SINGLE CELL ARRAYS FOR SENSITIVE KARYOTYPE ANALYSIS OF SMALL CELL AND TISSUE SAMPLES

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Abstract

Our work addresses the sensitive detection of chromosomal changes such as small translocations, rearrangements or genomic imbalances in apparently normal individuals, benign neoplasia, premalignant lesions and cancer. The vast majority of our study specimens are frozen or fixed archival tissues. Current techniques for full karyotype analysis of individual cells require metaphase cells; cells in interphase or non-viable cells can not be analyzed. The objective of this research project is the development of technologies to support the cytogenetic analysis of small amounts of fresh, fixed or archival tissues regardless of the cells' proliferative stage. A highly sensitive, fluorescence in situ hybridization (FISH)-based technology platform termed 'Single Cell Arrays (SCAs)' will allow the detection of small rearrange-ments in interphase and metaphase cells by combining the high-resolution DNA in situ analysis with sensitivity in the kb range. This will be achieved by immobilizing cell nuclei on glass slides and controlled stretching of chromatin in specially design micro-chambers followed by cytogenetic analysis using FISH. The aims of this NIH/NCI-funded feasibility study are 1. to demonstrate the feasibility that interphase cell nuclei can be immobilized in defined pattern and reproducibly extended for subsequent cytogenetic analysis and 2. to develop an optimized assay for the sensitive, high-resolution cytogenetic analysis of SCAs. The deliverable of this effort is a protocol for FISH-based multi-locus cytogenetic analysis of SCAs to provide near kilobase sensitivity for the detection of single copy nucleic acids with a resolution in the order of 10-20 kb, while minimizing the overall loss of DNA. While SCAs may become powerful tools in basic and clinical research, slight protocol changes may allow them to find widespread applications in the assessment of biospecimen quality.

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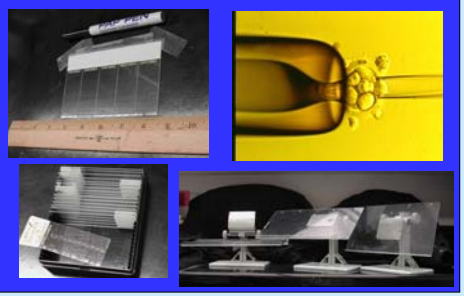
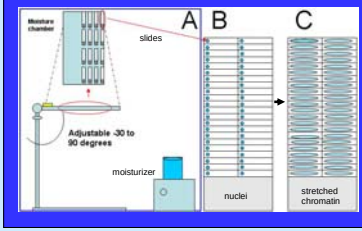
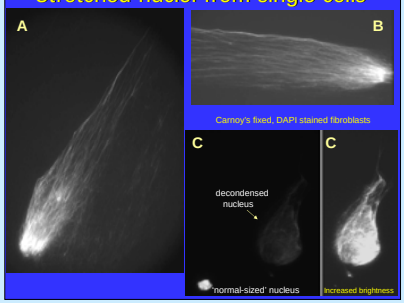
Preparation of Single Cell Arrays



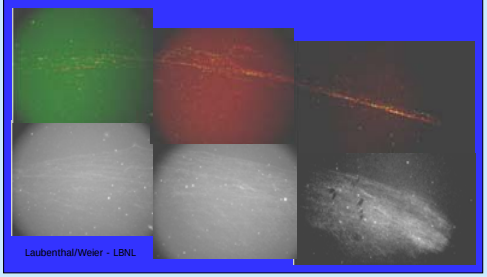
Simple stretching of nuclei after swelling in low salt buffer + detergent



Stretched nuclei from single cells

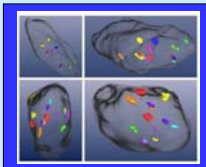


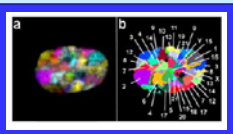
EGFR Amplification in MDA-MB-468



Problem - Solution

Spatial overlap limits multi-target FISH analysis of interphase cells

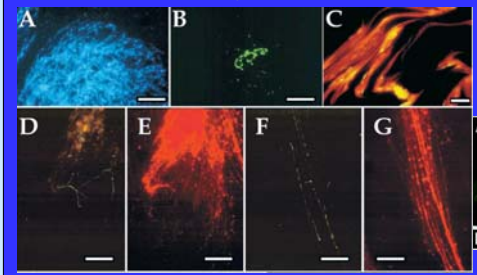




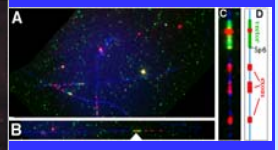
[Image credit: M. Speicher, M.Cremer http://www.science.ngfn/de8_52.htm]

From chromosomes to DNA molecules

Solution: Decondensation and spread of nuclear DNA

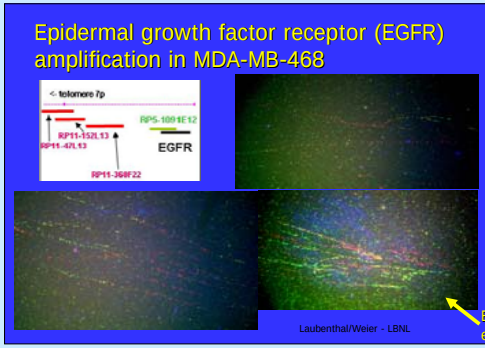


From interphase nuclei to decondensed chromatin and DNA fibers
(FISH signal are shown in green, DAPI or EB counterstain in A,C,E,G)



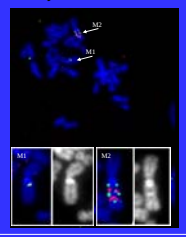
Example for high resolution and sensitivity: Hybridization of a cDNA probe to stretched BAC DNA molecules reveals the genomic organization of the gene

Epidermal growth factor receptor (EGFR) amplification in MDA-MB-468



Background due to RNA from highly expressed sequences

Application: Characterization of complex marker chromosomes in post-Chernobyl childhood thyroid cancers



Summary

1. Individual cells can be arrayed on glass slides inside specially designed micro-channels,
2. cells can be treated physico-chemically to release chromatin,
3. the entire chromatin can be stretched in a linear fashion,
4. the extent of stretching (from a few microns to 10-12 mm) can be adjusted by controlling the stretching force and environmental parameters,
5. stretched chromatin can be analyzed by FISH providing a resolution of up to 5-15 kb,
6. the method is suitable to address tumor heterogeneity by preparing chromatin arrays of 12-32 single cell spreads per slide, and
7. the method works well with fresh, frozen or fixed cells as starting material.