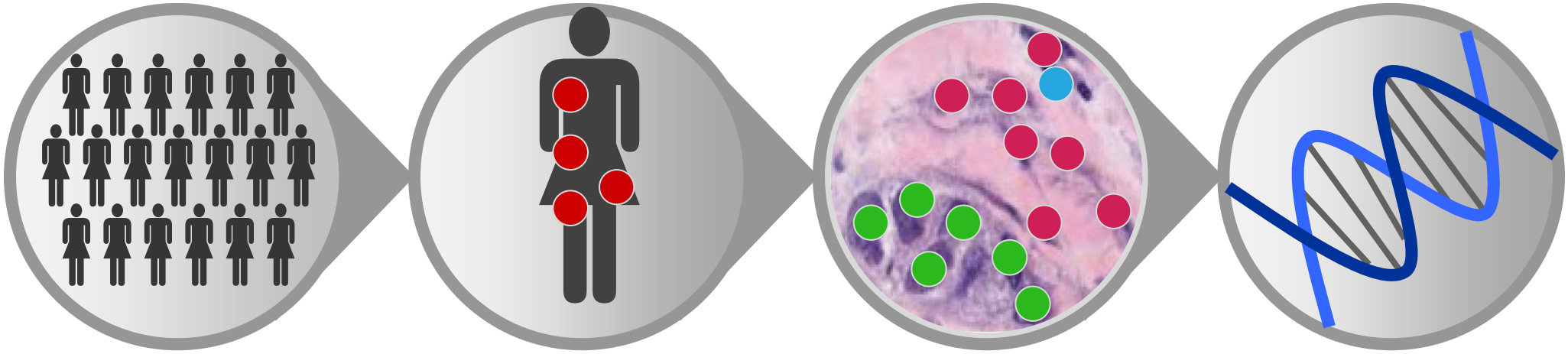
Dissecting cancer **heterogeneity**

Population > patient > tissue > genome

Florian Markowetz
CRUK Cambridge Institute
www.markowetzlab.org

Heterogeneity in cancer



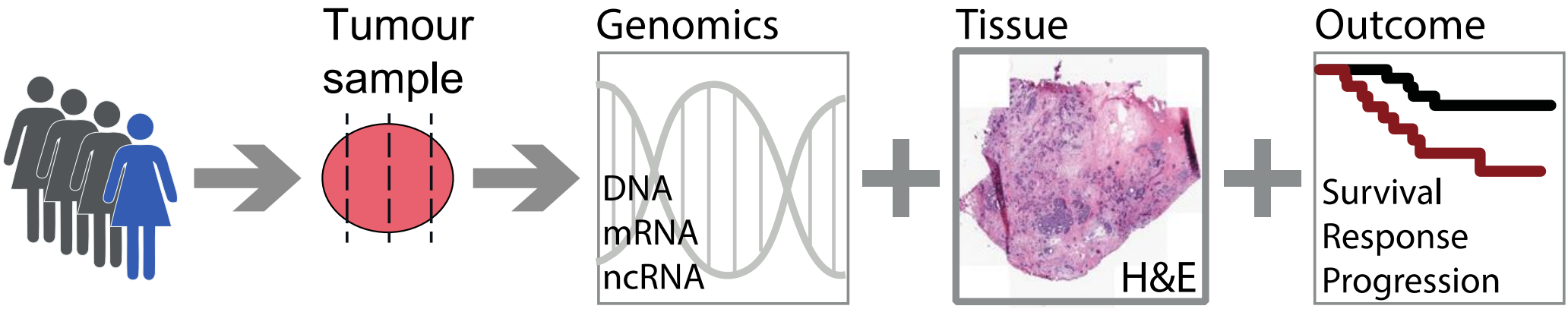
Inter-patient
population
subtypes

Intra-patient
spatial,
temporal

Intra-tumor
tissue

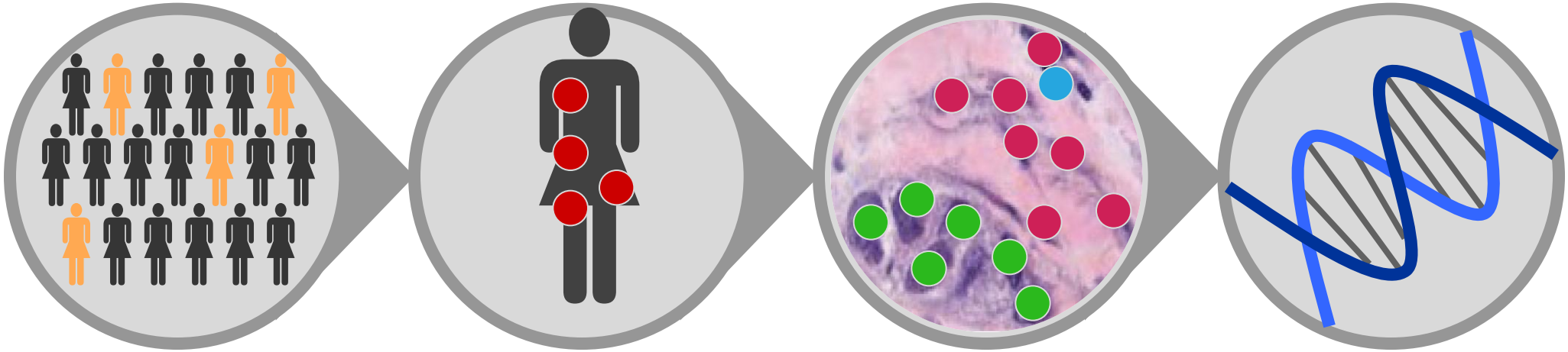
Intra-tumor
genetic

Systems Genetics of Cancer



- What are prognostic subtypes of cancer?
- Which genetic events drive tumour development?
- What are markers to predict disease progression?

Population heterogeneity



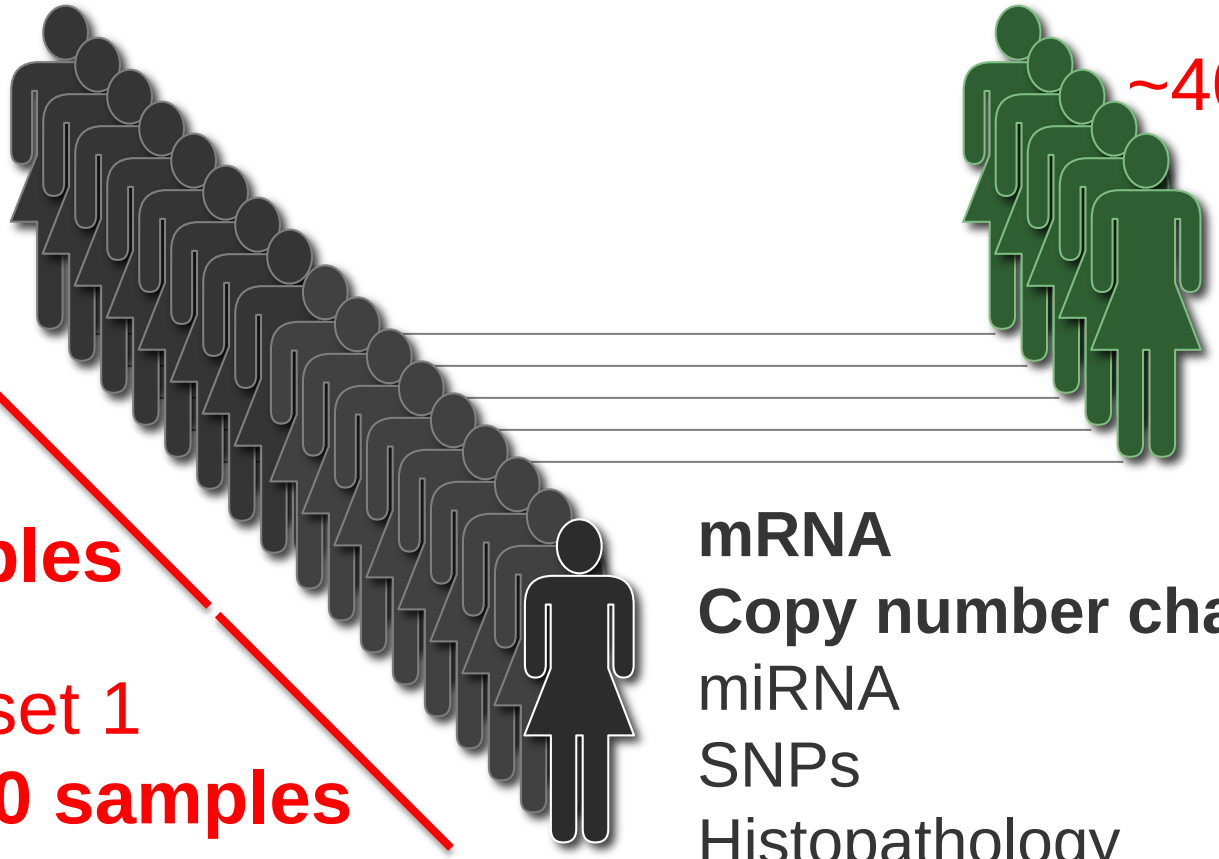
Curtis *et al*, Nature 201

ARTICLE

doi:10.1038/nature10983

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups

METABRIC – genomic and transcriptional landscape of breast cancer



~400 paired normals

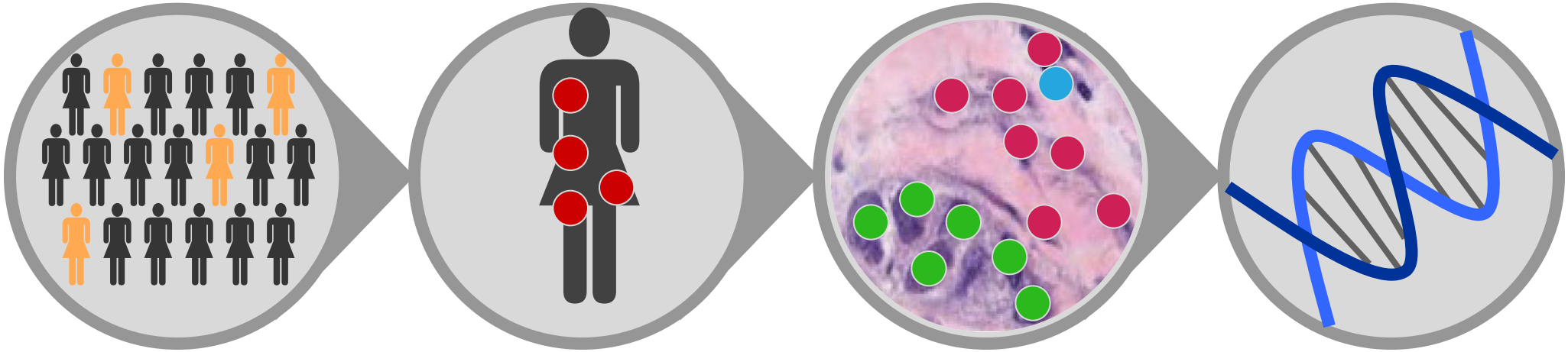
Dataset 2
~1000 samples

Dataset 1
~1000 samples

mRNA
Copy number changes
miRNA
SNPs
Histopathology
Clinical information

https://www.ebi.ac.uk/ega/studies/**EGAS00000000083**

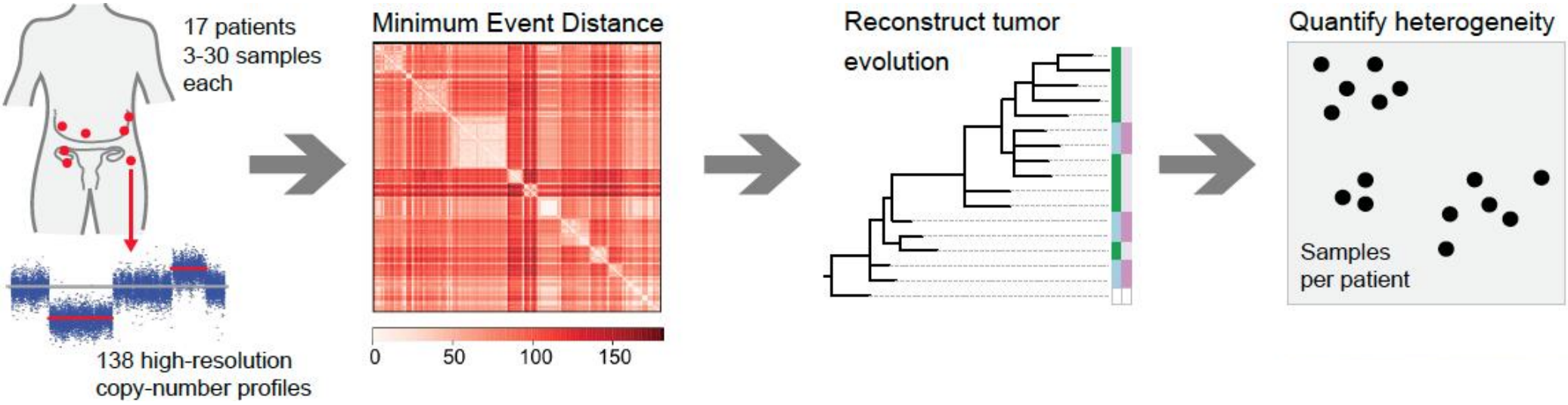
Intra-patient heterogeneity



Schwarz *et al*, submitted

Spatial and temporal heterogeneity in ovarian cancer predicts survival

Intra-patient heterogeneity in HGSOC

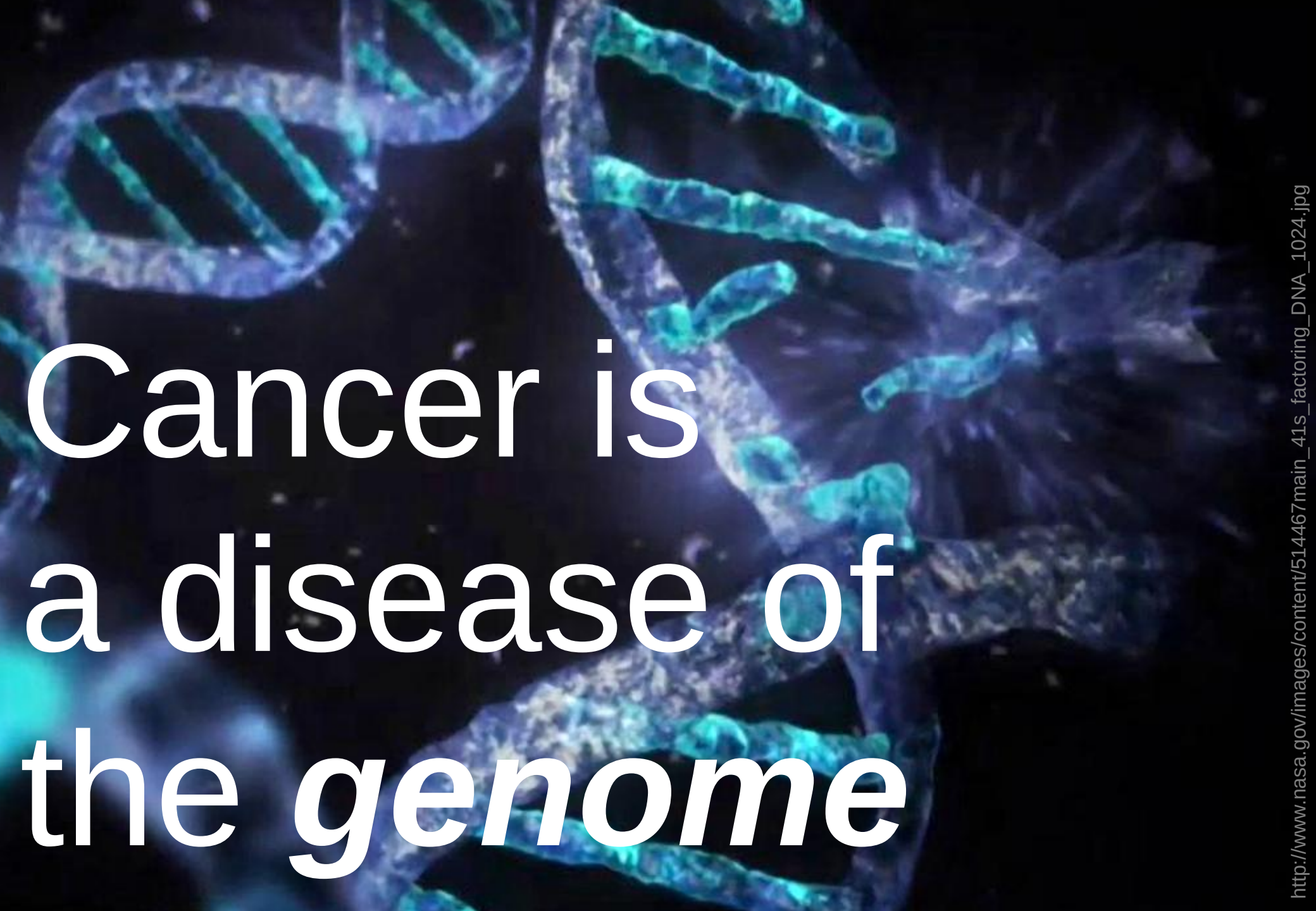


HGSOC

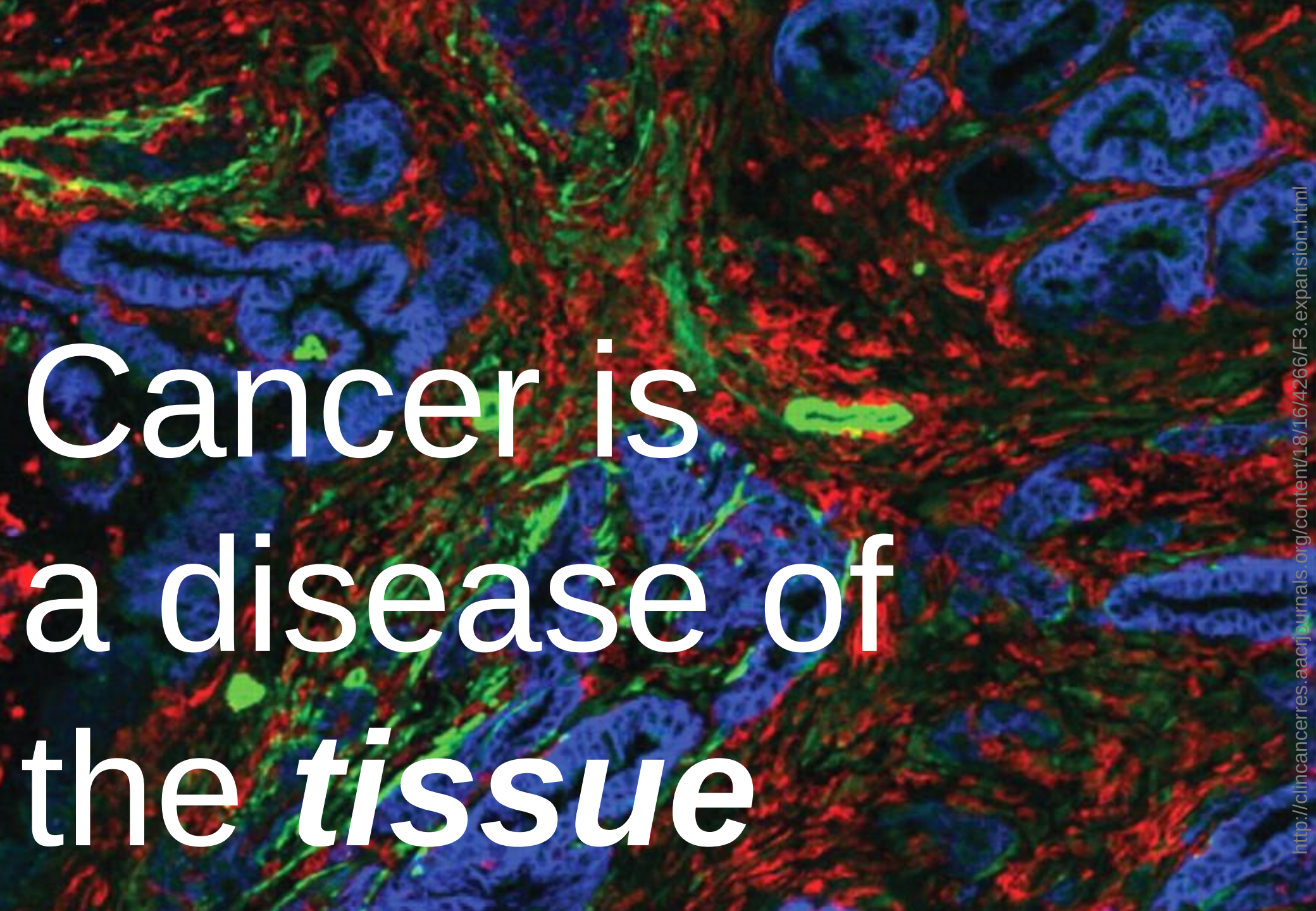
- Multiple metastases
- Good initial response
- Often resistant relapse
- Genomic rearrangements

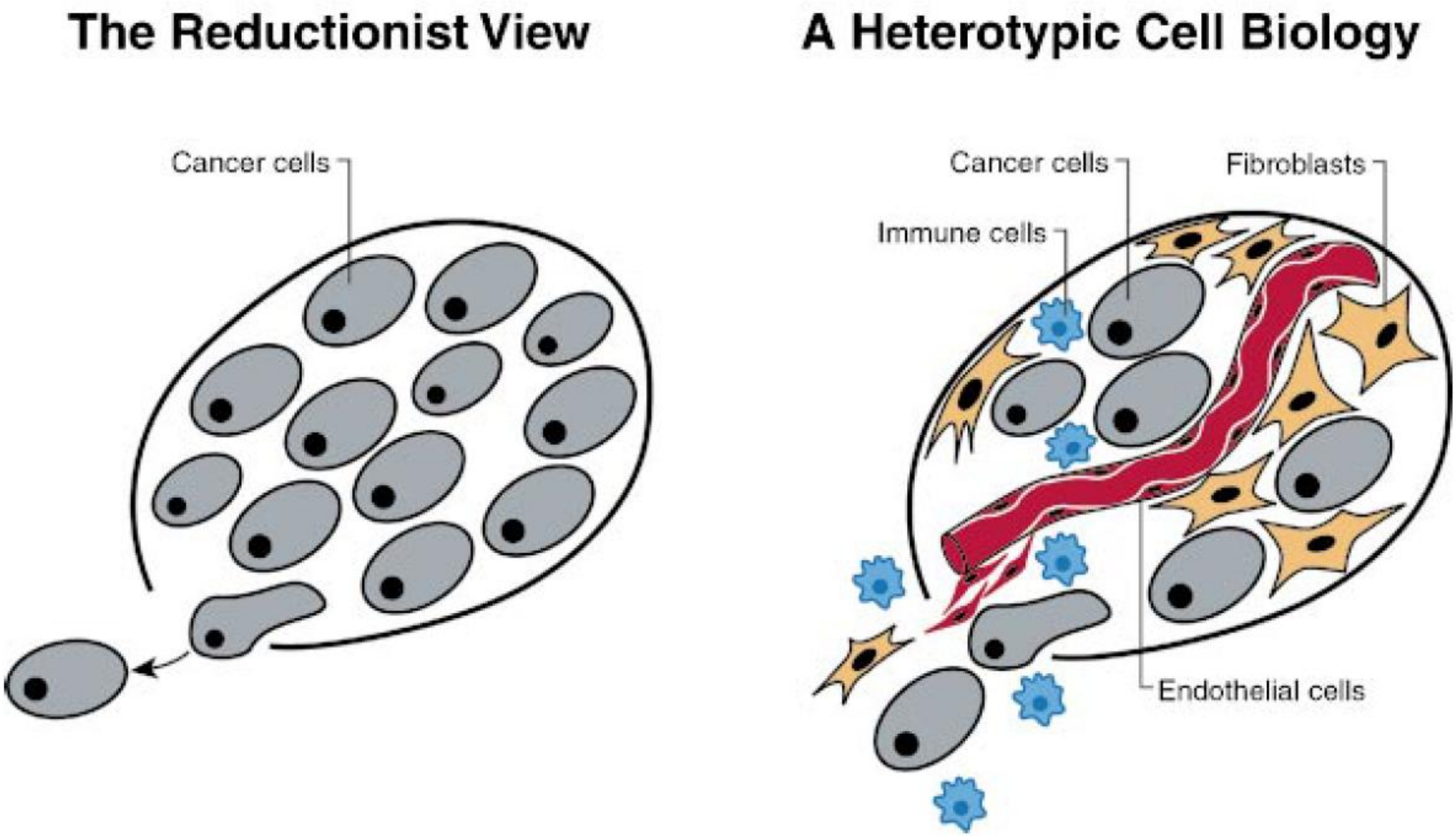
OV03/04 study

- 17 patients
- 3-30 samples per patient
- Biopsy, surgery and relapse
- Pre- and post-chemotherapy



http://www.nasa.gov/images/content/514467main_41s_factoring_DNA_1024.jpg

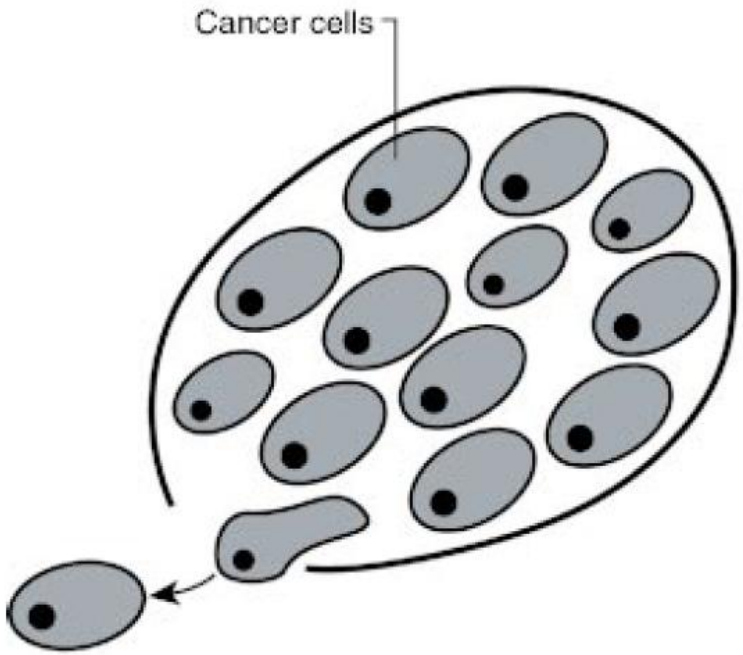




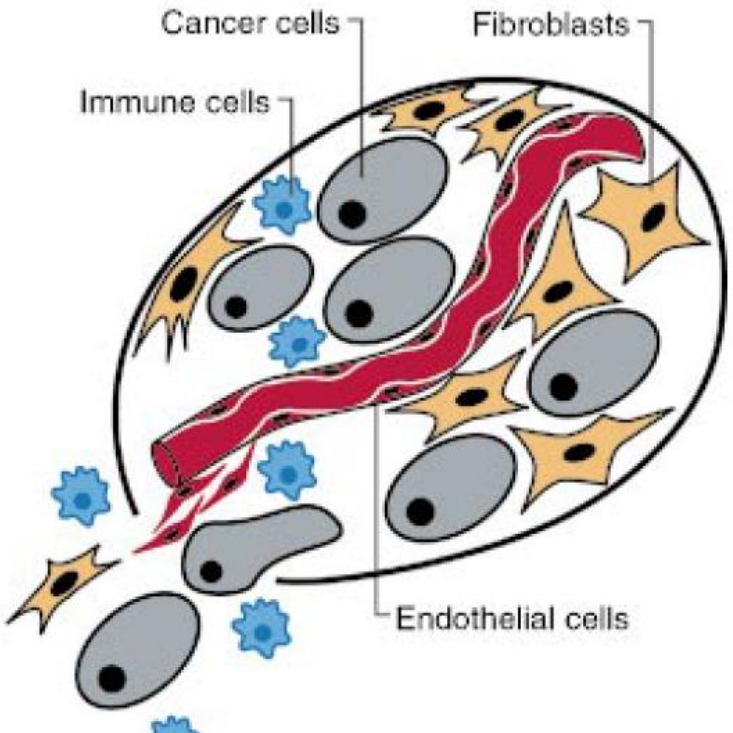
Hanahan and Weinberg (2001)

Comprehensive portraits of cancer

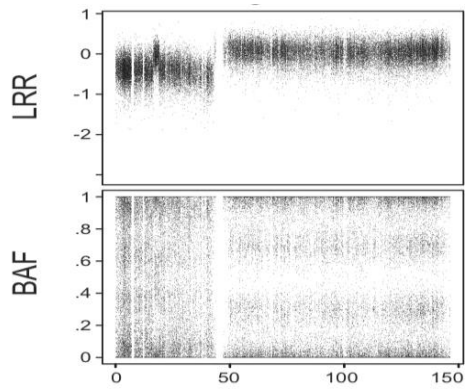
Genomics

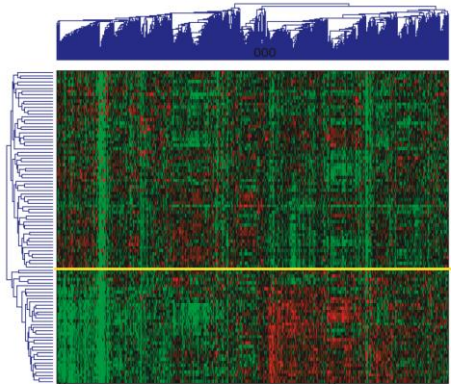


Tissue

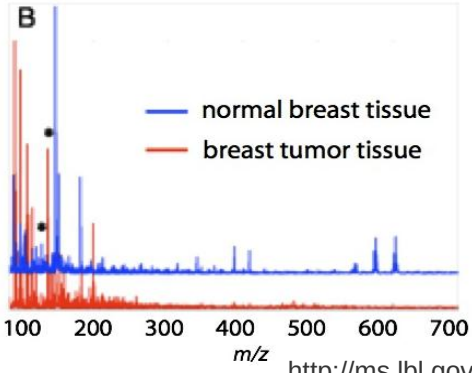


Tumors are complex tissues

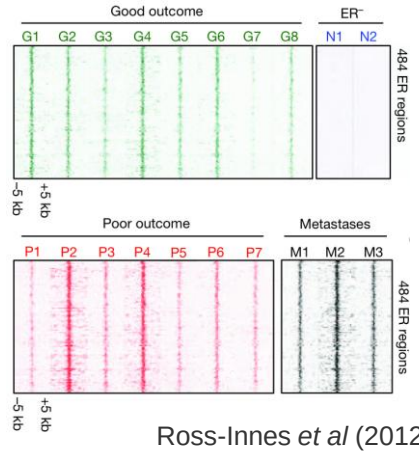




Van't Veer *et al* (2002)

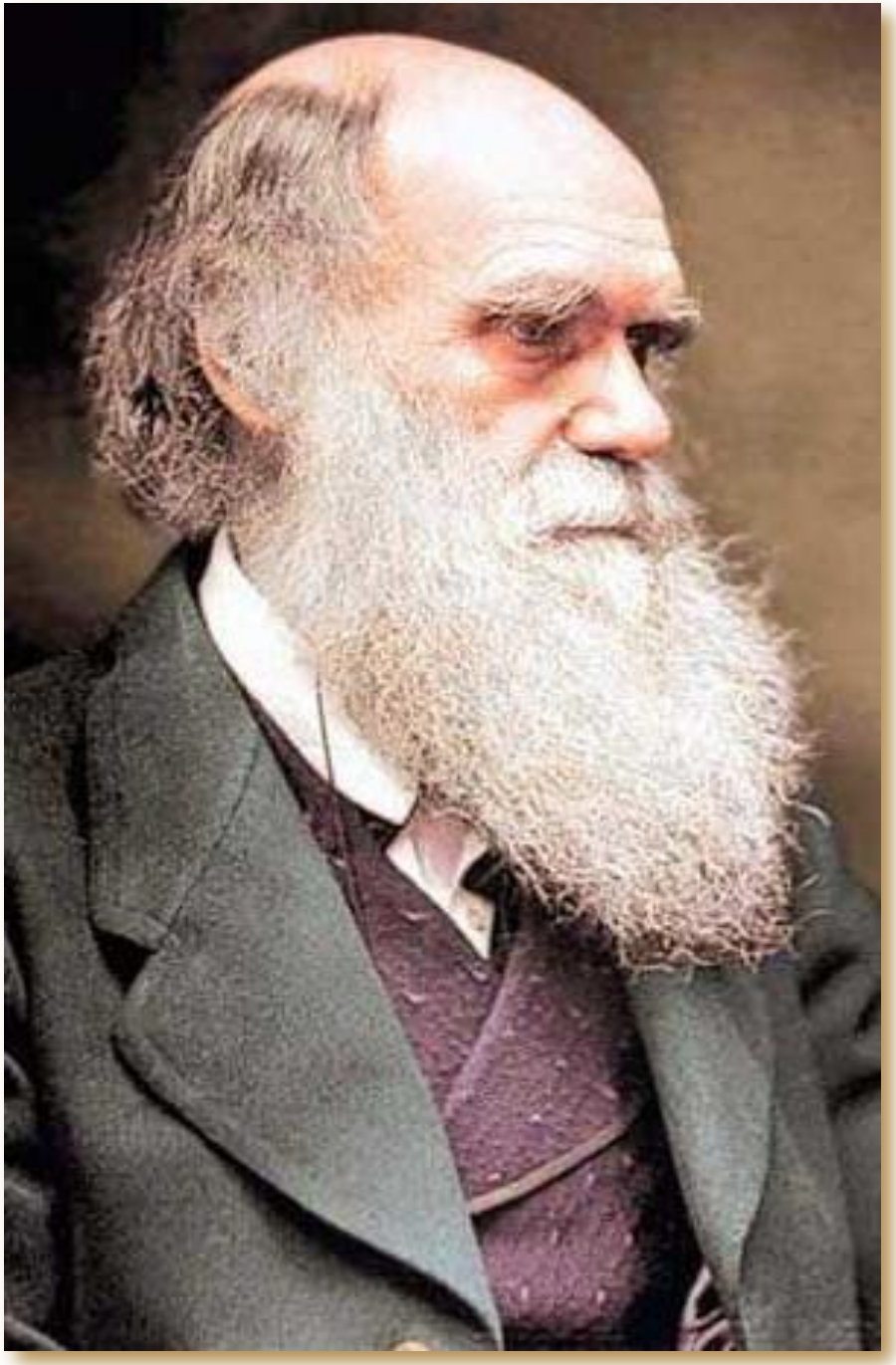


http://ms.lbl.gov

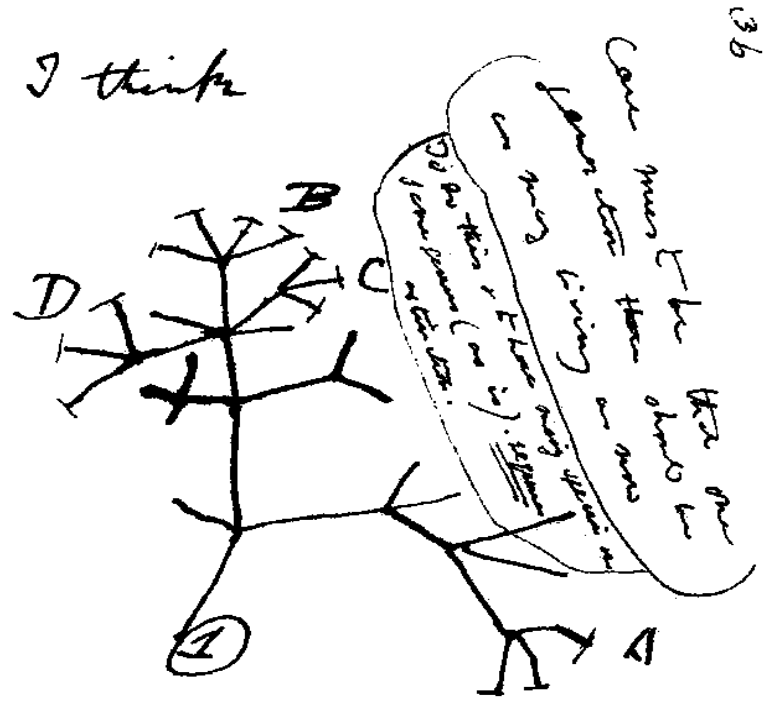


Ross-Innes *et al* (2012

DNA RNA Protein ChIP

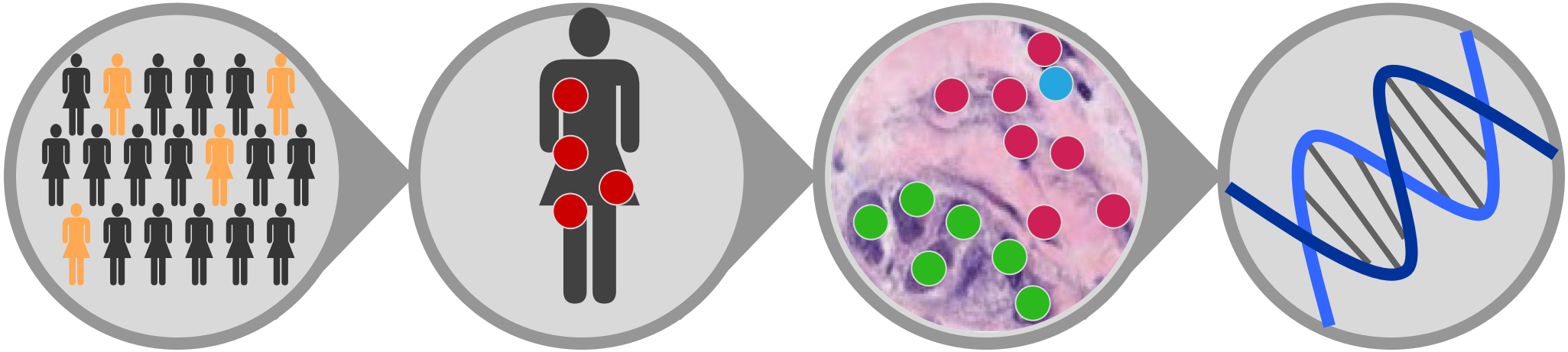


I think



Then between A & B. causing
beg & ulcers. C & B. the
first production, B & D
rather greater distinction
than genera would have
formed. — Hence ulcers

Intra-tumor heterogeneity



Yuan *et al*, Science Trans Med 2012

Quantitative image analysis of cellular heterogeneity complements genomics

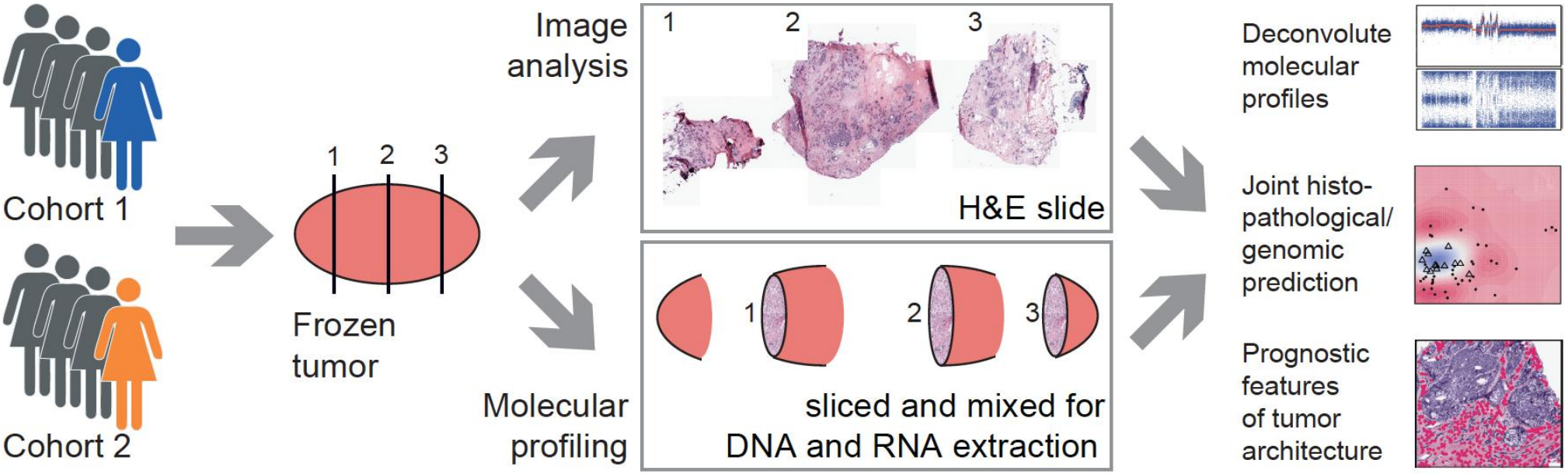
RESEARCH ARTICLE

Science Translational Medicine
AAAS

CANCER

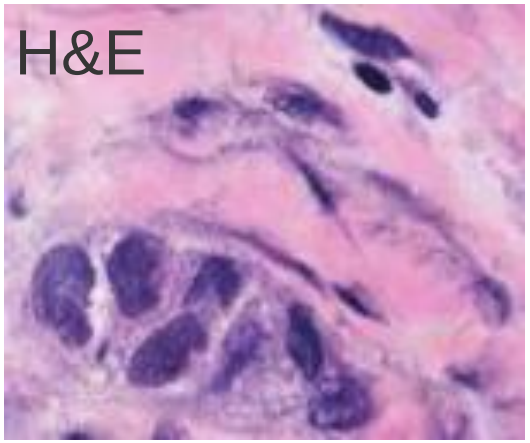
Quantitative Image Analysis of Cellular Heterogeneity in Breast Tumors Complements Genomic Profiling

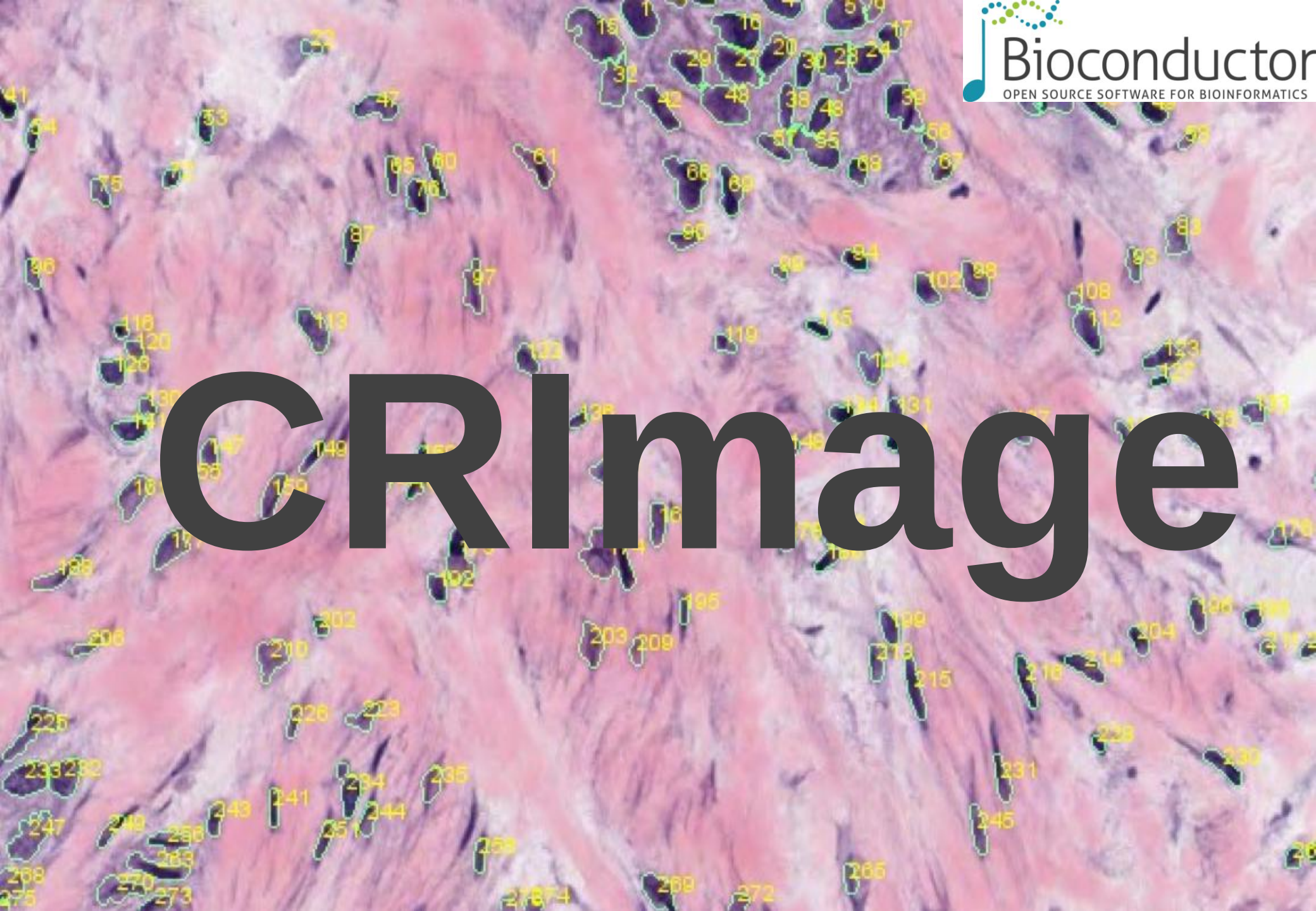
Yinyin Yuan,[1,2*†] Henrik Failmezger,[3,4‡] Oscar M. Rueda,[1,2‡] H. Raza Ali,[1,2‡] Stefan Gräf,[1,2§] Suet-Feung Chin,[1,2] Roland F. Schwarz,[1,2] Christina Curtis,[5] Mark J. Dunning,[1] Helen Bardwell,[1] Nicola Johnson,[6] Sarah Doyle,[6] Gulisa Turashvili,[7,8] Elena Provenzano,[9] Sam Aparicio,[7,8] Carlos Caldas,[1,2,9,10] Florian Markowetz[1,2*]



Automated image analysis

Yinyin Yuan

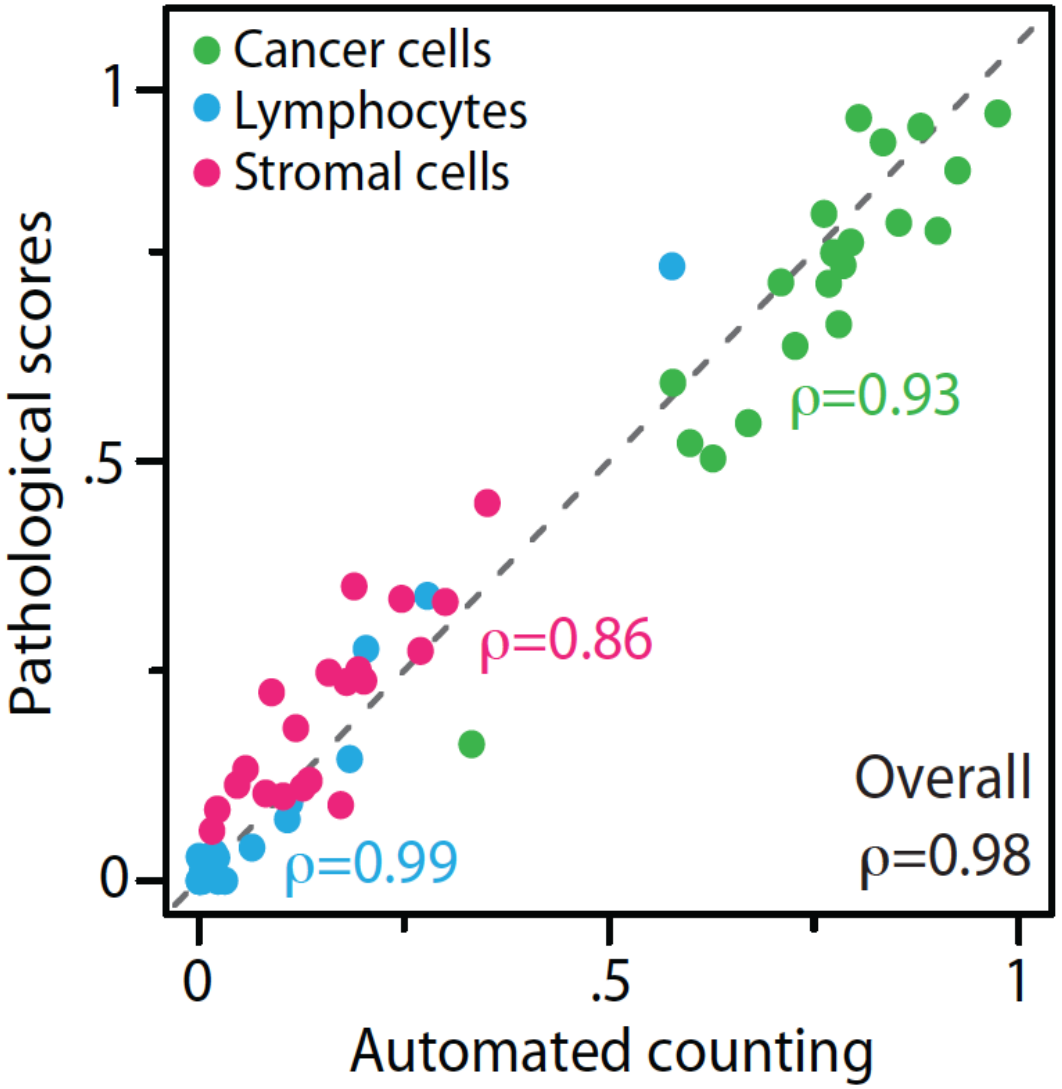




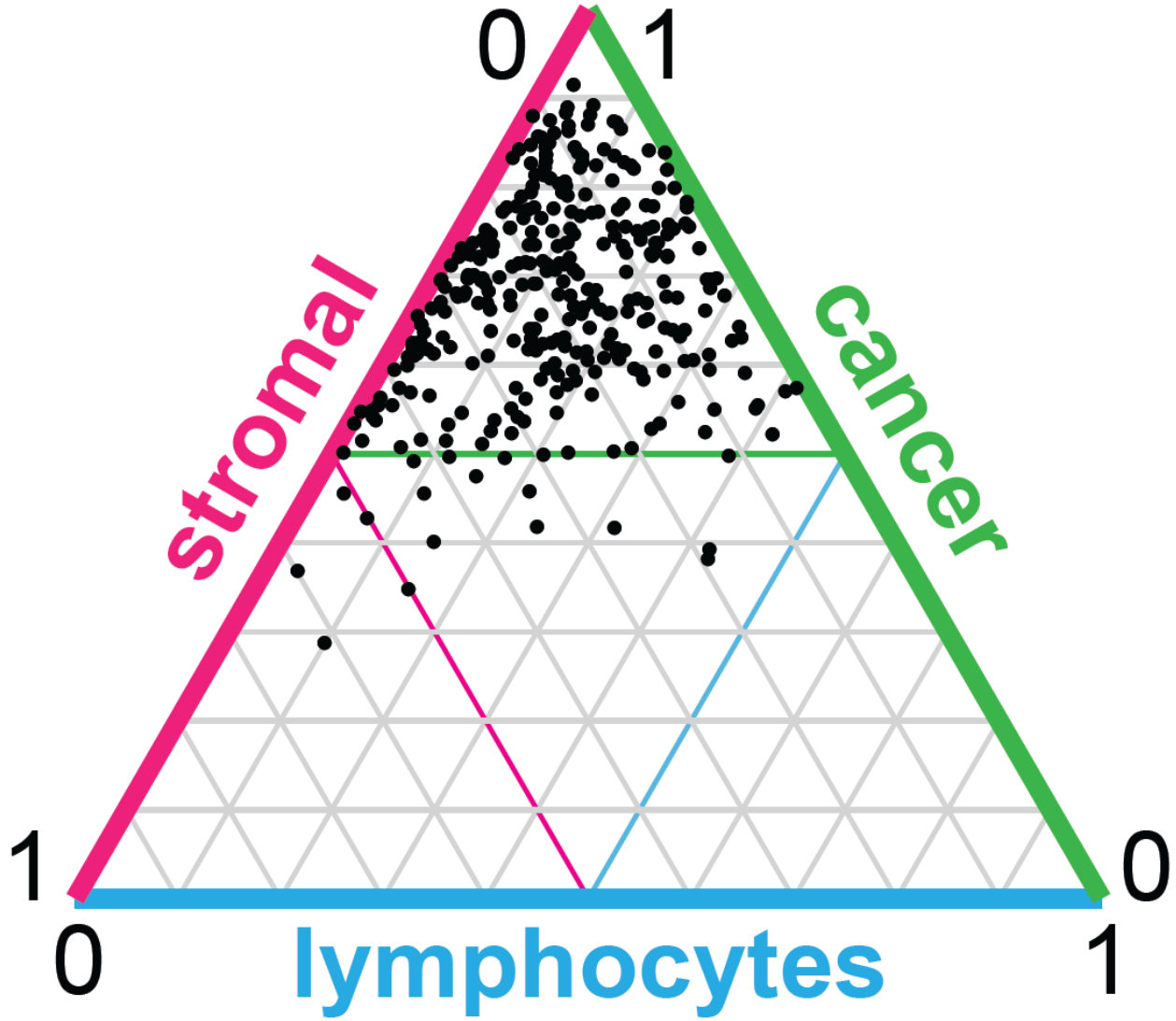
Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

Man vs Machine

Raza Ali
(Caldas lab)

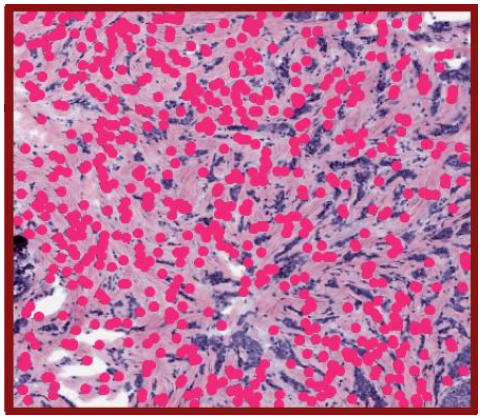


Quantitative analysis of tumour composition

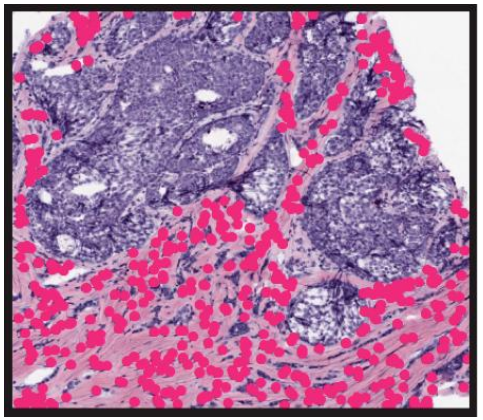


Spatial features of tissue **organisation**

Uniform

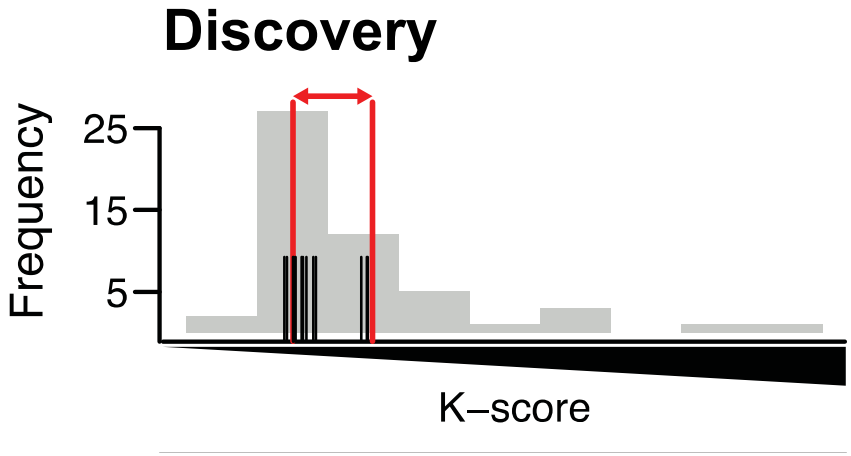


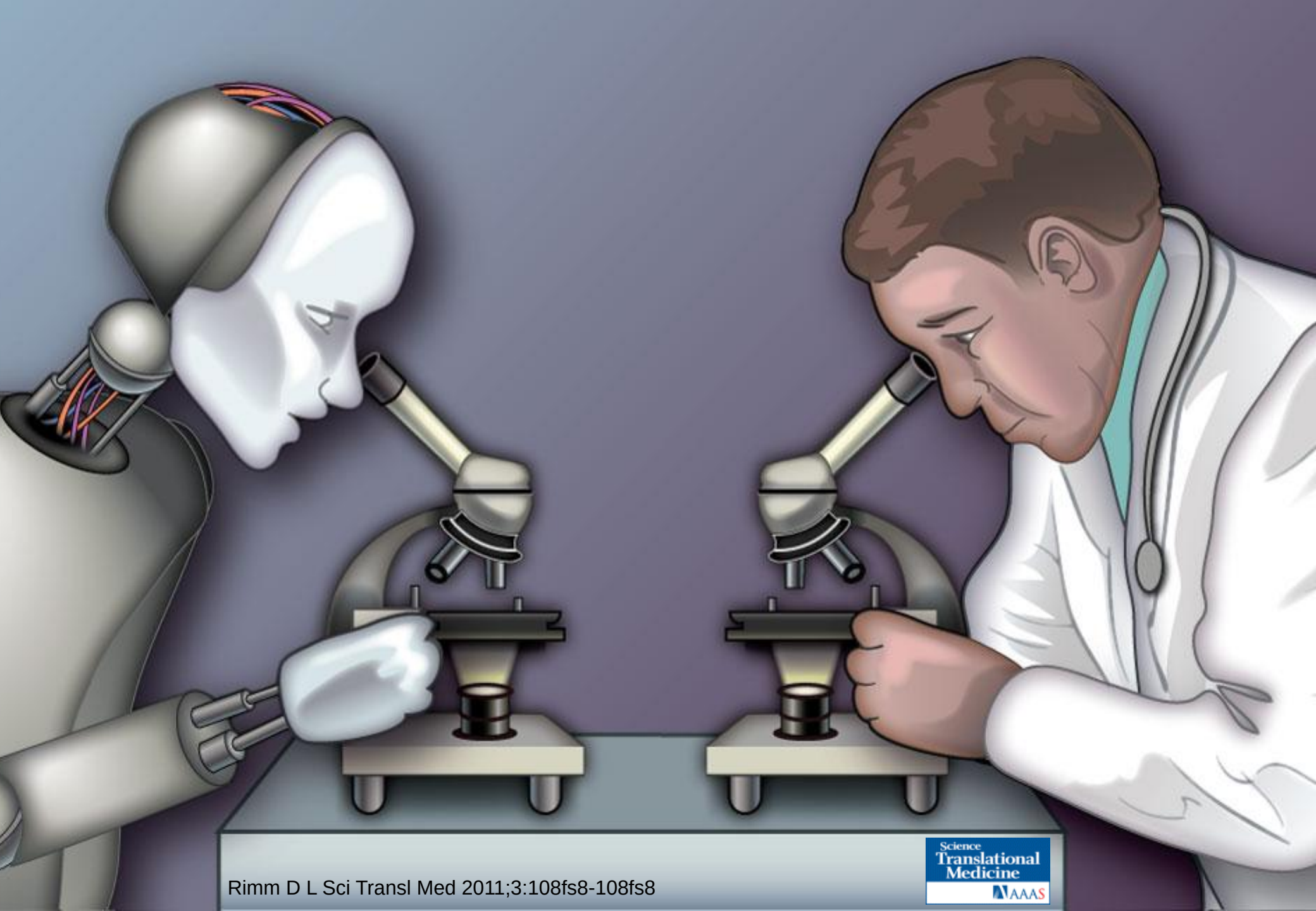
Clustered



Spatial statistics
(K-score)

Spatial features of tissue **organisation**



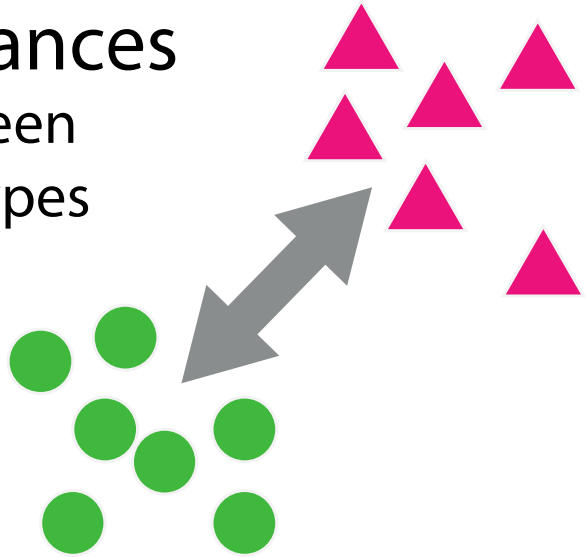


Rimm D L Sci Transl Med 2011;3:108fs8-108fs8

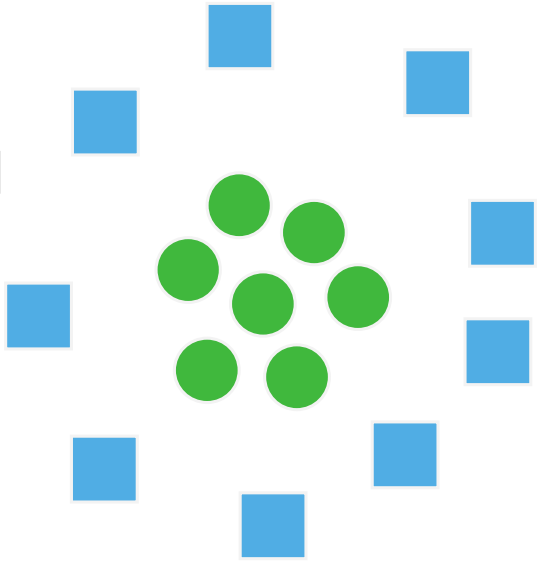
Science Translational Medicine AAAS

Spatial features of tumour tissue

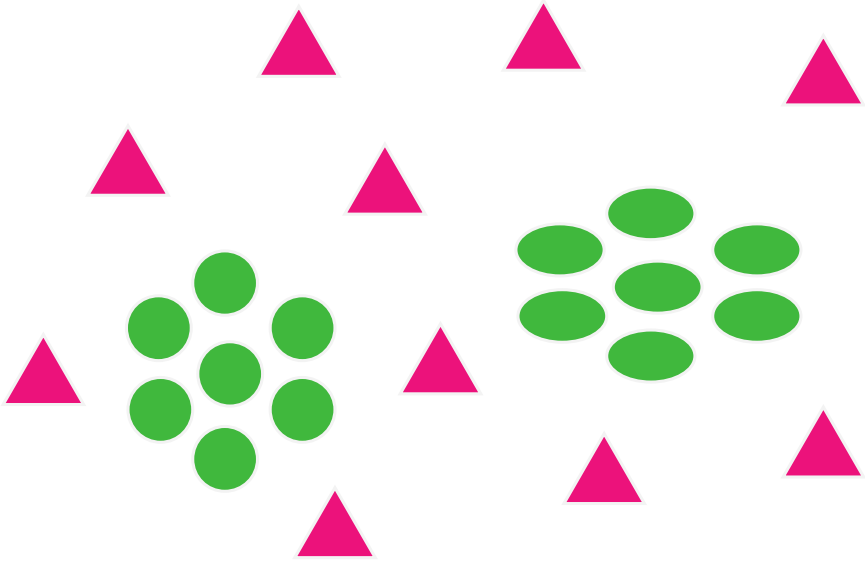
Distances
between
cell types



Co-location
e.g. are tumour
cells sourrounded
by immune cells

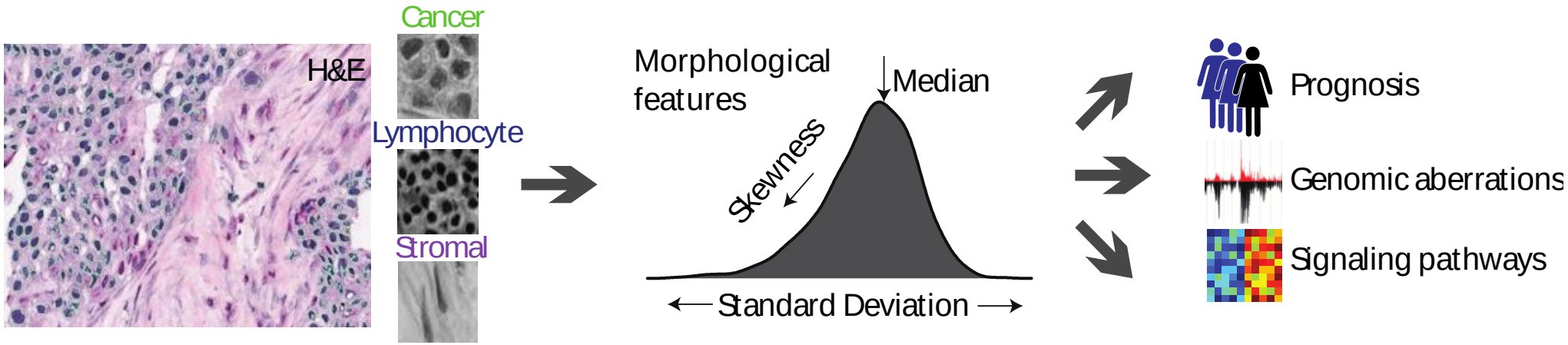


Distribution
of cells and
morphologies
across tumour



Morphological heterogeneity

Yinyin Yuan

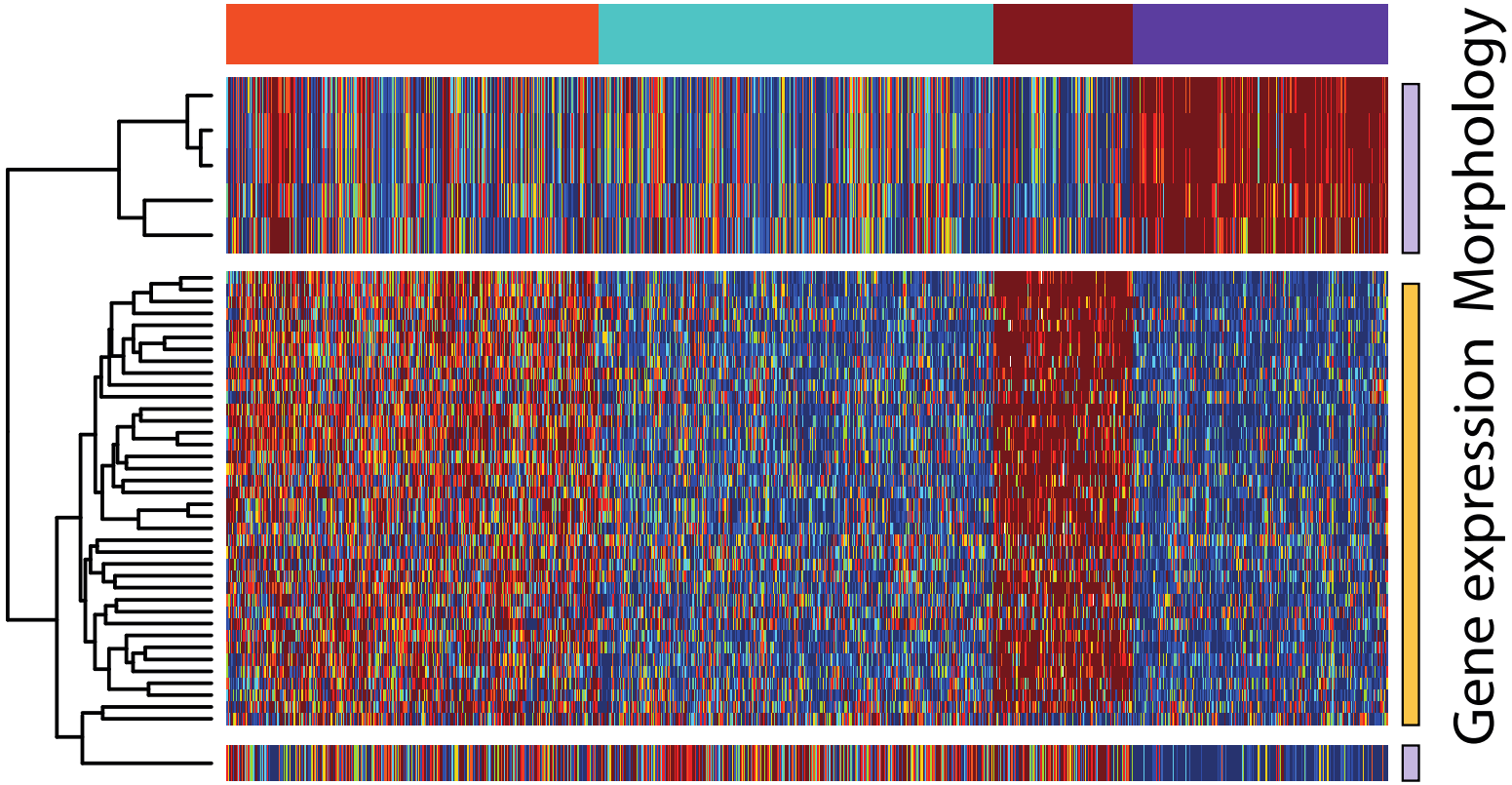


Morphological features

1. Fraction of pixels outside of the circle with radius effr
2. Shape factor,
3. 1st Hus translation/scale/rotation invariant moment
4. Eccentricity calculated based on geometric information
5. Eccentricity calculated based on image moments

Morpho-genomic subtypes

Yinyin Yuan

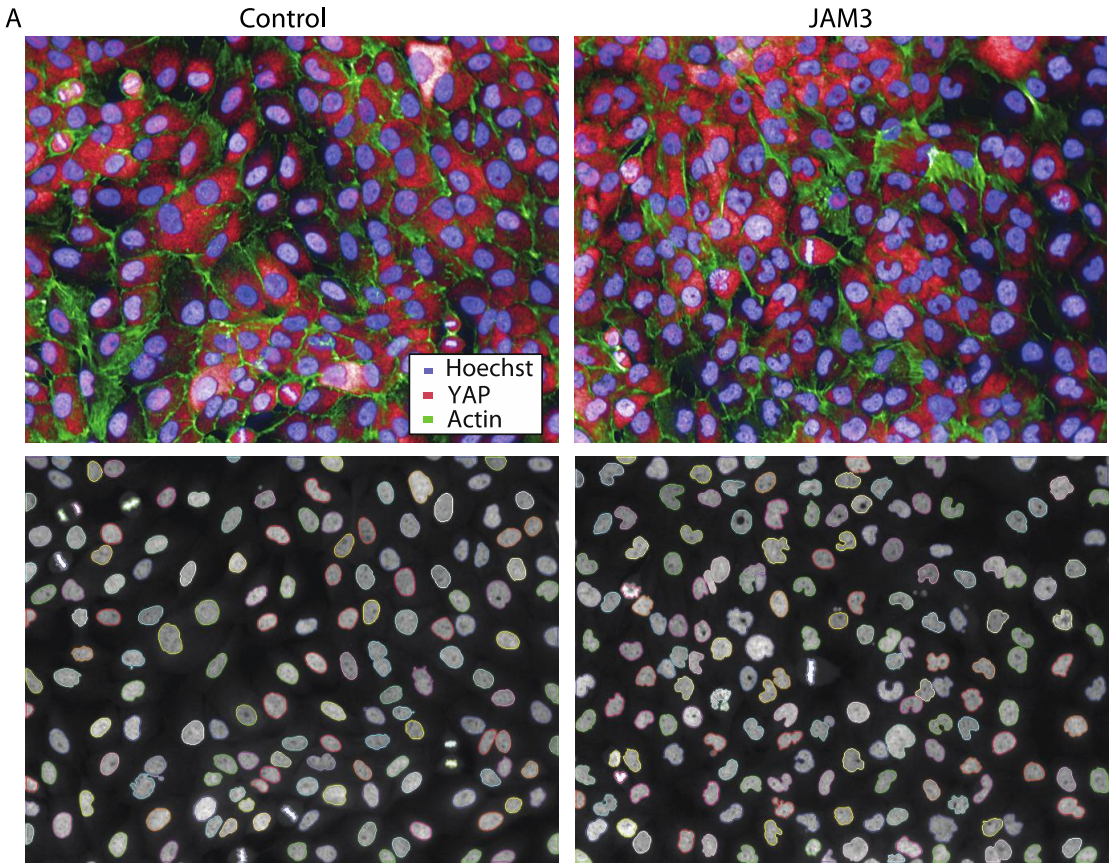


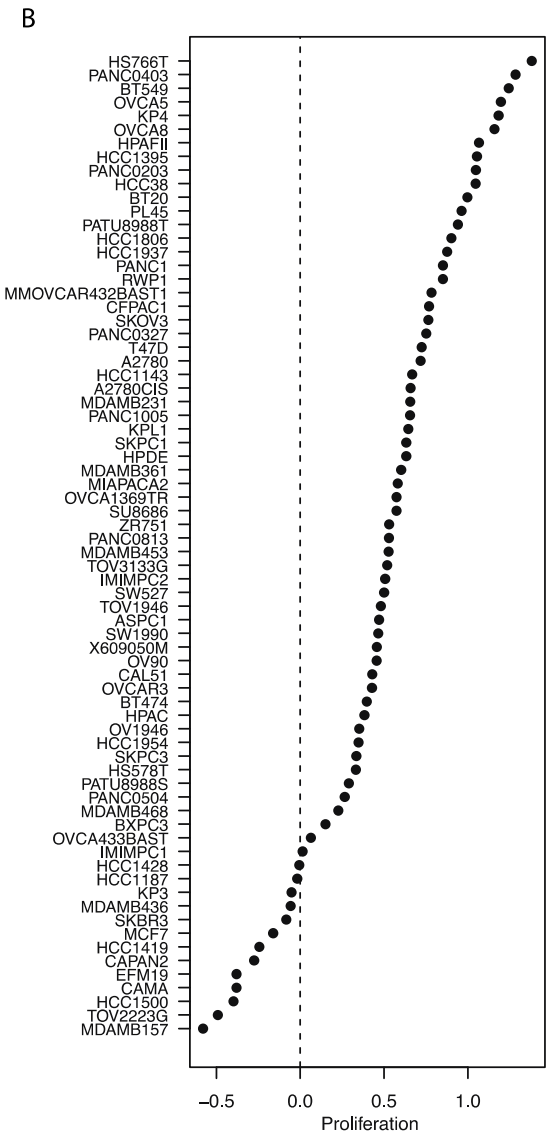
Morphology <-> Gene expression

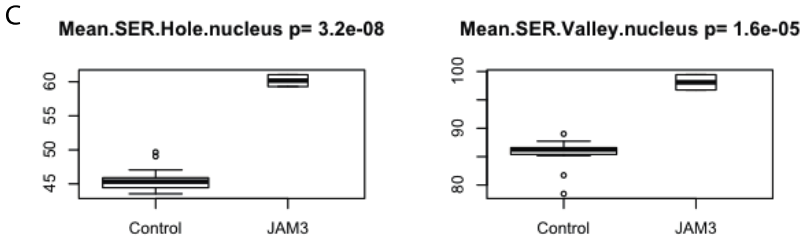
JAM3 – driver of cell morphology

Yinyin Yuan

Chris Bakal







nature medicine

Xin Wang

Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions

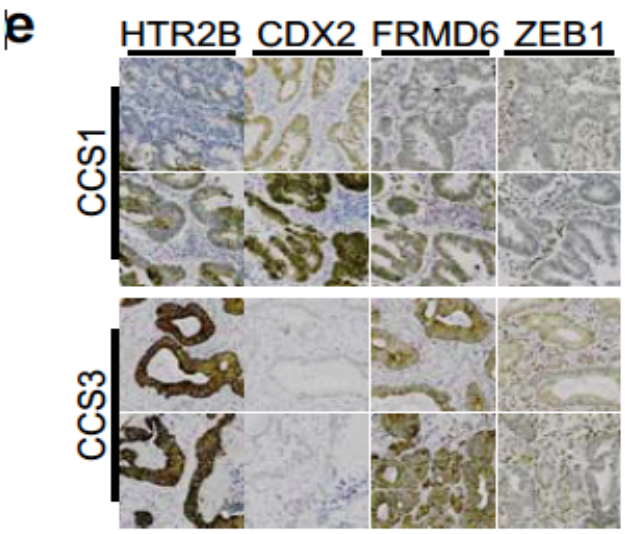
Felipe De Sousa E Melo[1,7], Xin Wang[2,7], Marnix Jansen[3], Evelyn Fessler[1], Anne Trinh[2], Laura P M H de Rooij[1], Joan H de Jong[1], Onno J de Boer[3], Ronald van Leersum[1], Maarten F Bijlsma[1], Hans Rodermond[1], Maartje van der Heijden[1,4], Carel J M van Noesel[3], Jurriaan B Tuynman[5], Evelien Dekker[6], Florian Markowetz[2], Jan Paul Medema[1,7] & Louis Vermeulen[1,4,7]

Colon cancer is a clinically diverse disease. This heterogeneity makes it difficult to determine which patients will benefit most from adjuvant therapy and impedes the development of new targeted agents[1]. More insight into the biological diversity of colon cancers, especially in relation to clinical features, is therefore needed. We demonstrate, using an unsupervised classification strategy involving over 1,100 individuals with colon cancer, that three main molecularly distinct subtypes can be recognized. Two subtypes have been previously identified and are well characterized (chromosomal-instable

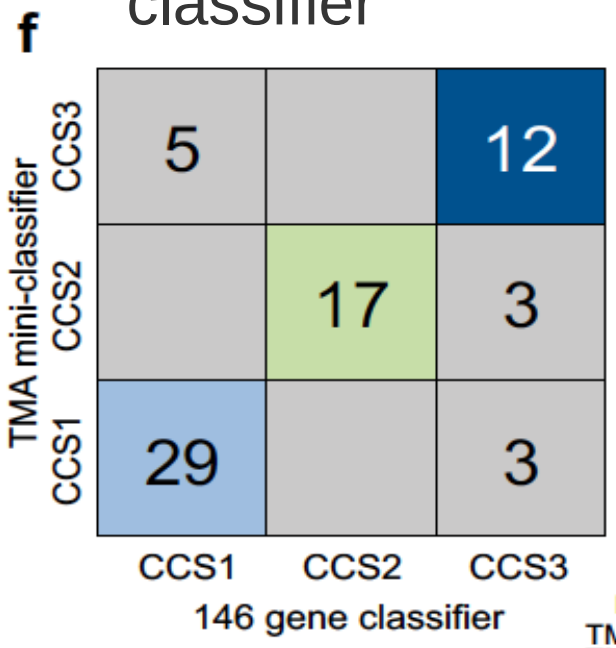
24 patients with CCS3 (49, 24, and 27%, respectively) (**Fig. 1b**). We validated this classifier in six independent data sets and found comparable proportions of patients being assigned to each subtype (**Fig. 1c** and **Supplementary Table 3**). We could also classify colorectal cancer cell lines into the three subtypes (**Fig. 1d**)[4]. Moreover, the subtypes were generally maintained upon xenografting of cell lines and primary tumors (**Supplementary Fig. 3d–g**)[5,6], suggesting they reflect persistent genetic or epigenetic features of tumor cells rather than differences in stroma or immune infiltrates, which have been used previously to stratify patients[7,8]. To further characterize the subtypes, we determined the

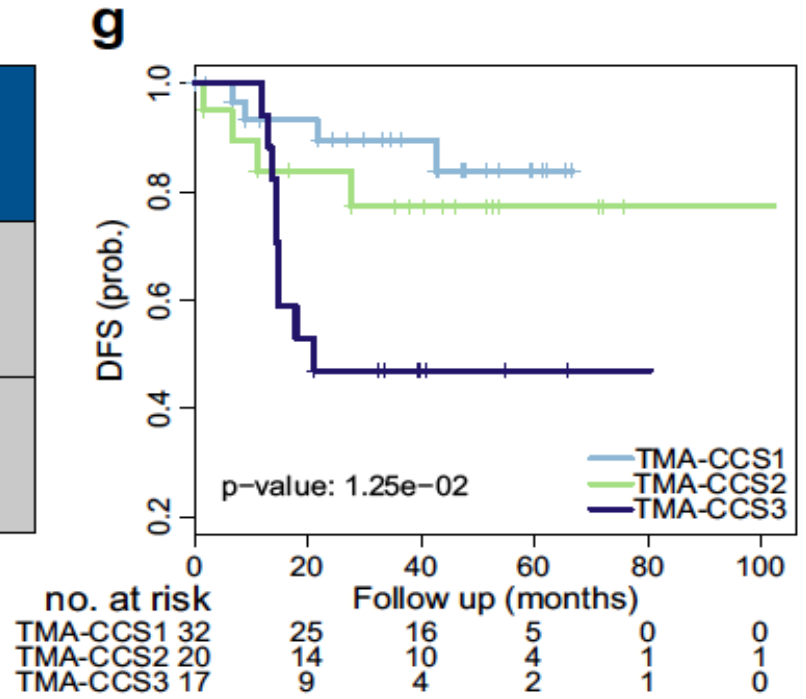
Anne Trinh

Stainings in tissue microarrays



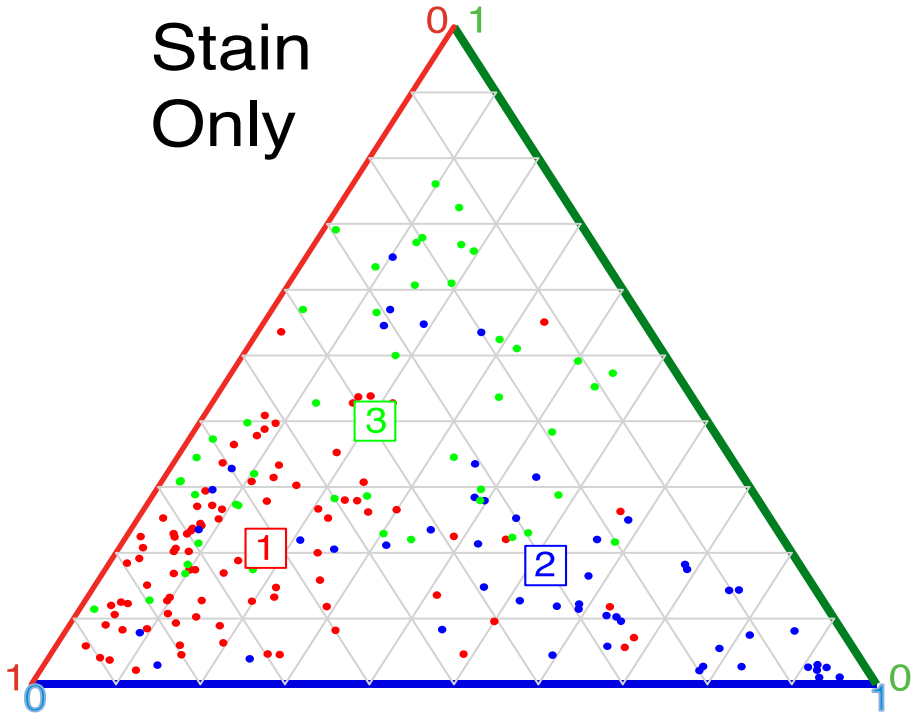
Comparison to gene expression classifier

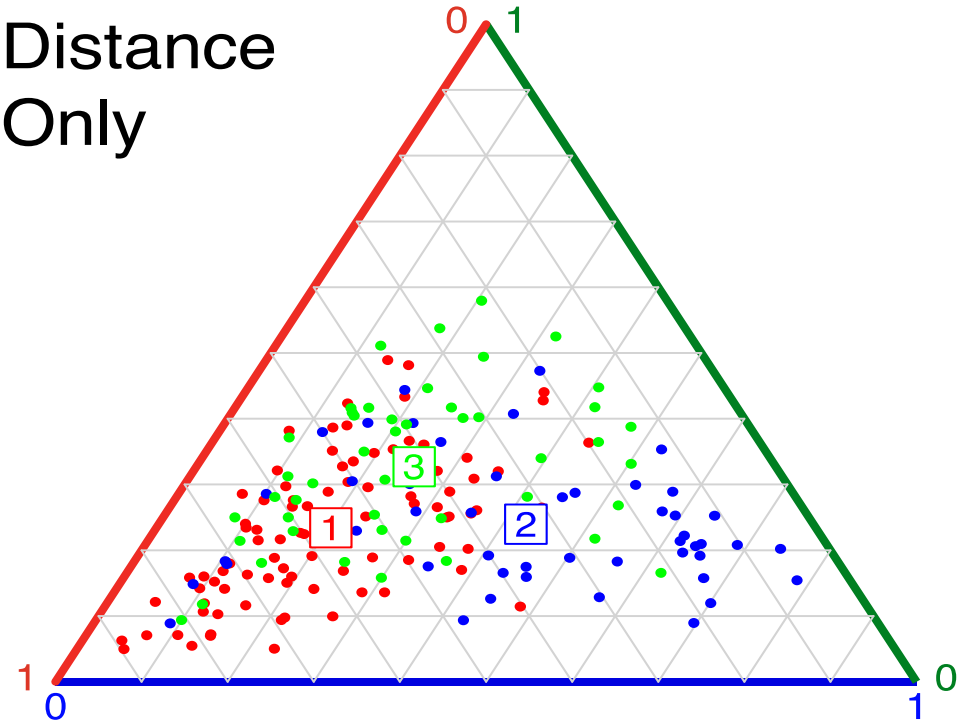




Spatial features are predictive

Anne Trinh





ASUMT: A Still Unnamed MATLAB Toolbox

Anne Trinh

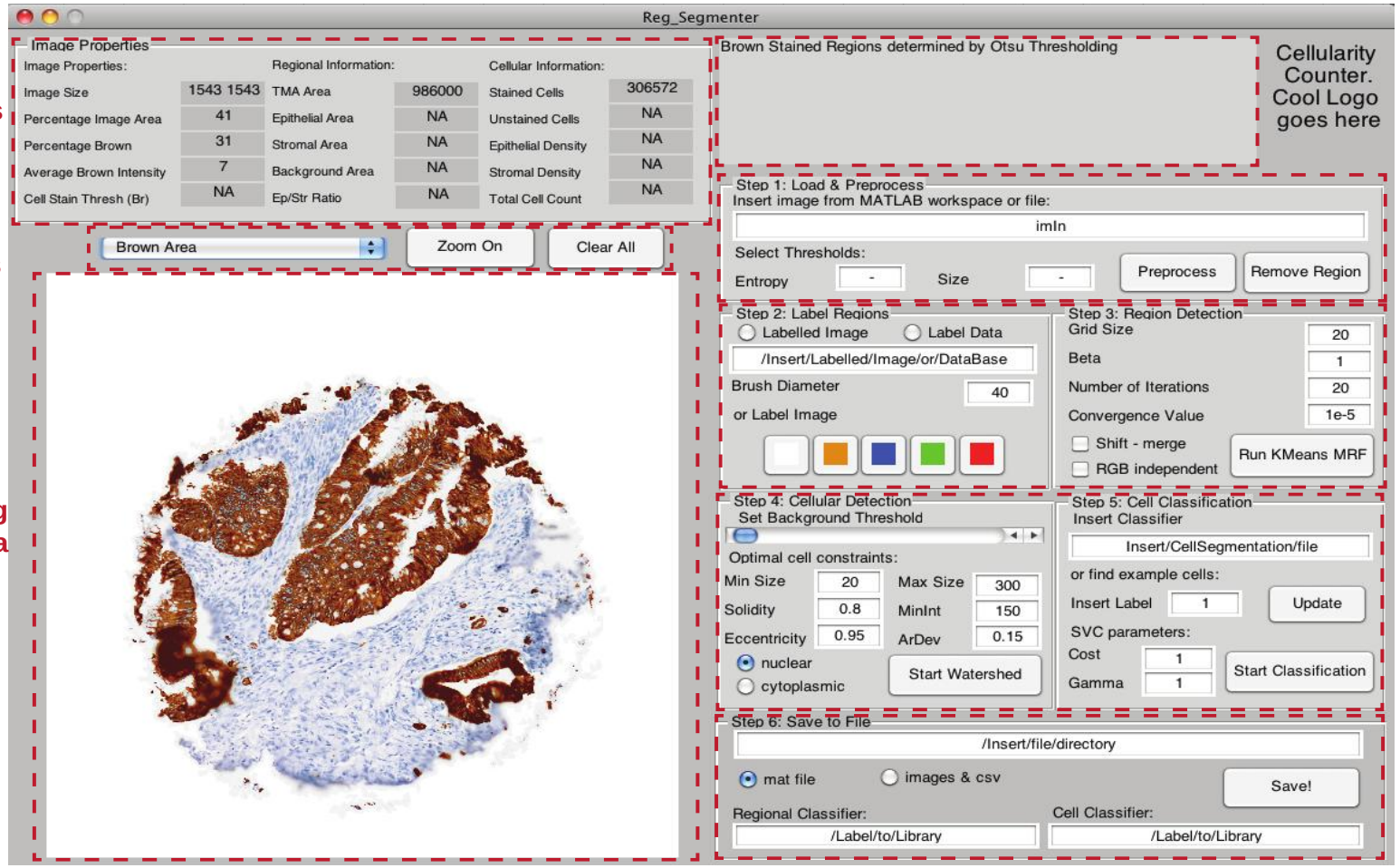


Image Properties

View Results

Plotting Area

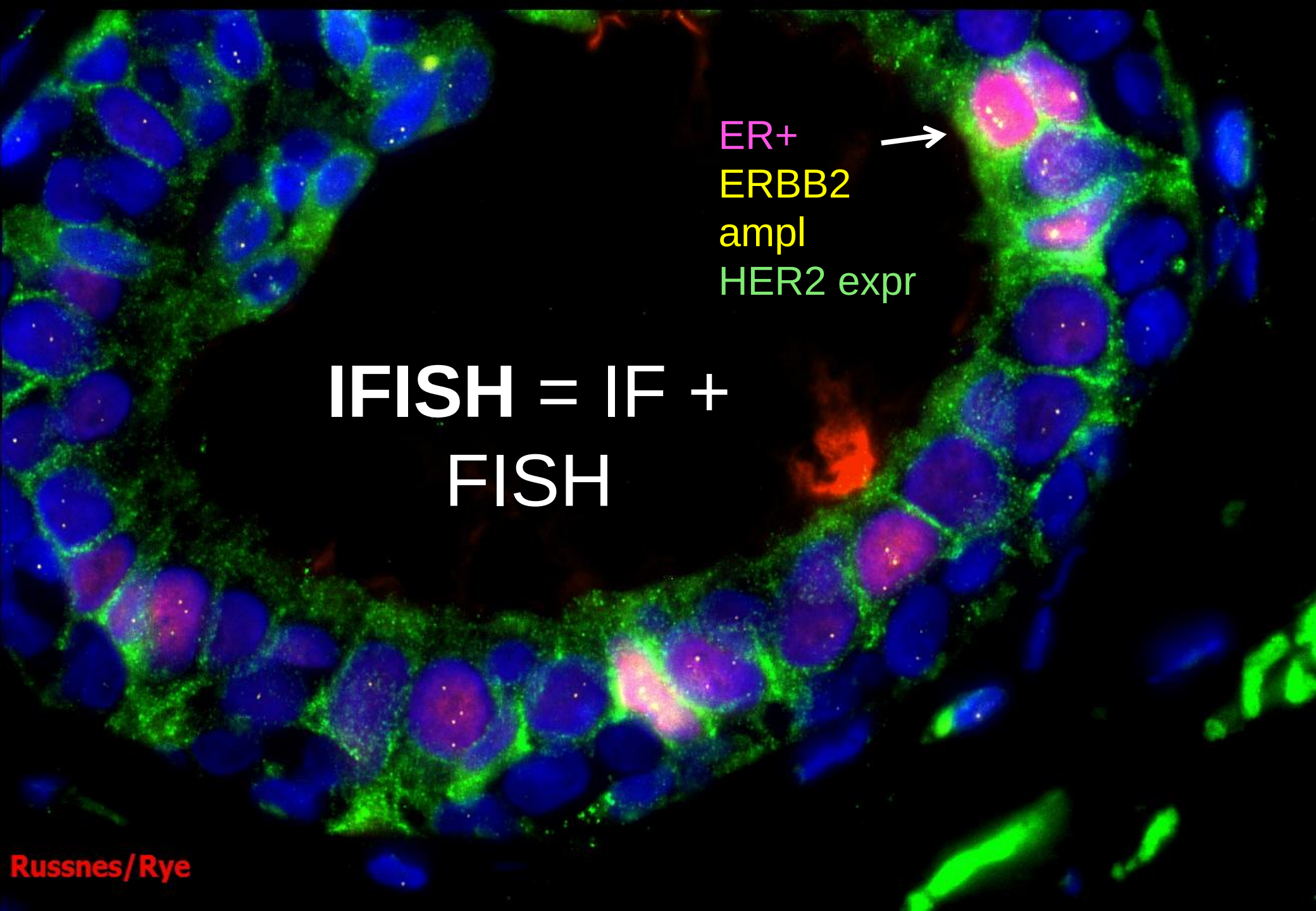
Feedback Box:
* Instructions
* Errors

Load & Preprocess:
* Load TMA
* Detect Outline
* Find Brown Area

Regional Segmentation:
* Label Dataset (file or directly)
* KMeans-MRF (grayscale & RGB)

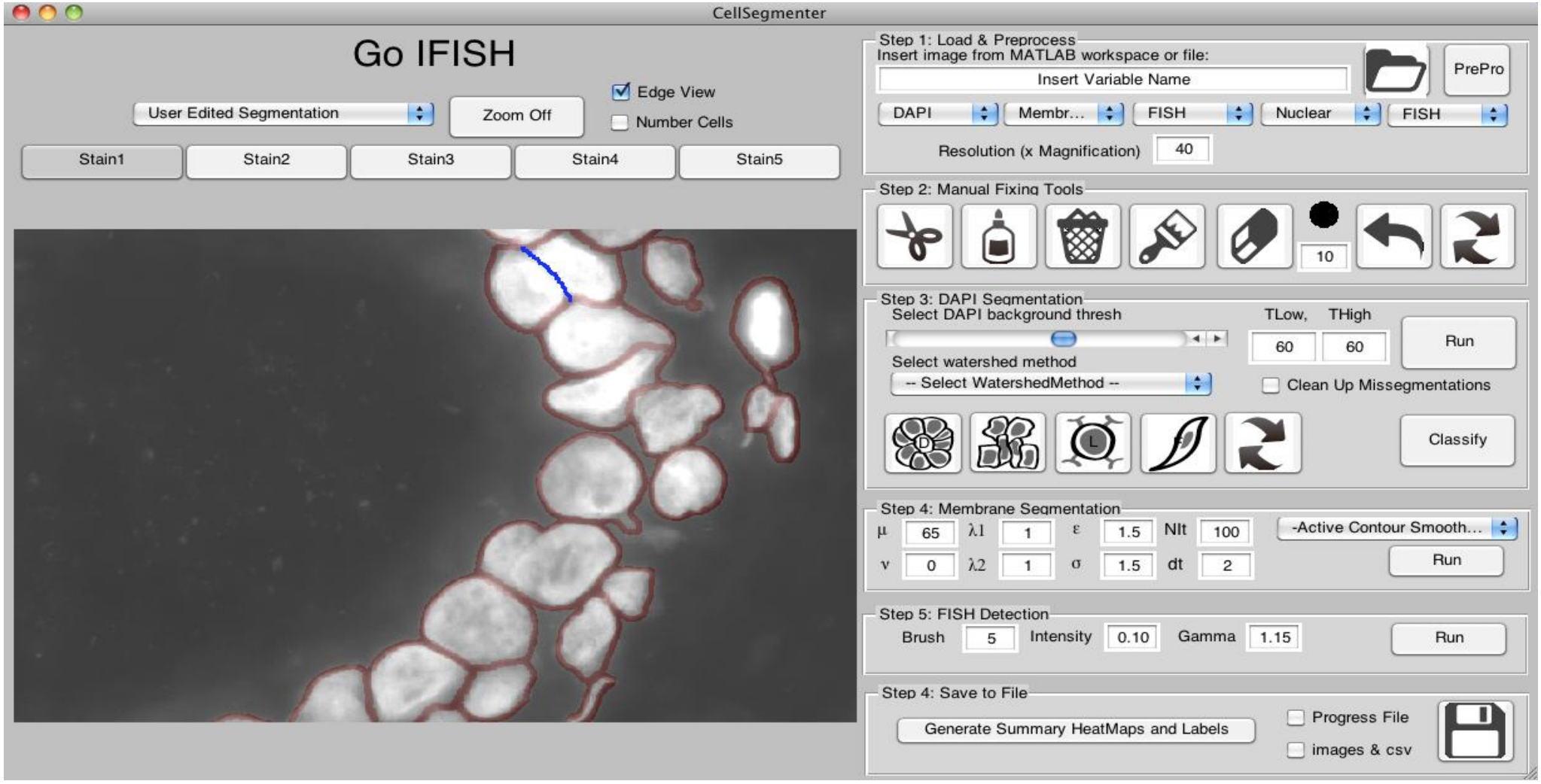
Cell Count:
* Background Thresholding
* H-minima Watershed
* SVM Cell Classification

Save:
* mat file
* csv & images
* classifiers


ER+
ERBB2
ampl
HER2 expr
IFISH = IF +
FISH
Russnes/Rye

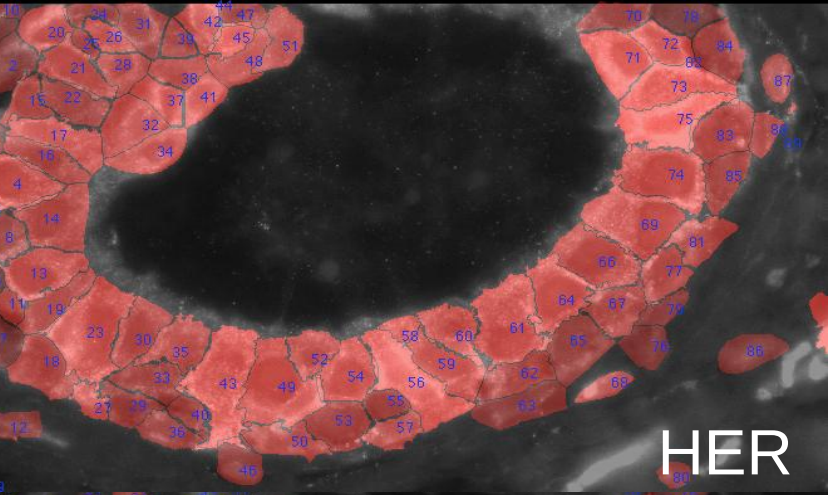
Go IFISH: a toolbox for semi-automated detection of nuclei, membrane and spots

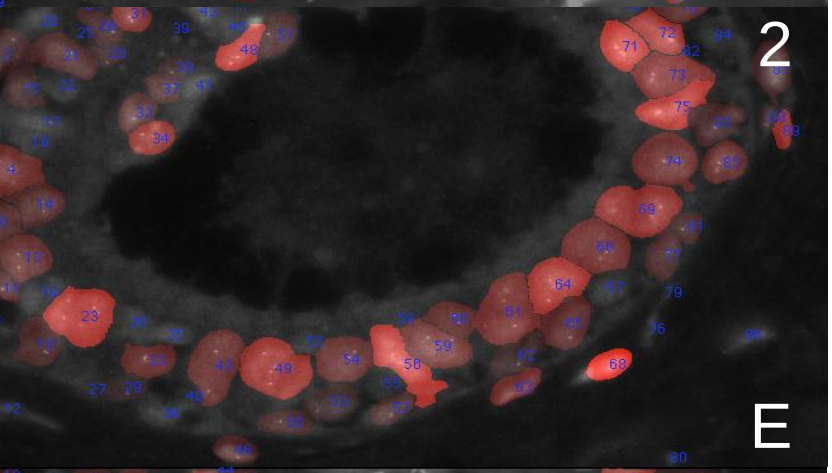
Anne Trinh

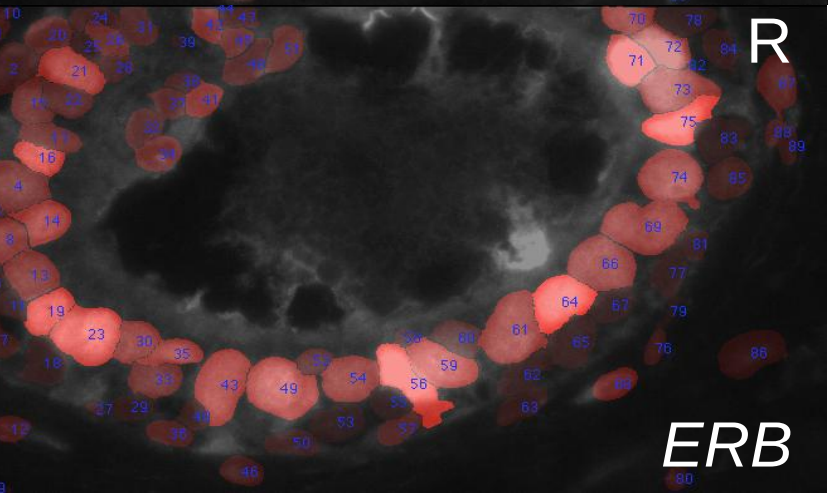


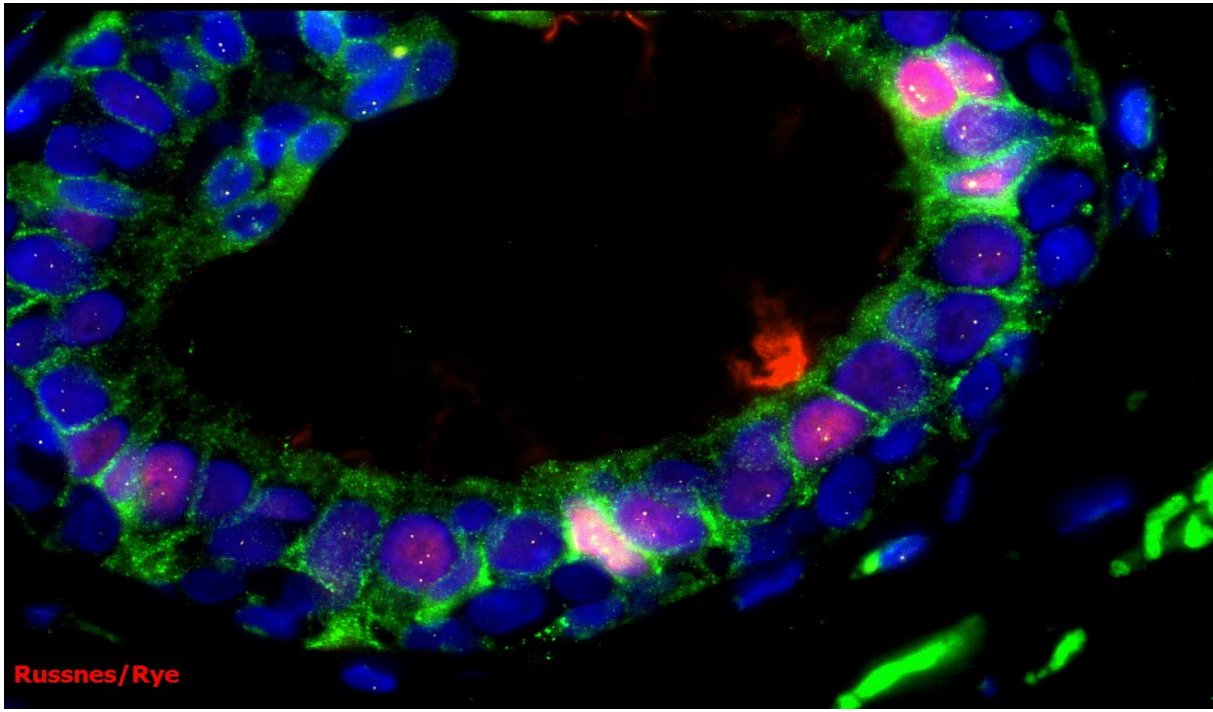
Single cell analysis of stain intensities

Anne Trinh









Key collaboration partners

- **Carlos Caldas,** Raza Ali, Suet-Feung Chin, Oscar Rueda, Stefan Gräf @ University of Cambridge
- **Yinyin Yuan** + lab @ Institute for Cancer Research
- **Chris Bakal** + lab @ Institute for Cancer Research
- **JP Medema**, Louis Vermeulen @ Amsterdam Medical Center
- **Anne-Lise Børresen-Dale**, Hege Russnes, Inga

the **team**

Alumni:
Xin Wang
Yinyin Yuan
Roland Schwarz
Mauro Castro
Gökmen Altay

UNIVERSITY OF CAMBRIDGE

breast cancer CAMPAIGN

wellcome trust

HWL Hutchison Whampoa

CANCER RESEARCH UK

CRUK Cambridge Institute

Paul Pharoah
Strangeways Laboratories, Cambridge
- Genetic epidemiology

Carlos Caldas
- Breast Cancer Functional Genomics
- Cambridge Breast Cancer Research Unit

FM*lab*

Jason Carroll
- ER biology
- ChIP-seq in tumors

Stephen Friend
Sage Bionetworks

Doug Fearon
- Tumor immunology
- Tumor microenvironment

Dissecting cancer heterogeneity

Thank you !

Florian Markowetz
CRUK Cambridge Institute
www.markowetzlab.org

Systems Genetics = genome × phenotypes × conditions