
Scott D. Tanner
atomic mass spectrometry

• aqueous samples:
14 elements: 30 isotopes

measured on the CyTOF™ Mass Cytometer
replace fluorophores and fluorescence …

with metals and atomic mass spectrometry

- detector channel resolution at least $10^4$
- dynamic range at least $10^4$
Stable Isotopes for MAXPAR Tags

Stable Isotopes instead of Fluorescent Dyes
atomic analytical methods pre-1980:

- graphite furnace atomic absorption
- X-ray fluorescence
- optical emission

particularly with Inductively Coupled Plasma excitation
Plasma is a fourth state of matter containing a substantial and equal number of positive ions and negative electrons that behaves like a gas, but its ionic state makes it additionally responsive to electromagnetic fields.

Why “plasma”?: Langmuir (1928) thought the soup of ions and electrons in a broth of residual neutrals “looked” like blood.
Yttrium particles injected into an ICP

Photo credit: High-speed digital photographic study of an inductively coupled plasma during laser ablation: comparison of dried solution aerosols from a microconcentric nebulizer and solid particles from laser ablation
many unique labels available

13 lanthanides: 37 stable isotopes
24 elements: 67 stable isotopes

CyTOF™ Mass Cytometer: single cell analysis

~ 7500 K
~ $10^{15}$ e-/cm$^3$
≈ sun’s surface

element labeled reagents

intensity

(stable isotope) mass
[Cp*Ir(dppz){(NH₂)₂CS}]²⁺

Metalointercalation

64% Live 83% Live 95% Live

64% Live
83% Live
95% Live
Heart of mass cytometry: ICP argon plasma

2mm dia cell ion cloud flowing @ 10 m/s
200 µs to pass sampler
~ 200 µs transient signal per cell event
CyTOF™ schematic

Bandura et al, Analytical Chemistry, 81, 6813-6822 (2009)
Fresh PBMc stained with 27 markers (mix I):

Lymp CD4+T

CD45

CD2  CD3  CD4

Lymp B

CD45RA; CD20;

CD45; CD38; CD71 CD19; CD40

Lymp CD8+ T

CD45RA

CD45

CD2  CD8
31 parameter BM assay -> 496 biaxial plots

- CD33
- CD20
- CD45
- CD61
- CD11b
- CD20
- CD8
- CD8+
- CD4+
- CD4
- Lymphocytes
- Platelets
- B cells
- Myeloids
- Macrophages
- T cells
Equivalence of fluorescence and mass cytometry

Bendall et al., Science (2011)
20-parameter cytometry

Two leukemia cell lines and one patient sample

enriched stable isotope labels
Unsupervised Neural Network: use multivariate “fingerprint” to detect rare cells

Ramos B-lymphoma cell line
Every Glyph has a fine structure

This part of the Glyph corresponds to the Iridium 191/193 intercalator.
Reproducibility studies of seven CB aliquots analyzed on different days

<table>
<thead>
<tr>
<th>CB1</th>
<th>CB2</th>
<th>CB3</th>
<th>CB4</th>
<th>CB5</th>
<th>CB6</th>
<th>CB7</th>
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</thead>
<tbody>
<tr>
<td>T cell 2</td>
<td>T cell 1</td>
<td>RBC</td>
<td>NK cells</td>
<td>Gran 1</td>
<td>Mono/Mac</td>
<td>B cells</td>
</tr>
<tr>
<td>Gran 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4+ T</td>
</tr>
</tbody>
</table>
High level Glyphs

Represents percentage of different cell populations in a sample

Represents percentage of different cell populations in batch of samples
seven aliquots of one CB were analyzed on different days.

Number of gates: 10

All samples in the batch were gated employing the same UNN
Seven Different CB donors

seven different CB were analyzed on the same day

Number of gates: 10

All samples in the batch were gated employing the same UNN
Stimulate cells *in vitro*, crosslink proteins, permeabilize cell membrane, stain with isotope tags, and measure by TOF. Ionize (7500K) and nebulize single-cell droplets. Compare cell signal between unstimulated and after 4 minutes for Cell 1 and Cell 2.
unsupervised clustering – SPADE analysis
Rediscovery of canonical signaling pathways validates method
First report of metal-encoded tetramer probes

**36-parameters:**
- 6 pMHC tetramers
- 16 surface antigens
- 10 intracellular stains
- 4 gating stains

<table>
<thead>
<tr>
<th>Surface stains:</th>
<th>Atomic Mass:</th>
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<tbody>
<tr>
<td>6 pMHC tetramers</td>
<td>156,158,159,159,1</td>
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<tr>
<td></td>
<td>64,165,168</td>
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<tr>
<td>CD3</td>
<td>QDot = Cd-112</td>
</tr>
<tr>
<td></td>
<td>and others</td>
</tr>
<tr>
<td>CCR7</td>
<td>142</td>
</tr>
<tr>
<td>CD11a</td>
<td>148</td>
</tr>
<tr>
<td>CD7</td>
<td>143</td>
</tr>
<tr>
<td>CD8</td>
<td>146</td>
</tr>
<tr>
<td>CD27</td>
<td>154</td>
</tr>
<tr>
<td>CD28</td>
<td>160</td>
</tr>
<tr>
<td>CD29 (β1 integrin)</td>
<td>162</td>
</tr>
<tr>
<td>CD43</td>
<td>167</td>
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<tr>
<td>CD45RA</td>
<td>166</td>
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<tr>
<td>CD45RO</td>
<td>144</td>
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<tr>
<td>CD49d (VLA-4)</td>
<td>172</td>
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<tr>
<td>CD57</td>
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<tr>
<td>CD62L</td>
<td>169</td>
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<tr>
<td>KLRG1</td>
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<tr>
<td>HLA-DR</td>
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<table>
<thead>
<tr>
<th>Intracellular stains:</th>
<th>Atomic Mass:</th>
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<tr>
<td>IL-2</td>
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<tr>
<td>GM-CSF</td>
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<tr>
<td>MIP-1α</td>
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<tr>
<td>MIP-1β</td>
<td>150</td>
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<td>Granzyme B</td>
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<tr>
<td>CD69*</td>
<td>149</td>
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<tr>
<td>Perforin</td>
<td>175</td>
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<tr>
<td>TNF-α</td>
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<tr>
<td>IFN-γ</td>
<td>170</td>
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<tr>
<td>CD107a/b**</td>
<td>153</td>
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<table>
<thead>
<tr>
<th>Other:</th>
<th>Atomic Mass:</th>
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</thead>
<tbody>
<tr>
<td>DNA content</td>
<td>191, 193</td>
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<tr>
<td>“cell length”</td>
<td>N/A</td>
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<tr>
<td>Live/dead stain</td>
<td>115</td>
</tr>
<tr>
<td>Mouse CD45 ***</td>
<td>139</td>
</tr>
</tbody>
</table>

Functional T-cell phenotyping

Memory cell progression – phenotypic and functional

A 3D-PCA view of CD8+ T cell 25 parameter data

B Memory cell phenotypic progression

C Memory cell functional capacity progression

extreme multi-parameter multiplex:
18,816 states simultaneously x 24 drugs (Bodenmiller, in press)
Logic Map data analysis:
plate analyzer image courtesy of J. Paul Robinson (Purdue)
data courtesy Bernd Bodenmiller (Nolan lab, Stanford)
http://www.cyto.purdue.edu/pa-demo

96 well plate Logic Map for data set from 43 variables including 7 barcoding parameters (screen shot)

Each drug box represents an 8-point response curve
Summary

• atomic mass spectrometry provides new and transformational analytical opportunity in cell biology

• 35-parameter biomarker assay at the single cell level at 1000 cells/s is now a reality, requires no compensation, and is easy – potential to grow to 100-parameters

• potential to identify rare diseased cells – perhaps early enough to provide non-aggressive, personalized therapy – and diagnostics through treatment, remission and relapse

• ability to measure multiple parallel translational pathway responses to agonist/antagonist intervention– potential to optimize drug discovery