Introduction

Our previous development of a single molecule, single cell mapping technology relies on nanofluidic confinement, and demonstrated usage on whole yeast genomes derived from gel plugs. Single cell sample handling remains problematic in a microfluidic context.

We present a simplified hydrogel microsphere encapsulation protocol for single cell handling in bulk intended to:

(i) bypass the physical limitations of mass transport in a microfluidic environment and
(ii) shield long molecules from shearing forces during sample manipulation.

Background

Cells are lysed in a microfluidic chamber. Chromatin strands are extended using hydraulic actuation via application of negative pressure.

Above Right: Time series of cell lysis and chromatin extension using a dedicated microfluidic device. Chromatin is imaged using an intercalating fluorescent dye.

Right: Chromatin strands imaged using an 100x objective. Dashed red line indicates the same field of view.

Alginate microsphere droplet generation

Above: (a) Water in oil droplet generation of alginate microspheres.
(b) Sample preparation (cell lysis) is carried out in a centrifuge tube following extraction from oil.
(c) Microspheres loaded with intact single cell genomes are delivered to the microfluidics chip.

Right: Alginate microspheres containing intact cells with a membrane-permeable fluorescent nuclear stain. Select microspheres containing cells are indicated by the arrows.

Mammalian cell lysis inside alginate microspheres

Right: Traps for alginate microspheres in a PDMS (polydimethylsiloxane) microfluidic device. Trapping regions are indicated by the arrows.

Below: Time series of mammalian cell lysis inside of a trapped alginate microsphere.
Cells are initially labelled with membrane-permeable fluorescent dye for identification.
Lysis buffer is added at t = 0 seconds. It contains a membrane-impermeable fluorescent intercalator.
Membrane lysis was identified by an increase in fluorescence intensity due to intercalator binding.
Yellow dashed line indicates alginate microsphere

Mbp-length DNA extraction on a microscope slide

Above: (a) Microspheres containing cellular chromatin are deposited onto a microscope slide.
(b) Solution containing an intercalating agent is added to the solution to disrupt the polymer network of the microspheres.
(c-d) A coverslip is placed over the sample and drawn across to induce fluid shear to separate and stretch chromatin strands.

Right: Stretched chromatin reaching a length of 1mm.

Below: Chromatin strands imaged using an 100x objective. Dashed red line indicates the same field of view.

Conclusion

We validated hydrogel microsphere encapsulation of single, cultured, human lymphoblast cells as a sample preparation protocol for whole single cell genomes.
We also demonstrate in-alginate lysis using time course analysis of fluorescence imaging of chromatin.

The present results show that single cell genome content can be handled off-chip using alginate microspheres before introduction into a device for single molecule sensing.

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References