Claims

1. A sample holder for use in an optical microscope, the sample holder including:

a plasmonic layer defining a periodic array of sub-micron structures,

wherein the sample holder is configured to support an object such that the periodic array of sub-micron structures is located on a same side of the sample holder as the object when supporting the object; and

an optically clear protective layer on an upper side of the plasmonic layer to isolate the plasmonic layer from the object such that the optically clear protective layer is located between the plasmonic layer and the object when the object is supported by the sample holder,

wherein the periodic array of sub-micron structures comprises an array of separated plasmonic regions.

2. The sample holder as claimed in claim 1 wherein:

the plasmonic regions are islands of plasmonic material; and/or

the periodic array is a 1-dimensional or 2-dimensional array; and/or

the plasmonic regions are shaped as any one or more of: a circle, a torus, an ellipse, a cross, a rectangle, a square, a line and a strip.

3. The sample holder as claimed in claim 1 or 2 wherein:

the sub-micron structures are arranged in a periodic array with a separation between the sub-micron structures in the range of 200 nm to 500 nm; and/or

the sub-micron structures have a largest dimension in the range of 50 nm to 300 nm; and/or

the sub-micron structures are regions of plasmonic material about between 100 nm and 200 nm across.

4. The sample holder as claimed in any one of the preceding claims wherein the plasmonic layer is formed from one or more metals selected from the group comprising Al, Ag, Au, Ni, Pt and Pd.

5. The sample holder as claimed in any one of the preceding claims wherein the plasmonic layer has a thickness in the range of 20 nm to 300 nm.

6. The sample holder as claimed in any one of the preceding claims wherein the plasmonic regions are arranged in an array with a first spacing in a first direction and a second spacing in a second direction, wherein the first spacing and second spacing are the same.

7. The sample holder as claimed in any one of claims 1 to 5, wherein the plasmonic regions are arranged in an array with a first spacing in a first direction and a second spacing in a second direction, wherein the first spacing and second spacing are different.

8. The sample holder as claimed in claim 6 or claim 7 wherein the first direction is orthogonal to the second direction.

9. The sample holder as claimed in any one of the preceding claims wherein the sample holder includes a substrate for providing mechanical support for the plasmonic layer, wherein the plasmonic layer is located on a first surface of the substrate and wherein the plasmonic layer is between the substrate and the optically transparent protective layer.

10. The sample holder as claimed in any one of claims 1 to 9 wherein the optically clear protective layer has a thickness less than 150 nm.

11. The sample holder as claimed in any one of claims 1 to 10 wherein the optically clear protective layer includes:

any one or more of: silicon oxide, silicon nitride, transparent metal oxide, and a polymer; and/or

a capping layer of hydrogen silsesquioxane (HSQ) or a metal oxide.

12. The sample holder as claimed in any one of the preceding claims, wherein the sample holder is arranged in use to enable light, which is transmitted through the sample to the plasmonic layer to be reflected from the sample holder for the creation of images comprising the reflected light, wherein incident light illuminates the sample and sample holder and interacts with the sample and the plasmonic layer.

13. The sample holder as claimed in claim 12 wherein the reflected light includes a characteristic spectra in which each colour is dependent on the localised dielectric constant of a sample held on the sample holder.

14. The sample holder of any one of claims 1 to 13, wherein the plasmonic layer extends over a region of the sample holder and the periodic array of sub-micron structures is arranged to fill, by area, not more than 80% of said region.

15. The sample holder of claim 14, wherein the region of the sample holder is the entire area of the sample holder.

16. The sample holder of any one claims 1 to 15, wherein the object to be supported is a biological sample, and wherein the optically clear protective layer provides a polar surface to aid in adherence of the biological sample to the support surface.

17. A method of imaging a sample comprising:

providing a sample holder having an upper surface and a lower surface, the upper surface having a plasmonic layer and an optically clear protective layer associated therewith, the plasmonic layer including a periodic array of sub-micron structures;

applying the sample to the upper surface of the sample holder;

illuminating the sample with light so that said light interacts with the sample and the plasmonic layer of the sample holder;

receiving light emitted from the sample by fluorescence and forming a first image thereof; and

receiving light after interaction with said sample and the plasmonic layer of the sample holder and forming a second image thereof,

wherein at least one localised structural property of the sample is visible in the second image based on the colour of the received light,

wherein the optically clear protective layer is arranged on an upper side of the plasmonic layer to isolate the plasmonic layer from the sample such that the optically clear protective layer is located between the plasmonic layer and the sample when the sample is supported by the sample holder.

18. The method of claim 17, wherein the first image formed from the light emitted from the sample by fluorescence is formed in a first time period, and the second image formed from light after interaction with said sample and the plasmonic layer of the sample holder is formed in a second time period.

19. The method as claimed in claim 18, including illuminating the sample using light having a first illumination spectrum in the first time period, and a second illumination spectrum in the second time period, wherein the first illumination spectrum is selected on the basis of a fluorescence property of the sample.

20. The method as claimed in any one of claims 17 to 19, wherein receiving light emitted from the sample by fluorescence for forming the first image includes filtering light

received from the sample and/or sample holder to minimise received light from sources other than said fluorescence.

21. The method as claimed in any one of claims 17 to 20 including: spatially correlating the first image and the second image.

22. The method of claim 21, wherein the method further includes forming a combined image by combining:

at least one first image, each first image formed from the light emitted from the sample by fluorescence, and at least one second image, each second image formed from light after interaction with said sample and the plasmonic layer of the sample holder.

23. A method of imaging a sample comprising:

providing a sample holder having an upper surface and a lower surface, the upper surface having a plasmonic layer and an optically clear protective layer associated therewith, the plasmonic layer including a periodic array of sub-micron structures;

applying the sample to the upper surface of the sample holder;

illuminating the sample with light so that said light interacts with the sample and plasmonic layer of the sample holder;

receiving light emitted from the sample by fluorescence and light reflected after interaction with said sample and the plasmonic layer of the sample holder and forming a combined image thereof,

wherein at least one localised structural property of the sample is visible in the combined image based on the colour of the light received after interaction with said sample and the plasmonic layer of the sample holder,

wherein the optically clear protective layer is arranged on an upper side of the plasmonic layer to isolate the plasmonic layer from the sample such that the optically clear protective layer is located between the plasmonic layer and the sample when the sample is supported by the sample holder.

24. The method as claimed in any one of claims 17 to 23, including illuminating the sample from the side of the sample holder on which the sample is positioned, and receiving light emitted from the sample by fluorescence and also light which has interacted with said sample and said plasmonic layer of the sample holder from the same side as said illumination.

25. The method as claimed in any one of claims 17 to 24 wherein the sample is a biological sample.

26. A system for imaging a sample, the system configured to carry out the method of any one of claims 17 to 25, the system including:

a reflected light microscope having an image forming system, an illumination system, and a sample holder according to any one of claims 1 to 16.