An overview of the (Flow) Cytometry Core Facility

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Newcastle University

THE Flow Cytometry Core Facility

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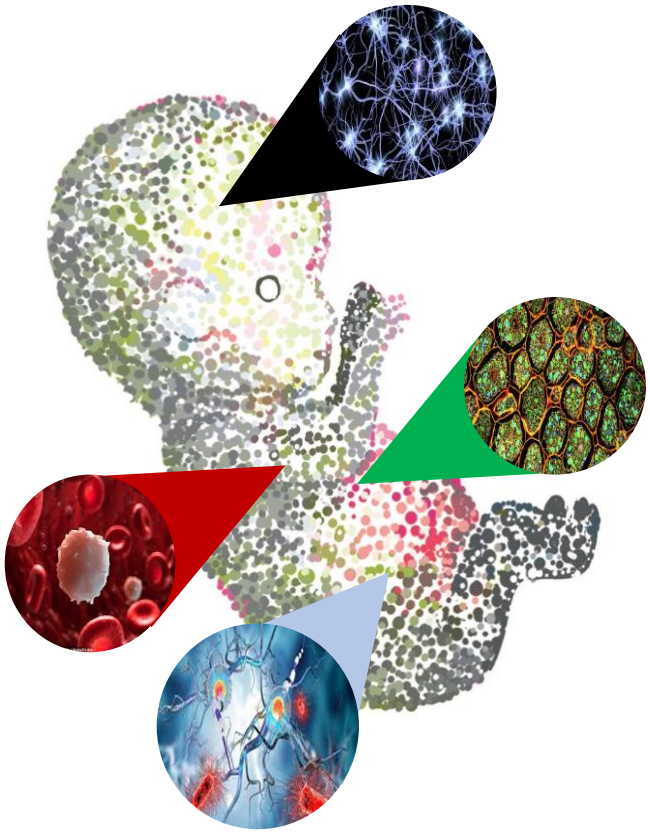
Presentation overview

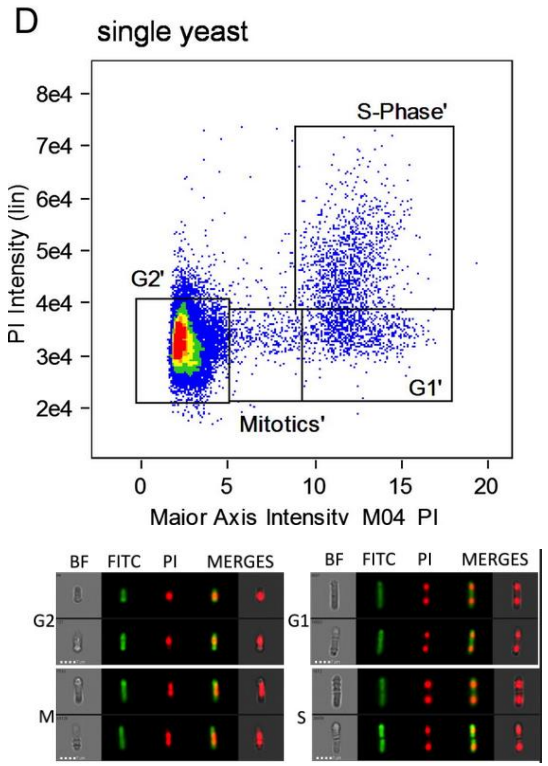
- Cellular heterogeneity: The **eternal common** problem
- What is (Flow) Cytometry and how can it help
- The Newcastle University FCCF:
  - Ethos
  - Technologies
  - Techniques/methods/services
  - Staff
- Summary

Heterogeneity: The biggest challenge to ALL cellular research

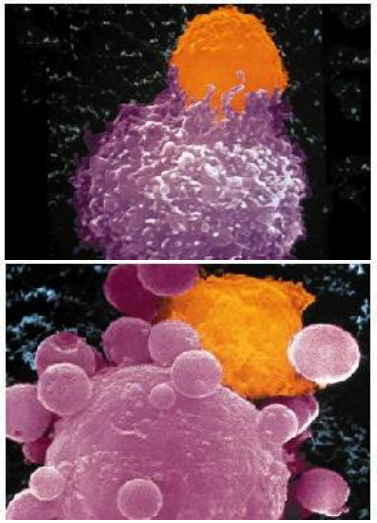
1. Different (stable) cell types



2. Transition states (temporal)



3. Functional states



Cells doing a "job" such as killing others

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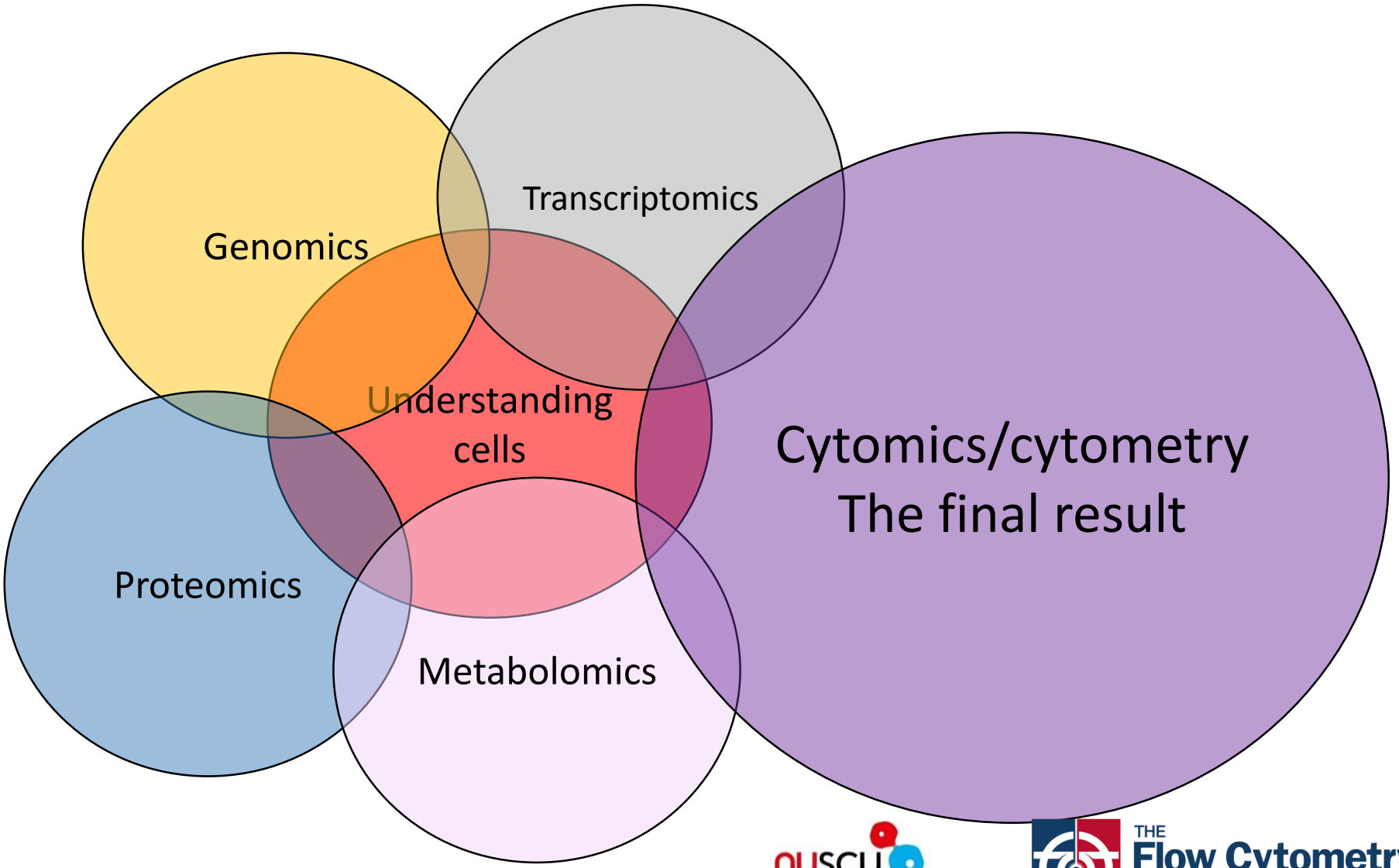
Understanding cells and cell systems is all about the Omics…..



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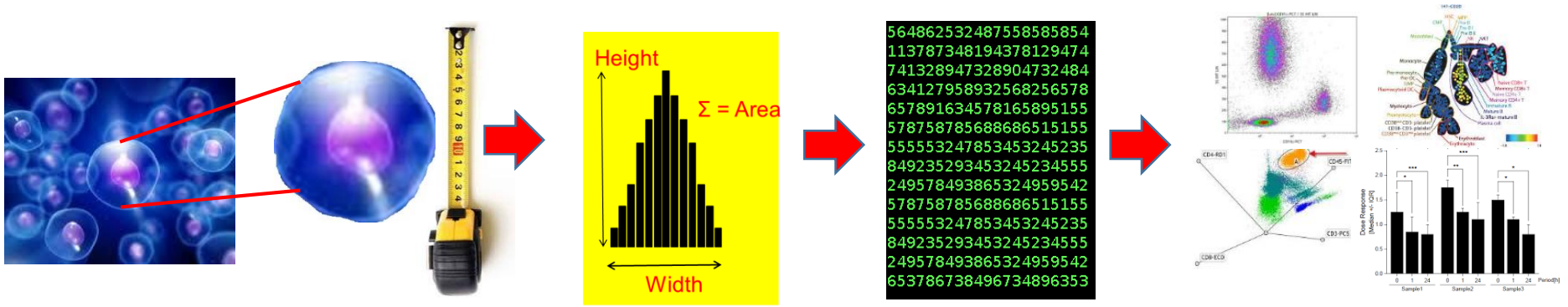
What is Cytometry?

Cytometry

Greek = “Kytos”
“hollow basket”
Relates to CELL

Greek = “Metria”
“Process of Measuring”

"**Cytometry** is the measurement of cell phenotype, form and function at the single object (cell) level conducted on a **population**-wide basis in order to understand and decode the heterogeneity inherent to **ALL** systems"



Cytometry is:

1. High throughput, capable of analysing many cells (statistical power)
2. Multi-parameter, can make multiple measures of single cells
3. Can be zero-resolution or image based, but always (semi) **QUANTITATIVE**
4. **POWERFUL for decoding cellular heterogenity**

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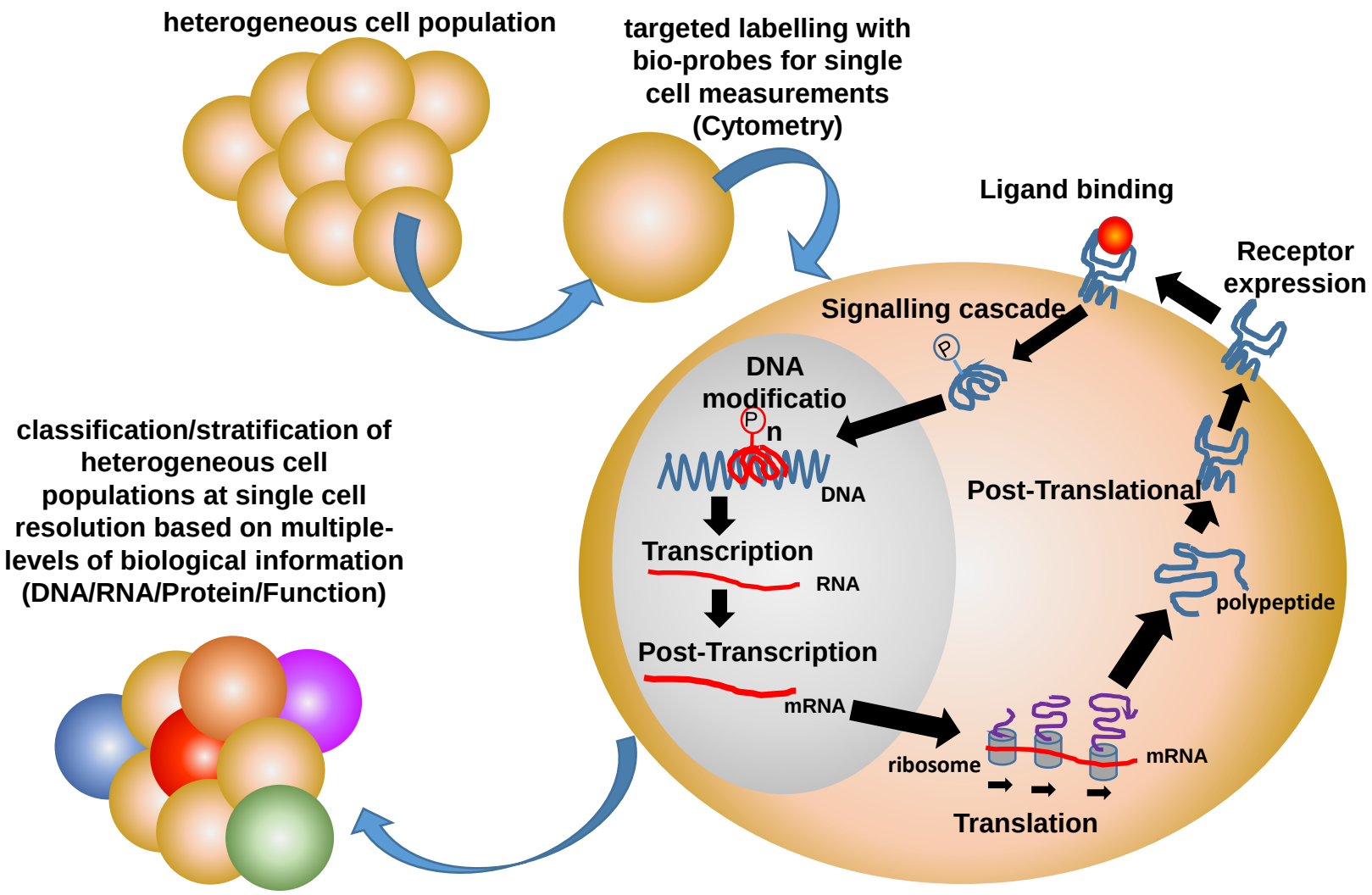
Cytometry is the study of EVERYTHING single cell



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The Flow Cytometry Core Facility (FCCF) @ Newcastle University: Multiple locations

NICR: Herschel

iCFL

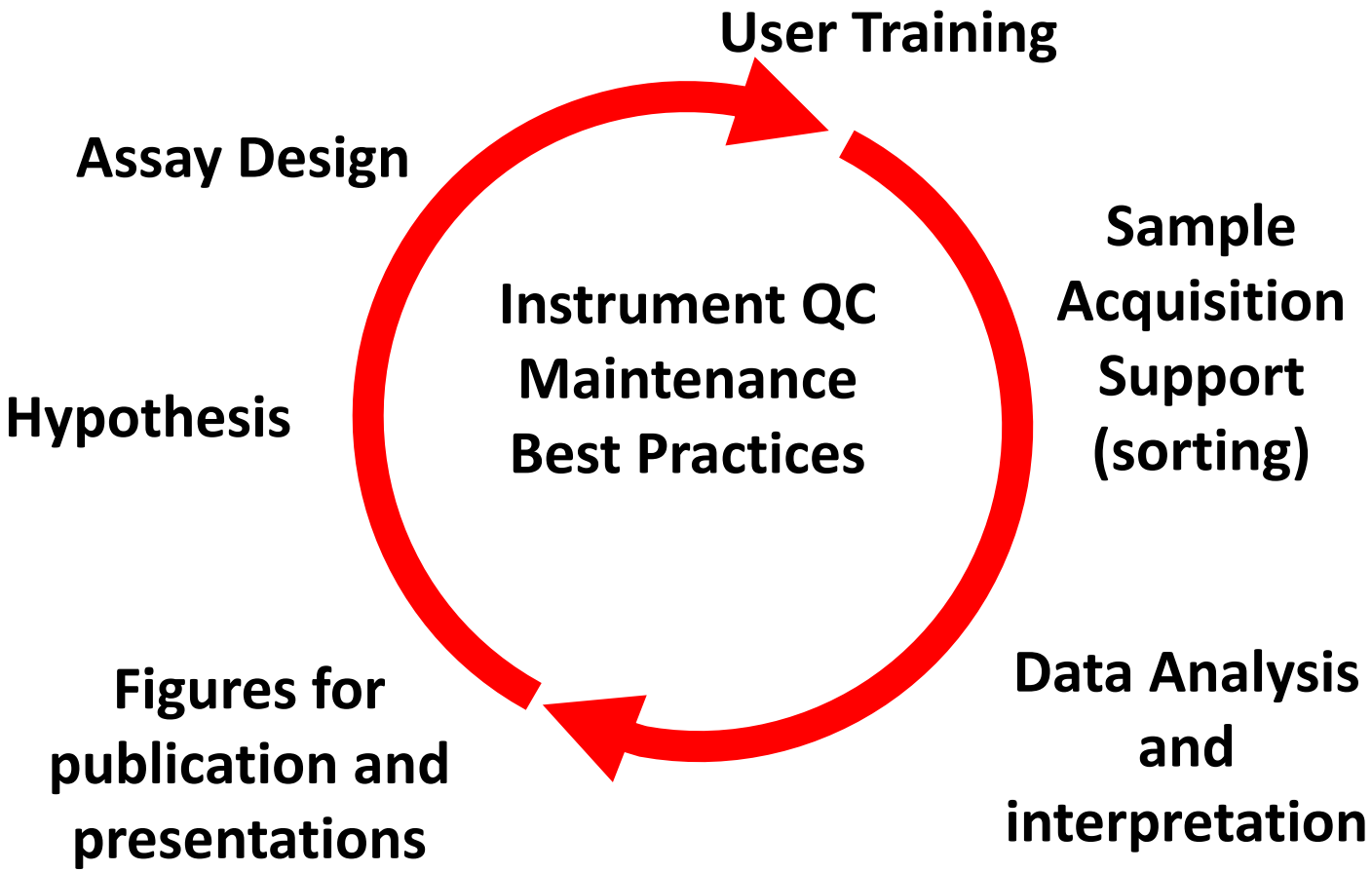
William Leech Medical School

Main HUB

NICR: POG

Meeting the cytometry needs of over 300 users across several different disciplines/institutes

FCCF Cycle of Support: From Hypothesis to result



DATA CONFIDENCE (negative or positive results)

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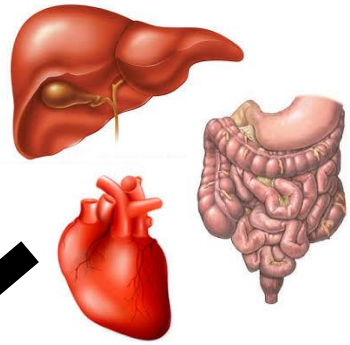
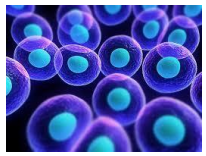
Work-FLOW of a "typical" Flow Cytometry Experiment

Liquid Biopsies

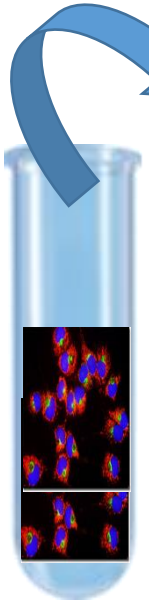
Tissue (need to digest)



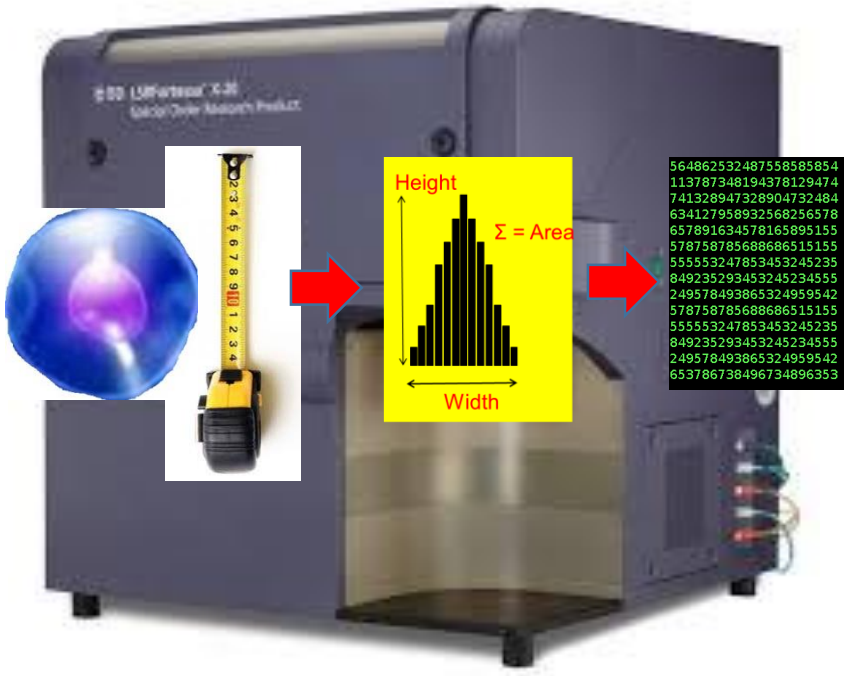
- **Gives us single cells**
- **Allows us to make many measurements per cell**
- **Can analyse lots of cells, quickly**



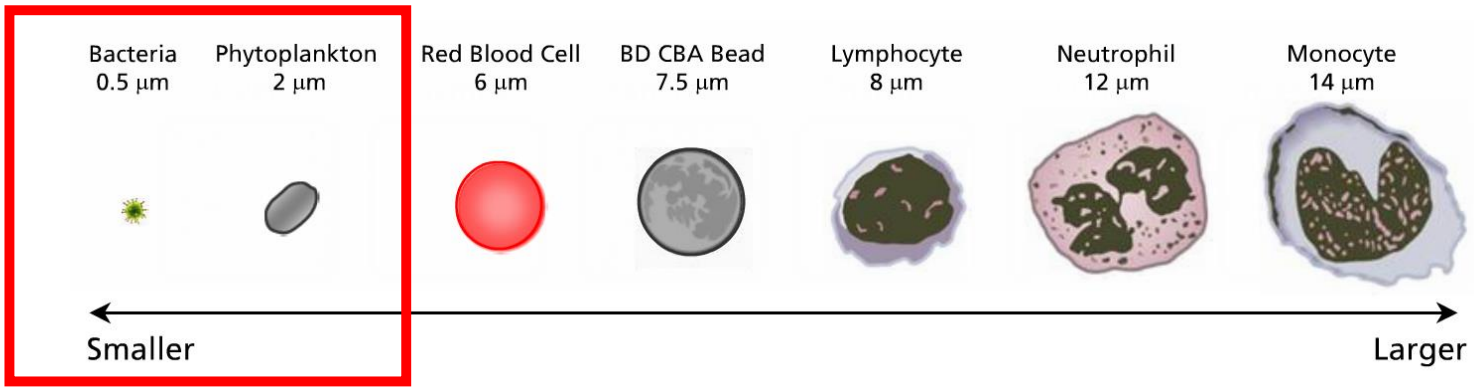
Label/mark cells



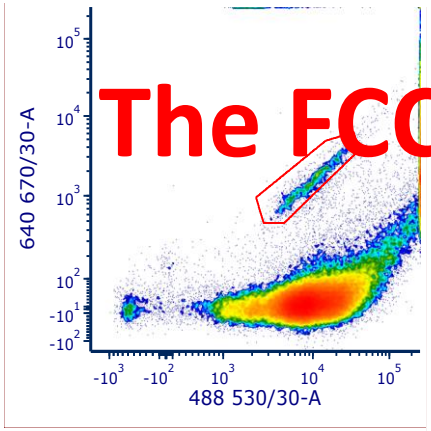
Flow Cytometer



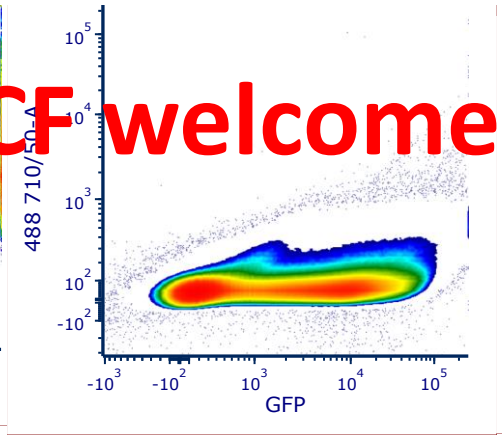
Cytometry is the study of all kinds/types of cells



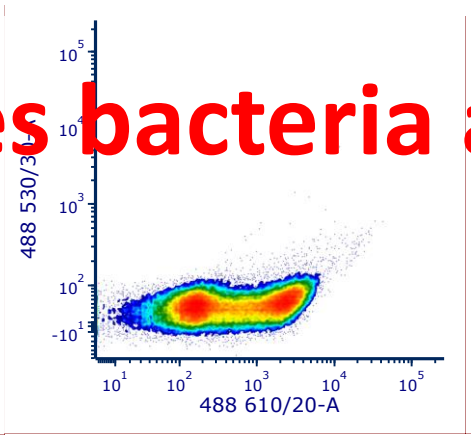
The FCCF welcomes bacteria and yeast



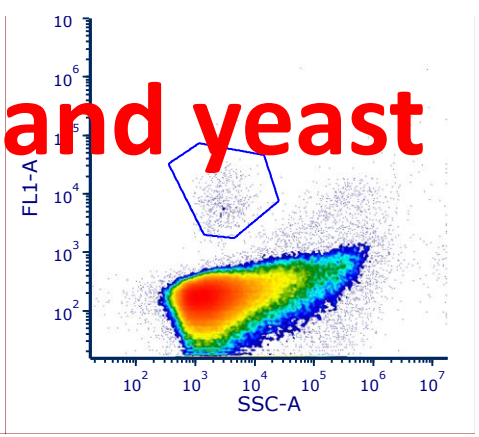
Rare bacteria identified by FISH probes and Syto9 staining from water treatment plants



GFP library tested in bacillus species and sorted using FACSFusion



DHE a superoxide indicator used with Candida species to test peroxide production and stress



Marine samples tested on portable cytometer for autofluorescent phytoplankton

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The technology and relationships within the FCCF for decoding single cell biology

Imaging Cytometry (n = ∞)

Mass-based

Fluorescence-based

Sorting:
6 way/single cell

Hypothesis

Analytical Cytometry:
Decoding and identifying heterogeneity

Cell Sorting:
Ability to control/limit/influence heterogeneity

Low dimensional Fluorescence-based (n = 10)

Mid-dimensional Fluorescence-based (n = 20)

Hi-dimensional Fluorescence-based (n = 30)

Sorting:
4 way/single cell

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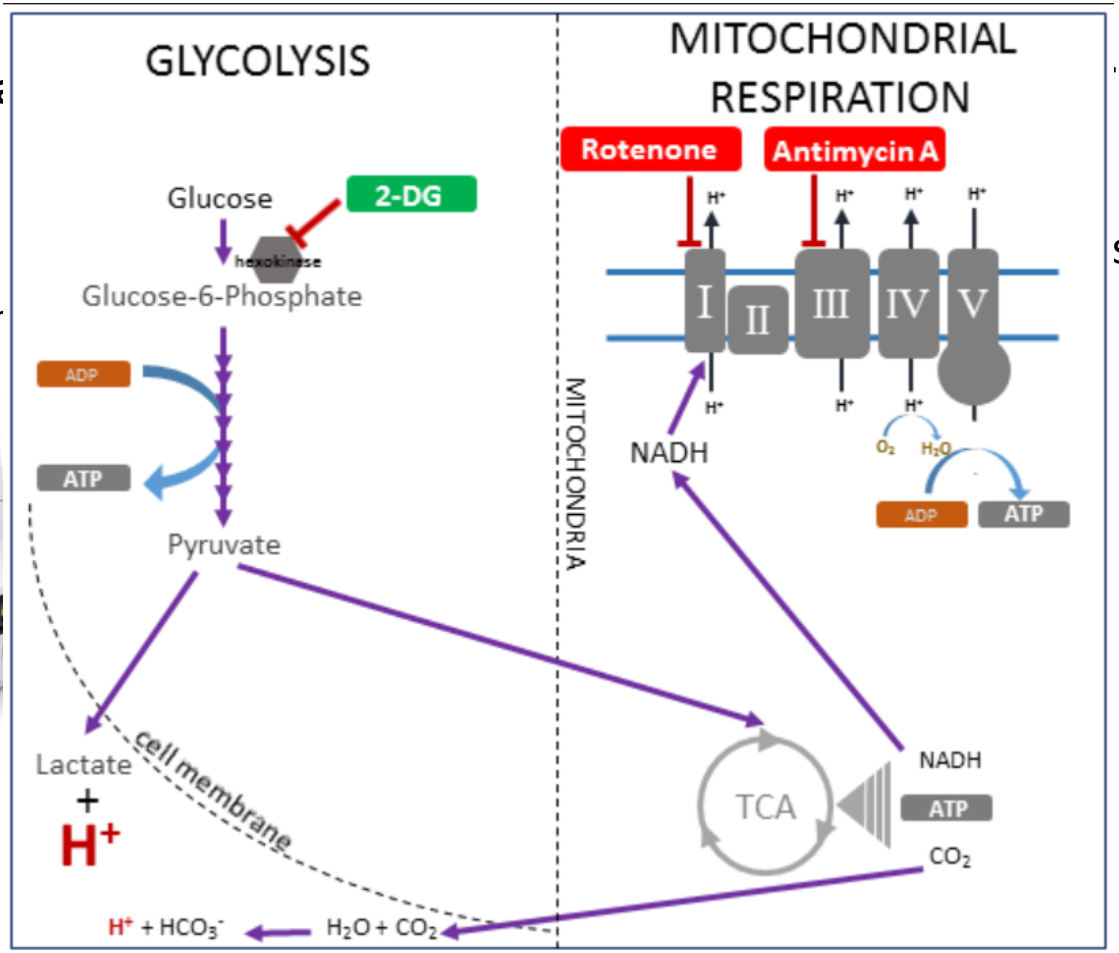
FCCF also offers Seahorse metabolomics technology

XF96 – at Medica **Ageing and Life**

- High throughp sual' samples
- Good for testir



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Work-FLOW of a "typical" Flow Cytometry Experiment

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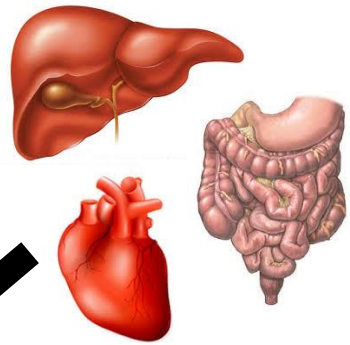
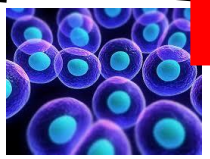
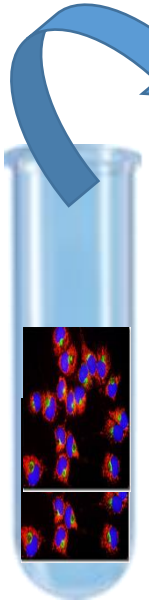
Tissue (need to digest)



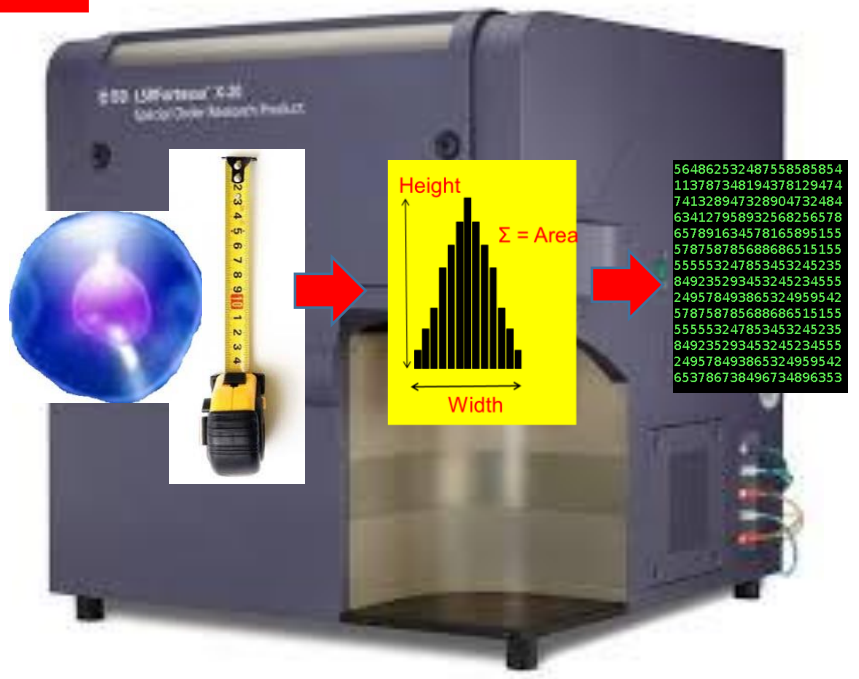
- Is digestion good?



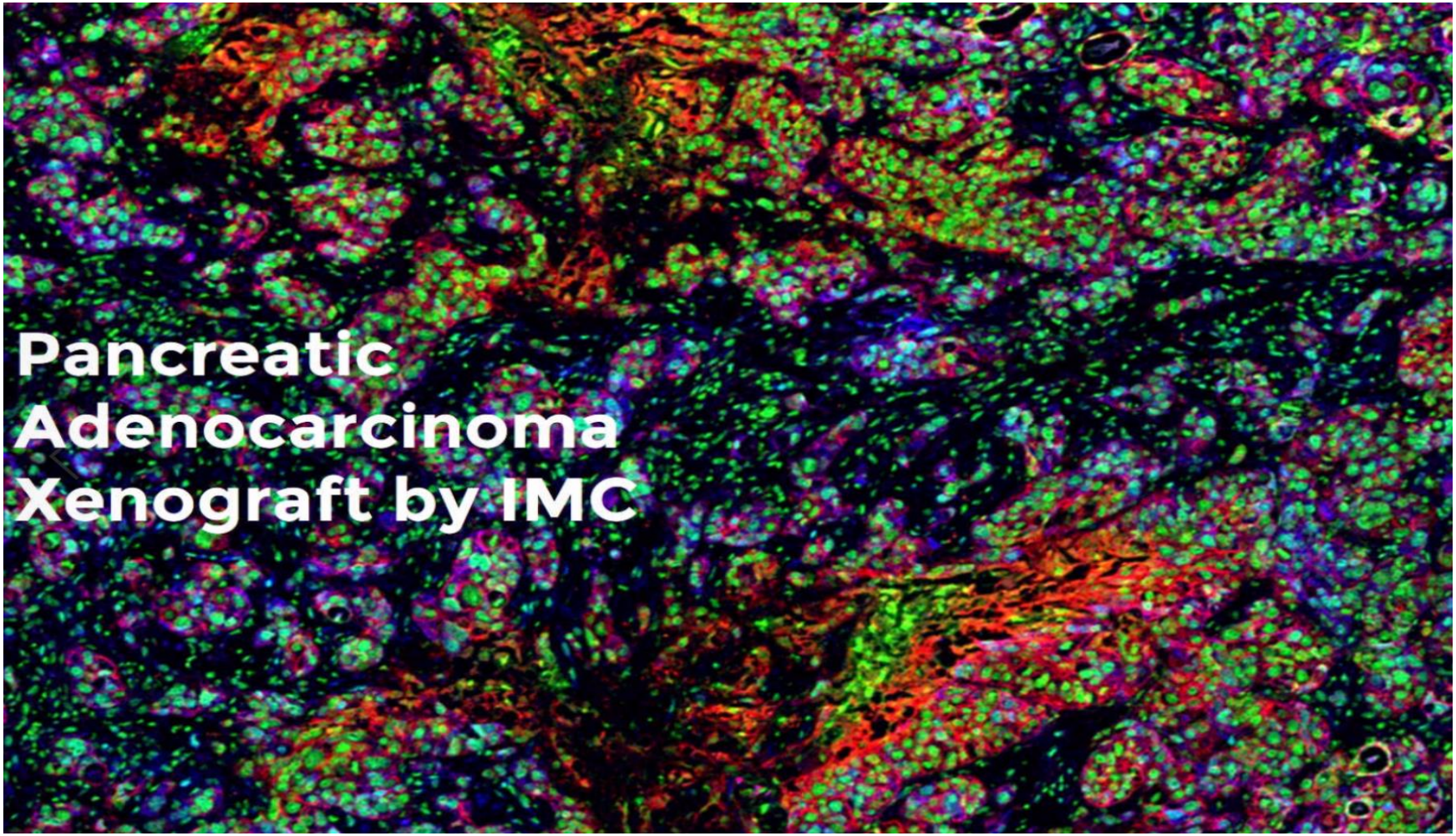
Label/mark cells



Flow Cytometer



Cells exist in structures/organs/systems….should we be breaking this down in to single cell suspensions..?



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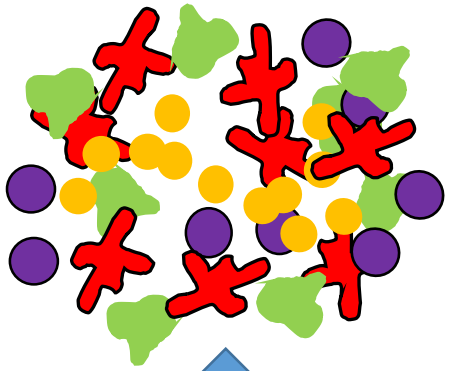
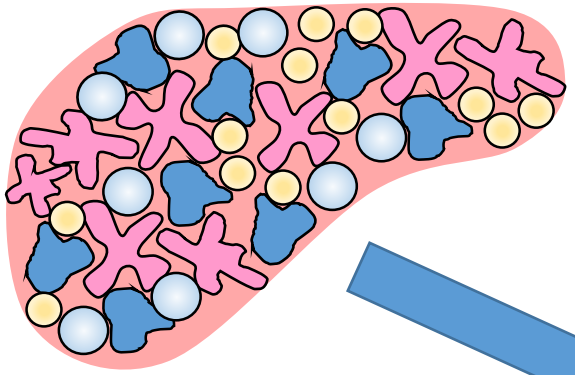
The pros and cons of digesting tissue

"Global" scan of phenotypes that "may" be present in tissue. Local relationships lost, BUT fast and multi-parameter



"Native" Tissue destined for single cell phenotypic "exploration"



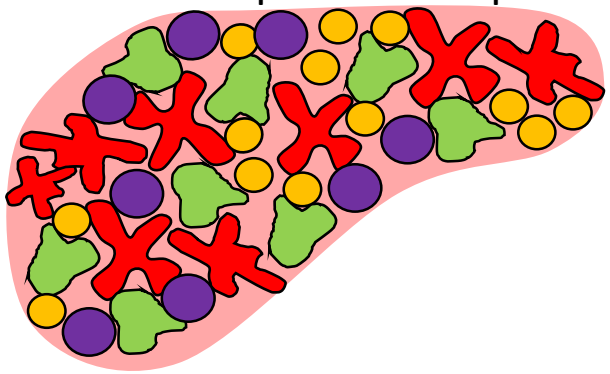
DIGESTION

Label/mark cells for identity

BOTH have use

SECTIONING

"local" phenotypes. Spatial relationships are preserved but throughput is reduced as well as parameter space



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Welcome the Hyperion to NU FCCF: 40+ directed measurements on tissue

1

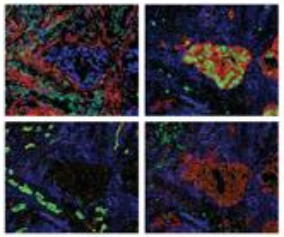
DESIGN
panels using pathologist-verified Maxpar antibodies conjugated to metal tags.

2

STAIN
tissues (FFPE or frozen) or fixed cells using familiar IHC protocols.

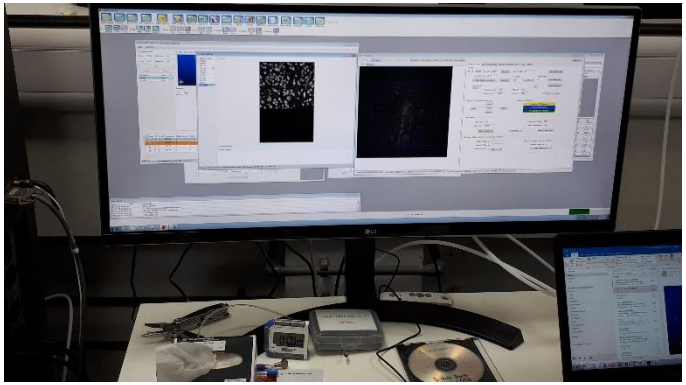
3

IMAGE
protein markers at subcellular resolution using the Hyperion Imaging System.



4

ANALYZE
images in minutes using the MCD Viewer and easily export for secondary analysis.



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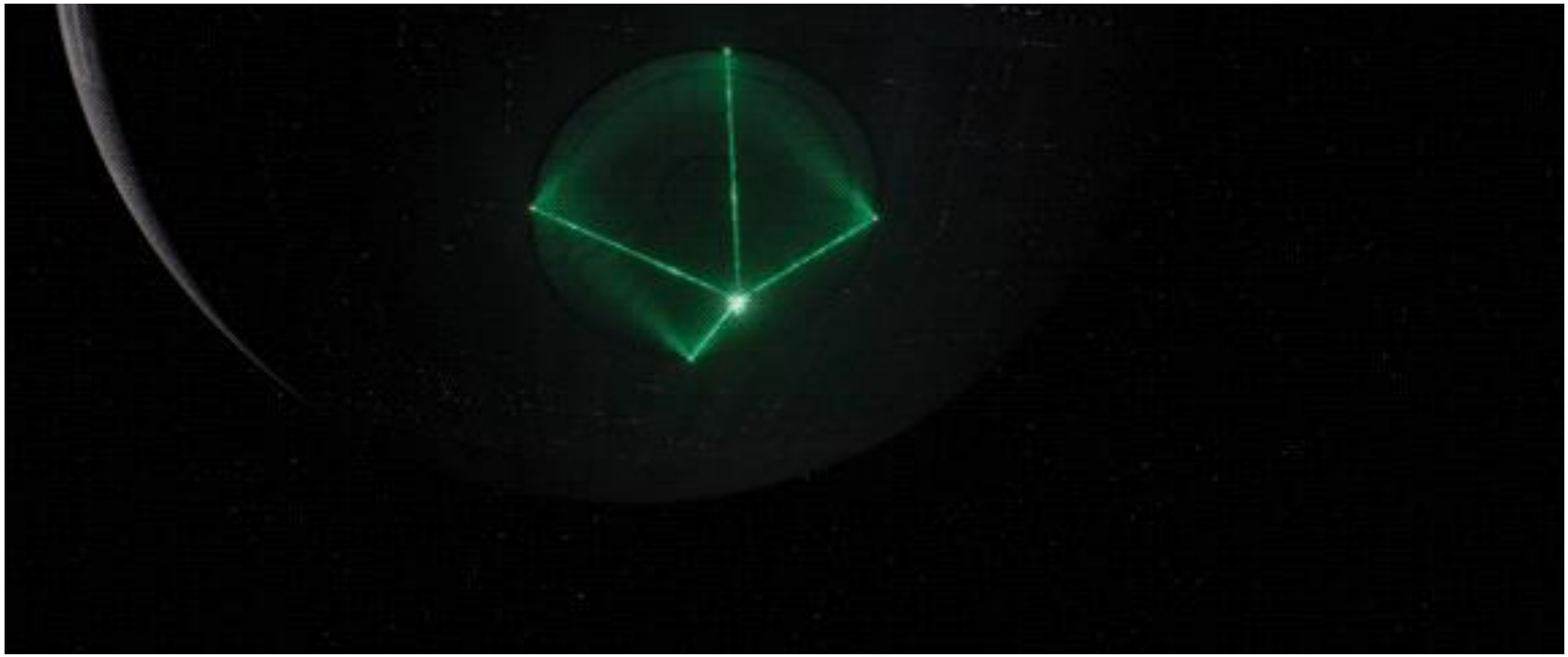
How does the system work?

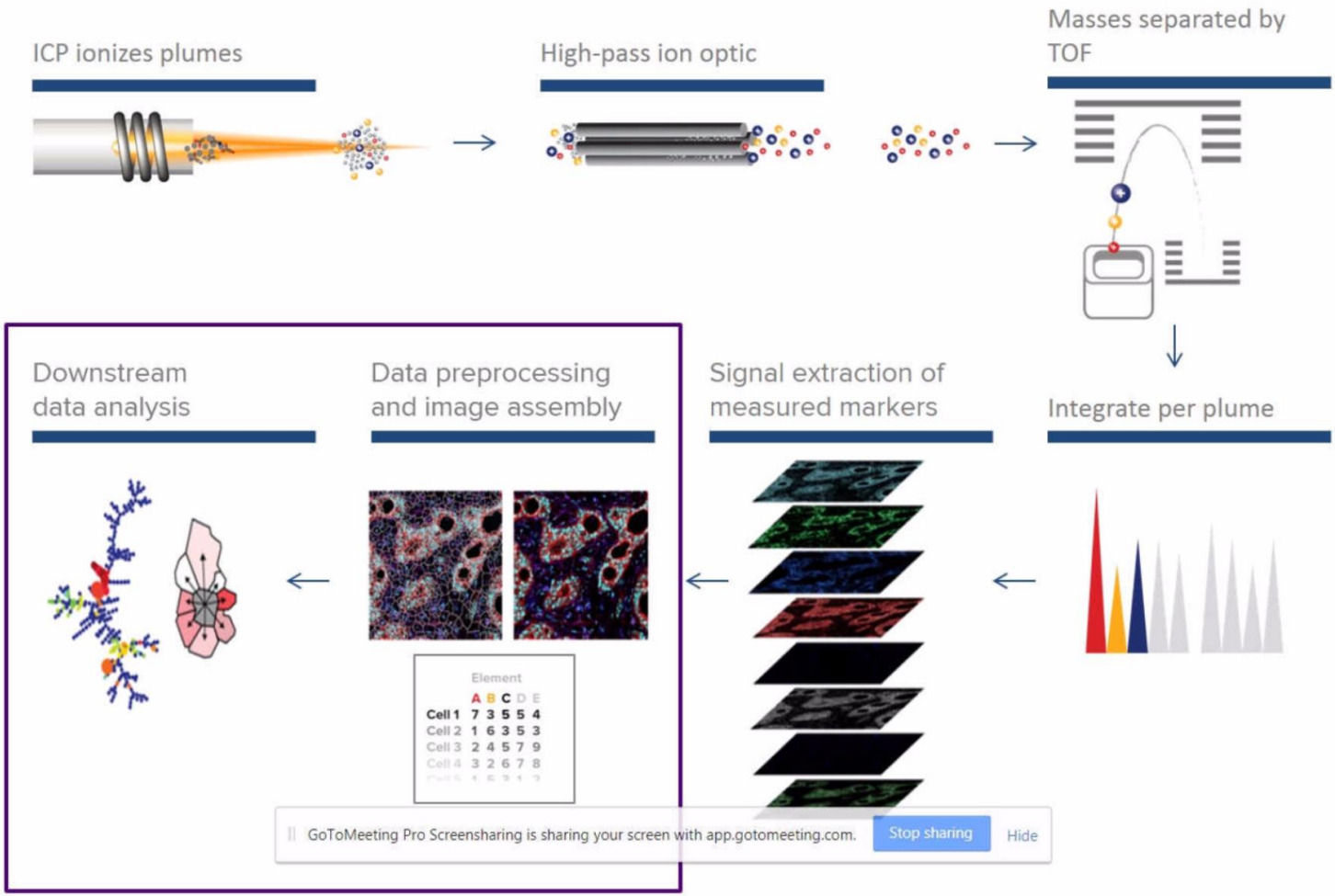
UV laser ablates tissue 1 μm^2 at a time



Not actual footage!

Motorized stage in sample ablation chamber

Ions are carried by Helium plumes in to the ICP



- Ablates 1mm^2/90 min
- Dynamic range = 32 bit
- Suggested to ablate 2mm^2 ROI at a time to allow detector recalibration to regain sensitivity. Simply then start on new ROI

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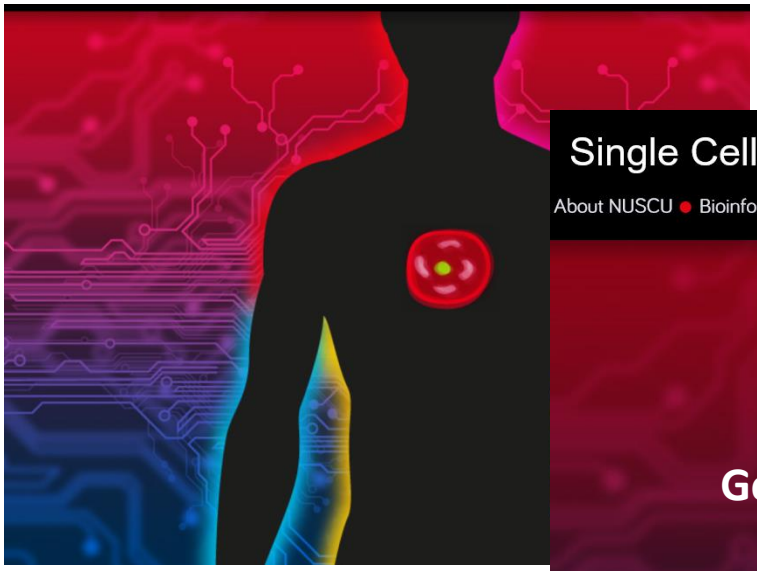
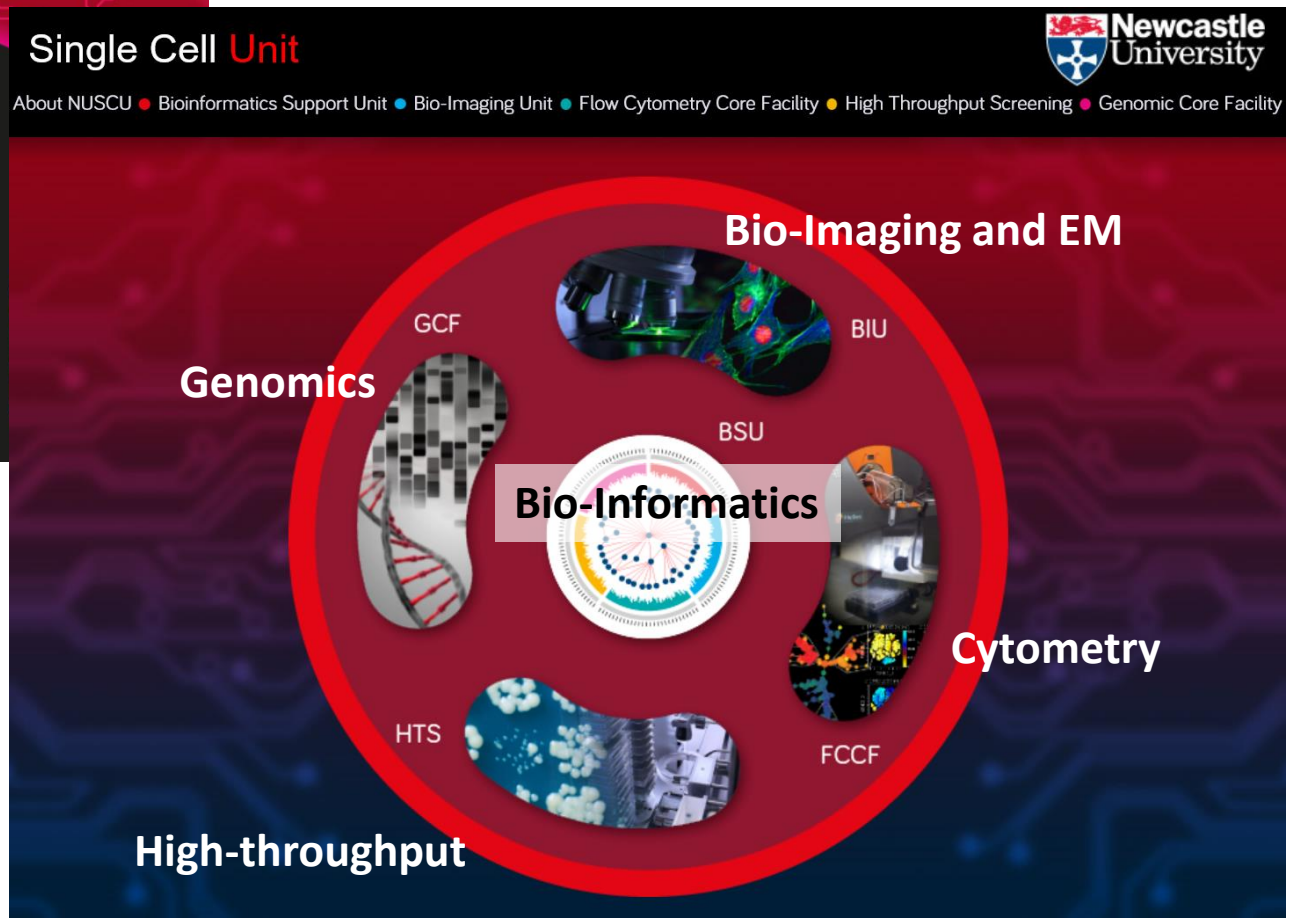


Putting the **Single Cell** at the heart of clinical/basic research



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Thanks for your attention: Questions?

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Arthritis Research UK

wellcome

bright red

Fighting blood cancer for a brighter future