DEVELOPMENT AND APPLICATION OF A 12-COLOUR MULTIPLEX FISH ASSAY FOR SUBTELOMERIC CHROMOSOME REARRANGEMENTS IN LEUKAEMIA. L. Kearney*, K. Saracoglu*, S.J.L. Knight*, S.J.A. Lucas*, M.A. Schulze*, R. Eils*, J. Brown*. (Intr. by S. Watt) MRC Molecular Haematology Unit, Institute of Molecular Medicine, Oxford, UK; Interdisciplinary Center of Scientific Computing, University of Heidelberg, Germany; Perceptive Scientific Instruments Inc, League City, Texas; Steinbeis-Transfer Center *Analytical methods in molecular cytogenetics,* Heidelberg, Germany.

Up to 50% of bone marrow karyotypes in AML are normal by conventional (G-banded) cytogenetic analysis. However, it is believed that a proportion of these may harbour cryptic translocations, particularly involving telomeric regions. Translocations involving telomeric regions are particularly difficult to identify by G-banding, as these regions are usually pale staining. The new multicolour karyotyping techniques, multiplex FISH (M-FISH) and spectral karyotyping (SKY), provide one approach to the identification of cryptic chromosome rearrangements in leukaemia. However, the resolution of these techniques for detecting subtle translocations is unknown. To provide a more sensitive approach to the identification of hidden chromosome rearrangements in apparently normal leukaemic karyotypes, we have developed a 12-colour M-FISH assay for subtelomeric rearrangements, which we have termed the multiplex telomere (M-TEL) assay. This uses a set of 41 chromosome-specific cosmid, PAC and P1 clones, all confirmed as within 500 kb of their respective chromosome end. Probes were combinatorially labelled using four fluorochromes, with both the short (p) and long (q) arms of each chromosome having the same labelling combination. This allows the identification of 12 pairs of chromosomes in one hybridization, with a full survey of all telomeric regions possible in only two hybridizations. Analysis of the results was performed using two innovative software programmes. The first used an automated karyotyping facility with the ability to manually review each of the fluorochrome channels in karyotype format. The second method used a region-oriented spectral imaging approach, with automated colour classification of the telomeric regions based on their unique fluorochrome combination. Firstly, we validated the M-TEL assay on a series of 8 patients with known telomeric rearrangements. In all cases, the rearrangement was correctly identified. Thereafter, we used the M-TEL assay to analyze 15 apparently normal karyotypes from a series of AML patients at diagnosis. No cryptic rearrangements were identified. The outcome of screening a much larger series of normal AML karyotypes is expected to address the question of how frequently cryptic translocations occur, and to identify new regions implicated in leukaemogenesis.