We introduce a novel amorphous silicon absorption filter that has high rejection for all angles of incident light for wavelengths below approximately 700 nm. This filter is used for microscopic cancer tissue detection in a small intraoperative contact fluorescence imaging system that requires excitation light at oblique angles. Our 15 \( \mu \)m thick filter presents over five orders of magnitude rejection at 633 nm, making it compatible with several clinically tested fluorophores, including IR700DX. We have demonstrated imaging of fluorescently labeled human epidermal growth factor receptor 2+ breast cancer tissue using the filter, and we can reliably detect microscopic clusters of breast cancer cells with only a 75 ms integration time.

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Intraoperative imaging is necessary to improve outcomes from cancer surgery by allowing the complete removal of all tumor cells and ensuring no microscopic residual disease (MRD) is left behind. Fluorescently tagged molecular agents can identify tumor cells down to the single-cell level, are non-toxic, and are ideal for tissue surface imaging. Yet intraoperative fluorescence imagers themselves limit surgeons ability to detect microscopic disease intraoperatively [1]. Conventional fluorescence microscopes can be mounted above the patient to illuminate the tissue surface, filter out the background and excitation light, gather and focus the fluorescent signal, and guide it to a camera. However, these imagers are restricted by rigid and bulky optics to line-of-sight operation outside the tumor bed and far away from the labeled cells on the tissue surface, as shown in Fig. 1(a). The sidewalls of the 1–3 cm diameter tumor cavities cannot be imaged at all. Clusters of larger than 200 cells are clinically significant in cancer staging and should be removed [2], but tumor cells are often undetected and left behind. A new method of intraoperative imaging is needed to completely detect all MRD.

Multiple efforts have been made to miniaturize conventional microscopes, but they are still several centimeters in length [3,4]. Scaling down the size of optical elements is challenging due to the increased fabrication difficulty, and micro-scale components will often have worse aberration and imperfections [5]. Fiber optic imagers are hampered by the fundamental trade-off between the bending radius and imaging area.

Contact imagers, as shown in Fig. 1(b), overcome the dual challenges of sensitivity and maneuverability by dispensing with focusing optics, significantly reducing physical size, and having physical proximity to the fluorescing tissue. In vitro contact imagers can use en face illumination as they are not physically restricted by human body tissue [6,7]. However, oblique illumination is required intraoperatively, as depicted in Fig. 1(c), due to the tissue proximity. The optical filter needs to not only have high rejection for the excitation light, but also needs to maintain its performance over all incident light angles. Any photon detected by the sensor that does not originate from

![Fig. 1](https://example.com/figure1.png)

**Fig. 1.** (a) Conventional fluorescence microscopes cannot image tumor cavity sidewalls. (b) En face illumination contact imagers can only be used in vitro. (c) Oblique illumination contact imagers can be used intraoperatively. (d) Thin-film interference filters’ pass-band blue-shifts as illumination angle is increased and cannot be used with oblique illumination.
a fluorophore, including excitation light that leaks through the filter, both consumes pixel capacity and increases shot noise.

Conventional interference filters make use of finely controlled layers of materials with different indices of refraction [8]. Although these filters have high performance for perpendicularly incident light, they suffer from a dependence on the incident light angle. As this angle increases, as shown in Fig. 1(d), the optical path length difference between path $p_1$, in orange, and path $p_2$, in red, blue-shifts the filter response. For small angles of incidence, the spectrum is shifted in wavelength according to $\lambda_{\text{new}} = \lambda \left( 1 - \sin \frac{\theta}{n_{\text{eff}}} \right)^{1/2}$, where $\lambda_{\text{new}}$ is the shifted wavelength, $n_{\text{eff}}$ is the effective index of refraction of the filter, and $\theta$ and $\lambda$ are the incident angle and wavelength of the illumination light, respectively. At large angles, this effect grows, and there is a reduction in filter rejection. Consequently, interference filters allow obliquely incident excitation light to pass directly through and cannot be used in miniature fluorescence imagers.

Alternatives to explicit filters including on-chip wavelength filtering methods [9,10] generally reduce sensitivity or do not have adequate excitation light rejection. Time-resolved methods [11] are not suitable for intraoperative use as the pico to nanosecond decay times of most organic fluorophores are similar to molecules inherently present in tissue, making cancer indistinguishable. Techniques using total internal reflection and compressive sampling [12] work well in vitro, but are hard to implement in vivo since a sparse signal is required.

In this Letter, we introduce a novel angle-insensitive optical filter compatible with organic fluorophores used in both animal and human clinical studies, relieving the requirement of tight control over the incident light angle. This filter enables a lens-free fluorescence contact imaging platform. We leverage the intrinsic absorptive properties of amorphous silicon with a bandgap of approximately 1.65 eV to create an angle-insensitive long-pass filter above 700–800 nm. This wavelength range is appropriate for clinically used fluorophores, such as IR700DX [13], IR800CW [1], and indocyanine green (ICG) [14], as well as commonly used quantum dots. A single thin-film layer achieves over five orders of magnitude of excitation light rejection and can be directly fabricated on top of a complementary metal–oxide–semiconductor (CMOS) chip. We demonstrate a fluorescence contact imager including a custom CMOS image sensor and the amorphous silicon absorption filter, building on our previous work using interference filters [15,16].

In order to determine the required filter rejection, we need to determine the fluorescence emission from a single fluorophore using the relation $F = Q \sigma I$, where $F$ is the fluorescence emission in photons/(s * molecule), $\sigma$ is the fluorophore absorption cross-section in cm$^2$/molecule, $Q$ is the fluorophore quantum yield, and $I$ is the incident light flux in photons/(s * cm$^2$). A typical fluorophore has an absorption cross-section of approximately $1 \times 10^{-16}$ cm$^2$ and a quantum yield of about 10% at these wavelengths [17–19]. The typical cell is approximately 10 µm in diameter and may bind one million fluorophores. Four orders of magnitude of excitation light rejection is necessary to suppress the intensity of the excitation light that reaches the image sensor to a factor of 10 below the signal level when trying to detect a cluster of one hundred cancer cells.

We can achieve this rejection level for all incident angles, while also eliminating the need for precision multi-layer depositions by using an absorption-based filter. The choice of material and thickness depends on the intrinsic bandgap and the steepness of the absorption spectrum fall-off, the Urbach tail, which needs to be considered for amorphous semiconductors. Many organic fluorophores are optimally excited at 30–50 nm below their maximal emission, but they often have broad absorption spectra, albeit at lower fluorescence efficiency. Depending on the fluorophore, for a 100 nm difference between excitation and emission wavelengths, only a 50% reduction in emitted photons is incurred. ICG is a commonly used, Food and Drug Administration approved, fluorophore with such properties. IR700DX and IR800CW present similar behavior. The bandgap of amorphous silicon can range from approximately 1.4–2 eV, depending on fabrication methods, but typically will be close to 1.65 eV [20,21], corresponding to a wavelength of 750 nm, for the thick layers needed for filtering applications. There is no precise cutoff frequency due to the exponential Urbach tail in the absorption spectrum of amorphous silicon, allowing the filter to be designed to work with different fluorophores depending on thickness [21]. Since amorphous silicon begins absorbing photons with energy approximately close to and above its bandgap, a thin layer can be designed to act like a long-pass filter ideally suited to standard optical fluorophores with emission wavelengths from 500 to 900 nm.

To demonstrate this concept, filters were fabricated at the University of Washington, Nanofabrication Facility. Hydrogenated amorphous silicon was deposited onto 500 µm thickness fused silica wafers at a rate of 120 nm/min, using a SPTS APM plasma-enhanced chemical vapor deposition with a process pressure and temperature of 1 Torr and 350 °C, respectively. Silane (SiH$_4$) and argon gas flow into the chamber at 300 sccm and 1500 sccm, respectively. 5, 10, and 15 µm thick filters were fabricated. Hillock density [22] increases with increasing film thickness over some areas of the wafer. The hillocks become noticeable for 10 and 15 µm thickness layers, as shown in Fig. 2, but remain sparse enough to obtain flat, uninterrupted sections for imaging.

Figure 3(a) shows the transmittance spectra for 5, 10, and 15 µm thickness amorphous silicon films taken on a Perkins Elmer Lambda 850 UV-Visible Spectrometer. Thicker films push the long-pass transmission wavelength above 700 nm with a sharper cutoff. At 15 µm thickness, over five orders of magnitude of optical rejection is achieved when illuminating at 633 nm and imaging at 733 nm. The rejection at 633 nm

![Fig. 2. Hillock size and density increase as amorphous silicon film thickness increases, but remain sparse enough to obtain areas suitable for imaging.](image-url)
is 60.7 dB. The transmission in the pass-band is approximately 54% with insertion loss of 2.7 dB, mainly due to the reflection off the high refractive index of 4.3 of amorphous silicon. The ripple in the pass-band is due to interference effects in the thin filter layer. Figure 3(b) displays the absorption coefficient versus wavelength extracted from the transmittance curve data. The spectra are fit to \( T = \exp(-d\alpha) \), where \( T \) is the transmittance, \( d \) is the layer thickness, and \( \alpha \) is the attenuation coefficient.

The optical properties of our amorphous silicon filter agree with previously published work [20,21,23]. Figure 4 shows the 10 \( \mu \)m film thickness transmittance data overlaid with the excitation point and emission curves for IR700DX. This demonstrates that our filter is applicable to commonly used and clinically tested fluorophores. Dye-based absorption filters typically have 20–30 dB lower rejection in the stopband for similar thicknesses [24–28]. While dye-based filters have 1–2 dB lower insertion loss in the passband, this can be compensated by increasing the excitation light power, and the amorphous silicon filter offers a higher contrast due to its superior stopband rejection. Additionally, the amorphous silicon filter can be readily fabricated and patterned with micron-precision at the wafer level.

To demonstrate the angle insensitivity of the amorphous silicon filter compared to that of two thin-film interference filters, ET700/75m and ET705/40m (Chroma), we epoxied 3 mm by 5 mm sections of the respective filters onto a CMOS imager with angle-selective gratings (ASG) [15,16] using EpoTek 301 epoxy. The amorphous silicon filter was 15 \( \mu \)m thick. We illuminated the sensor with 633 nm collimated light at incident angles from the perpendicular ranging from 0° to 90° and measured the received signal response. A small fraction of light penetrates the filter, providing the signal. The normalized signal versus incident angle for all three filters is plotted in Fig. 5. The rejection of the two conventional interference filters degrades gradually with an increasing angle below 30°. Above approximately 30°, their rejection degrades exponentially. This behavior makes these filters incompatible with applications requiring illumination at even modestly steep angles or where the illumination light may be scattered by human tissue. The signal received by the sensor covered with the amorphous silicon filter smoothly falls off as the angle is increased, making the filter suitable for intra-operative cancer imaging. The smooth fall off is mainly due to the presence of the ASGs.

The spatial resolution and sensitivity is set by the spacing between the imager and the tissue and angular response of the sensor [16]. An imaging distance of 0.5 mm and the sensor’s ±18° angle-of-view corresponds to a field-of-view for each pixel of 0.22 mm, containing approximately 120 cells, less than the 200 cell requirement [2]. The insertion loss slightly increases with angle due to a longer path length, but this effect is negligible due to the high refractive index of the filter that limits the path length increase to less than 1% for incident light in the ±18° angle-of-view. Since the sensor has residual response for large angles (Fig. 5), the excitation light needs to be filtered out in that range, and this is accomplished by our filter.

We choose to image a human epidermal growth factor receptor 2 positive human breast cancer patient tumor to demonstrate applicability to the tissue architecture associated with invasive tumors \textit{in vivo}. The tissue is mounted on a glass slide and is stained using an anti-HER2 antibody and a quantum dot, Qdot 705, secondary antibody. Figure 6(a) shows a 75 ms integration time image taken using the image sensor with ASG and a 15 \( \mu \)m thick amorphous silicon absorption filter. The corresponding microscope image taken with a 5 s integration time is shown for reference in Fig. 6(b), but this microscope image could not have been obtained in an operating room. The tumor infiltrating the normal breast tissue is seen as the brighter areas. The area labeled Slide is the background.

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**Fig. 3.** (a) Amorphous silicon filter transmittance spectra. (b) Attenuation coefficient extracted from the spectra.

**Fig. 4.** 10 \( \mu \)m amorphous silicon filter measured transmittance spectrum overlaid with the IR700DX dye emission spectrum and 633 nm excitation line.

**Fig. 5.** Normalized angular response of a CMOS image sensor with ASG and optical filter using a 633 nm collimated laser source. The performance using two interference filters, ET700/75m and ET705/40m, and the 15 \( \mu \)m amorphous silicon filter is shown.
from the glass mounting slide, corresponding to filter bleed through and nonspecific binding. The cancer tissue is clearly distinguishable from both healthy tissue and the slide background with an integration time of only 75 ms in the custom sensor image taken using the amorphous silicon filter. This allows our imaging system to be used intraoperatively and in real-time.

Our approach, using semiconductors as optical filters, is not limited to only amorphous silicon. While amorphous silicon is ideal for fluorophores that emit close to 700–800 nm, including Alexa Fluor 700 and IR700DX, it is possible to use other materials compatible with microfabrication, including gallium phosphide (2.25 eV bandgap, 550 nm cutoff), cadmium selenide (1.74 eV bandgap, 710 nm cutoff), gallium arsenide (1.43 eV bandgap, 870 nm cutoff), indium phosphide (1.27 eV bandgap, 980 nm cutoff), and crystalline silicon (1.11 eV bandgap, 1100 nm cutoff). These materials allow a range of fluorophores and imaging systems to be used depending on the application.

We have described and demonstrated a new system for fluorescence contact imaging. We have dispensed with conventional thin-film interference filters that have poor angular response and instead use an amorphous silicon absorption filter epoxyed onto a custom CMOS image sensor. The filter has over five orders of magnitude rejection at 633 nm, and our imaging system can reliably detect breast cancer with only a 75 ms integration time. No large optical elements or physically restricting optical fibers are necessary to control the illumination and emission angles. Without these components, the imager can be miniaturized and placed directly in contact with the tissue surface, enabling rapid intraoperative imaging of fluorescently labeled tumor cells.

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**REFERENCES**