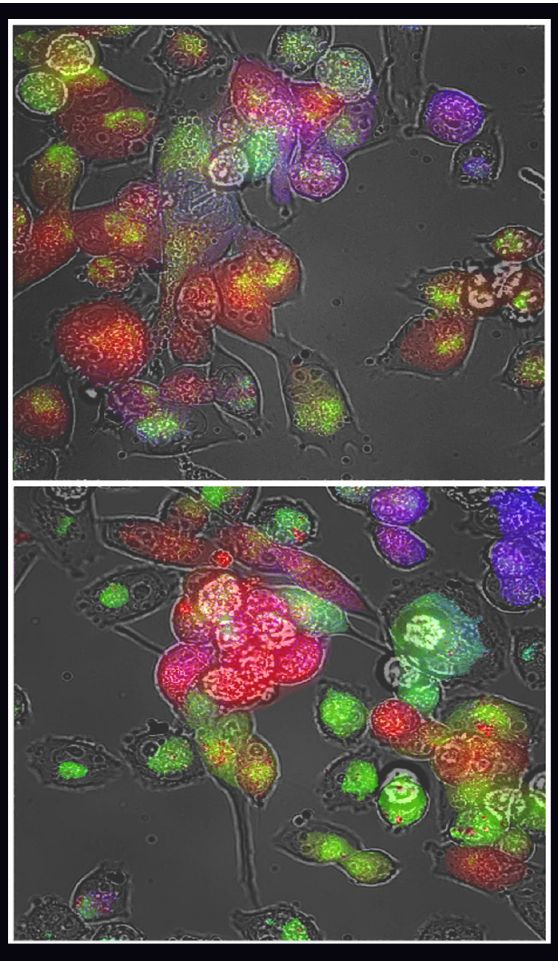

Hyperspectral imaging of cell populations

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Centre for Nanoscale BioPhotonics
ARC CENTRE OF EXCELLENCE

Need to identify cell subpopulations

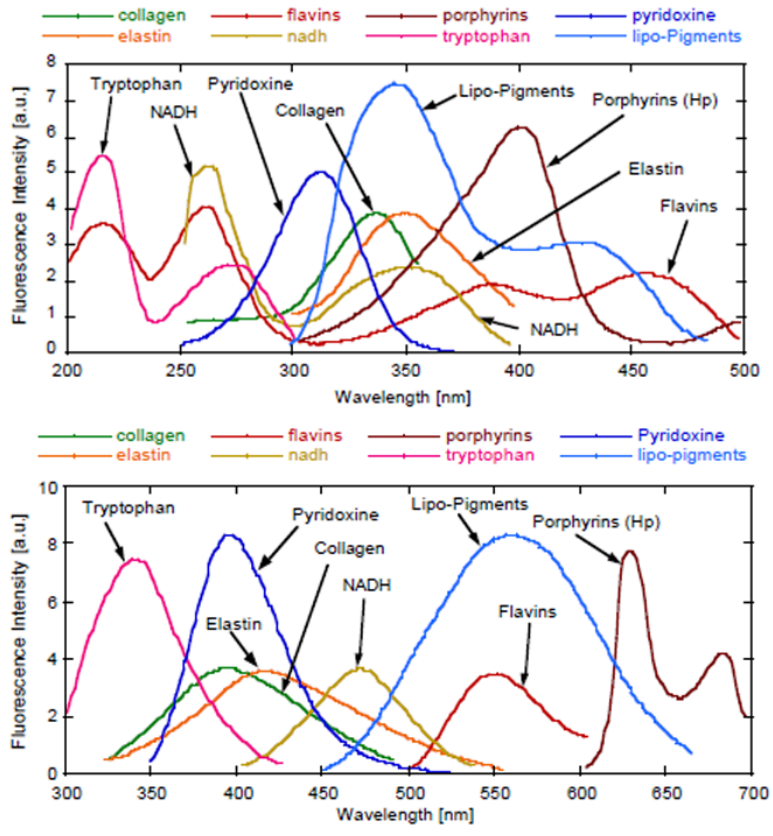
- Biological cells are very heterogeneous.
- Significant major subpopulations have been uncovered including in stem cells, neurons, cancer and immune cells [1-4].
- The understanding of these biochemically and functionally different cell subpopulations is a significant problem in biology and medicine.

There is a need to develop tools for identification and selection of cell populations with varying biochemistry.

[1] Nature, 496, 7444 229+; [2] Nature, 488, 7412, 522+; [3] Science, 327, 5965, 542-545; [4] Science, 317, 5836, 381-384.

Key features of our approach

- Use only native fluorescence of cells - non-invasive , no extraneous labels
- May be combined with established method of fluorescent labeling
- Analyse all acquired cell images - no subjective selection
- Fully automated computer based analysis - no subjective choices
- Derive maximum quantitative information from the cell images
- Extensively use mathematics and statistics to assess population properties

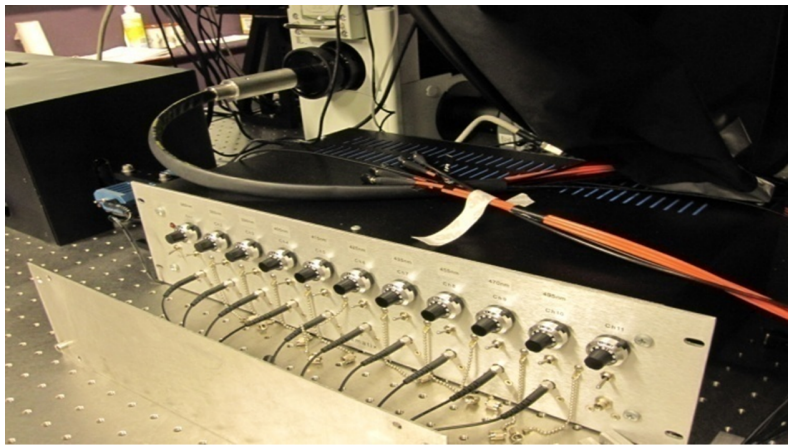


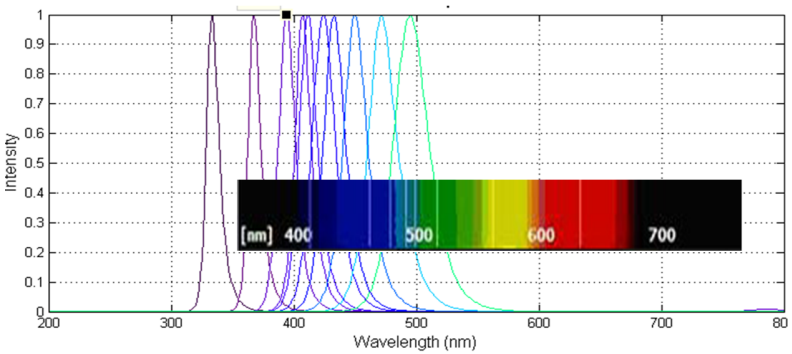
Top: excitation, bottom: emission of key native cell fluorophores.

Technicalities

Measurements

- Excite at a number of wavelengths (11-15) between 290 and 450 nm
- Detect cell autofluorescence at longer wavelength , around 385 and from 460 to 550 nm
- (In brief, sample as much of the excitation-emission matrix as possible)
- Subtract background, smooth noise, remove image correlations by Principal Component Analysis
- Use a variety of multivariate analyses coupled with subspace projections to highlight biologically interesting phenomena





Hardware

ANDOR IXON CCD + LED bank

Four methods

This presentation discusses four methods:

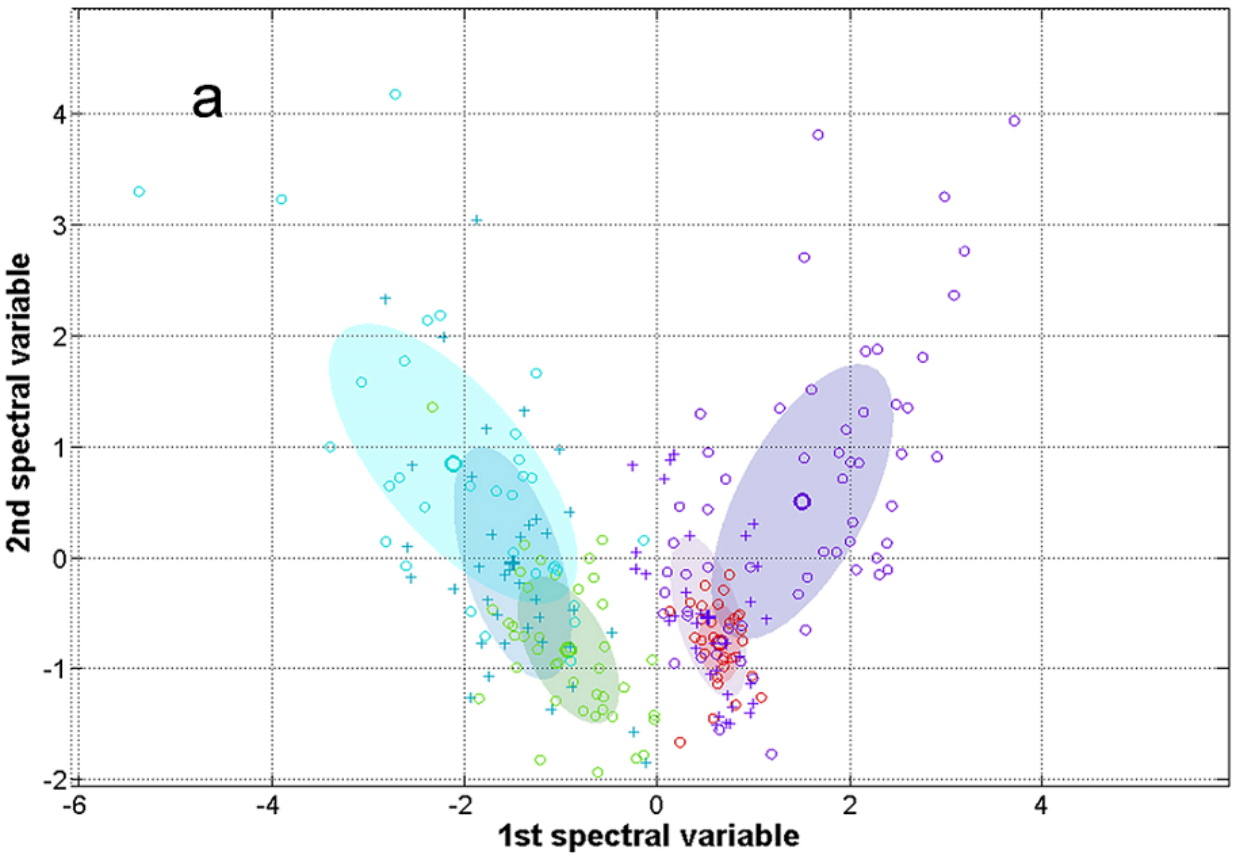
1. For differentiation of cell classes - "healthy controls" from "sick" from "sick treated"
2. For biochemical characterisation - cellular maps of key fluorophores, statistics of fluorophore contents in cell populations
3. For the detection of cell subpopulations - do cells form distinguishable clusters
4. For finding label-free signatures of cell subpopulations with respect to standard biomarkers - no need to use selected biomarkers, label-free characteristics is enough.

They have been applied to several cell types.

- Projection of high dimensional spectral features into a low dimension space involves finding a new set of basis vectors upon which the data is expressed, such that a particular criterion is satisfied. This can be done explicitly (PCA/LDA) or through a search and optimization method.
- Principal component analysis (PCA) find axes that maximally explain data variance, when correlative data is projected onto these new axes, a new set of uncorrelated variables is produced, usually with most of the variance captured by a few
- Discriminant analysis is similar to PCA, but is supervised in that the data is grouped into different classes and the new axes are chosen to show maximum separation between class types, within the new variable set
- Subpopulation analysis is unsupervised and uses a host of statistical tests to clusters data into Gaussian groups

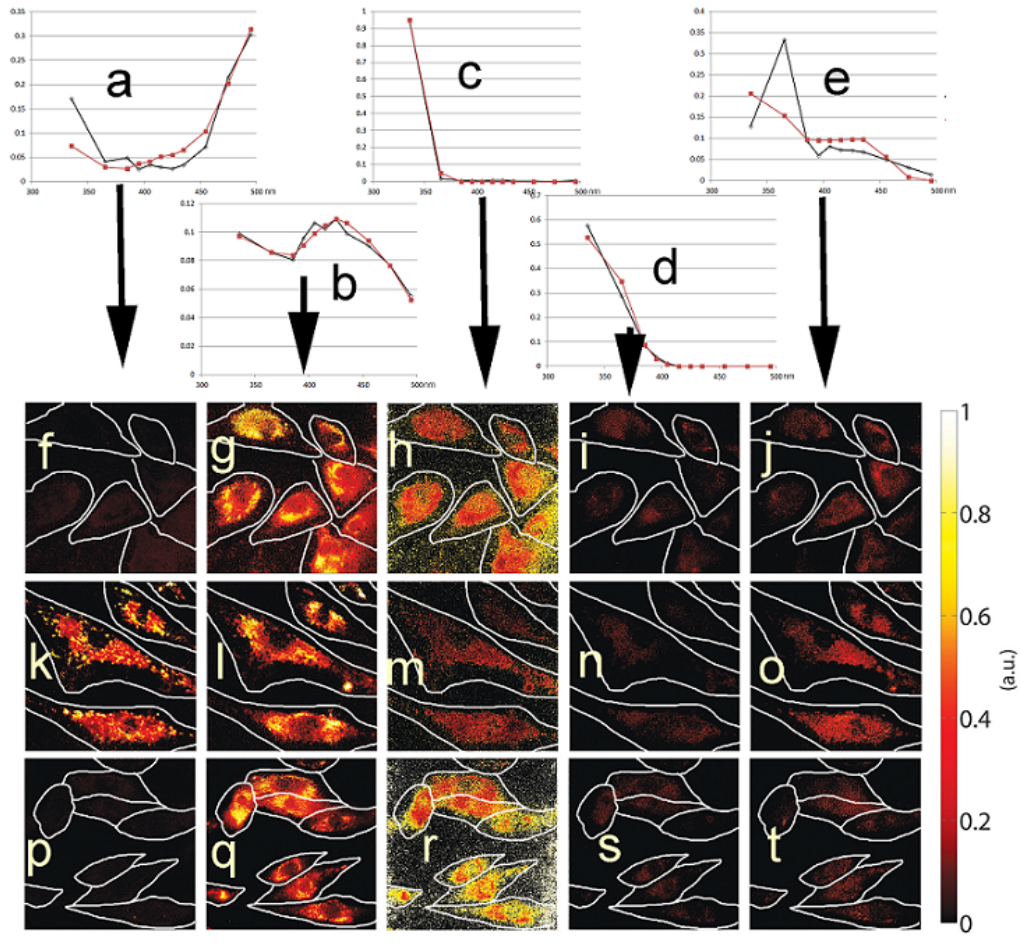
1. Mitochondrial disease (MELAS)

Cells: Olfactory neurospheres, accessible models of neurodegenerative disease



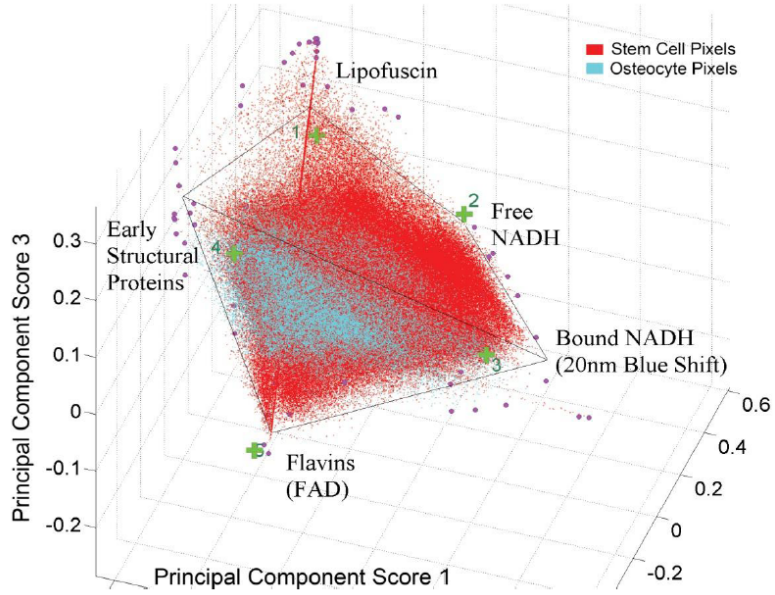
Differentiation of healthy - MELAS - MELAS treated cells. Data for two patients (and controls).

1. Mitochondrial disease (MELAS) ctd



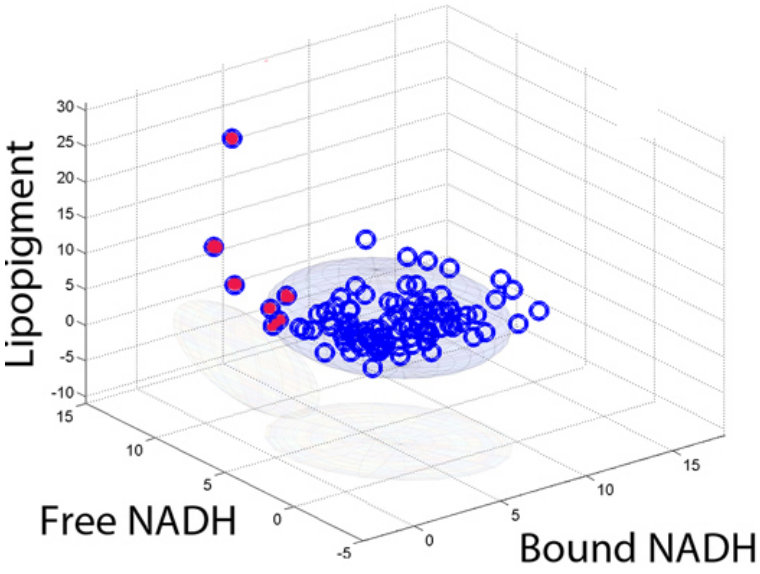
Biochemical content of cells

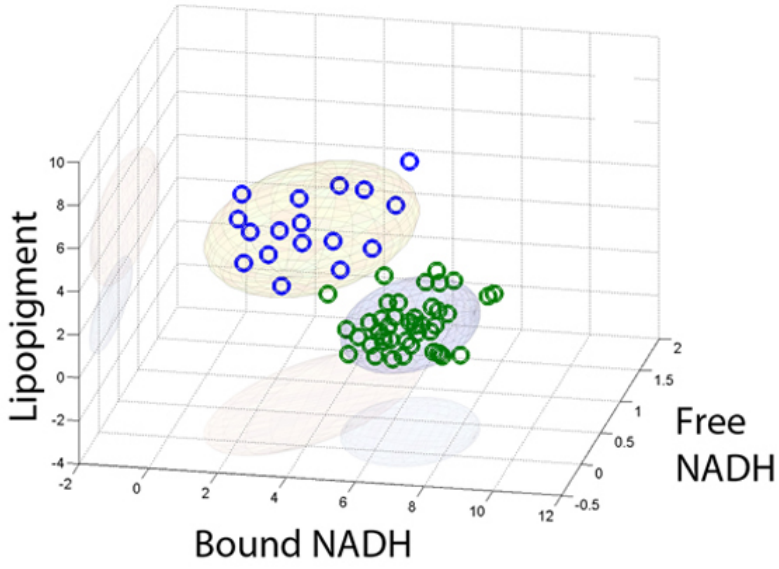
Healthy (top row)- MELAS (middle) - MELAS treated cells (bottom); a - flavins, b - A2E, c - bound NADH, d - free NADH, e - lipofuscin



The ratio of bound NADH to flavins reflects the metabolic rate of individual cells.

1. Mitochondrial disease (MELAS) ctd



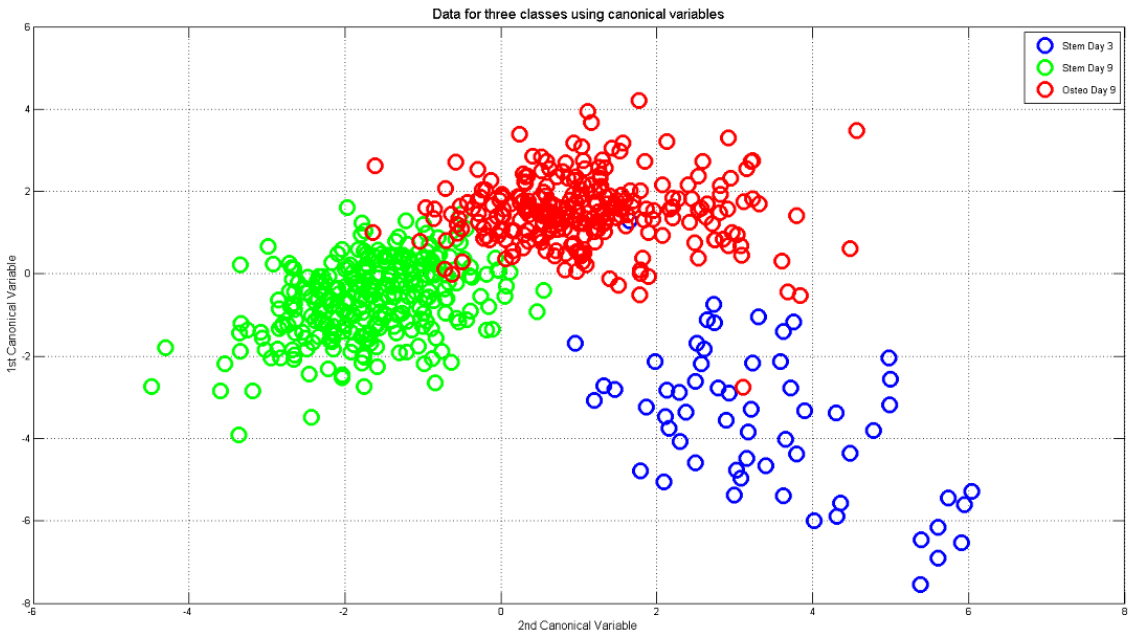


Data for two patients: 11% (left) and 44% (right) mutational load.

Automatic search for cell subpopulations with respect to biochemical content has unveiled very distinctive subpopulations of "healthy" and "diseased" cells in both patients.

2. Stem cell characterisation

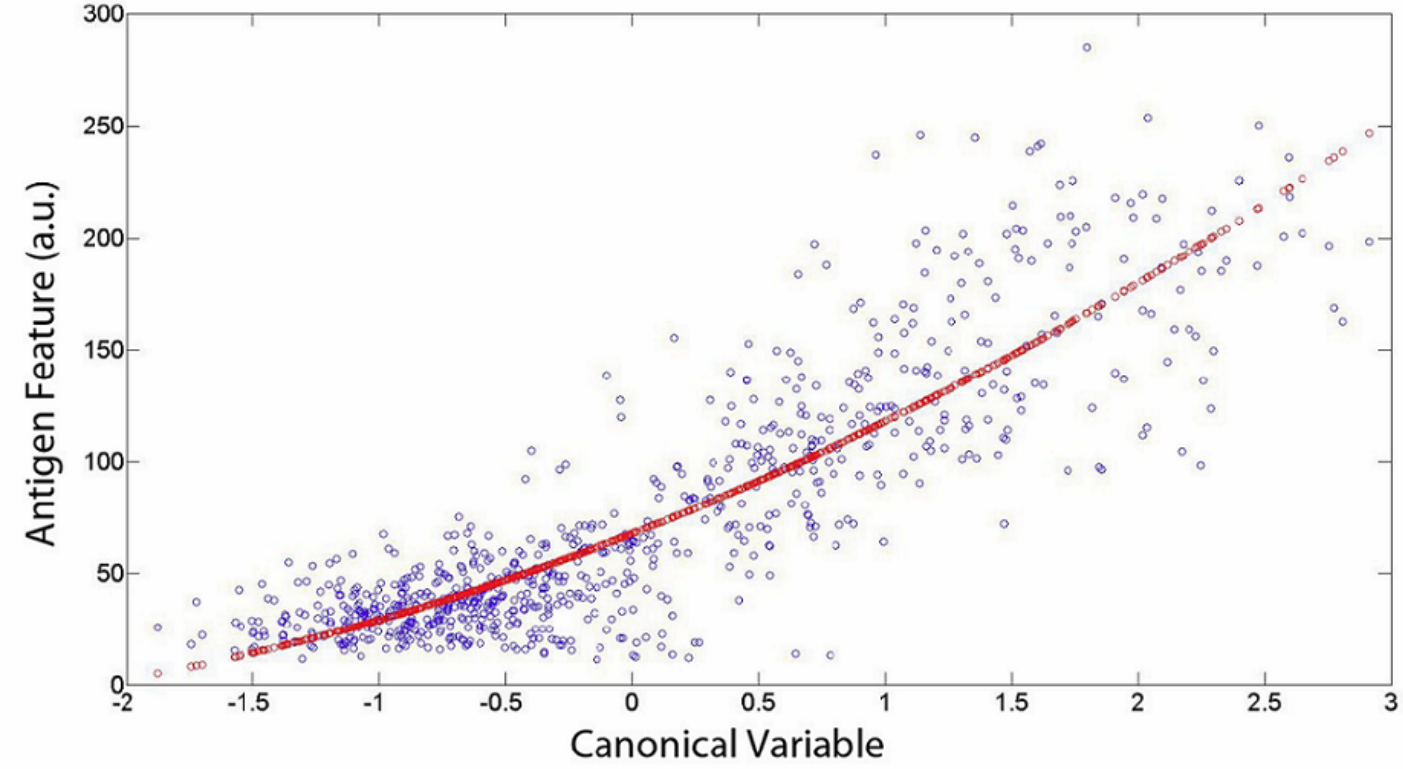
We can detect osteogenic differentiation of mesenchymal stem cells WITHOUT any fluorescent labeling



Blue - stem cells day 3 of culture, green - stem cells day 9, red - osteogenic cells day 9.

2. Stem cell characterisation ctd

We can measure biomarker expression WITHOUT fluorescent labeling with that biomarker



The CD90 antigen feature closely correlate with a suitably designed hyperspectral feature. High CD90 antigen cells can be selected by autofluorescence only.

3. Highly performing stem cell subpopulations

Background

- Commercial adipose derived cell cultures contain a mix of cells including fibroblasts, mesenchymal stem cells (MSC): preadipocytes, endothelial and a variety of immune cells such as macrophages
- CD166 biomarker is an MSC indicator.
  Its levels are significantly higher in MSCs than in fibroblasts.
- CD54 is an osteocyte indicator.
  Differentiating osteocytes have upregulated CD54 expression.

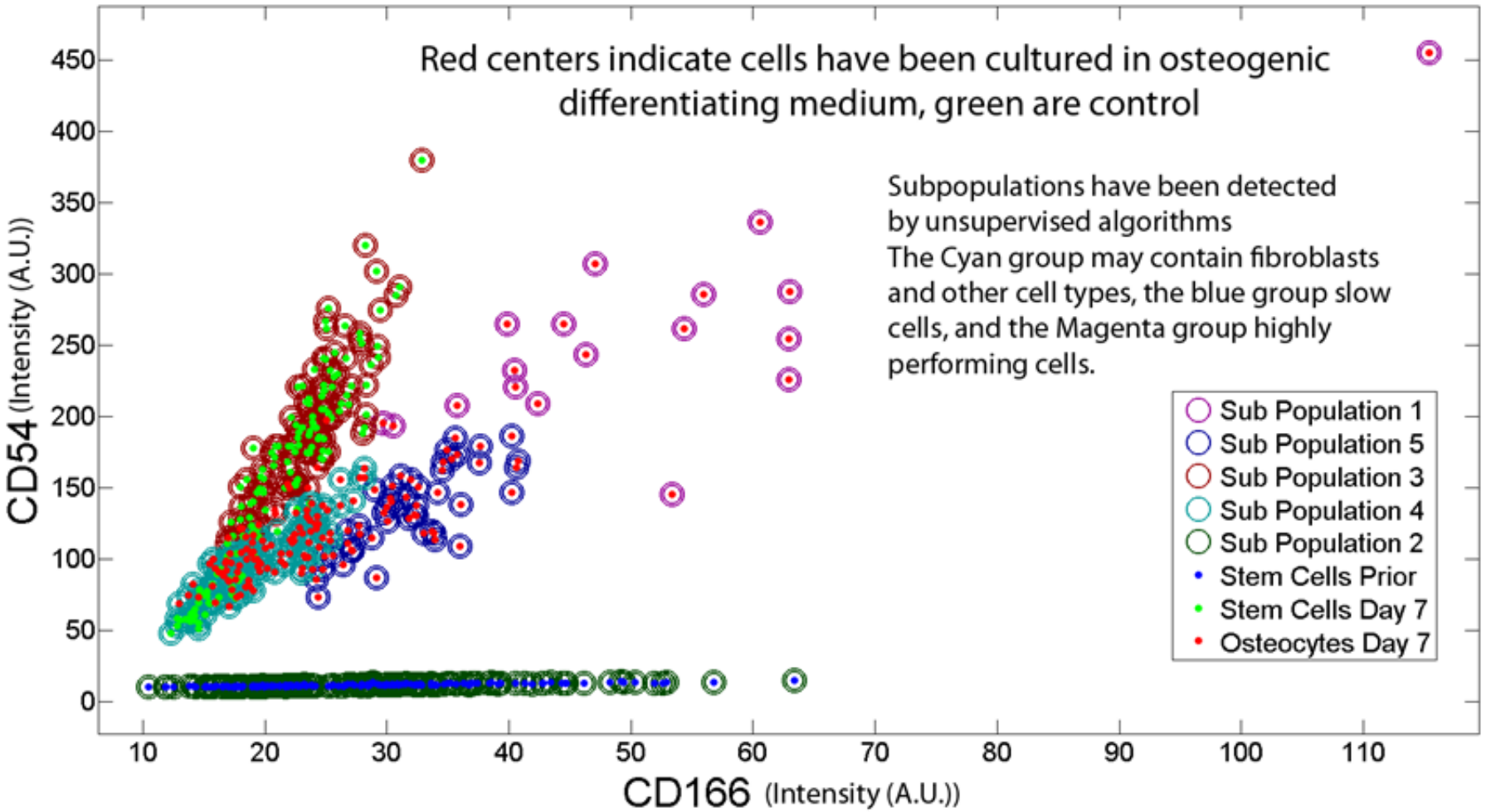
We can detect highly performing early osteocytes vs. fibroblasts noninvasively, WITHOUT fluorescent labeling with biomarkers

2. Highly performing stem cell subpopulations

Method

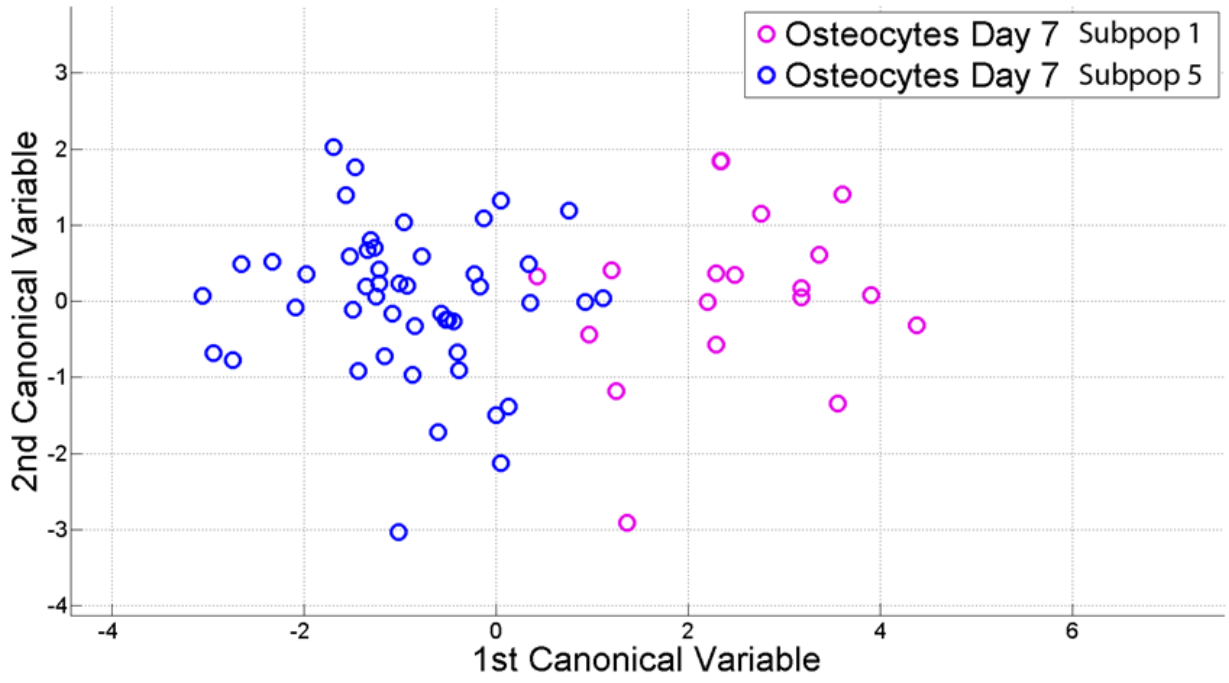
- Induce osteogenic differentiation of ADSC
- Carry out hyperspectral imaging
- Fluorescently label cells with CD166 and CD54 antibodies and image to detect biomarker levels.
- Identify subpopulations with respect to CD166 and CD54
- Identify individual cells from the highly performing population
- Perform hyperspectral features analysis of these cells
- Display results in a lower dimensional space

3. Finding highly performing stem cell subpopulations



Magenta circles, red centres: highly performing early osteocytes; blue circles, red centres: poorly performing cells

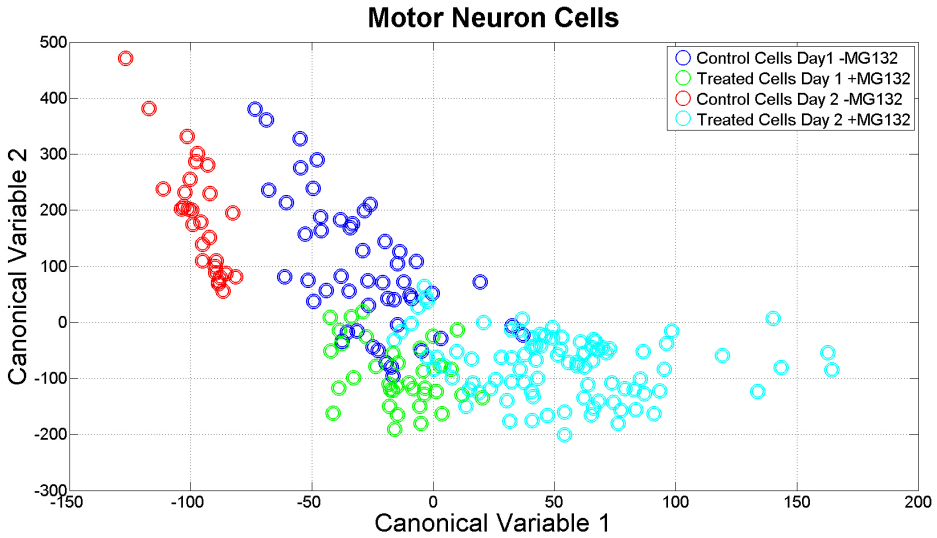
3. Finding highly performing stem cell subpopulations - ctd



The subpopulations of highly (magenta) and poorly (cyan) performing cells are clearly distinguished by label - free hyperspectral imaging.

Useful for (1) identification of the most potent stem cell populations, e.g. those that most effectively secrete anti-inflammatory cytokines for arthritis treatment; (2) identification of subpopulations which can be optimally expanded (major problem)

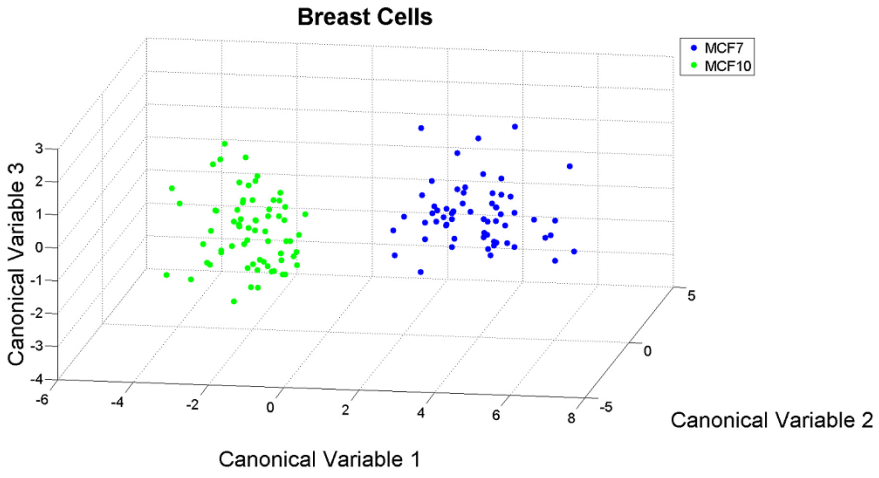
4. Motor neuron disease

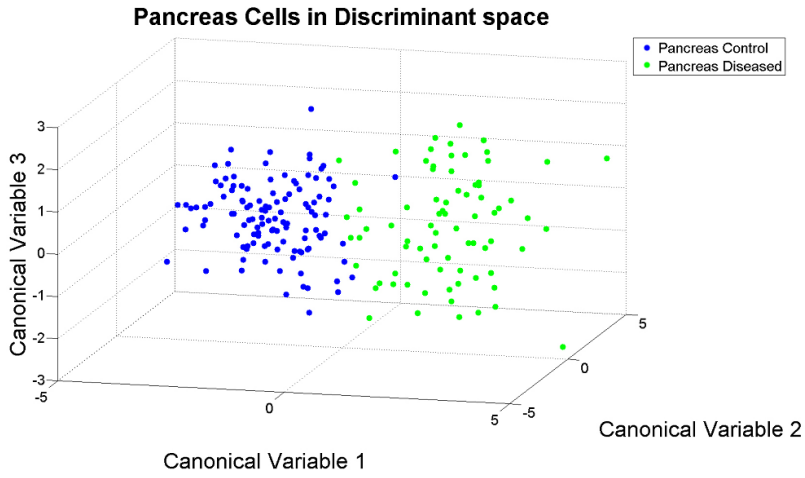


Hyperspectral analysis demonstrates the effect of treatment with MG132 on motor neurone disease cells. Data taken in 1 and 2 day cultures. The treatment significantly affects cell metabolism.

Proteasome inhibitor (MG132) treatment is known to effect gene expression (Rab24 mRNA), microtubule-associated protein light chain 3 (LC3), and accumulation of LC3-II, a processed form of LC3 and the most reliable marker for autophagy. Note that neurons can not be measured by flow cytometry

5. Cancer

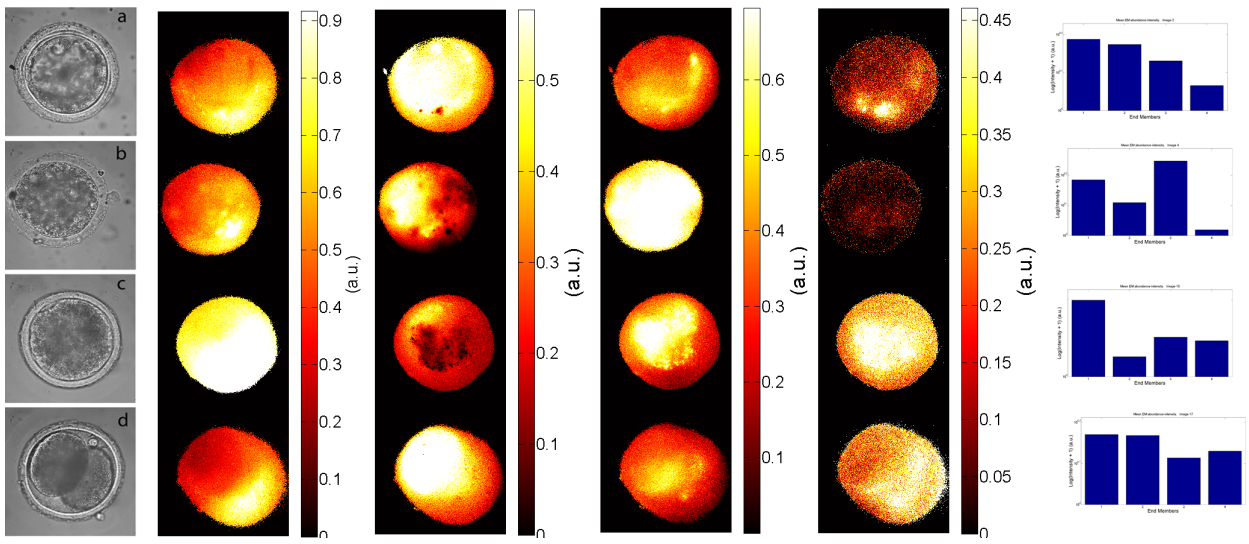




Cell discrimination in cancer: Left: breast, MCF7 - most common advanced breast cancer cell line, MCF10 - early cancer human mammary epithelial cells. Right: pancreas: green-cancer, blue-control.

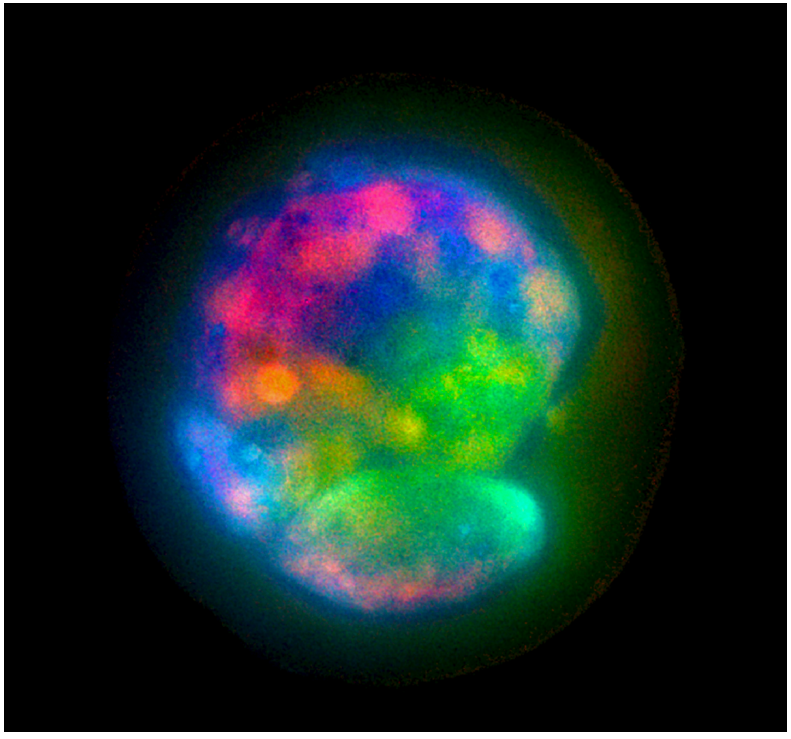
Method is useful for the assessment of glycolysis and other indicators of physiological status of samples in cell cultures

6. Embryo

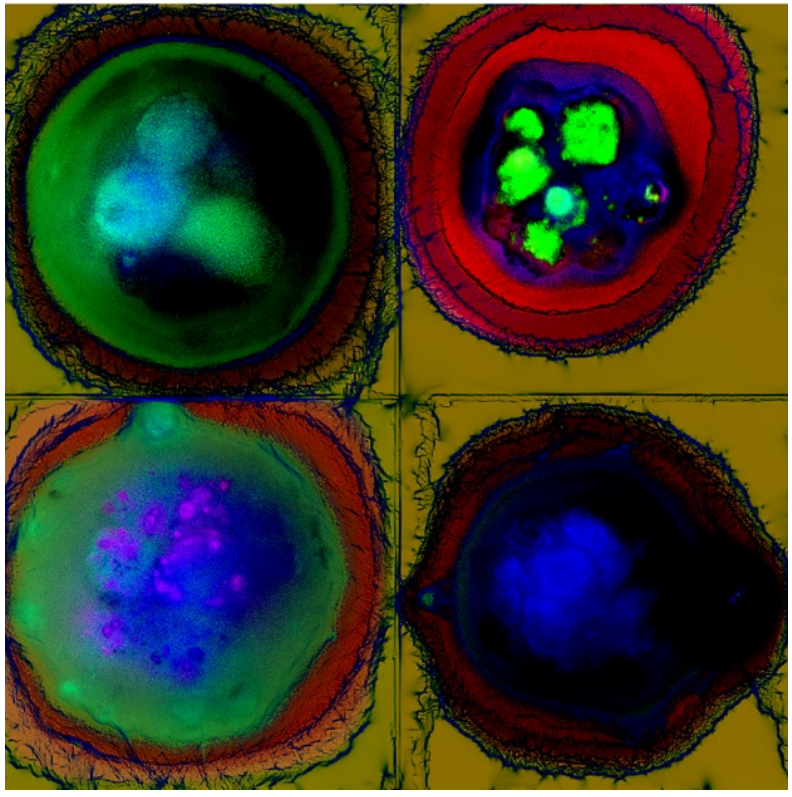


Biochemistry of living embryos. From left to right: Embryos (DIC), maps of bound NADH, flavins, unknown - possibly cytochrome c + structural protein, retinoids + free NADH. Summary histograms.

6. Embryo Continued



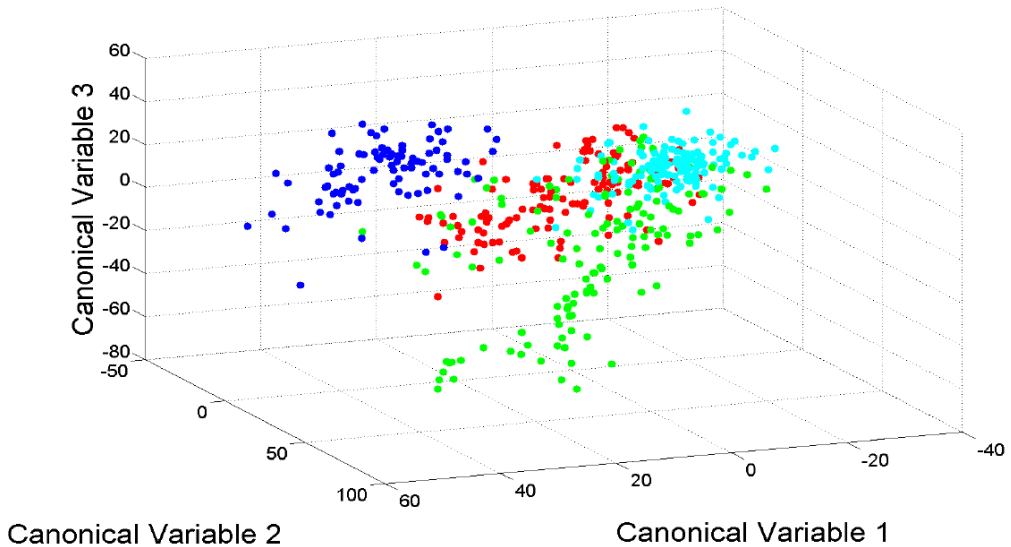
Fixed embryo. Early neuronal stem cells - pink



Fixed embryos at various stages, healthy and damaged by oxygen. Left: 7 % oxygen, right: 20% oxygen. Top: morula, bottom: cleaved.

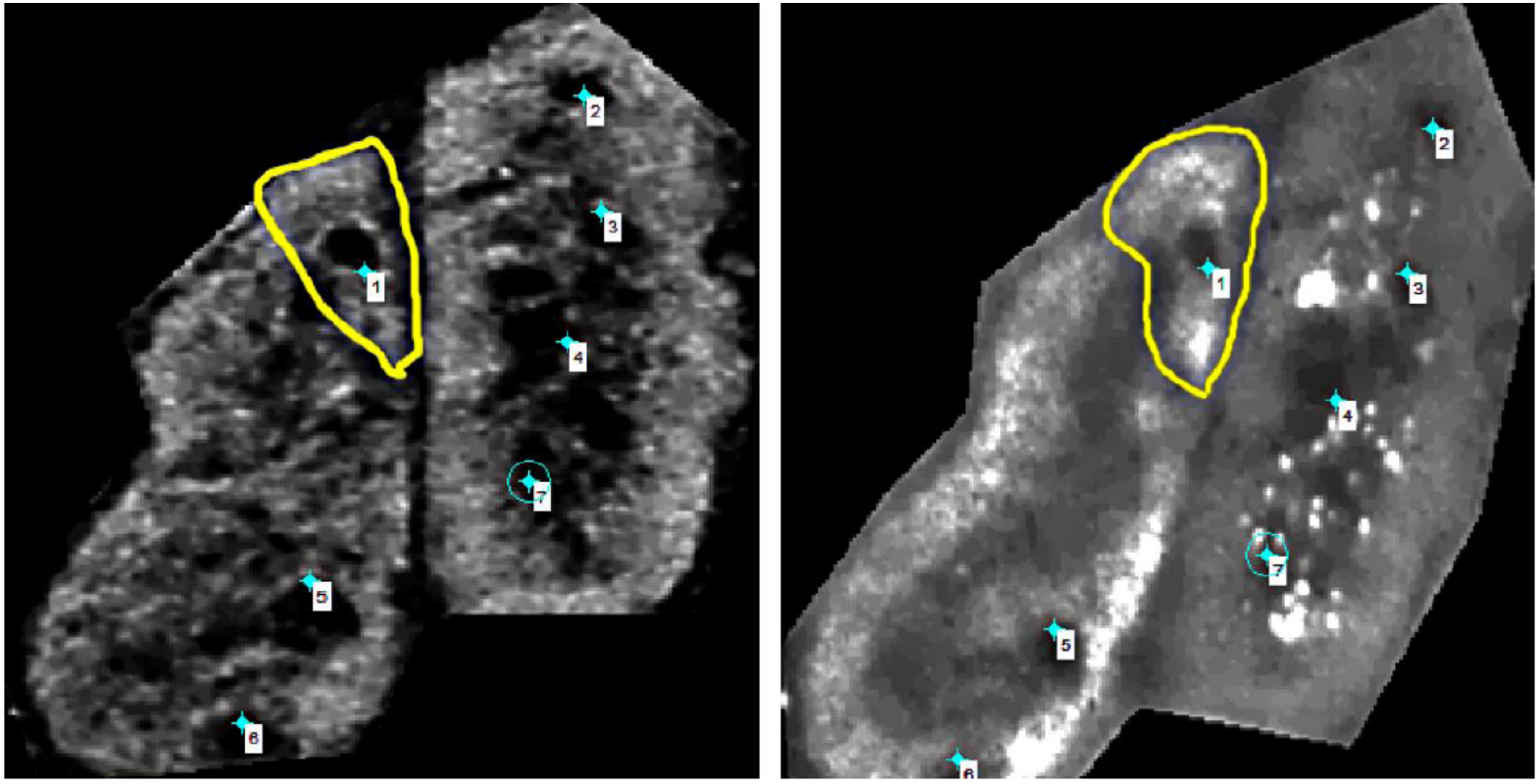
7. Genetic mutations in IPS cells

Induced Pluripotent Stem cells have embryo-like potential to differentiate into all other cell types.



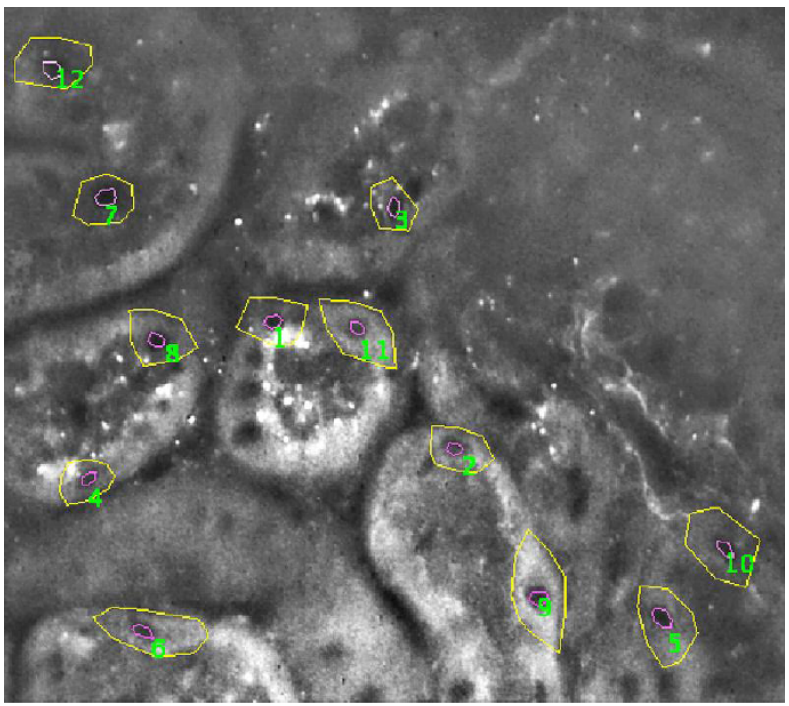
Healthy control cells (Cyan), protein kinase PINK1 mutation (green), ubiquitin ligase PARKIN mutation (Red) are both associated with Parkinson's disease. ATP13A2 mutation (Blue) known to cause KuforRakeb syndrome and dementia. ATP13A2 mutations have an impact on lysosomal function. Possible multiple populations in the PINK1 mutated cells (green).

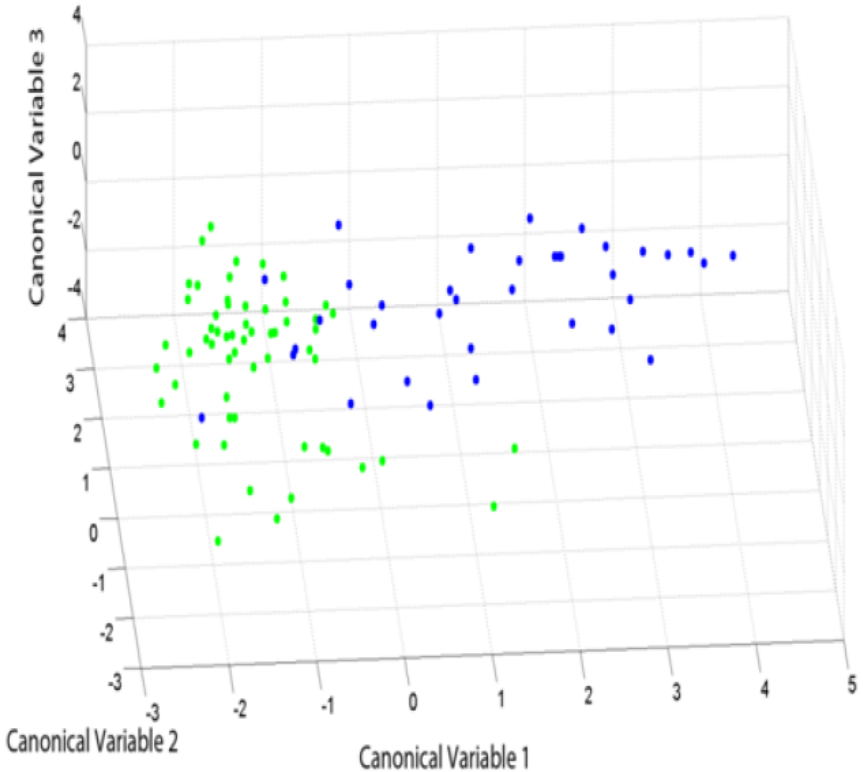
Imaging of tissue



Two kidney tissue corpuscules each containing tens of cells, imaged on confocal using Mitotracker (left) and autofluorescence (right). Here, seven obvious structural points are identified in each image allowing the images to be registered using an affine transform.

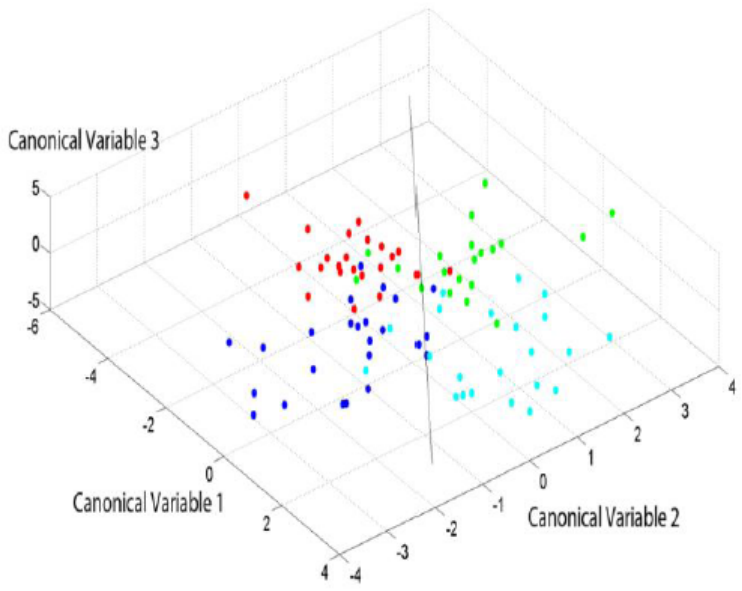
8. Diabetes

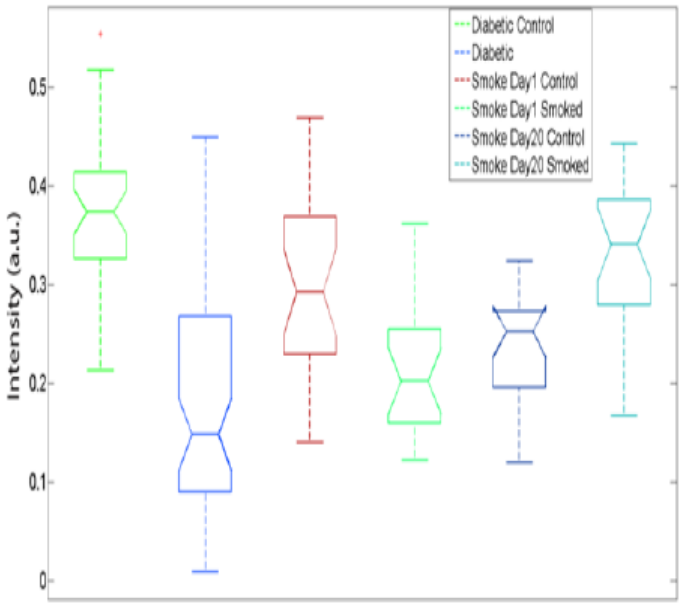




Left: Identified kidney cells in fresh frozen tissue. right: diabetic - green, control - blue

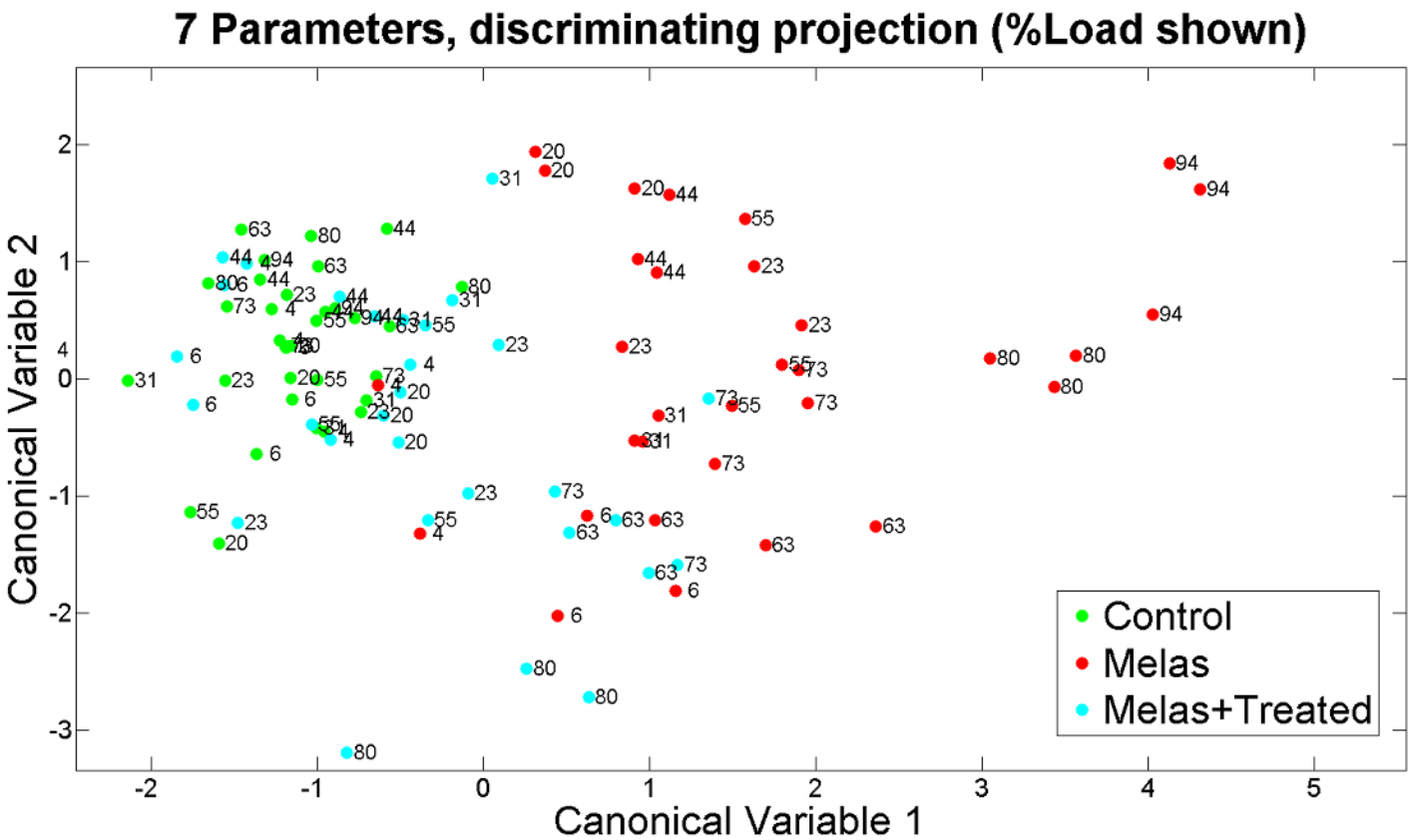
9. Effects of maternal smoking





Detecting kidney cells affected by maternal smoking. Left: Subpopulations of cell line of nonsmoker (control day 1-red, control day 20-blue) and smokers (smoker day 1-green, smoker day 20-cyan). Right: Our system identified flavin, showing its relative abundance to be highly informative.

Compatibility with alternative quantifications



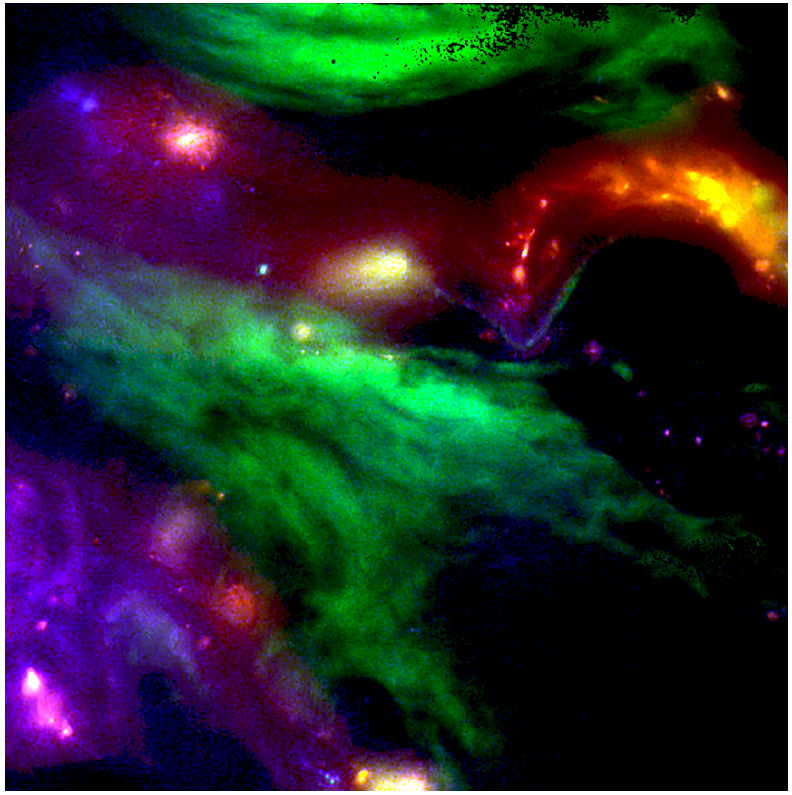
7 biochemical parameters in multiple patients. Patient groups form clear clusters.

Key applications of hyperspectral imaging

- In mineral exploration, resource management, and environmental monitoring
- In agriculture for monitoring of invasive species and the development and conditions of crops.
- In geology for rapidly mapping minerals of commercial interest
- In the military for chemical identification
- In the oil industry to identify oil deposits
- In forensics, food industry and food safety.

Key applications of hyperspectral imaging

- In cell biology and biomedical research



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Hyperspectral Team: Martin Gosnell, Quantitative Pty Ltd (custom code), Ayad Anwer, Saabah Mahbub, Aziz Rehmann, Jakub Tomczyk, Krystyna Drozdowicz-Tomsia

JOB OPPORTUNITIES

IN THE CENTRE OF NANOSCALE BIOPHOTONICS

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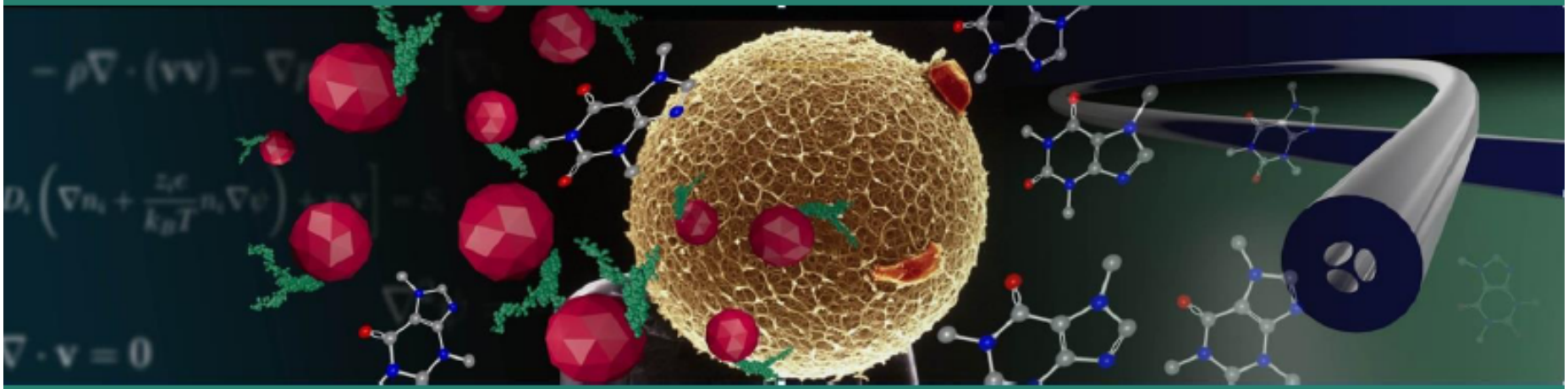

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The Centre will pursue fundamental science at the intersection of nanoscience,