Engineering Improved Approaches to Pancreatic Beta-cell Connectivity

B. Hansen, K. Suba, V. Salem

Department of Bioengineering, Imperial College London

1. Introduction

Pancreatic Islet Imaging

- Diabetes is a leading cause of early death, with half a billion cases worldwide, and results from failure in the coordinated beta cell insulin secretory response from pancreatic islets[1].
- Islet beta-cells form a functional unit and conventional cell culture struggles to model islet dynamics or physiological insulin secretion[2].

Organ-on-a-Chip as a Model for Islet Dynamics

- Organ-on-a-chip technology emulates 3D conditions present in living organs, with controlled microenvironment and constant perfusion.
- Current systems of functional calcium imaging of islets in vivo at the single cell resolution suffer from high degree of motion, making it difficult to track individual beta cells and infer functional connectivity[3].

Computational Automation and the three R's

- A more precise, efficient, and effective algorithm for analyzing islet-in-the-eye connectivity data will allow for the reduction in use of animals for islet imaging transplant.

2. Aims

Islet on a Chip Design and Refinement:

- Test current chip designs and refine into novel chips capable of trapping islets for reperfusable long-term experimentation ex vivo.

ROI detection and Motion Correction:

- Create an algorithm that, given few input parameters, automates region of interest (ROI) detection and motion correction for single cell resolution calcium imaging data.

3. ROI Detection and Motion Correction

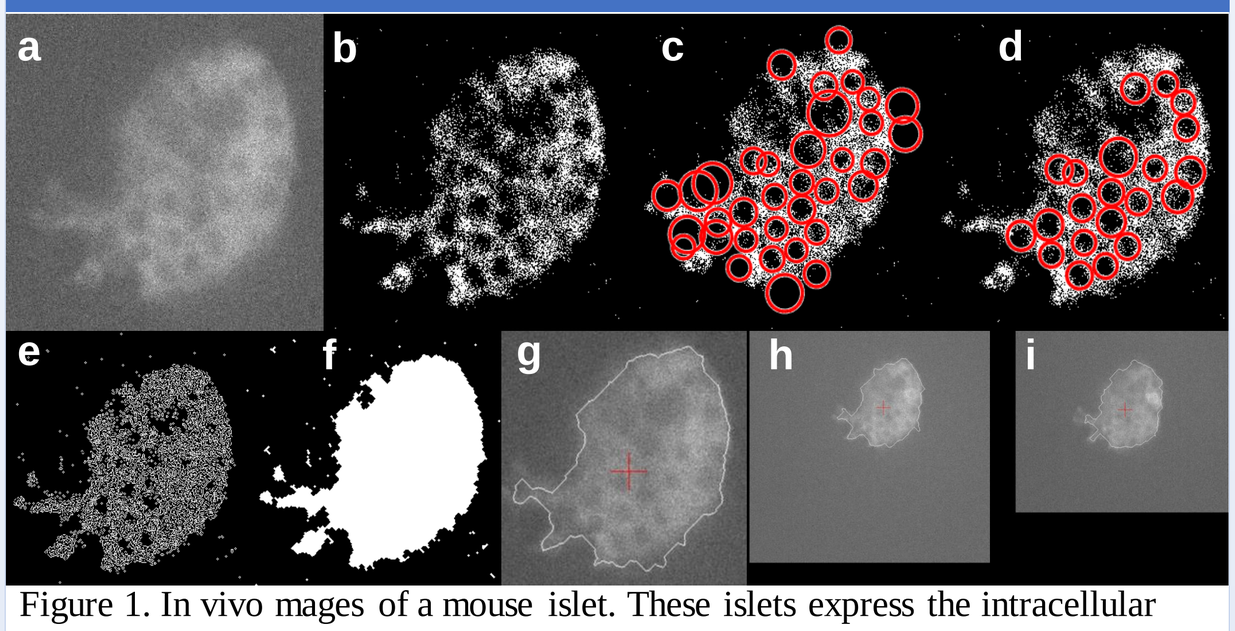


Figure 1. In vivo mages of a mouse islet. These islets express the intracellular calcium fluorophore GCaMP 6 in their beta cells and calcium imaging can be used to infer functional insulin secretory activity. (a-d) Automated ROI detection. (e-i) X-Y motion correction. (a) Normal image, pre-processing. (b) filtered image. (c) Initial ROI detection. (d) Filtered ROI selection. (e) Binary gradient mask. (f) Image with filled holes and cleared borders. (g) Image with outlined centroid, red cross marking center. (h) Motion corrected image at frame 68. (i) motion corrected image at frame 107.

4. Islet on a Chip Design and Refinement

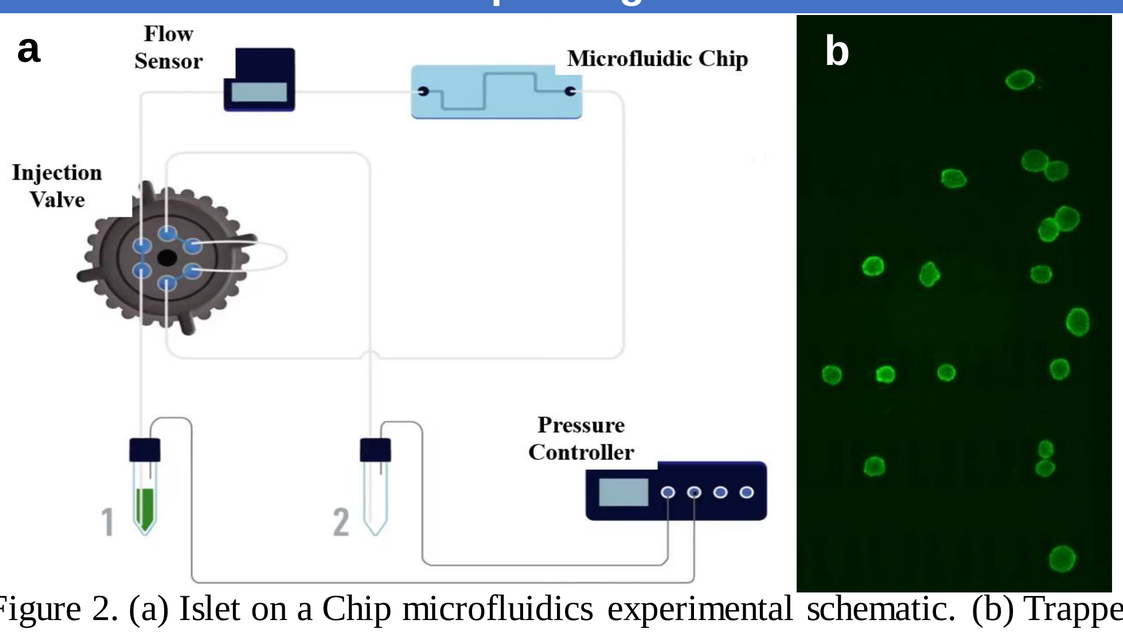


Figure 2. (a) Islet on a Chip microfluidics experimental schematic. (b) Trapped islets isolated from donor mice expressing fluorophore in beta cells stained with Cal-520.

5. Results

	150µm	100µm	70µm	50µm	30µm
Untrapped	10	10	5	3	1
Trapped	8	50	29	10	3
Trap Efficiency	44%	83%	85%	77%	75%

	Top Channel	Middle Channel	Bottom Channel
Untrapped	15	7	7
Trapped	34	43	22
Trap Efficiency	69%	86%	76%

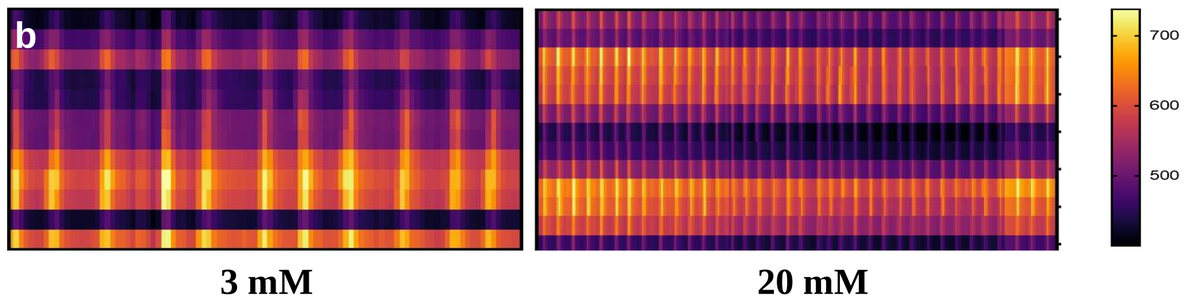


Figure 3. (a) Efficiency at various trap widths and for the three trap channels. (b) Segmental glucose readouts recorded from trapped islets perfused with 3mM and 20 mM glucose . (c) Calcium traces recorded from mouse islets via in vivo imaging processed manually (top) and using the detection and motion correction algorithm (bottom). Traces were standardized to f0. Pearson connectivity analyses between these two traces differed by ~3%.

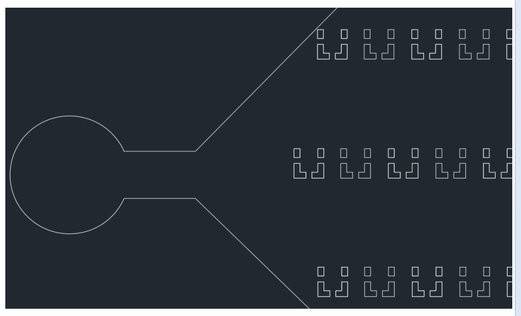
6. Conclusions

- The algorithm detects regions of interest with high fidelity, corrects deviation in the x and y planes, and saves in excess of 30 minutes of time per recording. The algorithm accurately recreates Pearson connectivity analyses generated manually.
- The current chip design traps islets at a maximum of 86% efficiency allowing for continuous perfusion, but not reperfusion, of media.

7. Future Work

- Improved chip design should maintain or increase trap efficiency while allowing for continuous reperfusion.
- Test more Pearson connectivity with data generated by automated algorithm and compare to manual data.

Figure 4. Novel Chip Design



8. References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 2019;157: 107843. https://doi.org/10.1016/J.DIABRES.2019.107843.
2. Salem V, Silva LD, Suba K, Georgiadou E, Neda Mousavy Gharavy S, Akhtar N, et al. Leader β-cells coordinate Ca2+ dynamics across pancreatic islets in vivo. *Nature Metabolism 2019 1:6*. 2019;1(6): 615–629. https://doi.org/10.1038/s42255-019-0075-2.
3. Bru-Tari E., Oropeza D., et al. Cell Heterogeneity and Paracrine Interactions in Human Islet Function: A Perspective Focused in β-Cell Regeneration Strategies. *Frontiers in Endocrinology*. 2021.