Single cell imaging for exploring the relationship between glucose uptake, proliferation and metastasis in cancer

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Single cell imaging

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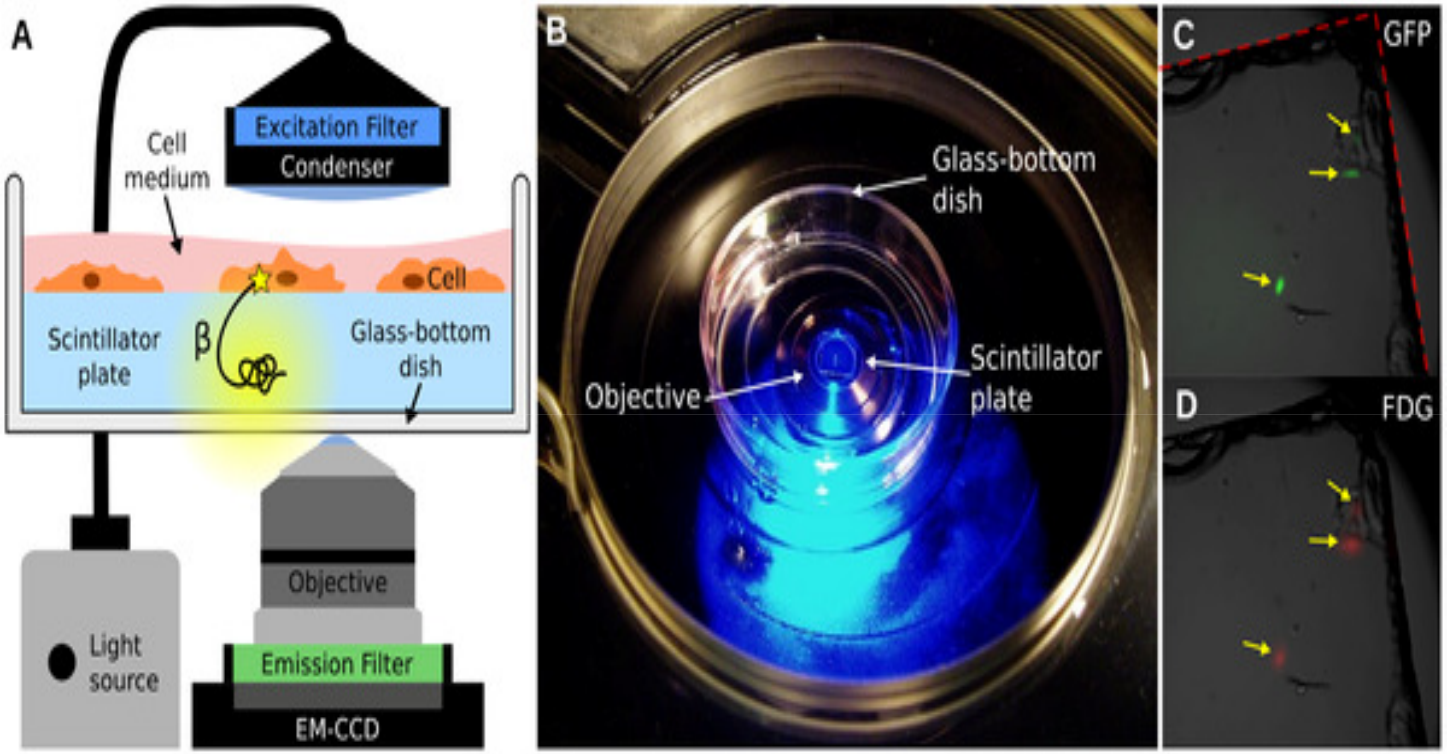
Radioluminescence Microscopy: Measuring the Heterogeneous Uptake of Radiotracers in Single Living Cells

Guillem Pratx[1]*, Kai Chen[1], Conroy Sun[1], Lynn Martin[1], Colin M. Carpenter[1], Peter D. Olcott[2], Lei Xing[1]

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Overview of the radioluminescence microscope.

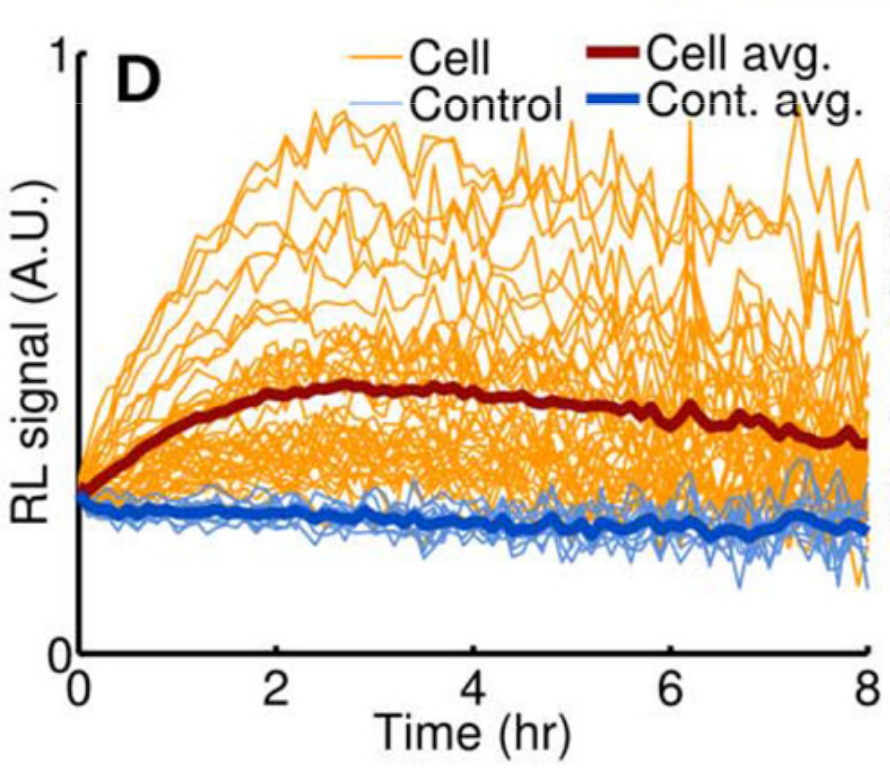
Ovarian Cancer Cells



Pratx G, Chen K, Sun C, Martin L, et al. (2012) Radioluminescence Microscopy: Measuring the Heterogeneous Uptake of Radiotracers in Single Living Cells. PLoS ONE 7(10): e46285. doi:10.1371/journal.pone.0046285
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0046285

Single cell imaging

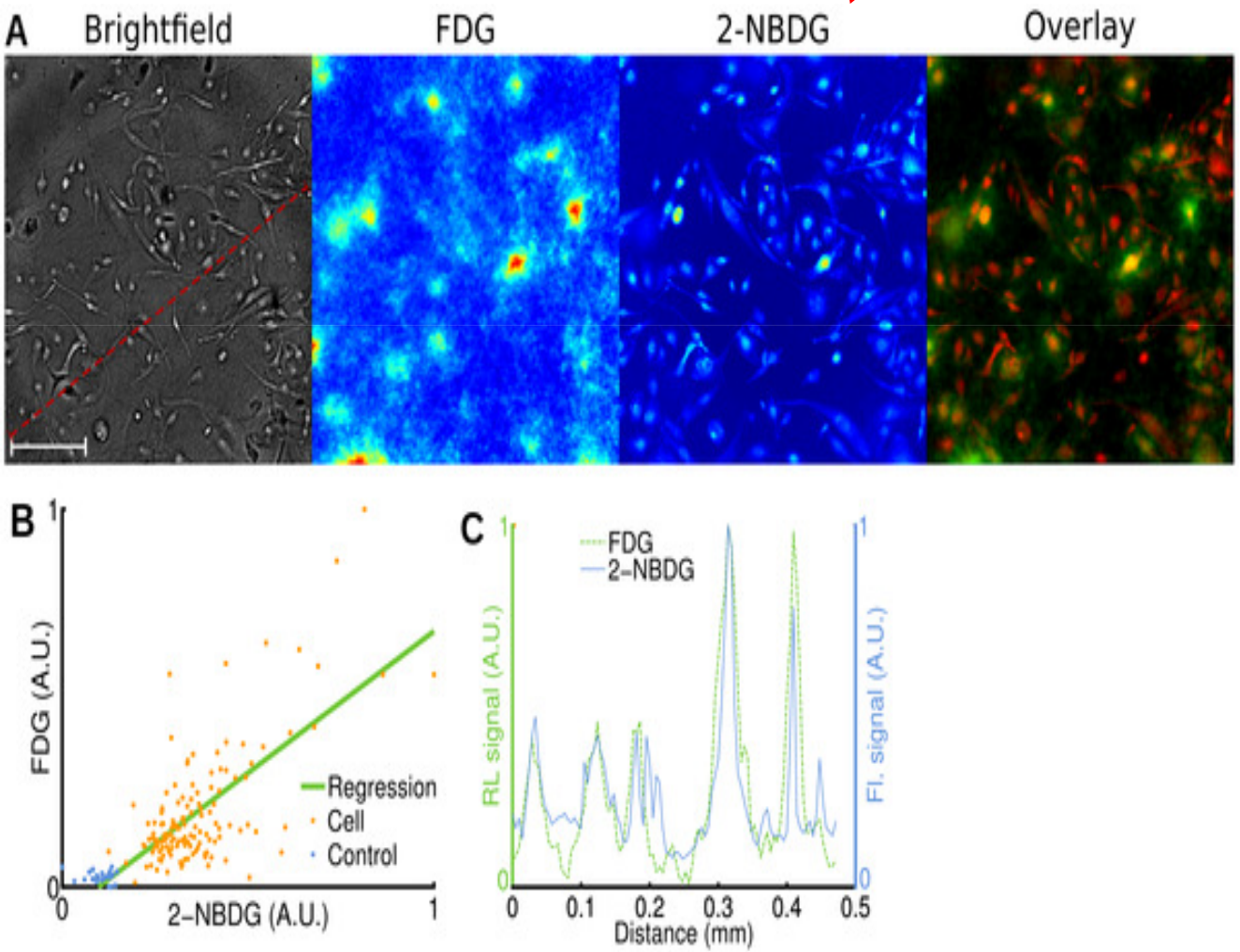
- "Radioluminescence Microscopy: Measuring the Heterogeneous Uptake of Radiotracers in Single Living Cells" (Pratx et al, 2012)
  - Visualization of radiotracer uptake at single-cell resolution
  - Heterogeneity in ^{18}F-FDG uptake of in single breast cancer cells



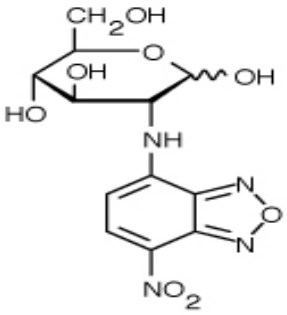
Radioluminescence imaging of FDG uptake in single cells.

Breast Cancer Cells

Fluorescent glucose analogue

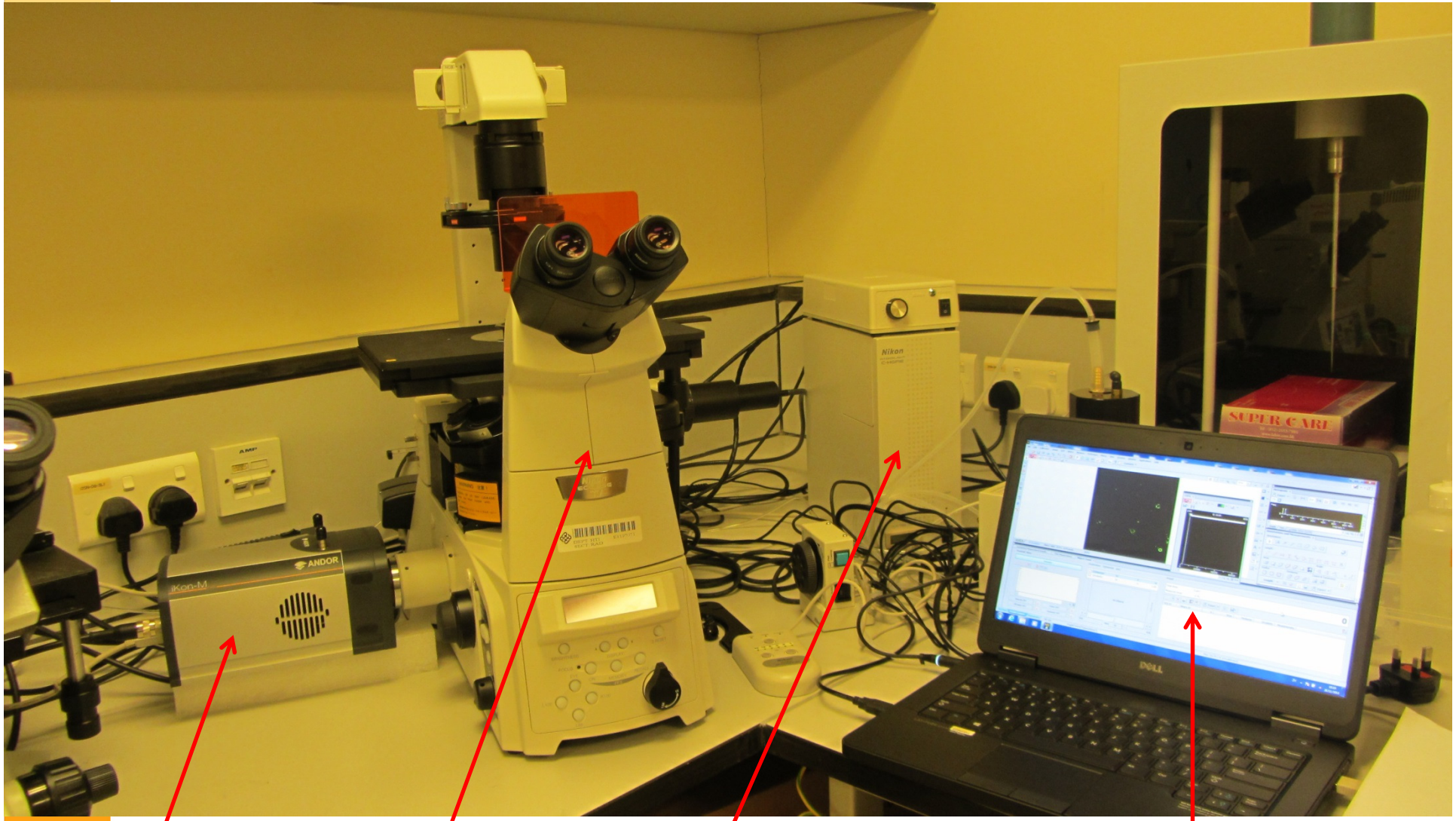


2-NBDG



- 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose): a fluorescent glucose analogue.
- 2-NBDG has been used to monitor glucose uptake in live cells, an indicator of cell viability.
- It displays excitation/emission maxima of ~465/540 nm, and can be visualized using fluorescence filters.
- 2-NBDG is rapidly accumulated in tumor cells, compared to nonmalignant cells (Roger G. O'Neil et al. 2005).
- An optical marker for detection of malignant cells.

Microscopy picture



Camera

Microscope

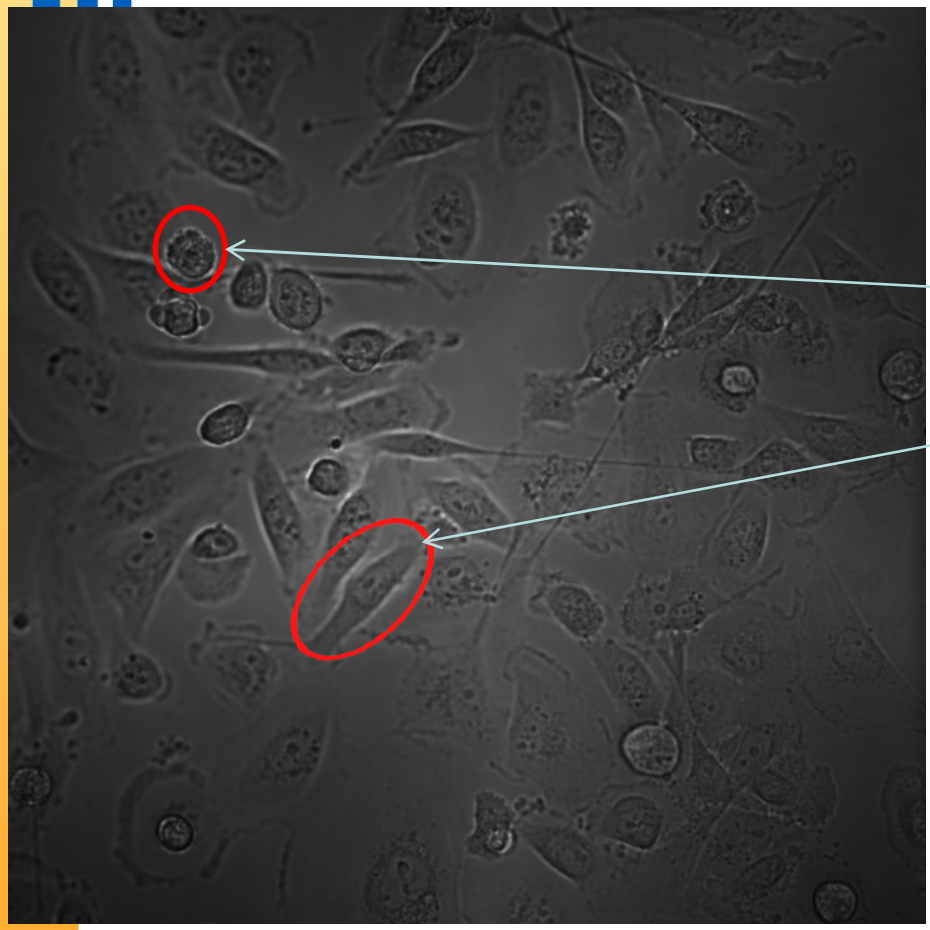
Laser exciter

Image control system

Protocol:

- **Cell culture** in L15 medium with 10% FBS, and incubation at 37 ℃;
- **Passage the cells** into glass bottom dishes with cell number of 10,000/ml (2ml in each dish);
- **24h incubation** at 37 ℃;
- 1h **glucose fasting** period in L-15 medium without FBS;
- **Add 2-NBDG and incubate** for 1h: the final concentration is 100M.
- **Image**: take one photo per 5mins and last for 12h.

MDA-MB-231 breast cancer cell line

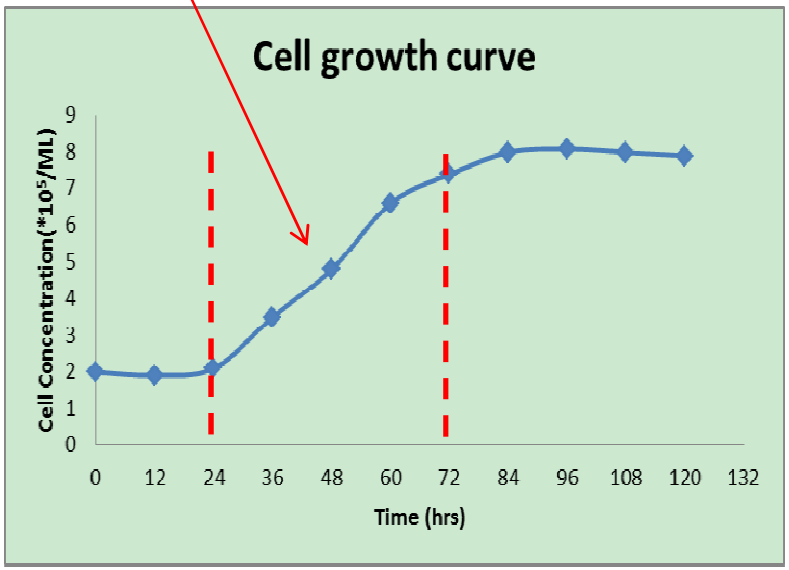


- **Morphology:**

Rounded cells

Spindle-shaped cells

Logarithmic phase – Cells for imaging



- **Growth chart:**

Result

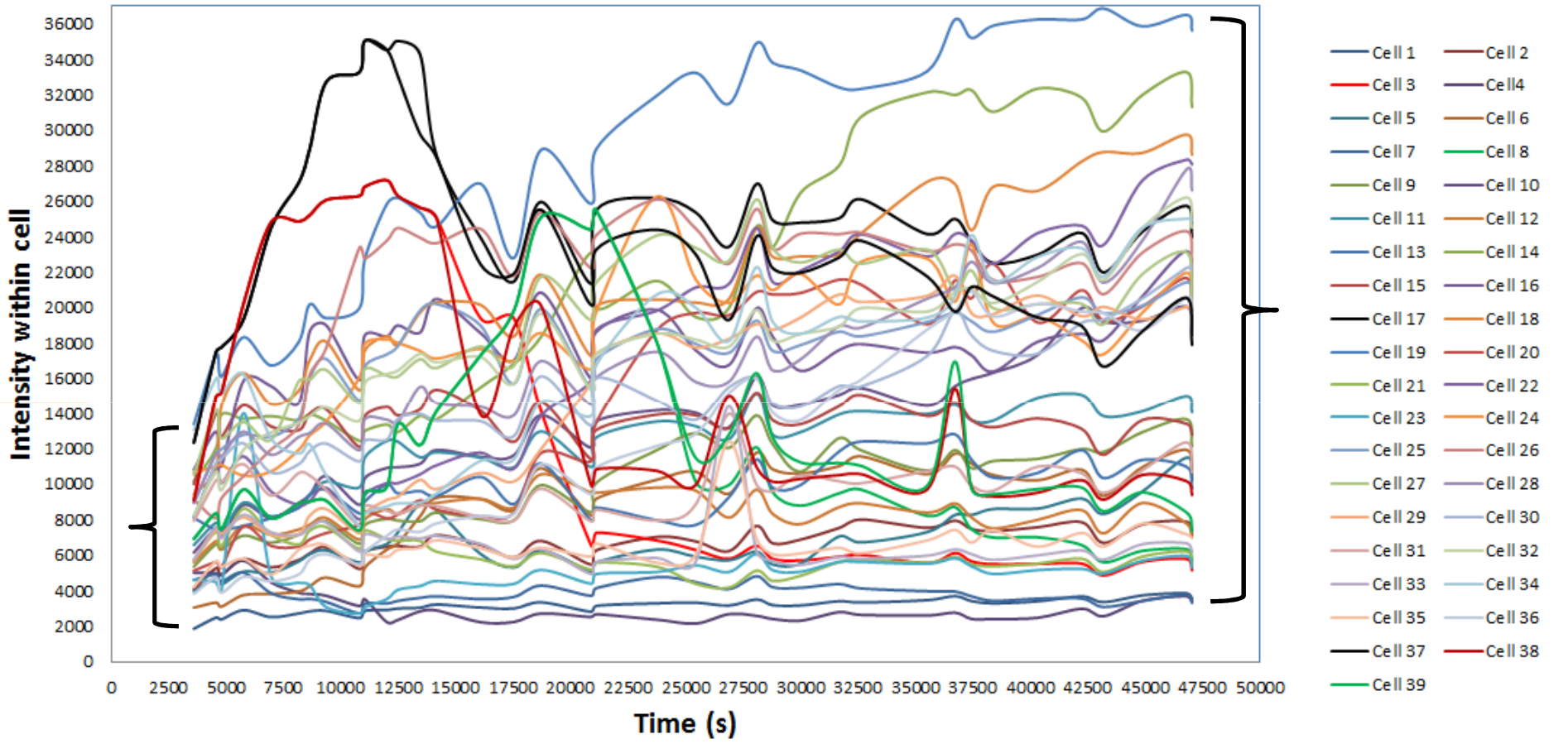


Figure 1. Heterogeneity of glucose uptake in individual cells.

Result

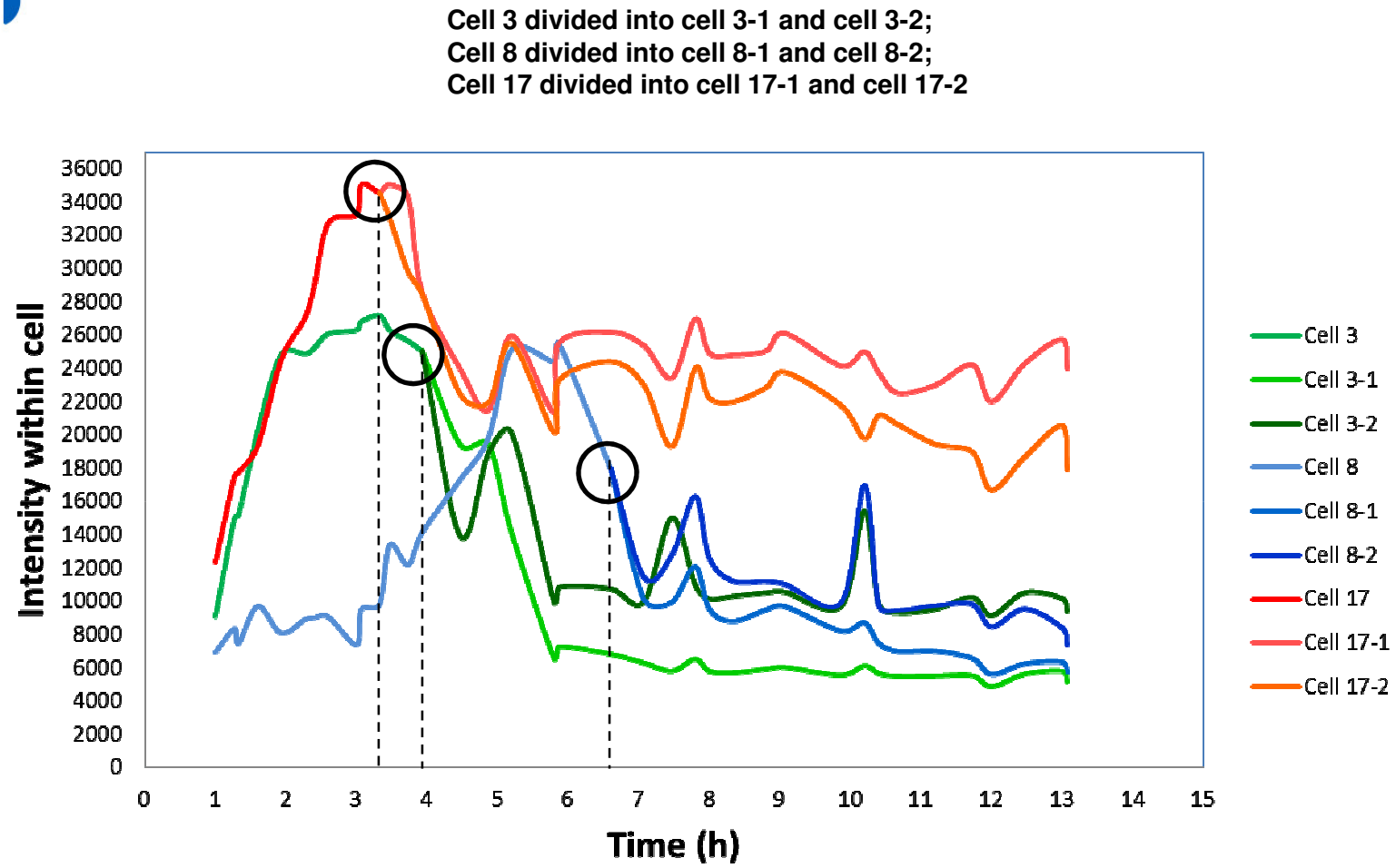


Figure 2. The relationship between cell division and glucose uptake. High glucose uptake before cell division and low glucose uptake after cell division.

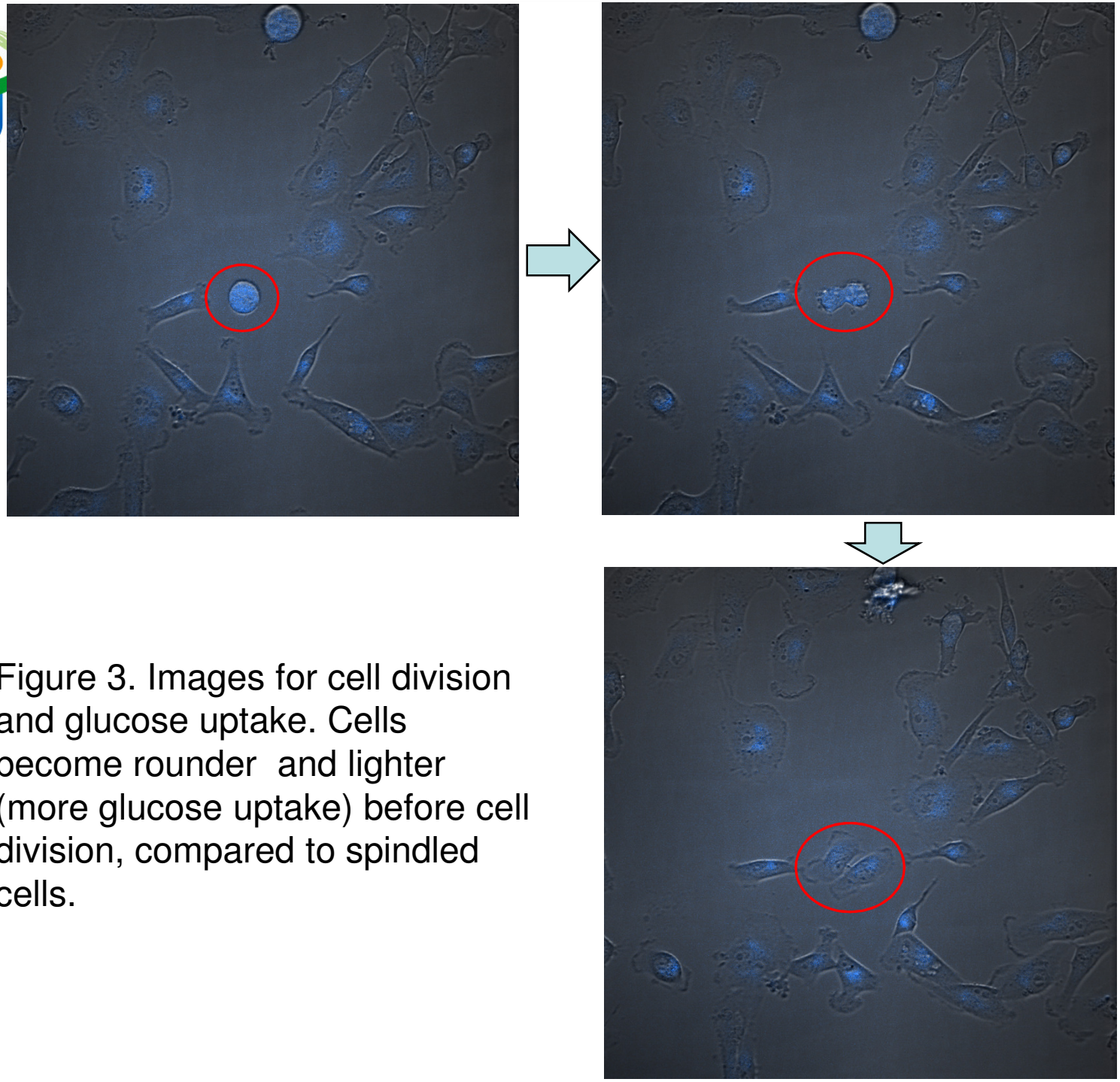
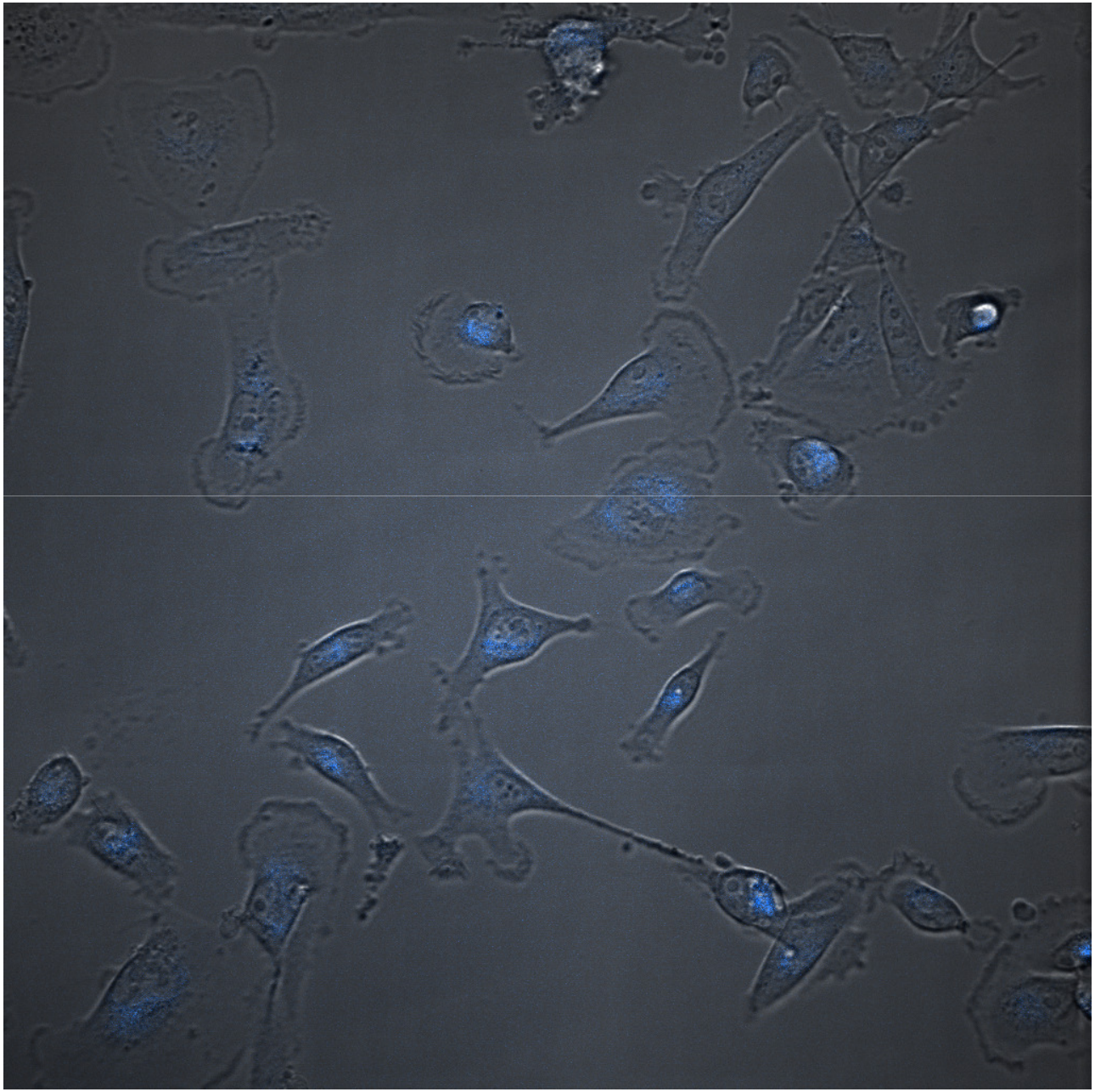
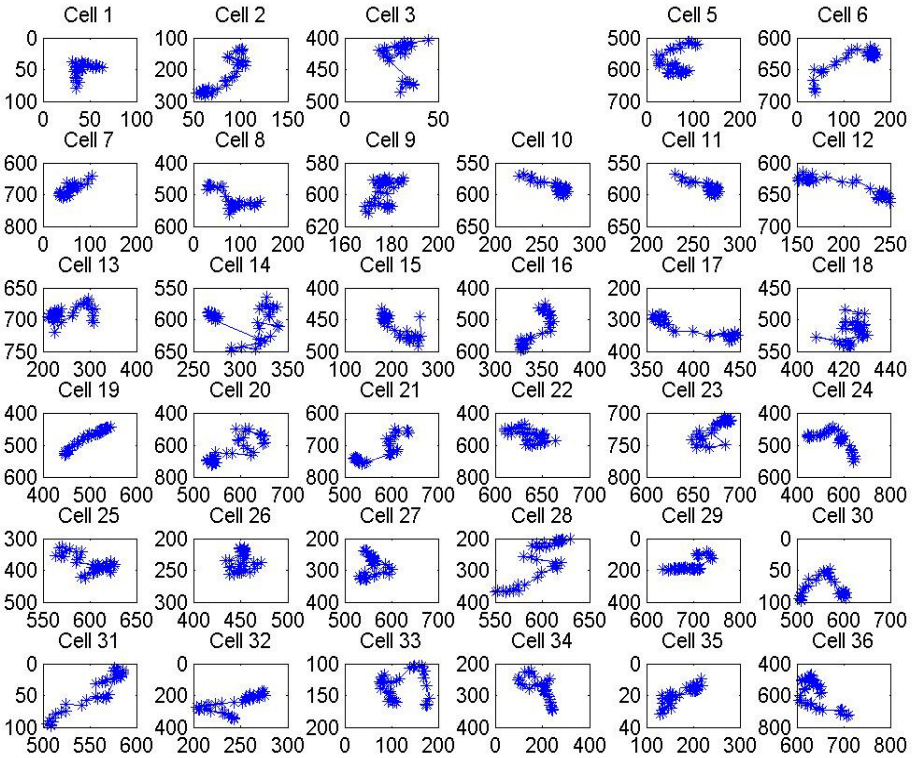


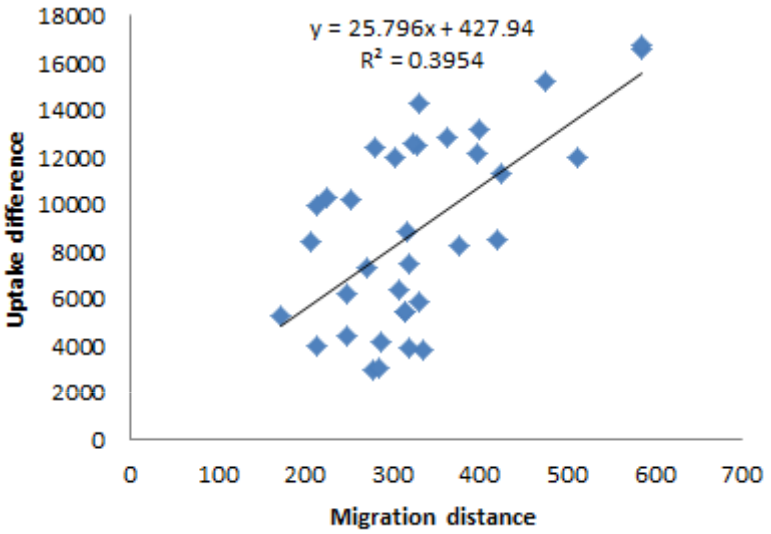
Figure 3. Images for cell division and glucose uptake. Cells become rounder and lighter (more glucose uptake) before cell division, compared to spindled cells.

Glucose uptake and cell division



Result





R=0.63, P=0.0001

Figure 4. Relationship between cell migration distance and glucose uptake. The longer cell migration distance, the more glucose uptake.

Conclusion

- Heterogeneity of glucose uptake exists in individual cell;
- High glucose uptake before cell division and low glucose uptake after cell division;
- Cells become rounder before cell division, compared to spindled cells;
- The longer cell migration distance, the more glucose uptake.

What's next?

We are using the live single cell imaging to:

- Classify cells into groups with respect to their metabolic features
- Compare the rates of cell divisions among the groups
- Identify candidate target genes that are related to glucose uptake and also the potential drug targets for cancer treatment.
- Identify genes involved in cell migration, the potential drug targets for metastasis.





Thank you