Wolfson School of Mechanical, Electrical and Manufacturing Engineering

Operator Measurement Uncertainty Contributions within Post-Analytical Flow Cytometry Data

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Research Aims

To investigate whether more detailed diagrammatical protocols can reduce the inter-operator variation seen within manual Flow Cytometry Data Analysis, using structured participant studies.

The manufacture of Cell and Gene Therapy products is a complex process, with many factors contributing variation to the final product [1, 2]. T-cells are a common biological source materials for Advanced Therapeutics, so a naïve T-Cell subset has been used as a data analysis target within this research as a representation of this analysis.

Flow Cytometry is a measurement technique used within various Quality Control release phases, as well as in-process to guide cell seeding densities. The process of obtaining a measure on this platform also contains variation sources from sample preparation, instrumental components and setup, analysis and post-analytical data evaluation [3]. Measurement Uncertainty is used to quantify and combine variation from sources within a measurement process. Through repeat measures, the variation of a process, gauge, operator or product can be quantified [4]. This has been used extensively within traditional manufacturing industries, and has been applied to Flow Cytometry data to quantify operator variation in result reporting, because this contains subjectivity which has not been focused on within the public body of knowledge.

Analysis Methodology

Figure 1 shows the analysis methodology to extract the Average Population Cell Counts and the Combined Uncertainties from each Participant. The Average Population Cell Counts are what would normally be presented as the final representative count for the cell product produced. The Combined Uncertainties measure variation from all gating stages used to refine the final cell count.

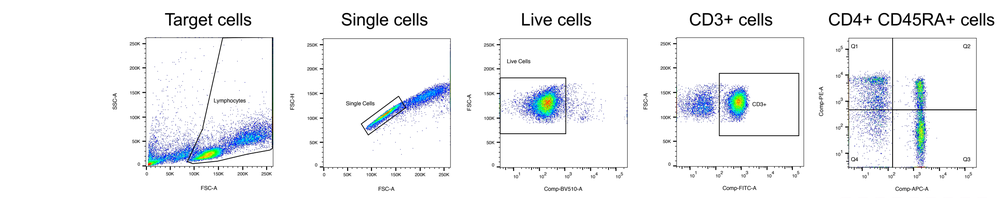
Study Structure

Session 1: Gating using Own Judgement → **Session 2:** Gating Following Protocol

To understand whether inter-operator variability can be reduced by more prescriptive protocols, participants took part in two analysis sessions.

- **Session 1**: Participants analysed data according to the instructed sequence, placing gates based on their own judgement.
- **Session 2**: Participants analysed the same data, but were provided with a diagrammatical protocol to follow.

In each session, participants completed the analysis sequence three times, to ensure variation could be calculated from the repeats.



Analysis sequence for naïve T-cells, identifying target cells, single cells, Live cells, CD3+ cells, CD4+ CD45RA+ cells

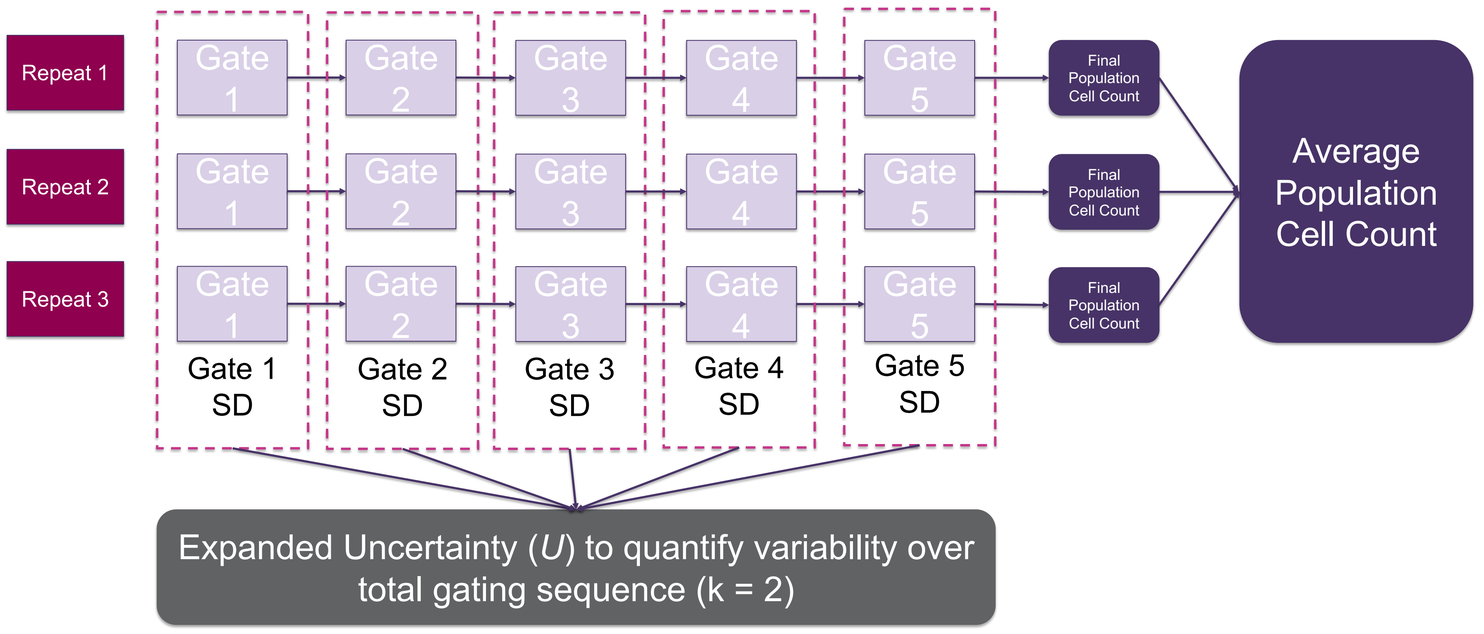


Figure 1: Analysis sequence provided for participants to follow, with description on how the Population Cell Counts and Uncertainty are calculated for each participant

Figure 1 shows the sequence of gates that participants used to refine the T cell count population in both sessions. The sequence images also show the gates participants copied in Session 2. The three operator repeats generated three cell counts, which are averaged to provide a representative cell count. The Standard Deviation (SD) from repeats of each gate (pink dashed line) was used to calculate a representative uncertainty for each operator. This is used for inter-operator comparison of Absolute results (average cell counts) and Inter-Operator uncertainty.

Average Population Results

- Same Mean and Median results for Average Population Cell Count when Participants use their own judgement or follow a protocol.
- Reduction in Range of Inter-Participant results, demonstrating a potential refinement of Cell Count variability when Participants copy a uniform protocol (Figure 2).
- CV of Participant Population Cell Counts becomes more positively skewed, however, more kurtosed when following a protocol to identify the correct target (Figure 3).

Personal Judgement	
Location Measures	
Mean	6.28
Median	6.01
Minimum	3.46
Maximum	7.99
Spread Measures	
Range	4.53
25th %ile	5.65
75 %ile	7.27
IQR	1.62
STDEV	1.14
CV	18.16

Following Protocol	
Location Measures	
Mean	6.12
Median	6.06
Minimum	4.91
Maximum	7.65
Spread Measures	
Range	2.74
25th %ile	5.96
75 %ile	6.25
IQR	0.30
STDEV	0.49
CV	7.94

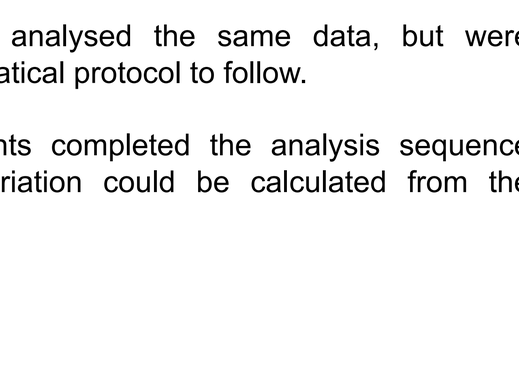


Figure 2: Personal Judgement and Following Protocol Results for Population Cell Count

Figure 3: Personal Judgement and Following Protocol Results for Population Cell Count CV.

Uncertainty Results

- Personal Judgement is more positively skewed than when participants follow a protocol, which appears to be bi-modal in distribution.
- Reduction in Range (3.1 %) in uncertainty of Inter-Participant results, implies a potential refinement of variability when using a protocol.
- Increased SDs are seen when using a protocol to try and gate a smeared cell population (final gate, CD4+ CD45RA+).

Personal Judgement	
Location Measures	
Mean	3.82
Median	2.12
Minimum	0.40
Maximum	15.80
Spread Measures	
Range	15.40
25th %ile	1.42
75 %ile	3.75
IQR	2.33
STDEV	4.22
CV	110.73

Following Protocol	
Location Measures	
Mean	5.83
Median	2.24
Minimum	0.54
Maximum	12.85
Spread Measures	
Range	12.30
25th %ile	1.40
75 %ile	10.90
IQR	9.50
STDEV	5.16
CV	88.36

Figure 4: Personal Judgement and Following Protocol Results for Expanded Uncertainty.

Conclusions

- A refinement of inter-participant variation of 1.8 % in absolute cell count results when participants follow a protocol shows that it may be possible to reduce variability in cell counts due to subjectivity.
- A refinement of inter-participant variation of 3.1 % in uncertainty when participants follow a protocol. However, a bimodal distribution appears due to high and low variance clusters.
- Protocols can potentially be used to reduce subjective components of biological measurement variability, but it has the the path of least resistance to ensure compliance. Further investigation is required to understand the two groups of participants who have higher and lower variances as a result of using a protocol.

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