Barcoding for CyTOF

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Revised:

- **Standard protocol (Dead cell barcoding)**
  - Simplified procedure
    - Stain single cell suspension with cisplatin
    - Fix/perm
    - Stain with Pd barcoding reagents
      - Up to 20 samples
    - Combine barcoded samples into one tube
    - Fc block/stain with antibodies
    - Stain with Ir-intercalator
  - Benefits
    - Faster sample acquisition
    - Less run time between sample 1 and sample 100, minimizes machine variability between samples
    - Can collect data on samples with low cell numbers
    - Saves on reagents for staining
    - Simplifies staining step to one tube
  - Precautions
    - Cells are fixed and permeabilized before staining with antibodies, which could change the staining pattern of some markers
      - It is possible to work out which antibodies and clones work on fix/permed cells using standard flow cytometry
    - After titrating antibodies on $3x10^6$ cells, the titration should be tested on the number of cells in the barcoded sample (ex: 20 samples x $3x10^6$ cells = $60x10^6$ cells)
- **Modified standard protocol**
  - Simplified procedure
    - Stain single cell suspension with cisplatin
    - Fc block/stain with antibodies
    - Fix/perm
    - Stain with Pd barcoding reagents
      - Up to 20 samples
    - Combine barcoded samples into one tube
    - Stain with Ir-intercalator
  - Benefits
    - Faster sample acquisition
    - Less run time between sample 1 and sample 100, minimizes machine variability between samples
    - Can collect data on samples with low cell numbers
    - Allows for staining of cells before fix/perm if the panel contains many markers that are susceptible to fix/perm
  - Precautions
    - If you are staining 20 samples, they must be stained and barcoded individually before combining – increases the possibility of error (unevenly stained samples, mixing up tubes, etc.)
    - Does not save reagents
- **Live cell barcoding**
  - Simplified procedure
    - Stain single cell suspension with cisplatin
    - Stain with CD45 antibodies – one per sample
      - Limited by number of CD45 antibodies with distinct metal tags

        - Combine CD45-tagged samples into one tube
        - Fc block/stain with antibodies
        - Fix/perm
        - Stain with Ir-intercalator
    - Benefits
        - Faster sample acquisition
        - Less run time between sample 1 and sample 100, minimizes machine variability between samples
        - Can collect data on samples with low cell numbers
        - Allows for staining of cells before fix/perm if the panel contains many markers that are susceptible to fix/perm
    - Precautions
        - Limited by number of CD45 antibodies with distinct metal tags
        - Each CD45 antibody takes away a channel that could be used for your markers
        - Pd barcoding metals can be conjugated to CD45 antibodies to avoid using up channels for markers, however special custom conjugations are required. Extra troubleshooting will be required.