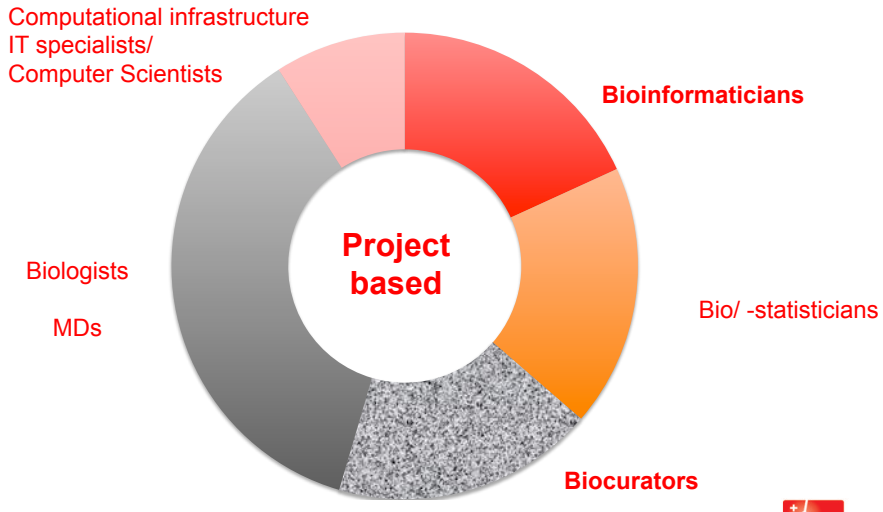
Genome, Genomics and Metagenomics

Sequencing the Universe

Swiss Institute of Bioinformatics

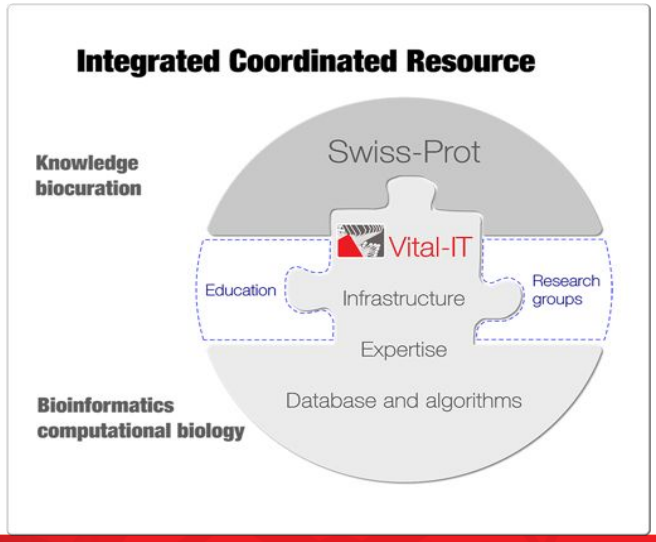
Dr. Nicolas Guex on behalf of Prof. Ioannis Xenarios
CERN, 28 May 2013 from 11:40 - 12:00

Most projects require a wide range of expertise



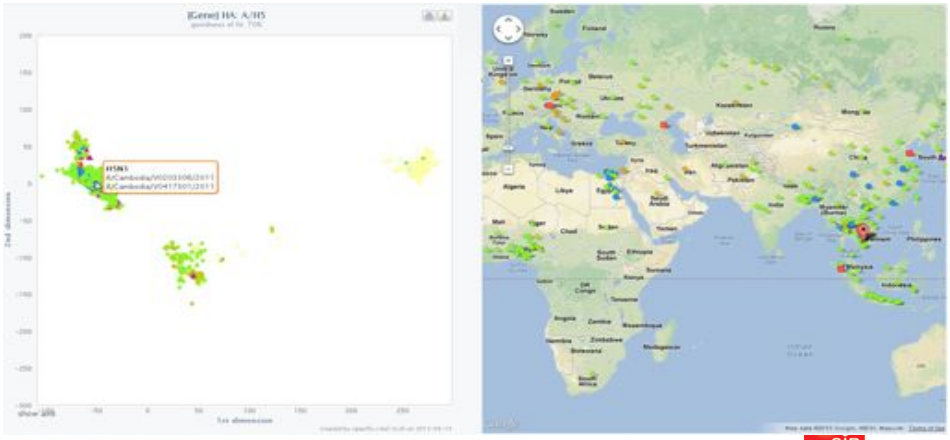
Swiss Institute of Bioinformatics

Prof. Ioannis Xenarios Ph.D.
Director Swiss-Prot/Vital-IT
Swiss Institute of Bioinformatics
Geneve-Lausanne, Switzerland



OpenFlu: large scale collection and analysis of *Influenza* genetic data

- Genetic landscape of H5Nx and cross-infection to humans



Avalanche of data

Every second a sequence informations (nucleotide) is deposited in one of the three worldwide nucleotide database (INSDC:Genbank-DDBJ-EMBL)

Every five minute a gene expression data (microarray) chip is deposited in one of the two worldwide databases (GEO-ArrayExpress)

Every day a new genome sequence is deposited (from bacteria mostly but more and more from higher eukaryotes) (Genbank-DDBJ-EMBL, ENA)

Every week a protein structure is deposited either originating from a structural biology project or from medium throughput crystallography project (PDB-RCSB)

Every month a new human genome is sequenced
In 2011, 1024 genomes completed…

In 2012…1 genome / week is produced; over 1000 exomes on Vital-IT

In 2013…8 genomes/ week.
over 3000 exomes and 150 complete genomes
on Vital-IT/Lausanne

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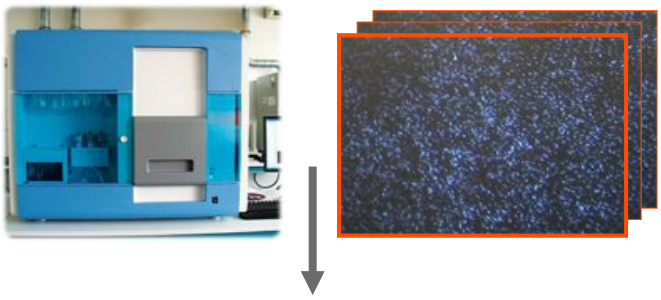
Next Generation Sequencing redefine Genomics (again)

Ultra High Throughput Sequencing (UHTS)
Solexa or other…



2007 – **1 Terabytes** of raw and processed data
2008 – **2.5 Terabytes** of raw and processed data
2009 – **7 Terabytes** of raw and processed data
2012 – **14 Terabytes** of raw data

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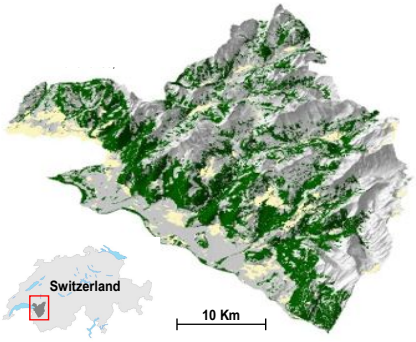
Characterization of the fungal biodiversity in the grassland soils of the western Swiss Alps



- 205 sampling sites, 4m^2 each
- DNA barcoding using Roche 454 pyro-sequencing
- Using pan-fungal primers to get a global picture
- How biotic and abiotic factors shape the distribution of the fungal biodiversity?

UNIL | Université de Lausanne
Prof. Jérôme Goudet
Prof. Antoine Guisan
Prof. Ian Sanders
Faculté de biologie et de médecine
Department of Ecology and Evolution

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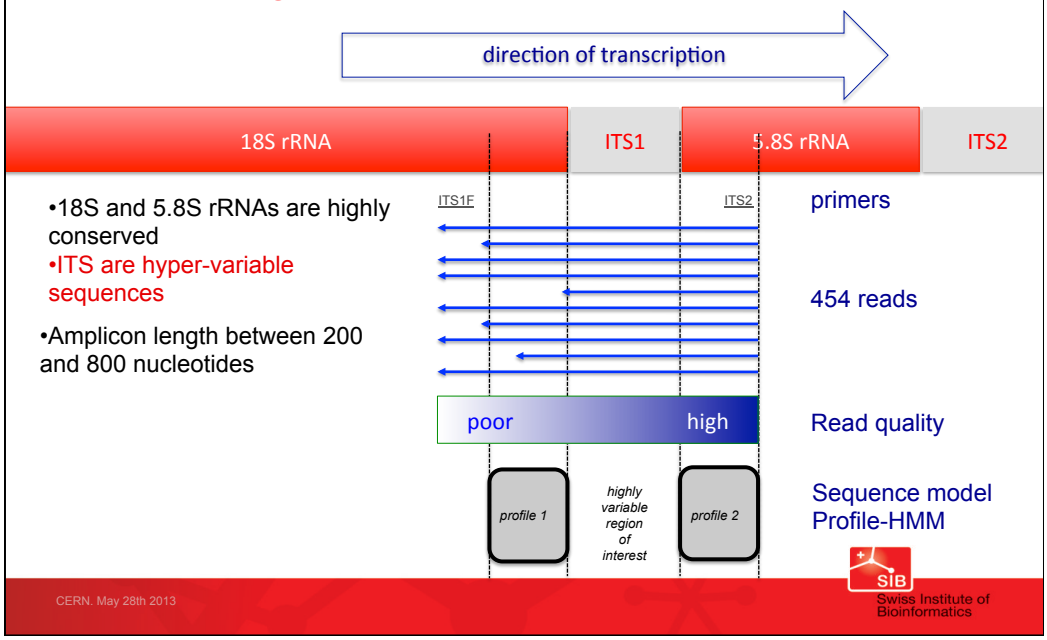
Sequencing ITS1 with Roche 454



- 18S and 5.8S rRNAs are highly conserved
- ITS are hyper-variable sequences
- Amplicon length between 200 and 800 nucleotides

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A big dataset from Alpine soil samples

Number of reads / sequences	Alpine soils Roche454 reads	EMBL FUN + PLN + INV + ENV
Total	1'649'376	NA
After profiles extraction	1'152'122	127'577
After profiles extraction, non redundant	341'585	72'501

- We were looking for a method to recognize/define Operational Taxonomic Units (OTUs) that is
  - unsupervised i.e. independent of any pre-existing taxonomy
  - robust with respect to the sequencing errors that exists in pyro-sequences
- There are just a few algorithms that can perform the task of clustering a million of sequences in a reasonable delay

CD-hit-454

- It uses a fast sequence comparison heuristic based on k-mers
- It produces spherical clusters with a center (a "seed" sequence) and a maximal radius (user supplied)

Otupipe

- It uses an even faster sequence comparison heuristic
- It uses a different selection of the seed sequences

ESPRIT-Tree

- Fast heuristics with a metrics related to the Jaccard distance with the sequences represented as vectors of k-mers
- Agglomerative hierarchical clustering
- Clusters are more or less spherical with variable radius

Our method: DBC454

- Extremely fast and fully parallelized implementation of a density-based clustering algorithm (explained following slides)
- Input: data points must be defined in a Euclididan space with *n*-dimensions, *n*

$$dist(\vec{A}, \vec{B}) = \sqrt{\sum_{i=1}^{n} (A_i - B_i)^2}$$

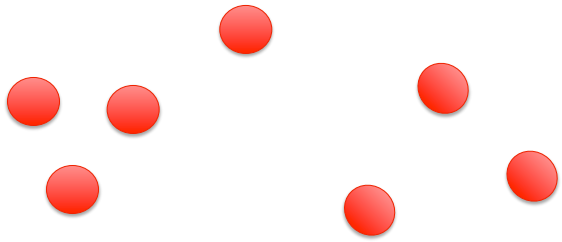
- How to represent a sequence as a point in an Euclidian space?
  - Counts of the dinucleotides XY (n = 16)
  - Counts of the dinucleotides X?Y (n =16)
  - Counts of the dinucleotides X??Y (n = 16)
- Total: 48 dimensions (16 XY + 16 X?Y + 16 X??Y)

Density-based hierarchical clustering

- Compute all pairwise distances and retain only those that are equal or smaller than a given distance threshold *T*
- A cluster is formed by single linkage and retained if it contains at least *N* points. Clusters too small are ignored
- Hierarchical clustering is obtained by repeating the clustering for increasing distance thresholds

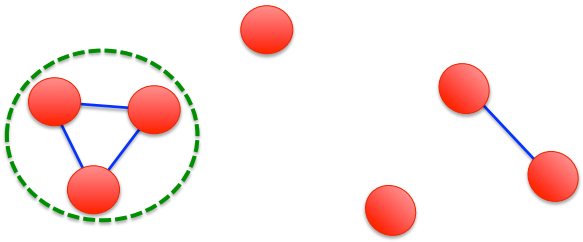
T from T_{min} to T_{max} in *s* steps

Density-based hierarchical clustering



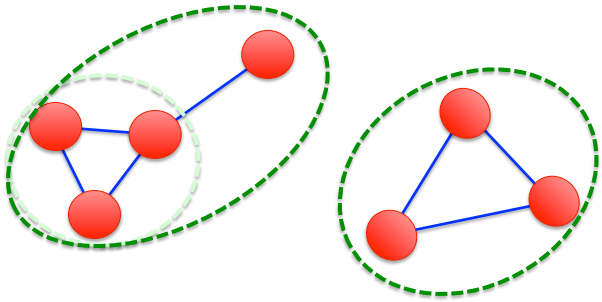
Density-based hierarchical clustering

- $T \leq 1$ and $N \geq 3$



Density-based hierarchical clustering

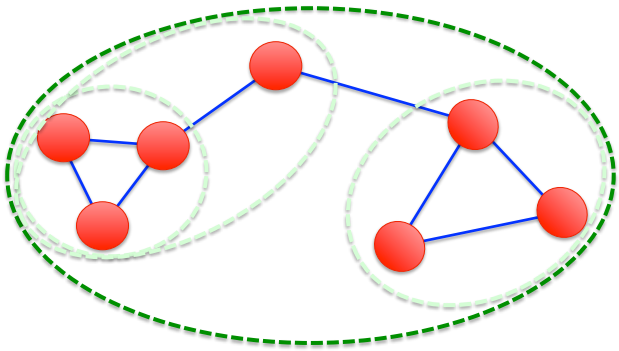
- $T \leq 2$ and $N \geq 3$



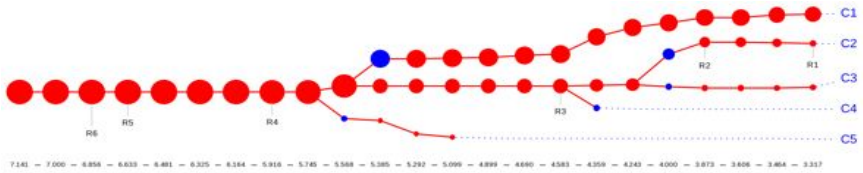
Density-based hierarchical clustering

- $T \leq 3$ and $N \geq 3$

Here, there is no such thing as a cluster center or a maximal cluster radius



Assignment to the final partition



- At the end of the hierarchical clustering, seed clusters are determined and the points are re-attributed to the closest seed
- Optionally, reference sequences can be attributed to the closest seed lusters

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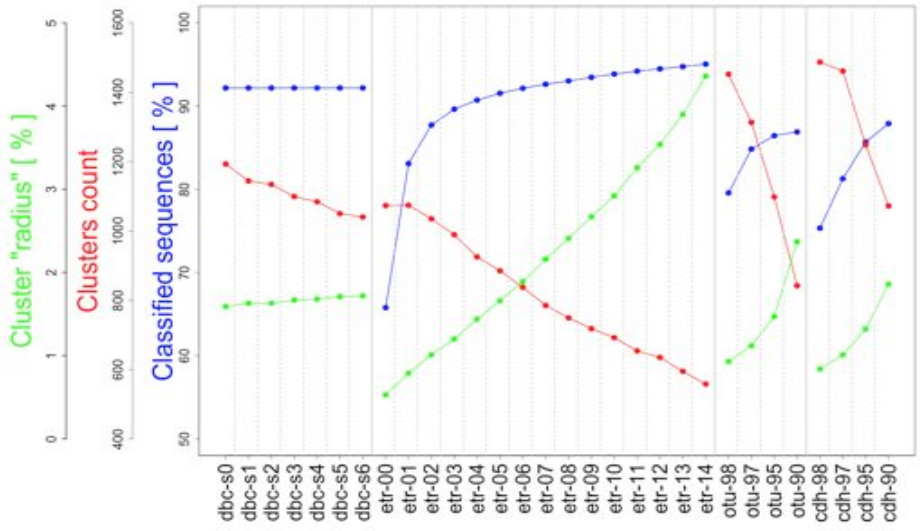
Algorithms comparison ($N \geq 100$)



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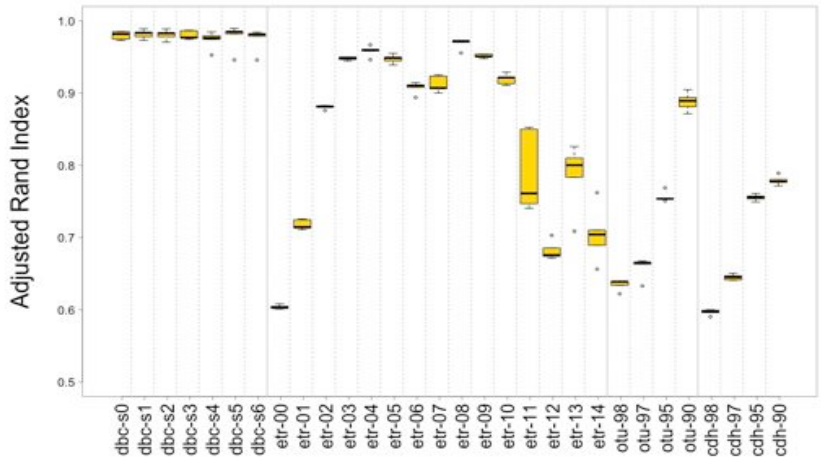
Five datasets with added errors

- Five mutated dataset were created to simulate the errors that occur during the 454 sequencing:
  - 18% of the original sequences were randomly selected
    - 61% were given a single error
    - 17% two errors
    - 22% three errors.
  - The types of errors introduced were
    - 46% insertions,
    - 33 % deletions
    - 21% substitutions

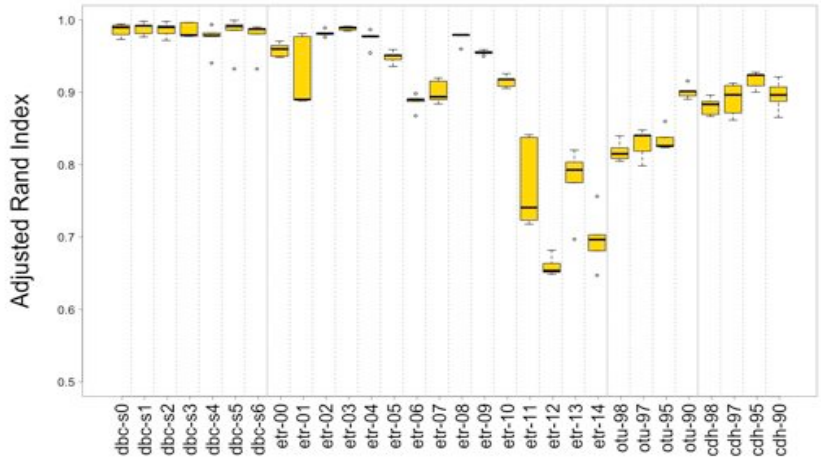
Robustness I: orginal *versus* mutated dataset

Noise was pooled as a single additional cluster



Robustness II: orginal *versus* mutated dataset

Sequences appearing in the noise were removed



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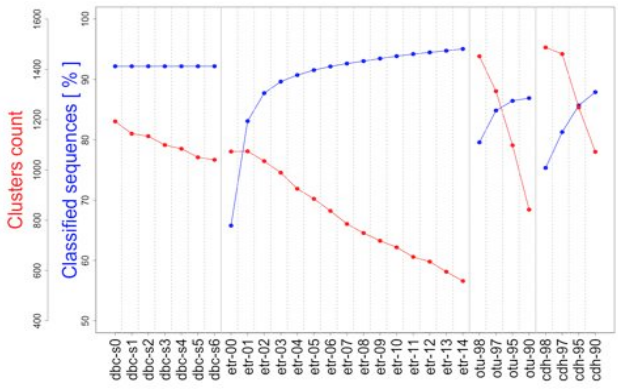
Conclusion

- Our algorithm is more robust to sequencing errors, and classifies more sequences than methods using fast heuristics



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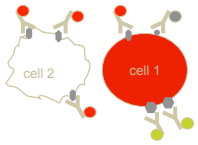
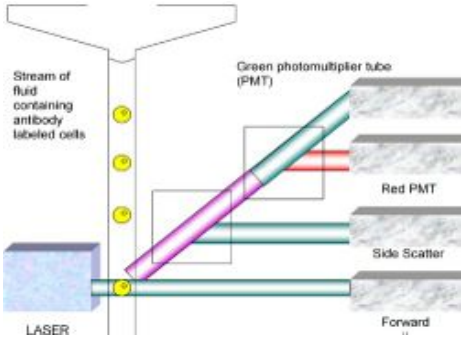
Sequences are not the only ones to be sorted



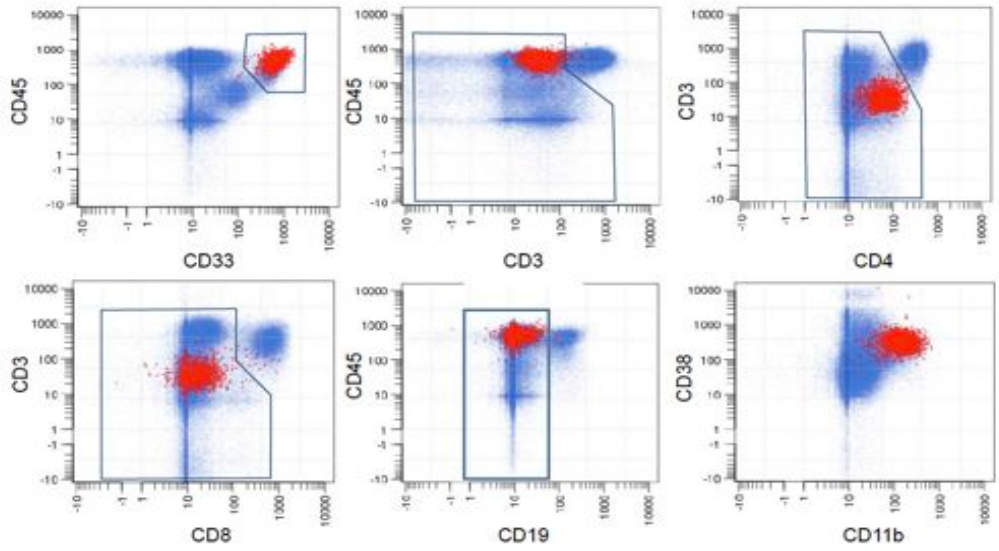
Fluorescent Activated Cell Sorting (FACS)



- Instruments can record >10 channels (colors)
- Measurements collected for 10^5 - 10^7 cells
- Analysis of cell populations is tedious and involves manual steps (biaised)

	FSC	SSC	color1	color2	color3
cell 1	100	100	100	100	200
cell 2	100	500	300	0	0

8 markers: CD45, CD33, CD3, CD4, CD8, CD19, CD38, CD11b



CERN. May 28th 2013

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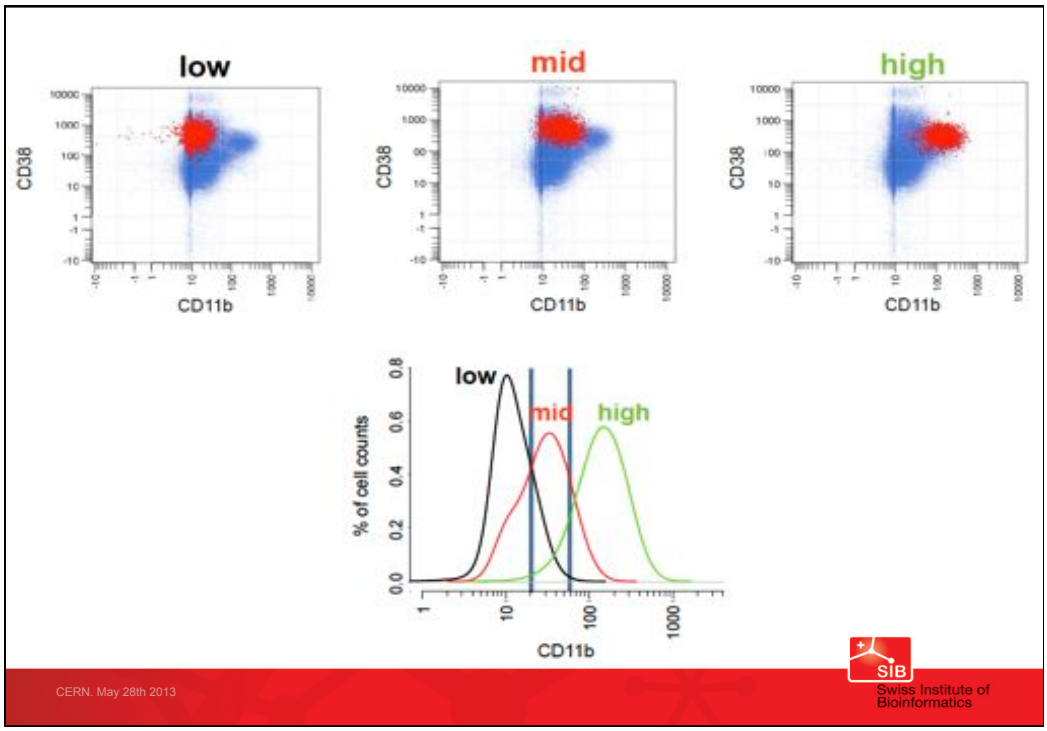
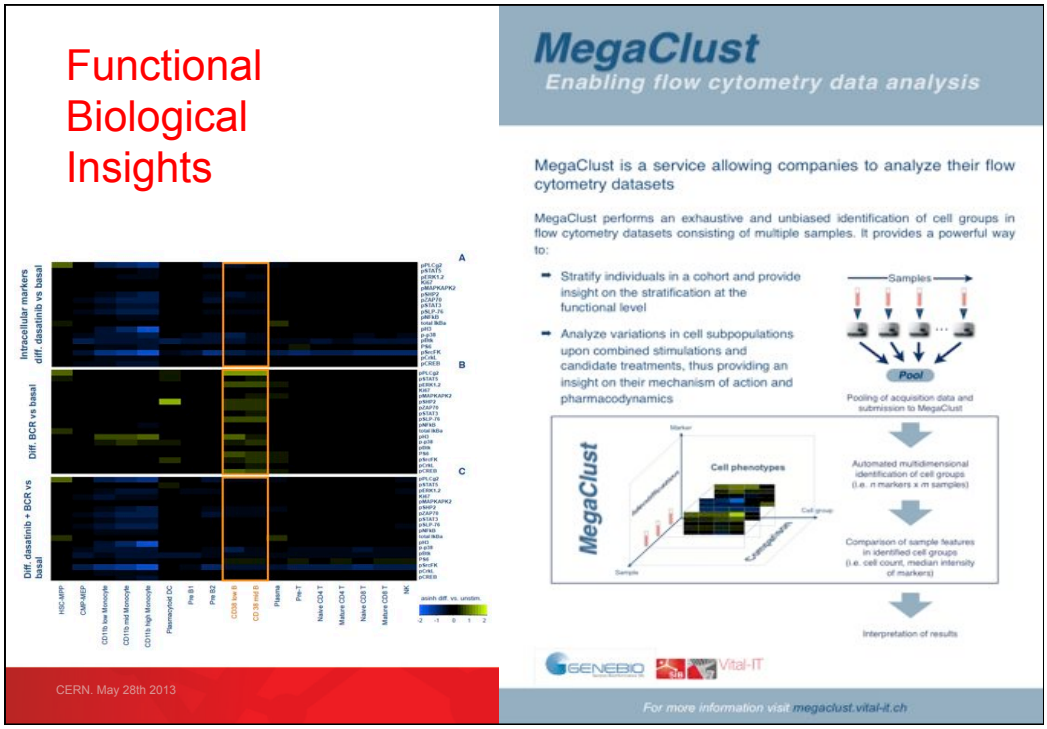
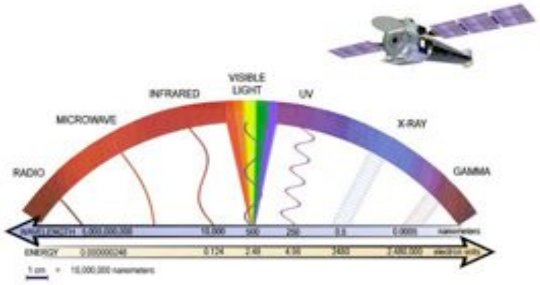


Functional Biological Insights



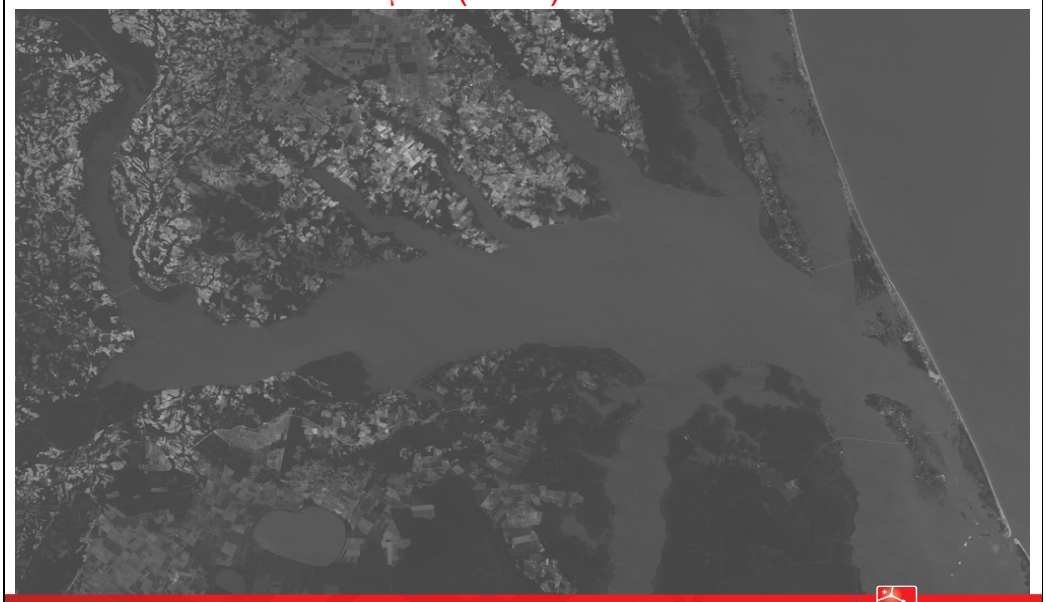
Applications to other large multidimensional datasets

- Small number of parameters measured
  - multi spectral data (various wavelengths, such as IR, visible, X-ray, ...)



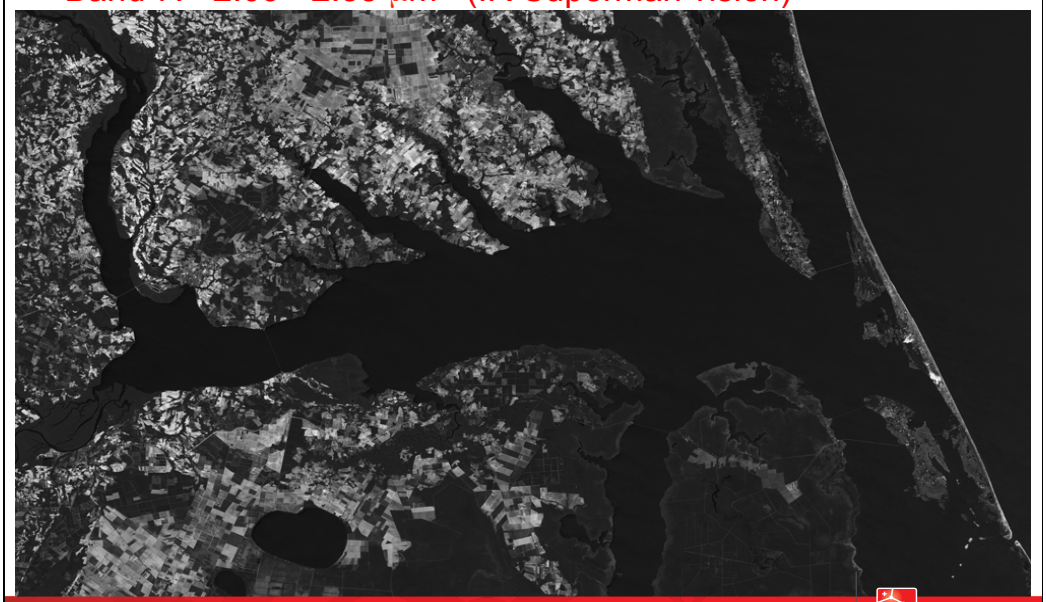
- Large number of measurements
  - pixels !

Band 1: 0.45 - 0.52 μm (violet)

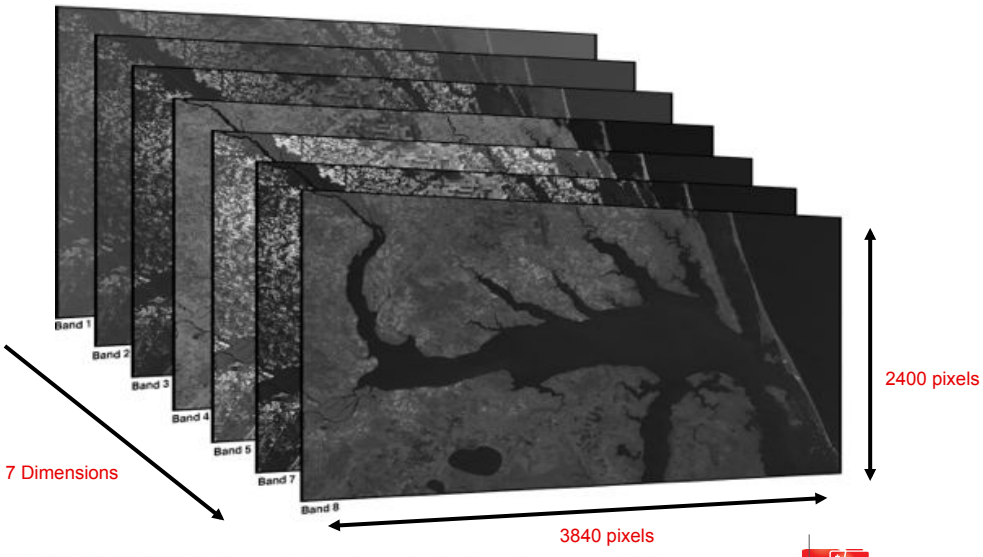


Band 8: 0.53 - 0.90 μm (green to red)

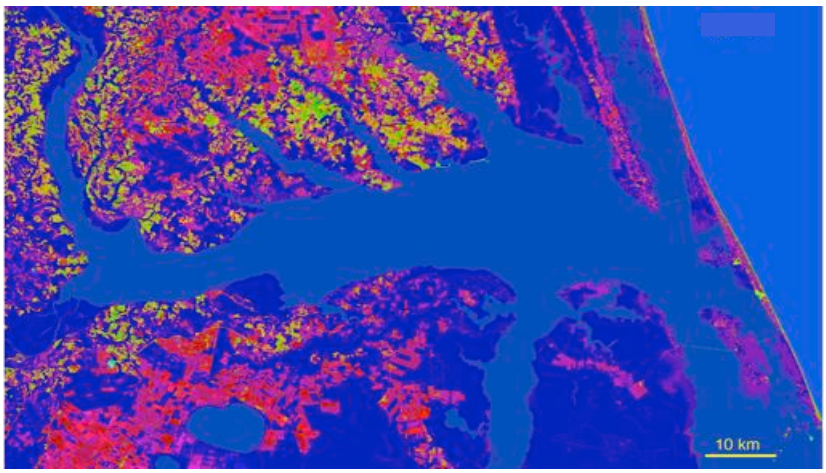
Band 7: 2.09 - 2.35 μm (IR Superman vision)



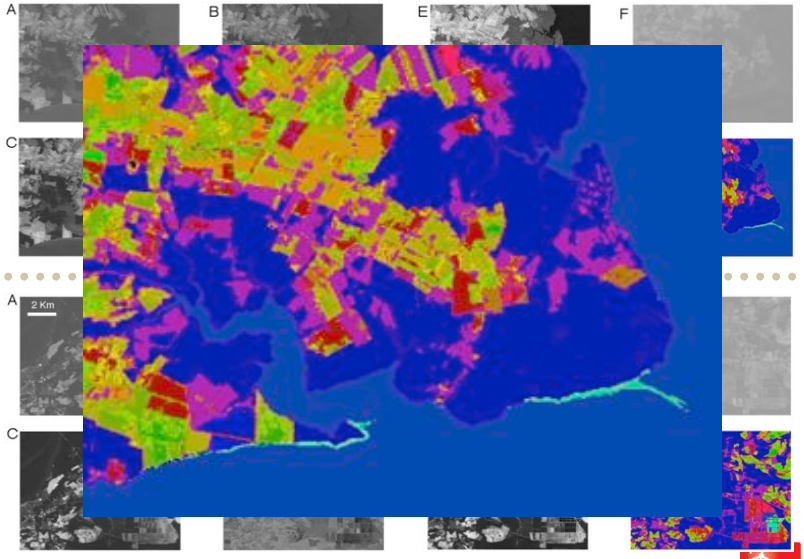
Multispectral image



Multispectral image Clustering Results



Enlargements



Funding sources

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NATIONAL INSTITUTES OF HEALTH

SIXTH FRAMEWORK PROGRAMME

SEVENTH FRAMEWORK PROGRAMME

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ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

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Innovative Medicines Initiative

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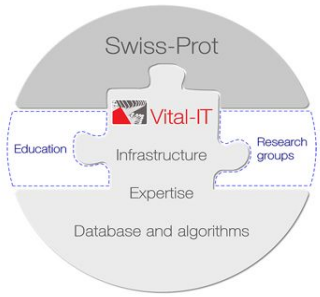

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