Electronic Supplementary Material (ESI) for Lab on a Chip.

Development and evaluation of inexpensive automated deep learning-based imaging systems for embryology

Authors

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Supplementary Information

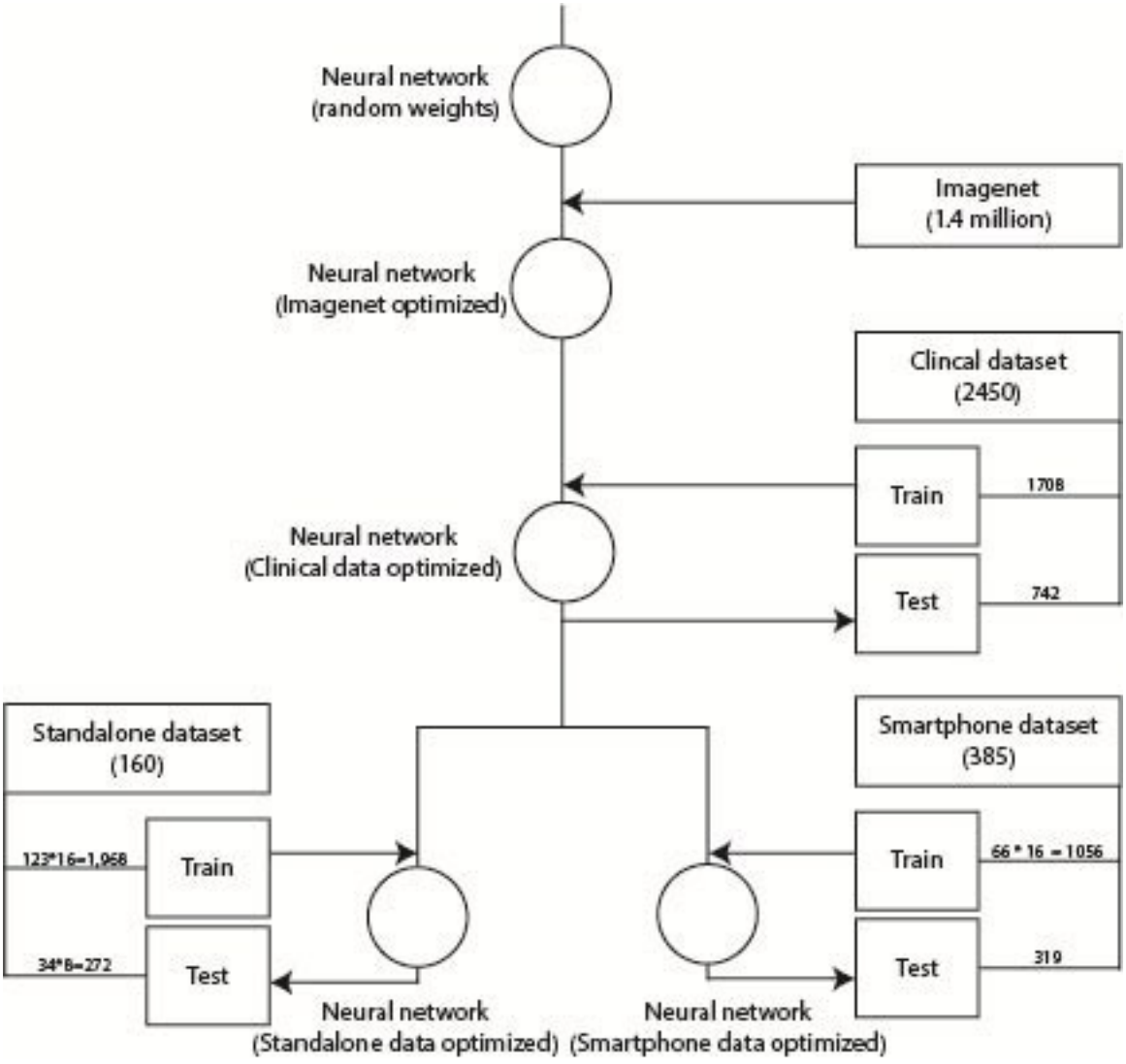

Figure S1. Data splits and flow in the layered learning process. Xception architecture is used in transfer learning feature maps of embryos at day 5 of development using embryo images acquired from different instruments. Training set is further split into training and validation in all cases and is used to optimize the network. Independent data sets were used during system evaluation and testing.

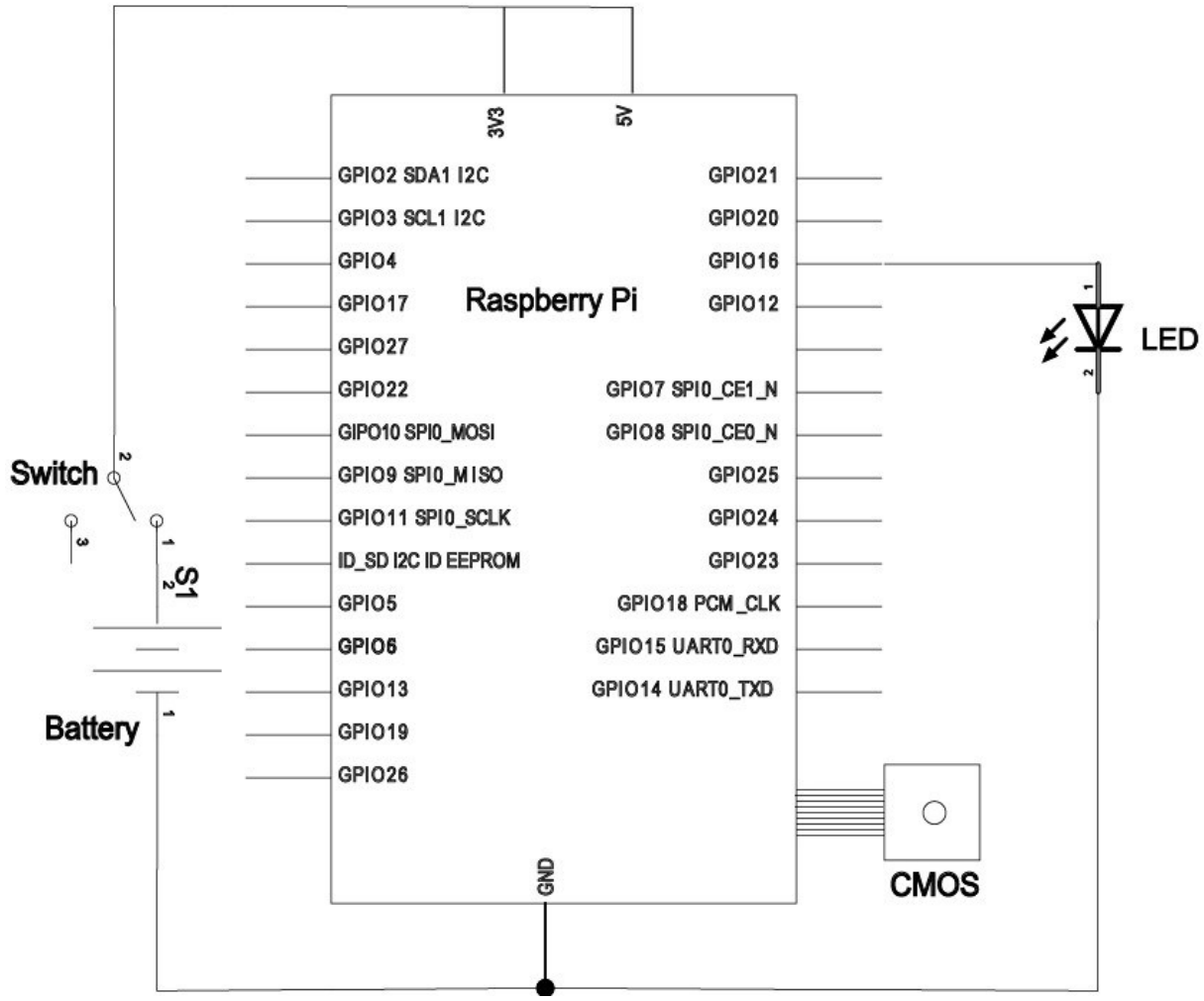

Figure S2. The circuit diagram of the stand-alone optical system. Wireless communication between the smartphone and the optical attachment was achieved through a wifi dongle attached to the raspberry Pi. The CMOS image sensor and an LED are connected to the controller, which controlled by the smartphone application.

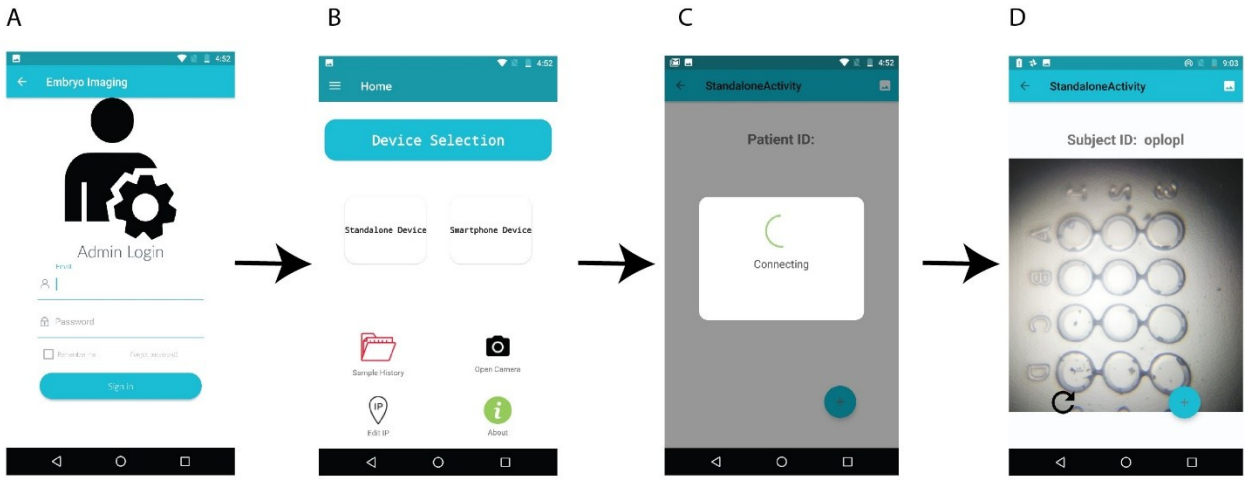

Figure S3. Smartphone application flow. These figures show the general process flow of the android application developed for the imaging systems developed for the study. (A) The smartphone application provides the user login screen so that data collection can be streamlined and better organized along with the added security. (B) The home screen allows for selection of the hardware imaging system between the stand-alone and the smartphone optical systems. (C) When the stand-alone optical system is selected, the application attempts to connect with the device via wifi and once a connection is established, (D) a live feed is presented which can be imaged by user at the press of a button. If the smartphone system is selected on the home screen of the application as shown in (B), the system automatically proceeds to the live camera feed through the cellphone's front camera.

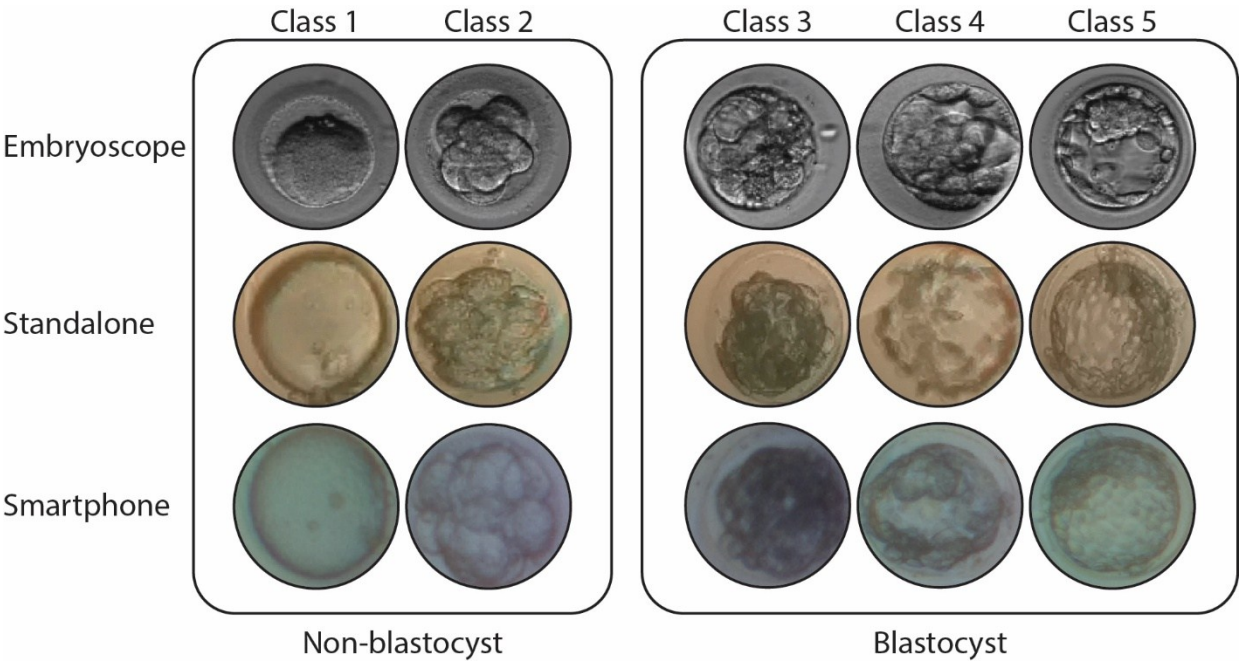

Figure S4. Embryo images collected at day 5 of development using different imaging systems. Classes 1 to 5 signify the quality of embryo development at day 5 of embryo culture (113 hours post-insemination). Classes 1 and 2 make up non-blastocysts and classes 3, 4 and 5 make up blastocysts.

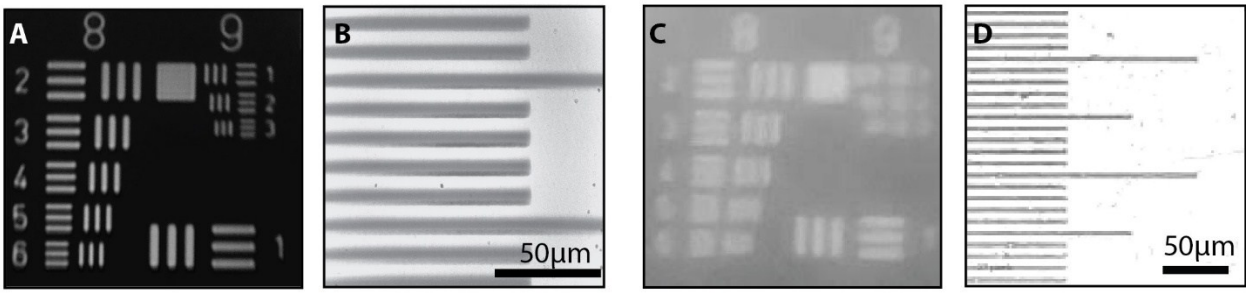

Figure S5. Image of a 1951 USAF resolution test chart and a micrometer scale with the optical systems. (A) and (B) are images acquired using the stand-alone system. (C) and (D) are images acquired using the smartphone system. **(A)** Image of the 1951 USAF test targets recorded with stand-alone optical device with the lowest resolvable limit of 0.78 microns. **(C)** Image of the 1951 USAF test targets recorded with the smartphone optical system with the lowest resolvable limit of 1.74 microns. The images (B) and (D) show the stage micrometer (Omax, B00FG89F0M) with 10 µm divisions. (B**)** Each micron imaged by the stand-alone device was represented by 25 pixels. (C) Each micron imaged by the smartphone system was represented by 2 pixels. The dimensions of the image shown here are 113 x 113 pixels.

Material costs		
Item	**Cost (USD)**	**Total (USD)**
Standalone imaging system		**84.5**
Objective Lens	30	
LED	0.1	
Camera	19	
Raspberry Pi	30	
3D printed parts	5	
Battery	0.4	
Smartphone imaging system		**2.5**
Lens	1	
LED	0.1	
Battery	0.4	
3D printed parts	1	

Table S1. Estimated material costs of the devices. Materials used for manufacturing the stand-alone and smartphone-based optical systems for embryo assessments. The costs do not include the smartphone and embryo culture dishes.

Non-blastocysts		Blastocysts		
Class 1	Class 2	Class 3	Class 4	Class 5
Degenerate[1]	Early Morula	Early Blastocyst[2]	Grade 2 <cc Blastocyst	Grade 3 >cc Blastocyst
	Morula		Grade 3 <cc Blastocyst	Grade 4 >cc Blastocyst
			Grade 4 <cc Blastocyst	Grade 5 >cc Blastocyst
			Grade 5 <cc Blastocyst	Grade 6 >cc Blastocyst
			Grade 6 <cc Blastocyst	

Notes:
Overall blastocyst grade syntax: Size (1-6), Inner cell mass grade (D-A), Trophectoderm grade (D-A)
The range is presented in ascending order of quality, i.e., 1 and D being the lowest and 6 and A being the highest
[1]Embryo failed to develop to at least the morula stage
[2]No ICM or TE score is given for Early Blastocysts

Table S2. Grading system used for the annotation of the embryos. Embryos were annotated using the modified Gardner system of blastocyst grading used by embryologists at Massachusetts General Hospital Fertility center. Neural networks were trained on 5 classes and a universally accepted 2-class system (blastocyst & non-blastocyst) was used to test the neural networks' performance.