Extra short incubation microfluidic assisted – fluorescence *in situ* hybridization (ESIMA-FISH)

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Fluorescence *in situ* hybridization (FISH) is a powerful technique for evaluating the *HER2* gene status in breast cancer specimen (1). However, most of FISH assays currently used in clinical laboratories are expensive and require long experimental time, up to two days with an overnight incubation (2). Indeed, despite the development of faster FISH probes (*HER2* IQFISH pharmDx™ from DAKO, Denmark) cutting the assay time to one day (3), the cost of these new probes is still high (more than 200$/test) and impedes the dissemination of this technique.

In this study, we present the extra short incubation microfluidic assisted- fluorescence *in situ* hybridization (ESIMA-FISH) technique that uses microfluidics to improve FISH for *HER2* assessment in breast cancer samples. ESIMA-FISH requires a very short incubation time (35 minutes) and uses 4-fold less probe solution per test. The system is based on a microfluidic chip, developed in our laboratory (4), that is clamped against a microscope slide containing a breast cancer tissue specimen (figure 1). A fluorescent DNA probe solution, specific to the target DNA, is then applied to the tissue section within a thin chamber using the microfluidic system. The probe solution used is obtained by diluting 4 times the standard *HER2* IQFISH pharmDx™ probe solution (DAKO, Denmark). Oscillating flows can then be implemented using syringe pumps to improve the delivery of the probe to the tissue. Thanks to this hydrodynamic enhancement of mass transport, the probe-target hybridization efficiency is increased, resulting in overall reductions in the cost and duration of the assay.

To validate the ESIMA-FISH technique, several tissue specimens were blindly tested with ESIMA-FISH and standard IQFISH. The results from these two techniques were comparable (figure 2, 3), supporting the possibility of a future clinical use of ESIMA-FISH.

References

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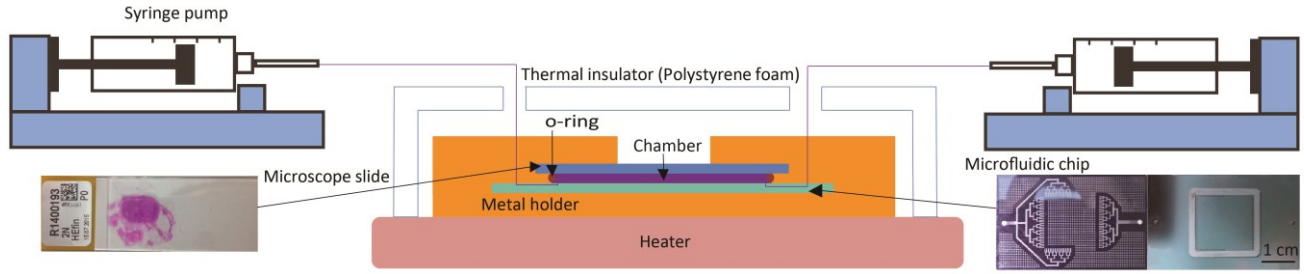

Figure 1. Illustration of the ESIMA-FISH microfluidic setup. The syringes are connected to automatic pumps and create flows inside the reaction chamber that is formed by clamping the microscope slide to the microfluidic chip.

A *B*

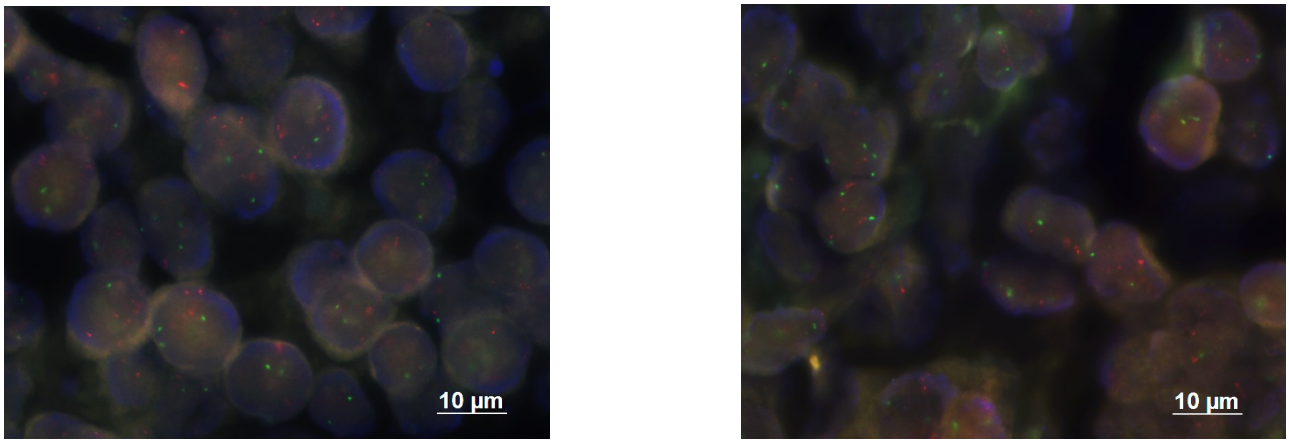

Figure 2. Fluorescent images of two biopsy tissue slides from the same HER2-positive patient implemented with the (A) ESIMA-FISH and (B) standard IQFISH protocols. For the sake of clarity, the contrasts of the two micrographs were modified.

		Standard FISH		
		Negative	Equivocal	Positive
ESIMA-FISH	Negative	3	1	0
	Equivocal	0	2	0
	Positive	0	0	3

Figure 3. Comparison of HER2 status assessment results of the ESIMA-FISH and the standard IQFISH techniques, showing a good agreement between these methods. The results were obtained from 9 tissue blocks using a blind counting of FISH signals (number of HER2 gene and the corresponding centromere per cell).