WSN-8

Dielectric Spectroscopy and RF and Microwave Passive Microsystems for Biological Applications and Discrimination of Cells

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HAWAI'I 5G Catch the Wave!

Presentation done during IMS 2017 conference

Outline

Content of the talk

- Motivation
- Dielectric RF spectroscopy challenges & issues
- Sensing methodologies for single cell analysis
- Cell electro-manipulation: another EM characterization approach
- Conclusion
- Acknowledgements

Motivation

- Dielectric Spectroscopy applied for material characterization

  -> *Electrical material properties measurement*

- Applied for biomaterial investigations

- Many works led on tissues/organs (ex & in-vivo)

From kHz up to tens of GHz-> Bio impedance library

- Used of macro size wave applicators

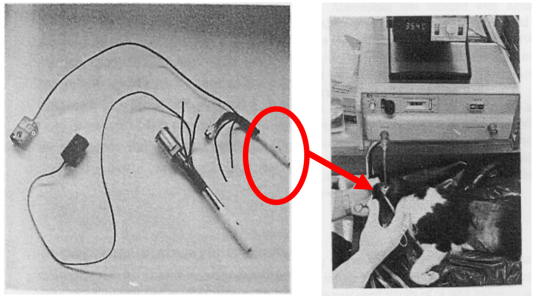
Wide band information ☺

Difficulties to overcome averaging effects or natural heterogeneity/variability of tissues sample ☹

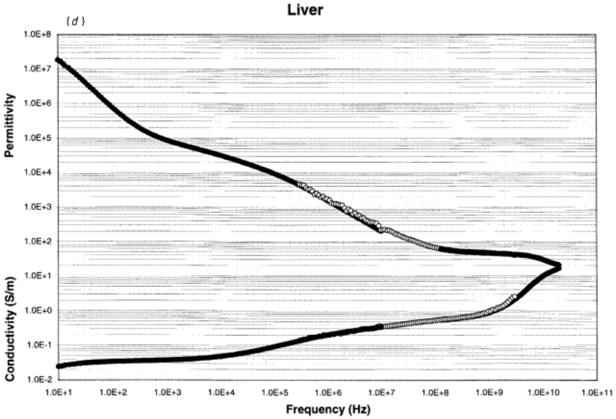
->Lack of accuracy at cellular level

- Investigation/characterization at cell or subcell level so requires dedicated micro applicators

➡ *Benefit of Microsystem technologies*



Maria A Stuchly et al., 1982



S. Gabriel et al., 1999

Connecting Minds. Exchanging Ideas.

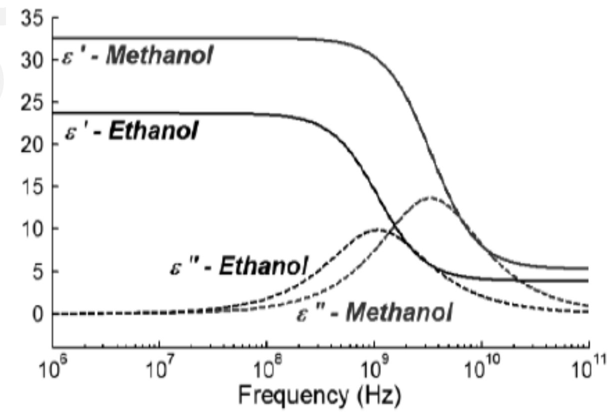
Motivation

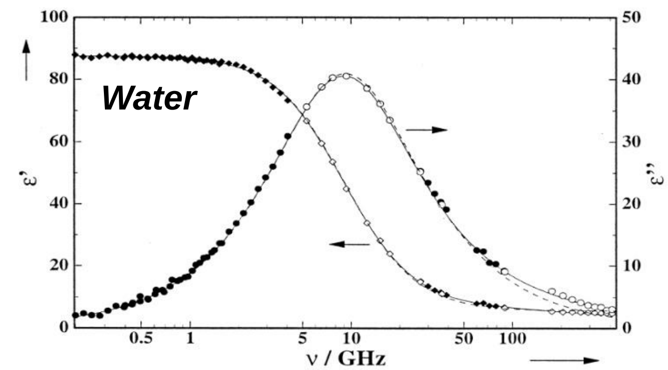
Interest of Dielectric Spectroscopy in bio investigations

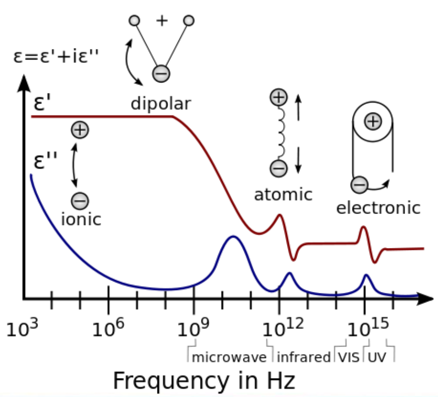
D.S allows measurements of sample complex permittivity (ε'-jε") over frequency

- The permittivity of many chemicals/bio-materials shows significant changes in RF/microwave spectrum

- Wideband measurement allows to image relaxation characteristics of various molecules especially at GHz







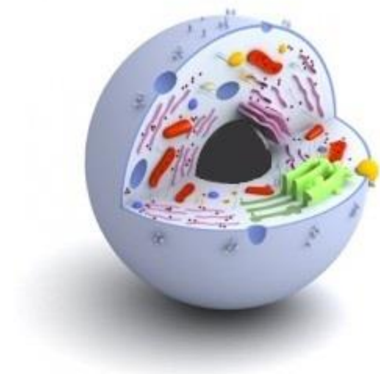
Current Applications:
- Moisture control
- Food safety
- Chemical/biological sensing
- *Disease diagnosis -> biological cell characterization*

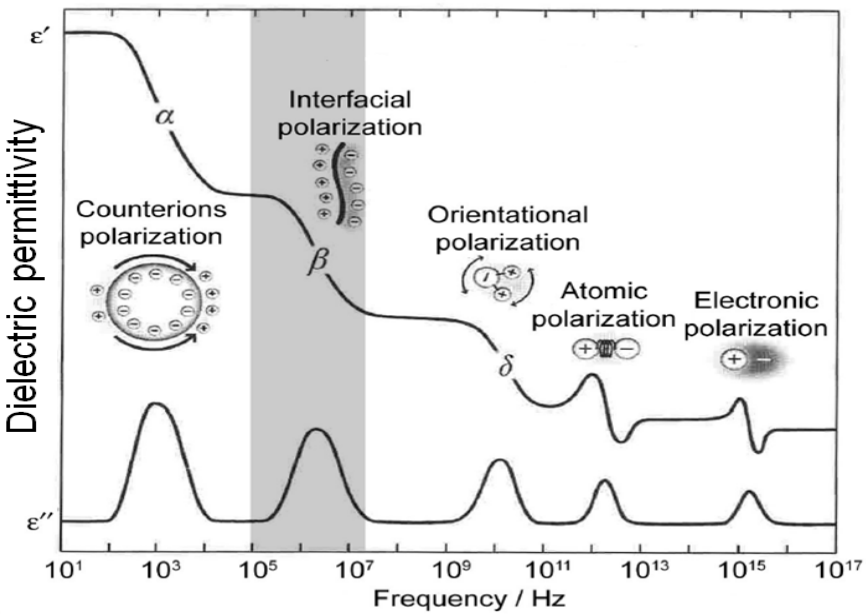
HAWAI'I 5G Catch the Wave!

Motivation

Why doing dielectric spectroscopy at Microwave on individual cells ?

Cells are very complex by nature (lipid, organelles, hydrated molecules…
In addition cells are immerged in a very dispersive (lossy) liquid



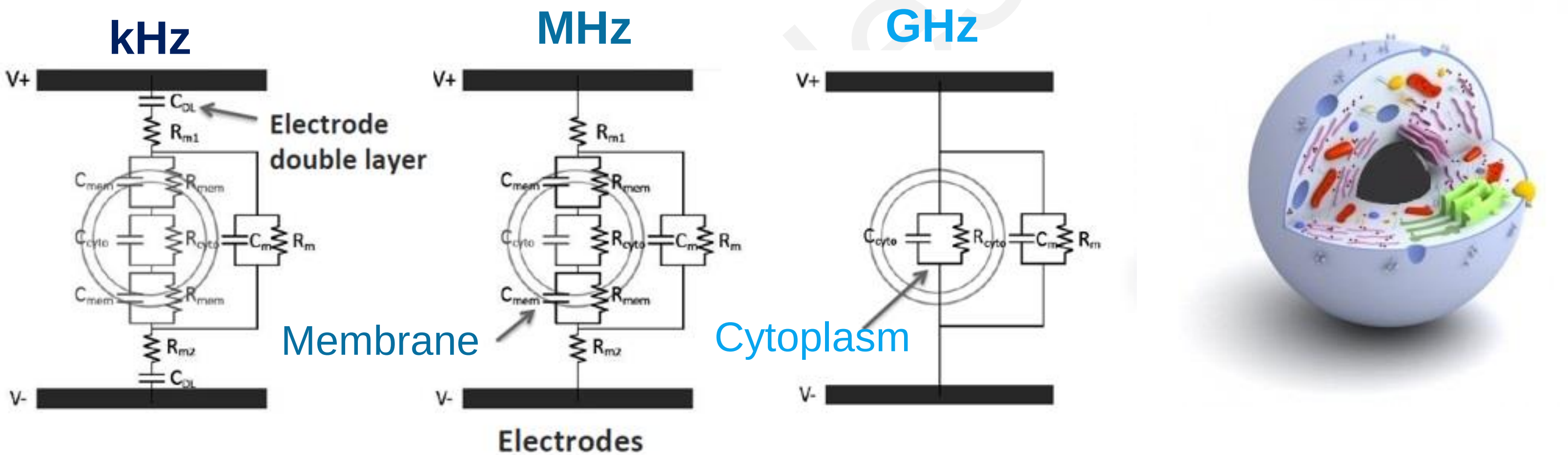


Cells impedance can change meaningfully related to applied frequency

Several phenomenon's are involved….

Motivation

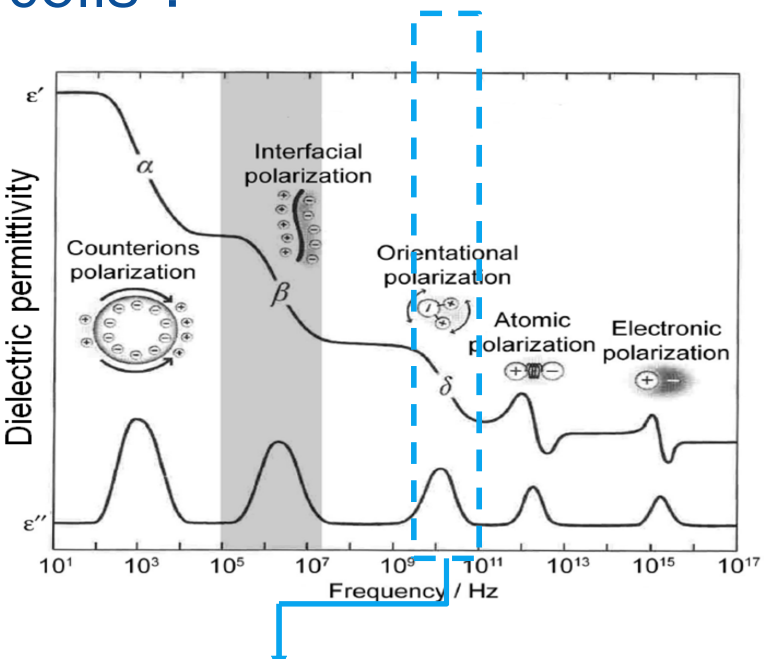
Why doing dielectric spectroscopy at Microwave on individual cells ?

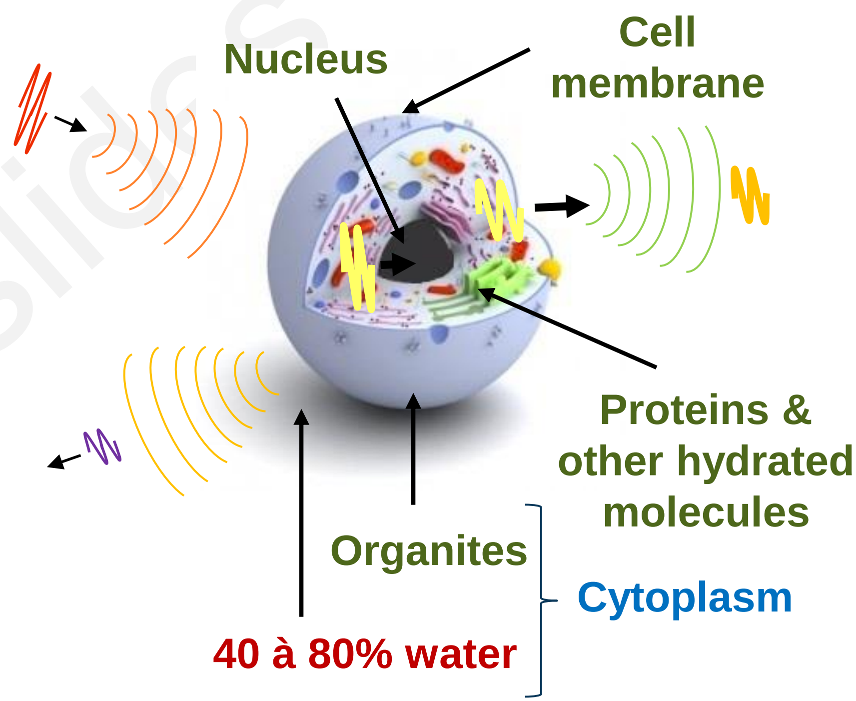


- kHz range: Application to size characterization and cell proliferation monitoring despites electrode issue effects, domination of ionic conduction & diffusion
- MHz range: Dominated by cell membrane properties: Interfacial characterization
- GHz range: Electric fields really penetrate the cytoplasm and allow to access to cell interior: Intracellular characterization

Motivation

Why doing dielectric spectroscopy at Microwave on individual cells ?





- Microwave spectrum:
  - Bypass of cell membrane
  - Less ionic effects (too slow to follow the HF field)
  - Relaxation of hydrated molecules

➢ Microwave bio-impedance
=
Own cell signature (Specific?)

Motivation

Potential interest of using GHz Diel Spectro for biologists

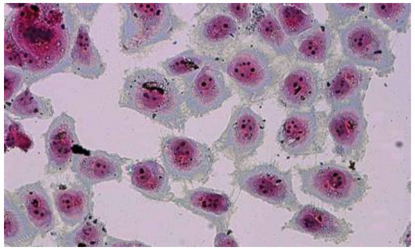
Non invasive, non destructive & label free technic
Simple, easy to implement and can allow fast (real time) analysis
Give insights on physical properties of cells

➡ *access to new information/data: complementary to conventional analysis*

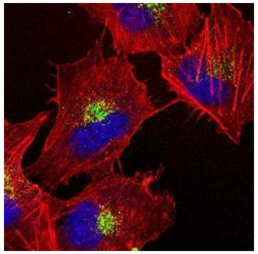
Common cell analysis techniques

Under microscope

- ✓ *Destructive or cell altering*
- ✓ *Time consuming*
- ✓ *Some time expensive (labels, proteome kits)*

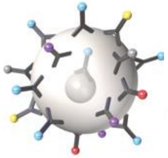


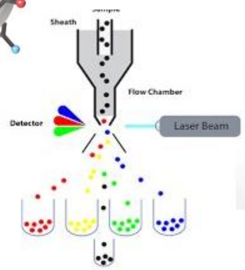
Staining



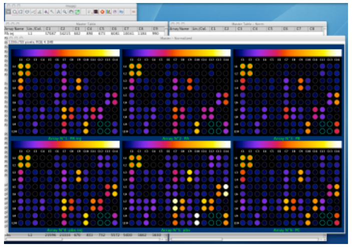
Fluorescence labeling

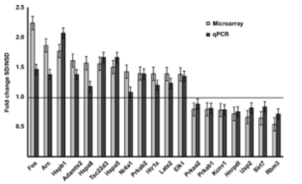
Flow cytometry





Protein Array analysis





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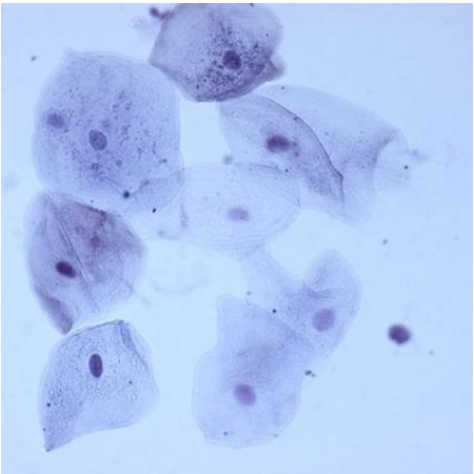
Dielectric RF spectroscopy challenges & issues

Measuring single cell characteristic is not so obvious

A single cell is :



- living, so is in continuous change …
- very heterogeneous by nature (origin, nature, differentiation, cycle, pathology…)
- can present various shape with different nucleus/cytoplasm ratio
- Needed to be suspended/maintained in liquid host medium
  - 5-20µm ranging diameter particle
  - few picoliter volume content

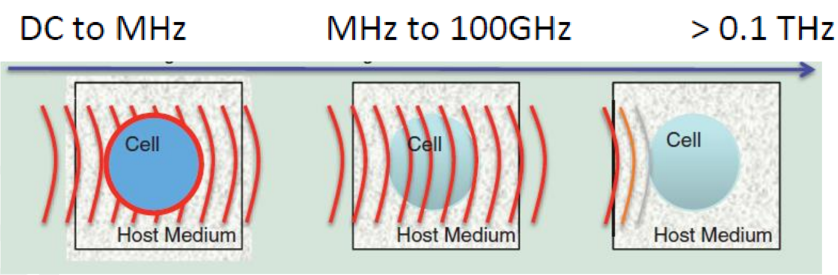
The dielectric effect of such small particle is so limited: ~100-600 attofarad perturbation

➢ Accurate and repeatable measurement technics are required using sensors with high sensitivity capabilities

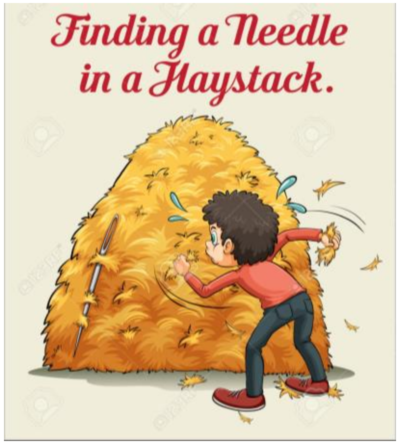
Dielectric RF spectroscopy challenges & issues

Impact of host medium

Host medium presents strong dielectric loss. On the electromagnetic point of view: *Cells can be seen as a dielectric medium hidden in a larger one with small differences*



K Grenier, IEEE microwave Magazine may 2015



Nevertheless microwave spectrum is more suitable for investigation than millimeter wave if:

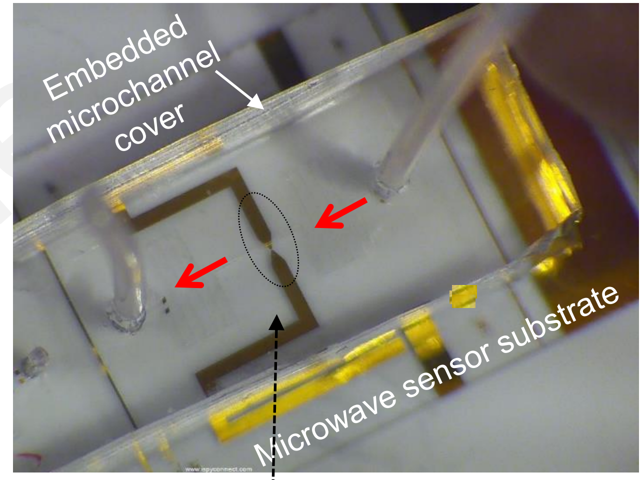
- EM signal is applied at the neighborhood to cell to characterize
- host medium volume considered is small in order to limit wave attenuation and measurement noise

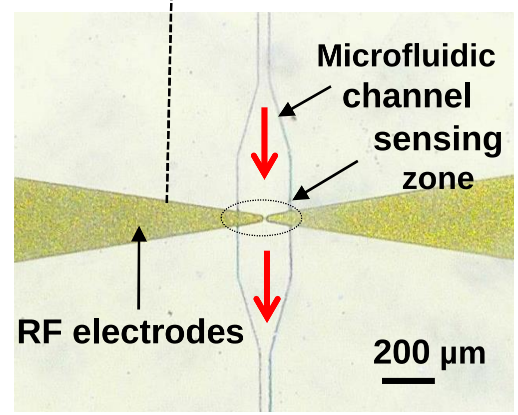
➡ *Benefit of mixing Microwave and Microsystem & Microfluidic technologies*

Dielectric RF spectroscopy challenges & issues

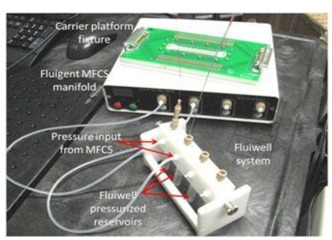
Sensors technologies

EM wave applicator & sensor:
-> *electrodes/array patterned by using lithography& thin film technologies*
Substrate:
-> *low loss glass (biological imaging compatible) or silicon*
Microfluidic packaging:
-> *Lab-on-chip technologies: PDMS (polydimethylsiloxane), polymers or glass based cover*
Cells manipulations
-> *Cell suspension injection and flow control outside chip*





Syringe pump

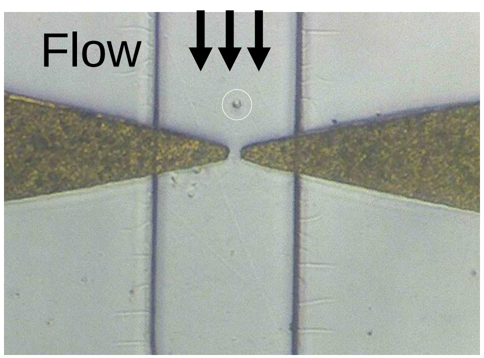


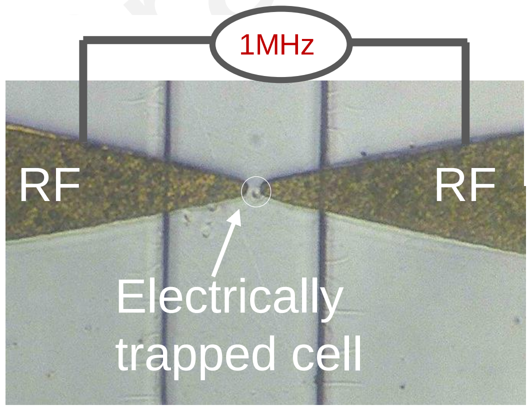
Microfluidic flow controller

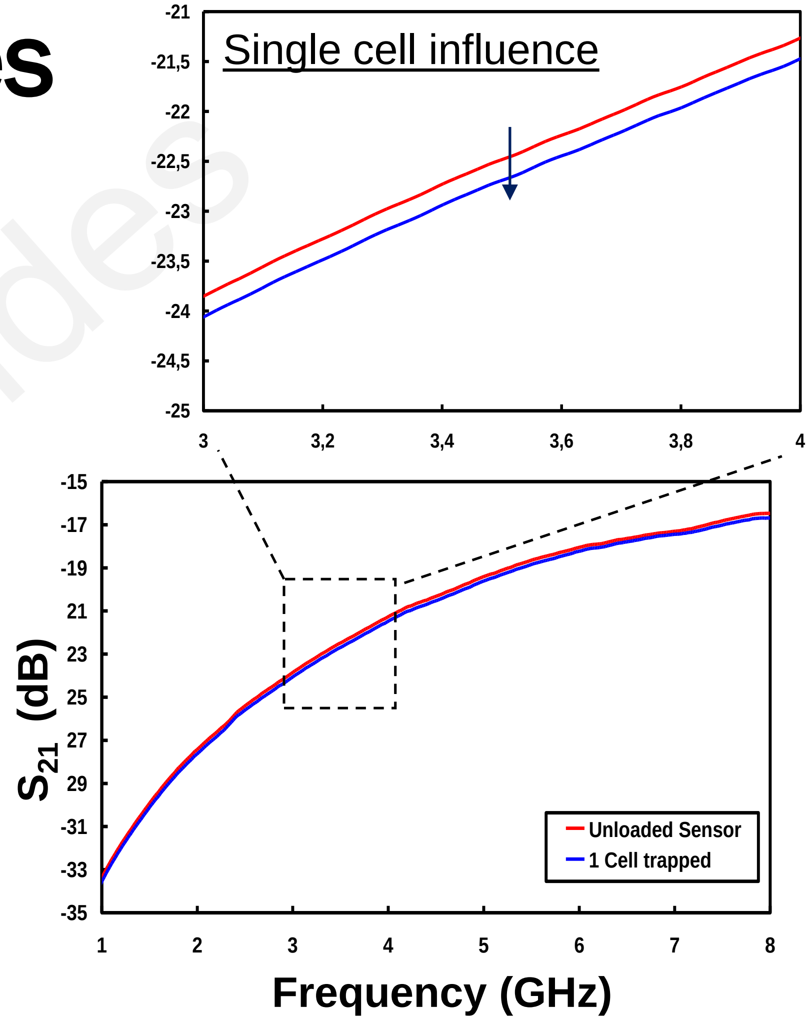
Dielectric RF spectroscopy challenges & issues

Sensitivity issues

- One single cell can have a limited impact on sensor
- Cell is subjected to motion: cell trapping can be required
- Reproducibility of measurements is the key: requires careful calibration, temperature stability…







Dielectric RF spectroscopy challenges & issues

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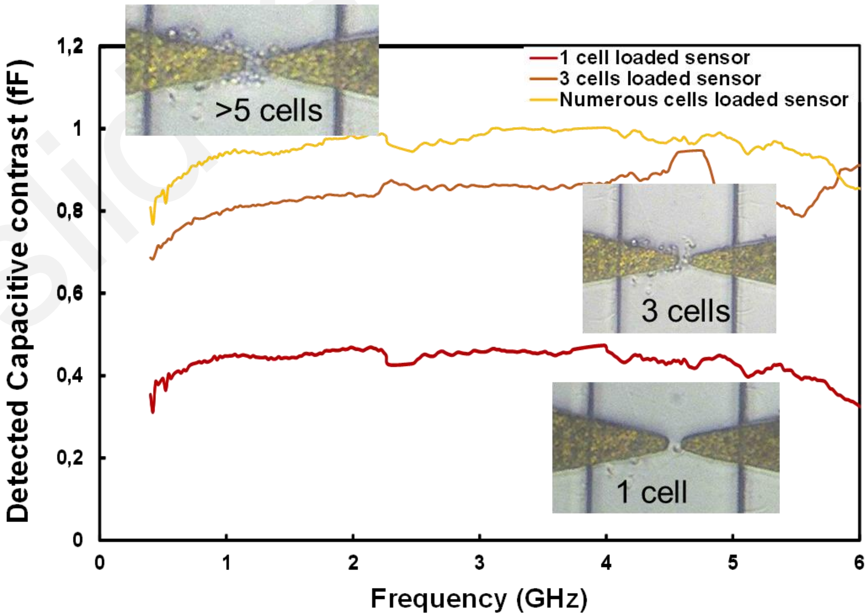

Single cell detection is achievable but own cell dielectric property analysis requires specific methodology

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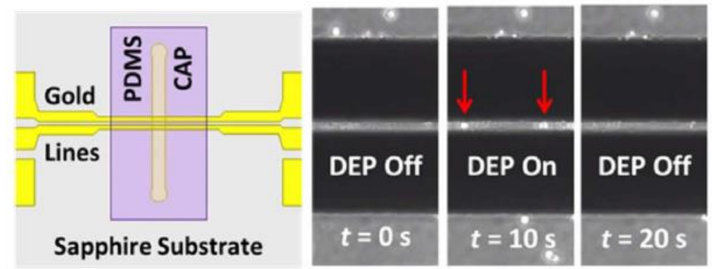
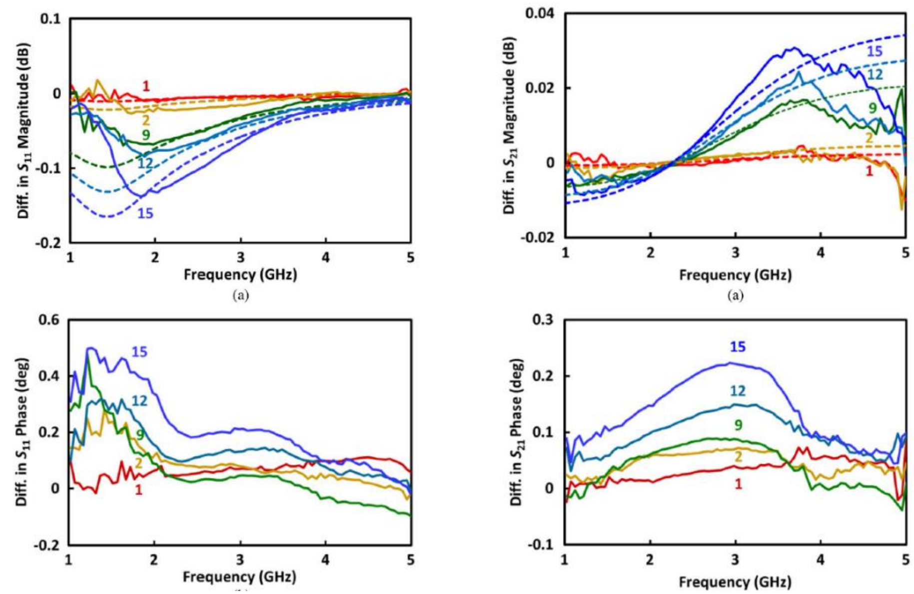
Broadband spectroscopy based on S-parameter measurement

Sensor design and operation

- CPW line implementation
- Cell interact with EM field through CPW gaps
- Optical imaging can be required to normalize measurement regarding number of cells involved
- Accurate measurement requires slow frequency scanning: cells need to be immobilized



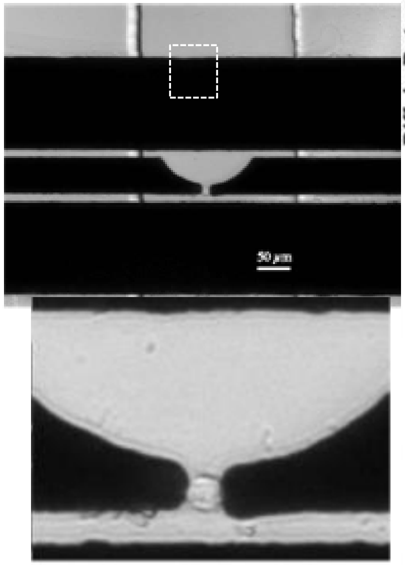


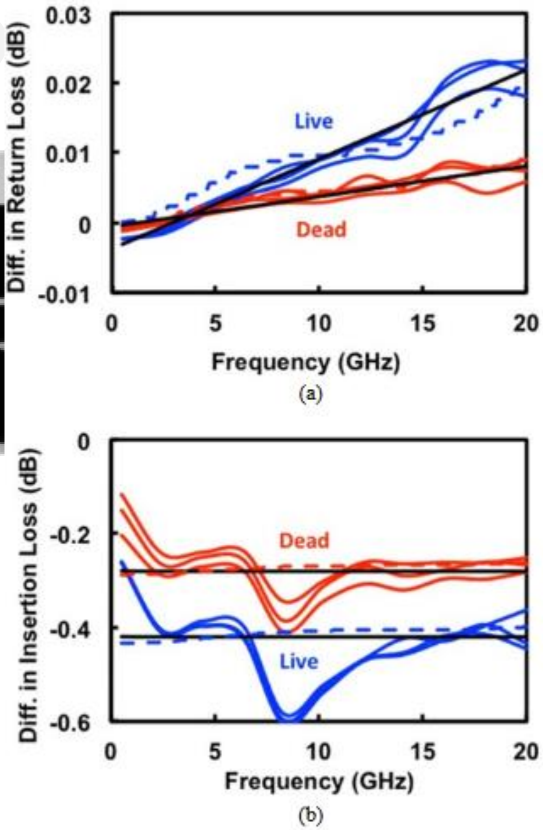
* J. C. M. Hwang et al, IEEE Transactions On Microwave Theory And Techniques, Vol. 62, No. 9, September 2014

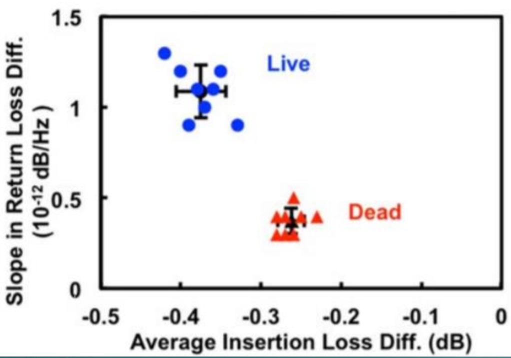
Broadband spectroscopy based on S-parameter measurement

Strategies for single cell analysis

- Narrowing of analysis zone
- Focusing EM field
- Proper sensor calibration is required
- Appropriated sensor & cell modeling to identify some threshold and handle cell variability







* J. C. M. Hwang et al, Reproducible Broadband Measurement for Cytoplasm Capacitance of a Biological Cell, IMS 2016

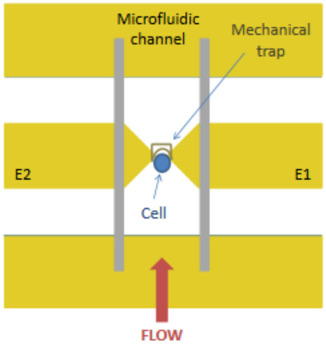
Broadband spectroscopy based on S-parameter measurement

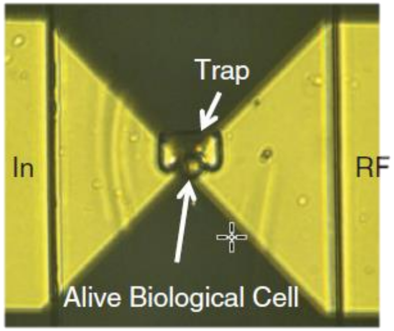
Strategies for single cell analysis

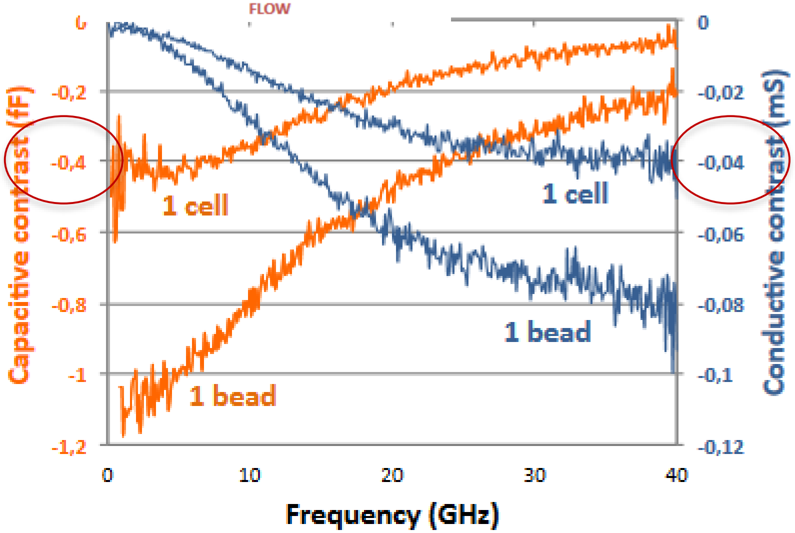
- Analysis of cell suspended in its culture medium is challenging but achievable thus differential measurements (with and without cell)

$$\Delta C = C_{cell} - C_{medium}$$

- Appropriated deembeding methodologies are required to lower measurement noise floor and access to low level signal variation





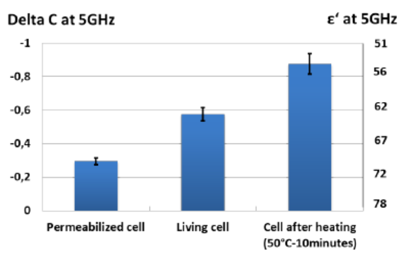


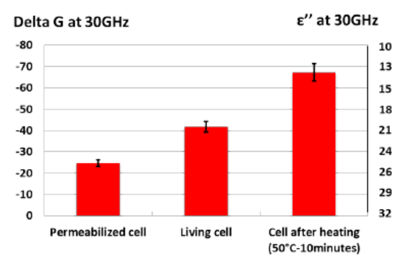
* K Grenier et al, Microwave Dielectric Spectroscopy of a Single Biological Cell with Improved Sensitivity up to 40 GHz, IMS 2016

Broadband spectroscopy based on S-parameter measurement

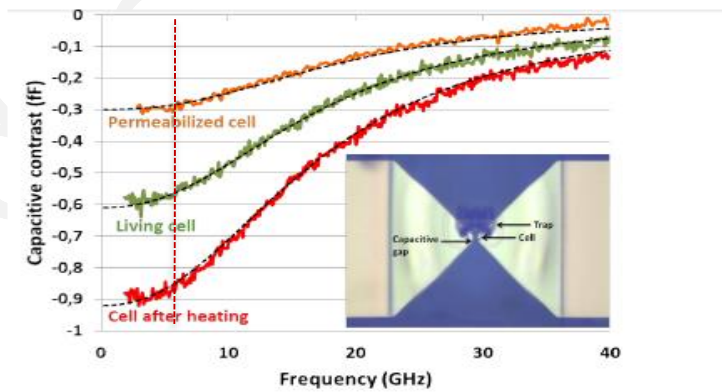
Strategies for single cell analysis

- Broadband measurement allows to identify specific frequencies where different electrical specificities can be exploited

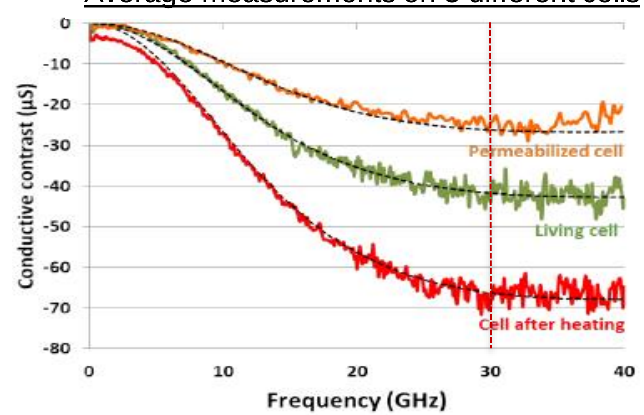




- Compatibility vs dynamic characterization of moving cells?



Average measurements on 3 different cells

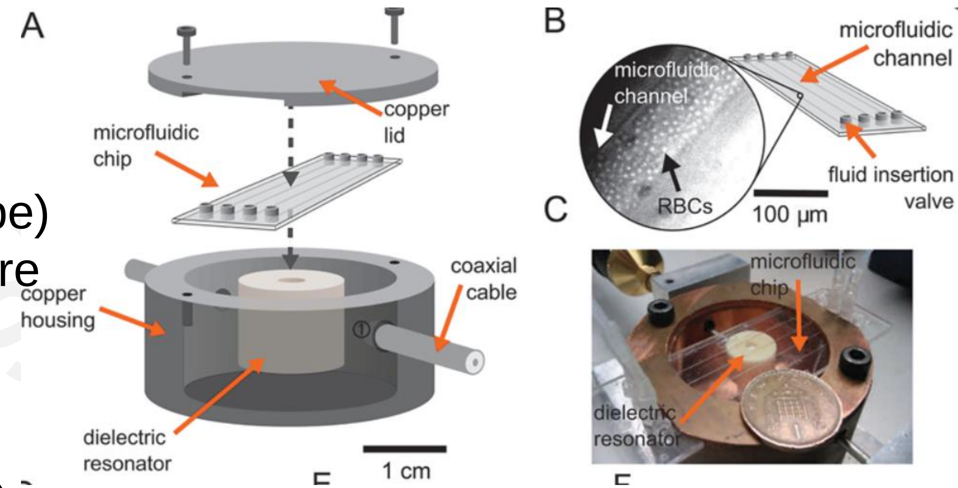


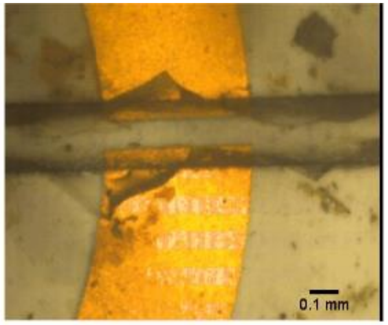
* K Grenier et al, Microwave permittivity extraction of individual biological cells submitted to different stimuli, IMS 2017

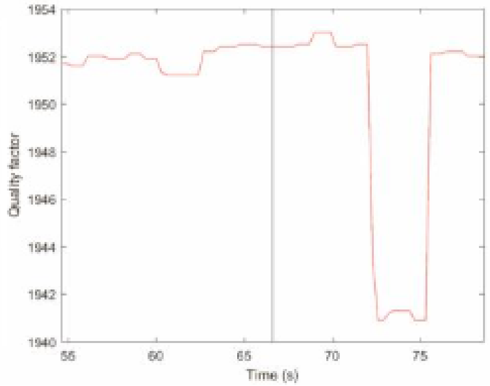
Narrowband spectroscopy using resonance effects

Resonator implementation

- A high Q resonator (whispering gallery type) resonator is coupled with split ring structure with embedded microchannel
- Host liquid loss has a limited impact on cavity resonator Q that stays high (>2000)
- Flowing particle effect change the own split ring resonance that translates in a change of cavity resonator Q
- Only Single frequency can be investigated





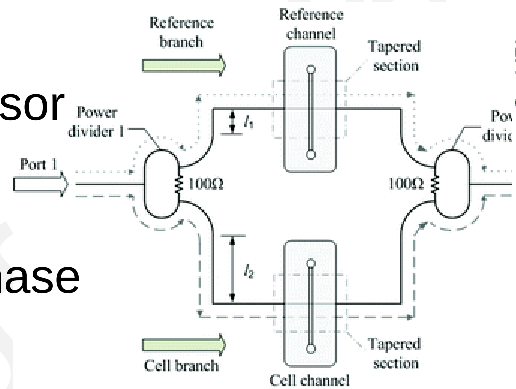


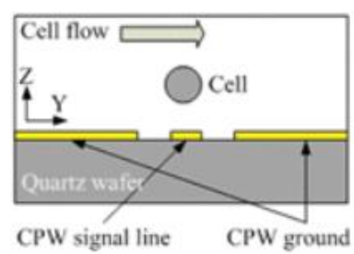
* Klein N et al, 2016, Microwave-to-te
sensing in microfluidic systems, : SPIE-INT SOC Optical Engineering

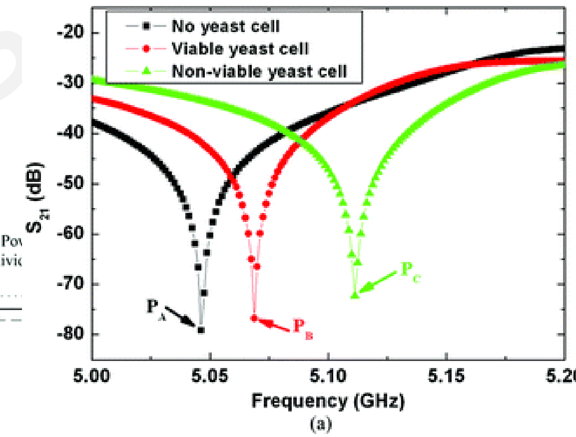
Narrowband spectroscopy using resonance effects

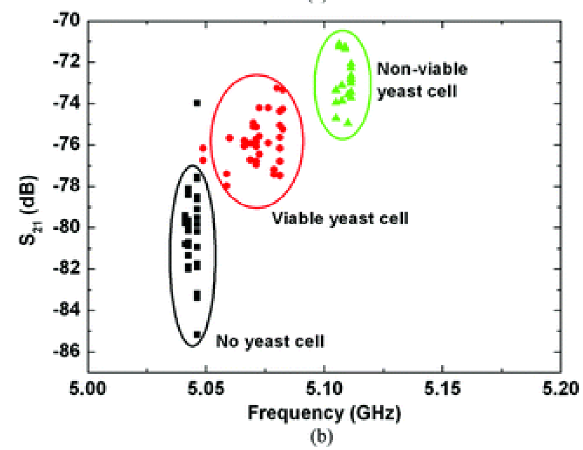
Interferometer implementation

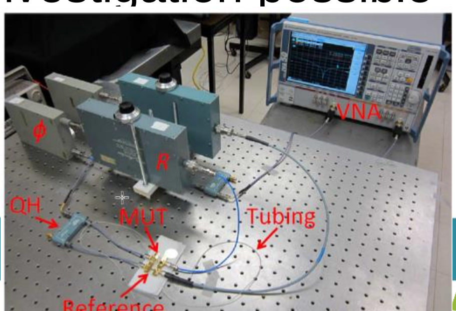
- Takes advantage of phase change induced by cell passing above sensor
- Interferometer enhance detection capability thanks to fine zeroing phase delay compared to a reference
- Narrow frequency band to address in measurement (fast measurement?)
- Multi frequency investigation possible but requires tunability and fine setting









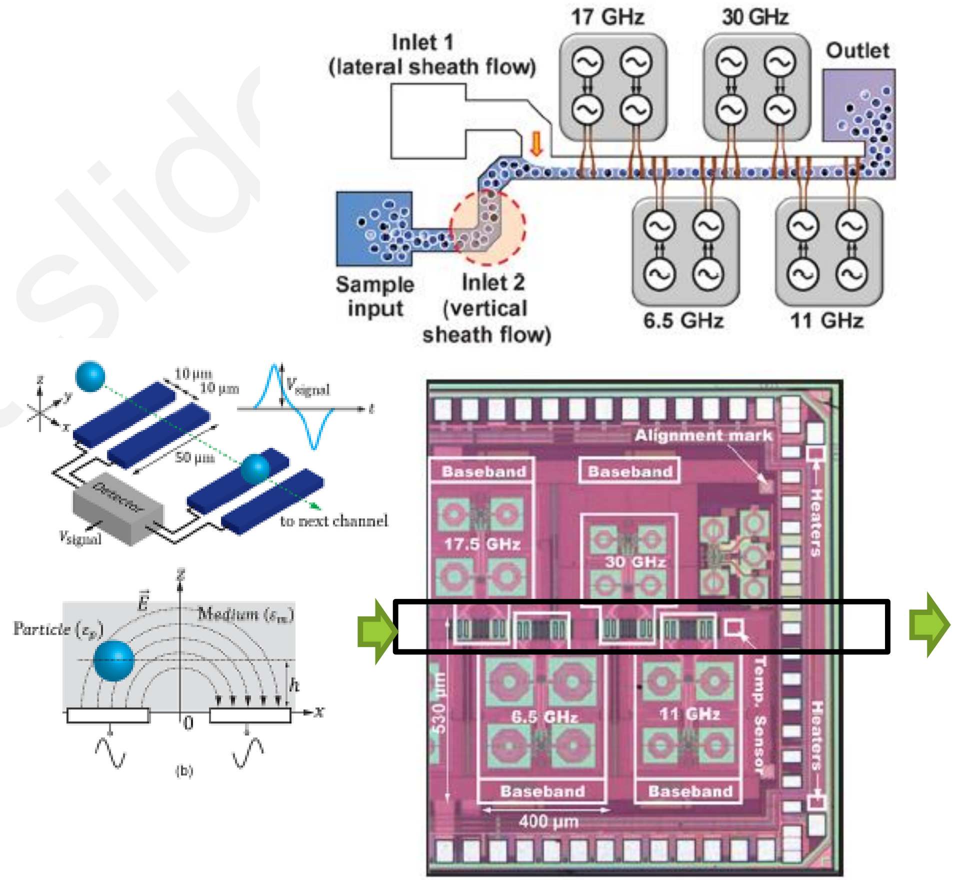


* P Wang et al, Distinguishing the viability of a single yeast cell with an ultra-sensitive radio frequency sensor, Lab Chip, 2010,

Narrowband spectroscopy using resonance effects

VCO implementation

- Monitoring the sudden induced frequency shift of VCO tank due to particle passing above pair of sensing electrodes
- Several discrete frequencies investigated
- Real time measurements
- CMOS implementation also allows easier feedback electronics on chip integration

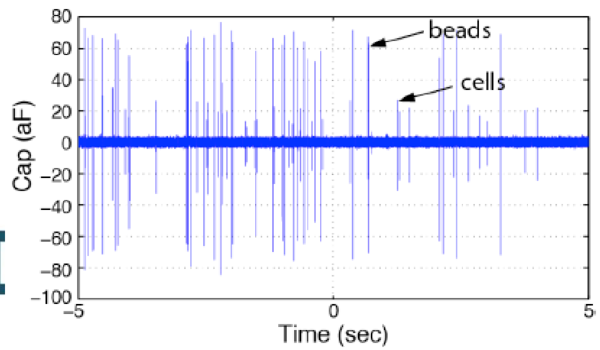


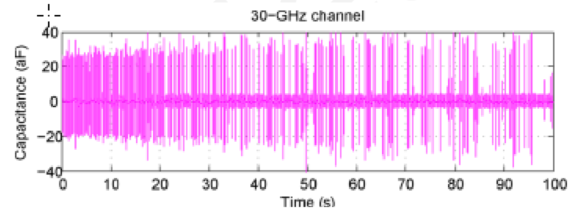
* JC Chien et al ,IEEE Journal Of Solid-state Circuits, Vol. 51, No. 2, February 2016,

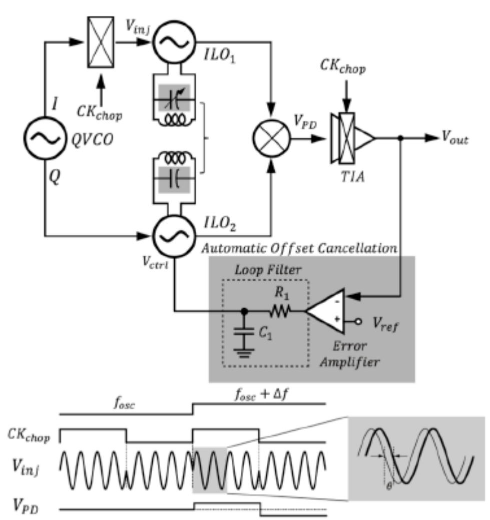
Narrowband spectroscopy using resonance effects

Low noise VCO highly sensitive

- Dedicated circuit architecture to strongly enhance detection sensitivity few attoF detection capability
- Rapid events capture/ detection & counting
- Real cytometer function allowing dynamic characterization of large population of flowing particles







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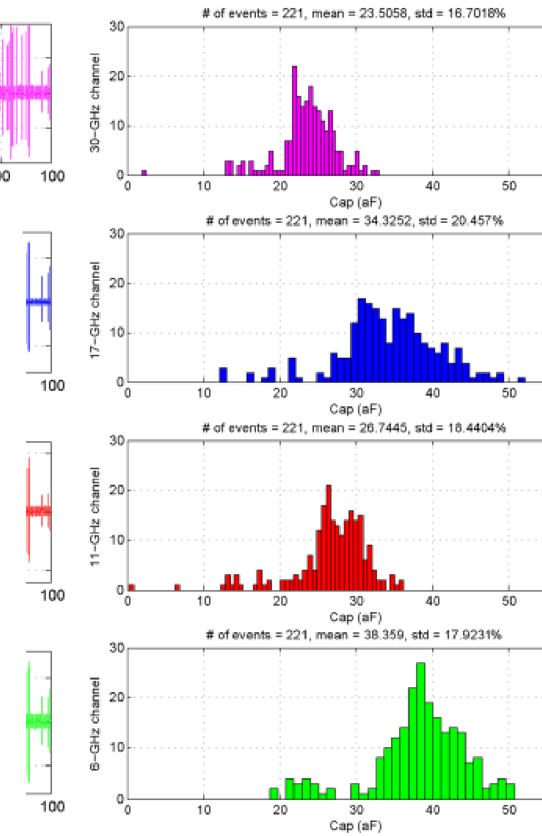


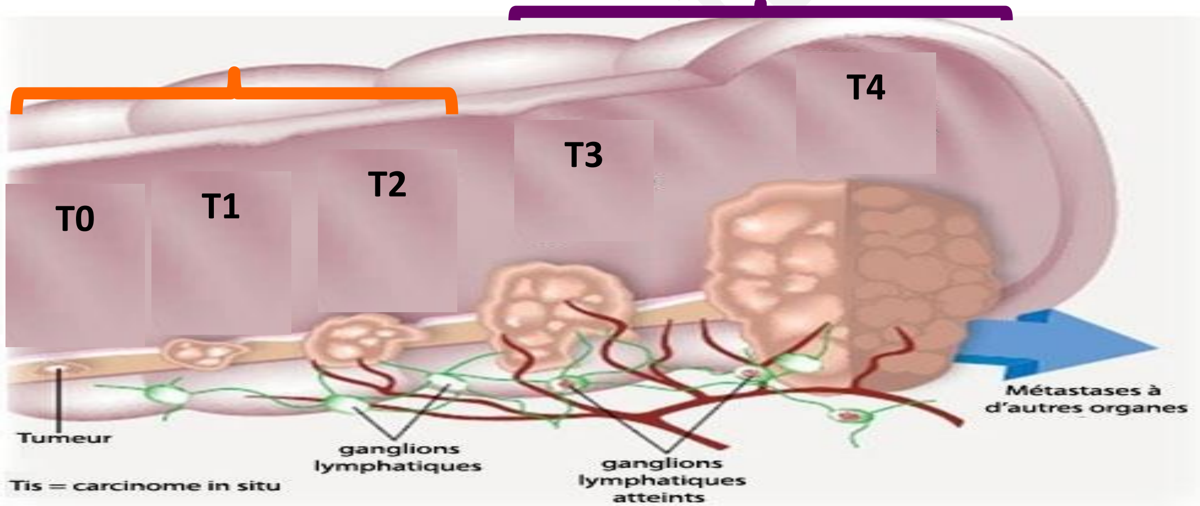
(b)

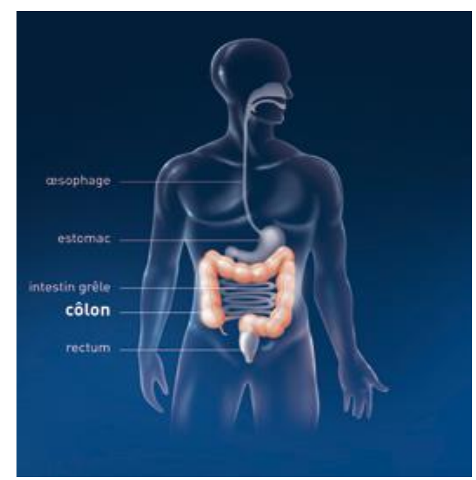
Example: Application to cell aggressiveness gradation

Colorectal cancer case

Early stages :Good chance to heal and good response to treatments
But early screening is rare since colonoscopy is needed

Advanced stages with numerous aggressive cell, tumor size is big and invasive: poor *prognostic*
Tumor cell can spread outside colon thanks to blood circulation: very aggressive cells

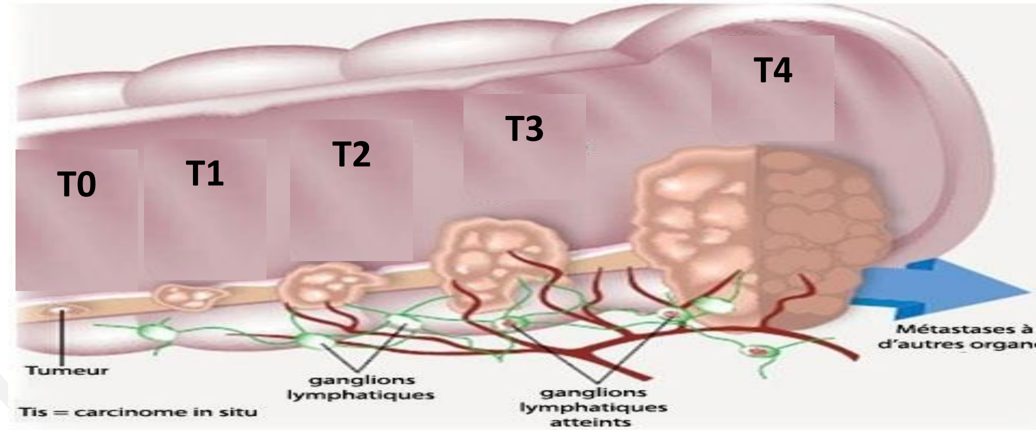


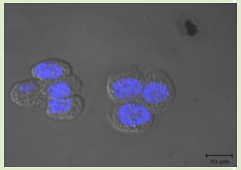
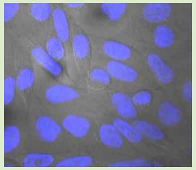
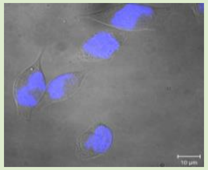
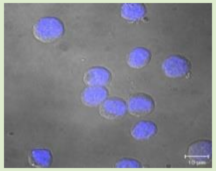


Example: Application to cell aggressiveness gradation

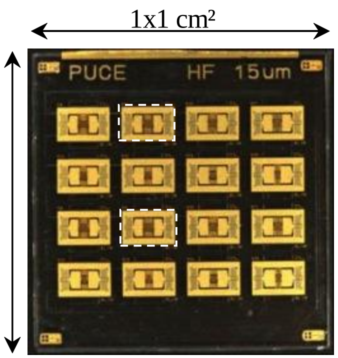
Colorectal cancer case

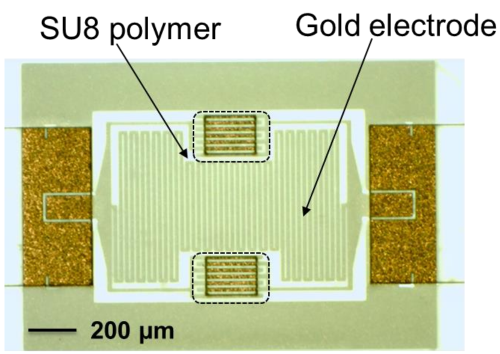
Comparative data measurements led on different cell lines from human colon cancer known to be representative of cancer development stage

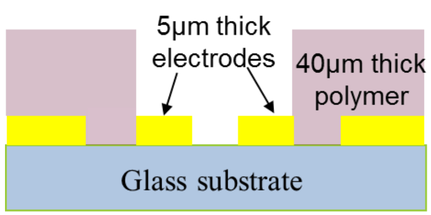


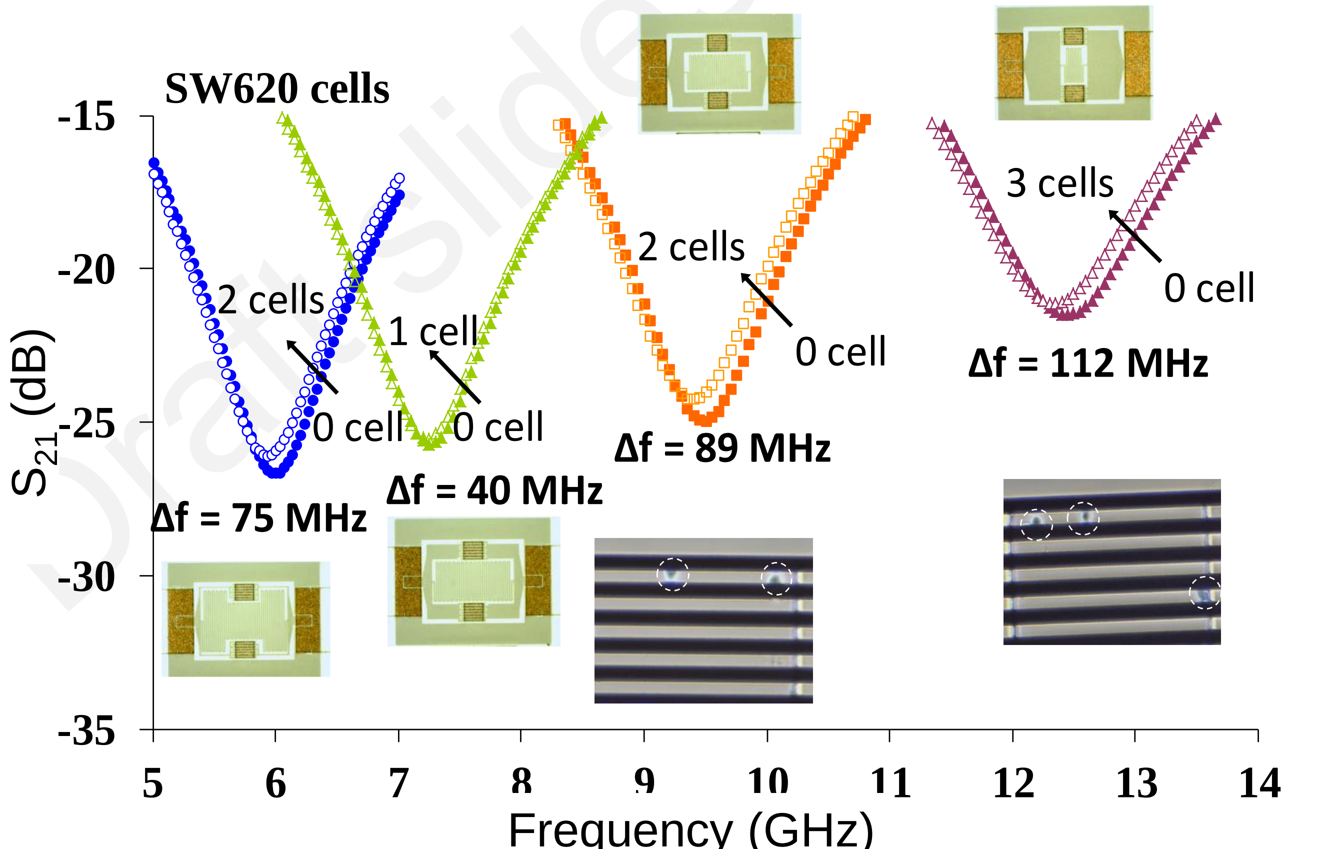
Cell Line	WiDr	SW480	SW620	DLD-1	Colo 205
Stage (Duke's classification)	II	II	III	III	IV
Diameter (mean value)	12,7 µm	13.1 µm	11.4 µm	13.6 µm	12.8 µm
Cell Morphology					

Measurement using resonant sensors





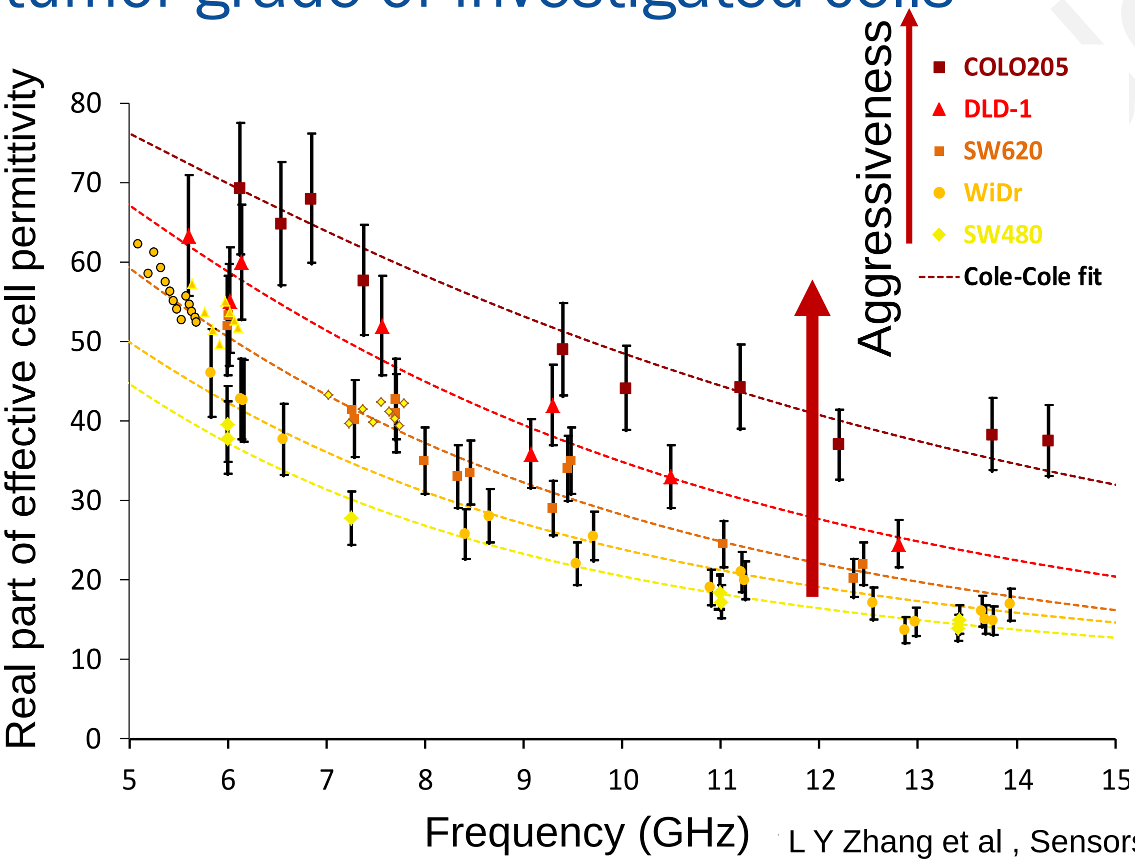




* L Y Zhang et al , Sensors & Actuators: A. Physical, Volume 216, 1 Sept 2014

EM signatures comparison

Correlation appears between measured EM signatures and tumor grade of investigated cells



Cole-Cole derived model:

$$\varepsilon_{r\_Cell}(\omega) = \frac{\Delta\varepsilon_r(1+(\omega\tau)^{1-\alpha}\sin(\alpha\pi/2))}{1+2(\omega\tau)^{1-\alpha}\sin(\alpha\pi/2)+(\omega\tau)^{2-\alpha}} + \varepsilon_{r.\infty}$$

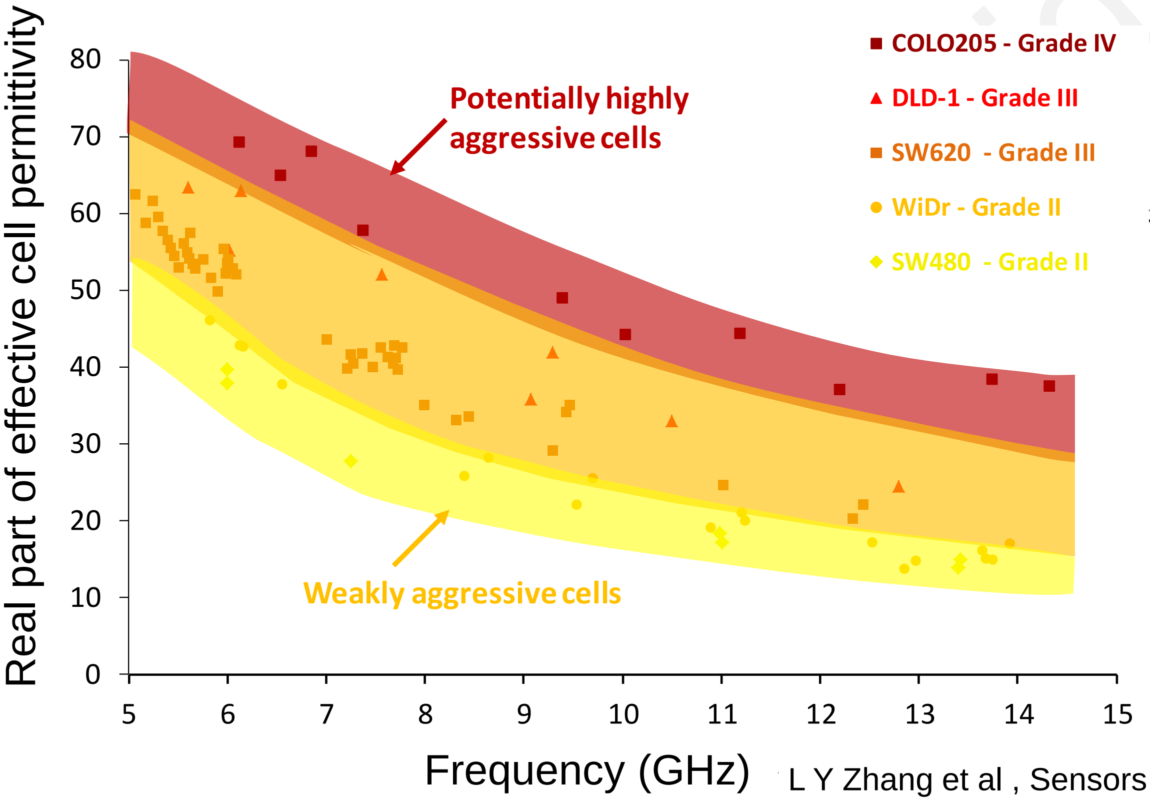
Extracted model parameters:

Para	SW480	WiDr	SW620	DLD-1	Colo 205
$\varepsilon_{r\infty\infty}$	3	3	3	3	3
$\Delta\varepsilon$	87	87	97	97	97
τ	34 ps	30 ps	27ps	23 ps	17 ps
α	0.005	0.005	0.005	0.005	0.05

L Y Zhang et al , Sensors & Actuators: A. Physical, Volume 216, 1 Sept 2014

EM signatures comparison

Correlation appears between measured EM signatures and tumor grade of investigated cells



Cole-Cole derived model:

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L Y Zhang et al , Sensors & Actuators: A. Physical, Volume 216, 1 Sept 2014

Outline

Content of the talk

- Motivation
- Dielectric RF spectroscopy challenges & issues
- Sensing methodologies for single cell analysis
- Cell electro-manipulation: another EM characterization approach
- Conclusion
- Acknowledgements

Cell electro-manipulation using high frequency signals

Translate dielectrophoresis principles to high frequencies

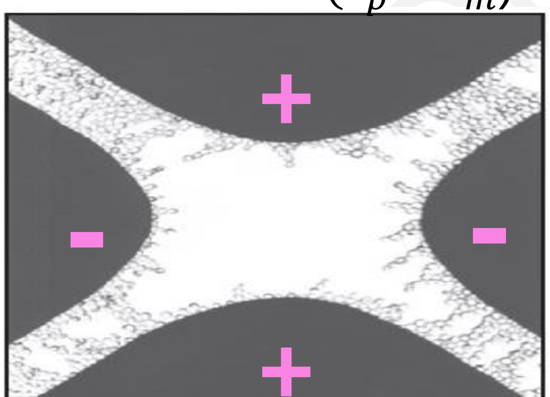
Electric fields can selectively attract or repeal suspended particles

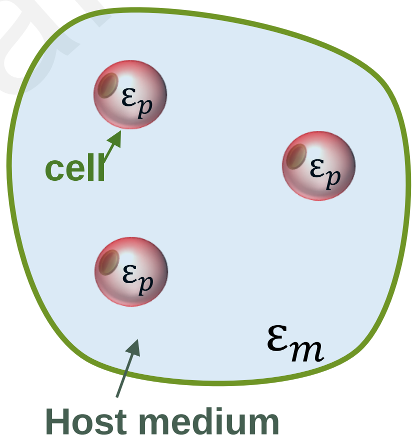
Dielectrophoresis force : $F_{DEP} = 2\pi\varepsilon_0\varepsilon_m r^3\, Re[K(\omega)]\nabla E^2$

Claussius-Mossotti factor : $K(\omega) = \dfrac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$

ε_p^*: particle complex permittivity
ε_m^*: host medium complex permittivity

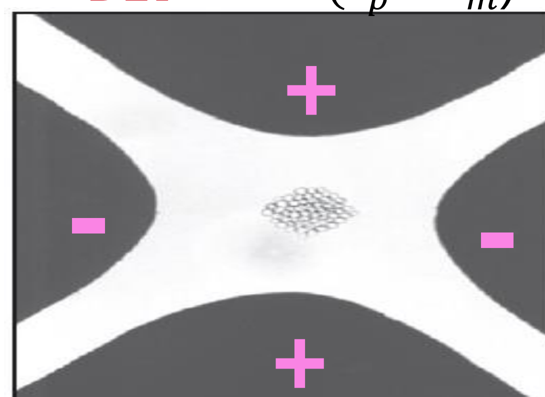
$F_{DEP} > 0$ $(\varepsilon_p^* > \varepsilon_m^*)$





R. Pethig, Advanced Drug Delivery Reviews, *2013*

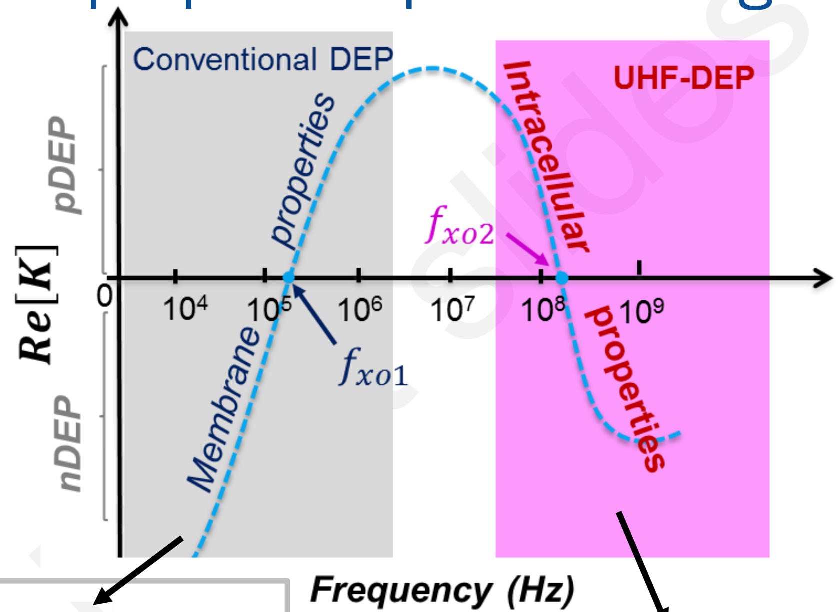
$F_{DEP} < 0$ $(\varepsilon_p^* < \varepsilon_m^*)$

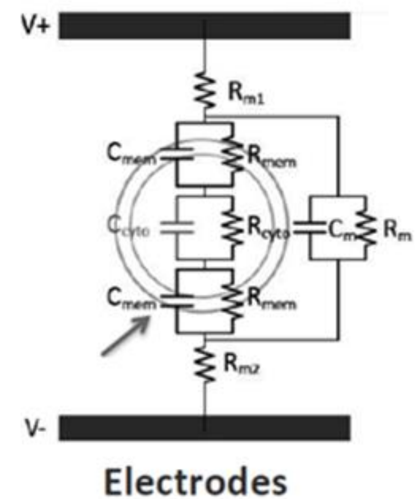


Cell dielectrophoresis behavior

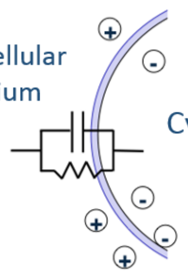
Cell & host medium properties presents slight differences

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$





- ***Conventional DEP:***

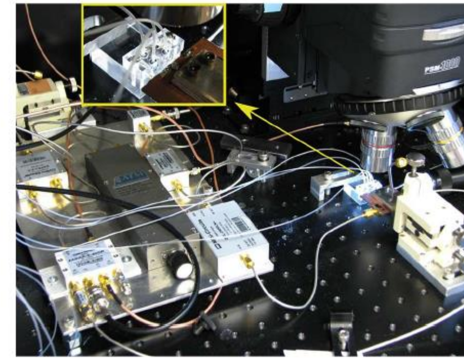


$$f_{xo1} = \frac{\sqrt{2}}{2\pi r C_m}\sigma_s$$

C_m: membrane capacitance
σ_s: surrounding electrolyte conductivity

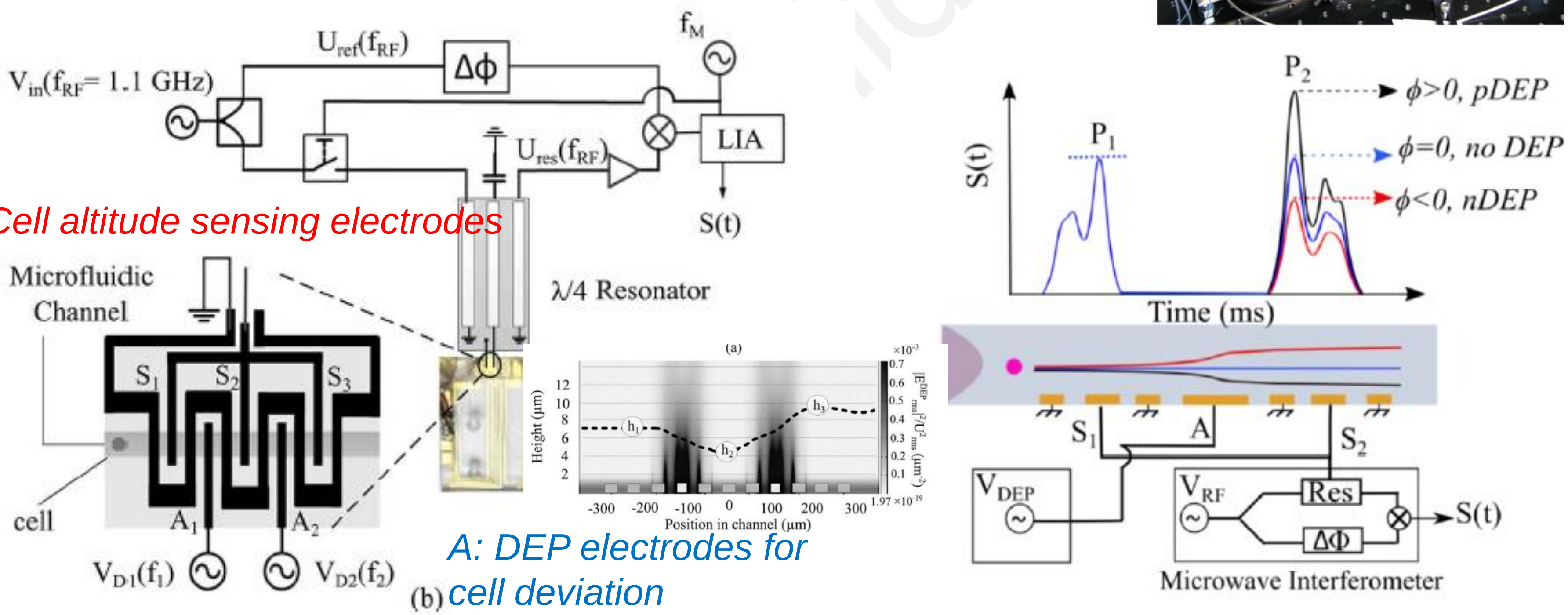
- ***UHF-DEP:***
  - Transparent cell membrane
  - Intracellular dielectric properties provide information on cell cytoplasm specificities...

Measuring cell deviation under electric field influence



Microwave interferometer implementation

Microwave resonator phase change as function as cell altitude



S: Cell altitude sensing electrodes

A: DEP electrodes for cell deviation

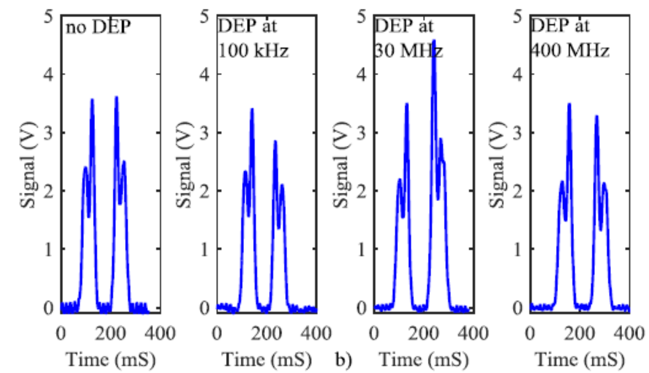
G Bridges et al, IEEE Transactions On Microwave Theory And Techniques, Vol. 64, No. 3, March 2016, Multi-Frequency DEP Cytometer Employing a Microwave Sensor for Dielectric Analysis of Single Cells

Measuring cell deviation under electric field influence

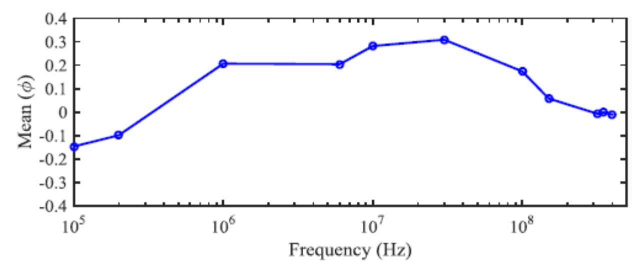
From cell deviation to useful cell dielectric signatures

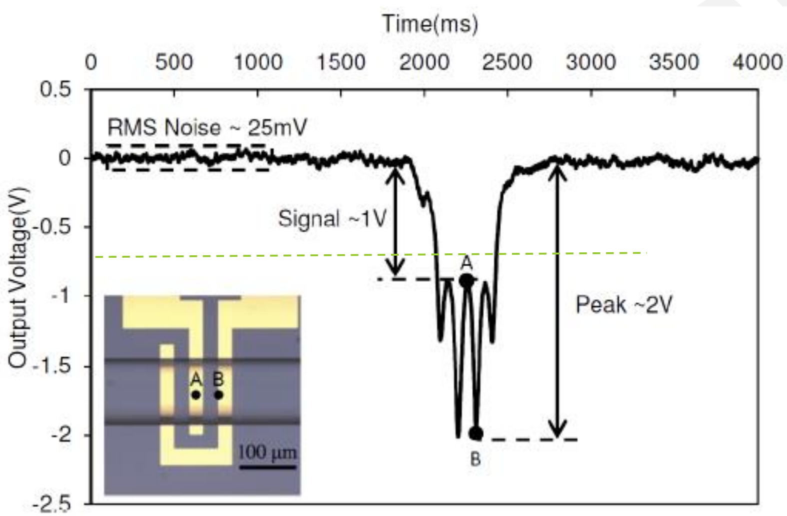
- Few attofarads detection capability allowed by interferometer architecture
- Single frequency RF sensing signal but different DEP signal frequencies to be investigated

Measured DEP behavior of single CHO cell



Wideband DEP signature of CHO cell population

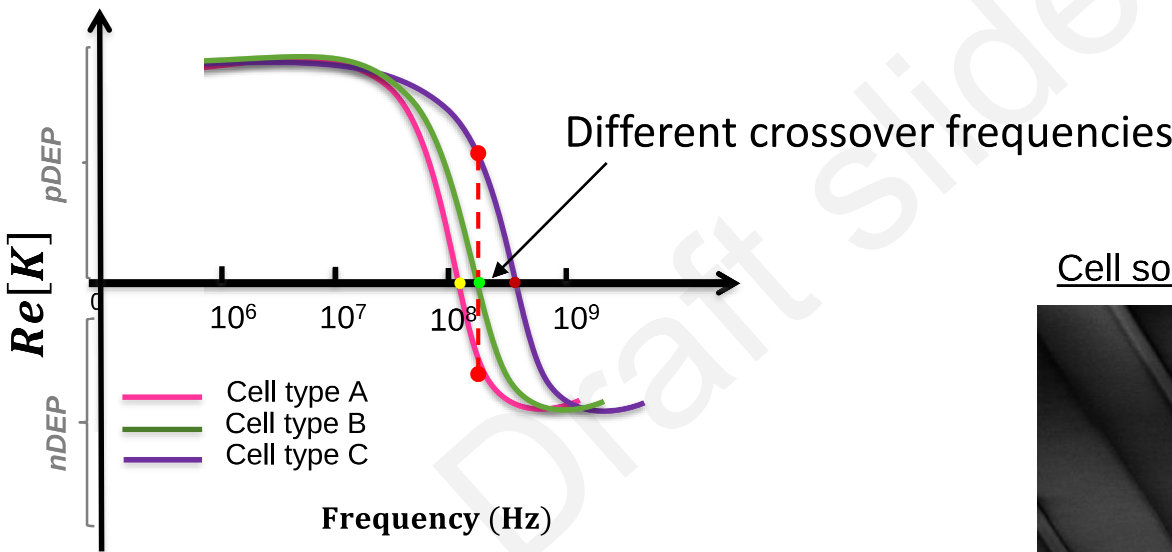




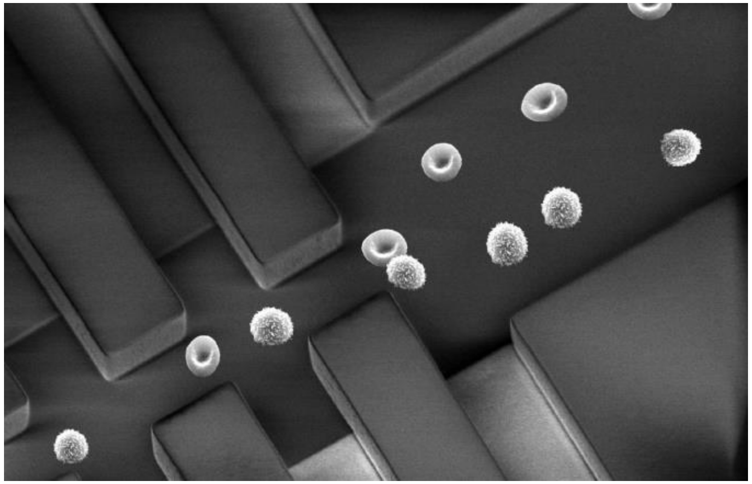
G Bridges et al, In-flow Dielectric Characterization of Single Biological Cells Using a Wideband DEP Cytometer, IMS 2016

Discriminating/Sorting cells using their UHF-DEP properties

Using cell crossover frequency specificities



Cell sorting concept



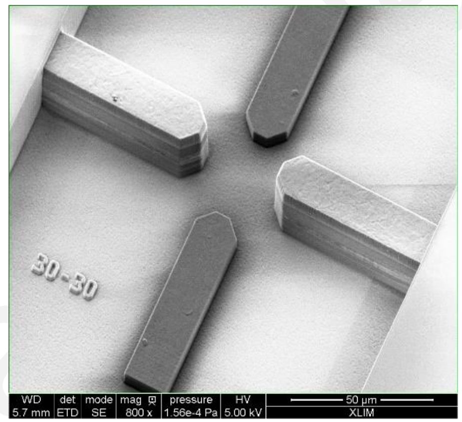
N Demierre, PhD Thesis EPFL 2008

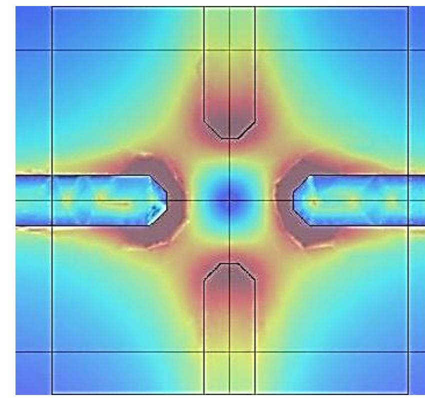
- One Single frequency DEP signal can induce different behavior if it is properly set
- Combination of DEP signals can be also considered

Discriminating/Sorting cells using their UHF-DEP properties

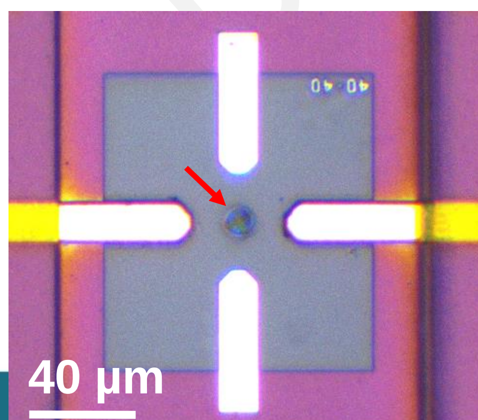
Sensor for single cell crossover frequency measurement

Quadrupole electrode device for efficient cell electrical trapping

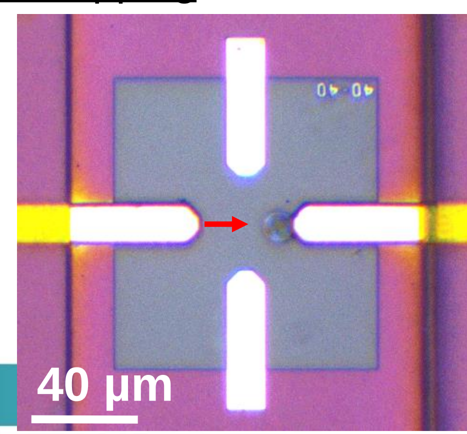




Negative DEP trapping



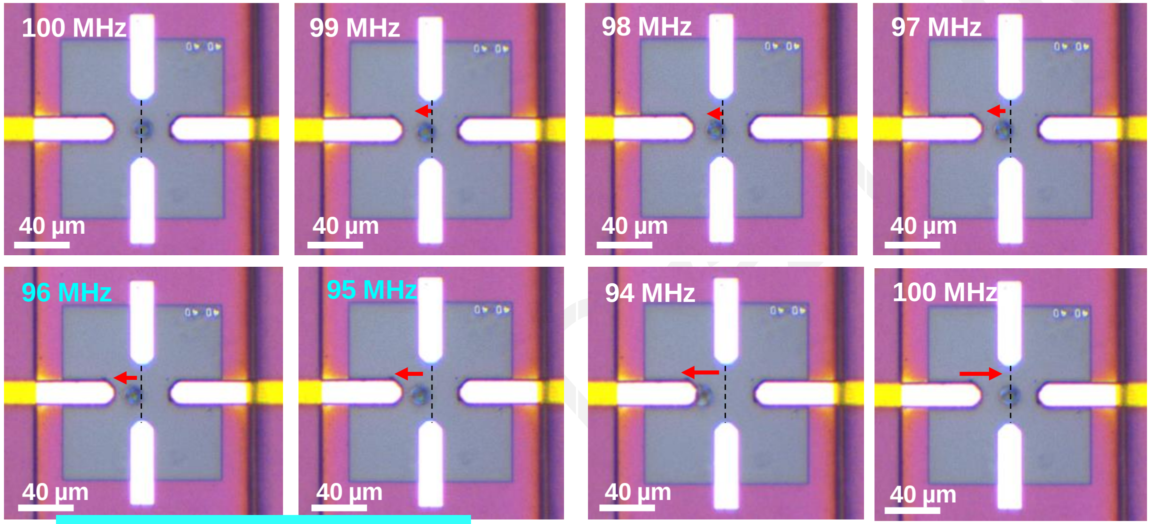
Positive DEP trapping



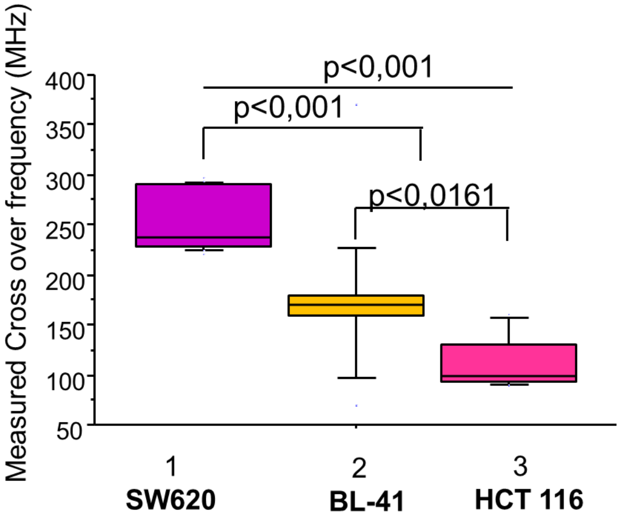
Discriminating/Sorting cells using their UHF-DEP properties

Sensor for single cell crossover frequency measurement

Trap efficiency monitoring when DEP signal frequency is tuned



Example of 3 cell population characterization



Crossover frequency

Specifies of cell behavior under UHF signals can be well identified

F Hjeij, Biological Cell Discrimination Based on Their High Frequency Dielectropheretic Signatures at UHF Frequencies, IMS 2017

Conclusion

- Exploiting dielectric specificities of cell content (cytoplasm) both in the Radiofrequency spectrum than UHF appears really relevant for cell label-less characterization and cell biological processes monitoring

- Microwave spectroscopy should be able provides new relevant data for biological investigation and characterization at the single cell level, complementary with conventional biological investigations: biologists are looking for new markers, new signatures!

- Novel biomedical instrumentation should emerge in the coming years , as the recently commercialized low frequency impedancemetry instruments, and our community might be involved…

Acknowledgments

Thanks for your attention

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