

DENSE: 2D Flow Gating

Phil De Jager

Aaron Brandes

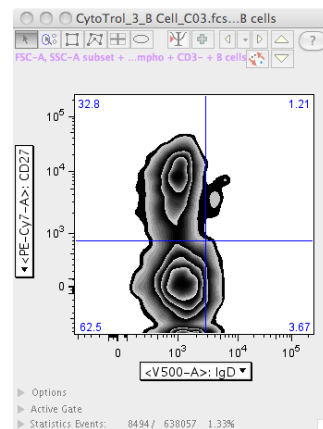
Nov. 30, 2012

FlowCAP III

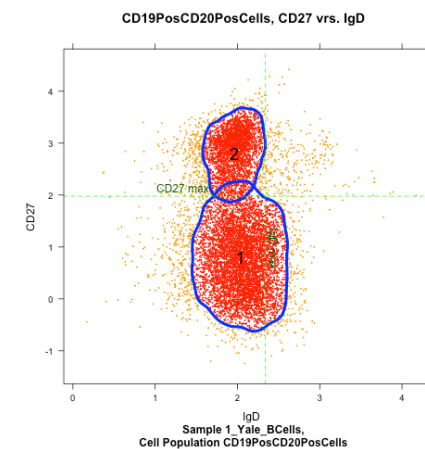
Introduction: DENSE

- Challenge 4: Cyto-trol Gating
- Goal: Method that finds gates based on landmark regions of high density
 - Similar to approach taken by biologists who use FlowJo zebra plots as an aid for tough decisions
 - Allows for feedback and iterative design
- Solution: DENSE

Zebra
Plot



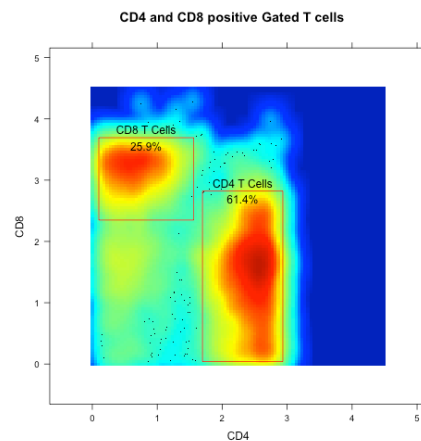
Strategy
Plot



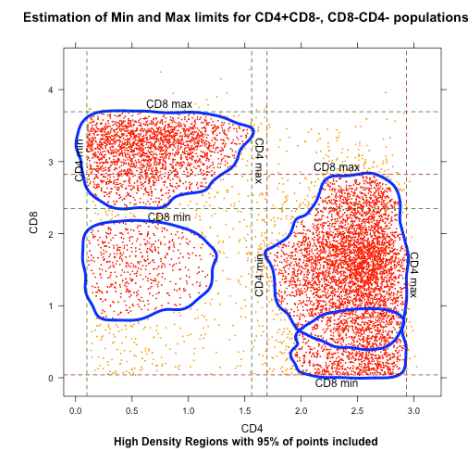
DENSE: 2D Flow Gating

- DENSE uses 1 & 2 D density info to create gates
- Implemented in R using flowStats, flowCore, curvHDR
- Preprocessing
 - Regularization of input
 - Logicle transformation
- Focus here on gating strategy for a few cell populations
- Visualization important for development and debugging

Gating Plot

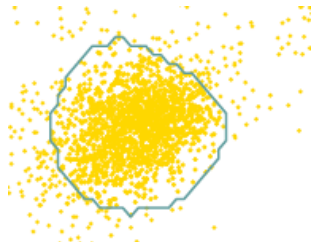


Strategy Plot



curvHDR Package M. Wand

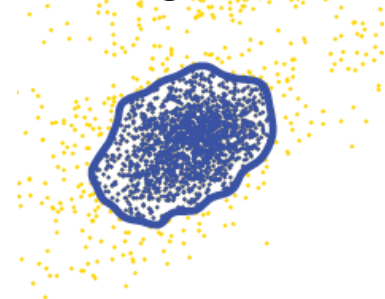
High Density
Region



Find
Contours



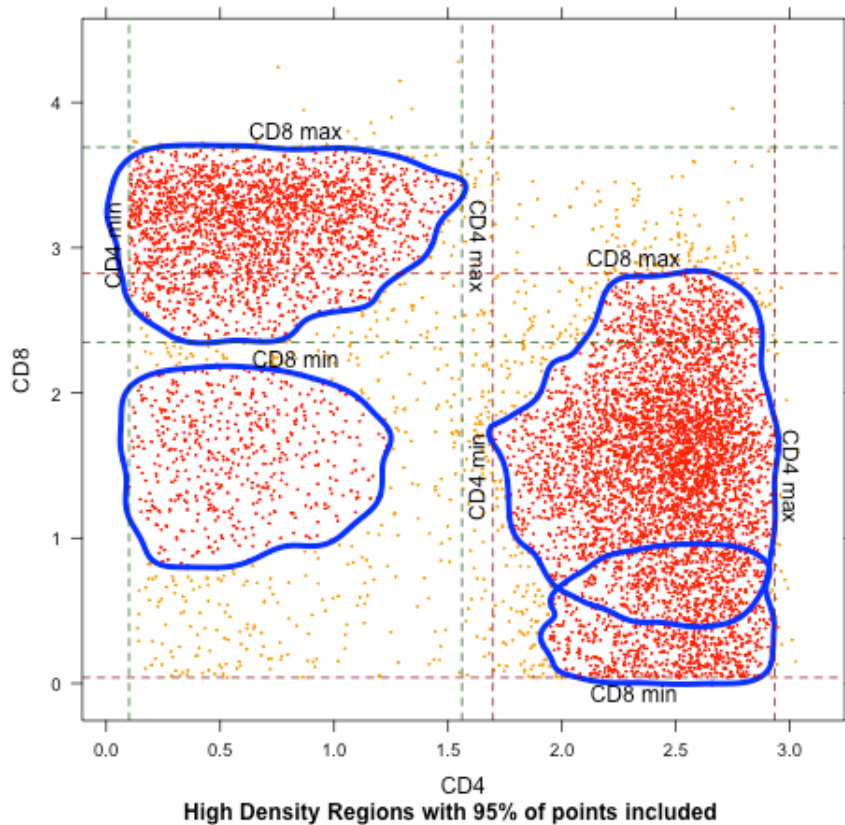
Trim to given %



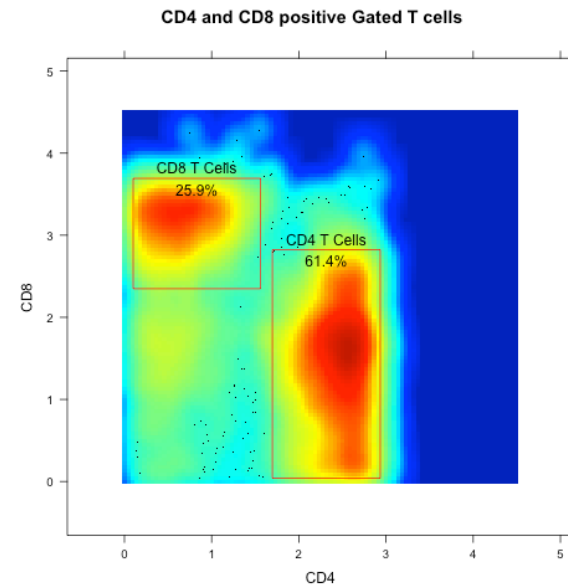
- curvHDR finds regions of high density
 - non-parametric
 - points may be in 0, 1 or more regions
 - returns requested %ile of entire region
 - I use region with estimated 95% of points
- I developed tools to:
 - compute statistics for the regions
 - min, max, mean, median, area %tile
 - select regions based on statistics
 - greatest, smallest, within a range
 - visualize gates

CD4 & CD8 Gating

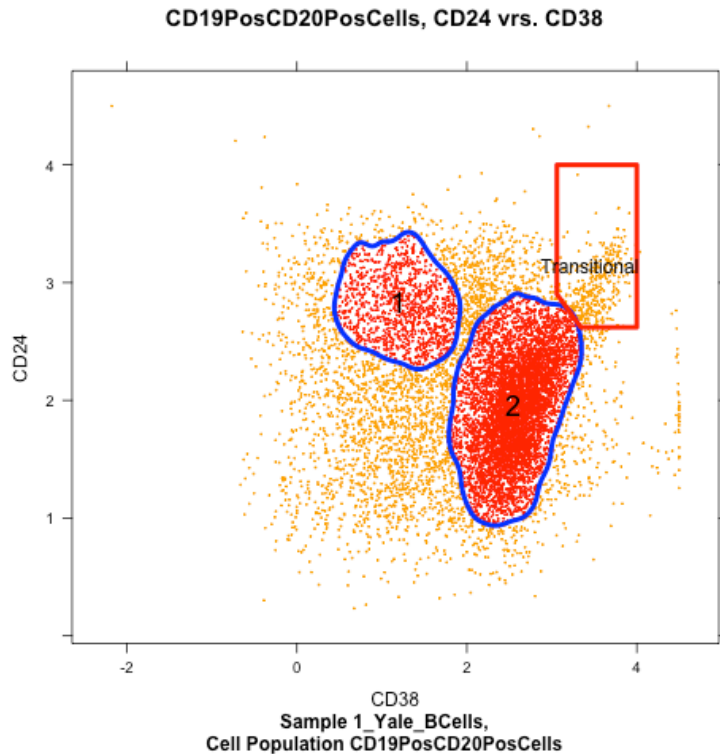
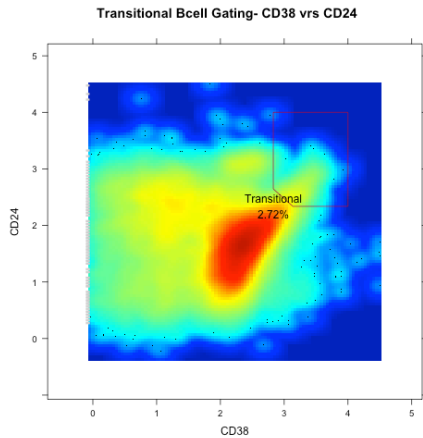
Estimation of Min and Max limits for CD4+CD8-, CD8-CD4- populations



- ID CD4+ components
- ID CD4-CD8+ comps.
- Drop CD4+CD8+ comps
- Average non-overlapping boundaries if desired

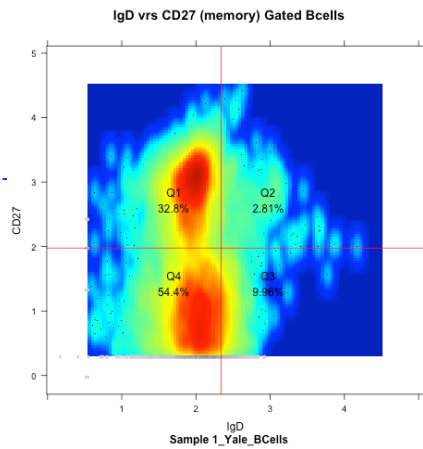


Transitional BCell Gating

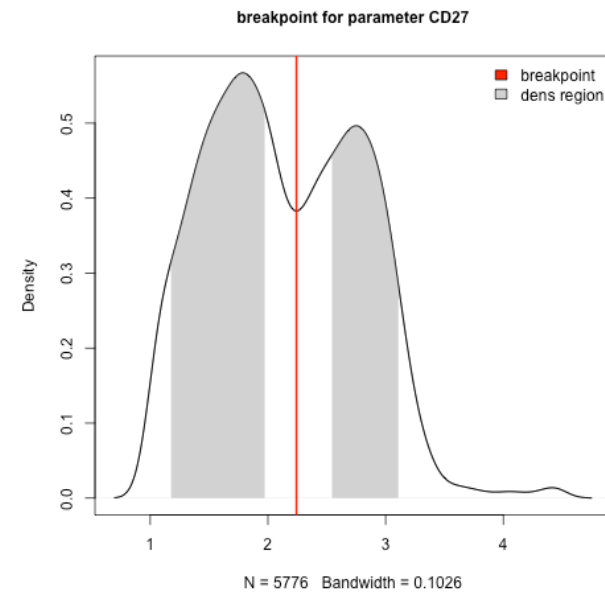
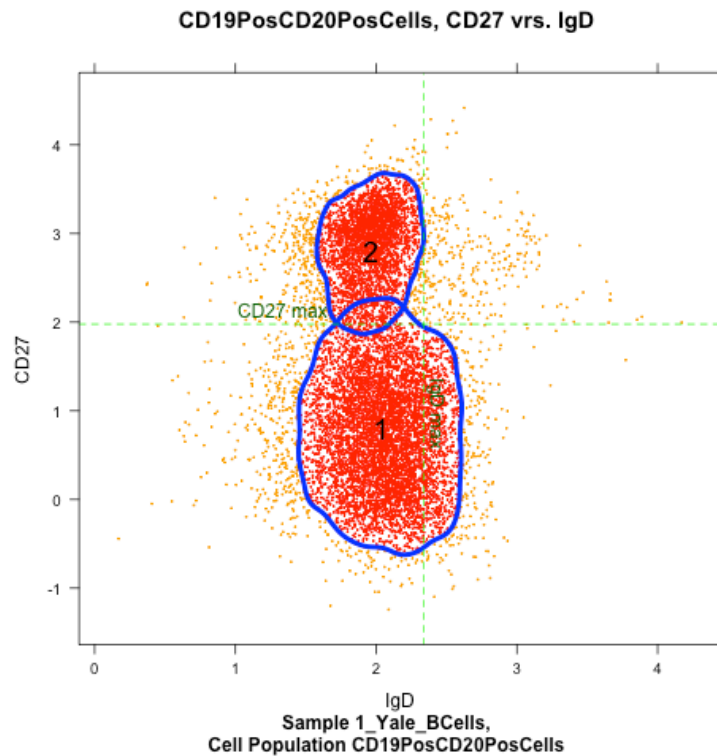


- A more complex example
- ID rightmost component
- ID northmost point
 - $\max(x + y)$ for comp
- Intersect line $x + y = \max$ with boundary box
- Find max CD38, CD 24
- Create polygon gate

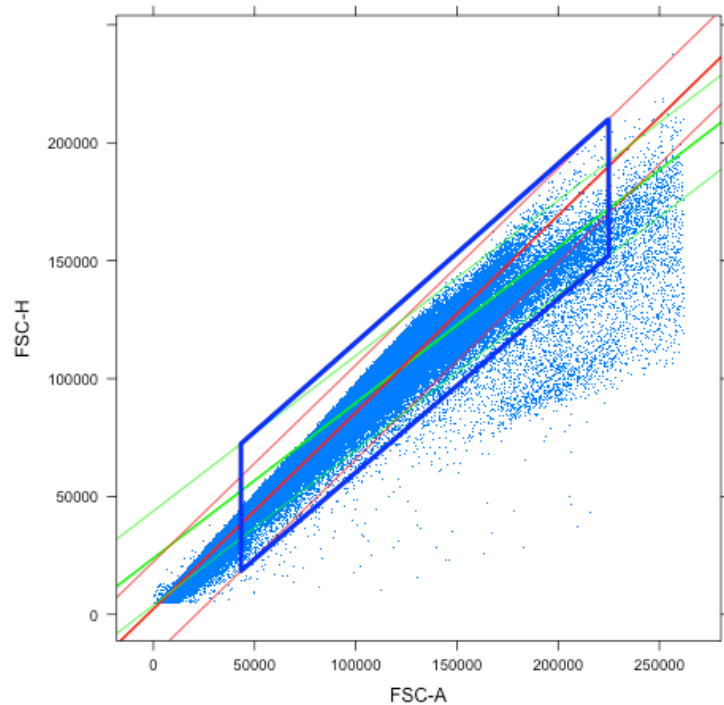
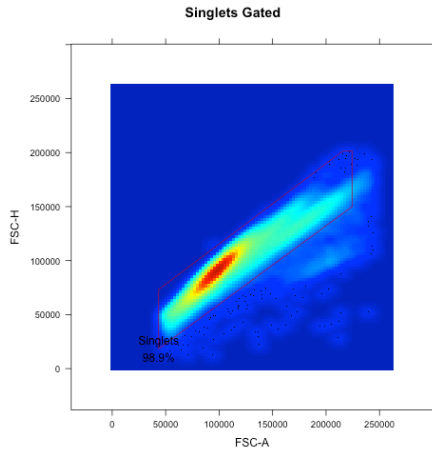
Memory BCell Gating



- Use 1D Gating for CD27 boundary
- Use 2D for IgD boundary



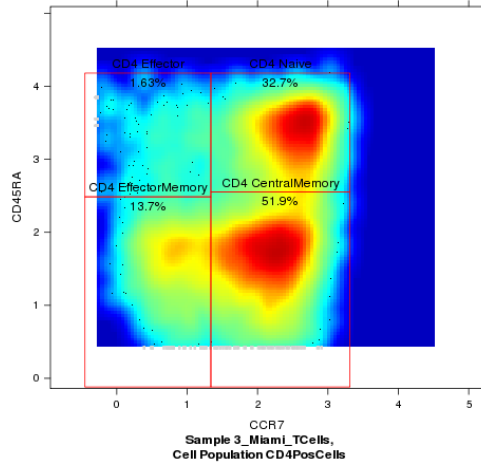
Singlet Gating



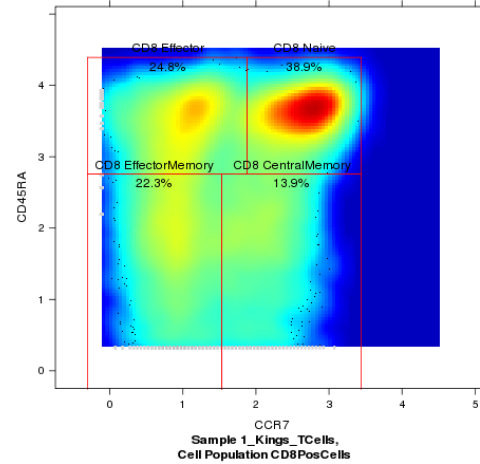
- regression lines
 - red – all cells
 - green – lymphocytes
- vertical offsets + and –
- create polygon
 - FSC-A lymph limits
 - intersect verticals w regression line
 - choose best (hi/low) one

Memory TCell Gating

CCR7 (effector) vrs CD45RA (memory) Gated CD4 Tcells

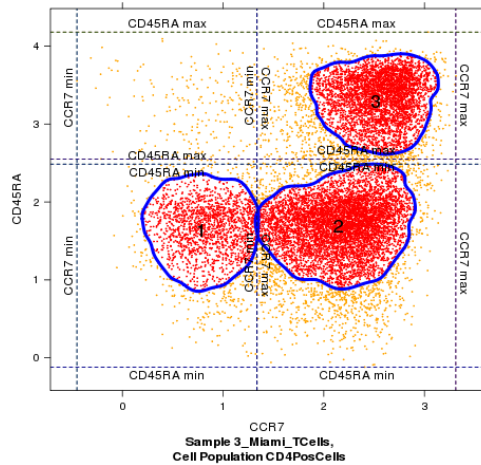


CCR7 (effector) vrs CD45RA (memory) Gated CD8 Tcells

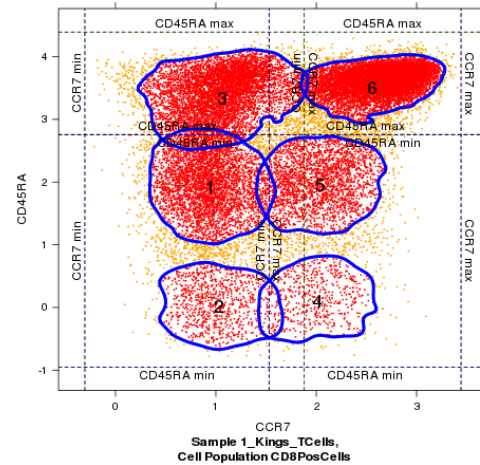


- adjustment of boundaries is vertical for CD4 cells, horizontal for CD8 cells

CD4PosCells, CD45RA vrs. CCR7



CD8PosCells, CD45RA vrs. CCR7



Conclusions

- DENSE uses 2D regions of high density for automated gate creation
 - each gating scheme created using DENSE utilities
 - Future possibilities:
 - framework to ease creation of new gating schemes
 - use of clustering or parametric modeling to create components
- curvHDR
 - creates intuitive looking 2D regions
 - I developed supplementary tools for greater utility
 - performs slowly on 100,000+ cells
- Visualization of results is important for
 - iterative design with biologists
 - debugging

Thanks To

- Hafler Lab Yale
 - Khadir Raddassi
 - Lesley Devine
- Phil De Jager
- Greg Finak

- Funding - NIH/NIAID
