Integration of automated image processing via a smartphone application with multicolor Rapid Tests for cutting-edge liquid biopsy applications

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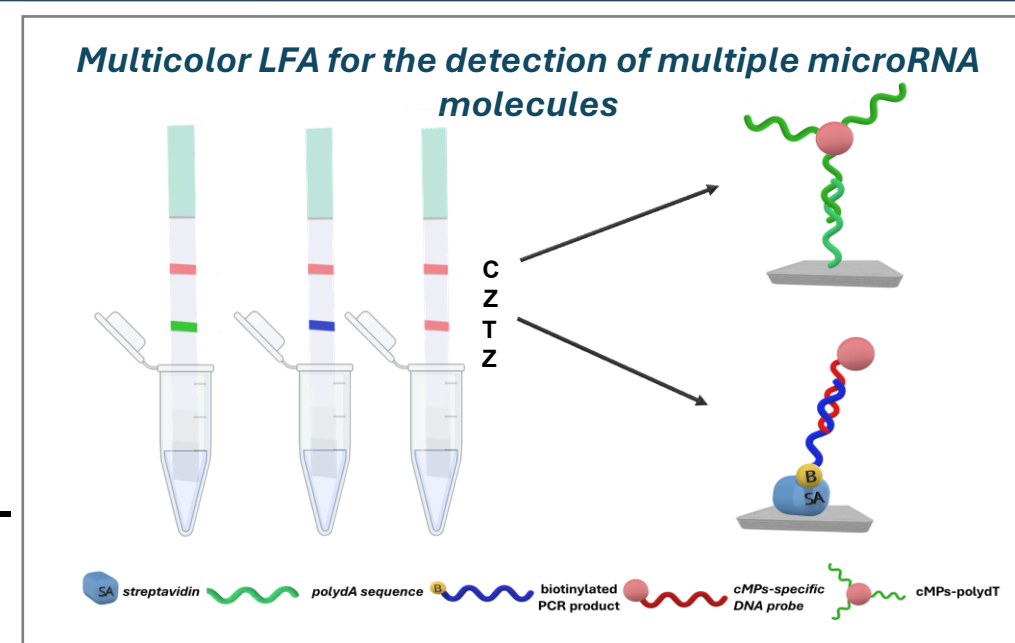[1]Department of Chemistry, University of Patras, Patras, GR26504, Greece
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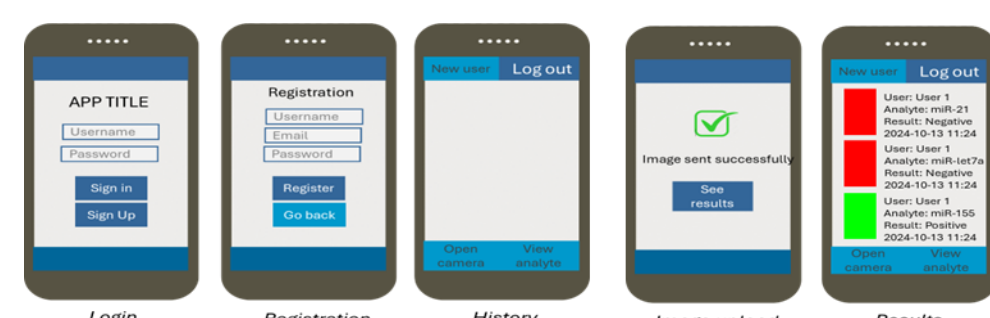
INTRODUCTION & AIM

Lateral flow assays (LFAs) are valuable diagnostic tools in various fields. To improve detection efficiency in Point-of-Care (POC) devices, AI and image analysis (IA) are increasingly utilized for enhanced accuracy in Analytical Chemistry and Biosensing Technology. A novel multicolor LFA system has been developed, using different colored beads to distinguish multiple targets, specifically for microRNA detection in urine samples. This integrated system has demonstrated high performance, achieving approximately 99.3% accuracy, 99.1% sensitivity, and 100% specificity, showcasing its potential for reliable diagnostic applications.
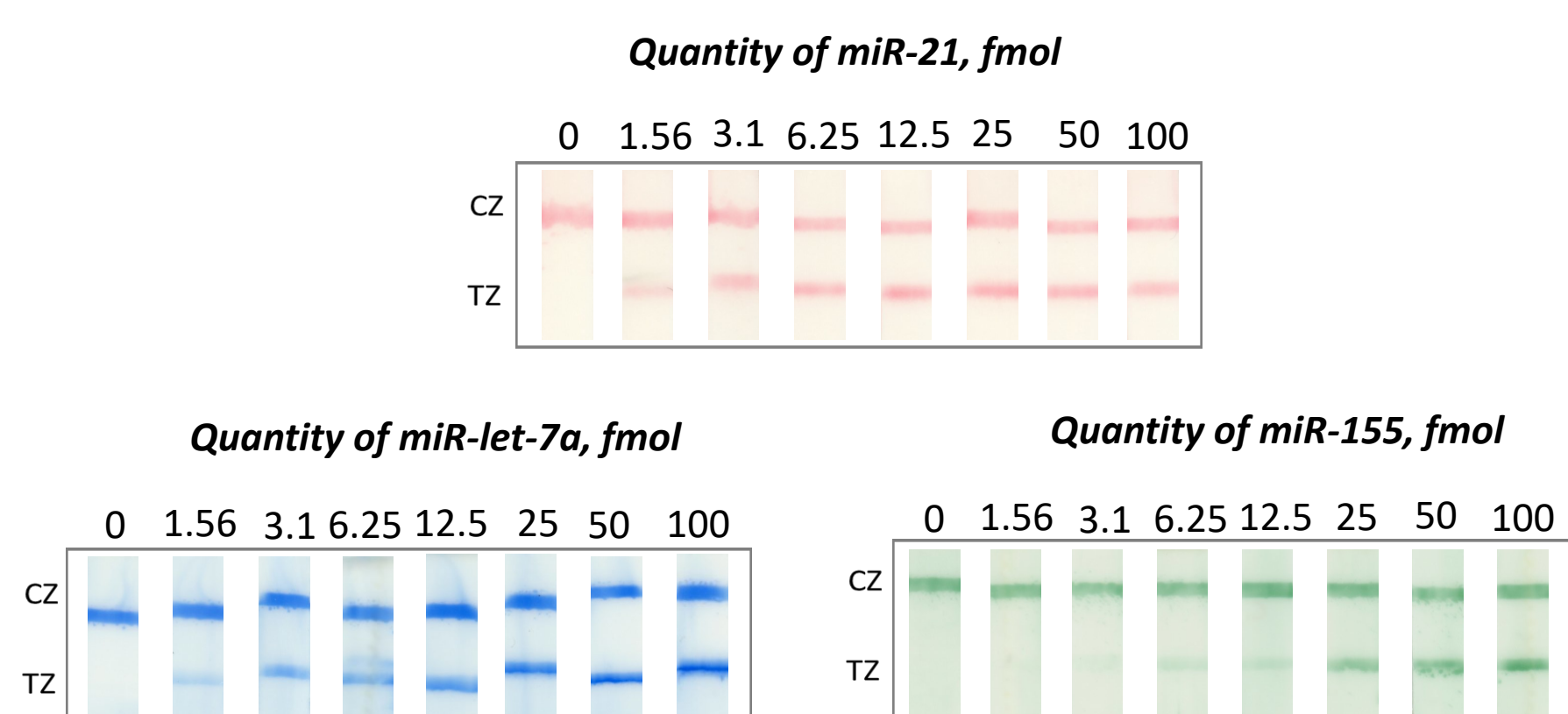
METHOD

1. Isolation of miRNA-21 and miRNA-let-7a from spiked synthetic urine and normal urine samples using a microRNA isolation kit.
2. Synthesis of cDNA using a stem-loop primer and PCR amplification.

Multicolor LFA for the detection of multiple microRNA molecules

3. Conjugation of colored carboxylate polystyrene beads with specific NH2-probes complementary to miRNA-21, miRNA-let-7a and miRNA- 155 and with NH2-dT(30).
4. Visual detection of microRNAs with the multicolor LFA using colored polystyrene beads as reporters.
5. Detection and characterization of random samples with the use of a mobile (smartphone)/web application and automated image analysis.
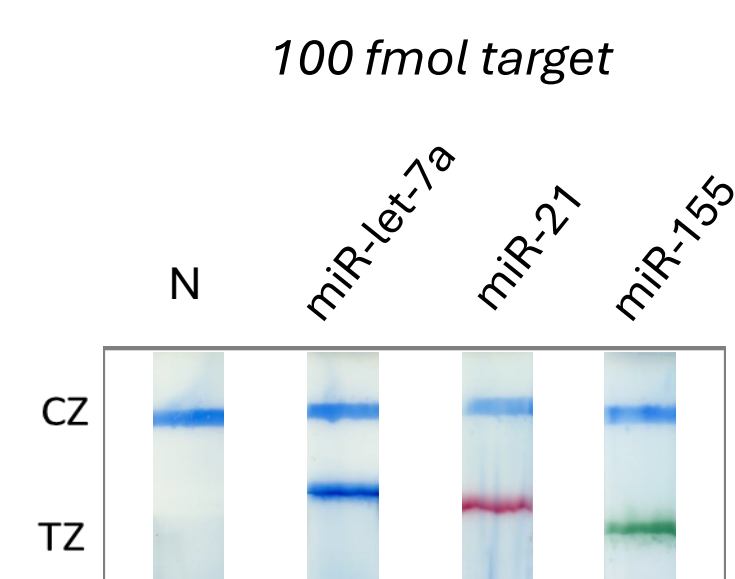
RESULTS & DISCUSSION

Login | Registration | History | Image upload | Results

Calibration Curves of the three targets

Quantity of miR-21, fmol
0 1.56 3.1 6.25 12.5 25 50 100
CZ
TZ

Quantity of miR-let-7a, fmol
0 1.56 3.1 6.25 12.5 25 50 100
CZ
TZ

Quantity of miR-155, fmol
0 1.56 3.1 6.25 12.5 25 50 100
CZ
TZ

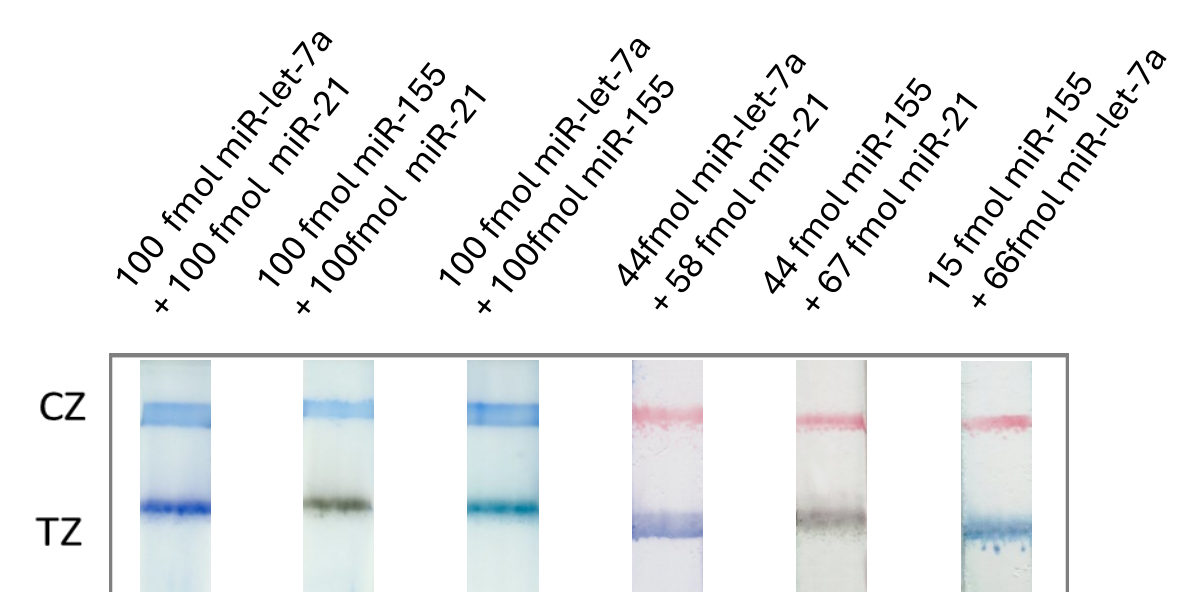### Detection of microRNAs with the multicolor lateral flow strip

Detection of microRNAs in mixture
Specificity of the lateral flow assay

100 fmol target
N | miR-let-7a | miR-21 | miR-155
CZ
TZ

Detection of microRNAs in mixture

100 fmol miR-let-7a + 100 fmol miR-21 | 100 fmol miR-155 + 100fmol miR-21 | 100 fmol miR-let-7a + 100fmol miR-155 | 44fmol miR-let-7a + 58 fmol miR-21 | 44 fmol miR-155 + 67 fmol miR-21 | 15 fmol miR-155 + 66mol miR-let-7a
CZ
TZ

Analysis of positive samples of various concentrations

miR-21: 15 fmol | 38 fmol | 53 fmol | 74 fmol | 83 fmol

miR-let-7a: 9 fmol | 17 fmol | 32 fmol | 67 fmol | 89 fmol

miR-155: 16 fmol | 27 fmol | 49 fmol | 78 fmol | 92 fmol

Confusion matrix
Interpretation

		Negative	Positive
Actual	Negative	41	0
	Positive	1	113
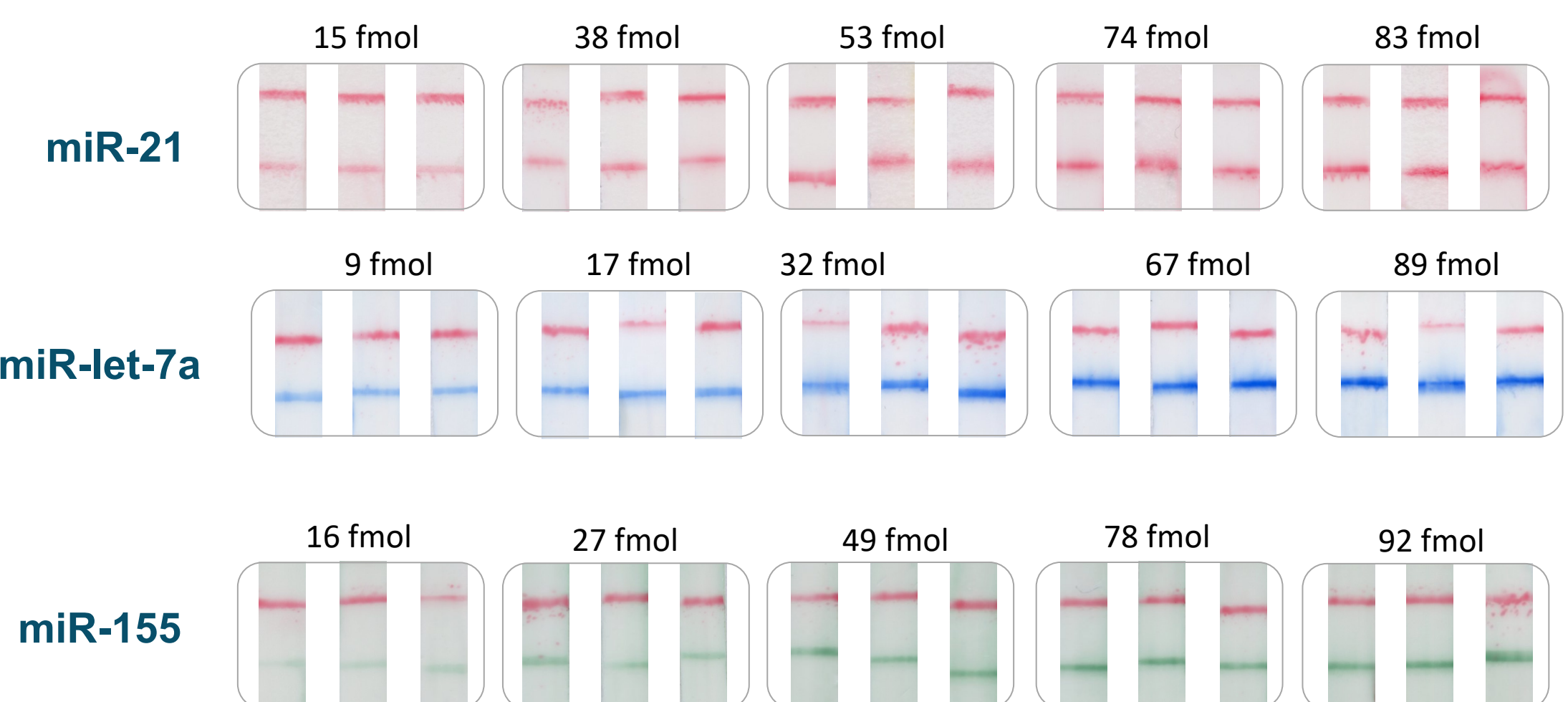
Images of the lateral flow strip tests acquired by the smartphone and used for further analysis by the web application.
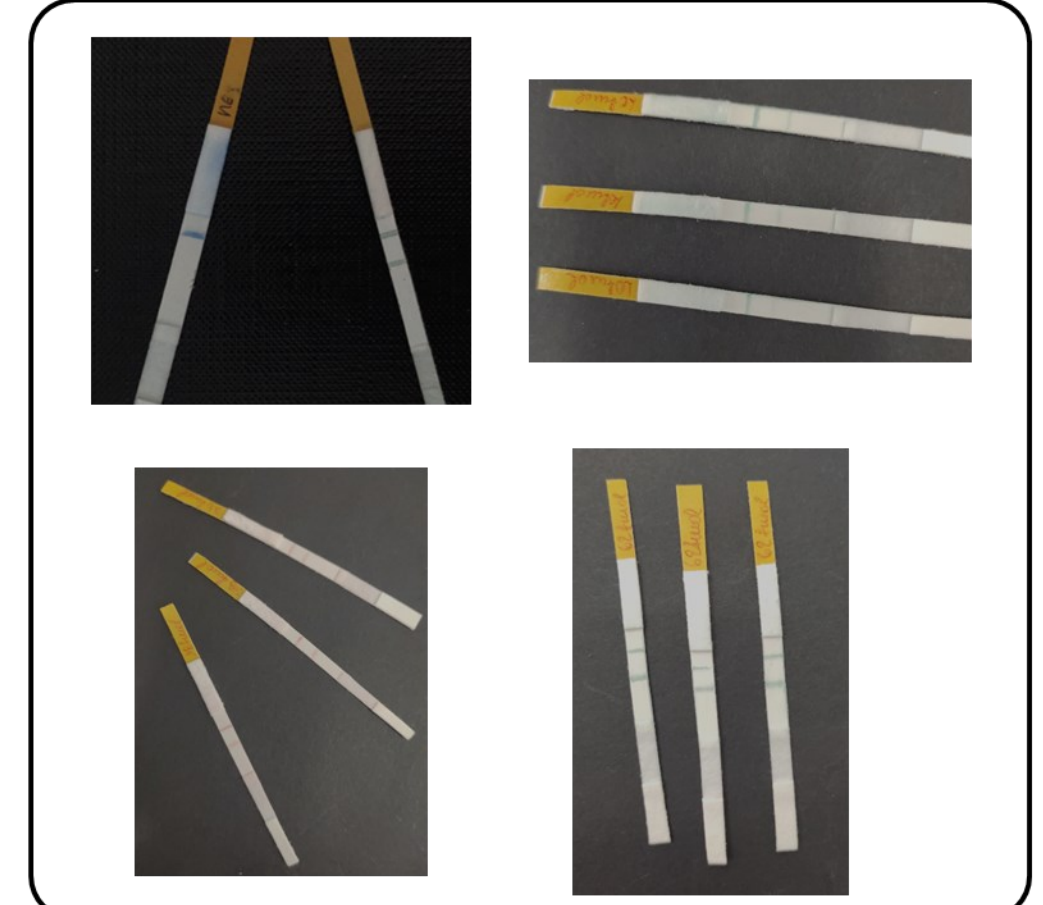
CONCLUSION

In this work, a combination of multicolor LFA with smartphone-based automated image analysis was developed for precise interpretation of the visual outcome of multiplex LFAs. The automated image-based application successfully detects multiple biomarkers with high accuracy, sensitivity, and specificity, significantly reducing human error of subjective interpratation. The system's performance indicates its potential for effective point-of-care diagnostics, making it a valuable tool in clinical settings..

FUTURE WORK / REFERENCES

Future developments will focus on enhancing multiplexing capabilities to broaden its application in real-world scenarios.