

Building analysis pipelines for antibody-based multiplexed images

Beštak Krešimir, Ibarra-Arellano Miguel A.

2.December.2024

Schapiro Lab, Heidelberg

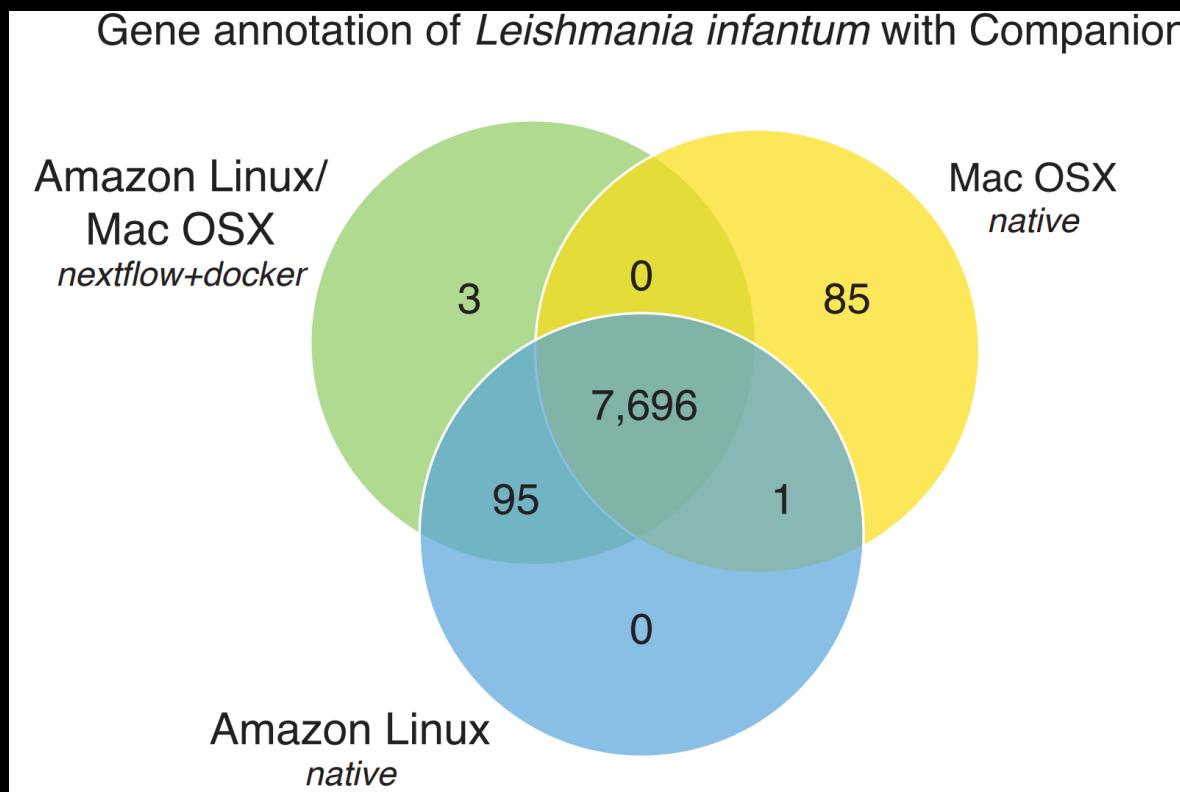


Hackathon: Towards Composable Modules for Standardized Analysis Pipelines in Nextflow, Leuven, Belgium 2024

Outlook

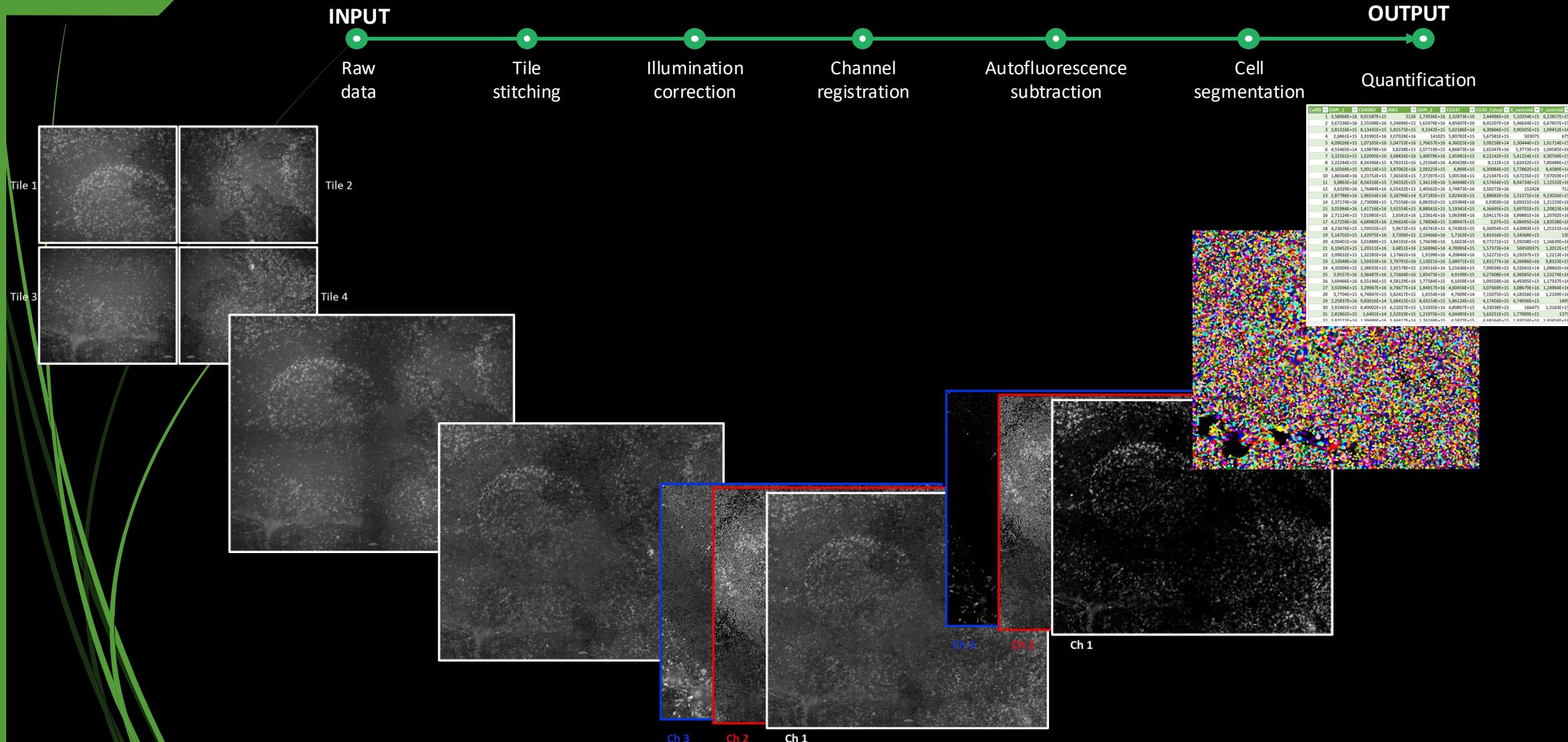
- ▶ Addressing computational reproducibility with workflow managers.
- ▶ What is Nextflow and nf-core.
- ▶ Image analysis pipelines in nf-core.
- ▶ Data specific use cases.
- ▶ Future outlook.

Computational reproducibility problem



4

An example of an analysis pipeline for multiplexed images



Dependency trees of common python packages used in the pipeline

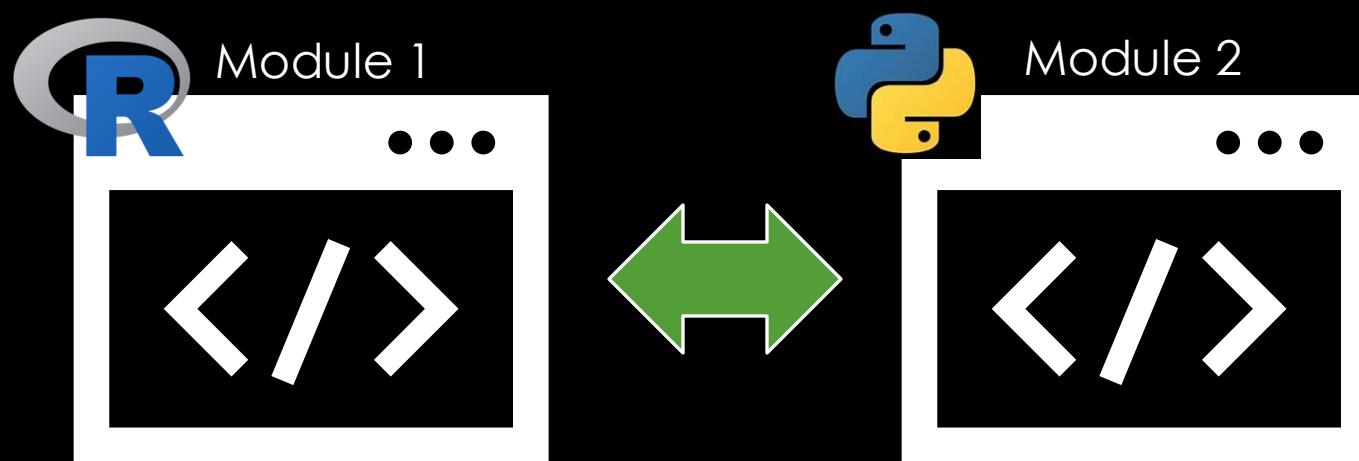


Workflow managers



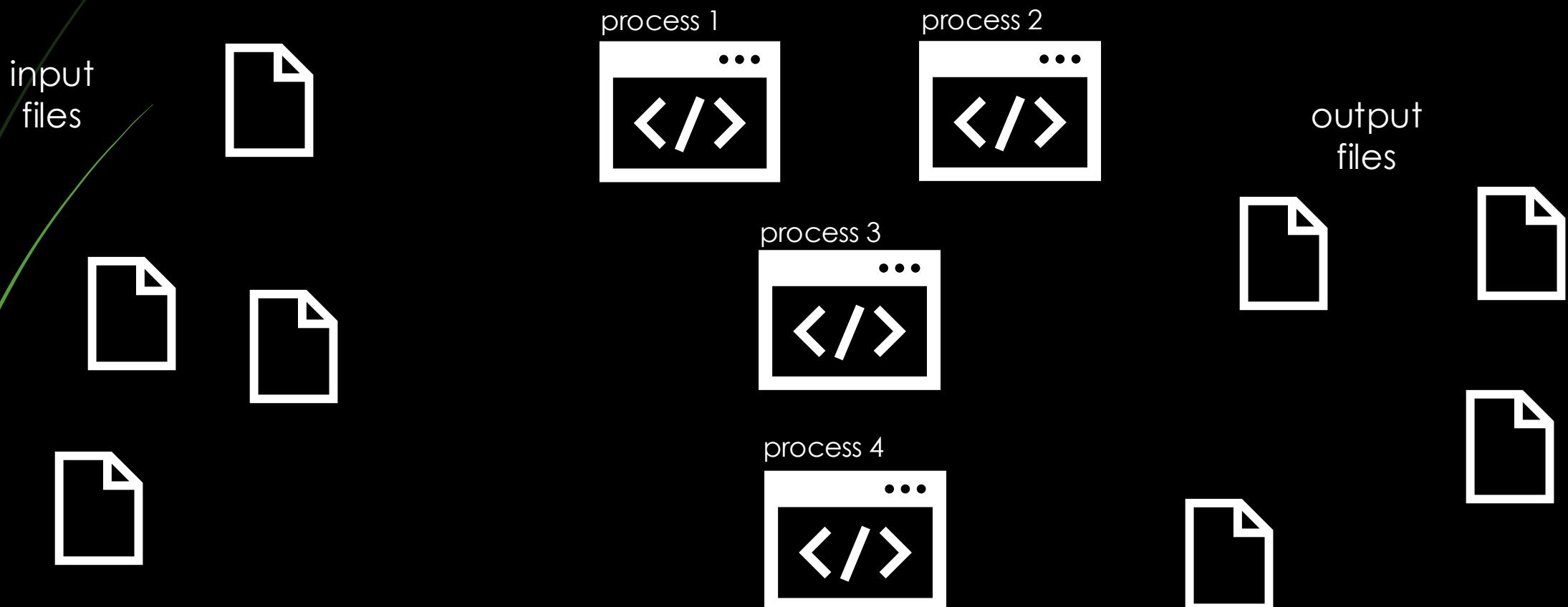
What do workflow managers do?

- ▶ **Facilitate putting together multiple modules even if written in different scripting languages.**



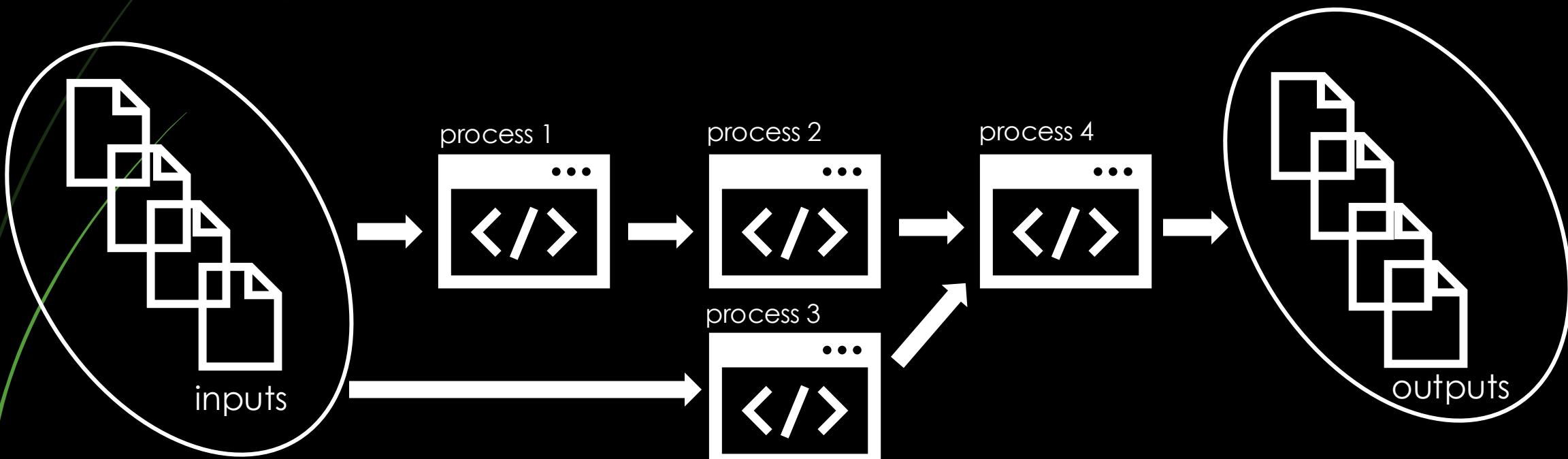
What do workflow managers do?

- ▶ **Orchestrate the efficient and parallel flow of inputs and outputs across different processes**



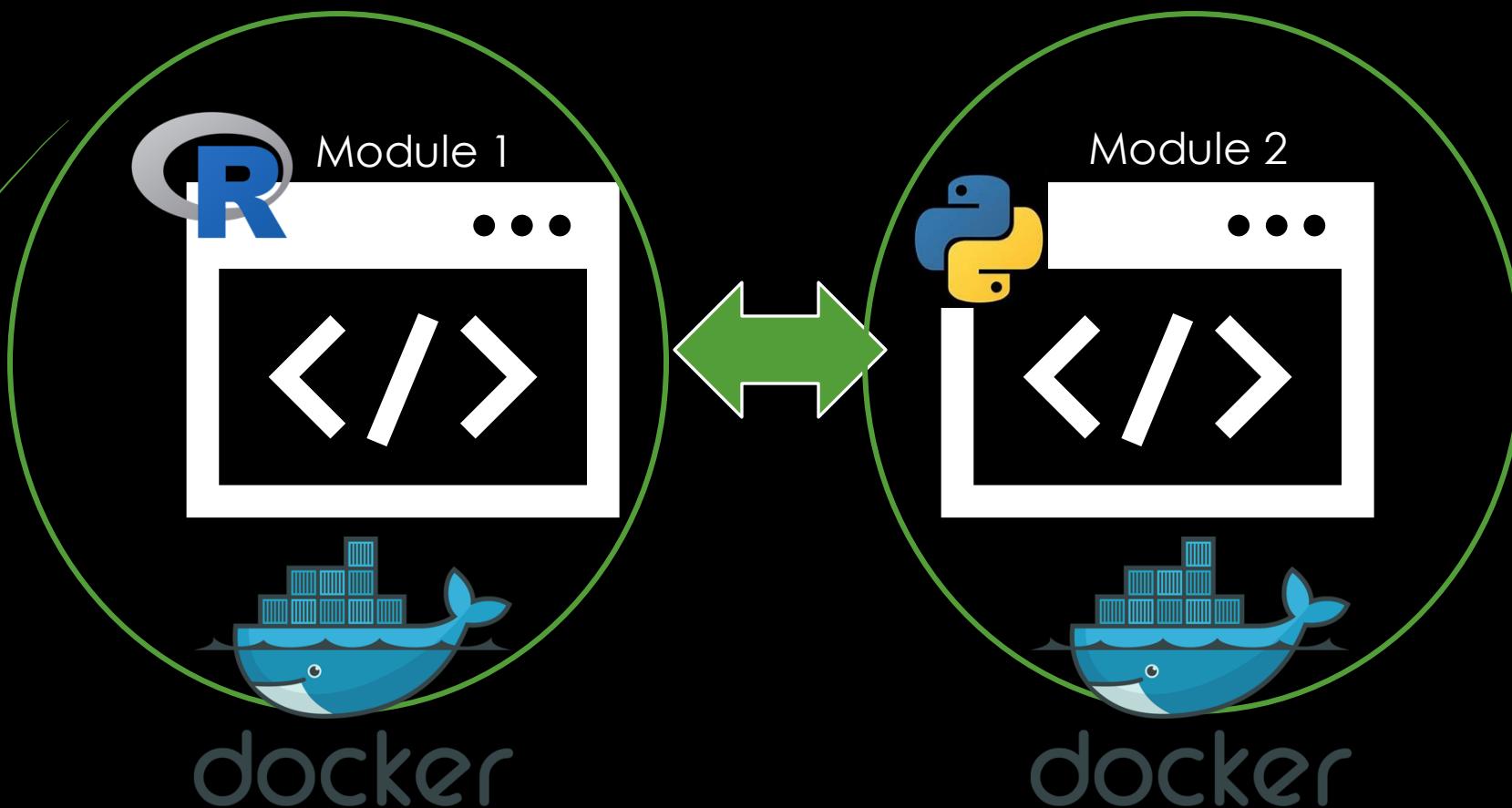
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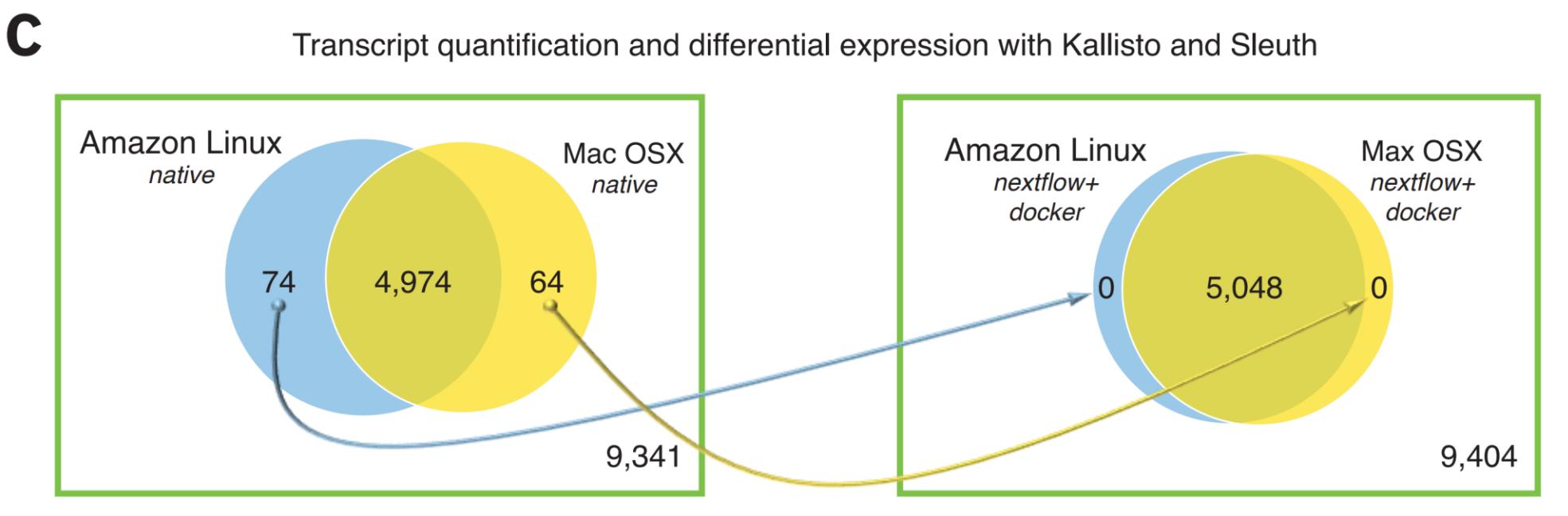
What do workflow managers do?

- ▶ Enable reproducibility when coupled with containerized modules.



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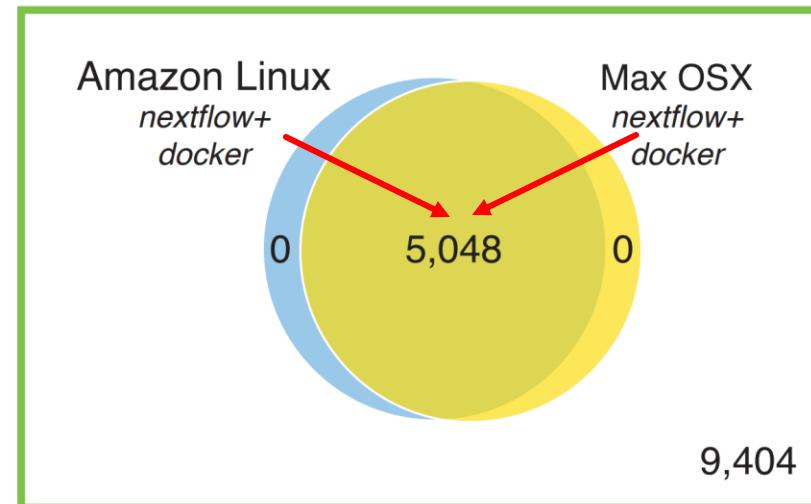
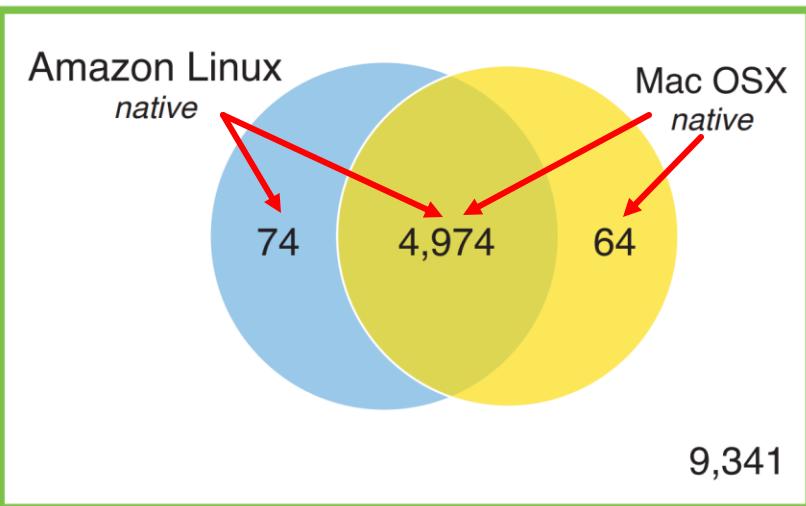


What do workflow managers do?

► Portability and Shareability

C

Transcript quantification and differential expression with Kallisto and Sleuth



What do workflow managers enable in a pipeline

- ▶ Interoperability across languages.
- ▶ Orchestrates inputs and outputs dependencies.
- ▶ Reproducibility.
- ▶ Portability and Shareability.

Overview of workflow managers

Table 1 | Overview of workflow managers for bioinformatics (top, editable version; bottom, image version)

Tool	Class	Ease of use ^a	Expressiveness ^b	Portability ^c	Scalability ^d	Learning resources ^e	Pipeline initiatives ^f
Galaxy	Graphical	●●●	●○○	●●●	●●●	●●●	●●○
KNIME	Graphical	●●●	●○○	○○○	●●○	●●●	●●○
Nextflow	DSL	●●○	●●●	●●●	●●●	●●●	●●●
Snakemake	DSL	●●○	●●●	●●○	●●●	●●○	●●●

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Workflow manager: Nextflow

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Programming
Language: Groovy

Workflow manager: Nextflow

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Container integration:
Docker, Singularity,
Shifter, Conda

Workflow manager: Nextflow

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Resources:

Slack community,
YouTube channel,
Hackathons, Tutorials

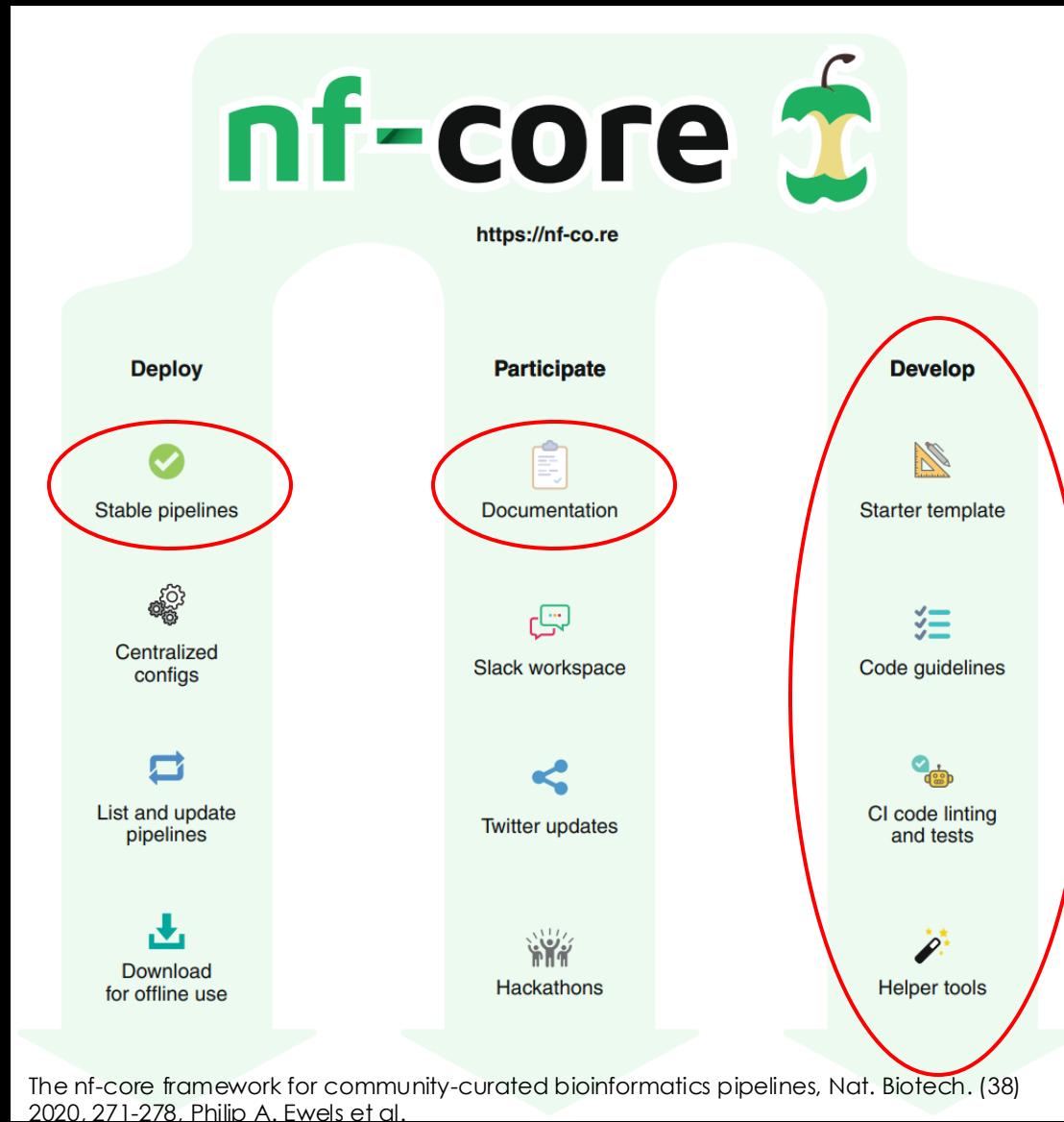
Workflow manager: Nextflow

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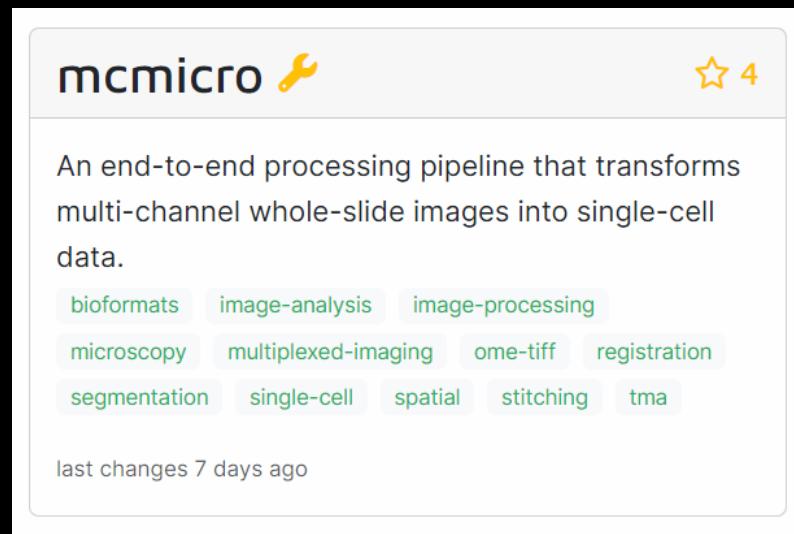
Pipeline community:
Pipeline templates,
Pipeline testing and
validation.

Pipeline development community: nf-core

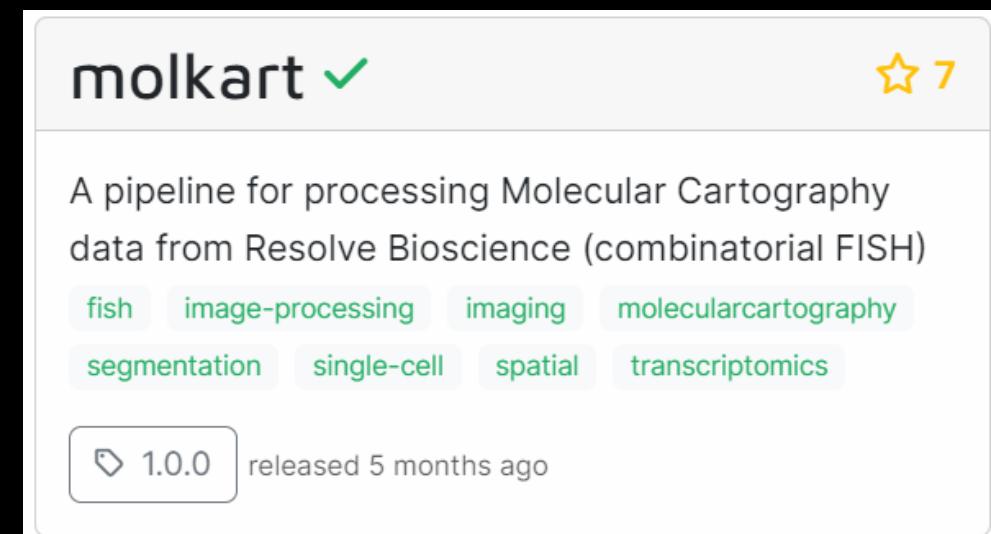


The pipelines: mcmicro and moltkart

► multiple-choice microscopy



► moltkart



mcmicro

<https://mcmicro.org/>



nature methods BRIEF COMMUNICATION
<https://doi.org/10.1038/s41592-021-01308-y>

OPEN

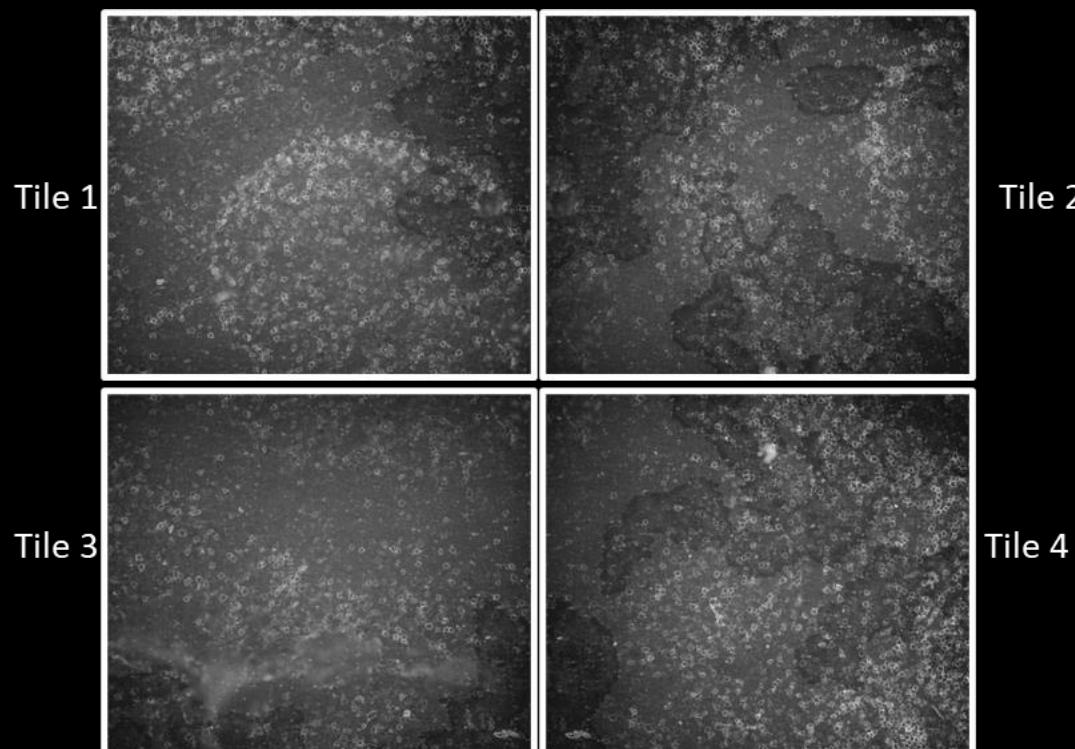
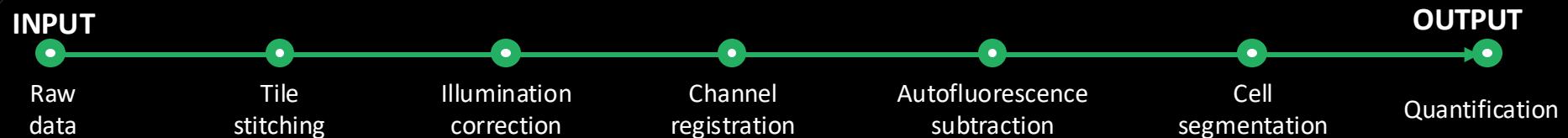
MCMICRO: a scalable, modular image-processing pipeline for multiplexed tissue imaging

Denis Schapiro^{1,2,3,18,20}, Artem Sokolov^{1,2,4,20}, Clarence Yapp^{1,2,5,20}, Yu-An Chen^{1,2}, Jeremy L. Muhlich^{1,2}, Joshua Hess^{1,6}, Allison L. Creason^{1,7}, Ajit J. Nirmal^{1,2,8}, Gregory J. Baker^{1,2}, Maulik K. Nariya^{1,2}, Jia-Ren Lin^{1,2}, Zoltan Maliga^{1,2}, Connor A. Jacobson^{1,2}, Matthew W. Hodgman^{2,9}, Juha Ruokonen^{1,2}, Samouil L. Farhi^{1,3}, Domenic Abbondanza³, Eliot T. McKinley^{10,11}, Daniel Persson^{7,12}, Courtney Betts¹³, Shamilene Sivagnanam¹³, Aviv Regev^{1,3,14,19}, Jeremy Goecks^{1,7,12}, Robert J. Coffey^{1,15}, Lisa M. Coussens^{1,12,13}, Sandro Santagata^{1,2,16} and Peter K. Sorger^{1,2,17}

A small rectangular button with a magnifying glass icon and the text "Check for updates". A green arrow points from this button towards the right edge of the slide.

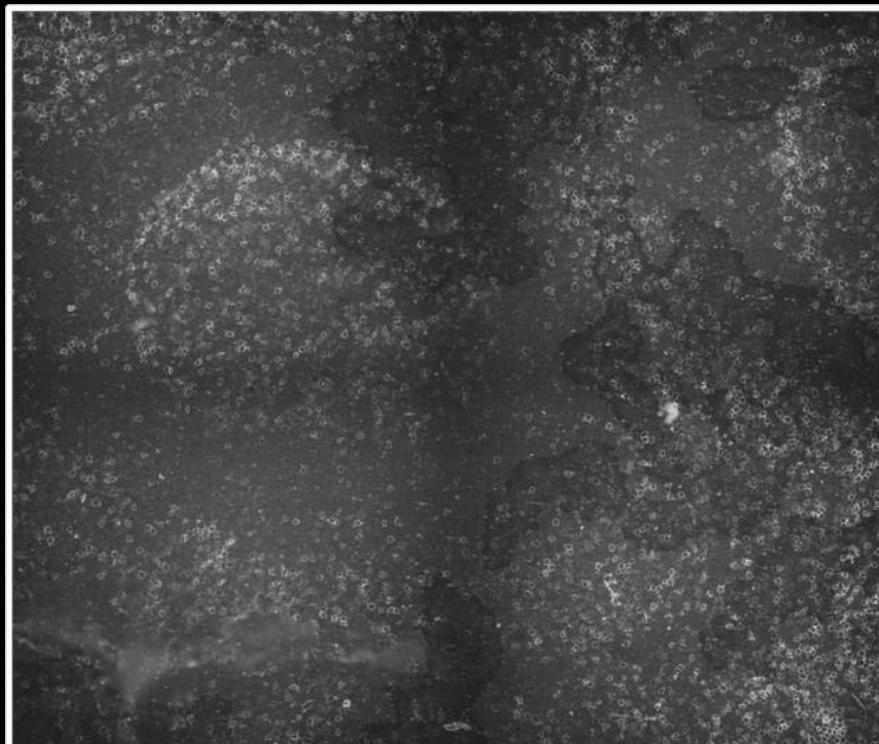
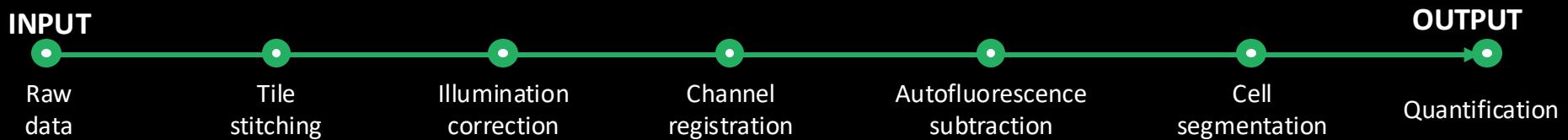
mcmicro

<https://mcmicro.org/>



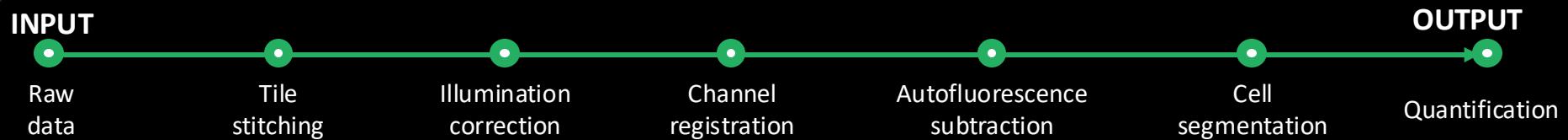
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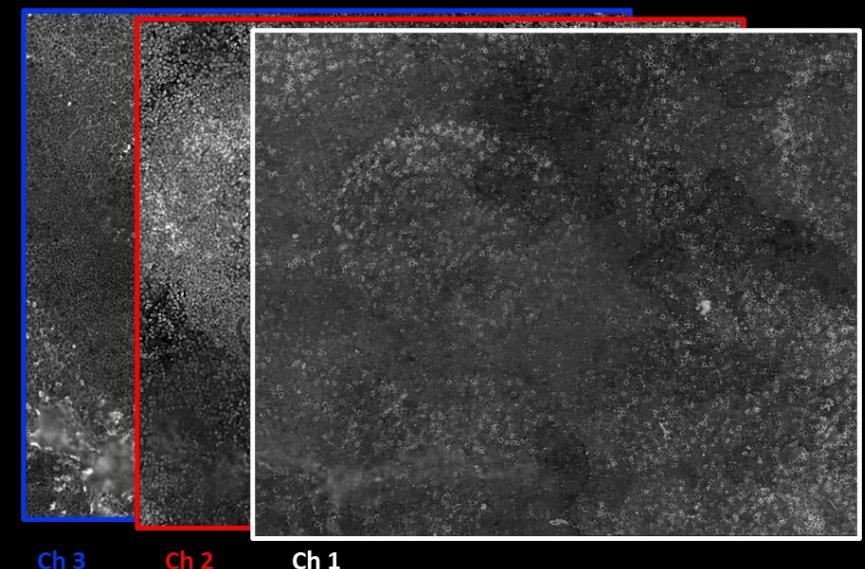
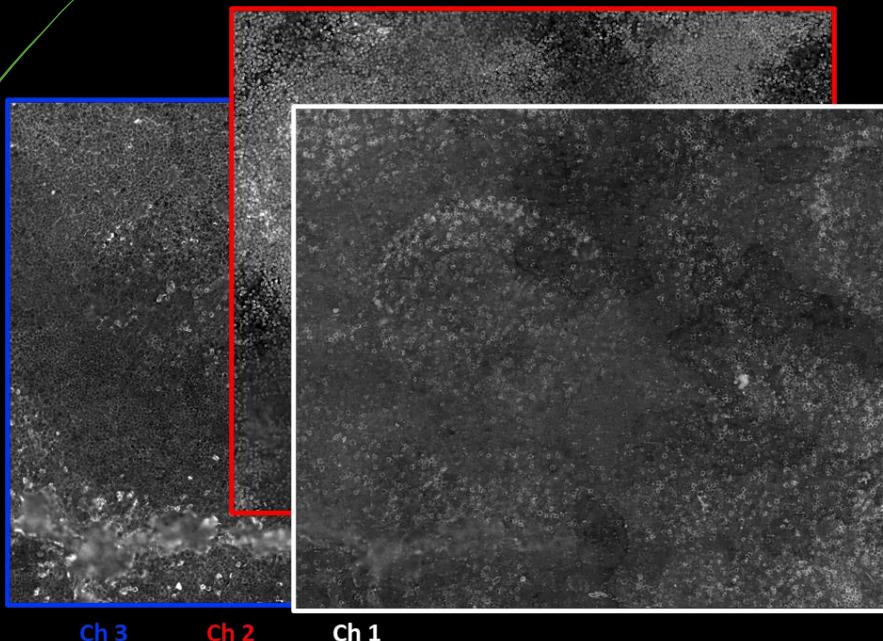
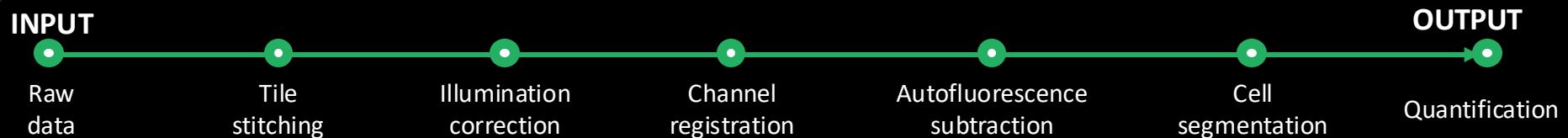
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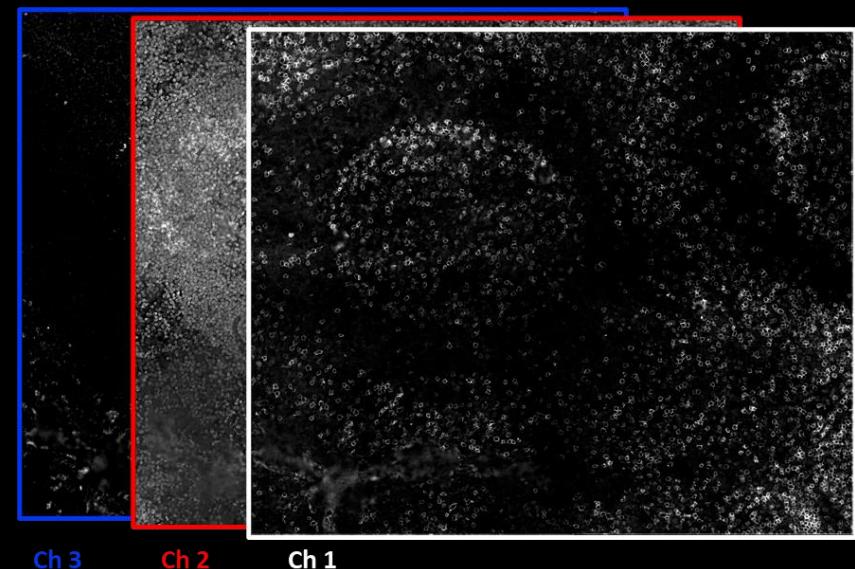
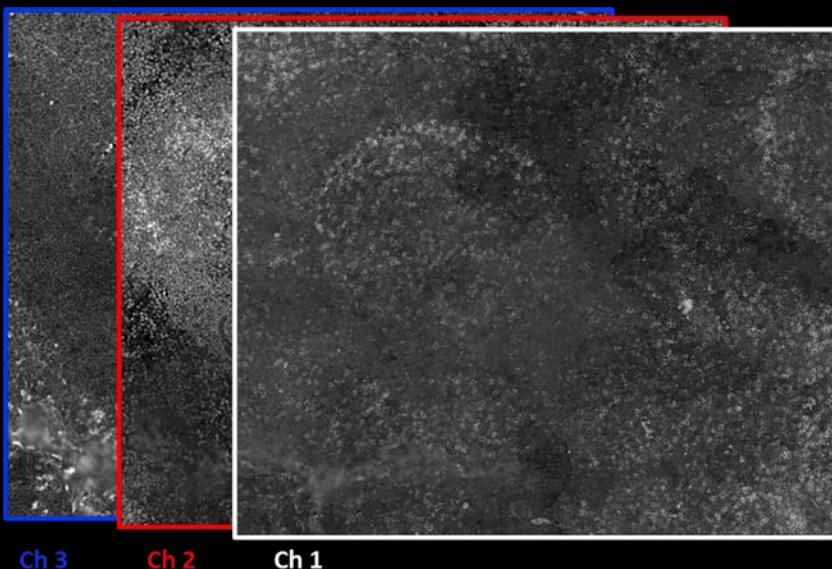
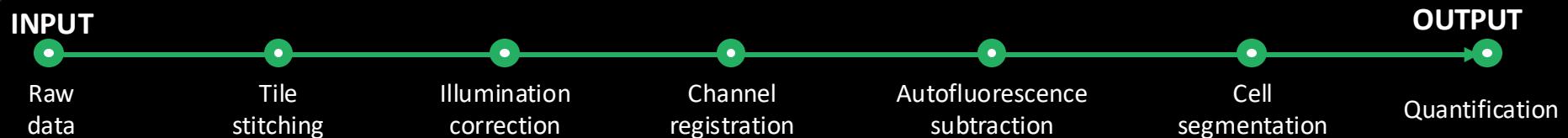
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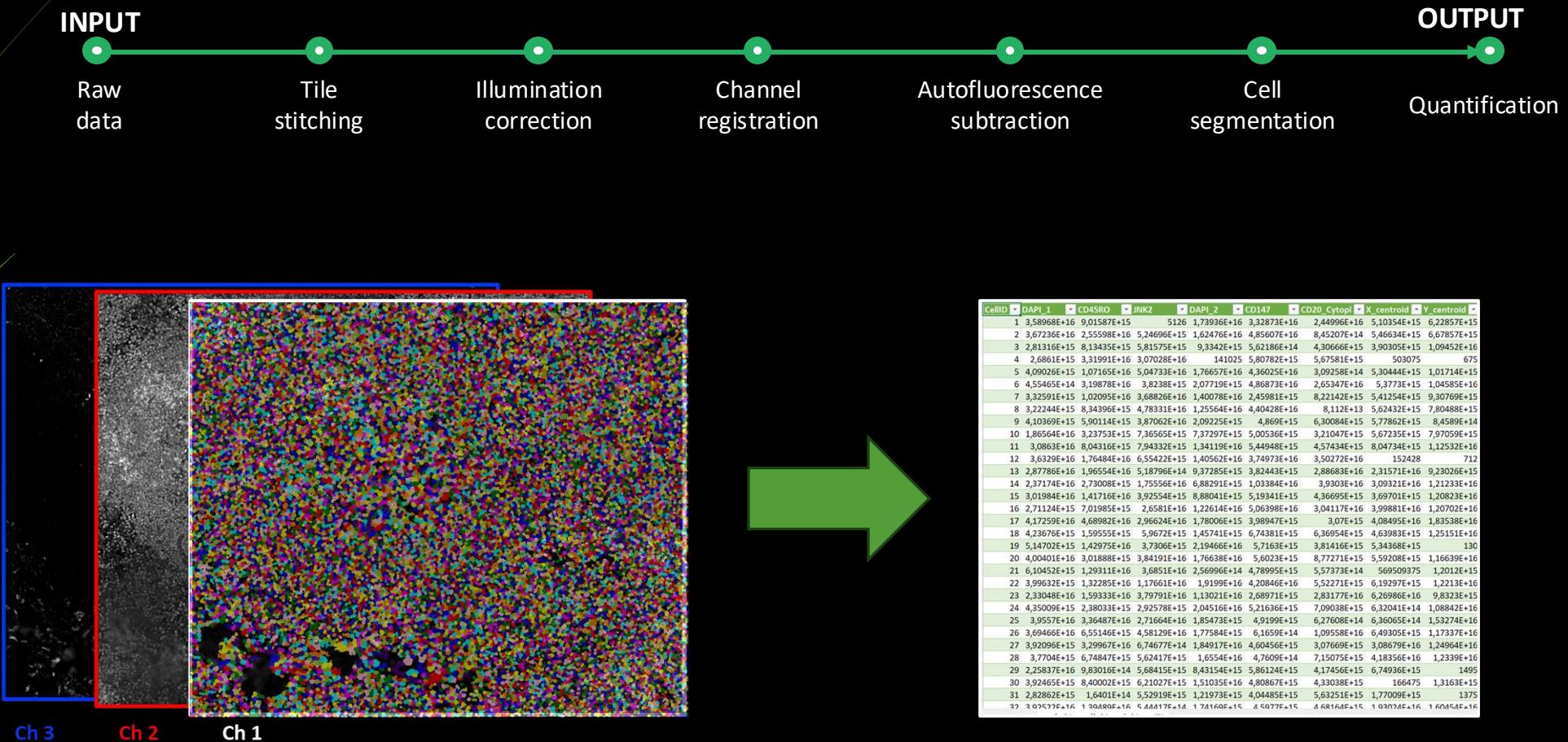
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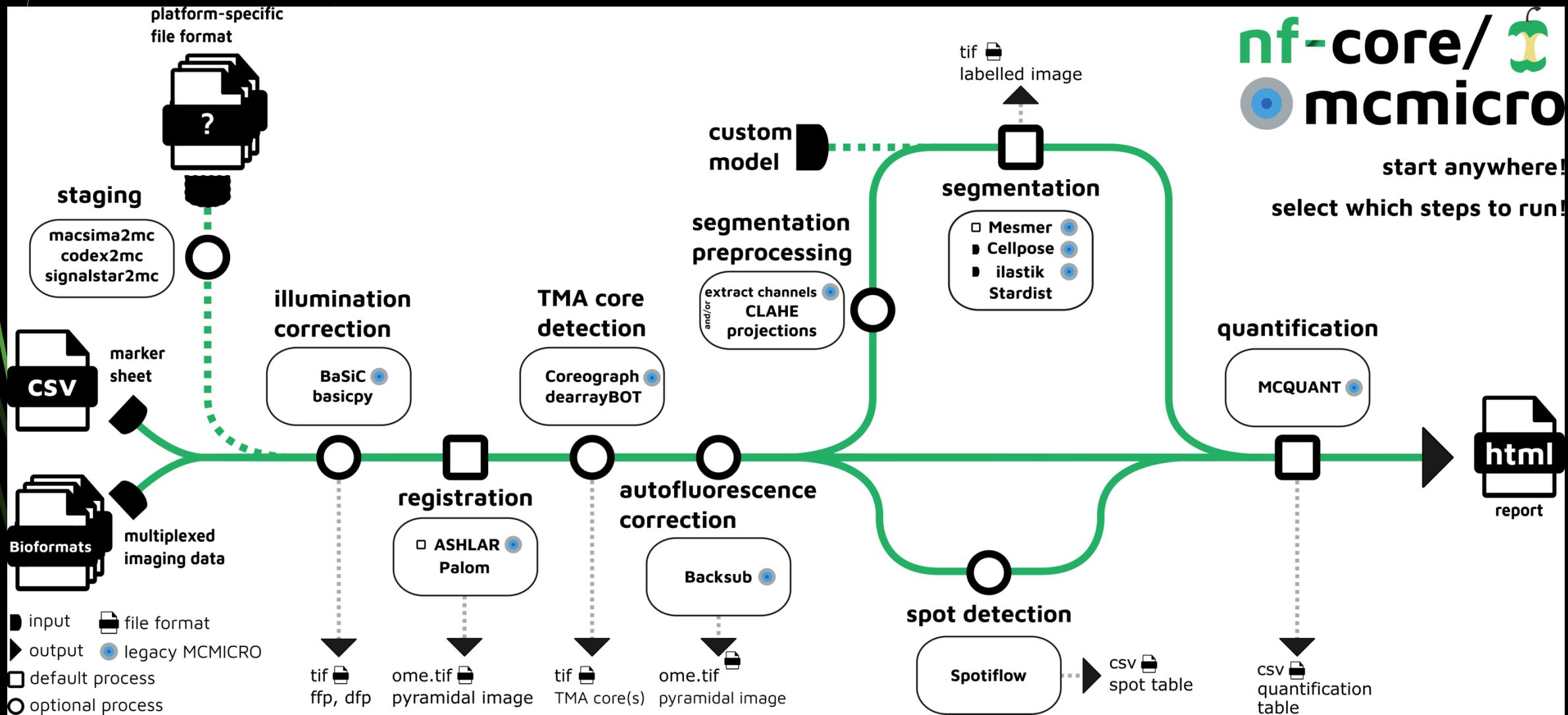


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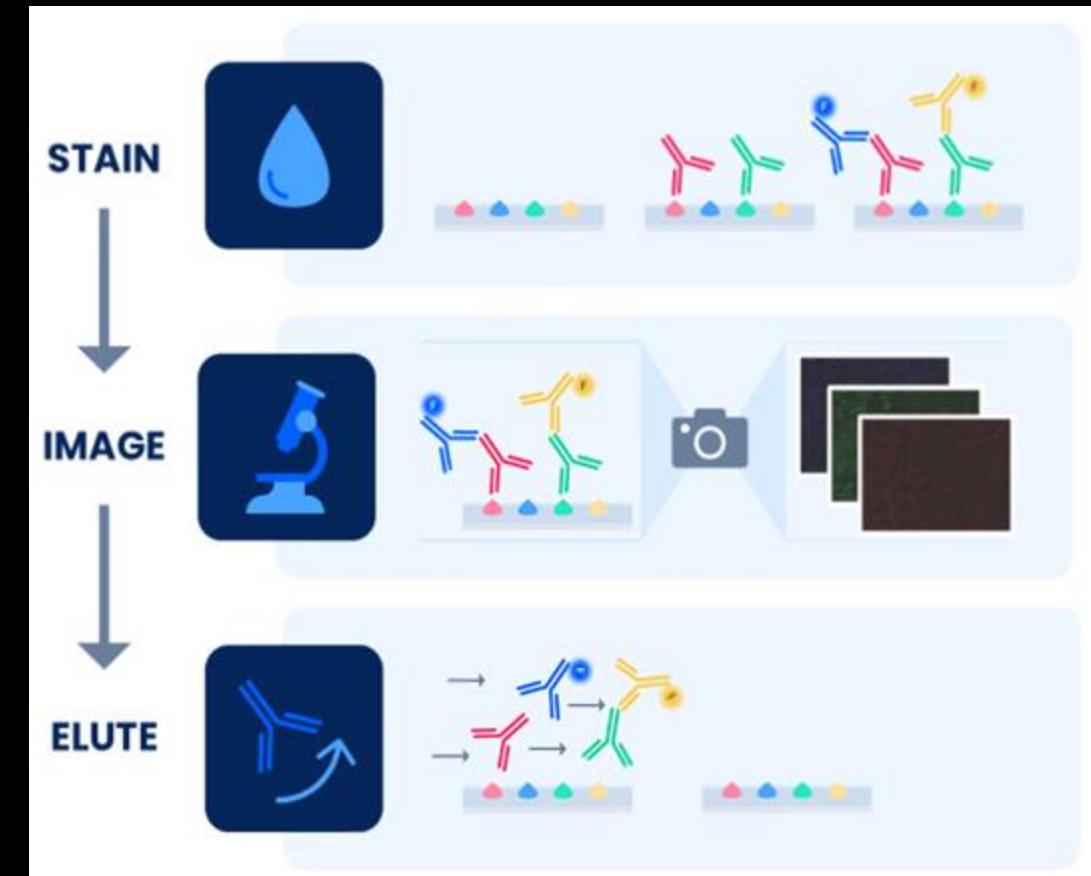
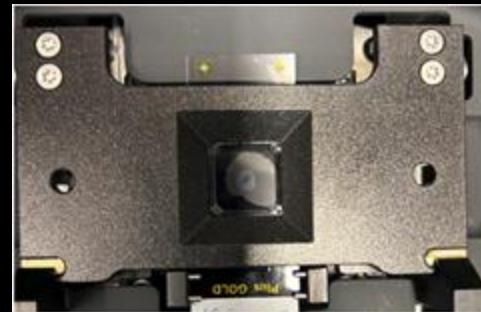


nf-core/mcmicro

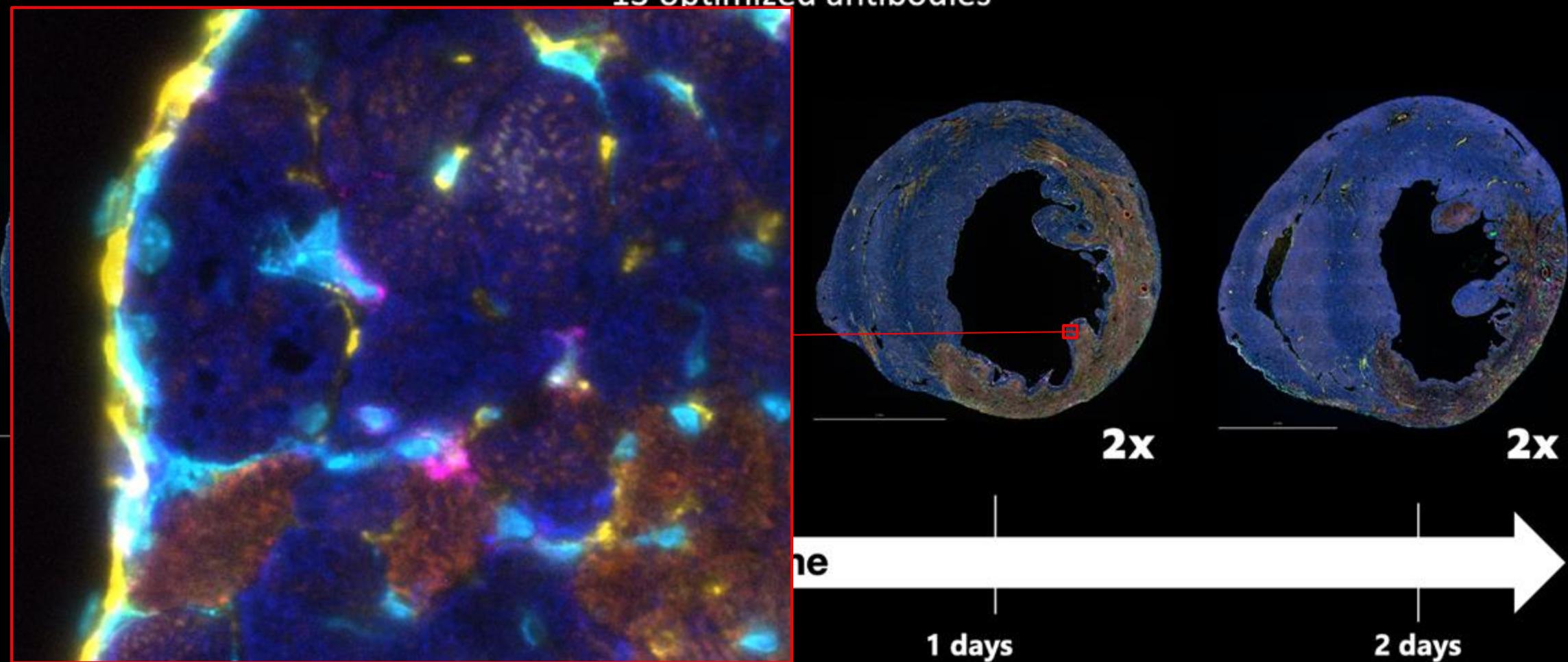


Murine MI Sequential immunofluorescence with the Lunaphore COMET

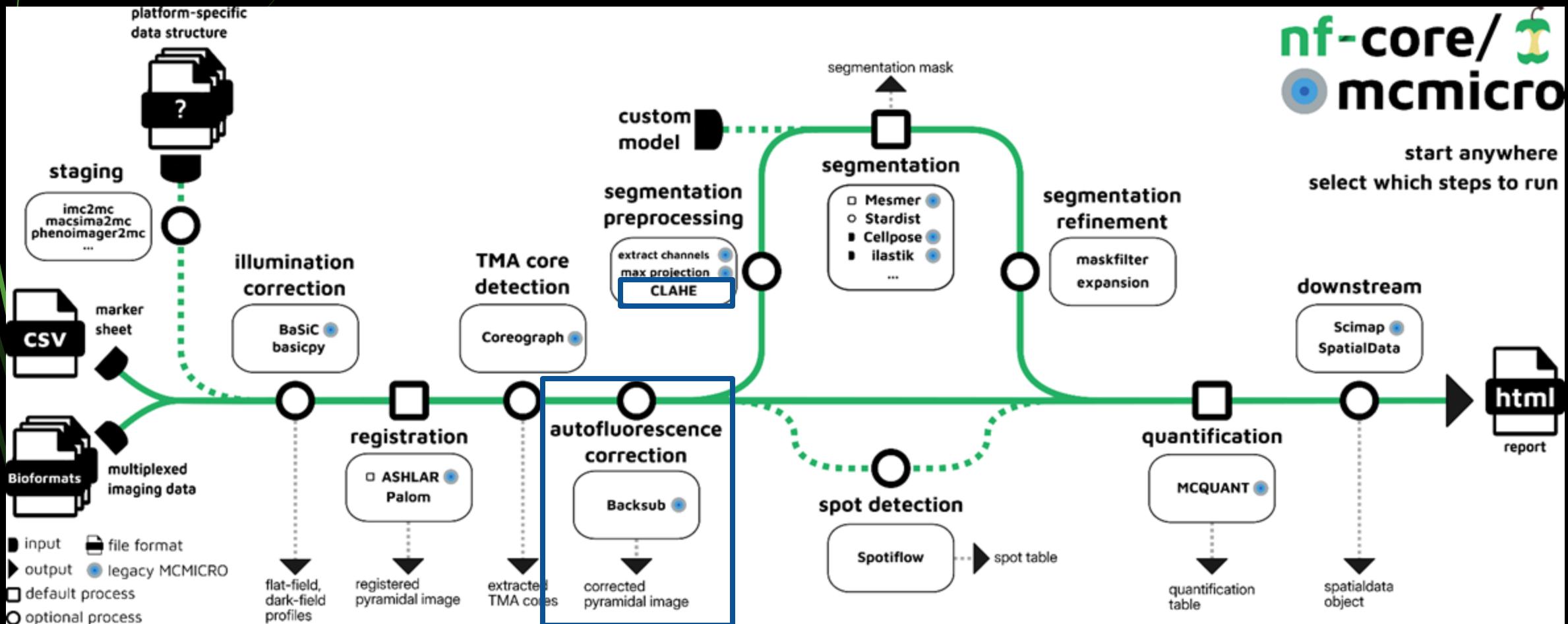
- ▶ up to 40 primary antibodies on the same slide
- ▶ up to 4 samples processed in the same run
- ▶ 2 markers per cycle + DAPI



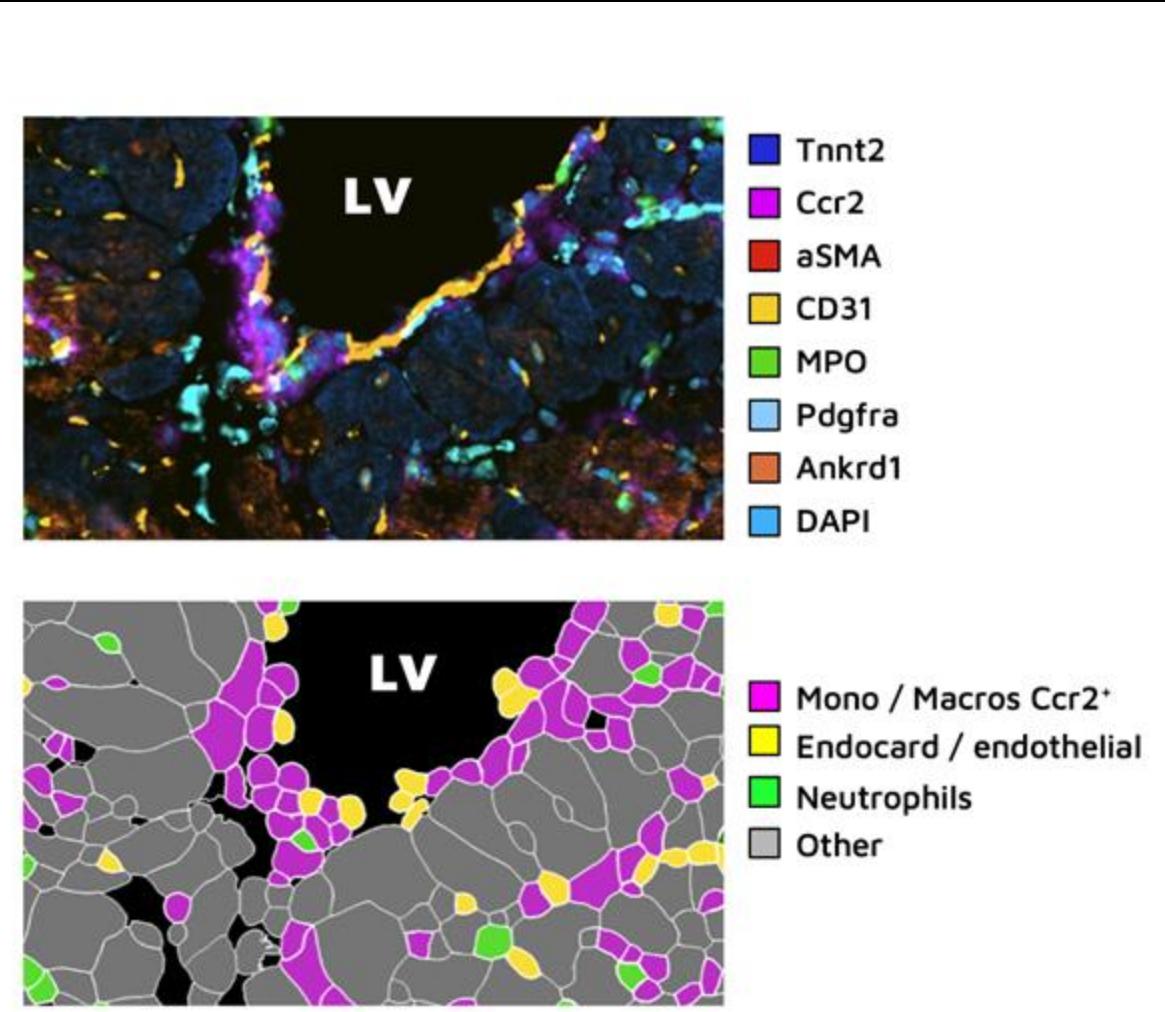
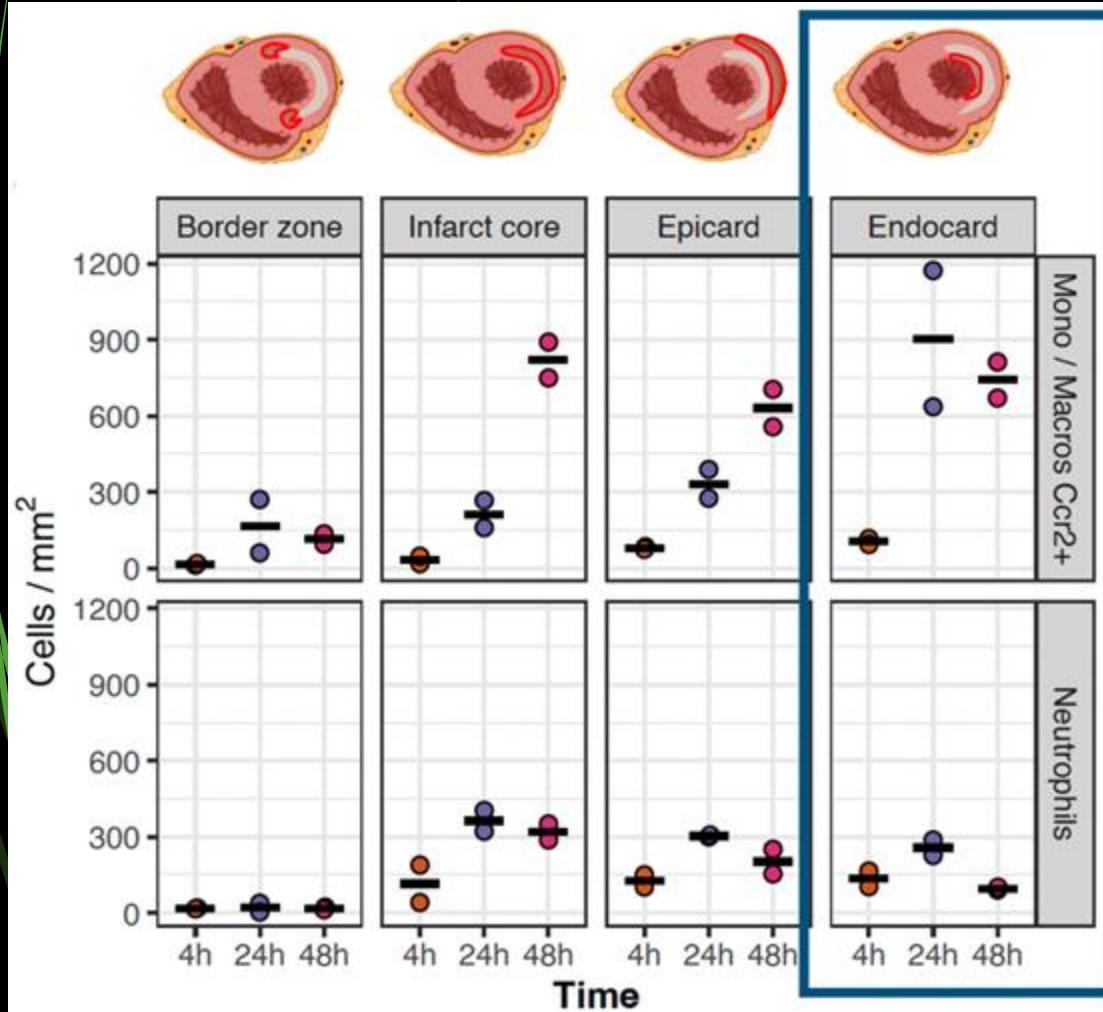
Murine MI Sequential immunofluorescence with the Lunaphore COMET



Multiplexed antibody-based imaging data processing with MCMICRO

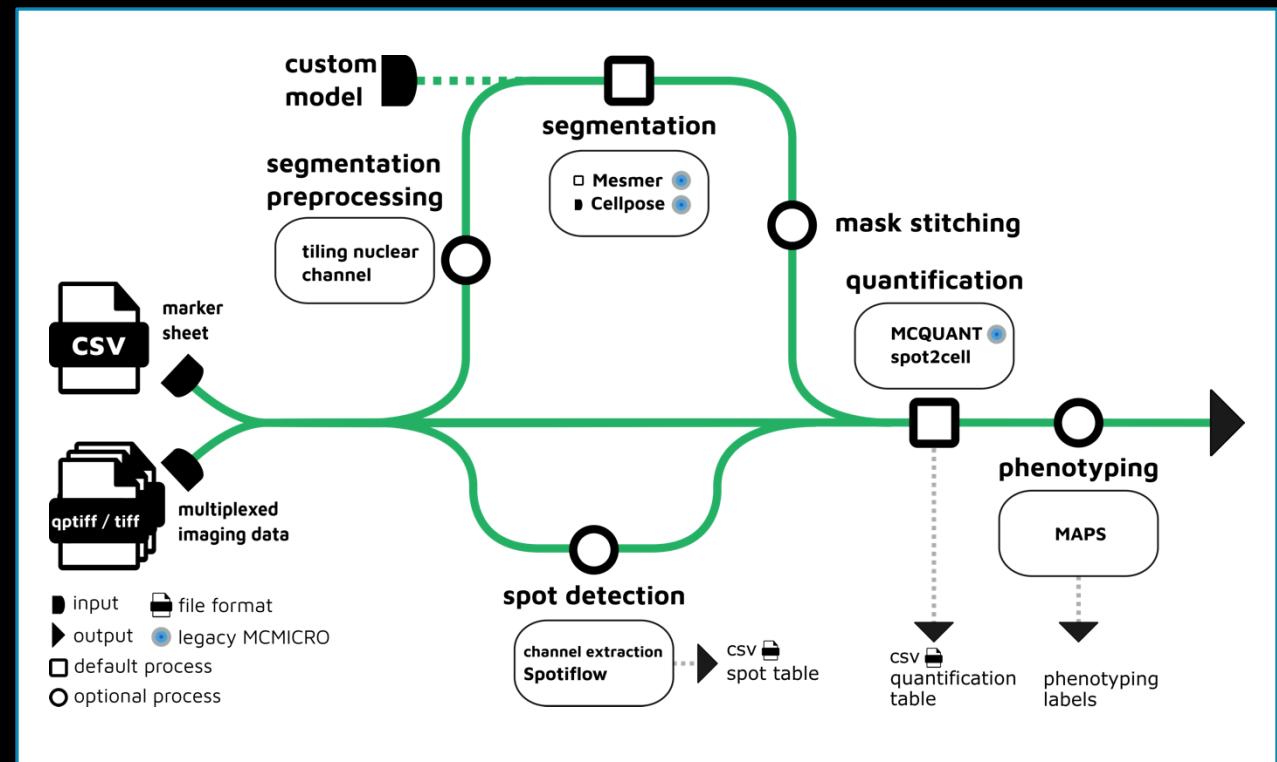


Quantification of immune infiltration to the infarct core through different routes



Use case – BrainMets (under revision)

- ▶ Challenges
 - ▶ 170 prestitched images with up to 3 000 000 cells per sample
 - ▶ Deepcell Mesmer wasn't performant on larger images
 - ▶ Phenotyping optimization and implementation
 - ▶ Punctate channel information extraction

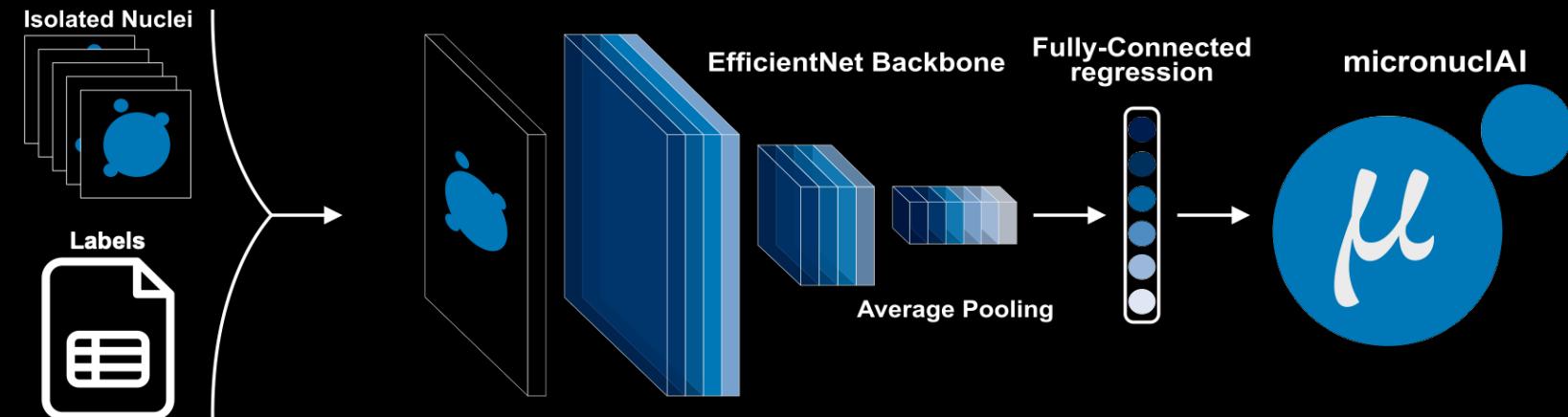
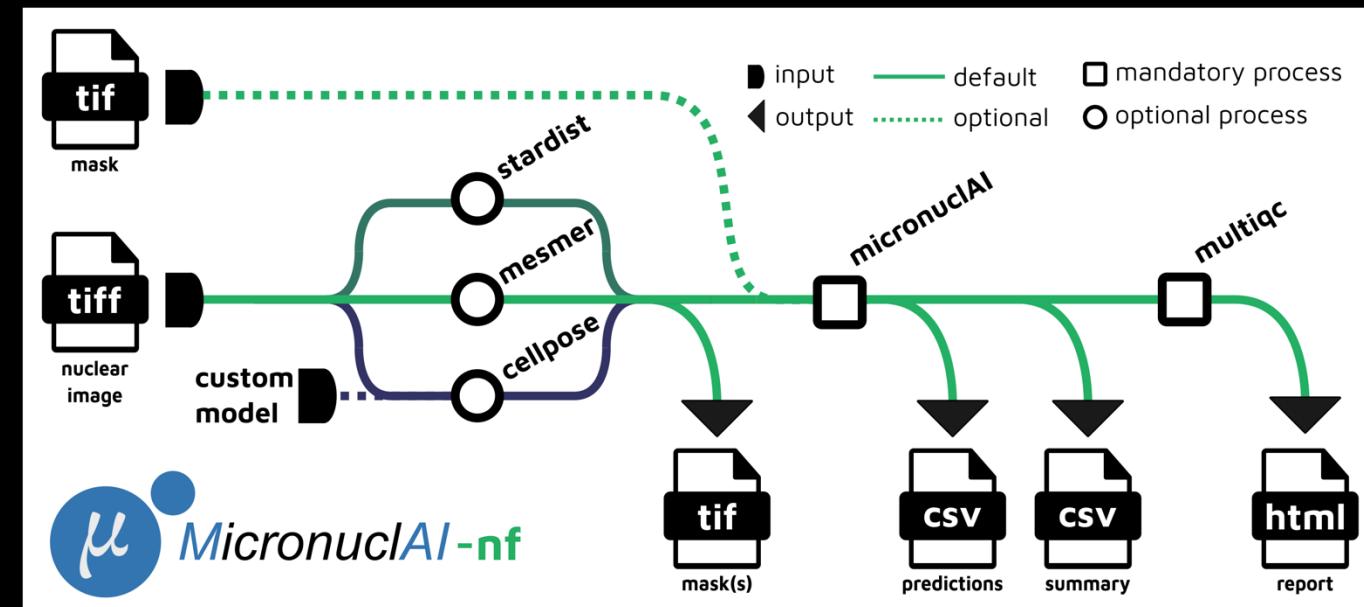




MicronuclAI



Miguel A.
Ibarra-Arellano



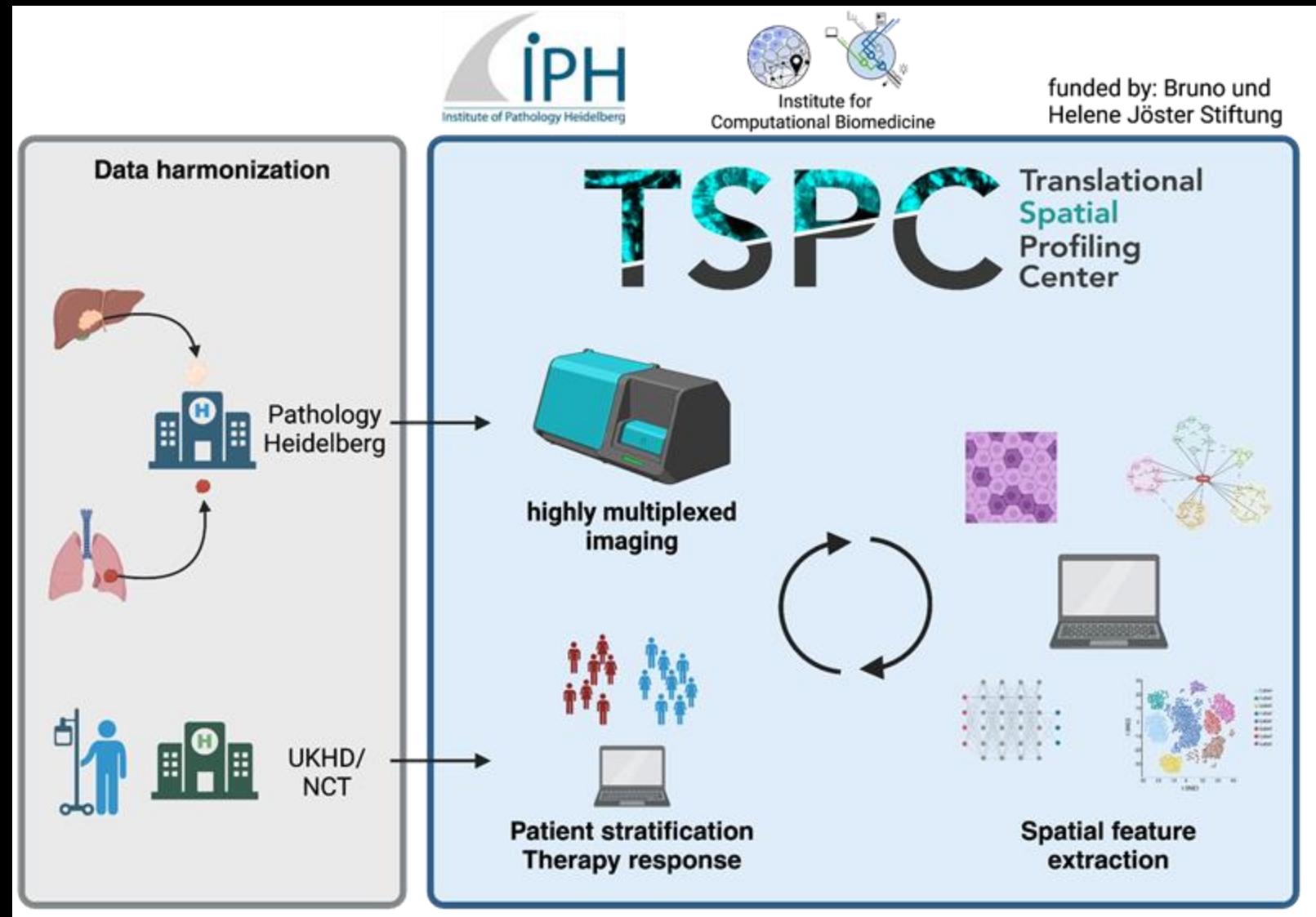
Translational Spatial Profiling Center (TSPC)



Chiara
Schiller

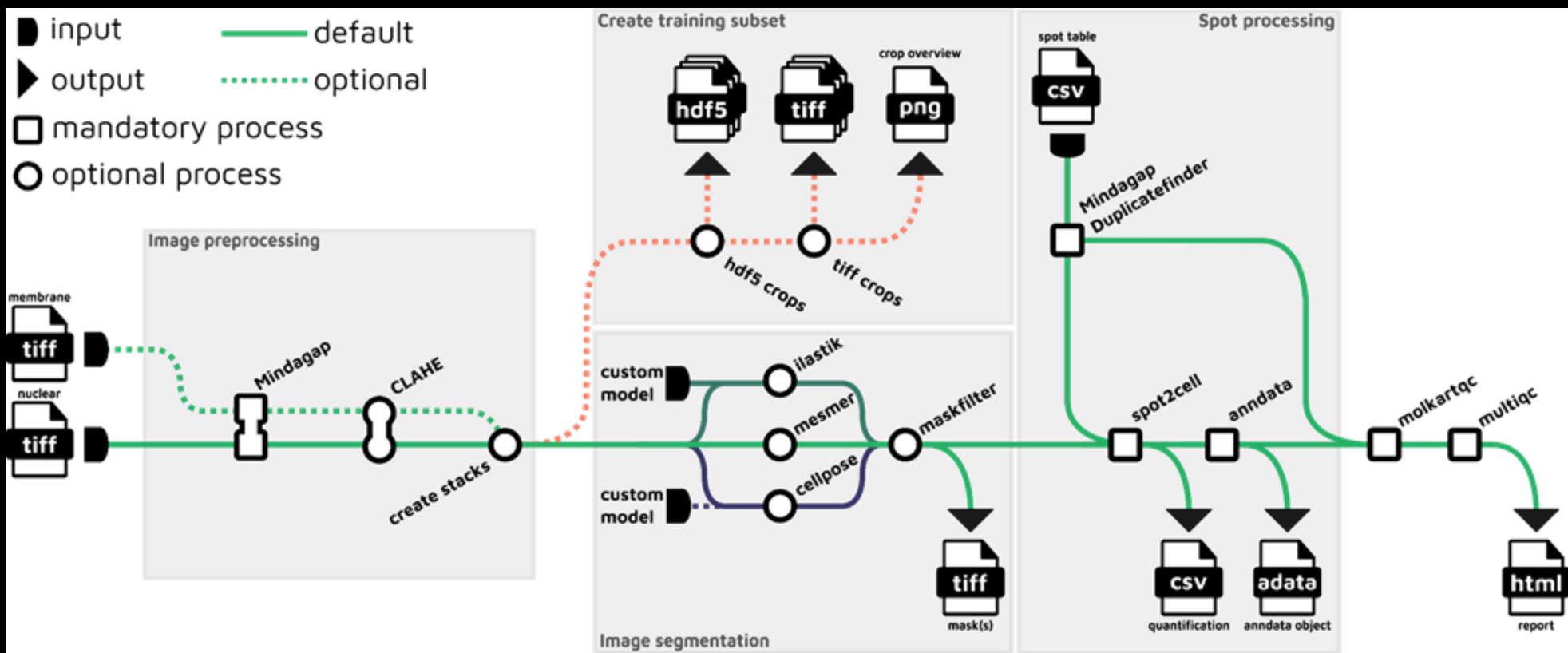


Lukas
Hatscher

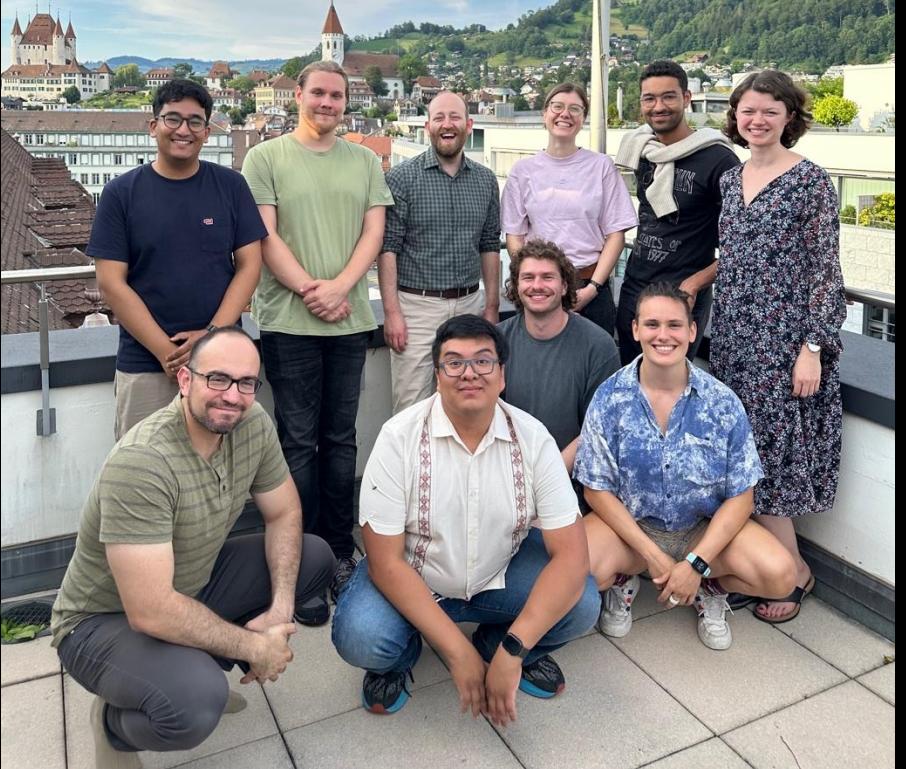


molkart

- ▶ Processing of single-molecule FISH data



Krešimir Beštak



**Schapiro
Lab**

<https://www.schapirolab.com/>



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MCMICRO <https://mcmicro.org/>

nf-core 

[nf-core/molkart](https://nf-co.re/molkart/1.0.0) <https://nf-co.re/molkart/1.0.0>

[nf-core/mcmicro](https://nf-co.re/mcmicro/dev) <https://nf-co.re/mcmicro/dev>



40

MCMICRO
/MOLKART

See you there

Inaugural conference of the
**European Society for
Spatial Biology**

12 – 13 December 2024
Berlin, Germany



Registration is open!

spatialbiologysociety.eu
spatialbiologysociety@gmail.com

