

Optimization of LC/MS-MS Parameters for Shotgun Proteoanalysis

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Introduction

- Human aging is a biological process
- Change in concentration of certain proteins in human serum correlates to biological aging².
- Optimize LC/MS² parameters for shotgun proteoanalysis

Methodology

- Micro-flow LC/MS² proteomics.
- Vanquish Horizon liquid chromatography system directly connected to a Q-Exactive HF-X with BioPharma option mass spectrometer
 - HeLa Digest
- Measuring effects on total protein identifications (IDs)
 - Spray voltage
 - column length
 - protein load
 - flow rate
 - gradient time length
 - gradient spread
- UniProtKB Human Reference Proteome database in Proteome Discoverer 2.4 software.

Results

- 932 protein IDs/ μ g of protein loaded
- 3729 proteins
- 4000ng of HeLa digest
- 3.50 kV spray voltage
- 180-minute gradient from 8% acetonitrile to 32% acetonitrile
- 2.1mm x 150mm column
- 50 μ l/min flow rate

Conclusion

- A decrease in protein IDs with a longer column length was an unanticipated finding
- Protein IDs continue to increase up to a certain limit, but too many can overwhelm the MS
 - e.g., serum proteins
- In terms of protein IDs, our data is comparable to (if not better than) that of the literature¹.
- We proceed by analyzing human serum samples

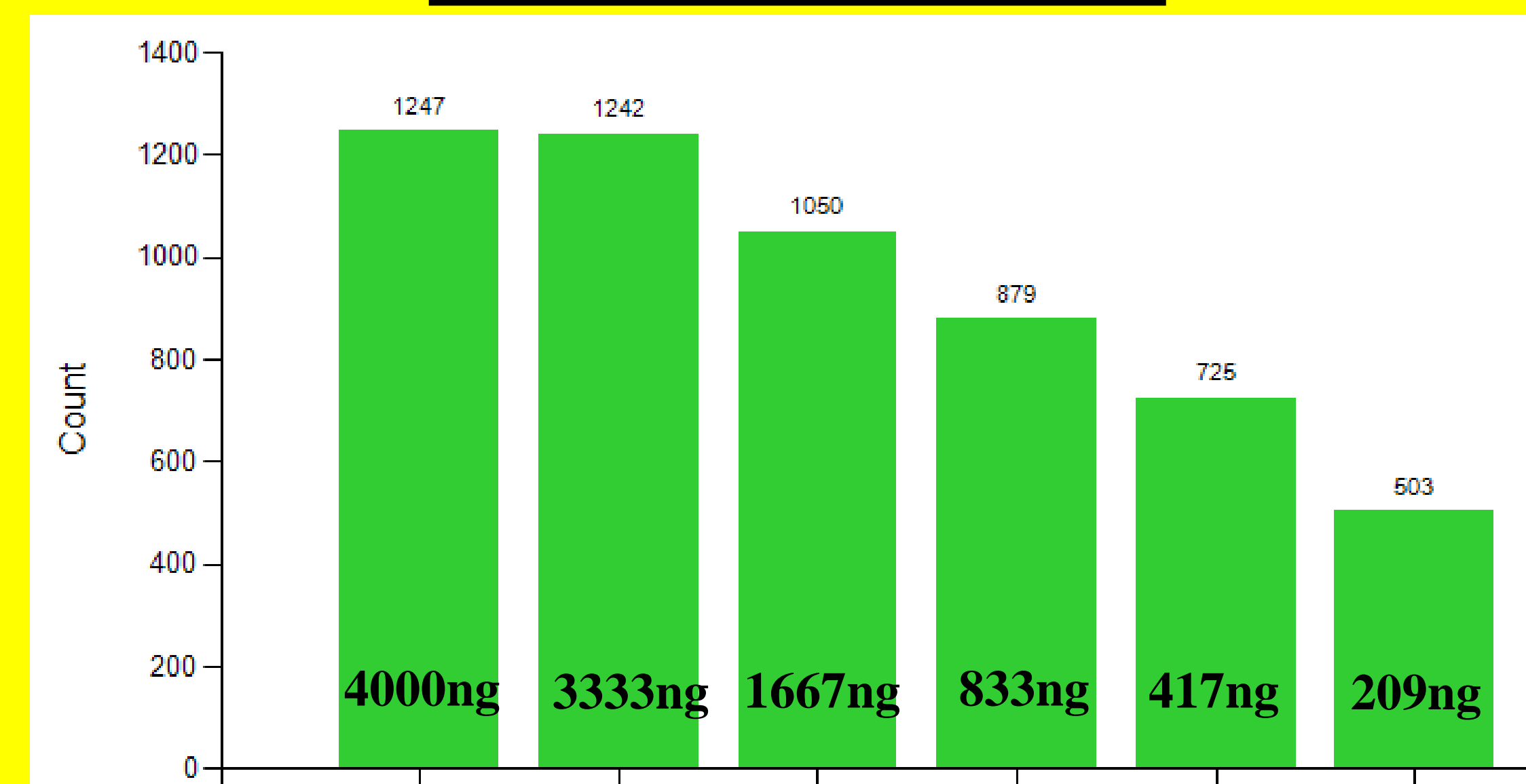
References

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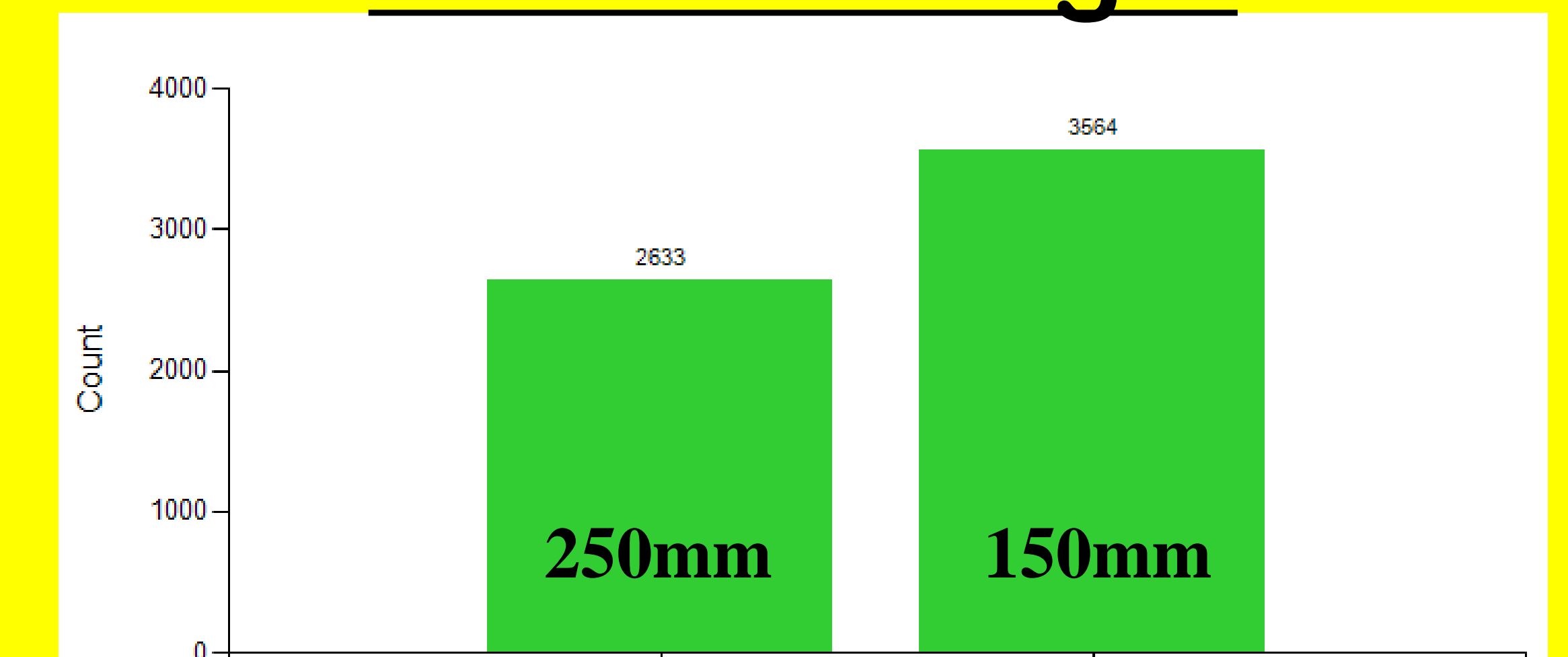
Acknowledgments

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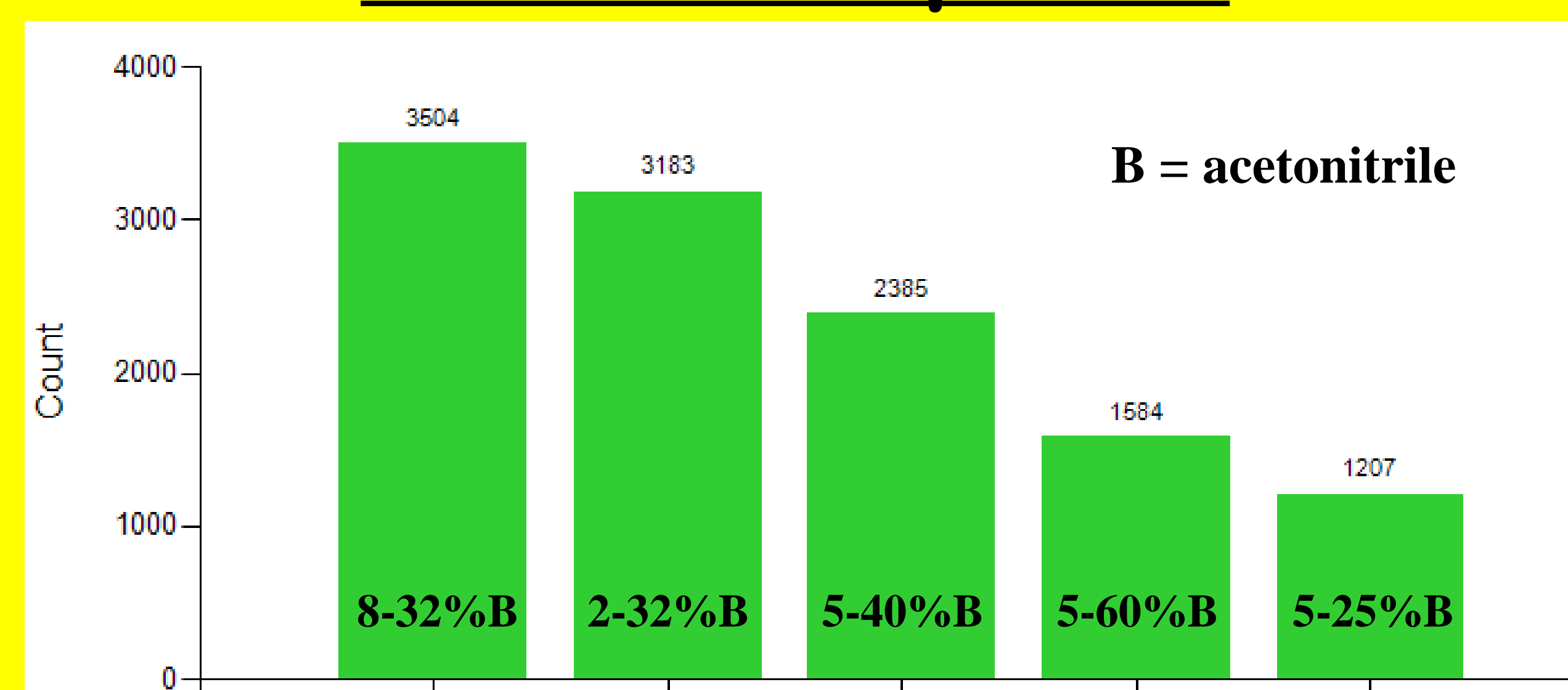
Protein Load



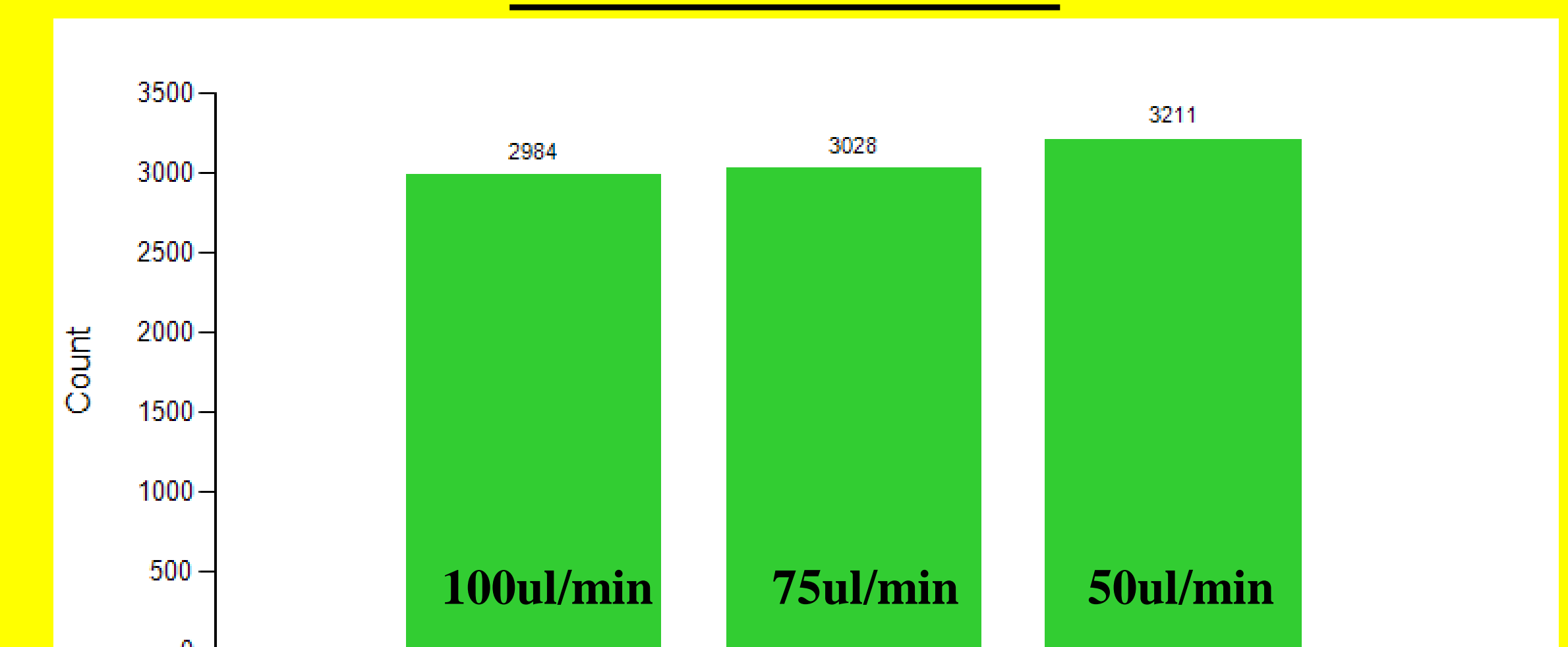
Column Length



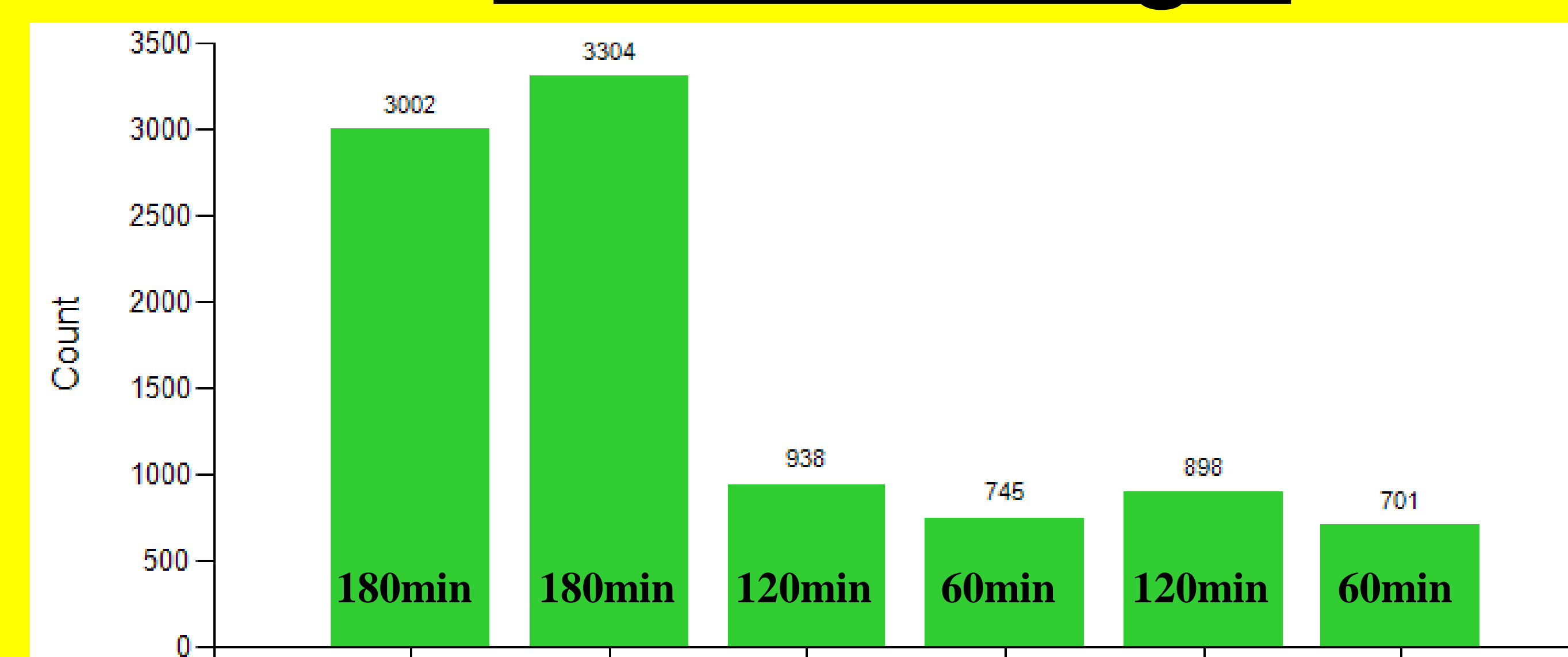
Gradient Spread



Flow Rate



Gradient Length



Spray Voltage

