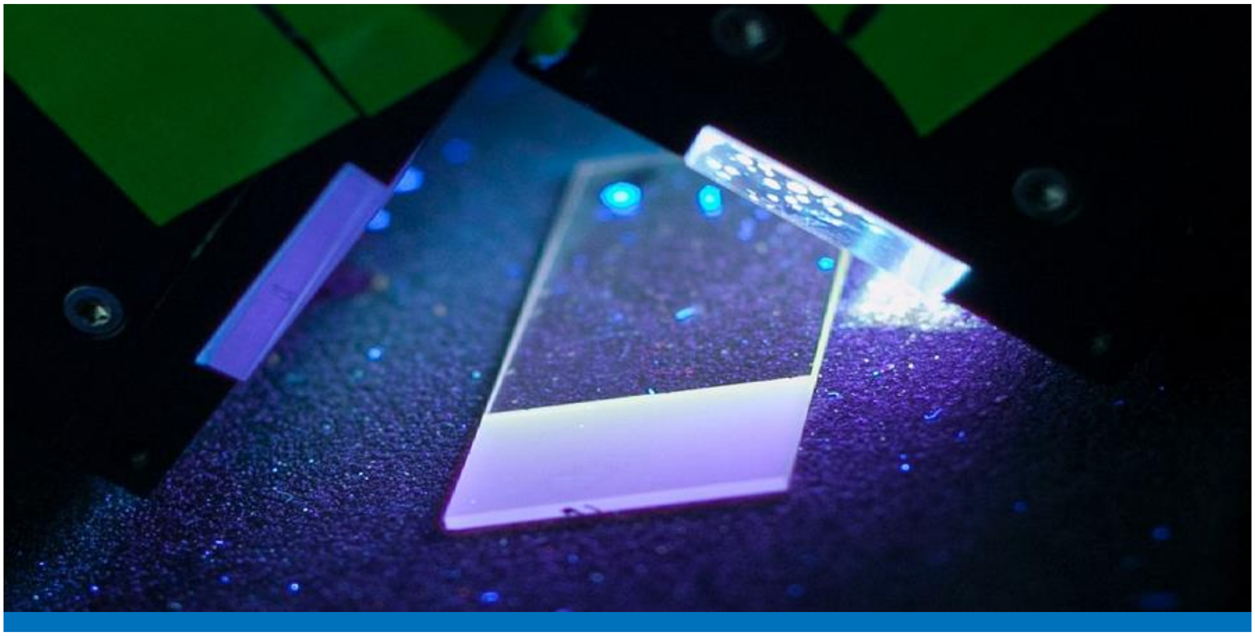

Best wavelengths for disinfection in the age of Sars-CoV-2 (corona-virus)

A Phoseon Technology White Paper
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UV LED radiation has proven to be an effective and innovative measure for disinfection and decontamination. Particular wavelengths affect different bonds within biological molecules. Therefore, determining what wavelengths are most effective against specific microorganisms and macromolecules can greatly impact the capability of a lab to ensure accurate and consistent results as far as maintaining experimental parameters goes.

What is the difference between disinfection and decontamination?

Disinfection	Decontamination
Inactivation of microorganisms: viruses, bacteria, and fungi	Inactivation of biological molecules: DNA, RNA, and Enzymes
Current Techniques: Chemicals, Heat, Ethlene Oxide, Steam	Current Techniques: Chemicals, Heat, Scrubbing, Rinsing
UV LEDs effectively inactivate microorganisms such as Influenza A, Clostridium difficile spores, Aspergillis brasiliensis, and Staphylococcus Aureus	UV LEDs effectively inactivate hard target biological molecules, even RNase A

Common applications for UV LED disinfection/decontamination

While UV LED technology can be a great ally for most laboratories by increasing accuracy and consistency of results while reducing time and cost in experiment trials, there are specific areas of study were UV LED can make a tremendous impact:

1. **Improving RNA driven protocols and results -**By completely inactivating RNase A[1], an enzyme present in most surfaces that degrades RNA samples.

2. **Vaccine development research and manufacturing -** Testing with UV LED can be a fast track to rapid and cost-effective virus inactivation; an essential technique when developing vaccines.

3. **Microbiology laboratories**- Whether the purpose is to reuse or to properly dispose of equipment, disinfection is an everyday necessity in microbiology labs. As opposed to other techniques, UV LED technology has proven to achieve complete levels of disinfection in microplates or pipettes[2] while leaving no residue behind. This zero-trace result is rarely seen by other disinfection approaches. Phoseon disinfection products have efficiently inactivated viruses, bacteria, and fungi from a variety of surfaces[3].

How to choose best wavelengths for disinfection?

The standard recommendation is a comparison model approach, starting with literature review: what have others done in similar situations and how successful were they? As you look for wavelengths that match your specific goals, make sure to keep in mind these characteristics about the target species for inactivation:

- Size
- Structure (including similar amino acids and ionic behavior)
- Kingdom and Family
- Biochemical information such as reaction pockets or active sites
- The microenvironment of chromophores

Wavelengths, dosage levels, and exposure time will all be impacted by the characteristics above. In addition, the object or organism that will be released from contaminants must be taken into consideration as well. For example, if mammalian cells are the ones that will be subject to disinfection, it's important to make sure that the dosage and wavelength do not impact relevant cellular functioning.

Wavelengths used for common and challenging contaminants

222 nm and below: studies surrounding the 200-222 nm wavelength range predict its ability to inactivate microbes while remaining safe for humans. This is due to the fact that light in the 200-222 nm range can only transverse small organisms such as bacteria or viruses and is not able to affect larger biological samples like mammalian cells[4]. There're not many conclusive and specific reports on the 222 nm mechanism of action. Most assume inactivation is due to DNA damage through dimer formation. In addition, protein UV absorption at this range is attributed to peptide bonds[5]. Given sufficient irradiance and dose, some argue protein damage could be possible.

254 nm: deactivates biomolecules by attacking the structure of nucleic acids. This is the common wavelength used by mercury UV lamps.

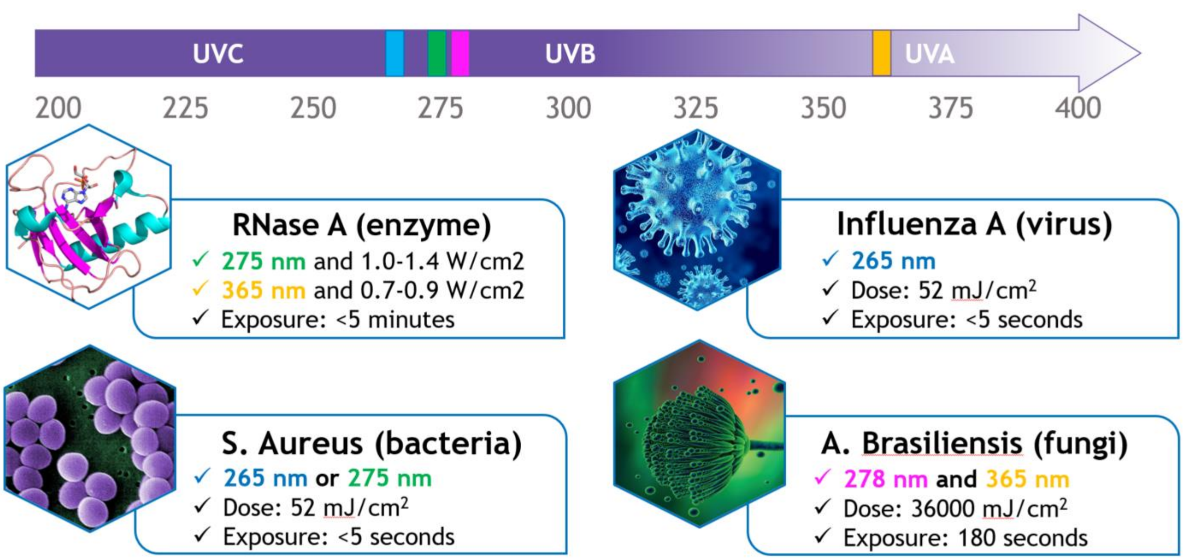
260 -265 nm: it is known as an effective bactericide and has shown great results against viruses like the Influenza A[3]. It also relies on nucleic acid damage as a mechanism of action and it is available in UV LED lamps.

The best absorbance range for nucleic acids is 240-275 nm, this is due to the $\pi \rightarrow \pi*$ transitions of the bases in purine and pyrimidine rings[5]

275-278 nm: this wavelength range is able to deactivate biomolecules through disturbing their protein structures. This is due to the peak absorption of the aromatic amino acids Tryptophan (W) and Tyrosine (T) at this wavelength range, as well as the absorption of Cysteine (making changes to disulfide bonds)[5]. These structural modifications interfere with protein functionality, therefore resulting in the inactivation of the target organisms. For example, this wavelength acts on RNase A via an effect on the aromatic amino acids proximal to disulfide bonds, reaching its complete inactivation (as shown in column 1 of Figure 1). It has shown great results against common bacteria (such as S. Aureus) and proven effective against fungi like *Aspergillus brasiliensis* and *Clostridium difficile* (in synergy with 365 nm)[3].

365 nm: this wavelength is thought to target the Lysine side chain and help destabilize the reaction pocket of enzymes like RNase A. When used in combination with 278nm light researchers at Phoseon have found complete, irreversible inactivation of RNaseA[11]. RNase A is one of the most challenging contaminants in laboratories. The complete inactivation of this enzyme in a matter of minutes opens up new possibilities, making research faster and more accurate. The 365nm synergy with 278nm has also been effective for fungal inactivation[3]. In addition, 365 nm has been shown to produce single-strand DNA breaks[6].

405 nm: This wavelength has shown the peak absorption within the 400-420 nm blue-purple range at 405 nm[7]. The mechanism of action suggested at this wavelength relies on the formation of oxygen radicals, highly reactive oxygen species that often lead to oxidative damage and cell death[8]. Research on visible light used for disinfection is at an early stage. Its germicidal efficiency is known to be less of that of UVC light, however with high enough dosages, complete inactivation of organisms could be possible.



220 nm wavelength in the market

LEDs with wavelengths below 250 nm are in an early research stage and have power in the nano-Watt range. With current technology the lowest commercially viable wavelength is 254nm. Our recommendation would be to use one of our 275nm high power systems and take the necessary precautions to avoid direct contact with the light.

Wavelengths synergy: a promising inactivation tool

Synergy between UV-A and UV-C for disinfection has been seen before in various microorganisms such as viruses and bacteria[9,10]. Phoseon's research team proved the power of 275nm and 365nm working in synergy for complete inactivation of RNase A (shown in Figure 1)[11].

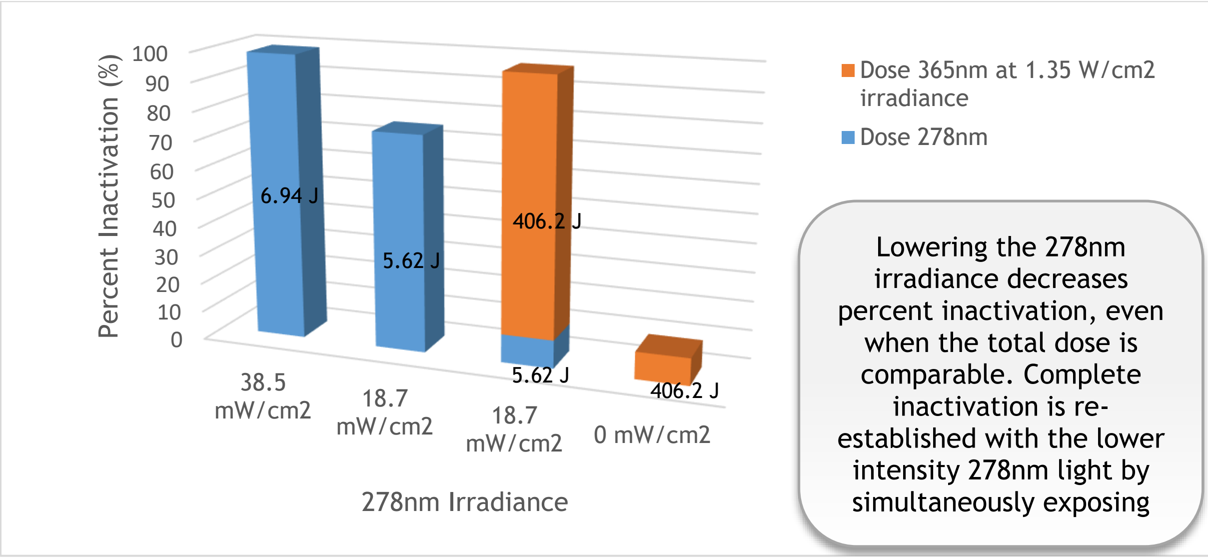


Figure 1. Complete inactivation of RNase A using a combination of 365 nm and 278 nm wavelengths. Distance from the lamp was 25 mm[11].

As Figure 1 shows, the 278 nm wavelength can inactivate RNase A at an irradiance of 38.5 mW/cm^2. However, the simultaneous use of 365 nm in combination with 278 nm reduces the irradiance needed for the same level of inactivation, potentially affecting the time needed for complete decontamination (given the direct relation between irradiance, dosage, and time). Highly resistant enzymes (RNase A) can be irreversibly inactivated by targeted wavelength UV LED sources and, further, the enzyme activity can be affected by specific wavelength combinations. This suggests a specific multi-wavelength approach to achieve inactivation of highly resistant organisms[11] and opens doors to further combinations that should be explored between UV-A, UV-C and even into the IR spectrum.

The mechanism for this synergistic effect is yet being elucidated, however preliminary data points to a few factors that might be contributing to the results seen in Figure 1. First, based on Phoseon's laboratory calculations, the 365 nm is an ideal absorption for the NH_3^+ group in the Lysine side chain, which is near the RNase A reaction pocket. Second, a hyperchromicity shift might be taking place. As shown in Figure 2, single-stranded or denatured DNA has a higher light absorption at the same wavelength. This is consistent with the results seen in Figure 1, where a much lower dose of 278 nm was needed to achieve the same level of RNase inactivation in the presence of 365 nm, indicating a greater absorption of 278 nm. In addition, 365 nm has been used as a DNA damaging agent to induce single-strand break in cancer cell studies[6]. While specific biochemical reports are needed to support this conclusion, the synergy between the simultaneous use of 365 nm and 278 nm could rely on the hyperchromicity shift of 278 nm (known as the maximum absorption for proteins) once 365 nm has destabilized DNA via the lysine side chain or by producing single-strand breaks in the molecule.

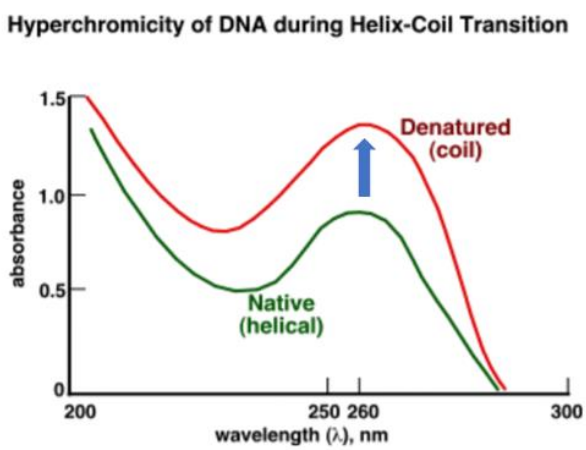


Figure 2. Hyperchromicity of DNA and absorbance peaks during Helix-Coil Transition[12]

While single-strand breaks are easily fixed by the cellular repair mechanisms such as base or nucleotide excision repair, the simultaneous attack by 278 nm (damaging essential amino acids such as Tryptophan or Tyrosine) could result in unrepairable damage and therefore complete inactivation.

How can UV LED inactivate Sars-CoV-2?

As mentioned above, UV LEDs have proven effective against some of the most challenging contaminants, raising the question of whether it would work against the biggest current global threat: SARS-CoV-2. A preliminary study in Boston University inactivated SARS-CoV-2 using UV LED with a dose of 22 mJ/cm^2. While data is limited in regard to this novel virus and the best wavelength for its complete inactivation, the information gathered to date can point us in the right direction. Many research efforts are focused on developing "information libraries". Whether they contain active sites, mutations, or genomic information; having a place where this knowledge is accessible to scientists is imperative for progress. Similarly, developing a wavelength library can be very useful to research labs. So far, we know that novel SARS-CoV-2 and Influenza A (inactivated at 265nm) are both enveloped RNA viruses and may be susceptible to UV inactivation under similar conditions.

Deep and highly controlled ultraviolet light is a key advantage to any disinfection/decontamination equipment. KeyPro™ products offer an innovative light source technology with an unmatched level of irradiance ($5W/cm^2$), allowing for greater dosages to act against the most resilient contaminants. Reliable technology, wide range of dosaging, great power, and excellent customer service.

What is the best wavelength for disinfection?

The answer to this question is highly dependent on experimental goals, however there are two competing candidates for the leading position. Traditionally, the standard peak wavelength for disinfection has been 265nm since this is the known absorption maxima for nucleic acids. Inactivation occurs due to dimer formation[13]. For many decades mercury lamp systems have been available for disinfection utilizing one of the emission peaks of mercury, 254nm (which also deactivates molecules by attacking nucleic acids). This wavelength is close enough to the absorption peak to be effective.

For decades 275nm-280nm has been known as the peak absorption for proteins, due to essential aromatic amino acids being most affected by this wavelength range. It is now shown that biomolecules can be inactivated by disturbing bonds at this protein level, therefore influencing secondary and tertiary structures. For example, the 280nm wavelength excites the aromatic group of the amino acid Tryptophan, which is preferentially placed near disulfide bonds; destabilizing these vital chemical structures (S-S bonds) will result in deactivation of molecules with minimal chance of reformation.

One of the greatest challenges for disinfection and decontamination is the ability of microorganisms and enzymes to reform after a period of time. Most cells contain a dimer repair mechanism and damaged proteins are generally recycled within cells. Viruses cannot directly repair themselves and must infect a host to take advantage of any cellular repair mechanisms. The main objective is then to damage the organism so much that the repair mechanism starts failing. Both greater dosages and the synergistic effect mentioned previously could contribute to the lack or reformation.

265nm and 275-280nm are the most effective wavelengths for disinfection. Depending on the assay in question one may be more beneficial than the other. If working at the DNA level, 265 nm would ideal; if at the protein level, 275-280nm works best. All in all, the high-power UV LED systems available at 275*nm have enhanced performance that positions them well above any mercury lamp.

*Phoseon Technology offers the possibility to customize equipment based on the wavelength needed

The transition made possible

Phoseon Technology has recently launched volume production of the KeyPro™ Explorer UVC LED evaluation kit, a compact UV LED disinfection and decontamination system that fits the needs of small or developing labs at an affordable cost.

Keypro Explorer is the perfect transition into UV LED light technology for life sciences purposes. It is a compact, air-cooled, UVC LED system that acts as the best stepping-stone to investigate new applications using UVC light. In addition, **its user-friendly approach can be great for introducing students and trainees to top-notch techniques** in disinfection, photochemical reactions, decontamination, and more…

UV Light Safety Precautions

Ultraviolet light has proven to be damaging to humans when in direct contact. Therefore, it is recommended to use protective measures such as wearing safety goggles, gloves, have restricted access to areas where UV light is in use, and make sure there is no skin exposure to the light source.

The best way to prevent UV light damage is to remove exposure altogether. Our KeyPro KP100 instrument for example has a built in safety switch on the door to turn off the light if the door is opened.

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About Phoseon Technology

The world leader since 2002, Phoseon Technology pioneered the use of LED technology for Life Science and Industrial Curing applications. Phoseon delivers innovative, highly engineered, patented LED solutions. The company is focused 100% on LED technology and provides worldwide support.

Contacts

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