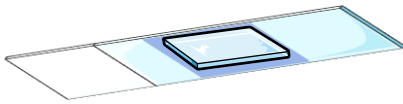


How to immobilize non-adherent cells for single molecule imaging

Single molecule imaging experiments are very sensitive to any movement, and therefore require cells to be immobile. Several techniques, including glass surface coating or cell trapping in microfluidic devices for example, have been developed to image non-adherent cells. However, these methods (i) are often tedious to prepare (ii) increase the fluorescence background (iii) can be toxic to the cells and (iv) are not compatible with STORM imaging. In this context, abbelight developed a commercial kit for cell immobilization: the *Blinking Pad kit*



Scheme of a sample mounted with a Blinking pad

What is a Blinking pad kit?

The blinking pad is a commercial kit developed and provided by abbelight. It:

- Is easy to manipulate
- Maintains the biological objects immobilized on the coverslip
- Induces photoswitching of the fluorophores.

What does the blinking pad kit contain?

- One vial containing a gel
- One green tube
- One red tube
- 10 glass slides with a small chamber
- 1 glass slide without any chamber
- 1 plastic razor blade

How long does it take to prepare a blinking pad?

- Whether you prepare 1 or 10 pads, you'll need about 20 to 25 min

What about the fluorescence background?

- The solutions have been optimized to reduce the fluorescence background as much as possible (see images below)

Is the blinking pad compatible with live cell imaging?

- Two kits are available: one for STORM imaging, compatible with organic fluorophores, and one for SPT and PALM imaging, compatible with photoactivatable and photoconvertible proteins.

Can I use it for other microscopy experiments?

- The blinking pad is compatible with every kind of fluorescence microscopy (confocal, spinning disk, widefield, ...)

In practice

What do I need to immobilize cells ?

- A blinking pad kit
- A heating plate or a microwave
- Tweezers
- Gloves
- Coverslips (1.5H, 22x22 mm)
- Non-adherent cells or structures (eukaryotic cells, yeast cells, bacteria, viruses or giant unilamellar vesicles for example)

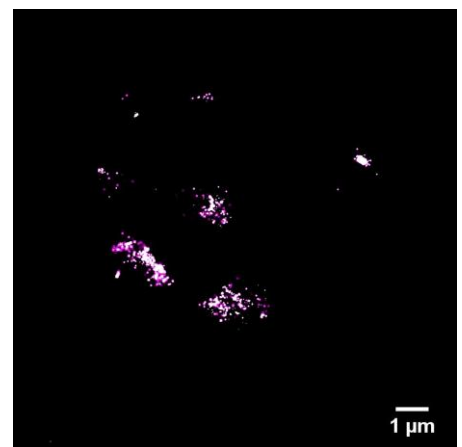
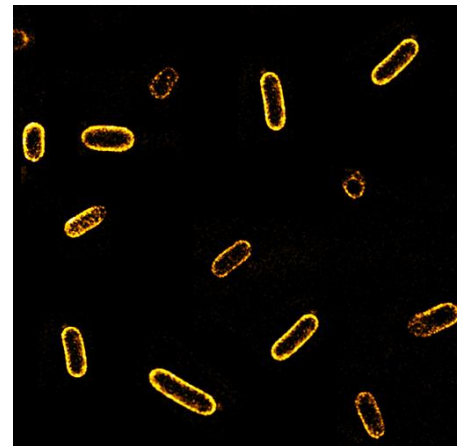


Fig. 1. Single molecule images of *E. coli* bacteria (top) and nuclear proteins in yeast cells (bottom) immobilized with the blinking pad kit