Effects of stray light on the fidelity of photodirected oligonucleotide array synthesis

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ABSTRACT

Fabrication of high density oligonucleotide arrays using metal on glass photolithographic masks is inflexible and expensive. Maskless methods using computer-controlled projection have been proposed and implemented, but associated stray light effects on photodirected oligonucleotide synthesis and their analysis have not been reported. We have developed a theoretical approach: it predicts that the stray light content of the output of digital micro-mirror devices and other spatial light modulators of similar performance (contrast ratio ~400) will cause extensive random base insertions. For example, use of a digital micromirror device for synthesis of a 20mer array will result in the majority of oligonucleotide chains being 21mers or 22mers. This chain lengthening effect of stray light would not be preventable when synthesis involves a directly photosensitive 5'-blocking group. If the 5'-blocking group is acid labile and released with photogenerated acid, the presence of low concentrations of weak base will prevent the effect of stray light. We have demonstrated experimentally the anticipated chain lengthening effect of stray light on photoacid-dependent synthesis of oligonucleotides and prevention of the effect by low concentrations of n-octylamine. The application of these findings should facilitate the development of maskless fabrication and availability of high density and high fidelity user-designed arrays for research applications.

INTRODUCTION

Photodirected synthesis of high density oligonucleotide arrays as described by Pease et al. (1) and industrialised by Affymetrix Inc. (www.Affymetrix.com) uses photolithographic masks of metal on glass or quartz to determine the pattern of illumination and hence the sequence of oligonucleotides at each array element. Fabrication and inflexibility of the masks are important contributors to high manufacturing costs, particularly for limited runs, as would be the case for many specific research needs. On the other hand, the contrast ratio of the masks is high (105) ensuring that during the illumination stages of photodirected synthesis those array elements not scheduled for illumination remain in the dark.

Garner (2), Singh-Gasson and colleagues (3,4), LeProust et al. (5) and Seo et al. (6) have described the use of computer-controlled projectors for determining the pattern of illumination. The patterns are generated in software and transferred as needed to a digital micromirror device (DMD). Physical masks are not required. Fodor et al. (7) suggested the use of liquid crystal displays (LCDs) as spatial light modulators and Quate and Stern (8) proposed the use of DMDs and also grating light valves. Neither report described implementation. The contrast ratio for DMDs given by the manufacturer is ~400 (9). Lower values apply to LCDs and grating light valves. None of these reports on the prospect or implementation of maskless photodirected synthesis of oligonucleotide arrays considered the possibility that stray light might cause significant discrepancies between the designed sequences and those synthesised.

We have made an analysis of the predicted effects of stray light on the fidelity of photodirected oligonucleotide array synthesis when physical masks of higher contrast ratio (105) are replaced with devices of lower contrast ratio (<103). We have also observed experimentally the effects of stray light on the fidelity of photodirected oligonucleotide synthesis: they can be considerable.

MATERIALS AND METHODS

Simulation studies

All programs for modelling the effects of stray light on photodirected oligonucleotide synthesis were written in HTBasic version 8.2 for Microsoft Windows (Transera Corp., Sarem, UT).

Synthesis of photoacid generator

The trichloroacetate ester (‘the ester’) of α-phenyl-4,5-dimethoxy-2-nitrobenzyl alcohol was synthesised as described elsewhere (P.J.Serafinowski and P.B.Garland, submitted for publication). The ester is photolysed by UV light to yield trichloroacetic acid and 4,5-dimethoxy-2-nitrosobenzophenone, with a quantum yield at 365 nm of 0.14 in dichloromethane. The ester has an $E_{\text{mM}}$ at 365 nm of 3.5 mM$^{-1}$ cm$^{-1}$.
Measurements of rate constants for acid-induced 5'-detritylation

Particles of controlled porosity glass (CPG; pore size 500 Å) with 5'-O-dimethoxytritylthymidine (DMTr-T, 50 μmol g dry wt\textsuperscript{-1}) attached through its 3'-OH by a long chain alkylamino linker were from Chem Genes Corp. (Ashland, MA). Approximately 30 mg of particles were first freed of any unbound DMTr species by suspension in 14 ml of dichloromethane and recovery by centrifugation, repeating the process twice. The particles were then resuspended in dichloromethane. Washed DMTr-T-CPG particles were suspended in 3.0 ml of dichloromethane in a 1 cm light path cuvette to give 5–10 μM DMTr-T. The suspension was continuously stirred with a magnetic bar and a VarioMag mini stirrer. The time course of detritylation following the addition of acid was measured spectrophotometrically at room temperature (22–25°C) with a Beckman DU-7 instrument, utilising the strong absorption band of the DMTr\textsuperscript{+} cation at 494 nm (10). Acid was added in at least 10-fold excess over DMTr-T. Recordings at any one acid concentration were made for sufficient time (1–4 min) to collect adequate data. The experiment was then terminated by adding trichloroactic acid to a final concentration of ~20 mM, to obtain the absorption reading at the full extent of detritylation.

The initial detritylation velocities, expressed in fractional yields of DMTr\textsuperscript{+} cation released from the DMTr-T-CPG precursor, were calculated from the spectrophotometer recordings. For a first order reaction each of these initial velocities (in fractional yield units per second) is equal to the rate constant for a given acid concentration. In addition semi-logarithmic graphs of time-dependent, acid-induced detritylation were made for the higher concentrations of acid (>2 mM), where the full time course of the reaction could be conveniently observed.

Measurements of rate constants for photolysis of the ester

A well stirred solution of ~50–100 μM ester solution in dichloromethane (3.0 ml) in a 1 cm light path cuvette was exposed to varying durations of 365 nm light isolated by filters from a 100 W Hg arc lamp assembly (Photon Technology Inc.). The 350–450 nm absorption spectrum of the solution was measured after each exposure. The absorption reading of interest is at 380 nm, corresponding to the main peak in the difference spectrum (\(E_{\text{abs}} = 6.5 \text{ M}^{-1} \text{ cm}^{-1}\)) of the nitroso photoproduct minus the ester. Experiments were done over a range of average light intensities incident on the cuvette surface from 0.1 to 35 mW cm\textsuperscript{-2}. Sets of 7–10 time-dependent spectra were collected for each illumination intensity, except that the last of each set was after exposure of the cuvette to the full intensity (35 mW cm\textsuperscript{-2}) for 2 min, to record the maximum extent (equated with 100% photolysis) of spectrophotometric change.

The fractional yields of photoproduct were calculated from the spectra and used to construct plots of \(\log_{10}(1 - \text{yield})\) against time. These plots were linear and their slopes were used to calculate the first order rate constant at each light intensity.

Photodirected synthesis of oligonucleotides

In this method the conventional addition of trichloroactic acid to effect 5'-detritylation is replaced by photogeneration of the acid from a photosensitive precursor. All other steps of conventional solid phase phosphoramidite-based synthesis (11,12), including capping of uncoupled 5'-OH groups with acetic anhydride, were unchanged. Stepwise yields for the two methods using either photogenerated or added trichloroactic acid are indistinguishable (P.J.Serafinowski and P.B.Garland, submitted for publication).

We modified a Millipore Expedite Model 8909 DNA synthesiser for use in semi-automated protocols of photodirected oligonucleotide synthesis at the 0.5 μmol level on CPG in flow columns. Full details have been submitted for publication elsewhere, but briefly the essential modifications were (i) conversion of the exterior of the flow column to a simple cylindrical surface by removal of flanges, (ii) addition of a small optical assembly constructed from microbench components holding filters and lenses to isolate and then focus the 365 nm emission of a mercury arc lamp on the flow column and (iii) coupling of the optical assembly to a shuttered 100 W Hg arc lamp with a flexible liquid light guide. The optical assembly was also equipped with a 1% transmission neutral density filter that could be rotated in or out of the light path. All optical components were from Linos Photonics Ltd.

The high absorption at 365 nm of both the ester and its photoproduc causes severe inner filter effects within the 3 mm internal diameter flow cell. We therefore devised a protocol in which the detritylation step consisted of a 30 min period of 365 nm illumination during which ~4.2 ml of 22 mM ester solution in dichloromethane was flowed through the flow column. The pulsatile nature of the flow (10 pulses min\textsuperscript{-1}) provided some mixing of the flow cell contents. According to the position of the neutral density filter the illumination could be either at full intensity (~25 mW cm\textsuperscript{-2}) or 1% of that.

Analysis of synthesised oligonucleotides

After the completion of an oligonucleotide synthesis the solid support was treated with concentrated ammonium hydroxide (2 ml) for 18 h at room temperature. The solid was filtered off and the filtrate concentrated \textit{in vacuo} on a Speedvac rotary concentrator. The residue was dissolved in 0.05 M aqueous \([\text{Et}_3\text{NH}]^+ [\text{CH}_3\text{COO}]^-/\text{MeCN} (95:5 \text{ v/v}, 0.4 \text{ ml})\) using an ultrasonic bath. Insoluble particles were filtered off and the filtrate was analysed by reverse phase HPLC of the \(5'\)-DMTr-oligonucleotides using a Waters chromatography system with a variable wavelength detector set at 254 and 280 nm. Waters Delta Pak 5μ C18 300 Å columns were used for both analytical and preparative scale chromatography. The mobile phases were (A) 0.05 M aqueous \([\text{Et}_3\text{NH}]^+ [\text{CH}_3\text{COO}]^-\) and (B) MeCN. Gradient elution was from 5 to 60% B over 30 min at a flow rate of 1 ml min\textsuperscript{-1}. The sequences synthesised were short and their retention times differed significantly with chain length.

We validated the use of the HPLC system by conventionally synhesising sequences from T\textsubscript{3} to T\textsubscript{7} and submitting them to both analytical HPLC to determine retention times and preparative HPLC followed by mass spectrometry (MS) to confirm molecular identities. Mass spectra were recorded using a ThermoFinnegan LCQ instrument. Samples were
analysed by electrospray ionisation LC-MS using Jones Chromatography APEX ODS WP columns, 15 cm × 4.6 mm i.d. (7 μm), with a wavelength detector set at 254 nm. In each instance the molecular ion identified by MS was that expected from the synthetic protocol. The HPLC retention times in minutes and molecular ions for the 5′-DMTr-oligonucleotides were as follows: T₇, 13.30, 1183.5 [M−2H]²⁻; T₆, 13.42, 1031.5 [M−2H]²⁻; T₅, 13.58, 1759.4 [M−H]⁻; 879.5 [M−2H]²⁻; T₄, 13.88, 1455.4 [M−H]⁻; T₃, 14.38, 1151.3 [M−H]⁻. We have demonstrated (P.J.Serafinowski and P.B.Garland, submitted for publication) that the stepwise yields, retention times and molecular ion masses as observed by MS were independent of whether synthesis was conventional or photodirected by photolysis of α-phenyl-4,5-dimethoxy-2-nitrobenzyl trichloroacetate.

Stray light and photodirected oligonucleotide synthesis

In order to study the effects of stray light, we treated the flow cell as a single array element and exposed it to a ‘dummy’ cycle between each scheduled synthetic cycle. The illumination intensity for the dummy cycles was 1% of that in the scheduled cycles; otherwise all conditions were identical for all cycles. The designed oligonucleotide that would result if the dummy cycles were without effect was a pentamer (T₅). The synthesis used DMTr-T-CPG as the starting material and so had already received the equivalent of one synthetic cycle, albeit not photodirected.

We therefore commenced with a dummy synthetic cycle (1% illumination intensity) followed by a scheduled synthetic cycle with full illumination, and so on until five dummy cycles and four scheduled cycles, alternating with each other, had been completed. The photodirected syntheses were performed either with or without n-octylamine (0.26–0.5 mM) present in the solution of photoacid generator. Following completion of synthesis overnight exposure to concentrated aqueous ammonia solution was used to deprotect the oligonucleotide bases and cleave the linkage to CPG. The released DMTr-oligonucleotides were then analysed by HPLC and MS, as described above.

RESULTS

Theory and modelling

Methods of photodirected oligonucleotide synthesis. The methods described to date use photo-removable protection of the oligonucleotide 5′-OH group (1,13–23) or, more recently, the 3′-OH group (24). The protecting group can be either directly sensitive to light (1,13–17,24) or indirectly, as with the 5′-O-dimethoxytrityl group (DMTr-) which can be removed by photogenerated acid (18–23,25). Irrespective of whether photodeprotection is direct or indirect, it must be complete, otherwise poor stepwise yields result. The synthetic steps otherwise follow those of conventional solid phase oligonucleotide synthesis (11,12).

The illumination patterns for photodirected array synthesis are illustrated in Figure 1. It shows that during extension of the array by one monomer, each array element is exposed to one period of scheduled illumination and three periods of stray light illumination. Thus the synthesis of an array of Nmers exposes every element to N periods of scheduled (full) light and 3N periods of stray light.

Kinetics: a model for deprotection at the oligonucleotide-5′-OH position. The photosensitive groups currently used for protecting the oligonucleotide 5′-OH during chemical synthesis respond to photo-excitation with an intramolecular rearrangement leading directly to their departure (1,13–17). The overall reaction kinetics for such a process are first order, with an apparent rate constant k proportional to the incident light intensity and a half-life given by t_half = ln2/k. The rate constant may also vary with local environment, solvent, oligonucleotide sequence and so on, but these factors are independent of light intensity and do not enter into our analysis of the kinetics of stray light effects. They do of course enter into the design of protocols for photodirected oligonucleotide synthesis, where success requires stepwise yields that are high and preferably no less than those achieved with conventional synthesis. To that end photolysis is usually extended over 8–10 times the shortest photolytic half-life to give >99% 5′-deprotection.

Indirect 5′-O-deprotection using photogenerated acid in the absence of chemical amplification (19,20) has kinetics based on two reactions: first, photocid generation, secondly, acid-dependent 5′-detritylation. The kinetics of photocid generation will vary with the choice of photocid generating system. Photolysis of substituted 2-nitrobenzyl esters proceeds by an intramolecular rearrangement and gives first order kinetics, whereas higher reaction orders may occur with systems involving intermolecular collisions. Either way it is unnecessary to pursue photocid generation over many lifetimes: what
In the presence of excess acid present in excess: it is a reactant, and a proton is consumed for detritylation as described in equation 1.

\[ \text{DMTr}^+ \text{+ cation formed.} \]

It is informative to examine a graph of the time-dependent product yields of a first order reaction under two sets of conditions with widely different rate constants, as would be the case for direct 5'-deprotection by scheduled or stray light. The graph (Fig. 2) is plotted on a time axis of 10 half-lives for the faster reaction, which has a rate constant 400-fold higher than that for the slower reaction. The faster reaction proceeds essentially to completion and effectively stops after 8–10 half-lives.

Figure 2. Time course of fractional product yield for two first order reactions where the rate constants differ by a factor of 400. The time scale is in half-lives for the reaction with the higher rate constant. The fractional product yield \( Y_T \) was calculated from the equation \( Y_T = 1 - (0.5)^T \), where \( T \) is the reaction time expressed in half-lives.

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In order to drive detritylation to completion, acid must be present in excess: it is a reactant, and a proton is consumed for each DMTr cation formed. In the presence of excess acid detritylation as described in equation 1 will follow first order kinetics, with rate constants proportional to acid concentration. As with the direct method, 5'-deprotection must proceed for 8–10 half-lives to achieve high synthetic stepwise yields.

The predicted outcomes are: (i) each unscheduled addition of monomer to a chain creates a base insertion, not a substitution, and increases the oligonucleotide chain length by one, (ii) there are on average 3Np stray light-induced insertions per chain in the completed array and (iii) insertions will be randomly distributed along the length of the chains.

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focuses on stray photoacid rather than stray light as the agent immediately responsible for unscheduled 5'-deprotection.

Figure 3 shows a graph, calculated for direct 5'-photodeprotection, giving the predicted fraction of oligonucleotide chains with the correct sequence, plotted against contrast ratio values from 10^1 to 10^5. Data are for designed chain lengths N = 5, 10, 20 or 40 and a light exposure period of 10 half-lives per synthetic cycle. Other causes of improper chain synthesis such as incomplete detritylation or coupling have not been taken into account.

The plots of Figure 3 show, as expected, that at a contrast ratio of 10^3, characteristic of metal on glass photolithographic masks, effects of stray light are negligible. However, the fraction of oligonucleotides with the correct sequence begins to fall rapidly at contrast ratios between 10^3 and 10^4, depending on the designed oligonucleotide chain length: the greater the length, the greater the sensitivity to stray light. In the case of a 20mer, at contrast ratios of 400 and 200 the fraction of correct sequences falls to 0.36 and 0.13, respectively. Thus changes in the contrast ratio in the region typical of DMDs and other spatial light modulators are predicted to have marked effects on the accuracy of photodirected oligonucleotide synthesis in array formats.

**Kinetics of 5'-detritylation using photoacid generation.** The two reactions are photoacid generation and photoacid-induced 5'-detritylation. When the rate constant for the former is much greater than for the latter, as would be the case with brief exposure to intense light, the overall kinetics for photoacid-induced 5'-detritylation are essentially those for detritylation in response to an instantaneous addition of acid, in which case the kinetics, statistics and effects of stray light for indirect 5'-photodeprotection are as described in Figures 2 and 3 for direct 5'-photodeprotection except that stray photoacid replaces stray light.

When the two reactions overlap in time there is not a simple analytical solution to the kinetics, although the statistical considerations of the effects of unscheduled 5'-photodeprotection on the fidelity of array synthesis are unchanged. The extreme case of reaction overlap is when photoacid generation continues throughout the time taken to achieve complete (i.e. 99.9%) detritylation. Figure 4 shows the outcome of a computer simulation using numerical methods of integration. The initial acceleration of the rate of 5'-OH-oligonucleotide formation is due to the growth of photoacid concentration. As expected, continuation of exposure to photoacid after completion of scheduled detritylation worsens the effect of stray light.

**Contrast ratio and yield ratios.** Figure 2 shows that the ratio of the product yields for a first order reaction proceeding with either higher or lower rate constants is initially equal to the ratio of the two rate constants. The ratio then falls as the reaction proceeds. If the reaction is photolytic, the ratio of the two rate constants is equal to the contrast ratio. Both the reactions of indirect 5'-photodeprotection are modelled as first order, and each contributes to reducing the final ratio of product yields to well below the contrast ratio. This undesirable effect in the case of the photoacid-generating first
reaction can be reduced by restricting the duration of scheduled photolysis to a single half-life or less.

Negation of the effects of stray light. The photoacid generated by stray light should be neutralisable by the presence of sufficient buffer, or weak base, to prevent accumulation of stray light-induced photoacid to levels that cause 5’-detritylation. The amounts of stray photoacid produced from precursor can be predicted from experimental data on precursor concentration, the light intensities and photolytic rate constants.

For example, with an initial concentration of 100 mM photoacid generator, an illumination period of 1 half-life and a contrast ratio of 400, the concentration of photoacid generated at scheduled array elements would be 50 mM, with ~0.17 mM stray photoacid elsewhere (see Figs 2 and 4). The presence of 2–3 mM base or buffer would be several-fold in excess of stray light-induced photoacid, without significantly reducing the 50 mM concentration of scheduled photoacid. We describe experimental proof of the principle for this approach below. However, when direct 5’-photoprotection is used, the effects of stray light are not preventable.

Experimental

Kinetics of photoacid production and acid-induced detritylation. We wished to test some of the assumptions made in creating a theoretical model for stray light effects. We confirmed that photolysis of the ester at 365 nm followed first order kinetics and that the rate constants were proportional to light intensity over a 350-fold range. First order rate constants at room temperature in dichloromethane with an illumination intensity of 1.5 mW cm⁻² were in the region of ~0.03 to ~0.04 s⁻¹.

We confirmed that DMTr-T-CPG could be detritylated by low concentrations of trichloroacetic acid. The time resolution with manual addition of acid was adequate at <20 mM acid. Slow rates could be measured down to 0.1 mM acid. Semi-logarithmic plots (data not shown) of the time course of detritylation, where it could be reasonably measured (acid concentration >1 mM) were linear, in keeping with first order kinetics. Figure 5 shows that the initial rate of acid-induced detritylation, expressed in fractional units, was approximately proportional to the acid concentration over the range 0.1–20 mM. The rates at lower concentrations (<1 mM) were somewhat lower than would be expected from the rates observed at higher concentrations (>2 mM), but this is probably due to errors in the rate measurements at low detritylation rates. There does not appear to be a significant deviation from our modelling assumption that the rate constant for acid-induced detritylation is proportional to the acid concentration.

Demonstration of stray light-induced chain lengthening and its prevention. Results of HPLC analyses for the products of conventional and photodirected T5 synthesis are reproduced in Figure 6. Peaks were identified according to retention times of the peaks assigned to T5, T6 and T7. The HPLC traces in Figure 6 show profiles for (a) conventionally synthesised T5, (b) photodirected synthesis of T5, with alternation of synthetic cycles (full light) with dummy cycles (1% light) resulting in the appearance of a significant proportion of T6 and even T7 oligonucleotides in addition to the main T5 peak and (c) the conditions as in the previous trace but with the inclusion of 0.26 mM n-octylamine in the photacid generator solution. A control experiment (data not shown) in which the five dummy cycles carried zero rather than 1% stray light demonstrated that the synthesis of T6 and T7 was dependent on the presence of stray light and so could not be attributed to pre-existing low levels of acid in the photacid generator solution. Similar results were observed using 0.5 mM in place of 0.26 mM n-octylamine.

The T4 peak is consistent with the known imperfections of oligonucleotide synthesis over the first few bases (28) and is common to both photodirected and conventional syntheses. The T6 and T7 peaks arising in the presence of stray light during dummy synthetic cycles are due to unscheduled chain lengthening. In over 100 photodirected syntheses of a range of oligonucleotides on CPG in the column format we have observed unscheduled chain lengthening only when (i) dummy synthetic cycles were included and either (ii) weak acid was present in the photacid generator solution or (iii) there was exposure to stray light during the dummy cycles.

Table 1 lists the relative areas under the UV absorption peaks from T4 to T7 for the HPLC profiles shown in Figure 6, as measured by the HPLC apparatus at 254 nm. Stray light reduced the fraction of T5 relative to the sum of T5 + T6 + T7 from 0.99 to 0.64. The fraction rose to 0.96 when 0.26 mM n-octylamine was included in the photacid generator solution.

DISCUSSION

Pirrung and Bradley (29) noted in 1995 that because photodirected synthesis of different sequences in an array is simultaneously conducted at many locations on the same
surface, light falling on unintended locations would result in the incorporation of extra monomer units. Despite this caveat, the consequences of moving from physical masks with high contrast ratios to devices with lower contrast ratios do not appear to have been explored. When stray light falls on a photosensitive material, it cannot be without an effect. How large that effect might be for both direct and indirect photodeprotection in photodirected oligonucleotide array synthesis, and its consequences for array fidelity, are questions that we have addressed by a combination of theory and computer simulation. We have also demonstrated in an experimental model the predicted large effect of stray light, and circumstances in which it can be negated.

Contrast and contrast ratio

The term ‘contrast ratio’ describes a property of photolithographic illumination, as illustrated in Figure 1, and differs from ‘contrast’, which is a property of the resulting image. Contrast in a photolithographic context is the gradient of the profile of the photoproduced effect at the boundary between an illuminated area and an immediately adjacent non-illuminated area (30): it gives a measure of the minimum achievable size and separation of photolithographically defined array elements.

The theoretical model

The assumptions made for the development of our model for the effects of stray light on photodirected oligonucleotide array synthesis are conservative and, apart from the photochemical aspects, are shared with conventional solid phase oligonucleotide synthesis. Where we have made unshared assumptions, such as first order kinetics for photolysis of photoacid generators or acid-induced detritylation, and for the dependence of their respective rate constants on the light intensity or acid concentration, we have demonstrated their validity experimentally either directly (photolysis) or in a model system (acid-induced detritylation of DMTr-T-CPG).

It is perhaps surprising that the predicted effects of stray light on photodirected synthesis should be so marked. For example, a contrast ratio of 400:1 corresponds to a stray light intensity of 0.25% of the scheduled intensity, yet we predict that it will cause 65% of the chains of a notional 20mer synthesis to be 21mers or longer due to random base insertions. However, the contrast ratio by itself does not give a sound basis for an intuitive estimate of stray light effects. There are two main reasons for this. First, as shown in Figure 2, the time courses of photolysis caused by stray and scheduled light are not parallel, with the result that the yield of...
photoproduction from stray light is several-fold higher than would be expected from a simple comparison of stray and scheduled light intensities. Second, each array element experiences 3N exposures to stray light during the photodirected synthesis of an N-mer array. As with Russian roulette, the cumulative chance of an insertion rises with the number of exposures.

Experimental demonstration

The level of stray light used in the experiments in Figure 6 was 1% (contrast ratio = 100) but only one dummy cycle and not three was used for each scheduled synthetic cycle. For values of \( p \ll 1 \), the term \((1-p)^{3N}\) used for calculating the fraction of correct length oligonucleotides is approximately equal to \((1-3p)^N\), so the use of 1% stray light for one period is therefore equivalent to 0.33% (contrast ratio = 300) for three periods. Reference to Figure 3, treating it as predictive for both direct and indirect 5′-deprotection, shows that for \( N = 5 \), contrast ratio = 300 and number of half-lives = 10, the predicted fraction of chains of designed length is 0.71. This value is also the predicted value when the first nucleotide is already in place on CPG before the start of photodirected synthesis and is followed by exposure to stray light prior to the next synthetic cycle. It is not incompatible with the value of 0.64 observed experimentally (Table 1).

Quantitative experimental exploration of the effects of stray light will require synthesis on planar surfaces or in thin films, where complications of inner filter effects and mixing are minimised, but product analysis is more difficult.

Validity of the models

The theoretical model is based on light intensity-dependent changes in the rate constants for a photolytic reaction, either direct 5′-deprotection or photogeneration of photogenerated acid, which controls oligonucleotide synthesis. The model is independent of physical format. It could be applied to photodirected synthesis of oligonucleotides in a variety of formats provided that the synthetic protocol has first been optimised for high yields. Although the physico-chemical features of the array support surface may affect the illumination exposure required to achieve complete 5′-deprotection or the time allocated for other reactions in the synthesis to occur, the contrast ratio does not change and therefore neither does the ratio of the photolytic rate constants under scheduled or stray light.

The model assumes 100% efficiency for the coupling step. Lower efficiencies resulting in chain truncation would reduce the size of the oligonucleotide pool proceeding to the full (or longer) design length, but not affect the fractional distribution of \( N \)-mer, \( N+1 \)-mer, \( N+2 \)-mer, etc. within the pool. The theoretical model for indirect 5′-deprotection utilising photogenerated acid has been developed for photogenerated generation reactions with first order kinetics and requires modification for reactions with higher orders. The rate constant could also change during photolysis due to the effects of product accumulation.

The experimental model with photodirected oligonucleotide synthesis on CPG differs from synthesis on planar glass surfaces in overall optical geometry, in diffusion path lengths and microenvironment. Nevertheless, the synthetic chemistry is unchanged and the optical path by which a photon arrives at an absorbing molecule is immaterial. Oligonucleotide synthesis can behave similarly on either planar or controlled porosity glass (25,28,31).

Practical application

Our description of the effects of stray light on photodirected synthesis is given for the 3′→5′ direction, but it will also apply in the opposite direction (34). The rate constants for 3′-deprotection might differ, but not the principles. The same general applicability applies to maskless photodirected synthesis of arrays of other polymers, such as oligopeptides and oligosaccharides.

In practice, the effective contrast ratio at the array may be lower than the manufacturer’s quoted device values because of two factors. First, contrast ratios for DMD devices and other light valves are usually obtained by comparing the full-field intensity in its on and off states. But what matters is the relative light intensity at the light and dark patterns in a 1:4 chequerboard pattern (Fig. 1). Values have been given for the contrast ratios for 50/50 chequerboard patterns: they are about a half of the full-field contrast ratios (32). Further degradation of the contrast ratio may arise from scattering and reflections at the surface of the array, with significant additional loss of array fidelity in the typical region of contrast ratios of \(<10^3\) (Fig. 3).

Obviously, stray light-induced insertions do not stop oligonucleotide synthesis. Provided that there is a sufficient fraction of correct sequence oligonucleotides amongst those that have been inadvertently extended by insertions or truncated by acetylation following incomplete coupling, a workable array for hybridisation studies may be obtainable (3) because the fractional contribution of any one extended sequence is low. But a high content of \( N + 1 \) or longer chains is not a property that one would wish for. It would cause two undesirable outcomes: a diminished signal to background noise ratio and diminished confidence in the data.

Prospects

Others have commented on the considerable expense and therefore relative unavailability of high density oligonucleotide arrays (33). Alternative means of making arrays are available, such as printing cDNAs, presynthesised oligonucleotides or reagents for oligonucleotide synthesis, but they fall well short of the density achieved by photodirected methods. There are several initiatives to develop and provide bench-top array synthesisers based on DMD devices (see www.Xeotron.com, www.Febit.de and www.nimblegen.com). The development of photoacid generators giving high stepwise yields, and of a method to negate the effects of stray light, should enable computer-controlled light valve devices of modest contrast ratio to successfully replace physical masks, leading to fabrication of high density oligonucleotide arrays that are not only more widely affordable but also of improved fidelity.

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REFERENCES


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