

CLAIMS

1. A method for patterning flow cell substrates, comprising:
preparing a flow cell for a photoinitiated chemical reaction, wherein the flow cell includes
5 a substrate having light coupling gratings formed thereon;
a first layer of material disposed over the substrate;
a second layer of material disposed over the first layer of material; and nanowells
formed in the second layer of material, wherein each nanowell includes a top portion
and a bottom portion, and wherein preparing the flow cell includes:
10 silanizing the second layer of material;
coating the silanized second layer of material and
nanowells with a first group of reactants;
introducing a second group of reactants into the nanowells, wherein the second group
of reactants includes at least one target reactant, a copper chelated ligand, and a light-
15 sensitive photoinitiator system; and
directing light internally within the flow cell through the light coupling gratings to only the
bottom portion of each nanowell for photo-initiating a chemical reaction between the first and
second groups of reactants,
wherein the photo-initiated chemical reaction covalently binds the target reactant to only
20 the bottom portion of each nanowell.
2. The method of claim 1, further comprising washing unreacted reactants out of the
nanowells.
- 25 3. The method of any of claims 1-2, further comprising using a polymer and azide moieties
that are bound to the polymer as the first group of reactants.
4. The method of claim 3, further comprising using poly(N-(5-azidoacetamidylpentyl)
acrylamide as the polymer.
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5. The method of any of claims 1-4, further comprising using a camphorquinone-amine
photosensitizing system using a light wavelength of about 470 nm as the light-sensitive
photoinitiator system.
- 35 6. The method of any of claims 1-5, further comprising using an alkyne-linked primer as the
target reactant.

7. The method of any of claim 1-6, further comprising using an alkyne-linked fluorophore as the target reactant.

8. The method of any of claims 1-7, further comprising using a laser as a source of light.

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9. The method of any of claims 1-8, further comprising using a material having a refractive index in the range of 1.0 to 1.3 for the substrate and a material having a refractive index in the range of 2.0 to 2.15 for the first layer.

10 10. A method for patterning flow cell substrates, comprising:

fabricating a planar waveguide flow cell, wherein fabricating the planar waveguide flow cell includes:

forming a layer of light coupling gratings on a glass substrate layer;

depositing a core layer on the layer of light coupling gratings;

15 depositing a cladding layer on the core layer; and

forming nanowell substrates in the cladding layer, wherein each nanowell substrate includes a top portion and a bottom portion, and wherein the nanowell substrates define interstitial regions therebetween;

silanizing the cladding layer;

20 coating the silanized cladding layer and nanowell substrates with a first group of reactants;

introducing a second group of reactants into the nanowell substrates, wherein the second group of reactants includes at least one target reactant, a copper chelated ligand, and a light-sensitive photoinitiator system; and

25 directing light internally within the planar waveguide flow cell through the light coupling gratings to only the bottom portion of each nanowell substrate for photo-initiating a chemical reaction between the first and second groups of reactants,

wherein the photo-initiated chemical reaction covalently binds the target reactant to only the bottom portion of each nanowell substrate.

30 11. The method of claim 10, further comprising washing unreacted reactants out of the nanowell substrates.

12. The method of any of claims 10 and 11, further comprising using a polymer and azide moieties that are bound to the polymer as the first group of reactants.

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13. The method of claim 12, further comprising using poly(N-(5-azidoacetamidylpentyl) acrylamide as the polymer.

14. The method of any of claims 10-13, further comprising using a camphorquinone-amine
5 photosensitizing system using a light wavelength of about 470 nm as the light-sensitive photoinitiator system.

15. The method of any of claims 10-14 further comprising using an alkyne-linked primer as
10 the target reactant.

16. The method of any of claims 10-15 further comprising using an alkyne-linked fluorophore
as the target reactant.

17. The method of any of claims 10-16, further comprising using a laser as a source of light.
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18. The method of any of claims 10-17 further comprising using a material having a refractive
index in the range of 1.0 to 1.3 for the layer of light coupling gratings and a material having a
refractive index in the range of 2.0 to 2.15 for the core layer.

20 19. A method for patterning flow cell substrates, comprising:
fabricating a planar waveguide flow cell, wherein fabricating the planar waveguide flow cell
includes:

forming a layer of light coupling gratings on a glass substrate layer;
depositing a core layer on the layer of light coupling gratings;
25 depositing a cladding layer on the core layer; and
forming nanowell substrates in the cladding layer, wherein each nanowell substrate
includes a top portion and a bottom portion, and wherein the nanowell substrates define interstitial
regions therebetween; and
silanizing the cladding layer;
30 coating the cladding layer and nanowell substrates with a first group of reactants, wherein
the first group of reactants further includes a polymer, azide moieties bound to the polymer, a
copper ligand, and a light-sensitive photoinitiator system;
directing light of a predetermined wavelength internally within the planar waveguide flow
cell to only the bottom portion of each nanowell substrate for photo-initiating a chemical reaction
35 between the reactants in the first group of reactants, wherein the photo-initiated chemical reaction
covalently binds the polymer to only the bottom portion of each nanowell substrate;

introducing a second group of reactants into the nanowell substrates, wherein the second group of reactants includes at least one target reactant, a copper ligand and a light-sensitive photoinitiator system; and

5 directing light of a predetermined wavelength internally within the planar waveguide flow cell to only the bottom portion of each nanowell substrate for photo-initiating a chemical reaction between the covalently bound polymer and the second group of reactants, wherein the photo-initiated chemical reaction covalently binds the target reactant to only the bottom portion of each nanowell substrate.

10 20. The method of claim 19, further comprising washing unreacted reactants out of the nanowell substrates after each photo-initiated chemical reaction.

21. The method of any of claims 19-20, further comprising using 3-azidopropyltrimethoxysilane for silanizing the cladding layer.

15 22. The method of any of claims 19-21, further comprising using poly(N-(5-azidoacetamidylpentyl) acrylamide as the polymer.

20 23. The method of any of claims 19-22, further comprising using a camphorquinone-amine photosensitizing system using a light wavelength of about 470 nm as the light-sensitive photoinitiator system.

24. The method of any of claims 19-23, further comprising using an alkyne-linked primer as the target reactant.

25 25. The method of any of claims 19-23, further comprising using an alkyne-linked fluorophore as the target reactant.

26. The method of any of claims 19-25, further comprising using a laser as a source of light.

30 27. The method of any of claims 19-26, further comprising using a material having a refractive index in the range of 1.0 to 1.3 for the layer of light coupling gratings and a material having refractive index in the range of 2.0 to 2.15 for the core layer.

35 28. The method according to any of the preceding claims 1-18 wherein silanizing is carried out by way of chemical vapor deposition of norbornene silane.

29. The method according to any of the preceding claims 4-9, 13-18, wherein poly(N-(5-azidoacetamidylpentyl) acrylamide is spin coated on the silanized layer by way of the following procedure: Step 1--600 rpm, 5 seconds, acceleration 1500 rpm/second; Step 2--1500 rpm, 30 seconds, acceleration 5000 rpm/second; Step 3--4000 rpm, 5 seconds, acceleration 5000 rpm/second; Step 4--600 rpm, 5 seconds, acceleration 5000 rpm/second, and subsequently preferably heating at 65-75°C for 1 hour.
30. The method according to any of the preceding claims 21-27 wherein silanizing is carried out by vapor deposition of 3-azidopropyltrimethoxysilane, whereafter poly(N-(5-azidoacetamidylpentyl) acrylamide is preferably crosslinked to azide groups using a photo-initiated reaction that uses a bi-functional crosslinker.
31. The method according to any of the preceding claims wherein the poly(N-(5-azidoacetamidylpentyl) acrylamide is covalently bound to surfaces at the bottom of the nanowells using the light, preferably laser light.
32. The method according to claims 30 or 31 wherein the copper is subsequently removed using a dilute solution of EDTA (0.1M).
33. The method according to any of the preceding claims, absent a polishing step to remove any existing surface chemistry found in interstitial areas between the nano wells.
34. The method according to any of the preceding claims wherein light is directed through the light coupling gratings to only a bottom portion of each nanowell for photo-initiating the chemical reaction between the first and second groups of reactants.
35. A flow cell for a photoinitiated chemical reaction, which flow cell comprises:
a substrate,
a light coupling grating layer arranged on the substrate, which light coupling grating layer has a refractive index,
a core layer arranged on the grating layer, which core layer has a refractive index, wherein the refractive index of the core layer is greater than the refractive index of the light coupling grating layer,
a silanized layer arranged on the core layer in which silanized layer multiple nanowells are present, and
wherein the nanowells are separated by interstitial areas.

36. The flow cell according to claim 35, wherein the substrate is glass.
37. The flow cell according to claims 35 or 36 wherein the light coupling grating layer and the core layer are formed from a resin.
- 5 38. The flow cell according to claim 37 wherein the light coupling grating layer and/or the core layer is formed from tantalum pentoxide.
39. The flow cell according to any of the claims 35-38, wherein the silanized layer is formed
10 from a water buffer or a polymer cladding layer.
40. The flow cell according to any of the preceding claims 35-39 wherein the refractive index of the light coupling grating layer is in the range of 0.5 to 2.0 and the refractive index of core layer is in the range of 1.5 to 2.5.
- 15 41. The flow cell according to any of the preceding claims 35-40 wherein the poly(N-(5-azidoacetamidylpentyl) acrylamide is covalently bound to surfaces at the bottom of the nanowells.
42. The flow cell according to claim 41 further comprising the first group of reactants arranged
20 on the poly(N-(5-azidoacetamidylpentyl) acrylamide covalently bound to surfaces at the bottom of the nanowells.
43. The flow cell according to claim 42 wherein the second group of reactants is arranged on the first group of reactants, and includes at least one target reactant, a copper chelated ligand, and a
25 light sensitive photoinitiator system.
44. A method of sequencing nucleic acids, using a flow cell made by any of the previous claims 1-34 and/or a flow cell according to any of the claims 35-43.
- 30 45. A system for photoinitiated chemical reactions comprising a flow cell according to any of the preceding claims 35-43 and a light source, preferably a laser light source.