# Investigating the Impact of Astrocyte-Melanoma Direct Junctions on Spatial GTP Distribution in Melanoma Cells Using GTP Biosensor

11/25/2024 BME milestone exam

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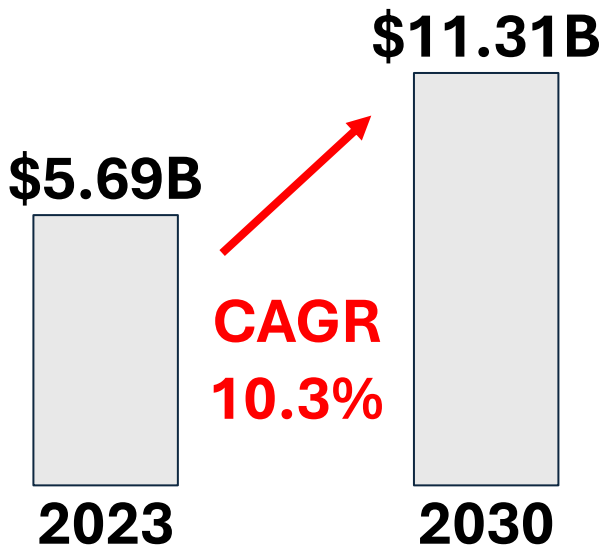
Department of Biomedical Engineering

# MBM exhibits high mortality & prevalence

- Melanoma represents **1%** skin cancer cases but **80%** skin cancer-related death due to high metastatic potential
- 50% metastatic melanoma patients develop brain metastasis (**MBM**)
- MBM patients' median survival = **12.8 month** with mortality rate = **80-85%**




Saginala, K., Barsouk, A., Aluru, J. S., Rawla, P. & Barsouk, A. Epidemiology of Melanoma. Med Sci (Basel) 9 (2021)
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Research, V. M. Global Drugs For Melanoma Market Size By Test (Chemotherapy, Immunotherapy, Targeted Therapy), By Application (Hospitals, Clinics), By Geographic Scope And Forecast. Verified Market Research (2023).

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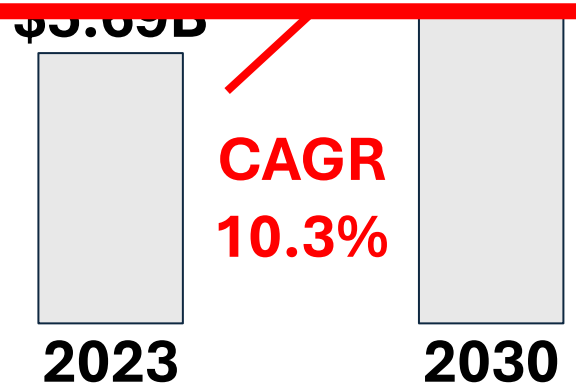
- 50% metastatic melanoma patients develop brain metastasis (**MBM**)

**Understanding acquisition of invasive phenotype (melanoma → MBM) is critical for developing effective therapeutic strategies**

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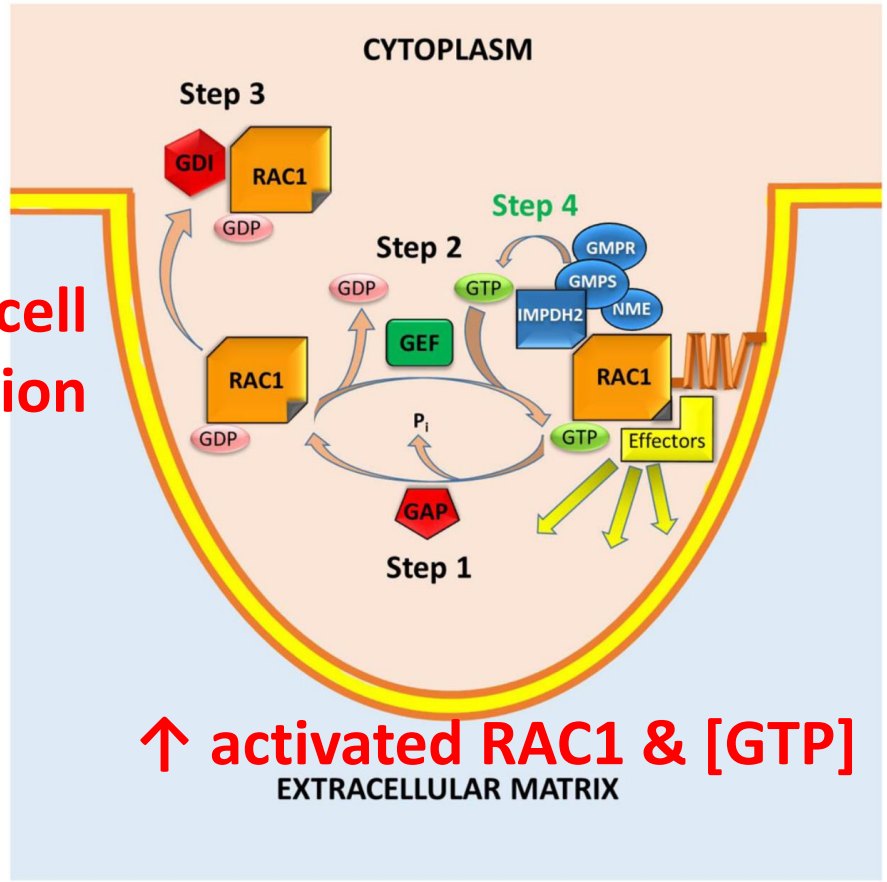
# Fluctuations in intracellular GTP levels regulate melanoma cell invasion & metastasis

**Melanoma cell protrusion**






Bianchi-Smiraglia, A., Wolff, D.W., Marston, D.J. *et al.* Regulation of local GTP availability controls RAC1 activity and cell invasion. *Nat Commun* **12**, 6091 (2021). https://doi.org/10.1038/s41467-021-26324-6

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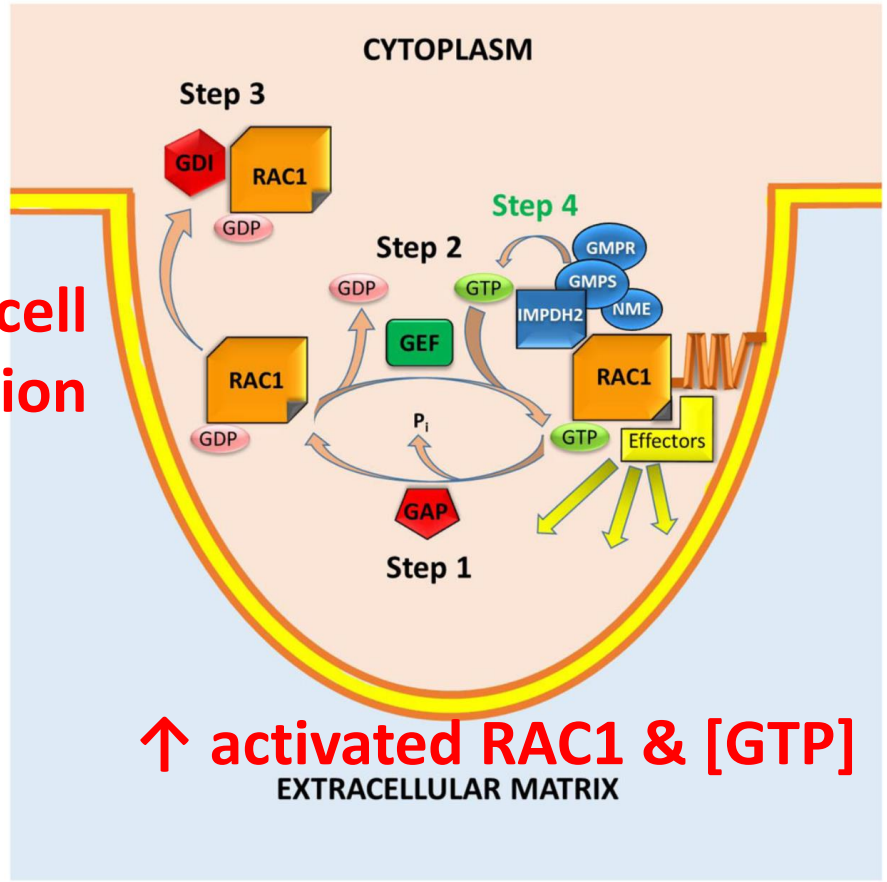


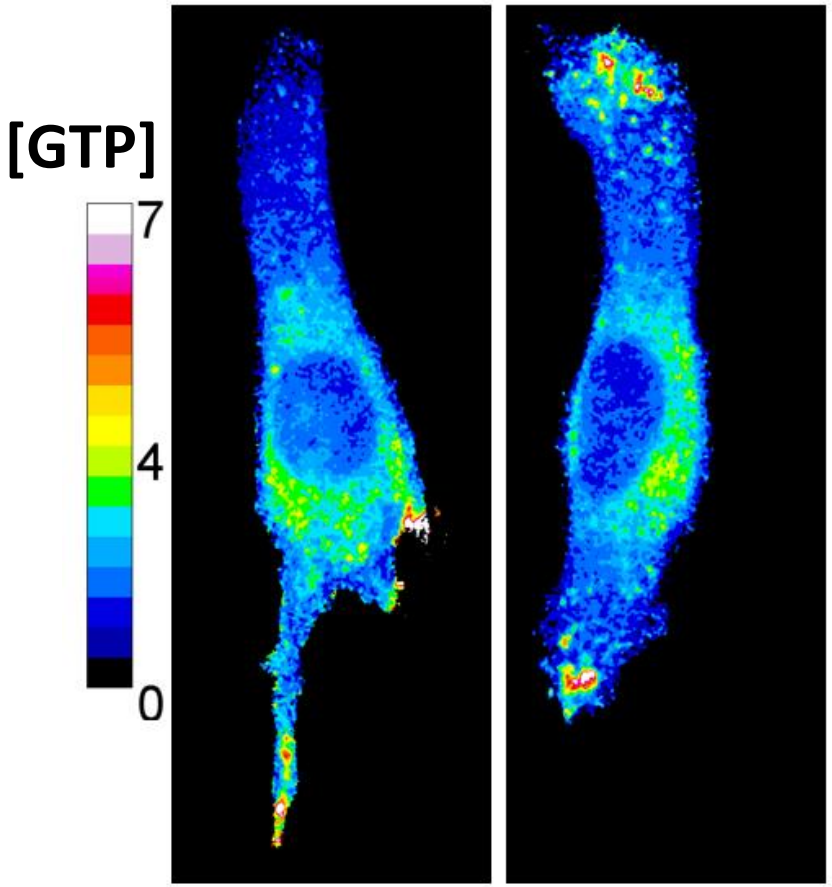




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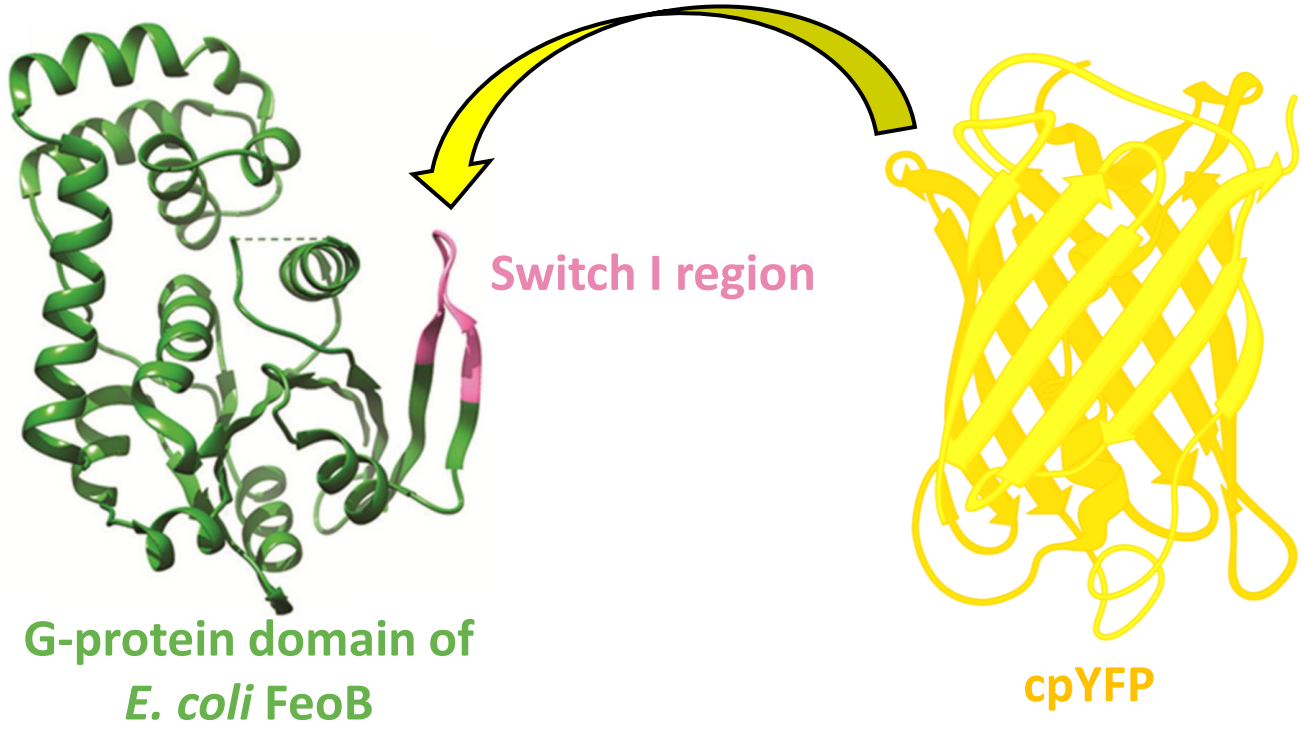
# GEVAL measures GTP distribution in melanoma cells




Anna Bianchi-Smiraglia, Ph.D.



Bianchi-Smiraglia, A., Rana, M., Foley, C. *et al.* Internally ratiometric fluorescent sensors for evaluation of intracellular GTP levels and distribution. *Nat Methods* **14**, 1003–1009 (2017). https://doi.org/10.1038/nmeth.4404

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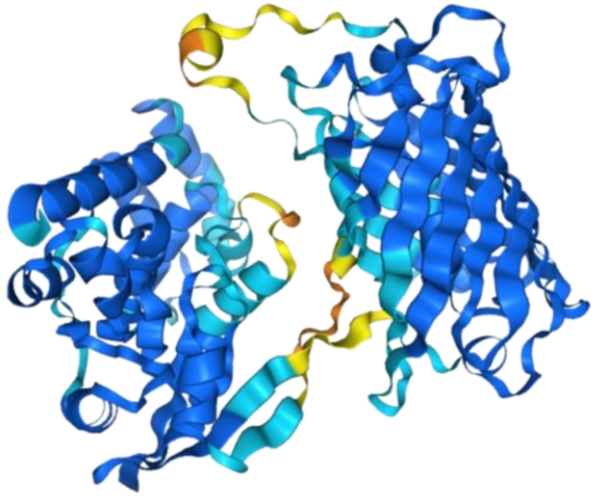

**GTP evaluators (GEVAL) structure**
**predicted by AlphaFold with confidence from high to low**



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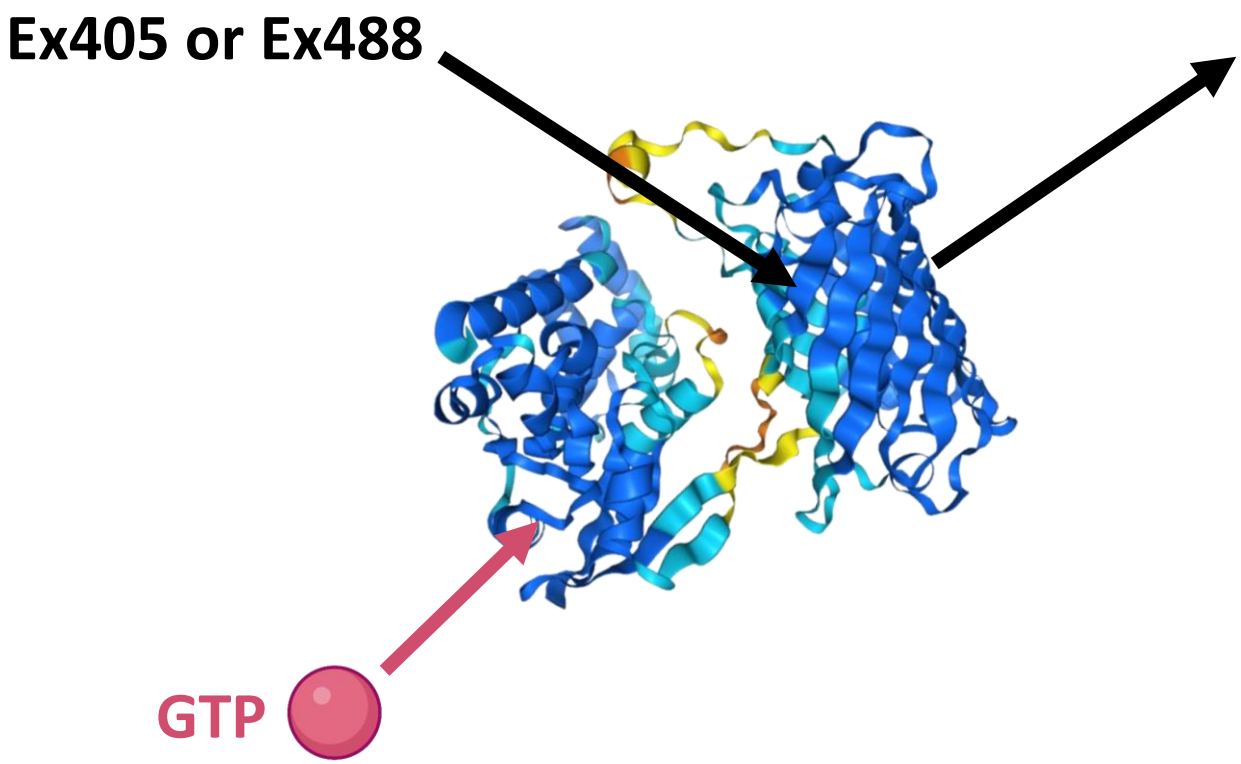


**Ratio between emissions from Ex405 & Ex488 proportional to local [GTP]**

**GTP evaluators (GEVAL) structure predicted by AlphaFold with confidence from high to low**



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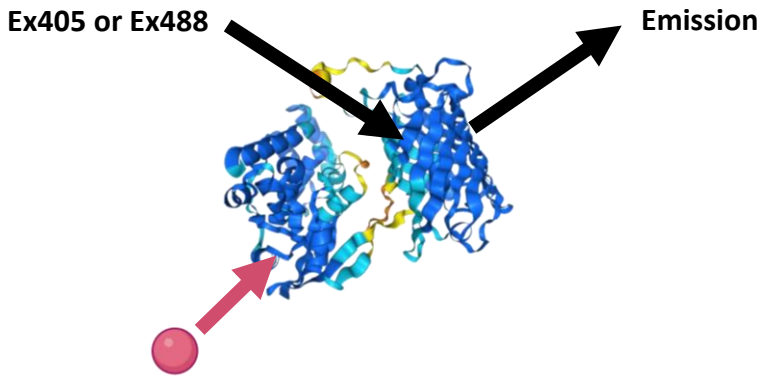
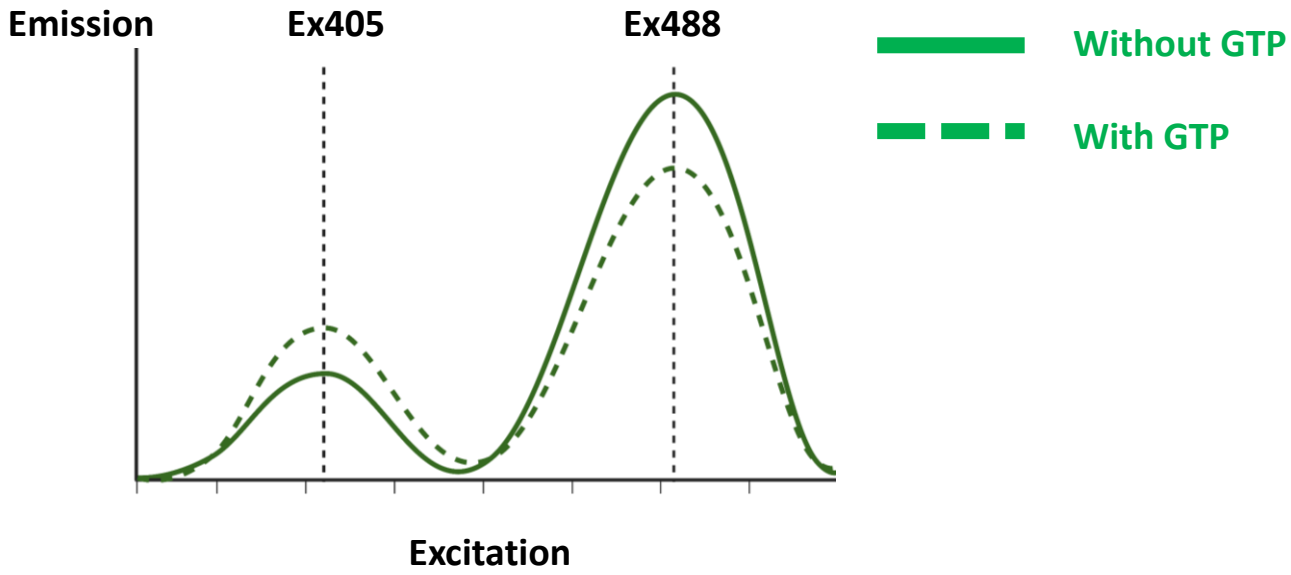
# GEVAL measures GTP distribution in melanoma cells

**GEVAL30**

**$K_{eff}$ = 32.3 µM**

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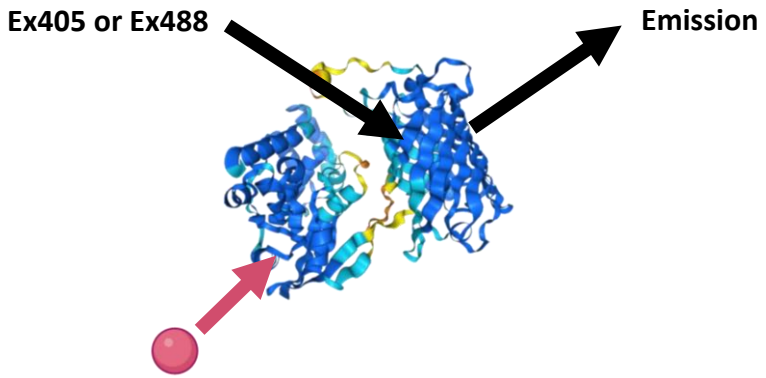
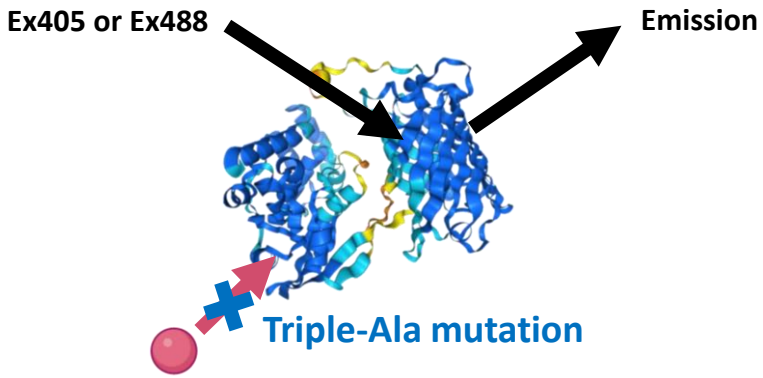
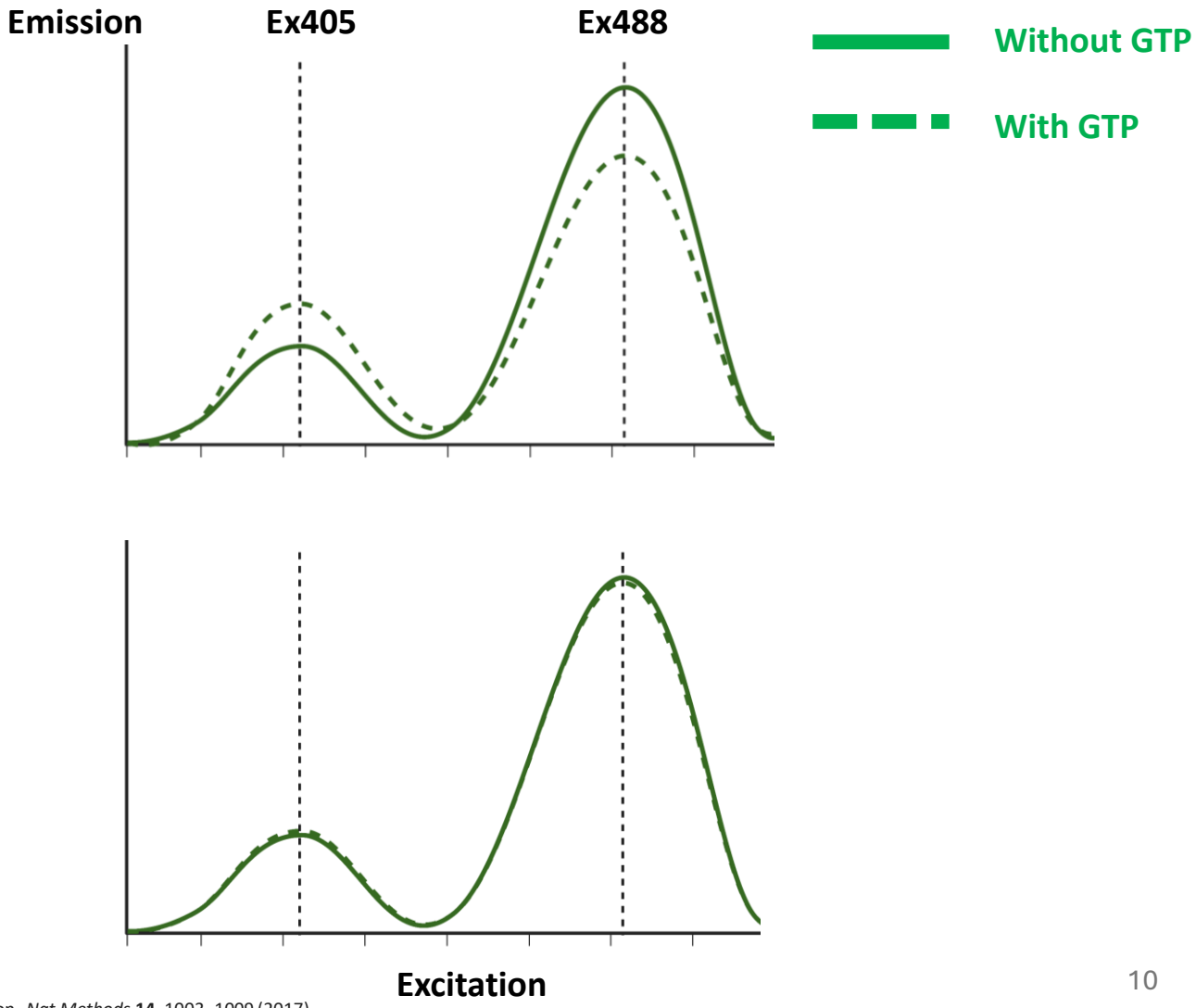



**GEVALNull**



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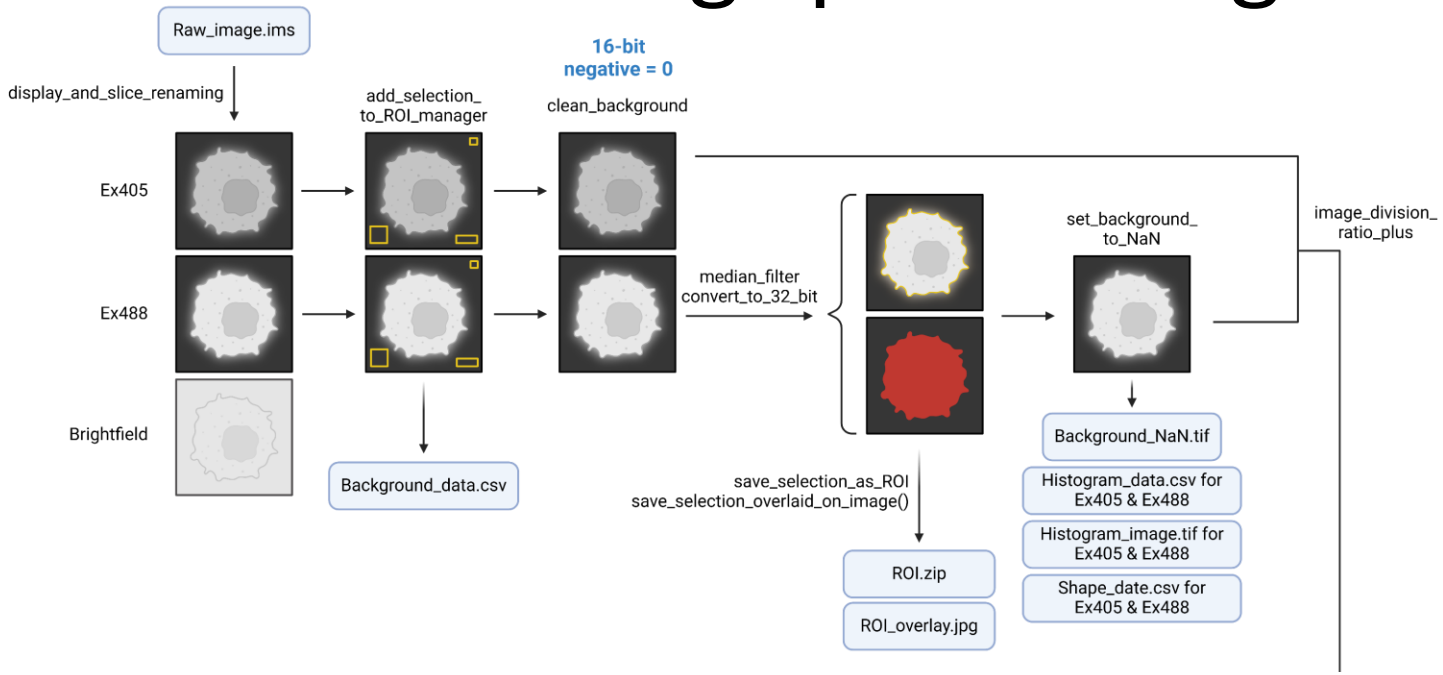
**Aim: use computational tools & AI to streamline GEVAL protocol & optimize analysis pipeline**

# GEVAL*Iris* accelerates image processing






https://github.com/junqilu/GEVALIris

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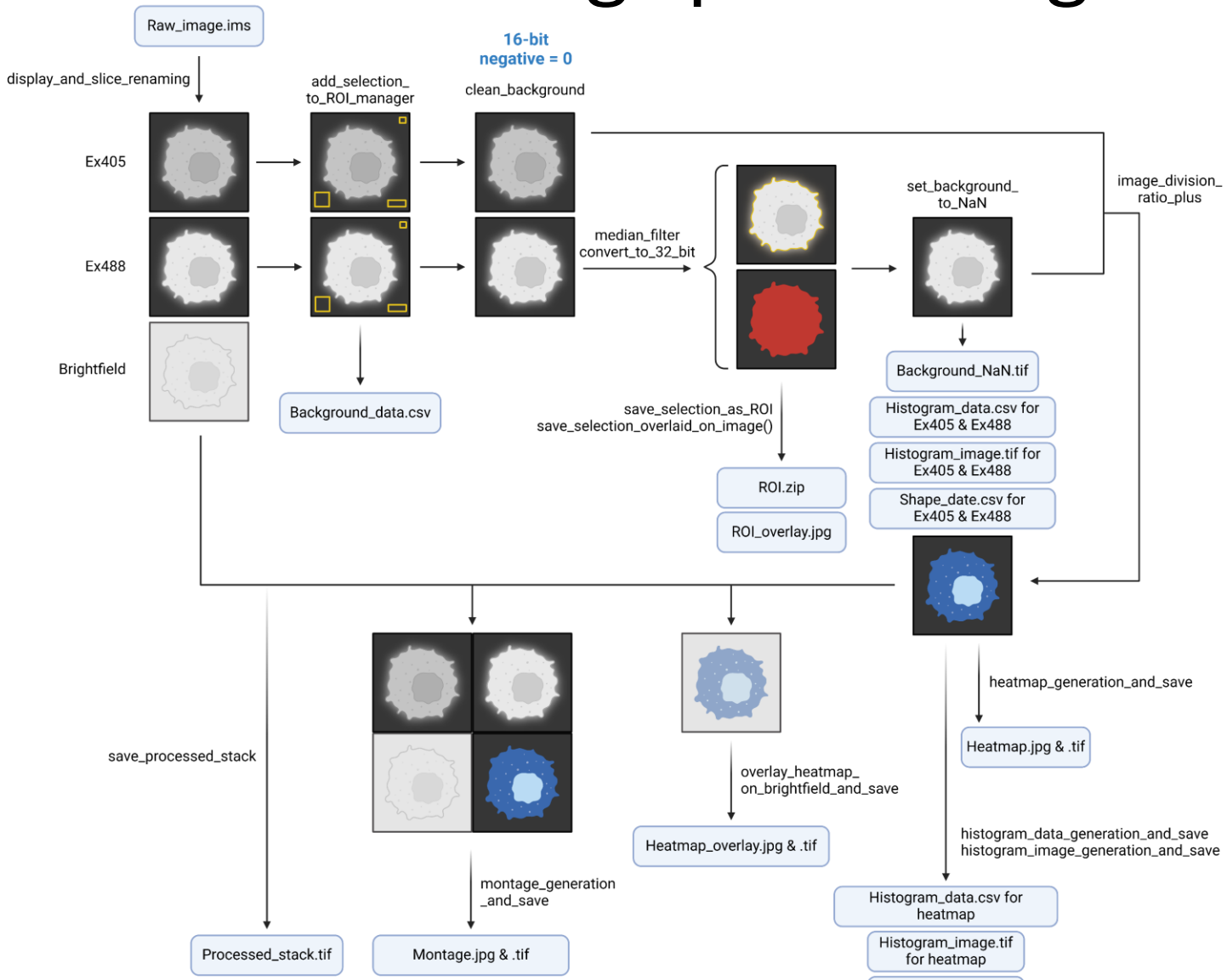


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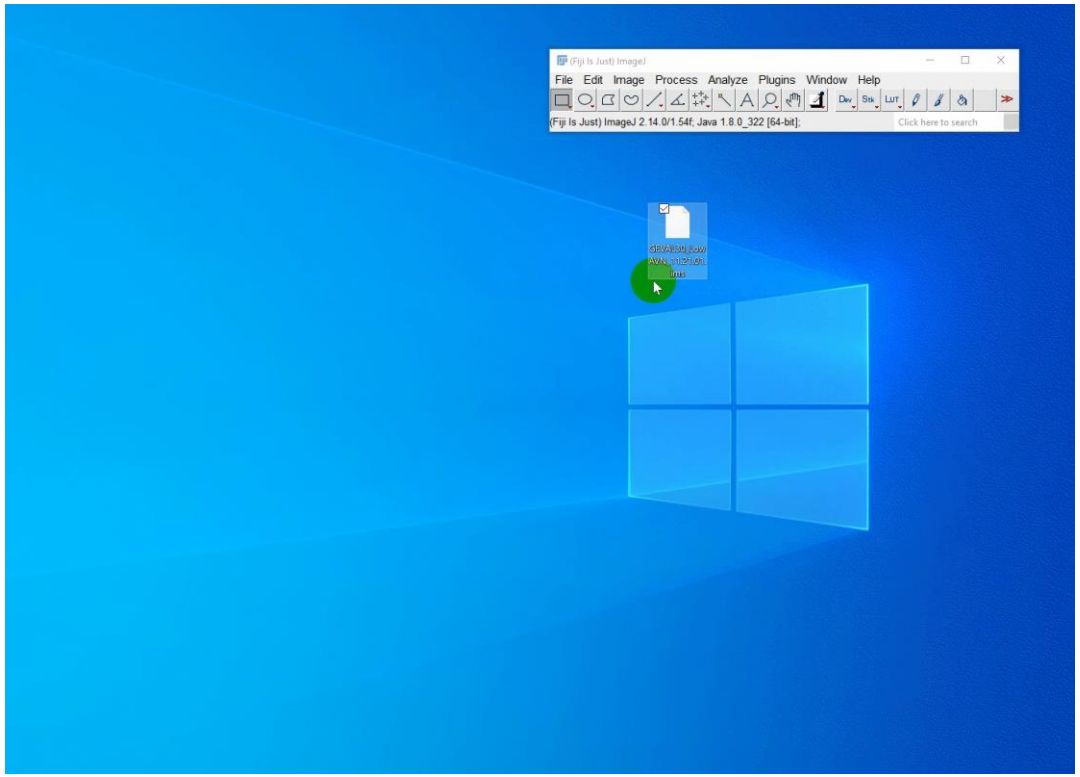
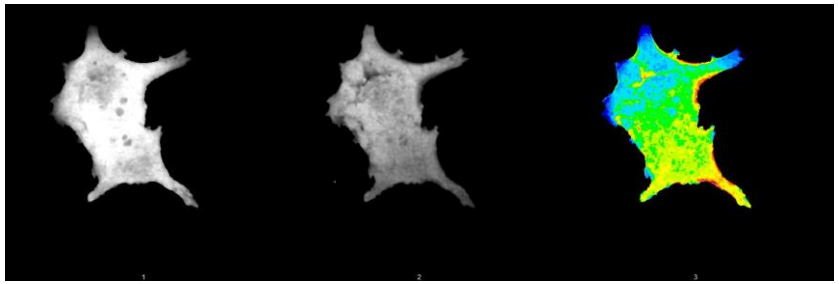
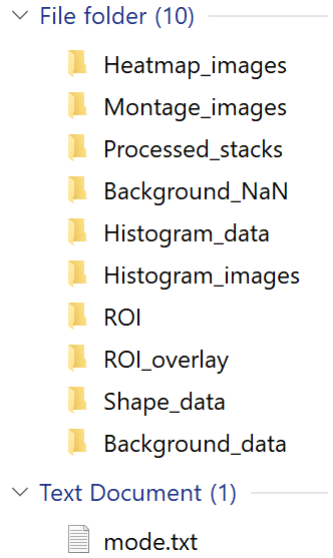
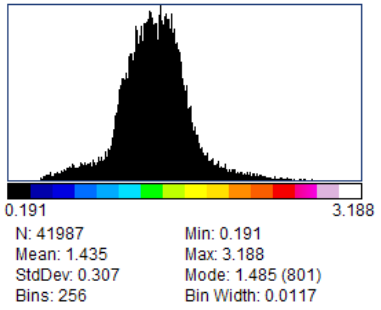



**https://youtu.be/aysFCivxEpw?t=1**





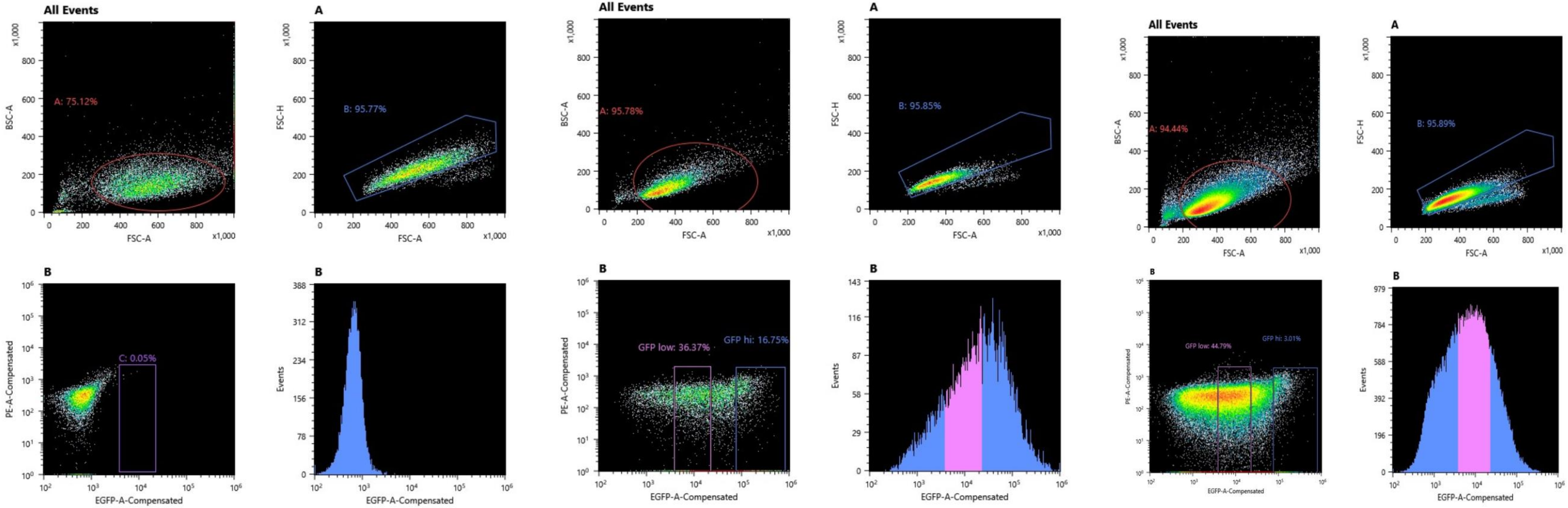

**Total 102,252 features**



https://github.com/junqilu/GEVALIris

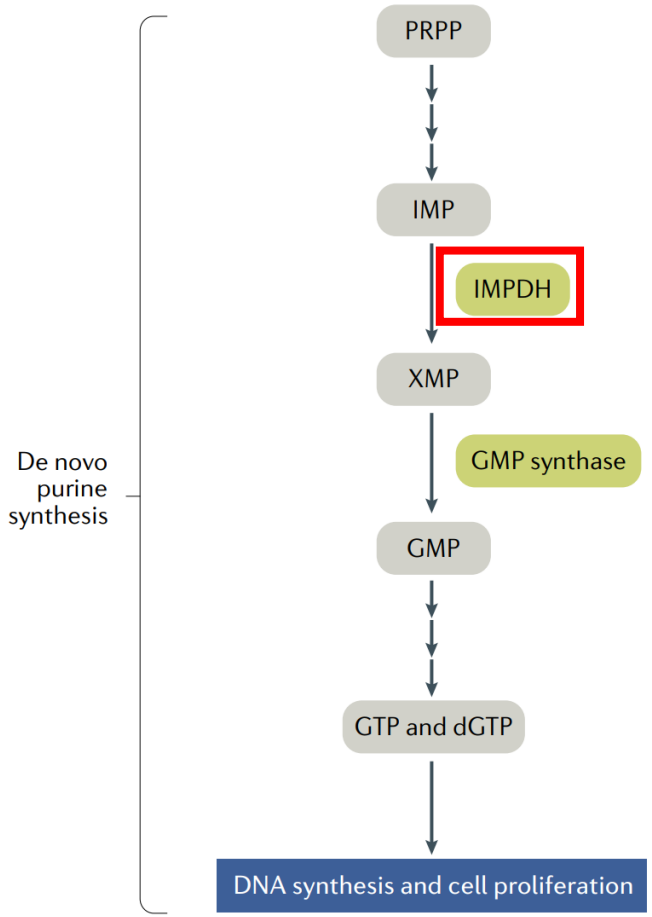
# Flowcytometry verified GEVALNull has higher mean GFP intensities

**Uninfected** | **GEVALNull** | **GEVAL30**




**GEVALNull population is brighter than GEVAL30**

# IMPDH is major GTP biosynthesis enzyme




Broen, J. C. A., & van Laar, J. M. (2020). Mycophenolate mofetil, azathioprine and tacrolimus: mechanisms in rheumatology. *Nature reviews. Rheumatology*, *16*(3), 167–178. https://doi.org/10.1038/s41584-020-0374-8
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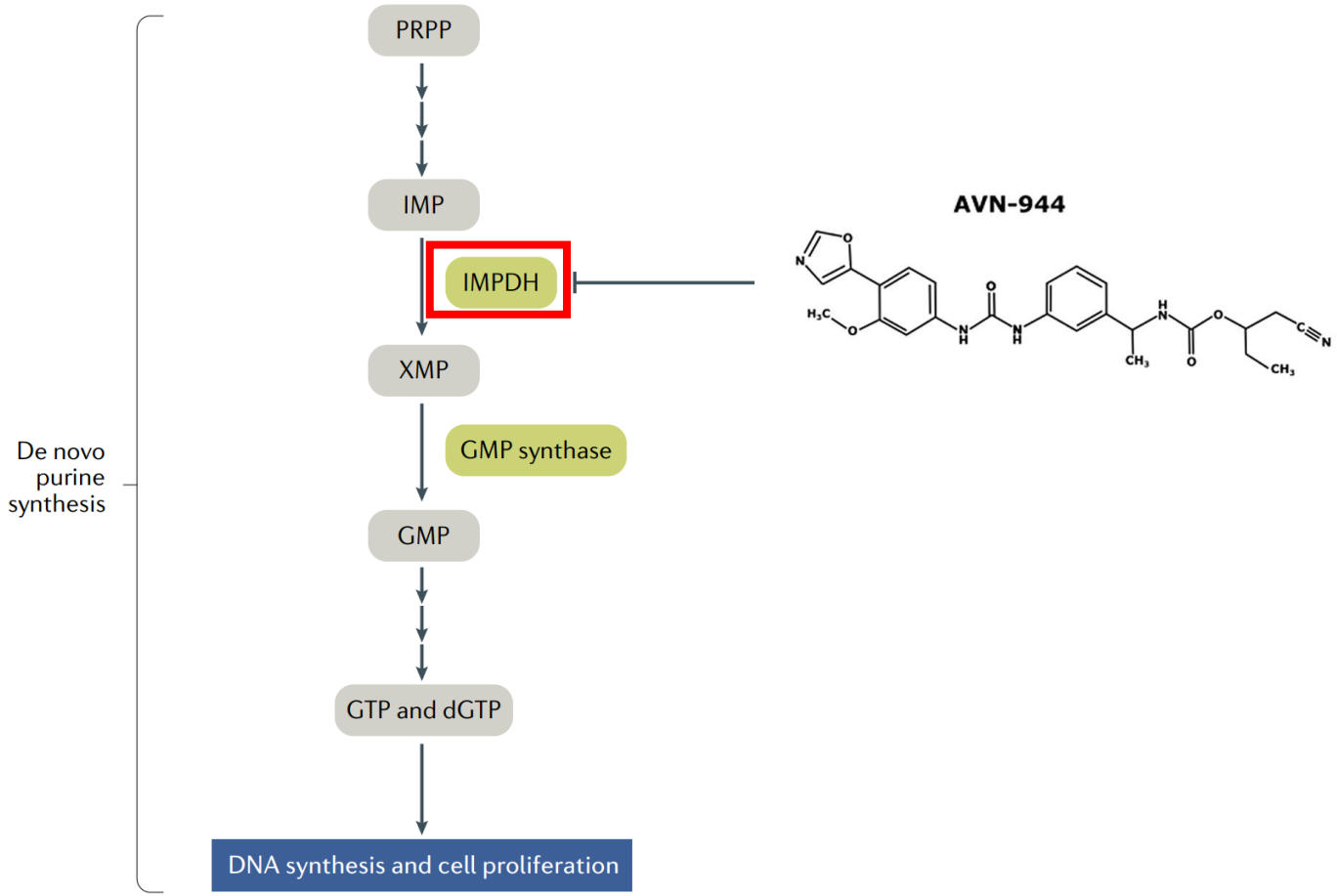
# MPA & AVN are IMPDH inhibitors

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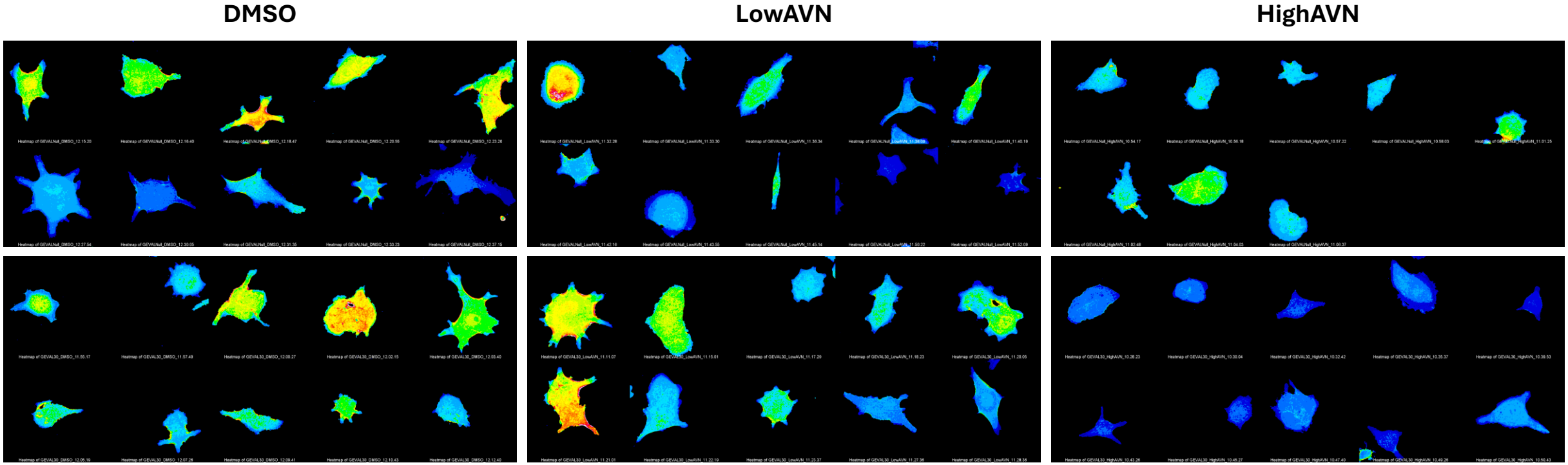
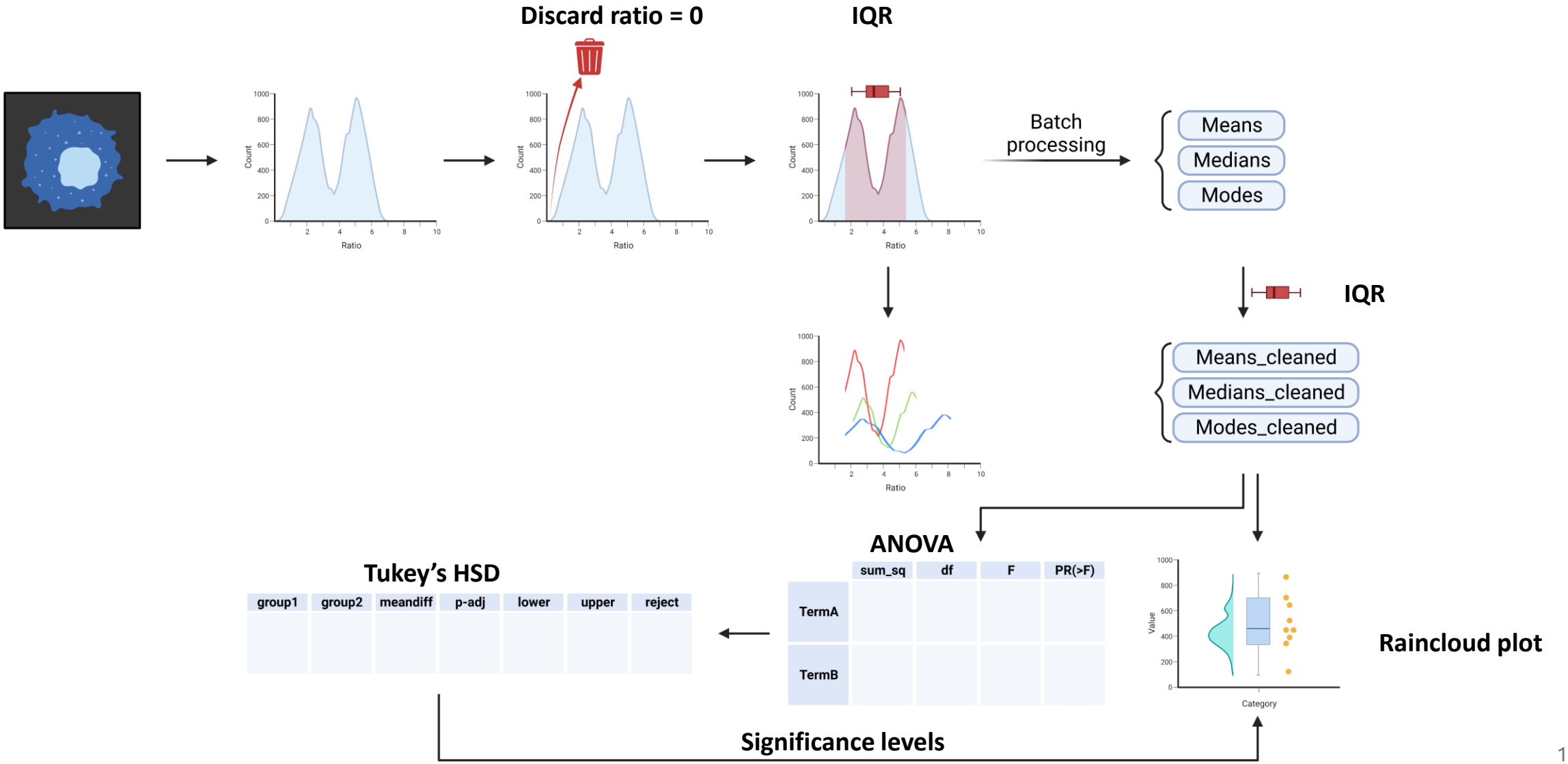
# AVN treatment doesn’t alter cell morphology




Color range normalized to [1E-4, 2.5]

**Low AVN = 0.025 µM**
**High AVN = 0.05 µM**

# GEVALytics heatmap data analysis pipeline



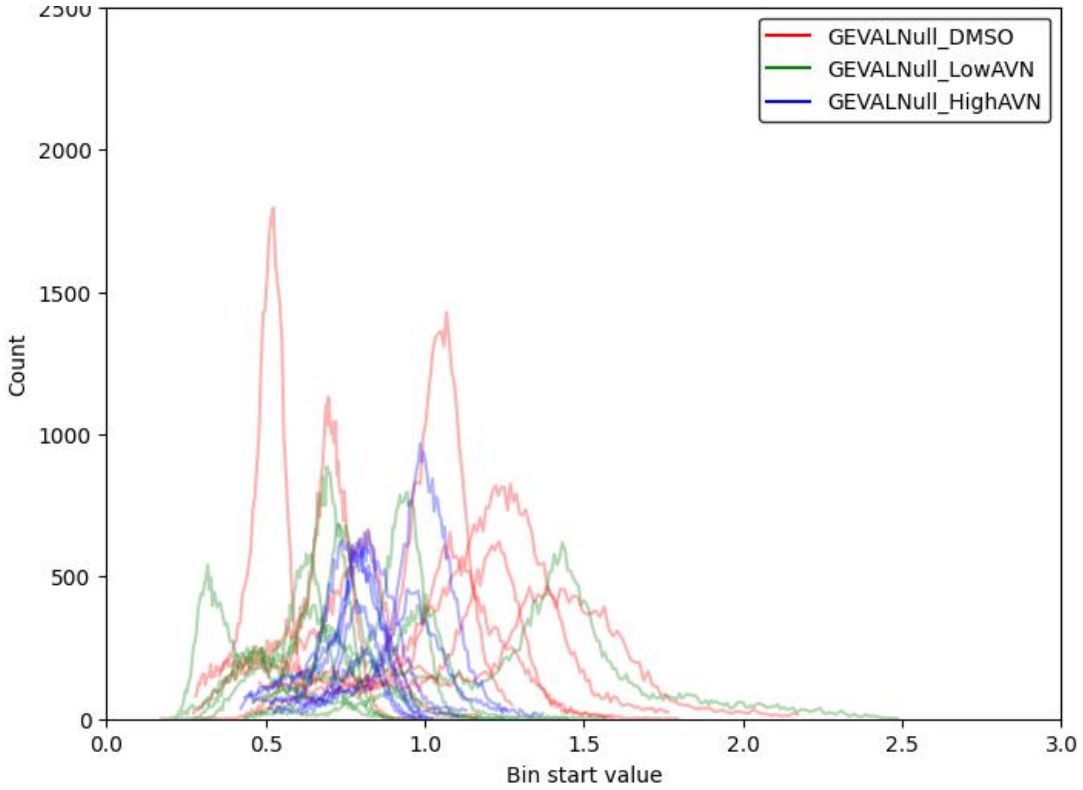



https://github.com/junqilu/GEVALytics

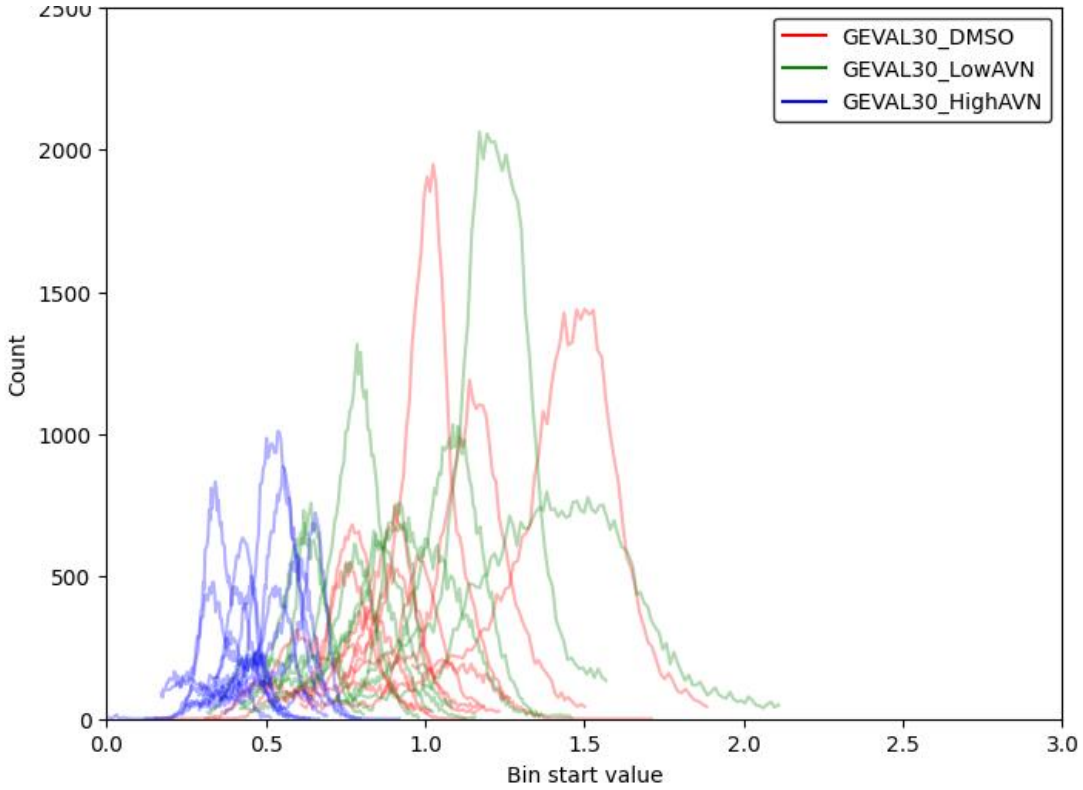
# AVN decreases GEVAL signals in GEVAL30

**Pooled histograms**

**GEVALNull**



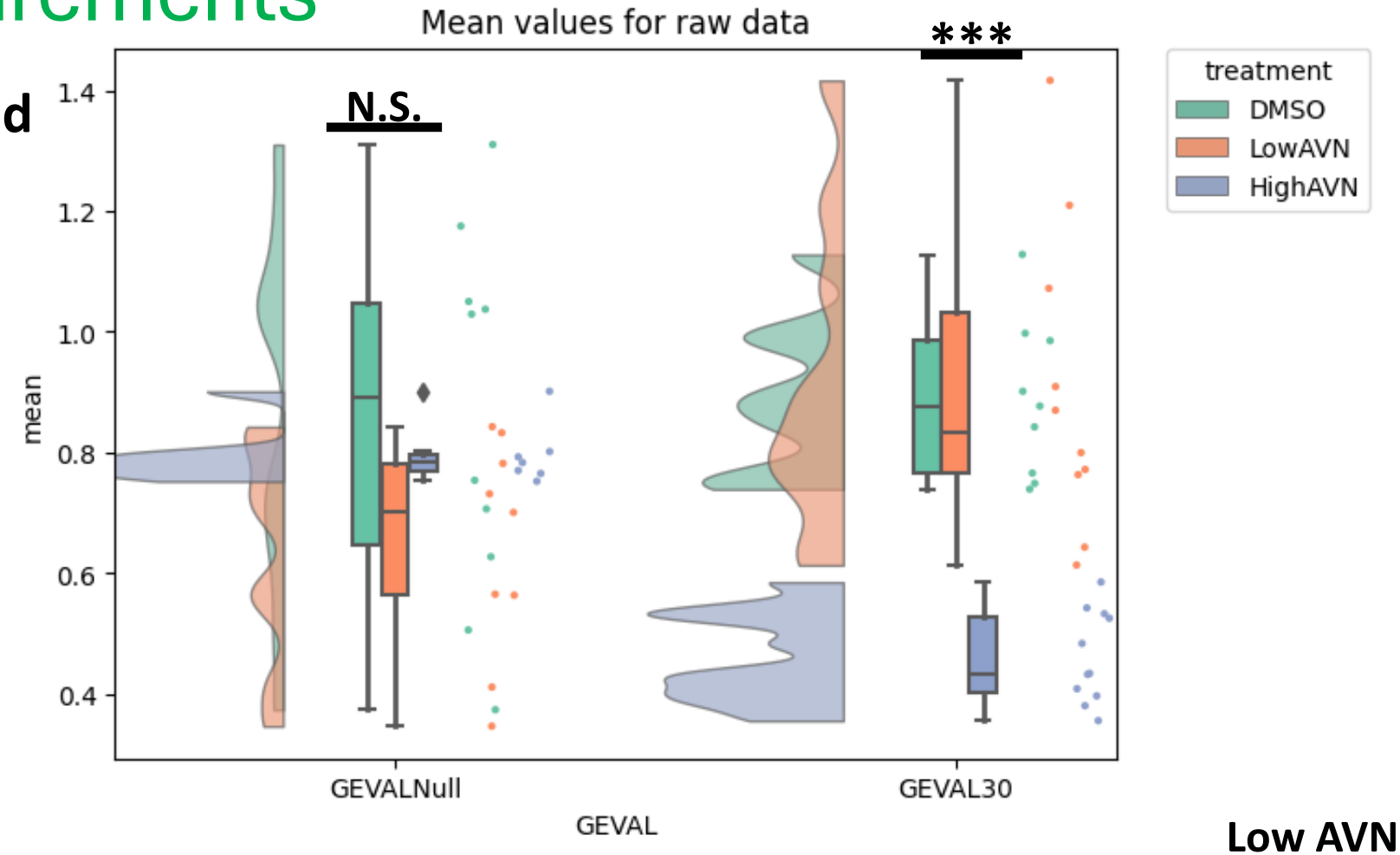

**GEVAL30**




**Low AVN = 0.025 µM**
**High AVN = 0.05 µM**

# GEVALNull not responsive to AVN treatment while higher AVN significantly dropped GEVAL30 measurements

**Pooled raincloud plot**

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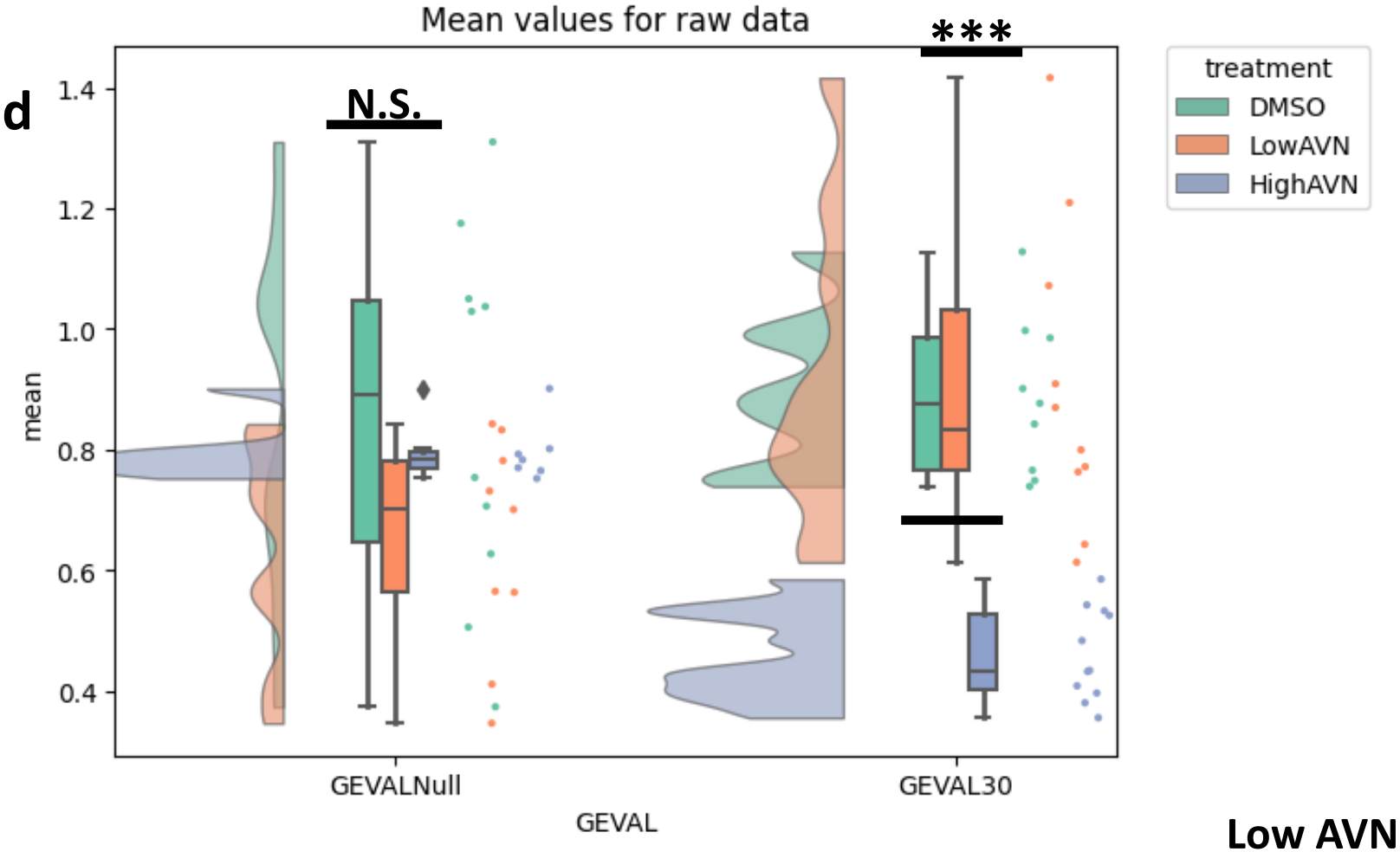
# Large variance may indicate suboptimal GEVAL performance

**Pooled raincloud plot**

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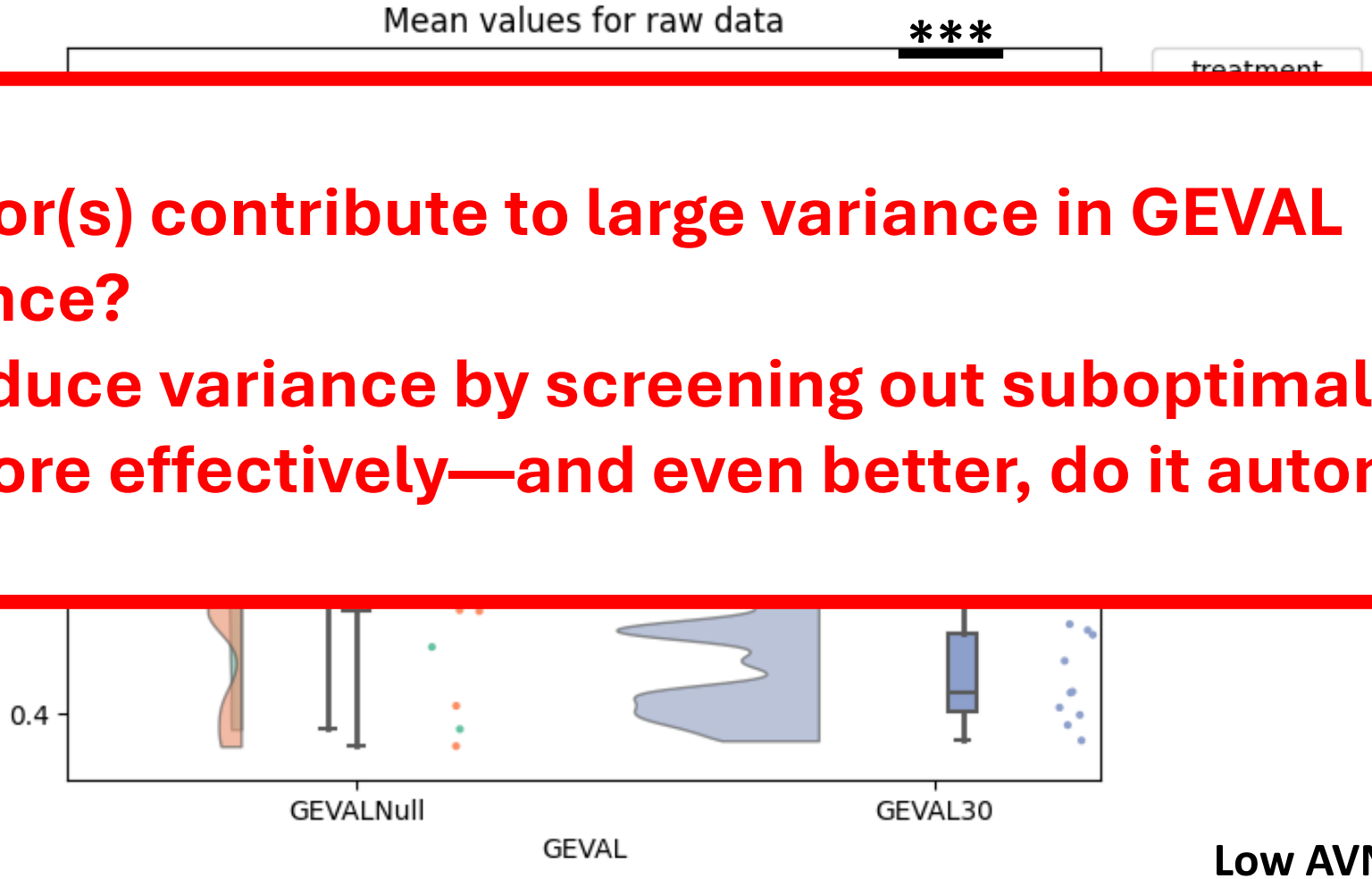
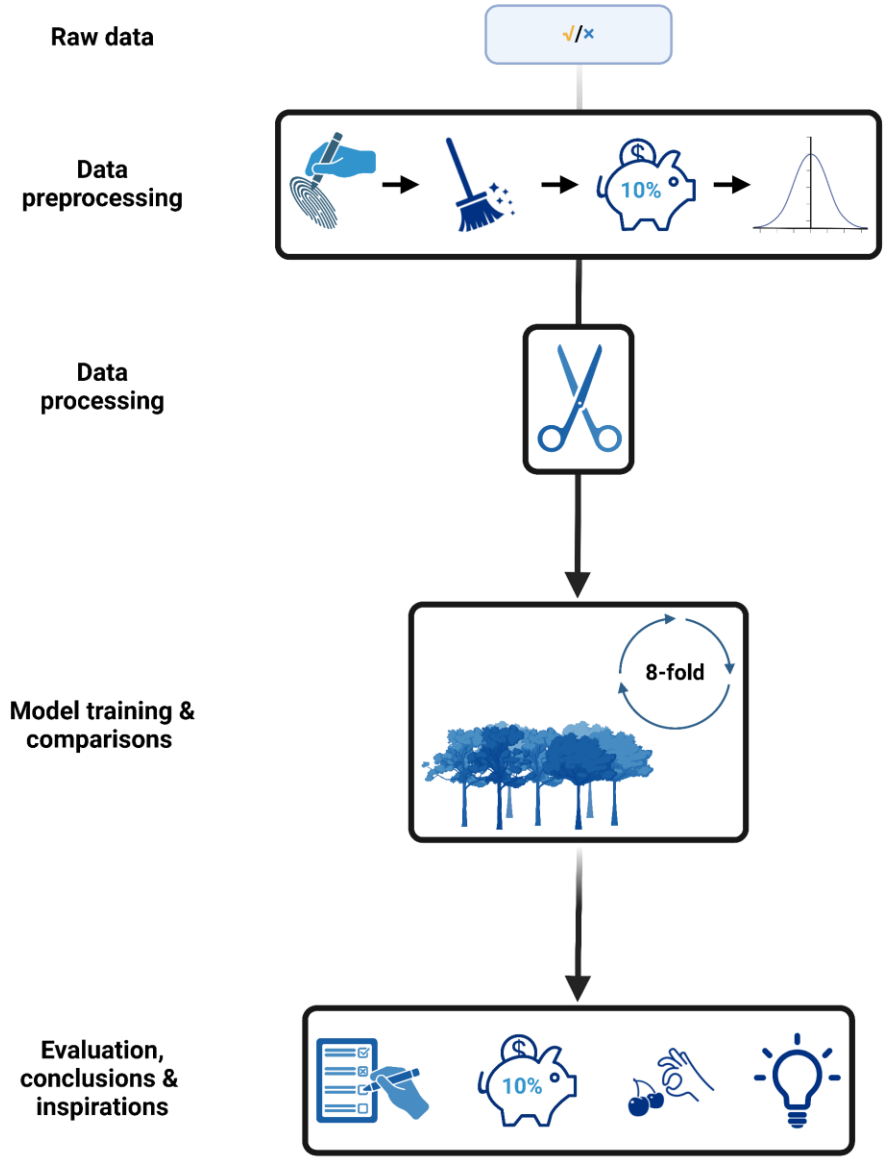



- **What factor(s) contribute to large variance in GEVAL performance?**
- **How to reduce variance by screening out suboptimal GEVAL images more effectively—and even better, do it automatically?**

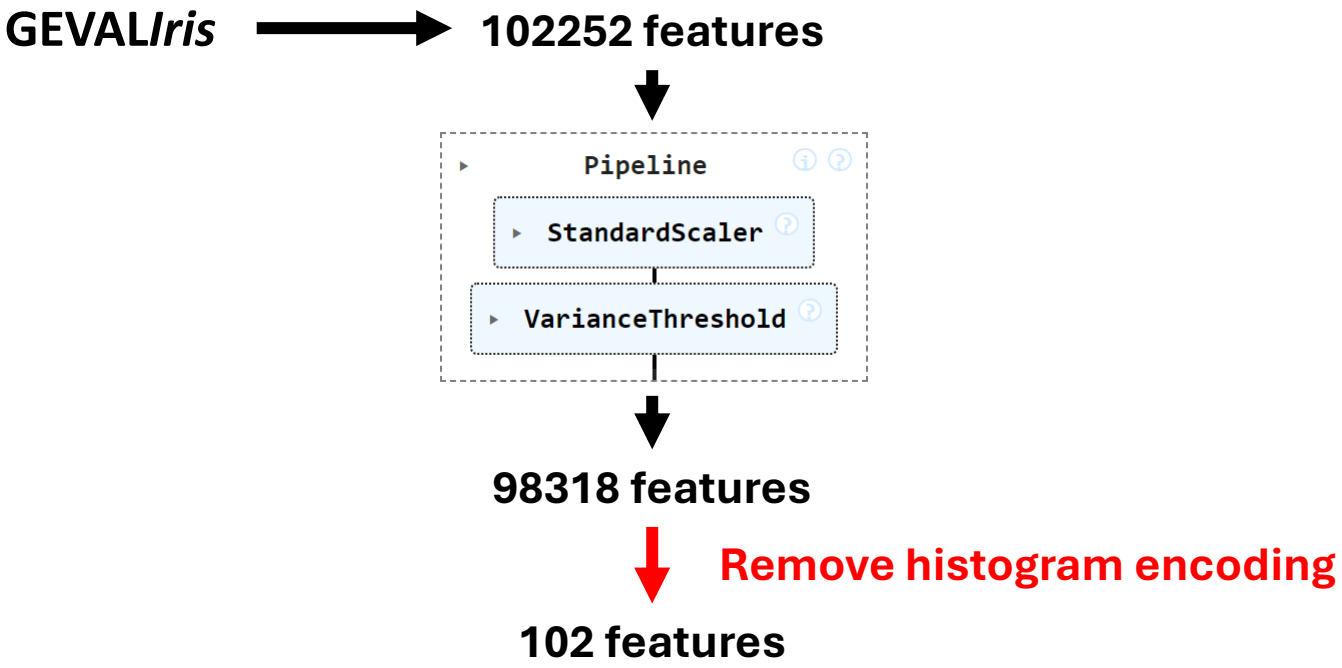
**Low AVN = 0.025 µM**
**High AVN = 0.05 µM**

# GEVALytics AI workflow




https://github.com/junqilu/GEVALytics

# Feature selection to reduce dimensionality



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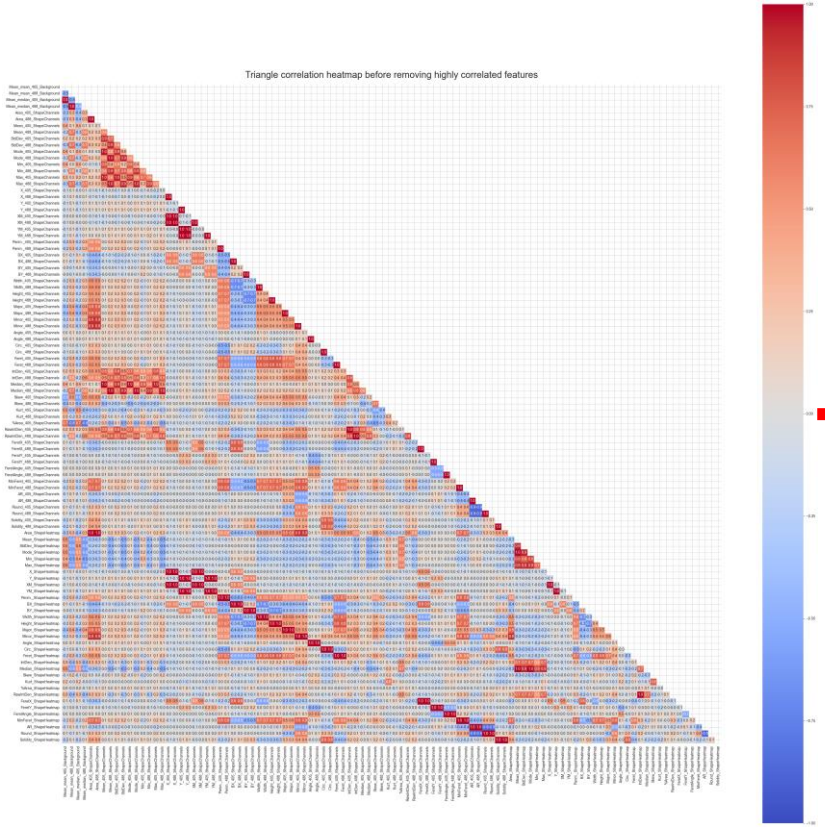
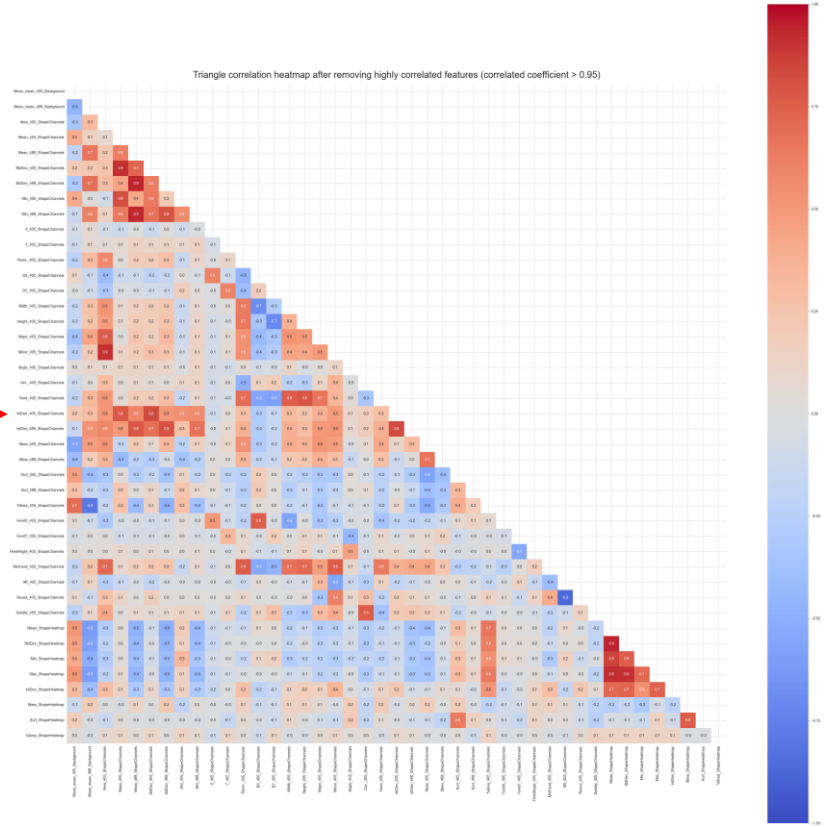
# Feature selection to reduce dimensionality

**102 features**




**Remove features with correlation coefficients ≥ 0.95**

**43 features**

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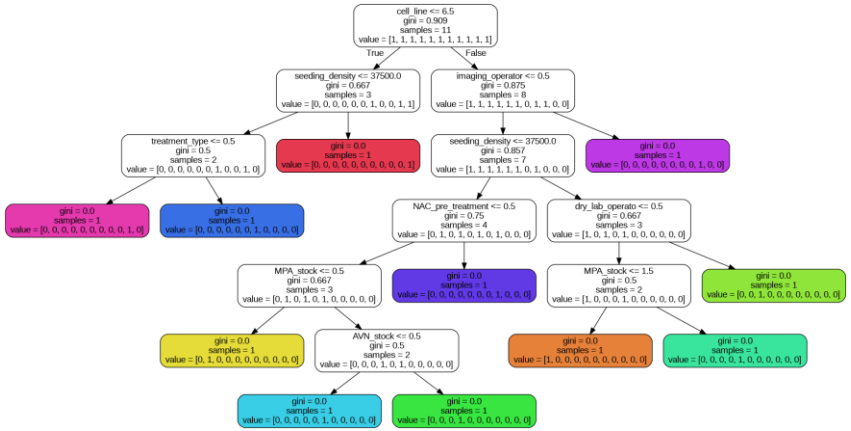
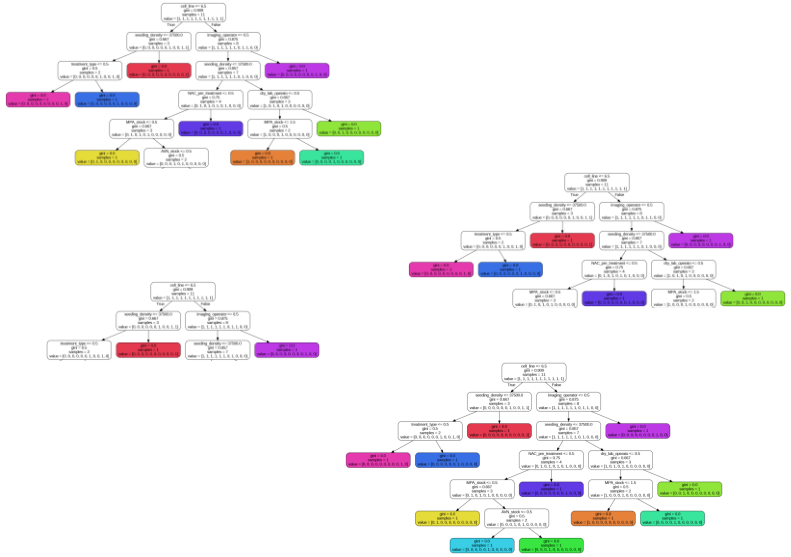
# Random forest (RF) classifier training & best model



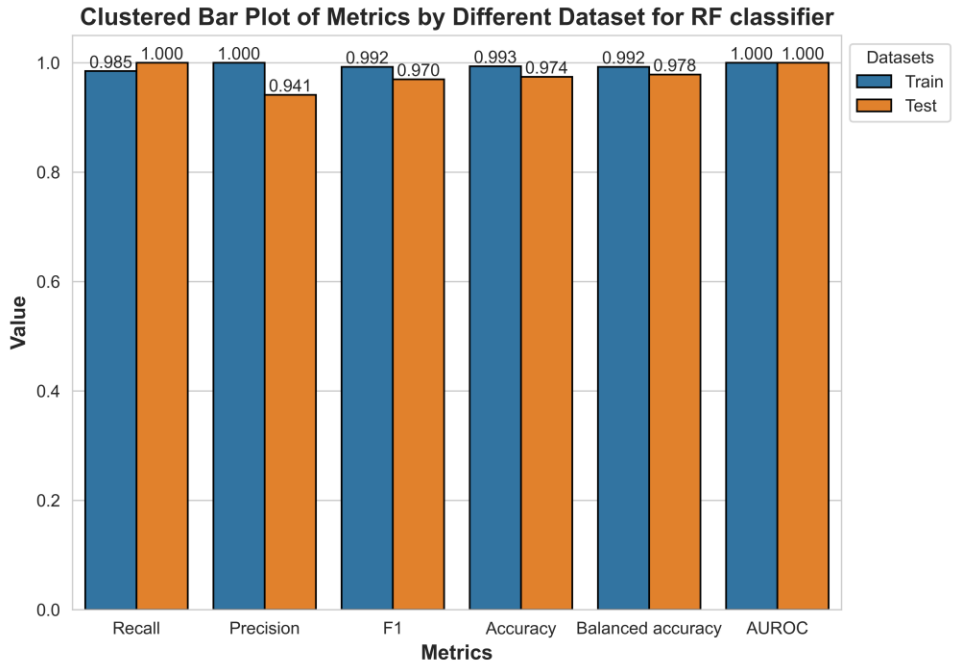



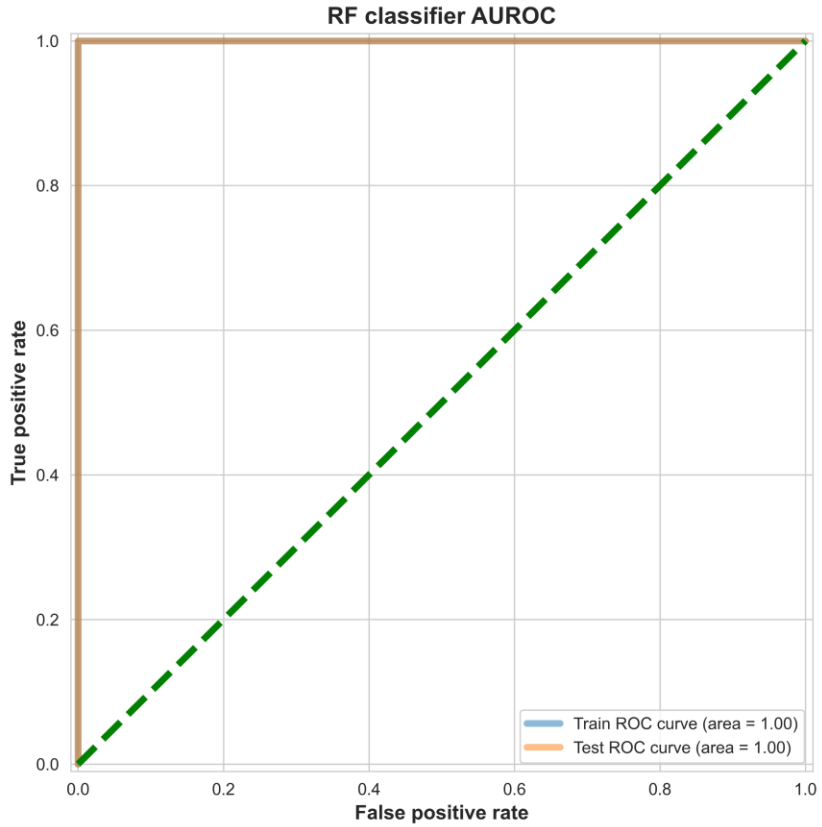
**Best RF classifier has 1000 decision trees**



https://github.com/junqilu/GEVALytics

# Best RF classifier is highly reliable & less likely to overfit

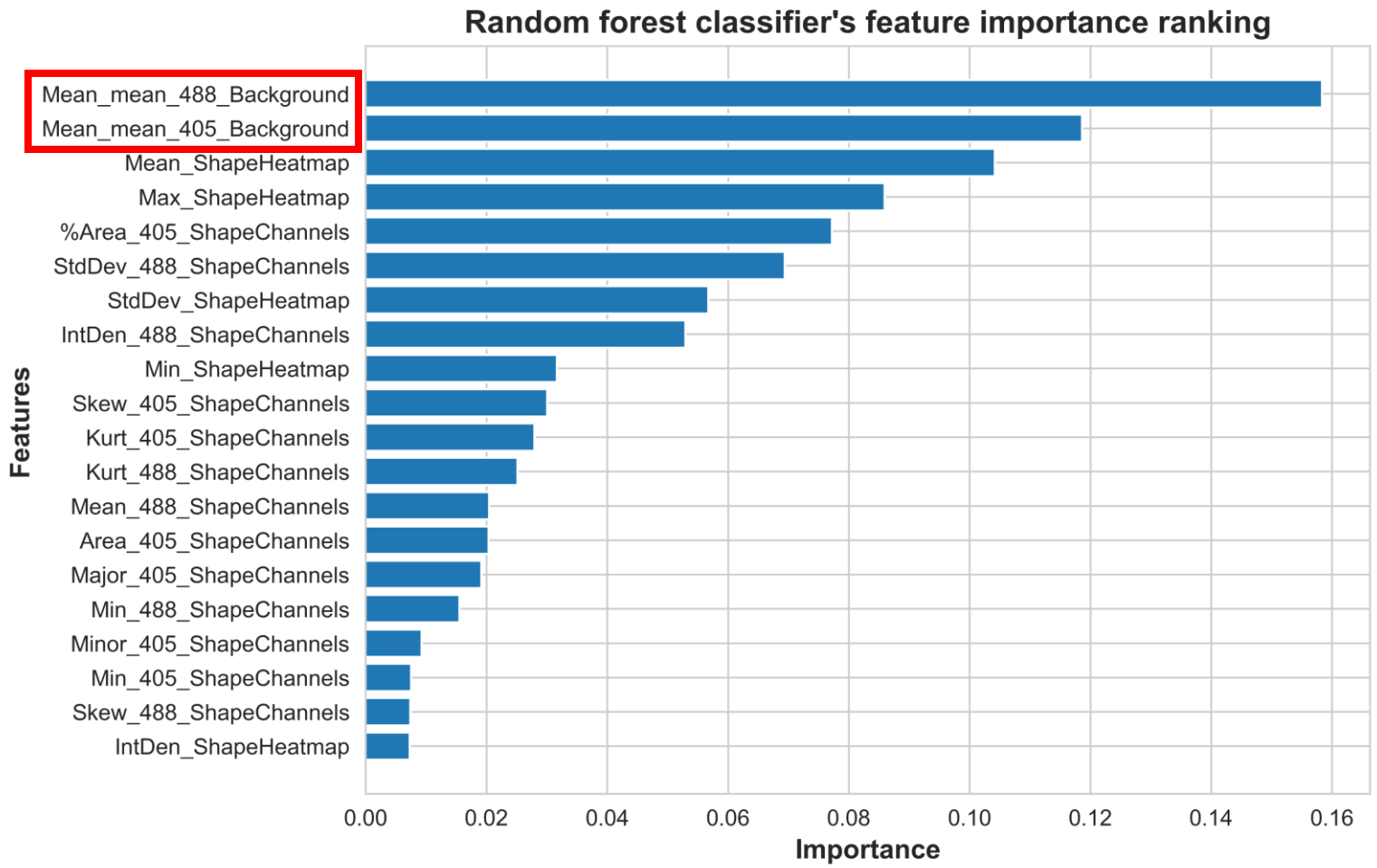





https://github.com/junqilu/GEVALytics

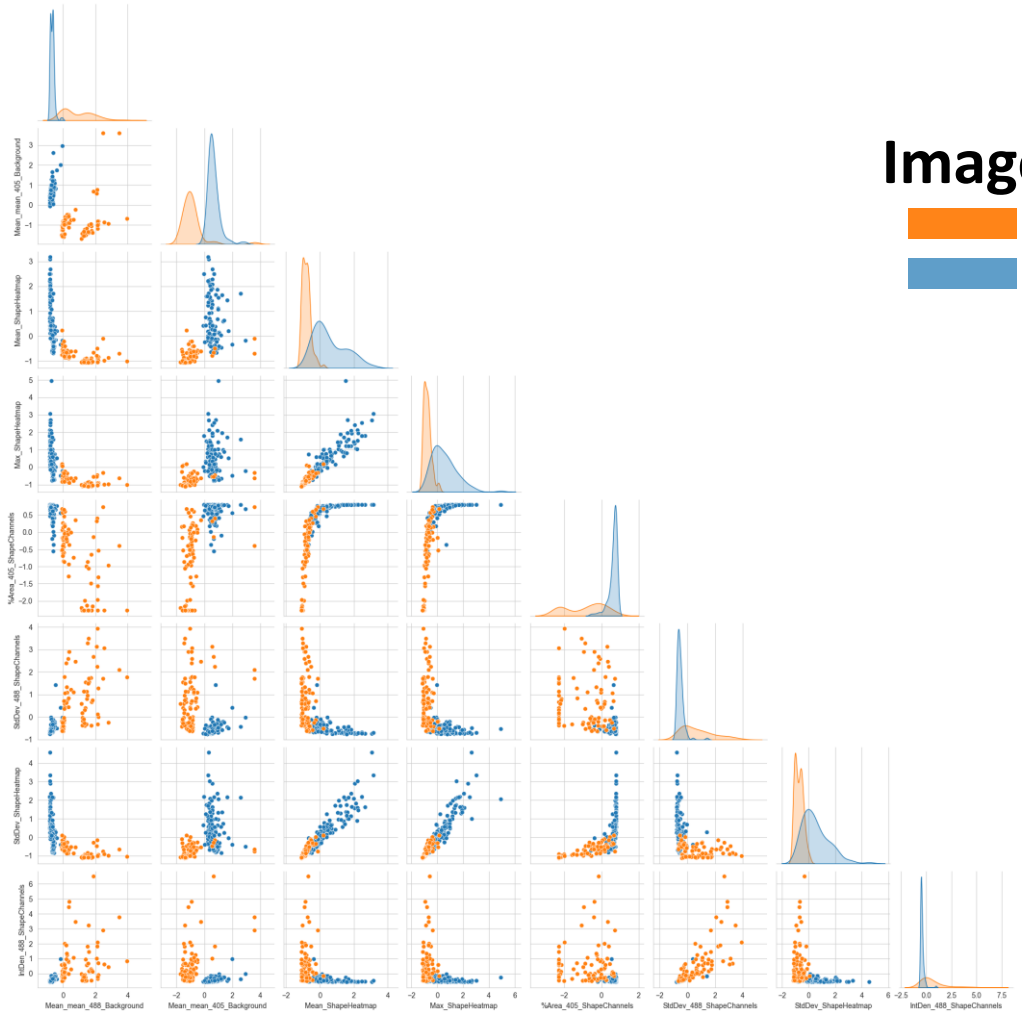
# Best RF classifier identifies laser settings as most important features contributing to large variance






https://github.com/junqilu/GEVALytics

# Most important features to distinguish aren't highly correlated = no leftover data structure to model




**Images**

- Optimal
- Suboptimal



https://github.com/junqilu/GEVALytics

# GEVAL*Iris* + GEVALytics = automatic screening eyes with expertise

- RF classifier highly trustworthy & generalizes very well
  - Optimal GEVAL performance requires fine-tuning on laser settings
  - Automatically screens out suboptimal images for future GEVAL users, **anytime & anywhere**
- Potential future directions
  - Bigger image set to avoid curse of dimensionality
  - Correlation ≠ causation so need controlled experiments to determine

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