of other SHOM applications have been tested as intermediate steps in the SHOM research.

1. Sequence analysis and sequencing

A number of technical problems should be solved for de novo sequencing although they are much less stringent for comparative sequence analysis than for de novo sequencing. Among these:

a) Reliable discrimination of perfect and mismatched duplexes. We have significantly improved the discrimination by decreasing the length of hybridized oligonucleotides to 6-and 8-mers (1, 7) and by using 5-mers in "contiguous stacking" hybridization (1,2). Essential improvement was also achieved by automatic measuring of the melting curves for duplexes formed in each microchip element and calculating their thermodynamic parameters, free energy, enthalpy and entrophy for different regions of the melting curves and by comparing them with these parameters for perfect duplexes. In addition, a highly reliable discrimination was achieved by using two-color fluorescence microscopy and by quantitative comparison of the hybridization pattern of a known DNA or synthetic oligonucleotides and DNA under study labeled with different fluorophores (8).

b) Difference in hybridization efficiency depends on the GC-content and the length of the duplex. We have equalized the efficiency by choosing proper concentration for the immobilized oligonucleotide (6,7) and also by increasing the effective length of immobilized oligonucleotides by adding at one or both their ends 5-nitroindole as a universal base or a mixture of four bases (2).

c) Interference of hairpins and other structures in DNA with less stable duplexes formed upon the DNA hybridization with comparatively short immobilized oligonucleotides of the microchip. This interference was decreased by fragmentation of the analysed sample of DNA and RNA in the course of incorporation of a fluorescence label (4). We have also tested incorporation by a chemical bond of an intercalator into immobilized oligonucleotides that stabilized its base paring with DNA over hairpin formation (10).

d) Necessity to increase the microchip complexity for sequencing long DNA stretches. As an alternative, further development of so-called contiguous stacking hybridization was shown to improve the efficiency of 8-mer microchip up to that of 13-mer microchip so that DNA of several kilobases in length could be sequenced by SHOM (2).

e) 6-mer microchips for sequencing and sequence analysis. We have now come to the stage of manufacturing microchips containing 4,096 (i.e. all possible) 6-mers. The control tests partly described above have shown that these microchips can be effectively used for sequence analysis, mutation diagnostics and detection of sequencing mistakes by conventional gel-sequencing methods. We hope that after demonstrating the efficiency of 6-mer microchips, we shall be able to get sufficient financial support for production of the microchip with all 65,536 8-mers.

2. Mutation diagnostics and gene polymorphism analysis

The improvements described above have been introduced for reliable ("Yes" or "No" mode) identification of single-base changes in human genomic DNA. The efficiency of SHOM has been demonstrated for identification of a number of b-thalassemia mutations (1,2,8) and HLA allele variations in the human genome.

3. Identification of microorganisms and gene expression monitoring

Bacterial microchips have been manufactured and tested. Their ability for reliable identification of a number of bacterial strains in the sample has been demonstrated (6). The chips containing oligonucleotides complementary to specific regions of 16S ribosomal RNA were hybridized with samples of rRNA, total RNA, DNA and RNA transcripts of PCR-amplified genomic rDNA. Similar preliminary experiments demonstrated the efficiency of SHOM for monitoring the gene expression.

III. Development of new approaches based on the SHOM technology

1. Enzymatic modification of nucleic acids on selected elements of the oligonucleotide chip. The gel pads of the oligonucleotide chip are separated from each other by hydrophobic glass surface. It prevents the cross-talking of the chip elements when a drop of solution is applied on specified elements. At the same time, a high porosity of the gel allows diffusion of large proteins into the gel. We have demonstrated that immobilized oligonucleotides can be enzymatically phosphorylated and ligated with contiguously stacked 5-mer after hybridization with DNA. A walking sequencing procedure by stacked pentanucleotides was proposed that is based on enzymatic ligation and phosphorylation on oligonucleotides chips (9).

2. DNA fractionation on oligonucleotide chips. Due to the same properties, the oligonucleotide chips are used for fractionation of DNA after DNA hybridization with some complementary oligonucleotides of the chip. A new procedure for sequencing long DNA pieces was proposed that is based on fractionation of DNA on fractionating oligonucleotide chips followed by sequencing of the isolated DNA by SHOM on sequencing microchips. The procedure allows the investigator to skip cloning and mapping of long DNA pieces (9).

Conclusions

It appears that the major technical problems of SHOM have been in most part solved, and this technology can al-