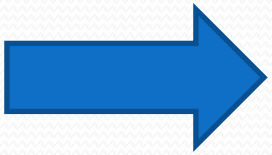
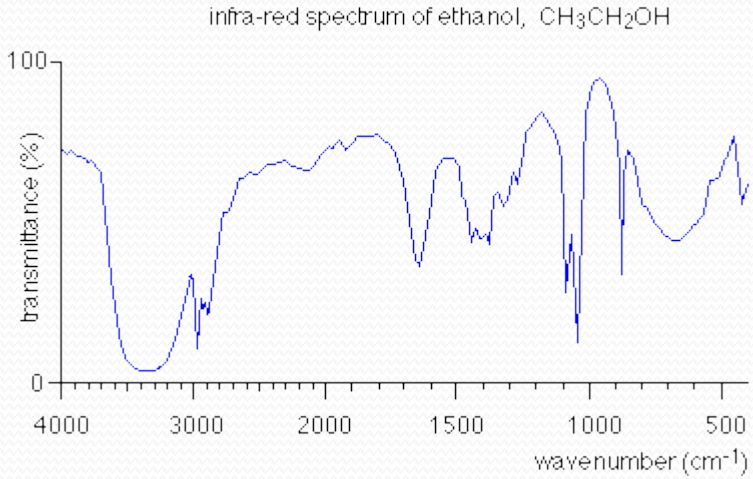
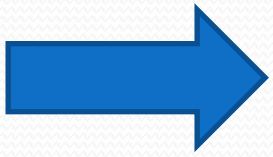
FTIR microspectroscopy and imaging on single cells: experimental procedures and data handling

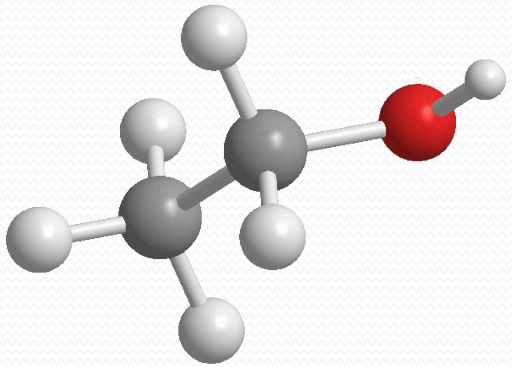
Mariangela Cestelli Guidi
INFN - Laboratori Nazionali di Frascati

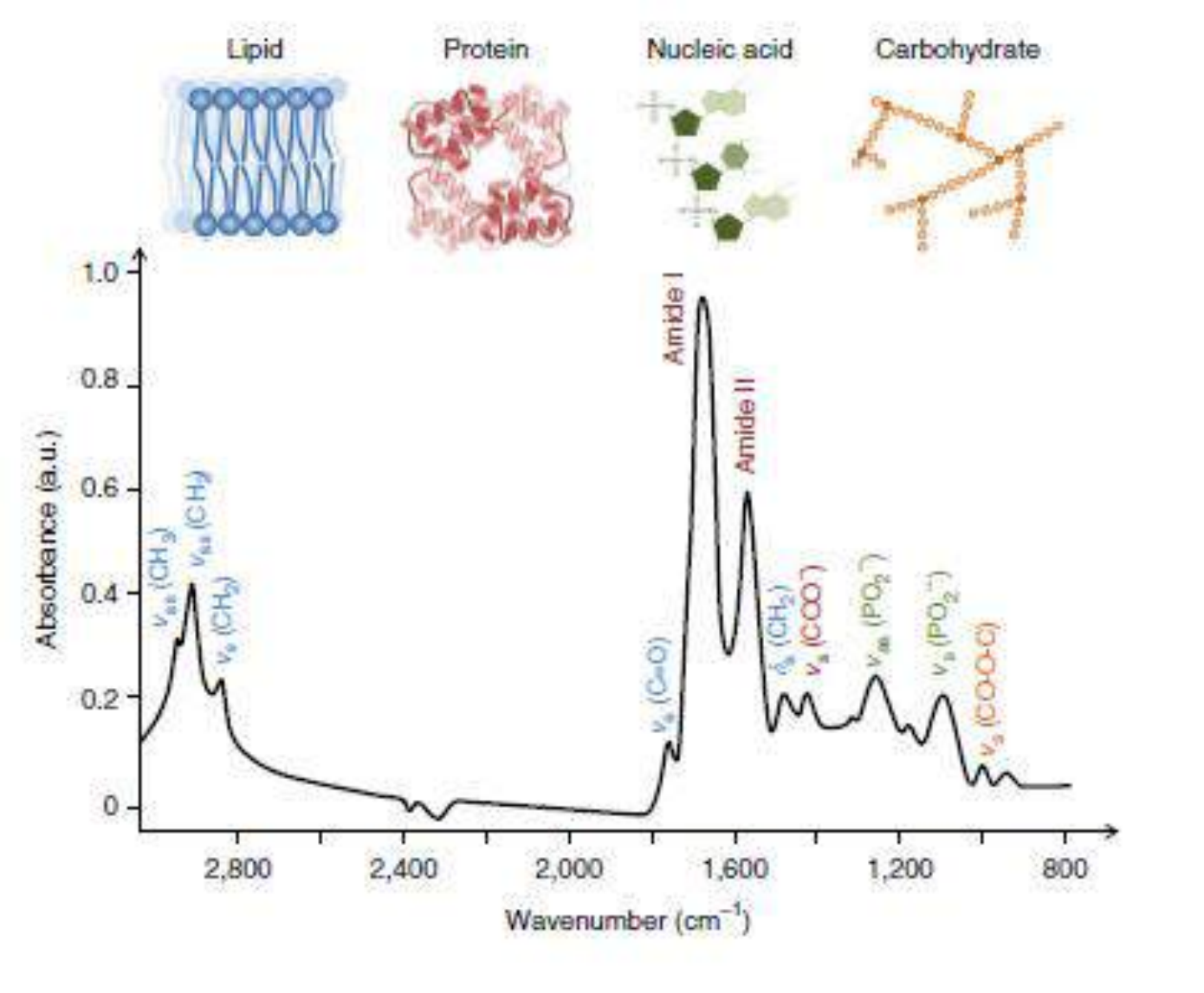
- Single cell FTIR spectroscopy: SR vs standard sources
- The importance of sample preparation: choosing the substrate , the fixation protocol and how to minimize diffraction effects
- Data analysis and classification tools. A case study: identification of cystic fibrosis (CF) cells



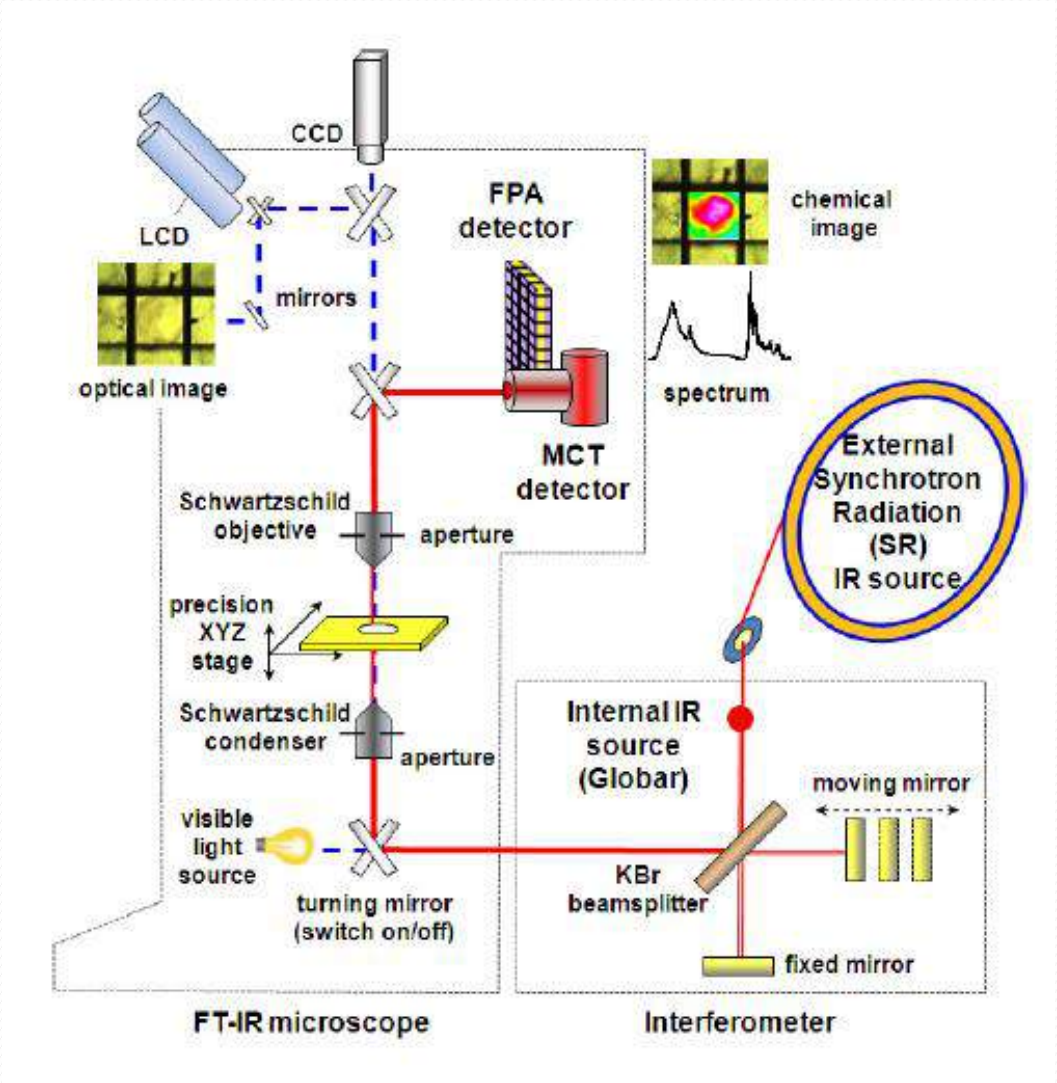

infra-red spectrum of ethanol, CH_3CH_2OH
100
0
transmittance (%)
4000
3000
2000
1500
1000
500
wavenumber (cm^{-1})





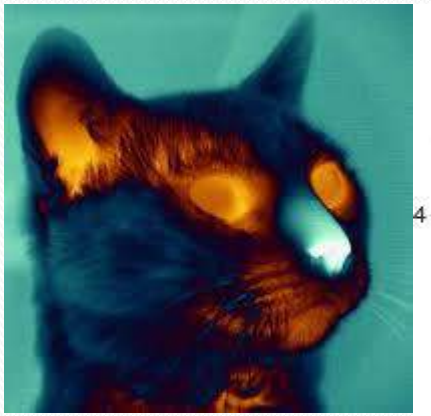


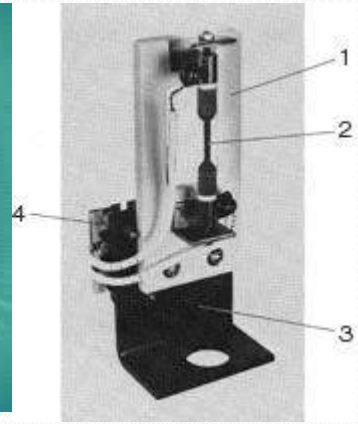
M. J. Baker et al, Nature protocols **9**, 8, 1771-1791 (2014)

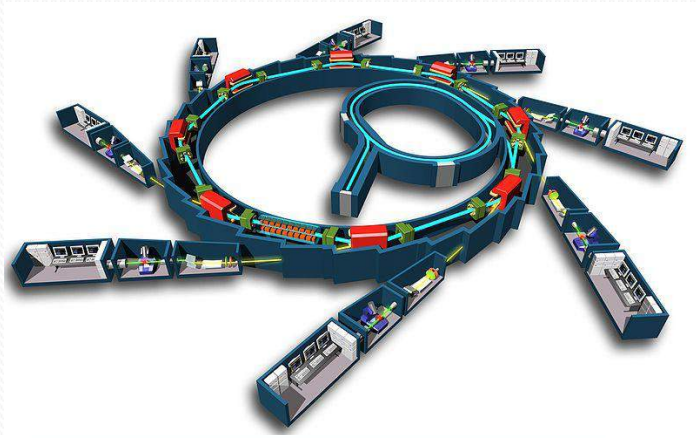


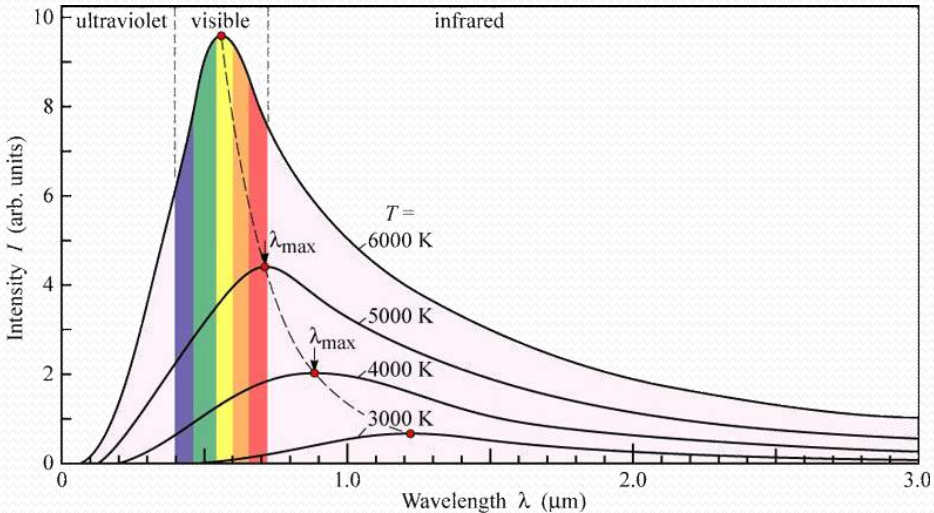
G. Bellisola et al, Am J Cancer Res 2012;2(1):1-21

IR radiation sources









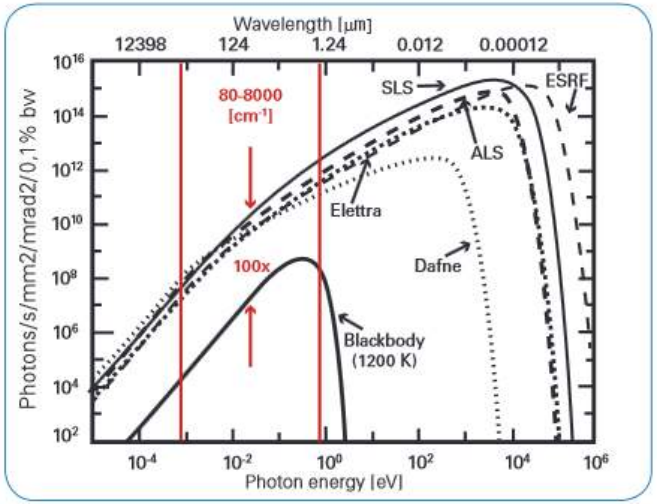
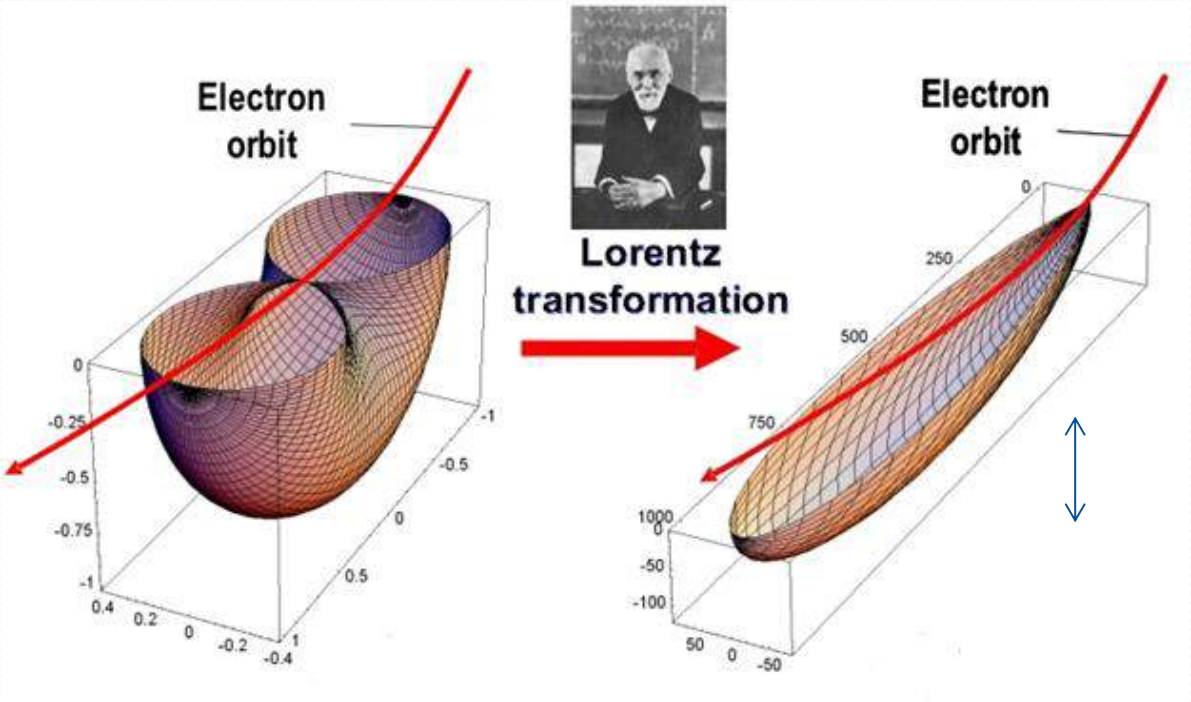


Fig. 1: Advantages of the e-Synchrotron radiation source



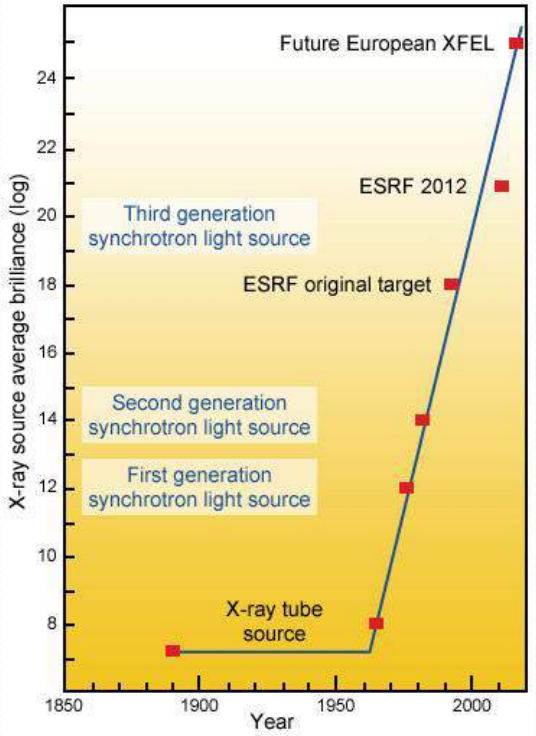
Classic ($v<<c$)

Relativistic ($v\approx c$)

$$\beta = v/c$$

$$\gamma = \frac{1}{\sqrt{1-\beta^2}}$$

For β=0.99 $1/\gamma$= 10 mrad



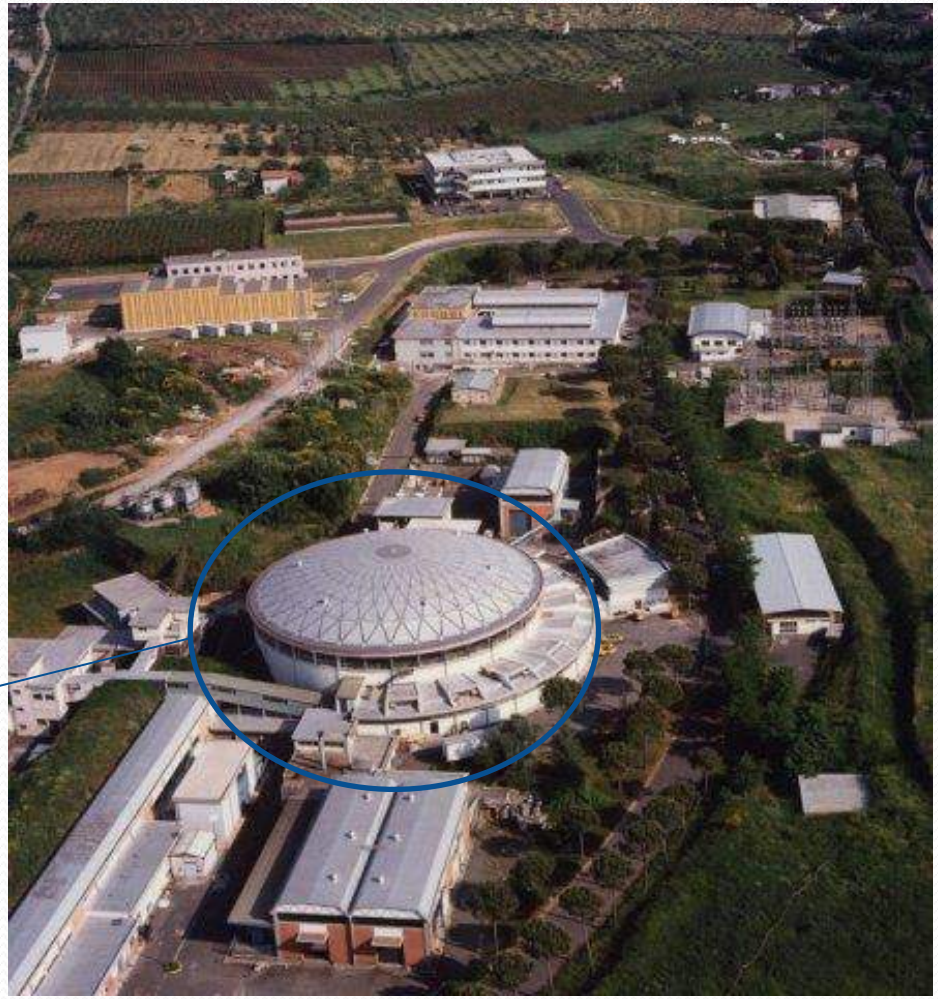
$$brilliance = \frac{photons}{second \cdot mrad^2 \cdot mm^2 \cdot 0.1\%BW}$$

National Institute of Nuclear Physics

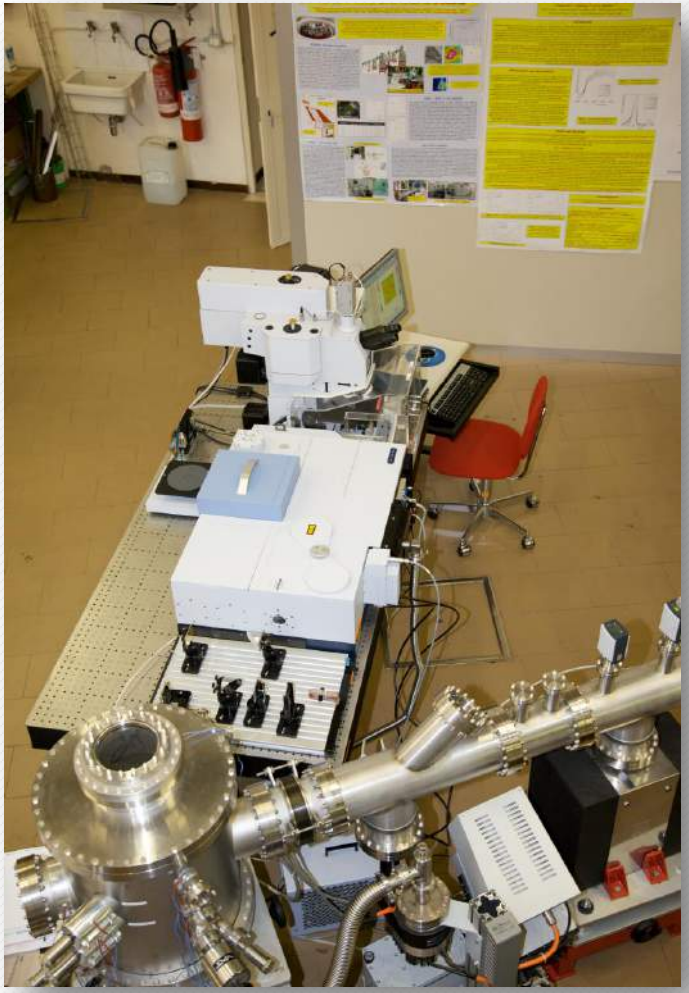
Laboratori Nazionali di Frascati

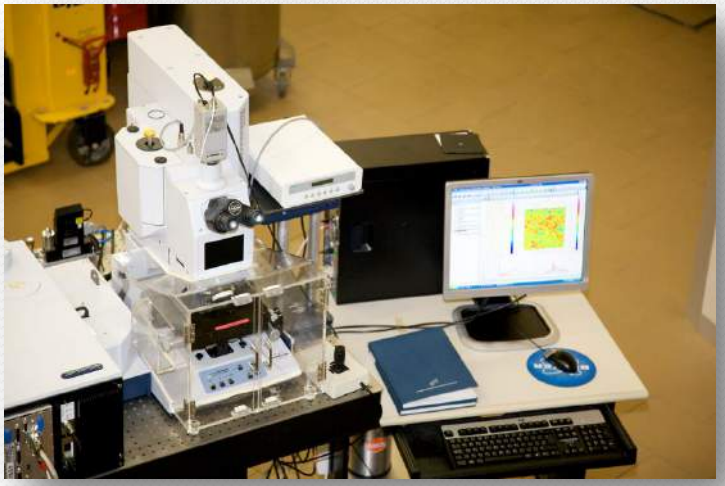
DaΦne storage ring

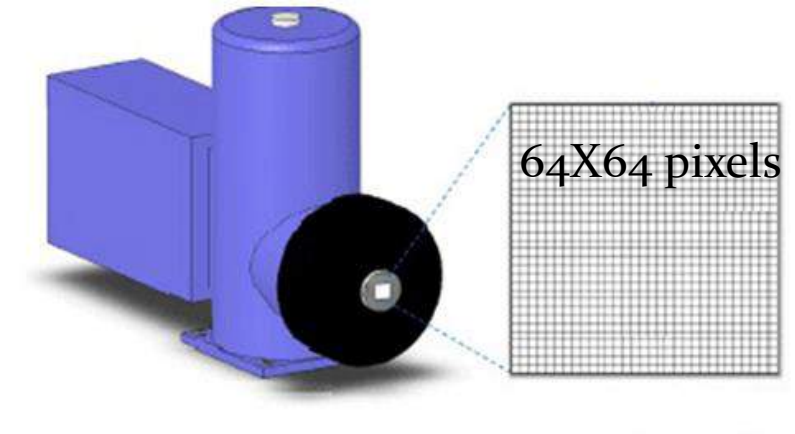
E=0,51GeV
I=1.5-2 A

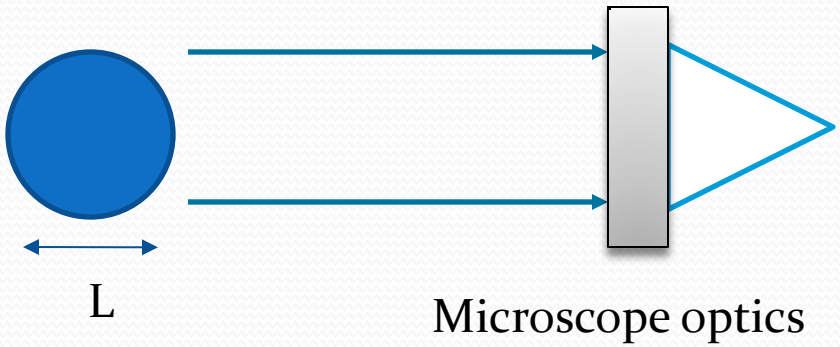


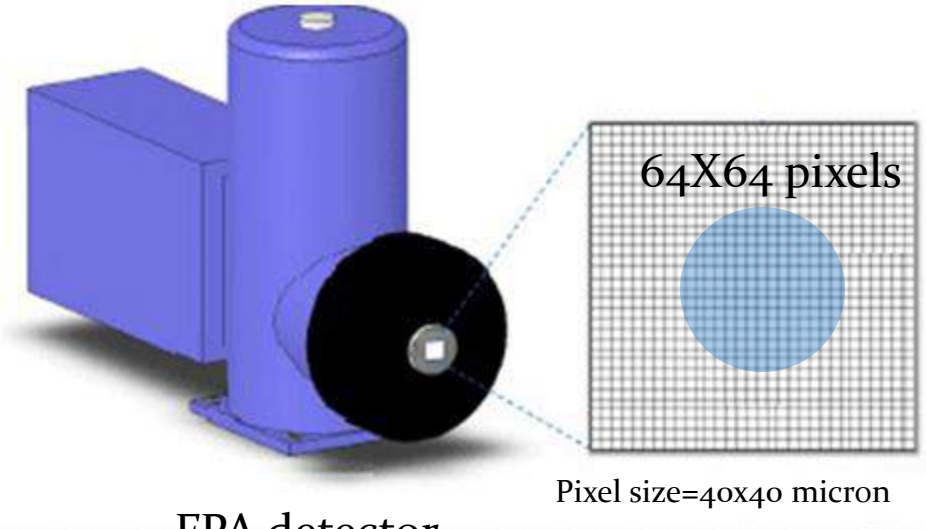
The Sinbad (Synchrotron INfrared Beamline At DaFne) beam line





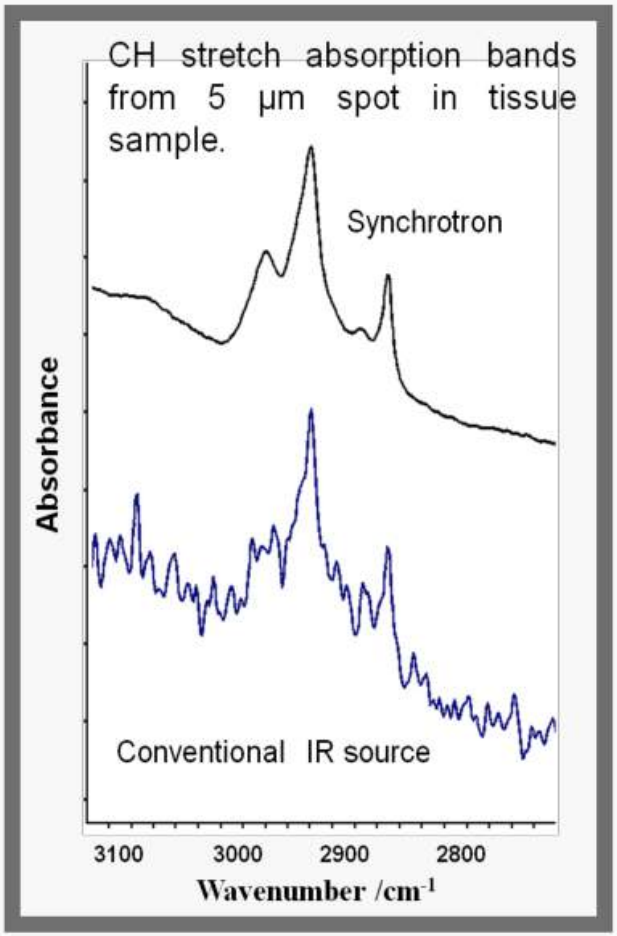




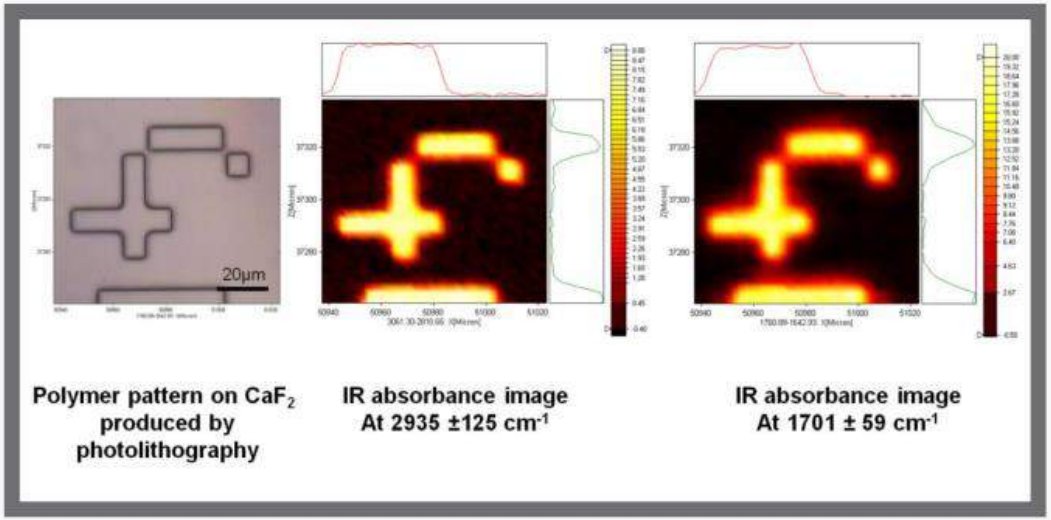


Microscope objective	NA	FPA pixel resolution	sample area covered
15X	0.4	40 μm/15 = 2.6 μm	170x170 μm²
20X	0.6	40 μm/20 = 2 μm	128x128 μm²
36X	0.5	40 μm/36 = 1.1 μm	102x102 μm²

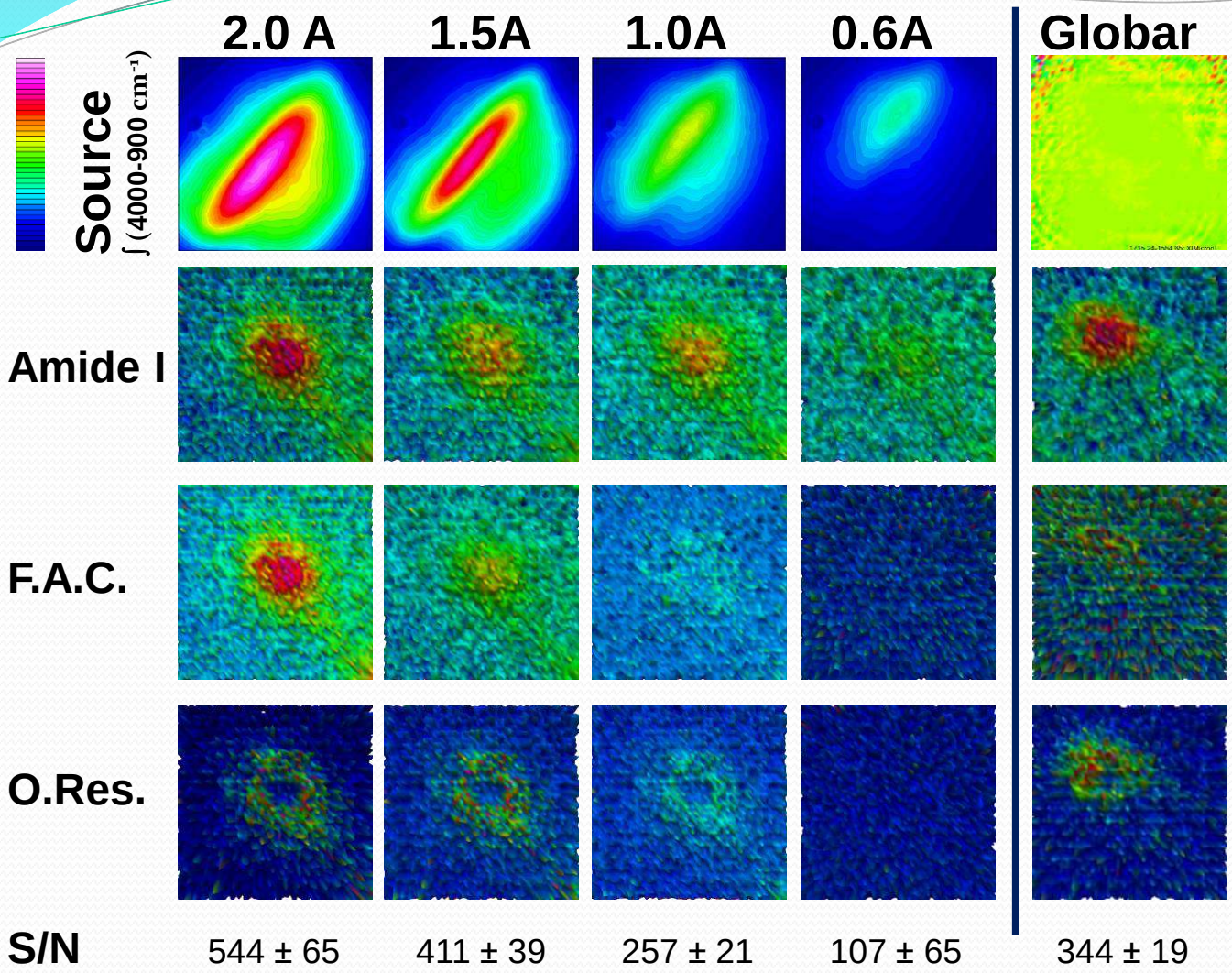
SR advantages in the IR domain

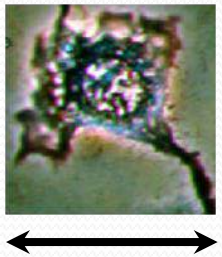


S/N ratio



Spatial resolution
(diffraction limited)



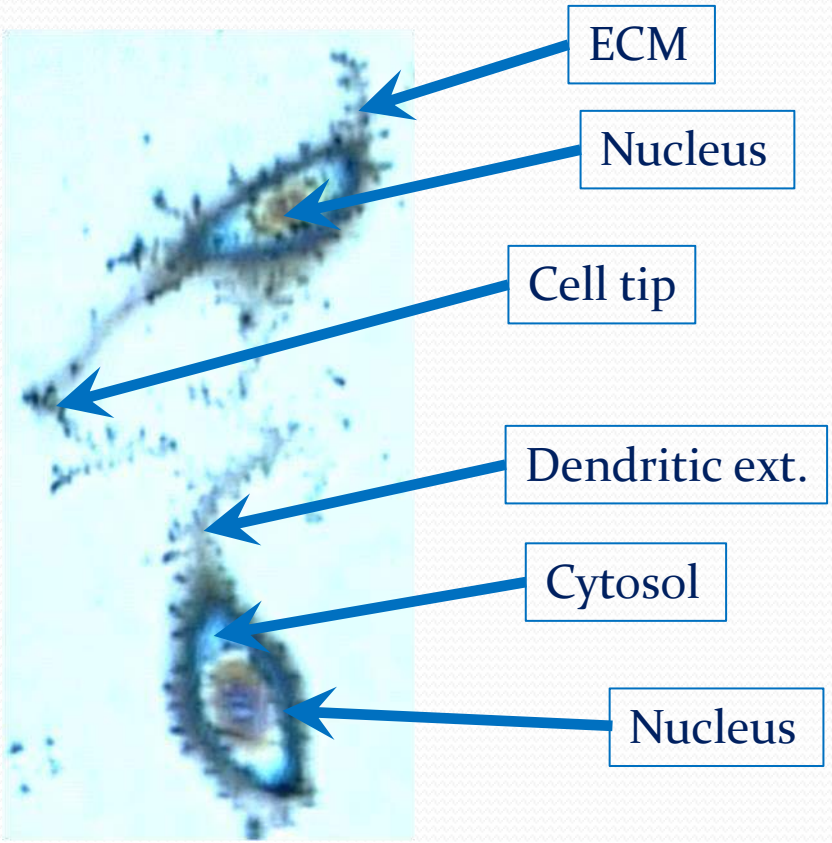


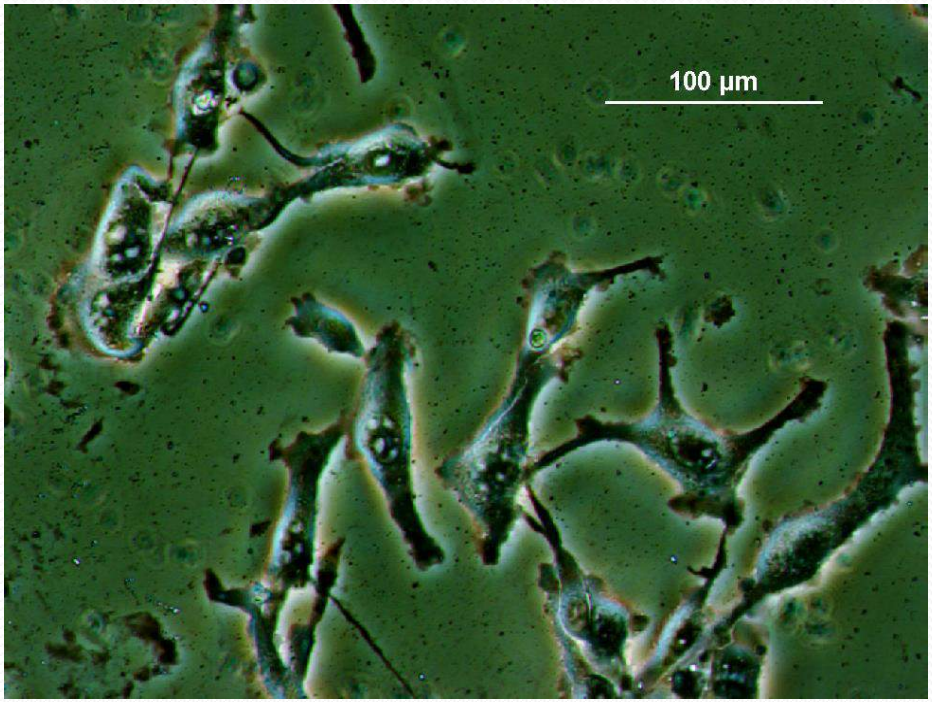
36 µm (32x32 pixels)

C. Petibois et al, Anal Bioanal Chem (2010) 397:2123–2129

Sample preparation: cell fixation protocol, choice of the substrate and acquisition method

- Obtaining ECM elements
- Maintaining cell morphology
- Obtaining cell-cell interactions
- Stopping culture at selected moments





In vitro vs. *ex vivo* after HP cryofixation

Maintaining morphological organization = no fixation, no staining, no detachment

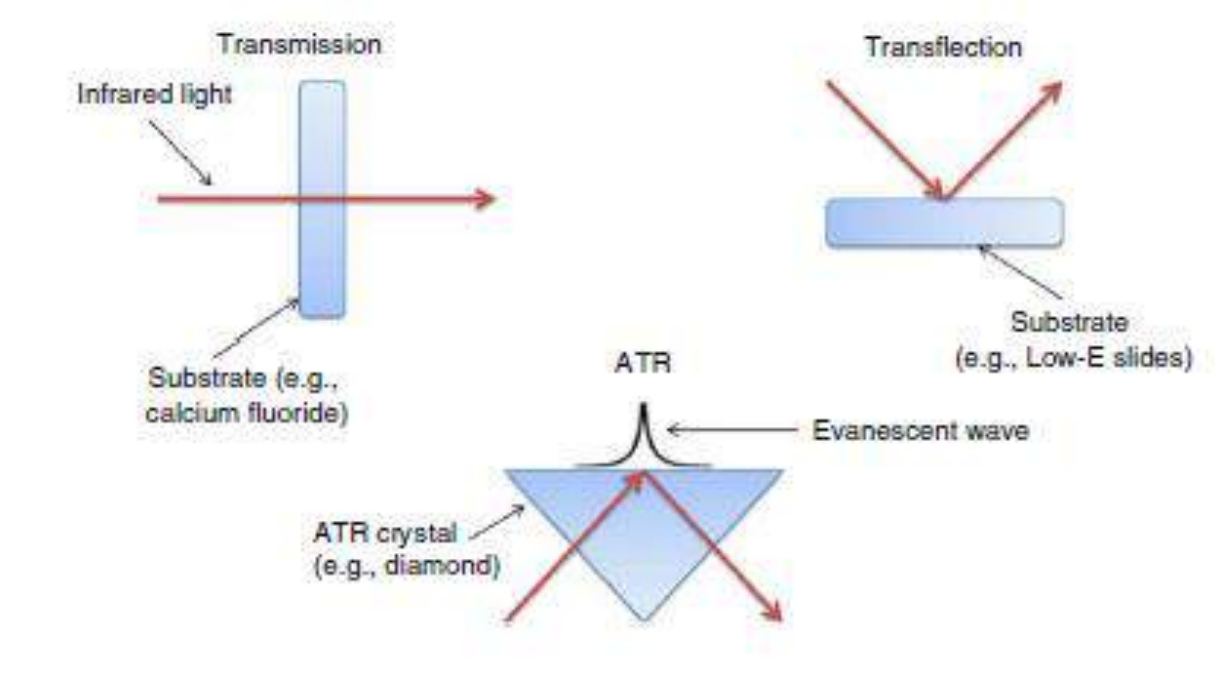
Which substrate to chose?

The most relevant parameter for cell biology is the biocompatibility of the substrate, which can be characterized both by **cell viability** and by **cell adhesiveness** on surface.

•Considering the **biotoxicity parameter**, it appeared that **Ge, Si, diamond, Si_3N_4, and LaF_3** were the only substrates to offer comparable conditions (less than 10% cell death) to the PC (5% cell death) or glass (3% cell death).

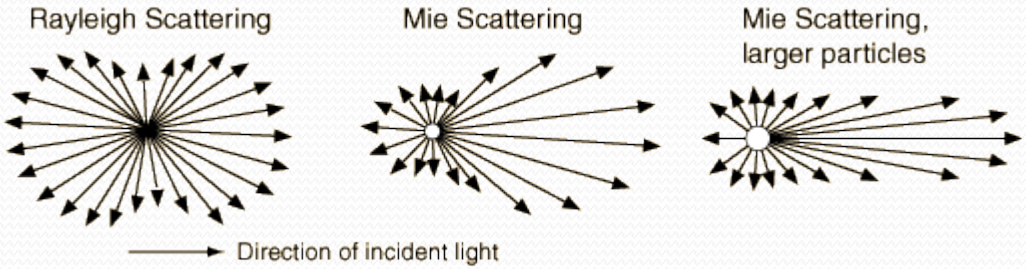
•The second parameter we took into account is **cell adhesiveness on substrates**.
Compared to PC (187 cell/mm²) or glass (171 cells/mm²), the IR-transparent substrates providing satisfying results were **Si_3N_4** > Si > LaF_3 > ZNS/C > Ge

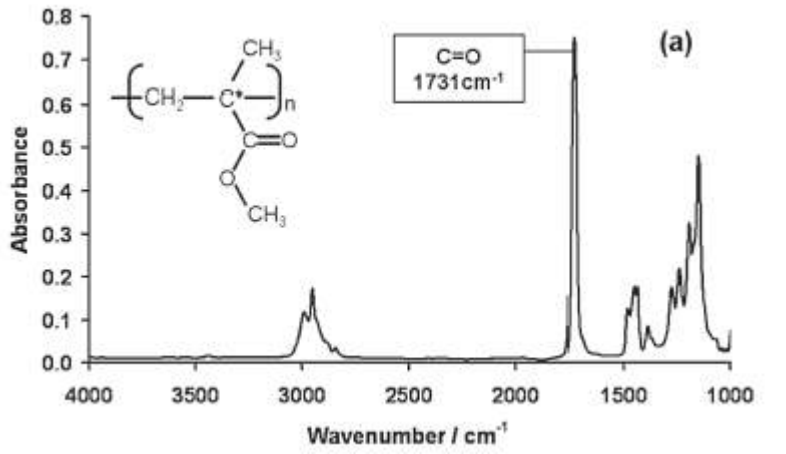
The acquisition method



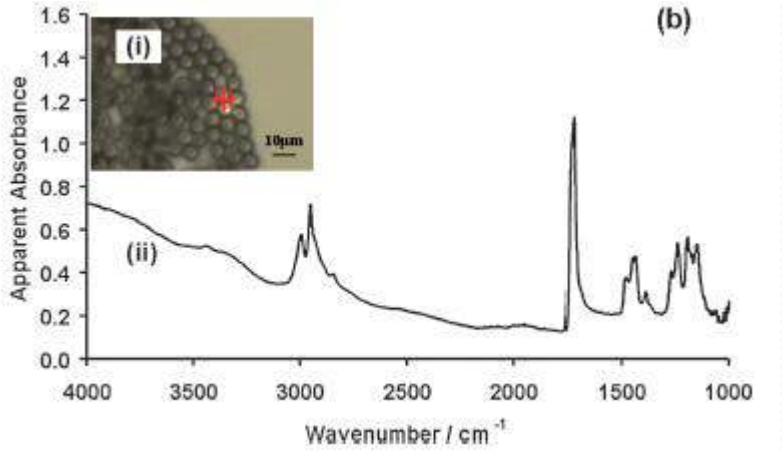
$$r = \frac{0.61\lambda}{\mathrm{NA}} \qquad \mathrm{NA} = n \sin \theta$$

Minimizing scattering effects





PMMA polymer film

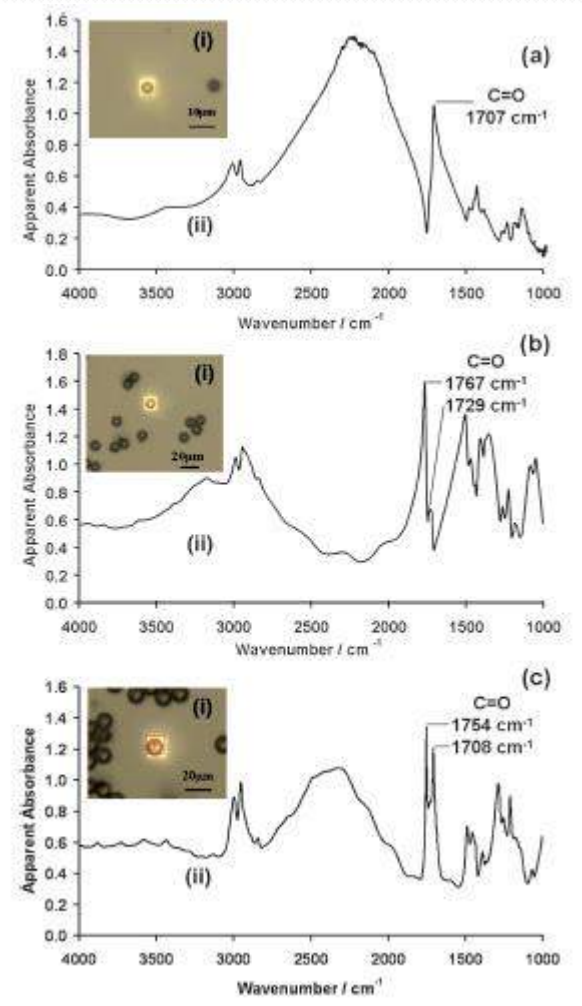


PMMA close-packed microspheres

$$Q = 2 - (4/\rho)\sin(\rho) + (4/\rho^2)[1 - \cos(\rho)]$$

where $\rho = 4\pi d(n - 1)/\lambda$ and $n = n_1/n_2$.

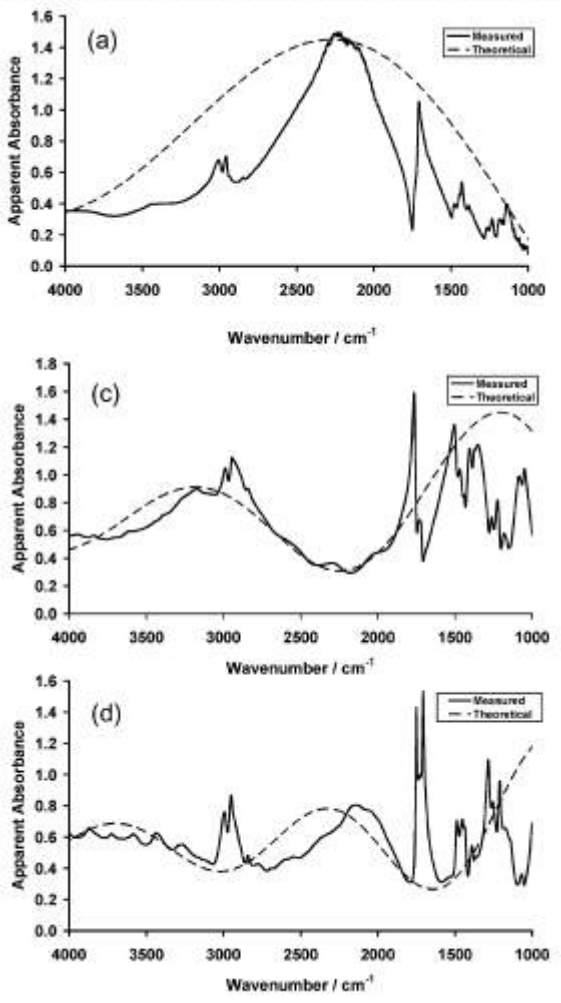
P. Bassan et al, Analyst, 2009, 134, 1586–1593



5.5 μm

10.8 μm

15.7 μm



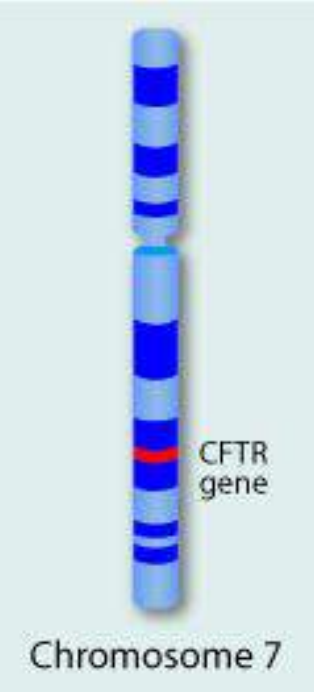
Mie scattering correction

P. Bassan et al, Analyst, 2009, 134, 1586–1593

The identification of cystic fibrosis (CF) cells and their pharmacological correction by mid-infrared microspectroscopy and unsupervised data analysis methods

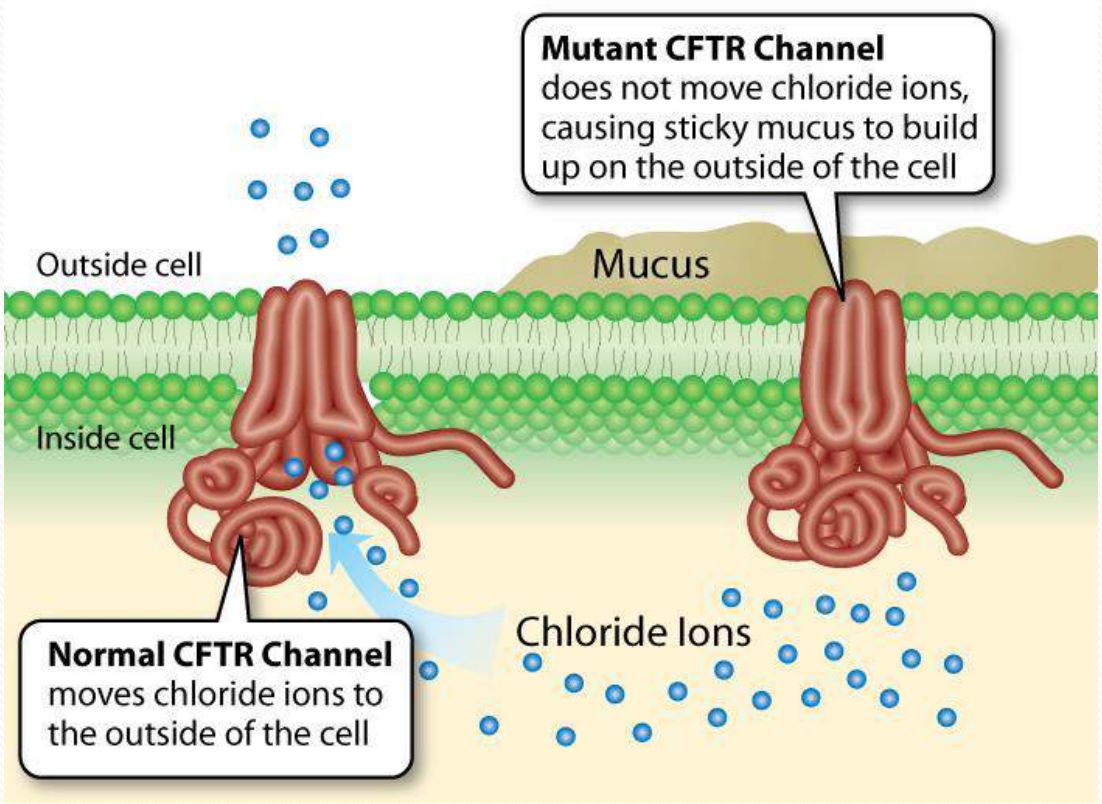
G. Bellisola, S. Caldrer, G. Cinque, M. Cestelli Guidi, B. M. Assael, P. Melotti, C. Sorio

What is cystic fibrosis?



Cystic fibrosis is a genetic disorder that affects the respiratory and digestive systems.

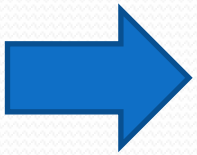
People with cystic fibrosis inherit a defective gene on chromosome 7 called *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator).



The protein produced by this gene normally helps **salt** (sodium chloride) **move in and out of cells**. If the protein doesn't work correctly, that movement is blocked and an abnormally thick sticky mucus is produced on the outside of the cell.
The cells most seriously affected by this are the lung cells. This mucus clogs the airways in the lungs, and increases the risk of infection by bacteria.

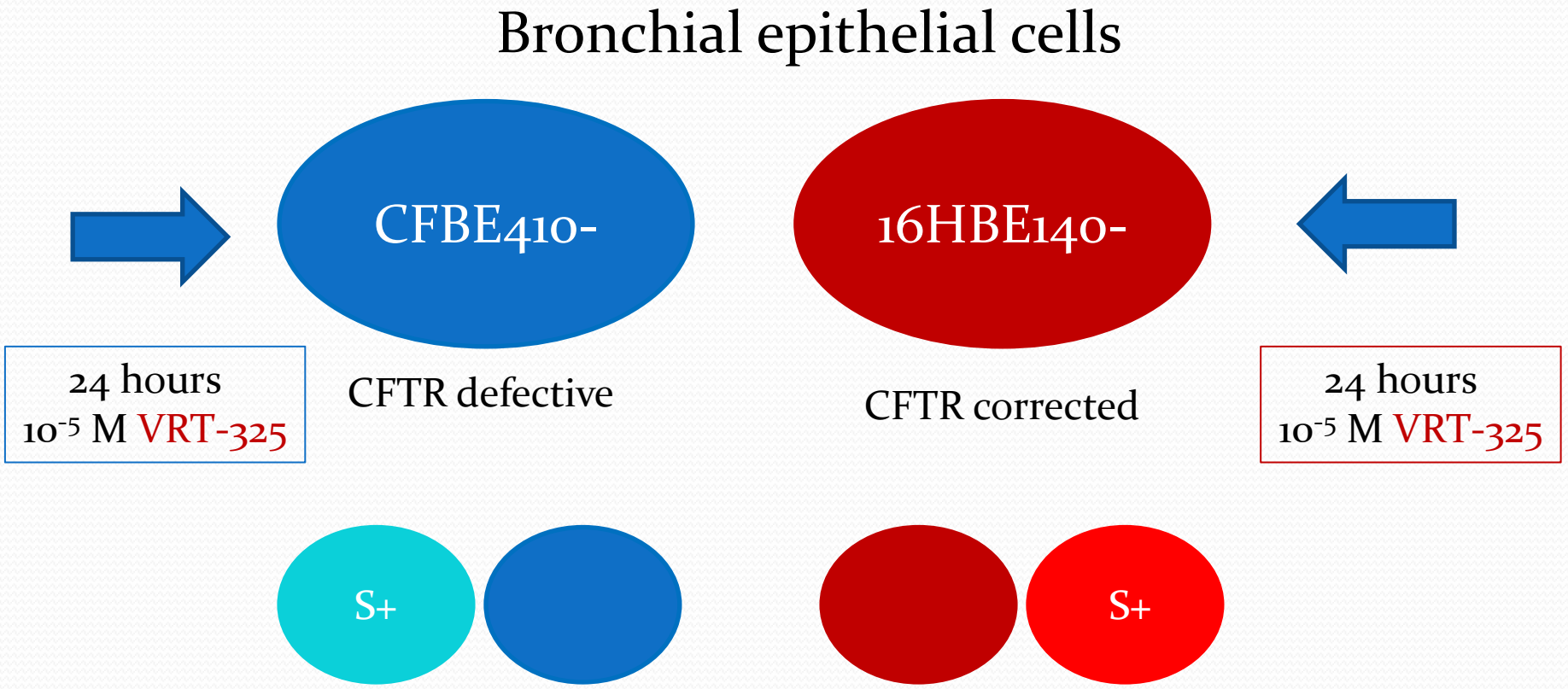
Genic therapy

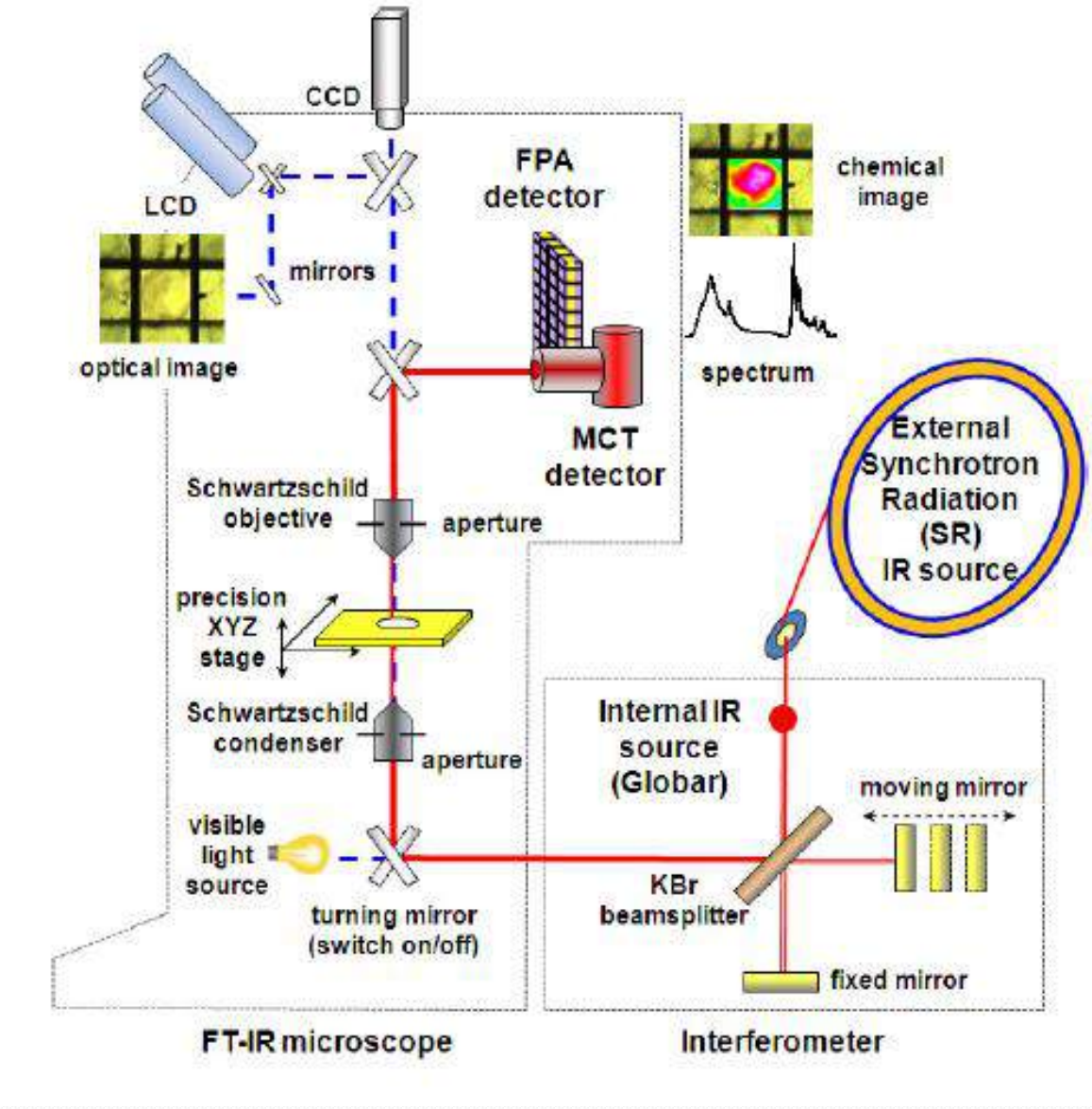
- Therapeutic strategies targeting defective CFTR protein rather than disease symptoms have been proposed
- Some encouraging results of ongoing clinical trials have already put in light clinical benefit of some drug molecules known as CFTR modulators

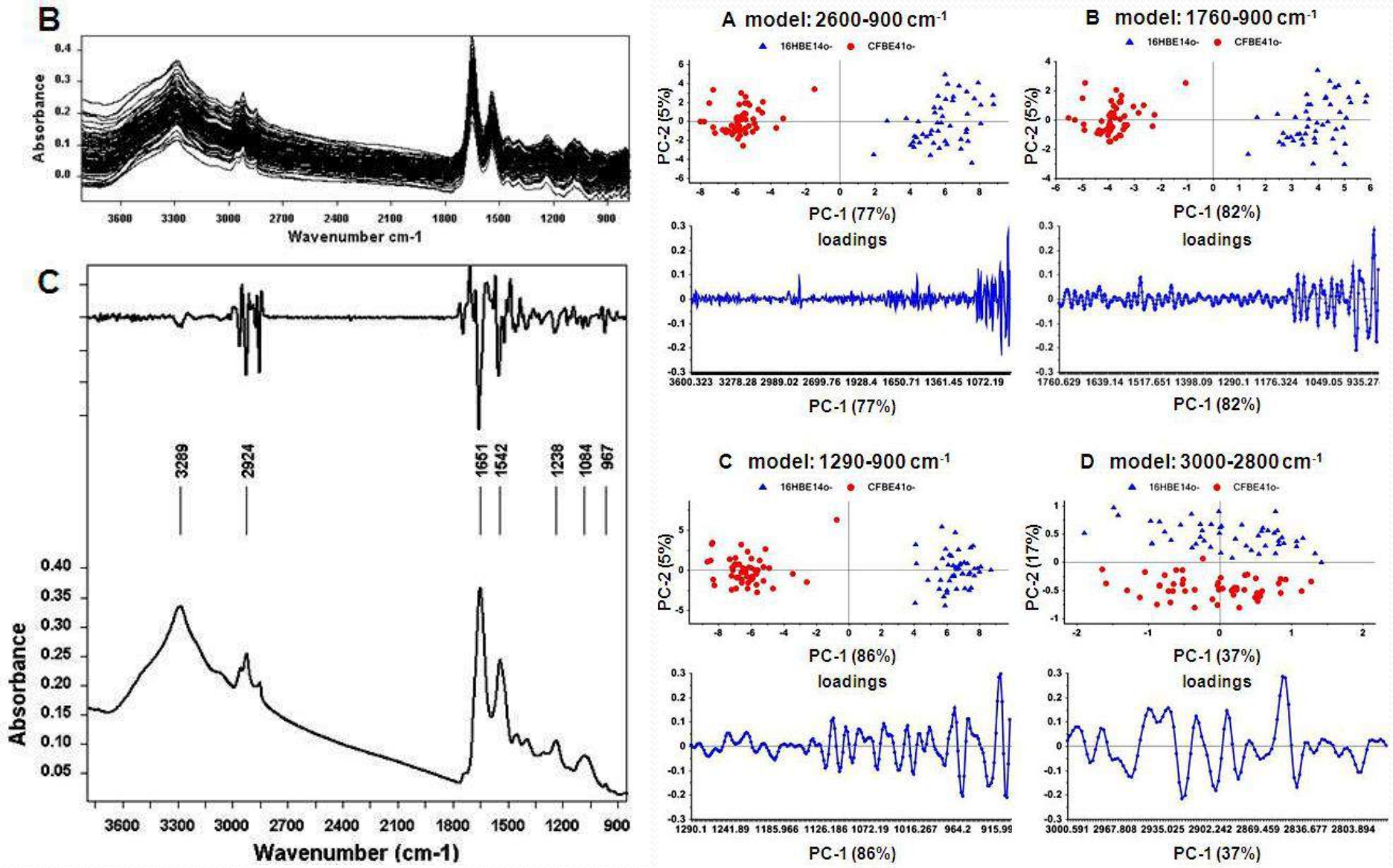


CF cells were exposed “ex vivo” to VRT-325, a chemical corrector of defective anion transporter CFTR

Treatment conditions

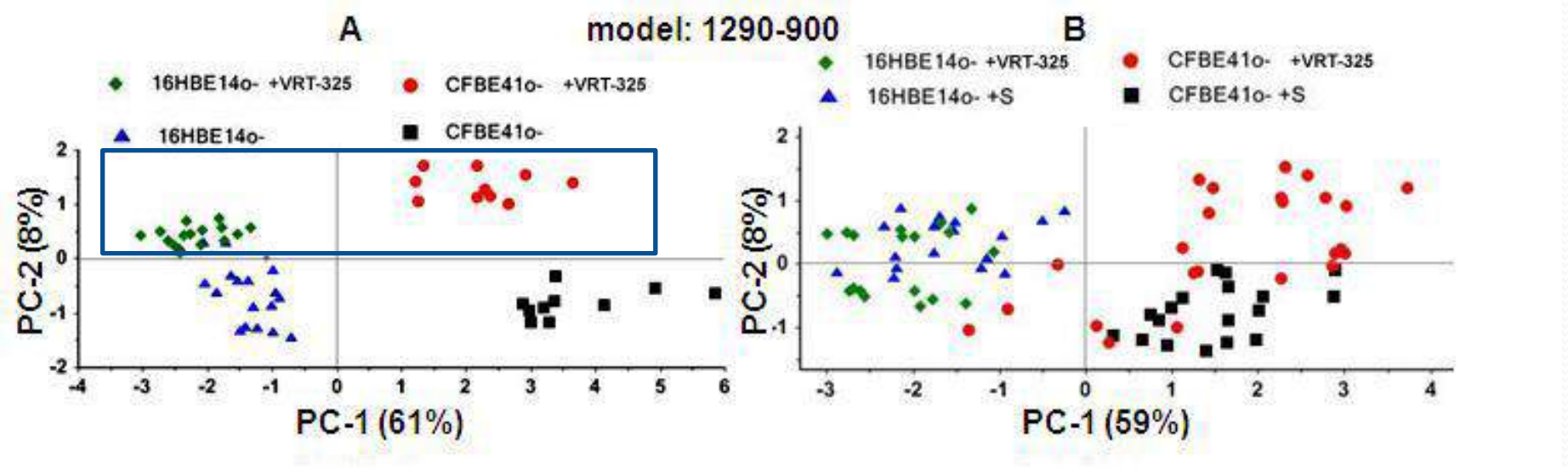



CCD
LCD
mirrors
optical image
FPA
detector
chemical
image
spectrum
MCT
detector
Schwartzschild
objective
aperture
precision
XYZ
stage
Schwartzschild
condenser
aperture
visible
light
source
turning mirror
(switch on/off)
External
Synchrotron
Radiation
(SR)
IR source
Internal IR
source
(Globar)
moving mirror
KBr
beamsplitter
fixed mirror
FT-IR microscope
Interferometer



Vector normalized+Mie scattering corrected

(G.Bellisola et al, ScienceJet 2014, 3: 51)

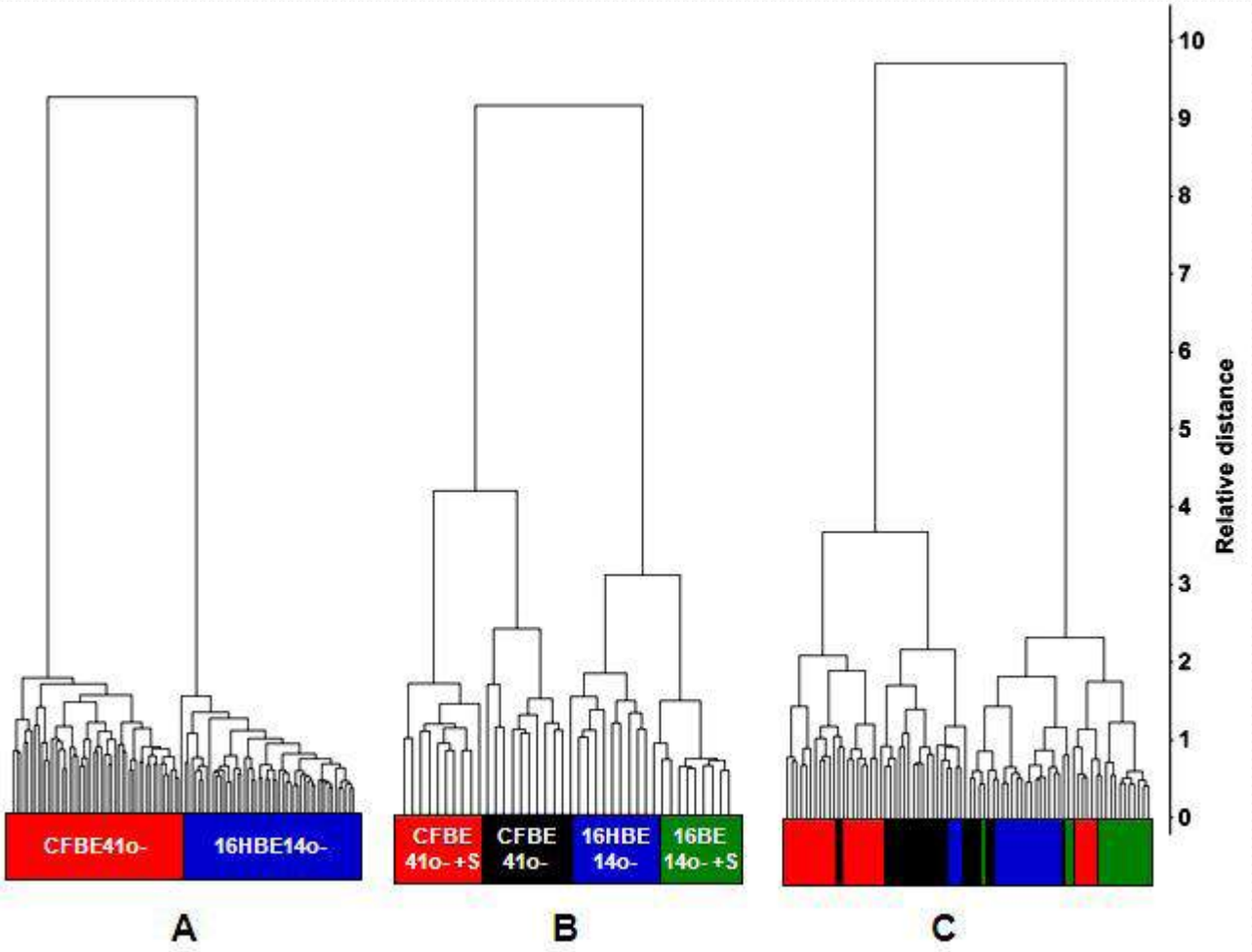


VRT-325 treated (top) and non-treated (bottom)

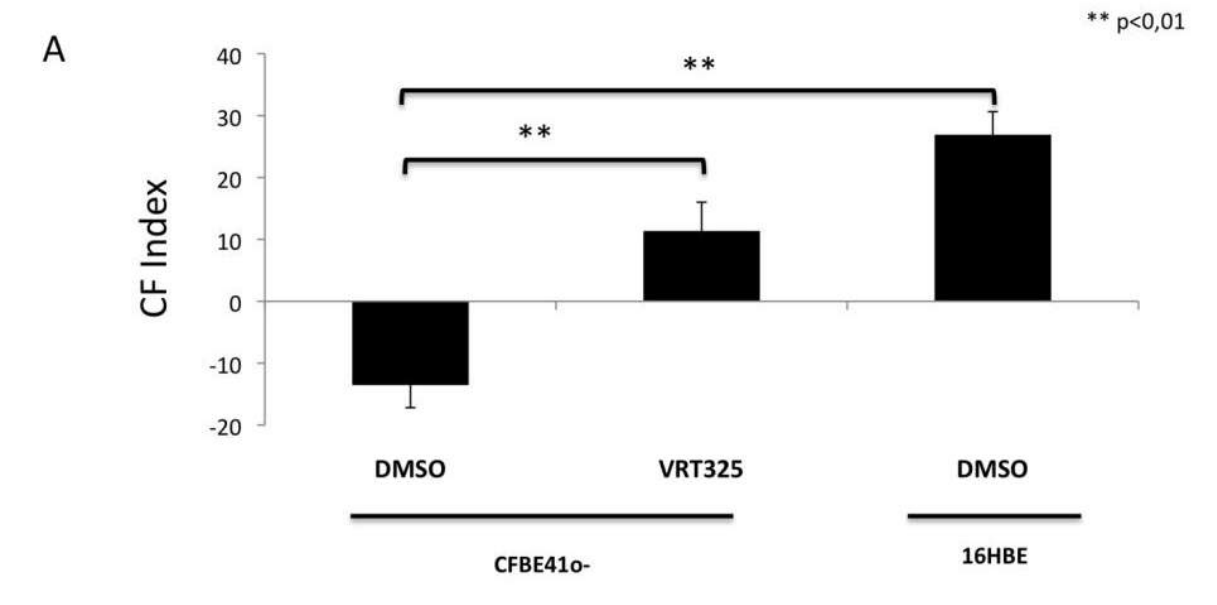
After stimulation

Some CF samples treated with VRT-325 and stimulated with the cocktail position within corresponding non CF controls thus suggesting that under stress conditions CF cells treated with VRT-325 can have similar behavior than the corresponding non-CF control cells

HCA cluster analysis



Measure of membrane depolarization following stimulation with cAMP agonists, before and after VRT-325 treatment. The treatment is able to revert CF index from negative (left) to positive (middle) values that are typical of non-CF cells (right).



Thank you for your attention!

G. Cinque - *MIRIAM beamline*

G. Bellisola,
S. Caldrer,
C. Sorio

C. Petibois
S. Yao

M. C. G.
A. Marcelli