



Hugh Green
**Technology
Centre**
at the Malaghan Institute

QuPath Spatial Analysis and Visualization

Extended Protocol

Cynthia Morgan

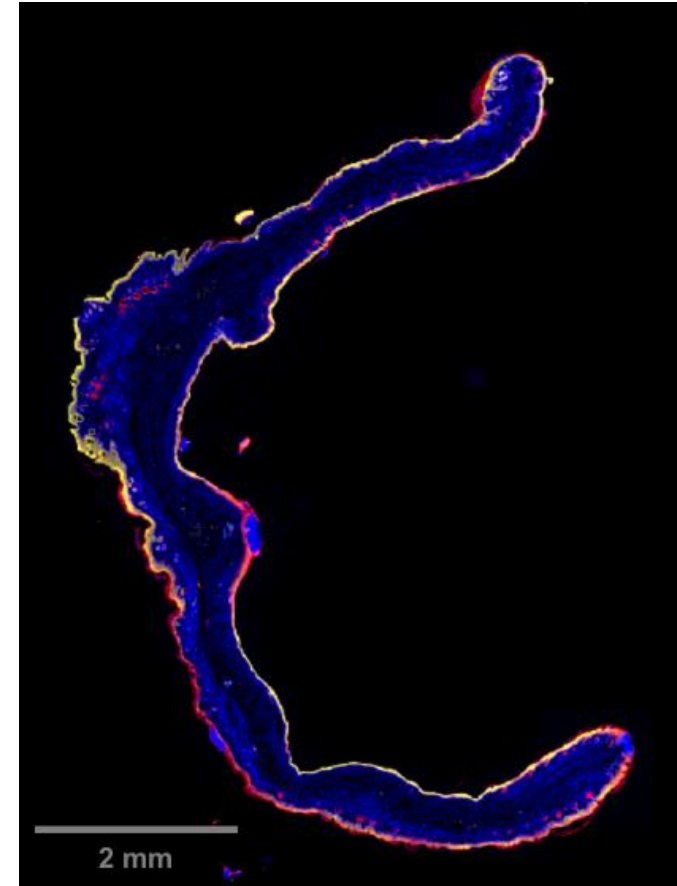
Spatial Biology

- Cell-to-cell interactions, protein expression, and the spatial arrangement of cells within tissue
- Preserves spatial context
 - Enables a deeper understanding of biological processes and disease states
- Crucial in fields such as cancer research, neuroscience, and immunology
- Multiplex Immunofluorescence (mIF) is a key technique to spatial biology that enables the analysis of entire microscope slides



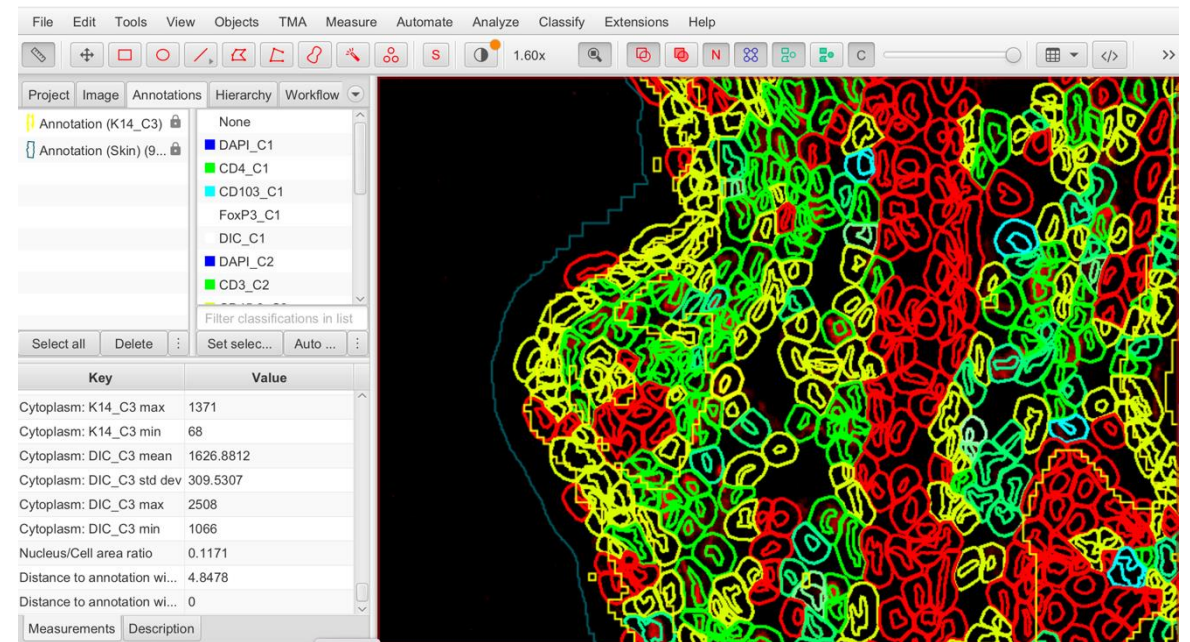
mIF and QuPath

- The ability to acquire and analyze entire microscopic slides has transformed tissue biomarker and diagnostic discovery
- Multiple open-source tools have been developed such as Fiji and ImageJ
- QuPath is a free, open-source software that is used for quantifying and spatially profiling multiplex immunofluorescence.



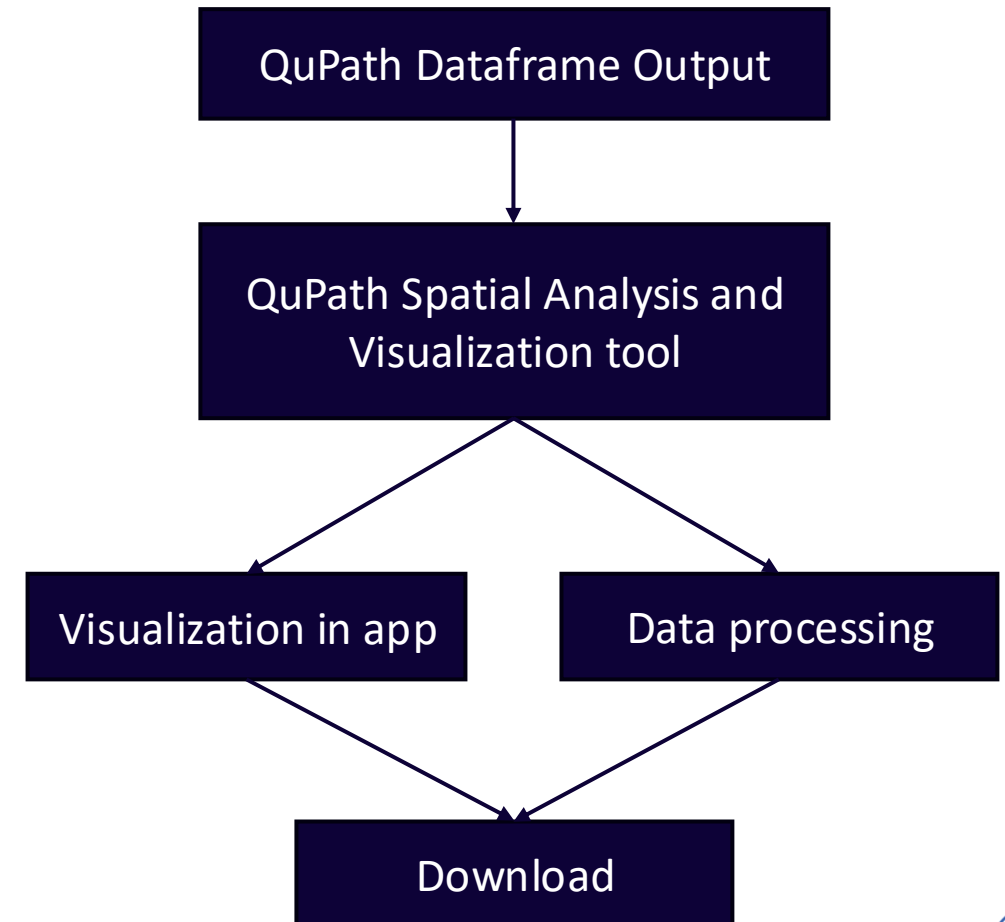
QuPath outputs

- QuPath offers numerous novel algorithms and users can implement their own algorithms
- Alisha Dabb developed pipeline for automated analysis of multiplex immunofluorescent samples
- A very large data frame is the end output

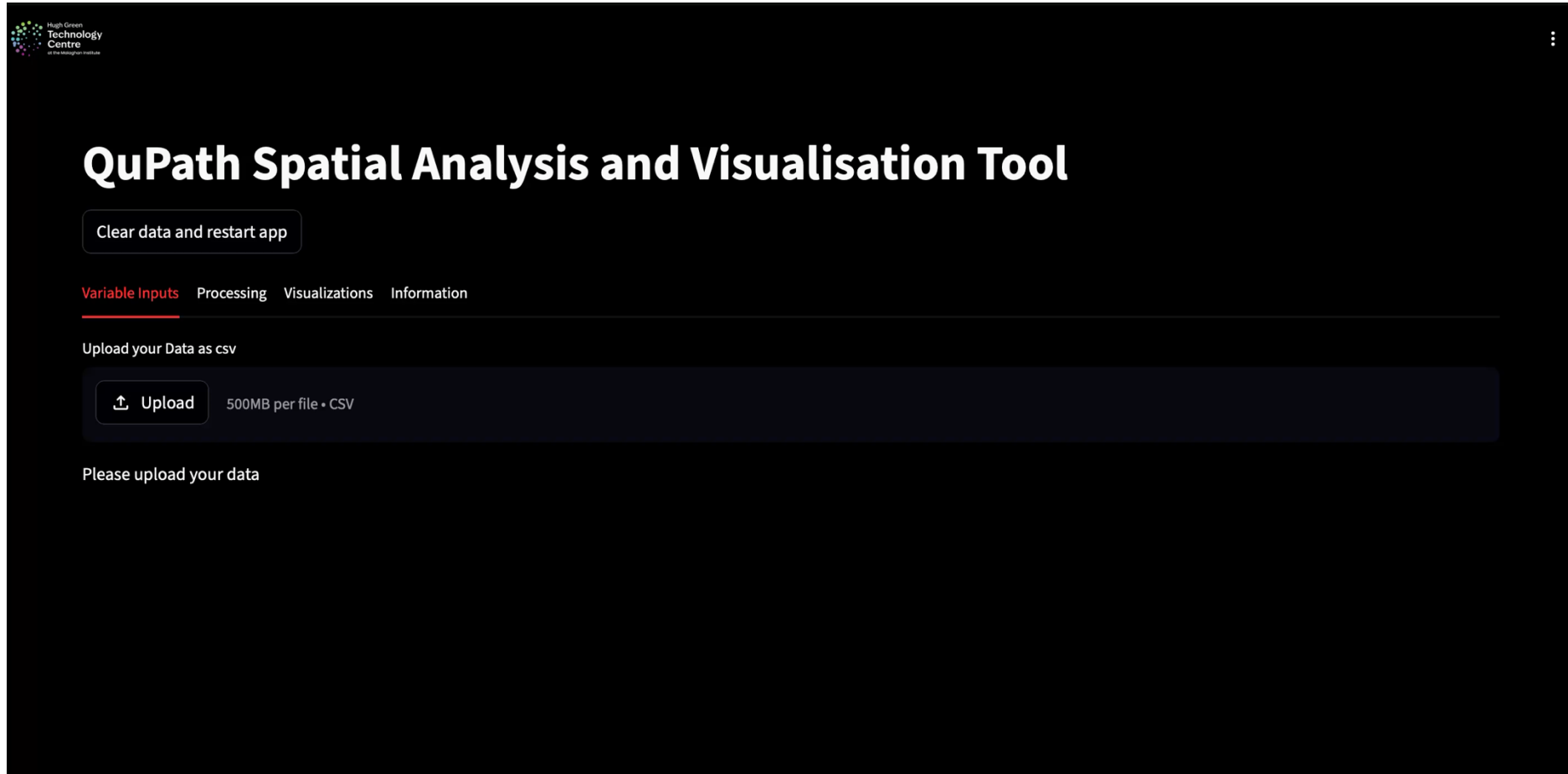


Python/Streamlit

- QuPath Spatial Analysis and Visualization developed as an extended protocol
- Written with Streamlit, an open-source Python framework used to develop interactive web applications
- Takes in large data frame outputs and provides processing and visualization capabilities.



Demonstration



The screenshot shows the QuPath web application interface. At the top left is the logo for the Hugh Green Technology Centre at the Malaghan Institute. The main heading is "QuPath Spatial Analysis and Visualisation Tool". Below the heading is a button labeled "Clear data and restart app". A navigation menu includes "Variable Inputs" (highlighted with a red underline), "Processing", "Visualizations", and "Information". The primary instruction is "Upload your Data as csv", followed by an "Upload" button with an upward arrow icon and the text "500MB per file • CSV". At the bottom of the interface, it says "Please upload your data".



Function in Simplicity

- With the sheer breadth of frameworks, packages, and technologies available today, this tool could have been developed a multitude of different ways.
- Simple development using only 3 packages and basic data frame manipulation kept things light and easy
- Stripping back development complexity relative to development is beneficial for reducing mental load
 - Should you add this rather than can you add this
 - Impact vs Cost



Conclusions

- QuPath offers open-source algorithms for Whole Slide Image analysis, alongside flexibility for users to develop their own analysis algorithms
- Multiplex immunofluorescent analysis pipelines within QuPath generate large data frames that are extremely slow to process conventionally
- QuPath Spatial Analysis and Visualization provides a computationally light web application for extremely fast data frame processing and additional visualizations of imaging data.
- Simplicity in development benefits everyone



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PROTOCOL

An Open-Source Workflow for Semi-Automated Spatial Profiling of Multiplex Immunofluorescent Images

[Alisha Dabb](#), [Cynthia Morgan](#), [Sophia Noble](#), [Alfonso J. Schmidt](#) ✉

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Questions?

