HIGH RESOLUTION SPRI FOR THE STUDY AND EARLY DETECTION OF BACTERIA

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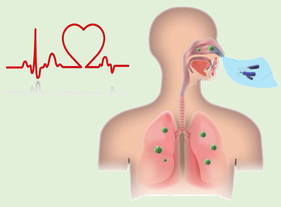
Context

Domains

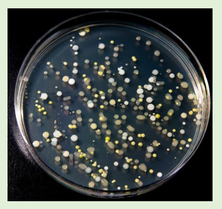
Food industry



Health

Need for faster, more effective bacteria detection tools, with lower detection limits [1]

Standard technics



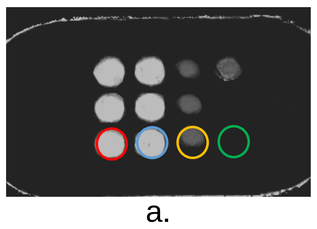
standard plate counts

✓ Precise ✓ Reliable
➢ Laborious
➢ Delay of detection
(up to 3 days *for L. monocytogenes* [2])

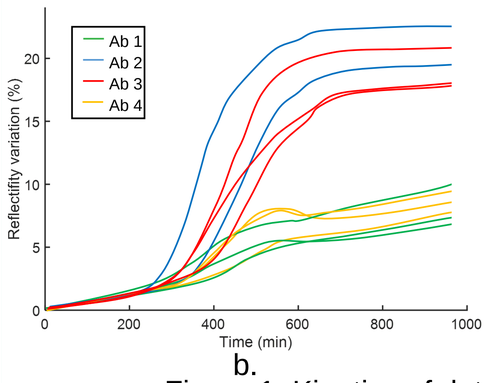
[1] Velusamy *et al,* 2010, *Biotechnology advances*

[2] Morlay *et al*, 2017, *Measurement*

Performances of classical SPR imaging



a.



b.

Figure 1. Kinetics of detection of bacteria with SPRi
a. Differential image at the end of the experiment b. SPR kinetic curves

Advantages of SPRi :

- Label-free
- Ease of use
- Spatial multiplexing
- Compatible with complex media
- Real time

<u>Delay of detection :</u> 10 cfu/mL of *L.monocytogenes* detected in 25h in complex medium in growth mode [2]

Principle of high resolution SPR imaging

Objective : study the dynamic behavior of individual bacteria on surfaces to identify early positive antibody recognition

Coupling high-resolution SPR imaging with classical microscopy

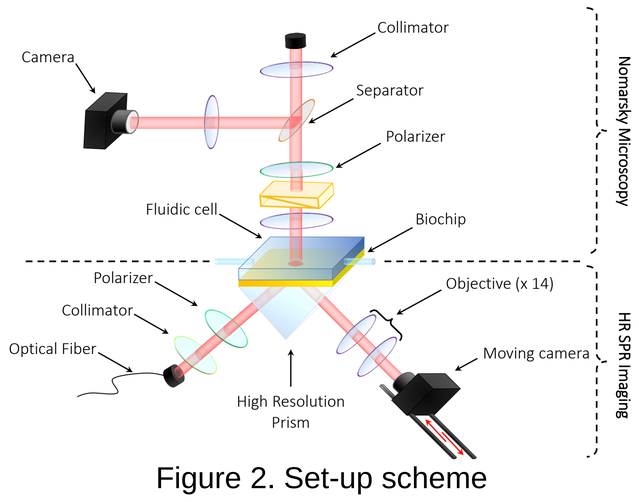
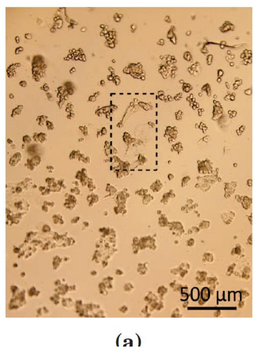
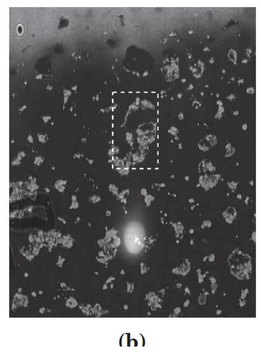


Figure 2. Set-up scheme

To study individual cells by bimodal imaging



(a) Bright field microscopy

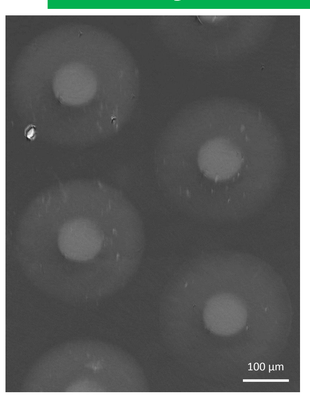


(b) SPR image at 632nm

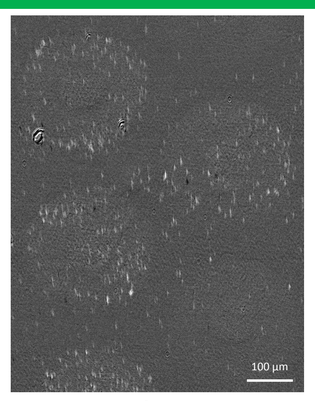
Figure 3. Wide FOV imaging of individual adherent cells [3]

[3] Laplatine et al, 2014, *Optics express*

Early detection of *Listeria spp* using HR SPRI



a.



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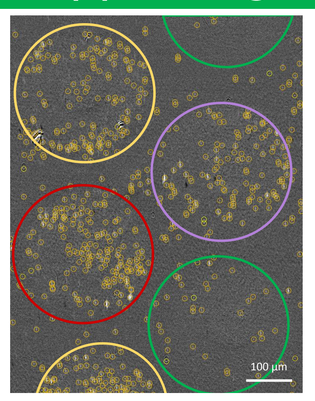


c.

Spot organisation :

Ab 1 : *L.* spp. Ab
Ab 2 : *L. mono.* Ab
Ab 3 : *L. mono.* Ab
Ab 4 : Negative ctrl

Figure 4. Wide FOV SPR imaging at 735 nm of *Listeria monocytogenes* and *Listeria innocua* (injected at 10^4 cfu.mL^{-1}) after 3h incubation into TSB at 37°C
a. Raw mage b. Reference substracted image c. Bacteria events coutning (using *FIJI Trakmate*)

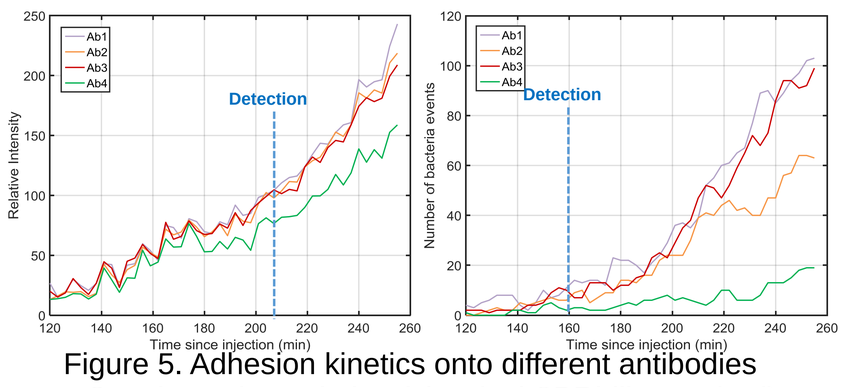
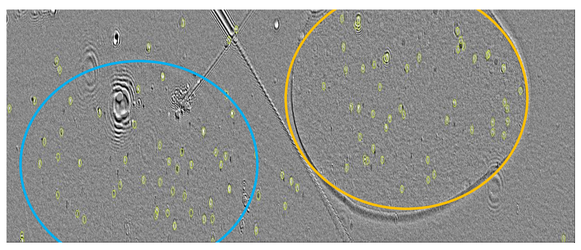


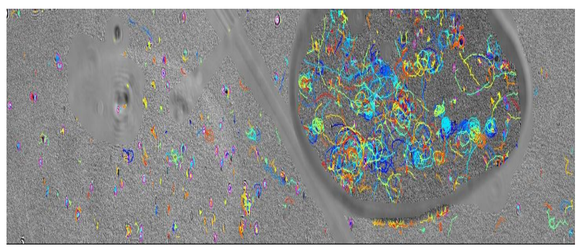
Figure 5. Adhesion kinetics onto different antibodies
a. Spot intensity variation (classical SPRi-like method)
b. Bacteria events counting using high resolution SPRi

Equivalent time of detection : ~ 24h

Study of *E.coli* trajectories using HR SPRI



a.



b.

Spot organisation :
Ab 1 : *E.coli directed Ab*
Ab 2 : *Negative control*

Figure 5. Wide FOV SPR imaging at 735 nm of *E.coli* (injected at 10^5 cfu.mL^{-1})
a. Event detection
b. b. Tarjectory analysis (using FIJI *Trackmate*)

- ✓ Countable individual phenomena
  (resolution of 2µm x 8µm at 735 nm)
- ✓ Specific negative/positive signature
  (Displacement, trajectory radius, …)
- ✓ High sensitivity signal
- ✓ High resolution in a large field of view
  (FOV of ~1x1.5 mm²)

Conclusion and Perspectives

These experiments show both the ability of our system to early detect pathogenic bacteria and to study interaction of individual bacteria with antibody coated surfaces.

Further statistical analysis will help us better understand the first steps of bacterial adhesion and develop a faster detection technique.

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