Bioluminescent whole-cell biosensors for point-of-need applications: new analytical devices exploiting 3D printing technology

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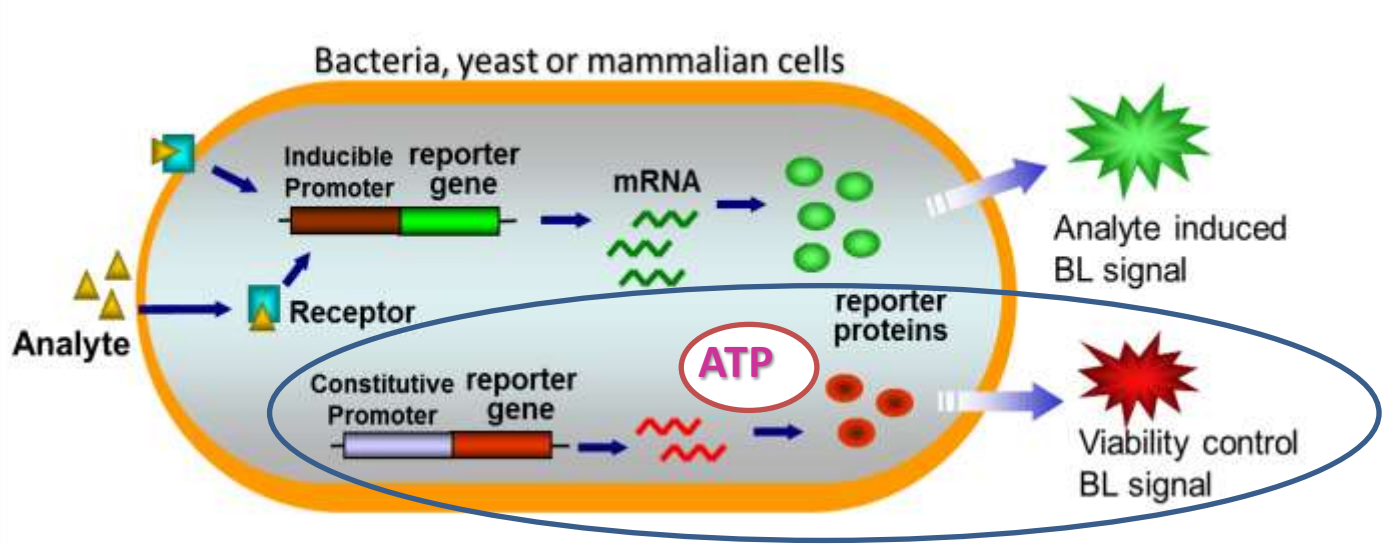
Bioluminescent whole-cell bioreporters



Peculiarities

- **Easy to engineer** to respond to different analytes
- Analyte **bioavailability**
- **Biological activity** of a sample
- **Low cost**, self replicating
- **Multiplexing** capability

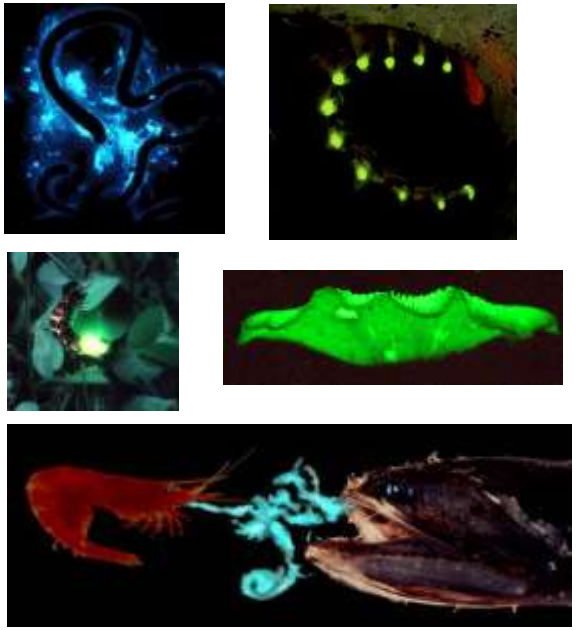
Main issues

- Genetic stability of engineered cells (**low robustness**)
- Long assay and **response time**
- Limited **dynamic range** for quantitative analysis

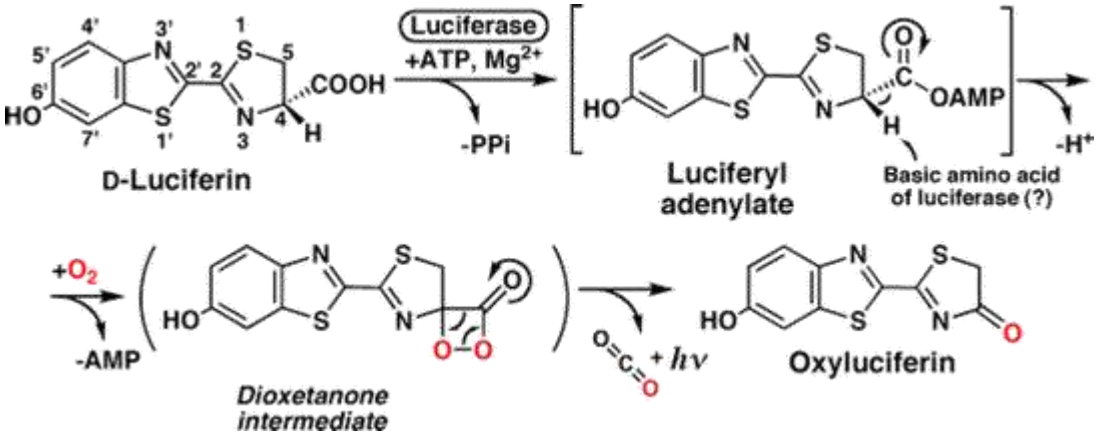
Bioluminescence as detection principle

Bioluminescent reactions:

- high quantum yield emission → **high detectability**
- high specific signal (low background) → **high S/N ratio**
- no excitation light source is required → **simple instrumentation**
- different BL systems & spectral tunable emission → **multiplexing**



e.g. Firefly Luciferase catalized BL reaction



λ_{max}: 560 nm, quantum yield: ~ 0.44

Analytical issue: development of portable BL biosensors

From lab...

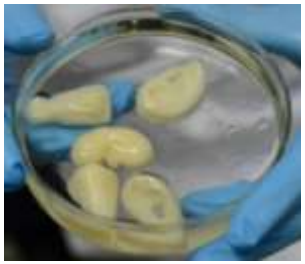
Miniaturization

... to on-site analysis

- Robustness
- Sensitivity
- Response time

- **Bioreporters integration with a detection system**
- **Cell movement/control on chip**

3D Printing Technology

Bioprinting: living tissue, organs


Guns

Bionic medical devices

Is 3D printing the future of everything?

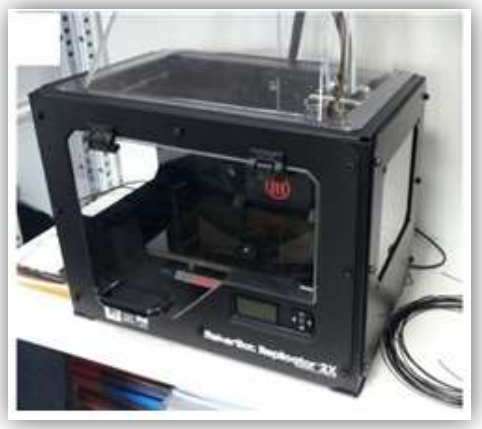
Food

Houses

Space station spare parts

3D-Printing Analytical Devices

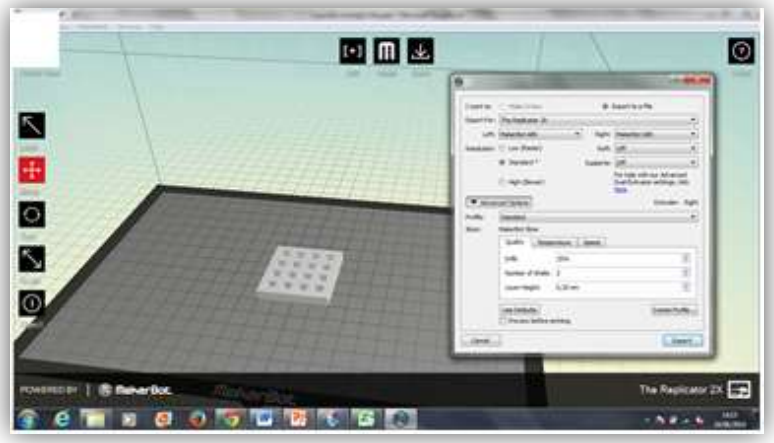
Desktop 3D printer



Makerbot Replicator 2X

- Fused polymer deposition
- Dual extrusion
- Resolution: 100µm

Filament (1.75mm)**:**
-**ABS** (1kg, 25€)
-**PLA**
-**Rubber**



Makerware software

Molds for PDMS casting

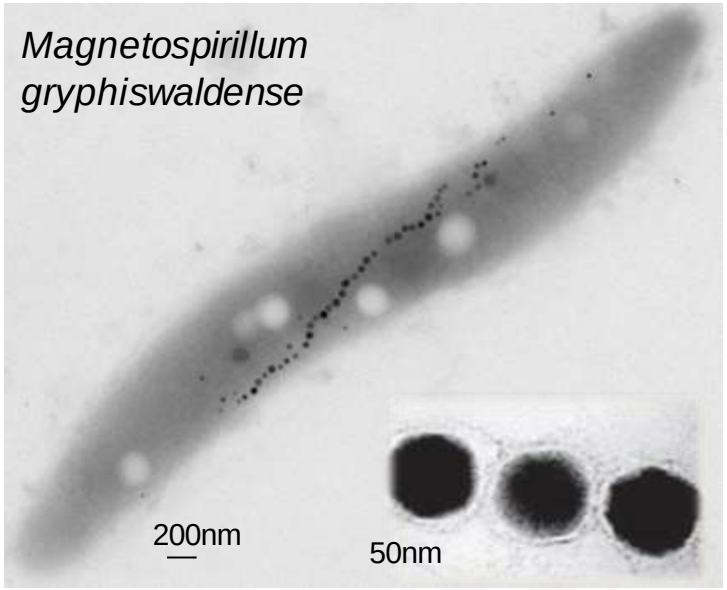
Microfluidic chips

Multiwell cartridges

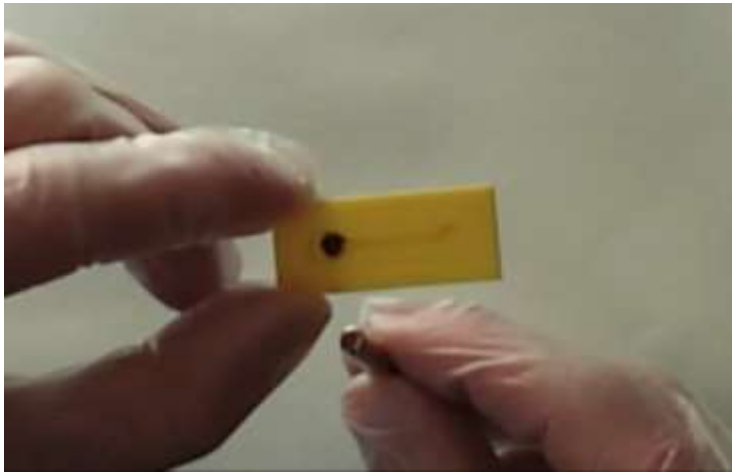
Development of magnetic/BL whole-cell bioreporters

Magnetotactic bacteria (MTB) are microorganisms (0.5x5µm) that can orient along geomagnetic field lines thanks to a **magnetosome** chain.

The magnetosome consists of **magnetite crystal** (Fe_3O_4; 30-50nm) surrounded by **lipid membrane**



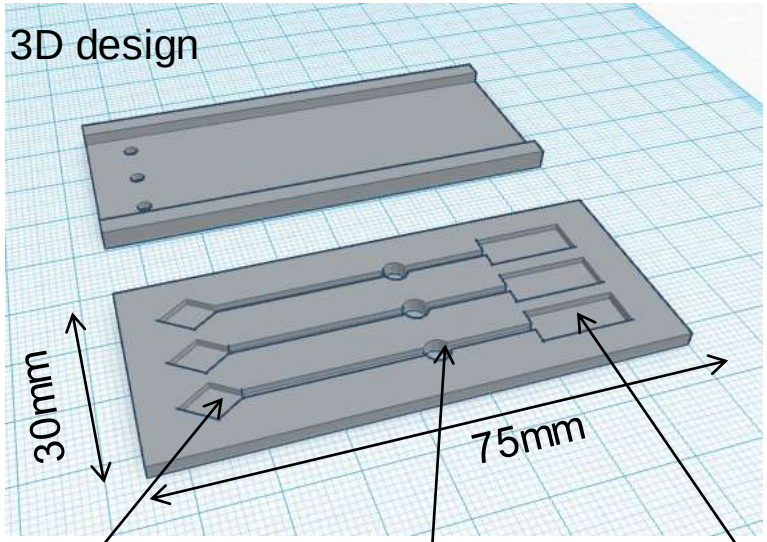
Magnetic control of BL-MTB

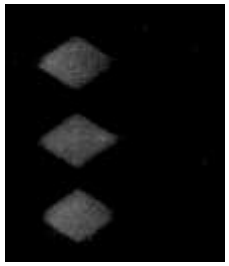


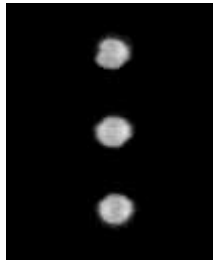
Cells can be easily moved/trapped inside a microfluidic chip by applying external magnetic field

3D-printed Magnetox chip prototype

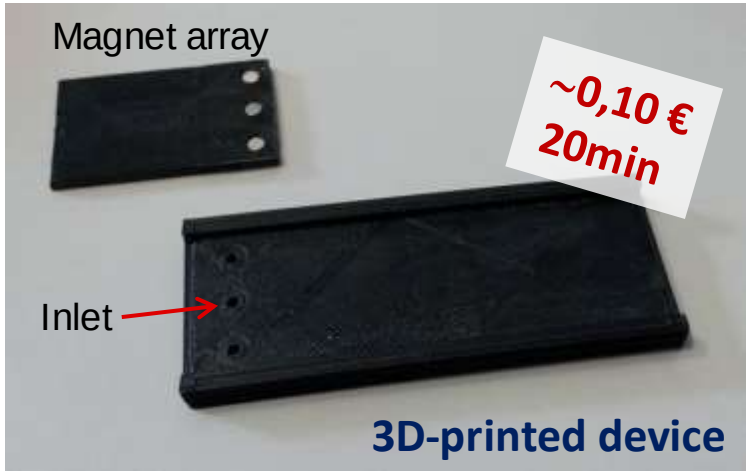
A **3D printed** microfluidic chip is created using the dual-extrusion mode: **black** and **transparent ABS** are fused to create a chip prototype.







BL-MTB can be moved to detection areas thanks to neodymium-iron-boron (NdFeB) magnets



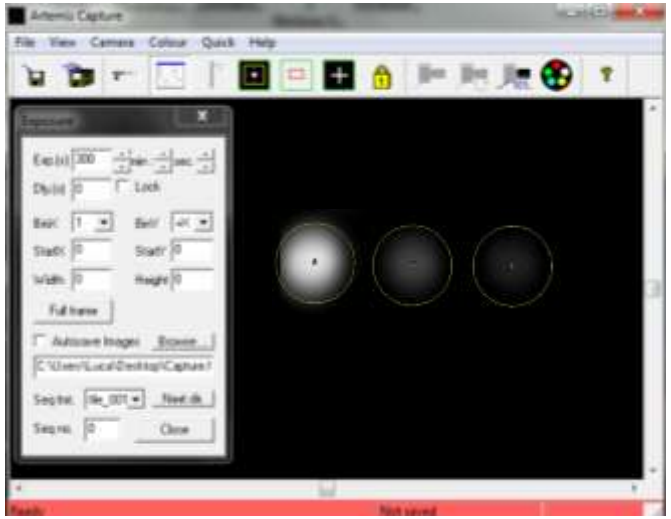
*[Roda A. et. al, **Lab Chip**, 2013]*

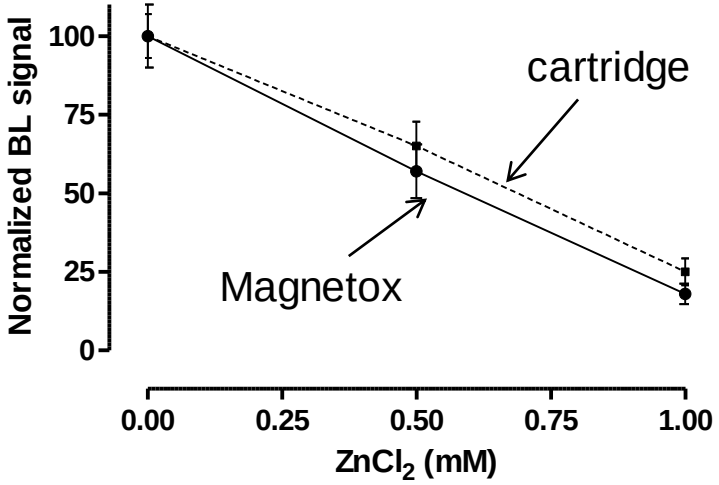
Integration with a portable detection system

ATIK 11000

Sensor Type: CCD - Kodak 11002
Sensor size: 37.25mm x 25.70mm
Resolution: 4008 pixels x 2672 pixels
Pixel Size: 9 µM x 9 µM
Cooling: Peltier with ΔT=-38°C

Magnetox biosensor prototype





Bioluminescence detection with Smartphones

Advantages

-Large diffusion & Connectivity

-Applications for data acquisition/handling

Limitations

-Field of view, macro focusing

-No cooling system

-Short exposure time (few sec)

POOR SENSITIVITY

Samsung Galaxy S2

How to increase sensitivity: new BL reporters

Cloning and mutagenesis of new BL genes

Synthetic luciferases

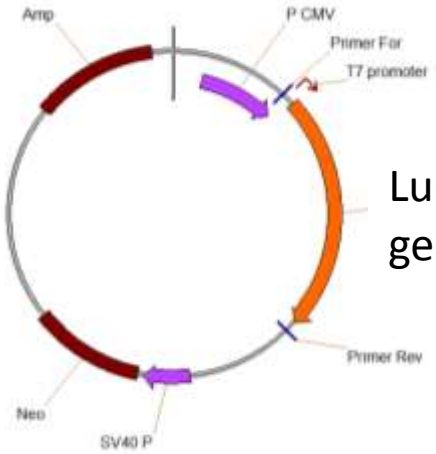
-Different emission
-Increased stability
-pH insensitive emission

Rational design

Luciferase	λmax (nm)	T½ (h, 37°C)
PpyWT	557	0,3
LucGREEN	548	> 5
LucRED	615	> 5

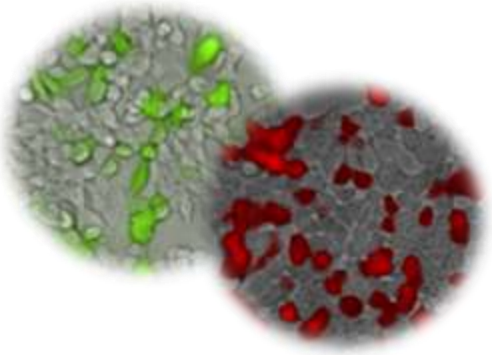
Generation of BL whole-cell bioreporters for smartphone

Constitutive expression vector

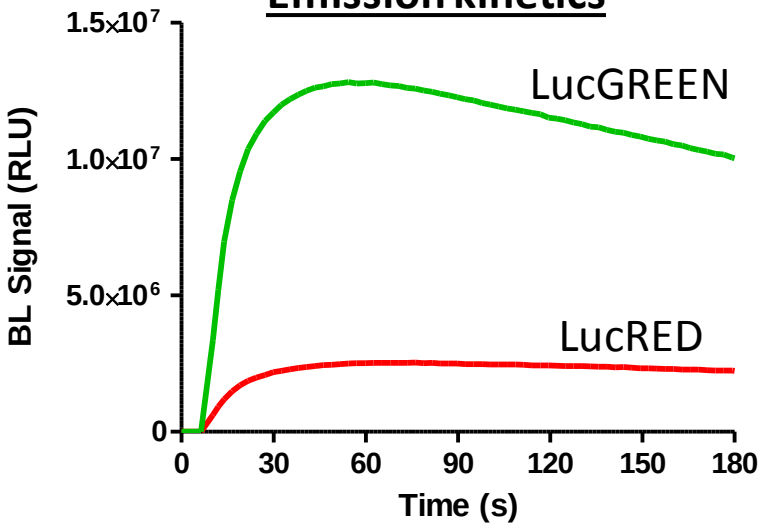


Transient transfections

Hek293T cell line



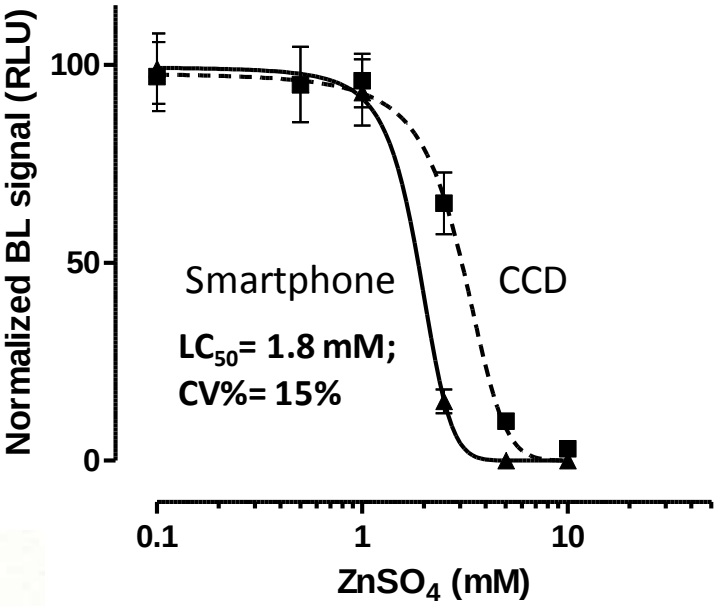
Emission kinetics



Toxicity test with smartphone

Method

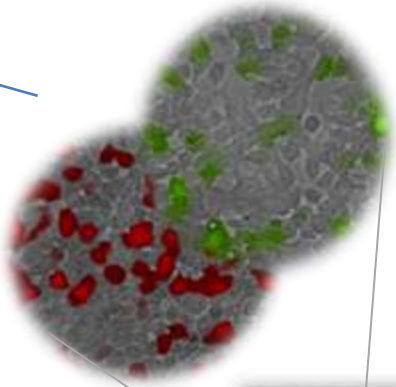
- Cells seeding (20µl) in the multiwell cartridge
- Incubation (30min) with increasing concentration of $ZnSO_4$ (5µl)
- Substrate addition: 10µl D-luciferin
- Signal acquisition: 4s
- Image elaboration : ImageJ
- Toxicity curve



Perspectives

Analytical applications of smartphone-based whole-cell biosensors

Heavy metal detection



Food contamination

Water analysis

Anti-doping test

Endocrine disruptors
Xenobiotics detection