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Combining Bacterial Fingerprints

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Who's world is this?

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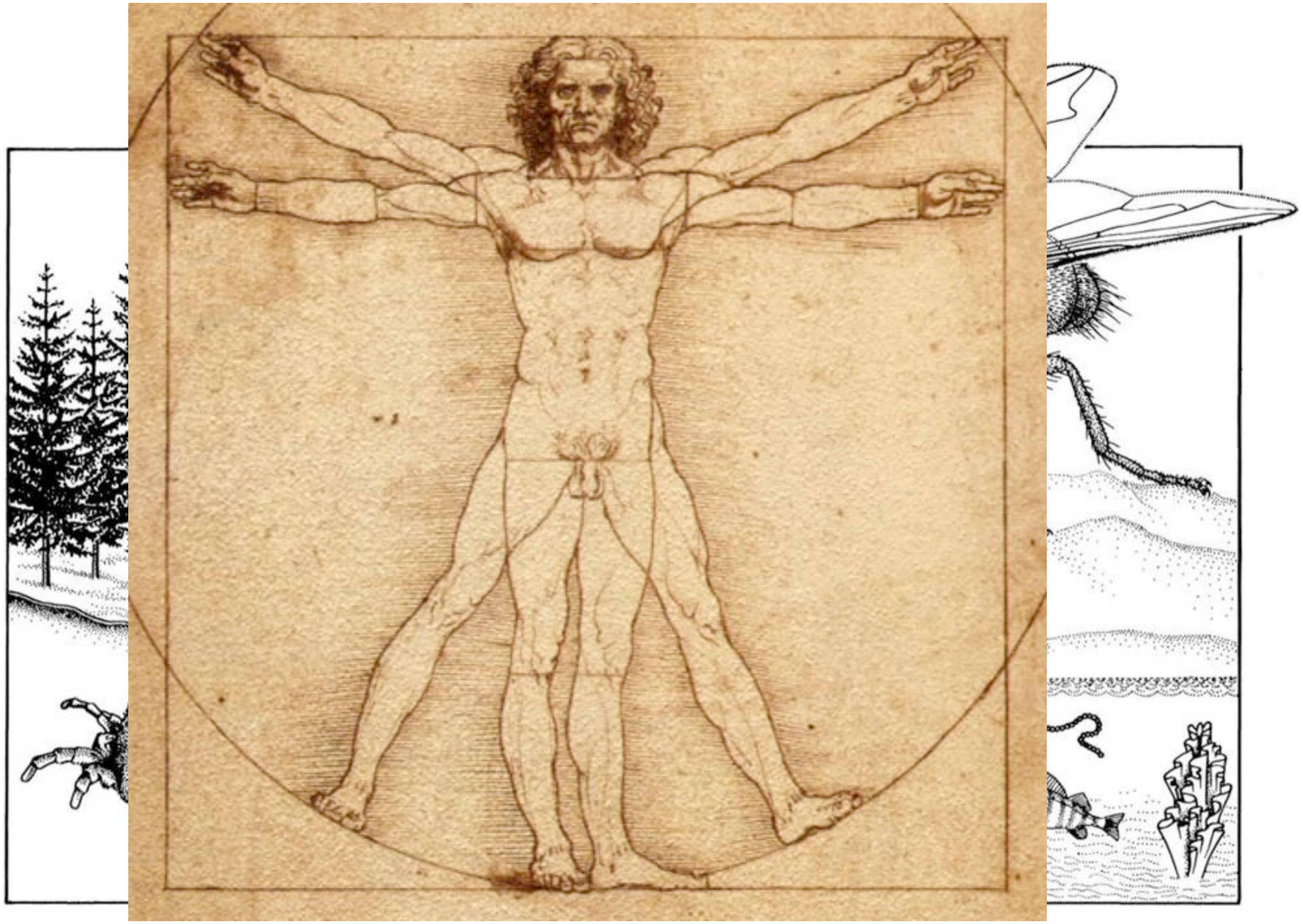
We are one out of 2 million named species (5-100m est.)



Ignores 1 Billion species Bacteria!

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Million years on earth

3600 Bacteria

500 Fish

130 Flowers

190 Mammals

0.2 Humans

Bacterial inventions

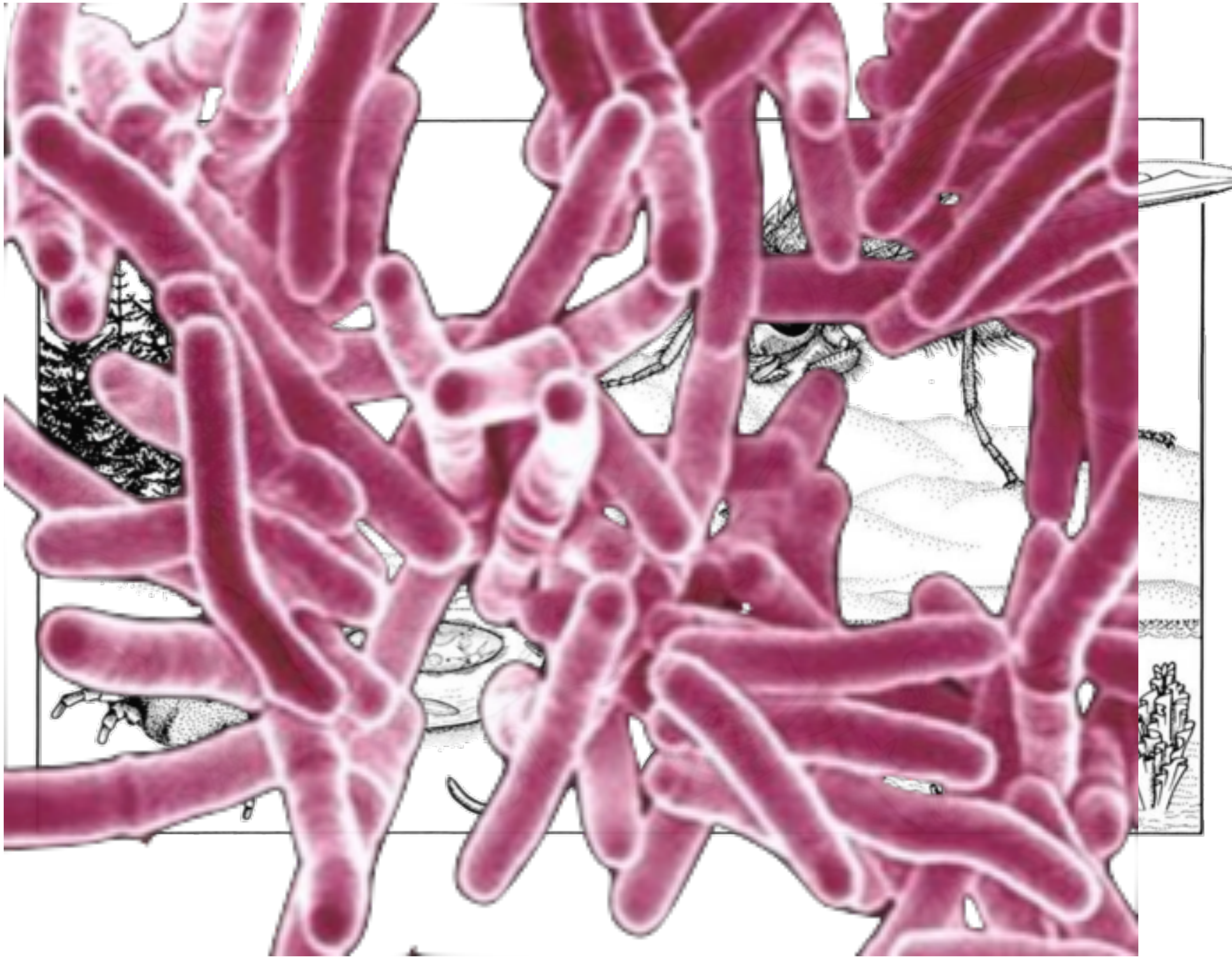
Oxygen

Photosynthesis

Nitrogenation

the Nucleus

Mitochondria



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Whose world are you?

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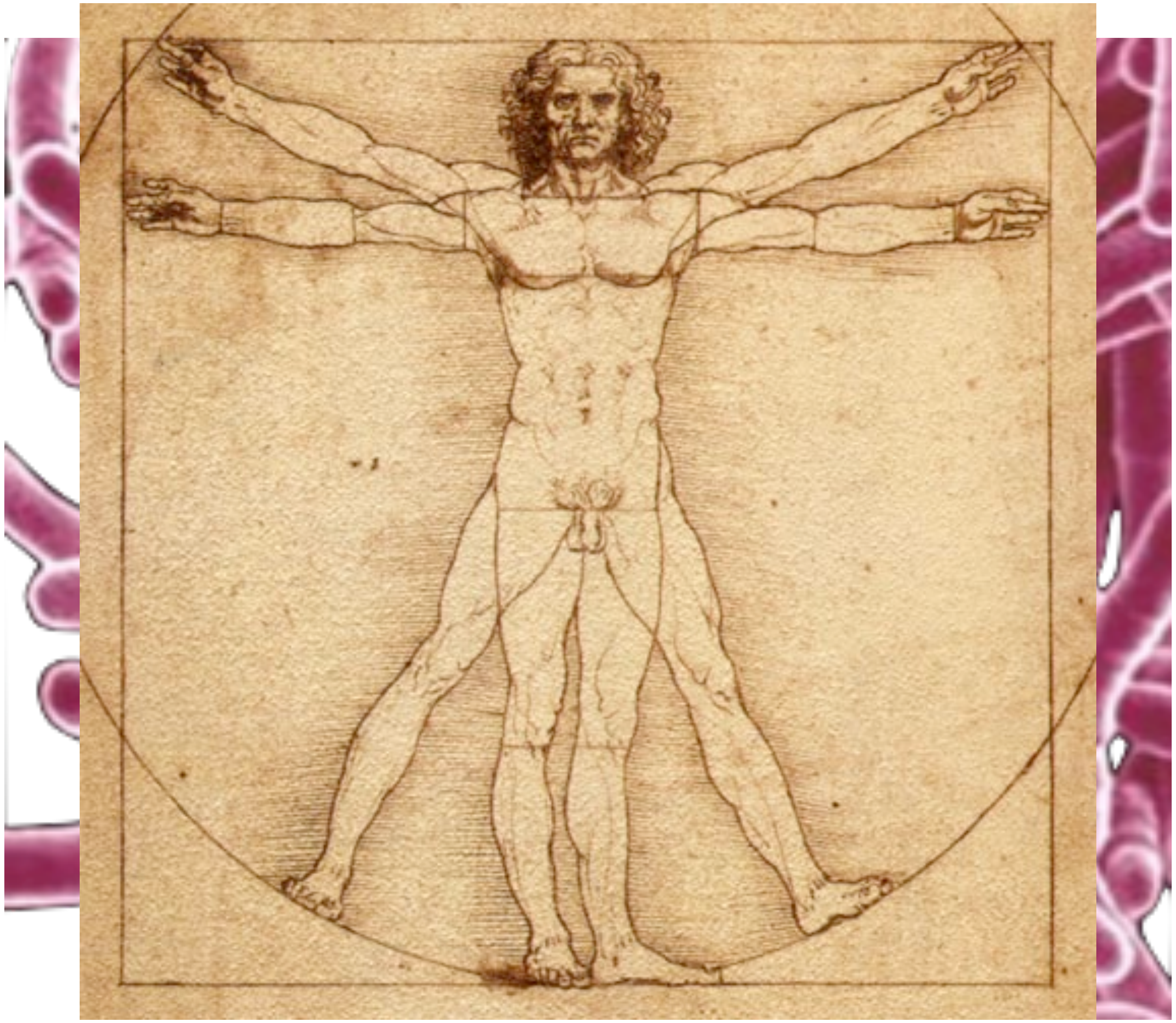
90% of the cells in your body are bacterial

≥ 99.99% of the gene products in your body are bacterial

Biologically, you are mostly a bacterial ecosystem



The dark microbiome

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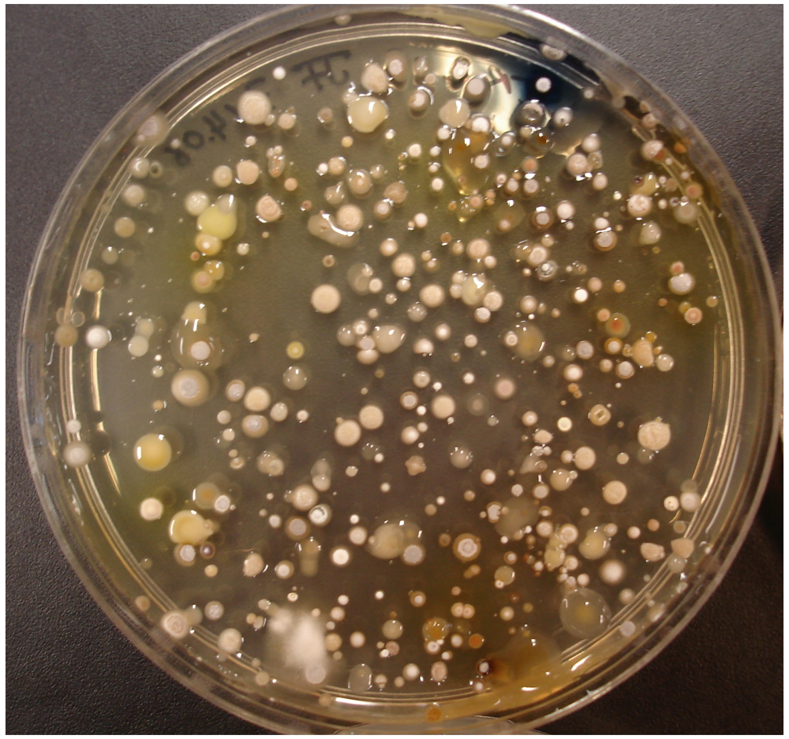
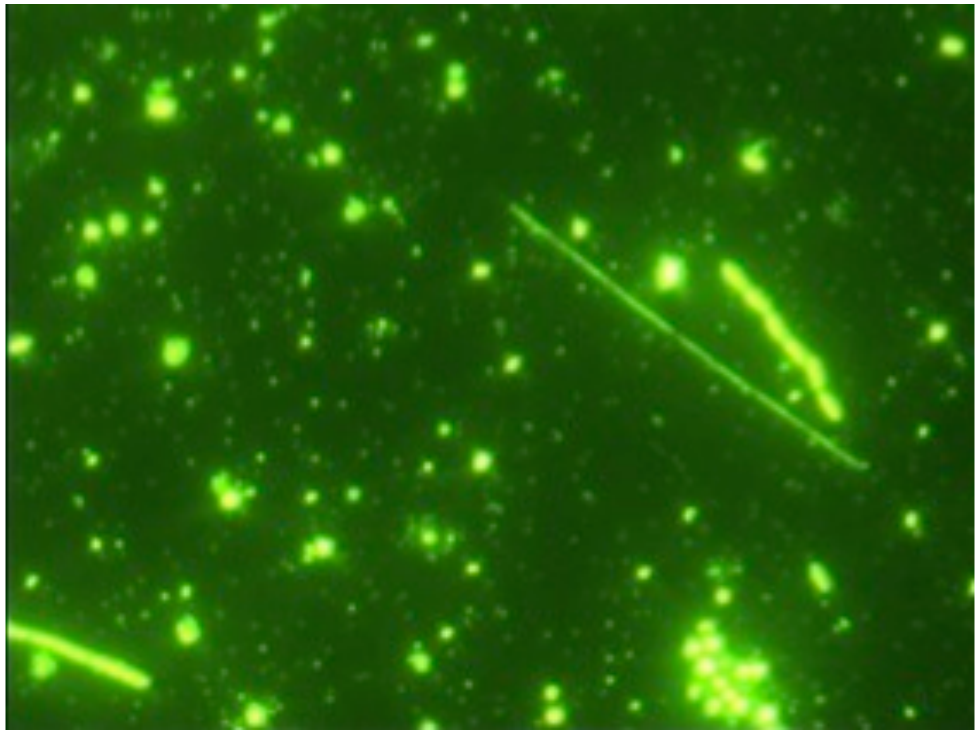
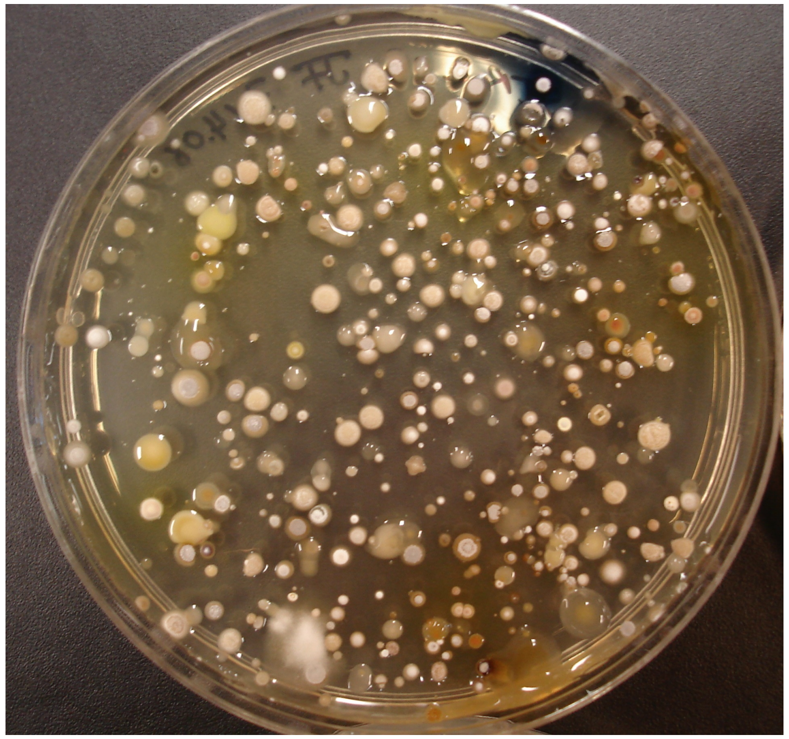
- Up to 1 Billion species, about 5,000 known
  - 10K species in a gram of soil
  - 1cm^2 intestine: bacteria > all humans, ever
  - Half of all Phyla undiscovered (human vs sponge)
- Great plate count anomaly: approx. 97% of bacteria cannot be grown



The dark microbiome

- Great plate count anomaly: approx. 97% of bacteria cannot be grown
- What we do know is *highly biased*

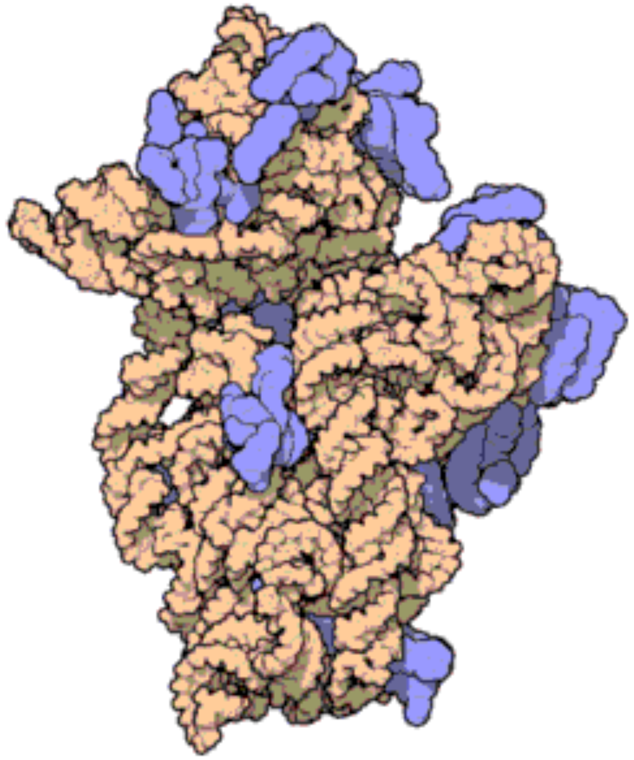


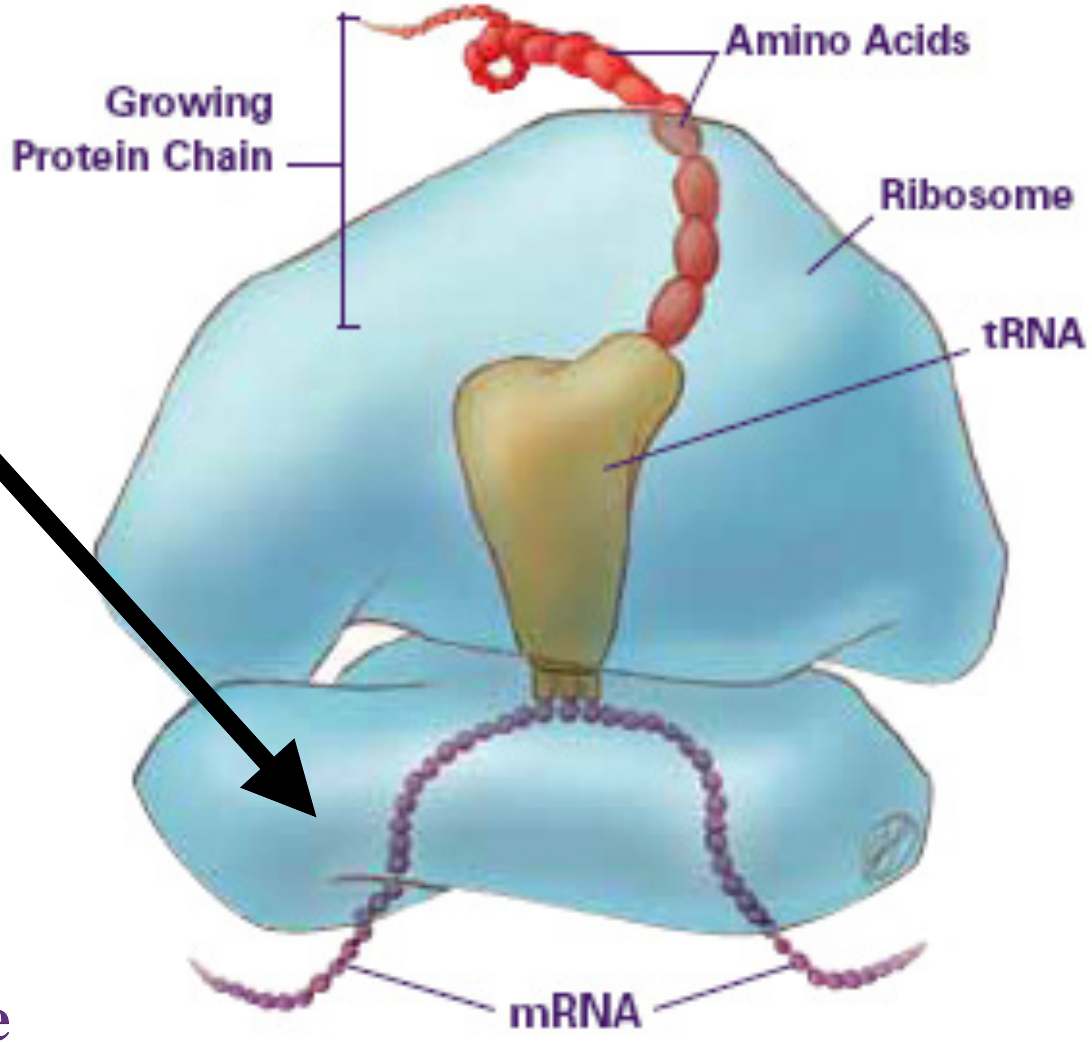


How to count the invisible

All bacteria:

- Translate genome to proteins
- Using *ribosome*: RNA + proteins
- Small subunit attaches first
- Coded by 16S gene





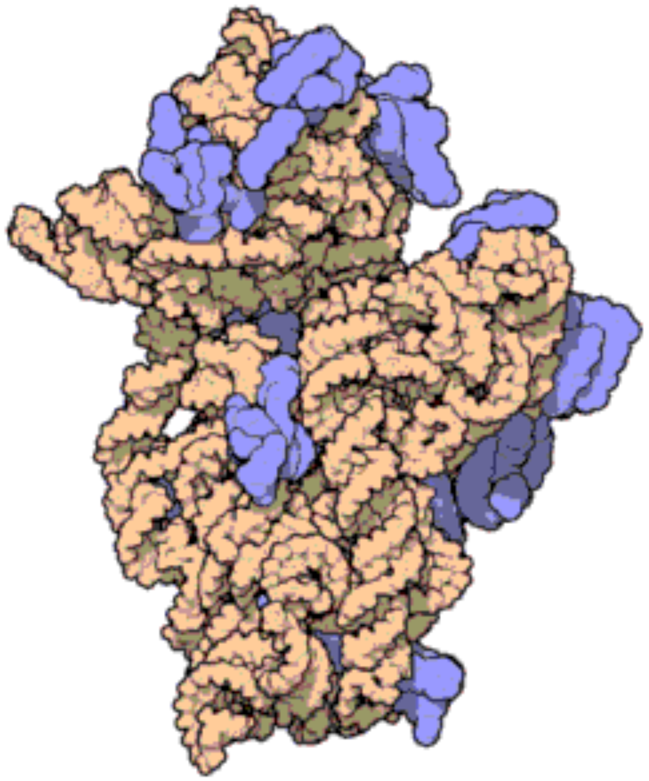
Genome

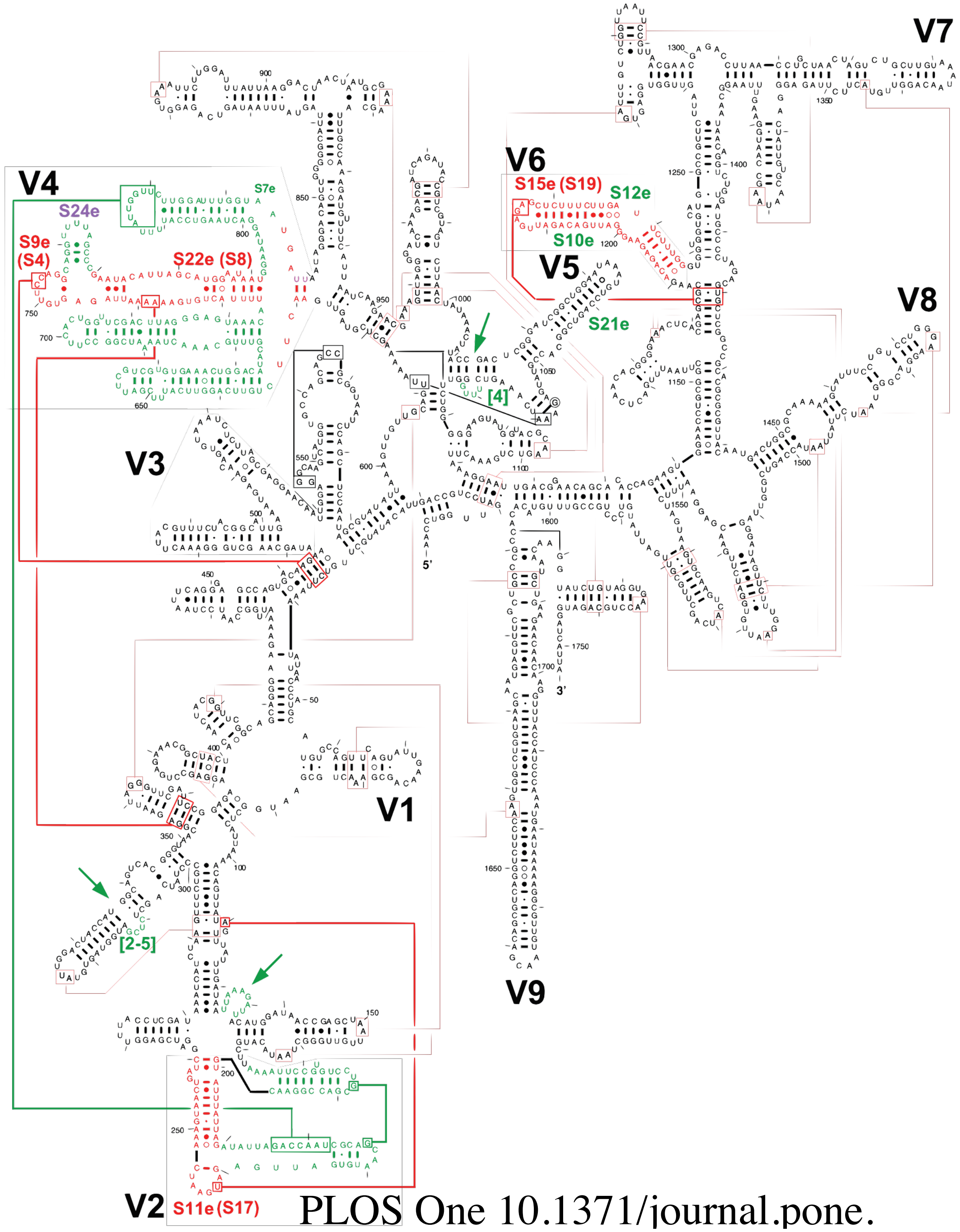
16S small subunit fingerprints

Secondary structure

Variable/conserved regions

V1 – V9: Fingerprints





PLOS One 10.1371/journal.pone.

High throughput fingerprinting

1. Get “*every*” DNA molecule in a sample: break cells up, wash, filter
2. Isolate fingerprint regions from *all* bacteria
3. Sequence them *all*

↓

10–20 million fingerprint sequences

↓

Infer how many of which species were there

Interpreting fingerprint data

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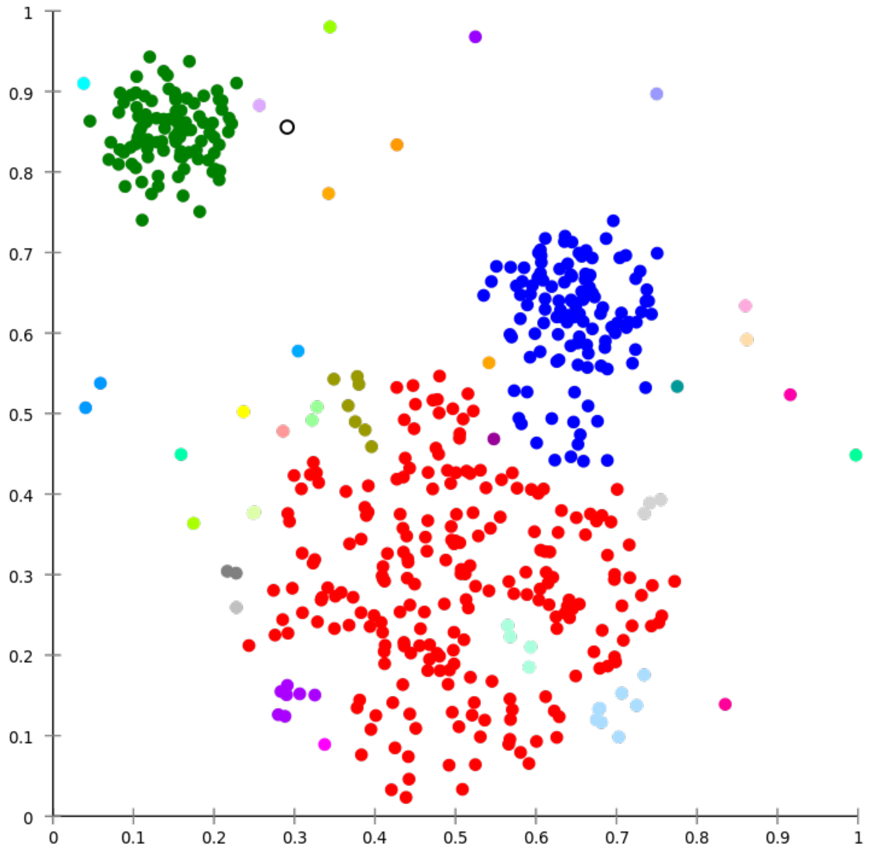

1. Compute similarity (distance) between fingerprint sequences
2. Cluster, call a cluster a “species”
3. Number of clusters is species richness
4. Size of clusters is species abundance

Problem – Solution

?

- Fingerprints evolve at different rates: which to use?
- Varies with species: need to know who is there to choose best fingerprint!

! Use multiple fingerprints!

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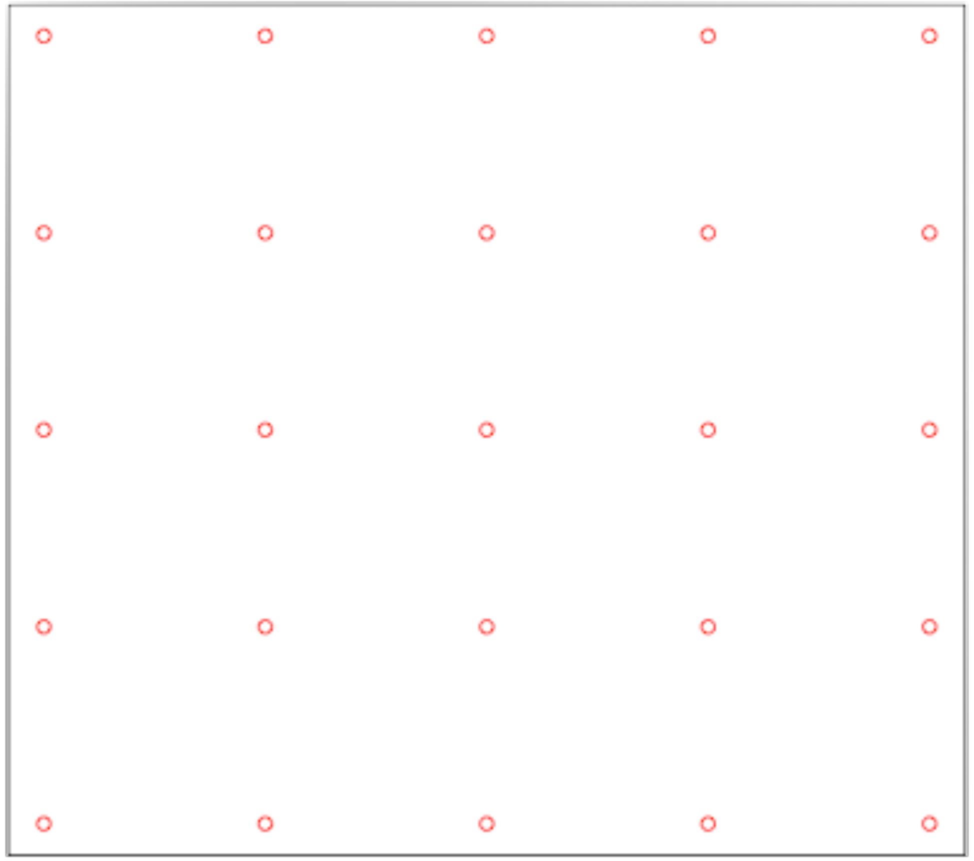
- How to compare distances between sequences from unknown species?
- With multiple fingerprints with unknown biases?

Solution: Find registration marks

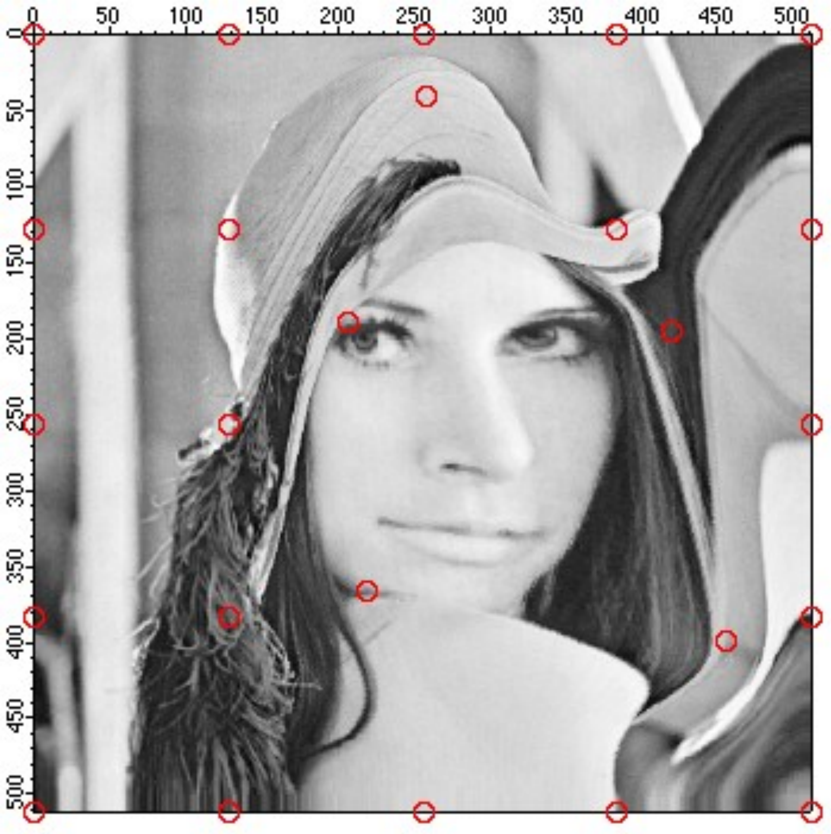
!

Translate distances using reference points from known fingerprints

Registration marks



Observed image



Solution: Use image registration

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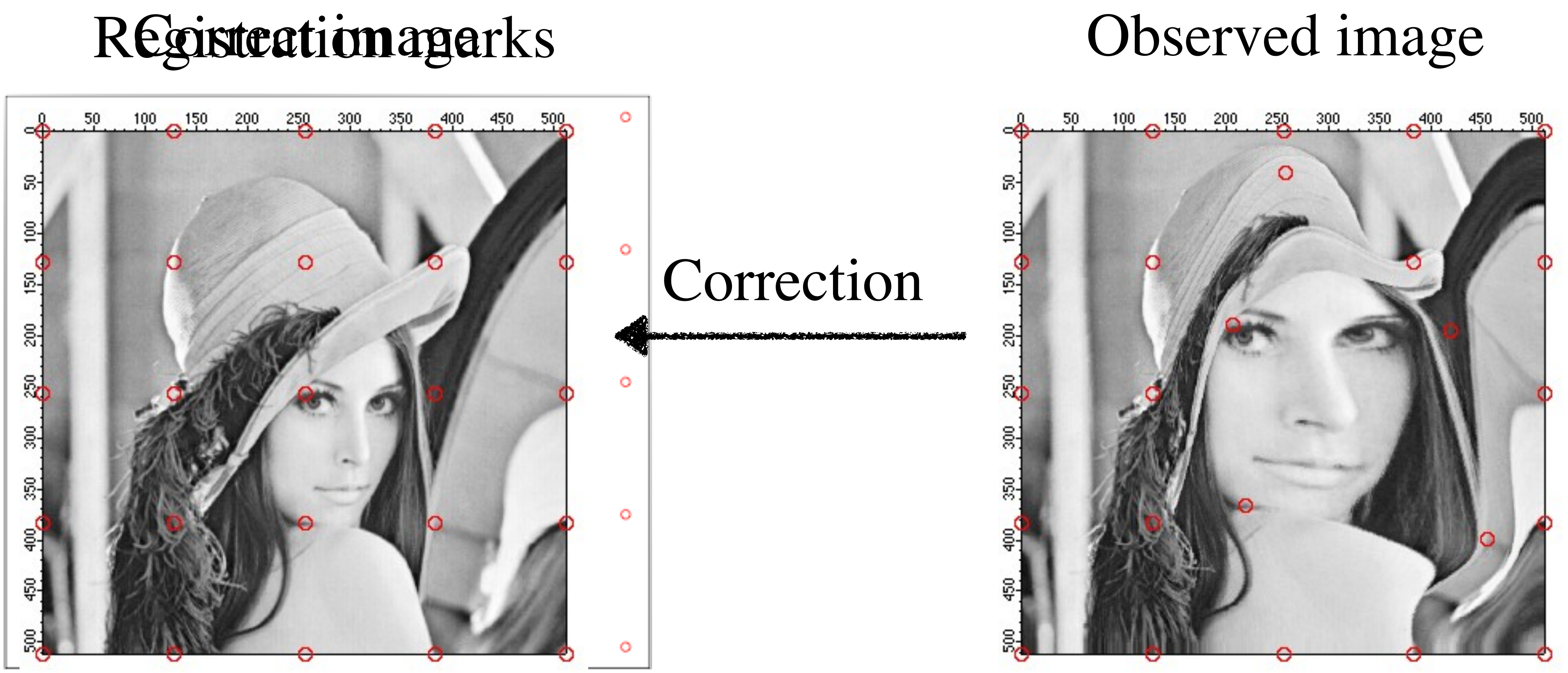
Translate distances using reference points from known fingerprints



Solution: Find registration marks

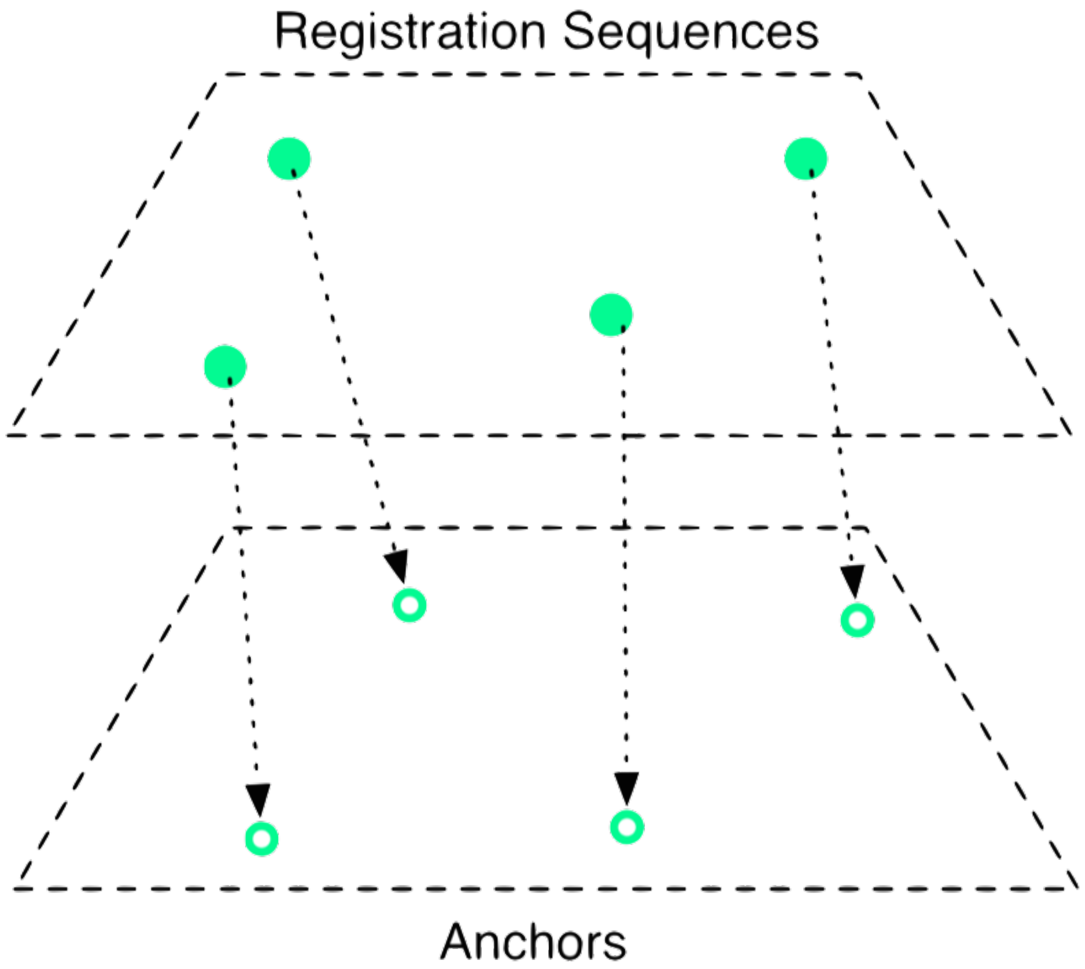
Determine distances for known full 16S sequences

Extract a fingerprint

Determine distances for reference fingerprints (anchors)

● Known full 16S sequences

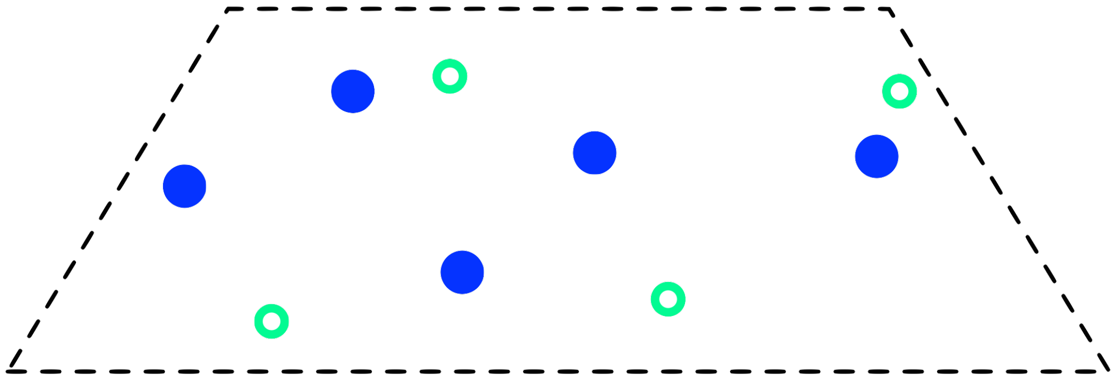
○ Fingerprint subsequences



Solution: add empirical reads

Combine DNA sequences with (known) anchors

Compute distances from reads to *all* sequences



Empirical Reads

● Actual reads

○ Fingerprint sequence

Observed image

Solution: match registration

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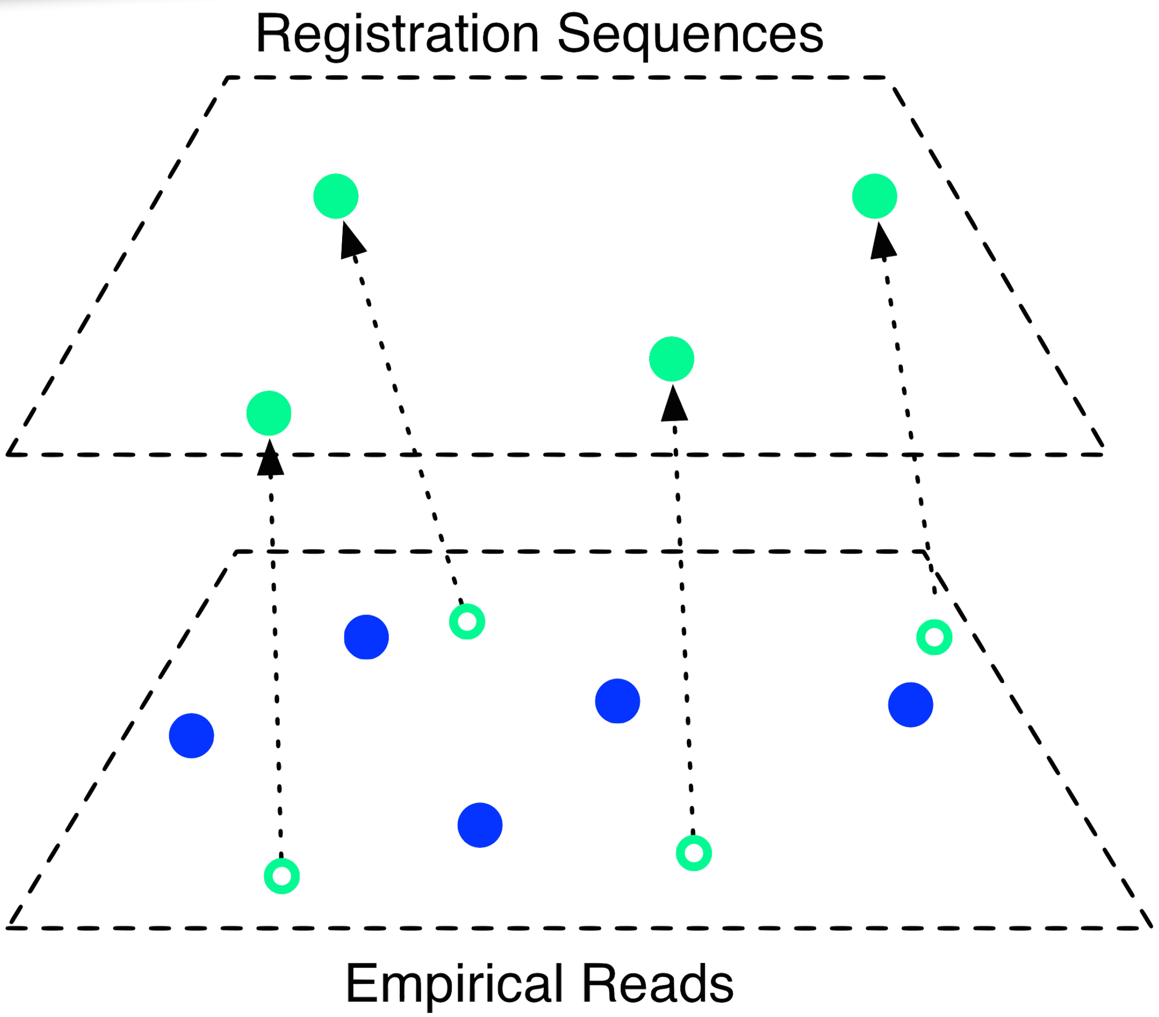
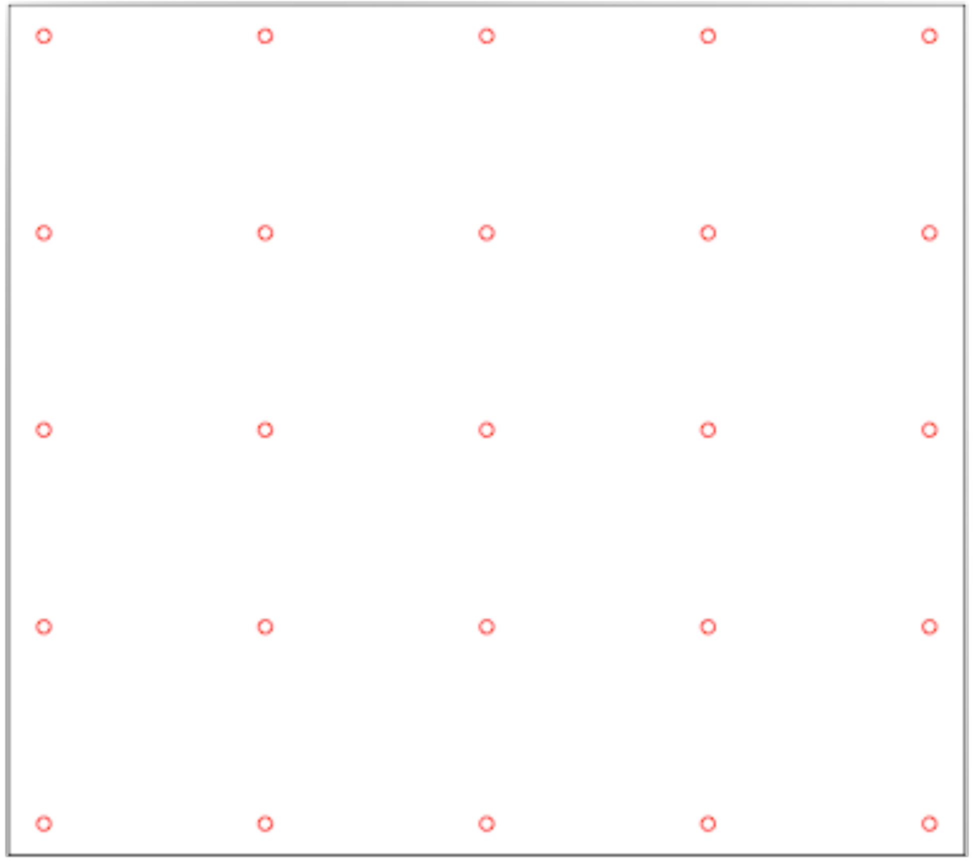
Map anchors back to known registration marks



Registration marks



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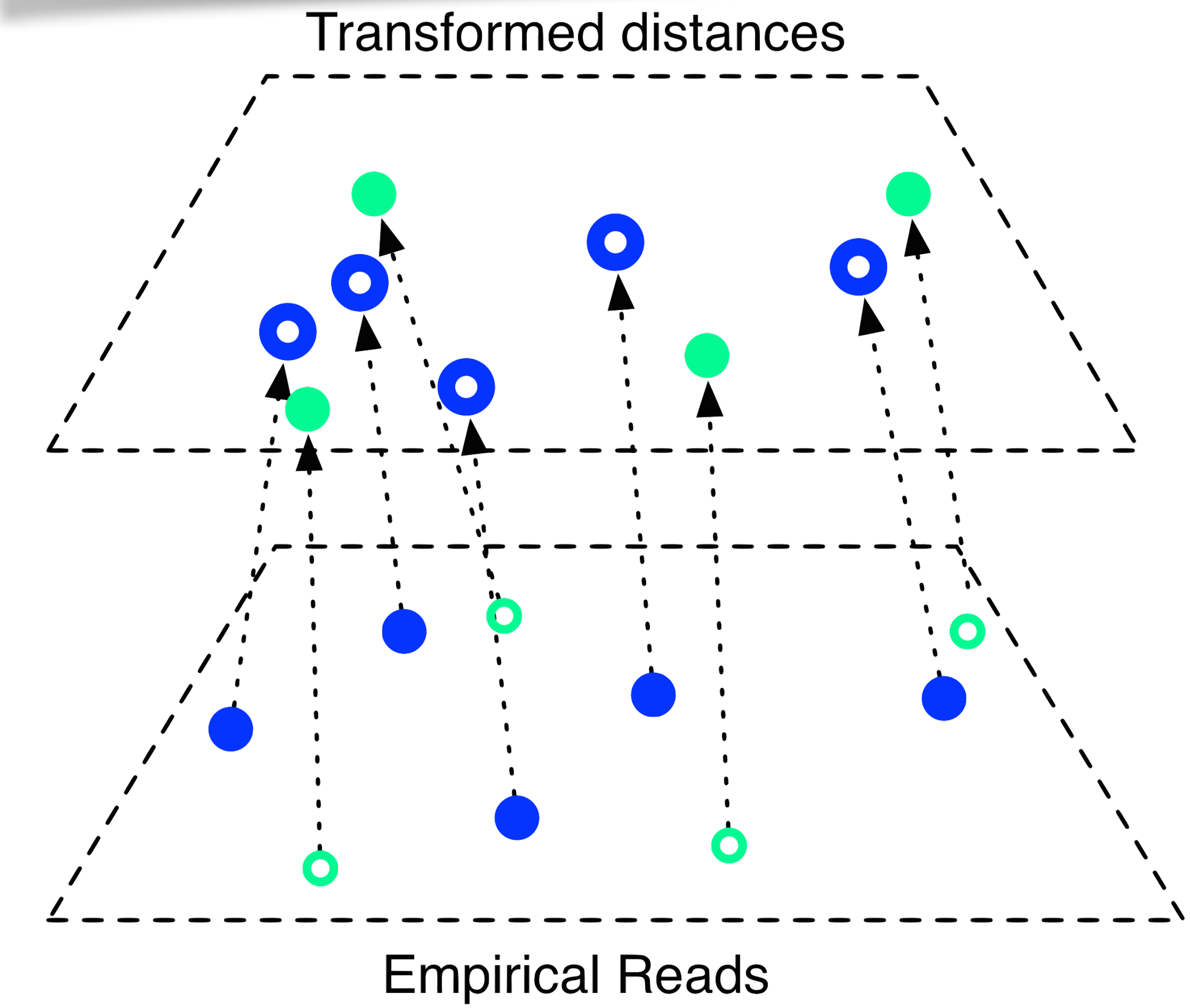
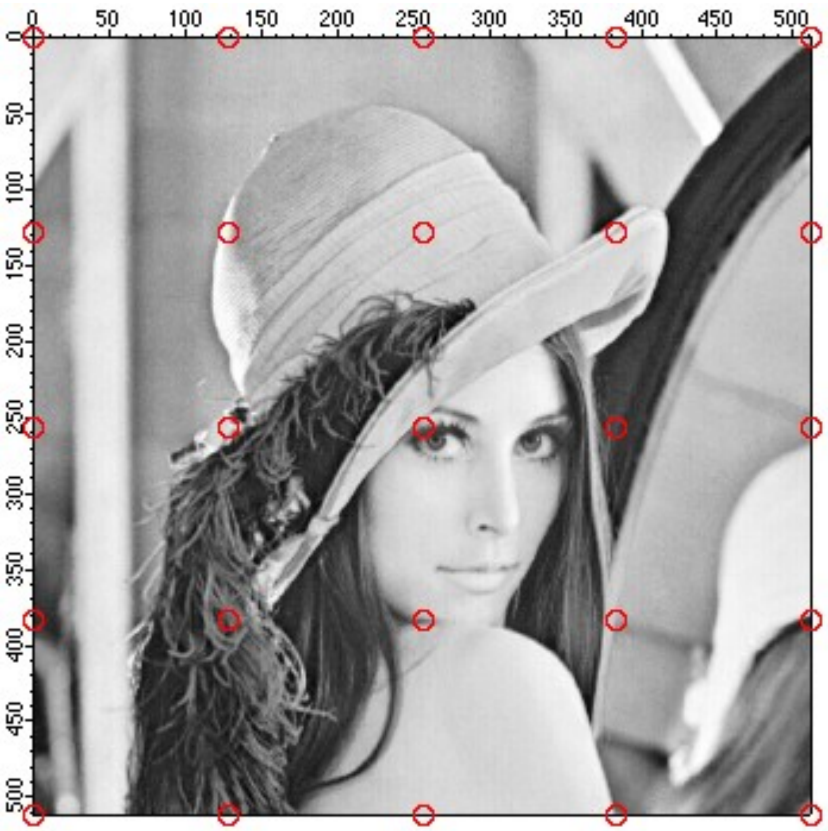
Solution: Move empirical data

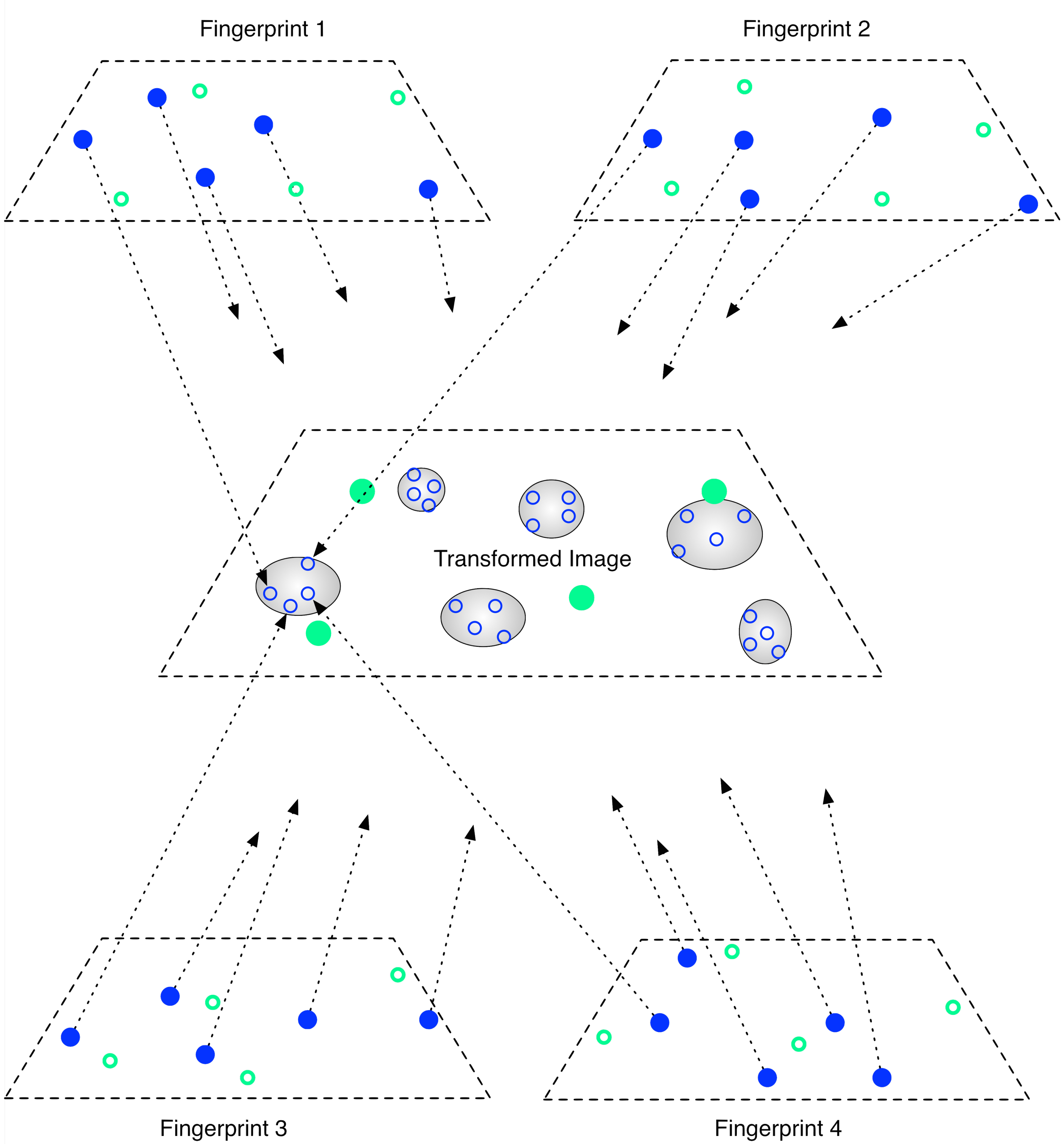
Carry empirical reads along



Correct image



Solution: combine fingerprints



Repeat for multiple fingerprints

Remove outliers

Cluster results

Current activity

- Find efficient 2D mapping: nonmetric multidimensional scaling (NMDS)
- Develop fast distance computation algorithms: pre clustering plus hashing
- Develop accuracy statistics: perturbation analysis
- Determine how many "registration sequences" are best for 20 million empirical points
- Precompute registration libraries for different sample types (soil, human microbiome, ocean, etc.)
- Determine accuracy with simulation and known sequences

Future work

- ✦Parallelize: use reference triangles
- ✦What to do with outliers?
- ✦What do cluster shapes/density say about fingerprints, species, ecology?
  - Which fingerprints are good for which species?
  - Which fingerprints are most misleading in given environments?
- ✦How are clusters and evolution related?
- ✦Application to empirical data: milk project
- ✦Many more!

Acknowledgements

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Institute for Bioinformatics and Evolutionary Studies (IBEST)

- IBEST Computational Resources Core

Students

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- Janet Williams, BCB PhD student
- Daniel Beck, BCB PhD