



High-throughput virtual slide scanning of fluorescence in situ hybridization (FISH)

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Abstract

Introduction: Fluorescence in situ hybridization (FISH) is currently considered to be the most specific and sensitive method for the assessment of HER-2 oncogene amplification in breast cancer. However, darkfield microscopy of FISH is tedious, and archiving of FISH specimens is not possible due to fading of the fluorescence signals. To date multicolour digital image capture of FISH has been used only to generate illustrations to scientific articles. Several automated dot counting software applications have been developed, but their use has been limited due to the difficulty to scan large enough tissue areas at high magnification.

Methods/Results: To overcome these limitations we have developed an effective virtual slide scanning system for breast tumor slides, which were hybridized using the Vysis triple colour FISH probe (HER-2 in green, TOP2A in red, CEP17 in light blue + DAPI counterstain). The scanning system is based on a fully motorized epifluorescence microscope, which is controlled with a PC equipped with OASIS controller card and Surveyor software (Objective Imaging, Cambridge, UK). Each fluorochrome is scanned at six different focal depths. The scanning software (Surveyor) constructs an extended focus image on the fly. This ensures that the gene dots present at different focal depths are properly visualised. Scanning of 10x10 fields using oil-63X objective is fast and takes less than 5 min. Image tiles of each fluorochrome scan are automatically converted into a montage. The montages are stored as a single multilayered JPEG2000 file. Viewing of FISH scans is done using JVSview software, which allows free panning and zooming of each fluorochrome layer individually, or viewing of the layer colours merged with each other. Gene dot counting can be done by transferring screenshots to ImageJ to be automatically analyzed by visual counting (click and count method), or by using a macro script enabling fully automated copy number assessment (see Konsti J et al. J Clin Pathol 2008).

Conclusion: This study shows that virtual slide scanning of multicolour FISH slides is feasible and yields comparable results to visual assessment done via microscope oculars. Virtual slide scanning provides much needed possibilities to archive the diagnostically important slides, and to perform copy number counting off-line on computer screen. The problem of fading fluorescence with FISH specimens can be avoided, and any part of a sample can be re-evaluated for gene copy number if needed.