# Pico-electro-spray device for single cell analysis

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## Single cell measurements with mass spectrometry

A single cell contains a vast amount of molecular species in a concentration range of at least six decades which react with one another in a highly dynamic manner. Some studies have measured on single cells [1,2,3,4,5]. Mass spectrometry (MS) is an ideal technique to obtain fast analysis of complex mixtures with high resolution[6]. Electrospray ionization (ESI,or electro-hydrodynamic atomization, EHDA) is a technique used to transfer solvated molecules to gas phase molecular ions which can be analyzed in MS. In order to measure samples with pico- to femtoliter volumes, an ESI device with miniaturized dimensions is used. Due to these dimensions some scaling laws predict a different behavior of this process. Negative effects observed in "normal" ESI, known as ion suppression could be decreased or be absent all over[7,8]. Also differences in MS response of multiple charged ions could be simplified[9,10]. The overall spray efficiency of nano-spray comes close to 12% at best[11]. More standard are efficiencies of 0,01% –1%[12].

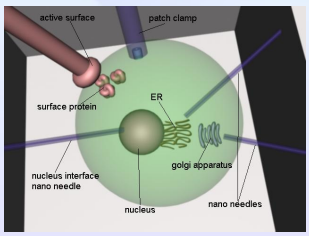


Fig1) schematic presentation of the lab-in-a-cell concept. Which envisions the use of functionality, selectivity and physiology of a single cell or cluster of single cells to perform complex actions/reactions related to chemical/pharmaceutical /medical applications.

## Chip-Electrode geometry

The electrode setup is determining the field 'shape'. The field strength at the tip of the capillary is calculated with the equation

$$E_c = \frac{2V_c}{r_c \ln(4d/r_c)}$$

$r_c$ = radius of the tip
d = distance to counter -electrode

A decrease of the tip radius $r_c$ results in an increase of the field strength at the tip at a given potential and distance. As liquid flows through the capillary and forms a meniscus on the tip it influences the field 'shape' and is shown in fig. 2. After incorporating fluid parameters in the equation above the following equation is used to determine the onset voltage at which the meniscus starts emitting a current

$$V_0 = \left(\frac{r_c \gamma \cos\theta}{2\varepsilon_o}\right)^{1/2} \ln\left(\frac{4d}{r_c}\right)$$

$\gamma$ = surface tension
$\varepsilon 0$ = permittivity of vacuum
$\cos\theta$ = Taylor angle
$V_0$ = onset voltage for jetting.

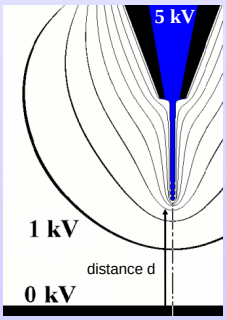


Fig 2) showing the field 'shape' of a typical point-gap-plane electrode setup with a jet perturbing the field

## Chip device used

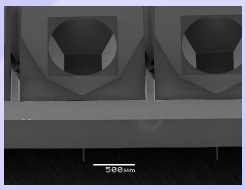

Fig 3a) top side chip with liquid well and hollow Micro-needles (200 x 8 x 0,4 µm) connected through a nano-channel[13]

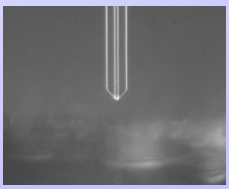

Fig 3b) top side single needle (ID 2 x 0,2 µm). channel is filled with liquid. Lower plane is counter-electrode

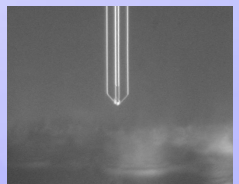

Fig 3c) channel almost completely dry after voltage increase

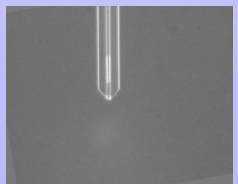

Fig 3d) channel filled, with typical bubble near tip. Spray plume can be seen

## Current-voltage measurements

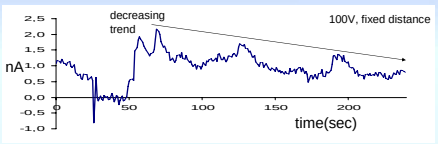


Fig 4) distance between tip and counter-electrode is constant. Voltage applied to the liquid was kept at 100V

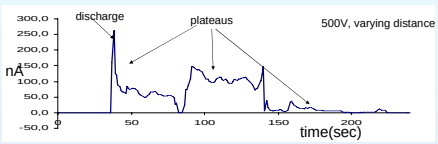


Fig 5) distance was changed until a steep change in current was detected. After steep change the distance was kept constant for ~ 50 sec.

## Current-regimes

In fig. 5 we observe three current plateaus

| | | |
|---|---|---|
| low voltage | 1 nA ←→ 4 nA | stable cone-jet regime |
| medium voltage | 20 nA ← → 50 nA | multi-jet regime |
| high voltage | 100 nA ← → 150 nA | unidentified high current regime |

Spikes can be seen near the highest plateau these indicate corona discharge, the height of these spikes is limited by the ampere meter which was set at 250 nA.

## Current-flowrate calculation

Based on literature a scaling of I ~ $Q^{1/2}$[14]can be found.

$$Q = \frac{I^2 \varepsilon}{324 \gamma K}$$

K = conductivity
Q = flowrate

For 1 nA a flowrate is 1,85 pL/min is calculated. Based on optical measurements we determined the flowrate of capillary filling action to be 1,8 pL/min. From this we assume the capillary filling flowrate is actually determining the lower limit in a stable cone-jet regime.

## Declining trend of current

In fig. 4 a decreasing trend is observed. A possible explanation is the evaporation from the spray liquid well (the liquid was administered trough a pipette and exposed to the open air) Another explanation is the pH effect which is seen in nano-ESI as well, the liquid left at the tip is depleted of positive ions including $H_3O^+$, leaving the negative couterions in the solution, in this case $OH^-$, thus alkalizing the liquid at the tip.

## 'Drying' of the channel

The transition from the 2nd to the 3rd regime is accompanied by an apparent drying rate of 6,7 pL/min (fig. 3c). the expected flowrate could be much higher corresponding to the current emitted by the tip. The calculated flowrate would be in the range of 20 nL/min, which seems unlikely, moreover the scaling law is based on the cone-jet regime and therefore cannot be used with accuracy for a different regime.

## Bubble formation/solvation

A striking feature was the bubble formation during the refilling of the channel 5 µm upstream of the tip (fig. 3d). The solvation speed of this bubble was determined to be 26 fL/min. The bubble formed in multiple chips and multiple measurements, ruling out the chance of a single artefact event.

## Conclusions

- Stable spray has been established from this device
- Lower voltages yield higher stabilities
- Onset voltages below 100V
- Flowrates in the 1-100 pL/min range thus sufficient for cells as small as 100µm
- Some phenomena are observed which point in the direction of 'nano'-effects
- Compared to nano-ESI, this device is more efficient in producing ions from liquids

## References

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