A / SUMMARY / FAREWELL

presented by Prof. Dr. Paul V. Lehmann