sampling live cells on a picoscale and its applications

technical journal club

merve avar

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importance

- individual cells are unique in structure, composition and function

- cell heterogeneity

→ differentiation during development
→ environmental changes result in differential response in cell behavior

- cell lysis provides a snapshot of the state of the cell

studying single cells

advantages

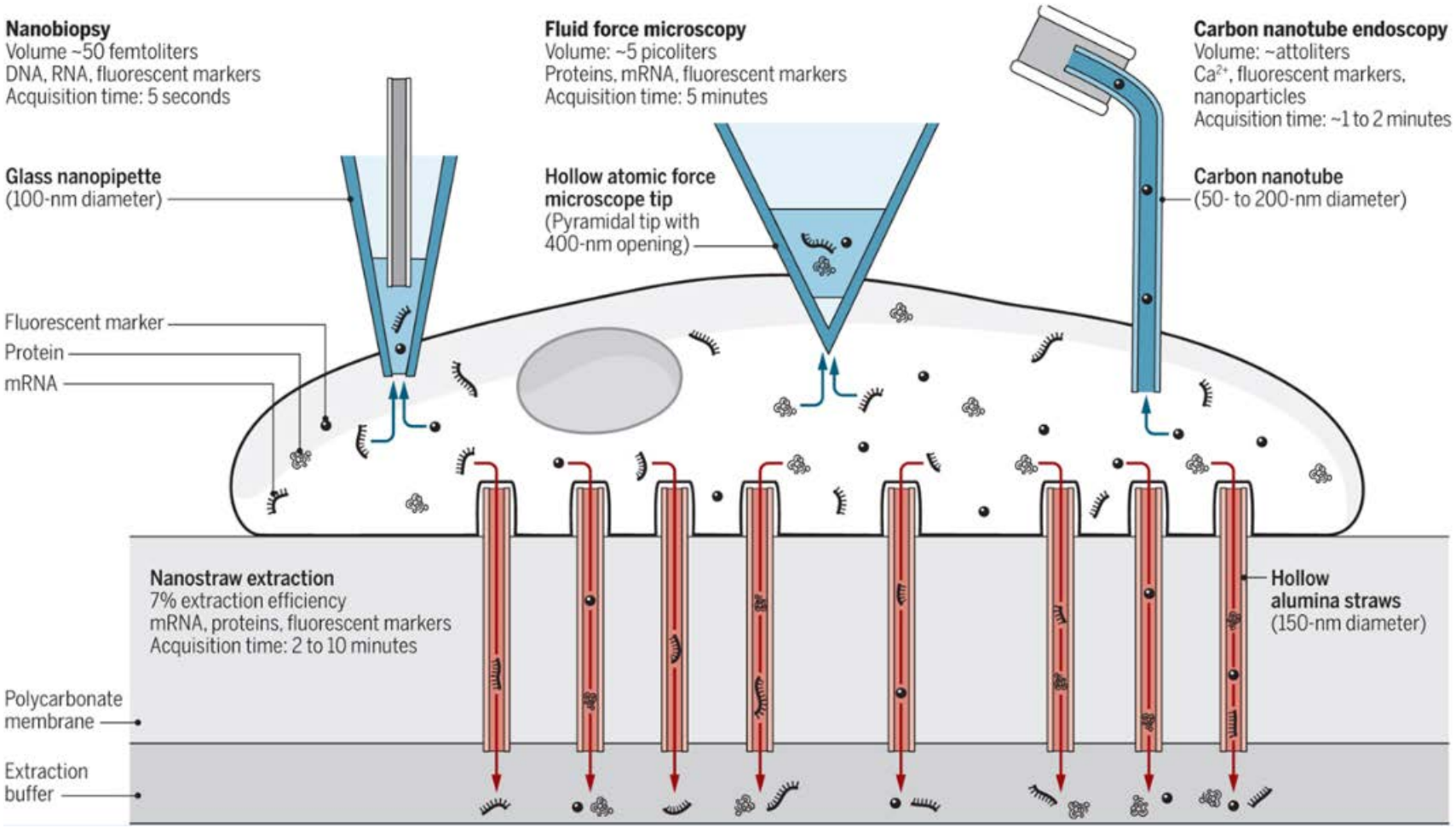
- information acquired is high in resolution; i.e. knowledge gain about heterogeneity
- profiling of rare cell populations possible
- investigation of subtle differences in signaling pathways possible
- high-throughput is achievable (i.e. microfluidics)

challenges

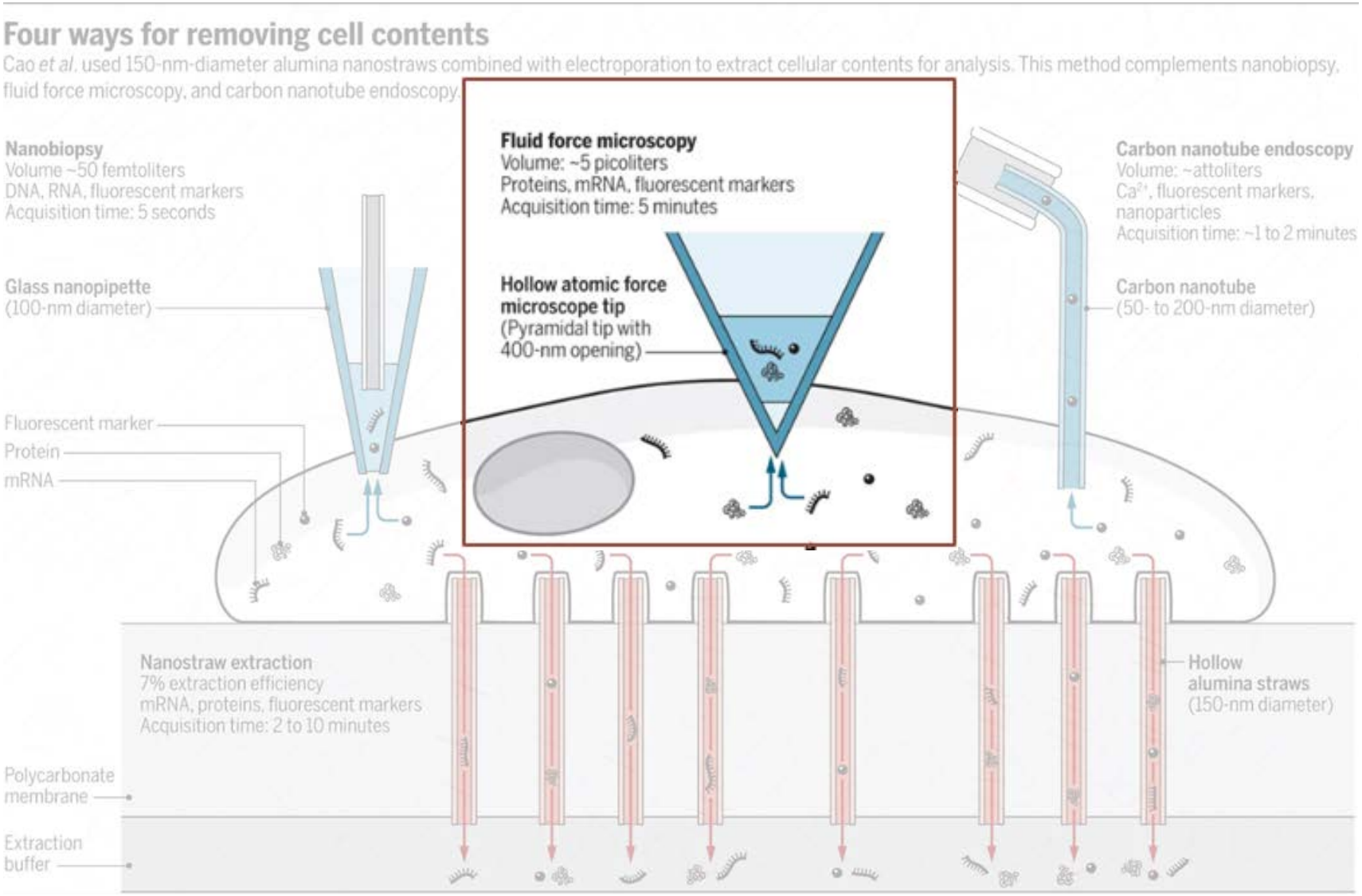
- most methods require lysis, therefore lack of spatial-temporal content
- batch effect; differentially collected samples may have drastically different results
- data analysis not trivial

Four ways for removing cell contents

Cao *et al*. used 150-nm-diameter alumina nanostraws combined with electroporation to extract cellular contents for analysis. This method complements nanobiopsy, fluid force microscopy, and carbon nanotube endoscopy.



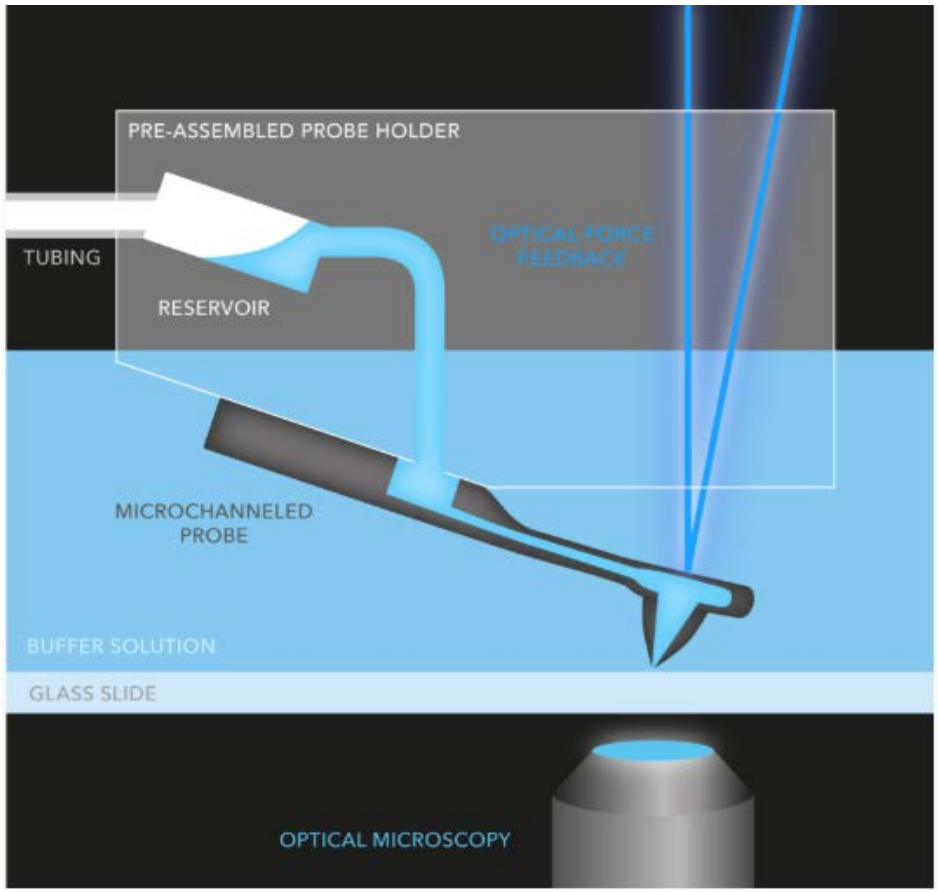
Higgins and Stevens, Science, 2017



Higgins and Stevens, Science, 2017

fluid force microscopy: fluidFM

- combination of atomic force microscopy (AFM) and closed microchannels for delivering/retrieval of fluids

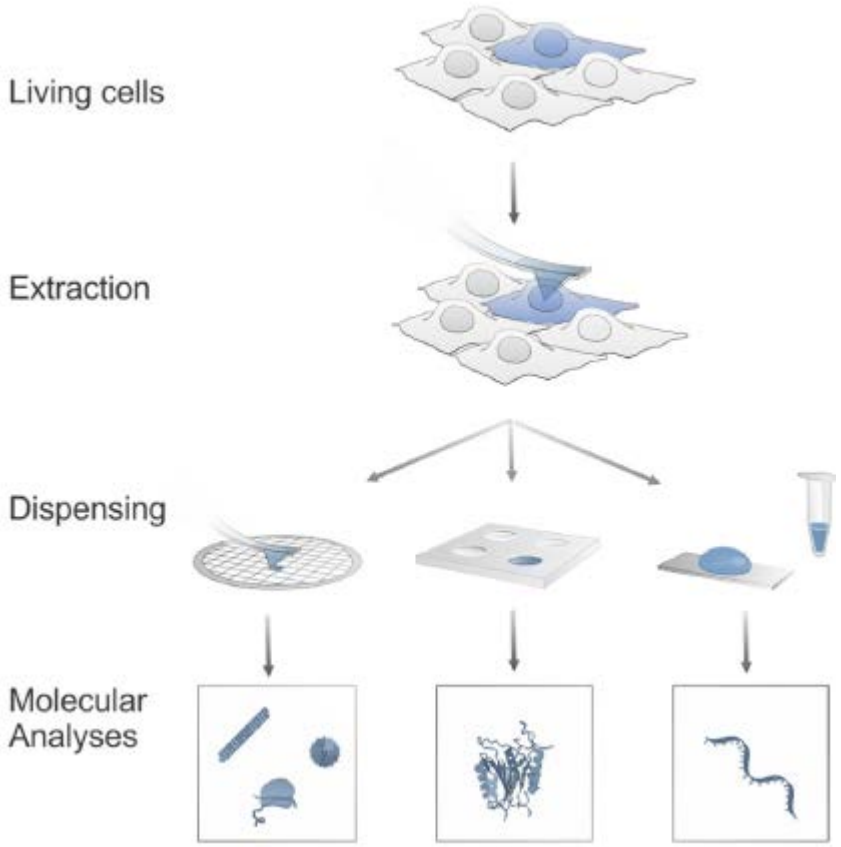


- minimally invasive
- allows realtime observation during manipulation of the cells
- cytoplasmic and nucleoplasmic sampling
- drawback of method: nanoprobe not capable of sampling from deeper tissue layers

adapted from: FluidFM: Meister & Gabi et al., Nano Lett. 9, 2009

Cell

Tunable Single-Cell Extraction for Molecular Analyses



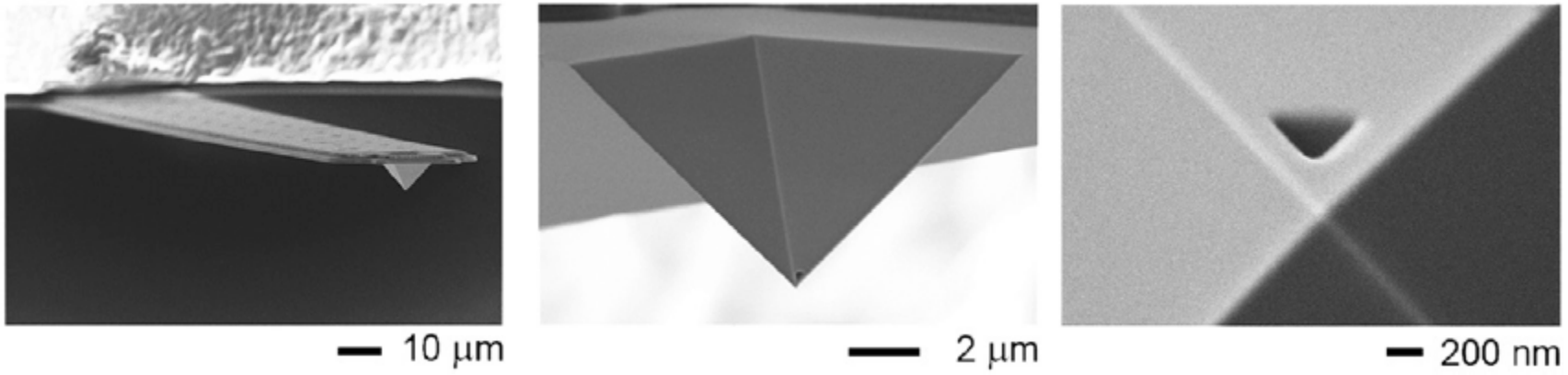
Authors

Orane Guillaume-Gentil, Rashel V. Grindberg, Romain Kooger, ..., Martin Pilhofer, Tomaso Zambelli, Julia A. Vorholt

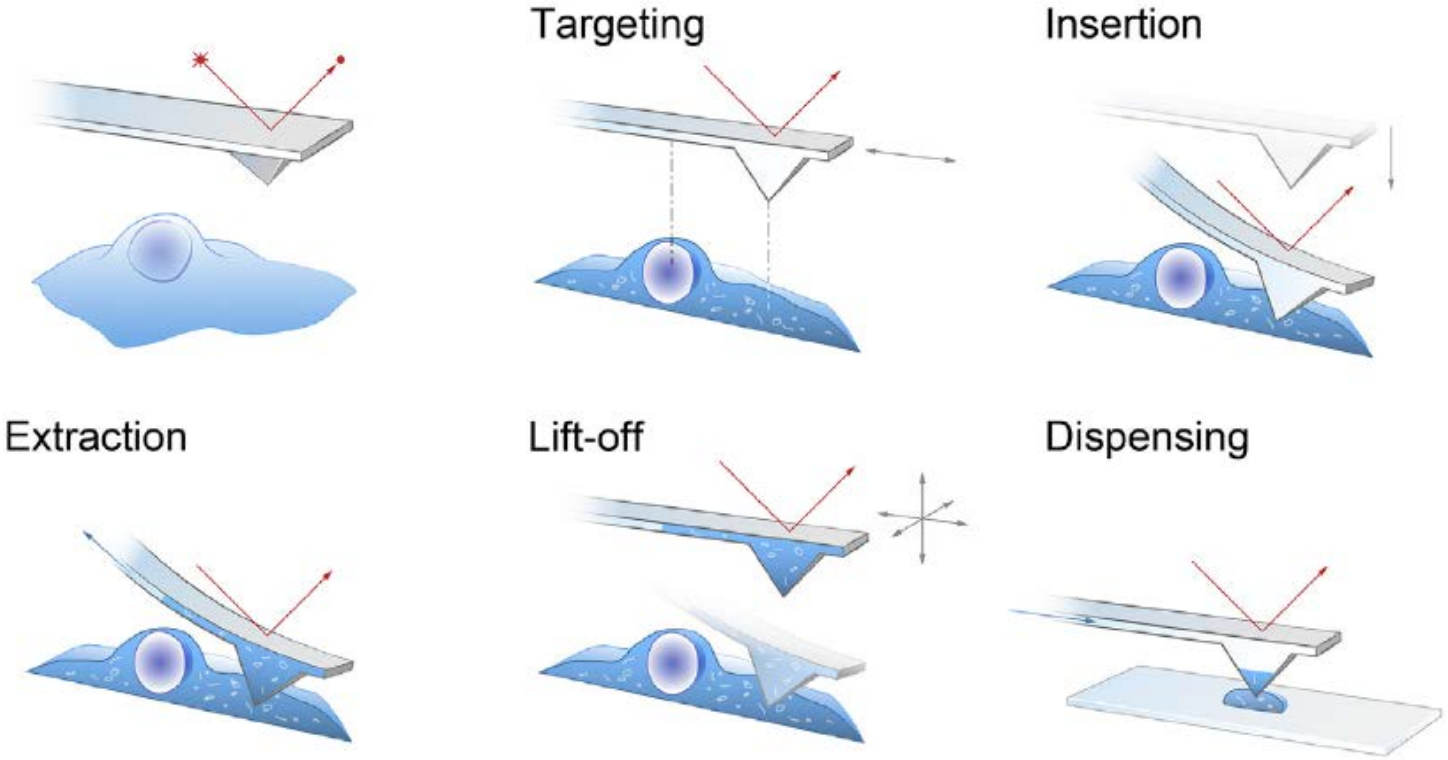
establishment of the system

- surface of the probe adjusted to prevent adsorption of cellular material (silicon based)

- to avoid evaporation an immiscible phase with mineral oil is filled inside the probing chamber

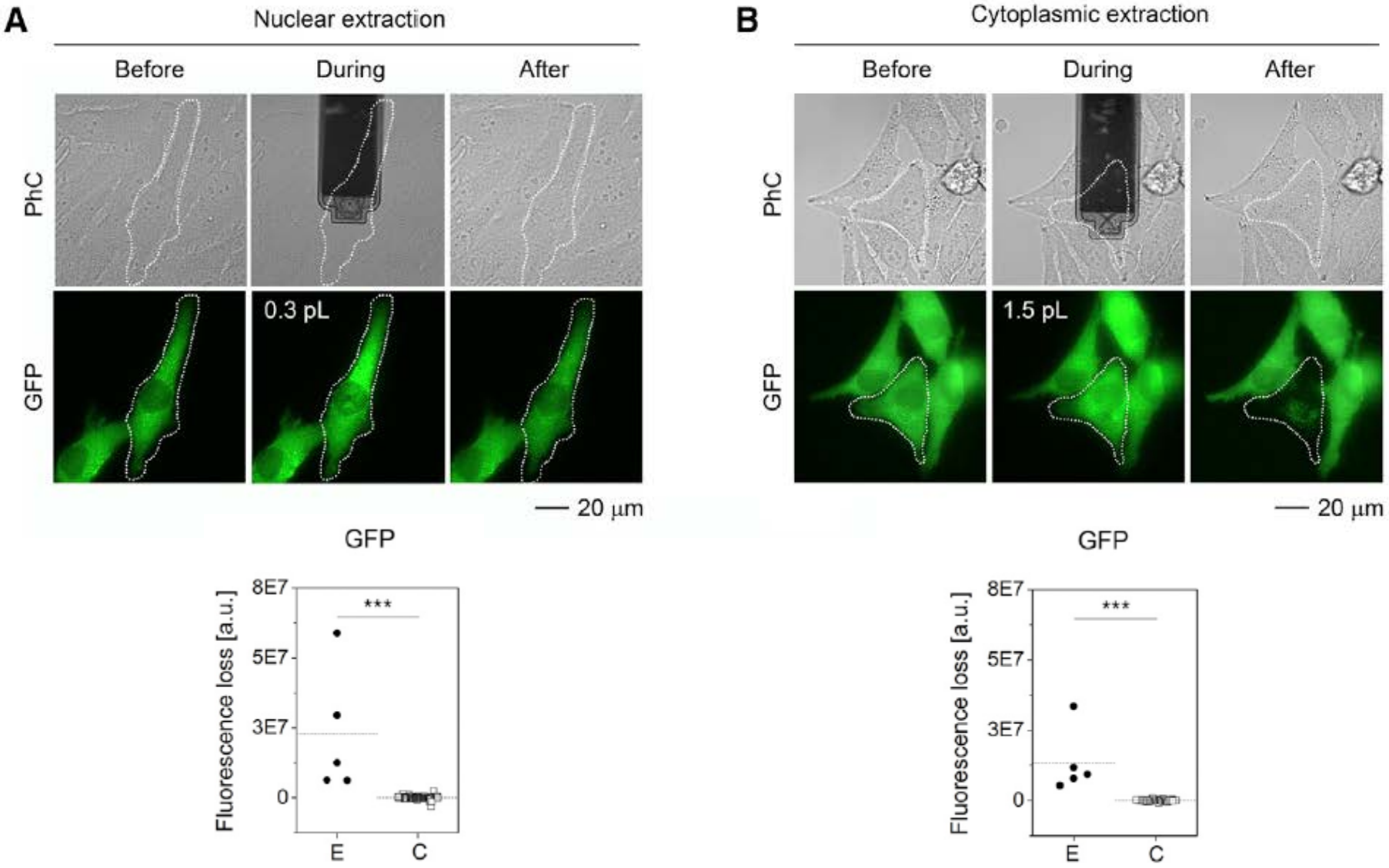


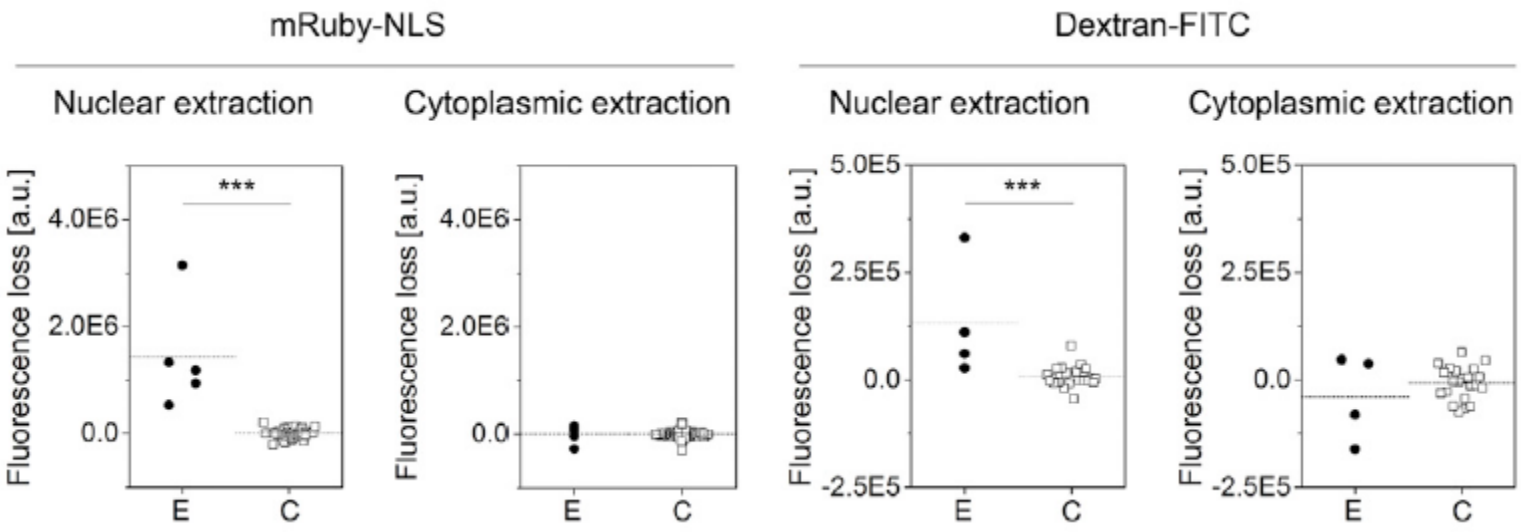
workflow

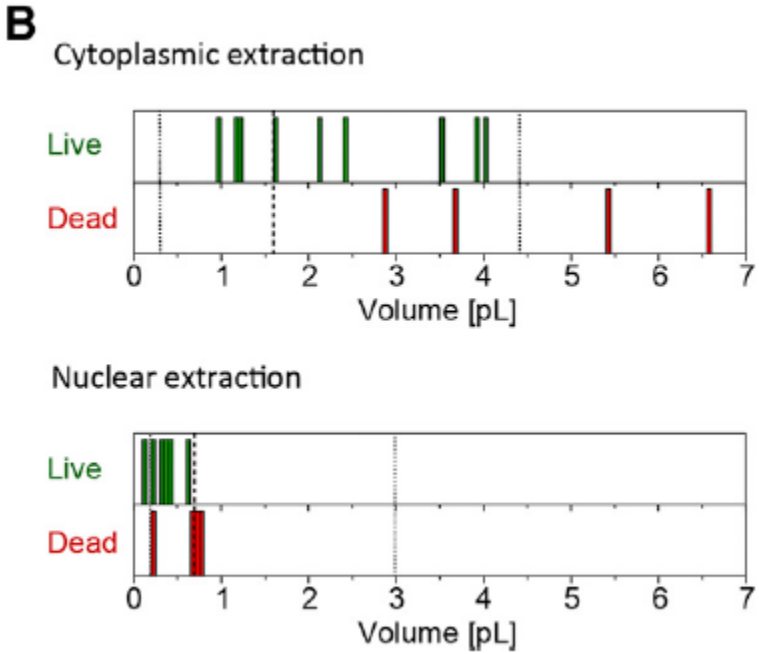


proof of principle

- HeLa cells stably transfected with GFP

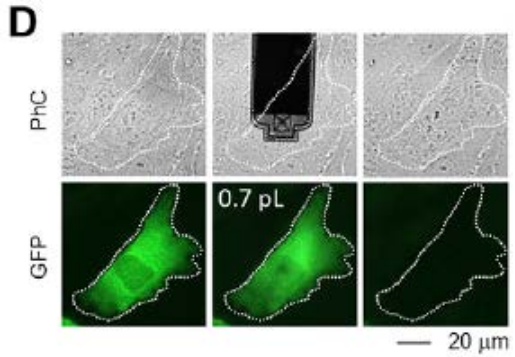






82% viability up to 4 pL (n= 13)

86% viability up to 0.6 pL (n= 10)



molecular analyses of live picoliter sampling

3 different approaches

imaging based – electron microsopy

biochemical analysis - detection of enzymatic activities

transcriptional analysis – qRT-PCR

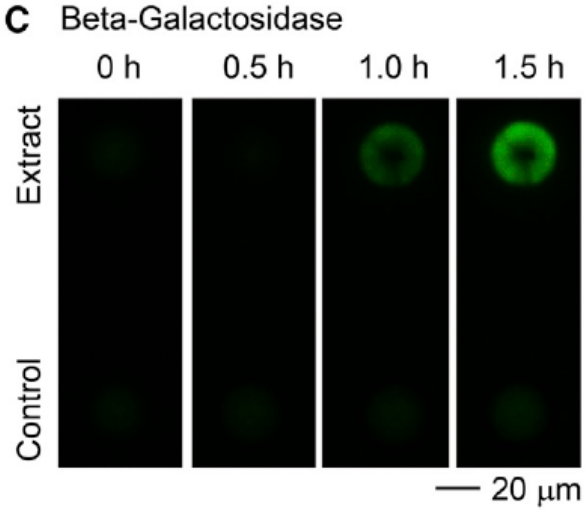
molecular analyses of live picoliter sampling – detection of enzymatic activities

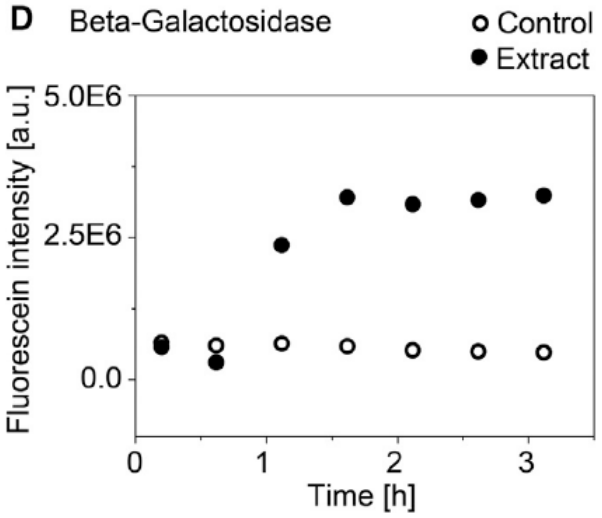
design of picowells to test whether the isolated β-gal is still active

wells are prefilled with the fluorogenic subtrate and then covered with oil to prevent evaporation

later on FluidFM probe is used to extract samples from cells and later on used to dispense into the wells using overpressure

wells are then assessed with fluorescent microscopy for the conversion of the substrate into the final product





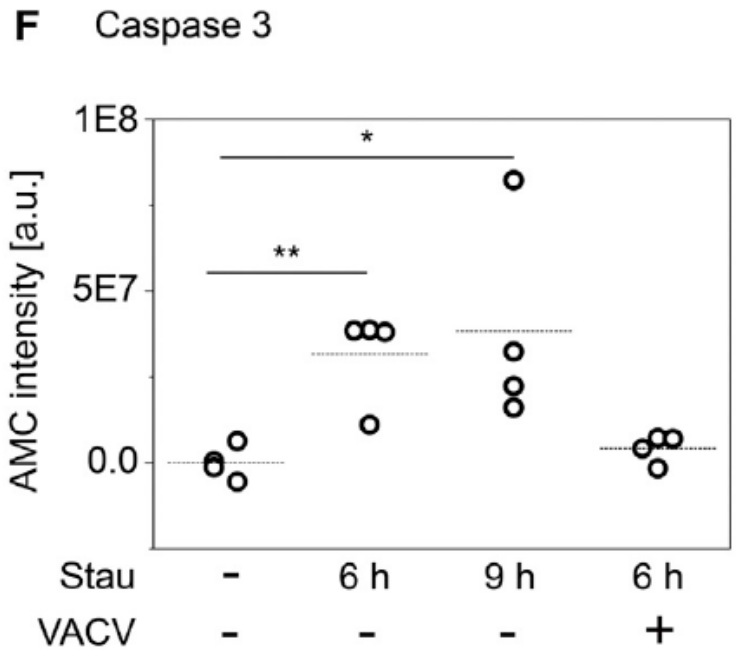
molecular analyses of live picoliter sampling – detection of enzymatic activities

HeLa cells

caspase 3 – apoptotic marker

staurosporine – inducer of apoptosis

VACV- viral pathogen capable of evading apoptosis



in VACV infected cells treated caspase activity abolishes

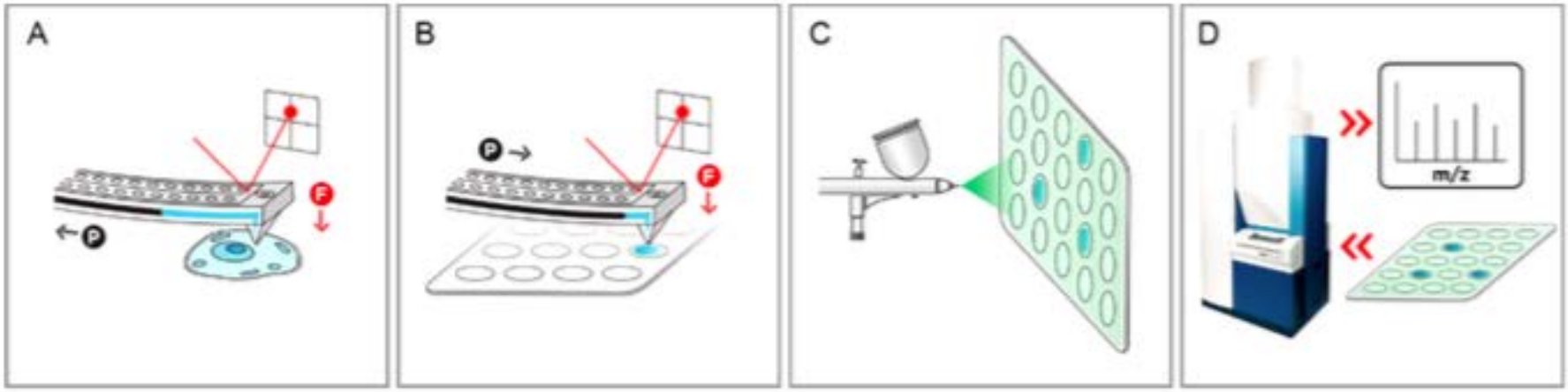
overall-

enzymatic activity of extracts is retained and testable in picoliter amounts

it is possible to distinguish between differentially treated cells

Single-Cell Mass Spectrometry of Metabolites Extracted from Live Cells by Fluidic Force Microscopy

Orane Guillaume-Gentil,[*,§,⊥] Timo Rey,[§,⊥] Patrick Kiefer,[§] Alfredo J. Ibáñez,[∥,†] Robert Steinhoff,[∥] Rolf Brönnimann,[‡] Livie Dorwling-Carter,[¶] Tomaso Zambelli,[¶] Renato Zenobi,[∥] and Julia A. Vorholt[*,§]



extraction

spotting on membrane suitable for MS

spraying of MALDI suitable matrix

MS spectra acquisition

proof of principle

- cytoplasmic extractions from HeLa cells (n=4)
- spots without cell extract are used for computing the background

compound	m/z_0	mean $m/z_0 \pm$ SD	Δm (ppm)	CID	$S/N \pm$ SD	N° out of 4
succinic acid	117.0193	117.024 ± 0.002	39.9	1110	69.3 ± 31.1	2
taurine	124.0074	124.010 ± 0.002	21.1	1123	54.5 ± 13.7	2
asparagic acid	132.0302	132.032 ± 0.001	13.4	5960	58.8 ± 5.1	3
threonic acid	135.0299	135.030 ± 0.004	0.7	151152	18.8 ± 14.1	2
glutamic acid	146.0459	146.046 ± 0.003	0.8	33032	92.8 ± 49.7	4
cysteic acid	167.9972	167.000 ± 0.002	16.6	25701	42.6 ± 18.4	3
formylglutamic acid	174.0408	174.042 ± 0.008	6.9	439376	39.0 ± 6.6	2
glycerophosphorylethanolamine	214.0486	214.056 ± 0.004	34.6	22833510	4.1 ± 1.5	2
cytidine	242.0782	242.080 ± 0.012	7.2	6175	22.0 ± 23.1	2
glutaconylcarnitine	272.1140	272.114 ± 0.002	0.1	53481620	6.4 ± 2.9	2
glutathione	306.0765	306.078 ± 0.006	4.8	124886	52.8 ± 25.4	2
lactaminic acid	308.0987	308.094 ± 0.006	−15.3	445063	14.1 ± 3.5	3
cyclic GMP	344.0402	344.040 ± 0.018	−0.5	24316	15.1 ± 11.3	2
UDP	402.9949	403.002 ± 0.005	17.6	6031	29.3 ± 6.6	3
ADP	426.0221	426.029 ± 0.002	16.1	6022	31.5 ± 8.4	2
ATP	505.9885	505.985 ± 0.004	−6.9	5957	9.4 ± 5.2	2
UDP-glucose	565.0478	565.054 ± 0.013	11.1	53477679	20.8 ± 4.9	3
UDP-glucuronate	579.0270	579.026 ± 0.008	−1.7	17473	18.1 ± 0.3	2
UDP-acetylglucosamine	606.0743	606.072 ± 0.005	−3.8	445675	29.8 ± 9.8	4
glutathione (oxidized)	611.1447	611.161 ± 0.007	26.7	975	13.3 ± 3.5	3

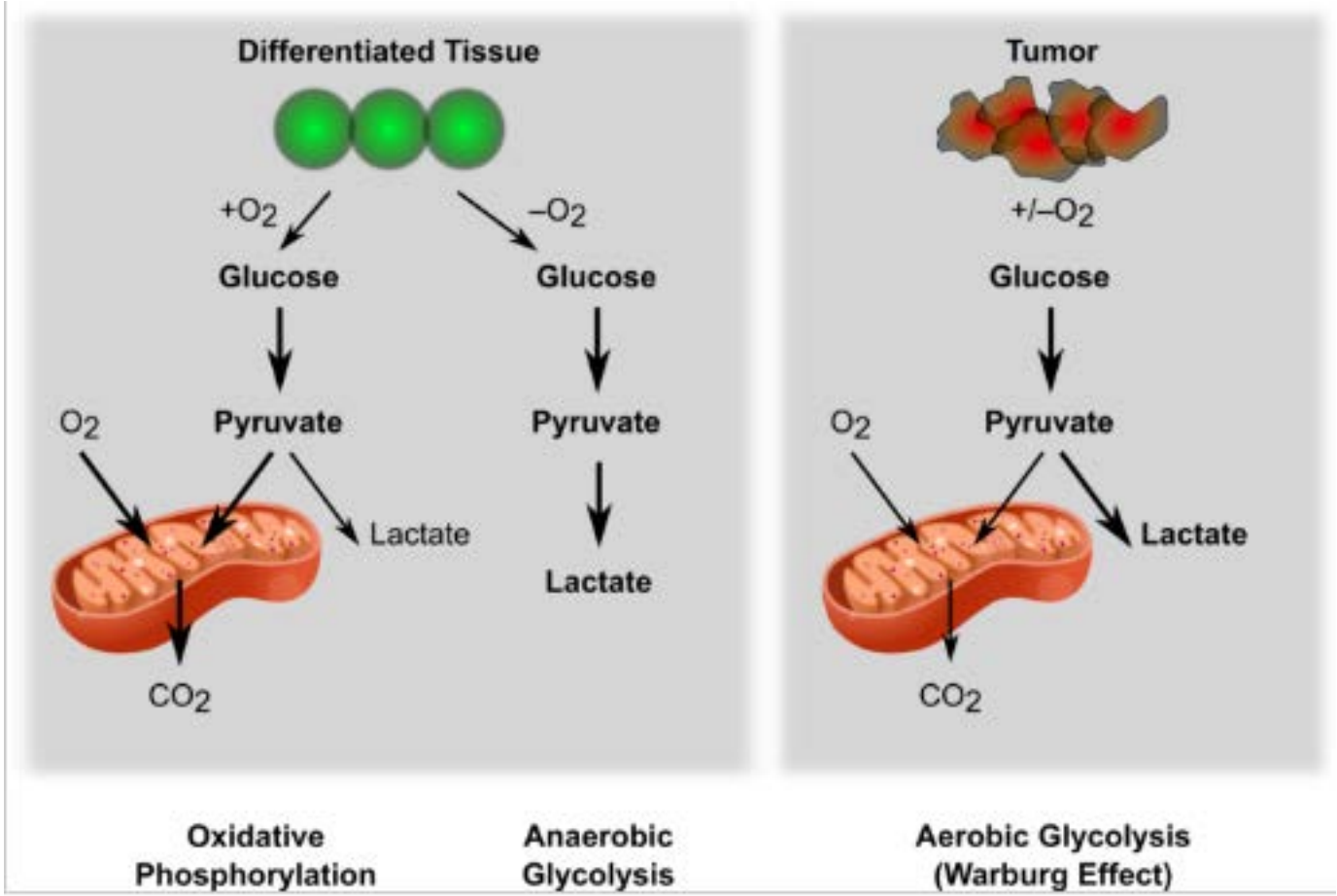
further validation of method- ^{13}C labeling

used to trace

- operation of metabolic pathways
- examination of intracellular fluxes

further validation of method- ^{13}C labeling

excursion to Warburg metabolism



Vander Heiden et al., 2009

further validation of method- ^{13}C labeling

after ^{13}C- glucose feeding the cells for 48h, FluidFM extraction and MS was followed

compound	m/z [M-H]$^-$	number of ^{13}C	replicate #1	measured Δm (ppm[a]) Replicate #2
glutamic acid	146.0459	0	146.043 (−19.7)	n.d.
GMP	367.0675	5	367.059 (−23.2)	367.075 (20.43)
UDP	408.0117	5	n.d.	408.0163 (11.3)
ADP	431.0389	5	431.039 (0.2)	431.037 (−4.4)
GDP	447.0338	5	447.033 (−1.9)	447.025 (−19.8)
UTP	487.9780	5	487.986 (16.3)	487.983 (10.2)
ATP	511.0052	5	511.003 (−4.4)	511.012 (13.2)
UDP-glucose	576.0847	11	576.079 (−9.8)	576.092 (12.7)
UDP-acetylglucosamine	617.1112	11	617.108 (−5.2)	n.d.
glutathione (oxidized)	611.1447	0	611.147 (3.8)	n.d.

[a]Parts per million values were calculated with accurate theoretical m/z values.

one isotopologue per compound captured for most cases

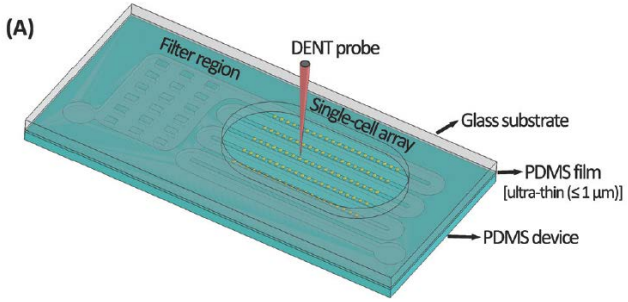
→ due to identical labeling of intermediates of glucose pentose phospate pathway

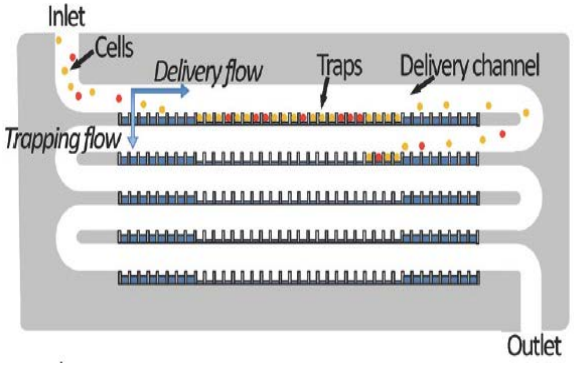
C numbers incorporated from heavy labeling are representative for their respective synthesis way in the cell

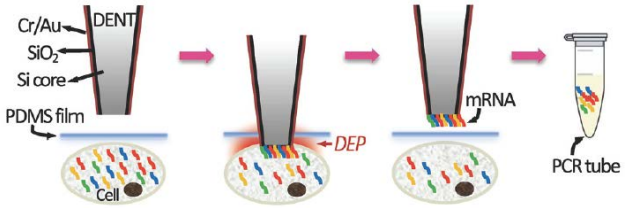
In situ mRNA isolation from a microfluidic single-cell array using an external AFM nanoprobe†

Xuan Li, ‡[a] Yinglei Tao, ‡[b] Do-Hyun Lee,[a] Hemantha K. Wickramasinghe*[abc] and Abraham P. Lee*[ad]

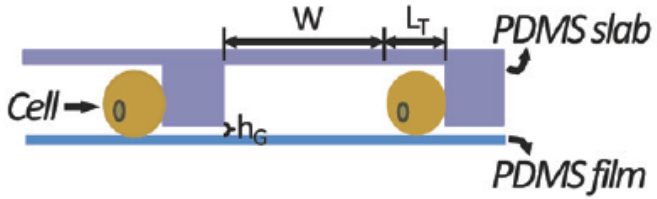
- based on single cell trapping by microfluidics
- followed by dielectrophoretic tweezer (DENT) application capturing RNA in a non destructive manner from single target cells
- after capturing the RNA is dispensed into PCR tubes, which subsequently undergo qRT-PCR



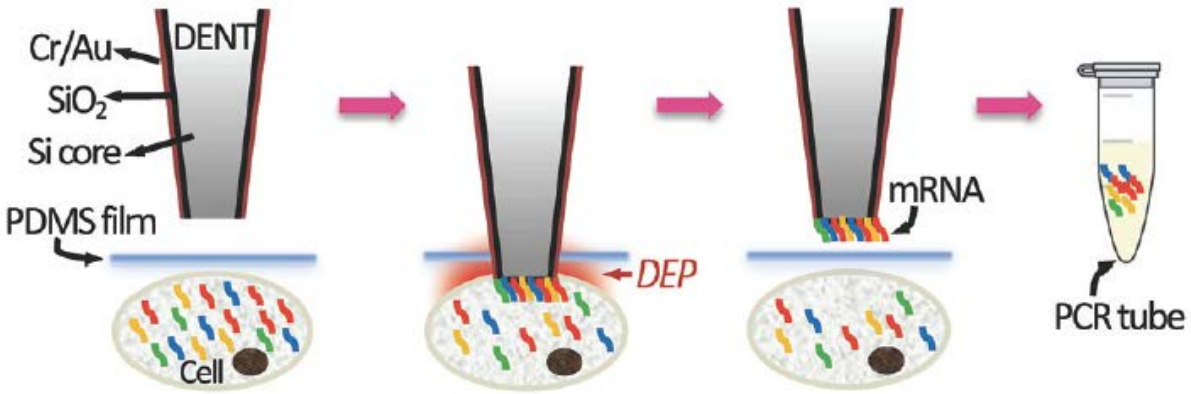




single cell capturing



design of the DENT probe



SiO_2: electrical insulation

Cr/Au layer: serves as outer electrode

tip is cut to expose the inner silicon core, diameter: 300nm to allow mRNA capture

amount of electricity applied is optimized for maximal RNA extraction

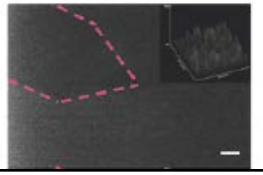
proof of principle

- 100 live HeLa cells are captured
- expression of 3 housekeeping genes analyzed;
  - GAPDH
  - ACTB
  - HPRT

- post extraction cells were investigated for viability with Calcein AM

- positive control: mRNAs extracted from bulk cell lysates- diluted

DENT application

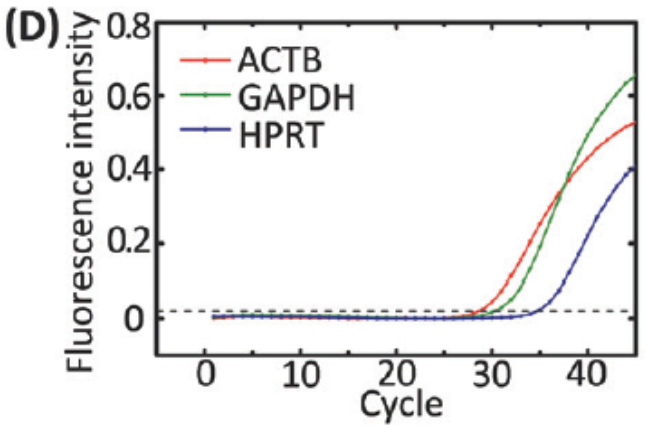


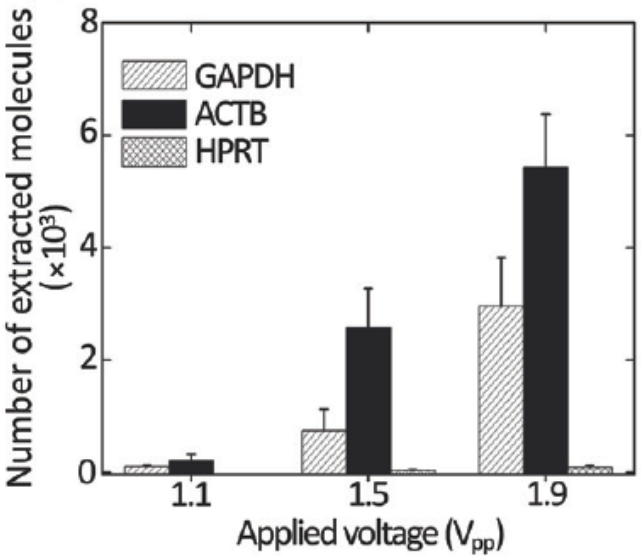
proof of principle

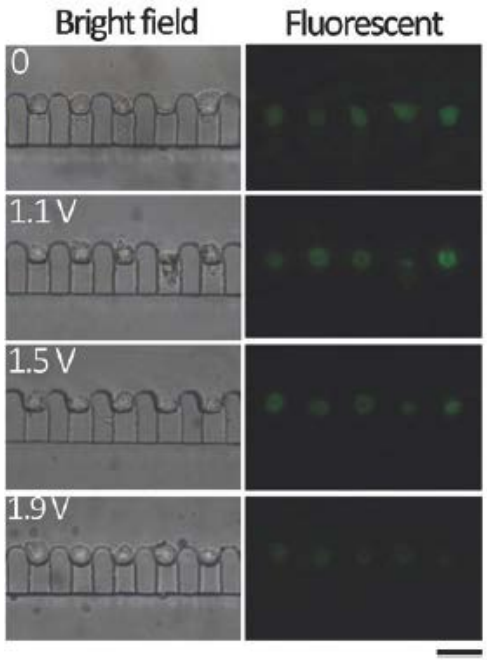
- 100 live HeLa cells are captured
- expression of 3 housekeeping genes analyzed;
  - GAPDH
  - ACTB
  - HPRT

- post extraction cells were investigated for viability with Calcein AM (on cip culturing for 12h)

- positive control: mRNAs extracted from bulk cell lysates- diluted



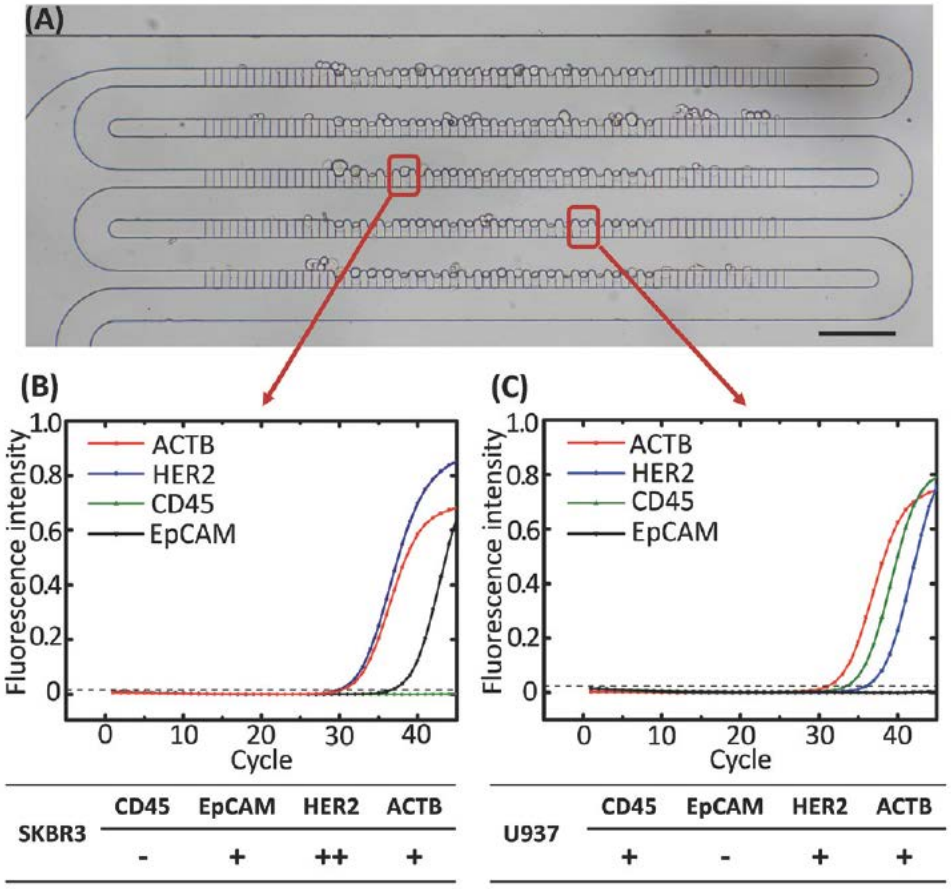


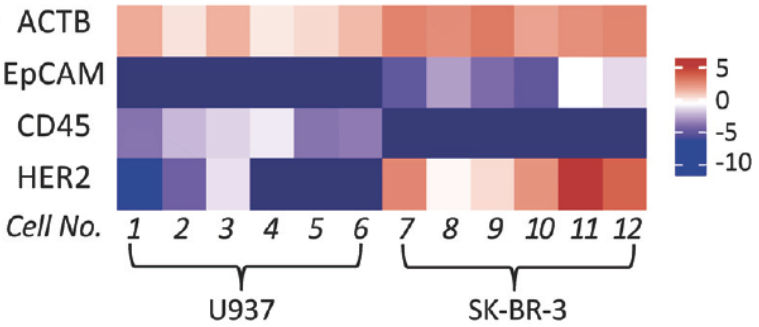


further validation of the method

application to detect cancer cells to develop a cancer screening platform

a mixture of SK-BR-3 cell line (human breast cancer) and U937 (human monocyte) cells were analyzed

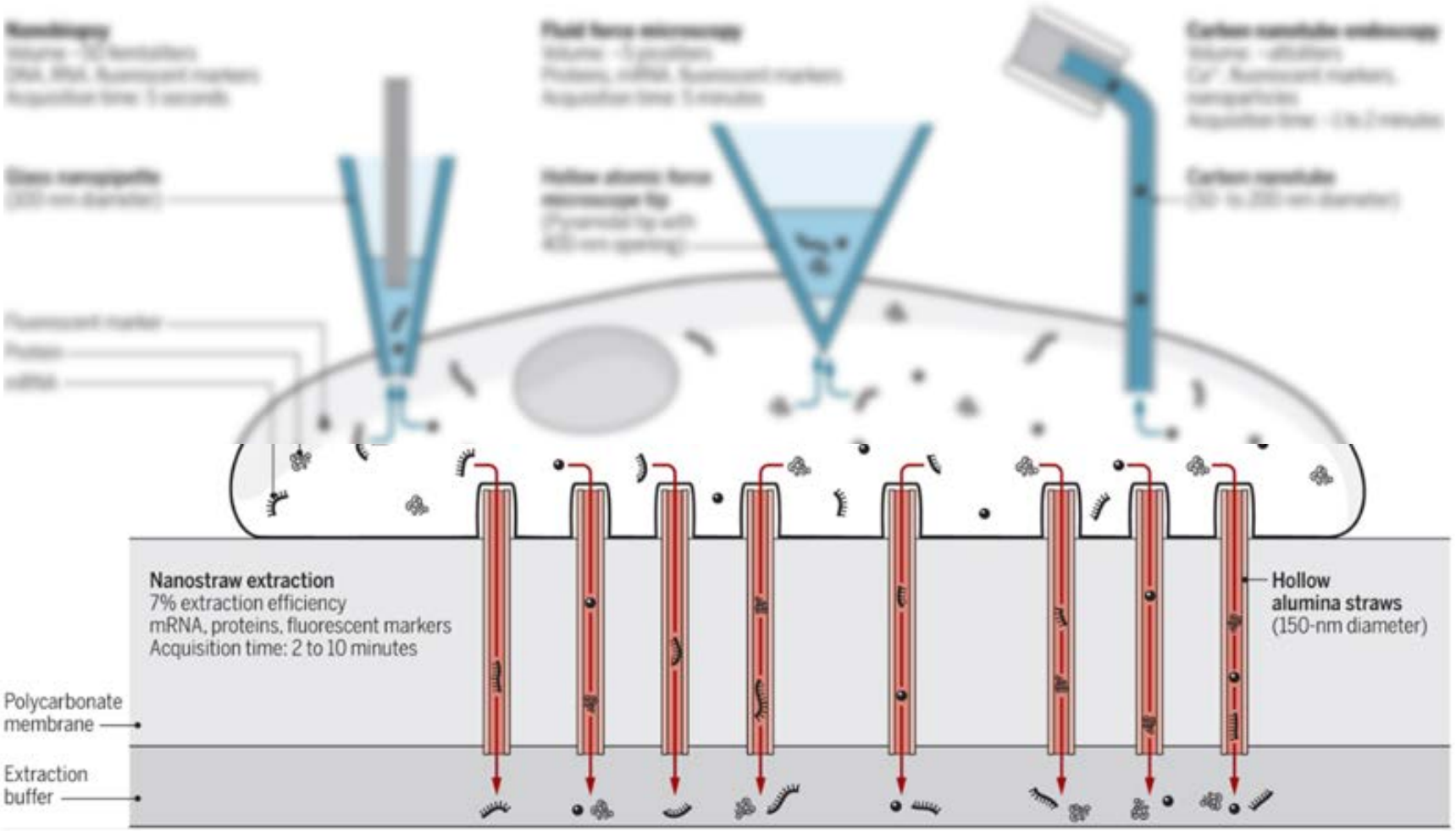




summary: single cell extraction with FluidFM

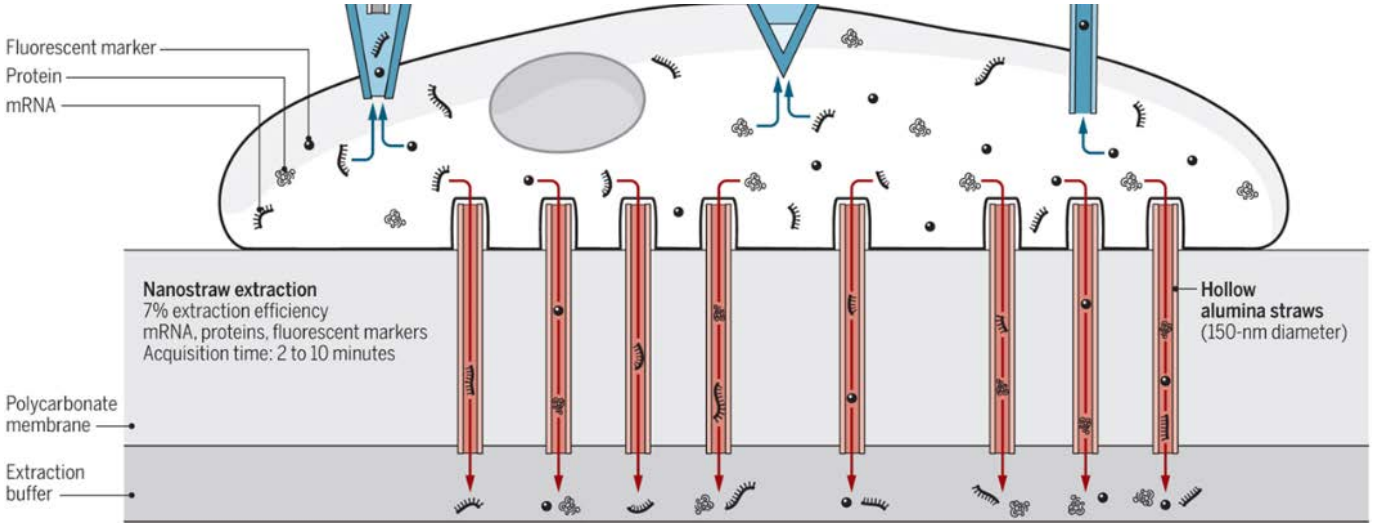
- proven feasible with a wide range of applications
- requires special training with the FluidFM probe
- possible high throughput applications need further exploring with different methods of cell content extraction

Four ways for removing cell contents



Nondestructive nanostraw intracellular sampling for longitudinal cell monitoring

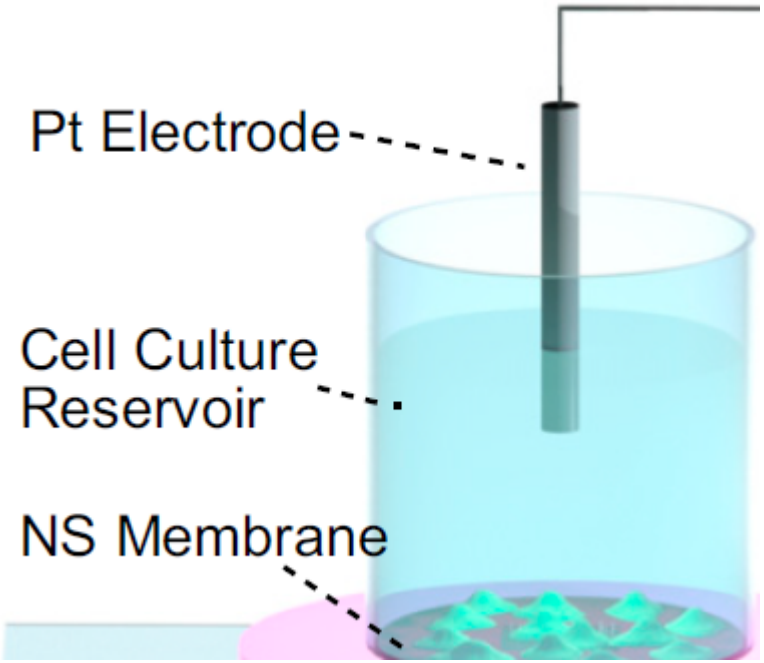
Yuhong Cao[a], Martin Hjort[a], Haodong Chen[b], Fikri Birey[c], Sergio A. Leal-Ortiz[a], Crystal M. Han[d], Juan G. Santiago[d], Sergiu P. Paşca[c], Joseph C. Wu[b], and Nicholas A. Melosh[a,1]



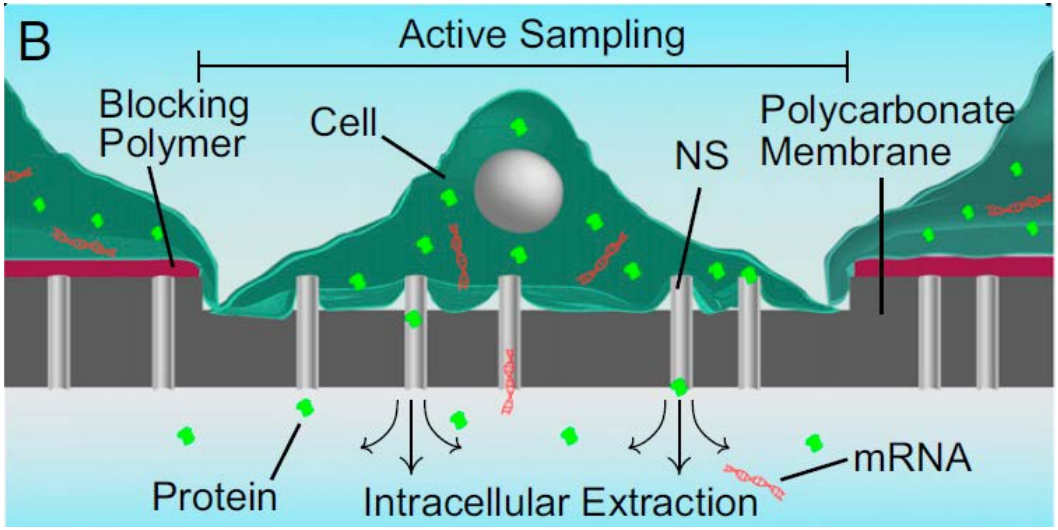
- sampling based on diffusion after electic pulsing
- usually for groups of cells as opposed to single cell sampling
- time-resolved, longitudinal sampling over says is possible

Higgins and Stevens, Science, 2017

the setup of nanostraw extraction (NEX) system



Polymer membrane and nanostraw apparatus is adjustable up to a 96 well setup

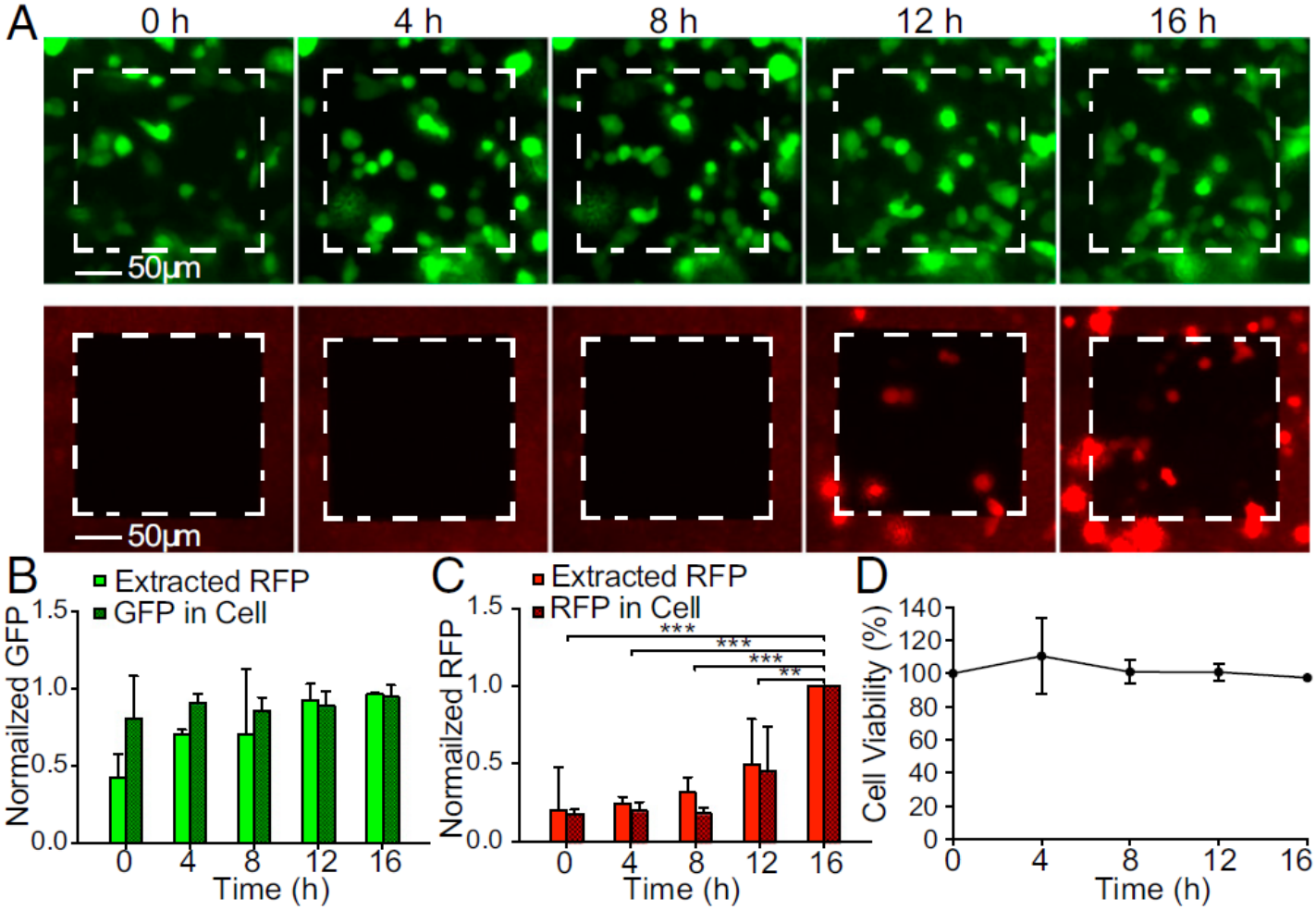


adjustable nanostraw size: gives control over how many cells are sampled

minimum size: >150 nm

proof of principle

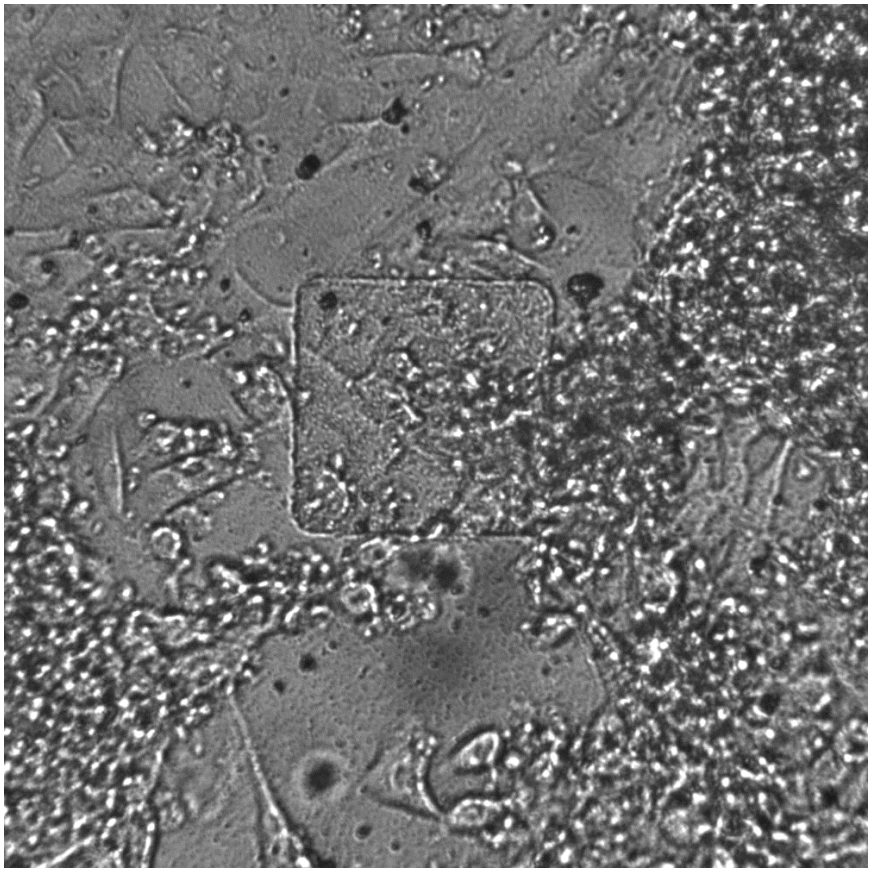
GFP expressing CHO cells transfected with RFP plasmid
extracted GFP/RFP vs GFP/RFP in the cell via fluorescence microscopy is measured



longitudinal sampling of hiPSC derived cardiomyocytes -ELISA

testing of the method with a transient process: heat shock is applied to the cells (44˚C, 30m)

upon induced heat shock HSP27 is upregulated



cardiomyocytes are cultured on the nanostraw platform began beating → sampling was still possible during beating

longitudinal sampling of hiPSC derived cardiomyocytes -ELISA

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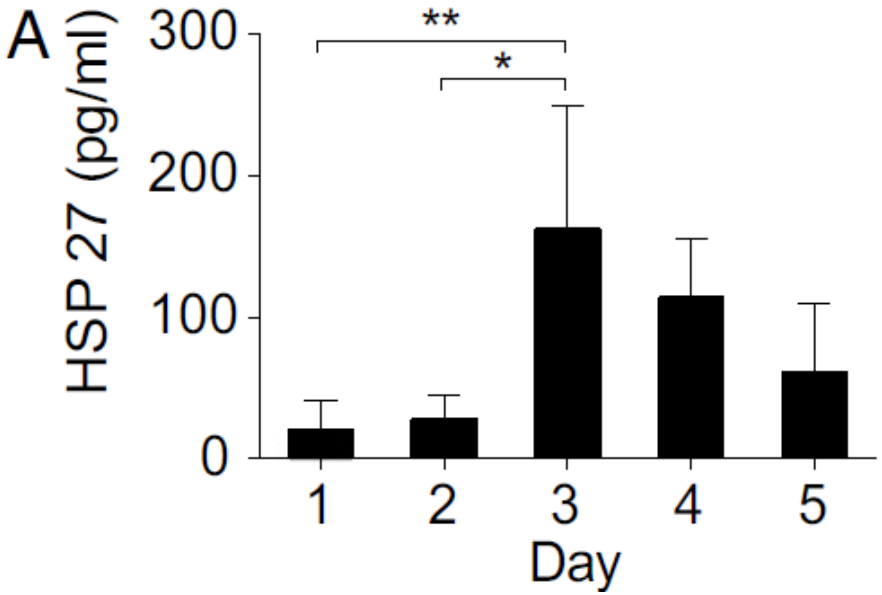
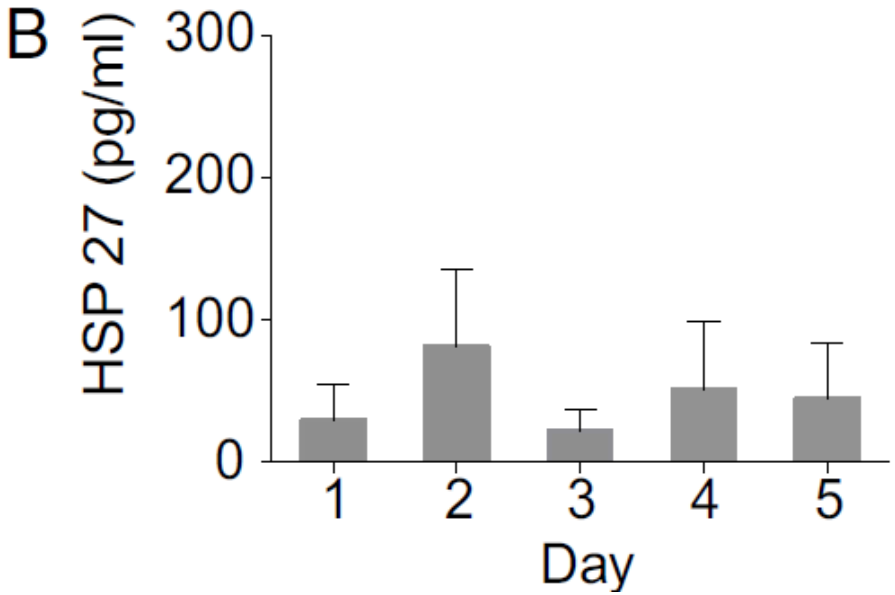


stressed



non- stressed

summary: single cell extraction

both methods of sampling from live cells have been validated with a number of applications:

- metabolomics
- qRT-PCR
- ELISA

possibility to apply to high throughput has been explored with a microfluidics approach