Nordic collaboration within biological dosimetry
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Mass casualty scenarios and biosimetry
- large number of exposed individuals with wide range of doses
- rapid and reliable dose assessment required
- physical dosimetry and clinical analysis (blood cell counts) may not give sufficient support for medical decision making
- essential to identify individuals with no or low exposure
- capacity of small biosimetry laboratories exceeds easily: collaboration and networking are key issues

Classic chromosome aberration assay
- based on dicentric chromosomes observed in blood lymphocytes
- sensitive: 100-200 mGy (low-LET); 10-20 mGy (high LET), with
- demanding, analysis requires excessive training
- upper dose limit 6-7 Gy
- dependent on mitogen sensitive cells (T-lymphocytes) reaching mitosis (high doses may block cell cycle)

NKS-project 2006-2007: Biodosimetry application in emergency preparedness (BIODOS)
- prematurely condensed chromosome (PCC) assay

Advantage of PCC with respect to dicentric assay
- scoring of radiation-induced damage in pre-mitotic cell cycle stages
- ability to assess very high doses
- potential for more rapid scoring

Induction of prematurely condensed chromosomes (PCC)
- original procedure: fusion of interphase lymphocytes and mitotic Chinese hamster ovary cells; mitotic factors induce the nucleus to condense into chromosomes within 1-2 hours
- relatively low yield of PCC, inconsistent assay
- more recently, chemically (okadaic acid and calyculin A) induced chromosome condensation of stimulated cells; requires 48 h culture
- increased yield of PCC
- induction of PCC in unstimulated cells within hours facilitated by cyclin B kinase and calyculin A / okadaic acid
- inadequate condensation
- the need for systematic evaluation of the different assays

PCC induction and analysis methods
Okadaic acid and Calyculin A
- Giemsa-staining
  - excess fragments
  - ring chromosomes
- Fluorescence in situ hybridization (FISH) with chromosome probes
  - exchange type aberrations
Protein kinase/Cyclin B + OA or CalA
- FISH
  - evaluation of painted chromosome “areas”
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- **Okadaic acid induced PCC in cells in different cell cycle stages**
- **Counting of excess fragments (>46 elements seen)**
- **Chromosome aberration analysis in FISH painted stimulated cells**
- **Okadaic acid treatment in stimulated cells, analysis of rings**

**Curve fitting of the PCC ring data**

- The data fit Poisson distribution.
- Data best described by linear relationship:
  \[ Y = C + \alpha \cdot D \]
- where:
  \[ Y = \text{freq. of PCC rings} \]
  \[ C = 0.002 (\pm 0.002) \]
  \[ \alpha = 0.049 (\pm 0.006) \]
  \[ D = \text{dose (Gy)} \]

- **Triage test with 3 doses; error bars represent 95% confidence intervals**
  - Dicentrics scoring: 50 metaphases
  - PCC ring scoring: 300 PCC cells
  - STUK1, STUK2, STUK3, NRPA

- **Given dose**
  - 2.5 Gy
  - 7.5 Gy
  - 10 Gy
Conclusions of BIODOS

- Okadaic acid treatment of lymphocyte cultures
- Evaluation of ring chromosomes
- Linear fit of data 0 - 20 Gy
- The PCC assay may be most applicable at doses above 5 Gy
- For emergency preparedness applications, the dicentric assay and PCC assay cultures could be run in parallel and evaluated in triage mode
- PCC ring assay requires less training than the classical dicentric assay
- Essential to maintain the analysis routine by arranging and participating in intra- and intercomparisons

Mass casualty exercise (BIOPEX) NKS 2008
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- Main aim is to evaluate the applicability of the PCC ring assay in comparison to the dicentric assay
- Simulated triage exercise involving a large number of exposed casualties = 60 blood samples
- \textit{In vitro} exposure with $^{60}\text{Co}$
  - wide range of doses, including non-uniform exposures
- Parallel cultures for both PCC ring and dicentric assays
- Dose estimation will be performed using the PCC ring curve and the routinely used dicentric curve