proper operation of the reader instrument. Positioning of cartridge holder assembly **1254** with respect to housing **1202** is illustrated in FIGS. **15** through **17**. While FIG. **12** shows the cartridge holder assembly with respect to the housing, an isolated cartridge holder assembly **1254** is indicated in perspective view in FIG. **15**, and side cross sectional views with cartridge absent and fully inserted are respectively seen in FIGS. **16** and **17**.

[0108] Cartridge holder assembly **1254** may be attached, at least in part, to housing **1202** by a front bracket **1253** and a side bracket **1259**. Cartridge holder assembly **1254** may include a door **1252**, which may be spring-loaded with a spring **1255** to block aperture **1250** in a normally closed position, protecting users from inadvertent exposure to laser light potentially emanating from reader instrument **1200**, and limiting admission of dust, dirt or other contaminants into reader instrument **1200**.

[0109] Elements that assist in proper positioning of a cartridge are illustrated in FIGS. **15**, and **16-17**. A cartridge may be mechanically held in proper position by cooperation of door **1252**, spring-loaded levers **1256**, and rollers **1278**. Spring pressure from spring-loaded levers **1256** and rollers **1278** holds a cartridge in position against laser illumination module **1204**. In the exemplary embodiment shown in FIG. **15**, cartridge holder assembly **1254** is formed of a lever plate **1272**, onto which door **1252**, spring-loaded levers **1256** and rollers **1278** are mounted, a guide plate **1274**, which includes features for guiding an inserted cartridge into proper position, and a base plate **1276**, onto which laser illumination module **1204** and guide plate **1274** are fastened. Guide plate **1274** and base plate **1276** are configured with openings therein so as to collectively define an aperture **1280** through which the inserted cartridge may be viewed and imaged.

[0110] The correct positioning of the cartridge within the reader instrument activates a safety interlock switch **1258**, which allows activation of the laser in laser illumination module **1204**. In other words, safety interlock switch **1258** (FIGS. **13** and **15**) monitors when a cartridge is properly inserted into reader instrument **1200**, thereby enabling laser illumination to occur. Proper positioning of the cartridge may be indicated to the user by, for example, an audible and/or tangible "click" produced by a pin **1266** (FIGS. **14** and **16**), which is configured to produce the "click" when compressed by a cartridge placed thereon. When the cartridge is extracted, switch **1258** physically disables the laser illumination circuitry, preventing possible accidental laser radiation exposure to the operator. Upon removal of the cartridge, door **1252** rotates back to a closed position.

Laser Illumination Module and Light Transmission Module

[0111] Precise and accurate illumination control is an important element of reader instrument **1200** operation. In one embodiment illustrated in part with respect to FIGS. **12** through **14**, laser illumination module **1204** produces illumination light capable of optically coupling to waveguide of cartridge after passing through the light transmission module, which is cooperatively composed of housing-mounted optical elements and cartridge-mounted integral lens elements.

[0112] Laser illumination module **1204** may provide light with suitable optical power, for example, between 10 and 100 milliwatts. To maintain calibration and desired reader instrument sensitivity, it may be advantageous for the optical power to have long-term stability better than 15% drift per month, along with a short-term stability better than 1% RMS variation at a 5-second integration time. In some embodiments, laser light may have wavelength equal to 660±5 nm. This wavelength specification may be changed (e.g., to 642 nm±5 nm) to accommodate different fluorescent tag systems, and in some advanced reader instrument embodiments multiple or tunable laser light wavelengths may be used.

[0113] In an embodiment, the laser light may be polarized orthogonal to plane of cartridge waveguide, to better than 10% polarization extinction ratio. The laser light may be directed toward the cartridge so that it propagates in a direction inside the waveguide that is within 1°, such as between 0.10° and 0.25° of being parallel to the long axis (centerline) in the plane of the assay surface of the waveguide. The laser light ordinarily propagates along the centerline of the waveguide. Ideally, the relative average power difference between waveguide surface locations that are equidistant from the centerline is less than 10%, such as between about 1% and 5%. When a cartridge is fully inserted into the reader instrument, laser light may enter the coupling lens of the cartridge waveguide at a height and angle relative to top surface of waveguide that optimizes assay performance. The laser light is collimated (e.g., to less than 2 to 5 degrees far-field divergence angle) so as to ensure uniformity of illumination throughout the length of the waveguide, particularly in the assay region. Additionally, collimation of the laser light may further reduce sensitivity of the reader instrument to longitudinal displacement (i.e., in a direction indicated by double-headed arrow **108** in FIG. **2**) of the waveguide with respect to laser illumination module **1204**. Beam width ($1/e^2$ full width) is parallel to polarization axis and selected to be about 1.7±0.2 mm (affects longitudinal uniformity). Beam width ($1/e^2$ full width) perpendicular to polarization axis may be, for example, 9±1 mm (this parameter affects transverse uniformity). In operation, far-field spatial deviations in the imaging region negligibly contribute to site-to-site variations of the assay. RMS variations of the optical power at the surface of the waveguide, with an averaging window of 60 μm, may be less than 10%. Finally, RMS variations of the optical power at the surface of the waveguide, with an averaging window of 600 μm, may be less than 10% (typically between 1% and 5%).

[0114] Additional light sources may be used to illuminate portions of the cartridge when inserted into the reader instrument. For instance, an LED **1268** may be used to illuminate features, such as a barcode or a fiducial mark, disposed on a bottom portion of the cartridge.

[0115] In an embodiment, a rotating diffuser system may be used to further improve the uniformity of the laser light illumination. For example, a diffuser (such as rotating diffuser **1270** of FIGS. **16** and **17**) may be used with rotation, in which the diffuser itself is rotated about an axis parallel to, but offset from the laser beam propagation direction. In this way, the need for any wedge and/or focusing lenses for the laser beam around the location of diffuser **1270** is eliminated, hence greatly simplifying the optical path compared to existing art (see, for example, Hard et al., "Phase-randomized laser illumination for microscopy," J. Cell Sci. 23:335-343, 1977).

[0116] It is recognized that the method, as disclosed herein, removes undesirable non-uniformities in the planar waveguide illumination, and thus in the fluorescence images of microarrays (including one or more reaction sites) illuminated by the laser beam inserted into the planar waveguide, thereby resulting in several positive benefits: 1) improved image quality; 2) reduced intra-site coefficient of variation