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Research Article

Assessing the Impact of Dynamic Software Environments (MATLAB) on Rural-Based Pre-Service Teachers' Spatial-Visualisation Skills

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Abstract

Dynamic visual tools such as MATLAB have inbuilt features which are believed to be able to empower students to learn through the visualisation of three-dimensional objects. While student learning through MATLAB has been investigated regarding students in urban settings, only a handful of studies have investigated how MATLAB can assist students in rural settings. Spatial visualisation (SV) as a measure or reflection of one's cognitive reasoning is affected by family social economic status (SES). For instance, it is argued that SES in combination with other components, do enhance cognitive development in different ways. What is meant is that components such as but not exclusively, economic and occupational components of SES may vary and hence provide opportunities for generating better understanding of education (cognition). In this study, we randomly selected 100 second-year rural-based pre-service teachers in a vector calculus class at University of Zululand (UNIZULU). Students need SV skills to learn vector calculus and the Purdue spatial-visualization test/rotations (PSVT/R) is well established for measuring individuals' spatial reasoning. In this study, spatial reasoning skills were assessed through a vector calculus pre-test and through a post-test using the Purdue spatial-visualization test/rotations (PSVT/R). The experimental group of students learned the vector calculus topics supported by activities and investigations using MATLAB. Duval's Theory of Register of Semiotic Representation (TRSR) was employed to comprehend the impact of MATLAB on rural-based pre-service teachers' spatial-visualisation skills. From using an independent sample t-test, our findings indicated that, for participants in this study, using MATLAB had positive impact on the rural-based pre-service teachers' SV skills.

Keywords: spatial-visualisation skills, dynamic visual tool and learning, spatial reasoning, vector calculus and mental rotation

INTRODUCTION

While spatial-visualisation (SV) skills are only one facet of a person's overall intelligence, research suggests that spatial reasoning is an important predictor of achievement in science, technology, engineering, and mathematics disciplines (STEM) (Stieff & Uttal 2015; Uttal et al., 2013; Wai et al., 2009). Thus, spatial skills have been considered to form the basis of several learning disciplines. Some mathematics psychology

researchers have argued that the ability to rotate, compose, and decompose two- and three-dimensional objects in space mainly describes spatial skills (Nguyen & Rebello, 2011; Price et al., 2013; Šipuš & Cizmešija, 2012; Sorby et al., 2013; Stieff & Uttal, 2016; Wai et al., 2009; Zhang et al., 2014). Also, studies have proven that spatial-visualization skills are trainable through visual interaction (e.g., mental rotation tasks) and dynamic software and other computer environments (Cheng & Mix, 2014; Ćurčić et al., 2018; Joshi & Rawal, 2021; Mailizar & Fan, 2021; Uttal et al., 2013). While similar conclusion has been suggested by Mailizar and Fann (2021), Ćurčić et al. (2018) revealed that the integration of software has a link with students' knowledge and hence increase not only the interest in studying content, but also the teaching.

Altogether, these interconnected notions have provided researchers rich opportunities to investigate student understanding and SV skills through the integration of dynamic software (e.g., GeoGebra, Maple, MATLAB, CAS and other computer environments) in various learning disciplines. While this research has previously predominately considered student learning in mostly urban settings, this study considers rural college student learning and growth in mental rotations in the context of vector calculus using MATLAB. For example, in comparing the ongoing studies in the investigation of impact of dynamic software environments (MATLAB) of pre-service teachers' spatial-visualisation skills, one key factor stands out. Thus, while ongoing research points to pervasive usage of dynamic software environments in urban settings, little to no evidence, this far exist in the context of rural settings particularly with the connections that link SV skills, mental rotations, and learning through the dynamic visual tool MATLAB. Consequently, in rural -settings, little to no evidence exist to unpack a) digital technology and learning, b) mental rotation skills as a measure of spatial reasoning skill, c) spatial reasoning and cognitive reasoning, and d) vector calculus.

In response to such limited body of knowledge, this study explores connections linking SV skills, mental rotations, and learning through the dynamic visual tool MATLAB. We do so through a range of conceptual underpinnings for a) digital technology and learning, b) mental rotation skills as a measure of spatial reasoning skill, c) spatial reasoning and cognitive reasoning, and d) vector calculus. To fully address the impact of rural-based pre-service teachers' spatial-visualisation using MATLAB, we draw from a theoretical perspective on spatial visualisation of Duval's (1995) theory of register of semiotic representation (TRSR).

While research reveals that computer-aided tools can enhance students' spatial visualisation (SV) (often believed to be one of the defining characteristics of intelligence), this has had limited attention in South Africa in respect to learning vector calculus. Vector calculus integrates vectors and calculus and is extensively applied in both mathematics and physics (Bollen, van Kampen, & De Cock, 2015; Ferrer, 2016; Fleisch, 2011; Gire & Price, 2012).

Gire and Price (2014) found that students made many errors when sketching graphical representations of vector fields, and they suggested that instructors should be aware that some representational features may have two potential meanings (e.g., length, meaning both distances between points and strength of a field) or do not match what is being represented (e.g., closer spacing in field line diagrams corresponds to a greater magnitude). This may result from insufficient instructional emphasis focusing on graphical representations through traditional instructional techniques. The conclusion drawn from the works of Törnkvist et al. (1993), and Gire and Price (2014) is that students need to grasp vector concepts and apply their spatial reasoning in vector calculus problem-solving situations.

Integral functions (i.e., integration techniques and theorems) are employed in vector calculus for the calculation of areas bounded by curves, the volume of solids revolution, the centroid, the moment of inertia, fluid pressure, work, etc. (Ferrer, 2016). These calculations are grounded on three theorems: Green's theorem, which relates to line integrals; Stokes' theorem, relating to surface integrals; and Guass' theorem, regarding flux through a given surface volume integrals involving field's divergence.

Integration of areas bounded by curves and volumes of solids of revolution require high spatial reasoning ability including the ability to; draw a sketch or visualized 3D mathematical object in space for a given algebraic expression; interpret a sketch with understanding; and use the 3D object as an aid to write out the integral function (Zazkis et al., 1996). Interestingly, while some students find it easy to evaluate a given vector integral using analytical techniques, some struggle to visualize and transform it from one coordinate system

to another – a skill requiring more sophisticated problem-solving. Research suggests that imagery, spatial skills, and multiple representations aid in cognitive reasoning enhancing problem-solving skills (Casey et al., 2015; Rebello et al., 2012).

Objective of the Study

This study explores connections linking SV skills, mental rotations, and learning through the dynamic visual tool MATLAB. We do so through a range of conceptual underpinnings for a) digital technology and learning, b) mental rotation skills as a measure of spatial reasoning skill, c) spatial reasoning and cognitive reasoning, and d) vector calculus. To fully address the impact of rural-based pre-service teachers' spatial-visualisation using MATLAB, we draw from a theoretical perspective on spatial visualisation of Duval's (1995) theory of register of semiotic representation (TRSR).

LITERATURE REVIEW

Digital Technology and Vector Calculus Challenges in Rural Setting

Mathematics education, like other learning disciplines, incorporates digital technology to explore and enhance teaching and learning (Andreatos et al., 2015; Phillips, Norris & Macnab, 2010). For instance: Phillips et al. (2010) examined concept of visualization in mathematics; Baltaci and Yildiz (2015) investigated elementary pre-service teachers use of GeoGebra in the context of 3D objects; Andreatos and Zagorianos (2009) explored the use MATLAB in teaching control systems; and others such as Hodanbosi (2001), and Baltaci and Yildiz (2015) offered insights to find the value of digital technology for learning.

Despite ongoing research, numerous dimensions remain ripe for further investigation, including: appropriate pedagogical uses of digital technologies; how digital technologies are evaluated in relation to the effective learning; and the critical analysis of teaching and learning of various mathematical topics with digital technology. Generally accepted is that the appropriate use of digital technology can be a catalyst to greater academic achievement. The value of technology on teaching and learning in mathematics education has been underscored by the National Council of Mathematics Teachers (NCTM) (NCTM, 2000). The ubiquitous nature of computers has increased the technological focus in many disciplines (e.g., STEM). Research has shown that computer-assisted instrumental materials have a major influence on teaching and learning (Baltaci et aL., 2001).

The noteworthy use of technology in mathematics education has led to the invention of dynamic computer tools such as MATLAB, GeoGebra, Maple, and other computer environments (Baltaci & Yildiz, 2015). These computer environments can empower students to think, learn, problem-solve, and grow in the visualisation of three-dimensional objects. These computer environments are constructed with tools through which well-designed activities can engage students to intuitively and visually investigate and manipulate various geometric relationships between surfaces, space curves, vectors, vector fields, and other calculus related shapes. Using these technology tools, more readily visualized are relationships between surfaces (space curve) and rotations to gain 3D perspective (Bollen et al., 2015).

Mental Rotation Skills as a Measure of Spatial Reasoning Skills

Measuring spatial reasoning skills through the ability to perform mental rotations has received considerable attention for decades (Hoffkamp et al., 2010; Jansen et al., 2013; Koch, 2006; Mix & Cheng, 2014; Shepard & Metzler, 1971; Utall et al., 2013; Wai et al., 2009). For example, hinged upon the work of Osman et al. (2018), the researchers investigated the relation in enhancing students' mathematical problem-solving Skills through Bar Model visualisation technique. Similar studies have been conducted (Joshi & Rawal, 2021; Mailizar & Fan, 2021). The conclusion was that students understanding, and motivation influenced performance. Additionally, students performed positively after the implementation of the Bar Model in mathematical problem-solving. However, one key component as suggested by Mailizar and Fann (2021) is that instructional practices coupled with technology has ramifications with the type of tasks they set in lessons, and example being mental rotation. Mental rotation is the ability to mentally rotate 2D or 3D object in a space. This ability

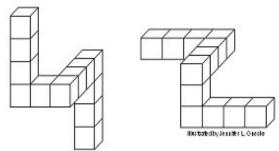


Figure 1. Isometric block

is measured through a number of recognized cognitive tasks: the mental rotation task (Shepard & Metzler, 1971); mental paper folding (Shephard & Feng, 1972); and the Purdue visualization rotation (Bodner & Guay, 1976). An illustrative example in **Figure 1** gives an overview of spatial measurement. The ability to accurately predict or imagine orientation of the cubes (refer to **Figure 1**) measures an individual's spatial skills by accurately providing answers to questions such as; are these two shapes different or are they identical and merely reoriented? The dependent time coupled with providing an accurate answer to this question is believed to measure an individual's spatial abilities.

Researchers have also found evidence to suggest that an individual's performance in mental rotation tasks correlate with his/her achievement in several STEM learning disciplines (Mix & Cheng, 2014). This may evidence that students who attain a high score in STEM disciplines tend to exhibit high spatial abilities (Hoffkamp, 2011; Höffler, 2010; Jansen et al., 2013; Koch, 2006; Utall et al., 2013; Wai et al., 2009). Wai et al. (2009, p. 817) advance the notion that mental rotation ability is a predictor of an individuals' future career. Contradicting opinions of some (Mix & Cheng, 2014; Wai et al., 2009), Sipus and Cizmesija (2012) argue that mental skills and achievement is gender independent.

Research also identified the connection between mental rotation skills and early age (Verdine et al., 2014). Development of mathematics aptitude and spatial reasoning are closely tied, and early spatial intelligence predicts a child's achievement in mathematics (Verdine et al., 2014). As a result, young children with high visualising skills tend to perform better in mathematics achievement. This relationship may be based on shared underlying processes.

Hence, it could be concluded that applications involving spatial skills may contribute to bridging the gaps in assessing the impact of dynamic software environments on rural-based pre-service teachers' spatial-visualisation skills.

THEORETICAL FRAMEWORK

In the context of addressing the impact of MATLAB on rural-based pre-service teachers' spatial-visualisation, the notion of representation plays a significant role. Duval's (1995) theory of register of semiotic representation activity" (p. 15). The idea drawn from this is that representations mediate cognitive reasoning. One of the distinctions of the semiotic representations is dependence on an organised system of signs such as linguistic, algebraic, symbolic writing, and cartesian graphs. This means that all semiotic representations must be interpreted in the context of the register in which they were formed and with an understanding of what they clearly represent and what they cannot represent. The TRSR recognizes two cognitive actions: treatments, which involves movement of a representation within the same registers where both registers are associated with the exact same mathematical concept (e.g., the symbolic expression $x^2 - 1$ is rewritten as (x + 1)(x - 1)) and conversions, where a representation in one register is translated to a representation in a different register (e.g., the equation $x^2 - 4$ is translated into a graph). Conservation among semiotic representation – whether in the same or a different register.

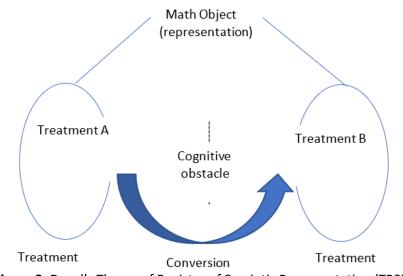


Figure 2. Duval's Theory of Register of Semiotic Representation (TRSR)

According to Duval's (1995) TRSR, students often struggle to perform conversions between registers, particularly if the conversion does not include an algorithm for mapping objects from the source to the target register. Fortunately, upon request, technological graphical tools such as GeoGebra, Applets, and MATLAB readily perform conversions among numeric, symbolic, and graphical representations. In the context of this study, the 3D solid figures associated with vector integration problems are readily translated from one coordinate system to another coordinate system, providing students with quick feedback in problem solving in vector calculus. Thus, MATLAB could be employed to serve as a tool through which to provide learning and assessment opportunities regarding the spatial reasoning of rural-based pre-service teachers.

RESEARCH METHOD

One hundred rural-based pre-service teachers in second year vector calculus class at UNIZULU (case site) were randomly selected into an experimental group (N = 50) and a control group (N = 50). A quantitative approach was employed in this study. A pre-test (vector calculus) covering the vector calculus topics of vector operations, double integral, and triple integral was given to all participants. These topics were chosen because, their contents demand students' spatial reasoning. This was then followed by teaching interventions for both the control and experimental groups. A two-hour lesson each day for three weeks were given to both groups by one of the researchers.

For the control group, lessons were delivered by using the lecturer's notes and textbook whereby 2D and 3D figures associated with vector integration were drawn on the board and explained. However, for the experimental group the dynamic software, MATLAB was employed in teaching and learning and participants experienced MATLAB activities covering, but not limited to; generating geometric 3D shapes (e.g., cubes, cylinder, rectangular etcetera); viewing 3D shapes in different perspectives; vector fields, vector operations, and performing vector integrations (refer to **Figure 2**).

Instruction was followed by all participants completing the post-test, the ten-items of Section A on the PSVT/R. The PSVT/R was designed to measure the participants' ability to visualise the rotation of threedimensional objects. The instrument was chosen because of its higher correlation with similar instruments measuring visualisation such as the Shepard-Metzler tests. According to Bodner and Guay (1997), these tests are "among the spatial test least likely to be confounded by analytic processing strategies" (p. 13). The format for each item on the PSVT/R is that an object is pictured in one position; it is then paired with another image which is reoriented to a different position. The participants were shown a second object and given five choices for a pairing, one of the choices matched the rotation of the original object example pair. From which data was collected from both groups. Ethics consideration of participants were at all times observed as well as privacy and confidentiality of informants.

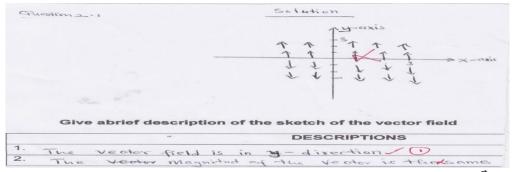


Figure 3. A solution provided by rural-based pre-service teacher for the vector function $\vec{F}(x, y) = x\vec{j}$.

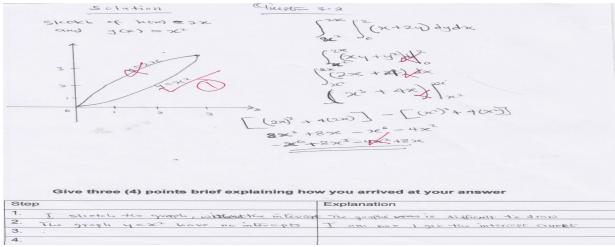


Figure 4. Solution presented by rural-based pre-service teacher for the conversion of the Cartesian coordinate $(x, y, z) = (1, \sqrt{3}, 2)$ to cylindrical coordinates and a graph in cylindrical coordinates

DATA ANALYSIS

Data was collected through a pre-test (vector calculus) and a post-test as defined below.

PRE-TEST (Vector Calculus Test)

In question 1, participants were to match the equations with their respective graphs. In all, 72% of the participants were able to match the equations: $y = x^3$ and xy = 3 to their respective graphs while nearly 28% of the students were able to match the equations $x^2 + y^2 = 3$, $z = x^2 + y^2$ and $\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$ to their respective graphs. Question 2(a) required participants to sketch and describe the vector field of the vector function $\vec{F}(x, y) = x\vec{j}$. Overall, only a few of the participants (10%) sketched the vector field correctly and provided adequate descriptions (refer to **Figure 3**).

Question 2.1(a) required participants to convert the coordinate $(1, \sqrt{3}, 2)$ in cartesian system to cylindrical coordinates system and question 2.2(b) required participants to sketch the cylindrical coordinates calculated in question 2.1a above. Interestingly, while a large number of the participants (85%) were able to do the conversion and provide the respective cylindrical coordinate equation, among these students only 15% were able to correctly sketch the cylindrical coordinates.

Question 3.1. 3.2, and 3.3 were based on multivariate calculus. Question 3.1 required participants to make a 3D sketch of the algebraic equation 2x + y + z = 8. In all, nearly 13% of the participants were able to calculate the x, y, and z intercepts and correctly sketch the respective 3D shape. Participants found it difficult to sketch the graph bounded by the region h(x) = 2x and $g(x) = x^2$ as requested in question 3.2.

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Table 1a. Group statistics on pre-test
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Pre-Test	Group	Ν	Mean	Std. Deviation	Std. Error Mean	
Score	Control Group	50	14.62	3.78	.535	
	Experimental Group	50	15.40	3.65	.516	

 Table 1b. Independent Sample Test for assessing the baseline of the groups

		Levene's Equality of	t-test for Equality of Means							
		F	F Sig.	Т	Df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% confidence interval of the Difference	
									Lower	Upper
Pre -Test Score	Equal variances assumed	.51	.48	-1.05	98	.29	78	.74	-2.26	695
	Equal variances not assumed			-1.05	97.87	.29	78	.74	-2.26	695
T_Value s	ignificant at $n < 0$	05								

T-Value significant at p < 0.05

In question 3.3, the last item of the pre-test, most participants were unable to visualise and give a correct sketch of a cube. However, all those who correctly sketched the cube also correctly performed the integration of the volume.

The pre-test was administered to all 100 participants including those in the control group (N = 50) and the experimental group (N = 50) as indicated in **Table 1a**. The mean pre-test score of the control group was $\bar{x} = 14.62$ with SD = 3.78 and the mean pre-test score for the experimental group was $\bar{x} = 15.40$ with SD = 3.65. An independent sampled test was further carried out to see whether or not there was significant difference of the pre-test score between the two groups (refer to **Table 1b**).

From **Table 1b**, the mean difference between the groups was 0.78 with a t-value 1.05. Since the *p*-value (2-tailled) was 0.29 (p > 0.05), we have reason to conclude that there was no significant difference between the mean pre-test score of the control group and the experimental group. This meant that control and experimental groups were both on a similar baseline before the teaching experiment (treatment).

The pre-test was then followed by teaching interventions for both the control and experimental groups. The control group had lessons using the lecturer's notes and text book. The experimental group employed MATLAB in teaching and learning activities and experienced MALTAB investigations involving, among others: generating 3D shapes (e.g., cubes, cylinder, rectangular etcetera); viewing 3D shapes through different perspectives; vector fields, vector operations, and performing vector integration (refer to **Figures 2a** and **2b**).

Post-Test -Purdue Spatial-Visualisation Test/Rotation (PSVT/R)

Research evidence from many disciplines supports the claim that dynamic environments such as MATHEMATICA, CAS, and MATLAB enhance spatial-visualisation skills. In the field of cognitive science, the ability to accurately predict or imagine orientation of cubes with respect to the time taken is a reflection or measure of one's spatial-visualisation skills. The Purdue Visualisation rotation (Bodner & Guay, 1976) has long been used to measure this aspect of spatial-visualisation skills.

The control group was used as a baseline since statistical evidence from **Tables 1a** and **1b** indicated that the two groups statistically possess similar prior knowledge. The ten-items of Section A on the PSVT/R was administered to both the experimental and the control groups after the treatments. The results were compared using the independent sampled t-test to evaluate if there was significant difference in the mean spatial-visualisation skills difference between the two groups (refer to **Tables 1a** and **1b**).

Table 2a shows the control group obtained the mean score of $\bar{x} = 5.8$ and SD = .85 whereas the experimental group obtained the mean score of $\bar{x} = 6.86$ with SD = 1.18. The independent sample test further revealed that, the mean score difference and the *p*-values of the control group and the experimental group (refer to **Table 2b**).

Spatial-	Group	Ν			Mean			Std. Deviatio	n Std.	Std. Error Mean	
Visualisation Control Group		50			5.86			.86		.12	
Test Score	Experimental Gro	oup 5		50		6.86		1.18	.17		
Table 2b. In	dependent Sam	•									
		Levene's	Test for				t-test for Eq	juality of Mea	ans		
		Equal	ity of								
		Varia	nces								
		F	Sig.	Т	Df	Sig. (2-	Mean	Std. Error	95% confid	dence interva	
						tailed)	Difference	Difference	of the Difference		
									Lower	Upper	
Spatial-	Equal variances	13.423	.000	-4.853	98	.000	-1.000	.206	-1.409	591	
Visualisation	assumed										
Score	Equal variances			-4.853	89.530	.000	-1.000	.206	-1.409	591	

(p < 0.05)

The findings presented in **Table 2b** show that the mean difference between the groups was 1.000 whereas the *p*-value was 0.00 which is lower(p < 0.05). Hence, there was a statistically significant difference between the control group and the experimental group, with the experimental group performing better than the control group.

DISCUSSION

Post-Test Students' Performance Against PSV/RT

The main aim of this study was to assess the impact of MATLAB-infused instruction on rural-based pre-service teachers' spatial-visualisation skills. The experimental group went through MATLAB activities as previously described.

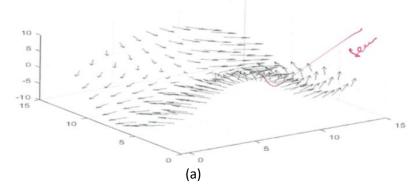
Laboratory Activities

Instructional treatments were followed by an assessment test to assess the impact of the MATLAB on the pre-service teachers' spatial-visualisation skills. After a post-test, descriptive statistics and an independent sample t-test were used and revealed that the experimental group performed significantly better than the control group (refer **Tables 2a** and **2b**). This finding is consistent with some relevant literature which have evidenced that computer-based 3D visualisations support spatial-visualisation skills (Arıcı et al., 2015; Cheng & Mix 2014; Joshi & Rawal, 2021; Mailizar & Fan, 2021). Generating and viewing geometric figures in space, cubes arrangement, and predicting the orientations of cubes are factors identified to have a link with spatial-visualisation skills (Arıcı et al., 2015; Cheng & Mix 2014; Uttal et al., 2013). The positive effects of using MATLAB may stem from the dynamic visual features that enable the creation and animation of 3D display on the screen. The results obtained from Purdue spatial-visualisation/rotation test revealed that the experimental group developed spatial-visualisation skills while learning vector calculus with MATLAB (refer to **Table 2a** and **2b**). This finding appears to support the theory by Cheng and Mix (2014), Uttal et al. (2013), Arıcı et al. (2015) that spatial-visualisation skills are trainable through dynamic visual tools in the context of manipulation and orientation of geometric shapes.

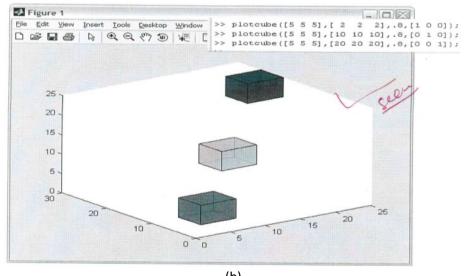
Previously, Shepard and Metzler (1971) made strong arguments about an association between physical manipulation or rotations of the cubes (i.e., mental rotation task) and spatial-visualisation skills. They further claimed that these two variables (mental rotation tasks and spatial-visualisation skills) both share a link with ones' cognitive reasoning. Hubbard et al. (2005), and Park and Brannon (2013) deepened this argument claiming that there are interconnections among mental rotation, spatial-visualisation, and mathematics achievement. This is consistent with the findings with this study.

Laboratory activity 2: Run the following code using the Matlab

figure quiver3(Z,U,V,W) view(-35,45) [Code adopted from mathworks.com in generating the vector field]



Laboratory activity 3: Use the Matlab to generate cube boxes



(b)

Figure 5. A worksheet presented by student (EG015) from the experimental class run the code given to generate vector field and the generating 3D shape with the MATLAB respectively

According to Duval (1995), representation plays a significant role regarding learning and cognitive reasoning. Drawing from this theory, pre-test questions and learning activities for both the control and experimental groups involved the translation of mathematical representations from one register to another. This led to increases in participants' spatial-visualisation skills as measured by their ability to correctly interpret rotations of solids on the post-test.

The experimental group's greater increase in spatial-visualisation score (refer **Table 2a** and **2b**) than the control group supports the claim by Hubbard et al. (2005) and Park and Brannon (2013) that there is a shared link between the dynamic visual tool, MATLAB, mental rotation skills, and spatial-visualisation. From the results obtained in this study, rural-based pre-service teachers can grow in spatial-visual skills.

CONCLUSION AND FUTURE RESEARCH

From the empirical evidence gathered from the research results, we found that the use of MATLAB as a pedagogical tool in traditional classrooms mediates spatial-visualisation skills. Hence, we can conclude that the use of computer and technology dynamic software can be equally employed in rural-based universities to enhance their spatial reasoning skills for smooth mediation of learning. Drawn from the results of the

current study and in relation to (1) the positive effects of using MATLAB as well as (2) the fact that findings tend to support the theory by Arici et al. (2015) and hence (3) improving SV skills: It is accordingly important to situate rural-setting teaching in a) digital technology (MATLAB) and learning, while drawing on spatial reasoning and cognitive reasoning and other mathematical concepts.

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Data availability: Data generated or analysed during this study are available from the authors on request.

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