

# X-ray Scattering of DNA During Agarose-gel Electrophoresis

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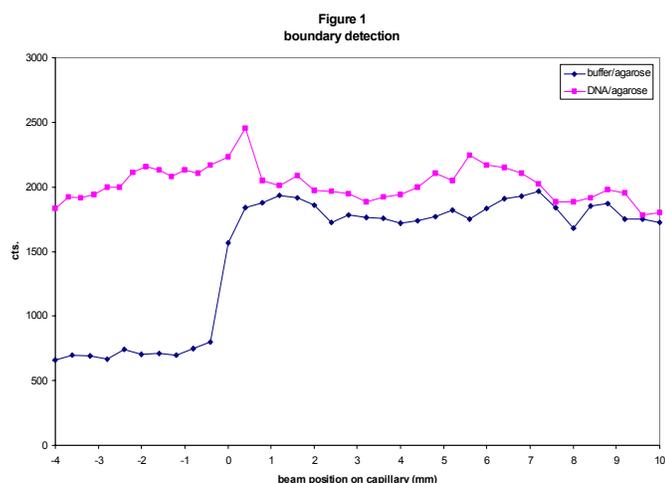
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Beamline: X16C

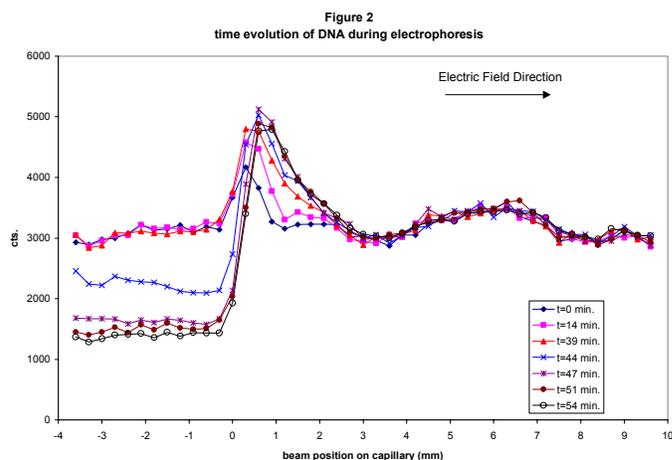
**Introduction:** Electrophoresis is the most commonly used technique for determining base-pair sequence in samples of DNA. In this experiment we attempted to observe the migration of groups of DNA molecules during agarose-gel electrophoresis by SAXS.

**Methods and Materials:** Electrophoresis was conducted using agarose-gel prepared in a 1.5mm diameter x-ray capillary. The DNA samples we studied are commercially available (Sigma) "ladder" standards, the typical size being 1000 base-pairs. We used a tris-borate EDTA running buffer consisting of 13mM tris, 4.5 mM boric acid, 0.25 mM EDTA. The DNA was loaded into one end of the capillary, corresponding to left-hand side of figures 1 and 2. An electric field was applied in the direction corresponding to the positive x-direction in figures 1 and 2. We performed SAXS at consecutive points along the capillary by translating the sample stage in the direction perpendicular to the beam. The measurements were performed during the electrophoretic process.

**Results:** Figure 1 indicates the position of the buffer-agarose interface, as well as the relative scattered intensities of buffer, agarose and DNA solution. As figure 2 indicates, we observed a monotonic decrease with time of scattering intensity in the DNA loading region of the capillary, suggesting that the DNA traveled into the gel. Correspondingly, the scattering maximum near the solution-gel interface moved in the direction of the field with time, from which the DNA mobility is estimated to be on the order of  $10^{-8}$  m<sup>2</sup>/V/s.



**Figure 1.** The buffer/agarose curve indicates the gel boundary. The DNA/agarose curve indicates that the DNA solution scatters much more strongly than buffer alone.



**Figure 2.** The scattered intensity decreases with time, suggesting that the DNA is leaving the loading region (left). The motion of the peak near the solution-gel interface indicates that the DNA is moving in the direction of the electric field.