121 QUANTIFYING STATISTICAL AND SYSTEMATIC UNCERTAINTIES IN PREDICTING CLINICAL OUTCOMES USING MULTIPLEX IMMUNOFLUORESCENCE

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Background Statistical analysis of multiplex immunofluorescence images can be used to predict patient outcomes. Recent advances have enabled complex analyses of marker expressions across every cell on a slide, as well as their spatial correlations.[1] [2] In parallel, calibration of the microscope slides to correct for errors in the imaging and for differences between different microscopes, staining batches, and slides, has also advanced.[3]

One critical missing piece is the connection between the two. If after calibration there remains a 0.8% uncertainty on the flatfielding, how does that translate into an uncertainty on the prediction of patient response?

Methods Using the AstroPath platform, we recently that CD8+FoxP3+ cells can be used to predict response to treatment. By examining the neighbors of CD8+FoxP3+ cells, we identified 'CD8+FoxP3+-like' neighborhoods that show similar predictive power but are much more abundant than the cells themselves.[2] Here, we reran this analysis, estimating several sources of statistical and systematic uncertainty and their application to the final result.

Statistical uncertainty on the cell count was estimated using a Poisson distribution. The effects of uncertainties in the scanning, processing, segmentation, and phenotyping calibration were estimated by examining cells in different parts of the high-powered field, and in particular by using the 20% overlap between adjacent high-power fields

Results The remaining systematic uncertainties from image processing, after applying flatfielding and other corrections, are negligible. We find them, conservatively, to be less than 0.3%. These uncertainties would likely be higher for an analysis that relied more heavily on marker expression levels.

Statistical uncertainties are more significant. The Poisson uncertainty on the count of relevant cells is especially important for rare phenotypes, such as actual CD8+FoxP3+ cells, where it is 25% or higher for many samples. Although we find the area under the receiving operator characteristic curve (AUC) is 0.79, the Poisson uncertainty gives (0.74, 0.83) at 68% confidence level (CL) and (0.66, 0.86) at 95% CL. For CD8+FoxP3+-like neighborhoods, which are more abundant, the Poisson error is 2–3% for most samples. The nominal AUC is 0.80, with a much smaller uncertainty: (0.79, 0.80) at 68% CL and (0.76, 0.81) at 95% CL.

Conclusions A quantitative approach to statistical and systematic uncertainties is shown in application to multiplex immunofluorescence analysis. Accounting for these uncertainties is not only necessary to properly understand the result, but also identifies which calibrations have the greatest effect on the analysis and should therefore be prioritized for further improvement.

REFERENCES

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