

illustrated example, extension arms 628 are elongate structural members attached, affixed, joined or otherwise connected to opposite sides of frame portion 622, and beam blocking member 626 extends across the distal ends of extension arms 628.

[00119] Frame portion 622 and extension arms 628 provide a mounting structure by which the beam blocking member 626 can be mounted in position at beam splitter 532 without interfering with reflections from surfaces S2 and S3. Beam blocker 620 can be cast, molded, machined or otherwise fabricated as a unitary structure. In other examples, the elements that make up beam blocker 620 can be separate components that are attached, joined, fastened or otherwise connected together to form the resulting assembly. Beam blocker 620 can be implemented using light-absorbing, opaque surfaces to avoid other unwanted reflections within the system. For example, beam blocker 620 can be made using black anodized aluminum or other light-absorbing, or light-absorbing-coated materials. Beam blocker 620 can be dimensioned for a particular application. In one example application, beam blocker 620 is dimensioned to provide: an aperture width of 30 mm and a height of 21 mm; extension arms 628 of 25 mm in length; and a blocking surface that is 2.8 mm wide and 21 mm in length.

[00120] With reference now to FIG. 16B, wherein the upper diagram 682 illustrates a top-down view of beam blocker 620, and the lower diagram 683 illustrates a cross-sectional side view at A of beam blocker 620. The front edge of extension arms 628 is tapered to conform to the angle of beam splitter 532 as further illustrated in FIG. 17 (described below). The beam blocking member has a triangular cross-section and is oriented to present flat blocking face 630 to the incoming beam. Although beam blocker 620 can be made using light-absorbing

materials, presenting a triangular cross-section to the unwanted beams can have the effect of reflecting any unabsorbed light out of the return path.

[00121] FIG. 17A presents a cutaway view of beam blocker 620 installed at beam splitter 532. With reference now to FIG. 17A, in operation, reflections of the focus tracking beams from surfaces S1, S2, S3 and S4 travel up from the objective lens, are reflected off of mirror 536 and directed toward beam splitter 532. Blocking face 630 (see FIGs. 16A and 16B) of blocking member 626 blocks the S4 reflections from continuing past beam splitter 532. It is shown in this example that extension arm 628 is dimensioned so as to place blocking member 626 at or near the surface of beam splitter 532. This figure also illustrates the tapered front angle of extension arm 628 to allow the blocking face 630 of blocking member 626 to be placed adjacent to and at substantially the same angle as beam splitter 532. In some examples, blocking member 626 is positioned such that blocking face 630 is in touching relation with beam splitter 532. In other examples, blocking member 626 is positioned such that blocking face 630 is separated from the face of beam splitter 532 by a small amount such as, for example, by 50 μm to 500 μm .

[00122] In alternative examples, a blocking element can be disposed on the back side of beam splitter 532 without the structure illustrated in FIGs. 16 and 17. For example, in some instances, a strip of opaque material can be attached to the rear surface of beam splitter 532. In other instances, an opaque or optically absorbent coating can be applied in a narrow stripe to the back of beam splitter 532.

[00123] For scanning operations, the imaging beams, which for example can be red and green imaging beams, enter the system from the right-hand side as illustrated by arrow

stability of +/- 139 nm with 95% confidence interval ($\sim 2 * \text{StDev}$ for a Gaussian population). This is calculated as shown in the figure as $(1.178 * 1.96)/16.36 \mu\text{m} = +/- 141 \text{ nm}$. The 16.36 factor represents the Focus tracking Gain in pixels/ μm . It represents how many pixels of spot separation are obtained for every 1 μm shift of objective-to-sample distance. It is used for the conversion of a delta in spot separation (pixels) to a delta in space in z direction (nm).

[00138] The inventors have discovered that the interference fringe patterns arise due to the multilevel structure of the sample container as shown in FIG. 3A. The inventors have further discovered that this is a result of the superimposition of multiple beams and/or scattered light within the multi-layer glass sample container. Changes in the position of the sample container (e.g., in the X and Y direction) with no other changes can result in movement of the fringes.

[00139] FIGS. 30A and 30B illustrate additional examples of spot movement with the laser diode operating in a hybrid mode. Particularly, FIGS. 30A and 30B illustrate a more optimal scenario with a stable laser that is not mode hopping. As illustrated in FIG. 30A, standard deviation of the left spot is down to 0.069 pixels, and the right spot is down to 0.064 pixels. As the upper two graphs on the figure indicate, spot movement from frame to frame is generally less than one pixel. Because the movement can be additive, the Delta X difference between the left and right spots can have a standard deviation of .122 pixels. This reduces the spot separation stability to +/- 14.6 nm $((0.122 * 1.96) / 16.36 \mu\text{m} = +/-14.6 \text{ nm})$. Here, 16.36 is the FTM gain in pixel/ μm . This is the amount of Delta X in pixel that is obtained when the objective moves 1 μm in the Z axis. This can be used to covert from pixels in Delta X to μm in the Z space and vice versa. Additionally, 1.96 is the multiplication factor for the standard deviation to

express 95% confidence interval of the error of the distribution (assuming it's a Gaussian distribution).

[00140] In the example of Fig 30B, the standard deviation of the left spot is down to 0.069 pixels, and the right spot is also down to 0.069 pixels. As the upper two graphs on the figure indicate, spot movement from frame to frame is generally less than one pixel. Because the movement can be additive, the Delta X difference between the left and right spots can have a standard deviation of .127 pixels. This reduces the spot separation stability to +/- 15.2 nm $((0.127 * 1.96) / 16.36 \mu\text{m} = \pm 15.2\text{nm})$.

[00141] As noted above, it is impractical to run the laser in a pre-existing ASE mode. As also just described, accuracy suffers with the laser diode running at a power level above the lasing threshold, and this is especially true if mode hopping occurs (such as, for example, via power variations). However, the inventors have discovered that operating the laser in a hybrid mode, between the ASE mode and a full lasing mode, provide sufficient beam intensity for measurement at the image sensor and increased spot placement stability for improved measurement accuracy. This mode can be achieved in some instances by operating slightly above the lasing threshold of the laser diode. For example, this might occur slightly past the knee of the lasing curve, but is still low enough that a significant portion of the power is in the ASE state. This produces an output where a large amount of the light still has a broader spectral width resulting in significantly reduced coherence.

[00142] Operating a laser in this hybrid mode can be advantageous as compared to other light sources that might be used to attempt to achieve the same effect. Laser diodes tend to be desirable light sources because they exhibit a high reliability and low cost, due to the high

CLAIMS

The claims defining the invention are as follows:

1. An imaging system, comprising:

a laser diode light source operable in an Amplified Spontaneous Emission ("ASE") mode and in a lasing mode, the ASE mode having frequency components across a range of wavelengths without a single dominant peak, the lasing mode having a single dominant peak at an operating frequency;

an objective lens positioned to direct a focus tracking beam from the laser diode light source onto a location in a sample container and to receive the focus tracking beam reflected from location in the sample container; and

an image sensor comprising a plurality of pixel locations to receive focus tracking beam that is reflected off of the location in the sample container, wherein the reflected focus tracking beam creates a spot on the image sensor;

wherein the laser diode light source is operated at a power level that is above a power level for operation in the ASE mode and below a power level for operation in the lasing mode.

2. The imaging system of claim 1, wherein the power level at which the laser diode light source is operated is at an input current that is between about 2%-10% above lasing threshold current for the laser diode light source.

3. The imaging system of claim 1 or 2, wherein a laser operating current is set between about 0.6 and 3.0 mA above threshold current.

4. The imaging system of any one of the preceding claims, wherein a laser power of the laser diode light source is set such that laser spectrum full width at about 5 % is greater than 2nm.

5. The imaging system of any one of the preceding claims, wherein the power level at which the laser diode light source is operated is selected such that a dominant peak in the laser diode light source at a given frequency has a normalized intensity of between about 15% - 100% greater than any secondary peaks output by the laser diode light source.

6. The imaging system of any one of claims 1 to 4, wherein the power level at which the laser diode light source is operated is selected such that a dominant peak output by the laser diode light source at a given frequency has a normalized intensity of between about 15% - 25% greater than a normalized intensity of a secondary peak output by the laser diode light source.

7. The imaging system of any one of claims 1 to 4, wherein the power level at which the laser diode light source is operated is selected such that a dominant peak output by the laser diode light source at a given frequency has a normalized intensity of between about 15% - 100% greater than a normalized intensity of a secondary peaks output by the laser diode light source.

8. The imaging system of any one of the preceding claims, further comprising a beam splitter to split the focus tracking beam from the laser diode light source into at least two focus tracking beams to be reflected off of the location in the sample container and provide at least two spots on the image sensor, wherein the power level at which the laser diode light source is

operated is selected such that spot separation of the at least two spots on the image sensor is less than 18 nm.

9. The imaging system of any one of the preceding claims, wherein the power level at which the laser diode light source is operated is selected such that an amount of fringing on the spot on the image sensor is less than about 10% peak to peak across the profile.

10. The imaging system of any one of the preceding claims, further comprising a windowed sinc filter or Blackman window filter communicatively coupled to the image sensor.

11. The imaging system of any one of the preceding claims, wherein the power level at which the laser diode light source is operated is selected as a power at which an exposure time is less than about 250 μ s for the image sensor to sense the focus tracking beam .

12. An imaging system, comprising:

a laser diode light source operable in an Amplified Spontaneous Emission ("ASE") mode and in a lasing mode, the ASE mode having frequency components across a range of wavelengths without a single dominant peak, the lasing mode having a single dominant peak at an operating frequency;

a beam splitter disposed in the imaging system at a position to receive a focus tracking beam from the laser diode light source and to split the focus tracking beam into at least two focus tracking beams;

an objective lens positioned to direct the at least two focus tracking beams from the laser diode light source onto a location in a sample container and to receive reflections of the at least two focus tracking beams reflected from the sample container;

an image sensor comprising a plurality of pixel locations to receive the focus tracking beams reflected off of the location in the sample container, wherein the at least two reflected focus tracking beams create at least two spots on the image sensor;

wherein a power level at which the laser diode light source is operated is selected above a power level for operation in the ASE mode and below a power level for operation in the lasing mode such that spot separation of the at least two spots on the image sensor is less than about 20 nm.

13. The imaging system of claim 12, wherein the power level at which the laser diode light source is operated is selected such that spot separation of the at least two spots on the image sensor is between about 15 and 17 nm.

14. The imaging system of claim 12, further comprising a windowed sinc filter circuit or a Blackman window filter circuit communicatively coupled to the image sensor.

15. The imaging system of any one of claims 12, wherein the power level at which the laser diode light source is operated is such that an amount of fringing on each of at least two spots on the image sensor is less than about 10% peak to peak across a profile of each of the at least two spots.

16. The imaging system of any one of claims 12 to 15, wherein the power level at which the laser diode light source is operated is selected such that a standard deviation of a position of each of the at least two spots on the image sensor is less than 10% of the pixel size of the image sensor.

17. The imaging system of any one of claims 12 to 16, wherein the power level at which the laser diode light source is operated is selected such that spot separation of the at least two spots on the image sensor is less than about 5% of the pixel size of the image sensor.

18. The imaging system of any one of claims 12, wherein the power level at which the laser diode light source is operated is selected as a power at which an exposure time required by the image sensor is less than about 250 μ s to sense a focus tracking beam of the at least two focus tracking beams.

19. The imaging system of claim 12, wherein the power level of the diode light source is set below that corresponding to the onset of fringes.

20. The imaging system of any one of claims 12, wherein a laser operating current of the laser diode light source is set between about 0.6 and 3.0 mA above a threshold current.

21. The imaging system of claim 19, wherein the laser power is set such that laser spectrum full width at about 5 % is greater than 2nm.